Supporting Information

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SI Materials and Methods

Slice Preparation. Acute hippocampal slices were prepared from wild-type C57BL/6 mice and from NogoA KO mice according to standard procedures. For LTP experiments 40- to 60-d-old mice (P40–P60) and for LTD experiments 14- to 21-d-old mice (P14–P21) were used. In brief, mice were anesthetized and decapitated, the brain was quickly transferred into ice-cold carbogenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF). Hippocampi were cut with a vibratome (400 μm; VT 1000S; Leica). The ACSF used for electrophysiological recordings contained 125.0 mM NaCl, 2.0 mM KCl, 1.25 mM NaH_2PO_4, 1.0 mM $MgCl_2$, 26.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$, and 25.0 mM glucose. Recordings were done in a submerged recording at 32 °C.

Antibody Treatment. For the LTP experiments with antibody treatment, three different highly purified mouse and goat monoclonal antibodies were used: A NogoA-specific blocking antibody (antibody 11C7, mIgG1), raised against an 18-aa peptide in the most active region of NogoA (NogoΔ20 region) (1), a control antibody (anticyclosporin, mIgG1, a gift from Novartis Pharma, Basel, Switzerland), and an antibody against the Nogo receptor subunit NgR1 (mNogo receptor affinity-purified goat IgG; R&D Systems). The antibody 11C7 was shown to block the NogoA-mediated neurite outgrowth inhibition in vitro and in vivo (1–3). Antibody solutions were freshly prepared in carbogenated ACSF at a final concentration of 5 μg/mL. To prevent sticking of the antibody to the tubing and the chamber, silicon tubing was used and prewashed with ACSF containing BSA (0.1 mg/mL). The slices were preincubated for 1 h with the anti-NogoA, anti-Nogo receptor, or control antibody in an incubation chamber maintaining a constant flow of the solution. For the electrophysiological recordings, the perfusion rate in the recording chamber was constantly kept at 1.5 mL/min. The same settings were used for the experiments with the active NogoΔ20 peptide. In two further experimental sets, the slices were either incubated for 1 h before the experiment with the NogoΔ20 peptide or the control, but not during the electrophysiological recordings or the NogoΔ20 peptide was only applied 5 min before and 5 min after LTP induction, for an overall time of 10 min. As control for the NogoΔ20 peptide the same peptide was boiled for 20 min and transferred onto ice before being used for the experiments.

Electrophysiological Recordings. After placing the slices in a submerged recording chamber fEPSPs were recorded in the stratum radiatum of the CA1 region with a glass micropipette (resistance 3–15 MΩ) filled with 3 M NaCl at a depth of ~150–200 μm. For the antibody experiments, the recording electrode was placed up to 100 μm below the surface, at a depth at which the antibody certainly penetrated. Monopolar tungsten electrodes were used for stimulating the Schaffer collaterals at a frequency of 0.1 Hz. Stimulation was set to elicit a fEPSP with a slope of ~40–50% of maximum for LTP recordings and ~50–60% for LTD recordings. After 20 min of baseline stimulation, LTP was induced by applying theta-burst stimulation (TBS), in which a burst consists of 4 pulses at 100 Hz, which were repeated 10 times in an 200-ms interval (5 Hz). Three such trains were used to induce LTP at 0.1 Hz. For LTD induction, a 1-Hz stimulus train was delivered for 15 min (900 pulses) after a 20-min baseline recording.

Basic synaptic transmission and presynaptic properties were analyzed via input-output (IO) measurements and paired pulse facilitation. The IO measurements were performed either by application of a defined value of current (25–250 μA in steps of 25 μA) or by adjusting the stimulus intensity to a certain current eliciting a fiber volley (FV) of desired voltage. Paired pulse facilitation was performed by applying a pair of two stimuli in different interstimulus intervals (ISI) ranging from 10, 20, 40, and 80–160 ms.

