**BRIEF COMMUNICATION**

**Amygdala Metabotropic Glutamate Receptor 1 Influences Synaptic Transmission to Participate in Fentanyl-Induced Hyperalgesia in Rats**

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**Abstract**

The underlying mechanisms of opioid-induced hyperalgesia (OIH) remain unclear. Herein, we found that the protein expression of metabotropic glutamate receptor 1 (mGluR1) was significantly increased in the right but not in the left laterocapsular division of central nucleus of the amygdala (CeLC) in OIH rats. In CeLC neurons, the frequency and the amplitude of mini-excitatory postsynaptic currents (mEPSCs) were significantly increased in fentanyl group which were decreased by acute application of a mGluR1 antagonist, A841720. Finally, the behavioral hypersensitivity could be reversed by A841720 microinjection into the right CeLC. These results show that the right CeLC mGluR1 is an important factor associated with OIH that enhances synaptic transmission and could be a potential drug target to alleviate fentanyl-induced hyperalgesia.

**Graphical Abstract**

**Keywords** Pain · Opioid-induced hyperalgesia · mGluR1 ·Amygdala · CeLC

**Abbreviations**

- OIH: Opioid-induced hyperalgesia
- mGluR: Metabotropic glutamate receptor
- CeLC: Laterocapsular division of central nucleus of the amygdala
- mEPSCs: Mini-excitatory postsynaptic currents
- ERK: Extracellular signal-regulated kinase (ERK)
- NMDA: N-Methyl-d-aspartate
- iGluR: Ionotropic glutamate receptor
- ACSF: Artificial cerebrospinal fluid
- RVM: Rostroventral medulla of the brainstem
- LA/BLA: Lateral/basolateral amygdala nuclei
- ITC: Intercalated cell mass of the amygdala
- CeA: Central nucleus of the amygdala
- mPFC: Medial prefrontal cortex

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**Introduction**

Opioids produce strong analgesia and are mainly used to treat acute and chronic pain. Paradoxically, the administration of opioids may elicit a lower threshold of pain, which is defined as opioid-induced hyperalgesia (OIH) (Celerier et al. 2000; Mao 2002; Vanderah et al. 2001; Lee and Yeomans 2014). Prolonged opioid exposure also produces analgesic tolerance characterized as opioid tolerance, which has the same clinical manifestations as OIH. However, opioid tolerance can usually be suppressed by increasing the dosage of the opioid, whereas it may exacerbate OIH. This contradictory phenomenon has led to the clinical use of opioids as a dilemma (Kim et al. 2014). As fentanyl, a potent synthetic opioid, and its derivatives are the most commonly used in clinical anesthesia and analgesia, more scientific research providing deeper insight into the mechanisms of OIH is needed.

All opioids induce adverse effects that include opioid tolerance and/or hyperalgesia, which are characterized in relation to analgesic approach, sex, and age. Sex differences in the magnitude of opioid effects upon hyperalgesia have been described in humans and in animal models for reasons that remain unclear (Barrett et al. 2003; Holtman and Wala 2005; Bodnar and Kest 2010). Male patients with long-term opioid use demonstrated hyperalgesic response with following fentanyl administration in comparison in females and healthy males (Wasserman et al. 2015). In addition, the use of opioids has increased in adolescents undergoing surgical procedures (Kotzer 2000; Maxwell et al. 2005; Engelhardt et al. 2008; Tripi et al. 2015). Most relevant studies on OIH emphasized the need to explore the mechanistic basis exclusively in adult subjects. Instead, our research highlights the molecular underpinnings in male adolescents.

Previous studies have implicated a prominent role for the laterocapsular division of the central amygdala (CeLC) in OIH development (Kissiwaa et al. 2019). Only elderly mice, not middle-aged or young mice, show elevated ERK-1 phosphorylation in the right CeA following peripheral damage. ERK1 activation may account for age-related variations in pain sensitivity (Sadler et al. 2017). Our team and our collaborators discovered that extracellular signal-regulated kinase (ERK) activation in CeLC may contribute to OIH development by strengthening synaptic communication between CeLC neurons (Li et al. 2017b). Calcium/calmodulin-dependent protein kinase II alpha (CaMKIIα) activity in the CeLC is also involved in OIH development (Li et al. 2016). We are still working on this field to reveal the potential integrate pathways to regulate signal transmission in CeLC during OIH occurs.

