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Paroxetine engenders analgesic effects through inhibition of p38 phosphorylation in a rat migraine model[☆]

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Abstract

In this study, a model of migraine was established by electrical stimulation of the superior sagittal sinus in rats. These rats were then treated orally with paroxetine at doses of 2.5, 5, or 10 mg/kg per day for 14 days. Following treatment, mechanical withdrawal thresholds were significantly higher, extracellular concentrations of 5-hydroxytryptamine in the periaqueductal grey matter and nucleus reticularis gigantocellularis were higher, and the expression of phosphorylated p38 in the trigeminal nucleus caudalis was lower. Our experimental findings suggest that paroxetine has analgesic effects in a rat migraine model, which are mediated by inhibition of p38 phosphorylation.

Key Words

paroxetine; migraine; 5-hydroxytryptamine; p38; phosphorylation; neural regeneration

Abbreviations

TNC, trigeminal nucleus caudalis; 5-HT, 5-hydroxytryptamine; NGC, nucleus reticularis gigantocellularis; PAG, periaqueductal grey matter; MAPK, mitogen-activated protein kinases

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INTRODUCTION

Migraines are often associated with a sensitized central pain syndrome, which causes an altered processing of sensory input in the brainstem, principally in the trigeminal nucleus caudalis (TNC)^[1]. Central sensitization impacts the efficacy of symptomatic therapy and is responsible for the maintenance of head pain in chronic migraine patients^[2-3]. A low 5-hydroxytryptamine (5-HT) tone facilitates the activation of trigeminovascular nociceptive pathways and may predispose patients to migraine attacks^[4]. Long-term antidepressant treatment has been shown to

increase extracellular 5-HT in several brain structures including the nucleus reticularis gigantocellularis (NGC) and the periaqueductal grey matter (PAG)^[5]. Thus, an enhancement of 5-HT neurotransmission at these postsynaptic areas could reduce the frequency of migraines, although few papers have examined the effects of chronic paroxetine treatment on migraines. TNC neurons in the brainstem are more excitable due to changes in receptor sensitivity^[6]. Among various second messenger systems associated with pain responses, the p38 family of mitogen-activated protein kinases contains likely candidates for the development and maintenance of central pain sensitization^[7-9].

One important consequence of p38 activation appears to be higher expression of genes important for synaptic remodeling and long-term changes in synaptic efficacy^[10-11]. It is furthermore known that the p38 is a stress induced kinase that plays a critical role in pathological pain. Therefore, the present study was designed to directly investigate extracellular concentrations of 5-HT in the NGC and PAG and phosphorylated p38 in the TNC after chronic paroxetine treatment, to elucidate how chronic paroxetine treatment potentiates serotonergic transmission, which may underlie its analgesic effects.

RESULTS

Quantitative analysis of experimental animals

A total of 90 rats were randomly assigned to five groups, including a sham-surgery group, a model group, and groups treated with paroxetine at doses of 2.5, 5 or 10 mg/kg per day. Six animals per group underwent the various experimental procedures. The migraine model was established by electrical stimulation in four groups, but not the sham-surgery group. All rats were orally treated with vehicle or paroxetine. One rat in the model group died at 10 days, and the remaining 89 rats were included in the final analysis.

Influence of paroxetine on mechanical withdrawal thresholds in a rat migraine model

The analgesic effects of paroxetine in a rat migraine model were tested using an electronic von Frey anesthesiometer. In the sham-surgery group, the mean baseline withdrawal threshold of the masseter muscle was 52.2 ± 3.3 g. After electrical stimulation of the superior sagittal sinus, there was a dramatic decrease in withdrawal thresholds in the model group and all three paroxetine-treated groups ($P < 0.05$). However, with paroxetine therapy at doses of either 2.5, 5, or 10 mg/kg, withdrawal thresholds against the tactile stimulation steadily increased in a time- and dose-dependent manner (Figure 1). Starting at 2 days and becoming evident after 7 days of paroxetine treatment, there was a significant increase in withdrawal thresholds in all three treatment groups ($P < 0.05$). When paroxetine was given at 10 mg/kg, withdrawal thresholds increased to about 80% of those in the sham-surgery group at 14 days of treatment ($P < 0.05$). In contrast, no changes in withdrawal thresholds were observed in sham-surgery or model groups throughout the experiment.

