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METABOTROPIC GLUTAMATE RECEPTORS SUBTYPE 5 ARE NECESSARY FOR THE ENHANCEMENT OF AUDITORY EVOKED POTENTIALS IN THE LATERAL NUCLEUS OF THE AMYGDALA BY TETANIC STIMULATION OF THE AUDITORY THALAMUS

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Abstract—The lateral nucleus of the amygdala (LA) receives axonal projections from the auditory thalamus, the medial geniculate nucleus (MGN), and mediates auditory fear conditioning. Tetanic electrical stimulation of the MGN can induce long-term potentiation of acoustically-evoked responses (AEPs) recorded in the LA of anesthetized rats. The present study investigated the temporal development of tetanus-induced AEP potentiation recorded in the LA of anesthetized rats during the recording time up to 120 min after tetanization. In addition, the present study investigated whether the artificially-induced AEP potentiation is mediated by the metabotropic glutamate receptors subtype 5 (mGluR5). The results show that AEPs recorded in the LA to a broadband-noise burst were significantly enhanced immediately after tetanic but not low-frequency stimulation of the MGN. The AEP potentiation was well retained up to 120 min after tetanization. High-dose (1.5 μg/4 μl) microinjection of the selective antagonist of mGluR5, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), into the ipsilateral lateral ventricle 30 min before tetanization completely blocked the AEP potentiation without affecting the baseline AEP. Low-dose (0.5 μg/4 μl) microinjection partially suppressed the AEP potentiation. When the high-dose MPEP was injected 40 min after tetanization, the AEP potentiation was not affected. These results indicate that in anesthetized rats mGluR5 receptors are necessary for the induction or early maintenance (40 min) of AEP potentiation in the LA by tetanic stimulation of the MGN.

Key words: acoustically evoked potentials, auditory thalamus, lateral nucleus of the amygdala, long-term potentiation, metabotropic glutamate receptors, tetanic stimulation.

The lateral nucleus of the amygdala (LA) receives auditory afferents from both the auditory thalamus (the medial geniculate nucleus, MGN) and auditory association cortex (LeDoux et al., 1990; Mascagni et al., 1993; Romanski and LeDoux, 1993; Woodson et al., 2000) and is critical for neural plasticity underlying auditory fear conditioning (Blair et al., 2001; Maren, 1999; Maren and Quirk, 2004). In the LA, neural responses evoked by an acoustic stimulus can be enhanced after the sound is paired with an unconditioned stimulus such as foot shock (Quirk et al., 1997; Rogan et al., 1997; Collins and Pare, 2000).

Long-term potentiation (LTP) induced in the LA is considered as a candidate of the cellular mechanism underlying auditory fear conditioning (Quirk et al., 1995, 1997; Rogan and LeDoux, 1995; Rogan et al., 1997). LTP in the LA occurs during auditory fear conditioning (Quirk et al., 1995, 1997; Rogan and LeDoux, 1995; McKeman and Shinnick-Gallagher, 1997; Rogan et al., 1997) and can be artificially induced by tetanic stimulation of the auditory thalamus or the auditory association cortex in either *in vitro* or *in vivo* preparations (Chapman et al., 1990; Bauer et al., 2002; Huang and Kandel, 1998; Doyère et al., 2003; Bauer and LeDoux, 2004). Particularly, naturally induced LTP and artificially induced LTP appear to share the same neural mechanisms (Bauer and LeDoux, 2004; Huang and Kandel, 1998; Quirk et al., 1995, 1997; Repa et al., 2001; Rodrigues et al., 2001, 2002; Rogan et al., 1997).

In the LA of urethane-anesthetized rats, acoustically evoked potentials (AEPs) to a frequency-modulated pure-tone burst can be enhanced by tetanic stimulation of the MGN, in a manner that is similar to the enhancement of electrically evoked potentials (Rogan and LeDoux, 1995). This finding indicates that both LTP of naturally evoked LA responses and LTP of electrically evoked LA responses cannot be prevented by general anesthesia induced by urethane. More importantly, this finding demonstrates that auditory responses recorded in the LA can be modulated by the neural process associated with LTP. Thus the tetanus-induced AEP potentiation in the LA can be used for studying both the temporal features of the development of LTP and related neural transmissions.

Glutamate exerts its actions as a type of excitatory neurotransmitters through both ionotropic and metabotropic receptors. The group I metabotropic glutamate receptors subtype 5 (mGluR5), which are coupled to various second messenger cascades, are involved in synaptic plasticity and memory formation associated with both the hippocampus and the LA (Balschun and Wetzel, 2002; Fendt and Schmid, 2002; Francesconi et al., 2004; Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005; Zou et al., 2007). mGluR5 are most localized in postsynaptic structures (Rodrigues et al., 2002), and the expression of mGluR5 protein can be up-regulated by fear conditioning (Riedel et al., 2000). Some

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Abbreviations: AEP, acoustically evoked potential; CREB, cAMP response-element binding protein; LA, lateral nucleus of the amygdala; LTP, long-term potentiation; mGluR5, metabotropic glutamate receptors subtype 5; MGN, medial geniculate nucleus; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; NMDARs, *N*-methyl-D-aspartate receptors.

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previous studies have suggested that mGluR5 are important to the formation of LTP both in the hippocampus and in the LA (Francesconi et al., 2004; Lee et al., 2002; Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005; Rodrigues et al., 2002). For example, using *in vitro* slice preparations, blocking mGluR5 during the time from 10 or 20 min before to 5 or 15 min after tetanic stimulation of the thalamic afferent fibers innervating the LA completely abolishes LTP in the LA without affecting baseline synaptic transmission (Lee et al., 2002; Rodrigues et al., 2002). However, blocking mGluR5 2.5–3 h after tetanization has no effects on established LTP (Lee et al., 2002). In addition, blocking mGluR5 during LTP induction by a pairing protocol in an *in vitro* preparation (low-frequency paired stimulation of the presynaptic and postsynaptic components of the thalamic afferents to the LA) also abolishes LTP without affecting baseline synaptic transmission (Fendt and Schmid, 2002). Thus mGluR5 must play a role in quickly developing LTP after the artificial induction.

