Original paper

Folia Neuropathologica

Alzheimer's amyloid-β peptide disturbs P2X7 receptor-mediated circadian oscillations of intracellular calcium

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Folia Neuropathol 2016; 54 (4): 360-368 DOI: 10.5114/fn.2016.64813

Abstract

Recent data indicate that Alzheimer's disease (AD) is associated with disturbances of the circadian rhythm in patients. We examined the effect of amyloid-β (Aβ) peptide, the main component of the senile plaques playing a critical role in the deregulation of calcium (Ca^{2+}) homeostasis in AD, on the circadian oscillation of cytosolic calcium (Ca^{2+}) levels in vitro. The experiments we carried out in human primary skin fibroblasts. This cell line was previously shown to exhibit circadian rhythms of clock genes. Moreover, the basic clock properties of these peripheral cells closely mimic those measured physiologically and behaviorally in human and do not change during aging. In this study we showed that i) cytosolic Ca^{2+} oscillations depend on the activation of purinergic P2X7 receptors; and ii) these oscillations are abolished in the presence of Aβ. In total, our new findings may help to deepen our understanding of the molecular mechanisms involved in AD-related circadian alterations.

Key words: *amyloid-β, Alzheimer's disease, calcium, P2X7 receptors, circadian rhythms.*

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder that is characterized by progressive neuronal loss, mainly in the brain cortex and hippocampus [28]. One of the main neuropathological hallmarks of this disease is the deposition of extracellular senile plaques containing amyloid-β (Aβ) peptides, derived from processing of the amyloid-β precursor protein (APP) [3,18]. Many previous data point out to a critical role for deregulation of calcium (Ca^{2+}) homeostasis in the pathogenesis of AD. Thus, the levels of intracellular Ca^{2+} concentration and calcium-regulated enzymes (e.g. calpains, proteases, phospholipases) were found to be elevated in animal models of AD [24] as well as the brains of AD patients [47,48]. The "calcium hypothesis" was further supported by demonstrations that, during brain aging, the molecular processes responsible for Ca^{2+} regulation were impaired. This involved the mechanisms of Ca^{2+} sequestration into its intracellular stores (endo(sarco)plasmic-reticulum (ER) and mitochondria) as well as Ca^{2+} influx into the cytoplasm by voltage-gated Ca^{2+} channels, ionotropic or metabotropic receptors [13,21,64]. In AD, disturbed Ca^{2+} homeostasis is not restricted

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to neurons but represents a global phenomenon affecting virtually all cells in the brain. AD-related aberrant Ca^{2+} signaling in astrocytes and microglia probably contributes profoundly to an inflammatory response that, in turn, impacts neuronal Ca^{2+} homeostasis and brain function [8]. It has been shown that Aβ release induces intracellular calcium overload and activates intracellular calcium-dependent events, leading to a decrease in learning and memory as well as cognitive dysfunction [27,43,62]. Previous findings suggested that *N*-methyl-D-aspartate receptors (NMDARs) are the main mediators of enhanced Ca^{2+} entry evoked by Aβ [19,35]. However, more recent discoveries showed that the soluble form of Aβ oligomers induce their toxic effects by disrupting the integrity of the cell plasma membrane leading to uncontrolled fluxes of Ca^{2+} into the cells [66].

Moreover, recent data indicate that AD progression is associated with disturbances of the circadian rhythm in patients. Circadian rhythms govern a wide variety of physical, behavioral and metabolic processes that follow a roughly 24-hour cycle, responding primarily to the light/dark cycle. These are controlled by the circadian clock machinery, in which rhythm-generating mechanisms are encoded by a transcription-translation feedback loop. The mammalian circadian clock machinery is regulated by a central pacemaker in the suprachiasmatic nucleus (SCN) of the brain that synchronizes oscillators in peripheral tissue [16]. The entrained signals from SCN neurons are distributed through different target organs by efferent neural and humoral mechanisms, such as circulating melatonin, producing changes in metabolism, core body temperature, and sleep. Calcium ions are a potent second messenger coupling the clock gene oscillation and the rhythmic firing of action potential in SCN neurons [33,37,58,60]. Calcium mediates intracellular clock signals, such as entrainment processes [6,23,30], clock gene expression [33,37,45,55], and output signaling [2]. Moreover a topological specificity of the circadian Ca^{2+} rhythm in SCN was observed, suggesting that calcium plays a role in the hierarchical organization of rhythmicity in the central pacemaker [25].

The alterations of the SCN as well as in melatonin secretion are the major factors of circadian clock disruption [72]. Insomnia, nocturnal behavioral changes, sundowning syndrome and excessive daytime sleepiness are the common to circadian disturbances observed in AD patients as well as patients with mild cognitive impairment [7,17,67,68,70]. The studies in animal and humans demonstrated that Aβ level in the cerebrospinal fluid is modulated by sleep-wake cycles [5,36,40]. This raises the possibility that disturbances in the circadian rhythm causes brain Aβ accumulation over time, suggesting a causative rather than an associative link between sleep loss and Aβ accumulation [52]. However early-stage AD events such Aβ aggregation and disturbances in calcium homeostasis may also induce molecular changes that lead to circadian clock disruption. Therefore the aim of this study was to deepen our understanding of the molecular mechanisms involved in AD-related circadian clock alterations, by investigating i) the clock-dependent regulation of intracellular calcium levels in a peripheral tissue, and; ii) the effect of Aβ peptides on the changes of cytosolic calcium levels around the clock. For this purpose, we used primary cultures of human fibroblasts because: i) fibroblasts coming from AD patients present a disturbed Ca^{2+} homeostasis [31,39]; and ii) fibroblasts are a valuable *in vitro* model of peripheral oscillators [10,56,57].

