NORTHWESTERN UNIVERSITY

Epigenetic Regulation in Aging and Alzheimer's Disease

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Abstract

Aging is the greatest known risk factor for Alzheimer's Disease (AD); however, the molecular mechanisms underlying aging and how it can initiate and or exacerbate AD, is still unknown. Epigenetic regulation has been widely accepted to play an essential role in aging or AD-related processes; however, whether dysregulations of histone modifications during aging initiate AD is not well understood. This dissertation project addressed this question by using the APP/PS1 transgenic mouse model combined with biochemical and behavioral measures to investigate the class 1 subtypes of histone deacetylases (HDACs) in the modulation of memory and AD-related pathologies in aging and AD. Results showed that class 1 HDACs modulated memory function and AD pathologies in a differential manner. More specifically, results showed HDAC 2 modulated synapse-related gene expression through dysregulations of H3K9ac levels at synapse-related gene promoters during aging and AD in the hippocampus.

Conversely, results showed HDAC 3 modulated synapse-related gene expression through dysregulations of H3K9ac levels at synapse-related gene promoters during AD in the prefrontal cortex. For AD pathologies, results showed HDAC 1 and HDAC 3 modulated amyloidosis throughout AD progression and class 1 HDAC's modulated microglia cell densities. These results helped dissect the dynamic changes of epigenetics that can occur during normal aging and AD provided evidence that indicates dysregulated histone modifications in aging may initiate or exacerbate AD onset and progression. Furthermore, these results helped identify several key epigenetic markers that play a critical role in aging and AD, and these markers could serve as potential future therapeutic targets for AD.

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My graduate school journey began 6 years ago. There is no way you could have told my younger high school self that I would not only get into a neuroscience PhD program, but I would attend Northwestern University to complete a PhD in neuroscience. As many know, this journey has been one met with many lows and highs. Graduate school has not been easy and have questioned my career in research way too many times as I can count. I came into the NUIN program fresh out of undergrad, with only ~1.5 years of neuroscience research under my belt, to say I was nervous was an understatement. However, so many people have made this journey possible and have helped me tremendously, and I am here to thank you all.

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Dedication

To my amazing mother who has sacrificed everything for me

This degree is for you

Love you, Mom.

List of Abbreviations

Aβ = Amyloid beta
AB_{40} = Amyloid beta 40
AB_{42} = Amyloid beta 42
AD = Alzheimer's Disease
ADAM-10 = A disintegrin and metalloproteinase domain-containing protein 10
ANOVA = Analysis of variance
APP = Amyloid precursor protein
ARC = Activity regulated cytoskeleton associated protein
ATAC-seq = Assay for transposase-accessible chromatin sequencing
BACE-1 = Beta secretase-1
BDNF = Brain-derived neurotropic factor
BPSD = Behavioral and psychological symptoms of dementia
cDNA = Complementary deoxyribonucleic acid
chIP = Chromatin immunoprecipitation
CI-994 = Tacedinaline
CoREST = Co-repressor element-1 silencing transcription
CR = caloric restriction
CUT&RUN-seq = Cleavage under targets & release using nuclease sequencing
DI = Discrimination index
DMSO = Dimethyl sulfoxide
DNA = Deoxyribonucleic acid
ELISA = Enzyme-linked immunosorbent assay
FAD = Familiar Alzheimer's disease
Fmn2 = Formin 2
H3 = Histone 3
H4 = Histone 4
HAM = Histone acetyltransferase modulator
HDAC = Histone deacetylase
HDAC inhibitor = Histone deacetylase inhibitor
HMI = Histone methyltransferase inhibitor
Hu = Human
i.p. = Intraperitoneally
Iba1 = Ionized calcium binding adaptor molecule 1
IC_{50} = Half maximal inhibitory concentration
IHC = immunohistochemistry
IDE = Insulin-degradation enzyme
ITI = Inter-trial interval
LAMP2 = Lysosome-associated membrane protein 2
LBD = Lewy body disease
MANOVA = Multivariate analysis of variance
Mo = Mouse
mRNA = Messenger ribonucleic acid
MS-275 = Entinostat
MWM = Morris water maze

NaB = Sodium butyrate
NaOH = Sodium hydroxide
NCOR = Nuclear receptor co-repressor
NEP = Neprilysin
NEUN = Neuronal nuclei
NOR = Novel object recognition
OCT = Optimal cutting temperature
OKSKM = Oct3/4, Sox2, Klf4, C-Myc
PBS = Phosphate buffer saline
PBS-T = Phosphate buffer saline-Tween
PCR = Polymerase chain reaction
PFC = Prefrontal cortex
PPA = Primary progressive aphasia
PS1 = Presenilen-1
qPCR = Quantitative polymerase chain reaction
RNA = Ribonucleic acid
ROI = Region of interest
SAD = Sporadic Alzheimer's disease
SAHA = Suberoylanilide hydroxamic acid
scRNA-seq = Single cell ribonucleic acid sequencing
SEM = Standard error of the mean
SMRT = Silencing mediator for retinoid and thyroid receptors
TSA = Trichostatin A
VPA = Valproic acid
WT = Wild type

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Chapter 1

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss combined with characteristic neuropathological changes. Presently, AD affects about 6 million Americans and by 2050, approximately 13 million people 65 years or older are projected to have the disease (Association, 2022). Current treatments for AD are based on mediating memory impairments at mild to moderate stages (Association, 2020; Rodda & Carter, 2012), but do not address the underlying pathological mechanisms. Numerous clinical trials of "disease-modifying" drugs have been conducted to target the neuropathological changes of AD, including Aβ plaques, neurofibrillary tangles, and neuroinflammation; however, these studies have been limited in success (Liu et al., 2019). Given these continuing gaps in clinical practice, there is a demand for more rigorous research into the molecular mechanisms that underlie AD.

Aging is the single largest risk factor for sporadic AD (Edwards Iii et al., 2019; Harman, 1991; Hou et al., 2019; Lindsay et al., 2002; Niccoli & Partridge, 2012; Wyss-Coray, 2016). While the rate of AD cases is strongly correlated with aging, the molecular and cellular mechanisms underlying the impact of aging on AD are still not well understood. Aging encompasses multiple biological changes. The causes of age-related changes are still uncertain, although many hypotheses have been suggested, including cellular reprogramming (Hou et al., 2019). Identifying aging factors that play a critical role in the decline of memory and the onset of AD-related neuropathologies are needed if the field hopes to develop biomarkers that aid in diagnosis and molecular targets for new therapies.

The causes of aging are uncertain, and the impact of aging on AD is still not well understood. The rate of AD cases is positively correlated with aging by 5-10 % at the age of 65 and 30 % at

the age of 85, showing how aging-related processes increase the vulnerability of disease development and progression (Hou et al., 2019; Niccoli & Partridge, 2012). Other factors such as physical activities, sleep, and diet significantly decline in aging and may underlie increased susceptibility to AD (Baker et al., 2010; Bonanni et al., 2005; Edwards Iii et al., 2019; Erickson et al., 2011; Gardener et al., 2012; Lindsay et al., 2002; Loewenstein et al., 1982; Tangney et al., 2011). Yet, it needs to be clarified how these factors play a critical role in the decline of normal cognition shifting to AD-related cognitive and memory deficits.

Epigenetic Regulation in Aging

Epigenetics is a biological process referring to the changes in gene expression without a change in the DNA sequence itself (Berger et al., 2009; Chuang & Jones, 2007). Epigenetic mechanisms mainly include DNA methylation, histone modifications, and RNA-associated silencing. DNA methylation and histone modifications remain the building blocks of the epigenome (Al Aboud et al., 2022; Houston et al., 2013). Many biological processes, including aging, are regulated by epigenetic mechanisms (Benayoun et al., 2015; Kane & Sinclair, 2019; Mansuy & Mohanna, 2011; Moosavi & Motevalizadeh Ardekani, 2016; Morris et al., 2019; Sidler et al., 2017). The dynamic changes of epigenetics can have functional consequences on the biological systems, including the brain. Therefore, it is critical to identify the epigenomic signatures underlying aging and aging-associated diseases to develop appropriate approaches to target these age and disease-associated epigenetic changes.

Various studies have shown that significant alterations in epigenetic regulation occur during aging. These epigenetic alterations include changes in DNA methylation, histone modifications,

and histone modifying enzymes that constitute epigenomic signatures in aging (Ciccarone et al., 2018; Johnson et al., 2012; Maleszewska et al., 2016; Molina-Serrano et al., 2019; Nativio et al., 2018a; Nativio et al., 2020; Pal & Tyler, 2016; Ryu et al., 2011; Wang et al., 2018). There are two different changes in the epigenome that are associated with aging: Epigenetic drift, and Epigenetic clock. Epigenetic drift refers to the random changes in the epigenome that occur during aging (Mendelsohn & Larrick, 2017; Teschendorff et al., 2013). Conversely, Epigenetic clock refers to changes of the epigenome during aging as programmed, and a natural result of aging (Fransquet et al., 2019; Lowe et al., 2016; Thompson et al., 2018). Both epigenetic drift and epigenetic clock results in aberrant changes in gene expression. Growing evidence suggests that the progressive accumulation of aging-associated epigenetic changes can lead to aberrant gene expression regulation, metabolic instability, stem cell senescence and or exhaustion and tissue homeostasis imbalance, all of which contribute to aging (Brunet & Rando, 2017; Fraga et al., 2005; Ohtani & Hara, 2013; Pal & Tyler, 2016; Pouikli & Tessarz, 2022; Ren et al., 2017). Additionally, epigenetic alterations can cause transcriptional noise (Bahar et al., 2006; Pal & Tyler, 2016; Warren et al., 2007) and alter transcriptional programs like cellular defense and inflammation, which in turn can recruit and upregulate transcriptional processes that govern aging (Adams, 2009; Coppe et al., 2008; Rai et al., 2014). However, how these epigenetic dysregulations may trigger and promote the development of neurodegenerative diseases such as AD, has not been well studied.

Epigenetics can regulate multiple biological processes, including memory (Day & Sweatt, 2011; Kane & Sinclair, 2019; Levenson & Sweatt, 2005; Lubin et al., 2011; Maity et al., 2021; Mansuy & Mohanna, 2011; Moosavi & Motevalizadeh Ardekani, 2016; Morris et al., 2019; Sidler et al.,

2017; Sultan & Day, 2011). Memory is the psychological process of acquiring, storing, retaining, and later retrieving information (Klein, 2015). With aging, various forms of memory have been shown to be impacted in humans and multiple animal models (Gallagher & Pelleymounter, 1988; Leslie et al., 1985; Neuner et al., 2017; Peleg et al., 2010; Petersen et al., 1992; Rubin et al., 1998; Zheng et al., 2018). The underlying causes of memory decline in normal aging are still not well understood; however, evidence suggests that deficits in long-term potentiation, a key mechanism of long-term memory process, is one of many systemic mechanisms for age-related cognitive decline (Barnes, 2003; Boric et al., 2008; Kumar, 2011; Lynch, 2004; Morris, 2003; Shankar et al., 1998). Additionally, synaptic plasticity, which is the activity-dependent modification of the strength or efficacy of synaptic transmission at pre-synapses (Martin et al., 2000; Silva, 2003), is modulated by epigenetic mechanisms. DNA methylation and histone modifications work in parallel to regulate transcriptional processes for the formation and storage of memories within the hippocampus (Lubin et al., 2008; Miller et al., 2008; Zovkic et al., 2013). During aging, changes in DNA methylation and histone modifications can dysregulate transcriptional programs and therefore result in a deficit in memory (Cui & Xu, 2018; Francis et al., 2009; Huls et al., 2022; Kushwaha & Thakur, 2020; Peleg et al., 2010). In regard to histone modifications, the “histone code” is a hypothesis that states that memory formation is regulated by a specific code of histone modifications. Multiple histone modifications are integrated and help regulate gene expression by either changing the state of chromatin or recruiting signaling complexes which can further influence memory processes (Peixoto & Abel, 2013; Turner, 2002). Various studies have shown that dysregulations of histone acetylation during aging can impact memory function (Castellano et al., 2012; Graff et al., 2012; Peleg et al., 2010). However, due to the vast and complex histone code, the exact histone modifications and their interactions with

other transcriptional machinery that play a role in memory processes and how they are impacted in aging is still unknown.

Given that aging is a major risk factor for multiple diseases, including AD, researchers have sought ways to slow or reverse epigenetic changes regulating aging-related mechanisms. Currently, two main themes of approaches have been studied: dietary interventions and cellular reprogramming. Caloric restriction (CR), a dietary intervention, has shown beneficial effects on aging (Anderson et al., 2009). Studies have shown that CR can increase lifespan, decrease retrotransposon, changes in DNA methylation patterns, and histone modifications, and prevent loss of heterochromatin in mouse models of aging (De Cecco et al., 2013; Jiang et al., 2013; Kane & Sinclair, 2019; Kim et al., 2016; Li et al., 2011). Additionally, clinical studies have shown that CR benefits humans and improves general health and memory (Acosta-Rodriguez et al., 2022; Spadaro et al., 2022; Witte et al., 2009). Cellular reprogramming is another approach that has been heavily investigated to help improve or reverse aging processes. In vitro studies have shown promising results with reprogramming. The usage of specific sets of transcription factors called Oct3/4, Sox2, Klf4, C-Myc (OSKM) can induce epigenetic remodeling through stochastic and deterministic phase changes, which can slow molecular markers of aging (Kane & Sinclair, 2019; Takahashi & Yamanaka, 2006). However, reprogramming heavily depends on the stability of the epigenome of the host.

Additionally, limited in vivo studies have utilized reprogramming methods to slow or reverse aging processes. Studies in animal models have shown promising reprogramming results on aging phenotypes; however, such studies have been met with lethal side effects (Kane & Sinclair,

2019; Mahmoudi & Brunet, 2012; Rando & Chang, 2012). Therefore, more rigorous, and innovative tools are needed to dig deeper into the histone modifications in transcriptional networks and other epigenetic changes influencing biological processes like memory. Understanding the mechanisms of epigenetic changes during aging could help reveal novel targets to consider for future therapeutics that can help slow or reverse aging processes.

Epigenetic Regulation of Alzheimer's Disease

Aging combined with genetics, epigenetics, and environmental factors determines the progression of AD (Edwards Iii et al., 2019; Sharma et al., 2020). Although evidence of epigenetic regulation involvement in aging has been reported, few studies have addressed whether epigenetic dysregulations in aging may influence AD development and progression. Epigenetic alterations impact on memory behaviors have been reported in animal models of aging and AD (Castellano et al., 2012; Janczura et al., 2018; Peleg et al., 2010; Ricobaraza et al., 2012; Ricobaraza et al., 2009). These studies have collectively shown how dysregulations of histone modifications, particularly histone acetylation, can greatly impact memory domains, such as recognition memory, long-term spatial reference memory, and contextual-fear memory. These studies provide a framework for the dynamic changes of the epigenome in aging and AD; however, we still lack knowledge of the epigenetic mechanisms that link aging and AD. One study suggested that a specific histone modification, H4K16ac, becomes dysregulated in aging and increases the vulnerability of AD pathogenesis (Nativio et al., 2018b). More specifically, this study performed genome-wide profiling of H4K16ac in aging and AD and found H4K16ac enrichment in normal aging postmortem tissue and dramatic loss of H4K16ac in AD postmortem tissues at genes linking aging and AD. (Nativio et al., 2018b). These results suggest that the

dysregulation of histone acetylation plays an important role in aging-associated neurodegeneration. Yet, it is still unclear whether alterations of histone modifications in aging could increase the susceptibility to AD development. Therefore, it is critical to determine the differences between histone modifications in normal aging and AD to identify age-specific vs. disease-specific histone acetylation changes that may be part of mechanisms underlying aging that increases the risk of AD.

Growing evidence indicates that epigenetic dysregulations play a critical role in the pathogenesis of AD. Different studies have integrated a genome-wide enrichment approach to analyze specific histone modifications and DNA methylation patterns across the genome in various brain regions to determine specific epigenomic signatures of AD (Altuna et al., 2019; Nativio et al., 2018b; Nativio et al., 2020; Persico et al., 2022; Santana et al., 2022; Zhang et al., 2020). For example, genome-wide studies have found that global decreases in DNA methylation and increases of histone acetylation at gene promoters are linked to memory loss and the neuropathology of AD (Nativio et al., 2018b; Nativio et al., 2020; Zhang et al., 2020). In addition, one study investigated the role of histone acetylation at genes associated with AD pathogenesis in N2a/APPswe cells and found increased H3 acetylation at PS1 and BACE1 promoters (Lu et al., 2014). Besides the impact of epigenetics on neuropathological changes, other studies have also found a significant reduction of global histone acetylation at gene promoters associated with learning and memory (Graff et al., 2012; Peleg et al., 2010). Important findings from these studies include downregulation at specific histone marks, such as H4K12ac, H3K9ac, and H4K16ac at memory genes like ARC, BDNF, Homer1, Myst5, and Fmn2, which has an impact on target gene expression (Graff et al., 2012; Peleg et al., 2010). These studies demonstrate the

important role of epigenetics in regulating memory and neuropathology in AD. However, to fully characterize the histone modifications that may regulate genes associated with memory and the neuropathology in AD and to determine whether correcting these changes could be a valid therapeutic approach for the prevention and/or treatment of AD, more translational studies are needed.

The epigenome contains many modifying enzymes that play a key role in epigenetic regulation. Of these enzymes, histone deacetylases (HDACs) are widely studied across multiple biological systems, including the central nervous system. HDACs are subdivided into five main classes (class 1, 2a, 2b, 3, and 4) based on structure, homology, and location within cells. Of the HDAC classes, class 1 (HDAC 1, 2 ,3,8), has been widely studied in the context of aging and disease. Studies have found altered class 1 HDAC expression and activity in aged rodents and have been associated with memory decline in aging (Chouliaras et al., 2013; Dos Santos Sant' Anna et al., 2013; Mahady et al., 2018). On the other hand, HDACs have been widely studied in human postmortem tissue from AD patients and AD mouse models. Such studies have found that HDAC 2 and HDAC 3 are primary modulators of memory-related processes. HDAC 2 is robustly increased in the entorhinal cortex and hippocampus of AD postmortem tissues and has been linked to memory formation and storage deficits in the hippocampus (Chouliaras et al., 2013; Dos Santos Sant' Anna et al., 2013; Guan et al., 2009; Yamakawa et al., 2017; Yu et al., 2018). However, whether HDAC 2 dysfunction is exclusively linked to AD-related memory decline in contrast to aging is unknown. Conversely, studies dissecting HDAC 3 involvement in memory processes has been limited. Mouse models using genetic manipulation or inhibition of HDAC 3 have shown significant changes in memory processes that are cortical and

hippocampal-dependent (Janczura et al., 2018; Malvaez et al., 2013; McQuown et al., 2011; Shu et al., 2018; Zhou et al., 2019), indicating HDAC 3 to potentially be an important player in various forms of memory. However, studies have yet to investigate class 1 HDACs spatially and temporally in aging and AD using mouse models. Therefore, it is essential to understand the dynamic changes of class 1 HDACs in aging and AD processes and the functional consequences of such changes spatially and temporally.

Usage of HDAC inhibitors in Preclinical Models of Aging and AD

Many preclinical studies have implemented HDAC inhibitors in aging and AD models to determine their potentially beneficial effects on the epigenetic regulation of memory and the pathogenesis of AD. These studies have used a range of broad-acting and specific HDAC inhibitors and found increased transcriptionally permissive histone modifications. Additionally, HDAC inhibitors have improved memory performance and decreased markers of the pathogenesis of AD (Kilgore et al., 2010; Long et al., 2013; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Xuan et al., 2015; Yao et al., 2014; Zeng et al., 2019; Zheng et al., 2018).

In aging, suberoylanilide hydroxamic acid (SAHA, a class 1 HDAC inhibitor) and VPA (broad acting) were able to ameliorate memory deficits, suggesting that HDAC inhibitors may have a potential to improve neuronal function, slow the progression, or reverse cognitive deficits in aged patients (Benito et al., 2015; Reolon et al., 2011; Zhao et al., 2018). Additionally, HDAC inhibitors have improved memory performance and decreased markers of the pathogenesis of AD (Kilgore et al., 2010; Long et al., 2013; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Xuan et al., 2015; Yao et al., 2014; Zeng et al., 2019; Zhang & Schluesener, 2013). These studies

have shown HDAC inhibitors, including VPA, TSA, MS-275, NaB, and 4-phenylbutyrate, have promising results on the pathogenesis of AD.

More specifically, these HDAC inhibitor treatments across multiple AD mouse models have shown promising results of improved neurodegenerative conditions, reduced amyloid and tau pathologies, reduced neuroinflammation, and improved cognitive functions; however, these studies have failed to establish whether any selective HDAC inhibitor could be a therapeutic candidate in AD, to help improve memory and disease modifications.

Do epigenetic alterations associated with aging increase the susceptibility to AD?

Epigenetic alterations have been investigated regarding their roles in modulating memory function in animal models of aging and AD, but mostly in separate studies. A few recent publications (Nativio, 2018, 2020; Klein, 2019) have used multiple approaches, including ChIP-seq and proteomics, and have revealed specific histone modification changes across the genome and linked them with AD. However, there currently needs to be more research examining the lifespan trajectory of the epigenetic differences in aging and AD at genes linked to memory and synaptic plasticity. Understanding how epigenetic alterations act as a bridge linking aging and AD is still in its infancy. Additionally, most genome-wide studies of postmortem brain tissues that profile a particular epigenetic marker are not rigorously compared to normal aging profiles, which makes it harder to dissect the complex interrelations of aging and neurodegenerative processes. Comparing epigenetic changes in normal aging to AD changes allows for investigating how aging affects AD's epigenetic profiles.

Recent pioneering studies suggested that the dysregulation of the histone landscape plays an important role in aging-associated neurodegeneration (Nativio et al., 2018a). Also, genome-wide profiling of H4K16ac reveals that H4K16ac increases in normal aging but decreases in AD (Nativio et al., 2018a). This study concluded that AD is not just an advanced stage of aging, but rather dysregulated aging, where specific disease-associated modifications can alter structural and/or transcriptional programs, leading to AD-related processes (Nativio et al., 2018b). Additional studies by the same group found H3K7ac and H3K9ac modifications throughout AD progression can impact disease pathways by dysregulating transcriptional and chromatin gene feedback loops (Nativio et al., 2020). However, the histone modification landscape relationships with other epigenetic modifiers are complex. These studies unraveling histone modifications and their impact on aging and AD processes do not consider histone-modifying enzymes and their modulation throughout aging and AD as a potential dysregulated factor in aging that may drive or exacerbate AD-related processes. Therefore, it is critical to 1) determine the histone modification differences that occur during normal aging and the development of AD, 2) identify age-related versus AD-related histone changes, and 3) their interaction so that the epigenetic component of aging as a risk factor for AD pathogenesis can be identified.

Potential mechanisms of epigenetic regulation in aging and AD.

The epigenome contains many modifying enzymes that play a key role in epigenetic regulation. Among these enzymes, HDACs are important and widely studied across multiple biological systems. HDAC activity involves the process of deacetylating histone proteins, which makes the DNA less accessible to transcriptional machinery, thereby impacting gene expression. In the

central nervous system, several human studies have shown that HDAC 1 and HDAC 3 are robustly increased in areas such as the frontal cortex and white matter during aging (Gilbert et al., 2019; Mahady et al., 2018). Additionally, altered HDAC expression and activity have been found in aged rodent models and have been associated with changes in memory function (Dos Santos Sant' Anna et al., 2013; Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Guan et al., 2009; Janczura et al., 2018; Yu et al., 2018). Class 1 HDACs have been studied in human postmortem tissues from AD patients, as well as in AD mouse models (Gilbert et al., 2019; Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Guan et al., 2009; Janczura et al., 2018; Mahady et al., 2018; Yu et

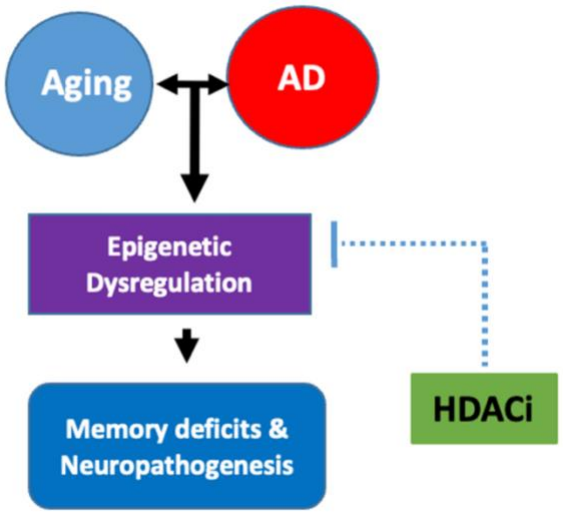

Figure 1. Hypothesis

Objective 1. Characterize the spatial and temporal epigenetic alterations in aging and AD.

This study examined the regional abundance of class 1 HDAC enzymes globally and at synapse-related genes. Then, histone acetylation levels at synapse-related gene promoters were measured to gain a better understanding of the epigenetic regulation of memory in a spatial and temporal manner. At the functional level, several memory domains were evaluated with the aim to understand how different forms of memory are impacted in aging and AD. Collectively, results showed a differential class 1 HDAC modulation of synapse-related gene expression through H3K9ac alterations at synapse-related gene promoters in aging and AD. Additionally, the finding of altered memory function in aging and AD is a foundation for continuing an investigation of how the subtype of class 1 HDACs plays a role in differential memory function.

Objective 2: Evaluate the beneficial effects of HDAC inhibitors on reversing memory deficits and decreasing neuropathogenesis in aging or AD mouse models.

This study tested the drug effects of the pan (VPA) and selective class 1 HDAC (MS-275 and CI-994) inhibitors on memory function in APP/PS1 mice at 3, 12, and 18 months of age. Following chronic treatment and behavioral testing, synapse-related gene expression, H3K9ac levels at synapse-related gene promoters, amyloidosis, and microgliosis were evaluated. The results confirmed that HDAC 2 modulated synapse-related gene expression in the hippocampus through decreased H3K9ac levels at gene promoters. Conversely, HDAC 3 modulated synapse-related gene expression through decreased H3K9ac levels at gene promoters in the PFC. These findings suggested that HDAC 1 and HDAC 3 are important modulators of amyloidosis, however, HDAC 1, HDAC 2, and HDAC 3 are important in the regulation of microgliosis. Collectively, these findings suggested that class 1 HDAC subtypes could be considered as a

therapeutical target and that HDAC inhibitors could be used for the prevention and treatment of memory deficits in aging and AD.

Chapter 2

Class 1 HDAC Modulate Synapse-Related Genes in Aging & Alzheimer's Disease

Abstract:

Studies have demonstrated that epigenetics plays a vital role in aging and Alzheimer's disease (AD) pathogenesis; however, whether epigenetic alterations during aging can initiate AD development and exacerbate AD progression remains unclear. Given this, it is critical to determine the epigenetic alterations occurring during aging and how these changes influence AD pathogenesis.

In this study, young (3 months old), middle-aged (12 months old), and aged (18 months old) APP/PS1 mice (APPswePsen1de9; B6.Cg-Tg(APPswe, PSEN1dE9)85Dbo/Mmjax transgenic strain, Jackson Laboratory), and WT littermates including males and females were used. Mice underwent a series of behavioral tests to evaluate different memory domains, including recognition memory (Novel Objective Recognition test), short-term working memory (Y-maze test), and long-term spatial reference memory (Morris water maze test). After behavioral testing, the hippocampus and prefrontal cortex (PFC) were collected for biochemical assessment to determine the dynamic changes of class 1 HDACs globally and at synapse-related gene promoters. Additionally, synapse-related gene expression and H3K9ac levels at synapse-related gene promoters were evaluated during aging and AD progression.

The molecular findings showed increased HDAC 2 global mRNA expression levels and increased abundance of HDAC 2 at synaptic gene promoters (*nr2a*, *glur1*, *glur2*, *psd95*) in the hippocampus in WT mice only at 18 months of age, but not at 3 and 12 months of age. However, HDAC 2 global levels and abundance at synapse-related gene promoters were significantly increased in 12- and 18-month-old APP/PS1 mice compared to age-matched WT controls. Additionally, 18-month-old APP/PS1 mice displayed the highest levels of HDAC 2 expression,

suggesting aging further exacerbated this effect. No changes in global expression or abundance were seen for HDAC 1 and 3 in the hippocampus. Additionally, downregulation of synapse-related gene expression for all target genes was seen during normal aging, 12-and 18-month APP/PS1 mice compared to age-matched WT controls, and 18-month APP/PS1 mice showed the most significant downregulation of all synapse-related gene expression. In the PFC, there were no significant changes in global mRNA expression levels of class 1 HDACs in normal aging. Still, a significant abundance of HDAC 3 was seen at synapse-related gene promoters in 12- and 18-month APP/PS1 mice compared to age-matched controls. Given that HDACs regulate histone acetylation, in this study, H3K9ac levels were measured at synapse-related gene promoters in the hippocampus and PFC. Results also showed significantly decreased H3K9ac levels at all synapse-related gene promoters in WT mice at 18 months of age. Additionally, results showed significantly decreased H3K9ac levels at all synapse-related gene promoters in 12- and 18-month APP/PS1 mice compared to age-matched WT controls and aging further exacerbated this effect. These findings suggest a potential differential HDAC modulation of synapse-related gene expression underlying memory in aging and AD.

Introduction:

Many studies have demonstrated the physiological importance of epigenetic regulation in biological processes, ranging from development, cancer, neurological, and cardiovascular systems (Chen et al., 2015; Haberland et al., 2009; Shukla & Tekwani, 2020). HDACs are not only important histone acetylation regulators but are also critical factors that directly influence a multitude of biological processes and diseases, such as neuronal development, axon regeneration, and memory formation, neuropsychiatric disorders, and neurological and neurodegenerative diseases (Bahari-Javan et al., 2017; Cho & Cavalli, 2014; Haberland et al., 2009; Machado-Vieira et al., 2011; Mahmoudi & Brunet, 2012; McQuown et al., 2011; Zhu et al., 2019). Learning and memory are processes that are subject to epigenetic control. HDACs, specifically class 1 and 2, have been shown to modulate memory processes during aging and disease states (Kilgore et al., 2010; Kim et al., 2012; Mahgoub & Monteggia, 2014; McClarty et al., 2021; McQuown et al., 2011; Montalvo-Ortiz et al., 2017; Zhu et al., 2019). More interestingly, many studies have dissected class 1 HDACs in memory processes. However, an abundance of genetic manipulation and pharmacological studies have examined HDACs 1-3 and have shown modulation of memory function, such as contextual fear and spatial memory (Burns et al., 2022; Fuller et al., 2019; Guan et al., 2009; Kilgore et al., 2010; McClarty et al., 2021; McQuown et al., 2011; Morris et al., 2019; Morris et al., 2013; Nakatsuka et al., 2021). Of class 1 HDACs, HDAC 2 has been most linked to memory changes. Various studies have used genetic manipulation approaches in aging to target HDAC 2 and have found HDAC 2 to be a negative regulator of memory within the hippocampus, making HDAC 2 an attractive target for hippocampal-dependent memory processes (Guan et al., 2009; Morris et al., 2019; Morris et al., 2013; Nakatsuka et al., 2021; Singh & Thakur, 2018).

HDAC 2 also plays a vital role in the response to memory impairment in AD. HDAC 2 is substantially increased in AD post-mortem brain samples and rodent brain tissues (Bie et al., 2014; Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Grinan-Ferre et al., 2016; D. Liu et al., 2017). Studies have dissected the functional role of this increase and have found HDAC 2 to modulate histone acetylation and gene expression changes of synaptic plasticity target genes underlying the memory (Graff et al., 2012; Guan et al., 2009; Yamakawa et al., 2017). Genetic knock-down of HDAC 2 in the hippocampus in AD mouse models has helped rescue synaptic plasticity deficits, increased histone acetylation, and improved memory function (Guan et al., 2009; Morris et al., 2013). However, whether HDAC 2 has the same modulatory effects during AD progression with aging and whether this effect is seen in other brain regions necessary for memory function, has yet to be well known.

HDACs modulate the balance of histone acetylation by removing acetyl functional groups from lysine residue groups of histones (Li et al., 2020; Seto & Yoshida, 2014). Histone acetylation is one of many epigenetic mechanisms most known underlying transcriptional regulation and is essential in many biological processes, including the memory (Graff & Tsai, 2013; Turner, 2002). The regulation of histone acetylation is essential for memory function (Burns & Graff, 2021; Levenson & Sweatt, 2005; Lopez-Atalaya & Barco, 2014; Peixoto & Abel, 2013). Studies have dissected changes in histone acetylation levels globally and at target gene promoter's hours after memory events (Itzhak et al., 2012; Peleg et al., 2010; Zhang et al., 2014; Zhao et al., 2012), concluding that changes in histone acetylation are critical for the activation of transcriptional programs underlying memory formation in the brain.

Changes in memory function during normal aging have been associated with age-related decline in specific histone acetylation markers (Castellano et al., 2012; Dagnas & Mons, 2013; McClarty et al., 2021; Montalvo-Ortiz et al., 2017; Peleg et al., 2010). During aging, histone modifications have been shown to impact transcriptional programs on memory formation through synaptic plasticity targets (Bousiges et al., 2010; Castellano et al., 2012; Nativio et al., 2018). Although evidence indicates age-induced epigenetic changes, how these changes are linked to age-related neurodegenerative diseases is not well understood.

There are conflicting reports regarding histone acetylation pathway changes and their relation to neurodegenerative diseases, specifically AD, both in human post-mortem tissue work and animal studies. For instance, some studies report global increases of histone acetylation, while others report global decreases or no changes at all in the histone acetylation (Castellano et al., 2012; Francis et al., 2009; Mahady et al., 2018; Narayan et al., 2015; Peleg et al., 2010). A possible explanation for the disparities in findings is due to the various brain areas, animal models, and experimental designs. We must also consider that histone acetylation pathways work in concert with an array of other transcriptional networks to regulate transcriptional processes (Agalioti et al., 2002). Therefore, we must consider other environmental factors, such as pathological hallmarks, to influence histone acetylation changes when comparing epigenetic profiles of a disease state in differing models. Each mouse model of AD encompasses different pathological hallmarks, which can present in a range of severities; therefore, it is essential to consider these variations when comparing global epigenetic changes. Although previous studies have shown differences of histone acetylation and methylation in relation to both aging and the development of the AD (Govindarajan et al., 2011; Levenson et al., 2004; Peleg et al., 2010; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Yao et al., 2014; Zhang & Schluesener, 2013), most of these

studies examined aging and the development of AD separately. To understand how aging increases the vulnerability to AD, it is essential to investigate epigenetic changes temporally and spatially during aging and the development of AD within the same experimental model first, before comparing across models. In this chapter, the APP/PS1 mouse model and WT controls were used at 3, 12, and 18 months of age to investigate the dynamic changes of a particular histone modification and histone modifying enzymes and their impact on memory function in aging and AD. Class 1 HDACs were the main focus of this study, with the intended goal to help understand the differential regulation of class 1 HDACs at synapse-related genes across aging and AD and their association with differential memory domains.

