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Original Article

Hippocampal ischemia causes deficits in local field potential and synaptic plasticity

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Abstract

The long-term enhancement in glutamate receptor mediated excitatory responses has been observed in stroke model. This pathological form of plasticity, termed post-ischemic long-term potentiation (i-LTP), points to functional reorganization after stroke. Little is known, however, about whether and how this i-LTP would affect subsequent induction of synaptic plasticity. Here, we first directly confirmed that i-LTP was induced in the endothelin-1-induced ischemia model as in other in vitro models. We also demonstrated increased expression of NR2B, CaMKII and p-CaMKII, which are reminiscent of i-LTP. We further induced LTP of field excitatory post-synaptic potentials (fEPSPs) on CA1 hippocampal neurons in peri-infarct regions of the endothelin-1-induced mini-stroke model. We found that LTP of fEPSPs, induced by high-frequency stimulation, displayed a progressive impairment at 12 and 24 hours after ischemia. Moreover, using *in vivo* multi-channel recording, we found that the local field potential, which represents electrical property of cell ensembles in more restricted regions, was also dampened at these two time points. These results suggest that i-LTP elevates the induction threshold of subsequent synaptic plasticity. Our data helps to deepen the knowledge of meta-synaptic regulation of plasticity after focal ischemia.

Keywords: long-term potentiation, local field potential, ischemia, endothelin-1, multi-channel *in vivo* recording

Introduction

Ischemic stroke is now one of the major causes of death and disability in the world[1]. Evidence shows that focal ischemia in the territory of the middle cerebral artery (MCA) induces widespread neuropathological changes both in the ischemic region and in areas remote from the original infarct[2]. The glutamate receptor mediated ischemic long-term potentiation (i-LTP) often occurs after ischemic stroke. This neural plasticity plays an important role in ischemic injury and recovery. Therefore, deepening the understanding of the i-LTP mechanism has a great significance for guiding the treatment of ischemic stroke.

LTP, a cellular model of synaptic plasticity that is now widely considered to share similar cellular mechanism with learning and memory, can be reflected by changes in the amplitude or slope of field excitatory postsynaptic potentials (fEPSPs). The low impedance and positioning of the electrode allows the activity of a large number of neurons to contribute to the signal. The unfiltered signal reflects the sum of action potentials

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from cells within approximately 50-350 μm from the tip of the electrode and represents the electrical property of cell ensembles in more restricted regions. It is generally known that the i-LTP is initiated by excessive calcium influx with the activation of NMDAR after stroke[3]. However, whether and how the i-LTP would affect subsequent induction of synaptic plasticity and the local field potential (LFP) remains less known.

Metaplasticity is a concept originally coined by W.C. Abraham and M.F. Bear to refer to synaptic plasticity[4]. The idea is that the synapse's previous history of activity determines the plasticity afterwards. Therefore, the metaplastic regulation of i-LTP on subsequent induction of synaptic plasticity may underlie impaired capability of learning and memory after ischemia. The CA1 area in the hippocampus is one of the most sensitive regions[5-6] to ischemic stroke. In the present study, we first infused endothelin-1 (ET-1) to the dorsal hippocampus to establish a mini-stroke model[7-9]; we then demonstrated the increase in expression of NR2B, CaMKII and p-CaMKII, which hints the occurrence of i-LTP. Using whole-cell patch-clamp recording, we found that LTP of fEPSPs induced by high-frequency stimulation (HFS) displayed a progressive impairment at 12 and 24 hours after ischemia. Moreover, using *in vivo* multi-channel recording, we found that the LFP, which represents electrical properties of cell ensembles in more restricted regions, was also dampened at these two time-points. These results point to the notion that i-LTP elevates the induction threshold of subsequent synaptic plasticity. Our data helps to deepen the knowledge on metasynaptic regulation of plasticity after focal ischemia.

Materials and methods

Animals

Four-week-old male Sprague-Dawley (SD) rats were used. All animal studies followed the guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals. Before the experiments, animals were randomly divided into two groups: the control group and the ischemia group. To construct the ischemic model, we applied ET-1 (15 pmol; Sigma-Aldrich, St. Louis, MO, USA; 0.8 μL in saline solution[10]; 10 nL/s) stereotaxically targeted to the dorsal hippocampus CA1 region (AP: -4.52 mm relative to the bregma; ML: ±3.0 mm; DV: 3.0 mm[11-12]). For intracranial injections, rats were deeply anesthetized with 3% chloral hydrate. The rats were then fixed on a stereotaxic frame (RWD Life Science, China) and the injection rate and volume were controlled by a microsyringe pump controller (WPI, USA). The needle was left in place for 5 additional minutes after injection. Rats in the ischemia group received bilateral infusion of ET-1 for all experiments. Rats in the control group received bilateral infusion an equivalent volume of saline through the same surgical procedure. Rats were returned to home cage after surgery.

Magnetic resonance imaging

The infarct size and location were detected by MRI at 6, 12 and 24 hours after ischemia. MRI was conducted by using 7.0-T MRI (Eclipse, Philips Medical Systems, The Netherlands) with a 21-cm bore magnet. The MRI sequences were a T2-weighted spin-echo [repetition time (msec)/echo time (msec), 500/17.9]. The rats were anesthetized with 3% chloral hydrate.