Material and methods

Ethical permission

Prior ethical consent to the use of human skin tissues was given by the Ethical Committee of Basel, and informed written consent to participation in this study was obtained from all human subjects.

Materials

Human recombinant $A\beta_{42}$ was purchased from Bachem (Germany). Coomassie Brilliant Blue G, DMSO, DMEM, Fetal Bovine Serum (FBS), Glutamax, Hank's Balanced Salt Solution (HBSS), Horse Serum, Penicillin/Streptomycin, Pluronic® F-68, Thapsigargin and ATP were obtained from Sigma (St. Louis, MO, USA). Liberase was from Roche Diagnostics GmbH (Mannheim, Germany). Fluo-4 AM was obtained from Invitrogen (Thermofisher Scientific, Waltham, Massachusetts, USA).

Tissue isolation and fibroblast culture

Two cylindrical cutaneous biopsies (2 mm diameter) were taken from the buttocks of a healthy male subject. Fibroblasts were isolated from biopsies by

4-h digestion of tissue in DMEM/1% penicillin streptomycin/1% Glutamax (DMEMc)/20% FBS/87.5 ng/ml Liberase, and cultured in DMEMc/20% FBS.

Synchronization of cells and timetable to study cellular circadian rhythms

For all experiments, cells were seeded onto collagen-coated 48-well or 96-well dark plates at the density of $1,4*10^5$ cells per ml. Cells were synchronized by treatment with DMEM containing 50% Horse Serum for 2 hours at 37°C. After the synchronization, cells were washed with PBS and the medium was changed into DMEM/2% FBS according to [1]. Experiments were performed every 4 hours, starting 4 h after synchronization and until 48 h.

Preparation of Aβ species and cell treatment

$A\beta_{42}$ was dissolved in PBS to make stocks of 500 µM and stored at –80°C until use. Aging of the peptides was induced by shaking the diluted solution (50 µM) at 1000 rpm overnight at 37°C. The cells were treated after synchronization at a final concentration of 0.5 µM of $A\beta_{42}$. In selected experiments, after measurement of basal Ca^{2+} levels, cells were treated either with a sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor (Thapsigargin; 10 nM), or with an agonist (ATP; 1 mM) of purinergic P2X7 receptors. The measurements were repeated immediately or after 5 min of incubation, respectively.

$[Ca^{2+}]_i$ measurements

$[Ca^{2+}]_i$ measurement was carried out using the fluorescent indicator Fluo-4 acetoxymethyl (AM) ester (200 µM stock solution in DMSO). At the specific time points after cell synchronization, fibroblasts were loaded with 4 µM Fluo-4 AM supplemented with 0.02% Pluronic® F-68 for 60 min at 37°C in a standard HBSS. The cells were washed 3 times with HBSS and, to ensure complete AM ester hydrolysis, kept for 30 min at 37°C in the dark. After a second washing step, the fluorescence was measured using Fluoroskan® counter at 485/520 nm. To study the involvement of purinergic receptors and endoplasmic reticulum (ER) stores on a cytosolic calcium level, cells were treated with an agonist (ATP, 1 mM) or an antagonist of purinergic P2X7 receptors (Coomassie Brilliant Blue G, 5 µM) for 1 min and a non-competitive SERCA inhibitor for 30 seconds, as described in figures' captions, and the fluorescence of Fluo-4 was measured.

Statistical analysis

The results were expressed as mean values ± SEM. Differences between means were analyzed using Student's two-tailed *t* test. $P < 0.05$ was considered statistically significant. Cosinor software analysis was used to evaluate and estimate the parameters of circadian rhythm (period plus mean, amplitude and acrophase).

Results

Since intracellular calcium was previously shown to exhibit a well-defined circadian rhythm in neuronal population of SCN [32], we first verified whether Ca^{2+} level exhibited a circadian rhythmicity in peripheral oscillators (Fig. 1). We used human primary fibroblasts since these cells are an excellent *in vitro* model of peripheral oscillators [10,56,57] and show a disturbed Ca^{2+} homeostasis in AD patients [31,39].

In the current study, fibroblasts presented changes in Ca^{2+} accumulation, with a peak 16 h post-synchronization (TP16) and a trough 28 h post-synchronization (TP28) (Fig. 1A). Under these conditions, relative Fluo-4 fluorescence in cell cultures was significantly different ($p < 0.001$) between the peak and the trough (Fig. 1D).

Since endoplasmic reticulum (ER) Ca^{2+} stores are important in the regulation of Ca^{2+} signaling in cells, we quantified $[Ca^{2+}]_i$ in fibroblasts after treatment with 10 nM thapsigargin (THAPS), an SERCA inhibitor. Depletion of ER Ca^{2+} stores with THAPS did not alter the circadian rhythm of $[Ca^{2+}]_i$ but increased the total Ca^{2+} level (Fig. 1B). The significant difference between $[Ca^{2+}]_i$ at TP16 and TP28 was still present (Fig. 1D).

Considering the controlling mechanisms of cytosolic Ca^{2+} fluctuations, it is possible that receptor-mediated Ca^{2+} influx is involved in the regulation of circadian rhythm of $[Ca^{2+}]_i$. Since primary human fibroblasts are electrically non-excitable and do not express voltage-gated Ca^{2+} channels, Ca^{2+} could be transported via purinergic P2X receptors, especially P2X7 subtype that is widely distributed in skin tissue [65]. Using the specific antagonist of P2X7 receptor-Brilliant Blue G (BBG), we showed that treatment with this compound abolished circadian oscillations

