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Supplementary Materials for

A vicious cycle of amyloid β-dependent neuronal hyperactivation

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Materials and Methods

All experimental procedures were performed in compliance with institutional animal welfare guidelines and were explicitly approved by the local government of upper bavaria.

Animals

All experiments were performed in nine days to two month-old C57 Bl/6N wildtype mice of both sexes or age-matched female APP23xPS45 mice (*12*). This double-transgenic mouse model expresses both the APP Swedish mutation (670/671) and the G384A mutation in the presenilin 1 (PS1) gene under the Thy-1 promotor. At this early age, APP23xPS45 mice exhibit neuronal hyperactivity of hippocampal neurons and increased levels of soluble Aβ in the brain but significant amyloid plaque formation occurs later (*11, 12*). All mice were housed in standard mouse cages under a 12-h dark/12-h light cycle and constant temperature and humidity. Food and water were provided ad libitum.

Virus injection of iGlu SnFr

AAV2/1.hSynapsin.iGluSnFR A184S (*29*) and AAV2/1.hSyn.FLEX-SF-iGluSnFrA184S was a gift from Loren Looger. AAV1.hSyn.Cre.WPRE.hGH was purchased from UPenn VectorCore. Four week-old wildtype-mice were anesthetized with isoflurane and analgesia was provided by the s.c. injection of metamizole and metacam as well as the injection of xylocaine under the scalp. The mouse was fixed in a stereotaxic frame and the coordinates of the CA1 region was identified using a mouse brain atlas (*42*). 500nl of AAV2/1.hSynapsin.iGluSnFR A184S ($2.4x10^{12}$ gc/ml) or AAV2/1.hSyn.FLEX-SF-iGluSnFrA184S ($1.8x10^{12}$ gc/ml) and AAV1.hSyn.Cre.WPRE.hGH ($7.3x10^{8}$ gc/ml) were injected at AP -2.75, ML 3.5 and DV 2-3 mm at a speed of 10-20 nl/min. After the retraction of the injection pipette, the wound was stitched and mice were transferred back to their home cage, where 2-3 weeks were allowed for viral expression.

Surgery

In vivo two-photon imaging was performed as described previously (*11, 12, 43*). In brief, mice were initially anesthetized with Isoflurane (2% vol/vol in pure O_2). The scalp was partially removed and a custom-made plastic recording chamber with a central opening was attached to the skull using dental cement. The skull was thinned with a dental drill (Meisinger, Neuss, Germany) in a circle with a diameter of approximately 2 mm with the center on top of the hippocampal CA1 region (AP -2.75, ML 3.5) and the recording chamber was filled with artificial cerebrospinal fluid (ACSF; 125 mM NaCl, 4.5 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM glucose, pH 7.4, when bubbled with 95 % O_2 and 5 % CO_2), which had been heated to 37°C. The bone was carefully removed with a thin cannula to open a cranial window directly above the imaged region. The dura and cortical tissue covering the hippocampus were removed by suction. After this, multi-cell bolus loading was performed with the organic Ca^{2+}-indicator Cal-520 AM as previously described (*44, 45*). The indicator was loaded either 200µm below the

hippocampal surface. After the surgery was completed, the concentration of Isoflurane was reduced to 0.8-1.0% vol/vol for imaging.

Hippocampal slice preparation

Mice were anesthetized and decapitated. The brain was surgically removed and submerged in ice-cold slicing solution (24.7 mM glucose, 2.48 mM KCl, 65.47 mM NaCl, 25.98 mM $NaHCO_3$, 105 mM sucrose, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 1.25 mM NaH_2PO_4, and 1.7 mM ascorbic acid) with an osmolarity of 290-300 mOsm and a pH of 7.4, which was stabilized by bubbling with carbogen gas. Horizontal slices (300µm) were cut using a vibratome. These were allowed to recover at room temperature for at least one hour in a recovering solution containing 2 mM CaCl2, 12.5 mM glucose, 2.5 mM KCl, 2 mM MgCl2, 119 mM NaCl, 26 mM NaHCO3, 1.25 mM NaH2PO4, 2 mM thiourea (Sigma, St. louis, USA), 5 mM Na-ascorbate (Sigma), 3 mM Na-pyruvate (Sigma), and 1 mM glutathion monoethyl ester. The pH value was adjusted to 7.4 with HCl, and the osmolality was 290 mOsm. Before the experiment, the slices were transferred into the recording setup and superfused with heated (37°C) ACSF. For calcium imaging experiments, bolus loading of Cal-520 AM was performed as described above.

Two-photon imaging and data analysis

In vivo and in vitro two-photon Ca^{2+} or glutamate imaging was performed in a custom-made multi-photon recording setup based on an upright microscope (Olympus) as described previously (*11*). Excitation light was provided by a tunable Ti:sapphire laser at a wavelength of 920 nm (Coherent). A resonant galvo mirror scanner operating at 8 or 12 kHz (GSI) as well as a 40x 0.8 NA objective (Nikon) was used. Full frames were acquired at 40-120 Hz. Spontaneous Ca^{2+} -transients were recorded from the pyramidal layer of hippocampal CA1 in vivo and in vitro under baseline conditions, during the application of drugs or Aβ in the bath solution or by pressure injection from a pipette and after a washout period of 5-10 minutes. Glutamate imaging was performed in the stratum radiatum of CA1 hippocampal slices, synaptic glutamate transients were elicited by electrical stimulation of the Schaffer collaterals (100 µsec, 30-40V) and recorded in the stratum radiatum of CA1 hippocampal slices.

Offline image analysis was performed as described previously (*11, 12, 43, 46*). In brief, Ca^{2+}-traces were extracted from the imaging data using custom-written software based on LabVIEW. In Ca^{2+}-imaging experiments, neurons were visually identified and regions of interest (ROIs) were drawn around their somata. Astrocytes were excluded from the analysis due to their morphology and their high fluorescence levels. In glutamate imaging, a ROI was painted over the full imaging frame. The fluorescence of each ROI over time was extracted, low-pass filtered and normalized to the baseline (df/f). Fluorescence changes were accepted as neuronal calcium or extracellular glutamate transients if their shape was similar to that observed previously (*11, 12, 29*) and their peak amplitude was three times larger than the standard deviation of the noise band. In drug application experiments, only cells that were visible in the field of view during baseline, drug application and washout were analyzed. To estimate the effect of the baseline activity on the

Aβ-induced hyperactivity in Figs. 1I, 5D and S4C, the Aβ-induced hyperactivity (ΔHyper) for cells with a baseline activity of 0-10 events/min (in vivo) or 1-5 events/min (in vitro) was calculated as follows: ΔHyper = neuronal activity during Aβ application - neuronal activity at baseline. Cells were binned according to their respective activity under baseline conditions and means and SEM for each bin were plotted. Because the EC50 estimate (Fig. 5C) was based on the concentration in the local application pipette, it is reasonable to assume that because of dilution in the surrounding extracellular volume, the effective in vivo concentration of Aβ dimers would be considerably lower.

Intracellular electrophysiology

For electrophysiological recordings, hippocampal slices were prepared as described above, transferred to the recording setup and perfused with ACSF containing 10 μM bicuculline (Enzo, U.S.A) to block $GABA_A$ receptor-mediated synaptic transmission. In NMDAR-epsc experiments, AMPA receptors were additionally blocked by 10 μM GYKI53655 (Tocris). To minimize recurrent excitation, the Schaffer collaterals between CA3 and CA1 were cut using a razor blade. An upright light microscope was used to identify CA1 pyramidal neurons, which were carefully approached by a glass pipette with a tip resistance of approximately 7MΩ connected to an EPC 9/2 patch-clamp amplifier (HEKA, Germany). The patch pipette was filled with an internal solution containing 122.5 mM cesium gluconate, 5 mM HEPES, 10 mM cesium BAPTA, 6 mM $MgCl_2$, and 10 mM phosphocreatine at a pH of 7.2, adjusted with gluconic acid (*30*). In NMDAR-epsc experiments, the membrane potential was held at +40 mV, without correcting for liquid junction potentials. In PPF experiments, the cell was held at resting membrane potential. A second glass electrode (5-7 MΩ) filled with 2 M NaCl was used to elicit synaptic responses in 5 second intervals (NMDAR-epsc) or two pulses with inter-pulse intervals of 30-500 msec (PPF experiments). The pulse amplitude was adjusted to approximately twice the value that gave the smallest synaptic response, typically around 10 V and the pulse-length was 100 μsec. After recording baseline data, Aβ (500 nM) or TBOA (5 μM) were applied in the bath solution and the recording was repeated after several minutes of superfusion of the drug. Data was collected with PULSE software (HEKA) at 10 kHz, Bessel-filtered at 2.9 kHz and stored for offline analysis.