Several possible molecular mechanisms of OIH include sensitization of the central glutamatergic system, disruption of Cl− homeostasis, and changes in opioid receptors (Lee and Yeomans 2014; Roeckel et al. 2016). It has been recently discovered that the activation of the N-Methyl-d-aspartate (NMDA) receptor is intimately linked to OIH. The cross-regulation between the ionotropic glutamate receptor N and the mu-opioid receptor contribute to the transmission and modulation of nociceptive signals, which result in the development of OIH (Sanchez-Blazquez et al. 2013). Most studies on glutamatergic pathways in the OIH field focused on the ionotropic glutamate receptor (iGluR), while studies on the central metabotropic glutamate receptor (mGluR) are rarely reported. There are previous evidences suggesting that mGluR7 and mGluR8 in the amygdala have emerged as novel targets in pain controls (Palazzo et al. 2008, 2017; Ren et al. 2011). mGluR8 within the CeA modulates nociceptive behavior in inflammatory pain conditions (Palazzo et al. 2011). Our collaborators are working on the contribution of mGluR subtypes in OIH, including mGluR7 and mGluR8.

Meanwhile, knockdown of spinal mGluR1 significantly diminishes cold hyperalgesia, heat hyperalgesia, and mechanical allodynia by restoring morphine sensitivity and reducing NMDA sensitivity in neuropathic rats (Fundytus et al. 2001). mGluR1 plays a vital role in the BLA-driven prefrontal cortical inactivation after carrageenan-induced inflammatory pain (Luongo et al. 2013). Further research is necessary to confirm the particular involvement of mGluR1 in OIH.

Here, we describe a hyperalgesia model prepared by repeated injection of fentanyl in rats. Notably, increased mGluR1 expression in CeLC was detected in OIH rats. The frequency and amplitude of mEPSCs in CeLC neurons were also increased during OIH and then recovered to the normal level by blocking mGluR1 in brain slices. Mechanical and heat sensitivities were recovered by treatment with an mGluR1 receptor antagonist in the right CeLC of the OIH model. We focused on the modulation of CeLC mGluR1 in fentanyl-stimulated hyperalgesia.

**Materials and Methods**

**Animals**

Male-adolescent Sprague–Dawley rats weighing 90–110 g were obtained from Hubei Research Center of Laboratory Animals. The rats were housed in cages under standard laboratory conditions of temperature (20–22 °C) and relative humidity (55–65%) with a 12 h light/dark cycle. All rats were provided with food and water-available ad libitum. Animal handling was approved by the Animal Care and Use Committee of South-Central Minzu University.
(2019-SCUEC-AEC-022) and conformed to the guidelines of the International Association for the Study of Pain (Zimmermann 1983). The rats were randomly grouped, and the experimenters were blinded to group assignments and data analysis.

Behavioral Assessments

The fentanyl citrate solution (Yichang Humanwell Pharmaceutical Co. Ltd., Yi Chang, Hubei, China) was administered as four subcutaneous bolus injections at 15 min intervals using a 60 µg/kg dose per injection (fentanyl citrate concentration 50 µg/ml, 120 µl/100 g, 4 times) (Celerier et al. 2000). The same volume of 0.9% saline was administered to the vehicle group. Mechanical and heat sensitivities were measured before injection as the baseline data. Mechanical allodynia was tested by measuring the withdrawal threshold for a series of von Frey filaments (beginning with 1.0 g; North Coast, San Jose, CA, USA). The thermal pain threshold was tested using a model BME-410C radiant thermal stimulator (Biomedical Engineering, Boerni Science and Technology Co. Ltd., Guangzhou, China).

Western Blot and Immunohistochemistry

Brain slices (1 mm, brain matrix 0–175 g) were prepared using a model BJK-001 device (J&K Seiko, DongGuan, China). CeLCs were collected using a patching technique guided by a dissecting microscope following the Allen Brain atlas (Zapala et al. 2005; Han et al. 2005). Then 6 CeLCs per one sample were lysed using radio-immunoprecipitation assay (RIPA) buffer, and protein concentrations were determined using the Coomassie (Bradford) assay kit (23200; Pierce Biotechnology, Rockford, IL, USA). Samples containing equal amounts of protein were separated using 10% SDS-PAGE and blotted onto polyvinylidene fluoride membranes, blocked with 5% (w/v) bovine serum albumin in Tris-buffered saline (TBST) at room temperature for 1 h, and then incubated with the primary antibody at 4 °C overnight. The antibody was anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:500 dilution; Boster, Wuhan, China) or anti-mGluR1 (1:2000 dilution, cat#07–617, lot #2207272; MilliporeSigma) (Shin et al. 2015). After several washes, the blots were incubated with the secondary antibody (1:10000 dilution; Boster). The immunoreactive bands corresponding to mGluR1 were analyzed using the ChemiDoc system (Bio-Rad, Hercules, CA, USA).