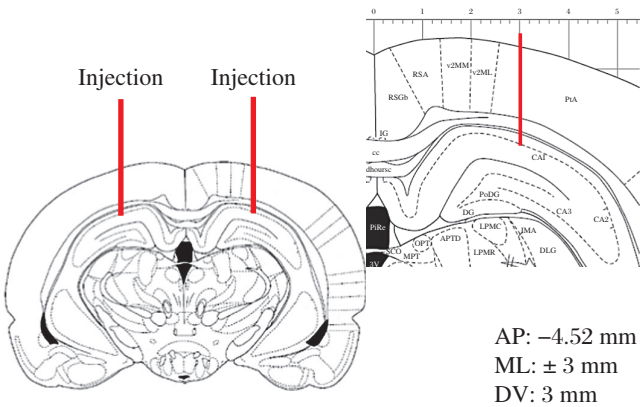
TTC staining

The infarct size and location were evaluated at 6, 12 and 24 hours after ischemia. After anesthetization and euthanasia, the brains were removed directly and frozen at -20 °C for 15 min. The whole brains of rats were incised to corresponding slices at 2 mm. The sections were immersed in 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma) at 37 °C for 20 min including an invert of the slices in light-blocking environment. The pale region representing the focal infarcts was distinctly visible by examining TTC-stained sections.

Electrophysiological recordings

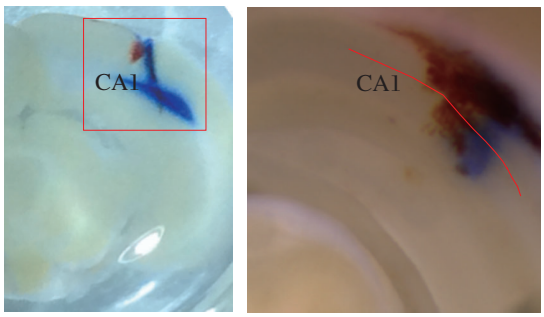
The protocol was adapted from previous studies[13]. Rats were anesthetized with 10% chloral hydrate and decapitated. Ice-cold artificial cerebrospinal fluid (ACSF) containing 126 mmol/L NaCl, 2.5 mmol/L KCl, 1 mmol/L MgCl2, 1 mmol/L CaCl2, 1.25 mmol/L KH2PO4, 26 mmol/L NaHCO3 and 20 mmol/L glucose was bubbled continuously with Carbogen (95% O_2/5% CO_2). The brain was immediately removed and 350 μm coronal hippocampal brain slices were prepared with a vibrating blade microtome (Leica VT1200S) in ACSF. Fresh slices were incubated in a chamber with carbogenated ACSF and recovered at 30 °C for at least 2 h. fEPSP responses were evoked at 0.05 Hz with a 125 μm electrode placed in the middle of the stratum radiatum of CA1. A 2-3 MΩ glass recording electrode filled with 2mol/L NaCl was positioned orthodromic (200 μm) from the stimulating electrode. High frequency stimulation protocol: Weak HFS (wHFS), 1 train, consisting of 10 bursts in 100 Hz; HFS, 4 trains, consisting of 100 bursts in 100 Hz with 20 seconds inter-burst interval. Responses were set to -60% max for LTP experiments.

A



AP: −4.52 mm
ML: ± 3 mm
DV: 3 mm

B



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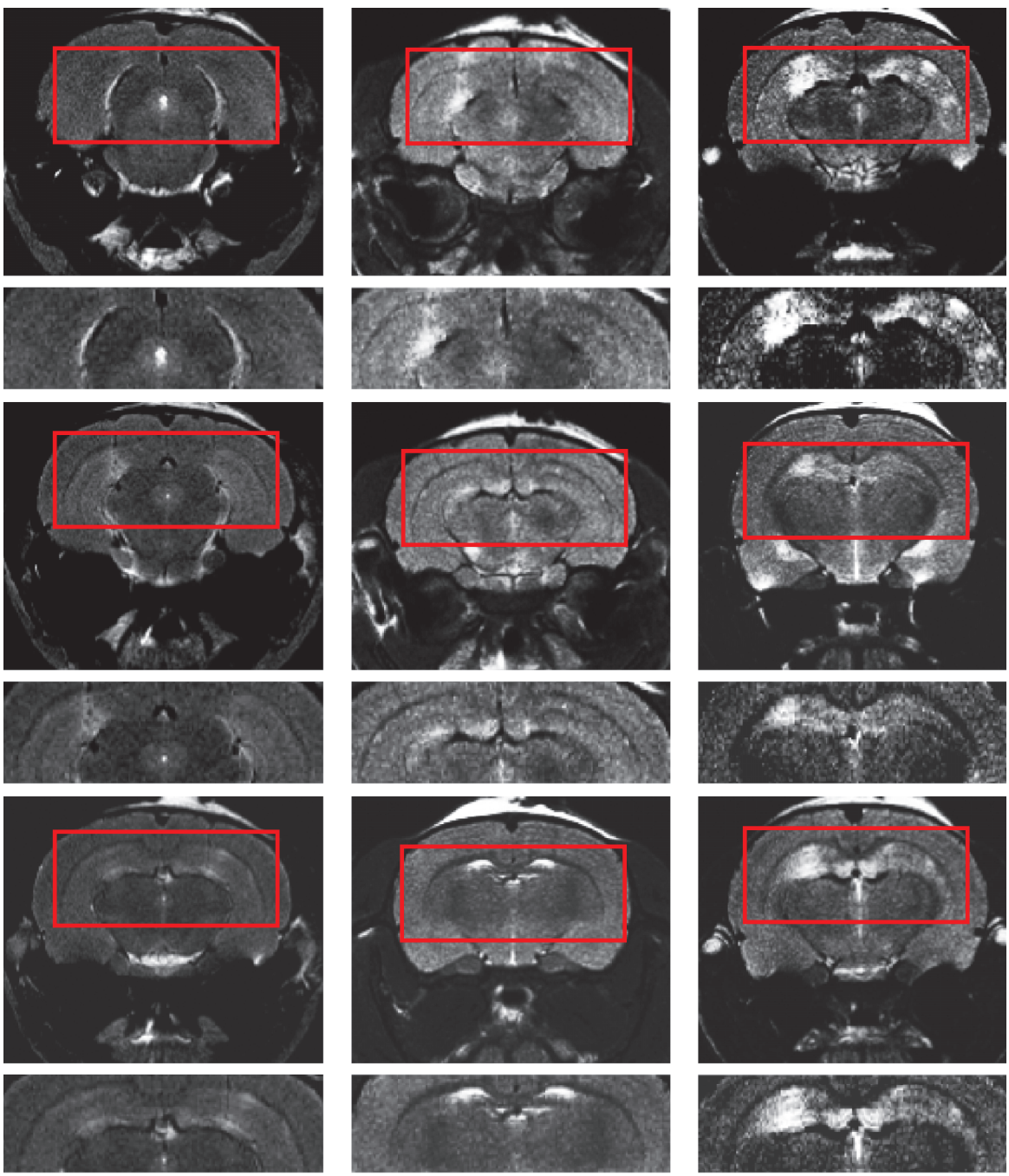