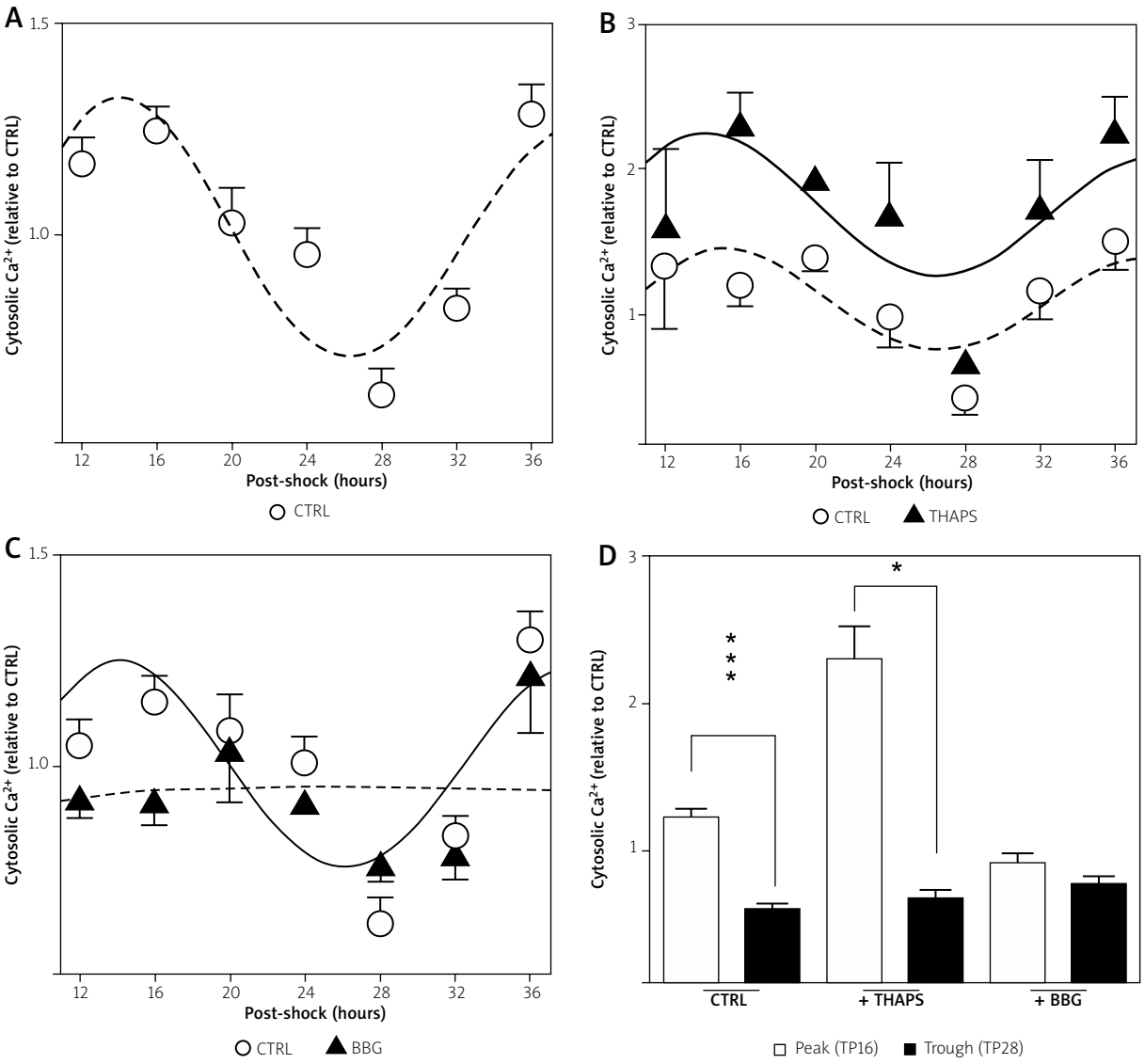

Fig. 1. Circadian oscillations of cytosolic calcium depend on activation of P2X7 receptors but not calcium uptake through SERCA. **A)** Cytosolic calcium levels were evaluated by using fluorescent dye, Fluo-4 (4 µM), in synchronized human skin fibroblasts from 12 hours post-synchronization time point every 4 hours for 7 time points (n = 5). **B-C)** Cytosolic calcium levels were evaluated by using fluorescent dye, Fluo-4 (4 µM), in synchronized human skin fibroblasts from 12 hours post-synchronization time point every 4 hours for 7 time points in presence of **(B)** thapsigargin (THAPS, 10 nM), inhibitor of SERCA, or **(C)** Coomassie Brilliant Blue G, antagonist of purinergic P2X7 receptors (BBG, 5 µM). **D)** Relative cytosolic calcium level at 16 hours post-shock (peak: TP16) and at 28 hours (trough: TP28) compared to non-treated cells (CTRL) (n = 3-5). The emitted fluorescence is linearly related to the cytosolic calcium content. *p < 0.05, ***p < 0.001; Student's two-tailed t test comparing single time points. Data are represented as average ± SEM.

of $[Ca^{2+}]_i$ (Fig. 1C). Thus, the significant difference between $[Ca^{2+}]_i$ at TP16 and TP28 was not observed anymore (Fig. 1D).

