Enhanced dendritic inhibition and impaired NMDAR activation in a mouse model of Down syndrome

Jan M. Schulz¹, Frederic Knoflach², Maria-Clemencia Hernandez² and Josef Bischofberger¹

¹Department of Biomedicine, University of Basel, Pestalozzistr. 20, CH-4056 Basel, Switzerland
²Pharma Research and Early Development, Discovery Neuroscience Department, F. Hoffmann-La Roche Ltd, Basel, Switzerland

https://doi.org/10.1523/JNEUROSCI.2723-18.2019

Received: 22 October 2018
Revised: 9 April 2019
Accepted: 10 April 2019
Published: 18 April 2019

Author contributions: J.M.S., M.C.H., and J.B. designed research; J.M.S. and F.K. performed research; J.M.S. analyzed data; J.M.S. and J.B. wrote the first draft of the paper; J.M.S. and J.B. wrote the paper; F.K., M.C.H., and J.B. edited the paper.

Conflict of Interest: The authors declare no competing financial interests.

We would like to thank Tom Otis for helpful comments on the manuscript. We thank Selma Becherer and Martine Schwager for mouse genotyping, histochemical stainings and technical assistance, Marie-Claire Pfimlin for some electrophysiological recordings and Andrew Thomas for RO4938581 supply. This work was supported by a Roche Postdoctoral Fellowship and by the Swiss National Science Foundation (SNSF, Project 31003A_176321). The authors declare no competing financial interests.

Correspondence: Dr. Josef Bischofberger, Department of Biomedicine, University of Basel, Pestalozzistr. 20, CH-4046 Basel, Switzerland, Phone: +41-61-2672729, E-mail: Josef.Bischofberger@unibas.ch

Cite as: J. Neurosci 2019; 10.1523/JNEUROSCI.2723-18.2019

Alerts: Sign up at www.jneurosci.org/alerts to receive customized email alerts when the fully formatted version of this article is published.
Enhanced dendritic inhibition and impaired NMDAR activation in a mouse model of Down syndrome

Jan M. Schulz, Frederic Knoflach, Maria-Clemencia Hernandez, and Josef Bischofberger

1Department of Biomedicine, University of Basel, Pestalozzistr. 20, CH-4056 Basel, Switzerland
2 Pharma Research and Early Development, Discovery Neuroscience Department, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Abbreviated title: α5-GABAA receptor modulation recues impaired NMDAR activation in DS

Address correspondence to:
Dr. Josef Bischofberger
Department of Biomedicine
University of Basel
Pestalozzistr. 20
CH-4046 Basel, Switzerland
Phone: +41-61-2672729
E-mail: Josef.Bischofberger@unibas.ch

Key words: Down syndrome, Ts65Dn mice, dendritic inhibition, alpha5 GABAA receptors, NMDA receptors, hippocampus, CA1 pyramidal cells

Acknowledgements. We would like to thank Tom Otis for helpful comments on the manuscript. We thank Selma Becherer and Martine Schwager for mouse genotyping, histochemical stainings and technical assistance, Marie-Claire Pfimlin for some electrophysiological recordings and Andrew Thomas for RO4938581 supply. This work was supported by a Roche Postdoctoral Fellowship and by the Swiss National Science Foundation (SNSF, Project 31003A_176321). The authors declare no competing financial interests.
Down syndrome (DS) or Trisomy 21 is a developmental disorder leading to cognitive deficits including disruption of hippocampus-dependent learning and memory. Enhanced inhibition has been suggested to underlie these deficits in DS based on studies using the Ts65Dn mouse model. Here we show that in this mouse model, GABAergic synaptic inhibition onto dendrites of hippocampal pyramidal cells is increased. By contrast, somatic inhibition was not altered. In addition, synaptic NMDA receptor (NMDAR) currents were reduced. Furthermore, dendritic inhibition was mediated via nonlinear α5-subunit containing GABA_ARs that closely matched the kinetics and voltage dependence of NMDARs. Thus, enhanced dendritic inhibition and reduced NMDA currents strongly decreased burst-induced NMDAR-mediated depolarization and impaired LTP induction. Finally, selective reduction of α5-GABA_A-mediated inhibition rescued both, burst-induced synaptic NMDA receptor activation and synaptic plasticity. These results demonstrate that reduced synaptic NMDAR activation and synaptic plasticity in the Ts65Dn mouse model of DS, can be corrected by specifically targeting nonlinear dendritic inhibition.

Significance statement (<120 words)

Mild to moderate intellectual disability is a prominent feature of Down Syndrome. Previous studies in mouse models suggest that increased synaptic inhibition is a main factor for decreased synaptic plasticity, the cellular phenomenon underlying memory. The present study shows that increased inhibition specifically onto dendrites together with reduced NMDA receptor content in excitatory synapses may be the cause. Reducing a slow nonlinear component that is specific to dendritic inhibitory inputs and mediated by α5 subunit-containing GABA_A receptors rescues both NMDA receptor activation and synaptic plasticity.
**Introduction**

Trisomy of chromosome 21, also known as Down syndrome (DS), is the most common genetic neurodevelopmental disorder associated with mild-to-moderate intellectual disability (Lott and Dierssen 2010). While implicit learning is relatively preserved, there are strong deficits in explicit learning capabilities including hippocampus-dependent tasks. Triplication of the human Chromosome 21 might increase expression of developmental genes regulating size and fate of neural progenitor cell pools (Dierssen 2012). Besides smaller brain size and reduced neurogenesis, an imbalance between the cortical excitatory and inhibitory synaptic transmission (E/I balance) has been implicated as a major cause of cognitive deficits in DS (Kleschevnikov et al. 2004, Costa and Grybko 2005, Fernandez et al. 2007, Kleschevnikov et al. 2012, Dierssen 2012, Bartesaghi et al. 2015) similar to other neurodevelopmental disorders including autism and schizophrenia (Lewis 2014, Braat and Kooy, 2015, Pocklington et al. 2015). In the mouse, about 50% of the orthologue genes of the human chromosome 21 including the Down Syndrome critical region are located on the chromosome 16 (Reeves et al. 1995, Charkrabarti et al. 2007). Several mouse models of DS have been developed including the widely used Ts65Dn mouse, which has an additional fragment of the murine chromosome 16 containing about 100 orthologue genes (Charkrabarti et al. 2007, Gardiner 2014) and recapitulates the hallmarks of the DS phenotype including cognitive impairments (Reeves et al., 1995, Dierssen 2012). Although the precise contributions of all triplicated genes have not been clearly identified, there is evidence that the number of medial ganglionic eminence (MGE)-derived somatostatin (SOM) and parvalbumin (PV)-expressing GABAergic interneurons is increased due to enhanced expression of the transcription factors Olig1 and Olig2 in interneuron progenitors (Chakrabarti et al. 2010). In addition, there may be an increased number of NO-synthase (NOS)/NPY-positive neurogliaform cells, which derive from the same progenitor pool in the MGE (Tricoire et al. 2010, Raveau et al. 2018). Similarly, the triplicated expression of the serine/threonine kinase DYRK1A affects interneuron differentiation and shifts the E/I-balance towards more inhibition (Souchet et al. 2014).

