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RESEARCH

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Astrocytes respond to a neurotoxic A β fragment with state-dependent Ca²⁺ alteration and multiphasic transmitter release

Cuong Pham¹, Karine Hérault², Martin Oheim³, Steeve Maldera¹, Vincent Vialou¹, Bruno Cauli¹ and Dongdong Li^{1*} 

Abstract

Excessive amounts of amyloid β (A β) peptide have been suggested to dysregulate synaptic transmission in Alzheimer's disease (AD). As a major type of glial cell in the mammalian brain, astrocytes regulate neuronal function and undergo activity alterations upon A β exposure. Yet the mechanistic steps underlying astrocytic responses to A β peptide remain to be elucidated. Here by fluorescence imaging of signaling pathways, we dissected astrocytic responses to A β 25–35 peptide, a neurotoxic A β fragment present in AD patients. In native health astrocytes, A β 25–35 evoked Ca²⁺ elevations via purinergic receptors, being also dependent on the opening of connexin (CX) hemichannels. A β 25–35, however, induced a Ca²⁺ diminution in A β -preconditioned astrocytes as a result of the potentiation of the plasma membrane Ca²⁺ ATPase (PMCA). The PMCA and CX protein expression was observed with immunostaining in the brain tissue of hAPPJ20 AD mouse model. We also observed both Ca²⁺-independent and Ca²⁺-dependent glutamate release upon astrocytic A β exposure, with the former mediated by CX hemichannel and the latter by both anion channels and lysosome exocytosis. Our results suggest that A β peptide causes state-dependent responses in astrocytes, in association with a multiphasic release of signaling molecules. This study therefore helps to understand astrocyte engagement in AD-related amyloidopathy.

Keywords: ATP, Glutamate, Hemichannel, Lysosome, Alzheimer's disease

Introduction

Toxic A β peptides are implicated in the development of cognitive deficits of AD [10]. In the mammalian brain, information processing is sustained by dynamic interactions between neurons and glial cells [13]. Emerging evidence suggests that A β dysregulates neuron-glia communication thereby impairing synaptic transmission [33]. In the meanwhile, therapeutics targeting neuronal dysfunctions yield only limited effects [32], urging the need

to examine A β -caused pathological adaptations in glial signaling.

Astrocytes are the major glial cell-type in the mammalian brain [34]. Albeit electrically non-excitable, their activity is encoded by intracellular Ca²⁺ signaling [43], which in turn modulates neuron activity, via for instance regulating ambient transmitter and ion recycling [19, 91], the delivery of energy fuels [76], the peri-synaptic structural remodeling [69] as well as the release of transmitter substances [5]. Astrocyte Ca²⁺ activity has been shown to be upregulated by A β peptides [2, 11, 85], and near amyloid plaques in AD mouse models [17, 47]. A β -caused Ca²⁺ hyperactivity was found to compromise neuronal survival [1]. Accordingly, inhibiting the Ca²⁺-dependent protein phosphatase calcineurin in astrocytes ameliorates

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synaptic function in AD mouse model [27]. In addition, the cognitive deficit during AD progression has been attributed to synapse excitotoxicity, a process involving aberrated astrocytic handling of neurotransmitter recycling and signaling molecule release [63, 64, 90, 98]. While astrocytes are being recognized to participate in AD amyloidopathy, the dynamically weaved signaling cascades remain to be delineated.

Here, we imaged astrocytic signaling cascades in response to A β 25–35, a neurotoxic A β fragment found in AD patients [4, 46, 57]. We observed that A β 25–35 upregulated Ca²⁺ signals in primary astrocytes derived from mouse cortex, which involved the activation of metabotropic P2Y receptor and the opening of CX hemichannel. In contrast, A β 25–35 caused a Ca²⁺ diminution in A β -preconditioned astrocytes that involved the potentiated Ca²⁺ extrusion via PMCA and the activation of cAMP signal. We further observed both Ca²⁺-independent and -dependent glutamate release in astrocytes upon A β 25–35 exposure, which relied respectively on CX hemichannel, anion channels and lysosome exocytosis. These results show a state-dependent adaptation in astrocyte responses to neurotoxic A β peptide, and suggest molecular targets to control astrocyte functions in AD amyloidopathy.

Materials and methods

Animals and preparation of primary cortical astrocytes

Our laboratory follows the European Union and institutional guidelines for the care and use of experimental animals (Council directive 86/609EEC). The care of experimental animals was also in conformity with the French National Charter on the ethics of animal experimentation. Primary astrocytes were cultured from the neocortex of P0-1 NMRI mice of either sex adapted from the published protocol [52, 56]. Astrocytes were plated in Petri dishes for 1 week prior to being transferred to cover slips (#1, BK-7, 25-mm, Menzel-Gläser) coated with poly-ornithine (Sigma). Cells were kept at 37 °C in a humidified 5% CO₂ atmosphere in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 5% fetal bovine serum (FBS, HyClone), penicillin (5 U/ml, Sigma), and streptomycin (5 µg/ml, Sigma). Recordings were made during the following week at room temperature (RT, 22–23 °C) in the standard extracellular saline containing (in mM): 140 NaCl, 5.5 KCl, 1.8 CaCl₂, 1 MgCl₂, 20 glucose, 10 HEPES (pH 7.3, adjusted with NaOH). The hAPPJ20 AD mouse model was obtained from The Jackson Lab (No: 34836-JAX), expressing mutated human amyloid precursor protein (hAPP) monogene comprising the Swedish (K670N/M671L) and the Indiana (V717F) mutations [44, 60]. The overexpression of the mutated hAPP was controlled

under the human platelet-derived growth factor beta polypeptide promoter. This AD mouse model displays diffusive A β peptides at age ~5–7 months and plaques by age ~8–10 months [44, 60]. Breeding was made between hemizygote males and C57BL/6 females to obtain hAPPJ20 mice (~7 month old) for slice immunohistochemistry as stated below.

Fluorophores and drugs

The chemical Ca²⁺-indicator dyes Oregon Green BAPTA-1 AM (OGB-1 AM) or Xrhod-1 AM (Invitrogen) were loaded into astrocytes by incubating them in dye-containing extracellular solutions (2 µM, 40 min for OGB-1; 200 nM, 10 min for Xrhod-1, respectively). To label lysosomes, astrocytes were incubated in 6.7 µM FM4-64 (Invitrogen) for 30 min. To fluorescently label ATP accumulation compartments in live cells, astrocytes were incubated in 50 µM MANT-ATP (Invitrogen) for 1 h. Prior to live cell imaging, cells were thoroughly washed during at least 30 min, and they were continuously perfused by dye-free solution (~0.5 ml/min) during imaging. BAPTA AM (Invitrogen; 100 µM for 50 min) was used to chelate astrocyte intracellular Ca²⁺. Plasmids encoding fluorescent sensors were obtained from Addgene unless otherwise indicated. Lipofectamine 2000 (Invitrogen) was used for transfecting cDNA plasmids into astrocytes following the standard protocol provided by the supplier. Cells were used ~24 h after transfection. Suramin, MPEP, thapsigargin and Gap26 were purchased from Tocris, 2-APB from Ascent Scientific, forskolin from Abcam, A β 25–35 from Bachem, and all other compounds from Sigma-Aldrich. Dual-channel local perfusion system was used to switch smoothly between control solution and specific pharmacological manipulations, and controlled by electric valves operated by a TTL trigger box commanded by MetaMorph (Molecular Devices). Immunostaining for mouse brain slices was performed following the standard protocol as previously described [70]. As for immunostaining of astrocyte primary cultures, cells were fixed with 1% paraformaldehyde (PFA, Sigma-Aldrich) for 10 min at RT, then washed three times with phosphate buffered saline (PBS, 5 min, at RT). After permeabilization and blockage of unspecific sites with PBS, 0.3% Triton X-100 and 2% bovine serum albumin (PBS-BT, 1 h at RT), astrocytes were probed with respective primary antibodies in the same solution overnight at 4 °C. After being washed with PBS three times at RT, cells were incubated with secondary antibodies in PBS-BT (2 h, RT). After three times of final washing (PBS, 5 min, RT) and rinsed afterwards with triple distilled water, cells were mounted with Mowiol (Millipore, Darmstadt, Germany) onto microscope

slides. Combinations of the primary and secondary antibodies used for fluorescence immunostaining are listed in Additional file 1: Table S1.

Fluorescence imaging and analysis

Total internal reflection fluorescence (TIRF) imaging was performed on a custom-made inverted microscope via the through-the-objective configuration (PlanApo TIRF $\times 60$ /NA1.45 oil objective, Olympus) [61]. The 488- and 568-nm excitation wavelengths were isolated from the beam of an Ar⁺/Kr⁺ multi-line laser (CVI Melles Griot) with an acousto-optical tunable filter (AA.Opto). Laser beam was directed onto the glass/water interface at a super-critical angle, thereby enabling the total reflection of the excitation beam and the generation of evanescent field on the side of astrocyte substrate. The penetration depth ($1/e^2$ -intensity decay) of the evanescent field was estimated of the order of 200 nm [61], thereby allowing ultrathin optical sectioning in astrocyte subplasmalemmal region for dynamic signal recording. Emission fluorescence was further magnified ($\times 2$) and acquired by an electron multiplying charge-coupled device (EMCCD, QuantEM 512, Princeton Instruments), and the effective pixel size in fluorescent images was 133 nm. The imaging hardwares were all controlled by MetaMorph software (Molecular Devices). For TIRF imaging, each field of view in general contains the footprint of a single astrocyte. In our recording, two to three separate astrocyte culture preparations were used and about three independent coverslips of each preparation for TIRF imaging per condition. Results were derived from signals of all recorded cells.

Background was estimated from the autofluorescence signal in non-labeled cells of the same preparation, and then subtracted from the fluorescent images. The contour of the footprint of single astrocytes was delineated with the ImageJ plugin Cell Outliner or with MetaMorph segmentation tool, from which the mean fluorescence was measured over time to generate the time courses of specific signals. The FRET ratio of the cAMP sensor GFP(nd)-EPAC1(dDEP)-mCherry and the donor/acceptor bleed-through control was obtained as previously reported [71]. During TIRF imaging, the 488-nm laser line was used to excite the cAMP sensor, while both GFP and mCherry fluorescence were simultaneously collected and projected by a custom image splitter side-by-side onto a single EMCCD camera. Details of the optical filter set are listed in Additional file 1: Table S2. Corrected by the amount of acceptor direct excitation and donor bleed-through [71], the FRET signal was calculated from the GFP/mCherry ratio and normalized to the pre-stimulation basal level as fractional changes.

