Pre and post synaptic mechanisms about the peripheral nerve-injured plasticity of nociceptive transmission in the superficial dorsal horn of mouse spinal cord.

Yuya Sato[1)], Takaaki Ajima[1)], Toshifumi Takasusuki[1)], Eiko Kato[2)], Yuuichi Hori[2)], Shigeki Yamaguchi [1)]

Dokkyo Medical University, School of Medicine, Departments of Anesthesiology[1)] and Physiology[2)]

Correspondent author: Shigeki Yamaguichi
Address: 880 Kitakobayashi, Mibu, Tochigu 321-0293, Japan
Tel: 81-282-86-1111
Fax: 82-282-86-0478
E-mail: shigeki@dokkyomed.ac.jp

SUMMURY

Long-term potentiation (LTP) and long-term depression (LTD) in synaptic transmission have been not only observed the hippocampus, but also in the superficial dorsal horn. LTP and LTD in the superficial dorsal horn play an important role of central sensitization, leading to hyperalgesia and allodynia in neuropathic pain. In order to clarify its mechanisms, we evaluated evoked excitatory postsynaptic currents (eEPSCs) and asynchronous EPSCs (aEPSCs) in the superficial dorsal horn of sciatic nerve-ligated neuropathic pain mice (nerve-ligated mice) using whole cell patch clamp recording.

Three types of neuron in the superficial dorsal horn of mouse spinal cord, including eEPSC amplitude-increased (LTP neuron), decreased (LTD neuron) and unchanged neurons were observed after tetanus stimulation. After tetanus stimulation, cumulative probability of aEPSCs amplitude did not change in LTP neuron, but shifted to the left in LTD neuron. In nerve-ligated mice, the ratio of LTP neuron was high. On the other hand, the ration of both LTD neuron and unchanged neuron was high in sham-operated control mice.

These findings may suggest both pre- and post-synaptic mechanisms of central sensitization in the superficial dorsal horn contribute neuropathic pain and LTP or LTD may be important role of pre- or post-synaptic action, respectively.

Key Words: neuropathic pain, central sensitization, neuroplasticity, long-term potentiation, long-term depression

INTRODUCTION

Neuropathic pain is now defined by the International Association for the Study of Pain (IASP) as 'pain caused by a lesion or disease of the somatosensory nervous system' [1]. Neuropathic pain has many serious effects on quality of life and is associated with a high economic burden for both individuals and society, and makes from a heterogeneous group disorders that affect the peripheral and central somatosensory nervous systems [2]. It is now regarded as a distinct clinical entity despite a large variety of causes. People with neuropathic pain may experience altered pain sensation, areas of numbness or burning, and continuous or intermittent evoked or spontaneous pain. Thus, neuropathic pain is an unpleasant sensory and emotional experience.

For establishing suitable treatments of neuropathic pains, such as pharmacological approach, it must be important to know about detail of mechanisms of neuropathic pain. However, its mechanisms are complicated and have been still unclear. Central sensitization is considered as one of it mechanisms, and may make hyperalgesia and allodynia. Recent reports suggested a relationship between central sensitization and neuroplasticity in the spinal cord dorsal horn[3].

Long-lasting changes in the efficacy of synaptic transmission, such as long-term potentiation (LTP) and long-term depression (LTD) play an important role of neuroplasticity and is observed in the hippocampus and cerebellum [4]. Both LTP and LTD are also observed in the superficial dorsal horn, which is an important area for transmission and regulation on nociception [5-10]. However, the detail mechanisms of neuroplasticity, involved in the pathogenesis of neuropathic pain, are not still clear.

In the present study, to clarify mechanism of neuroplasticity on neuropathic pain, we evaluated evoked excitatory postsynaptic currents (eEPSCs) and asynchronous EPSCs (aEPSCs) in the superficial dorsal horn of peripheral nerve-ligated neuropathic pain mice using whole cell patch clamp recording.

MATERIALS AND METHODS

1. Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Dokkyo Medical University. The care and use of the animals were in accordance with the National Institutes of Health guidelines on animal care and with the guidelines of the IASP. Experiments were performed on male ICR mice aged 6–8 weeks. Mice were maintained in a temperature-controlled room on a 12- h light–dark cycle with

food and tap water available ad libitum.