On a functional level, it has been shown that hippocampal long-term potentiation (LTP) is reduced in Ts65Dn mice in a GABA\textsubscript{A} receptor dependent manner (Kleschevnikov et al. 2004, Costa and Grybko 2005, Fernandez et al. 2007,
Furthermore, partial block of GABA\textsubscript{A} receptors did not only rescue hippocampal LTP, but also reversed learning deficits in Ts65Dn mice (Fernandez et al. 2007). However, it is largely unknown which functional properties of inhibitory transmission onto hippocampal and cortical pyramidal neurons are altered in Ts65Dn mice.

Pharmacological evidence indicates that specific subtypes of GABA\textsubscript{A}-receptors may be involved in DS phenotypes. Behavioral studies have shown that pharmacological modulation of α5-containing GABA\textsubscript{A} receptors (α5-GABA\textsubscript{A}Rs) ameliorates cognitive deficits in Ts65Dn mice and rescues impaired LTP induction (Braudeau et al. 2011, Martinez-Cue et al. 2014, Rudolph and Möhler 2014). Furthermore, it was shown that genetic ablation of the α5-subunit of GABA\textsubscript{A}Rs in TS mice partially rescues learning as well as LTP deficits (Vidal et al. 2018) pointing to a specific role of these GABA receptors in DS. Hence, selective modulation of α5-GABA\textsubscript{A}R by negative allosteric modulators (α5-NAM) may represent an attractive treatment strategy, as these drugs do not generate proconvulsant or anxiogenic side effects (Rudolph and Knoflach 2011, Rudolph and Möhler 2014).

Immunohistochemistry indicates that α5-GABA\textsubscript{A}Rs are localized in hippocampal pyramidal cell dendrites in the synaptic as well as in extrasynaptic membrane (Serwanski et al. 2006). We recently showed that these receptors mediate synaptic inhibition of pyramidal cell dendrites evoked by SOM- and NOS-positive interneurons (Schulz et al. 2018), in addition to the contribution to tonic inhibition (Caraiscos et al., 2004; Prenosil et al., 2006; Brickley and Mody, 2012). By contrast, PV-interneuron-mediated inhibition targeting pyramidal cell somata was independent of α5-GABA\textsubscript{A}Rs. Together with results from in vivo pharmacological studies in Ts65Dn mice, this suggests that SOM- and/or NOS-interneuron mediated dendritic inhibition may contribute to cognitive deficits in DS. However, the role of dendritic inhibition in DS models has not been addressed.

In the current study, we show for the first time that reduced NMDAR-EPSPCs and increased dendritic inhibition converge towards reduced NMDAR-function during burst PSPs in Ts65Dn mice. We further elucidate the mechanisms by which an α5-NAM counteract the increased dendritic inhibition and rescue disturbed NMDAR-dependent LTP.
Methods

Animals. Ts65Dn mice (B6EiC3Sn a/A-Ts(17<16>)65Dn) were obtained from The Jackson Laboratory and bred by backcrossing of Ts65Dn females with B6C3F1/OlaHsd males (F1 hybrid males from C57BL/6J x C3H/HeNHsd obtained from Harlan). Breeding was performed at the animal facilities of the University of Basel. The supernumerary Chromosome 17<16> in offsprings was identified by standard PCR procedures as described previously (Reinholdt et al. 2011). Some Ts65Dn mice plus wt littermates were kindly provided by Carmen Martinez-Cue from the University of Cantabria for pilot experiments. For most experiments, mice were young adults of 8 to 15 weeks. For some experiments (Fig. 3C-F), some younger C57BL/6 mice were used in addition (6 to 10 weeks of age).

Mice were housed in groups of up to 5 animals in standard Individually Ventilated Cages in standard laboratory conditions with a 12 h light/dark cycle, and access to food and water ad libitum. Wet chow was fed to weaned mice to ensure sufficient growth in Ts65Dn mice. All experiments were approved by the Basel Cantonal Committee on Animal Experimentation according to federal and cantonal regulations.

Slice preparation for patch-clamp recordings. Mice were anaesthetized with isoflurane (4% in O2, Vapor, Draeger) and killed by decapitation, in accordance with national and institutional guidelines. In order to increase cell viability for single cell patch-clamp recordings, animals were exposed to oxygen-enriched atmosphere for 10 min prior to decapitation. Slices were cut as previously described (Geiger et al. 2002; Bischofberger et al. 2006). Briefly, the brain was dissected in ice-cold sucrose-based solution at about 4 °C. Horizontal 350-μm-thick hippocampal brain slices were cut at an angle of 20° to the dorsal surface of the brain along the dorso–ventral axes of the hippocampus using a Leica VT1200 vibratome. For cutting and storage, a sucrose-based solution was used, containing (in mM) 87 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 75 sucrose, 0.5 CaCl2, 7 MgCl2 and 10 glucose (equilibrated with 95% O2/ 5% CO2). Slices were kept at 35°C for 30 min after slicing and subsequently stored at room temperature until experiments were performed at 32-33°C.
**Slice preparation for field potential recordings.** Wild-type and Ts65Dn mice were anaesthetized using a mixture of 2.5% isoflurane in O₂ and decapitated as approved by the local institutional animal welfare committee. After removing the brain, the left side of the hippocampal formation was dissected out in a solution containing (in mM):

- 124 NaCl, 2.5 KCl, 1.25 KH₂PO₄, 2 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃, 10 Glucose at room temperature. Transverse slices (400 μm-thick) from the medium part of the hippocampus were cut with a TC-2 Sorvall tissue chopper (MTS, Liverpool, NY, USA), transferred to the recording chamber and stored at room temperature for one hour prior to the experiment.

Patch-clamp recordings. CA1 pyramidal neurons were visually identified in the pyramidal cell layer close to the border of stratum radiatum using infrared differential interference contrast (IR-DIC) video microscopy. Slices were continuously superfused with artificial cerebrospinal fluid (ACSF) at near physiological temperature (32-33°C). The ACSF contained (in mM): 125 NaCl, 25 NaHCO₃, 25 glucose, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂ (equilibrated with 95% O₂/ 5% CO₂). Patch pipettes were pulled from borosilicate glass tubing with a 2.0 mm outer diameter and 0.5 mm wall thickness (Hilgenberg GmbH, Malsfeld, Germany) on a Flaming-Brown P-97 puller (Sutter Instruments, Novato, USA).

For most current-clamp recordings, patch-pipettes (4–7 MΩ) were filled with a solution containing (in mM): 120 KMeSO₄, 4 KCl, 10 EGTA, 10 Heps, 2 MgCl₂, 10 Na₂-phosphocreatine, 2 Na₂ATP, 0.3 GTP, and 0.2% biocytin adjusted to pH 7.3 with KOH. For voltage-clamp recordings, patch-pipettes (2–4 MΩ) were filled with a Cs gluconate-based solution containing (in mM): 135 CsGluc, 4 CsCl, 10 EGTA, 10 Heps, 2 MgCl₂, 2 Na₂ATP, 2 TEA-Cl, 5 QX314 adjusted to pH 7.3 with CsOH.

