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RESEARCH ARTICLE

Supraspinal kappa-opioid receptors: new therapeutic strategies for pain, pruritus, and negative emotions

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Abstract

Research on kappa-opioid receptor (KOR) regulation of pain and itching has focused primarily on spinal and peripheral levels. However, the role of central KOR in this process, as well as the mechanisms exacerbating negative emotional responses to pain and itching, remains unknown. Therefore, this study aimed to utilize the advantages of intracerebroventricular (*i.c.v.*) administration of U50488H to explore supraspinal KOR activation on pain, itching, and negative emotions. U50488H, a prototypical KOR agonist, was administered *i.c.v.*, with physiological saline as the control. The Hargreaves test and intradermal injection of histamine and chloroquine were conducted to assess thermal pain and itch behavior, respectively. The elevated plus maze (EPM), open field test (OFT), and tail suspension test (TST) were performed to evaluate negative emotions. *i.c.v.* administration of U50488H increased thermal pain latencies, reduced scratching behavior, and decreased locomotor activity in the central zone of the OFT and in the open arms of the EPM, while increasing immobility in the TST. *i.c.v.* pretreatment with the KOR antagonist nor-Binaltorphimine dihydrochloride reversed all of the above behaviors. In conclusion, central administration of U50488H can exhibit analgesic and antipruritic effects while also inducing negative emotional responses. Our results highlight the potential of supraspinal KOR as a promising therapeutic target in the combined treatment of pain, pruritus, and negative emotions.

Keywords U50488H · Pain · Itch · Anxiety · Depression · Kappa-opioid receptor

Introduction

Dynorphins (DYNs) and their endogenous target, the kappa-opioid receptor (KOR), constitute the DYNs/KOR system. Understanding this system is essential for developing potential therapeutic interventions for reward, motivation, addiction, pain, and mood disorders (Cahill et al. 2021; Khan et al. 2022). Understanding this system is essential in developing potential therapeutic interventions for pain and mood disorders (Aldrich and McLaughlin 2021). U50488H is a synthetic selective KOR agonist, mimicking the effects of endogenous dynorphins and is widely utilized in KOR preclinical research on the pharmacological functions of KORs (Kuhn et al. 2017; Zhang et al. 2022b). KOR activation by DYNs induces analgesia, but it is also associated with negative emotions, distinguishing it from the effects of other opioid receptor systems (Malfliet et al. 2017; Machelska and Celik 2018). However, the acting target and causal relationships between KOR-induced pain, itching, and negative emotions remain under debate, necessitating further investigation.

Li Zhang and Shuai Zhou contributed equally to this work.

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KOR is expressed in the central nervous system (CNS) and is distributed throughout the body in various peripheral organs, including the heart, lungs, spleen, kidneys, liver, and small intestine (Wittert et al. 1996; Peng et al. 2012). This widespread distribution pattern of KORs in peripheral tissues underscores their importance in modulating numerous physiological processes beyond those of the brain (Wu et al. 2021). U50488H can be explored for its effects on pain and itch perception through systemic administration via intraperitoneal (*i.p.*) and intravenous (*i.v.*) routes. However, systemic administration can also affect numerous non-neural internal organs (Zhou et al. 2015). Typically, U50488H is investigated via intrathecal (*i.t.*) and subcutaneous (*s.c.*) administration, which primarily targets the spinal cord and peripheral nerves (Mansikka and Uhl 2004; Mika et al. 2014). Therefore, intracerebroventricular (*i.c.v.*) administration, which directly targets the higher CNS, would offer greater specificity and minimize interference from other peripheral factors as well as the lower CNS.

Anxiety and depression often accompany intractable pain and itching, constituting common mental health complications. They exhibit certain overlaps and connections at the neurotransmitter, peptide, and neural circuit levels in the brain (Malfliet et al. 2017; Michaelides and Zis 2019). Pharmacological strategies solely focused on pain management may not yield optimal efficacy, underscoring the need for further investigation into combined intervention strategies targeting negative emotions, pain, and itching (Docherty et al. 2023). U50488H, administered via *i.t.*, *i.v*, and *s.c.* routes, attenuates neuropathic pain (Mansikka and Uhl 2004; Rutten et al. 2018; Zhang et al. 2022a) and itching (Ko et al. 2003; Yamamoto and Sugimoto 2010). However, *i.p.* administration of U50488H can induce anxiety- and depression-like behaviors in mammals (Wiley et al. 2009; Wittmann et al. 2009). The pharmacological effects and underlying mechanisms of KOR activation in the brain on pain and itching, and the related negative emotions remain unclear. Therefore, this study aimed to leverage the advantages of the *i.c.v* administration of U50488H to explore the effects of KOR on pain, itching, and associated negative emotions. To explore the effects of U50488H at the supraspinal level, we used *i.c.v.* administration at graded doses, based on a previous study (Roerig 1989).

Methods

Animals

Male C57BL/6J mice (RRID: IMSR_JAX:000664), 8–10 weeks old and weighing 20–27 g, were obtained from Jinan Pengyue Company and housed in a specific-pathogen-free facility under controlled conditions, maintained at a temperature of 22℃–25℃, with a 12-h dark-light cycle. The animals had *ad libitum* access to food and water. All animal experiments strictly adhered to the ethical guidelines established by the Binzhou Medical University Animal Ethics Committee (Ethics approval number: 2020−238) and complied with the principles of the Declaration of Helsinki.

Drug

U50488H [trans-(1 S,2 S)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl] benzene acetamide hydrochloride hydrate] was purchased from Sigma-Aldrich Incorporation (U111, sigma, St. Louis, MO, USA). The drugs were dissolved in 0.9% (w/v) sterile saline. Based on the solubility of U50488H (13 mg/mL) and its ED50 value for *i.c.v.* administration (Roerig 1989), the drug concentration was divided into three gradient concentrations—3.25, 6.5, and 13 μg/μL—with physiological saline used as the control. U50488H was administered via *i.c.v.* injection at a volume of 5 μL over a 10 min duration. After injection, a waiting period of 30 min was observed before the relevant experimental tests were conducted. Nor-Binaltorphimine dihydrochloride (nor-BNI), an antagonist of KOR, was purchased from Aladdin Incorporation (105618-26-6, Aladdin, shanghai, China). The nor-BNI was dissolved in 0.9% (w/v) sterile saline and prepared into a working solution of 0.5 μg/μL. All the drugs were manually administered at a rate of 0.25 μL per 30 s with a 10 μL syringe to prevent an increase in intracranial pressure.