For immunohistochemistry, 7-µm-thick CeLC sections were cut on a freezing sliding microtome and rinsed in anti-mGluR1 antibody (1:100) overnight at 4 °C. Detection was performed using a DAB Substrate kit (Boster) following the manufacturer’s instructions. Images were taken using a digital camera (IX-81, Olympus Corp, Tokyo, Japan, magnification, x400).

Cannula Construction and Implantation

The rats were intraperitoneally injected with 10% chloral hydrate (3 ml/kg) and then positioned on a stereotaxic frame (Zenda, Austin, TX, USA). The right CeLC was determined according to the rat brain atlas (Paxinos and Watson 2006), and infusion cannulas (RWD Life Science, Shenzhen, China) were implanted into it. The final stereotaxic coordinates in young rats should be 2.2 mm caudal to Bregma, 4.2 mm lateral to midline, and 7.5 mm ventral to dura (Li et al. 2016, 2017a; Butler et al. 2011). Each cannula was fitted with a dummy cannula (RWD Life Science) to reduce the incidence of occlusion. After cannulation, rats were housed individually and allowed to recover for 7 days.

The noncompetitive mGluR1 antagonist, A841720 (SML0818, MilliporeSigma, Darmstadt, Germany) was completely dissolved in dimethyl sulfoxide (DMSO) and then diluted to 20 nmol/ml with saline (El-Kouhen et al. 2006; More et al. 2007; Gao et al. 2016, 2017). DMSO (0.1%) was used as a vehicle control. A841720 was microinjected 6 h after fentanyl administration. Timeline diagrams were constructed for illustrative purposes (see the details in Figs. 2A, 3A, and 4A). An infusion volume of 0.3 μL was pumped into the right CeLC over the course of 2 min at a rate of 0.15 μL/min. The needle was kept in place for 2 min and then gently removed. Success of the injection in the CeLC was histologically verified at the conclusion of the procedures by injecting 0.3 μL of eosin. The data were excluded if the cannula locations were outside the right CeLC.

Measurement of CeLC Glutamate Levels

The glutamate concentrations of right CeLC were determined separately 30 min after the microinjection of A841720 using an ELISA kit (RA21320; Bioswamp, Wuhan, China). Following the manual directions, each sample including seven CeLCs was transferred into a well, incubated with biotinylated detection antibody, and then labeled with horseradish peroxidase-conjugated (HRP) substrate. The optical density at 450 nm was recorded using a spectrophotometer (Varioskan LUX, Thermo Scientific).
This value was proportional to the glutamate concentration.

**Brain Slice Preparation and Electrophysiological Recording**

**Slice Preparation**

The rats were prepared 6 h after the last injection of fentanyl and killed by decapitation. Brain tissue was sectioned into coronal slices containing the amygdala by a vibrating slicer (VT1000S, Leica Nussloch, Germany). The components of the dissection buffer (in mM) were 213 sucrose, 3 KCl, 1 NaH₂PO₄, 0.5 CaCl₂, 5 MgCl₂, 26 NaHCO₃, and 10 glucose. The artificial cerebrospinal fluid (ACSF) contained (in mM) 125 NaCl, 5 KCl, 1.2 NaH₂PO₄, 2.6 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃, and 10 glucose. Both solutions were equilibrated to a pH of 7.3–7.4 and oxygenated with a mixture of 95% O₂ and 5% CO₂. The right hemisphere coronal slices (300 μm) containing CeLC were cut with a vibrating microtome in pre-cooled dissection buffer. The slices were incubated with ACSF at room temperature for 1 h and transferred to the recording chamber with a continuous perfusion of 2 mL/min of ACSF. Only one neuron was recorded from each brain slice.

