Supporting Information

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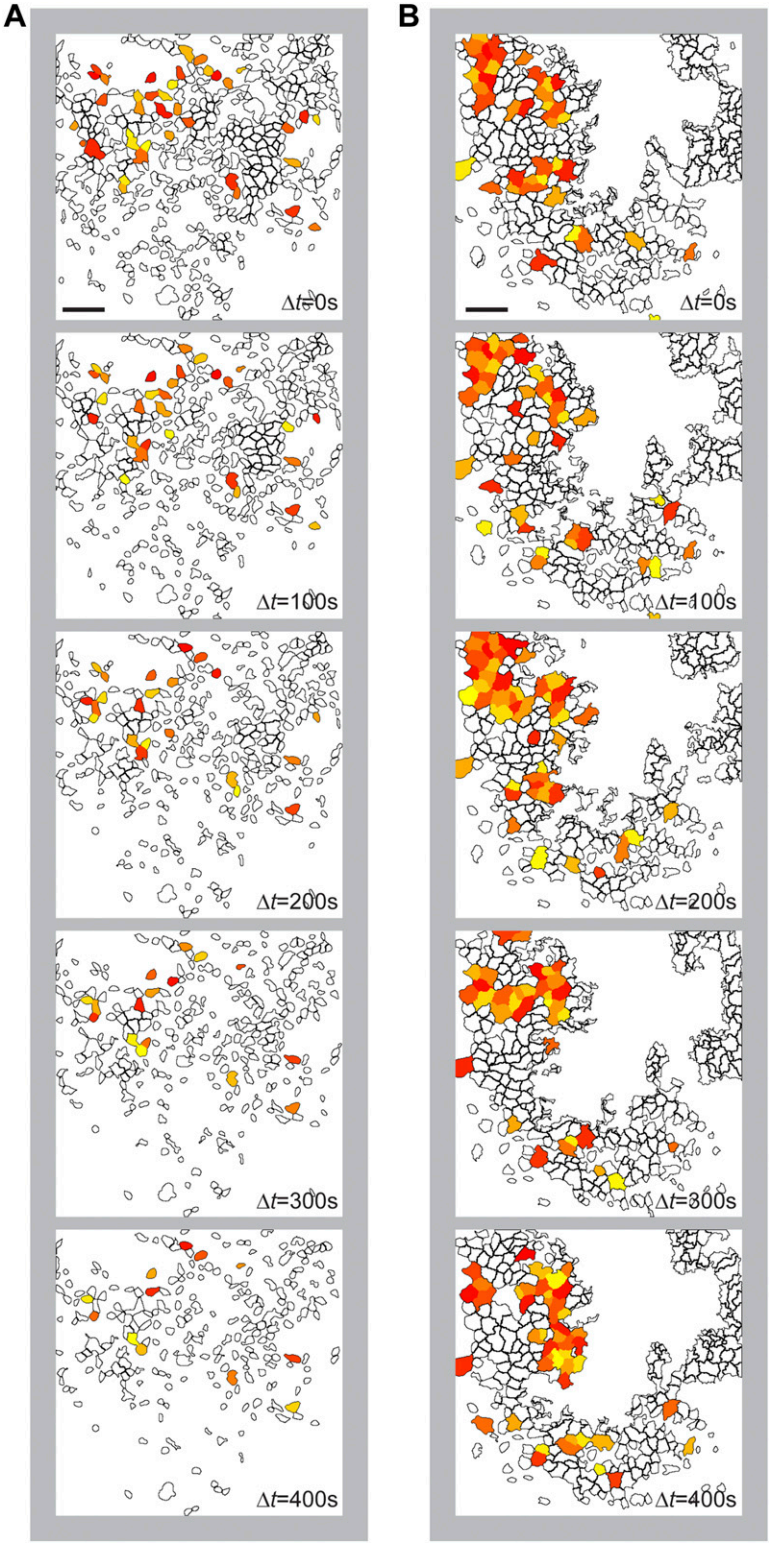


Fig. S1. Related to Fig. 1. (*A* and *B*) Proliferating mouse ES cells exhibited sparse and disperse spontaneous calcium ion (Ca^{2+}) activity (*A*), whereas neural progenitor cells exhibited vivid and clustered spontaneous Ca^{2+} activity (*B*). Cells with a cytosolic Ca^{2+} concentration 25% above baseline are pseudocolored (hot) at indicated time points. (Scale bars, 50 μm.)