Statistics

All data are expressed as mean \pm standard deviation (SD), and the t-test was used for assessing the significance. Comparison of non-normally distributed data was also validated using their median \pm absolute deviation and the non-parametric tests (Kolmogorov–Smirnov or Mann–Whitney U-test). All statistical operations were performed with Matlab (The MathWorks), with n.s., denoting non significant, * $p < 0.05$, and ** $p < 0.01$.

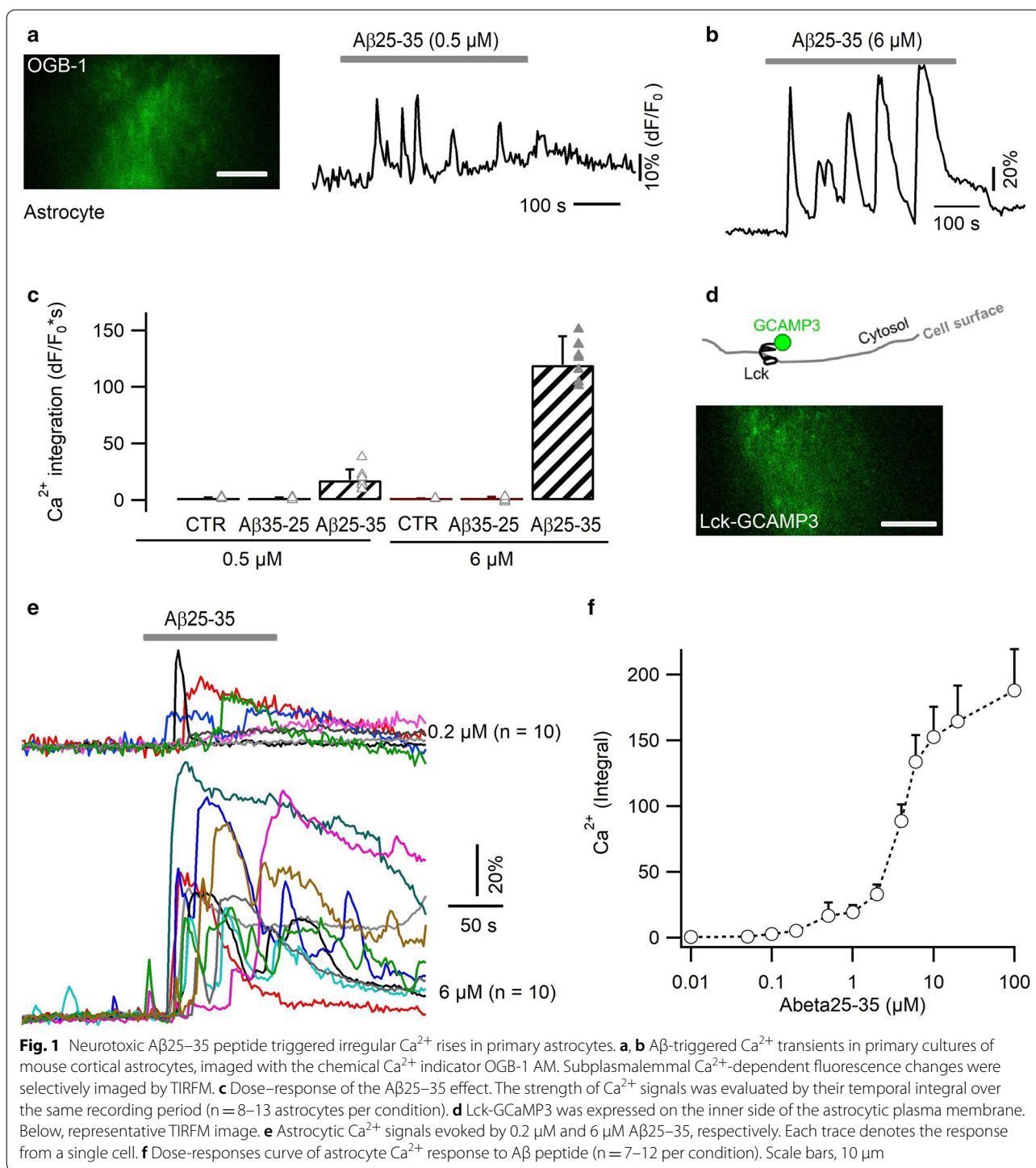
Results

Astrocytic Ca²⁺ elevation induced by neurotoxic A β 25–35

To study the acute response of astrocytes to A β , we used TIRF microscopy (TIRFM) to image near-membrane Ca²⁺ transients in primary astrocytes cultured from mouse cortex. With the cytosolic Ca²⁺ indicator OGB-1 AM, we observed an oscillatory Ca²⁺ increase upon the local application of submicromolar A β 25–35 (0.5 μ M, temporal integral = 17.6 ± 9.5 dF/F₀*s; Fig. 1a). Higher doses of A β evoked stronger (6 μ M, integral = 119.5 ± 25.4 dF/F₀*s) and longer-lasting Ca²⁺ signals following a temporal delay (43.5 ± 21.6 s; Fig. 1b, c). This signal was absent in response to extracellular control solutions, either without A β peptide or containing the sequence-reversed peptide A β 35–25 (6 μ M, Additional file 1: Fig. S1a). To further confine Ca²⁺ detection in subplasmalemmal region, we also used the plasma membrane-targeted Ca²⁺ sensor Lck-GCaMP3 [80] (Fig. 1d, e). As before, Ca²⁺ elevations could be detected in response to submicromolar A β 25–35 and gradually reached to a plateau level with increased doses (Fig. 1f). The similarity in Ca²⁺ profiles detected with the bulk indicator OGB-1 and the subplasmalemmal sensor Lck-GCaMP3, suggests that the neurotoxic A β 25–35 causes Ca²⁺ elevations throughout astrocyte cytosol.

Purinergic activation contributes to A β -evoked astrocytic Ca²⁺ rise

A β peptides have been suggested to induce Ca²⁺ influx [1, 18], while other studies showed the contribution of intracellular Ca²⁺ release from the endoplasmic reticulum (ER) store [3, 31, 85]. We therefore examined in primary astrocytes the mechanism for A β 25–35-evoked Ca²⁺ rise. Compared to control condition (temporal integral dF/F₀*s = 31.2 ± 11.9 ; Fig. 2a, j), removing Ca²⁺ from extracellular solution diminished the A β 25–35-evoked Ca²⁺ signal (integral = 9.7 ± 10.1 , $p < 0.01$; Fig. 2b, j). We then kept the extracellular Ca²⁺ at normal level while pre-depleting the internal ER Ca²⁺ store. To this end, the ER-resident Ca²⁺ ATPase was inhibited by thapsigargin (TG, 0.5 μ M), which caused a prominent discharge of Ca²⁺ from ER store (Fig. 2c, top). This treatment



significantly decreased the subsequent responses to Aβ₂₅₋₃₅ (integral = 18.5 ± 13.7%, *p* < 0.05; Fig. 2c, bottom; j). Since Ca²⁺ release from ER store is mediated by the inositol 1,4,5-trisphosphate (IP₃) receptor, we examined the effect of its blocker 2-Aminoethoxydiphenyl borate (2-APB, 200 μM) and we also observed an

inhibition impact (integral = 17.8 ± 9.6, *p* < 0.05; Fig. 2d, j). Thus, both Ca²⁺ influx and release from the ER store contribute to the Aβ-evoked astrocytic Ca²⁺ signal.

Ca²⁺ release from the internal store is recruited by the activation of metabotropic receptors. Astrocytes express a variety of receptors, among which

metabotropic glutamate receptor 5 (mGluR5) and purinergic P2 receptors respond with Ca^{2+} rises to glutamate and ATP, respectively [6]. While antagonizing mGluR5 with the group I mGluR antagonist MPEP showed no effect (50 μM , $p=0.6$; Fig. 2e, j), $\text{A}\beta$ -evoked Ca^{2+} signals were fully abolished by the combination of P2 receptor antagonists PPADS (100 μM) and suramin (50 μM , $p<0.01$; Fig. 2f, j). This indicates that $\text{A}\beta$ -induced Ca^{2+} increase requires the activation of astrocytic P2 receptors. Although both ionotropic P2X and metabotropic P2Y receptors were suggested to regulate astrocytic Ca^{2+} signal, we observed that the wide-spectrum P2X antagonist TNP-ATP [41] failed to inhibit $\text{A}\beta$ -triggered Ca^{2+} rise (10 μM , $p=0.53$, Fig. 2j). In contrast, the $\text{A}\beta$ response was inhibited by antagonizing the P2Y1 receptor (5 μM MRS2179, integral = 17.5 ± 7.8 , $p<0.01$; Fig. 2g, j), in line with its contribution to astrocyte Ca^{2+} hyperactivities in AD mouse model [17].

Metabotropic receptor activation triggers Ca^{2+} release from internal ER store, which then activates store-operated channels (SOCs) to induce Ca^{2+} influx [83]. Lipophilic molecules including $\text{A}\beta$ peptide were suggested to facilitate Ca^{2+} influx through astrocytic SOCs [74, 88]. To image SOC-mediated Ca^{2+} upon, we used a standard protocol to image SOC-mediated Ca^{2+} influx [83]. ER store was first depleted by thapsigargin in Ca^{2+} -free solution, and then Ca^{2+} added back to generate SOC-mediated Ca^{2+} influx (Fig. 2h). Presence of $\text{A}\beta_{25-35}$, indeed, facilitated SOC Ca^{2+} influx (peak amplitude, $dF/F_0 = 1.46 \pm 0.36$ vs. CTR 0.77 ± 0.27 , $p<0.05$; Fig. 2h), suggesting its contribution to the Ca^{2+} oscillations following P2Y1 receptor activation. These results corroborate the dual dependence of $\text{A}\beta$ -evoked Ca^{2+} signal on both the internal store and Ca^{2+} influx (Fig. 2b, c, j).