The electrophysiological data was analyzed using Igor Pro software (WaveMetrics, U.S.A). Statistical analysis was performed with Matlab. The full width at half maximum (FWHM) of NMDAR-epscs was measured manually. The decay time was fitted as a bi-exponential fit with a fast component τ_1 and a slow component τ_2 and $\tau_{weighted}$ was calculated as previously described (*47, 48*) $\tau_w = (A_1\tau_1 + A_2\tau_2)/(A_1 + A_2)$ A_1 and A_2 were the amplitudes of the first and second decay components, respectively. The PPR was calculated as $PPR = A_{P2}/A_{P1}$. A_{P1} and A_{P2} were the amplitude of the first and the second EPSC, respectively.

Immunostaining and confocal imaging of hippocampal slices

Hippocampal slices were fixed in 4% PFA for 30 minutes and washed in PBS. Neurotrace staining was performed as previously described (*49*). Slices were mounted in a confocal microscope (Olympus) and Z-stacks were made.

Human brain tissue

Specimens were obtained from the Massachusetts ADRC Neuropathology core at the Massachusetts General Hospital in accordance with Partners Institutional Review (Walsh BWH2011). Tissue from a 67 year old male AD patient was used to prepare an aqueous brain extract and cortical tissue from a 69 year old female AD patient was used to isolate amyloid plaques. Both patients met clinical (CERAD C) and pathological criteria for AD (the male patient had Braak VI, and the female Braak V).

Preparation of extracts

Ten grams of cortical gray matter was Dounce-homogenized in 5 volumes of ice-cold artificial cerebrospinal fluid base buffer (aCSF-B) (124 mM NaCl, 2.8 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, pH 7.4) supplemented with protease inhibitors (5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethyleneglycoltetraacetic acid, 5 μg/mL leupeptin, 5 μg/mL aprotinin, 2 μg/mL pepstatin, 120 μg/mL pefabloc and 5 mM NaF) (Mengel et al 2018, Wang et al 2017). The resulting homogenate was centrifuged at 200,000 g for 110 minutes and 4°C in a SW41 Ti rotor (Beckman Coulter, Fullerton, CA) and the upper 80% of the supernatant was removed and dialyzed against fresh aCSF-B, with 3 buffer changes over a 72 hour period. Dialysates were removed to clean tubes, aliquoted and stored at -80 °C until required.

Immunoprecipitation/Immunoblot analysis of amyloid β-protein

To minimize non-specific binding to protein A, brain extracts were pre-cleared with protein A sepharose (PAS) beads. Half milliliter aliquots of extracts were incubated with 15 μl PAS beads for 1 hour at 4°C with gentle shaking. PAS beads were removed by centrifugation (4,000 g for 5 minutes), the supernatant removed and incubated with 10 μl of AW7 and 15 μl PAS beads overnight at 4°C with gentle shaking. Aβ-antibody-PAS complexes were collected by centrifugation and washed as previously described (Hong et al 2018). Sample treated with AW7 is referred to as immunodepleted (ID) and sample treated with pre-immune serum as mock immunodepleted (mock). The immunoprecipitated (IP'd) Aβ was eluted by boiling in 15 μl of 2× sample buffer (50 mM Tris, 2% w/v SDS, 12% v/v glycerol with 0.01% phenol red) and electrophoresed on hand poured, 15 well 16% polyacrylamide tris-tricine gels. Proteins were transferred onto 0.2 μM nitrocellulose at 400 mA and 4°C for 2 hours. Blots were microwaved in PBS and Aβ detected using the anti-Aβ40 and anti-Aβ42 antibodies, 2G3 and 21F12, and bands visualized using a Li-COR Odyssey infrared imaging system (Li-COR, Lincoln, NE).

MSD Aβx-42 immunoassay

This assay was performed using the Meso Scale Discovery (MSD) platform and reagents from Meso Scale (Rockville, MD) as described previously (Mably et al 2015). m266 (3 μg/ml) was used for capture and biotinylated 21F12 (1 μg/ml) for detection. Samples, standards and blanks were loaded in triplicate and analyzed as described previously (Hong et al 2018). Since GuHCl effectively disaggregates high molecular weight Aβ species, samples were analyzed both with and without incubation in 5 M GuHCl. Analysis of samples in the absence of GuHCl allows the measurement of native Aβ monomer, whereas, analysis of samples treated with GuHCl allows detection of disassembled aggregates with the Aβx-42 assay. To dissociate aggregates, 20 μl of extract was incubated overnight with 50 μl of 7 M GuHCl at 4°C. Thereafter, samples were diluted 1:10 with assay diluent, so that the final GuHCl concentration was 0.5 M. Aβ standards were prepared in tris-buffered saline, pH 7.4 containing 0.5 M GuHCl, 0.05% Tween 20 and 1% Blocker A so that both standards and samples contained the same final concentration of GuHCl.

Amyloid plaque isolation and Aβ purification

Amyloid plaques were isolated as described previously (*36*). Approximately 50 grams of cortical gray matter was dissected from a whole hemi-brain and incubated with 5 volumes of 2% (w/v) SDS in 50 mM Tris-HCl, pH 7.6, containing 0.1 M β-mercaptoehanol at room temperature for 2 hours. The suspension was then Dounce-homogenized and incubated in a boiling water bath for 15 minutes. Samples were allowed to cool to room temperature and then passed through a 112 μm nylon mesh. The flow-through was then centrifuged at 300 g for 30 minutes and the resulting pellet was washed 3 times with 0.1% SDS in 150 mM NaCl. The final pellet was Dounce-homogenized in 0.1% SDS in 150 mM NaCl and passed through a 38 μm nylon mesh. The flow-through was then applied to a sucrose step gradient composed of layers of 1.2 M, 1.4 M, 1.6 M, and 1.8 M sucrose in 1% SDS, 50 mM Tris-HCl, pH 7.6 and centrifuged at 72,000 g for 1 hour at 26°C in a SW28 rotor (Beckman Coulter, Fullerton, CA). The 1.6 M sucrose interface was collected and diluted 1:5 with 0.1% SDS in 150 mM NaCl and plaques were pelleted by centrifugation at 300 g for 30 minutes in a bench top centrifuge. To remove SDS, plaques were resuspended in 1 mL of MilliQ water and centrifuged at 10,000 g. The supernatant was removed, and the pellet washed again with 1 mL of MilliQ water. Finally, the pelleted plaques were re-suspended in 100 μL of MilliQ water and a portion (10 μL) was stained with 0.2% Congo red and visualized using polarized microscopy.

The remaining plaque suspension was freeze-dried and the lyophilizate dissolved in 88% formic acid and incubated with gentle agitation for 12-14 hours. The solution was then centrifuged at 12,000 g for 15 minutes. The upper 90% of supernatant was loaded on to a Superdex 75 10/300 GL SEC column eluted with 50 mM ammonium bicarbonate, pH 8.5 at a flow rate of 0.5 mL/minute. Fractions (0.5 mL) were collected and a small portion (5 μL) of each was analyzed by WB. Fractions containing ~7 kDa Aβ were pooled to yield a dimer stock, and fractions containing ~4 kDa Aβ were pooled to produce a monomer stock. The concentration of Aβ in the monomer and dimer stocks was estimated and concentration-matched samples used for bioactivity studies.

Application of Aβ, drugs and antibodies

Synthetic Aβ(1-40)S26C dimers, to which we refer to as $[A\beta S26C]_2$ were purchased from JPT. In these dimers, the Serine in position 26 was replaced by a Cysteine that was used for dimerization via a disulfide-bond. The molar weight of the dimer was used for the calculation of the molar concentration of $[A\beta S26C]_2$. The samples were dissolved in DMSO and frozen (-20°C). Human Aβ samples were prepared as described above and stored at -80°C. Immediately before the experiments, the aliquots were thawed and diluted in fresh ACSF. Drugs were stored in aliquots and diluted in fresh ACSF to the following concentrations: DL-TBOA (Tocris), 10-250µM; Dihydrokainic acid (DHK, Tocris), 250µM. EAAT-2/GLT-1 polyclonal antibodies were purchased from Novus Biologicals (NBP1-20136) and diluted 1:100 in ACSF (*47*). Equivalent concentrations of IgG- antibodies (Thermo Fischer Scientific) were used as controls. The substanmces were filled into a glass patch pipette (tip resistance 2-3 MΩ), which was carefully lowered into the brain or the hippocampal slice and maneuvered into the center of the field of view. After a baseline recording with the pipette in place, the drug was the carefully pressure applied (100 mBar, 30 sec) into the brain while monitoring the neuronal activity. After a washout period of 5-10 minutes, another recording was made while the pipette was left in place. In bath application experiments, the following substances were added to the ACSF: Aβ1-40 S26C dimer (JPT, 500µM), Bicuculline (Enzo, 10-100µM), CNQX (Tocris, 50µM), D-APV (Abcam, 50µM) DL-TBOA (Tocris, 5-250µM), L-glutamic acid (Sigma, 40-80 µM), Gyki53655 (Tocris, 10 µM) or TTX (Abcam, 1µM).