Experimental design and animal grouping

We selected 110 C57BL/6J mice, which were randomly divided into 16 groups. All mice were anesthetized with 1.5% sodium pentobarbital (0.06 mL/10 g, *i.p.*) and fixed in a stereotaxic apparatus. A metal cannula (62002, RWD, Shenzhen, China) was carefully positioned into the right lateral ventricle using the following coordinates: anteroposterior, −0.34 mm; lateral to midline, 1.0 mm; dorsoventral, 2.5 mm. They were fixed in place using dental cement and underwent 1 week of postoperative recovery. Of these, 64 mice were assigned to the eight U50488H groups (8 mice per group). Mice in groups 1–4 were treated with saline or U50488H at 3.25, 6.5, or 13 μg/μL, and underwent rotarod, pain, and itch behavior assessments. Mice in groups 5–8 were treated with saline or U50488H at 3.25, 6.5, or 13 μg/μL, and underwent negative emotional behavior assessments. The remaining 56 mice were assigned to the 9–16 groups (nor-BNI + U50488H group, 7 mice per group). For each group, 1 μg/2 μL of nor-BNI was pretreated via *i.c.v.* 30 min prior to the experiment (Narita et al. 1993). Mice

in groups 9–12 were treated with saline or U50488H at 3.25, 6.5, or 13 μg/μL, and underwent rotarod, pain, and itch behavior assessments. Mice in groups 13–16 were treated with saline or U50488H at 3.25, 6.5, or 13 μg/μL, and underwent negative emotional behavior assessments. To exclude potential drug accumulation effects from *i.c.v* administration, each group of mice was tested once every 7 days (Fig. 1).

Hargreaves test

Paw withdrawal latencies (PWL) to radiant heat were assessed using the method originally described by Hargreaves (Hargreaves et al. 1988) and measured with the IITC Plantar Meter (IITC Life Science, Woodland Hills, CA, USA). The mice were allowed to acclimatize to the testing environment for 30 min before the experiment commenced. The temperature of the glass plate was maintained at 30°C. Radiant heat intensity, emitted by a 150-watt bulb, was calibrated to achieve a suitable light intensity that maintained the paw withdrawal threshold of normal mice between 8 and 12 s. Following *i.c.v.* administration of U50488H, the mice were individually positioned in transparent plastic boxes on the glass floor. The radiant heat was regulated using the start button. The termination signal for the latency period was recorded when mice displayed evident reflexive paw withdrawal or licking. A cutoff time of 30 s was used to prevent potential harm. The experiment comprised five trials, with measurements conducted at 10-min intervals within each trial.

Acute itch test

At 3 days before the experiment, mice had their necks shaved to prepare the skin. Subsequently, they were placed in a behavior room inside transparent plastic boxes for a 30-min acclimation period, which continued for 3 days. A small amount of bedding material was placed inside the acrylic boxes to mimic the cage environment. Histamine (H7250, Sigma, St. Louis, MO, USA) or chloroquine (C6628, Sigma, St. Louis, MO, USA) was dissolved in sterile saline (0.9%) to prepare a working solution at a

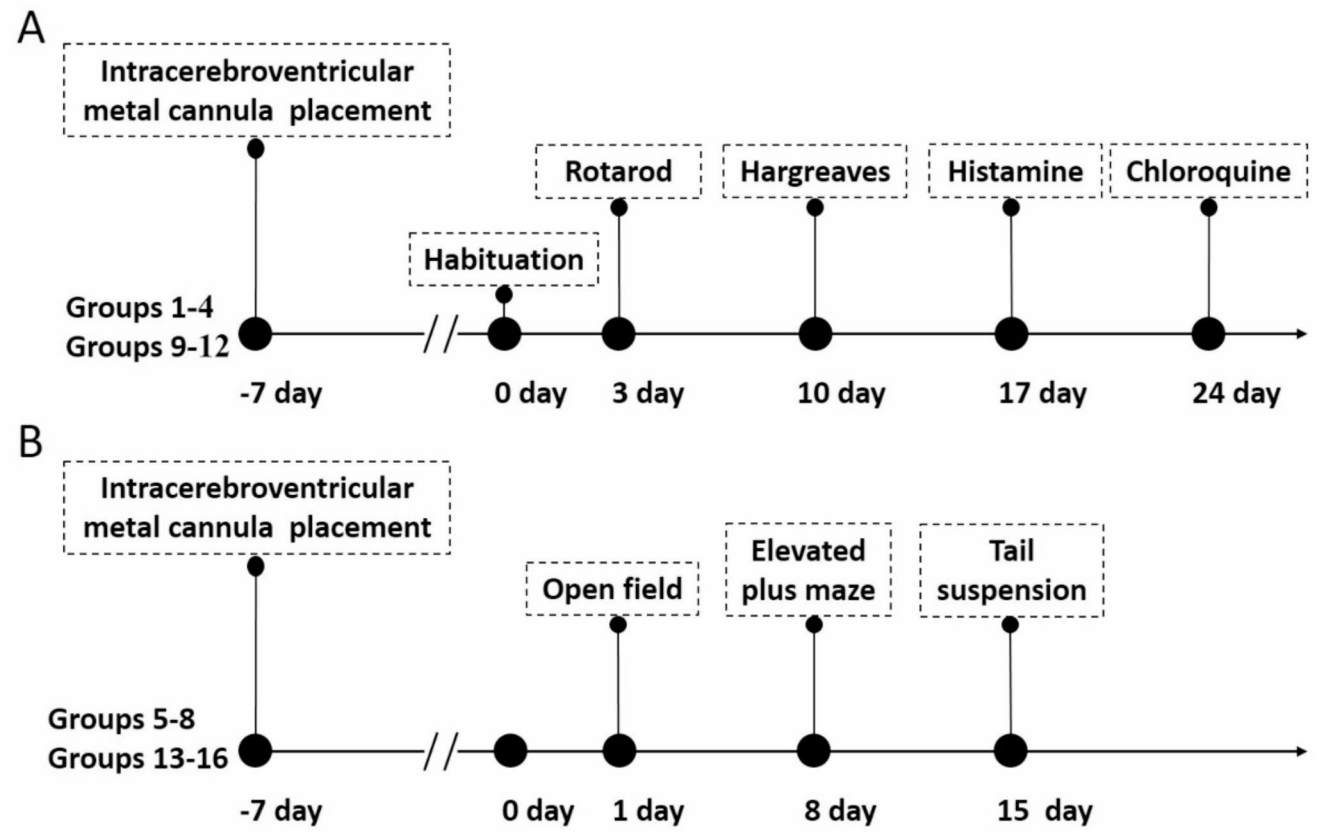

Fig. 1 Experimental design. (**A**) Schedule of the rotarod, pain, and itch behavioral tests. After a 7-day recovery following cranial cannula placement, mice in groups 1–4 (U50488H groups, 8 mice per group) and groups 9–12 (nor-BNI+U50488H groups, 7 mice per group) sequentially underwent the rotarod, Hargreaves, histamine, and chloroquine tests on days 3, 10, 17, and 24, respectively. (**B**) Schedule of negative emotions test. After a 7-day recovery, mice in groups 5–8 (U50488H groups, 8 mice per group) and groups 13–16 (nor-BNI+U50488H groups, 7 mice per group) sequentially underwent the OFT, EPM, and TST experimental assessments on days 1, 8, and 15, respectively

concentration of 4 μg/μL. Following *i.c.v.* administration of U50488H, intradermal injections of 50 μL of histamine or chloroquine were administered at the back of the neck. After the intradermal injections, the scratching behavior of the mice inside the transparent plastic box was recorded for 30 min (Kamei and Nagase 2001).

