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# Original Reports

## $GABA_A$ Receptors in the Central Nucleus of the Amygdala Are Involved in Pain- and Itch-Related Responses

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**Abstract:** **Itch and pain are unpleasant sensations that distress many patients with disease. However, most studies have focused on the neural mechanisms of pain, and much less effort has been devoted to itch. It has been reported that itch and pain might share a common pathway, and γ-aminobutyric acid type A ($GABA_A$) receptors in the central nucleus of the amygdala (CeA) are involved in pain modulation. However, the contribution of $GABA_A$ receptors in the CeA to the modulation of itch remains poorly understood. Herein, we report that bilateral intra-CeA microinjection of a selective $GABA_A$ receptor agonist muscimol hydrochloride (Mus; 50 ng per side), but not a selective $GABA_A$ receptor antagonist bicuculline (Bic; 20 ng per side) or vehicle, showed significant analgesic effects, reflected by an increase in tail-flick latency and a decrease in allyl isothiocyanate (mustard oil)–evoked ipsilateral forelimb wipes. More importantly, rats subjected to intra-CeA infusion of Bic showed a significantly greater number of scratching bouts and time in acute and chronic pruritus animal models than control rats. Conversely, intra-CeA infusion of Mus in animal models dramatically decreased the number of scratching bouts and time compared with control rats. In addition, intra-CeA infusion of Bic or Mus at the current dose had no obvious effects on other behaviors including locomotor activity and spontaneous facial grooming in rats subjected to cheek microinjection of 5-hydroxytryptamine. Taken together, these results indicate that the $GABA_A$ receptor–mediated inhibitory system in the CeA is involved in itch modulation as well as is known in pain control.**
***Perspective:*** ***Itch, especially chronic itch, remains a challenge in clinic. Results of this study showed that the $GABA_A$ receptors in the CeA play an important role in itch modulation, which might help us to better understand the mechanisms of itch and subsequently develop novel mechanisms-based strategies to treat chronic itch in clinic.***



***Key words:*** *Itch, pain, amygdala, scratching, γ-aminobutyric acid type A.*

Itch, also known as pruritus, is a common symptom of skin diseases, which elicits the desire to scratch and is associated with impaired sleep quality and a considerable reduction in productivity at work and quality of life.[19,33] Although a number of topical and systemic antipruritic drugs are available, the optimal therapy is hampered by the fact that our understanding of crucial itch mediators and receptors in the various subforms of itch is poor.[30]

The amygdala is a forebrain structure that is involved in pain modulation[18,20,21] in addition to being a key region involved in the modulation of emotional and defensive reactions.[13] In particular, the central nucleus

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of the amygdala (CeA) has been called the 'nociceptive amygdala' and has an important role in pain control.[21,22] For instance, bilateral intra-CeA infusions of morphine elicit dramatic suppression of nociceptive behavior, whereas naloxone microinjections into the CeA reduce the analgesic effects of morphine.[26,27] Furthermore, lesions of the CeA largely eliminate the antinociceptive effects of systemic morphine in tail-flick and formalin tests in rats.[14,15] Because itch and pain are similar in that they signal the organism of potentially dangerous

