Bri2 BRICHOS chaperone rescues impaired fast-spiking interneuron behavior and neuronal network dynamics in an AD mouse model in vitro

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ABSTRACT

Synchronized and properly balanced electrical activity of neurons is the basis for the brain's ability to process information, to learn, and to remember. In Alzheimer's disease (AD), which causes cognitive decline in patients, this synchronization and balance is disturbed by the accumulation of neuropathological biomarkers such as amyloid-beta peptide (Aβ42). Failure of Aβ42 clearance mechanisms as well as desynchronization of crucial neuronal classes such as fast-spiking interneurons (FSN) are root causes for the disruption of the cognition-relevant gamma brain rhythm (30–80 Hz) and consequent cognitive impairment observed in AD. Here we show that recombinant BRICHOS molecular chaperone domains from ProSP-C or Bri2, which interfere with Aβ42 aggregation, can rescue the gamma rhythm. We demonstrate that Aβ42 progressively decreases gamma oscillation power and rhythmicity, disrupts the inhibition/excitation balance in pyramidal cells, and desynchronizes FSN firing during gamma oscillations in the hippocampal CA3 network of mice. Application of the more efficacious Bri2 BRICHOS chaperone rescued the cellular and neuronal network performance from all ongoing Aβ42-induced functional impairments. Collectively, our findings offer critical missing data to explain the importance of FSN for normal network function and underscore the therapeutic potential of Bri2 BRICHOS to rescue the disruption of cognition-relevant brain rhythms in AD.

1. Introduction

Alzheimer's disease (AD) is one of the most devastating neurodegenerative diseases of our time with late-life dementia as a hallmark symptom. In AD the severity of cognitive decline correlates with the disruption of cognition-relevant neuronal network rhythms. Particularly, fast neuronal electrical activity in the gamma-frequency band (30–80 Hz; gamma oscillations) is altered in the hippocampus, a brain structure whose function is greatly affected by the neurodegeneration typical of AD.

The crucial role of gamma oscillations for cognitive functions has been extensively documented in pre-clinical and clinical studies (Buzsáki, 2011; van Vugt et al., 2010; Womelsdorf and Fries, 2007). Gamma oscillations emerge from the rhythmic pacing activity driven by perisomatic-targeting fast-spiking interneurons (FSN) onto the excitatory pyramidal cell (PC) population (Cardin et al., 2009). They depend on a proper balance between excitation and inhibition (Atallah and Scanzian, 2009) and the recurrent excitation among CA3 PCs (Fisahn et al., 1998).

The amyloid cascade hypothesis posits amyloid-β peptide 1–42 (Aβ42) aggregates as a major trigger of the toxic effects on brain function observed in AD, including neuroinflammation, synaptic and neuronal loss, tau-associated pathology (De Strooper and Karran, 2016; Selkoe and Hardy, 2016) as well as early cognitive decline (Mucke et al., 2000; Selkoe, 2002; Walsh and Selkoe, 2007). In AD, disturbances in the systems involved in protein homeostasis lead to an imbalance between Aβ42 release and clearance (Frere and Slutsky, 2018). The clearance of Aβ42 from the extracellular space in vivo depends on several mechanisms with experimental evidence suggesting that molecular chaperones play an important role in the maintenance of protein homeostasis under...
a wide range of circumstances (Cohen et al., 2015; Hartl et al., 2011; Hermansson et al., 2014; Oksarsson et al., 2018).

The BRICHOS domain of about 100 amino acid residues is present in a set of proproteins that all have similar overall architecture and harbor regions prone to form β-sheets and to misfold into amyloid fibrils (Buxbaum and Johansson, 2017; Knight et al., 2013). The BRICHOS domain has been proposed to assist the amyloid-prone region of their respective proproteins to fold correctly, and also shows strong inhibitory effects on fibril formation of amyloidogenic peptides that are not part of their precursors (Hedlund et al., 2009; Sánchez-Pulido et al., 2002). Recombinant BRICHOS domains from human prosurfactant protein C (proSP-C) and Bri2 are efficient inhibitors of amyloidogenic non-client proteins such as medin, islet amyloid polypeptide, Aβ40 and Aβ42 (Nerclius et al., 2009; Oksarsson et al., 2018; Willander et al., 2012). The BRICHOS domain of ProSP-C, which is exclusively expressed in lung tissue, specifically binds to fibril surface and blocks the surface-catalyzed secondary nucleation during Aβ fibril formation, which leads substantial decrease in formation of toxic Aβ oligomers (Cohen et al., 2015; Hermansson et al., 2014; Törnquist et al., 2020). In the Central Nervous System (CNS), Bri2 is produced in neurons of the hippocampus and cortex in humans (Akiyama et al., 2004; Vidal et al., 1999), colocalizes with AD plaques (Del Campo et al., 2014; Dolfie et al., 2018), and affects the Aβ precursor protein (Aβ42PP) processing in cell models (Del Campo et al., 2014; Matsuda and Senda, 2019).

In cell models the BRICHOS domain is released by proteolysis from the Bri2 precursor protein (Del Campo et al., 2014; Martin et al., 2008; Oksarsson et al., 2018). Recombinant human (rh) Bri2 BRICHOS efficiently inhibits Aβ42 fibril formation in vitro, in particular generation of toxic Aβ42 oligomers (Cohen et al., 2015; Hermansson et al., 2014; Törnquist et al., 2020). In the Central Nervous System (CNS), Bri2 is produced in neurons of the hippocampus and cortex in humans (Akiyama et al., 2004; Vidal et al., 1999), colocalizes with AD plaques (Del Campo et al., 2014; Dolfie et al., 2018), and affects the Aβ precursor protein (Aβ42PP) processing in cell models (Del Campo et al., 2014; Matsuda and Senda, 2019).

In previous work we have reported that on the network level acutely administered Aβ42 degrades gamma oscillations in the in vitro hippocampal network in a time- and concentration-dependent manner. On the cellular level Aβ42 shifts the balance of inhibition and excitation needed for proper neuronal network function and leads to the desynchronization of action potential (AP) firing in hippocampal PCs (Balleza-Tapia et al., 2018; Kurudenkandy et al., 2014). We thus hypothesized that recombinant Bri2 and/or ProSP-C BRICHOS may be able to rescue Aβ42-induced degradation of gamma oscillations, inhibitory/excitatory imbalance, AP firing desynchronization of PCs and potential Aβ42-induced changes of FSN behavior. In the present study, we investigated the efficacy of rh Bri2 BRICHOS and ProSP-C BRICHOS to rescue functional network dynamics and physiological FSN behavior in the hippocampal network after Aβ42-induced degradation using acute slice preparations from WT mice. Our results show that Aβ42 alters FSN excitability leading to desynchronization of FSN and PC action potential firing. Based on the critical role that FSNs play for network stability the disruption of their behavior provides a root cause for the disruption of gamma rhythms in animal models as well as AD patients. This makes it a relevant focus for therapeutic intervention. Our results reveal that Bri2 BRICHOS is most efficacious at rescuing functional network dynamics as well as FSN and PC firing behavior from Aβ42-induced degradation. It emphasizes the potential of this chaperone peptide as a possible treatment against AD and other amyloidogenic brain disorders.