2. Partial ligation of the sciatic nerve

All mice were anesthetized with sevoflurane for the sciatic nerve-ligation surgery and for the sham operation. The left sciatic nerve was partially ligated according to the protocol described by Seltzer et al. (nerve-ligated mice) [11]. In sham-operated control mice, the sciatic nerve was exposed, but not ligate (sham-operated mice).

3. Behavioral assessment

To assess the effects of sciatic nerve ligation on the nociceptive behavior, the withdrawal threshold on mechanical stimulation was determined by using an electronic von Frey device (Model 1601; IITC Life Science, Woodland Hills, CA, USA). The use of the electronic von Frey device has been validated for investigations on nociceptive behaviors (Moller et al. [12], 1998; Cunha et al., 2004 [13]). The probe of the electronic von Frey device was manually applied with the force increasing at a rate of 1.6–8.1 g/s. Each trial of stimuli was composed of 10 applications of mechanical stimulation at approximately 10-s intervals; each trial was repeated three times at approximately 3-min intervals. Behavioral assessments were performed from 3 days prior to 10 days after surgery [13].

4. Electrophysiological experiments

1) Preparation of spinal cord slices

Transverse spinal cord slices were obtained on post-surgical day 10. Briefly, segments of the lumbosacral (L4-S1) spinal cord were removed under ketamine/xylazine anesthesia. Slices (450 μm thick) were cut on a vibratome (Dosaka EM, Japan) in Krebs solution at 4°C. The Krebs' solution was equilibrated with 95% O_2 and 5% CO_2. The solution contained the following ingredients (in mM): NaCl, 113; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4, 1; $CaCl_2$, 2; $MgCl_2$, 1; d-glucose, 11. Before electrophysiological experiments, spinal cord slices were incubated in Krebs solution at 37°C for 60 minutes.

2) Tight-seal whole-cell recordings

The spinal slices were mounted in a recording chamber on a microscope stage (Axioskop FS-II, Zeiss), and continuously perfused with Krebs' solution. Conventional tight-seal whole-cell recordings were obtained from neurons located in the superficial dorsal horn (lamina II) under visual control using infrared-differential interference contrast optics and a CCD video camera (IR-CCD 2741; Hamamatsu Photonics), as described previously [14, 15]

The pipette were filled with a solution of the following composition (in mM): K gluconate, 123; KCl, 14; Na gluconate, 2; EGTA, 1; HEPES, 10; and the pH of the solution was neutralized to 7.4 with KOH. The currents were recorded in the voltage-clamp mode at a holding potential of −70 mV, using an Axopatch 200B patch-clamp amplifier (Axon Instruments). The data were sampled using a Digidata 1440 interface (Axon Instruments). A PCLAMP 10 (Axon Instruments) and Mini Analysis 6.0.3 (SynaptoSoft) were used to analyze the data.

3) Electrical stimulation-evoked EPSCs (eEPSCs)

To record eEPSCs, the external solution routinely contained strychnine (Sigma,2–5 μM) and bicuculline (Sigma, 10 μM). Electrical stimulation was applied using a glass pipette filled with 1 M NaCl with its tip (diameter, ca 3 μm) placed at the dorsolateral margin of the spinal cord,
100–200 μm away from the recorded neuron. With a square pulse of 0.1 ms duration, stimulus intensity was adjusted so that an EPSC of similar amplitude was evoked in each experiment.

4) LTP and LTD

For induction of LTP or LTD, tetanus stimulation (100Hz, 5s) was applied at the dorsolateral margin of the spinal cord. The external solution routinely contained strychnine (Sigma, 2–5 μM) and bicuculline (Sigma, 10 μM). Electrical stimulation was applied using a glass pipette filled with 1 M NaCl with its tip (diameter, ca 3 μm) placed at the dorsolateral margin of the spinal cord, 100–200 μm away from the recorded neuron.

