Rapid Communication

Stress Evoked by Opiate Withdrawal Facilitates Hippocampal LTP In Vivo

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ABSTRACT: Stress impairs hippocampal long-term potentiation (LTP), but it is unknown whether the stress evoked by opiate withdrawal has the same effect. Here the authors report that opiate withdrawal for 4 days does not influence basal synaptic transmission, but results in a greatly increased LTP in hippocampal CA1 area in anesthetized rats. Elevated-platform stress enabled a large LTP in rats withdrawn for only 18 h, but the glucocorticoid receptor antagonist RU38486 (twice per day for 3 days) prevented the large LTP on 4 days withdrawal. Moreover, 4 days withdrawal enhanced the NMDAR-mediated EPSCs, in which the NR2A-containing NMDAR-mediated EPSC was increased but the NR2B-containing NMDAR-mediated EPSC was decreased. These results suggest that adaptive changes of the NMDAR and glucocorticoid receptor functions during 4 days of opiate withdrawal may enable stress to facilitate hippocampal LTP, potentially contributing to the opiate withdrawal experience-dependent modifications of hippocampal functions.

KEY WORDS: opiate withdrawal; long-term potentiation; hippocampal CA1; stress

INTRODUCTION

Hippocampus is enriched with glucocorticoid receptors (GRs) (McEwen et al., 1986) and plays crucial roles in regulating the stress effects on learning and memory as well as synaptic plasticity (McEwen, 1999; Kim and Diamond, 2002). Evidence has documented that stress activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to elevated glucocorticoid (GC) level, which is tacitly used as the indication of stress (Kim and Diamond, 2002). On the other hand, addictive drugs cause maladaptive changes of the brain stress circuits (Sinha, 2001). It is known that addictive drugs are able to elevate GC level (Buckingham and Cooper, 1984). Repeated opiate use causes a complete tolerance of this effect (Buckingham and Cooper, 1984; Pechnick, 1999), but opiate withdrawal evokes severe stress response as indicated by elevated GC level again (Ho et al., 1977). Consistent with these findings, stress is known to increase the propensity of an individual to drugs of abuse (Piazza and Le Moal, 1996) and trigger drug seeking and relapses after drug abstinence (Shaham et al., 2000; Sinha, 2001).

Hippocampal synaptic plasticity such as long-term potentiation (LTP) is believed to be the mechanism underlying certain types of learning and memory (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Martin et al., 2000). Evidence suggests that stress impairs hippocampus-dependent learning and memory (de Quervain et al., 1998; Yang et al., 2003). Consistent with this notion, stress impairs LTP (Foy et al., 1987; Shors et al., 1989; Diamond et al., 1996) and facilitates long-term depression (LTD) (Kim et al., 1996; Xu et al., 1997; Yang et al., 2005). Recent evidence has suggested that NR2A- and NR2B-containing NMDAR receptors (NMDARs) govern the direction of synaptic plasticity in the hippocampus (Liu et al., 2004). Consistent with these findings, the stress-facilitated hippocampal LTD is completely prevented by the antagonist to NR2B-containing NMDARs or GRs (Yang et al., 2005), and the stress-impaired hippocampal LTP is restored by the NR2B-containing NMDAR antagonist (Wang et al., 2006). On the other hand, evidence demonstrates that repeated opiate use impairs hippocampal LTP, which is recovered during the first 3 days of opiate withdrawal (Pu et al., 2002). Meanwhile, repeated opiate use attenuates the ability of stress to facilitate hippocampal LTD, but additional stress in tandem with morphine still elicits a large synaptic depression (Yang et al., 2004). These findings suggest that stress

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may influence total synaptic weight by breaking the balance of LTP and LTD in hippocampal network, a homeostatic theory of synaptic plasticity suggested previously (Royer and Pare, 2003). Our recent report demonstrates that low frequency stimulation induces Schaffer-CA1 LTD and temporoammonic-CA1 LTP (Yang et al., 2006), a phenomenon consistent with the homeostatic theory of synaptic plasticity (Royer and Pare, 2003), but stress disrupts the balance that enables LTD in both pathways (Yang et al., 2006). Here, we have examined whether and how the stress evoked by opiate withdrawal influences hippocampal LTP during a longer withdrawal period.

