Cellular/Molecular

# The Neuropeptide Nociceptin Is a Synaptically Released Endogenous Inhibitor of Hippocampal Long-Term Potentiation

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**Hippocampal long-term potentiation (LTP) of excitatory synaptic transmission has been regarded as a cellular model of learning and memory. Its induction is regulated by many functional molecules at synapses, including the neuropeptide nociceptin, which is identified as an endogenous ligand for the orphan opioid receptor. Mutant mice lacking the receptor exhibit enhanced LTP and hippocampus-dependent memory formation; however, the precise molecular and cellular mechanism is largely unknown. Here, we show that LTP in the hippocampal CA1 region is inhibited by nociceptin synaptically released from interneurons by tetanic stimulation. This endogenous nociceptin downregulates the excitability of pyramidal cells by the hyperpolarization induced by the activation of $K^+$ channels, which are the common target shared with $GABA_B$ receptors, although the mode of action is considerably different. Interestingly, the modulation of LTP by endogenous nociceptin is not observed when theta-burst stimulation is used instead of tetanic stimulation, suggesting that relatively longer high-frequency synaptic activation is required for the release of endogenous nociceptin. These results indicate that, in addition to GABA, nociceptin released from interneurons by their high-frequency activation is a novel endogenous neuromodulator that negatively regulates LTP induction in the hippocampus through direct modulation of pyramidal cells.**

*Keywords:* **hippocampus; long-term potentiation (LTP); plasticity; memory formation; G-protein-coupled receptor (GPCR); neuropeptide**

## Introduction

The hippocampus is a critical brain structure associated with certain types of memory (Milner et al., 1998), and synaptic plasticity in the hippocampus, represented by long-term potentiation (LTP), has been regarded as a cellular model of memory formation (Lynch, 2004). It is generally agreed that LTP in the hippocampal CA1 region is induced by activation of the postsynaptic NMDA receptor, which results in an increase in postsynaptic $Ca^{2+}$ concentrations (MacDermott et al., 1986). The increased $Ca^{2+}$ activates biochemical processes in the postsynaptic cell, such as the calcium/calmodulin-dependent protein kinase II signaling pathway, leading to long-lasting synaptic strengthening (Lisman et al., 2002). Thus, the NMDA receptor plays an essential role in LTP induction in the hippocampal CA1 region (Mori and Mishina, 1995).

Although the main signaling pathway for LTP induction has been elucidated precisely, there are various functional molecules at the synapse that regulate the induction and expression of LTP, such as receptors for neurotransmitters and neuromodulators, intracellular signaling molecules, and adhesion molecules. As one of these molecules, the neuropeptide nociceptin (Noc; also called orphanin FQ) has been shown to be associated with LTP as well as learning and memory. Nociceptin was identified as an endogenous ligand for the orphan opioid receptor (Meunier et al., 1995; Reinscheid et al., 1995). Although all of the endorphins except nociceptin exert their effects through interneurons, nociceptin is the only endorphin that directly acts on the cell body of pyramidal cells (Nicoll et al., 1980; Ikeda et al., 1997), suggesting that the nociceptin–nociceptin receptor pathway is likely associated closely with synaptic plasticity. In fact, we have demonstrated previously that knock-out mice lacking the nociceptin receptor exhibit enhanced hippocampal LTP as well as better performance in the Morris water maze and passive avoidance task (Manabe et al., 1998). In addition, it has been reported that exogenously applied nociceptin inhibits LTP in the CA1 region (Yu et al., 1997) and the dentate gyrus (Yu and Xie, 1998). Furthermore, a few reports have suggested that nociceptin modifies spatial learning in a dose-dependent manner (Sandin et al., 1997; Sandin et al., 2004) as well as the passive avoidance task and spontaneous alteration in the Y-maze (Hiramatsu and Inoue, 1999). Thus, these results suggest that nociceptin may regulate the process of LTP induction and memory formation in normal animals; however, the molecular and cellular mechanism is almost totally unknown. Furthermore, the origin of nociceptin is also undetermined.

In this study, we demonstrated that LTP in the hippocampal CA1 region was inhibited by endogenous nociceptin in an activity-dependent manner. Thus, nociceptin was synaptically

Received Dec. 7, 2006; revised March 28, 2007; accepted March 28, 2007.

This work was supported by Grants-in-Aid for Scientific Research (T.M.) and the Center for Brain Medical Science, 21st Century Center of Excellence Program (S.B.-p., T.M.) from the Ministry of Education, Science, Sports, Culture, and Technology of Japan, by Research Institute of Science and Technology for Society, Japan Science and Technology Agency (T.M.), by The Novartis Foundation (Japan) for the Promotion of Science, and by The Uehara Memorial Foundation. We are grateful to Drs. Yuko Sekino, Shizuka Kobayashi, and Ayako M. Watabe (University of Tokyo, Tokyo, Japan) for their helpful comments on this manuscript.

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DOI:10.1523/JNEUROSCI.0876-07.2007

 0270-6474/07/274850-09$15.00/0

released from interneurons by high-frequency stimulation in the stratum radiatum, which then evoked hyperpolarizing currents in CA1 pyramidal cells. As a result, the excitability of pyramidal cells was drastically downregulated, which resulted in the suppression of LTP induction. This suppression is similar to that caused by $GABA_B$ receptors, although the time course of the suppression was much slower in the case of nociceptin, and is likely to share the responsible ionic channel for the hyperpolarization with the $GABA_B$ receptor-mediated LTP suppression. Therefore, in addition to GABA, nociceptin released by high-frequency synaptic activation is another critical endogenous factor that negatively regulates LTP induction in the hippocampus.

## Materials and Methods

*Animals.* All experiments were performed according to the guidelines of the Animal Care and Experimentation Committee of University of Tokyo. C57BL/6J mice (male; 6–10 weeks of age) were used in all experiments.

*Slice preparations.* Mice were deeply anesthetized with halothane, and both hippocampi were quickly removed from the brain after decapitation. Transverse hippocampal slices (400 $\mu$m thick) were prepared with a vibratome tissue slicer and were placed in a humidified interface-type holding chamber for at least 1 h before electrophysiological experiments.

*Solutions.* The artificial CSF (ACSF) contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.0 $NaH_2PO_4$, 26.2 $NaHCO_3$, and 11 glucose. The ACSF was used for preparing slices. The ACSF containing 100 $\mu$M picrotoxin, a $GABA_A$ receptor antagonist, was used for all of the experiments except for the recording of population spikes and $GABA_A$ receptor-mediated IPSCs ($GABA_A$-IPSCs).

