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Picomolar Amyloid- β Peptides Enhance Spontaneous Astrocyte Calcium Transients

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Abstract

Amyloid- β (A β) peptides are constitutively produced in the brain throughout life via mechanisms that can be regulated by synaptic activity. Although A β has been extensively studied as the pathological plaque-forming protein species in Alzheimer's disease (AD), little is known about the normal physiological function(s) and signaling pathway(s). We previously discovered that physiologically-relevant, low picomolar amounts of A β can enhance synaptic plasticity and hippocampal-dependent cognition in mice. In this study, we demonstrated that astrocytes are cellular candidates for participating in this type of A β signaling. Using calcium imaging of primary astrocyte cultures, we observed that picomolar amounts of A β peptides can enhance spontaneous intracellular calcium transient signaling. After application of 200 pM A β_{42} peptides, the frequency and amplitude averages of spontaneous cytosolic calcium transients were significantly increased. These effects were dependent on $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ -nAChRs), as the enhancement effects were blocked by a pharmacological $\alpha 7$ -nAChR inhibitor and in astrocytes from an $\alpha 7$ deficient mouse strain. We additionally examined evoked intercellular calcium wave signaling but did not detect significant picomolar A β -induced alterations in propagation parameters. Overall, these results indicate that at a physiologically-relevant low picomolar concentration, A β peptides can enhance spontaneous astrocyte calcium transient signaling via $\alpha 7$ -nAChRs. Since astrocyte-mediated gliotransmission has been previously found to have neuromodulatory roles, A β peptides may have a normal physiological function in regulating neuron-glia signaling. Dysfunction of this signaling process may underlie glia-based aspects of AD pathogenesis.

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SUPPLEMENTARY MATERIAL

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Keywords

$\alpha 7$ nicotinic acetylcholine receptor; amyloid- β ; astrocyte; calcium imaging; glia; gliotransmission

INTRODUCTION

Although amyloid- β (A β) peptides have been extensively characterized as the primary neurotoxic molecules in Alzheimer's disease (AD), A β is constitutively produced in the brain throughout life. A β peptides are present in the central nervous systems (CNS) of various mammalian species, including humans, in the absence of pathology [1, 2]. Direct measurements of interstitial fluid in the brain as well as cerebrospinal fluid indicate that the physiological concentrations for A β normally lie in the picomolar range [3, 4]. In addition, previous studies indicate that A β secretion can be regulated by neuronal activity in an endocytosis-dependent manner and appears to correlate with neurological status in humans [5–7]. In the AD field, a large amount of research has focused on high concentrations (nanomolar to micromolar) and/or aggregated assemblies, usually of the A β_{42} peptide that is the more aggregation-prone cleavage product of amyloid- β protein precursor (A β PP) (reviewed in [8]). However, the question of the normal physiological function(s) of A β , which likely occur with much lower concentrations, remains an important issue that has not been fully addressed.

Our laboratory and others have previously demonstrated that A β can have positive modulatory effects on synaptic plasticity and cognition at non-pathological, low picomolar concentrations [9–11]. Specifically, we demonstrated that both hippocampal long-term potentiation (LTP) and hippocampal-dependent learning and memory can be enhanced by the acute administration of 200 pM A β_{42} [9]. These positive nootropic effects were found to be dependent on the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ -nAChR), which has been previously described to bind A β with picomolar affinity and lead to calcium signaling effects [12–15]. While neurons do express $\alpha 7$ -nAChRs, astrocytes also express functional $\alpha 7$ -nAChRs that lead to calcium influx [16, 17] and potential release of signaling molecules such as glutamate [18].

Astrocytes are a type of glia cell that has multiple neuromodulatory functions, including the regulation of synaptic plasticity (reviewed in [19–21]). With intimate contact around a large multitude of synaptic clefts in the brain (“tripartite synapses”), astrocytes are well-positioned to sense and respond to the activity-dependent synaptic release of A β peptides. Previous studies in the AD field have shown that astrocytes can detect high concentrations of A β , undergo reactive gliosis, surround amyloid plaques and exhibit complex pathological signaling interactions with neurons and other glial cells—astrocytes are well-established as cellular participants in AD pathology (reviewed in [22]). However, it is currently unknown whether astrocytes can also sense and respond to the normal non-pathological release of picomolar amounts of A β peptides that occurs in healthy brains.

To investigate how picomolar A β affects astrocytes, we used primary murine astrocyte cultures for calcium imaging experiments. Using well-characterized A β preparation methods, we applied picomolar amounts of A β , in either predominantly monomeric or

oligomeric forms. Since intracellular calcium signaling constitutes the primary mechanism of activation in astrocytes, which are electrically silent, this experimental paradigm allowed us to directly visualize the ability of astrocytes to detect low picomolar amounts of A β peptides.

MATERIALS AND METHODS

Animals

Breeding colonies for wild-type C57BL/6J and $\alpha 7$ nAChR subunit deficient mice [23] were housed in animal facilities at Columbia University and maintained on a 12-h light/dark cycle. All experimental protocols involving animals were approved by Columbia University and the Institutional Animal Care and Use Committee.

Primary cultures

Astrocyte cultures were prepared from P0–P1 mouse pups as previously described [24, 25]. Briefly, meninges-free forebrains were dissected out and dissociated with trypsin, followed by trituration and plating in culture-treated flasks. Culture media: high-glucose Dulbecco's Modified Eagle Medium plus GlutaMax (Invitrogen), supplemented with 10% heat-inactivated fetal calf serum plus penicillin (100 U/ml) and streptomycin (100 μ g/ml). After the cells reached confluence, the flasks were shaken for 2 h on an orbital shaker. The media was replaced, and the sealed flasks were shaken again overnight (~16 h). The following day, the cultures were extensively washed and passaged onto poly-D-lysine-coated glass coverslips. In all experiments, ~12–16 DIV confluent cultures that underwent only one passage were used. Cultures were regularly checked for contamination by other cell types. The standard overnight shaking method for purifying astrocytes removes most remaining neurons/microglia/oligodendrocytes, as previously described [24, 25]. When examined by immunocytochemistry for an astrocyte marker, glial fibrillary acid protein (GFAP), the purified cultures were almost completely GFAP+ (data not shown). By light microscopy examinations, no neurons and nominal microglia and oligodendrocytes were present in the purified cultures.

