

Supplemental Materials and Methods

Annexin V detection

Oligodendrocytes were exposed to agonists for 15 min and early apoptosis were determined by incubation for 10 min with Annexin V-FITC (1:100; Molecular Probes, Invitrogen) at room temperature in the dark. In all cases, cells were counterstained with Hoechst 33258 (20 μ M; Sigma-Aldrich, St. Louis, MO, USA) to simultaneously evaluate nuclear condensation. After washing, cells were observed under a fluorescence microscope and apoptotic cells showed the presence of Annexin V-FITC on the cell membranes (green colour).

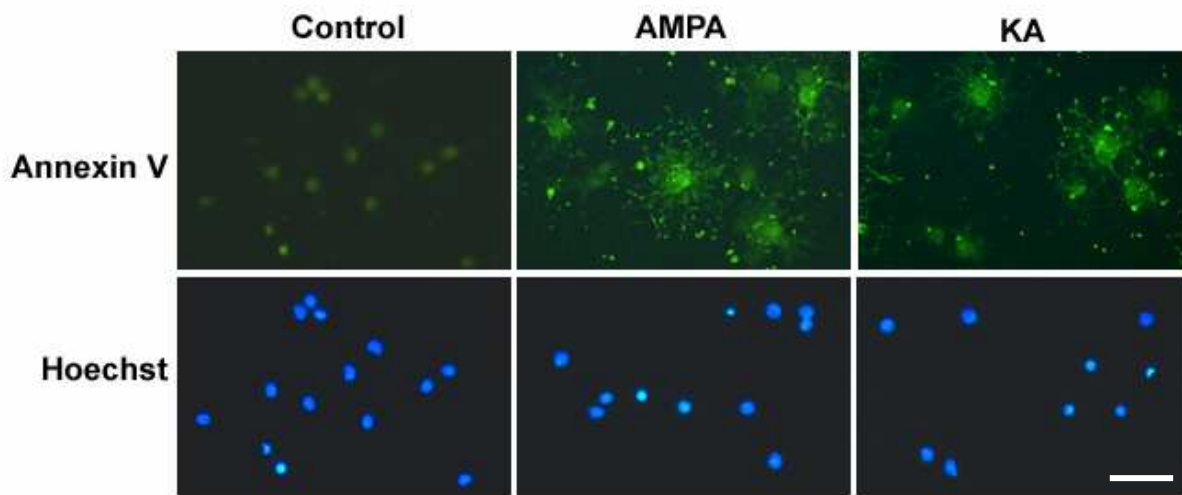
Hoechst 33258 staining and TUNEL assay

Late apoptosis was evaluated by Hoechst 33258 staining and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling) method. Oligodendrocytes were treated with agonists for 15 min, maintained for 24 h in fresh medium and, to analyze the nuclear condensation, cells were incubated with 20 μ M Hoechst 33258 for 10 min. The cells were then washed twice with phosphate-buffered saline (PBS) and examined using the fluorescence microscopy system. Apoptotic feature was assessed by observing chromatin condensation and fragments staining.

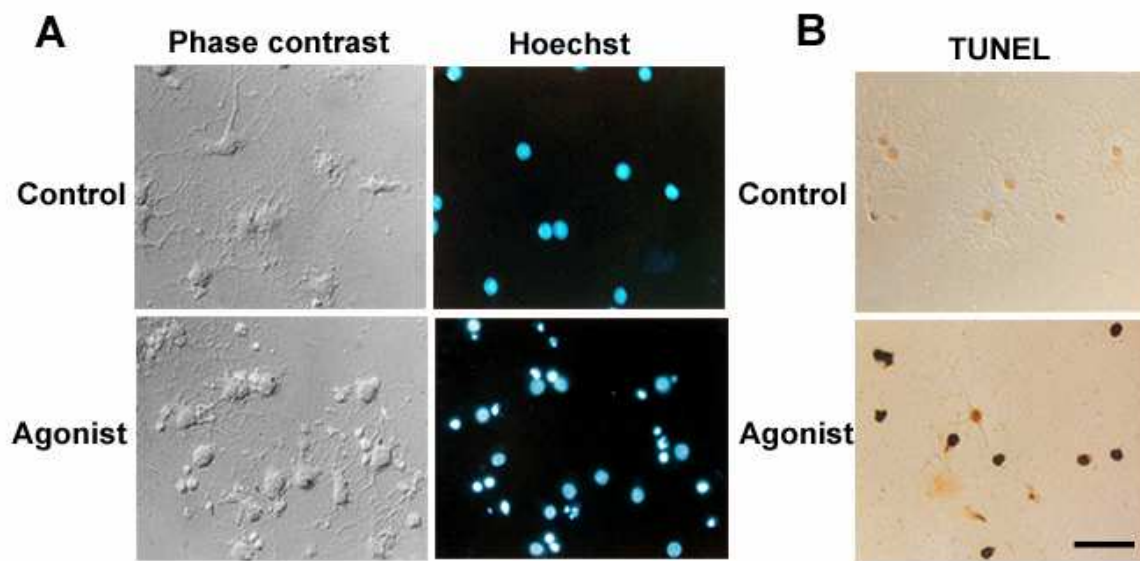
For TUNEL method, we used an in situ apoptosis detection kit and the assay was carried out as indicated by the supplier (Roche Applied Science). Briefly, cells were exposed to agonists and fixed with 4% formaldehyde in PBS at 24 h after stimulation. Endogenous peroxidase was blocked with 3% H₂O₂ and cells were incubated with equilibration buffer, biotin-dUTP and terminal deoxynucleotidyl transferase (TdT) enzyme, for 1 h. The cells were rinsed with PBS, incubated with streptavidin-HRP solution for 30 min and the peroxidase activity was shown by the application of DAB

substrate for 10-15 min. The positive cells were identified and analyzed under the light microscopy.

