Amyloid-β_{1-42} oligomers enhance $mGlu_5R$-dependent synaptic weakening via NMDAR activation and complement C3aR/C5aR signaling

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Abstract

Synaptic dysfunction, weakening, and loss of synapses are well-correlated with the pathology of Alzheimer's disease (AD). Recent studies have implicated hyper-activation of the complement cascade as the driving force for loss of synapses in AD. However, the synaptic cues to trigger pathological complement activity remain elusive. To address this, we studied the actions of oligomeric, 42 residue length amyloid β (oAβ) on metabotropic glutamate receptor (mGluR) long-term depression (mGluR-

LTD) at hippocampal CA1 synapses, a form of synaptic plasticity that is disrupted in rodent models of AD. We found that exogenous application of oAβ to mouse hippocampal slices enhanced the magnitude of mGluR subtype 5 ($mGlu_5R$)-dependent LTD. This enhanced synaptic weakening occurred via both NMDARs and complement C3aR/C5aR signaling, as revealed by pharmacological inhibition experiments. Our findings reveal a novel mechanistic interaction between $mGlu_5R$, NMDARs, and the complement cascade in synaptic weakening induced by oAβ, which could represent an early trigger of synaptic degeneration in AD.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss, cognitive deficits and changes to personality and behavior (Breijyeh & Karaman, 2020). Although it is the most common cause of dementia affecting the aging population worldwide, therapeutic options are limited. The development of better treatments necessitates a deeper understanding of pathophysiological mechanisms. Weakening and subsequent loss of synapses are early events in AD progression that precede the deterioration of memory and cognitive functions (Koffie, Hyman, & Spires-Jones, 2011; Li & Selkoe, 2020). As such, targeting these events has the potential to prevent AD before irreversible neuronal damage can occur. Normally, synaptic strength is balanced by long-term potentiation (LTP) and long-term depression (LTD), which are thought to provide the cellular and molecular basis of learning and memory (Bliss & Collingridge, 1993). Substantial evidence implicates amyloid precursor protein (APP) in the pathogenesis of AD (O'brien & Wong, 2011). APP is cleaved by secretases to yield amyloid beta (Aβ) peptides of 30-51 amino acids, including $A\beta_{1-42}$

which is highly prone to aggregation and rapidly forms oligomeric Aβ (oAβ) which accumulates in the brain (Lue, et al., 1999). *In vitro* and *in vivo* studies of AD models have found that oAβ mediates a shift in the LTP/LTD balance, favoring LTD over LTP, leading to net synapse loss (Kim, Anwyl, Suh, & Djamgoz, 2001; Hsieh, et al., 2006; Shankar, et al., 2007; Shankar, et al., 2008; Li, et al., 2009; Jo, et al., 2011; Cline, Bicca, Viola, & Klein, 2018; Li & Selkoe, 2022). Therefore, understanding the mechanisms by which oAβ alters synaptic plasticity to bias towards LTD and synapse elimination is essential to determining the pathological events underlying AD.

The complement cascade is an innate immune pathway which has emerged as a central driver of synapse loss in AD (Wu, Dissing-Olesen, MacVicar, & Stevens, 2015; Hammond, Robinton, & Stevens, 2018). Complement cascade activation leads to phagocytosis by microglia via C3b/CR3, as well as chemotactic and inflammatory signaling via C3a-C3aR and C5a-C5aR axes (Ricklin, Hajishengallis, Yang, & Lambris, 2010). Tissue from post-mortem samples of patients with AD demonstrates activation of complement activity, including increased deposition of C1q and proteolytic cleavage of C3 (Afagh, Cummings, Cribbs, Cotman, & Tenner, 1996; Yasojima, Schwab, McGeer, & McGeer, 1999; Wu, et al., 2019). Further, oAβ acts as a potent activator of the complement cascade and oAβ-mediated synapse loss is dependent on the complement pathway (Hong, et al., 2016; Bie, Wu, Foss, & Naguib, 2019). Finally, in multiple mouse models of AD, there is aberrant activation of complement-mediated synapse phagocytosis. Genetic deletion or pharmacological inhibition of complement cascade proteins prevents synapse and neuronal loss, preserving cognitive function (Fonseca, et al., 2009; Hong, et al., 2016; Dejanovic, et al., 2018; Litvinchuk, et al., 2018; Wu, et al., 2019; Carvalho, et al., 2022). However,

the upstream synaptic signals that lead to aberrant activation of complement and subsequent synapse loss are not well understood.