Extracellular concentrations of 5-HT in the PAG and NGC after administration of paroxetine in a rat migraine model

Extracellular concentrations of 5-HT in the PAG and

NGC were evaluated by microdialysis and high performance liquid. As presented in Figure 2, the mean dialysate concentrations of 5-HT in the PAG and NGC were significantly lower in the model group compared with the sham-surgery group ($P < 0.05$). After 14 days of paroxetine administration at 2.5 mg/kg per day, there were higher 5-HT levels in dialysates from both the PAG and NGC. At 5 or 10 mg/kg, 5-HT levels were even higher in the PAG and NGC dialysates. One-way analysis of variance showed that the paroxetine-treated animals show significantly higher 5-HT levels compared with the sham-surgery and model controls ($P < 0.05$).

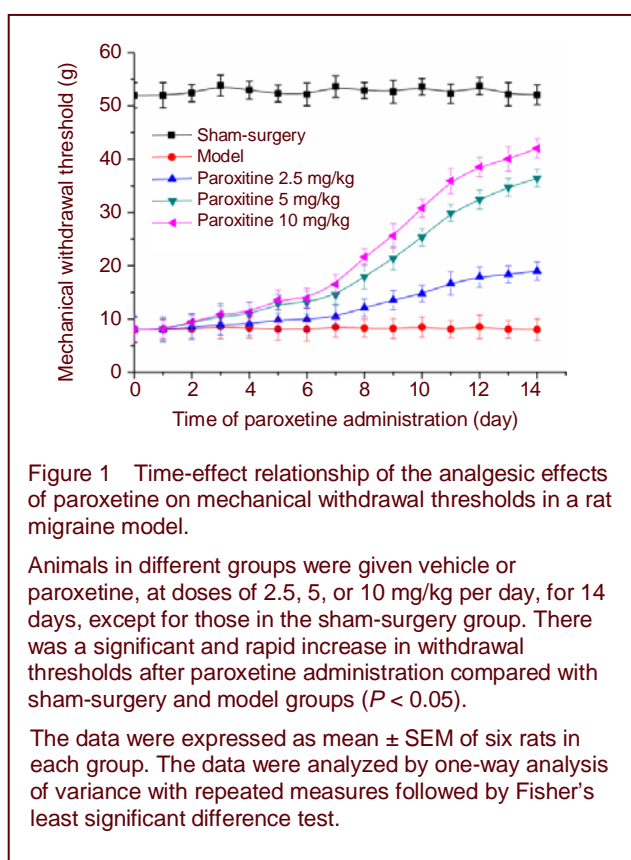


Figure 1 Time-effect relationship of the analgesic effects of paroxetine on mechanical withdrawal thresholds in a rat migraine model.

Animals in different groups were given vehicle or paroxetine, at doses of 2.5, 5, or 10 mg/kg per day, for 14 days, except for those in the sham-surgery group. There was a significant and rapid increase in withdrawal thresholds after paroxetine administration compared with sham-surgery and model groups ($P < 0.05$).

The data were expressed as mean \pm SEM of six rats in each group. The data were analyzed by one-way analysis of variance with repeated measures followed by Fisher's least significant difference test.

Phosphorylated p38 expression in the TNC after administration of paroxetine in a rat migraine model

The p38 activity in TNC neurons was detected using immunofluorescence and western blot assay. Electrical stimulation of the superior sagittal sinus induced p38 phosphorylation in the TNC of model rats. However, this activation was markedly smaller by 14 days of administration of paroxetine at 2.5, 5, or 10 mg/kg. One-way analysis of variance showed that phosphorylated p38 expression was significantly lower in the paroxetine-treated animals compared with the sham-surgery or model controls ($P < 0.05$; Figures 3, 4). Our data demonstrated that p38 was activated in the TNC of migraine animals, and it was inhibited by paroxetine treatment.