Open-field test (OFT)

The OFT was used to assess locomotor activity and anxiety-like behaviors, following the method described previously (Liebsch et al. 1998). The test was conducted in a dimly lit room at 22–25 °C. The OFT apparatus (Xinruan, Shanghai, China) consisted of a large blue acrylic wall placed on a white plastic bottom plate measuring 50 × 50 × 40 cm, and was divided into 16 squares (12.5 × 12.5 cm) with two zones: an outer zone with 12 peripheral squares and a center zone with 4 inner squares. Before testing each mouse, the OFT apparatus was cleaned with 75% ethanol to maintain hygiene and prevent potential interference between mice. The behavior of the mice was recorded using a camera mounted above the arena, and the recordings were subsequently analyzed using the VisualTrack software (Xinruan, Shanghai, China) for 5 min. The following behaviors were assessed during the OFT, including distance traveled and time spent in each zone (outer and center).

Elevated plus maze (EPM) test

The EPM test was used to assess anxiety-like behaviors and was conducted according to the methods (Yoshizaki et al. 2020). The testing room was kept quiet and dimly lit at 22–25 °C. The test apparatus comprised four blue acrylic arms (35 × 5 cm) elevated on 60 cm aluminum alloy legs from Xinruan Instruments (Xinruan, Shanghai, China). The EPM was divided into five zones: two open arms featuring 0.5 cm ledges, two closed arms with 15 cm walls, and a center zone measuring 5 × 5 cm. Behavioral analysis was automatically conducted using the VisualTrack software for 5 min. The software automatically recorded the following behavioral parameters: distance traveled, time spent, and number of entries into the open arms, closed arms, and center zone.

Tail suspension test (TST)

The TST was employed to evaluate depression-like behavior (Can et al. 2011). Acoustic suspension and visual isolation were attained in the testing room. Mice were suspended approximately 50 cm above the floor using a testing apparatus (Xinruan, Shanghai, China) set at a height of 20–25 cm above the ground. This was achieved by affixing adhesive tape with a length of 15 cm to the tail, with the tail tip left exposed at 2–3 mm. During the 5-min test, the total immobility time and mobility time induced by the TST were measured. Behavioral analysis was automatically conducted using the VisualTrack software.

Rota-rod test

Following a previously described method, the rotarod was employed to assess potential neurological impairments affecting the motor capacity of the animals, such as sedation, ataxia, and muscle relaxation (Dunham and Miya 1957). To minimize stress during the testing period, the animals underwent a 3-day acclimation period to the model. Mice were placed on a rotating roller set at a constant speed of 6 rpm. The duration for which the mice remained on the rotating roller without falling was recorded over 60 s. The primary objective of this test was to assess potential neurological impairments affecting the motor capacity of the animals, such as sedation, ataxia, and muscle relaxation.

Software and data analysis

Data analysis was conducted using IBM SPSS 27.0 and GraphPad Prism 6.0. Before conducting statistical tests, the normal distribution of the data was assessed. Data that did not follow a normal distribution were analyzed using the Kruskal–Wallis test. Data that were normally distributed but did not meet the assumption of homogeneity of variances were analyzed using Tamhane's test. Data that were normally distributed and met the assumption of homogeneity of variances were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Comparisons between the U50488H and nor-BNI + U50488H groups were performed using an unpaired t-test. The results are presented as the mean ± standard error of the mean (S.E.M.). A p-value of <0.05 was considered statistically significant. Statistical significance in figures is indicated as $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$.

Results

Intracerebroventricular U50488H administration mitigates radiant heat-induced thermal hyperalgesia in hargreaves testing

The effects of *i.c.v.* U50488H administration on radiant heat-induced thermal pain were examined in this study. Mice were administered saline (0 μg/μL) or one of the three concentrations—3.25, 6.5, and 13 μg/μL—of U50488H via *i.c.v.* injection, followed by evaluation of hind PWL using

the Hargreaves test (Fig. 2). As the concentration of administration increased, a distinct dose-dependent elevation trend was observed in bilateral PWL assessments. Significant statistical differences were observed when the 13 μg/μL U50488H groups were compared with the 0 and 3.25 μg/μL U50488H groups (right: $p<0.0001$ and $p<0.01$, respectively; left: $p<0.0001$ and $p<0.0001$, respectively; Fig. 2A and B). An increase in bilateral PWL values was observed between the 6.5 and 0 μg/μL U50488H groups (right: $p<0.05$, left: $p<0.0001$, Fig. 2A and B). Significant statistical differences were observed between the left 6.5 μg/μL U50488H group and both the 3.25 and 13 μg/μL U50488H groups ($p<0.0001$ and $p<0.0001$, respectively; Fig. 2B). The increase in bilateral PWL values was reversed by the antagonist nor-BNI in the comparison between the 6.5 μg/μL U50488H and 6.5 μg/μL nor-BNI+U50488H groups (right: $p<0.01$, left: $p<0.001$, Fig. 2A and B), as well as between the 13 μg/μL U50488H and 13 μg/μL nor-BNI+U50488H groups (right: $p<0.0001$, left: $p<0.001$, Fig. 2A and B). The PWL values showed no statistically significant difference in the comparison between the 0 μg/μL U50488H group and the four nor-BNI+U50488H groups. These findings suggest that *i.c.v.* administration of U50488H can effectively suppress radiant heat-induced thermal pain.

Intracerebroventricular U50488H administration suppresses scratching responses induced by Histamine and chloroquine

To explore the effects of *i.c.v.* U50488H administration on scratching responses induced by histaminergic and nonhistaminergic pruritogens, four U50488H groups of mice were administered saline or varying concentrations—3.25, 6.5, and 13 μg/μL—of U50488H via *i.c.v.* injection, followed by an assessment of scratching responses after intradermal injections of histamine or chloroquine (Fig. 3). In the chloroquine and histamine tests, the scratching frequency of the 13 μg/μL U50488H group exhibited a significant decrease compared with that in the 0, 3.25, and 6.5 μg/μL U50488H groups (chloroquine: $p<0.0001$, $p<0.0001$, and $p<0.0001$, respectively; histamine: $p<0.0001$, $p<0.0001$, and $p<0.01$, respectively; Fig. 3A and B). Furthermore, in the histamine tests, the 6.5 μg/μL U50488H group exhibited significant statistical differences compared with the 0 μg/μL U50488H group ($p<0.01$, Fig. 3B). The decrease in scratching frequency in the chloroquine and histamine tests was reversed by the antagonist nor-BNI in the comparison between the 13 μg/μL U50488H and nor-BNI+U50488H groups (chloroquine: $p<0.001$, histamine: $p<0.001$, Fig. 3A and B), as well as between the 6.5 μg/μL U50488H and nor-BNI+U50488H groups in the histamine test ($p<0.05$,