Methods

Animal Models

Double transgenic APP/PS1 mice expressing a chimeric Mo/Hu APP695swe mutation and a mutant Hu PS1-de9 mutation, were used for this project. APP/PS1 males (Jackson Laboratory, Bar Harbor, ME) were bred with C57BL/6L females (Jackson Laboratory, Bar Harbor, ME). The APP/PS1 and their wildtype (WT) littermate offspring were housed in groups of 3-5 per cage starting at 21 days until 3 months, 12 months, and 18 months of age for this study. Animals were group housed on a 12-h light/dark cycle and given food and water ad libitum. All procedures in animals were performed according to NIH guidelines and the Current Guide for the Care and Use of Laboratory Animals (2011, eight edition) under a protocol approved by the Northwestern University Animal Care and Use Committee.

Tissue Collection

Methods for tissue collection were done in a similar matter to a previous published study in my lab (McClarty et al., 2021). Before brain tissues were collected for biochemical and immunohistochemical studies, a cardiac perfusion was performed with 0.1M PBS solution for 1 min, to wash-out the blood from blood vessels in the mouse brain. The mouse brains were then removed and quickly dissected under ice with a dissecting scope. The hippocampus and prefrontal cortex were dissected and then froze at −80°C until ready for processing for molecular analysis. Half brains were used for immunohistochemical studies. Half-brains were collected and fixed in 4% paraformaldehyde overnight at 4 °C, followed by dehydration in 30% sucrose

solution until the tissue sank to the bottom of the bottles. After fixation and dehydration, the half-brains were dissected and embedded in OCT compound.

Chromatin Immunoprecipitation

Methods for tissue collection were done in a similar matter to a previous published studies in my lab (McClarty et al., 2021; Montalvo-Ortiz et al., 2017). The commercially available Magna ChIP™ G Tissue Kit (17-20000, Millipore) was used. For ChIP, tissue samples were homogenized in cell lysis buffer containing proteinase inhibitor and chromatin was sonicated using a Branson Digital Sonifier with 3 rounds of 10 s at 75% power per sample on ice. Fragmented chromatin lysate was immunoprecipitated with 5 µg of antibody directed against HDAC 1 (ab 53091), HDAC 2 (ab32117), HDAC 3 (ab 32369), H3K9ac (ab32129). The DNA-histone complex was incubated with Protein G Magnetic Beads overnight at 4°C. The DNA-histone complex was eluted from the beads and dissociated at 65 4°C for 2 h under high salt conditions. Proteins were digested using proteinase K treatment and the associated DNA was precipitated with 100% ethanol and resuspended in 75 µL of PCR grade water.

RNA Extraction

Methods for tissue collection were done in a similar matter to a previous published study in my lab (McClarty et al., 2021). In order to access synaptic-related gene expression, RNA extractions were performed. For gene expression analysis, messenger RNA (mRNA) was extracted (Qiagen), reverse transcribed (QuantaBio) and quantitatively amplified on a thermal cycler (ThermoFischer), yielding complementary DNA.

Real-time Quantitative Polymerase Chain Reaction (qPCR)

Methods for tissue collection were done in a similar matter to a previous published studies in my lab (McClarty et al., 2021; Montalvo-Ortiz et al., 2017). Input, immunoprecipitated DNA, and cDNA amplification reactions were run in triplicate in the presence of SYBR Green (Applied Biosystems) using QuantStudio 6 Flex Real Time PCR System (Applied Biosystems, Foster City, CA, United States). Primers for each gene target can be found in **Table 1.** Ct values from each sample were obtained using the Sequence Detector 1.1 software. Ct values were normalized to endogenous gene, beta actin, to obtain a percent input. Fold differences were then determined using the delta delta C_T method as a previous report (Livak & Schmittgen, 2001).

Gene Target (mouse)	Method	Forward primer (5' → 3')	Reverse Primer (5' → 3')
HDAC 1	mRNA	TTCACCATGGCGATGGCGTGG	CCCCAATGTCCCGTAGGTCCCC
HDAC 2	mRNA	GGGACAGGCTTGGTTGTTTC	GAGCATCAGCAATGGCAAGT
HDAC 3	mRNA	TGCTTCAATCTCAGCATTCG	GTAGCCACCACCTCCCAGTA
β-actin	mRNA	GCACCACACCTTCTACAATG	TGCTTGCTGATCCACATCTG
Nr2a	mRNA	TGCAAGTTACACAGCCAACC	ATCGGAAAGGCGGAGAATAG
GluR1	mRNA	GTGGTGGTGGACTGTGAATC	TTGGCGAGGATGTAGTGGTA
GluR2	mRNA	TGTGTTTGTGAGGACTACGGCA	GGATTCTTTGCCACCTTCATTC
PSD95	mRNA	GCCCTGTTTGATTACGACAA	CTCATAGCTCAGAACCGAGT
β-actin	chIP (promoter)	GAGACATTGAATGGGGCAGT	ATGAAGAGTTTTGGCGATGG
Nr2a	chIP (promoter)	TCGGCTTGGACTGATACGTG	AGGATAGACTGCCCCTGCAC
GluR1	chIP (promoter)	GGAGGAGAGCAGAGGGAGAG	TTCCTGCAATTCCTTGCTTG
GluR2	chIP (promoter)	GCGGTGCTAAAATCGAATGC	ACAGAGAGGGGCAGGCAG
PSD95	chIP (promoter)	GGAGGGGTGAGAACCCACCGA	CTCCCCCTCCCCACTGCTCC

Table 1: Primers used for qPCR experiments

Immunohistochemistry

Methods for tissue collection were done in similar matter to a previous published study in my lab (Cao et al., 2021). For immunofluorescence staining, brain slices were washed in PBS containing 0.3% Triton X-100 and blocked for 2 h at room temperature with 7% donkey. Slices were then incubated overnight at 4 °C with primary antibody NEUN (1:500, ab177487, Abcam). Slices were then washed with PBS containing 0.3% Triton and incubated for 2 h at room temperature with anti-rabbit Alexa Fluor® 488 (1:500, ab150073, Abcam) secondary antibody. Slices were then washed in PBS containing 0.3% Triton before being mounted with mounting medium ProLong™ Glass Antifade Mountant (P36980, Thermo Fischer). Immunofluorescence pictures were taken with a fluorescence microscope (80i, Nikon, Japan) and CoolSNAP DYNO CCD (Photometrics, Canada). To quantify NEUN expression, the brain images (magnification = × 4) were outlined with the size-standardized regions of interest (ROIs) by the Image J software (v.1.52a, NIH, USA), and the percentage of area with fluorescence, and cell count was quantified using this program. In particular, the threshold was set and standardized across images to maximize true protein expression signal for quantification; then, the total pixel number of target protein were recorded, and the percentage was calculated by dividing the pixel number with the total unfiltered pixel number in the ROI (Cao et al., 2021). Three sections for each brain region per immunostaining marker were averaged and analyzed.

Behavioral Testing:

Methods for behavioral testing were done in similar matter to previous studies in my lab (Locci et al., 2021; McClarty et al., 2021). Mice were acclimated to a soundproof behavioral testing room 30 min prior to testing, and assays were performed during the light part of the 12-h light/dark cycle. The order of the behavioral test was as follow: Novel object recognition (NOR), Y-maze, and Morris Water Maze (MWM). The behavioral tests and data analysis were conducted by one investigator that was blinded to age and treatment conditions.

Novel Object Recognition

Recognition memory was tested in an open plexiglass box (40 wide cm × 40 long cm × 40 high cm). Two sets of objects were used, and they were consistent in height and volume, but different in shape, color, and texture. Mice were individually habituated to the test arena for 10 min on each of the 3 days prior to data acquisition. On the first day of habituation, an open field test for mouse locomotor activity and anxiety behavior were recorded to ensure the mice had no severe impairments in locomotor function and anxiety behavior due to antipsychotic drug and CI-994 treatment. The experimental session consisted of 3 phases: acquisition trial (10 min), inter-trial interval (ITI; home-cage, 24 h), and retention trial (10 min). During acquisition, animals were recorded while exploring the arena with two glass, cylinder-shaped identical objects that are dark gray color (height of 8 cm and a diameter of 4 cm) placed diagonally across from each other. Following the ITI, during which one of the objects was replaced by a novel object that is plastic, prism-shaped with a yellow and blue color (6 cm length × 6.5 cm width × 6 cm height), in a counterbalanced manner, animals were placed back in the arena for the retention trial. The amount of time spent exploring each object during the acquisition and retention trial was scored

by an experimenter blinded to the condition using two milliseconds' stopwatches for precision. Exploration was defined as touching, leaning on the object, or orienting the head towards the object and sniffing within <1.0 cm for at least 20 s to make sure the sensorial perception was not impaired. Climbing on top of the object was not counted as exploration. Between each trial, the arena and the objects were cleaned with 70% alcohol to eliminate olfactory traces. The time spent with each object during the retention trial was used to calculate the discrimination index (DI), which represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects. To calculate DI, the total time spent exploring the novel object was subtracted by the total time spent exploring the familiar object, divided by the total time spent exploring during the retention trial. The formula used to calculate DI is as follows:

$$DI = \frac{(Time\ spent\ exploring\ novel\ object) - (Time\ spent\ exploring\ familair\ object)}{(Total\ time\ spent\ exploring)}$$

Y-maze

The Y-maze apparatus consisted of three-arms (5 cm wide × 21 cm long × 15.5 cm high) with three different special cues positioned in the top inner part of each arm. The apparatus was placed on a stable table with overhead video recording. Mice were placed in arm "A" (starting point, excluded from the analysis) facing the end of the arm and were allowed to freely explore the apparatus for 5 mins without investigator presence while a camera recorded their movements (Any-Maze, Stoelting, Wood Dale, IL). Spontaneous alternation was defined as discrete and successive entries into each open arm, including events where the animal directly progresses from one arm to the next in consecutive fashion (i.e., ABC, ACB, BAC, BCA, CAB, and CBA)

without reentering the two previously visited arms. The spontaneous alternation percentage was calculated by dividing the number of total successful alternations by the total number of possible alternations (i.e., the number of total entries minus two) multiplied by 100.

Morris Water Maze

In this study, we used a modified Morris water maze to test reference memory (Vorhees & Williams, 2006). Reference memory is a long-term memory that is associated with hippocampus function (Vorhees & Williams, 2006). Morris water maze is performed in a water tank with a moveable platform equipped with a video camera and computerized data analysis software (Any Maze). The water temperature was maintained at 23ºC. Prior to testing, the mice were acclimated to the Morris maze room for 30 minutes. Mice then underwent acquisition and test trails as described below. Upon completion of these trials, mice were removed from the tank and put into a drying cage. To assess reference memory, each mouse was tested for a total of 5 days with 4 trials/day (Vorhees & Williams, 2006). The interval between trials was1 hour each day. Day 1 consists of a training day during, which the mice were allowed to find a platform that protrudes above the water's surface. Then, the mice were trained to find a submerged platform. During the testing days (days 2-5) the water was opaque, and the platform was submerged. For each trial, the mouse started at a different position for each trial (N, E, S, W), but the platform remained in the same location across the trials (Vorhees & Williams, 2006). Visual cues were placed on the N, E, and W facing walls for the mice to use for spatial reference. The swimming distance and time were used as indicators of spatial reference learning. We provided a maximum time of 90 seconds for the mice to find the platform. During the test days, measured the swimming distance and time to find the platform. On day 6 (probe trial), mice were probed for spatial reference

memory. All mice had a start position in the complete opposite quadrant of the target quadrant, and the platform was removed. The swimming distance, and total time spent in target quadrant were used as indicators of reference memory. Mice underwent only 1 trial for 60 seconds.

Statistical Analysis

All statistical analyses were conducted using the GraphPad prism software (San Diego, CA, United States). Data are expressed as mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) was used to detect age, genotype, and age x genotype interaction effects followed by a multiple comparison's analysis using Tukey's post hoc method.

Results:

I. Differential Global Class 1 HDAC and Synapse-Related Gene Expression in Aging and AD

Global class 1 HDAC (HDAC 1-3) mRNA expression in the hippocampus and PFC in 3-, 12-, and 18-month-old APP/PS1 and WT mice (**Figure 2**). Two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 35.48, p < 0.0001), genotype ($F_{1,30}$ = 52.71, p < 0.0001), and an age × genotype interaction ($F_{2,30}$ = 9.139, p = 0.0008) of *HDAC 2* mRNA expression in the hippocampus (**Figure 2B**). Post-hoc analysis revealed global mRNA expression of HDAC 2 increased with normal aging (p = 0.0291) but is more significantly increased in 12- and 18-month-old APP/PS1 mice compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in our AD model exacerbated the increased HDAC 2 levels (p = 0.0479). No significant changes in global mRNA expression of HDAC 1 and HDAC 3 in aging and AD were found (**Figure 2A,C**).

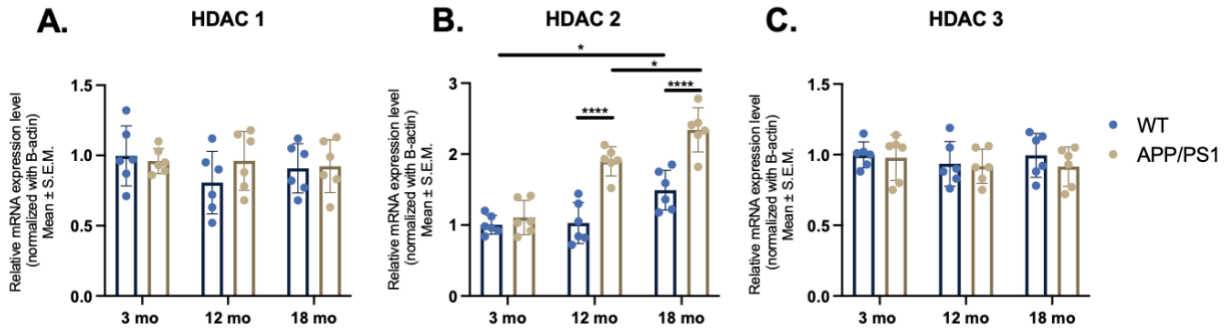

Figure 2. Class 1 HDAC Global Landscape in the Hippocampus in Aging and AD. A: mRNA expression of *HDAC 1* **B:** mRNA expression of *HDAC 2*. **C:** mRNA expression of *HDAC 3*. *HDAC 2* was significantly increased during aging and AD globally in the hippocampus. No significant changes were seen for *HDAC 1* and *HDAC 3*. Data represent mean ± SEM (n = 6/group). Blue circles represent WT mice, gold circles represent APP/PS1 mice. *p < 0.05, **p < 0.01.

Then, class 1 HDAC (HDAC1-3) mRNA expression was measured in the PFC in 3-, 12-, and 18-month-old APP/PS1 and WT mice (**Figure 3**). Two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 25.53 $p < 0.0001$), genotype ($F_{1,30}$ = 10.17, p = 0.0033), and an age × genotype interaction ($F_{2,30}$ = 11.57, p = 0.0002) of *HDAC 3* mRNA expression in the PFC (**Figure 3C**). Post-hoc analysis revealed no significant changes in expression levels in normal aging (p = 0.2880), however, both 12- and 18-month-old APP/PS1 mice showed significantly increased *HDAC 3* mRNA expression compared to age-matched controls (p = 0.0041, p = 0.0148), and aging in our APP/PS1 mice did not significantly impact *HDAC 3* expression levels, although a trend was seen (p = 0.0514). No significant changes in global mRNA expression of *HDAC 1* and *HDAC 2* in aging and AD were found (**Figure 3A,B**).

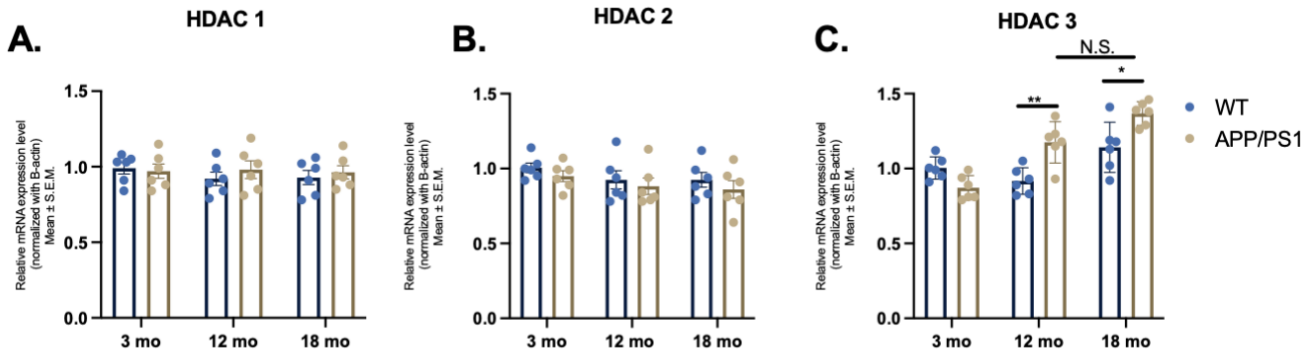

Figure 3. Class 1 HDAC Global Landscape in the PFC in Aging and AD. A: mRNA expression of *HDAC 1* **B:** mRNA expression of *HDAC 2*. **C:** mRNA expression of *HDAC 3. HDAC 3* was significantly increased only in our AD mice. No significant changes were seen for *HDAC 1* and *HDAC 2*. Data represent mean ± SEM (n = 6/group). Blue circles represent WT mice, gold circles represent APP/PS1 mice. *p < 0.05, **p < 0.01.

Before evaluating class 1 HDACs and their abundance at synapse-related gene promoters underlying memory function, global mRNA expression of synapse-related gene targets was measured to understand how these targets (*nr2a, glur1, glur2, psd95*) are regulated in aging and AD.

Hippocampus:

Two-way ANOVA revealed significant effects of age ($F_{2,30} = 26.13$, $p < 0.0001$), genotype ($F_{1,30} = 24.73$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 4.265$, $p = 0.0234$) of *nr2a* mRNA expression in the hippocampus (**Figure 4A**). Post-hoc analysis revealed significantly decreased levels of *nr2a* mRNA expression in normal aging (p = 0.0027). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *nr2a* mRNA levels compared to age-matched controls (p = 0.0047, p = 0.0034), and aging in APP/PS1 mice exacerbated the decreased *nr2a* mRNA expression levels (p = 0.0373) in the hippocampus. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 35.72$, $p < 0.0001$), genotype ($F_{1,30} = 16.72$, $p = 0.0004$), and an age × genotype interaction ($F_{2,30} = 6.101$, $p = 0.0006$) of *glur1* mRNA expression in the hippocampus (**Figure 4B**). Post-hoc analysis revealed significantly decreased levels of *glur1* mRNA expression in normal aging (p = 0.0061). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *glur1* mRNA levels compared to age-matched controls (p = 0.0051, p = 0.0140), and aging in APP/PS1 mice exacerbated the decreased *glur1* mRNA expression levels (p = 0.0264) in the hippocampus. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 36.65$, $p < 0.0001$), genotype ($F_{1,30} = 30.56$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 7.657$, $p = 0.0021$) of *glur2* mRNA expression in the hippocampus (**Figure 4C**). Post-hoc analysis revealed significantly decreased levels of *glur2* mRNA expression in normal aging (p = 0.0096). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *glur2* mRNA levels compared to age-matched controls (p = 0.04, p = 0.0008), and aging in APP/PS1 mice exacerbated the decreased *glur2* mRNA expression levels (p = 0.0428) in the hippocampus. For *psd95*, two-way ANOVA

revealed significant effects of age ($F_{2,30}$ = 33.68, p < 0.0001), genotype ($F_{1,30}$ = 26.48, p < 0.0001), and an age × genotype interaction ($F_{2,30}$ = 7.008, p = 0.0032) of *psd95* mRNA expression in the hippocampus (**Figure 4D**). Post-hoc analysis revealed significantly decreased levels of *psd95* mRNA expression in normal aging (p = 0.0171). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *psd95* mRNA levels compared to age-matched controls (p = 0.0013, p = 0.0012), and aging in APP/PS1 mice exacerbated the decreased *psd95* mRNA expression levels (p = 0.0371) in the hippocampus.

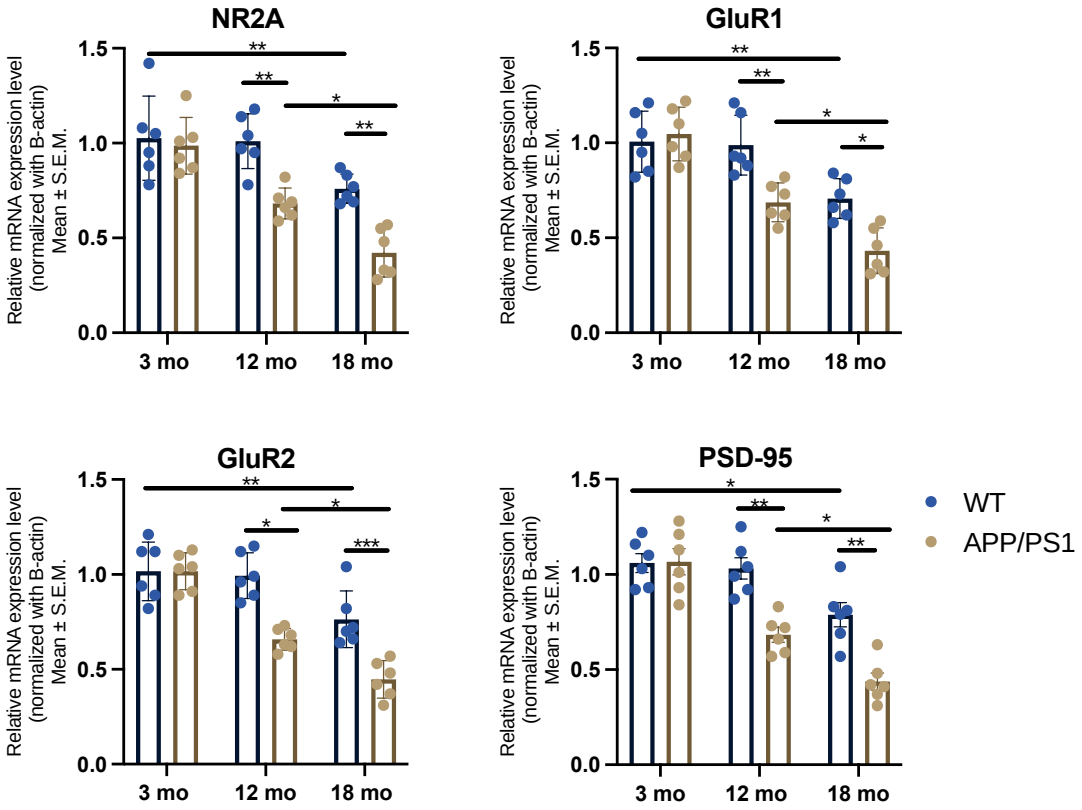

Figure 4. Synapse-related Gene Expression in the Hippocampus in Aging and AD. A: mRNA expression of *nr2a* **B:** mRNA expression of *glur1*. **C:** mRNA expression of *glur2* **D:** mRNA expression of *psd95*. All synapse-related genes were downregulated in normal aging and AD; however, all genes showed the most significant downregulation in our aged AD mice. Data represent mean ± SEM (n = 6/group). Blue circles represent WT mice, gold circles represent APP/PS1 mice. *p < 0.05, **p < 0.01, ***p < 0.001.

PFC:

Again, global mRNA expression of the gene targets was measured in the PFC (**Figure 5**). Two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 38.41, p < 0.0001), genotype ($F_{1,30}$ = 11.43, p = 0.0020), and an age × genotype interaction ($F_{2,30}$ = 9.766, p = 0.0005) of *nr2a* mRNA expression in the PFC (**Figure 5A**). Post-hoc analysis revealed significantly decreased levels of *nr2a* mRNA expression in normal aging (p = 0.0214). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *nr2a* mRNA levels compared to age-matched controls (p = 0.0337, p = 0.0024), and aging in APP/PS1 mice exacerbated the decreased *nr2a* mRNA expression levels (p = 0.0037) in the PFC. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 33.91, p < 0.0001), genotype ($F_{1,30}$ = 13.25, p = 0.0010), and an age × genotype interaction ($F_{2,30}$ = 9.909, p = 0.0005) of *glur1* mRNA expression in the hippocampus (**Figure 5B**). Post-hoc analysis revealed significantly decreased levels of *glur1* mRNA expression in normal aging (p = 0.0414). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *glur1* mRNA levels compared to age-matched controls (p = 0.0016, p = 0.0216), and aging in APP/PS1 mice exacerbated the decreased *glur1* mRNA expression levels (p = 0.0086) in the PFC. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 43.74, p < 0.0001), genotype ($F_{1,30}$ = 19.83, p = 0.0001), and an age × genotype interaction ($F_{2,30}$ = 11.01, p = 0.0003) of *glur2* mRNA expression in the hippocampus (**Figure 5C**). Post-hoc analysis revealed significantly decreased levels of *glur2* mRNA expression in normal aging (p = 0.0215). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *glur2* mRNA levels compared to age-matched controls (p = 0.0017, p = 0.0010), and aging in APP/PS1 mice exacerbated the decreased *glur2* mRNA expression levels (p = 0.0004) in the PFC. For *psd95*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 41.03, p < 0.0001), genotype

($F_{1,30}$ = 22.03, p < 0.0001), and an age × genotype interaction ($F_{2,30}$ = 1.91, p = 0.0002) of *psd95* mRNA expression in the hippocampus (**Figure 5D**). Post-hoc analysis revealed significantly decreased levels of *psd95* mRNA expression in normal aging (p = 0.0252). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *psd95* mRNA levels compared to age-matched controls (p = 0.0002, p = 0.0026), and aging in APP/PS1 mice exacerbated the decreased *psd95* mRNA expression levels (p = 0.018) in the PFC.

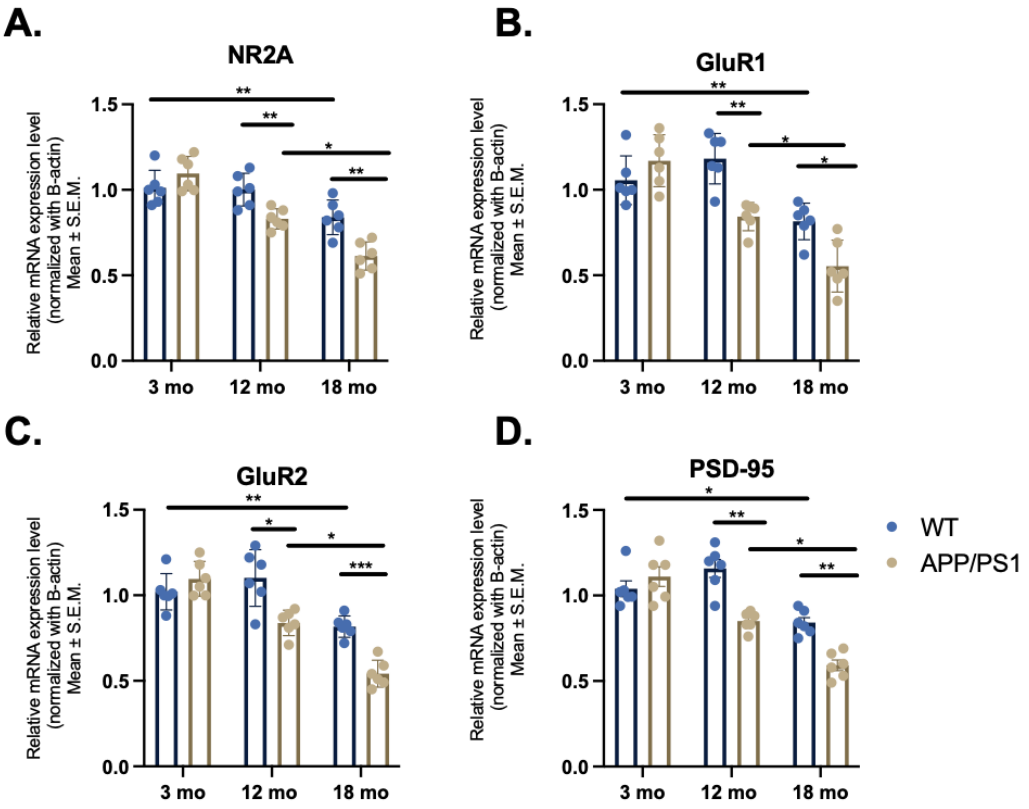

Figure 5. Synapse-related Gene Expression in the PFC in Aging and AD. A: mRNA expression of *nr2a* **B:** mRNA expression of *glur1*. **C:** mRNA expression of *glur2* **D:** mRNA expression of *psd95*. All synapse-related genes were downregulated in normal aging and AD; however, all genes showed the most significant downregulation in our aged AD mice. Data represent mean ± SEM (n = 6/group). Blue circles represent WT mice, gold circles represent APP/PS1 mice. *p < 0.05, **p < 0.01, ***p < 0.001.

II. Differential Class 1 HDAC Abundance at Synapse-Related Gene Promoters in Aging and AD

Previous experiments found differential global expression of class 1 HDACs and synapse-related gene expression in aging and AD. Given HDACs function mostly at gene promoters, class 1 HDAC abundance at the synapse-related gene promoters in the hippocampus and PFC was measured to reveal the relationship between these enzymes in the regulation of our target genes.

Hippocampus:

After the immunoprecipitated cDNA of HDAC 1-3 was isolated and amplified at synapse-related gene promoters through chIP + qPCR, only HDAC 2 was found to have significant changes at synapse-related gene promoters in aging and AD (**Figure 6**). More specifically, two-way ANOVA revealed significant effects of age ($F_{2,30} = 55.36$, $p < 0.0001$), genotype ($F_{1,30} = 36.59$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 7.329$, $p = 0.0026$) of HDAC 2 abundance at the *nr2a* gene promoter in the hippocampus (**Figure 6A**). Post-hoc analysis revealed a significant increased abundance of HDAC 2 at the *nr2a* promoter in normal aging ($p = 0.0004$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 2 at the *nr2a* promoter compared to age-matched controls ($p = 0.0005$, $p = 0.0001$), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 2 at the *nr2a* promoter ($p = 0.0365$) in the hippocampus. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 78.55$, $p < 0.0001$), genotype ($F_{1,30} = 68.46$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 19.39$, $p < 0.0001$) of *HDAC 2* abundance at the *glur1* gene promoter in the hippocampus (**Figure 6B**). Post-hoc analysis revealed significantly increased abundance of HDAC 2 at the *glur1* promoter in normal aging ($p = 0.0005$). Both 12- and 18-month-old

APP/PS1 mice showed significantly increased abundance of HDAC 2 at the *glur1* promoter compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 2 at the *glur1* promoter ($p = 0.0004$) in the hippocampus. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 125.0$, $p < 0.0001$), genotype ($F_{1,30} = 68.61$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 17.37$, $p < 0.0001$) of *HDAC 2* abundance at the *glur2* gene promoter in the hippocampus (**Figure 6C**). Post-hoc analysis revealed a significantly increased abundance of HDAC 2 at the *glur2* promoter in normal aging ($p < 0.0001$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 2 at the *glur2* promoter compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging APP/PS1 mice exacerbated the increased abundance of HDAC 2 at the *glur2* promoter ($p = 0.0012$) in the hippocampus. For *psd95*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 44.85$, $p < 0.0001$), genotype ($F_{1,30} = 29.16$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 12.18$, $p = 0.0001$) of *HDAC 2* abundance at the *psd95* gene promoter in the hippocampus (**Figure 6D**). Post-hoc analysis revealed a significantly increased abundance of HDAC 2 at the *psd95* promoter in normal aging ($p = 0.0341$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 2 at the *psd95* promoter compared to age-matched controls ($p = 0.0374$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 2 at the *psd95* promoter ($p = 0.0003$) in the hippocampus.

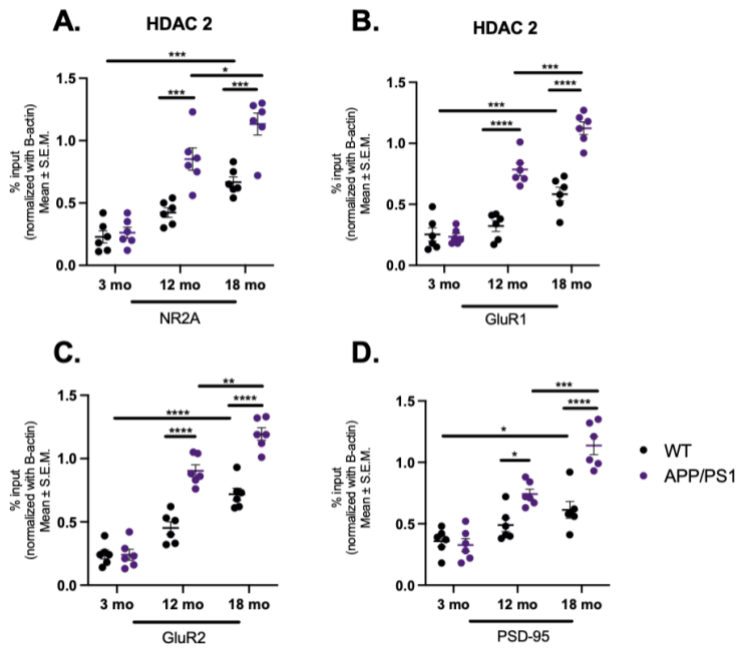

Figure 6. HDAC 2 Abundance at Synapse-Related Gene Promoters in the Hippocampus in Aging and AD. A: HDAC 2 abundance at the NR2A promoter. **B:** HDAC 2 abundance at the GluR1 promoter. **C:** HDAC 2 abundance at the PSD95 promoter **D:** HDAC 2 abundance at the GluR2 promoter. HDAC 2 showed increased abundance at all synapse-related gene promoters in normal aging and AD; however, all genes showed the most significant downregulation in our aged AD mice. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice. *p<0.05, **p<0.01 ***p<0.001.

When HDAC 1 and HDAC 3 abundance were assessed at synapse-related gene promoters in the hippocampus, no significant differences were found in aging and AD (**Figure 7A,B**).

