AB42 alters glutamatergic transmission in the CA1 region of the mouse hippocampus

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Aβ42 alters glutamatergic transmission in the CA1 region of the mouse hippocampus

by

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Abstract

Alzheimer’s disease (AD) is a neurodegenerative disease affecting 4.5 million people in the US, a number expected to increase 3-fold over the next 30 years. One of the hallmarks of AD is the extracellular accumulation of the peptide Aβ₄₂ throughout in the brain, particularly in limbic structures like the temporal cortex and hippocampus, key regions for learning and memory. Currently available animal models for AD aim to reproduce key genetic traits of the familial variant of the disease, but >90% of AD patients are affected by idiopathic AD. To overcome this potential limitation, we use a different approach to study AD, based on the use of viral induction of Aβ₄₂ accumulation in the mouse hippocampus. Our findings show that this leads to a reduction in the glutamate uptake capacity of hippocampal astrocytes, with no change in their potassium buffering ability. We find that the NMDA/AMPA ratio in CA1 pyramidal cells (CA1-PCs) is reduced in mice injected with the AAV-Aβ₄₂. These data identify astrocytic glutamate uptake and NMDA/AMPA receptors as key targets of Aβ₄₂ accumulation in the hippocampus, paving the way to a deeper understanding of the onset and progression of idiopathic AD.
Introduction

Alzheimer’s disease: main traits

AD is a progressive neurodegenerative disease that has been designated as the most common form of dementia throughout the world (Su et al., 2021). AD onset has been seen in individuals beginning at 60 years old, and risk is known to increase with age (Matthews et al., 2019). According to the Alzheimer’s Association, today, more than 5.8 million cases of AD have been reported in the United States in adults aged 65 and older, with it being labeled as the 5th leading cause of death within the same age bracket. According to the National Institute on Aging (NIA), AD is defined symptomatically by memory loss, loss of cognition, reduction in spatial awareness and disrupted sleep pattern. These symptoms are brought on by a combination of two different neurological factors, the accumulation of beta amyloid-42 ($\text{A}_{\beta 42}$) and tau protein entanglements (Yeung et al., 2020). $\text{A}_{\beta 42}$ is a peptide of the beta amyloid family; however, unlike the physiologically functional peptides in this family, it is 42 residues long (Hsu et al., 2018). This large size leads to a neurotoxic effect and the formation of insoluble deposits, referred to as plaques, in the brain (De et al., 2019). These deposits are known to become more prevalent as the disease progresses (Yan et al., 2009).

Tau protein entanglements are produced when tau/microtubule binding is interfered with by mutations, as well as phosphorylation, that disturb localization of the protein. The disturbance in tau associated with AD is an increase in tau protein. This tauopathy leads to neuronal death and dementia (Drummond et al., 2020). The accumulation of tau entanglements and amyloid plaques are hallmarks of AD pathogenesis.
Genetic and idiopathic AD: Converging symptoms between different AD variants

There are several different variations of AD (Spina et al., 2021). While the symptoms of said variants remain somewhat consistent, the genesis of each specific form of AD is diverse. AD variants can be arranged by two distinct methods of pathogenesis: genetic and idiopathic.

Genetic AD has been heavily associated with a number of known genetic mutations and polymorphisms. Missense mutations in presenilin 1 and 2, as well as in amyloid precursor protein (APP) result in a dysregulation of gamma-secretase (Zhou et al., 2020; Marei et al., 2021). This dysregulation leads to enhanced proteolysis of βAPP, which has been shown to lead to the accumulation of Aβ₄₂ and is linked to genetic variants of AD (Kouchi et al., 1998). Inheritance of an e4 allele of Apolipoprotein E, referred to as ApoE4 polymorphism, is linked to late onset genetic AD (Lin et al., 2019). This polymorphism is linked to an increased density and Aβ₄₂ plaques and vascular deposits within the brain. ApoE4 polymorphism is the strongest risk factor for developing late onset genetic AD. There are animal models that have been developed to research each of the genetic mutations mentioned that are associated with AD. The most prominent models are in rats and mice (Sarasa & Pesini., 2009; Benedikz et al., 2009). While the use of animal models in studying AD has been incredibly productive, they are limited in their capacity to present a reliable model for the idiopathic form of AD.

