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Rostral ventromedial medulla astrocytes regulate chronic itch and anxiety-related behaviors

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ABSTRACT

Itch (pruritus), a maladaptive and debilitating cutaneous symptom, is commonly associated with many skin conditions; however, the available therapies with sufficient efficacy are lacking. The role of astrocytes in the rostral ventromedial medulla (RVM), a crucial brain region in the descending pain modulation system, in chronic itch remains uncertain. In this study, we examined the chronic itch behavior and itch-related anxiety behavior in the diphenylcyclopripenone (DCP)-induced contact dermatitis mice, and also observed the activation of astrocytes in the RVM in the DCP mice. Reducing calcium signaling in astrocytes through global IP3R2 gene knockout, conditional astroglial IP3R2 gene knockout in the RVM, or microinjection of AAV-GfaABC1D-hPMCA2 w/b into the RVM, exhibited an anti-pruritic effect on the chronic itch. These findings suggest that RVM astrocytes play a role in regulating chronic itch, and interventions targeting astrocytic activation may offer potential relief for chronic itch.

1. Introduction

Itch (pruritus) is a bothersome sensation that frequently elicits the urge or reflex to scratch. Chronic pruritus, lasting for a duration exceeding six weeks, represents a challenging symptom linked to a diverse array of medical conditions, encompassing dermatological disorders (e.g., atopic dermatitis, psoriasis), systemic illnesses (e.g., hepatic or renal dysfunction), neurological disorders, and certain malignancies (Stander et al., 2022; Yosipovitch et al., 2018). Recalcitrant and severe chronic pruritus may additionally precipitate adverse psychological states, such as anxiety, depression, and insomnia, thereby significantly impacting the quality of life of patients (Sanders and Akiyama, 2018; Silverberg, 2019). Currently, the management of chronic pruritus poses significant challenges within the clinical setting. Therefore, a deeper comprehension of the underlying mechanisms of itch is essential for the advancement of efficacious therapeutic interventions.

Substantial advancements in the understanding of itch have been achieved over the past two decades. It is widely acknowledged that pruritic stimuli are detected, transduced, and transmitted from the dorsal root ganglion (DRG) to the dorsal horn (Mahmoud et al., 2023). Subsequently, the processed signal is transmitted to various brain regions, encompassing those involved in sensory discrimination and emotional reactions to itch (Mu and Sun, 2022).

In addition to ascending transmission, research has revealed the involvement of various supraspinal neural circuits in the descending modulation of itch, including the periaqueductal gray (PAG), parabrachial nucleus, nucleus accumbens, and anterior cingulate cortex (ACC) (Mu and Sun, 2022; Nguyen et al., 2023). The rostral ventromedial medulla (RVM), a brainstem region known for its pivotal role in pain modulation, has also been implicated in itch modulation (Follansbee et al., 2022; Nguyen et al., 2022).

Nevertheless, the precise function of the RVM in itch modulation

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remains uncertain. It is noteworthy that glial cells, particularly astrocytes and microglia, play a significant role in central sensitization, contributing to chronic pruritus (Gao et al., 2023; Ji et al., 2019). Astrocytes, a type of glial cell in the central nervous system, have been implicated in the central sensitization of itch, potentially contributing to the development of chronic itch conditions (Green and Dong, 2015; Ji et al., 2019). The activation of astrocytes can enhance and prolong itch signals, thereby perpetuating the sensation. Prolonged activation of astrocytes in the spinal cord has been observed in mouse models of atopic and contact dermatitis (Du et al., 2019; Shiratori-Hayashi et al., 2015). Administration of the astroglial inhibitor l-α-aminoadipate via intrathecal injection reduced chronic itch following dry skin injury in mice (Liu et al., 2016). However, research on the cerebral effects of astrocytes in the development of chronic itch remains limited. The potential role of astrocytes in the RVM in the pathogenesis of chronic itch and related conditions warrants further investigation.

In this study, utilizing a multidimensional approach, we aim to investigate the involvement of astrocytes in the RVM in chronic itch and associated anxiety-like behavior in a mouse model of atopic contact dermatitis induced by diphenylcyclopripenone (DCP). It will provide valuable insights into understanding the neural mechanisms underlying itch sensation and may inform the development of novel therapeutic approaches for chronic itch disorders.

2. Materials and methods

2.1. Animals and ethical statement

Adult male C57BL/6J mice (aged 8–10 weeks and weighing 20–25 g) were purchased from the Experimental Animal Center, Chinese Academy of Sciences, Shanghai, China. IP3R2 KO mice (C57BL/6-Itpr2em1Smoc, NM-KO-190976, Shanghai Model Organisms) were provided by Dr. Fengfei Ding at Basic Medical College of Fudan University. IP3R2 flox mice were kindly shared by Kunfu Ouyang from Peking University. The animals were housed in groups of four mice per cage, with food and water ad libitum, and maintained under controlled conditions at a temperature of 22 ± 1 °C with a standard 12-h light/12-h dark cycle. The mice were habituated for at least one week prior to the experimental manipulations. All experimental procedures were carried out in compliance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ethical standards set forth by the International Association for the Study of Pain, as well as the Animal Research Welfare Council of the School of Basic Medical Science, Fudan University (20230301-012). All efforts were made to minimize the number of animals used and their suffering.

2.2. DCP-induced allergic contact dermatitis model

DCP was utilized to induce an allergic contact dermatitis model of chronic itch in accordance with established protocols (Yang et al., 2023). Briefly, the nape of mice was shaved at least three days prior to the experiment. During the sensitization stage, 200 μl of a 1% DCP solution in acetone was applied to the shaved skin. Five days later, a 0.5% DCP solution was applied for seven consecutive days as the challenge stage. Sham mice underwent the same procedure with acetone alone. Spontaneous scratching behaviors were automatically recorded for 60 min using the magnetic induction method following DCP application.