LTP and LTD of glutamatergic synapses is mediated by activation of N-methyl-D-aspartate receptors (NMDARs) and/or metabotropic glutamate receptors (mGluRs; Collingridge, Peineau, Howland, & Wang, 2010; Nicoletti, et al., 2011; Volianskis, et al., 2015). LTD, induced either by NMDARs or mGluRs, can trigger subsequent synapse elimination (Shinoda, Tanaka, Tominaga-Yoshino, & Ogura, 2010; Wiegert & Oertner, 2013; Hasegawa, Sakuragi, Tominaga-Yoshino, & Ogura, 2015; Wiegert, Pulin, Gee, & Oertner, 2018). Both mGluR- and NMDAR-dependent signaling are affected in AD, leading to synaptic plasticity impairments and aberrant synapse loss (Hsieh, et al., 2006; Shankar, et al., 2007; Shankar, et al., 2008; Jo, et al., 2011; Li, et al., 2011; Cavallucci, et al., 2013; Beraldo, et al., 2016; Jackson, et al., 2019; Li & Selkoe, 2020; Abd-Elrahman, et al., 2020). While it is known that mGluRs form protein complexes with NMDARs (Tu, et al., 1999; Perroy, et al., 2008), the role of this interaction in mediating synaptic impairments in AD is not well understood. Interestingly, the complement cascade has recently been implicated in synapse loss mediated by mGluRs in AD mouse models. Intra-hippocampal activation of mGluRs with the group I ($mGlu_1R$ and $mGlu_5R$) agonist (S)-3,5-dihydroxyphenyl glycine (S isomer, abbreviated herein as DHPG) *in vivo* increased C1q synaptic tagging and synapse elimination. Additionally, knockout of $mGlu_1R$ in a mouse model of AD prevented microglial synapse phagocytosis (Bie, Wu, Foss, & Naguib, 2019). A silent allosteric modulator of $mGlu_5R$ has also recently been found to restore synapse density through reduced tagging by C1q and microglia engulfment in a mouse model of AD (Spurrier, 2022).

However, the interaction between NMDARs and mGluRs in promoting complement activation in AD is not defined. To address this, we used hippocampus slices treated with oAβ as an *in situ* model of AD. We found that oAβ enhanced $mGlu_5R$-mediated LTD via a mechanism that involves both the activation of NMDARs and the C3aR and C5aR arms of the complement cascade. These findings have implications pertinent to the understanding of the mechanism that leads to synaptic deficits that may underlie the initial stages of dementia.

Methods

Animals

Adult C57BL/6JOlaHsd male mice (10-16 weeks old) from Envigo (U.K.) or $mGlu_5R$ knockout line (Lu, et al., 1997) backcrossed more than 10 generations to the C57BL/6J strain were used in this study. We chose to use male mice due to the recently reported sex specificity of oAβ-$mGlu_5R$ binding which is present only in males (Abd-Elrahman, et al., 2020). Animals were housed four per cage in a room with designated 12 h light/dark cycle (lights on at 08:15/ off at 20:15) with temperature at 21 °C ± 2 °C and access to food and water *ad libitum.* All procedures were performed in accordance with the U.K. Animals (Scientific Procedures) Act of 1986. Genomic DNA from $mGlu5_R$ knockout litters were isolated from tail tips with DNeasy blood and tissue kit (Qiagen; cat. no. 69504) and amplified as described (Lu, et al., 1997). The amplified products were resolved and visualized in ethidium bromide-stained agarose gel.

Oligomeric amyloid-$\beta_{1\text{-}42}$ (oAβ) preparation

oAβ was prepared as described (Whitcomb, et al., 2015). Briefly, Aβ peptide$_{(1\text{-}42)}$ (Millipore; TFA recombinant human, ultra-pure; cat. no. AG912) was dissolved in 100

% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma Aldrich; cat. no. 105228) to a final concentration of 1 mg/mL. The HFIP/peptide mixture was incubated at room temperature for 1 h and sonicated for 10 min in a water-bath sonicator. The mixture was then air-dried under a gentle stream of nitrogen gas for 1.5 h. The peptide crystal was re-suspended in 100 % DMSO, aliquoted to 10 µL per tube and stored at -80 °C until experimental use (see next section).

Hippocampal slice preparation

Mice were deeply anaesthetized via inhalation with a mixture of 5 % isofluorane / 95 % oxygen and euthanized by cervical dislocation. The brain was rapidly removed (<1 min) and chilled in cold ACSF solution (in mM: 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 D-glucose, 2 $MgSO_4$ and 2 $CaCl_2$; bubbled continuously with 95% O_2 /5% CO_2). Hippocampi were isolated from both hemispheres in cold ACSF. Transverse slices of 400 µm in thickness were prepared from the dorsal ends of hippocampi using a McIlwain tissue chopper. The CA3 region was left intact for LTP but removed via a surgical cut in LTD experiments. Slices were transferred to a holding chamber with room temperature ACSF and allowed to recover for at least 1 h. On the day of experiment, the Aβ peptide/DMSO mixture was diluted to 100 µM in D-PBS, vortexed briefly and allowed to aggregate for 2 h at room temperature. The heterogenous mixture of oAβs was diluted to a final concentration of 500 nM in oxygenated ACSF and acutely applied to hippocampal slices for 2 h at room temperature. As an inclusion criterion to account for batch-to-batch variability, we routinely assessed the bioactivity of our aggregated oAβs by confirming the ability of each oAβ batch to impair hippocampal LTP (Figure S1A,B).