*Electrophysiology.* A single slice was transferred into a submerge-type recording chamber, fixed with a nylon net, and submerged beneath the continuously perfusing ACSF (at a rate of 1.5–2.0 ml/min) that had been saturated with 95% $O_2$/5% $CO_2$. A cut was made to separate the CA3 region from the CA1 region to avoid epileptiform activity from the CA3 region. All experiments were performed at 25°C unless otherwise stated. To evoke synaptic responses, a bipolar tungsten stimulating electrode was placed in the stratum radiatum at 200–300 $\mu$m from the recording site, and Schaffer collaterals were stimulated at 0.1 Hz. An Axopatch-1D amplifier (Molecular Devices, Union City, CA) was used in all experiments, and the records were stored on a personal computer for later analyses. Field-potential recordings were made using a glass recording pipette filled with 3 M NaCl placed in the stratum radiatum or the pyramidal cell layer of the CA1 region. The stimulus strength was adjusted so that EPSPs of 0.10–0.15 mV/ms slopes or population spikes of 0.7–1.2 mV in amplitude were evoked. Whole-cell patch-clamp recordings were made from CA1 pyramidal cells with the blind technique at −70 mV in the voltage-clamp mode. Recording patch pipettes were filled with an internal solution containing the following (in mM): either 122.5 Cs gluconate, 17.5 CsCl, 10 HEPES, 0.2 EGTA, 8 NaCl, 2 MgATP, and 0.3 $Na_3GTP$ (pH 7.2, 290–310 mOsm) for recording $GABA_A$-IPSCs and NMDA receptor-mediated EPSCs (NMDA-EPSCs); or 122.5 K gluconate, 17.5 KCl, 10 HEPES, 0.2 EGTA, 8 NaCl, 2 MgATP, and 0.3 $Na_3GTP$ (pH 7.2, 290–310 mOsm) for recording $GABA_B$-IPSCs and hyperpolarizing currents. The values of the membrane potential were compensated for the liquid junction potential at the electrode tip. The stimulus strength was adjusted so that it gave rise to $GABA_A$-IPSCs of the amplitude value between 100 and 200 pA and $GABA_B$-IPSCs of the amplitude value between 10 and 50 pA. For IPSC recordings, the perfusing ACSF contained blockers for ionotropic glutamate receptors: 10 $\mu$M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA receptor antagonist, and 25 $\mu$M D-2-amino-5-phosphonovaleric acid (D-APV), an NMDA receptor antagonist; for hyperpolarizing-current recordings, the perfusing ACSF contained 10 $\mu$M CNQX, 50 $\mu$M D-APV, and 2 $\mu$M CGP55845 [(2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl] (phenylmethyl)phosphinic acid] (CGP), a $GABA_B$ receptor antagonist. NMDA-EPSCs were recorded with whole-cell patch-clamp recording techniques in the voltage-clamp mode at +20 mV in the presence of 10 $\mu$M CNQX. All of the representative traces shown in the figures are the averages of 10 consecutive responses. Nociceptin, [Nphe$^1$,Arg$^{14}$,Lys$^{15}$]nociceptin-NH2 [UFP-101 (UFP)], enkephalin, D-APV, CNQX, and CGP were obtained from Tocris Cookson (Bristol, UK), and baclofen was obtained from Sigma-Aldrich (St. Louis, MO).

*Statistical analysis.* All values are expressed as the mean ± SEM. Statistical analysis was performed using Student's *t* test to determine whether there was a statistically significant difference between the means.

## Results

### Synaptically released endogenous nociceptin inhibits LTP through the regulation of excitability of CA1 pyramidal cells

EPSPs were recorded in the CA1 region of hippocampal slices with extracellular field-potential recording techniques. Exogenously applied nociceptin (100 nM) had no effect on LTP induced by tetanic stimulation (100 Hz for 1 s) (control, 164.4 ± 5.8% of baseline; $n = 7$; Noc, 161.8 ± 8.2% of baseline; $n = 7$; $p = 0.784$) (Fig. 1*A*). On the contrary, LTP was enhanced in the presence of the nociceptin receptor antagonist UFP-101 (1 $\mu$M) (control LTP, 162.9 ± 2.9% of baseline; $n = 9$; UFP LTP, 187.1 ± 2.1% of baseline; $n = 9$; $p = 0.000320$) (Fig. 1*B*), which is consistent with our previous report showing the enhanced LTP in nociceptin receptor mutant mice (Manabe et al., 1998). UFP alone without tetanic stimulation had no effect on basal synaptic transmission (UFP control, 30–40 min; 95.5 ± 3.0% of baseline; $n = 6$; $p = 0.233$) (Fig. 1*B*, open squares). Similar effects of UFP (1 $\mu$M) on LTP were also observed at higher temperature (32°C) (control, 149.8 ± 9.6% of baseline; $n = 5$; UFP, 183.8 ± 12.3% of baseline; $n = 5$; $p = 0.0135$). These results indicated that endogenous nociceptin suppressed LTP, but exogenous nociceptin had no suppressing effect on LTP induced by tetanic stimulation.

The induction of LTP in the hippocampal CA1 region is dependent on NMDA receptors (Lynch, 2004), and the enhanced LTP in the presence of UFP might be caused by the modification of NMDA-EPSCs. Amplitudes of NMDA-EPSCs recorded with the whole-cell patch-clamp recording technique were not affected by either nociceptin (100 nM; 100.6 ± 1.7% of baseline; $n = 4$; $p = 0.929$) or UFP (1 $\mu$M; 101.2 ± 1.6% of baseline; $n = 4$; $p = 0.912$). Furthermore, the kinetics of NMDA-EPSCs was not affected by nociceptin (100 nM) (time to peak, control, 15.6 ± 1.0 ms; Noc, 15.4 ± 0.9 ms; $n = 4$; $p = 0.402$; decay time constant, control, 85.4 ± 8.3 ms; Noc, 87.7 ± 9.4 ms; $n = 4$; $p = 0.414$). UFP had also no effect on the kinetics of NMDA-EPSCs (data not shown). These results indicated that the enhancement of LTP in the presence of UFP was not associated with the modification of properties of the NMDA receptor.

We have previously shown that nociceptin activates G-protein-activated inward rectifier $K^+$ (GIRK) channels in hippocampal pyramidal cells (Ikeda et al., 1997). Thus, endogenous nociceptin may suppress LTP induction by activating GIRK channels that hyperpolarize pyramidal cells during tetanic stimulation, resulting in less activation of NMDA receptors. In fact, the depolarizing envelope during the tetanic stimulation in the presence of UFP (1 $\mu$M; 1020 ms from the onset of tetanic stimulation; 152.5 ± 17.7% of baseline EPSP amplitude; $n = 8$) was significantly larger than in its absence (92.3 ± 19.2% of baseline EPSP amplitude; $n = 8$; $p = 0.0185$) (Fig. 1*C*), suggesting that endogenous nociceptin reduced the depolarization of pyramidal cells. To elucidate whether nociceptin affected the excitability of pyramidal cells, we examined the effect of nociceptin on population spikes recorded in the pyramidal cell layer that represent the excitability of pyramidal cells (Yu et al., 1997), which is closely related to the induction of LTP (Malinow and Miller, 1986). We found that exogenously applied nociceptin reversibly reduced population spike amplitudes in a dose-dependent manner (10

