Amelioration of Memory Deficits in a Transgenic Mouse Model of Alzheimer's Disease by Manipulation of the Histone Acetyltransferase p300/CBP Associated Factor (PCAF)

by

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ABSTRACT

AMELIORATION OF MEMORY DEFICITS IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE BY MANIPULATION OF THE HISTONE ACETYLTRANSFERASE P300/CBP ASSOCIATED FACTOR (PCAF)

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Acetylation of histone and non-histone proteins by histone acetyltransferases (HATs) supports many mnemonic processes. As there is growing evidence that dysregulation of acetylation plays a role in cognitive deficits and neuropathology in Alzheimer's disease (AD), increasing HAT activity has emerged as a promising therapeutic strategy. However, acetylation patterns in AD are likely multifarious and memory deficits may not always be ameliorated by simply activating HATs. Indeed, the HAT, PCAF, may function atypically in AD. While PCAF activation enhances memory in normal rodents, in Aβ-treated rats, PCAF inhibition or knockout attenuates AD-like cognitive deficits, suggesting that PCAF activity may actually be detrimental. By longitudinally characterizing object (object recognition; OR) and spatial (object location; OL) memory deficits at 3, 6, 9, and 12-months-of-age, we show that male and female triple transgenic mice (3xTG) develop progressive cognitive impairments. Acute PCAF activation and inhibition remediated these impairments in an age-related bidirectional manner. At 3 and 6 months of age, prior to the development of OR deficits, the PCAF activator, SPV106, enhanced short- (5min) and long-term (3h) OR, whereas the PCAF inhibitor, embelin, impaired. At 9 months of age, when OR impairment was first observed, SPV106 ameliorated the long-term OR

deficit. At 12 months of age, however, SPV106 induced a short-term OR impairment, while embelin ameliorated the long-term OR deficit. A similar, albeit accelerated, pattern of results was observed for spatial memory using the object location task (OL). OL impairments were first observed at 3 months of age. At both 3 and 6 months of age, SPV106 ameliorated short- and long-term OL deficits. At 9 months of age, SPV106 had no effect on OL, whereas embelin ameliorated OL deficits. This work reveals a complex role for PCAF throughout AD progression, initially benefitting memory but detrimental as neuropathology becomes more severe. Therefore, memory deficits in AD may not always be ameliorated by HAT activation, and greater insight into the interactions between HATs, AD pathology, and cognition is necessary.

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LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

3xTG	Triple transgenic
ADAM	A disintegrin and metalloproteinase
AC	Acetyl
AD	Alzheimer's disease
AICD	Amino acid intracellular domain
ANOVA	Analysis of variance
aMCI	Amnesic mild cognitive decline
APP	Amyloid precursor protein
BACE	β-site APP cleavage enzyme
BDNF	Brain derived neurotrophic factor
CA	Cornu ammonis
CBP	Cyclic adenosine monophosphate response element-binding protein (CREB) binding protein
CFC	Contextual fear conditioning
CNS	Central nervous system
DMSO	Dimethylsulfoxide
DMS48	Delayed matching to sample recognition memory task
DR	Discrimination ratio
Elp3	Elongator acetyltransferase complex subunit 3
ER	Estrogen receptor
fAD	Familial AD
FBS	Fetal bovine serum
GNAT	Gcn5-N-acetyltransferases

HAT	Histone acetyltransferase
HDAC	Histone deacetylase
Hsp90	Heat shock protein 90
HPC	Hippocampus
K	Lysine
LTP	Long-term potentiation
MAPT	Microtubule-associated protein tau
MCI	Mild cognitive impairment
MSO	Multisensory object oddity
MTL	Medial temporal lobe
MWM	Morris water maze
NaBut	Sodium butyrate
NMDA-R	N-Methyl-D-Aspartate Receptor
NF-κB	Nuclear factor-κB
Nut1	Negative regulator of URS2 protein 1
OL	Object location
OR	Object recognition
P300	EA1-associated protein
PCAF	P300/CBP associated factor or PCAF
PHC	Parahippocampal cortex
PHD	plant homeodomain-linked
PRh	Perirhinal cortex
PVDF	Polyvinylidene difluoride
PS1, PS2	Presenilin 1, 2

STAT3	Signal transducer and activator of transcription 3
TAFT1	TATA-Box Binding Protein Associated Factor 1
Tip60	Tat-interacting protein of 60 kDa
TSA	Trichostatin A
TO	Temporal order
Wt	Wild-type

1 Literature Review

1.1 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder initially defined by Alois Alzheimer as a progressive senile dementia with the presence of amyloid plaques and neurofibrillary tangles in the brain (Alzheimer, 1911; Alzheimer, Förstl, & Levy, 1991; Maurer, Volk, & Gerbaldo, 1997; Stelzmann, Schnitzlein, & Murtagh, 1995). The plaques are extracellular and consist primarily of insoluble beta-amyloid (Aβ) peptide while the tangles are intracellular deposits of the microtubule-associated protein tau. In addition to these protein aggregates, AD is characterized by the loss of neurons and synapses in the cortex and multiple sub-cortical regions and the progressive impairment of cognition and function.

1.1.1 Alzheimer's Disease Pathology

1.1.1.1 Amyloid Plaques

The transmembrane amyloid precursor protein (APP) is cleaved by α-, β -, and γ-secretases to yield a variety of peptides (sAPPα, sAPP β, C83, C99, p3, APP intracellular domain (AICD), and Aβ) with important physiological functions, including regulation of synaptic activity, transcriptional regulation, stem cell development, neuronal survival, long-term potentiation and blood clotting (Dawkins & Small, 2014; Turner, O'Connor, Tate, & Abraham, 2003; Whitson, Selkoe, & Cotman, 1989). Under normal physiological conditions, APP is predominantly cleaved though the non-amyloidogenic pathway by α-secretases (a disintegrin and metalloproteinase (ADAM) 9, 10, 11) to produce C83 (and AICD and P3 following γ-secretase cleavage) and the sAPPα ectodomain. In AD, there is increased amyloidogenic processing of APP by β -, and γ-secretases, resulting in

excessive Aβ production (McLaurin, Yang, Yip, & Fraser, 2000; Sisodia & St George-Hyslop, 2002). Specifically, APP is cleaved by β-secretase (β-site APP-cleaving enzyme 1, or BACE1), yielding C99 and the sAPPβ ectodomain. C99 is subsequently cleaved to produce the 50 amino acid AICD and Aβ peptides by the γ-secretase complex (complex components include: presenilin (PS) 1 or 2, nicastrin, anterior pharynx defective and presenilin enhancer 2; LaFerla, Green, & Oddo, 2007; McLaurin et al., 2000; Sisodia & St George-Hyslop, 2002; Thinakaran & Koo, 2008). Early onset (familial) AD is caused by mutations in the APP PS1 or PS2) genes which all result in the increased pathogenic production of Aβ by: inhibiting α-secretase, potentiation of β-secretase and γ-secretase splice alterations, competitive binding, and endoplasmic reticulum trafficking (Ertekin-Taner, 2007; Haass, 1997; Hardy, 1997; Selkoe, 1998).

Although the function of Aβ peptides is not well understood, they have a variety of intracellular consequences, including lysosome up-regulation, mitochondrial dysfunction, dysregulation of cAMP response element (CRE)-directed gene expression and tau phosphorylation (Cuello, 2005). Aβ aggregates to form amyloid fibrils, which can rupture the membrane of neuronal cells causing cell death and the extracellular accumulation of Aβ (Friedrich et al., 2010). Extracellular Aβ can trigger cell death and is specifically associated with synaptic dysfunction, altered cholinergic tone, oxidative stress and abnormal glucose metabolism (Bossy-Wetzel, Schwarzenbacher, & Lipton, 2004; Cuello, 2005; Laursen, Mørk, Plath, Kristiansen, & Bastlund, 2013; Nizzari et al., 2012; Rossor et al., 2015; Thinakaran & Koo, 2008).

1.1.1.2 Neurofibrillary Tangles

Tau phosphoproteins play a critical role in the stabilization of microtubules in central nervous system (CNS) axons. Normally tau proteins are phosphorylated at approximately 30 serine/threonine (Ser/Thr) residues to promote association with microtubules (composed of α and β tubulin; Buée, Bussière, Buée-Scherrer, Delacourte, & Hof, 2000; Filippakopoulos & Knapp, 2014). In AD, serine/threonine residues become hyperphosphorylated by various kinases (including GSK3β, CDK5 and ERK2), which causes destabilization of microtubules and misfolding and aggregation of hyperphosphorylated tau proteins into neurofibrillary tangles (Avila, 2006; Filippakopoulos & Knapp, 2014; J. Wang, Xia, Grundke-Iqbal, & Iqbal, 2012).

The phosphorylation of distinct sites differentially affects tau pathogenesis. Specifically, Ser199/Ser202/Thr205, Thr212, Thr231/Ser235, Ser262/Ser356, and Ser422 sites contribute to microtubule dissociation; Thr231, Ser396, Ser422 are associated with tau aggregation; and Ser396 and Ser404 sites are linked with fibrillogenesis (Gong & Iqbal, 2008). The consequences of tau hyperphosphorylation and aggregation include inhibition of axonal trafficking, impaired synaptic function, and the initiation of neurodegeneration (Alonso, Grundke-Iqbal, & Iqbal, 1996; Ballatore, Lee, & Trojanowski, 2007; Bancher, Braak, Fischer, & Jellinger, 1993; Gong, Liu, Grundke-Iqbal, & Iqbal, 2006).

1.1.1.3 Other Pathological Features of Alzheimer's Disease

We now understand that presence of amyloid plaques and neurofibrillary tangles is accompanied by many other pathological changes, including: oxidative stress,

inflammation, dysregulation of gene expression, reduced synaptic responsiveness, and cell death (Braak & Braak, 1991, 1995; Cuello, 2005; Duyckaerts, Delatour, & Potier, 2009; Griffin et al., 1998; Honda & Casadesus, 2004; Jacobsen et al., 2006; McLaurin et al., 2000; Selkoe, 1991).

1.1.1.4 Spread of Alzheimer's Disease Pathology in Human Patients

Although the clinical staging of AD pathology is heterogeneous, histopathological and imaging studies report pathological changes that begin in the medial temporal lobe (MTL) prior to AD diagnosis and progress to widespread cortical and sub-cortical regions later in the disease (Braak & Braak, 1991; Hyman, Hoesen, Damasio, & Clifford, 1984; Scahill, Schott, Stevens, Rossor, & Fox, 2002; Schönheit, Zarski, & Ohm, 2004; Whitwell et al., 2007). Braak & Braak (1991, 1995) describe an Aβ and tau deposition pattern that begins in limbic regions, specifically the hippocampus (HPC), association cortices, basal forebrain, thalamus and hypothalamus and spreads to neocortex and various subcortical nuclei. It has been suggested that AD pathology deposits in a non-random fashion following signaling pathways via cell-to-cell transmission of Aβ and tau (Hyman et al., 1984; Saper, Wainer, & German, 1987; Steiner, Angot, & Brundin, 2011). The distribution of brain atrophy is consistent with patterns of Aβ and tau deposition. Specifically, gray matter atrophy is present in the HPC, amygdala, entorhinal cortex and fusiform gyrus prior to AD diagnosis; atrophy of the MTL becomes quite extensive as mild cognitive impairment (MCI) advances, and at AD diagnosis atrophy extends to frontal and parietal lobes (Scahill et al., 2002; Whitwell et al., 2007).

1.1.2 Rodent Models of Alzheimer's Disease

Analysis of cognition in human patients and postmortem neuropathology have provided valuable insights into AD. Pre-clinical AD models, nevertheless, have become popular for the characterization of AD-like phenotypes, as well as the evaluation of pharmacological interventions. The disease can be modelled in rodents via injection of Aβ into the CNS or using transgenic mice with genetic mutations implicated in AD (Ashe & Zahs, 2010; Bilkei-Gorzo, 2014; LaFerla & Green, 2012; Lecanu & Papadopoulos, 2013; Van Dam & De Deyn, 2011; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014).

