Supporting Information

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SI Materials and Methods

Design and Testing of the microRNA Constructs. To determine the optimal design for the coexpression of microRNAs (miRNAs) together with EGFP, we used CMV-based vectors expressing a miRNA-targeting firefly luciferase (fluc). The miRNA sequence was inserted either 3′ of *EGFP* between the ORF and the polyadenylation signal (pUTR) or 5′ of *EGFP* in an intron (pINTRON) (1). Details on the cloning procedure can be obtained on request. The constructs were tested by transient cotransfections together with luciferase constructs in HeLa cells as described before (1). Briefly, 15–20 μg respective DNA mixture were added to 2 million HeLa cells in 300 μL serum-free DMEM in a 4-mm GenePulser cuvette (BioRad). The cells were electroporated at 200 V, 25 Ω, and 975 μF at room temperature and spread on six-well Tissue Cultures Plates (Cell+; Sarstedt). The DNA mixture consisted of equal amounts of a vector for constitutive fluc production (pCMV-luc; can be obtained on request from D.B.), a vector for constitutive expression of the renilla luciferase (rluc) gene (pRL-SV40; Promega), and pUTR or pINTRON, respectively. After 36 h, cells were washed two times with PBS and lysed on tissue culture plate surface with passive lysis buffer (Promega). From the lysates, after centrifugation at 16,000 × *g* for 5 min at 4 °C, the fluc and rluc activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Cloning of the Transgenic miRNA Construct Targeting Nogo-A. siRNAs targeting Nogo-A–specific exon 3 of *Rtn4* (ENSRNOT-0000000644) were designed to silence both rat and mouse Nogo-A expression using established algorithms (2). For expression of the siRNAs in vitro and later, in vivo, we designed the expression vector cytomegalovirus (CMV) immediate enhancer/β-actin (pCAG)-INTRON-EGFP, in which the ubiquitous CMV enhancer/chicken β-actin promoter (3) controls the expression of EGFP. The construct was assembled using the CAG promoter of the plasmid pCAβ, which was cloned upstream of the *EGFP* ORF and an SV40 polyA signal. An artificial intron containing a *Bsa*I-flanked stuffer fragment surrounded by sequences optimized for polymerase II-controlled miRNA expression and procession (1) was placed in front of the *EGFP* expression cassette. Subsequently, the most efficient siRNA targeting Nogo-A as determined by mRNA knockdown percentage was inserted in the *Bsa*I-digested vector as annealed oligonucleotides. Details on the various intermediate cloning steps can be obtained on request.

To test the knockdown efficiency of the final miRNA construct, it was transfected into 3T3 cells using Lipofectamine LTX according to manufacturer's instructions (Invitrogen). Knockdown of Nogo-A mRNA levels after 3 d was assessed using quantitative real-time PCR (qPCR) as described before (4, 5) and below. The plasmid showing the highest knockdown efficiency was denominated pCAG-INTRON-(miRNA Nogo-A)-EGFP and used for generation of transgenic animals.

Generation of Transgenic Rats. Nogo-A miRNA transgenic rats were generated by microinjection of 2 ng/μL into of a *Pme*I-SacI fragment released from the pCAG-INTRON-(miRNA Nogo-A)-EGFP vector backbone into fertilized Sprague–Dawley rat oocytes as described previously (6). Founder rats and their offspring were analyzed by PCR of tail DNA using primers for *EGFP* (EGFP_for 5′-TTC AAG GAC GAC GGC AAC TAC AAG-3′, EGFP_rev 5′-CGG CGG CGG TCA CGA ACT CC-3′). Genomic DNA was prepared using the Qiagen Blood & Tissue Kit. Heterozygous rats were officially named SD-Tg(CAG-RNAi:Nogo-A,EGFP)#ZI, where # stands for the number of the transgenic line (in the following text, it is abbreviated as L#). The DNA microinjection experiments were performed in accordance with the local Animal Welfare Acts (AZ: 35–9185.81/G-104/09).

Culturing Rat Ear Fibroblasts. The isolation and culture of primary rat ear fibroblasts were carried out according to standard protocols (7). In brief, ear biopsies were taken from founder animals and digested overnight with 2 mg/mL collagenase (Sigma) in DMEM (Invitrogen). The resulting cells were cultured in DMEM supplemented with 20% (vol/vol) FBS followed by microscopic examination (DM-IRB; Leica) with filter settings for visualization of EGFP.