The idiopathic pathogenesis AD is far less defined compared to the genetic variants. According to the National Institute on Aging, there is no specific gene that leads to late-onset AD. While the general characteristics of AD remain the same, accumulation of Aβ₄₂ in plaques and tau neurofibrillary entanglements, the cause of these inefficiencies remains unknown (United Kingdom National Health Service 2021). There are environmental risk factors that have been linked to idiopathic AD, which include metabolic disorders, stress and disrupted sleep patterns (Silva et al.,
The limited knowledge regarding the pathogenesis of idiopathic AD had drastically handicapped research on this variation of the disease.

**The hippocampus and its implication in AD**

The hippocampus is a brain region present in the brain of all mammals, located ventral with respect to the medial temporal lobe (Donato et al., 2020). The hippocampus is composed of the dentate gyrus, the subiculum, and the CA1-3 fields. Excitatory inputs reach the hippocampus via the perforant path, which connects the entorhinal cortex to the DG. From the DG, another excitatory path sends information to the CA3 region, and this is then relayed to the CA1 region, the major output center of the hippocampus. The CA1 region of the hippocampus relays information to the subiculum and back to the entorhinal cortex. These connections and projections involving the hippocampus are what give it its function as a major learning and memory center for the brain.

The hippocampus is implicated in AD pathogenesis (Fjell et al., 2014). The hippocampus is an especially sensitive brain region to the early effects of Aβ₄₂ aggregate formation. AD is associated with hippocampal volume loss and deterioration of neurological function. This loss of function results in the onset of some of the classic AD symptoms such as disrupted circadian rhythm, reduced cognitive ability and memory loss.

**Glutamatergic transmission in AD**

Glutamate is a neurotransmitter that is responsible for numerous excitatory activities throughout the brain (Zhou & Danbolt., 2014). Glutamate’s function in the hippocampus is related to neuroplasticity and cognitive functions surrounding learning and memory. Its associated post
synaptic receptors are excitatory amino acid transporters (EAAT) 1-5, AMPA receptors and NMDA receptors (AMPAR and NMDAR, respectively).

The function of glutamate transporters to regulate glutamate concentration in the extracellular space and maintain it at levels that are ideal for biological function, without leading to a high concentration of glutamate that would cause toxicity. There are two types of glutamate transporters expressed in hippocampal astrocytes, GLT-1 and GLAST. GLT1 is the main astrocytic glutamate transporter in the adult brain (Danbolt., 2001).

AMPARs are ionotropic transmembrane glutamate receptors that are associated with fast glutamatergic transmission. NMDARs are ionotropic transmembrane glutamate receptors that are associated with slow glutamatergic transmission. It is in regard to these receptors that detrimental changes are observed in AD disease states. A number of studies have shown that alterations in receptors involved in glutamatergic transmission are associated with AD disease progression. The function and expression of GLT1, but not GLAST, are impaired in AD (Scimemi et al., 2013). Others have shown that there is a significant downregulation of AMPARs and NMDARs in AD (Zhang et al., 2018). NMDARs are heteromers comprising a number of different subunits, particularly NR1 and NR2 subunits (Gielen et al., 2009). The subunit composition on NMDARs can determine both the localization of the receptor as well as the kinetic properties (McQuail et al., 2016). It is for these reasons that we were determined to investigate the implications that changes in glutamatergic transmission have on AD.

**Spines and the anatomical correlates of glutamatergic synapses**

A primary structure involved in glutamatergic transmission are dendritic spines (Yao et al., 2008). Dendritic spines are protrusions of dendrites that function as a point of synaptic contact in a
postsynaptic manner for excitatory glutamatergic activity. Spines grow off of the parent dendrite and make contact with a presynaptic specialization in order to form an excitatory glutamatergic synapse. The loss of dendritic spine density, or reduced function, has been shown to be directly related to the loss of synaptic function (Aguilar-Hernández et al., 2020). In turn, loss of synaptic function is associated with memory and cognitive decline, which are two major known effects of AD. It is due to this relationship that a variety of dendritic spine analysis were performed in the context of this thesis.

**Astrocytes and glutamate uptake**

Astrocytic activity is a particularly vital component of glutamatergic transmission. Astrocytes contain two major glutamate transporters: GLT1 and GLAST (Shen et al., 2020). Both transporters collect glutamate from the extracellular space. After glutamate is taken up into the astrocyte cytoplasm, glutamate is converted into glutamine and eventually transported back to the presynaptic terminal, where it is converted into glutamate, stored into synaptic vesicles ready to be released in the synaptic cleft (Bak et al., 2006). Glutamatergic transporter currents in whole cell voltage clamp electrophysiology can give insight into the glutamate transport being done by astrocytes via this mechanism.
Materials and methods

Ethics statement

All experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the State University of New York (SUNY) Albany and guidelines described in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Mice

All experiments were performed on C57BL/6NCrl mice of either sex, aged 3-8 weeks. Food and water were available ad libitum to all mice throughout the 24-hour period. Unless otherwise stated, all mice were group housed and kept under 12 h:12 h L/D conditions (lights on at 7 AM; lights off at 7 PM).

