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Anteromedial thalamic nucleus to anterior cingulate cortex inputs modulate histaminergic itch sensation

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HIGHLIGHTS

- Anteromedial thalamic nucleus (AM) was activated during histaminergic itch.
- Photoactivation of AM induced spontaneous scratching behavior.
- There existed reciprocal neuronal projections between AM and ACC in the brain.
- Disconnection of AM and ACC circuit attenuated histaminergic-induced itch.
- Photoactivation of AM-ACC inputs evoked histaminergic itch sensation.

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ABSTRACT

Itch is an unpleasant feeling that triggers scratching behavior. Much progress has been made in identifying the mechanism of itch at the peripheral and spinal levels, however, itch circuits in the brain remain largely unexplored. We previously found that anterior cingulate cortex (ACC) to dorsal medial striatum (DMS) inputs modulated histamine-induced itch sensation, but how itch information was transmitted to ACC remained unclear. Here, we demonstrated that the anteromedial thalamic nucleus (AM) was activated during histaminergic itch, and there existed reciprocal neuronal projections between AM and ACC. Disconnection between AM and ACC resulted in a significant reduction of histaminergic, but not nonhistaminergic, itch-related scratching behavior. Optogenetic activation of AM-ACC, but not ACC-AM, projections evoked histaminergic itch sensation. Thus, our studies firstly reveal that AM is critical for histaminergic itch sensation and AM-ACC projections modulate histaminergic itch-induced scratching behavior.

1. Introduction

Itch is an uncomfortable sensation that elicits the desire to scratch (Dong and Dong, 2018; Ikoma et al., 2006). Physiological itch protects the body from irritants or invaders by scratching, however, pathological chronic itch causes long-term scratching behavior that results in severe skin and tissue damage (Bautista et al., 2014). Itch is characterized as either histaminergic or nonhistaminergic itch according to the responsiveness to histamine (Bautista et al., 2014; Ikoma et al., 2006; Yosipovitch et al., 2018). The histaminergic itch is induced by histamine that activates histamine receptor 1 (H1R) and histamine receptor 4 (H4R) (Dong and Dong, 2018; Dunford et al., 2007; Shim and Oh, 2008). Both H1R-deficient mice and H4R-deficient mice show a significant defect in histamine-induced scratching behavior, and chemical blockade of H1R or H4R also leads to a remarkable deficit in scratching behavior in response to histamine (Dunford et al., 2007; Roßbach et al., 2009; Sugimoto et al., 2003). To date, the circuits for supraspinal itch modulation underlying histaminergic itch are poorly understood, although much efforts have been devoted to identify the mechanism at the spinal and peripheral levels.

The anterior cingulate cortex (ACC) is implicated in the regulation of emotion, motivation, cognition and encoding reward information

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(Allman et al., 2001; Amiez et al., 2006; Bush et al., 2000; Hadland et al., 2003; Shima and Tanji, 1998). A series of recent studies indicated that ACC was activated during pruritogenic stimulation (Mochizuki et al., 2003; Wang et al., 2015), and the activity of ACC decreased during repeated scratching behavior (Herde et al., 2007; Yosipovitch et al., 2008). Our subsequent studies provided a more nuanced view of ACC function, demonstrating that the neural projections from ACC to dorsal medial striatum (DMS) constituted a critical circuit element for regulating histaminergic itch. At the circuit level, how itch information is transmitted to ACC remains unclear.

Thalamus plays a critical role in sensory conduction that replays sensory information from the spinal cord to the cerebral cortex. Previous studies indicate that itch information is transmitted to the contralateral thalamus from spinal cord (Akiyama et al., 2015; Andrew and Craig, 2001a; Davidson et al., 2009b, 2012b). Spinothalamic tract neurons respond to pruritogen and transmit itch information to the brain (Andrew and Craig, 2001a; Davidson et al., 2009b, 2012a, 2012b). These evidence suggest that thalamus may be a potential upstream brain region of ACC, and transmit pruritic information from spinal cord to ACC. Indeed, recent studies have reported that some pruritogen-induced itch activates both ACC and thalamus (Leknes et al., 2007a; Mochizuki et al., 2015; Papoiu et al., 2013a; Vierow et al., 2009). Thalamus is a heterogeneous structure and anatomically divided into the anterior nucleus (ATN), the medial nucleus and the lateral nucleus, and ATN are composed of anterior ventral nuclei (AV), anterior dorsal nuclei (AD) and anterior medial nuclei (AM) (Child and Benarroch, 2013; Jung, 1938). ATN is known to be activated by histamine and non-histamine pruritogen (Leknes et al., 2007b; Papoiu et al., 2013a). How thalamic subregions integrate into a central neural circuit for itch sensation is not known. In the present study, we investigate the role of AM in itch sensation and study whether projections between AM and ACC constitute a neural circuit that modulate histamine-elicited scratching behavior.

2. Materials and methods

2.1. Animals

Adult male C57BL/6J mice weighing 22–24 g were housed in a temperature controlled room (21–24 ˚C) on a 12 h light/dark cycle (lights on from 7 a.m. to 7 p.m.). All mice were group-housed (four mice/cage) and allowed free access to food and water. All animal experiments were performed under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals using an approved animal protocol (2019-01-LJG-41).

