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Co-activation of selective nicotinic acetylcholine receptors is required to reverse beta amyloid–induced Ca^{2+} hyperexcitation

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ABSTRACT

Beta-amyloid (Aβ) peptide accumulation has long been implicated in the pathogenesis of Alzheimer's disease (AD). Hippocampal network hyperexcitability in the early stages of the disease leads to increased epileptiform activity and eventually cognitive decline. We found that acute application of 250 nM soluble Aβ42 oligomers increased Ca^{2+} activity in hippocampal neurons in parallel with a significant decrease in activity in Aβ42-treated interneurons. A potential target of Aβ42 is the nicotinic acetylcholine receptor (nAChR). Three major subtypes of nAChRs (α7, α4β2, and α3β4) have been reported in the human hippocampus. Simultaneous inhibition of both α7 and α4β2 nAChRs mimicked the Aβ42 effects on both excitatory and inhibitory neurons. However, inhibition of all 3 subtypes showed the opposite effect. Importantly, simultaneous activation of α7 and α4β2 nAChRs was required to reverse Aβ42-induced neuronal hyperexcitation. We suggest co-activation of α7 and α4β2 nAChRs is required to reverse Aβ42-induced Ca^{2+} hyperexcitation.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deficits in learning and memory (Selkoe, 2011). The hippocampus plays crucial roles in learning and memory and is one of the first brain regions to display AD pathological hallmarks including beta-amyloid (Aβ) peptide–containing senile plaques (Querfurth and LaFerla, 2010). Despite considerable progress in deciphering the molecular pathology underlying neurodegeneration in AD over the last 3 decades, current understanding of the physiological basis of memory loss in AD remains limited (Holtzman et al., 2012).

Neuronal hyperexcitability and abnormal hippocampal network rewiring is strongly implicated in the early stages of AD pathogenesis (Kazim et al., 2017; Noebels, 2011; Palop et al., 2007; Palop and Mucke, 2009). As it follows, patients with AD exhibit an increased risk for developing seizures and epilepsy (Friedman et al., 2012; Palop and Mucke, 2009). Notably, Aβ leads to both neuronal and Ca^{2+} hyperexcitation in cortical and hippocampal neurons (Brown et al., 2011; Busche et al., 2008, 2012; Harris et al., 2010; Hartley et al., 1999; Kuchibhotla et al., 2008; Minkeviciene et al., 2009; Palop et al., 2007; Palop and Mucke, 2009; Roberson et al., 2011; Verret et al., 2012). Although picomolar levels of Aβ can strengthen glutamatergic synapses (Puzzo et al., 2008), a large body of studies have shown that nanomolar levels of Aβ induce synaptic depression and impair plasticity in hippocampal excitatory synapses (Hsieh et al., 2006; Kamenetz et al., 2003; Querfurth and LaFerla, 2010; Walsh et al., 2002), which is inconsistent with the ability of Aβ to induce hyperexcitability. Interestingly, it has been suggested that Aβ-induced dysfunction of inhibitory interneurons contributes to hyperexcitation in hippocampal networks and cognitive decline in the AD mouse model (Busche et al., 2008; Verret et al., 2012). However, the mechanism of how Aβ disrupts interneuron function and induces hyperexcitation in hippocampal pyramidal cells is not fully understood.

Another prominent AD pathology is the loss of cholinergic neurons and nicotinic acetylcholine receptors (nAChRs) throughout the brain (Kadir et al., 2006; Nordberg, 2001). Activation of neuronal nAChRs modulates neurotransmission by altering both inhibitory interneurons and pyramidal excitatory neurons in the hippocampus, thus affecting neuronal circuits at multiple levels (Dani and Bertrand, 2007; Jurgensen and Ferreira, 2010). Notably, cholinergic signaling in GABAergic inhibitory networks is generally

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more impactful than direct effects on glutamatergic neurons because nAChRs are more densely expressed in inhibitory interneurons than excitatory cells in the hippocampus (Ji et al., 2001; Son and Winzer-Serhan, 2008). Therefore, cholinergic modulation of hippocampal synaptic activity is mainly mediated by activation of inhibitory interneurons, which act to reduce the net output of pyramidal neurons and depress plasticity mechanisms in glutamatergic cells. Accordingly, AD-associated loss of nAChRs may impair GABAergic neuron function, leading to disruption of inhibitory and excitatory balance at a circuit level, which ultimately contributes to hippocampal hyperexcitation.

Molecular interactions between Aβ and nAChRs affect receptor function in the early stages of AD (Arora et al., 2013; Auld et al., 1998; Lambert et al., 1998; Liu et al., 2009; McLean et al., 1999). Although nearly 30 subtypes of neuronal nAChRs have been reported, the 3 major nAChR subtypes in the hippocampus are composed of α7, α4β2, and α3β4 subunits (Albuquerque et al., 2009; Clementi et al., 2000; Lindstrom, 2003). In particular, the interaction between α7 nAChRs and Aβ has been extensively demonstrated. α7 subunits colocalize with Aβ in senile plaques of human brain slices as well as in cultured cells (Hahm et al., 2018; Wang et al., 2000a). Reciprocal immunoprecipitation experiments confirm Aβ physically binds to α7 nAChRs (Wang et al., 2000a,b). A recent study using a quantitative time-resolved fluorescence resonance energy transfer (TR-FRET)—based binding assay also confirms the specific binding of Aβ to α7 nAChR (Cecon et al., 2019). In addition, β_2-containing nAChRs interact with Aβ42 in several heterologous expression systems (Lamb et al., 2005; Liu et al., 2009, 2012; Wu et al., 2004). Nonetheless, the role of Aβ in the pathophysiology of AD is not yet precisely understood. In particular, the interaction between Aβ and nAChRs is still controversial. In fact, contradicting studies show Aβ produces either a functional inhibitory effect, receptor activation, or no effect (Liu et al., 2001a; Lombardo and Maskos, 2015; Pettit et al., 2001; Pym et al., 2005). Therefore, how Aβ specifically interacts with each subtype of receptor to produce an overall impact on neuronal function, particularly in GABAergic inhibitory networks, is still unknown.

Here, we show soluble Aβ42 oligomers selectively affect both α7 and α4β2 nAChRs but not α3β4 nAChRs in hippocampal interneurons, resulting in hyperexcitation in pyramidal neurons. We further reveal co-activation of α7 and α4β2 nAChRs is required to abolish Aβ42-induced hyperexcitation. For this reason, understanding the interaction of Aβ42 with inhibitory neurons through selective nAChR inhibition may yield potential therapeutic targets for AD.

2. Materials and methods

2.1. Mouse hippocampal neuron culture

Mouse hippocampal neuron cultures were prepared as described previously (Kim et al., 2015a,b; Kim and Ziff, 2014; Sztukowski et al., 2018). Hippocampi were isolated from postnatal day 0 (P0) C57Bl6J mouse (#000664; Jackson laboratory, Bar Harbor, ME) brain tissues and digested with 10 U/mL papain (Worthington Biochemical Corp., Lakewood, NJ, USA). For Ca^{2+} imaging and in vivo calcineurin activity assay, mouse hippocampal neurons were plated on polylysine-coated glass bottom dishes (500,000 cells) and imaged on day in vitro (DIV). For immunocytochemistry, neurons were plated on 12-mm polylysine-coated cover slips (200,000 cells) and fixed on DIV 14. For biotinylation assays, neurons were plated in 6-well dishes (500,000 cells) and biotinylated on DIV 14. Cells were grown in Neurobasal Medium (Life Technologies, Carlsbad, CA, USA) with B27 supplement (Life Technologies, Carlsbad, CA, USA), 0.5 mM GlutaMAX (Life Technologies), and 1% penicillin/streptomycin (Life Technologies). Colorado State University's Institutional Animal Care and Use Committee reviewed and approved the animal care and protocol (16—6779A).