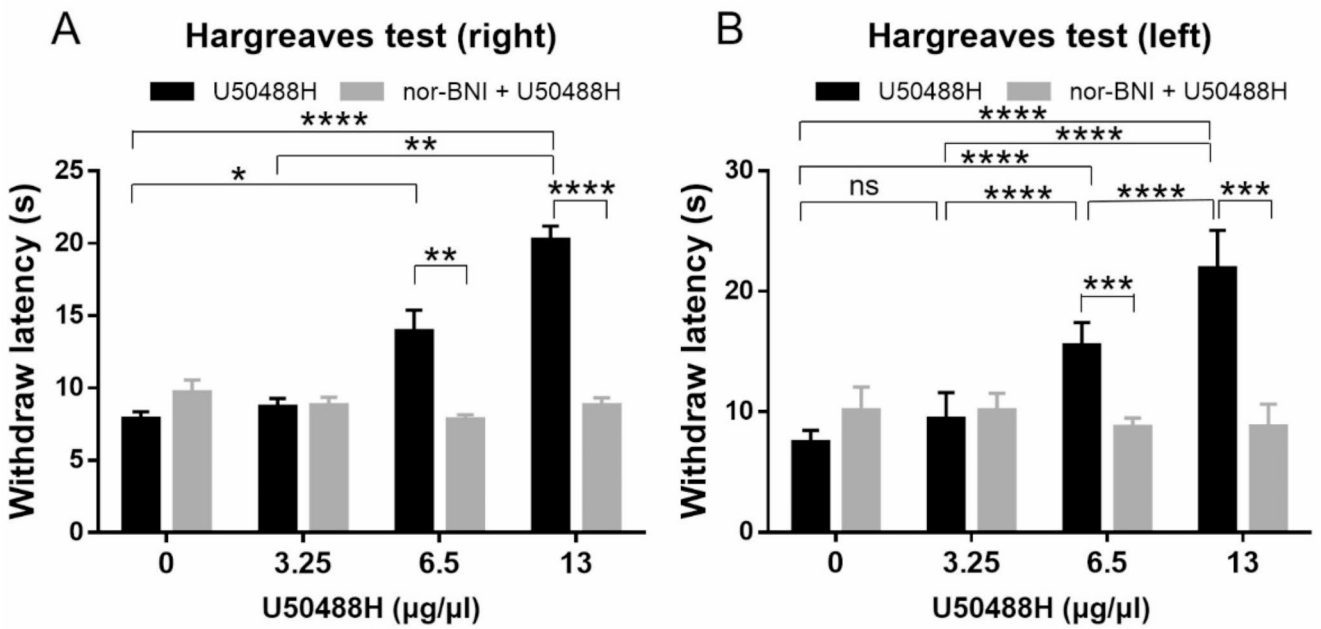

Fig. 2 Intracerebroventricular administration of U50488H exhibits analgesic effects on radiant heat-induced pain and is reversed by nor-BNI. Hargreaves PWL of the right hind paw (**A**) [13 vs. 0 μg/μL $P<0.0001$; 13 vs. 3.25 μg/μL $P<0.01$; 6.5 vs. 0 μg/μL $P<0.05$] and left hind paw (**B**) [13 vs. 0 μg/μL $P<0.0001$; 13 vs. 3.25 μg/μL $P<0.0001$; 13 vs. 6.5 μg/μL $P<0.0001$; 6.5 vs. 0 μg/μL $P<0.0001$; 6.5 vs. 3.25 μg/μL $P<0.0001$] in mice were compared between different U50488H groups ($n=8$). Data are presented as the mean±S.E.M. and significance was assessed by the Kruskal–Wallis test in (**A**) and one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test in (**B**) between different U50488H groups. Comparisons between the U50488H and nor-BNI+U50488H groups were performed using an unpaired t-test ($n=7$ mice per nor-BNI+U50488H group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

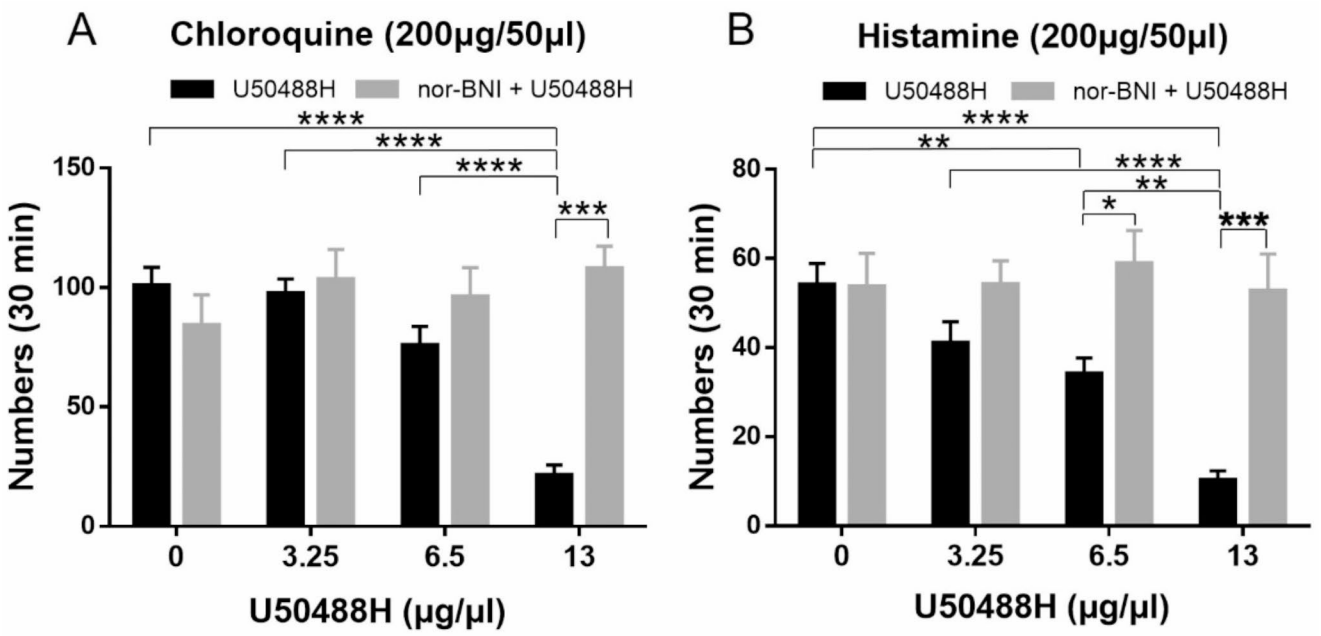

Fig. 3 Intracerebroventricular administration of U50488H inhibits itch behavior and is reversed by nor-BNI. (**A**) After *i.c.v.* administration of saline and U50488H at concentrations of 3.25, 6.5, and 13 μg/μL, followed by intradermal injections of chloroquine, scratching numbers were compared between different U50488H groups for 30 min [13 vs. 0 μg/μL $P<0.0001$; 13 vs. 3.25 μg/μL $P<0.0001$; 13 vs. 6.5 μg/μL $P<0.0001$] ($n=8$ mice per U50488H group). (**B**) After *i.c.v.* administration of saline and U50488H at concentrations of 3.25, 6.5, and 13 μg/μL, followed by intradermal injections of histamine, scratching numbers were compared between different U50488H groups for 30 min [13 vs. 0 μg/μL $P<0.0001$; 13 vs. 3.25 μg/μL $P<0.0001$; 13 vs. 6.5 μg/μL $P<0.01$; 6.5 vs. 0 μg/μL $P<0.01$] ($n=8$ mice per U50488H group). Data are presented as the mean ± S.E.M. and significance was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test between different U50488H groups. Comparisons between the U50488H and nor-BNI + U50488H groups were performed using an unpaired t-test ($n=7$ mice per nor-BNI + U50488H group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

Fig. 3B). These findings suggest that *i.c.v.* U50488H administration can inhibit pruritogen-induced scratching responses.

