Supplementary Information for: Amyloid-β-induced amyloid-β secretion: A possible feed-forward mechanism in Alzheimer disease by Ian T. Marsden, Laurie S. Minamide, and James R. Bamburg published in Journal of Alzheimer Disease

Rodent total Aβ ELISA

$RA\beta_{1\text{-}40}$ and $RA\beta_{1\text{-}42}$ pellets were solubilized in 10 uL of DMSO and diluted further with a phosphate buffered saline containing Tween and BSA (PBSTB; Covance). Wells to be used on the assay plate were washed 1x with PBSTB and standards or samples (100 µL per well) were applied and plates incubated overnight at 4°C. Wells were washed 5x with 300µL PBSTB. SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific) was added at 300uL to each well. Chemiluminescence was quantified by photon counting between 5 and 10 min after substrate addition using a Perkin-Elmer Victor V multi-mode microplate reader operating at room temperature with no filter. The optimal concentration of capture antibody that allowed for maximal binding of rodent Aβ to the plate was determined to be 5 µg/mL. Standard curves using 1.6 µg/mL detection antibody and synthetic $RA\beta_{1\text{-}40}$ and $RA\beta_{1\text{-}42}$ between 0 and 1,200 pg/mL were identical for both forms of RAβ and were linear from 10-600 pg/mL (Supplementary Figure 2).

Organotypic hippocampal slice cultures

Ethanol washed coverslips (12x22mm) were treated with 2% 3-aminopropyltriethoxy-silane in acetone (10 sec dip), rinsed in water, air dried and UV sterilized. Chick plasma (2 x 4 µL aliquots) was spread into two 5 mm diameter circles

near one end of the coverslip, two slices were placed side by side on the plasma and the slices were each covered with 4 µL of fresh plasma/thrombin mixture (4 µL chick plasma (Cocalico Biologicals Inc., Reamstown, PA) and 4 µL thrombin (150 NIH units/mL; MP Biomedicals, Inc.) in Gey's BSS/glucose (per 100 ml: 97 ml Gey's BSS (Sigma), 2 ml 25% glucose, 1 mL Pen/Strep). After the plasma clotted, the coverslip was inserted into a flat sided tube (Nunclon Delta Tubes, Nalge Nunc, Rochester, NY) and 600 µL slice culture medium added. Tubes were placed at a 5° angle in a roller incubator (10 revolutions per hour) at 35^oC. The original slice medium (per 205 mL: 50 mL horse serum, 50 mL Hanks BSS, 4 mL 25% glucose, 100 mL minimum essential medium containing glutaMAX (250 µL/100 mL), HEPES (4.76g/ L) and Pen/Strep (1 mL)) was replaced on day 2 with Neurobasal A medium containing (per 50 mL): 48 mL Neurobasal A, 180 µL 25% glucose, 625 µL GlutaMAX 1, 1 mL B27 supplements and 250 µL Pen/Strep. The Neurobasal A medium was replaced every 2-3 days. Slices were allowed to recover for at least 1 week after dissection before treatment.