The Journal of Headache and Pain

RESEARCH

Open Access

Optogenetic cortical spreading depression originating from the primary visual cortex induces migraine-like pain and anxiety behaviors in freely moving C57BL/6 J mice

Huijuan Yuan[1,2,3], Weinan Na[1,2,3], Bozhi Li[2,3], Shuai Miao[2,4], Wenjing Tang[2,3], Li Kang[2], Chenghui Pi[2], Chunxiao Yang[5], Wei Xie[2], Tao Wang[6], Deqi Zhai[2,3,4], Dengfa Zhao[2,3], Ruozhuo Liu[2,3] and Shengyuan Yu[1,2,3*]

Abstract

Background Migraine is the second disabling neurological disorder with a high prevalence. Aura occurs in one-third of migraineurs and visual aura accounts for over 90%. Cortical spreading depression (CSD) underlies aura and might trigger migraine headaches. Compared with CSD induction by invasive electrical, chemical, or mechanical stimulation, optogenetics avoids direct influences on meninges in the stimulation process. However, previous optogenetic CSD models mainly use *Thy1-ChR2-YFP* or *CaMKIIa*-cre transgenic mice. They are limited when the pathogenesis study requires transgenic mice to express other specific promotor, such as the dopamine or serotonin transporter promotor. In addition, reported behavioral paradigms were based on CSD induction under anesthesia. This study aimed to establish an optogenetic CSD-induced migraine model originating in the primary visual cortex (VISp) in C57BL/6 J mice and presented the behavioral paradigm when CSD induction was under awake condition.

Methods We performed viral transduction for the expression of light-sensitive channelrhodopsin-2 in pyramidal neurons of VISp in C57BL/6 J mice. Regional cerebral blood flow (rCBF) was measured by laser speckle flowmetry to confirm CSD induction. The von Frey, light–dark box, elevated plus maze, and open field test were conducted to verify migraine-related behaviors in freely moving mice.

Results An optogenetic stimulus induced typical spreading triphasic rCBF change with a reduction of over 20%, confirming CSD induction. A single unilateral CSD in freely moving C57BL/6 J mice triggered bilateral periorbital and hind-paw allodynia lasting for 4–24 h. Notably, the ipsilateral periorbital mechanical threshold was significantly lower than the contralateral at 1 h. It also generated photophobia and anxiety behaviors persisting for 24–48 h. Furthermore, cutaneous allodynia and anxiety behaviors were alleviated by sumatriptan.

Conclusions This study proposes an optogenetic CSD-induced migraine model originating from VISp in awake and freely moving C57BL/6 J mice and presents the behavioral paradigm in detail. The CSD model in wild-type mice is promising to be wildly used to study the pathogenesis of MwA.

Keywords Migraine, Optogenetic, Cortical spreading depression, Cerebral blood flow, Allodynia, Anxiety, Primary visual cortex

*Correspondence:
Shengyuan Yu
yusy1963@126.com
Full list of author information is available at the end of the article

Background

Migraine is a primary headache, typically manifesting as a unilateral pulsatile headache lasting for 4–72 h concomitant with nausea, vomiting, photophobia, phonophobia, and cutaneous allodynia [1, 2]. Migraine is classified into migraine with aura (MwA), migraine without aura (MwoA), and other subtypes [3]. Aura occurs in a third of migraine patients and usually comes 5–60 min before headache [2]. Visual symptoms account for over 90% of migraine aura [4].

Migraine is the second disabling neurological disorder next to stroke [5], with a high prevalence of 14% worldwide [6]. Several medications, for instance, nonsteroidal anti-inflammatory drugs, triptans, ditans, and gepants, are utilized for abortive and prophylactic treatment of migraine [7]. Nevertheless, there remains a certain non-response rate of migraineurs [7] and most of these drugs have a high risk of medication overuse headache [8, 9]. Moreover, the underlying pathogenesis of migraine is still blurred. So there is an urgent demand for further research on the pathophysiology and therapeutic targets of migraine. The nitroglycerin-induced migraine model is widely used for researching MwoA, and cortical spreading depression (CSD)-induced migraine model for MwA.

CSD underlies migraine aura and is possibly related to migraine headaches [10]. It is an electrical activity featured as a depolarization wave of neurons and glial cells propagating across the cortex at a rate of 2–5 mm/min, followed by prolonged suppression of neuronal activity [11, 12]. Functional magnetic resonance imaging has proven the occurrence of CSD and corresponding changes in regional cerebral blood flow (rCBF) in MwA patients [13–15]. The direct current shift recorded by an electrode is time-locked with hemodynamic changes in the cortex of CSD mice [16]. In general, a potential shift of direct current over 5 mV amplitude or an rCBF change of over 20% compared with baseline spreading along the cortex was defined as a CSD induction [17].

Traditional methodologies of CSD induction, electrical, chemical (e.g. potassium chloride), and mechanical (e.g. pinprick) stimuli, are invasive and damage meningeal tissues and the cortex in the process. Recently, several studies proposed a non-invasive optogenetic CSD model in transgenic *Thy1-ChR2-YFP* mice expressing channelrhodopsin-2 (ChR2) in neurons [17, 18]. Houben et al. [17] induced CSD through the intact skull over the primary visual cortex (VISp), while Chung et al. [18] found that the threshold of light stimulation on VISp was too high to induce CSD via intact skull in *Thy1-ChR2-YFP* mice. Harriott et al. [19] proved that CSDs from the motor cortex under anesthesia triggered periorbital allodynia and anxiety-like behaviors in *Thy1-ChR2-YFP* mice. Masvidal-Codina et al. [20] then proposed a CSD model by viral transduction of light-sensitive ChR2 to neurons of the motor cortex in non-transgenic mice. Pi et al. [21] transfected ChR2 to the unilateral VISp in *CaMKIIα-cre* mice and triggered periorbital allodynia and anxiety behaviors by CSD induction under anesthesia. The feasibility of the optogenetic CSD mouse model has been verified sufficiently.

However, there are several issues to address. Firstly, CSD-induced migraine model on the basis of *Thy1-ChR2-YFP or CaMKIIα-cre* mice is limited when the study requires transgenic mice to express other specific promotor, such as the dopamine or serotonin transporter promotor. It is necessary to build an optogenetic CSD-induced model in wild-type mice. Additionally, reported behavioral paradigms were based on CSD induction under anesthesia [19, 21]. It brings up a need to research on behavioral paradigm for optogenetic CSD induced under awake state. Here, we established an optogenetic CSD-induced migraine model originating from VISp in awake and freely moving C57BL/6 J mice, confirmed by sumatriptan administration.

Methods

Animals

Male C57BL/6 J wild-type mice (8–12 weeks) were purchased from SiPeiFu Biotechnology Co., Ltd (Beijing, China). Mice got access to food and water ad libitum and housed under 12 h light–dark cycling conditions with controlled temperature and humidity. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Chinese People's Liberation Army General Hospital. The following experimental procedures were summarized and picturized in Fig. 1.

Virus injection

The procedures of virus injection referred to reported studies with some modifications [20–22]. Mice were anesthetized intraperitoneally by 1.25% avertin (0.02 ml/g of body weight; T48402, 152,463, Sigma, St. Louis, MO, USA) and positioned onto the stereotaxic apparatus (69105, RWD Life Science, Shenzhen, China). A heating pad was set at 37℃ to maintain the mouse body temperature. The erythromycin ointment was applied to protect the conjunctiva. For virus injection, the skull was exposed and cleaned. A right occipital craniotomy was undergone over the VISp (3.5 mm posterior and 2 mm lateral to the bregma) with a 0.8-mm burr hole. The dura mater here remained intact for the dorsoventral coordinate positioning of its surface. Then the dura mater over the injection site was punctured by a sterilized needle. The tip of a glass capillary connected with a nanoliter microinjection pump (R-480, RWD Life Science, Shenzhen, China) was lowered 0.5 mm under the dura mater. Then 300 nl