2. Materials and methods

2.1. Animals

Experiments were performed in accordance with the ethical permit granted by Norra Stockholm’s Djurörsöketiska Nämnd (dnr N45/13) to AF. C57BL/6 male mice supplied from Charles River, Germany, were used for electrophysiological studies in brain slices at postnatal days 14–28. For brain dissection mice were deeply anaesthetized with isoflurane before being sacrificed by decapitation.

2.2. Hippocampal slice preparation

Hippocampal slices were prepared as previously described (Andrade-Talavera et al., 2020; Balleza-Tapia et al., 2018). Briefly, the brain was quickly dissected out and placed in ice-cold artificial cerebrospinal fluid (ACSF) prepared for dissection and containing (in mM): 80 NaCl, 24 NaHCO3, 25 glucose, 1.25 NaH2PO4, 1 ascorbic acid, 3 Na-pyruvate, 2.5 KCl, 4 MgCl2, 0.5 CaCl2, 75 sucrose and bubbled with carbogen (95% O2 and 5% CO2). Horizontal hippocampal sections (350 μm thick) from both hemispheres were obtained with a Leica VT1200S vibratome (Leica Microsystems). Slices were then transferred into an interface holding chamber containing standard recording ACSF (in mM): 124 NaCl, 30 NaHCO3, 10 glucose, 1.25 NaH2PO4, 3.5 KCl, 1.5 MgCl2, 1.5 CaCl2, and kept for at least 1 h prior to the recordings. The holding chamber was held at 37 °C for the first 15 min and then allowed to cool down to room temperature at least for 1 h total prior to any recordings being performed. Slices in the holding chamber were continuously supplied with humidified carbogen gas (5% CO2, 95% O2). For local field potentials (LFP)-only experiments slices were transferred to an interface-type recording chamber, where they were continuously superfused with oxygenated ACSF at a perfusion rate of 4.5 ml/min at 34 °C and continuously supplied with humidified carbogen gas. Neuronal dynamics studies were performed in submersed-type recording chambers, where slices were continuously superfused with oxygenated ACSF at a perfusion rate of 3 ml/min at 34 °C.

2.3. Electrophysiological recordings

LFP recordings were carried out in str. Pyramidal of hippocampal area CA3 with borosilicate glass microelectrodes, pulled to a resistance of 3–5 MΩ and filled with standard ACSF. All single-cell experiments were carried out in a submersed recording chamber. Different intracellular solutions were used according to the configuration of the patch recordings. Action potentials and EPSCs from PCs were recorded in whole-cell mode with an internal recording solution containing (in mM): 122.5 K+-glutamate, 8 KCl, 4 Mg2+, ATP, 0.3 Na+ GTP, 10 HEPES, 0.2 EGTA, 2 MgCl2 or (in mM) 140 CsMetSO4, 4 Mg2+, ATP, 0.3 Na+ GTP, 10 HEPES, and 0.6 EGTA for IPSC recordings, both with pH set to 7.2–7.3 and osmolality to 270–280 mosmol/l. EPSCs and IPSCs were recorded in voltage-clamp configuration holding the membrane potential at -70 mV or 0 mV, respectively. Cells were visualized under an upright microscope using IR-DIC microscopy (Axioskop, Carl Zeis AG, Göttingen, Germany).

PCs and FSN were identified based on their morphology and location in the hippocampal str. Pyramidal and str. Radiatum respectively. For FSN recordings in whole-cell patch clamp configuration different electrophysiology protocols were applied to confirm the interneuron subtype (FSN) based on its unique electrophysiological characteristics (Andrade-Talavera et al., 2020; Gulyas et al., 2010) (Fig. S1). Internal recording solution used was a K+-glutamate-based solution as used for PC recordings where 2 mM Mg2+ ATP was used. Either for PC or FSN recordings in whole-cell configuration access resistance was monitored. Cells were discarded from the study if more than 20% of change was observed throughout the experiment.

LFP gamma oscillations were induced by applying 100 nM kainic acid (KA, Tocris) (Andrade-Talavera et al., 2020; Balleza-Tapia et al., 2018).
2018) and were allowed to stabilize for 30 min before any recordings were performed. LFP recordings in interface-type recording chambers were performed with a 4-channel M102 amplifier (University of Cologne, Germany). Data was sampled at 10 kHz, conditioned using a HumBug 50 Hz noise eliminator (Quest Scientific), low-pass filtered at 1 kHz, digitized using a Digidata 1440A, Molecular Devices, CA, USA and stored using pCLAMP 9.6 software (Molecular Devices, CA, USA). LFP and patch clamp recordings in submerged-type recording chambers were performed with a patch clamp amplifier (Multiclamp 700B), and data were acquired using pCLAMP 10.4 software (Molecular Devices). LFPs were also conditioned using a HumBug 50 Hz noise eliminator (Quest Scientific). All signals recorded in submerged configuration were low-pass filtered at 1 kHz, acquired at 5 kHz, digitized and stored using Digidata 1322A and pCLAMP 10.4 software (Molecular Devices, CA, USA).

To test the ability and mechanisms of the chaperones to counteract and rescue β2-fibril-mediated toxicity three different approaches were followed:

1) For prevention of β2-fibril-mediated toxicity slices were incubated with β2-fibrils, incubated with chaperone-coated β2-fibrils or co-incubated with β2-fibrils and chaperones prior to gamma oscillations induction (see β2-fibril coating treatment below). A control KA-induced gamma oscillation was recorded where slices were not incubated with β2-fibrils, with chaperones-coated β2-fibrils or co-incubated with β2-fibrils and chaperones prior to gamma oscillations induction.