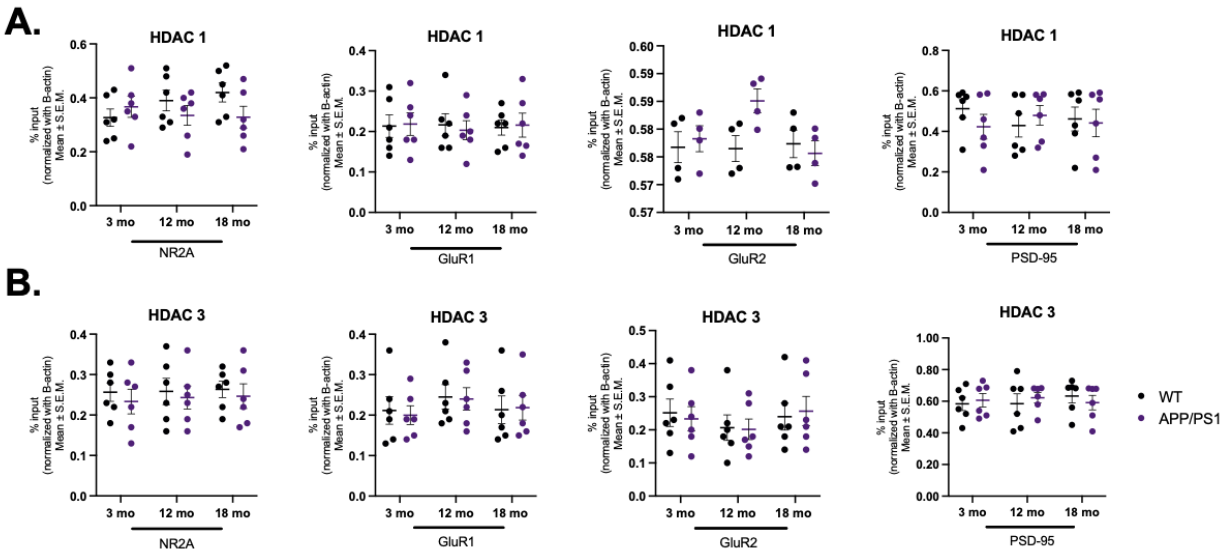

Figure 7. HDAC 1 and HDAC 3 Abundance at Synapse-Related Gene Promoters in the Hippocampus in Aging and AD. A: HDAC 1 abundance at the *nr2a*, *glur1*, *glur2*, and *psd95* gene promoters **B:** HDAC 3 abundance at the *nr2a*, *glur1*, *glur2*, and *psd95* gene promoters. HDAC 1 and HDAC 3 showed no significant changes in abundance at synapse-related gene promoters in normal or AD. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice.

PFC:

I also measured class 1 HDACs at target gene promoters in the PFC. Only HDAC 3 was found to have significant changes in synapse-related gene promoters in AD (**Figure 8**). More specifically, two-way ANOVA revealed significant effects of age ($F_{2,30} = 50.32$, $p < 0.0001$), genotype ($F_{1,30} = 77.69$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 14.25$, $p = 0.0026$) of HDAC 3 abundance at the *nr2a* gene promoter in the PFC (**Figure 8A**). Post-hoc analysis revealed a significant increased abundance of HDAC 3 at the *nr2a* promoter in normal aging (p = 0.0179). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 3 at the *nr2a* promoter compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 3 at the *nr2a* promoter (p = 0.4330) in the PFC. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 46.27$, $p < 0.0001$), genotype ($F_{1,30} = 50.92$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 10.06$, $p = 0.0005$) of HDAC 3 abundance at the *glur1* gene promoter in the PFC (**Figure 8B**). Post-hoc analysis revealed a significant increased abundance of HDAC 3 at the *glur1* promoter in normal aging (p = 0.0177). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 3 at the *glur1* promoter compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 3 at the *glur1* promoter (p = 0.0017) in the PFC. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 24.70$, $p < 0.0001$), genotype ($F_{1,30} =$

39.46, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 3.346$, $p = 0.0458$) of HDAC 3 abundance at the *glur2* gene promoter in the PFC (**Figure 8C**). Post-hoc analysis revealed no significant increased abundance of HDAC 3 at the *glur2* promoter in normal aging (p = 0.0389). Both 12- and 18-month-old APP/PS1 mice showed a significantly increased abundance of HDAC 3 at the *glur2* promoter compared to age-matched controls (p = 0.0215, p < 0.0001), and aging in APP/PS1 mice exacerbated the increased abundance of HDAC 3 at the *glur2* promoter (p = 0.0130) in the PFC. For *psd95*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 46.27$, $p < 0.0001$), genotype ($F_{1,30} = 50.92$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 10.06$, $p = 0.0005$) of HDAC 3 abundance at the *psd95* gene promoter in the PFC (**Figure 8D**). Post-hoc analysis revealed no significant increased abundance of HDAC 3 at the *psd95* promoter in normal aging (p = 0.00013). Both 12- and 18-month-old APP/PS1 mice showed significantly increased abundance of HDAC 3 at the *psd95* promoter compared to age-matched controls (p < 0.0001, p = 0.0002), and aging in APP/PS1 mice exacerbated did not show an increased abundance of HDAC 3 at the *psd95* promoter, however, a trend was seen (p = 0.0713) in the PFC.

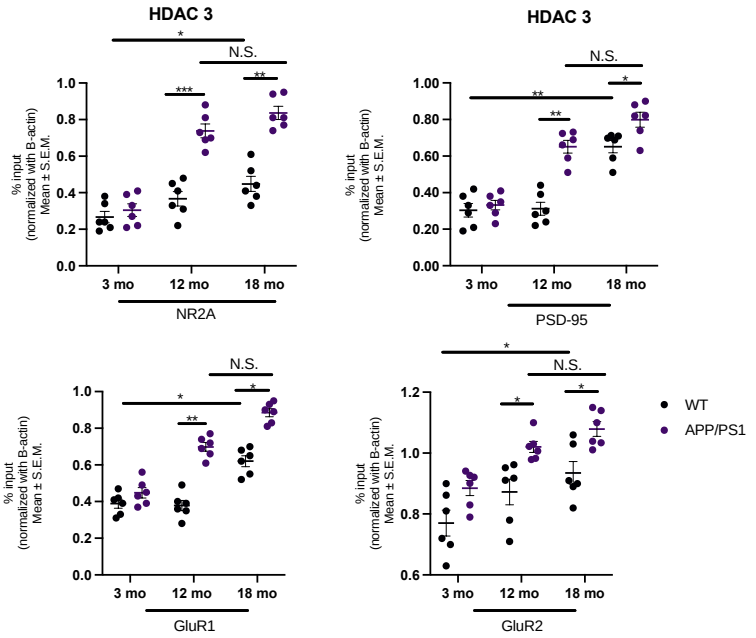

Figure 8. HDAC 3 Abundance at Synapse-Related Gene Promoters in the PFC in Aging and AD. A: HDAC 3 abundance at the NR2A promoter. **B:** HDAC 3 abundance at the GluR1 promoter. **C:** HDAC 3 abundance at the PSD95 promoter **D:** HDAC 3 abundance at the GluR2 promoter. HDAC 3 showed no significant changes in abundance at synapse-related gene promoters in normal or AD. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice.

When HDAC 1 and HDAC 2 abundance were assessed at synapse-related gene promoters in the PFC, no significant differences were found in aging and AD (**Figure 9A,B**).

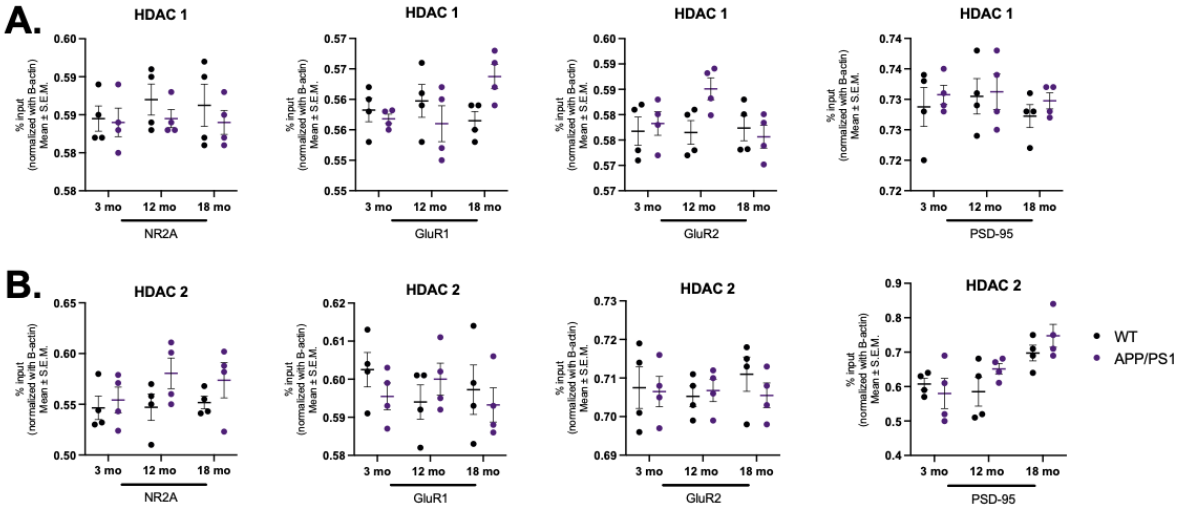

Figure 9. HDAC 1 and HDAC 2 Abundance at Synapse-Related Gene Promoters in the PFC in Aging and AD. A: HDAC 1 abundance at the *nr2a*, *glur1*, *glur2*, and *psd95* gene promoters **B:** HDAC 2 abundance at the *nr2a*, *glur1*, *glur2*, and *psd95* gene promoters. HDAC 1 and HDAC 2 showed no significant changes in abundance at synapse-related gene promoters in normal or AD. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice.

III. Alterations of Histone Acetylation at Synapse-Related Gene Promoters in AD mice

HDACs are enzymes that are responsible for the balance of histone acetylation. Changes in acetylation can alter the chromatin landscape and therefore transcription. Most importantly, HDACs impact gene expression through regulate histone acetylation. Therefore, to understand

how histone acetylation is regulated in aging and AD, H3K9ac levels at synapse-related gene promoters in our WT and APP/PS1 mice in the hippocampus and PFC were measured.

Hippocampus:

Two-way ANOVA revealed significant effects of age ($F_{2,30} = 30.36$, $p < 0.0001$), genotype ($F_{1,30} = 19.86$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 6.480$, $p = 0.0046$) of H3K9ac levels at the *nr2a* gene promoter in the hippocampus (**Figure 10A**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *nr2a* promoter in normal aging (p = 0.0004). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *nr2a* promoter compared to age-matched controls (p = 0.0006, p = 0.0372), and aging in APP/PS1 mice did not exacerbate the already existing decreased H3K9ac levels at the *nr2a* promoter (p = 0.1512) in the hippocampus. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 27.31$, $p < 0.0001$), genotype ($F_{1,30} = 28.35$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 5.189$, $p = 0.0116$) of H3K9ac levels at the *glur1* gene promoter in the hippocampus (**Figure 10B**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *glur1* promoter in normal aging (p = 0.0163). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur1* promoter compared to age-matched controls (p = 0.0005, p = 0.0065), and aging in APP/PS1 mice did not exacerbate the already existing decreased H3K9ac levels at the *glur1* promoter (p = 0.1301) in the hippocampus. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30} = 28.42$, $p < 0.0001$), genotype ($F_{1,30} = 27.48$, $p < 0.0001$), and an age × genotype interaction ($F_{2,30} = 4.401$, $p = 0.0211$) of H3K9ac levels at the *glur2* gene promoter in the hippocampus (**Figure 10C**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *glur2* promoter in

normal aging (p = 0.0141). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur2* promoter compared to age-matched controls (p = 0.0017, p = 0.0037), and aging in APP/PS1 mice did not exacerbate the already existing decreased H3K9ac levels at the *glur2* promoter, however, a trend was seen (p = 0.0692) in the hippocampus. For *psd95* two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 28.61, p < 0.0001), genotype($F_{1,30}$ = 15.67, p = 0.0004), and an age × genotype interaction ($F_{2,30}$ = 9.268, p = 0.0007) of H3K9ac levels at the *psd95* gene promoter in the hippocampus (**Figure 10D**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *psd95* promoter in normal aging (p = 0.0325). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *psd95* promoter compared to age-matched controls (p = 0.0005, p = 0.0464), and aging in APP/PS1 mice did not exacerbate the already existing decreased H3K9ac levels at the *psd95* promoter (p = 0.2000) in the hippocampus.

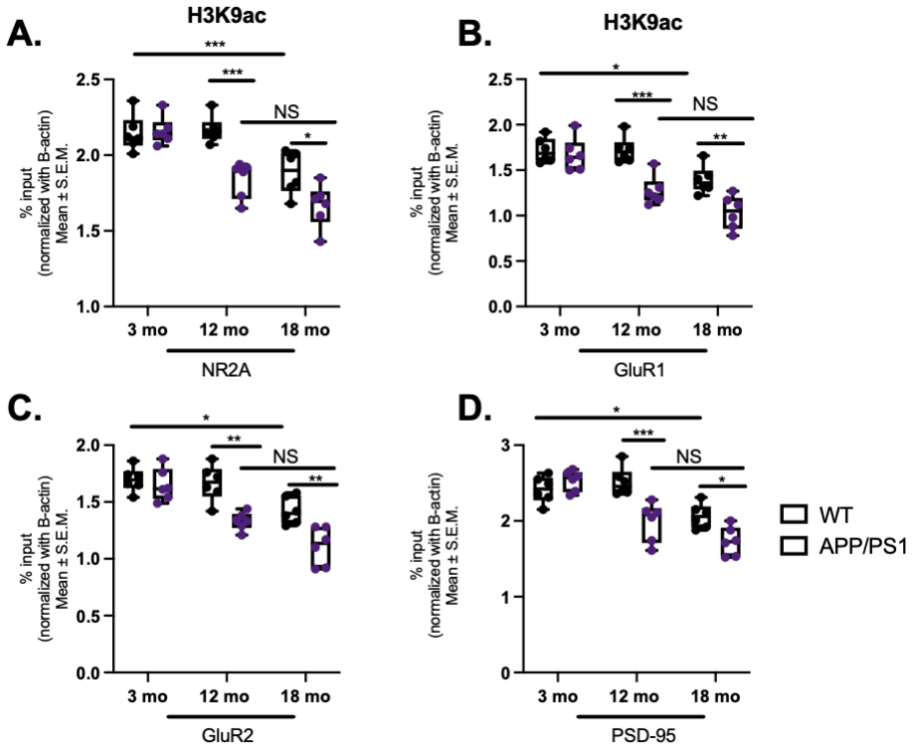

Figure 10. Alterations of H3K9ac Levels at Synapse-Related Gene Promoters in the Hippocampus in Aging and AD. A: H3K9ac levels at the *nr2a* promoter. **B:** H3K9ac levels at the *glur1* promoter. **C:** H3K9ac levels at the *glur1* promoter **D:** H3K9ac levels at the *psd95* promoter. Decreased H3K9ac levels were shown at all synapse-related gene promoters in normal aging and AD. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice. *p < 0.05, **p < 0.01 ***p < 0.001.

PFC:

To understand H3K9ac changes in aging and AD in a spatial manner, H3K9ac levels at synapse-related gene promoters in the PFC were measured (**Figure 11**). Two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 32.11, p < 0.0001), genotype ($F_{1,30}$ = 14.22, p = 0.0007), and an age × genotype interaction ($F_{2,30}$ = 6.442, p = 0.0047) of H3K9ac levels at the *nr2a* gene promoter in the PFC (**Figure 11A**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *nr2a* promoter in normal aging (p = 0.0198). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *nr2a* promoter compared to age-matched controls (p < 0.0001, p = 0.0223), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *nr2a* promoter (p = 0.0103) in the PFC. For *glur1*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 29.54, p < 0.0001), genotype ($F_{1,30}$ = 29.24, p < 0.0001), and an age × genotype interaction ($F_{2,30}$ = 4.977, p = 0.0136) of H3K9ac levels at the *glur1* gene promoter in the PFC (**Figure 11B**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *glur1* promoter in normal aging (p = 0.0134). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur1* promoter compared to age-matched controls (p = 0.0007, p = 0.0034), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *glur1* promoter (p = 0.0390) in the PFC. For *glur2*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 27.41, p < 0.0001), genotype ($F_{1,30}$ = 21.80, p < 0.0001), and an age × genotype interaction ($F_{2,30}$ = 3.993, p =

0.0290) of H3K9ac levels at the *glur2* gene promoter in the PFC (**Figure 11C**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *glur2* promoter in normal aging (p = 0.0134). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur2* promoter compared to age-matched controls (p = 0.0007, p = 0.0034), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *glur2* promoter (p = 0.0390) in the PFC. For *psd95*, two-way ANOVA revealed significant effects of age ($F_{2,30}$ = 44.79, $p < 0.0001$), genotype ($F_{1,30}$ = 32.65, $p < 0.0001$), and an age × genotype interaction ($F_{2,30}$ = 9.670, $p = 0.0006$) of H3K9ac levels at the *psd95* gene promoter in the PFC (**Figure 11D**). Post-hoc analysis revealed significantly decreased H3K9ac levels at the *psd95* promoter in normal aging (p = 0.0044). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *psd95* promoter compared to age-matched controls (p = 0.0002, p = 0.0003), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *psd95* promoter (p = 0.0075) in the PFC.

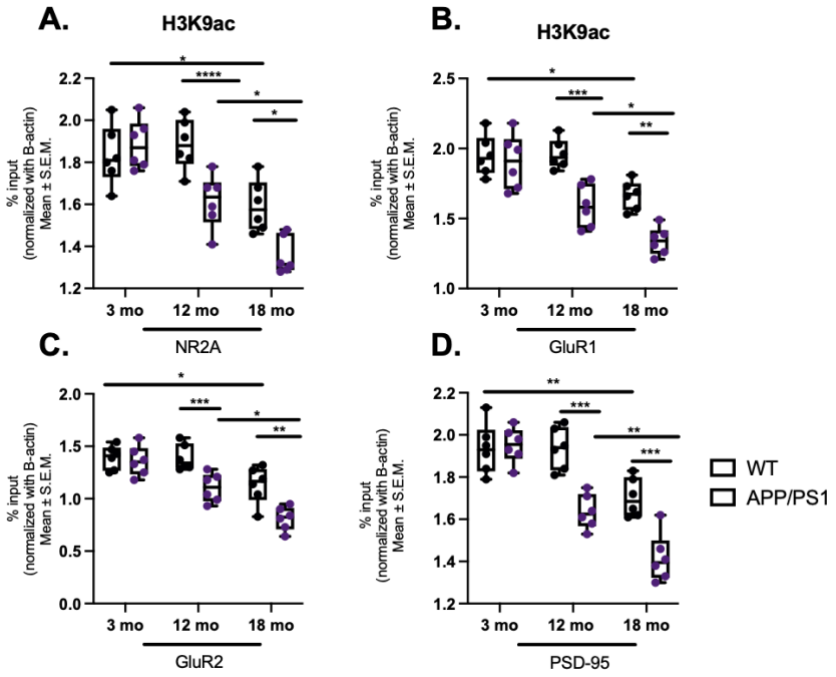

Figure 11. Alterations of H3K9ac Levels at Synapse-Related Gene Promoters in the PFC in Aging and AD. A: H3K9ac levels at the *nr2a* promoter. **B:** H3K9ac levels at the *glur1* promoter. **C:** H3K9ac levels at the *glur2* promoter **D:** H3K9ac levels at the *psd95* promoter. Decreased H3K9ac levels were shown at all synapse-related gene promoters in normal aging and AD; however, all genes showed the most significant downregulation in our aged AD mice. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, purple circles represent APP/PS1 mice. *p < 0.05, **p < 0.01 ***p < 0.001.

IV. Evaluating Neuronal Densities in Aging and AD

Previous results found a differential class 1 HDAC distribution in the hippocampus and PFC at synapse-related gene promoters, which may regulate target gene expression through alterations of histone acetylation. However, whether these changes in synapse-related genes could eventually influence neuronal structure and function are unknown. To address this, immunohistochemical staining of neuronal nuclei was performed. Neuronal nuclei densities were quantitatively measured by investigating the % area and cell count in the hippocampus and PFC in WT and APP/PS1 mice at 3-, 12-, and 18 months of age.

Hippocampus:

Two-way ANOVA revealed no significant effects of age ($F_{2,18} = 0.02181$, $p = 0.9785$, genotype ($F_{1,18} = 0.02342$, $p = 0.8801$), and an age × genotype interaction ($F_{2,18} = 0.1737$, $p = 0.8420$) of NEUN+ cell count in the hippocampus (**Figure 12**). Additionally, two-way ANOVA revealed no significant effects of age ($F_{2,30} = 0.2669$, $p = 0.7687$), genotype ($F_{1,30} = 0.01171$, $p = 0.9150$), and an age × genotype interaction ($F_{2,30} = 0.7654$, $p = 0.4797$) of NEUN+ % area in the hippocampus.

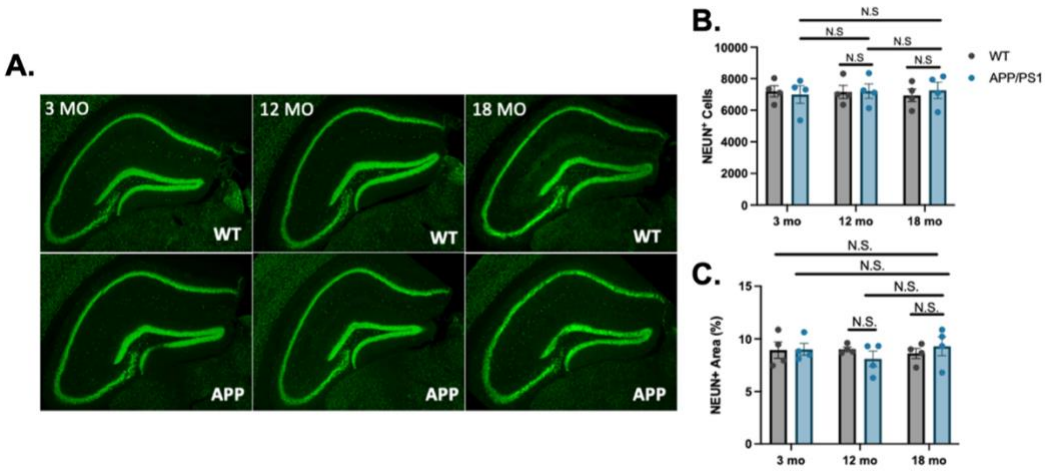

Figure 12. Evaluation of Neuronal Densities in the Hippocampus in Aging and AD. A: Immunostaining of NEUN+ cell densities in the hippocampus. **B:** Total number of NEUN+ cells. **C:** % Area of NEUN+ cells. No significant changes in neuronal densities found in the hippocampus our aging and AD mice. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, blue circles represent APP/PS1 mice.

PFC:

For the PFC, two-way ANOVA revealed no significant effects of age ($F_{2,18} = 0.7241$, $p = 0.4984$, genotype ($F_{1,18} = 2.206$, $p = 0.1548$), and an age × genotype interaction ($F_{2,18} = 0.03924$, $p = 0.9616$) of NEUN+ cell count in the PFC (**Figure 13**). Additionally, our two-way ANOVA revealed no significant effects of age ($F_{2,30} = 0.7724$, $p = 0.4766$), genotype ($F_{1,30} = 2.919$, $p =$

0.1048), and an age × genotype interaction ($F_{2,30}$ = 0.6817, p = 0.5184) of NEUN+ % area in the PFC.

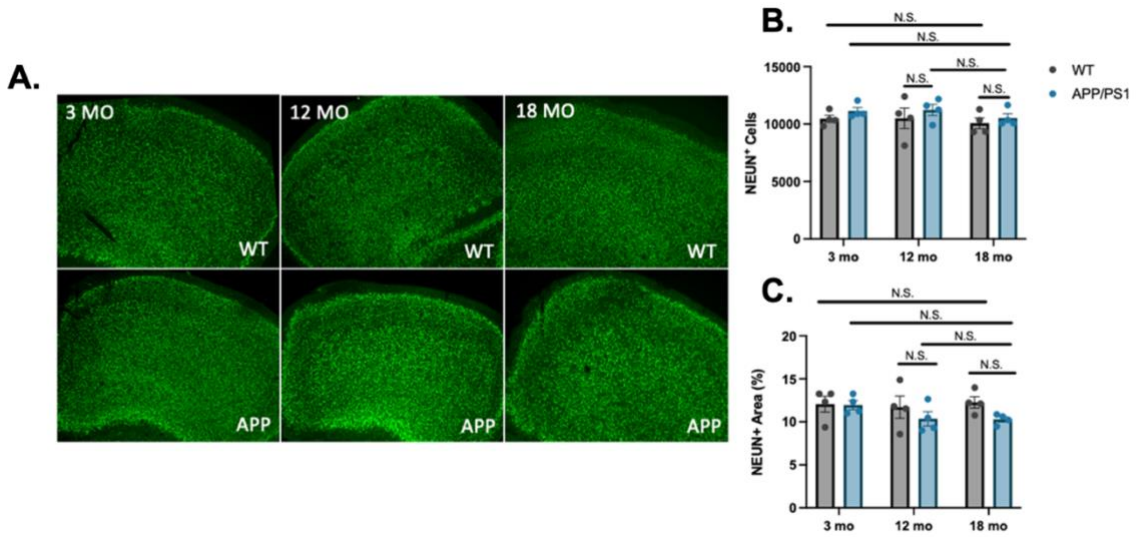

Figure 13. Evaluation of Neuronal Densities in the PFC in Aging and AD. A: Immunostaining of NEUN+ cell densities in the hippocampus. **B:** Total number of NEUN+ cells. **C:** % Area of NEUN+ cells. No significant changes in neuronal densities found in the hippocampus our aging and AD mice. Data represent mean ± SEM (n = 6/group). Black circles represent WT mice, blue circles represent APP/PS1 mice.

V. Memory Domains are Differentially Modulated in Aging and AD

Molecular findings from study 1 revealed a potential differential epigenetic regulation of synapse-related gene expression through alterations of histone acetylation due to class 1 HDAC changes. However, these findings did not parse out the HDAC contributions, therefore, it is important to understand how HDACs impact memory processes at the functional level, which entails measuring the memory function of our WT and APP/PS1 mice through the treatment of HDAC inhibitor. Before understanding the impact HDAC inhibitors have on memory function throughout aging and AD, it is important to first understand the baseline how memory function is modulated throughout aging and AD. Therefore, recognition memory

through NOR, short-term working memory through Y-maze, and long-term spatial reference memory through MWM were assessed (**Figure 14**).

For NOR testing, which measures recognition memory, two-way ANOVA revealed significant effects of age ($F_{2,75} = 66.78$, $p < 0.0001$, genotype ($F_{1,75} = 68.86$, $p = 0.0300$), and an age × genotype interaction ($F_{2,75} = 17.10$, $p < 0.0001$) of the discrimination index in the NOR behavior test (**Figure 14A**). Post-hoc analysis revealed significantly declined recognition memory in normal aging (p = 0.0003). Both 12- and 18-month-old APP/PS1 mice showed significantly declined recognition memory compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decline in recognition memory (p = 0.0300). For Y-maze testing, which measures short-term working memory, two-way ANOVA revealed significant effects of age ($F_{2,75} = 6.981$, $p = 0.0017$, genotype ($F_{1,75} = 47.06$, $p < 0.0001$), and an age × genotype interaction ($F_{2,75} = 5.004$, $p = 0.0091$) of the % alteration in the Y-maze behavior test (**Figure 14B**). Post-hoc analysis revealed no significant changes were seen in short-term working memory in normal aging (p = 0.9949). Both 12- and 18-month-old APP/PS1 mice showed significantly declined short-term working memory compared to age-matched controls (p < 0.0001, p < 0.0001), however, aging in APP/PS1 mice did not exacerbate the decline in short-term working memory (p = 0.9777). For MWM testing, which measures long-term spatial reference memory, two-way ANOVA revealed significant effects of age ($F_{2,75} = 47.19$, $p < 0.0001$, genotype ($F_{1,75} = 32.72$, $p < 0.0001$), and an age × genotype interaction ($F_{2,75} = 4.115$, $p = 0.0201$) of the total time spent in the target quadrant in the MWM behavior test (**Figure 14C**). Post-hoc analysis revealed significantly declined long-term spatial reference memory (p = 0.0008). Both 12- and 18-month-old APP/PS1 mice showed significant declined

long-term spatial reference memory compared to age-matched controls ($p = 0.0282$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the decline in long-term spatial reference memory ($p < 0.0001$).

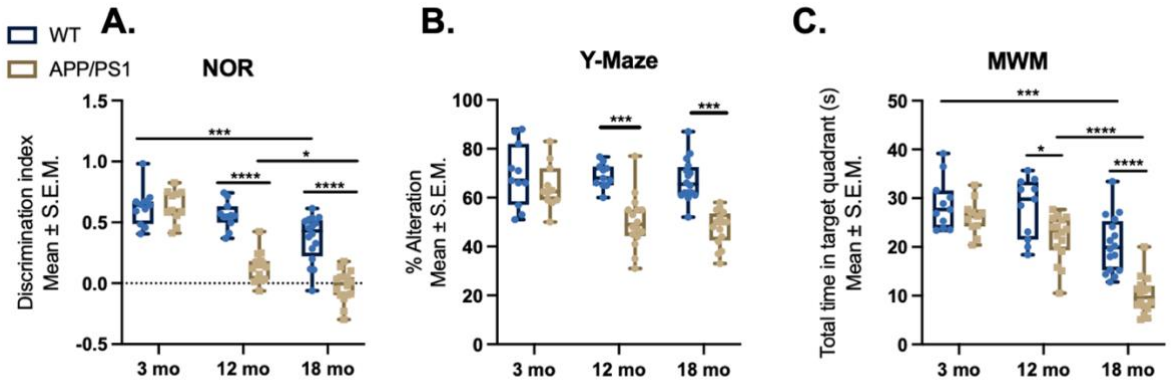

Figure 14. Characterization of Memory Function in Aging and AD. A: Novel object recognition measured by discrimination index. **B**: Y-maze measured by % spontaneous alteration **C:** Memory assessment of the Morris water maze measured by total time spent in target quadrant. In normal aging, recognition and long-term spatial memory declined. All memory domains showed deficits in 12-and 18-month-old APP/PS1 mice. Aging in APP/PS1 mice exacerbated recognition and long-term spatial reference memory deficits. Data represent mean ± SEM (n = 11-16/group). Blue circles represent WT mice, gold circles represent APP/PS1 mice. *p < 0.05, ***p < 0.001, ****p < 0.0001.

Discussion:

In this study, results showed differential global class 1 HDAC and synapse-related gene expression in aging and AD, followed by altered levels of H3K9ac at synapse-related gene promoters in APP/PS1 mice. More specifically, results showed that HDAC 2 in the hippocampus and HDAC 3 in the PFC are important modulators of synapse-related gene expression in aging and AD.

Class 1 HDACs, compared to the other classes, have shown promising findings in their role in learning and memory processes (Acosta-Rodriguez et al., 2022; Kilgore et al., 2010; Kim et al., 2012; Mahgoub & Monteggia, 2014; McClarty et al., 2021; McQuown et al., 2011; Montalvo-Ortiz et al., 2017; Zhu et al., 2019). Therefore, this study dissected global class 1 HDACs as a starting point to help understand the regulation of these enzymes in aging and AD. In the hippocampus, results showed only HDAC 2 to be significantly expressed during normal, APP/PS1 mice, and aging in APP/PS1 mice exacerbated this increase, compared to HDAC 1 and HDAC 3. These results are consistent with literature surrounding HDAC 2 in memory processes, which has shown that during normal aging, HDAC 2 increases expression and has been linked to changes in the cognition (Bie et al., 2014; Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Grinan-Ferre et al., 2016; Guan et al., 2009; D. Liu et al., 2017; Yamakawa et al., 2017). Similarly, few studies have dissected HDAC 2's role in AD processes and have found that HDAC 2 is significantly increased in the hippocampus, and at promoters for synapse genes (Graff et al., 2012; Yamakawa et al., 2017).

On the contrary, results showed HDAC 3 to be increased in APP/PS1 mice, compared to HDAC 1 and 2 in the PFC. However, one study on post-mortem tissues of AD subjects has shown no changes of HDAC 3 in the PFC. Instead, this study showed reductions of HDAC 2 in the PFC (Mahady et al., 2018). In this study, results showed no changes in HDAC 2 globally in the PFC. More interestingly, HDAC 3 abundance was increased at all synapse-related gene promoters, indicating that the increase of HDAC 3 globally is occurring at the target gene promoters. It is clear there are discrepancies between this preclinical mouse study and previous postmortem tissue work, partly due to the complex variations between brain regions with different neuropathological progression. Even though there is no clear evidence of HDAC 3 expression and abundance at synapse-related gene promoters, studies have shown HDAC 3 to play an important role in the PFC (Alaghband et al., 2017; Janczura et al., 2018). These results expand the existing literature regarding HDAC 3 impact on synapse-related genes in the PFC in AD.

This study also showed that synapse-related target genes were differentially expressed in a spatial manner during aging and AD. The specific genes *nr2a, glur1, glur2, and psd95* we selected because these gene sets are present at the synapse, but are known to be reduced and or dysregulated in aging and AD. Given the evidence surrounding the target genes, these results are consistent with the literature that changes in synapse-related gene expression through epigenetic mechanisms may impact memory function (Dickey et al., 2003; Graff et al., 2012; Yamakawa et al., 2017). However, no studies dissect the impact of synapse-related gene expression in a temporal and spatial manner, therefore these results are important in not only understanding the regulation of expression but further demonstrating that the expression of these important genes is being modulated by epigenetic mechanisms.

Given HDACs directly affect histone acetylation, it was important to measure histone acetylation at synapse-related gene promoters. Results showed altered levels of H3K9ac at synapse-related gene promoters in APP/PS1 mice in both the hippocampus and PFC. H3K9ac is a commonly studied histone acetylation marker and has been shown to be important for many neurological processes, including synaptic plasticity (Geng et al., 2021; Thatcher & LaSalle, 2006). More interestingly, H3K9ac has been shown to be dysregulated in aging and AD processes, however, no study dissects H3K9ac in a spatial and temporal manner. These results provide evidence for a mechanism detailing class 1 HDACs to modulate synapse-related gene expression through altered levels of H3K9ac at synapse-related gene promoters.

Lastly, to understand whether the study results are due to changes in neuronal numbers, neuronal densities were measured in the hippocampus and PFC in WT and APP/PS1 mice at 3,12, and 18 months old. Results showed no significant changes in neuronal densities in WT and APP/PS1 mice at all ages. These results are consistent with the literature, even though several studies have reported significant reductions in neuronal densities in the APP/PS1 mouse model (Hou et al., 2020; Schroder et al., 2020; Zhu et al., 2018). However, results vary depending on the AD model and age (Eimer & Vassar, 2013; Rupp et al., 2011; S. Zhu et al., 2017). Even though the number of neuronal densities did not change in this study, does not mean the number of synapses has. Results reflecting global mRNA expression of synapse-related genes found all synapse-related genes to be significantly decreased in normal aging and AD. Therefore, it is likely that changes in synaptic densities in aging and AD occur, and additional immunohistochemical and western blotting experiments are needed to further validate this.