Live-cell imaging of human induced pluripotent stem cells (iPSCs) derived neurons (iNs)

Human iPSCs-derived neurons were induced and cultured as described previously (*37*). Briefly, the effects of Aβ on neuritic integrity were tested at iN day 21. Neural Track was used on phase contrast images to automatically define neurite processes and cell bodies. Total neurite length and branch point number were quantified and normalized to the average value measured during the 6 hour period prior to sample addition (*36*).

Statistical analysis

All statistical tests were performed in Matlab. Wilcoxon signed-rank test, Wilcoxon rank sum test, Kolmogorov-Smirnov test, student's t-test, Kruskal Wallis test and ANOVA were used as indicated and multiple comparison testing was employed where appropriate. Linear regression was performed in Matlab and neurons with similar baseline activities were binned. p-values of < 0.05 were considered to be statistically significant.

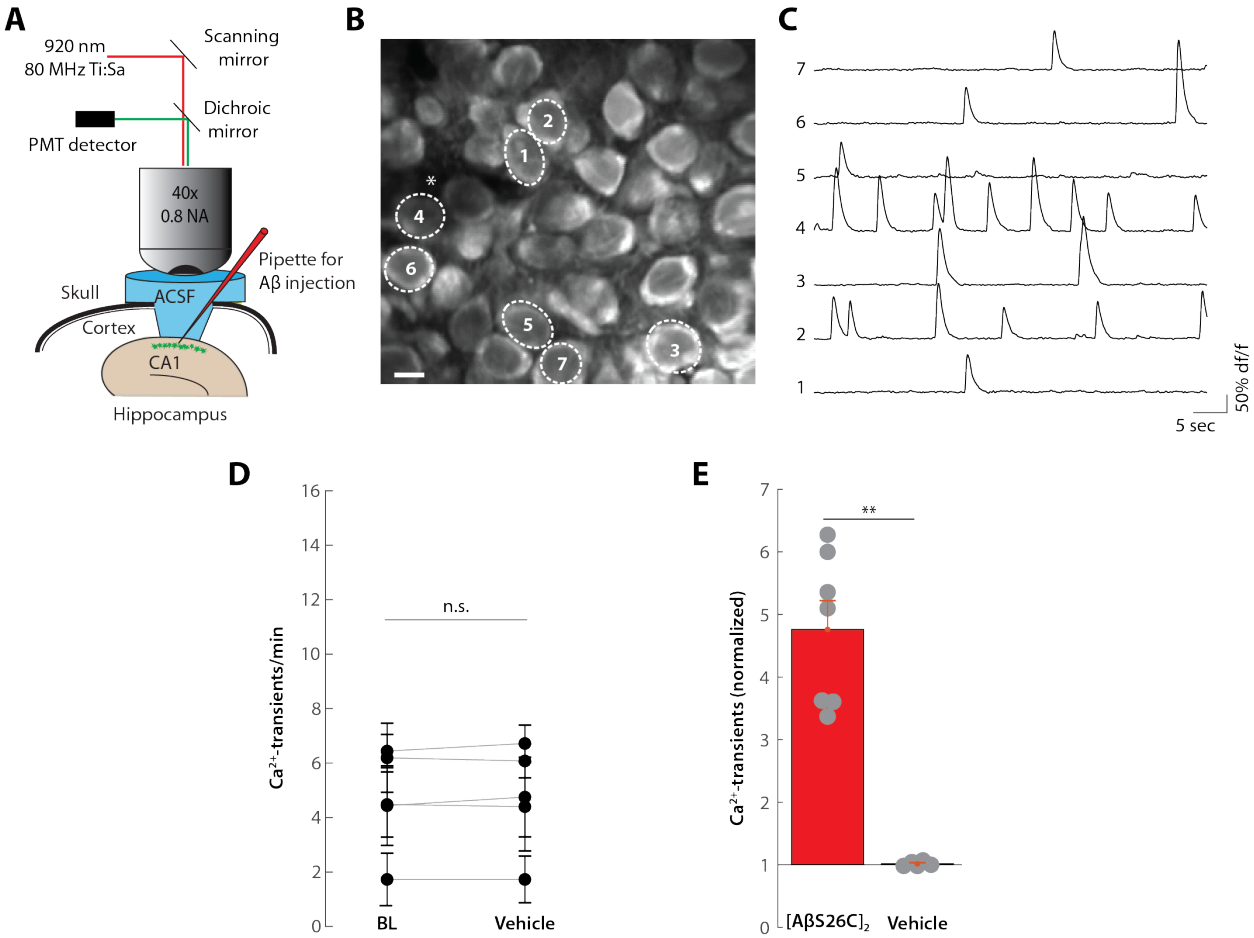

Fig. S1. Two-photon Ca^{2+}-imaging of hippocampal CA 1 neurons in vivo

(**A**) Experimental arrangement for *in vivo* two-photon imaging. (**B**) Representative two-photon image of the hippocampal CA1 pyramidal layer of a wildtype mouse after multi-cell bolus loading of the fluorescent Ca^{2+}-indicator dye Cal-520. The asterisk marks the shadow of the dye application pipette. Scale bar: 5µm. (**C**) Ca^{2+}-traces extracted from the seven cells cycled in (B). (**D**) Summary data for experiments, in which a vehicle solution (ACSF + 5% DMSO) was applied. Data points represents the mean number of Ca^{2+}- transients for all recorded cells in one mouse for baseline (left) and vehicle application (right) conditions. (**E**) Bar graph showing the normalized number of Ca^{2+}-transients for $[A\beta S26C]_2$ (left, n = 7) and vehicle (right, n = 5) application. Each point represents the mean number of Ca^{2+}-transients in one mouse, normalized to baseline. Error bars show SEM. Wilcoxon signed rank test (D, E) or Wilcoxon rank sum test (F), **P<0.005, *P<0.05; n.s. not significant.

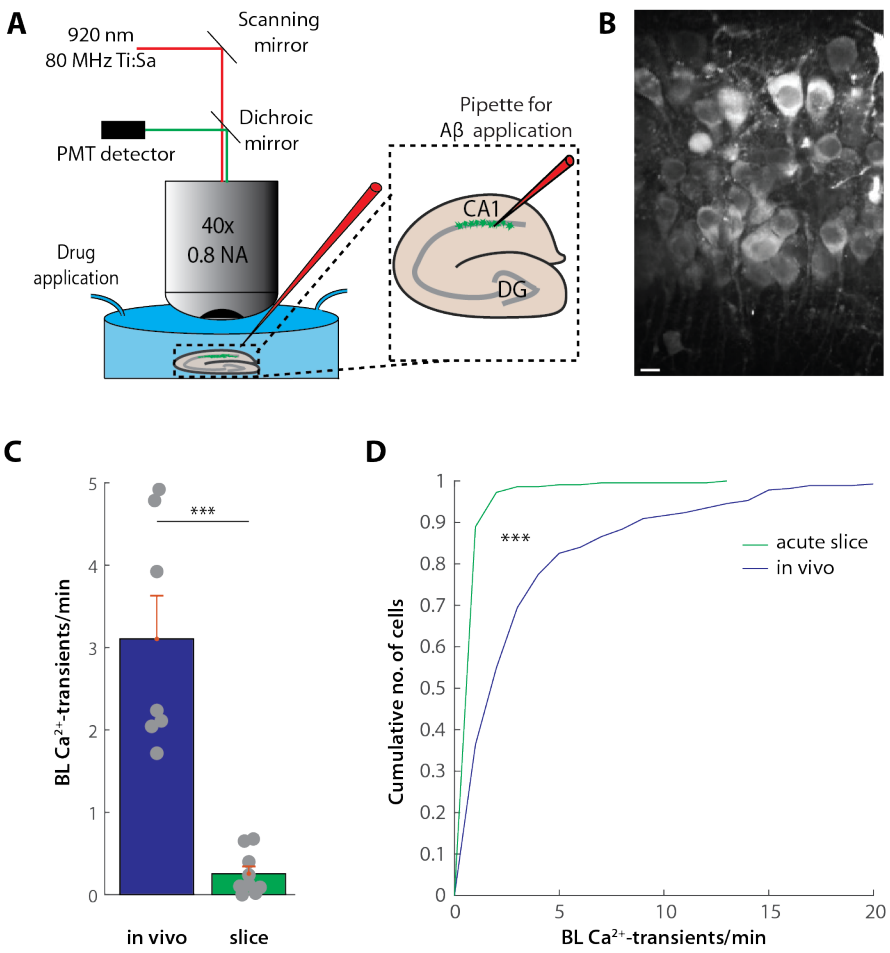

Fig. S2. Two-photon Ca^{2+}-imaging of hippocampal CA1 neurons in vitro

(**A**) Experimental arrangement for two-photon imaging in an *in vitro* hippocampal slice. (**B**) Representative two-photon image of the hippocampal CA1 pyramidal layer of an acute hippocampal slice after staining with the fluorescent Ca^{2+}-indicator Cal-520. Scale bar: 5 µm. (**C**) Bar graph of the average number of Ca^{2+}-transients in vivo (left, n = 7 mice) and in slice preparation (right, n = 9 slices). Each point represents the mean obtained in a mouse or a slice, respectively. (**D**) Cumulative distribution of neuronal baseline activities in vivo (blue, n = 248 cells) and in acute hippocampal slices in vitro (green, n = 217). Error bars show SEM. Wilcoxon rank sum test (C) or Kolmogorov-Smirnov test (D). ***P<0.001.