Electrophysiology

After vehicle or oAβ incubation, a dorsal hippocampal slice was submerged in a Slicemate recording chamber (Scientifica, U.K.) with ACSF perfusing at a rate of 2.5 mL/min and temperature maintained at 30.0 ± 0.5 °C. Extracellular field potentials were recorded from the stratum radiatum area of CA1 using a glass microelectrode (~2-3 MΩ) filled with ACSF. Responses were evoked by stimulating the Schaffer collaterals using a platinum-iridium bipolar electrode (FHC) positioned at the border between area CA2-CA1. Stimuli of 100 µs in duration were delivered once every 15 s via a DS2 constant voltage isolator (Digitimer, U.K.). Responses were amplified using a Multiclamp 700B amplifier (Molecular Devices, U.S.A.), digitized at 40 kHz and monitored in real time using WinLTP (WinLTP Ltd, U.K.) software (Anderson & Collingridge, 2007). After a period of stable baseline recordings, datapoints for input/output (I/O) plots were obtained with fixed stimulus intensity at 1x, 1.5x, 2x, 3x, and 4.5x. The lowest intensity at 1x was defined as the threshold for evoking a visually detectable field excitatory postsynaptic potential (fEPSP) (≤ 0.05 mV). fEPSPs for plasticity experiments were recorded using a stimulus intensity of 3x the threshold intensity. LTP was induced by a theta-burst stimulation (TBS) protocol, with four stimuli delivered at 100 Hz, repeated 10 times at a frequency of 5 Hz. LTD was chemically induced by bath-application of (S)-3,5-DHPG (50 µM; Abcam; cat. no. ab120007) for 10 min. For offline analysis, responses were filtered at 2 kHz and expressed as the mean of four consecutive responses.

Quantification and statistical analysis

Data in the time course plots were normalized to the mean of the entire baseline period, defined as 100 % and presented as mean ± standard error of mean (SEM). The magnitude of LTP or LTD was presented as percentage of potentiation or depression of fEPSP from baseline and quantified as mean of the last 5 min of time

course recordings. Fiber volleys (FVs) and fEPSPs were quantified using peak amplitude and initial slope measurements, respectively, using WinLTP. The I/O plots were analysed using Excel linear regression software (Microsoft). The respective slope values from a fitted line between FV *versus* stimulation intensity and fEPSP slope *versus* FV were used for quantification. Statistical comparisons between -oAβ and +oAβ conditions were assessed using unpaired two-tailed Student's t-test (Microsoft Excel). The level of significance was set at $p<0.05$.

Results

Acute oAβ exposure enhances mGlu$_5$R-LTD

To investigate the impact of oAβs on synaptic function, we incubated acute hippocampus slices for 2 h with oAβ. We subsequently measured synaptic plasticity with field potential recordings at Schaffer collateral-CA1 synapses. As a positive control for the bioactivity of our oAβ preparation, we confirmed previous findings (Shankar, et al., 2008; Li, et al., 2011) that oAβ was able to impair LTP in slices pre-treated with oAβ (+oAβ) *versus* interleaved non-treated slices (-oAβ; Figure S1A,B). Although LTP was impaired by acute application of oAβ, basal synaptic transmission was unaltered, indicated by the overlapping input-output relationship of +oAβ compared to -oAβ slices (Figure S1C-E). As such, this paradigm allowed us to study early disruptions to plasticity that precedes the loss of synapses.

To investigate how oAβ alters mGluR-dependent synaptic plasticity mechanisms, we induced LTD using DHPG (S isomer), a selective agonist for group I mGluRs (mGlu$_1$R and mGlu$_5$R), that is widely used to study mGluR function. A short application of DHPG (10 min) induced a stable LTD in -oAβ slices from WT mice (Figure 1A,B). The

magnitude of DHPG-LTD was significantly enhanced in the interleaved +oAβ slices (Figure 1A, B), consistent with published observations (Hsieh, et al., 2006; Shankar, et al., 2008; Li, et al., 2009; Cavallucci, et al., 2013). To determine the group I mGluR subtype-specificity of our observed phenotype, we used hippocampal slices from $mGlu_5R^{-/-}$ mice (Lu, et al., 1997) which were littermates to the $mGlu_5R^{+/+}$ mice in Figure 1A, B. DHPG-LTD was absent in both -oAβ and +oAβ slices (Figure 1C,D). This indicates that the form of LTD which is enhanced by oAβ requires the activation of $mGlu_5R$ receptors (referred to hereafter as $mGlu_5R$ LTD).

NMDARs are required for oAβ-mediated enhancement of $mGlu_5R$-LTD

There are two distinct forms of DHPG-LTD that can be distinguished by their dependence upon the activation of NMDARs (Palmer, Irving, Seabrook, Jane, & Collingridge, 1997; Sanderson, et al., 2022). To determine whether NMDARs are required for either $mGlu_5R$-LTD under control conditions or the enhanced $mGlu_5R$-LTD observed in the presence of oAβ, we used a selective NMDAR antagonist, L689,560. L689,560 was used as it acts at the glycine site of the NMDAR and so is independent of L-glutamate concentration, which might be altered by oAβ treatment (Li, et al., 2009). In these experiments, we interleaved a new set of controls and again observed the potentiation of $mGlu_5R$-LTD by oAβ (Figure 1E,F). We found that NMDAR activation was not required for $mGlu_5R$-LTD in control mice. However, the enhancement of DHPG-LTD by oAβs was completely prevented by L689,560 (Figure 1G,H) and therefore requires NMDARs.