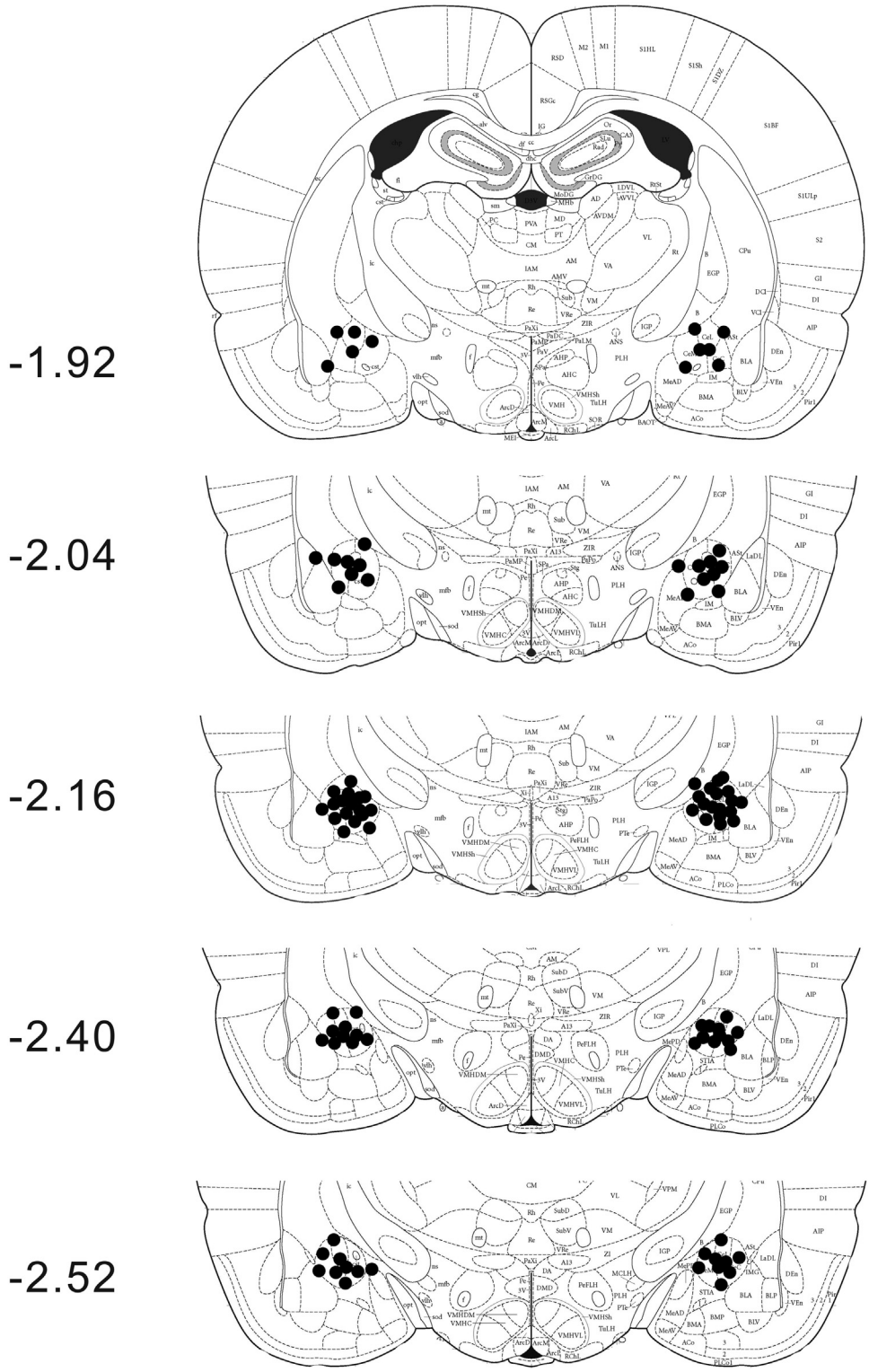


**Figure 1.** Histological representation of bilateral injection sites within the proximity of the CeA. The tip of the injection cannula is represented by a black dot and the localization described within coronal sections of rat brain (1.92–2.52 mm posterior to bregma) according to the atlas of Paxinos and Watson.[24]

stimuli, and are associated with protective motor responses, it is reasonable to propose that CeA might play an important role in itch modulation.

It is well known that the γ-aminobutyric acid type A ($GABA_A$) receptor, the main mediator of inhibitory neurotransmission in the central nervous system, is expressed in high concentration in the CeA.[6,16] Evidence accumulated from recent studies has suggested that $GABA_A$ receptors in the CeA are involved in pain modulation. For example, intra-CeA administration of muscimol hydrochloride (Mus), a selective $GABA_A$ receptors agonist, could inhibit mechanical allodynia,[11,25] whereas intra-CeA application of bicuculline (Bic), a selective $GABA_A$ receptor antagonist, showed no influence on mechanical allodynia[25] or induced mechanical hyperalgesia.[11] Nonetheless, several recent studies report contradictory results that indicated that microinjection of Bic and Mus into the CeA increased and decreased antinociceptive response, respectively.[10,26] Thus, it is necessary to determine the role of $GABA_A$ receptors in the CeA in pain modulation. Furthermore, in contrast to pain, it is not clear whether $GABA_A$ receptors in the CeA contribute to the modulation of itch. In the present study, therefore, we investigated the effects of $GABA_A$ receptor in the CeA on scratching behavior in 5-hydroxytryptamine–elicited acute itch and chronic dry skin itch, which appears to well discriminate between itch and pain elicited by chemical stimuli.[2,12]

## Methods

### *Animals*

Adult male Sprague Dawley rats (250–320 g) were obtained from Chongqing Medical University Animal House Center and maintained at Children's Hospital of Chongqing Medical University Animal Care Centre. Animals were pair-housed in plastic cages in a temperature-controlled (21°C) colony room on a 12/12 hour light/dark cycle. Food and water were available ad libitum. All experiments and procedures were approved by Chongqing Medical University Animal Care and Use Committee. All efforts were made to minimize the number of animals used.

### *Drugs*

All drugs including Bic, Mus, 5-HT, and allyl isothiocyanate (AITC; mustard oil) were obtained from Sigma Chemicals (St. Louis, MO). Bic was dissolved in 1 drop of glacial acetic acid and diluted with 0.9% sterile saline (Sal) to a concentration of 40 ng/μL. Mus was dissolved in the 0.9% sterile Sal at a concentration of 100 ng/μL. 5-HT was dissolved in the 0.9% sterile Sal at a concentration of 47 mM. AITC was diluted in 7% Tween 80 and Sal to a concentration of 10%. The drug application and behavioral test were performed in a double-blinded manner.

### *Cannula Implantation*

Rats received cannulas implanted above the CeA as previously described.[31] Briefly, rats were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg) and atropine (0.4 mg/kg), which was also given to help relieve respiratory congestion. Scalp skin was shaved using a clipper and disinfected using iodine before the rat was mounted on a stereotaxic instrument. After opening the scalp skin and exposing the skull, two 26-gauge stainless steel guide cannulas (11 mm; Plastics One, Roanoke, VA) were implanted above the CeA (2.2 mm posterior to bregma, 4.2 mm lateral to the midline, and 8.1 mm below the surface of the dura) and fixed to the skull with 4 jeweler's screws and dental cement. Sterile dummy cannula (30-gauge stainless steel rod; 11 mm in length; Plastics One) were inserted into guide cannula to avoid bacterial infection and cerebrospinal fluid leakage