2) For the rescue of β2-mediated disruption of gamma oscillations in an interface-type recording chamber, slices were incubated with β2, then transferred to the recording chamber for gamma induction. The chaperones were bath applied for 3 h, starting 30 min after gamma oscillations induction to allow stabilization of oscillations. To further test that the increase of gamma power was mediated by the application of the chaperone, slices were incubated with β2, then transferred to the interface recording chamber and gamma oscillations were recorded for 3 h after induction without adding any chaperone. A control KA-induced gamma oscillation was recorded where slices were not incubated with β2, nor any chaperone was added during the 3 h of control gamma recording.

3) For the rescue of β2-mediated disruption of gamma oscillations and neuronal network dynamics in a submerged-type recording chamber, 5 min of stable baseline (control-KA) were recorded 30 min after the induction of gamma oscillations, β2 was applied and 15 min after its application Br2 BRICHOS was subsequently applied. β2 stayed in the batch solution throughout the experiment.

2.4. Rh Bri2 BRICHOS and ProSP-C BRICHOS preparation

Rh Bri2 BRICHOS (corresponding to residues 113–231 of full-length Bri2) was expressed and purified as described (Chen et al., 2017). Briefly, Shuffe T7 E. coli cells, transformed with a construct coding for a His6-NT*-Bri2 BRICHOS fusion protein were incubated at 30 °C in LB medium containing 100 μg/ml ampicillin until OD600 nm reached to 0.8–1.2, then the temperature was lowered to 25 °C and IPTG was added to 0.5 mM. The expression lasted for another 4 h. The cells were harvested by centrifugation at 7000 × g for 20 min at 4 °C and resuspended in 20 mM Tris-HCl pH 8.0. The cells were lysed by 3 min sonication on ice (2 s on, 2 s off, 65% of max power), and the pellets were collected by 24,000 × g centrifugation at 4 °C for 30 min. The pellets were resuspended in 20 mM Tris-HCl pH 8.0 containing 0.1 M NaCl and 2 M urea, and sonicated for 10 min on ice (2 s on, 2 s off, 80% of max power). Then the supernatant, containing the fusion protein His6-Trx-ProSP-C BRICHOS, was collected by 24,000 × g centrifugation at 4 °C for 30 min, which were purified by a Ni-NTA column. To remove the His6-Trx part, the fusion protein was cleaved with thrombin (1:1000 enzyme to substrate w/w ratio) at 4 °C overnight and loaded on a Ni-NTA column. The cleaved-off ProSP-C BRICHOS was further purified by anion exchange chromatography.

2.5. β2 fibril preparation

Met-β2 residues 1–42 (referred to as β2 herein) was produced in E. coli BL21* pLysS cells and purified by ion exchange chromatography as described (Chen et al., 2017). Briefly, the crude β2 protein was lyophilized and re-dissolved in 7 M Gdn-HCl. The β2 monomers were isolated by a Superdex 75 column (GE Healthcare, UK) in 20 mM sodium phosphate pH 8.0 with 0.2 mM EDTA. For preparation of amyloid fibrils, 80 μl solution containing 3 μM β2 monomer were incubated under quiescent conditions at 37 °C for 12 h. For preparation of BRICHOS-coated fibrils, 3 μM rh Bri2 BRICHOS or ProSP-C BRICHOS were added to preformed β2 fibrils and incubated at 37 °C for 12 h.

2.6. Immunogold staining of BRICHOS-coated β2 fibrils

5 μM β2 monomer was incubated at 37 °C in the presence of 5 μM rh Bri2 BRICHOS or ProSP-C BRICHOS overnight, and the fibrils were collected by centrifugation for 1 h at 22,000 × g at 4 °C. The fibrils were resuspended in 20 μl TBS, and 2 μl were applied to form coated nickel grids. Blocking was performed by incubation in 1% BSA in TBS for 30 min, after which the grids were washed 3 × 10 min by TBS. The grids were then incubated with goat anti-Bri2 BRICHOS or anti ProSP-C BRICHOS antibodies over night at 4 °C, and washed 3 × 10 min with TBS. Finally the grids were incubated with anti-goat IgG-gold secondary antibody for 2 h at room temperature, and washed 5 × 10 min with TBS. For staining, 2 μl of 2.5% uranyl acetate was added to each grid. The grids were dried and analyzed by transmission electron microscopy (TEM, Hitachi H7100 TEM operated at 75 kV). One square per grid was selected at random and used for EM analysis. Within each selected square 8 fibril ends were selected at random and the immunogold particles covering those 8 selected ends (if any) were counted.

2.7. Data analysis

For oscillations power spectra Fast Fourier Transformation was obtained from 60s of LFP recording (segment length 8192 points) using Axograph X software (Kagi, Berkeley, CA, USA). Gamma power was calculated by integrating the power spectral density from 20 to 80 Hz using KagiX software. Correlation coefficient (Cr) was calculated from the auto-correlograms as a measure of the quality of gamma oscillations. It was defined as Cr = (∑(α·β))/(α + β) including (1 + α) and (1 + β) corrections prior to Cr calculation. Alpha corresponds to the value of the height of the second peak and β to the first trough in the auto-correlogram (counting the first peak at zero lag). Auto-correlograms were performed from filtered LFP (20–40 Hz, RC-single pole) using a 100 ms lag in Clampfit 10.2. Cr ranges between 0 and 1 with higher coefficient values denoting more rhythmic activity. Only recordings having a Cr ≥ 0.01 were considered rhythmic and included in the study (Balleza-Tapia et al., 2018). EPSCs and IPSCs were detected off-line.
using a custom-made template in Clampfit 10.2 software including no less than 20 averaged representative events. EPSCs and IPSCs parameters like charge transfer, event amplitude and inter-event-interval (IEI) distributions were performed and analyzed using GraphPad Prism (GraphPad Software, USA).