Quantification of mRNA by qPCR. Total RNA obtained from fresh frozen brain tissue was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's recommendations; 1 μg total RNA was used for reverse transcription (RT) with SuperScript III reverse transcriptase and oligo(dT)20 primers (Invitrogen), 1 μL resulting cDNA was subjected to real-time PCR analysis in triplicates, and 20-μL real-time PCR reactions were performed using Taqman Universal PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol on an Applied Biosystems 7900 HT fast real-time PCR system. For detection of Nogo-A mRNA, the following primers were used together with rat probe #18 (Universal Probe Library for Rat; Roche Applied Science): Nogo-A_for 5′-TGT CAG CAG AGC TGA GTA AAA CTT-3′ and Nogo-A_rev 5′-CAA TGC TGA ACA CTG TCA GAG A-3′. For normalization, the following primers/probe systems were applied: Ywhaz_for 5′-GCA GTT ACT GAG AGA CAA CTT GAC A-3′, Ywhaz_rev 5′-TGG AAG GCC GGT TAA TTT T-3′, rat probe #7; CycA_for 5′-CTT CCC AAA GAC CAC ATG CT-3′, CycA_rev 5′-TGC TGG ACC AAA CAC AAA TG-3′, rat probe: #42. Final concentrations of primers were 300 nM, and final concentrations of probes were 250 nM. Quantification was done as described before (5, 8).

Quantification of Mature Transgenic miRNA by qPCR. Detection of processed miRNA was performed using a Custom Taqman Small RNA Assay (AssayID: CS6RMYI; Applied Biosystems; target sequence: UCCCUACUUCCCUCAUAAGUC) according to the manufacturer's protocol. Briefly, 10 ng total RNA were dissolved in 5 μL RNase-free water and then mixed with 7 μL recommended RT-master mix (containing SuperScript III RT) and 3 μL 5RT primer provided with the Taqman Small RNA Assay. RT reactions were run in a thermocycler and programmed for 30 min at 16 °C, 30 min at 42 °C, and 5 min at 80 °C. All qPCR reactions were run with undiluted or 1/2- or 1/4-diluted RT reactions using an Applied Biosystems 7900 HT Fast qPCR System in accordance with the recommended protocol. Each PCR was performed in triplicate and consisted of 1 μL RT reaction product, 1 μL Custom Taqman Small RNA Assay, and 10 μL Taqman Universal PCR Master Mix (Applied Biosystems) in a total volume of 20 μL.

Comparison of Endogenous miRNA Expression Levels in L2 Vs. WT Animals by qPCR. Total RNA was extracted from adult L2 cortices using the RNeasy Kit (Qiagen) according to the manufacturer's instructions. Expression levels of 370 abundantly expressed and well-characterized miRNAs annotated by the Sanger miRBase (release 14) were investigated using the rat

miRNome miRNA PCR Array (MAR-3100G; SABiosciences) according to the manufacturer's instructions.

Western Blotting. Cell lysates were obtained from frozen rat brain and spinal cord tissue by homogenization in sample buffer (50 mM CHAPS, 20 mM Tris, 1 mM EDTA, 1 mM PMSF, 1 Roche cOmplete protease inhibitor mixture tablet) for 3 min on ice with mechanical disruption using a Teflon-coated, motor-driven tissue grinder (Büchi). Samples were incubated on ice for 40–60 min, and debris was pelleted by centrifugation (10,000 × *g* for 20 min at 4 °C). Total protein concentration of cleared lysates was measured by DC Protein Assay (BioRad) in a Multiskan Ascent ELISA Reader (Thermo Scientific). Serial dilutions of two WT samples per CNS region were used to determine the optimal protein amount per lane, resulting in bands that were within the dynamic range of the detection system (Fig. S3). Equal protein concentrations (cortex, 18 μg per lane; hippocampus, 12 μg per lane; spinal cord, 3 μg per lane) from three animals per genotype were separated on 4–12% (wt/vol) Bis·Tris NuPAGE Gels (Invitrogen) and transferred to polyvinylidene difluoride (PVDF). Membranes were blocked in a blocking buffer designed for fluorescent Western blotting (Rockland) and incubated with rabbit anti-Nogo antiserum Bianca (1:20,000) (9) and mouse anti-GAPDH antibody (1:20,000; Abcam) overnight at 4 °C. Membranes were then washed in tris-buffered saline/Tween and subjected to fluorophore-conjugated secondary antibodies IRDye 800CW goat anti-rabbit and IRDye 680 goat anti-mouse (1:10,000; LI-COR) diluted in blocking buffer for 1 h at room temperature. Detection was performed on an Odyssey imaging system (LI-COR). Densitometry of bands was conducted in AIDA image analysis software version 4.15 (Raytest).

