Supporting Online Material

Material and Methods

Animals. Male adult (8-16 weeks) mice were used for all experiments. C57BL/6N mice were purchased from Charles River (Germany). $CB1^{-/-}$ and $CB1^{+/+}$ littermates were described previously (*1*). $CB1^{f/f}$ mice were obtained by crossing mice carrying the CB1-floxed-neo allele (*1*) with flipase-deleter mice (*2*), carrying the germ-line expression of the recombinase flipase (*2*) in order to delete the FRT-PGK-Neo selection cassette. $CB1^{f/f;CaMKII\alpha Cre}$ mice were obtained by crossing $CB1^{f/f}$ mice with mice expressing the improved Cre recombinase from a bacterial artificial chromosome containing the regulatory sequences of Ca^{2+}/calmodulin-dependent kinase IIα (CaMKIIα iCre BAC) (ref. [*3*], here named $CB1^{CaMKII\alpha Cre}$), in order to obtain $CB1^{f/f}$ x $CB1^{f/f;CaMKII\alpha Cre}$ breeding pairs. Littermates were used for each experiment. All lines were in mixed genetic background, with a predominant C57BL/6N contribution (6-7 backcrossings). Genotyping was performed as described for Cre transgene (*3*) and by PCR using the primers G50 and G51 described in ref. (*1*). All experimental procedures were approved by the Committee on Animal Health and Care of local Government.

Seizure scoring and pharmacology. Kainic acid (KA; Sigma) was dissolved in saline and administered intraperitoneally at 10 ml/kg body weight. SR141716A (NIMH Chemical Synthesis and Drug Supply Program, U.S.A.) and UCM707 (*4, 5*) were dissolved in vehicle solution (1 drop of Tween-80 in 3 ml of 2.5% dimethylsulfoxide in saline) and injected subcutaneously at 3 mg/20 ml/kg body weight under light isoflurane anesthesia 30 min and 1 h before KA injection, respectively. Mice were monitored for 2 h

and behavioral scores were recorded every 15 min. Scores were quantified by trained observers blind to genotype and drug treatment according to ref. (*6*), with stage 7 indicating death. Scores were analyzed by 2-way repeated measures ANOVA with genotype or treatment as between subjects factors and time as within subject factor (followed by Tukey's test, when applicable), and death rates by Fisher Exact test.

Measurement of endocannabinoids. C57BL/6N mice were sacrificed at different time points after the injection of 30 mg/kg KA. Hippocampi were rapidly dissected, snap-frozen in liquid nitrogen and stored at –80°C. Endocannabinoids were extracted, and their levels were measured by isotope-dilution liquid chromatography-mass spectrometry as described (*1*). Data were analyzed with ANOVA, followed by Bonferroni's *post-hoc* test.

Electrophysiology. Preparation of coronal brain slices (400 µm thick) and whole-cell voltage-clamp recording (-70 mV holding potential) was performed essentially as described (*7*). The pipette solution consisted of (in mM): K-gluconate, 105; KCl, 30; Mg-ATP, 4; phosphocreatine, 10; GTP, 0.3; HEPES, 10; QX314, 5 (pH 7.3). All experiments were performed at room temperature (22-24°C). As a measure of the excitation of the recorded neuron by spontaneous synaptic activity, the charge transfer (in Coulomb) across the neuronal membrane mediated by spontaneous excitatory postsynaptic currents (EPSCs) was calculated. For statistical analysis, values from each neuron were collected 15 to 20 min after KA application, averaged and normalized to baseline (last 5 min before KA application). Frequencies of spontaneous EPSCs were calculated for 1 minute immediately before and 20 minutes after KA application. Data are expressed as means ± s.e.m. Significance was tested using the Student`s *t*-test.

***In situ* hybridization.** Brains were isolated and snap-frozen on dry ice. Single and double *in situ* hybridization were performed as described (*8, 9*). Sources of cDNA clones, restriction enzymes for linearization and RNA polymerases (NEB) for synthesis of antisense riboprobes are listed: CB1, as described (*8*); glutamic acid decarboxylase 65kD (GAD65), as described (*8*); c-fos, I.M.A.G.E. clone 2647069 (Research Genetics) *EcoRV*, T7; zif268, 2370 bp corresponding to 3' region, *EcoRV*, T7; BDNF, I.M.A.G.E. clone 1397218 (Research Genetics), *XhoI*, T3. Identity of all cDNA clones was checked by sequencing. Densitometric analyses were performed on autoradiographic films using the NIH Image software (http://rsb.info.nih.gov/nih-image/Default.html). Data were analyzed with ANOVA, followed by Bonferroni's *post-hoc* test.

