Journal of Alzheimer's Disease 23 (2011) 673–687
DOI 10.3233/JAD-2011-091717
IOS Press

Soluble Aβ$_{1\text{-}40}$ Peptide Increases Excitatory Neurotransmission and Induces Epileptiform Activity in Hippocampal Neurons

Magdalena E. Cuevas[a], Henny Haensgen[a], Fernando J. Sepúlveda[a,b], Gabriela Zegers[a], Jorge Roa[c], Carlos Opazo[c] and Luis G. Aguayo[a,b,*]
[a]*Laboratory of Neurophysiology, Department of Physiology, University of Concepción, Concepción, Chile*
[b]*Centro de Investigación Avanzada en Educación, University of Concepción, Concepción, Chile*
[c]*Laboratory of Neurobiometals, Department of Physiology, University of Concepción, Concepción, Chile*

Accepted 19 October 2010

Abstract. It is believed that amyloid-β peptide (Aβ), in its aggregated-oligomeric state, constitutes one of the neurotoxic factors involved in the pathogenesis of Alzheimer's disease. With the objective of studying a potential role of the peptide on synaptic transmission, we studied the effect of soluble Aβ$_{1\text{-}40}$ on synaptic transmission in rat hippocampal neurons. Neurons incubated with 500 nM of Aβ$_{1\text{-}40}$ peptide for 3 days presented higher levels of intracellular calcium transients, as evaluated by fluorimetric techniques. These effects of Aβ were time and concentration dependent and were accompanied by increases in glutamatergic (0.8 ± 0.2 Hz to 2.9 ± 0.6 Hz), but not GABAergic, transmission. The analysis of pharmacologically isolated currents in treated neurons showed increases in both AMPA- and NMDA-mediated currents as compared to control. The effects of the peptide on the frequency of synaptic currents correlated well with increases in the number of SV2 puncta and of FM1-43 destaining, suggesting a presynaptic locus for the peptide. The data also shows that application of either Aβ or bicuculline alone for 24 h was without effects on neurotransmission. However, their co-application induced an increase in synaptic transmission which was accompanied by synchronous discharges reminiscent to those produced by pro-convulsive drugs, such as bicuculline. In conclusion, these results suggest that the soluble form of Aβ$_{1\text{-}40}$ participates in the regulation of synaptic transmission increasing excitability and producing a pre-epileptogenic state in hippocampal neurons.

Keywords: Alzheimer's disease, AMPA receptors, amyloid-β, bicuculline, epilepsy, GABA$_A$ receptors, NMDA receptors, synaptic plasticity

INTRODUCTION

Alzheimer's disease (AD) is a progressive dementia characterized by a deficiency in learning and memory. One of the characteristics of this disease is the presence of extracellular senile plaques principally composed of aggregated species of amyloid-β peptide (Aβ), a 39 to 42 amino acid peptide produced by the cleavage action of β and γ secretases on the amyloid-β protein precursor (AβPP). The oligomeric and aggregated states of Aβ have been implicated in the synaptotoxicity and neurodegeneration found in the brain of AD patients [1, 2]. It has been demonstrated that aggregated Aβ inhibits synaptic plasticity and decreases long term potentiation (LTP) and dendritic spine density in rat hippocampal slices [3–6]. Interestingly, these effects do not occur in presence of the monomeric form of the peptide [7, 8], and studies in rat hippocampal slices

*Correspondence to: Dr. Luis G. Aguayo, Department of Physiology, University of Concepción, P.O. Box 160-C, Concepción, Chile. Tel.: +56 41 2203380; Fax: +56 41 2245975; E-mail: laguayo@udec.cl.

ISSN 1387-2877/11/$27.50 © 2011 – IOS Press and the authors. All rights reserved

showed that a low concentration of the peptide can facilitate LTP [9]. In addition, it was shown that soluble $A\beta_{1\text{-}40}$ promotes neurogenesis in neural stem cells increasing the expression of neuronal markers such as NeuN and Tuj1 [10]. Overall, these results suggest that less aggregated forms of Aβ might have neurotrophic effects on the central nervous system.

Therefore, in order to elucidate the role of these more soluble forms of Aβ on synaptic transmission, we evaluated the effects of $A\beta_{1\text{-}40}$ on synaptic function in hippocampal neurons. The neurons treated with the soluble form of Aβ ($A\beta_{s1\text{-}40}$) for 72 h showed that the peptide facilitates synaptic transmission, increasing the immunoreactivity for SV2, a synaptic vesicle protein, and glutamatergic neurotransmission, leading to an *in vitro* pre-convulsive state contrary to that reported with the oligomeric forms of the peptide.

MATERIALS AND METHODS

Rat hippocampal cultures

Sprague Dawley rats were treated and manipulated according to the ethical guide for the use and care of experimental animals established by NIH (NIH, Maryland, USA) and the Ethic Committee at the University of Concepcion. Female rats pregnant with 18–19 day old embryos were euthanized. The embryos were removed and the hippocampi dissected. The neurons were dissociated enzymatically and mechanically and plated at a density of 350,000 cells/ml on 35 mm coverglass slips coated with poly-L-lysine (0.25% P/V; MW > 350 kDa, Sigma, USA). The cultures were maintained at 37ºC with 5% CO_2, and the culture medium consisting of 90% minimal essential media (MEM, GIBCO, Rockvile, USA), 5% horse serum (Hyclone), 5% fetal bovine serum and N3 (a mixture of nutrients) was replaced every 3 days. Chronic treatment (24–72 h) with soluble peptide ($A\beta_{s1\text{-}40}$, 0.1–1 μM) was done on neurons having 8–9 days *in vitro* (DIV) and similar results were obtained with older neurons (17 DIV). Unlike longer applications, acute applications (min–hr) of $A\beta_{s1\text{-}40}$ did not produce any noticeable effects on synaptic transmission or intracellular calcium.

Peptide preparation

Human $A\beta_{1\text{-}40}$ peptide was purchased from Tocris (MO, USA). $A\beta_{1\text{-}40}$ and the reverse peptide $A\beta_{40\text{-}1}$ were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml and immediately stored in aliquots at −20ºC until its use. For the preparation of Aβ oligomers/aggregates (80 μM), aliquots of peptide stock (250 μg in 25 μl of DMSO) were added to 700 μl of PBS (Gibco, USA) and stirred continuously in a vertical manner (200 RPM at 37ºC) for 90 min and stored at 4ºC. Aβ peptide aggregation was followed by turbidity measurements (O.D. 405 nm). Final products of aggregation assays were kept at 4ºC until a particular experiment.

Whole cell patch clamp

Tissue culture media was replaced with an external solution containing (in mM): 150 NaCl, 5.4 KCl, 2.0 $CaCl_2$, 10 glucose and 10 HEPES (pH 7.4). The internal solution of the recording pipet contained (in mM): 120 KCl, 2.0 $MgCl_2$, 2 $ATPNa_2$, 10 BAPTA, 0.5 GTP, 10 HEPES (pH 7.4). Electrodes were made using borosilicate capillaries (WPI, Sarasota, FL) on a horizontal puller (Sutter Instruments, Novato, CA). The pipette and series resistances were 4 and 10 MΩ, respectively. The series resistance was continuously monitored and cells were discarded if the resistance increased by more than 15%. The membrane potential was adjusted to −60 mV and the data was displayed and stored using a 1322A Digidata acquisition board (Axon Instruments, Inc.) and analyzed with electrophysiological software (pClamp9, Axon Instruments, Inc.). At this holding potential, excitatory (cationic driven) and inhibitory (anionic driven) currents were inward and displayed higher amplitudes facilitating the analysis since their reversal potentials were near 0 mV. Changes in synaptic currents were detected using the whole cell patch clamp technique as previously described [11] with an Axopatch-200B amplifier (Axon Instruments, Inc., Burlingame, CA) and an inverted microscope (Nikon, Eclipse, TE200-U, Japan). In order to study miniature synaptic currents (mEPSCs and mIPSCs), TTX (50 nM) was added to inhibit action potentials. Fresh stock solutions were prepared weekly in deionized-distilled water and kept refrigerated at 4ºC. In order to rapidly apply the ligands, we used an array of external tubes (200 μm in internal diameter) which was placed to within 50 μm from the neuron. The solutions containing the ligands flowed continuously from the tubes by gravity. To obtain pharmacologically isolated neurotransmissions, we used bicuculline (5 μM) to block $GABA_A$ receptors, CNQX (4 μM) for AMPA receptors, and AP-V (50 μM) for NMDA receptors (Tocris Bioscience, MO, USA).

Intracellular calcium fluorimetry

Neurons were loaded (30 min, 36.5°C) with Fluo 4-AM (0.5 µM in pluronic acid/DMSO (0.1%), Molecular Probes) and washed with PBS (0.1 M) for 30 min at 36.5°C. After washing, the cells were placed in a perfusion bath and put on an inverted Nikon microscope (Eclipse TE, Nikon). The bath solution contained (in mM): 150 NaCl, 5.4 KCl, 2.0 $CaCl_2$, 1.0, 10 HEPES (pH 7.4, 330 mOsm) and 10 glucose. No external Mg^{2+} was used to detect NMDA dependent responses. The neurons were briefly illuminated (200 ms, 480 nm) with a Xenon lamp at 2–5 s intervals for 5 min using a computer-controlled Lambda 10–2 filter wheel (Sutter Instruments) and Axon Workbench 2.1 software (Axon Instruments). The images of Fluo 4-AM bound to Ca^{2+} were obtained using a Sensicam CCD camera (PCO, Kelheim, Germany) having 12 bit resolution and stored on a computer for subsequent analysis. Regions of interest (ROI) to measure levels of fluorescence were positioned in the soma of identified neurons.