Tissue Processing and Immunohistochemistry. For immunohistological analyses, rats were deeply anesthetized and perfused intracardially [4% (wt/vol) paraformaldehyde, 5% (wt/vol) sucrose, 0.1 M phosphate buffer]. Brains and spinal cords were excised and postfixed in the same fixative overnight at 4 °C. For initial screening of different transgenic lines, EGFP expression was characterized on 50-μm free floating vibratome sections by immunohistochemistry using 3,3-diaminobenzidine (DAB) staining (Vectastain Elite ABC Kit) with a rabbit anti-GFP primary antibody (1:5,000; Invitrogen). L2 was further characterized with dual-label fluorescent immunohistochemistry using the primary antibodies rabbit anti-GFP (1:1,000; Invitrogen), mouse anti-GFP (1:2,000; Invitrogen), mouse anti-NeuN (1:4,000; Millipore), rabbit anti-Olig2 (1:500; Millipore), mouse anti-GAD67 (1:500; Millipore), and mouse anti-PV (1:200; Sigma). Secondary antibodies were AF488 donkey anti-rabbit (1:5,000 for EGFP; Invitrogen), AF488 donkey anti-mouse (1:200 for NeuN; Invitrogen), AF555 donkey anti-rabbit (1:1,000; Invitrogen), and Cy3 anti-mouse (1:200; Jackson ImmunoResearch). Sections were examined using a Leica TCS SP5 Confocal Laser-Scanning Microscope with a 20× objective and sequential acquisition of separate channels.

Nogo-A expression was examined in various CNS regions of L2 and WT rats by quantitative immunohistochemistry. Tissue was fixed as described above, washed in 0.1 M phosphate buffer, and incubated in 30% (wt/vol) sucrose for 3 d at 4 °C. Brains and spinal cords were separated, embedded in Tissue-Tek O.C.T. Compound (Sakura), and frozen at −40 °C; 50-μm coronal and transversal sections were obtained from brain and spinal cord, respectively, in Kryostat 1720 digital (Leitz) and washed in 0.1 M phosphate buffer on an orbital shaker overnight at 4 °C. Sections were stored in an antifreeze solution containing phosphate buffer, glucose, and ethylene glycol at −20 °C until use. Free-floating sections were washed in PBS, transferred to 0.1 M Tris and 50 mM glycine, and incubated 30 min at room temperature, 3 min at 80 °C, and 30 min at room temperature for antigen retrieval. For EtOH/AcOH treatment, mounted spinal cord sections were subjected to 95% (vol/vol) EtOH and 5% (vol/vol) AcOH for 25 min, 70% (vol/vol) EtOH for 5 min, and 0.1 M PB for 10 min at 4 °C. In this case, antibody incubations were conducted in a wet chamber.

Sections were incubated with primary antibodies against Nogo-A [Laura rabbit serum 1:500 (9) or 11C7 mouse affinity purified at 2 μg/mL (10)] and AF488-conjugated rabbit anti-GFP antibody (1:1,000; Molecular Probes) in tris-buffered saline/Triton containing 4% (vol/vol) normal goat serum (NGS) overnight at 4 °C. After additional washing, they were incubated for 1 h with high affinity-purified secondary antibodies (Jackson ImmunoResearch): Cy3-conjugated goat anti-rabbit (1:200) and Cy3 or Dylight 649-conjugated goat anti-mouse (1:400) in tris-buffered saline/Triton containing 4% (vol/vol) NGS. Sections were mounted, dried overnight at 4 °C, and coverslipped.

