Effect of electroacupuncture pretreatment at GB20 on behaviour and the descending pain modulatory system in a rat model of migraine

Pei Pei, Lu Liu, Luopeng Zhao, Yingxue Cui, Zhengyang Qu, Linpeng Wang

Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, PR China

Correspondence to
Professor Linpeng Wang, Acupuncture and Moxibustion Department, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, No. 23 Meishuguanhou Street, Dongcheng District, Beijing 100010, PR China; wlp5558@sina.com

Accepted 16 September 2015
Published Online First 5 October 2015

To cite: Pei P, Liu L, Zhao L, *et al*. *Acupunct Med* 2016;**34**:127–135.

ABSTRACT

Background While electroacupuncture (EA) pretreatment has been found to ameliorate migraine-like symptoms, the underlying mechanisms remain poorly understood. Emerging evidence suggests that the brainstem descending pain modulatory system, comprising the periaqueductal grey (PAG), raphe magnus nucleus (RMg), and trigeminal nucleus caudalis (TNC), may be involved in migraine pathophysiology. We hypothesised that EA would ameliorate migraine-like symptoms via modulation of this descending system.

Methods We used a conscious rat model of migraine induced by repeated electrical stimulation of the dura. Forty male Sprague-Dawley rats were randomly assigned to one of four groups: an EA group, which received EA at GB20 following dural stimulation; a sham acupuncture (SA) group, which received manual acupuncture at a non-acupuncture point following dural stimulation; a Model group, which received dural stimulation but no acupuncture; and a Control group, which received neither dural stimulation nor acupuncture (electrode implantation only). HomeCageScan was used to measure effects on behaviour, and immunofluorescence staining was used to examine neural activation (c-Fos immunoreactivity) in the PAG, RMg, and TNC.

Results Compared to the Model group, rats in the EA group showed a significant increase in exploratory, locomotor and eating/drinking behaviour ($p<0.01$) and a significant decrease in freezing-like resting and grooming behaviour ($p<0.05$). There was a significant increase in the mean number of c-Fos neurons in the PAG, RMg, and TNC in Model versus Control groups ($p<0.001$); however, this was significantly attenuated by EA treatment ($p<0.001$). There were no significant differences between the SA and Model groups in behaviour or c-Fos immunoreactivity.

Conclusions EA pretreatment ameliorates behavioural changes in a rat model of recurrent migraine, possibly via modulation of the brainstem descending pathways.

INTRODUCTION

Migraine is a debilitating neurological disease that affects millions worldwide, including up to 12% of American and 9.3% of Chinese people.[1 2] It is characterised by recurrent attacks of unilateral headache that can be aggravated by routine physical activity,[3] resulting in reduced function.[4] The brainstem descending pain modulatory system has been shown to play a vital role in migraine pathophysiology via effects on trigeminovascular nociceptive information transmission and central sensitisation.[5]

Current drug treatments include migraine-specific medications (ergotamine, 5-$HT_{1B/1D/1F}$ receptor agonists) and prophylactic treatments including antiepileptic drugs, lisuride, β-blockers, and ergot derivatives. However, these pharmacological agents have been associated with suboptimal efficacy, poor adherence and side effects, and have many contraindications.[6 7] Overall, an efficacious clinical treatment for migraine is lacking, particularly for prophylaxis and to delay progression of recurrent to chronic migraine.

A recent review has shown that acupuncture is effective for the treatment of migraine, especially prophylaxis.[8] Indeed, we have also found that acupuncture pretreatment is effective at decreasing the frequency of migraine attacks.[9]

Moreover, Gao and colleagues found that electroacupuncture (EA) is able to regulate the peripheral excitatory neurotransmitter glutamate in plasma.[10] Nevertheless, the exact mechanisms underlying the analgesic effect of acupuncture (including pretreatment) on migraine have not yet been determined.

We hypothesised that acupuncture pretreatment would ameliorate migraine-like symptoms in recurrent migraine via modulation of the brainstem descending pain modulatory system: the periaqueductal grey (PAG), raphe magnus nucleus (RMg), and trigeminal nucleus caudalis (TNC). To test this hypothesis we chose a rat model of recurrent migraine induced by repeated electrical stimulation of the dura surrounding the superior sagittal sinus (SSS), which is an established paradigm of migraine-associated pain.[11 12] The aim of the present study was to examine the effect of EA on behaviour (using HomeCageScan technology) and neuronal activation in the PAG, RMg, and TNC (by immunofluorescence staining).

METHODS

Ethics statement

All experiments were performed in accordance with the International Association for the Study of Pain guidelines and Guideline for the Care and Use of Laboratory Animals (State Council of China, 2013). The study was approved by the Beijing Institutional Review Board for Animal Experiments (Use Committee of Capital Medical University, Beijing, reference number AEEI 2015-075). All surgeries were performed under anaesthesia, and every possible effort was made to reduce suffering.

Animals

Male, Sprague-Dawley rats weighing 210±10 g were purchased from Vital River Laboratories. They were individually housed in a controlled environment (room temperature 23±2°C, humidity 50±10%, 12 h light-dark cycle with the lights turned on at 08:00) with food and water freely available. After 1 week of habituation, rats underwent brain surgery to establish the migraine model.