2.3. Virus injection

Mice were anesthetized and placed onto a stereotaxic frame (RWD, Shenzhen, China). A 100-nL virus solution was administered via microinjection into the RVM at coordinates of bregma −5.9 mm, lateral 0.0 mm, and ventral −5.9 mm. This procedure was performed using a calibrated glass microelectrode connected to an infusion pump (UMP3-1, WPI, Berlin, Germany) at a rate of 20 nL per minute. Following completion of the infusion, the micropipette remained in the injection site for an additional 10 min before being slowly retracted. Mice were then placed on a heating pad and closely monitored until fully recovered. The experiments were carried out no sooner than three weeks post-virus injection. The viruses used are as following: rAAV-GfaABC1D-mCherry-WPRE-SV40 polyA, rAAV-GfaBC1D-mCherry-hPMCA2w/b-SV40 polyA, rAAV-GfaBC1D-mCherry-hPMCA2w/b-SV40 polyA (BrainVTA, Wuhan, China).

2.4. Scratching behavior recording

The scratching behavior through video recording and automatic assessment were conducted as described previously (Yang et al., 2023). Through video recording, the mice were habituated for 30 min in the plastic chambers with transparent glass floor. The video was analyzed and the number of scratching bouts were quantified. A scratching bout was defined by a period of continuous rubbing of the injection site by the hind paw, from lifting the paw to placing it back to the mouth or on the ground.

For magnetic automatic assessment, the cylindrical magnets (diameter, 1 mm; height, 1 mm) were surgically implanted in the right hindpaw of each mouse under anesthesia at least 7 days prior to the administration of pruritogens, enabling automated assessment of scratching behavior. Transparent cylindrical recording barrels (diameter, 15 cm; height, 20 cm) were positioned within the induction coil. Mice were acclimated to the recording barrels for 30 min over two consecutive days before experimental testing. During the experimental procedure, the movement of the mouse hindpaw containing a magnet resulted in variations in amplitude within the recorded traces from the induction coil. The analysis of scratching behavior was conducted utilizing custom-written code in MATLAB.

2.5. Rota-rod test

Motor coordination was evaluated through the rota-rod test, in which mice underwent daily training on a rotating rod for two days to prevent accidental falls, followed by testing on an accelerated-speed program ranging from 4 to 40 revolutions per minute for a maximum duration of 300 seconds per trial. Each mouse was tested three times to determine the latency and speed to fall from the rotating rod at a 10-min interval.

2.6. Open field test

Mice were subjected to locomotor activity testing within polystyrene enclosures measuring 40 × 40 × 40 cm. The mice were positioned at the center of the enclosure and recorded individually for a duration of 5 min.

2.7. EPM test

The experimental setup included a behavioral apparatus consisted of two open arms and two closed arms (5 cm in width and 30 cm in length), elevated 50 cm above the floor and illuminated at a brightness of 300 lux. Each mouse was individually placed in the center of the maze, facing an open arm, and allowed to explore freely for a period of 5 min. The activity of the mice in each arm was recorded and analyzed using a video tracking system. The maze was sanitized with 70% ethanol after each experimental session.

2.8. Immunofluorescence

Mice were deeply anesthetized by intraperitoneal injection of pentobarbital (100 mg/kg) and perfused transcardially with 0.1 M PBS (pH 7.4), followed by ice-cold 4% paraformaldehyde (PFA) in 0.1 M PBS. The brains were removed, post-fixed at 4 °C overnight, and placed in 20% sucrose solution for 24 h and 30% sucrose solution for 48 h at

4 °C. The brain segments were sliced using a freezing microtome (Leica 2000; Germany) at a thickness of 30 μm. The bregma ranges from −5.68 to −6.48 mm was considered as the RVM section coordinates. Following this, the sections underwent three washes and were blocked with 10% donkey serum in 0.3% Triton X-100 for 1 h at 37 °C. Subsequently, the sections were incubated overnight at 4 °C with primary antibodies including rabbit anti-GFAP (1:400, Cell Signaling Technology, 12389), and goat anti-SOX9 (1:200, R&D Systems, AF3075). After the incubation period, the tissue sections were washed and then exposed to a secondary antibody (Alexa 488/594-conjugated secondary antibodies; 1:1000; Invitrogen) solution for 1 h at room temperature. Following three rinses, the sections were cover-slipped using a mixture of 80% glycerin in 0.01 M PBS. Fluorescent images were visualized using All-in-One fluorescence microscopy system (BZ-X800; Keyence, Japan), and mean fluorescence intensity was quantified with Image J (http://rsbweb.nih.gov/ij/).

2.9. Sholl analysis

Sholl analysis was performed to evaluate astrocyte morphology as previously described (Wang et al., 2024). Confocal images immunostained with GFAP antibody were utilized for analysis. Astrocytes from the RVM region were selected to delineate serial concentric circles around the cell, originating from the center of the SOX9 signal and extending outward at radial increments of 1 μm. The Sholl analysis plugin in ImageJ was employed to automatically quantify the number of intersections of GFAP processes within each circle and the total intersections.

2.10. Histological analysis

The shaved part of the nape skin was collected, and the samples were fixed with 4% PFA for 3 days and embedded. The skins were sectioned into 5 μm slices and stained with hematoxylin and eosin (H&E) to assess the thickness of the epidermis and dermis. Histopathological evaluation of the skin sections was visualized using a fluorescence microscope (Nikon, Japan).

2.11. Statistical analysis

All data were expressed as mean ± SEM. All data were analyzed using the Unpaired *t*-test, Two-way repeated measured ANOVA with post hoc Bonferroni test was used to analyze 2 group data with multiple time points. For all statistical analyses, the criterion for statistical