The aim of the present study was to investigate the effect of tetanic stimulation of the MGN on AEPs recorded in the LA in chloral-hydrate anesthetized rats. The recording time was extended to 120 min after tetanization. Moreover, the role of mGluR5 in mediating the tetanus-induced AEP potentiation was examined.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (320–380 g) provided by Beijing Vital River Experimental Animals Technology Ltd. (Beijing, China) were used in this study. They were housed individually in a 12-h light/dark cycle (lights on at 7:00 AM) with food and water freely available. All efforts were made to minimize animal suffering (e.g. pain or discomfort) and to use only the number of animals necessary to produce reliable scientific data. Animals were treated in accordance with the Guidelines of the Beijing Laboratory Animal Center, the Guidelines of the Canadian Council of Animal Care, and the Policies on the Use of Animals and Humans in Neuroscience Research approved by the Society for Neuroscience (2006).

Surgery

The rat was anesthetized deeply with 10% chloral hydrate (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) (400 mg/kg, intraperitoneally) and placed in a Kopf stereotaxic head holder (Model 902; David Kopf Instrument, Tujunga, CA, USA). The state of anesthesia was carefully maintained throughout the experiment by supplemental injection of the same anesthetic at the dose of 0.2 ml/h. A midline incision was made in the scalp, and the skin and temporal muscles were retracted laterally. The rat's head was positioned with bregma and lambda at the same horizontal plane. A hole was drilled and a stainless steel recording electrode insulated by a silicon tube (10–20 kΩ, 0.3 mm in diameter) was implanted through the hole in the right LA (AP=−2.8 to −3.8 mm; ML=5.3–5.5 mm; DV=−7.5 to −8.0 mm, relative to bregma. AP: anterior-posterior dimension; ML: medial-lateral dimension; DV: dorsal-ventral dimension). The second hole was made and a monopolar stimulating electrode (Li and Yeomans, 2000) was implanted through the hole in the area of the medial division of the MGN and posterior intralaminar nuclei (AP=−5.4 mm; ML=3.0–3.2 mm; DV=−6.0 to −7.0 mm, relative to bregma), ipsilateral to the recorded LA. The third hole was made and a guide cannula (C317G guide cannula; Plastics One, Roanoke, VA, USA) was implanted through the hole aimed to the cerebral ventricle (AP=−0.8 mm; ML=1.6 mm; DV=−3.6 mm, relative to bregma), ipsilateral to the recorded LA (also see Naie and Manahan-Vaughan, 2004). The recording electrode, stimulating electrode, and guide cannula were all mounted on the skull with dental acrylic cement. Each animal was used only for one experiment.

Stimulus delivery

Electrical stimuli were generated by a Grass S-88F stimulator (Grass, Quincy, MA, USA), which provided monophasic cathodal rectangular pulses (pulse duration=0.2 ms) via a constant-current, photoelectric stimulus-isolation unit (Model PSIU6). Before electrophysiological recordings, a single-pulse stimulation of the MGN was used for determining the current threshold that evoked the minimal and reliable whole-body startle-like response (Li and Yeomans, 2000; Lin et al., 2002; He et al., 2005). The current for tetanic stimulation of the MGN was first set at a level 100 μA below the threshold, so that single-pulse stimulation of the MGN did not evoke a startle-like response, and then increased in the step of 20 μA between stimulation series (Huang et al., 2005). Tetanic stimulation consisted of four series of pulse trains (pulse frequency=100 Hz; train length=100 ms; number of trains=10; train interval=100 ms) with an inter-series interval of 1 min (Huang et al., 2005). The stimulation treatment for the low-frequency control group included the same number of electrical pulses but had the pulse frequency of 5 Hz (Huang et al., 2005).

The acoustic stimulus was a single broadband-noise burst (duration=1 ms, sound pressure level=52.4 dB), which was generated from a TDT System II (Tucker-Davis Technologies, FL, USA) using TDT Sigen software. The acoustic signal was transduced by a custom-made analog switch, amplified by an amplifier, and presented through an ED1 earphone. One end of a TDT sound-delivery tube with the length of 12 cm was connected to the ED1 earphone, and the other end was inserted into the rat's left ear canal with a fixed position close to the tympanic membrane.

Electrophysiological recordings

Ten minutes after surgery, both the stereotaxic instrument and the rat were transferred to a sound-attenuating chamber. The recording electrode, reference electrode, and ground wire were connected to a TDT head stage, and then to the TDT system DB4 amplifier. Neural potentials were amplified by 1000× and filtered via the 5–1000 Hz band pass. A 50-Hz notch filter was also applied. Neural signals were digitized at 20 kHz and stored on disk for both online and off-line analyses. Online EEG and averaged field potentials were processed with TDT Biosig software (Tucker-Davis Technologies).

Drug injection

The noncompetitive, selective and systemically active antagonist of mGluR5 (Gasparini et al., 1999), 2-methyl-6-(phenylethynyl)-pyridine (MPEP) ($C_{14}H_{11}N$·HCl, Sigma-Aldrich Corporation, St. Louis, MO, USA), was dissolved in Locke's solution, which contained 154 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, and 5.6 mM glucose. MPEP solution or vehicle solution was injected with the fixed volume of 4 μl at the constant rate of 1 μl/min via a Hamilton microsyringe (5 μl) (W-103; Anting Microsyringe Co. Ltd., Shanghai, China). The microsyringe was connected to the injection cannula (30-gauge; Plastics One) via a flexible polyethylene tubing. The injection cannula was then inserted into the guide cannula. MPEP or vehicle was administered into the lateral cerebral ventricle 30 min prior to tetanic stimulation of the MGN in order to allow sufficient diffusion from the lateral cerebral ventricle to the LA (Naie and Manahan-Vaughan, 2004;

Manahan-Vaughan and Braunewell, 2005). Alternatively, in some rats MPEP was injected 40 min after tetanic stimulation to investigate the effect of MPEP on the maintenance of AEP potentiation. After injection, the injection cannula was left in place for 1 min.