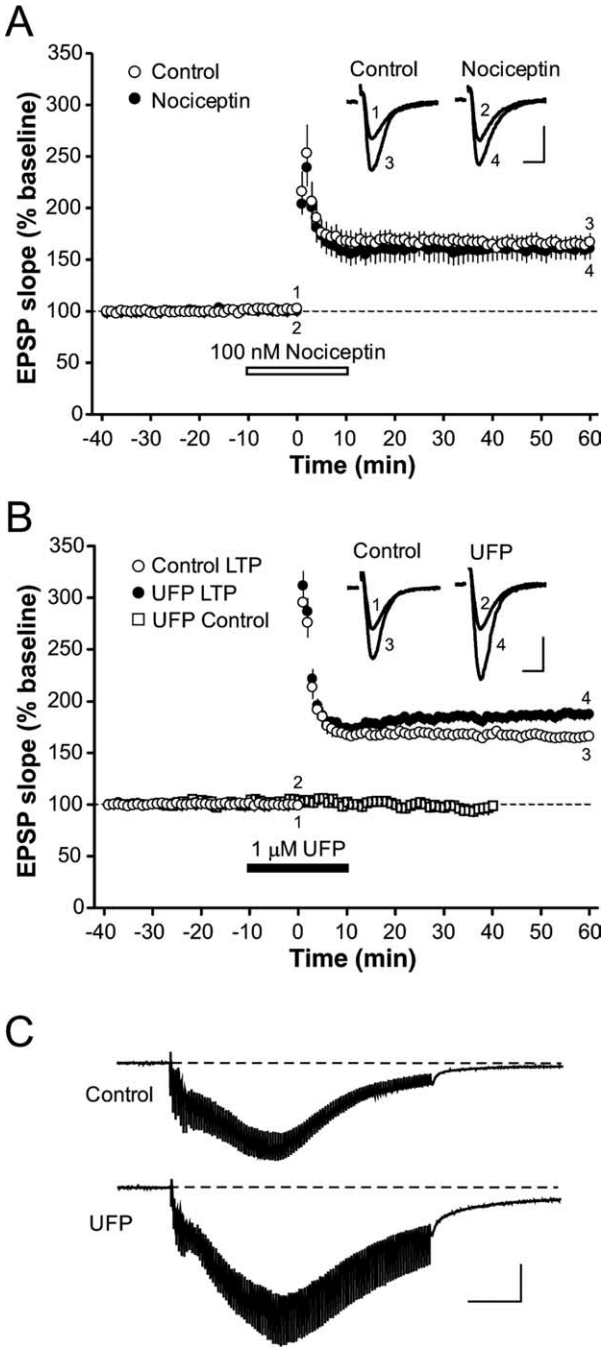


**Figure 1.** LTP is enhanced in the presence of a nociceptin receptor antagonist but not in the presence of exogenously applied nociceptin. ***A***, LTP induced by tetanic stimulation was not altered in the presence of exogenously applied nociceptin. Nociceptin (100 nM) was applied from −10 to 10 min, as shown by the horizontal open bar. Tetanic stimulation (100 Hz for 1 s) was applied at time 0. Inset, Representative traces recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. The potentiation ratio was calculated by dividing the averaged EPSP slope values from 50 to 60 min by those from −30 to 0 min. ***B***, LTP was enhanced in the presence of the nociceptin receptor antagonist UFP (1 μM; UFP LTP) compared with LTP in the absence of UFP (Control LTP). UFP alone had no effect on basal synaptic transmission (UFP Control). UFP was applied from −10 to 10 min, as shown by the horizontal closed bar. Tetanic stimulation (100 Hz for 1 s) was applied at time 0. Inset, Representative traces of the LTP experiment recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. ***C***, Depolarization envelope during tetanic stimulation (100 Hz for 1 s) in field-potential recordings in the absence (Control) and presence (UFP) of 1 μM UFP. Calibration: 200 ms, 0.5 mV. The value of the potential at 1020 ms after the onset of tetanic stimulation was normalized to the mean amplitude of baseline EPSPs (average of 10 consecutive EPSPs just before the tetanus). Depolarization just after the tetanic stimulation was significantly larger in the presence of UFP than in its absence.

nM, 98.0 ± 2.7% of baseline, $n = 5$, $p = 0.416$; 50 nM, 79.3 ± 2.8% of baseline, $n = 5$, $p = 0.00727$; 100 nM, 53.7 ± 6.3% of baseline, $n = 5$, $p = 0.00531$; 1 μM, 45.9 ± 3.8% of baseline, $n = 5$, $p = 0.00109$) (Fig. 2*A*,*B*). In contrast, initial EPSP slopes were not affected at all in the same experiments, confirming that nociceptin had no effect on basal synaptic transmission (10 nM, 99.2 ± 2.4% of baseline, $n = 5$, $p = 0.729$; 50 nM, 99.4 ± 3.4% of baseline, $n = 5$, $p = 0.865$; 100 nM, 99.1 ± 2.6% of baseline, $n = 5$, $p = 0.626$; 1 μM, 99.3 ± 2.4% of baseline, $n = 5$, $p = 0.923$) (Fig. 2*C*,*D*). The inhibition in population spikes was almost completely blocked by the specific nociceptin receptor antagonist UFP (1 μM) (Noc, 53.7 ± 6.3% of baseline; $n = 5$; UFP plus Noc, 95.2 ± 3.1% of baseline; $n = 5$; $p = 0.00180$) (Fig. 2*E*,*F*), indicating that the excitability of pyramidal cells was dynamically regulated by nociceptin receptors. It should be noted that population spikes were not affected by UFP alone (101.1 ± 2.7% of baseline; $n = 6$; $p = 0.877$) (Fig. 2*F*), suggesting that ambient nociceptin in slices, if any, had little or no effect on the pyramidal cell excitability. Furthermore, we failed to observe any significant change in the holding current when UFP (1 μM) was applied to the slice (change in the holding current, −4.8 ± 2.7 pA; $n = 6$; $p = 0.133$), confirming that ambient nociceptin had little or no effect on the pyramidal cell excitability and likely exhibited little effect on LTP induction. Thus, it was strongly suggested that synaptically released nociceptin played a critical role in the regulation of LTP induction.

**Modulation of inhibitory synaptic transmission by nociceptin**

Although nociceptin has a direct effect on the excitability of pyramidal cells, it is also possible that LTP induction is indirectly suppressed by the modification of inhibitory synaptic transmission caused by nociceptin receptor activation (Wigström and Gustafsson, 1983). We first examined the action of nociceptin on $GABA_A$-IPSCs with whole-cell patch-clamp recordings from CA1 pyramidal cells. Neither nociceptin (100 nM; 97.7 ± 2.1% of baseline; $n = 7$; $p = 0.903$) (Fig. 3*A*) nor the nociceptin receptor antagonist UFP (1 μM; 102.7 ± 2.0% of baseline; $n = 5$; $p = 0.957$) (Fig. 3*B*) showed any significant effect on $GABA_A$-IPSCs, suggesting that $GABA_A$ receptor-mediated inhibitory synaptic transmission is not associated with the nociceptin-induced LTP suppression.

$GABA_B$ receptor-mediated synaptic transmission has also been reported to hyperpolarize pyramidal cells by activating GIRK channels (Andrade et al., 1986) and to suppress LTP induction in the hippocampal CA1 region (Stäubli et al., 1999). Thus, we next examined whether $GABA_B$-IPSCs are modulated by nociceptin using the internal solution based on $K^+$ gluconate (Fig. 4). Nociceptin (100 nM) inhibited $GABA_B$-IPSCs (to 76.0 ± 1.3% of baseline; $n = 5$; $p = 0.000120$), and this inhibition was significantly suppressed ($p < 0.0001$) by 1 μM UFP (99.9 ± 1.8% of baseline; $n = 7$; $p = 0.861$) (Fig. 4*A*,*B*). Application of UFP alone had no significant effect on $GABA_B$-IPSCs (98.9 ± 0.6% of baseline; $n = 6$; $p = 0.107$) (Fig. 4*B*, UFP). Thus, $GABA_B$ receptor-mediated synaptic transmission was inhibited by nociceptin receptor activation; however, this finding is inconsistent with the decreased excitability of pyramidal cells caused by nociceptin (Fig. 2) because $GABA_B$ receptor-mediated synaptic transmission should hyperpolarize pyramidal cells through an increase in the $K^+$-channel conductance (Newberry and Nicoll, 1984; Dutar and Nicoll, 1988a), and the reduction of $GABA_B$-IPSCs should increase the excitability of pyramidal cells. Therefore, $GABA_B$ receptor-mediated synaptic transmission should not play a significant role in the LTP modulation via the nociceptin system.