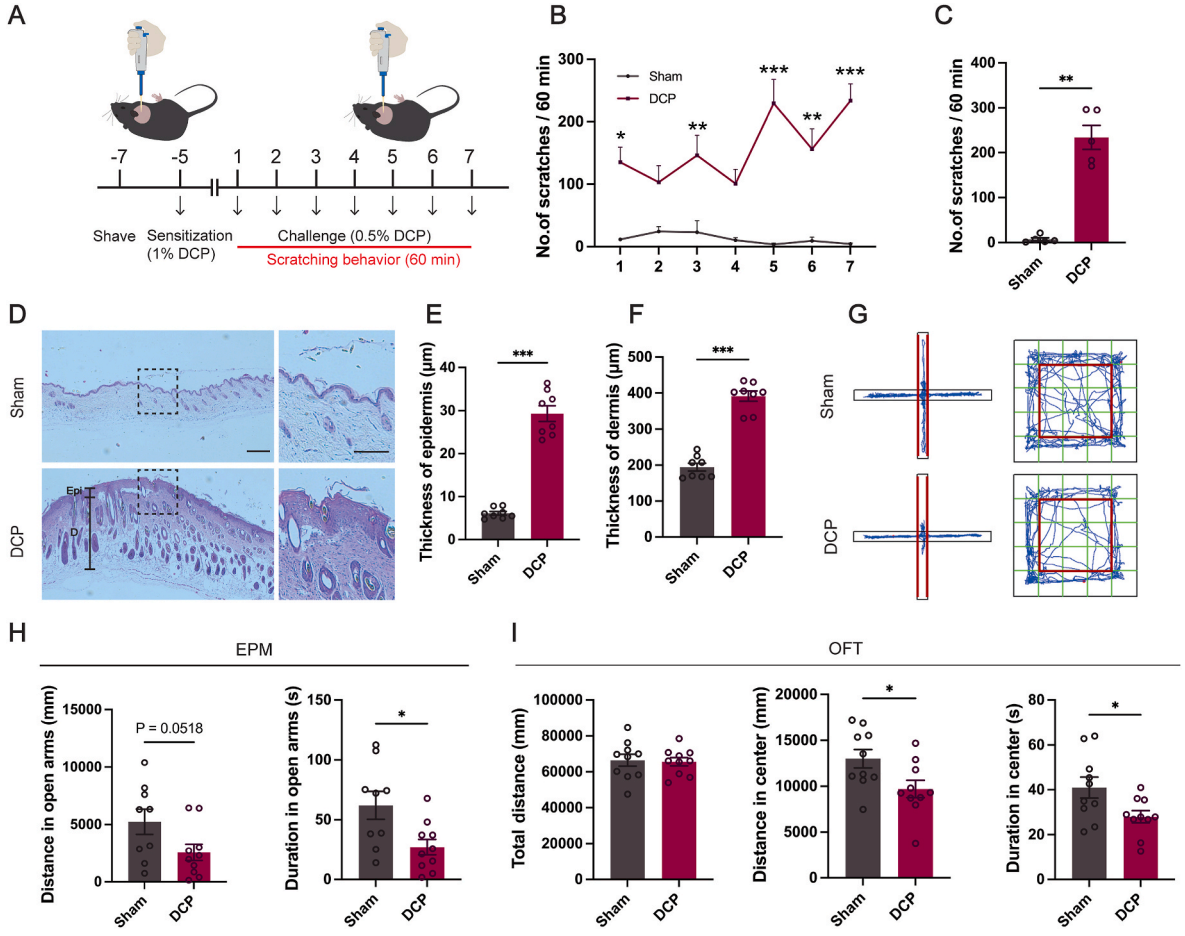

Fig. 1. DCP-induced chronic itch and anxiety-like behavior. (A) Scheme of the establishment of DCP-induced ACD mouse model, and the behavior testing. (B) Time course of the scratching numbers in DCP-treated mice. *p < 0.05, **p < 0.01, ***p < 0.001, n = 5 mice per group (two-way ANOVA with post hoc Bonferroni test). (C) Scratching behavior analysis in 60 min on day 7 after the final application by DCP. **p < 0.01, n = 5 mice per group (Unpaired *t*-test). (D) Hematoxylin and Eosin (H&E) staining of the neck skin in the Sham and DCP groups on day 8. Scale bar = 100 μm, 50 μm. (E, F) Quantification of dermis and epidermis thickness of Sham and DCP mice. The thickness of the epidermises randomly selected and analyzed in each slice. ***p < 0.001. n = 8 slices from 4 mice (Unpaired *t*-test). (G) Typical graphs of motion trajectories of Sham and DCP groups in elevated plus maze test (left) and open field test (right). (H) The traveled distance and the time spent of open arms area in DCP mice in the elevated maze test. *p < 0.05, n = 10 mice per group (Unpaired *t*-test). (I) The total traveled distance, the traveled distance in center area and the time spent in center area in the DCP mice in open field test. *p < 0.05, n = 10 mice per group (Unpaired *t*-test). All data are shown as mean ± SEM.

significance was set at $P < 0.05$. The statistical analyses were performed using GraphPad Prism 8.0 software (San Diego, CA, USA)

3. Results

3.1. DCP-induced chronic itch and anxiety-like behavior

DCP, a topical immunotherapeutic agent utilized in the treatment of alopecia areata, often induces significant adverse reactions in both human patients and murine models, such as eczematous skin, contact dermatitis, and intense pruritus. Consistently, we found that repeated application of DCP to the nape skin resulted in a progressive escalation of scratching behavior (Fig. 1A–C). Furthermore, histological examination of the DCP-treated nape skin was conducted using H&E staining, and the thickening of the epidermis and dermis was analyzed. Compared with the sham group, there was marked epidermal hyperplasia and atopic dermatitis in the DCP group (Fig. 1D–F).

In addition, chronic itch can lead to poor sleep and mental health issues such as anxiety (Sanders and Akiyama, 2018). We next evaluated the anxiety-like behaviors in the chronic itching mice using EPM and OFT. The distance and duration in open arms during the EPM were significantly decreased in the DCP group (Fig. 1G and H). While there were no notable variances in total distance, the DCP mice exhibited reduced exploratory behavior in the OFT, as evidenced by decreased distance and duration in the center (Fig. 1I). These findings collectively suggest that DCP elicits pronounced scratching behavior and anxiety-like behaviors.

3.2. Astrocyte reactivity in the RVM of the DCP mice

To assess the activation of astrocytes in the RVM of mice with chronic pruritus induced by DCP, immunofluorescence staining of GFAP, a widely utilized astrocyte marker, was performed. We found that the astrocytes displayed enlarged cell somas and arborized processes, with a significant increase in the mean fluorescence intensity (MFI) of GFAP in the RVM of the DCP mice (Fig. 2A and B), indicating the astrocyte activation in the RVM of the DCP mice.

