

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, 1mM 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 \pm 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 \pm 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).

Weinmann O, Schnell L, Ghosh A, Montani L, Wiessner C, Wannier T, Rouiller E, Mir A, Schwab ME (2006) Intrathecally infused antibodies against Nogo-A penetrate the CNS and downregulate the endogenous neurite growth inhibitor Nogo-A. *Mol Cell Neurosci* 32:161-173.

WT

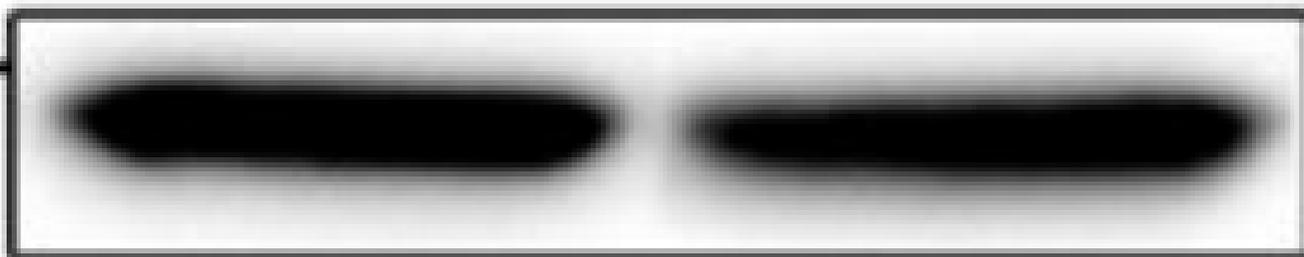
KO

191

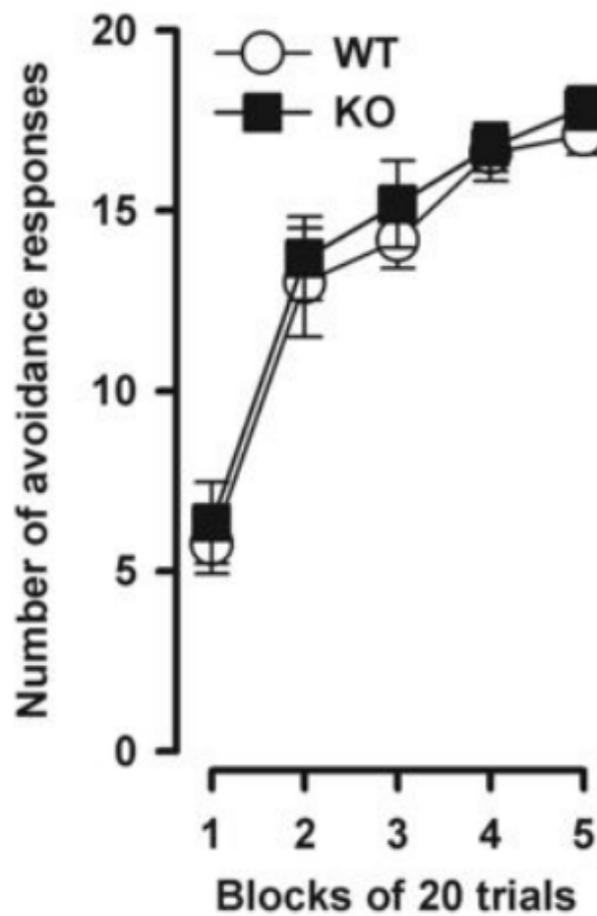
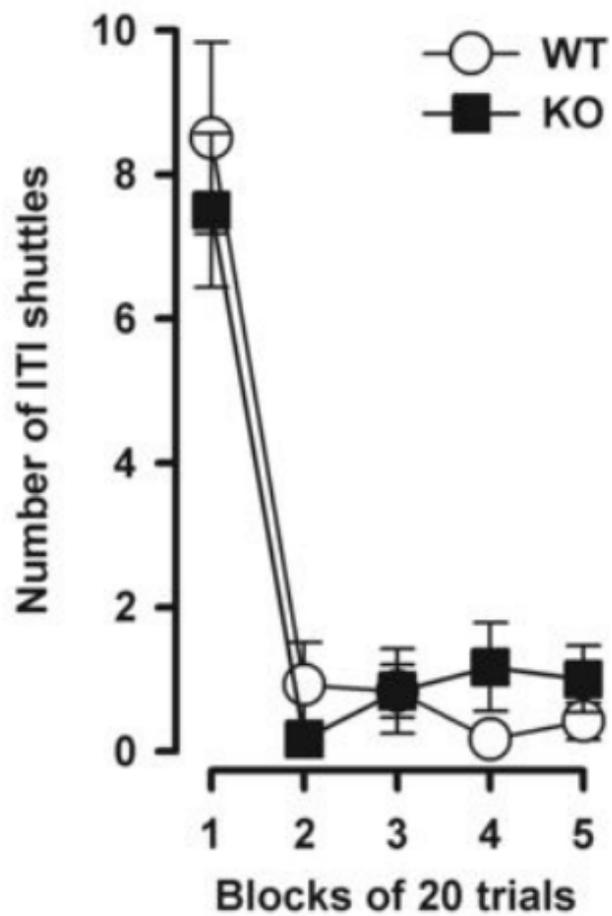