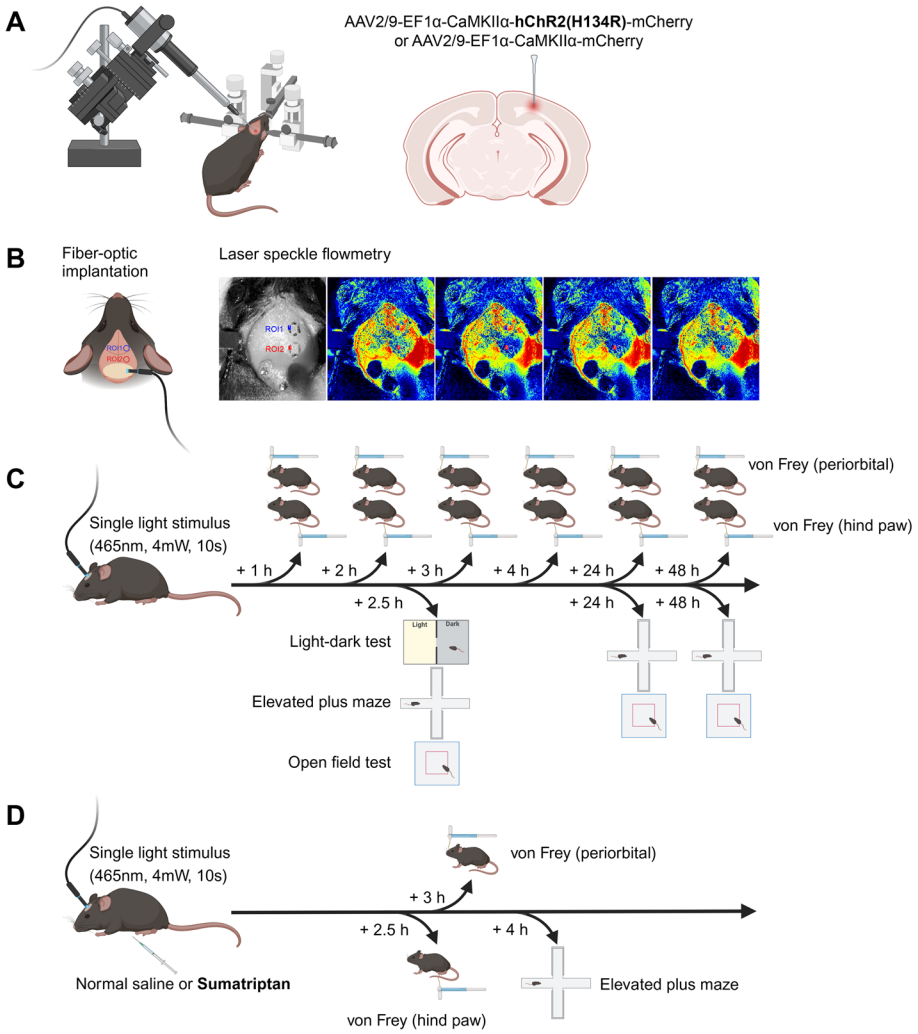

Fig. 1 The experimental flow graph. **A** A right occipital craniotomy over the primary visual cortex (3.5 mm posterior and 2 mm lateral to the bregma) and virus injection 0.5 mm below the dura mater via a glass microelectrode. **B** After at least 5 weeks, a fiber optic cannula was implanted 0.1 mm over the dura mater at the injection site. Regional cerebral blood flow (rCBF) was recorded to confirm the occurrence of cortical spreading depression (CSD). **C** The time course of von Frey tests on bilateral periorbital skins and hind paws, light–dark test, elevated plus maze (EPM), and open field test (OFT) after a single light stimulus (465 nm, 4 mW, 10 s). In each mouse, optogenetic stimulation was implemented under awake and freely moving condition. Eleven mice in each of VEH and CSD group were used for periorbital von Frey tests, and twelve mice for hind paw von Frey tests. Twenty-seven mice in each group were used for light–dark box, EPM, and OFT, and each mouse was conducted different tests at different time points (2.5 h, 24 h, and 48 h). **D** After a two-week washout period, sixteen mice in the CSD group were evenly divided into CSD + NS and CSD + Suma group, suffering an illumination (465 nm, 4 mW, 10 s) immediately followed by 0.9% normal saline and sumatriptan injection, respectively. The hind-paw and periorbital withdrawal thresholds were assessed at 2.5 h and 3 h and the EPM test at 4 h. Figure 1 was created in BioRender.com with permission

rAAV-EF1α-CaMKIIα-mCherry-WPRE-hGH polyA (AAV2/9, PT-0108, BrainVTA, Wuhan, China) or rAAV-EF1α-CaMKIIα-hChR2(H134R)-mCherry-WPRE-hGH polyA (AAV2/9, PT-0297, BrainVTA, Wuhan, China) was injected into the VISp (0.5 mm ventral to the dura mater for viral transduction in layer 5 pyramidal neurons [20, 22]) at a rate of 30 nl/min in the VEH and CSD group, respectively. Waiting for 10 min after injection prevented virus reflux. The scalp was stitched and mice were put on a hot plate at 37℃ until freely moving. The mice were sacrificed after all behavioral tests. The brain tissues were collected to observe the actual sites of virus expression (Fig. S1).

Fiber-optic implantation and optogenetic CSD induction

The interval between virus injection and fiber-optic implantation was at least 5 weeks for abundant ChR2 expression at the apical dendrite of layer 5 pyramidal

neurons [23]. Mice were anesthetized intraperitoneally and positioned on the stereotaxic apparatus as above. After the skull was exposed and cleaned, the screw head was adhered to the skull surface using glue. A fiber optic cannula (200 μm core diameter, 1.25 mm outer diameter, 0.37 numerical aperture; Inper, Zhejiang, China) was placed 0.1 mm above the dura mater over the injection site and secured to the occipital skull using dental cement. The frontal and parietal bones were kept clean and moist. The markers on the right frontal and parietal skull were respectively defined as a region of interest (ROI) 1 over the motor cortex (1.5 mm anterior and 2 mm lateral to the bregma) and 2 over the somatosensory cortex (1 mm posterior and 2 mm lateral to the bregma) for the rCBF recording. The fiber optic cannula was connected with a flexible cable, a 465 nm LED light device (2009, Plexon, USA), and an optogenetic controller (184000NQV, Plexon, USA) in sequence. A photometer (PM100D Thorlabs, Dachau Germany) was employed to calibrate the output power at the tip of the fiber optic cannula. The skull remained still and a non-invasive laser speckle flowmetry (LSF; PeriCam PSI HR, PrimedAB, Sweden) was positioned about 10 cm high from the skull and focused on a 1.5 × 1.5 cm monitoring area.

After recording the stable baseline of rCBF at least 5 min, light intensity was set at 4 mW in 1-s steps between 1–10 s, and stimulation duration was set for 10 s in 1-mW steps between 1–4 mW to detect CSD threshold. A propagating rCBF change over 20% compared with baseline was defined as a CSD. A triphasic rCBF change with over 20% reduction at the initial hypoperfusion was defined as a first CSD. To analyze CSD characteristics, mice were exposed to a uniform illumination (465 nm, 4 mW, 10 s). The rCBF was also detected in the VEH group for the exclusion of CSD induction.

The fiber optic cannula was reinforced on the frontal and parietal skulls using dental cement after the rCBF recording. Mice recovered from surgery over 10 days. Fifty mice in each of VEH and CSD group were enrolled for behavioral tests and the 465 nm-LED illumination was uniformly set as "4 mW, 10 s". In each mouse, CSD was induced under awake and freely moving condition. Eleven mice in each group were used for periorbital von Frey measurements, and twelve mice for hind paw von Frey measurements. Other twenty-seven mice in each group were used for light–dark box, elevated plus maze (EPM), and open field test (OFT), and each mouse was conducted different tests at different time points (2.5 h, 24 h, and 48 h).

After a two-week washout period, sixteen mice in the CSD group were evenly divided into CSD + NS and CSD + Suma group, suffering a 465 nm-LED illumination (4 mW, 10 s) immediately followed by an intraperitoneal injection of 0.9% normal saline (0.02 ml/g of body weight) and sumatriptan (diluted in 0.9% normal saline, 2 mg/kg, 0.02 ml/g of body weight), respectively. The hind-paw and periorbital withdrawal thresholds were assessed at 2.5 h and 3 h and the EPM test at 4 h.

von Frey test

Cutaneous allodynia often accompanies migraine attacks [2]. The calibrated von Frey filaments (Aesthesio, Danmic Global, CA, USA), the gold standard for mechanical withdrawal pain thresholds in rodents [24], were used to evaluate periorbital and hind-paw allodynia. The mice were allowed 3 consecutive days to adapt to the new environment and experiment operation. To assess the periorbital mechanical withdrawal thresholds, mice were held on the palm of researchers without constraint for at least 10 min daily. On the formal test day, mice stayed calmly on the palm and the monofilament was forced perpendicularly on bilateral periorbital skins in ascending order. The filaments remained bent as "C" or "S" for 3 s. Positive responses included head withdrawal, head shaking, facial grooming, or orbital tightening [25, 26]. To assess the hind-paw mechanical pain thresholds, mice were put in a coated acrylic chamber (10 × 7 × 16 cm) on a mesh for 30 min daily to acclimate. The monofilament was forced perpendicularly onto the plantar surface of bilateral hind paws. Positive responses included paw licking and paw withdrawal. The 50% withdrawal thresholds of periorbital areas and hind paws were assessed at the baseline and 1, 2, 3, 4, 24, and 48 h after an LED stimulation in the VEH and CSD group. The "up-down" method was used to determine the mechanical withdrawal thresholds as previously described [24]. The XO patterns were converted to continuous variables on a freely accessible website address (https://bioapps.shinyapps.io/von_frey_app/) [27].