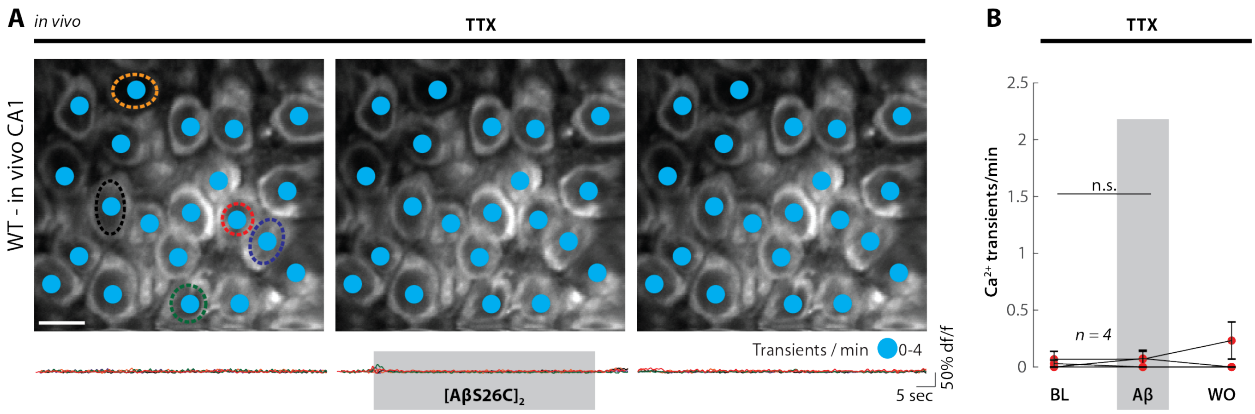

Fig. S3. The application of TTX prevents the hyperactivity-inducing effect of [AβS26C]$_2$ in vivo

(**A**) Top: representative two-photon images of the hippocampal CA1 region of a wild-type mouse *in vivo* during superfusion of TTX (1µM) under baseline conditions (left), during the application of [AβS26C]$_2$ (500 nM, middle) and after washout (right). The colored dots on the neurons represent the number of Ca^{2+}-transients per minute. Bottom: Overlaid Ca^{2+}-traces of the five neurons circled in the top panel. The color of the circle corresponds to the color of the respective Ca^{2+}-trace. The grey shaded area represents the time of [AβS26C]$_2$ application. Scale bar: 5 µm. (**B**) Summary data of the experiment in (A). Each dot represents the mean under baseline (BL), [AβS26C]$_2$ application and washout (WO) conditions. Error bars show SEM. Wilcoxon rank sum test; n.s., not significant.

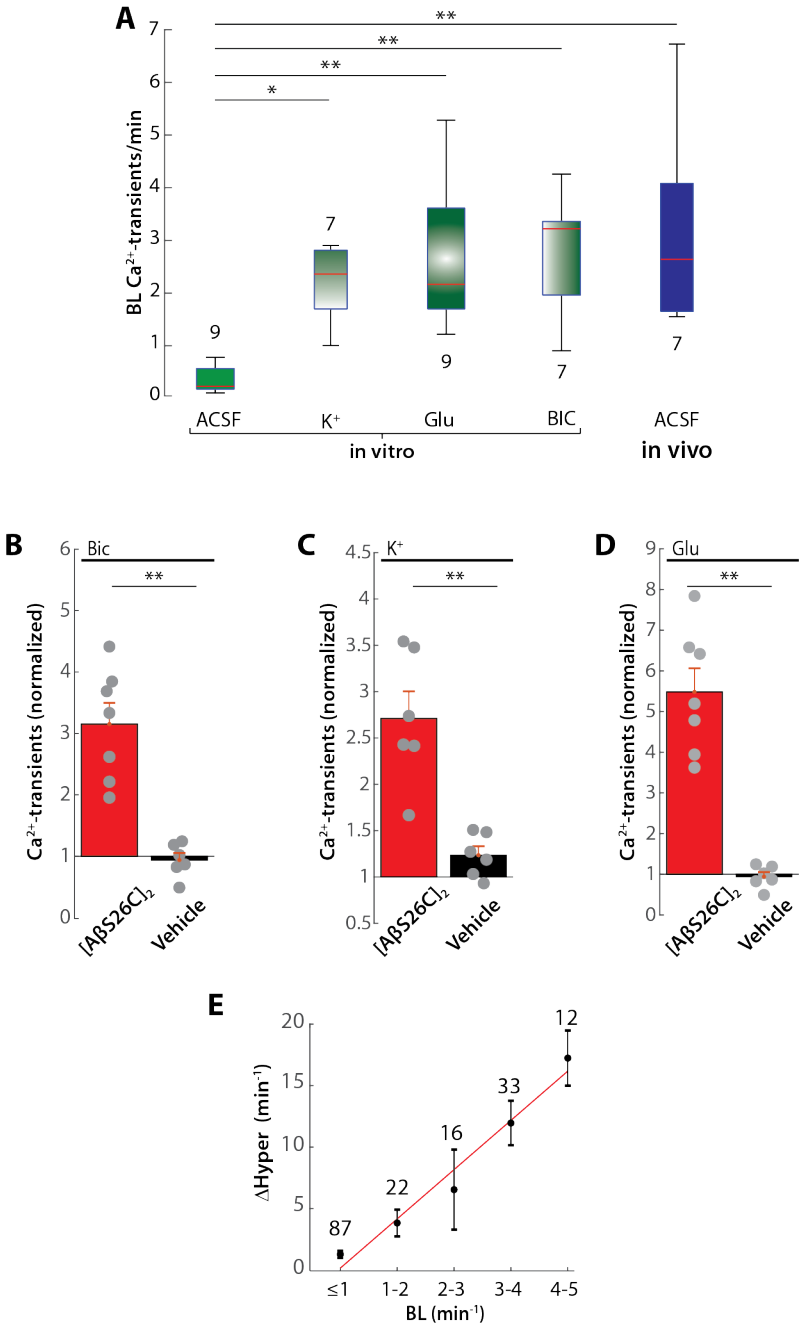

Fig. S4. [AβS26C]$_2$-dependent hyperactivation in hippocampal slices with enhanced baseline activity

(**A**) Boxplot of the average baseline activities under different conditions. The N-numbers for each experiment are indicated next to the respective boxes. (**B**) Normalized changes of activity for applications of [AβS26C]$_2$ (left, n = 7 slices) or vehicle (right, n = 7) in hippocampal slices in which the neuronal baseline activity had been enhanced by the superfusion of bicuculline in ACSF containing elevated K^+-concentrations (5.5 - 6.5 mM). Each dot represents the mean of values obtained from one slice. (**C**) Same as (B) for slices, in which baseline activity had been induced

by the superfusion of ACSF containing elevated levels of K^+ (7.5-8.5 mM) for $[A\beta S26C]_2$ (N = 6), or vehicle (N = 6) application. (**D**) Same as (B) for slices, in which baseline activity had been elevated by the superfusion of glutamate (40-60 µM, $[A\beta S26C]_2$ n = 7, vehicle n = 6). (**E** Plot of baseline activity (BL) vs. $[A\beta S26C]_2$-dependent relative increase in activity in vitro (ΔHyper) for individual neurons. The numbers for neurons for each bin of BL activity is indicated in the graph. Red line: linear fit. Error bars show SEM. Kruskal Wallis test with Dunn-Sidak post-hoc comparison (A) or Wilcoxon rank sum test (B-D). **P<0.005, *P<0.05.