oAβ enhanced $mGlu_5R$-LTD via complement cascade signaling

Early studies of the effects of oAβ on LTP found that inhibition of LTP was prevented by application of minocycline, an inhibitor of microglia activation (Wang, Rowan, & Anwyl, 2004). Further, it has recently been found that agonism of group I mGluR *in vivo* activates the complement cascade leading to synapse loss (Bie, Wu, Foss, & Naguib, 2019). We therefore hypothesized that complement signaling is necessary for the enhancement of $mGlu_5R$-LTD by oAβs. We antagonized either C3aR or C5aR, as both receptors have been implicated in the pathology of mouse models of AD (Fonseca, et al., 2009; Lian, et al., 2016; Hernandez, et al., 2017; Litvinchuk, et al., 2018; Carvalho, et al., 2022; Gomez-Arboledas, et al., 2022). For these experiments we also interleaved a new set of controls (Figure 2A,B) and again observed the potentiation of $mGlu_5R$-LTD by oAβ. To inhibit C5aR, we bath-applied PMX205, a peptide-based antagonist (March, et al., 2004). PMX205 had no effect on the level of $mGlu_5R$-LTD induced under control conditions but eliminated the potentiation induced by oAβ (Figure 2C,D). We next used W54011, a non-peptide based C5aR antagonist (Sumichika, et al., 2002). As with PMX205, bath-application of W54011 had no effect on the level of $mGlu_5R$-LTD induced under control conditions but eliminated the potentiation induced by oAβ (Figure 2E,F). Lastly, we investigated the role of C3aR using the antagonist SB290157 (Ames, et al., 2001). As with the C5aR antagonists, SB290157 abolished the enhanced $mGlu_5R$-LTD induced by oAβ without affecting the level of $mGlu_5R$-LTD under control conditions (Figure 2G,H). Together, these data indicate that the oAβ-mediated enhancement of $mGlu_5R$-LTD occurs via activation of complement C3aR/C5aR signaling.

Discussion

The complement cascade is emerging as a key innate immune pathway for shaping glutamatergic synapses during brain development, as well as driving synapse loss in various disease states. However, the synaptic signals that lead to complement activation remain poorly understood. In the present study, we uncovered a novel signaling mechanism through which both complement C3aR and C5aR activation amplified LTD of glutamatergic synapses. As oAβ has been identified as a key mediator of synaptic toxicity in AD (Cline, Bicca, Viola, & Klein, 2018; Li & Selkoe, 2020), we used exogenous application of oAβ to acute hippocampus slices to model the early stages of oAβ-mediated synaptic impairment. Our findings that basal synaptic transmission is unaltered confirms that we engaged an early stage of synaptic plasticity impairment (Figure S1). Thus, our findings reveal mechanisms which are upstream of overt synapse loss. We uncovered that $mGlu_5R$-dependent LTD, induced by DHPG application, was enhanced in slices treated with oAβ (Figure 1). Unlike vehicle treated slices, the oAβ-mediated enhanced portion of $mGlu_5R$-LTD was dependent on NMDAR signaling. Similarly, antagonism of either C3aR or C5aR prevented oAβ from enhancing DHPG-LTD (Figure 2), without affecting the underlying $mGlu_5R$-LTD *per se*. Together, our results have uncovered a novel interaction between mGluRs, NMDARs, and the complement cascade, which drive pathological levels of synaptic depression.

Glutamatergic synapse dysfunction in AD

A foundational hypothesis in AD research over the past two decades has centered around synaptic failure, which posits that oAβs initially reduce synaptic efficacy and subsequently cause neuronal loss (Selkoe, 2002; Li & Selkoe, 2020). Reductions in AMPAR- and NMDAR-mediated synaptic transmission, as well as a shift in the LTP-LTD balance favoring LTD, caused by oAβ (Cullen, Suh, Anwyl, & Rowan, 1997;

Walsh, et al., 2002; Hsieh, et al., 2006; Shankar, et al., 2007; Shankar, et al., 2008; Li, et al., 2009; Jo, et al., 2011; Li, et al., 2011; Cavallucci, et al., 2013) has provided converging evidence supporting the idea that a primary triggering event in AD is excess synapse weakening. Altered activity of both group I mGluRs and NMDARs by oAβ and in AD mouse models is central to glutamatergic synaptic failure. Interestingly, $mGlu_5R$ was identified as a co-receptor of cellular prion protein (PrP_c) for binding oAβ and is necessary for oAβ-induced intracellular signal transduction and synapse loss (Um, et al., 2013; Haas, Kostylev, & Strittmatter, 2014; Beraldo, et al., 2016; Brody & Strittmatter, 2018). Additionally, antagonists of $mGlu_5R$ and genetic deletion have both been found to reduce synaptic and behavioral deficits in AD mouse models (Hamilton, Esseltine, DeVries, Cregan, & Ferguson, 2014; Hamilton et al., 2016; Haas, et al., 2017; Abd-Elrahman, et al., 2020; Spurrier, 2022). Evidence that altered NMDAR signaling is causally related to the human condition is the use of memantine, a non-competitive NMDAR antagonist, for the treatment of AD. Memantine slows the progression of dementia and its underlying mechanism has been attributed to the normalization of synaptic plasticity (Parsons, Stöffler, & Danysz, 2007; Wang & Reddy, 2017; Liu, Chang, Song, Li, & Wu, 2019).

Despite these important advances, how NMDARs and mGluRs, individually or synergistically, are involved in triggering synaptic weakening and loss in AD is poorly understood. Of relevance to the present study, at least two distinct forms of DHPG-LTD exist, which can be distinguished by the dependence on NMDARs (Palmer, Irving, Seabrook, Jane, & Collingridge, 1997; Sanderson, et al., 2022). In our current study, DHPG-LTD under control conditions was not affected by NMDAR antagonism. In contrast the oAβ-enhanced portion of the LTD totally depended on the activation of

NMDARs. As such, promotion of mGluR-NMDAR signaling during synaptic depression may be a key aspect of the shift in LTP/LTD balance and may constitute a trigger towards AD.