Most transgenic AD mice model aspects of familial AD by expressing various mutations in APP and PS1 genes. One of the first transgenic AD models, the PDAPP strain, was developed by Games et al. (1995). PDAPP mice express the V717F APP mutation, which results in an age-dependent elevation in human APP protein (Games et al., 1995), synaptic loss (Dodart et al., 2000), and cognitive deficits (Chen et al., 2000; Dodart et al., 1999). Similarly, Hsiao et al. (1996) expressed the APP Swedish familial AD (FAD) mutation (K670N/M671L) in the Tg2576 model which develop age-dependent increases in APP, Aβ40/Aβ42, gliosis and cognitive deficits (Arendash et al., 2004; Hsiao et al., 1996; Oulès et al., 2013; Westerman et al., 2002; Yassine et al., 2013). Others have developed mutant PS1 mice, which have increased Aβ42 but generally do not develop amyloid plaques (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996). In order to create a more robust amyloid pathology, subsequent models (e.g. APP/PS1; Jankowsky et al., 2001) expressed multiple mutations in APP and PS1 that increase

amyloid pathology in an additive fashion (Citron et al., 1998; Eckman et al., 1997; Oakley et al., 2006).

No single transgenic AD model perfectly recapitulates the neuropathological staging and cognitive decline seen in human AD, and many models do not exhibit neuronal loss, tau hyperphosphorylation, or the formation of neurofibrillary tangles (Elder, Gama Sosa, & Gasperi, 2010; Webster, Bachstetter, & Van Eldik, 2013). To address these limitations, frontotemporal dementia and parkinsonism mutations that produce neurofibrillary tangles (e.g. microtubule associated protein tau (MAPT) P301L; Barghorn et al., 2000) have been combined with APP and PS1 mutations to induce both amyloid and tau pathology in transgenic AD mice (Lewis et al., 2001; Oddo, Caccamo, Shepherd, et al., 2003). Lewis et al. (2001) first crossed Tg2576 mice with JPNL3 (transgenic mice with a P301L mutation) to yield TAPP mice. Oddo et al. (2003) developed triple-transgenic (3xTG) mice by co-injection of APPswe (KM670/671NL; associated with increases in total Aβ; Ancolio et al., 1999; Cai, Golde, & Younkin, 1993; Citron et al., 1992; Citron et al., 1994; Johnston et al., 1994; Nilsberth et al., 2001; Scheuner et al., 1996) and tauP301L (promotes the formation of paired helical filaments that constitute neurofibrillary tangles; Barghorn et al., 2000), under the control of Thy1.2 promoter, into PS1 knock-in mouse embryos (M146V; associated with increased Aβ42; (Li et al., 2016; Murayama et al., 1999; Figure 1.1). 3xTG mice develop intracellular Aβ accumulation and amyloid plaques first in the cortex beginning at 3 to 4-months-of-age and in the HPC by 6-months-of-age (Belfiore et al., 2019; Oddo, Caccamo, Shepherd, et al., 2003). However, there is some evidence that male 3xTG do not exhibit the extensive Aβ pathology (Creighton et al., 2019). Tau

hyperphosphorylation is evident in the HPC between 6 and 12-months-of-age and neurofibrillary tangles or paired helical filaments form between 12 and 18-months-of-age (Belfiore et al., 2019; Mastrangelo & Bowers, 2008; Oddo, Caccamo, Kitazawa, Tseng, & LaFerla, 2003; Oddo, Caccamo, Shepherd, et al., 2003). With progressive age 3xTG mice also exhibit other pathological features of AD, including: cholinergic and noradrenergic neuronal death, increased microglia activity, inflammation, and alterations in glucose metabolism (Creighton et al., 2019; Da Cruz et al., 2012; Manaye et al., 2013; Mastrangelo & Bowers, 2008; Nicholson et al., 2010; Oddo, Caccamo, Kitazawa, et al., 2003; Sy et al., 2011). However, gross neuronal loss has not been observed. Because 3xTG mice express many pathological features of human AD, this model is used in this thesis.

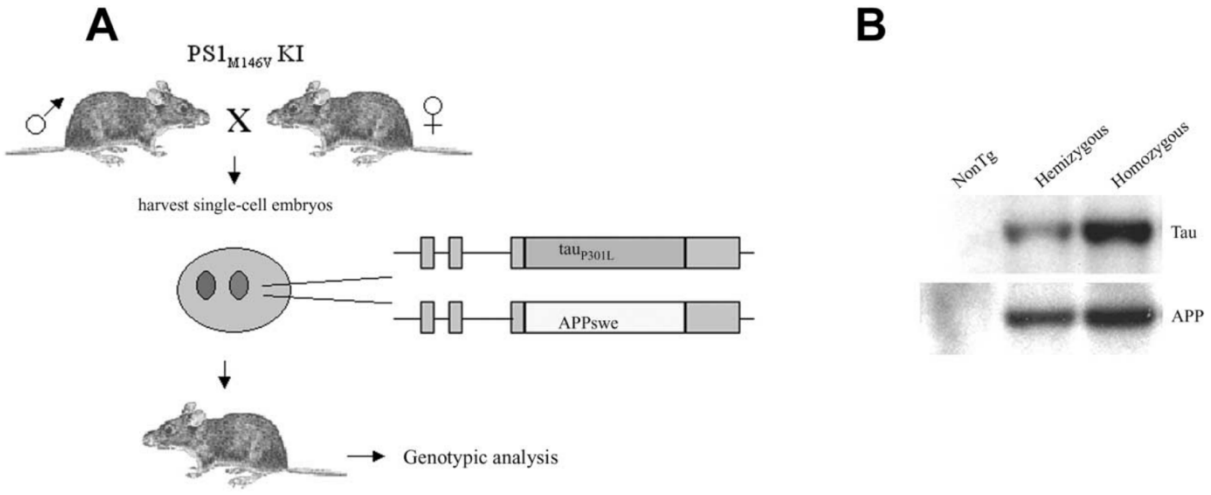

Figure 1.1 Generation of 3xTG-AD mouse model. a) Using pronuclear injection, the APPswe and tauP301L transgenes, under the control of the mouse Thy1.2 regulatory elements, were co-injected into single-cell embryos harvested from PS1 knockin mice. Injected embryos were then implanted into foster mothers and offspring were genotyped. b) Southern blot comparing gene dosage of human tau and APP transgenes. From Oddo et al. (2003).

1.1.3 Cognition in Alzheimer's Disease

1.1.3.1 Cognition in Human Alzheimer's Disease Patients

The most prominent symptom of AD is the progressive development of cognitive impairment. Alzheimer's patients can have deficits in a wide range of cognitive facets including attention, memory, language, and executive function (Henneges, Reed, Chen, Dell'Agnello, & Lebrec, 2016; Zhao et al., 2014). Typically, impairments in attention, orientation in time/place, executive function, and memory are observed in the early stages of AD (Carlesimo & Oscar-Berman, 1992; Henneges et al., 2016; Jahn, 2014; Zhao et al., 2014). As the disease progresses, global cognitive impairments are observed. Of these prominent cognitive impairments, memory deficits are the most likely to be reported by patients and are the best characterized. Episodic memory, the conscious memory for facts and events, is strongly affected by AD (Bäckman, Small, & Fratiglioni, 2001; Hodges, 1998; Jahn, 2014).

Human AD patients have extensive atrophy of MTL structures, including the parahippocampal cortex (PHC), entorhinal cortex, and perirhinal cortex (PRh), that is associated with impairment in visual recognition memory, a facet of episodic memory (Barbeau et al., 2008; Didic, Ranjeva, Barbeau, Confort-gouny, & Le, 2010; Snowden et al., 2011; Stopford, Snowden, Thompson, & Neary, 2007; Wolk, Signoff, & DeKosky, 2008). For example, Barbeau et al. (2008) evaluated visual recognition memory in patients with amnesic MCI (aMCI; often a pre-cursor to AD) to assess the relationship between cortical gray matter atrophy and memory impairment using a delayed matching to sample recognition memory (DMS48) task. In the DMS48 task, patients learn 48

images and, following a 1h retention delay, are evaluated on their ability to identify a learned image amongst distractors. aMCI patients impaired in DMS48 had gray matter loss in the MTL and temporal-parietal regions, including the PRh (Barbeau et al., 2008). Similarly, Didic et al. (2010) evaluated visual recognition memory in patients with aMCI to determine if visual recognition memory deficits, using DMS48, are associated with metabolic abnormalities in the MTL. Patients with impaired visual recognition had decreased bilateral MTL metabolism, including regions of the HPC, and visual recognition memory deficits correlated with MTL metabolism (Didic et al., 2010).

1.1.3.2 Cognition in Mouse Models of Alzheimer's Disease

Mouse models of AD display progressive cognitive deficits similar to those in human patients (Janus & Westaway, 2001). Indeed several mouse models of AD have impairments in attention (Palmer & Winters, 2016; Romberg, Mattson, Mughal, Bussey, & Saksida, 2011), visual spatial learning (Palmer & Winters, 2016), spatial memory assessed using the Morris Water Maze (MWM; Bromley-Brits, Deng, & Song, 2011; Janus, 2004), spatial memory assessed using contextual fear conditioning (CFC; Corcoran, Lu, Scott Turner, & Maren, 2002), episodic-like memory (Good, Hale, & Staal, 2007), and object recognition (OR; Creighton et al., 2019; Grayson et al., 2014).

Spontaneous OR memory tasks (Ennaceur & Delacour, 1988) for rodents are both procedurally and neurobiologically similar to human tests of visual recognition memory; therefore evaluation of OR memory in transgenic AD mouse models has significant translational potential (Grayson et al., 2015; Winters, Saksida, & Bussey, 2010). The OR task assesses rodents' ability to distinguish between previously encountered and novel

objects by exploiting their innate preference for novel stimuli (Ennaceur & Delacour, 1988). The most popular version of the OR task consists of a sample (learning) phase, in which rodents explore two identical objects; a variable retention delay, to manipulate the mnemonic demands of the task; and a choice phase, in which rodents explore one object from the sample phase and a novel object (Figure 1.2a). Intact memory for object identity is inferred from preferential exploration of the novel object during the choice phase. By modifying the nature of the OR task it is also possible to tax different forms of object processing with relevance to AD, such as spatial processing (Creighton et al., 2019). Spatial memory can be evaluated using the object location (OL) task. In OL, the location of one object, rather than its identity, is novel (Figure 1.2b; Dix & Aggleton, 1999; Abdelkader Ennaceur, Neave, & Aggleton, 1997).The one-trial nature of these OR/OL tasks does not require extensive training, aversive stimuli, or reward; therefore memory can be evaluated in a manner similar to daily human interaction with objects (Dere, Huston, & De Souza Silva, 2007; Ennaceur & Delacour, 1988). This thesis focused on characterizing impairments in OR and OL tasks.

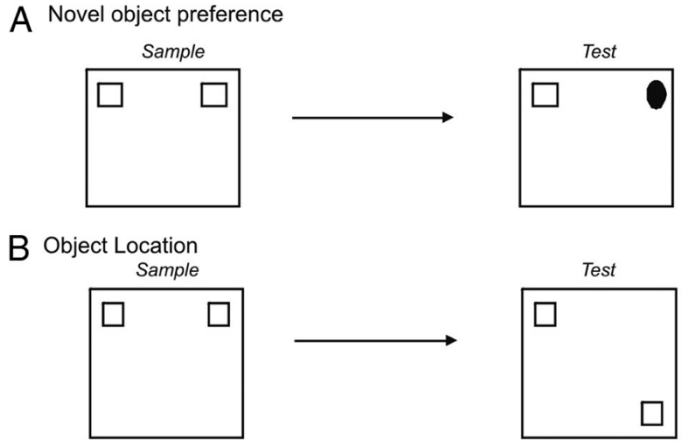

Figure 1.2 Schematic representation of spontaneous object tasks. a) Spontaneous object recognition. b) Object location. From Barker & Warburton (2011).