Surgical procedures

Exposure of dura mater

Rats were anaesthetised with 60 mg/kg intraperitoneal sodium pentobarbitone (Sigma-Aldrich) and placed into a stereotactic frame. To access the dura mater, the parietal bone was exposed and two cranial windows (4 mm anterior and 6 mm posterior to the bregma on the midline suture, 1 mm in diameter) were carefully made using a saline-cooled drill (78001, RWD Life Science, Shenzhen, China). The cranial windows were then opened to expose the dura mater adjacent to the SSS, as previously described.[13]

Electrode fixation

A pair of tailored electrode fixtures (Beijing JiAnDeEr Ltd, China), each composed of a bipolar electrode (1.9–2.1 mm in length, 0.8 mm in diameter), a plastic holder with two screw holes and an obturator cap, were inserted into the cranial windows so that they touched the surface of the dura mater surrounding the SSS. Two stainless steel screws (M1.4×2.8 mm) were then placed into the screw holes as anchors for the dental cement (Shanghai New Century Dental Materials Co, Ltd, Shanghai, China) that was then used to stabilise the electrode fixtures. Finally the obturator cap was placed over the external stimuli line interface to prevent clogging. All surgery was performed under direct visual control using an operating microscope. Finally, penicillin (0.04 million IU/100 g, Harbin Pharmaceutical Group Co, Ltd, China) was applied to the lesion in order to prevent infection. All rats were allowed to recover for 7 days before experiments began.

Experimental design

After recovering from surgery, 40 animals were randomly divided into four groups: an EA group, which received EA at GB20 (*Fengchi*) following electrical stimulation of the dura; a sham acupuncture (SA) group, which received acupuncture (manual not EA) at a distant non-acupuncture point following electrical stimulation of the dura; a Model group, which received electrical dural stimulation but no EA; and a Control group, which received neither electrical dural stimulation nor EA (only electrode implantation). The EA, SA, and Model groups received repeated electrical stimulation using a stimulator (YC-2 stimulator Chengdu) every other day for a total of three sessions (day 1, 3, and 5). Before electrical stimulation, the EA group received EA at GB20 once a day from day 0 to day 5 for a total of six sessions. The number of rats used was based on the power calculation of a previous study.[10] A schematic representation of the experimental protocol is given in figure 1.

EA at GB20

Before dural stimulation, the EA and SA group received EA/SA pretreatment from days 0 to 5, for a total of six sessions. Conscious rats were placed into tailored fixtures that exposed their heads and necks. GB20 is a commonly used acupuncture point for the treatment of migraine.[9] Its anatomical location in rats is similar to the human, being 3 mm lateral to the centre of a line joining the two ears at the back of the head.[14] In the EA group, two stainless steel acupuncture needles (diameter 0.25 mm; length 25 mm; Suzhou Medical Appliance Factory, Suzhou, China) were inserted bilaterally at GB20 to a depth of 2–3 mm in the direction of the opposite eye. The needle handle was then connected to an electrical stimulator (Han's acupuncture point nerve stimulator

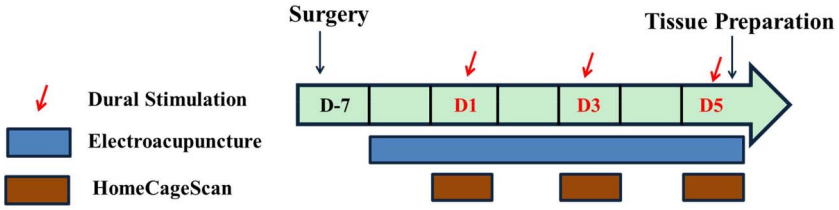

Figure 1 Schematic representation of the experimental protocol. One week after surgery to implant electrodes, rats received electrical dural stimulation (or no stimulation in the case of the Control group) every 2 days from day 1 to day 5 (on days 1, 3, and 5). Electroacupuncture (or sham acupuncture) pretreatment was performed daily from day 0 to day 5 (on days 0, 1, 2, 3, 4 and 5) in subgroups of rats. Behavioural analysis was carried out using HomeCageScan immediately after dural stimulation on days 1, 3, and 5. c-Fos immunofluorescence was measured in brain tissues prepared following necropsy on day 5.

HNAS-200, Nanjing) between 08:00 and 10:00 for 15 min/day. EA was given at 2/15 Hz frequency (amplitude-modulated wave)[15] and 0.5–1.0 mA intensity (depending on the reaction of the rat). In the SA group, needles were placed bilaterally at distant non-acupuncture points (about 10 mm above the iliac crest),[16] but no stimulation was given. Animals in the Control and Model groups were similarly placed into fixtures for 20 min, but no acupuncture was provided.

Repeated electrical stimulation of the dura

Before electrical stimulation, rats were individually placed into an observation cage (40 cm diameter, 17.5 cm height) and habituated for 20 min to minimise stress. The obturator cap was then removed from the electrode fixtures and an internal delivery electrode tip was inserted, which was connected to the current external output port of an electrical stimulator (YC-2 stimulator, Chengdu). Based on previous studies[12] and our own optimisation of the stimulation parameters, effective dural stimuli consisted of monophasic square-wave pulses with a 0.5 ms pulse duration at 1.8–2.0 mA intensity and 20 Hz frequency, and were administered for a 15 min period every other day for a total of three sessions for rats in the EA, SA, and Model groups. Rats in the Control group were also connected to the stimulator but received no electrical stimulation.

Behavioural analysis by HomeCageScan

Spontaneous behaviour was monitored by the automated behavioural acquisition and analysis software 'HomeCageScan' (Clever Systems Inc, Reston, Virginia, USA). Before real/sham electrical dural stimulation, rats were acclimatised for 20 min in an individual transparent acrylic observation cage with an automatic real-time video surveillance and image analysis system, which was lit to model daytime conditions. Subsequently, animals received electrical dural stimulation for 15 min in another cage before being individually placed back into the observation cage for a 1 h recording.