2.2. Reagents

Soluble Aβ42 oligomers were prepared as previously described (Stine et al., 2003). 1 mg of lyophilized human Aβ42 (AnaSpec, Fremont, CA, USA) was dissolved in 1 mL of 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma, St. Louis, MO, USA) to prevent aggregation, portioned into 10 μg aliquots, air-dried, and stored at −80 °C. For use in experiments, an aliquot was thawed at room temperature and then dissolved in dimethyl sulfoxide and phosphate-buffered saline (PBS) to make a 100 μM solution. The solution was incubated for 16 hours at 4 °C and then diluted to a final concentration for use in experiments. The following antagonists were used in this study: 50 nM α-bungarotoxin (αBTx) (Alomone labs, Jerusalem, Israel), 1 μM dihydro-β-erythroidine hydrobromide (DHβE) (Tocris Bioscience, Bristol, UK), and 3 μM α-conotoxin AuIB (Alomone labs). The following agonists were used in this study: 25 nM muscimol (MUS; MP Biomedicals, Santa Ana, CA, USA), 1 μM PNU-120596 (Alomone labs), 2 μM RJR-2403 oxalate (Alomone labs), and 1 μM carbamoylcholine chloride (carbachol) (Tocris Bioscience).

2.3. GCaMP Ca^{2+} imaging

GCaMP Ca^{2+} imaging was carried out by the previously reported method (Sztukowski et al., 2018). DIV 4 neurons were transfected with pCMV-GCaMP5 (a gift from Douglas Kim and Loren Looger, Addgene plasmid # 31788; http://n2t.net/addgene:31788; RRID:Addgene_31788) (Akerboom et al., 2012) or pGP-CMV-GCaMP6f (a gift from Douglas Kim, Addgene plasmid # 40755; http://n2t.net/addgene:40755; RRID:Addgene_40755) (Chen et al., 2013) for imaging hippocampal pyramidal cells or pAAV-mDlx-GCaMP6f-Fishell-2 (a gift from Gordon Fishell, Addgene plasmid # 83899; http://n2t.net/addgene:83899; RRID:Addgene_83899) (Dimidschstein et al., 2016) for imaging interneurons by using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. For Ca^{2+} imaging selectively in hippocampal excitatory neurons, cells were prepared from P0 transgenic mice expressing Cre recombinase under the control of the excitatory neuron-specific alpha calcium/calmodulin-dependent protein kinase II (CaMKIIα) promoter (#005359; Jackson Laboratory), and GCaMP6f was expressed selectively in CaMKIIα-positive neurons by infection with adeno-associated virus (AAV1.CAG.Flex.GCaMP6f.WPRE.SV40; Penn Vector Core, Philadelphia, PA, USA). Neurons were imaged DIV 12—14. The transfection efficiency was around 2%, and no obvious cellular toxicity has been observed. Neurons were grown in Neurobasal Medium without phenol red (Life Technologies) and with B27 supplement (Life Technologies), 0.5 mM GlutaMAX (Life Technologies), and 1% penicillin/streptomycin (Life Technologies) for 8—10 days after transfection and during the imaging. Glass bottom dishes were mounted on a temperature-controlled stage on an Olympus IX73 microscope and maintained at 37 °C and 5% CO_2 using a Tokai-Hit heating stage and digital temperature and humidity controller. For GCaMP5, the images were captured with a 50-ms exposure time using a 60x oil immersion objective (NA = 1.42). A total of 100 images were obtained with a 1-second interval, and Ca^{2+} activity in the cell body (excluding dendrites) was analyzed using the Olympus cellSens software. For mDlx-GCaMP6f and AAV1-GCaMP6f, the images were captured with a 10-ms exposure time and a total of 100 images were obtained with a 500-ms interval. F_{min} was determined as the minimum fluorescence value during the imaging. Total Ca^{2+} activity was obtained by 100 values of $\Delta F/F_{min} = (F_t - F_{min})/F_{min}$ in each image,

and values of $\Delta F/F_{min} < 0.1$ were rejected due to bleaching. 10 to 20 neurons were used for imaging in one individual experiment, and one individual neuron was assayed in an image.

2.4. FRET-based in vivo calcineurin activity assay

In vivo calcineurin activity was determined by an FRET-based calcineurin activity sensor as shown previously (Kim and Ziff, 2014; Newman and Zhang, 2008). Neurons were transfected with the calcineurin activity biosensor, and FRET activity was measured at DIV 14 according to a modification of the previously described method. Neurons were pre-treated with 250 nM soluble Aβ42 oligomers or scrambled Aβ42 for 1 hour. Images were captured by using an Olympus IX73 microscope. The following formula was used to calculate emission ratio.

$$\text{Yellow} - \text{to} - \text{cyan emission ratio}(\text{FRET channel intensity} / \text{CFP channel intensity}) = \frac{\text{FRET channel emission intensity} - \text{FRET channel emission intensity of background}}{\text{CFP channel emission intensity} - \text{CFP channel emission intensity of background}}$$

Pseudocolor images of the emission ratio were generated by the Olympus cellSens software.

2.5. Immunocytochemistry

Immunocytochemistry was carried out by a modification of the previously reported method (Hahm et al., 2018). Cultured hippocampal neurons were fixed in 4% formaldehyde in PBS for 10 minutes, blocked in 1% bovine serum albumin, and 0.1% sapponin for 30 minutes, and then incubated overnight with an anti-choline acetyltransferase (ChAT) antibody (1:250; MilliporeSigma, Burlington, MA, USA) to identify cholinergic neurons. After 4 washes with 0.1% triton-X in PBS for 5 minutes each, cells were incubated with Alexa-Fluor-594 conjugated secondary antibody (Life Technologies 1:1000) for 1 hour, washed, and mounted in p-phenylenediamine in 90% glycerol. Neurons were imaged with a 20x objective using an Olympus BX51 microscope.

2.6. Surface biotinylation and immunoblots

Surface biotinylation was performed according to the previous studies (Kim et al., 2015a,b; Kim and Ziff, 2014; Sztukowski et al., 2018). Equal amounts of protein were loaded on 10% SDS-PAGE gel and transferred to nitrocellulose membranes. Membranes were blotted with anti-NR1 (1:1000; Millipore), anti-GluA1 (1:2000; Millipore), anti-GluA2 (1:2000; Abcam, Cambridge, UK), anti-phosphorylated GluA1 S845 (1:1000, Millipore), and anti-actin (1:2000, Abcam) antibodies and developed with enhanced chemiluminescence (Thermo Fisher Scientific, Waltham, MA, USA). Immunoblots were at least duplicated for quantitative analysis.

2.7. Statistics

Statistical comparisons were analyzed with the GraphPad Prism 7 software. Unpaired two-tailed Student t-tests were used in single comparisons. For multiple comparisons, a one-way analysis of variance followed by the Tukey test was used to determine statistical significance. Results are represented as mean ± SEM, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Soluble Aβ42 oligomer–induced Ca^{2+} hyperexcitation in cultured hippocampal neurons

There are contrasting findings concerning the effect of Aβ on neuronal excitability and synaptic function (Morris et al., 2014). This is partially due to the fact that Aβ exists in multiple forms from monomers to oligomers to fibrils and varies in conformation (Jarosz-Griffiths et al., 2016; Rushworth and Hooper, 2010; Zott et al., 2019). Among these conformations, soluble Aβ42 oligomers appear to be the most neurotoxic species, triggering various processes that underlie AD pathogenesis including synaptic dysfunction and Ca^{2+} deregulation (Ferreira and Klein, 2011; Walsh and Selkoe, 2007). We thus prepared soluble Aβ42 oligomers as described previously (Stine et al., 2003) to examine the action of Aβ on neuronal activity. As neuronal Ca^{2+} is the second messenger responsible for transmitting depolarization status and synaptic activity (Gleichmann and Mattson, 2011), we measured Ca^{2+} activity in cultured DIV 12–14 mouse hippocampal neurons transfected with GCaMP5 as described previously (Sztukowski et al., 2018). We acutely treated neurons with 100 nM, 250 nM, and 500 nM soluble Aβ42 oligomers and determined Ca^{2+} activity in hippocampal neurons immediately after Aβ42 treatment. We found active spontaneous Ca^{2+} transients in both scrambled Aβ42 (sAβ42) and soluble Aβ42 oligomer (oAβ42)–treated neurons (Fig. 1A). However, total Ca^{2+} activity in 250 nM and 500 nM oAβ42-treated cells was significantly higher than that in sAβ42-treated controls (250 nM sAβ42, 1.00 ± 0.04 $\Delta F/F_{min}$, and 250 nM oAβ42, 1.70 ± 0.13 $\Delta F/F_{min}$, $p = 0.0007$; 500 nM sAβ42, 1.00 ± 0.13 $\Delta F/F_{min}$, and 500 nM oAβ42, 3.08 ± 0.35 $\Delta F/F_{min}$, $p < 0.0001$), confirming that soluble Aβ42 oligomers at these concentrations were sufficient to increase neuronal Ca^{2+} activity, while 100 nM oAβ42 treatment slightly elevated Ca^{2+} activity but was not significantly different from control cells (100 nM sAβ42, 1.00 ± 0.10 $\Delta F/F_{min}$, and 100 nM oAβ42, 1.23 ± 0.10 $\Delta F/F_{min}$, $p = 0.112$) (Fig. 1A). We thus decided to use 250 nM soluble Aβ42 oligomers for further experiments. We also confirmed that both the average frequency (sAβ42, 24.93 ± 1.07 events, and oAβ42, 39.85 ± 1.55 events, $p < 0.0001$) and amplitude (sAβ42, 0.97 ± 0.02 $\Delta F/F_{min}$, and oAβ42, 1.52 ± 0.062 $\Delta F/F_{min}$, $p < 0.0001$) were significantly elevated in 250 nM oAβ42-treated neurons (Fig. 1B). Taken together, 250 nM soluble Aβ42 oligomers were sufficient to induce Ca^{2+} hyperexcitation in cultured hippocampal neurons.

