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Abstract

Neuroglial contribution to Alzheimer's disease (AD) is pathologically relevant and highly heterogeneous. Reactive astrogliosis and activation of microglia contribute to neuroinflammation, whereas astroglial and oligodendroglial atrophy affect synaptic transmission and underlie the overall disruption of the central nervous system (CNS) connectome. Astroglial function is tightly integrated with the intracellular ionic signalling mediated by complex dynamics of cytosolic concentrations of free Ca^{2+} and Na^+. Astroglial ionic signalling is mediated by plasmalemmal ion channels, mainly associated with ionotropic receptors, pumps and solute carrier transporters, and by intracellular organelles comprised of the endoplasmic reticulum and mitochondria. The relative contribution of these molecular cascades/organelles can be plastically remodelled in development and under environmental stress. In AD astroglial Ca^{2+} signalling undergoes substantial reorganisation due to an abnormal regulation of expression of Ca^{2+} handling molecular cascades.

1. Calcium hypothesis of ageing and AD

The main risk factor for the development of neurodegeneration and Alzheimer's disease (AD) is ageing [1]. There are several stages of AD, but the most familiar one, a late stage of AD with severe dementia, is linked to the presence of extracellular deposits of fibrillar β-amyloid peptide and intraneuronal accumulation of aggregates of hyper-phosphorylated Tau protein [2]. Neurodegeneration occurs gradually and dementia may reflect the end stage of an accumulation of pathological changes that start to develop decade(s) before the onset of the clinical symptoms [3].

The calcium hypothesis of ageing and neurodegeneration, inspired by pioneering experiments of Philipp Landfield, emerged more than 30 years ago [4-6]. This hypothesis postulated that slow and mounting deregulation in Ca^{2+} homeostatic cascades with age progressively affects cellular homeostasis, cellular signalling, synaptic transmission and ultimately cell survival; in neurodegeneration this process of Ca^{2+} deregulation is accelerated, which leads to massive cell death, brain atrophy and dementia, such as are observed in AD [7, 8].

Several Ca^{2+} regulating molecular cascades seem to be deregulated in neurones undergoing degenerative transformations. These changes arise either from direct interactions between β-amyloid and Ca^{2+} handling molecules or can develop independently of the former, being probably a part of pathological evolution. The ability of β-amyloid to form plasmalemmal Ca^{2+} permeable channels and cause massive and deleterious Ca^{2+} influx into neuronal cells have been noted in the early 1990s ([9], for further details see [10-12]). The mechanism for this Ca^{2+} influx was arguably associated with ionophoretic abilities of β-amyloid that forms Ca^{2+} permeable transmembrane pores; Ca^{2+} influx can be further amplified through Ca^{2+} release from the endoplasmic reticulum (ER) Ca^{2+} store [13]. The "β-amyloid calcium pore" hypothesis has evolved into β-amyloid-induced modulation of physiologically-relevant Ca^{2+} homeostatic/signalling pathways. Exposure to β-amyloid affects several types of Ca^{2+} permeable membrane channels. In particular β-amyloid binds to and modulates Ca^{2+}-permeable neuronal acetylcholine receptors in α7 (homomeric), α7β2 and α4β2 compositions; β-amyloid activates α7 receptors in picomolar concentrations, while inhibits the both of above heteromeric receptors in nanomolar concentrations [14, 15]. There are certain arguments that β-amyloid excitotoxicity is mediated by extra-synaptic NMDA receptors [16], although this might be an indirect effect (for example, through an inhibition of astroglial glutamate clearance systems [17]). Polymorphism of the encoding gene of the recently identified Ca^{2+} homeostasis modulator 1 channel (CALHM1), which is present in neuronal plasmalemma and endomembranes [18, 19], has been associated with some forms of AD [20]; the aberrant form of CALHM1 arguably deregulated neuronal Ca^{2+} homeostasis [21, 22]. In the animal models bearing AD-associated mutations of presenilins, depletion of the ER Ca^{2+} stores and modification of inositol 1,4,5 trisphosphate ($InsP_3$) receptors gating are a common finding [23, 24]. It is generally agreed that mutated presenilins increase Ca^{2+} release from the ER through both $InsP_3$ receptors and ryanodine receptors [25-27]. Finally, mutant presenilins affect Ca^{2+} handling by mitochondria and ER-mitochondria cross-talk [28]. All these changes in the status of Ca^{2+} handling may be relevant for progression of the AD; nonetheless, mostly Ca^{2+} handling cascades were studied in neurones. In this review we shall focus on astrocytes, the homeostatic cells of CNS which significantly contribute to the AD pathology.