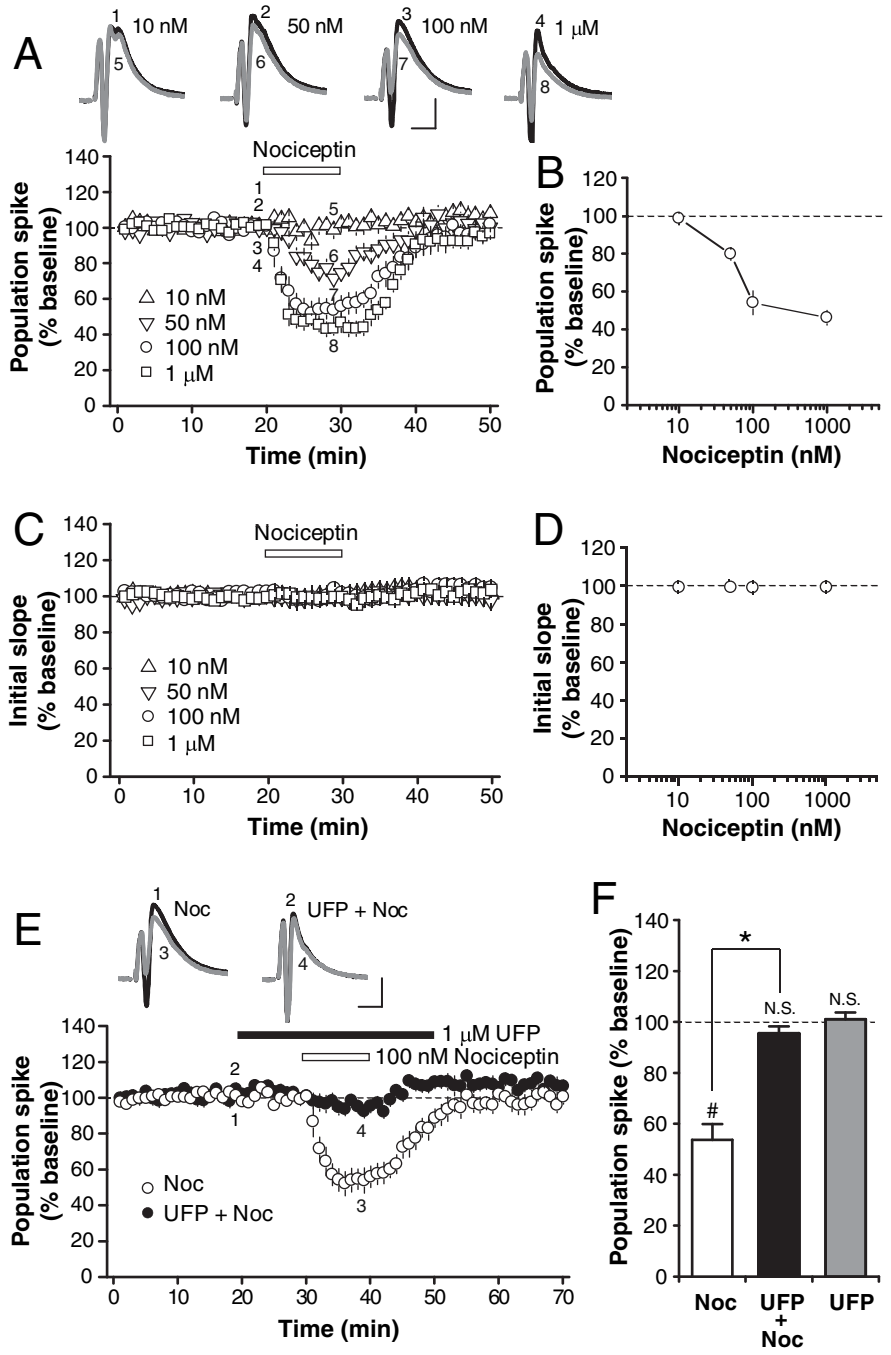


**Figure 2.** Nociceptin suppresses the excitability of CA1 pyramidal cells in a dose-dependent manner. ***A***, Population spikes recorded in the pyramidal cell layer were reduced in amplitude in the presence of nociceptin in a dose-dependent manner. Inset, Representative traces at each concentration of nociceptin recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. ***B***, A dose–response curve of nociceptin-induced suppression of population spike amplitudes. The ratio of averaged population spike amplitudes from 25 to 30 min to those from −20 to 0 min is plotted against nociceptin concentrations. ***C***, Nociceptin had no effect on initial EPSP slopes at any concentrations in the same experiments shown in ***A***. ***D***, Summary of the effect of nociceptin on initial EPSP slopes. ***E***, Nociceptin-induced suppression of population spikes was inhibited by the specific nociceptin receptor antagonist UFP. Nociceptin (100 nM) was applied from 30 to 40 min, as shown by the horizontal open bar, and UFP (1 μM) was applied from 20 to 50 min, as shown by the horizontal closed bar. Inset, Representative traces recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. ***F***, Summary of the effects of nociceptin, UFP, and nociceptin in the presence of UFP (UFP + Noc) on population spike amplitudes. Nociceptin significantly inhibited population spikes (Noc), whereas neither nociceptin in the presence of UFP (UFP + Noc) nor UFP alone (UFP) had any effect on population spikes. $^{*}p < 0.05$ (unpaired *t* test); $^{\#}p < 0.05$ (paired *t* test); N.S., not significant.

Rather, these findings suggest that the nociceptin and $GABA_B$ receptors share the downstream GIRK channel as a final common pathway (Andrade et al., 1986), because nociceptin activates GIRK channels in advance, and available GIRK channels to $GABA_B$ receptors become small in number. To show that $GABA_B$ and nociceptin receptors in the pyramidal cell share the same GIRK channels, we performed the occlusion experiments: the outward $K^+$ current evoked by simultaneous application of 3 μM baclofen, a selective $GABA_B$ receptor agonist, and 100 nM nociceptin (Noc plus Bac, 24.8 ± 3.1 pA; $n = 7$) was no larger than the current evoked by application of either nociceptin (23.8 ± 3.3 pA; $p = 0.383$) or baclofen (22.7 ± 2.1 pA; $p = 0.367$) alone (Fig. 4*C*,*D*). Furthermore, as reported previously (Stäubli et al., 1999), the $GABA_B$ receptor antagonist CGP (2 μM) enhanced LTP (control, 145.6 ± 7.7% of baseline; CGP, 169.4 ± 6.9% of baseline; $n = 7$; $p = 0.00252$) (Fig. 5*A*), indicating that $GABA_B$-IPSCs decreased the excitability of pyramidal cells and inhibited LTP induction; however, when both CGP (2 μM) and UFP (1 μM) were applied, there was no additive enhancement of LTP (control, 145.8 ± 5.5% of baseline; $n = 7$; UFP plus CGP, 171.6 ± 7.9% of baseline; $n = 7$; $p = 0.000410$) (Fig. 5*B*). These results strongly suggested that $GABA_B$ and nociceptin receptors inhibited LTP induction through the same mechanism.