Light–Dark Box test

To evaluate photophobia associated with a migraine attack, a modified light–dark box (30 × 30 × 30 cm) with infrared beam tracking (XR-XB120, Xinruan, Shanghai, China) was employed. The whole box was evenly divided into the light (1000 lx) and black (< 5 lx) compartment. The two compartments communicated via a hole (7 × 7 cm). The test was performed for 10 min at 2.5 h after optogenetic stimulation in the VEH and CSD group. Mice were positioned in the same corner of the light compartment facing the wall. The primary outcomes of photophobia were distance and time in the light box. Total distance and speed were counted to evaluate the motor ability.

Elevated Plus Maze (EPM) test

The EPM (XR-XG201, Xinruan, Shanghai, China) test was implemented for anxiety-like behaviors. The EPM contained two open arms (35 × 5 cm, about 60 lx), two closed arms (35 × 5 cm × 15 cm, about 10 lx), and a central platform (5 × 5 cm). It was 60 cm above the ground. Mice were positioned in the central platform facing an open arm and explored freely for 10 min at 2.5, 24, and 48 h after optogenetic stimulation in the VEH and CSD group. The maze was also conducted for 10 min at 4 h after optogenetic stimulation in the CSD + NS and CSD + Suma group. The primary outcomes were time in open arms and open arm time/(open + closed arm time) × 100% [OT/(OT + CT) %]. Total distance and speed were counted to evaluate the motor ability.

Open Field Test (OFT)

The OFT (XR-XZ301, Xinruan, Shanghai, China) was also conducted to measure comorbid anxiety. The apparatus was 30 × 30 × 30 cm with a light intensity of 30 lx. Mice were positioned in the center area facing the same quadrant and explored freely for 10 min at 2.5, 24, and 48 h after optogenetic stimulation in the VEH and CSD group. The primary outcomes were absolute and percent time in the center. Total distance and speed were counted to evaluate the motor ability.

Statistical analysis

The study was conducted by randomized grouping (random number table) and blindness for behavioral tests. Data were expressed as mean ± standard error of the mean (mean ± SEM) or median with quartiles. Two-tailed unpaired and paired t-test and two-way repeated-measures ANOVA were achieved by IBM SPSS Statistics V22.0. The analytical details were described in the article text and figure legend.

Results

Optogenetic stimulation induces a single propagating CSD

CSD is an electrophysiological event and is strictly coupled with hemodynamic changes [16]. Non-invasive LSF was used to assess rCBF as the verification of CSD. As shown in Fig. 2, a single optogenetic stimulation stably induced triphasic rCBF response in the right (ipsilateral) hemisphere of the CSD group, initial hypoperfusion (Fig. 2A, B; II, III), transient normalization (Fig. 2A, B; IV), and post-CSD oligemia (Fig. 2A, B; V). The premier hypoperfusion and transient normalization of rCBF were coincidental with the direct current shift in electrophysiological recording, confirming the induction of CSD [16, 28]. The CSD originated from VISp and spread towards the somatosensory cortex (Fig. 2; ROI 2) and the motor cortex (Fig. 2; ROI 1) in order. Simultaneously, the rCBF recordings of contralateral motor cortex (ROI 3) and somatosensory cortex (ROI 4) showed no significant change after CSD induction (Fig. S2). When the light power was set to 4mW, the stimulation duration of the CSD threshold was 4.13 ± 0.58 s ($n = 15$; range 1–10 s); when the stimulation duration was set to 10 s, the light power of the CSD threshold was 1.65 ± 0.17 mW ($n = 26$; range 1–4 mW). To analyze the CSD characteristics, the optogenetic stimulation was uniformly set as "4 mW, 10 s" in other mice ($n = 20$). The propagation rate of CSD waves was 4.71 ± 0.19 mm/min. At the initial hypoperfusion, the percentage of rCBF reduction was 34.84 ± 1.71% and 39.11 ± 1.44% in ROI 1 and 2 relative to baseline, similar to previous reports [17]. The VEH group had no profound hemodynamic response after optogenetic stimulus (465 nm, 4 mW, 10 s; Fig. 2C).

A single optogenetic CSD triggers migraine-like pain and photophobia

Peripheral and central sensitization mediate throbbing headaches and cutaneous allodynia during migraine [2, 29]. The von Frey tests were executed to assess mechanical pain thresholds in a time-dependent pattern. Bilateral mechanical thresholds of the periorbital skins and hind paws were evaluated at baseline and 1, 2, 3, 4, 24, and 48 h after a single light stimulus. A unilateral CSD triggered bilateral periorbital (Fig. 3A, B) and hind-paw allodynia (Fig. 3C, D). The allodynia lasted for 4 h and recovered to the baseline within 24 h. Interestingly, the ipsilateral (right) periorbital withdrawal thresholds at 1 h was significantly lower than the contralateral (left) in the CSD group ($p = 0.005$, Fig. 3A).

Photophobia was the most common symptom simultaneous with migraine attacks [1]. The light-aversion behavior was evaluated at 2.5 h after a light stimulation in the light–dark box (Fig. 3E). The total distance and speed of movement had no difference between the CSD and VEH group (Fig. 3F, G). Compared with the VEH group, the distance ($p = 0.011$, Fig. 3H) and duration time ($p = 0.018$, Fig. 3I) in the light box were significantly reduced in the CSD group, suggesting that a single optogenetic CSD generated light aversion.

A single optogenetic CSD elicits anxiety behaviors

Migraine often coexists with mood disorders, and the anxiety is the most relevant psychiatric comorbidity [30, 31]. The EPM (Fig. 4A) and OFT (Fig. 5A) tests were conducted at 2.5, 24, and 48 h to assess the anxiety-like behaviors. The total distance (Fig. 4B, 5B) and speed of movement (Fig. 4C, 5C) showed no significant differences between the VEH and CSD group at all testing periods, suggesting the similar locomotor ability. The absolute time spent in open arms of the CSD group displayed