## RESULTS

### Opiate Withdrawal Facilitated Hippocampal LTP

We first recorded the field EPSP in CA1 area of the hippocampus in anesthetized rats that were subjected to morphine withdrawal for 2–18 h, and 4, 7, and 20 days. We found that input–output relationship and baseline EPSP amplitude remained unchanged following opiate withdrawal (Figs. 1a,b). After baseline EPSP recordings, three episodes of high-frequency stimulation (HFS) (HFS, 10 trains of 20 pulses at 200 Hz) with 60-min intervals were used to examine the maximal capacity of LTP. One-way analysis of variance (ANOVA) analysis revealed that there was a significant difference among groups ($F_{(5,37)} = 26.90, P < 0.05$; Fig. 2). The HFS protocol induced a reliable LTP in repeated saline group (Sal, $n = 3,131.9\% \pm 2.1\%; P < 0.05$ vs. baseline; Fig. 2a, see also insets). Then, a reliable but smaller LTP was induced by given HFS at about 2 h after the last injection of morphine (2 h, $n = 8,121.5\% \pm 1.7\%; P < 0.05$ vs. baseline or Sal; Fig. 2b). It is consistent with previous reports (Pu et al., 2002; Salmanzadeh et al., 2003). Compared with Sal group, the impaired LTP was quickly recovered at 18 h withdrawal (18 h, $n = 8,121.5\% \pm 1.7\%; P < 0.05$ vs. baseline, $P > 0.05$ vs. Sal; Fig. 2c), consistent with a previous report (Pu et al., 2002). Then, we examined LTP on 4 days withdrawal and found that HFS induced a large LTP compared with other groups (4 days, $n = 8,148.8\% \pm 2.6\%; P < 0.05$ vs. baseline, $P < 0.05$ among all groups; Fig. 2d, see also insets). Since then, the magnitude of LTP on 7 days withdrawal was declined to a level similar to that found in Sal group (7 days, $n = 8,133.7\% \pm 2.0\%; P < 0.05$ vs. baseline, $P > 0.05$ vs. Sal; Fig. 2e). The magnitude of LTP on 20 days withdrawal became even smaller (20 days, $n = 8,120.1\% \pm 1.6\%; P < 0.05$ vs. baseline or Sal, $P > 0.05$ vs. 2 h; Fig. 2f). We summarized these data and found that the magnitude of LTP showed an inverted-U curve and the largest magnitude occurred on 4 days withdrawal (**$P < 0.01$, *$P < 0.05$ vs. Sal; Fig. 2g).

### The GR Antagonist RU38486 Prevented the Facilitation of Hippocampal LTP on 4 Days Withdrawal

We then examined whether additional stress impaired hippocampal LTP. One-way ANOVA analysis revealed that there was a significant difference among groups ($F_{(4.24)} = 7.18, P < 0.05$; Fig. 3). Elevated-platform stress remarkably facilitated LTP at 18-h withdrawal (Str-18h, $n = 4,152.9\% \pm 3.4\%; P < 0.05$ vs. baseline; Fig. 3a, see also insets), similar to the large LTP found on 4 days withdrawal (Fig. 2d). This result disagrees with previous reports that stress impairs hippocampal LTP in naive rats (Foy et al., 1987; Shors et al., 1990; Diamond et al., 1992; Radecki et al., 2005), but consistent with the notion in a recent report that chronic mild stress facilitates LTP in fimbria-prefrontal cortex pathway (Kessal et al., 2006). Furthermore, when the GR antagonist RU38486 (20 mg/kg, sc.) was given twice per day for the first 3 days of withdrawal, the large LTP on 4 days withdrawal was suppressed (RU-4d, $n = 4,134.4\% \pm 1.6\%; P < 0.05$ vs. baseline; Fig. 3b, see also insets), to a level similar to that found at 18 h withdrawal (Fig. 2b). An extinction
FIGURE 2. Opiate withdrawal facilitated hippocampal LTP.

