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ACC Plasticity Maintains Masseter Hyperalgesia Caused by Occlusal Interference

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Abstract

Acute occlusal interference following improper occlusal alteration in dental practice can induce chronic masticatory muscle pain. The underlying mechanism has not been clarified. Synaptic plasticity in the anterior cingulate cortex (ACC) plays a key role in the chronic pain state. This study investigated the role of synaptic plasticity in the ACC in acute occlusal interference–induced chronic masticatory muscle pain. A rat model of experimental occlusal interference (EOI) was established. In vivo local field potential (LFP) recording was conducted to evaluate the change of synaptic strength and plasticity from the medial thalamus (MT) to the ACC after EOI application. The effects of microdialysis of antagonists of glutamate receptors into the ACC on synaptic transmission from the MT to the ACC were examined. Furthermore, the influence of inhibiting glutamate receptors in the ACC on EOI-induced mechanical hyperalgesia in the masseter muscles of rats was investigated. The amplitude of LFP in the ACC evoked by MT stimulation was significantly potentiated since 14 d of EOI application. Long-term potentiation of LFP in the ACC was reliably induced by theta burst stimulation to the MT in control rats but was occluded in 14-d EOI rats. Microdialysis of AMPA/kainate receptor antagonist CNQX into the ACC attenuated LFP in the ACC evoked by stimulating the MT in control and EOI rats. Administration of NMDA receptor subunit NR2B antagonist Ro 25-6981 into the ACC significantly alleviated the potentiation of MT stimulation-evoked LFP in the ACC of EOI rats without affecting that in control rats. EOI-induced hyperalgesia in the bilateral masseter muscles of rats was dose-dependently relieved after microdialysis of Ro 25-6981 into ACC. These findings provide direct evidence that prolonged acute occlusal interference potentiates synaptic transmission in the ACC, which in turn mediates chronic masticatory muscle pain.

Keywords: occlusal interference, chronic masticatory muscle pain, anterior cingulate cortex, synaptic transmission, long term potentiation, glutamate receptor

Introduction

Patients usually suffer from discomfort in the orofacial region, especially within the masticatory muscle area, following acute occlusal interference induced by inappropriate prosthodontic treatment. Sometimes this condition persists, and chronic masticatory muscle pain (CMMP) develops. The underlying mechanism has not been clarified, and the available clinical strategy is rare especially when the acute pain has turned to chronicity. We previously developed a rat model of experimental occlusal interference (EOI) by applying an artificial crown on the first molar to produce premature occlusal contact in posterior dentition, and we demonstrated that EOI directly leads to persistent and irreversible hyperalgesia in bilateral masseter muscles (MMs) in rats (Cao et al. 2009). Furthermore, we explored the neural mechanisms underlying this condition and revealed that EOI induces neural plastic changes in trigeminal ganglion (upregulated expression of nociceptive receptors, such as TRPV1, ASIC3, and P2X; Xu et al. 2016) and spinal trigeminal subnucleus caudalis (hypersensitivity of nociceptive neurons and activation of glial cells; Cao et al. 2013). These pathologic changes in the lower-level nuclei in the trigeminal nociceptive pathway are indicative of amplified nociceptive inputs that will probably be projected upward and further evoke plastic changes in the higher-level neural circuitry, which may account for the sustained behavioral sensitivity. Cerebral cortex is highly plastic and plays vital roles in the chronic pain state (Zhuo 2008). However, whether EOI-induced sensitization of the lower-level nociceptive neural circuitry leads to cortical plastic change has not been determined.

The anterior cingulate cortex (ACC) is the frontal part of the cingulate cortex. Extensive research, including human and animal studies, demonstrated that ACC plays key roles in pain processing, especially for the affective-motivational aspects of pain (Sikes and Vogt 1992; Rainville et al. 1997; Wong et al. 1997; Lenz et al. 1998; Johansen et al. 2001). Furthermore, by

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retrograde transsynaptic labeling with rabies virus, ascending multisynaptic pathways from the trigeminal ganglion, spinal trigeminal subnucleus caudalis, and medial thalamus (MT) to the ACC were identified, providing anatomic evidence that ACC receives sensory inputs from orofacial areas via the relay of the MT (Iwata et al. 2011). Activation of the ACC was observed among patients with atypical orofacial pain in a functional magnetic resonance imaging study (Jiang et al. 2006). In addition, the role of the ACC was identified in a rat model of acute orofacial pain induced by experimental tooth movement (Xin et al. 2014). Long-term potentiation (LTP) is an activity-dependent plasticity involving consistent enhancement in synaptic potency, which serves vital cellular mechanisms underlying the shaping of neural circuitries and the storing of information for a long-lasting period. Numbers of studies confirmed that plastic changes in synaptic substrates exist in the ACC by in vitro (LJ Wu et al. 2005; Xu et al. 2008; Li et al. 2010; Bie et al. 2011; Koga et al. 2015) or in vivo (Wei and Zhuo 2001; MF Wu et al. 2005; Li et al. 2013; Wang et al. 2015) approaches, indicating that synaptic plasticity may act as one of the fundamental neuronal mechanisms for highly adaptable brain functions, such as learning and memory, as well as the formation of chronic pain. Heretofore, the role of synaptic plasticity in the ACC in EOI-induced CMMP has not been clarified.

