Supporting Information

Tzingounis et al. 10.1073/pnas.1004644107

SI Materials and Methods

Slice Electrophysiology. Transverse hippocampal slices (300 μm) were prepared from either 12- to 19-day-old WT or *Kcnq5*$^{dn/dn}$ mice. After animals were deeply anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (Sigma), they were rapidly decapitated and the hippocampus was dissected out and placed into the stage of a vibroslicer (Leica Instruments). The hippocampus was cut in cold solution containing 50 mM NaCl, 25 mM $NaHCO_3$, 150 mM sucrose, 10 mM glucose, 2.5 mM KCl, 1 mM NaH_2PO_4, 0.5 mM $CaCl_2$, and 7 mM $MgCl_2$. Slices were incubated at 35 °C for 30 min, then moved to room temperature for 30 min, and finally stored at room temperature in the recording extracellular solution containing artificial cerebrospinal fluid (ACSF) (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose equilibrated with 95% O_2 and 5% CO_2 [vol/vol]). Whole-cell recordings were obtained using glass electrodes (2–3 MΩ). Hippocampal pyramidal cells were visually identified with infrared differential interference contrast optics using a 40× water-immersion objective on an upright microscope (BX50; Olympus). The series resistance as measured by the instantaneous current response to a 4-mV 50-ms step with only the pipette capacitance canceled was measured continuously. The recording internal pipette solution consisted of the following: 150 mM potassium methylsulfate, 10 mM KCl, 10 mM Hepes, 4 mM NaCl, 4 mM Mg_2ATP, and 0.4 mM Na_4GTP. Osmolarity was adjusted to 300–305 mOsm and pH to 7.25–7.35 with potassium hydroxide. To block voltage-gated sodium channels, the ACSF contained 500 nM TTX during recordings. Current responses were collected with an Axopatch-1D amplifier (Axon Instruments), filtered at 2 kHz, digitized at 5 kHz, and analyzed online using custom-written Igor Pro software (Wavemetrics).

Western Blots. For preparation of brain membrane fractions, tissues were homogenized mechanically (Ultra Turrax; IKA-Werke) in ice-cold homogenization buffer [1 M Tris, 1 M NaCl, 0.5 M EDTA, 1 mM Pefabloc (Roche), 1× Complete protease inhibitor mix (Roche)]. After homogenization and centrifugation at 1,000 × *g* for 10 min at 4 °C, the supernatant was recovered and subsequently centrifuged at 132,000 × *g* for 30 min at 4 °C. The pellet was resuspended in Ripa buffer (1 M Tris, 0.5 M EDTA, 10% (wt/vol) SDS, 1 mM Pefabloc, 1× Complete protease inhibitor mix), and the protein concentration was determined using the bicinchoninic acid assay (BCA) method (Thermo Scientific). A total of 50–100 μg of total protein per lane was separated on reducing 8.5% (wt/vol) SDS-polyacrylamide gels and then transferred to PVDF membranes (Roth) using a semidry blotting system (TE77; Hoefer). Western blots were probed with affinity-purified anti-KCNQ2 (1:1,000), anti-KCNQ3 (1:200), anti-KCNQ5 (1:200), and anti-synaptophysin (1:5,000; Synaptic Systems). Detection used ECL Western blotting substrate.

Morphology. For immunohistochemistry, *Kcnq5*$^{+/+}$ and *Kcnq5*$^{dn/dn}$ P18 and P65 animals were deeply anesthetized with ketamin/rompun and perfused transcardially with 0.01% heparin in PBS, followed by 1% paraformaldehyde (PFA) in PBS. Brains were dissected and postfixed for 1 h in 1% PFA at 4 °C. After dehydration in a graded ethanol series, Roti-Histol (Carl Roth, Karlsruhe) incubation for 15 min, and paraffin embedding, 7-μm sagittal sections were cut (Cool-cut; Microm) and incubated in 20 mM citric acid, 60 mM disodium phosphate, and 1.5% (vol/vol) H_2O_2 at room temperature for 15 min. After boiling in 40 mM Tris and 1 mM EDTA (pH 9.0), cooling to room temperature (1 h), and washing in PBS, sections were blocked with 1% BSA in PBS and 0.1% Triton X-100 (PBST) for 1 h and incubated in 0.05% BSA/PBST with the appropriate antibodies: anti-KCNQ2 (1:500), anti-KCNQ3 (1:200 and 1:1,000 for DAB staining), anti-KCNQ5 (1:500), and anti-ankyrinG (1:20; Zymed). After washing in PBST, the sections were incubated for 1 h at room temperature with biotin-conjugated anti-mouse IgG for ankyrinG staining. Finally, sections were incubated with Alexa Fluor 488 goat anti-rabbit IgG and streptavidin Alexa Fluor 555 conjugate in 0.05% BSA/PBST, including 1 μg/mL DAPI (Sigma). For DAB peroxidase staining, HRP-conjugated secondary antibodies were used (Envision+ HRP system; DAKO). The peroxidase reaction was started using 500 mg/mL diaminobenzidine, 10 mM imidazol, 0.3% ammonium nickel sulfate, and 0.003% H_2O_2 in 50 mM Tris (pH 7.6). The sections were washed and mounted in Kaiser's Glycerol Gelatin (Merck). Pictures were taken by confocal laser-scanning microscopy (LSM510; Zeiss) and assembled using Adobe Photoshop (Adobe Systems). For Nissl and DAB staining, *Kcnq5*$^{+/+}$ and *Kcnq5*$^{dn/dn}$ PD18 and PD65 animals were perfused with 4% (wt/vol) PFA, and brains were postfixed overnight in 4% (wt/vol) PFA at 4 °C, dehydrated, and paraffin-embedded as described, and 7-μm sections were cut. Sections were then examined with a Zeiss Axiophot or Zeiss Stemi-2000-c microscope.

