Co-activation of selective nicotinic acetylcholine receptor subtypes is required to reverse hippocampal network dysfunction and prevent fear memory loss in Alzheimer's disease

Rahmi Lee, Gayeon Kim, Seonil Kim

Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA

*Corresponding author: Seonil Kim

Email: seonil.kim@colostate.edu

Abstract

Alzheimer's disease (AD) is the most common form of dementia with no known cause and cure. Research suggests that a reduction of GABAergic inhibitory interneurons' activity in the hippocampus by beta-amyloid peptide (Aβ) is a crucial trigger for cognitive impairment in AD via hyperexcitability. Therefore, enhancing hippocampal inhibition is thought to be protective against AD. However, hippocampal inhibitory cells are highly diverse, and these distinct interneuron subtypes differentially regulate hippocampal inhibitory circuits and cognitive processes. Moreover, Aβ unlikely affects all subtypes of inhibitory interneurons in the hippocampus equally. Hence, identifying the affected interneuron subtypes in AD to enhance hippocampal inhibition optimally is conceptually and practically challenging. We have previously found that Aβ selectively binds to two of the three major hippocampal nicotinic acetylcholine receptor (nAChR) subtypes, α7- and α4β2-nAChRs, but not α3β4-nAChRs, and inhibits these two receptors in cultured hippocampal inhibitory interneurons to decrease their activity, leading to hyperexcitation and synaptic dysfunction in excitatory neurons. We have also revealed that co-activation of α7- and α4β2-nAChRs is required to reverse the Aβ-induced adverse effects in hippocampal excitatory neurons. Here, we discover that α7- and α4β2-nAChRs predominantly control the nicotinic cholinergic signaling and neuronal activity in hippocampal parvalbumin-positive (PV+) and somatostatin-positive (SST+) inhibitory interneurons, respectively. Furthermore, we reveal that co-activation of these receptors is necessary to reverse hippocampal network dysfunction and fear memory loss in the amyloid pathology model mice. We thus suggest that co-activation of PV+ and SST+ cells is a novel strategy to reverse hippocampal dysfunction and cognitive decline in AD.

Introduction

About 5.7 million Americans currently have Alzheimer's disease (AD); that figure is projected to reach 14 million people by 2050[1]. In 2020, the direct costs to American society of caring for those with AD and related dementias are a total of an estimated $305 billion, which makes AD the most expensive disease in America[1]. However, there is no known cause and cure for AD[2]. Therefore, understanding the neural mechanisms of AD pathogenesis is urgently needed for better diagnostic and therapeutic development.

Both preclinical and clinical studies have shown that cortical and hippocampal hyperactivity is found in the early stages of AD[3]. Increased neuronal activity enhances the release of both beta-amyloid peptide (Aβ)[4, 5] and tau[6, 7], which contributes to AD pathology. Additionally, in patients, hyperexcitability is strongly linked to cognitive decline[8-10]. Hyperactivity in the brain is thus most likely a driver of AD pathology and cognitive impairment. Hence, it could be a potential target for both symptomatic and disease-modifying therapies. However, there is still much to learn about hyperexcitability in AD, including the neuronal circuits that are affected in AD, as well as their composite cell types, and the mechanistic link between hyperactivity and cognitive impairment.

Considering AD is a multifactorial disease, it is likely that several factors, rather than a single cause, act in conjunction to cause neuronal hyperexcitability[3]. Importantly, increased neuronal excitability is observed in the vicinity of Aβ plaques[11, 12]. Studies have also shown that neuronal hyperactivity can promote Aβ accumulation in mice[4, 5]. Besides, reducing neuronal excitability decreases Aβ deposition in mice[5, 8]. This suggests that Aβ is a major trigger of hyperexcitability, which in turn initiates a destructive feedback loop for the cells. However, the mechanisms of how Aβ participates in hyperexcitability are still not fully understood. Importantly, cholinergic regulation of GABAergic inhibitory network is generally stronger than direct actions on excitatory neurons in the hippocampus since nicotinic acetylcholine receptors (nAChRs) are more densely expressed in inhibitory interneurons than excitatory cells[13-18]. The loss of cholinergic neurons and nAChR expression in the hippocampus is a prominent AD pathology[19-26]. This suggests that cholinergic dysfunction is likely a primary cause for hippocampal inhibition deficits in AD. Although nAChRs are high-affinity targets for Aβ, contradictory results have been reported describing effects of Aβ on nAChRs[27-29]. For example, Aβ appears to bind to nAChRs producing functional inhibitory effects or receptor activation, depending on Aβ concentration, type of preparation (i.e., monomers, soluble oligomers or fibrils), and incubation times[27-29].

Moreover, nearly 30 subtypes of neuronal nAChRs have been reported in the human brain[30-32]. In fact, the U.S. Food and Drug Administration (FDA)-approved current drugs for AD mostly attempt to delay the general breakdown of acetylcholine, which potentially stimulates all types of acetylcholine receptors. Thus, it is not surprising that these non-selective activators suffer from modest efficacy[21, 33, 34]. This suggests, in turn, that distinct nAChR subtypes are differentially affected in AD. Thus, it is challenging to comprehend how Aβ impacts nAChR functions in AD.

The three major nAChR subtypes in the hippocampus are composed of α7, α4β2, and α3β4 subunit combinations[30-32]. Importantly, our previous work using Ca^{2+} imaging in cultured hippocampal neurons has shown that Aβ selectively inhibits α7- and α4β2-nAChRs together, but not α3β4-nAChRs, which reduces overall neuronal activity in interneurons, resulting in neuronal hyperexcitation and synaptic dysfunction in excitatory cells[35, 36]. Additionally, our published data using co-immunoprecipitation have shown that Aβ can specifically bind to α7- and α4-containing nAChRs, but not α3-containing receptors[36]. We have also identified two amino acids that are critical for the interaction of Aβ with α7 and α4 subunits, but not the α3 subunit, providing the molecular mechanism underlying the selective interaction of Aβ with specific nAChRs[36]. In line with these findings, considerable evidence suggests that Aβ is shown to exert subtype-specific inhibition of α7- and/or α4β2-nAChR function without affecting α3β4-nAChRs[27-29, 35, 37-40]. α7 and α4 subtypes are also more significantly reduced in the brains of AD patients compared to α3-type receptors[41]. This supports the idea that Aβ predominantly reduces neuronal activity in inhibitory neurons via selective inhibition of α7- and α4β2-nAChRs, but not α3β4-nAChRs[35]. In fact, we have revealed that co-activation of α7- and α4β2-nAChRs is required to reverse the Aβ-induced adverse effects in hippocampal excitatory and inhibitory cells[35, 36]. Moreover, non-selective stimulation of acetylcholine receptors exacerbates Aβ effects on cultured neurons[35, 36]. In sum, our published works have shown that selective co-activation of α7- and α4β2-nAChRs is required to reverse the Aβ effects on the activity of inhibitory interneurons in AD.

The hippocampal memory system is particularly vulnerable to AD-related cellular pathology[42]. Distinct subtypes of GABAergic inhibitory interneurons differentially regulate hippocampal activity at the cellular, circuit, and network levels[43, 44]. Therefore, maintaining proper inhibition within the hippocampus is essential to ensure normal cognition. Parvalbumin-positive (PV+) and somatostatin-positive (SST+) cells, two major subtypes of inhibitory interneurons in the hippocampus, mainly provide somatic and dendritic inhibition onto pyramidal cells,

respectively, to differentially regulate hippocampal network activity and cognitive processes[44]. Aβ is shown to decrease the activity of inhibitory cells to increase excitation in pyramidal cells[45]. This results in hippocampal hyperexcitability and network dysfunction, leading to cognitive decline in AD[3, 45, 46]. This suggests that the reduction of hippocampal inhibition by Aβ is a crucial trigger for hyperexcitability-induced cognitive impairment in AD. Hence, enhancing hippocampal inhibition is thought to be protective against AD[47]. However, Aβ unlikely affects all subtypes of hippocampal interneurons equally[48-50]. Therefore, identifying the affected interneuron subtypes in AD to enhance hippocampal inhibition optimally is conceptually and technically challenging. Furthermore, the important question of how Aβ-induced subtype-specific disruptions in hippocampal interneuron functions are linked to distinct nAChR subtypes remains unanswered.

Here, we discover that α7-, α4β2-, and α3β4-nAChRs regulate the cholinergic activity mainly in PV+, SST+, and excitatory cells, respectively. We further reveal that hippocampal network activity is disrupted following fear learning in a widely used amyloid pathology mouse model, 5XFAD mice[51]. Most importantly, we show that co-activation of these receptors is necessary to reverse hippocampal network dysfunction and fear memory loss in 5XFAD mice. This suggests that co-activation of PV+ and SST+ cells is a novel strategy to reverse hippocampal network dysfunction and cognitive decline in AD.

Results

Nicotinic cholinergic regulation of cultured hippocampal PV+ and SST+ interneurons is mainly mediated by α7- and α4β2-nAChRs, respectively.