Voltage and current signals were measured with a Multiclamp 700A amplifier (Molecular Devices, Palo Alto, CA, USA), low-pass filtered with cut-off frequencies of 8 kHz and digitized at 20 kHz using a CED Power 1401 interface (Cambridge Electronic Design, Cambridge, UK). Recorded cells were only included if the initial seal exceeded a resistance of 1 GΩ, the initial membrane potential was well below -55 mV and there were no signs of cellular deterioration (e.g. decreasing input resistance) or rundown of synaptic transmission during the recording. Bridge balance was used to compensate the series resistance \( R_s = 10-40 \) MΩ in current clamp recordings. Series resistance in voltage-clamp experiments \( R_s = 5-20 \) MΩ was
Data acquisition was controlled using IGOR Pro 6.31 (WaveMetrics, Lake Oswego, Oregon) and the CFS library support from CED (Cambridge Electronic Design, Cambridge, UK).

For the assessment of cellular excitability in interneurons and pyramidal neurons (Fig. 3), we used a potassium gluconate-based internal solution (in mM): 135 potassium gluconate, 21 KCl, 2 MgCl₂, 2 Na₂ATP, 0.3 Na-GTP, 10 HEPES, 0.5 EGTA adjusted to pH 7.3 with KOH. These experiments were performed in the presence of ionotropic receptor blockers 10 μM NBQX, 25 μM AP5, 100 μM picrotoxin. Interneurons were identified by their location in stratum radiatum (SR) or stratum oriens (SO). In SO, only interneurons with a horizontally elongated soma and without prominent apical dendrites were chosen according to properties of dendrite-targeting somatostatin positive interneurons (Maccaferri et al. 2000, Lien et al. 2002). A few interneurons with a prominent fast-spike phenotype indicative of parvalbumin positive interneurons, which mainly target axon initial segments and peri-somatic compartments, were excluded from analysis.

To evaluate cellular properties 1 s-long current steps of increasing amplitude (steps of 25 pA) were injected during current-clamp recordings. In all current-clamp protocols other than the initial assessment of cellular properties, the resting membrane potential was kept constant close to the initial potential of -67 to -70 mV by small constant current injections throughout the experiment. During pharmacological manipulations, the input resistance was regularly assessed by small current steps of 25 pA amplitude.

**Extracellular synaptic stimulation.** For stimulation of synaptic inputs, 4-6 MΩ pipettes filled with HEPES-buffered Na²⁺-rich solution were used to apply brief negative current pulses (200 μs). To stimulate Schaffer Collaterals, the pipettes were placed into SR close to the CA2-CA1 border at a distance of ≥500 μm (500-800 μm) from the recorded neuron, and stimulation was applied at intermediate intensity (50-100 μA). To record NMDA-AMPA ratios, the pipettes were placed close to the recorded cell at a distance of <200 μm in either SR or SO, and stimulation was applied at low intensity (30 μA). GABAergic IPSCs were evoked at a range of intensities (10-40 μA). To avoid the confounding influence of potentially altered GABA₆ receptor-signalling in Ts65Dn mice (Kleschevnikov et al., 2012), 1 μM
CGP54626 was added to block GABA\textsubscript{B} signaling except for field potential recording experiments (Fig.6). Stimulus artifacts have been truncated in most Figures for clarity.

**NMDA-AMPA ratios.** To measure the balance between NMDAR- and AMPAR-mediated currents, the neuron was clamped to -70 mV and stepped alternatingly to -90 mV and to +50 mV. Voltage steps were of 1 s duration and a single stimulus was applied 750 ms after the voltage step onset. At -90 mV, AMPAR-mediated inward currents were recorded. At +50 mV, the NMDAR-mediated outward currents were recorded. The inter-stimulus interval was ≥ 50 s. Experiments were performed in the presence of 10 \(\mu\)M Nimodipine to reduce dendritic Ca\textsuperscript{2+} channel activation, 100 \(\mu\)M picrotoxin to block GABA\textsubscript{A}R, and the NMDAR co-agonist 10 \(\mu\)M glycine to avoid potential wash-out of endogenous glycine and ensure optimal synaptic NMDAR activation. PSCs were recorded in whole cell configuration. The series resistance (5-12 M\textOmega) was compensated (50-90\%, 5 kHz bandwidth). The conductance was calculated as the PSC amplitude divided by the driving force, i.e. the difference between membrane potential and synaptic reversal potential. The synaptic reversal potential for glutamatergic synapses was assumed to be 0 mV.

**Voltage-dependence of NMDA-PSCs.** NMDAR-PSCs were recorded at increasing membrane potentials with an Axopatch 200 B (Fig.3C). Series resistance was compensated at 90-95\%. For the analysis of the voltage-dependence, only experiments with a maximal PSC amplitude of >0.5 nA were included. Membrane potentials were corrected offline by the calculated liquid junction potential of -15.7 mV (JPCalcWin; Barry, 1994).

**Measurement of \(\alpha5\)-GABA\textsubscript{A}R-mediated IPSCs.** All IPSCs were recorded at 80\% series resistance (5-12 M\textOmega) compensation. To assess the decay phase of outward currents, IPSCs were recorded at about -20 mV before and after a wash-in phase of >5 min of RO4938581 (1 \(\mu\)M). Experiments were performed at 32-33°C, except for the assessment of the voltage-dependence (Fig.3F), which was performed at room temperature (22-24°C) for greater recording stability.
Local field potential recordings and LTP induction. Field excitatory postsynaptic potentials (fEPSP) were recorded from hippocampal slices of wt and Ts65Dn mice using glass micropipettes (Clark GC 120F; 1-4 MΩ) filled with 2 M NaCl and placed in the stratum radiatum (SR) of the CA1 region. Slices were constantly superfused with an artificial cerebrospinal fluid (ACSF) at 30 °C containing (in mM): 120 NaCl, 3.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose equilibrated with 95% O₂/ 5% CO₂. fEPSPs were evoked by stimulating the SR (0.033 Hz, 100 μs) with insulated bipolar platinum/iridium electrodes (RDM, Basel). The stimulus strength was adjusted to evoke fEPSPs equal to 30% of the relative maximum amplitude without superimposed population spike. This was determined from separate input-output experiments recorded for each slice. After stable baseline recordings, long term potentiation (LTP) was induced using a theta burst stimulation paradigm (TBS) consisting of a pattern of 5 stimuli at 100 Hz repeated 10 times at 200 ms intervals. The duration of the stimulation pulses was doubled during TBS without modifying the stimulus strength.

fEPSPs were amplified with a Cyberamp 380 amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2.4 kHz and digitized at 20 kHz with a Digidata 1322 acquisition board (Molecular Devices). The pClamp data acquisition software (Molecular Devices) was used to record and to analyze the signals.

Drugs. Nimodipine (Sigma) was dissolved at 20 mM in DMSO on the day of the experiment. All other drugs were stored as aliquots at -20°C. D-AP5 (50 mM; Tocris) was dissolved in water. Picrotoxin was dissolved at 50 mM in ethanol. CGP 54626 hydrochloride (10 mM; Tocris), NBQX (20 mM; Tocris), L-655,708 (1mM; Tocris) and the α₅-NAM RO4938581 (10 mM; F. Hoffmann-La Roche) were dissolved in DMSO.