Immunofluorescence

Neurons were fixed for 10 min with methanol at −20ºC. The cells were then permeabilized with Triton X-100 (0.1%) and blocked with 10% bovine serum albumin (BSA, Fisher, Fraction V) for 30 min to reduce nonspecific binding. The cells were subsequently incubated overnight with the following primary antibodies: rabbit polyclonal antibody against Microtubule Associated Protein 2 (MAP-2, 1 : 200) or mouse monoclonal antibody against Synaptic Vesicle 2 (SV2, 1 : 200). In order to visualize the binding of the primary antibody to the neurons, the cells were incubated with a second antibody from goat conjugated to Cy3 for MAP-2 and FITC for SV2 (1 : 200; Jackson ImmunoResearch Laboratories, West Grove, PA). Finally, the cells were mounted onto slides using Dako Fluorescent Mounting Media (Dako, North America, Inc, CA, USA) and the images were obtained using a Nikon confocal microscope (60X water immersion objective, Nikon, Tokyo, Japan).

Triethylammonium(propyl)-4-(2-(4-dibutylaminophenyl)vinyl)pyridinium (FM1-43) loading and unloading

FM1-43 (15 mM; Molecular Probes, Eugene, OR, USA) was loaded in presynaptic terminals of control neurons and neurons treated with Aβ_{s1-40} incubated in a high potassium solution (30 mM) for 5 min at room temperature [12]. The loading was dependent on extracellular calcium and TTX, indicating a complete dependence on synaptic vesicle recycling. After washing with external solution for 10 min, discharge was induced from the active zones by rapid perfusion of a high potassium solution (30 mM) to the bath [13]. Higher concentrations of KCl were avoided since they were associated to slow and partial recoveries on the reuptake of FM1-43. The FM1-43 emission was obtained using an inverted microscope (Eclipse TE, Nikon, Melville, NY, USA) equipped with an oil immersion objective (100X NA 1.45), long-pass filters (>510 nm) and a Sensicam CCD camera (PCO, Kelheim, Germany). The FM1-43 fluorescence intensity was measured continuously during 5 min using an area of 2X2 pixels. All intensity values were analyzed using the Imaging Axon Workbench 2.2 program (Axon instruments).

Immunogold labeling for Aβ_{1-40} and Aβagg_{1-40}

Soluble and aggregated Aβ were adsorbed onto nickel grids covered by carbon-stabilized Formvar film and air-dried. After washing in buffer (PBS 1X, pH 7.4) for 2 min, nonspecific binding was blocked by incubation with 3% ovalbumin in PBS 1X pH 7.4, for 1 h. The grids were then placed on a droplet of anti-Aβ 1 : 500 (Santa Cruz Biotechnology, CA, USA) in PBS (pH 7.4) with 3% ovalbumin, incubated at 4°C overnight, and passed under five droplets of washing solution (PBS 1X, pH 7.4, with 0.05% Tween 20) for 5 min each time. A droplet of anti-rabbit IgG conjugated to 10 nm colloidal gold particles for 1 h (Sigma; diluted 1 : 20 PBS, pH 7.4, with 3% ovalbumin) was added and washed [14]. Before final examination under a JEOL 100-B electron microscope, the specimens were negatively stained with 2% uranyl acetate in water (Fig. 2).

Thioflavine-S staining

Aβ soluble and aggregates were stained with thioflavine-S (ThS, 0.5% w/v) for 10 min at 22–24°C. ThS fluorescence was acquired after exciting the samples at 488 nm.

Western blots

Standard Western blotting procedures were followed [15]. Equal amounts of protein were separated on 10–12% SDS-PAGE gels. Protein bands were transferred onto nitrocellulose membranes, blocked with 5% low fat milk, and incubated with anti-Aβ 1 : 500

(Santa Cruz Biotechnology, CA, USA). Immunoreactive bands were visualized with ECL Plus Western Blotting Detection System (PerkinElmer, MA, USA).

Dot blot

Aβ was diluted to 0.5 μM in culture media and samples of 100 μl were taken at various times of incubation (0–72 h). The samples were centrifuged at 10,000 rpm for 15 min to separate supernatant and pellet and applied onto nitrocellulose membranes. Non specific binding was blocked with non fat milk before incubation with anti-Aβ 1 : 500 (Santa Cruz Biotechnology, CA, USA).

Charge transfer analysis

Charge transfer was calculated by integration of membrane currents using the area under the curve of synaptic events pharmacologically isolated.

Data analysis

Experiments were carried out in parallel sister cultures in order to reduce the variability between control and treated neurons. Control cells were incubated with 0.1% DMSO in order to show that the solvent had no effect on the neurons. The data was obtained from at least 3 distinct experiments. The results are expressed as mean ± SEM (standard error mean). Statistical analysis was done using Student's *t* test or ANOVA followed by the Bonferroni post test. (*) $p < 0.05$ was considered statistically significant, (**) $p < 0.01$, (***) $p < 0.005$.

RESULTS

The soluble form of the Aβ peptide facilitates neurotransmission in hippocampal neurons

The oligomeric forms of the Aβ peptide have been described as factors responsible for the neurodegeneration observed in patients with AD [16, 17]. This deterioration has been associated to the depression in glutamatergic neurotransmission with consequent modifications in functional and structural synaptic plasticity, also leading to loss of synapses and memory in rodent AD models, as well as humans [18, 19]. However, the function of the monomeric and simpler forms of Aβ in synaptic activity and long term potentiation (LTP) are still largely unknown [7, 20]. In the present study, rat hippocampal neurons were incubated for 72 h with increasing concentrations of soluble $A\beta_{s1\text{-}40}$ (0.1–1 μM), and the frequency of intracellular calcium transients were evaluated using the fluorescent indicator Fluo-4AM (Fig. 1A). The data in Fig. 1A shows that the soluble form of $A\beta_{1\text{-}40}$ increased the frequency of intracellular calcium transients in a concentration-dependent manner that was statistically significant from control at 0.5 μM (0.06 ± 0.004 Hz, $n = 99$ cells; vs 0.08 ± 0.004 Hz, $n = 126$; $p<0.05$). Lower concentrations did not produce any effect on neuronal activity at 72 h. On the other hand, neurons treated with the same concentration of the aggregated form of the $A\beta_{1\text{-}40}$ peptide for 24 h displayed a marked decrease in the frequency of calcium transients (0.023 ± 0.003 Hz, $n = 136$, $p < 0.001$, Fig. 1A). These data are in agreement with results recently published and show that soluble and aggregated forms of $A\beta_{1\text{-}40}$ have different activities [7, 20]. In addition, patch clamp experiments done to evaluate synaptic currents using the same conditions as above demonstrated the same trend, with the soluble peptide significantly increasing the frequency of these events, whereas aggregated Aβ produced a decrease (Fig. 1B). On the other hand, cultures chronically incubated with the $A\beta_{40\text{-}1}$ reverse peptide or with acute applications of $A\beta_{1\text{-}40}$ showed no effect in synaptic transmission (Fig. 1C). The modification in synaptic transmission by chronic $A\beta_{s1\text{-}40}$ was likely produced by non-aggregated forms of the peptide. Ultra structural and biochemical analysis of the soluble peptide with electron microscopy (Fig. 2A–C), β-sheets detecting thioflavin-S (Fig. 2D), Western blot and dot blots showed significant differences to the pre-aggregated form, composed of fibrillar/amyloid structures found in the pellet fraction (Fig. 2E, F).

In order to determine the time course of the positive effects of Aβ on neuronal activity, electrophysiological recordings of overall miniature synaptic activity were carried out at different incubation times. The data revealed a time-dependent increase in the levels of synaptic current frequency with statistically significant effects only at 72 h of treatment with the peptide, compared with parallel sister cultures having the same developmental age (Fig. 3A). The treatment produced alterations in miniature current amplitude, 36 ± 3 pA ($n = 20$) for control and 24 ± 3 pA ($n = 19$, $p < 0.05$) for Aβ. Analysis of pharmacologically isolated glutamatergic synaptic currents showed that they were significantly increased by Aβ (Fig. 3B), without significant changes in the amplitude (29 ± 4 pA vs 21 ± 2 pA, $p > 0.05$).