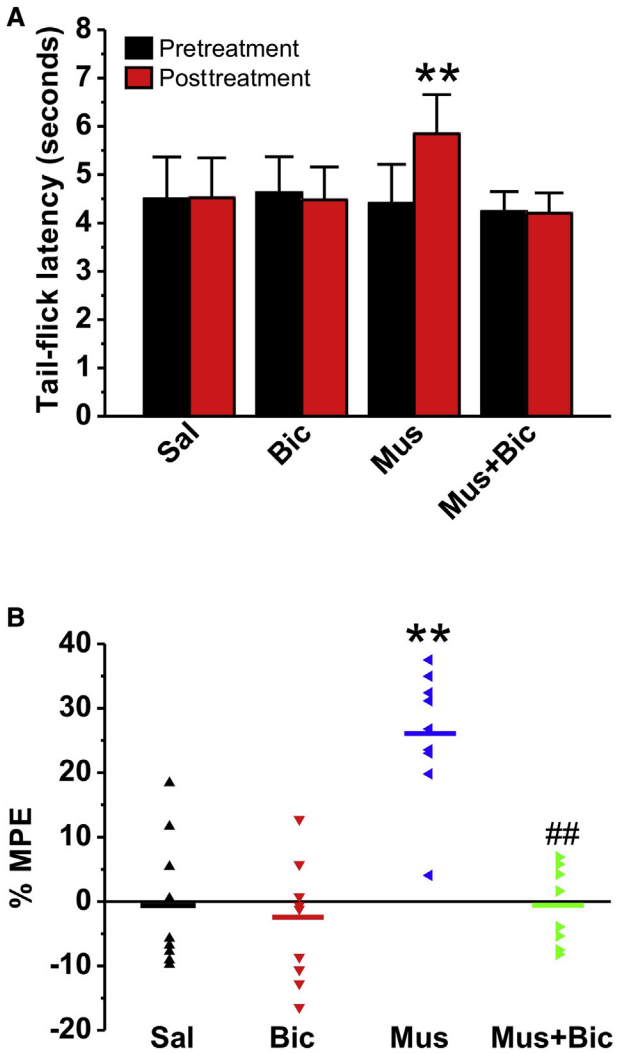


**Figure 2.** $GABA_A$ receptors in the CeA are associated with pain modulation in the tail-flick test. **(A)** The baseline tail-flick latencies measured before intra-CeA drug microinjection remained unchanged. Intra-CeA microinjection of Mus (n = 9, 50 ng per side), but not Bic (n = 9, 20 ng per side), or the same volume of sterile Sal (n = 9, 0.5 μL per side), 30 minutes before the tail-flick test significantly increased test latencies. The increased test latency induced by Mus was fully blocked by coapplication of Bic (Mus + Bic, n = 8). $^{**}P < .01$ versus BL. **(B)** Mus, but not Bic, produced dramatic antinociceptive effects, reflected by the maximum possible effect (% MPE), compared with Sal control. $^{**}P < .01$ versus Sal, $^{\#\#}P < .01$ versus Mus, post hoc Tukey test after ANOVA ($F_{3,31} = 20.265$, $P < .001$).

through the cannula. All rats were allowed to recover for 7 to 10 days before experiments.

## Intra-CeA Microinjection

On the day before experiments, the animals were placed in the experiment room and given a sham intra-CeA injection to become acclimatized to the injection procedure. Dummy cannulas were removed and the rats were placed into a Plexiglas injection box (25 × 45 × 25 cm; same as home cage) with 30-gauge injection cannulas in their guide cannulas. Injection cannulas (11 mm; Plastics One) were connected to a microsyringe pump (Harvard Apparatus, Holliston, MA) with PE-50 tubing, which were 1 mm beyond the tip of the guide cannulas.

On the experimental day, animals were divided randomly into 4 groups. Each group received bilateral microinjections of Mus, Bic, Mus and Bic (Mus+Bic), or Sal into the CeA in a volume of 0.5 μL per side with a microsyringe pump at 0.1 μL/min for 5 minutes. After injection, the injection cannulas were left in place for an additional minute to allow the diffusion of the drug away from the cannula tips. The rats were then removed from the injection box, their dummy cannulas were replaced, and the rats were placed back in their home cages. The cannula placement was verified in a histological examination of the brain after methylene blue injection (0.5 μL per side), and only data obtained from rats with correctly inserted cannulas were included in statistical analysis (approximately 10% of the animals were excluded from the experiment because of nonfunctional cannulas or postoperative complications). Fig 1 shows a schematic diagram that depicts the areas of acceptance for cannula placements in the CeA, as defined by Paxinos and Watson.[24]

## Tail-Flick Test

The tail-flick test was used to determine the effect of GABAergic modulation in the CeA on analgesia as in our previous report.[7] Briefly, the room temperature was maintained at 24°C ± 0.5°C throughout the experiment. Tail-flick latencies were examined in response to noxious heat stimulus (hot water at 52°C), in which the tail of a rat was immersed into water 2 to 3 cm from the tip and we observed the latency until a rapid tail-flick. The cutoff time for latencies was set at 10 seconds to avoid skin damage. The latency was assessed 3 times at 5-minute intervals and the mean value was taken as baseline latency (BL). Then, the rats were microinjected with Mus (n = 9), Bic (n = 9), Mus+Bic (n = 8), or Sal (n = 9) into the CeA and tail-flick latency was examined 30 minutes after the injection. The percentage of the maximal possible antinociceptive effects (% MPE) was calculated using tail-flick latencies before (BL) and after injection (test latency) using the equation: % MPE = [(test latency − BL)/(10 − BL)] × 100%.