Data Analysis. Analysis of patch-clamp data was performed offline using the open source analysis software Stimfit (https://neurodroid.github.io/stimfit, Guzman et al. 2014) and customized scripts written in Python. For calculations of the integral, traces were low-pass filtered (3rd order Butterworth, 100 Hz cut off) to determine the start and endpoint of the integral as the intersection of the smoothed trace with the baseline. The integral was then calculated as the sum of all values of the original trace minus the baseline in between start and endpoint. The analysis of voltage-clamp data was performed on mean waveforms. The waveform of the IPSCs
mediated by α5-GABAARs was obtained by subtracting the mean IPSC waveform after the addition of an α5-NAM from the waveform recorded under control conditions. The analysis of current-clamp data was performed on single trial data to avoid distortion by occasional action potential (AP) discharge. The analysis of the AP5-sensitive burst components after distant SC stimulation was restricted to the last PSP of the burst, where the strongest NMDAR contribution was expected. Before the calculation of the integral, occasional APs were cut off at the AP threshold defined as the membrane potential at which the voltage slope reached 10 V/s. The median of all single episode measurements of a whole epoch (defined by the drug treatment) was then saved.

Standard electrophysiological parameters were determined from a family of 1 s steps of increasing amplitudes (25 pA step size). Input resistance was determined from the slope of a regression line fitted to four mean membrane potentials produced by a series of subthreshold current pulses (-25, 0, +25, +50 pA). The rheobase current was defined as the minimal current amplitude necessary to evoke an action potential within 1s pulses. AP frequency versus current relationship (F-I curve) for somatic current injections was fitted by the logarithmic function F=gain*ln(I/I_0), where I_0 denotes the threshold current (Engel et al, 1999). Action potential properties were measured on the first spike elicited by the rheobase current.

The slope of the fEPSP was measured by fitting a straight line from 40% to 70% of the peak amplitude using the Clampfit program.

Experimental Design and Statistical Analysis. Statistical analyses were performed in GraphPad Prism 6. Before statistical evaluation, data was always tested for normality by the Shapiro-Wilk normality test. In most instances, statistical estimations of significance of paired data were derived from paired two-tailed Student’s t-tests. For comparisons between groups, statistical tests were two sample two-tailed Student’s t-tests. Data sets that failed the Shapiro-Wilk normality test were subsequently analyzed with the nonparametric Wilcoxon Signed Rank and the Mann-Whitney tests for paired and unpaired data, respectively. The significance level was set to P=0.05. The number of cells per group varied for different experiments typically ranging from n=10-18 cells. Assuming an effect size of one standard deviation, the statistical power ranged from 0.62 to 0.96 for comparison between genotypes, and from 0.58 to 0.99 for repeated measures comparisons. Due to the phenotypical
features of Ts65Dn mice (i.e. smaller head and brain size), a blinding of the experimenter to the genotype of mice was not possible. No randomization was performed. Ts65Dn mice and wildtype (wt) litter mates were used alternately from the same litters. All data are shown as mean ± s.e.m.. Unless stated otherwise, the number (n) of observations indicated reflects the number cells recorded from.

Results

Normal excitability of CA1 pyramidal neurons in Ts65Dn mice
CA1 pyramidal neurons were examined in hippocampal brain slices from adult Ts65Dn mice and wildtype (wt) littermates. Fundamental electrophysiological parameters such as membrane potential, capacitance, input resistance, action potential (AP) threshold and other AP parameters (rheobase, AP amplitude, half width, rise and decay slope) were normal (Table 1). Therefore, it is unlikely that altered cellular excitability might contribute to the potential differences in hippocampal synaptic plasticity, learning and memory.

Reduced NMDAR activation in CA1 pyramidal neurons of Ts65Dn mice
Hippocampal LTP induced by theta-burst stimulation (TBS) is decreased in Ts65Dn mice (Costa and Grybko 2005, Martinez-Cue et al. 2013). This could be due to reduced activation of synaptic NMDARs or reduced down-stream signaling during LTP expression. On the other hand, memantine has been shown to counteract cognitive deficits in Ts65Dn mice during fear conditioning indicating that NMDARs might be hyperactive in DS (Costa et al. 2008). To directly measure synaptic NMDAR and AMPAR activation, EPSCs were recorded after stimulation of Schaffer collateral (SC) inputs in stratum radiatum (SR; Fig. 1AB) in the presence of GABA\textsubscript{A}-receptor antagonists. AMPAR-mediated PSCs evoked in SR were of similar amplitude in Ts65Dn and wt mice independent of stimulation intensity (Fig. 1C). In contrast, NMDAR-mediated PSCs tended to be smaller in Ts65Dn mice (Fig.1D), although this effect was not statistical significant (P=0.37, F(1, 32) = 0.8224, two-way ANOVA; n\textsubscript{1}=16, n\textsubscript{2}=18) probably due to the large cell-to-cell variability of evoked current amplitudes. To increase statistical power, we normalized the NMDAR-mediated conductance by the AMPAR-mediated conductance recorded in the same cell. The
NMDA-AMPA conductance ratio was 0.84 ± 0.04, (n=27) in Ts65Dn mice and thus about 30% smaller than in wt mice (1.18 ± 0.06, n=31, P<0.0001, two sample t-test; Fig. 1E). To test whether the decrease in the synaptic NMDAR content was synapse specific, we recorded also evoked synaptic inputs onto basal dendrites by stimulating in stratum oriens (SO) in a subset of neurons. The NMDA-AMPA ratio of SO inputs was not different between Ts65Dn and wt mice (1.24 ± 0.17, n=9 versus 1.31 ± 0.08, n=15; P=0.7; Fig. 1F-H). Therefore, these results indicate that glutamatergic synapses show a subcellular compartment-specific reduction of the synaptic NMDAR complement.

Next, we studied the contribution of NMDARs to burst EPSPs evoked by SC activation in SR in the absence of GABAAR antagonists. Burst stimulation (5 pulses at 50 Hz) revealed a significant NMDAR contribution in wt mice to both, the peak amplitude and integral of the 5th burst PSP of 6.2 ± 2.5% (P=0.038; one sample t-test, n=10) and 15.5 ± 2.5% (P<0.001), respectively (Fig. 1G). However, in Ts65Dn mice the contribution of NMDARs to amplitude and integral of burst PSPs was virtually absent (both: P>0.6, n=15, Fig. 1G, H). Together, these results show that NMDAR recruitment is deficient in response to inputs from CA3, the major input source of CA1 pyramidal neurons.

Increased dendritic inhibition in Ts65Dn mice

Increased inhibition has been proposed to be the main cause for impaired synaptic plasticity in Ts65Dn mice. Although there is substantial evidence showing that reduction of GABA signaling rescues LTP deficits in Ts65Dn mice (Kleschevnikov et al., 2004; Costa and Grybko, 2005; Fernandez et al. 2007, Martinez-Cue et al. 2013), GABAAR-mediated IPSCs have rarely been investigated in CA1 pyramidal neurons of Ts65Dn mice (Chakrabarti et al. 2010, Best et al. 2012). Therefore, we systematically analyzed GABAAR-mediated IPSCs in CA1 pyramidal cells, evoked either in dendritic layers (SR) or close to the soma (SP) with different stimulation intensities. As shown in Figure 2, dendritic IPSCs evoked in SR increased with increasing stimulation intensity (P<0.0001, F(3, 72) = 77.46, two-way ANOVA; Fig. 2C). In addition, there was a significant genotype-by-stimulation-intensity interaction (P<0.0001, F(3,72) = 8.316). Post-hoc tests revealed that SR-IPSCs were larger in Ts65Dn mice as compared to littermate controls at strong stimulation intensities of 30 μA (610.1 ± 75.5 pA, n=12 versus 395.0 ± 60.8 pA, n=14, P=0.043) and 40 μA (713.1 ± 83.9 pA,
By contrast, for somatic IPSCs evoked in SP there was neither a significant main effect of genotype \((P=0.35, F(1,21)=0.9191, n_1=9, n_2=14)\), nor a significant genotype-by-stimulation-intensity interaction \((P=0.74, F(3,63)=0.4194; \text{Fig. 2D})\). These results show that dendritic inhibition is significantly increased in Ts65Dn mice, while somatic inhibition is unchanged.