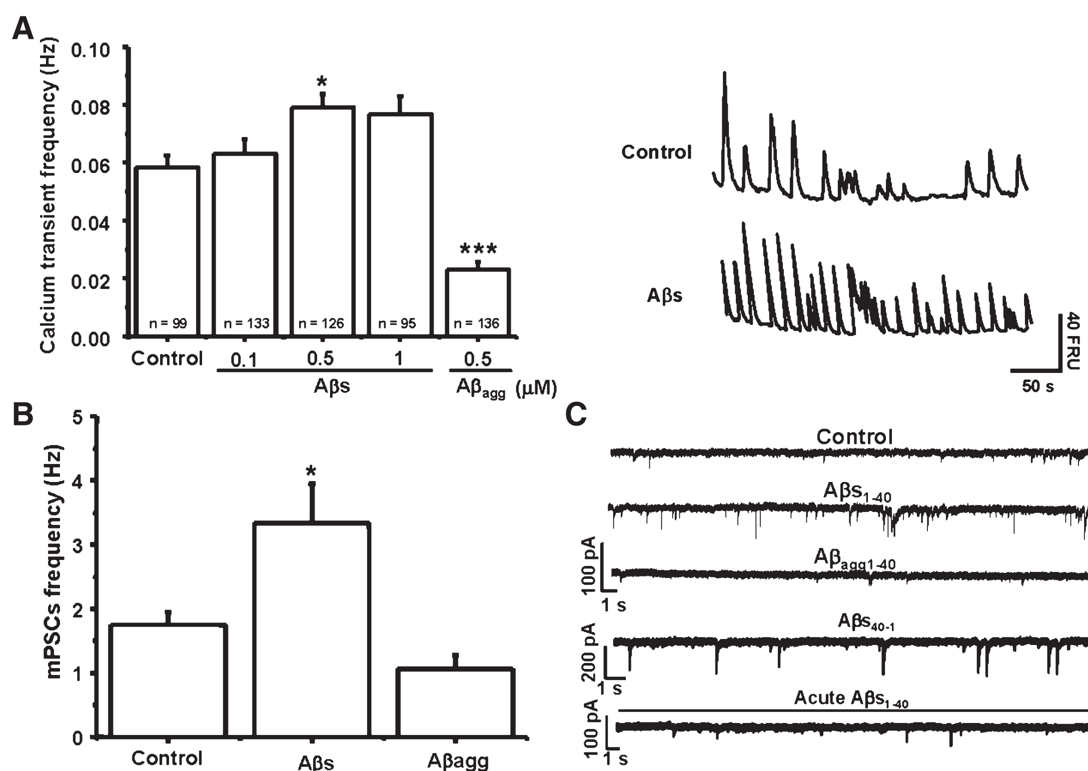

Fig. 1. $A\beta_{s1\text{-}40}$ facilitates neurotransmission in hippocampal neurons. A) The bar graph shows the frequency of intracellular calcium transients recorded from 11 DIV rat hippocampal neurons treated with the soluble form of $A\beta_{1\text{-}40}$ (0.1–1.0 μM). For comparison, aggregated peptide (0.5 μM) was included. Representative traces are included on the right. B) Averaged frequencies of total miniature synaptic currents recorded in the indicated conditions. C) Representative current traces for each condition from graph in B. The data also show that the reverse $A\beta_{40\text{-}1}$ (72 h) and acute application of $A\beta_{s1\text{-}40}$ were without effects. The values represent the mean ± SEM from at least 9 neurons (*$p < 0.05$; ***$p < 0.001$).

Next, we wanted to determine if these increases in synaptic current frequency were accompanied by morphological changes and if they were dependent on the time of incubation. For this, changes in a presynaptic component were analyzed using an antibody that recognizes SV2, a synaptic vesicle protein, and was quantified by counting the number of immunoreactive puncta for 20 μm of primary process in hippocampal neurons treated for 72 h with $A\beta_{s1\text{-}40}$. Analysis of the effect produced by $A\beta_{s1\text{-}40}$ at different times of exposure (12–72 h) is shown in Fig. 4. The quantitative immunofluorescent analysis of confocal images (Fig. 4A, B) revealed that $A\beta_{1\text{-}40}$ produced a sustained increase in SV2 staining with time and was statistically significant at 72 h (7.8 ± 0.65 puncta/20 μm vs 14.2 ± 1.05, $p < 0.01$). To determine if the increase in SV2 was accompanied by a functional increase in synaptic release upon presynaptic depolarization with high K^+, we carried out experiments using the fluorescent probe FM1-43 (Fig. 4C), a functional synaptic marker having an amphipatic nature that interacts with the lipid component of the membrane through its hydrophobic region [21]. Control and $A\beta_{1\text{-}40}$ treated neurons were charged with FM1-43 and subsequently exposed to a pulse of high potassium solution which caused a decrease in the measured fluorescence. The concentration of 30 mM K^+ was chosen because it has been shown that it depolarizes the presynaptic terminal sufficiently enough to produce an increase in the frequency of miniature currents [22] and the release of synaptic vesicles loaded with FM1-43 [11]. Additionally, a group of neurons treated with the reverse peptide showed no differences to control neurons in FM destaining which is in agreement with the lack of synaptic activity of this form (Fig. 4D).

The data showed that after treatment with $A\beta_{s1\text{-}40}$, hippocampal neurons displayed a faster decay of fluorescence associated to FM1-43 (Fig. 4D), indicating that the soluble form of the $A\beta_{1\text{-}40}$ peptide facilitates the release of synaptic vesicles. The values for the depleted fraction ($\Delta F/Fi$) of FM1-43 after 20 min of recording were 0.80 ± 0.02 in control condition

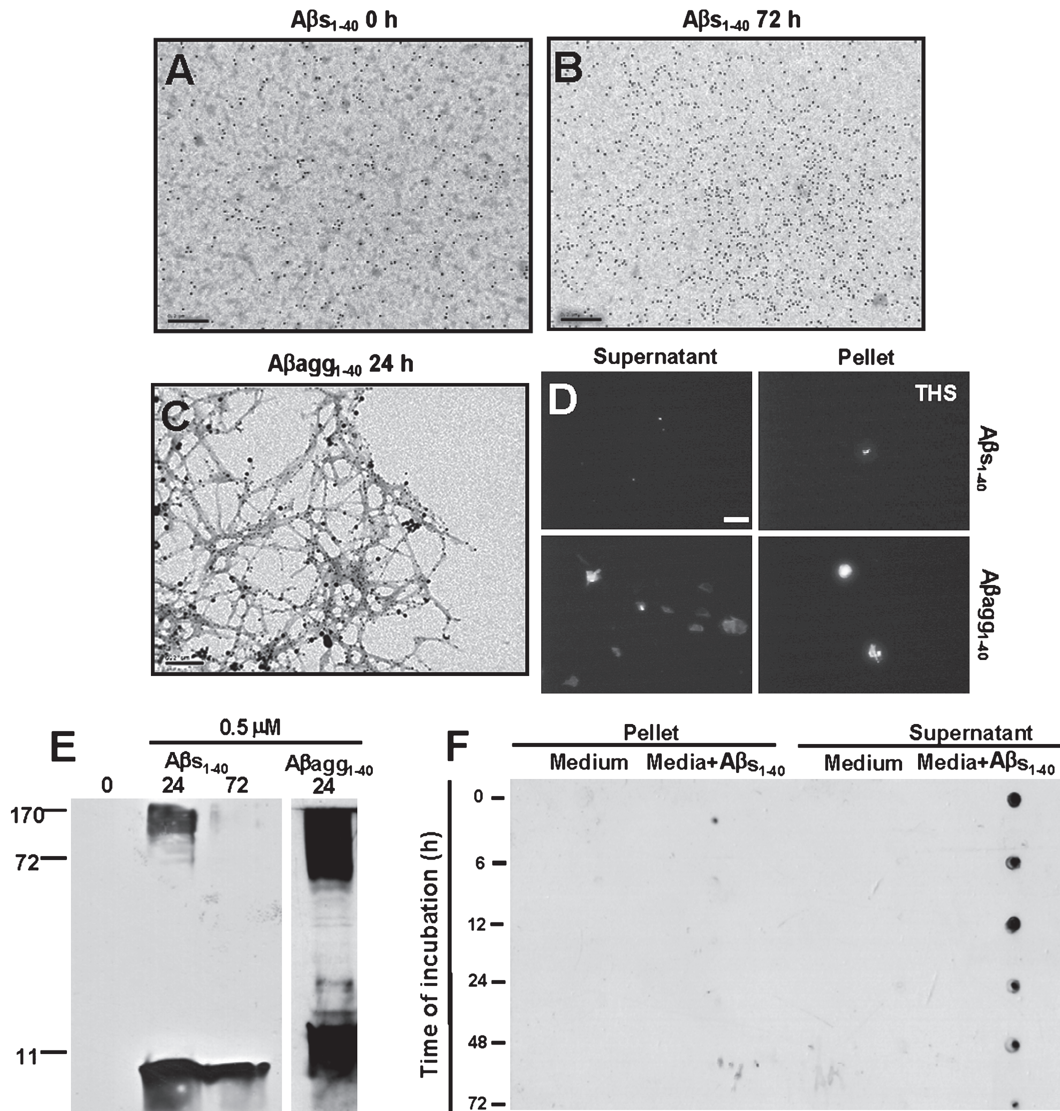

Fig. 2. Ultrastructural and biochemical characterization of $A\beta_{s1\text{-}40}$ and $A\beta_{agg1\text{-}40}$. A, B) The electron micrographs show immunogold of $A\beta_{s1\text{-}40}$ at 0 and 72 h incubation with culture media (37ºC). C) Shows $A\beta_{1\text{-}40}$ aggregates. D) Illustrates TH-S staining of soluble (left) and insoluble (right) fractions of $A\beta_{s1\text{-}40}$ and $A\beta_{1\text{-}40}$ aggregated after 72 ($A\beta s_{1\text{-}40}$) and 24 h ($A\beta agg_{1\text{-}40}$) incubation. E) Western blot from $A\beta_{s1\text{-}40}$ and aggregated preparations. F) Shows dot blots from soluble and insoluble fractions of $A\beta_{s1\text{-}40}$. Calibration bars are 0.2 μm for A and C, and 25 μm for B.

and 0.94 ± 0.01 ($p < 0.01$) for neurons treated with $A\beta_{s1\text{-}40}$. In addition, the fluorescent decay constant value for control neurons was 61 ± 4.8 s, which was significantly higher than in neurons treated with the soluble peptide (39 ± 4.4 s; $n = 3$, $p < 0.01$, Fig. 4D). Taken together, these results suggest that nanomolar concentrations of soluble $A\beta_{1\text{-}40}$ can positively modify presynaptic components, thus facilitating synaptic neurotransmission. Quantification of SV2 punctaes in age-matched neurons showed an increase at 11 and 20 DIV neurons after 72 h of $A\beta_{s1\text{-}40}$ treatment (Fig. 5A, B).