6 hours 12 hours 24 hours

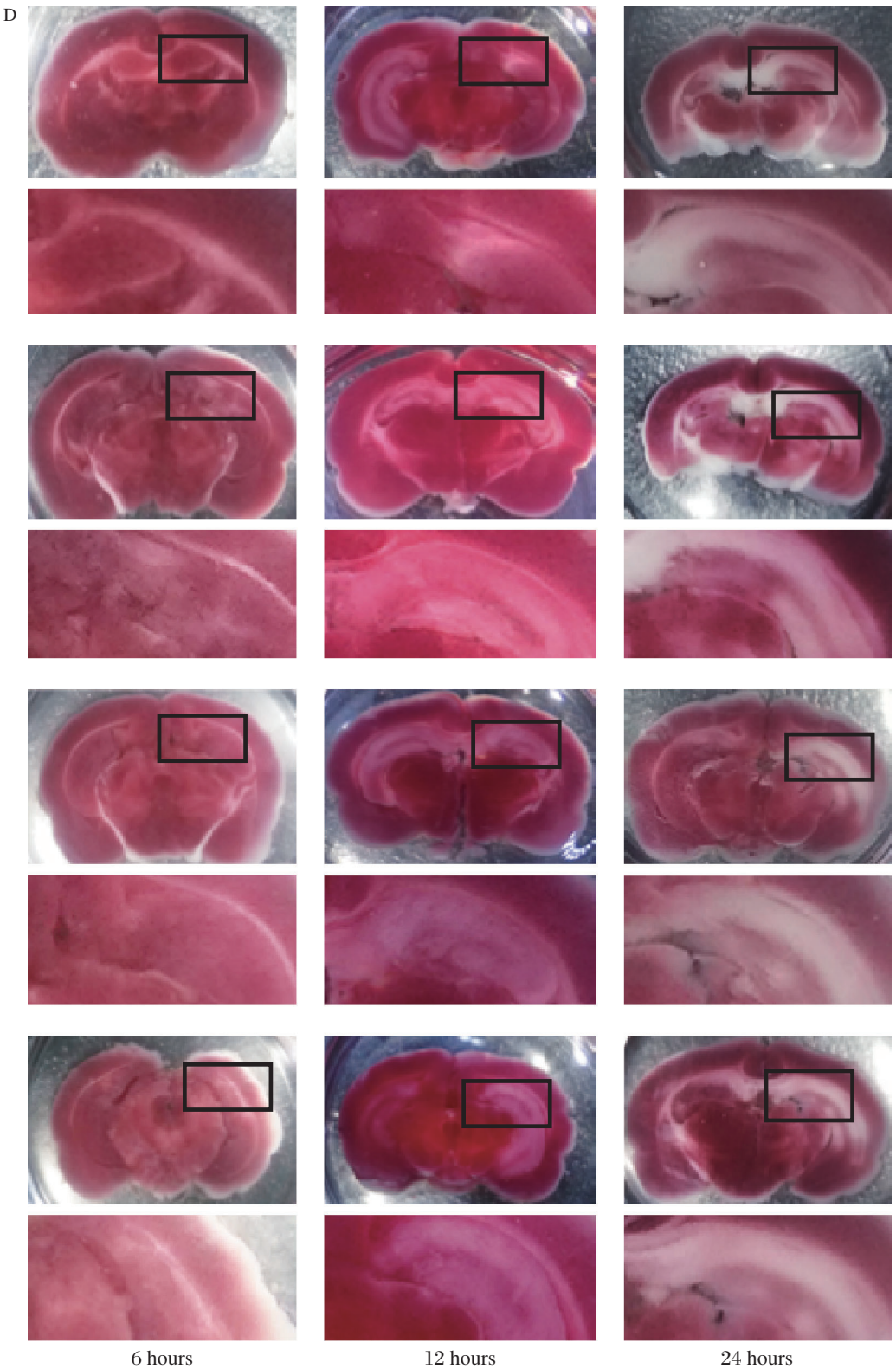

Fig. 1 **MRI and 2,3,5–triphenyltetrazolium chloride (TTC) staining confirms the ischemic region after local endothelin–1 (ET–1) injection.** A: Schematic diagram shows the site of injection. B: Methylthionine chloride solution is applied to display the accuracy of the injection site. Left, the area in the red box is the methylthionine chloride solution diffusion region, showing accurate localization of the dorsal hippocampus CA1 region; Right: another sample showing injection sites in magnified image. The line indicates the pyramidal cell layer in CA1 region. C: Sequential brain T2-w MRI in rats with ischemic lesion. The image in the red box and the magnified image shown underneath indicate the infarct regions. D: A series of brain slices stained with TTC after ischemic lesion. Pale staining in the black box and the magnified image shown underneath indicates the infarct region. C and D also show progressive increase of the infarct regions.

