Neuronal Gap Junctions Are Required for NMDA Receptor-Mediated Excitotoxicity: Implications in Ischemic Stroke

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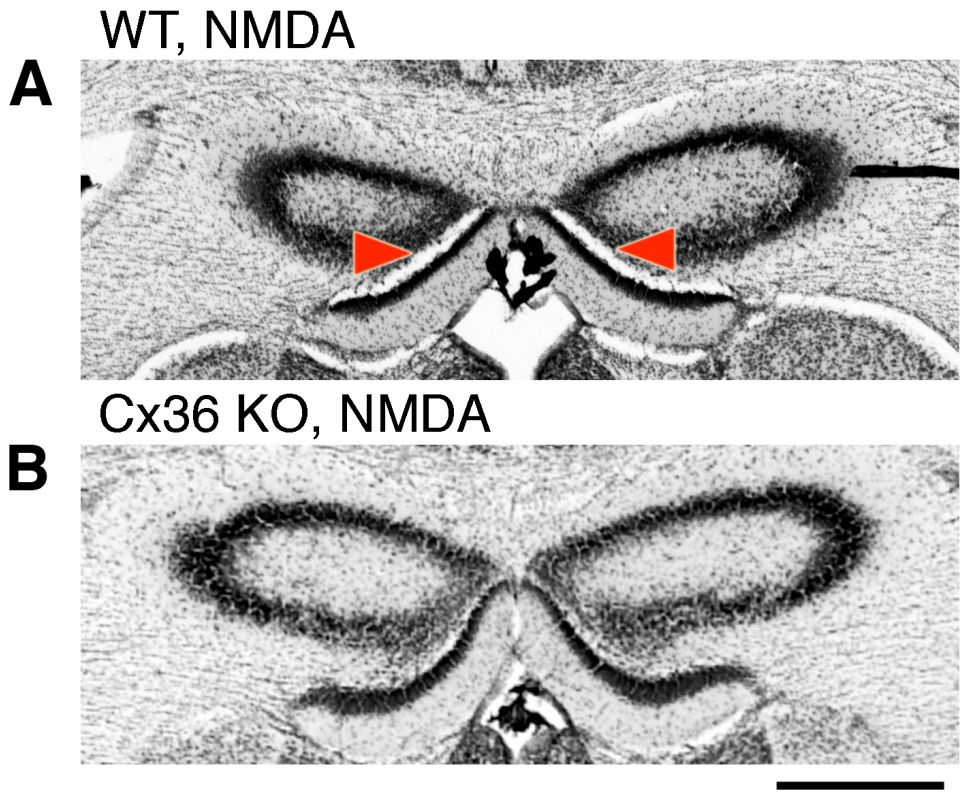
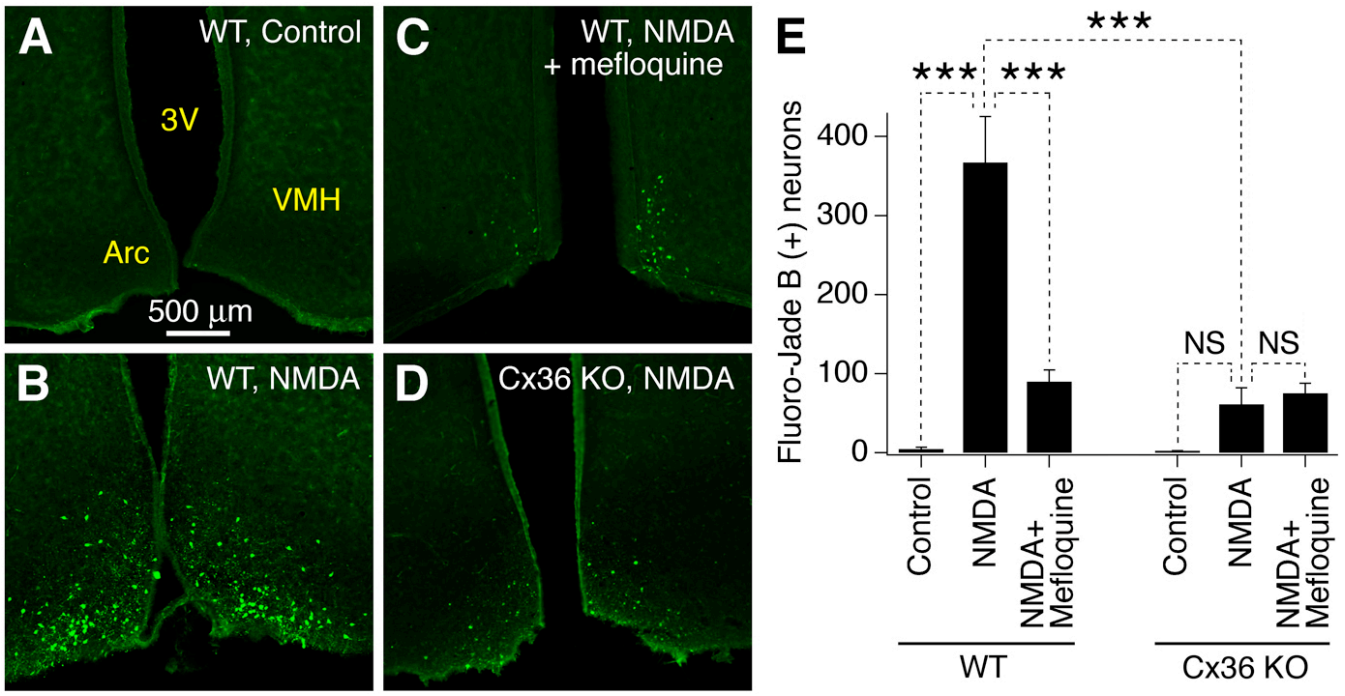
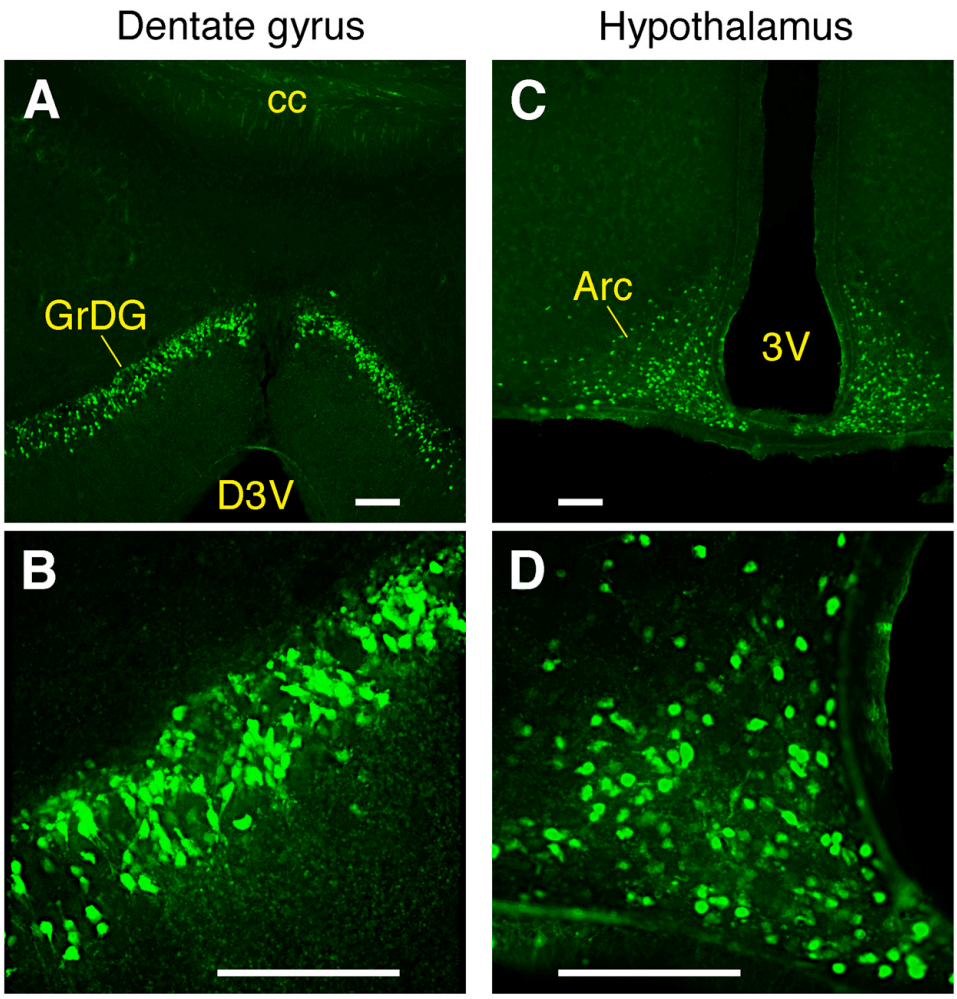
SUPPLEMENTAL FIGURES