It has been reported that injection of 1.0 μg MPEP into the LA attenuates the acquisition of conditioned fear as indicated by a loss of fear-potentiated startle (Fendt and Schmid, 2002). Moreover, injection of MPEP at the dose of 1.8 μg into the lateral cerebral ventricle impairs the induction and expression of LTP in the hippocampus (Naie and Manahan-Vaughan, 2004). Accordingly, in the present study, both the lower (0.5 μg/4 μl) and higher (1.5 μg/4 μl) doses of MPEP were used.

Procedures

In experiment 1, 24 rats with both the MGN stimulating electrode and LA recording electrode were assigned into two groups: (1) tetanic stimulation (n=14), and (2) low-frequency stimulation (n=10). At the beginning of the experiment, the rat was first exposed to the acoustic stimulus for 15 min. Then, AEPs in the LA were recorded for examining the stability of AEPs for 30 min. When AEPs were stable, AEP recordings were made as the pre-tetanization or pre–low-frequency-stimulation baseline responses. Following the baseline recordings, either tetanic or low-frequency stimulation was delivered to the MGN. AEPs were then measured at the time points of 5, 15, 30, 45, 60, 90, and 120 min after tetanic or low-frequency stimulation. In this and the next experiments, a recording session for a time point consisted of 100 stimulation trials with the inter-stimulus interval of 1 s, and all recordings were conducted during the light phase (8:00 AM–6:00 PM) of the light/dark cycle.

In experiment 2, 49 animals with the MGN stimulating electrode, LA recording electrode, and guide cannula were divided into the following four groups: (1) vehicle control (dose=0 μg/4 μl)/early injection (12 rats), (2) low-dose (0.5 μg/4 μl)/early MPEP injection (12 rats), (3) high-dose (1.5 μg/4 μl)/early MPEP injection (14 rats) and (4) high-dose (1.5 μg/4 μl)/late MPEP injection (11 rats). For the three early-injection groups, the injection was made 30 min before tetanic stimulation, and for the late-injection group, based on the time course of the development of the AEP enhancement following tetanization in experiment 1, the injection was made 40 min after tetanic stimulation.

Similar to experiment 1, in experiment 2 rats were first exposed to the acoustic stimulus for 15 min. Then, recordings were carried out for 30 min to acquire baseline AEP responses. Following the measurement of baseline AEPs, MPEP or vehicle was injected into the cerebral ventricle in the three early-injection groups. Then AEP measurements were collected at 5, 15, and 30 min after injection. Subsequently, 30 min following injection, tetanic stimulation was delivered to the MGN. AEP measurements were then collected at the time points up to 120 min after tetanization. For rats in the late-injection group, baseline recordings were collected for 30 min before tetanization and high-dose MPEP was injected 40 min after tetanization.

Statistical analyses

The amplitude of AEPs was determined by measuring the peak-to-peak amplitude of the primary response (see Fig. 4A). To make results comparable across animals, the amplitude of AEP for each rat was normalized with respect to the mean baseline amplitude before MPEP injection or before tetanic stimulation. In experiment 1, for each rat in either the tetanic stimulation group or the low-frequency stimulation group, the mean baseline amplitude was the average of the AEP amplitudes recorded before tetanic or low-frequency stimulation. In experiment 2, for each rat with early injection, the mean baseline amplitude was the average of the AEP amplitudes at the time points between 20 and 10 min before injection. For the comparisons between the high-dose/late injection group, high-dose/early injection group and vehicle injection group, the mean baseline amplitude was the average of the AEP amplitudes at the time points between 20 and 10 min before tetanization.

The following equation was used to calculate the normalized amplitude of AEP:

$$\text{Normalized amplitude of AEP} = 100\,\% \times (\text{amplitude of AEP at a time after treatment} / \text{mean baseline amplitude of AEP})$$

ANOVAs were performed using SPSS 13.0 software. The null-hypothesis rejection level was set at 0.05.

Histology

At the end of testing, the rat was killed with an overdose of chloral hydrate. Lesion marks were made via the stimulating electrode and the recording electrode by an anodal DC current (500 μA for 10 s). Brains were removed, stored in 10% formalin with 30% sucrose until they sank and then sectioned at 40 μm in the frontal plane in a cryostat (−20 °C). Sections were examined to determine locations of stimulation, recording and injection.

RESULTS

Histology

The sites of stimulating and recording electrodes in rats used in experiment 1 are shown in Fig. 1. Correct placements of both stimulating electrodes (Fig. 1, upper panel) and recording electrodes (Fig. 1, lower panel) were found in 18 rats, 10 in the tetanic-stimulation group and 8 in the low-frequency-stimulation group.

The sites of stimulating and recording electrodes in the four groups of rats used in experiment 2 are shown in Fig. 2 and Fig. 3, respectively. Correct placements of both stimulating and recording electrodes were found in 9 rats in the vehicle control group, 10 rats in the low-dose/early MPEP group, 11 rats in the high-dose/early MPEP group, and 8 rats in the high-dose/late MPEP group. All the rats with correct placements of electrodes were also confirmed to have correct placements of injection cannula.

Data analyses were only based on results of rats with correct placements of both stimulating electrodes and recording electrodes.

Experiment 1: Effects of tetanic or low-frequency stimulation

A single broadband-noise burst could elicit manifest AEP responses in the LA in chloral-hydrate-anesthetized rats (Fig. 4A). The AEP waveform contains a large negative peak with the latency between 12 and 20 ms following the stimulus onset.

