

## Supplemental Methods

**Reagents.** Amyloid- $\beta$  was purchased from Tocris, Biosource and Calbiochem and 42-1 reverse peptide was purchased from Biosource. Peptides were diluted according to manufacturer's instructions. We observed no difference between sources of amyloid- $\beta$  with comparable results from all manufacturers. MLA, BTX and TTX were purchased from Sigma. NMDA and DAPT were purchased from Calbiochem.

**Antibodies.** We purchased CREB and Phospho-CREB antibodies from Cell Signaling Technology; Synapsin 1, NR1 (monoclonal) and GABA<sub>A</sub> from Chemicon; NR1 (polyclonal), NR2B from Upstate; Actin from Sigma; STEP antibody from Novus; The Phospho-STEP antibody has been previously described<sup>36</sup>. Phospho-NR2B antibody was raised in the laboratory of Michael Greenberg as described<sup>41</sup>.

**Neuronal cultures.** Rat and mouse primary neuronal cultures were prepared from embryonic day 18 animals. Cortices were dissected out and exposed to 0.25% trypsin for 10 minutes and triturated to isolate cells. Neurons were plated in Neurobasal media with B-27 and N-2 supplements (Invitrogen). For biochemistry, neurons were plated at 300,000 cells per well in plastic 6 well plates. For immunocytochemistry, neurons were plated on glass coverslips pre-coated with poly-d-lysine (Fisher Bio-coat) at a density of 50,000 cells per 18 mm coverslip.

**N2A cell experiments.** Neuroblastoma (N2a) cells doubly transfected with human APP695 were maintained in medium containing 50% DMEM, 50% Opti-MEM, supplemented with 5% fetal bovine serum, 200 µg/ml of G418, and antibiotics (GIBCO/BRL). Secreted amyloid-β was analyzed by immunoprecipitating culture media (10 ml) with 3 µl of 4G8 antibody (Roche) and protein A-Sepharose (Pierce). Sepharose pellets were washed twice, resuspended and heated for 3 min at 100 °C in 25 µl of tricine loading buffer (Novex, San Diego, CA). Samples were subjected to SDS-polyacrylamide gel electrophoresis on 10-20% tricine gels, transferred to PVDF and probed with antibody 6E10 (Roche). For conditioned media experiments, N2A cultures were grown to 80% confluency and then transferred to serum-free Neurobasal media for 24 hours. Media was then harvested and spun at 10,000g for 10 minutes. 50% of neuron media was then replaced with N2A cell media and biotinylation assays were performed. Consistent with previous reports, media from APP<sub>SWE</sub> 9 cells contained much more amyloid-β than untransfected cells (Fig. 3c,d).

**Quantitative Western blots.** Proteins were separated using 7.5 or 10% tris-glycine gels and transferred to Immobilon P membranes (Millipore). Immunoreactive bands were visualized by enhanced chemiluminescence (ECL; Amersham or Pierce), captured on autoradiography film (Kodak Biomax film). Digital images were quantified using NIH Image 1.63 software.

**Immunocytochemistry.** Cultured neurons were fixed successively with 4% paraformaldehyde, 4% sucrose in PBS (4°C, 10 min), and then with 100% methanol (incubated at 4°C, 5 min); next the neurons were permeabilized with 0.2% Triton X-100 (4°C, ~10-15 min). To block non-specific staining, coverslips were incubated in 10% goat serum in PBS for 60 min and then incubated with primary antibodies dissolved in 10% goat serum in PBS. Neurons were exposed to antibodies against NR1 (Chemicon MAB363 1:200), and synapsin 1 (Chemicon AB1543p, 1:500) overnight at 4°C. Neurons were then rinsed 3x 10 min in 10% goat serum in PBS and exposed to Alexa-conjugated fluorescent secondary antibodies for one hour (Molecular Probes, 1:300). Cultures were rinsed 3x 10 min in block and coverslipped in Gel/Mount (Biomed). Stained neurons were visualized on a Zeiss Confocal Microscope.

**Immunoprecipitation.** Cells or frozen brain cortices were lysed in a buffer containing 50 mM HEPES, pH 7.4, 0.5 M NaCl, 0.5% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, and a complete set of protease inhibitors (Roche). Lysates were centrifuged for 10 min at 14,000 r.p.m. and then were precleared with protein G-sepharose. Samples were incubated for 1 hr with specific antibodies and then incubated with 50µl protein G-sepharose for 12 hr at 4 °C. Beads were collected by centrifugation at 1000 r.p.m. for 5 min and washed 3 times with lysis buffer containing NP-40. Proteins were eluted using SDS sample buffer.