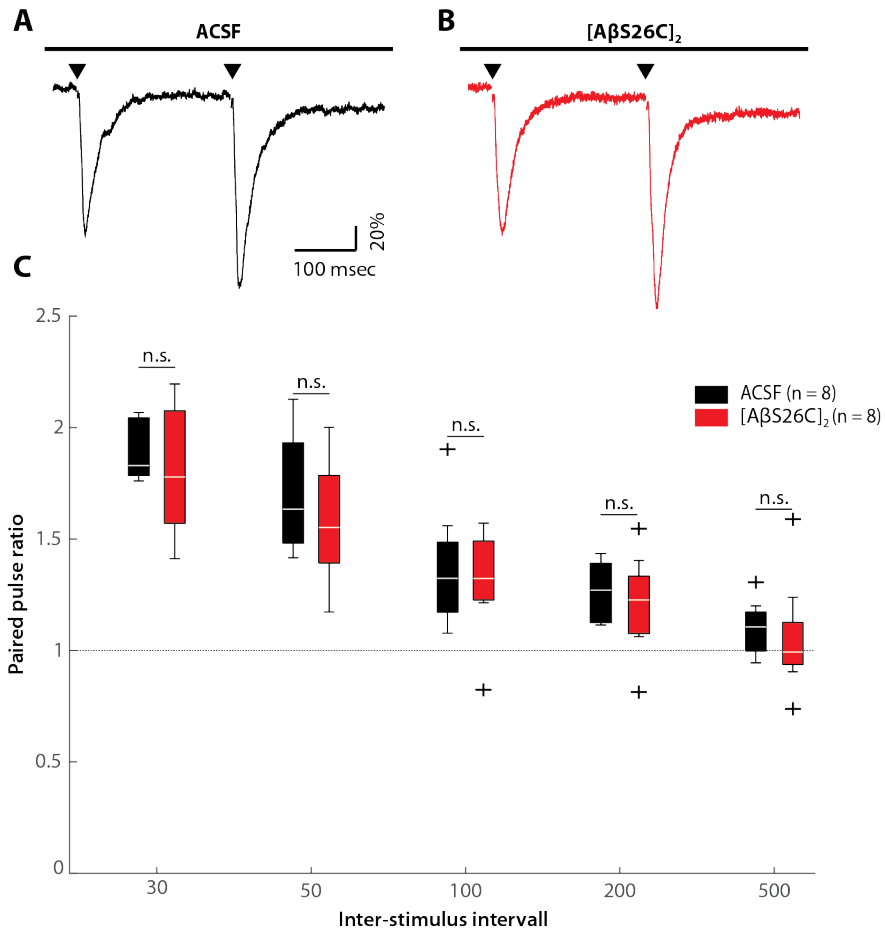

Fig. S5. Paired-pulse facilitation is not affected by the application [AβS26C]$_2$
(**A and B**) Normalized excitatory post-synaptic currents (epscs) recorded from voltage-clamped (+40 mV) in vitro CA1 pyramidal neurons during paired-pulse stimulation (interval 20 ms) of the Schaffer collateral pathway (arrows). Superfusion with artificial cerebrospinal fluid (ACSF) (**A**) and 1 µM [AβS26C]$_2$ (**B**). (**C**) Paired-pulse ratio (PPR) for inter-pulse intervals ranging from 30 to 500 msec for neurons in slices superfused with ACSF (black, n = 8) or [AβS26C]$_2$ (red, n = 8). Wilcoxon rank sum test. n.s. not significant.

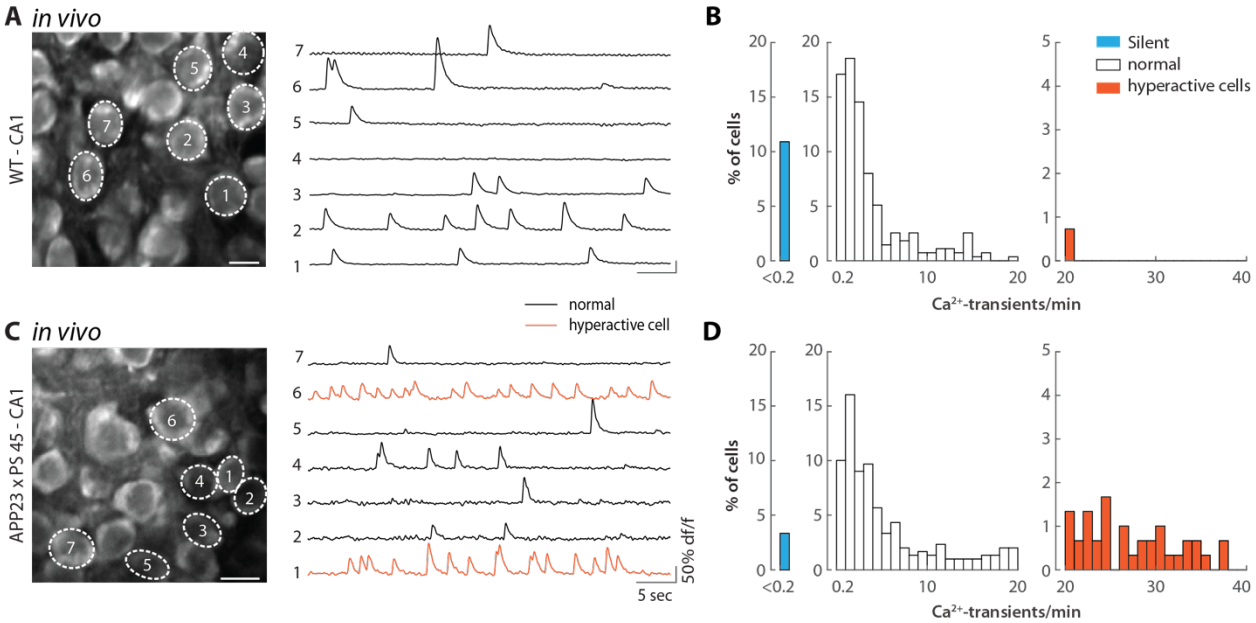

Fig. S6. Neuronal hyperactivity in the APP23 x PS45 mouse model of Aβ-amyloidosis

(**A**) Left: Representative two-photon image of the hippocampal CA1 region of a wild-type mouse in vivo. Right: Ca^{2+}-transients from the seven cycled neurons on the right side under baseline conditions. (**B**) Activity histogram of all neurons in wild type mice (n = 275). Silent cells are indicated in blue, normal cells in white and hyperactive cells in orange. (**C**) Same as (A) for an APP23 x PS45 mouse. Traces from hyperactive neurons are indicated in red. (**D**) Same as (B) for all neurons in APP23 x PS45 mice (n = 299). Scale bars: 5 µm.

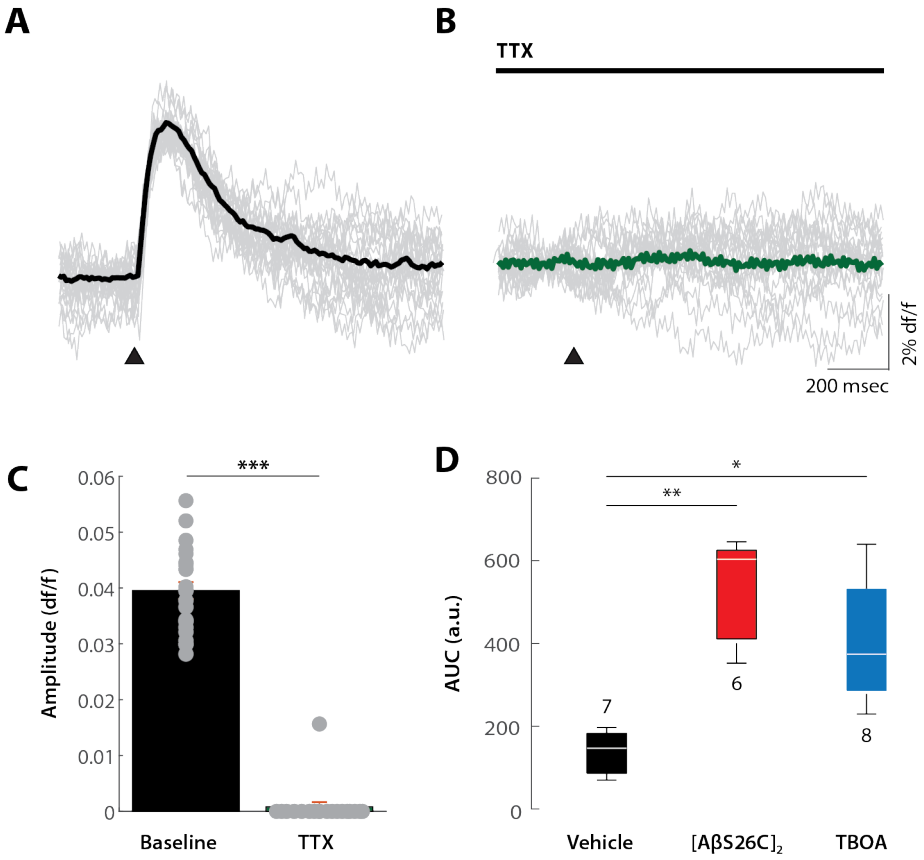

Fig. S7. Synaptic stimulation-evoked glutamate transients in the hippocampal slices in vitro (**A**) Individual glutamate transients (grey) and mean (black) in the stratum radiatum of the hippocampal CA1 region after single-shock electrical stimulation of the Schaffer collateral pathway in control conditions. Experimental arrangement similar to that shown in **Fig. 3D, left.** (**B**) Block of glutamate transients by TTX (5 μM). (**C**) Bar graph of the amplitudes of glutamate transients under baseline conditions (left) and in the presence of TTX (right). (**D**) Box plot of the area under the curve (AUC) of synaptically induced glutamate transient after the applications of ACSF (left), $[A\beta S26C]_2$ (500 nM, middle) and TBOA (10 μM, right). Error bars show SEM. n-numbers are indicated next to the boxes. One sample student's t-test (C) or Kruskal wallis test with Dunn-Sidak post-hoc comparison (D). ***P<0.001, *P<0.05; n.s. not significant.