One possible mechanism underlying P2Y1 activation by $\text{A}\beta$ might be that it activated ATP-releasing pathways in astrocytes. In spinal cord and hippocampal

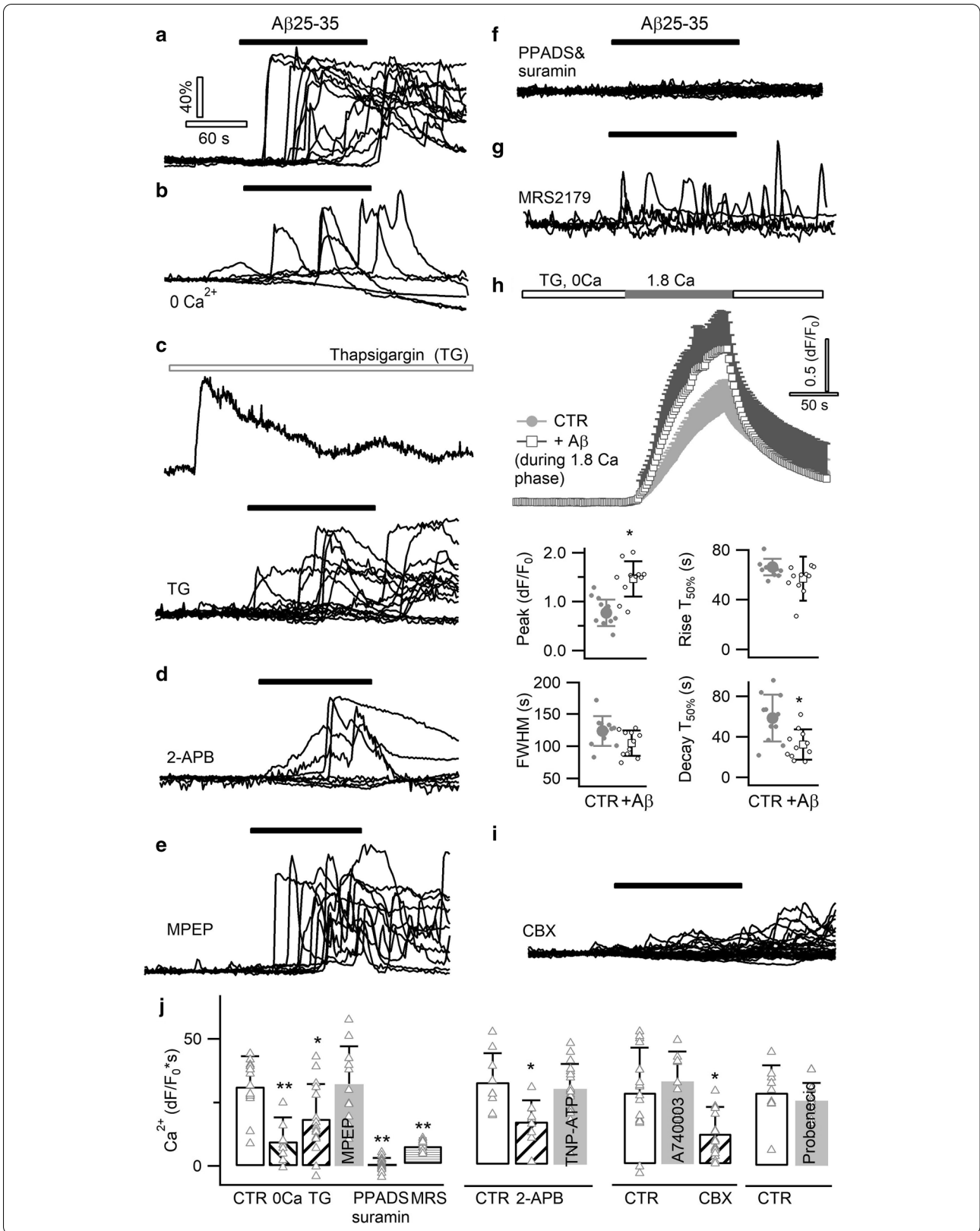
astrocytes, ATP release was suggested to be mediated by the pore-forming P2X7 receptor [95], although its expression in astrocytes of specific regions was called into reconsideration [59]. We observed that P2X7 antagonist A740003 (20 μM) [35] failed to affect $\text{A}\beta$ -triggered Ca^{2+} signal, echoing the absence of an effect of the wide-spectrum P2X blocker TNP-ATP (Fig. 2j). Alternatively, astrocytes express connexin (CX) hemichannels that mediate ATP release in physiological and pathological conditions [28, 98]. Immunostaining of CX43 protein was observed on the surface of cortical astrocytes in culture and in the cortex of hAPPJ20 AD mouse model [44, 60] (Additional file 1: Fig. S2). We found that blocking CX hemichannels with carbenoxolone (CBX, 50 μM) reduced $\text{A}\beta$ -elicited Ca^{2+} oscillation (integral = 13.2 ± 15.5 vs. CTR 37.5 ± 24.9 , $p<0.05$; Fig. 2i, j). Although astrocyte ATP was also shown to be released by pannexin hemichannel [36], its blocker probenecid (500 μM) failed to alter the $\text{A}\beta_{25-35}$ effect (Fig. 2j). Hence, $\text{A}\beta$ -caused Ca^{2+} oscillations depend on the opening of CX hemichannels, by which ATP might be released to activate the P2Y1 purinergic receptor.

$\text{A}\beta$ diminishes Ca^{2+} level in preconditioned astrocytes

Astrocytes change status over chronic $\text{A}\beta$ exposure [4, 64]. In AD patients and mouse models, astrocytes become reactive near $\text{A}\beta$ plaques [55]. To examine potential response alterations in $\text{A}\beta$ -conditioned astrocytes, we pre-incubated primary astrocyte cultures with sub-micromolar $\text{A}\beta_{25-35}$ (0.5 μM) for different durations. Such preconditioning reduced the number of astrocytes displaying Ca^{2+} rises ('Rise' type response, Fig. 3a left) in response to the subsequent acute $\text{A}\beta_{25-35}$ challenge, and over time started diminishing the basal Ca^{2+} level ('Mix' type response, Fig. 3a middle). After a 2-h preconditioning, most astrocytes responded to $\text{A}\beta_{25-35}$ with a diminution in basal Ca^{2+} level ('Drop' type response, Fig. 3a right; Fig. 3b, versus control Additional file 1: Fig.

(See figure on next page.)

Fig. 2 A purinergic pathway underlies $\text{A}\beta$ -evoked Ca^{2+} signal. Representative responses evoked by $\text{A}\beta_{25-35}$ in astrocytes loaded with the chemical Ca^{2+} indicator OGB-1 AM, in control condition (a), in Ca^{2+} -free extracellular solution (b), following thapsigargin (TG, 0.5 μM) depletion of ER Ca^{2+} store (c, top trace reflecting the Ca^{2+} leak signal upon TG application), in the presence of the IP3 receptor antagonist 2-APB (d, 200 μM), and of the mGluR5 antagonist MPEP (e, 50 μM). Each trace represents the response of a single astrocyte. f $\text{A}\beta$ -evoked Ca^{2+} responses were fully abolished by blocking purinergic P2 receptors with the combination of wide-spectrum antagonists PPADS (100 μM) and suramin (50 μM). g The P2Y1 antagonist MRS2179 (5 μM) attenuated $\text{A}\beta$ -induced Ca^{2+} responses. h $\text{A}\beta_{25-35}$ enhanced Ca^{2+} influx via store-operated channels (SOCs). SOCs were activated by fully depleting the ER store with TG in Ca^{2+} -free solution. SOC-mediated Ca^{2+} influx was induced by re-supplying Ca^{2+} in the extracellular solution. Ca^{2+} influx was significantly increased in the presence of $\text{A}\beta_{25-35}$ ($n=11$ cells per condition). i Effect of blocking connexin hemichannels with CBX (50 μM). j $\text{A}\beta$ -evoked astrocyte Ca^{2+} responses in different conditions. Ca^{2+} signal strength was derived from the temporal integral of individual normalized traces (dF/F_0 's). Wide-spectrum P2X receptor antagonist TNP-ATP, P2X7 antagonist A740003 and pannexin blocker probenecid were applied at 10 μM , 20 μM and 500 μM , respectively. Control experiments were performed for a defined set of experiments as shown ($n=9-20$ cells per condition)