Effects of $A\beta_{s1\text{-}40}$ on pharmacologically isolated synaptic transmissions

We then evaluated the effect of Aβ on miniature excitatory and inhibitory synaptic activities, mEPSC, and mIPSC, respectively. To accomplish this, miniature synaptic currents associated to AMPA, NMDA, and $GABA_A$ receptors were pharmacologically isolated (see Methods). Analysis of electrophysiological recordings indicated that $A\beta_{s1\text{-}40}$ significantly increased the frequency of currents associated to AMPA receptors from 0.56 ± 0.21 Hz ($n = 5$) to

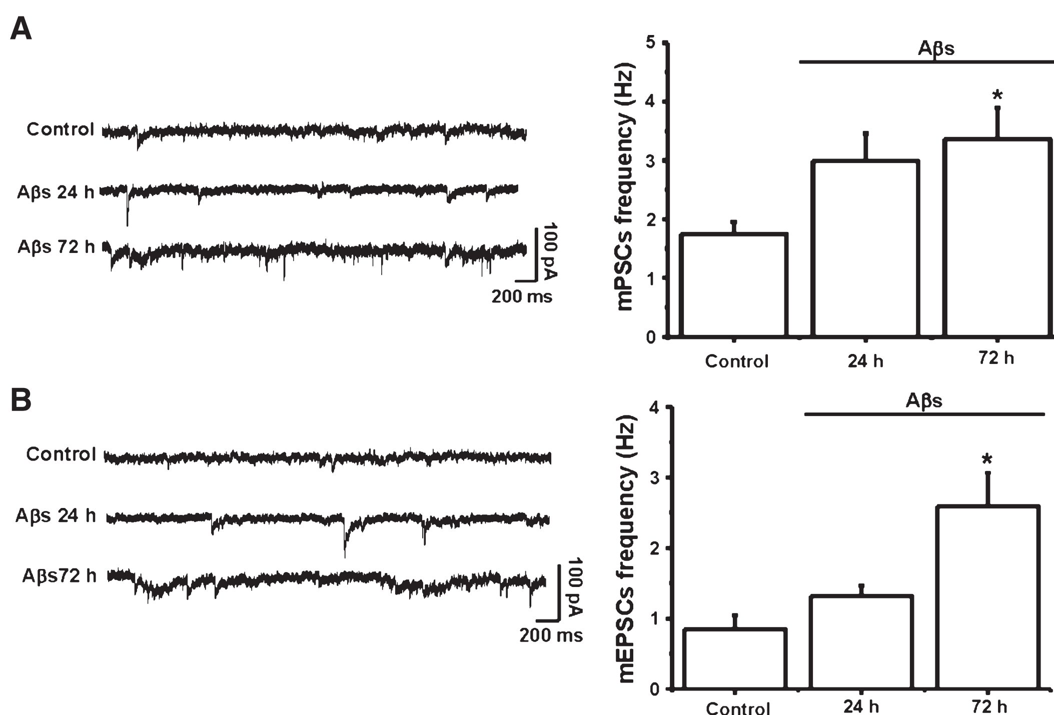

Fig. 3. $A\beta_{s1\text{-}40}$ produced a time-dependent increase in synaptic currents. A) The traces show whole miniature current recorded in control (age matched) and neurons treated with the peptide at several times at a concentration of 0.5 μM. The bar graph shows average frequencies of total synaptic events for each condition. B) The traces show miniature glutamatergic currents in control and treated neurons at different times. The bar graph shows average frequencies for each condition. The values represent the mean ± SEM ($^*p < 0.05$). The data shows results from at least 9 neurons.

1.65 ± 0.20 Hz after 72 h of $A\beta_{s1\text{-}40}$ 0.5 μM ($n = 8$) ($p < 0.01$), without changes in the time constant of decay (5.20 ± 0.7 ms, $n = 5$ and 4.5 ± 0.4 ms, $n = 11$) or peak amplitude of these fast excitatory currents (Fig. 6A, D).

Figure 6E shows electrophysiological recordings of miniature NMDA synaptic activity isolated in the presence of a cocktail of inhibitors for AMPA-R and $GABA_A$-R, 4 μM CNQX, and 5 μM bicuculline, respectively. The acute application of 50 μM AP-V resulted in a complete blockade of these synaptic currents, confirming that these low amplitude currents corresponded to synaptic events associated to the NMDA-R (Fig. 6E, lower trace). Subsequently, the properties of these synaptic currents were analyzed and the values of frequency and peak amplitude were compared in both conditions (Fig. 6F, G). Of these parameters, we found that the frequency of miniature NMDA synaptic currents was significantly increased (Fig. 6F). Furthermore, applications of NMDA to the postsynaptic membrane revealed that the current density induced by activation of NMDA receptors was not significantly affected by Aβ (Fig. 6 H). Evaluation of the GABAergic inhibitory activity, on the other hand, showed no significant variations in the frequency (0.63 ± 0.14 Hz, $n = 11$ vs 0.55 ± 0.13 Hz, $n = 9$).

The previous results suggest that the soluble form of the $A\beta_{1\text{-}40}$ peptide at nanomolar concentrations induces selective facilitation of glutamatergic neurotransmission. Since it is widely known that the activation of excitatory receptors can produce an increase in calcium permeability, we decided to examine if the frequency of intracellular calcium transients, and associated AMPA or NMDA receptors, could be altered after treatment with the soluble peptide. Therefore, we analyzed calcium transients in the presence of 50 μM AP-V or 4 μM CNQX acutely applied and the percentage of inhibition evoked by the acute blockade was obtained (Fig. 7A). The results demonstrated that treatment with the soluble form of the $A\beta_{1\text{-}40}$ peptide induced a significant increase in the percentage of inhibition by AP-V, but not by CNQX, suggesting that $A\beta_{s1\text{-}40}$ facilitates the NMDA-type phenotype at the network level (57 ± 4% vs 88 ± 4%, $p < 0.05$, Fig. 7B).

The data previously presented suggest that the soluble form of the $A\beta_{1\text{-}40}$ peptide facilitates glutamatergic

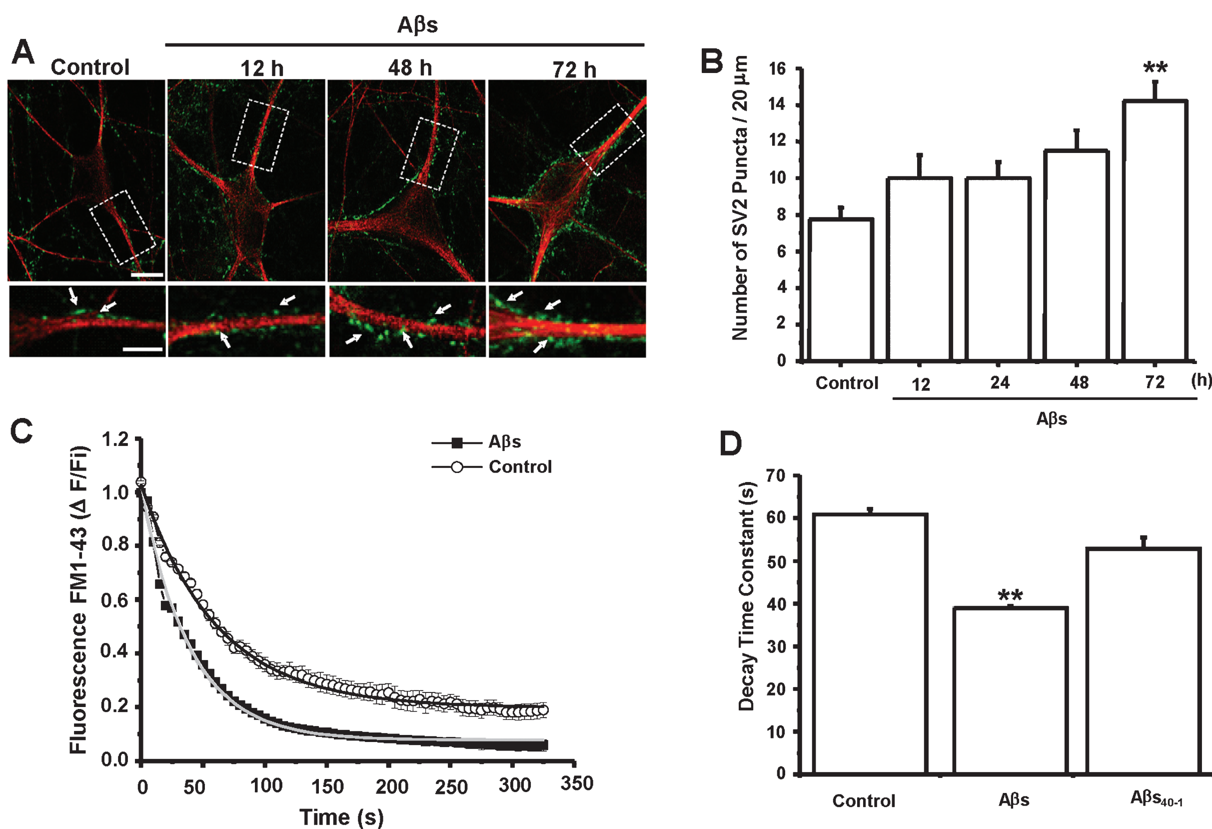

Fig. 4. $A\beta_{s1\text{-}40}$ caused an increase in SV2 and vesicular release. A) Neurons stained for neurofilament protein MAP-2 (red) and the synaptic vesicle protein SV2 (green) in control (age matched) and after different times with $A\beta_{s1\text{-}40}$. The lower micrographs are expanded views of the enclosed regions above. Calibration bars are 10 µm and 5 µm for the upper and lower panel, respectively. B) The graph shows the number of SV2 positive puncta (see arrows in A) per 20 µm of primary process from three experiments. C) The data illustrate the destaining of FM1-43 in control (white symbols) and after treatment with 0.5 µM $A\beta_{s1\text{-}40}$ (black symbols). D) The graph shows the decay time constant for FM1-43 destaining in control and after 72 h treatment with $A\beta_{1\text{-}40}$ and $A\beta_{40\text{-}1}$. Note that the reverse peptide was without effects. The values represent the mean ± SEM ($^*p < 0.05$; $^{**}p < 0.01$) from more than three separate experiments.

transmission, previously shown to support neuronal connectivity and excitability that can be associated to LTP [23, 24], learning and memory [25–27]. Also, it has been reported that high levels of glutamatergic activity sustained for a long time can induce excitotoxicity and pre-convulsive states affecting network homeostasis [28, 29]. Therefore, the following question arises: Is it possible that, aside from increasing excitatory transmission, the soluble form of the $A\beta_{1\text{-}40}$ peptide has pro-convulsive activity?