The observation cage was cleaned with 10% ethanol between sessions to eliminate odour traces. Behaviours were analysed by identifying body parts such as the mouth, head, tail, forelimb, hindlimb, upper/lower back and abdomen, and using sequence data to recognise and analyse movements automatically. During the test, rats had free access to food and water.

According to previously described classifications,[17 18] we divided behaviours into the following six categories:

1. Exploratory behaviour: rearing up and sniffing
2. Locomotor behaviour: turning, stretching, walking, jumping and walking slowly
3. Freezing-like behaviour (frozen, immobile posture): twitching, 'remaining low'
4. Resting behaviour: sleeping (head rested on flexed forepaws with eyes open/closed)
5. Eating and drinking behaviour
6. Grooming behaviour: face and body grooming.

All behaviours were verified and calibrated before recording and analysis.

c-Fos immunofluorescence and image analysis

After the last HomeCageScan recording session, animals were anaesthetised with 10% chloral hydrate (15 ml/kg, intraperitoneally) and transcardially perfused through the ascending aorta with 100 ml 0.1 M phosphate-buffered saline (PBS), followed by 500 mL of 4% paraformaldehyde (PFA) in PBS. Brains were removed and postfixed overnight at 4°C in 4% PFA/PBS, then transferred to 30% sucrose/PBS for 72 h for cryopreservation. Coronal sections (25 μm thick) through the PAG, RMg, and TNC were cut using a cryostat (Leica CM3050S, Leica Inc, Wetzlar, Germany), incubated with rabbit anti-c-Fos antiserum (sc-52, 1:200, Santa Cruz Biotechnology Inc, USA) overnight at 4°C, and then treated with Cy3 goat anti-rabbit secondary antibodies (111-165-003, 1:500, Jackson ImmunoResearch Laboratories Inc, Pennsylvania, USA) for 2 h at room temperature before being mounted, dehydrated, and cover-slipped in anti-fade reagent (AR1109, Boster Bioengineering Inc, Wuhan, China).

Section images were captured using a Leica semi-automatic light microscope (Leica DM5500 B), and cells were counted in squares at 20× magnification.

Cell density in the PAG, RMg, and TNC was calculated by a blinded assessor using Image J software. Anatomical boundaries were determined according to a rat brain atlas.[19]

Statistical analysis

Data are presented as mean±SD. Groups were compared by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences Software (SPSS) V.12.0 (SPSS Inc, Chicago, Illinois, USA). Post-hoc testing was performed using Bonferroni (homogeneity of variance) or Tamhane (heterogeneity of variance) tests. Statistical significance was defined as $p<0.05$.

RESULTS

Behavioural analysis

The results at baseline and following establishment of the recurrent migraine model ±EA/SA treatment are shown in table 1. Before dural stimulation, during the acclimatisation period (20 min), there were no significant differences between groups in any of the analysed behaviours (all $p>0.05$).

Exploratory behaviour, locomotor behaviour and eating/drinking behaviour

Across all subsequent time periods (D1–D5), rats in the Model group spent less time in exploratory behaviours (rearing up and sniffing), locomotor behaviours (turning, stretching, walking, and jumping) and eating/drinking behaviours than the Control group ($p<0.001$–0.011). However, by day 5, these effects were all attenuated by pretreatment with EA ($p<0.001$–0.006). There were no statistically significant differences between groups in the 'walking slowly' behaviour. There were very few significant differences between the Model and SA groups for any parameter, except for turning (D5) and walking (D1 and D5), where a greater amount of time was spent on these activities in the SA group ($p=0.024$, $p=0.027$ and $p=0.001$, respectively).

Freezing-like behaviour, resting behaviour, and grooming behaviour

Across all three time periods, freezing-like (twitching and remaining low) and resting (sleep) behaviours were significantly increased in the Model group compared to the Control group ($p<0.001$–0.002). Grooming behaviour was similarly increased on D3 and D5 ($p<0.001$ and $p=0.005$, respectively) but not D1 ($p=0.887$). These effects were also mitigated on D3 and D5 (but not D1) for freezing-like behaviour ($p<0.001$–0.047 and $p=0.085$–1, respectively) and at all time points for sleeping ($p<0.001$–0.011). Rats in the EA group spent less time grooming on D1 and D3 ($p=0.042$ and $p=0.018$, respectively) and tended to spend less time grooming on D5 ($p=0.079$) compared to the Model group. By contrast, there were no significant differences between the Model and SA groups for any of these parameters ($p>0.05$).

c-Fos immunofluorescence analysis

Representative images and the density of c-Fos-immunoreactive cells in the PAG, RMg, and TNC are shown in figures 2–4, respectively. Rats in the Model group showed greater c-Fos neuron expression than that the Control group within the PAG (all $p<0.001$). In the EA group, the mean number of c-Fos positive neurons was significantly reduced in all three brain regions compared to the Model group ($p<0.001$), while SA had no significant effect on dural stimulation-induced c-Fos activation compared to the Model group (PAG/RMg: $p=1.0$; TNC: $p=0.64$).

DISCUSSION

The results of the present study suggest that acupuncture pretreatment at GB20 can ameliorate behavioural responses to electrical stimulation of the dura in rats, and that the positive effects may, at least in part, result from modulation of brainstem descending pathways in this model.