To determine whether nicotinic cholinergic regulation was stronger in GABAergic inhibitory cells than in excitatory neurons, we first compared nicotinic cholinergic activity between excitatory cells (EX) and inhibitory interneurons (IN) using Ca^{2+} imaging with nicotine uncaging in 12-14 days *in vitro* (DIV) cultured mouse hippocampal neurons as neuronal nAChRs are ligand-gated ion channels, and when activated, they induce membrane depolarization and increase somatic Ca^{2+} levels[52, 53]. We found that nicotinic cholinergic activity measured by Ca^{2+} transients in inhibitory interneurons was significantly higher than in excitatory neurons (EX, 1.000 ± 0.879 $\Delta F/F_0$ and IN, 1.575 ± 1.208 $\Delta F/F_0$, $p = 0.0109$) (**Fig. 1a**). This is consistent with the previous reports that cholinergic regulation of inhibitory cells is stronger than excitatory neurons in the hippocampus[13-18].

We next treated 12-14 DIV cultured mouse hippocampal neurons with nAChR subtype-specific antagonists and conducted Ca^{2+} imaging with nicotine uncaging to identify which subtypes of nAChRs played a crucial role in nicotinic cholinergic regulation of hippocampal excitatory and inhibitory cells. In excitatory cells, when compared to controls (CTRL), acute treatment of 3 μM α-Conotoxin AuIB (αCTx), an α3β4-receptor inhibitor, significantly reduced nicotinic cholinergic activity, while 50 nM α-Bungarotoxin (αBTx), an α7-receptor inhibitor, or 1 μM Dihydro-β-erythroidine hydrobromide (DHβE), an α4β2-receptor inhibitor, had no effects (CTRL, 1.000 ± 0.4921 $\Delta F/F_0$, αBTx, 0.870 ± 0.328 $\Delta F/F_0$, DHβE, 1.042 ± 0.644 $\Delta F/F_0$, and αCTx, 0.325 ± 0.362 $\Delta F/F_0$) (**Fig. 1b and Table S1**). Conversely, in inhibitory cells, when compared to controls (CTRL), αBTx or DHβE treatment significantly reduced nicotine-induced activity, but the α3β4 antagonist had no effect (CTRL, 1.000 ± 0.525 $\Delta F/F_0$, αBTx, 0.398 ± 0.290 $\Delta F/F_0$, DHβE, 0.335 ± 0.300 $\Delta F/F_0$, and αCTx, 0.871 ± 0.330 $\Delta F/F_0$) (**Fig. 1c and Table S2**). This data show that α7- and α4β2-nAChRs are mainly responsible for nicotinic cholinergic activity in hippocampal inhibitory cells, while α3β4-nAChRs are important for nicotinic cholinergic activity in hippocampal excitatory cells. These findings are consistent with the previous reports demonstrating that postsynaptic α7- and α4β2-nAChRs are mainly localized on GABAergic interneurons in the hippocampus and mediate nicotine currents[14, 16], while they are expressed in the presynaptic area of excitatory cells[54-58] to regulate neurotransmitter release without neuronal activation such as action potentials. This suggests that stimulation of α7- and α4β2-nAChRs has more profound effects on neuronal activation in inhibitory cells than

excitatory neurons in the hippocampus.

We next analyzed subtype-specific roles of nAChRs in cultured hippocampal PV+ and SST+ interneurons. Since α3β4-nAChRs are shown to mainly regulate excitatory neurons, we focused on the role of α7- and α4β2-nAChRs in these inhibitory cells. In PV+ interneurons, when compared to controls (CTRL), acute treatment of αBTx significantly reduced nicotinic cholinergic activity, while DHβE had no effects (CTRL, 1.000 ± 0.590 $\Delta F/F_0$, αBTx, 0.507 ± 0.374 $\Delta F/F_0$, and DHβE, 1.076 ± 0.613 $\Delta F/F_0$) (**Fig. 1d and Table S3**). Importantly, co-treatment of αBTx and DHβE had no additional effect (αBTx + DHβE, 0.607 ± 0.448 $\Delta F/F_0$) (**Fig. 1d and Table S3**). Conversely, in SST+ interneurons, when compared to controls (CTRL), acute treatment of DHβE significantly reduced nicotinic cholinergic activity, while αBTx had no effects (CTRL, 1.000 ± 0.426 $\Delta F/F_0$, αBTx, 0.917 ± 0.519 $\Delta F/F_0$, and DHβE, 0.524 ± 0.262 $\Delta F/F_0$) (**Fig. 1e and Table S4**). Importantly, co-treatment of αBTx and DHβE had no additional effect (αBTx + DHβE, 0.533 ± 0.272 $\Delta F/F_0$) (**Fig. 1e and Table S4**). This shows that α7- and α4β2-nAChRs play important roles in nicotinic cholinergic regulation of PV+ and SST+ cells, respectively.

The Aβ-induced decrease in the neuronal activity of PV+ and SST+ interneurons is reversed by stimulation of α7- and α4β2-nAChRs, respectively.

We next examined whether Aβ affected nicotinic cholinergic activity of PV+ and SST+ interneurons using nicotine uncaging as described in **Fig. 1**. We found that 250 nM soluble Aβ42 oligomers (oAβ42) treatment significantly reduced nicotine-induced activity in both PV+ (sAβ42, 1.000 ± 0.772 $\Delta F/F_0$ and oAβ42, 0.448 ± 0.233 $\Delta F/F_0$, p = 0.0038) and SST+ interneurons (sAβ42, 1.000 ± 0.391 $\Delta F/F_0$ and oAβ42, 0.600 ± 0.237 $\Delta F/F_0$, p = 0.0002) compared to neurons treated with 250 nM scrambled Aβ42 (sAβ42) (**Fig. 2a-b**). We further determined how Aβ affected neuronal activity in PV+ and SST+ interneurons. As somatic Ca^{2+} levels in neurons indicate neuronal activity[59], we measured spontaneous somatic Ca^{2+} activity without tetrodotoxin (TTX) in 12-14 DIV cultured hippocampal neurons as carried out previously[35, 36, 60, 61]. 250 nM oAβ42 treatment significantly decreased neuronal activity in both PV+ and SST+ interneurons (PV+ cells; sAβ42, 1.000 ± 0.379 $\Delta F/F_{min}$ and oAβ42, 0.589 ± 0.223 $\Delta F/F_{min}$, p = 0.0371 and SST+ cells; sAβ42, 1.000 ± 0.500 $\Delta F/F_{min}$ and oAβ42, 0.650 ± 0.330 $\Delta F/F_{min}$, p = 0.0206) (**Fig. 2c-d and Table S5-6**). To determine subtype-specific roles of

nAChRs in the Aβ effect on PV+ and SST+ cells' activity, we treated these cells with 250 nM oAβ42 or 250 nM sAβ42 and subtype-specific nAChR agonists - 1 μM PNU-282987 (PNU), an α7 agonist, 2 μM RJR-2403 Oxalate (RJR), an α4β2 agonist, or 1 μM NS-3861 (NS), an α3β4 agonist. In PV+ interneurons, when compared to controls (CTRL), activation of α7-nAChRs was sufficient to reverse the Aβ effects, while stimulation of other nAChR subtype had no effects (oAβ42:CTRL, 0.589 ± 0.223 $\Delta F/F_{min}$, oAβ42:PNU, 1.408 ± 0.806 $\Delta F/F_{min}$, and oAβ42:RJR, 0.528 ± 0.299 $\Delta F/F_{min}$) (**Fig. 2c and Table S5**). In SST+ interneurons, when compared to controls (CTRL), stimulation of α4β2-nAChRs reversed the Aβ effects, but other agonists had no effects (oAβ42:CTRL, 0.650 ± 0.328 $\Delta F/F_{min}$, oAβ42:PNU, 0.557 ± 0.271 $\Delta F/F_{min}$, and oAβ42:RJR, 0.994 ± 0.358 $\Delta F/F_{min}$) (**Fig. 2d and Table S6**). Activation of α3β4 receptors had no effects on the activity of both PV+ and SST+ interneurons (PV+ Cells; oAβ42:NS, 0.550 ± 0.384 $\Delta F/F_{min}$ and SST+ cells; oAβ42:NS, 0.561 ± 0.249 $\Delta F/F_{min}$) (**Fig. 2c-d and Table S5-6**), which is consistent with the findings that α3β4-nAChRs are important for nicotinic cholinergic activity in excitatory cells (**Fig. 1b-c**). This suggests that the Aβ-induced decrease in the neuronal activity of PV+ and SST+ interneurons is reversed by stimulation of α7- and α4β2-nAChRs, respectively. This further shows that stimulation of α7- and α4β2-nAChRs is a novel and ideal way to stimulate PV+ and SST+ interneurons' activity rather than excitatory neurons' activity.

Deficits in hippocampal network activity in 5XFAD mice following fear learning.