Amyloid- β peptide preparation

Synthetic human A β peptides (American Peptide Company) were prepared as previously described [9, 26, 27]. Briefly, lyophilized peptides were dissolved to 1 mM in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to remove any preexisting structures [27] and dried by SpeedVac to produce peptide films. Prior to use, films were fully dissolved in anhydrous dimethylsulfoxide (DMSO) to 5 mM and briefly bath sonicated at room temperature. For fresh (predominantly monomeric) preparations, the DMSO solution was immediately diluted down to the picomolar concentration range in imaging buffer prior to an experiment. For oligomeric preparations, the DMSO solution was diluted to 100 μ M in PBS and incubated overnight at 4°C; the formation of oligomeric A β species was confirmed through western blotting with 6E10 monoclonal antibody (Covance), as previously described [9] (Supplementary Fig. 1).

Spontaneous calcium transient imaging

Confluent astrocyte cultures on glass coverslips were removed into imaging buffer. Buffer composition (in mM): NaCl (140), KCl (5), MgCl₂ (2), CaCl₂ (2), glucose (5), HEPES (10), pH 7.4. Cells were loaded with Fluo-4 AM esters (Molecular Probes) diluted in imaging buffer with Pluronic F-127 (Invitrogen). The astrocyte cultures were readily loaded with Fluo-4 dye and consistently exhibited even, homogenous loading distributions. A Nikon D-Eclipse C1 confocal microscope (488 nm argon laser) was used for all experiments. Time-lapse movies in all experiments were acquired at 0.33 Hz, generally in continuous 10 min blocks for 60 min total. In analyses of spontaneous transients, the first 10-min imaging block was performed under basal control conditions and was used to normalize subsequent data from that experiment. Movies were imported into MetaMorph software (Molecular Devices) for automated segmentation and quantification of cell body regions of interest. The entire imaging field was analyzed to minimize manual selection bias. The quantified fluorescence intensity data were analyzed in Igor Pro (Wavemetrics). All fluorescent data values were normalized to the global minimum value for that trace. Traces were plotted with these normalized data as fold changes. The basic threshold used for the identification of calcium transients was a minimum 40% rise in normalized fluorescent signal intensity. This threshold reliably detected all distinct calcium transient peaks with minimal contamination by noise. Amplitudes were calculated as (peak maximum) – (base minimum). The frequency measure reflects the absolute number of calcium transients per 10 min imaging block. Data were averaged and binned for each 10 min block during an experiment and represented as fold changes from the baseline control.

Calcium wave imaging

Intercellular calcium waves were induced as previously described by deformation of the cell membrane via a light mechanical stimulus using a glass micropipette electrode [28–30]. Glass micropipette electrodes were pulled from thick-walled borosilicate glass tubing, filled with imaging buffer, and mounted on a piezoelectric micromanipulator. With constant monitoring of the resistance, the electrode was slowly lowered toward the cell layer, briefly contacting the cell surface followed by immediate withdrawal. This mechanical stimulus reliably induced intercellular calcium waves in the astrocyte cultures. Movies were imported into Igor Pro software (Wavemetrics). All fluorescent data were normalized to the global maximum value and plotted as a percentage of this value. The radial distribution of intensities was calculated based on the manually-identified wave center initiation point. Distance and time measures were based on a threshold intensity of 50%.

Immunofluorescence

Immunostaining was performed according to standard protocols. Briefly, cultured cells were washed and fixed in paraformaldehyde followed by permeabilization with Triton X-100. Blocking was done in 1% BSA plus 5% normal goat serum. Primary antibodies were diluted in blocking solution. Antibodies used include: GFAP (Affinity Bioreagents), $\alpha 7$ nAChR subunit (Chemicon/Millipore, Santa Cruz Biotechnology). Analyses were performed with ImageJ (NIH) software.

α -Bungarotoxin surface labeling

Alexa Fluor 594-conjugated α -bungarotoxin (Invitrogen) was diluted in cell culture media to 0.1 μ M and applied to cultures for 15 min at 37°C. Cultures were fixed in paraformaldehyde, mounted with Vectashield (Vector Labs), and examined by confocal microscopy.

Western blot

Cell cultures were washed and directly lysed in 2x standard Laemmli sample buffer. Equal amounts of lysate or synthetic A β solutions were loaded in tris-glycine or tricine gels for SDS-PAGE. Proteins were transferred onto 0.2 μ m nitrocellulose membranes and subsequently blocked in 5% bovine serum albumin. Primary antibodies were diluted in blocking solution. The antibodies used include: α 7 nAChR subunit (Chemicon/Millipore, Santa Cruz Biotechnology), β -actin (Abcam), A β 6E10 (Covance). Blots were developed with enhanced chemiluminescence (ECL) substrate or scanned using the Odyssey system (Licor) for infrared labeling detection. Digitized blot images were analyzed using ImageJ (NIH) software.

ELISA

A $\beta_{(x-42)}$ ELISAs (Invitrogen) were performed according to the manufacturer's protocols. Tissue culture media was collected, supplemented with protease inhibitors (Roche), and immediately used for the ELISAs.

Statistical analyses

All data in figures are presented as means $\pm$ SEM. For the calcium imaging experiments, data were normalized to the global minimum fluorescence intensity of that experiment. Figure data are represented as fold-changes from the baseline control imaging block. Data were analyzed by two-tailed Student's *t*-test or two-way ANOVA plus *post-hoc* Tukey's multiple comparisons test using Prism (GraphPad) software. The threshold for significance was set at $p < 0.05$ in all analyses.

RESULTS

Primary murine astrocyte cultures do not secrete A β peptides under basal conditions

Prior to investigating the effects of exogenous A β peptides, we first measured the basal A β levels of the purified astrocyte cultures to ensure that levels during experiments do not exceed the range of physiologically-occurring A β concentrations (picomolar). The issue of whether or not astrocytes can express β -secretase (BACE) and cleave A β PP to produce A β remains unclear, with a few studies reporting A β production by astrocytes under certain conditions [31–33]. We found that confluent astrocyte cultures, either before (7–8 DIV) or after purification (12–16 DIV), did not secrete detectable amounts of A β_{42} (Fig. 1A). This is in contrast to mixed neuron-astrocyte cultures (14–21 DIV), which did have significant amounts of A β_{42} in the culture supernatants (50–60 pM). During a calcium imaging experiment with purified astrocyte cultures in imaging buffer, no significant amounts of endogenous A β_{42} peptides are produced throughout the duration of the 1 h experiment (Fig. 1B).

Basal spontaneous intracellular calcium transient characteristics

Spontaneous oscillating calcium transients have been observed in astrocytes *in vitro* and *in vivo* and are involved in modulating neuronal activity [34–36]. In the purified astrocyte cultures, we observed variation in the types of spontaneous calcium transients. While some cells were relatively quiescent, a significant proportion (~20–30% out of an average of 288 analyzed cells per imaged field) displayed distinct spontaneous oscillatory-type calcium transients (Fig. 2A). On average, under basal conditions, these spontaneously active astrocytes exhibited 0.24 transients/minute, with an average amplitude of 1.52 fold increase over baseline. Over the course of an hour-long experiment, there was some decay in the Fluo-4 signal amplitudes over time, particularly in the high-frequency oscillating astrocytes, and likely reflects photo-bleaching effects (Fig. 2B).