2.2. Materials

Histamine, 5-HT, NMDA, and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, USA). AAV was purchased from OBiO Technology Corp., Ltd. (Shanghai, China). C-fos antibody (rabbit anti c-fos) and Diphenhydramine were purchased from Santa Cruz Bio Biotechnology (Santa Cruz, CA, USA). CTB-Alexa 488 and secondary antibody (Alexa Fluor 488 goat anti-rabbit) were purchased from Thermo Fisher Scientific (Rockford, IL). Vetbond Tissue Adhesive was purchased from 3M Animal Care Products (St Paul, MN, USA). TritonX-100, saline, NaCl, KCl, Na2HPO4, and sucrose were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Normal goat serum was purchased from Wuhan Boster Biological Technology, Ltd. (Wuhan, China). Sodium pentobarbital was purchased from Shanghai Westang Bio-Tech Co, Ltd. (Shanghai, China). JNJ 7777120 and chloroquine were purchased from Selleck (Shanghai, China).

2.3. Animal behavior tests

Before formal experiment, mice were allowed to explore the chamber for 15 min to acclimate the chamber. The recording chamber was surrounded by four mirrors and the scratching behavior in mice was recorded by a digital video camera (C525; Logitech). A lifting of either hindpaw to scratch any part of the body followed by replacing it back to the floor was defined as one bout of scratching.

2.4. Pruritogen-induced itch response

Scratching behavior was acutely induced by subcutaneous injection of 50 μl pruritogens histamine (10 μg/μl in saline), chloroquine (4 μg/μl in saline), 5-HT (1.5 μg/μl in saline) into the nape of neck in mice. The number of scratching bouts was assessed within 30 min after pruritogens injection.

2.5. Locomotor activity

Mice were placed into the locomotor chambers (30 × 30 × 30 cm) equipped with video recorder for 30 min. The distance (in millimeter) moved were analyzed using locomotor activity analysis software.

2.6. Balance beam test

A horizontal flat beam of 12 mm width was selected for the experiment. The two ends of the balance beam were settled 50 cm above the floor. One end of the balance beam was the initial area, and there was strong light stimulation in the initial area to exert an aversive stimulus. The terminal point of the balance beam was a platform, on which a black cardboard box with material from the home cage is placed. Two days before test, mice were trained to pass the balance beam three times daily. On the third day, the time of mice successfully passing the beam was recorded. Mice passed the beam twice. The average value of time was taken as the final result.

2.7. Cannula implantation and microinjection

Mice were anesthetized with sodium pentobarbital (70 mg/kg, i.p.) and fixed on a stereotaxic apparatus and then the skulls were exposed. The cannulas were implanted above the bilateral AM (anteroposterior (AP), −0.66 mm; mediolateral (ML), ± 0.50 mm; dorsoventral (DV), −2.80 mm), and cemented to two anchoring screws on the skull. After one week recovering from surgery, mice were microinjected with NMDA (0.06 mM, 0.2 μl) into AM. Bilateral microinfusions were made through a 33 gauge extended 1 mm beyond the tip of the guide cannula needle connected to a 10 μl Hamilton microsyringe mounted on a microinfusion pump (Harvard apparatus) into the AM. The rate of infusion was 0.2 μl/min and the total infusion volume was 0.2 μl per side. After microinjection, the needle was retained in place for 3 min to ensure reagent diffusion. Then the mice were put into the recording chamber and the scratching behavior was recorded immediately for 30 min.

2.8. CTB-Alexa 488 tracing

Mice were anesthetized with sodium pentobarbital (70 mg/kg, i.p.) and fixed on a stereotaxic apparatus and then the skulls were exposed. Microinjections were made through a 33 gauge needle connected to a 10 μl Hamilton microsyringe mounted on a microinfusion pump (Harvard apparatus) into the corresponding nuclei. The retrograde tracer CTB-Alexa 488 was microinjected into the ACC (AP, +0.66 mm; ML, +0.25 mm; DV, −2.0 mm) or the AM (AP, −0.66 mm; ML, +0.50 mm; DV, −3.80 mm). The rate of infusion was 0.1 μl/min and the total infusion volume was 0.2 μl per side. Then the needle was retained in place for 10 min for CTB-Alexa 488 diffusion. Mice were allowed to recover from surgery in their home cage for about 3 weeks, and the signals of CTB-Alexa 488 were detected by using an Olympus FV1000 confocal laser scanning microscope. The excitation wavelength was 488 nm for green channel. High-resolution 12-bit images (1024 × 1024 pixels) were set using a 10× or 20× objective lens.

2.9. Disconnection of AM and ACC circuit

Mice were microinjected with excitotoxin quinolinic acid (15 μg/μl), one side into unilateral ACC (AP, +0.66 mm; ML, +0.25 mm; DV, −2.0 mm) and the other side into contralateral AM (AP, −0.66 mm; ML, −0.50 mm; DV, −3.80 mm). The rate of infusion was 0.2 μl/min and the total infusion volume was 0.2 μl per side. Then the needle was retained in place for 6 more minutes to ensure reagent diffusion. After one week recovering from surgery, mice were subcutaneously injected with pruritogen. The scratching behavior was recorded for 30 min immediately after pruritogen injection.