Disturbances of the Ca^{2+} homeostasis have been demonstrated to be associated with Aβ neurotoxicity. Therefore, we investigated the effect of Aβ peptides on the circadian fluctuation of Ca^{2+} levels (Fig. 2). Our data showed that extracellular treatment with $Aβ_{42}$ (aged peptide) completely abolished circadian oscillations of intracellular Ca^{2+} and impacted the levels of $[Ca^{2+}]_i$ at

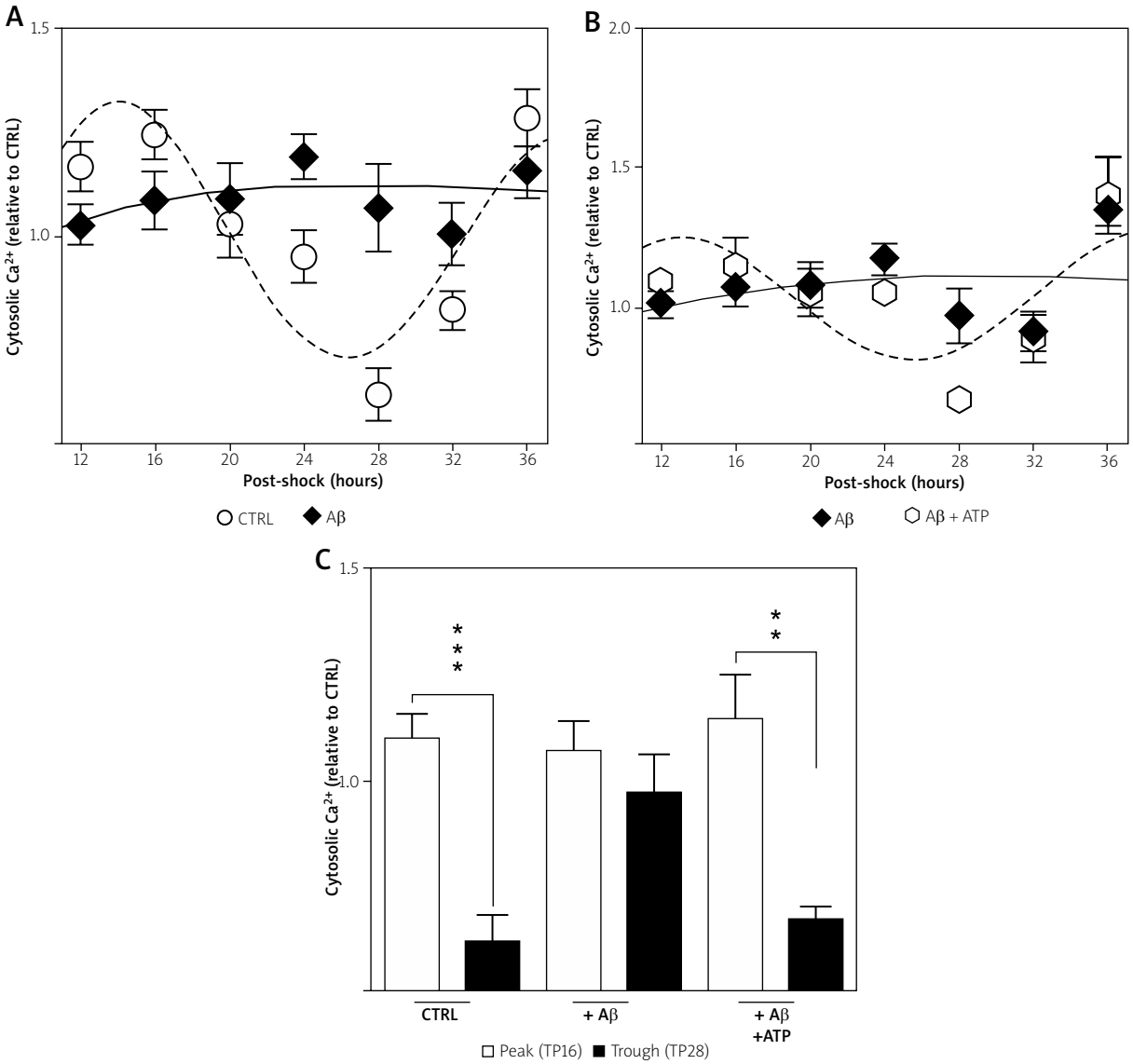

Fig. 2. Cytosolic calcium oscillations are abolished in the presence of Aβ. **A-B)** Cytosolic calcium levels were evaluated by using fluorescent dye, Fluo-4 (4 μM), in synchronized human skin fibroblasts from 12 hours post-synchronization time point every 4 hours for 7 time points in presence of **(A-B)** $A\beta_{42}$ (aged peptide) at 0.5 μM (n = 3). **(B)** Aβ treated cells were co-treated with ATP (1 mM) to overcome the dampening due to Aβ presence (n = 3). **(C)** Relative Cytosolic Calcium level at 16 hours post-shock (peak: TP16) and at 28 hours (trough: TP28) compared to non-treated cells (CTRL) (n = 3). Aβ treatment completely abolished differences in $[Ca2+]_i$ concentration between peak and trough time points while ATP treatment rescued the circadian oscillation of cytosolic calcium. The emitted fluorescence is linearly related to the cytosolic calcium content. $^{**}p < 0.01$, $^{***}p < 0.001$; Student's two-tailed t test comparing single time points. Data are represented as average ± SEM.

peak and trough time points (Fig. 2A and 2C). Those Aβ-evoked disturbances were not mediated by deregulation of ER Ca^{2+} stores, because THAPS treatment did not restore rhythmic oscillations of $[Ca^{2+}]_i$ (Supplementary Figure 3 online). Interestingly, treatment with adenosine triphosphate (ATP) abolished the negative effect of Aβ and restored oscillation of $[Ca^{2+}]_i$ (Fig. 2B and 2C). These data suggest that Aβ peptides altered the P2X7 receptor-mediated circadian rhythm of $[Ca^{2+}]_i$, leading to disturbances of calcium homeostasis.

Discussion

Accumulating evidence has suggested that sleep disturbances may be early indicators of dementia and may actually precede the onset of cognitive symptoms in AD [53]. Moreover, the sleep–wake cycle was shown to be a critical regulator of Aβ release and loss of slow wave sleep resulted in higher cumulative levels of neuronal activity and higher Aβ concentration in CSF [12]. It was previously suggested that intracellular Ca^{2+} may be a coordinator of the circadian timing system and biochemical reactions due to its ubiquitous role as a metabolic regulator [4]. Therefore the disturbances in Ca^{2+} homeostasis observed in AD brains could be partly associated with deregulation of patients' circadian rhythms. However the role of Ca^{2+} in regulating the clock function in pathophysiology is unknown. In this study, we showed for the first time that Alzheimer's Aβ peptides could negatively influence circadian fluctuations of Ca^{2+} in peripheral oscillators. This subsequently may alter calcium-dependent molecular processes involved in circadian clock regulation in AD.

