

Investigation of hippocampal synaptic transmission and plasticity in mice deficient in the actin-binding protein Drebrin

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Supplementary Information

Table S1. Primary antibodies for immunohistochemistry and immunoblotting

Antigen	Species	Dilution	Source
actin	m	1:3000 (WB)	Genetex – GTX82559
α -actinin	m	1:800 (WB)	Sigma – A5044
Cofilin	rb	1:500 (WB)	Cell Signaling – #3312
Cofilin Phospho S3	rb	1:500 (WB)	Cell Signaling – #3311
DBN	m	1:1000 (WB) 1:200 (IHC)	Genetex - GTX12350
DBN	rb	1:1000 (WB)	homemade (see below)
GAPDH	m	1:4000 (WB)	Calbiochem – CB1001
Homer	rb	1:1000 (WB)	kind gift of Teiichi Furuichi (Laboratory for Molecular Neurogenesis, RIKEN Brain Science Institute, Japan)
MAP2	gp	1:200 (IHC)	Synaptic systems – 188004
Myosin Va	rb	1:1000 (WB)	Cell Signaling – 3402
PSD95	m	1:1000 (WB)	Thermo Scientific – MA 1-045
Synaptophysin	m	1:2000 (WB)	Synaptic systems – 101011
Neuronal Class III β -Tubulin	rb	1:500 (IHC)	Covance - PRB-435P

Pan-DBN Peptide Antibodies

Pan-DBN Peptide Antibodies were generated from rabbits immunized with a peptide comprising the conserved phospho-site S647 of DBN A (H₂N-CGYFSQS(PO₃H₂)QEEEF-CONH₂). Antibodies were purified as described previously⁵⁷ and antibodies that bound the non-phosphorylated control peptide (H₂N-CGYFSQSQEEEF-CONH₂) were referred to as pan-DBN.