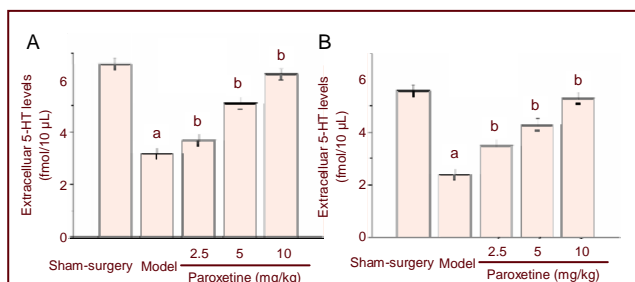


Figure 2 Extracellular concentrations of 5-hydroxytryptamine (5-HT) in rat nucleus reticularis gigantocellularis (NGC) and the periaqueductal grey matter (PAG) after paroxetine administration. The extracellular concentrations of 5-HT in rat PAG (A) and NGC (B) decreased markedly in the model group compared with the sham-surgery group ($P < 0.01$, $n = 6$). After 14 days of paroxetine treatment at doses of 2.5, 5, or 10 mg/kg, 5-HT levels were significantly higher compared with the model group.

The data were expressed as mean \pm SEM of dialysate 5-HT levels. ^a $P < 0.01$, vs. sham-surgery group; ^b $P < 0.05$, vs. model group. The data were analyzed by one-way analysis of variance with a post-hoc Scheffe's test.

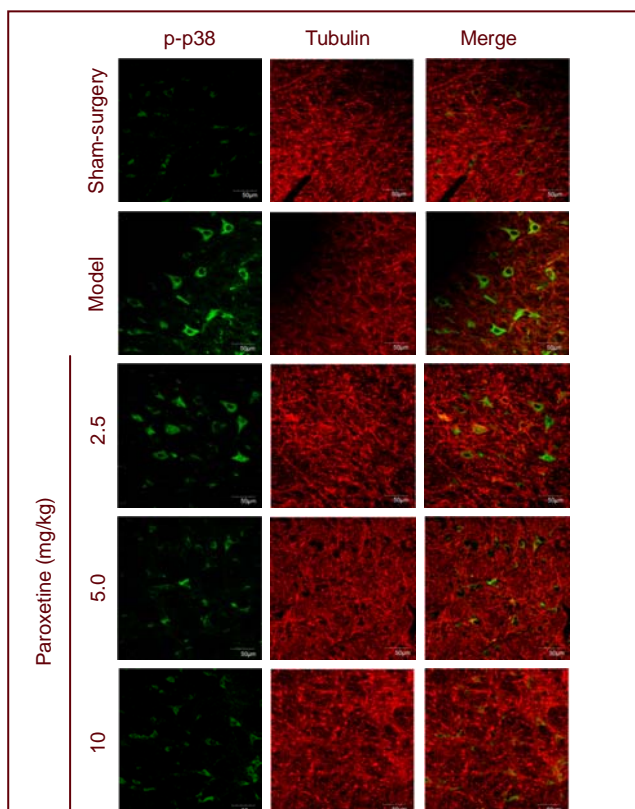


Figure 3 Phosphorylated p38 and tubulin expression in the trigeminal nucleus caudalis after administration of paroxetine in a rat migraine model.

Tubulin staining was used to localize the cytoplasm (red). Double-immunofluorescent staining verified the localization of phosphorylated p38 within the cytoplasm of trigeminal nucleus caudalis neurons (green), suggesting the activation of p38 in these neurons (confocal laser-scanning microscope). Scale bars: 50 μ m.

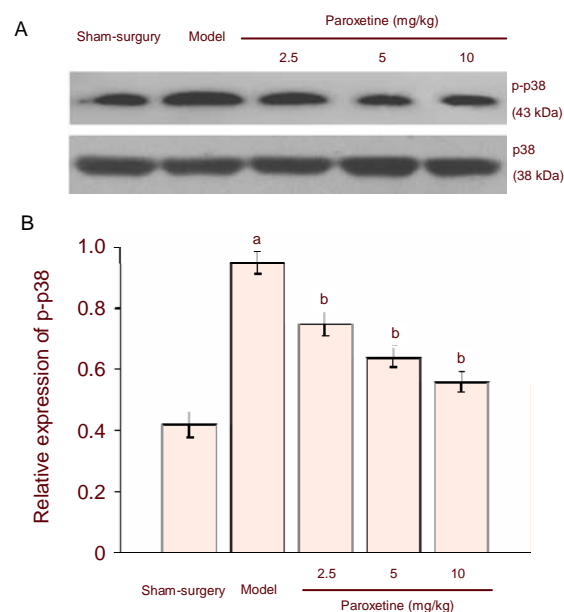


Figure 4 Paroxetine inhibited p38 activation in the trigeminal nucleus caudalis (TNC) in a rat migraine model.