At the functional level, results showed memory domains to be differentially impacted in WT and APP/PS1 mice. More specifically, results showed recognition and long-term spatial reference memory declined in normal aging. Recognition, short-term, and long-term spatial reference memory declined in APP/PS1 mice, and aged APP/PS1 mice showed the most significant decline in recognition and long-term spatial reference memory. The behavioral phenotyping of memory function in AD models can vary; however, the results regarding memory deficits in all memory domains are consistent with the literature for the APP/PS1 mouse model (Guo et al., 2016; Han et al., 2019; Hu et al., 2020; Li et al., 2019; Locci et al., 2021; Perez-Gonzalez et al., 2014; Silva, 2003; Wu et al., 2017; Xu et al., 2021; Zhong et al., 2018). This study is the first to demonstrate the impairment of different memory domains in a temporal manner in APP/PS1 mouse model. The 3-, 12- and 18-month-old age time points were selected to cover the whole life span in aging and to investigate the dynamic changes of AD-like pathogenesis throughout AD progression in the APP/PS1 mice. Additionally, previous studies studying memory deficits in AD models typically range from 4-12 months of age. Various studies relate molecular and behavioral processes with aging, however, the WT control rodents used in these studies are not truly “aged”. The usage of 18-month WT mice in this study is considered aged mice (Flurkey K, 2007; Jackson et al., 2017), which provides evidence that the molecular and behavioral findings reflect aging and disease processes. Therefore, in this study, the aging process continues to decline existing memory deficits, specifically recognition and long-term spatial memory function in aged APP/PS1 mice.

In summary, this study, results showed a spatial and temporal differential HDAC modulation of synapse-related gene expression underlying memory in aging and AD. Given AD is an age-associated neurodegenerative disease, it is critical to understand how epigenetic changes and pathways impact aging processes, and the functional consequences of such impact on the initiation or exacerbation of AD. These results help us understand how epigenetic changes are regulated throughout 1) normal aging and 2) aging within a disease state, and can further help pinpoint specific epigenetic targets for future therapeutic purposes along with disease progression in AD.

Chapter 3

Histone Deacetylase Inhibitors Improve Memory Function and Decrease Neuropathologies in Alzheimer's Disease

Abstract:

Histone deacetylases (HDACs) have been involved in the regulation of memory, and previous work from my research laboratory and others has shown promising beneficial effects of HDAC inhibitors in aging and neurodegenerative disorders, including Alzheimer's disease. However, currently, there are no reports on the cross-comparison of none-selective and selective histone deacetylase inhibitors of their beneficial effects on memory and AD-like pathologies in both aging and AD models.

In this study, a broad-acting histone deacetylase inhibitor valproic acid (VPA), and two selective class 1 histone deacetylase inhibitors entinostat (MS-275, targeting HDAC 1 and 3) and tacedinaline (CI-994, targeting HDAC 1, 2 and 3) were administered to 3-, 12-, and 18-months of age WT and APP/PS1 mice for 30 days. After HDAC inhibitor treatment, mice underwent three memory tests: novel object recognition (NOR) for recognition memory, Y-maze for short-term working memory, and Morris water maze (MWM) for long-term spatial reference memory. After memory assessment, the hippocampus and PFC were collected for biochemical measures, to investigate synapse-related gene expression, H3K9ac levels at synapse-related gene promoters, and AD pathologies after chronic HDAC inhibitor treatments.

The molecular and biochemical results showed that CI-994 significantly increased synapse-related gene expression, and H3K9ac levels at all synapse-related gene promoters in the hippocampus of 12- and 18-month APP/PS1 mice compared to age-matched controls. Conversely, MS-275 significantly increased all synapse-related gene expression and H3K9ac levels at *nr2a* and *psd95* gene promoters in the PFC of 12- and 18-month APP/PS1 mice

compared to age-matched controls. Immunohistochemical measures showed that MS-275 significantly reduced amyloidosis in both the hippocampus and PFC of 12- and 18-month APP/PS1 mice compared to age-matched controls. MS-275 and CI-994 significantly reduced microglia cell densities of 12- and 18-month APP/PS1 mice compared to age-matched controls. Behavioral tests demonstrated that both MS-275 and CI-994 improved recognition memory, and short-term spatial reference memory in 12- and 18-month-old APP/PS1 mice compared to age-matched controls. however, only CI-994 was sufficient to improve long-term spatial reference memory in 12- and 18-month APP/PS1 mice compared to age-matched controls.

These results also suggested that changes in memory during aging and AD that involve hippocampal processes (NOR, MWM), are likely to be affected by HDAC 2 modulatory effects of synapse-related gene targets through regulation of H3K9ac levels at target gene promoters. Furthermore, the significantly increased synapse-related gene expression through increased H3K9ac levels at synapse-related gene promoters after CI-994 treatment in the hippocampus were correlated with improved recognition, short-term working, and long-term spatial reference memory in 12- and 18-month-old APP/PS1 mice. In comparison, changes in memory during aging and AD that involve prefrontal cortex processes (NOR, Y-maze) are likely to be affected by HDAC 3 modulatory effects of synapse-related gene targets through the regulation of H3K9ac levels at target gene promoters. Furthermore, the significantly increased synapse-related gene expression through increased H3K9ac levels at target gene promoters after MS-275 treatment in the PFC were correlated with improved recognition, and short-term working memory in 12- and 18-month APP/PS1 mice.

In parallel, amyloid pathology, specifically, Aβ plaque burden seen throughout AD progression, seems to be modulated through HDAC 1 and or 3-related mechanism(s) in both the hippocampus and PFC. However, results showed a correlation between the significant reduction of Aβ plaque load after chronic MS-275 treatment in the PFC with improved recognition and short-term working memory. The significant reductions of Aβ plaque burden after chronic MS-275 treatment showed no correlation with long-term spatial reference memory, indicating that changes in amyloid pathology impact recognition and short-term working memory, and not long-term spatial reference memory. Therefore, a differential HDAC modulation of memory and AD pathological processes in aging and AD exist in a temporal and spatial manner.

Introduction:

Class 1 HDACs play a major role in many biological processes (Chen et al., 2015; Haberland et al., 2009), however, class 1 HDAC's impact on aging and AD processes is still not well understood. Pharmacological intervention is one common approach to investigating the function of HDACs in aging and AD processes. Many studies have shown that class 1 and 2 HDAC inhibitor treatment can restore memory function in rodents during normal aging (Dagnas et al., 2013; Dagnas et al., 2015; McClarty et al., 2021; Wu et al., 2019; C. X. Yang et al., 2020) and can also restore memory function and reduce AD-related pathologies (Badrikoohi et al., 2022; Govindarajan et al., 2011; Janczura et al., 2018; Kilgore et al., 2010; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Sung et al., 2013). Although HDAC inhibitors have been administered to aged mouse models of AD and have shown beneficial effects, to our knowledge, no study has cross-compared pan and selective HDAC inhibitors to evaluate their benefits on memory function and AD-like pathologies in a temporal and spatial manner.

Valproic acid (VPA), a broad-acting HDAC inhibitor, targets multiple HDACs from class 1 and 2 and is also used as an antiepileptic medication. VPA has been shown to help improve memory function and decrease AD pathologies in multiple AD mouse models (Kilgore et al., 2010; Long et al., 2016; Noh & Seo, 2014; Qing et al., 2008; Wang et al., 2014; Xuan et al., 2015; Zeng et al., 2019). However, most of these reports are seen in younger age-timepoints and administered with higher doses. Entinostat (MS-275), a class 1 HDAC inhibitor, targets HDAC 1 and HDAC 3 and has shown promising preclinical findings. MS-275 has helped decrease multiple pathologies across various diseases (Du et al., 2022; Kalin et al., 2019; K. Ma et al., 2018; Zhang & Schluesener, 2013), including AD (Zhang & Schluesener, 2013). MS-275 has also been shown to

decrease amyloidosis and gliosis densities in several AD mouse models. Tacedinaline (CI-994), another class 1 HDAC inhibitor, targets HDAC 1, 2, and 3, and is a newer HDAC inhibitor that has been shown to help improve memory function in normal aging (Burns et al., 2022; Graff et al., 2014; McClarty et al., 2021), and pathologies in various diseases and disorders (Kim et al., 2021; Lin et al., 2022; S. Zhang et al., 2018). However, we still do not know whether CI-994 could have any benefits in improving memory function and decreasing AD pathologies in an AD mouse model.

In this chapter, APP/PS1 and WT control mice were used at 3, 12, and 18 months of age. Mice were administered broad (VPA) and class 1 (MS-275, CI-994) histone deacetylase inhibitors to investigate the HDAC modulatory effects on synapse-related gene expression, H3K9ac levels at synapse-related gene promoters, as well as AD pathologies. Results showed a differential class 1 HDAC modulation of synapse-related gene expression through changes of H3K9ac at synapse-related gene promoters after chronic HDAC inhibitor treatment in aging and AD. These findings further support that class 1 HDAC's involvement in AD pathologies, such as amyloidosis and gliosis in AD and that histone modifications play an important role in aging and AD progression.

Methods

Animal Models

Double transgenic APP/PS1 mice expressing a chimeric Mo/Hu APP695swe mutation and a mutant Hu PS1-de9 mutation, were used for this project. APP/PS1 males (Jackson Laboratory, Bar Harbor, ME) were bred with C57BL/6L females (Jackson Laboratory, Bar Harbor, ME). The APP/PS1 and their wildtype (WT) littermate offspring were housed in groups of 3-5 per cage starting at 21 days until 3 months, 12 months, and 18 months of age for this study. Animals were group housed on a 12-h light/dark cycle and given food and water ad libitum. All procedures in animals were performed according to NIH guidelines and the Current Guide for the Care and Use of Laboratory Animals (2011, eight edition) under a protocol approved by the Northwestern University Animal Care and Use Committee.

Drugs

VPA was purchased from Sigma Aldrich (St. Louis, MO, United States), MS-275 was purchased from biotechne (Minneaplois, MN) with inhibition of IC_{50} of 0.18, 0.74 μM for recombinant HDAC 1 and 3, and CI-994 was purchased from MedChemExpress (Monmouth Junction, NJ, United States) with inhibition of IC_{50} of 0.9, 0.9, 1.2 recombinant HDAC 1, 2 and 3. Drugs were prepared freshly on the day of treatment. All histone deacetylase inhibitors were first dissolved in 2% DMSO, and then 33.3% of PEG-300 was added to MS-275 and CI-994. The final dose volume for all histone deacetylase inhibitors were volumed up in 0.9% saline with pH adjusted to ~6-7 with 0.1 M NaOH. All HDAC inhibitor dosages were selected based on previously published reports, and unpublished preliminary data. All compounds and vehicles were administered intraperitoneally (i.p.) at a constant volume of 10 μL/g of body weight once a day

for 30 consecutive days. Immediately after drug treatment, animals were monitored for physical condition and behaviors, including respiratory stress, locomotor function and body weight. Respiratory distress was determined by breathing patterns in mice. If fast/short, labored breathing was observed for more than 30 min after injections, and this symptom persisted 1 day post injections was marked as respiratory distress. General locomotor activity was monitored by observation 5, 10, and 20 min after first day of injections. Then locomotor activity was measured after 30 min of injections on the first day of the study using the open field test. Locomotor function impairments were considered if a mouse movement was greatly reduced, or no movement was shown compared to control mice for more than 30 min after drug treatment. Body weight was measured daily, and any weight loss (>2 g) within 2–3 days was considered a significant side effect.

Tissue Preparation and Collection

Methods for tissue collection were done in similar matter to a previous published study in my lab (McClarty et al., 2021). Before brain tissues were collected for biochemical and immunohistochemical studies, a cardiac perfusion was performed with 0.1M PBS solution for 1 min, to wash-out the blood from blood vessels in the mouse brain. The mouse brains were then removed and quickly dissected under ice with a dissecting scope. The hippocampus and PFC were dissected and then froze at −80°C until ready for processing for molecular analysis. Half-brains were used for immunohistochemical studies. Half-brains were collected and fixed in 4% paraformaldehyde overnight at 4 °C, followed by dehydration in 30% sucrose solution until the tissue sank to the bottom of the bottles. After fixation and dehydration, the whole brain and spinal cord were dissected and embedded in OCT compound.

Chromatin Immunoprecipitation

Methods for tissue collection were done in similar matter to a previous published studies in my lab (McClarty et al., 2021; Montalvo-Ortiz et al., 2017). The commercially available Magna ChIP™ G Tissue Kit (17-20000, Millipore) was used. For ChIP, tissue samples were homogenized in cell lysis buffer containing proteinase inhibitor and chromatin was sonicated using a Branson Digital Sonifier with 3 rounds of 10 s at 75% power per sample on ice. Fragmented chromatin lysate was immunoprecipitated with 5 μg of antibody directed against H3K9ac (ab32129). The DNA-histone complex was incubated with Protein G Magnetic Beads overnight at 4°C. The DNA-histone complex was eluted from the beads and dissociated at 65 4°C for 2 h under high salt conditions. Proteins were digested using proteinase K treatment and the associated DNA was precipitated with 100% ethanol and resuspended in 75 μL of PCR grade water.

RNA Extraction

Methods for tissue collection were done in similar matter to a previous published study in my lab (McClarty et al., 2021). In order to access synaptic-related gene expression, RNA extractions were performed. For gene expression analysis, mRNA was extracted (Qiagen), reverse transcribed (QuantaBio) and quantitatively amplified on a thermal cycler (ThermoFischer), yielding complementary DNA.

Real-time Quantitative Polymerase Chain Reaction (qPCR)

Methods for tissue collection were done in similar matter to a previous published studies in my lab (McClarty et al., 2021; Montalvo-Ortiz et al., 2017). Input, immunoprecipitated DNA, and cDNA amplification reactions were run in triplicate in the presence of SYBR Green (Applied Biosystems) using QuantStudio 6 Flex Real Time PCR System (Applied Biosystems, Foster City, CA, United States) using primers found in **Table 2.** Ct values from each sample were obtained using the Sequence Detector 1.1 software. Ct values were normalized to endogenous gene, beta actin, to obtain a percent input. Fold differences (drug treated versus control) were then determined using the delta delta C_T method as a previous report (Livak & Schmittgen, 2001).

Gene Target (mouse)	Method	Forward primer (5' → 3')	Reverse Primer (5' → 3')
β-actin	mRNA	GCACCACACCTTCTACAATG	TGCTTGCTGATCCACATCTG
Nr2a	mRNA	TGCAAGTTACACAGCCAACC	ATCGGAAAGGCGGAGAATAG
GluR1	mRNA	GTGGTGGTGGACTGTGAATC	TTGGCGAGGATGTAGTGGTA
GluR2	mRNA	TGTGTTTGTGAGGACTACGGCA	GGATTCTTTGCCACCTTCATTC
PSD95	mRNA	GCCCTGTTTGATTACGACAA	CTCATAGCTCAGAACCGAGT
ADAM-10	mRNA	TGCACCTGTGCCAGCTCTGATG	GATAGTCCGACCACTGAACTGC
BACE-1	mRNA	ACCGACGAAGAGTCGGAGGAG	CACAATGCTCTTGTCATAG
NEP	mRNA	AGCCTCTCTGTGCTTGTCTTGC	CACTCATAGTAGCCTCTGGAAGGG
β-actin	chIP (promoter)	GAGACATTGAATGGGGCAGT	ATGAAGAGTTTTGGCGATGG
Nr2a	chIP (promoter)	TCGGCTTGGACTGATACGTG	AGGATAGACTGCCCCTGCAC
GluR1	chIP (promoter)	GGAGGAGAGCAGAGGGAGAG	TTCCTGCAATTCCTTGCTTG
GluR2	chIP (promoter)	GCGGTGCTAAAATCGAATGC	ACAGAGAGGGGCAGGCAG
PSD95	chIP (promoter)	GGAGGGGTGAGAACCCACCGA	CTCCCCCTCCCCACTGCTCC

Table 2: Primers used for qPCR

Immunohistochemistry

Methods for tissue collection were done in similar matter to a previous published study in my lab (Cao et al., 2021). For immunofluorescence staining, brain slices were washed in PBS containing 0.3% Triton X-100 and blocked for 2 h at room temperature with 10% donkey. Slices were then incubated overnight at 4 °C with primary antibody for amyloid beta plaques (6E10, 1:250, 803001, Biolegend), for microglia (Iba1, 1:500, 019-19741, Wako). Slices were then washed with PBS containing 0.3% Triton and incubated for 2 h at room temperature with anti-rabbit Alexa Fluor® 488 (1:500, ab150073, Abcam) or Alexa Cy3® (1:250, ab97035, Abcam) secondary antibody. Slices were then washed in PBS containing 0.3% Triton before being mounted with mounting medium ProLong™ Glass Antifade Mountant (P36980, Thermo Fischer). Immunofluorescence pictures were taken with a fluorescence microscope (80i, Nikon, Japan) and CoolSNAP DYNO CCD (Photometrics, Canada). To quantify Aβ plaque burden and Iba1 cell densities, the brain images (magnification = × 4) were outlined with the size-standardized regions of interest (ROIs) by the Image J software (v.1.52a, NIH, USA), and the percentage of area with fluorescence, and cell count was quantified using this program. In particular, the threshold was set and standardized across images to maximize true protein expression signal for quantification; then, the total pixel number of target protein were recorded, and the percentage was calculated by dividing the pixel number with the total unfiltered pixel number in the ROI. Three sections for each brain region per immunostaining marker were averaged and analyzed.

Behavioral Testing:

Methods for tissue collection were done in similar matter to a previous published study in my lab (Locci et al., 2021; McClarty et al., 2021). Mice were acclimated to a soundproof behavioral testing room 30 min prior to testing, and assays were performed during the light part of the 12-h light/dark cycle. The order of the behavioral test was as follow: Novel object recognition (NOR), Y-maze, and Morris Water Maze (MWM). The behavioral tests and data analysis were conducted by one investigator that was blinded to age and treatment conditions.

Novel Object Recognition

Recognition memory was tested in an open plexiglass box (40 wide cm × 40 long cm × 40 high cm). Two sets of objects were used, and they were consistent in height and volume, but different in shape, color, and texture. Mice were individually habituated to the test arena for 10 min on each of the 3 days prior to data acquisition. On the first day of habituation, an open field test for mouse locomotor activity and anxiety behavior were recorded to ensure the mice had no severe impairments in locomotor function and anxiety behavior due to antipsychotic drug and CI-994 treatment. The experimental session consisted of 3 phases: acquisition trial (10 min), inter-trial interval (ITI; home-cage, 24 h), and retention trial (10 min). During acquisition, animals were recorded while exploring the arena with two glass, cylinder-shaped identical objects that are dark gray color (height of 8 cm and a diameter of 4 cm) placed diagonally across from each other. Following the ITI, during which one of the objects was replaced by a novel object that is plastic, prism-shaped with a yellow and blue color (6 cm length × 6.5 cm width × 6 cm height), in a counterbalanced manner, animals were placed back in the arena for the retention trial. The amount of time spent exploring each object during the acquisition and retention trial was scored

by an experimenter blinded to the condition using two milliseconds' stopwatches for precision. Exploration was defined as touching, leaning on the object, or orienting the head towards the object and sniffing within <1.0 cm for at least 20 s to make sure the sensorial perception was not impaired. Climbing on top of the object was not counted as exploration. Between each trial, the arena and the objects were cleaned with 70% alcohol to eliminate olfactory traces. The time spent with each object during the retention trial was used to calculate the discrimination index (DI), which represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects. To calculate DI, the total time spent exploring the novel object was subtracted by the total time spent exploring the familiar object, divided by the total time spent exploring during the retention trial. The formula used to calculate DI is as follows:

$$DI = \frac{(Time\ spent\ exploring\ novel\ object) - (Time\ spent\ exploring\ familair\ object)}{(Total\ time\ spent\ exploring)}$$

Y-maze

The Y-maze apparatus consisted of three-arms (5 cm wide × 21 cm long × 15.5 cm high) with three different special cues positioned in the top inner part of each arm. The apparatus was placed on a stable table with overhead video recording. Mice were placed in arm "A" (starting point, excluded from the analysis) facing the end of the arm and were allowed to freely explore the apparatus for 5 mins without investigator presence while a camera recorded their movements (Any-Maze, Stoelting, Wood Dale, IL). Spontaneous alternation was defined as discrete and successive entries into each open arm, including events where the animal directly progresses from one arm to the next in consecutive fashion (i.e., ABC, ACB, BAC, BCA, CAB, and CBA) without reentering the two previously visited arms. The spontaneous alternation percentage was

calculated by dividing the number of total successful alternations by the total number of possible alternations (i.e., the number of total entries minus two) multiplied by 100.

Morris Water Maze

In this study, we used a modified Morris water maze to test reference memory (Vorhees & Williams, 2006). Reference memory is a long-term memory that is associated with hippocampus function (Vorhees & Williams, 2006). Morris water maze is performed in a water tank with a moveable platform equipped with a video camera and computerized data analysis software (Any Maze). The water temperature was maintained at 23ºC. Prior to testing, the mice were acclimated to the Morris maze room for 30 minutes. Mice then underwent acquisition and test trails as described below. Upon completion of these trials, mice were removed from the tank and put into a drying cage. To assess reference memory, each mouse was tested for a total of 5 days with 4 trials/day(Vorhees & Williams, 2006). The interval between trials was1 hour each day. Day 1 consists of a training day during, which the mice were allowed to find a platform that protrudes above the water's surface. Then, the mice were trained to find a submerged platform. During the testing days (Days 2-5) the water was opaque, and the platform was submerged. For each trial, the mouse started at a different position for each trial (N, E, S, W), but the platform remained in the same location across the trials (Vorhees & Williams, 2006). Visual cues were placed on the N, E, and W facing walls for the mice to use for spatial reference. The swimming distance and time were used as indicators of spatial reference learning. We provided a maximum time of 90 seconds for the mice to find the platform. During the test days, measured the swimming distance and time to find the platform. On day 6 (probe trial), mice were probed for spatial reference memory. All mice had a start position in the complete opposite quadrant of the target quadrant,

and the platform was removed. The swimming distance, and total time spent in target quadrant were used as indicators of reference memory. Mice underwent only 1 trial for 60 seconds.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Multivariate Analysis of Variance (MANOVA) was used in our assessment of age, genotype, treatment, and age x genotype x treatment interaction effects, followed by a multiple comparisons analysis using Tukey's post hoc method. All MANOVAs were conducted using IBM SPSS Statistics software (Chicago, IL). Two-way ANOVA was used in our assessment of AD pathologies to detect age, treatment, and age x treatment interaction effects followed by a multiple comparison's analysis using Tukey's post hoc method. All two-way ANOVAs were conducted using the GraphPad Prism software (San Diego, CA, United States).

Results:

I. Chronic HDAC Inhibitor Treatment Rescues Synapse-Related Gene Expression in the Hippocampus and PFC of AD Mice.

Hippocampus

nr2a:

MANOVA revealed significant effects of age ($F_{2,144}$ = 76.780, $p < 0.001$, genotype ($F_{1,144}$ = 83.006, $p < 0.0001$), treatment ($F_{3,144}$ = 14.127, $p < 0.0001$), and an age × genotype x treatment inhibitor interaction ($F_{6,144}$ = 4.194, $p = 0.001$) on the *nr2a* mRNA expression in the hippocampus (**Figure 15**). Post-hoc analysis revealed significantly decreased *nr2a* mRNA expression in normal aging at 18 months of age (p =0.047). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *nr2a* mRNA expression as compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased *nr2a* mRNA expression (p = 0.009). After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased *nr2a* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated APP/PS1 mice (p < 0.0001, p < 0.0001). Both VPA and MS-275 did not show any effects on nr2a mRNA expression in WT and APP/PS1 mice.

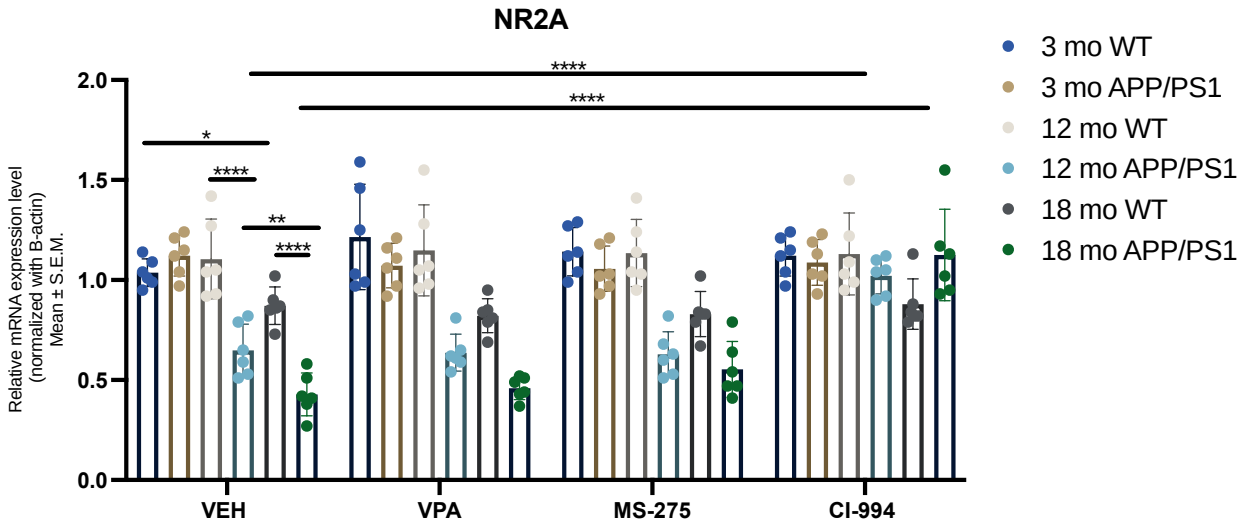

Figure 15. Chronic HDAC Inhibitor Impact on *nr2a* Gene Expression in the Hippocampus in Aging and AD. mRNA expression of nr2a. *nr2a* expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. CI-994 was the only chronic HDAC inhibitor treatment to significantly rescued *nr2a* expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *p < 0.05, **p < 0.01, ****p < 0.0001.

glur1:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 203.368, p < 0.0001, genotype ($F_{1,144}$ = 310.574, p < 0.0001), HDAC inhibitor ($F_{3,144}$ = 17.779, p < 0.0001), and an age × genotype x HDAC inhibitor interaction ($F_{6,144}$ = 2.426, p = 0.030) of the *glur1* mRNA expression in the hippocampus (**Figure 16**). Post-hoc analysis revealed significantly decreased levels of *glur1* mRNA expression in WT mice at 18 months of age (p < 0.0001). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased *glur1* mRNA expression compared to age-matched controls (p < 0.001, p < 0.001), and aging in APP/PS1 mice exacerbated the decreased *glur1* mRNA expression (p < 0.001). After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased *glur1* mRNA expression in both 12- and 18-month-old

APP/PS1 mice compared to VEH-treated mice ($p < 0.001$, $p < 0.001$). However, both VPA and MS-275 did not show any effects on *glur1* mRNA expression in WT and APP/PS1 mice.

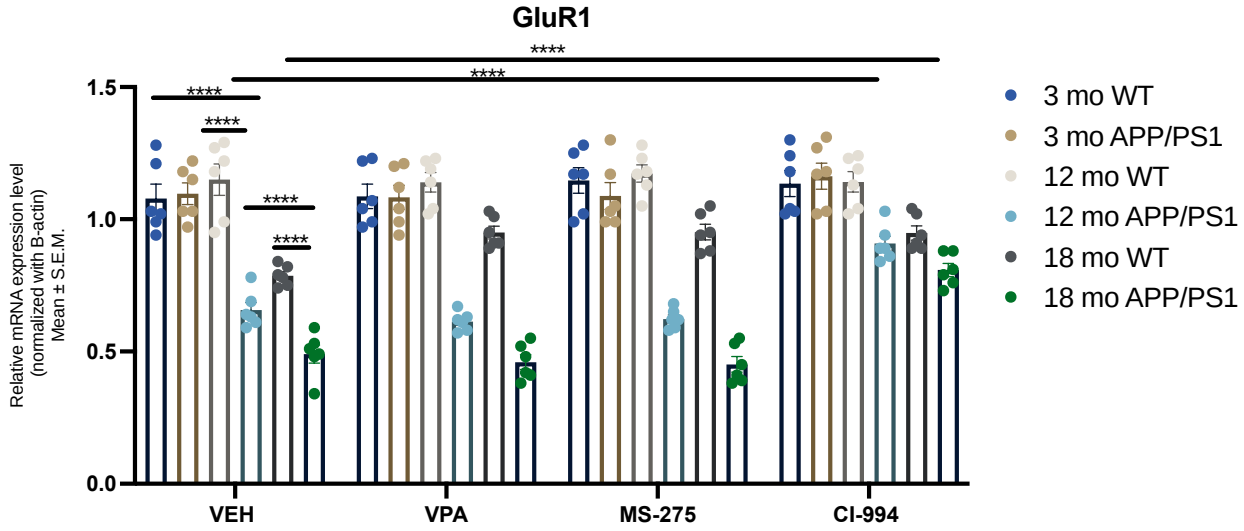

Figure 16. Chronic HDAC Inhibitor Impact on *glur1* Gene Expression in the Hippocampus in Aging and AD. mRNA expression of *glur1*. *glur1* expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. CI-994 was the only chronic HDAC inhibitor treatment to significantly rescued *glur1* expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ****p < 0.0001.

glur2:

MANOVA results revealed a significant effect of age ($F_{2,144} = 259.900$, $p < 0.001$, genotype ($F_{1,144} = 239.152$, $p < 0.001$), treatment ($F_{3,144} = 6.045$, $p = 0.001$), and an age × genotype x treatment interaction ($F_{6,144} = 3.179$, $p = 0.006$) of the *glur2* mRNA expression in the hippocampus (**Figure 17**). Post-hoc analysis revealed significantly decreased *glur2* mRNA expression in WT mice at 18 months of age ($p < 0.0001$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *glur2* mRNA expression compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the decreased *glur2* mRNA

expression (p = 0.002) in the hippocampus. After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased *glur2* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.0001, p < 0.0001). However, both VPA and MS-275 did not show any significant effects on *glur2* mRNA expression in WT and APP/PS1 mice.

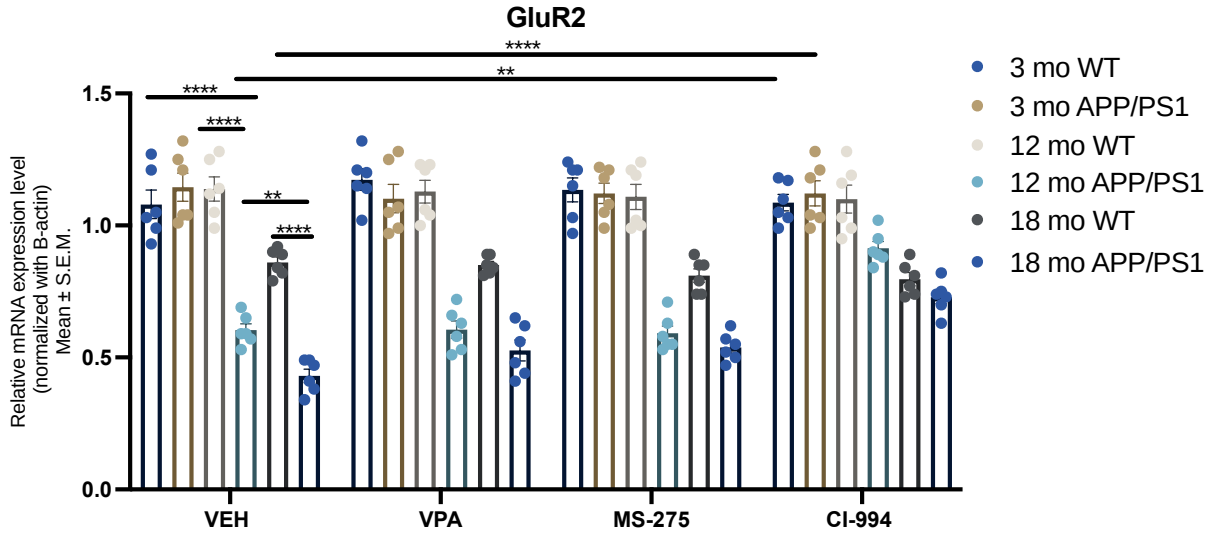

Figure 17. Chronic HDAC inhibitor Impact on *glur2* gene expression in the Hippocampus in Aging and AD. mRNA expression of *glur2*. *glur2* expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. CI-994 was the only chronic HDAC inhibitor treatment to significantly rescued *glur2* expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ****p < 0.0001

psd95:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 154.579, p < 0.001, genotype ($F_{1,144}$ = 76.364, p < 0.001), treatment ($F_{3,144}$ = 14.771, p < 0.001), and an age × genotype x treatment interaction ($F_{6,144}$ = 2.665, p = 0.018) of the *psd95* mRNA expression in the hippocampus (**Figure 18**). Post-hoc analysis revealed significantly decreased levels of *psd95*

mRNA expression in WT mice at 18 months of age ($p < 0.0001$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *psd95* mRNA expression compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the decreased *psd95* mRNA expression ($p = 0.001$) in the hippocampus. After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased *psd95* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p < 0.0001$). However, both VPA and MS-275 did not show any significant effects on *psd95* mRNA expression in WT and APP/PS1 mice.

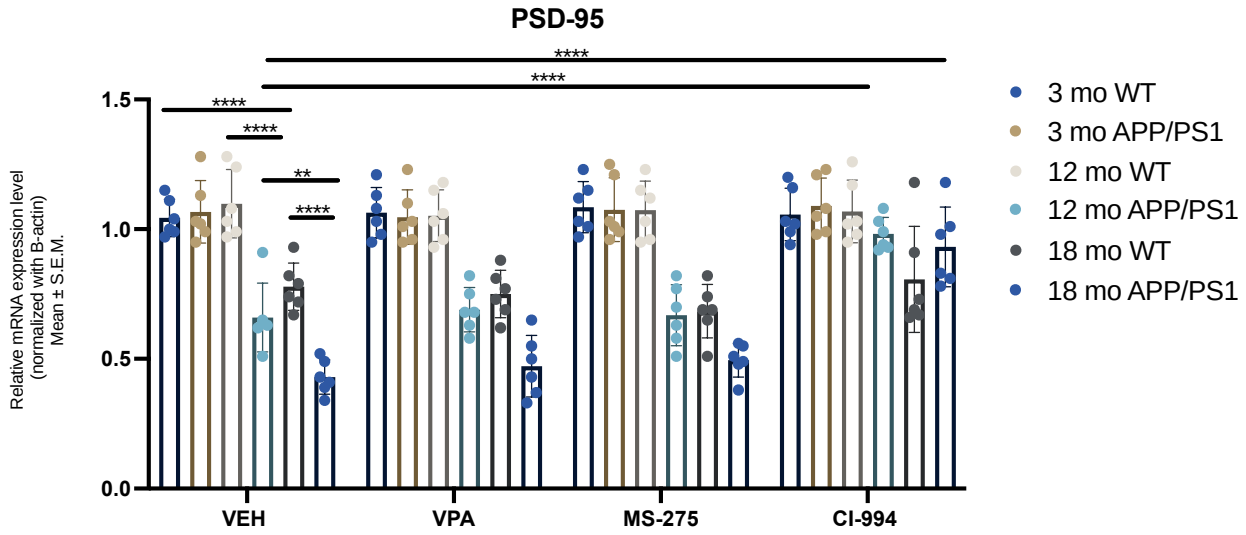

Figure 18. Chronic HDAC inhibitor Impact on *psd95* Gene Expression in the Hippocampus in Aging and AD. mRNA expression of *psd95*. *psd95* expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. CI-994 was the only chronic HDAC inhibitor treatment to significantly rescued *psd95* expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p < 0.01, ****p < 0.0001.