2.10. Virus injection

In optical activation of AM experiments, 0.3 μl of AAV-CaMK2α-ChR2-EYFP or AAV-CaMK2α-EYFP was microinjected into the unilateral AM (AP, −0.66 mm; ML, −0.50 mm; DV, −3.80 mm) at a rate of 0.1 μl/min. After microinjection, the needle was retained in place for 10 min to ensure virus diffusion. After the AAV infusion, an optic fiber (diameter, 200 μm; NA, 0.37) was implanted above the ipsilateral AM (AP, −0.66 mm; ML, −0.50 mm; DV, −3.30 mm). The optic fiber was cemented to two anchoring screws on the skull and tissue adhesive (Vetbond Tissue Adhesive; 3M) was applied to the skull surface, and then the skull surface was covered with dental cement immediately. In optogenetic activation of AM-ACC projections experiments, 0.3 μl of AAV-CaMK2α-ChR2-EYFP or AAV-CaMK2α-EYFP was microinjected into the unilateral AM (AP, −0.66 mm; ML, −0.50 mm; DV, −3.80 mm), and an optic fiber (diameter, 200 μm; NA, 0.37) was implanted above the ipsilateral ACC (AP, +0.66 mm; ML, −0.25 mm; DV, −1.5 mm). In optogenetic activation of ACC-AM projections experiments, 0.3 μl of AAV-CaMK2α-ChR2-EYFP or AAV-CaMK2α-EYFP was microinjected into the unilateral ACC (AP, +0.66 mm; ML, −0.25 mm; DV, −2.0 mm), and an optic fiber (diameter, 200 μm; NA, 0.37) was implanted above the ipsilateral AM (AP, −0.66 mm; ML, −0.50 mm; DV, −3.30 mm).

2.11. Optical stimulation

Fiber-coupled laser source (Anilab) was connected to the implanted fiber optic via a rotatory joint patch cable (NA 0.37; Anilab). Optical illumination was modulated by a stimulus generator software (AniOptover 2.0). Mice received 473 nm blue-light illumination (5 mW, 20 Hz, 5 ms pulses) for 3 min and then rested for 2 min without light illumination. This “light-rest” cycle was defined as one S-R cycle (5 min) (Lu et al., 2018). After total six S-R cycles for 30 min, the scratching bouts were recorded for 30 min. All mice were killed and the brains were sectioned to verify an appropriate virus expression and optical fiber implantation after the behavioral tests. If the virus extended to the nearby regions, the data were excluded from further analysis.

2.12. Immunohistochemistry

Mice were subcutaneously injected with pruritogen histamine or saline. Then mice were anesthetized by sodium pentobarbital after injection and perfused with 4% PFA (paraformaldehyde). Then the brains were removed and kept in 4% PFA overnight for postfixation. After postfixation, the brains were kept in 30% sucrose solution for dehydration. Coronal sections (30 mm in thickness) were cut on a cryostat microtome and immunostaining was applied to detect the expression of c-fos in AM. The sections were incubated in PBS with 0.3% TritonX-100 and 3% normal goat serum and primary antibody (rabbit anti-c-fos, 1:500; Santa Cruz Bio Biotechnology) overnight at 4 ˚C. Then the sections were incubated with the secondary antibody (Alexa Fluor 488 goat anti-rabbit, 1:500; Invitrogen). The optical sections were captured by using Olympus FV-1000 confocal laser scanning microscope. High-resolution 12-bit images (1024 × 1024 pixels) were set using a 20× objective lens. The excitation wavelength was 405 nm for blue channel and 488 nm for green channel.

2.13. Histology

After the behavioral test, mice were killed and the brains were sectioned to detect the cannulas/injection sites by Nissl staining. All images were recorded by using Olympus inverted fluorescence microscope. High-resolution images (1360 × 1024 pixels) were acquired using a 4× objective lens. Animals were only kept for analysis when the cannulas/injection were placed in the right sites.

2.14. Data analysis

All data were expressed as mean ± SEM. Single-variable differences were analyzed by two-tailed and unpaired Student's t tests. Grouped differences were analyzed with One- or Two-way analysis of variance (ANOVA) followed by Bonferroni posttest. Statistical analyses were conducted using GraphPad Prism 7 software. Statistical significance was defined as $p < 0\ 0.05$.

3. Results

3.1. AM was involved in itch-related scratching behavior

Thalamus as a heterogeneous structure is subdivided into different regions, and the function of these subregions in itch sensation is little known. After subcutaneous injection of histamine into the nape of neck (Lu et al., 2018), we found that mice exhibited significant scratching bouts (Fig. 1A) and c-fos expression was significantly increased in the AM (Fig. 1B and C). We further microinjected with glutamate receptor agonist NMDA into AM, and found that NMDA significantly increased spontaneous scratching behavior (Fig. 1D). To confirm the function of AM in itch sensation, we used optogenetic approach to activate AM and detected scratching behavior in mice. AM mainly expresses glutamatergic neurons (Barroso-Chinea et al., 2007; Zakowski, 2017). Since CaMK2α expresses selectively in excitatory glutamatergic neurons rather than inhibitory interneurons in the cortex and thalamus (Liu and Jones, 1996; Lu et al., 2018; Tye et al., 2011), CaMK2α can be used as a promoter of virus to specifically express the channelrhodopsin in glutamatergic neurons of AM. We thus microinjected adeno-associated virus (AAV) carrying CaMK2α-ChR2-EYFP into AM, and implanted the optic fiber above the AM. The schematics of AAV vector and the representative injection sites were shown in Fig. 1E. After 5 weeks, the green fluorescence signals (EYFP) were detected in AM (Fig. 1F). A 30-minute “light-rest” cycle (Lu et al., 2018) was set and a 473 nm laser was applied (Fig. 1G). We found that illumination of AM significantly increased spontaneous scratching behavior (Fig. 1H), but did not affect mice locomotor activity and motor coordination (Fig. 1I and J).

3.2. Neuronal projections between AM and ACC were required for histaminergic itch sensation

Functional image studies have reported that pruritogen-induced itch activates both ACC and thalamus. To verify whether neural projections between ACC and AM exist in the brain, CTB-Alexa 488 is applied for retrograde tracing. As shown in Fig. 2, when CTB-Alexa 488 was microinjected into ACC, abundant tracer signals were detected in the AM (Fig. 2A and B). Similarly, when CTB-Alexa 488 was injected into AM, robust tracer signals were also detected in the ACC (Fig. 2C and D). These results indicate that there are reciprocal neural projections between AM and ACC.