Stereotaxic surgeries

We utilized a Neurostar motorized stereotaxic apparatus (Neurostar, Tubingen, Germany), controlled through Neurostar software, to perform all stereotaxic surgical procedures. Along with the Neurostar stereotaxic apparatus, a hand-held drill was used to bore holes into the mouse skull during the surgical procedures. The apparatus was equipped with a 1ul Hamilton syringe (Hamilton, Reno, NV), which was manually loaded with the AAV construct that was to be injected in the mouse brain. We used the following AAVs for the stereotaxic injections:

AAV-1-hEF1a-EGFP-\( \text{A}\beta_{42} \)-WPRE-bGHp(A) - here referred to as AAV-\( \text{A}\beta_{42} \)

AAV-1/2- hEF1a-EGFP-WPRE-bGHp(A) - here referred to as AAV-Sham
The AAV-Aβ42 was a generous gift from Dr. Martin Darvas (Washington University, Seattle, WA). The AAV-Sham was purchased from the Viral Vector Facility at the Neuroscience Center (Zurich, Switzerland). This virus was used as a control, as it had the same viral type (adeno) and serotype as the experimental AAV-Aβ42. Each virus was injected separately on different mice during surgical procedures.

To begin each stereotaxic surgery, mice were initially anesthetized with 3-5% isoflurane. Soon after the mice were under the effects of the anesthetic, the hair on their scalp was removed with nair. Each mouse was then transferred to the stereotaxic frame where their skull was positioned with the use of ear bars. At this point, 2% lidocaine was administered with a cotton tip applicator to the bald scalp of the mouse. After a waiting period of 5 min, the scalp was sterilized with the use of 70% ethanol and betadine. Once sterilized, an incision was made along the midline of the mouse skull in a rostral to caudal manner. Neurostar software was used to position the tip of the Hamilton syringe on bregma, which was set as the origin for the surgical coordinates. The injections done for this procedure were hippocampal, specifically targeting the CA1 region of the hippocampus. The coordinates were: AP: -1.8 mm, ML: ±1.3 mmL, DV: 1.4 mm. Once the needle was positioned correctly, a hole was bored into the skull of the mouse, allowing the needle to directly penetrate brain tissue. 0.2 μl of the viral construct was injected into the mouse hippocampus at a rate of 0.05 μl/min. The procedure involved a bilateral injection of the viral construct into both the left and right hemispheres of the mouse brain. Once the viral injection was completed, the incision site was closed with veterinary tissue adhesive and the mouse was allowed to recover for 3 weeks before euthanasia.

**Electrophysiology**
We performed whole-cell patch-clamp electrophysiology from cells (neurons and astrocytes) identified under differential interference contrast (DIC) in transverse slices of the mouse hippocampus. The slices were prepared from brains isolated after transcardial perfusion of a sucrose based artificial cerebrospinal fluid (ACSF) containing (in mM): 248 sucrose, 2 KCl, 2 MgSO₄·7H₂O, 1.25 NaH₂PO₄, 1 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 D-glucose. The slices (250 μm thick) were prepared using a vibrating blade microtome (VT1200, Leica Microsystems, Buffalo Grove, IL). The slicing solution was kept at 4°C and contained (in mM): 26.2 NaHCO₃, 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgSO₄·7H₂O, 20 D-glucose, 3CaCl₂, 4 MgCl₂ 300 mOsm, pH 7.4. The slices were allowed to recover in this solution at 36°C for 30 min and at RT for up to 6 hrs. Whole cell patch clamp electrophysiology was performed on both CA1-PCs and astrocytes using an intracellular solution containing (in mM) 120 CsCH₃SO₃. The external solution used to patch CA1-PCs contained (in mM): 26.2 NaHCO₃, 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgSO₄·7H₂O, 20 D-glucose, 1.2 CaCl₂. We used 100 mM picrotoxin (PTX) as an antagonist to GABAergic activity and 50 mM 2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) to antagonize AMPA receptors. The external solution used to patch astrocytes contained (in mM): 26.2 NaHCO₃, 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgSO₄·7H₂O, 20 D-glucose, 2.5 CaCl₂.

