Supplementary Methods

Preparation of membrane and soluble fractions from brain, and western blotting. Membrane and soluble fractions from brains were prepared as described[1] with the following modifications: briefly, brains from E14 and E18 and cortices from adult Sprague-Dawley rats were homogenized using a Teflon homogenizer in 5 ml ice-cold buffer (320 mM sucrose, 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1:100 Protease Inhibitor Cocktail (Sigma)) per 1 g of tissue. The homogenates were centrifuged at 700*g* for 10 min at 4 °C, followed by centrifugation of the supernatant at 28,000*g* for 16 min at 4 °C. The high-speed pellet was resuspended in 500 l ice-cold TE buffer (10 mM Tris-HCl, 5 mM EDTA, pH 7.4). Samples containing 20–40 g protein were diluted 1:1 in loading buffer[2], boiled for 5 min, then separated on 16.5% Tris-Tricine gels[3]. Before antibody staining, the blot was incubated for 2 min with Ponceau S solution (Sigma) to determine protein content as a loading control.

Hoechst staining and caspase 3 immunocytochemistry. Fixed cells were incubated for 10 min with Hoechst 33342 (1:1,000 in PBS, Sigma), rinsed three times in PBS and mounted onto slides with Fluoromount G (Southern Biotechnology). For immunocytochemistry, fixed cells were washed with PBS for 5 min and then permeabilized with 0.3% Triton X-100 for 5 min at 4 °C. Neurons were washed again with PBS, incubated with blocking solution (10% goat serum, 0.1% Triton X-100 in PBS) for 1 h at 4 °C and then incubated with an antibody to cleaved caspase 3 (1:100, Cell Signaling Technology) in blocking solution overnight at 4 °C. After rinsing three times with PBS, an anti-rabbit secondary antibody coupled to rhodamine (1:500, Jackson ImmunoResearch) was added for 1 h at approximately 25 °C. Chromatin staining with Hoechst was done simultaneously with the last antibody wash, and neurons were rinsed and mounted as described above.

Immunocytochemistry of neural progenitor cultures. For immunocytochemistry, cells were fixed for 20 min with 4% paraformaldehyde. Cultures were then washed with PBS, permeabilized for 5 min in 0.3% Triton X-100 in PBS and then blocked for 1 h with buffer containing 10% goat serum and 0.3% Triton X-100. Cells were then incubated at 4 °C overnight with primary antibodies in 0.1% Triton X-100 in PBS containing 10% goat serum. After three washes with 0.1% Triton X-100 in PBS, cells were incubated at room temperature for 60 min with Alexa-555-conjugated goat anti-mouse (1:2,000, Molecular Probes) and Alexa-647-conjugated anti-rabbit (1:2,000, Molecular Probes) secondary antibodies prepared in 0.1% Triton X-100 PBS containing 10% goat serum. Finally, samples were washed three times with PBS and counterstained for 10 min with Hoechst 33258. Fluorescent images were imported into Spot 3.5 (Diagnostic Instruments) with a Diagnostic Instruments Spot2 digital camera mounted on a Nikon Eclipse E600 using a 20×/0.75 Plan Apo objective (Nikon).

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2. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685 (1970).
3. Schagger, H. & von Jagow, G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368–379 (1987).