Many studies have demonstrated object memory deficits in transgenic AD mouse models (e.g. Arsenault, Julien, Tremblay, & Calon, 2011; Blanchard et al., 2010; Chiquita et al., 2019; Creighton et al., 2019; Davis, Easton, Eacott, & Gigg, 2013; Davis, Eacott, Easton, & Gigg, 2013; Feld et al., 2014; Filali et al., 2012; Gulinello et al., 2009; Guzmán-Ramos et al., 2012; Janczura et al., 2018; Kazim et al., 2014; Masciopinto et al., 2012; Onishi et al., 2011; Parachikova, Vasilevko, Cribbs, LaFerla, & Green, 2010; Shih et al., 2018; St-Amour et al., 2014; Stover, Campbell, Van Winssen, & Brown, 2015). In 3xTG mice, behavioural impairments map onto what is known about the development of hallmark pathological features of AD in this model. Immunohistochemical analysis of pathology in male 3xTG mice demonstrated changes in Aβ1-42 and phosphorylation of tau in the hippocampus, primary motor cortex, and amygdala, as early as 2-months-of-age (Mastrangelo & Bowers, 2008). Correspondingly, impairments in spatial memory have been observed at 3-months-of-age (examples in triple transgenic (3xTG) mice: Billings, Oddo, Green, McGaugh, & LaFerla, 2005; Caccamo et al., 2010; Clinton et al., 2007; Stover et al., 2015). Object recognition impairments, however, are not typically seen around 6-months-of-age. This likely reflects a difference in the staging and severity of pathology in task-relevant brain regions. For example, an earlier accumulation of intracellular Aβ1-42 in the HPC (Mastrangelo & Bowers, 2008), than in the entorhinal cortex, Aβ plaques are detected in the HPC prior to the cortex (Belfiore et al., 2019), higher levels of Aβ in the HPC than the PRh of 5-month-old female 3xTG mice (K. E. Davis et al., 2013), and we have observed more Aβ in the HPC, compared to the cortex, of 12-month-old 3xTG male and female mice (Creighton et al., 2019).

However, there are often discrepancies in the specific nature of object memory deficits reported (Grayson et al., 2014; Simón et al., 2009; Taglialatela, Hogan, Zhang, & Dineley, 2009), and some studies fail to find impairments (Chen et al., 2014; Davis et al., 2013; Davis et al., 2013; Fragkouli, Tsilibary, & Tzinia, 2014; Good, Hale, & Staal, 2007; Karl, Bhatia, Cheng, Kim, & Garner, 2012; Yassine et al., 2013). Inconsistent performance on OR tasks is likely related to the lack of a standardized OR paradigm and systematic evaluation of sex, age and mnemonic delay. Furthermore, procedural differences can alter the conceptual nature of the task and thus tax distinct behavioural processes (Barker & Warburton, 2011; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). For example, spatial memory impairments, evaluated using the OL task, are more severe and observed at earlier ages in transgenic AD mice (Bergin & Liu, 2010; Bollen et al., 2013; Creighton et al., 2019; Dodel et al., 2011; Frye & Walf, 2008; Gulinello et al., 2009). To clarify the nature of OR deficits, we recently systematically analyzed various facets of object memory and perception in two strains of transgenic AD mice (Creighton et al., 2019). At 12-months-of-age, memory and multisensory perceptual impairments were observed, with dissociations between transgenic AD strains and sex that paralleled neuropathological changes. 3xTG mice, specifically, had selective impairments in Y-apparatus OR at 3h, indicative of a more selective long-term memory deficit. When OR was evaluated in the open-field, 3xTG females were also selectively impaired at 3h, whereas males were impaired at both 5min and 3h. Object location memory was impaired at both 5min and 3h delays in both 3xTG males and females, suggesting that spatial memory is severely impaired. This study highlighted clinically significant differences in the

behavioural characterization of object memory possible when using different variations of the OR task (Creighton et al., 2019). Given the differential performance seen across object tasks in mouse models of AD, one major aim of this thesis is to systematically clarify the longitudinal nature of OR and OL deficits in male and female 3xTG mice, before evaluating the therapeutic potential of epigenetic mechanisms.

1.2 Epigenetics

The term ‘epigenetics’ was first used by Conrad Waddington to describe the interactions between genetic and environmental factors that produce heritable changes in cellular phenotype (Waddington, 1942). Specifically, Waddington deduced that there must be a set of mechanisms which act above (epi) the level of the DNA sequence, to control which genes are expressed and repressed (Waddington, 1942). This definition has been refined by Allis et a. (2007) to “…the sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome”. Now, especially in the neurosciences, the term epigenetics is used more broadly to describe the regulation of gene expression that is linked to mechanisms that act on the DNA while leaving the DNA sequence intact (Stilling & Fischer, 2011).

Many different biochemical modifications are currently classified as epigenetic, including covalent modification of DNA, post-translational modification of histones, histone subunit exchange, and non-coding RNAs (Sweatt, 2013). Of these, one of the

most extensively studied epigenetic mechanisms is the post-translational modification of histone proteins.

1.2.1 Chromatin Structure

DNA is a double-stranded helix composed of pairs of repeating nucleotide bases: adenine, thymine, cytosine and guanine bonded to a sugar-phosphate backbone (Luger, Dechassa, & Tremethick, 2012). For compaction and regulation of gene expression, DNA is wrapped around protein complexes called histones (Luger et al., 2012; Robertson & Wolffe, 2000). Specifically, 147 base pairs of DNA are tightly wrapped around an octamer of canonical histone proteins, containing two copies each of the histones H2A, H2B, H3 and H4 (Figure 1.3a; Luger et al., 2012). Each segment of DNA wrapped around a histone octamer is called a nucleosome. Occasionally, during histone subunit exchange, canonical histone monomers can be exchanged for histone variants (e.g. H2A.Z and H3.3; Maze et al., 2015; Zovkic, Paulukaitis, Day, Etikala, & Sweatt, 2014; Zovkic & Sweatt, 2015). Collectively, the DNA and nuclear histone proteins are called chromatin. The linker histone H1 binds nucleosomes with linker DNA to stabilize the three-dimensional structure of chromatin (Luger et al., 2012).

Flexible amino acid ‘tails’ protrude from histone proteins; it is at these histone ‘tails’ that many post-translational epigenetic modifications occur, including lysine mono/di/tri methylation, arginine mono/di methylation, lysine acetylation, serine/threonine phosphorylation, mono-ubiquitination, and poly ADP-ribosylation (Jenuwein & Allis, 2001; Kouzarides, 2007; Luger et al., 2012). The histone code hypothesis postulates that these modifications act in a coordinated fashion – (or act together/collectively/jointly) to regulate

specific patterns of gene transcription (Jenuwein & Allis, 2001); this thesis, however, will focus exclusively on histone acetylation.

1.2.2 Histone (Lysine) Acetylation

Histone acetylation almost exclusively promotes gene transcription and involves the addition of acetyl groups (Ac; $-C_2H^3O$) from acetyl coenzyme A (acetyl CoA) to lysine (K) residues on histone protein tails (Kuo & Allis, 1998). This process is catalyzed by histone acetyltransferases (HATs). There are a number of lysine residues that are known to be acetylated on canonical histones and histone variants (Figure 1.3b; Corujo & Buschbeck, 2018; Gräff & Tsai, 2013; McKittrick, Gafken, Ahmad, & Henikoff, 2004; Tweedie-Cullen et al., 2012). The exact mechanism through which histone acetylation promotes gene transcription likely involves the modification of chromatin structure (open or closed sate) and/or the recruitment of transcriptional machinery. Initially, the addition of negatively charged acetyl groups to histones was thought to weaken the electrostatic interaction between negatively charged DNA and positively charged histones, allowing transcriptional machinery to access the DNA (Peixoto & Abel, 2013; Strahl & Allis, 2000). However, some evidence suggests that neutralization of electrostatic charges between DNA and histones is improbable (Choi & Howe, 2009; Mutskov et al., 1998), and it is more likely that acetylated lysine residues on histone tails function as molecular tags that are recognized by transcription factors and machinery (Peixoto & Abel, 2013; Schneider et al., 2013; Strahl & Allis, 2000).

The acetylation level of lysine residues on histone tails is dynamically balanced by histone acetylation and deacetylation enzymes.

1.2.2.1 Histone Acetyltransferases

Histone acetyltransferases are grouped into two categories: cytoplasmic and nuclear (Kuo & Allis, 1998). Cytoplasmic HATs are believed to acetylate new histones during DNA replication, whereas nuclear HATs acetylate histones to promote transcriptional activation (Kuo & Allis, 1998). There are three major families of nuclear HATs: EA1-associated protein (p300)/cyclic adenosine monophosphate response element-binding protein (CREB) binding protein (CBP) (p300/CBP); Gcn5-N-acetyltransferases (GNAT) which includes Gcn5, p300/CBP associated factor (PCAF), histone acetyltransferase 1, Histone acetyltransferase 2, Elongator Acetyltransferase Complex Subunit 3 (Elp3) and Negative regulator of URS2 protein 1 (Nut1); and the MYST family Morf, Ybf2/Sas3, Sas2 and Tat-interacting protein of 60 kDa (Tip60), as well as TATA-Box Binding Protein Associated Factor 1 (Taf1) and other nuclear proteins with intrinsic HAT activity but which lack HAT domains (Kuo & Allis, 1998; K. K. Lee & Workman, 2007; A. Schneider et al., 2013).

Individual HATs are known to acetylate at not entirely overlapping lysine residues (Kouzarides, 2007). However, HATs, often function synchronously in multiprotein complexes with a wide variety of subunits (e.g. bromodomains, chromodomains, WD40 repeats, Tudor domains and plant homeodomain-linked (PHD) fingers). The composition of HAT complexes contributes to the specificity of biological outcomes (K. K. Lee & Workman, 2007). For example, HATs that contain bromodomains can also recognize acetylated lysine resides.

1.2.2.2 Histone Deacetylases

Histone acetylation is reversible, and the removal of acetyl groups from histones is catalyzed by histone deacetylases (HDACs). Histone deacetylation typically induces transcriptional repression. HDACs can be categorized into two main classes: class 1 HDACs are predominantly localized to the nucleus (HDAC1, HDAC2, HDAC3, HDAC8), and class 2 HDACs shuttle between the nucleus and cytoplasm in response to cellular signaling cascades (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9a/b/c, HDAC10; De Ruijter, Van Gennip, Caron, Kemp, & Van Kuilenburg, 2003). HDACs function in multiprotein complexes to remove acetyl groups via a charge-relay system that consists of two adjacent histidine residues, two aspartic residues, and a tyrosine residue and are dependent on Zn^{2+} ions (De Ruijter et al., 2003). Deacetylation promotes a tighter interaction between DNA and histones, thereby restricting access of transcription factors to DNA and inducing transcriptional repression (De Ruijter et al., 2003).