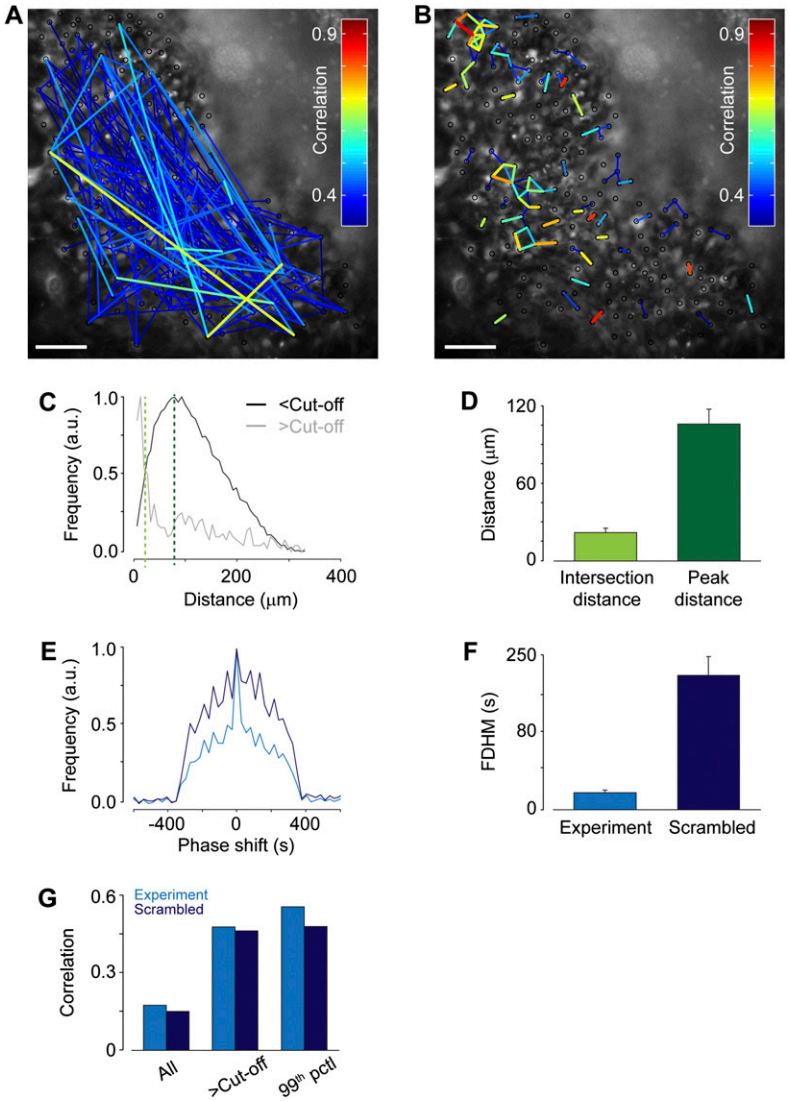


Fig. S2. Related to Fig. 2. (*A*) Network plot of cross-correlation coefficients from time-shifted scrambled Ca^{2+} recordings on neural progenitor cells greater than cutoff (color coded as indicated). (*B*) Network plot of cross-correlation coefficients from Ca^{2+} recordings of neural progenitor cells greater than cutoff (color coded as indicated) and cell-to-cell distances shorter than 22.1 μm. (Scale bars, 50 μm.) (*C*) Plot of distance distribution for the experiment depicted in Fig. 2*A* showing correlation coefficients less than (black trace) and greater than (gray trace) the cutoff of 0.39. (*D*) Mean intersection value (light green bar) and mean peak value (dark green bar) of distance distributions from six experiments, indicated in C by light green and dark green lines, respectively. (*E*) Cross-correlation analysis of Ca^{2+} activity using time lag for six experiments shows significantly shorter phase-shift for experimental data (light blue) compared with scrambled signals (dark blue). (*F*) Quantification of full duration at half maximum of phase shift from experimental data and scrambled data. (*G*) Statistical analyses of correlation coefficients derived from in vivo Ca^{2+} imaging of whole embryo and scrambled data. The entire data set (All), and filtered data sets greater than cutoff (>Cut-off) or the 99th percentile (99^{th} pctl) are presented. Values are mean ± SEM.