2. Ionic excitability of astroglia

The substrate of astroglial excitability is associated with dynamic spatio-temporal changes in intracellular ionic concentrations, with Ca^{2+} and Na^+ being the main players. Astroglial calcium signalling has been identified in the early 1990s when it become obvious that chemical (neurotransmitters) or mechanical stimulation of astrocytes *in vitro* triggers elevations of free cytosolic Ca^{2+} ($[Ca^{2+}]_i$) that spread through astroglial syncytia in a form of propagating Ca^{2+} waves [29-32]. Astroglial calcium signals have been subsequently characterised in *in vitro* and *in vivo* settings (see [33-35] for details and critical discussion).

Calcium ions are the most ubiquitous and evolutionary ancient intracellular second messenger that controls various vital cellular functions and regulate physiological responses in virtually every living organism [36-40]. Generation of astroglial Ca^{2+} signals utilises several tightly coordinated molecular pathways that include Ca^{2+} release from the ER stores, plasmalemmal Ca^{2+} entry, cytosolic Ca^{2+} buffering, Ca^{2+} sequestration into the ER and mitochondria and Ca^{2+} extrusion mainly via plasmalemmal Ca^{2+} pumps [33]. Expression of molecular components of Ca^{2+} homoeostatic/signalling system can be dynamically modified, being an important part of cellular plasticity. Specific combinations of Ca^{2+} handling molecules, generally referred to Ca^{2+} signalling toolkits [41], may also be a part of the pathological development, with aberrant Ca^{2+} handling contributing to cellular pathology [42]. Intracellular Ca^{2+} release from the ER, predominantly mediated by activation of type II $InsP_3$ receptors, was generally considered to be the leading mechanism for astroglial Ca^{2+} signalling; indeed, activation of various metabotropic receptors may trigger this pathway [33, 43, 44]. The ER Ca^{2+} release, however, is mainly responsible for generation of global Ca^{2+} signals and propagating Ca^{2+} waves; local Ca^{2+} signalling in perisynaptic astroglial processes generally devoid of the ER seems to rely mostly on plasmalemmal Ca^{2+} entry. This latter is mediated by a plethora of ionotropic receptors (i.e. glutamatergic, purinergic and cholinergic [45-47]), transient receptor potential (TRP) channels [48, 49] and sodium calcium exchanger (NCX), operating in the reverse mode [50]. The TRPC-containing channels [51] also contribute to the store-operated Ca^{2+} entry initiated by the depletion of the ER store of releasable Ca^{2+} [52, 53].

The role for sodium signalling in astroglial excitability has been considered rather recently [54-57]. It appeared that stimulation of astrocytes with neurotransmitters released from neuronal terminals triggers long lasting elevation of cytosolic Na^+ ($[Na^+]_i$) with amplitudes of 5 - 20 mM [58-61]; this $[Na^+]_i$ increase resulted from Na^+ entry through plasmalemmal channels (mainly ionotropic receptors) and transporters (mainly Na^+-dependent glutamate transporters). Astroglial Na^+ signals are also able to spread trough astroglial syncytia in a form of propagating waves; this propagation is mediated by connexin channels forming gap junctions [62]. Fluctuations in astrocytic $[Na^+]_i$ control multiple membrane transporters and pumps responsible for astroglial homeostatic functions, including plasamallemal neurotransmitter (glutamate, GABA and adenosine) and glutamine transporters, NCX, sodium-potassium ATPase, etc. (see [55, 56] for details and references).