$A\beta_{s1\text{-}40}$ facilitates generation of pre-convulsant activity in vitro

To answer the previous question, we employed a paradigm used to study the potential of a variety of compounds to induce pro-epileptogenic activity [30]. For example, the pro-convulsant action of bicuculline, a competitive antagonist of $GABA_A$ receptors, has been well documented [31, 32]. Hippocampal neurons cultured with 5 µM bicuculline showed increases in neuronal excitability and bursting activity (Fig. 8C, inset). This excitation resulted from the blockade on inhibition and was mainly produced by an increase in AMPA transmission [30, 33]. On the other hand, a lower concentration of bicuculline (1 µM) was unable to induce such a state of overt hyperactivity (Fig. 8A). For example, analysis of spontaneous and pharmacologically isolated miniature synaptic currents revealed that there was no significant increase in the frequency of overall synaptic currents and those mediated by NMDA, AMPA, and $GABA_A$ (data not shown). After determining that neither Aβ nor bicuculline, applied for 24 h, produced hyper excitability, we evaluated if their co-incubation was able to increase synaptic transmission. To assess this point, we compared the

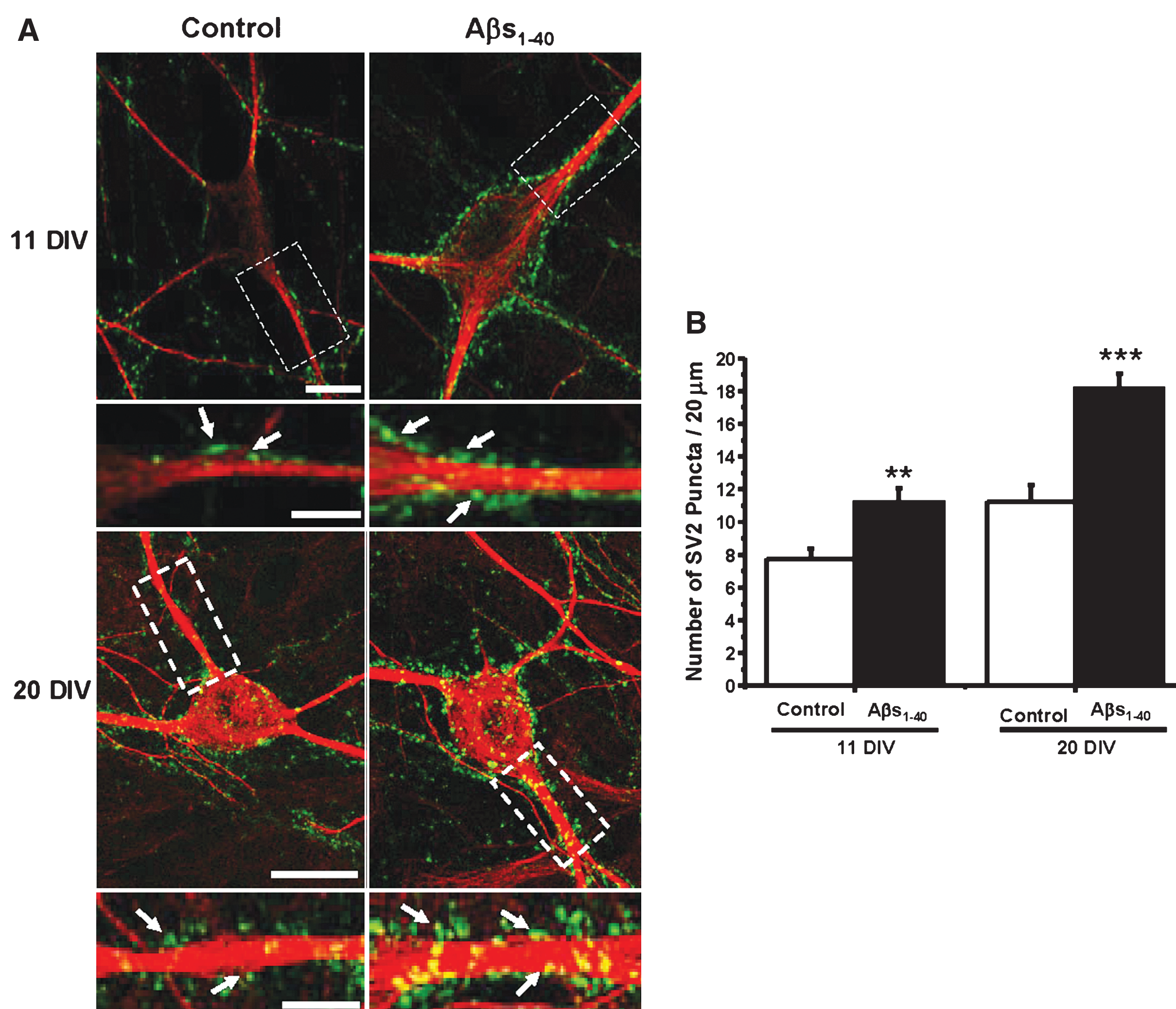

Fig. 5. $A\beta_{s1\text{-}40}$ increases the expression of SV2 at two developmental states. A) Confocal images show neurofilament protein MAP-2 (red) and SV2 (green) in 11 and 20 DIV neurons in control and after 72 h with $A\beta_{s1\text{-}40}$. B) The graph shows the number of punctas per 20 μm of primary process. The data was obtained from sister cultures. Each bar shows data obtained from 20 neurons from 3 experiments.

spontaneous synaptic activity obtained under these conditions. Neurons co-incubated with Aβ and the $GABA_A$ receptor antagonist showed more spontaneous postsynaptic currents (1.7 ± 0.2 vs 5.1 ± 0.8 Hz) than control neurons or those treated with $A\beta_{s1\text{-}40}$ or bicuculline alone (Fig. 8C). Additionally, quantification of synaptic events in presence of a cocktail to detect NMDA currents showed that co-incubation with soluble $A\beta_{1\text{-}40}$ peptide and bicuculline produced an increase in current frequency (0.4 ± 0.1 vs 1.8 ± 0.1 Hz, NMDA ($n = 5$), $^{**}p < 0.01$, Fig. 8B, D). Analysis of charge transfer during the synaptic currents also showed that the combination of Aβ and bicuculline increased the function of AMPA and NMDA receptors (Fig. 8E, F, $p < 0.05$). Furthermore, analysis of immunoreactivity associated to SV2, under the same conditions, revealed an increase in the fluorescence for this presynaptic marker that correlated with the increase in frequency (Fig. 9).

DISCUSSION

Facilitation of glutamatergic neurotransmission mediated by the soluble form of $A\beta_{1\text{-}40}$

Aβ peptide is secreted by several cell types explaining its presence, at picomolar concentration, in plasma and cerebral spinal fluid [34]. However, the functional relevance of Aβ formation in the brain is still under