3.2. Soluble Aβ42 oligomer reduces surface AMPA receptor expression

As soluble Aβ42 oligomers were sufficient to induce Ca^{2+} hyperexcitation (Fig. 1), it was possible that Aβ42 directly activated excitatory synapses. To examine the direct effect of soluble Aβ42 oligomers on excitatory synapses, we measured surface expression of synaptic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors (AMPARs and NMDARs), by biotinylation

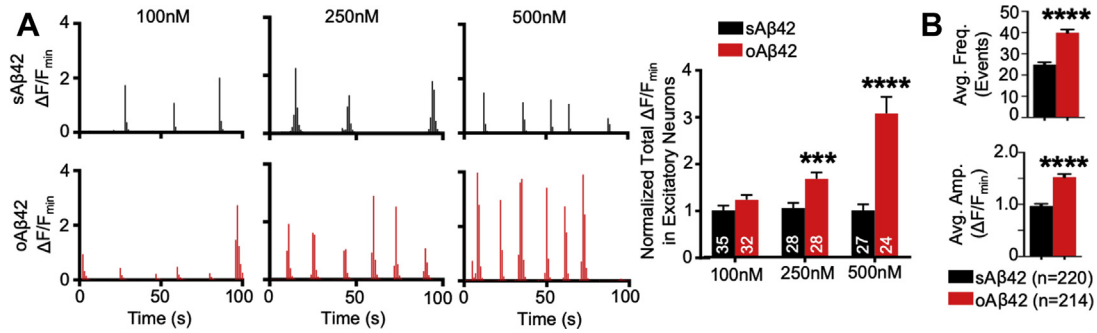

Fig. 1. Soluble Aβ42 oligomers induce hyperexcitability in hippocampal cells. (A) Representative traces of GCaMP5 fluorescence intensity in hippocampal cells and a summary graph of the normalized average of total Ca^{2+} activity in neurons treated with either sAβ42 (black) or oAβ42 (red) at concentrations of 100 nM, 250 nM, and 500 nM showing oAβ42 significantly increases Ca^{2+} activity at concentrations of 250 nM and 500 nM (n = number of neurons, $^{***}p < 0.001$ and $^{****}p < 0.0001$, two-tailed student's t-test). (B) Average frequency and amplitude of Ca^{2+} activity in sAβ42- or oAβ42-treated neurons showing 250 nM oAβ42 significantly elevates both frequency and amplitude of Ca^{2+} activity in cultured hippocampal neurons (n = number of neurons, $^{****}p < 0.0001$, two-tailed Student's t-test). Abbreviations: oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

after 250 nM oAβ42 was applied for one hour. Consistent with the previous findings in which Aβ induces synaptic depression at hippocampal excitatory synapses (Hsieh et al., 2006; Kamenetz et al., 2003; Querfurth and LaFerla, 2010; Walsh et al., 2002), 250 nM oAβ42 treatment was sufficient to reduce surface expression of AMPAR subunits GluA1 (sAβ42, 1.00, and oAβ42, 0.64 ± 0.08, $p = 0.003$) and GluA2 (sAβ42, 1.00, and oAβ42, 0.74 ± 0.08, $p = 0.0146$), an indication of decreased activity at glutamatergic synapses, yet oAβ42 treatment had no effect on NMDAR subunit NR1 surface expression (sAβ42, 1.00, and oAβ42, 0.76 ± 0.16, $p = 0.182$) (Supplementary Fig. 1A). To further investigate how oAβ42 treatment reduced surface AMPAR levels, we measured phosphorylation of GluA1 at serine 845 (pGluA1), which is important for activity-dependent trafficking of GluA1-containing AMPARs (Kim and Ziff, 2014). Consistent with our biotinylation data (Supplementary Fig. 1A), 1 hour of 250 nM oAβ42 treatment was sufficient to reduce pGluA1 (sAβ42, 1.00, and oAβ42, 0.54 ± 0.12, $p = 0.0013$) (Supplementary Fig. 1B). Phosphorylation of GluA1 can be regulated by kinase and phosphatase activity (Diering and Huganir, 2018). In particular, Ca^{2+}/calmodulin-dependent protein phosphatase, calcineurin, dephosphorylates pGluA1, which enables GluA1-containing AMPARs to be endocytosed from the plasma membrane during long-term depression (Lee et al., 1998; Sanderson et al., 2012). Given that oAβ42 elevated Ca^{2+} activity, we hypothesized that Aβ-induced Ca^{2+} hyperexcitation enhanced calcineurin activity, resulting in reduction of pGluA1 and surface expression of AMPARs. To measure in vivo calcineurin activity directly, we employed an FRET-based calcineurin activity sensor as shown previously (Kim et al., 2015a,b; Kim and Ziff, 2014). As expected, oAβ42 treatment for 1 hour was sufficient to increase FRET activity (assayed by measuring the emission ratio) as compared with sAβ42 treatment (sAβ42, 1.0 ± 0.03, and oAβ42, 1.12 ± 0.04, $p = 0.012$) (Supplementary Fig. 1C). This suggests soluble Aβ42 oligomers increase calcineurin activity, which leads a decrease in pGluA1, ultimately resulting in a reduction of surface AMPAR expression. Taken together, Aβ42-induced Ca^{2+} hyperexcitation is not solely caused by enhanced excitatory activity in cultured hippocampal neurons.

3.3. Soluble Aβ42 oligomer–induced Ca^{2+} hyperexcitation is dependent on GABAergic input

Because it has been suggested that disruption of inhibitory inputs onto hippocampal pyramidal cells contributes to network hyperexcitation and consequent cognitive decline in the AD mouse model (Busche et al., 2008; Verret et al., 2012), we acutely treated neurons with 250 nM oAβ42 together with a $GABA_A$ receptor ($GABA_AR$) agonist, MUS, to examine whether Aβ42-induced Ca^{2+} hyperexcitation is mediated by GABAergic disinhibition (Fig. 2A). To avoid affecting basal Ca^{2+} activity, we used 25 nM MUS, a concentration that had no effect on Ca^{2+} activity in control neurons