3. Astroglia in neurological diseases

Pathological potential of astroglia in various neurological diseases has been widely recognised by Rudolf Virchow, Ramón y Cajal, Pío del Río Hortega and Alois Alzheimer [63-65]; the interest in neuroglia in neuropathology has been greatly reinvigorated in the most recent decade [66-71]. Astrocytes contribute to every type of neuropathology, and this contribution is complex and context specific. Conceptually, depending on neuropathology,

astroglia may undergo structural or functional atrophy with a loss of function, pathological remodelling or reactivity; these changes can develop on their own or in combination [70, 72]. Reactive astrogliosis is an evolutionary conserved defensive reaction, which results in a complex structural, biochemical and functional remodelling of astrocytes leading to an appearance of multiple reactive phenotypes, which again seem to be context/disease specific. Astroglial reactivity is a *bona fide* defensive response; inhibition of astroglial reactivity, as a rule, exacerbates neuropathology.

The sequence of pathological metamorphosis of astroglia often follows the evolution of the disease. Astroglial pathological remodelling, for example, is a characteristic of Alexander's disease or epilepsy [73, 74], when astrocytes acquire a new pathological phenotype that drives these diseases. In neuropsychiatric disorders the astroglial atrophy is manifested by a decrease in the number of astrocytes, which contributes to disbalance in excitatory/inhibitory neurotransmission [75-77]. In brain trauma or stroke, astroglial reactivity dominates, often leading to formation of a glial scar; reactive astrocytes, however, are obligatory feature of post-lesion regeneration [68]. In neurodegenerative diseases all forms of astrocytopathy (i.e., atrophy, reactivity and pathological remodelling) are observed [1, 78]. In amyotrophic lateral sclerosis (ALS) the early loss of astroglial ability to contain glutamate loads results from astrodegeneration and astroglial atrophy that occur before clinical symptoms and neuronal death. This deficient astroglial phenotype may be associated with changes in the traffic of intracellular vesicles. In astrocytes exposed to antibodies isolated from ALS patients, a massive alteration in vesicle dynamics and Ca^{2+} homeostasis has been reported [79, 80]. In the animal model of ALS expressing human mutant superoxide dismutase 1 (Tg(SOD1*G93A)1Gur mice), the emergence of atrophic astrocytes is the earliest pathological signature [81-83]; these atrophic astrocytes down-regulate glutamate uptake and become vulnerable to glutamate by themselves. Deficient astrocytes, therefore, provide a background for developing glutamate excitotoxicity that leads to neuronal death [72]. Incidentally, a cell-specific silencing of the mutant SOD1 gene in astrocytes significantly delayed development of clinical symptoms [84]. Reactive astrocytes appear at the later stages of ALS; their reactivity is most likely triggered by dying neurones, although atrophic forms of thses cells also remain. Pathological suppression of astroglial glutamate uptake also plays the leading role in the development of Wernicke encephalopathy, a thalamo-cortical neurodegeneration, which represents the substrate for Korsakoff syndrome. Expression of astroglial glutamate transporters decreases by ~ 70% resulting in massive glutamate excitotoxicity [85, 86]. In Huntington disease (HD) the hampered astroglial glutamate uptake is concomitant with an aberrant release of glutamate from astrocytes, both processes instigating excitotoxic damage to neurones [87]. Astroglial reactivity is also observed in HD, while suppression of astrogliotic response by inhibition of JAK/STAT3 signalling cascade increases the number of huntingtin aggregates [88]. Similarly astroglial reactivity seems to be decreased in Parkinson's disease as judged by a decreased expression of glial fibrillary acidic protein (GFAP) in human tissue samples [89].

4. Astroglia in AD

The extent and detailed characterisation of astrogliopathology in AD remains virtually unknown. Astroglial reactivity mainly documented by hypertrophy and an increase in expression of GFAP and S100B proteins, has been generally mentioned in morphological analysis of post-mortem tissues from AD patients [90-93]. Sporadic reports claimed a degree of correlation between an increase in GFAP expression and the Braak stage of AD, although no correlation between astrogliotic changes and β-amyloid load were found [94]. Reactive astrocytes were found to be associated with some senile plaques, but they were also identified

in plaque free regions of the grey matter [94].To the contrary, no differences in GFAP expression was found between demented and non-demented brains [95]. Of note, reactive astrogliosis in AD is quite mild; astrocytes in the grey matter preserve their domain organisation and there are no indications of anisomorphic gliosis and formation of glial scars [78, 96].