Furthermore, in addition to the significantly upregulated expression of GFAP in reactive astrocytes, these cells may also exhibit typical morphological changes including process extension and hypertrophy, which are recognized as phenotypic characteristics of reactive astrocytes (Li et al., 2020). Subsequently, we quantified these morphological differences among astrocytes using Sholl analysis. The Sholl analysis of individual reactive astrocytes demonstrated that the number of intersections at each ring (Fig. 2C) and the total number of intersections (Fig. 2D) was remarkably increased in the astrocytes of RVM of DCP mice, as compared to sham mice. These findings suggest that activated RVM astrocytes in DCP mice exhibit greater process complexity than those in sham mice.

3.3. IP3R2 KO suppresses the DCP induced chronic pruritus

Astrocytic activation is characterized by heightened intracellular Ca^{2+} signals primarily mediated by the inositol 1,4,5-trisphosphate (IP3) pathway, with IP3 receptor type 2 (IP3R2) being the predominant functional IP3R isoform in astrocytes. IP3R2 knockout mice demonstrate significantly reduced Ca^{2+} signals in astrocytes, but not in neurons (Petravicz et al., 2008; Wang et al., 2021). Subsequent investigation focused on the impact of astrocyte-specific functional impairment on scratching behaviors, skin lesions, and anxiety-related behaviors in DCP mice (Fig. 3A). Behavioral assessments indicated that IP3R2 knockout mice experienced relief from chronic pruritus (Fig. 3B

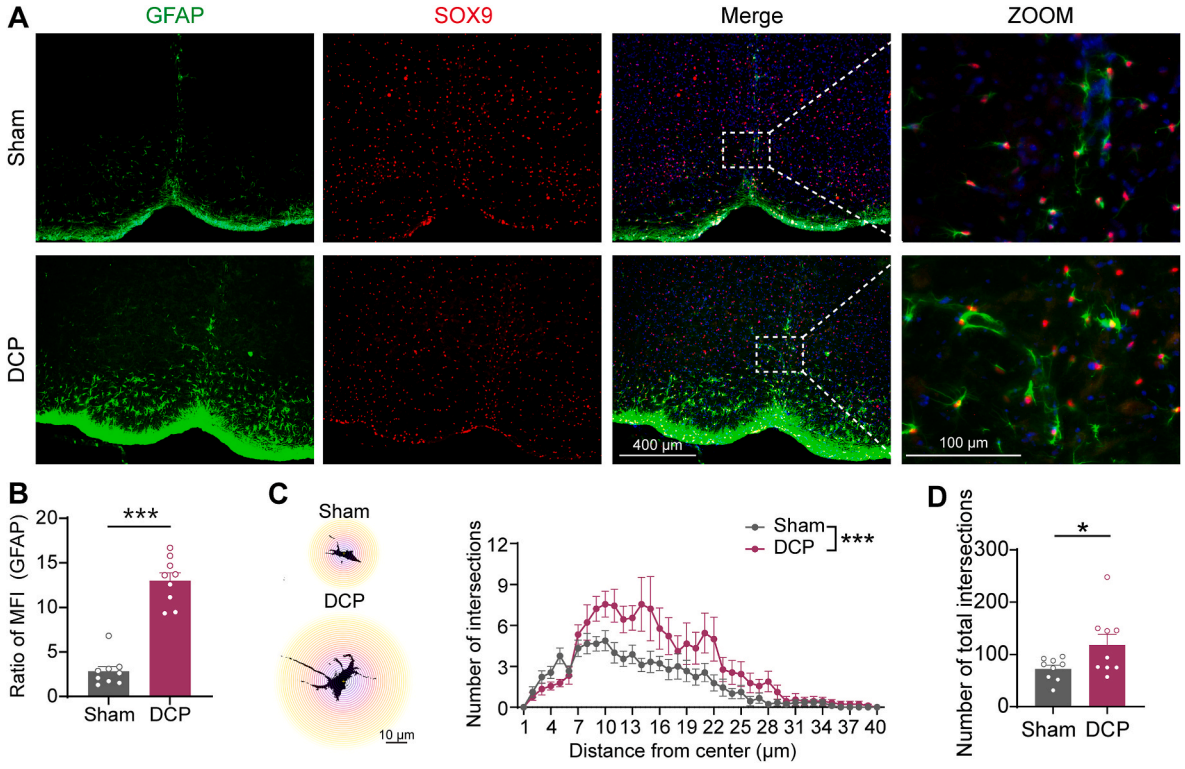

Fig. 2. Astrocyte reactivity in the RVM of the DCP mice. (A) Representative immunofluorescence staining of GFAP and SOX9 in the RVM of DCP and sham mice on day 8; GFAP (green), SOX9 (red), Merge (the co-expression of GFAP with SOX9); Scale bars = 400 μm, 100 μm (zoom). (B) Quantification of the mean fluorescence intensity (MFI) of GFAP expression in RVM region (Unpaired *t*-test). (C) Typical image of Sholl analysis of astrocytes in the RVM of sham and DCP mice (Left). The number of intersection points between GFAP protrusions and concentric circles of different radii in DCP mice (Right) (two-way ANOVA with post hoc Bonferroni test). (D) The number of total intersections in the DCP and sham mice. *p < 0.05. ***p < 0.001. n = 9 slices from 3 mice per group (Unpaired *t*-test). All data are shown as mean ± SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