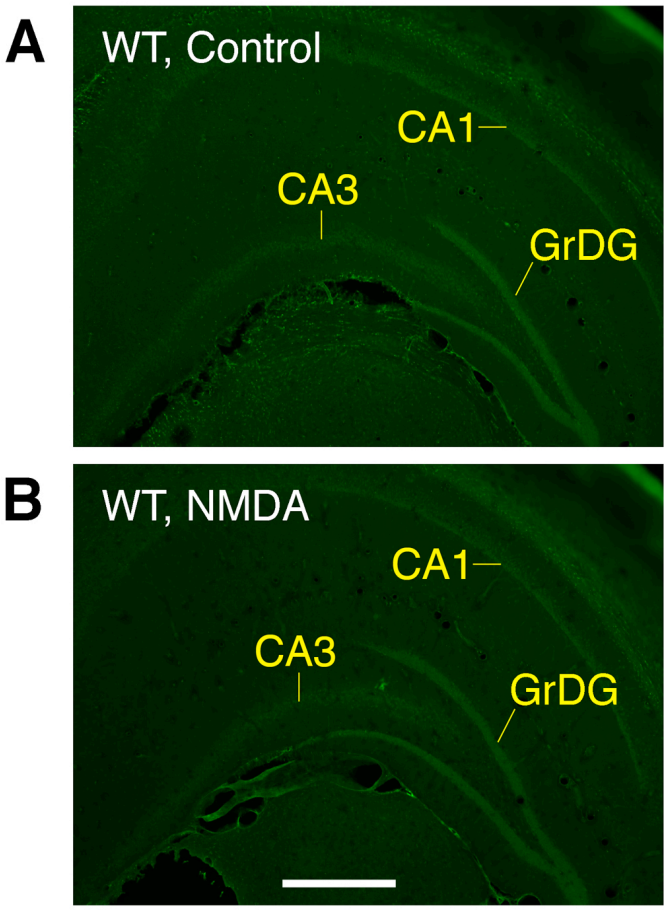
Supplemental Figure 1. Inactivation of neuronal gap junctions prevents NMDA receptor (NMDAR)-mediated neuronal death. *A, B;* Shown are images of Nissl staining in brain sections from NMDA-treated wild-type (WT; *A*) and NMDA-treated connexin 36 knockout (Cx36 KO; *B*) mice. The images are representative of 4 animals in each group. NMDAR-mediated neuronal death is seen in the inner part of the rostral dentate gyrus granule cell layer (red arrowheads) in WT, but not in Cx36 knockout mice. The analysis was done 3 weeks after NMDA administration. Calibration bar: 500 μm.