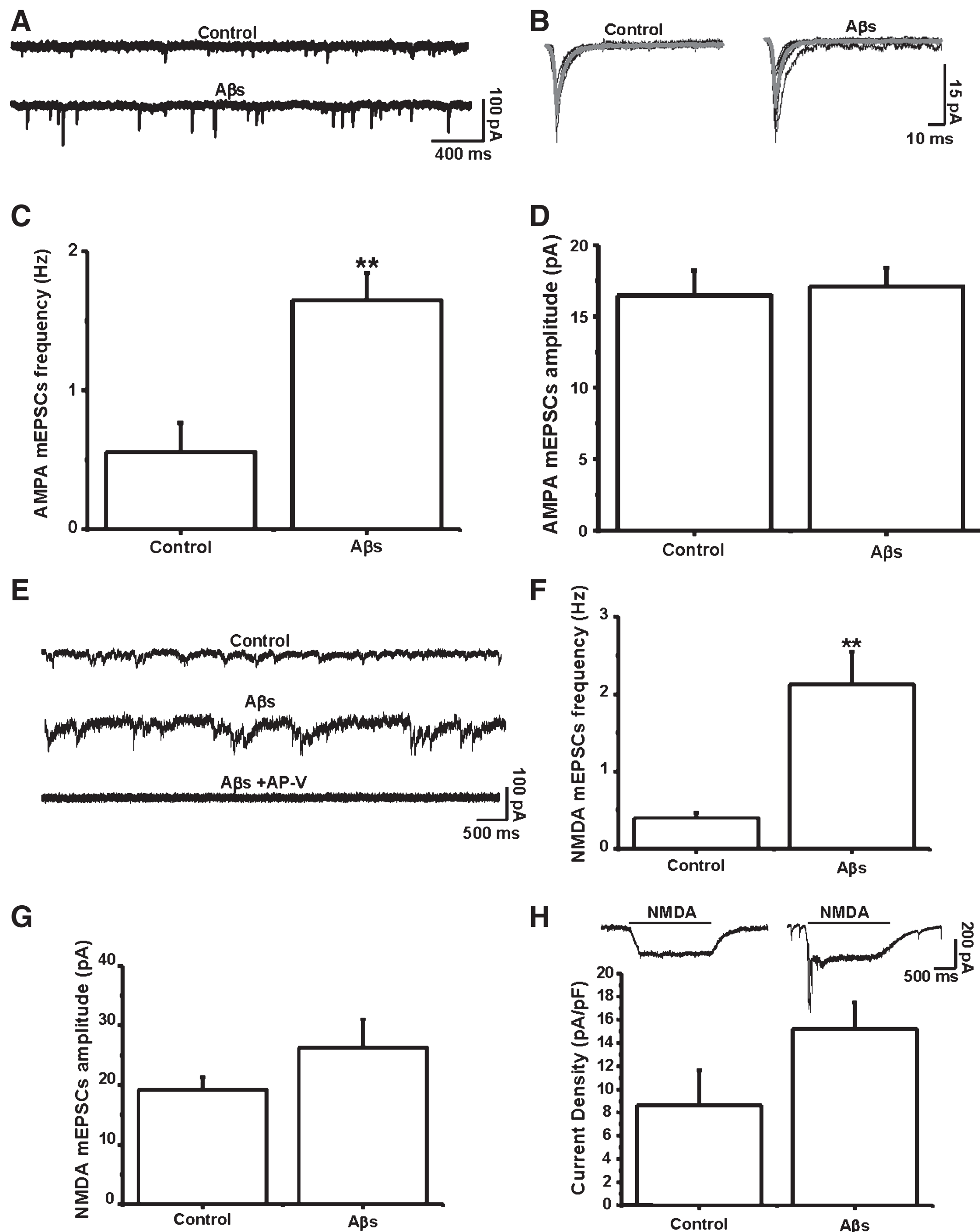

Fig. 6. Effect of $A\beta_{s1\text{-}40}$ on glutamatergic neurotransmission. A) The traces show typical miniature glutamatergic currents in control and treated sister neurons. B) Traces show AMPA-receptor mediated synaptic currents ($n = 5$, control; $n = 11$, treated neurons). The gray line is the averaged current under both conditions. C, D) The bars show AMPAergic current properties. E) NMDA synaptic currents were blocked by 50 μM AP-V. F-G) The graphs show NMDA mEPSC properties. H) The graph shows current density induced by application of 100 μM NMDA. The values represent the mean ± SEM (** $p < 0.01$).

investigation. Existing evidence supports its action in the maintenance of neuronal survival. For example, it was demonstrated that incubation of neuron cultures with β or γ secretase inhibitors induced neuronal death [35], a phenomenon that was reversed by incubation with low concentrations of $A\beta_{1\text{-}40}$. However, the mechanism by which Aβ regulated this survival process is not understood.

In agreement with a neurotrophic action, we report that $A\beta_{s1\text{-}40}$ facilitates synaptic transmission and

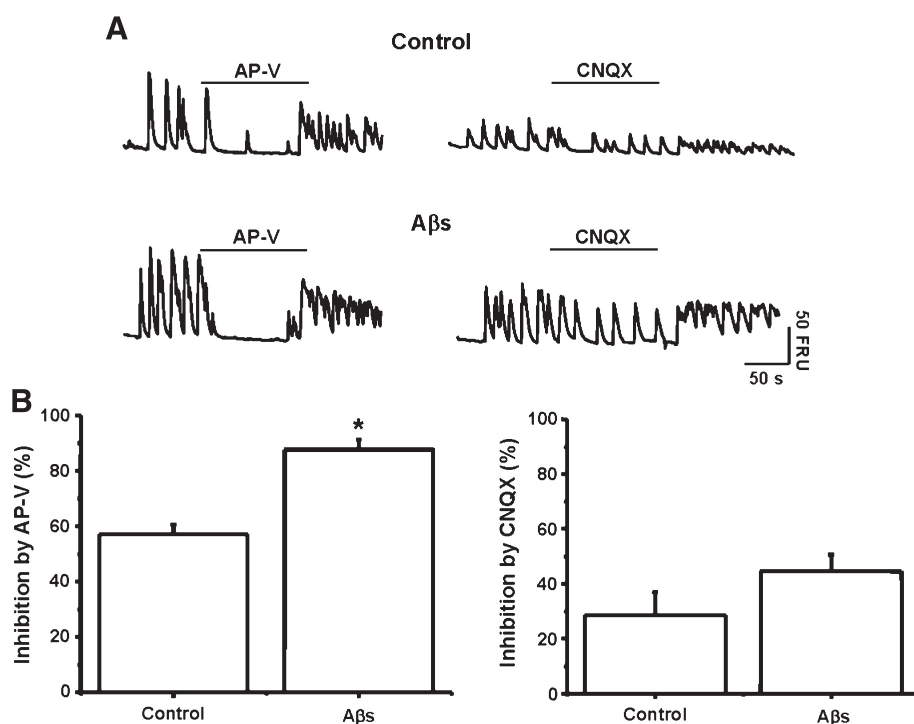

Fig. 7. $A\beta_{s1\text{-}40}$ increased NMDA receptor-dependent calcium transients. A) Representative traces of intracellular calcium transients before and during application of either AP-V (50 μM) or CNQX (4 μM). B) The bar graphs show the percentage inhibition of intracellular calcium transients induced by the receptor antagonists. The values represent the mean ± SEM (* $p < 0.05$). Control neurons, $n = 99$; treated neurons, $n = 126$.

increases the frequency of intracellular calcium transients. Although the concentration of Aβ used was above its reported value in the brain [34], it is justified to accelerate its effects in our working *in vitro* cellular model. In addition, it is likely that the concentration of Aβ at the synaptic cleft is much higher than in plasma or cerebral spinal fluid thus allowing its nucleation and subsequent aggregation. Although it was reported that Aβ clearance from CSF and blood in normal individuals has a time course of only a few hours [36], we believe that this does not contradict our interpretation that synaptic Aβ reaches a higher concentration in the synaptic cleft to exert its physiological and pathological actions. It is very well accepted that Aβ will accumulate and eventually cause brain damage [37].

Since previous studies have shown that augmentations in intracellular calcium are related to increases in neuronal excitability and therefore to network connectivity [38], we can hypothesize that soluble $A\beta_{1\text{-}40}$ increases network excitability. Distinctly, this action was divergent to that of aggregated $A\beta_{1\text{-}40}$, which under similar conditions displayed inhibitory effects (Fig. 1). This supports the conclusion that soluble and aggregated forms of Aβ have differential actions at the synapses. Additionally, Aβ was not extensively aggregated [39] at the nanomolar concentrations used in our experiments, a conclusion that was largely confirmed by ultra structural and biochemical results (Fig. 2). Additionally, the time course of the soluble Aβ effects on synaptic transmission found in this study are different to those reported using protofibrils and a neprelysin inhibitor [40, 41] and this is likely due to the Aβ forms and differences in the experimental models used in the studies. While in the first study, protofibrils might be increasing activity by disrupting the cell membrane [11], the pharmacological blockade of peptidases in the second study would increase synaptic release by accelerating the onset of Aβ actions. In any case, less aggregated forms of Aβ facilitate synaptic release.

The increase in intracellular calcium transients correlated well with the augmentation in miniature synaptic currents suggesting that Aβ increases network excitability augmenting the release of neurotransmitters. The finding that Aβ increases glutamatergic transmission is in agreement with previous studies showing that Aβ can enhance the concentration of extracellular glutamate by interfering with glutamate transporters in cultured cortical neurons and glia [42]. We also found an increase in the immunoreactivity associated with SV2 that was maintained in time and statistically significant after 72 h. Additionally, experiments