5) Asynchronous EPSCs (aEPSCs)

aEPSCs was recorded under prefusion of Krebs' solution, replaced $CaCl_2$ with $SrCl_2$ (2mM), which can effectively substitute for calcium in driving synaptic transmission[16].

5. Statistical analysis

All data are presented as the mean ± SEM. Statistical analyses of the data were performed by one-way or two-way analysis of variance (ANOVA) followed by post hoc multiple comparison (Tukey test). To compare frequencies of LTP and LTD between ligated and control mouse, chi-square test was performed using 3x2 contingency table. Cumulative frequency distribution of aEPSCs amplitude was evaluated by Kolmogorov–Smirnov test. The level of significance was set at $p < 0.05$.

RESULTS

1. Behavioral assessment of mechanical allodynia induced by partial ligation of the sciatic nerve

Mechanical withdrawal thresholds of the paw ipsilateral to the sciatic nerve ligation were determined using von Frey filaments. Partial sciatic nerve ligation elicited a significant decrease in the threshold for evoking hindpaw withdrawal (Fig. 1, filled circles). In nerve-ligated mice, the decrease in withdrawal thresholds appeared within 1 day after sciatic nerve-ligated mice, and lasted throughout the period of investigation up to 10 days after sciatic nerve-ligation. On the other hand, in sham-operated mice, no significant alterations in withdrawal thresholds were observed (Fig. 1A, open circles).

2. Tight-seal whole-cell recordings

1) Various patterns of eEPSCs after tetanus stimulation

Three types of neuron in the superficial dorsal horn of mouse spinal cord, including eEPSC amplitude-increased (LTP neuron, Fig. 2A, before; a, after; b), decreased (LTD neuron, Fig. 3A, before; a, after; b) and unchanged neurons were observed after tetanus stimulation. Fig. 4A and B show changes in averages of percentage to baseline values of eEPSC amplitude before and after tetanus stimulation in both LTP and LTD neurons, respectively.

2) Effects of sciatic nerve ligation on the incidence of various patterns of eEPSCs

In both nerve-ligated and sham-operated mice, all types of neurons were observed (Table). However, the ratio of LTP neuron in nerve-ligated mice (74%) was significantly more than that in sham-operated mice (29%). On the other hand, the ratio of both LTD and unchanged neurons in nerve-ligated mice (17%, 9%, respectively) was significantly less than that in sham-operated mice (32% and 39%, respectively).

3) Effect of tetanus stimulation in aEPSCs.

Figs. 5A and 6A show effect of tetanus stimulation on aEPSCs in LTP and LTD neurons, respectively. In LTP neuron, after tetanus stimulation, cumulative probability of aEPSCs amplitude did not changed, resulted in no change in aEPSCs amplitude due to tetanus stimulation (Fig. 5B). However, in LTD neuron, after tetanus stimulation, cumulative probability of aEPSCs amplitude shifted to the left, resulted in decrease in aEPSCs amplitude due to tetanus stimulation (Fig. 5B).

DISCUSSION

In the present study, before electrophysiological experiment, we evaluated the threshold for evoking hindpaw withdrawal in both mice with sciatic nerve-ligation by Seltzer method

[10] and sham operation. The threshold in nerve-ligated mice significantly decreased after sciatic nerve-ligation and lasted for ten days, but not in sham-operated mice. It is well known that neuroplasticity in the superficial dorsal horn contributes to hyperalgesia and allodynia. LTP, evoked by high frequent pre-synaptic stimulation, is one of mechanism of neuroplasticity. It is not observed in the hippocampus, but also in the superficial dorsal horn [5, 17].

Therefore, we performed whole cell patch clump recording on neurons in the superficial dorsal horn of spinal cord slices in both mice after sciatic nerve-ligation or sham operation. Then, three types of neuron in the superficial dorsal horn of mouse spinal cord, including LTP (eEPSC amplitude-increased), LTD (eEPSC amplitude-decreased) and unchanged neurons after tetanus stimulation in both mice. These results consist with reports [18-19]. However, there was a significant difference in the ratio of these neurons between both mice. The sciatic nerve-ligation changed the ratio of LTP and LTD neurons in the superficial dorsal horn. Our results suggest that increase in LTP of excitatory synaptic transmission in the superficial dorsal horn may play an important role of neuropathic pain.