Data Analysis. Data were collected, stored, and analyzed with Labview software (National Instruments). The initial slope of fEPSPs elicited by stimulation of the Schaffer collaterals was measured over time, normalized to baseline, and plotted as average ± SEM.

Immunohistochemistry. The acute slices used for LTP or LTD experiments underwent specific immunohistochemistry to analyze the binding, location, and penetration of the 11C7 antibody. The slices were fixed in 4% PFA overnight and transferred in a 30% sucrose solution (phosphate buffer 0.1 M) until they sank. The 400-μm thick slices were cut into 30-μm thin sections with a freezing microtome, by maintaining the serial order of the sections to be able to judge the penetration depth. The sections were first blocked for 1 h (1× PBS, 0.2% Triton, 10% NGS, 1% BSA). For quantifying the amount of antibody penetrating the tissue the Cy2-conjugated AffiniPure goat antimouse IgG, Fcγ subclass 1 antibody was used (1:200 in blocking solution, Jackson Immuno Research). After washing, the sections were embedded in a water-based mounting medium (Biomeda). The slices were imaged with an Axioplan microscope equipped with an ApoTome module (Zeiss).

NogoΔ20 Peptide Internalization. Acute hippocampal slices, prepared as above and fixed 10 min after incubation with either the T7 tagged NogoΔ20-T7 peptide or the boiled T7 tagged NogoΔ20 peptide, as control, were used to detect the internalization of the NogoΔ20 peptide. Slices were fixed for 2 h at 4 °C in 4% PFA containing 5% sucrose and cryoprotected in 30% sucrose. After cutting the slices at 25 μm, they were blocked in PB with 4% NGS containing 0.3% Triton for 10 min. Primary antibodies (mouse anti-T7, EMD, 1:500; rabbit anti–EEA-1, Abcam, 1:400; Anti Map2, Sigma, 1:300) were incubated at 4 °C overnight and secondary antibodies (Dianova, 1:300) were incubated 1 h at room temperature in blocking solution containing 0.05% Triton. After washing, the slices were mounted with a water-based antifading medium. Images were acquired on a Zeiss laser scanning microscope (LSM510) using a C-Apochromat 40×/1.2 W objective and a zoom 8 with an optical slice thickness of <1μm. Images were processed with the use of ImageJ. The cytoplasmatic localization of the NogoΔ20 peptide was judged visually on the basis of the T7, EEA-1, and MAP2 staining. The experimenter was blind to the treatment. Data are given as the mean value ± SEM. Data analysis was performed by Prism 4.0 (GraphPad Software) using a paired Student's t test (two-tailed and two sample unequal variance).

Pharmacology. The $GABA_A$-receptor antagonist picrotoxin (PTX) was added in a 50-μM concentration 10 min before LTP induction until the end of the experiments.

1. Oertle T, et al. (2003) Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. *J Neurosci* 23:5393–5406.
2. Liebscher T, et al. (2005) Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. *Ann Neurol* 58:706–719.
3. Maier IC, et al. (2009) Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain* 132:1426–1440.