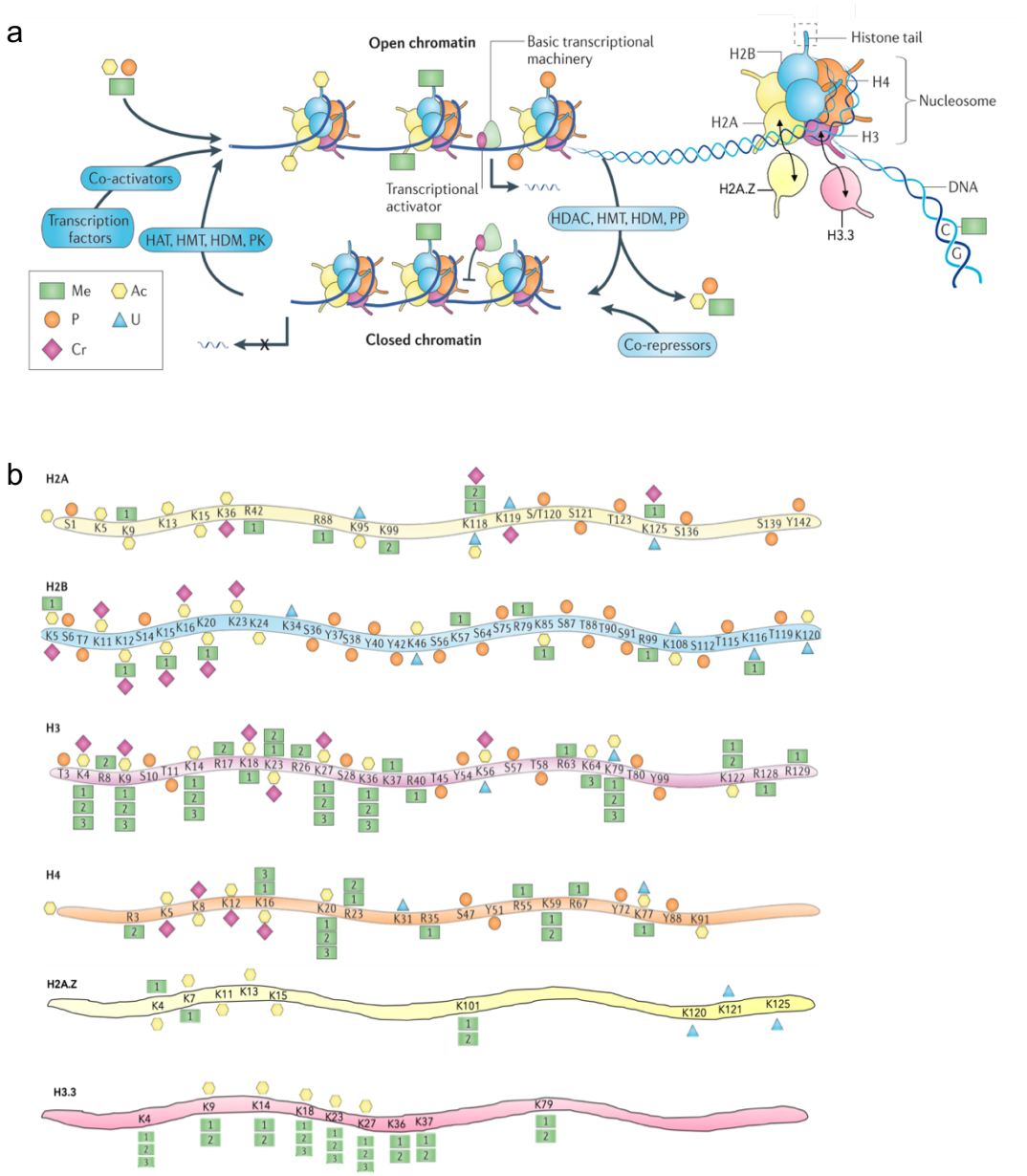

Figure 1.3 Schematic representation of chromatin and post-translational modification of histones. a) DNA is wrapped around an octamer of histone proteins. Post-translational modification of histone proteins alters the state of chromatin. In an open state, chromatin is transcriptionally active. The active state is characterized by hyperacetylation and hyperphosphorylation of histones. In a closed state, chromatin is transcriptionally repressed. The closed state is characterized by hypoacetylation and hypophosphorylation of histones. b) Known post-translational modifications to specific residues on histone protein tails. Modified from Gräff & Tsai (2013).

1.2.3 Acetylation of Non-Histone Proteins

It is important to note that many HATs and HDACs can also modify non-histone proteins including various transcription factors (p53, YY1, HMG proteins, signal transducer and activator of transcription 3 (STAT3), c-MYC, androgen receptor, estrogen receptor (ER), SHP, GATA binding factors, EKLF, MyoD, E2F/Rb, nuclear factor-κB (NF-κB), HIF-1α, and Smad7) and other cellular proteins (α-tubulin, importin-α, Ku70, heat shock protein 90 (Hsp90); Glozak, Sengupta, Zhang, & Seto, 2005). Therefore, HATs and HDACs can also regulate non-genomic cellular mechanisms and are more generically referred to as lysine acetyltransferases and lysine deacetylases, respectively.

1.2.4 Neuroepigenetics

Histone acetylation, and other epigenetic modifications, have long been implicated in the storage of information at a cellular level (Henderson, Shindo, & Dean, 2004; Jablonka & Lamb, 1998; Jablonka & Lamb, 1989; Smale, 2003). For example, some epigenetic patterns that are established during cellular differentiation, in response to environmental cues, are maintained for the lifespan of that cell and renewed in daughter cells. In this case, these 'stable' epigenetic modifications allow for the maintenance of distinct cellular phenotypes (e.g. liver or skin cell) in new generations of genotypically identical cells (Ehrenhofer-Murray, 2004; Levenson & Sweatt, 2005; Rakyan, Preis, Morgan, & Whitelaw, 2015). The identification of histone acetylation, and other epigenetic modifications, in the brain (Bondy, Roberts, & Morelos, 1970; Caspary & Sewell, 1968) led to the hypothesis that epigenetic mechanisms may be also involved in the storage of long-term behavioural memories (Crick, 1984; Griffith & Mahler, 1969; Holliday, 1999;

Levenson & Sweatt, 2005). David Sweatt's group (2010) coined the term 'neuroepigenetics' to refer to this adaptation of epigenetic modifications in the brain for the formation and storage of behavioural memories, which they specifically defined as a "…subfield of epigenetics that deals with the unique mechanisms and processes allowing dynamic experience-dependent regulation of the epigenome in nondividing cells of the nervous system, along with the traditionally described developmental epigenetic processes involved in neuronal differentiation and cell-fate determination." In agreement with this theory, histone acetylation has been shown to increase in response to neuronal activity (Crosio, Heitz, Allis, Borrelli, & Sassone-corsi, 2003; Maharana, Sharma, & Sharma, 2010); is involved in long-term potentiation (LTP), a cellular model of memory-related synaptic plasticity (Alarcon et al., 2004; Barrett et al., 2011; Levenson et al., 2004; Miller & Sweatt, 2007; Vecsey et al., 2007; Wood et al., 2005; Yeh, Lin, & Gean, 2004); is altered during memory consolidation (Schmitt & Matthies, 1979); and now has a very well-established role in the learning-induced formation of long-term behavioural memories (Day & Sweatt, 2011; Fischer, 2014; Gräff & Tsai, 2013; Sweatt, 2009).

1.2.4.1 Histone Acetylation and Memory

Levenson et al. (2004) provided the first demonstration that histone acetylation is induced in response to a learning experience. Contextual fear conditioning and latent inhibition increased the acetylation of H3 and H4, respectively, suggesting dissociable roles of epigenetic modifications for different facets of memory (Levenson et al., 2004). Changes in histone acetylation were shown to return to baseline 24h following fear conditioning. By increasing histone acetylation via the administration of histone

deacetylase inhibitors (trichostatin A (TSA) or sodium butyrate (NaBut)), Levenson et al. (2004) also demonstrated enhanced LTP and long-term contextual fear memory. These results suggest a specific role for histone acetylation during the time window for memory consolidation. Subsequently, others demonstrated that learning-induced increases in acetylation are accompanied by the increased expression of memory promoting genes (e.g. brain-derived neurotrophic factor (BDNF), CREB, Zif268; (Bredy et al., 2007; Koshibu et al., 2009; Lubin, Roth, & Sweatt, 2008) implicated in supporting various facets of long-term memory (Peixoto & Abel, 2013), including OR. Genetic and pharmacological manipulations of HDACs/HATs have broadly established that increased levels of histone acetylation (HDAC inhibition/HAT activation) enhance object memory, whereas decreased levels of histone acetylation (HDAC activation/ HAT inhibition) impair object memory (Fontán-lozano et al., 2008; Haettig et al., 2011; McQuown et al., 2011; Mitchnick et al., 2016; Oliveira et al., 2011; Roozendaal et al., 2010; Scott, Smith, Barker, Uney, & Warburton, 2017; Stefanko, Barrett, Ly, Reolon, & Wood, 2009; Zhao, Fan, Fortress, Boulware, & Frick, 2012; Zhao, Fan, & Frick, 2010). As the regulation of object memory by HATs is a major focus of this thesis, the involvement of three prominent HATs CBP, p300, and PCAF, in object memory will be discussed in greater detail.

CBP is a transcription factor named for its ability to bind c-AMP response element (CRE) sequences (Kalkhoven, 2004). CBP also demonstrates HAT activity and has HAT, KIX, bromo, and steroid receptor coactivator-1 interaction domains. Object recognition has been evaluated in a variety of different CBP mutant mice. For example, long-term OR impairments were observed in several different lines of CBP mutant mice (Alarcon et al.,

2004; Barrett et al., 2011; Korzus, Rosenfeld, & Mayford, 2004; Valor et al., 2011). Interestingly, CBP may have dissociable functions in HPC-dependent and independent memory. Wood, Attner, Oliveira, Brindle, & Abel (2006) and Stefanko et al. (2009) demonstrated that $CBP^{KIX/KIX}$ mice had impaired OR that was restored by treatment with the HDAC inhibitor NaBut, whereas object location impairments were not (Haettig et al., 2011; Roozendaal et al., 2010). Pharmacological manipulation of HATs has also been used to investigate the function of histone acetylation in object memory; these studies address some of the common limitations associated with the use of HAT mutants, including lack of regional specificity and selective inhibition of HAT activity. Mitchnick et al. (2016) demonstrated that selective inhibition of CBP (with C646) in the PRh and HPC impaired long-term object-in-place memory (24h retention delay).

A HAT highly homologous with CBP is p300. Like CBP, p300 has HAT, KIX, bromo, and steroid receptor coactivator-1 interaction domains and functions as a transcriptional coactivator and HAT (Kalkhoven, 2004). Oliveira et al. (2007, 2011) found OR impairments (24h delay) in p300Δ1 mutants, which lack carboxy-terminal HAT and activation domains, and in conditional knockout mice in which deletion of p300 was restricted to the post-natal forebrain. Zhao et al. (2012) corroborated a role of p300 in object memory by demonstrating that intra-HPC administration of garcinol (a semi-selective p300/PCAF inhibitor) is able to block estradiol-mediated enhancement of OR memory with a 48h retention delay. Furthermore, intra-HPC, but not intra-PRh, administration of plumbagin (a selective p300 inhibitor) impaired object-in-place memory (Mitchnick et al 2016).

PCAF, is a co-transcription factor and HAT with HAT, bromo, ADA2-interaction and CBP/p300 binding domains. PCAF knockout mice have impaired long-term OR memory; however, unlike CBP and P300 mutants, PCAF knockouts have impairments in short-term memory as well (Duclot, Jacquet, Gongora, & Maurice, 2010; Maurice et al., 2008). Specifically, $PCAF^{+/-}$ mice had impaired short-term object-in-place memory (3min delay) but intact short-term OR memory (Maurice et al., 2008). Since it is unlikely that changes in gene expression modulated by epigenetic mechanisms are established and able to modify behaviour during a 3min retention delay, these findings suggest PCAF modulate facets of short-term object memory via acetylation of non-histone proteins. Findings from our group also support this premise, as intra-HPC administration of embelin (a selective PCAF inhibitor) impaired both short- and long-term object-in-place memory in rats (20min delay, 24h delay; Mitchnick et al., 2016). Interestingly, intra-PRh embelin selectively impaired long-term object-in-place memory (Mitchnick et al., 2016).

These results establish a critical role for HATs in long-term object memory and suggest distinct roles for individual HATs in aspects of object memory that are mediated by different brain regions.

1.3 Histone Acetylation as a Therapeutic Target in Alzheimer's Disease

Neuropathological hallmarks of AD have been associated with dysregulated gene expression (Caldeira, Ferreira, & Rego, 2013). Specifically, increased expression of apoptotic genes (Selvi, Cassel, Kundu, & Boutillier, 2010) and decreased expression of learning and memory genes (Ding, Dolan, & Johnson, 2008; Sananbenesi & Fischer,

2009; Sanchez-Mut & Gräff, 2015; Zhang et al., 2012) have been reported; these aberrant patterns of gene expression in AD may be related to changes in histone acetylation.

1.3.1 Dysregulation of Histone Acetylation and HATs in Alzheimer's Disease

There is growing evidence that global levels of lysine acetylation and HAT proteins are abnormal in both human AD patients and transgenic AD mice (Cacabelos & Torrellas, 2014; Sanchez-Mut & Gräff, 2015). It is somewhat unclear, however, whether acetylation is increased or decreased, but reported inconsistencies may be related to differences in the brain regions examined as well as the stage of the disease in humans and the age and sex of the transgenic mice. Increased histone acetylation was observed in two studies, the first in post-mortem AD brains (Narayan, Lill, Faull, Curtis, & Dragunow, 2015) and the second in 3xTG primary neuron cultures (Walker, Laferla, Oddo, & Brewer, 2013), while others have reported no change in AD human brains (Lu et al., 2014; Rao, Keleshian, Klein, & Rapoport, 2012) and 3xTG mice (Cadena-del-Castillo et al., 2014). The bulk of the evidence however is consistent with decreased acetylation and repressed gene expression in AD as demonstrated by reduced HAT levels in AD mice (reference), increased HDAC levels in AD mice and post-mortem human brains (Gräff, Rei, et al., 2012; Rouaux, Jokic, Mbebi, & Boutillier, 2003; Saura et al., 2004) and decreased histone acetylation in post-mortem AD brains and transgenic AD mice (Francis et al., 2009; Govindarajan, Agis-Balboa, Walter, Sananbenesi, & Fischer, 2011; Zhang et al., 2012).