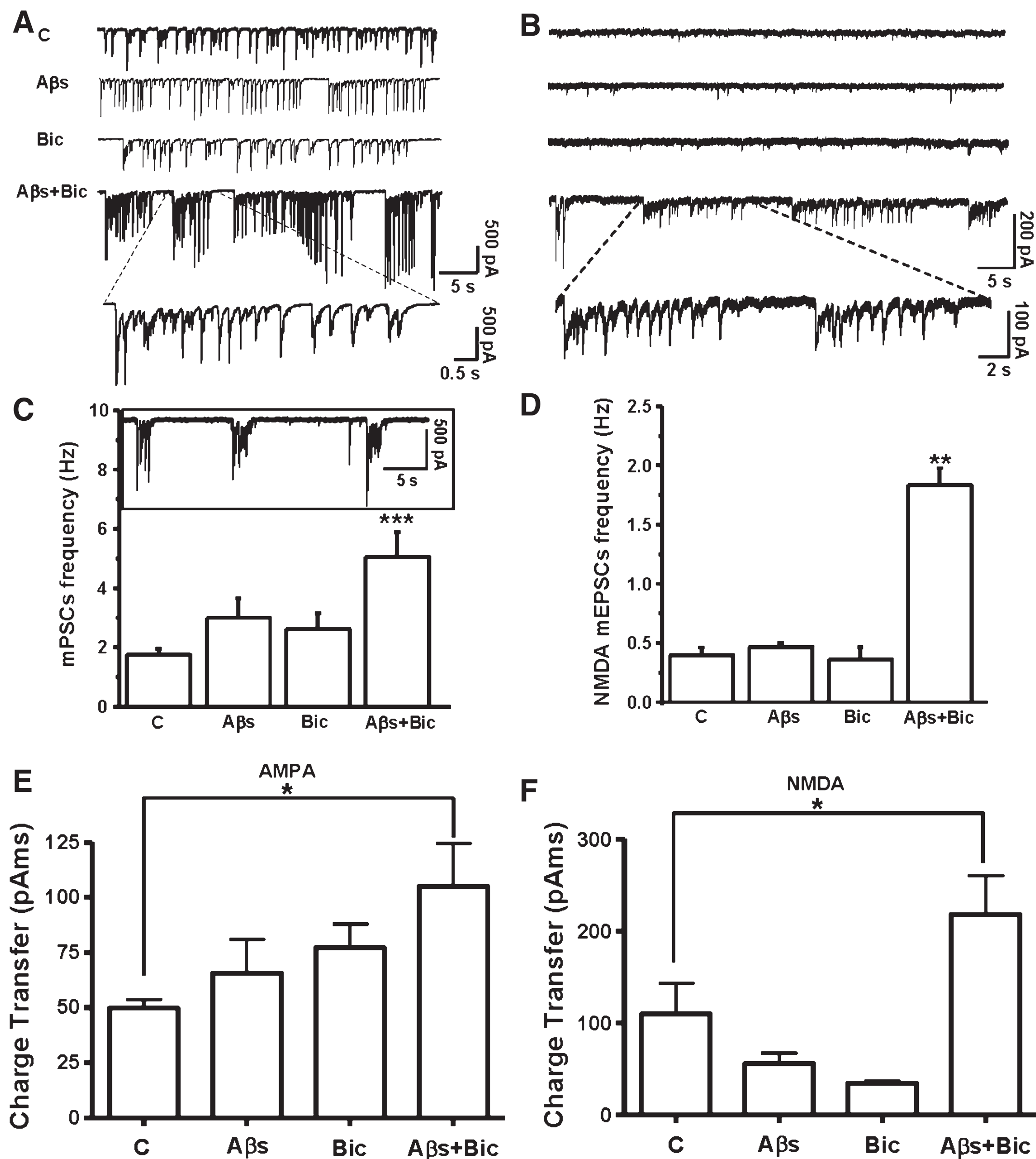

Fig. 8. $A\beta_{s1\text{-}40}$ facilitates pre-convulsive activity. A) Spontaneous synaptic currents under the indicated conditions: 0.5 μM $A\beta_{s1\text{-}40}$ 24 h, 1 μM bicuculline for 24 h and a combination of 0.5 μM $A\beta_{s1\text{-}40}$ and 1 μM bicuculline. B) NMDA mEPSCs for the same conditions shown in A. C, D) Frequencies for the conditions in A and B, respectively. The inset in C shows spontaneous synaptic activity of neurons treated with bicuculline 5 μM. E, F) Charge transfer associated to AMPA and NMDA synaptic currents recorded over a 2 min period. Bicuculline (5 μM), CNQX (4 μM) and AP-V (50 μM) were used to block $GABA_A$, AMPA, and NMDA receptors, respectively. The data shows mean ± SEM ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$).

to evaluate neurotransmitter exocytosis by means of FM1-43 fluorescence destaining revealed that neurotransmitter release was significantly higher in neurons treated with $A\beta_{s1\text{-}40}$. Altogether, these results demonstrate that incubation of hippocampal neurons with $A\beta_{s1\text{-}40}$ induces a phenomenon of synaptic facilitation that stimulates presynaptic release. Interestingly, the amplitude of the synaptic currents was smaller in the treated neurons and this might reflect a reduced density of postsynaptic receptors, as opposed to the presynaptic regions enhanced by Aβ. The overall effect of Aβ, nevertheless, is an increase in synaptic transmission as correlated to enhancements in calcium transients, repetitive firings and synaptic transfer charge.

This study also showed a significant increase in AMPAergic and NMDA mEPSC frequencies after

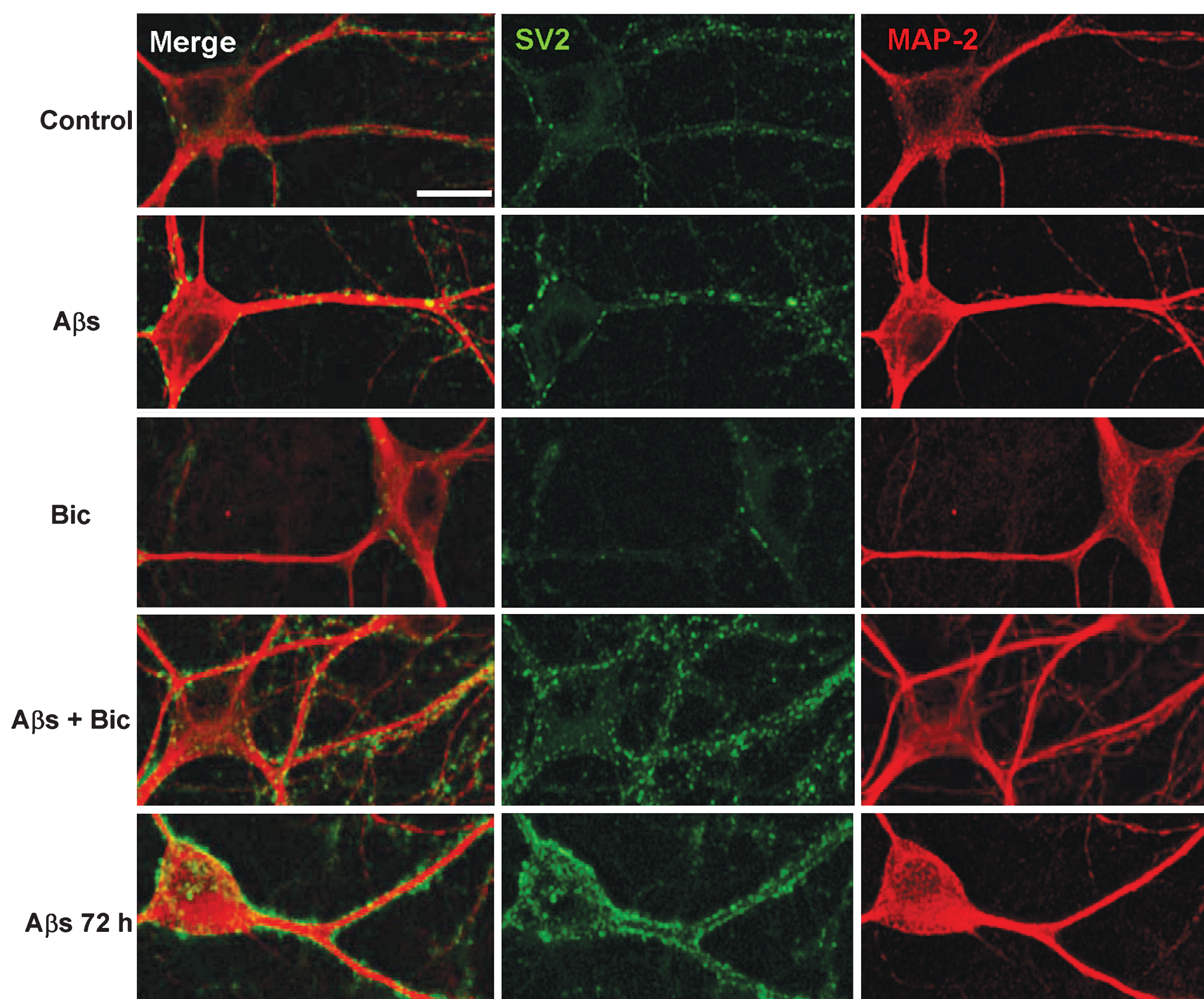

Fig. 9. Effect of combined $A\beta_{s1\text{-}40}$ and bicuculline on SV2. Confocal microscopy shows immunoreactivity associated to MAP2 (Cy3, Red) and SV2 (FITC, green) obtained in hippocampal neurons in control condition and after the indicated treatments. Channel superposition is shown at the left while the immunoreactivity associated to each channel in each condition is shown separately. Size of calibration bar is 10 μm.

chronic treatment. Furthermore, calcium fluorimetry analysis indicated that $A\beta_{s1\text{-}40}$ induced the appearance of a glutamatergic NMDA-like phenotype that through activation of signaling pathways likely participate in synaptic remodeling [43, 44]. We are currently examining potential mechanisms for this synaptic facilitation.

$A\beta_{s1\text{-}40}$ facilitates the generation of pro-convulsant activity

The present data indicate that the soluble form of Aβ enhances neuronal connectivity, events that have been implicated in learning and memory processes, but also in excitotoxicity [28, 45, 46]. Therefore, is it possible that these effects of Aβ lead hippocampal neurons to a pro-convulsant-like behavior? This question was answered using a classic paradigm which blocks GABAergic activity leading to an increase in neuronal excitability that leads to epileptogenesis [47–49]. Co-incubation with $A\beta_{s1\text{-}40}$ and bicuculline, at concentrations that are not active alone, produced an increase in the frequency of excitatory synaptic transmission as well as repetitive firing activity. Evaluation of pharmacologically isolated synaptic activity revealed that the transfer charge of NMDA mEPSCs was significantly increased (Fig. 8D), suggesting increased excitation and synaptic plasticity [50]. In addition, the analysis of SV2 demonstrated that co-incubation of $A\beta_{s1\text{-}40}$ and bicuculline increased this presynaptic protein. These results show that Aβ facilitates glutamatergic transmission, which has been documented as important for epileptogenesis and pro-convulsant activity

[51]. Our results agree with experiments showing that overexpression of Aβ in transgenic mice produced epileptiform activity in the hippocampal-cortex enthorinal circuit [52], and it is possible that these electrical alterations in the brain could lead to impairments in memory and learning in AD.

Regarding the potential mechanism by which soluble Aβ enhances synaptic activity, we believe that intracellular signaling activated by calcium is important. Our preliminary results show that the effect of Aβ was inhibited by KN-62 suggesting the involvement of CaMK-IV and initiated by AMPA and NMDA receptors (Cuevas, unpublished results). This is in agreement with the role of calcium on synaptic plasticity previously suggested [53].