Spike phase-coupling analysis was performed on concomitant LFP recordings and single-cell recordings using custom-made routine in MATLAB to relate the pyramidal or fast-spiking cells spiking activity to ongoing gamma oscillations (Andrade-Talavera et al., 2020). LFP traces were previously filtered using a band pass filter set to 20–40 Hz (low and high-pass filters: RC-single pole) using Clampfit 10.2 and action potentials were detected using an amplitude threshold. The instantaneous phase-angle of gamma oscillations at which action potential occurred was determined by using a Hilbert transform. AP phase-angles frequency-distributions were normalized by the total of AP, a Gaussian function was fitted and the half-width at half-maximum was calculated–

**Fig. 1.** Interference with Aβ42 aggregation rescues gamma oscillations in the CA3 hippocampal circuitry of mice. (A): Gamma oscillation power from control slices (gray, $3.81 \pm 1.14 \times 10^{-9} \mu V^2$, $n = 12$), slices incubated with 50 nM Aβ42 fibrils (light magenta, $1.08 \pm 0.39 \times 10^{-9} \mu V^2$, $n = 12$), 50 nM ProSP-C-coated Aβ42 fibrils (orange open bar, $3.26 \pm 1.03 \times 10^{-9} \mu V^2$, $n = 8$), 50 nM Aβ42 fibrils co-incubated with 1 μM ProSP-C (orange, $2.72 \pm 1.0 \times 10^{-9} \mu V^2$, $n = 8$) and slices incubated with 50 nM Bri2-coated Aβ42 fibrils (blue open bar, $4.73 \pm 0.36 \times 10^{-9} \mu V^2$, $n = 6$) and 50 nM Aβ42-fibrils co-incubated with 1 μM Bri2 (blue, $2.86 \pm 1.18 \times 10^{-9} \mu V^2$, $n = 8$); statistics summary is provided in Table S1. (B): Electron micrograph of fibrillar Aβ42-only treated slices recorded in parallel. Bri2 BRICHOS vs. ProSP-C: control vs. Aβ42, $p = 0.0256$. (D) Time course of gamma oscillation power recorded during 3 h. of ProSP-C application (left, orange: $4.31 \pm 0.05 \times 10^{-9} \mu V^2$, $n = 13$) and Bri2 BRICHOS application (blue, right: $6.02 \pm 0.73 \times 10^{-9} \mu V^2$, $n = 13$) from slices previously incubated with 50 nM Aβ42 and time course for slices incubated with 50 nM Aβ42 without chaperones application (magenta, $1.99 \pm 1.18 \times 10^{-9} \mu V^2$, $n = 13$). The corresponding time course of gamma oscillations power recorded in control conditions is shown in gray (ProSP-C, control: $4.97 \pm 1.15 \times 10^{-9} \mu V^2$, $n = 13$; Bri2, control: $5.49 \pm 0.32 \times 10^{-9} \mu V^2$, $n = 13$). Inset: Representative example traces showing the Bri2 BRICHOS-driven rescue of gamma oscillations. Gamma power corresponds to the last minute of the time course for each condition. ProSP-C: control vs. Aβ42, $p < 0.0001$; ProSP-C vs. Bri2, $p < 0.0001$; ProSP-C vs. control, $p = 0.0922$, ordinary one-way ANOVA followed by Holm-Sidak’s post hoc multiple comparisons test. Bri2 BRICHOS: control vs. Aβ42, $p < 0.0001$; Bri2 vs. Aβ42, $p < 0.0001$; Bri2 vs. control, $p = 0.0146$, ordinary one-way ANOVA followed by Holm-Sidak’s post hoc multiple comparisons test. (E): Summary of the rescued gamma power shown in D 3 h. after ProSP-C and Bri2 BRICHOS wash-in and gamma power of Aβ42-only treated slices recorded in parallel. Bri2 BRICHOS vs. ProSP-C BRICHOS $p < 0.0001$, ordinary one-way ANOVA followed by Holm-Sidak’s post hoc multiple comparisons test. Data is presented as means ± SD. Significance levels were set as $p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
as a measure of the synchronization level. Accordingly, we assume that the narrower the firing window is, the more AP the cell fires within the same phase-angle hence the spiking activity is more synchronized.

To further confirm the spike phase-coupling analysis described above, phase-angles of all APs and gamma oscillation-phases were represented in polar-plots for each cell and condition. Phase-angles were expressed in radians with the peak of the oscillation cycle corresponding to 0 π and the trough to ±π in the polar plots. Each action potential was assigned a vector of length 1 with an angle corresponding to the phase of the field at the same time. Once all the vectors are assigned, an averaged resultant phase-density vector was used to describe the preferred phase of firing (phase-angle) and how recurrent the firing in that angle is (vector length), meaning that the longer the vector is the more synchronized the firing is. Vector length is shown normalized by the total number of APs for each cell and condition (control, Aj42, Bri2 BRICHOS). The preferred phase-angle was extracted from the resultant vector for each cell and calculated by averaging the AP phase-angles where each cell fired for each condition. All concomitant recordings were tested for circular uniformity using Rayleigh’s test in order to test whether neurons fired in a phase-related manner. Only recordings with Rayleigh’s p < 0.05 were considered for the analysis.

2.8. Statistical analysis

In experiments performed in interface-type recording chamber (Fig. 1) the last minute of recording was used to estimate changes in gamma power compared to baseline (set as the zero-minute point of time course) when the chaperones were bath-applied. As for the experiments performed with BRICHOS-coated Aj42 fibrils gamma power was calculated from 1 min recorded at 30 min after 100 nM KA application. For the study of Aj42 and subsequent Bri2 application effects on neuronal dynamics parameters in submerged-type recording chamber, comparisons were performed between the last minute of the control, 1 min taken 15 min after Aj42 and 35 min after subsequent Bri2 applications. In order to minimize the variation between slices a paired/ repeated measures statistical design was used. Statistics were performed either on raw values and/or data normalized to 5 min of recorded baseline (control KA) for each parameter and reported as means ± SD. All the data were subjected to outlier’s detection in GraphPad Prism (GraphPad Software, USA) with the ROUT method. Detected outliers were also removed out of the study. D’Agostino & Pearson omnibus normality test was carried out before any statistics were performed. For unpaired statistical analysis of non-parametric distributed data Mann-Whitney U test was performed. Multiple comparisons of parametric data were assessed either with repeated measures or ordinary one-way ANOVA followed by Holm-Sidak’s post hoc multiple comparisons test depending on the paired or not paired nature of the experimental approach respectively. Multiple comparisons of non-parametric or relatively small sample-size data were assessed with matched-pairs Friedman’s test followed by Dunn’s post hoc multiple comparisons test.

3. Results

3.1. BRICHOS chaperone interference with Aj42 aggregation rescues gamma oscillations in area CA3 of the mouse hippocampus

In order to test the ability of two recombinant BRICHOS chaperones, from ProSP-C or Bri2, to rescue cognition-relevant network dynamics we first performed two different experiments to gain more insight into the mechanisms underlying their previously described preventative efficacy (Kurudenkandy et al., 2014; Chen et al., 2017). Firstly, we tested whether prevention of Aj42-induced decrease of gamma oscillation power is different for chaperone proteins either 1) co-incubated with Aj42 fibrils or 2) allowed to bind to the Aj42 fibril surface prior to exposing the hippocampal network to them (Aj42 fibril coating treatment, see Methods). Thus, we induced gamma oscillations in slices incubated for 15 min with 50 nM of fully-fibrillized Aj42 (Aj42 fibrils), 50 nM ProSP-C BRICHOS-coated Aj42 fibrils or 50 nM Aj42-fibrils co-incubated with 1 μM ProSP-C. The same experiment was repeated with Bri2 BRICHOS-coated Aj42 fibrils and 50 nM Aj42 fibrils co-incubated with 1 μM Bri2 BRICHOS (Fig. 1A).