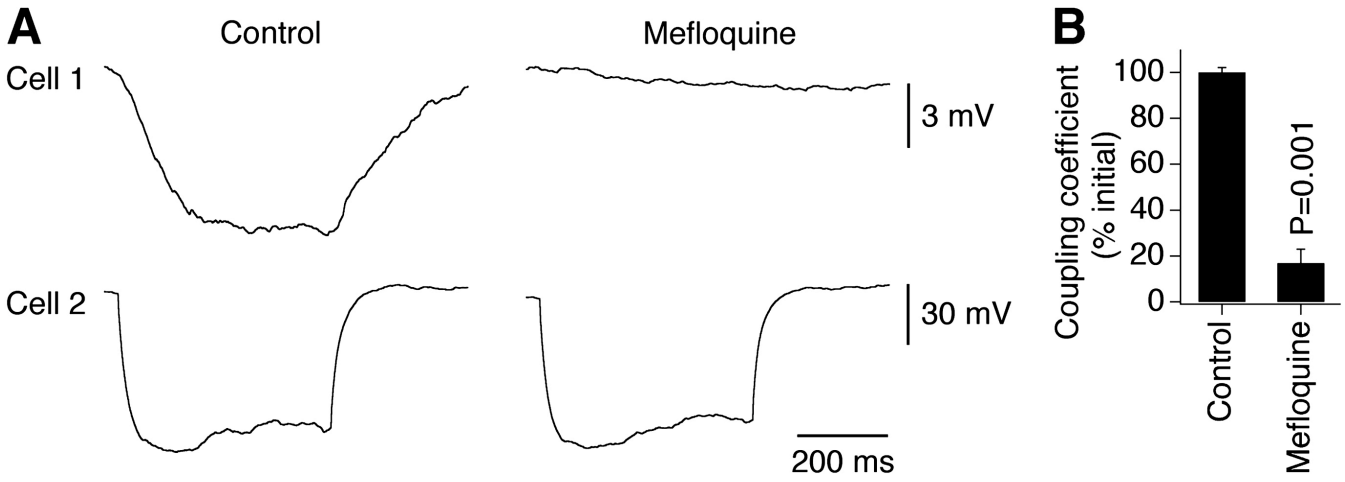
Supplemental Figure 2. Inactivation of neuronal gap junctions prevents NMDAR-mediated neuronal death in the hypothalamus. *A-D;* Representative images of Fluoro-Jade B staining in brain sections from control WT (*A*), NMDA-treated WT (*B*), NMDA plus mefloquine-treated WT (*C*) and NMDA-treated Cx36 knockout (*D*) mice are shown. Administration of NMDA induces neuronal death in the medial hypothalamus (B) that is prevented by pharmacological (C) or genetic (D) inactivation of Cx36-containing gap junctions. *E;* Graph presents statistical analysis of the number of Fluoro-Jade B-positive neurons in the hypothalamus (ANOVA; n=4-7 mice per group; ***P<0.001; NS, non-significant; mean ± s.e.m.). The analysis was done 24 hrs after saline or drug administrations (i.p.). The total number of stained neurons was counted in the whole hypothalamic region, in both hemispheres, in 7 sections in each brain (including -0.6, -0.8, -1.0, -1.2, -1.4, -1.6 and -1.8 mm from bregma) and was averaged for 7 sections. 3V, 3rd ventricle; Arc, arcuate nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus.