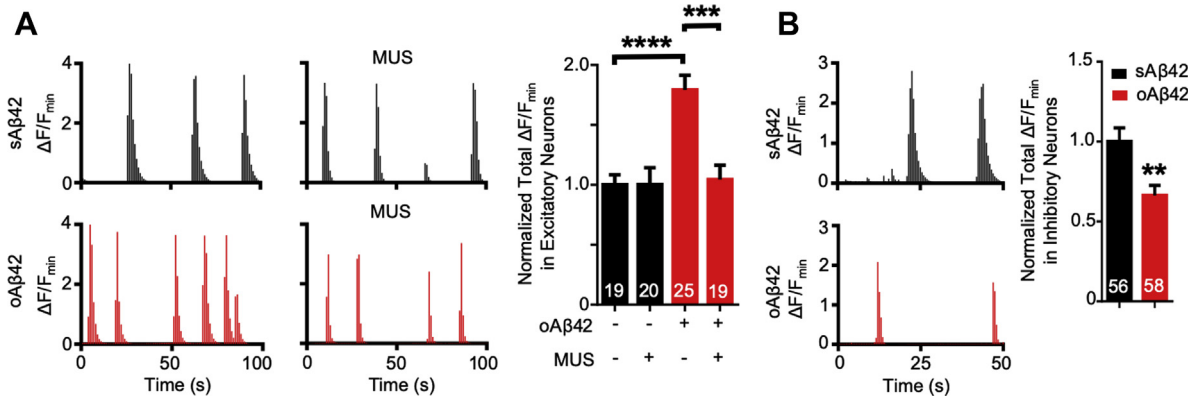

Fig. 2. Soluble Aβ42 oligomer–induced hyperexcitability is mediated by GABAergic disinhibition. (A) Representative traces of GCaMP5 fluorescence intensity in hippocampal neurons and a summary graph of normalized average of total Ca^{2+} activity in each condition showing that a $GABA_AR$ agonist, 25 nM MUS, abolishes oAβ42-induced hyperexcitability (n = number of neurons, $^{****}p < 0.0001$, $^{***}p < 0.001$, one-way ANOVA, Tukey Test). (B) Representative traces of GCaMP6f fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in hippocampal interneurons treated with 250 nM sAβ42 (black) or 250 nM oAβ42 (red) showing that oAβ42 treatment significantly reduces neuronal Ca^{2+} activity (n = number of neurons, $^{**}p < 0.01$, two-tailed Student's t-test). Abbreviations: MUS, muscimol; oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(sAβ42, 1.00 ± 0.08 $\Delta F/F_{min}$, and sAβ42 + MUS, 1.00 ± 0.14 $\Delta F/F_{min}$, $p > 0.999$) (Fig. 3A), whereas 5 μM MUS treatment was sufficient to reduce activity (sAβ42, 1.0 ± 0.14 $\Delta F/F_{min}$, and sAβ42 + MUS, 0.18 ± 0.10 $\Delta F/F_{min}$, $p < 0.0001$) (Supplementary Fig. 2). We also confirmed that 250 nM oAβ42 significantly elevated Ca^{2+} activity (sAβ42, 1.00 ± 0.08 $\Delta F/F_{min}$, and oAβ42, 1.79 ± 0.12 $\Delta F/F_{min}$, $p < 0.0001$) (Fig. 2A). Significantly, 25 nM MUS was sufficient to abolish the oAβ42 effects on Ca^{2+} activity in hippocampal neurons (oAβ42, 1.79 ± 0.12 $\Delta F/F_{min}$, and oAβ42 + MUS, 1.04 ± 0.12 $\Delta F/F_{min}$, $p = 0.0001$) (Fig. 2A). This suggests the oAβ42 effects on Ca^{2+} activity are mediated by inhibitory inputs. We thus examined whether soluble Aβ42 oligomers affect Ca^{2+} activity in hippocampal interneurons by expressing GCaMP6f under the control of the GABAergic neuron-specific enhancer of the mouse *Dlx* gene (Dimidschstein et al., 2016). In contrast to excitatory neurons, 250 nM oAβ42 treatment significantly reduced Ca^{2+} activity in interneurons (sAβ42, 1.00 ± 0.09 $\Delta F/F_{min}$, and oAβ42, 0.66 ± 0.06 $\Delta F/F_{min}$, $p = 0.0018$) (Fig. 2B). To confirm whether oAβ42 induced Ca^{2+} hyperexcitation selectively in hippocampal excitatory neurons, GCaMP6f was expressed under the control of the excitatory neuron-specific CaMKIIα promoter (Jones et al., 1994; Ochiishi et al., 1994). Indeed, we found 250 nM oAβ42 treatment was sufficient to elevate Ca^{2+} activity in CaMKIIα-positive neurons (sAβ42, 1.00 ± 0.10 $\Delta F/F_{min}$, and oAβ42, 1.31 ± 0.10 $\Delta F/F_{min}$, $p = 0.0347$) (Supplementary Fig. 3), as seen in Fig. 1A. This is consistent with the fact that the majority of cells in hippocampal cultures are excitatory neurons (Benson and Cohen, 1996). In addition, pharmacological activation of $GABA_A$Rs was able to block Aβ42-induced Ca^{2+} hyperexcitation in CaMKIIα-positive excitatory neurons (oAβ42, 1.31 ± 0.10 $\Delta F/F_{min}$, and oAβ42 + MUS, 0.73 ± 0.11 $\Delta F/F_{min}$, $p = 0.0005$) (Supplementary Fig. 3). Taken together, this suggests that soluble Aβ42 oligomers reduce inhibitory inputs to hippocampal excitatory neurons, indicating that network dysfunction can lead to hyperexcitability.

3.4. Selective inhibition of nAChRs mimics the oAβ42 effects on Ca^{2+} activity

Molecular interactions between Aβ and nAChRs play important roles in AD pathogenesis (Arora et al., 2013; Auld et al., 1998; Lambert et al., 1998; Liu et al., 2009; McLean et al., 1999). It has previously reported that about 10% of neurons in primary hippocampal cultures exhibit acetylcholine (ACh) currents after rapid application of ACh, and nearly all of the responsive cells are GABAergic interneurons (Liu et al., 2001b). Furthermore, there is a study showing the existence of intrinsic cholinergic interneurons in the hippocampus (Yi et al., 2015). Consistently, we also identified that cholinergic neurons were present in our culture system by immunocytochemistry using an anti-choline acetyltransferase (ChAT) antibody (Supplementary Fig. 4). Because there is conflicting evidence concerning whether Aβ42 works in an nAChR