Our results show that coating is more efficient than mere co-incubation, presumably because coating more efficiently prevents secondary nucleation and the consequent increase in the neurotoxic water-soluble oligomeric form of Aj42 as we have previously demonstrated (Cohen et al., 2015). Additionally, coating Aj42 fibrils with Bri2 BRICHOS prevented the degradation of gamma oscillations more efficiently compared to the presence of 1 μM ProSP-C BRICHOS or Bri2 BRICHOS during exposure of the hippocampal network to 50 nM uncoated Aj42 fibrils (Fig. 1A, Table S1). This suggests that the most efficacious agent at preventing the neurotoxicity of fibrillar Aj42 on gamma oscillations is the Bri2 BRICHOS domain.

A potential cause for the differential preventative efficacy could be different effectiveness of the two chaperone proteins in coating Aj42 fibril surfaces. To test this hypothesis, we analyzed preparations of ProSP-C-coated Aj42 fibrils and Bri2-coated Aj42 fibrils with immunogold labeling and electron microscopy. Our results show that Bri2 BRICHOS chaperone covers the Aj42 fibril surface more extensively than ProSP-C BRICHOS, and also binds more efficiently to fibril ends (Fig. 1B, C; see Methods).

An effective potential treatment should be able to rescue already impaired function rather than merely preventing impairment from occurring. In order to test the rescue ability of the two BRICHOS chaperones, hippocampal slices were incubated 15 min with 50 nM Aj42, then transferred to an interface-type recording chamber and gamma oscillations induced by applying 100 nM KA. After 30 min of stable gamma oscillations we applied 1 μM Bri2 BRICHOS or 1 μM ProSP-C BRICHOS for 3 h. For each treatment, control gamma oscillations and gamma oscillations from slices incubated with Aj42 without subsequent BRICHOS application were also recorded (Fig. 1D, E).

Both, Bri2 BRICHOS and ProSP-C BRICHOS rescued the power of gamma oscillations (Fig. 1D, E). Bri2 BRICHOS treatment prompted a rapid rescue of gamma power that reached levels significantly higher than the corresponding non-Aj42-treated control. It was also higher than the rescue driven by ProSP-C BRICHOS (Fig. 1D, E). We noted that the Bri2 BRICHOS chaperone, endogenous to the CNS, is a more effective rescue treatment compared to the ProSP-C BRICHOS chaperone, endogenous to lung tissue.

3.2. Bri2 BRICHOS rescues Aj42-induced degradation of gamma oscillations by increasing both gamma power and rhythmicity

Because of its higher efficacy in rescuing cognition-relevant network activity from the established Aj42-induced degradation and its expression in the CNS, we proceeded to use Bri2 BRICHOS when investigating the network and cellular mechanisms underlying the chaperone-driven gain-of-function. Hereafter all recordings were performed in a submerged-type recording chamber and 1 μM Aj42 was used to induce functional deviations of LFP (Andrade-Talavera et al., 2020; Balleza-Tapia et al., 2018; Kurudenkandy et al., 2014). Gamma oscillations were elicited by applying 100 nM KA and after a period of 30 min of stable gamma oscillations a 5 min baseline period was recorded. Application of Aj42 induced a drastic decrease of gamma oscillation power and the subsequent application of 1 μM Bri2 BRICHOS failed to rescue it in 6 out of 13 slices (Fig. 2A–C). Interestingly, Bri2 BRICHOS treatment rescued the Aj42-induced loss of rhythmicity as measured by the coefficient of rhythmicity (Cr) (Fig. 2D). These discrepancies could be caused by the fact that an equimolar concentration of Bri2 BRICHOS vs. Aj42 is not enough to robustly interfere with Aj42 aggregation and rescue the network from the ongoing neurotoxicity. Thus, hereafter we decided to repeat the rescue experiments with a Bri2 BRICHOS concentration increased to 3 μM.
Using this 1:3 concentration ratio, Aβ42 application led to the usual significant degradation of gamma oscillation power and the subsequent application of Bri2 BRICHOS rescued gamma power almost immediately and reached a stable long-lasting plateau (Fig. 2E–G). Again, the rescue effect of Bri2 BRICHOS on gamma oscillation power was paralleled by a rescue of gamma rhythmicity. Notably, using 3 μM Bri2 BRICHOS only 1 out of 23 slices did not show a chaperone-mediated partial or total rescue (Fig. 2E, H).

### 3.3. Bri2 BRICHOS rescues action potential firing synchrony in PCs

Next, we proceeded to study the cellular and synaptic mechanisms underlying the Bri2 BRICHOS-mediated rescue. Since Bri2 BRICHOS rescues the Aβ42-degraded gamma oscillation rhythmicity we hypothesized that this may be driven by rescuing the synchronization of AP firing of relevant hippocampal neuron classes. To test this we first performed concomitant whole-cell current-clamp recordings of PC-APs and gamma oscillation LFP (Fig. 3A). Parallel to the gamma oscillation degradation (Fig. 3A, D), Aβ42 significantly decreased the firing rate of...
A

B

C

D

E

F

G

H

(caption on next page)
No Aβ application of Bri2 BRICHOS on the inhibitory input. We observed that inhibitory input levels (Fig. 4D). Split amplitudes were notably higher than control after Bri2 BRICHOS application (Fig. 3B, H).

Inhibitory inputs onto PCs while subsequent Bri2 BRICHOS treatment rescued it (Fig. 3A, E). No Aβ42-induced changes were observed regarding the firing-preferred phase-angle and Bri2 BRICHOS did not exert further changes (Fig. 3F).

In addition, Aβ42 caused a widening of the PC firing window consistent with Aβ desynchronization (Fig. 3A–C). Bri2 BRICHOS application rescued this desynchronization as evidenced by a decrease of the Gaussian half-width of the AP firing distribution (Fig. 3G), and observable as a tightening of the firing window (Fig. 3A–C). Moreover, Aβ42 wash-in reduced the vector length indicating a desynchronization of PC-AP firing, which is rescued by subsequent Bri2 BRICHOS application (Fig. 3B, H).