Next we asked whether the neuronal projections between AM and ACC are involved in histaminergic itch. We carried out the lesion of AM in one hemisphere and ACC in the other by using excitotoxin quinolinic

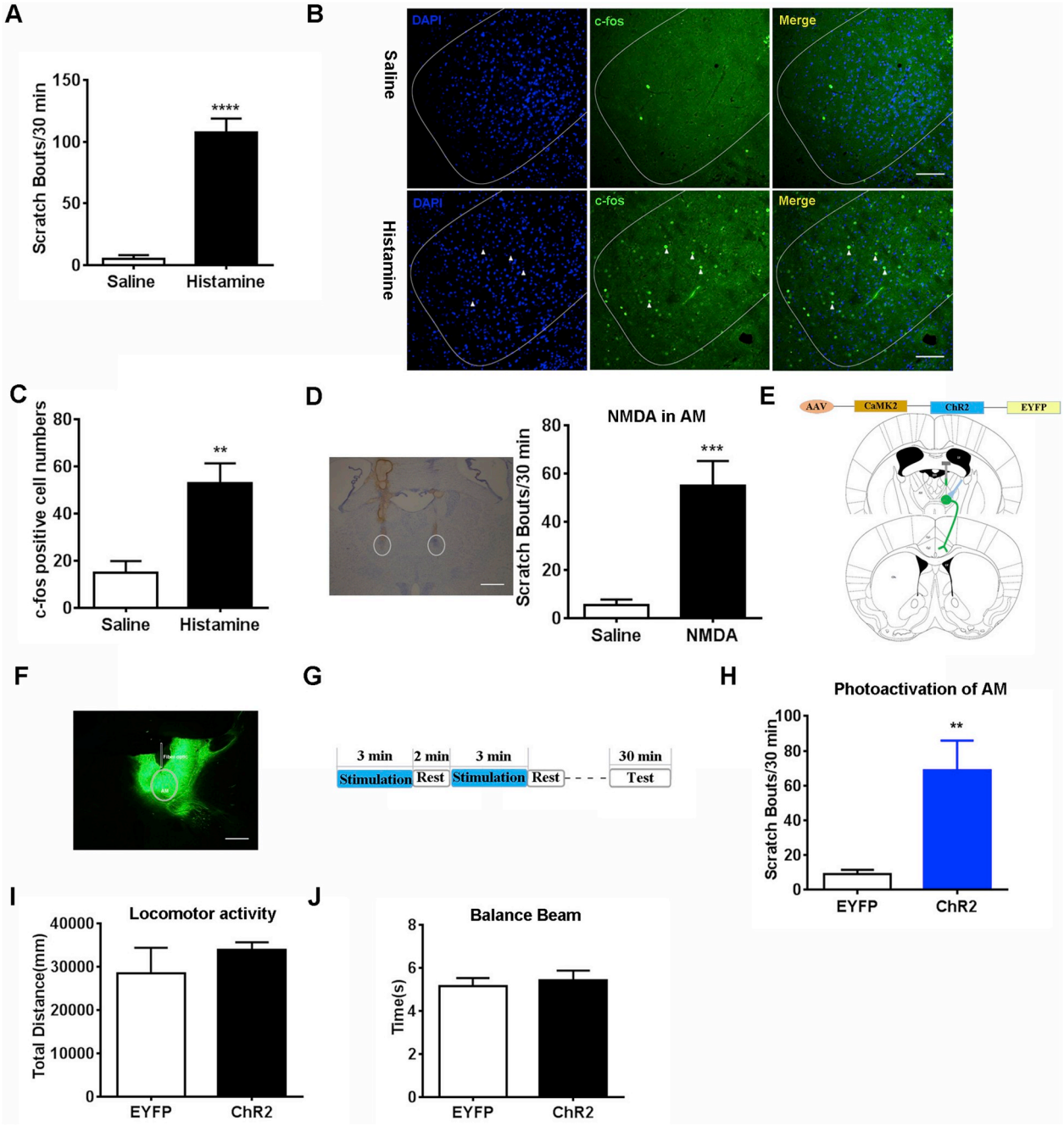

Fig. 1. Activation of AM significantly increased scratching behavior.
A, After subcutaneous injection of histamine into the nape of neck, mice exhibited significant increased scratching bouts. B, High resolution images of c-fos. Scale bar, 100 μm. C, C-fos positive cell numbers were significantly increased in the AM after histamine injection. D, Microinjection of NMDA into AM increased spontaneous scratching behavior. Left, the brain sections of AM for Nissl staining. Scale bar, 100 μm. Right, the scratching behavior was recorded. E, Schematic illustration of injection of AAV carrying ChR2-EYFP into AM and optical stimulation of AM. F, ChR2-EYFP expression in AM. Scale bar, 100 μm. G, Experiment paradigm of optical illumination and behavior test. Light illumination for 3 min, followed by 2 min of rest time without light illumination, and total six cycles for 30 min. H, Mice expressing ChR2-EYFP displayed significant increased spontaneous scratching behavior after 30 min of repeated stimulation–rest cycles. I, Photoactivation of the AM has no effect on mice locomotor activity. J, Photoactivation of the AM has no effect on mice motor coordination. All data are shown as mean ± SEM. **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with Saline group, **p < 0.01 compared with EYFP group. n = 6 to 11 mice for each group.

acid to bilaterally disrupt interhemispheric neural communication between AM and ACC (Fig. 3A and B). As shown in Fig. 3, AM and ACC disconnection significantly decreased histamine-induced scratching behavior (Fig. 3C) but did not affect chloroquine- and 5-HT-induced non-histaminergic itch (Fig. 3D and E). These results suggest that projections between ACC and AM play a key role in histaminergic itch sensation.

