A fluorescence assay for detecting amyloid-β using the cytomegalovirus (CMV) enhancer/promoter

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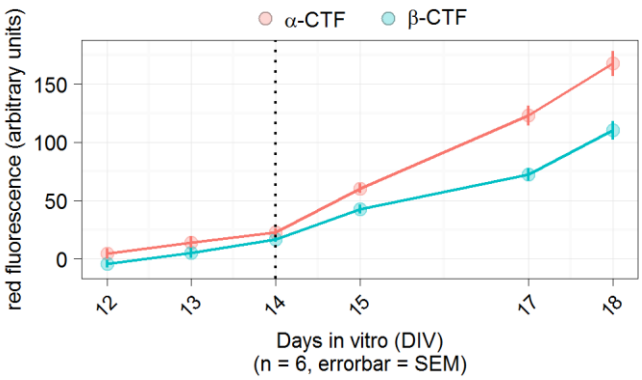
Supplementary information:



Supplementary Information Figure 1. AAV2(CMV:tdTomato) shows a decrease in fluorescence in response to overexpressed α-CTF relative to β-CTF. Primary neuronal cultures grown in 384-well plates were infected with reporter-containing AAV virus added at 9 DIV. β- or α-secretase carboxyl terminal products (β-CTF or α-CTF) of amyloid-precursor protein (APP) were overexpressed using Sindbis virus infections at 14 DIV. β-CTF leads to production of Aβ, while α-CTF does not and thus serves as a negative control. This method of detecting Aβ is noteworthy, but was not pursued further because synthetic Aβ peptide was more experimentally tractable. n indicates number of wells used for each condition and the error-bars are standard-error of the mean (SEM).

General reagents/conditions:

Unless otherwise stated, all general chemicals were from Sigma-Aldrich, and all neuroactive reagents from Tocris Bioscience. All procedures involving animals were approved by the Institutional Animal Care and Use Committees of the University of California, San Diego.

Primary neuronal cultures

Primary hippocampal neurons were made according to previously described protocols with minor modifications [1,2]. Hippocampi from P0-P2 Sprague-Dawley rat pups were first dissected in ice-cold dissection media (in mM: 82 Na2SO4, 30 K2SO4, 5.8 MgCl2, 0.25 CaCl2, 8 glucose, 1 HEPES buffer; 2% Phenol Red solution) before being cut into fine pieces using a scalpel. The hippocampal tissue was then resuspended in dissociation media (dissection media supplemented with 2 mM L-cysteine hydrochloride, ~10-20 units papain, pH adjusted to ~7.4) and further dissociated using a serological pipette. The suspension was left at room temperature for 30 minutes with gentle rocking. 1 µL DNaseI was added to digest precipitated DNA from dead cells. Neurons were then filtered through a 70 µm cell strainer to remove undissociated tissue and spun at 1000x *g*. After removal of most of the supernatant the neurons were resuspended in plating media (Neurobasal-A, 10% FBS, 0.5% Pen/Strep and 0.25% Glutamax)

and counted. 45 µL of 35000-50000 neurons/ml were added to each well of Corning Bio-Coat poly-D-lysine flat-bottomed clear 384-well plates (Corning part number 3845). After 1 h at 37 °C and 5% CO_2, 45 uL of reduced FBS media was added (Neurobasal-A, 5% FBS, 2% B-27, 0.5% Pen/Strep, 0.25% Glutamax) was added. Thereafter, half the media was replaced each time (Neurobasal-A, 2% B-27, 0.5% Pen/Strep, 0.25% Glutamax) beginning on day 2, day 5 (along with 10 µM Cytosine β-D-arabinofuranoside to inhibit non-neuronal replication), and every 2-4 days afterwards

References:

1. Aow J, Dore K, Malinow R (2015) Conformational signaling required for synaptic plasticity by the NMDA receptor complex. Proc Natl Acad Sci U S A 112: 14711-14716.
2. Dore K, Aow J, Malinow R (2015) Agonist binding to the NMDA receptor drives movement of its cytoplasmic domain without ion flow. Proc Natl Acad Sci U S A 112: 14705-14710.