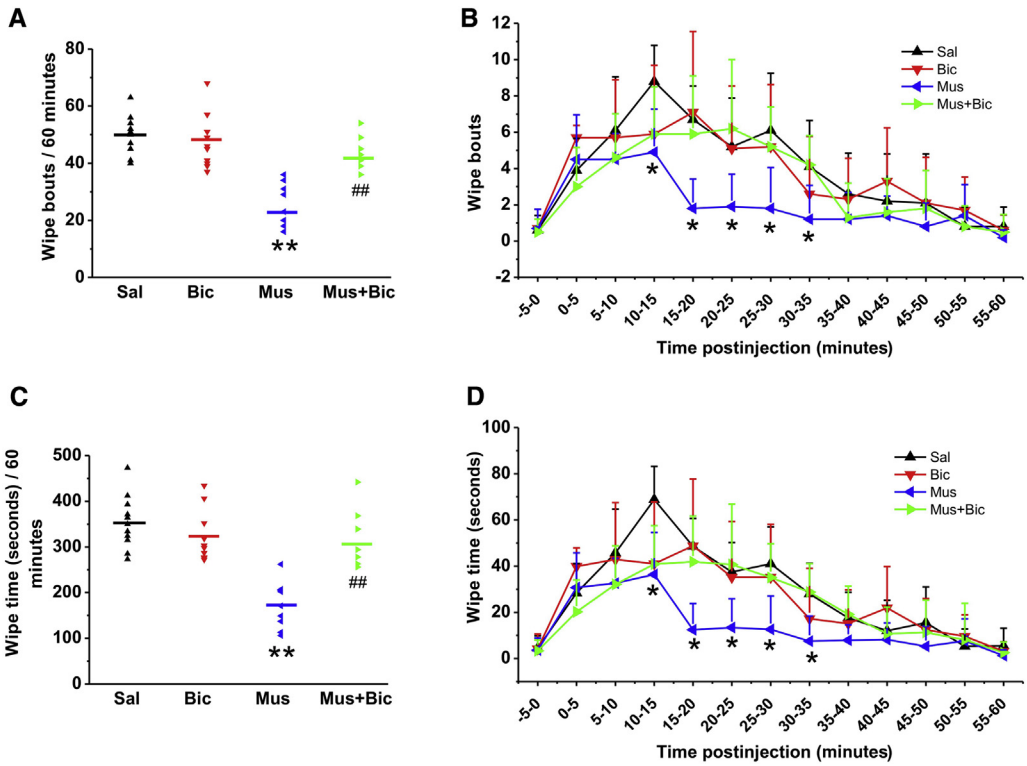


**Figure 3.** $GABA_A$ receptors in the CeA are associated with pain modulation in the cheek AITC injection model. **(A)** Scatter plot shows individual number of AITC-elicited wipe bouts in rats subjected to intra-CeA microinjection of Mus (n = 10, 50 ng per side), Bic (n = 10, 20 ng per side), Mus + Bic (n = 10), or the same volume of sterile Sal (n = 11). $^{**}P < .01$ versus Sal, $^{\#\#}P < .01$ versus Mus, post hoc Tukey test after ANOVA ($F_{3,37} = 20.304$, $P < .001$). **(B)** Time course of AITC-evoked wipe bouts. $^{*}P < .05$ versus Sal. **(C)** Individual wipe time after cheek injection of AITC. $^{**}P < .01$ versus Sal, $^{\#\#}P < .01$ versus Mus, post hoc Tukey test after ANOVA ($F_{3,37} = 17.725$, $P < .001$). **(D)** Time course of AITC-evoked wipe time. $^{*}P < .05$ versus Sal.

## AITC-Elicited Wiping Behavior

The methods for intradermal (i.d.) cheek injections were similar to those described previously.[12] In brief, an observation chamber (30 × 30 × 40 cm) was used for video recording in the present experiment. Rats were first habituated in 3 daily 1-hour sessions to the recording box. The fur at the right face of each rat was carefully clipped at least 3 days before receiving i.d. AITC microinjection. Twenty minutes before i.d. injection, rats received an intra-CeA microinjection of Mus (n = 10), Bic (n = 10), Mus+Bic (n = 10), or Sal (n = 11) through the guide cannula. Immediately after cheek injection of AITC (10 μL, 10% in 7% Tween 80 and Sal) into the previously-shaved right side cheek, the rats were placed into the recording box and videotaped for 60 minutes. The videotapes were subsequently reviewed by observers who were blinded to treatment, and the number and time of ipsilateral forelimb wipes directed to the injected cheek were counted in 5-minute intervals over the 60-minute period.

## 5-HT–Evoked Acute Scratching Behavior

The procedure was similar to i.d. cheek injection of AITC, and the only difference is that AITC was replaced with 5-HT in the acute scratching behavioral test. Twenty minutes before i.d. injection, rats received intra-CeA microinjection of Mus (n = 12), Bic (n = 10), Mus+Bic (n = 8), or Sal (n = 12) through the guide cannula. The following behavioral responses were counted in 5-minute intervals over the 60-minute testing period after i.d. injection: 1) total number of bouts of hind limb scratches directed to the injected cheek (off-site scratches, such as ears, were excluded), 2) number of scratch bouts occurring in a sequence/series, 3) total time of each rat spent scratching, 4) time of each rat spent scratching in a sequence or series, 5) total number of bouts of facial grooming behavior, which consisted of discrete episodes of head- or face-washing using the forepaws, and 6) total time each rat spent in facial grooming. We only scored facial grooming and did not consider licking, scratching, and other grooming behaviors directed to the lower body. The total distance and rearing bouts of each rat in the chamber were also recorded to determine the locomotor activity of each rat after different drug treatment.