**Increased dendritic inhibition is not caused by increased excitability of dendrite-targeting interneurons Ts65Dn mice**

Increased inhibition during local electric stimulation could be potentially the result of an enhanced recruitment of inhibitory fibers due to altered cellular excitability of interneurons in Ts65Dn mice. To test for this possibility, we recorded from interneurons located in SR in the presence of ionotropic receptor blockers (10 \(\mu\)M NBQX, 25 \(\mu\)M AP5, 100 \(\mu\)M picrotoxin; \text{Fig. 3}). There was no difference in resting membrane potential (\text{Fig. 3A, B and E}), AP voltage threshold (\text{Fig. 3F}), input resistance (\text{Fig. 3G}), or rheobase \((100.0 \pm 11.3 \text{ pA}, n=17 \text{ versus } 92.5 \pm 8.8 \text{ pA}, n=10; P=0.65, \text{unpaired two-sample } t\)-test) between SR interneurons in Ts65Dn and wt animals. Surprisingly, the analysis of the relationship of AP frequency versus current \((\text{F-I curve})\) for somatic current injections (\text{Fig. 3C-D, H}) showed a decreased frequency gain per e-fold current increase in SR interneurons in Ts65Dn mice \((41.3 \pm 2.2 \text{ Hz}, n=17 \text{ versus } 52.6 \pm 3.6 \text{ Hz}, n=10; P=0.008, \text{unpaired two-sample } t\)-test). This difference indicates that excitability of SR interneurons is not increased in Ts65Dn mice, but instead decreased during continuous current injection. Under the same recording conditions, we did not find any indication for increased intrinsic excitability in pyramidal neurons or dendrite-targeting interneurons located in SO (\text{Fig. 3E-H}), either. Taken together, these results indicate that increased dendritic inhibition in Ts65Dn mice is not due to increased cellular excitability and differential recruitment of dendrite-targeting interneurons.

**Dendritic \(\alpha_5\)-GABA\(_A\)-mediated IPSCs match NMDAR properties**

We have previously shown that dendritic inhibition in CA1 pyramidal cells is largely mediated via \(\alpha_5\)-GABA\(_A\)-R \cite{Schulz2018}. By contrast, peri-somatic IPSCs evoked in SP were not affected by \(\alpha_5\)-GABA\(_A\)-R-selective modulators \cite{Schulz2018}. Therefore, we aimed to reduce enhanced dendritic inhibition in Ts65Dn mice.
by application of the α5-NAM RO4938581 (0.6 μM), resulting in a decrease of
dendritic IPSCs by 19.7 ± 3.8% at a membrane potential of -70 mV (n=9, Ballard et
al. 2009, Schulz et al. 2018). These receptors were shown to have slow gating
kinetics and non-linear voltage dependence. Therefore, we compared the decay time
course of NMDAR-mediated currents with the time course of α5-GABA<sub>A</sub>R-mediated
dendritic inhibition. As shown in Figure 4, the decay time constant of NMDA EPSCs
in Ts65Dn mice were not significantly different from wt littermates (46.2 ± 2.2 ms,
n=9, versus 46.9 ± 2.5 ms, n=15, P=0.85, two sample t-test; Fig. 4A,B). Dendritic
IPSCs evoked in SR showed a faster time course (28.0 ± 0.8 ms, n=5, Fig. 4D, E)
than NMDARs. However, the α5-NAM sensitive component was significantly slower
(P=0.001, paired t-test) and showed a similar decay time constant (56.2 ± 3.1 ms,
n=5) to NMDAR-mediated currents (Fig. 4B black versus 4E light blue). We further
analyzed the current-voltage relationship of GABAergic currents evoked in SR,
revealing a pronounced nonlinear voltage dependence. The maximal slope of the IV-
curve equivalent to the maximal conductance was reached at about -50 mV, very
similar to the voltage-dependent activation threshold of NMDA receptors (Fig. 4C
versus 4F).

This suggests that the absence of NMDAR activation during burst PSPs in
pyramidal cells in Ts65Dn mice (Fig. 1D,E) is due to a synergistic action of reduced
synaptic NMDAR currents (Fig. 1B,C) and increased dendritic IPSCs (Fig. 2).

**Rescue of impaired NMDAR activation in Ts65Dn mice by α5-NAM**

To directly study, whether dendritic inhibition interferes with NMDAR activation in
Ts65Dn mice via slow α5-GABA<sub>A</sub>R, we tested the effect of 1μM of the α5-NAM
RO4938581 on SC-evoked burst PSPs, which was reported to block about 50% of
the α5-GABAR-mediated currents (Ballard et al. 2009, Schulz et al. 2018). Strikingly,
application of the α5-NAM restored the NMDAR activation during burst PSPs in
Ts65Dn mice up to the level found in wt littermates under control condition (Fig. 5A).
The presence of the α5-NAM allowed NMDARs to contribute with 9.7 ± 3.7% (n=8)
significantly to the PSP amplitude in Ts65Dn mice (P=0.035, one sample t-test; Fig.
5B). Likewise, the reduction of slow α5-GABA<sub>A</sub>R mediated currents resulted in a
significant NMDAR contribution to the integral in Ts65Dn mice (11.8 ± 4.8%,
P=0.008, Wilcoxon Signed Rank test), comparable to the one in wt mice under
control conditions (15.5 ± 2.5%, n=10). This shows that blocking 50% of the α5-
The application of the α5-NAM effectively increased firing frequency by a factor of two (13.9 ± 2.5 Hz versus 6.7 ± 2.0 Hz, n=5, P=0.022, paired t-test; Fig. 5D). As the input resistance in pyramidal cells of Ts65Dn mice was not significantly increased by the α5-NAM (P=0.76, n=8, paired t-test; Fig. 5E), this indicates that decreased dendritic inhibition powerfully increase postsynaptic depolarization and output firing.

Finally, we measured TBS-induced LTP of synaptic field potentials in SR (Fig. 6; Bliss and Collingridge, 1993; Raymond and Redman, 2006). Consistent with previous studies (Costa and Grybko, 2005; Martinez-Cue et al., 2013), the relatively mild TBS paradigm induced LTP in slices from wt littermates (127.1 ± 7.2% at 80 min, n=14), but failed to induce LTP in Ts65Dn mice (110.1 ± 3.1% of baseline control, n=19, P<0.05, two-tailed Student’s t-test; Fig. 6A,B). Reducing α5-GABAA-R-mediated inhibition by acute application of the α5-NAM (0.3 μM) compensated for both, dendritic overinhibition and smaller NMDAR-mediated currents, and rescued the deficit in LTP in Ts65Dn mice (122.9 ± 6.2%, n=11; Fig 6C). Together, these results demonstrate that synaptic plasticity in Ts65Dn mice can be effectively rescued by targeting α5-GABAA-Rs to counterbalance increased dendritic inhibition as well as decreased synaptic NMDAR availability.