Fig. 4B shows the group-mean normalized AEPs to the noise burst for rats with tetanic stimulation (solid symbols) and those with low-frequency stimulation (open symbols) at various recording time points. For rats receiving tetanic stimulation, compared with the baseline AEP (before tetanic stimulation), the AEP amplitude at each of the seven post-tetanization time points was markedly enhanced. Specifically, there was an enhancement of AEPs shortly

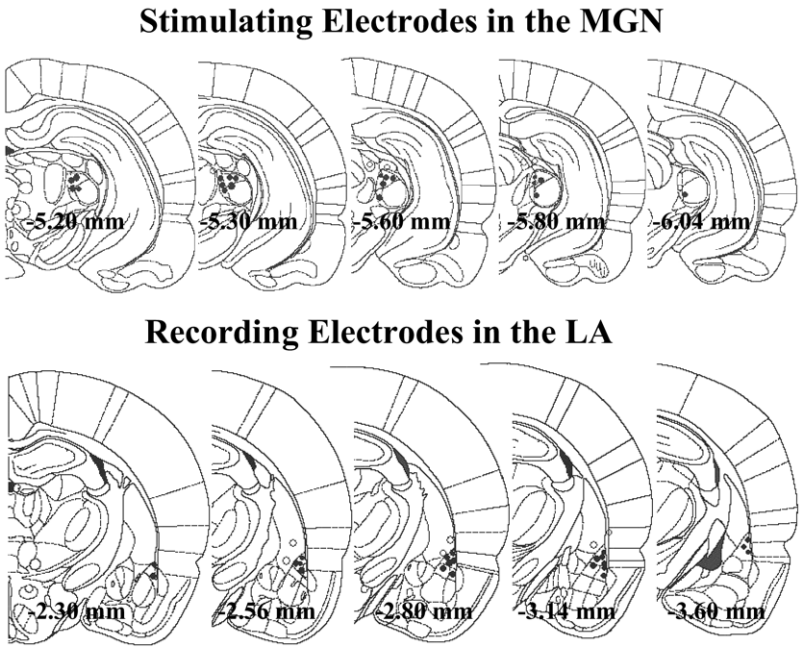

Fig. 1. Upper panel: placements of stimulating electrodes aimed to the area of the medial division of the MGN for electrical (tetanic or low-frequency) stimulation in experiment 1 for 24 rats. Lower panel: placements of recording electrodes aimed to the LA for recording AEPs to a noise burst for the same 24 rats. In this and following figures, filled circles indicate electrode locations within the target areas, and open circles indicate electrode locations outside the target areas, and the distance of each brain frontal plane to bregma is indicated at the bottom of the panel.

(5 min) after tetanization. Then, a slight decrease in the degree of enhancement occurred at the time point of 15 min, and subsequently the AEP amplitude remained relatively stable during the period of 30–45 min after tetanization. An increase of the AEP potentiation started from the time point of 45 min and lasted toward the time point of 120 min.

However, for rats receiving low-frequency stimulation of the MGN, compared with the baseline AEP before low-frequency stimulation, the AEP amplitude at each of the seven time points after low-frequency stimulation generally remained at the same level.

A two (group: tetanic group, low-frequency group) by eight (recording time: one before tetanization, seven after tetanization) two-way mixed between-and-within-group ANOVA shows that the interaction between group and recording time was significant ($F_{7, 84}=6.033$, $P<0.05$), the main effect of recording time was significant ($F_{7, 84}=5.706$, $P<0.05$), and the main effect of group was significant ($F_{1, 12}=24.832$, $P<0.05$).

A separate one-way within-group ANOVA shows that for rats with tetanic stimulation, AEPs were significantly different across the recording time points ($F_{7, 42}=7.029$, $P<0.01$). Separate ANOVAs show that the AEP at of each of the recording times after tetanization was significantly different from the AEP at the recording time just before tetanization ($P<0.05$).

Another separate one-way within-group ANOVA shows that for rats with low-frequency stimulation there were no significant differences in AEP amplitude across recording times ($F_{7, 42}=0.703$, $P>0.05$).

A two (group: tetanic group, low-frequency group) by seven (seven recording time points after tetanization) two-way mixed between-and-within-group ANOVA shows that the interaction between group and recording time was not significant ($F_{6, 72}=2.174$, $P>0.05$), the main effect of recording time was not significant ($F_{6, 72}=2.734$, $P>0.05$), but the main effect of group was significant ($F_{1, 12}=24.832$, $P=0.000$).

These statistical analyses confirm that tetanic stimulation but not low-frequency stimulation of the MGN had a significant enhancing effect on the AEP recorded from 5 to 120 min after tetanization.

Experiment 2: Effects of MPEP injection

The effects of injection of low- or high-dose MPEP injection or Locke's solution 30 min before tetanic stimulation of the MGN on AEPs recorded in the LA are shown in Fig. 5. Immediately after injection of Locke's solution or low-dose MPEP, a transient increase in AEP amplitude occurred. But the AEP amplitude returned to the baseline level at the time points of 15–25 min after injection. One potential reason that caused the transient increase in AEPs following vehicle infusion may be that infusion of solution into the lateral cerebral ventricle transiently changed the pressure and temperature of the lateral cerebral ventricle, and the brain gave a transient response to the stress. This assumed response might be associated with activity of mGluR5 and could be reduced by MPEP.

As shown in Fig. 5, Locke's solution did not prevent the AEP from being enhanced following tetanization. Similar to the results of the tetanic-stimulation group in experiment 1, the AEP potentiation in this vehicle-injection group continued to increase toward the time point of 120 min.