It was demonstrated that intracellular Ca^{2+} concentration exhibits circadian rhythms in pacemaker neurons of the SCN [15,38]. The oscillatory physiology of Ca^{2+} was shown to be regulated by the circadian fluctuations in the Ca^{2+} currents generated by voltage-dependent calcium channels (VDCC) [41,60]. Calcium fluctuations were also shown in astrocytes of SCN, but, unlike in neurons, they were regulated by the Ca^{2+} release from ER stores [11,20]. Our study extends previous results by showing the existence of daily fluctuations in cytosolic calcium in peripheral oscillators, the human skin fibroblasts. This cell line was previously shown to exhibit circadian rhythms of clock genes, and the clock properties of these peripheral cells closely mimic those measured physiologically and behaviorally in humans [10,57]. Therefore, skin fibroblasts are a good *in vitro* model for studying molecular mechanisms of circadian rhythms. Moreover, it was shown that aging does not alter the basic clock properties (period length, amplitude and phase) of fibroblasts [56]. We observed that calcium oscillations in fibroblasts correspond to the previously demonstrated changes in transcript levels of the clock genes Per2, Bmal1, Rev-Erb, and Cry1 in those cells [9]. Former data showed that Ca^{2+} mobilized from internal deposits modulates the molecular circadian clock of hepatic cells *ex vivo*, in a manner that did not depend on the entrainment cue (meal or light) [4]. This suggests that Ca^{2+} signaling is a key regulator of circadian rhythms in peripheral tissues in contrast to the central pacemaker mediating hierarchical organization of rhythmicity [25].

Calcium signaling in non-excitable cells is initiated by mobilization of Ca^{2+} from intracellular ER stores through IP3 and ryanodine receptors. In our experiments, inhibition of SERCA significantly elevated cytosolic Ca^{2+} level, but did not alter calcium fluctuations. A similar effect was observed in SCN astrocytes [11]. Besides, in SCN neurons, the expression of SERCA was shown to follow a circadian pattern [50]. Together, all these data suggested that ER stores are not necessary for controlling $[Ca^{2+}]_i$ daily oscillations in non-neuronal cells.

Previous data reported the crosstalk between circadian oscillation of intracellular Ca^{2+} and rhythmic extracellular ATP accumulation in SCN astrocytes [11]. Exogenous ATP was shown to be a mediator of intercellular communication in physiology and neurodegeneration, by acting on the cell surface receptors, including ligand-gated ion channels (P2X) and G-protein coupled (P2Y) receptor subtypes. It was demonstrated that ATP selectively promotes the expression of the clock gene Per1 through gene transactivation after stimulation of P2X7 purinergic receptors in microglial cells [54]. Moreover, the endogenous purinergic receptors were shown to determine the local clock activity in the urinary bladder cells [69]. Therefore, the ATP-signaling may be also involved in changes of Ca^{2+} fluctuations in peripheral oscillators. Indeed, our study demonstrated that the circadian rhythm of the calcium level in fibroblasts depend on activation of the ATP-binding receptor, P2X7.

In AD pathology, changes of neuronal Ca^{2+} concentration are responsible for the oxidative stress as well as altered metabolism of APP and overproduction of Aβ peptides. On the other hand, Aβ neurotoxicity has been associated with the disturbances of intracellular Ca^{2+} homeostasis in neurons as well as in glial cells. The studies using APP transgenic mouse models of AD identified significantly elevated numbers of neurites with overloaded cytosolic Ca^{2+} and this effect was positively correlated with the distance from Aβ plaques [43]. Many previous studies demonstrated impaired Ca^{2+} regulation in fibroblasts of AD patients [31,39]. Our study confirms those reports, by demonstrating that exposure

to $A\beta_{42}$ (aged peptide) abolished Ca^{2+} fluctuations in the cytosol of fibroblasts.

A disruption of the Ca^{2+} regulation in the ER was previously shown to mediate signal-transduction alterations associated with AD [44]. Moreover, mutations that cause familial Alzheimer's disease have been linked to disturbances in intracellular calcium signaling pathways [34]. Skin fibroblasts from humans that harbor a mutation in presenilin 1 (PS1-A246E) showed exaggerated Ca^{2+} release from IP3-gated stores compared to controls after treatment with bombesin and bradykinin [39]. The elevated Ca^{2+} release from the ER, evoked by activation of the IP3 [14] or ryanodine [61] receptors, was shown to increase Aβ level. Overexpression of SERCA was also shown to increase Aβ production [29]. Furthermore, ER is also a potential intracellular target for Aβ protein [26,71], which disrupts the function of the intracellular Ca^{2+} stores. In our study, thapsigargin treatment did not restore the physiological oscillations of $[Ca^{2+}]_i$ that were significantly altered by Aβ. These data suggest that Aβ-mediated disruption of intracellular Ca^{2+} homeostasis may be evoked by an excess of calcium influx across the plasma membrane.

Furthermore, previous studies indicated that an altered activity of the purinergic P2X7 receptor mediates the pro-inflammatory processes in a transgenic AD model and in brains of AD patients [46,49,59]. The observation that Aβ may cause ATP release from microglia, and that P2X7 receptor is an obligate participant in microglia activation by Aβ, put the role of ATP and P2 receptors as a key event in neurodegeneration [42,63]. Recent data demonstrated that *in vivo* inhibition of P2X7 receptors significantly reduces the amyloid plaques formation in brain hippocampal structures through activation of α-secretase activity [51]. The mechanism of P2X7R-specific cleavage of APP was shown to be independent of ADAM9, -10, and -17 activity, but involved Erk1/2 and JNK phosphorylation [22]. In our study, we demonstrated that Aβ peptide significantly interferes with the P2X7-receptor mediated circadian oscillations of intracellular Ca^{2+}, however the mechanism underlying this phenomenon needs to be further investigated.