**Discussion**

In the present study, we found a reduced NMDAR complement in SC inputs and reduced NMDAR-mediated depolarization during afferent burst activity in hippocampal CA1 pyramidal cells of Ts65Dn mice. This was due to a combination of decreased NMDAR-EPSCs as well as increased dendritic inhibition mediated by nonlinear α5-GABAA-R. Both, NMDAR-mediated depolarization and NMDAR-dependent LTP deficits could be normalized by acute application of an α5-NAM, showing that dendritic inhibition is a major contributor to disturbed NMDAR recruitment during physiologically relevant activity patterns.
Control of NMDAR-dependent plasticity and learning by dendritic inhibition

The NMDAR component of the field EPSP recorded in the dentate gyrus during brief burst activation was previously shown to be reduced in Ts65Dn mice relative to control. Complete blockade of GABARs with picrotoxin rescued the NMDAR component up to control levels (Kleschevnikov et al. 2004), suggesting that synaptic NMDA receptor expression is normal in dentate gyrus in Ts65Dn mice. Hence, different mechanisms can result in NMDAR hypoaivation. Besides reduced NMDAR content (CA1 radiatum) a major cause is increased GABAergic inhibition (CA1 radiatum and DG).

The present study demonstrates that not all forms of inhibition are equally relevant. There was a specific increase of dendritic inhibition of pyramidal cells in Ts65Dn mice mediated via α5-GABAR, which compromise postsynaptic integration of EPSPs (Fig. 7). By contrast, somatic inhibition was not significantly different in Ts65Dn mice. The identity of the presynaptic interneurons mediating the dendritic inhibition is unclear at present. However, it is likely, that the increased number of dendrite targeting SOM- and NOS-interneurons contribute to enhanced inhibition in DS (Chakrabarti et al. 2010, Raveau et al. 2018). Dendritic depolarization is critically important for the activity-dependent opening of voltage-dependent NMDARs. Our results show that the reduced NMDAR currents together with the exaggerated dendritic inhibition in Ts65Dn mice powerfully interferes with the NMDAR-activation process via the nonlinear slow α5-GABARs (Fig. 7B-C). However, the disturbed excitation-inhibition balance in DS animals can be effectively rectified by an α5-NAM via reduction of dendritic inhibition up to a level that enables relatively normal NMDAR activation during brief burst EPSPs (Fig. 7D). Therefore, our new results highlight the importance of dendritic inhibition in this DS model and may explain why a selective down-modulation of α5-GABARs by about 50% rescues both NMDAR-dependent LTP in CA1 as well as learning behavior (Martinez-Cue et al. 2013).

Dendritic inhibition is crucially important for plasticity and learning. In CA1 pyramidal cells it was shown, that synchronous pairing of Schaffer-collateral stimulation with distal entorhinal inputs generates NMDAR-dependent dendritic plateau potentials and burst firing, which can induce long-lasting changes in synaptic strength (Takahashi and Magee 2009). Consequently, the generation of plateau potentials and burst firing during spatial exploration can induce the formation of new
place fields in CA1 pyramidal cells (Bittner et al. 2015). Dendritic inhibition in particular is ideally placed to regulate the local membrane potentials in the dendrites. Using optogenetic silencing, it has been shown that PV-positive soma-targeting basket cells affect spike timing of CA1 pyramidal cells relative to extracellular theta oscillations without a large effect on firing frequency during spatial exploration. By contrast, silencing of dendrite-targeting SOM interneurons strongly increase NMDAR-dependent burst firing in pyramidal cells (Lovett-Barron et al. 2012, Royer et al. 2012). Similarly, it has been shown that neurogliaform cells, which also target distal dendrites (Bloss et al. 2016), prevent the activation of dendritic calcium spikes in CA1 pyramidal cells (Milstein et al. 2015). This demonstrates that dendrite-targeting interneurons effectively control NMDAR activation and synaptic plasticity.

Both, SOM-interneurons and neurogliaform cells control NMDAR activation via α5-GABARs (Zarnowska et al. 2009, Capogna and Pearce 2011, Schulz et al. 2018). What are the mechanisms underlying the α5-GABAR-mediated control of NMDA receptor opening? The powerful inhibitory effect of α5-GABA<sub>δ</sub>R containing synapses has been attributed to the slow time course of synaptic currents which is probably mediated by a slow GABA transient together with the slow gating kinetics of the high-affinity α5-GABA<sub>δ</sub>Rs (Karayannis et al. 2010). Furthermore, the nonlinear voltage dependence provides large inhibitory conductance on demand, specifically when synaptic burst activity depolarizes distal dendrites (Schulz et al. 2018). Thus, α5-GABA<sub>δ</sub>R-mediated inhibition in the hippocampus is in a powerful position to control the generation of dendritic calcium spikes, synaptic plasticity and the formation of new spatial memories.

Using in vivo tetrode recordings in another mouse model of Down syndrome (Dp(16)1Yey), specific deficits in firing of CA1 pyramidal cells were recently reported (Raveau et al. 2018). Burst firing during spatial exploration and memory consolidation was reduced. By contrast, spike timing relative to the hippocampal theta cycle was normal. Furthermore, the firing rate within place fields was lower and the spatial information content of the pyramidal cell population was lower in DS mice compared to wt controls. As all these parameters are dependent on proper synaptic NMDAR activation, our results suggest that these deficits would be improved or rescued by targeting dendritic inhibition via modulators of α5-GABA<sub>δ</sub>Rs. Further pharmacological studies in this mouse line will be necessary to confirm this hypothesis.
NMDAR deficits in Down syndrome mouse models

Deficits in NMDAR-dependent LTP have been reported previously in mouse models of DS. However, since these deficits could be corrected by blockade of GABAergic inhibition (Costa and Grybko, 2005; Belichenko et al., 2009; Kleschevnikov et al. 2004), they have been mainly ascribed to pathologically increased inhibition. The present study demonstrates in addition a reduction of NMDAR-mediated currents relative to AMPAR-mediated currents. These results are in line with a 20% reduction in protein levels of NR1 NMDAR subunits in the hippocampus of Ts65Dn mice (Souchet et al. 2014, 2015). A similar reduction of NR1 subunits relative to GluA1/A2 receptors was also found in Dyrk1a overexpressing mice (Souchet et al. 2014), suggesting that the triplication of Dyrk1a contributes to the reduced NMDAR expression in DS. By contrast, levels of GluA1 AMPAR subunits were not significantly different from control. Another potential pathway could involve APP, as triplication of APP, also located on human chromosome 21 and mouse chromosome 16, has been suggested to promote increased endocytosis of NMDARs in Ts65Dn mice (Snyder et al. 2005, Netzer et al. 2010). As we observed a reduced NMDA-AMPA ratio only of SC inputs stimulated in SR, but not of inputs onto basal dendrites stimulated in SO, there may be other factors in addition to APP and Dyrk1a triplication contributing to the observed differentially localized deficits. Overall, a relatively large network of proteins related to NMDAR signaling is potentially modified in DS (Block et al. 2018).