Intracerebroventricular U50488H administration induces anxiety-like behavior in mice

The study aimed to explore the potential of *i.c.v.* U50488H administration to induce anxiety-like behavior using the EPM and OFT (Figs. 4 and 5). The total distance traveled in the OFT showed no statistical significance between the 3.25, 6.5, and 13 μg/μL U50488H groups compared with that of the 0 μg/μL U50488H group (Fig. 4A and E). No statistically significant difference was observed among the U50488H groups in peripheral zone distances (Fig. 4F). However, a decrease in the central zone distance was observed when comparing the 3.25, 6.5, and 13 μg/μL U50488H groups with the 0 μg/μL U50488H group ($p<0.01$, $p<0.05$, and $p<0.0001$, respectively; Fig. 4G). Compared with the 0 μg/μL U50488H group, the 13 μg/μL U50488H group showed an increased peripheral zone duration ($p<0.05$, Fig. 4H) and a decreased central zone duration ($p<0.05$, Fig. 4I). Compared with the 13 μg/μL nor-BNI + U50488H group, the 13 μg/μL U50488H group showed a decreased total distance, peripheral zone distance, central zone distance, and central zone duration (all $p<0.01$, Fig. 4E, G and I), as well as an increased peripheral zone duration ($p<0.05$, Fig. 4H). In the EPM, the total distance of the 13 μg/μL U50488H group decreased compared with that of the 0 and 3.25 μg/μL U50488H groups ($p<0.0001$ and $p<0.0001$, respectively; Fig. 5A and E). The 6.5 μg/μL U50488H groups exhibited a significant decrease in total distance compared with that of the 0 and 3.25 μg/μL U50488H groups ($p<0.05$ and $p<0.05$, respectively; Fig. 5B and C, and 5E). Compared with the 0 μg/μL U50488H group, the 13 μg/μL U50488H group showed a reduction in the distance traveled in the closed arms ($p<0.05$, Fig. 5F). Furthermore, in the open arm, a decrease in the locomotor distance was observed in the 13 μg/μL U50488H group compared with that in the 0 and 3.25 μg/μL U50488H groups ($p<0.001$ and $p<0.0001$, respectively; Fig. 5G). Similarly, a decrease in open arm distance was observed in the 6.5 μg/μL U50488H group compared with those in the 0 and 3.25 μg/μL U50488H groups ($p<0.05$ and $p<0.05$, respectively; Fig. 5G). Compared with the 0 and 3.25 μg/μL U50488H groups, the 13 μg/μL U50488H group showed an increased closed arm duration ($p<0.01$ and $p<0.001$, respectively; Fig. 5H) and decreased open arm duration ($p<0.001$ and $p<0.001$, respectively;

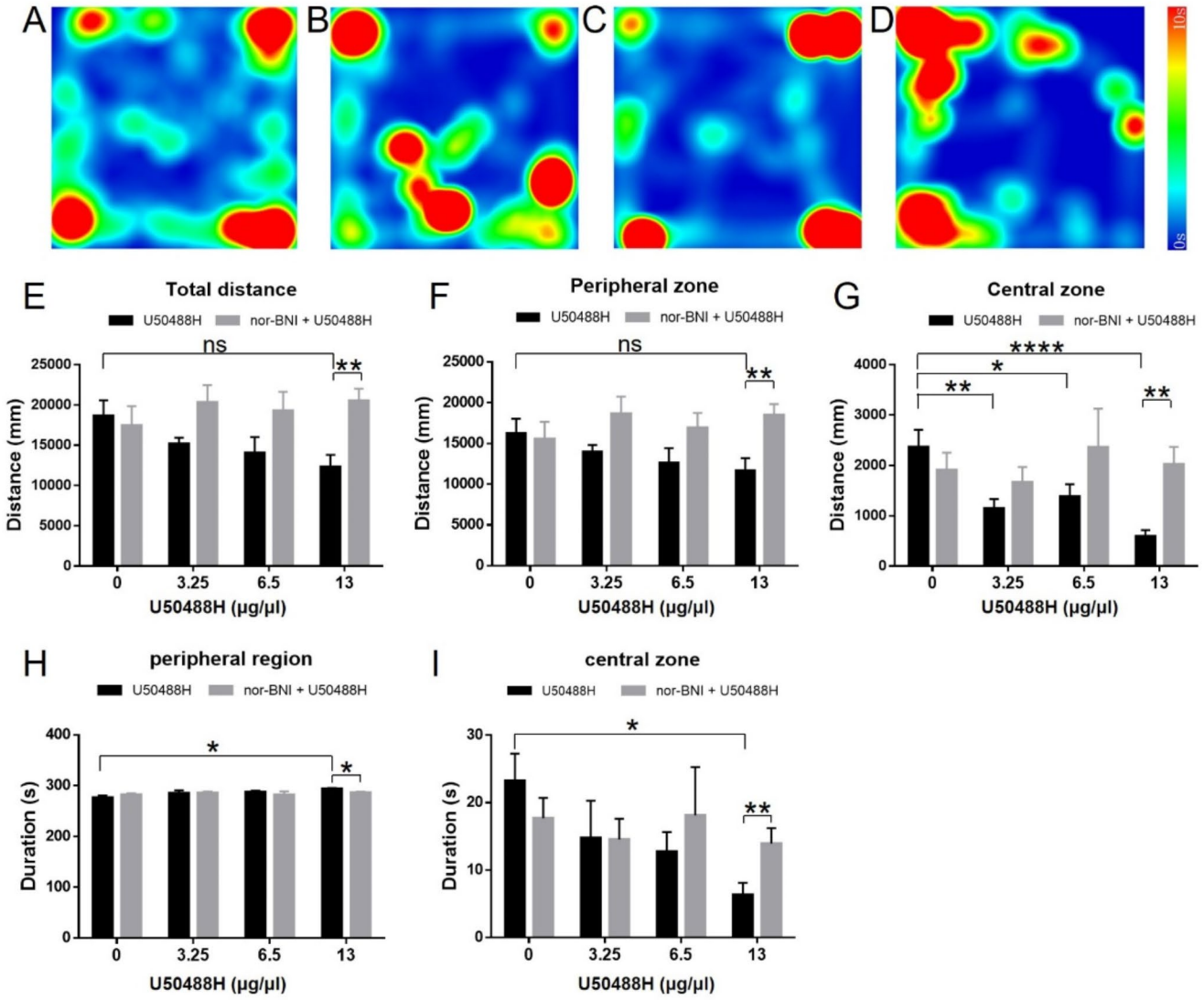

Fig. 4 Effects of U50488H on open-field test behaviors in mice. Representative two-dimensional heat maps showing movement trajectories in the OFT after *i.c.v.* administration of saline (**A**) and U50488H at concentrations of 3.25 (**B**), 6.5 (**C**), and 13 μg/μL (**D**). Total distance (**E**), peripheral area distance (**F**), and central area distance (**G**) in the different U50488H concentration groups and saline control group (**G**: 13 vs. 0 μg/μL $P<0.0001$; 6.25 vs. 0 μg/μL $P<0.05$; 3.25 vs. 0 μg/μL $P<0.01$). Durations in the peripheral zone (**H**) and central zone (**I**) were recorded (**H**: 13 vs. 0 μg/μL $P<0.05$; **I**: 13 vs. 0 μg/μL $P<0.05$) between the different U50488H groups. Significance was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test between different U50488H groups ($n=8$). Comparisons between the U50488H and nor-BNI+U50488H groups were performed using an unpaired t-test ($n=7$ mice per nor-BNI+U50488H group). * $p<0.05$, ** $p<0.01$, **** $p<0.0001$, no significance (ns)