3.4. Bri2 BRICHOS rescues Aβ42-induced impairment of excitatory and inhibitory inputs onto PCs

Gamma oscillations depend on a tightly-regulated balance of inhibitory and excitatory synaptic activity in the neuronal network. Here, recordings of EPSCs and IPSCs from PCs in a KA-activated network with ongoing gamma oscillations showed that Aβ42 application disrupts both excitatory and inhibitory inputs. The subsequent application of Bri2 BRICHOS rescues such synaptic activity impairment (Fig. 4A, B). As such, Aβ42 application decreased EPSCs amplitude and subsequent Bri2 BRICHOS application restored it (Fig. 4A–C).

In contrast, Aβ42 and subsequent Bri2 BRICHOS treatment did not affect the overall mean of the inter-event-intervals (IEI). Consequently, no overall alterations in EPSCs mean frequency were observed. However, analysis of cumulative-frequency distribution showed that after Aβ42 application the EPSC-IEI distribution is significantly different from both control and Bri2 BRICHOS (Fig. 4A, B).

Moreover, the EPSC amplitude distribution histogram revealed that Aβ42 disrupts EPSC amplitude distribution and frequency (Fig. 4C). It shifts to the left with a drastic increase in the occurrence of small amplitude events while the frequency of larger amplitudes components is notably decreased. Bri2 BRICHOS counteract this imbalance: It restores the occurrence of Aβ42-increased small events to frequencies lower than control. Additionally, the larger components of EPSC amplitudes were notably higher than control after Bri2 BRICHOS application. As a reflection of both the amplitude and frequency changes observed here, this behavior was also evidenced in EPSC charge transfer analysis: Aβ42 induced a decrease of the overall excitatory drive onto PC and Bri2 BRICHOS counteracted such decrease back to control excitatory input levels (Fig. 4D).

Next, we proceeded to study the effects of Aβ42 and subsequent application of Bri2 BRICHOS on the inhibitory input. We observed that Aβ42 application decreases IPSC amplitude and Bri2 BRICHOS treatment rescues it (Fig. 4E, F). Overall IPSCs frequency was not significantly affected either by Aβ42 application or subsequent Bri2 BRICHOS wash-in. However, analysis of cumulative IPSC-frequency distribution revealed significant differences after Aβ42 application compared with control, as well as after Bri2 BRICHOS application. IPSC amplitude distribution evidenced a similar behavior to that observed for EPSC amplitude distribution. Bri2 BRICHOS caused a further increase on the occurrence of larger IPSC components over the Aβ42-induced decrease (Fig. 4G). As such, IPSC charge transfer was significantly decreased by Aβ42 and increased back to control levels after Bri2 BRICHOS application (Fig. 4H).

FSN are the major source of perisomatic inhibitory input onto PCs and play a central role in the entrainment of the network into gamma oscillations (McBain and Fisahn, 2001). Accordingly, the observed rescue is likely caused by the rescue of perisomatic inhibition driven by FSN. Overall, our results show that the Bri2 BRICHOS treatment is able to rescue the excitatory and inhibitory inputs onto PCs from ongoing Aβ42-induced impairment of synaptic transmission.

3.5. Bri2 BRICHOS rescues action potential firing synchrony of FSNs

Next, we investigated whether rescue of gamma oscillation rhythmicity (see Fig. 1), PC firing synchrony (Fig. 3) and IPSC parameters (Fig. 4) could result from the rescue of FSN activity. To address this, we performed concomitant recordings of gamma oscillation LFP and whole-cell current-clamp recordings of APs from FSNs. FSNs were recorded with 2 mM ATP-containing internal solution and were identified by their location, morphology and distinctive firing properties (Andrade-Talavera et al., 2020; Gulyas et al., 2010). Concomitantly to the LFP changes, Aβ42 significantly increased the firing rate of FSNs as we have recently observed (Andrade-Talavera et al., 2020). The subsequent application of Bri2 BRICHOS completely rescued this functional impairment (Fig. 5A, D, E).

In parallel, Aβ42 significantly changed the AP preferred phase-angle, evidenced by a shift to the left in the AP-phase distribution. Bri2 BRICHOS treatment changed the AP preferred phase-angle set by Aβ42 by shifting the AP-phase to the right towards control phase of firing values (Fig. 5B, C, F). Moreover, Aβ42 caused a widening of the FSN firing window (blue), consistent with AP desynchronization. Bri2 BRICHOS rescued FSN firing desynchronization as evidenced by the tightening of the firing window and assessment of the Gaussian half-width of the AP firing distribution (Fig. 5B, C, G). In addition, the efficacy of Bri2 BRICHOS at rescuing FSN firing synchrony was confirmed by the rescue of the vector length (Fig. 5B, H).
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A  EPSCs (-70 mV)
CONTROL
Aβ42
+ Bri2

B  Cumulative fraction
Amplitude (pA)
0.0  0.5  1.0
CONTROL  Aβ42  + Bri2

C  EPSCs #
Amplitude (pA)
0  200  400  600  800
CONTROL  Aβ42  + Bri2

D  Change transfer (%)

E  IPSCs (0 mV)
CONTROL
Aβ42
+ Bri2

F  Cumulative fraction
Amplitude (pA)
0.0  0.5  1.0
CONTROL  Aβ42  + Bri2

G  IPSCs #
Amplitude (pA)
0  200  400  600
CONTROL  Aβ42  + Bri2

H  Change transfer (%)

(caption on next page)
4. Discussion

4.1. BRICHOS domain molecular chaperones rescue cognition-relevant brain activity

Gamma oscillations underlie higher cognitive functions and are modulated according to memory load (Buzsáki, 2011; van Vugt et al., 2010; Womelsdorf and Fries, 2007). These neuronal fast network oscillations are disrupted in brain disorders with cognitive deficits as hallmark symptoms such as schizophrenia and AD, with the degree of disruption correlating with the severity of the experienced cognitive decline. Often, the development of cognitive deficits correlates with changes in the hippocampus, where gamma oscillations are a product of recurrent excitation among CA3 PCs (Csicsvari et al., 2003; Fisahn et al., 2016). Here, the effectiveness of Bri2 BRICHOS at rescuing the network activity from Aβ42-induced degradation was dependent on the concentration used, which is in line with the previous behavior observed against Aβ42 fibrillation in vitro at molecular levels (Chen et al., 2020, 2017; Cohen et al., 2015). The rescue effects of Bri2 BRICHOS on Aβ42-induced degradation were very rapid and occurred within minutes. The high binding affinity (Kd values in the nanomolar range) (Cohen et al., 2015; Leppert et al., 2021) and rapid association of proSP-C and Bri2 BRICHOS to Aβ42 fibrils (Bivertstal et al., 2020; Cohen et al., 2015; Leppert et al., 2021) allow fast and stable binding of BRICHOS to the fibril surface, which, markedly reduces ongoing Aβ42 oligomer formation via surface-catalyzed secondary nucleation. The rapid recovery observed in this study strongly suggest that Bri2 BRICHOS reduction of Aβ42 toxicity and rescue of network activity is primarily mediated by blocking oligomers formation and their activity.