### Activity-dependent release of nociceptin from the interneuron

Because UFP had no effect on the holding current in the whole-cell patch-clamp recording, as shown above, and on the pyramidal cell excitability in the field-potential recording (Fig. 2*C*,*D*), the modification of LTP by nociceptin is most likely mediated by synaptically released nociceptin. As we have shown previously (Ikeda et al., 1997), nociceptin receptor activation evokes a hyperpolarizing current mediated by the GIRK channel, and thus, we examined whether tetanic stimulation in the stratum radiatum evoked membrane currents sensitive to the nociceptin receptor antagonist with the whole-cell patch-clamp recording technique. When the tetanic stimulation (100 Hz for 1 s) was delivered in the stratum radiatum, a hyperpolarizing current lasting a few seconds was evoked, which was partially and reversibly inhibited by 1 μM UFP (control, 9.5 ± 0.8 pA; UFP, 4.5 ± 0.6 pA; wash, 8.0 ± 0.6 pA; $n = 9$; control vs UFP, $p < 0.0001$; UFP vs wash, $p = 0.000191$) (Figure 6*A*,*B*), indi-

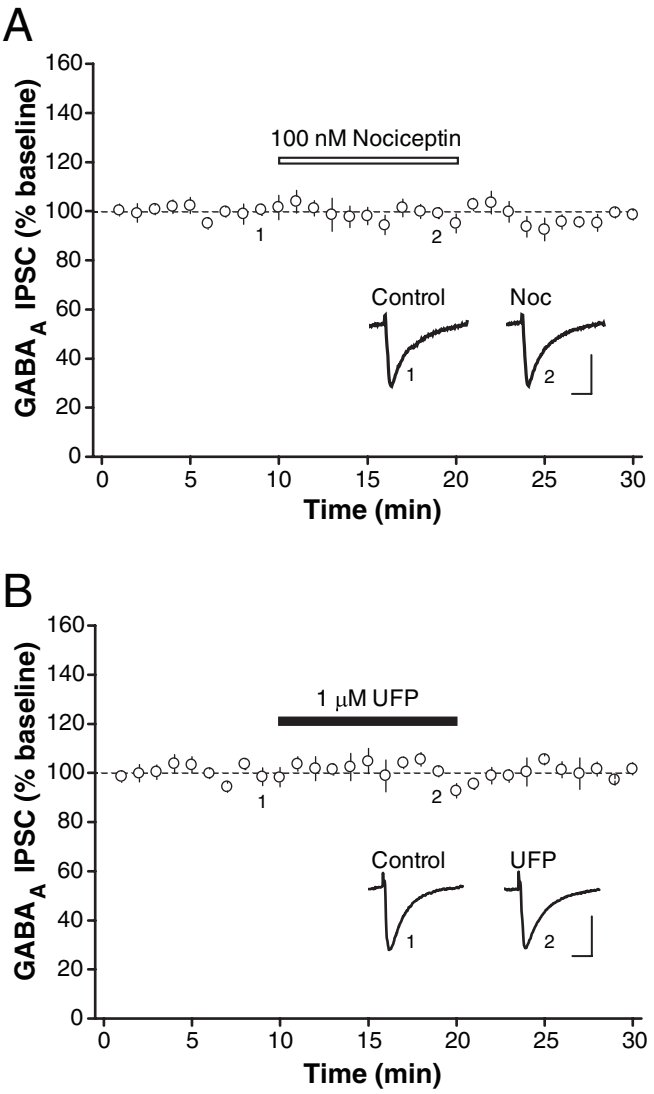


**Figure 3.** GABA$_A$-IPSCs are not affected by nociceptin or the nociceptin receptor antagonist. ***A***, ***B***, GABA$_A$-IPSCs recorded in the CA1 pyramidal cell were unaltered in the presence of 100 nM nociceptin (***A***) or 1 μM UFP (***B***). Calibration: 10 ms, 100 pA. Nociceptin and UFP were applied from 10 to 20 min, as shown by the horizontal bar. Insets, Representative traces recorded at the times indicated by the numbers in the graph. Neither nociceptin nor UFP had any effect on GABA$_A$-IPSCs. Averaged GABA$_A$-IPSC amplitudes from 15 to 20 min were compared with the baseline GABA$_A$-IPSC amplitudes. We confirmed that these synaptic currents were abolished by the GABA$_A$ receptor antagonist picrotoxin (data not shown).

cating that the tetanic stimulation that was used for LTP induction actually released nociceptin, which then evoked the hyperpolarizing current. These results strongly suggested that nociceptin was synaptically released in an activity-dependent manner.

We have reported previously that nociceptin precursors are expressed in interneurons as well as in pyramidal cells in the hippocampus (Ikeda et al., 1997; Manabe et al., 1998). Thus, we next examined the possibility that nociceptin was released from the interneuron. Inhibitory interneurons, but not pyramidal cells, are known to express the opioid receptor in the hippocampus (Stumm et al., 2004), and enkephalin, a μ- and δ-opioid receptor agonist, selectively decreases the excitability of GABAergic interneurons (Madison and Nicoll, 1988; Manzoni et al., 1994). When 10 μM enkephalin was applied to the slice, the hyperpolarizing current was partially and reversibly inhibited (control, 9.4 ± 0.7 pA; enkephalin, 5.2 ± 0.7 pA; wash, 8.7 ± 0.6 pA; $n = 10$; control vs enkephalin, $p = 0.000907$; enkephalin vs wash, $p = 0.00193$) (Fig. 6*C*,*D*), suggesting that nociceptin was released at least from interneurons firing during the tetanic stimulation.

### Release of nociceptin is dependent on the pattern of synaptic activation

Synaptic release of peptides usually requires high-frequency activation of the synapse (Jan and Jan, 1982; Weisskopf et al., 1993), and thus, we finally examined whether the modulation of LTP by the nociceptin system was dependent on the pattern of conditioning for LTP induction. In contrast to the LTP induced by 100 Hz tetanic stimulation, LTP induced by theta-burst stimulation (a train consisted of five bursts at 5 Hz, each burst was composed of four pulses at 100 Hz, and three trains were delivered at 10 s intervals) was significantly reduced by exogenously applied nociceptin (100 nM) (control, 174.6 ± 7.4% of baseline; $n = 7$; Noc, 144.9 ± 5.4% of baseline; $n = 7$; $p = 0.00754$) (Fig. 7*A*), whereas it was not affected by UFP (1 μM) (control, 173.6 ± 8.1% of baseline; $n = 7$; UFP, 172.6 ± 10.0% of baseline; $n = 7$; $p = 0.936$) (Fig. 7*B*). These results suggested that the modulation of LTP by endogenous nociceptin required relatively longer high-frequency stimulation.

## Discussion

### The origin of nociceptin

Because LTP is enhanced in mutant mice lacking nociceptin receptors (Manabe et al., 1998), endogenous nociceptin should have some action on LTP through nociceptin receptor activation; however, it has not been elucidated whether ambient or synaptically released nociceptin plays a role in this regulation. In this study, we demonstrated that ambient nociceptin in the hippocampal slice, if any, had apparently no effect on synaptic transmission and excitability of pyramidal cells. First, as shown in Figure 2*F* (UFP), the nociceptin receptor antagonist UFP had no effect on population spikes, whereas exogenous nociceptin reduced them. Second, as shown in Figure 4*B* (UFP), UFP exhibited no effect on GABA$_B$-IPSCs, whereas exogenous nociceptin decreased them. Last, the holding current in the whole-cell patch-clamp recordings was unchanged by UFP application, although exogenous nociceptin evoked hyperpolarizing currents (Ikeda et al., 1997) (Fig. 4*C*). All of these findings strongly suggest that synaptically released, but not ambient, nociceptin has physiological action on synaptic transmission and postsynaptic excitability.