Acupuncture pretreatment can ameliorate the behavioural responses to migraine

The rat model of migraine used herein was developed to mimic clinical pain conditions experienced by migraineurs, based upon clinical diagnostic criteria from the International Classification of Headache Disorders (ICHD)-II and ICHD-IIIβ classification including a decrease in physical activity during migraine attacks.[3 20] Analysis of behavioural changes using HomeCageScan offers a new way to study migraine.[18] However, it should be remembered that there are clear differences in pathophysiology between this model and the human condition, which may limit the ability to extrapolate our results.

Similar to previous studies,[17 18] we found that repeated stimulation of the dura produced an increase in nociceptive behaviour, such as freezing and resting. There was also a decrease in exploratory, locomotor and eating/drinking behaviour. Collectively, these reflect the clinical symptoms of migraine attacks, which includes a reduction in routine physical activity, loss of motivation, and decreased intake of food and drink.[3 4 21] Interestingly, these behavioural changes were reversed by EA pretreatment, as the EA group exhibited decreased nociceptive behaviour and increased exploratory, locomotor and eating/drinking behaviour.

It has been suggested that grooming in rats may reflect the cutaneous allodynia that migraineurs often suffer during an episode.[18] Cutaneous allodynia arises as a consequence of central sensitisation of the TNC and higher structures, such as the thalamus, PAG, and RMg.[5 22] It is an independent predictor of migraine progression and is closely related with imbalances in the brainstem descending pain modulatory system.[22] In the current study, EA pretreatment significantly

Table 1 Behavioural analysis results by group at baseline and on days 1–5

	EA	SA	Model	Control	p Value
Rearing up					
Baseline	3.2±0.61	3.4±1.06	3.8±1.05	3.0±0.51	0.195
D1	2.4±0.21[a]	2.0±0.10[a]	2.0±0.25[a]	4.0±1.02[b]	<0.001
D3	1.8±0.34[a]	1.4±0.13[a]	1.2±0.35[a]	4.0±1.14[b]	<0.001
D5	2.3±0.35[a]	1.3±0.26[b]	1.0±0.37[b]	3.6±0.35[c]	<0.001
Sniffing					
Baseline	490.7±124.17	518.8±93.98	522.0±120.27	527.5±125.92	0.898
D1	589.5±36.74[a]	306.4±20.87[b]	247.2±39.57[b]	1211.3±93.22[c]	<0.001
D3	482.9±30.93[a]	197.6±16.68[bc]	179.9±28.42[b]	1074.9±102.94[c]	<0.001
D5	501.9±34.56[a]	145.0±10.39[b]	129.4±17.74[b]	1096.1±123.87[a]	<0.001
Turning					
Baseline	24.3±4.96	22.6±5.62	29.8±6.08	22.9±7.13	0.039
D1	50.7±5.04[a]	25.9±4.77[b]	25.8±4.69[b]	66.2±9.2[c]	<0.001
D3	27.2±4.55[a]	15.7±1.93[b]	11.1±1.43[b]	64.1±8.83[c]	<0.001
D5	22.7±3.47[a]	13.4±2.13[b]	7.0±0.96[c]	50.7±6.07[d]	<0.001
Stretching					
Baseline	45.5±9.74	42.0±5.20	43.5±7.64	38.4±5.06	0.178
D1	59.3±3.2[a]	56.5±5.76[a]	50.4±9.48[a]	99.2±10.21[b]	<0.001
D3	61.9±6.71[a]	50.6±5.01[ab]	43.6±8.75[b]	100.1±10.92[c]	<0.001
D5	60.4±9.04[a]	47.6±4.71[ab]	41.2±7.8[b]	107.9±13.48[c]	<0.001
Walking					
Baseline	34.1±9.37	39.1±9.08	37.0±10.11	37.0±8.65	0.699
D1	50.3±7.53[a]	36.4±4.1[b]	27.7±4.16[c]	87.5±13.12[d]	<0.001
D3	48.8±4.08[a]	22.6±4.4[b]	22.4±4.36[b]	89.0±8.03[c]	<0.001
D5	31±4.15[a]	23.9±3.67[a]	17.0±3.5[b]	72.1±9.09[c]	<0.001
Jumping					
Baseline	10.1±2.23	10.6±3.36	11.8±3.89	10.0±3.43	0.593
D1	16.1±2.45[a]	12.6±2.41[a]	11.9±2.65[a]	20.9±1.85[b]	<0.001
D3	18.3±3.7[a]	12.5±1.93[b]	10.8±1.95[b]	21.2±1.51[a]	<0.001
D5	16.8±2.83[a]	10.1±1.09[b]	8.1±1.53[b]	22.8±5.04[c]	<0.001
Walking slow					
Baseline	21.1±3.70	19.5±5.73	17.1±5.30	18.0±4.51	0.776
D1	51.1±6.96	49.1±5.76	46.5±9.2	55.3±4.9	0.3
D3	48.2±7.05	45.9±5.01	44.7±9.14	53.0±6.22	0.33
D5	48±6.49	45.1±5.39	43.7±5.71	52.2±7.01	0.179
Twitching					
Baseline	15.0±3.01	17.4±2.91	14.8±1.85	15.6±2.62	0.150
D1	51.9±6.91[ab]	61.1±8.22[a]	64.6±7.77[a]	40.1±9.82[b]	0.001
D3	47.8±9.08[a]	68.0±5.66[b]	73.9±7.51[b]	36.0±5.04[a]	<0.001
D5	36±5.84[a]	101.7±15.62[b]	106.2±11.28[b]	26.8±5.42[a]	<0.001
Remaining low					
Baseline	49.0±13.75	56.7±17.93	56.9±16.92	53.1±19.21	0.698
D1	399.8±20.09[a]	418.7±54.11[a]	421.9±58.2[a]	181.0±49.88[b]	<0.001
D3	351.8±50.97[a]	423.6±42.77[ab]	438.3±31.89[b]	175.6±42.9[c]	<0.001
D5	398.9±30.84[a]	471.1±65.86[ab]	492.3±67.89[b]	229.5±32.04[c]	<0.001
Sleeping					
Baseline	87.6±14.77	78.3±7.31	82.8±13.57	83.4±13.99	0.456
D1	699.8±102.83[ac]	1101.5±271.2[ab]	1215.2±357.9[b]	509.7±64.79[c]	0.001
D3	719.7±148.45[a]	1366.2±209.97[b]	1466.5±210.69[b]	486.2±65.56[a]	<0.001
D5	937.0±95.41[a]	1303.8±206.5[b]	1496.5±202.86[b]	624.9±117.77[a]	<0.001
Drinking					
Baseline	6.7±2.35	5.8±1.56	6.0±2.16	7.6±2.99	0.288