In this study, we explore the role of synaptic plasticity in the ACC in EOI-induced CMMP. Our hypotheses are that EOI can enhance synaptic transmission in the ACC and that the reverse of synaptic potentiation in the ACC can relieve masticatory muscle pain evoked by EOI. In vivo local field potential (LFP) recording, pharmacologic microdialysis, and nociceptive behavioral testing were carried out to verify these hypotheses.

Materials and Methods

Male Sprague-Dawley rats weighing between 220 and 250 g were used (n = 66). Rats were housed in a temperature- and light-controlled laboratory animal room (25 °C, 12-h diurnal cycle) with food and water ad libitum. The study design was authorized by the Animal Care and Use Committee of the Peking University Health Science Center. This study was conducted in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) for preclinical animal studies.

Experimental Occlusal Interference

The procedures of establishing an EOI model were described previously (Cao et al. 2009). In brief, the impression of the upper dentition of the rat was made with silicone rubber in a prefabricated individual tray under isoflurane anesthesia. The conventional dewaxing casting technique was performed to manufacture a 0.4-mm-thick metal crown of the upper-right first molar. For the EOI group, rats were injected intraperitoneally with pentobarbital sodium (50 mg/kg), and crowns were cemented intraorally with dental resin cement (Panavia F; Kuraray). For the control group, the same procedures were conducted without an actual EOI.

In Vivo LFP Recording in the ACC

To test the change of synaptic strength from the MT to the ACC after EOI application, in vivo LFP recordings were performed in control rats and rats with EOI for 7, 14, and 21 d (n = 6 each). Detailed procedures are described in the Appendix Methods. The synaptic strength from the MT to the ACC was evaluated through plotting the input-output curves by recording the LFP amplitudes in the ACC responding to incremental MT stimulation (square wave, from 100 to 1000 μA in increments of 100 μA, 0.2-ms duration, 10-s intervals). The mean of the LFP amplitudes, as recorded 10 times at each current intensity level, was used to generate the input-output curves.

The plasticity of the MT-ACC synapse was evaluated via induction of LTP by applying theta burst stimulation (TBS) to the MT (3 sets of 10 trains at 0.1 Hz, with each train containing 10 bursts at 5 Hz and each burst including 5 pulses at 100 Hz). This stimulus paradigm closely simulates the activity of ACC neurons and was proven to be able to artificially induce long-lasting potentiation in ACC synapses (Zhuo 2007). To evaluate the plasticity of the MT-ACC synapse after EOI application, we applied TBS to the MT of control rats and 14-d EOI rats (n = 5 each). LFP in the ACC was recorded 15 min before TBS and lasted 1 h after TBS (current intensity was set with the value that evoked 50% of the maximum LFP amplitude based on input-output curves, 0.2-ms duration, 10-s intervals). The percentage change of LFP amplitude after TBS was calculated as compared with the baseline level.

When the recording was finished, a small electrical lesion (50 μA for 30 s) was made at the microelectrode tip. The brain was acquired and fixed in 4% paraformaldehyde, then sliced at 50 μm with a microtome (CM1950; Leica) to identify the locations of microelectrode tips related to the targeted area. A standard brain atlas of the rat (Paxinos and Watson 2009) was employed to reconstruct the stimulating and recording sites.

Effects of Antagonists of Glutamate Receptors on MT-ACC Synaptic Transmission

Glutamate receptors are responsible for excitatory synaptic transmission in the ACC. To test the role of glutamate receptors involved in synaptic transmission from the MT to the ACC after EOI application, in vivo LFP recording in the ACC was performed simultaneously with microdialysis of AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or NMDA receptor 2B subunit (NR2B) inhibitor Ro 25-6981 into the ACC in control rats (n = 5) and 14-d EOI rats (n = 6). Microdialysis technique is able to deliver desired drugs into localized area without changing tissue volume or pressure, and avoid intervening electrophysiologic recording (West et al. 2002). These antagonists were dissolved in artificial cerebrospinal fluid containing 147mM NaCl, 3.0mM KCl, 0.8mM $MgCl_2$, 1.2mM $CaCl_2$, 2.0mM NaH_2PO_4, and 2.0mM Na_2HPO_4

(CNQX: 1 mM, Ro 25-6981: 0.5 mM). The same dosage was used in other research (Wang et al. 2015). Detailed procedures are described in the Appendix Methods.