Densitometry Analysis of Nogo-A in Brain Sections and Confocal Microscopy. Densitometry measurements of Nogo-A immunoreactivity in different brain regions were performed to assess the degree of Nogo-A expression in L2 and WT rats. Images were acquired using a 12-bit CoolSnap HQ Camera (Photometrics) attached to a Zeiss Axiophot Microscope and collected using image analysis software designed for densitometry: "MCID Elite" version 7.0 (Imaging Research). Immunoreactivity in regions of interest was measured using the software ImageJ (National Institutes of Health), and background-corrected ODs were averaged per brain region. The mean gray value of WT sections was set as 100% for each brain region. Exemplary sections and tissues processed for double and triple immunofluorescence staining were visualized by confocal laser-scanning microscopy (TCS SP2 AOBS; Leica). Only identical minimal contrast adjustments were applied. Sections from three animals per genotype were analyzed (cortices: $n = 5$–6 sections; hippocampus: $n = 3$ sections; standard retrieval oligodendrocyte and motoneuron: $n = 6$ cells from six sections; ethanol/acetic acid oligodendrocyte: $n > 40$ cells from three sections; ethanol/acetic acid motoneuron: ≥15 cells from three sections).

Quantification of Transgene Integrations Per Cell by qPCR. Copy number quantification of transgenes per cell was done by genomic qPCR. *Tyrosine hydroxylase* (*Th*; ENSRNOG00000020410) was used as single copy number reference gene. The following primers were used: TransgeneFW 5′-TTC AAG GAC GAC GGC AAC TAC AAG-3′; TransgeneRV 5′-CGG CGG CGG TCA CGA ACT CC-3′; ThFW 5′-CTC AAG AAT CCT GTC ACC AG-3′; ThRV 5′-ACT GCC TTT CAG GGT ATG TC-3′. SYBR Green I qPCR reactions were performed on the Roche LightCycler 480 under the following conditions: 95 °C (10 min), 45 cycles at 95 °C (15 s) and 60 °C (1 min) and ramp rate of 1 °C/s followed by a melting curve analysis. Products were verified using agarose gel electrophoresis. Quantification was performed as described before (11).

Behavioral Analysis. For the behavioral assessment, L2 ($n = 10$) or WT littermate ($n = 10$) heterozygous male Sprague–Dawley rats were used. Animals were 6 mo old at the start of the experiments. Three to four rats were housed per cage in a temperature-controlled room (22 °C) under 12-h light cycles with ad libitum access to food and drinking water. All experimental procedures were performed during the light phase. Handling of the animals was done for 1 wk (5 min/d) before the start of the tests. All experiments were performed in accordance with the local Animal Welfare Acts (AZ: 35–9185.81/G-5/11) and the European Communities Council Directive of November 24th, 1986 (86/609/EEC).

Basal locomotor activity was assessed in an open field (four equal areas, 51 × 51 × 50 cm). Distance traveled (centimeters) in the open field apparatus was digitally recorded for 30 min at a light intensity of 50 lx. The test was started by placing the rats

in the center of the box. For the analysis of locomotor activity, the observation program Viewer2 (Biobserve) was used.

Object recognition testing was performed in the open field setting described above at a light intensity of 50 lx (12). All stimulus objects were made out of ceramic or glass and applied in duplicates. Preliminary experiments indicated comparable attraction of all objects chosen for this test; 24 h before testing, the rats were habituated to the open field for 30 min. The test consisted of an initial 3-min sample phase (P1) and a 3-min discrimination phase (P2), which were separated by an intertrial interval of 15 min. During P1, the rats were placed in the center of the open field and exposed to two identical objects (A1 and A2). After cessation of P1, the rats were returned to the home cage, and the objects were removed. The rat was placed back in the open field after 15 min for object discrimination in P2 and now exposed to the familiar object (A′, an identical copy of the objects presented in P1) and a novel test object (B). Exploration of the objects (sniffing or licking) was recorded during P1 and P2. Sitting beside or standing on top of the objects was not scored as object investigation. Animals were videotaped during P1 and P2, and videos were analyzed by an observer blind to the genotype. For the calculation of object discrimination, the exploration time of the novel object was expressed as percentage of the total exploration time of both objects during P2.