Prominent HATs also appear to directly interact with various elements of AD neuropathology including transcriptional regulation by Aβ, Aβ degradation, and inflammation (Fischer, 2014; Lu et al., 2014). Indeed, CBP and p300 levels are decreased

during APP signaling pathway-induced apoptosis (Rouaux et al., 2003), and PS1/PS2 dysfunction reduces the expression of CBP (Saura et al., 2004). In contrast, AD pathology can also drive activity of CBP and p300. For example, familial mutations in PS1 upregulate CBP and p300 activity (Marambaud et al., 2003). PCAF has also been implicated in the expression of genes that regulate Aβ degradation (Baek, 2002; Duclot, Meffre, Jacquet, Gongora, & Maurice, 2010) and has been shown to induce NF-kB-mediated inflammation (Duclot et al., 2010; Park et al., 2015, 2013).

Given the critical role of histone acetylation in memory formation and the apparent dysregulation of acetylation in AD, targeting histone acetylation in AD may be a promising therapeutic avenue (Cacabelos & Torrellas, 2014; Day & Sweatt, 2011; Fischer, 2014; Fischer, Sananbenesi, Mungenast, & Tsai, 2010; Gräff & Tsai, 2013; Peixoto & Abel, 2013; Selvi et al., 2010). Further, the clear interactions between HATs and AD pathology strongly suggests that, in addition to cognitive enhancement, the reinstatement of HAT activity has the potential to attenuate AD pathology.

1.3.2 Targeting Histone Acetylation for Attenuation of Cognitive Deficits in Rodent Models of Alzheimer's Disease

Histone acetylation was first shown to be a promising therapeutic target for cognitive impairment in neurodegenerative disease by Fischer, Sananbenesi, Wang, Dobbin, & Tsai, (2007) using the p25 mouse model of AD. p25 mice have pathological deregulation of cyclin-dependent kinase 5 (CDK5) and display amyloid and tau pathology, neurodegeneration and memory impairments (Fischer, Sananbenesi, Pang, Lu, & Tsai, 2005). In p25 mice, systemic treatment with the HDAC inhibitor, NaBut, restored

contextual fear learning and the retrieval of contextual fear memories (Fischer et al., 2007). Subsequent work continued to demonstrate memory enhancing effects of non-selective HDAC inhibitors in transgenic familial AD models (with mutations in APP and/or PS1 and MAPT): TSA restored H4 acetylation and improved contextual fear conditioning in APP/PS1 mice (Francis et al., 2009); phenylbutyrate restored H4 acetylation, enhanced dendritic spine density, improved spatial learning in the MWM, and reduced tau phosphorylation in Tg2576 mice (Ricobaraza, Cuadrado-tejedor, Marco, Perez-Otano, & Garcia-Osta, 2012; Ricobaraza et al., 2009); and NaBut increased histone acetylation in the HPC, increased expression of learning and memory genes in the HPC and cortex and restored contextual and cue fear memory, but did not reduce amyloid levels in aged APPPS1-21 mice (Govindarajan et al., 2011). Notably, HDAC inhibition appears to have a robust effect on memory enhancement; memory impairments were attenuated even in 15 month old mice with very advanced stages of AD pathology (Govindarajan et al., 2011).

More recent work has employed semi-selective or selective HDAC inhibitors in order to examine the role of individual HDACs and reduce toxic effects associated with broad HDAC inhibition (Boutillier, Trinh, & Loeffler, 2003; Salminen, Tapiola, Korhonen, & Suuronen, 1998). For example: HDAC inhibitors relatively selective for class 1 HDACs restored contextual fear in APP/PS1 mice (Kilgore et al., 2010); the semi-selective HDAC1 inhibitor entinostat improved nesting behaviour and reduced amyloid pathology in APP/PS1 mice (Zhang & Schluesener, 2013); the moderate HDAC1/ phosphodiesterase 5 (PDE5) inhibitor decreased amyloid and tau pathology, restored

dendritic spine density in the HPC, increased the expression of genes involved in synaptic transmission, and attenuated deficits in contextual fear conditioning and MWM (Cuadrado-Tejedor et al., 2015; Cuadrado-Tejedor et al., 2016); novel HDAC1 and 2 inhibitors W2 and I1 improved spatial memory and reduce both amyloid and tau pathology in 3xTG mice (Sung et al., 2013); and short hairpin RNA (shRNA) for HDAC2 increased H4 acetylation, expression of neuroplasticity genes, and enhanced spatial learning in the MWM in CK-p25, 5xFAD, and Cdk5 knockout mice (Gräff, Rei, et al., 2012).

Despite success in rodent models of AD, there are several limitations associated with using HDAC inhibitors to treat cognitive impairment. The mechanism of action of many HDAC inhibitors is poorly understood, broad spectrum HDAC inhibition can be toxic, few selective HDAC inhibitors are commercially available, memory enhancing effects of HDACs can be dependent on proper HAT function (Chen, Zou, Watanabe, Deursen, & Shen, 2010; Vecsey et al., 2007), and the primary function of HDACs is to remove acetyl groups added by HATs (Day & Sweatt, 2011). Therefore, perhaps modulation of specific HATs would be a more promising and direct therapeutic avenue.

Although selective HAT activators are commercially available (Chatterjee et al., 2013; Sbardella et al., 2008) and increasing lysine acetylation has positive effects on cognition, very few studies have evaluated the therapeutic potential of HATs in pre-clinical AD models. Caccamo, Maldonado, Bokov, Majumder, & Oddo (2010) increased CBP expression via a CBP-expressing lentivirus that was infused into the dorsolateral ventricles. Increased CBP expression attenuated MWM impairments in 6-month-old 3xTG mice, which was related to increased BDNF expression (Caccamo et al., 2010).

Similarly, Chatterjee et al. (2018) demonstrated that CSP-TTK21, a CBP and p300 activator, restores long-term depression (LTD), dendritic spine density, and MWM performance in 8-month-old THY-Tau22 transgenic mice. These restorative effects were shown to be related to increased acetylation of H2B and increased expression of plasticity and memory enhancing genes (Chatterjee et al., 2018). These results demonstrate that increasing the activity of the HATs CBP and p300 positively regulates cognition in mouse models of AD and is likely an effective therapeutic strategy.

Interestingly, PCAF may function atypically in AD. While PCAF activation enhances memory in normal rodents (Mitchnick & Winters, 2018; Wei et al., 2012), in Aβ-treated rodents, PCAF inhibition or KO attenuates AD-like cognitive deficits (Duclot et al., 2010; Park et al., 2015, 2013), suggesting that PCAF activity may actually be detrimental when disease is present. Indeed, the PCAF inhibitor, C-30-27, decreased Aβ-induced inflammation and cell death in BV-1 cells (Park et al., 2013); inhibited the non-histone acetylation of NF-κB in Aβ-treated rats and improved spatial water maze performance in Aβ-treated rats (Park et al., 2015). Similarly, Duclot et al. (2010) demonstrated that PCAF KO mice were protected against Aβ-induced toxicity following intra-ventricular Aβ treatment. PCAF KO prevented Aβ-induced changes in PS transcription and decreased neprilysin activity; increased somatostain expression, which may promote Aβ degradation; and prevented Aβ-induced impairments in spontaneous alteration and passive avoidance. These studies suggest that HAT activity and acetylation patterns in AD are likely complex and memory deficits may not always be ameliorated by simply

activating HATs. We hypothesize that PCAF functions bidirectionally in AD, initially benefitting memory in young/cognitively unimpaired mice but is detrimental as neuropathology/cognitive impairments become more severe.

1.4 Current Study

The major objective of this thesis was to determine if PCAF bidirectionally regulations object and spatial memory in an age-dependent manner in male and female 3xTG mice. To answer this question, four main behavioural objectives were developed. First, a longitudinal object memory phenotype was established by evaluating OR at 3, 6, 9, and 12-months-of-age (Chapter 3). As other groups have demonstrated progressive impairments in other object tasks, we predicted that 3xTG mice would display age-related OR impairments. Second, the effects of acute PCAF activation and inhibition on OR memory at 3, 6, 9, and 12 months of age were evaluated (Chapter 4). Based on our previous studies in healthy rats (Mitchnick et al., 2016; Mitchnick & Winters, 2018), we predicted that PCAF activation would be beneficial for OR memory and PCAF inhibition would impair OR memory in Wt mice and young/unimpaired 3xTG mice. Conversely, we predicted that PCAF activation would worsen OR memory in 3xTG mice as they developed OR impairments, whereas PCAF inhibition would ameliorate OR deficits (Duclot et al., 2010; Park et al., 2015). Third, a longitudinal spatial memory phenotype was established by evaluating OL at 3, 6, and 9-months-of-age (Chapter 5), where we predicted that 3xTG mice would develop age-related OL impairments. Because others have demonstrated spatial memory deficits prior to OR deficits (Billings, Green, McGaugh, & LaFerla, 2007; Gulinello et al., 2009) it was predicted that OL impairments

would be observed at earlier ages than OR impairments. Fourth, the effects of acute PCAF activation and inhibition on OL memory at 3, 6, and 9-months-of-age was evaluated (Chapter 6). Again, we predicted that PCAF activation would be beneficial for OL in Wt and young/unimpaired 3xTG mice, whereas PCAF inhibition would be beneficial in older/cognitively impaired 3xTG mice. In addition to the behavioural objectives, we began to probe the epigenetic underpinnings of the selective PCAF activator (SPV106) and inhibitor (embelin) used in our behaviour experiments (Chapter 7). We predicted that acute systematic SPV106 administration would increase histone acetylation, whereas embelin would decrease histone acetylation.

2 General Behavioural Materials & Methods

This chapter describes the methodology used in the behavioural experiments presented in Chapters 3-6.

2.1 Animals, Breeding, & Housing

Triple transgenic (3xTG) mice have a knockin of the familial Alzheimer's disease (AD) amyloid precursor protein (APP)Swe and PS2M146V genes as well as TauP301L, which, respectively, recapitulate amyloid and tau pathology in the central nervous system (CNS). Briefly, 3xTG mice were generated by co-injection of APPSwe and TauP301L, under the Thy1.2 promoter, into presenilin-1 (M146V) knock-in embryos (Oddo, Caccamo, Shepherd, et al., 2003). Original breeders were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) at approximately 2-months-of-age. Mice were bred homozygously in-house by Central Animal Facility staff at the University of Guelph. Offspring were earpunched for identification and subsequently weaned at 28 days of age.

Wild-type (B6129SF2/J male n=60, female n=60) and 3xTG (B6;129 Psen1tm1Mpm Tg(APPSwe,tauP301L)1Lfa/Mmjax; male n=60, female n=60) mice were used for behavioural testing. Given the sexual dimorphism in AD pathology (Carroll et al., 2007, 2010; Corder et al., 2004; Li & Singh, 2015; Mielke, Vemuri, & Rocca, 2014; Rocca, Amaducci, & Schoenberg, 1986; Ruitenberg, Ott, Van Swieten, Hofman, & Breteler, 2001), both male and female mice were used. An initial sample size of n=12 per experimental group was selected to compensate for behavioural variability and attrition; our previous work indicates power of 0.80 can be achieved with a sample size of 8 mice ($\alpha = 0.05$). Due to time and monetary constraints, most cohorts of mice were used for multiple experiments (Table 2.1).