Picomolar amounts of A β ₄₂ peptides enhance spontaneous astrocyte calcium transients

To investigate the effects of A β peptides on spontaneous astrocyte calcium transient properties, we acutely applied 200 pM A β and continuously imaged the cells in 10 min blocks for a total of 60 min. The initial block served as the baseline control against which later measurements were compared to (as fold changes). We tested both freshly-prepared A β ₄₂ as well as aged, oligomerized A β ₄₂ [27] (Supplementary Fig. 1) and observed that the fresh A β preparation had significant potentiating effects on two of the analyzed calcium transient parameters: frequency and amplitude (Fig. 3A, B). While we observed that there were some nominal increases in spontaneous calcium signaling in the vehicle control experiments (Fig. 3), possibly due to stimulation from the imaging procedure and/or buffer changes, the calcium transients were significantly enhanced above control levels with the presence of picomolar A β . 200 pM fresh A β ₄₂ peptides facilitated increases in the frequencies and amplitudes of spontaneous calcium transients, evident in the imaging blocks after A β application (Fig. 3A, B, G, H), with no significant changes in the proportions of astrocytes exhibiting calcium transients (data not shown). In contrast, aged A β ₄₂ peptides did not have any modulatory effects on the analyzed transient parameters (Fig. 3A, B). Since the fresh A β ₄₂ peptides were prepared and diluted down to the picomolar range immediately prior to each experiment, the majority of the peptides were likely in predominantly monomeric form [27]. In contrast, as verified by western blot analyses, aged A β ₄₂ peptide preparations had multiple oligomeric species (and potentially additional SDS-unstable assemblies not visible by blotting) (Supplementary Fig. 1).

In the brain, A β ₄₂ is a relatively minor A β species while A β ₄₀ is the predominant form that is present at roughly ten times higher concentrations *in vivo* [3, 37, 38]. We investigated the potential role of A β ₄₀ peptides in modulating astrocyte calcium signaling and found that, in comparison to A β ₄₂ peptides, A β ₄₀ peptides do not have the same long-term enhancement effects on spontaneous calcium transients (Fig. 3C, D). Application of 200 pM freshly-prepared A β ₄₀ did not significantly impact the amplitudes of the calcium signals (Fig. 3D, H). However, A β ₄₀ did significantly facilitate calcium transient frequency at the 30-min time point (Fig. 3C, G). This was a temporally-constrained effect that decreased to baseline levels while the A β ₄₂-mediated frequency enhancement persisted for the duration of the experiment. Together, these results demonstrate that picomolar amounts of the A β ₄₂ peptide,

likely in the monomeric state, can have long-term enhancement effects on spontaneous astrocyte calcium signaling.

Enhancement of spontaneous calcium transients is specific for A β peptides

In order to verify the specificity for A β peptides in the observed enhancements of spontaneous astrocyte calcium signaling, we used an anti-A β monoclonal antibody (6E10) for immunodepletion-based neutralization experiments. 6E10 is a widely-used A β antibody that has been demonstrated to bind to monomeric and aggregated A β [26, 39]. The antibody by itself did not significantly affect astrocyte calcium signaling (Fig. 3E, F). However, concurrently washing in 6E10 together with 200 pM freshly-prepared A β ₄₂ peptides fully inhibited the enhancement effects (Fig. 3E–H). Thus, A β peptides are the specific molecules responsible for the observed enhancements of astrocyte calcium transients.

A β -mediated enhancement of calcium transients is dependent on astrocytic α 7-nAChRs

Astrocyte calcium signaling has been shown to involve various mechanisms and calcium sources, including intracellular calcium stores regulated by IP₃ and calcium-induced calcium release (CICR) [16, 40]. In elucidating a calcium signaling pathway in the observed A β -mediated enhancements, we focused on α 7-nAChRs since we previously discovered that these receptors are necessary for the facilitation of synaptic plasticity and memory following picomolar A β administration [9]. As potential receptors for mediating calcium influx, functional α 7-nAChRs that result in CICR have been previously demonstrated in astrocytes [16, 17].

We first verified that our primary astrocyte cultures do express the α 7 nAChR subunit with western blot analyses (Fig. 6A). Of note, while adult brain tissues had a single band at the expected molecular weight (~56 kDa), we observed that astrocyte cultures had a doublet band pattern; while it is unclear what the extra band may represent, this α 7 subunit doublet has been previously observed in mammalian cell cultures [41]. We further confirmed expression with immunofluorescent and live α -bungarotoxin cell-surface labeling. Both methods demonstrated a clear punctate labeling in the astrocytes (Fig. 6B, C) that is similar to that observed in cultured hippocampal neurons (Fig. 6C). Together with the previous studies which demonstrated functional α 7-nAChR-mediated currents in astrocytes, we concluded that the purified astrocyte cultures do express cell-surface α 7-nAChRs.

To investigate whether these receptors are involved in the A β -mediated enhancements of astrocyte calcium signaling, we used both a pharmacological agent and an α 7 subunit knockout mouse strain. Methyllycaconitine (MLA) is a potent α 7-nAChR antagonist which has a stronger affinity and higher-specificity binding compared to α -bungarotoxin [42]. When we first pretreated cultures with MLA and then concurrently washed in 200 pM A β ₄₂ peptides, the enhancement effects on calcium transients were abolished (Fig. 6D–G). However, MLA appeared to have a depressive effect on calcium transient frequency (Fig. 6D, F), which may be due to non-specific effects.

To further test this issue and confirm the data using another experimental approach, we also used astrocyte cultures obtained from an α 7 knockout (α 7-KO) mouse line. This α 7-KO has a targeted deletion of the last three exons of the α 7 subunit gene and lack α -bungarotoxin

binding sites [23]. This $\alpha 7$ -KO line was previously used to demonstrate that the picomolar A β -mediated enhancement of LTP and memory is dependent on $\alpha 7$ -nAChRs [9]. However, the cellular location of the necessary $\alpha 7$ -nAChRs was not determined at the time. To test the hypothesis that astrocytic $\alpha 7$ -nAChRs may be involved in picomolar A β signaling and confirm the MLA data, we used astrocyte cultures prepared from $\alpha 7$ -KO mice and littermate wild-type controls. Under basal conditions, $\alpha 7$ -KO cultures did not differ significantly from wild-type cultures (data not shown). However, confirming our results with MLA, 200 pM A β_{42} peptides did not significantly facilitate astrocyte calcium transients, in either the frequency or amplitude parameters, when $\alpha 7$ -nAChRs were absent (Fig. 6D–G).

