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Supplementary Data

Amyloid-β-Induced Amyloid-β Secretion: A Possible Feed-Forward Mechanism in Alzheimer's Disease

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RODENT TOTAL Aβ ELISA

$RA\beta_{1\text{-}40}$ and $RA\beta_{1\text{-}42}$ pellets were solubilized in 10 μL 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 1× with PBSTB and standards or samples (100 μL per well) were applied and plates incubated overnight at 4°C. Wells were washed 5× with 300 μL PBSTB. SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific) was added at 300 μL 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 to 600 pg/mL (Supplementary Fig. 2).

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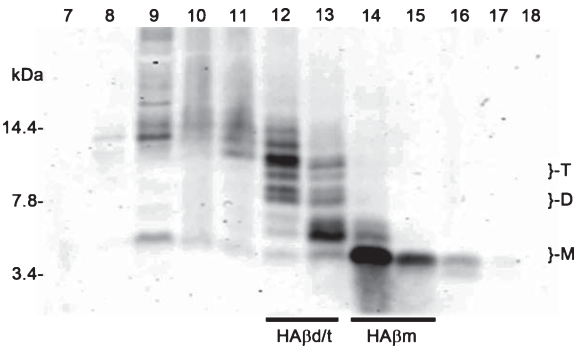

Supplementary Fig. 1. Western blot showing fractions of the HAβm and HAβd/t from 10× concentrated culture medium of 7PA2 cells. Of each 1 mL fraction, 800 μL was removed and stored at −80°C. The remaining 200 μL was lyophilized, resuspended in 2× sample buffer, and electrophoresed on a 10–20% Tris-tricine gel. Proteins were transferred onto 0.1 μm nitrocellulose and, after boiling the blot for 10 min, Aβ was detected by Western blotting with 6E10 mouse monoclonal antibody and DyLight secondary antibodies. Band fluorescence was captured with a LI-Cor Odyssey Infrared Imaging System. Fractions enriched in HAβm (fractions 14–15) or HAβd/t (fractions 12–13) were pooled separately, lyophilized, and stored at −80°C.

ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

Ethanol washed coverslips (12 × 22 mm) were treated with 2% 3-aminopropyltriethoxy-silane in ace-

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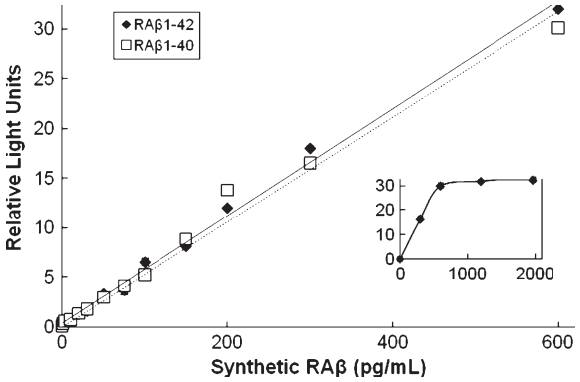
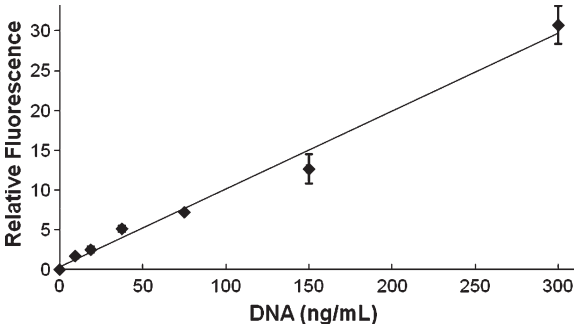


Supplementary Fig. 2. RAβ ELISA standard curves comparing $RA\beta_{1\text{-}40}$ and $RA\beta_{1\text{-}42}$. There is no significant difference between the two RAβ species used in this assay. Points are triplicate samples; error bars = standard deviation (less than the symbol size). Inset: The assay is linear between 10 pg/mL and 600 ng/mL. $n = 8$.



Supplementary Fig. 3. DNA standard curves using calf thymus DNA dissolved in TE buffer as standard. Standards and cell lysates are diluted with TE buffer containing SybrGreen I and then assayed using a microtiterplate reader operating in the fluorescence mode. The assay is linear between 1 and 300 ng/mL of DNA, but linearity can be expanded by adjusting the intensity of the excitation source or exposure time. Points are triplicate samples; error bars = standard deviation.

tone (10 sec dip), rinsed in water, air dried and UV sterilized. Chick plasma (2×4 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 5º angle in a roller incubator (10 revolutions per hour) at 35ºC. 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 (4.76 g/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.