Prefrontal Cortex (PFC)

nr2a:

MANOVA revealed a significant effect of age ($F_{2,144}$ = 169.927, p < 0.001, genotype ($F_{1,144}$ = 43.866, p < 0.001), treatment ($F_{3,144}$ = 11.500, p < 0.001), and an age × genotype x treatment interaction ($F_{6,144}$ = 3.908, p = 0.001) of the *nr2a* mRNA expression in the PFC (**Figure 19**). Post-hoc analysis revealed significantly decreased *nr2a* mRNA expression in WT mice at 18 months of age (p < 0.0001). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *nr2a* mRNA compared to age-matched controls (p < 0.001, p < 0.001), and aging in APP/PS1 mice exacerbated the decreased *nr2a* mRNA expression (p < 0.001) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased *nr2a* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.001, p < 0.001). However, both VPA and CI-994 did not show any significant effects on *nr2a* mRNA expression in WT and APP/PS1 mice.

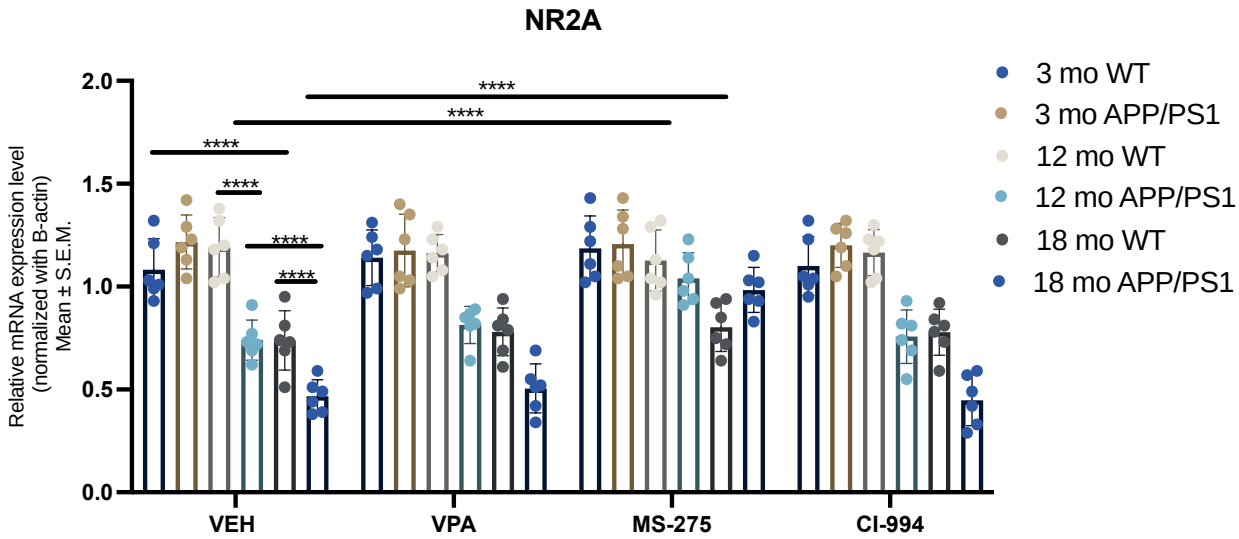

Figure 19. Chronic HDAC inhibitor Impact on *nr2a* Gene Expression in the PFC in Aging and AD. mRNA expression of NR2A. NR2A expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment that significantly rescued NR2A expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ****p < 0.0001.

glur1:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 207.972, $p < 0.0001$, genotype ($F_{1,144}$ = 114.907, $p < 0.0001$), treatment ($F_{3,144}$ = 21.986, $p < 0.0001$), and an age × genotype x treatment interaction ($F_{6,144}$ = 8.923, $p < 0.0001$) of the *glur1* mRNA expression in the PFC (**Figure 20**). Post-hoc analysis revealed significantly decreased *glur1* mRNA expression in WT mice at 18 months of age (p = 0.005). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *glur1* mRNA expression compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased *glur1* mRNA expression (p = 0.002) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased *glur1* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.0001, p < 0.0001). However, both VPA and CI-994 did not show any significant effects on *glur1* mRNA expression in WT and APP/PS1 mice.

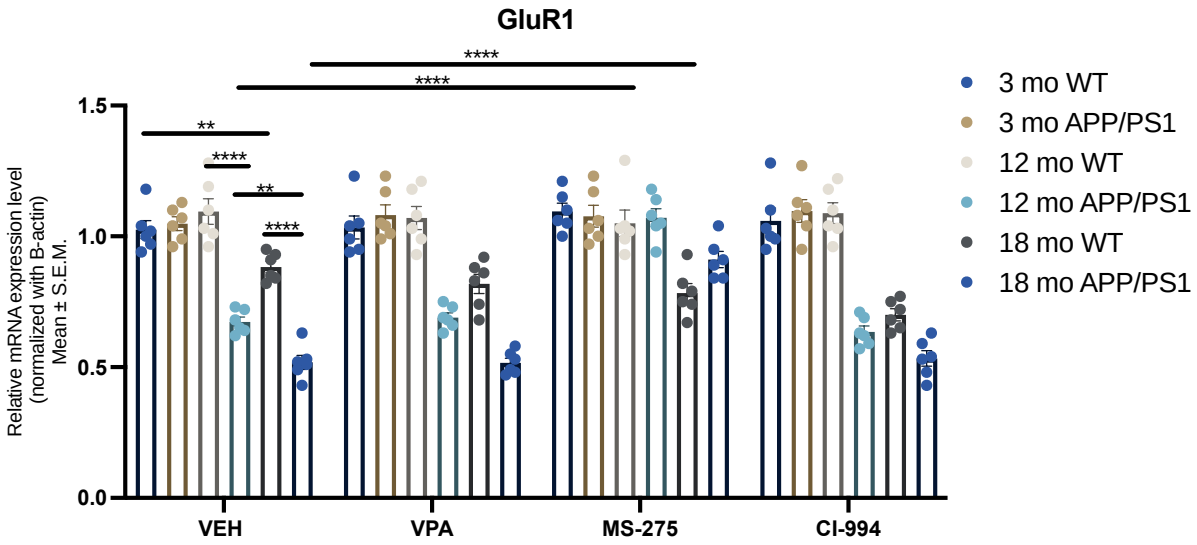

Figure 20. Chronic HDAC inhibitor Impact on *glur1* Gene Expression in the PFC in Aging and AD. mRNA expression of GluR1. GluR1 expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment that significantly rescued GluR1 expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p<0.01, ****p<0.0001.

glur2:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 200.183, p < 0.0001, genotype ($F_{1,144}$ = 123.136, p < 0.0001), treatment ($F_{3,144}$ = 15.525, p < 0.0001), and an age × genotype x treatment interaction ($F_{6,144}$ = 5.348, p < 0.0001) of the *glur2* mRNA expression in the PFC

(**Figure 21**). Post-hoc analysis revealed significantly decreased levels of *glur2* mRNA expression in WT mice at 18 months of age ($p = 0.001$). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *glur2* mRNA expression compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the decreased *glur2* mRNA expression ($p = 0.002$) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased *glur2* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p < 0.0001$). However, both VPA and CI-994 did not show any significant effects on *glur2* mRNA expression in WT and APP/PS1 mice.

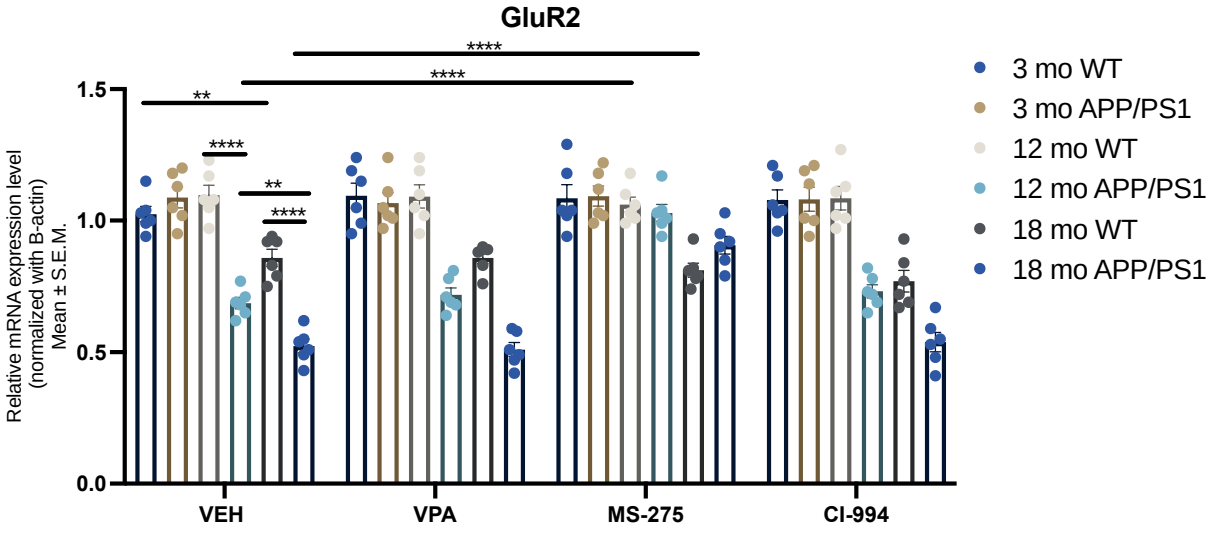

Figure 21. Chronic HDAC inhibitor Impact on *glur2* Gene Expression in the PFC in Aging and AD. mRNA expression of GluR2. GluR2 expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued GluR2 expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p<0.01, ****p<0.0001.

psd95:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 174.003, p < 0.0001, genotype ($F_{1,144}$ = 74.961, p < 0.0001), treatment ($F_{3,144}$ = 17.871, p < 0.0001), and an age × genotype x treatment interaction ($F_{6,144}$ = 5.388, p < 0.0001) of the *psd95* mRNA expression in the PFC (**Figure 22**). Post-hoc analysis revealed significantly decreased *psd95* mRNA expression in WT mice at 18 months of age (p < 0.0001). Both 12- and 18-month-old APP/PS1 mice showed significantly increased *psd95* mRNA expression compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased *psd95* mRNA expression (p < 0.0001) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased *psd95* mRNA expression in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.0001, p < 0.0001). However, both VPA and CI-994 did not show any significant effects on *psd95* mRNA expression in WT and APP/PS1 mice.

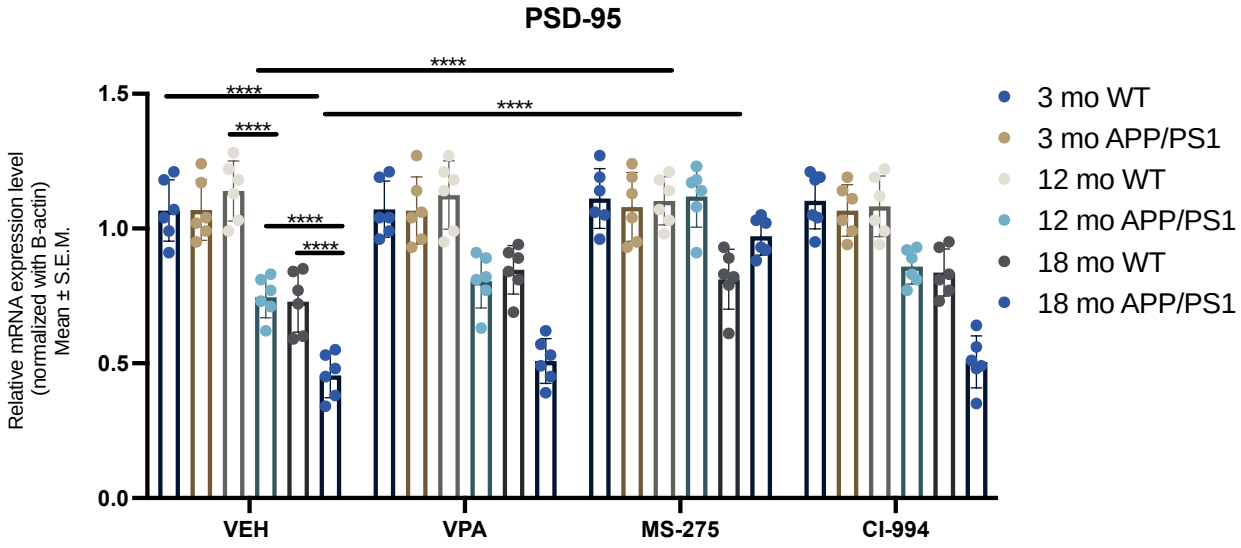

Figure 22. Chronic HDAC inhibitor Impact on *psd95* Gene Expression in the PFC in Aging and AD. mRNA expression of PSD95. PSD95 expression was downregulated in normal aging and AD; however, all genes showed the most significant downregulation in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued PSD95 expression in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ****p < 0.0001.

II. Chronic HDAC Inhibitor Treatment Rescues H3K9ac at Synapse-Related Gene Promoters in the Hippocampus and PFC of AD Mice

Previous results displayed multiple synapse-related gene expression levels were rescued after HDAC inhibitor treatment. To understand whether such changes are being regulated through histone acetylation levels at synapse-related gene promoters, H3K9ac levels were measured at synapse-related gene promoters in the hippocampus and PFC in aging and AD.

Hippocampus:

nr2a:

MANOVA results revealed a significant effect of age ($F_{2,144} = 283.467$, $p < 0.0001$, genotype ($F_{1,144} = 202.008$, $p < 0.0001$), treatment ($F_{3,144} = 14.077$, $p < 0.0001$), and an age × genotype x treatment interaction ($F_{6,144} = 2.181$, $p = 0.049$) on the H3K9ac levels at the *nr2a* gene promoter in the hippocampus (**Figure 23**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *nr2a* promoter in WT mice at 18 months of age ($p < 0.0001$). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *nr2a* promoter compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice did not exacerbate the decreased H3K9ac levels at the *nr2a* promoter, however, a trend was seen ($p = 0.070$). After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased H3K9ac levels at the *nr2a* promoter in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p < 0.0001$). However, both VPA and MS-275 did not show any effects on H3K9ac levels at the *nr2a* promoter in WT and APP/PS1 mice.

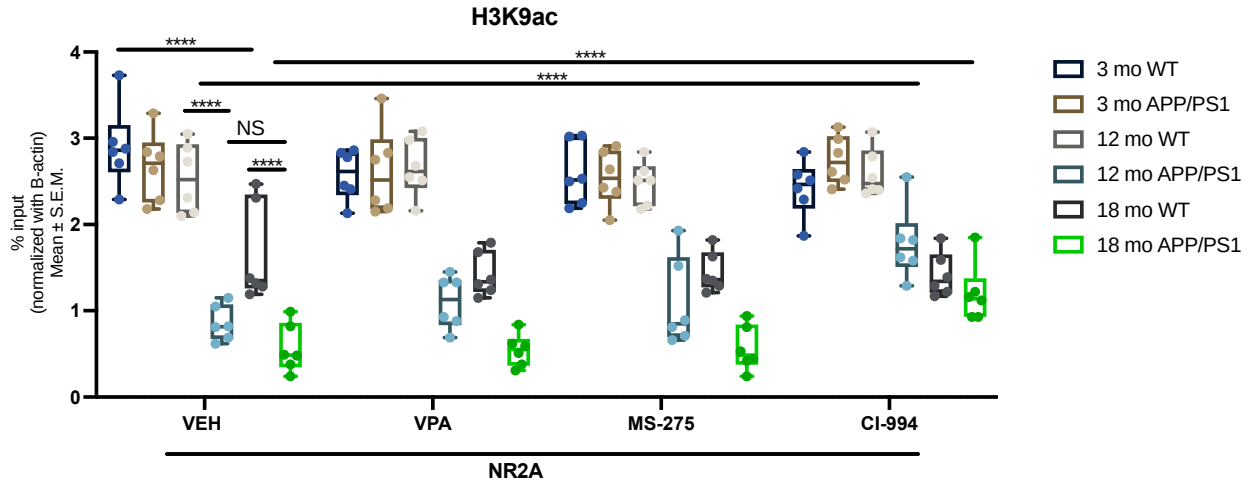

Figure 23. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *nr2a* Gene Promoter in the Hippocampus in Aging and AD. H3K9ac Levels at the *nr2a* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ****p < 0.0001.

glur1:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 156.332, p < 0.0001, genotype ($F_{1,144}$ = 45.630, p < 0.0001), treatment ($F_{3,144}$ = 7.218, p < 0.0001), and an age × genotype x treatment interaction ($F_{6,144}$ = 2.418, p = 0.031) of the H3K9ac levels at the *glur1* gene promoter in the hippocampus (**Figure 24**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *glur1* promoter in WT mice at 18 months of age (p < 0.0001). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur1* promoter compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *glur1* promoter (p < 0.0001). After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased H3K9ac levels at the *glur1* promoter in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p

< 0.0001, p < 0.0001). However, both VPA and MS-275 did not show any effects on H3K9ac levels at the *glur1* promoter in WT and APP/PS1 mice.

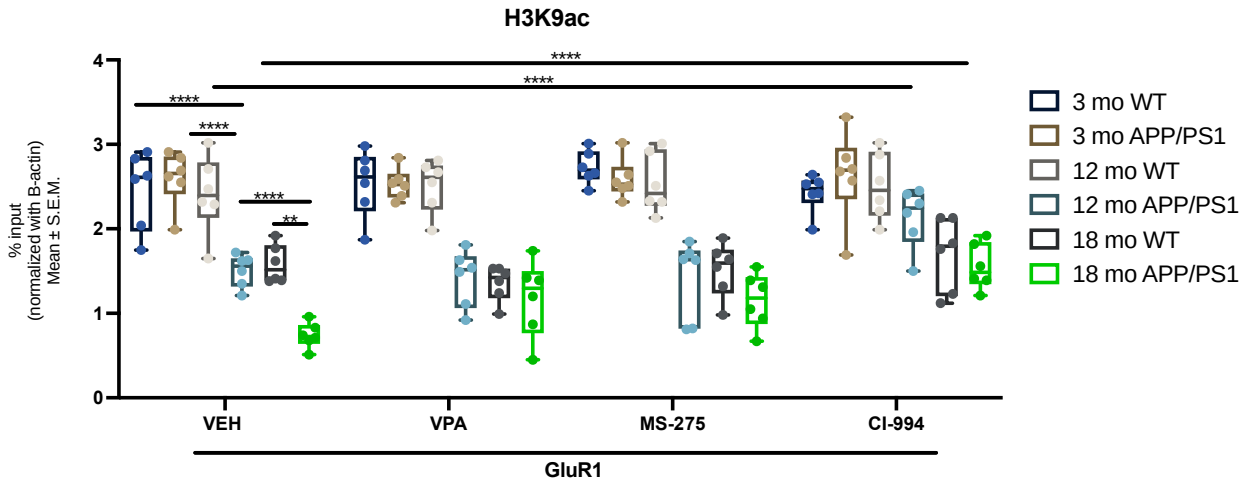

Figure 24. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *glur1* Gene Promoter in the Hippocampus in Aging and AD. H3K9ac Levels at the *glur1* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p < 0.01, ****p < 0.0001.

glur2:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 57.234, p < 0.001, genotype ($F_{1,144}$ = 117.441, p < 0.001), treatment ($F_{3,144}$ = 4.172, p = 0.008), and an age × genotype x treatment interaction ($F_{6,144}$ = 3.313, p = 0.005) of the H3K9ac levels at the *glur2* gene promoter in the hippocampus (**Figure 25**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *glur2* promoter in WT mice at 18 months of age (p = 0.021). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur2* promoter compared to age-matched controls (p < 0.001, p < 0.001), and aging in APP/PS1 mice

exacerbated the decreased H3K9ac levels at the *glur2* promoter ($p = 0.004$). After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased H3K9ac levels at the *glur2* promoter in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.026$, $p < 0.001$). However, both VPA and MS-275 did not show any effects on H3K9ac levels at the *glur2* promoter in WT and APP/PS1 mice.

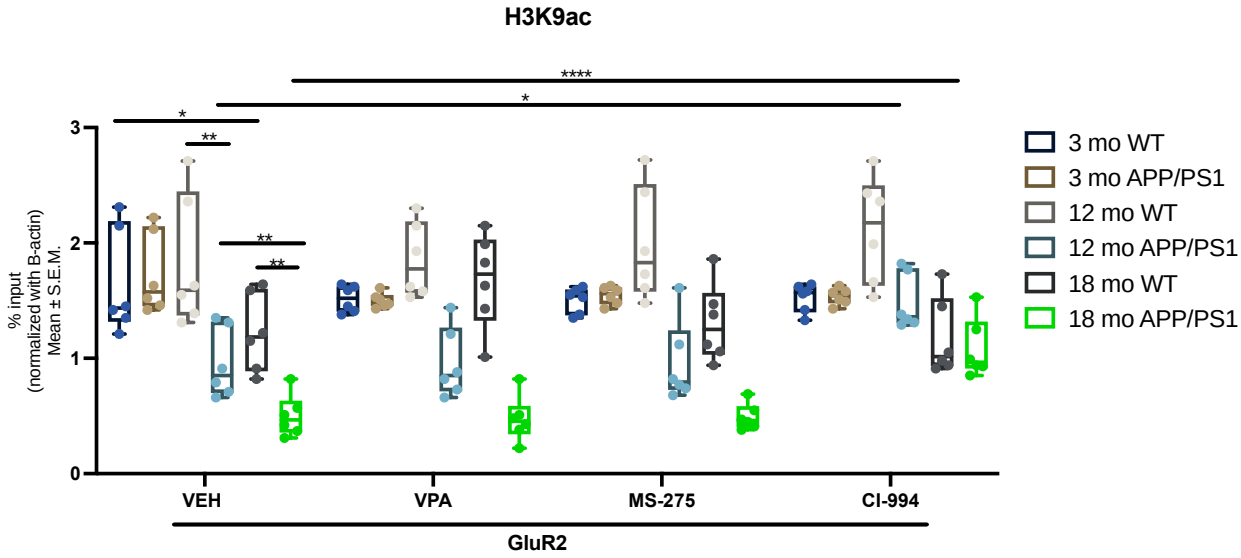

Figure 25. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *glur2* Gene Promoter in the Hippocampus in Aging and AD. H3K9ac Levels at the *glur2* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *$p < 0.05$, **$p < 0.01$,****$p < 0.0001$.

psd95:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 134.852, p < 0.0001, genotype ($F_{1,144}$ = 129.471, p < 0.0001), treatment ($F_{3,144}$ = 17.169, p < 0.0001), and an age × genotype x treatment interaction ($F_{6,144}$ = 2.405, p = 0.031) of the H3K9ac levels at the *psd95* gene promoter in the hippocampus (**Figure 26**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *psd95* promoter in WT mice at 18 months of age (p = 0.001). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *psd95* promoter compared to age-matched controls (p < 0.001, p < 0.001), and aging in APP/PS1 mice did not exacerbate the decreased H3K9ac levels at the *psd95* promoter. After chronic HDAC inhibitor treatment, only CI-994 showed significantly increased H3K9ac levels at the *psd95* promoter in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.0001, p < 0.0001). However, both VPA and MS-275 did not show any effects on H3K9ac levels at the *psd95* promoter in WT and APP/PS1 mice.

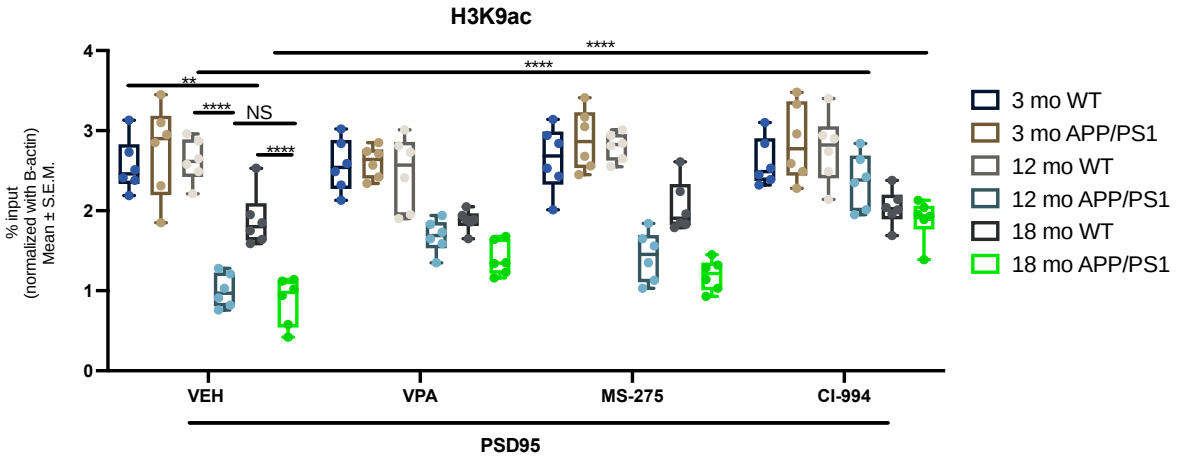

Figure 26. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *psd95* Gene Promoter in the Hippocampus in Aging and AD. H3K9ac Levels at the *psd95* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *p<0.05, **p<0.01, ***p<0.001.

Prefrontal Cortex (PFC)

nr2a:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 107.501, p < 0.0001, genotype ($F_{1,144}$ = 76.076, p < 0.0001), treatment ($F_{3,144}$ = 11.314, p < 0.0001), and an age × genotype x treatment interaction ($F_{6,144}$ = 2.242, p = 0.044) of the H3K9ac levels at the *nr2a* gene promoter in the PFC (**Figure 27**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *nr2a* in 18-month-old WT mice (p = 0.021). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *nr2a* promoter compared to age-matched controls (p < 0.0010, p < 0.0001) and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *nr2a* gene promoter (p = 0.0004) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased H3K9ac levels at the *nr2a* in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p < 0.0001, p < 0.0001). However, both VPA and CI-994 did not show any effects on H3K9ac levels at the *nr2a* promoter in WT and APP/PS1 mice.

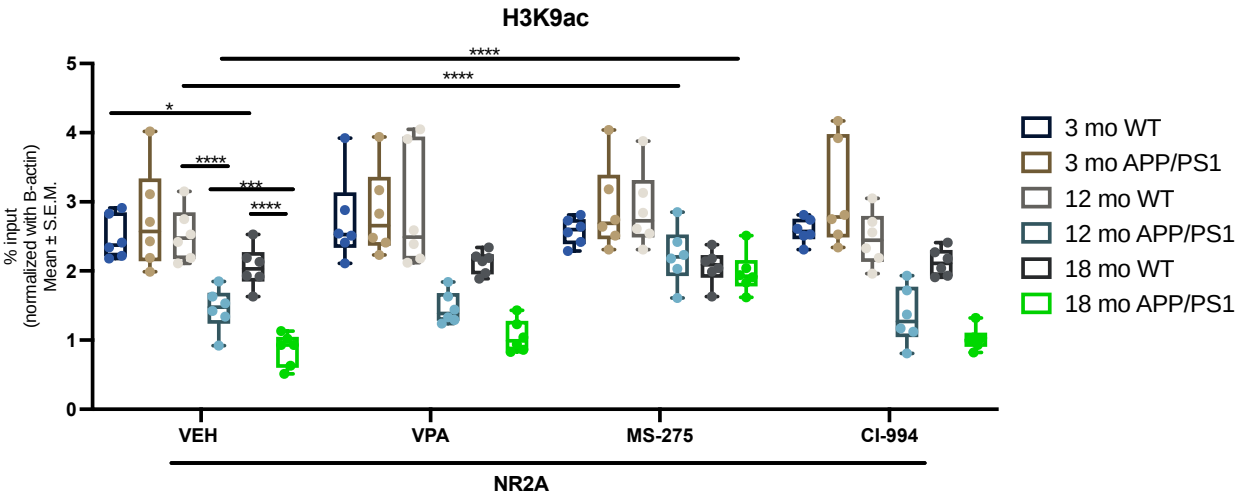

Figure 27. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *nr2a* Gene Promoter in the PFC in Aging and AD. H3K9ac Levels at the *nr2a* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *p < 0.05, ***p < 0.001, ****p < 0.0001

glur1:

MANOVA results revealed a significant effect of age ($F_{2,144} = 89.852$, $p < 0.001$, genotype ($F_{1,144} = 204.029$, $p < 0.001$), treatment ($F_{3,144} = 8.555$, $p < 0.001$), and an age × genotype x treatment interaction ($F_{6,144} = 3.081$, $p = 0.008$) of the H3K9ac levels at the *glur1* gene promoter in the PFC (**Figure 28**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *glur1* in 18-month-old WT mice (p = 0.001). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur1* promoter compared to age-matched controls (p < 0.0001, p < 0.0001) and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *glur1* gene promoter (p = 0.012) in the PFC. After chronic HDAC inhibitor treatment, only MS-275 showed significantly increased H3K9ac levels at the *glur1* in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p = 0.0001, p = 0.0001). However, both VPA and CI-994 did not show any effects on H3K9ac levels at the *glur1* promoter in WT and APP/PS1 mice.

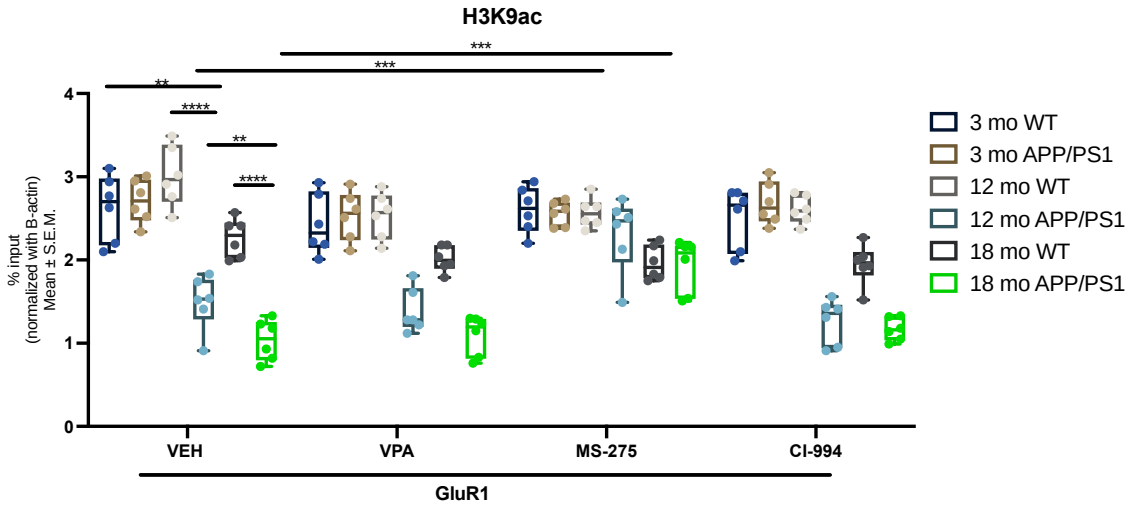

Figure 28. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *glur1* Gene Promoter in the PFC in Aging and AD. H3K9ac Levels at the *glur1*gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. MS-275 was the only chronic HDAC inhibitor treatment to significantly rescued H3K9ac levels in 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *p < 0.05, **p < 0.01, ****p< 0.0001.

glur2:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 166.161, p < 0.0091, genotype ($F_{1,144}$ = 163.161, p < 0.0001), treatment ($F_{3,144}$ = 0.228, p = 0.887), and an age × genotype x treatment interaction ($F_{6,144}$ = 0.113, p = 0.995) of the H3K9ac levels at the *glur2* gene promoter in the PFC (**Figure 29**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *glur2* in 18-month-old WT mice (p = 0.004). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *glur2* promoter compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *glur2* gene promoter (p < 0.0001) in the PFC. After chronic

HDAC inhibitor treatment, no treatment showed a significant effect of H3K9ac levels at the *glur2* in APP/PS1 and WT mice.

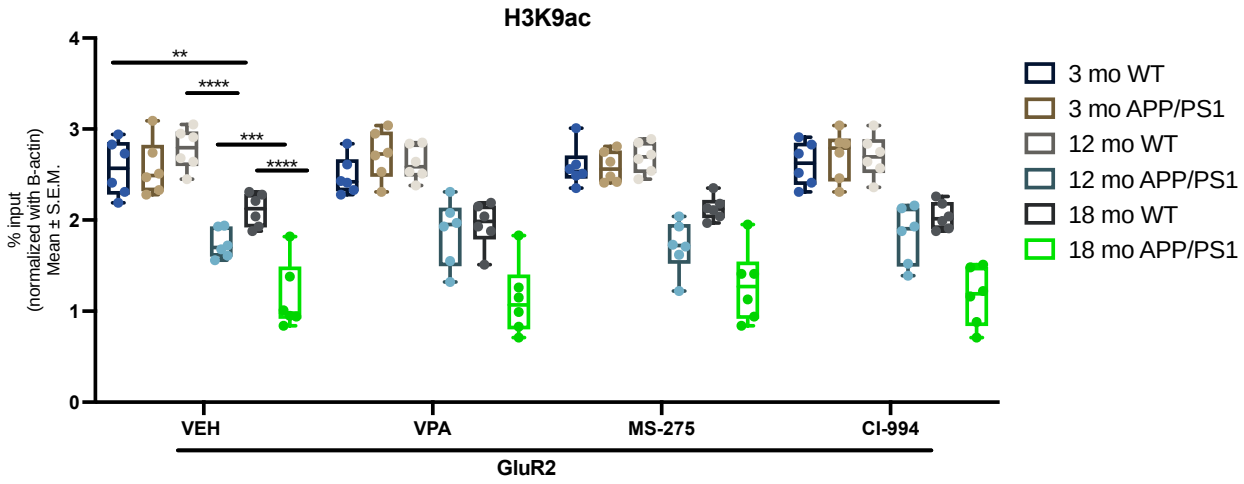

Figure 29. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *glur2* Gene Promoter in the PFC in Aging and AD. H3K9ac Levels at the *glur2* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. No chronic HDAC inhibitor treatment had an effect on H3K9ac levels. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p < 0.01, ****p < 0.0001.

psd95:

MANOVA results revealed a significant effect of age ($F_{2,144}$ = 67.620, p < 0.0001, genotype ($F_{1,144}$ = 77.994, p < 0.0001), treatment ($F_{3,144}$ = 6.116, p = 0.001), and an age × genotype x treatment interaction ($F_{6,144}$ = 2.255, p = 0.043) of the H3K9ac levels at the *psd95* gene promoter in the PFC (**Figure 30**). Post-hoc analysis revealed significantly decreased levels of H3K9ac levels at the *psd95* in 18-month-old WT mice (p = 0.010). Both 12- and 18-month-old APP/PS1 mice showed significantly decreased H3K9ac levels at the *psd95* promoter compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice exacerbated the decreased H3K9ac levels at the *psd95* gene promoter (p = 0.040) in the PFC. After chronic

HDAC inhibitor treatment, only MS-275 showed significantly increased H3K9ac levels at the *psd95* in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p = 0.001$). However, both VPA and CI-994 did not show any effects on H3K9ac levels at the *psd95* promoter in WT and APP/PS1 mice.