Multichannel recordings

Rats were treated in accordance with surgical procedures. Sprague Dawley rats (300-350 g) were anaesthetized with 0.015 mL/g, 3% chloral hydrate as needed, and body temperature was maintained with a heating pad. LFP was recorded with an 8-channel electrode array in the hippocampal CA1 pyramidal cell layer. LFP (sampling rate 800 Hz) was filtered online at 0.5-250 Hz[14,15].

Western blotting

Hippocampal slices were prepared from the animals injected with ET-1. We choose the slices with an injection hole and then the slices were homogenized in cold 0.32 mol/L sucrose solution containing 1 mmol/L HEPES, 1 mmol/L $NaHCO_3$, 1 mmol/L $MgCl_2$, 0.2 mmol/L dithiothreitol, 20 mmol/Lβ-phosphogrycerol, 20 mmol/L sodium pyrophosphate, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L Na_3VO_4, 50 mmol/L NaF, 1 mmol/L and p-nitrophenyl phosphate (PNPP) (pH=7.4), in the presence of protease and phosphatase inhibitors. The 40-60 μg samples were subjected to SDS-PAGE and transferred to PVDF membranes. Antibodies against the following proteins were used: CaMKIIα (SC-13141), p-CaMKII (SC-32289), NMDAε2 (NR2B, SC-365597), and β-tubulin (CW0098, Beyotime, China).

Data analysis

Data were presented as mean±SEM. Differences between groups were compared using independent-sample *t*-test (two populations) and one-way ANOVA for comparisons. Statistic differences were considered to be significant when P was <0.05.