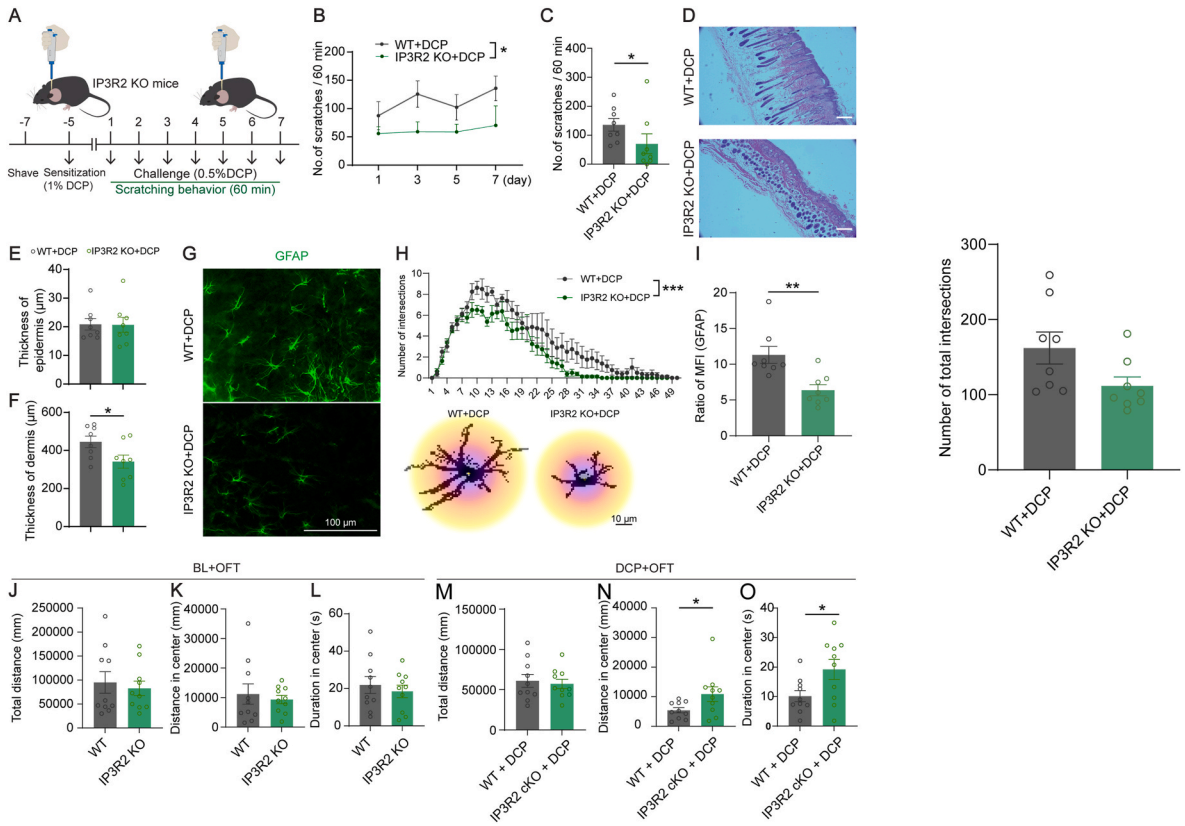

Fig. 3. IP3R2 KO suppresses the DCP induced chronic pruritus. (A) Timeline for DCP/acetone applications, and behavior tests in IP3R2 KO mice. (B) Time course of the number of scratches in DCP-treated IP3R2 KO mice and WT mice. *p < 0.05, n = 8 mice per group (two-way ANOVA with post hoc Bonferroni test). (C) Scratching behavior analysis in 60 min on day 7 after the final application by DCP in the IP3R2 KO mice and WT mice. **p < 0.01, n = 8 mice per group (Unpaired *t*-test). (D) Hematoxylin and Eosin (H&E) staining of the nape skin in the IP3R2 KO + DCP and WT + DCP groups on day 8. Scale bar = 100 μm. (E, F) Quantification of dermis and epidermis thickness. *p < 0.05. n = 8 slices from 4 mice per group. (Unpaired *t*-test). (G) Representative immunofluorescence staining of GFAP (green), Scale bars = 100 μm. (H) The number of intersection points between GFAP protrusions and concentric circles of different radii in DCP mice (Upper) (two-way ANOVA with post hoc Bonferroni test). Typical image of Sholl analysis of astrocytes in the RVM (Bottom). (I) Quantification of the mean fluorescence intensity (MFI) of GFAP expression in RVM region in the IP3R2 KO DCP mice on day 8. **p < 0.01. n = 8 slice from 4 mice per group (Unpaired *t*-test). (J–O) Effect of IP3R2 KO on the total traveled distance, the traveled distance in center arena and the time spent in center arena in the open field test in the DCP mice (DCP + OFT), and prior to DCP treatment (BL + OFT). *p < 0.05, n = 10 mice per group (Unpaired *t*-test). All data are shown as mean ± SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and C). Skin pathological staining further demonstrated that IP3R2 KO mice exhibited improvements in dermal thickening within the affected area of chronic pruritus mice (Fig. 3D–F). Immunofluorescence analysis and Sholl analysis revealed that IP3R2 knockout effectively suppressed abnormal activation of RVM astrocytes in chronic pruritus mice (Fig. 3G–I). Additionally, there were no statistically significant variances in the total distance, center distance and center duration in the IP3R2 KO mice (Fig. 3J–L), indicating that the IP3R2 deficiency doesn't affect the motor function of mice. The IP3R2 deficiency hindered the reduction of center time and center distance in the DCP mice in the OFT, while the total distance was unchanged (Fig. 3M–O), suggesting the attenuation of the anxiety behavior in the DCP mice by IP3R2 deficiency. These findings suggest that the inhibition of astrocytic calcium activity mitigated the scratching behavior, skin lesions, and anxiety-like behavior induced by chronic pruritus.

We further deleted the IP3R2 receptors from RVM astrocytes by injecting a viral vector expressing GfaABC1D-Cre into the IP3R2 flox mice to observe the effect of IP3R2 conditional knockout (cKO) in the RVM (Fig. 4A and B). The motor function was not affected after the virus injection for three weeks (Fig. 4C). Following IP3R2 cKO, the astrocyte activation was inhibited as indicated by the decreased MFI of GFAP (Fig. 4D and E). The DCP-induced chronic pruritus was significantly attenuated in the IP3R2 cKO mice, compared with the WT mice (Fig. 4F). However, there are no significant improvements in the thickness of dermis and epidermis within the affected area of chronic pruritus mice (Fig. 4G–I). And the anxiety behavior of DCP mice was unaffected by the IP3R2 cKO (Fig. 4J, K, N, O), with the baseline unaffected (Fig. 4L, M, P, Q).