Effect of Microdialysis of NR2B Antagonist into the ACC on Mechanical Sensitivity of MM

To verify the role of ACC synaptic potentiation in EOI-induced MM hyperalgesia, we detected the change of head withdrawal thresholds (HWTs) of bilateral MMs in control and 14-d EOI rats (n = 5 each) after microdialysis of NR2B antagonist Ro 25-6981 (0.1, 0.5 mM) into the ACC. Detailed procedures are described in the Appendix Methods.

Statistics

Statistical analyses were performed with SPSS 20.0 (IBM). All data were expressed as mean ± SEM. Results of 1) LFP in the ACC of different groups responding to incremental MT stimulation, 2) LFP in the ACC of control and EOI rats before and after application of TBS to the MT, and 3) HWTs of bilateral MMs of control and EOI groups at different time points were compared by repeated measures 2-way analysis of variance, followed by the Bonferroni post hoc test. Multivariate analysis of variance was used for comparison among groups at each time point where appropriate. LFP in the ACC of control and EOI rats before and after microdialysis was compared with paired t tests. Differences were considered significant at $P < 0.05$.

Results

Enhancement of LFP in the ACC Evoked by Stimulating the MT in EOI Rats

Increased MT stimulation steadily elicited gradually enhanced LFP amplitudes in the ACC of control and EOI rats. Representative tracings of LFP in the ACC are shown in Figure 1A. Rats with EOI for 14 and 21 d exhibited dramatically enhanced LFP in the ACC responding to MT stimulation, as compared with the control rats and 7-d EOI rats ($P < 0.01$; Fig. 1C, D). No difference was observed between 14- and 21-d EOI rats, as well as between the control rats and 7-d EOI rats ($P > 0.05$; Fig. 1C, D). The results suggest that MT-ACC synaptic transmission was potentiated since 14 d after EOI application. The histologic sections confirmed the stimulating sites located in the MT (including the central lateral, mediodorsal lateral, and paracentral regions) and the recording site located in the ACC (cingulate cortex, area 1; Fig. 1B).

Blockage of TBS-Induced LTP of LFP in the ACC in EOI Rats

In control rats, TBS to the MT reliably elicited a marked potentiation of LFP in the ACC in response to MT stimulation for at least 1 h (129.9% ± 0.675% over the baseline level, $P < 0.01$; Fig. 2). However, in 14-d EOI rats, TBS to the MT failed to evoke LTP of MT stimulation-evoked LFP in the ACC (101.4% ± 0.269% over the baseline value, $P > 0.05$; Fig. 2). The results suggest that the capability of MT-ACC synaptic plasticity is suppressed in 14-d EOI rats.

Effects of Antagonists of Glutamate Receptors on MT Stimulation-Evoked LFP in the ACC of Control and EOI Rats

Application of CNQX into the ACC significantly decreased LFP in the ACC responding to MT stimulation in control and EOI rats ($P < 0.05$; Fig. 3). Microdialysis of Ro 25-6981 into the ACC significantly suppressed LFP in the ACC responding to MT stimulation in EOI rats ($P < 0.05$) but exhibited no influence on that in control rats (Fig. 4). These observations indicate that AMPA/kainate receptors mediate MT-ACC synaptic transmission under normal and EOI conditions, whereas NR2B exclusively plays a role in synaptic transmission from the MT to the ACC under the EOI condition rather than the normal state.

Effect of Suppression of the ACC Synaptic Potentiation on EOI-Induced MM Hyperalgesia in Rats

In control rats, as compared with the baseline level, there was no significant change of HWT of bilateral MMs 5 d after cannula implantation, as well as after microdialysis of artificial cerebrospinal fluid or Ro 25-6981 into the ACC (Fig. 5). In EOI rats, the HWT of bilateral MMs significantly decreased at 14 d after EOI application as compared with the baseline level (right MM: baseline, 164.0 ± 2.2 g; 14-d EOI, 87.2 ± 2.4 g; left MM: baseline, 160.1 ± 3.1 g; 14 d EOI, 87.5 ± 1.4 g, $P < 0.01$; Fig. 5). No significant difference of HWT was observed before and after microdialysis of artificial cerebrospinal fluid into the ACC. However, application of Ro 25-6981 into the ACC partially increased the HWT of bilateral MMs in a dose-dependent way (right MM: 0.1 mM, 110.8 ± 3.1 g; 0.5 mM, 131.6 ± 3.9 g; left MM: 0.1 mM, 108.9 ± 2.8 g; 0.5 mM, 132.5 ± 4.8 g, $P < 0.01$; Fig. 5). These data demonstrate that suppression of synaptic potentiation in the ACC can relieve EOI-induced MM hyperalgesia.