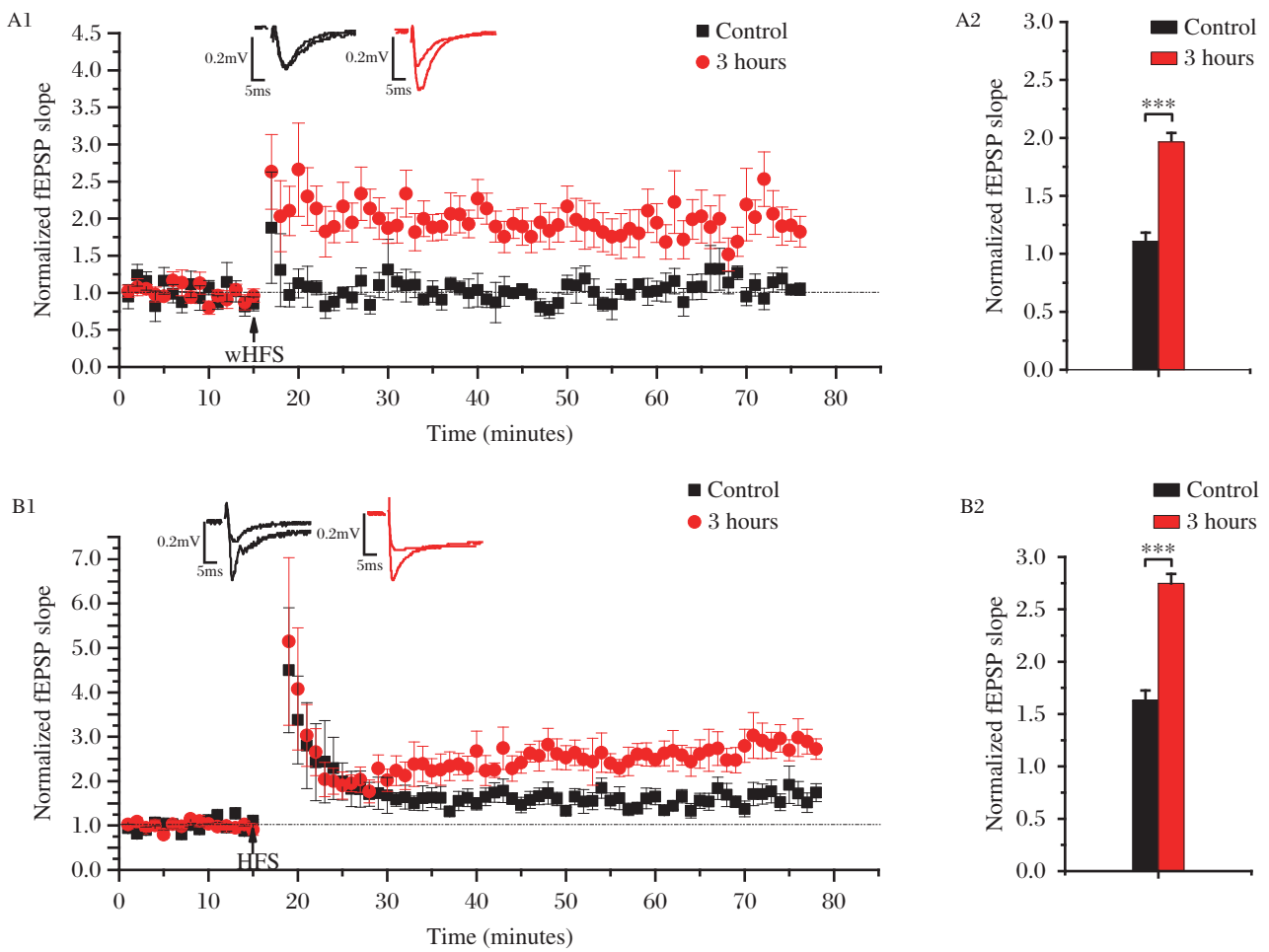

***Fig. 2* The occurrence of i–LTP in ET–1–induced mini–stroke model.** A: Weak high-frequency stimulation (HFS) induced i-LTP at 3 hours after ischemia. When a weak HFS (wHFS, 1 train, consisting of 10 bursts in 100 Hz) was delivered to Schaffer fibers in control slices, no LTP was induced. In contrast, a persistenpotentiation was induced upon stimulation with wHFS in slices obtained from ET-1-treated animals (A2, Control: 1.11±0.04, P<0.001, n=5 at 61-75 minutes; 3 hours: 1.97±0.08, P<0.001, n=8). B: HFS faciliates LTP in ET-1-induced stroke model. Compared with normal LTP in control brain slices, LTP in ET-1-treated slices displayed LTP with enhanced potentiation magnitude (B2, Control: 1.63±0.06, P<0.001, n=5 at 61-75 minutes; 3 hours: 2.75±0.08, P<0.001, n=5). Data represents mean±SEM. Data represents mean±s.e.m. Statistic differences were compared using independent-sample *t*-test.

Results

MRI and TTC staining reveal progressive infarct regions following ET–1 infusion

We first stereotaxically infused ET-1 with middle concentration and volume (15 pmol, 0.8 μL dissolved in sterile saline) into the CA1 region of the dorsal hippocampus to establish mini-stroke model (***Fig. 1A***)[12]. The location of ET-1 injection sites was then confirmed (***Fig. 1B***) by application of methylthionine chloride solution. We also used the MRI and TTC to examine and confirm the establishment of the mini-stroke model. We found that the region subjecting to ischemic attack was progressively enlarging. At 6 hours post-ischemia, the ischemic region was just around the injection site; 12 hours post-ischemia, the infarct region was slightly increased as indicated by MRI and TTC staining. At 24 hours post-ischemia, the infarct region significantly increased in both MRI and TTC staining experiments (***Figs. 1C, 1D***).

Post-ischemic long-term potentiation

We then examined, in the ET-1-induced ischemia model, whether i-LTP can be induced as in other in vitro models[16-17]. Three hours after ET treatment, acute hippocampal brain slices were obtained. When a weak HFS (wHFS, 1 train, consisting of 10 bursts in 100 Hz) was delivered to Schaffer fibers in control slices, no LTP was induced. In contrast, a persistent potentiation was induced upon stimulation with wHFS in slices obtained from ET-1-treated animals (Control: 1.11 ± 0.04, $P<0.001$, $n=5$, at 61-75 minutes; 3 hours: 1.97 ± 0.08, $P<0.001$, $n=8$; ***Fig. 2A***). Consistently, compared with normal LTP in control brain slices, LTP in ET-1-treated slices displayed LTP with enhanced potentiation magnitude (Control: 1.63 ± 0.06, $P<0.001$, $n=5$, at 61-75 minutes; 3 hours: 2.75 ± 0.08, $P<0.001$, $n=5$; ***Fig. 2B***), suggesting that LTP was facilitated in ET-1-treated stroke model. Taken together, these results confirm the occurrence of i-LTP in the ET-1-induced stroke model.

Post-ischemic increase in expression of NR2B, CaMKII and p-CaMKII

I-LTP is usually accompanied by biochemical changes in LTP-related signaling molecules. Following activation of NMDA receptor[18] and Ca^{2+} influx through the receptor channel, increased intracellular Ca^{2+}/CaM