Glutamate uncaging electrophysiology experiments were performed on hippocampal CA1-PCs. The slices for these experiments were prepared from brains isolated after transcardial perfusion of a sucrose based artificial cerebrospinal fluid (ACSF) containing (in mM): 248 sucrose, 2 KCl, 2 MgSO₄·7H₂O, 1.25 NaH₂PO₄, 1 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 D-glucose. The slices (250 μm thick) were prepared using a vibrating blade microtome (VT1200, Leica Microsystems, Buffalo Grove, IL). The slicing solution was kept at 4°C and contained (in mM): 26.2 NaHCO₃, 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgSO₄·7H₂O, 20 D-glucose, 3CaCl₂, 4 MgCl₂ 300 mOsm, pH 7.4. The
slices were allowed to recover in this solution at 37°C for 30 min and at RT for up to 6 hrs. Rubi glutamate uncaging was performed using an intracellular solution containing (in mM) 120 CsCH$_3$SO$_3$. The external solution used to patch CA1-PCs contained (in mM): 26.2 NaHCO$_3$, 119 NaCl, 2.5 KCl, 1 NaH$_2$PO$_4$, 1.3 MgSO$_4$•7H$_2$O, 20 D-glucose, 50 Rubi glutamate, 1.2 CaCl$_2$.

**Biocytin fills**

Neurons and astrocytes that were patched in the electrophysiology procedure previously mentioned were filled with biocytin. biocytin was incorporated into the internal solution (120 mM CsCH$_3$SO$_3$ added with 0.2-0.4% biocytin) that was used throughout electrophysiology procedures. The slices in which cells were patched and filled were left in 4% paraformaldehyde for 24 hr, then transferred to a 30% sucrose solution for another 24 hr until it was finally transferred once again to a 1x phosphate buffered saline solution to be stored until imaging.

**Confocal imaging**

Biocytin-filled brain slices were washed with 1× PBS twice for 5 min before incubating the slices in 0.3% streptavidin AlexaFluor 488 in 0.1% Triton X-100 in 1X PBS for 24 hr at RT. Sections were washed and mounted with DAPI mounting medium. Serial optical sections (z-stacks) were acquired using a confocal-LSM (Zeiss inverted LSM 710 microscope) with a 40× oil-immersion (numerical aperture = 1.4) DIC M27 objective at 2 μm intervals as an average of 4 consecutive images. Images were stored at a resolution of 512 × 512 pixels (1257.85 μm × 1257.85 μm × 1 μm). Online stitching was performed using Zen Black software (https://fiji.sc/) plugin and analyzed data using IgorPro (WaveMetrics, OR). For dendritic spine analysis, confocal-LSM710 z-stacks of labeled neurons were acquired at a 8-10× zoom onto the dendritic branches. Z-stack files were
stored at a resolution of 1024 × 1024 pixels (21.25 μm × 21.25 μm × 1 μm) as an average of 4 to 8 consecutive images with the objective as described above. The z-stacks were used to generate maximum intensity projections, which were used to analyze the morphology of CA1-PCs.

**Data acquisition and analysis**

Experimental data from electrophysiology experiments were analyzed with Igor Pro 6.37. Paired t-tests were done with Igor Pro 6.37 (Wavemetrics, Lake Oswego, OR) to determine statistical significance; p<0.05 (*p<0.05; **p<0.01; ***p<0.001) was deemed statistically significant throughout the experiments conducted in this thesis.
Results

**AAV- Aβ₄₂ leads to the accumulation of Aβ₄₂ peptide in the mouse brain**

Stereotaxic injection of AAV-Aβ₄₂ and SHAM constructs were done on mice aged P12-P19 (Fig. 1A). Through tissue analysis, we were able to determine that there was a significant increase in Aβ₄₂ in injected animals after three weeks of transfection (Fig. 1B). We were able to determine through RIPA analysis that a substantial portion of the Aβ₄₂ peptide produced was insoluble. These data provided our team with a basis to delve deeper into an investigation surrounding potential effects that the AAV-Aβ₄₂ could have on the mouse hippocampus.