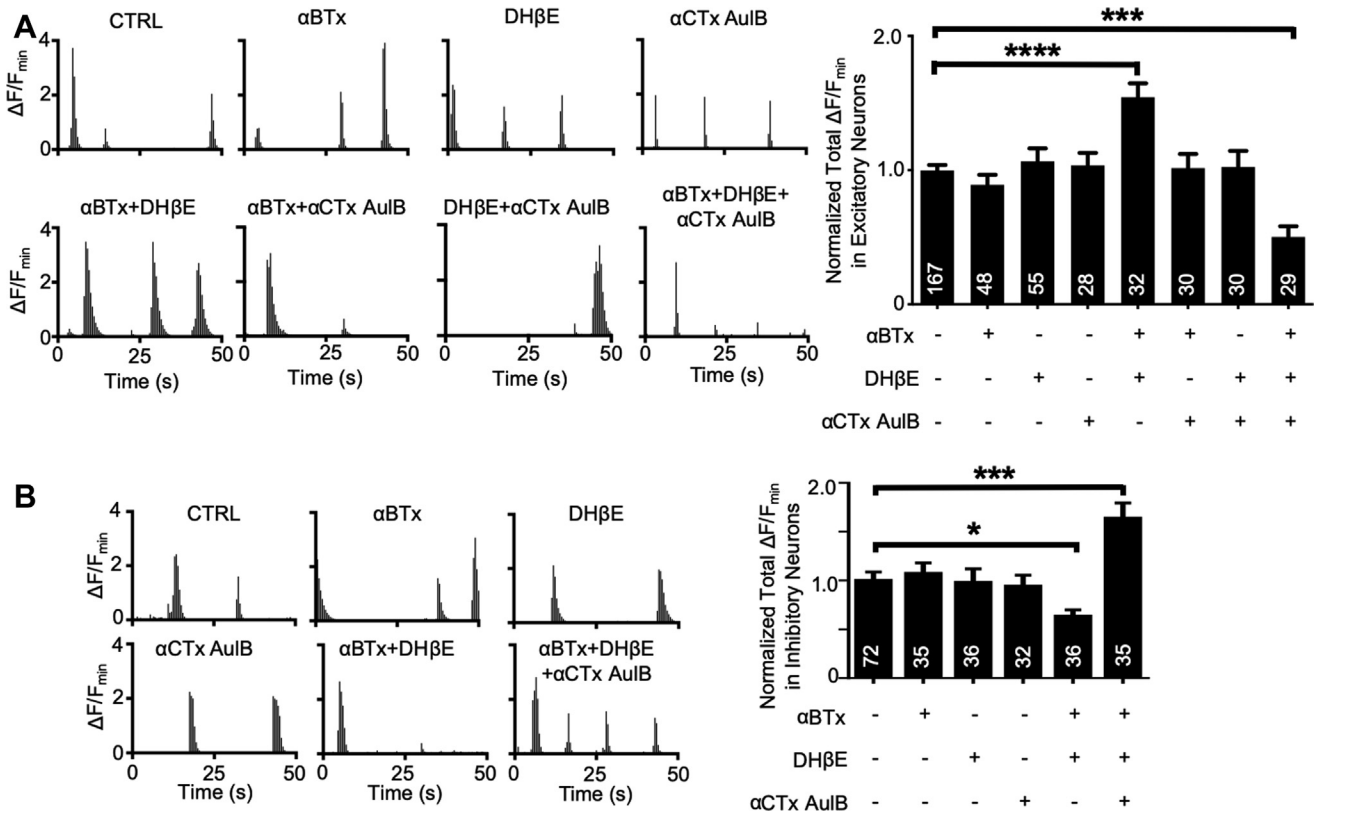

Fig. 3. Selective inhibition of α7 and α4β2 nAChRs mimics Aβ42-induced hyperexcitability in hippocampal pyramidal and inhibitory neurons. (A) Representative traces of GCaMP6f fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in each condition showing application of 50 nM αBTX, an α7 nAChR antagonist, and 1 μM DHβE, an α4β2 nAChR antagonist, together significantly increases neuronal Ca^{2+} activity, similar to oAβ42 treatment. Opposingly, treatment of all 3 antagonists together, 50 nM αBTX, 1 μM DHβE, and 3 μM αCTX AuIB, an α3β4 antagonist, significantly decreases Ca^{2+} activity. Application of each antagonist individually, 50 nM αBTX and 3 μM αCTX AuIB together, or 1 μM DHβE and 3 μM αCTX AuIB together had no effect on Ca^{2+} activity (n = number of neurons, ***$p < 0.001$, ****$p < 0.0001$, Tukey Test). (B) Representative traces of GCaMP6f fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in each condition showing application of 50 nM αBTX and 1 μM DHβE together significantly decreases Ca^{2+} activity in interneurons, similar to oAβ42 treatment. Opposingly, treatment of all 3 antagonists together, 50 nM αBTX, 1 μM DHβE, and 3 μM αCTX AuIB, significantly increases Ca^{2+} activity in inhibitory cells. Application of each antagonist individually had no effect on Ca^{2+} activity (n = number of neurons, *$p < 0.05$, ***$p < 0.001$, one-way ANOVA, Tukey Test). Abbreviations: αBTX, α-bungarotoxin; DHβE, dihydro-β-erythroidine hydrobromide; nAChR, acetylcholine receptor; oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42.

subtype–specific manner, we treated neurons with nAChR subtype–specific antagonists to identify which major subtypes of nAChRs are involved in the oAβ42 effects (Fig. 3A). Interestingly, acute treatment of 50 nM αBTx, an α7 receptor inhibitor, 1 μM DHβE, an α4β2 receptor inhibitor, or 3 μM α-conotoxin AuIB (αCTx AulB), an α3β4 receptor inhibitor, by themselves had no effect on hippocampal Ca^{2+} activity compared with control neurons (CTRL) (CTRL, 1.00 ± 0.04 $\Delta F/F_{min}$; αBTx, 0.89 ± 0.07 $\Delta F/F_{min}$, $p = 0.931$; DHβE, 1.06 ± 0.09 $\Delta F/F_{min}$, $p = 0.995$, and αCTx AulB, 1.04 ± 0.10 $\Delta F/F_{min}$, $p > 0.999$) (Fig. 3A). Inhibition of α7 and α3β4 nAChRs together or α4β2 and α3β4 nAChRs together also yielded no effect on Ca^{2+} activity (CTRL, 1.00 ± 0.04 $\Delta F/F_{min}$; αBTx + αCTx AulB, 1.02 ± 0.11 $\Delta F/F_{min}$, $p > 0.999$; αBTx + DHβE, 1.02 ± 0.12 $\Delta F/F_{min}$, $p > 0.999$). However, inhibition of both α7 and α4β2 nAChRs together significantly elevated GCaMP5 activity (αBTx + DHβE, 1.54 ± 0.10 $\Delta F/F_{min}$, $p < 0.0001$) (Fig. 3A) similar to oAβ42 treatment (Fig. 1A). Conversely, blocking all 3 nAChR subtypes resulted in significantly reduced hippocampal Ca^{2+} activity, the opposite effect of oAβ42 treatment (αBTx + DhβE + αCTx AulB, 0.50 ± 0.08 $\Delta F/F_{min}$, $p = 0.0003$) (Fig. 4A). This suggests oAβ42 effects may be due to selective inhibition of both α7 and α4β2 nAChR subtypes.

Next, we measured the effects of the nAChR antagonists on Ca^{2+} activity in hippocampal interneurons by using Dlx-GCaMP6f as shown in Fig. 2B. In contrast to excitatory neurons (Fig. 3A), acute treatment of 50 nM αBTx and 1 μM DHβE significantly reduced interneuron-specific GCaMP6f signals (CTRL, 1.00 ± 0.09 $\Delta F/F_{min}$ and αBTx + DHβE, 0.57 ± 0.06 $\Delta F/F_{min}$, $p = 0.041$) (Fig. 3B), mimicking the oAβ42 effects in interneurons (Fig. 3B). Moreover, treating neurons with all 3 antagonists showed significant elevation of interneuron Ca^{2+} activity, the opposite effect of oAβ42 treatment (αBTx + DhβE + αCTx AulB, 1.64 ± 0.16 $\Delta F/F_{min}$, $p = 0.0003$) (Fig. 3B). Notably, each antagonist by themselves had no effect on GCaMP6f activity (αBTx, 1.07 ± 0.11 $\Delta F/F_{min}$, $p = 0.997$; DHβE, 0.98 ± 0.14 $\Delta F/F_{min}$, $p > 0.999$; and αCTx AulB, 0.94 ± 0.11 $\Delta F/F_{min}$, $p = 0.999$) (Fig. 3B). Taken together, selective inhibition of α7 and α4β2 nAChRs induces hyperexcitability and hypoactivity in hippocampal excitatory and inhibitory neurons, respectively, as seen in oAβ42 treatment (Figs. 1A and 2B).

3.5. Combination treatment of selective nAChR agonists is required to abolish oAβ42-induced hyperexcitation via elevation of inhibitory neuronal activity

Because inhibition of α7 and α4β2 nAChRs mimicked the oAβ42-induced effects seen in both hippocampal excitatory and inhibitory neurons (Fig. 3), we hypothesized that concurrent activation of both receptor types would abolish the oAβ42-induced effects. Indeed, when we treated neurons with 250 nM oAβ42 and concurrently activated α7 and α4β2 nAChRs using 1 μM PNU-120596 (PNU) and 2 μM RJR-2403 oxalate (RJR), oAβ42-induced Ca^{2+} hyperexcitation was significantly reduced in pyramidal cells (sAβ42, 1.00 ± 0.06 $\Delta F/F_{min}$; oAβ42, 1.87 ± 0.13 $\Delta F/F_{min}$, $p < 0.0001$; oAβ42 + PNU + RJR, 0.81 ± 0.09 $\Delta F/F_{min}$, $p < 0.0001$) (Fig. 4A). Importantly, activation of either α7 or α4β2 nAChR singularly had no effect on oAβ42-induced hyperexcitation (oAβ42 + PNU, 1.58 ± 0.12 $\Delta F/F_{min}$, $p = 0.786$ and oAβ42 + RJR, 1.72 ± 0.17 $\Delta F/F_{min}$, $p = 0.996$) (Fig. 4A). Next, we examined whether combination treatment of α7 and α4β2 nAChR agonists was capable of rescuing the oAβ42 effects in GABAergic interneurons. Notably, stimulation of both α7 and α4β2 nAChRs was sufficient to rescue oAβ42-induced Ca^{2+} hypoactivity in interneurons (sAβ42, 1.00 ± 0.11 $\Delta F/F_{min}$; oAβ42, 0.56 ± 0.05 $\Delta F/F_{min}$, $p = 0.0436$; oAβ42 + PNU + RJR, 1.36 ± 0.15 $\Delta F/F_{min}$, $p < 0.0001$) (Fig. 4B). This suggests that combinatorial activation of α7 and α4β2 nAChRs is required to rescue the oAβ42 effects in both hippocampal excitatory and inhibitory neurons.