Taken together, in addition to enhanced dendritic inhibition, NMDAR-mediated EPSCs in CA1 pyramidal cells are also reduced in DS. Although triplication of Dyrk1a probably plays a major role in down-regulation of synaptic NMDARs, several converging pathways may be involved. This may include dynamic regulation by activity-dependent associative or homeostatic synaptic plasticity during development.

Treatment options to restore NMDAR-dependent plasticity and learning

Chronic treatment with negative modulators of α5-GABA<sub>A</sub>Rs not only restored NMDAR-dependent LTP but also ameliorated disturbed hippocampus-dependent learning in Ts65Dn mice (Braudeau et al. 2011, Martinez-Cue et al. 2013). In addition, decreased inhibitory markers were observed in the hippocampus of Ts65Dn.
mice treated with the α5-NAM RO4938581 (Martinez-Cue et al. 2013). Similarly, in earlier studies, low concentrations of the nonselective GABA_A antagonist PTZ rescued disturbed LTP in the dentate gyrus and deficits in hippocampus-dependent learning (Fernandez et al. 2007). These encouraging preclinical data supported the initiation of clinical trials in DS with basmisanil, a selective GABA_A α5-NAM (www.clinicaltrials.gov). However, the Phase 2 trials were terminated due to lack of efficacy (Roche Press Release, June 28, 2016; http://www.roche.com/media/store/statements.htm), indicating that our understanding of the cognitive deficits in DS is incomplete.

The reasons for the inconclusive clinical results may be manifold. First, the reduction in synaptic NMDAR currents, which we have found in Ts65Dn mice, might be larger in patients relative to the mouse model. Therefore, in addition to counterbalancing dendritic inhibition via an α5-NAM, further treatment to upregulate NMDAR-responses might be useful to restore the E/I balance and neuronal plasticity in the DS brain. Alternatively, intervention might be necessary at much earlier time points in brain development. Furthermore, it is also possible that the Ts65Dn mouse model does not fully recapitulate the brain deficits in DS and more triplicated genes contribute to the human condition. Proof for increased inhibitory transmission in individuals with DS is still missing and therefore additional studies are needed. In case GABAergic inhibition is indeed increased in DS patients, additional medication on top of an α5-NAM might be necessary to successfully improve cognitive deficits in patients.

In conclusion, the present study indicates that deficits in synaptic plasticity in Ts65Dn mice is the combined effect of a smaller NMDAR complement and increased nonlinear dendritic inhibition. Hence, pharmacological intervention specifically targeting dendritic inhibition via α5-GABA_ARs may be well suited to restore the fundamentally important mechanism of synaptic plasticity in the hippocampus without adverse side effects.

Author contributions:
Study concept and design: J.M.S., M.C.H and J.B.. Acquisition and analysis of data: J.M.S. and F.K.. Drafting of the manuscript: J.M.S. and J.B.. Interpretation of data, and critical revision of the manuscript: all authors.

References


hippocampal CA1 neurons. Nat Neurosci 18:1133-42. (Coincident EC-CA3 input generate dendritic spikes and place field formation)


LEGENDS

Figure 1. Impaired synaptic NMDAR activation in Ts65Dn mice.

A) Experimental design. Schaffer Collaterals were locally stimulated in stratum radiatum (SR) in the presence of the GABA\textsubscript{A} antagonist picrotoxin (100 \textmu M). B) AMPAR-mediated PSCs and NMDAR-mediated PSCs were measured in pyramidal neurons at -90 mV and +50 mV, respectively. C) Input-output relationship of AMPAR PSC amplitude evoked in SR versus stimulating current strength. D) Input-output relationship of NMDAR PSCs. E) The mean NMDA-AMPA conductance ratio of PSCs evoked in SR using 30 \textmu A pulses was significantly smaller in Ts65Dn mice (red; n=27) than in wt littermates (P<0.0001; two sample t-test, n=31). F) Scatter plot of the mean NMDA-AMPA conductance ratio measured during SR versus stratum oriens (SO) stimulation (30 \textmu A) in the same wt neurons (n=15 neurons). There was no positive correlation of the NMDA-AMPA conductance ratios between stimulation sites (R\textsuperscript{2}=0.24, P=0.06, slope= -0.48 ± 0.24) indicating that largely independent sets of inputs had been activated. Group means of NMDA-AMPA conductance ratios measured in SR and SO are indicated by horizontal and vertical dashed lines, respectively. G) The same scatter plot for NMDA-AMPA conductance ratios recorded in neurons from Ts65Dn brain slices (red; n=9). For comparison, group means and values from individual wt neurons are shown in light grey. Note that SO NMDA-AMPA conductance ratios in Ts65Dn mice scatter around the group mean measured in wt slices (vertical dashed line), while the SR NMDA-AMPA conductance ratios in Ts65Dn mice lie consistently below the group mean measured in wt slices (horizontal dashed line). H) There was no statistically significant difference of NMDA-AMPA conductance ratio of PSCs evoked in SO between neurons in Ts65Dn and wt littermates (P=0.71; two sample t-test). I) Postsynaptic potentials evoked by burst stimulation (5 pulses, 50 Hz) in SR in control condition (black) and after the application of the NMDAR-antagonist AP5 (50 \textmu M, green) reveal a NMDAR-mediated PSP component (shaded area) in wt (n=10 neurons) but not in Ts65Dn mice (n=13). Experiments were performed in the presence of 1 \textmu M CGP54626. J) Group means of the NMDAR-mediated contribution to the amplitude and integral of the last PSP. Statistical significant contributions are indicated (P<0.05, one sample t-tests).
Figure 2. Increased dendritic but not somatic inhibition in Ts65Dn mice.

A) Left, experimental design. Axon collaterals of local interneurons targeting the dendritic compartment were stimulated in in stratum radiatum (SR). IPSCs evoked at increasing stimulation strength were recorded at 0 mV. Right, representative mean IPSCs recorded from pyramidal neurons in Ts65Dn and wt mice. B) The same for local stimulation of interneuron axon collaterals targeting the perisomatic compartment in stratum pyramidale (SP). C) Input-output relationship of IPSC amplitude evoked in SR versus stimulating current strength. A two-way ANOVA revealed a significant genotype-by-stimulation-intensity interaction (P<0.0001, F(3,72) = 8.316). Statistical significant differences between neurons from Ts65Dn (n=12) and wt mice (n=14) are indicated for specific stimulation current intensities (P<0.05; Sidak's multiple comparisons test). D) Input-output relationship of IPSC amplitude evoked in SP versus stimulating current strength (wt, n=14; Ts65Dn, n=9).

Figure 3. Excitability of dendrite-targeting interneurons is not increased in Ts65Dn mice.