The hippocampal memory system is particularly vulnerable to AD-related cellular pathologies[42]. Neural network activity in the hippocampus plays important roles in memory processes[62-66]. Studies have shown that altered hippocampal network activity can impair learning and memory processes, which contributes to the development of AD[10, 67-70]. In people at high risk of developing AD, abnormal activation and/or deactivation of specific network activities during hippocampal memory processes are detected decades before the onset of clinical disease[10, 67-70]. This suggests that monitoring hippocampal network activity may be a useful tool for early detection and diagnosis of AD. Hippocampal inhibitory interneurons regulate network oscillations of local field potentials (LFPs), which are thought to play a mechanistic role in various aspects of hippocampus-dependent memory processes[44, 62-66]. In mice, it has been reported that theta (4-8 Hz) synchrony is increased between the hippocampal CA1 and lateral amygdala during the consolidation and reconsolidation of fear memory[62, 63]. Research also suggests that gamma rhythms (30-100 Hz) are two functionally distinct oscillatory

activities, slow (30-50 Hz) and fast (50-100 Hz) gamma[65, 71, 72]. Fast gamma rhythms promote the transmission of current sensory information to the hippocampus during new memory encoding[65, 72]. Slow gamma oscillations are likely involved in storing memory[73-75]. It has been shown that spatial learning increases theta, slow gamma, and fast gamma powers in the hippocampal CA1 area[76-78]. We thus first recorded hippocampal LFPs before and following contextual fear conditioning (CFC) to determine if hippocampal network activity was disrupted in 5XFAD mice after fear learning. Prior to learning, basal LFPs were recorded for an hour (PRE) (**Fig. 3a**). Consolidation of hippocampus-dependent fear memory occurs between 1 and 3 hours after training[79, 80]. Therefore, test animals were returned to their home cage for 90 minutes after fear learning, and then we recorded hippocampal LFPs for an hour (POST) (**Fig. 3a**). We then compared hippocampal theta, slow gamma, and fast gamma powers between 5XFAD mice and their wild-type (WT) littermates. We found that theta (Female; WT PRE, 2.826e-051 ± 3.388e-051 μV^2 and WT POST, 4.793e-051 ± 4.387e-051 μV^2, $p = 0.0029$, Male; WT PRE, 1.031e-051 ± 1.310e-051 μV^2 and WT POST, 1.985e-051 ± 2.936e-051 μV^2, $p = 0.0029$), slow gamma (Female; WT PRE, 2.783e-053 ± 3.403e-053 μV^2 and WT POST, 4.519e-053 ± 3.950e-053 μV^2, $p = 0.0031$, Male; WT PRE, 9.813e-054 ± 1.103e-054 μV^2 and WT POST, 2.229e-053 ± 2.406e-053 μV^2, $p < 0.0001$), and fast gamma powers (Female; WT PRE, 8.722e-054 ± 9.474e-054 μV^2 and WT POST, 1.403e-053 ± 1.197e-053 μV^2, $p = 0.0008$, Male; WT PRE, 3.188e-054 ± 3.349e-054 μV^2 and WT POST, 7.863e-054 ± 7.924e-054 μV^2, $p < 0.0001$) were significantly elevated following CFC in WT female and male mice (**Fig. 3e-g**). Conversely, 5XFAD mice showed significant reduction in powers of theta (Female; 5XFAD PRE, 6.203e-051 ± 1.726e-050 μV^2 and 5XFAD POST, 6.359e-052 ± 5.929e-052 μV^2, $p = 0.0018$, Male; 5XFAD PRE, 2.292e-051 ± 3.545e-051 μV^2 and 5XFAD POST, 6.515e-052 ± 1.235e-051 μV^2, $p < 0.0001$), slow gamma (Female; 5XFAD PRE, 8.752e-053 ± 2.161e-052 μV^2 and 5XFAD POST, 1.336e-053 ± 9.528e-054 μV^2, $p = 0.0007$, Male; 5XFAD PRE, 2.096e-053 ± 3.009e-053 μV^2 and 5XFAD POST, 7.592e-054 ± 1.101e-053 μV^2, $p < 0.0001$), and fast gamma bands (Female; 5XFAD PRE, 2.668e-053 ± 6.334e-053 μV^2 and 5XFAD POST, 4.301e-054 ± 2.416e-054 μV^2, $p = 0.0005$, Male; 5XFAD PRE, 6.650e-054 ± 8.634e-054 μV^2 and 5XFAD POST, 2.682e-054 ± 3.187e-054 μV^2, $p < 0.0001$) (**Fig. 3e-g**). This suggests that a learning-induced increase in hippocampal oscillations is impaired in 5XFAD mice.

Co-activation of α7- and α4β2-nAChRs is required to restore normal hippocampal network activity in 5XFAD mice.

We next compared hippocampal oscillatory activity during memory consolidation between WT and 5XFAD mice. When compared to WT mice, 5XFAD mice exhibited a significant decrease in the powers of theta (Female; WT, 4.793e-051 ± 1.726e-051 μV^2 and 5XFAD, 6.359e-052 ± 5.929e-052 μV^2, $p < 0.0001$, Male; WT, 1.985e-051 ± 2.936e-051 μV^2 and 5XFAD, 6.515e-052 ± 1.235e-051 μV^2, $p < 0.0001$), slow gamma (Female; WT, 4.519e-053 ± 3.950e-053 μV^2 and 5XFAD, 1.336e-053 ± 9.528e-054 μV^2, $p = 0.0026$, Male; WT, 2.229e-053 ± 2.406e-053 μV^2 and 5XFAD, 7.592e-054 ± 1.101e-053 μV^2, $p = 0.0012$), and fast gamma bands (Female; WT, 1.403e-053 ± 1.197e-053 μV^2 and 5XFAD, 4.301e-054 ± 2.416e-054 μV^2, $p = 0.0027$, Male; WT, 7.863e-054 ± 7.924e-054 μV^2 and 5XFAD, 2.682e-054 ± 3.187e-054 μV^2, $p < 0.0001$) (**Fig. 4a-c**). This indicates impaired fear memory processing in 5XFAD mice. Acetylcholine modulates hippocampal oscillations to control memory processes[81-83]. We thus examined if stimulating α7- and α4β2-nAChRs together was required to reverse hippocampal network deficits in 5XFAD mice after fear conditioning. We found that activation of single receptors by themselves using PNU or RJR had no effect on the powers of theta (Female; 5XFAD:PNU, 6.619e-052 ± 1.216e-051 μV^2, $p = 0.9673$, and 5XFAD:RJR, 9.233e-052 ± 1.663e-051 μV^2, $p = 0.6696$, Male; 5XFAD:PNU, 2.441e-052 ± 2.723e-052 μV^2, $p = 0.3112$, and 5XFAD:RJR, 1.795e-052 ± 2.118e-052 μV^2, $p = 0.2312$), slow gamma (Female; 5XFAD:PNU, 1.377e-053 ± 1.895e-053 μV^2, $p = 0.9721$, and 5XFAD:RJR, 1.167e-053 ± 3.295e-053 μV^2, $p = 0.8221$, Male; 5XFAD:PNU, 6.075e-054 ± 3.491e-054 μV^2, $p = 0.8194$, and 5XFAD:RJR, 3.159e-054 ± 2.720e-054 μV^2, $p = 0.4959$), and fast gamma bands (Female; 5XFAD:PNU, 3.767e-054 ± 5.506e-054 μV^2, $p = 0.8834$, and 5XFAD:RJR, 3.752e-054 ± 5.583e-051 μV^2, $p = 0.8868$, Male; 5XFAD:PNU, 2.067e-054 ± 1.531e-054 μV^2, $p = 0.7265$, and 5XFAD:RJR, 1.113e-054 ± 1.089e-054 μV^2, $p = 0.3619$) (**Fig. 4a-c**). This shows that stimulation of either α7- or α4β2-nAChRs is insufficient to improve hippocampal network activity in 5XFAD mice. However, co-stimulation of α7- and α4β2-nAChRs significantly elevated the powers of theta (Female; 5XFAD:PNU+RJR, 2.483e-051 ± 3.995e-051 μV^2, $p = 0.0005$, Male; 5XFAD:PNU+RJR, 1.356e-051 ± 1.955e-051 μV^2, $p = 0.0229$), slow gamma (Female; 5XFAD:PNU+RJR, 4.783e-053 ± 7.379e-053 μV^2, $p = 0.0005$, Male; 5XFAD:PNU+RJR, 2.898e-053 ± 5.295e-053 μV^2, $p < 0.0001$), and fast gamma bands (Female; 5XFAD:PNU+RJR, 1.758e-053 ± 2.693e-053 μV^2, $p <$

0.0001, Male; 5XFAD:PNU+RJR, 9.915e-054 ± 1.386e-053 μV^2, $p < 0.0001$) (**Fig. 4a-c**). In sum, 5XFAD mice showed significant deficits in hippocampal network activity following fear conditioning. Co-activation of α7- and α4β2-nAChRs, potentially stimulating PV+ and SST+ cells together, is required to reverse AD-related hippocampal network dysfunction after fear learning.

Co-activation of α7- and α4β2-nAChRs is required to improve contextual fear memory in 5XFAD mice.