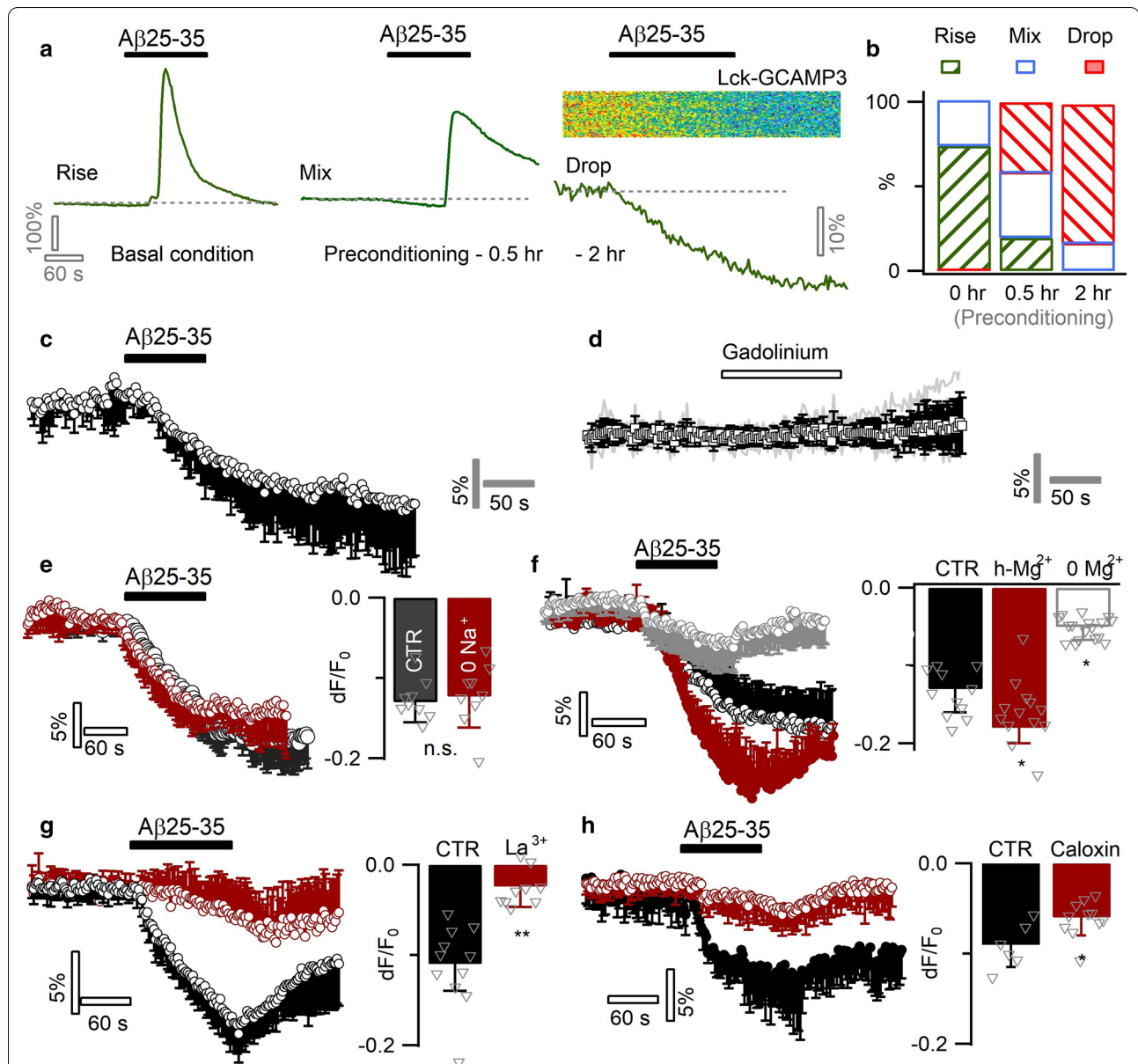


Fig. 3 $A\beta_{25-35}$ inhibits Ca^{2+} levels in preconditioned astrocytes by potentiating PMCA Ca^{2+} extrusion. **a** Effect of preconditioning on the acute responses of $A\beta_{25-35}$ ($6\ \mu M$) in cultured mouse cortical astrocytes. Left, Ca^{2+} rise was triggered in intact astrocytes ('Rise' type response). Middle, after a short term incubation (i.e., preconditioning) of astrocytes with submicromolar $A\beta_{25-35}$ ($0.5\ h, 0.5\ \mu M$), acute application of $6\ \mu M\ A\beta_{25-35}$ caused a basal line drop mixed with Ca^{2+} rise ('Mix' type response). Right, following a $\sim 2\ h$ preconditioning in $0.5\ \mu M\ A\beta_{25-35}$, astrocytes exhibited only a drop in the intracellular Ca^{2+} level ('Drop' type response). **b** The percentage of three types of astrocytes that either displayed a Ca^{2+} rise ('Rise'), an initial diminution followed by rise ('mix'), or only a drop in the basal Ca^{2+} levels (Drop'; $n = 19-7$ cells per condition). **c** Average of $A\beta$ -induced Ca^{2+} diminution in $A\beta_{25-35}$ -preconditioned ($2\ h$) astrocytes ($n = 11$). **d** Blocking spontaneous Ca^{2+} influx by gadolinium ($100\ \mu M$) failed to mimic $A\beta$ -evoked Ca^{2+} diminution ($n = 8$). **e** Inactivating NCX by Na^+ -free solution showed no effect ($n = 9$ per condition). **f** $A\beta$ -induced Ca^{2+} diminution affected by ambient Mg^{2+} concentration, implying the recruitment of an ATP-dependent pathway. Astrocytes were incubated in defined solutions $1\ h$ prior to imaging ($n = 11-17$ per condition). **g, h** Inhibiting PMCA by La^{3+} ($50\ \mu M$) or Caloxin 3A1 ($500\ \mu M$) counterbalanced the $A\beta$ -evoked astrocytic Ca^{2+} diminution ($n = 6-11$ per condition)

S1b). Spontaneous Ca^{2+} rises that occurred in a subpopulation of pre-conditioned astrocytes (16/117 cells), were also inhibited by an acute application of A β 25–35 (Additional file 1: Fig. S1c, d).

We then probed potential mechanisms underlying the inhibition effect of A β . Spontaneous Ca^{2+} entry via SOC and the transient receptor potential cation channel A1 (TRPA1) channel regulates basal Ca^{2+} level in astrocytes [81, 83]. The inhibition effect of A β 25–35 might be due to the blockade of this spontaneous Ca^{2+} entry. Yet blocking SOC and TRPA1 channels by the wide-spectrum blocker gadolinium (Gd^{3+} , 100 μM) [72, 81] failed to mimic the effect of A β 25–35 (Fig. 3c, d). Alternatively, the Ca^{2+} diminution might be due to the potentiation of Ca^{2+} extrusion. We noted that chronic pre-conditioning of astrocytes with low A β 25–35 gradually elevated intracellular Ca^{2+} level (Additional file 1: Fig. S1e). The overload of Ca^{2+} would likely facilitate its extrusion due to the increased efflux driving force. Potential extrusion pathways include $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) that utilizes the Na^+ gradient to export intracellular Ca^{2+} and the plasma membrane Ca^{2+} ATPase (PMCA) driven by ATP hydrolysis [7, 26, 73]. Inhibiting NCX with Na^+ -free external solution showed no effect on A β -caused Ca^{2+} diminution ($dF/F_0 = -12.6 \pm 6.7\%$ vs. CTR $dF/F_0 = -12.9 \pm 5.6\%$, $p = 0.7$; Fig. 3e). We then examined the role of PMCA ATPase. As ATP requires the binding to Mg^{2+} to become biologically active in the form Mg-ATP [30, 92], we sought to up- and down-regulate PMCA activity by bathing astrocytes, respectively, in high (20 mM) or zero concentration of extracellular Mg^{2+} (vs. CTR, 1 mM). This manipulation proportionally altered A β -induced Ca^{2+} decline (Fig. 3f). Further, the wide-spectrum PMCA blocker La $^{3+}$ (50 μM) [7, 14, 82], largely antagonized the Ca^{2+} diminution induced by A β 25–35 ($dF/F_0 = -2.4 \pm 3.7\%$ vs. CTR $dF/F_0 = -11.3 \pm 4.9\%$, $p < 0.01$; Fig. 3g). A similar effect was observed with the PMCA-blocking peptide caloxin 3A1 [22, 65] (500 μM , $dF/F_0 = -5.9 \pm 1.9\%$ vs. CTR $dF/F_0 = -9.1 \pm 2.3\%$, $p < 0.05$; Fig. 3h). As PMCA exports Ca^{2+} against its transmembrane gradient, we expected this process to be facilitated by the removal of extracellular Ca^{2+} . Indeed, Ca^{2+} -free external solution largely augmented the A β -potentiated Ca^{2+} efflux (Additional file 1: Fig. S1f). The expression of PMCA in astrocytes was observed by immunohistochemistry. Astrocytes characterized by GFAP immunostaining could be observed in the cortical region of a hAPPJ20 AD mouse model [44, 60] (Additional file 1: Fig. S3a). We observed PMCA immunostaining in GFAP-positive astrocytes in culture (Additional file 1: Fig. S3b). PMCA expression was also present in cortical astrocytes identified by a wide-spectrum marker S100 β (Additional file 1: Fig. S3c), suggesting a general involvement

of PMCA in astrocyte activity regulation. These results, together, suggest that A β 25–35 potentiates PMCA-mediated Ca^{2+} extrusion in preconditioned astrocytes, thereby diminishing the intracellular Ca^{2+} level.

A β activates PMCA Ca^{2+} -H $^+$ exchange via cAMP signal

We next examined the signaling link between A β 25–35 and PMCA potentiation. PMCA is activated upon intracellular rise of Ca^{2+} so as to prevent its overload [7, 62]. In preconditioned astrocytes, A β 25–35 diminished basal Ca^{2+} level independent of its elevation, suggesting other signaling pathways than Ca^{2+} had been recruited. Another second messenger cAMP is also known to potentiate PMCA activity [16, 40]. Indeed, elevating cytosolic cAMP by forskolin (100 μM) induced a diminution in basal Ca^{2+} level (peak $dF/F_0 = -7.5 \pm 3.1\%$) in primary cultured astrocytes, which was counteracted by the PMCA blocker La $^{3+}$ (50 μM , $dF/F_0 = -0.82 \pm 0.7\%$, $p < 0.01$; Fig. 4a). Forskolin also inhibited the spontaneous Ca^{2+} transients observed in a subpopulation of preconditioned astrocytes (Additional file 1: Fig. S1g), as seen with A β 25–35 (Additional file 1: Fig. S1c, d). By a fluorescent FRET sensor GFP $^{\text{nd}}$ -EPAC(dDEP)-mCherry [49, 87], astrocytic cAMP increase was detected upon A β 25–35 application (Fig. 4b). These results suggest that cAMP signal is involved in A β potentiation of PMCA Ca^{2+} extrusion.

PMCA couples Ca^{2+} export with H $^+$ influx [7, 14]. In preconditioned astrocytes, we therefore performed dual-color imaging to simultaneously follow intracellular pH and Ca^{2+} dynamics during A β application. As the fluorescence of GFP protein can be quenched by H $^+$ [45], we used it here as a pH sensor. Meanwhile, we co-expressed a red-fluorescent Ca^{2+} sensor protein GECO-R [102] to image cytoplasmic Ca^{2+} . We observed a concomitant decrease in GFP fluorescence ($dF/F_0 = -4.0 \pm 3.7\%$) upon A β 25–35-induced Ca^{2+} diminution ($dF/F_0 = -7.1 \pm 2.3\%$; Fig. 4c), indicating PMCA-mediated H $^+$ influx accompanying the Ca^{2+} extrusion. Consistently, such phenomenon was abolished by chelating cytosolic Ca^{2+} with 100 μM BAPTA AM (GFP, $dF/F_0 = 1.1 \pm 1.7\%$, $p < 0.01$; Fig. 4c). In preconditioned astrocytes that displayed mixed Ca^{2+} responses (i.e., *an initial Ca^{2+} diminution followed by elevation*) to A β 25–35, we observed stepwise H $^+$ influx recorded by GFP quenching (Additional file 1: Fig. S1h) confirming the recruitment of PMCA by both Ca^{2+} phases. It is worth noting that H $^+$ is a potent inhibitor of astrocytic CX hemichannels [77]. In preconditioned astrocytes, A β 25–35 potentiates PMCA causing Ca^{2+} diminution and H $^+$ influx that would have inhibited CX hemichannels, an effect found to inhibit Ca^{2+} rises (Fig. 2i, j). Astrocytes hence display state-dependent Ca^{2+} responses to the neurotoxic A β 25–35.