**Aβ₄₂ speeds glutamate clearance from hippocampal astrocytes**

GLAST and GLT-1 are the main glutamate transporters, broadly expressed in astrocytes throughout the brain. Their main function is to clear glutamate from the extracellular space. Since glutamate uptake is electrogenic, we can record currents generated via the glutamate transport process using whole-cell patch clamp recordings from astrocytes (Fig. 2A). We call these currents Synaptically-activated Transporter Currents (STCs). Experimentally, STCs can be evoked by delivering extracellular stimulations to the neuropil. Here we asked how Aβ₄₂ alters glutamate clearance. We performed whole cell patch clamp recordings from astrocytes in stratum radiatum of the mouse hippocampus of wild type (WT) mice and WT mice three weeks after AAV-Aβ₄₂ transfection. Aβ₄₂ peptide reduced the amplitude of the astrocytic transporter currents relative to control. This reduction, however, was not associated with changes in the sustained potassium current, due to uptake of extracellular potassium following action potential propagation (WT n=11, AAV-Aβ₄₂ n=17, *p=0.021; Fig. 2D). Notably, only 40% of the astrocytes recorded from AAV-Aβ₄₂ injected mice had measurable STCs, as opposed to 100% of astrocytes from WT mice (Fig.
Interestingly, AAV-Aß₄₂ astrocytes had a relatively faster rate of glutamate clearance in when compared to control astrocytes, suggesting that AAV-Aß₄₂ changes the time course of glutamate uptake by astrocytes (Fig. 2F).

**Aß₄₂ reduces the NMDA/AMPA ratio in CA1-PCs**

We asked whether the detected changes in glutamate uptake could lead to changes in glutamate receptor activation. To test this hypothesis, we measured the NMDA/AMPA ratio in CA1-PCs. We performed whole cell voltage clamp electrophysiology on CA1-PCs in WT, AAV-Sham and AAV-Aß₄₂ injected mice. We obtained whole-cell patch-clamp recordings from CA1-PCs and used paired pulses to evoke AMPA EPSCs (recorded at -70 mV) or NMDA EPSCs (recorded at 40 mV; Fig. 3A). There was no statistically significant difference in the paired-pulse ratio of AMPA EPSCs between the three groups (WT n=15, AAV-Sham n=10, AAV-Aß₄₂ n=12; (Fig. 3B)), suggesting no difference existed in the release probability of glutamate due to Aß₄₂. The AMPA EPSC amplitude, rise and decay time were similar across the groups (Fig. 3C). We obtained paired pulse NMDA EPSCs evoked at +40 mV during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aß₄₂ injected mice. A pair pulse TTL was used in the whole cell voltage clamp recordings of pyramidal neurons in the CA1 region of the hippocampus (Fig. 3E). There was also no statistically significant difference in NMDA EPSC amplitude, rise and decay time across the three groups (Fig. 3F). However, in these experiments, we have no control over the number of fibers we activate in different slices. To overcome this limitation and gain further insights into whether there is a disproportionate activation of NMDARs versus AMPARs, we measured the NMDA/AMPA ratio in each cell. In these experiments, GABAₐ receptors were blocked by adding picrotoxin (100 µM) to the recording solution. Surprisingly, we detected a
statistically significant reduction in the NMDA/AMPA ratio in the AAV-Aβ42 injected group compared to both the control group and AAV-Sham group (*p=0.034 & *p=0.044; Fig. 3G, H). By contrast, no difference in the NMDA/AMPA ratio was detected between the AAV-Sham group and the control group. Together, these findings suggest that Aβ42 changes the proportionate recruitment of AMPARs and NMDARs at the synapse.

A glutamate uncaging readout of the functional pool of AMPARs/NMDARs in CA1-PCs

The results described above do not provide information on whether the reduction in NMDA/AMPA ratio induced by Aβ42 is due to different levels of expression of AMPARs or NMDARs. To address this concern, we bypassed presynaptic terminals and performed a series of uncaging experiments. Here, we used blue light stimuli to uncage Rubi-glutamate over stratum radiatum to evoke AMPA and NMDA EPSCs. Each light stimulation consisted of a 50 µs long pulse, repeated every 30 s. AMPA EPSCs were recorded at a holding potential of -70 mV (Fig. 4A). We then blocked AMPARs with NBQX (10 µM) and switched the holding potential to 40 mV to record NMDA EPSCs (Fig. 4C, E). These uncaging experiments were performed on WT, AAV-Sham and AAV-Aβ42 injected mice. Although the data set is preliminary data, it shows that there was no difference in AMPA and NMDA EPSC amplitude or rise time across the three groups (Fig. 4B). A statistically significant increase in NMDA EPSC half decay time (t50) was detected in the AAV-Aβ42 when compared to the WT and AAV-Sham groups (Fig. 4D). No difference in the NMDA/AMPA ratio across the three groups (Fig. 4F).

