Supplementary Material

1. Original cell-attached patch-clamp recordings

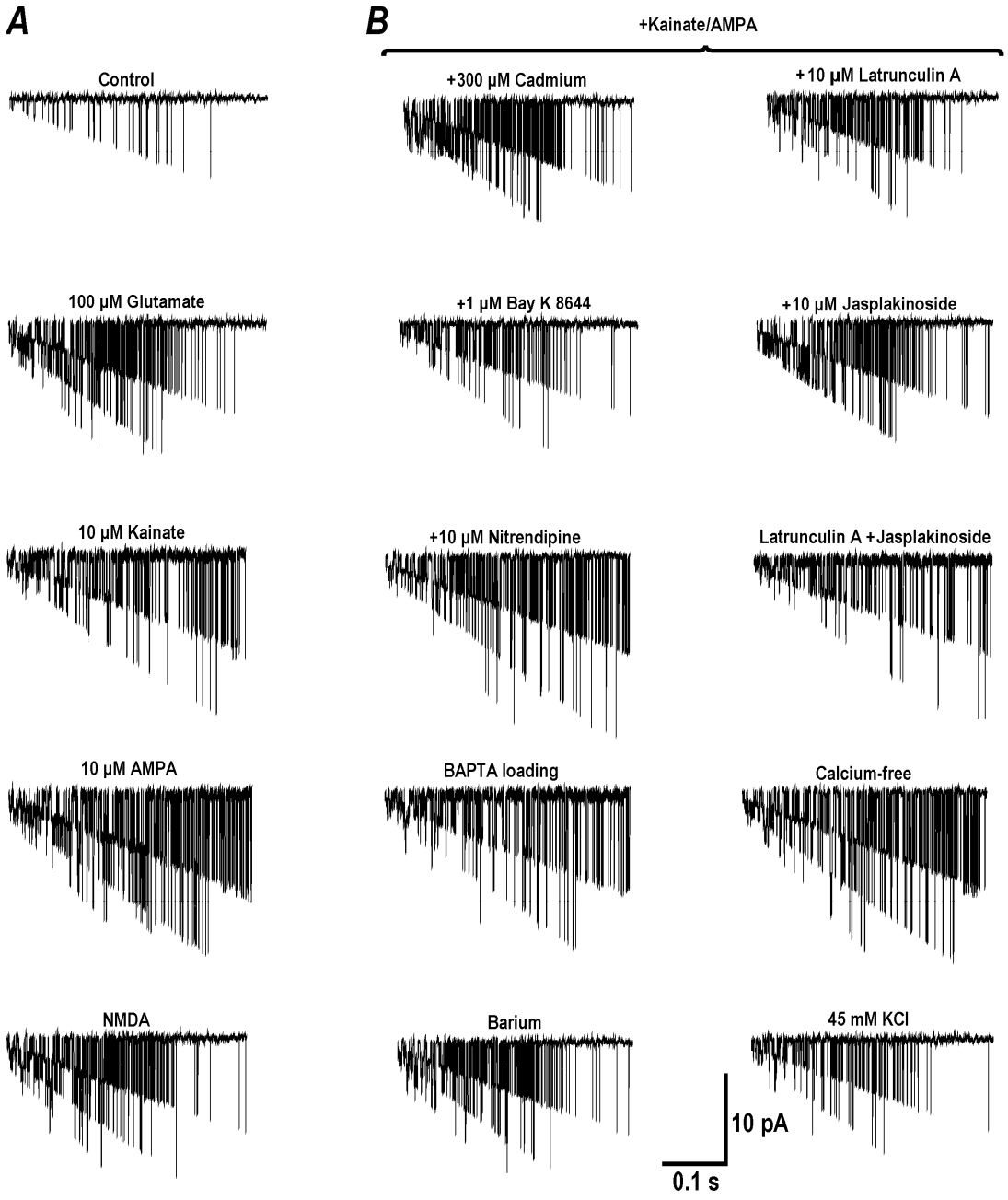

Fig. S1. GluR-mediated potentiation of KATP channels.

A - Representative traces show KATP channel activities in the control and 2 min after applications of 100 μM glutamate, 10 μM kainate or AMPA and 100 μM NMDA. ***B*** – Specimens of KATP channel activity obtained 2 min after testing applications of 10 μM AMPA or kainate in the presence of 1 μM Bay K 8644 and 10 μM Nitrendipine to modulate

L-type calcium channels, 1 mM cadmium (to block calcium entry into the cell); after replacement of external calcium with barium, and in calcium-free solutions. Neurons were incubated with agents for 3 to 30 min and they were left in the bath during AMPA/kainate applications. GluR effects were also tested in neurons loaded with the calcium chelator BAPTA (incubation with 10 μM AM-ester for 30 min; washout, 30 min); after modification of actin turnover with 10 μM Latrunculin A and 10 μM Jasplakinoside (applied also in combination, Section 4).

2. Osmotic effects

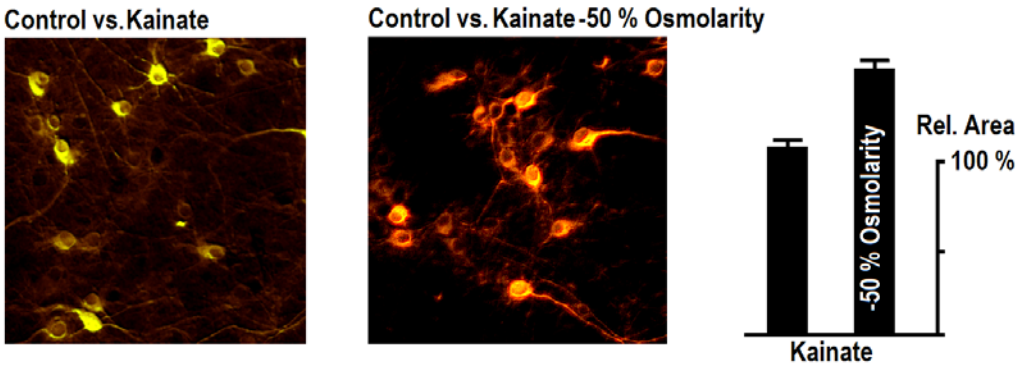

Fig. S2. Kainate and osmolarity effects.