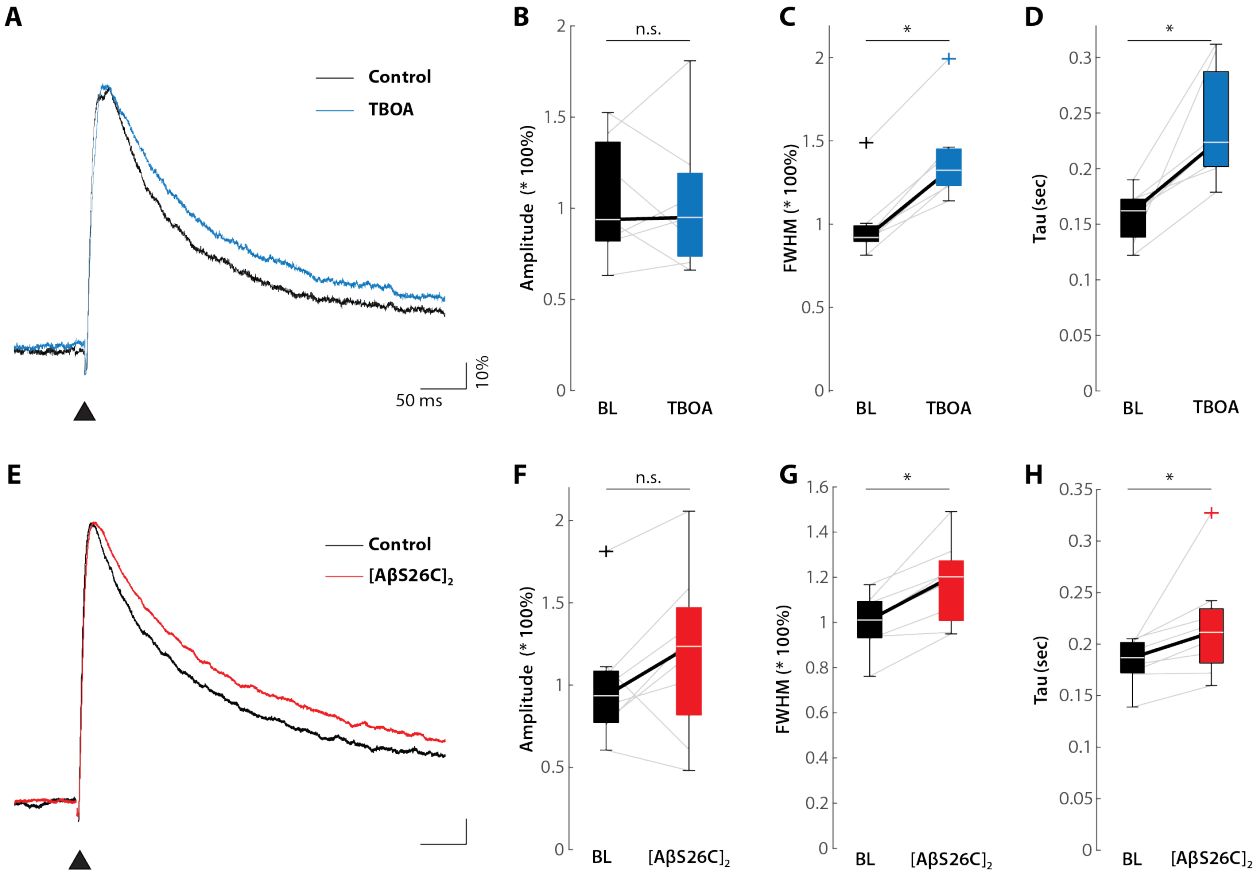

Fig. S8. Alteration of NMDA-epscs by [AβS26C]$_2$ and TBOA

(**A**) NMDA-epscs recorded in a voltage-clamped CA1 pyramidal neuron (+40 mV) in a hippocampal slice. Baseline (black), TBOA superfusion (10 µM, blue). (**B-D**) Bar graph of the amplitudes (**B**), the normalized full-width at half maximum (FWHM, **C**) or the decay time τ (**D**) of NMDA-epscs under baseline conditions (BL, left) and after the application of TBOA (right). Each grey line corresponds to one cell (n = 7), the black line indicates the median of all cells. (**E**) Same arrangement as in **A** but application of [AβS26C]$_2$. Baseline (black), [AβS26C]$_2$ superfusion (1 µM, red). (**F - H**) Bar graph of the amplitudes (**F**), the normalized full width at half maximum (FWHM, **G**) or the decay time τ (**H**) of the synaptically evoked NMDA-receptor currents under baseline conditions (BL, left) and after the application of [AβS26C]$_2$ (right). Each grey line corresponds to one cell (n = 8), the black line indicates the median of all cells. Wilcoxon signed rank test. *P<0.05; n.s. not significant.

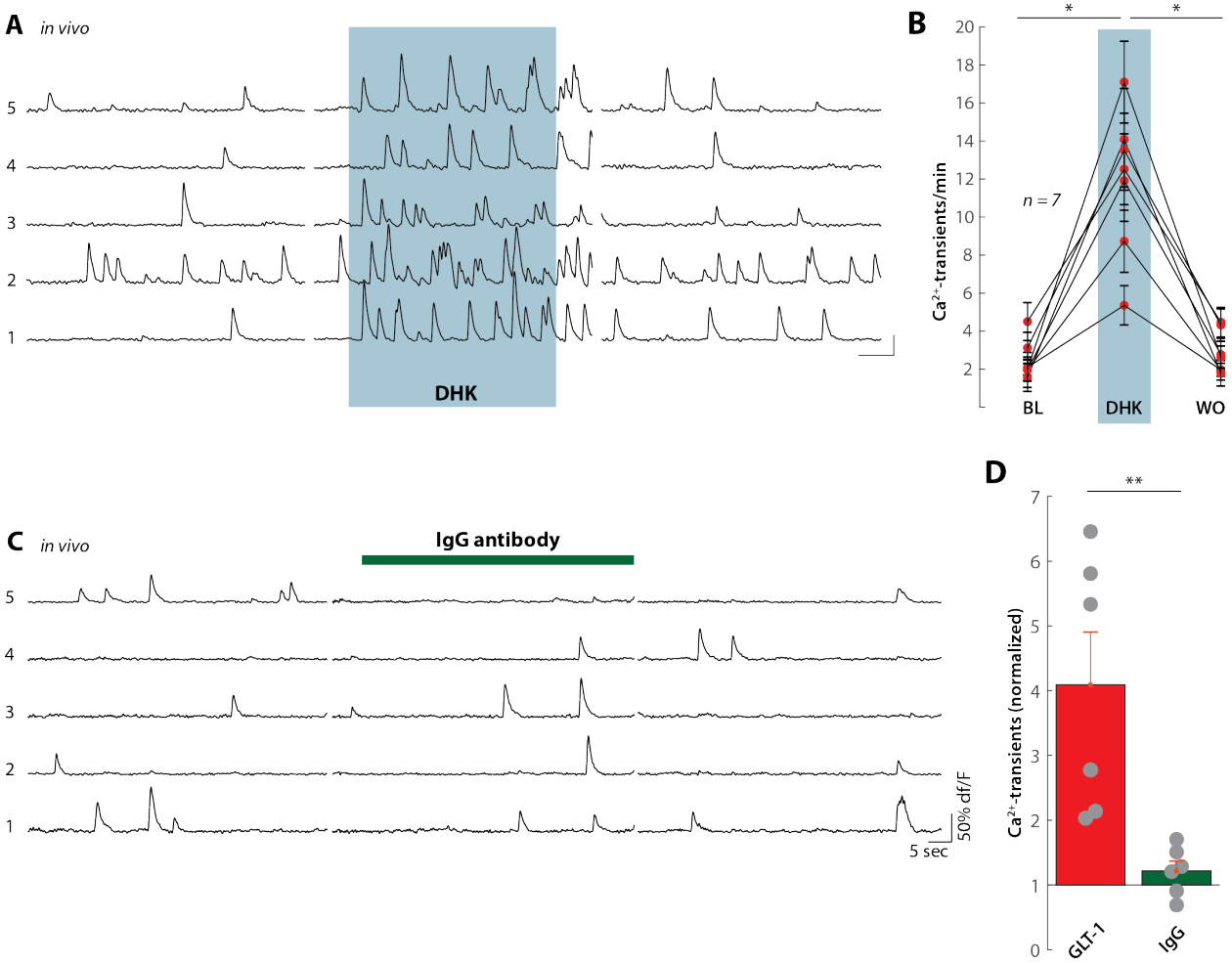

Fig. S9. GLT-1 -dependent block of glutamate reuptake

(**A**) Ca^{2+}-traces from in vivo two-photon imaging experiments for five representative hippocampal CA1 neurons in a wild-type mouse under baseline conditions (left), during the application of DHK (250 µM, middle) and after washout (right). The blue shaded area represents the time of DHK application. (**B**) Summary data for the experiment in (A). (**C**) Same arrangement as in **A** but application of IgG antibodies as controls. (**D**) Bar graph showing the normalized number of Ca^{2+}-transients for the application of the antibody against GLT-1 (left, n = 6 mice) or the antibody against IgG (right, n = 6). Each point represents the mean number of Ca^{2+}-transients in one mouse, normalized to baseline. Error bars show SEM. Wilcoxon signed rank test (B) or Wilcoxon rank sum test (D), **P<0.005, *P<0.05.