In animal models of AD, both astroglial atrophy and reactive astrogliotic remodelling were described. Treatment of rodent astrocytes *in vitro*, in dissociated cell cultures and *in situ* in organotypic slices with β-amyloid in concentrations ranging between 100 nM and > 5 μM trigger astrogliotic response [97, 98]. In the brains of transgenic AD mouse models, reactive astrocytes are generally associated with β-amyloid depositions and β-amyloid plaques in the hippocampus [96]. Astroglial reactivity was not uniform throughout the brain; formation of extracellular β-amyloid deposits and emergence of senile plaques failed to induce reactive astrogliosis in entorhinal and prefrontal cortices [99, 100]. In the triple transgenic (3xTG) AD mice over-expressing mutant genes for amyloid precursor protein (APP_{Swe}), presenilin 1 ($PS1_{M146V}$) and microtubule-associated protein Tau (Tau_{P301L}), [101]and in PDAPP-J20 mice carrying the Swedish and Indiana human mutations of APP [102] astroglial atrophy was identified throughout the brain [99, 100, 103-106]. Astrodegenerative changes were found at the early pre-symptomatic stages (i.e. before considerable accumulation of extracellular β-amyloid and formation of senile plaques) and they were characterised by decreased complexity of astrocytes (which had less primary and secondary processes) and by reduction on the size of profiles of astrocytes labelled with antibodies against GFAP or glutamine synthetase. [99, 100, 103, 104]. The atrophic changes in astrocytes developed in a particular spatio-temporal pattern with the earliest signs of atrophy observed in the entorhinal cortex (at 1 months of age); at 3 months of age morphological atrophy of astrocytes was identified in the prefrontal cortex, whereas in the hippocampus the atrophic changes were evident from 9 to 12 months of age [99, 100, 103]. Expression of glutamine synthetase was generally decreased in hippocampal reactive astrocytes, indicating possible deficits in glutamate-glutamine shuttle [107]; in the entorhinal cortex, however, expression of glutamine synthetase seems to be preserved [108].

Astroglial atrophy that emerges at the early stages of neurodegeneration may have an important contribution to the launching of the AD pathology, as, indeed, a loss of astroglial coverage and a loss of astroglial function can each have dire consequences. Atrophy of astroglial perisynaptic processes, which foster and maintain synaptic transmission (as embellished by the concept of the astroglial cradle - [109]) can be an important (if not the leading) mechanism of synaptic weakening and synaptic loss that signals the beginning of AD pathology [110]. General decrease in the astroglial homeostatic reserve reduces neuroprotection and may be detrimental for the neuro-vascular unit and may affect the glial ability to metabolically support neuronal networks. Furthermore, the astroglial loss of function manifests in suppressed reactivity in certain brain regions such as entorhinal and prefrontal cortices; deficits in the astrogliotic response could be an important factor determining higher sensitivity of these regions to AD pathology. In summary, astroglial asthenia and functional paralysis may define the landscape permissive for AD evolution and, hence, determine the depth of cognitive deficit and clinical progression of the disease [78].

5. Astroglial Ca^{2+} homoeostasis and Ca^{2+} signalling in AD

Conceptually, pathological remodelling of astroglial Ca^{2+} homeostatic/signalling toolkits may either be cell-autonomous (i.e. reflecting intrinsic cellular pathology, for example, associated with the expression of specific mutated genes) or it can develop in response to extrinsic stress

associated, for example, with the accumulation of aberrant molecules in the extracellular space. In the context of AD, the latter mechanism was intensely scrutinised because of the general belief that this pathology is associated with the accumulation of β-amyloid in the brain parenchyma.

