Supplemental Material

Willi et al.

Supplemental Methods

Western Blotting

Mice were killed by cervical dislocation. Whole brains were quickly dissected out and transferred into CHAPS lysis buffer [60 mM CHAPS, 20 mM Tris pH 8.0, 1 mM EDTA, Complete Mini EDTA-free protease inhibitor cocktail (Roche, Basel, Switzerland), 1% PMSF in ethanol] on ice. The fresh brain tissue was then immediately homogenized using a EUROSTAR power basic stirrer (with a Teflon pestle set at 700 rpm; IKA, Staufen, Germany), incubated for 30 min on ice and centrifuged for 15 min at 4°C and 15000 g. The total protein concentration was determined using the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA). Samples (10 µg) were resolved by 4-12% NuPAGE gels (Invitrogen, Carlsbad, CA) and transferred onto nitrocellulose membranes (Whatman, Dassel, Germany). After blocking, membranes were incubated with primary antibodies [Nogo-A antiserum Bianca (1:20,000), α-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:20,000)] overnight at 4°C, washed, and incubated with horseradish peroxidase-conjugated secondary antibodies for 1h at room temperature. Protein bands were detected using a chemiluminescent substrate system (SuperSignal West Pico, Pierce Biotechnology, Rockford, IL) and images were captured with a Stella imaging system (Stella 3200, Raytest, Straubenhardt, Germany).

Supplemental Figures and Tables

Figure S1. Endogenous Nogo-A protein levels in Nogo-A+/+ and Nogo-A−/− mice. Immunoblotting with antiserum Bianca that recognizes Nogo-A and an anti-GAPDH antibody as internal standard (see Supplemental Methods). Total brain lysates from Nogo-A+/+ and Nogo-A−/− mice are loaded in each lane. As expected, Nogo-A is absent in Nogo-A−/− mice. KO, Nogo-A−/−; WT, Nogo-A+/+.

Figure S2. Unaltered conditioned avoidance learning in Nogo-A−/− mice. (A) Number of avoidance responses per block and (B) number of ITI crossings per block in the active avoidance paradigm. Nogo-A−/− mice showed similar performance compared with Nogo-A+/+ mice. All values are mean ± SEM. KO, Nogo-A−/− (n = 12); WT, Nogo-A+/+ (n = 12).
Figure S3. Immunoreactivity of GAP-43 in adult mice acutely treated with anti-Nogo-A antibodies for 2 weeks. Quantitative analysis of the immunostainings with GAP-43. The relative optical density of GAP-43 was slightly enhanced in the dentate gyrus (DG; \( *p < 0.05 \)) of mice treated with Nogo-A antibodies, whereas it was not altered in the other regions investigated. All values are mean ± SEM. 11C7, anti-Nogo-A antibody treated \((n = 5)\); IgG, control IgG treated \((n = 5)\).

Table S1. List of primary antibodies used in the present study.

a) heat sections in 0.1 M Tris (pH 8.0) at 80°C for 20 min
b) heat sections in microwave oven in 0.1 M citrate buffer (pH 4.5) at 650 W for 30 s
c) MeOH Kryofix 10 min at 4°C (containing Methanol (50%), PEG 400 (7%), and H2O)
  * reacts strongly with S100B
  ** for specifics, see (Weinmann et al., 2006).

Table S1. List of primary antibodies used in the present study.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Company</th>
<th>Species</th>
<th>Dilution</th>
<th>Antigen retrieval prior IHC</th>
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<td>Rabbit</td>
<td>1:100</td>
<td>a)</td>
</tr>
<tr>
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<td>Rabbit</td>
<td>1:250</td>
<td>b)</td>
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<td>11C7 **</td>
<td>In house</td>
<td>Mouse</td>
<td>1:300</td>
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