3.3. Optogenetic activation of AM-ACC, but not ACC-AM, inputs evoked histamine-related itch responses

Although our results demonstrate that the projections between AM and ACC are involved in itch sensation, whether there is direct neural pathway between AM and ACC is unclear. We therefore used optogenetic approach to specifically activation of AM-ACC or ACC-AM neural pathway. We first microinjected AAV carrying CaMK2α-ChR2-EYFP

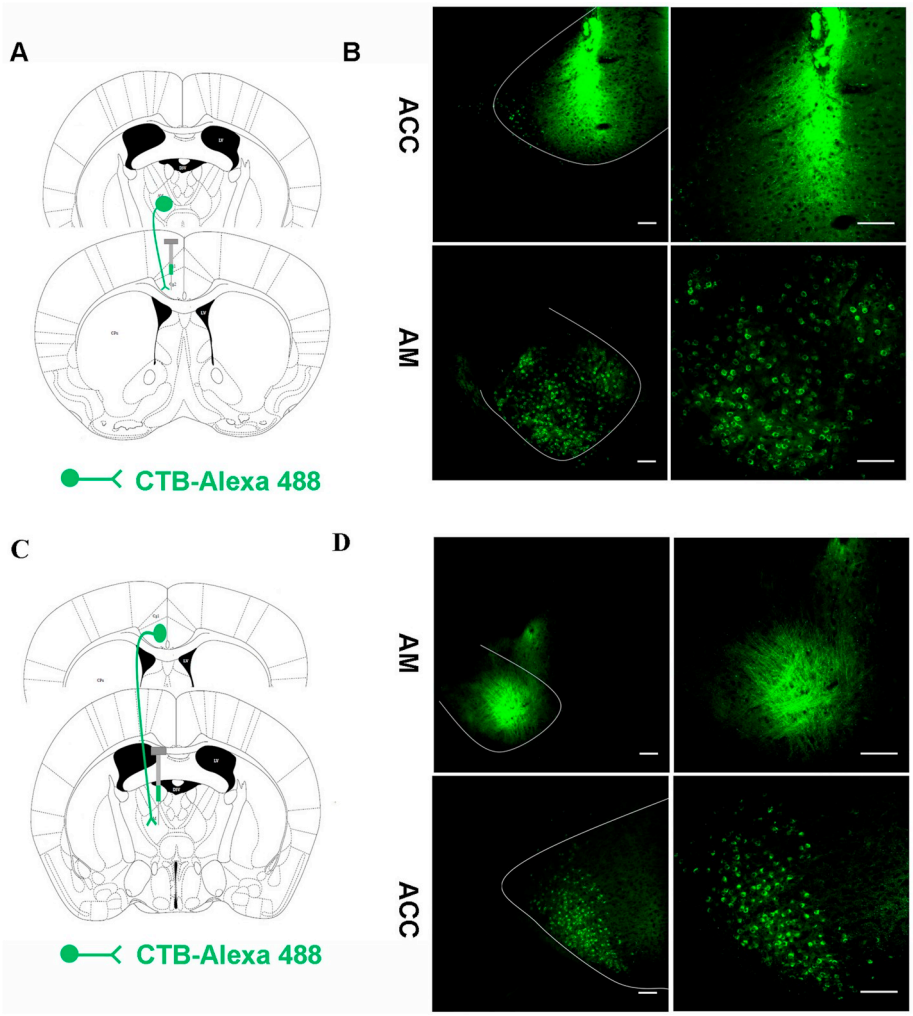

Fig. 2. Reciprocal Neuronal projections between AM and ACC.
A, Schematic depiction of neural projections from AM to ACC. B, AM neurons project to ACC revealed by CTB-Alexa 488. Upper, CTB-Alexa 488 was microinjected into ACC. Lower, tracer signals in AM by CTB-Alexa 488 retrograding from ACC. Scale bar, 100 μm. C, Schematic depiction of neural projections from ACC to AM. D, ACC neurons project to AM revealed by CTB-Alexa 488. Upper, CTB-Alexa 488 was microinjected into AM. Lower, tracer signals in ACC by CTB-Alexa 488 retrograding from AM. Scale bar, 100 μm n = 3 mice for each group.

into AM, and implanted an optic fiber above the ACC (Fig. 4A). Green fluorescence signals were detected in ACC 5 weeks later (Fig. 4B). A 473 nm laser was used to stimulate the projections. After a 30 min "light-rest" cycle (Lu et al., 2018), photoactivation of AM-ACC inputs significantly increased spontaneous scratching behavior (Fig. 4C), but did not affect athletic ability of mice (Fig. 4D and E). Both H1R and H4R are involved in histaminergic itch (Dong and Dong, 2018; Dunford et al., 2007; Roßbach et al., 2009; Shim and Oh, 2008; Sugimoto et al., 2003; Thurmond et al., 2015). We further found that photoactivation of AM-ACC inputs-induced scratching behavior was significantly inhibited by H1R antagonist diphenhydramine (Fig. 4F) and H4R antagonist JNJ 7777120 (Fig. 4G), which indicate that photoactivation-induced scratching behavior was due to histaminergic itch sensation. H1R antagonist diphenhydramine did not affect mice locomotor activity and motor coordination (Supplementary Fig. 1). Next, we microinjected adeno-associated virus carrying CaMK2α-ChR2-EYFP into ACC, and implanted the optic fiber above the AM (Fig. 5A). 5 weeks later, the green fluorescence signals were also detected in the AM (Fig. 5B). However, optogenetic activation of ACC-AM pathway did not cause any scratching behavior (Fig. 5C). Thus, these findings suggest that AM-ACC, but not ACC-AM, projections mediate histaminergic itch-related scratching behaviors.