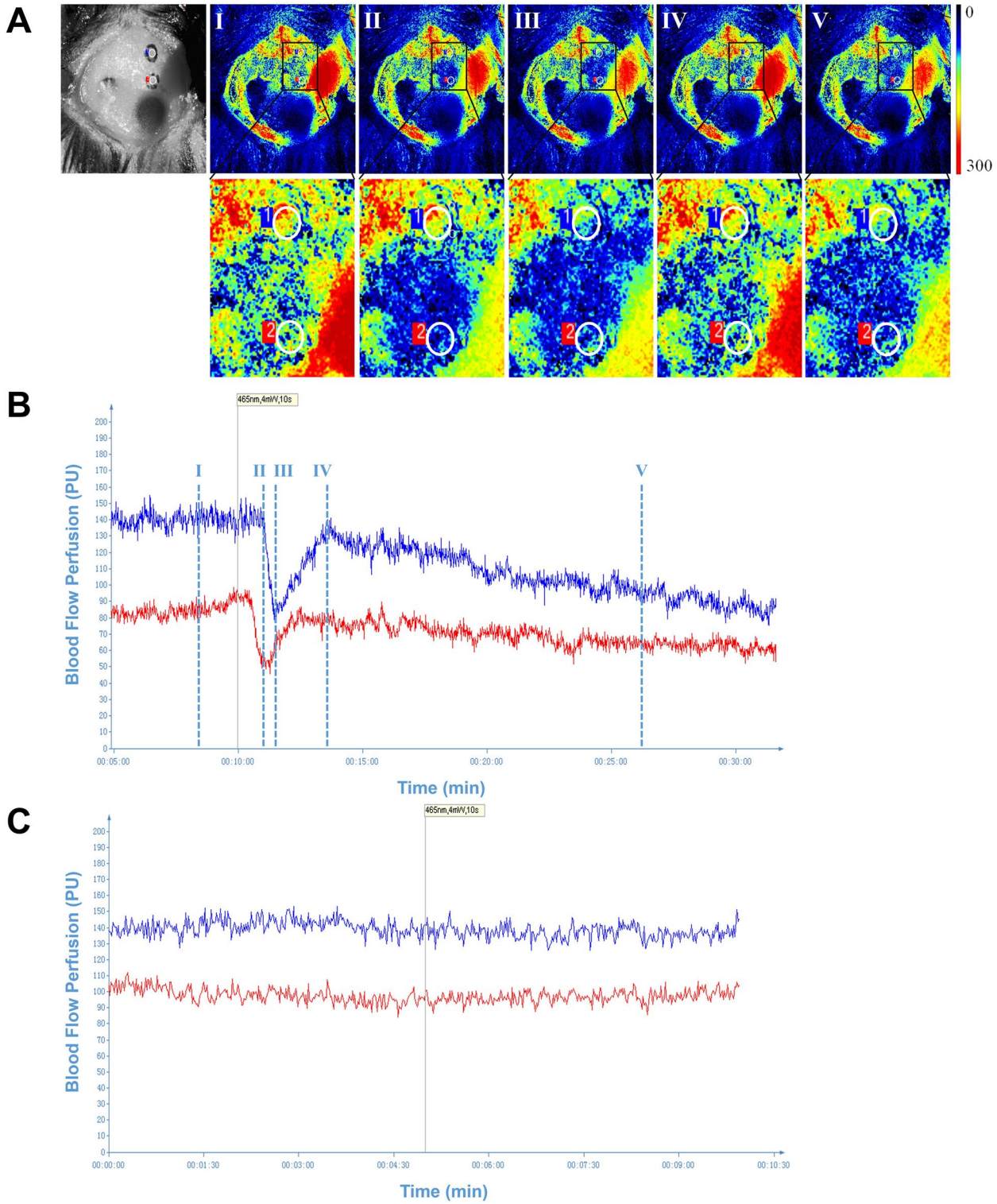

Fig. 2 An LED light stimulation induces spreading triphasic rCBF changes detected by laser speckle flowmetry. **A** The positions of ROI 1 (the motor cortex, blue) and ROI 2 (the somatosensory cortex, red) were manually marked. ROIs were amplified for display. **A** and **B** After recording the stable baseline over 5 min (**I**), optogenetic stimulus in the right hemisphere evoked initial hypoperfusion (**II, III**), transient normalization (**IV**), and post-CSD oligemia (**V**) at the same side. Triphasic rCBF changes spread from the somatosensory cortex (ROI 2, red) towards the motor cortex (ROI 1, blue). **C** The VEH group had little changes in the rCBF after optogenetic stimulus

a significant reduction at 2.5 h ($p=0.014$, Fig. 4D) and 24 h ($p=0.028$, Fig. 4D) and resumed to normal level at 48 h ($p=0.946$, Fig. 4D), as well as the percentage of time in open arms [OT/(OT + CT)%, 2.5 h: $p=0.012$; 24 h: $p=0.024$; 48 h: $p=0.907$; Fig. 4E] in the EPM test. The absolute time spent in the center areas (Fig. 5D) of the CSD group significantly decreased at 2.5 h ($p=0.027$) and 24 h ($p=0.039$) and got back to normal at 48 h ($p=0.674$), as same as the percentage of time in the center areas relative to the total test time (2.5 h: $p=0.027$; 24 h: $p=0.039$; 48 h: $p=0.674$; Fig. 5E) in the OFT. Both results in the EPM and OFT revealed a single CSD increased the thigmotaxis and anxiety-like behaviors [32].

Sumatriptan alleviates optogenetic-induced migraine-like pain and anxiety behaviors

Sumatriptan, a selective agonist of 5-hydroxytryptamine receptors, has been proven to be an effective and specific treatment for acute migraine attacks in numerous preclinic and clinical research [33]. In this study, the normal saline (CSD + NS group) or sumatriptan (CSD + Suma group) was administrated by peritoneal injection immediately after a single light stimulus. Compared to the baseline, the CSD + NS group showed a significant decrease in bilateral hind-paw (2.5 h, left: $p<0.001$, right: $p<0.001$; Fig. 6A) and periorbital (3 h, left: $p<0.001$, right: $p<0.001$; Fig. 6B) withdrawal thresholds. Both hind-paw (2.5 h, left: $p=0.008$, right: $p=0.028$; Fig. 6A) and periorbital (3 h, left: $p=0.028$, right: $p=0.011$; Fig. 6B) allodynia were alleviated at varying degrees in the CSD + Suma group, relative to the CSD + NS group. The mechanical pain thresholds of periorbital areas recovered to the baseline in the CSD + Suma group, while hind paws not.

Furthermore, the EPM was executed at 4 h in both groups. Time in open arms ($p=0.042$, Fig. 6F) and OT/(OT + CT)% ($p=0.038$, Fig. 6G) increased in the CSD + Suma group. It suggested that the CSD + Suma group tended to spend more time exploring the open arms and anxiety-like behaviors were relieved by sumatriptan. Given the poor central penetration of sumatriptan, we considered the possibility of anxiety-like behaviors secondary to pain. While the anxiety produced by CSD itself could not be excluded completely owing to lack of baseline level of thigmotaxis. The factors contributing to anxiety-like behaviors are likely multifaceted and complex.

Discussion

Our study proposed an optogenetic CSD-induced migraine model originating from VISp in freely moving C57BL/6 mice. A single optogenetic stimulation at the unilateral VISp elicited spreading triphasic rCBF change with the reduction of 20% or more at the initial perfusion in the ipsilateral hemisphere, which proved a first light-evoked CSD. A single unilateral CSD triggered bilateral periorbital and hind-paw allodynia, which were alleviated by sumatriptan administration. More notably, the ipsilateral periorbital mechanical threshold was significantly lower than the contralateral at 1 h. Furthermore, the migraine-like pain was accompanied by photophobia and anxiety behaviors. The behavioral pattern above was in accord with the clinical features of migraine [3].

The corresponding relationship between the laterality of rCBF changes and cutaneous allodynia in this study was congruent with previous reports. Olesen et al. [34] summarized the laterality of rCBF abnormality, headache, and aura symptoms in 63 migraine patients, 56 of which reported unilateral rCBF change, 2 bilateral rCBF change, and 5 without rCBF abnormality. In 56 migraineurs with unilateral rCBF abnormality, the aura symptoms were usually contralateral to rCBF abnormality (92.86%, 52/56) and bilateral (7.14%, 4/56) occasionally. While migraine headaches were found haphazardly ipsilateral to the side of rCBF changes in 58.93% (33/56), contralateral in 7.14% (4/56), bilateral in 28.57% (16/56),

(See figure on next page.)

Fig. 3 A single optogenetic CSD triggers prolonged allodynia and photophobia. Time sequential variation of bilateral periorbital (**A**, $n=11$ per group) and hind paw (**C**, $n=12$ per group) withdrawal pain thresholds after a single optogenetic stimulus. The data were shown as mean ± SEM. The difference was analyzed by two-way repeated measures ANOVA with post hoc analysis. **A** Left (contralateral): $F=2.657$, $p=0.116$ for group; $F=10.106$, $p<0.001$ for time; $F=4.505$, $p=0.003$ for group × time interaction. Right (ipsilateral): $F=11.234$, $p=0.003$ for group; $F=13.541$, $p<0.001$ for time; $F=8.375$, $p<0.001$ for group × time interaction. **C** Left (contralateral): $F=16.062$, $p=0.001$ for group; $F=19.656$, $p<0.001$ for time; $F=5.353$, $p<0.001$ for group × time interaction. Right (ipsilateral): $F=21.002$, $p=0.001$ for group; $F=31.214$, $p<0.001$ for time; $F=9.168$, $p<0.001$ for group × time interaction. Solid lines for VEH, dotted lines for CSD. VEH_left versus CSD_left, # $p<0.05$, ## $p<0.01$, ### $p<0.001$; VEH_right versus CSD_right, * $p<0.05$, ** $p<0.01$, *** $p<0.001$; CSD_left versus CSD_right, ▲▲ $p<0.01$. Individual time-sequential changes of bilateral periorbital (**B**) and hind-paw (**D**) withdrawal thresholds in the CSD group (blue, left side; red, right side). The significance between post-stimulation time points and the baseline was assessed by the post-hoc pairwise comparison. * $p<0.05$, *** $p<0.001$, ns = no significance. **E** Sample trajectories of 10-min light–dark box test at 2.5 h (VEH, upper panel; CSD, lower panel) after a single light stimulus. The differences in total distance (**F**), speed of movement (**G**), distance in the light (**H**), and time in the light (**I**) were presented as bar graphs (mean ± SEM) and were assessed by the unpaired t-test between the VEH and CSD group ($n=9$ per group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns = no significance