Fig. 5I). Compared with the 13 μg/μL nor-BNI+U50488H group, the 13 μg/μL U50488H group showed a decreased total distance, closed arm distance, open arm distance, and open arm duration ($p<0.001$, $p<0.01$, $p<0.01$, and $p<0.05$, respectively; Fig. 5E, G and I). Compared with the nor-BNI+U50488H group at the same concentration, the 3.25 μg/μL U50488H group showed a decreased closed arm duration, while the 13 μg/μL U50488H group showed an increased closed arm duration ($p<0.05$ and $p<0.05$, respectively; Fig. 5H). These findings indicate that *i.c.v.* U50488H administration can induce anxiety-like behavior in mice.

Intracerebroventricular U50488H administration induces depression-like behavior: evidence from TSTs

Next, we investigated the potential of *i.c.v.* U50488H administration to induce depression-like behavior using the TST. After *i.c.v.* administration of U50488H, the results of the TST showed a statistically significant decrease in mobility time ($p<0.05$, Fig. 6A) and an increase in immobility time ($p<0.01$, Fig. 6B) in the 13 μg/μL U50488H group compared with that in the 0 μg/μL U50488H group. Compared

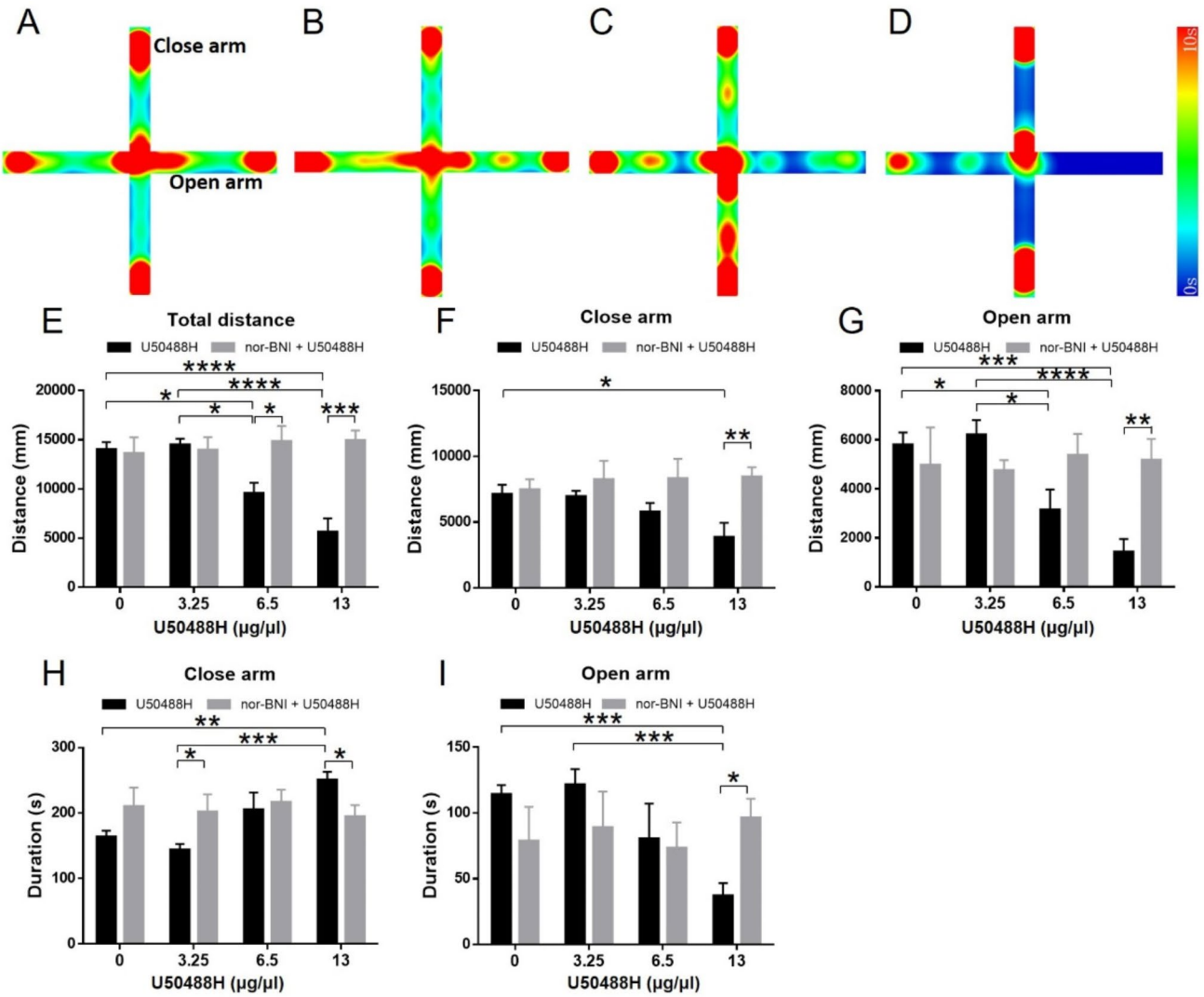

Fig. 5 Effects of U50488H on elevated plus maze behaviors in mice. Representative heat maps showing movement trajectories in the EPM after *i.c.v.* administration of 0.9% saline (**A**) and U50488H at concentrations of 3.25 (**B**), 6.5 (**C**), and 13 μg/μL (**D**). Total distance (**E**) [13 vs. 0 μg/μL $P<0.0001$; 13 vs. 3.25 μg/μL $P<0.0001$; 6.5 vs. 0 μg/μL $P<0.05$; 6.5 vs. 3.25 μg/μL $P<0.05$], closed arm distance (**F**) [13 vs. 0 μg/μL $P<0.05$], and open arm distance (**G**) [13 vs. 0 μg/μL $P<0.001$; 13 vs. 3.25 μg/μL $P<0.0001$; 6.5 vs. 0 μg/μL $P<0.05$; 6.5 vs. 3.25 μg/μL $P<0.05$] traveled in the different U50488H groups and saline control group. Closed arm duration (**H**) [13 vs. 0 μg/μL $P<0.01$, 13 vs. 3.25 μg/μL $P<0.001$] and open arm duration (**I**) [13 vs. 0 μg/μL $P<0.001$, 13 vs. 3.25 μg/μL $P<0.001$] were recorded between different U50488H groups ($n=8$). Data are presented as the mean ± S.E.M. and significance was assessed by Tamhane's test in (**I**) and one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test in (**A–H**) between the different U50488H groups. An unpaired t-test was used for the U50488H and nor-BNI + U50488H groups ($n=7$ mice per nor-BNI + U50488H group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

with the 0 μg/μL U50488H group, the 6.5 μg/μL U50488H group showed an increased immobility time ($p<0.05$, Fig. 6B). No statistically significant difference was observed in immobility time or a decrease in mobility time between the 3.25 and 6.5 μg/μL U50488H groups (Fig. 6A and B). The immobility time showed a dose-dependent increase, while the mobility time demonstrated a dose-dependent decrease. Compared with the nor-BNI + U50488H group at the same concentration, the 6.5 and 13 μg/μL U50488H groups showed a decreased mobility duration ($p<0.05$ and $p<0.01$, respectively; Fig. 6A) and an increased immobility duration ($p<0.05$ and $p<0.01$, respectively; Fig. 6B). These findings indicate that *i.c.v.* U50488H administration can induce depression-like behavior in mice.