Nogo-A

39



GAPDH

A**B**

GAP-43-immunoreactivity
(relative regional optical density)

1.5
1.0
0.5
0.0

□ IgG
■ 11C7

CA1 CA3 DG mPFC CPU NAcC NAcS

*

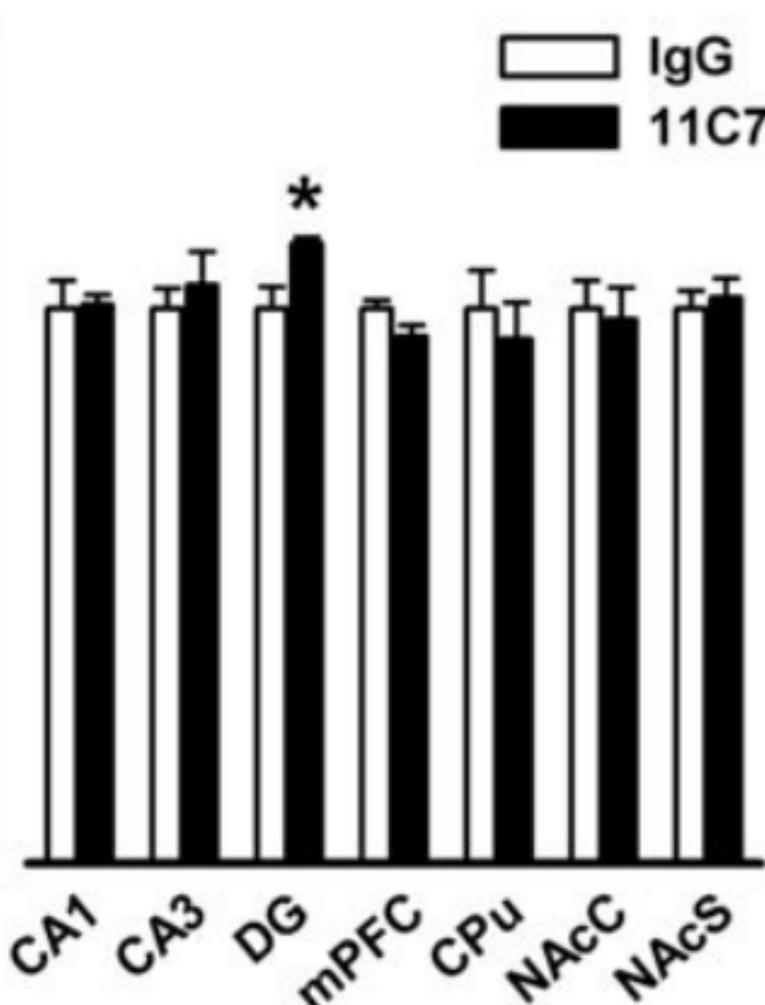


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Antibody	Company	Species	Dilution	Antigen retrieval prior IHC
D2R	Chemicon	Rabbit	1:100	a)
GAP-43	Chemicon	Rabbit	1:250	b)
S100 *	Dako	Rabbit	1:250	none
GFAP	Chemicon	Mouse	1:1000	none
11C7 **	<i>In house</i>	Mouse	1:300	c)