Western blotting. Hippocampi were dissected and homogenized by sonication in protease inhibitor solution (Roche) containing phosphatase inhibitors (phosphatase inhibitor cocktails I and II; Sigma). After determination of protein content, 15 µg protein samples were electrophoresed on a 10% SDS-polyacrylamide mini-gel and blotted electrophoretically to Immobilon-P (Millipore), following standard procedures. Immunodetection was performed by incubating membranes with the polyclonal antibody anti-phospho-Erk1/2 (Thr202/Tyr204; 1:1000; #9101; Cell Signaling) overnight at 4°C. After washing, blots were incubated at room temperature for 1-2 h with secondary antibody conjugated to horseradish peroxidase (1:2000; DAKO) and developed using the enhanced chemoluminescence method (ECLplus; Amersham). After stripping (in 2% SDS, 50 mM DTT, 50 mM Tris/HCl pH 7.0 for 30 min at 70°C), blots were incubated with the polyclonal antibody anti-Erk1/2 (1:1000; #9102; Cell Signaling). Densitometric

analysis of bands was performed using the Quantity One software (BioRad). Normalized values were analyzed by Student's *t*-test.

TUNEL staining and immunohistochemistry. Cell damage was detected on frozen sections by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling of DNA double strand breaks (TUNEL method) using an *in situ* cell death detection kit (POD; Roche) following manufacturer's instructions. TUNEL staining was evaluated by densitometric analysis. Gliosis was evaluated by immunohistochemistry on frozen sections by overnight incubation at 4°C with a polyclonal anti-GFAP antibody (1:1000; DAKO) followed by immunoperoxidase staining (Vectastain ABC kit, Vector Laboratories). Staining was evaluated by subjective scoring by 3 independent observers blind of genotype and treatment. Student's *t*-test was used for statistical analysis.

References and notes

1. G. Marsicano *et al.*, *Nature* 418, 530 (2002).

2. S. Dymecki, *Proc. Natl. Acad. Sci. U. S. A.* 93, 6191 (1996).

3. E. Casanova *et al.*, *Genesis* 31, 37 (2001).

4. E. de Lago *et al.*, *Eur. J. Pharmacol.* 449, 99 (2002).

5. M. L. Lopez-Rodriguez *et al.*, *J. Med. Chem.* 46, 1512 (2003).

6. P. E. Schauwecker, O. Steward, *Proc. Natl. Acad. Sci. U. S. A.* 94, 4103 (1997).

7. M. Eder, W. Zieglgänsberger, H. U. Dodt, *J. Neurosci.* 22, 7558 (2002).

8. G. Marsicano, B. Lutz, *Eur. J. Neurosci.* 11, 4213 (1999).

9. H. Hermann, G. Marsicano, B. Lutz, *Neuroscience* 109, 451 (2002).

Supporting Online Figures

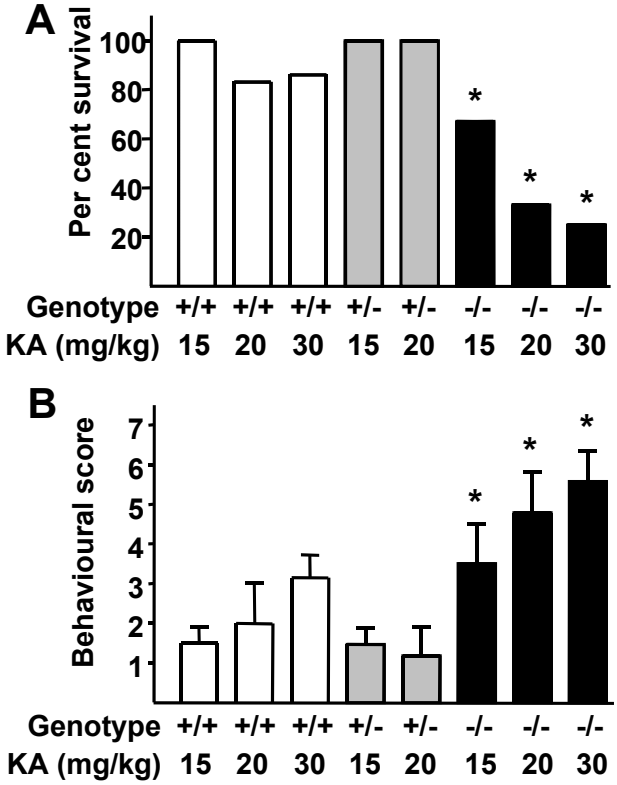

Fig. S1. Responses of $CB1^{+/+}$, $CB1^{+/-}$ and $CB1^{-/-}$ mice to the administration of different doses of KA. (**A**) Survival after injection of 15, 20 and 30 mg/kg KA (n=6-8/group), and (**B**) averaged seizure scoring of $CB1^{+/+}$, $CB1^{+/-}$, and $CB1^{-/-}$ 1 h after injection of KA. Means ± s.e.m.; *, $p<0.05$ versus $CB1^{+/+}$.