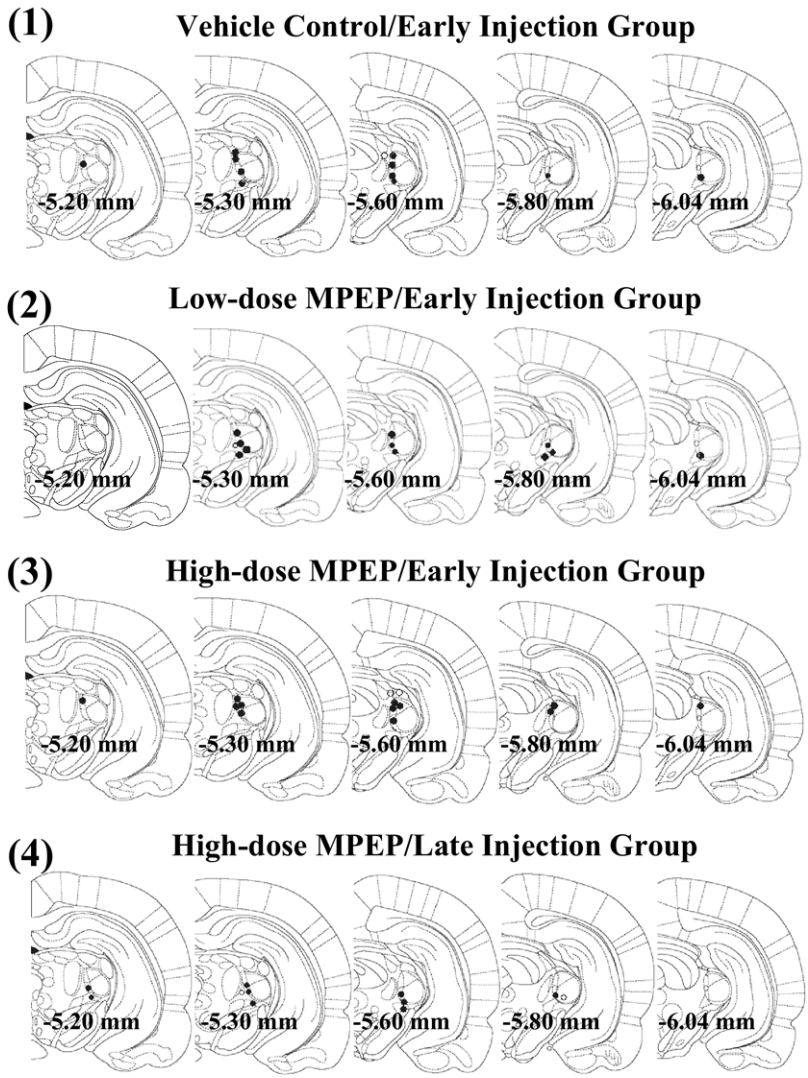

Fig. 2. Placements of stimulating electrodes aimed to the area of the MGN for tetanic stimulation in experiment 2 for the following four groups: (1) vehicle control (dose=0 μg/4 μl)/early injection group (12 rats); (2) low-dose MPEP (0.5 μg/4 μl)/early injection group (12 rats); (3) high-dose MPEP (1.5 μg/4 μl)/early injection group (14 rats); (4) high-dose MPEP (1.5 μg/4 μl)/late injection group (11 rats). Early-injection rats were injected with the vehicle or MPEP 30 min before tetanization; late-injection rats were injected with MPEP 40 min after tetanization.

In the low-dose/early MPEP injection group, the AEP potentiation appears to occur during the early period (before the time point of 30 min) after tetanization. However, there was no continued increase in the AEP amplitude from the time point of 45 min to the time point of 120 min.

In the high-dose/early MPEP injection group, the AEP was not affected by tetanization. The tetanus-induced AEP potentiation did not occur during the whole course (5–120 min) of post-tetanization. In other words, the tetanus-induced AEP potentiation was completely blocked by high-dose/early MPEP injection.

A three (early injection group) by ten (recording time: one just before tetanization, nine after tetanization) two-way mixed between-and-within-group ANOVA shows that the main effect of group was significant ($F_{2, 27}=9.236$, $P<0.05$), the main effect of recording time was significant ($F_{9, 243}=4.041$, $P<0.05$), and the interaction between group and recording time was significant ($F_{18, 243}=2.782$, $P<0.05$).

For rats with injection of Locke's solution, a one-way within-group ANOVA shows that AEPs were significantly different across the recording time points ($F_{9, 72}=6.111$, $P<0.01$). Further separate one-way within-group ANOVAs show that the AEP at each of the post-tetanization recording times was significantly different from the AEP that was recorded just before tetanization ($P<0.01$).

For rats with injection of low-dose of MPEP, a one-way within-group ANOVA shows that the recording-time effect on the AEP was not significant (or marginally significant) ($F_{9, 81}=1.774$, $P=0.086$).

For rats with injection of high-dose of MPEP, a one-way within-group ANOVA shows that the recording-time effect on the AEP was not significant ($F_{9, 90}=1.042$, $P=0.414$).