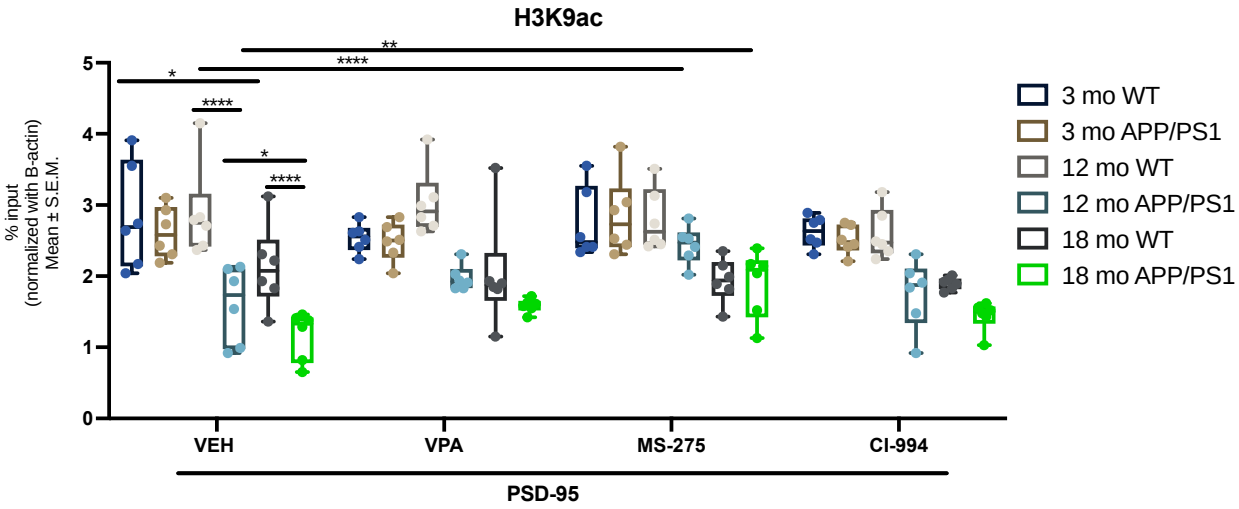

Figure 30. Chronic HDAC inhibitor Impact on H3K9ac Levels at the *psd95* Gene Promoter in the PFC in Aging and AD. H3K9ac Levels at the *psd95* gene promoter. H3K9ac levels were decreased in normal aging and AD; however, the most significant decline of H3K9ac levels was found in aged APP/PS1 mice. Chronic MS-275 treatment significantly rescued H3K9ac levels in 12-and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

III. Chronic MS-275 Treatment Reduces Amyloidosis During AD Progression in the Hippocampus and PFC

To evaluate amyloidosis, Aβ plaque burden was measured by immunostaining of 3-, 12- and 18-month-old APP/PS1 hippocampal and PFC slices. Additionally, soluble $A\beta_{42}$ levels were measured by ELISA in the hippocampus and PFC lysates.

Two-way ANOVA revealed a significant effect of age ($F_{2,48} = 190.9$, $p < 0.0001$, treatment ($F_{3,48} = 15.12$, $p < 0.0001$), and an age × treatment interaction ($F_{6,48} = 4.495$, $p = 0.0011$) of the % area of Aβ plaques in the hippocampus (**Figure 31A,B**). Post-hoc analysis revealed a significantly increased Aβ plaque load in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice ($p < 0.0001$, $p < 0.0001$). However, no significant differences were found in Aβ plaque load between 12- and 18-month-old APP/PS1 mice ($p = 0.9987$). After chronic HDAC inhibitor treatment, only MS-275 showed a significant reduction in Aβ plaque load in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated APP/PS1 mice ($p = 0.0273$, $p = 0.0008$). Chronic treatment of VPA and CI-994 did not show any significant reductions on Aβ plaque load in the hippocampus. In terms of $A\beta_{42}$ levels, two-way ANOVA revealed a significant effect of age ($F_{2,36} = 96.39$, $p < 0.0001$, treatment ($F_{3,36} = 15.96$, $p < 0.0001$), and an age × treatment interaction ($F_{6,36} = 4.633$, $p = 0.0014$) on $A\beta_{42}$ levels in the hippocampus (**Figure 31C**). Post-hoc analysis revealed significantly increased $A\beta_{42}$ levels in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice ($p < 0.0001$, $p < 0.0001$). However, no significant differences were found between $A\beta_{42}$ levels between 12- and 18-month-old APP/PS1 mice ($p > 0.9999$). After chronic HDAC inhibitor treatment, only MS-

275 showed a significant reduction in Aβ$_{42}$ levels in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice APP/PS1($p < 0.0001$, $p < 0.0001$). Both VPA and CI-994 did not show any significant reductions in Aβ$_{42}$ levels across AD progression in the hippocampus.

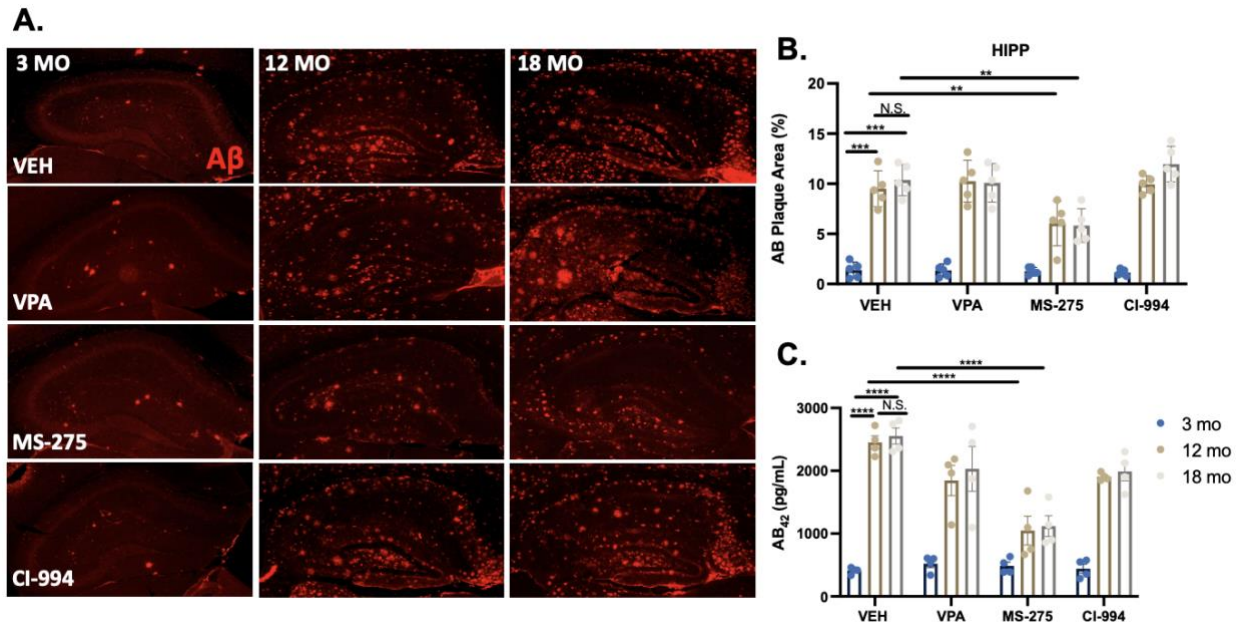

Figure 31. Chronic HDAC inhibitor Impact on Amyloidosis in the Hippocampus throughout AD Progression. A: Immunostaining of Aβ in the hippocampus in AD progression. **B:** Quantification of Aβ immunostaining. Chronic MS-275 treatment significantly reduced AB plaque area. **C:** Aβ$_{42}$ ELISA in the hippocampus in AD progression. Chronic MS-275 treatment significantly reduced Aβ$_{42}$ levels. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. **p < 0.01, ***p < 0.001.

In addition to the hippocampus, Aβ plaque burden and Aβ$_{42}$ levels were measured in the PFC of 3,12-, and 18-month-old APP/PS1 mice. Two-way ANOVA revealed a significant effect of age ($F_{2,48} = 201.6$, $p < 0.0001$, treatment ($F_{3,48} = 18.55$, $p < 0.0001$), and an age × treatment interaction ($F_{6,48} = 5.551$, $p = 0.0002$) of the % area of Aβ plaques in the PFC (**Figure 32A,B**). Post-hoc analysis revealed a significantly increased Aβ plaque load in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001).

However, no significant differences were found in Aβ plaque load between 12- and 18-month-old APP/PS1 mice (p = 0.1027). After chronic HDAC inhibitor treatment, MS-275 showed a significant reduction in Aβ plaque load in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p = 0.0020, p < 0.0001). In terms of $A\beta_{42}$ levels, two-way ANOVA revealed a significant effect of age ($F_{2,36} = 205.0$, $p < 0.0001$, treatment ($F_{3,36} = 16.74$, $p < 0.0001$), and an age × treatment interaction ($F_{6,36} = 6.675$, $p < 0.0001$) of $A\beta_{42}$ levels in the PFC (**Figure 32C**). Post-hoc analysis revealed significantly increased $A\beta_{42}$ levels in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001), and a significant difference of $A\beta_{42}$ levels were found between 12- and 18-month-old APP/PS1 mice (p = 0.0402). After chronic HDAC inhibitor treatment, only MS-275 showed a significant reduction in $A\beta_{42}$ levels in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice (p = 0.0285, p < 0.0001). Both VPA and CI-994 did not show any significant reductions in $A\beta_{42}$ levels across AD progression in the PFC.

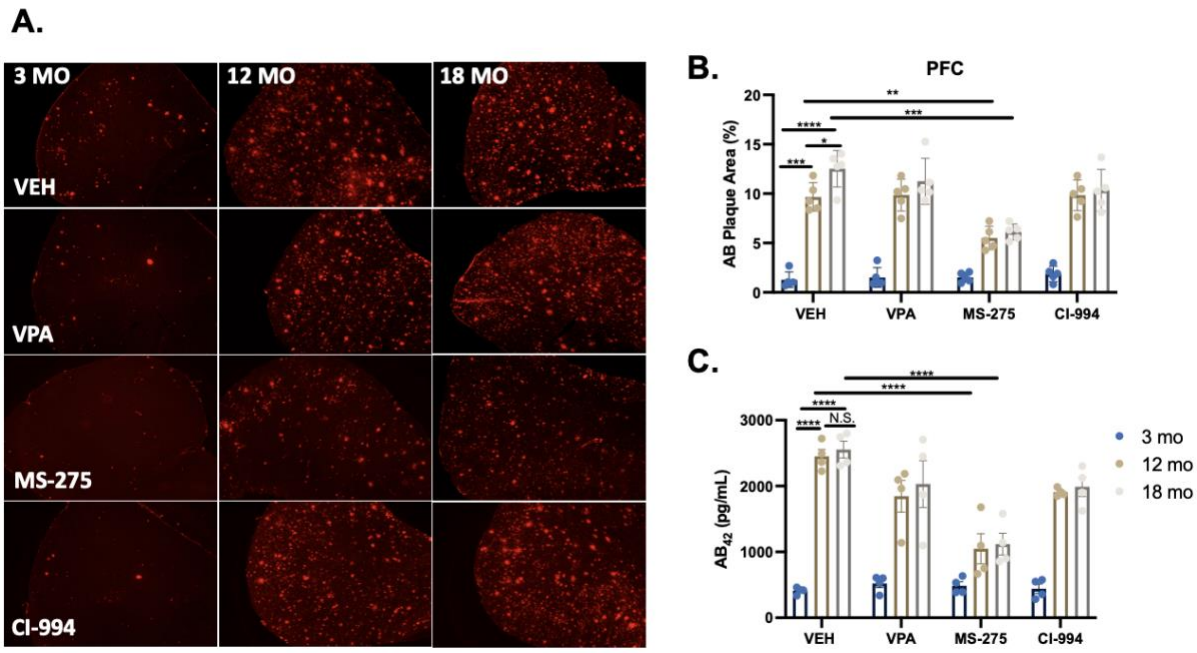

Figure 32. Chronic HDAC inhibitor Impact on Amyloidosis in the PFC throughout AD Progression. A: Immunostaining of Aβ in the PFC in AD progression. **B:** Quantification of Aβ immunostaining. Chronic MS-275 treatment significantly reduced Aβ plaque area. **C:** $A\beta_{42}$ ELISA in the PFC in AD progression. Chronic MS-275 treatment significantly reduced $A\beta_{42}$ levels. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. **p < 0.01, ***p < 0.001.

Given both amyloid plaque burden and $A\beta_{42}$ levels were significantly reduced after chronic MS-275 treatment, the next steps were to identify potential mechanisms regulating the changes in amyloidosis. Given APP processing is the initial step in the formation of Aβ, it was necessary to further investigate the amyloidogenic and non-amyloidogenic pathways of APP processing. First, the global mRNA expression of ADAM-10 was measured (**Figure 33**). ADAM-10 is a major alpha protease in neurons responsible for cleaving APP and results in the promotion of non-amyloidogenic pathway processing. An increase in ADAM-10 activity can shift the balance of APP processing and decrease Aβ plaque burden (Li et al., 2014; Yuan et al., 2017; Zhang et al., 2010). As expected, two-way ANOVA revealed a significant effect of age ($F_{2,60} = 185.3$, $p < 0.0001$), but no treatment ($F_{3,60} = 1.196$, $p = 0.3189$), and no age × treatment interaction ($F_{6,48} = 1.481$, $p = 0.2000$) was found for the expression of ADAM-10 in the hippocampus (**Figure 33A**). Post-hoc analysis revealed significantly decreased ADAM-10 mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice ($p < 0.0001$, $p < 0.0001$), however, no significant differences were found of ADAM-10 expression between 12- and 18-month-old APP/PS1 mice ($p > 0.9999$). Chronic HDAC inhibitor treatment did not affect ADAM-10 mRNA expression when compared to 12- and 18-month-old APP/PS1 VEH-treated mice in the hippocampus.

For the PFC, two-way ANOVA revealed a significant effect of age ($F_{2,60}$ = 200.1, p < 0.0001), but no effect of treatment ($F_{3,60}$ = 1.410, p = 0.2487), and no age × treatment interaction ($F_{6,48}$ = 0.5221, p = 0.7893) of the expression of ADAM-10 in the PFC (**Figure 33B**). Post-hoc analysis revealed significantly decreased ADAM-10 mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001), however, no significant differences were found in ADAM-10 expression between 12- and 18-month-old APP/PS1 mice (p = 0.9993). Chronic HDAC inhibitor treatment did not affect ADAM-10 mRNA expression when compared to 12- and 18-month-old APP/PS1 VEH-treated mice in the PFC.

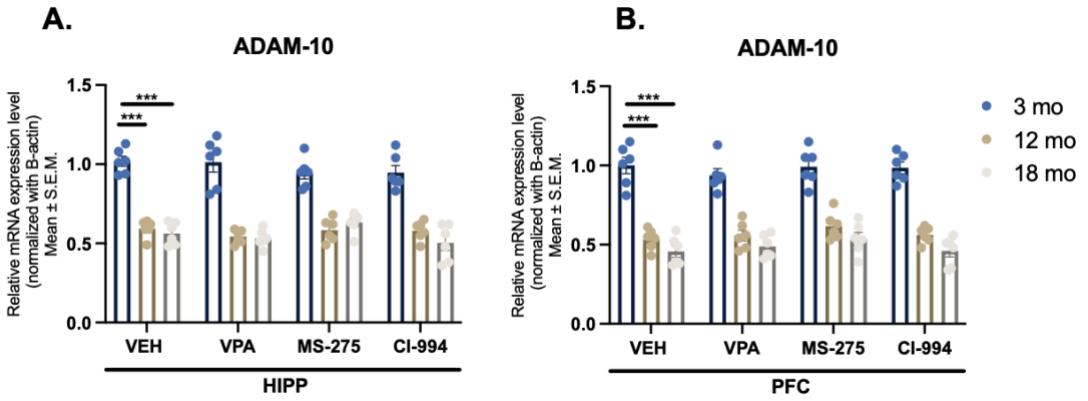

Figure 33. Chronic HDAC inhibitor Impact on Non-Amyloidogenic Processing in the Hippocampus and PFC throughout AD Progression. A: mRNA expression of ADAM-10 in the hippocampus. Chronic HDAC inhibitor treatment had no impact on ADAM-10 mRNA expression. **B:** mRNA expression of ADAM-10 in the PFC. Chronic HDAC inhibitor treatment had no impact on ADAM-10 mRNA expression. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. ***p < 0.001.

Conversely, BACE-1 is an enzyme that plays a key role in amyloidogenic processing and is essential for the generation of Aβ. Changes in BACE-1 activity can shift the balance of APP processing and increase Aβ plaque burden (Hussain et al., 2007; Miners et al., 2011; Vassar,

2004). Therefore, global BACE-1 mRNA expression levels were measured after chronic HDAC inhibitor treatment. In the hippocampus, two-way ANOVA revealed a significant effect of age ($F_{2,60}$ = 28.63, p < 0.0001), but no significant effect of treatment ($F_{3,60}$ = 0.07230, p = 0.9746), and no age × treatment interaction ($F_{6,60}$ = 1.194, p = 0.3217) of the expression of BACE-1 in the hippocampus (**Figure 34A**). Post-hoc analysis revealed significantly increased BACE-1 mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p = 0.0002, p = 0.0001), however, no significant differences were found in BACE-1 expression between 12- and 18-month-old APP/PS1 mice (p > 0.9999). Chronic HDAC inhibitor-treated mice showed no significant impact on BACE-1 mRNA expression when compared to VEH-treated mice in the hippocampus. In the PFC, after chronic HDAC inhibitor treatment, two-way ANOVA revealed a significant effect of age ($F_{2,60}$ = 66.37, p < 0.0001), but no significant effect of treatment ($F_{3,60}$ = 0.2105, p = 0.8887), and no age × treatment interaction ($F_{6,48}$ = 0.7189, p = 0.6359) of the expression of BACE-1 in the PFC (**Figure 34B**). Post-hoc analysis revealed a significant increase of BACE-1 mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p = 0.0483, p = 0.0021), however, no significant increase of BACE-1 mRNA expression was seen between 12- and 18-month-old APP/PS1 (p = 0.9961). Chronic HDAC inhibitor treated mice showed no significant impact on BACE-1 mRNA expression when compared to VEH treated mice in the PFC.

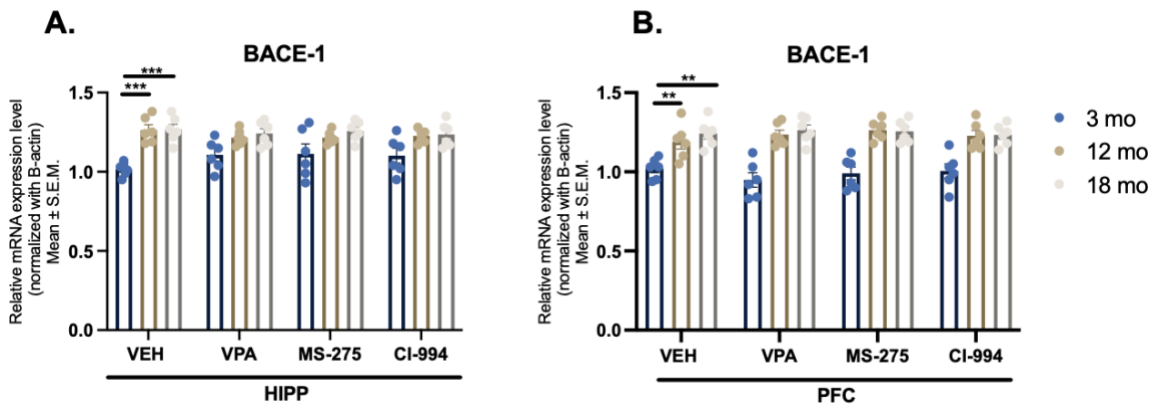

Figure 34. Chronic HDAC inhibitor Impact on Amyloidogenic Processing in the Hippocampus and PFC throughout AD Progression. A: mRNA expression of BACE-1 in the hippocampus. Chronic HDAC inhibitor treatment had no impact on BACE-1 mRNA expression. **B:** mRNA expression of BACE-1 in the PFC. Chronic HDAC inhibitor treatment had no impact on BACE-1 mRNA expression. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. ***p < 0.001.

Given the results showed no changes in APP processing, both in amyloidogenic and non-amyloidogenic pathways, investigation of mRNA expression of Aβ degrading enzymes in aging and AD was needed to help understand changes in amyloidosis. Nephrylsin (NEP) and Insulin Degradation Enzyme (IDE) are enzymes known to degrade Aβ, and are downregulated during AD progression (Numata & Kaplan, 2010; Wang et al., 2006). Therefore, the goal was to know how NEP regulates amyloid in the APP/PS1 mouse model, and whether chronic HDAC inhibitor treatment could impact NEP gene expression (**Figure 35**). Two-way ANOVA revealed a significant effect of age ($F_{2,60}$ = 184.1, p < 0.0001), treatment ($F_{3,60}$ = 7.954, p = 0.0002), and an age × treatment interaction ($F_{6,60}$ = 2.406, p = 0.0377) of the expression of NEP in the hippocampus (**Figure 35A**). Post-hoc analysis revealed significantly increased NEP mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001), however, no significant differences were found in NEP expression

between 12- and 18-month-old APP/PS1 mice (p > 0.9999). Chronic HDAC inhibitor-treated mice with MS-275 in both 12- and 18-month-old APP/PS1 mice showed significantly increased NEP mRNA expression (p = 0.0100, p = 0.0206) when compared to age-matched VEH treated control mice in the hippocampus.

NEP mRNA expression was also evaluated in the PFC after chronic HDAC inhibitor treatment. Two-way ANOVA revealed a significant effect of age ($F_{2,60}$ = 259.7, p < 0.0001), treatment ($F_{3,60}$ = 10.20, p = 0.0002), and an age × treatment interaction ($F_{6,60}$ = 2.794, p = 0.0183) of the expression of NEP in the PFC (**Figure 35B**). Post-hoc analysis revealed significantly increased NEP mRNA expression in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001), however, no significant differences were found in NEP expression between 12- and 18-month-old APP/PS1 mice (p = 0.6671). Chronic HDAC inhibitor-treated mice with MS-275 in both 12- and 18-month-old APP/PS1 mice showed significantly increased NEP mRNA expression (p = 0.0239, p = 0.0021) when compared to age-matched VEH-treated control mice in the PFC.

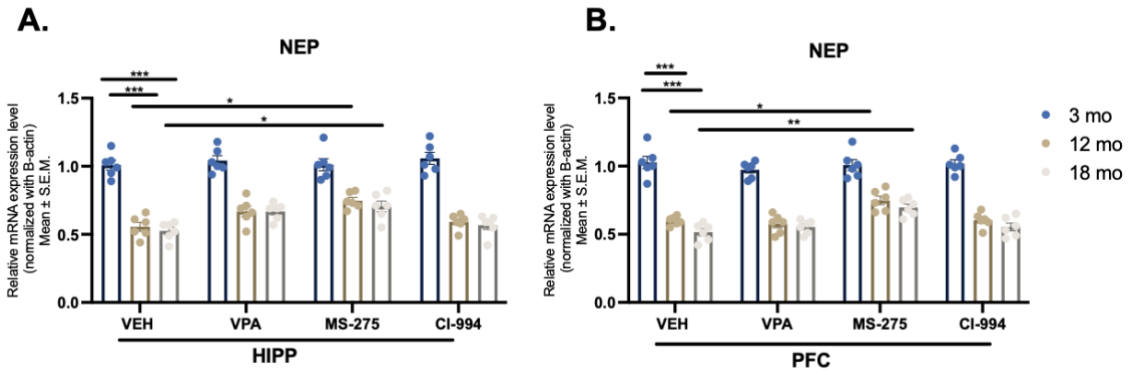

Figure 35. Chronic HDAC inhibitor Impact on Aβ Degradation Enzymes in the Hippocampus and PFC throughout AD Progression. A: mRNA expression of NEP in the hippocampus. Chronic MS-275 treatment significantly increased NEP expression. **B:** mRNA expression of NEP in the PFC. Chronic MS-275 treatment significantly increased NEP expression. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. ***p < 0.001.

IV. Chronic MS-275 & CI-994 Treatment Reduces Microglia Cell Densities During AD Progression in the Hippocampus and PFC

Microglia play an important role in inflammation, and contribute to the chronic neuroinflammatory state, a key hallmark in AD progression. Ionized calcium-binding adaptor molecule 1 (Iba1), is a marker that has actin-bundling activity and participates in membrane ruffling and phagocytosis in activated microglia. Therefore, to evaluate microglia cell densities, Iba1microglia cells were labeled using immunohistochemical staining in the hippocampus and PFC of 3-, 12-, and 18-month-old APP/PS1 mice after chronic HDAC inhibitor treatment (**Figure 36**). Two-way ANOVA revealed significant effects of age ($F_{2,24} = 48.75$, $p < 0.0001$, treatment ($F_{3,24} = 27.44$, $p < 0.0001$), and an age × treatment interaction ($F_{6,24} = 8.640$, $p < 0.0001$) of the % area of iba1+ cells in the hippocampus (**Figure 36A,B**). Post-hoc analysis revealed significantly increased iba1+ cells in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice ($p < 0.0001$, $p < 0.0001$), however, no significant differences were found in iba1+ cell densities between 12- and 18-month-old APP/PS1 mice ($p = 0.9615$). After chronic HDAC inhibitor treatment, MS-275 showed a significant reduction in iba1+ cell densities in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p = 0.0009$, $p < 0.0001$). Additionally, CI-994 showed a significant reduction in iba1+ cells in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p =$

0.0008). VPA did not show any effects on iba1+ cell densities across AD progression in the hippocampus.

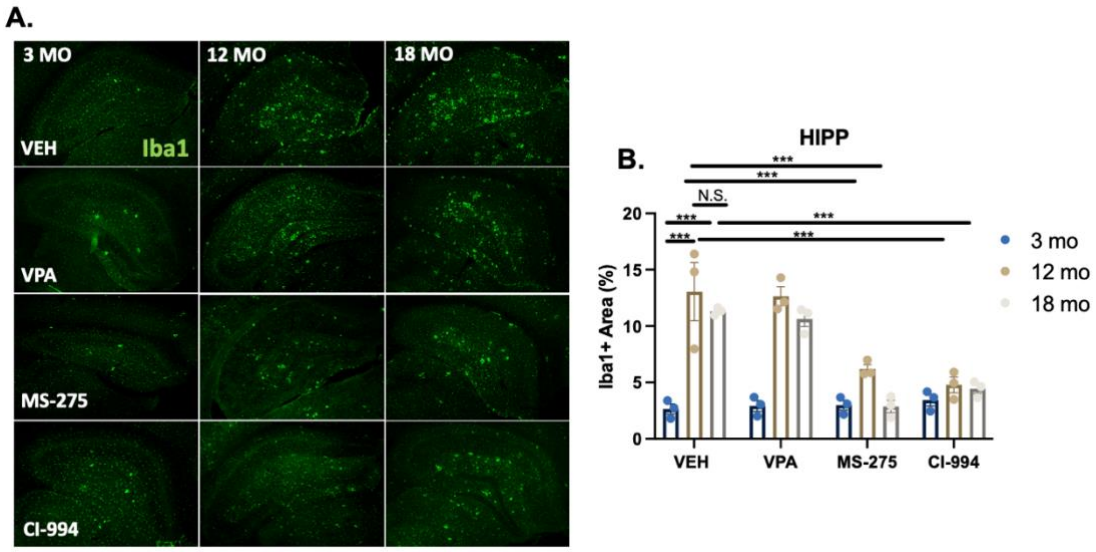

Figure 36. Chronic HDAC inhibitor Impact on Microglia Cell Densities in the Hippocampus throughout AD Progression. A: Immunostaining via Iba1 of microglia cell densities in the hippocampus. **B:** Quantification of Iba1+ cell densities. Chronic MS-275 and CI-994 treatment significantly decreased Iba1+ cell densities. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. ***p < 0.001.

For the PFC, two-way ANOVA revealed significant effects of age ($F_{2,24}$ = 141.5, p < 0.0001, treatment ($F_{3,24}$ = 20.57, p < 0.0001), and an age × treatment interaction ($F_{6,24}$ = 13.29, p < 0.0001) of the % area of iba1+ cells in the PFC (**Figure 37A,B**). Post-hoc analysis revealed significantly increased iba1+ cell densities in 12- and 18-month-old APP/PS1 mice when compared to 3-month-old APP/PS1 mice (p < 0.0001, p < 0.0001). However, no significant differences were found in iba1+ cell densities between 12- and 18-month-old APP/PS1 mice (p = 0.3636). After chronic HDAC inhibitor treatment, MS-275 showed a significant reduction in iba1+ cell densities in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice

($p < 0.0001$, $p < 0.0001$). Additionally, CI-994 showed a significant reduction in iba1+ cell densities in both 12- and 18-month-old APP/PS1 mice compared to VEH-treated mice ($p < 0.0001$, $p = 0.0424$). VPA did not show any effects on iba1+ cell densities across AD progression in the PFC.

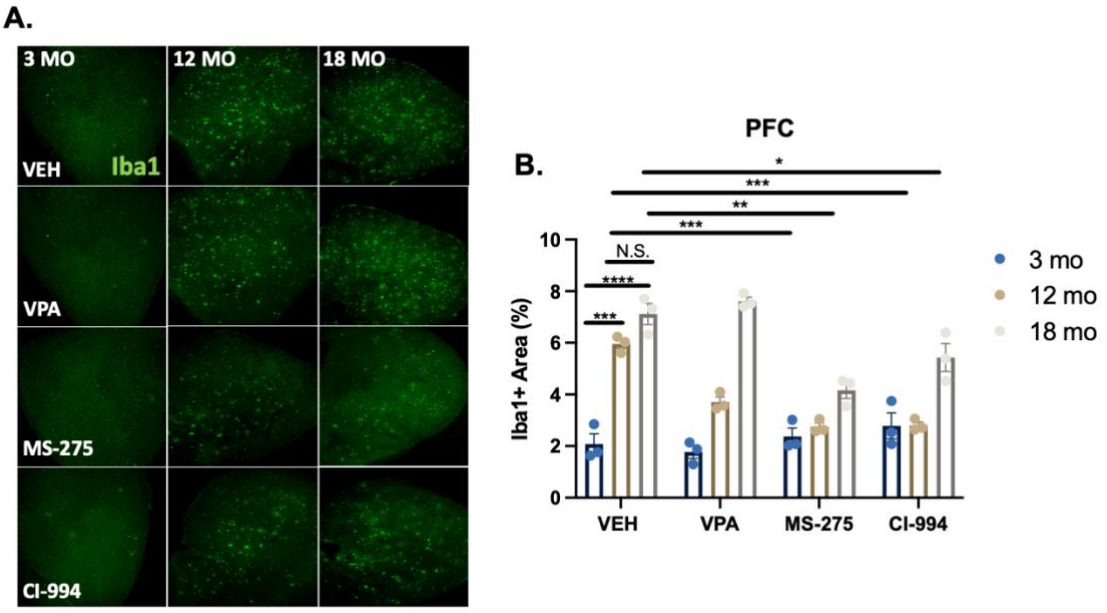

Figure 37. Chronic HDAC inhibitor Impact on Microglia Cell Densities in the PFC throughout AD Progression. A: Immunostaining via Iba1 of microglia cell densities in the PF. **B:** Quantification of Iba1+ cell densities. Chronic MS-275 and CI-994 treatment significantly decreased Iba1+ cell densities. Data represent mean ± SEM (n = 6/group). Blue circles represent 3 mo APP/PS1 mice, gold circles represent 12 mo APP/PS1 mice, white circles represent 18 mo APP/PS1 mice. ***$p < 0.001$.

V. Chronic HDAC inhibitor Treatment Improves Memory Function in APP/PS1 and Aged mice

Previous results found different memory domains to be impaired in aged and APP/PS1 mice. However, to dissect class 1 HDAC modulatory effects on memory, this study dissected HDAC contributions to memory through the pan and selective HDAC inhibitors. This study also wanted to determine whether chronic treatment of HDAC inhibitors can improve or restore memory function and decrease AD-related pathologies. This work further confirmed HDAC regulation of memory in aging and AD.

For recognition memory function, which was tested by NOR, MANOVA revealed a significant effect of age ($F_{2,243} = 149.847$, $p < 0.001$), genotype ($F_{1,243} = 5.409$, $p < 0.001$), treatment ($F_{3,243} = 19.409$, $p < 0.001$), and an age x genotype x treatment interaction ($F_{6,243} = 2.230$, $p = 0.041$) of the discrimination index (DI) (**Figure 38**). Post-hoc analysis revealed significantly decreased recognition memory in 18-month WT mice ($p = 0.001$). Both 12- and 18-month-old APP/PS1 mice showed significantly reduced recognition memory compared to age-matched controls ($p < 0.001$, $p < 0.001$), and aging in APP/PS1 mice exacerbated the decreased recognition memory ($p < 0.001$). After chronic HDAC inhibitor treatment, MS-275 and CI-994 showed improved recognition memory in 12- and 18-month-old APP/PS1 mice ($p < 0.001$, $p < 0.001$) compared to age-matched VEH-treated control mice. VPA-treated APP/PS1 mice did not show any significant changes in recognition memory compared to VEH-treated control mice. No

significant effects were seen in aged WT mice after chronic HDAC inhibitor treatment on recognition memory.

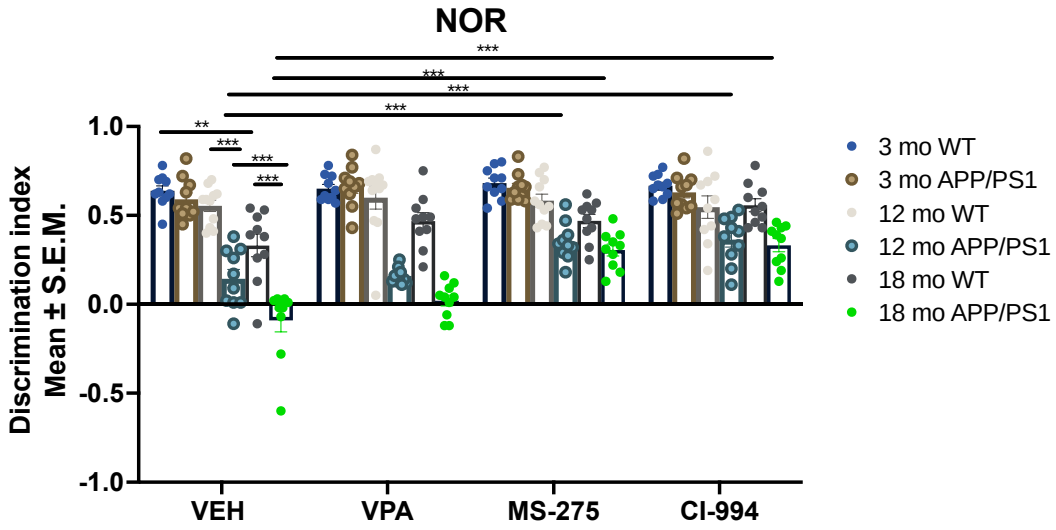

Figure 38. Chronic HDAC inhibitor Impact on Recognition Memory in Aging and AD. Novel object recognition was used to measure discrimination index. Chronic MS-275 and CI-994 treatments significantly improved recognition memory in both 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 9-12/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. **p<0.01, *** p < 0.001, **** p < 0.0001.