Picomolar A β_{42} peptides do not affect evoked intercellular calcium wave propagation

Besides spontaneous calcium transients and oscillations, intercellular calcium waves have also been observed in astrocyte cultures and induced in brain slices [28, 43]. A large increase in cytosolic calcium levels spreads radially from the stimulation point to neighboring astrocytes (Fig. 7A). To investigate how the presence of picomolar A β peptides may impact astrocyte calcium wave propagation, we applied 200 pM A β_{42} for 20 min to the culture prior to inducing waves with a light mechanical stimulus. We did not detect any significant differences between the A β and vehicle groups in several calcium wave propagation parameters: distance travelled, time span, velocity, and maximal calcium signal intensity (Fig. 7B–E). We conclude that picomolar A β_{42} peptides do not significantly affect the propagation of evoked intercellular astrocyte calcium waves.

Summary of picomolar A β_{42} -mediated effects on astrocyte calcium signaling

In conclusion, we discovered that 200 pM A β_{42} peptides can enhance spontaneous cytosolic calcium transients but not evoked intercellular calcium waves. After application of A β , the frequency and amplitude of spontaneous calcium transients were significantly increased starting from 10–20 min after A β exposure, with the frequency facilitation persisting until the end of the 1 h experiment (Fig. 3). These facilitation effects were prevented by the 6E10 A β antibody, MLA $\alpha 7$ -nAChR antagonist and in $\alpha 7$ -KO astrocytes (Figs. 3, 4). Overall, these data support the existence of an $\alpha 7$ -nAChR-dependent astrocyte calcium signaling pathway that is responsive to A β peptides at physiologically-relevant picomolar concentrations.

DISCUSSION

Despite the wealth of research on the pathological properties of A β , little is known about the normal physiological functions of this peptide that is ubiquitously present throughout the CNS. Here, we have demonstrated that one possible role for A β is as a signaling molecule in neuron-glia communication. The ability of astrocytes to detect and respond to synaptically-released molecules is well-established. The most prominent example is the neurotransmitter glutamate, which has been shown by multiple studies to be detectable by astrocytes *in vitro* and *in situ*; activation of glutamate receptors on astrocytes can lead to intracellular calcium elevations observed at both the whole-cell and microdomain levels [44–46]. We hypothesized that astrocytes may similarly sense physiological A β release, which can also be regulated by neuronal activity and has been linked with synaptic vesicle exocytosis [6].

Our data presented here support this hypothesis and demonstrate that astrocytes can detect A β at the low picomolar concentrations that are typically found in brain interstitial fluid. When exposed to 200 pM A β ₄₂, a concentration previously found to enhance synaptic plasticity and spatial learning and memory [9], spontaneous cytosolic calcium signaling was significantly enhanced. Specifically, the frequency and amplitude averages of spontaneous calcium transients were positively modulated after picomolar A β application. Our results are consistent with previous data demonstrating astrocyte detection of other neuro-active molecules such as glutamate and adenosine. As described above, when astrocytes are activated by glutamate application, cytosolic calcium signaling is increased [44, 47, 48]. Similarly, adenosine has also been shown to trigger long-lasting facilitation of spontaneous calcium transients in astrocytes *in situ* [49]. We have now discovered that astrocytes are additionally capable of detecting physiologically-relevant amounts of A β and responding with enhanced spontaneous calcium oscillations.

Spontaneous astrocyte calcium transients have been characterized in multiple studies *in vitro*, *in situ*, and *in vivo* [34–36, 48, 50]. Of note, the magnitude, frequency, and patterns of the spontaneous cytosolic calcium events observed *in vivo* in the cortex were found to be qualitatively similar to those observed in cultures and acute brain slices [36]. Our characterization of basal spontaneous calcium signaling in purified astrocyte cultures (Fig. 2) are consistent with these data; for example, the frequencies of the observed calcium transients are within the range of what was previously observed *in vivo* [36]. We take such similarities to indicate that our results are relevant for extrapolating to astrocytes in the context of the brain.

Neuron-glia communication is bidirectional; astrocyte calcium elevations have direct functional impact on neurotransmission via the release of gliotransmitters such as glutamate, ATP, and D-serine. These gliotransmitters signal at multiple neuronal receptors including AMPA receptors [51], NMDA receptors [34], and purinergic receptors [46]. Functionally, astrocytes exert multiple effects including the modulation of inhibitory transmission [52], presynaptic release properties [53], and hippocampal LTP [54]. Based on our previous discovery that picomolar A β can positively modulate hippocampal LTP, we hypothesize that astrocytes serve as cellular mediators in this A β signaling pathway. With regards specifically to spontaneous astrocyte calcium oscillations, one of the functions of these events is the triggering of NMDAR-mediated slow inward currents in nearby neurons [34], which may facilitate the synchronization of neuronal activity [55, 56]. By modulating spontaneous calcium oscillations in astrocytes, A β may thus function as a neuron-glia signal that provides feedback regulation contributing to the enhanced LTP and memory we have previously discovered with picomolar A β . The astrocyte-mediated regulation of LTP was previously determined to depend on D-serine gliotransmitter [54] while astrocyte-induced slow inward currents are caused by glutamate gliotransmitter [55]. It remains to be investigated in a future study whether the picomolar A β -mediated enhancements of astrocyte calcium signaling results in the release of D-serine, glutamate, and/or other gliotransmitters.

We observed in this study that fresh, HFIP-treated A β ₄₂ preparations, but not aged oligomerized A β ₄₂ preparations, were capable of enhancing astrocyte calcium transients (Fig. 3). However, we would like to note that while monomeric A β ₄₂ may be a primary A β

species involved in the calcium signaling facilitation, we cannot exclude the presence of transient (and potentially SDS-unstable) oligomeric species in the fresh preparations that may also contribute to the observed effects on calcium transients. It is well-established that high concentrations (nanomolar to micromolar) of soluble, SDS-stable A β ₄₂ aggregates are pathological and constitute a primary neurotoxic species in AD (reviewed in [8]). However, the normal physiological conformation(s) of A β in the healthy brain is currently unclear and may include monomers and/or possibly soluble oligomers. Future experiments beyond the scope of this study will be necessary to fully define this aspect.