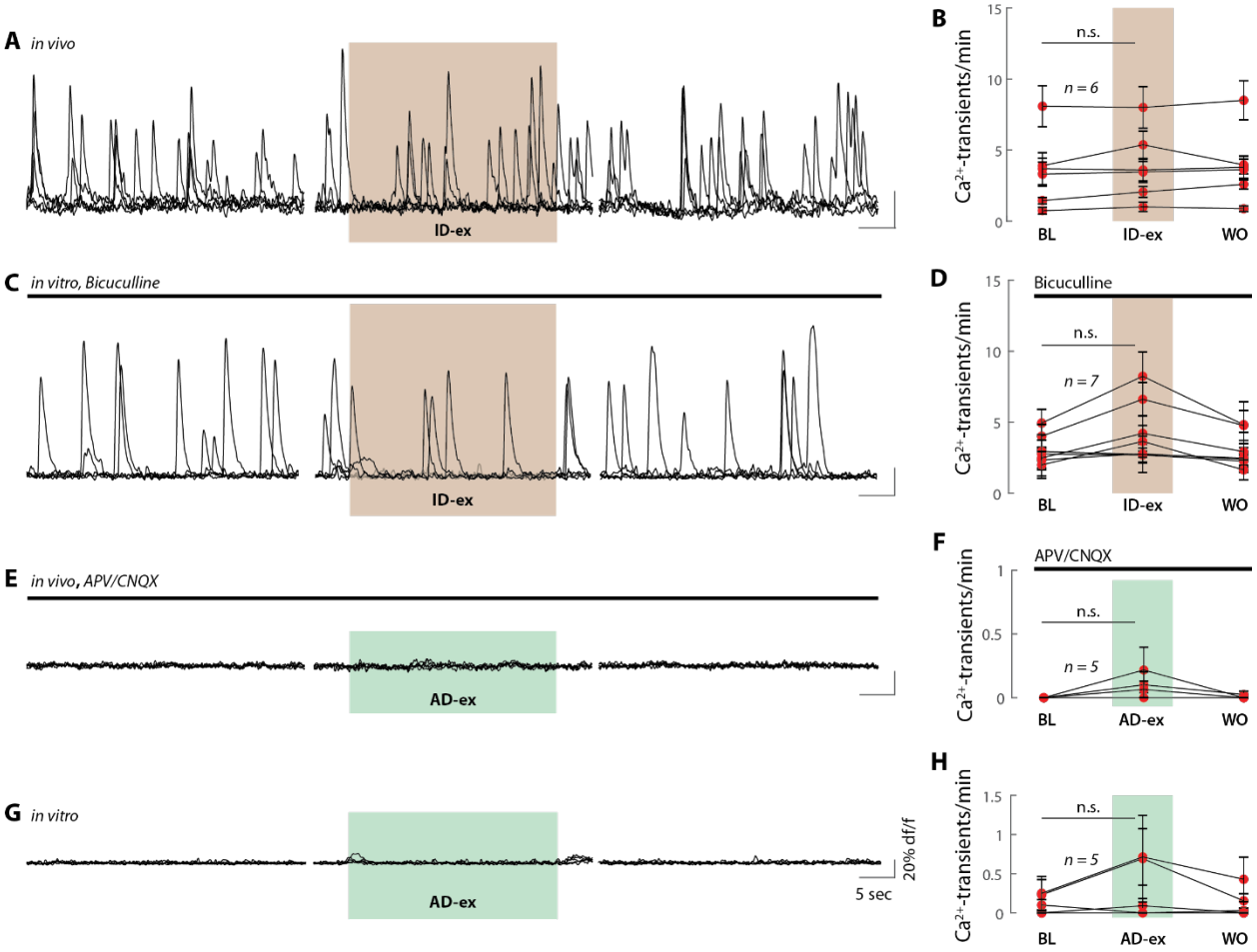

Fig. S10. No hyperactivation through Aβ-free immunodepleted (ID) brain extract

(**A**) Overlaid Ca^{2+}-traces from five representative neurons recorded *in vivo* under baseline (left), ID extract application (middle) and washout conditions (right). The brown shaded area corresponds to the time of ID extract application. (**B**) Summary data for the experiment in (**A**). Each dot indicates the mean number of Ca^{2+}-transients per minute for all neurons in one mouse under baseline (BL), ID extract application and washout (WO) conditions. Results from N = 6 animals. (**C**) Overlaid Ca^{2+}-traces from neurons recorded in an acute hippocampal slice, in which the neuronal baseline activity had been elevated by the superfusion of bicuculline under baseline conditions (left), ID extract application (middle) and washout conditions (right). (**D**) Summary data for the experiment in (C), results from n = 7 slices. (**E**) Overlaid Ca^{2+}-traces from neurons recorded in vivo during the superfusion of APV and CNQX under baseline conditions (left), AD extract application (middle) and washout conditions (right). (**F**) Summary data for the experiment in (E), results from n = 5 animals. (**G**) Overlaid Ca^{2+}-traces from neurons recorded in untreated hippocampal slices under baseline conditions (left), AD extract application (middle) and washout conditions (right). (**H**) Summary data for the experiment in (**G**), results from n = 5 animals. Error bars show SEM. Wilcoxon signed rank test. n.s. not significant.

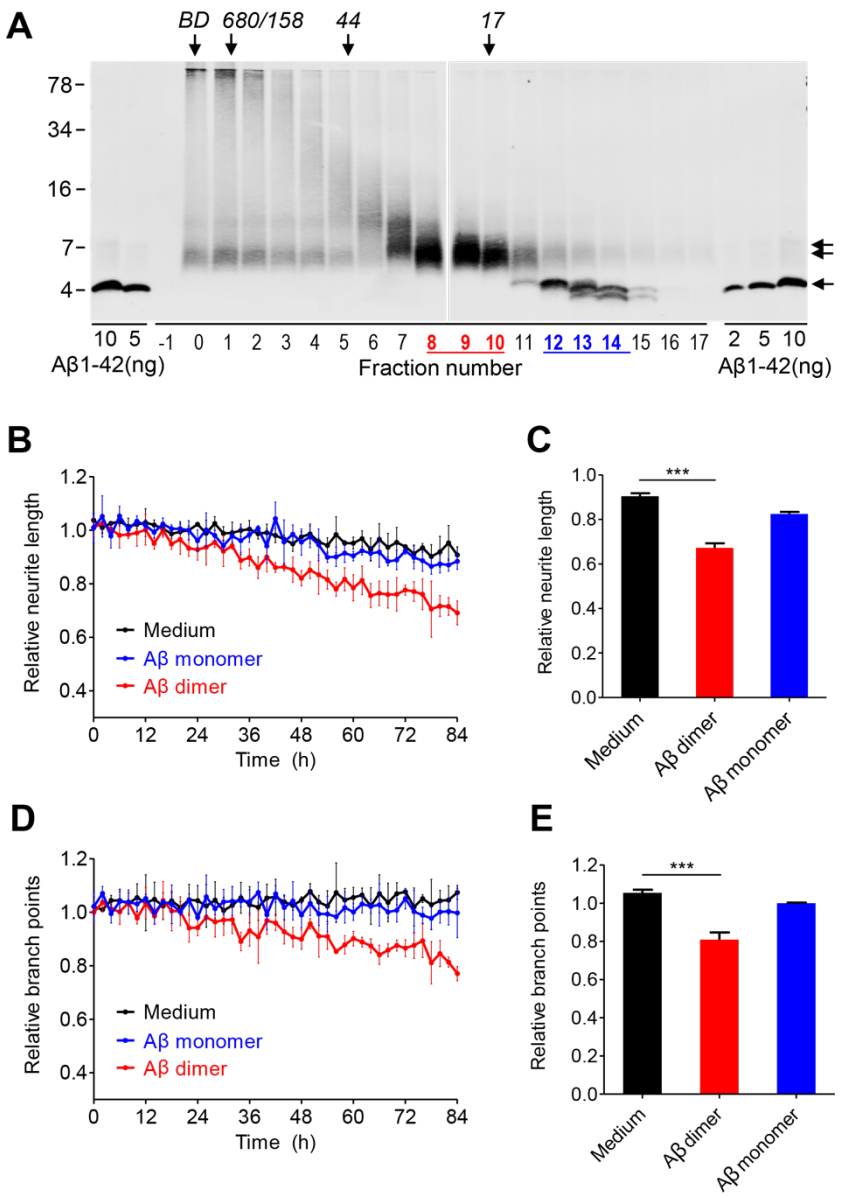

Fig. S11. Purification and characterization of Aβ dimer and monomer from AD amyloid plaques.