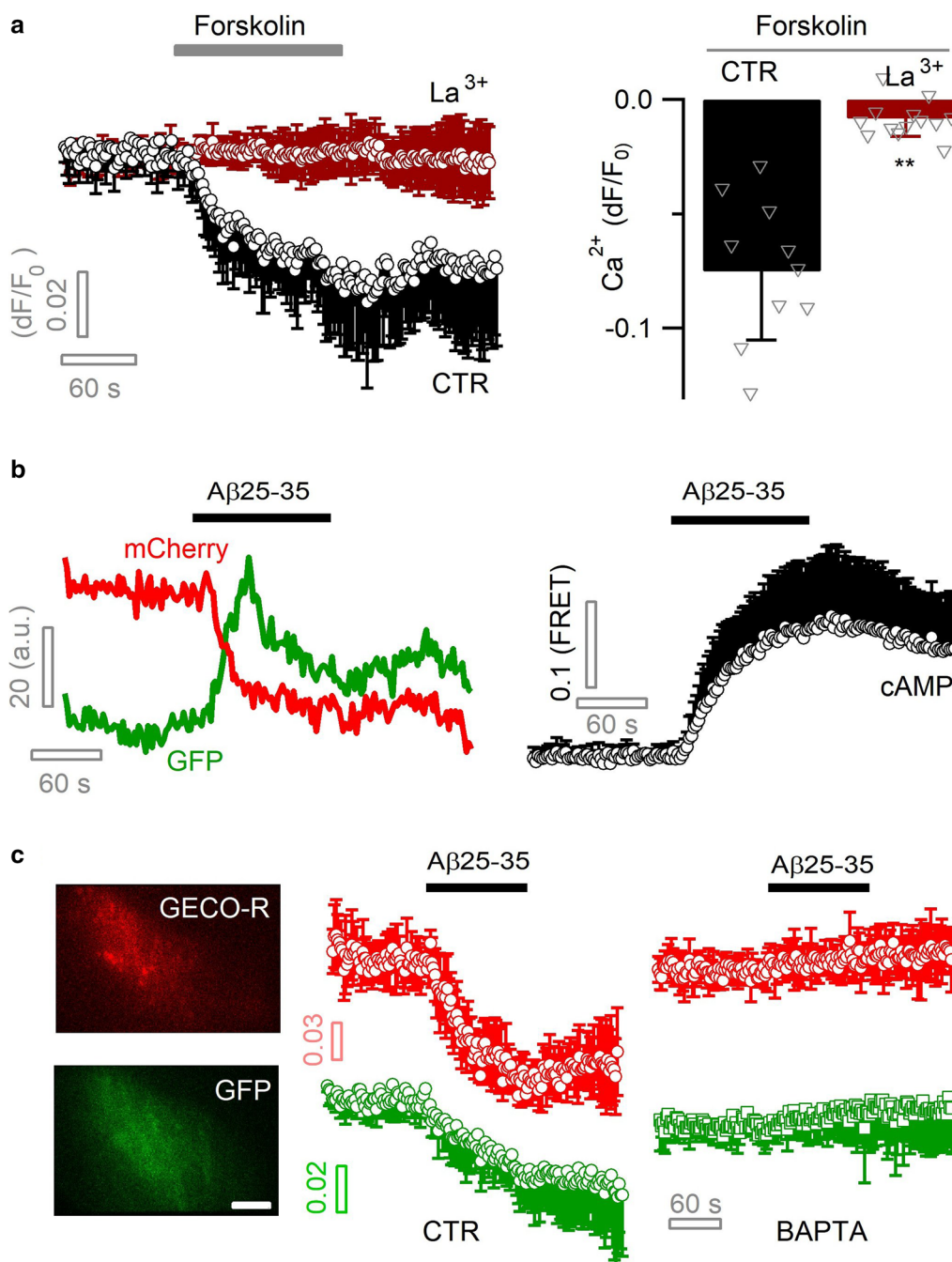
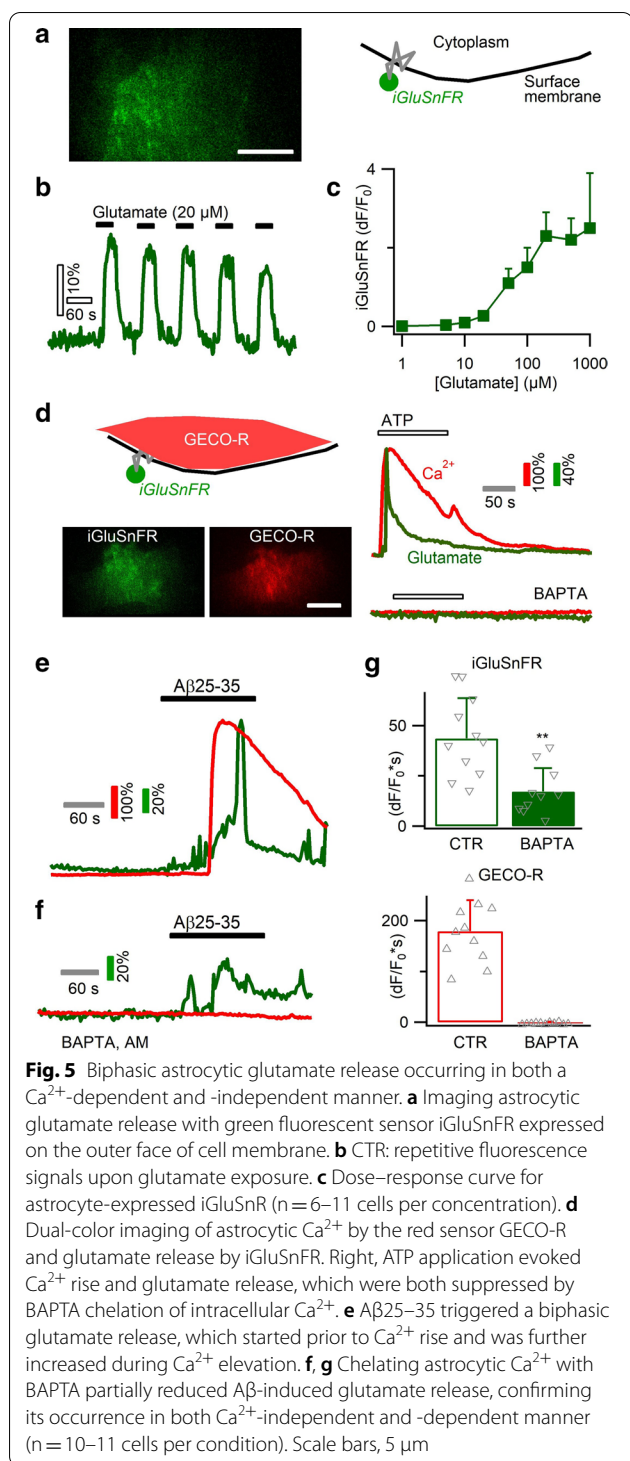


Fig. 4 $A\beta$ potentiates astrocytic PMCA via cAMP signaling. **a** Elevation of astrocytic cAMP level increased PMCA-mediated Ca^{2+} extrusion. cAMP was elevated by forskolin (100 μM) and sub-cellular Ca^{2+} level imaged with Lck-GCaMP3 and TIRFM. Ca^{2+} diminution was blocked by the PMCA blocker La^{3+} (50 μM ; $n = 9-13$ cells per condition). **b** Intracellular cAMP level was monitored with the FRET sensor GFPnd-EPAC(dDEP)-mCherry and dual-color TIRFM. Left, representative dual-color recording of fluorescence change for EGFP and mCherry of the FRET sensor. Right, averaged astrocytic cAMP rise induced by $A\beta_{25-35}$ (6 μM ; $n = 6$). **c** PMCA-mediated astrocytic Ca^{2+} diminution was coupled with H^+ influx. Dual-color TIRFM recorded concomitant cytosolic Ca^{2+} and pH diminution, by expressing the red genetically encoded Ca^{2+} sensor GECO-R and the pH-sensitive GFP protein in same astrocytes. Both effects were abolished by chelating astrocyte cytoplasmic Ca^{2+} with BAPTA AM (100 μM ; $n = 7-9$ cells per condition). Scale bar, 5 μm



$\text{A}\beta_{25\text{--}35}$ evokes biphasic glutamate release from astrocytes

Astrocyte Ca^{2+} signals have been suggested to trigger the release of signaling molecules and affect neuronal activity [5]. During pathological $\text{A}\beta$ accumulation, neurotoxicity

has been attributed to excessive buildup of extracellular glutamate [37, 54, 63]. To examine how astrocytes contribute to such glutamate buildup, we imaged astrocyte glutamate release in response to $\text{A}\beta_{25\text{--}35}$. We expressed the genetically encoded glutamate sensor iGluSnFR in primary cortical astrocytes [51], which showed repetitive fluorescence change upon glutamate puff (Fig. 5a, b). The dose–response curve reveals a dynamic range of $\sim 10\text{--}200$ μM glutamate (Fig. 5c). Triggering astrocyte Ca^{2+} elevation by ATP caused glutamate release, which was inhibited by the Ca^{2+} chelator BAPTA AM (100 μM , Fig. 5d). We then observed that $\text{A}\beta_{25\text{--}35}$ application also induced glutamate release as reflected by the green fluorescence increase of iGluSnFR (Fig. 5e). We noted that during $\text{A}\beta$ application, a fraction of glutamate was released before the onset of Ca^{2+} elevation and the Ca^{2+} signal then accelerated the release (Fig. 5e). We then performed similar experiments in astrocytes loaded with the Ca^{2+} chelator BAPTA AM. While intracellular Ca^{2+} signal was fully inhibited, a portion of glutamate was still released upon $\text{A}\beta_{25\text{--}35}$ application, thereby validating the presence of a Ca^{2+} -independent release component (Fig. 5f, g). The presence of BAPTA, meanwhile, also reduced the total amount of glutamate release, showing the co-expression of Ca^{2+} -dependent release (integral $\text{dF}/\text{F}_0 \cdot \text{s} = 17.2 \pm 16.2$ vs. CTR 43.6 ± 22.4 ; $p < 0.01$; Fig. 5g). Thus, $\text{A}\beta_{25\text{--}35}$ induced astrocytic glutamate release via both Ca^{2+} -dependent and -independent mechanisms.