Nociceptin is generated by the cleavage of its precursor prepronociceptin, which is abundantly expressed in pyramidal cells and interneurons in the hippocampus (Ikeda et al., 1997; Manabe et al., 1998). Thus, nociceptin can be released from the nerve terminal of Schaffer collaterals originating in CA3 pyramidal cells or released from inhibitory interneurons in the CA1 region. In this study, we have identified interneurons as a likely source of released nociceptin (Fig. 6*C*,*D*), although we cannot exclude the possibility that nociceptin is also released from the nerve terminal of Schaffer collaterals. Thus, the electrical stimulation in the stratum radiatum may activate not only Schaffer collaterals but also interneurons located in the layer, although it is rather unlikely, because the stimulating electrode is considerably far away from the recording site (200–300 μm) (Kauer, 1999). In more physiological conditions in the presence of excitatory synaptic transmission, interneurons may be repetitively activated synaptically by Schaffer collaterals. The release of nociceptin required high-frequency activation of the interneuron, because the baseline frequency of electrical stimulation (0.1 Hz) failed to induce noci-

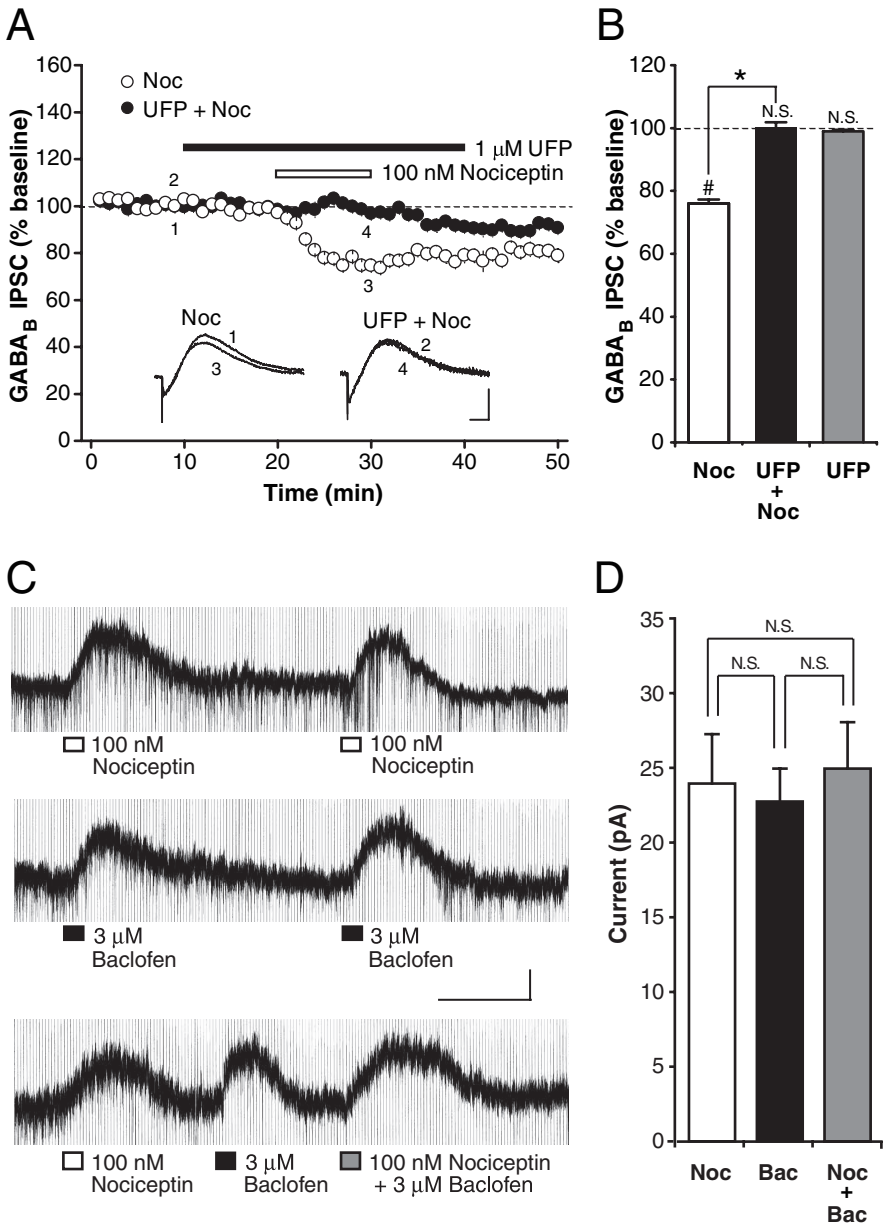


**Figure 4.** Nociceptin suppresses $GABA_B$-IPSCs. ***A***, $GABA_B$-IPSCs were significantly suppressed by 100 nM nociceptin, and this suppression was inhibited by the nociceptin receptor antagonist UFP (1 μM). Nociceptin was applied from 20 to 30 min, as shown by the horizontal open bar, and UFP was applied from 10 to 40 min, as shown by the horizontal closed bar. Inset, Representative traces recorded at the times indicated by the numbers in the graph. Calibration: 100 ms, 10 pA. We confirmed that these synaptic currents were abolished by the $GABA_B$ receptor antagonist CGP (data not shown). ***B***, Summary of the effects of nociceptin, UFP, and nociceptin in the presence of UFP (UFP + Noc) on $GABA_B$-IPSC amplitudes. Nociceptin significantly inhibited $GABA_B$-IPSCs (Noc), whereas neither nociceptin in the presence of UFP (UFP + Noc) nor UFP alone (UFP) had any effect on $GABA_B$-IPSCs. Averaged $GABA_B$-IPSC amplitudes from 25 to 30 min were compared with the baseline $GABA_B$-IPSC amplitudes. ***C***, Representative traces of the hyperpolarizing $K^+$ current in the CA1 pyramidal cell in voltage-clamp recordings (holding potential, −70 mV). Top, Nociceptin (100 nM for 1 min)-evoked outward currents. Middle, Baclofen (3 μM for 1 min)-evoked outward currents. Bottom, Outward currents evoked by nociceptin and baclofen applied alone or together. The second application of nociceptin (nociceptin first application, 20.4 ± 3.3 pA; nociceptin second application, 20.3 ± 3.0 pA; $n = 6$; $p = 0.964$) (top) or baclofen (baclofen first application, 23.5 ± 3.3 pA; baclofen second application, 23.7 ± 4.8 pA; $n = 6$; $p = 0.943$) (middle) evoked amplitudes of outward currents similar to those evoked by the first application. Calibration: 5 min, 10 pA. The currents evoked by voltage steps to calculate series and input resistances were truncated for clarity. ***D***, Summary of the outward currents evoked by nociceptin and baclofen. Outward currents evoked by simultaneous application of nociceptin and baclofen (Noc + Bac) was no larger than the current evoked by application of either nociceptin (Noc) or baclofen (Bac) alone. $^*p < 0.05$ (unpaired *t* test); $^\#p < 0.05$ (paired *t* test); N.S., not significant.

ceptin receptor-dependent suppression of population spikes (Fig. 2*F*, UFP) or $GABA_B$-IPSCs (Fig. 4*B*, UFP). Synaptic release of peptides usually requires high-frequency activation of the synapse (Jan and Jan, 1982; Weisskopf et al., 1993), and this also seems to be the case in the process of nociceptin release, and thus, LTP modulation by endogenous nociceptin is dependent on the pattern of the conditioning for LTP induction (Fig. 7).

### The role of nociceptin in synaptic transmission

Although a previous report indicates that nociceptin inhibits EPSPs in the rat hippocampal slices (Yu et al., 1997), we have never observed any effect of nociceptin on basal excitatory synaptic transmission (Yu and Xie, 1998; Higgins et al., 2002) (Figs. 1*A*, 2*C*,*D*, 7*A*). We have also shown that nociceptin inhibits population spikes (Fig. 2) and induces hyperpolarizing currents in pyramidal cells (Ikeda et al., 1997) (Fig. 4*C*). If nociceptin hyperpolarizes pyramidal cells in field-potential recordings, EPSPs should become larger, because the hyperpolarization must increase the driving force for EPSPs. However, this is not the case, and thus, nociceptin may not have apparent action on pyramidal cells at the resting membrane potential but inhibit the excitability of pyramidal cells depolarized by repetitive stimulation (Fig. 1*C*) and/or action potentials evoked with stronger stimulus strengths (Fig. 2). Because inhibitory interneurons are heavily innervated by Schaffer collaterals (McBain and Fisahn, 2001), interneurons are likely to fire repetitively when CA1 pyramidal cells are repeatedly activated by Schaffer collaterals. Thus, the modification of LTP induction by the nociceptin system can occur even in more physiological conditions. Because nociceptin is released only when interneurons repeatedly fire, this inhibitory mechanism will function as the frequency-dependent gating of information flow from the CA3 region, in contrast to the GABA-mediated inhibition, which is functional even when interneurons fire only once.

