

DISSERTATION

SELECTIVE CO-ACTIVATION OF NICOTINIC ACETLYCHOLINE RECEPTOR
SUBTYPES RESTORES ALZHEIMER'S RELATED HIPPOCAMPAL DYSFUNCTION
AND PATHOLOGY

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ABSTRACT

SELECTIVE CO-ACTIVATION OF NICOTINIC ACETLYCHOLINE RECEPTOR SUBTYPES RESTORES ALZHEIMER'S RELATED HIPPOCAMPAL DYSFUNCTION AND PATHOLOGY

Alzheimer's disease (AD) is the most common form of dementia. It is a serious disease that not only affects the patient but also their caregivers because it is irreversible with many deteriorating symptoms with limited treatments that are very expensive. Most importantly, these treatments are not very effective. AD has two main pathological characteristics, amyloid plaques, whose main components are misfolded beta amyloid peptides ($A\beta$), and neurofibrillary tau tangles, where hyperphosphorylated tau proteins misfold and aggregate together to make helical filaments. Unfortunately, the cause of the disease is still unknown, and there is no cure. Previous preclinical and clinical trials for the treatment of AD have not been very successful due to a few reasons. For example, most patients join the study after their diagnosis has been made (the progression of the disease is too far for treatments to make an impact on the disease). Progress in the field is further constrained by the lack of reliable biomarkers for early detection of AD. Previous literature has shown that $A\beta$ induces hyperexcitation in neurons, which can happen in multiple layers. At the neuronal level, $A\beta$ can be seen to induce neuronal hyperexcitability. At the circuit level, an imbalance between the excitation and inhibition in excitatory cells and inhibitory interneurons induces hyperexcitation, which leads to impaired information processing. This can cause

network dysfunction, resulting in cognitive decline. Unfortunately, the underlying mechanisms are unknown. Our previous findings, using cultured mouse hippocampal neurons show A β selectively interacts with α 7- and α 4 β 2-nicotinic acetylcholine receptors (nAChRs), but not α 3 β 4-nAChRs, and decreases activity in inhibitory interneurons, leading to hyperexcitation in excitatory neurons. Moreover, we have shown that co-activation of α 7- and α 4 β 2-nAChRs is required to reverse the A β -induced adverse effects in hippocampal excitatory neurons. Here, we discover that α 7- and α 4 β 2-nAChRs predominantly control the nicotinic cholinergic signaling and neuronal activity in hippocampal parvalbumin-positive (PV+) and somatostatin-positive (SST+) inhibitory interneurons, respectively. We further reveal that selective co-activation of α 7- and α 4 β 2-nAChRs is necessary to reverse hippocampal network dysfunction, amyloid pathology, and fear memory loss in the amyloid pathology mouse model by enhancing hippocampal inhibition.

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CHAPTER 1 – INTRODUCTION

History of Alzheimer's Disease

Alzheimer's disease (AD) is characterized as the most expensive disease in America, as of 2020 direct costs have been estimated at \$305 billion, with about 5.8 million Americans that suffer from this disease (1). Despite decades of research, the exact cause of AD remains unknown, and there is currently no cure (2). Therefore, understanding the biological mechanisms underlying AD is essential for developing more effective diagnostic tools and therapeutic strategies.

AD is characterized by two major pathological hallmarks: extracellular accumulation of beta-amyloid peptide ($A\beta$) and intracellular aggregation of hyperphosphorylated tau into neurofibrillary tangles (2). AD is named after the German psychiatrist and neurologist Dr. Alois Alzheimer. In 1906, he examined a patient named Auguste D., who experienced severe memory loss, confusion, and unusual behavioral changes. After the patient passed away, Dr. Alzheimer examined her brain and made two important observations. He found abnormal protein clumps, now known as amyloid plaques, and twisted fibers inside nerve cells, called neurofibrillary tangles. These two pathological features are now recognized as the hallmark characteristics of AD. In 1907, Alzheimer reported his findings after studying several additional cases of what was then called "senile dementia". These characteristic plaques in 12 out of 16 patients were found later, providing strong evidence that these brain abnormalities were consistently associated with the disease (3).

Cholinergic Hypothesis

The first major hypothesis proposed to explain AD, known as the cholinergic hypothesis, which was introduced in 1976 (4). This hypothesis suggests that many of the cognitive symptoms of AD result from a loss of cholinergic neurons in the basal forebrain. These neurons produce acetylcholine, a neurotransmitter that plays a critical role in learning, memory, attention, and other cognitive functions (5, 6). Acetylcholine is synthesized in the presynaptic neuron, released into the synapse, and binds to receptors on the postsynaptic neuron to transmit nerve signals (7). Normally, the enzyme acetylcholinesterase (AChE) rapidly breaks down acetylcholine after it has delivered its signal (7). However, in AD, degeneration of cholinergic neurons reduces acetylcholine levels, which weakens communication between neurons and contributes to memory loss and cognitive decline (5). The cholinergic hypothesis was the basis of many first-generation therapy drugs such as acetylcholinesterase inhibitors, which block the breakdown of acetylcholine, allowing it to remain in the synapse longer and enhance neurotransmission (5). These drugs can temporarily improve symptoms in some patients, they do not stop or reverse the progression of AD, suggesting the over production of acetylcholine is not solely the cure of the disease.

Amyloid Hypothesis

After the discovery of A β , researchers proposed the amyloid hypothesis, introduced in 1992 (8). This hypothesis suggests that the accumulation of A β , especially the A β 42 form, is the initiating event that triggers the development of AD. A β is produced when a larger protein called amyloid precursor protein (APP) is cleaved by

specific enzymes (9). In healthy brains, small amounts of A β are produced, moderated, and regularly cleared from the brain (10). However, genetic mutations, such as mutations in the APP, presenilin 1 (PSEN1), or presenilin 2 (PSEN2) genes, or the extra copy of the APP gene in Down syndrome (Trisomy 21), increase the production of the more aggregation-prone A β 42 peptide (11). A β 42 molecules then begin to aggregate, first forming soluble oligomers, which are considered the most toxic form because they interfere with communication between neurons (10). These oligomers accumulate further to form insoluble amyloid plaques, which are deposited in the extracellular space of the brain (10). According to the amyloid hypothesis, this accumulation creates aggregate stress, which then triggers a cascade of downstream events. These include the formation of tau filaments, also known as neurofibrillary tangles, followed by neuronal dysfunction, brain cell death, and ultimately the development of dementia (12). A β accumulation is thus considered the driving event leading to a pathological cascade, making it a major target for AD drug development.

To better understand the amyloid hypothesis, it's important to know how A β is produced. There are two different pathways of APP processing. Amyloid precursor protein is a protein found on the surface of neurons. APP can be processed through two different pathways: the non-amyloidogenic pathway and the amyloidogenic pathway (10). The non-amyloidogenic pathway, which is considered the normal and protective pathway, APP is first cleaved by the enzyme α -secretase. This cut occurs within the A β region, preventing A β production. The remaining fragment is then cleaved by γ -secretase, producing harmless fragments such as sAPP α , p3, and AICD. No A β is made, thus, this pathway does not contribute to AD (**Fig. 1**).

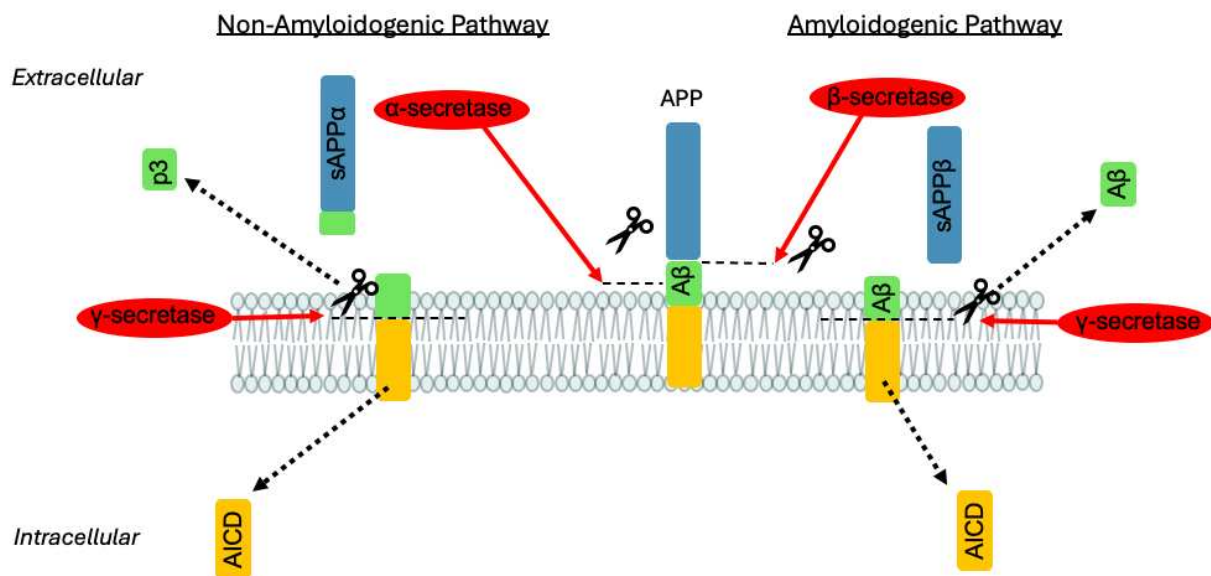


Figure 1. Illustration of the non-amyloidogenic and amyloidogenic pathway with cleavage sites.

The non-amyloidogenic pathway APP is processed and cleaved by α -secretase within the Aβ sequence not allowing for Aβ formation while releasing sAPPα. Remaining fragment cleaved by γ -secretase forming fragments p3 and AICD. The amyloidogenic pathway APP is processed and cleaved by β secretase at the N-terminus of the Aβ region, releasing sAPPβ. Remaining fragment cleaved by γ -secretase forming fragments Aβ peptides and AICD.

The amyloidogenic pathway on the other hand is associated with AD. In this pathway, APP is first cleaved by β -secretase, also known as BACE1. Remaining fragment is then cut by γ -secretase, releasing Aβ peptides, particularly Aβ42. Aβ42 is identified as the more toxic form of Aβ because it is more likely to stick together and aggregate than other forms, such as Aβ40 (10). These peptides first form small soluble oligomers, which are highly toxic to neurons, and over time they accumulate into amyloid plaques, one of the hallmark features of AD (**Fig. 1**).

FDA-approved medications currently available for treatment can be divided into two main categories: symptomatic drugs or disease modifying drugs (13). The first group of drugs are cholinesterase inhibitors, such as Donepezil (Aricept), Rivastigmine (Exelon), and Galantamine (Razadyne) (14). As discussed earlier with the cholinergic hypothesis, these drugs work by blocking the enzyme acetylcholinesterase, which normally breaks down acetylcholine. By increasing acetylcholine levels in the brain, they can provide temporary improvements in memory, thinking, and daily functioning. However, they do not slow or stop disease progression; they only help manage symptoms, thus a symptomatic drug (15, 16). The second group is NMDA receptor antagonists, such as Memantine (Namenda) (17). Memantine works by regulating glutamate signaling. Excessive glutamate activity can damage neurons through excitotoxicity. By blocking excessive activation of NMDA receptors, memantine helps protect neurons and improve symptoms, again a symptomatic drug (18). There is also a combination therapy, Memantine plus Donepezil (Namzaric), which combines both mechanisms (19, 20). The third and newest group of drugs targets the underlying disease process making them disease modifying drugs. These are A β monoclonal antibodies, such as, Lecanemab (Leqembi) and Donanemab (Kisunla) (21, 22). These drugs are designed to remove A β from the brain, with the goal of slowing disease progression. While these newer therapies seem optimistic they are very expensive, costing approximately \$26,500 to \$32,000 per year, and they require regular intravenous infusions and careful patient monitoring (23). In addition, they carry the risk of amyloid-related imaging abnormalities (ARIA) which include brain swelling and small

brain hemorrhages (21). Patients require periodic MRI scans during treatment to monitor these risks.

Alternative Tau Hypotheses

Even after decades of research, it is still unclear what the primary cause of AD is. Although the amyloid hypothesis has dominated the field for many years, other hypotheses also emerged trying to uncover the singular or multifactorial mechanisms underlying AD. As tau filaments are also a hallmark of AD, it was also investigated as a primary trigger. While tau normally binds and stabilizes microtubules in axons (24-26). When tau gets hyperphosphorylated, tau detaches from microtubules and conformational changes or misfolding occurs (27, 28). These misfolded aggregates then can further form into neurofibrillary tangles (28, 29). The original tau hypothesis characterized mature neurofibrillary tangles as the primary target (30, 31) however with more research it has shifted the field to soluble tau as the toxic species (32-34). Soluble tau oligomers impair synaptic function, promote aggregation of endogenous tau, and propagate pathology (35, 36). Although soluble tau oligomers are increasingly recognized as key elements of neurotoxicity, the tau oligomer hypothesis has not replaced the amyloid hypothesis because human genetic evidence strongly supports A β accumulation as the initiating event in AD (8, 10).

Another hypothesis is the tau propagation hypothesis, also known as the “prion-like” or “seeding” hypothesis. This hypothesis proposes that misfolding spreads through the brain in a manner like prion proteins (37, 38). Following hyperphosphorylation and misfolding, pathological tau is released from affected neurons into the extracellular

space, where it can be taken up by neighboring neurons (38, 39). Once internalized, the misfolded tau acts as a “seed”, converting native soluble tau into the same abnormal conformation (37-39). As tau pathology continues to spread, it also self-amplifies. Tau is not infectious like the prion protein that is responsible for prion disease. With tau’s ability to undergo templated misfolding, self-amplification, and propagate itself, it has become known as a prion-like protein (37-39). This hypothesis, however, is generally considered a model of disease progression rather than disease initiation and the prion-like tau propagation drives neurodegeneration and cognitive decline (40-42).

Amyloid Cascade Hypothesis

Compared with other tau hypotheses, the amyloid cascade hypothesis model takes a multifactorial approach by integrating the two pathological hallmarks of AD. Rather than considering A β and tau as different mechanisms, it proposes that A β initiates the disease process, while pathological tau drives neurodegeneration and cognitive decline (10, 43-45). The current view of Alzheimer's disease is that the amyloid and tau hypotheses are complementary (43, 44, 46). The amyloid cascade hypothesis, A β accumulation triggers a series of downstream events, including neuroinflammation and activation of kinases that promote tau hyperphosphorylation and misfolding (43, 47, 48). The resulting pathological tau then spreads from neuron to neuron, leading to synaptic dysfunction, neuronal loss, and cognitive decline (43, 49, 50). The amyloid cascade hypothesis is further supported by evidence showing that amyloid accumulation occurs many years before symptoms appear, while tau pathology correlates much more closely to clinical symptoms with neuronal damage and the

severity of cognitive decline (43, 51, 52). The amyloid hypothesis is generally considered the stronger model because mutations that increase A β production are sufficient to cause familial AD, and amyloid accumulation consistently precedes widespread tau pathology (10, 11, 51). The amyloid cascade hypothesis seems the most compelling because it acknowledges the multifactorial nature of Alzheimer's disease.

Limitations in Current AD Biomarkers

Even with currently available treatments, AD remains one of the greatest challenges in medicine. Despite decades of research, the exact cause of AD is still not fully understood (53, 54). We know that factors such as amyloid, tau, genetics, inflammation, and aging all contribute, but no single mechanism can completely explain the disease. As a result, there is still no cure that can stop or reverse AD. Most treatments are not highly selective and often target only one disease mechanism, such as acetylcholine or A β . AD is a complex, multifactorial disorder, so targeting a single pathway may produce only limited therapeutic effects (55). Hence, the challenges we face in clinical trials are also further complicated with patient enrollment for the study (56, 57). Many patients are diagnosed and enrolled in clinical trials after significant and irreversible brain damage has already occurred. At this stage, treatments are much less likely to be effective. There is still no reliable biomarkers for detecting AD at its earliest stages for diagnosis and for monitoring treatment responses (58, 59). To achieve better outcomes, we need earlier diagnosis, more reliable biomarkers, and therapies that target multiple disease mechanisms rather than a single pathway (60).

AD begins many years before symptoms become noticeable enough within clinics (52). A β accumulation is one of the earliest detectable changes, beginning during the preclinical stage, long before cognitive impairment occurs. As the disease further progress, A β levels gradually plateau (**Fig. 2**). Tau pathology develops after amyloid accumulation and becomes more prominent during the prodromal or the mild cognitive impairment stage (**Fig. 2**). This then leads to neurodegeneration, which refers to the gradual loss of neurons and brain tissue (**Fig. 2**). This results in cognitive decline (**Fig. 2**). Cognition remains relatively normal during the early stages, despite significant amyloid accumulation. Memory loss and cognitive symptoms only become obvious after substantial brain pathology has already occurred, thus the difficulty in early diagnosis. Perhaps a solution to this can be the detection of changes in brain function, or neurophysiology, potentially as a reliable biomarker for AD. As discussed previously, the biological changes of AD occur long before cognitive symptoms develop. Therefore, early biomarkers are essential for early diagnosis, patient selection, and improving the effectiveness of future drug development.

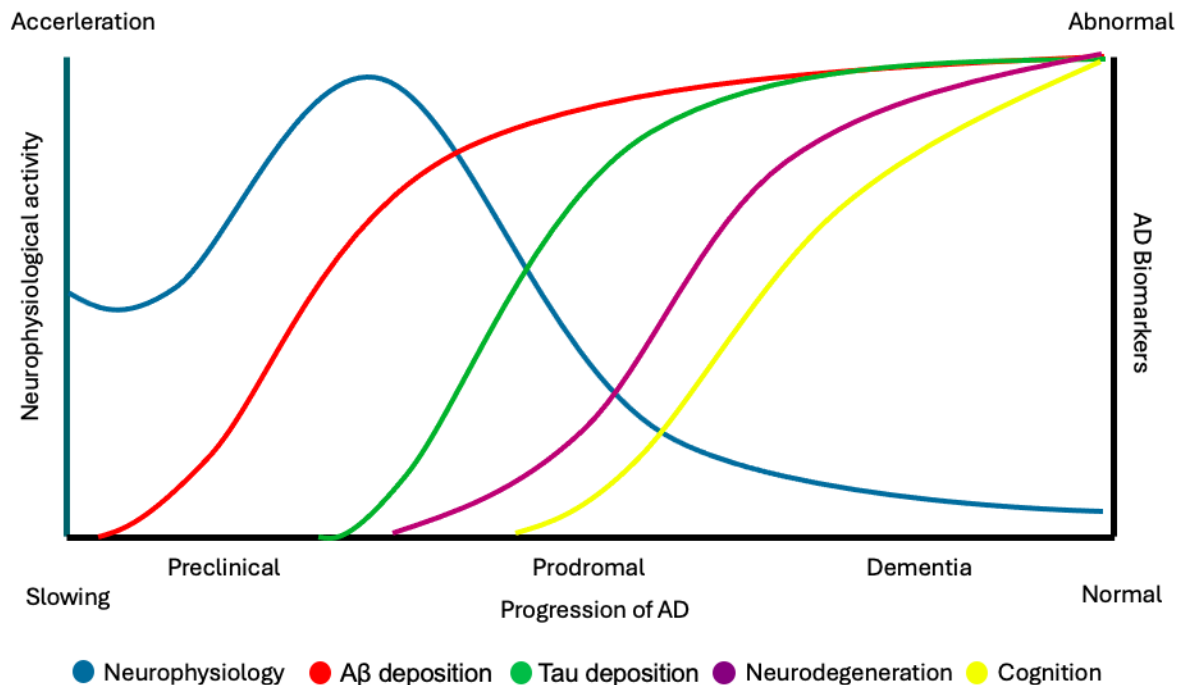


Figure 2. Illustrative graph of the progression of AD with pathological markers.

Adapted from Gallego-Rudolf *et al.*

Progression of disease stages along time course categorized as preclinical, prodromal (mild incognitive impairment), and dementia. Left axis corresponds to acceleration and slowing of neurophysiological activity. Right axis corresponds to AD pathological changes from normal to abnormal. During preclinical stage, Aβ deposition changes occur. During prodromal stage tau deposition, neurodegeneration, and cognition changes occur. Neurophysiology activity accelerates occur early in preclinical stage and rapidly declines in prodromal stage as pathological changes occur.

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A β Disrupts Neuronal Activity and Excitation-Inhibition Balance

A critical unanswered question is how A β causes cognitive impairment. Increasing evidence suggests that these pathological changes begin many years before the onset of clinical symptoms (61). Both preclinical and clinical studies have demonstrated that cortical and hippocampal hyperactivity is present during the earliest stages of AD and strongly correlates with subsequent cognitive decline (61-64). Interestingly, neuronal activity itself enhances the release of both A β (65, 66) and tau (67), suggesting the existence of a pathological positive-feedback loop in which increased neuronal activity promotes AD pathology, which in turn further increases neuronal hyperexcitability. At the neuron level, soluble A β oligomers interact directly with neurons and synapses (10). Rather than immediately killing neurons, these A β oligomers first disrupt synaptic function and make neurons hyperexcitable (61, 68). In other words, neurons begin firing more frequently than they should. This excessive activity disrupts the balance between excitation and inhibition (E/I) (61). Excitatory neurons stimulate brain activity, while inhibitory neurons suppress excessive firing. A healthy balance between these two systems is essential to regulate information processing (61, 69). A β is known to disrupt E/I balance to induce neuronal hyperexcitation (70). The resulting E/I imbalance impairs communication within neuronal circuits, making it more difficult for the brain to process information efficiently (70). Local circuit abnormalities then spread to the brain network level (70). Since different brain regions work together through interconnected networks, disrupted communication eventually affects the entire network (70). As these networks become dysfunctional, patients begin to experience memory loss, impaired learning, and other cognitive

impairments (70). A β doesn't only accumulate as plaques, it also disrupts neuronal function by causing hyperexcitability and E/I imbalance. This suggests that restoring normal neuronal activity and rebalancing E/I could be a promising therapeutic strategy for AD.

The Role of Nicotinic Acetylcholine Receptors in AD

Understanding the molecular mechanisms underlying A β -induced neuronal hyperexcitability and finding potential therapeutically targetable pathways have become major foci of AD research (70). My research addresses this critical knowledge gap. One potential molecular target that may explain how A β causes neuronal hyperexcitability are nicotinic acetylcholine receptors (nAChRs) (71, 72). The cholinergic system is essential for learning and memory. nAChRs are one of the major receptors that respond to the neurotransmitter acetylcholine and play an important role in regulating synaptic transmission and neuronal excitability (73). Research has shown that beta-amyloid binds to these receptors with very high affinity, making them one of its important molecular targets (72, 74). When A β binds to these receptors, it can alter Ca²⁺ signaling within neurons (75, 76). This abnormal Ca²⁺ influx affects several intracellular signaling pathways, leading to changes in synaptic plasticity, which is the ability of synapses to strengthen or weaken during learning and memory (77). These signaling changes ultimately impair long-term potentiation (LTP), which is essential for memory formation, while promoting long-term depression (LTD), which help refine memories (78). Nonetheless, the exact role of nAChRs is still debated (79). Some studies suggest that A β activates nicotinic receptors, causing excessive Ca²⁺ entry and neuronal

hyperexcitability (66, 74). While other studies report that A β blocks or desensitizes these receptors, reducing cholinergic signaling and impairing cognition (66). Conflicting findings suggest that the effects of A β may depend multiple factors such as concentration, aggregation state, receptor subtype, or stage of disease.

nAChRs are a large family of receptors made up of different combinations of alpha (α) and beta (β) subunits (80). Neuronal nicotinic acetylcholine receptors (nAChRs) are a diverse family of ligand-gated ion channels assembled from nine α subunits (α 2- α 10) and three β subunits (β 2- β 4), each encoded by a distinct gene (CHRNA2-CHRNA10 and CHRNA2-CHRNA4), allowing for many different subunits (81, 82). All nAChRs share a conserved pentameric architecture composed of five subunits surrounding a central ion-conducting pore (83). Each subunit contains a large extracellular N-terminal ligand-binding domain, four transmembrane helices (M1-M4), a large intracellular loop between M3 and M4, and a short extracellular C-terminal domain (80, 83). The M2 helices from each subunit line the pore and form the ion channel (83, 84). Depending on how these subunits assemble, they form receptors with different structures, functions, and distributions throughout the human body (83). Each receptor subtype has different physiological roles and pharmacological properties, beta-amyloid may affect each subtype differently. This diversity helps explain why previous studies have produced conflicting results regarding the interaction between A β and nicotinic receptors. We cannot treat all nAChRs the same, it is essential to determine which receptor subtype is involved in AD to identify the subtype could lead to more selective therapies with improved efficacy (85). Additionally, nicotinic cholinergic system is highly diverse (83). Different receptor subtypes have distinct structures, locations, and

functions, meaning that their responses to A β are likely to differ (76, 79). It is thus important to understand this complexity in further developing targeted AD therapies.

Our lab has previously studied how the molecular mechanism underlying A β -induced neuronal hyperexcitability by nAChR inhibition-mediated disinhibition (71, 72). In healthy brains, inhibitory interneurons release GABA, which suppresses excessive activity in excitatory neurons. This balance between excitation and inhibition is essential for normal brain function. A β preferentially targets inhibitory interneurons (86, 87). Specifically, it inhibits two nAChR subtypes: α 7 and α 4 β 2 nAChRs (71, 72). α 7-nAChRs are composed of five identical α 7 subunits (88). They are highly permeable to Ca²⁺ and are widely expressed in the hippocampus and cortex, where they regulate neurotransmitter release, synaptic plasticity, learning and memory (89, 90). The high calcium permeability enables activation of intracellular signaling pathways involved in neuronal survival and long-term potentiation (LTP) (89). α 4 β 2-nAChRs are composed of α 4 and β 2 subunits and represent the most abundant nAChR subtype in the mammalian brain (81, 83). It is expressed throughout the cortex, hippocampus, and thalamus and exhibit high affinity for acetylcholine and nicotine (81, 91). Activation of α 4 β 2-nAChRs regulates neuronal excitability, neurotransmitter release, and cognitive processes, including attention, learning, and working memory (73, 92). Unlike α 7 receptors, α 4 β 2-nAChRs have lower Ca²⁺ permeability but contribute strongly to fast cholinergic neurotransmission (89, 93). Reduced expression and impaired function of α 4 β 2-nAChRs have been reported in AD, suggesting that dysfunction of this receptor subtype contributes to cognitive decline (94, 95).

As a result, the inhibitory interneurons become less active, releasing less GABA onto excitatory neurons via disinhibition (71, 72). This reduction of inhibitory activity makes excitatory neurons hyperexcitable, disrupting normal neuronal communication, which results in cognitive dysfunction which is also observed in AD. Importantly, we found that when both $\alpha 7$ and $\alpha 4\beta 2$ receptors are co-activated using selective agonists, the A β -induced hyperexcitability was reversed (71, 72). Interestingly, it was found that non-selective activation of all nicotinic receptors was harmful and accelerated pathological changes (71, 72). This suggests that targeting specific receptor subtypes is much more beneficial than broadly activating the entire cholinergic system.

A β selectively binds to the $\alpha 7$ and $\alpha 4\beta 2$ -nAChRs, but not to the $\alpha 3\beta 4$ receptor (71). $\alpha 3\beta 4$ -nAChRs are composed of $\alpha 3$ and $\beta 4$ subunits (83, 96). It is expressed predominantly in autonomic ganglia and, within the central nervous system, are highly enriched in the medial habenula and interpeduncular nucleus (83, 97). Compared with $\alpha 7$ -nAChRs, $\alpha 3\beta 4$ receptors exhibit lower Ca^{2+} permeability and mediate fast cholinergic neurotransmission (89, 98). A β fits into a specific binding pocket on the receptor, a direct physical interaction between the peptide and the receptor (99, 100). A β directly interacts with $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs through residues within Loop C of the extracellular ligand-binding domain (99, 100). Mutational analysis identified Arg208 and Glu211 as critical determinants of A β binding, suggesting that A β binds at or near the orthosteric ligand-binding pocket (99, 100). We have further compared the amino acid sequences of different nicotinic receptor subunits from both humans and mice. Several conserved amino acid residues that are present in the $\alpha 7$ and $\alpha 4$ subunits but are absent or different in the $\alpha 3$ subunit (71). Arg208 and Glu211 are likely responsible for the high-

affinity binding of A β to α 7 and α 4 β 2 receptors. Because α 3 β 4 receptors lack these critical residues, A β cannot bind, explaining why this receptor subtype is not affected. This study shows how A β selectively affects the cholinergic system in AD. Thus, selective co-activation of α 7 and α 4 β 2 receptors may represent a promising therapeutic strategy for AD.

The hippocampus is highly vulnerable in AD (101). A key component that is associated with hyperexcitation are hippocampal GABAergic inhibitory interneurons (102, 103), especially parvalbumin-positive (PV+) and somatostatin-positive (SST+) cells (**Fig. 3**).