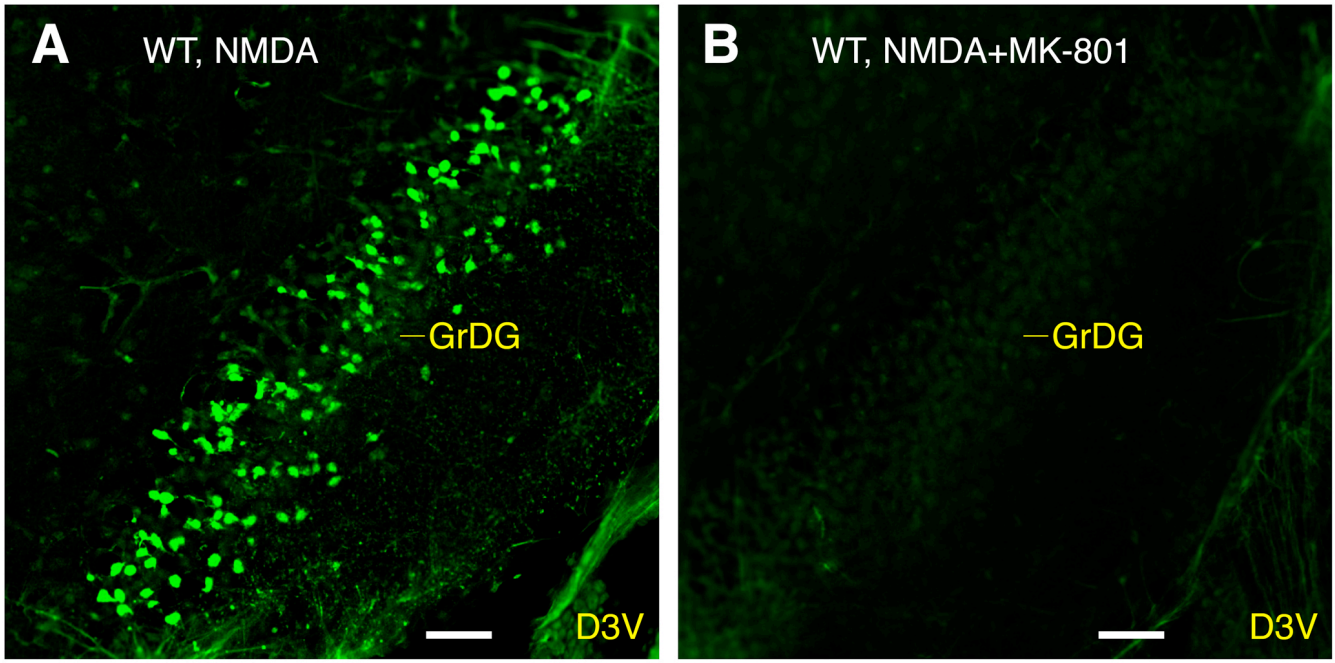
Supplemental Figure 3. NMDAR-mediated neuronal death is detected in the rostral dentate gyrus (*A, B)* and hypothalamus (*C, D*) of WT mice 24 hrs after NMDA administration. Representative images of Fluoro-Jade B staining in coronal brain sections are shown. Fragments of images in *A* and *B* are shown at a higher magnification as figures *B* and *D,* respectively. In all images, calibration bar: 100 μm. cc, corpus callosum; D3V, dorsal 3rd ventricle; GrDG, granule cell layer of dentate gyrus.



Supplemental Figure 4. NMDA administration in WT mice does not induce neuronal degeneration in the caudal dentate gyrus. *A, B;* Fluoro-Jade B staining images are representative for control (*A*) and NMDA-treated (*B*) WT mice (n=4 and 7 mice, respectively). The analysis was done in caudal parts of the dentate gyrus 24 hrs after saline or drug administrations (i.p). Calibration bar: 500 μm. CA1, field CA1 hippocampus; CA3, field CA3 hippocampus.



Supplemental Figure 5. Mefloquine reduces electrical coupling between neurons. *A, B;* Whole-cell current-clamp recording was done from pairs of neurons in rostral dentate gyrus acute slices. Traces of recordings from a representative pair of neurons (*A*) and statistical analysis of coupling coefficient (*B*) are shown. Test current steps (500 ms, -100 pA) were applied to cell 2 (injected cell) and electrotonic responses were detected in cell 1 (non-injected cell). Illustrated traces are average voltage responses from 5 sequential steps. The coupling coefficient was calculated as the response amplitude in the non-injected cell divided by the amplitude in the injected cell. Measurements were done before (control) and 30-50 min after beginning of mefloquine administration (50 μM; bath application). Statistical analysis: paired Students *t*-test; n=4 pairs; mean ± s.e.m.



Supplemental Figure 6. NMDAR blocker, MK-801, prevents NMDAR-mediated neuronal death. *A, B;* Fluoro-Jade B staining images are representative for NMDA-treated (*A*) and NMDA plus MK-801-treated (*B*) WT mice (n=4 mice in each group). Images of sections from the rostral dentate gyrus are shown. D3V, dorsal 3rd ventricle; GrDG, granule cell layer of dentate gyrus. Calibration bar: 100 μm.