Aβ42-Induced regulation of the structural properties of CA1-PCs

We analyzed the morphology of patched CA1-PCs by adding biocytin (2%) to the internal solution. We then incubated the slices with streptavidin-AF488 and acquired confocal images to
analyze the structure of these cells.

We conducted a sholl analysis on CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice (Fig. 5A). The individual morphology on each biocytin filled CA1-PC was determined and analyzed (WT n=16, AAV-Sham n=16, AAV-Aβ42 n=13) (Fig. 5B)). There was no relative change observed in the distribution of dendritic intersections in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice (Fig. 5C, D). We also found no change in total number of intersections, average number of intersections or maximum radius of dendrite arbors in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 CA1-PCs (Fig. 5E-G). A statistically significant increase in spine density was observed in the AAV-Aβ42 group when compared to the WT and AAV-Sham group (AAV-Aβ42 vs. WT **p=8.0e-3, AAV-Aβ42 vs. AAV-Sham **p=8.0e-3; WT vs. AAV-Sham p=0.61 Fig. 6A). However, the proportion distribution of spines with different shapes and sizes was similar across groups. Specifically, mushroom, thin and stubby spines were equally represented in the three experimental groups (Fig. 6B). We did not detect significant changes in spine area, neck length, head volume, or head diameter across groups (Fig. 6C).
**Discussion**

In this work, we asked how accumulation of the peptide $\text{A}^\beta_{42}$ alters the functional properties of excitatory synaptic transmission in the mouse hippocampus. Currently available transgenic mouse models of AD provide information on genetic variants of AD (Lok et al., 2013; Ano et al., 2020). However, most AD patients suffer from idiopathic AD, and there are limited tools to study this form of the disease. The use of a viral approach like the one used here provides an effective tool to increase $\text{A}^\beta_{42}$ production. It also allows us to gain insights into idiopathic AD, specifically to the initial phases of the disease. This is because our analysis is currently limited to the effects detected 3 weeks after the stereotaxic injections, as opposed to chronic effects of $\text{A}^\beta_{42}$ accumulation. Therefore, our model does not currently recapitulate all aspects of AD that may affect human patients. We focused specifically on $\text{A}^\beta_{42}$ even though this is not the only hallmark of AD. For example, there is extensive literature showing that intracellular accumulation of the phosphoprotein tau is another hallmark of AD in the brain of patients, which leads to the formation of tangles (Selkoe., 2001). Since this is an important aspect of the pathophysiology of AD, we plan to extend our analysis to the phosphoprotein tau in our future experiments.

There are four main findings in this work: *(1)* there is a large subset of astrocytes that do not exhibit STCs; *(2)* astrocytes with STCs show faster rates of glutamate clearance; *(3)* these results are associated with no changes in CA1-PC morphology, spine size and AMPAR/NMDAR expression, but with an increase in the spine density; *(4)* however, $\text{A}^\beta_{42}$ might lead to changes in the NMDAR subunit composition in CA1-PCs, and a reduced activation of NMDARs relative to AMPARs.
Glutamate uptake in AD

Our structural and functional analysis of astrocytes show that a smaller proportion of astrocytes exhibit STCs in AAV-Aβ₄₂ injected mice (~40%). In the remaining astrocytes, those with STCs, glutamate uptake is faster. It is important to note that while the transporter current was reduced, the sustained potassium current was unaffected and remained unchanged between WT and AAV-Aβ₄₂ mice. This is important, because the fact that the sustained K⁺ current was still detectable indicated that the cells were not dead. The faster rate of glutamate clearance in a subset of astrocytes suggests that in these cells there might be an increased expression of glutamate transporters. Overall, however, AD has been suggested to induce a decrease in the expression of the glial glutamate transporter GLT1, without altering that of GLAST. Work from our own lab supports this finding, showing that exogenous application of Aβ₄₂ to hippocampal slices selectively reduces GLT1 expression. Whether these effects increase or decrease the uptake capacity of the hippocampal neuropil also depends on how Aβ₄₂ changes the diffusion properties of the neuropil. We plan to test this hypothesis with future experiments, to determine how Aβ₄₂ alters the extracellular space fraction tortuosity, etc. Because of the opposite consequences that each one of these effects could have (i.e., fewer astrocytes with STCs, and faster STCs), it is hard to conclude whether these cells are affected by or are reacting to Aβ₄₂ accumulation.