5.1. Effects of β-amyloid on astroglial Ca^{2+} signalling

Numerous studies on cultured astrocytes have demonstrated that exogenous β-amyloid alters $[Ca^{2+}]_i$. For example, incubation of astrocytes with 5 µM β-amyloid$_{1-42}$ for 2 hours led to a two-times increase in resting $[Ca^{2+}]_i$ [111]. In another study the effect of β-amyloid on resting Ca^{2+} was even more profound; incubation of cultured rat hippocampal astrocytes with 100 nM oligomeric β-amyloid$_{1-42}$ for 4 - 6 hours elevated increased resting $[Ca^{2+}]_i$ from ~50 nM to 100-150 nM [112]. However, longer exposures (48 hours) of cultured astrocytes to β-amyloid in concentrations ranging between 200 nM and 20 mM did not affect basal $[Ca^{2+}]_i$ levels [113, 114]. The data on acute effects of β-amyloid on astroglial cells *in vitro* and in slices are controversial. While several laboratories reported that β-amyloid (at 100 nM - 5 µM) concentrations triggered transient $[Ca^{2+}]_i$ increases or $[Ca^{2+}]_i$ oscillations [98, 115-119], others have not found acute $[Ca^{2+}]_i$ responses to β-amyloid [112-114]. This seemingly incongruent findings could possibly be attributed to variability of β-amyloid species used. The properties of astrocytes in a given experimental preparation may also contribute. Ffor example, β-amyloid in exceedingly low concentrations (200 - 300 pM) was reported to activate α7 nicotinic cholinoreceptors and trigger $[Ca^{2+}]_i$ responses in hippocampal astrocytes *in situ* [120]; yet, not every astrocyte expresses this receptor type [121].

Indeed, more careful analysis showed that β-amyloid affects only a sub-population of astrocytes. Challenging cultured astrocytes with 1 µM β-amyloid$_{1-40}$ induced transient $[Ca^{2+}]_i$ responses only in 17% of astrocytes, although a challenge with 1 µM of β-amyloid$_{25-35}$ evoked $[Ca^{2+}]_i$ response in 36% astrocytes [118]. In another study, the exposure of primary astrocytes to 1 µM of β-amyloid$_{25-35}$ triggered $[Ca^{2+}]_i$ a response in 27% astrocytes, whereas at 2-5 µM β-amyloid$_{25-35}$ produced $[Ca^{2+}]_i$ transients in ~ 60% of cells [122].

Chronic treatment of astrocytes with β-amyloid resulted in pathological remodelling of the Ca^{2+} signalling toolkit [123], with changes in the expression of neurotransmitter receptors linked to Ca^{2+} signalling being probably best documented. Already after 1 hour of incubation of cultured astrocytes with 100 nM of β-amyloid, it induced clustering and diffusional trapping of metabotropic glutamate receptor 5 (mGluR5); this in turn activated the receptor and induced release of ATP [124]. At longer exposure times (24-72 h), β-amyloid up-regulated the expression of astroglial mGluR5 [125]. Incidentally, the up-regulation of mGluR5 has been also found in plaque-surrounding cortical astrocytes associated with senile plaques in the APPswe/PS1dE9 mouse model, as well as in post-mortem human tissues [112, 113]. As alluded to earlier, α7 nicotinic cholinoreceptor (α7nAChR) binds β-amyloid with high affinity and can be modulated by ιτ is [126]. Chronic treatment with low β-amyloid concentrations (0.1 - 100 nM), however, up-regulates several cholinoreceptors including those containing α7, α4 and β2 subunits [127]. Increase in astroglial expression of α 7AChR was also detected in human post-mortem samples obtained from both sporadic and familial forms of AD [128]. Another receptor which seems to be positively modulated by β-amyloid is G-protein coupled metabotropic Ca^{2+}-sensing receptor (CaSR). Exposure to β-amyloid was shown to acutely activate CaSR in primary astrocytes and this activation triggered CaSR-dependent signalling pathway that stimulates the expression of nitric oxide synthase-2 (NOS-2) followed by an excessive release of nitric oxide increased expression of Vascular

Endothelial Growth Factor (VEGF)-A, and instigated production of β-amyloid; all these effects were suppressed by specific CaSR antagonists [129-132].

Chronic treatment with β-amyloid also effects ER Ca^{2+} handling and signalling in astroglia. After 48 hours of incubation with 100 nM of β-amyloid$_{1-42,}$ a significant increase in the expression of mRNA for InsP$_3$ receptors type 1 and 2 (InsP$_3$R1 and InsP$_3$R2) was detected in rat hippocampal primary astrocytes [112]. In contrast, the same treatment did not affect the expression of InsP$_3$R1 protein in the entorhinal cortex astrocytes, indicating a regional heterogeneity of astrocytes [125]. In human post-mortem tissues, however, an overall decrease in the expression of InsP$_3$ receptors is observed [133-135], which may, however, indicate the overall cell loss and, hence, a decrease in the total expression of receptors. Finally, a chronic treatment with β-amyloid was also reported to increase the store-operated Ca^{2+} entry in astrocytes [136, 137].