Conditioned fear responses have been demonstrated to be impaired in patients with mild to moderate AD[84, 85]. Fear memory loss in 5XFAD mice starts at the age of 5 months[86]. We thus carried out contextual fear conditioning (CFC) as described previously[87, 88] with 5-month-old 5XFAD and WT female and male mice. In consistent with the previous finding[5], 5XFAD female and male mice intraperitoneally given saline displayed significant less freezing in contextual fear conditioning compared to WT controls, indicating impaired hippocampus-dependent fear memory (Female, WT:Saline, 36.785 ± 10.866% and 5XFAD:Saline, 14.188 ± 12.202%, $p < 0.0001$, and Male, WT:Saline, 38.938 ± 13.066% and 5XFAD:Saline, 16.177 ± 8.761%, $p < 0.0001$) (**Fig. 5**). Given that co-activation of α7- and α4β2-nAChRs is sufficient to reverse fear learning-associated hippocampal network activity (**Fig. 4**), we examined the effects of stimulating these receptors on contextual fear memory in 5XFAD mice (**Fig. 3a**). We thus intraperitoneally injected PNU or RJR into 5XFAD and WT mice to see if activation of either α7- or α4β2-nAChRs reversed impaired contextual fear memory in AD model mice. Notably, neither PNU nor RJR single injection reversed fear memory loss in 5XFAD mice (Female, 5XFAD:PNU, 15.751 ± 10.332%, $p > 0.9999$, and 5XFAD:RJR, 14.836 ± 10.400%, $p > 0.9999$, and Male, 5XFAD:PNU, 15.558 ± 6.577%, $p > 0.9999$, and 5XFAD:RJR, 14.648 ± 6.018%, $p > 0.9999$) (**Fig. 5**). This shows that stimulation of either α7- or α4β2-nAChRs is insufficient to improve fear memory in 5XFAD mice. We thus intraperitoneally injected PNU and RJR together into 5XFAD and WT littermates and examined whether co-activation of α7- and α4β2-nAChRs improved contextual fear memory in 5XFAD mice. We found that co-activation of α7- and α4β2-nAChRs significantly enhanced contextual fear memory in 5XFAD male and female mice (Female, 5XFAD:PNU+RJR, 43.664 ± 13.580%, $p < 0.0001$, and Male, 5XFAD:PNU+RJR, 43.434 ± 21.002%, $p = 0.0101$) (**Fig. 5**). Taken all together, we discover that co-activation of α7- and α4β2-nAChRs (potentially stimulates hippocampal PV+ and SST+ cells together) is required to improve hippocampus-

dependent fear memory in 5XFAD mice.

Discussion

A large body of research has suggested that Aβ-induced inhibitory dysfunction in the hippocampus can be a crucial trigger for disruptions in neural oscillations and consequent cognitive function in AD[89-95]. However, the molecular mechanisms underlying Aβ-induced hippocampal interneuron dysfunctions in AD have not been studied in depth. Cholinergic dysfunction may be a prime suspect for Aβ-induced inhibitory dysfunction because cholinergic signaling has greater effects on hippocampal inhibitory cells than excitatory neurons[13-18], and there are diverse lines of evidence that molecular interactions between Aβ and nAChRs impair receptor functions in the early stage of AD[53, 96-98]. Aβ thus likely inhibits nAChR activities in inhibitory interneurons, which reduces hippocampal inhibition and oscillations to trigger cognitive decline in the early stage of AD. However, hippocampal inhibitory cells are highly diverse, and distinct interneuron subtypes differentially contributes to hippocampal oscillations and memory formation[44]. In addition, various neuronal nAChRs have been found in the human brain[30-32], and distinct nAChR subtypes differentially affect hippocampal inhibition and oscillations[56, 99-101]. Therefore, it is challenging to elucidate the nAChR-mediated mechanisms by which Aβ affects hippocampal neural network and memory processing in AD at the interneuron subtype levels. Our published works have discovered that Aβ selectively binds to two of the three major hippocampal nAChR subtypes, α7- and α4β2-nAChRs, but not α3β4-nAChRs, and inhibits these two receptors in cultured hippocampal inhibitory interneurons to decrease their activity, leading to hyperexcitation and synaptic dysfunction in excitatory neurons[35, 36]. We have also revealed that co-activation of α7- and α4β2-nAChRs is required to reverse the Aβ-induced adverse effects in hippocampal neurons[35, 36]. In the current study, we further identify that α7-, α4β2-, and α3β4-nAChRs regulate the cholinergic activity mainly in PV+, SST+, and excitatory cells, respectively. Additionally, we discover that co-activation of α7- and α4β2-nAChRs is necessary to reverse hippocampal network dysfunction and prevent fear memory loss in the amyloid pathology model mice. In sum, our study provides the mechanistic link between hippocampal disinhibition-induced hyperexcitability via altered cholinergic activity, network dysfunction, and memory loss in AD.

Based on our findings, we propose the following model. α7-, α4β2-, and α3β4-nAChRs predominantly control the nicotinic cholinergic signaling in PV+ interneurons that provide somatic inhibition, SST+ interneurons that provides dendritic inhibition, and pyramidal excitatory neurons, respectively, in the hippocampus, which is supported by the previous findings[14, 16, 17, 56, 102-110] (**Fig. 6a**). These distinct cholinergic

regulations of inhibitory activity contribute to inhibition onto pyramidal cells, which controls hippocampal network activity and memory processes (**Fig. 6a**). Aβ selectively inhibits α7- and α4β2-nAChRs in PV+ and SST+ cells, respectively, but not α3β4-nAChRs in excitatory neurons, which reduces inhibition to induce hyperexcitability and network dysfunction in the hippocampus, leading to memory loss in AD (**Fig. 6b**). Stimulation of PV+ or SST+ cells by the activation of each nAChR subtype-specific agonists, PNU-282987 (PNU), an α7 agonist, or RJR-2403 Oxalate (RJR), an α4β2 agonist, is insufficient to reverse the adverse effects of Aβ (**Fig. 6c-d**). Therefore, co-stimulation of hippocampal PV+ and SST+ inhibitory interneurons via activation of α7- and α4β2-nAChRs together is required to be protective against AD (**Fig. 6e**).

Given that cholinergic deficiency is associated with AD, strategies aiming to restore normal cholinergic function have been developed as therapeutic drugs for AD, including several acetylcholinesterase inhibitors[111]. Therefore, all cholinergic synapses can be intensified by these drugs. However, some subtypes of nAChRs that may not be affected by Aβ (e.g. α3β4) can also be activated when attempts are made to use such drugs in the treatment of AD, resulting in unexpected side effects. Moreover, acetylcholinesterase inhibitors have been shown to be only partially effective in reversing experimental deficits in hippocampal-dependent memory in rodents[112]. Consistently, we have revealed a non-selective cholinergic agonist exacerbates Aβ effects on hippocampal neurons[35, 36]. Furthermore, overstimulation of the M1 type metabotropic AChRs adversely affect neuronal activity in the prefrontal cortex, related to working memory[113]. Therefore, acetylcholinesterase inhibitors are not very effective in slowing AD progression due to non-selective stimulation of acetylcholine receptors[114, 115]. There are also 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 non-human primate studies[116], while several other studies have failed to find significant enhancement of learning and memory by nicotine treatment[117]. Nonetheless, nAChR agonists have consistently suggested promising approaches in the treatment of AD[118]. However, clinical trials thus far have been challenged by adverse effects or minimal improvement[119]. Additionally, stimulating one type of nAChRs by using a subtype specific agonist enhances cognitive performance, but other studies find no beneficial effect. For instance, selective α7 nAChR agonists have been reported to improve cognition in a variety of animal models[120-122], while another study has found almost no beneficial effect on learning and memory in mice[123]. An α4β2-nAChR agonist alone can improve working memory only in young rats but not older animals[124]. It is thus

not yet clear whether single activation of specific nAChR subtypes provide optimal efficacy in AD[118]. Research also shows that co-activation of α7- and α4β2-nAChRs has substantially greater neuroprotective effects against a ketamine-based model of schizophrenia-like cognitive impairments in rats, when compared to single receptor stimulation[125]. Moreover, pharmacological inhibition of each α7- or α4β2-nAChR subtype has no effect on working memory in nonhuman primates[126]. These studies suggest that proper cholinergic modulation in hippocampal functions requires both α7- and α4β2-nAChRs. Our published works[35, 36] and current study further support this idea. Therefore, the idea that selective co-activation of nAChRs in the hippocampus can reverse Aβ effects on AD pathology is a new concept, which may lead to innovative and novel therapeutic strategies. In fact, several subtype-specific agonists have been developed for clinical trials, but co-activation of nAChRs has not been applied for clinical trials yet[118]. The current work thus suggests that activation of α7- and α4β2-nAChRs as an innovative and selective way to stimulate hippocampal PV+ and SST+ cells in the hippocampal local circuits, which has potentials for a novel therapeutic strategy in AD.

Materials and Methods

Animals

C57Bl6J (Jax 000664), PV-Cre (Jax 017320), SST-Cre (Jax 018973), and 5XFAD (Jax 034840) mice were obtained from Jackson laboratory and bred in the animal facility at Colorado State University (CSU). Animals were housed under a 12:12 hour light/dark cycle. CSU's Institutional Animal Care and Use Committee (IACUC) reviewed and approved the animal care and protocol (3408).