Discussion

The ACC was shown to be involved in various pathologic pain models, such as inflammation (LJ Wu et al. 2005; Bie et al. 2011), peripheral nerve injury (Li et al. 2010; Chen et al. 2014), digit amputation (Wei and Zhuo 2001; MF Wu et al. 2005), and visceral hypersensitivity (Fan et al. 2009; Wang et al. 2015). In this study, we performed in vivo LFP recording to explore the role of the ACC in EOI-induced CMMP. LFP is the summed potential across the extracellular space of multiple neurons within a local volume, and it fluctuates in response to neural activity. Evoked LFP by afferent stimulation mainly reflects

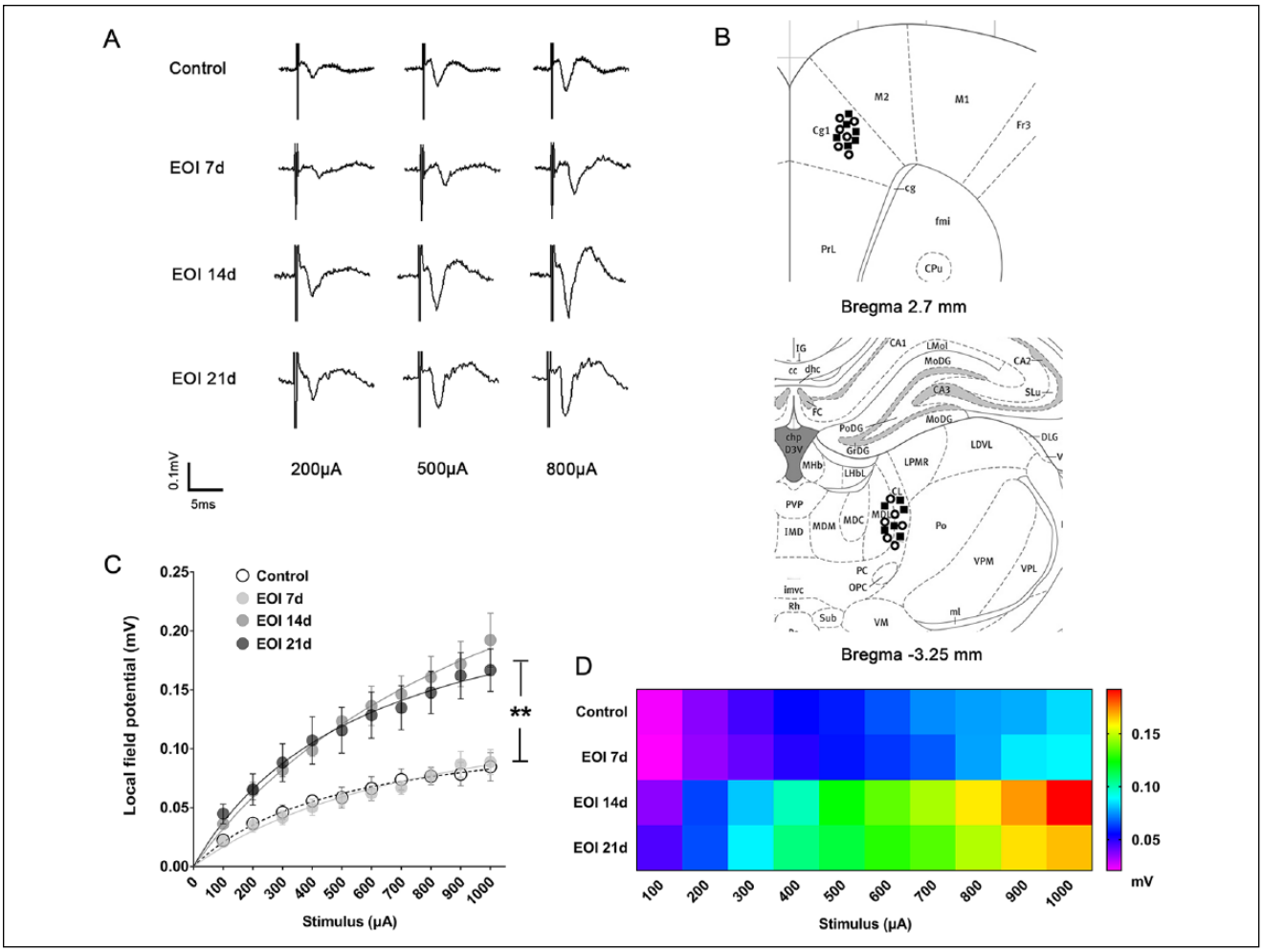

Figure 1. In vivo local field potential (LFP) recordings in the anterior cingulate cortex (ACC) of control rats and rats with experimental occlusal interference (EOI) for 7, 14, and 21 d. (**A**) Representative tracings of LFP in the ACC responding to incremental stimuli to the medial thalamus (MT). (**B**) Reconstructed stimulating sites in the MT and recording sites in the ACC of the control rats (circles) and 14-d EOI rats (black squares) in a standard rat brain atlas. Cg1, cingulate cortex, area 1; CL, central lateral; MDL, mediodorsal lateral; PC, paracentral; PrL, prelimbic cortex. (**C**) Input-output curves of synaptic transmission from the MT to the ACC, as generated from the LFP recordings in the ACC. Note that the left shift of input-output curves occurred among 14- and 21-d EOI rats as compared with control rats and 7-d EOI rats. Values are presented as mean ± SEM. $^{**}P < 0.01$. Multivariate analysis of variance. (**D**) Heat map indicates the mean LFP amplitudes in the ACC responding to graded increased MT stimuli in the control rats as well as EOI rats (7, 14, and 21 d). $n = 6$ in each group.

the globally synchronous synaptic activity in grouped postsynaptic neurons. As compared with intracellular or whole-cell recordings, LFP recording is more readily obtained and stably maintained for a longer duration. Although it has inherent limitations—such as being unable to clarify the components of the overall effect or identify the synaptic properties at the single-neuron level—LFP recording is widely employed to provide information for the average synaptic strength and long-term synaptic plasticity in various brain regions (Zhou and Poon 2000). In vivo LFP recording in the ACC was first adopted by researchers exploring digit amputation–induced potentiation of the sensory response (Wei and Zhuo 2001) and the feature of synaptic projection from the MT to the ACC (Kung and Shyu 2002). By using in vivo LFP recording in the ACC as a quantitative measurement of synaptic efficacy, Wang et al. (2015) recently exhibited facilitation of synaptic transmission in the ACC in viscerally