3.4. RVM astrocytes modulate the DCP induced scratching behavior

To validate the role of astrocyte calcium activities in the RVM in DCP mice, we administered adeno-associated virus (AAV) encoding hPMCA2w/b (AAV-GfaABC1D-hPMCA2w/b-mCherry) into the RVM, and the expression of the virus was confirmed by immunostaining (Fig. 5A and B). There was no significant difference in the latency to falling off and maximum speed on the rotarod between the hPMCA2w/b and mCherry groups (Fig. 5C and D), suggesting that the virus does not induce motor abnormalities. Immunofluorescence analysis and Sholl analysis further confirmed the ability of the virus to suppress the aberrant activation of RVM astrocytes in the DCP mice (Fig. 5E–H).

Three weeks post virus injection, the scratching behaviors were not affected (Fig. 5I), indicating that the virus injection did not affect the itching behaviors. However, a notable decrease in scratching behavior was observed in AAV-GfaABC1D-hPMCA2w/b-injected DCP mice (Fig. 5J and K). The anxiety-like behavior in DCP mice was not significantly affected in the OFT test and EPM test, when the RVM astrocyte Ca^{2+} activation was blocked with hPMCA2w/b (Fig. 5L–O). These

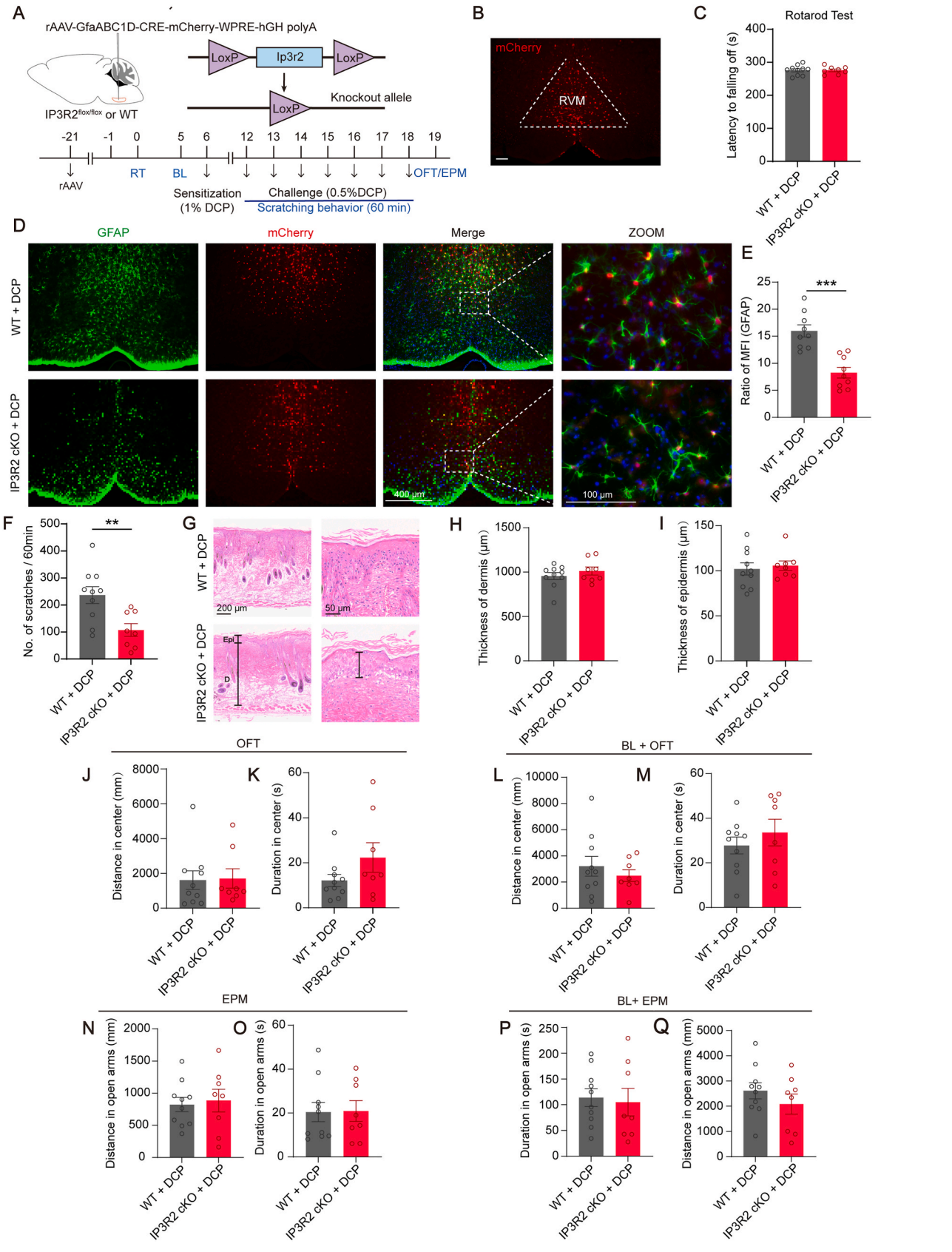

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Fig. 4. Conditional deletion of astroglial IP3R2 in the RVM alleviates DCP-induced chronic pruritus behavior. (A, B) Schematic illustration and representative image of viral delivery into the RVM of IP3R2 flox or WT mice, along with the experimental timeline for DCP application and behavioral tests. (C) Rotarod test of IP3R2 cKO and WT mice. (D) Representative immunofluorescence images showing GFAP (green) and mCherry (red) staining in the RVM region. Scale bars: 400 μm, 100 μm (zoom). (E) Quantification of the mean fluorescence intensity (MFI) of GFAP expression in the RVM of IP3R2 cKO and WT mice on day 8 post-DCP treatment. ***$p < 0.001$; $n = 9$ slices from 3 mice per group. (F) Quantification of scratching behavior over 60 min on day 7 following the final DCP application. **$p < 0.01$. (G) Representative hematoxylin and eosin (H&E) staining of the nape skin in IP3R2 cKO + DCP and WT + DCP groups on day 8. Scale bars: 200 μm, 50 μm (zoom). (H, I) Quantification of dermal and epidermal thickness based on H&E staining. (J–M) Open field test (OFT) results, assessing the distance traveled in the center arena and time spent in the center arena for IP3R2 cKO and WT mice pre- (BL + OFT) and post-DCP treatment. (N–Q) Elevated plus maze (EPM) results, analyzing the distance traveled and duration spent in open arms for IP3R2 cKO and WT mice pre- (BL + EPM) and post-DCP treatment. n = 8-10 mice per group. Unpaired *t*-test. All data are presented as mean ± SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