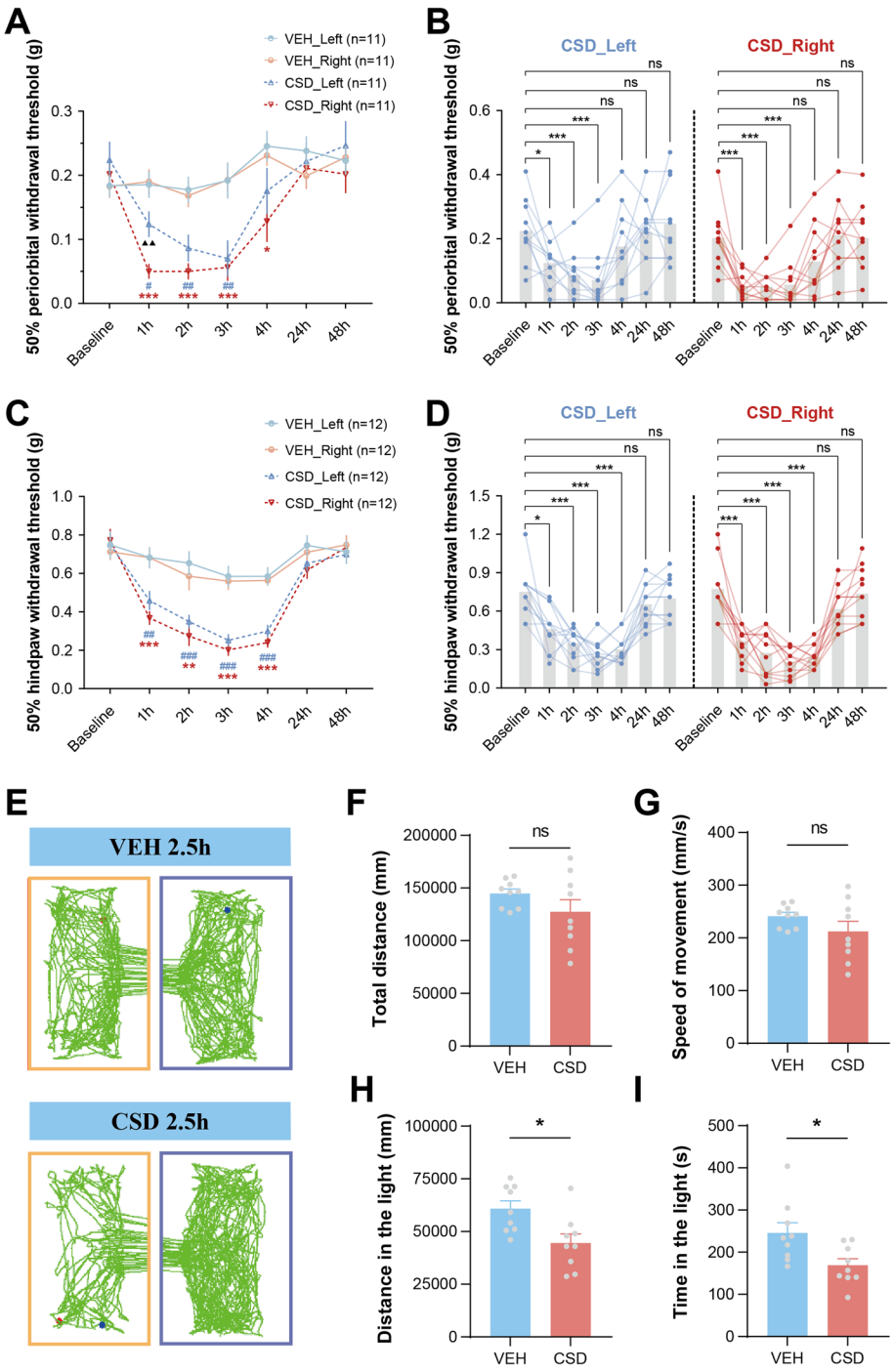

Fig. 3 (See legend on previous page.)

absence in 3.57% (2/56), and no recording in 1.79% (1/56). Migraine headaches usually occurred about 1 h after the onset of aura and about 30 min after the end of aura [34]. Burstein et al. [35] measured the mechanical pain thresholds of the head and forearms bilaterally before a migraine attack and at 1, 2, and 4 h after the onset of migraine attack following aura symptoms. The mechanical allodynia emerged in the head ipsilateral to headache at 1 h, extended to the contralateral head and ipsilateral forearm at 2 h, and persisted at 4 h. It was consistent with the interesting phenomenon in our study that the ipsilateral periorbital mechanical threshold decreased

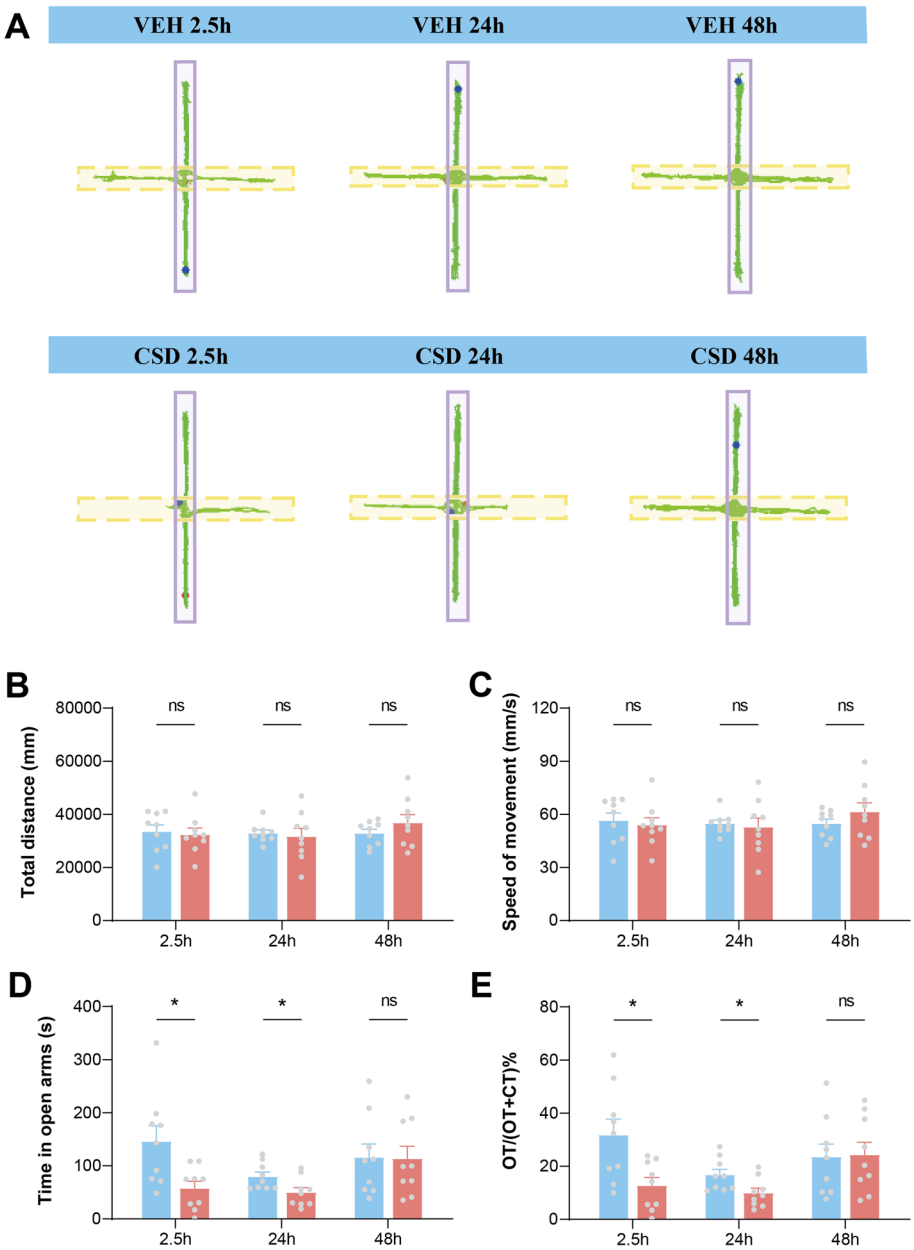

Fig. 4 A single optogenetic CSD produces anxiety behaviors in EPM. **A** Sample trajectories of 10-min EPM test at 2.5 h, 24 h, and 48 h (VEH, upper panel; CSD, lower panel) after a single light stimulus. The differences in total distance (**B**), speed of movement (**C**), time in open arms (**D**), and OT/(OT + CT)% (**E**) were presented as bar graphs (mean ± SEM) and were assessed by unpaired t-test between the VEH and CSD group ($n = 9$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significance

dramatically and was significantly lower than the contralateral at 1 h. In summary, the unilateral rCBF changes in the CSD-induced migraine mice or migraineurs were usually ipsilateral to headache and contralateral to aura symptoms [34], and a unilateral CSD generated bilateral cutaneous allodynia [2].