4. Discussion

In the present study, we firstly present evidence that AM-ACC projections modulate histaminergic itch-related scratching behavior. The result is important because it implicates a critical requirement for AM in itch sensation, and provides a precise functional connectivity between AM and ACC in controlling histaminergic itch.

One major finding of the present study is that AM is the key subregion of thalamus that modulates itch-related scratching behavior. The conclusion is supported by the following evidences. Firstly, AM was detected with significantly increased c-fos expression after histamine stimulation. Second, microinjection of NMDA into AM caused significant spontaneous scratching bouts. Thirdly, photoactivation of AM neuronal bodies induced significant scratching bouts. Our result is consistent with previous study indicating that thalamus is a central relay for itch sensation (Andrew and Craig, 2001b; Davidson et al., 2009a, 2012a; Leknes et al., 2007a; Papoiu et al., 2013b).

Another major finding of the present study is that AM-ACC, but not ACC-AM, projections evoke histaminergic itch-related scratching behavior. We firstly showed that disconnection of AM and ACC projections with excitotoxin quinolinic acid attenuated histamine-induced itch, but not affecting chloroquine- and 5-HT-induced non-

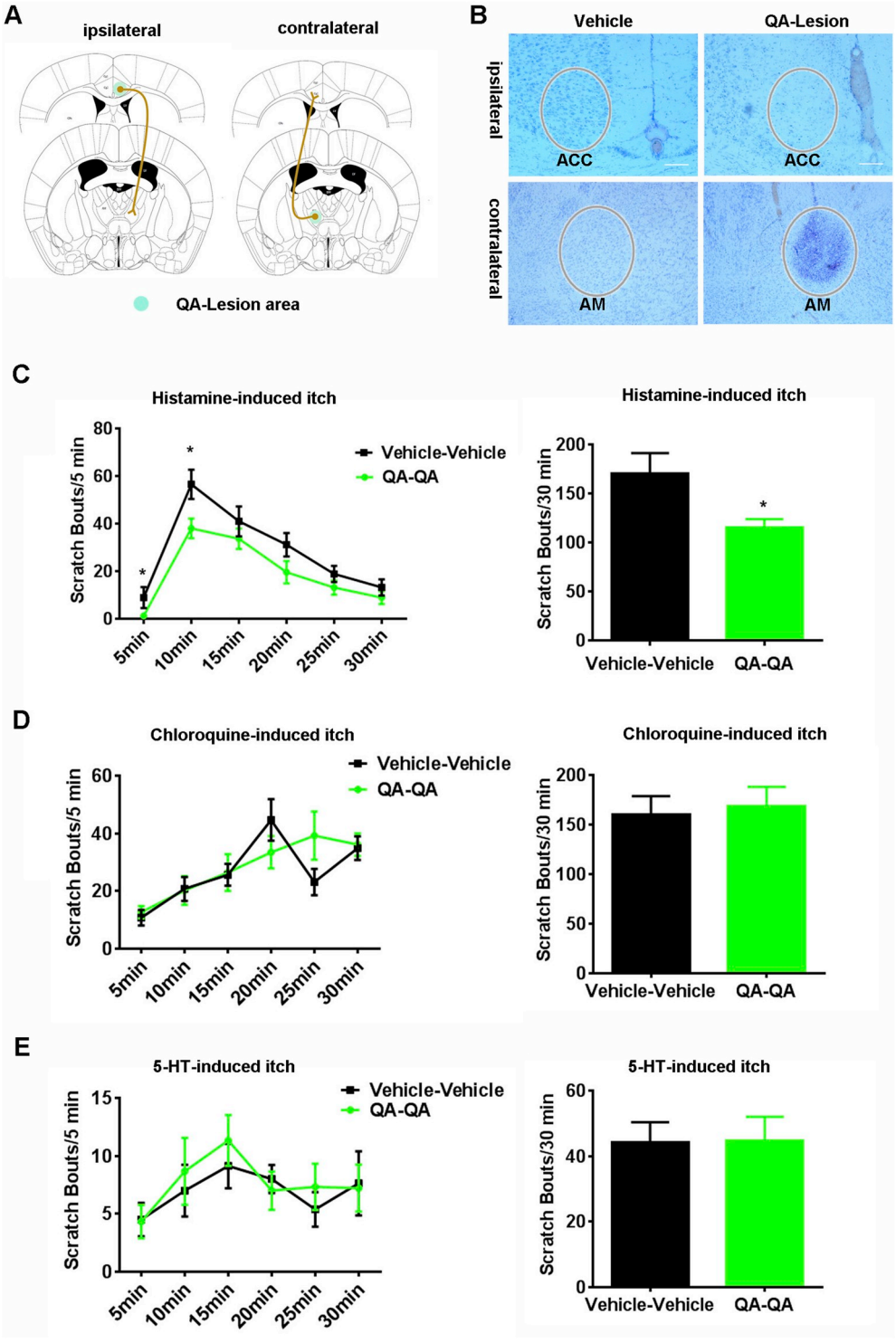

Fig. 3. Disconnection of AM and ACC projections with quinolinic acid attenuated histaminergic itch, without affecting non-histaminergic itch.
A, Schematic illustration of contralateral lesions of AM and ACC. B, The brain sections of AM and ACC for Nissl staining to verify neural lesion. Scale bar, 100 μm. C, Disconnection of AM and ACC circuit attenuated the scratching behavior induced by subcutaneous injection of histamine into the nape of neck. Histamine was dissolved in saline at the concentration of 10 μg/μl, and then 50 μl was given to each animal. D, E, Disconnection of AM and ACC circuit did not affect the scratching behaviors induced by subcutaneous injection of 50 μl chloroquine (4 μg/μl in saline) or 50 μl 5-HT (1.5 μg/μl in saline). All data are shown as mean ± SEM. Two-way ANOVA. *P < 0.05 compared with Vehicle group, n = 8 to 15 mice for each group.

histaminergic itch. Secondly, photoactivation of AM-ACC projections evoked itch-related scratching behavior, and this effect could be attenuated by H1R antagonist diphenhydramine and H4R antagonist JNJ 7777120, while photoactivation of ACC-AM did not induce any spontaneous itch-related scratching behavior. Previous studies have identified that AM is the only nucleus of ATN that has extensive reciprocal projections with ACC (Child and Benarroch, 2013). Our work provides direct evidence demonstrating that the reciprocal neuronal projections between AM and ACC exist in the brain, and these projections are involved in modulation of histaminergic itch. One possible explanation for modulation of histaminergic itch by AM-ACC projections but not ACC-AM projections is that AM may process itch signal transmitted from itch-sensing neurons in spinal cord and convey it to cortex (Akiyama et al., 2012, 2015; Davidson et al., 2007, 2009a, 2012a; Moser and Giesler, 2014). All information that reaches the cerebral cortex must pass through the thalamus first, and the thalamus is the only provider of sensation and subcortical information to cortex (Sherman, 2017; Sherman and Guillery, 2002). Together with our previous findings showing that anterior cingulate cortex (ACC) to dorsal medial striatum (DMS) inputs modulate histaminergic itch sensation, it is reasonable to conclude that ACC-DMS inputs might be the downstream target of AM in modulating histaminergic itch-elicited