In summary, our data provide first evidence that Alzheimer's $A\beta_{42}$ peptides induce disturbances of P2X7 receptors mediated Ca^{2+} oscillation in peripheral oscillators. These findings may be therefore helpful for a better understanding of the circadian rhythms disruption related to AD.

Acknowledgments

Financial support was provided by the following grants: Sciex-NMSch to A.W. as well as Swiss National foundation (SNF #310030_122572) and Synapsis Foundation to A.E. The sponsors of this study were not involved in the study design; in collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Disclosure

Authors report no conflict of interest.

References

1. Acosta-Rodríguez VA, Márquez S, Salvador GA, Pasquaré SJ Gorné LD, Garbarino-Pico E, Giusto NM, Guido ME. Daily rhythms of glycerophospholipid synthesis in fibroblast cultures involve differential enzyme contributions. J Lipid Res 2013; 54: 1798-1811.
2. Aguilar-Roblero R, Mercado C, Alamilla J, Laville A, Diaz-Munoz M. Ryanodine receptor Ca2+-release channels are an output pathway for the circadian clock in the rat suprachiasmatic nuclei. Eur J Neurosci 2007; 26: 575-582.
3. Armstrong RA. A critical analysis of the 'amyloid cascade hypothesis'. Folia Neuropathol 2014; 52: 211-225.
4. Baez-Ruiz A, Diaz-Munoz M. Chronic inhibition of endoplasmic reticulum calcium-release channels and calcium-atpase lengthens the period of hepatic clock gene per1. J Circadian Rhythms 2011; 9: 6.
5. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of csf amyloid-beta levels: Implications for a diagnostic and therapeutic biomarker. Neurology 2007; 68: 666-669.
6. Baz el-S, Wei H, Grosshans J, Stengl M. Calcium responses of circadian pacemaker neurons of the cockroach Rhyparobia maderae to acetylcholine and histamine. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 2013; 199: 365-374.
7. Bonanni E, Maestri M, Tognoni G, Fabbrini M, Nucciarone B, Manca ML, Gori S, Iudice A, Murri L. Daytime sleepiness in mild and moderate Alzheimer's disease and its relationship with cognitive impairment. J Sleep Res 2005; 14: 311-317.
8. Brawek B, Garaschuk O. Network-wide dysregulation of calcium homeostasis in Alzheimer's disease. Cell Tissue Res 2014; 357: 427-438.
9. Brown SA, Kunz D, Dumas A, Westermark PO, Vanselow K, Tilmann-Wahnschaffe A, Herzel H, Kramer A. Molecular insights into human daily behavior. Proc Natl Acad Sci U S A 2008; 105: 1602-1607.
10. Brown SA, Fleury-Olela F, Nagoshi E, Hauser C, Juge C, Meier CA, Chicheportiche R, Dayer JM, Albrecht U, Schibler U. The period length of fibroblast circadian gene expression varies widely among human individuals. PLoS Biol 2005; 3: e338.
11. Burkeen JF, Womac AD, Earnest DJ, Zoran MJ. Mitochondrial calcium signaling mediates rhythmic extracellular atp accumu-