Our functional study also provides evidence of the mechanism by which BRICHOS molecular chaperone domains exert their beneficial effect. The Aβ42-fibril-coating treatments evidenced that the blocking of the Aβ42-fibril surface by the chaperones may underlie the prevention of the secondary nucleation mechanisms that we previously described (Cohen et al., 2015). Our data also show that the BRICHOS chaperone expressed in the CNS (Br2 BRICHOS) can cover Aβ42 fibrils to a greater extent compared to the BRICHOS chaperone found in lung tissue (ProSP-C BRICHOS). This likely results in a larger reduction of the available catalytic surface needed for the production of the toxic oligomeric form of Aβ42 (Cohen et al., 2015). This finding also explains why in our previous studies fibrillar Aβ42 was the conformational state causing the strongest degradation of gamma oscillations and desynchronization of AP firing (Kurdenkandy et al., 2014): The increased amount of available fibrillar surface acting as a catalyst for the increased production of toxic oligomeric Aβ42 species.

4.2. Bri2 BRICHOS rescues Aβ42-disrupted FSN activity and neuronal network dynamics

In addition to the Aβ42-induced desynchronization of PC-AP firing...
**A**  
Neurophysiological recordings from control (left) and Aβ42-treated (middle) and Bri2-treated (right) animals.  

**B**  
Vector densities of phase-averaged amplitudes normalized to control in control (left), Aβ42-treated (middle), and Bri2-treated (right) conditions.  

**C**  
Normalized amplitude probability distributions (NAPDs) for control (left), Aβ42-treated (middle), and Bri2-treated (right) conditions.  

**D**  
Gamma power (left) and Cr (right) across experimental conditions.  

**E**  
AP firing (%) across experimental conditions.  

**F**  
Half-width of Gaussian map (left) and phase-angle (right) across experimental conditions.  

**G**  
Vector length across experimental conditions.  

**H**  
Vector length across experimental conditions.
Fig. 5. Bri2 BRICHOS rescues Aβ42-induced desynchronization of AP firing in CA3 FSNs. (A): Schematic diagram (left) showing the recording electrode position in a hippocampal slice for whole-cell current-clamp recordings of FSN-AP firing (upper example traces), and concomitant recording of gamma oscillation LFP (lower example traces) and the corresponding effect of 1 μM Aβ42 and 3 μM Bri2 application. Representative example of the waveform transforms corresponding to the three experimental conditions are shown as heat-maps. (B): Example polar plots showing AP firing distribution (left) and preferred firing phase and magnitude (right) with a vector displaying the normalized magnitude in the conditions described in A. Inset: Example traces showing two cycles of a gamma oscillation for each condition. Note that the Aβ42-induced desynchronization of FSN firing causes a widening of the firing window (increase of half-width of the Gaussian fit) and shifts the mean preferred firing phase. (D): Summary bar histograms of normalized gamma power (control: 103.5 ± 4.0%, Aβ42: 60.6 ± 12.8%, p = 0.0099 vs. control, Bri2 BRICHOS: 121.4 ± 43%, p = 0.0226 vs. Aβ42, p > 0.9999 vs. control; n = 7) and coefficient of rhythmicity (control: 0.48 ± 0.04, Aβ42: 0.4 ± 0.08, p = 0.0226 vs. control, Bri2 BRICHOS: 0.5 ± 0.07; p = 0.0099 vs. Aβ42, p > 0.9999 vs. control; n = 7) from experiments described in A. (E): Bri2 BRICHOS rescues the AP firing rate (control: 106.9 ± 14.1%, Aβ42: 159.1 ± 33.5%, p = 0.0095 vs. control, Bri2 BRICHOS: 110 ± 21.1%, p = 0.0095 vs. Aβ42, p = 0.6758 vs. control; n = 8, repeated measures one-way ANOVA followed by Holm’s Sidak post hoc multiple comparisons test). (F): Bri2 BRICHOS shifts the mean preferred firing phase-angle towards control values (control: 5 ± 0.3 rad, Aβ42: 4.7 ± 0.4 rad, p = 0.0485 vs. control, Bri2 BRICHOS: 5.1 ± 0.4 rad, p = 0.0485 vs. Aβ42, p > 0.9999 vs. control, n = 7). (O): Summary of the half-width of the Gaussian fit to the firing window as a measure of firing synchrony (control: 2.47 ± 1.35 rad, Aβ42: 5 ± 2 rad, p = 0.0040 vs. control, Bri2 BRICHOS: 3.4 ± 1.7, p = 0.0485 vs. Aβ42, p > 0.9999 vs. control, n = 7). (H): Quantification of the vector length confirming that Bri2 BRICHOS efficiently rescue AP firing synchronization of FSNs: (control: 0.47 ± 0.15, Aβ42: 0.24 ± 0.1, p = 0.0040 vs. control, Bri2 BRICHOS: 0.38 ± 0.14, p = 0.0485 vs. Aβ42, p > 0.9999 vs. control, n = 7). Data is presented as means ± SD. Significance levels were set as *p < 0.05, **p < 0.01, ***p < 0.001. Otherwise stated, statistics were assessed with Friedman’s test followed by Dunn’s post hoc multiple comparisons test.

(Kurudendkandy et al., 2014; Balleza-Tapia et al., 2018) we have shown here that Aβ42 also desynchronizes the AP firing of FSNs in hippocampus. FSNs fire synchronously and play a central role in determining PC firing rate and phase during ongoing gamma oscillations (Tükleđi-Hamburyan et al., 2015), and are critical to maintain network stability (Verret et al., 2012). Notably, Bri2 BRICHOS rescued the FSN firing phase-locking and hence the overall entrainment of the neuronal network back to proper gamma oscillations. This is conducive to a stronger coordinated activity within the network and hence to proper information processing. Here, enhancement of gamma phase-locked activity of FSN could underlie the rescue of the network entrainment within the gamma range observed.