Primary hippocampal neuronal culture

Postnatal day 0 (P0) male and female C57Bl6J, PV-Cre, or SST-Cre pups were used to produce mouse hippocampal neuron cultures as shown previously[60, 127, 128]. Hippocampi were isolated from P0 C57Bl6J mouse brain tissues and digested with 10 U/mL papain (Worthington Biochemical Corp., LK003176). Mouse hippocampal neurons were plated on following poly lysine-coated dishes for glass bottom dishes (500,000 cells) for Ca^{2+} imaging. Neurons were grown in Neurobasal Medium without phenol red (Thermo Fisher Scientific, 12348017) with B27 supplement (Thermo Fisher Scientific, 17504044), 0.5 mM Glutamax (Thermo Fisher Scientific, 35050061), and 1% penicillin/streptomycin (Thermo Fisher Scientific, 15070063). The previous study evaluates maturation, aging, and death of mouse cortical cultured neurons for 60 days *in vitro* (DIV), which demonstrates that synaptogenesis is prominent during the first 15 days and then synaptic markers remained stable through 60 DIV[129]. In particular, the levels of glutamate receptors increase to a maximum by 10-15 DIV and then remain unchanged through 60 DIV. This indicates that 12-14 DIV neurons that we used here are mature cells, and their maturity is likely comparable to that of older neurons. Additionally, neuronal cultures are shown to contain excitatory and inhibitory cells, cholinergic neurons, as well as glia[35, 36, 130].

Reagents

Soluble Aβ42 oligomers (oAβ42) were prepared as previously described[35, 131]. 1 mg of lyophilized human Aβ42 (Anaspec) was dissolved in 1mL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma, St. Louis, MO) 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 DMSO and 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. Scrambled Aβ42 oligomers (sAβ42) were prepared the same way for controls. The following nAChR antagonists were used in this study: α-Bungarotoxin (αBTx) (Alomone labs)), an α7 antagonist, Dihydro-β-erythroidine hydrobromide (DHβE) (Tocris Bioscience), an α4β2 antagonist, and α-Conotoxin AuIB (Alomone labs), an α3β4 antagonist. The following nAChR agonists were used in this study: PNU-282987 (PNU) (Tocris Bioscience), an α7 agonist, RJR-2403 Oxalate (RJR) (Tocris Bioscience), an α4β2 agonist, and NS-3861 (NS) (Tocris Bioscience), an α3β4 agonist. Tetrodotoxin (TTX) (Abcam) was used to prevent action potential-dependent spontaneous network activity.

GCaMP Ca^{2+} imaging with nicotine uncaging

For Ca^{2+} imaging with nicotine uncaging, a genetically encoded Ca^{2+} indicator, GCaMP, was used. As the majority of cells in hippocampal cultures are excitatory neurons[132], we expressed GCaMP6f (Addgene #40755)[133] under the control of the CMV promoter by transfection for imaging excitatory cells, and GCaMP6f under the control of the GABAergic neuron-specific enhancer of the mouse *Dlx* (mDlx) gene (Addgene #83899)[134] was transfected for imaging inhibitory interneurons as shown previously[35]. 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 nicotine uncaging, 1 μM photoactivatable nicotine (PA-Nic) (Tocris Bioscience) was added to the culture media, and epi-illumination photolysis (390 nm, 0.12 mW/mm^2, 1 sec) was used as shown previously[52]. TTX (1 μM) was added in culture media to prevent action potential-dependent spontaneous network activity. A baseline average (F_0) of 20 frames (50 ms exposure) were captured in the soma prior to nicotine uncaging, and 50 more frames (50 ms exposure) were obtained after photostimulation. The fractional change in fluorescence intensity relative to baseline ($\Delta F/F_0$) was calculated. For **Fig. 1a**, the average peak amplitude in excitatory cells (EX) was used to normalize the peak amplitude in each cell and was compared to the inhibitory cells' average (IN). For **Fig. 1b-c**, the images were captured right after each antagonist was added to the media. The average peak amplitude in the control group without drug treatment (CTRL) was used to normalize the peak amplitude in each cell, and the control group's average peak amplitude was compared to the experimental groups' average. For **Fig. 1d-e**, PV-Cre and SST-Cre mice were used to culture hippocampal neurons. We infected 4

DIV neurons prepared from PV-Cre or SST-Cre mice with adeno-associated virus (AAV) expressing Cre-dependent GCaMP7s (Addgene# 104495-AAV1) - pGP-AAV-CAG-FLEX-jGCaMP7s-WPRE was a gift from Douglas Kim & GENIE Project (Addgene plasmid #104495 ; http://n2t.net/addgene:104495 ; RRID:Addgene_104495)[135] to determine nicotinic cholinergic activity in PV+ and SST+ cells using nicotine uncaging. For GCaMP7s, the images were captured right after each antagonist was added to the media. A baseline average (F_0) of 20 frames (5 ms exposure) were captured prior to nicotine uncaging, and 100 more frames (5 ms exposure) were obtained after photolysis. The average peak amplitude in the control group (CTRL) was used to normalize the peak amplitude in each cell, and the control group's average peak amplitude was compared to the experimental groups' average. For Fig. 2, PV-Cre and SST-Cre mice were also used to culture hippocampal neurons. For **Fig 2a-b**, Ca^{2+} imaging with nicotine uncaging was conducted as described in **Fig. 1d-e**. The images were captured right after 250 nM sAβ42 or 250 nM oAβ42 was added to the media. The average peak amplitude in sAβ42-treated cells was used to normalize the peak amplitude in each cell, and the sAβ42 group's average peak amplitude was compared to the oAβ42-treated cells' average.

GCaMP Ca^{2+} imaging

We measured spontaneous Ca^{2+} activity in cultured hippocampal excitatory neurons because It has been shown that networks of neurons in culture can produce spontaneous synchronized activity[136]. In fact, network activity emerges at 3-7 DIV independent of either ongoing excitatory or inhibitory synaptic activity and matures over the following several weeks in cultures[136]. Therefore, the somatic Ca^{2+} signals we observed are from the spontaneous network activity in cultured cells. To do this, we used AAV to express Cre-dependent GCaMP7s (Addgene# 104495-AAV1)[135] in cells prepared from PV-Cre or SST-Cre mice. We then measured Ca^{2+} activity in the soma of 14 DIV cultured hippocampal excitatory neurons with a modification of the previously described method[35, 36, 60, 61, 137-139]. For GCaMP7s, the images were captured right after 250 nM sAβ42 or 250 nM oAβ42 was added to the media in the presence or absence of each agonist. A 10 ms exposure time and a total of 100 images were obtained with a one-second 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 potential photobleaching. The average total Ca^{2+} activity in the control group (CTRL) was used to normalize total Ca^{2+} activity in each cell.

The control group's average total Ca^{2+} activity was compared to the experimental groups' average as described previously[35, 36, 60, 137-139].

Contextual fear conditioning (CFC)

Contextual fear conditioning (Habitest Modular System, Coulbourn Instrument) was carried out as described previously[87, 88]. In Day 1 (encoding), the test mouse was placed in a novel rectangular chamber with a grid floor. After a 3-min baseline period, the test animal was given one shock (a 2 sec, 0.5 mA shock) and stayed in the chamber for an additional 1 min after the shock before being returned to the home cage for overnight (consolidation). A contextual memory test was conducted the next day (Day 2 - retrieval) in the same conditioning chamber for 3 min. Fear memory was determined by measuring the percentage of the freezing response (immobility excluding respiration and heartbeat) using an automated tracking program (FreezeFrame).

Stereotaxic surgery

5-month-old mice were anaesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotactic frame. The target craniotomy site for local field potential (LFP) recordings was marked on the skull, and electrodes were implanted bilaterally into the dorsal hippocampal CA1 area (Bregma coordinates: AP: −1.95 mm, ML: ± 1.12 mm, DV: −1.20 mm). One small screw for the ground anchored at the anterior edges of the surgical site and a wire for a reference in the posterior part of the craniotomy site were bound with dental cement (GC America) to secure the implant in place. The electrodes were constructed by twisting 16 Formvar-insulated NiCh wires (75 μm, California Fine Wire) and attached to a Mill-Max connector. After surgery, mice were treated with antibiotics and analgesic drugs daily, and their health was closely monitored as described in our approved IACUC protocol.

Hippocampal LFP recording

We conducted contextual fear conditioning one week after the surgery as a learning and memory paradigm. Basal LFPs were recorded before learning for one hour (**Fig. 3a**). According to a large body of studies, consolidation of hippocampus-dependent fear memory occurs between 1 and 3 hours after training[79,

[80]. Therefore, after fear learning, test animals were returned to their home cage for 90 minutes, and then we recorded hippocampal LFPs for one hour, a time for fear memory consolidation (**Fig. 3a**). LFP data were collected through electrodes which connect to a 16-channel head-stage (Intan Technologies) that digitizes the raw analog LFP signals via an onboard analog-to-digital converter. We used the Open Ephys Acquisition Board[140] to acquire the digitized signal at 2 kHz. The electrode locations were verified histologically at the end of recordings (**Fig. 3b**). If the electrode was placed outside the CA1 area, the animal was excluded in analysis.