Phosphorylation of p38 in the TNC was measured in rats that underwent electrical stimulation of the superior sagittal sinus during 14 days of administration of vehicle or paroxetine at a dose of 2.5, 5, or 10 mg/kg per day.

Western blot assays were used to evaluate the level of phosphorylated p38 (p-p38) in the TNC (A).

Relative protein expression was expressed as the ratio of phosphorylated p38/p38 gray values. In comparison with sham surgery group, the model rats showed higher phosphorylated p38 levels after electrical stimulation of the superior sagittal sinus (B). This difference was significantly attenuated by paroxetine treatment.

^a $P < 0.01$, vs. sham-surgery group; ^b $P < 0.05$, vs. model group. The data were expressed mean \pm SEM from three independent experiments. Six rats were included in each group. The data were analyzed by one-way analysis of variance with a post-hoc Scheffe's test.

DISCUSSION

5-HT plays a pivotal role in migraines that do not include an aura^[12-13]. A previous study documented that selective serotonin reuptake inhibitors were more effective than conventional migraine medications, and therefore could be used in patients if conventional therapy fails^[14-16]. The current study showed that the extracellular concentrations of 5-HT decreased markedly after electrical stimulation of the superior sagittal sinus compared with sham-surgical animals. In contrast, after 14 days of paroxetine administration, 5-HT levels increased significantly in both the PAG and NGC. This is consistent with previous studies, which showed that long term antidepressant treatment enhances extracellular 5-HT levels in brain structures, including the PAG and

NGC^[17]. Three doses of paroxetine were tested in our migraine model, all of which showed significant up-regulation of mechanical withdrawal thresholds and 5-HT levels in a dose-dependent manner, and down-regulation of phosphorylated p38. However, other work reported that drug challenges did not alter extracellular levels of 5-HT in the rat brainstem following chronic treatment with paroxetine, perhaps because dose of paroxetine used was too low. In this study, potential adverse effects were not detected^[18]. Our data support the potential application of paroxetine in the treatment of mild to moderate migraines at doses lower than those used for depression. However, high doses are indicated in patients with serious migraines. It is widely believed that central sensitization reflects a cascade of events that is initiated, in part, by the release of excitatory amino acids and peptides^[19]. Persistent p38 activation plays a key role in intracellular pathways involved in neuronal hyperexcitability, by providing substrates for sustained central neuropathic pain^[20-22]. In this investigation, immunofluorescence staining showed a very low but detectable level of phosphorylated p38 in the TNC of control rats, even when they did not undergo electrical stimulation of the superior sagittal sinus. In our induced neuropathic pain rats, there was phosphorylation and activation of p38 in the TNC following 14 days of electrical stimulation of the superior sagittal sinus, implying that TNC p38 was phosphorylated as a result of TNC excitotoxic lesions. Our results lent strong support to the hypothesis that the activation of TNC p38 is essential to further processing of downstream events in response to electrical stimulation of the superior sagittal sinus. This finding is consistent with a literature that suggests p38 in the TNC is important in pain processing, which leads to behaviorally defined hyperalgesia^[23]. More importantly, with paroxetine treatment over 14 days at various doses, there was a significant suppression of TNC phosphor-p38 compared with in the model group. These results indicate that chronic administration of paroxetine attenuated activation of p38 in the TNC in our migraine model. Other studies have reported there are 5-HT receptors in the TNC. Moreover, these studies have reported that there is dual modulation of excitatory synaptic transmission by 5-HT in the nucleus tractus solitarius in rat brain stem, with presynaptic inhibition of the peripheral inputs synapsing to the relay neurons *via* 5-HT(2) receptors and presynaptic excitation of inputs from the intrinsic local network *via* 5-HT(3) receptors^[24-25]. Our works suggest 5-HT concentration-dependently decreased phosphorylation of p38 in TNC following 14 days of paroxetine treatment, which was accompanied by an increase in mechanical withdrawal thresholds. However, the types of 5-HT receptors that are involved

remain unclear.