a: High frequency stimulation at 200 Hz (HFS, arrows) induced a reliable LTP in rats repeatedly treated with saline (Sal).
b: HFS induced a smaller LTP in rats repeatedly exposed to morphine and subjected to morphine withdrawal for 2 h, compared with Sal.
c: However, LTP was restored at 18 h withdrawal, similar as that found in Sal.
d: Remarkably, HFS induced a large LTP on 4 days withdrawal. 
e and f: Since then, LTP became smaller on 7 days withdrawal and the smallest on 20 days withdrawal.
g: Summary of the magnitude of hippocampal LTP following opiate withdrawal, and compared with that of rats repeatedly treated saline (Sal). The hippocampal LTP showed an inverted-U curve and peaked on 4 days withdrawal. **P < 0.01, *P < 0.05 vs. Sal.

Insets: representative traces of the field EPSP (average of six consecutive sweeps) at the times indicated by the numbers in a and d. Calibration bars: horizontal = 5 ms, vertical = 1 mV.
dose of morphine (15 mg/kg, s.c.) was given at 12 h before the first HFS on 4 days withdrawal, to avoid its acute effect. Now, the large LTP on 4 days withdrawal was suppressed (EM-4d, \( n = 5, 135\% \pm 1.5\% \); \( P < 0.05 \) vs. baseline; Fig. 3c), to a level similar to that found at 18 h withdrawal (Fig. 2b). Thus, opiate withdrawal may activate GRs, leading to a large LTP.

Stimulation at Lower Frequencies Induced Hippocampal LTP on 4 Days Withdrawal

We examined whether frequency response in LTP induction was changed on 4 days withdrawal. In Sal group, we found that low-frequency stimulation (LFS) at 1 Hz (900 pulses), and stimulation at 10–50 Hz (10 trains of 20 pulses) failed to affect synaptic efficacy (open circles, \( n = 3 \) for each panel, 99.2% ± 1.3%, 100.6% ± 2.0%, and 103.3% ± 2.7% for 1, 10, and 50 Hz; \( P > 0.05 \) vs. baseline; Figs. 4a–c), whereas HFS at 100 or 200 Hz induced reliable LTP (open circles, \( n = 3 \) for each panel, 124.0% ± 1.5% and 131.9% ± 2.0% for 100 and 200 Hz; \( P < 0.05 \) vs. baseline; Figs. 4d,e). However, in rats on 4 days withdrawal, stimulation at 1 Hz failed to affect synaptic efficacy (black circles, \( n = 3, 101.5\% \pm 0.9\% \); \( P > 0.05 \) vs. baseline; Fig. 4a). Stimulation at 10 Hz induced a small LTP (black circles, \( n = 3, 106.0\% \pm 1.8\% \); \( P > 0.05 \) vs. baseline; Fig. 4b), and those at 50, 100, and 200 Hz induced robust LTP (black circles, \( n = 3 \) for each panel except \( n = 5 \) for e panel, 120.7% ± 2.6%, 126.0% ± 2.6%, and 151.4% ± 1.3% for 50, 100, and 200 Hz, \( P < 0.05 \) vs. baseline; Figs. 4c–e). The stimulus frequencies at 10, 50 but not 100 Hz might be more appropriate to examine the threshold for LTP induction since 100 Hz protocol induced similar LTP in Sal and 4 days withdrawal rats. These findings suggested that the reduced threshold for LTP induction could be responsible for a large LTP on 4 days withdrawal.