For reversal learning, animals were trained in a black circular water maze (1.5 m diameter, 50 cm height; 25 °C water temperature), into which a plastic plus maze (arms wall: 38 cm arm height, 15 cm arm width, and 51 cm arm length) was inserted. The plus maze was elevated 5 cm from the bottom of the water maze. Final water level in the tank was 35 cm. The maze was surrounded by visual cues consisting of various geometric shapes. None of these cues were placed in proximity to the ends of the plus maze arms to avoid spatial bias. During training, an invisible escape platform (14 × 11cm) was located at the end of one arm of the maze (north) 1 cm below water level. The arm opposite to the start arm was blocked off by a guillotine door, resulting in a T configuration of the maze. Swimming behavior was recorded using the tracking software Ethovision v.3 (Noldus). Rats were trained in the plus maze for a total of 4 d. Each training session consisted of five swim trials, during which rats were placed in the start arm of the maze (south) and allowed to swim to the escape platform, which was consistently located in one arm of the maze (east). Rats remained on it for 10 s before being returned to their cages and being replaced in the maze after an intertrial interval of 30 s. Rats that failed to find the escape platform within 60 s were manually guided to it. On the fifth day, a reversal learning trial was performed. Rats were placed into the same start arm, but the location of the escape platform was switched to the west arm. Each east- or start arm-entering during test trial was scored as an error.

Prepulse inhibition of the acoustic startle response was measured using the Startle Response System (SR-LAB Startle Response System; San Diego Instruments) as described before (12). A white noise pulse was used as the startle stimulus, with an intensity of 110 dB sound pressure level (SPL) and 40 ms duration (0 ms rise/fall times). Four different white noise intensities (66, 70, 74, and 78 dB SPL, duration of 20 ms) were used as prepulses. An acclimatization time of 5 min to the background noise (60 dB) was followed by the presentation of five initial startle stimuli. After this habituation program, the test program was started with six different trial types presented in a pseudorandom order: 1, pulse alone; 2, no stimulus; 3–6, pulse with preceding prepulse 100 ms before (prepulse 66, 70, 74, or 78 dB SPL). A total of 10 presentations of each trial type was given, with an intertrial interval randomized between 10 and 20 s. Prepulse inhibition was calculated as percent decrease of the acoustic startle response when the pulse was preceded by a prepulse.

Social interaction was assessed in the open field at a light intensity of 50 lx. Young male rats were used as social partners to exclude confounding effects of aggressive or sexual behavior (12, 13); 24 h before testing, the social partners were habituated to the test arena for 10 min. For testing, rats were placed in the open field and exposed to an unknown social partner for 5 min. Animals were videotaped, and the following behavioral elements were quantified only for the experimental rats by a trained observer blind to group assignment: (*i*) social behavior: contact behavior, social exploration, and approach/following were scored as social behaviors; (*ii*) evade, which is normally defined as a defensive behavior in the context of social play, was scored in the social interaction test as an active withdrawal from social contact; and (*iii*) self-grooming behavior (12).

Investigation of Changes in Hippocampal Long-Term Potentiation Between L2 and WT Rats. Four hundred-micrometer horizontal slices were prepared from 6- to 8-wk-old L2 rats or control littermates according to standard procedures. Deeply anesthetized (pentobarbital, 50 mg/kg) L2 transgenic rats and their WT littermates, respectively, were decapitated, and the brains were quickly removed and immersed in cold (5–7 °C) oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF; 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM dextrose); 400-μm horizontal slices were prepared using a Leica vibratome. Slices were transferred to a temperature-controlled (34 ± 0.5 °C) interface chamber and superfused with oxygenated ACSF at a rate of 1–2 mL/min. Slices were allowed to recover for at least 1 h. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum of the CA1 region with a glass micropipette filled with ACSF at a depth of ∼150–200 μm. Concentric bipolar electrodes were used to stimulate the Schaffer collaterals at a frequency of 0.03 Hz. Stimulation was set to evoke an fEPSP with a slope of 30–40% of maximum. After 20 min of baseline recording, long-term potentiation (LTP) was induced by applying a θ-burst stimulation, which consisted of 10 trains of 5 Hz stimuli, each composed of four (200 μs) pulses at 100 Hz repeated three times every 10 s. Basic synaptic transmission and presynaptic properties were analyzed through input/output (IO) measurements and paired pulse facilitation. IO measurements were performed by applying a defined value of current (0–100 μA in steps of 10 μA). Paired pulse facilitation was performed by applying a pair of two stimuli with an interstimulus interval of 40 ms. Data were collected and analyzed using 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.

Investigation of Changes in Cortical LTP Between L2 and WT Rats. Five hundred-micrometer coronal slices (1.5–3.5 mm anterior to Bregma, 2–4 mm lateral), including the forelimb area of the primary motor cortex, were prepared from 12- to 15-wk-old rats using a vibratome.