In conclusion, our results show that chronic administration of paroxetine suppresses central neuropathic pain in a migraine model. This suppression presented as higher mechanical allodynia thresholds after paroxetine treatment in rats undergoing electrical stimulation of the superior sagittal sinus. Our results support the use of paroxetine as an analgesic agent for migraine treatment. It also provided insights into potential therapeutic targets for migraine prophylaxis.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

The experiment was performed at the Medical Research Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China, from March 2010 to June 2011.

Materials

Animals

Experiments were performed on adult male Sprague-Dawley rats weighing 250–300 g. All rats were supplied by the Experimental Animal Center of Sun Yat-Sen University, China (license No. SCXK (Yue) 2011-0029). They were housed one animal per cage and maintained on a 12-hour light-dark cycle in a temperature-controlled environment (22–24°C), with food and water continuously available. All animal procedures were in strict accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China^[26].

Drugs

Paroxetine was kindly donated by Lilly Corporate Center (Indianapolis, IN, USA). Paroxetine was dissolved in distilled water. Drugs were daily administered between 11:00 and 12:00.

Methods

Establishment of a migraine model by electrical stimulation of the superior sagittal sinus

All rats except those in the sham surgery group were immobilized in a Stereotaxic Alignment System (David Kopf, Tujunga, CA), and a craniotomy was performed to expose the sagittal sinus. Bipolar stimulating electrodes were placed on the dura mater. Single pulse, constant current electrical stimulation (1 mA, 0.25 ms, 1 Hz) was delivered by a bipolar stimulating electrode made from a silver ball (interpolar distance: 2 mm) and placed on the exposed sagittal sinus, as described in previous studies^[27].

Electrical stimulation of the superior sagittal sinus was performed in the model group and all three paroxetine-treated groups for 30 minutes per day for 14 days^[28].

Mechanical withdrawal thresholds

Rats were tested for their response to mechanical stimulation of the masseter muscle region using an electronic von Frey anesthesiometer (IITC Inc. Life Science, Woodland Hills, Canada). All behavioral assessments were carried out in a quiet room, generally between 09:00 and 16:00. Rats were habituated using a methodology described previously, and a continuously variable force transducer with a fixed contact area was used to measure the withdrawal thresholds^[29]. Mechanical withdrawal thresholds were tested by probing the masseter muscle through the skin surrounding the mystacial vibrissae. The force needed to elicit a withdrawal of the head was recorded following five stimulus presentations at approximately one minute intervals, and the mean values of the five readings in grams were used for analysis^[30]. Repeated measures were conducted in sham control animals to determine the variability in head withdrawal upon repeated testing on different days. The right masseter muscle was tested following unilateral electrical stimulation of the superior sagittal sinus.

Guide cannula implants for microdialysis

The microdialysis surgeries were conducted after 14 days of paroxetine treatment. The rats were initially anesthetized with sodium pentobarbital at 45 mg/kg. The head of each rat was positioned in a Stereotaxic Alignment System (David Kopf, Model 1911, Kent, UK) with the incisor bar 3 mm below the horizontal zero. Small craniotomies were performed^[31]. Two holes were drilled on the surface of the skull over the brain stem for inserting the guide cannulae (CMA 11 Guide Cannula 8309017, Solna, Sweden) into the PAG and NGC for microdialysis. The locations of the PAG (7.32 mm caudal to Bregma, 0.5 mm left of the midline sagittal plane, and 5.5 mm below the horizontal plane passing through bregma and lambda on the surface of the skull) and the NGC (11.40 mm caudal to bregma, 1 mm left of the midline sagittal plane, and 9 mm below the cortical surface) were determined according to *The Rat Brain in Stereotaxic Coordinates, 6th Edition*^[32]. In all cases, the guide cannulae were lowered to 1 mm above the intended sites. After insertion, the probes were secured with dental cement^[33]. After completion of the surgeries, the rats were housed individually and maintained under standard laboratory conditions.

Extracellular concentrations of 5-HT

The microdialysis probes (CMA11 8309581, Solna, Sweden) were inserted into the unilateral PAG and NGC

via the guide cannula to 1 mm beyond the tip of the guide cannula. The dialysis probe was connected to a microdialysis pump (CMA/100, CMA, Sweden) and the outlet cannula was connected to the microfraction collector (CMA/200, Solna, Sweden). The perfusion fluid was infused at 2 µL/minute using a CMA/100 microdialysis pump as previously described^[34]. After the dialysate levels stabilized (about 60 minute), 30-minute samples of the PAG and NGC were collected. Aliquots were frozen at -80°C for later analysis. Extracellular concentrations of 5-HT were evaluated by high performance liquid chromatography (Beckman Fullerton, CA, USA) with an electrochemical detector (Beckman Fullerton). Standard solution or samples (20 µL) were injected into the column, separated using a mobile phase, detected by a chemical detector at an oxidation potential of 700 mV against an Ag/AgCl electrode, and quantified using BAS Chromgraph programs. Comparisons among the groups were performed with one-way analysis of variance.