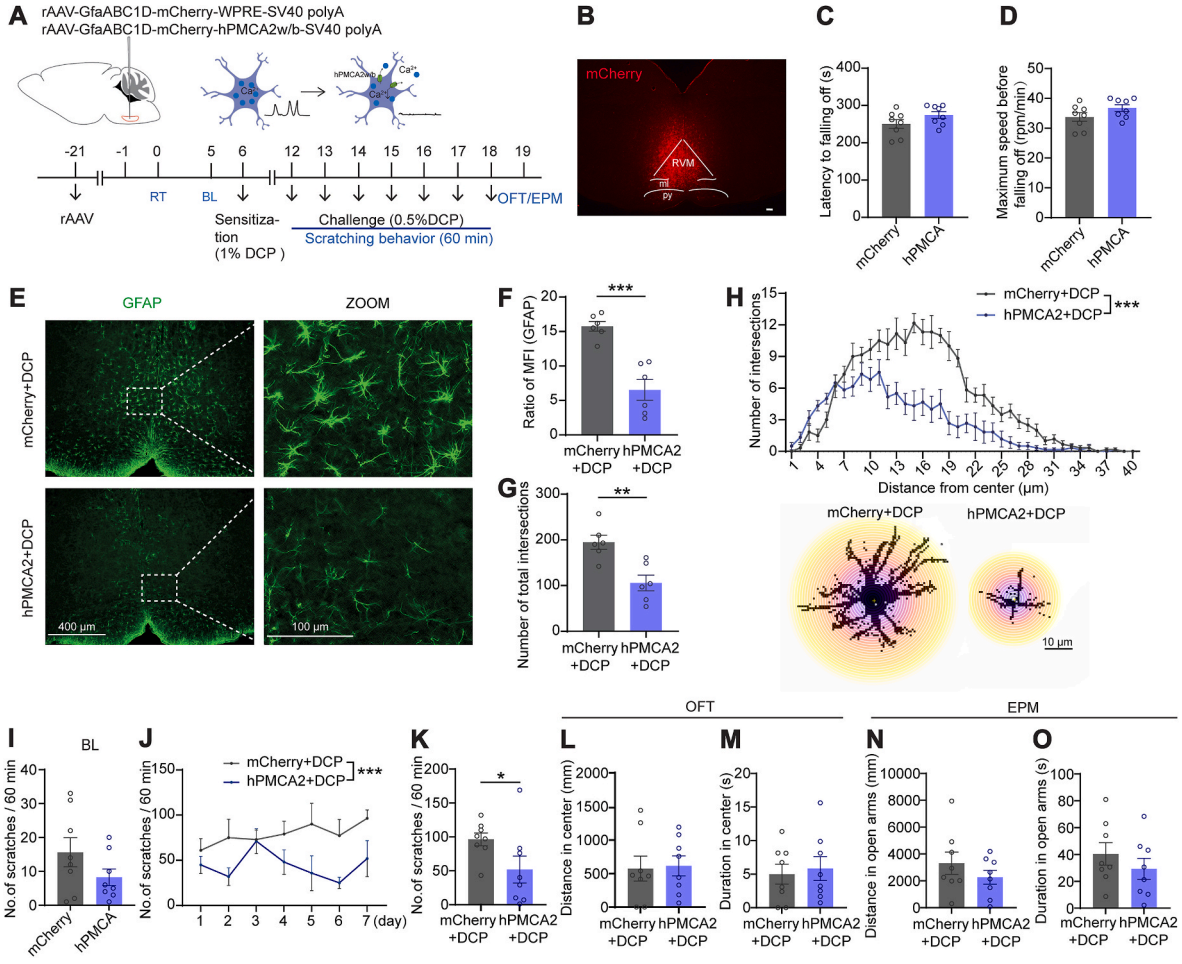

Fig. 5. Inhibition of the RVM astrocytes activity attenuated the DCP induced scratching behavior. (A, B) Illustration and representative image of viral delivery into the RVM of C57BL/6 mice. Scale bar = 100 μm. (C, D) Behavioral effects of inhibition of the RVM astrocytes activity 3 weeks after the virus injection in the Rotarod test. n = 8 per group (Unpaired *t*-test). (E, F) Representative immunofluorescence staining of GFAP (green) and quantification of the mean fluorescence intensity (MFI) in RVM region after virus injection. Scale bar = 400 μm, 100 μm. n = 6 slice from 3 mice per group (Unpaired *t*-test). (G, H) The number of intersection points between GFAP protrusions and concentric circles of different radii in DCP mice (two-way ANOVA with post hoc Bonferroni test) and the total number of intersection points (Unpaired *t*-test). (I) The scratching behaviors 3 weeks after virus injection. n = 8 mice per group. (J) Inhibition of the RVM astrocyte attenuated the DCP-induced scratching behavior. ***$p < 0.001$, n = 8 mice per group (two-way ANOVA with post hoc Bonferroni test). (K) Effect of the inhibition of the RVM astrocytes activity on the scratching behavior in 60 min on day 7 after the final application by DCP. *$p < 0.05$, n = 8 mice per group (Unpaired *t*-test). (L–O) Behavioral effects of inhibition of the RVM astrocytes activity on the DCP mice by the OFT and EPM test. n = 8 mice per group. All data are shown as mean ± SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

findings suggested that inhibition of the RVM astrocyte activation attenuated the DCP-induced itch but not anxiety-like behaviors.