lation in suprachiasmatic nucleus astrocytes. J Neurosci 2011; 31: 8432-8440.
12. Cedernaes J, Osorio RS, Varga AW, Kam K, Schioth HB, Benedict C. Candidate mechanisms underlying the association between sleep-wake disruptions and Alzheimer's disease. Sleep Med Rev 2016; doi: 10.1016/j.smrv.2016.02.002 [Epub ahead of print].
13. Chakroborty S, Stutzmann GE. Calcium channelopathies and Alzheimer's disease: insight into therapeutic success and failures. Eur J Pharmacol 2014; 739: 83-95.
14. Cheung KH, Shineman D, Muller M, Cardenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foskett JK. Mechanism of Ca2+ disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. Neuron 2008; 58: 871-883.
15. Colwell CS. Circadian modulation of calcium levels in cells in the suprachiasmatic nucleus. Eur J Neurosci 2000; 12: 571-576.
16. Colwell CS. Linking neural activity and molecular oscillations in the SCN. Nat Rev Neurosci 2011; 12: 553-569.
17. Craig D, Hart DJ, Passmore AP. Genetically increased risk of sleep disruption in Alzheimer's disease. Sleep 2006; 29: 1003-1007.
18. Crews L, Rockenstein E, Masliah E. APP transgenic modeling of Alzheimer's disease: mechanisms of neurodegeneration and aberrant neurogenesis. Brain Struct Funct 2010; 214: 111-126.
19. De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 2007; 282: 11590-11601.
20. Deitmer JW, Verkhratsky AJ, Lohr C. Calcium signalling in glial cells. Cell Calcium 1998; 24: 405-416.
21. Del Prete D, Checler F, Chami M. Ryanodine receptors: physiological function and deregulation in Alzheimer disease. Mol Neurodegener 2014; 9: 21.
22. Delarasse C, Auger R, Gonnord P, Fontaine B, Kanellopoulos JM. The purinergic receptor P2X7 triggers alpha-secretase-dependent processing of the amyloid precursor protein. J Biol Chem 2011; 286: 2596-2606.
23. Ding JM, Buchanan GF, Tischkau SA, Chen D, Kuriashkina L, Faiman LE, Alster JM, McPherson PS, Campbell KP, Gillette MU. A neuronal ryanodine receptor mediates light-induced phase delays of the circadian clock. Nature 1998; 394: 381-384.
24. Dorszewska J, Oczkowska A, Suwalska M, Rozycka A, Florczak-Wyspianska J, Dezor M, Lianeri M, Jagodzinski PP, Kowalczyk MJ, Prendecki M, Kozubski W. Mutations in the exon 7 of Trp53 gene and the level of p53 protein in double transgenic mouse model of Alzheimer's disease. Folia Neuropathol 2014; 52: 30-40.
25. Enoki R, Kuroda S, Ono D, Hasan MT, Ueda T, Honma S, Honma K. Topological specificity and hierarchical network of the circadian calcium rhythm in the suprachiasmatic nucleus. Proc Natl Acad Sci U S A 2012; 109: 21498-21503.
26. Fonseca AC, Ferreiro E, Oliveira CR, Cardoso SM, Pereira CF. Activation of the endoplasmic reticulum stress response by the amyloid-beta 1-40 peptide in brain endothelial cells. Biochim Biophys Acta 2013; 1832: 2191-2203.
27. Fonseca AC, Moreira PI, Oliveira CR, Cardoso SM, Pinton P, Pereira CF. Amyloid-beta disrupts calcium and redox homeostasis in brain endothelial cells. Mol Neurobiol 2015; 51: 610-622.
28. Goedert M, Spillantini MG. A century of Alzheimer's disease. Science 2006; 314: 777-781.
29. Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM. SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production. J Cell Biol 2008; 181: 1107-1116.
30. Hamada T, Liou SY, Fukushima T, Maruyama T, Watanabe S, Mikoshiba K, et al. The role of inositol trisphosphate-induced Ca2+ release from IP3-receptor in the rat suprachiasmatic nucleus on circadian entrainment mechanism. Neurosci Lett 1999; 263: 125-128.
31. Hirashima N, Etcheberrigaray R, Bergamaschi S, Racchi M, Battaini F, Binetti G, Govoni S, Alkon DL. Calcium responses in human fibroblasts: A diagnostic molecular profile for Alzheimer's disease. Neurobiol Aging 1996; 17: 549-555.
32. Hong JH, Jeong B, Min CH, Lee KJ. Circadian waves of cytosolic calcium concentration and long-range network connections in rat suprachiasmatic nucleus. Eur J Neurosci 2012; 35: 1417-1425.
33. Honma S, Honma K. The biological clock: Ca2+ links the pendulum to the hands. Trends Neurosci 2003; 26: 650-653.
34. Huang HC, Tang D, Lu SY, Jiang ZF. Endoplasmic reticulum stress as a novel neuronal mediator in Alzheimer's disease. Neurol Res 2015; 37: 366-374.
35. Huang HC, Chang P, Lu SY, Zheng BW, Jiang ZF. Protection of curcumin against amyloid-β-induced cell damage and death involves the prevention from NMDA receptor-mediated intracellular Ca2+ elevation. J Recept Signal Transduct Res 2015; 35: 450-457.
36. Huang Y, Potter R, Sigurdson W, Santacruz A, Shih S, Ju YE, Kasten T, Morris JC, Mintun M, Duntley S, Bateman RJ. Effects of age and amyloid deposition on Aβ dynamics in the human central nervous system. Arch Neurol 2012; 69: 51-58.
37. Ikeda M. Calcium dynamics and circadian rhythms in suprachiasmatic nucleus neurons. Neuroscientist 2004; 10: 315-324.
38. Ikeda M, Sugiyama T, Wallace CS, Gompf HS, Yoshioka T, Miyawaki A, Allen CN. Circadian dynamics of cytosolic and nuclear Ca2+ in single suprachiasmatic nucleus neurons. Neuron 2003; 38: 253-263.
39. Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL. Internal Ca2+ mobilization is altered in fibroblasts from patients with Alzheimer disease. Proc Natl Acad Sci U S A 1994; 91: 534-538.
40. Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, Fujiki N, Nishino S, Holtzman DM. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science 2009; 326: 1005-1007.
41. Kim DY, Choi HJ, Kim JS, Kim YS, Jeong DU, Shin HC, Kim MJ, Han HC, Hong SK, Kim YI. Voltage-gated calcium channels play crucial roles in the glutamate-induced phase shifts of the rat suprachiasmatic circadian clock. Eur J Neurosci 2005; 21: 1215-1222.
42. Kim SY, Moon JH, Lee HG, Kim SU, Lee YB. ATP released from beta-amyloid-stimulated microglia induces reactive oxygen species production in an autocrine fashion. Exp Mol Med 2007; 39: 820-827.
43. Kuchibhotla KV, Goldman ST, Lattarulo CR, Wu HY, Hyman BT, Bacskai BJ. Abeta plaques lead to aberrant regulation of calci-