hypersensitive rats. Here, we observed significant enhancement of synaptic transmission from the MT to the ACC 14 d after EOI application. Our previous data reflected that MM hyperalgesia peaked at 5 to 7 d following EOI and was maintained for >28 d. Thus, synaptic potentiation in the ACC occurred at the late phase of EOI-induced behavioral sensitization, suggesting its potential role in the maintenance of hyperalgesia. Noteworthy, the excitatory postsynaptic potentials of ACC neurons were shown to increase at 3 d after digit amputation and 7 d after Complete Freund's Adjuvant injection into the hind paw of rats (Li et al. 2013). Increased synaptic transmission in the ACC was triggered at 7 to 14 d following ligation of the common peroneal nerve in mice (Xu et al. 2008). Therefore, it is likely that EOI may act more mildly than the

aforementioned noxious stimuli and require more time to trigger synaptic plastic change in the ACC. In addition, TBS-evoked LTP of LFP in the ACC was reliably observed in control rats but not in 14-d EOI rats, suggesting that EOI-evoked enhancement of synaptic transmission and TBS-induced LTP expression in the ACC probably share common intracellular signaling pathways so that TBS-induced LTP will be occluded if the system has already been initiated and the maximal potentiation has already been achieved following EOI application. In line with these observations, the similar blocking effect on LTP at ACC synapses was demonstrated in several pathologic pain conditions, such as visceral hypersensitization (Wang et al. 2015), chronic inflammation (Koga et al. 2015), and nerve injury (Li et al. 2010).

Glutamate is the major excitatory neurotransmitter in the ACC region. The roles of glutamate receptors in basal synaptic transmission and plasticity in the ACC have been well elucidated. In detail, activation of the NMDA receptor subunits NR2B and NR2A is vital for induction of LTP in the ACC (Zhao et al. 2005), and AMPA receptor subunit GluA1 is crucial for expression of LTP in the ACC (Toyoda et al. 2009). GluA1 in the ACC was proven to be involved in chronic inflammatory pain (Bie et al. 2011) and neuropathic pain (Chen et al. 2014), while NR2B in the ACC was shown to play key roles in chronic inflammatory pain (LJ Wu et al. 2005), neuropathic pain (Yang et al. 2015), and visceral hypersensitization (Fan et al. 2009). Moreover, NR2B is located predominantly in pain-related neural structures, such as the dorsal spinal horn, thalamus, and cortex, making it a promising candidate as a target of analgesic drugs with less adverse effects (Wu and Zhuo 2009). We previously showed that systemic application of NMDA antagonist MK801 can reverse EOI-induced bilateral MM hyperalgesia (Cao et al. 2009). In the present study,