## Chronic Dry Skin Itching Test

Chronic dry skin was induced as previously reported.[3,29] Briefly, a mixture of acetone and diethylether (1:1) was applied daily for 29 days on the foot sole of the left hind paw for 30 seconds immediately followed by the application of distilled water for 30 seconds using cotton gauze. Scratching behavior was assessed on days 0, 7, 14, 21, 28, and 29 for a period of 60 minutes by placing the rat in the

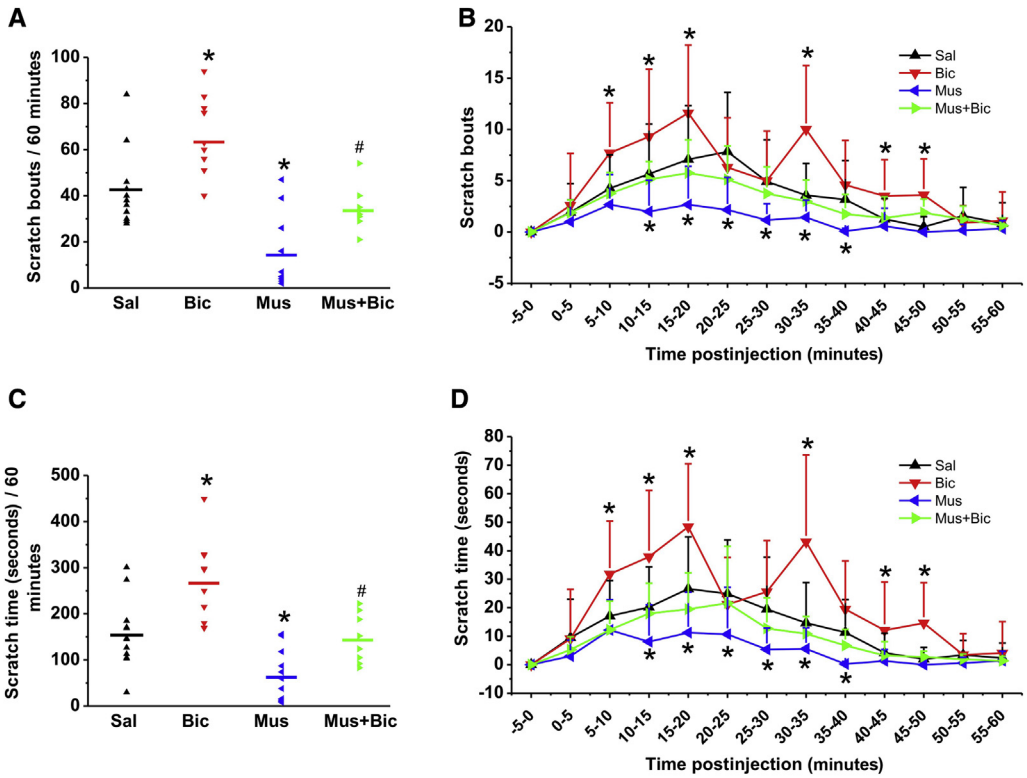


**Figure 4.** $GABA_A$ receptors in the CeA modulate 5-HT–elicited acute itching behavior. **(A)** Scatter plot shows individual number of 5-HT–elicited hind limb scratch bouts directed to the cheek injection site in rats subjected to intra-CeA microinjection of Mus (n = 12, 50 ng per side), Bic (n = 10, 20 ng per side), Mus + Bic (n = 8), or the same volume of sterile Sal (n = 12). *$P$ < .05 versus Sal, #$P$ < .05 versus Mus, post hoc Tukey test after ANOVA ($F_{3,38}$ = 9.812, $P$ < .001). **(B)** Time course of 5-HT-evoked scratching bouts. **(C)** Total mean scratching time after cheek injection of 5-HT. *$P$ < .05 versus Sal, #$P$ < .05 versus Mus, post hoc Tukey test after ANOVA ($F_{3,38}$ = 10.520, $P$ < .001). **(D)** Time course of 5-HT-evoked scratching time.

recording box. Twenty minutes before the last soak on day 29, rats received an intra-CeA microinjection of Mus (n = 11), Bic (n = 10), Mus+Bic (n = 11), or Sal (n = 10) through the guide cannula.

### Statistical Analysis

For pain-related behaviors, data were expressed as mean ± SD. The % MPE in the tail-flick test was analyzed using 1-way analysis of variance (ANOVA), with drug treatment as the between-subjects factor. The wiping bouts and time at different periods were analyzed using a 2-way between- and within-subjects factorial ANOVA, with drug treatment as the between-subjects factor and session as the within-subjects factor. All significant main effects and interactions were further analyzed using Turkey comparisons.

For itch-related behaviors, data were expressed as mean ± SD. The scratch bouts and time at different periods elicited by acute 5-HT were analyzed using a 2-way between- and within-subjects factorial ANOVA, with drug treatment as the between-subjects factor and session as the within-subjects factor. All significant main effects and interactions were further analyzed using Turkey comparisons. The scratch bouts and time in the chronic dry skin model were analyzed using 1-way ANOVA, with drug treatment as the between-subjects factor.

For spontaneous motor activity, 1-way ANOVA was used in statistical comparisons for facial grooming time, total walking distance, and rearing bouts.

## Results

### Effects of $GABA_A$ Receptors in the CeA on Pain Modulation in the Tail-Flick Test

Previous study has shown that microinjection of the $GABA_A$ receptor agonist Mus into the CeA inhibits mechanical allodynia, whereas intra-CeA application of the $GABA_A$ receptor antagonist Bic induces significant mechanical allodynia.[11] Thus, we first wanted to confirm the effects of $GABA_A$ receptors in the CeA on pain modulation in the tail-flick test. As shown in Fig 2A, bilateral intra-CeA microinjection of Mus (50 ng per side) induced a dramatic increase in tail-flick latency, whereas Bic (20 ng per side) or Sal administration had no effect on tail-flick latency compared with baseline tail-flick latency (Sal: n = 9, 4.52 ± 0.83 seconds, $P > .05$ vs BL; Bic: n = 9, 4.48 ± 0.68 seconds, $P > .05$ vs BL; Mus: n = 9, 5.85 ± 0.81 seconds, $P < .01$ vs BL; Fig 2A). In addition, the analgesia produced by intra-CeA injection of Mus was attenuated when Bic was injected into the CeA 5 minutes earlier (Mus+Bic: n = 8, 4.20 ± 0.42 seconds, $P > .05$ vs BL; Fig 2A). Similarly, % MPE analysis also showed that Mus application produced significantly antinociceptive effects and these effects were fully prevented by coapplication of Mus and Bic (Sal: n = 9, −0.25 ± 10.06%; Bic: n = 9, −3.38 ± 9.44%, $P > .05$ vs Sal; Mus: n = 9, 25.92 ± 10.02%, $P < .01$ vs Sal; Mus+Bic: n = 8, −0.81 ± 6.13%, $P > .05$ vs Sal, $P < .01$ vs Mus; Fig 2B).