Normal

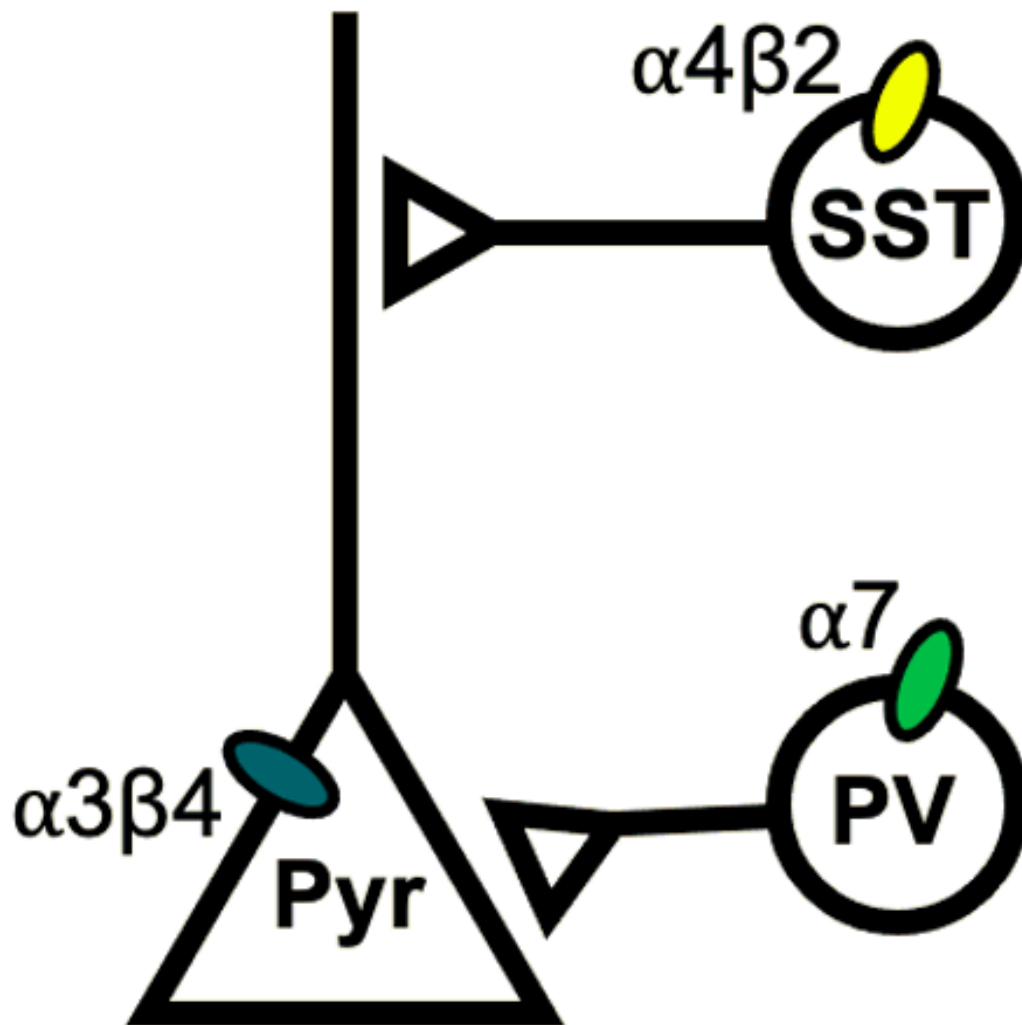


Figure 3. A schematic model of local hippocampal circuit.

$\alpha 7$ -, $\alpha 4 \beta 2$ -, and $\alpha 3 \beta 4$ -nAChRs predominantly control the nicotinic cholinergic signaling in parvalbumin-positive (PV+), somatostatin-positive (SST+), and pyramidal cells (Pyr), respectively, in the hippocampus.

These cells are critical for maintaining network balance and cognitive function (69, 101-104), however when A β disrupts inhibition of these interneurons leading to increased excitation (69), it leads to network dysfunction and memory loss (86, 104-107).

Hyperexcitability causes a disruption in the net neuronal network, which in turn disrupts the many processes carried out. Alterations or changes in the hippocampus network can impact changes to the learning and memory processes (108). These abnormal changes in the network activity, hyperexcitability, can be observed many years prior to behavior onsets (108) and thus suggesting hippocampal network activity as a biomarker to detect AD. To test this, we can measure the network oscillations, local field potentials (LFP), that are regulated by PV+ and SST+ hippocampal inhibitory interneurons (69, 103, 104).

There are three major nAChRs in the hippocampus: α 7, α 4 β 2, and α 3 β 4 (83, 109, 110). α 7, α 4 β 2, and α 3 β 4 subtypes expressed predominantly in PV+, SST+, and excitatory neurons, respectively (83, 109-116). PV+ cells regulate somatic inhibition and SST+ cells regulate dendritic inhibition (103). This coordinated activation enhances overall network inhibition. It has been found that A β binds selectively to the α 7- and α 4 β 2- nAChRs subtypes, but not to α 3 β 4-nAChRs (72) (**Fig. 3**).

Clinical studies are observational and limited in the mechanistic information that can be tested in living patients. In contrast, animal studies permit the manipulation of variables and the investigation of effects on an entire organism. The animal model, however, is disadvantaged by species differences. AD is a strictly human disease, and we are aware that rodents do not develop true AD (117). Nonetheless, multiple transgenic mouse models develop the pathological hallmarks of the disease as they

age, like those found in humans (117, 118). We thus employ a widely used amyloid pathology mouse model of AD, 5XFAD. The 5XFAD mouse is a transgenic model with five different familial Alzheimer's mutations that overexpresses the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations, human PSEN1 carrying the M146L and L286V mutations (119, 120). These five mutations accelerate A β 42 production, resulting in rapid amyloid plaque deposition, neuroinflammation, synaptic dysfunction, neuronal loss, and cognitive impairment beginning as early as 2-4 months of age (119, 120) but do not show cognitive deficits beginning at approximately 4-5 months of age and aggressive impairments in hippocampal-dependent learning and memory observed by 6 months of age (120, 121). In fact, it is found that co-activation of α 7- and α 4 β 2-nAChRs is necessary to restore hippocampal inhibition, reduce hyperexcitability, improve memory, and reduce amyloid pathology in the 5XFAD mouse model of AD. This suggests that precise targeting specific nAChR subtypes on interneuron populations is a promising therapeutic strategy for AD.

Several critical questions remain unclear regarding the mechanisms underlying hippocampal hyperexcitability and cognitive impairments and how they can be therapeutically reversed. This dissertation will investigate whether α 7- and α 4 β 2-nAChRs differentially regulate distinct hippocampal inhibitory interneuron populations to restore the excitation-inhibition balance that has been disrupted by A β in AD. It will further examine whether fear learning reveals deficits in hippocampal network activity in the 5XFAD mouse model and whether co-activation of α 7- and α 4 β 2-nAChRs will be required to restore normal hippocampal network function. Finally, it will investigate whether restoring hippocampal circuit activity through co-activation of α 7- and α 4 β 2-

nAChRs subtypes translates into improved memory and reduce AD pathology in 5XFAD mice.

CHAPTER 2 - SUBTYPE-SPECIFIC NICOTINIC REGULATION OF HIPPOCAMPAL INTERNEURONS

It is crucial to first investigate the roles of nAChR subtypes in regulating hippocampal inhibitory interneurons and restoring network function that is disrupted by A β . Specifically, to examine whether $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs differentially regulate PV+ and SST+ interneurons and whether the activation of these receptor subtypes can counteract A β -induced neuronal dysfunction. Then to investigate if selective activation of nAChRs reverses the A β -induced reduction in the activity of their corresponding interneuron populations. By identifying the receptor subtypes involved in regulating inhibitory interneuron function, to determine whether subtype-specific nicotinic modulation could provide a more targeted therapeutic strategy for restoring hippocampal circuit function in AD.

Nicotinic cholinergic regulation of cultured hippocampal PV+ and SST+ interneurons is mainly mediated by $\alpha 7$ - and $\alpha 4\beta 2$ -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 Ca²⁺ imaging with nicotine uncaging in 12-14 days *in vitro* (DIV) cultured mouse hippocampal neurons as neuronal nAChRs are ligand-gated ion channels, when activated, they induce membrane depolarization and increase somatic Ca²⁺ levels (122). We found that nicotinic cholinergic activity measured by Ca²⁺ transients in inhibitory interneurons was

significantly higher than in excitatory neurons (EX, $1.000 \pm 0.879 \Delta F/F_0$ and IN, $1.575 \pm 1.208 \Delta F/F_0$, $p = 0.0109$) (**Fig. 4 and Table 1**). This finding is consistent with the previous reports that cholinergic regulation of inhibitory cells is stronger than excitatory neurons in the hippocampus (111-116).

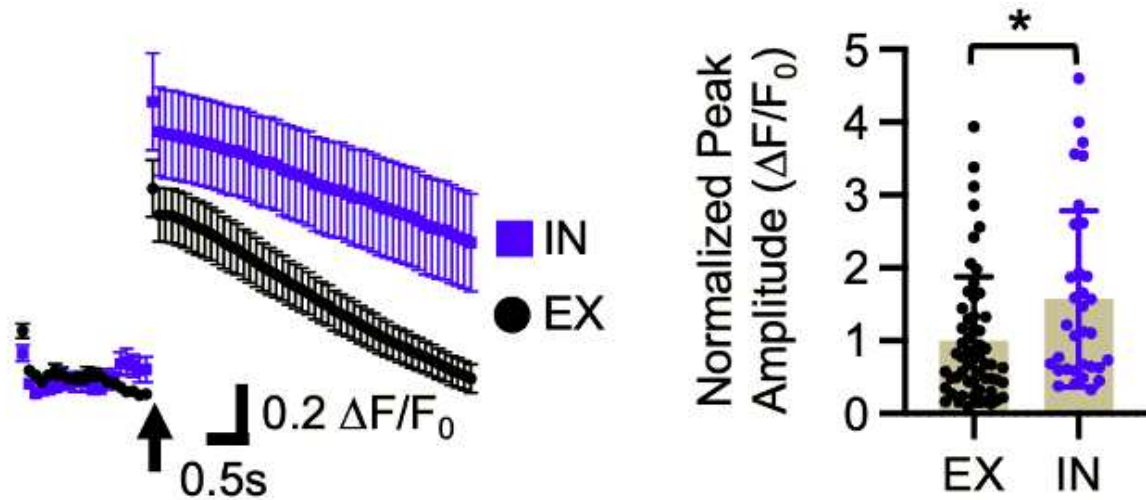


Figure 4. More nicotinic cholinergic activity in cultured hippocampal in inhibitory interneurons.

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). * $p < 0.05$, unpaired two-tailed student's t-test.

We 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 AulB (αCTx), $\alpha 3\beta 4$ -receptor inhibitor, significantly reduced nicotinic cholinergic activity, while 50 nM α -Bungarotoxin (αBTx), an $\alpha 7$ -receptor inhibitor, or 1 μM Dihydro- β -erythroidine hydrobromide (DH β E), an $\alpha 4\beta 2$ -receptor inhibitor, had no effects (CTRL, 1.000 ± 0.492 $\Delta\text{F}/\text{F}_0$, αBTx , 0.870 ± 0.328 $\Delta\text{F}/\text{F}_0$, $p = 0.8881$, DH β E, 1.042 ± 0.644 $\Delta\text{F}/\text{F}_0$, $p = 0.9932$, and αCTx , 0.325 ± 0.362 $\Delta\text{F}/\text{F}_0$, $p = 0.0003$) (**Fig. 5a and Table 2**).

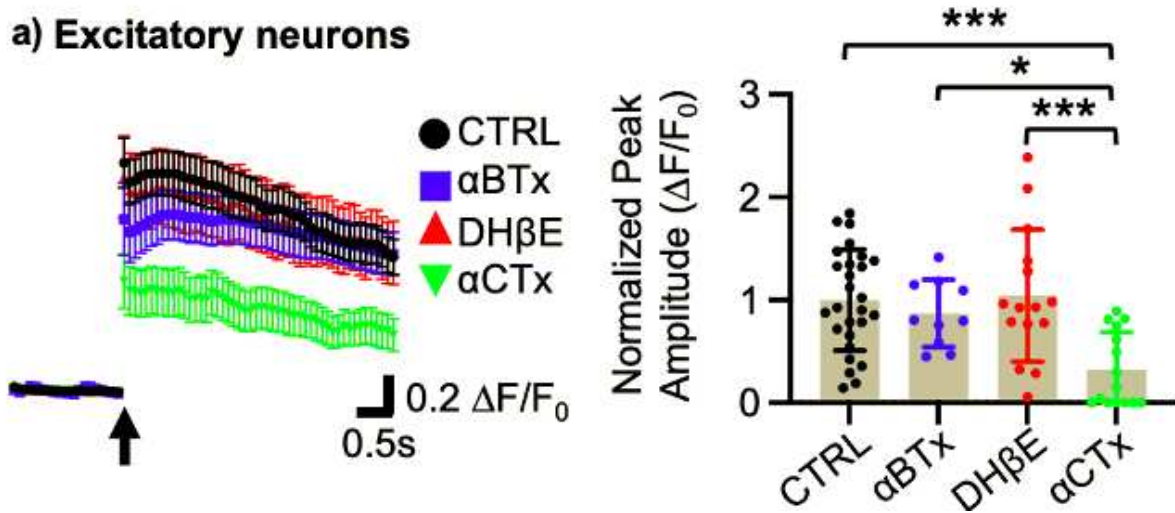


Figure 5. Nicotinic cholinergic regulation of cultured hippocampal excitatory cells is mediated by $\alpha 3\beta 4$ -nAChRs and inhibitory interneurons is mainly mediated by $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs.

Average traces of GCaMP6f signals and summary graphs of normalized peak amplitude in each condition in **a)** excitatory neurons (n = number of cells, CTRL = 27, αBTx = 10, DH β E = 15, and αCTx = 15 from 4 pups in 2 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. Work done by Seonil Kim.

In inhibitory cells, when compared to controls (CTRL), α BTx or DH β E treatment significantly reduced nicotine-induced activity, but the α 3 β 4 antagonist had no effect (CTRL, $1.000 \pm 0.525 \Delta F/F_0$, α BTx, $0.398 \pm 0.290 \Delta F/F_0$, $p = 0.0025$, DH β E, $0.335 \pm 0.300 \Delta F/F_0$, $p < 0.0001$, and α CTx, $0.871 \pm 0.330 \Delta F/F_0$, $p = 0.7866$) (**Fig. 5b and Table 2**). This data show that α 7- and α 4 β 2-nAChRs are mainly responsible for nicotinic cholinergic activity in hippocampal inhibitory cells, while α 3 β 4-nAChRs are important for nicotinic cholinergic activity in hippocampal excitatory cells. These findings are consistent with the previous reports that postsynaptic α 7- and α 4 β 2-nAChRs are mainly localized on GABAergic interneurons in the hippocampus (112, 114), while they are expressed in the presynaptic area of excitatory cells (123-127) to regulate neurotransmitter release without neuronal activation. This suggests that stimulation of α 7- and α 4 β 2-nAChRs has more profound effects on neuronal activation in inhibitory cells.

b) Inhibitory interneurons

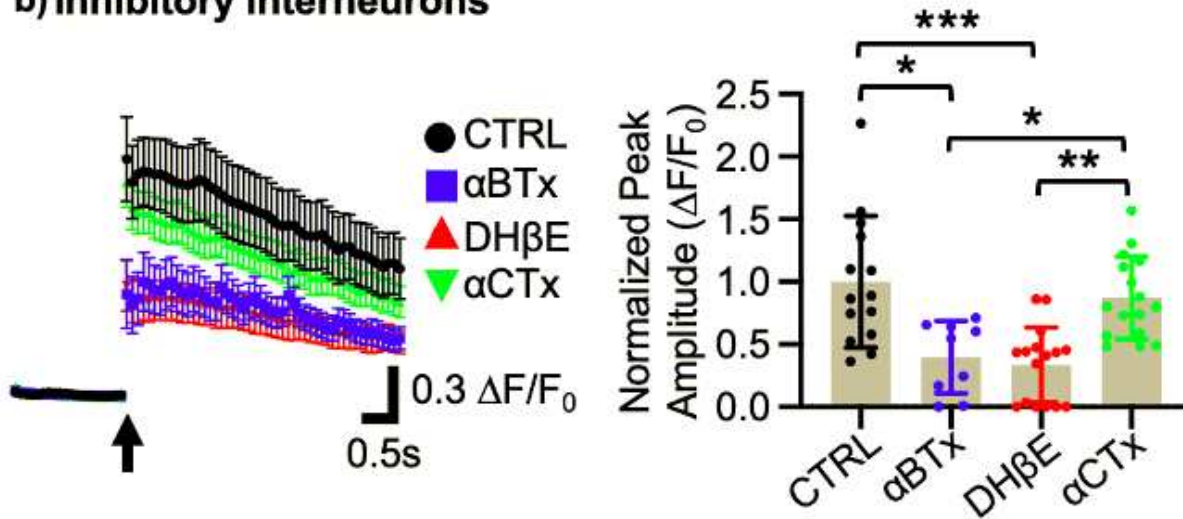


Figure 5. Nicotinic cholinergic regulation of cultured hippocampal excitatory cells is mediated by α 3 β 4-nAChRs and inhibitory interneurons is mainly mediated by α 7- and α 4 β 2-nAChRs.

Average traces of GCaMP6f signals and summary graphs of normalized peak amplitude in each condition in **b)** inhibitory interneurons (n = number of cells, CTRL = 14, α BTx = 9, DH β E = 16, and α CTx = 16 from 4 pups in 2 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. Work done by Seonil Kim.

Subtype-specific roles of nAChRs in cultured hippocampal PV+ and SST+ interneurons was analyzed. α 3 β 4-nAChRs are shown to mainly regulate excitatory neurons, the role of α 7- and α 4 β 2-nAChRs in inhibitory cells was looked at more closely. PV+ interneurons, when compared to controls (CTRL), acute treatment of α BTx reduced nicotinic cholinergic activity, while DH β E treatment had no effect (CTRL, 1.000 ± 0.590 $\Delta F/F_0$, α BTx, 0.507 ± 0.374 $\Delta F/F_0$, p = 0.0006, and DH β E, 1.076 ± 0.613 $\Delta F/F_0$, p = 0.9340) (**Fig. 6a and Table 3**). Co-treatment of α BTx and DH β E also had no additional effect (α BTx + DH β E, 0.607 ± 0.448 $\Delta F/F_0$) (**Fig. 6a and Table 3**).

a) PV+ interneurons

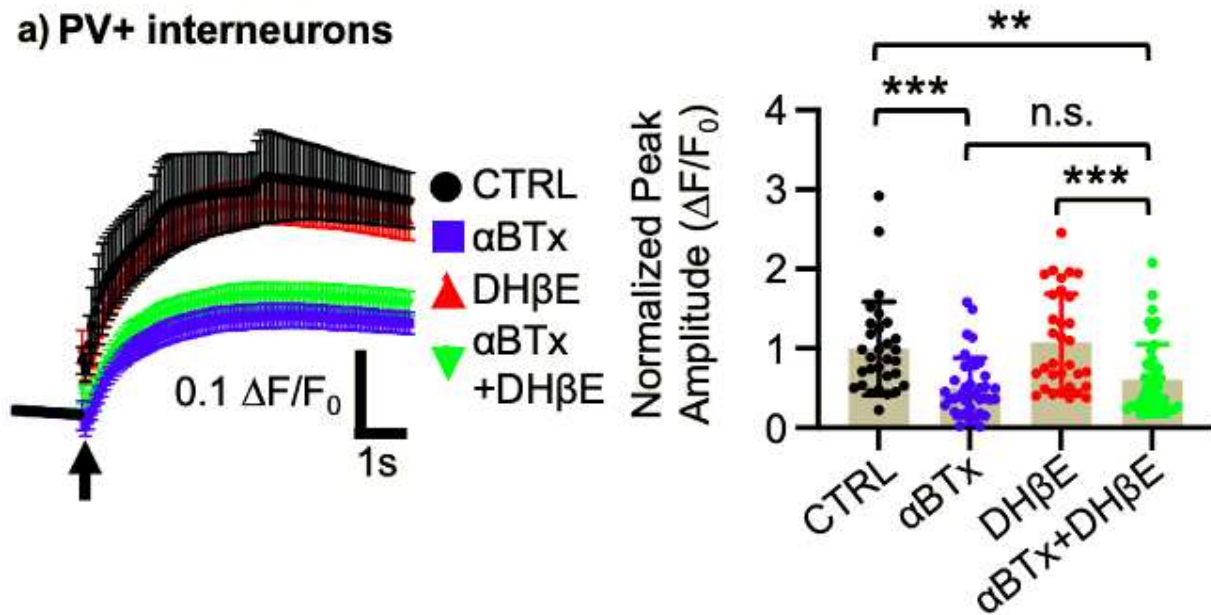


Figure 6. Nicotinic cholinergic regulation of cultured hippocampal PV+ and SST+ interneurons is mainly mediated by $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, respectively.

Average traces of GCaMP7s signals and summary data of normalized peak amplitude in each condition in **a)** PV+ interneurons (n = number of cells, CTRL = 30, α BTx = 38, Dh β E = 32, and α BTx + Dh β E = 43 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. Work done by Seonil Kim.

SST+ interneurons, when compared to controls (CTRL), acute treatment of DH β E reduced nicotinic cholinergic activity, while α BTx treatment had no effect (CTRL, $1.000 \pm 0.426 \Delta F/F_0$, α BTx, $0.917 \pm 0.519 \Delta F/F_0$, $p = 0.8707$, and DH β E, $0.524 \pm 0.262 \Delta F/F_0$, $p < 0.0001$) (**Fig. 6b and Table 3**). Co-treatment of α BTx and DH β E also had no additional effect (α BTx + DH β E, $0.533 \pm 0.272 \Delta F/F_0$) (**Fig. 6b and Table 3**). This shows that $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs play important roles in nicotinic cholinergic regulation of PV+ and SST+ cells, respectively.

b) SST+ interneurons

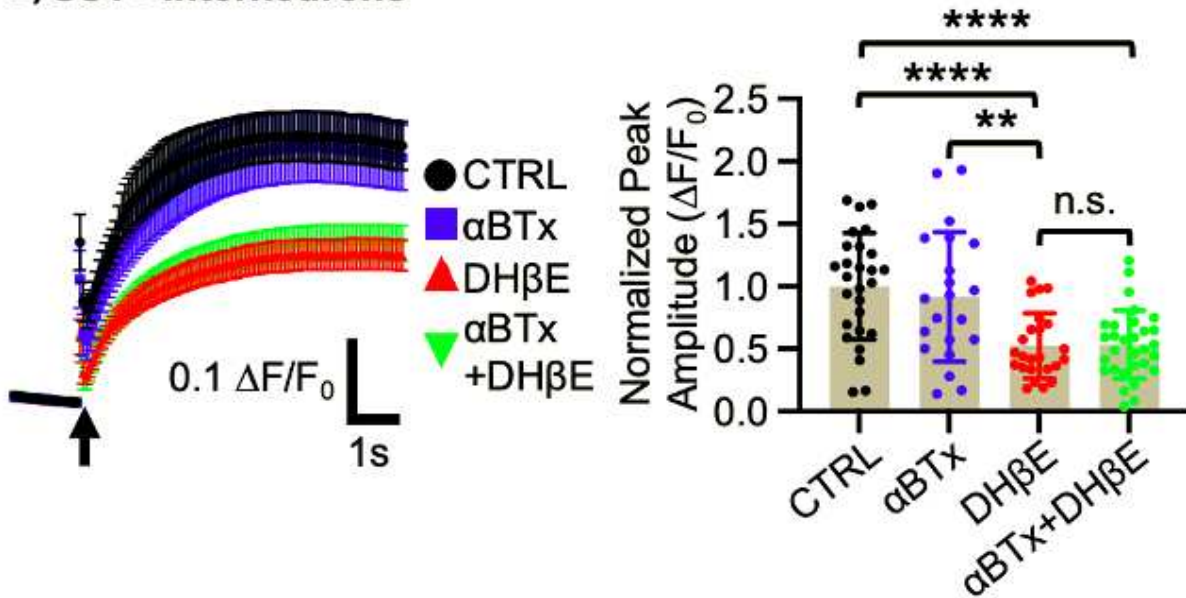


Figure 6. Nicotinic cholinergic regulation of cultured hippocampal PV+ and SST+ interneurons is mainly mediated by $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, respectively.

Average traces of GCaMP7s signals and summary data of normalized peak amplitude in each condition in **b)** SST+ interneurons (n = number of cells, CTRL = 27, α BTx = 21, Dh β E = 24, and α BTx + Dh β E = 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. Work done by Seonil Kim.

The A β induced decrease in the neuronal activity of PV+ and SST+ interneurons is reversed by stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, respectively.

We examined whether A β affected nicotinic cholinergic activity of PV+ and SST+ interneurons using nicotine uncaging as described in **Fig. 1**. We found that 250 nM soluble A β 42 oligomers (oA β 42) treatment significantly reduced nicotine-induced activity in both PV+ and SST+ interneurons compared to neurons treated with 250 nM scrambled A β 42 (sA β 42) (PV+ interneurons, sA β 42, $1.000 \pm 0.772 \Delta F/F_0$ and oA β 42, $0.448 \pm 0.233 \Delta F/F_0$, $p = 0.0038$ and SST+ interneurons, sA β 42, $1.000 \pm 0.391 \Delta F/F_0$ and oA β 42, $0.600 \pm 0.237 \Delta F/F_0$, $p = 0.0002$) (**Fig. 7 and Table 4**).

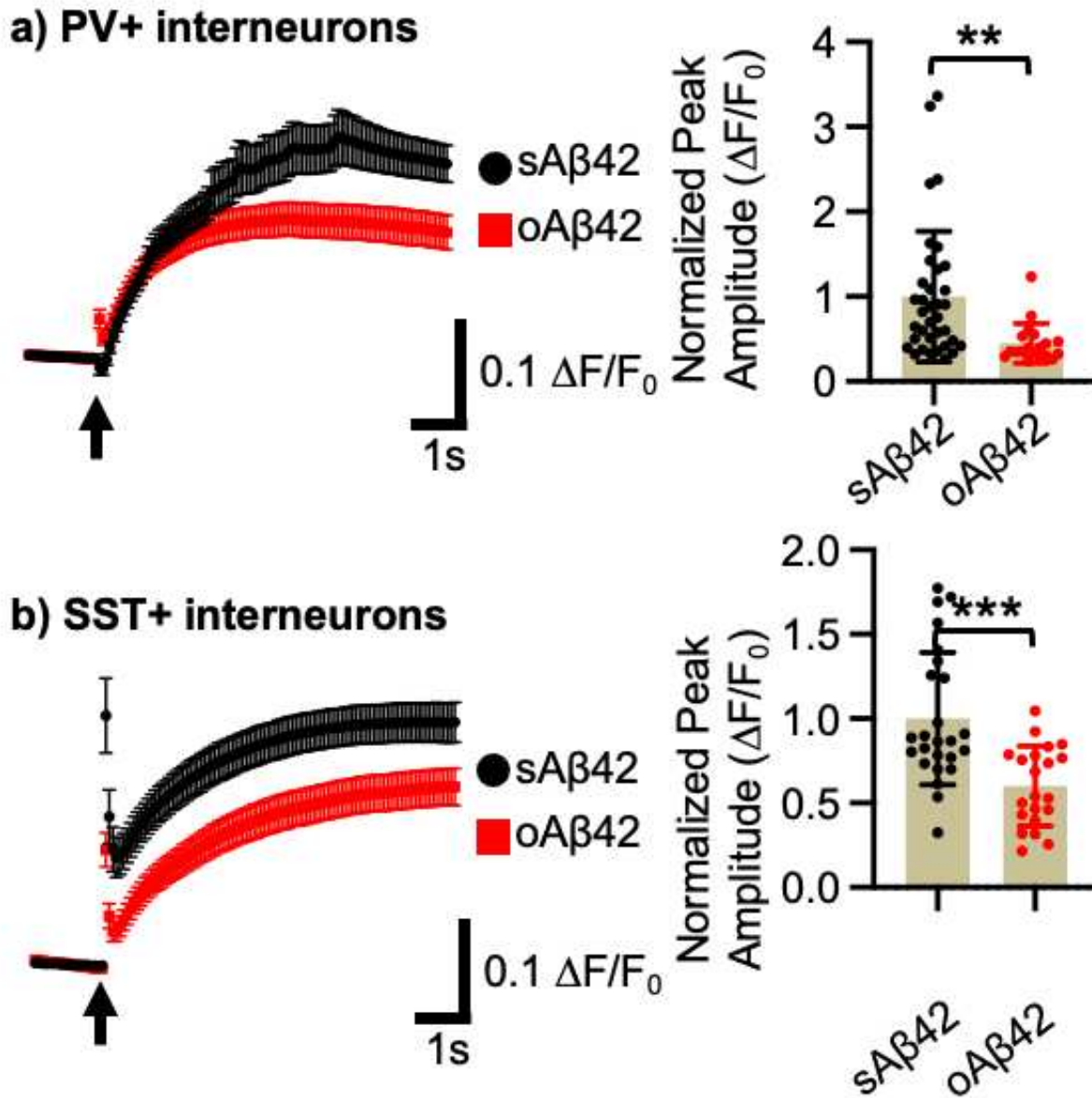


Figure 7. The Aβ-induced decrease in the neuronal activity of PV+ and SST+ interneurons.

Average traces of GCaMP7s signals and summary data of normalized peak amplitude in each condition in **a)** PV+ interneurons (n = number of cells, sAβ42 = 36 and oAβ42 = 19 from 6 pups in 3 independent cultures) and **b)** SST+ interneurons (n = number of cells, sAβ42 = 25 and oAβ42 = 21 from 6 pups in 3 independent cultures). ** $p < 0.01$ and *** $p < 0.001$, unpaired two-tailed student's t-test. An arrow indicates photostimulation.