A) Membrane potential responses of an interneuron located in stratum radiatum (SR) in a wt mouse to linear current steps. The inset shows the relationship between membrane potential and injected current. The input resistance was derived from the slope of the regression line. B) The same for a representative SR interneuron in Ts65Dn mice. C-D) AP frequency versus current relationship (F-I curve) for current injections into the SR interneurons shown in A) and B). The AP frequency was fitted by the logarithmic function F=gain*ln(I/I0), where I0 denotes the threshold current. The estimated F-I gains from the fits are indicated. E) Scatter plots of the resting membrane potential for the group of pyramidal neurons (PN), SR interneurons (SR IN) and interneurons located in stratum oriens (SO IN). F-H) Scatter plots of AP threshold, input resistance and F-I gain. The statistically significant difference in the F-I gain of SR interneurons in wt versus Ts65Dn mice are indicated (P=0.008, unpaired two-sample t-tests),
Figure 4. α5-GABAR-mediated dendritic inhibition matches slow decay and voltage dependence of NMDARs.

A) Mean NMDAR-mediated PSCs recorded from a wt and a Ts65Dn pyramidal neuron after stimulation in SR. Biexponential fits to the decay phase (lighter thick lines) and weighted decay taus are indicated. B) Group data show that there was no difference in the weighted decay tau between wt (n=15) and Ts65Dn (P=0.8, n=9; two sample t-test). C) Current-voltage relationship for NMDAR-mediated PSCs recorded in the presence of picrotoxin (100 μM) and NBQX (10 μM). D) Mean IPSCs evoked in the outer third of SR before and after the addition of an α5-NAM (blue; RO4938581, 0.6 μM) in the presence of NBQX (10 μM) and AP5 (25 μM). Biexponential fits to the decay phase (lighter thick lines) and weighted decay taus are indicated. Bottom, the difference between the mean IPSCs before and after addition of the α5-NAM reveals the slow dynamics of the component that is mediated by α5-GABARs. E) Group data show that the α5-NAM significantly reduced the decay tau (P<0.001; paired t-test, n=5). The α5-GABAR-mediated IPSCs had a much slower decay tau than the control IPSCs (P=0.001; paired t-test). F) Current-voltage relationship for pharmacologically isolated α5-GABAR-IPSCs (blue, 0.6 μM RO4938581, n=6; turquoise, 50 nM L-655,708, n=3). A linear fit to the outward IPSCs above -50 mV is indicated (dashed line). Note the strong deviation of inward currents at more hyperpolarized membrane potentials, indicating outward rectification. Inset, the grand mean of α5-GABAR-IPSCs from experiments with application of 50 nM L-655,708. The membrane potential was corrected by the calculated liquid junction potential of -15.7 mV in C) and F).

Figure 5. Reduction of α5-GABAR-dependent inhibition increases synaptic NMDAR activation and spike output in Ts65Dn mice.

A) Postsynaptic potentials evoked by burst stimulation (5 pulses, 50 Hz) of the SC in control condition (grey) were enhanced by the acute application of the α5-NAM (1 μM RO4938581; blue). Under this condition, application of the NMDAR-antagonist AP5 (50 μM, green) revealed a NMDAR-mediated component in both wt and Ts65Dn mice (blue shaded area). B) Group means of the NMDAR-mediated contribution to the amplitude and integral of the last PSP. All significant contributions by NMDAR to
the PSP in wt (n=7) and Ts65Dn mice (n=8) are indicated by asterisks (P<0.05; one sample t-test). For comparison, the NMDAR-mediated component in the absence of the α5-NAM is indicated by the dashed lines (from Fig. 1E). C) Example voltage traces of theta-burst evoked AP firing in pyramidal cells during Schaffer-Collateral (SC) stimulation before and after the addition of the α5-NAM (1 μM) in Ts65Dn mice. D) Group means show a significantly increased spike rate (P=0.022; paired t-test, n=5). E) The input resistance was not significantly increased by the α5-NAM (P=0.76, n=8; paired t-test) in Ts65Dn mice. However, there was a small increase of the input resistance in wt pyramidal neurons (P<0.005; paired t-test, n=17).

Figure 6. Acute application of a α5-NAM rescues NMDAR-dependent synaptic plasticity.

A) The mean fEPSPs recorded in stratum radiatum before (light color) and after TBS (10 x 5 pulses @ 100 Hz every 200 ms; dark color) of the SC from a slice of a wt (grey), a Ts65Dn mouse (red) and a Ts65Dn mouse in the presence of the α5-NAM (blue). B) Time courses of the fEPSP slope. TBS was applied at time 0. The fEPSP slope was significantly less elevated in Ts65Dn slices (110.1 ± 3.1%, n=19; red) compared to wt litter mates (127.1 ± 7.2%, n=14; P<0.05, two sample t-test; black) at 80 min post induction. C) Application of the α5-NAM (0.3 μM) enhanced LTP in Ts65Dn mice up to normal levels. The potentiation of the fEPSP slope in Ts65Dn slices was increased in the presence of the α5-NAM (122.9 ± 6.2%, n=11; blue) relative to control conditions (P<0.05; two sample t-test), and comparable to wt slices in control condition (127.1 ± 7.2%, n=14; black, same experiments as in B).

Figure 7. Mechanisms underlying the reduced NMDA receptor activation in Ts65Dn mice.

A) Schematic drawing of Schaffer-collateral activated synapses onto CA1 pyramidal cells. B) Brief burst activation of SC inputs engages a positive feedback loop between membrane depolarization and voltage-dependent NMDAR activation in CA1-pyramidal cell dendrites of wt mice. This positive feedback is regulated by α5-GABAR-mediated voltage-dependent inhibition. C) Both, increased dendritic
inhibition and reduced NMDAR expression disrupts NMDAR activation in Ts65Dn mice. D) Application of the α5-NAM reduces dendritic inhibition and restores dendritic E/I balance to enable normal NMDAR activation in Ts65Dn mice.

Table. 1. Cellular properties of CA1 pyramidal neurons in Ts65Dn mice and wt littermates. Data presented are means ± S.D.

<table>
<thead>
<tr>
<th>Response to current steps</th>
<th>Ts65Dn</th>
<th>wildtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane potential (mV)</td>
<td>-67.7 ± 3.0</td>
<td>-67.2 ± 1.9</td>
</tr>
<tr>
<td>Capacitance (pF)</td>
<td>231.0 ± 19.3</td>
<td>241.4 ± 12.5</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>95.2 ± 20.0</td>
<td>90.3 ± 22.6</td>
</tr>
<tr>
<td>$\tau_{\text{membrane}}$ (ms)</td>
<td>20.9 ± 7.3</td>
<td>21.4 ± 6.5</td>
</tr>
<tr>
<td>$I_h$ dependent sag (mV)</td>
<td>2.3 ± 0.5</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>Rheobase current (pA)</td>
<td>154.6 ± 45.0</td>
<td>168.0 ± 53.8</td>
</tr>
</tbody>
</table>

Spike waveform

<table>
<thead>
<tr>
<th>Response to current steps</th>
<th>Ts65Dn</th>
<th>wildtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firing threshold (mV)</td>
<td>-41.8 ± 2.5</td>
<td>-41.1 ± 2.0</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>88.5 ± 8.7</td>
<td>88.4 ± 9.0</td>
</tr>
<tr>
<td>Half-width (ms)</td>
<td>0.83 ± 0.12</td>
<td>0.82 ± 0.12</td>
</tr>
</tbody>
</table>