Table 2.1: Cohorts of animals used for behavioural and molecular experiments

Cohort	Behavioural Experiments	Molecular Experiments
1	• Longitudinal OR (3, 6, 9, and 12-months-of-age) • Longitudinal OR with acute SPV16 (3, 6, 9, and 12-months-of-age) • OR with acute embelin (12+-months-of-age)	• 12+-months-of-age acute embelin
2	• OR with acute embelin (3-months-of-age)	• 6-months-of-age acute SPV106
3	• Longitudinal OL (3, 6, and 9-months-of-age • Longitudinal OL with acute SPV106 (3, 6, and 9-months-of-age) • OL with acute embelin (9+-months-of-age)	• 12-months-of-age acute SPV106
4	• OR pilot experiments with PCAF inhibitor L-Moses (data not shown)	• 9-months-of-age acute SPV106
5		• 3-months-of-age acute SPV106

Mice were group housed, when possible, in clear polyethylene cages (16x12x26 cm), with minimal environmental enrichment (corncob bedding, crink-l'Nest and cotton nest squares) and maintained on a 12h light/dark cycle (0800h lights on; 2000h lights off) at 26 ±2°C. Rodent chow (16% Tekland Global Protein Rodent Maintenance Diet, Harlan, Tekland, WI) and water were available ad libitum. All procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of Guelph.

2.2 Drugs

All compounds were administered via intraperitoneal (ip) injection. Drugs were given systemically to mimic systemic drugs administration that is used therapeutically. Mice were habituated to the injection procedure with two injections of physiological saline prior to the start of each behavioural experiment.

2.2.1 SPV106

SPV106 (2-Pentadecylidene-Propanedioic acid 1,3-diethyl ester, Pentadecylidenemalonate 1b; donated by Gianluca Sbardella, University of Salerno, Italy) is a selective PCAF activator and CBP inhibitor. While the exact mechanism of action is unclear, SPV106 has been shown to increase H3 and H4 acetylation (Milite et al., 2011; Sbardella et al., 2008). Acute SPV106 or vehicle (1% DMSO) treatments were administered three days pre-sample at 25 mg/kg. Six days were left between injections in within-subjects designs. This dosing regimen was based on our pilot data demonstrating OR impairment in aged 3xTG mice and findings from another group demonstrating behavioural enhancements following systemic SPV106 administration in healthy mice (Wei et al., 2012).

2.2.2 Embelin

Embelin (2,5-Dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione; Abcam) is a non-competitive specific PCAF antagonist. Embelin inhibits H3K9 acetylation by altering the catalytic activity of PCAF via acetyl-CoA-PCAF interactions (Modak et al., 2013). Acute embelin or vehicle (5% DMSO + 5%Tween 20) treatments were administered immediately post-sample (Mitchnick et al., 2016) at 20 mg/kg (3-months-of-age OR) or

10 mg/kg (9+ or 12+-months-of-age OR; 9+-months-of-age OL). A lower dose was used in aged mice because some toxic effects were observed with the 20mg/kg dose. At least three days were left between injections.

2.3 Behavioural Testing

Behavioural testing was conducted at 3, 6, 9, and 12-months-of-age. These timepoints were chosen based on previous experiments in the lab (Palmer & Winters, 2016), and the observation of bidirectional regulation of object and spatial memory by PCAF manipulation by 9 and 12-months-of-age.

2.3.1 Y-Apparatus Object Recognition

Object recognition (OR) was run in a modified Y-apparatus with walls 30.5 cm high, and arms 15 cm long and 7 cm wide constructed from white Plexiglas (Figure 2.1). The start arm of the Y-apparatus has a guillotine door 11 cm from the back of the arm that was closed at the beginning of each trial. When OR is conducted in the Y-apparatus, spatial information is minimized, allowing for direct evaluation of object identity processing (B. Winters et al., 2004). In the sample phase, mice were presented two identical objects to explore for 10 min. Following the sample phase, there was a 5-min or 3-h retention delay, to assess short- and long-term memory, respectively. At the end of the retention delay, mice underwent a 3-min choice phase, in which mice were presented with one object from the sample phase and a novel object. The order of object pairs, the designated sample and choice novel object within each pair, and the side of the apparatus (left or right) where the novel object was placed during the choice phase were counterbalanced.

2.3.1.1 Sub-Optimal Object Recognition Task

In order to evaluate the memory enhancing effects of pharmacological compounds in animals with intact OR (Chapter 4) sub-optimal OR was carried out. In sub-optimal OR, rodents did not receive sufficient exposure to objects during the sample phase to learn and express OR memory in the choice phase. We reduced the sample phase duration to either 2min or 4min for 5min and 3h retention delays. Under these conditions, in pilot experiments, Wt mice did not demonstrate OR memory.

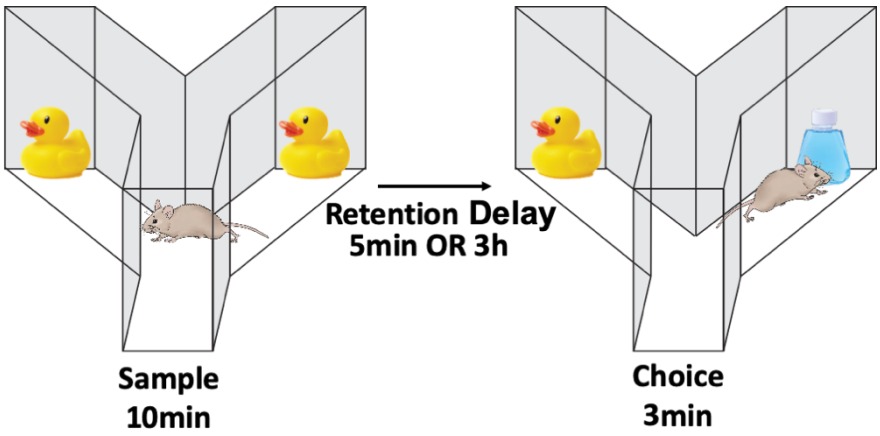

Figure 2.1 Schematic representation of Y-appartaus OR. In the sample phase the rodent explores two identical objects. Following a variable retention delay, in the choice phase, the rodent may explore an identical copy of the sample object or a novel object. Memory is inferred by preferential exploration of the novel object in the choice phase. Our group favours the use of a modified Y-shaped apparatus to minimize extraneous spatial and contextual cues.

2.3.2 Object Location

The object location (OL) task was used to evaluate spatial object memory in the open-field using the same testing parameters as OR, except that one object from the sample phase was moved to an adjacent corner of the arena in the choice phase (Figure

2.2); in this case, the location of one object, rather than its identity, is novel. In OL, the order of object pairs and the side of the apparatus (left or right) where the novel object was placed during the choice phase were counterbalanced.

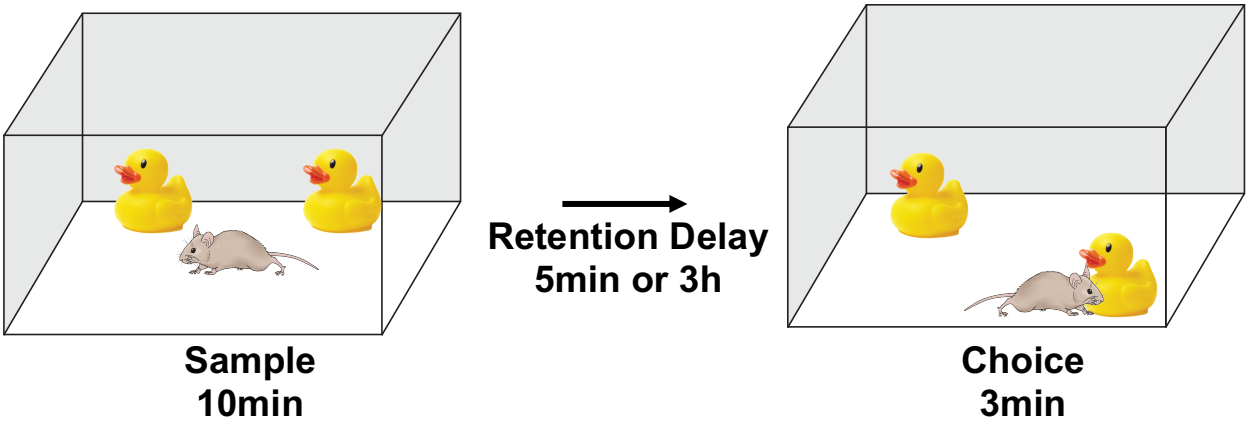

Figure 2.2 Schematic representation of OL. In the sample phase the rodent explores two identical objects. Following a variable retention delay, in the choice phase, the rodent explores copies of the sample objects. One object remains in the sample position as the sample phase, while the other object is positioned in a novel location. Memory is inferred by preferential exploration of the object in the novel location during the choice phase.

2.3.3 Objects

Objects had no apparent biological significance to mice and were distinct in size (5-15 cm tall), material (glass, metal, and plastic), and colour (Figure 2.3). To prevent object displacement by mice during testing all objects were fixed to the floor of the testing apparatus with white adhesive putty. Prior to each behavioural trial objects were wiped with 50% ethanol using a paper towel.

Figure 2.3 Examples of objects used in OR and OL tasks.

2.3.4 General Behavioural Procedure

All behavioural testing was conducted under white fluorescent light in a 190 cm by 145 cm room with white walls and an orange door. A television, computer modem, and computer monitor sat atop metal shelving. A few additional visual cues were fixed to the walls. The apparatuses were placed on the floor away from the walls.

Prior to behavioural testing all mice were extensively handled and habituated to an empty testing apparatus for 10 min on two consecutive days. Behavioural testing began at least 24 h after the second habituation day. Immediately prior to testing, mice were brought into the testing room in their home cage. Mice were placed in either the start arm of the Y-apparatus or in a start box placed in the center of the open field. The trial began when the start arm or box was opened or removed, respectively, and the mouse began to explore. During testing, an experimenter blind to the experimental condition, viewed the mouse on a television screen and pressed a key corresponding to a given object at the onset and end of an exploratory bout (sniffing within 1 cm of the object and/ or touching the object with the nose). Between trials, objects were wiped with 50% ethanol (to eliminate olfactory cues), the testing apparatus was wiped with dry paper towel and the mouse was returned to its home cage.

2.3.5 Behavioural Data Analysis

For both OR and OL tasks, the novelty preference in the choice phase was quantified by calculating a discrimination ratio (DR = (novel object exploration – familiar object exploration)/(total object exploration)). Split-plot analysis of variance (ANOVA) was used to analyze DRs with retention delay and/drug as within-subjects factors and sex and

genotype as between-subjects factors. Only significant effects are reported. Where appropriate, post-hoc *t*-tests were used to analyze group differences. In the sample phase, objects should be equally novel and a DR of approximately zero is expected. The sample DR was calculated as DR = (object in the location that the novel object in the choice phase will be placed in – object in the location that the familiar object in the choice phase will be placed in)/(total object exploration). In the choice phase, a DR significantly greater than the sample DR indicates novelty preference, from which we infer intact memory. Paired samples t-tests were used as a complementary analysis to compare sample and choice DRs, as a significant increase in the DR from sample to choice is indicative of intact memory.

We also examined total exploratory behaviour (left object exploration + right object exploration) using Split-plot ANOVAs. Only significant effects on total exploration are reported. Pearson product moment correlation coefficients were used to examine the relationship between total exploration (sample or choice) and novelty preference (choice DR).

Outliers (>2 SD ± mean) were excluded from analyses. All statistical analyses were conducted with a significance level of $\alpha = .05$, unless otherwise specified, using IBM SPSS statistics. Where appropriate, the Bonferroni correction was applied.

3 Longitudinal Object Memory Phenotype

3.1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment and hallmark pathological accumulation of amyloid beta (Aβ) and phospho-tau (Alzheimer, 1911). Early onset, familial, AD is caused by mutations in the amyloid precursor protein (APP) or presenilin (PS1, PS2) genes, which initiate a pathological cascade by inducing excessive Aβ production (Ertekin-Taner, 2007). Many transgenic mouse models of AD express one or more of these causative human AD transgenes and can provide valuable insight into molecular and cognitive abnormalities seen in human patients. The triple transgenic (3xTG) mouse model of AD expresses human APP (K670N/M671L) and PS1 (M146V) mutations associated with amyloid pathology in addition to a human tauopathy mutation (tauP301L; Oddo, Caccamo, Shepherd, et al., 2003). As the mice age, their cognitive abilities decline, and their brains display progressive amyloid and tau pathology and neurodegeneration which suggests this model can provide valuable insight into molecular and cognitive abnormalities seen in human patients.