Two overlays of images (green, before; red, 3 min after 10 µM kainate) were obtained in normal and hypotonic ACSF. Neurons for experiments were transduced with GFP-synapsin (Hartelt et al. 2008). Histogram on the right shows mean increases in the somatic area after kainate in the control and hypotonic solution ($n = 24$, $p = 0.056$).

3. ROS imaging

ROS levels were imaged with ro-GFP1 (Hanson et al. 2004). Sensor distribution within cytoplasm was non-uniform (inset in Fig. S3) and possibly reflected locations of mitochondria, a major source of ROS. Oxidizing agent H_2O_2 increased fluorescence ratio (380/470 nm excitation) and reducing agent dithiotreitol (DTT) reversed the effect with undershoot (Fig. S3). Application of 10 μM kainate for 3 min did not change signals (n = 6).

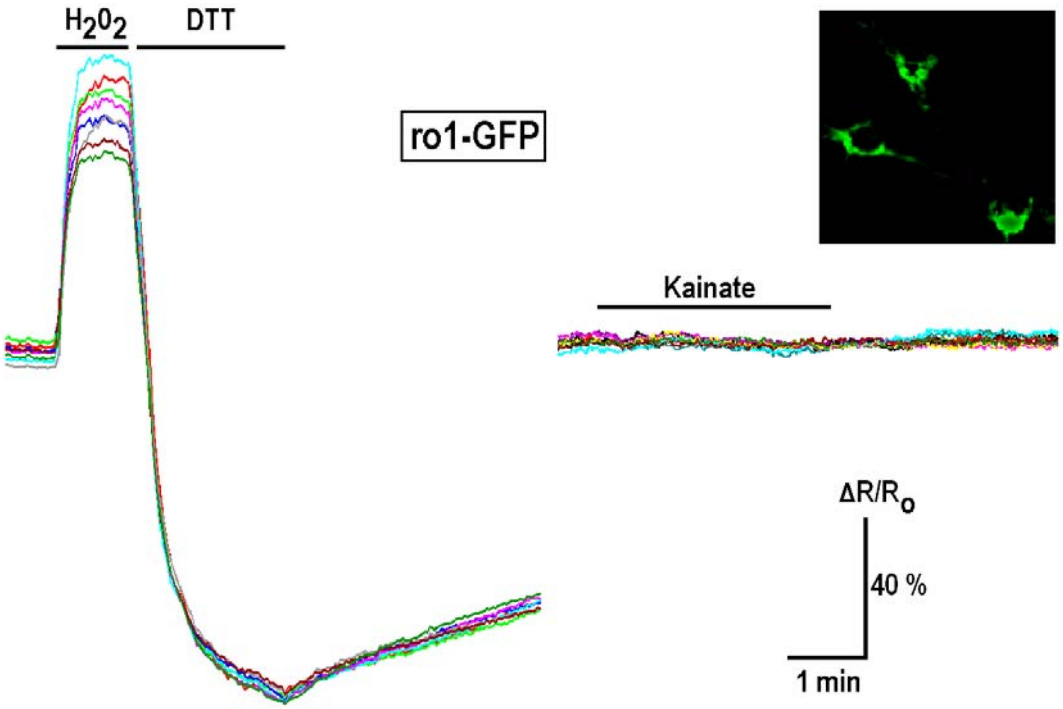

Fig. S3. ROS changes and GluR effects.

Differently colored traces present responses of neurons and the inset shows neurons transduced with ROS sensor ro-GFP1. Traces present changes in the ratio of fluorescence excited at 380 and 470 nm (emission, 515 nm) in response to 0.1 mM H_2O_2 and 1 mM dithiotreitol indicating dynamic range of ROS sensing. 10 μM kainate did not change FRET/CFP ratio as shown by traces in the right panel.

4. Actin turnover is not involved in GluR effects on KATP channels

Contribution of cytoskeleton dynamics to ATP consumption was assessed using jasplakinolide to slow down actin filament turnover by inhibiting actin disassembly and latrunculin A to prevent actin assembly by sequestering actin monomers. Both drugs (alone and in combination) did not modify increases in KATP channel opening after AMPA/kainate (Fig. S4; original recordings, Fig. S1).

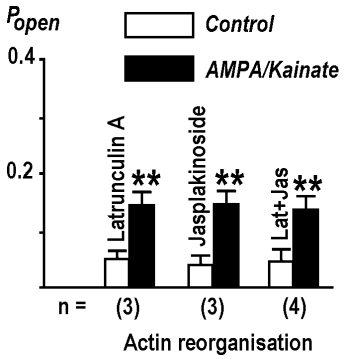

Fig. S4. Actin-reorganization is not involved in AMPA/kainate effects.

Experiments were performed after modification of actin turnover with Latrunculin A (10 μM) and Jasplakinolide (10 μM, both applied for 30 min). The experimental protocols reproduced that used by Bernstein and Bamburg (2003).

Bernstein, B.W, and Bamburg, J.R. (2003). Actin-ATP hydrolysis is a major energy drain for neurons. J. Neurosci. 23, 1-6.