Implications of Aβ₄₂ accumulation for synaptic function and plasticity in AD

The increase in spine density, and the lack of any effect on spine size are surprising, because they have not been previously detected in AD (DeKosky & Scheff., 1990; Wu et al., 2004). In fact, spine loss is typically thought to be associated with AD (Merlini et al., 2019; Tsai et al., 2004). One way to reconcile this apparent discrepancy is to consider that we are only analyzing the contribution of Aβ₄₂ and likely overlooking the implication of other pathophysiological hallmarks of AD, including
phosphoprotein tau accumulation. We therefore speculate that spine loss in AD might be mainly attributed to phosphoprotein tau deposition, as opposed to Aβ42. Another possibility is that perhaps spine loss only occurs after prolonged exposure to Aβ42, which is something we plan to analyze in the future.

NMDARs are implicated with synaptic plasticity, in particular the induction of long-term potentiation (LTP) (Hunt & Castillo., 2012; Dudek & Bear., 1992). This finding is consistent with previous works showing that loss of synaptic plasticity occurs in AD (Malenka & Bear., 2004; Auffret., 2009). Here, we show that this is one of the first molecular events that takes place during Aβ42 accumulation. By using whole-cell patch-clamp recordings, we plan to validate this finding, to test the hypothesis that loss of LTP also occurs in our own experimental conditions. Some studies have shown that the NR2A-containing NMDARs are predominantly expressed synaptically, whereas those containing the NR2B subunit are more abundantly expressed extrasynaptically (Slutsky et al., 2010; Yang et al., 2017; Sanz-Clemente et al., 2013). The precise roles that NMDAR subunits play in synaptic plasticity are hotly debated (Bartlett et al., 2007, Liu et al., 2004; but see Berberich et al., 2005, Morishita et al., 2007). Whereas NR2A-NMDARs have been suggested to promote LTP, NR2B-NMDAR activation appears to induce LTD, due to the fact that NR2A- and NR2B-NMDARs are coupled to different intracellular signaling cascades (Massey et al., 2004). We hypothesize that an increase in NR2B-NMDARs expression occurs in response to Aβ42 accumulation based on the fact that the NMDA EPSCs evoked by glutamate uncaging have a slower decay time, and the decay time of NR2B-NMDA EPSCs is slower than that of NR2A-NMDA EPSCs. Ifenprodil is the first and best-characterized NMDAR antagonist shown to discriminate between NR2A- and NR2B-NMDARs, being more than 100-fold more selective for NR2B- over NR2A-containing receptors (Williams, 1993). However, the effect of ifenprodil on triheteromeric
receptors (i.e., NR2A/B-NMDARs) is not entirely clear. Hatton and Paoletti (Hatton and Paoletti, 2005) suggest that triheteromeric receptors have high affinity for ifenprodil but are inhibited to only a small extent. Our goal is to use ifenprodil to test our hypothesis that Aß₄₂ accumulation induces a switch in the subunit composition of extrasynaptic receptors. Perhaps the lack of a detectable effect on the subunit composition of synaptic NMDARs is due to the fact that Aß₄₂ accumulation occurs in extrasynaptic regions, outside the synaptic cleft.

Conclusions

Overall, our work shows that the use of a viral approach provides an effective tool to induce insoluble Aß₄₂ accumulation in vivo. Our findings identified key abnormalities in neuron structure and function that may occur in vivo during the initial accumulation of Aß₄₂ in the hippocampus of patients affected by AD. The effects we detected provide only a glimpse into the pathology of AD. Changes in peptide expression and composition outside of Aß₄₂ plaques are not addressed by our viral vector. A significant portion of AD pathology that is not included in our work thus far is the formation of tau entanglements, which are intimately related to the onset and progression of AD. While our viral vector has shown to be sufficient in producing a model to study Aß₄₂ plaque formation and subsequent effects, there are still aspects of AD pathology for us to address in order to form an accurate model for idiopathic AD.
Figures and figure legends