**Patch-Clamp Recordings**

The whole-cell voltage clamp was used to record mEPSCs in the amygdala slices. The A841720 solution (20 nmol/mL) was added to brain slices 10 min after the baseline recording. The pipette (4–6 MΩ) was made from borosilicate glass capillaries (WPI, Sarasota, FL, USA) filled with the inner solution, containing (in mM): 145 KCl, 5 NaCl, 10 HEPES, 5 EGTA, 4 Mg-ATP, and 0.3 Na₃-GTP. Data were acquired using an EPC-10 amplifier (HEKA, Lambrecht, Germany) and PATCHMASTER software (HEKA), and analyzed using Clampfit (Molecular Devices, Sunnyvale, CA, USA). High (> 2 GΩ) seal resistances and low (< 20 MΩ) series resistances were checked throughout the experiment to ensure high-quality recordings. Picrotoxin (50 μM) and tetrodotoxin (1 μM) were used to block the gamma aminobutyric acid A (GABAₐ) and sodium channels, respectively, during the recording of the mEPSCs at a holding potential of -70 mV 10 min before and after drug administration. The Mini Analysis Program 6.0 (Synaptosoft Inc., Fort Lee, NJ, USA) and Clampfit software were used to analyze the frequency and amplitude of mEPSCs.

**Statistical Analyses**

The effect of A841720 on the change in glutamate concentrations (Fig. 2C), frequency and amplitude of mEPSCs (Fig. 3C, D), and mechanical and heat sensitivities (Fig. 4B, C) were all analyzed by ordinary one-way ANOVA followed by Tukey test. Cumulative distributions of mEPSC inter-event interval and amplitude were analyzed with a Kolmogorov–Smirnov test. All statistical analyses and graphs were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). All data were presented as Mean±SD. Significant differences were defined as P<0.05. All statistical analyses were blinded with respect to treatment groups.

Sample size was estimated after data were collected base on partial eta squared observed in the experiments (Kadam and Bhalerao 2010). The minimal number of animals was determined to obtain a power of 80% at an alpha level of 0.05. It was calculated with G*Power 3.1.

**Results**

**Fentanyl Increased the Expression of mGluR1 in CeLC**

In the previous study, we used OIH models induced by Fentanyl administration (Li et al. 2017b, 2016), and in this study, we continued to use this model. OIH models were prepared by consequent injections of fentanyl. Compared with the control, fentanyl administration exhibited a significant increase initially (an acute analgesic effect), followed by a substantial decrease in the mechanical and thermal nociceptive threshold (hyperalgesia). The mechanical or thermal pain threshold reached the lowest point 5 ~ 6 h after the last injection. The behavior hyperalgesia was reinstated to the normal level on the 5th day. So sampling time point was set at 6 h after the last fentanyl administration.

The effect of fentanyl on mGluR1 protein expression was investigated using Western blot and immunohistochemistry. Western blot analysis showed mGluR1 protein levels in the right CeLC were significantly increased in rats receiving fentanyl compared with those receiving the same volume of saline (Fig. 1B). The experiment was repeated independently and the similar results were obtained. And an increased expression of mGluR1 was observed around the plasma membrane (arrows) in right CeLC in OIH condition (Fig. 1C).
mGluR1 Blockage by A841720 can Decrease Glutamate Concentration in CeLC Under OIH Condition and Normal Condition

The CeLC glutamate concentration was elevated in OIH rats compared to that in normal ones. The microinjection of the mGluR1 antagonist A841720 into the right CeLC inhibited glutamate release in OIH models (Fig. 2), suggesting that the glutamate content associated with mGluR1 may facilitate nociceptive responses of CeLC neurons to fentanyl stimulation. In addition, A841720 can significantly down-regulate the glutamate content under the normal condition as well (Fig. 2).

mGluR1 Blockage can Inhibit the Increased Frequency and Amplitude of mEPSCs in CeLC Slices Induced by Fentanyl Injection

Spontaneous mEPSCs can be used to characterize synaptic transmission changes responsible for inflammatory and neuropathic pain. mEPSCs were recorded at −70 mV from CeLC neurons after fentanyl treatment in the presence or absence of the mGluR1 antagonist A841720. Normalized cumulative distribution analysis showed that A841720 caused an increase in the inter-event interval in fentanyl groups (Fig. 3). Compared to the control group, both the frequency and amplitude of mEPSCs of the right CeLC neurons in the fentanyl-treated group were initially higher which were reversed after A841720 was directly applied to the brain slices (20 nmol/ml in the recording chamber) (**P < 0.001) (Fig. 3). The recording of mGluR1-mediated mEPSCs, reflecting the release of neurotransmitters, revealed that CeLC mGluR1 is critical for the persistence of OIH by influencing synaptic transmission.