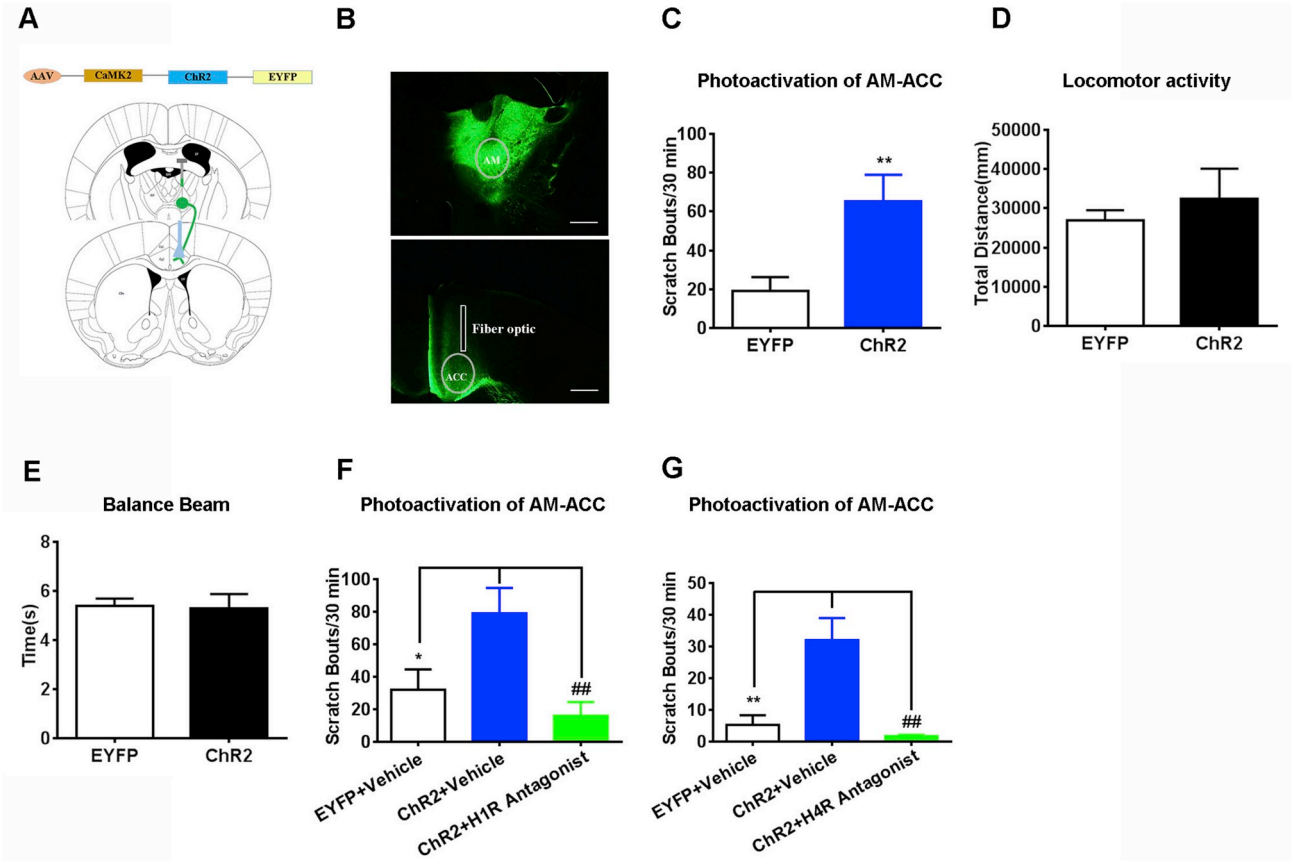

Fig. 4. Photoactivation of AM-ACC projections evoked histamine-related scratching behavior.
A, Schematic illustration of injection of AAV carrying ChR2-EYFP into AM and optical illumination of ACC. B, ChR2-EYFP expression in AM and ACC. Scale bar, 100 μm. C, Mice expressing ChR2-EYFP displayed scratching behavior after 30 min of repeated stimulation–rest cycles. D, E, Photoactivation of AM-ACC inputs has no effect on mice locomotor activity and motor coordination. F, G, Intraperitoneal injection of diphenhydramine (H1R antagonist, 10 mg/kg) and JNJ 7777120 (H4R antagonist, 15 mg/kg) greatly attenuated scratching behavior induced by photoactivation of AM-ACC inputs. All data are shown as mean ± SEM. $^{**}p < 0.01$ compared with EYFP group, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with ChR2 + Vehicle group, $^{\#\#}p < 0.01$ compared with ChR2 + H1R Antagonist group, $^{\#\#}p < 0.01$ compared with ChR2 + H4R Antagonist group. n = 6 to 11 mice for each group.

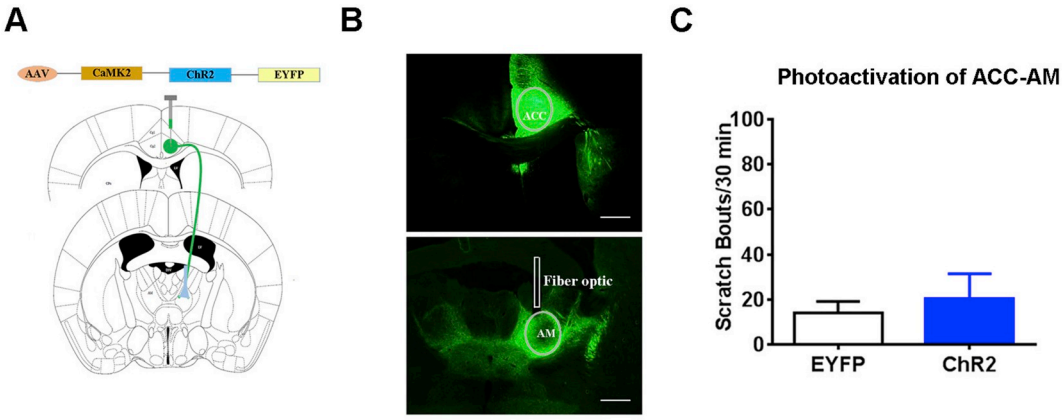

Fig. 5. Photoactivation of ACC-AM projections did not induce scratching behavior.
A, Schematic illustration of injection of AAV carrying ChR2-EYFP into ACC and optical illumination of AM. B, ChR2-EYFP expression in ACC and AM. Scale bar, 100 μm. C, Photoactivation of ACC-AM projections did not induce spontaneous scratching behavior. All data are shown as mean ± SEM. n = 6 mice for each group.

scratching behavior. Itch behavior is determined by the interaction between the cerebral excitatory itch circuit and the spinal inhibitory itch circuit (Dong and Dong, 2018; Kardon et al., 2014; Lu et al., 2018; Ross et al., 2010). ACC to DMS projections modulate histaminergic itch sensation, and dorsal spinal cord exerts an in inhibitory effect on itch signal from ACC-DMS projections through inhibitory interneurons (B5–I neurons) (Lu et al., 2018). Inhibitory of B5–I neurons in the dorsal spinal cord may induce the release of endogenous pruritogens