We further determined how A β affected neuronal activity in PV+ and SST+ interneurons. As somatic Ca²⁺ levels in neurons indicate neuronal activity (128), we measured spontaneous somatic Ca²⁺ activity without tetrodotoxin (TTX) in 12-14 DIV cultured hippocampal neurons as carried out previously (71, 72, 129, 130). 250 nM oA β 42 treatment significantly decreased neuronal activity in both PV+ and SST+ interneurons (PV+ interneurons, sA β 42, $1.000 \pm 0.379 \Delta F/F_{\min}$ and oA β 42, $0.589 \pm 0.223 \Delta F/F_{\min}$, $p = 0.0371$, and SST+ interneurons, sA β 42, $1.000 \pm 0.499 \Delta F/F_{\min}$ and oA β 42, $0.604 \pm 0.295 \Delta F/F_{\min}$, $p = 0.0044$) (**Fig. 8-9, and Table 5-6**). To determine subtype-specific roles of nAChRs in the A β effect on PV+ and SST+ cells' activity, we treated these cells with 250 nM oA β 42 or 250 nM sA β 42 and subtype-specific nAChR agonists - 1 μ M PNU-282987 (PNU), an $\alpha 7$ agonist, 2 μ M RJR-2403 Oxalate (RJR), an $\alpha 4\beta 2$ agonist, or 1 μ M NS-3861 (NS), an $\alpha 3\beta 4$ agonist, as carried out previously (71). In PV+ interneurons, when compared to controls, activation of $\alpha 7$ -nAChRs significantly reversed the A β effects, while stimulation of other nAChR subtype had no effects (oA β 42 + PNU, $1.408 \pm 0.806 \Delta F/F_{\min}$, $p < 0.0001$, oA β 42 + RJR, $0.528 \pm 0.299 \Delta F/F_{\min}$, $p > 0.9999$, and oA β 42 + NS, $0.550 \pm 0.384 \Delta F/F_{\min}$, $p > 0.9999$) (**Fig. 8 and Table 5**). In SST+ interneurons, when compared to controls, stimulation of $\alpha 4\beta 2$ -nAChRs reversed the A β effects, but other agonists were unable to reverse the A β effects (oA β 42 + PNU, $0.557 \pm 0.271 \Delta F/F_{\min}$, $p = 0.9900$, oA β 42 + RJR, $0.994 \pm 0.358 \Delta F/F_{\min}$, $p = 0.0103$, and oA β 42 + NS, $0.561 \pm 0.249 \Delta F/F_{\min}$, $p > 0.9999$) (**Fig. 9 and Table 6**). We discover that the A β induced decrease in the neuronal activity of PV+ and SST+ interneurons is reversed by stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, respectively. Additionally, our findings show that $\alpha 3\beta 4$ -nAChRs are important for nicotinic cholinergic

activity in excitatory cells. This suggests that stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is a novel and ideal way to stimulate PV+ and SST+ interneurons' activity rather than excitatory neurons' activity.

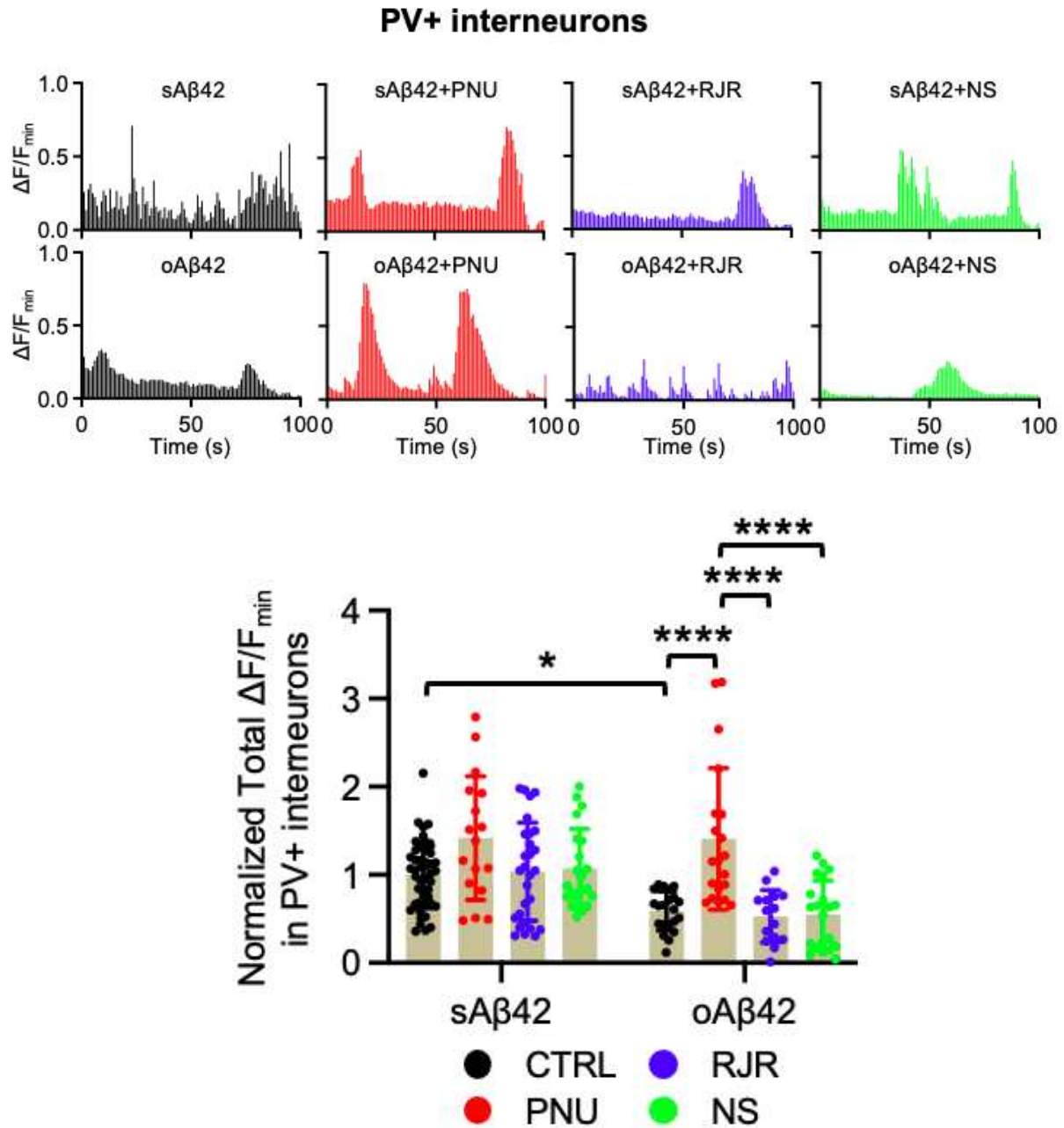


Figure 8. The Aβ-induced decrease in the neuronal activity of PV+ interneurons is reversed by stimulation of α7-nAChRs.

Representative traces of GCaMP7s fluorescence intensity and summary graphs of normalized total Ca²⁺ activity in each condition in PV+ interneurons (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) **p* < 0.05, ***p* < 0.01, and *****p* < 0.0001, Two-way ANOVA, Tukey test. Work done by Seonil Kim.

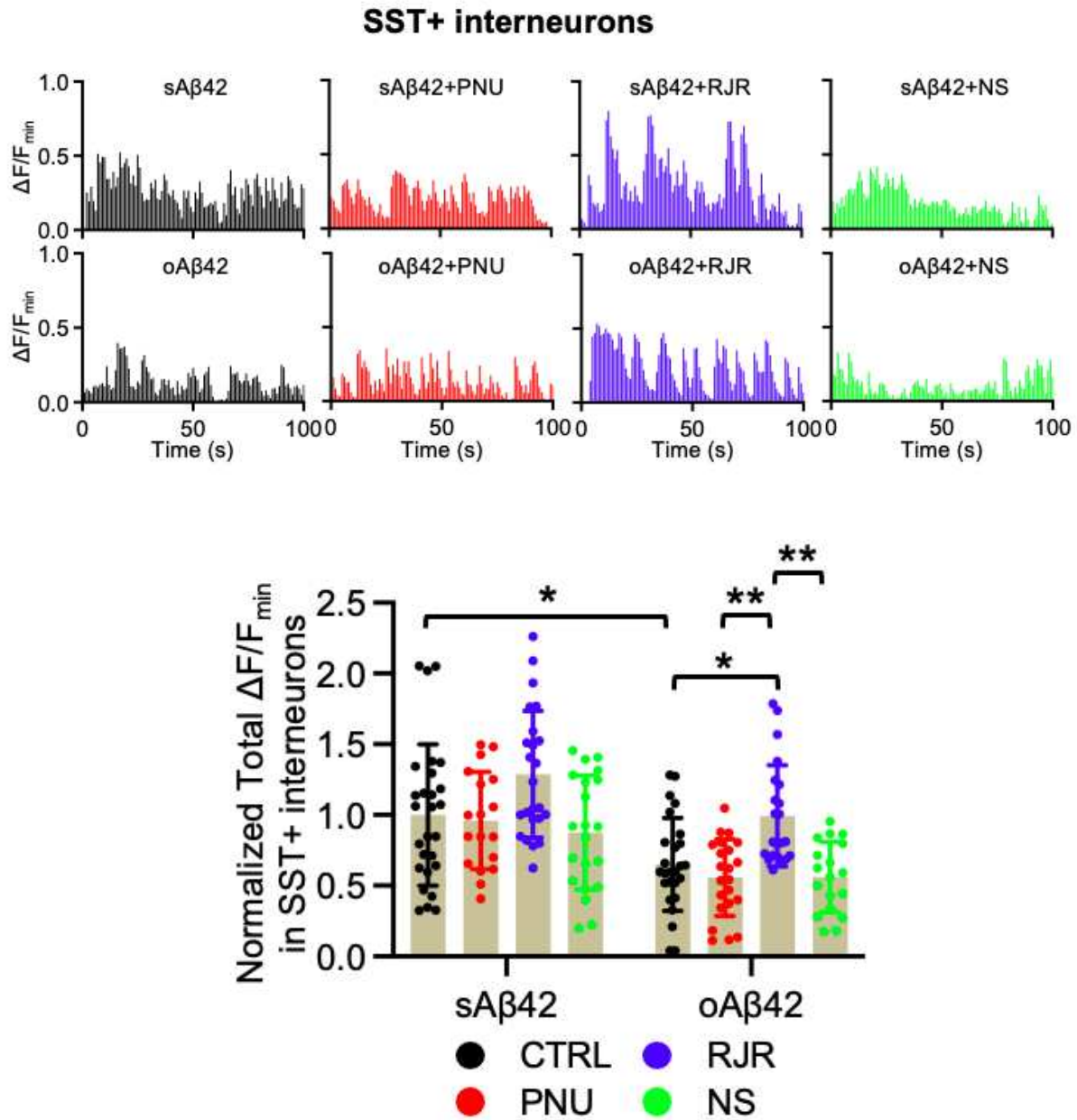


Figure 9. The A β -induced decrease in the neuronal activity of SST+ interneurons is reversed by stimulation of $\alpha 4\beta 2$ -nAChRs.

Representative traces of GCaMP7s fluorescence intensity and summary graphs of normalized total Ca^{2+} activity in each condition in SST+ interneurons (n = number of cells, sA β 42 (CTRL) = 27, sA β 42 + PNU = 18, sA β 42 + RJR = 24, sA β 42 + NS = 18, oA β 42 (CTRL) = 24, oA β 42 + PNU = 22, oA β 42 + RJR = 22, and oA β 42 + NS = 18 from 6 pups in 3 independent cultures). * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$, Two-way ANOVA, Tukey test. Work done by Seonil Kim.

Co-stimulation of PV+ and SST+ interneurons by activating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to reverse hippocampal hyperexcitability in 5XFAD mice.

Previous studies suggest that a reduction of GABAergic inhibitory interneurons' activity in the hippocampus by A β is one of the main causes of neuronal hyperexcitability in AD (52, 61, 69, 104). However, cell-type specificity is absent in these studies, thus it is essential to determine cell-type specific changes of neuronal activity in the AD hippocampus. We measured the expression of the activity-regulated gene, c-Fos (131), to determine if the activity of PV+ and SST+ cells was reduced while pyramidal neurons were hyperexcitable in the hippocampus in AD pathology animals. We then examined whether co-stimulation of PV+ and SST+ cells via activating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is necessary for reducing hyperexcitability in hippocampal excitatory cells in the AD hippocampus in mice. Widely used amyloid pathology model mice, 5XFAD, were used because they show hippocampal hyperexcitability as early as 2.5 months of age (132). Hippocampal sections of 5-month-old 5XFAD and wild-type (WT) female and male were stained with anti-PV, anti-SST, and anti-c-Fos antibodies, and DAPI was used to identify nuclei in pyramidal cells (**Fig. 10a, 11a, 12a**).

We then counted hippocampal c-Fos+ cells in pyramidal, PV+, and SST+ cells per $5.0 \times 10^6 \mu\text{m}^2$ and compared them in each condition. First, we found a significant elevation of the number of c-Fos+ pyramidal cells in the hippocampus of both female and male 5XFAD mice when compared to WT animals (Female; WT + Saline, 69.845 ± 27.943 and 5XFAD + Saline, 150.818 ± 56.931 , $p < 0.0001$, Male; WT + Saline, 65.166 ± 28.820 and 5XFAD + Saline, 143.918 ± 47.784 , $p < 0.0001$) (**Fig. 10b and Table 7**), indicating hyperexcitability. To test whether co-activation $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs was

required to reverse hippocampal hyperexcitation in 5XFAD mice, we used subtype-specific nAChR agonists, PNU and RJR. We first treated PNU to animals for examining whether stimulating $\alpha 7$ -nAChRs was sufficient to reverse hippocampal hyperexcitability. Interestingly, PNU treatment was unable to reverse hyperexcitation in the female 5XFAD hippocampus while it had a slight but significant effect on the activation of hippocampal pyramidal cells in 5XFAD mice (Female; 5XFAD + PNU, 121.873 ± 76.511 , $p = 0.6233$ and Male; 5XFAD + PNU, 110.495 ± 34.132 , $p = 0.0013$) although these neurons were still hyperexcitable when compared to cells in the WT hippocampus ($p = 0.0104$) (**Fig. 10b and Table 7**). RJR treatment for activation of $\alpha 4\beta 2$ -nAChRs was incapable of reversing hippocampal hyperexcitability in 5XFAD mice (Female; 5XFAD + RJR, 127.829 ± 59.897 , $p = 0.2577$ and Male; 5XFAD + RJR, 131.236 ± 53.291 , $p = 0.3558$) (**Fig. 10b and Table 7**). As single receptor activation was unable to reverse hyperexcitation, we next treated PNU and RJR together and found that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs was required to reduce hippocampal hyperexcitation in 5XFAD mice (Female; 5XFAD + PNU + RJR, 79.067 ± 47.185 , $p = 0.0001$ and Male; 5XFAD + PNU + RJR, 78.509 ± 43.314 , $p < 0.0001$) (**Fig. 10b and Table 7**). Importantly, agonist treatments had no major effect on the activity of hippocampal pyramidal cells in WT mice (**Fig. 10b and Table 7**). Notably, in the male 5XFAD hippocampus, PNU + RJR treatment had a stronger effect on neuronal hyperexcitation than PNU alone ($p = 0.0175$) (**Table 7**). This thus suggests that A β induces neuronal hyperactivation in hippocampal pyramidal neurons in 5XFAD mice, and co-stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to reverse hippocampal hyperexcitability in 5XFAD mice.

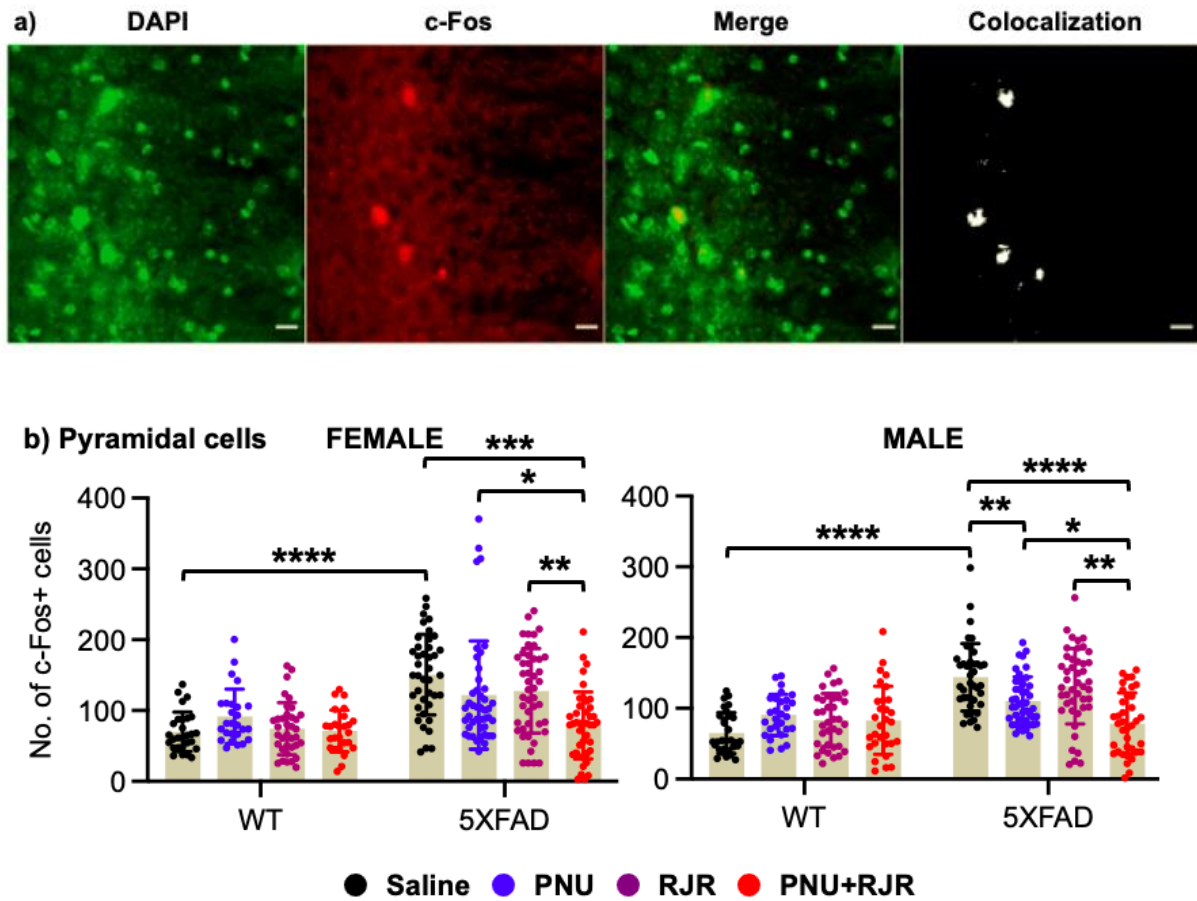


Figure 10. Co-stimulation of PV+ and SST+ interneurons by activating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to reverse hippocampal hyperexcitability in 5XFAD mice.

a) Representative images of DAPI (green), c-Fos (red), merge, and colocalization in the hippocampus. A scale bar represents 10 μ m. **b)** Summary data of an average of the total number of c-Fos+ pyramidal neurons in each condition (n = number of sections [number of animals]; Female, WT + Saline = 28 [7], WT + PNU = 26 [7], WT + RJR = 36 [8], WT + PNU + RJR = 26 [6], 5XFAD + saline = 40 [9], 5XFAD + PNU = 44 [11], 5XFAD + RJR = 46 [10], and 5XFAD + PNU + RJR = 42 [10], Male, WT + Saline = 30 [6], WT + PNU = 28 [6], WT + RJR = 36 [7], WT + PNU + RJR = 30 [6], 5XFAD + saline = 40 [9], 5XFAD + PNU = 44 [11], 5XFAD + RJR = 45 [11], and 5XFAD + PNU + RJR = 37 [10]). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Mixed-effects models / repeated-measures ANOVA, Tukey test.

We next examined whether hippocampal disinhibition was linked to hyperexcitation in 5XFAD mice. The number of cFos+ PV+ cells was significantly decreased in the 5XFAD hippocampus (Female; WT + Saline, 3.256 ± 1.730 and 5XFAD + Saline, 0.788 ± 0.929 , $p < 0.0001$, Male; WT + Saline, 2.513 ± 1.294 and 5XFAD + Saline, 0.733 ± 0.624 , $p < 0.0001$) (**Fig. 11b and Table 8**). PNU treatment significantly increased the hippocampal PV+ cells' activity in 5XFAD mice (Female; 5XFAD + PNU, 3.827 ± 2.703 , $p < 0.0001$ and Male; 5XFAD + PNU, 3.487 ± 2.980 , $p = 0.0005$) (**Fig. 11b and Table 8**). Conversely, RJR had no effect on PV+ cells' activity in the 5XFAD hippocampus (Female; 5XFAD + RJR, 1.627 ± 1.735 , $p = 0.0735$ and Male; 5XFAD + RJR, 1.067 ± 1.580 , $p = 0.8721$) (**Fig. 11b and Table 8**). Notably, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs markedly elevated the activity of hippocampal PV+ cells to the same degree as PNU treatment itself (Female; 5XFAD + PNU + RJR, 4.554 ± 2.889 , $p < 0.0001$ and Male; 5XFAD + PNU + RJR, 4.391 ± 3.143 , $p < 0.0001$) (**Fig. 11b and Table 8**). Notably, agonist treatments had no major effect on PV+ cells' activity in the WT hippocampus (**Fig. 11b and Table 8**). This suggests that A β reduces the activity of hippocampal PV+ cells, but activation of $\alpha 7$ -nAChRs by PNU selectively simulates these cells in 5XFAD mice, which is consistent with our findings in cultured neurons (**Fig. 7a and 8**).

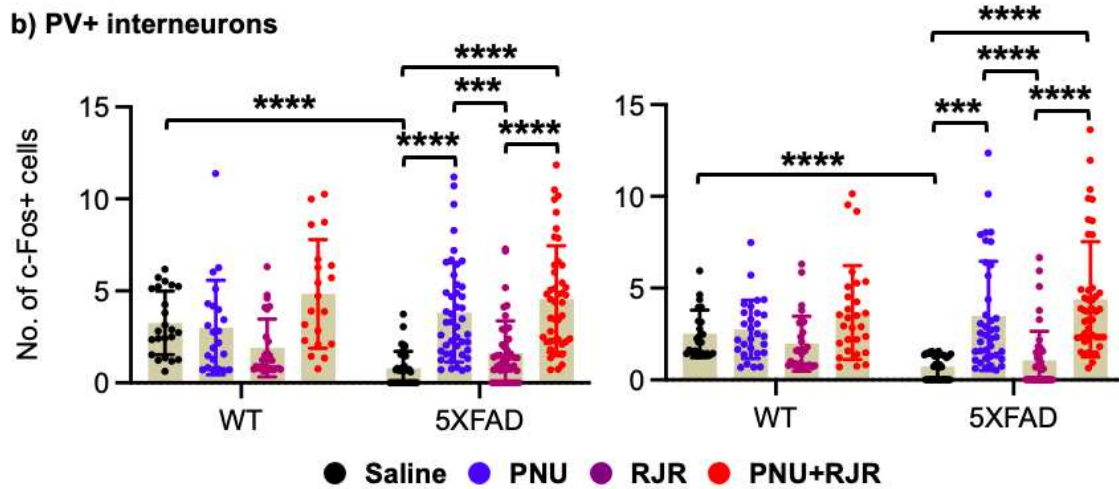
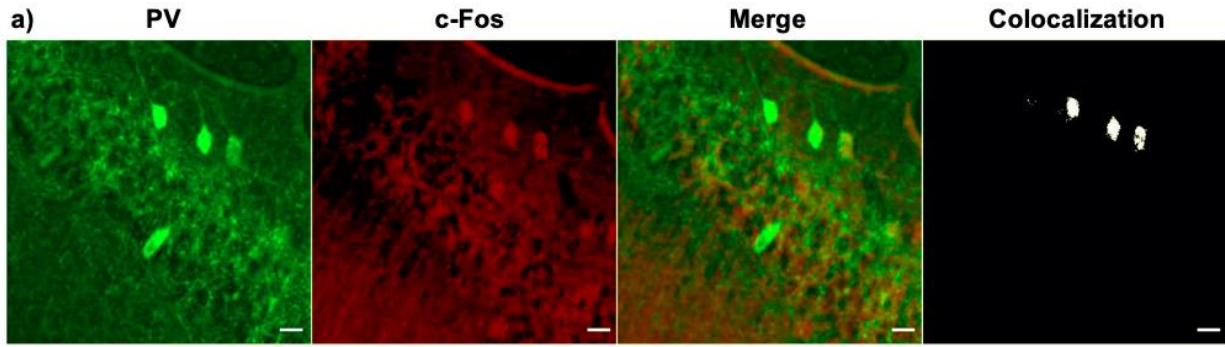


Figure 11. Stimulation of PV+ interneurons by activating $\alpha 7$ - nAChRs alone and $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to increase neuronal activity in 5XFAD mice.

a) Representative images of PV (green), c-Fos (red), merge, and colocalization in the hippocampus. A scale bar represents 10 μ m. **b)** Summary data of an average of the total number of c-Fos+ PV+ interneurons in each condition (n = number of sections [number of animals]; Female, WT + Saline = 23 [7], WT + PNU = 22 [7], WT + RJR = 28 [8], WT + PNU + RJR = 20 [6], 5XFAD + saline = 40 [9], 5XFAD + PNU = 45 [11], 5XFAD + RJR = 45 [10], and 5XFAD + PNU + RJR = 44 [10], Male, WT + Saline = 23 [6], WT + PNU = 28 [6], WT + RJR = 31 [7], WT + PNU + RJR = 28 [6], 5XFAD + saline = 35 [9], 5XFAD + PNU = 39 [11], 5XFAD + RJR = 46 [11], and 5XFAD + PNU + RJR = 44 [10]). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Mixed-effects models / repeated-measures ANOVA, Tukey test.

Finally, we examined the contribution of SST+ cells to hyperexcitability. The hippocampal SST+ cells' activity was markedly reduced in 5XFAD mice (Female; WT + Saline, 1.868 ± 1.527 and 5XFAD + Saline, 0.781 ± 0.854 , $p = 0.0037$, Male; WT + Saline, 1.699 ± 1.238 and 5XFAD + Saline, 0.511 ± 0.669 , $p = 0.0001$) (**Fig. 12b and Table 9**). Notably, PNU was unable to affect SST+ cells' activity in the 5XFAD hippocampus (Female; 5XFAD + PNU, 0.911 ± 0.934 , $p = 0.6740$ and Male; 5XFAD + PNU, 1.001 ± 0.903 , $p = 0.2145$) (**Fig. 12b and Table 9**). However, RJR treatment markedly elevated the activity of hippocampal SST+ cells in 5XFAD mice (Female; 5XFAD + RJR, 2.876 ± 2.408 , $p = 0.0006$ and Male; 5XFAD + RJR, 2.650 ± 2.216 , $p < 0.0001$) (**Fig. 12b and Table 9**). Importantly, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs significantly elevated the hippocampal SST+ cells' activity to the same degree as RJR treatment itself in 5XFAD mice (Female; 5XFAD + PNU + RJR, 2.219 ± 1.498 , $p = 0.0002$ and Male; 5XFAD + PNU + RJR, 1.967 ± 1.413 , $p = 0.0004$) (**Fig. 12b and Table 9**). Notably, agonist treatments had no effect on hippocampal SST+ cells' activity in WT mice (**Fig. 12b and Table 9**). This suggests that A β reduces the hippocampal SST+ cells' activity, but stimulation of $\alpha 4\beta 2$ -nAChRs by RJR selectively activates these cells in 5XFAD mice, which is consistent with our findings in cultured neurons (**Fig. 7b and 9**). These findings all together suggest that A β significantly reduces both PV+ and SST+ interneurons' activity to induce hyperexcitation in pyramidal cells in 5XFAD hippocampus, and co-stimulation of PV+ and SST+ cells via activating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to reduce disinhibition-induced hippocampal hyperexcitability in 5XFAD mice.