Phosphorylated p38 and tubulin expression in the TNC

Rats were anaesthetized by overdose of chloral hydrate (80 mg/kg) and perfused transcardially with normal saline followed by 4% paraformaldehyde. Coronal brainstem sections (20 µm) in were cut in a cryostat (Leica 1900, Solms, Germany) and processed for immunofluorescence^[35]. Briefly, the sections were incubated overnight at 4°C with a mixture of rabbit anti-phospho-p38 MAPK (1:800; CST, MA, USA) and mouse anti-tubulin antibodies (1:1 000; Millipore, MA, USA) in phosphate buffered saline containing 1% normal goat serum and 0.3% Triton X-100. Following three rinses (15 minutes each) in phosphate buffered saline, the sections were incubated with a mixture of Alexa Fluor 488 goat anti-rabbit IgG (H+L) (1:500; Jackson) and Alexa Fluor 594 goat anti-mouse IgG (H+L) (1:500; Jackson) in phosphate buffered saline containing 1% normal goat serum, for 1 hour at room temperature followed by 2 hours at 4°C. All sections were coverslipped with a mixture of 50% glycerin in phosphate buffered saline, and observed under a ZEISS LSM710 confocal laser-scanning microscope (ZEISS, Oberkochen, Germany).

Phosphorylated p38 protein expression

At 24 hours after the last behavioral evaluation of mechanical thresholds (14 days of paroxetine treatment), rats were sacrificed for western immunoblotting^[36]. The brainstems from five groups of rats were isolated and mechanically homogenated in ice-cold tris-buffered saline (Roche, Indianapolis, Indiana, USA). Homogenates were centrifuged at 10 000 r/minute for

10 minutes. The supernatant was collected and centrifuged again at 10 000 r/min for 10 minutes and then stored at -80°C . Protein concentrations of the homogenate were determined using the BCA Protein Assay Kit (Pierce, IL, USA). 40 μg protein sample per lane in an equal volume of sample buffer was loaded onto a polyacrylamide gel. The stacking gel was 4% acrylamide, prepared in 0.13 M Tris, pH 6.8, and 0.1% sodium dodecyl sulfate. The separating gel was 10% acrylamide, prepared in 0.38 M Tris, pH 8.8, and 0.1% sodium dodecyl sulfate. Samples were separated by electrophoresis in Tris-glycine buffer (25 mM Tris, 250 mM glycine, 0.1% sodium dodecyl sulfate) at 100 V for approximately 150 minutes. Proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, MA, USA) in transfer buffer at 175 mA for 1.5 hours at 4°C . Membranes were incubated for 1 hour at room temperature in blocking buffer containing 5% non-fat powdered milk in Tris-buffered saline Tween-20, then washed for 10 minutes in Tris-buffered saline Tween-20. Membranes were incubated with primary rabbit anti-phospho-p38 polyclonal antibodies (1:1 000; CST, Upton, MA, USA), rabbit anti-p38 polyclonal antibodies (1:1 000; CST) overnight at 4°C . Primary antibodies were detected with horseradish peroxidase-conjugated secondary antibodies (1:5 000; CST) and blots were visualized using ECL reagents (Millipore, Billerica, MA, USA) and X-ray film (Kodak, Rochester, NY, USA). Band intensities were analyzed using alpha DigiDoc gel analysis software (Alpha Innotech, San Leandro, CA, USA). Relative protein expression was expressed as the ratio of phospho-p38/p38 gray values. Comparisons among groups were performed with one-way analysis of variance.

Statistical analysis

All values were presented as mean \pm SEM. All statistical calculations were performed with the SPSS 13.0 software (SPSS, Chicago, IL, USA). Mechanical withdrawal thresholds were analyzed using Fisher's least significant difference test. One-way analysis of variance and post-hoc Scheffe test were used to compare the difference among the groups with different treatments, and values were considered to be significantly different when $P < 0.05$.