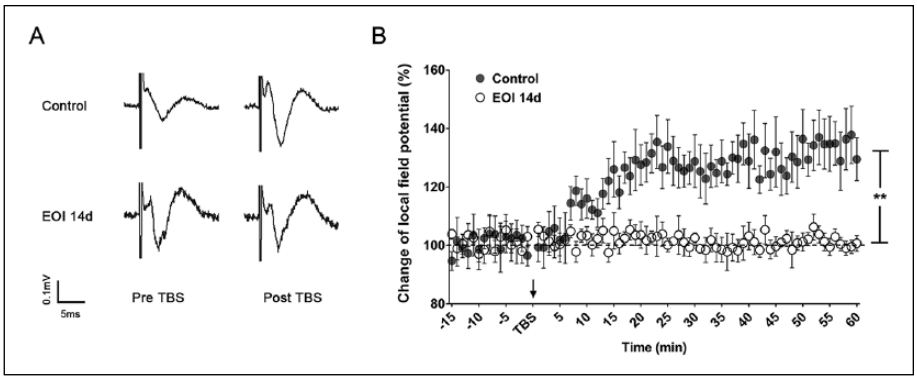

Figure 2. TBS-induced long-term potentiation (LTP) of local field potential (LFP) in the anterior cingulate cortex (ACC) in control and 14-d EOI rats. (**A**) Representative tracings of LFP in the ACC before and after TBS application to the medial thalamus (MT). (**B**) Change of LFP in the ACC after TBS to the MT in control and 14-d EOI rats. Note that LTP of LFP in the ACC was induced by TBS to the MT in control rats. However, TBS-induced LTP of LFP in the ACC was blocked in 14-d EOI rats. Values are presented as mean ± SEM. $n = 5$ in each group. $^{**}P < 0.01$. Repeated measures 2-way analysis of variance. EOI, experimental occlusal interference; TBS, theta burst stimulation.