In the previous reports [18, 20], LTP and LTD were observed in spinal thalamic tract neurons and GABAergic neurons, respectively. In our results, the ratio of eEPSC amplitude-increased neuron was significantly increased after sciatic nerve-ligation. It means that nociceptive synaptic transmission is sensitized and inhibitory synaptic transmission is depressed. Our results consist with the previous reports [20-22].

Furthermore, to confirm pr- or post- synaptic changes in LTP and LTD, we evaluated aEPSC in the superficial dorsal horn [23, 24]. In LTD neuron, amplitude of aEPSC was decreased after initiation of LTD. It may suggest that decrease in sensitivity of post synaptic neurons contributes expression of LTD. On the other hand, amplitude of aEPSC was not changed in LTP neuron. It may suggest that sensitivity in post synaptic neurons dose not contribute to expression of LTP. Therefore, frequency of aEPSC was evaluated in LTP neuron and its increase was observed. It may suggest that increase in sensitivity of pre synaptic neurons contributes expression of LTP in the superficial dorsal horn.

Limitations in this study is that we only investigated pre and post-synaptic electro physiological mechanisms in the in the superficial dorsal horn using neuropathic pain model mouse. Therefore, in the next study, to confirm its theory and to establish suitable pharmacotherapy for neuropathic pain, another study using presynaptic ligand, Ca^{2+} channel $\alpha 2$ ligands, such as pregabalin, must be performed.

CONCLUSIONS

Our results, such as no change in aEPSCs after LTP and decrease in aEPSCs after LTD, suggest that both pre synaptic mechanism for LTP and post synaptic mechanism for LTD may play an important rule of central sensitization in the superficial dorsal horn after peripheral nerve injury.

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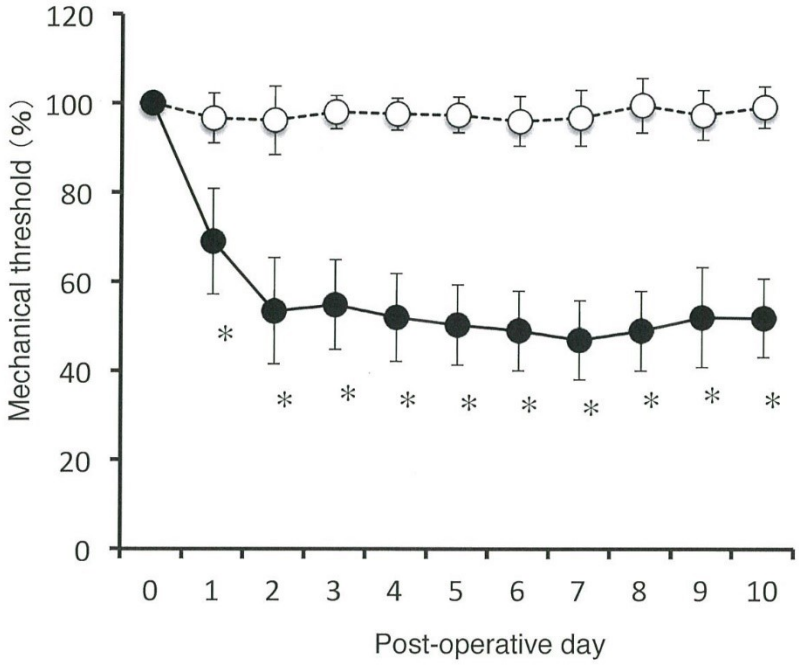
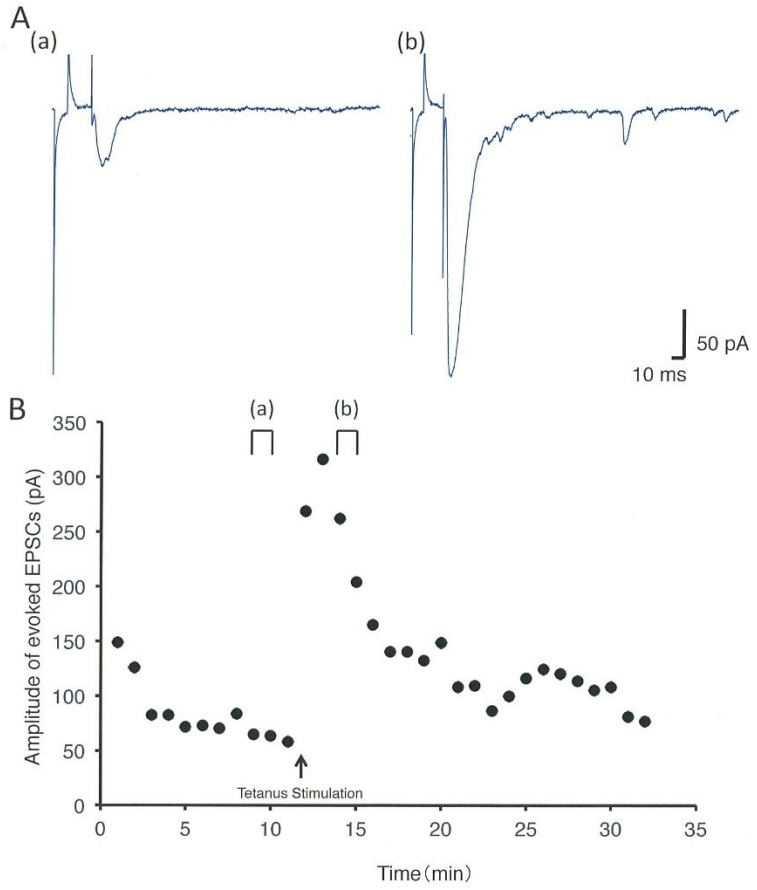
Figures and table