Combined application of α7 and α4β2 nAChR antagonists mimicked oAβ42 effects, whereas simultaneous inhibition of all 3 nAChRs showed the opposite results (Fig. 3). This suggests it is possible that inhibition of α3β4 nAChRs can reverse oAβ42 effects in both excitatory and inhibitory cells. To test this idea, we first treated neurons with 250 nM oAβ42 and 3 μM αCTx AulB and measure GCaMP5 activity (Supplementary Fig. 5A). As seen before, oAβ42 treatment was sufficient to induce Ca^{2+} hyperexcitation (sAβ42, 1.00 ± 0.10 $\Delta F/F_{min}$, and oAβ42, 2.14 ± 0.27 $\Delta F/F_{min}$, $p < 0.0001$) (Supplementary Fig. 5A). However, inhibition of α3β4 nAChRs had no significant effect on oAβ42-induced Ca^{2+} hyperexcitation in excitatory neurons (oAβ42, 2.14 ± 0.27 $\Delta F/F_{min}$ and oAβ42 + αCTx AulB, 1.68 ± 0.25 $\Delta F/F_{min}$, $p = 0.3309$) (Supplementary Fig. 5A). Next, we measured Ca^{2+} activity in inhibitory neurons by using mDlx-GCaMP6f (Supplementary Fig. 5B). As seen before, oAβ42 treatment decreased GCaMP6f activity significantly (sAβ42, 1.00 ± 0.01 $\Delta F/F_{min}$, and oAβ42, 0.55 ± 0.06 $\Delta F/F_{min}$, $p = 0.0012$) (Supplementary Fig. 5B). However, inhibition of α3β4 nAChRs had no significant effect on Ca^{2+} activity in the presence or absence of oAβ42 treatment (sAβ42 + αCTx AulB, 0.91 ± 0.10 $\Delta F/F_{min}$, $p = 0.8817$, and oAβ42 + αCTx AulB, 0.67 ± 0.07 $\Delta F/F_{min}$, $p = 0.7633$) (Supplementary Fig. 5B). This suggests inhibition of α3β4 nAChRs by itself is unable to reverse oAβ42 effects.

Of clinical relevance, currently the U.S. Food and Drug Administration–approved drugs for AD mostly delay the general breakdown of ACh, which potentially stimulates various types of AChRs. To mimic this effect, we used a cholinergic agonist, carbachol. Interestingly, we showed 1 μM carbachol was unable to rescue oAβ42-induced Ca^{2+} hyperexcitation but instead exacerbated the oAβ42 effects in hippocampal neurons (sAβ42, 1.00 ± 0.08 $\Delta F/F_{min}$; oAβ42, 2.17 ± 0.28 $\Delta F/F_{min}$, $p < 0.0001$; oAβ42 + carbachol, 2.93 ± 0.28 $\Delta F/F_{min}$, $p = 0.0462$) (Fig. 5). We further confirmed that in the absence of Aβ, carbachol significantly elevated Ca^{2+} activity (sAβ42, 1.00 ± 0.08 $\Delta F/F_{min}$, and sAβ42 + carbachol, 1.63 ± 0.14 $\Delta F/F_{min}$, $p = 0.04$) (Fig. 5). This suggests nonselective stimulation of AChRs may have unintended consequences that limit agonist impact. Taken together, selective coactivation of α7 and α4β2 nAChRs is required to abolish the oAβ42 effects in both inhibitory and excitatory neurons.

4. Discussion

Although increased epileptiform activity has been shown to occur in the early stages of AD, a cellular mechanism for this aberrant activity has not yet been detailed. We show here a novel cellular mechanism for hippocampal hyperactivity in AD (Fig. 6). Aβ42 induces hypoactivity in hippocampal interneurons and consequent hyperexcitability in pyramidal cells (Figs. 1A and 2B). Furthermore, we show that selective inhibition of α7 and α4β2 nAChRs but not α3β4 receptors in hippocampal interneurons and excitatory neurons mimics oAβ42 effects on neuronal activity (Fig. 3). More importantly, selective coactivation of α7 and α4β2 receptors is required for a rescue of both Aβ42-induced hypoactivity in interneurons and hyperactivity in excitatory cells (Fig. 4). However, we show that general activation of AChRs by carbachol does not lessen pyramidal cell hyperactivity and instead exacerbates hyperactivity further (Fig. 5). Congruently, current AD medications are only mildly effective at treating symptoms and slowing disease progression, possibly due to nonselective stimulation of receptors (Ferreira-Vieira et al., 2016). Therefore, the idea that Aβ selectively affects α7 and α4β2 nAChRs in hippocampal inhibitory interneurons to induce hyperexcitation is a fundamentally new concept and could have impactful implications on drug discovery.

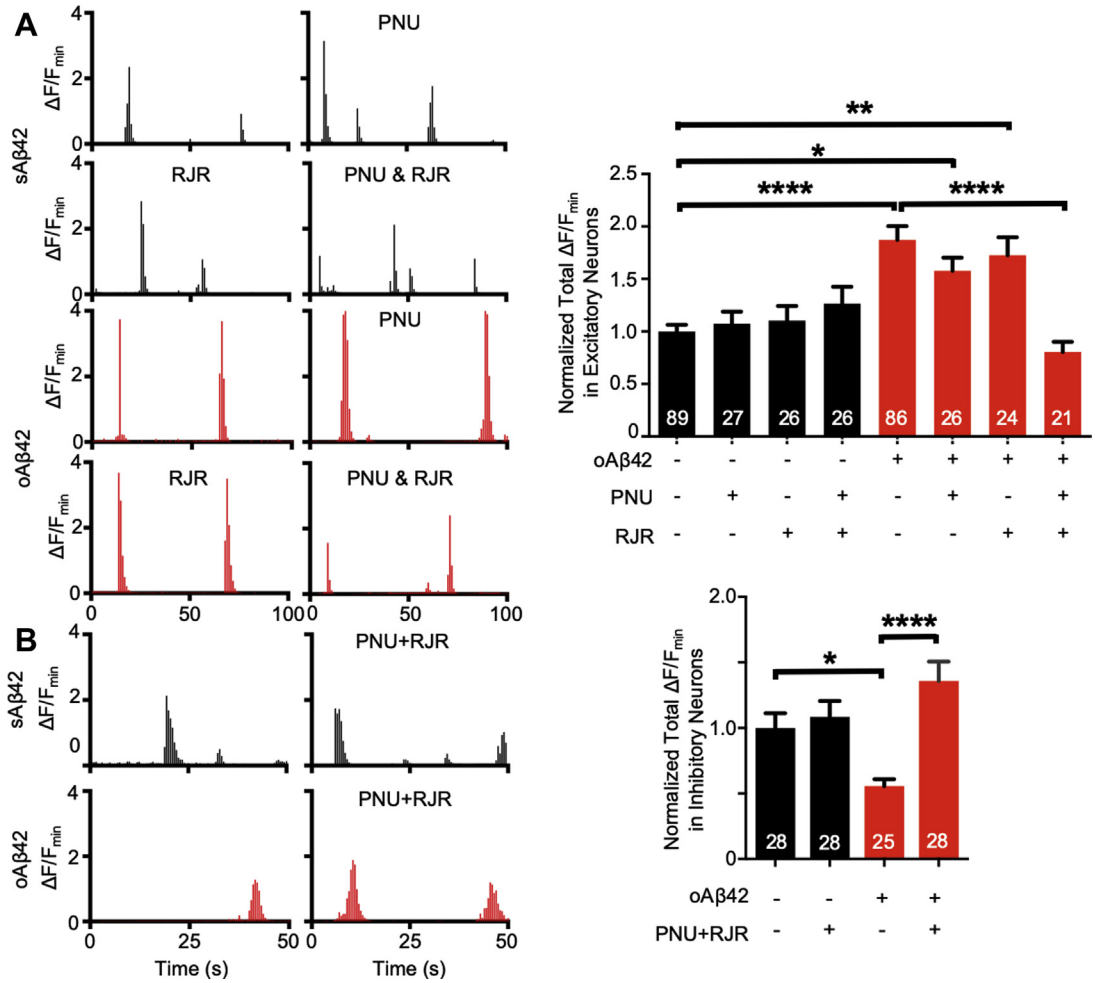

Fig. 4. Combination treatment of α7 and α4β2 nAChRs abolishes oAβ42-induced hyperexcitation in hippocampal pyramidal and inhibitory cells. (A) Representative traces of GCaMP5 fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in each condition showing that treatment of 1 μM PNU-282987 (PNU), an α7 agonist, and 2 μM RJR-2403 oxalate (RJR), an α4β2 agonist, together significantly decreases oAβ42-induced Ca^{2+} hyperactivity. Notably, activation of either α7 or α4β2 singularly does not decrease oAβ42-induced Ca^{2+} hyperactivity (n = number of neurons, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, one-way ANOVA, Tukey Test). (B) Representative traces of GCaMP6f fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in each condition showing that treatment of 1 μM PNU and 2 μM RJR together significantly increases oAβ42-induced Ca^{2+} hypoactivity in interneurons (n = number of neurons, $^{*}p < 0.05$, $^{****}p < 0.0001$, one-way ANOVA, Tukey Test). Abbreviations: αBTX, α-bungarotoxin; DHβE, dihydro-β-erythroidine hydrobromide; nAChR, acetylcholine receptor; oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42.