We further determined that α 7 nicotinic receptors on astrocytes mediate the picomolar A β effects on calcium signaling. This is consistent with previous data demonstrating that the A β -mediated enhancement of hippocampal LTP and cognition is specifically blocked by α 7-nAChR pharmacological antagonists and is absent in α 7 subunit knockout mice [9]. In this study, we have now determined a likely cellular location of the requisite α 7-nAChRs—astrocytes—although the involvement of neuronal α 7-nAChRs cannot be ruled out.

In support of the view that α 7-nAChRs can mediate A β signaling, other studies have found that A β peptides can either directly bind or indirectly affect α 7-nAChRs with high picomolar affinity; depending on A β concentration and experimental conditions, A β may consequently act as either an α 7-nAChR agonist or antagonist [12–14, 57–60]. Particularly relevant to this study, picomolar amounts of A β ₄₂ were found to bind to the receptor with very high affinity and induce sustained calcium increases in hippocampal and neocortical nerve endings [13]. In *Xenopus* oocytes, low picomolar A β ₄₂ has also been found to activate α 7-nAChRs and lead to inward currents with a calcium component [14]. It is important to note that at higher nanomolar to micromolar concentrations, A β peptides have been found to have the opposite or no effects on calcium dynamics [59, 60], indicating that there are likely different mechanisms of action for different A β concentrations and/or aggregation states. With toxic pathological A β exposure, alternative signaling mechanisms may underlie an inhibition of α 7-nAChR-mediated calcium signaling.

In cultured astrocytes and gliosome preparations, α 7-nAChR activation has been shown to lead to calcium elevations via calcium-induced-calcium-release [16] and can result in glutamate gliotransmitter release [18], respectively. These findings are supported by experiments with acute hippocampal slices, in which synaptic acetylcholine release leads to intracellular store-dependent calcium elevations in astrocytes [61]. Therefore, given that A β peptides can be synaptically regulated and bind to α 7-nAChRs with picomolar affinity, and given that these receptors are able to lead to astrocyte calcium signaling and gliotransmitter release, the α 7-nAChR is well-poised to be a molecular link between physiological A β signaling and astrocyte-mediated neuromodulation.

In neurodegenerative states such as AD, our proposed neuromodulatory A β signaling pathway could become dysregulated due to alterations in A β processing/aggregation, astrocyte functioning, and/or expression of cholinergic receptors [8]. In particular, as potent participants in the innate immune system of the CNS, astrocytes have been well-characterized for their involvement in the inflammation-related aspects of AD. Under pathological A β conditions, astrocytes undergo reactive gliosis, surround amyloid plaques,

and produce pro-inflammatory molecules [62, 63]. In addition to these inflammation-related responses, the expression of cholinergic receptors is also altered. In human AD patient brain tissue, there is a selective increase in the expression of the $\alpha 7$ subunit on astrocytes despite a general decrease in overall nicotinic receptor expression [64–67]. This finding was supported by an *in vitro* study which demonstrated a significant upregulation of the $\alpha 7$ receptor subunit in cultured astrocytes when exposed to exogenous $A\beta_{42}$ peptides [68]. These studies demonstrate the impact of pathological $A\beta$ signaling on astrocytic nicotinic receptors. Based on the data from our current study, we hypothesize that such effects could be based on the derailment and dysregulation of a normal physiological $A\beta$ - $\alpha 7$ -nAChR signaling pathway in astrocytes.

Astrocyte calcium signaling has also been shown to be affected by pathological $A\beta$ conditions. Previous studies have shown that very high concentrations of aggregated $A\beta$ can lead to abnormal intracellular calcium signaling in astrocytes *in vitro* and *in vivo*. In astrocyte cultures, pathological $A\beta$ can cause abnormal calcium influx and intracellular signaling [69, 70]. Live calcium imaging of the $A\beta$ PP/PS1 double transgenic AD mouse model provided further support for these findings; in older mice with amyloid plaques, the proportion of astrocytes with calcium oscillations was increased along with the amplitudes of those oscillations [71]. In light of our current data, these findings could be interpreted to indicate that pathological $A\beta$ -mediated calcium signaling dysfunction in astrocytes represents a “hijacking” of an underlying physiological $A\beta$ signaling pathway. In the case of the transgenic AD mice, the chronically high concentrations of $A\beta$ peptides could lead to dysregulated activation of astrocytes. In addition, the specific upregulation of $\alpha 7$ -nAChRs on astrocytes with AD pathology would further exacerbate this pathological effect. This type of dysfunction would be expected to significantly contribute to AD pathology, given the importance of astrocytes in both synaptic physiology and immune regulation.

Another calcium signaling phenomenon that was observed in the $A\beta$ PP/PS1 mice was intercellular astrocyte calcium waves. These waves were initially described for cultures and brain slices and can be triggered by mechanical stimulation as well as strong electrical stimulation of nearby neurons [28, 43]. However, the physiological relevance of these calcium waves remains unclear. In the $A\beta$ PP/PS1 imaging study, intercellular calcium waves were rare and only occurred in the older transgenic mice with extensive plaque pathology [71]. This is supported by another *in vivo* astrocyte imaging study which did not detect calcium waves in wild-type mice [36]. Since the only observed instances of astrocyte calcium waves *in vivo* were in aged $A\beta$ PP/PS1 mice, calcium waves likely represent a pathological signaling event and thus are more likely to be affected by high $A\beta$ levels rather than low picomolar amounts. This is supported by our data which demonstrate that low physiologically-relevant amounts of $A\beta$ do not impact evoked calcium wave parameters (Fig. 5). At a much higher, pathological concentration (5 μ M), $A\beta_{42}$ has been previously described to increase several wave parameters [72], which further supports the idea that intercellular waves are pathology-related phenomenon facilitated by abnormal $A\beta$ levels.

In conclusion, we have demonstrated that picomolar amounts of $A\beta$ peptides, such as occurs *in vivo* in the healthy brain, can be detected via an $\alpha 7$ -nAChR-dependent mechanism in astrocytes, which respond with enhanced spontaneous calcium transient signaling. This

physiologically-relevant pathway may underlie certain glia-related aspects of AD pathology, including aberrant astrocyte activation and ensuing deleterious downstream effects on synaptic physiology. By understanding the basic biology of A β peptides and the role(s) of astrocytes in normal A β signaling, we can gain insight into the disease process and enlighten AD research and drug discovery efforts.