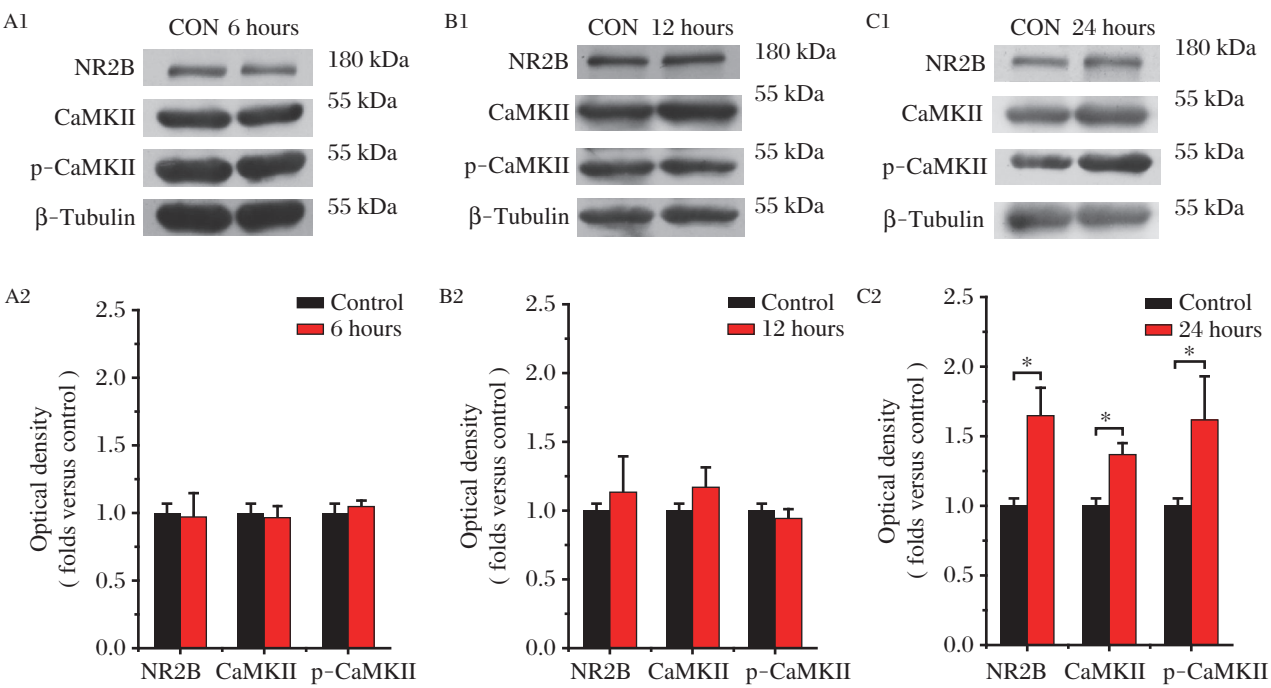

Fig. 3 **Increased expression level of NR2B, CaMKII and p–CaMKII after ischemia.** A: Absence of change in these proteins at 6 hours after ischemia (NR2B, 0.97 ± 0.18, $P>0.05$, $n=4$; CaMKII, 0.97 ± 0.09, $P>0.05$, $n=4$; p-CaMKII, 1.05 ± 0.04, $P>0.05$, $n=4$). B: At 12 hours after ischemia, a slight but not significant increase in the expression of NR2B and CaMKII was detected (NR2B, 1.13 ± 0.26, $P>0.05$, $n=4$; CaMKII, 1.17 ± 0.14, $P>0.05$, $n=4$). No increases of p-CaMKII was detected (0.94 ± 0.07, $P>0.05$, $n=4$). C: At 24 hours after ischemia, a significant increase in the NR2B, CaMKII and p-CaMKII was detected (NR2B, 1.65 ± 0.20, $P<0.05$, $n=4$; CaMKII, 1.16 ± 0.13, $P<0.05$, $n=3$; p-CaMKII, 1.62 ± 0.31, $P<0.05$, $n=4$). Data represents mean ± SEM. Statistic differences were compared using one-way ANOVA. CON: control, h: hours.

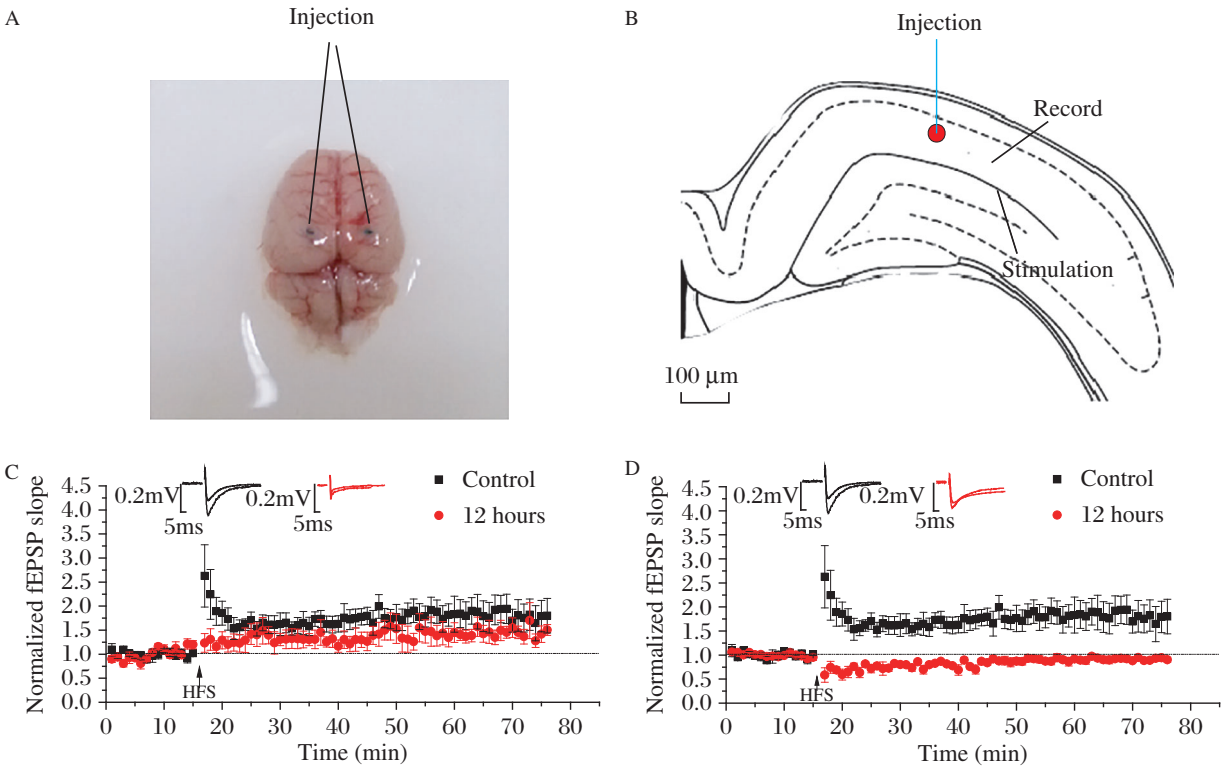

***Fig. 4* Ischemia impairs LTP induction in CA1 hippocampal neuorns.** A: ET-1 injection sites. B: Schematic diagram showing position of stimulating and recording electrodes. Recording and stimulation sites are at a distance of 150-200 μm from the injection point. C: Sample traces showing LFP recorded in 3 of 8 channels. Scale bar: 200 ms, 0.5 mV. LTP was slightly but significantly decreased at 12 hours after ischemia (Control: 1.82±0.02 at 61-75 minutes, n=5; 12 hours: 1.46±0.03, n=5; P<0.001). D: LTP was completely abolished at 24 hours after ischemia (Control: 1.82±0.02 at 61-75 minutes, n=5; 24 hours: 0.92±0.01, n=5; P<0.001). Data represents mean±SEM. C and D share the same data of control group. Statistic differences were compared using independent-sample t-test.