CX hemichannel affects Ca^{2+} -independent glutamate release

Glutamate is known to permeate through hemichannels [64, 97]. We confirmed this by artificially opening CX hemichannels in cultured astrocytes with Ca^{2+} -free solution [97], which indeed induced glutamate release (Additional file 1: Fig. S4). This occurred in the absence of intracellular Ca^{2+} increase, suggesting that CX hemichannels may contribute to the glutamate release preceding the Ca^{2+} rises.

We next imaged $\text{A}\beta$ -evoked glutamate release in the absence and presence of the CX hemichannel blocker CBX (100 μM). As expected, this treatment reduced the glutamate release during the phase prior to Ca^{2+} increase ($\text{dF}/\text{F}_0 = 0.03 \pm 0.7$ vs. 0.31 ± 0.24 of CTR; $p < 0.01$; Fig. 6a). The overall Ca^{2+} signal and glutamate release throughout the recording period were also reduced (Fig. 6a), corroborating that hemichannel opening contributes to the $\text{A}\beta$ -evoked Ca^{2+} signal. Applying during $\text{A}\beta_{25\text{--}35}$ stimulation the mimetic peptide Gap26 (200 μM), a selective blocker of connexin43 hemichannel [20, 64], also inhibited the Ca^{2+} elevation and glutamate release as compared to control and to the inactive

scrambled peptide of Gap26 (Fig. 6b). Upon the washing of Gap26 and A β , glutamate release and Ca²⁺ signal reappeared (Fig. 6b), suggesting a post-inhibition rebound response. Applying Gap26 throughout recording (i.e., pre-, during- and post-A β application) inhibited the post-A β response (Fig. 6c). We then suppressed astrocyte Ca²⁺ signal with BAPTA and isolated the A β -induced Ca²⁺-independent glutamate release that was found to be affected by Gap26 (temporal integral dF/F₀*s = 3.7 ± 3.4 vs. 19.7 ± 16.1 of CTR, *p* < 0.01; Fig. 6d). Hence, astrocyte CX hemichannel contributes to the Ca²⁺-independent glutamate release induced by A β 25–35.

Anion channel and lysosome exocytosis modulate Ca²⁺-dependent glutamate release

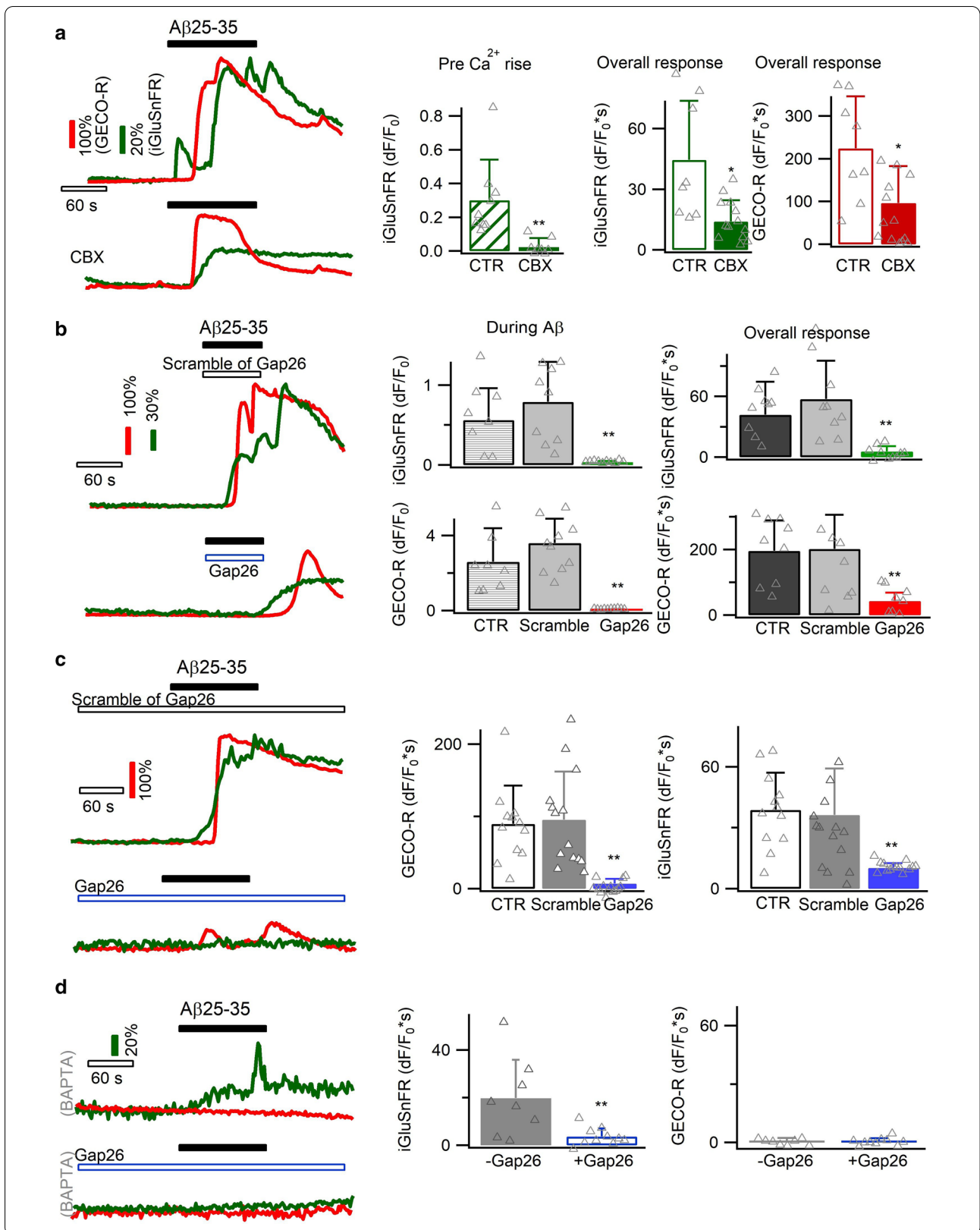
Astrocyte CX hemichannel opening was suggested to be Ca²⁺-sensitive [15], and may modulate A β -evoked Ca²⁺-dependent glutamate release. One way to clarify this issue would be to block CX hemichannels, which however is known to interfere with A β -induced Ca²⁺ signal (Figs. 2, 6). To bypass this problem, we mimicked A β -evoked Ca²⁺ signals by ATP stimulation (30 μ M, Fig. 7a) that partially recapitulated the purinergic receptor activation by A β 25–35 (Fig. 2). ATP-triggered Ca²⁺ oscillations showed comparable patterns as the A β 25–35-induced signals (GECO-R, Fig. 7a) and triggered glutamate release (iGluSnFR, Fig. 7a). Yet contrary to our expectation, Ca²⁺-activated glutamate release persisted in the presence of the CX hemichannel blocker CBX (100 μ M, Fig. 7b, d), suggesting its dispensable involvement in this process. Alternatively, Ca²⁺-sensitive anion channels represent another route for Ca²⁺-activated glutamate release in astrocytes [29, 86, 93, 96]. Indeed, the anion channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB, 100 μ M) reduced the Ca²⁺-activated glutamate release (temporal integral dF/F₀*s = 1.3 ± 1.5 vs. 4.2 ± 1.9, *p* < 0.05; Fig. 7c, d). Also, during the response to A β 25–35, astrocyte glutamate release over the phase of Ca²⁺ rise was reduced by another anion channel blocker DCPIB (50 μ M, Fig. 7e), corroborating a role for anion channels in the Ca²⁺-dependent glutamate release.

Another pathway for glutamate release could be Ca²⁺-regulated vesicular exocytosis. In astrocytes, lysosomes are known vesicular compartments undergoing

Ca²⁺-activated exocytosis [38, 50, 101], though the involvement of small synaptic like vesicles is still debated [23, 75]. The vesicular glutamate transporter sialin [58] was observed on astrocyte lysosomes [50], suggesting their contribution to Ca²⁺-dependent glutamate release. We therefore imaged with TIRFM lysosome exocytosis from primary astrocytes in response to A β 25–35. Lysosomes were labeled with the red fluorescent dye FM4-64 [50, 101], and the concomitant Ca²⁺ signals monitored with the green fluorescent indicator OGB1 AM (Fig. 8a1). Following A β -evoked Ca²⁺ elevation, we observed an asynchronous lysosome exocytosis as reflected by FM dye destaining (Fig. 8a2, a3). We also used a pH-sensitive sensor to image exocytosis, where the GFP mutant pHluorin is conjugated to the intraluminal site of the lysosomal membrane protein CD63 [49] (Fig. 8b). CD63-pHluorin is quenched in the acidic lysosome lumen, and becomes fluorescent upon its exocytotic exposure to extracellular neutral solution. We observed that A β 25–35 induced CD63-pHluorin brightening on astrocyte surface, thus corroborating the occurrence of lysosome exocytosis (Fig. 8b). A similar temporal distribution was found with the two exocytotic probes (*p* = 0.6; Fig. 8c), consistent with the co-localization of FM4-64 and CD63 in astrocyte lysosomes [50]. Next, to examine the potential glutamate storage in astrocyte lysosomes, we performed glutamate staining in cultured astrocytes that resulted in a punctuate labeling distributed across the cytoplasm (Additional file 1: Fig. S5a). Glutamate staining was diminished by the cathepsin C substrate glycyl-L-phenylalanine 2-naphthylamideto (GPN, 200 μ M), a compound permeabilizing lysosomes by osmotic swelling [50, 101] (Additional file 1: Fig. S5a). On the other hand, the fluorescent nucleotide marker MANT-ATP showed little colocalization with FM4-64-labeled lysosomes (Additional file 1: Fig. S5b). It was also observed that permeabilization of lysosomes by GPN reduced the A β 25–35-induced glutamate release (Fig. 8d), and the presence of anion channel blocker DCPIB (50 μ M) showed no significant effect on astrocyte lysosome release (Fig. 8e). These data suggest the astrocyte lysosomes, in parallel with anion channels, contribute to A β -induced Ca²⁺-dependent glutamate release.