Supplementary Material

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References

1. Shoji M, Kanai M. Cerebrospinal fluid Abeta40 and Abeta42: Natural course and clinical usefulness. *J Alzheimers Dis.* 2001; 3:313–321.
2. Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid *in vivo*. *Nat Med.* 2006; 12:856–861. [PubMed: 16799555]
3. Cirrito JR, May PC, O'Dell MA, Taylor JW, Parsadanian M, Cramer JW, Audia JE, Nissen JS, Bales KR, Paul SM, DeMattos RB, Holtzman DM. *In vivo* assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *J Neurosci.* 2003; 23:8844–8853. [PubMed: 14523085]
4. Ramsden M, Nyborg AC, Murphy MP, Chang L, Stanczyk FZ, Golde TE, Pike CJ. Androgens modulate beta-amyloid levels in male rat brain. *J Neurochem.* 2003; 87:1052–1055. [PubMed: 14622134]
5. Cirrito JR, Yamada KA, Finn MB, Sloviter RS, Bales KR, May PC, Schoepp DD, Paul SM, Mennerick S, Holtzman DM. Synaptic activity regulates interstitial fluid amyloid-beta levels *in vivo*. *Neuron.* 2005; 48:913–922. [PubMed: 16364896]
6. Cirrito JR, Kang JE, Lee J, Stewart FR, Verges DK, Silverio LM, Bu G, Mennerick S, Holtzman DM. Endocytosis is required for synaptic activity-dependent release of amyloid-beta *in vivo*. *Neuron.* 2008; 58:42–51. [PubMed: 18400162]
7. Brody DL, Magnoni S, Schwetye KE, Spinner ML, Esparza TJ, Stocchetti N, Zipfel GJ, Holtzman DM. Amyloid-beta dynamics correlate with neurological status in the injured human brain. *Science.* 2008; 321:1221–1224. [PubMed: 18755980]
8. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol.* 2007; 8:101–112. [PubMed: 17245412]
9. Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci.* 2008; 28:14537–14545. [PubMed: 19118188]
10. Puzzo D, Privitera L, Fa M, Staniszewski A, Hashimoto G, Aziz F, Sakurai M, Ribe EM, Troy CM, Mercken M, Jung SS, Palmeri A, Arancio O. Endogenous amyloid-beta is necessary for hippocampal synaptic plasticity and memory. *Ann Neurol.* 2011; 69:819–830. [PubMed: 21472769]
11. Garcia-Osta A, Alberini CM. Amyloid beta mediates memory formation. *Learn Mem.* 2009; 16:267–272. [PubMed: 19318468]

12. Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. Beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. *J Biol Chem.* 2000; 275:5626–5632. [PubMed: 10681545]
13. Dougherty JJ, Wu J, Nichols RA. Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex. *J Neurosci.* 2003; 23:6740–6747. [PubMed: 12890766]
14. Dineley KT, Bell KA, Bui D, Sweatt JD. Beta-Amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J Biol Chem.* 2002; 277:25056–25061. [PubMed: 11983690]
15. Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD. Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: *In vitro* and *in vivo* mechanisms related to Alzheimer's disease. *J Neurosci.* 2001; 21:4125–4133. [PubMed: 11404397]
16. Sharma G, Vijayaraghavan S. Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. *Proc Natl Acad Sci U S A.* 2001; 98:4148–4153. [PubMed: 11259680]
17. Oikawa H, Nakamichi N, Kambe Y, Ogura M, Yoneda Y. An increase in intracellular free calcium ions by nicotinic acetylcholine receptors in a single cultured rat cortical astrocyte. *J Neurosci Res.* 2005; 79:535–544. [PubMed: 15635597]
18. Patti L, Raiteri L, Grilli M, Zappettini S, Bonanno G, Marchi M. Evidence that alpha7 nicotinic receptor modulates glutamate release from mouse neocortical gliosomes. *Neurochem Int.* 2007; 51:1–7. [PubMed: 17462791]
19. Perea G, Navarrete M, Araque A. Tripartite synapses: Astrocytes process and control synaptic information. *Trends Neurosci.* 2009; 32:421–431. [PubMed: 19615761]
20. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: The revolution continues. *Nat Rev Neurosci.* 2005; 6:626–640. [PubMed: 16025096]
21. Halassa MM, Fellin T, Haydon PG. The tripartite synapse: Roles for gliotransmission in health and disease. *Trends Mol Med.* 2007; 13:54–63. [PubMed: 17207662]
22. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. *Neurobiol Aging.* 2000; 21:383–421. [PubMed: 10858586]
23. Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW, Beaudet AL. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci.* 1997; 17:9165–9171. [PubMed: 9364063]
24. Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci U S A.* 1995; 92:3948–3952. [PubMed: 7732010]
25. Parpura V, Fang Y, Basarsky T, Jahn R, Haydon PG. Expression of synaptobrevin II, cellubrevin and syntaxin but not SNAP-25 in cultured astrocytes. *FEBS Lett.* 1995; 377:489–492. [PubMed: 8549782]
26. Dahlgren KN, Manelli AM, Stine WB Jr, Baker LK, Krafft GA, LaDu MJ. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem.* 2002; 277:32046–32053. [PubMed: 12058030]
27. Stine WB Jr, Dahlgren KN, Krafft GA, LaDu MJ. *In vitro* characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. *J Biol Chem.* 2003; 278:11612–11622. [PubMed: 12499373]
28. Charles AC, Merrill JE, Dirksen ER, Sanderson MJ. Intercellular signaling in glial cells: Calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron.* 1991; 6:983–992. [PubMed: 1675864]
29. Scemes E, Giaume C. Astrocyte calcium waves: What they are and what they do. *Glia.* 2006; 54:716–725. [PubMed: 17006900]