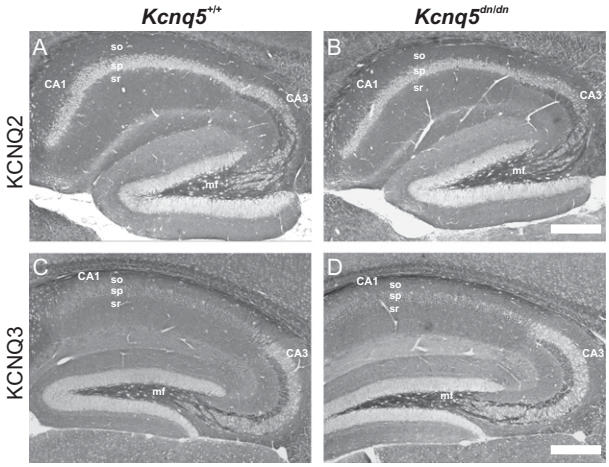

Fig. S1. Unchanged expression and cellular distribution of KCNQ2 and KCNQ3 in the hippocampus of adult *Kcnq5*$^{dn/dn}$ mice. DAB immunohistochemistry revealed normal expression of KCNQ2 (*B*) and KCNQ3 (*D*) compared with *Kcnq5*$^{+/+}$ mice (*A* and *C*). mf, mossy fibers; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. (Scale bars: 500 μm.)

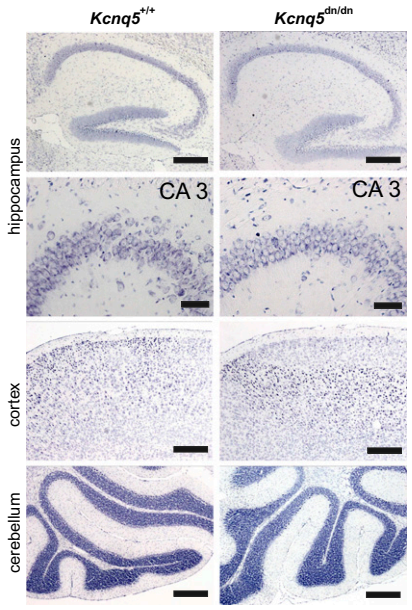

Fig. S2. Nissl staining of sagittal sections of hippocampus, cortex, and cerebellum show normal general histomorphology in *Kcnq5*$^{dn/dn}$ mice at PD18. Higher magnification of CA3 area is shown. (Scale bars: 500 μm; for higher magnification of CA3, 100 μm.)

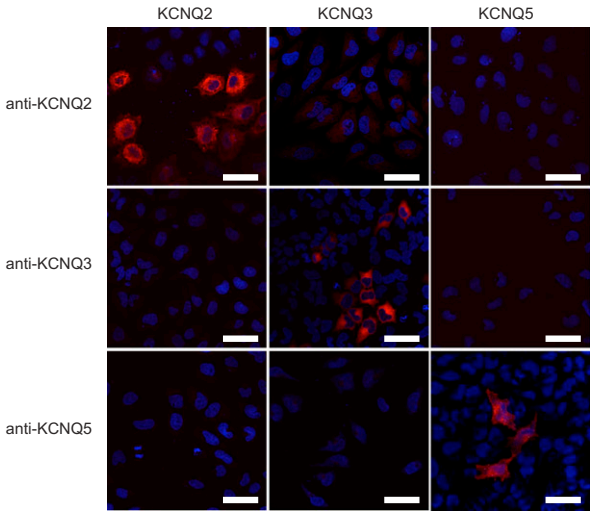

Fig. S3. No cross-reactivity of newly generated KCNQ antibodies in transfected HeLa cells. HeLa cells were transiently transfected with KCNQ2, KCNQ3, and KCNQ5 expression vectors and immunostained with affinity-purified KCNQ2, KCNQ3, and KCNQ5 antibodies (all 1:500) and secondary Alexa Fluor 555 goat anti-rabbit (1:1,000). (Scale bars: 50 μm.)