(See figure on next page.)

Fig. 6 Involvement of CX hemichannels in Ca²⁺-independent glutamate release. **a** Glutamate release prior to the Ca²⁺ elevation (top, CTR) was reduced by the CX hemichannel blocker CBX (100 μ M; present throughout the recording; *n* = 8–13 cells per condition). **b** During the A β application phase, Ca²⁺-independent glutamate release was blocked by another CX hemichannel blocker Gap26 peptide (200 μ M). The inactive scramble peptide of Gap26 showed no effect (*n* = 8–10 cells per condition). **c** A more pronounced inhibition effect of Gap26 was observed when applying it throughout the entire imaging period (i.e., pre-, during- and post-A β ; *n* = 12–14 per condition). **d** Chelating astrocytic Ca²⁺ with BAPTA AM isolated Ca²⁺-independent astrocytic glutamate release, which was inhibited by CX hemichannel blocker Gap26 (*n* = 8–10 per condition)



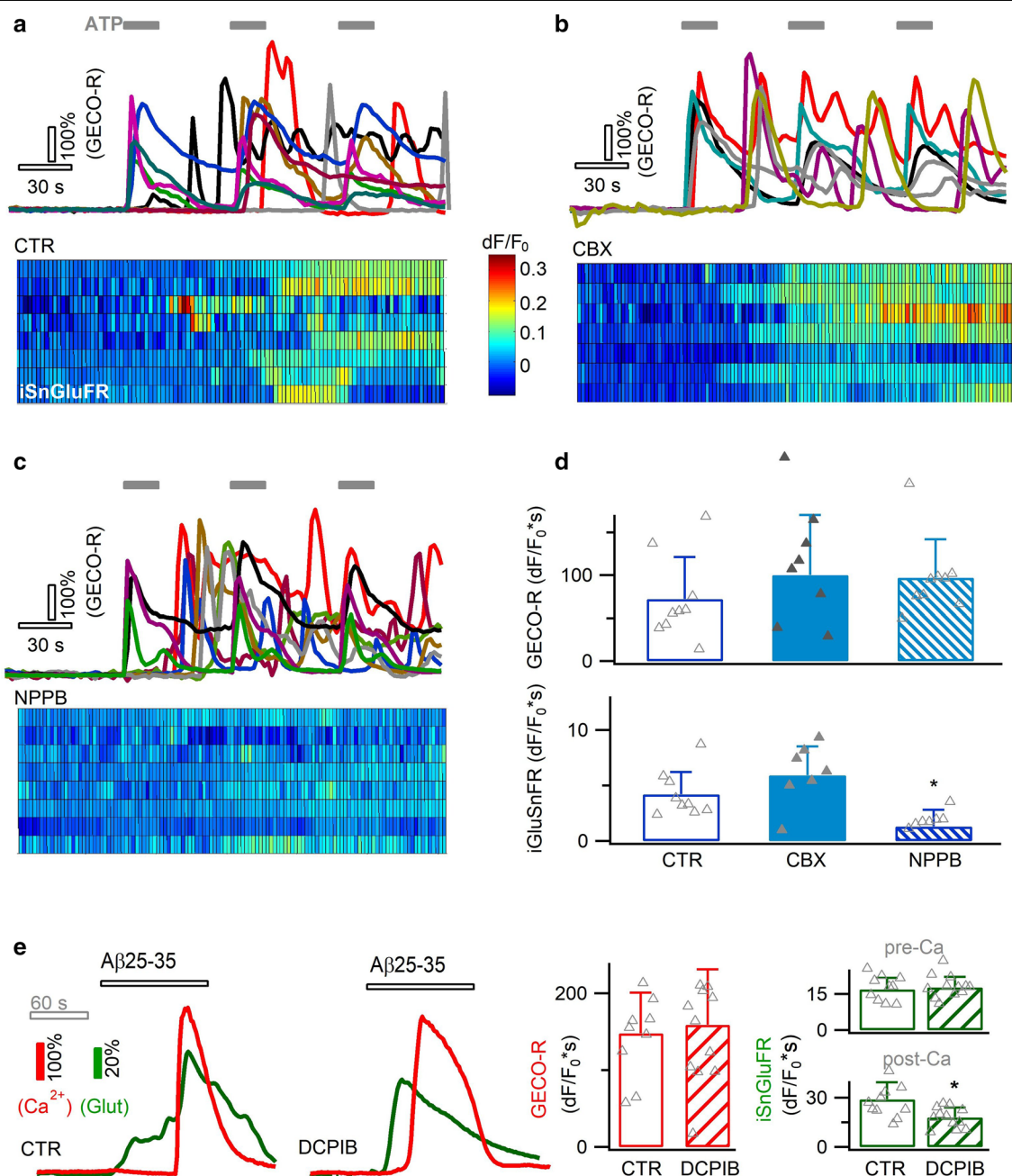
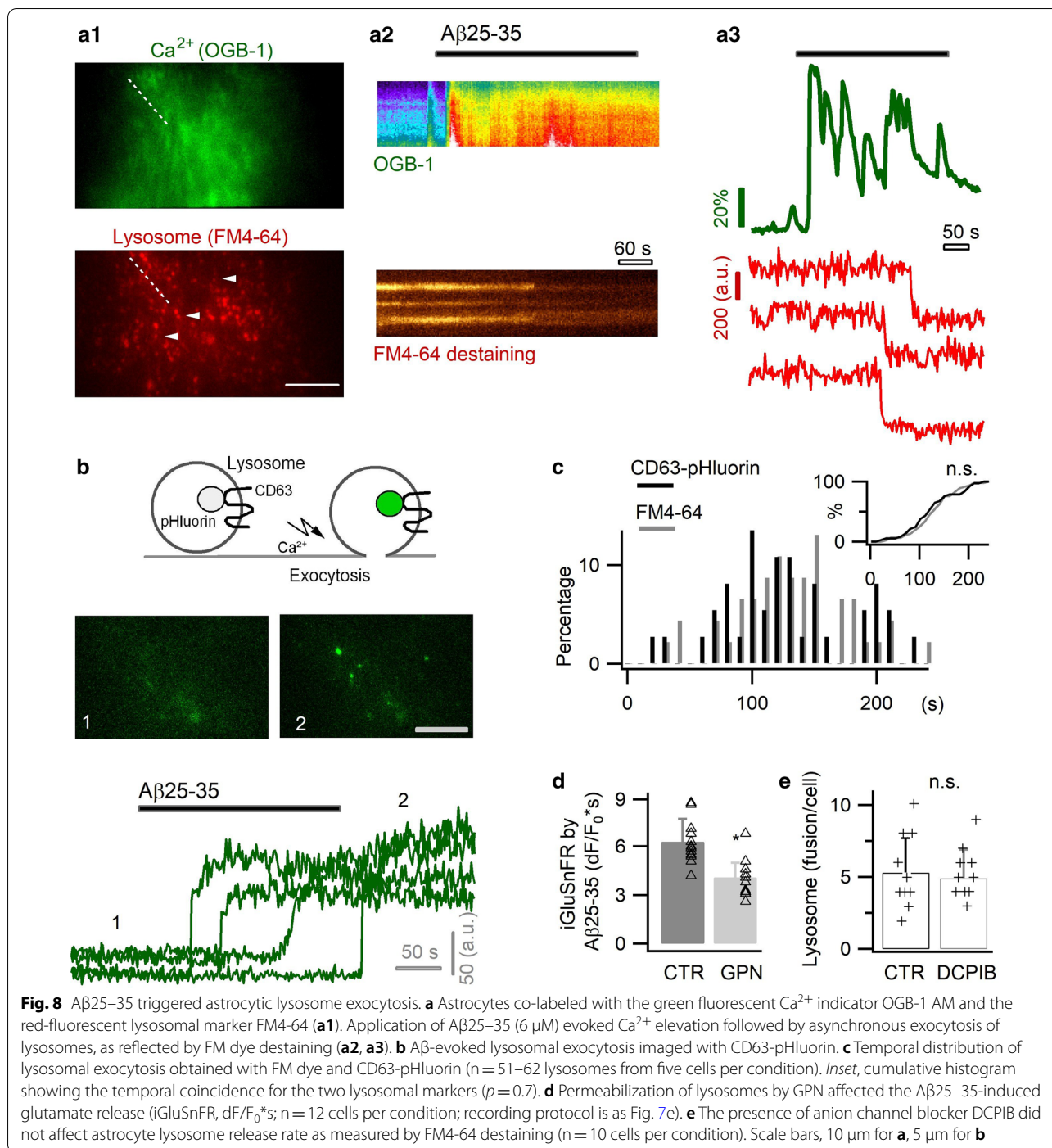


Fig. 7 Contribution of anion channels to Ca²⁺-dependent glutamate release. **a** Aβ-evoked astrocytic Ca²⁺ rises were due to purinergic receptor activation. To examine Ca²⁺-dependent glutamate release, we applied ATP (30 μM) to trigger astrocytic Ca²⁺ and glutamate release. **b–d** Ca²⁺-dependent glutamate release was unaffected by inhibition of CX hemichannel (CBX, 100 μM), but reduced by blocking anion channels with NPPB (100 μM) (n = 7–9 cells per condition). **e** During the response to Aβ₂₅₋₃₅ (6 μM), inhibiting anion channels with DCPIB (50 μM) influenced the glutamate release during Ca²⁺ elevation phase (n = 10–12 cells per condition)

Discussion

In this study, we examined the sub-cellular mechanisms underlying the astrocytic response to the neurotoxic amyloid beta fragment. A state-dependent alteration of Ca²⁺ homeostasis in association with a multiphasic

release of signaling transmitters have been observed in primary cortical astrocytes. As illustrated in Additional file 1: Fig. S6a, the free cytosolic Ca²⁺ concentration is maintained at physiological low level (~ 100 nM) in astrocytes in basal conditions [62], thereby restraining



the initial PMCA reaction to Aβ25-35 application. One possibility is that Aβ25-35 opens CX hemichannels leading to glutamate and ATP co-release, the latter triggering Ca²⁺ elevation to cause further glutamate release. In basal conditions, Aβ25-35 plays an excitatory role in upregulating astrocyte Ca²⁺ signals. In Aβ-preconditioned astrocytes (Additional file 1: Fig.