$GABA_B$ receptor-mediated synaptic transmission is an effective inhibitor of LTP in the hippocampal CA1 region (Stäubli et al., 1999). In fact, we have confirmed that the $GABA_B$ receptor antagonist CGP significantly enhances LTP (Fig. 5*A*). We found that nociceptin inhibits $GABA_B$-IPSCs (Fig. 4*A*,*B*), which should cause disinhibition, and LTP should be enhanced when nociceptin receptors are activated. However, our present results

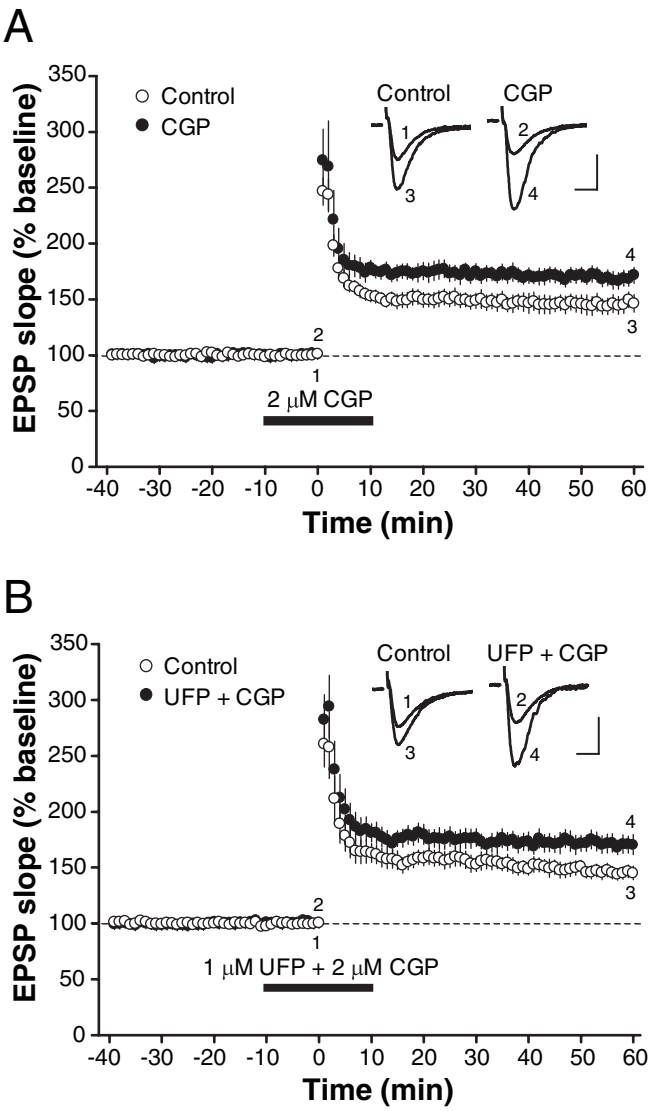


**Figure 5.** LTP is enhanced in the presence of a $GABA_B$ receptor antagonist. ***A***, LTP was enhanced in the presence of the $GABA_B$ receptor antagonist CGP (2 $\mu$M). CGP was applied from −10 to 10 min, as shown by the horizontal bar. Inset, Representative traces in the absence (Control) and presence of CGP recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. ***B***, LTP was enhanced in the presence of both CGP and UFP (1 $\mu$M). CGP and UFP were applied from −10 to 10 min, as shown by the horizontal bar. Inset, Representative traces in the absence (Control) and presence of both CGP and UFP (UFP + CGP) recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV.

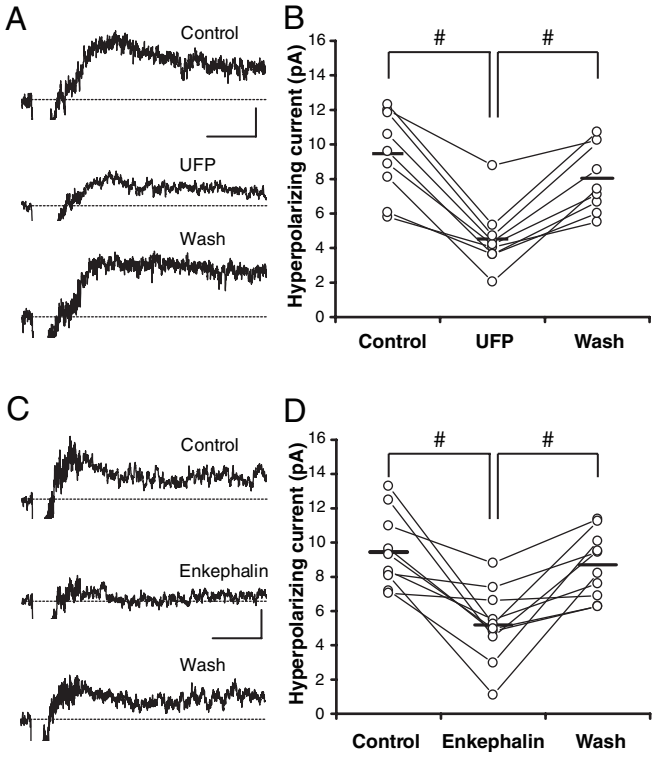


**Figure 6.** Nociceptin is synaptically released from interneurons. ***A***, Representative traces of hyperpolarizing currents evoked by tetanic stimulation (100 Hz for 1 s) in the absence (Control) and presence of UFP (1 $\mu$M) and after the washout of UFP (Wash). Calibration: 1 s, 5 pA. ***B***, The hyperpolarizing currents were reversibly suppressed by UFP. The data points obtained from the same cell are connected with the thin lines. The horizontal bars represent the mean values. ***C***, Representative traces of hyperpolarizing currents evoked by tetanic stimulation in the absence (Control) and presence of enkephalin (10 $\mu$M) and after the washout of enkephalin (Wash). Calibration: 1 s, 5 pA. ***D***, The hyperpolarizing currents were reversibly suppressed by enkephalin. $^{\#}p < 0.05$ (paired *t* test).

strongly suggest that nociceptin inhibits LTP through activation of GIRK channels. This apparent contradiction can be explained as follows. Bath application of nociceptin continuously and strongly activates nociceptin receptors, resulting in massive activation of GIRK channels on the pyramidal cells. $GABA_B$ receptors are also known to activate the GIRK channel and to share the same channel with other G-protein-coupled receptors such as serotonin and adenosine receptors (Andrade et al., 1986; Sodickson and Bean, 1998). In the present study, we found that $GABA_B$ and nociceptin receptors share the same GIRK channels in the CA1 pyramidal cell (Fig. 4*C*,*D*). Thus, strong and sustained activation of nociceptin receptors by bath-applied nociceptin recruits an enormous number of the shared GIRK channel in advance, and synaptic currents evoked by $GABA_B$ receptors are consequently reduced. However, in more physiological conditions, nociceptin is released transiently, and $GABA_B$ receptors may not compete with nociceptin receptors for the GIRK channel, and thus, $GABA_B$-IPSCs are intact even if nociceptin is released, which is consistent with the fact that LTP is enhanced by CGP (Fig. 5*A*).