It can be difficult to reconcile hyperexcitability in the context of reduced surface AMPAR expression in the presence of oAβ42. A large body of studies have confirmed that Aβ oligomers cause synaptic depression at specific excitatory synapses in the hippocampus as evidenced by impaired synaptic plasticity, reduced synaptic transmission, and a decrease in surface AMPAR expression

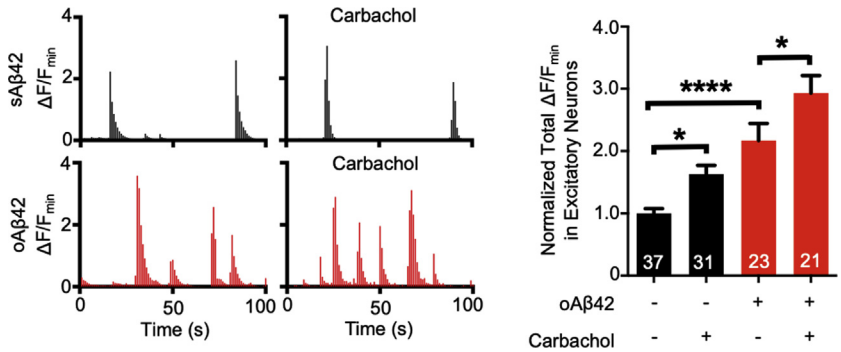

Fig. 5. Carbachol is unable to rescue oAβ42-induced Ca^{2+} hyperactivity. Representative traces of GCaMP5 fluorescence intensity and a summary graph of normalized average of total Ca^{2+} activity in each condition showing that application of a nonselective AChR agonist, 1 μM carbachol, does not rescue oAβ42-induced Ca^{2+} hyperactivity and exacerbates Ca^{2+} activity in the presence or absence of oAβ42 (n = number of neurons, $^{*}p < 0.05$, $^{****}p < 0.0001$, one-way ANOVA, Tukey Test). Abbreviations: AChR, acetylcholine receptor; oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42.

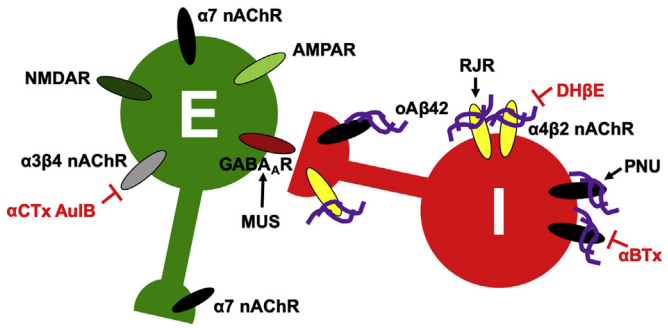

Fig. 6. Soluble Aβ42 oligomer-induced hyperexcitability via selective inhibition of nAChRs in hippocampal interneurons. Excitability of hippocampal excitatory neurons (E) is regulated by a balance between AMPAR- (light green) and NMDAR- (dark green) mediated glutamatergic excitation and GABAergic inhibition via GABA$_A$Rs (red). nAChRs are densely expressed in hippocampal inhibitory interneurons (I) and regulated by cholinergic inputs. oAβ42 (purple) selectively inhibits both α7 (black) and α4β2 (yellow) nAChR subtypes in interneurons but not α3β4 receptors (gray) in excitatory cells to reduce neuronal activity in interneurons, leading to hyperexcitation in hippocampal pyramidal neurons. Therefore, combination treatment of α7 and α4β2 nAChR agonists, PNU and RJR, in the hippocampus can be neuroprotective in AD. Abbreviations: αBTX, α-bungarotoxin; DHβE, dihydro-β-erythroidine hydrobromide; AD, Alzheimer's disease; nAChR, acetylcholine receptor; oAβ42, soluble Aβ42 oligomer; sAβ42, scrambled Aβ42. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Chapman et al., 1999; Hsia et al., 1999; Hsieh et al., 2006; Jurgensen et al., 2011; Kamenetz et al., 2003; Shankar et al., 2007; Whitcomb et al., 2015). However, at the network level, Aβ oligomers cause epileptiform activity and seizures (Minkeviciene et al., 2009; Palop et al., 2007; Sperling et al., 2009). Cellular mechanisms of these contradictory Aβ-dependent effects have not been completely determined (Palop and Mucke, 2010). Importantly, in addition to excitatory synaptic inputs, neural activity is dependent on inhibition, which regulates overall activity level and shapes the temporal pattern of activity (Isaacson and Scanziani, 2011; Kullmann, 2011). In fact, loss of the tuberous sclerosis complex (TSC) 1 in CA1 pyramidal cells of the hippocampus results in disrupted inhibitory synaptic function, which alters the balance of excitatory and inhibitory synaptic transmission, leading to hippocampal hyperexcitability (Bateup et al., 2013). More importantly, such hyperexcitation in the hippocampus persists when surface AMPAR expression is reduced (Bateup et al., 2013), consistent with our findings. Thus, neural activity can be enhanced by repressing inhibitory synapses onto excitatory neurons even if glutamatergic synapses on excitatory cells are depressed. Another hypothesis is that hyperexcitability can be driven by the Aβ-induced suppression of glutamate reuptake. It has been shown that there is reduction of glutamate clearance rates in synapses close to amyloid deposits, and chronic states of elevated glutamate levels are found near amyloid plaques (Hefendehl et al., 2016). Moreover, a recent study using two-photon imaging to measure in vivo Ca^{2+} and glutamate transients shows that soluble human Aβ dimers are sufficient to induce hyperexcitation in the hippocampus (Zott et al., 2019). As glutamate is the major excitatory neurotransmitter, it is possible that an increase in glutamate levels can induce hippocampal hyperexcitation. Indeed, elevation of glutamate uptake alleviates nerve root-mediated pain-induced neuronal hyperexcitability (Nicholson et al., 2014). Another possible explanation of this inconsistency is the different kinetics of oAβ42 effects on neuronal hyperexcitability. One study shows that 300 nM oAβ42 in cultured neurons causes a gain of NMDAR function within seconds after application (De Felice et al., 2007), whereas prolonged (>45 minutes) exposure of high concentration (500 nM-5 μM) oAβ42 in cultured neurons is sufficient to induce GluA1 and NR1 internalization (Guntupalli et al., 2017; Sinnen et al., 2016). Thus, it is possible that Ca^{2+} hyperexcitation can be induced by acute treatment of 250 nM oAβ42. It is also possible that chronic exposure (~1 hour) of 250 nM oAβ42 is sufficient to reduce surface expression of AMPA receptors. Although we started recording Ca^{2+} activity within seconds after oAβ42 treatment, we continued to record more than one hour under the same condition, yet we have not found any significant differences in Ca^{2+} activity throughout the duration of each experiment. This thus rules out the possibility that our findings can be caused by the different kinetics of oAβ42 effects. Taken together, it remains to be determined if Aβ-induced synaptic depression and network abnormality are mechanistically related.

A large body of studies support that Aβ can physically interact with α7, α4, and β2 receptors in various different model systems (Dineley et al., 2015; Jurgensen and Ferreira, 2010; Lombardo and Maskos, 2015; Parri and Dineley, 2010), whereas Aβ is unable to affect α3 and β4 receptor function when heterologously expressed in *Xenopus* oocytes (Pym et al., 2005). This suggests Aβ may not be able to interact with α3β4 receptors. Interestingly, one study shows Aβ40 inhibits α3β4 receptors in human embryonic kidney (HEK) cells (Nery et al., 2013). We used a soluble oligomeric form of Aβ42 rather than Aβ40, which may lend to the different response. In fact, much of literature describes that different forms of Aβ interact with different receptors (Smith and Strittmatter, 2017). Thus, the discrepancy observed in our data and the study by Nery et al. may be due to different forms of Aβ used in the experiments. Although α7 and α4β2 nAChRs are expressed in both pyramidal cells and inhibitory neurons in the hippocampus and are able to modulate hippocampal synaptic plasticity (Jones and Yakel, 1997; Vizi and Lendvai, 1999), α7- and β2-containing nAChRs are mainly expressed in hippocampal GABAergic interneurons, demonstrated by double in situ hybridization with glutamate decarboxylase 67 (GAD67) and nAChRs (Son and Winzer-Serhan, 2008). However, α3 and β4 subunits are rarely detected in GABAergic cells (Son and Winzer-Serhan, 2008). It has also been suggested that low levels of α3β4 receptors are present in hippocampal glutamatergic neurons (Feduccia et al., 2012). This supports the idea that soluble Aβ42 oligomers reduce neuronal activity in inhibitory neurons via selective inhibition of α7 and α4β2 receptors, but not α3β4 nAChRs.