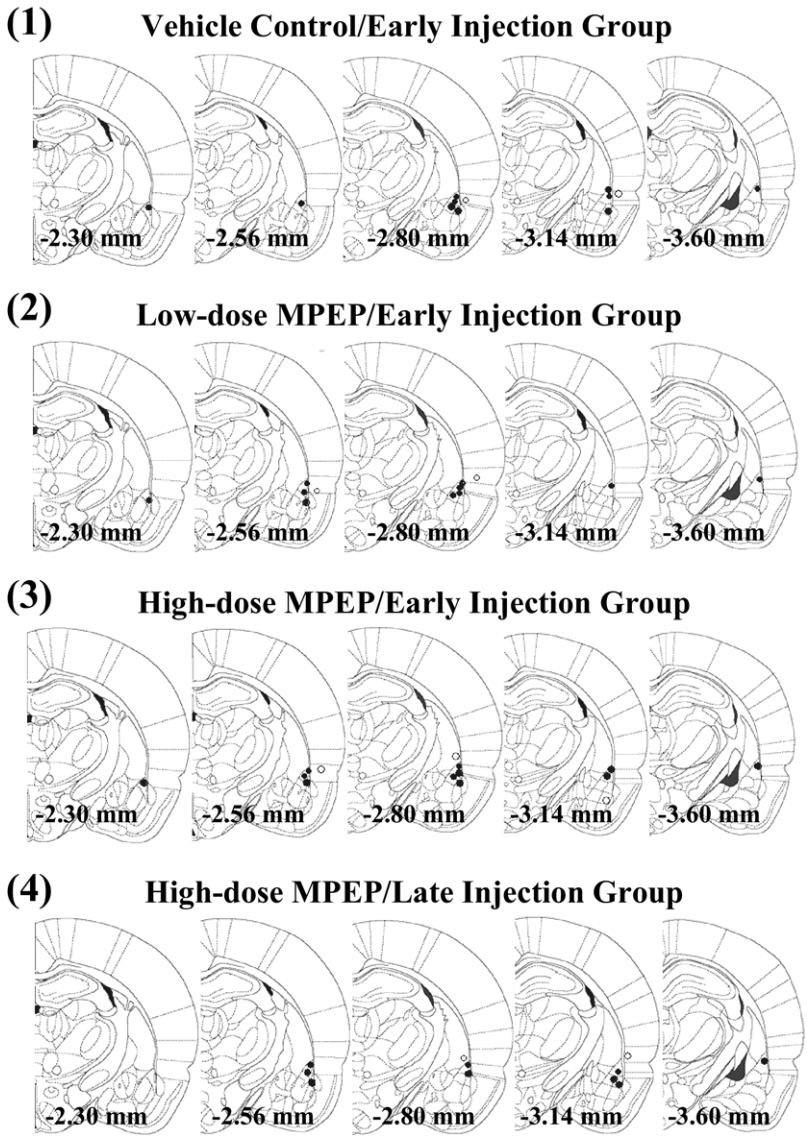

Fig. 3. Placements of recording electrodes aimed to the LA for recording AEPs in experiment 2 for the following groups: (1) vehicle control (dose=0 μg/4 μl)/early injection group (12 rats); (2) low-dose MPEP (0.5 μg/4 μl)/early injection group (12 rats); (3) high-dose MPEP (1.5 μg/4 μl)/early injection group (14 rats); (4) high-dose MPEP (1.5 μg/4 μl)/late injection group (11 rats).

These statistical analyses indicate that high-dose MPEP injections completely blocked the tetanization-induced enhancement of the AEP, low-dose MPEP injection partially blocked the tetanization-induced enhancement of the AEP, and Locke's solution injection had no effects on the tetanization-induced enhancement of the AEP.

A three (three early injection groups) by nine (nine recording time points after tetanization) two-way mixed between-and-within-group ANOVA shows that the interaction between group and recording time was not significant ($F_{16, 216}=1.667$, $P>0.05$), the main effect of recording time was not significant ($F_{8, 216}=0.954$, $P>0.05$), but the main effect of group was significant ($F_{2, 27}=10.155$, $P<0.001$). Fisher's (LSD) tests show that the group with injection of Locke's solution was not significantly different from the group with injection of low-dose MPEP ($P>0.05$), but the group with injection of high-dose MPEP was significantly different from both the group with injection of Locke's solution ($P<0.001$) and the group with injection of low-dose MPEP ($P<0.01$). These analyses confirm that low-dose MPEP injection was less effective than high-dose MPEP injection.

To examine the effect of high-dose/late MPEP injection on the tetanus-induced AEP potentiation, comparisons were made between the following three groups: (1) vehicle control, (2) high-dose/early MPEP injection, and (3) high-dose/late MPEP injection (Fig. 6). As shown in Fig. 6, injection of high-dose of MPEP 40 min after the tetanization had no any effects on the AEP potentiation.

Also as shown in Fig. 6, the AEP curve for the group of high-dose/late MPEP injection is markedly different from

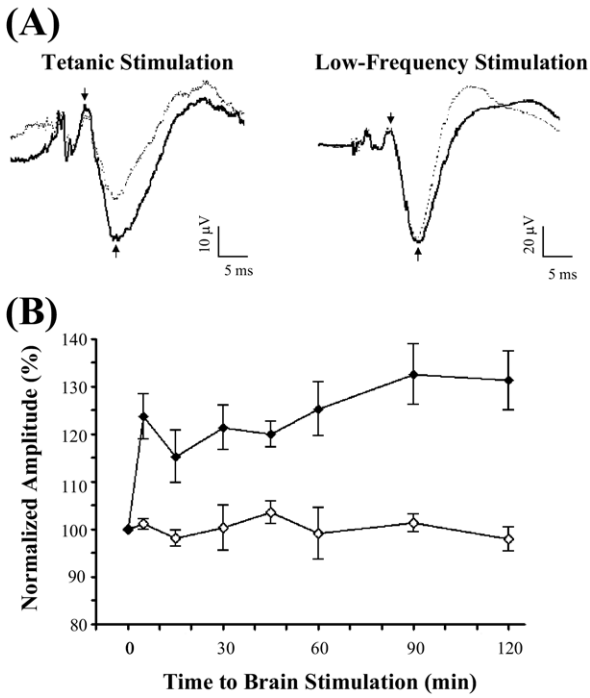

Fig. 4. (A) Examples of AEP waveforms recorded in the LA in response to a single broadband-noise burst. AEP waveforms were obtained before (broken curves) and after (solid curves) tetanic stimulation of the MGN (waveforms on the left) or low-frequency stimulation of the MGN (waveforms on the right). Arrows indicate the onset and the peak of the primary AEP response. (B) Group mean AEP amplitudes recorded in the LA in response to the noise burst before and 5, 15, 30, 45, 60, 90, and 120 min after tetanic (filled diamonds) or low-frequency (open diamonds) stimulation of the MGN in experiment 1.

that for the group of high-dose/early MPEP injection but very similar to that for the group of Locke's solution injection. To confirm the absence of any effects of high-dose/late MPEP injection on AEP, a two (group: group of high-dose/late MPEP injection, group of Locke's solution injection) by ten (recording time: one just before tetanization, nine after tetanization) two-way mixed between-and-within-group ANOVA is conducted. The ANOVA shows that the recording-time effect was significant ($F_{9, 135}=15.775$, $P<0.05$), but the interaction between group and recording time was not significant ($F_{9, 135}=0.843$, $P>0.05$), and group effect was not significant ($F_{1, 15}=0.007$, $P>0.05$), confirming that the AEP curve for the group of high-dose/late MPEP injection is highly similar to that for the group of Locke's solution injection. Thus, late MPEP injection had no effects on AEP amplitude after the AEP potentiation had been induced.