It is well-known that the meninges and their feeding vessels are the only intracranial pain-sensitive structures [36]. A past study observed that direct stimulation to the dura mater could activate the trigeminovascular pathway and evoke cutaneous allodynia, even in the absence of CSD events [37]. Thus, traditional CSD animal models induced by electrical, chemical, or mechanical stimulus could not attribute cutaneous allodynia to CSD itself. It is important to avoid direct stimulation of meningeal tissues in the process of CSD induction. Owing to the

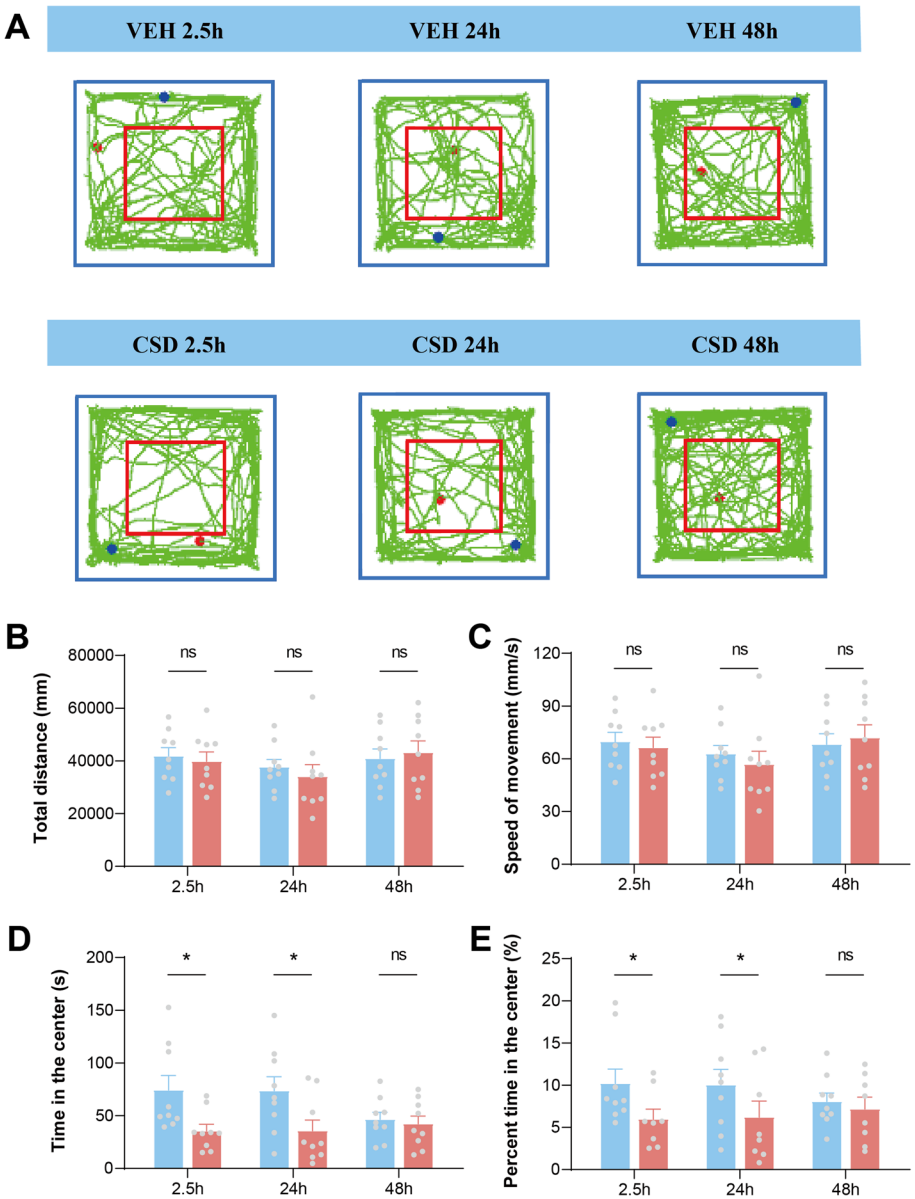

Fig. 5 A single optogenetic CSD generates anxiety behaviors in OFT. **A** Sample trajectories of 10-min OFT at 2.5 h, 24 h, and 48 h (VEH, upper panel; CSD, lower panel) after a single light stimulus. The differences in total distance (**B**), speed of movement (**C**), time in the center (**D**), and percent time in the center (**E**) were presented as bar graphs (mean ± SEM) and were assessed by unpaired t-test between the VEH and CSD group ($n = 9$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significance

minimal invasiveness of optogenetics, it is a great choice to induce CSD. Yet *Thy1-ChR2-YFP* mice [19], *CaMKIIα-cre* mice [21], and wild-type mice [20] have been used to establish the CSD model. Avoiding direct stimulation to meningeal tissues in the optogenetic process increases the reliability of optogenetic CSD-induced migraine model.

Recently, Rasmussen et al. [38] found that the trigeminal ganglion was directly exposed to the cerebral spinal fluid (CSF). Six CSDs were provoked by topical potassium chloride at 10-min intervals and cortical extracellular solutes such as calcitonin gene-related peptide (CGRP) increased at 1 h after the first CSD. The CSF solutes flowed towards the ipsilateral trigeminal ganglion primarily and firstly, which might drive the unilateral migraine headache. A smaller portion of CSF solutes also flowed to the contralateral trigeminal ganglion and dura mater. This theory might elucidate the reason for the