From a potential treatment standpoint, Bri2 BRICHOS could reverse Aβ42-driven early cognitive impairment by decreasing the newly generated Aβ42 toxic oligomers while rescuing FSN activity and, consequently, overall network performance. Particularly, it will be helpful to study whether Bri2 BRICHOS counteracts the functional deviations of neuronal dynamics recently found in the AppNL-G-F AD mouse model at very early stages showing that FSN impairment precedes the emergence of Aβ plaques and cognitive impairment (Arroyo-Garcia et al., 2021). Furthermore, particular players such as microglia and astrocytes should also be considered in an ongoing in vivo study in order to further increase our knowledge of the therapeutic potential of Bri2 BRICHOS.

In this regard, artificial external stimulation of FSN at gamma frequencies in an AD mouse model has shown to result in a decreased Aβ42 load. This was accompanied by a shift from pro-inflammatory to phagocytic phenotype of the microglia, a cell population that also contributes to Aβ42 clearance in the brain (Iaccarino et al., 2016). Here, by interfering with Aβ42 aggregation using the chaperone Bri2 BRICHOS we have been able to reverse the desynchronization of the FSN activity and restore normal overall neuronal network dynamics.

Several findings indicate that FSNs utilize much more energy than other cortical neurons (Kann, 2016). This likely renders them highly vulnerable to conditions of metabolic stress, another deleterious effect of Aβ42. Here, the use of 2 mM ATP in the internal recording solution of FSN recordings was not arbitrary. Similar to previous reports of Aβ42-induced increase of PC firing rate (Kurudendkandy et al., 2014), we observed that the FSN firing rate drastically increases after acute exposure to Aβ42. Moreover, while Aβ42 induces an increase of PC (Kurudendkandy et al., 2014) and FSN firing rates (Andrade-Talavera et al., 2020) reduced with 2 mM ATP, the toxic peptide induces a decrease of the PC firing rate when 4 mM ATP is used in the internal recording solution. This is in line with the previous reported decrease of the excitatory input onto PCs (Balleza-Tapia et al., 2018). Both, excitatory input and PC firing rate were rescued by the subsequent application of Bri2 BRICHOS during ongoing Aβ42 toxicity. These results points to the impact of Aβ42 on neuronal metabolic rate putatively dependent on the energy supply and its effect on hippocampal networks dynamic. Future experiments including simultaneous monitoring of neuronal activity (i.e. calcium dynamics) and metabolic dynamics in specific neuronal compartments (i.e. somatic and dendritic) should further our understanding of Aβ42-induced disruption of cellular parameters rescued by Bri2 BRICHOS.

Tightly-regulated synaptic activity is crucial for the proper emergence and maintenance of the gamma brain rhythm. Here, we go beyond previous analyses of Aβ42 effects on both EPSC and IPSC behavior (Andrade-Talavera et al., 2020; Balleza-Tapia et al., 2018; Kurudendkandy et al., 2014). Beyond the study of overall frequency and amplitude we observed that Aβ42 shifts the IPSC and EPSC amplitude distributions. Particularly, the behavior of PC-EPSCs could indicate that more heterogeneous excitatory cell types contribute to the overall excitatory input. It also reveals the possibility that Aβ42 could differentially affect different subpopulations of PCs in the hippocampus during ongoing gamma oscillations. Supporting this notion it has been observed ex vivo that there are distinct classes of PCs with mutually exclusive firing patterns in the hippocampal CA3 area (Hemond et al., 2008). Hippocampal neuron types in vivo differ from each other in the characteristics of input-output transformation, suggesting cell type-specific information processing in the hippocampal network (Kowalski et al., 2016). Regardless of this possible cell type heterogeneity underlying our results, the application of Bri2 BRICHOS notably reversed Aβ42-induced imbalance of EPSC distribution.

It is intriguing that Aβ42 increases FSN firing rate while no changes were observed for the overall PC-IPSCs frequency. The strong reduction of larger amplitude components observed here in PCs could be masking a drastic increase of FSN excitationality caused by Aβ42, likely observed as a decrease in frequency of perisomatic-occurring inhibition. In turn, the increase of smaller IPSC components could be reflecting the observed increase of FSN firing rate or the overall amplitude decrease. Bri2 BRICHOS-driven rescue of the occurrence of larger IPSC amplitudes appears remarkable since FSNs are the major source of perisomatic inhibition onto PCs during gamma oscillations. A possible mechanism could be found in a previous study showing that Aβ42-induced decrease of inhibitory drive onto CA3 PCs is mediated by downregulation of GABAA receptors (Ulrich, 2015). The increase of smaller IPSC components could also be either caused by an offline detection of more small amplitudes corresponding to the decrease of perisomatic amplitudes or could be a result of an increase in the activity of O-LM interneurons which target basal and distal dendritic arbour fields (Gioveli et al., 2005, Sihm). Simultaneous dendritic and somatic patch-clamp recordings coupled to dynamic calcium imaging could shed light on this hypothesis.

In this regard, during field gamma oscillations a more prominent

Kurudendkandy et al., 2014; Balleza-Tapia et al., 2018)
theta frequency inhibitory input to distal apical dendrites has been reported. In vivo oscillatory activity in the hippocampus frequently takes the form of a gamma rhythm nested in a theta rhythm, called cross-frequency coupling. This non-trivial cross-frequency coupling allows for a hierarchical organization of the rhythms that leads to precise neuronal firing patterns (Canolty et al., 2006). In this scenario, where different interneuron populations can work simultaneously in the same network providing different inhibitory inputs to PCs to generate temporal patterns with functional relevance (Park et al., 2020), Bri2 BRICHOS rescues the IPSC distribution from the Aβ42-induced increase of small amplitude components. Consequently, it provides evidence for the efficacy of the molecular chaperone to reverse the Aβ42-induced disruption of cognition-relevant neuronal dynamics.

5. Concluding remarks

Our model can serve as a prototype and drug discovery assay for cognition-relevant gamma oscillation behavior during AD progression in humans (Andrade-Talavera et al., 2021, 2020; Iaccarino et al., 2016; Kurudenkandy et al., 2014; Park et al., 2020; Sellke, 2002). The model has been cross-validated in a recent study from our laboratory where several commonalities have been found between the in vitro acute Aβ-induced functional deviations and the chronic AD mouse model AppNL-G-F (particularly in early stages of disease progression). The present study bridges a gap between previous studies at the molecular level on G-F (particularly in early stages of disease progression). The present study and analyzed data. YAT and AF wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2021.105514.

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