LFP data analysis

LFP data were analyzed in MATLAB (The Mathworks Inc.). The data were formatted for importation into the brainstorm platform[141]. Recorded traces were downsampled to 2 kHz and then bandpass filtered between 0.1 and 500 Hz (**Fig. 3c**). Power spectral density was calculated by a Fast Fourier Transform (FFT) algorithm (**Fig. 3d**). The mean absolute power spectra of the theta (4-8 Hz), slow gamma (30-50 Hz), and fast gamma (50-100 Hz) bands were calculated using Welch's method in the brainstorm platform[141]. The power of each frequency band was first compared before and after fear conditioning in WT and 5XFAD mice. We also compared them between WT and 5XFAD mice during memory consolidation.

***In vivo* activation of nAChRs**

For *in vivo* activation of nAChRs, we used PNU-282987 (PNU), an α7-nAChR agonist, and RJR-2403 Oxalate (RJR), an α4β2-nAChR agonist. These agonists have a number of advantages that can be used to test our hypothesis; 1) they are blood-brain barrier (BBB) permeable[122-124, 142, 143], 2) they do not require endogenous acetylcholine to stimulate receptors since they are direct agonists rather than positive allosteric modulators[122, 123, 142, 144-147], and 3) repeated injection of these agonists is sufficient to show receptor activation in the rodent brains without causing receptor desensitization[145, 146, 148-150]. PNU and RJR were prepared at concentrations of 1 mg/ml and intraperitoneally injected into 5-month-old 5XFAD and WT female and male mice at a dose of 5 mg/kg by themselves or together once per day for 7 days, a condition that is sufficient to stimulate each nAChR subtype *in vivo*[122-124, 142]. Saline was given to mice as a control.

Statistical analysis

The Franklin A. Graybill Statistical Laboratory at CSU has been consulted for statistical analysis in the current study, including sample size determination, randomization, experiment conception and design, data analysis, and interpretation. We used the GraphPad Prism 9 software to determine statistical significance (set at $p < 0.05$). Grouped results of single comparisons were tested for normality with the Shapiro-Wilk normality or Kolmogorov-Smirnov test and analyzed using an unpaired two-tailed Student's t-test when data are normally distributed. Differences between multiple groups were assessed by N-way analysis of variance (ANOVA) with the Tukey test or Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli. The graphs were presented as mean ± Standard Deviation (SD).

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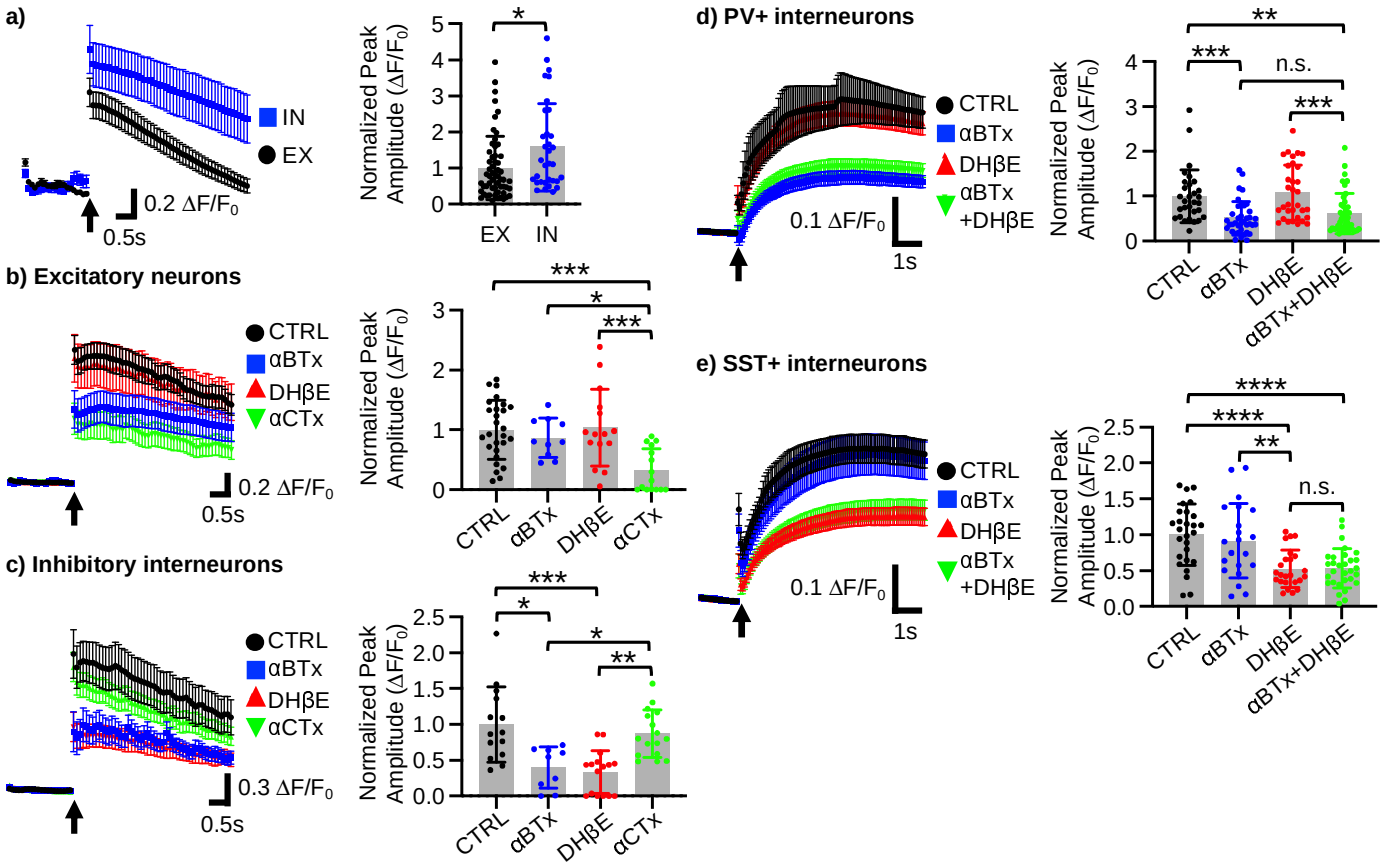
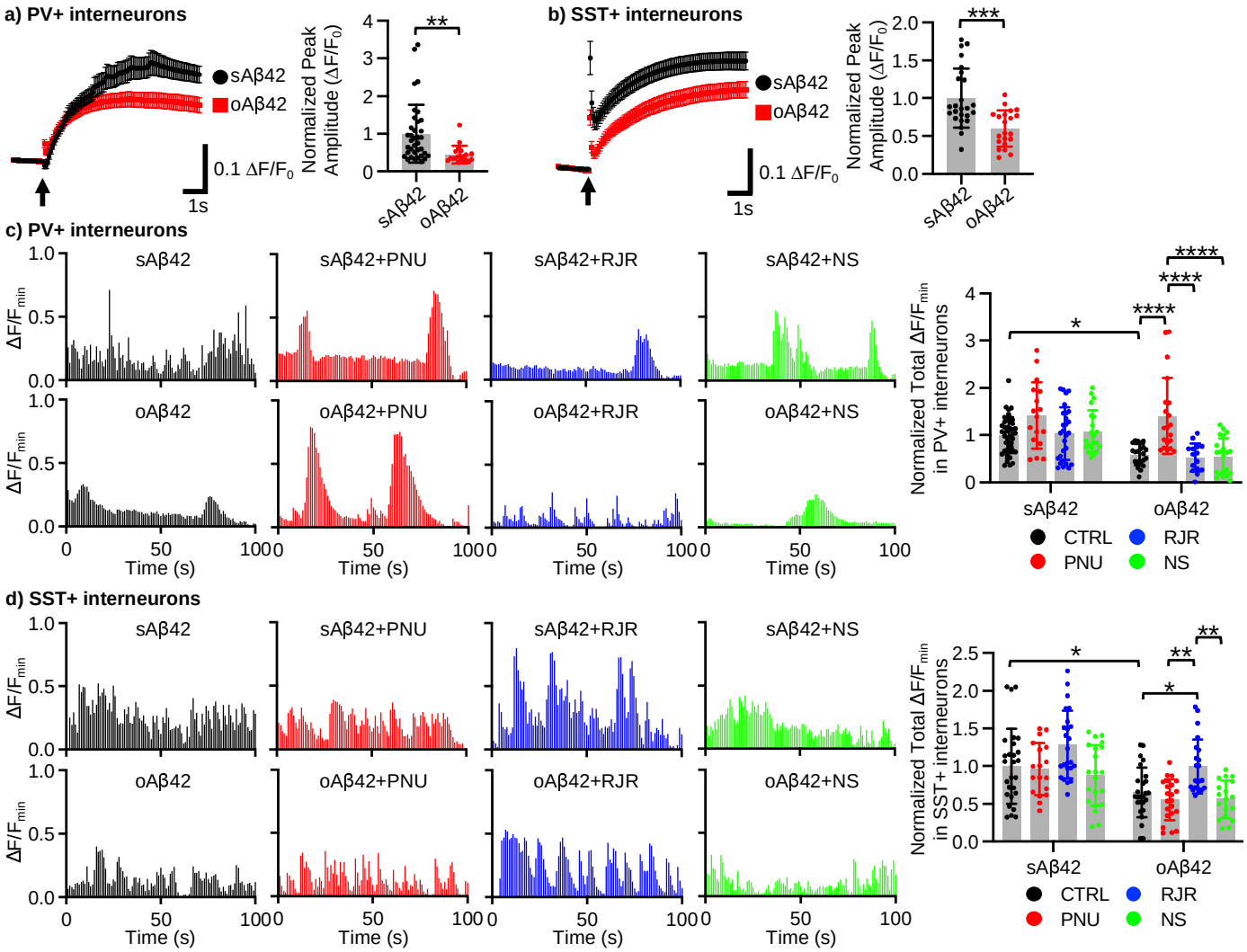
Figure 1. Nicotinic cholinergic regulation of cultured hippocampal PV+ and SST+ interneurons is mainly mediated by α7- and α4β2-nAChRs, respectively.