(**A**) Amyloid plaques were isolated from an end-stage AD brain, and dissolved in formic acid, chromatographed on SEC and used for WB with 2G3 and 21F12. The elution of Blue dextran (BD) and globular protein standards is indicated by downward point arrows and SDS-PAGE molecular weight standards are on the left. (**B-E**) SEC-isolated Aβ dimer (pool of fractions 8-10) and monomer (pool of fractions 12-14) were used for bioactivity assay. was used to estimate the Aβ monomer and Aβ dimer concentrations and approximately equal amounts were applied to iNs. Time-course plots of relative neurite length (**B**) and branch points (**D**) show that plaque-derived Aβ dimer (red) causes neurito-toxicity, whereas plaque-derived Aβ monomer (blue) does not. The values shown in Error bars are the average of triplicate wells for each treatment ± SD. Plots of normalized neurite length (**C**) and branch points (**E**) are derived from the last 6 h of the traces

shown in (B) and (D. The medium vehicle control is shown in black. Error bars show SEM. One-way ANOVA, ***P < 0.001.

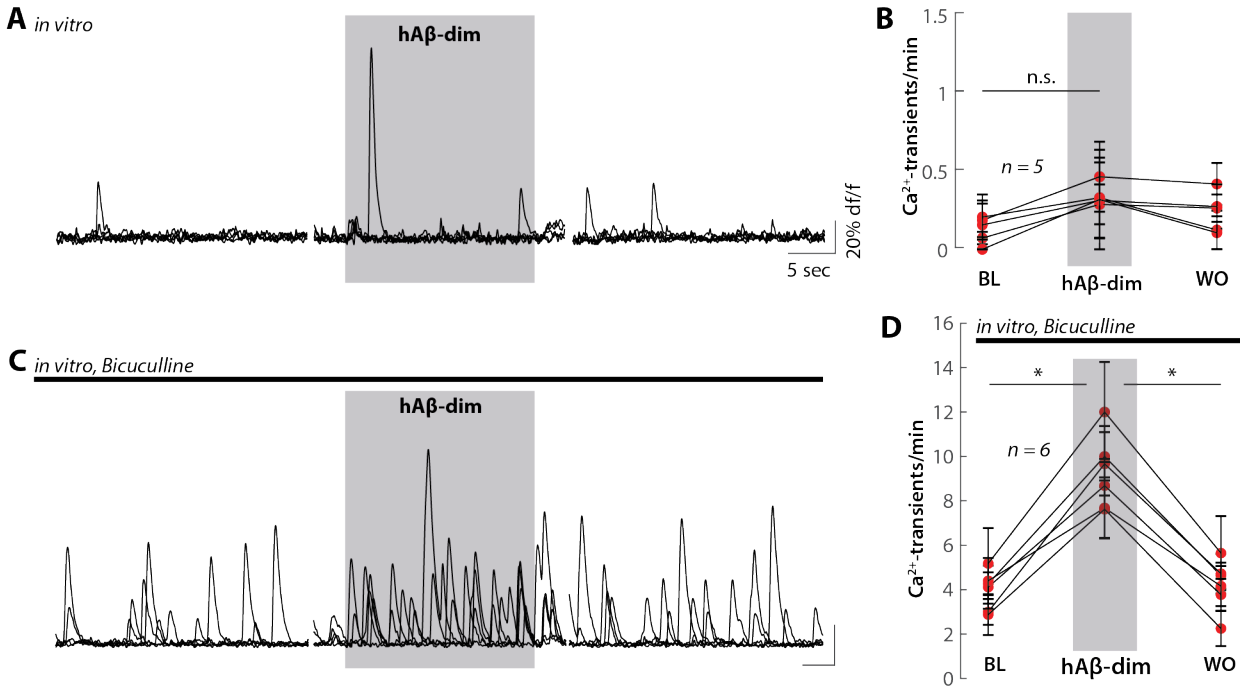

Fig. S12. Roles of human Aβ dimers in hippocampal slices in vitro

(**A**) Overlaid Ca^{2+}-traces from five representative neurons recorded in a naïve hippocampal slice under baseline (left), human Aβ dimer application (hAβ-dim, middle) and washout conditions (right). The grey shaded are corresponds to the time of hAβ-dim application. (**B**) Summary data for the experiment in (A). Each dot represents the mean number of Ca^{2+}-transients per minute for all neurons in one slice under baseline (BL), hAβ-dim application and washout (WO) conditions. (**C**) Overlaid Ca^{2+}-traces from five representative neurons recorded in a hippocampal slice in which neuronal baseline activity had been induced by the superfusion of bicuculline under baseline (left), hAβ-dim application (middle) and washout conditions (right). The grey shaded are corresponds to the time of hAβ-dim application. (**D**) Summary data for the experiment in (C). Error bars show SEM. Wilcoxon signed rank test. n.s. not significant.

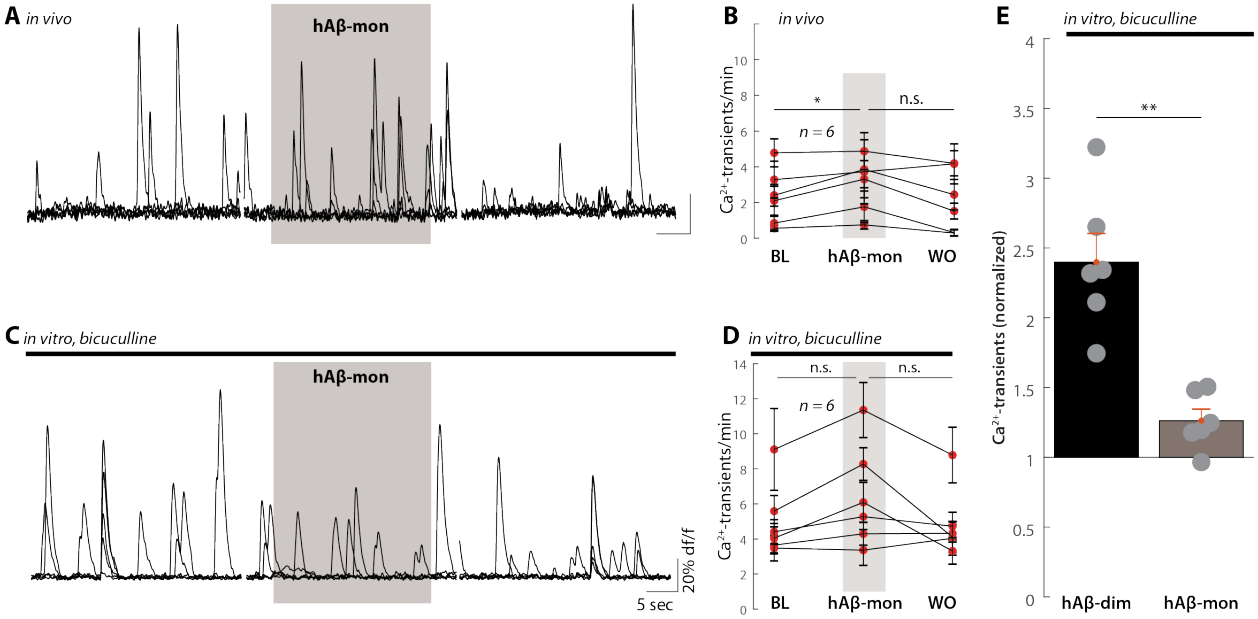

Fig. S13. Human Aβ monomers are ineffective

(**A**) Overlaid Ca^{2+}-traces from five representative neurons recorded in vivo under baseline (left), application of human Aβ monomer (hAβ-mon, middle) and washout conditions (right). The grey shaded are corresponds to the period of hAβ-mon application. (**B**) Summary data for the experiment in (**A**). Each dot represents the mean number of Ca^{2+}-transients per minute for all neurons in one slice under baseline (BL), hAβ-dim application and washout (WO) conditions. (**C**) Overlaid Ca^{2+}-traces from five representative neurons recorded in a hippocampal slice in which neuronal baseline activity had been elevated by the superfusion of bicuculline under baseline (left), hAβ-mon application (middle) and washout conditions (right). (**D**) Summary data for the experiment in (C). (**E**) Bar graph of the normalized activity during the application of hAβ-dim (left, n = 6) and hAβ-mon (right, N = 6). Each point represents the mean number of Ca^{2+}-transients in one mouse during the application, normalized to baseline. Error bars show SEM. Wilcoxon signed rank test (B, D) or Wilcoxon rank sum test (E), **P<0.005, *P<0.05; n.s. not significant.