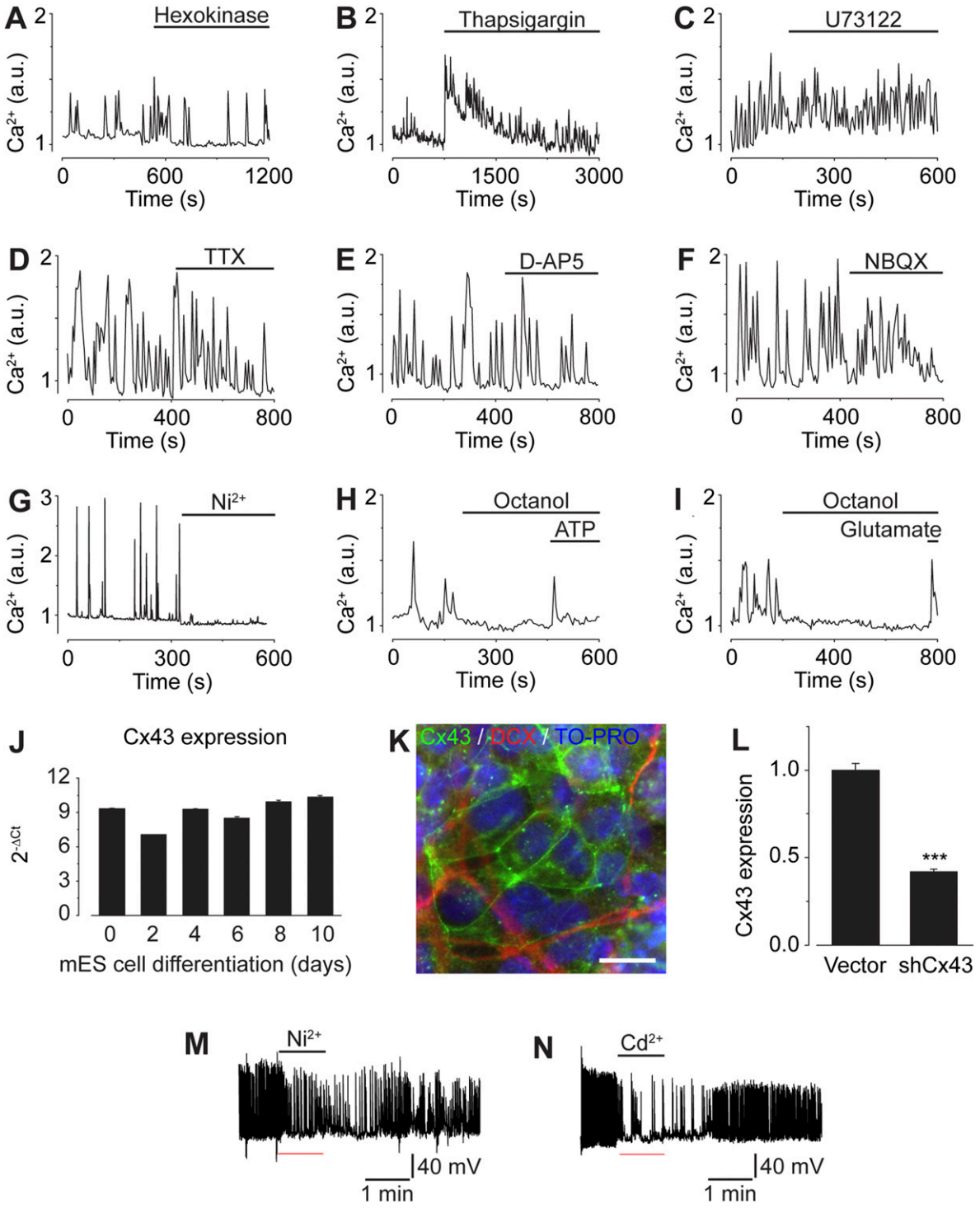


Fig. S3. Related to Fig. 4. (*A*–*F*) Hexokinase (10 U/mL) for consumption of extracellular ATP (*A*), thapsigargin (1 μM) for depleting intracellular endoplasmic reticulum Ca^{2+} stores (*B*), U73122 (5 μM) for inhibiting phospholipase C (*C*), TTX (3 μM) for voltage-dependent sodium channel blockade (*D*), D(–)-2-amino-5-phosphonovaleric acid (D-AP5; 100 μM) for NMDA receptor blockade (*E*), and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX; 50 μM) for AMPA receptor inhibition (*F*), all failed to abolish spontaneous Ca^{2+} activity in neural progenitor cells. (*G*) Voltage-gated Ca^{2+} channel blockade with Ni^{2+} (50 μM) inhibited spontaneous Ca^{2+} activity. (*H* and *I*) Cells treated with octanol (1 mM) were still responsive with Ca^{2+} transients following treatment with ATP (10 μM) (*H*) or glutamate (100 μM) (*I*). (*J*) Real-time PCR analysis of *Cx43* expression relative to the housekeeping gene (TATA-box binding protein) in differentiating neural progenitors. (*K*) Immunocytochemistry of neural progenitor cells (day 8) stained for Cx43 and the neural progenitor marker doublecortin (DCX). Nuclei were counterstained with TO-PRO-3. (Scale bar, 20 μm.) (*L*) Real-time PCR analysis of *Cx43* in mouse embryonic NIH 3T3 fibroblasts transfected with empty vector (Vector) or shRNA/Cx43 (shCx43) for 48 h. (*M* and *N*) Spontaneous electrical activity in neural progenitor cells was suppressed by blocking voltage-gated Ca^{2+} channels with Ni^{2+} (50 μM) (*M*) or Cd^{2+} (0.1 mM) (*N*). Line under name of applied drug indicates time of drug administration. Values are mean ± SEM; ***$P < 0.001$.

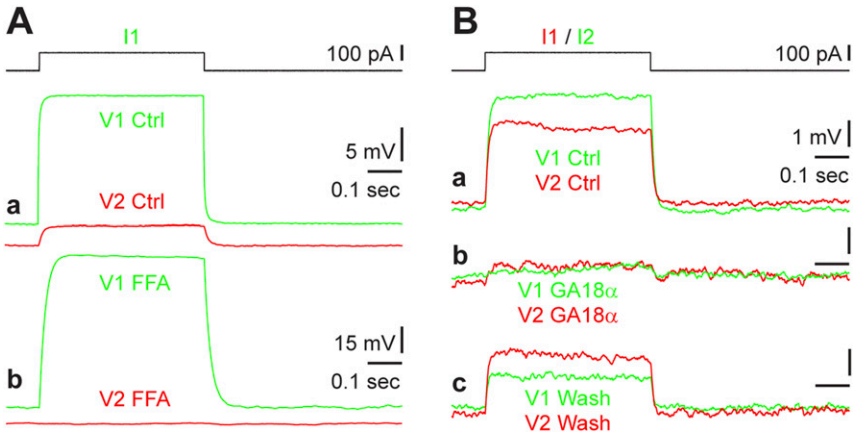


Fig. S4. Related to Fig. 5. (*A*, *a*) Injecting a 100-pA pulse (I1) into one cell (E1) revealed electrical coupling with a neighboring cell (E2). (*A*, *b*) Adding flufenamic acid (FFA; 100 μM) to the medium blocked the electrical coupling between cells. Note the increased input resistance of the recorded cells, as evidenced by the larger voltage response in cell 1 (green traces: V1 control vs. V1 FFA). (*B*, *a*) Injecting a 100 pA-pulse into one cell revealed electrical coupling with a neighboring cell (I1 to V2, red trace), and vice versa (I2 to V1, green trace). (*B*, *b*) Adding 18α-glycyrrhetinic acid (GA18α; 50 μM) to the medium blocked the electrical coupling between cells, and vice versa. (*B*, *c*) After washing out GA18α, the electrical coupling recovered.

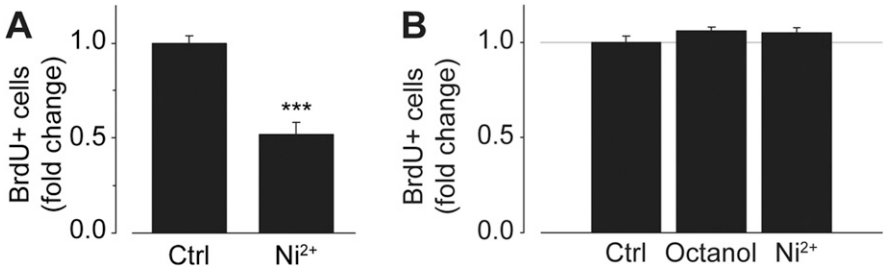


Fig. S5. Related to Fig. 6. (*A* and *B*) Quantification of BrdU-positive neural progenitor cells (*A*) and proliferating ES cells (*B*) treated with Ni^{2+} (50 μM) or octanol (1 mM) for 7 h normalized to control. Values are mean ± SEM; ***$P < 0.001$.