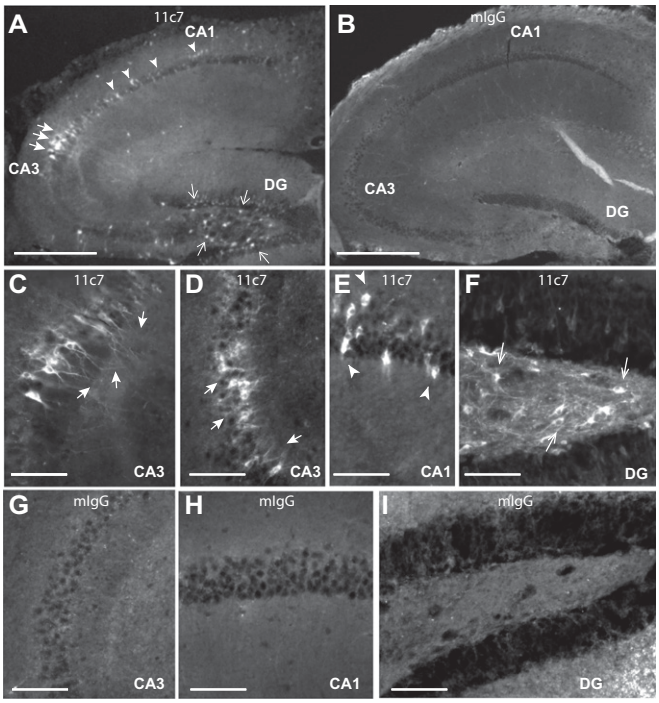

Fig. S1. Immunohistochemistry against NogoA blocking functional antibody. Representative images of slices treated with 11c7 antibody (*A* and *C–F*) and a control mIgG antibody (*B* and *G–I*). (*A*) Closed arrows indicate CA3 pyramidal neurons, arrowheads indicate CA1 pyramidal neurons, and open arrows indicate cells in the Hilus positive for the 11c7 antibody. (Scale bar, 100 μm.) (*C* and *D*) Closed arrows indicate the dendrites of CA3 pyramidal neurons. (*E*) Arrowheads indicate the cell bodies of CA1 neurons, (*F*) open arrows indicate the cell bodies of cells in the polymorphic layer of the dentate gyrus labeled with the 11c7 antibody. (Scale bars, 100 μm.) (*B*) mIgG control antibody treatment revealed no staining in the acute slices. (Scale bars, 100 μm.) mIgG staining in *F*, CA3; *G*, CA1; and *H*, dentate gyrus. (Scale bars, 100 μm.)

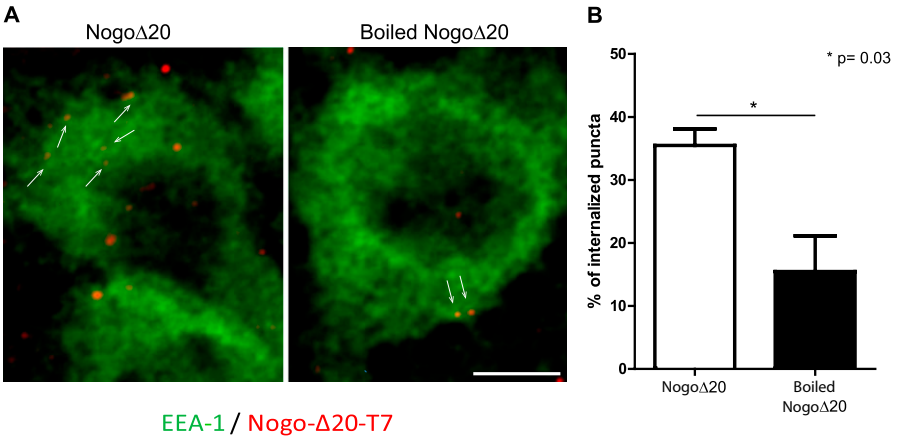

Fig. S2. NogoΔ20 peptide internalization (*A*) Representative images of slices treated with NogoΔ20 peptide (*Left*) or the boiled NogoΔ20 peptide (*Right*). The arrows point to some of the internalized NogoΔ20-T7 particles (red) in the cytoplasm of hippocampal pyramidal cells, identified by the EEA-1 staining (green). Please note that the extracellular space does not show any diffuse staining, indeed suggesting that what is here detected are peptide aggregates forming upon binding to the receptor or internalization. (Scale bar, 5 μm.) (*B*) The graph shows the percentage of internalized NogoΔ20 peptide particles, respectively, in slices treated with NogoΔ20 peptide or boiled NogoΔ20 peptide as control. The data are the result of three independent experiments each of them with 32 cells from four slices. $*P < 0.05$.