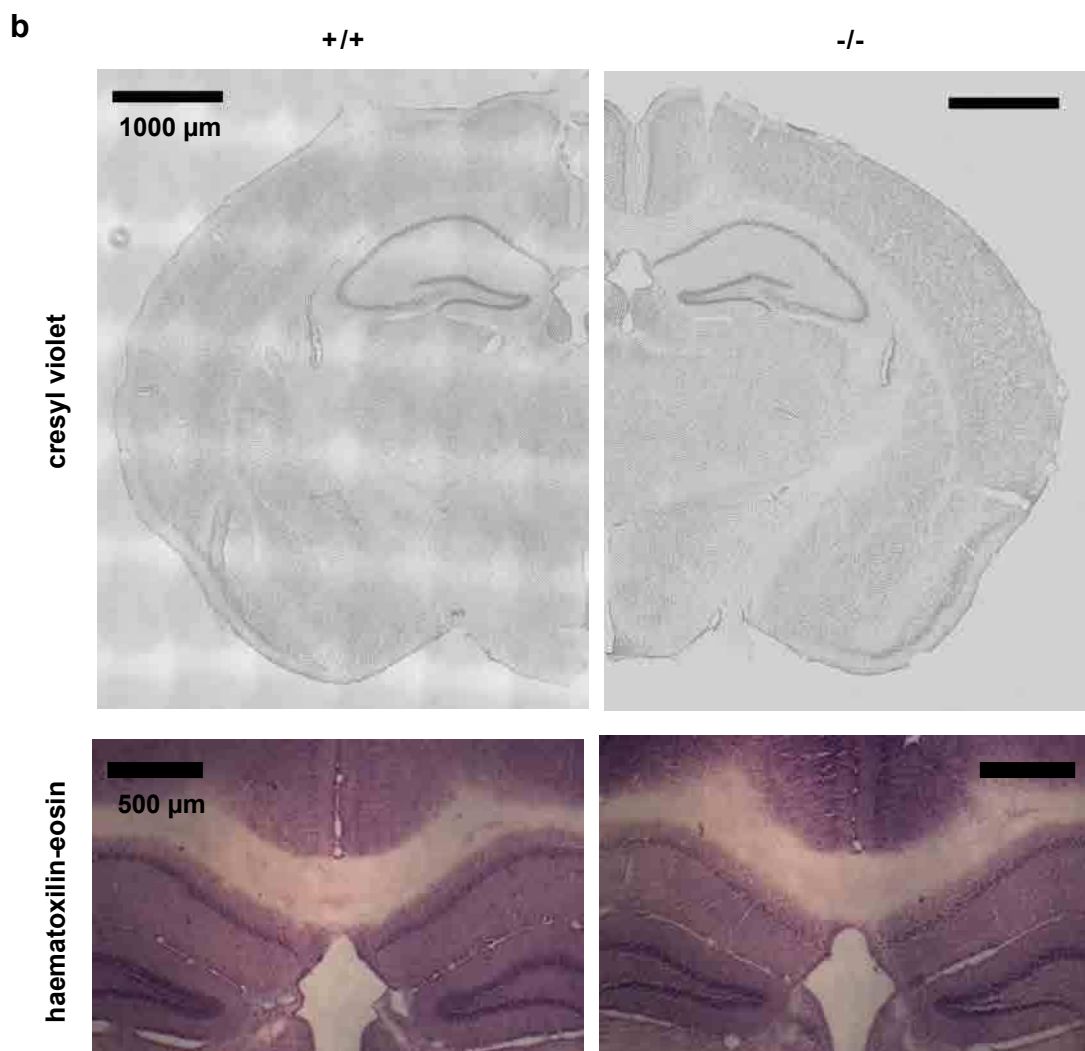
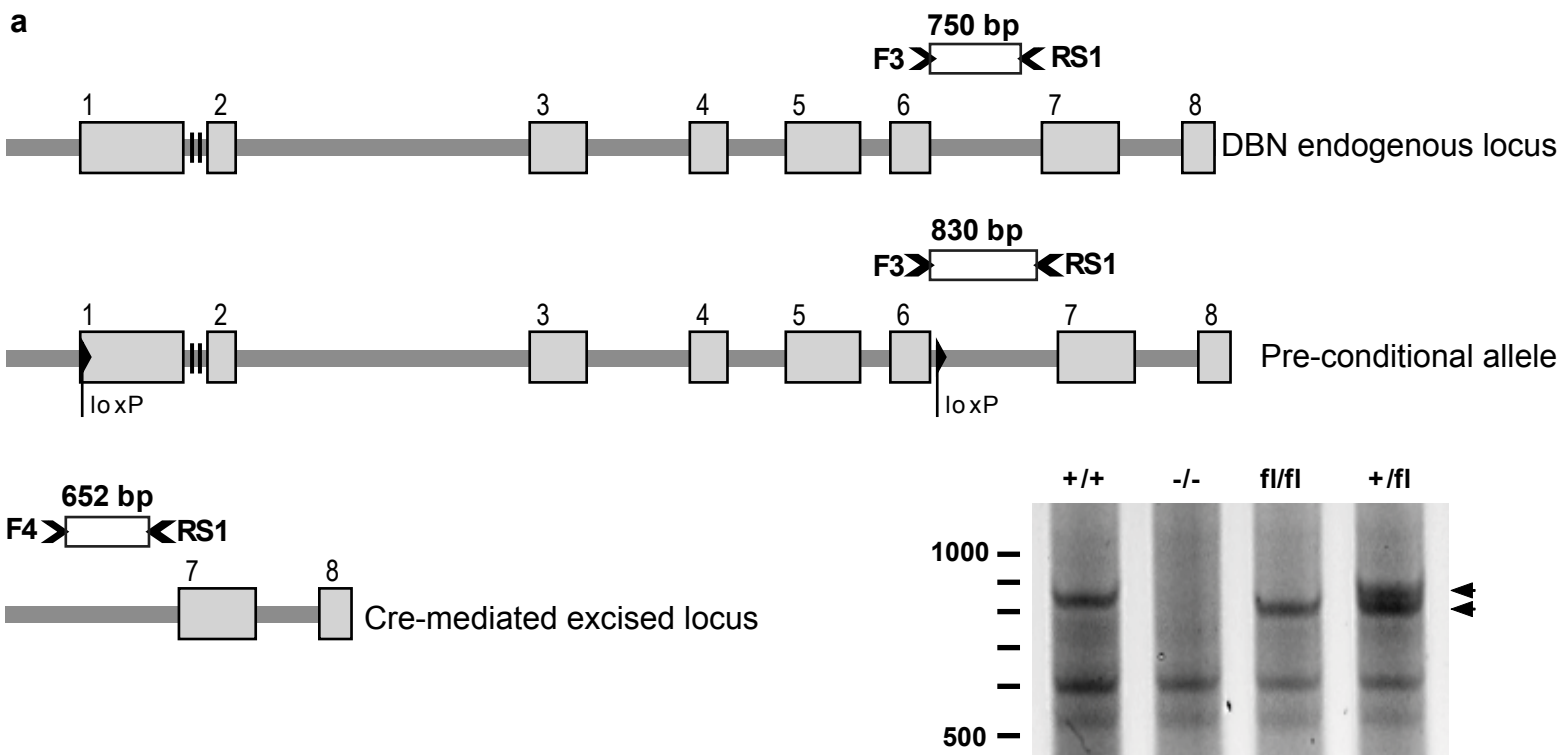


Fig. S1. Generation of DBN knockout mice.

(a) Magnification of DBN exon 1 – 8. Genotyping primers are indicated as black arrows, calculated PCR products are indicated as rectangles, bp: base pairs. Pre-conditional alleles were detected by PCR products with forward primer in exon 6. In contrast, null alleles produced no PCR product under these conditions, but were recognized using a forward primer upstream of exon 1. Agarose gels shows WT PCR with Primer pair F3-RS1 for WT (+/+), DBN KO (-/-), double floxed (fl/fl) and heterozygous WT, floxed (+/fl) mice. The PCR product of the floxed gene is shorter than the estimated size. PCR results for WT allele show 845 bp and for floxed allele show 830 bp. Heterozygous mice presented bands in both PCR condition. Lines indicate DNA ladder in base pairs.