### The role of nociceptin in synaptic plasticity

In our present study, endogenous nociceptin suppressed LTP induced by tetanic stimulation (Fig. 1*B*), but exogenously applied nociceptin failed to inhibit the LTP (Fig. 1*A*). There are two possible explanations for this finding. The concentration of synaptically released nociceptin may be higher than that of the exogenously applied nociceptin (100 nM), which is not sufficiently high to affect LTP, but UFP can block the action of endogenous nociceptin. However, this possibility may not be so likely, because 1 $\mu$M nociceptin, which exhibited a nearly maximal effect on GIRK channel currents and population spikes (Fig. 2*B*), still failed to show any effect on LTP (data not shown). Alternatively, the concentration of synaptically released nociceptin may be lower than 100 nM but sufficient to suppress LTP, and thus, additional exogenous nociceptin of higher concentrations may exert no more influence on LTP. However, previous studies have shown that exogenous nociceptin inhibited LTP induction (Yu et al., 1997; Yu and Xie, 1998; Higgins et al., 2002). We do not know exactly what causes the inconsistency, but the difference in animal species used and/or brain regions examined may introduce

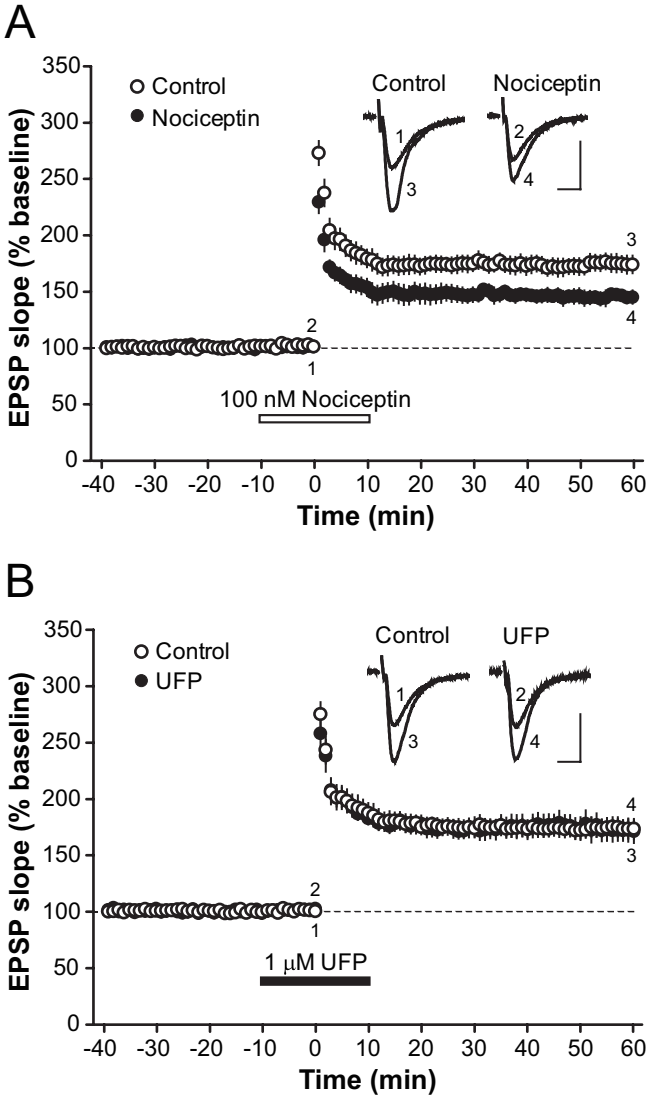


**Figure 7.** LTP modulation by nociceptin is dependent on the pattern of synaptic activation. ***A***, LTP induced by theta-burst stimulation was inhibited by exogenously applied nociceptin. Nociceptin (100 nM) was applied from −10 to 10 min, as shown by the horizontal open bar. Theta-burst stimulation (a train consisted of 5 bursts at 5 Hz, each burst was composed of 4 pulses at 100 Hz, and 3 trains were delivered at 10 s intervals) was applied at time 0. Inset, Representative traces recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV. The potentiation ratio was calculated by dividing the averaged EPSP slope values from 50 to 60 min by those from −30 to 0 min. ***B***, LTP induced by theta-burst stimulation was unaltered by UFP. UFP (1 μM) was applied from −10 to 10 min, as shown by the horizontal closed bar. Theta-burst stimulation was applied at time 0. Inset, Representative traces recorded at the times indicated by the numbers in the graph. Calibration: 10 ms, 0.2 mV.

the contradiction. In one of these studies (Higgins et al., 2002), LTP in mutant mice lacking nociceptin are examined, and there are no significant differences in LTP induced by either high-frequency stimulation or theta-burst stimulation between mutant and wild-type mice. This finding indicates that endogenous nociceptin has no effect on LTP, although it is possible that the knock-out of nociceptin also affects the expression of nocistatin, which is generated by the cleavage of the same precursor and has opposite action in pain transmission (Okuda-Ashitaka et al., 1998), and that the lack of nocistatin may complicate the results of LTP experiments. Alternatively, all of these discrepancies may be brought about by the difference in experimental conditions: the tetanic stimulation for inducing LTP used in our present study may be more suitable for the release of nociceptin from interneurons than that in previous studies. In fact, in our present study, LTP induced by theta-burst stimulation, which is less effective in releasing peptides from the nerve terminal, was not affected by UFP (Fig. 7). In any case, our findings clearly show that nociceptin released by tetanic stimulation inhibits LTP induction in physiological conditions.

Endorphins identified so far except nociceptin have no direct action on hippocampal pyramidal cells (Stumm et al., 2004), and their actions are mediated mostly by interneurons (Nicoll et al., 1980). Nociceptin is the first endorphin acting directly on pyramidal cells, and the effect of nociceptin is mainly mediated by GIRK channels (Ikeda et al., 1997). $GABA_B$ receptors seem to exert their action through the same GIRK channels, but there are a couple of differences in the mode of actions. First, $GABA_B$ receptor-mediated synaptic responses usually last for several hundred milliseconds (Dutar and Nicoll, 1988b) (Fig. 4*A*), whereas hyperpolarizing currents evoked by synaptically released nociceptin last for a few seconds (Fig. 6). This difference in the current kinetics may be accounted for by the difference in the time course of neurotransmitter release and/or in the kinetics of receptor activation. Second, GABA can be released in response to only one firing of interneurons, but nociceptin release requires their repetitive firings. Although both $GABA_B$ (Fig. 5*A*) and nociceptin (Fig. 1*B*) receptors seem to inhibit LTP induction through the same GIRK channels, the mode of LTP inhibition may differ: $GABA_B$ receptors may preferentially suppress an early phase of the enhanced activity of pyramidal cells during the tetanic stimulation, which should inhibit the activation process of NMDA receptors, whereas nociceptin receptors may predominantly suppress a late phase, which should make opening NMDA receptor channels close. In this case, the effects of the activation of $GABA_B$ and nociceptin receptors converge on the final common pathway NMDA receptor, which would explain the result that the degree of LTP enhancement in the simultaneous presence of UFP and CGP seems to be similar to that in the presence of either antagonist alone (Fig. 5).

The main conclusion in this study demonstrating that LTP is inhibited by endogenous nociceptin in normal mice is in accord with our previous report showing that mutant mice lacking nociceptin receptors exhibit enhanced LTP (Manabe et al., 1998). Our present results also indicate that possible developmental modification of the hippocampal neural circuit and/or compensation of the lack of nociceptin receptors in mutant mice are not associated with the phenotypes of the mutant mice. These mutant mice also show better performance in the hippocampus-dependent learning task, suggesting that the local regulation of neural activities in the hippocampal network through the nociceptin system may play a significant role in memory formation. The nociceptin receptor could be the target of pharmaceutical approaches for treating psychiatric and neurological disorders accompanying memory impairments such as Alzheimer's disease.

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