**Figure 1:** AAV-Aβ42 induces insoluble Aβ42 accumulation in the mouse hippocampus. (A) Schematic representation of a sagittal section of the mouse brain. The syringe points to the injection site in hippocampal area CA1. The viral constructs used for the stereotaxic injections are reported above the scheme. (B) *Left,* Summary graph of the Aβ42 peptide expression levels detected using western blot analysis from the hippocampus of mice injected with AAV-Sham and AAV-Aβ42, three weeks after surgery. *Right,* As in the left panel, retrieval of insoluble material using Radio-Immunoprecipitation Assay (RIPA).
Figure 2: Aβ₄₂ speeds glutamate clearance in a subset of astrocytes. (A) Confocal images of biocytin-filled astrocytes in stratum radiatum of WT and AAV-Aβ₄₂-injected mice. (B) Representative trace of whole-cell patch-clamp recordings of STCs in astrocytes in stratum radiatum of WT mice. (C) Representative trace of whole-cell patch-clamp recordings of STCs in astrocytes in stratum radiatum of AAV-Aβ₄₂-injected mice. (D) Summary graph of transporter currents and sustained potassium currents recorded from astrocytes of WT and AAV-Aβ₄₂-injected mice. (E) Summary of the proportion of astrocytes from which STCs could be recorded. (F) Left, Peak-normalized glutamate clearance in hippocampal astrocytes from WT mice. Right, As in the left panel, for astrocytes in AAV-Aβ₄₂-injected mice. (G) Centroid of glutamate clearance from the traces shown in panel F.
Figure 3: Aβ42 reduces the NMDA/AMPA ratio in CA1-PCs of the mouse hippocampus. (A) Representative trace of paired pulse AMPA EPSCs evoked at -70 mV during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (B) AMPA EPSC paired pulse ratio in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (C) Left AMPA EPSC amplitude, Middle AMPA EPSC rise time, Right AMPA EPSC t50 in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (D) Representative trace of paired pulse NMDA EPSCs evoked at +40 mV during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (E) NMDA EPSC paired pulse ratio in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (F) Left NMDA EPSC amplitude, Middle NMDA EPSC rise time, Right NMDA EPSC t50 in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (G) Representative trace of NMDA and AMPA EPSCs evoked at +40 mV and -70 mV respectively during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice. (H) NMDA/AMPA ratio of CA1-PCs from WT, AAV-Sham and AAV-Aβ42 injected mice.
Figure 4: Aβ_{42} does not change total AMPA and NMDAR activity in CA1-PCs (A)
Representative trace of single pulse AMPA EPSCs evoked at -70 mV during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (B) Left, AMPA EPSC amplitude. Right, AMPA EPSC t_{50} in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (C) Representative trace of single pulse NMDA EPSCs evoked at +40 mV during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (D) Left, NMDA EPSC amplitude. Middle, NMDA EPSC rise time. Right, NMDA EPSC t_{50} in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (E) Representative trace of NMDA and AMPA EPSCs evoked at +40 mV and -70 mV respectively during whole cell patch clamp electrophysiology recordings in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (F) NMDA/AMPA ratio of CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice.
Figure 5: Aβ_{42} does not change dendritic spine arborization in CA1-PCs

(A) Confocal images of biocytin filled WT, AAV-Sham and AAV-Aβ_{42} CA1-PCs in the hippocampus of the mouse brain. (B) Representative image of biocytin filled CA1-PCs. (C) Dendritic intersections in sholl analysis in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (D) Cumulative number of intersections of dendrites from soma in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (E) Total number of dendritic intersections from the soma in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (F) Average number of intersections of dendrites from the soma of CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice. (G) Maximum radius of dendrite arbors from soma in CA1-PCs from WT, AAV-Sham and AAV-Aβ_{42} injected mice.
Figure 6: Aβ42 alters the Spine density, but not the spine type distribution of CA1-PCs. (A) Left, Representative image of thin, stubby and mushroom spines distributed on dendrites of CA1-PCs in WT, AAV-Sham and AAV-Aβ42 injected mice. Right, y-axis being total spine density in WT, AAV-Sham and AAV-Aβ42 injected mice. (B) Pie charts depicting dendritic spine composition in WT, AAV-Sham and AAV-Aβ42 injected mice (Mushroom: blue; thin: green; stubby: yellow). (C) Left, spine area of mushroom, thin and stubby dendritic spines in WT, AAV-Sham and AAV-Aβ42 injected mice. Right spine head volume of mushroom, thin and stubby dendritic spines in WT, AAV-Sham and AAV-Aβ42 injected mice. (D) Left, spine head diameter of mushroom, thin and stubby dendritic spines in WT, AAV-Sham and AAV-Aβ42 injected mice. Right, spine head length of mushroom, thin and stubby dendritic spines in WT, AAV-Sham and AAV-Aβ42 injected mice.
References


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