4. Discussion

Pruritus is a maladaptive and incapacitating symptom present in numerous dermatological and systemic conditions. Despite this, limited therapeutic strategies are available for pruritus in clinical practice. The current investigation has identified a pivotal role of RVM astrocytes in the development of chronic itch induced by DCP. Activation of RVM astrocytes was observed in DCP mice, and the inhibition of RVM astrocytes through global IP3R2 gene knockout, conditional astroglial IP3R2 gene knockout in the RVM, and AAV-GfaABC1D-hPMCA2 injection into the RVM, resulted in the suppression of local astrocyte activation, leading to a reduction in chronic itch. These findings suggest that RVM astrocytes play a crucial role in the pathogenesis of chronic itch.

The DCP-induced chronic itch model was initially established, with subsequent observation of scratching behaviors and thickening of the

epidermis and dermis in the DCP-treated dorsal skin. Given the potential for negative emotional responses including anxiety-like behavior, in the chronic itch mice, the OFT and EPM were conducted. Results indicated a decrease in time spent in the center area of the OFT and in the open arms of the EPM in DCP mice, suggesting the presence of anxiety behavior in the context of DCP-induced chronic itch. Consistently, the induction of atopic dermatitis by MC903 in mice results in anxiety- and depression-like behaviors (Yeom et al., 2022), while chronic itch-induced anxiety-like behavior can be induced by DNFB (Li et al., 2023).

The RVM, a key hub in pain modulation, has the ability to either inhibit or facilitate the transmission of pain signals (Nguyen et al., 2023). Recent research indicates that the RVM is also involved in regulating itch (Follansbee et al., 2022; Gao et al., 2021). Specifically, studies have shown that targeted lesioning of the descending serotonergic pathway within the RVM using the serotonin neurotoxin 5, 7-dihydroxytryptamine can reduce pruritic behavior induced by compound 48/80, suggesting a modulatory role of serotonergic pathway at the RVM for pruritic behavior (Liu et al., 2014). Moreover, a subset of RVM neurons that specifically express the G-protein-coupled estrogen receptor (GPER) has been shown to facilitate the inhibition of both acute and chronic itch (Gao et al., 2021). Additionally, the Tacr1-expressing ON cells within the RVM are known to play a crucial role in the regulation of pruriceptive transmission (Follansbee et al., 2022).

In addition to the neuronal roles, studies have extensively shown that astrocytes located in the RVM also play a significant role in the modulation of descending pain pathway (Dubovy et al., 2018; Roberts et al., 2009). However, the specific role of RVM astrocytes in the pathogenesis of chronic itch remains unclear, despite recent preclinical studies indicating the significant involvement of spinal cord astrocytes in the development of pathological chronic itch (Gao et al., 2023). Previous research has demonstrated the activation of spinal astrocytes in various mouse models, including 2,4-dinitrofluorobenzene (DNFB)- or DCP-induced ACD mice, and acetone, diethyl ether, and water (AEW) induced dry skin mice (Du L et al., 2019; Liu et al., 2016). Further investigations have revealed that STAT3 plays a role in astrogliosis, which enhances central itch transmission through LCN2-signaling with $GRPR^{+}$ neurons (Shiratori-Hayashi et al., 2015). This study presents novel findings indicating that RVM astrocytes play a significant role in chronic itch. By manipulating the activity of RVM astrocytes, this research confirms their involvement in chronic itch and offers new perspectives on the treatment of chronic itch and related anxiety-like behaviors.

Nevertheless, this study is subject to certain limitations. Firstly, it only identifies morphological changes in astrocytes, necessitating further investigation into their functional activity within the RVM. Secondly, the global deletion of IP3R2 reduced both chronic itch and associated anxiety-like behaviors, whereas conditional knockout of IP3R2 specifically in RVM astrocytes mitigated only the chronic itch behavior, without affecting anxiety-like behaviors. This suggests that the reduction of DCP-induced anxiety in IP3R2-deficient mice is likely mediated by IP3R2 in regions outside the RVM. Furthermore, the attenuation of DCP-induced anxiety behavior in IP3R2-deficient mice was evaluated using the open field test, and further validation using the EPM test is warranted. Thirdly, the potential mechanisms driving IP3R1-dependent calcium signaling in astrocytes is also reported to be involved in the chronic itch (Shiratori-Hayashi and Tsuda, 2023; Shiratori-Hayashi et al., 2021). And the role of IP3R2-independent calcium signaling, specifically involving IP3R1, in chronic itch-like behavior warrants investigation. Fourthly, astrocytes are demonstrated to undergo reactive changes in response to central nervous system disorders, resulting in alterations in their morphology, molecular profile, and physiological functions. It is imperative to delve deeper into the molecular mechanisms and signaling pathways involved in astrocyte activation, such as the transcription factors like STAT3. Finally, glia-neuron interactions play a critical role in the modulation of itch sensation (Koga et al., 2020; Liu et al., 2023). Astrocytes release neurotransmitters and immune mediators that enhance neuronal excitability within neural circuits. Further research is needed to determine the impact of astrocyte activation on neuronal circuits involved in mediating the pruritic effect.

In conclusion, the present study emphasizes the importance of reactive astrocytes in the RVM for chronic itch, suggesting that targeting these astrocytes could be a promising therapeutic approach for treating chronic itch. Exploration of the intricate pathways and interactions of astrocytes in the RVM could present novel avenues for managing chronic itch in patients.

CRediT authorship contribution statement

Ting Yi: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mengping Lou:** Investigation, Formal analysis. **Xinyi Gao:** Methodology, Formal analysis. **Liyuan Bao:** Data curation. **Heting Yan:** Formal analysis. **Teng Lin:** Formal analysis. **Yayue Yang:** Methodology, Data curation. **Tianchi Gao:** Methodology. **Chenghao Wang:** Methodology, Data curation. **Jianyu Zhu:** Methodology, Data curation. **Yanqing Wang:** Writing – review & editing, Project administration, Conceptualization. **Wenli Mi:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

None of the authors declare the financial and personal relationships with other people or organizations that could inappropriately influence (bias) the work.

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Data availability

No data was used for the research described in the article.

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