Therefore, based on the above, we suggest that $A\beta_{1\text{-}40}$, in simple non-aggregated forms, induces synaptic facilitation of glutamatergic transmission. This could lead to a pre-convulsive condition and probably to alterations in the homeostasis of neuronal network, a phenomenon that has been widely documented in AD [52, 54]. Nevertheless, the mechanisms that originate these changes and the targets of Aβ in the membrane still need to be determined.

ACKNOWLEDGMENTS

We would like to thank Lauren Aguayo, Claudia Lopez, Juan C. Urrutia and Mario Reyes for technical assistance. F.J.S. funded by Ph.D. fellowship from CONICYT. This work was supported by FONDECYT grant 1100502, Ring of Research PBCT ACT-04 (L.G.A. and C.O.) and CIE-05 (L.G.A.).

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=660).

REFERENCES

[1] Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K (1985) Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J* **4**, 2757-2763.

[2] Poling A, Morgan-Paisley K, Panos JJ, Kim EM, O'Hare E, Cleary JP, Lesne S, Ashe KH, Porritt M, Baker LE (2008) Oligomers of the amyloid-beta protein disrupt working memory: confirmation with two behavioral procedures. *Behav Brain Res* **193**, 230-234.

[3] Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R (2006) AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* **52**, 831-843.

[4] Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A* **95**, 6448-6453.

[5] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842.

[6] Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* **8**, 1051-1058.

[7] Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. *J Physiol* **572**, 477-492.

[8] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* **416**, 535-539.

[9] Wu J, Anwyl R, Rowan MJ (1995) beta-Amyloid-(1-40) increases long-term potentiation in rat hippocampus *in vitro*. *Eur J Pharmacol* **284**, R1-R3.

[10] Chen Y, Dong C (2009) Abeta40 promotes neuronal cell fate in neural progenitor cells. *Cell Death Differ* **16**, 386-394.

[11] Parodi J, Sepulveda FJ, Roa J, Opazo C, Inestrosa NC, Aguayo LG (2010) Beta-amyloid causes depletion of synaptic vesicles leading to neurotransmission failure. *J Biol Chem* **285**, 2506-2514.

[12] Chen G, Harata NC, Tsien RW (2004) Paired-pulse depression of unitary quantal amplitude at single hippocampal synapses. *Proc Natl Acad Sci U S A* **101**, 1063-1068.

[13] Ryan TA, Reuter H, Wendland B, Schweizer FE, Tsien RW, Smith SJ (1993) The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. *Neuron* **11**, 713-724.

[14] Naslund J, Thyberg J, Tjernberg LO, Wernstedt C, Karlstrom AR, Bogdanovic N, Gandy SE, Lannfelt L, Terenius L, Nordstedt C (1995) Characterization of stable complexes involving apolipoprotein E and the amyloid beta peptide in Alzheimer's disease brain. *Neuron* **15**, 219-228.

[15] Garrido JL, Godoy JA, Alvarez A, Bronfman M, Inestrosa NC (2002) Protein kinase C inhibits amyloid beta peptide neurotoxicity by acting on members of the Wnt pathway. *FASEB J* **16**, 1982-1984.

[16] Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* **8**, 79-84.

[17] Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gouras GK (2004) Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci* **24**, 3592-3599.

[18] Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R (2003) APP processing and synaptic function. *Neuron* **37**, 925-937.

[19] Roselli F, Tirard M, Lu J, Hutzler P, Lamberti P, Livrea P, Morabito M, Almeida OF (2005) Soluble beta-amyloid 1-40 induces NMDA-dependent degradation of postsynaptic density-95 at glutamatergic synapses. *J Neurosci* **25**, 11061-11070.

[20] Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, Betts V, Spooner ET, Jiang L, Anwyl R, Selkoe DJ, Rowan MJ (2005) Amyloid beta protein immunotherapy neutralizes

Abeta oligomers that disrupt synaptic plasticity *in vivo*. *Nat Med* **11**, 556-561.
[21] Betz WJ, Bewick GS, Ridge RM (1992) Intracellular movements of fluorescently labeled synaptic vesicles in frog motor nerve terminals during nerve stimulation. *Neuron* **9**, 805-813.
[22] Calupca MA, Prior C, Merriam LA, Hendricks GM, Parsons RL (2001) Presynaptic function is altered in snake K^+-depolarized motor nerve terminals containing compromised mitochondria. *J Physiol* **532**, 217-227.
[23] Anwyl R, Mulkeen D, Rowan MJ (1989) The role of N-methyl-D-aspartate receptors in the generation of short-term potentiation in the rat hippocampus. *Brain Res* **503**, 148-151.
[24] Fitzjohn SM, Pickard L, Duckworth JK, Molnar E, Henley JM, Collingridge GL, Noel J (2001) An electrophysiological characterisation of long-term potentiation in cultured dissociated hippocampal neurones. *Neuropharmacology* **41**, 693-699.
[25] Sharma K, Fong DK, Craig AM (2006) Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. *Mol Cell Neurosci* **31**, 702-712.
[26] De Roo M, Klauser P, Garcia PM, Poglia L, Muller D (2008) Spine dynamics and synapse remodeling during LTP and memory processes. *Prog Brain Res* **169**, 199-207.
[27] Muller D, Nikonenko I, Jourdain P, Alberi S (2002) LTP, memory and structural plasticity. *Curr Mol Med* **2**, 605-611.
[28] Liu Y, Wong TP, Aarts M, Rooyakkers A, Liu L, Lai TW, Wu DC, Lu J, Tymianski M, Craig AM, Wang YT (2007) NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both *in vitro* and *in vivo*. *J Neurosci* **27**, 2846-2857.
[29] DeLorenzo RJ, Pal S, Sombati S (1998) Prolonged activation of the N-methyl-D-aspartate receptor-Ca^{2+} transduction pathway causes spontaneous recurrent epileptiform discharges in hippocampal neurons in culture. *Proc Natl Acad Sci U S A* **95**, 14482-14487.
[30] Arnold FJ, Hofmann F, Bengtson CP, Wittmann M, Vanhoutte P, Bading H (2005) Microelectrode array recordings of cultured hippocampal networks reveal a simple model for transcription and protein synthesis-dependent plasticity. *J Physiol* **564**, 3-19.
[31] Pong SF, Graham LT (1972) N-methyl bicuculline, a convulsant more potent than bicuculline. *Brain Res* **42**, 486-490.
[32] Meldrum BS, Horton RW (1971) Convulsive effects of 4-deoxypyridoxine and of bicuculline in photosensitive baboons (*Papio papio*) and in rhesus monkeys (*Macaca mulatta*). *Brain Res* **35**, 419-436.
[33] Sepulveda FJ, Opazo C, Aguayo LG (2009) Alzheimer beta-amyloid blocks epileptiform activity in hippocampal neurons. *Mol Cell Neurosci* **41**, 420-428.
[34] Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, et al. (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* **359**, 325-327.
[35] Plant LD, Boyle JP, Smith IF, Peers C, Pearson HA (2003) The production of amyloid beta peptide is a critical requirement for the viability of central neurons. *J Neurosci* **23**, 5531-5535.
[36] Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM (2006) Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid *in vivo*. *Nat Med* **12**, 856-861.
[37] Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* **8**, 101-112.
[38] Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. *Neuron* **59**, 882-901.
[39] Harper JD, Wong SS, Lieber CM, Lansbury PT Jr (1999) Assembly of A beta amyloid protofibrils: an *in vitro* model for a possible early event in Alzheimer's disease. *Biochemistry* **38**, 8972-8980.
[40] Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I (2009) Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. *Nat Neurosci* **12**, 1567-1576.
[41] Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ (1999) Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci* **19**, 8876-8884.
[42] Fernandez-Tome P, Brera B, Arevalo MA, de Ceballos ML (2004) Beta-amyloid25-35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. *Neurobiol Dis* **15**, 580-589.
[43] Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* **48**, 289-301.
[44] Mattson MP, Barger SW (1993) Roles for calcium signaling in structural plasticity and pathology in the hippocampal system. *Hippocampus* **3**, 73-87.
[45] Bausch SB, He S, Petrova Y, Wang XM, McNamara JO (2006) Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. *J Neurophysiol* **96**, 2151-2167.
[46] Zeng LH, Ouyang Y, Gazit V, Cirrito JR, Jansen LA, Ess KC, Yamada KA, Wozniak DF, Holtzman DM, Gutmann DH, Wong M (2007) Abnormal glutamate homeostasis and impaired synaptic plasticity and learning in a mouse model of tuberous sclerosis complex. *Neurobiol Dis* **28**, 184-196.
[47] Campbell AM, Holmes O (1984) Bicuculline epileptogenesis in the rat. *Brain Res* **323**, 239-246.
[48] de Feo MR, Mecarelli O, Ricci GF (1985) Bicuculline- and allylglycine-induced epilepsy in developing rats. *Exp Neurol* **90**, 411-421.
[49] Ueda H, Ge M, Satoh M, Takagi H (1987) Subconvulsive doses of intracisternal bicuculline methiodide, a GABAA receptor antagonist, produce potent analgesia as measured in the tail pinch test in mice. *Eur J Pharmacol* **136**, 129-131.
[50] Berberich S, Jensen V, Hvalby O, Seeburg PH, Kohr G (2007) The role of NMDAR subtypes and charge transfer during hippocampal LTP induction. *Neuropharmacology* **52**, 77-86.
[51] McNamara JO, Huang YZ, Leonard AS (2006) Molecular signaling mechanisms underlying epileptogenesis. *Sci STKE* **2006**, re12.
[52] Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* **55**, 697-711.
[53] Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O (2008) Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci* **28**, 14537-14545.
[54] Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH, Haass C, Staufenbiel M, Konnerth A, Garaschuk O (2008) Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science* **321**, 1686-1689.