This poses the question: Why do soluble Aβ42 oligomers predominantly affect interneurons but not excitatory cells? GABAergic cells contain both presynaptic α7 receptors and somatodendritic expression of α7 and α4β2 nAChRs (Alkondon and Albuquerque, 2001; Zarei et al., 1999), whereas excitatory neurons have mainly presynaptic α7 nAChRs (Alkondon and Albuquerque, 2002; Gray et al., 1996; Ji et al., 2001). Importantly, somatodendritic α7 nAChRs in rodent hippocampal interneurons are functionally coupled with GABA$_A$Rs and as it follows, stimulation of α7 receptors downregulates GABA currents (Zhang and Berg, 2007). This suggests GABA$_A$R activity can be elevated when α7 nAChRs are inhibited. However, inhibition of α7 nAChRs by itself is not sufficient to reduce neuronal excitability in GABAergic neurons possibly due to the presence of functional α4β2 nAChRs. Accordingly, co-inhibition of α7 and α4β2 receptors is sufficient to decrease neuronal activity in inhibitory cells, leading to hyperexcitation in pyramidal neurons (Fig. 3). In contrast to GABAergic neurons, the direct cholinergic modulation of tonic firing in excitatory neurons is notably mediated by muscarinic AChRs, although α7 nAChRs can contribute to pyramidal cell activity through potential interaction with NMDARs in presynaptic terminals (Bali et al., 2017). This suggests that nAChR-mediated cholinergic activity in the hippocampus may mainly affect excitability in inhibitory interneurons. More importantly, nearly all of ACh responsive neurons in rat hippocampal cultures are GABAergic neurons (Liu et al., 2001b), consistent with previous findings that GABAergic interneurons in

hippocampal slices are extremely sensitive to ACh (Alkondon et al., 1997; Frazier et al., 1998; McQuiston and Madison, 1999), further implicating predominant cholinergic effects on neuronal activity in hippocampal interneurons. Taken together, these previous studies and our current findings led us to the idea that soluble Aβ42 oligomers inhibit α7 and α4β2 receptors primarily in interneurons, contributing to hyperexcitation via disinhibition.

Cholinergic interneurons intrinsic to the hippocampus have been found and may contribute to a hippocampal source of ACh (Liu et al., 2001b; Yi et al., 2015). However, cellular function of intrinsic hippocampal cholinergic neurons has not been fully understood yet. Importantly, our study focuses on the contribution of intrinsic hippocampal cholinergic inputs to disrupted neuronal activity in AD. Nonetheless, the majority of cholinergic inputs to both pyramidal cells and interneurons in the hippocampus originate from the basal forebrain, which have crucial roles in cognition by modulating properties of the hippocampal network (Frotscher et al., 2000; Frotscher and Leranth, 1985). Moreover, many studies indicate the strong correlation between dysfunction of basal forebrain cholinergic neurons and cognitive deficits in patients with AD (Mattson and Pedersen, 1998). Importantly, Aβ has adverse effects on the basal forebrain cholinergic systems in cultured neuron and animal models that could be crucial for understanding AD pathogenesis (Mattson and Pedersen, 1998). Thus, we are still unable to rule out the possibility that Aβ can differentially affect basal forebrain–originated cholinergic function in the hippocampus.

In the presymptomatic stages of AD, neuronal hyperexcitability may play a role in increased network and epileptiform activity leading to eventual cognitive decline, yet current medications may act to increase hyperexcitability. In fact, there are discrepancies involving the use of nicotine treatment to stimulate nAChRs to alter cognitive function. For example, nicotine agonists have been found to improve performance in a variety of memory tasks in rodents and nonhuman primate studies (Levin and Simon, 1998), whereas several other studies have failed to find significant enhancement of learning and memory by nicotine treatment (Vicens et al., 2003). Furthermore, some results show that stimulating one type of nAChR by using a specific agonist enhances cognitive performance, but other studies find no beneficial effect. For instance, selective α7 nAChR agonists have recently been reported to improve cognition in a variety of animal models (Bitner et al., 2007; Cincotta et al., 2008; Redrobe et al., 2009), whereas another study has found almost no beneficial effect on learning and memory in mice (Vicens et al., 2011). Moreover, an α4β2 nAChR agonist alone can improve working memory only in young rats but not older animals (Levin and Christopher, 2002). Notably, we reveal that inhibition of α3β4 receptors has no effect on oAβ42-induced alteration of Ca^{2+} transients in both excitatory and inhibitory cells (Supplementary Fig. 5). This suggests oAβ42 may affect different subtypes of nAChRs other than the α7, α4β2, and α3β4 subtypes and possibly receptors other than nAChRs to alter neuronal activity. In fact, it has been shown that Aβ can interact with α7β2, α2β2, and α4α5β2 nAChRs (Lamb et al., 2005; Liu et al., 2009). Notably, nAChR agonists have consistently suggested promising approaches in the treatment of AD. However, clinical trials thus far have been challenged by adverse effects or minimal improvement (Hoskin et al., 2019). Interestingly, many drugs that have been used in clinical trials target either the α7 receptor or a broad range of nAChRs, including α7, α4β2, α6β2, and α3β4 (Hoskin et al., 2019). Our finding that nonselective stimulation of nAChRs has adverse effects (Fig. 5) may explain why these drugs are failed. Importantly, we have confirmed combination treatment of α7 and α4β2 nAChR agonists is required to restore normal Ca^{2+} activity in Aβ-treated cells (Fig. 4). Thus, combination treatment of selective agonists leads to innovative and novel therapeutic strategies. Nonetheless, further studies are needed to better understand the role of each receptor agonist or combination treatment of agonists in cognitive function to develop more effective pharmacological treatments for AD.

Currently, there are 5 Alzheimer's drugs approved by U.S. Food and Drug Administration that treat the symptoms of AD, temporarily improving memory and cognitive processing concerns. However, these medications do not treat the underlying causes of the disease or slow its progression. Markedly, there are several drugs in development for AD with billions of dollars invested. Despite the massive investment in AD drugs targeting Aβ, there have been exponentially more failures than treatment successes, suggesting that the amyloid hypothesis can no longer be used in AD therapeutics (Huang et al., 2019). However, an argument has been made against whether these trials failed because Aβ actually is the wrong target by countering that the interventions were started too late in the progression of the disease (McDade, 2019). One possible reason is that the pharmacological intervention may be too late for the patients because the pathophysiological process of AD is thought to begin decades before the diagnosis (Mehta et al., 2017). Therefore, there is a need to establish valid early AD biomarkers. Importantly, Aβ-induced hyperexcitability has interesting implications especially for seizure-like activity and epilepsy at the early stage of the disease pathogenesis, which are potentially linked to AD-associated cognitive impairment (Bakker et al., 2012; Minkeviciene et al., 2009; Palop et al., 2007; Palop and Mucke, 2009). In fact, in people at high risk of developing AD, abnormal activation of specific networks during memory processing can be detected decades before the predicted onset of clinical disease (Bateman et al., 2012; Filippini et al., 2009; Mondadori et al., 2006; Reiman et al., 2012). This suggests hyperexcitation may be a preclinical AD biomarker. Thus, our data provide a potential cellular mechanism underlying Aβ-induced hyperexcitability, which may lead to a novel therapeutic target for preclinical AD. Moreover, in the AD mouse model, Aβ can negatively affect GABAergic interneuron function in the hippocampus, which can lead to network hyperexcitability and eventual cognitive decline (Verret et al., 2012). Nonetheless, the mechanism of how Aβ disrupts interneuron function has not been fully understood. The present study provides not only a previously unidentified cellular mechanism underlying Aβ-induced dysfunction in interneurons but also a novel early therapeutic approach of combination treatment of α7 and α4β2 nAChR agonists.

Disclosure

All authors have stated no conflicts of interest exist. All authors have reviewed the contents of the manuscript being submitted, approved of its contents, and validated the accuracy of the data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.09.005

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