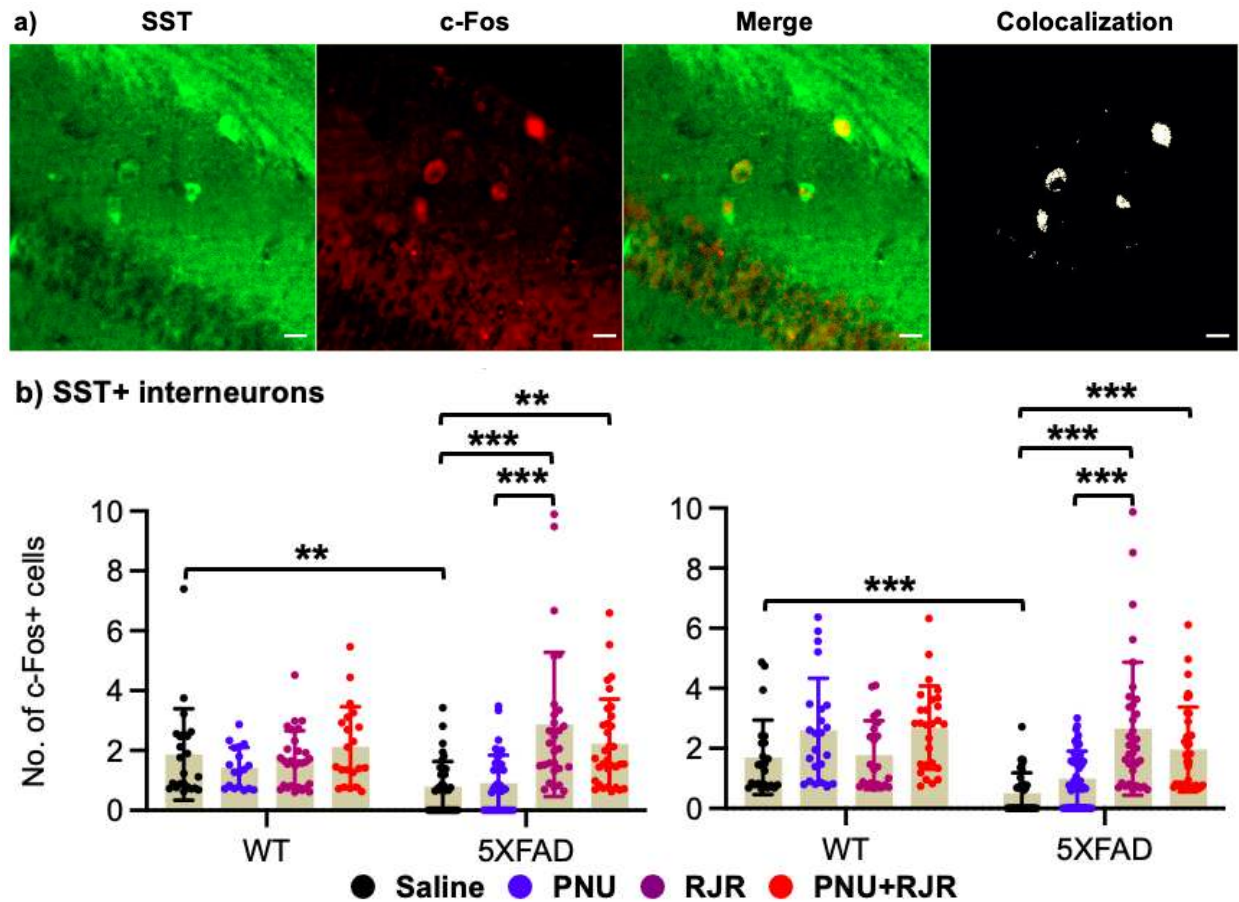


Figure 12. Stimulation of SST+ interneurons by activating $\alpha 4\beta 2$ - nAChRs alone and $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to increase neuronal activity in 5XFAD mice.

a) Representative images of SST (green), c-Fos (red), merge, and colocalization in the hippocampus. A scale bar represents 10 μ m. **b)** Summary data of an average of the total number of c-Fos+ SST+ interneurons in each condition (n = number of sections [number of animals]; Female, WT + Saline = 23 [7], WT + PNU = 18 [7], WT + RJR = 30 [8], WT + PNU + RJR = 21 [6], 5XFAD + saline = 40 [9], 5XFAD + PNU = 43 [11], 5XFAD + RJR = 28 [10], and 5XFAD + PNU + RJR = 33 [10], Male, WT + Saline = 24 [6], WT + PNU = 23 [6], WT + RJR = 22 [7], WT + PNU + RJR = 27 [6], 5XFAD + saline = 39 [9], 5XFAD + PNU = 47 [11], 5XFAD + RJR = 35 [11], and 5XFAD + PNU + RJR = 36 [10]). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Mixed-effects models / repeated-measures ANOVA, Tukey test.

In conclusion, Chapter 2 demonstrates that distinct nAChR subtypes regulate specific populations of hippocampal inhibitory interneurons and play complementary

roles in maintaining hippocampal network activity. $\alpha 7$ -nAChRs primarily mediate cholinergic regulation of PV+ interneurons, whereas $\alpha 4\beta 2$ -nAChRs predominantly regulate SST+ interneurons. Selective activation of these receptor subtypes effectively reversed the A β -induced reduction in the activity of their respective interneuron populations, indicating that A β disrupts inhibitory circuit function through subtype-specific mechanisms. Importantly, simultaneous activation of both $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs was required to restore excitation-inhibition balance and reverse hippocampal hyperexcitability in 5XFAD mice, demonstrating that targeting a single interneuron population is insufficient to fully normalize network function. We then wanted to address this in a *in vivo* mouse model, and the role of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs plays in hippocampal network activity.

CHAPTER 3 - ALTERED HIPPOCAMPAL NETWORK ACTIVITY FOLLOWING FEAR LEARNING IS REVERSED BY CO-ACTIVATION OF $\alpha 7$ - AND $\alpha 4\beta 2$ -NACHRS IN 5XFAD MICE

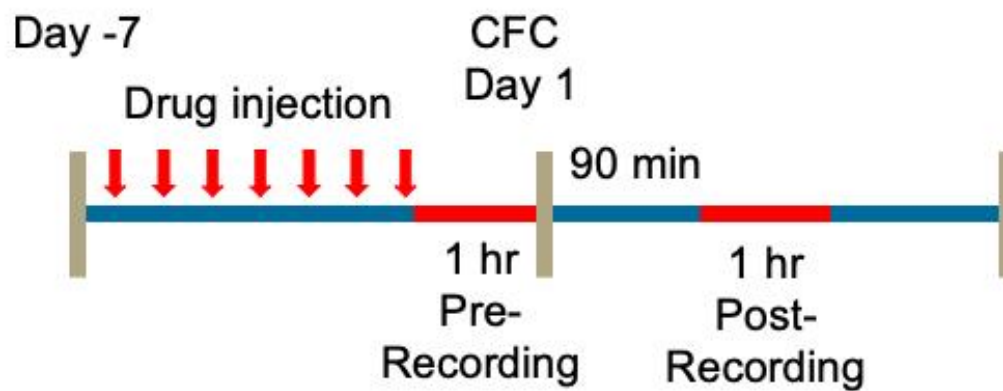
The hippocampus plays a central role in learning and memory by coordinating the activity of neuronal ensembles that encode and retrieve information. Proper hippocampal function depends on a precise balance between excitatory and inhibitory signaling, allowing neurons to generate coordinated network activity during memory formation. In AD, A β disrupts this balance, leading to hippocampal hyperexcitability, impaired neuronal synchronization, and deficits in memory. In Chapter 2 we demonstrated that A β alters the activity of inhibitory interneurons, it remains unclear how these cellular changes affect hippocampal network activity during memory processing and whether this dysfunction can be restored. Therefore, this chapter will investigate hippocampal network activity in 5XFAD mice following fear learning and examines whether co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs can reverse network dysfunction.

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

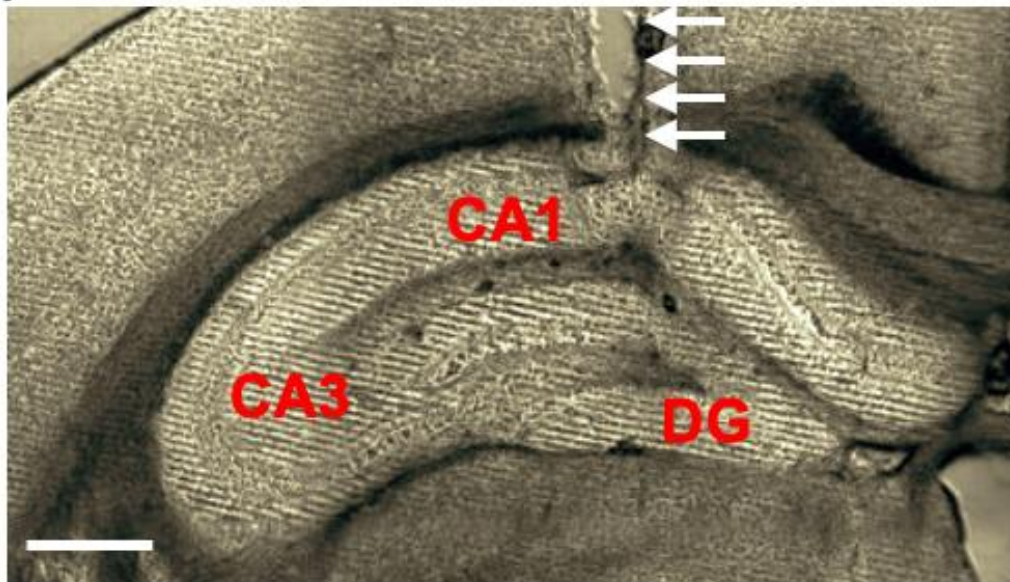
Neural network activity in the hippocampus plays important roles in memory processes (133-137). Studies have shown that altered hippocampal network activity can impair learning and memory processes, which contributes to the development of AD (64, 138-141). In people at high risk of developing AD, abnormal activation and/or deactivation of specific network activities during hippocampal memory processes are

detected decades before the onset of clinical disease (64, 138-142). This suggests that monitoring hippocampal network activity may be a useful tool for early detection and diagnosis of AD. Especially, 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 (103, 133-137). 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 (133, 134). 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 (143, 144). Fast gamma rhythms promote the transmission of current sensory information to the hippocampus during new memory encoding (136, 145). Slow gamma oscillations are likely involved in storing memory (146-148). It has been shown that spatial learning increases theta, slow gamma, and fast gamma powers in the hippocampal CA1 area (149-151). 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. 13a**). Especially, the excitatory/inhibitory (E/I) balance within the hippocampus is critical for neural network stability and is precisely regulated during sleep to facilitate memory consolidation (152). Consolidation of hippocampus-dependent fear memory is known to occur between 1 and 3 hours after training (153, 154). Therefore, test animals were returned to their home cage for 90 minutes after fear learning, and then we recorded hippocampal LFPs for an additional one hour (POST) (**Fig. 13a**).

a) Experimental Timeline



b) Electrode location



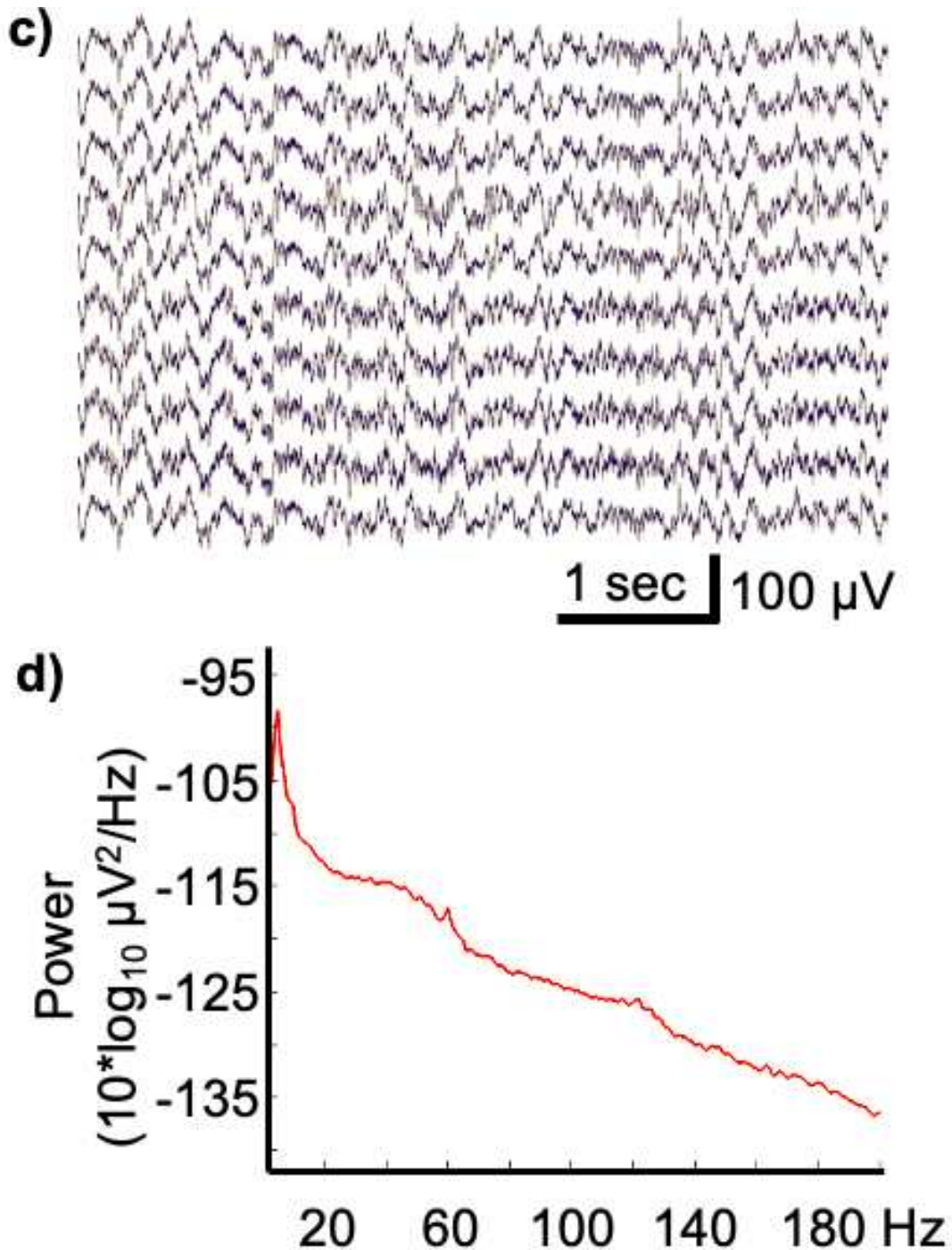


Figure 13. Hippocampal network activity in 5XFAD mice following fear learning.
a) Experimental timeline. **b)** A representative image of a brain section after electrolytic lesioning indicates electrode placement (arrows) in the hippocampus CA1 area. A scale bar represents 1 mm. **c)** Representative section of LFP traces in CA1 of a freely behaving mouse. **d)** Representative power spectral density.

We then compared hippocampal theta, slow gamma, and fast gamma powers between 5XFAD mice and WT littermates. We found that theta (Female; WT PRE, 1.000 ± 1.199 and WT POST, 1.696 ± 1.552 , $p = 0.0029$, Male; WT PRE, 1.000 ± 1.270 and WT POST, 1.926 ± 2.849 , $p = 0.0001$), slow gamma (Female; WT PRE, 1.000 ± 1.223 and WT POST, 1.624 ± 1.420 , $p = 0.0031$, Male; WT PRE, 1.000 ± 1.124 and WT POST, 2.049 ± 2.413 , $p < 0.0001$), and fast gamma powers (Female; WT PRE, 1.000 ± 1.086 and WT POST, 1.601 ± 1.372 , $p = 0.0009$, Male; WT PRE, 1.000 ± 1.051 and WT POST, 2.467 ± 2.486 , $p < 0.0001$) were significantly elevated following CFC in WT female and male mice (**Fig. 14a-c and Table 10**). Conversely, 5XFAD mice showed significant reduction in powers of theta (Female; 5XFAD PRE, 2.195 ± 6.108 and 5XFAD POST, 0.225 ± 0.210 , $p = 0.0015$, Male; 5XFAD PRE, 2.168 ± 3.446 and 5XFAD POST, 0.539 ± 1.019 , $p < 0.0001$), slow gamma (Female; 5XFAD PRE, 3.145 ± 7.767 and 5XFAD POST, 0.480 ± 0.342 , $p = 0.0007$, Male; 5XFAD PRE, 2.095 ± 3.080 and 5XFAD POST, 0.690 ± 1.940 , $p < 0.0001$), and fast gamma bands (Female; 5XFAD PRE, 3.059 ± 7.262 and 5XFAD POST, 0.493 ± 0.284 , $p = 0.0005$, Male; 5XFAD PRE, 2.055 ± 2.724 and 5XFAD POST, 0.770 ± 0.855 , $p < 0.0001$) (**Fig. 14a-c and Table 10**). Interestingly, we found significant higher basal powers in 5XFAD mice than WT animals in all frequency bands (**Fig. 14a-c and Table 10**), which is likely related to A β induced early hippocampal hyperexcitation. These findings suggest that the learning-induced increase in hippocampal oscillations is impaired in 5XFAD mice.

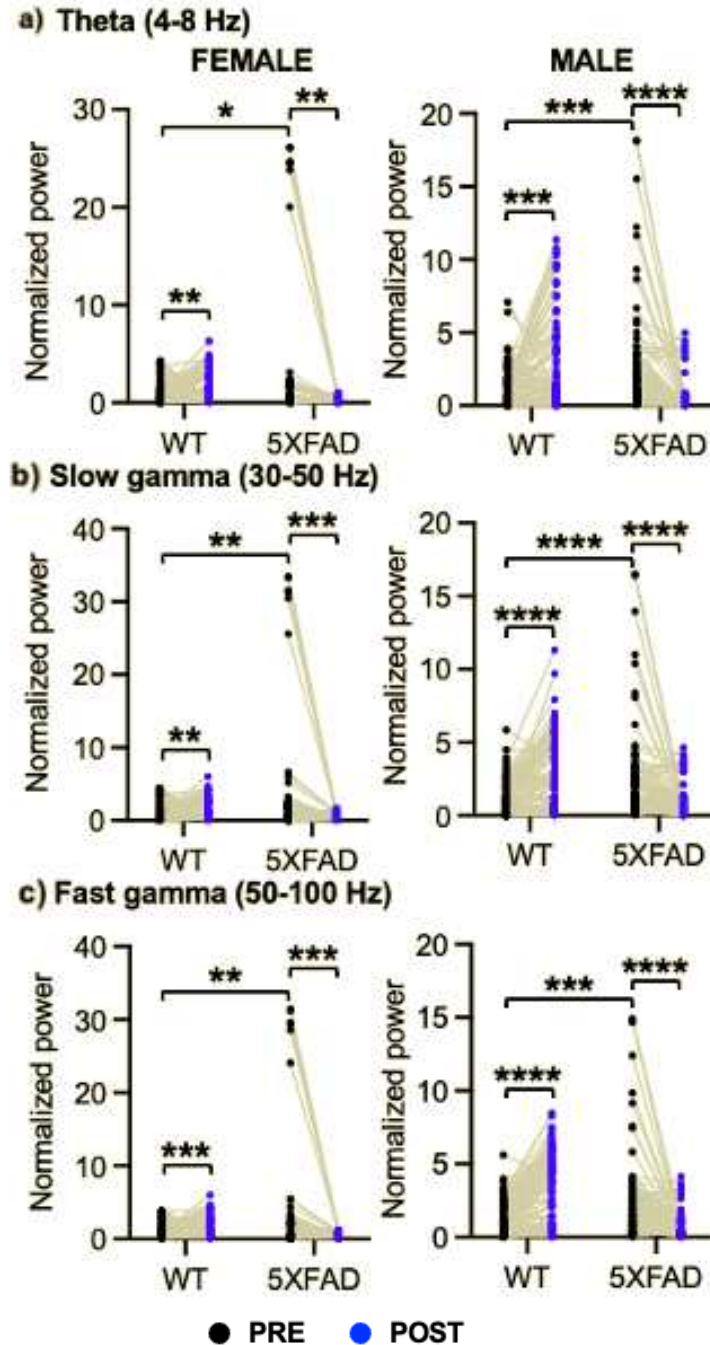


Figure 14. Deficits in hippocampal network activity in 5XFAD mice following fear learning.

Summary graphs of normalized powers of **a)** theta, **b)** slow gamma and **c)** fast gamma frequencies 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 = 106 [10]. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. n.s. – not significant. Paired two-tailed student's t-test was used to compare between PRE and POST data. Two-way ANOVA, Šídák's test was used to compare between WT PRE and 5XFAD PRE data.

In healthy rodents and humans, the hippocampus exhibits prominent gamma oscillations that emerge at specific phases of theta oscillations known as theta-gamma coupling (155). The strength of theta-gamma coupling in the hippocampus is correlated with the task load of memory in humans (156). This coupling is impaired in hippocampal slices in AD model mice (157). Appropriate phase-amplitude coupling (PAC) and entrainment to hippocampal oscillations are thought to be essential for cognition and have been directly linked to memory performance in humans (156, 158, 159). We thus calculated the mean vector length (MVL) as an entrainment strength. We found that the MVL in both theta-slow gamma and theta-fast gamma was significantly higher following learning than before learning in WT female and male mice (Theta-slow gamma, Female; WT PRE, 0.036 ± 0.027 and WT POST, 0.067 ± 0.044 , $p < 0.0001$, Male; WT PRE, 0.041 ± 0.032 and WT POST, 0.070 ± 0.053 , $p < 0.0001$. Theta-fast gamma, Female; WT PRE, 0.059 ± 0.087 and WT POST, 0.076 ± 0.070 , $p = 0.0004$, Male; WT PRE, 0.036 ± 0.042 and WT POST, 0.061 ± 0.061 , $p < 0.0001$) (**Fig. 15a-b and Table 11**). In contrast, the theta-slow gamma MVL in 5XFAD mice was significantly lower following learning when compared to the basal condition (Female; 5XFAD PRE, 0.055 ± 0.021 and 5XFAD POST, 0.042 ± 0.018 , $p < 0.0001$, Male; 5XFAD PRE, 0.060 ± 0.067 and 5XFAD POST, 0.043 ± 0.047 , $p = 0.0157$) (**Fig. 15a-b and Table 11**). Moreover, fear learning was unable to elevate 5XFAD animals' theta-fast gamma PAC in the hippocampus (Female; 5XFAD PRE, 0.028 ± 0.015 and 5XFAD POST, 0.028 ± 0.022 , $p = 0.9556$, Male; 5XFAD PRE, 0.041 ± 0.046 and 5XFAD POST, 0.030 ± 0.039 , $p = 0.0180$) (**Fig. 15a-b and Table 11**). This data suggest that fear learning strengthens PAC entrainment in WT mice, consistent with the previous report (160). However, fear

learning is unable to increase theta-gamma coupling in 5XFAD mice. These findings thus show deficits in hippocampal network activity in 5XFAD mice following fear learning.

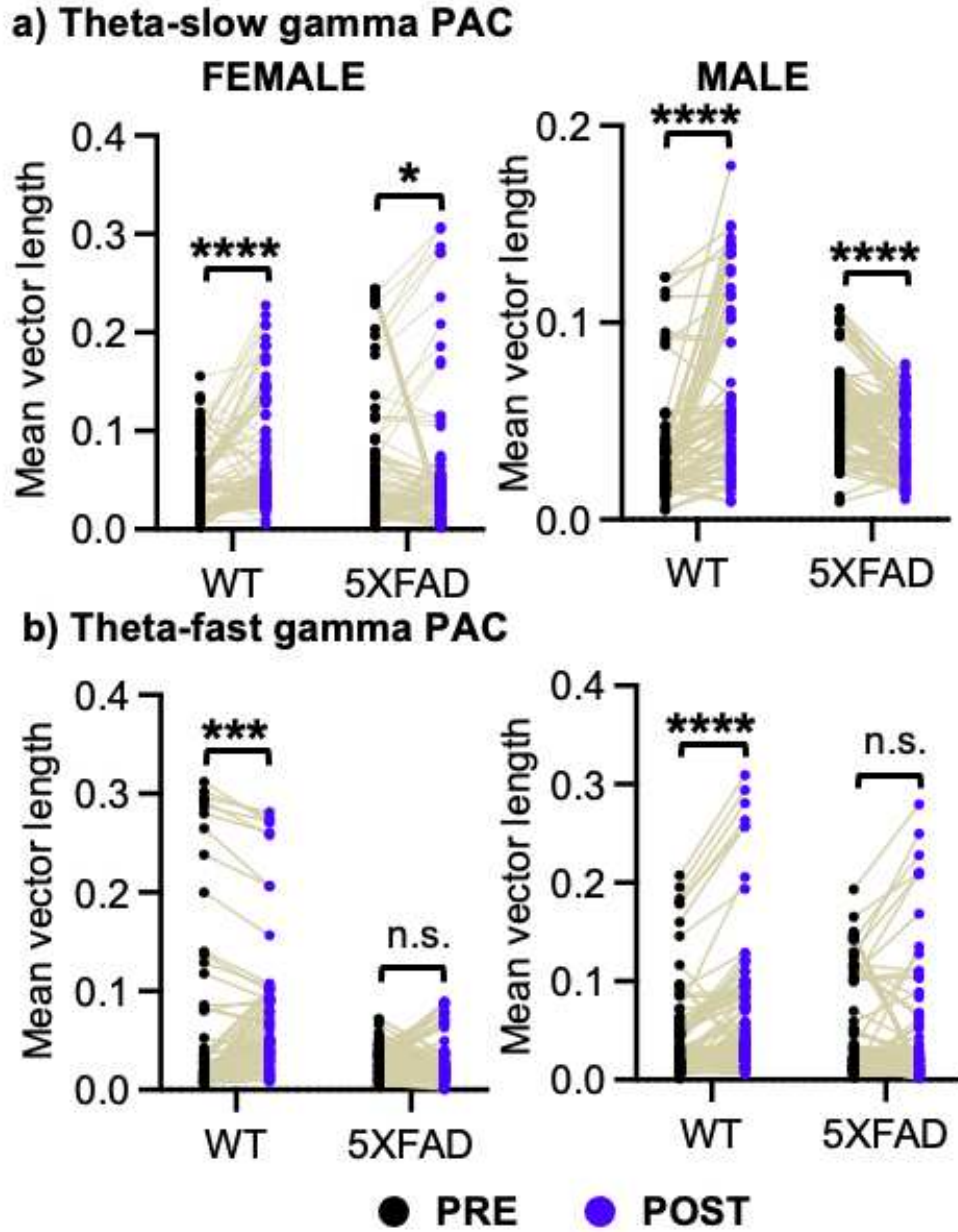


Figure 15. Deficits in hippocampal network coupling in 5XFAD mice following fear learning.

Summary of **a)** Theta-slow gamma PAC and **b)** Theta-fast gamma PAC in each condition. n = number of channels [number of animals]; Female, WT = 74 [7] and 5XFAD = 101 [9], Male, WT = 125 [12] and 5XFAD = 106 [10]. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. n.s. – not significant. Paired two-tailed student's t-test was used to compare between PRE and POST data. Two-way ANOVA, Šídák's test was used to compare between WT PRE and 5XFAD PRE data.

Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to restore normal hippocampal network activity in 5XFAD mice.

We next compared hippocampal oscillatory activity following fear learning between WT and 5XFAD mice. When compared to WT mice, 5XFAD mice exhibited a significant decrease in the powers of theta (Female; WT, 1.000 ± 0.915 and 5XFAD, 0.151 ± 0.148 , $p < 0.0001$, Male; WT, 1.000 ± 1.479 and 5XFAD, 0.280 ± 0.529 , $p < 0.0001$), slow gamma (Female; WT, 1.000 ± 1.874 and 5XFAD, 0.268 ± 10.167 , $p = 0.0015$, Male; WT, 1.000 ± 1.079 and 5XFAD, 0.304 ± 0.414 , $p = 0.0007$), and fast gamma bands (Female; WT, 1.000 ± 0.853 and 5XFAD, 0.297 ± 0.162 , $p = 0.0024$, Male; WT, 1.000 ± 1.008 and 5XFAD, 0.312 ± 0.347 , $p < 0.0001$) (**Fig. 16a-c and Table 12-14**).

This indicates impaired fear memory processing in 5XFAD mice. Acetylcholine modulates hippocampal oscillations to control memory processes (161-163). We thus examined if stimulating $\alpha 7$ - and $\alpha 4\beta 2$ -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, 0.138 ± 0.234 , $p = 0.9997$, and 5XFAD + RJR, 0.127 ± 0.174 , $p = 0.9984$, Male; 5XFAD + PNU, 0.123 ± 0.137 , $p = 0.8638$, and 5XFAD + RJR, 0.090 ± 0.107 , $p = 0.7720$), slow gamma (Female; 5XFAD + PNU, 0.305 ± 0.419 , $p = 0.9988$, and 5XFAD + RJR, 0.214 ± 0.297 , $p = 0.8501$, Male; 5XFAD + PNU, 0.272 ± 0.157 , $p = 0.9995$, and 5XFAD + RJR, 0.142 ± 0.122 , $p = 0.9405$), and fast gamma bands (Female; 5XFAD + PNU, 0.269 ± 0.392 , $p = 0.9995$, and 5XFAD + RJR, 0.198 ± 0.240 , $p = 0.9849$, Male; 5XFAD + PNU, 0.263 ± 0.195 , $p = 0.9962$, and 5XFAD

+ RJR, 0.142 ± 0.139 , $p = 0.8634$) (**Fig. 16a-c and Table 12-14**). This data show that stimulation of either $\alpha 7$ - or $\alpha 4\beta 2$ -nAChRs is insufficient to improve hippocampal network activity in 5XFAD mice. However, co-stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs significantly elevated the powers of theta (Female; 5XFAD + PNU+RJR, 0.518 ± 0.834 , $p = 0.0057$, Male; 5XFAD + PNU+RJR, 0.684 ± 0.986 , $p = 0.0460$), slow gamma (Female; 5XFAD + PNU + RJR, 1.058 ± 1.633 , $p = 0.0006$, Male; 5XFAD + PNU + RJR, 1.300 ± 2.375 , $p = 0.0001$), and fast gamma bands (Female; 5XFAD + PNU + RJR, 1.253 ± 1.919 , $p < 0.0001$, Male; 5XFAD + PNU + RJR, 1.261 ± 1.763 , $p < 0.0001$) (**Fig. 16a-c and Table 12-14**). Interestingly, agonist treatment significantly reduced theta power only in WT mice (Female; WT + PNU, 0.490 ± 0.950 , $p = 0.0077$, WT + RJR, 0.566 ± 0.600 , $p = 0.0138$, WT + PNU + RJR, 0.457 ± 1.209 , $p < 0.0001$, Male; WT + PNU, 0.422 ± 0.365 , $p = 0.0031$, WT + RJR, 0.489 ± 0.256 , $p = 0.0173$, WT + PNU + RJR, 0.609 ± 1.648 , $p = 0.0382$) while drugs had no major effect on slow and fast gamma powers in WT animals (**Fig. 16a-c and Table 12-14**). These findings suggest that a memory consolidation-mediated increase in hippocampal oscillations is disrupted in 5XFAD mice, which is reversed only by co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs.