Figure 1. Mechanical allodynia induced by the partial ligation of the sciatic nerve.



Mechanical withdrawal thresholds of the hindpaw ipsilateral to the sciatic nerve ligation, expressed as percentage to baseline values, are plotted as a function of postoperative days. Filled circles represent sciatic nerve-ligated mice (n=13), and open circles represent sham-operated control mice (n=14). Data are expressed as the mean±SEM. * Indicates a significant decrease in the withdrawal threshold compared with the values before the surgical procedure.

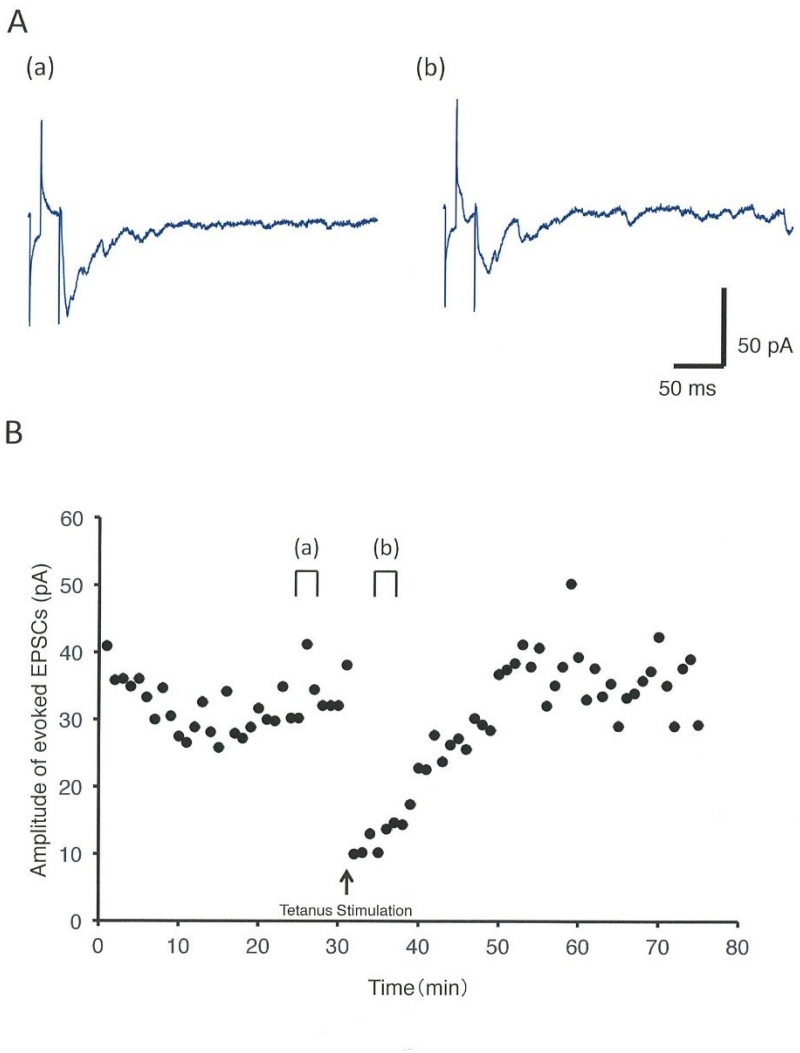
Figure 2. A sample recoding in neuron with long term potentiation