in the periphery (Kardon et al., 2014; Ross et al., 2010). Thus, our observation that histamine H1 and H4 receptor antagonists injections into the mice abolished photoactivation of AM-ACC inputs-induced scratching behaviors may suggest that light activation of AM-ACC inputs results in inhibition of inhibitory B5–I neurons in the dorsal spinal cord, which in turn causes activation of pruriceptors via increasing histamine release

in the peripheral, while whether AM-ACC projections modulate scratching behavior by acting on the dorsal spinal cord is not explored in the present work, and a disynaptic circuit from AM through ACC to DMS is not identified yet. More studies are needed and are ongoing in our laboratory.

Whether the neuronal circuits between AM and ACC also involved in chronic itch are not explored in the present work. In addition, itch and pain are two different types of sensation, but they share some common neural basis and molecular mechanism (Bautista et al., 2014; Davidson and Giesler, 2010; Ikoma et al., 2006; Lu et al., 2018). The role of AM-ACC inputs in pain responses needs further investigation.

In conclusion, our study demonstrates that histamine-induced itch-related scratching behavior is modulated by AM-ACC projections and this work provides new insight into the mechanism of itch at the supraspinal level. This study firstly reveals that AM-ACC inputs constitute a supraspinal circuit for modulating histaminergic itch sensation.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

CRediT authorship contribution statement

Ying-Zhi Deng: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Yu-Chen Lu:** Investigation, Methodology. **Wei-Wei Wu:** Methodology. **Li Cheng:** Methodology. **Gui-Ying Zan:** Methodology. **Jing-Rui Chai:** Methodology. **Yu-Jun Wang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Zhong Chen:** Conceptualization. **Jing-Gen Liu:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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