### Effects of $GABA_A$ Receptors in the CeA on Pain Modulation in the Cheek AITC Injection Model

To further determine the effects of $GABA_A$ receptors in the CeA on pain modulation, we next introduced another pain-related behavioral model: cheek microinjection of AITC. The results showed that bilateral intra-CeA microinjection of Mus, but not Bic, significantly decreased the number (Sal: n = 11, 49.5 ± 6.8; Mus: n = 10, 25.6 ± 7.1, $P < .01$ vs Sal; Bic: n = 10, 47.3 ± 9.5, $P > .05$ vs Sal; Fig 3A) and time (Sal: n = 11, 354.0 ± 58.0 seconds; Mus: n = 10, 175.8 ± 49.3 seconds, $P < .01$ vs Sal; Bic: n = 10, 322.3 ± 57.1 seconds, $P > .05$ vs Sal; Fig 3C) of forelimb wipes evoked with i.d. cheek microinjection of AITC, compared with intra-CeA microinjection of Sal. Similar to the tail-flick test, the analgesic effect of Mus was attenuated by intra-CeA Bic injection (Mus+Bic: n = 10, 42.0 ± 7.0 and 301.4 ± 69.9 seconds for wiping number and time, $P > .05$ vs Sal, $P < .01$ vs Mus; Figs 3A and 3C). Detailed analysis of wipe bouts on each 5-minute session revealed that there were low levels of spontaneous wipe bouts (−5 to 0 minutes), and AITC injection primarily elicited a significant increase in wipe bouts in rats subjected to intra-CeA microinjection of Sal, which peaked 10 to 15 minutes after injection and persisted for up to 40 minutes (Figs 3B and 3D). Compared with Sal treatment,

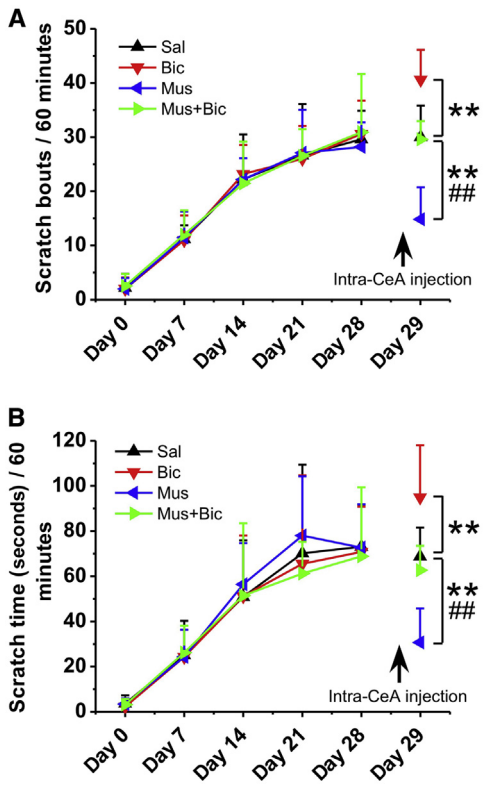


**Figure 5.** $GABA_A$ receptors in the CeA modulate chronic dry skin itching behavior. The number **(A)** and time **(B)** of scratch bouts after intra-CeA microinjection of Mus (n = 11, 50 ng per side), Bic (n = 10, 20 ng per side), Mus + Bic (n = 11) or the same volume of sterile Sal (n = 10). $^{**}P < .01$ versus Sal, $^{\#\#}P < .01$ versus Mus, post hoc Tukey test after ANOVA ($F_{3,38} = 43.706$, $P < .001$ for scratch number; $F_{3,38} = 28.942$, $P < .001$ for scratch time).

Mus, but not Bic, dramatically decreased wipe bouts from 10 to 40 minutes after AITC injection (Figs 3B and 3D).

Combined with the aforementioned results from the tail-flick test, these results suggest that the $GABA_A$ receptors in the CeA are involved in pain modulation.

## *Effects of $GABA_A$ Receptors in the CeA on Acute Itch Modulation*

Because itch and pain are unpleasant sensations and might share a common pathway, we next wanted to determine the effects of $GABA_A$ receptors in the CeA on itch modulation. We found that intra-CeA microinjection of Bic dramatically increased the number (Sal: n = 12, 42.0 ± 24.3; Bic: n = 10, 62.9 ± 33.0, $P < .05$ vs Sal; Fig 4A) and time (Sal: n = 12, 155.8 ± 74.1 seconds; Bic: n = 10, 268.3 ± 131.1 seconds, $P < .05$ vs Sal; Fig 4C) of hind limb scratch bouts elicited using i.d. cheek microinjection of 5-HT, compared with intra-CeA microinjection of Sal. In contrast, Mus infusion dramatically reduced the number (Mus: n = 12, 14.3 ± 15.4, $P < .05$ vs Sal; Fig 4A) and time (Mus: n = 12, 62.3 ± 55.6 seconds, $P < .05$ vs Sal; Fig 4C) of hind limb scratch bouts. Furthermore, the effect of Mus on scratch behavior was attenuated by coapplication of Bic (Mus+Bic: n = 8, 34.3 ± 9.6 and 146.8 ± 65.8 seconds for wiping number and time, $P > .05$ vs Sal, $P < .05$ vs Mus; Figs 4A and 4C). Detailed analysis of scratch bouts in each 5-minute session revealed that there were low levels of spontaneous hind limb scratch bouts (−5 to 0 minutes), and 5-HT injection primarily elicited a significant increase in hind limb scratch bouts directed to the injection site in rats subjected to intra-CeA microinjection of Sal, which peaked 10 to 20 minutes after injection and persisted for up to 40 minutes (Figs 4B and 4D). Compared with Sal treatment, Bic and Mus dramatically increased and decreased hind limb scratch bouts from 10 to 50 minutes after 5-HT injection, respectively (Figs 4B and 4D).