5.2. Ca^{2+} signalling in AD astrocytes

Gene profiling of human astrocytes, laser dissected from the post-mortem temporal cortex obtained from three groups of patients with different Braak scores, found 32 genes associated with Ca^{2+} signalling and homeostasis to be abnormally expressed [138]. In astrocytes purified (by fluorescence activated cell sorting) from the 15-18 month old APPswe/PS1dE9 mice, a substantial increase in "calcium ion binding" gene ontology class genes was detected [139].

Modified calcium signalling was observed in astrocytes from various transgenic models of the AD (Fig. 1). Rather profound (~100%) increase in resting $[Ca^{2+}]_i$ as well as aberrant $[Ca^{2+}]_i$ activity linked to a generation of abnormal long-projecting Ca^{2+} waves were found in astrocytes associated with senile plaques in APP/PS1 mice [140]. Similar, high-frequency Ca^{2+} waves were also monitored in astrocytes from APP$_{Swe}$ mice even before the formation of β-amyloid deposits [141]. In hippocampal astrocytes isolated and cultured from neonatal 3xTg-AD mice, the amplitude of ATP-induced $[Ca^{2+}]_i$ transients as well as the store-operated Ca^{2+} entry were significantly increased [125, 137]. In contrast, store-operated Ca^{2+} entry in astrocytes from APP-over-expressing Tg5469 AD mice was not affected, although the deletion of APP inhibited SOCE, possibly due to down-regulation of Orai1 and TRPC1 channels [142].

Astroglial calcium signalling and in particular Ca^{2+} release form ER stores via InsP$_3$ receptors controls astroglial reactivity, which is fundamental for tissue protection against various pathological insults including AD pathology. As dicussed earlier, in 3xTG-AD mice astrogliotic response is present in the hippocampus but not in the entorhinal and prefrontal cortices. The role for astroglial Ca^{2+} signalling in triggering astrogliosis in response to β-amyloid was demonstrated in cultured astrocytes and in organotypic slices [98]. Exposure of these cells to β-amyloid triggered $[Ca^{2+}]_i$ oscillations originating from InsP$_3$-mediated Ca^{2+} release from the ER; pharmacological inhibition of these oscillations suppressed both $[Ca^{2+}]_i$ dynamics and astroglial reactivity [98]. When hippocampal astrocytes were compared to the astrocytes from the entorhinal cortex, it turned out that β-amyloid significantly up-regulated the expression of mGluR/InsP$_3$ Ca^{2+} signalling pathway in the former but did not affect this cascade in the latter. This difference in the sensitivity of Ca^{2+} signalling toolkit to pathological stress may account for heterogeneity of astrogliotic response and hence distinct vulnerability of different brain regions to AD pathology.

6. Conclusions

Astrocytes, the homeostatic and defensive cells of the CNS exhibit a form of excitability associated with spatio-temporally controlled fluctuations of cytosolic concentrations of ionised Ca^{2+} and Na^+. Glial Ca^{2+} handling can be affected either by b-amyloid, or through intrinsic mechanisms associated with AD pathology, which in turn affects various cell functions manifesting in altered morphology and astroglial-neuronal communications. Distinct brain regions exhibit different pathological manifestations, likely due to specific deficits in astroglial reactivity, which can accelerate, exacerbate or slow-down AD pathology. Moreover, differential sensitivity of Ca^{2+} signalling toolkit to the pathological environment may define the vulnerability of brain regions to the pathological process.

6. Acknowledgements

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Figure Legend

Figure 1: ATP-evoked oscillatory calcium responses in wild type and 3xTg-AD astrocytes.