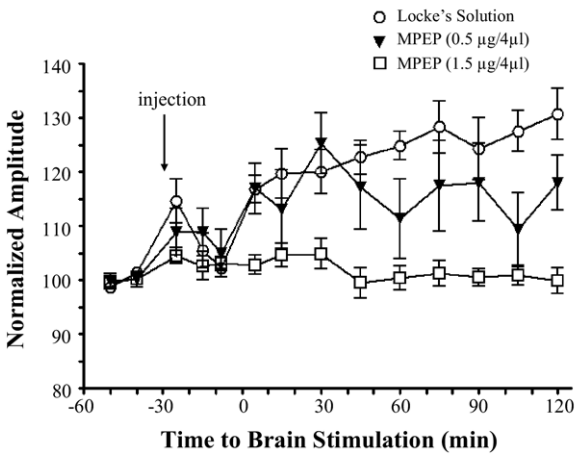

Fig. 5. Group mean AEP amplitudes recorded in the LA in response to the noise burst 50, 40, 25, 15 and 8 min before, and 5, 15, 30, 45, 60, 75, 90, 105, and 120 min after tetanic stimulation of the MGN for the following three groups in experiment 2: (1) vehicle control (open circles), (2) low-dose/early MPEP injection (filled triangles), and (3) high-dose/early MPEP injection (open squares).

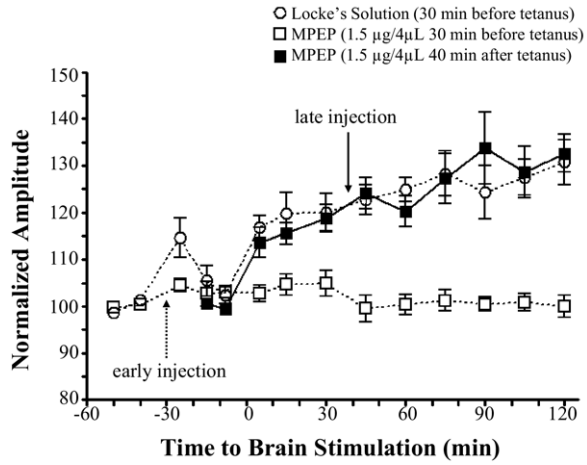

Fig. 6. Group mean AEP amplitudes recorded in the LA in response to the single noise burst before and after tetanic stimulation of the MGN for the following three groups in experiment 2: (1) vehicle control (open circles), (2) high-dose/early MPEP injection (open squares), and (3) high-dose/late MPEP injection (filled squares).

DISCUSSION

Tetanic stimulation of the MGN enhances AEPs recorded in the LA

The results of the present study show that the AEP recorded in the LA to a noise burst was significantly enhanced after tetanic, but not low-frequency, stimulation of the MGN in chloral-hydrate-anesthetized rats. Our results are consistent with the results reported by Rogan and LeDoux (1995), in which both AEPs recorded in the LA to a frequency-modulated tone burst and evoked potentials recorded in the LA to an electrical stimulus delivered to the MGN were enhanced by tetanic stimulation of the MGN in urethane-anesthetized rats. Thus the animal model with general anesthesia is useful for studying mechanisms underlying pavlovian fear conditioning (Weinberger et al., 1984; Gold et al., 1985; Pang et al., 1996).

Tetanic stimulation of the MGN causes unspecific and prolonged excitation of both presynaptic fibers and postsynaptic cells in the LA at the same time, simulating an integration of inputs of conditioned stimulus and inputs of unconditioned stimulus. However, auditory fear condition-

ing requires the time-precisely concurrent activation of presynaptic auditory inputs to the LA and strong depolarization of the same neurons induced by pain signals. Thus it should be noted that although tetanic stimulation of the MGN obeys the Hebbian rule, tetanization-induced LTP patterns at the circuitry level may be different from natural LTP patterns that occur during auditory fear conditioning.

mGluR5 are involved in the tetanus-induced enhancement of AEPs

Behavioral results have confirmed the requirement of mGluR5 for the acquisition of fear conditioning (Balschun and Wetzel, 2002; Fendt and Schmid, 2002; Francesconi et al., 2004; Zou et al., 2007). However, mGluR5 may not be essential to the expression and consolidation of fear conditioning (Rodrigues et al., 2002; Naie and Manahan-Vaughan, 2004).

As mentioned in the introduction, the MGN directly transmits auditory information to the LA by its axonal projections (LeDoux et al., 1991; Turner and Herkenham, 1991; Bordi and LeDoux, 1994). The LA contains principal neurons and interneurons with different morphological, immunohistochemical, and physiological characteristics (Lang and Paré, 1998; Mahanty and Sah, 1998; McDonald, 1982; McDonald and Augustine, 1993; Millhouse and de Olmos, 1983; Rainnie et al., 1991; Sugita et al., 1993; Washburn and Moises, 1992). Previous studies have suggested that the direct excitatory impact of MGN afferents may be much stronger onto principal neurons than onto interneurons (Farb and LeDoux, 1997; Mahanty and Sah, 1999; McDonald, 1982; Szinyei et al., 2000; Tsvetkov et al., 2004; Woodson et al., 2000). Glutamatergic transmissions in the MGN-LA pathway are involved in neural physiological changes during fear conditioning (Blair et al., 2001; Rodrigues et al., 2002). The enhancing effect induced by tetanic stimulation of the MGN (but not low-frequency stimulation of the MGN) on AEPs recorded in the LA is possibly due to the increase in excitatory-glutamate synaptic transmissions in the MGN–LA pathway.