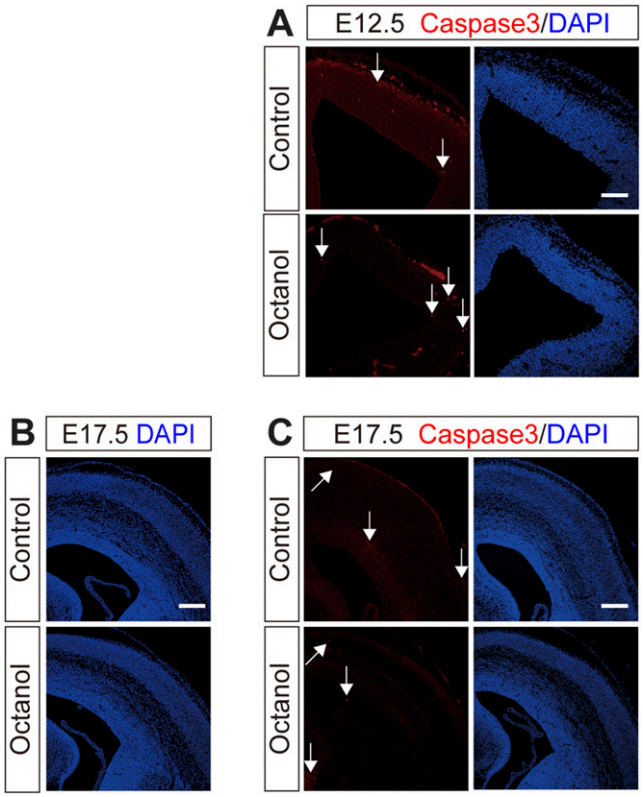
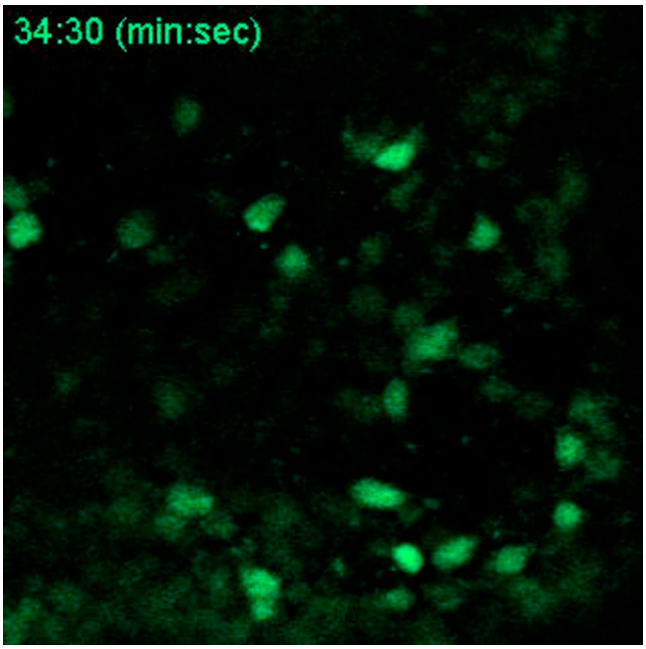


Fig. S6. Related to Fig. 7. (*A*) Immunohistochemistry of cleaved Caspase-3 in embryonic day 12.5 (E12.5) brain treated with control or octanol for 7 h. Nuclei were counterstained with DAPI (blue). (*B*) Cortical thickness in brains from E17.5 animals injected with control or octanol 5 d earlier at E12.5. (*C*) Immunohistochemistry of cleaved Caspase-3 in E17.5 brain injected with control or octanol 5 d earlier at E12.5. Nuclei were counterstained with DAPI (blue). (Scale bars, 200 μm.)



Movie S1. In vivo imaging of spontaneous Ca^{2+} activity in mouse embryo brain at E9.5. Related to Fig. 5. Movie showing spontaneous Ca^{2+} activity in in-vivo experiments on whole-mouse embryos at E9.5 loaded with Fluo-4/AM and time-lapse recording every 10 s. Frame size is 260 × 260 μm.

Movie S1