Object recognition (OR) tasks evaluate declarative-like memory in rodents and are both procedurally and neurobiologically similar to human tests of recognition memory, which are impaired in human AD patients (Didic et al., 2013, 2010; Ennaceur & Delacour, 1988). Object recognition deficits have been reported in numerous mouse models of AD (Grayson et al., 2015), including 3xTG mice (for example: Arsenault et al., 2011; Blanchard et al., 2010; Chen et al., 2014; Chiquita et al., 2019; Creighton et al., 2019; Davis et al., 2013; Davis et al., 2013; Feld et al., 2014; Filali et al., 2012; Gulinello et al.,

2009; Guzmán-Ramos et al., 2012; Janczura et al., 2018; Kazim et al., 2014; Masciopinto et al., 2012; Onishi et al., 2011; Parachikova et al., 2010; Shih et al., 2018; St-Amour et al., 2014; Stover et al., 2015; Tournissac et al., 2018). However, there are often discrepancies in the specific nature of object memory deficits reported (Grayson et al., 2014; Simón et al., 2009; Taglialatela, Hogan, Zhang, & Dineley, 2009), and some studies have failed to find impairments (Cheng, Low, Logge, Garner, & Karl, 2014; Davis, Eacott, Easton, & Gigg, 2013; Davis, Easton, et al., 2013; Fragkouli, Tsilibary, & Tzinia, 2014; Good, Hale, & Staal, 2007; Gulinello et al., 2009; Hale & Good, 2005; Karl, Bhatia, Cheng, Kim, & Garner, 2012; Yassine et al., 2013). Differential performance on OR tasks may be related to procedural differences that alter the conceptual nature of the task and thus tax distinct behavioural processes. Discrepancies in the mnemonic index, the length of the retention delay, the age of behavioural testing and/or the choice of sex and transgenic model may also explain discrepant OR findings. We have recently addressed some of these limitations by performing a systematic analysis of “object processing” in two prominent mouse models of AD, 5xFAD and 3xTG. Specifically, we evaluated performance on several tasks that manipulate different types of object information: object identity (i.e., object recognition: OR), spatial processing (object location; OL), temporal processing (temporal order; TO), and multisensory perception (multisensory object oddity; MSO) using consistent retention delays in 12-month-old male and female 5xFAD and 3xTG mice (Creighton et al., 2019). Results demonstrated a multifaceted impairment in object processing. Memory for object identity was impaired in 5xFAD males and females when the retention delay was 5min or 3h. 3xTG males were impaired on open-

field OR at 5min and 3h, whereas females were selectively impaired at 3h; when spatial and contextual cues were minimized during OR, using a modified Y-apparatus, 5xFAD males and females are impaired at 5min and 3h, and 3xTG males and females were selectively impaired on Y-apparatus OR at 3h. OL was impaired in 5xFAD and 3xTG males and females with 5min and 3h delays. TO was also impaired in wild-type (Wt) and transgenic 5xFAD females and transgenic 3xTG females at 3min. Lastly, multisensory perception was impaired in 5xFAD females as well as 3xTG males and females, despite intact basic visual and tactile object perception. 5xFAD males, however, were also impaired on visual and tactile object perception and 5xFAD Wt males had impaired tactile perception. These results highlight the advantages of using object-based tasks to evaluate cognition in transgenic AD models, as interesting dissociations across transgenic AD strains, sex, and type of object task corresponded with underlying alterations in synaptic markers, Aβ, tau hyperphosphorylation, and gliosis in task-relevant brain regions (Creighton et al., 2019). However, these experiments were exclusively performed in aged animals with severe AD pathology, and more information is required regarding the development and progression of the cognitive phenotype in 3xTG mice as they age.

Relatively few studies have evaluated object memory at more than one age in transgenic AD mice (Otalora et al., 2012; Chiquita et al., 2019; Davis et al., 2013; Dodart et al., 2002; Dodart et al., 1999; Guzmán-Ramos et al., 2012; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006; Mori et al., 2012; Mouri et al., 2007; Ryan et al., 2013; Simón et al., 2009; Simón et al., 2009; St-Amour et al., 2014; Webster et al., 2013;

Zhang et al., 2014, 2006). Because AD pathology becomes more severe with progressive age (examples in 3xTG mice: Da Cruz et al., 2012; Manaye et al., 2013; Mastrangelo & Bowers, 2008; Nicholson et al., 2010; Oddo et al., 2003; Sy et al., 2011), longitudinal analysis of object memory should provide valuable insight into the relationship between AD pathology and behavioural deficits. Generally, in 3xTG mice, object memory deficits are reported around 6-months-of-age (Chen et al., 2014; Davis et al., 2013; Feld et al., 2014), but are dependent on the specific nature of the task. These tasks were all conducted in an open-area where spatial information may be involved in task performance. Previous findings suggest that object memory impairments can become more severe when the spatial nature of the task is increased (Creighton et al., 2019). For this reason, our group favours the use of a modified Y-apparatus to selectively evaluate object memory by minimizing potentially confounding spatial and contextual cues (Winters et al., 2004). Here, we longitudinally evaluated OR, using the Y-apparatus, in male and female 3xTG mice at 3,6,9 and 12-months-of-age. We report an age-dependent impairment in OR memory in 3xTG mice that was first observed at 9-months-of-age.

3.2 Methods

Object recognition was longitudinally evaluated every 3 months from 3- to 12-months-of-age in male and female Wt and 3xTG mice. Methodological details for behavioural testing and data analysis are described in chapter 2.

3.2.1 Experiments

Experiment 1 was designed to evaluate OR memory at 3-months-of-age. Using the Y-apparatus, during the sample phase, mice were presented two identical objects to

explore for 10-min. Following the sample phase, there was a 5-min or 3-h retention delay, to assess short- and long-term memory, respectively. At the end of the retention delay, mice underwent a 3-min choice phase, in which mice were presented with one object from the sample phase and a novel object.

Experiment 2 was designed to evaluate OR memory at 6-months-of-age. Experiment 2 was run identically to experiment 1.

Experiment 3 was designed to evaluate OR memory at 9-months-of-age. Experiment 3 as run identically to experiments 1 and 2.

Experiment 4 was designed to evaluate OR memory at 12-months-of-age. Experiment 4 was run identically to experiments 1, 2, and 3.

3.3 Results

3.3.1 Longitudinal OR Phenotype in 3xTG mice

Object recognition was intact at 3-months-of-age in male and female 3xTG mice, a 2×2×2 split-plot analysis of variance (ANOVA) revealed a significant main effect of sex ($F_{1,44}$ = 5.261, p = .027), but not genotype or delay; Figure 3.1a. Paired samples t-tests between sample and choice discrimination ratio (DR) suggest intact memory in all Wt and 3xTG mice (Wt males, 5min: t_{11} = –6.430, p < .001; Wt males 3h: t_{11} = –6.228, p < .001; Wt females, 5min: t_{11} = –6.601, p <.0001; Wt females, 3h: t_{11} = –3.159, p = .009; 3xTG males, 5min: t_{11} = –7.084, p < .001; 3xTG males, 3h: t_{11} = –4.776, p = .001; 3xTG females,

5min: $t_{11} = -2.993$, $p = .012$; 3xTG females, 3h: $t_{11} = -3.821$, $p = .003$), as they significantly discriminated between novel and sample objects.

Object recognition remained intact at 6-months-of-age in male and female 3xTG mice, a 2×2×2 split-plot ANOVA revealed no significant interactions or main effects of genotype, sex, or delay; Figure 3.1b. Paired samples t-tests between sample and choice DR suggest intact memory in all Wt and 3xTG mice (Wt males, 5min: $t_{11} = -4.659$, $p = .001$; Wt males 3h: $t_{11} = -7.082$, $p < .001$; Wt females, 5min: $t_{11} = -2.846$, $p = .016$; Wt females, 3h: $t_{11} = -4.573$, $p = .001$; 3xTG males, 5min: $t_{11} = -5.913$, $p < .001$; 3xTG males, 3h: $t_{11} = -7.143$, $p < .001$; 3xTG females, 5min: $t_{10} = -5.453$, $p < .001$; 3xTG females, 3h: $t_{10} = -2.789$, $p = .019$), as they significantly discriminated between novel and sample objects.

At 9-months-of-age OR was delay-dependently impaired in male and female 3xTG mice, a 2×2×2 split-plot ANOVA revealed a significant genotype × delay interaction ($F_{1,42} = 16.319$, $p < .001$), a significant main effect of genotype ($F_{1,42} = 51.384$, $p < .001$), a significant main effect of sex ($F_{1,42} = 6.359$, $p = .016$), and a significant main effect of delay ($F_{1,42} = 18.271$, $p < .001$); Figure 3.1c. *Post hoc* t-tests revealed a significant difference between 3xTG mice at 3h and all other groups (Wt, 5min: $t_{44} = 6.631$, $p < .001$; Wt, 3h: $t_{44} = 6.543$, $p < .001$; 3xTG, 5min: $t_{21} = 5.021$, p < .001). Paired samples t-tests between sample and choice DR indicate intact memory in Wt mice (males, 5min: $t_{11} = -7.287$, $p < .001$; males 3h: $t_{11} = -10.970$, $p < .001$; females, 5min: $t_{11} = -5.787$, $p < .0001$; females, 3h: $t_{11} = -3.737$, $p = .003$, and 3xTG mice at 5min (males: $t_{10} = -5.475$, $p < .001$;

females: $t_{10} = -6.381$, $p < .001$), but impaired memory in 3xTG mice at 3h, as they failed to significantly discriminate between novel and sample objects (males: $t_{10} = .262$, $p = .799$; females: $t_{10} = 1.232$, $p = .246$).

At 12-months-of-age OR was still delay-dependently impaired in male and female 3xTG mice, a 2×2×2 split-plot ANOVA revealed a significant genotype × delay interaction ($F_{1,40} = 12.202$, $p = .001$), a significant main effect of genotype ($F_{1,40} = 11.548$, $p = .002$), and a significant main effect of delay ($F_{1,40} = 34.460$, $p < .001$); Figure 3.1d. *Post hoc t*-tests revealed a significant difference between 3xTG mice at 3h and all other groups (Wt, 5min: $t_{42} = 5.775$, $p < .001$; Wt, 3h: $t_{26.783} = 3.886$, $p = .008$; 3xTG, 5min: $t_{19} = 5.309$, p < .001). Paired samples t-tests between sample and choice DR suggest intact memory in Wt mice (males, 5min: $t_{11} = -10.675$, $p < .001$; males, 3h: $t_{11} = -10.717$, $p < .001$; females, 5min: $t_{11} = -6.974$, $p < .001$; females, 3h: $t_{11} = -3.942$, $p = .002$), and 3xTG mice at 5min (males: $t_9 = -6.402$, $p < .001$; females: $t_9 = -6.834$, $p < .001$), but impaired memory in 3xTG mice at 3h (males: $t_9 = -.106$, $p = .918$; females: $t_9 = -1.271$, $p = .236$).