Continued

Table 1 Continued

	EA	SA	Model	Control	p Value
D1	50.3±4.86[a]	49.0±5.75[a]	48.1±3.89[a]	97.5±8.25[b]	<0.001
D3	39.3±4.9[a]	37.8±4.41[a]	33.7±3.83[a]	96.5±7.45[b]	<0.001
D5	43.8±3.24[a]	27.1±3.56[b]	24.6±3.08[b]	108.9±13.28[c]	<0.001
Eating					
Baseline	2.2±1.52	2.7±1.97	2.7±1.71	2.8±1.79	0.879
D1	2.9±0.57[a]	1.7±0.35[b]	1.4±0.34[b]	4.2±0.94[c]	<0.001
D3	2.3±0.31[a]	1.7±0.36[ab]	1.5±0.31[b]	5.2±0.71[a]	<0.001
D5	2.2±0.22[a]	1.0±0.2[b]	0.8±0.09[b]	4.5±0.48[c]	<0.001
Grooming					
Baseline	79.9±12.56	73.0±16.46	69.2±17.65	77.7±15.4	0.413
D1	899.8±63.37[a]	1054.8±121.15[ab]	1083.7±128.2[b]	797.6±85.31[b]	0.001
D3	896±47.72[ac]	1036.9±108.54[ab]	1077.9±121.86[b]	775.1±49.01[c]	<0.001
D5	910.9±94.07[ab]	1041.1±152.53[a]	1115.4±161.76[a]	783.9±50.06[b]	0.004

Data are time spent per behaviour per hour (s/h) and are expressed as mean±SD.
Overall p values are for overall (one-way) analysis of variance comparing across the four groups at each individual time point. Mean values within a row with *unlike* superscripts (a, b and c) are significantly different (p<0.05) on post-hoc testing using Bonferroni/Tanhame tests.
EA, electroacupuncture; SA, sham acupuncture.

decreased grooming behaviour, which suggests that cutaneous allodynia and central sensitisation were attenuated.

A possible mechanism underlying the effects of EA pretreatment

We found that EA at GB20 reversed c-Fos immunoreactivity changes in migraine-related brain areas (compared to the Model group). These results provide neurophysiological support for the assertion that the effects of acupuncture pretreatment are mediated via the regulation of imbalances in the descending pain modulatory system.

c-Fos is a protein marker of neuronal activation that has been used in diverse nociception research, including migraine.[23] The TNC, which receives input from peripheral sensory nerves, is the second-order neuron of the trigeminovascular system. Activation of the