A. Representative recordings showing evoked EPSCs from an evoked EPSC amplitude-increased neuron in superficial dorsal horn of mouse spinal cord before (a) and after (b) tetanus stimulation.

B. Change in amplitude of evoked EPSCs in an evoked EPSC amplitude-increased neuron before (a) and after (b) tetanus stimulation. Inset traces show examples of eEPSCs recorded at the time indicated by (a) before and (b) after tetanus stimulation.

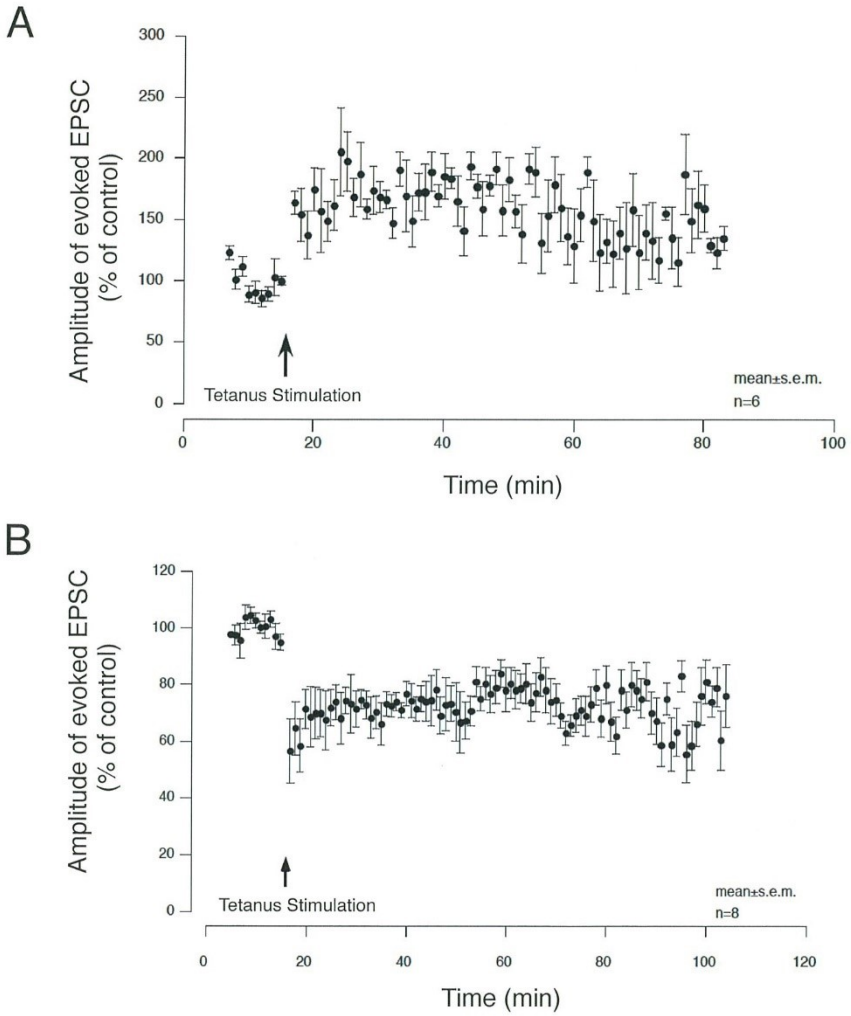
Figure 3. A sample recoding in neuron with long term depression



A. Representative recordings showing evoked EPSCs from an evoked EPSC amplitude-decreased neuron in superficial dorsal horn of mouse spinal cord before (a) and after (b) tetanus stimulation.

