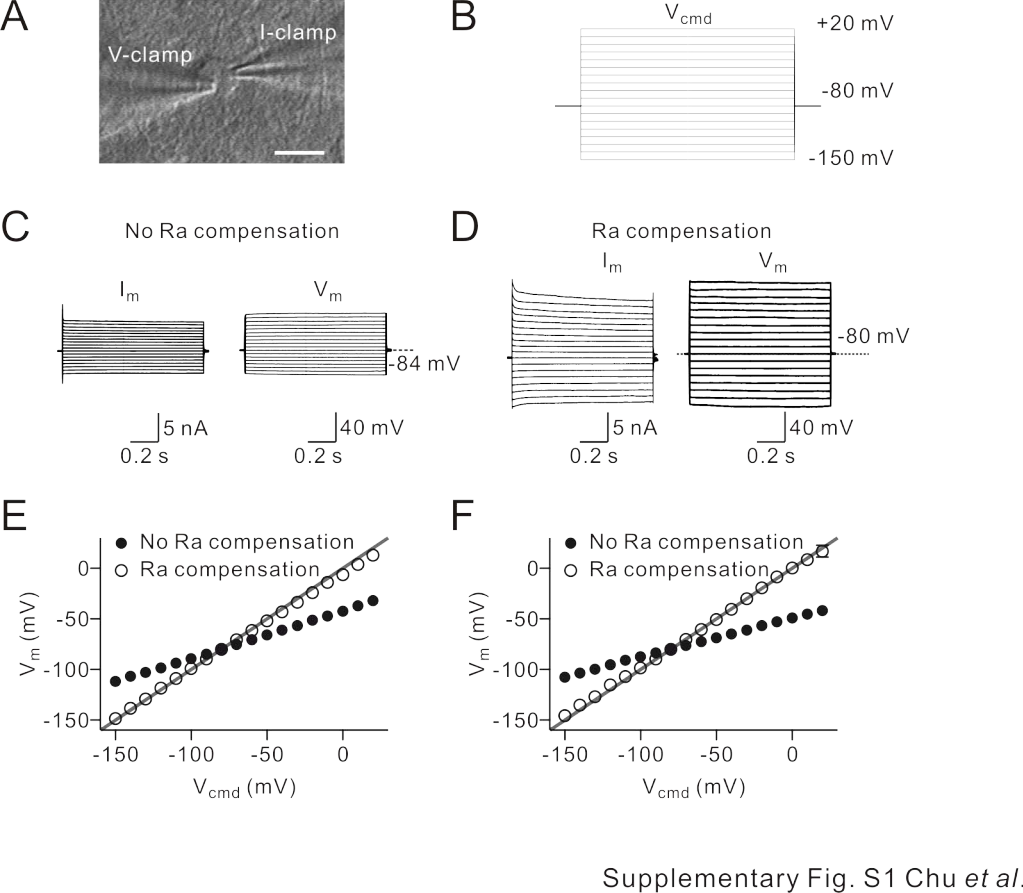
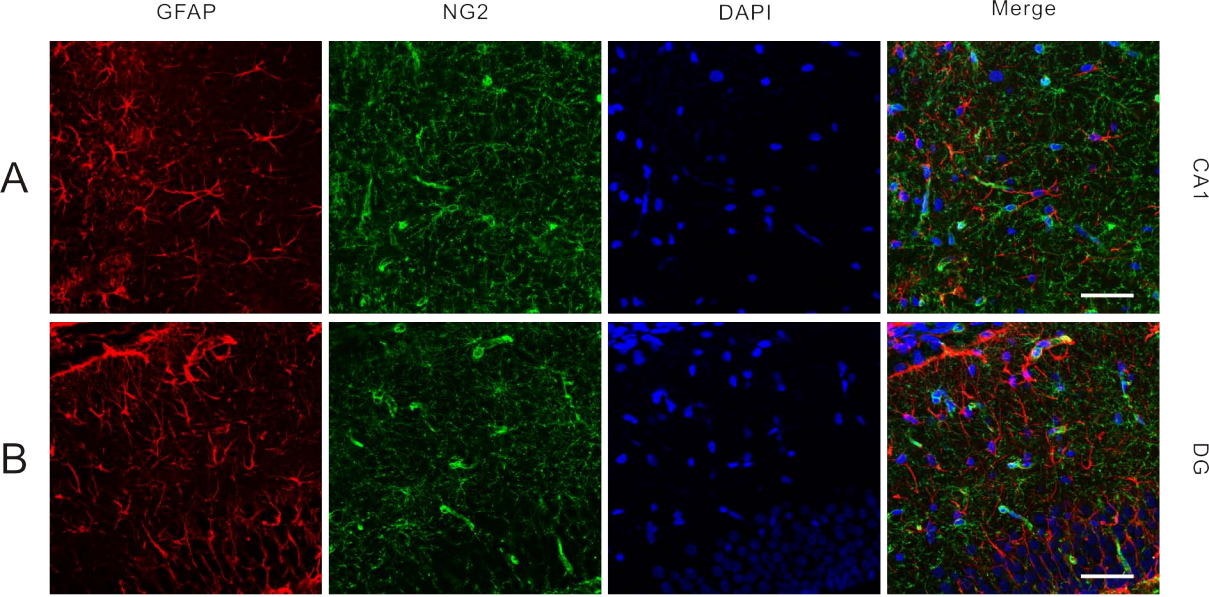


Supplementary Fig. S1 Chu *et al.*



Supplementary Fig. S2 Chu *et al*.