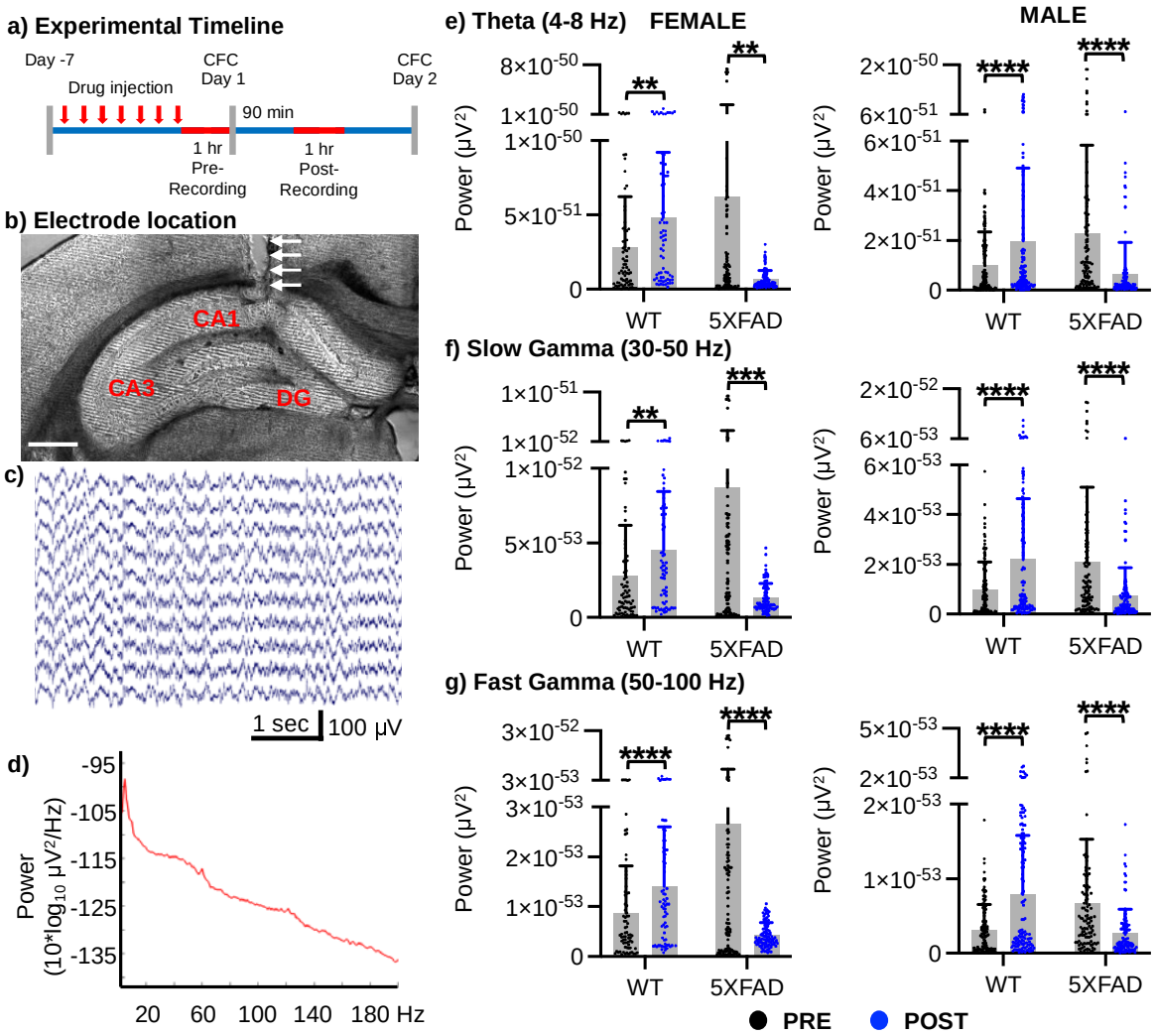
a) Average traces of GCaMP6f signals in excitatory (EX) and inhibitory (IN) cells and summary data of normalized peak amplitude in each condition (n = number of cells excitatory cells = 57 and inhibitory cells =33 from 4 pups in 2 independent cultures). *$p < 0.05$, two-tailed student's t-test. Average traces of GCaMP6f signals and summary graphs of normalized peak amplitude in each condition in **b)** excitatory (n = number of cells, CTRL = 27, αBTx = 10, DhβE = 15, and αCTx = 15 from 4 pups in 2 independent cultures) and **c)** inhibitory cells (n = number of cells, CTRL = 14, αBTx = 9, DhβE = 16, and αCTx = 16 from 4 pups in 2 independent cultures). Average traces of GCaMP7s signals and summary data of normalized peak amplitude in each condition in **d)** PV+ (n = number of cells, CTRL = 30, αBTx = 38, DhβE = 32, and αBTx+DhβE =43 from 6 pups in 3 independent cultures) and **e)** SST+ cells (n = number of cells, CTRL = 27, αBTx = 21, DhβE = 24, and αBTx+DhβE = 30 from 6 pups in 3 independent cultures). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. one-way ANOVA, Tukey test. An arrow indicates photostimulation. n.s.: not significant.

Figure 2. The Aβ-induced decrease in the neuronal activity of PV+ and SST+ interneurons is reversed by stimulation of α7- and α4β2-nAChRs, respectively.



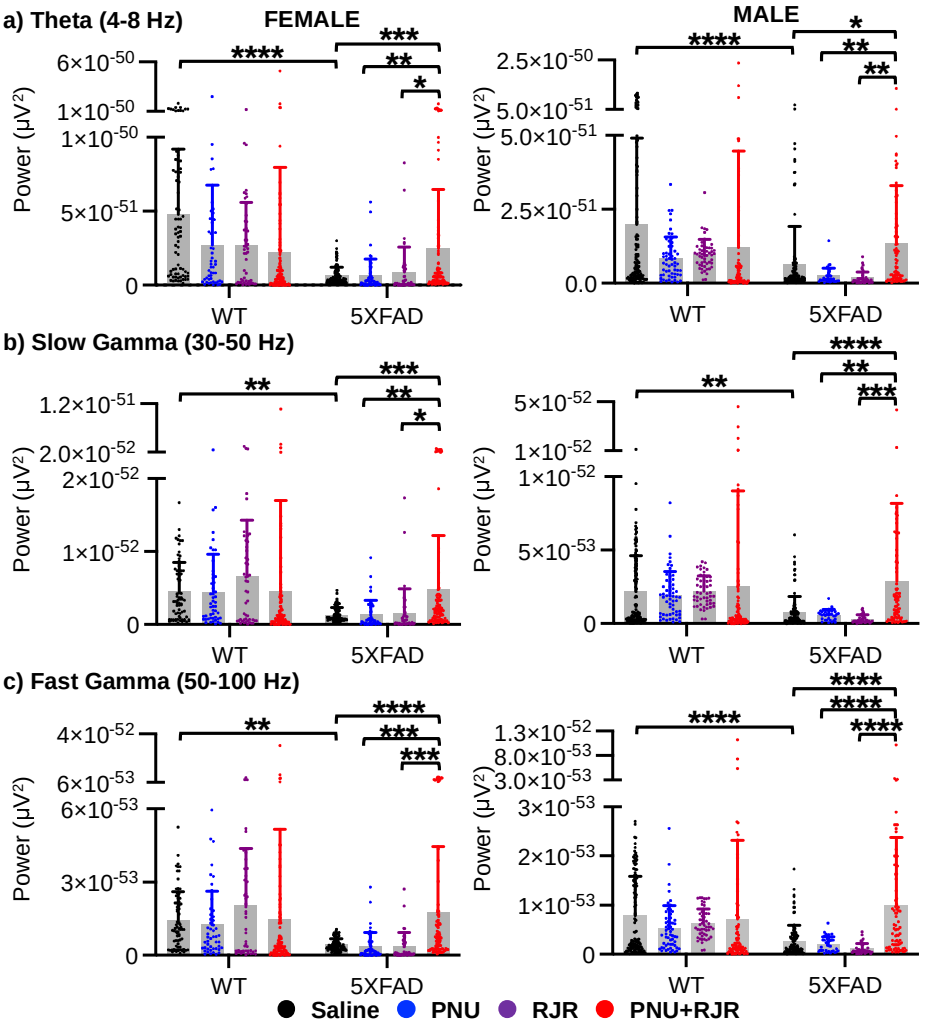
Average traces of GCaMP7s signals and summary data of normalized peak amplitude in each condition in **a)** PV+ cells (n = number of cells, sAβ42 = 36 and oAβ42 = 19 from 6 pups in 3 independent cultures) and **b)** SST+ cells (n = number of cells, sAβ42 = 25 and oAβ42 = 21 from 6 pups in 3 independent cultures). $^{**}p < 0.01$ and $^{***}p < 0.001$, two-tailed student's t-test. An arrow indicates photostimulation. Representative traces of GCaMP7s fluorescence intensity and summary graphs of normalized total Ca^{2+} activity in each condition in **c)** PV+ (n = number of cells, sAβ42:CTRL = 47, sAβ42:PNU = 17, sAβ42:RJR = 28, sAβ42:NS = 21, oAβ42:CTRL = 21, oAβ42:PNU = 20, oAβ42:RJR = 15, and oAβ42:NS = 21 from 6 pups in 3 independent cultures) and **d)** SST+ interneurons (n = number of cells, sAβ42:CTRL = 27, sAβ42:PNU = 18, sAβ42:RJR = 24, sAβ42:NS = 21, oAβ42:CTRL = 26, oAβ42:PNU = 22, oAβ42:RJR = 22, and oAβ42:NS = 18 from 6 pups in 3 independent cultures). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$, Two-way ANOVA, Tukey test.