(**A**) Confocal images of astrocytes containing the fluorescent Ca^{2+} indicator Fluo-2. The images display astrocytes before (0 s, left) and after stimulation with 100 µM ATP (30 and 180 s; middle and right, respectively). ATP evoked strong increases in intracellular calcium activity as indicated by the pseudocoloured intensity scale (right, 0 – 255 intensity levels). Scale bars, 50 µm. ATP (white rectangle) evoked two types of calcium responses in wt (**B**) and 3xTg-AD (**C**) astrocytes: (i) biphasic transients and (ii) oscillatory calcium responses. The peak (p, mean ± SEM) and the time-integrated $[Ca^{2+}]_i$ (S) evoked by 100 µM ATP. The horizontal dotted line indicates the baseline fluorescence level (F_0).The downward (black) and upward (white) arrowheads indicate successive minima and maxima (p_{osc}), respectively, in $[Ca^{2+}]_i$ during oscillatory responses. (**D**, **E**) Plots displaying the relationship between the ratio of the sum of follow-up peak calcium amplitudes – oscillations (Sum p_{osc}) and the first calcium peak amplitude (p), and the number of oscillations within 4 min (No. p_{osc}/4 min) in calcium responses evoked by 100 µM ATP in wt (**D**) and 3xTg-AD (**E**) astrocytes. The non-oscillatory (i) calcium responses are confined to the grey shaded areas delineated with dashed lines, while the white zone of the plots show the oscillatory (ii) responses (the details are described in the Results section). The relative proportion (%) of non-oscillatory (i, left) and oscillatory (ii, right) responses in wt (n = 115) and 3xTg-AD (n = 150) astrocytes are displayed at the top of the plots. Note that ATP evoked three times more oscillatory responses in wt than in 3xTg-AD astrocytes. With permission reprinted from [143].
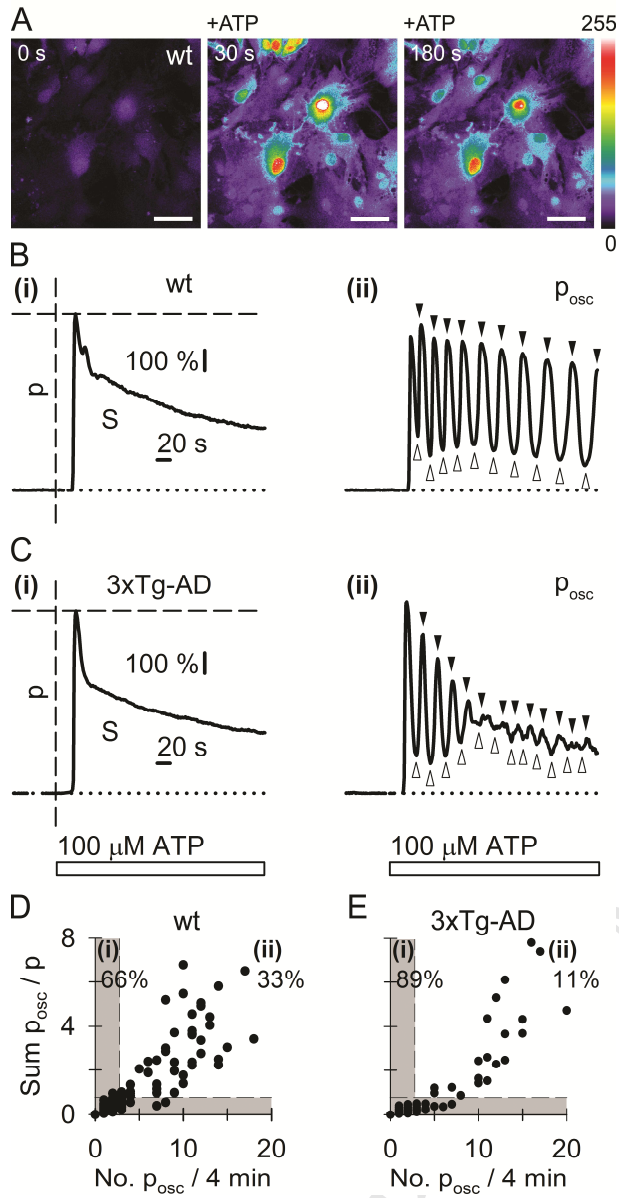
Highlights

Astrocytes undergo complex pathological remodelling ranging from atrophy to reactivity in the Alzheimer disease.

Exposure of astrocytes to β-amyloid disturbs Ca^{2+} homeostasis and Ca^{2+} signalling.

Astrocytes carrying AD-associated pathological genes display aberrant Ca^{2+} signalling