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Author contributions: Chuanming Wang and Wei Bi had full access to all data and evaluated the data integrity and data analysis accuracy. Yanran Liang and Xiuna Jing participated in data collection. Songhua Xiao and Yannan Fang participated in

data analysis and interpretation. Qiaoyun Shi and Enxiang Tao were responsible for study design, study supervision, and manuscript development.

Conflicts of interest: None declared.

Ethical approval: The project received full ethical approval by the Animal Ethics Committee, Sun Yat-sen University in China.

REFERENCES

- [1] Knight YE, Goadsby PJ. The periaqueductal grey matter modulates trigeminovascular input: a role in migraine? *Neuroscience*. 2001;106(4):793-800.
- [2] Burstein R, Jakubowski M. Unitary hypothesis for multiple triggers of the pain and strain of migraine. *J Comp Neurol*. 2005;493(1):9-14.
- [3] Caudle RM, King C, Nolan TA, et al. Central sensitization in the trigeminal nucleus caudalis produced by a conjugate of substance P and the A subunit of cholera toxin. *J Pain*. 2010;11(9):838-846.
- [4] Sakai Y, Dobson C, Diksic M, et al. Sumatriptan normalizes the migraine attack-related increase in brain serotonin synthesis. *Neurology*. 2008;70(6):431-439.
- [5] Sargent PA, Williamson DJ, Cowen PJ. Brain 5-HT neurotransmission during paroxetine treatment. *Br J Psychiatry*. 1998;172:49-52.
- [6] Hegarty DM, Tonsfeldt K, Hermes SM, et al. Differential localization of vesicular glutamate transporters and peptides in corneal afferents to trigeminal nucleus caudalis. *J Comp Neurol*. 2010;518(17):3557-3569.
- [7] Di Cesare ML, Ghelardini C, Toscano A, et al. The neuropathy-protective agent acetyl-L-carnitine activates protein kinase C-gamma and MAPKs in a rat model of neuropathic pain. *Neuroscience*. 2010;165(4):1345-1352.
- [8] Lee MK, Han SR, Park MK, et al. Behavioral evidence for the differential regulation of p-p38 MAPK and p-NF-kappaB in rats with trigeminal neuropathic pain. *Mol Pain*. 2011;7:57.
- [9] Svensson CI, Medicherla S, Malkmus S, et al. Role of p38 mitogen activated protein kinase in a model of osteosarcoma-induced pain. *Pharmacol Biochem Behav*. 2008;90(4):664-675.
- [10] Gould TD, Quiroz JA, Singh J, et al. Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. *Mol Psychiatry*. 2004;9(8):734-755.
- [11] Liu MG, Wang RR, Chen XF, et al. Differential roles of ERK, JNK and p38 MAPK in pain-related spatial and temporal enhancement of synaptic responses in the hippocampal formation of rats: multi-electrode array recordings. *Brain Res*. 2011;1382:57-69.
- [12] Domingues RB, Silva AL, Domingues, et al. A double-blind randomized controlled trial of low doses of propranolol, nortriptyline, and the combination of propranolol and nortriptyline for the preventive treatment of migraine. *Arq Neuropsiquiatr*. 2009;67(4):973-977.
- [13] Cassidy EM, Tomkins E, Dinan T, et al. Central 5-HT receptor hypersensitivity in migraine without aura. *Cephalalgia*. 2003;23(1):29-34.