um homeostasis in vivo resulting in structural and functional disruption of neuronal networks. Neuron 2008; 59: 214-225.
44. LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci 2002; 3: 862-872.
45. Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB, Evans RM. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 2009; 326: 437-440.
46. Lee HG, Won SM, Gwag BJ, Lee YB. Microglial $P2X_7$ receptor expression is accompanied by neuronal damage in the cerebral cortex of the APPswe/PS1dE9 mouse model of Alzheimer's disease. Exp Mol Med 2011; 43: 7-14.
47. Lim D, Ronco V, Grolla AA, Verkhratsky A, Genazzani AA. Glial calcium signalling in Alzheimer's disease. Rev Physiol Biochem Pharmacol 2014; 167: 45-65.
48. Mairet-Coello G, Polleux F. Involvement of 'stress-response' kinase pathways in Alzheimer's disease progression. Curr Opin Neurobiol 2014; 27: 110-117.
49. McLarnon JG, Ryu JK, Walker DG, Choi HB. Upregulated expression of purinergic $P2X_7$ receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. J Neuropathol Exp Neurol 2006; 65: 1090-1097.
50. Menger GJ, Lu K, Thomas T, Cassone VM, Earnest DJ. Circadian profiling of the transcriptome in immortalized rat SCN cells. Physiol Genomics 2005; 21: 370-381.
51. Miras-Portugal MT, Diaz-Hernandez JI, Gomez-Villafuertes R, Diaz-Hernandez M, Artalejo AR, Gualix J. Role of P2X7 and P2Y2 receptors on alpha-secretase-dependent app processing: Control of amyloid plaques formation "in vivo" by P2X7 receptor. Comput Struct Biotechnol J 2015; 13: 176-181.
52. Moghekar A, O'Brien RJ. Con: Alzheimer's disease and circadian dysfunction: Chicken or egg? Alzheimers Res Ther 2012; 4: 26.
53. Musiek ES, Xiong DD, Holtzman DM. Sleep, circadian rhythms, and the pathogenesis of Alzheimer disease. Exp Mol Med 2015; 47: e148.
54. Nakazato R, Takarada T, Yamamoto T, Hotta S, Hinoi E, Yoneda Y. Selective upregulation of Per1 mRNA expression by ATP through activation of P2X7 purinergic receptors expressed in microglial cells. J Pharmacol Sci 2011; 116: 350-361.
55. Oh-hashi K, Naruse Y, Tanaka M. Intracellular calcium mobilization induces period genes via MAP kinase pathways in NIH3T3 cells. FEBS Lett 2002; 516: 101-105.
56. Pagani L, Schmitt K, Meier F, Izakovic J, Roemer K, Viola A, Cajochen C, Wirz-Justice A, Brown SA, Eckert A. Serum factors in older individuals change cellular clock properties. Proc Natl Acad Sci U S A 2011; 108: 7218-7223.
57. Pagani L, Semenova EA, Moriggi E, Revell VL, Hack LM, Lockley SW, Arendt J, Skene DJ, Meier F, Izakovic J, Wirz-Justice A, Cajochen C, Sergeeva OJ, Cheresiz SV, Danilenko KV, Eckert A, Brown SA. The physiological period length of the human circadian clock in vivo is directly proportional to period in human fibroblasts. PLoS One 2010; 5: e13376.
58. Park D, Lee S, Jun K, Hong YM, Kim DY, Kim YI, Shin HS. Translation of clock rhythmicity into neural firing in suprachiasmatic nucleus requires mGluR-PLCbeta4 signaling. Nat Neurosci 2003; 6: 337-338.
59. Parvathenani LK, Tertyshnikova S, Greco CR, Roberts SB, Robertson B, Posmantur R. P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. J Biol Chem 2003; 278: 13309-13317.
60. Pennartz CM, de Jeu MT, Bos NP, Schaap J, Geurtsen AM. Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. Nature 2002; 416: 286-290.
61. Querfurth HW, Jiang J, Geiger JD, Selkoe DJ. Caffeine stimulates amyloid beta-peptide release from beta-amyloid precursor protein-transfected HEK293 cells. J Neurochem 1997; 69: 1580-1591.
62. Rege SD, Geetha T, Broderick TL, Babu JR. Resveratrol protects β amyloid-induced oxidative damage and memory associated proteins in H19-7 hippocampal neuronal cells. Curr Alzheimer Res 2015; 12: 147-156.
63. Sanz JM, Chiozzi P, Ferrari D, Colaianna M, Idzko M, Falzoni S, Fellin R, Trabace L, Di Virgilio F. Activation of microglia by amyloid {beta} requires P2X7 receptor expression. J Immunol 2009; 182: 4378-4385.
64. Saravanaraman P, Chinnadurai RK, Boopathy R. Why calcium channel blockers could be an elite choice in the treatment of Alzheimer's disease: a comprehensive review of evidences. Rev Neurosci 2014; 25: 231-246.
65. Solini A, Chiozzi P, Morelli A, Fellin R, Di Virgilio F. Human primary fibroblasts in vitro express a purinergic P2X7 receptor coupled to ion fluxes, microvesicle formation and IL-6 release. J Cell Sci 1999; 112 (Pt 3): 297-305.
66. Ullah G, Demuro A, Parker I, Pearson JE. Analyzing and modeling the kinetics of amyloid beta pores associated with Alzheimer's disease pathology. PLoS One 2015; 10: e0137357.
67. Volicer L, Harper DG, Manning BC, Goldstein R, Satlin A. Sundowning and circadian rhythms in Alzheimer's disease. Am J Psychiatry 2001; 158: 704-711.
68. Weldemichael DA, Grossberg GT. Circadian rhythm disturbances in patients with Alzheimer's disease: A review. Int J Alzheimers Dis 2010; 2010.
69. Wu C, Sui G, Archer SN, Sassone-Corsi P, Aitken K, Bagli D, Chen Y. Local receptors as novel regulators for peripheral clock expression. Faseb J 2014; 28: 4610-4616.
70. Yesavage JA, Friedman L, Kraemer H, Tinklenberg JR, Salehi A, Noda A, et al. Sleep/wake disruption in Alzheimer's disease: APOE status and longitudinal course. J Geriatr Psychiatry Neurol 2004; 17: 20-24.
71. Yoo Y, Byun K, Kang T, Bayarsaikhan D, Kim JY, Oh S, Kim YH, Kim SY, Chung WI, Kim SU, Lee B, Park YM. Amyloid-beta-activated human microglial cells through ER-resident proteins. J Proteome Res 2015; 14: 214-223.
72. Zee PC, Attarian H, Videnovic A. Circadian rhythm abnormalities. Continuum (Minneap Minn) 2013; 19: 132-147.