C3aR/C5aR signaling in AD

In both post-mortem human samples as well as numerous AD mouse models, many components of the complement cascade have been found to be upregulated (Hammond, Robinton, & Stevens, 2018; Schartz, 2020). Importantly, pharmacological and genetic manipulations of both C3aR and C5aR have provided evidence that both receptors are necessary for synaptic dysfunction and cognitive impairments in AD mouse models. SB290157, the C3aR antagonist used in our current study, reduces amyloid pathology in the APP/TTA mouse model (Lian, et al., 2016), and synapse engulfment by microglia and neuron loss are reduced in $C3aR^{-/-}$ mice in the PS19 tauopathy AD model (Litvinchuk, et al., 2018). Similarly, C5aR1 genetic ablation is protective in AD mouse models (Hernandez, et al., 2017; Carvalho, et al., 2022). Additionally, the C5aR antagonist used in our study, PMX205, improved amyloid pathologies, reactive gliosis, and behavioral outcomes in multiple AD mouse models (Fonseca, et al., 2009; Gomez-Arboledas, et al., 2022). However, the way in which glutamatergic synapses communicate with the complement cascade to cause this dysfunction was not understood. Our study provides insight into this communication, by demonstrating that oAβ enhances synaptic weakening, via NMDAR and $mGlu_5R$ synergistic mechanisms that requires both C3aRs and C5aRs.

One important question to be addressed by future studies pertains to which arm of the complement cascade initiates the cleavage of C3 following mGluR-LTD in the presence of oAβ? Proteolytic cleavage of C3 generates both C3a, as well as

components of the C5 convertase which produces C5a. C3 conversion can be driven by either the classical arm, initiated by C1q, the lectin arm via mannose-binding lectin (MBL) or the alternative pathway through C3 “tickover” (Ricklin, Hajishengallis, Yang, & Lambris, 2010). Hong et al. (2016) found that the ability of oAβ to inhibit LTP requires the classical complement pathway, as co-incubation of hippocampal slices with oAβ and a function-blocking antibody of C1q restored LTP. Further, *in vivo* injections of oAβ into rat hippocampus upregulated C1q, and increased synapse phagocytosis by microglia (Bie, Wu, Foss, & Naguib, 2019). C1q upregulation by oAβ and subsequent synapse phagocytosis required group I mGluR signaling, and *in vivo* DHPG administration alone increased C1q. Finally, a recent study found that a silent allosteric modulator of $mGlu_5R$ reduced C1q co-localization with PSD-95 and microglia engulfment of synapses in a mouse model of AD (Spurrier, 2022). As such, our findings suggest that NMDAR and C3aR/C5aR activation link C1q induction via mGluRs to downstream synaptic depression and elimination. Cleavage of C3 also generates the C3b fragment, which can covalently attach to synapses where it is converted to iC3b and binds to the phagocytic receptor CR3 (Ricklin, Hajishengallis, Yang, & Lambris, 2010). Genetic deletion of CR3 has also been found to spare synapses and improve memory in AD models (Hong, et al., 2016), indicating that phagocytosis through iC3b-CR3 binding is also critical for the deleterious effects of complement in pathology. As weaker synapses are targeted by complement for phagocytic removal (Schafer, et al., 2012), an intriguing question is whether synapse weakening by C3aR/C5aR activation also tags those synapses with iC3b for subsequent engulfment by microglia. CR3 can also impact synapses by non-phagocytic mechanisms in neuroinflammatory conditions. LPS and hypoxia activated CR3 which induces LTD of AMPAR transmission in the hippocampus (Zhang, et al.,

2014). Thus, microglia and the complement cascade have a multi-faceted role in synaptic dysfunction in disease conditions, being critical for both changes to AMPAR-mediated transmission strength as well as structural synapse elimination.

Relevance to other neurodegenerative diseases

Upregulation of complement cascade proteins and activity is not exclusive to AD and has been detected in several other neurodegenerative diseases. For example, A1 reactive astrocytes, which express high levels of C3, have been identified in human post-mortem tissue from patients with amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and multiple sclerosis (Liddelow, et al., 2017). Further, studies from *in vitro* and mouse models of these diseases have demonstrated the necessity of C3 and/or C5 for their pathology (Woodruff, et al., 2008; McGeer, Lee, & McGeer, 2017; Wang, Lee, Lee, Woodruff, & Noakes, 2017; Hammond, et al., 2020; Bourel, et al., 2021; Chen, Hu, Ding, Du, & Hu, 2021; Gregersen, et al., 2021; Lopez-Sanchez, et al., 2022). Interestingly, $mGlu_5R$ has separately been identified as an emerging therapeutic target in these complement-associated neurodegenerative diseases (Geurts, et al., 2003; Abd-Elrahman, et al., 2017; Bonifacino, et al., 2017; Doria, et al., 2018; Bonifacino, et al., 2019; Farmer, et al., 2020; Su, Wang, Han, & Shen, 2021; Zhang, et al., 2021; Azam, et al., 2022; Li, Colson, Abd-Elrahman, & Ferguson, 2022). We therefore speculate that the $mGlu_5R$-NMDAR-C3aR/C5aR signaling axis identified in our study may be a common feature of many brain disorders.