Intracerebroventricular U50488H administration does not impair motor function in mice

We investigated the potential effects of *i.c.v.* U50488H administration on motor function in mice using the rotarod

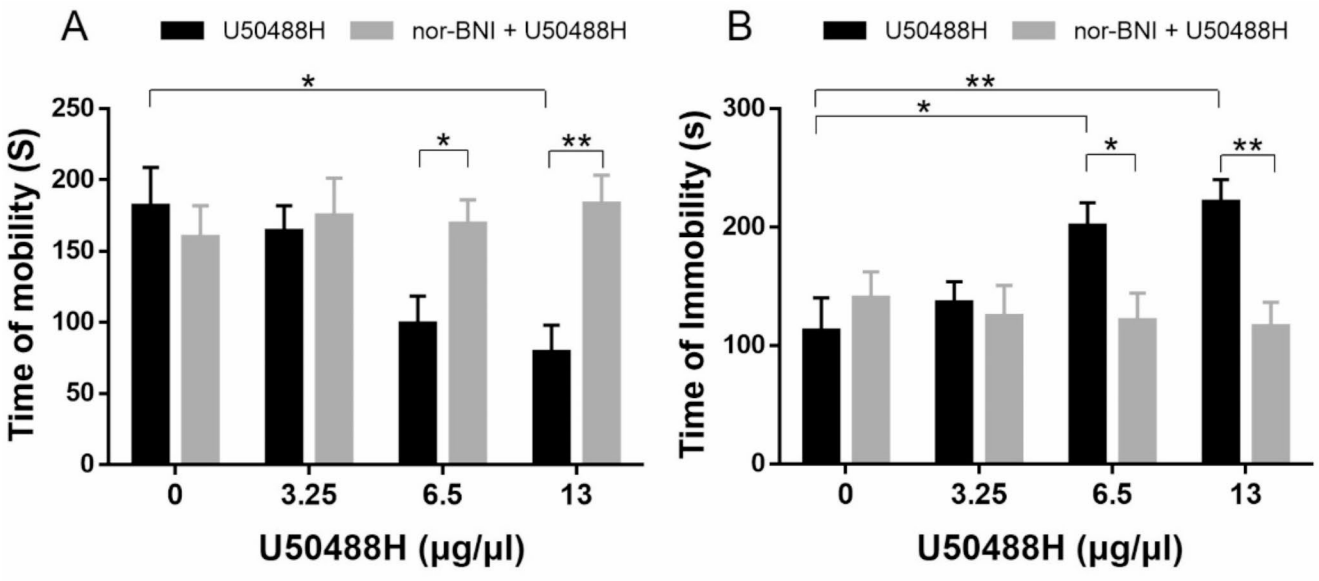

Fig. 6 Effects of U50488H on tail suspension test behaviors in mice. (**A**) Mobility time and (**B**) immobility time in different U50488H groups and the saline control group (**A**: 13 vs. 0 μg/μL $P<0.05$; **B**: 13 vs. 0 μg/μL $P<0.01$, 6.5 vs. 0 μg/μL $P<0.05$). Data are presented as the mean ± S.E.M. and significance was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test between the different U50488H groups ($n=8$). An unpaired t-test was used for the U50488H and nor-BNI + U50488H groups ($n=7$ mice per nor-BNI + U50488H group). * $p<0.05$, ** $p<0.01$

test. The results showed no statistically significant difference in the duration for which the mice could remain on the rotating rod for more than 60 s among all groups. This indicates that i.c.v. administration of U50488H did not induce sedation, ataxia, or muscle relaxation, which could potentially influence experimental results related to motor function.

Discussion

Prior experiments have demonstrated that KOR agonists can alleviate itching and pain at the peripheral nervous system and spinal cord levels (Inan and Cowan 2020; Aldrich and McLaughlin 2021). However, KORs are also widely distributed in the claustrum, medial prefrontal cortex, anterior cingulate cortex, insular cortex (IC), amygdala, periaqueductal gray, nucleus accumbens, and rostral ventromedial medulla (RVM) [Cahill et al. 2021]. Whether the supraspinal KOR contributes to the regulation of pain, itching, and negative emotion regulation remains unclear. In this study, we utilized the prototypical KOR agonist U50488H administered via *i.c.v.* to explore its supraspinal effect. Our results showed that *i.c.v.* administration of U50488H (1) suppressed histamine- and nonhistaminergic (chloroquine)-induced itching behavior, (2) suppressed radiant heat-induced pain triggered by the Hargreaves test, and (3) elicited anxiety and depressive behaviors. Additionally, pretreatment with nor-BNI via i.c.v. injection reversed pain, itching, and negative emotional behaviors in mice.

The DYNs/KOR system serves as a potential therapeutic target for the treatment of clinical pruritus (Kardon et al. 2014). KOR agonists, including U50488H, nalfurafine, CR 845, triazole 1.1, and nalbuphine, have been found to inhibit itch behavior in rodent and non-human primate models via *i.t.*, *s.c.*, *i.v.*, or *i.p.* administration (Wakasa et al. 2004; Inan and Cowan 2020; Huskinson et al. 2022). However, whether supraspinal KOR activation has an identical effect remains unknown. Limited evidence suggests that central mechanisms, rather than peripheral systems, might be involved in cholestatic pruritus (Andoh et al. 2020). The present study, for the first time, demonstrated that the activation of supraspinal KORs dose-dependently inhibits itch behavior. Human brain imaging studies show that different brain areas are activated extensively by histamine and cowhage, respectively (Papoiu et al. 2012). Therefore, such an antipruritic effect probably targets multiple different brain regions. Our experimental results demonstrated that activation of supraspinal KOR not only inhibits histamine-induced itch but also alleviates nonhistaminergic itch induced by chloroquine. This indicates that the activation of supraspinal KOR can simultaneously inhibit pruritic behaviors induced by different pruritogens. This finding is highly significant for the treatment of refractory pruritic disorders with multifactorial itch, such as itch associated with end-stage renal disease. Additionally, our results demonstrated that

30 min after U50488H administration, all mice successfully completed the rotarod test. This suggests that the reduction in scratching induced by U50488H was not due to motor abnormalities. In conclusion, U50488H has an antipruritic effect at the supraspinal level, consistent with its action at peripheral and spinal levels.