Stimulation and field potential recording. Concentric bipolar stimulation electrodes were positioned in layer II/III 2–2.5 mm lateral to the midline, and recording electrodes filled with 0.9% w/v NaCl were placed 500 μm laterally. Extracellular field potentials were evoked by 0.2 ms 0.03 Hz stimulation. Stimulation intensity was adjusted until a response of 0.2 mV was recorded, which was defined as the threshold intensity. Multiples of this intensity were used for determination of IO relationships to assess the baseline synaptic strength.

Induction of LTP. The stimulus intensity eliciting 50% of the maximum response amplitude was used for all measurements before and after LTP induction. Baseline amplitudes were recorded using single stimuli applied every 30 s. After a 30-min stable baseline period, LTP was induced by θ-burst stimulation, con-

sisting of 10 trains of 5 Hz stimuli, each composed of four (200 μs) pulses at 100 Hz repeated five times every 10 s. During θ-burst stimulation, the stimulation intensity was doubled; θ-burst stimulation was applied immediately after transient local application of the $GABA_A$ receptor antagonist bicuculline methiodide (3.5 mM) at the field potential recording site until response amplitude increased to 150–200% of baseline. LTP was attempted multiple times until responses were saturated. Saturated LTP was averaged and expressed as percentage of baseline. Average ± SEM was calculated for the last 10 min of stable LTP and compared with 10 min of baseline.

Statistical Analysis. Data evaluation and statistical analysis were conducted in Microsoft Excel and GraphPad Prism. Statistical significance was calculated by means of unpaired two-tailed Student *t* tests if not indicated otherwise.

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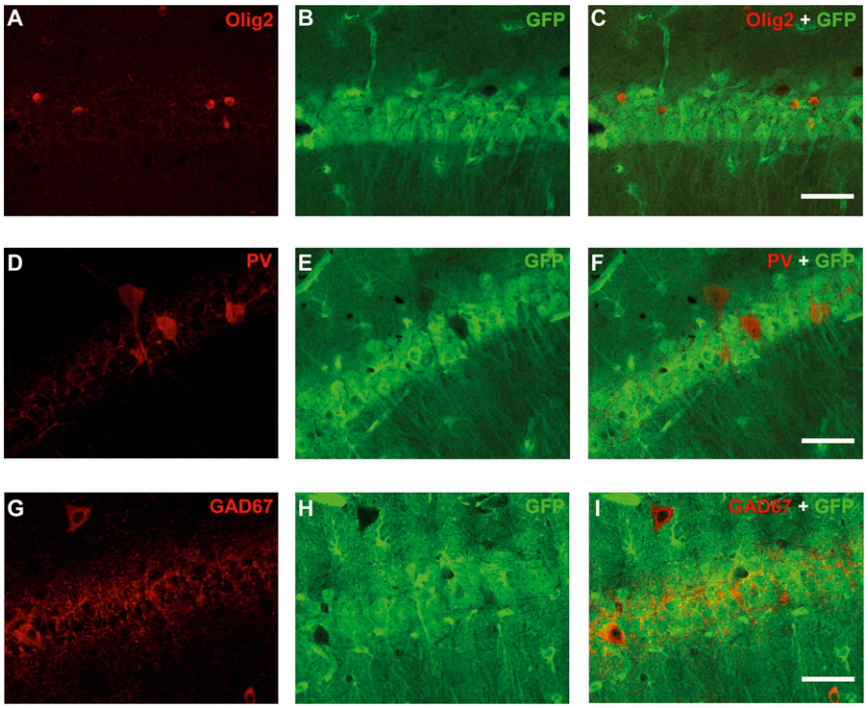

Fig. S1. Pattern of transgenic EGFP expression in L2 rat hippocampi. Immunofluorescence of the oligodendrocytic marker Oligodendrocyte transcription factor 2 (Olig2; *A*) or interneuronal markers Parvalbumin (PV; *D*) and Glutamate decarboxylase 67 (GAD67; *G*). EGFP expression is shown in *B*, *E*, and *H*. The merged pictures (*C*, *F*, and *I*) show the absence of transgenic expression in oligodendrocytes and interneurons. (Scale bars: 50 μm.)