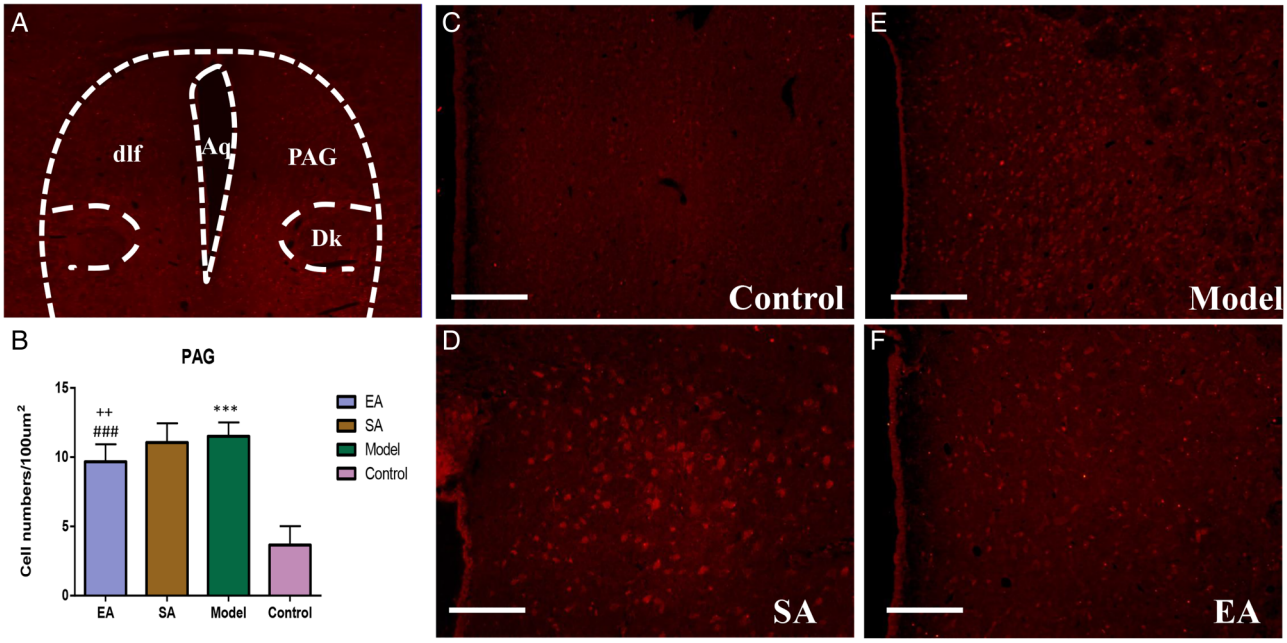

Figure 2 Immunocytochemical distribution of c-Fos positive cells in the periaqueductal grey (PAG) region (A) and numbers of positive cells per 100 μm^2 (B) in 40 rats that underwent implantation of electrodes followed by no stimulation (Control group, n=10) or repeated electrical dural stimulation (n=30) with no treatment (Model group, n=10), electroacupuncture pretreatment (EA group, n=10) or sham acupuncture pretreatment (SA group, n=10). Representative images show relatively sparse c-Fos labelling in the Control group (C) and EA group (F) and relatively intense c-Fos labelling in the SA group (D) and Model group (E). Scale bar=200 μm. Data are presented as mean±SD. ***p<0.001 vs Control group. ### p<0.001 vs Model group. ++ p<0.01 vs SA group. Aq, aqueduct (Sylvius); Dk: nucleus of Darkschewitsch.; dlf, dorsal longitudinal fasciculus.

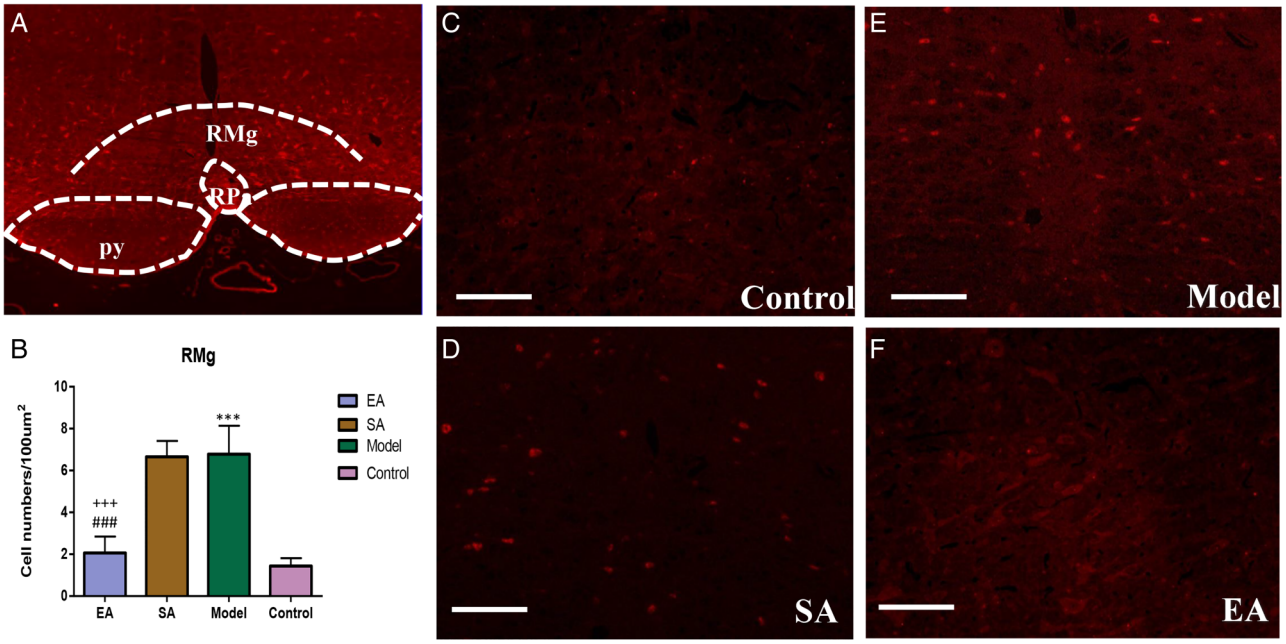

Figure 3 Immunocytochemical distribution of c-Fos positive cells in the raphe magnus nucleus (RMg) region (A) and numbers of positive cells per 100 μm^2 (B) in 40 rats that underwent implantation of electrodes followed by no stimulation (Control group, n=10) or repeated electrical dural stimulation (n=30) with no treatment (Model group, n=10), electroacupuncture pretreatment (EA group, n=10) or sham acupuncture pretreatment (SA group, n=10). Representative images show relatively sparse c-Fos labelling in the Control group (C) and EA group (F) and relatively intense c-Fos labelling in the SA group (D) and Model group (E). Scale bar=200 μm. Data are presented as mean±SD. *p<0.05 vs Control group. ###p<0.001 vs Model group. +++p<0.001 vs SA group. py, pyramidal cell layer of the hippocampus; RP, raphe pallidus nucleus.

TNC is regarded as a standard for activation and sensitisation of the trigeminovascular system and a reliable marker for the successful establishment of experimental models of headache.[23–25] Meanwhile, TNC inhibition has proven to be predictive of therapeutic anti-migraine efficacy.