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The NR2A-Containing NMDAR-Mediated EPSC was Enhanced on 4 Days Withdrawal

Finally, we examined EPSCs in hippocampal CA1 area of slices of rats on 4 days withdrawal. We did not find any changes of the AMPAR-mediated EPSC (Sal, $n = 7,493.2 \pm 70.5$ pA; 4d, $n = 9,410.9 \pm 64.9$ pA; $P > 0.05$ Sal vs. 4d; Fig. 5a), but the NMDAR-mediated EPSCs were increased compared with saline control (Sal, $156.2 \pm 36.3$ pA; 4d, $343.4 \pm 54.5$ pA; $P < 0.05$ Sal vs. 4d; Fig. 5c), suggesting that the induction mechanism of LTP rather than the basal synaptic transmission may be involved. Furthermore, the increased NMDAR-mediated EPSCs were accompanied by the increased NR2A-containing NMDAR-mediated EPSC and the decreased NR2B-containing NMDAR-mediated EPSC. This resulted in a large increase of $I_{NR2A}/I_{NR2B}$ ratio (Sal, $n = 8,1.93 \pm 0.19$; 4d, $n = 8, 3.71 \pm 0.38$; $P < 0.05$; Fig. 5b). Thus, the adaptive changes of NMDAR complex may also contribute to the facilitation of hippocampal LTP on 4 days withdrawal, since the NR2A- and NR2B-containing NMDARs are believed to govern the direction of hippocampal LTP and LTD, respectively (Liu et al., 2004).

**DISCUSSION**

Major finding was that 4 days of opiate withdrawal facilitated hippocampal LTP, which might be due to the adaptive changes of GRs and NMDARs that reduced the threshold for...
LTP induction. These findings may be relevant to withdrawal experience-dependent modifications of the hippocampal functions.

Opiate Withdrawal Altered the Effect of Stress on Hippocampal LTP

It is known that stress impairs LTP (Foy et al., 1987; Shors et al., 1990; Diamond et al., 1992) but facilitates LTD (Kim et al., 1996; Xu et al., 1997; Yang et al., 2005) in the hippocampus. The underlying mechanisms are believed to engage the activation of GRs or NR2B-containing NMDARs (Xu et al., 1998; Kim and Diamond, 2002; Yang et al., 2004, 2005). Contrarily, we reported here that the stress evoked by opiate withdrawal facilitated hippocampal LTP, reflecting remarkable alterations of the brain stress circuits during 4 days of opiate withdrawal (Sinha, 2001). Our recent report demonstrates that low frequency stimulation induces Schaffer-CA1 LTD and temporoparaminic-CA1 LTP (Yang et al., 2006), a phenomenon consistent with homeostatic theory of synaptic plasticity (Royer and Pare, 2003), but stress disrupts the balance that enables LTD in both pathways (Yang et al., 2006). Moreover, our recent report demonstrates that repeated morphine treatment prevents the facilitation effect of stress on hippocampal LTD induction (Yang et al., 2004), and here we failed to induce LTD with or without additional stress in rats withdrawn for 4 days (data not shown). Thus, the facilitated hippocampal LTP on 4 days withdrawal may also reflect the disruption of homeostasis of synaptic plasticity, unique modifications of hippocampal function depended on opiate withdrawal.

Both GCs and GRs may be changed during 4 days of morphine withdrawal. Evidence demonstrates that withdrawal symptoms persist for the first 4 days of morphine withdrawal (Dong et al., 2006). Similarly, corticosterone, the major GC hormones in the rat, remains elevated levels for over 3 days of morphine withdrawal (Houshyar et al., 2001). Maybe, because of selectively downregulation of GR expression in the hippocampus after acute morphine withdrawal (McNally and Akil, 2003), animals may become more sensitive to stress, by which evokes a higher and prolonged elevation of plasma corticosterone level (Houshyar et al., 2001). Furthermore, evidence demonstrates that GRs are able to regulate the expression and function of NMDARs (Wang et al., 2005). Therefore, the stress evoked by 4 days of morphine withdrawal may be a chronic stress, which has been activating GRs and NR2B-containing NMDARs for 4 days, leading to downregulation of NR2B-containing NMDARs to facilitate LTP induction. Consistent with this view, we report that the NR2B-containing NMDAR antagonist restores the acute stress-impaired hippocampal LTP (Wang et al., 2006). Thus, acute stress may influence synaptic plasticity but chronic stress may affect metaplasticity, which is termed by a phenomenon that prior cellular or synaptic activity (e.g., the activation of NMDARs) influences the direction or threshold for the subsequent induction of synaptic plasticity (Coan et al., 1989; Huang et al., 1992).