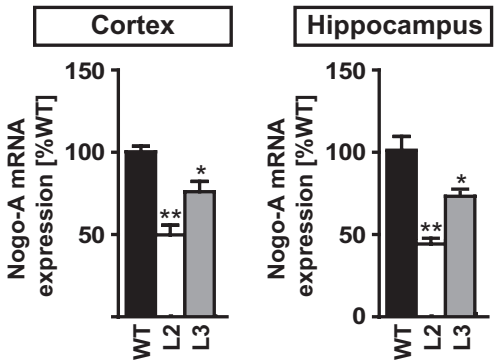

Fig. S2. Expression of Nogo-A mRNA in cortex and hippocampus. Relative expression of Nogo-A determined by qPCR in genotypes L2 and 3 as well as WT. *$P <$ 0.05; **$P < 0.01$.

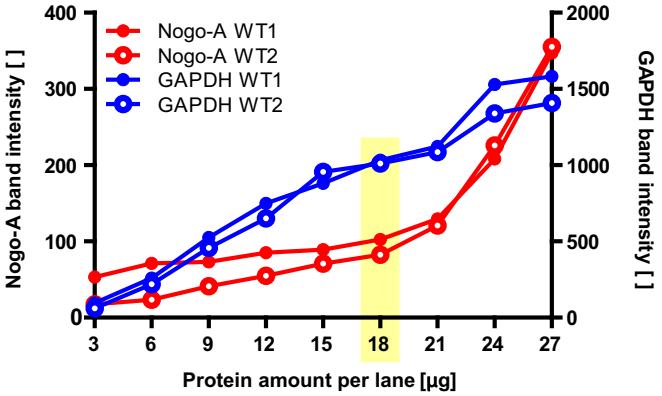

Fig. S3. Determination of the dynamic range for protein quantification by Western blotting. Serial dilutions of homogenized cortex from two WT animals were loaded on separate gels and analyzed by fluorescence Western blotting. The protein amount for subsequent experiments was selected such that differences between adjacent dilution factors were still visible (i.e., outside the saturation range of the detection system; yellow).

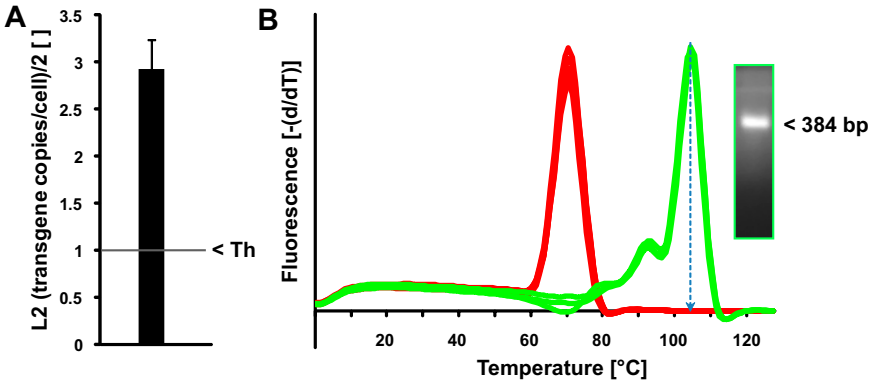

Fig. S4. Quantification of transgenic integration site. (*A*) qPCR copy number quantification of transgene/cell relative to *Tyrosine hydroxylase*. (*B*) Melting curve analysis of resulting PCR products: *Tyrosine hydroxylase* in red and transgene in green. *Inset* shows an agarose gel electrophoresis of the PCR product of the transgene.