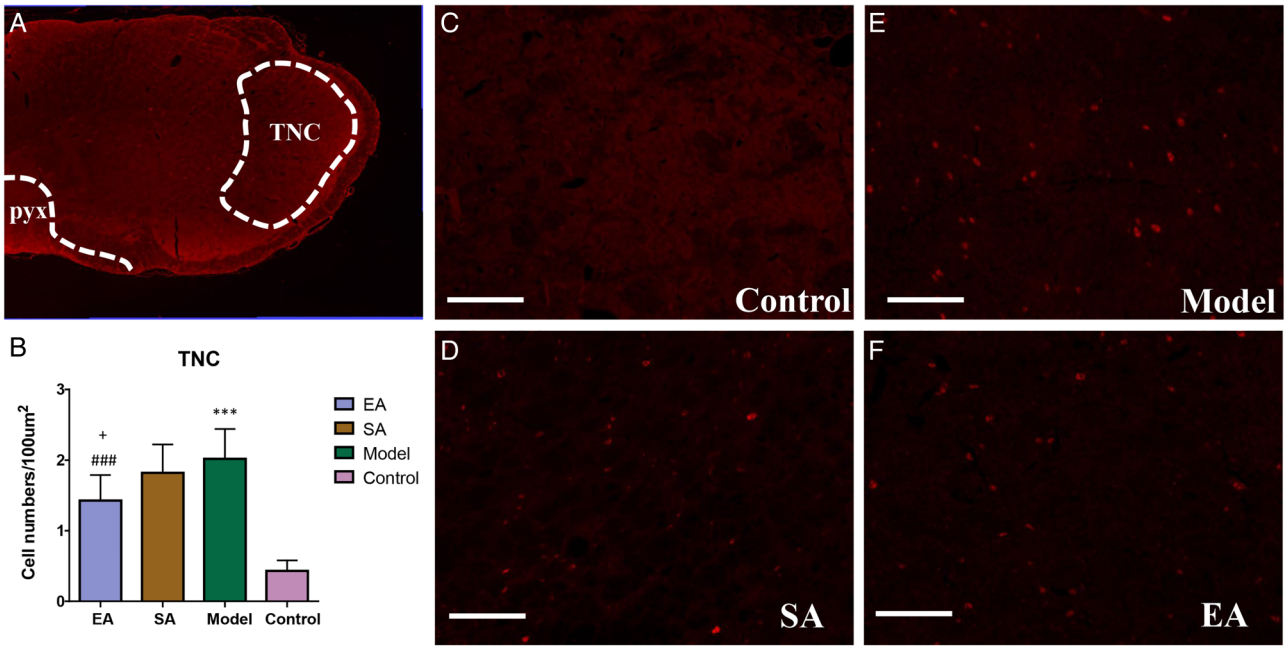

Figure 4 Immunocytochemical distribution of c-Fos positive cells in the trigeminal nucleus caudalis (TNC) region (A) and numbers of positive cells per 100 μm^2 (B) in 40 rats that underwent implantation of electrodes followed by no stimulation (Control group, n=10) or repeated electrical dural stimulation (n=30) with no treatment (Model group, n=10), electroacupuncture pretreatment (EA group, n=10) or sham acupuncture pretreatment (SA group, n=10). Representative images show relatively sparse c-Fos labelling in the Control group (C) and EA group (F) and relatively intense c-Fos labelling in the SA group (D) and Model group (E). Scale bar=200 μm. Data are presented as mean±SD. ***p<0.001, versus Control group. ###p<0.001 vs Model group. +p<0.05 vs SA group. pyx, pyramidal decussation.

Numerous investigations over the past half-century have established that the descending pain modulatory system arises in the brainstem from the PAG-rostral ventrolateral medulla (RVM) circuit to the spinal and medullary dorsal horn, and can exert bidirectional effects leading to both inhibition and facilitation of pain.[22 26] Electrophysiological studies by Fields and colleagues[27] have demonstrated that there are two distinct groups of RVM neurons producing opposite pain modulatory effects: (1) 'on'-cells mediating inhibition; and (2) 'off'-cells mediating facilitation. On-cells and off-cells form the neural basis for bidirectional control by the RVM and imbalance between these two cells types is implicated in the pathogenesis and progression of pain disorders, including migraine. For migraine, the descending pain modulatory pathway that is mainly involved is believed to be the PAG-RMg circuit to the TNC. The RMg is one of the important pain modulatory nuclei in the RVM region. On-cells and off-cells of the RMg project descending fibres to the TNC, suppressing or enhancing transmission of peripheral nociceptive information to the higher brain structures.[5 22]

In agreement with previous studies,[25 28] our results showed significant increases in c-Fos immunoreactivity within the PAG, RMg, and TNC after repeated dural stimulation, supporting the notion that activation of the PAG-RMg pathway in the descending pain modulatory system contributes to migraine pathophysiology through impaired descending inhibition and/or enhanced descending facilitation.[22] Pretreatment with EA strikingly decreased c-Fos immunoreactivity within these structures. The alterations in c-Fos expression and positive behavioural changes in the EA group may reflect either an improvement of impaired descending inhibition and an enhancement of antinociception—that is, decreased descending pain facilitation (through inhibition of off-cells) and/or increased descending pain inhibition (through facilitation of on-cells). This supports previous experimental work suggesting that acupuncture analgesia is mediated in part by the descending pain modulatory system.[29]

CONCLUSION

Our study has shown that EA pretreatment at GB20 can attenuate behavioural responses to electrical stimulation of the dura in a rat model of recurrent migraine and can mitigate the increased expression of c-Fos neurons within the PAG, RMg, and TNC that is characteristic of this model. Overall, this evidence supports the idea that EA pretreatment can ameliorate migraine-like symptoms by altering the descending pain modulatory system; however, further molecular and electrophysiological studies are required to better understand the central mechanisms of EA treatment of migraine.