30. Innocenti B, Parpura V, Haydon PG. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *J Neurosci*. 2000; 20:1800–1808. [PubMed: 10684881]
31. Rossner S, Apelt J, Schliebs R, Perez-Polo JR, Bigl V. Neuronal and glial beta-secretase (BACE) protein expression in transgenic Tg2576 mice with amyloid plaque pathology. *J Neurosci Res*. 2001; 64:437–446. [PubMed: 11391698]
32. Rossner S. New players in old amyloid precursor protein-processing pathways. *Int J Dev Neurosci*. 2004; 22:467–474. [PubMed: 15465276]
33. Grolla AA, Fakhfour G, Balzaretto G, Marcello E, Gardoni F, Canonico P, Diluca M, Genazzani AA, Lim D. Abeta leads to Ca(2+) signaling alterations and transcriptional changes in glial cells. *Neurobiol Aging*. 2013; 34:511–522. [PubMed: 22673114]
34. Parri HR, Gould TM, Crunelli V. Spontaneous astrocytic Ca²⁺ oscillations *in situ* drive NMDAR-mediated neuronal excitation. *Nat Neurosci*. 2001; 4:803–812. [PubMed: 11477426]
35. Nett WJ, Oloff SH, McCarthy KD. Hippocampal astrocytes *in situ* exhibit calcium oscillations that occur independent of neuronal activity. *J Neurophysiol*. 2002; 87:528–537. [PubMed: 11784768]
36. Hirase H, Qian L, Bartho P, Buzsaki G. Calcium dynamics of cortical astrocytic networks *in vivo*. *PLoS Biol*. 2004; 2:E96. [PubMed: 15094801]
37. Dovey HF, Suomensaaari-Chrysler S, Lieberburg I, Sinha S, Keim PS. Cells with a familial Alzheimer's disease mutation produce authentic beta-peptide. *Neuroreport*. 1993; 4:1039–1042. [PubMed: 8241460]
38. Asami-Odaka A, Ishibashi Y, Kikuchi T, Kitada C, Suzuki N. Long amyloid beta-protein secreted from wild-type human neuroblastoma IMR-32 cells. *Biochemistry*. 1995; 34:10272–10278. [PubMed: 7640283]
39. Kaye R, Head E, Sarsoza F, Saing T, Cotman CW, Necula M, Margol L, Wu J, Breydo L, Thompson JL, Rasool S, Gurlo T, Butler P, Glabe CG. Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Mol Neurodegener*. 2007; 2:18. [PubMed: 17897471]
40. Fiacco TA, McCarthy KD. Astrocyte calcium elevations: Properties, propagation, and effects on brain signaling. *Glia*. 2006; 54:676–690. [PubMed: 17006896]
41. Williams ME, Burton B, Urrutia A, Shcherbatko A, Chavez-Noriega LE, Cohen CJ, Aiyar J. Ric-3 promotes functional expression of the nicotinic acetylcholine receptor alpha7 subunit in mammalian cells. *J Biol Chem*. 2005; 280:1257–1263. [PubMed: 15504725]
42. Davies AR, Hardick DJ, Blagbrough IS, Potter BV, Wolstenholme AJ, Wonnacott S. Characterisation of the binding of [3H]methyllycaconitine: A new radioligand for labelling alpha7-type neuronal nicotinic acetylcholine receptors. *Neuropharmacology*. 1999; 38:679–690. [PubMed: 10340305]
43. Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron*. 1992; 8:429–440. [PubMed: 1347996]
44. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science*. 1990; 247:470–473. [PubMed: 1967852]
45. Bernardinelli Y, Salmon C, Jones EV, Farmer WT, Stellwagen D, Murai KK. Astrocytes display complex and localized calcium responses to single-neuron stimulation in the hippocampus. *J Neurosci*. 2011; 31:8905–8919. [PubMed: 21677174]
46. Panatier A, Vallee J, Haber M, Murai KK, Lacaille JC, Robitaille R. Astrocytes are endogenous regulators of Basal transmission at central synapses. *Cell*. 2011; 146:785–798. [PubMed: 21855979]
47. Fatatis A, Russell JT. Spontaneous changes in intracellular calcium concentration in type I astrocytes from rat cerebral cortex in primary culture. *Glia*. 1992; 5:95–104. [PubMed: 1349589]
48. Zur Nieden R, Deitmer JW. The role of metabotropic glutamate receptors for the generation of calcium oscillations in rat hippocampal astrocytes *in situ*. *Cereb Cortex*. 2006; 16:676–687. [PubMed: 16079243]
49. Kawamura M Jr, Kawamura M. Long-term facilitation of spontaneous calcium oscillations in astrocytes with endogenous adenosine in hippocampal slice cultures. *Cell Calcium*. 2011; 49:249–258. [PubMed: 21402407]

50. Parri HR, Crunelli V. The role of Ca²⁺ in the generation of spontaneous astrocytic Ca²⁺ oscillations. *Neuroscience*. 2003; 120:979–992. [PubMed: 12927204]
51. Fiacco TA, McCarthy KD. Intracellular astrocyte calcium waves *in situ* increase the frequency of spontaneous AMPA receptor currents in CA1 pyramidal neurons. *J Neurosci*. 2004; 24:722–732. [PubMed: 14736858]
52. Kang J, Jiang L, Goldman SA, Nedergaard M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat Neurosci*. 1998; 1:683–692. [PubMed: 10196584]
53. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci*. 2007; 10:331–339. [PubMed: 17310248]
54. Henneberger C, Papouin T, Oliet SH, Rusakov DA. Long-term potentiation depends on release of D-serine from astrocytes. *Nature*. 2010; 463:232–236. [PubMed: 20075918]
55. Angulo MC, Kozlov AS, Chrapak S, Audinat E. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. *J Neurosci*. 2004; 24:6920–6927. [PubMed: 15295027]
56. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron*. 2004; 43:729–743. [PubMed: 15339653]
57. Wang HY, Lee DH, Davis CB, Shank RP. Amyloid peptide Abeta(1–42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors. *J Neurochem*. 2000; 75:1155–1161. [PubMed: 10936198]
58. Oddo S, LaFerla FM. The role of nicotinic acetylcholine receptors in Alzheimer's disease. *J Physiol Paris*. 2006; 99:172–179. [PubMed: 16448808]
59. Pettit DL, Shao Z, Yakel JL. Beta-Amyloid(1–42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J Neurosci*. 2001; 21:RC120. [PubMed: 11150356]
60. Liu Q, Kawai H, Berg DK. beta-Amyloid peptide blocks the response of alpha7-containing nicotinic receptors on hippocampal neurons. *Proc Natl Acad Sci U S A*. 2001; 98:4734–4739. [PubMed: 11274373]
61. Araque A, Martin ED, Perea G, Arellano JJ, Buzo W. Synaptically released acetylcholine evokes Ca²⁺ elevations in astrocytes in hippocampal slices. *J Neurosci*. 2002; 22:2443–2450. [PubMed: 11923408]
62. Dong Y, Benveniste EN. Immune function of astrocytes. *Glia*. 2001; 36:180–190. [PubMed: 11596126]
63. Johnstone M, Gearing AJ, Miller KM. A central role for astrocytes in the inflammatory response to beta-amyloid; chemokines, cytokines and reactive oxygen species are produced. *J Neuroimmunol*. 1999; 93:182–193. [PubMed: 10378882]
64. Picciotto MR, Zoli M. Nicotinic receptors in aging and dementia. *J Neurobiol*. 2002; 53:641–655. [PubMed: 12436427]
65. Teaktong T, Graham AJ, Court JA, Perry RH, Jaros E, Johnson M, Hall R, Perry EK. Nicotinic acetylcholine receptor immunohistochemistry in Alzheimer's disease and dementia with Lewy bodies: Differential neuronal and astroglial pathology. *J Neurol Sci*. 2004; 225:39–49. [PubMed: 15465084]
66. Yu WF, Guan ZZ, Bogdanovic N, Nordberg A. High selective expression of alpha7 nicotinic receptors on astrocytes in the brains of patients with sporadic Alzheimer's disease and patients carrying Swedish APP 670/671 mutation: A possible association with neuritic plaques. *Exp Neurol*. 2005; 192:215–225. [PubMed: 15698636]
67. Teaktong T, Graham A, Court J, Perry R, Jaros E, Johnson M, Hall R, Perry E. Alzheimer's disease is associated with a selective increase in alpha7 nicotinic acetylcholine receptor immunoreactivity in astrocytes. *Glia*. 2003; 41:207–211. [PubMed: 12509811]
68. Xiu J, Nordberg A, Zhang JT, Guan ZZ. Expression of nicotinic receptors on primary cultures of rat astrocytes and up-regulation of the alpha7, alpha4 and beta2 subunits in response to nanomolar concentrations of the beta-amyloid peptide(1–42). *Neurochem Int*. 2005; 47:281–290. [PubMed: 15955596]