## *Effects of $GABA_A$ Receptors in the CeA on Chronic Itch Modulation*

Because itch manifests in acute and chronic forms and chronic itch remains a challenge in the clinic, we next introduced a chronic-itching animal model, dry skin itch, to further determine the effects of $GABA_A$ receptors in the CeA on chronic itch modulation. The results showed that intra-CeA microinjection of Bic dramatically increased the number (Sal: n = 10, 29.9 ± 5.8; Bic: n = 10, 40.5 ± 5.5, $P < .01$ vs Sal; Fig 5A) and time (Sal: n = 10, 68.7 ± 12.8 seconds; Bic: n = 10, 94.9 ± 23.0 seconds, $P < .01$ vs Sal; Fig 5B) of scratch bouts in rats subjected to the chronic treatment with a mixture of acetone and diethylether for 29 days, compared with intra-CeA microinjection of Sal. In contrast, Mus infusion dramatically reduced the number (Mus: n = 11, 14.7 ± 5.9, $P < .01$ vs

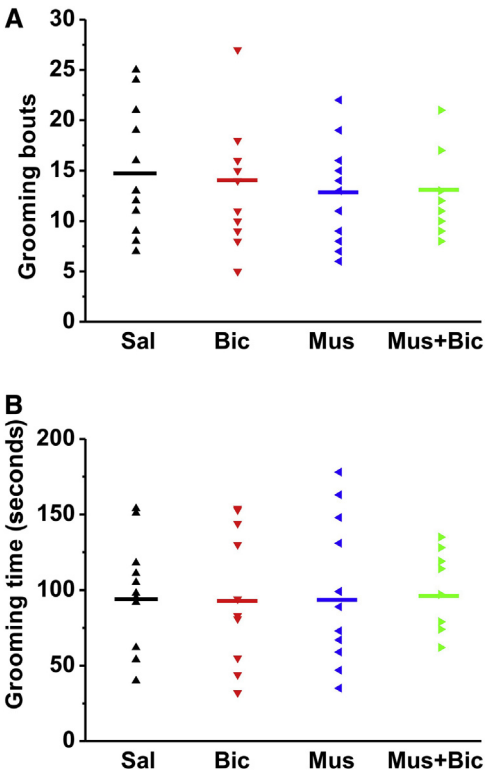


**Figure 6.** $GABA_A$ receptors in the CeA have no effects on facial grooming behavior. Total grooming bouts **(A)** and grooming time **(B)** remain unchanged in rats subjected to intra-CeA microinjection of Mus (n = 12), Bic (n = 10), or Mus + Bic (n = 8) compared with Sal control (n = 12). No difference among these groups, post hoc Tukey test after ANOVA ($F_{3,38} = .411$, $P > .05$ for grooming bouts; $F_{3,38} = 0.050$, $P > .05$ for grooming time).

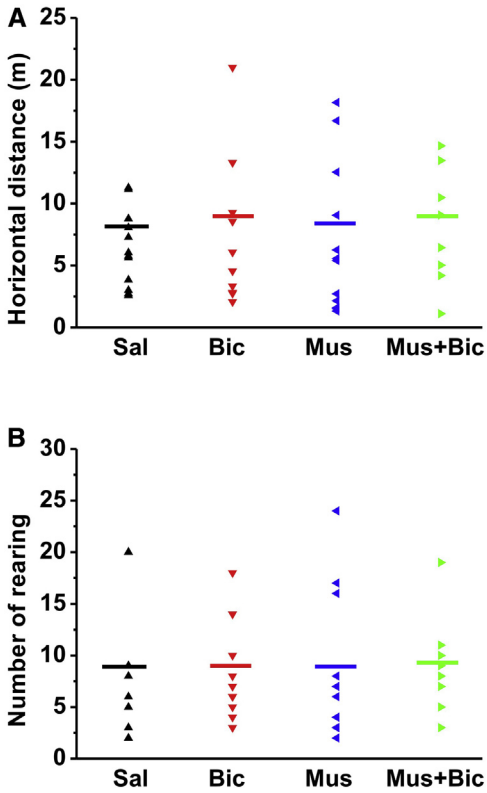


**Figure 7.** $GABA_A$ receptors in the CeA have no effects on locomotor activity. Horizontal distance **(A)** and rearing bouts **(B)** remain unchanged in rats subjected to intra-CeA microinjection of Mus (n = 12), Bic (n = 10), or Mus + Bic (n = 8), compared with Sal control (n = 12). No difference among these groups, post hoc Tukey test after ANOVA ($F_{3,38} = .199$, $P > .05$ for horizontal distance; $F_{3,38} = .055$, $P > .05$ for rearing bouts).