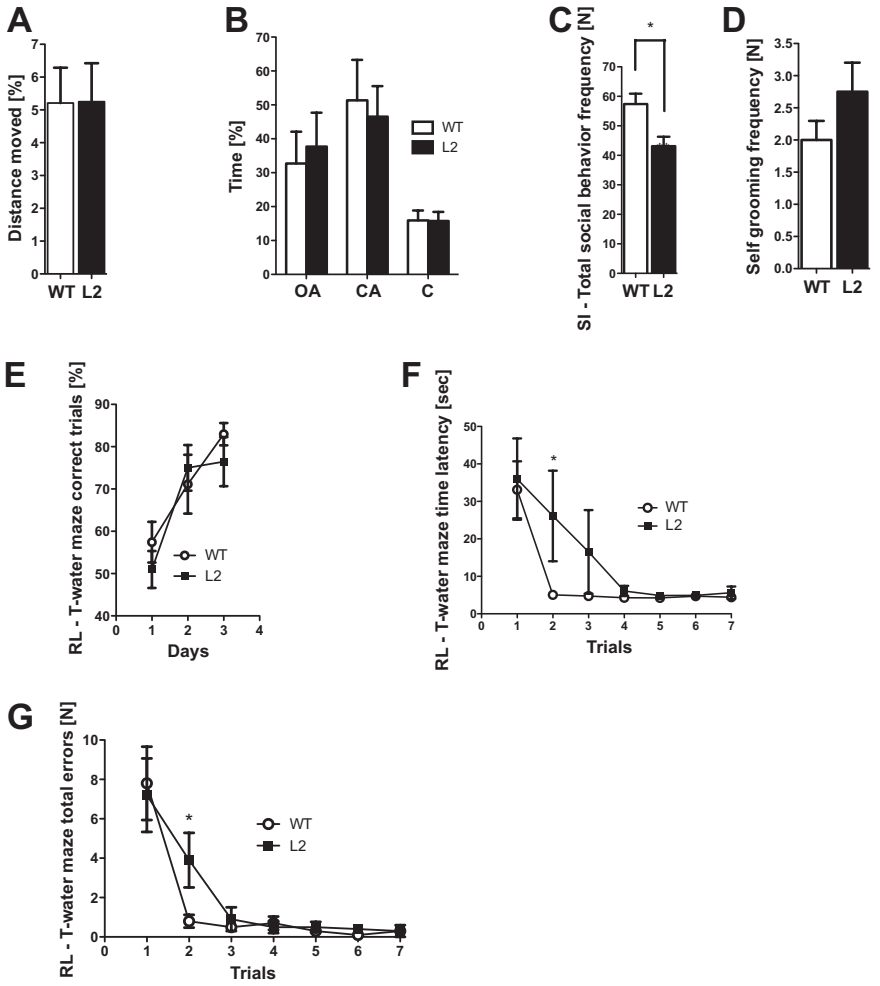

Fig. S5. (*A*) Percent of total distance moved in the center of the open field arena. Nogo-A knockdown rats (L2) show no difference compared with WT control rats. (*B*) Elevated plus maze. No difference in anxiety-related behavior could be observed. The percentage of time L2 rats spend in the closed arms (COs), open arms (OAs), and center of the maze (C) is not different compared with WT animals. (*C*) Total behavioral frequency. The sum of anogenital, nonanogenital, and following/approach was significantly different between L2 and control rats. (*D*) Self-grooming during social interaction. No difference is observed between L2 and WT rats. (*E*) Learning curve for the T water maze. No difference is observed between L2 and WT rats in five trials during 3 d of learning. (*F* and *G*) Reversal learning in the water T maze. Two-way ANOVA revealed that there was no significant difference between animals of both genotypes in the reversal of their escape strategy in the water T maze measured as escape latencies [*F*; L, $F_{Genotype}(1,108) = 1.53$; $P = 0.233$] and number of total errors [*G*; L, $F_{Genotype}(1,108) = 0.93$; $P = 0.348$]. However, Bonferroni posthoc testing revealed that, on trial 2, L2 rats have a significant impairment in finding the escape platform (time latency and total mistakes). All data presented are mean values + SEM or mean values ± SEM. *P* values were obtained by comparing L2 and WT rats with either unpaired *t* or Bonferroni posthoc test after two-way ANOVA of repeated measures: $*P < 0.05$.

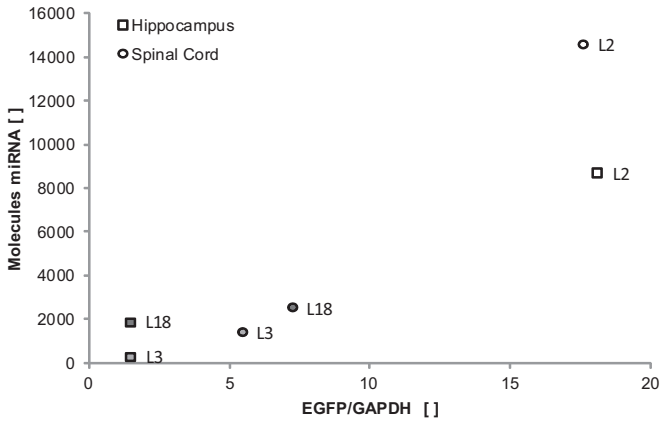

Fig. S6. Correlation of transgenic miRNA expression with EGFP expression for two exemplary regions from three transgenic rat lines.