69. Abramov AY, Canevari L, Duchen MR. Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. *J Neurosci.* 2003; 23:5088–5095. [PubMed: 12832532]
70. Chow SK, Yu D, Macdonald CL, Buibas M, Silva GA. Amyloid beta-peptide directly induces spontaneous calcium transients, delayed intercellular calcium waves and gliosis in rat cortical astrocytes. *ASN Neuro.* 2010; 2:e00026. [PubMed: 20001968]
71. Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ. Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science.* 2009; 323:1211–1215. [PubMed: 19251629]
72. Haughey NJ, Mattson MP. Alzheimer's amyloid beta-peptide enhances ATP/gap junction-mediated calcium-wave propagation in astrocytes. *Neuromolecular Med.* 2003; 3:173–180. [PubMed: 12835512]

Fig. 1.

Primary astrocyte cultures do not secrete significant amounts of Aβ₄₂ peptides. A) Aβ_(x-42) ELISA with culture supernatants from purified astrocyte cultures (12–16 DIV; $n = 4$), initial pre-purification astrocyte cultures (7–8 DIV; $n = 2$), and mixed neuron-astrocyte co-cultures (14–21 DIV; $n = 4$). B) Aβ_(x-42) ELISA with a time-course of supernatants collected: before the start of an experiment (baseline), immediately after media removal and imaging buffer wash-in (time-point 0), 30 min into an imaging experiment, and 60 min into an imaging experiment. Data presented as means ± SEM.

Fig. 2. Spontaneous intracellular calcium transients in cultured astrocytes. A) Example calcium imaging traces from individual cells (normalized to baseline). B) Decay of signal amplitude over time in oscillating cells. Data represented as normalized fluorescence intensity values (F/F_0).

Fig. 3.

Picomolar Aβ₄₂ peptides enhance spontaneous astrocyte calcium transients. A) Frequency of spontaneous calcium transients. 200 pM Aβ₄₂ (freshly prepared or aged oligomerized) were applied at the beginning of the second (20 min) imaging block (indicated by arrow). Vehicle *n* = 16 (4554 cells), fresh Aβ₄₂ 200 pM *n* = 18 (5241 cells), aged Aβ₄₂ 200 pM *n* = 17 (4798 cells). B) Amplitude of spontaneous calcium transients corresponding to A. C) Frequency of spontaneous calcium transients. 200 pM Aβ peptides (freshly prepared, Aβ₄₂, or Aβ₄₀) were applied at the beginning of the second (20 min) imaging block (arrow). Vehicle *n* = 16 (4554 cells), Aβ₄₂ 200 pM *n* = 18 (5241 cells), Aβ₄₀ 200 pM *n* = 6 (1723 cells). D) Amplitude of spontaneous calcium transients corresponding to C. E) Frequency of spontaneous calcium transients. 200 pM Aβ₄₂ (freshly prepared) and/or 6E10 antibodies were applied at the beginning of the second (20 min) imaging block (arrow). Vehicle *n* = 16 (4554 cells), Aβ₄₂ 200 pM *n* = 18 (5241 cells), 6E10 *n* = 4 (1209 cells), Aβ₄₂ 200 pM + 6E10 *n* = 8 (2395 cells). F) Amplitude of spontaneous calcium transients corresponding to E. G) Summary of frequency data at the 30 and 40 min imaging blocks. H) Summary of amplitude data at the

30 and 40 min imaging blocks. Data presented as means $\pm$ SEM, normalized as fold changes from the baseline control block. * p $\leq$ 0.05, ** p $\leq$ 0.01, *** p $\leq$ 0.001 compared to vehicle control by ANOVA.

Fig. 4.

Enhancement of calcium transients by A β ₄₂ peptides requires α 7-nAChRs. A) Western blot of astrocyte cultures and adult mouse brain tissues. Lanes 1 and 2: purified astrocyte cultures, lane 3: cortex, lane 4: hippocampus. B) α 7 nAChR subunit immunocytochemistry with purified astrocyte culture (red: α 7 subunit, blue: DAPI nuclear stain). C) Live cell-surface labeling of hippocampal neuron (left) and astrocyte (right) cultures with Alexa Fluor 594-conjugated α -bungarotoxin. D) Frequency of spontaneous calcium transients. 200 pM A β peptides (freshly prepared) and/or MLA were applied at the beginning of the second (20 min) imaging block (arrow). Vehicle $n = 16$ (4554 cells), A β ₄₂ 200 pM $n = 18$ (5241 cells), A β ₄₂ 200 pM + MLA $n = 13$ (3929 cells), α 7 knockout + A β ₄₂ 200 pM $n = 9$ (2801 cells). E) Amplitude of spontaneous calcium transients. F) Summary of frequency data at the 30 and 40 min imaging blocks. G) Summary of the amplitude data at the 30 and 40 min imaging blocks. Data presented as means $\pm$ SEM, normalized as fold changes from the baseline control block. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to vehicle control by ANOVA.

Fig. 5. Evoked intercellular calcium wave propagation is not significantly affected by picomolar Aβ₄₂ peptides. A) Representative time-lapse series of an evoked intercellular calcium wave in an astrocyte culture (psuedocolored). Labels indicate time elapsed in seconds from stimulation (B is baseline). B) Distance travelled by calcium waves. Vehicle $n = 11$, Aβ₄₂ 200 pM $n = 12$. C) Time span of calcium waves. D) Radial velocity of calcium waves. E) Maximal fluorescence intensity of calcium waves (normalized to baseline). *n.s.*=not significant. Data presented as means ± SEM.