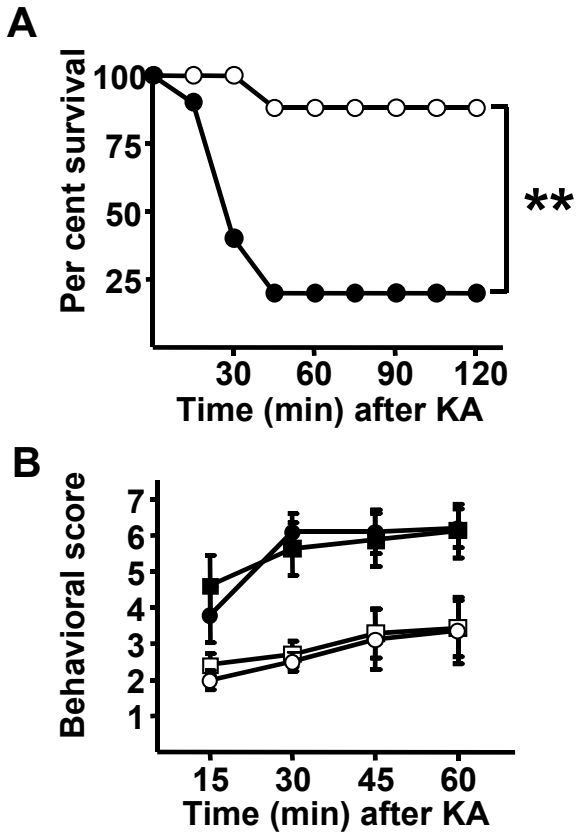

Fig. S2. (**A**) Survival of $CB1^{f/f}$ and $CB1^{f/f;CaMKII\alpha Cre}$ littermates over the course of the experiment depicted in Fig. 2F of main text. (**B**) Comparison between seizure scoring (30 mg/kg) of $CB1^{-/-}$ (filled squares) and $CB1^{f/f;CaMKII\alpha Cre}$ (filled circles) and respective control littermates ($CB1^{+/+}$, open squares, and $CB1^{f/f,}$ open circles). Same data as in Fig. 1A and 2F of main text. Means ± s.e.m.; **, $p<0.01$.

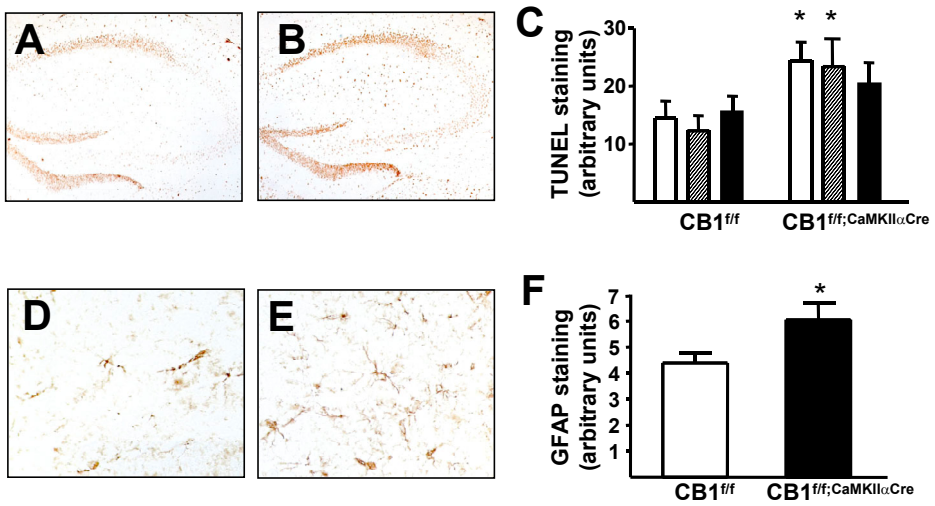

Fig. S3. (**A-C**) Increased degree of KA-induced neuronal death in CB1$^{f/f;CaMKII\alpha Cre}$ hippocampi as compared to CB1$^{f/f}$ littermates. (**A, B**) Representative TUNEL staining of hippocampi from CB1$^{f/f}$ (**A**) and CB1$^{f/f;CaMKII\alpha Cre}$ (**B**), 4 days after KA injection (20 mg/kg). (**C**) Densitometric quantification of TUNEL staining in CA1 (open bars), CA3 (hatched bars) and dentate gyrus (filled bars) of hippocampus in KA-treated CB1$^{f/f}$ (n=7 mice) and CB1$^{f/f;CaMKII\alpha Cre}$ (n=4 mice). *, $p<0.05$ versus respective region of CB1$^{f/f}$ mice. **(D-F)** Increased degree of KA-induced gliosis in CB1$^{f/f;CaMKII\alpha Cre}$ hippocampi as compared to CB1$^{f/f}$ littermates. (**D, E**) Representative GFAP immunostaining of hippocampi from CB1$^{f/f}$ (**D**) and CB1$^{f/f;CaMKII\alpha Cre}$ (**E**), 4 days after KA injection (20 mg/kg). (**F**) Quantification of GFAP staining in hippocampi from KA-treated CB1$^{f/f}$ (open bar, n=7) and CB1$^{f/f;CaMKII\alpha Cre}$ (filled bar, n=4). Means ± s.e.m.; *, $p<0.05$ versus CB1$^{f/f}$.