Microinjection of A841720 to Right CeLC Alleviated Fentanyl-Induced Hyperalgesia

To further explore the role of CeLC mGluR1 in OIH, the mGluR1 blocker A841720 was microinjected into the right CeLC following fentanyl administration. Mechanical and heat sensitivity were significantly reduced 30 min after A841720 microinjection compared with the vehicle-injected fentanyl group (Fig. 4). Inhibition of mGluR1 by A841720 in CeLC directly alleviated pain. These results implied that the activation of CeLC mGluR1 is critical for the development of hyperalgesia.
Fig. 2 The CeLC glutamate content is significantly increased in the case of fentanyl treatment and is decreased by A841720 microinjection. A Flow diagram of the experimental design. Seven days after CeLC cannulas, rats were given four injections of fentanyl and received A841720 at 6 h after the fentanyl treatment. Rat glutamic acid ELISA analysis was performed 30 min later. B Cannula location. CeLC was histologically verified with eosin. Rat Brain Atlas is from http://labs.gaidi.ca/rat-brain-atlas/ (Paxinos and Watson 2006). C Representative schematic illustration of the location for A841720 application into the CeLC. D The CeLC glutamate content measured by ELISA. Data were presented as mean ± SD. Each sample was collected from 7 rats. Number means biological replicates, n = (7, 6, 6, 6). One-way ANOVA followed by the Tukey Post hoc, F (3, 21) = 40.03, p < 0.0001. *p < 0.05, **p < 0.01, ***p < 0.001, exact p value was marked if it > 0.001. Total sample size = 8. The data are representatives of three independent experiments.
Discussion

Genders have been shown to influence response to hyperalgesia for reasons unknown. Interestingly, several male-specific genes have been implicated in the regulation of peripheral and spinal nociceptive processing during OIH (unpublished data, manuscript in preparation). For research continuity, our team attempted to delineate the molecular basis underlying male sensitivity to opioid antinociception. The aim was to better understand the distinct mechanisms of hyperalgesia between gender and therapeutic consequences.

And opioid administration is one of the most common methods of managing moderate to severe pain in patients of all ages. Remifentanil infusion has a strong association with OIH in patients undergoing adolescent idiopathic scoliosis repair (Kars et al. 2019). The present finding demonstrates that the use of an mGluR1 antagonist in adolescents may increase the pain threshold during OIH in an animal model. Further studies will be informative to elucidate the clinical efficacy and significance of this approach.

The amygdala is located in the medial temporal lobe and is comprised of the lateral-basolateral complex (LA/BLA), intercalated cell mass (ITC), and central nucleus (CeA). The LA/BLA neurons receive polymodal sensory signals from the cortical and thalamic regions. ITC cells receive excitatory inputs from the medial prefrontal cortex (mPFC) (Thompson and Neugebauer 2017; Neugebauer et al. 2020). Nociceptive information can be delivered to the CeA, which serves as the major output nucleus, and glutamatergic synaptic responses are involved in pain transmission (Tully et al. 2007; Neugebauer et al. 2004). CeLC, defined as the nociceptive amygdala, has been identified as a target of the spino-parabrachial-amygdaloid tract. The CeLC receives effect-related inputs from the LA/BLA and contributes critically to pain modulation via projections to the brainstem (Gauriau and Bernard 2002; Carrasquillo and Gereau 2007; Ji and Neugebauer 2008). The CeLC may serve as a central site for the modulation of pain perception in the OIH process.

Interestingly, there is evidence to suggest pain-related hemispheric lateralization in the amygdala. A predominant role of the right, but not left, amygdala has been observed in responses to inflammatory and neuropathic pain stimuli (Thompson and Neugebauer 2017). ERK activation occurs in the right CeA and plays a dominant role in inflammation-induced peripheral hypersensitivity (Carrasquillo and Gereau 2008). Engagement of opioid receptor signaling in the right CeA contributes to pain responses produced by morphine (Nation et al. 2018; Navratilova et al. 2020). An inhibitor of protein kinase A decreased the activity of right CeLC neurons after the induction of arthritis (Ji and Neugebauer 2009). This agrees with the previous finding that the modulation of pain by mGluR1 activation is functionally lateralized to the right hemisphere.