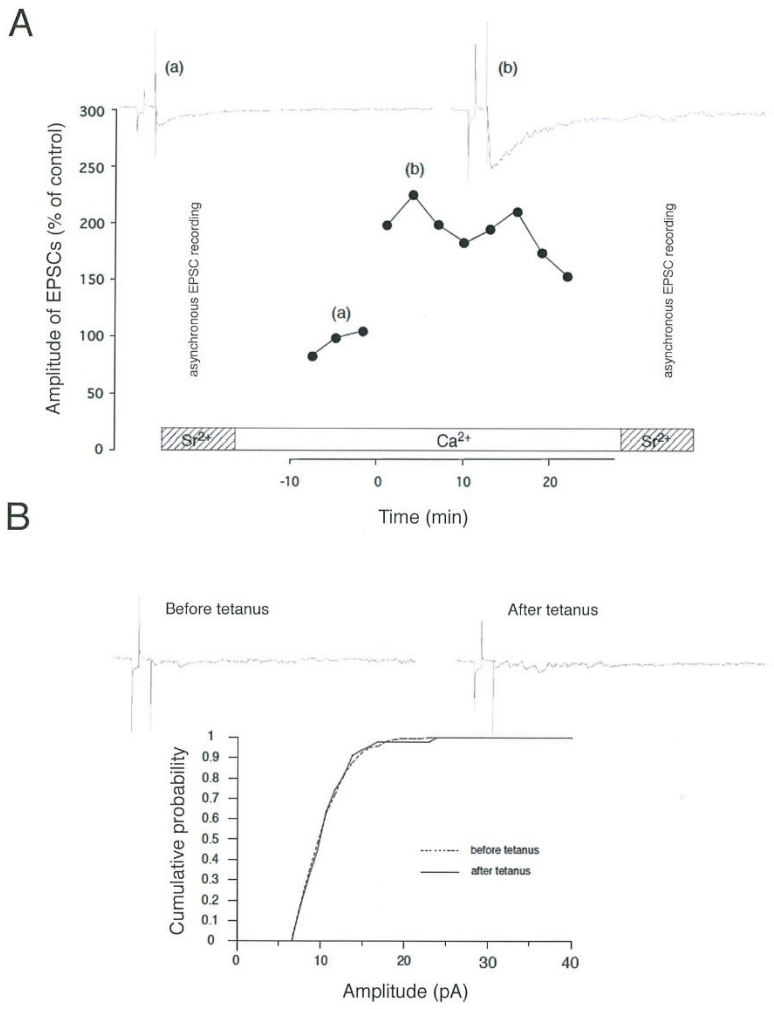
B. Change in amplitude of evoked EPSCs in an evoked EPSC amplitude-decreased neuron before (a) and after (b) tetanus stimulation. Inset traces show examples of eEPSCs recorded at the time indicated by (a) before and (b) after tetanus stimulation.

Figure 4. Long term potentiation and long term depression



Changes in averages of percentage to baseline values of eEPSC amplitude before and after tetanus stimulation in eEPSC amplitude-increased (A) and -decreased neurons (B).