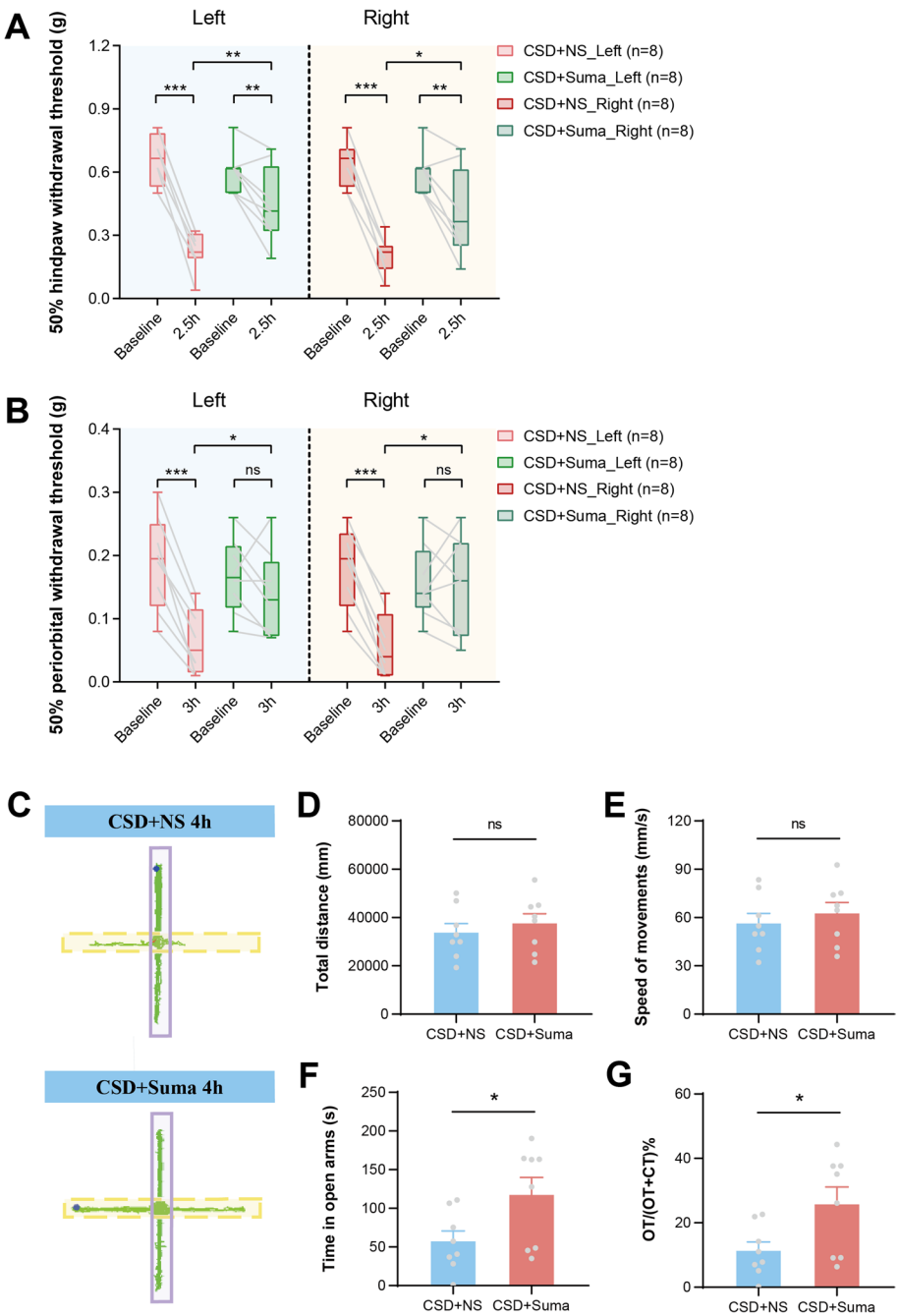

Fig. 6 Sumatriptan alleviates cutaneous allodynia and anxiety behaviors induced by a single optogenetic CSD. The hind-paw (**A**) and periorbital (**B**) withdrawal thresholds before and after normal saline and sumatriptan injection following optogenetic CSD induction in the CSD + NS ($n = 8$) and CSD + Suma ($n = 8$) group. The data were shown as box-whisker plots (median, the first quartile, and the third quartile). The difference was assessed by unpaired t-test (CSD + NS versus CSD + Suma) or paired t-test (post-stimulation time points versus baseline). **C** Sample trajectories of 10-min EPM test at 4 h (CSD + NS, upper panel; CSD + Suma, lower panel). The differences in total distance (**D**), speed of movements (**E**), time in open arms (**F**), and OT/(OT + CT)% (**G**) were presented as bar graphs (mean ± SEM) and were assessed by unpaired t-test between the CSD + NS and CSD + Suma group ($n = 8$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significance

ipsilateral periorbital allodynia being much more severe than the contralateral at 1 h after a single CSD in this study [2, 29]. Moreover, periorbital pain thresholds at 3 h after a single CSD recovered by sumatriptan administration to the baseline level in our study. Evidences above pointed together that CSD elevated the CSF proteins and

triggered the trigeminovascular system and that peripheral sensitization might underlie migraine pain and periorbital allodynia. The histological evidences in our model needed further investigation.

It was an interesting finding that, unlike both periorbital and hind-paw allodynia in this study, Pi et al. [21] and Harriott et al. [19] reported that optogenetic CSD triggered only bilateral periorbital allodynia but no hind-paw allodynia. The major difference was CSD induction under anesthesia in the research of Pi et al. [21] but under awake condition in our study. Tsurugizawa et al. [39] investigated the shift of the functional network from awake state to anesthesia in mice. The results showed that isoflurane reduced the functional connectivity between subcortex and cortex and suppressed the neuronal activity of thalamus, for example central medial thalamic nucleus. Fu et al. [40] found that the CSD activated glutamatergic neurons in the thalamus only in awake mice but not in anesthetized mice. Accordingly, whole brain c-FOS mapping showed that a single optogenetic CSD did not activate thalamus nuclei in anesthetized mice [21]. However, we found c-FOS activation in the thalamus including paraventricular nucleus and intralaminar thalamic nuclei (Fig. S3) on visual inspection at 2.5 h after a single optogenetic CSD. We supposed that optogenetic stimulation under anesthesia or awake state was a key factor for hind-paw allodynia in the mice model. In our study, intraperitoneal injection of sumatriptan partially alleviated hind-paw allodynia in mice of the CSD group, but pain thresholds of hind paws were still significantly lower than the baseline. So we considered hind-paw allodynia was mainly induced by central sensitization especially subcortical activations.

The importance of CSD induction at the VISp was emphasized in this study, primarily due to the most frequent visual aura in migraineurs [4]. The innate susceptibility and ChR2 expression in different brain regions were also relevant to CSD thresholds [18]. Besides, the cytoarchitecture (e.g. glia-neuron ratio) [41] and anatomical structures of the gyrencephalic cortex (e.g. sulci and vessels) [42] might change CSD characteristics. Additionally, several studies reported optogenetic stimulation initially excited pyramidal neurons in layer 5 of the cortex, postsynaptic responses of which contributed to subsequent excitatory responses in layer 2/3 and surrounding layer 5 and the activation of local inhibitory circuits [22, 43]. Thus, different CSD induction sites might activate diverse postsynaptic brain regions. Harriott et al. [19, 44] reported that a single optogenetic CSD from the motor cortex under anesthesia activated paraventricular nucleus of the thalamus (PVN) and supraoptic nucleus (SON). While PVN was not activated by CSD from VISp in anesthetic mice, and SON was not activated by CSD from VISp in awake mice in this study (Fig. S4). Although we could not explain the underlying mechanisms of different subcortical activations now, awake state and CSD induction sites indeed made a difference in outcomes.

There are several limitations in the study. We did not include female mice to avoid the estrous cycle in the experimental design. Estrogen could enhance CSD susceptibility [45] while testosterone suppress it [46]. And sex differences in expression of CGRP receptors [47] and in CGRP-induced vasodilation of human meningeal arteries [48] have been confirmed. It is vital to balance sex factors in basic research of migraine mechanisms. In addition, even though the CSDs for behavioral tests were induced under awake condition, we recorded the rCBF changes under anesthesia. The possible effects of anesthetics cannot be ruled out. Moreover, compared with optogenetic stimulation via intact skull in transgenic *Thy1-ChR2-YFP* mice, the craniotomy in the occipital bone for viral injection was invasive in this report.

Conclusions

This study proposes an optogenetic CSD-induced migraine model originating from VISp in freely moving C57BL/6 J mice. A single unilateral CSD under awake condition triggered bilateral periorbital and hind-paw allodynia, as well as photophobia and anxiety behaviors. The model in wild-type mice is promising to be wildly used to study the pathogenesis of MwA.

Abbreviations

CGRP	Calcitonin gene-related peptide
ChR2	Channelrhodopsin-2
CSD	Cortical spreading depression
CSF	Cerebral spinal fluid
EPM	Elevated plus maze
LSF	Laser speckle flowmetry
MwA	Migraine with aura
MwoA	Migraine without aura
OFT	Open field test
PVN	Paraventricular nucleus of the thalamus
rCBF	Regional cerebral blood flow
ROI	Region of interest
SON	Supraoptic nucleus
VISp	Primary visual cortex

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-025-01983-8.

Supplementary Material 1

Acknowledgements

Not applicable.

Authors' contributions

HJY, SYY, BZL, SM, WJT, LK, CHP, and RZL contributed to the study conceptualization and design. HJY performed the virus injection, fiber-optic implantation, and recording of cerebral blood flow. HJY and WNN carried out the behavioral

testing. HJY drew the graphs and wrote the manuscript. HJY, BZL, WNN, and SYY revised the manuscript. All authors participated in the data analysis and read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (grants 81471147, 81901145, 82271242).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Chinese People's Liberation Army General Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]School of Medicine, Nankai University, Tianjin 300071, China. [2]Department of Neurology, the First Medical Center, Chinese PLA General Hospital, Fuxing Road 28, Haidian District, Beijing 100853, China. [3]Neurology Institute of Chinese PLA General Hospital, the First Medical Center, Chinese PLA General Hospital, Fuxing Road 28, Haidian District, Beijing 100853, China. [4]Medical School of Chinese PLA, Beijing 100853, China. [5]Department of Neurology, the Second Medical Center, Chinese PLA General Hospital, Beijing 100853, China. [6]Department of Critical Care Medicine, The Fourth Medical Centre, Chinese PLA General Hospital, Beijing 100048, China.