Opiate Withdrawal Altered NMDARs

NMDARs play crucial roles in synaptic plasticity (Morris et al., 1986). Recent reports suggest that NR2A- and NR2B-
containing NMDARs govern the direction of synaptic plasticity (Liu et al., 2004). Here we found that neither input–output relationship nor the basal synaptic transmission was changed during opiate withdrawal. Consistent with this finding, the AMPAR-mediated EPSC remained unchanged but the NMDAR-mediated EPSCs were increased on 4 days withdrawal. Furthermore, the EPSC mediated by NR2A-containing NMDARs was increased, but that mediated by NR2B-containing NMDARs was decreased, resulted in increased INR2A/INR2B ratio. The NR2B-containing NMDAR antagonist was Ro25–6981 at 0.5 μM, which produced an effect similar to that of ifenprodil at 3 μM (data not shown), on the NMDAR-mediated EPSCs. Furthermore, intrahippocampal infusion of Ro25–6981 (2.3 μg in 6 μl, i.c.v. for 10 min) completely prevents the stress effect on hippocampal LTP and LTD (Wang et al., 2006). These findings suggest that changes in the subunit composition of NMDARs (or the ratio of INR2A/INR2B) after 4 days of morphine withdrawal results in a shift in the frequency threshold for LTP induction, consistent with both the sliding threshold hypothesis (Malenka and Bear, 2004) and the finding of the selective NMDAR subunit requirement for LTP vs. LTD (Liu et al., 2004).

In conclusion, the present study provides evidence that opiate withdrawal turns impairment effect of stress into facilitating hippocampal LTP, in which both GRs and subtypes of NMDARs are involved, contributing to the adaptive modifications of hippocampal functions during opiate withdrawal.

**DETAILED METHODS**

Male Sprague Dawley rats, weighing 200–250 g, were used. Animal care and experimental protocol was approved by Chinese Academy of Sciences, People's Republic of China.

**Electrophysiology In Vivo**

Animals were under pentobarbitone anesthesia (50–60 mg/kg, i.p.). Recordings of the field EPSPs were made from the CA1 stratum radiatum of the hippocampus in response to stimulation of the Schaffer collateral/commissural pathway using techniques described previously (Xu et al., 1998; Yang et al., 2004). Recording and stimulating electrodes were made by gluing together a pair of twisted Teflon-coated 90% platinum/10% iridium wires (50 μm inner diameter, 75 μm outer diameter, World Precision Instruments, Sarasota, FL). Test EPSPs were evoked at a frequency of 0.033 Hz and at a stimulus intensity adjusted to give an EPSP amplitude 50% of the maximum. Low frequency stimulation protocol consisted of 900 pulses at 1 Hz, and conditioning stimulation protocols for inducing LTP consisted of 10 trains of 20 pulses at 10, 50, 100, or 200 Hz, at 2 s intertrain intervals. EPSP amplitude was expressed as mean ± SEM% of the baseline EPSP amplitude recorded over at least a 40-min baseline period, and those in the last 5 min recordings were averaged in one animal and then cross animals to give a magnitude for the group. The sites of stimulation and recording electrodes were routinely verified by postmortem examination.