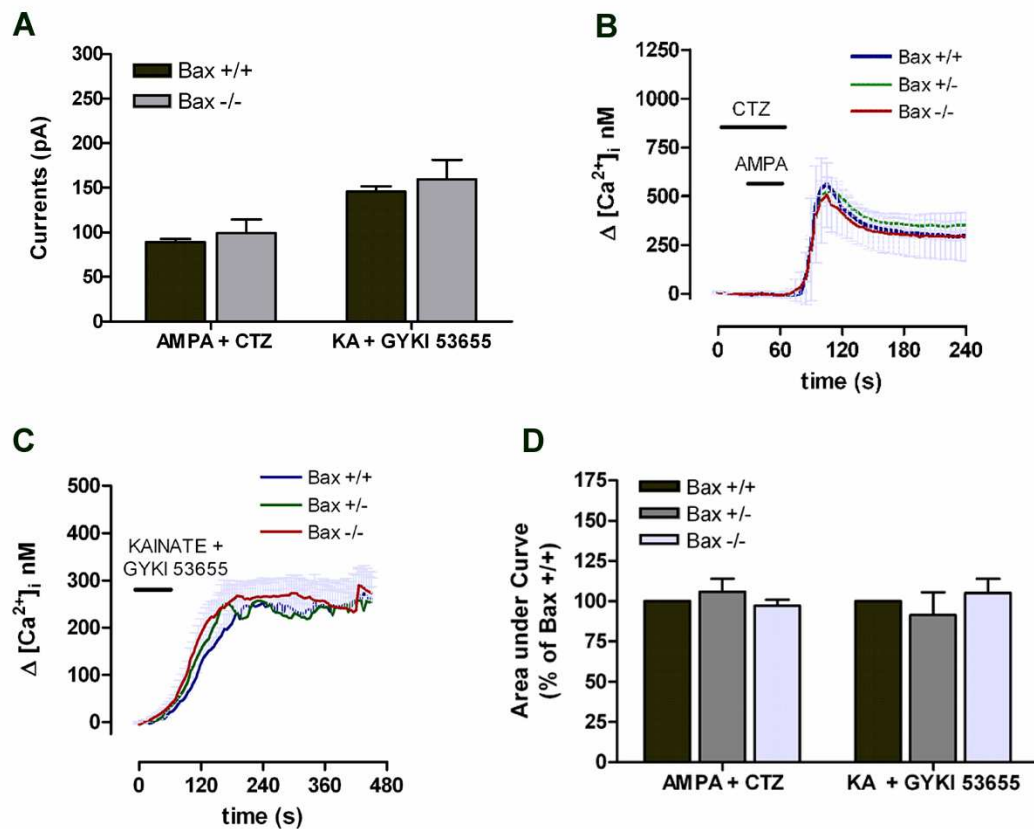
Supplemental Figures



Supplemental Figure 1. Activation of AMPA and kainate receptors induces early apoptosis in oligodendrocyte. Cells were exposed to agonists for 15 min and apoptotic features were early detected by labelling with Annexin V-FITC. Immediately after stimulus, cells were incubated with Annexin V-FITC and Hoechst 33258 for 10 min and were observed under a fluorescence microscope. Cells treated with AMPA or kainate bound Annexin V-FITC and showed green staining in the plasma membrane, an effect that was not observed in control cells (*top*). Nuclear staining with Hoechst 33258 corresponds to the field shown in the top panel (*bottom*). Scale bar = 25 μ m.



Supplemental Figure 2. Excitotoxicity-mediated oligodendroglial cell death has apoptotic features. (A) Phase-contrast photomicrographs of oligodendrocytes in control and treated cultures, at 24 h after of stimulation (*left*). Agonists induced chromatin condensation that was not observed in control cultures, as revealed with Hoechst 33258 nuclear staining (*right*). Fields shown in the images in the left correspond to those illustrated in the right. (B) Agonists caused DNA fragmentation as revealed by TUNEL (black cells), an effect that was absent in control cultures. Oligodendrocytes were processed for TUNEL 24 h after stimulation with agonists. Scale bar = 25 μm .



Supplemental Figure 3. Responses by AMPA and kainate receptors are similar in Bax-deficient and wild type oligodendrocytes. Cultured oligodendrocyte obtained from optic nerves of wild-type and *bax* knockout mice were monitored using whole-cell patch-clamp recording as previously described (Domercq et al., 2005, *Glia* 52:36-46), and Ca^{2+} imaging (see Materials and Methods). Activation of AMPA or kainate receptors was done in the presence of CTZ or GYKI 53655, respectively. (A) The responses of each receptor subtype evoked currents with similar amplitude in both Bax +/+ and Bax -/- oligodendrocytes ($p = 0.704$ and $p = 0.566$ for responses to AMPA and kainate receptor activation, respectively). (B, C) AMPA and kainate receptor activation induced a rapid and sustained increase in $[\text{Ca}^{2+}]_i$ which was similar in the three genotypes analyzed. (D) Histogram depicts the values of the area under the curve

corresponding to $[Ca^{2+}]_i$ increases following receptor activation in Bax +/- and Bax -/- oligodendrocytes with respect to Bax +/+ cells.