S6b), the chronically overloaded intracellular Ca²⁺ sets a greater driving force for its efflux. Hence, PMCA Ca²⁺ export is readily activated by subsequent acute Aβ challenge, leading to an overshoot drop in the basal Ca²⁺ diminution and concomitant H⁺ influx. H⁺ then exerts an inhibitory effect on CX hemichannel opening, thereby blocking the hemichannel and the purinergic activation

of Ca^{2+} elevation. In this situation, $\text{A}\beta$ tends to exert an inhibitory effect on astrocyte Ca^{2+} signal. Immunostaining of PMCA and CX43, a major hemichannel protein expressed in astrocytes, was observed in cultured astrocytes and in the cortex of hAPPJ20 AD mouse model.

Dysregulation of neuron-glia interaction emerges as an important aspect in $\text{A}\beta$ pathology and the evolution of AD [33]. Aberrant Ca^{2+} signals have been noted as a hallmark of astrocyte functional remodeling in AD mouse models [17, 47]. Our current data support the potential contribution of astrocytes to the dysregulated neuroglial activities in amyloidopathy, for instance via the interference with purinergic and/or glutamatergic communications [54]. The primary culture of astrocytes is an *in vitro* model to study the sub-cellular mechanism involved in AD pathophysiology. Primary astrocytes were reported to display a portion of properties different from their *in vivo* counterparts, like the genes featuring the reactive state [9, 100]. In the current study, preconditioning primary astrocytes with submicromolar $\text{A}\beta_{25-35}$ caused appreciable alteration in their subsequent response to high-dose $\text{A}\beta$, indicating a malleable adaptability in their functional status. This suggests that the cultured astrocytes used in this study were not fully reactivated, likely mirroring an early state during brain $\text{A}\beta$ deposition.

We show that $\text{A}\beta_{25-35}$ activates Ca^{2+} elevation via purinergic P2Y1 receptor activation that confirms the *in vivo* finding in AD mouse model [17]. The involvement of the Ca^{2+} release from the internal ER store is also in line with the previous *in vitro* study [85]. ER Ca^{2+} depletion is followed by Ca^{2+} influx via SOC channel [66], which was here observed to be facilitated by $\text{A}\beta_{25-35}$, as previously reported with $\text{A}\beta_{42}$ [74]. This therefore provides an additional mechanism for the upregulated astrocyte Ca^{2+} signal and explains in part its dependence on Ca^{2+} influx. We also show that $\text{A}\beta_{25-35}$ -caused Ca^{2+} signals depend on the opening of CX hemichannels, a major pathway for ATP release from astrocytes [28, 98]. It is possible that $\text{A}\beta_{25-35}$ triggers ATP release from CX hemichannels that then activates astrocyte P2Y1 receptor to cause Ca^{2+} elevation. Interestingly, purinergic autocrine stimulation and subsequent glutamate release has also been observed following optogenetic activation of astrocytes with channelrhodopsin 2 [79]. Optical activation of astrocytes with light-gated GPCRs optoAR and melanopsin also triggered ATP release and autocrine activation of astrocytic purinergic receptors [25, 53]. Nevertheless, our current data could not fully exclude other possible mechanisms underlying $\text{A}\beta_{25-35}$ -induced Ca^{2+} signals. For instance, $\text{A}\beta_{25-35}$ may directly activate astrocyte purinergic receptors, which could be mitigated by CX hemichannel blocking.

Besides the generally observed excitatory effect on astrocytic Ca^{2+} of $\text{A}\beta$ peptides or plaques, we observed

an inhibitory effect of $\text{A}\beta_{25-35}$ in astrocytes preconditioned by submicromolar concentrations of $\text{A}\beta$. It was reported that $\text{A}\beta_{25-35}$ not only triggered Ca^{2+} elevation, but also inhibited ATP-evoked Ca^{2+} elevation in primary cultures of rat astrocytes [85], implying a mixed status of the astrocytes used therein. We here attributed the mechanism of inhibition to the potentiation of PMCA-mediated Ca^{2+} extrusion from the cytoplasm, modulated by $\text{A}\beta$ -triggered cAMP elevation. As ATP-driven pumps, PMCA exports cytosolic Ca^{2+} in a calmodulin-dependent manner to maintain its physiological low level [8, 62]. Overexpression of a human PMCA in striatal astrocytes was used to inhibit Ca^{2+} signals [99]. In our study, the ready activation of PMCA by $\text{A}\beta$ in preconditioned astrocytes implies that their cytosolic Ca^{2+} concentration, due to the gradual overload upon the chronic $\text{A}\beta$ exposure, has been hyper-shifted from the physiological level. As H^+ is a hemichannel inhibitor [77], the H^+ influx that was coupled with PMCA-mediated Ca^{2+} extrusion would have inhibited CX hemichannel, an effect that we found to attenuate Ca^{2+} elevations. This thus resulted in a dominant inhibitory effect in preconditioned astrocytes. Ca^{2+} export by PMCA likely represents a protective mechanism to counterbalance the early Ca^{2+} upregulation in astrocytes caused by $\text{A}\beta$. Nevertheless, PMCA activity is often impaired by recurrent activation and metabolic stresses [8], as it would be encountered as a consequence of long-term $\text{A}\beta$ accumulation in AD [48]. Hence, Ca^{2+} hyperactivity could become eventually prevalent in astrocytes at the time when $\text{A}\beta$ plaques are formed [17, 47].

In an AD mouse model, astrocytes Ca^{2+} hyperactivity occurs globally independent of their proximity to $\text{A}\beta$ plaques, suggesting that the local $\text{A}\beta$ pathology is transmitted by intercellular mechanisms [47]. We here observed $\text{A}\beta_{25-35}$ -caused multiphasic release of glutamate from astrocytes, which could activate adjacent astrocytes and neurons *in situ*. Our data also suggest that $\text{A}\beta_{25-35}$ likely causes ATP release via CX hemichannels to activate astrocyte Ca^{2+} elevation (Additional file 1: Fig. S6a). CX hemichannels are known to release signaling molecules from astrocytes regulating neural activity in physiological and pathological conditions [28]. In AD mouse models, CX hemichannels have been implicated in the release of ATP and glutamate, which dysregulate synaptic transmission [98]. We here suggest the mechanistic steps underlying $\text{A}\beta$ -induced glutamate and ATP release. CX hemichannels may initiate the ATP release that subsequently activated purinergic autoreceptor to elevate Ca^{2+} signal. Consistent with CX hemichannels being nonselective channel pores [28], they were here also observed to mediate Ca^{2+} -independent glutamate efflux. In addition, we noted that $\text{A}\beta$ -evoked

Ca²⁺ signal further increased glutamate release. While Ca²⁺-dependent glutamate release from astrocytes is being debated under physiological conditions [24, 78], it has been observed upon the hyper-regulated astrocyte Ca²⁺ signals in pathological conditions [89].

Our results suggest that A β -evoked Ca²⁺-dependent glutamate release occurs via astrocytic anion channels and lysosome exocytosis. Astrocyte glutamate release was suggested to be mediated by mouse Bestrophin 1 channel in a Ca²⁺-activated manner [67] (but see [94]). In APP/PS1 AD mouse model, excessive GABA release from astrocytic Bestrophin 1 channel was also observed to impair memory and learning [39]. In addition, SWELL-1 (i.e., LRRC8A) channel has been shown to constitute the anion channels that mediate glutamate release from astrocytes in association with cell swelling [96]. The relative roles of Bestrophin 1 and SWELL-1 in A β -evoked glutamate release needs to be further evaluated. We also observed asynchronous lysosome exocytosis following A β -triggered Ca²⁺ signal, likely contributing to signaling molecule release. Lysosomes represent a population of vesicular compartments having a larger size than small secretory vesicles [49]. Although the physiological role of small secretory vesicles in astrocytes remains debated [75], lysosome exocytosis has been observed in response to pathological stimulations [21, 50, 84]. It is therefore plausible that lysosome exocytosis plays a role in modulating astrocytic signals in A β pathologies. Astrocytes could bidirectionally control synaptic transmission, for example via glutamatergic potentiation and purinergic/adenosinergic inhibition [12]. Hence, A β -evoked ATP and glutamate release would affect neuronal activities *in situ* during AD progression, with specific outputs depending on the receptor expression profiles of the local circuitry and their spatial correlation with astrocytic release sites. A β -induced glutamate release likely contributes to the glutamate neurotoxicity seen in AD context [90, 98]. Memantine, the uncompetitive antagonist with moderate affinity for the glutamate *N*-methyl-D-aspartate (NMDA) receptor, has been approved for the treatment of moderate to severe AD [68].

It has been noted that globally ablating pathologically altered astrocytes in AD mouse model worsens the disease [42]. Thus, understanding and hence being able to target dysregulations in specific signaling pathways in astrocytes holds the potential to ameliorate A β pathology. In this context, the current results provide testable targets to control astrocyte responses to neurotoxic A β peptide and will help to understand the astrocytic contributions.

Supplementary Information

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Additional file 1. Figure S1–S6 and Table S1–S2.

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Authors' contributions

This work was initiated while DL, KH and MO were with the Neurophysiology and New Microscopies lab (INSERM U603) at Université Paris Descartes. CP, KH, SM, VV, BC and DL performed the experiments. CP and BC performed the experiments using the hAPPJ20 transgenic mice. CP and DL analyzed the data. DL supervised the study and wrote the manuscript with input from co-authors. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon request.

Ethics approval and consent to participate

All animal care and experimental procedures are in conformity with the French National Charter on the ethics of animal experimentation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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