Concluding Remarks

mGluRs, NMDARs, and the complement cascade have been independently studied for their role in the pathogenesis of AD. Our study has found a novel link which places

glutamate receptor-innate immune interactions at the center of early synaptic dysfunction mediated by oAβ, which is overproduced in AD. As such, a deeper understanding of the cellular and molecular mechanisms of these interactions has the potential to elucidate therapeutic targets which prevent synapse loss to halt AD progression.

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Competing interests

The authors have no competing interests to declare.

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Figures

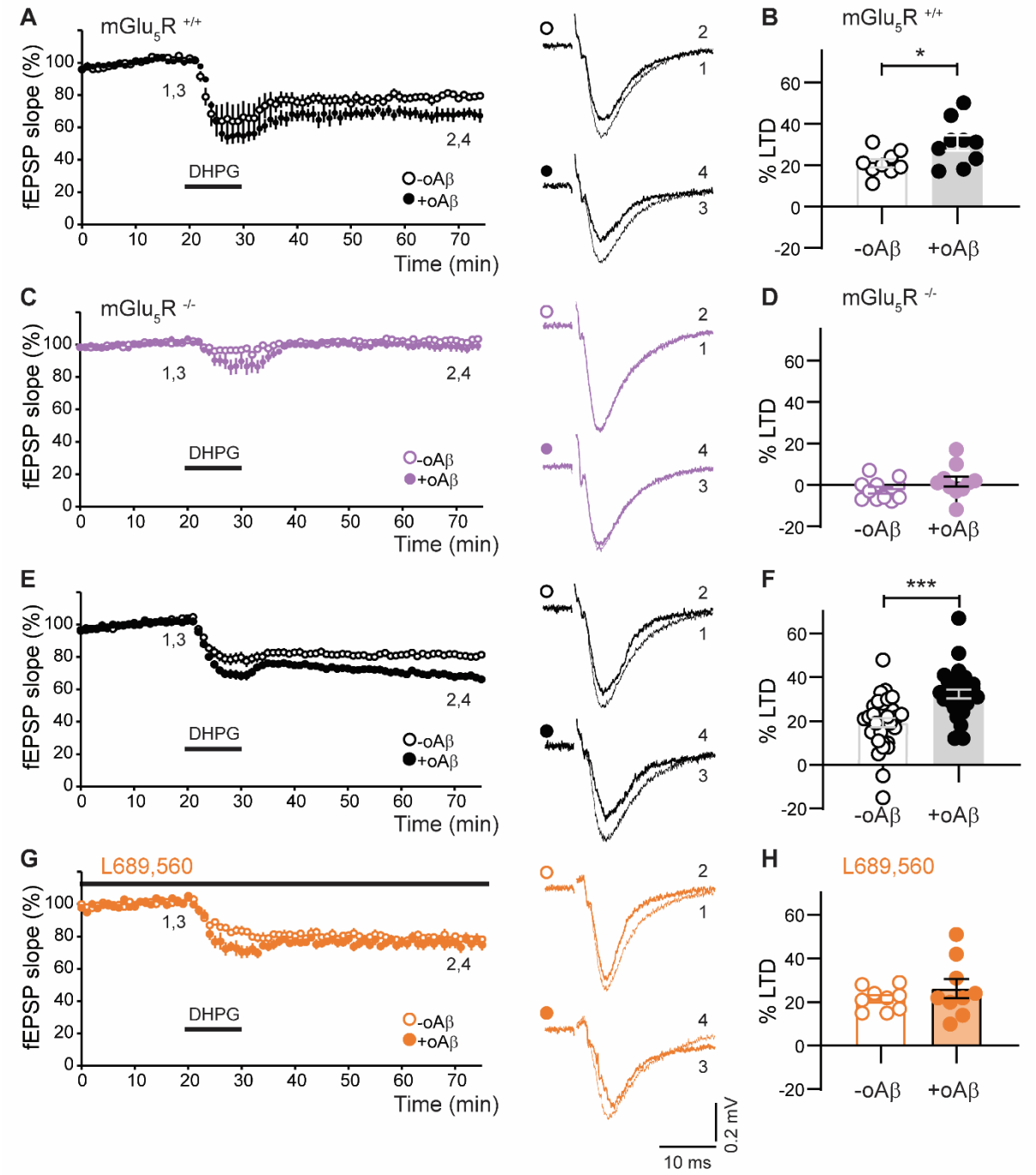


Figure 1: oAβs enhances $mGlu_5R$-LTD at CA1 synapses.