- [14] Krymchantowski AV, Silva MT, Barbosa JS, et al. Amitriptyline versus amitriptyline combined with fluoxetine in the preventative treatment of transformed migraine: a double-blind study. *Headache*. 2002;42(6):510-514.
- [15] Moja PL, Cusi C, Sterzi RR, et al. Selective serotonin re-uptake inhibitors (SSRIs) for preventing migraine and tension-type headaches. *Cochrane Database Syst Rev*. 2005(3):D2919.
- [16] Molyneux PD. Tricyclic antidepressants reduce frequency of tension-type and migraine headaches compared with placebo, and intensity of headaches compared with SSRIs, but cause greater adverse effects. *Evid Based Med*. 2011;16(3):75-76.
- [17] Harsing LJ. The pharmacology of the neurochemical transmission in the midbrain raphe nuclei of the rat. *Curr Neuropharmacol*. 2006;4(4):313-339.
- [18] Malagie I, Deslandes A, Gardier AM. Effects of acute and chronic tianeptine administration on serotonin outflow in rats: comparison with paroxetine by using in vivo microdialysis. *European journal of pharmacology*. 2000; 403(1-2):55-65.
- [19] Black JA, Nikolajsen L, Kroner K, et al. Multiple sodium channel isoforms and mitogen-activated protein kinases are present in painful human neuromas. *Ann Neurol*. 2008; 64(6):644-653.
- [20] Crown ED, Gwak YS, Ye Z, et al. Activation of p38 MAP kinase is involved in central neuropathic pain following spinal cord injury. *Exp Neurol*. 2008;213(2):257-267.
- [21] Svensson CI, Fitzsimmons B, Azizi S, et al. Spinal p38beta isoform mediates tissue injury-induced hyperalgesia and spinal sensitization. *J Neurochem*. 2005; 92(6):1508-1520.
- [22] Peng XM, Zhou ZG, Glorioso JC, et al. Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury. *Ann Neurol*. 2006;59(5):843-851.
- [23] Sweitzer SM, Peters MC, Ma JY, et al. Peripheral and central p38 MAPK mediates capsaicin-induced hyperalgesia. *Pain*. 2004;111(3):278-285.
- [24] Takenaka R, Ohi Y, Haji A. Distinct modulatory effects of 5-HT on excitatory synaptic transmissions in the nucleus tractus solitarius of the rat. *Eur J Pharmacol*. 2011; 671(1-3):45-52.
- [25] Yeung LY, Kung HF, Yew DT. Localization of 5-HT1A and 5-HT2A positive cells in the brainstems of control age-matched and Alzheimer individuals. *Age (Dordr)*. 2010;32(4):483-495.
- [26] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
- [27] Sokolov A, Amelin AV, Ignatov Iu D, et al. Effect of GABA-positive drugs on the background and superior sagittal sinus-electrostimulated activity of neurons in the nucleus trigeminalis caudalis of rats. *Eksp Klin Farmakol*. 2008;71(5):3-7.
- [28] Wang RF, Yu SY, Liu RZ, et al. NF- κ B expression in midbrain periaqueductal gray induced by electrical stimulation of dura near superior sagittal sinus in rats. *Zhonghua Shenjingke Zazhi*. 2007;40(11):771-774.
- [29] Nakai K, Nakae A, Oba S, et al. 5-HT_{2C} receptor agonists attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. *Eur J Pain*. 2010;14(10): 999-1006.
- [30] Piao L, Li HY, Park CK, et al. Mechanosensitivity of voltage-gated K⁺ currents in rat trigeminal ganglion neurons. *J Neurosci Res*. 2006;83(7):1373-1380.
- [31] Juckel G, Mendlin A, Jacobs BL. Electrical stimulation of rat medial prefrontal cortex enhances forebrain serotonin output: implications for electroconvulsive therapy and transcranial magnetic stimulation in depression. *Neuropsychopharmacology*. 1999;21(3):391-398.
- [32] Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates: Compact*. 6th ed. San Diego: Academic Press. 2008.
- [33] Ogaya T, Song Z, Ishii K, et al. Changes in extracellular kynurenic acid concentrations in rat prefrontal cortex after D-kynurenine infusion: an in vivo microdialysis study. *Neurochem Res*. 2010;35(4):559-563.
- [34] Guilloux JP, David DJ, Guiard BP, et al. Blockade of 5-HT_{1A} receptors by (+/-)-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioral approaches in 5-HT_{1A} receptor knockout mice. *Neuropsychopharmacology*. 2006;31(10):2162-2172.
- [35] Benzekroufa K, Liu B, Tang F, et al. Adenoviral vectors for highly selective gene expression in central serotonergic neurons reveal quantal characteristics of serotonin release in the rat brain. *BMC Biotechnol*. 2009;9: 23.
- [36] Budziszewska B, Szymanska M, Leskiewicz M, et al. The decrease in JNK- and p38-MAP kinase activity is accompanied by the enhancement of PP2A phosphate level in the brain of prenatally stressed rats. *J Physiol Pharmacol*. 2010;61(2):207-215.

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