The results of the present study demonstrate that the selective mGluR5 antagonist, MPEP, completely blocked the tetanus-induced AEP potentiation in the LA without affecting the baseline AEP when high-dose MPEP was injected 30 min before tetanization. Thus mGluR5 must be involved in the tetanus-induced enhancement of the AEP.

It has been documented that mGluR5 are critical for synaptic plasticity and conditioning associated with both the hippocampus and the LA (Balschun and Wetzel, 2002; Fendt and Schmid, 2002; Francesconi et al., 2004; Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005; Zou et al., 2007). LTP in the LA can be induced during fear conditioning (Quirk et al., 1995, 1997; McKeman and Shinnick-Gallagher, 1997), and MPEP, which dose-dependently impairs the acquisition of auditory fear conditioning (Fendt and Schmid, 2002; Zou et al., 2007), impairs LTP at thalamic input synapses to the LA *in vitro* (Rodrigues et al., 2002). Especially, the important contribution of mGluR5 to LTP induction has been confirmed in both the LA and the hippocampus (Fendt and Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002; Francesconi et al., 2004; Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005).

Previous *in vitro* studies have shown that application of MPEP during the time when LTP-induction stimulation is made can completely block LTP in the LA, indicating that mGluR5 play a role in mediating LTP in the LA (Fendt and Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002). The present study for the first time shows that intra-ventricle injection of MPEP, when in a high dose, completely blocks the AEP potentiation in the LA, which is induced by tetanic stimulation of the MGN in anesthetized rats. Thus LTP that is induced in the LA requires mGluR5 in such *in vivo* preparations. In addition, injection of low-dose MPEP before tetanization partially inhibits the AEP, indicating a dosage effect. Because blocking mGluR5 40 min after tetanic stimulation does not affect established LTP, mGluR5 may not be necessary for the maintenance of LTP.

It should be noted that in this study and previous studies (Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005), MPEP administered into the lateral cerebral ventricle affected not only the amygdala but also other parts of the brain. In addition, tetanic stimulation of the MGN may also cause long-term enhancement of auditory responses in other brain regions in addition to the LA. Thus further studies would be needed to examine whether there are brain-region-specific roles played by mGluR5 in establishing tetanization-induced LTP.

Possible mechanisms and future studies

mGluR5 and N-methyl-D-aspartate receptors (NMDARs). It has long been known that NMDARs are important for synaptic plasticity and memory formation. LTP in the LA induced by tetanic stimulation of the MGN can be abolished by the antagonist of NMDARs, (D)-2-amino-5-phosphonovaleric acid (APV) (Huang and Kandel, 1998; Bauer and LeDoux, 2004). Infusion of this antagonist of NMDARs into the LA also impairs both acquisition of auditory fear conditioning (Fanselow and Kim, 1994; Maren et al., 1996; Lee and Kim, 1998) and expression of fear-potentiated startle (Fendt, 2001).

mGluR5 have both structural and functional connections with NMDARs (for a recent review see Simonyi et al., 2005), and activation of mGluR5 results in a potentiation of NMDAR currents (Yu et al., 1997; Pisani et al., 2001) which can be suppressed by MPEP (Mannaioni et al., 2001). Moreover, NMDAR-dependent LTP in the CA1 region of the hippocampus is significantly reduced in the mice lacking mGluR5 (Lu et al., 1997). Synaptic plasticity of NMDARs is also partially dependent on activation of mGluR5 (Harney et al., 2006). In mGluR5-deficient mice, the NMDAR-mediated component of LTP disappears, but the AMPAR-mediated component of LTP is normal (Jia et al., 1998). Thus, MPEP-caused reduction of AEP potentiation is possibly, in part, due to the attenuated activation of NMDAR, which is tightly associated with mGluR5. NMDAR and mGluR5 seem to interact with each other to co-modulate synaptic plasticity (De Blasi et al., 2001). It is reason-

able to assume that high-dose MPEP may inhibit the AEP potentiation by suppressing NMDAR currents, and low-dose MPEP mainly disrupts the development of the AEP potentiation in a NMDAR-independent manner, which is possibly related to RNA or/and protein synthesis. In the future, more pharmacological experiments are needed to clarify how NMDARs and mGluR5 contribute the AEP potentiation in the LA.

mGluR5 and intracellular signaling. LTP induced by tetanic stimulation depends on the influx of calcium into the postsynaptic neurons (Malenka, 1991). The rise in calcium triggers a series of intracellular signaling processes which ultimately induce synaptic enhancement (Malenka, 1991; Carew, 1996; Kandel, 1997; Elgersma and Silva, 1999; Sweatt, 1999, 2000). The formation of early-phase LTP requires calcium entry through NMDA receptors which activate α-calcium/calmodulin-dependent kinase II (αCaMKII) and calcium/phospholipid-dependent protein kinase (PKC) (Huang and Kandel, 1994). This process may serve as a cellular mechanism for short-term synaptic plasticity and underlie some forms of short-term memory of fear conditioning (Mayford et al., 1996; Weeber et al., 2000). Different from early-phase LTP, late-phase LTP which is dependent on RNA and protein synthesis requires activation of the cAMP-dependent protein kinase and the extracellular-regulated kinase/mitogen-activated protein kinase. These protein kinases in turn activate transcription factors, such as the cAMP response-element binding protein (CREB), which induces the transcription of genes and protein synthesis that determine the formation of some long-term memory (for reviews see Schafe et al., 2001). CREB is considered as an important factor for the switch from short-term memory to long-term memory. It has been confirmed that mGluR5 initiate an intracellular cascade that conveys extracellular signals to induce phosphorylation of CREB, and subsequently, gene expression (Mao and Wang, 2002, 2003). Thus, the contribution of CREB to AEP potentiation at the thalamic–LA pathway is certainly worthy of further investigation in future studies using the experiment paradigm established by the present study.

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