Sal; Fig 5A) and time (Mus: n = 11, 30.6 ± 15.0 seconds, $P < .01$ vs Sal; Fig 5B) of scratch bouts. Furthermore, the effect of Mus on scratch behavior was attenuated by coapplication of Bic (Mus+Bic: n = 11, 29.5 ± 3.4 and 62.6 ± 10.8 seconds for wiping number and time, respectively, $P > .05$ vs Sal, $P < .01$ vs Mus; Figs 5A and 5B).

Combined with the aforementioned results from 5-HT–elicited acute itch, these results suggest that $GABA_A$ receptors in the CeA are involved in itch modulation.

### *Effects of $GABA_A$ Receptors in the CeA on Spontaneous Behaviors*

To further determine whether the effects of $GABA_A$ receptors in the CeA on pain and itch modulation were attributed to spontaneous motor activity, we next measured facial grooming behavior and locomotor activity in rats subjected to intra-CeA microinjection of Bic, Mus, or Sal. Facial grooming bouts (Sal: n = 12, 14.9 ± 6.5; Bic: n = 10, 13.3 ± 6.5, $P > .05$ vs Sal; Mus: n = 12, 12.7 ± 4.9, $P > .05$ vs Sal; Mus+Bic: n = 8, 12.5 ± 4.5, $P > .05$ vs Sal; Fig 6A) and time (Sal: 94.3 ± 36.9 seconds; Bic: 97.0 ± 45.9 seconds, $P > .05$ vs Sal; Mus: 99.0 ± 46.7 seconds, $P > .05$ vs Sal; Mus+Bic: 101.0 ± 27.1 seconds, $P > .05$ vs Sal; Fig 6B) were not affected by Bic or Mus. Furthermore, locomotor activity also remained unchanged, reflected by horizontal distance (Sal: n = 12, 6.3 ± 3.1 m; Bic: n = 10, 7.2 ± 6.3 m, $P > .05$ vs Sal; Mus: n = 12, 6.9 ± 6.0 m, $P > .05$ vs Sal; Mus+Bic: n = 8, 8.1 ± 4.7 m, $P > .05$ vs Sal; Fig 7A) and rearing bouts (Sal: 8.0 ± 6.2; Bic: 8.1 ± 4.7, $P > .05$ vs Sal; Mus: 8.1 ± 7.1, $P > .05$ vs Sal; Mus+Bic: 9.0 ± 4.8, $P > .05$ vs Sal; Fig 7B).

## Conclusions

In the present study, we confirmed previous reports that $GABA_A$ receptors in the CeA are associated with pain modulation, and demonstrated that intra-CeA infusion of a selective $GABA_A$ receptor antagonist Bic, increased acute and chronic itch-related response, whereas infusion of a selective $GABA_A$ receptor agonist Mus, decreased itch-related response. We have therefore provided evidence that $GABA_A$ receptors in the CeA are also involved in itch modulation.

There is a growing body of evidence has implicated that the amygdala, particularly the CeA, is a main pain center.[32] The CeA receives multiple forms of nociceptive information from the thalamus and the cerebral cortex as well as from the parabrachial nucleus,[5,8,17,28] and then integrates and delivers this nociceptive information to output neurons that influence effector centers of the brainstem.[4] Some of these centers such as periaqueductal gray[23] and parabrachial nucleus are key parts of the nociceptive descending controls.[4] Therefore, pharmacological suppression of CeA neurons excitability via activation of GABAergic neurons inhibits sensory nociceptive processing, as reflected by an increase in tail-flick latency (Fig 2) and a decrease in AITC-elicited forelimb wipes (Fig 3). These findings are supported by recent reports that activation of $GABA_A$ receptors in the CeA attenuates hind paw mechanical allodynia.[11,25] It is, however, interesting to note that inactivation of $GABA_A$ receptors in the CeA by intra-CeA microinjection of Bic, a selective $GABA_A$ receptor antagonist, had no effect on pain modulation (Figs 2 and 3). One possible explanation is that the dose of Bic (20 ng per side) that we used in the present study was not enough to induce an obvious pain response. Indeed, this hypothesis is supported by a recent report. Pedersen and colleagues reported that intra-CeA administration of 10 and 25 ng Bic has no effect on nociceptive behaviors.[25] However, to further understanding of the role of CeA $GABA_A$ receptors in pain modulation, future experiments to determine the effects of different doses of Mus and Bic on pain modulation are indeed necessary.

In contrast to pain, there have been until recently few studies of the spinal processing and modulation of itch, despite the fact that itch can significantly impair sleep quality and reduce the quality of life.[19,33] In the present study, we reported that activation of $GABA_A$ receptors in the CeA significantly inhibited itch-related scratching behavior in acute (Fig 4) and chronic (Fig 5) itching animal models. In contrast, inactivation of $GABA_A$ receptors in the CeA significantly increased itch-related scratching behavior (Figs 4 and 5). These influences are not likely attributable to generalized motor alteration, because rats did not exhibit a significant change in locomotor activity (Fig 7) and facial grooming behavior (Fig 6). However, it is noteworthy that there are a number of pruritogens that are used to create experimental animal models of itching besides 5-HT, such as histamine, compound 48/80, substance P, and H-Ser-Leu-Ile-Gly-Arg-Leu-NH2.[1,9] Thus, further experiments on the effects of $GABA_A$ receptors in the CeA on acute and chronic itch elicited by these pruritogens will help in understanding of the basic mechanisms of itch.

In summary, our study showed that $GABA_A$ receptors in the CeA are involved in pain- and itch-related responses. To our knowledge, this is the first study to examine the effects of $GABA_A$ receptors in the CeA on itch modulation, which might be helpful for us to better understand the basic mechanisms of itch and subsequently develop novel mechanism-based strategies to treat itch.

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