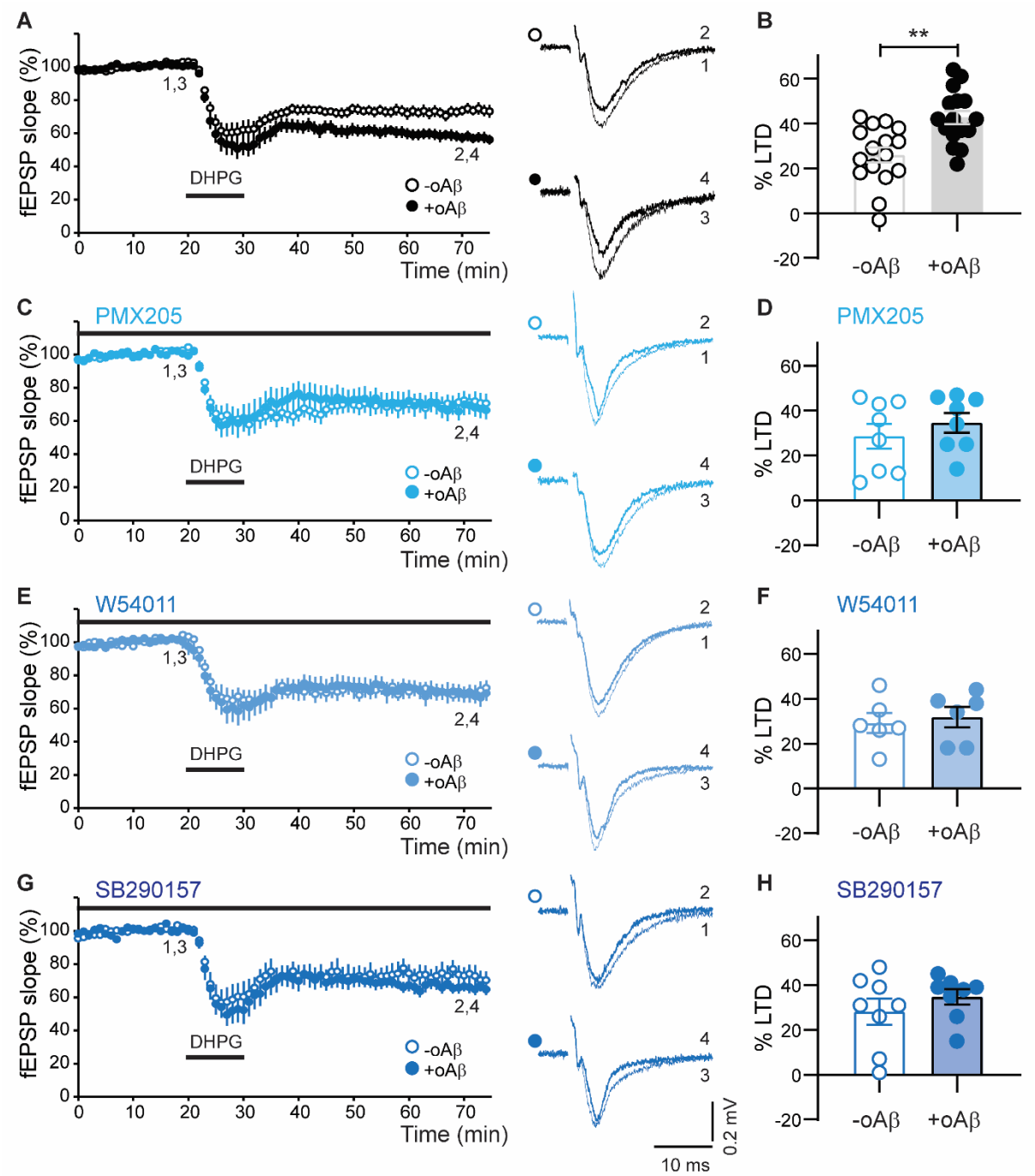
(**A-B**) Application of s-DHPG (50 µM, 10 min), induced LTD in -oAβ slices from $mGlu_5R^{+/+}$ mice (21 ± 2 %, n = 9). Interleaved +oAβ slices showed an enhancement of DHPG-LTD (31 ± 4 %, n= 9) with summary of LTD experiments in (**B**). p=0.032 *versus* -oAβ. Representative fEPSP traces showing enhanced DHPG-LTD in +oAβ compared to -oAβ slices.

(**C-D**) In slices from littermate $mGlu_5R^{-/-}$ mice, DHPG failed to induce LTD responses in both conditions (+oAβs = 2 ± 3 %, n=10; -oAβ= -3 ± 2 %, n=10) with summary in (**D**). Representative fEPSP traces showing the absence of DHPG-LTD in both conditions.

(**E-F**) In a separate cohort from (**A**), pre-treatment of oAβs also resulted in an enhancement of DHPG-LTD (34 ± 2 %, n=22) compared to the interleaved -oAβ slices (21 ± 2 %, n=22). p=0.00031, +oAβ *versus* -oAβ.

(**G-H**) Bath application of L689,560 prevented the enhancement of DHPG-LTD in +oAβ slices (26 ± 4 %, n=9) *versus* the interleaved -oAβ slices (22 ± 2 %, n=9).

Sample fEPSP traces are the mean of 4 consecutive responses at the indicated time points (1-4). The number of slices (n) used for each experimental condition is shown. For each experiment, sample traces before and after DHPG treatment are superimposed. Two-tailed t-test was used in **B, D, F, H**. Scale bar: 10 ms / 0.2 mV. Source data are provided as a Source Data file.