Received: 7 October 2024 Accepted: 18 February 2025
Published online: 26 February 2025

References

1. Ashina M, Terwindt GM, Al-Karagholi MA, de Boer I, Lee MJ, Hay DL et al (2021) Migraine: disease characterisation, biomarkers, and precision medicine. Lancet 397(10283):1496–1504
2. Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH (2000) An association between migraine and cutaneous allodynia. Ann Neurol 47(5):614–624
3. Headache Classification Committee of the International Headache Society (IHS) (2018) The International Classification of Headache Disorders, 3rd edition. Cephalalgia 38(1):1–211
4. Russell MB, Olesen J (1996) A nosographic analysis of the migraine aura in a general population. Brain 119(Pt 2):355–361
5. GBD (2016) Neurology Collaborators (2019) Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18(5):459–480
6. Jensen R, Stovner LJ (2008) Epidemiology and comorbidity of headache. Lancet Neurol 7(4):354–361
7. Argyriou AA, Mantovani E, Mitsikostas DD, Vikelis M (2022) Tamburin S (2022) A systematic review with expert opinion on the role of gepants for the preventive and abortive treatment of migraine. Expert Rev Neurother 22(6):469–488
8. Ferrari MD, Goadsby PJ, Burstein R, Kurth T, Ayata C, Charles A et al (2022) Migraine. Nat Rev Dis Primers 8(1):2
9. Saengjaroentham C, Strother LC, Dripps I, Sultan Jabir MR, Pradhan A et al (2020) Differential medication overuse risk of novel anti-migraine therapeutics. Brain 143(9):2681–2688
10. Karatas H, Erdener SE, Gursoy-Ozdemir Y, Lule S, Eren-Koçak E, Sen ZD et al (2013) Spreading depression triggers headache by activating neuronal Panx1 channels. Science 339(6123):1092–1095
11. AAP. L, (1944) Spreading depression of activity in the cerebral cortex. J Neurophysiol 7:359–390
12. Lauritzen M, Jørgensen MB, Diemer NH, Gjedde A, Hansen AJ (1982) Persistent oligemia of rat cerebral cortex in the wake of spreading depression. Ann Neurol 12(5):469–474
13. Olesen J, Larsen B, Lauritzen M (1981) Focal hyperemia followed by spreading oligemia and impaired activation of rCBF in classic migraine. Ann Neurol 9(4):344–352
14. Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D, Bakker D, Fischl B et al (2001) Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci U S A 98(8):4687–4692
15. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S (2017) Pathophysiology of migraine: a disorder of sensory processing. Physiol Rev 97(2):553–622
16. Ayata C, Shin HK, Salomone S, Ozdemir-Gursoy Y, Boas DA, Dunn AK et al (2004) Pronounced hypoperfusion during spreading depression in mouse cortex. J Cereb Blood Flow Metab 24(10):1172–1182
17. Houben T, Loonen IC, Baca SM, Schenke M, Meijer JH, Ferrari MD et al (2017) Optogenetic induction of cortical spreading depression in anesthetized and freely behaving mice. J Cereb Blood Flow Metab 37(5):1641–1655
18. Chung DY, Sadeghian H, Qin T, Lule S, Lee H, Karakaya F et al (2019) Determinants of optogenetic cortical spreading depolarizations. Cereb Cortex 29(3):1150–1161
19. Harriott AM, Chung DY, Uner A, Bozdayi RO, Morais A, Takizawa T et al (2021) Optogenetic spreading depression elicits trigeminal pain and anxiety behavior. Ann Neurol 89(1):99–110
20. Masvidal-Codina E, Smith TM, Rathore D, Gao Y, Illa X, Prats-Alfonso E et al (2021) Characterization of optogenetically-induced cortical spreading depression in awake mice using graphene micro-transistor arrays. J Neural Eng 18:05502. https://doi.org/10.1088/1741-2552/abecf3
21. Pi C, Tang W, Li Z, Liu Y, Jing Q, Dai W et al (2022) Cortical pain induced by optogenetic cortical spreading depression: from whole brain activity mapping. Mol Brain 15(1):99
22. Tang Q, Tsytsarev V, Yan F, Wang C, Erzurumlu RS, Chen Y (2020) *In vivo* voltage-sensitive dye imaging of mouse cortical activity with mesoscopic optical tomography. Neurophotonics 7(4):041402
23. Foutz TJ, Arlow RL, McIntyre CC (2012) Theoretical principles underlying optical stimulation of a channelrhodopsin-2 positive pyramidal neuron. J Neurophysiol 107(12):3235–3245
24. Deuis JR, Dvorakova LS, Vetter I (2017) Methods used to evaluate pain behaviors in rodents. Front Mol Neurosci 10:284
25. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S et al (2010) Coding of facial expressions of pain in the laboratory mouse. Nat Methods 7(6):447–449
26. Elliott MB, Oshinsky ML, Amenta PS, Awe OO, Jallo JI (2012) Nociceptive neuropeptide increases and periorbital allodynia in a model of traumatic brain injury. Headache 52(6):966–984
27. Christensen SL, Hansen RB, Storm MA, Olesen J, Hansen TF, Ossipov M et al (2020) Von Frey testing revisited: provision of an online algorithm for improved accuracy of 50% thresholds. Eur J Pain 24(4):783–790
28. Eikermann-Haerter K, Ayata C (2010) Cortical spreading depression and migraine. Curr Neurol Neurosci Rep 10(3):167–173
29. Noseda R, Burstein R (2013) Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. Pain 154(Suppl 1):S44-53
30. Peres MFP, Mercante JPP, Tobo PR, Kamei H, Bigal ME (2017) Anxiety and depression symptoms and migraine: a symptom-based approach research. J Headache Pain 18(1):37
31. Louter MA, Pijpers JA, Wardenaar KJ, van Zwet EW, van Hemert AM, Zitman FG et al (2015) Symptom dimensions of affective disorders in migraine patients. J Psychosom Res 79(5):458–463
32. Simon P, Dupuis R, Costentin J (1994) Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res 61(1):59–64
33. Subcutaneous Sumatriptan International Study Group (1991) Treatment of migraine attacks with sumatriptan. N Engl J Med 325(5):316–321
34. Olesen J, Friberg L, Olsen TS, Iversen HK, Lassen NA, Andersen AR et al (1990) Timing and topography of cerebral blood flow, aura, and headache during migraine attacks. Ann Neurol 28(6):791–798
35. Burstein R, Cutrer MF, Yarnitsky D (2000) The development of cutaneous allodynia during a migraine attack clinical evidence for the sequential

recruitment of spinal and supraspinal nociceptive neurons in migraine. Brain 123(Pt 8):1703–1709
36. Levy D, Moskowitz MA (2023) Meningeal mechanisms and the migraine connection. Annu Rev Neurosci 46:39–58
37. Fioravanti B, Kasasbeh A, Edelmayer R, Skinner DP Jr, Hartings JA, Burklund RD et al (2011) Evaluation of cutaneous allodynia following induction of cortical spreading depression in freely moving rats. Cephalalgia 31(10):1090–1100
38. Kaag Rasmussen M, Møllgård K, Bork PAR, Weikop P, Esmail T, Drici L et al (2024) Trigeminal ganglion neurons are directly activated by influx of CSF solutes in a migraine model. Science 385(6704):80–86
39. Tsurugizawa T, Yoshimaru D (2021) Impact of anesthesia on static and dynamic functional connectivity in mice. Neuroimage 241:118413
40. Fu X, Chen M, Lu J, Li P (2022) Cortical spreading depression induces propagating activation of the thalamus ventral posteromedial nucleus in awake mice. J Headache Pain 23(1):15
41. Eiselt M, Giessler F, Platzek D, Haueisen J, Zwiener U, Röther J (2004) Inhomogeneous propagation of cortical spreading depression-detection by electro- and magnetoencephalography in rats. Brain Res 1028(1):83–91
42. Santos E, Schöll M, Sánchez-Porras R, Dahlem MA, Silos H, Unterberg A et al (2014) Radial, spiral and reverberating waves of spreading depolarization occur in the gyrencephalic brain. Neuroimage 99:244–255
43. Lo SQ, Koh DX, Sng JC, Augustine GJ (2015) All-optical mapping of barrel cortex circuits based on simultaneous voltage-sensitive dye imaging and channelrhodopsin-mediated photostimulation. Neurophotonics 2(2):021013
44. Harriott AM, Kaya M, Ayata C (2024) Oxytocin shortens spreading depolarization-induced periorbital allodynia. J Headache Pain 25:152
45. Chauvel V, Multon S, Schoenen J (2018) Estrogen-dependent effects of 5-hydroxytryptophan on cortical spreading depression in rat: Modelling the serotonin-ovarian hormone interaction in migraine aura. Cephalalgia 38(3):427–436
46. Eikermann-Haerter K, Baum MJ, Ferrari MD, van den Maagdenberg AM, Moskowitz MA, Ayata C (2009) Androgenic suppression of spreading depression in familial hemiplegic migraine type 1 mutant mice. Ann Neurol 66(4):564–568
47. Maddahi A, Edvinsson JCA, Edvinsson L (2024) Sex differences in expression of CGRP family of receptors and ligands in the rat trigeminal system. J Headache Pain 25(1):193
48. de Vries T, Boucherie DM, Chan KY, Rubio-Beltrán E, Labastida-Ramírez A, Labruijere S et al (2024) Sex differences in CGRP-induced vasodilation of human middle meningeal arteries but not human coronary arteries: implications for migraine. Cephalalgia 44(7):3331024241254088

Publisher's Note