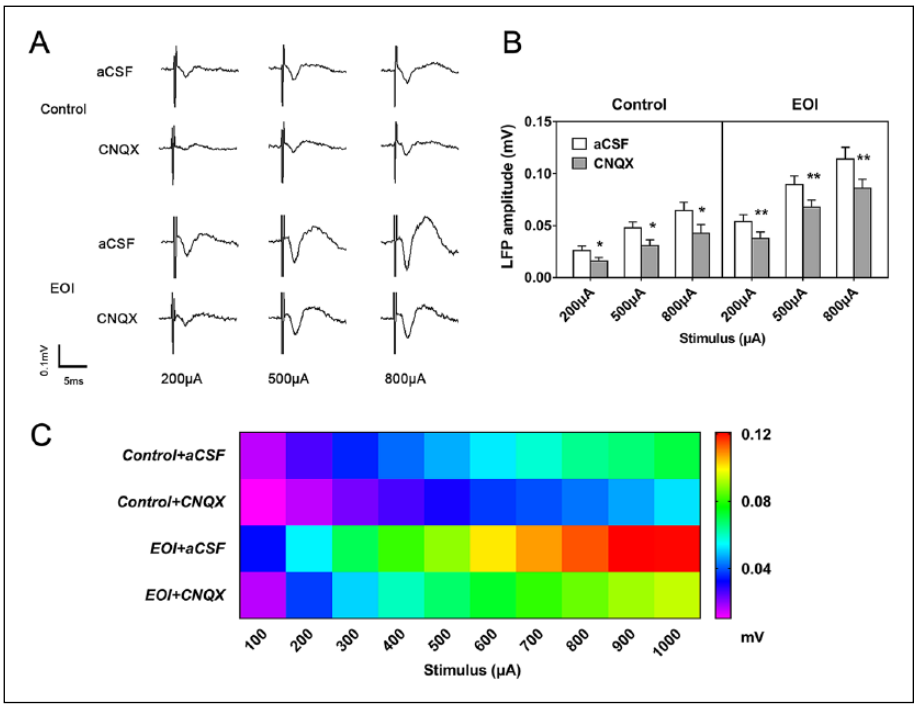

Figure 3. Effect of microdialysis of AMPA/kainate receptor inhibitor CNQX into the anterior cingulate cortex (ACC) on medial thalamus (MT)–ACC synaptic transmission in control ($n = 5$) and 14-d EOI ($n = 6$) rats. (**A**) Representative tracings of LFP in the ACC responding to different MT stimuli in control and 14-d EOI rats after microdialysis of aCSF or CNQX. (**B**) LFP amplitudes in the ACC responding to MT stimuli (200, 500, or 800 μA) were significantly decreased after CNQX administration as compared with that after application of aCSF in control and 14-d EOI rats. Values are presented as mean ± SEM. $^{*}P < 0.05$. $^{**}P < 0.01$. Paired t test. (**C**) Heat map indicates the mean LFP amplitudes in the ACC responding to graded increased MT stimuli in control and 14-d EOI rats after microdialysis of aCSF or CNQX. aCSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EOI, experimental occlusal interference; LFP, local field potential.

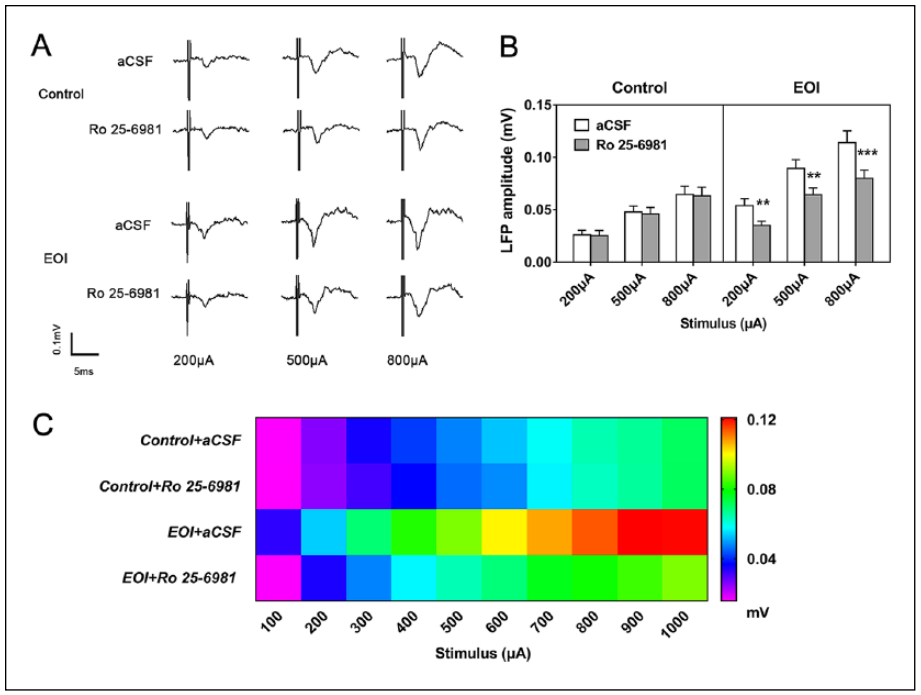

Figure 4. Effect of microdialysis of NR2B inhibitor Ro 25-6981 into the anterior cingulate cortex (ACC) on medial thalamus (MT)–ACC synaptic transmission in control ($n = 5$) and 14-d EOI ($n = 6$) rats. (**A**) Representative tracings of LFP in the ACC responding to different MT stimuli in control and 14-d EOI rats after microdialysis of aCSF or Ro 25-6981. (**B**) LFP amplitudes in the ACC responding to MT stimuli (200, 500, or 800 μA) were significantly decreased after Ro 25-6981 administration as compared with that after application of aCSF in 14-d EOI rats. In control rats, no change of LFP amplitudes in the ACC after microdialysis of Ro 25-6981 was observed. Values are presented as mean ± SEM. $^{**}P < 0.01$. $^{***}P < 0.001$. Paired *t* test. (**C**) Heat map indicates the mean LFP amplitudes in the ACC responding to graded increased MT stimuli in control and 14-d EOI rats after microdialysis of aCSF or Ro 25-6981. aCSF, artificial cerebrospinal fluid; EOI, experimental occlusal interference; LFP, local field potential.