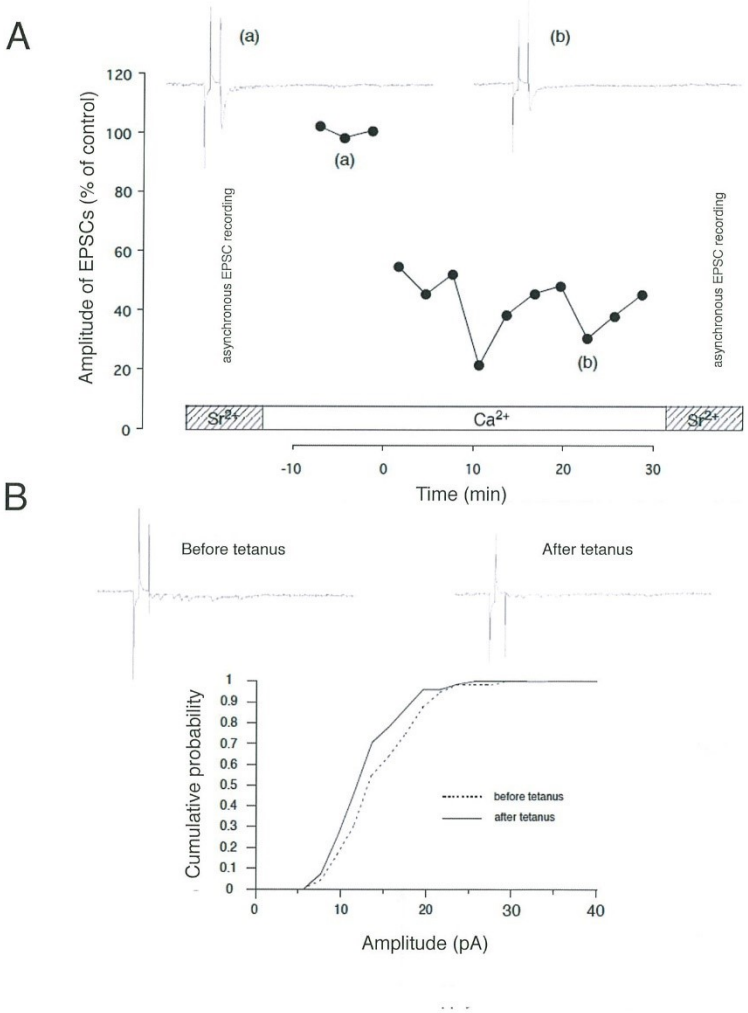
Figure 5. Amplitude of aEPSCs in the superficial dorsal horn with long term potentiation



A. Representative recordings showing asynchronous EPSCs from an evoked EPSC amplitude-increased neuron in superficial dorsal horn of mouse spinal cord before (a) and after (b) tetanus stimulation. Inset traces show examples of asynchronous EPSCs recorded at the time indicated by (a) before and (b) after tetanus stimulation.

B. Cumulative probability of asynchronous EPSCs amplitude. There was no difference between before and after tetanus stimulation.

Figure 6. Amplitude of sEPSCs in the superficial dorsal horn with long term depression



A. Representative recordings showing asynchronous EPSCs from an evoked EPSC amplitude-decreased neuron in superficial dorsal horn of mouse spinal cord before (a) and after (b) tetanus stimulation. Inset traces show examples of asynchronous EPSCs recorded at the time indicated by (a) before and (b) after tetanus stimulation.

B. Cumulative probability of asynchronous EPSCs amplitude. There was no difference between before and after tetanus stimulation. Cumulative probability of aEPSCs amplitude shifted to the left, resulted in decrease in aEPSCs amplitude due to tetanus stimulation.

Table

	LTP	LTD	no change
sciatic nerve-ligated mice (n=23 cells)	17(74%)	4(17%)	2(9%)
sham operated mice (n=31 cells)	9(29%)	10(32%)	12(39%)

LTP: long term potentiation (evoked EPSC amplitude-increased neuron)
LTD: long term potentiation (evoked EPSC amplitude-decreased neuron)

The ratio of evoked EPSC amplitude-increased, -decreased and unchanged neurons in sciatic-ligated and sham-operated mice. The ratio of eEPSC amplitude-increased neuron in sciatic nerve ligation mice was significantly more than that in sham controlled mice. On the other hand, the ratios of both eEPSC amplidute- decreased and -unchanged neuron in sciatic nerve ligation mice were significantly less than those in sham-controlled mice.