Although the mechanism of OIH is unclear, it is conceivable that the activation of the glutamatergic system is involved. Glutamate is a major excitatory neurotransmitter in the mammalian nervous system. The compound is important in pain pathways, where it participates in transmitting nociceptive signals from the peripheral nocceptors to the central nervous system. There are two types of glutamate receptors: the ionotropic type (iGluRs) and the metabotropic type (mGluRs). To date, eight subtypes of mGluR have been identified in the nervous system. They include group I mGluR1/mGluR5, group II mGluR2/mGluR3, group III mGluR4, and mGluR6-8 (Palazzo et al. 2019). Group I mGluR-induced nociceptive processing in amygdala neurons participates in emotional-affective pain modulation through a mechanism that involves reactive oxygen species (Ji and Neugebauer 2010). There are the enhanced synaptic transmission at the nociceptive parabrachial (PB) → CeA synapse and the increased excitability which results in the altered output from the CeA in the arthritis model. The enhanced synaptic transmission and altered excitability in the CeA neurons were accompanied by the upregulation of mGluR1 and mGluR5 (Neugebauer et al. 2003). Blocking mGluR1 can reverse pain-related alterations in excitatory and inhibitory transmission in CeLC neurons from arthritic rats (Ren and Neugebauer 2010). Rescue of impaired endocannabinoid-dependent mGluR5 facilitation can inhibit pain behaviors in arthritic rats (Kiritoshi et al. 2016). Activation of group II mGluR2 and mGluR3 subtypes can decrease neurotransmitter release in the synaptic cleft and inhibit spinal nociceptive processing in arthritis pain model (Mazzitelli and Neugebauer 2019; Mazzitelli et al. 2018). Recent evidence revealed that group III mGluR7 (pain enhancing) acts as a gatekeeper to regulate the flow of information to CeLC neurons under normal circumstances, but not during pain.
mGluR8 (pain inhibiting) inhibits excitatory transmission in the CeLC in the state of pain (Ren et al. 2011).

In this study, the potential role of mGluR1 in the OIH process was explored. A proportion of the cellular glutamate pool was stored in the synaptic gap, and the protein expression of mGluR1 was increased after fentanyl exposure. We noticed that application of A841720 reduced glutamate content both under the normal condition and in OIH, suggesting unknown influences on normal mGluR1-mediated function in the amygdala when mGluR1 antagonist was used in the treatment of OIH. The potential side effects should be closely investigated.

Presynaptic function was measured by mEPSC frequency, and postsynaptic responsiveness was determined by mEPSC amplitude. CeLC cells displayed significantly increased mEPSC frequency and amplitude in the OIH process, which could be restored by blockade of mGluR1 using A841720. It is not common that mGluR1 blockade reduces both the frequency and amplitude of mEPSCs. Our data are consistent with the previous study. The frequency and amplitude of mEPSCs were significantly increased in CeLC neurons in OIH rats and could be decreased by a CaMKIIα inhibitor KN93, suggesting that inhibition of CeLC CaMKIIα was able to attenuate fentanyl-induced hyperalgesia through pre- and postsynaptic mechanisms of action (Li et al. 2016). There are also some similar cases. The mEPSC frequency and amplitude recorded in anterior cingulate cortex neurons were obviously increased after peripheral nerve ligation (Xu et al. 2008). Tumor necrosis factor-alpha significantly increased mEPSC frequency and amplitude in the immature superficial dorsal horn neurons after the spared nerve injury (Li et al. 2009). Our findings indicated that CeLC mGluR1 may upregulate presynaptic neurotransmitter release and postsynaptic responsiveness as well to induce behavioral hyperalgesia.

The collective findings indicate the involvement of mGluR1 in the fentanyl-induced behavioral hyperalgesia, which may be related to the enhanced synaptic transmission in the right CeLC. Further prospective data are needed, which may help patients recover from OIH.
Fig. 4 Microinjection of A841720 into CeLC blocks hyperalgesia induced by fentanyl. A flow diagram of the experimental design. Seven days after CeLC cannulas, rats received a total of four doses of fentanyl and then, respectively, A841720 at 6 h after the fentanyl treatment. Nociceptive tests were performed 30 min later. The paw withdraw threshold B and the paw withdraw latency C were determined 30 min after A841720 microinjection (20 nmol/ml) into CeLC.

Data were presented as mean ± SD. One-way ANOVA followed by Tukey Post hoc. n = (7, 9, 5, 5), F (3, 22) = 11.71, p < 0.0001(B), n = (6, 9, 6, 6), F (3, 23) = 20.55, p < 0.0001(C). *p < 0.05, **p < 0.01, ***p < 0.001, exact p value was marked if it > 0.001. Total sample size = 12 (B, C). Number means biological replicates. The data are representatives of three independent experiments.
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