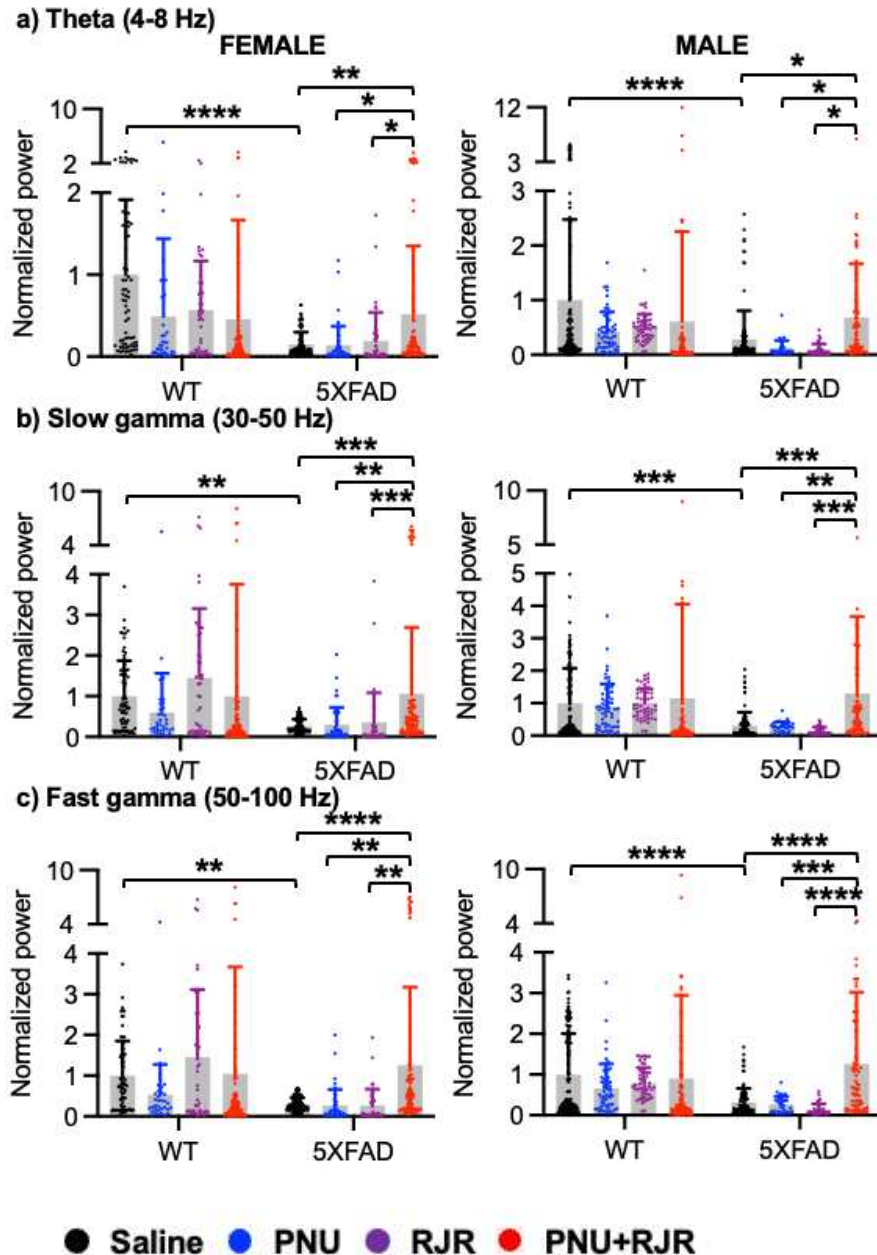


Figure 16. Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to restore normal hippocampal network activity in 5XFAD mice.

Summary graphs of normalized powers of **a)** theta, **b)** slow gamma and **c)** fast gamma frequencies in female and male mice in each condition. n = number of channels [number of animals]; Female, WT + Saline = 74 [7], WT + PNU = 35 [4], WT + RJR = 48 [4], WT + PNU + RJR = 97 [9], 5XFAD + saline = 101 [9], 5XFAD + PNU = 51 [4], 5XFAD + RJR = 41 [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 = 106 [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 **** $p < 0.0001$. Mixed-effects models / repeated-measures ANOVA, Tukey test.

We next analyzed the strength of theta-gamma coupling in the hippocampus. We found that the MVL in both theta-slow gamma and theta-fast gamma was significantly lower in 5XFAD mice following learning than WT animals (Theta-slow gamma, Female; WT, 0.067 ± 0.044 and 5XFAD, 0.042 ± 0.018 , $p = 0.0004$, Male; WT, 0.070 ± 0.053 and 5XFAD, 0.043 ± 0.047 , $p = 0.0003$. Theta-fast gamma, Female; WT, 0.076 ± 0.070 and 5XFAD, 0.028 ± 0.022 , $p < 0.0001$, Male; WT, 0.061 ± 0.061 and 5XFAD, 0.030 ± 0.039 , $p < 0.0001$) (**Fig. 17a-b and Table 15 and 16**). Interestingly, we found that activation of single receptors or two receptors together was sufficient to increase MVL in 5XFAD mice (Theta-slow gamma, Female; 5XFAD + PNU, 0.072 ± 0.053 , $p = 0.0005$, 5XFAD + RJR, 0.100 ± 0.062 , $p < 0.0001$, and 5XFAD + PNU + RJR, 0.120 ± 0.040 , $p < 0.0001$, Male; 5XFAD + PNU, 0.122 ± 0.054 , $p < 0.0001$, 5XFAD + RJR, 0.107 ± 0.032 , $p < 0.0001$, and 5XFAD + PNU + RJR, 0.071 ± 0.053 , $p = 0.0055$. Theta-fast gamma, Female; 5XFAD + PNU, 0.055 ± 0.039 , $p = 0.0008$, 5XFAD + RJR, 0.086 ± 0.036 , $p < 0.0001$, and 5XFAD + PNU + RJR, 0.078 ± 0.041 , $p < 0.0001$, Male; 5XFAD + PNU, 0.097 ± 0.047 , $p < 0.0001$, 5XFAD + RJR, 0.077 ± 0.057 , $p < 0.0001$, and 5XFAD + PNU + RJR, 0.056 ± 0.046 , $p = 0.0052$) (**Fig. 17a-b and Table 15 and 16**). This data show that activation of $\alpha 7$ - and/or $\alpha 4\beta 2$ -nAChRs is sufficient to strengthen fear learning-related PAC entrainment in 5XFAD mice. In sum, 5XFAD mice showed significant deficits in hippocampal network activity following fear conditioning. While stimulation of $\alpha 7$ - and/or $\alpha 4\beta 2$ -nAChRs is sufficient to increase PAC in 5XFAD mice, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, perhaps stimulating PV+ and SST+ cells together, is essential to restore normal powers of AD-related hippocampal oscillations following fear learning.

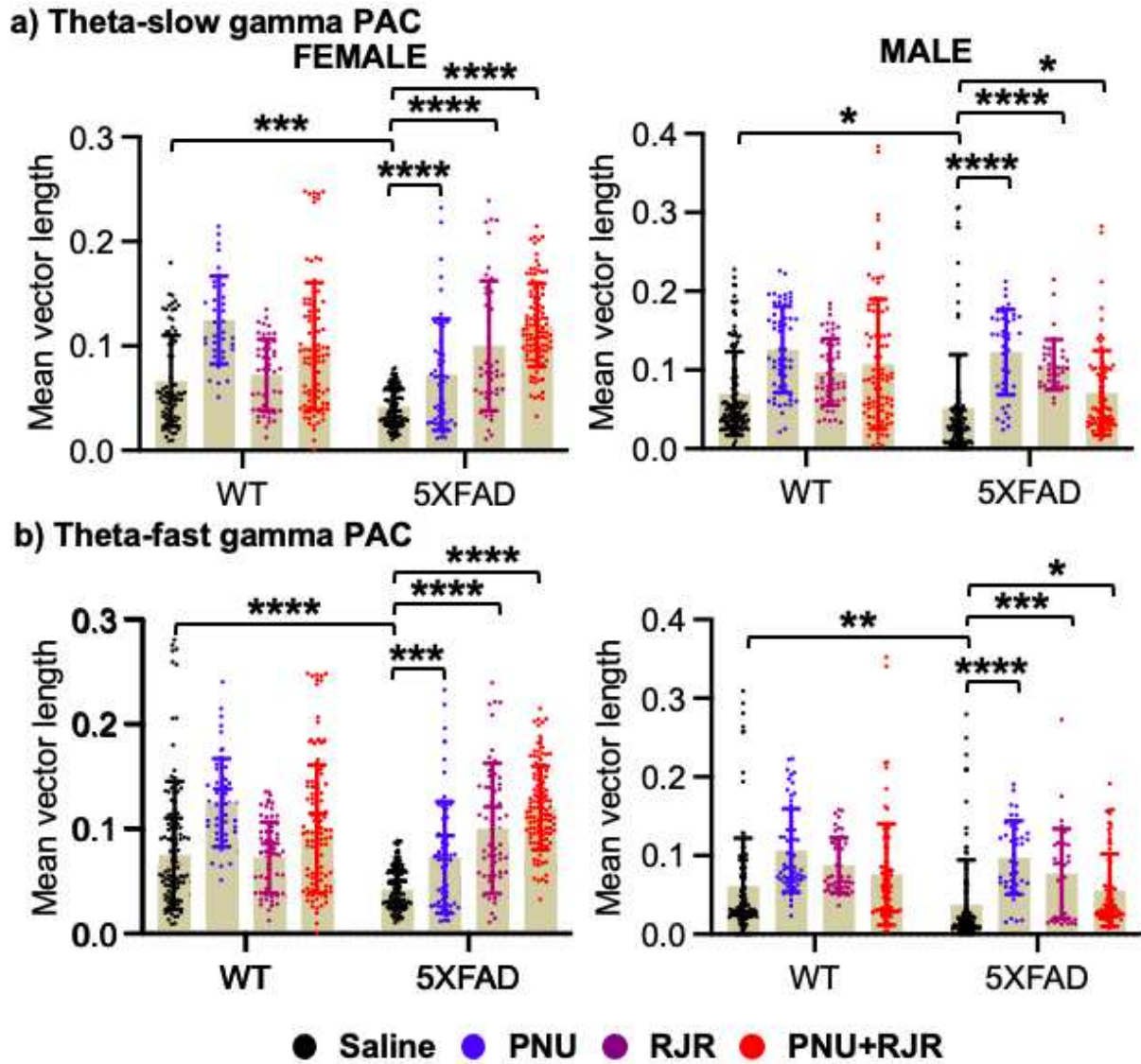


Figure 17. Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to restore normal hippocampal network coupling in 5XFAD mice.

Summary of **a)** Theta-slow gamma PAC and **b)** Theta-fast gamma PAC in each condition. n = number of channels [number of animals]; Female, WT + Saline = 74 [7], WT + PNU = 35 [4], WT + RJR = 48 [4], WT + PNU + RJR = 97 [9], 5XFAD + saline = 101 [9], 5XFAD + PNU = 51 [4], 5XFAD + RJR = 41 [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 = 106 [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 **** $p < 0.0001$. Mixed-effects models / repeated-measures ANOVA, Tukey test.

In conclusion, Chapter 3 demonstrates that hippocampal network activity is impaired in 5XFAD mice following fear learning, providing evidence that A β -induced circuit dysfunction also leads to disrupt memory-related network function. Importantly, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs reverses normal hippocampal network activity, whereas activation of either receptor subtype alone was insufficient to fully rescue network deficits. These findings indicate that complementary modulation of distinct inhibitory interneuron populations is required to re-establish excitation-inhibition balance and normalize hippocampal circuit activity.

CHAPTER 4 - CO-ACTIVATION OF $\alpha 7$ - AND $\alpha 4\beta 2$ -NACHRS IMPROVES MEMORY AND REDUCES A β PATHOLOGY IN 5XFAD MICE

Understanding the cellular and network mechanism of how the co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs has been investigated in Chapter 2 and 3. However, whether co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs can produce functional and pathological benefits in the 5XFAD mouse model remains an important question. Demonstrating that this mechanism is effective in a living, complex organism is essential, as findings from isolated cells or experimental systems have limited therapeutic value unless they translate to improvements *in vivo*. Therefore, this chapter investigates whether co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs can improve contextual fear memory and reduce AD pathology in the 5XFAD mouse model.

Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -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 (164, 165). Fear memory loss in 5XFAD mice starts at the age of 5 months (166). We thus carried out contextual fear conditioning (CFC) as described previously (167, 168) with 5-month-old 5XFAD and WT female and male mice. In consistent with the previous finding (166), 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, $36.785 \pm 10.866\%$ and 5XFAD + Saline, $14.188 \pm$

12.202%, $p < 0.0001$, and Male, WT + Saline, $38.938 \pm 13.066\%$ and 5XFAD + Saline, $16.177 \pm 8.761\%$, $p < 0.0001$) (Fig. 18 and Table 17).

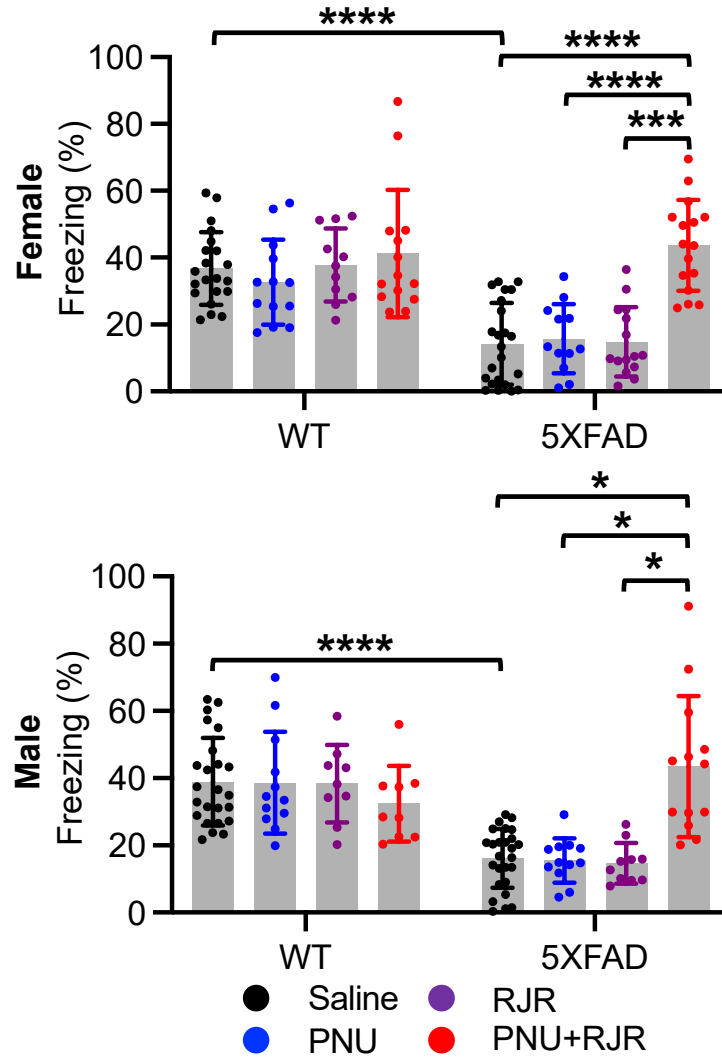


Figure 18. Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to improve contextual fear memory in 5XFAD mice.

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.

Given that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to reverse fear learning-associated hippocampal network activity (**Fig. 16-17**), we examined the effects of stimulating these receptors on contextual fear memory in 5XFAD mice. We intraperitoneally injected PNU or RJR into 5XFAD and WT mice to see if activation of either $\alpha 7$ - or $\alpha 4\beta 2$ -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, $15.751 \pm 10.332\%$, $p > 0.9999$, and 5XFAD + RJR, $14.836 \pm 10.400\%$, $p > 0.9999$, and Male, 5XFAD + PNU, $15.558 \pm 6.577\%$, $p > 0.9999$, and 5XFAD + RJR, $14.648 \pm 6.018\%$, $p > 0.9999$) (**Fig. 18 and Table 17**). This shows that stimulation of either $\alpha 7$ - or $\alpha 4\beta 2$ -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 $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs improved contextual fear memory in 5XFAD mice. We found that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs significantly enhanced contextual fear memory in 5XFAD male and female mice (Female, 5XFAD + PNU + RJR, $43.664 \pm 13.580\%$, $p < 0.0001$, and Male, 5XFAD + PNU + RJR, $43.434 \pm 21.002\%$, $p = 0.0101$) (**Fig. 18 and Table 17**). These findings suggest that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs (potentially stimulates hippocampal PV+ and SST+ cells together) is required to improve hippocampus-dependent fear memory in 5XFAD mice.

Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to reduce the A β buildup in the 5XFAD hippocampus.

Amyloid plaques are aggregation of A β in the extracellular space of the brain, central to the AD pathology. It has been shown that *in vivo* extracellular A β levels are dynamically and directly influenced by neuronal activity (65). Importantly, neuronal hyperexcitability increases A β secretion and accumulation (65, 66). Therefore, it is possible that beta-amyloid induced hippocampal hyperexcitation promotes the *in vivo* rapid growth of amyloid plaques, which can be prevented by reducing neuronal activity through enhancing hippocampal inhibition. To test this idea, we examined if enhancing PV+ and SST+ activity together by co-stimulating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs was required to reduce the A β buildup in the 5XFAD hippocampus. Notably, longitudinal *in vivo* imaging studies in AD pathology model mice have demonstrated that amyloid plaques form and grow quickly over the course of a day and one week (169, 170). We thus intraperitoneally injected PNU and RJR on their own or together into 5-month-old 5XFAD mice for 7 days. Saline was given to mice as a control. We then visualized amyloid plaques and measured the total number of amyloid plaques in the hippocampus. We found that stimulation of each receptor had no effect on amyloid pathology in the hippocampus of both female and male 5XFAD mice when compared to the saline-injected controls (Female, Saline, 50.62 ± 13.21 , PNU, 49.71 ± 11.17 , $p = 0.9978$, and RJR, 47.71 ± 10.28 , $p = 0.9359$, and Male, Saline, 38.95 ± 13.53 , PNU, 38.66 ± 8.85 , $p > 0.9999$, and RJR, 47.88 ± 5.97 , $p = 0.2469$) (**Fig. 19 and Table 18**). However, we revealed that co-stimulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs significantly reduced the total number of amyloid plaques in the hippocampus of both female and

male 5XFAD mice when compared to the saline-injected controls (Female, PNU + RJR, 32.19 ± 7.19 , $p = 0.0013$, and Male, PNU+RJR, 24.82 ± 9.89 , $p = 0.0042$) (**Fig. 19 and Table 18**). This data show that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to reduce the rapid growth of amyloid plaques in 5XFAD mice.

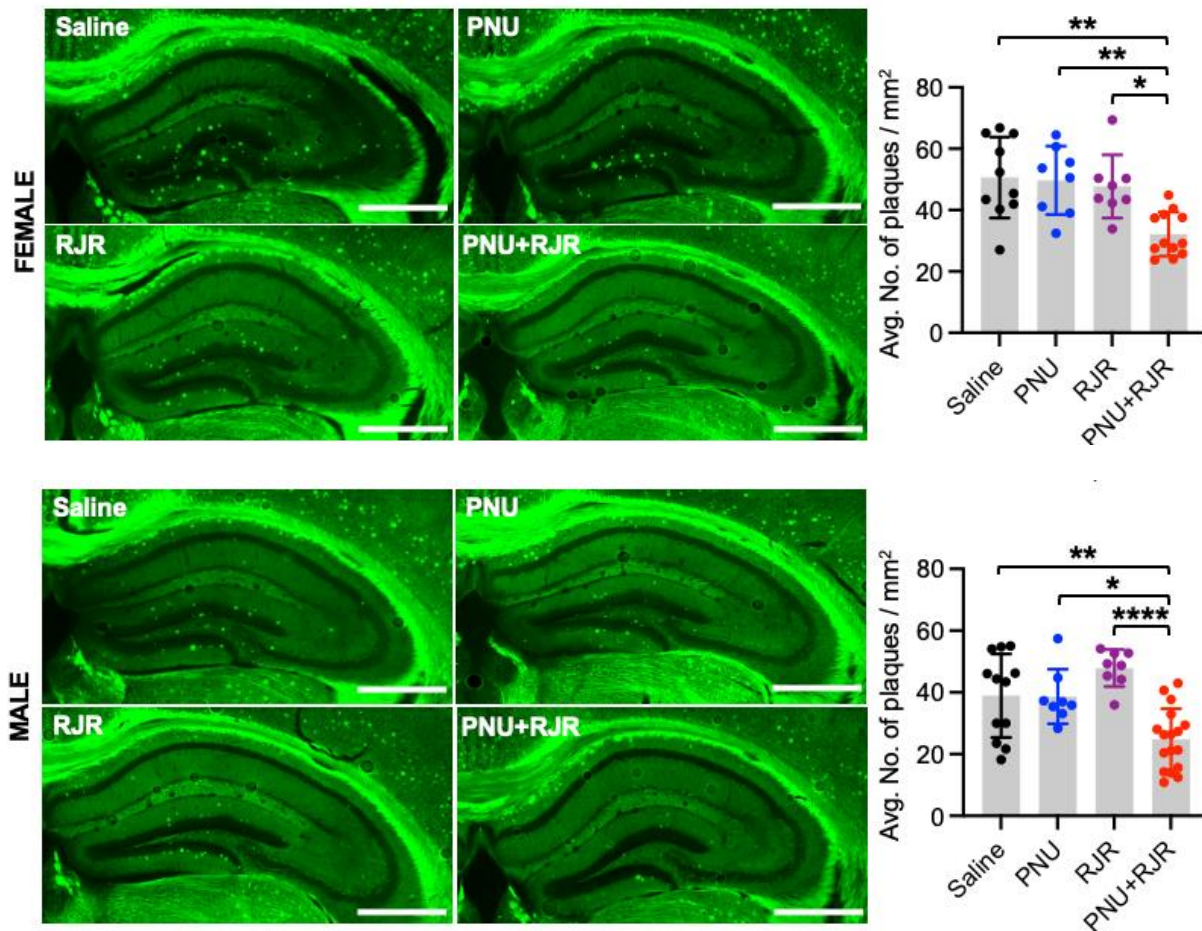


Figure 19. Co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs prevents the *in vivo* growth of amyloid plaques.

Representative images of Thioflavin S-positive amyloid plaques (Green puncta) in the hippocampus in 5-month-old 5XFAD female (n = number of animals, Saline = 10, PNU = 8, RJR = 8, and PNU + RJR = 12) and male (n = number of animals, Saline = 12, PNU = 8, RJR = 8, and PNU + RJR = 17) mice in each condition. A scale bar represents 1 mm. Summary graphs of the total number of amyloid plaques per mm² in each condition. * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$, One-way ANOVA, Tukey test.

In conclusion, Chapter 4 demonstrates that co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs produces both functional and pathological improvements in the 5XFAD mouse model. Co-activation of these receptor subtypes significantly improved contextual fear memory and reduced hippocampal A β buildup, indicating that restoring inhibitory circuit function can translate into meaningful cognitive benefits while also attenuating disease pathology. Together with the findings from the preceding chapters, these results support a model in which coordinated modulation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs restores hippocampal function across multiple levels of organization, from interneuron activity and network dynamics to behavior and pathological progression.

CHAPTER 5 - DISCUSSION

This study demonstrates that nicotinic cholinergic signaling is differentially regulated across hippocampal neuronal populations. The data shows that $\alpha 7$ -, $\alpha 4\beta 2$ -, and $\alpha 3\beta 4$ -nAChRs predominantly regulate PV+, SST+, and excitatory neurons, respectively. Furthermore, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs restores hippocampal inhibition, normalizes network oscillations, improves fear memory, and reduces amyloid pathology in 5XFAD mice. Together, these findings establish a mechanistic link between A β mediated cholinergic dysfunction, hippocampal disinhibition, neuronal hyperexcitability, network dysfunction, and cognitive decline in Alzheimer's disease.

A large body of research has suggested that A β -induced inhibitory dysfunction in the hippocampus can be a crucial trigger for disruptions in neural oscillations and consequent cognitive function in AD (87, 142, 171-176). However, the molecular mechanisms underlying A β -induced hippocampal interneuron dysfunctions in AD have not been fully understood. Cholinergic dysfunction may be a prime suspect for A β -induced inhibitory dysfunction because cholinergic signaling has greater effects on hippocampal inhibitory cells than excitatory neurons (111, 112, 115, 116, 177, 178), and there are diverse lines of evidence that molecular interactions between A β and nAChRs impair receptor functions in the early stage of AD (179-182). This suggests that A β 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 contribute to hippocampal oscillations and memory formation (103). In

addition, various neuronal nAChRs have been found in the human brain (83, 109, 110), and distinct nAChR subtypes differentially affect hippocampal inhibition and oscillations (125, 183-186). Therefore, it is challenging to elucidate the nAChR-mediated mechanisms by which A β affects hippocampal neural network and memory processing in AD at the interneuron subtype levels. Our published works have discovered that A β selectively binds to two of the three major hippocampal nAChR subtypes, α 7- and α 4 β 2-nAChRs, but not α 3 β 4-nAChRs, and inhibits these two receptors in cultured hippocampal inhibitory interneurons to decrease their activity, leading to hyperexcitation in excitatory neurons (72, 187). We have also revealed that co-activation of α 7- and α 4 β 2-nAChRs is required to reverse the A β -induced adverse effects in hippocampal neurons (72, 187). In the current study, we further identify that α 7-, α 4 β 2-, and α 3 β 4-nAChRs regulate the cholinergic activity mainly in PV+, SST+, and excitatory cells, respectively. Additionally, we discover that co-activation of α 7- and α 4 β 2-nAChRs is necessary to reverse hippocampal hyperexcitability, network dysfunction, and fear memory loss and reduce amyloid pathology in 5XFAD mice. In sum, our study provides the mechanistic link between hippocampal disinhibition-induced hyperexcitability via altered cholinergic activity, network dysfunction, pathology, and memory loss in AD.

The hippocampus contains a diverse population of inhibitory interneurons that regulate excitatory neuronal activity through distinct mechanisms. PV+ and SST+ interneurons are two of the principal inhibitory cell populations involved in controlling hippocampal network activity and memory formation (103, 112, 114, 115, 125, 186, 188-196). PV+ interneurons primarily target the soma and axon initial segment of pyramidal neurons, providing fast perisomatic inhibition that synchronizes neuronal

firing and contributes to gamma oscillations. SST+ interneurons innervate the distal dendrites of pyramidal neurons, regulating dendritic integration of excitatory inputs, synaptic plasticity, and information processing (103, 112, 114, 115, 125, 186, 188-196). These complementary inhibitory circuits maintain the balance between excitation and inhibition that is essential for normal hippocampal function.

Our findings indicate that $\alpha 7$ -nAChRs predominantly regulate PV+ interneurons, whereas $\alpha 4\beta 2$ -nAChRs primarily regulate SST+ interneurons. In contrast, $\alpha 3\beta 4$ -nAChRs are mainly expressed on pyramidal excitatory neurons, where they contribute to excitatory cholinergic signaling rather than inhibitory control. The segregation of these receptor subtypes provides a mechanistic explanation for the selectivity of inhibitory interneurons to A β . Our previous studies demonstrated that A β binds directly to $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs but not to $\alpha 3\beta 4$ -nAChRs because conserved charged amino acid residues required for A β binding are present in the $\alpha 7$ and $\alpha 4$ subunits but lacking in the $\alpha 3$ subunit (71, 72). Consequently, A β preferentially suppresses cholinergic signaling in PV+ and SST+ interneurons but not in pyramidal neurons. This selective inhibition reduces both somatic and dendritic inhibition, producing an excitation-inhibition imbalance that ultimately results in hippocampal hyperexcitability, disrupted network oscillations, and impaired memory.

Importantly, our findings also explain why activation of a single nAChR subtype is insufficient to restore normal hippocampal function. Activation of $\alpha 7$ -nAChRs alone mainly enhances PV+ interneuron activity and restores somatic inhibition, whereas activation of $\alpha 4\beta 2$ -nAChRs alone mainly enhances SST+ interneuron activity and restores dendritic inhibition. These two inhibitory pathways regulate distinct

hippocampal circuit function: activation of only one interneuron population leaves the complementary inhibitory pathway compromised. As a result, E/I balance cannot be fully restored, and hippocampal network dysfunction persists. Simultaneous co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs restores both PV+ and SST+ mediated inhibition, thereby restoring coordinated inhibitory control over neighboring pyramidal neurons. This coordinated restoration normalizes hippocampal network oscillations, reduces neuronal hyperexcitability, improves fear memory, and decreases amyloid pathology in 5XFAD mice.