Figure 3. Deficits in hippocampal network activity in 5XFAD mice following fear learning.



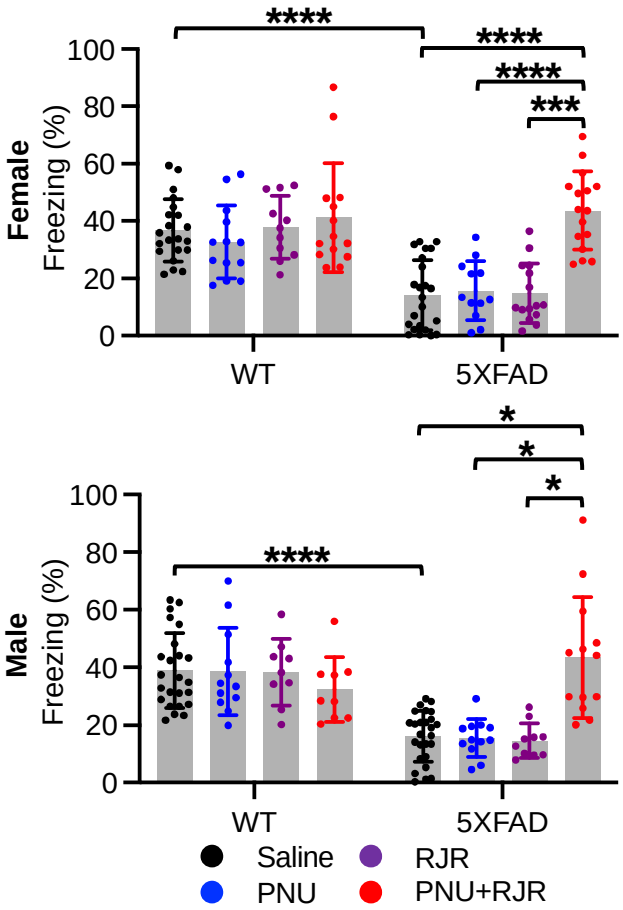
a) Experimental timeline. **b)** A representative image of a brain section after electrolytic lesioning indicates electrode placement (arrows) in the hippocampus CA1 area. A scale bar represents 1 mm. **c)** Representative section of LFP traces in CA1 of a freely behaving mouse. **d)** Representative power spectral density. Summary graphs of **d)** theta, **f)** slow gamma and **g)** fast gamma activity in female and male mice in each condition (n = number of channels [number of animals]; Female, WT =74 [7] and 5XFAD = 101 [9], Male, WT =125 [12] and 5XFAD = 108 [10]). $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, Two-Way ANOVA, Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli test.

Figure 4. Co-activation of α7- and α4β2-nAChRs is required to restore normal hippocampal network activity in 5XFAD mice.



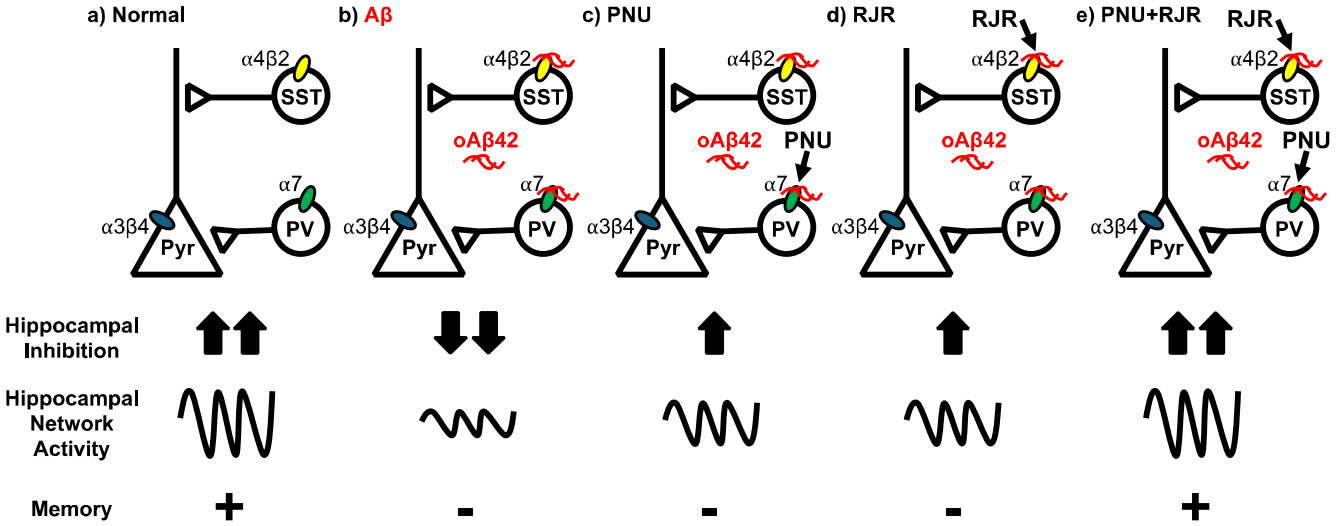
Summary graphs of **d)** theta, **f)** slow gamma and **g)** fast gamma activity in female and male mice in each condition (n = number of channels [number of animals]; Female, WT:Saline =74 [7], WT:PNU = 49 [4], WT:RJR = 48 [4], WT:PNU+RJR = 97 [9], 5XFAD:saline = 101 [9], 5XFAD:PNU = 51 [4], 5XFAD:RJR = 43 [4], and 5XFAD:PNU+RJR = 93 [8], Male, WT:Saline =125 [12], WT:PNU = 57 [5], WT:RJR = 51 [4], WT:PNU+RJR = 86 [8], 5XFAD:saline = 108 [10], 5XFAD:PNU = 36 [4], 5XFAD:RJR = 38 [4], and 5XFAD:PNU+RJR = 79 [7]). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, Two-Way ANOVA, Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli test.

Figure 5. Co-activation of α7- and α4β2-nAChRs is required to improve contextual fear memory in 5XFAD mice.



Summary data of freezing behavior of WT and 5XFAD female (n = number of animals, WT:Saline = 21, WT:PNU = 13, WT:RJR = 11, WT:PNU+RJR = 14, 5XFAD:Saline = 23, 5XFAD:PNU = 12, 5XFAD:RJR = 15, 5XFAD:PNU+RJR = 16) and male mice (n = number of animals, WT:Saline = 24, WT:PNU = 12, WT:RJR = 9, WT:PNU+RJR = 9, 5XFAD:Saline = 26, 5XFAD:PNU = 12, 5XFAD:RJR = 10, 5XFAD:PNU+RJR = 13) in each condition. *$p < 0.05$ and ****$p<0.0001$, Two-way ANOVA, Tukey test.

Figure 6. A schematic model of nAChR-mediated mechanisms of Aβ-induced dysfunction in hippocampal inhibition, network activities, and memory in AD.



a) A simplified local inhibitory circuit in the hippocampus. PV+ interneurons mainly provide somatic inhibition to pyramidal excitatory neurons (Pyr), while SST+ interneurons provide dendritic inhibition. This contributes to hippocampal oscillations and cognitive function. α7-, α4β2-, and α3β4-nAChRs predominantly control nicotinic cholinergic signaling in PV-, SST-, and pyramidal cells, respectively. **b)** Aβ selectively inhibits α7- and α4β2-nAChRs in inhibitory cells, but not α3β4-nAChRs, in turn reduces inhibitory inputs to excitatory cells, which contributes to disruptions in hippocampal oscillations and consequent learning and memory in AD. **c-d)** Single stimulation of each nAChR subtype by PNU-282987 (PNU), an α7 agonist, or RJR-2403 Oxalate (RJR), an α4β2 agonist, is unable to reverse the Aβ-induced adverse effects on network activity and memory. **e)** Co-stimulation of α7- and α4β2-nAChRs sufficiently restores normal hippocampal oscillations and memory.