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702 Figure 2: Complement C5aR and C3aR signaling is necessary for oAβs to enhance $mGlu_5R$-

703 LTD.

704 (**A-B**) In a third, separate cohort from Figure 1A and Figure 1E, pretreatment with oAβ

705 enhanced DHPG-LTD (43 ± 3 %, n=16) compared to the interleaved -oAβ slices (26 ± 3 %,

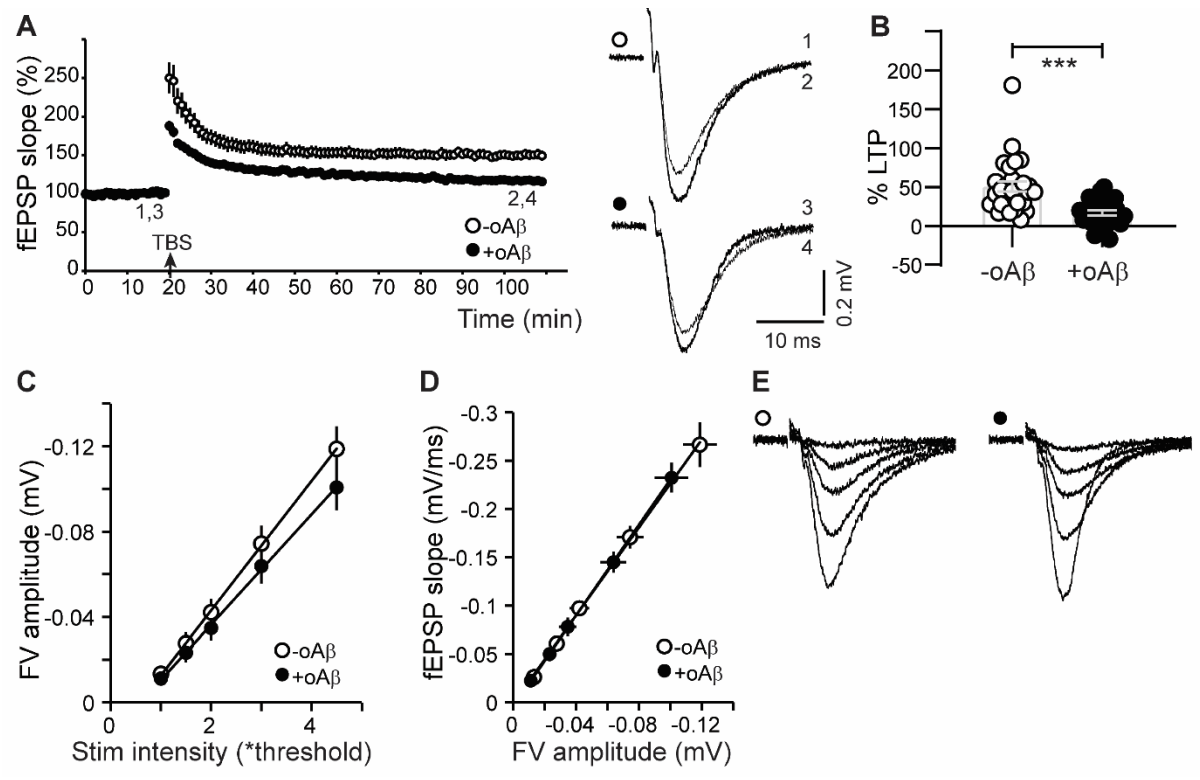
706 n=16). p=0.00064, +oAβ compared to -oAβ.

(**C-D**) Antagonism of C5aRs with PMX205 (0.3 µM) prevented the enhancement of DHPG-LTD in +oAβ slices (35 ± 4 %, n=8) without affecting the level of DHPG-LTD in interleaved -oAβ slices (29 ± 6 %, n=8).

(**E-F**) Antagonism of C5aRs with W54011 (0.3 µM) prevented the enhancement of DHPG-LTD in +oAβ slices (32 ± 5 %, n=6) without affecting the level of DHPG-LTD in interleaved -oAβ slices (29 ± 4 %, n=6).

(**G-H**) Antagonism of C3aRs with SB290157 (1 µM) prevented the enhancement of DHPG-LTD in +oAβ slices (35 ± 3 %, n=8) without affecting the level of DHPG-LTD in interleaved -oAβ slices (28 ± 6 %, n=8).

Sample fEPSP traces are the mean of 4 consecutive responses at the indicated time points. The number of slices (n) used for each experimental condition is shown. Scale bar: 10 ms / 0.2 mV. Source data are provided as a Source Data file.



Supplemental Figure 1: oAβs impair LTP at CA1 synapses.

(**A-B**) Delivery of a theta-burst protocol (TBS; see Methods), at the time indicated by the arrow, induced LTP in -oAβ slices (51 ± 7 %, n = 28). LTP was impaired in the interleaved +oAβ slices (17 ± 3 %, n = 28). Representative fEPSP traces for both conditions are shown to the right of the time course plot (superimposed for sample experiment, before and after TBS, at times indicated by the numbers 1-4). **B** Bar graph shows the level of LTP in each experiment. Two-tailed t-test was used in **B**. p = 0.00002. Scale bar: 10 ms / 0.2 mV. Source data are provided as a Source Data file.

(**C-E**) Basal synaptic transmission is similar in +oAβ slices and -oAβ slices, as assessed by input-output curves. **C** Fiber volley (FV) amplitude *versus* stimulus intensity (line slopes: +oAβ = -0.026 ± 0.003, n = 13; -oAβ = -0.030 ± 0.003, n = 13). **D** fEPSP slope *versus* FV amplitude (line slopes: +oAβ = 2.517 ± 0.237, n = 13; black, *versus* -oAβ = 2.513 ± 0.362, n = 13; white). **E** Representative fEPSP traces at different stimulus intensity (multiples of threshold) for both conditions.