Supporting Information

Functional identification of an outwardly rectifying pH- and anesthetic-sensitive leak K^+ conductance in hippocampal astrocytes

Supporting Materials and Methods

Dua- patch whole-cell recording

Whole-cell patch recordings were made using an Axopatch 200B amplifier (Molecular Devices, Union City, CA, USA). Pipette capacitance was compensated. The access resistance (≤ 12 MΩ) was carefully compensated (correction ~ 90% with lag 35 μs). Dual-patch whole-cell recording was used to test the voltage clamp condition (supplementary Fig. S1, A). The left electrode in V-clamp mode was used to deliver the command voltage (V_{cmd}; supplementary Fig. S1, B) and the right electrode in I-clamp mode was used as a potentiometer to record the actual measured membrane potential (V_m) without injecting any current. The V-clamping error, as defined by (ΔV_{cmd}-ΔV_m)/ΔV_{cmd}, was tested by dual-patch recording and was < 10% (3.7 ± 2.1 %, n = 7; supplementary Fig. S1) after the access resistance compensation. Data acquisition (low-pass filtered at 5 kHz and digitized at 10 kHz) and pulse generation were performed using a Digidata 1440A and pClamp 10.2 software (Molecular Devices). The recording temperature was 21-24 ˚C.

Fluorescent immunohistochemistry

Immunostaining was performed on transverse hippocampal slices that were prepared as described for electrophysiology. They were fixed overnight at 4 ˚C with 4% paraformaldehyde in 0.1 M PB and washed with PB for 10 min, 3 times. Free-floating slices were permeabilized for 30 min in 0.1 M PB containing 0.3% Triton X-100 and then incubated in the blocking solution (0.1 M PB, 10 % normal goat serum) for 4 hrs. Slices for double immunostaining (Fig. S2) were then incubated in PB containing 0.3% Triton X-100 and 5% goat serum at 4˚C for 2 days with mouse anti-NG2 antibody (Abcam; Cambridge, UK) at 1:200 and mouse anti-GFAP antibody (Cell Signaling

Technology; Danvers, MA) at 1:200. Sections were washed in 0.1 M PB for 10 min, 3 times and were then incubated at 4°C for 2 hrs with secondary fluorescent antibodies (Alexa Fluor 488 goat anti-mouse IgG at 1:500 for NG2 and Alexa Fluor 594 goat anti-mouse IgG at 1:500 for GFAP) in PB containing 0.3% Triton X-100 and 2% goat serum. For nuclei staining, sections were counterstained with DAPI at 1:5000 for 15 min and followed by wash in PB for 10 min, 6 times. Secondary antibodies and DAPI were from Invitrogen. After washing, slices were mounted on glass coverslips and viewed on a confocal/two confocal microscope (DM6000 CFS, Leica, Wetzlar, Germany). To show co-localization, digitized images were overlaid using Adobe Photoshop CS3 EXTENDED v. 10.0.1 software.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Estimation of voltage-clamping errors in astrocytes

(A) IR-DIC image of an astrocyte in the CA1 *stratum radiatum* during dual-patch whole-cell recording. The left electrode was used to deliver command voltages (V_{cmd}) in the V-clamp mode and the actual V_m was measured via the right electrode in the I-clamp mode. Scale bar, 5 μm.

(B) Command voltage (V_{cmd}) pulse protocol: 1 s test voltages were stepped from -150 mV to +20 mV with 10 mV increments; the holding potential was -80 mV.

(C) Without the access resistance (R_a, 11 MΩ here) compensation, the whole-cell currents (I_m) recorded from the left electrode (V-clamp at -80 mV) and the actual V_m recorded from the right electrode, respectively. Note the actual holding potential was -84 mV (dashed line) and the changes of V_m were greatly smaller than the V_{cmd} in (B).

(D) After a proper R_a compensation, the I_m recorded from the left electrode were largely increased and the V_m were close to the V_{cmd}. Note the actual holding potential was -80 mV (dashed line).

(E) The plot of V_m against V_{cmd} of the same astrocyte in (C) & (D) showed that V_m recorded before the R_a compensation (filled symbols) were markedly deviated from the identity line. The voltage-clamping error was approximately 54%. V_m recorded after the R_a compensation (open symbols) were very close to the V_{cmd}; the voltage-clamping error was about 6%.

(F) Summary of actual V_m before and after the R_a compensation from 7 cells. The mean voltage-clamping error was 63 ± 2% before the R_a compensation and 4 ± 2% with the proper R_a compensation, respectively. Error bars are shown only when they

exceed the size of the symbol.

Figure S2. No co-localization of GFAP and NG-2 immunoreactivties

(A) Labeling of GFAP, NG2, DAPI and the merged image in the CA1 region. No co-localization of GFAP and NG2 labeling. Scale bar, 50 μm.

(B) Labeling of GFAP, NG2, DAPI and the merged image in the dentate gyrus. Scale bar, 50 μm.