For short-term working memory, tested by Y-Maze, MANOVA revealed a significant effect of age ($F_{2,243}$ = 14.425, p < 0.001), genotype ($F_{1,243}$ = 101.921, p < 0.001), treatment ($F_{3,243}$ = 8.027, p < 0.001), and an age x genotype x treatment interaction ($F_{6,243}$ = 2.416, p = 0.049) of the % spontaneous alteration (**Figure 39**). Post-hoc analysis revealed no significant changes in short-term working memory in 18-month WT mice. Both 12- and 18-month-old APP/PS1 mice showed significantly decreased short-term working memory compared to age-matched controls (p < 0.0001, p < 0.0001), and aging in APP/PS1 mice did not exacerbate the decreased short-term working memory. After chronic HDAC inhibitor treatment, MS-275 and CI-994 showed improved short-term working memory in 12- and 18-month-old APP/PS1 mice (p < 0.001, p <

0.001) compared to age-matched VEH-treated control mice. VPA-treated APP/PS1 mice did not show any significant changes in short-term working memory compared to VEH control-treated mice. No significant effects were seen in aged WT mice after chronic HDAC inhibitor treatment on short-term working memory.

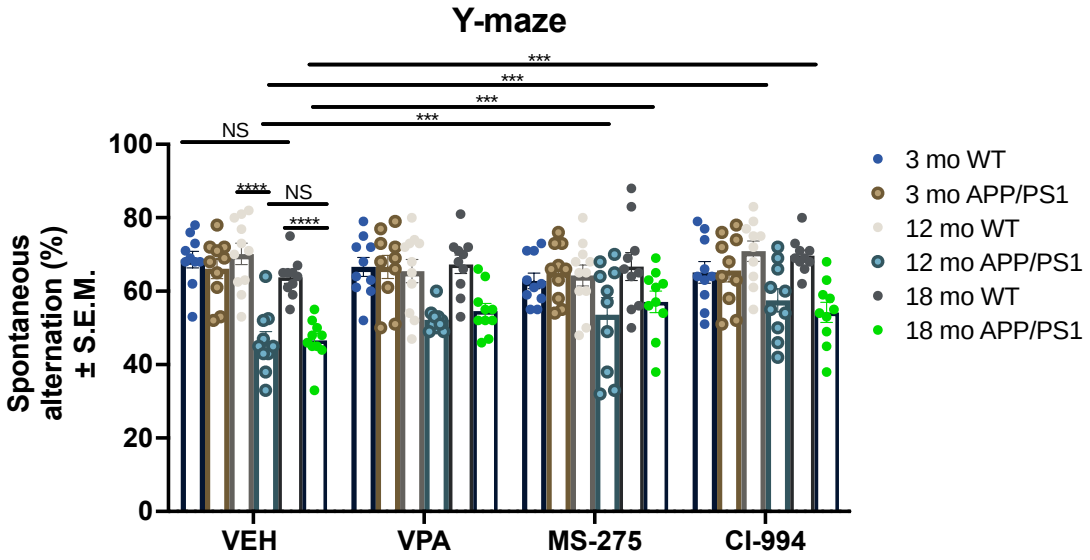

Figure 39. Chronic HDAC inhibitor Impact on Short-term Working Memory in Aging and AD. Y-maze was used to measure percent spontaneous alteration Chronic MS-275 and CI-994 treatments significantly improved short-term working memory in both 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 9-12/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ***p < 0.001, ****p < 0.0001.

For long-term spatial reference memory, tested with MWM, our MANOVA revealed significant effects of age ($F_{2,243}$ = 247.640, p < 0.0001), genotype ($F_{1,243}$ = 88.879, p < 0.0001), treatment ($F_{3,243}$ = 4.235, p < 0.0001), and an age x genotype x treatment interaction ($F_{6,243}$ = 2.172, p = 0.047) of the total time spent in target quadrant in the MWM behavior test (**Figure 40**).

Post-hoc analysis revealed a significant decrease in total time spent in the target quadrant in 18-month WT mice. APP/PS1 mice at 12- and 18-month-old showed significantly decreased long-term spatial reference memory compared to age-matched controls ($p < 0.0001$, $p < 0.0001$), and aging in APP/PS1 mice exacerbated the long-term spatial reference memory. After chronic HDAC inhibitor treatment, CI-994 showed improved long-term spatial reference memory in 12- and 18-month-old APP/PS1 mice ($p < 0.0001$, $p < 0.0001$) compared to age-matched VEH-treated control mice.

VPA-treated APP/PS1 mice did not show any significant changes in long-term spatial reference memory compared to VEH control-treated mice. No significant effects were seen in aged WT mice after chronic HDAC inhibitor treatment on long-term spatial reference memory.

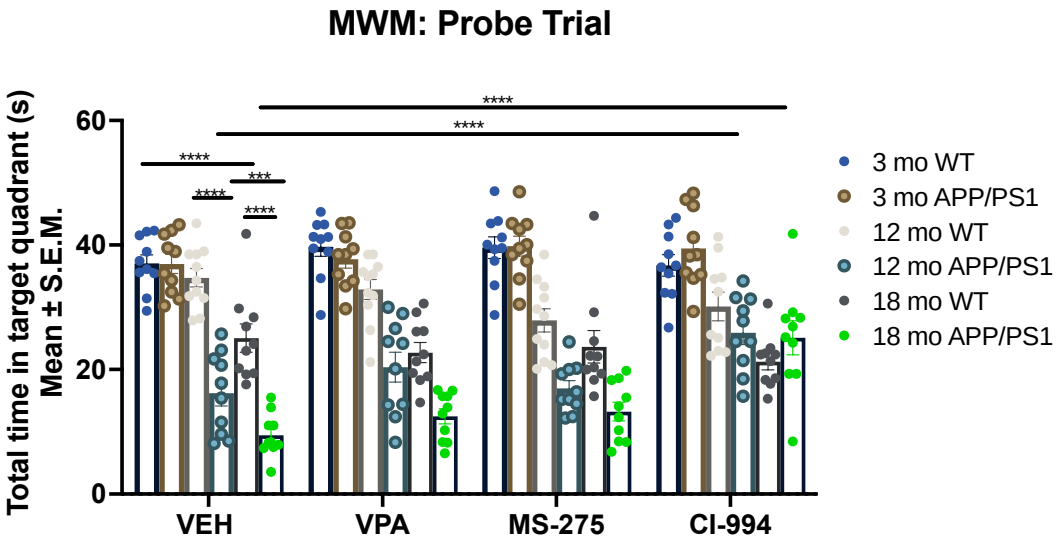

Figure 40. Chronic HDAC inhibitor Impact on Long-term Spatial Reference Memory in Aging and AD. MWM was used to measure total time spent in target quadrant. Chronic CI-994 treatment significantly improved long-term spatial reference memory in both 12- and 18-month-old APP/PS1 mice. Data represent mean ± SEM (n = 9-12/group). Blue circles represent 3 mo WT mice, gold circles represent 3 mo APP/PS1 mice, white circles represent 12 mo WT mice, light blue circles represent 12 mo APP/PS1 mice, gray circles represent 18 mo WT mice, green circles represent 18 mo APP/PS1 mice. ***$p < 0.001$, ****$p < 0.0001$

In the hippocampal data, results showed that chronic treatment of CI-994 significantly rescued synapse-related gene expression, and improved long-term spatial reference memory, recognition memory, and short-term working memory. To further understand how these molecular results associate with changes in the memory domains, several correlation analyses were performed using the significant hippocampal synapse-related gene expression changes in each brain region after chronic HDAC inhibitor treatment with each memory domain the hippocampus is involved in.

For long-term spatial reference memory, the analysis revealed a significant positive correlation between each synapse-related mRNA expression vs. total time spent in the target quadrant in 12 month-old (NR2A: r = 0.9444, p < 0.0001; GluR1: r = 0.8686, p = 0.0011; GluR2: r = 0.9127, p = 0.0002; PSD95: r = 0.9207, p = 0.0002) and 18-month-old (NR2A: r = 0.7586, p = 0.010; GluR1: r = 0.8785, p = 0.0008; GluR2: r = 0.8427, p = 0.0022; PSD95: r = 0.8711, p = 0.0010) APP/PS1 mice after chronic CI-994 treatment (**Figure 41**).

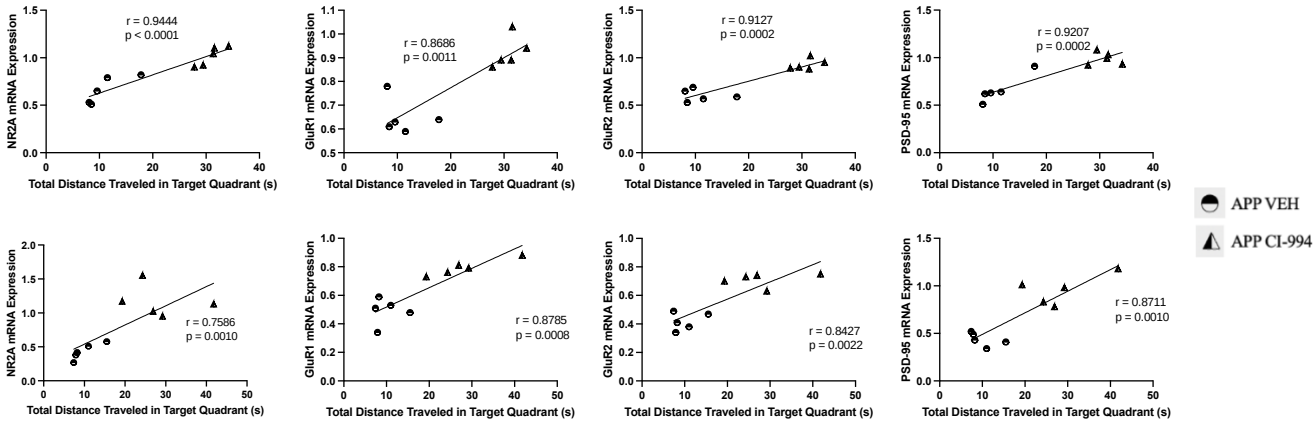

Figure 41. Correlation Analysis of Synapse-related mRNA Expression vs. Long-term Spatial Memory after Chronic Treatment of CI-994 in the Hippocampus of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant hippocampus data, where chronic treatment of CI-994 rescued synapse-related gene expression and improved long-term spatial reference memory. The analysis revealed significant positive correlations between the synapse-related gene expression and long-term spatial reference memory in both 12 (top row)- and 18-month-old (bottom row) APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 CI-994 treated mice.

For recognition memory, the analysis revealed a significant positive correlation between each synapse-related mRNA expression vs. discrimination index in 12-month-old (NR2A: r = 0.6400, p = 0.0462; GluR1: r = 0.7022, p = 0.0236; GluR2: r = 0.7279, p = 0.0170; PSD95: r = 0.6617, p = 0.0372) and 18-month-old (NR2A: r = 0.7499, p = 0.0125; GluR1: r = 0.7592, p = 0.0109; GluR2: r = 0.9115, p = 0.0002; PSD95: r = 0.0.7864, p = 0.0070) APP/PS1 mice after chronic CI-994 treatment (**Figure 42**).

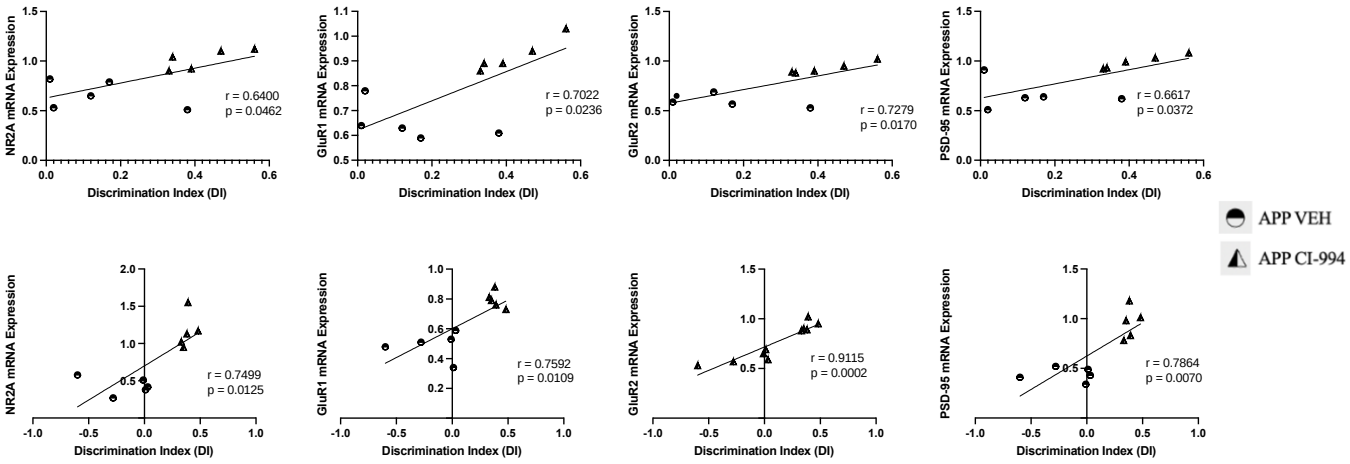

Figure 42. Correlation Analysis of Synapse-related mRNA Expression vs. Recognition Memory after Chronic Treatment of CI-994 in the Hippocampus of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant hippocampus data, where chronic treatment of CI-994 rescued synapse-related gene expression and improved recognition memory. The analysis revealed significant positive correlations between the synapse-related gene expression and recognition memory in both 12 (top row)- and 18-month-old (bottom row) APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 CI-994 treated mice.

For short-term working memory, the analysis revealed a significant positive correlation between each synapse-related mRNA expression vs. % spontaneous alteration in 12-month-old (NR2A: r = 0.8532, p = 0.0017; GluR1: r = 0.8080, p = 0.0047; GluR2: r = 0.8636, p = 0.0013; PSD95: r = 0.8893, p = 0.0006) and 18-month-old (NR2A: r = 0.8657, p = 0.0012; GluR1: r = 0.7660, p = 0.0098; GluR2: r = 0.8561, p = 0.0016; PSD95: r = 0.6323, p = 0.0498) APP/PS1 mice after chronic CI-994 treatment (**Figure 43**).

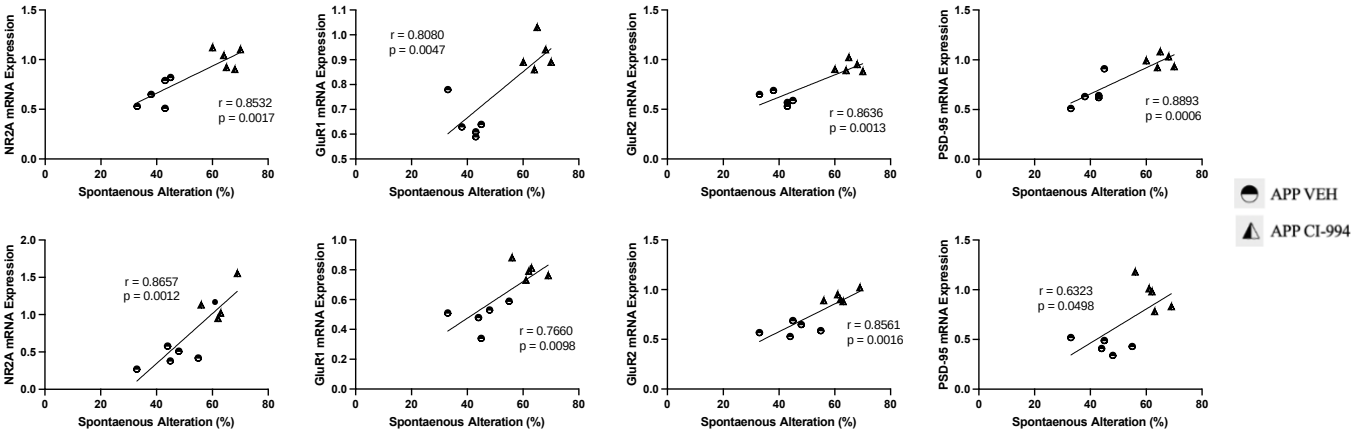

Figure 43. Correlation Analysis of Synapse-related mRNA Expression vs. Short-term Working Memory after Chronic Treatment of CI-994 in the Hippocampus of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant hippocampus data, where chronic treatment of CI-994 rescued synapse-related gene expression and improved short-term working memory. The analysis revealed significant positive correlations between the synapse-related gene expression and short-term working memory in both 12 (top row)- and 18-month-old (bottom row) APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 CI-994 treated mice.

The PFC data showed significant rescue of synapse-related gene expression after chronic MS-275 treatment, improved recognition memory, and short-term working memory. To further understand how these molecular results associate with changes in the memory domains, several correlation analyses' were performed using the significant PFC synapse-related gene expression

changes in each brain region after chronic HDAC inhibitor treatment with each memory domain in which the PFC is involved in.

For recognition memory, the analysis revealed a significant positive correlation between each synapse-related mRNA expression vs. discrimination index in 12-month-old (NR2A: $r = 0.9684$, $p < 0.0001$; GluR1: $r = 0.8104$, $p = 0.0045$; GluR2: $r = 0.7730$, $p = 0.0057$; PSD95: $r = 0.7863$, $p = 0.0070$) and 18-month-old (NR2A: $r = 0.8841$, $p = 0.0006$; GluR1: $r = 0.8893$, $p = 0.0006$; GluR2: $r = 0.9116$, $p = 0.0002$; PSD95: $r = 0.8709$, $p = 0.0010$) APP/PS1 mice after chronic MS-275 treatment (**Figure 44**).

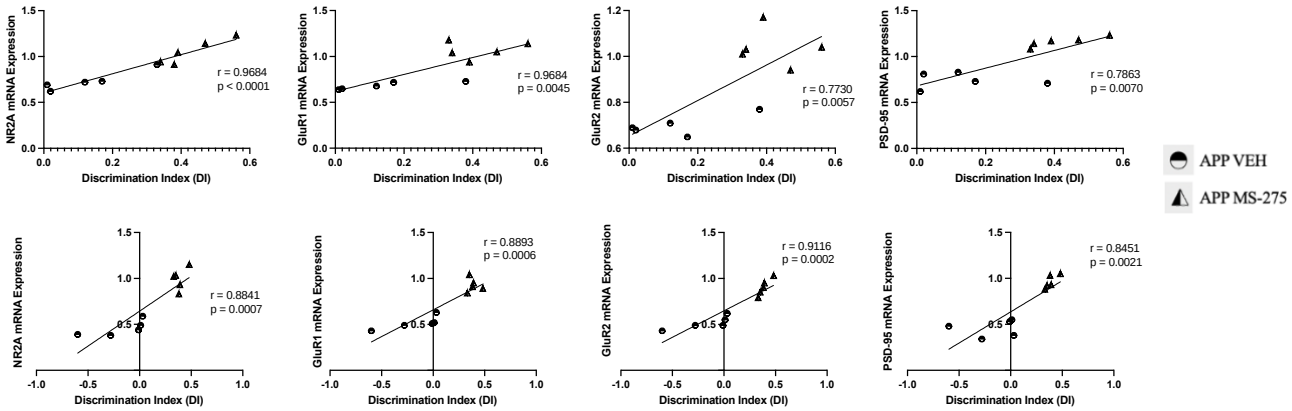

Figure 44. Correlation Analysis of Synapse-related mRNA Expression vs. Recognition Memory after Chronic Treatment of MS-275 in the PFC of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant PFC data, where chronic treatment of MS-275 rescued synapse-related gene expression and improved recognition memory. The analysis revealed significant positive correlations between the synapse-related gene expression and recognition memory in both 12 (top row)- and 18-month-old (bottom row) APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 MS-275 treated mice.

For short-term working memory, our analysis revealed a significant positive correlation between each synapse-related mRNA expression vs. discrimination index in 12-month-old (NR2A: r = 0.9100, p = 0.0003; GluR1: r = 0.9850, p < 0.0001; GluR2: r = 0.9891, p < 0.0001; PSD95: r = 0.8709, p = 0.0010) and 18-month-old (NR2A: r = 0.8723, p = 0.0010; GluR1: r = 0.8650, p = 0.0012; GluR2: r = 0.8411, p = 0.0023; PSD95: r = 0.7944, p = 0.0060) APP/PS1 mice after chronic MS-275 treatment (**Figure 45**).

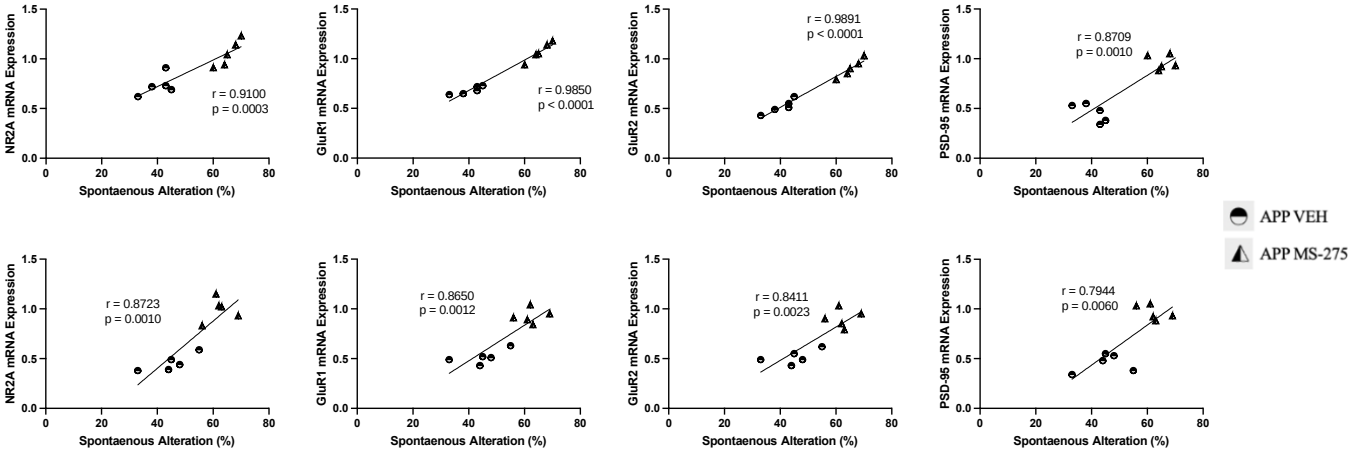

Figure 45. Correlation Analysis of Synapse-related mRNA Expression vs. Short-term Working after Chronic Treatment of MS-275 in the PFC of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant PFC data, where chronic treatment of MS-275 rescued synapse-related gene expression and improved short-term working memory. The analysis revealed significant positive correlations between the synapse-related gene expression and short-term working memory in both 12 (top row)- and 18-month-old (bottom row) APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 MS-275 treated mice.

To understand how changes in Aβ plaque burden after chronic HDAC inhibitor treatment are associated with changes in memory domains, several correlation analyses were performed using the significant Aβ plaque burden changes in each brain region after chronic HDAC inhibitor treatment with each memory domain. Chronic MS-275 treatment was the only treatment that significantly decreased Aβ plaque load in the hippocampus and PFC. Long-term spatial reference memory mainly involves hippocampal processes, therefore, the analysis revealed no

significant correlation between the changes in Aβ plaque load after chronic HDAC inhibitor treatment vs. total time spent in the target quadrant (MWM) in 12-month-old (r = -0.6024, p = 0.0653), and 18-month-old (r = -0.4679, p = 0.1727) APP/PS1 mice after chronic MS-275 treatment (**Figure 46**).

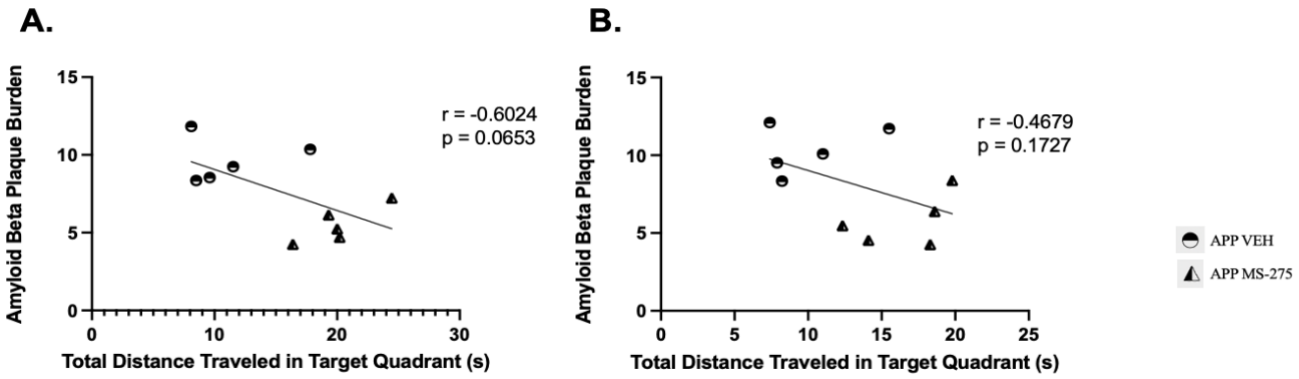

Figure 46. Correlation Analysis of Aβ Plaque Load vs. Long-term Spatial Reference Memory after Chronic Treatment of MS-275 in the Hippocampus of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant PFC data, where chronic treatment of MS-275 reduced Aβ plaque load, however, did not significantly improve long-term spatial reference memory. The analysis revealed no significant correlations between the Aβ plaque load and recognition memory in both **A:** 12-month-old and **B:** 18-month-old APP/PS1 mice (n = 5/group). Half-open circles represent APP/PS1 VEH treated mice, and half-open triangles represent APP/PS1 MS-275 treated mice.

Additionally, results showed that MS-275 significantly decreased Aβ plaque load in the PFC, and both recognition and short-term working memory are regulated by PFC-dependent processes. For recognition memory, the analysis revealed a significant negative correlation between changes in Aβ plaque load vs. discrimination index in 12-month-old (r = -0.9352, p < 0.0001), and 18-month-old (r = -0.9137, p = 0.0002) APP/PS1 mice. For short-term working memory, the analysis revealed a significant negative correlation between changes in Aβ plaque

load vs. % spontaneous alteration in 12-month-old ($r = -0.9691$, $p < 0.0001$), and 18-month-old ($r = -0.9460$, $p < 0.0001$) APP/PS1 mice after chronic MS-275 treatment (**Figure 37**).

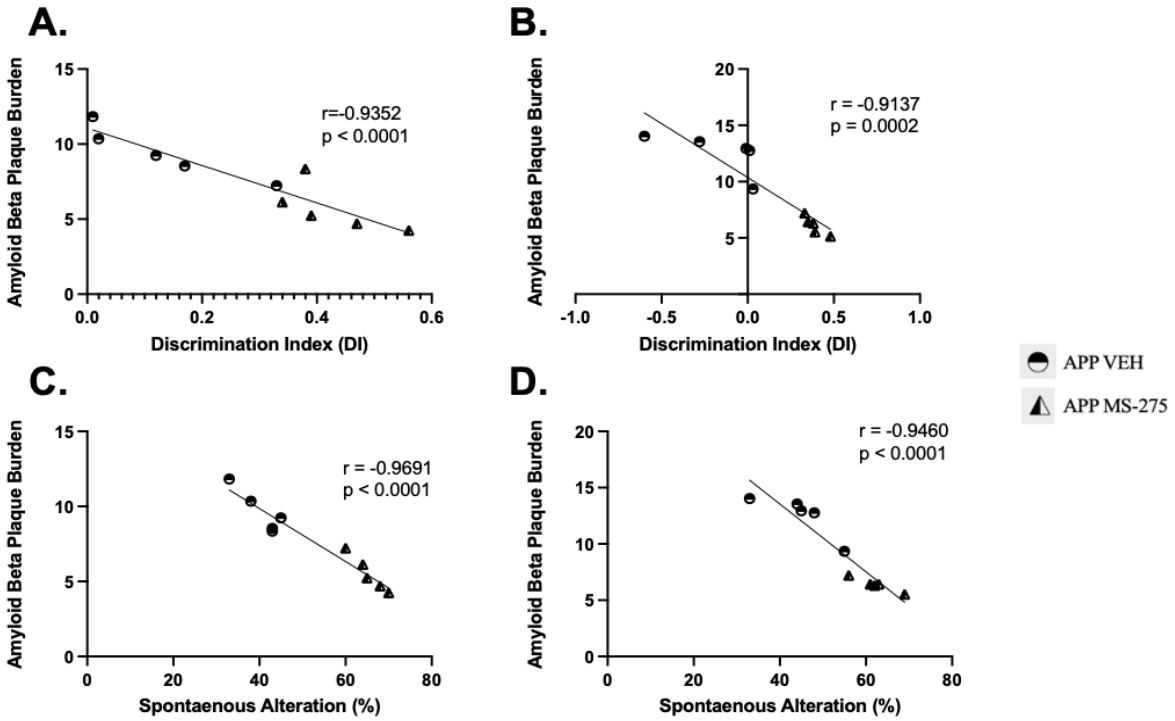

Figure 47. Correlation Analysis of AB Plaque Load vs. Recognition Memory and Short-term Working Memory after Chronic Treatment of MS-275 in the PFC of 12- and 18-month-old APP/PS1 Mice. A Pearson's correlation analysis was performed on the significant PFC data, where chronic treatment of MS-275 reduced AB plaque load, however, did not significantly improve long-term spatial reference memory. The analysis revealed significant correlations between the AB plaque load and recognition memory in both **A:** 12-month-old and **B:** 18-month-old APP/PS1 mice, and significant correlations between our AB plaque load and short-term working memory in **C:** 12-month-old and **D:** 18-month-old APP/PS1 mice (n = 5/group). Half-open circles represent APP VEH treated mice, and half-open triangles represent APP MS-275 treated mice.

Discussion:

In this study, a pharmacological approach targeting class 1 HDACs was used, demonstrating, and confirming that class 1 HDACs modulate memory function in aging and AD, and AD-related pathologies, in a spatial and temporal differential manner. This data shows that HDAC inhibitor CI-994, targeting HDAC 2, could improve long-term spatial reference memory through increased synapse-related gene (*nr2a*, *glur1*, *glur2*, *psd95*) expression and restored H3K9ac levels at all synapse-related gene promoters. Conversely, results also showed that HDAC inhibitor MS-275, targeting HDAC 1 and 3, could improve recognition and short-term working memory through increased synapse-related gene (nr2a, glur1, glur2, psd95) expression and restored H3K9ac levels at these genes (*nr2a*, *glur2*, *psd95*) promoters. No significant changes were seen in aged WT mice after chronic HDAC inhibitor treatment for overall memory function. Additionally, the results showed that MS-275 decreased Aβ plaque burden and $A\beta_{42}$ in both the hippocampus and PFC of 12- and 18-month-old APP/PS1 mice. Furthermore, MS-275 and CI-994 significantly reduced iba1+ cell densities in the hippocampus and PFC of 12- and 18-month-old APP/PS1 mice. These results suggest a differential HDAC modulation of the molecular mechanisms underlying memory function and AD pathologies throughout aging and AD and that this differential HDAC modulation exists spatially and temporally.

Results also showed that CI-994 was the only HDAC inhibitor treatment that significantly increased synapse-related gene expression, restored H3K9ac levels at synapse-related gene promoters in the hippocampus, and improved all memory domains that involve hippocampal processes. Given the previous findings that showed an increased abundance of HDAC 2 at synapse-related gene promoters, and that CI-994 is a potent inhibitor for HDAC 1 and 2, these

results showcased and confirmed that HDAC 2 is an essential modulator for memory, perhaps more specifically for hippocampal-related memory. These results are also consistent with the literature, which has found higher HDAC 2 expression in normal aging and in AD that negatively regulates memory function (Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Grinan-Ferre et al., 2016; Guan et al., 2009; D. Liu et al., 2017; Yamakawa et al., 2017).

Conversely, the selective HDAC inhibitor MS-275 significantly rescued synapse-related gene expression restored H3K9ac levels at synapse-related gene promoters in the PFC, and improved recognition and short-term working memory. Given the previous findings of increased abundance of HDAC 3 at synapse-related gene promoters in the PFC and that MS-275 is a potent inhibitor targeting HDAC 1 and 3, these results showcased that HDAC 3 is an important modulator for recognition, and short-term working memory, specifically for prefrontal cortex dependent memory processes. Although, unlike HDAC2, previous studies regarding HDAC 3 involvement in memory processes is not as well known (Janczura et al., 2018; McQuown et al., 2011; Shu et al., 2018; Suelves et al., 2017; Zhao et al., 2022), studies have used genetic and pharmacological approaches targeting HDAC 3 and have shown HDAC 3 to be involved in recognition and short-term working memory (Acosta-Rodriguez et al., 2022; Bieszczad et al., 2015; Janczura et al., 2018; McQuown et al., 2011), suggesting HDAC 3 may be more important in memory processes that are related to cortical function.

Interestingly, results regarding amyloidosis showed that only MS-275 significantly decreased both Aβ plaque burden and $A\beta_{42}$ levels in the hippocampus and PFC of 12- and 18-month APP/PS1 mice. HDAC inhibitor effects on amyloidosis have been reported, suggesting that

HDAC 1 and 3 are important modulators of amyloidosis. Such studies have shown increased HDAC 1 and 3 abundances at the NEP promoter, which suppresses the expression of NEP, a key enzyme for Aβ degradation (Belyaev et al., 2009; Kerridge et al., 2014). Results also showed that NEP expression was increased after chronic MS-275 treatment. Therefore, it is possible that MS-275 decreased amyloidosis through regulating NEP expression.

Neuroinflammation is a hallmark of AD (Acosta-Rodriguez et al., 2022; Frost et al., 2019; Thakur et al., 2022) and is commonly seen in postmortem tissues of AD subjects and AD mouse models (Acosta-Rodriguez et al., 2022; Cai et al., 2018; Cao et al., 2021; Fan et al., 2021; Gong et al., 2019; Lopez-Gonzalez et al., 2015). Microglia are specific innate immune cells in the CNS that act as macrophages and have a biphasic neurotoxic-neuroprotective role in the pathogenesis of AD, depending on the state of microglia, which largely depends on other glia communication and environmental stimuli/factors. Iba1 is a marker of activated and resting microglia. Therefore, evaluation of microgliosis by measuring iba1+ densities could reflect the status of neuroinflammation. Results showed that chronic MS-275 and CI-994 treatment significantly decreased iba1+ cell densities in 12- and 18-month-old APP/PS1 mice, suggesting HDACs 1-3 may be involved in regulating microglia cell activity. Previous studies have shown that after class 1 HDAC inhibition or genetic knockdown of class 1 HDACs decreased microglia cell densities and overall neuroinflammation (Choo et al., 2010; Durham et al., 2017; Kuboyama et al., 2017; Leus et al., 2016; Morioka et al., 2016; Winkler et al., 2012; Zhang & Schluesener, 2013; S. Zhu et al., 2017). However, given that iba1 is a marker for both resting and activated microglia, additional staining approaches are needed to help identify specific activated (e.g., CD68) vs. resting microglia cell types (e.g., CD11b) in order to further understand how class 1

HDAC inhibitors impact the state and function of microglia and the functional relationship on AD.