Identity of the PCR products was identified by sequencing (data not shown). (b) Nissl staining showed no gross abnormalities in brain morphology, haematoxylin and eosin staining revealed no differences in the morphology of corpus callosum.

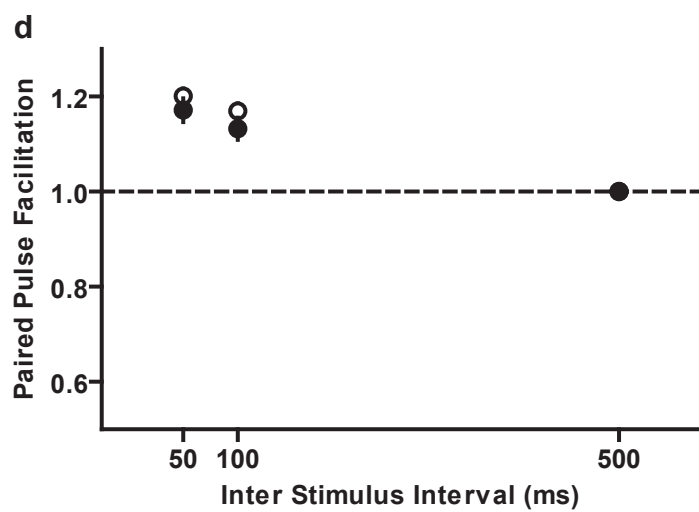
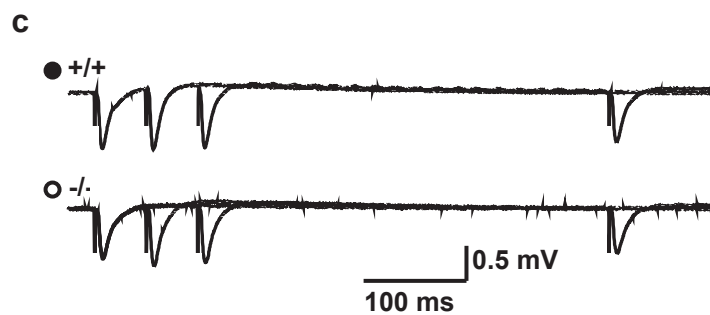
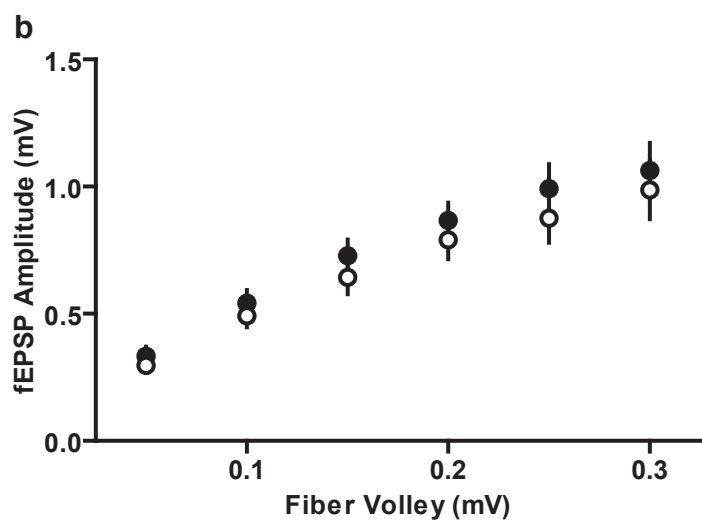
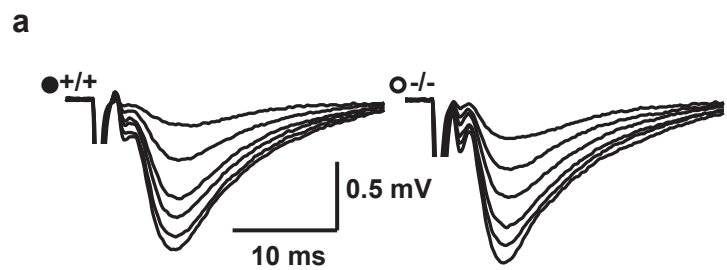


Fig. S2. Synaptic responses in area CA1 in young WT and DBN KO mice.

(a) Sample traces of input-output curves in area CA1 of the hippocampus for WT (+/+) and DBN KO (-/-) mice. (b) No significant difference was found between WT and DBN KO mice in the fEPSP slopes at various afferent volley amplitudes (WT, N=3 n=18; KO, N=3 n=14; $P > 0.05$, two-way ANOVA, Bonferroni post-hoc test). Data show mean $\pm$ s.e.m. (C) Sample traces for Paired-pulse intervals of 50, 100 and 500 ms. (D) Paired-pulse facilitation is unchanged in DBN KO mice compared to WT (WT, N=3 n=18; KO, N=3 n=14; $P > 0.05$, two-way ANOVA, Bonferroni post-hoc test).