In healthy brains, $\alpha 7$ -, $\alpha 4\beta 2$ -, and $\alpha 3\beta 4$ -nAChRs mainly regulate cholinergic signaling in PV+ interneurons, SST+ interneurons, and pyramidal neurons, respectively, thereby maintaining balanced hippocampal inhibition and normal cognitive function (**Fig. 20a**). Patients with Alzheimer's disease, A β selectively inhibits $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs on PV+ and SST+ interneurons without affecting $\alpha 3\beta 4$ -nAChRs on excitatory neurons (**Fig. 20b**). This selective disruption reduces inhibitory drive onto pyramidal neurons, leading to hippocampal hyperexcitability, impaired network oscillations, amyloid accumulation, and cognitive decline. Pharmacological stimulation of either $\alpha 7$ - or $\alpha 4\beta 2$ -nAChRs singularly partially restores inhibitory function (**Fig. 20c-d**). However, co-activation of both receptor subtypes effectively restores somatic and dendritic inhibition, normalizes hippocampal network activity, and protects against AD pathology (**Fig. 20e**). Collectively, these findings establish coordinated $\alpha 7$ - and $\alpha 4\beta 2$ -nAChR activation as a novel mechanism for restoring hippocampal inhibitory function and propose a new for therapeutic strategy for AD.

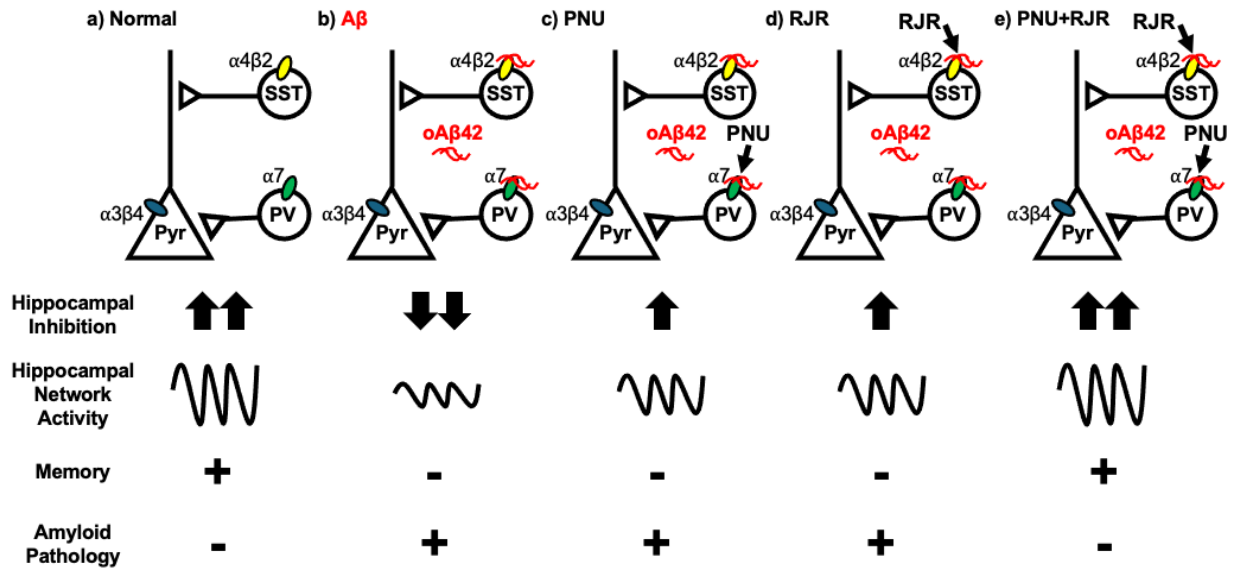


Figure 20. A schematic model of nAChR-mediated mechanisms of Aβ-induced dysfunction in hippocampal inhibition, network activities, amyloid pathology, and memory in AD.

a) $\alpha 7$ -, $\alpha 4\beta 2$ -, and $\alpha 3\beta 4$ -nAChRs predominantly control the nicotinic cholinergic signaling in parvalbumin-positive (PV+), somatostatin-positive (SST+), and pyramidal cells (Pyr), respectively, in the hippocampus. The cholinergic regulation of inhibitory activity contributes to inhibition onto pyramidal cells, which controls hippocampal network activity and memory processes. **b)** Aβ selectively inhibits $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs in PV+ and SST+ cells, respectively, but not $\alpha 3\beta 4$ -nAChRs in excitatory neurons, which reduces inhibition to induce hyperexcitability and network dysfunction in the hippocampus, leading to memory loss in AD. Additionally, Aβ-induced hippocampal hyperexcitability increases Aβ secretion to enhance the amyloid pathology. Stimulation of PV+ or SST+ cells by the activation of each nAChR subtype-specific agonists, **c)** PNU-282987 (PNU), an $\alpha 7$ agonist, or **d)** RJR-2403 Oxalate (RJR), an $\alpha 4\beta 2$ agonist, is insufficient to reverse the adverse effects of Aβ. **e)** Co-stimulation of hippocampal PV+ and SST+ inhibitory interneurons via activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs together is required to be protective against AD.

Current pharmacological therapies for AD primarily aim to compensate for the cholinergic deficits associated with disease progression. The most widely prescribed symptomatic treatments are acetylcholinesterase inhibitors, including donepezil, rivastigmine, and galantamine, which increase synaptic acetylcholine concentrations by inhibiting its enzymatic degradation (197). Although these drugs provide varied improvements in cognition, they neither halt disease progression nor alter AD pathology. One possible explanation for their limited efficacy is that acetylcholinesterase inhibitors enhance cholinergic signaling throughout the brain, resulting in the activation of all cholinergic receptor subtypes regardless of whether they contribute to AD pathology. Our previous data demonstrated that non-selective cholinergic stimulation worsens A β -induced dysfunction in hippocampal neurons (71, 72). A β selectively targets α 7- and α 4 β 2-nAChRs, global enhancement of acetylcholine signaling will also stimulate receptor populations that are not directly involved in A β pathology. Thus, non-selective cholinergic activation may disrupt normal circuit function and contribute to undesirable side effects. Similar observations have been reported for muscarinic receptors, where excessive activation of M1 receptors adversely affects neuronal activity within the prefrontal cortex and impairs working memory (198). These findings provide a mechanistic explanation for why acetylcholinesterase inhibitors produce only moderate clinical benefit and fail to substantially slow AD (199, 200).

Several studies have reported improvements in learning and memory following nicotine administration in rodents and non-human primates (201). However, other investigations have failed to demonstrate significant cognitive enhancement (202). Yet nicotinic receptor agonists continue to be represented as promising therapeutic

candidates because of their ability in regulating synaptic plasticity, neuronal excitability, and cognitive function (203). However, clinical trials evaluating nicotinic agonists have generally demonstrated varied efficacy and side effects (204). A possible explanation for these inconsistent outcomes may have been due to the focus on activation of single nicotinic receptor subtypes. Selective $\alpha 7$ -nAChR agonists have been shown to improve cognition in several models (205-207), other studies have reported little improvement in learning and memory (208). Likewise, selective activation of $\alpha 4\beta 2$ -nAChRs has been reported to improve working memory in young animals but not in aged animals (209). Collectively, these findings indicate that stimulation of either $\alpha 7$ - or $\alpha 4\beta 2$ -nAChRs alone is insufficient to consistently restore cognitive function, suggesting that activation of a single receptor subtype cannot fully compensate for the widespread disruption of hippocampal inhibitory circuits that occurs with AD.

This study supports a shift from first generation cholinergic therapies to receptor specific modulation. Rather than increasing cholinergic signaling through acetylcholinesterase inhibition or stimulating individual nicotinic receptor subtypes, co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs represents a more targeted strategy that directly addresses the cell-specific mechanisms disrupted by A β . Although several subtype-selective nicotinic agonists have entered clinical development, therapeutic strategies based on coordinated activation of multiple nicotinic receptor subtypes have not yet been systematically explored in AD (203). This study therefore proposes developing selective combination therapies that restore hippocampal inhibitory circuit function while minimizing the limitations associated with non-selective cholinergic stimulation.

A β is suggested to have profound effects on NMDA and AMPA receptors (NMDARs and AMPARs) via the direct interaction. However, there is insufficient data supporting this idea. First, only immunocytochemistry data supports that A β can bind to NMDARs (210). Additional studies have shown effects of A β on NMDARs (211, 212). However, one study heavily depends on a heterologous expression system using *Xenopus laevis* oocytes, which lacks the synaptic complexity, potentially limiting the physiological relevance of the observed direct activation of NMDARs by A β . The conclusion of direct receptor activation is also based primarily on electrophysiological and pharmacological data without fully excluding indirect mechanisms, such as interactions with membrane components or intermediary proteins. The use of simplified receptor compositions (NR1/NR2A and NR1/NR2B) also does not capture the diversity of receptor assemblies present *in vivo*. Second, there are no confirmed studies demonstrating direct physical binding of A β oligomers to AMPAR subunits. One study shows the roles of intracellular A β on AMPARs by injecting A β directly into cytosol (213), which does not accurately reflect the endogenous production, localization, and trafficking of intracellular A β in neurons. Importantly, we demonstrate that AMPAR dysfunction is downstream of A β -induced imbalance of excitation and inhibition by blocking $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs in hippocampal neurons (187). Finally, it is known that A β affects excitatory and inhibitory neurons differently (105). Since both excitatory and inhibitory cells express AMPARs and NMDARs, it is unknown how A β modifies these receptors to influence excitatory and inhibitory neurons differently. Conversely, multiple experimental data using immunocytochemistry (74), co-immunoprecipitation (74, 187), and a specific and quantitative time-resolved FRET (TR-FRET)-based binding assay

(100) demonstrate that A β binds to nAChRs, particularly the α 7- and α 4 β 2-subtypes. In fact, we provide computational and biochemical evidence showing that A β directly associates with α 7- and α 4 β 2-nAChRs (187). According to these findings, the effects of A β on AMPARs and NMDARs are unlikely to be the main pathogenic mechanism in AD.

How A β disrupts synaptic function is still unknown. It is hypothesized that there are direct molecular targets for A β such as nAChRs, glutamatergic receptors (NMDARs and AMPARs). Dysregulation of such receptors has been implicated in impaired synaptic plasticity, excitotoxicity, and cognitive dysfunction in AD. However, the mechanistic link between A β remains unclear. Studies have suggested that A β alters NMDAR function through receptor internalization, changes in receptor phosphorylation, or indirect modulation of intracellular signaling pathways. There is not very much biochemical evidence demonstrating high-affinity physical binding between A β oligomers and NMDAR subunits (210). Likewise, AMPAR dysfunction has consistently been observed in AD, particularly in association with impaired LTP, enhanced long-term depression LTD, and reduced surface receptor expression. However, the mechanistic link between A β remains unclear.

More evidence supports direct interactions between A β and nAChRs. Multiple approaches, such as immunocytochemistry, co-immunoprecipitation, computational molecular modeling, and quantitative time-resolved fluorescence resonance energy transfer (TR-FRET) assays, have demonstrated direct physical binding of A β to receptor subtypes (72, 74, 100). Furthermore, our previous work identified conserved charged amino acid residues within the α 7 and α 4 subunits that mediate this interaction and

demonstrated that these residues are absent from the $\alpha 3$ subunit, providing a structural explanation for receptor selectivity (72).

We previously showed that selective inhibition of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs by A β reduces the activity of hippocampal inhibitory interneurons, producing excitation-inhibition imbalance and neuronal hyperexcitability (71, 72). This loss of inhibitory control subsequently alters excitatory synaptic transmission. Therefore, the observed abnormalities in AMPAR function are more consistent with a consequence of hippocampal disinhibition than mediation by A β .

This model also provides a more comprehensive explanation for the selective vulnerability of different neuronal populations. Both excitatory pyramidal neurons and inhibitory interneurons express AMPARs and NMDARs, making it difficult to suggest that glutamate receptor be the explanation of why A β disrupts inhibitory interneuron function during the early stages of AD (105).

This study further supports why nicotinic receptor dysfunction is a more probable hypothesis as the mechanistic model for synaptic dysfunction to network abnormalities and cognitive impairment in AD. While nicotinic receptor dysfunction represents an upstream pathogenic mechanism, glutamatergic receptor dysfunction arises as downstream consequences of disrupted hippocampal inhibitory circuitry. This study integrates where A β first impairs cholinergic regulation of inhibitory interneurons, leading to excitation-inhibition imbalance that disrupts AMPAR- and NMDAR-dependent synaptic plasticity.

Theta oscillations in the hippocampus are generated by cholinergic input from the medial septum (MS) and is highly sensitive to nAChRs. Thus, they can be more

responsive to agonist stimulation than other frequencies (214, 215). In contrast, the local inhibitory interneuron networks play important roles in both slow and fast gamma oscillations, which makes them less sensitive to cholinergic inputs (215). In fact, we find that PNU or RJR treatment reduces theta power but have no major effect on slow and fast gamma powers in WT mice (**Fig. 16a**). This reduction may have been observed due to excessive nAChR activation. Importantly, we do not see this same reduction of theta rhythm in 5XFAD mice because the baseline cholinergic signaling is already impaired, and we are able to see that co-activation of $\alpha 7$ and $\alpha 4\beta 2$ nAChRs is able to restore to the optimal cholinergic tone. Therefore, agonist treatment likely enhances normal cholinergic activity in the WT hippocampus, resulting in this selective reduction in theta power, whereas local interneuron networks remain stable and resilient apart from this pharmacological activation.

Many $\alpha 7$ -nAChR agonists including PNU are known to act as serotonin 5-HT₃ receptor antagonists due to high structural homology between the receptors. However, the previous study has demonstrated that PNU is unable to affect hippocampal network activity in $\alpha 7$ -nAChR knockout mice, suggesting that effects of PNU on hippocampal oscillatory activity are exclusively mediated via $\alpha 7$ -nAChRs rather than 5-HT₃ receptors (216). Therefore, effects of PNU via 5-HT₃ receptor antagonism are unlikely.

Although this study provides novel insights into the role of subtype-specific nAChR signaling in AD, several limitations should be considered when interpreting these findings.

All *in vivo* experiments were performed using the 5XFAD mouse model, which rapidly develops amyloid pathology and cognitive impairment. Although this model

reproduces many key features of familial AD, it does not fully encompass the complex pathology of sporadic AD, nor in relation to the progression of the disease with tau pathology. Therefore, validation of these findings in additional AD models will be important. While 5XFAD mice that are based on overexpression of mutated transgenes have yielded key insights in mechanisms of disease, the model is subject to artifacts, including random genetic integration of the transgene, ectopic expression and non-physiological protein levels. An alternative model would be using APP KI mice that express APP containing a humanized A β region, including the Swedish, Iberian, and Arctic mutations (APP^{NL-G-F} KI) (217). APP^{NL-G-F} KI animals also show amyloid pathology, cholinergic dysfunction, hippocampal hyperexcitability, and memory loss by 6 months (217-219). Therefore, 5-month-old APP^{NL-G-F} KI mice would be suitable to overcome the limitations of the 5XFAD model.

Cognitive function was primarily evaluated using contextual fear conditioning, which predominantly assesses hippocampus-dependent associative memory. While our findings demonstrate significant improvements in memory following co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs, additional behavioral assays, for example the Morris water maze, Barnes maze, Y-maze, novel object recognition, and radial arm maze, would provide a broader assessment of hippocampal cognitive function.

The study relied primarily on pharmacological activation of nAChRs, PNU-282987 and RJR-2403. Genetic approaches would therefore provide stronger evidence for establishing causal relationships between individual receptor subtypes and hippocampal network function. Receptor subtype specificity was inferred primarily from pharmacological manipulation rather than cell type-specific genetic deletion. Conditional

knockout models targeting $\alpha 7$ - or $\alpha 4\beta 2$ -nAChRs selectively in PV+ or SST+ interneurons could establish the contribution of each receptor subtype within individual inhibitory circuits clearly. We found that intraperitoneal injection of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChR agonists together was required to improve fear memory in 5XFAD mice (220). However, systemic administration of nAChR agonists can affect many different brain regions. It is thus critical to determine whether the hippocampus is a prime site for the drugs' effects. Using bilaterally implant cannulas to locally inject nAChR agonists into the hippocampus and measure animals' memory using multiple hippocampus-dependent behavioral assays would test if selective *intrahippocampal* nAChR activation is sufficient to improve memory.

A β can inhibit glial nAChRs on astrocytes and microglia rather than neuronal receptors to contribute to AD pathology. Therefore, stimulation of nAChR on glial cells may induce the protective effects. Although roles of astrocytic and microglial nAChRs in AD have not been fully understood to date (221), it has been shown that stimulation of astrocytic and microglial $\alpha 7$ -nAChRs enhances A β phagocytosis in astrocytes and microglia to reduce A β levels (222, 223). Hippocampal A β in AD model mice can thus inhibit glial $\alpha 7$ -nAChRs to reduce phagocytic activity in these cells, resulting in an increase in A β levels to promote disease progress. nAChR agonist treatment would stimulate glial $\alpha 7$ -nAChRs to reduce A β levels in the AD hippocampus by enhancing phagocytosis, leading to the protective effects. In addition, activation of astrocytic $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs promotes the expression of glial cell-derived neurotrophic factor (GDNF) (224-226) that has been shown the protective roles in neurodegenerative disorders characterized mainly by damage of cholinergic neurons, such as AD (227).

Thus, A β can inhibit astrocytic $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs to reduce GDNF levels, resulting in the pathology, while activation of these receptors may induce the protective effects by increasing GDNF levels. To test these alternative ideas, we could measure phagocytosis by assaying phagocytic marker levels, such as CD206 and CD68 (228), and GDNF levels using a mouse GDNF ELISA kit in the AD hippocampus in the presence or absence of nAChR agonists. The results of these approaches would allow us to differentiate between the potential contributions of neuronal and glial nAChRs to AD pathology.

Importantly, direct involvement of rapid eye movement (REM) sleep in spatial and contextual memory consolidation is confirmed in mice (229, 230). Cholinergic activity is the highest during REM sleep, compared to non-REM sleep and wakefulness, while other neurotransmitter systems are almost silent (231-233). In fact, we recently found that co-stimulating $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs was necessary for reversing hippocampal oscillatory dysfunction following fear learning in 5XFAD mice (220). However, the link between cholinergic activity and memory consolidation-associated hippocampal oscillations is unknown. Further studies should determine if hippocampal network activity is significantly disrupted during REM sleep following fear learning in AD model mice. Further work should whether examine if co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs is required to reverse hippocampal network deficits during REM sleep after fear conditioning in AD model mice.

Experimental data was collected from cultured neurons and mouse models. Although these models provide important mechanistic insights, further studies using

clinical samples will be necessary to first determine whether the same receptor-specific mechanisms regulate hippocampal dysfunction in patients with AD.

Finally, the current work provides an opportunity to revisit the cholinergic hypothesis. Unfortunately, attempts to restore normal cholinergic activity using acetylcholinesterase inhibitors have only been moderately successful. Most of the previous research has focused on single nAChR subtype stimulation or non-selective activation in several brain disorders (203, 205-208, 234-236). It is also difficult to identify optimal combinations of nAChR agonists, thus co-activation of selective nAChRs has not been applied for both preclinical studies and clinical trials yet in AD (203). Identifying co-activation of $\alpha 7$ - and $\alpha 4\beta 2$ -nAChRs as a protective approach on hippocampal functions and memory in AD will thus provide a new perspective on the cholinergic hypothesis.

CHAPTER 6 - MATERIALS AND METHODS

Animals

C57Bl6J (Jax 000664), PV-Cre (Jax 017320), SST-Cre (Jax 018973), B6SJLF1/J (Jax 100012), and 5XFAD (Jax 034840) mice were purchased from Jackson laboratory and bred in the animal facility at Colorado State University (CSU). Clinical studies are observational and limited in the mechanistic information that can be tested in living patients. In contrast, animal studies permit the manipulation of variables and the investigation of effects on an entire organism. The animal model, however, is disadvantaged by species differences. AD is a strictly human disease, and we are aware that rodents do not develop true AD (117). Nonetheless, multiple transgenic mouse models develop the pathological hallmarks of the disease as they age, like those found in humans (117, 118). We thus employed a widely used amyloid pathology 5XFAD mouse models of AD. 5XFAD mice express human amyloid precursor protein (APP) and human presenilin 1 with the Familial Alzheimer's Disease (FAD) mutations (119, 120). Interestingly, biphasic changes in hippocampal circuit excitability are documented along the course of AD, with hyperactivity in the disease's preclinical stages and hypoactivity in its later stages (237-241). Importantly, 5XFAD mice show hippocampal hyperexcitability as early as 2.5 months of age (132). Additionally, at the age of 5 months, A β levels are significantly elevated in 5XFAD mice, which is associated with neuronal dysfunction and memory loss (119, 166, 242-245). Moreover, deep proteome profiling of the hippocampus shows no major difference in nAChR expression between 5XFAD mice and wild-type (WT) littermate controls at the

age of 5 months (246). However, cholinergic neuron loss is found after the age of 9 months in 5XFAD mice (247, 248). These findings suggest that in 5-month-old 5XFAD mice, A β -induced nAChR dysfunction occurs at the functional levels as opposed to the receptor expression levels. Hippocampal network dysfunction also reported in this model at the age of 3-6 months (172, 249). 5-month-old 5XFAD mice are thus the ideal amyloid pathology animal model for the current study. To collect brain tissues from animals, mice were deeply anesthetized and euthanized by CO₂ asphyxiation. 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). Results were reported following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (250).

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 (129, 251, 252). Hippocampi were isolated from P0 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 Ca²⁺ 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 (253). 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 (72, 187, 254).

Reagents

Soluble A β 42 oligomers (oA β 42) were prepared as previously described (72, 255). 1 mg of lyophilized human A β 42 (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 μ g aliquots, air-dried and stored at -80 °C. For use in experiments, an aliquot was thawed at room temperature and then dissolved in DMSO to make a 100 μ M solution. The solution was incubated for 16 hours at 4°C and then diluted to a final concentration for use in experiments. Scrambled A β 42 oligomers (sA β 42) were prepared the same way for controls. The following nAChR antagonists were used in this study: α -Bungarotoxin (α BTx) (Alomone labs), an α 7 antagonist, Dihydro- β -erythroidine hydrobromide (DH β E) (Tocris Bioscience), an α 4 β 2 antagonist, and α -Conotoxin AulB (Alomone labs), an α 3 β 4 antagonist. The following nAChR agonists were used in this study: PNU-282987 (PNU) (Tocris Bioscience), an α 7 agonist, RJR-2403 Oxalate (RJR) (Tocris Bioscience), an α 4 β 2 agonist, and NS-3861 (NS) (Tocris Bioscience), an α 3 β 4

agonist. Tetrodotoxin (TTX) (Abcam) was used to prevent action potential-dependent spontaneous network activity.

GCaMP Ca²⁺ Imaging with Nicotine Uncaging

For Ca²⁺ imaging with nicotine uncaging, a genetically encoded Ca²⁺ indicator, GCaMP, was used. As the majority of cells in hippocampal cultures are excitatory neurons (256), we expressed GCaMP6f (Addgene #40755) (257) 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) (258) was transfected for imaging inhibitory interneurons as shown previously (72). Glass-bottom dishes were mounted on a temperature-controlled stage on an Olympus IX73 microscope and maintained at 37°C and 5% CO₂ 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/mm², 1 sec) was used as shown previously (122). 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 and 1c, 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 and 1e, 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) (259) 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 and 2b, Ca^{2+} imaging with nicotine uncaging was conducted as described in Fig. 1d and 1e. The images were captured right after 250 nM sA β 42 or 250 nM oA β 42 was added to the media. The average peak amplitude in sA β 42-treated cells was used to normalize the peak amplitude in each cell, and the sA β 42 group's average peak amplitude was compared to the oA β 42-treated cells' average.

GCaMP Ca²⁺ Imaging

We measured spontaneous Ca²⁺ activity in cultured hippocampal excitatory neurons because It has been shown that networks of neurons in culture can produce spontaneous synchronized activity (260). 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 (260). Therefore, the somatic Ca²⁺ 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) (259) in cells prepared from PV-Cre or SST-Cre mice. We then measured Ca²⁺ activity in the soma of 14 DIV cultured hippocampal excitatory neurons with a modification of the previously described method (72, 129, 130, 187, 261-263). For GCaMP7s, the images were captured right after 250 nM sA β 42 or 250 nM oA β 42 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 Ca²⁺ 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 Ca²⁺ activity in the control group (CTRL) was used to normalize total Ca²⁺ activity in each cell. The control group's average total Ca²⁺ activity was compared to the experimental groups' average as described previously (72, 129, 187, 261-263).

Immunohistochemistry

c-Fos labeling was used to measure cell-type specific neuronal activity as shown previously (264, 265) because this provides activity patterns across multiple cell types simultaneously. We conducted immunohistochemistry with 5-month-old 5XFAD mice and WT littermates. Brain tissues were isolated and sectioned at 40 μm by using a vibratome. 10 consecutive sections containing the hippocampus (from Bregma: -1.64mm) in each mouse were stained with anti-PV, anti-SST, and anti-c-Fos antibodies. Nuclei was also labeled with DAPI as shown previously (264, 265). We imaged these sections using Olympus inverted microscope IX73 with the CellSens software. The colocalization analysis function in the CellSens program was utilized to identify c-Fos-positive (c-Fos+) signals on DAPI-positive (DAPI+) cells but PV and SST-negative cells as the activity of pyramidal cells. Similarly, we measured interneurons' activity by identifying c-Fos+ signals on PV+ or SST+ cells. The total number of c-Fos+ cells on each neuronal subtype in the hippocampus was counted using the particle analysis function in ImageJ (threshold = 100 μm^2). In addition, we measured the size of the hippocampus using ImageJ and normalize the total number of c-Fos+ cells on each neuronal subtype per $5.0 \times 10^6 \mu\text{m}^2$. As no overt hippocampal neuron loss is found by 12-month-old 5XFAD mice (266), cell-type specific neuronal activity was measured as an average of the total number of c-Fos+ cells on each cell subtype/ $5.0 \times 10^6 \mu\text{m}^2$ from each section. The average counts per section across all experimental conditions were then compared because multiple immunostainings were performed on the same anatomical sections, analyses were conducted at the section level to assess within-section differences in marker-defined cell populations.

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: $\pm$ 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 μ m, 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. 4a). According to a large body of studies, consolidation of hippocampus-dependent fear memory occurs between 1 and 3 hours after training (153, 154). 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. 4a). 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 (267) to acquire the digitized signal at 2 kHz. The electrode locations were verified histologically at the end of recordings (Fig. 4b). 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 (268). Recorded traces were downsampled to 2 kHz and then bandpass filtered between 0.1 and 500 Hz (Fig. 4c). We applied a notch filter at 60 Hz to reduce powerline artifacts. Power spectral density was calculated using Welch's method in the Brainstorm platform (268), which involves segmenting the signals and applies a Fast Fourier Transform (FFT) algorithm to each segment before averaging the power spectra (Fig. 4d). The power spectra were then processed by averaging frequency bins into larger predefined bands, including theta (4-8 Hz), slow gamma (30-50 Hz), and fast gamma (50-100 Hz). The power of each frequency band was normalized to WT controls and then compared before and after fear conditioning in WT and 5XFAD mice. We also compared the powers in each condition between WT and 5XFAD mice following learning. LFP power was quantified for each electrode and frequency band because multiple electrodes were obtained from the same animal. For theta-gamma phase amplitude coupling (PAC) analysis, we measured the high-frequency bands modulated by theta bands in epochs with a matched, filtered LFP amplitude envelope. Data were filtered into theta, slow gamma,

and fast gamma using a zero-phase FIR filter implemented with the eegfilt function in the EEGLAB toolbox for MATLAB (269). The analytic signal for each was computed using the Hilbert Transform (MATLAB function hilbert.m), resulting in instantaneous theta phase and gamma band amplitude. The peaks of the gamma-band amplitudes were defined as local maxima at or exceeding the mean + 1 standard deviation (SD) of the baseline gamma-band amplitude from the Hilbert Transform envelopes. The instantaneous theta phase for each peak was extracted. The mean vector length (MVL) was calculated as an entrainment strength. We compared the MVL before and after fear conditioning. The MVL in each condition was also compared in WT and 5XFAD mice following fear learning.

In vivo Activation of nAChRs

For *in vivo* activation of nAChRs, we used PNU-282987 (PNU), an $\alpha 7$ -nAChR agonist, and RJR-2403 Oxalate (RJR), an $\alpha 4\beta 2$ -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 (207, 208, 270-272), 2) they do not require endogenous acetylcholine to stimulate receptors since they are direct agonists rather than positive allosteric modulators (207, 208, 270, 273-276), and 3) repeated injection of these agonists is sufficient to show receptor activation in the rodent brains without causing receptor desensitization (274, 275, 277-279). 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* (207, 208, 270, 271). Saline was given to mice as a control.

Contextual Fear Conditioning (CFC)

Contextual fear conditioning (Habitest Modular System, Coulbourn Instrument) was carried out as described previously (167, 168). 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).