**Electrophysiology In Vitro**

EPSCs in rat slices were recorded by using techniques similar as those described previously (Ungless et al., 2001; Zhang et al., 2005). The rat brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (ACSF) in vibroslicer chamber. Coronary-sectioned 400-μm-thick hippocampal slices were cut and transferred into a submersion-type incubation chamber with 300 ml ACSF heated to 35°C ± 1°C for 1 h recovery. ACSF contained (in mM): NaCl 120, KCl 2.5, NaHCO3 26, NaH2PO4 1.25, CaCl2 2.0, MgSO4 2.0, and d-glucose 10 and saturated by continuously perfusion of the gas mixture of 95% O2 and 5% CO2. Then, the slice was gently transferred into a recording chamber, and held submerged between two nylon nets and maintained at room temperature (22–25°C). Recording chamber was placed on the stage of an upright Nikon microscope equipped with a 10× objective and a 10× ocular (600FN, Japan). The microscope was used to identify the CA1 region of the hippocampus, and EPSCs were recorded by using blind-patch approach in the pyramidal cell in response to electrical stimulation of the Schaffer pathway. Stimulating electrode was made by gluing together a pair of twisted Teflon-coated 90% platinum/10% iridium wires (50 μm inner diameter, 75 μm outer diameter, World Precision Instruments, Sarasota, FL). Patch pipette was pulled from boro-silicate glass tubing (1.5 mm outer diameter, 0.84 mm inner diameter, World Precision Instruments, Sarasota, FL) with a Brown-Flaming micropipette puller (P-87; Sutter Instruments Company, USA). The whole-cell recordings were obtained by using electrodes (3–6 MΩ) containing (in mM): potassium glutamate 130, KCl 10, CaCl2 1, NaCl 6, HEPES 20, EGTA 10, Mg-ATP 3, Na-GTP 0.5, and QX-314 5, pH 7.2. Recording of EPSCs was made by bath application of 100 μM picrotoxin to block the GABA A receptor-mediated currents, and were adjusted at a stimulus intensity to evoke about 50% of the maximal EPSCs. The NMDAR mediated–EPSCs were isolated by bath application of 10 μM CNQX and measured at a holding potential of +60 mV. Once stable NMDAR mediated–EPSCs were obtained, the selective NR2B subunit antagonist Ro25–6981 (0.5 μM) was applied to assess the NR2A-component EPSC. The NR2B-component EPSC was obtained by the difference between the NMDAR mediated-EPSCs and NR2A-component EPSC. Recordings were made by using an Axopatch 200B amplifier; signals were filtered at 5 kHz and digitized to 20 kHz and stored on computer. Each type of the EPSCs was recorded for 5 min (10 sweeps), which are reported as mean ± SEM, n being the number of slice.

**Drug Treatment**

Animals were treated with morphine (10 mg/kg, s.c.) or saline (0.6 ml/kg, i.p.) twice per day at 12 h intervals for 12 days similar as those described previously (Trujillo and Akil, 1991; Pu et al., 2002; Yang et al., 2004). Then, the rats were

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subjected to morphine withdrawal for 2–18 h, and 4, 7, and 20 days, while the animals showed obvious signs of stress such as grooming, diarrhea etc. on 18 h, 4 and 7 days of morphine withdrawal (Dong et al., 2006). Some animals were treated with the GR antagonist RU38486 (20 mg/kg, s.c.) twice per day for the first 3 days of withdrawal, and the others were treated with an extinction dose of morphine (15 mg/kg, s.c.) 12 h before the first HFS on 4 days withdrawal, to avoid its acute effect.

**Stress Protocol**

Behavioral stress was given at 16 h withdrawal, and after about 2 h spent in the preparations for EPSP recordings, and the first HFS was applied to induce LTP on 18 h withdrawal. Behavioral stress was evoked similar as those described previously (Xu et al., 1997; Rocher et al., 2004; Yang et al., 2004). Briefly, animals were placed on an elevated platform (10 × 10 cm² and 1.6 m high in the middle of a bright room) for 30 min.

**Data Analysis**

LTP comparisons were made by using t-test compared with 40-min baseline. The magnitude of LTP was the average of the last 10-min recordings. Between-groups comparisons were conducted by one-way ANOVA followed by least significance difference test (SPSS 11.0). Significance level was set at *P* < 0.05.

**References**


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