activates CaMKII[19-21], which in turn activates downstreaming signaling molecules like Ras, PI3K[22-23] and PKCλ[24-25][26-29]. As a result, AMPA receptor is phosphorylated and incorporated into postsynaptic membrane[30] which underlies LTP of AMPA receptor-mediated synaptic currents. Therefore, the possible alterations in these LTP-related molecules may be reminiscent of pathological i-LTP. We thus employed Western blotting assays to examine the expression of NR2B, CaMKII and p-CaMKII at 6, 12 and 24 hours after ischemia. As shown in ***Fig. 3***, there was no change in these proteins at 6 hours after ischemia (NR2B, 0.97±0.18, P>0.05; CaMKII, 0.97±0.09, P>0.05; p-CaMKII, 1.05±0.04, P>0.05, ***Fig. 3A***). Twelve hours after ischemia, the expression of NR2B and CaMKII showed a slight but statistically insignificant increase when compared with control (NR2B, 1.13±0.26, P>0.05; CaMKII, 1.17±0.14, P>0.05, ***Fig. 3B***), while at this time there was no increase of p-CaMKII (0.94±0.07, P>0.05, ***Fig. 2***). However, at 24 hours after ischemia, the expression of NR2B, CaMKII and p-CaMKII increased significantly (NR2B, 1.65±0.20, P<0.05; CaMKII, 1.16±0.13, P<0.05; p-CaMKII, 1.62±0.31, P<0.05, ***Fig. 3C***). These results indicate that ischemic attack can increase the expression of these i-LTP-associated molecules and indirectly demonstrate the occurrence of i-LTP.

ET-1-induced mini-stroke impairs LTP

To examine whether LTP induction, after i-LTP, upon ischemic attack is altered, we induced LTP of fEPSPs in CA1 neurons of the peri-infarct regions on acute hippocampal slices. The hippocampal slices were prepared at 12 and 24 hours after ischemia (***Fig. 4A***). We only selected the slices containing injection sites and set the recording and stimulation electrode at a distance of 150-200 μm from the injection point (***Fig. 4B***). We found that LTP induced by HFS decreased slightly, but significantly in CA1 hippocampal neurons at 12 hours after ischemia (Control: 1.82±0.02 at 61-75 min; 12 hours: 1.46±0.03; P<0.001, ***Fig. 4C***). At 24 hours after ischemia, LTP was totally reversed (Control: 1.82±0.02 at 61-75 min; 24 hours:

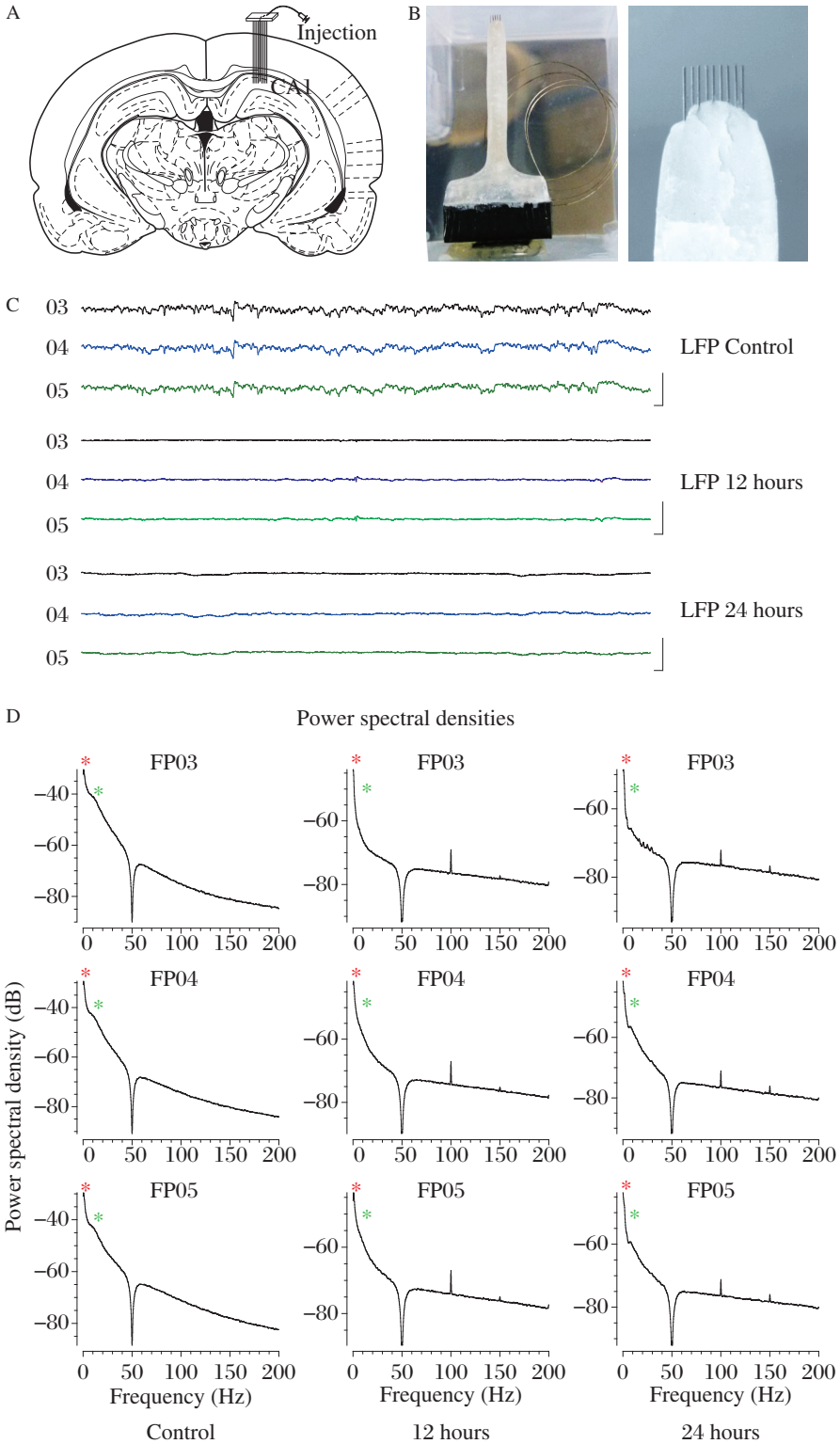

Fig. 5 **Ischemia impairs LFP in dorsal hippocampus.** A: Schematic diagram showing position of rejection and 8-channel recording electrodes. Recording electrodes are surrounded with the injection point. B: Schematic pictures of 8-channel recording electrodes. C: Sample traces showing LFP recorded in 3 of 8 channels. Scale bar: 200 ms, 0.5 mV. LTP was significantly suppressed at 12 hours and 24 hours time-points after ischemia. D: Power spectral density (PSD) of LFP in A. Note the decrease in the peaks at low frequency rhythm in the PSD of LFP(At the first peak marked with red asterisk: Control: -29.54±0.24, n=8; 12 hours: -44.01±0.48, n=8, P<0.001 compared with control; 24 hours: -43.86±1.08, n=8, P<0.001 compared with control; At the sencond peak marked with green asterisk: Contol: -42.08±0.33, n=8; 12 hours: -58.95±0.96, n=8, P<0.001 compared with control; 24 hours: -60.55±1.01, n=8, P<0.001 compared with control). Statistic differences were compared using one-way ANOVA.

0.92±0.01; $P<0.001$, ***Fig. 4D***). In addition, we also found that the maximal amplitude of excitatory postsynaptic potentials that could be achieved gradually decreased (data not shown). Further studies are necessary to examine whether it is a general phenomenon.

LFP declines after ischemia

Compared to conventional fEPSPs, LFP reflects electrical property of cell ensembles in more restricted regions. To examine possible alterations in LFP, we employed in vivo multi-channel recording to study the effect of ischemia on LFP in hippocampal CA1 pyramidal neurons. We continuously recorded LFP signals with 8-channel electrodes at different time-points after ischemia on anesthesized rats. We found that the amplitude of the LFP decreased dramatically at both 12 and 24 hours after ischemia (***Fig. 5A***). The power spectral density (PSD) analysis of LFP showed that the low frequency rhythms were also impaired significantly at these time points after ischemia (At the first peak: Control: -29.54±0.24; 12 hours: -44.01±0.48, $P<0.001$ compared with controls; 24 hours: -43.86±1.08, $P<0.001$ compared with controls; At the second peak: Control: -42.08±0.33; 12 hours: -58.95±0.96, $P<0.001$ compared with control; 24 hours: -60.55±1.01, $P<0.001$ compared with controls, ***Fig. 5B***).

Discussion

The pathological form of plasticity, i-LTP, is usually accompanied by alterations in plasticity-related proteins. First of all, we directly confirmed that i-LTP can be induced in ET-1-induced ischemia model as described previously in other in vitro models. We monitored the expression of CaMKII, p-CaMKII and NMDA receptor NR2 subtype NR2B. These molecules have been reported to be associated with both LTP and synaptic plasticity. Under ET-1induced mini-stroke condition[31,32], we detected an increase in CaMKII, phospho-CaMKII and NR2B, which is consistent with previous findings showing similar enhancement in NR2B subunit. These results provide both electrophysiological and biochemical evidence that hints the occurrence of i-LTP.

LTP and LTD are widely considered to share similar mechanisms with learning and memory and thus are used to study cellular synaptic plasticity[33-39]. The hippocampus is one of the brain regions most associated with learning and memory. However, this region is also very sensitive to ischemic attack. Although i-LTP is indicated as long-term enhancement in AMPA- and NMDA-receptor-mediated excitatory responses, what we actually observed in real life is the impaired capability on hippocampus-dependent learning and memory. This may be caused by the altered induction threshold of subsequent LTP. In the present study, we indeed observed detrimental effect by prior i-LTP. The magnitude of HFS-induced LTP was significantly inhibited at 12 hours after ischemia and was totally abolished at 24 hours after ischemia. Although ET-1 is usually used to mimic mini-stroke, we can still detect deficit on LTP induction. Compared to the MCAO and PT methods that are more often used, ET-1 infusion only produces local ischemia in a very restricted region within the dorsal hippocampus. Therefore, LFP, which represents electrical properties of cell ensembles in more restricted regions, seems more suitable to detect slight change in the peri-infarct region. Consistent with the findings on fEPSPs, we also detected suppression of LFP at 12 and 24 hours after ischemia. These results provide solid evidence to the notion that focal ischemia in the hippocampus causes deficits in local field potential and synaptic plasticity.

In summary, we first demonstrated the presence of metaplastic regulation in the mini-stroke model and then demonstrated how the i-LTP affected subsequent synaptic plasticity. These results enrich our understandings of metaplasticity in cerebral ischemia and provide new ideas for potential clinical treatment of cerebral ischemia.

Acknowledgement

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