Amyloid Plaque Analysis

10 hippocampal sections (from Bregma: -1.64mm) in each mouse were obtained and stained with 1% Thioflavin S. Amyloid plaques were identified as Thioflavin S-positive puncta. The total number of amyloid plaques in the hippocampus was counted using the particle analysis function in ImageJ (threshold = 10 μm^2). We also measured the size of the hippocampus using ImageJ and normalized these measurements per 1 mm^2 . Amyloid plaques were measured as an average of these measurements per 1 mm^2 from 10 sections (total 20 hemisphere/animal). The average counts per animal across all experimental conditions were then compared between the conditions.

Statistical Analysis

The Franklin A. Graybill Statistical Laboratory at CSU was 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 10 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 or paired 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 Šídák's test. Statistical analyses for LFP and immunostaining data were performed using mixed-effects models / repeated-measures ANOVA with Tukey test, and animal identity included as a random effect. Electrodes and sections in LFP and c-Fos analysis, respectively, were treated as repeated measures nested within animals, and animal identity was included as a random effect. This approach accounts for the nested structure of the data, avoids pseudoreplication, and accommodates unequal electrode counts across animals. The graphs were presented as mean $\pm$ Standard Deviation (SD).

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APPENDICES

Table 1. Statistical analysis of Ca^{2+} imaging with nicotine uncaging in Fig. 4.

Normalized Peak Amplitude ($\Delta F/F_0$)	
EX	$1.000 \pm 0.879 \Delta F/F_0$
IN	$1.575 \pm 1.208 \Delta F/F_0$
Unpaired t test	
P value	0.0109
t, df	t=2.601, df=88

Table 2. Statistical analysis of Ca²⁺ imaging with nicotine uncaging in Fig. 5a and 5b.

Excitatory cells			
Normalized Peak Amplitude ($\Delta F/F_0$)			
	CTRL α BTx Dh β E α CTx	1.000 $\pm$ 0.492 $\Delta F/F_0$ 0.870 $\pm$ 0.328 $\Delta F/F_0$ 1.042 $\pm$ 0.644 $\Delta F/F_0$ 0.325 $\pm$ 0.362 $\Delta F/F_0$	
ANOVA summary			
F	7.453		
P value	0.0002		
P value summary	***		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.2619		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
CTRL vs. α BTx	0.1299	- 0.3451 to 0.6048	0.8881
CTRL vs. DH β E	-0.04198	- 0.4552 to 0.3712	0.9932
CTRL vs. α CTx	0.6746	0.2615 to 1.088	0.0003
α BTx vs. DH β E	-0.1719	- 0.6957 to 0.3519	0.8224
α BTx vs. α CTx	0.5448	0.02098 to 1.069	0.0385
DH β E vs. α CTx	0.7166	0.2481 to 1.185	0.0008
Inhibitory interneurons			
Normalized Peak Amplitude ($\Delta F/F_0$)			
	CTRL α BTx Dh β E α CTx	1.000 $\pm$ 0.525 $\Delta F/F_0$ 0.398 $\pm$ 0.290 $\Delta F/F_0$ 0.335 $\pm$ 0.300 $\Delta F/F_0$ 0.871 $\pm$ 0.330 $\Delta F/F_0$	
ANOVA summary			
F	10.91		

P value	<0.0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.3908		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
CTRL vs. αBTx	0.6021	0.1752 to 1.029	0.0025
CTRL vs. DHβE	0.6648	0.2991 to 1.031	<0.0001
CTRL vs. αCTx	0.1287	- 0.2371 to 0.4944	0.7866
αBTx vs. DHβE	0.06266	- 0.3538 to 0.4791	0.9782
αBTx vs. αCTx	-0.4735	-0.8899 to - 0.05707	0.0200
DHβE vs. αCTx	-0.5361	-0.8895 to - 0.1828	0.0010

Table 3. Statistical analysis of Ca²⁺ imaging with nicotine uncaging in Fig. 6a and 6b.

PV+ interneurons			
Normalized Peak Amplitude ($\Delta F/F_0$)			
	CTRL α BTx Dh β E α BTx + Dh β E	1.000 $\pm$ 0.590 $\Delta F/F_0$ 0.507 $\pm$ 0.374 $\Delta F/F_0$ 1.076 $\pm$ 0.613 $\Delta F/F_0$ 0.607 $\pm$ 0.448 $\Delta F/F_0$	
ANOVA summary			
F	10.97		
P value	<0.0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.1914		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
CTRL vs. α BTx	0.4929	0.1728 to 0.8130	0.0006
CTRL vs. DH β E	-0.07598	-0.4090 to 0.2571	0.9340
CTRL vs. α BTx + Dh β E	0.3934	0.08163 to 0.7052	0.0071
α BTx vs. DH β E	-0.5689	-0.8833 to -0.2544	<0.0001
α BTx vs. α BTx + Dh β E	-0.09949	-0.3913 to 0.1923	0.8118
DH β E vs. α BTx + Dh β E	0.4694	0.1634 to 0.7754	0.0006
SST+ interneurons			
Normalized Peak Amplitude ($\Delta F/F_0$)			
	CTRL α BTx Dh β E α BTx + Dh β E	1.000 $\pm$ 0.426 $\Delta F/F_0$ 0.917 $\pm$ 0.519 $\Delta F/F_0$ 0.524 $\pm$ 0.262 $\Delta F/F_0$ 0.533 $\pm$ 0.272 $\Delta F/F_0$	

ANOVA summary			
F	11.56		
P value	<0.0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.2614		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
CTRL vs. αBTx	0.08337	-0.2022 to 0.3689	0.8708
CTRL vs. DHβE	0.4764	0.2011 to 0.7518	<0.0001
CTRL vs. αBTx + DhβE	0.4675	0.2071 to 0.7278	<0.0001
αBTx vs. DHβE	0.3931	0.09978 to 0.6863	0.0038
αBTx vs. αBTx + DhβE	0.3841	0.1048 to 0.6634	0.0028
DHβE vs. αBTx + DhβE	-0.008950	-0.2777 to 0.2598	0.9998

Table 4. Statistical analysis of Ca²⁺ imaging with nicotine uncaging in Fig. 7.

PV+ interneurons	
Normalized Peak Amplitude ($\Delta F/F_0$)	
sA β 42	$1.000 \pm 0.772 \Delta F/F_0$
oA β 42	$0.448 \pm 0.233 \Delta F/F_0$
Unpaired t test	
P value	0.0038
t, df	t=3.032, df=53
SST+ interneurons	
Normalized Peak Amplitude ($\Delta F/F_0$)	
EX	$1.000 \pm 0.391 \Delta F/F_0$
IN	$0.600 \pm 0.237 \Delta F/F_0$
Unpaired t test	
P value	0.0002
t, df	t=4.101, df=44

Table 5. Statistical analysis of Ca²⁺ imaging in Fig. 8.

PV+ interneurons					
Normalized Total $\Delta F/F_{\min}$					
		sA β 42 sA β 42 + PNU sA β 42 + RJR sA β 42 + NS oA β 42 oA β 42 + PNU oA β 42 + RJR oA β 42 + NS		$1.000 \pm 0.379 \Delta F/F_{\min}$ $1.417 \pm 0.702 \Delta F/F_{\min}$ $1.036 \pm 0.558 \Delta F/F_{\min}$ $1.070 \pm 0.451 \Delta F/F_{\min}$ $0.589 \pm 0.223 \Delta F/F_{\min}$ $1.408 \pm 0.806 \Delta F/F_{\min}$ $0.528 \pm 0.299 \Delta F/F_{\min}$ $0.550 \pm 0.384 \Delta F/F_{\min}$	
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	1.647	3	0.5491	F (3, 182) = 2.246	P=0.0845
Row Factor	5.593	1	5.593	F (1, 182) = 22.88	P<0.0001
Column Factor	11.07	3	3.691	F (3, 182) = 15.10	P<0.0001
Residual	44.50	182	0.2445		
Tukey's multiple comparisons test	Mean diff.		95.00% CI of diff.		Adjusted P Value
sA β 42 vs. sA β 42 + PNU	-0.4166		0.8458 to 0.01249		0.0639
sA β 42 vs. sA β 42 + RJR	-0.03628		-0.3983 to 0.3257		>0.9999
sA β 42 vs. sA β 42 + NS	-0.07011		-0.4681 to 0.3279		0.9994
sA β 42 vs. oA β 42	0.4115		0.01348 to 0.8094		0.0371
sA β 42 vs. oA β 42 + PNU	-0.4079		-0.8127 to -0.003091		0.0468
sA β 42 vs. oA β 42 + RJR	0.4722		0.02258 to 0.9219		0.0320
sA β 42 vs. oA β 42 + NS	0.4500		0.05205 to 0.8480		0.0148

sAβ42 + PNU vs. sAβ42 + RJR	0.3804	- 0.08584 to 0.8466	0.2013
sAβ42 + PNU vs. sAβ42 + NS	0.3465	-0.1482 to 0.8412	0.3884
sAβ42 + PNU vs. oAβ42	0.8281	0.3334 to 1.323	<0.0001
sAβ42 + PNU vs. oAβ42 + PNU	0.008748	-0.4914 to 0.5089	>0.9999
sAβ42 + PNU vs. oAβ42 + RJR	0.8889	0.3517 to 1.426	<0.0001
sAβ42 + PNU vs. oAβ42 + NS	0.8667	0.3720 to 1.361	<0.0001
sAβ42 + RJR vs. sAβ42 + NS	-0.03383	-0.4715 to 0.4039	>0.9999
sAβ42 + RJR vs. oAβ42	0.4477	0.01004 to 0.8854	0.0409
sAβ42 + RJR vs. oAβ42 + PNU	-0.3716	- 0.8155 to 0.07230	0.1750
sAβ42 + RJR vs. oAβ42 + RJR	0.5085	0.02335 to 0.9937	0.0326
sAβ42 + RJR vs. oAβ42 + NS	0.4863	0.04862 to 0.9240	0.0180
sAβ42 + NS vs. oAβ42	0.4816	0.01364 to 0.9495	0.0387
sAβ42 + NS vs. oAβ42 + PNU	-0.3378	-0.8115 to 0.1360	0.3648
sAβ42 + NS vs. oAβ42 + RJR	0.5423	0.02975 to 1.055	0.0298
sAβ42 + NS vs. oAβ42 + NS	0.5201	0.05222 to 0.9881	0.0179
oAβ42 + CTRL vs. oAβ42 + PNU	-0.8194	-1.293 to -0.3456	<0.0001
oAβ42 + CTRL vs. oAβ42 + RJR	0.06077	-0.4518 to 0.5734	>0.9999
oAβ42 + CTRL vs. oAβ42 + NS	0.03858	-0.4293 to 0.5065	>0.9999
oAβ42 + PNU vs. oAβ42 + RJR	0.8801	0.3622 to 1.398	<0.0001
oAβ42 + PNU vs. oAβ42 + NS	0.8579	0.3842 to 1.332	<0.0001
oAβ42 + RJR vs. oAβ42 + NS	-0.02219	-0.5348 to 0.4904	>0.9999

Table 6. Statistical analysis of Ca²⁺ imaging in Fig. 9.

SST+ interneurons					
Normalized Total $\Delta F/F_{\min}$					
		sA β 42 sA β 42 + PNU sA β 42 + RJR sA β 42 + NS oA β 42 oA β 42 + PNU oA β 42 + RJR oA β 42 + NS			$1.000 \pm 0.499 \Delta F/F_{\min}$ $0.960 \pm 0.344 \Delta F/F_{\min}$ $1.286 \pm 0.448 \Delta F/F_{\min}$ $0.973 \pm 0.345 \Delta F/F_{\min}$ $0.604 \pm 0.295 \Delta F/F_{\min}$ $0.557 \pm 0.271 \Delta F/F_{\min}$ $0.994 \pm 0.358 \Delta F/F_{\min}$ $0.561 \pm 0.249 \Delta F/F_{\min}$
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	0.1032	3	0.03441	F (3, 165) = 0.2529	P=0.8592
Row Factor	5.984	1	5.984	F (1, 165) = 43.98	P<0.0001
Column Factor	4.458	3	1.486	F (3, 165) = 10.92	P<0.0001
Residual	22.45	165	0.1360		
Tukey's multiple comparisons test	Mean diff.		95.00% CI of diff.		Adjusted P Value
sA β 42 vs. sA β 42 + PNU	0.04027		-0.3043 to 0.3849		>0.9999
sA β 42 vs. sA β 42 + RJR	-0.2864		-0.6041 to 0.03128		0.1104
sA β 42 vs. sA β 42 + NS	0.02724		-0.3173 to 0.3718		>0.9999
sA β 42 vs. oA β 42	0.3965		0.07877 to 0.7141		0.0044
sA β 42 vs. oA β 42 + PNU	0.4431		0.1179 to 0.7684		0.0012
sA β 42 vs. oA β 42 + RJR	0.006344		-0.3189 to 0.3316		>0.9999
sA β 42 vs. oA β 42 + NS	0.4390		0.09440 to 0.7836		0.0033
sA β 42 + PNU vs. sA β 42 + RJR	-0.3267		-0.6798 to 0.02641		0.0922

sA β 42 + PNU vs. sA β 42 + NS	-0.01303	-0.3905 to 0.3644	>0.9999
sA β 42 + PNU vs. oA β 42	0.3562	0.003097 to 0.7093	0.0464
sA β 42 + PNU vs. oA β 42 + PNU	0.4029	0.04296 to 0.7628	0.0167
sA β 42 + PNU vs. oA β 42 + RJR	-0.03392	-0.3938 to 0.3260	>0.9999
sA β 42 + PNU vs. oA β 42 + NS	0.3987	0.02124 to 0.7762	0.0303
sA β 42 + RJR vs. sA β 42 + NS	0.3137	-0.03944 to 0.6667	0.1218
sA β 42 + RJR vs. oA β 42	0.6829	0.3560 to 1.010	<0.0001
sA β 42 + RJR vs. oA β 42 + PNU	0.7295	0.3953 to 1.064	<0.0001
sA β 42 + RJR vs. oA β 42 + RJR	0.2928	-0.04149 to 0.6270	0.1333
sA β 42 + RJR vs. oA β 42 + NS	0.7254	0.3723 to 1.078	<0.0001
sA β 42 + NS vs. oA β 42	0.3692	0.01613 to 0.7223	0.0334
sA β 42 + NS vs. oA β 42 + PNU	0.4159	0.05599 to 0.7758	0.0116
sA β 42 + NS vs. oA β 42 + RJR	-0.02089	-0.3808 to 0.3390	>0.9999
sA β 42 + NS vs. oA β 42 + NS	0.4117	0.03427 to 0.7892	0.0220
oA β 42 + CTRL vs. oA β 42 + PNU	0.04667	-0.2876 to 0.3809	0.9999
oA β 42 + CTRL vs. oA β 42 + RJR	-0.3901	-0.7244 to - 0.05587	0.0103
oA β 42 + CTRL vs. oA β 42 + NS	0.04252	-0.3106 to 0.3956	>0.9999
oA β 42 + PNU vs. oA β 42 + RJR	-0.4368	-0.7782 to - 0.09535	0.0031
oA β 42 + PNU vs. oA β 42 + NS	-0.004149	-0.3641 to 0.3558	>0.9999
oA β 42 + RJR vs. oA β 42 + NS	0.4326	0.07273 to 0.7925	0.0072

Table 7. Statistical analysis of c-Fos counting in hippocampal pyramidal cells in Fig. 10b.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	69.845 ± 27.943 91.705 ± 38.469 74.321 ± 36.753 71.036 ± 29.632 150.818 ± 56.931 121.873 ± 76.511 127.829 ± 59.897 79.067 ± 47.185	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	<0.0001	F (1, 280) = 47.12	
Section	0.0024	F (2.069, 193.1) = 6.102	
Genotype x Section	0.0028	F (2.069, 193.1) = 5.939	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	-23.58	-45.28 to -1.888	0.0295
Saline vs. RJR	3.554	-16.01 to 23.11	0.9590
Saline vs. PNU+RJR	-1.488	-23.19 to 20.21	0.9975
PNU vs. RJR	24.46	-1.677 to 50.60	0.0725
PNU vs. PNU+RJR	15.69	-15.33 to 46.71	0.5072
RJR vs. PNU+RJR	-3.890	-17.41 to 9.632	0.8577
5XFAD			
Saline vs. PNU	25.63	-31.71 to 82.97	0.6233
Saline vs. RJR	20.98	-9.221 to 51.18	0.2577

Saline vs. PNU+RJR	80.71	36.29 to 125.1	0.0001
PNU vs. RJR	-5.344	-57.42 to 46.73	0.9926
PNU vs. PNU+RJR	29.44	2.300 to 56.59	0.0292
RJR vs. PNU+RJR	54.08	17.14 to 91.01	0.0019
Saline			
WT vs. 5XFAD	-80.97	-101.8 to -60.10	<0.0001
PNU			
WT vs. 5XFAD	-30.17	-57.68 to -2.656	0.0321
RJR			
WT vs. 5XFAD	-53.51	-74.91 to -32.10	<0.0001
PNU+RJR			
WT vs. 5XFAD	-8.031	-26.63 to 10.57	0.3918
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	65.166 ± 28.820 90.577 ± 29.214 83.423 ± 37.648 82.915 ± 48.144 143.918 ± 47.784 110.495 ± 34.132 131.236 ± 53.291 78.509 ± 43.314	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	<0.0001	F (1, 88) = 47.12	
Section	0.0013	F (2.723, 176.1) = 5.796	
Genotype x Section	<0.0001	F (2.723, 176.1) = 12.81	

Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	-23.28	-41.89 to -4.670	0.0101
Saline vs. RJR	-15.24	-39.74 to 9.274	0.3452
Saline vs. PNU+RJR	-17.75	-49.61 to 14.12	0.4404
PNU vs. RJR	9.457	-17.82 to 36.73	0.7790
PNU vs. PNU+RJR	3.777	-28.83 to 36.39	0.9887
RJR vs. PNU+RJR	-2.513	-28.81 to 23.78	0.9937
5XFAD			
Saline vs. PNU	33.18	11.61 to 54.74	0.0013
Saline vs. RJR	22.93	-14.28 to 60.14	0.3558
Saline vs. PNU+RJR	72.86	42.96 to 102.8	<0.0001
PNU vs. RJR	-21.87	-48.29 to 4.555	0.1352
PNU vs. PNU+RJR	28.26	3.998 to 52.52	0.0175
RJR vs. PNU+RJR	45.18	12.21 to 78.14	0.0040
Saline			
WT vs. 5XFAD	-78.75	-97.14 to -60.37	<0.0001
PNU			
WT vs. 5XFAD	-19.92	-35.00 to -4.842	0.0104
RJR			
WT vs. 5XFAD	-47.81	-67.97 to -27.66	<0.0001
PNU+RJR			
WT vs. 5XFAD	4.406	-18.23 to 27.04	0.6983

Table 8. Statistical analysis of c-Fos counting in hippocampal PV+ cells in Fig. 11b.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	3.256 ± 1.730 3.008 ± 2.573 1.898 ± 1.580 4.833 ± 2.944 0.788 ± 0.929 3.827 ± 2.703 1.627 ± 1.735 4.554 ± 2.889	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0672	F (1, 88) = 3.436	
Section	<0.0001	F (2.312, 131.8) = 23.36	
Genotype x Section	0.0017	F (2.312, 131.8) = 6.135	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	0.01388	-1.889 to 1.917	>0.9999
Saline vs. RJR	1.315	-0.5405 to 3.170	0.2165
Saline vs. PNU+RJR	-0.9393	-3.511 to 1.632	0.7117
PNU vs. RJR	0.3299	-0.9866 to 1.646	0.8867
PNU vs. PNU+RJR	-2.642	-5.111 to -0.1730	0.0343
RJR vs. PNU+RJR	-3.339	-4.656 to -2.021	<0.0001
5XFAD			
Saline vs. PNU	-3.151	-4.533 to -1.769	<0.0001
Saline vs. RJR	-0.9984	-2.065 to 0.06850	0.0735

Saline vs. PNU+RJR	-4.092	-5.662 to -2.522	<0.0001
PNU vs. RJR	2.241	0.9280 to 3.554	0.0003
PNU vs. PNU+RJR	-0.6088	-2.352 to 1.134	0.7857
RJR vs. PNU+RJR	-2.875	-4.177 to -1.572	<0.0001
Saline			
WT vs. 5XFAD	2.468	1.672 to 3.264	<0.0001
PNU			
WT vs. 5XFAD	-0.8187	-2.191 to 0.5531	0.2354
RJR			
WT vs. 5XFAD	0.2716	-0.5180 to 1.061	0.4942
PNU+RJR			
WT vs. 5XFAD	0.2792	-1.321 to 1.880	0.7256
Male			
	WT + Saline	2.513 ± 1.294	
	WT + PNU	2.751 ± 1.591	
	WT + RJR	1.977 ± 1.495	
	WT + PNU + RJR	3.664 ± 2.559	
	5XFAD + Saline	0.733 ± 0.624	
	5XFAD + PNU	3.487 ± 2.980	
	5XFAD + RJR	1.067 ± 1.580	
	5XFAD + PNU + RJR	4.391 ± 3.143	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.2528	F (1, 266) = 1.313	
Section	<0.0001	F (2.110, 187.1) = 21.23	
Genotype x Section	0.0054	F (2.110, 187.1) = 5.214	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	-0.2465	-1.816 to 1.323	0.9708
Saline vs. RJR	0.6232	-0.3278 to 1.574	0.2873
Saline vs. PNU+RJR	-1.085	-2.757 to 0.5883	0.2959
PNU vs. RJR	1.167	0.2022 to 2.131	0.0138
PNU vs. PNU+RJR	-0.7766	-2.477 to 0.9238	0.6000

RJR vs. PNU+RJR	-1.779	-3.343 to -0.2149	0.0218
5XFAD			
Saline vs. PNU	-3.241	-5.156 to -1.326	0.0005
Saline vs. RJR	-0.2224	-1.021 to 0.5759	0.8721
Saline vs. PNU+RJR	-4.131	-5.909 to -2.352	<0.0001
PNU vs. RJR	2.772	1.454 to 4.090	<0.0001
PNU vs. PNU+RJR	-0.5985	-2.772 to 1.575	0.8783
RJR vs. PNU+RJR	-3.420	-5.025 to -1.816	<0.0001
Saline			
WT vs. 5XFAD	1.779	1.187 to 2.372	<0.0001
PNU			
WT vs. 5XFAD	-0.7365	-1.864 to 0.3913	0.1965
RJR			
WT vs. 5XFAD	0.9094	0.2000 to 1.619	0.0128
PNU+RJR			
WT vs. 5XFAD	-0.7273	-2.079 to 0.6245	0.2866

Table 9. Statistical analysis of c-Fos counting in hippocampal SST+ cells in Fig. 12b.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.868 ± 1.527 1.418 ± 0.687 1.725 ± 0.939 2.124 ± 1.333 0.781 ± 0.854 0.911 ± 0.934 2.876 ± 2.408 2.219 ± 1.498	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.6570	F (1, 89) = 0.1986	
Section	<0.0001	F (2.625, 121.6) = 10.77	
Genotype x Section	0.0002	F (2.625, 121.6) = 7.943	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	0.08190	-0.6761 to 0.8399	0.9880
Saline vs. RJR	0.2914	-0.7407 to 1.324	0.8544
Saline vs. PNU+RJR	-0.4847	-1.888 to 0.9181	0.7495
PNU vs. RJR	-0.4226	-1.194 to 0.3487	0.4188
PNU vs. PNU+RJR	-1.021	-2.428 to 0.3855	0.1911
RJR vs. PNU+RJR	-0.6521	-1.962 to 0.6577	0.5031
5XFAD			
Saline vs. PNU	-0.2318	-0.7893 to 0.3257	0.6740
Saline vs. RJR	-2.364	-3.771 to -0.9579	0.0006
Saline vs. PNU+RJR	-1.486	-2.281 to -0.6912	0.0002

PNU vs. RJR	-1.491	-2.485 to -0.4966	0.0022
PNU vs. PNU+RJR	-1.297	-2.238 to -0.3554	0.0042
RJR vs. PNU+RJR	0.8887	-0.6802 to 2.458	0.3994
Saline			
WT vs. 5XFAD	1.087	0.3811 to 1.794	0.0037
PNU			
WT vs. 5XFAD	0.5070	0.07205 to 0.9420	0.0234
RJR			
WT vs. 5XFAD	-1.151	-2.139 to -0.1632	0.0237
PNU+RJR			
WT vs. 5XFAD	-0.09475	-0.8809 to 0.6914	0.8094
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.699 ± 1.238 2.593 ± 1.741 1.776 ± 1.143 2.687 ± 1.386 0.511 ± 0.669 1.001 ± 0.903 2.650 ± 2.216 1.967 ± 1.413	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0012	F (1, 86) = 11.28	
Section	<0.0001	F (2.480, 131.4) = 10.15	
Genotype x Section	<0.0001	F (2.480, 131.4) = 9.251	

Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	-0.6092	-1.853 to 0.6344	0.5206
Saline vs. RJR	0.01845	-0.9760 to 1.013	>0.9999
Saline vs. PNU+RJR	-0.9301	-1.901 to 0.04050	0.0634
PNU vs. RJR	0.9116	-0.7084 to 2.532	0.3859
PNU vs. PNU+RJR	0.04961	-1.265 to 1.364	0.9996
RJR vs. PNU+RJR	-0.9559	-2.158 to 0.2457	0.1454
5XFAD			
Saline vs. PNU	-0.3339	-0.7896 to 0.1217	0.2145
Saline vs. RJR	-2.518	-3.814 to -1.222	<0.0001
Saline vs. PNU+RJR	-1.420	-2.237 to -0.6040	0.0004
PNU vs. RJR	-1.704	-2.770 to -0.6375	0.0008
PNU vs. PNU+RJR	-0.8153	-1.531 to -0.09946	0.0206
RJR vs. PNU+RJR	0.1945	-1.036 to 1.425	0.9714
Saline			
WT vs. 5XFAD	1.188	0.6284 to 1.748	0.0001
PNU			
WT vs. 5XFAD	1.592	0.8010 to 2.383	0.0003
RJR			
WT vs. 5XFAD	-0.8740	-1.770 to 0.02214	0.0557
PNU+RJR			
WT vs. 5XFAD	0.7206	0.007995 to 1.433	0.0476

Table 10. Statistical analysis of LFP data in Fig. 14a-c.

Female					
Theta power		Slow gamma power		Fast gamma power	
WT PRE	1.000 ± 1.199	WT PRE	1.000 ± 1.223	WT PRE	1.000 ± 1.086
WT POST	1.696 ± 1.552	WT POST	1.624 ± 1.420	WT POST	1.601 ± 1.372
Paired t test		Paired t test		Paired t test	
P value	0.0029	P value	0.0031	P value	0.0009
t, df	t=3.084, df=73	t, df	t=3.057, df=73	t, df	t=3.467, df=73
5XFAD PRE		5XFAD PRE		5XFAD PRE	
5XFAD POST	2.195 ± 6.108	5XFAD POST	3.145 ± 7.767	5XFAD POST	3.059 ± 7.262
	0.225 ± 0.210		0.480 ± 0.342		0.493 ± 0.284
Paired t test		Paired t test		Paired t test	
P value	0.0015	P value	0.0007	P value	0.0005
t, df	t=3.260, df=100	t, df	t=3.494, df=100	t, df	t=3.609, df=100
WT PRE vs 5XFAD PRE		WT PRE vs 5XFAD PRE		WT PRE vs 5XFAD PRE	
Šídák's multiple comparisons test		Šídák's multiple comparisons test		Šídák's multiple comparisons test	
p=0.0445 (t=2.292, df=346)		p=0.0022 (t=3.285, df=346)		p=0.0017 (t=3.373, df=346)	
Male					
Theta power		Slow gamma power		Fast gamma power	
WT PRE	1.000 ± 1.270	WT PRE	1.000 ± 1.124	WT PRE	1.000 ± 1.051
WT POST	1.926 ± 2.849	WT POST	2.049 ± 2.413	WT POST	2.467 ± 2.486
Paired t test		Paired t test		Paired t test	
P value	0.0001	P value	< 0.0001	P value	< 0.0001
t, df	t=3.926, df=124	t, df	t=5.816, df=124	t, df	t=8.187, df=124
5XFAD PRE		5XFAD PRE		5XFAD PRE	
5XFAD POST	2.168 ± 3.446	5XFAD POST	2.095 ± 3.080	5XFAD POST	2.055 ± 2.724
	0.632 ± 1.227		0.777 ± 1.221		0.841 ± 1.000
Paired t test		Paired t test		Paired t test	
P value	< 0.0001	P value	< 0.0001	P value	< 0.0001
t, df	t=4.906, df=105	t, df	t=4.684, df=105	t, df	t=4.857, df=105
WT PRE vs 5XFAD PRE		WT PRE vs 5XFAD PRE		WT PRE vs 5XFAD PRE	
Šídák's multiple comparisons test		Šídák's multiple comparisons test		Šídák's multiple comparisons test	
p=0.0004 (t=3.739, df=458)		p<0.0001 (t=4.002, df=458)		P=0.0001 (t=4.078, df=458)	

Table 11. Statistical analysis of LFP data in Fig. 15 a-b.