Contributors PP and LPZ conducted the experiments. PP and LL interpreted the data and wrote the manuscript. YXC and ZYQ revised the manuscript. LPW supervised the research programme and contributed to integration of the research team. All authors have read and approved the final manuscript.

Funding This study was supported by the Ministry of Science and Technology of the People's Republic of China (grant number: Major State Basic Research Development Program 2014CB543203), Beijing Natural Science Foundation (7154205) and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201412).

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Hepp Z, Bloudek LM, Varon SF. Systematic review of migraine prophylaxis adherence and persistence. *J Manag Care Pharm* 2014;20:22–33.
2 Liu R, Yu S, He M, *et al*. Health-care utilization for primary headache disorders in China: a population-based door-to-door survey. *J Headache Pain* 2013;14:47.
3 Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013;33:629–808.
4 Steiner TJ, Stovner LJ, Al Jumah M, *et al*. Improving quality in population surveys of headache prevalence, burden and cost: key methodological considerations. *J Headache Pain* 2013;14:87.
5 Noseda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. *Pain* 2013;154(Suppl 1):S44–53.
6 Ramírez Rosas MB, Labruijere S, Villalón CM, *et al*. Activation of 5-hydroxytryptamine1B/1D/1F receptors as a mechanism of action of antimigraine drugs. *Expert Opin Pharmacother* 2013;14:1599–610.
7 Semenov IA. Migraine headaches. *Dis Mon* 2015;61:218–22.
8 Da Silva AN. Acupuncture for migraine prevention. *Headache* 2015;55:470–3.
9 Wang LP, Zhang XZ, Guo J, *et al*. Efficacy of acupuncture for migraine prophylaxis: a single-blinded, double-dummy, randomized controlled trial. *Pain* 2011;152:1864–71.
10 Gao Z, Liu X, Yu S, *et al*. Electroacupuncture at acupoints reverses plasma glutamate, lipid, and LDL/VLDL in an acute migraine rat model: A (1) H NMR-based metabolomic study. *Evid Based Complement Alternat Med* 2014;2014:659268.
11 Akerman S, Holland PR, Lasalandra MP, *et al*. Endocannabinoids in the brainstem modulate dural trigeminovascular nociceptive traffic via CB1 and "triptan" receptors: implications in migraine. *J Neurosci* 2013;33:14869–77.
12 Zhang M, Dai W, Liang J, *et al*. Effects of UCMS-induced depression on nociceptive behaviors induced by electrical stimulation of the dura mater. *Neurosci Lett* 2013;551:1–6.

13 Dong Z, Jiang L, Wang X, *et al*. Nociceptive behaviors were induced by electrical stimulation of the dura mater surrounding the superior sagittal sinus in conscious adult rats and reduced by morphine and rizatriptan benzoate. *Brain Res* 2011;1368:151–8.
14 Siu FK, Lo SC, Leung MC. Electro-acupuncture potentiates the disulphide-reducing activities of thioredoxin system by increasing thioredoxin expression in ischemia-reperfused rat brains. *Life Sci* 2005;77:386–99.
15 Chen XH, Han JS, Linde K, *et al*. All three types of opioid receptors in the spinal cord are important for 2/15 Hz electroacupuncture analgesia. *Eur J Pharmacol* 1992;211:203–10.
16 Li QQ, Shi GX, Yang JW, *et al*. Hippocampal cAMP/PKA/CREB is required for neuroprotective effect of acupuncture. *Physiol Behav* 2015;139:482–90.
17 Vos BP, Strassman AM, Maciewicz RJ. Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *J Neurosci* 1994;14(5 Pt 1):2708–23.
18 Melo-Carrillo A, Lopez-Avila A. A chronic animal model of migraine, induced by repeated meningeal nociception, characterized by a behavioral and pharmacological approach. *Cephalalgia* 2013;33:1096–105.
19 Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press, 2007.
20 Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* 2004;24(Suppl 1):9–160.
21 Stang PE, Osterhaus JT. Impact of migraine in the United States: data from the National Health Interview Survey. *Headache* 1993;33:29–35.
22 Boyer N, Dallel R, Artola A, *et al*. General trigeminospinal central sensitization and impaired descending pain inhibitory controls contribute to migraine progression. *Pain* 2014;155:1196–205.
23 Harris JA. Using c-fos as a neural marker of pain. *Brain Res Bull* 1998;45:1–8.
24 Akerman S, Holland PR, Goadsby PJ. Cannabinoid (CB1) receptor activation inhibits trigeminovascular neurons. *J Pharmacol Exp Ther* 2007;320:64–71.
25 Wang X, Yu S, Dong Z, *et al*. The Fos expression in rat brain following electrical stimulation of dura mater surrounding the superior sagittal sinus changed with the pre-treatment of rizatriptan benzoate. *Brain Res* 2011;1367:340–6.
26 Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 2014;8:143–51.
27 Fields HL, Vanegas H, Hentall ID, *et al*. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature* 1983;306:684–6.
28 Bohár Z, Fejes-Szabó A, Tar L, *et al*. Evaluation of c-Fos immunoreactivity in the rat brainstem nuclei relevant in migraine pathogenesis after electrical stimulation of the trigeminal ganglion. *Neurol Sci* 2013;34:1597–604.
29 Kuner R. Central mechanisms of pathological pain. *Nat Med* 2010;16:1258–66.