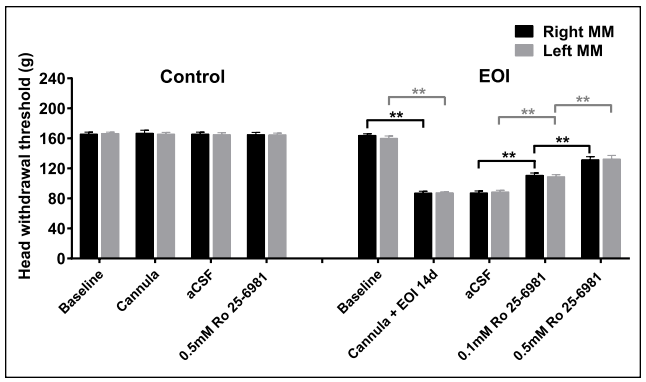

Figure 5. Effect of microdialysis of NR2B inhibitor Ro 25-6981 into the anterior cingulate cortex (ACC) on EOI-induced hyperalgesia in bilateral MMs. In control rats, no difference in head withdrawal threshold of bilateral MMs was detected after cannula implantation as well as after administration of aCSF or Ro 25-6981 as compared with the baseline level ($n = 5$). In EOI rats, there was significant decrease in head withdrawal threshold in bilateral MMs at 14 d after EOI as compared with baseline. Microdialysis of Ro 25-6981, not aCSF, into the ACC dose dependently alleviated EOI-induced hyperalgesia in the bilateral MMs of rats ($n = 5$). Values are presented as mean ± SEM. $^{**}P < 0.01$. Repeated measures 2-way analysis of variance, followed by Bonferroni post hoc test. aCSF, artificial cerebrospinal fluid; EOI, experimental occlusal interference; MM, masseter muscle.

inhibiting AMPA/kainate receptors in the ACC significantly decreased MT-ACC synaptic potency in control and EOI rats, suggesting that AMPA/kainate receptors in the ACC mediate basal synaptic transmission. However, antagonizing NR2B in the ACC did not affect MT-ACC synaptic efficacy in control rats but significantly suppressed synaptic transmission in the ACC of EOI rats, indicating that NR2B in the ACC is involved in the potentiation of MT-ACC synaptic strength under the EOI condition rather than basal synaptic transmission in the normal state. These results suggest that NR2B in the ACC can be exclusively targeted for intervening EOI-induced MM hyperalgesia, without affecting normal synaptic transmission in ACC circuitry. Given these findings, we further tested whether inhibition of synaptic potentiation in the ACC by administration with NR2B inhibitor into the ACC influences EOI-induced MM hyperalgesia. Blocking NR2B in bilateral ACC dose-dependently alleviated MM hyperalgesia in EOI rats, without affecting the mechanical sensitivity of MMs in control rats. These results provide direct evidence for the role of NR2B-mediated enhancement of synaptic transmission in the ACC in EOI-induced CMMP. The activation of NR2B was shown to evoke an increase in Ca^{2+} influx and the initiation of postsynaptic calcium-sensitive signal cascades that play essential roles in the facilitation of excitatory synaptic transmission in the ACC (Bliss et al. 2016). For the limitation of this study, we did not exhibit the specific downstream molecular interactions following the activation of NR2B in the ACC under the EOI condition. Detailed studies are being conducted to clarify the relevant pathologic process on the molecular level.

Although no corroborative clinical evidence confirms the association between congenital malocclusion or occlusal disharmony and CMMP, acute acquired occlusal interference was demonstrated to be one of the potential risk factors for this condition (Le Bell et al. 2002). Our results reflect that long-term EOI can directly induce plastic change in the ACC, which in turn mediates CMMP. These findings highlight the clinical significance of removing iatrogenic occlusal interference as early as possible when dealing with patients with improper acute occlusal alteration, to prevent the pathologic change in pain-related cortical circuitry. If the pain condition

has already become chronic, erasing the enhanced synaptic efficacy in ACC circuitry may serve as a new treatment strategy. NR2B and its downstream signal pathway in the ACC may act as the potential targets to intervene against this stubborn condition.

Furthermore, the ACC is a multifunctional cortical region, and the essential role of the ACC in emotional disorders such as anxiety (Kim et al. 2011) and depression (Bissiere et al. 2006) was previously demonstrated. Specifically, the coexistence of pre- and postsynaptic LTP in the ACC was shown to be involved in the interaction between anxiety and chronic pain (Koga et al. 2015). Occlusal alteration, such as unilateral molar loss, was shown to influence the decision making of rats by disrupting the interaction between the ACC and basolateral amygdala (Xu et al. 2015) and lead to anxiety and depression-like behaviors (unpublished data). Clinically, patients with CMMP are frequently accompanied with emotional disorders, significantly aggravating the difficulty in dealing with this clinical dilemma. Exploration of the role of the ACC in the interaction of chronic orofacial pain and emotional disorders may bring more explicit insights toward the underlying neural mechanisms and provide more effective intervention strategies.

Author Contributions

X.X. Xu, contributed to conception, design, data acquisition and interpretation, drafted the manuscript; Y. Cao, contributed to data analysis and interpretation, critically revised the manuscript; S.Y. Mo, Y. Liu, contributed to data acquisition and analysis; Q.F. Xie, contributed to design, data interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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