KOR agonists—as alternatives to the addictive mu-opioid receptor agonist morphine—exhibit analgesic and anti-hypersensitivity effects in numerous pain models (Aldrich and McLaughlin 2021). In previous experiments, U50488H has demonstrated analgesic effects on hot plates, cold pain, and tail-flick tests via *i.p.*, *s.c.*, and *i.t.* administration (Mansikka and Uhl 2004; Abraham et al. 2018; Zhang et al. 2022b). Additionally, human positron emission computed tomography neuroimaging studies have shown increased endogenous opioid release in the anterior cingulate cortex and IC during acute experimental heat-induced pain (Sprenger et al. 2006). However, the direct effect of *i.c.v.* administration of U50488H on radiation-induced heat pain remains unknown. Additionally, *i.c.v.* administration of amitriptyline resulted in contrasting effects, inhibiting heat-induced pain in a hot plate test, but facilitating heat-induced pain in a tail flick test. Our data revealed that *i.c.v.* administration of U50488H has a dose-dependent analgesic effect on the Hargreaves test. Consistent with these findings, administration of the KOR agonist Salvinorin A into the IC and chemogenetic excitation of KOR-expressing neurons in the RVM have shown analgesic effects on Hargreaves radiation-induced thermal hyperalgesia (Coffeen et al. 2018; Nguyen et al. 2022). Radiation-induced heat pain, like the tail flick test, primarily assesses the basic spinal reflex arc, whereas the hot plate test reflects supraspinal level responses (Dirksen et al. 1994). This is consistent with results showing that KOR agonists, such as HS665, administered via *i.c.v.*, exhibited antinociceptive effects in a 55 °C warm-water tail-flick assay (Spetea et al. 2017). Additionally, *i.c.v*. administration of U50488H similarly produced analgesic effects on neuropathic pain, tail pinch, and hot plate tests (Pieretti et al. 1994; Huong et al. 1997; Sounvoravong et al. 2004). Based on the above, our experimental results complement the understanding of the effects of U50488 on pain at supraspinal levels and confirm that it, consistent with systemic and intrathecal administration, can produce analgesic effects.

Previous studies demonstrate that *i.p.* administration of U50488H can induce depression-like behavior in rodents, which can be blocked by KOR antagonists (Dogra et al. 2016; Page et al. 2019). Similarly, in rodent models of anxiety-like behavior induced by alcohol withdrawal and methylphenidate exposure, *i.p.* administration of U50488H exacerbates this behavior (Privette and Terrian 1995; Wiley et al. 2009). These findings suggest the contribution of KOR to negative emotional processes. However, pharmacological studies on pain and itching often neglect to address the concurrent emergence of negative emotions simultaneously. Our experimental results are the first to show that *i.c.v.* administration of U50488H induces anxiety and depressive symptoms. In acute and chronic pain animal models, as well as in patients, pain and itching can elicit negative emotions, including anxiety and depression (Michaelides and Zis 2019; Silverberg 2019; Kremer et al. 2021). Conversely, escalating negative emotions exacerbate pain and itching (Wang et al. 2018; Docherty et al. 2023). The central mechanism underlying the bidirectional regulation of pain/itch perception and negative emotions remains unclear. Therefore, based on the current results, pharmacological treatments targeting central KOR may offer a new promising strategy for mitigating negative emotions. This is consistent with findings from previous research, demonstrating the antidepressant and anxiolytic effects of KOR antagonists (Carlezon and Krystal 2016). Moreover, antidepressants have also been utilized in pruritus treatment (Kuhn et al. 2017). Therefore, the mechanism by which systemic *i.p.* administration of U50488H can induce depression-like behavior may involve the activation of supraspinal KOR. Compared with studies on spinal and peripheral nervous system KORs, our findings identify a new therapeutic target for addressing the clinical issue of the vicious cycle between pain, itching, and negative emotions. Further investigations targeting supraspinal brain regions may help us determine the crucial role of KOR in simultaneously treating pain, itching, and related negative emotions.

Our experimental results show that nor-BNI pretreatment via *i.c.v.* injection reversed pain, itching, and negative emotional behaviors induced by *i.c.v.* administration of U50488H in mice, implying that these behaviors were mediated through KOR activation. Previous studies have demonstrated that U50488H exerts consistent analgesic effects at the spinal and supraspinal levels when administered via *i.t.* and *i.c.v.* injection methods, respectively (Murray and Cowan 1991; Aldrich and McLaughlin 2021). In the mouse tail-flick pain test, administration of U50488H via both *i.c.v.* and *i.t.* routes produced analgesic effects, with the peak efficacy time for both routes being 12 min (Takemori et al. 1988). This suggests that both spinal and supraspinal KORs are involved simultaneously in its action in terms of temporal effects. In the formalin pain test in mice, administration of U50488H via *i.c.v.* or *i.t.* routes resulted in A50 values of 3.3 and 12 μg/mouse, respectively (Murray and Cowan 1991). This suggests that, in terms of drug dosage, the i.c.v.

route requires a lower dose than the i.t. route, indicating that supraspinal KORs act independently and have a different analgesic mechanism from spinal KORs.

The current study utilized the prototypical KOR agonist U50488H as the primary systemic agent, enabling a comprehensive delineation of central KOR actions. However, our study has some limitations. First, KOR agonist application can also lead to side effects such as sedation, motor impairment, dysphoria, and psychotomimesis. In this present study, we demonstrated that central administration of U50488H is relatively safe, with no obvious motor impairment. However, triazole 1.1, a G protein-biased ligand at the KOR, did not induce sedation or motor impairment at the tested doses, suggesting it lacks certain adverse effects typical of KOR compared with U50488H (Brust et al. 2016). Nevertheless, we still utilized the prototypical KOR agonist U50488H as the primary systemic agent to systematically characterize its supraspinal effects on pain, itching, and negative effects. This approach enables a comprehensive delineation of its central actions—both advantageous and disadvantageous—paving the way for subsequent research. Second, *i.c.v.* administration can only reflect the overall effects on the brain and does not specifically reflect the effects on critical brain regions. Activation of supraspinal KOR can produce analgesic and antipruritic effects; however, it may also elicit anxiety and depression. This may be due to the differential effects of KOR activation in various brain regions. Further research should focus on identifying the key brain regions and specific neural circuits of KOR to address this discrepancy.

Conclusions

This study demonstrated that the activation of supraspinal KOR exhibits analgesic and antipruritic effects, but also induces anxiety- and depression-like behaviors. These findings provide further insights into potential treatment strategies for refractory pruritus and pain, as well as for combined intervention strategies targeting associated negative emotions. Compared with interventions targeting the spinal cord and peripheral nerves, direct modulation of central KOR can simultaneously treat both physiological and psychological disorders in patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00221-025-07066-z.

Author contributions Li Zhang and Shuai Zhou were responsible for itch behavior and negative emotional behavior assessments. Lujin Yan, Xinyu Li, and Yunqi Ju were responsible for pain behavior assessments. Bo Wu and Hongjie Wang conducted data analysis. Jian Wang and Yi Sun were responsible for project design, supervision, and manuscript writing.

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Data availability The data produced in this study can be obtained from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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