Chapter 4

Final Discussion, Limitations & Future Directions

Final Discussion:

Aging is the greatest risk factor for AD, however, the mechanisms underlying aging and its impact on AD processes are not well understood. Aging processes impact AD in many ways, including through epigenetic mechanisms (Cole et al., 2017; Gensous et al., 2019; Gravina & Vijg, 2010; Han et al., 2018; L. Liu et al., 2009; Wang et al., 2017; Xie et al., 2018). Current studies have investigated epigenetic alterations and their impact on aging and/or AD pathogenesis, however, whether these epigenetic changes during aging could initiate and/or exacerbate AD-related processes, needs to be better understood. My proposal fills in this knowledge gap by addressing the following research questions: 1) What are the main differences of epigenetic alterations during aging, and AD progression, 2) do epigenetic alterations display a spatial (cortex and hippocampus) and temporal (i.e. 3-, 12- and 18 months of age) pattern; 3) how epigenetic alterations impact specific memory domains between normal aging and AD; 4) whether HDAC inhibition can improve memory function in aging and AD, and AD pathologies; and most importantly, 5) whether the interaction of aging and AD could accelerate the above changes. I used a well-established mouse model, APP/PS1 mice, at 3-, 12, and 18 months of age, and WT littermates at the same ages as controls that serve as the normal aging model. The central hypothesis was that alterations of histone modifications through histone-modifying enzymes during aging can initiate and/or exacerbate memory deficits and AD neuropathogenesis, therefore, histone deacetylase (HDAC) inhibitors could alleviate memory impairments and neuropathogenesis induced by aging- and/or AD-related histone modifications.

In chapter 1, results showed a differential HDAC modulation of synapse-related gene expression through alterations of histone acetylation in the hippocampus and PFC. Within the hippocampus,

HDAC 2 levels were significantly increased at *nr2a, glur1, glur2, and psd95* gene promoters during normal aging and in AD, which was associated with decreased levels of H3K9ac levels at synapse-related gene promoters and synapse-related gene expression. Various studies have shown that HDAC 2 is an important modulator of memory processes. More specifically, previous reports have shown increased levels of HDAC 2 in the hippocampus of postmortem tissues and animal models of aging and AD (Bie et al., 2014; Gonzalez-Zuniga et al., 2014; Graff et al., 2012; Grinan-Ferre et al., 2016; Singh & Thakur, 2018). These findings of increased HDAC 2 levels in the hippocampus during normal aging and AD progression is consistent with previous reports, moreover, this study was the first to cross-compare HDAC 2 expression and its abundance at synapse-related gene promoters in a spatial and temporal manner. More interestingly, in chapter 3, results showed that only CI-994, a particular class 1 HDAC inhibitor that targets HDAC 1 and 2, rescued H3K9ac levels at synapse-related gene promoters in the hippocampus, increased synapse-related gene expression, and improved recognition, short-term working, and long-term spatial reference memory in both aged WT mice and APP/PS1 mouse models. CI-994 is the only HDAC inhibitor used in this study that strongly targets HDAC 2, and CI-994 was the only chronic treatment that improved all memory domains, which involve hippocampal processes (Broadbent et al., 2010; Cinalli et al., 2020; Jarrard et al., 1984; Sarnyai et al., 2000; Van der Borght et al., 2007). Additionally, one previous study indicated overexpressed HDAC 2 in adult mice experienced a decline in memory (Guan et al., 2009). It is possible that HDAC 2 levels and function in the hippocampus are increased during aging. However, in a disease state such as AD, the synergetic actions or interaction of age and disease pathology may promote HDAC 2 to become dysregulated and exacerbate memory decline. Together, these results further confirmed that HDAC 2 is an important modulator of memory

processes that involve hippocampal function in aging and AD by regulating synapse-related gene expression through alterations of H3K9ac at synapse-related gene promoters (**Figure 48**).

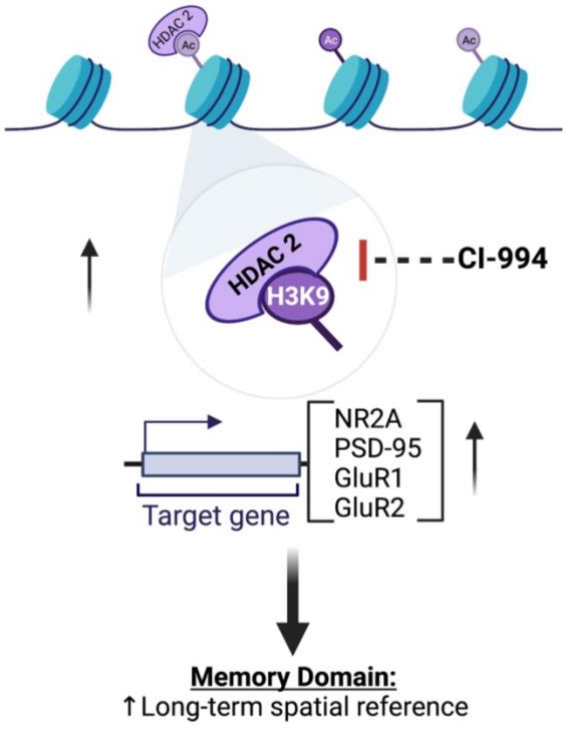

Figure 48. Chronic CI-994 Treatment Improves Long-Term Spatial Reference Memory through Rescuing H3K9ac Levels at Synapse-Related Gene Promoters and Increasing Synapse-Related Gene Expression in the Hippocampus of APP/PS1 mice.

In contrast, results also showed that HDAC 3 was significantly increased in global expression and abundance at synapse-related gene promoters in the PFC of AD mice. This increase was associated with a decreased H3K9ac levels at synapse-related gene promoters, and decreased synapse-related gene expression. Although HDAC 3 is known to be an important modulator in memory and AD processes (Alaghband et al., 2017; Janczura et al., 2018; Kwapis et al., 2019; McQuown et al., 2011; Shu et al., 2018; X. Zhu et al., 2017), our findings of increased global

HDAC 3 expression and abundance at synapse-related gene promoters in the PFC in APP/PS1 mice, is novel. Moreover, results showed that HDAC inhibitor MS-275 (targets HDAC 1 and 3) significantly improved recognition and short-term working memory, which is consistent with previous reports (Acosta-Rodriguez et al., 2022; Janczura et al., 2018; McQuown et al., 2011; Suelves et al., 2017). These studies have administered HDAC inhibitors specific for HDAC 3 and have found improvements in recognition and short-term working memory. Together, these results suggest that HDAC 3 modulates memory associated with PFC processes through changes of histone acetylation at synapse-related gene promoters, and synapse-related gene expression (**Figure 49**). Given the lack of molecular work on the modulation of HDAC 3 underlying molecular mechanisms of aging and AD, more rigorous research is needed to understand how HDAC 3 impacts aging and AD.

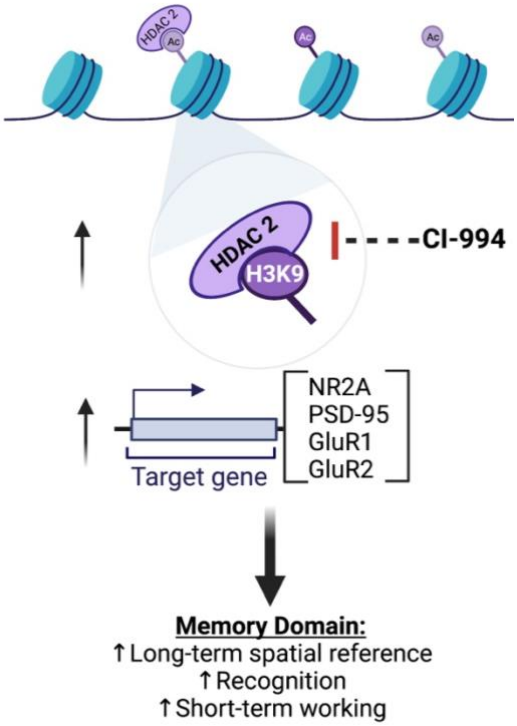

Figure 49. Chronic MS-275 Treatment Recognition and Short-Term Working Memory through Rescuing H3K9ac Levels at Synapse-Related Gene Promoters and Increasing Synapse-Related Gene Expression in the PFC of APP/PS1 mice.

To further understand how HDACs modulate synapse-related gene expression, it is important to dissect the relationships HDACs have with other epigenetic-modifying proteins and or complexes. HDACs are part of protein repressor complexes, and the two most common complexes are HDAC co-pressor of repressor element-1 silencing transcription factor (CoREST), and nuclear receptor co-repressor 1 (NCOR1). HDAC 1 and/or 2 are part of the CoREST repressor complex, whereas HDAC 3 is part of the NCOR repressor complex (Maksour et al., 2020; Zhang et al., 2002). Studies have shown that inhibition of the CoREST complex in the hippocampus significantly improves memory. More specifically, a study used a CoREST inhibitor, Rodin-A, which is similar to class 1 HDAC inhibitor CI-994, in a 5XFAD mouse model, and showed increased spine density and improved long-term spatial reference memory in the MWM behavior test (Fuller et al., 2019). This study indicated that the CoREST complex is important in memory processes in AD, specifically memory related to hippocampal function. These results are similar to the findings of study 2, which showed HDAC inhibitor, CI-994, improved hippocampal memory function by restoring histone acetylation levels and increasing of synapse-related gene expression. Therefore, developing new compounds that target other CoREST complex proteins with CI-994 may be an alternative strategy for improving hippocampal-dependent memory processes in aging and AD.

Results also showed that HDAC 3 increased its global expression and abundance at synapse-related promoters in the PFC in APP/PS1 mice only. There is limited evidence of NCOR/SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) complex and HDAC 3 regulation of aging and or AD-related processes. However, a study has shown global expression of the SMRT complex, which contains NCOR and HDAC 3, in neuronal cells and found high

densities in the neocortex (Iemolo et al., 2020). More interestingly, when HDAC 3 in the NCOR complex is lost, it enhances recognition memory, specifically in the NOR behavior test. These results indicate that the NCOR/HDAC 3 repressor complex may be important in the regulation of cortical-dependent processes (McQuown et al., 2011). HDAC inhibitor results showed that MS-275, targeting HDAC 1 and 3, significantly improved long-term recognition memory by restoring histone acetylation and by increasing synapse-related gene expression. Therefore, these results suggest that the NCOR/HDAC 3 complex may be a potential therapeutic target to consider for improving PFC-dependent memory processes in AD. Future work will need to dissect the regional and cellular distributions of repressor complexes through further genetic studies to understand the regulation of these repressor complexes in aging and AD.

At the pathological level, both Aβ plaque burden and $A\beta_{42}$ levels were significantly increased throughout AD progression in APP/PS1 mice. Aβ pathological progression is commonly seen in the APP/PS1 mouse model (Acosta-Rodriguez et al., 2022; Chen et al., 2014; Harach et al., 2017; Hou et al., 2020; S. Liu et al., 2017; Shi et al., 2017; Tachibana et al., 2019). More interestingly, results showed MS-275 significantly reduced both Aβ plaque burden and $A\beta_{42}$ levels in the hippocampus and PFC. A previous study also reported that treatment of MS-275 in the APP/PS1 mouse model significantly reduced amyloidosis (Zhang & Schluesener, 2013), and HDAC 3 could be involved in amyloid-mediated mechanisms through pharmacological and genetic manipulation approaches (Janczura et al., 2018; X. Zhu et al., 2017). Amyloid formation and degradation in the brain is initiated through amyloidogenic and non-amyloidogenic pathways, which include several key factors, such as BACE-1 (amyloidogenic), and ADAM-10 (non-amyloidogenic). To better understand the mechanisms underlying MS-275 ability to

decrease Aβ plaque load and $A\beta_{42}$ levels, BACE-1, and ADAM-10 gene expression was measured. Results showed significantly increased levels of BACE-1 expression and decreased levels of ADAM-10 expression in APP/PS1 mice at both 12- and 18-month-old APP/PS1 mice. However, none of the selected HDAC inhibitors influenced BACE-1 and ADAM-10 gene expression. The discrepancy between these results with previous findings may be due to the timing, dosing, and selectivity of the HDAC inhibitors. Additionally, various AD mouse models showcase altered amyloid processing in differing degrees, therefore, this may play a significant role in the differential findings of HDAC inhibitor impact on amyloid processing. More rigorous research is needed to further understand the interplay of amyloid processing and epigenetic changes before ruling it out as a potentially important mechanism and or target for future therapeutics.

Another important key factor determining Aβ accumulation in the brain is the function of Neprilysin (NEP), and Insulin-Degradation Enzyme (IDE), which are two enzymes that have been shown to degrade Aβ in the brain (Acosta-Rodriguez et al., 2022; Numata & Kaplan, 2010; Wang et al., 2006). NEP works by cleaving small peptides on the N terminal side of hydrophobic residues of Aβ (Numata & Kaplan, 2010). NEP expression and/or activity has been shown to be decreased in the brains of patients with MCI and AD (Hellstrom-Lindahl et al., 2008; Miners et al., 2006; Wang et al., 2010). In animal studies, NEP has been virally delivered in AD mouse models and has reduced amyloid pathology (Carty et al., 2013; Iwata et al., 2013; Y. Liu et al., 2009; Marr et al., 2003), confirming the importance of NEP reducing amyloidosis. In this study, results showed NEP expression was reduced in APP/PS1 mice in both 12- and 18-month-old APP/PS1 mice, which is consistent with the literature surrounding the APP/PS1 mouse model

(Drews et al., 2021; Fu et al., 2016; Jiang et al., 2021; You et al., 2018). However, after chronic HDAC inhibitor treatment, results showed MS-275 significantly increased NEP mRNA expression in both 12- and 18-month-old APP/PS1 mice. MS-275 impact on enzyme degradation of Aβ has not been studied, however, HDAC 1 and HDAC 3 are known to be part of a mechanism impacting NEP expression (Belyaev et al., 2009; Kerridge et al., 2014). Various studies have found HDAC 1 and HDAC 3 to be present at NEP gene promoters, both in cell lines and mouse models of AD (Belyaev et al., 2009; Kerridge et al., 2014). Given MS-275 is an HDAC 1 and 3 inhibitor, these results further support that NEP expression and function are modulated by HDAC 1 and 3. Therefore, inhibition of HDAC 1 and 3 can significantly increase NEP expression and reduce Aβ in the brain (**Figure 50A**). However, whether epigenetic mechanisms also affect another key enzyme of the Aβ degradation, such as IDE, is unknown, but worth investigating for future research.

As mentioned before, microgliosis is another common hallmark of AD, which is commonly found in postmortem tissues of AD subjects and the mouse models of AD, including APP/PS1 mice (Cai et al., 2018; Cao et al., 2021; Fan et al., 2021; Gong et al., 2019; Jardanhazi-Kurutz et al., 2011; Lopez-Gonzalez et al., 2015) and other mouse models of AD (Cuddy et al., 2020; Jin et al., 2013; Kantarci et al., 2020; Mezo et al., 2020). Microglia are innate immune cells that are responsible for various processes, such as CNS tissue maintenance, injury response, and pathogen defense (Soulet & Rivest, 2008; van Rossum & Hanisch, 2004). However, microglia are also known to be dysfunctional in AD, which can lead to decreased neuronal protection, increased neuroinflammation, and impaired phagocytosis of Aβ (Sarlus & Heneka, 2017; Streit, 2004). To determine the involvement of microglia in AD progression and how it can be

modulated HDACs, in this study, microglia were stained using the iba1+ marker, a cell surface marker of microglia, and evaluated whether changes of iba1+ densities in APP/PS1 after chronic HDAC inhibitor treatment.

As expected, results showcased increased iba1+ densities in AD at both 12- and 18-month-old APP/PS1 mice. Additionally, both MS-275 and CI-994 treatment significantly reduced iba1+ densities in the hippocampus and PFC (**Figure 50B**). Increased microglia densities have been reported in the APP/PS1 mouse model (Cao et al., 2021; Kiyota et al., 2018; Velazquez et al., 2020; Y. Yang et al., 2020) as well as other mouse models of AD (Cuddy et al., 2020; Kantarci et al., 2020; Shin et al., 2019). Previous studies also showed that MS-275 treatment can reduce Iba1+ densities (Wu et al., 2019; Zhang & Schluesener, 2013). However, this study is the first to report that CI-994 could have an impact on microgliosis. Microglia also play a significant role in Aβ signaling mechanisms. Both Aβ plaques, and microglia rely on communication to further exacerbate signaling cascade mechanisms to induce a chronic neuroinflammatory state in AD (Hansen et al., 2018; Lee & Landreth, 2010). Therefore, it is possible that the reductions in amyloidosis after chronic MS-275 treatment reduced the amount of cellular signaling between Aβ and microglia cells, resulting in reduced microglia cell densities. However, levels of inflammatory mediators, such as proinflammatory and anti-inflammatory cytokines, are needed to gain a better understanding of the reduced iba1+ densities after chronic MS-275 and CI-994 treatment. Additionally, a recent study has shown that HDACs 1 and HDAC 2 in microglia cells regulate the phagocytotic activity (Datta et al., 2018). Based on these results, it is possible that class 1 HDACs are modulating amyloidosis through microglia-mediated phagocytosis of Aβ plaques. However, future studies will need to quantify markers like lysosomal-associated

membrane protein 2 (LAMP2), a protein important for microglia phagocytic activity (Acosta-Rodriguez et al., 2022; Datta et al., 2018; He et al., 2022; Notomi et al., 2019; Tremblay et al., 2019), as well as the co-localization and 3D image rendering of microglia and Aβ plaque to understand the phagocytic function of microglia after chronic HDAC inhibitor treatment.

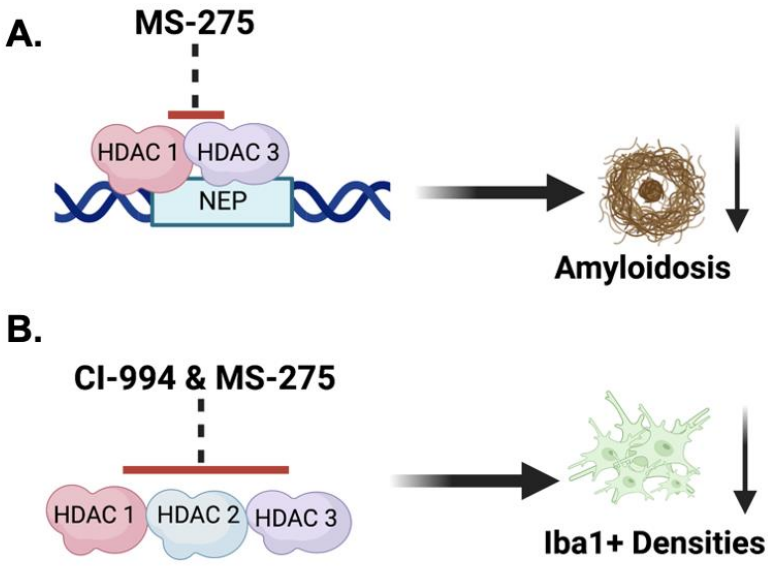

Figure 50. Chronic Class 1 HDAC inhibitor Treatment Decreases Amyloidosis and Microglia Cell Densities in AD Progression.

Memory is a complicated biological and psychological process with many intertwining mechanisms that are impacted differently across humans ‘ over time’ (Crystal & Glanzman, 2013; Kandel et al., 2014). With normal aging, experiencing loss in memory is common, which is known as age-associated memory impairment and does not significantly impact the elderly daily lives. When normal activities become harder to accomplish due to reduced cognitive abilities, dementia is still not a concern, however, these changes are classified as mild cognitive

impairment (MCI) (Gauthier et al., 2006; Petersen & Negash, 2008). Patients with MCI are at higher risk for developing AD or other types of dementia (Boyle et al., 2006; Serrano et al., 2013). Memory impairment is the most common and debilitating cognitive impairment associated with mild to moderate AD (Greene et al., 1996; Jahn, 2013; Sperling et al., 2010; Widmann et al., 2012). This memory impairment is different from normal age-associated memory impairments. Clinical evidence suggests episodic memory, specifically spatial and recognition memory, are the greatest memory domains impacted in AD patients (Baudic et al., 2006; Carlesimo et al., 1994; de Toledo-Morrell et al., 2000; Greene et al., 1996; Moodley et al., 2015; Schwindt & Black, 2009). Results from this study found that recognition and long-term spatial reference memory were impacted in APP/PS1 mice, and only recognition and long-term spatial reference memory impairments were exacerbated in APP/PS1 mice with aging.

These two memory deficits in aged APP/PS1 mice are among those commonly impacted in AD patients. Therefore, the aging and AD mouse models used in this study allowed for the molecular and cellular dissections of these memory processes to be translatable. Further investigation of the epigenetic regulation of these memory domains could help better understand the epigenetic regulation of memory and AD-related processes. It is also important to mention the findings regarding changes in the memory domains are consistent with previous reports (Han et al., 2019; Hong et al., 2015; Huang et al., 2021; Locci et al., 2021; Shi et al., 2018; Viana da Silva et al., 2016; Xu et al., 2015), although the variations of behavioral outcomes are largely dependent on the animal models of AD selected. Therefore, variation in behavioral changes, like memory for

example, is expected. Nevertheless, these results represent an informational guide of memory domains impacted in normal aging and AD, specifically an amyloidosis model of AD.

In previous studies, results showed that recognition and long-term spatial reference memory declined in normal-aged mice. Additionally, results showed decreased synapse-related gene expression, HDAC abundance at synapse-related gene promoters, and H3K9ac levels at synapse-related gene promoters during normal aging in both the hippocampus and PFC. Therefore, it is interesting to find that treatment of HDAC inhibitors did not increase synapse-related gene expression, H3K9ac levels at synapse-related gene promoters, and recognition and long-term spatial reference memory in normal aging. Even though results showed age-related molecular and behavioral changes in aged WT mice, the severity of these declines is not as drastic as they are in the APP/PS1 mice. These striking differences in findings between aged WT and APP/PS1 mice may in part be the reason why HDAC inhibitor treatment did not significantly improve the molecular and behavioral changes in aged WT mice. Furthermore, additionally, cellular factors may also play a role in the findings of aged WT mice. APP/PS1 mice experience age-related, amyloid, and neuroinflammatory changes, all of which can impact the molecular and behavioral outcomes in our studies (Benito et al., 2015). Given the findings of HDAC regulation in aging and AD-related pathologies, it is possible that the HDAC inhibitor treatments are modulating several cellular processes contributing to the age-AD state, which is why we see improvements in our APP/PS1 mice. Regardless, the results showcased age-related changes in synapse-related gene expression, H3K9ac levels at synapse-related gene expression, and declined recognition and long-term spatial memory, all changes which may interact with disease-associated processes

to exacerbate the molecular and behavioral changes. Therefore, dissecting age-related changes and understanding their impact in AD-related processes is critical.

The relationship between amyloid and memory is complicated and controversial. Plaques have been shown in normal cognitive individuals with AD, which represents the increased variation of behavioral symptoms of sporadic AD (Primavera et al., 1999; Zolochevska & Taglialatela, 2016). Tau mouse models of AD, which display the absence of amyloid pathology, but showed memory deficits (Blackmore et al., 2017; Lasagna-Reeves et al., 2011; Schindowski et al., 2006; Staff, 2015; Yue et al., 2011), indicated that amyloid may not be the root cause of memory deficits in AD. However, evidence has provided key insights into the relationship between the presence of amyloid and the decline of memory function in a significant population of AD patients (Mormino & Papp, 2018; Naslund et al., 2000; van der Kall et al., 2021). The correlation analysis showed a significant negative correlation between amyloid beta plaque burden with recognition and short-term working memory in both 12- and 18-month-old APP/PS1 mice. Moreover, results also showed that the reduction of plaque burden after chronic MS-275 treatment in the PFC was correlated with improved recognition and short-term working memory, but not long-term spatial reference memory function in both 12-and 18-month-old APP/PS1 mice. Interestingly, CI-994 also helped improve long-term spatial memory, but did not significantly lower Aβ plaque load and $A\beta_{42}$ levels. These results confirmed that amyloid pathology is not necessarily related to memory deficits, especially long-term spatial reference memory. Nevertheless, in the correlation analysis between synapse-related gene expression changes and memory function in the hippocampus, results showed that rescued synapse-related gene expression after chronic CI-994 treatment was correlated with all memory domains. These

results strengthen the idea that HDAC 2 is an important modulator of memory related to hippocampal function in AD. Additionally, in the PFC, findings of rescued synapse-related gene expression after chronic MS-275 treatment were correlated with recognition and short-term working memory. Again, these results further strengthen the previous results that show HDAC 3 is an important modulator of memory processes within the PFC in AD. Given Aβ oligomers are toxic to synapses through disrupted synaptic proteins (Ding et al., 2019; Marcantoni et al., 2020; Shankar et al., 2007; Tu et al., 2014), therefore, future studies investigating synaptic transmission are needed to better understand the relationships between amyloid, synaptic plasticity, and memory function in AD.

Memory is just one of many behavioral symptoms presented in patients. Often, AD patients experience the Behavioral and Psychological Symptoms of Dementia (BPSD), which are clinically classified into 5 domains: cognitive/perceptual, motor, verbal, emotional, and vegetative symptoms (Cerejeira et al., 2012). BPSD symptoms often can present before memory deficits associated with dementia, and if not treated, can significantly reduce the quality of life (Cerejeira et al., 2012). In this study, memory was the only behavioral change investigated, however, BPSD has been widely studied in the APP/PS1 mouse model (Locci et al., 2021; Q. Zhang et al., 2018), and other mouse models of AD (Alexander et al., 2011; Bories et al., 2012; Locci et al., 2021; Vloeberghs et al., 2006). Given the results identified specific memory domains impacted throughout normal aging and AD, it is worthwhile to further investigate BPSD symptoms impacted in normal aging and AD, and whether these symptoms are regulated through epigenetic mechanisms.

The treatment options for AD are limited and the therapeutic outcomes are controversial (Rabinovici, 2021). Currently, there is only one disease-modifying medication, aducanumab, which has been approved to treat AD (Dunn et al., 2021; Rabinovici, 2021). Aducanumab is an Aβ monoclonal antibody that targets aggregated forms of Aβ. Aducanumab is intended to treat patients with mild cognitive impairments (MCI) and mild dementia due to AD. The fast-track approval of aducanumab remains controversial due to the endpoint measure of Aβ reduction and its impact on cognition. Clinical trial data of aducanumab showed a significant reduction of Aβ in the brain, but the data did not point in any direction of a clinical benefit, rather it was assumed the reduction would improve cognition (Tampi et al., 2021). The pharmacoeconomics of aducanumab also poses a concern for clinicians when prescribing these medications to patients who fit ambiguous criteria. Currently, the cost to prescribe aducanumab is very costly, making treatment hard and inaccessible for many patients. Alternatively, other medications are FDA-approved drugs used to treat AD, such as cholinesterase inhibitors and Memantine, both of which moderately mitigate symptoms and behavioral problems associated with AD (Birks, 2006; Robinson & Keating, 2006), but are unable to prevent and target the pathologies of AD. Therefore, new targets are needed for the prevention and treatment of AD is urgent.

The pharmacogenomics and pharmacogenomics of epigenetic studies for drug development have been initiated, however, no clinical trials have been conducted with epigenetic drugs for the treatment of AD. Common epigenetic targets include HDAC inhibitors and histone acetyltransferase modulators (HAMs), and histone methyltransferase inhibitors (HMIs). Growing preclinical evidence suggests that HDAC inhibitors display neuroprotective effects (Burns et al., 2022; Cao et al., 2018; Fuller et al., 2019; K. Ma et al., 2018; McClarty et al., 2021; Montalvo-

Ortiz et al., 2017; Reolon et al., 2011; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Suelves et al., 2017; Sung et al., 2013; Wang et al., 2014; S. Zhang et al., 2018; Zhang & Schluesener, 2013). Additionally, the current results reflect beneficial effects of HDAC inhibitors by restoring histone acetylation, synapse-related gene expression, memory function, and AD-related pathologies. Although more rigorous investigation is needed to understand the mechanisms underlying aging and AD processes, these results help build a foundation to strengthen or innovate new epigenetic pharmacological treatment interventions to slow the progression of AD.

Limitations

This study investigated the epigenetic changes in aging and AD using only the APP/PS1 genetic mouse model. The model contains human transgenes for both APP, which bears the Swedish mutation and PSEN1, containing the L166P mutation, all under the control of the Thy1 promoter (Yokoyama et al., 2022). In this mouse line, the rate of APP production is higher than endogenous APP, which results in the overproduction of $A\beta_{42}$ and $A\beta_{40}$ (Jankowsky & Zheng, 2017), making this a good model for the study of amyloid and amyloid-associated neuroinflammation pathology, however; this model has several limitations. First, the APP and PS1 mutations are from the familiar AD subjects that only occupy less than 5% of AD patients and are not representative of most AD patients, which have sporadic AD. Second, the model does present tau pathology, another common hallmark of AD, which plays a significant role in AD development and progression. Studies have found that epigenetics could regulate tau pathology (J. Ma et al., 2018; Ricobaraza et al., 2009). Additionally, there is no single model that encompasses all behavioral and molecular changes and pathologies seen clinically, and any common changes seen across the models occur at various times and severities. Therefore,

interpretation and translation of the results should be used cautiously. Additionally, the usage of multiple AD mouse models is needed to help validate our results, but also explore the variations of epigenetic regulation in aging and AD-related processes. Another limitation of these studies is the selection of synapse-related genes. Although the targets selected are important in the of regulation synaptic plasticity, many other targets exist that are vital in the process of synaptic plasticity underlying memory function. Future work may consider using a chip-seq approach to investigate class 1 HDACs abundance across the entire mouse genome in aging and AD. However, antibody validation of class 1 HDACs using such techniques remains a growing effort, which may unravel additional synapse-related gene targets worth further investigating.

HDAC inhibitors have been shown to be promising for various neurological disorders and diseases (Cao et al., 2018; McClarty et al., 2021; Montalvo-Ortiz et al., 2017; Peleg et al., 2010; Qing et al., 2008; Ricobaraza et al., 2012; Ricobaraza et al., 2009; Zhang & Schluesener, 2013), however, the target specificity of HDAC inhibitors remains a significant issue. In the second study, two class 1 HDAC inhibitors: MS-275 (targets HDAC 1 and HDAC 3), and CI-994 (targets HDAC 1 and 2) were used. Both inhibitors are more selective than the broad-acting inhibitor VPA. Limiting the specificity of HDAC inhibitors reduces the non-specific blockage of HDACs, which could potentially induce more side effects and reduce the chances of clinical considerations (Cengiz Seval & Beksac, 2019; Subramanian et al., 2010). With the rapid development of epigenetic research and demanding requests for more powerful HDAC inhibitors, hopefully, the field is able to have more of a selection of HDAC inhibitors to better dissect the mechanisms of the histone modifications in aging and AD.

Final Concluding Remarks & Future Directions

Overall, this study was the first to rigorously characterize and compare epigenetic changes in aging and AD in a temporal and spatial manner that may drive AD development during aging in mouse models. The hypothesis that aging triggers AD development through epigenetic alterations is conceptually novel, which could help to identify a potential target at the epigenetic level for the prevention and treatment of AD. In this study, results showed a novel mechanism of differential class 1 HDAC modulation of memory and AD pathologies (**Figure 51**). These results provide a framework for epigenetic alterations that occur during normal aging that may impact AD progression. Based on the results obtained from this study, future research will cross-compare epigenetic impact in AD using multiple mouse models: APP/PS1, 5XFAD, NLGF, and Tg-2576. Using various models, future work will use multi-omic experiments (ATAC-seq, CUT&RUN-seq, scRNA-seq, and mass spec-proteomics) to understand the genome-wide changes in chromatin availability, histone modifications, RNA and protein expression spatially and temporally at the single cell level across aging and AD using WT mice. At the behavioral level, future work will also encompass affective and apathetic behaviors to understand BPSD across various AD mouse models better. This work will help to better understand the dynamic changes of epigenetics during aging and their impact on AD pathogenesis.

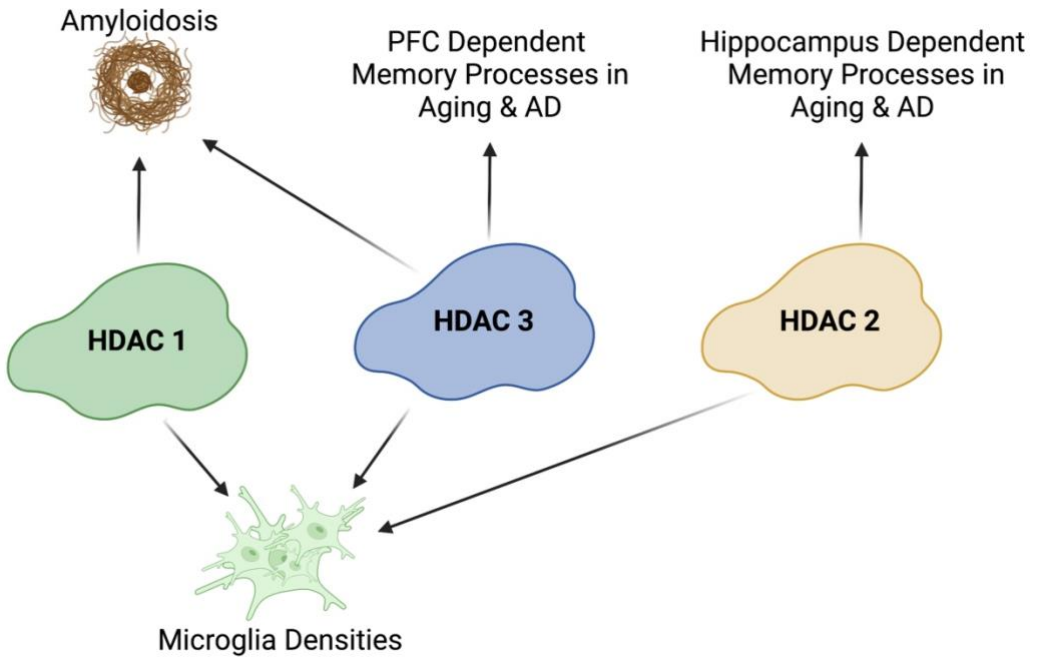

Figure 51. Differential Class 1 HDAC Modulation Work in Concert to Impact Aging and AD-Related Processes.

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