Female			
Theta-slow gamma PAC		Theta-fast gamma PAC	
WT PRE	0.036 ± 0.027	WT PRE	0.059 ± 0.087
WT POST	0.067 ± 0.044	WT POST	0.076 ± 0.070
Paired t test		Paired t test	
P value	< 0.0001	P value	0.0004
t, df	$t=6.667, df=73$	t, df	$t=4.128, df=73$
5XFAD PRE	0.055 ± 0.021	5XFAD PRE	0.028 ± 0.015
5XFAD POST	0.042 ± 0.018	5XFAD POST	0.028 ± 0.022
Paired t test		Paired t test	
P value	$p < 0.0001$	P value	0.9556
t, df	$t=6.032, df=100$	t, df	$t=0.056, df=100$
Male			
Theta-slow gamma PAC		Theta-fast gamma PAC	
WT PRE	0.041 ± 0.032	WT PRE	0.036 ± 0.042
WT POST	0.70 ± 0.053	WT POST	0.061 ± 0.061
Paired t test		Paired t test	
P value	< 0.0001	P value	< 0.0001
t, df	$t=7.153, df=124$	t, df	$t=9.003, df=124$
5XFAD PRE	0.060 ± 0.067	5XFAD PRE	0.041 ± 0.046
5XFAD POST	0.043 ± 0.047	5XFAD POST	0.030 ± 0.039
Paired t test		Paired t test	
P value	0.0157	P value	0.0180
t, df	$t=2.455, df=104$	t, df	$t=2.404, df=104$

Table 12. Statistical analysis of LFP theta power data in Fig. 16a.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR		1.000 ± 0.915 0.490 ± 0.950 0.566 ± 0.600 0.457 ± 1.209 0.151 ± 0.148 0.138 ± 0.234 0.127 ± 0.174 0.518 ± 0.834
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	<0.0001	F (1, 532) = 30.14	
Electrode	0.0323	F (3, 532) = 2.949	
Genotype x Electrode	<0.0001	F (3, 532) = 10.40	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	0.5098	0.1003 to 0.9193	0.0077
Saline vs. RJR	0.4344	0.06440 to 0.8043	0.0138
Saline vs. PNU+RJR	0.5429	0.2347 to 0.8510	<0.0001
PNU vs. RJR	-0.07542	-0.5191 to 0.3683	0.9719
PNU vs. PNU+RJR	0.03306	-0.3606 to 0.4267	0.9964
RJR vs. PNU+RJR	0.1085	-0.2438 to 0.4608	0.8574
5XFAD			
Saline vs. PNU	0.01254	-0.3304 to 0.3555	0.9997
Saline vs. RJR	0.02343	-0.3462 to 0.3931	0.9984
Saline vs. PNU+RJR	-0.3674	-0.6543 to -0.08049	0.0057
PNU vs. RJR	0.01089	-0.4079 to 0.4296	0.9999
PNU vs. PNU+RJR	-0.3799	-0.7278 to -0.03208	0.0260
RJR vs. PNU+RJR	-0.3908	-0.7650 to -0.01657	0.0368
Saline			
WT vs. 5XFAD	0.8494	0.6165 to 1.082	<0.0001
PNU			
WT vs. 5XFAD	0.3521	0.01810 to 0.6861	0.0388

RJR			
WT vs. 5XFAD	0.4384	0.1148 to 0.7620	0.0080
PNU+RJR			
WT vs. 5XFAD	-0.06087	-0.2817 to 0.1600	0.5884
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.000 ± 1.479 0.422 ± 0.365 0.489 ± 0.256 0.609 ± 1.648 0.280 ± 0.529 0.123 ± 0.137 0.090 ± 0.107 0.684 ± 0.986	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0004	F (1, 570) = 12.50	
Electrode	0.0024	F (3, 570) = 4.872	
Genotype x Electrode	0.0030	F (3, 570) = 4.696	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	0.5778	0.1486 to 1.007	0.0031
Saline vs. RJR	0.5111	0.06496 to 0.9573	0.0173
Saline vs. PNU+RJR	0.3909	0.01470 to 0.7671	0.0382
PNU vs. RJR	-0.06664	-0.5842 to 0.4509	0.9874
PNU vs. PNU+RJR	-0.1869	-0.6455 to 0.2718	0.7201
RJR vs. PNU+RJR	-0.1202	-0.5948 to 0.3544	0.9146
5XFAD			
Saline vs. PNU	0.1567	-0.3613 to 0.6747	0.8638
Saline vs. RJR	0.1893	-0.3185 to 0.6970	0.7720
Saline vs. PNU+RJR	-0.4040	-0.8031 to -0.004831	0.0460
PNU vs. RJR	0.03258	-0.5920 to 0.6571	0.9991
PNU vs. PNU+RJR	-0.5606	-1.101 to -0.02066	0.0384
RJR vs. PNU+RJR	-0.5932	-1.123 to -0.06308	0.0212
Saline			

WT vs. 5XFAD	0.7203	0.4500 to 0.9906	<0.0001
PNU			
WT vs. 5XFAD	0.2992	-0.1366 to 0.7350	0.1780
RJR			
WT vs. 5XFAD	0.3984	-0.04022 to 0.8371	0.0750
PNU+RJR			
WT vs. 5XFAD	-0.07455	-0.3935 to 0.2444	0.6464

Table 13. Statistical analysis of LFP slow gamma power data in Fig. 16b.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.000 ± 0.874 0.601 ± 0.963 1.452 ± 1.705 0.998 ± 2.758 0.268 ± 0.167 0.305 ± 0.419 0.214 ± 0.297 1.058 ± 1.633	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	<0.0001	F (1, 268) = 18.24	
Electrode	0.0240	F (3, 264) = 3.197	
Genotype x Electrode	<0.0001	F (3, 264) = 10.11	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	0.1377	-0.5836 to 0.8590	0.9605
Saline vs. RJR	-0.7994	-1.467 to -0.1323	0.0115
Saline vs. PNU+RJR	0.08354	-0.4661 to 0.6332	0.9794
PNU vs. RJR	-0.9371	-1.668 to -0.2060	0.0057
PNU vs. PNU+RJR	-0.05416	-0.7321 to 0.6237	0.9969
RJR vs. PNU+RJR	0.8830	0.2784 to 1.488	0.0011
5XFAD			
Saline vs. PNU	0.03497	-0.5617 to 0.6317	0.9988
Saline vs. RJR	0.2035	-0.4467 to 0.8536	0.8501
Saline vs. PNU+RJR	-0.7432	-1.231 to -0.2554	0.0006
PNU vs. RJR	0.1685	-0.5122 to 0.8492	0.9190
PNU vs. PNU+RJR	-0.7782	-1.365 to -0.1916	0.0039
RJR vs. PNU+RJR	-0.9467	-1.583 to -0.3099	0.0009
Saline			
WT vs. 5XFAD	0.7555	0.2897 to 1.221	0.0015
PNU			
WT vs. 5XFAD	0.6528	0.01591 to 1.290	0.0446
RJR			

WT vs. 5XFAD	1.758	1.137 to 2.380	<0.0001
PNU+RJR			
WT vs. 5XFAD	-0.07124	-0.5170 to 0.3745	0.7537
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.000 ± 1.079 0.879 ± 0.716 0.996 ± 0.461 1.149 ± 2.906 0.304 ± 0.414 0.272 ± 0.157 0.142 ± 0.122 1.300 ± 2.375	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0004	F (1, 260) = 12.70	
Electrode	0.0005	F (3, 310) = 6.133	
Genotype x Electrode	0.0225	F (3, 310) = 3.237	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	0.1095	-0.5254 to 0.7445	0.9704
Saline vs. RJR	-0.005204	-0.6656 to 0.6552	>0.9999
Saline vs. PNU+RJR	-0.1470	-0.7031 to 0.4092	0.9037
PNU vs. RJR	-0.1147	-0.8790 to 0.6496	0.9802
PNU vs. PNU+RJR	-0.2565	-0.9344 to 0.4215	0.7626
RJR vs. PNU+RJR	-0.1418	-0.8437 to 0.5602	0.9539
5XFAD			
Saline vs. PNU	0.03240	-0.7349 to 0.7997	0.9995
Saline vs. RJR	0.1664	-0.5856 to 0.9184	0.9405
Saline vs. PNU+RJR	-0.9977	-1.588 to -0.4077	0.0001
PNU vs. RJR	0.1340	-0.7904 to 1.058	0.9821
PNU vs. PNU+RJR	-1.030	-1.829 to -0.2311	0.0054
RJR vs. PNU+RJR	-1.164	-1.948 to -0.3797	0.0009
Saline			
WT vs. 5XFAD	0.6978	0.2971 to 1.099	0.0007

PNU			
WT vs. 5XFAD	0.6207	-0.02533 to 1.267	0.0597
RJR			
WT vs. 5XFAD	0.8694	0.2191 to 1.520	0.0089
PNU+RJR			
WT vs. 5XFAD	-0.1529	-0.6258 to 0.3200	0.5257

Table 14. Statistical analysis of LFP fast gamma power data in Fig. 16c.

Female			
	WT + Saline	1.000 ± 0.853	
	WT + PNU	0.532 ± 0.746	
	WT + RJR	1.455 ± 1.660	
	WT + PNU + RJR	1.052 ± 2.620	
	5XFAD + Saline	0.297 ± 0.162	
	5XFAD + PNU	0.269 ± 0.392	
	5XFAD + RJR	0.198 ± 0.240	
	5XFAD + PNU + RJR	1.253 ± 1.919	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0003	F (1, 532) = 13.01	
Electrode	0.0005	F (3, 532) = 5.948	
Genotype x Electrode	0.0010	F (3, 532) = 5.522	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	0.4679	-0.3300 to 1.266	0.4314
Saline vs. RJR	-0.4552	-1.176 to 0.2656	0.3640
Saline vs. PNU+RJR	-0.05159	-0.6520 to 0.5488	0.9962
PNU vs. RJR	-0.9232	-1.788 to -0.05858	0.0311
PNU vs. PNU+RJR	-0.5195	-1.287 to 0.2475	0.3012
RJR vs. PNU+RJR	0.4037	-0.2828 to 1.090	0.4289
5XFAD			
Saline vs. PNU	0.02842	-0.6398 to 0.6966	0.9995
Saline vs. RJR	0.09868	-0.6216 to 0.8190	0.9849
Saline vs. PNU+RJR	-0.9564	-1.515 to -0.3974	<0.0001
PNU vs. RJR	0.07027	-0.7456 to 0.8862	0.9961
PNU vs. PNU+RJR	-0.9848	-1.663 to -0.3071	0.0011
RJR vs. PNU+RJR	-1.055	-1.784 to -0.3259	0.0012
Saline			
WT vs. 5XFAD	0.7031	0.2494 to 1.157	0.0024
PNU			
WT vs. 5XFAD	0.2635	-0.3873 to 0.9143	0.4267
RJR			
WT vs. 5XFAD	1.257	0.6265 to 1.887	0.0001
PNU+RJR			

WT vs. 5XFAD	-0.2018	-0.6321 to 0.2285	0.3574
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	1.000 ± 1.008 0.667 ± 0.595 0.794 ± 0.380 0.904 ± 2.040 0.312 ± 0.347 0.263 ± 0.195 0.142 ± 0.139 1.261 ± 1.763	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0011	F (1, 570) = 10.80	
Electrode	<0.0001	F (3, 570) = 8.428	
Genotype x Electrode	<0.0001	F (3, 570) = 7.248	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	0.3329	-0.1438 to 0.8095	0.2747
Saline vs. RJR	0.2064	-0.2892 to 0.7019	0.7062
Saline vs. PNU+RJR	0.09575	-0.3221 to 0.5136	0.9350
PNU vs. RJR	-0.1265	-0.7014 to 0.4483	0.9418
PNU vs. PNU+RJR	-0.2371	-0.7465 to 0.2723	0.6274
RJR vs. PNU+RJR	-0.1106	-0.6377 to 0.4165	0.9490
5XFAD			
Saline vs. PNU	0.04936	-0.5260 to 0.6247	0.9962
Saline vs. RJR	0.1708	-0.3931 to 0.7347	0.8634
Saline vs. PNU+RJR	-0.9487	-1.392 to -0.5054	<0.0001
PNU vs. RJR	0.1214	-0.5722 to 0.8151	0.9694
PNU vs. PNU+RJR	-0.9980	-1.598 to -0.3983	0.0001
RJR vs. PNU+RJR	-1.119	-1.708 to -0.5307	<0.0001
Saline			
WT vs. 5XFAD	0.6877	0.3875 to 0.9879	<0.0001
PNU			
WT vs. 5XFAD	0.4041	-0.07985 to 0.8881	0.1015

RJR			
WT vs. 5XFAD	0.6521	0.1649 to 1.139	0.0088
PNU+RJR			
WT vs. 5XFAD	-0.3567	-0.7110 to - 0.002441	0.0484

Table 15. Statistical analysis of LFP theta-slow gamma PAC data in Fig. 17a.

Female			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	0.067 ± 0.044 0.125 ± 0.042 0.072 ± 0.034 0.100 ± 0.061 0.042 ± 0.018 0.072 ± 0.053 0.100 ± 0.062 0.120 ± 0.040	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0789	F (1, 546) = 3.100	
Electrode	<0.0001	F (3, 546) = 48.48	
Genotype x Electrode	<0.0001	F (3, 546) = 19.41	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	-0.05811	-0.08203 to -0.03419	<0.0001
Saline vs. RJR	-0.005962	-0.02757 to 0.01565	0.8927
Saline vs. PNU+RJR	-0.03313	-0.05112 to -0.01513	<0.0001
PNU vs. RJR	0.05215	0.02624 to 0.07807	<0.0001
PNU vs. PNU+RJR	0.02499	0.001997 to 0.04798	0.0270
RJR vs. PNU+RJR	-0.02716	-0.04774 to -0.006587	0.0040
5XFAD			
Saline vs. PNU	-0.03056	-0.05046 to -0.01066	0.0005
Saline vs. RJR	-0.05830	-0.07920 to -0.03741	<0.0001

Saline vs. PNU+RJR	-0.07795	-0.09432 to - 0.06158	<0.0001
PNU vs. RJR	-0.02774	-0.05148 to - 0.004002	0.0144
PNU vs. PNU+RJR	-0.04739	-0.06726 to - 0.02752	<0.0001
RJR vs. PNU+RJR	-0.01965	- 0.04051 to 0.001218	0.0733
Saline			
WT vs. 5XFAD	0.02470	0.01110 to 0.03830	0.0004
PNU			
WT vs. 5XFAD	0.05226	0.03282 to 0.07169	<0.0001
RJR			
WT vs. 5XFAD	-0.02764	-0.04608 to - 0.009195	0.0034
PNU+RJR			
WT vs. 5XFAD	-0.02012	-0.03273 to - 0.007517	0.0018
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	0.070 ± 0.053 0.126 ± 0.055 0.097 ± 0.042 0.108 ± 0.082 0.043 ± 0.047 0.122 ± 0.054 0.107 ± 0.032 0.071 ± 0.053	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0045	F (1, 574) = 8.128	
Electrode	<0.0001	F (3, 574) = 38.17	
Genotype x Electrode	0.0059	F (3, 574) = 4.209	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	-0.05610	-0.07907 to - 0.03314	<0.0001
Saline vs. RJR	-0.02726	-0.05113 to - 0.003381	0.0178

Saline vs. PNU+RJR	-0.03771	-0.05784 to - 0.01757	<0.0001
PNU vs. RJR	0.02885	0.001149 to 0.05654	0.0375
PNU vs. PNU+RJR	0.01840	- 0.006146 to 0.04294	0.2161
RJR vs. PNU+RJR	-0.01045	-0.03585 to 0.01495	0.7140
5XFAD			
Saline vs. PNU	-0.07905	-0.1057 to -0.05238	<0.0001
Saline vs. RJR	-0.06323	-0.09040 to - 0.03606	<0.0001
Saline vs. PNU+RJR	-0.02743	-0.04879 to - 0.006075	0.0055
PNU vs. RJR	0.01582	-0.01673 to 0.04837	0.5939
PNU vs. PNU+RJR	0.05162	0.02373 to 0.07950	<0.0001
RJR vs. PNU+RJR	0.03580	0.007430 to 0.06417	0.0067
Saline			
WT vs. 5XFAD	0.02650	0.01204 to 0.04097	0.0003
PNU			
WT vs. 5XFAD	0.003558	-0.01904 to 0.02615	0.7572
RJR			
WT vs. 5XFAD	-0.009470	-0.03294 to 0.01400	0.4285
PNU+RJR			
WT vs. 5XFAD	0.03678	0.01971 to 0.05385	<0.0001

Table 16. Statistical analysis of LFP theta-fast gamma PAC data in Fig. 17b.

Female			
	WT + Saline	0.076 ± 0.070	
	WT + PNU	0.090 ± 0.048	
	WT + RJR	0.053 ± 0.033	
	WT + PNU + RJR	0.065 ± 0.051	
	5XFAD + Saline	0.028 ± 0.022	
	5XFAD + PNU	0.055 ± 0.039	
	5XFAD + RJR	0.086 ± 0.036	
	5XFAD + PNU + RJR	0.078 ± 0.041	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0107	F (1, 267) = 6.604	
Section	<0.0001	F (3, 279) = 10.02	
Genotype x Section	<0.0001	F (3, 279) = 26.71	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
WT			
Saline vs. PNU	-0.02545	-0.04790 to -0.003008	0.0191
Saline vs. RJR	0.01421	-0.005747 to 0.03417	0.2567
Saline vs. PNU+RJR	0.01091	-0.005441 to 0.02726	0.3129
PNU vs. RJR	0.03967	0.01695 to 0.06239	<0.0001
PNU vs. PNU+RJR	0.03636	0.01550 to 0.05722	<0.0001
RJR vs. PNU+RJR	-0.003305	-0.02182 to 0.01521	0.9673
5XFAD			
Saline vs. PNU	-0.02663	-0.04447 to -0.008802	0.0008
Saline vs. RJR	-0.05564	-0.07446 to -0.03682	<0.0001
Saline vs. PNU+RJR	-0.04664	-0.06140 to -0.03188	<0.0001
PNU vs. RJR	-0.02900	-0.04958 to -0.008429	0.0018
PNU vs. PNU+RJR	-0.02001	-0.03811 to -0.001901	0.0237
RJR vs. PNU+RJR	0.008998	-0.009927 to 0.02792	0.6090
Saline			
WT vs. 5XFAD	0.04454	0.03117 to 0.05792	<0.0001
PNU			
WT vs. 5XFAD	0.04336	0.02460 to 0.06212	<0.0001

RJR			
WT vs. 5XFAD	-0.02531	-0.04313 to -0.007486	0.0055
PNU+RJR			
WT vs. 5XFAD	-0.01301	-0.02546 to -0.0005567	0.0406
Male			
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	0.061 ± 0.061 0.107 ± 0.053 0.088 ± 0.076 0.076 ± 0.064 0.030 ± 0.039 0.097 ± 0.047 0.077 ± 0.057 0.056 ± 0.032	
Fixed effects (type III)	P value	F (DFn, DFd)	
Genotype	0.0002	F (1, 574) = 14.12	
Section	<0.0001	F (3, 574) = 29.44	
Genotype x Section	0.2437	F (3, 574) = 1.394	
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value
WT			
Saline vs. PNU	-0.04548	-0.06696 to -0.02401	<0.0001
Saline vs. RJR	-0.02654	-0.04887 to -0.004218	0.0122
Saline vs. PNU+RJR	-0.01467	-0.03350 to 0.004156	0.1862

PNU vs. RJR	0.01894	-0.006960 to 0.04484	0.2360
PNU vs. PNU+RJR	0.03081	0.007864 to 0.05376	0.0033
RJR vs. PNU+RJR	0.01187	-0.01187 to 0.03562	0.5708
5XFAD			
Saline vs. PNU	-0.06721	-0.09214 to -0.04228	<0.0001
Saline vs. RJR	-0.04695	-0.07236 to -0.02155	<0.0001
Saline vs. PNU+RJR	-0.02577	-0.04574 to -0.005795	0.0052
PNU vs. RJR	0.02026	-0.01018 to 0.05069	0.3170
PNU vs. PNU+RJR	0.04144	0.01537 to 0.06752	0.0003
RJR vs. PNU+RJR	0.02119	-0.005340 to 0.04771	0.1683
Saline			
WT vs. 5XFAD	0.03098	0.01746 to 0.04451	<0.0001
PNU			
WT vs. 5XFAD	0.009258	-0.01187 to 0.03038	0.3898
RJR			
WT vs. 5XFAD	0.01057	-0.01138 to 0.03252	0.3445
PNU+RJR			
WT vs. 5XFAD	0.01989	0.003924 to 0.03585	0.0147

Table 17. Statistical analysis of fear conditioning in Fig. 18.

Female					
		WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR			$36.785 \pm 10.866\%$ $41.245 \pm 19.035\%$ $32.724 \pm 12.744\%$ $37.833 \pm 10.913\%$ $14.188 \pm 12.202\%$ $15.751 \pm 10.332\%$ $14.836 \pm 10.400\%$ $43.664 \pm 13.580\%$
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	3321	3	1107	F (3, 117) = 6.834	P=0.0003
Row Factor	6665	1	6665	F (1, 117) = 41.15	P<0.0001
Column Factor	6665	3	2222	F (3, 117) = 13.72	P<0.0001
Residual	18951	117	162.0		
Tukey's multiple comparisons test					
		Mean diff.	95.00% CI of diff.	Adjusted P Value	
WT + Saline vs. WT +PNU		-4.460	-18.01 to 9.094	0.9712	
WT + Saline vs. WT + RJR		4.061	-9.801 to 17.92	0.9851	
WT + Saline vs. WT + PNU+RJR		-1.048	-15.67 to 13.57	>0.9999	
WT + Saline vs. 5XFAD + Saline		22.60	10.74 to 34.45	<0.0001	
WT + Saline vs. 5XFAD + PNU		-6.879	-19.91 to 6.156	0.7320	
WT + Saline vs. 5XFAD + RJR		21.03	6.819 to 35.25	0.0003	
WT + Saline vs. 5XFAD + PNU+RJR		21.95	8.670 to 35.23	<0.0001	
WT + PNU vs. WT + RJR		8.521	-6.609 to 23.65	0.6624	
WT + PNU vs. WT + PNU+RJR		3.412	-12.42 to 19.24	0.9977	
WT + PNU vs. 5XFAD + Saline		27.06	13.74 to 40.37	<0.0001	
WT + PNU vs. 5XFAD + PNU		-2.419	-16.79 to 11.96	0.9995	
WT + PNU vs. 5XFAD + RJR		25.49	10.04 to 40.95	<0.0001	
WT + PNU vs. 5XFAD + PNU+RJR		26.41	11.81 to 41.01	<0.0001	
WT + RJR vs. WT + PNU+RJR		-5.109	-21.20 to 10.98	0.9764	
WT + RJR vs. 5XFAD + Saline		18.54	4.906 to 32.17	0.0013	
WT + RJR vs. 5XFAD + PNU		-10.94	-25.61 to 3.727	0.3014	
WT + RJR vs. 5XFAD + RJR		16.97	1.248 to 32.70	0.0248	
WT + RJR vs. 5XFAD + PNU+RJR		17.89	3.003 to 32.77	0.0075	
WT + PNU+RJR vs. 5XFAD + Saline		23.65	9.245 to 38.05	<0.0001	
WT + PNU+RJR vs. 5XFAD + PNU		-5.831	-21.22 to 9.555	0.9389	
WT + PNU+RJR vs. 5XFAD + RJR		22.08	5.685 to 38.48	0.0016	

WT + PNU+RJR vs. 5XFAD + PNU+RJR	23.00	7.404 to 38.59	0.0003		
5XFAD + Saline vs. 5XFAD + PNU	-29.48	-42.26 to -16.69	<0.0001		
5XFAD + Saline vs. 5XFAD + RJR	-1.563	-15.55 to 12.43	>0.9999		
5XFAD + Saline vs. 5XFAD + PNU+RJR	-0.6480	-13.68 to 12.39	>0.9999		
5XFAD + PNU vs. 5XFAD + RJR	27.91	12.91 to 42.91	<0.0001		
5XFAD + PNU vs. 5XFAD + PNU+RJR	28.83	14.71 to 42.95	<0.0001		
5XFAD + RJR vs. 5XFAD + PNU+RJR	0.9153	-14.30 to 16.13	>0.9999		
Male					
	WT + Saline WT + PNU WT + RJR WT + PNU + RJR 5XFAD + Saline 5XFAD + PNU 5XFAD + RJR 5XFAD + PNU + RJR	38.938 ± 13.066% 32.414 ± 11.302% 38.651 ± 15.155% 38.346 ± 11.517% 16.177 ± 8.761% 15.558 ± 6.577% 14.648 ± 6.018% 43.434 ± 21.002%			
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	5022	3	1674	F (3, 107) = 10.82	P<0.0001
Row Factor	5304	1	5304	F (1, 107) = 34.29	P<0.0001
Column Factor	1997	3	665.8	F (3, 107) = 4.305	P=0.0066
Residual	16549	107	154.7		
Tukey's multiple comparisons test		Mean diff.	95.00% CI of diff.	Adjusted <i>P</i> Value	

WT + Saline vs. WT +PNU	6.524	-8.505 to 21.55	0.8804
WT + Saline vs. WT + RJR	0.2876	-13.31 to 13.88	>0.9999
WT + Saline vs. WT + PNU+RJR	0.5926	-14.44 to 15.62	>0.9999
WT + Saline vs. 5XFAD + Saline	22.76	11.88 to 33.65	<0.0001
WT + Saline vs. 5XFAD + PNU	-4.496	-17.74 to 8.745	0.9654
WT + Saline vs. 5XFAD + RJR	23.38	9.786 to 36.97	<0.0001
WT + Saline vs. 5XFAD + PNU+RJR	24.29	9.818 to 38.76	<0.0001
WT + PNU vs. WT + RJR	-6.237	-23.19 to 10.72	0.9470
WT + PNU vs. WT + PNU+RJR	-5.932	-24.06 to 12.19	0.9717
WT + PNU vs. 5XFAD + Saline	16.24	1.366 to 31.11	0.0221
WT + PNU vs. 5XFAD + PNU	-11.02	-27.69 to 5.653	0.4581
WT + PNU vs. 5XFAD + RJR	16.86	-0.09964 to 33.81	0.0525
WT + PNU vs. 5XFAD + PNU+RJR	17.77	0.09869 to 35.43	0.0477
WT + RJR vs. WT + PNU+RJR	0.3050	-16.65 to 17.26	>0.9999
WT + RJR vs. 5XFAD + Saline	22.47	9.055 to 35.89	<0.0001
WT + RJR vs. 5XFAD + PNU	-4.784	-20.18 to 10.61	0.9788
WT + RJR vs. 5XFAD + RJR	23.09	7.395 to 38.79	0.0004
WT + RJR vs. 5XFAD + PNU+RJR	24.00	7.539 to 40.47	0.0004
WT + PNU+RJR vs. 5XFAD + Saline	22.17	7.298 to 37.04	0.0003
WT + PNU+RJR vs. 5XFAD + PNU	-5.089	-21.76 to 11.58	0.9809
WT + PNU+RJR vs. 5XFAD + RJR	22.79	5.832 to 39.74	0.0016
WT + PNU+RJR vs. 5XFAD + PNU+RJR	23.70	6.031 to 41.36	0.0017
5XFAD + Saline vs. 5XFAD + PNU	-27.26	-40.32 to -14.20	<0.0001
5XFAD + Saline vs. 5XFAD + RJR	0.6189	-12.80 to 14.04	>0.9999
5XFAD + Saline vs. 5XFAD + PNU+RJR	1.529	-12.78 to 15.84	>0.9999
5XFAD + PNU vs. 5XFAD + RJR	27.88	12.48 to 43.27	<0.0001
5XFAD + PNU vs. 5XFAD + PNU+RJR	28.79	12.61 to 44.96	<0.0001

5XFAD + RJR vs. 5XFAD + PNU+RJR	0.9101	-15.55 to 17.37	>0.9999
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Table 18. Statistical analysis of plaques in Fig. 19.

Female			
Average number of plaques / mm ²			
	Saline PNU RJR PNU + RJR	50.62 ± 13.21 49.71 ± 11.17 47.71 ± 10.28 32.19 ± 7.19	
ANOVA summary			
F	7.497		
P value	0.0006		
P value summary	***		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.3981		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
Saline vs. PNU	0.9117	-12.55 to 14.37	0.9978
Saline vs. RJR	2.916	-10.54 to 16.37	0.9359
Saline vs. PNU + RJR	18.43	6.280 to 30.58	0.0013
PNU vs. RJR	2.004	-12.18 to 16.19	0.9808
PNU vs. PNU + RJR	17.52	4.566 to 30.47	0.0046
RJR vs. PNU + RJR	15.51	2.562 to 28.46	0.0137
Male			
Average number of plaques / mm ²			

	Saline PNU RJR PNU + RJR	38.95 ± 13.53 38.66 ± 8.85 47.88 ± 5.97 24.82 ± 9.89	
ANOVA summary			
F	10.57		
P value	<0.0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.4360		
Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Adjusted P Value
Saline vs. PNU	0.2931	-12.33 to 12.92	>0.9999
Saline vs. RJR	-8.926	-21.55 to 3.700	0.2469
Saline vs. PNU + RJR	14.14	3.706 to 24.57	0.0042
PNU vs. RJR	-9.220	-23.05 to 4.612	0.2951
PNU vs. PNU + RJR	13.84	1.982 to 25.70	0.0165
RJR vs. PNU + RJR	23.06	11.20 to 34.92	<0.0001