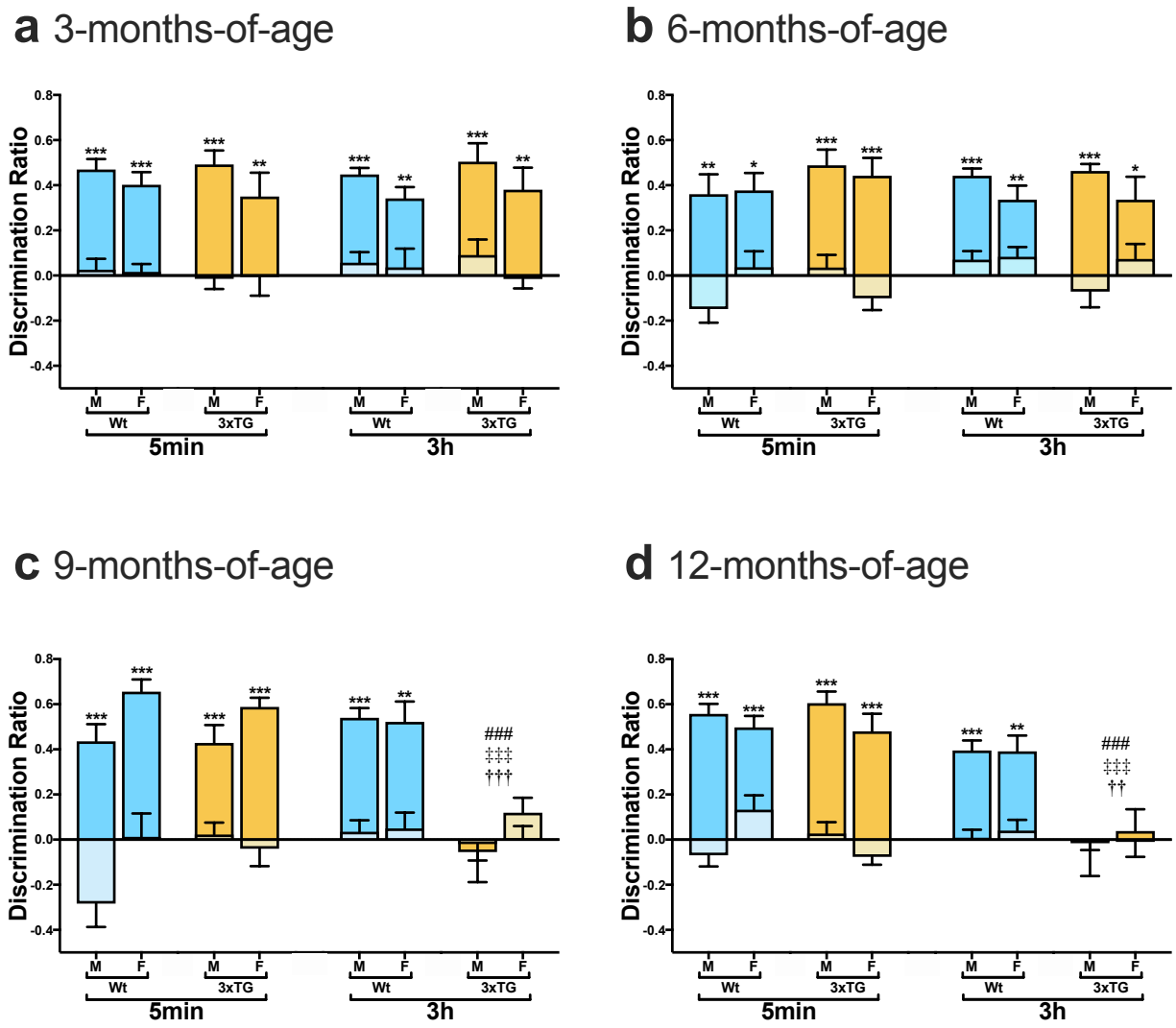

Figure 3.1 Longitudinal OR phenotype. a) 3xTG mice have intact OR at 3-months-of-age. b) 3xTG mice have intact OR at 6-months-of-age. c) 3xTG mice are delay-dependently impaired on OR at the 3h, but not 5min, delay at 9-months-of-age. d) 3xTG mice are delay-dependently impaired on OR at the 3h delay at 12-months-of-age. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR. ### p< .001 significantly different from Wt mice at 5min, ‡‡‡ p < .001 significantly different from 3xTG mice at 5min, and †† p< .01, ††† p< .001 significantly different from Wt mice at 3h.

3.3.1.1 Control Measure: OR Exploratory Behaviour

At 3-months-of-age a 2×2×2 split-plot ANOVA revealed significant main effects of genotype ($F_{1,44}$ = 41.353, p < .001) and sex ($F_{1,44}$ = 9.151, p = .004) on exploratory behaviour during the sample phase; Table 3.1. 2×2×2 split-plot ANOVA also revelated a significant main effect of genotype ($F_{1,44}$ = 40.954, p < .001) on exploratory behaviour during the sample phase.

At 6-months-of-age a 2×2×2 split-plot ANOVA revealed no significant interactions or main effects of genotype, sex, or delay on exploration during the sample phase. However, 2×2×2 split-plot ANOVA revealed a significant genotype interaction × delay ($F_{1,43}$ = 5.261, p = .027) and a main effect of genotype ($F_{1,\ 43}$ = 8.298, p = .006) on exploration during the choice phase. *Post hoc* tests revealed a significant difference between Wt exploration with 5min and 3h retention delays (t_{23} = 4.059, p < .001).

At 9-months-of-age a 2×2×2 split-plot ANOVA revealed a significant genotype × delay interaction ($F_{1.42}$ = 9.112, p = .004) on exploration during the sample phase. *Post hoc* analyses revealed a significant difference between Wt and 3xTG exploration at 3h ($t_{40.076}$ = 2.899, p = .048). A 2×2×2 split-plot ANOVA revealed a significant genotype × delay interaction ($F_{1,42}$ = 4.277, p = .045) and a sex × delay interaction ($F_{1,42}$ = 5.137, p = .029).

At 12-months-of-age a 2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,40}$ = 5.970, p = .019) on exploration during the same phase. A

2×2×2 split-plot ANOVA revealed no significant interactions or main effects on exploration during the choice phase.

Table 3.1 Longitudinal OR exploratory behaviour

		Mean Sample Exploration				Mean Choice Exploration			
		Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	63.28 (4.65)	45.84 (4.92)	29.46 (4.07)	25.78 (3.07)	17.64 (2.03)	13.20 (1.71)	7.82 (1.35)	6.22 (0.83)
	3h	53.25 (3.93)	38.43 (5.67)	31.60 (6.04)	26.35 (2.53)	13.72 (1.34)	12.00 (1.99)	7.09 (0.74)	6.98 (0.88)
6	5min	35.35 (6.23)	32.28 (3.85)	36.41 (5.97)	29.41 (4.70)	17.46 (2.55)	15.37 (1.98)	14.22 (2.36)	13.63 (1.82)
	3h	39.92 (7.47)	27.34 (2.56)	36.77 (3.98)	32.79 (4.75)	10.63 (1.71)	10.37 (1.71)	13.89 (2.35)	12.61 (3.80)
9	5min	28.86 (5.98)	22.70 (5.36)	32.88 (5.25)	25.64 (4.28)	3.76 (1.11)	7.43 (1.31)	8.27 (1.39)	10.49 (2.20)
	3h	30.98 (4.01)	30.68 (4.31)	20.80 (2.44)	20.59 (3.24)	10.09 (2.20)	7.34 (1.14)	8.98 (1.69)	8.03 (1.10)
12	5min	30.74 (3.66)	19.86 (3.27)	24.67 (4.32)	30.87 (2.82)	8.53 (2.66)	6.75 (1.32)	7.71 (2.21)	6.37 (0.71)
	3h	27.09 (3.13)	19.50 (2.47)	31.34 (5.83)	25.16 (4.22)	9.19 (1.63)	6.87 (1.33)	5.58 (0.84)	8.03 1.17)

Note. Data are mean exploration (s) ± SEM.

3.3.1.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Despite the differences in exploratory behaviour that were observed at each timepoint, exploration did not consistently correlate with OR performance (Choice DR; Table 3.2). Though, there was a significant positive correlation between total sample exploratory behaviour and choice DR for Wt females at 3h (r_{12} = .807, p = .016) at 3-months-of-age as well as a significant negative correlation between sample exploratory

behaviour and choice DR for 3xTG females at 5min (r_{11} = −.772, p = .04) at 9-months-of-age.

Table 3.2 Correlations between exploration and longitudinal OR phenotype

		Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
		Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	−.462	−.280	.083	−.087	−.389	−.401	−.663	−.222
	3h	.187	.807*	−.593	−.167	−.140	.235	.119	.252
6	5min	.222	.467	−.101	−.498	.481	.304	−.249	−.158
	3h	.015	−.119	.034	−.444	.191	.304	−.327	−.244
9	5min	−.310	.065	−.096	−.772*	.147	.120	−.083	−.566
	3h	−.297	.393	.430	.008	−.370	−.002	.330	.225
12	5min	.056	−.310	−.231	−.785	−.441	−.452	.087	−.204
	3h	−.078	−.115	.092	.256	.013	.251	.630	−.555

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR). * p < .05.

3.4 Discussion

These experiments demonstrate progressive impairments in OR memory in male and female 3xTG mice. Specifically, OR was intact in 3xTG mice at 3 and 6-months-of-age but was delay-dependently impaired at 9 and 12-months-of-age. The selective OR impairments that we observed with a 3h, but not 5min, retention delay are suggestive of a selective deficit in long-term memory that is consistent with our previous work in aged 3xTG mice (Creighton et al., 2019).

In agreement with previous work in transgenic AD mice, we have observed age-dependent impairments in OR (Otalora et al., 2012; Chiquita et al., 2019; Davis et al., 2013; Dodart et al., 2002; Dodart et al., 1999; Guzmán-Ramos et al., 2012; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006; Mori et al., 2012; Mouri et al., 2007; Ryan et al., 2013; Simón et al., 2009; Simón et al., 2009; St-Amour et al., 2014; Webster et al., 2013; Zhang et al., 2014, 2006). However, the age at which we first observed OR impairments (9-months-of-age) was significantly later than what other groups typically report (6-months-of-age in 3xTG mice; Blanchard et al., 2010; Chen et al., 2014; Davis et al., 2013; Davis et al., 2013; Feld et al., 2014; Stover et al., 2015). It is likely that procedural differences contributed to this discrepancy. For example, most groups evaluate OR in an open-field area but our group favours the use of a modified Y-apparatus. We have previously suggested that impairments on OR tasks become more severe in 3xTG mice when the spatial nature of the task is increased, and that testing OR in a Y-apparatus likely increases the focus on objects and provides fewer potentially interfering contextual stimuli (Creighton et al., 2019). Although the hippocampus (HPC) appears to be necessary for OR in mice (Cohen et al., 2013; Hammond, Tull, & Stackman, 2004), we believe the open-field likely places a greater demand on the HPC, which is affected by amyloid and tau pathology more severely and at earlier ages than other brain regions (e.g. perirhinal cortex) involved in OR (Creighton et al., 2019; Davis et al., 2013; Oddo, Caccamo, Shepherd, et al., 2003).

Given the established behavioural and pathological sex differences in 3xTG mice (Carroll & Pike, 2008; Carroll et al., 2007, 2010; Clinton et al., 2007; Fertan, Wong,

Vienneau, & Brown, 2019; Hirata-Fukae et al., 2008; Levin-allerhand, Lominska, Wang, & Smith, 2002), it was somewhat surprising that we did not observe any major sex differences between male and female 3xTG mice. Since we have observed subtle sex differences in 3xTG mice using a similar object task (Creighton et al., 2019), it is possible that the specific task or timepoints used in these experiments were not able to capture these differences. We did, however, observe subtle sex differences between male and female mice (Wt and 3xTG), where males generally performed better on OR than females (3 and 6-months-of-age). This is in agreement with previous work demonstrating superior OR performance in males (Frick & Gresack, 2003).

Overall, this systematic characterization of OR across age and sex has clarified the nature of OR deficits in 3xTG mice and defined relevant time points for the evaluation of novel therapeutic compounds in 3xTG mice.

4 Age-dependent Bidirectional Regulation of Object Memory by PCAF

4.1 Introduction

Histone acetylation, one of the most extensively studied epigenetic modifications, promotes gene expression via the addition of acetyl groups, by histone acetyltransferases (HATs), to lysine residues on histone tails around which DNA is wrapped, thereby promoting access of transcriptional machinery to the DNA (Kuo & Allis, 1998). Histone deacetylation, catalyzed by histone deacetylases (HDACs), removes acetyl groups from histone proteins and induces gene repression (De Ruijter et al., 2003). Both HAT activation and HDAC inhibition increase histone acetylation, which regulates the expression of genes that support many facets of learning and memory (Gräff & Tsai, 2013; Peixoto & Abel, 2013).

There is increasing evidence that dysregulation of histone acetylation plays a role in cognitive deficits and neuropathology in neurological disorders like Alzheimer's disease (AD; Fischer, 2014; Selvi et al., 2010; Stilling & Fischer, 2011). Research into the memory-enhancing effects of increased histone acetylation in AD has predominantly focused on HDAC inhibition. In transgenic mouse models of AD, global and selective HDAC inhibition have been shown to attenuate behavioural deficits in contextual fear conditioning (Francis et al., 2009), Morris water maze (MWM; Ricobaraza et al., 2009; Sung et al., 2013), and nesting behaviour (Zhang & Schluesener, 2013), as well as restore levels of histone acetylation (Francis et al., 2009; Ricobaraza et al., 2009) and decrease amyloid and tau pathology (Sung et al., 2013; Zhang & Schluesener, 2013). However, global HDAC inhibition provides limited mechanistic insight and can be toxic (Salminen et al., 1998). Since the primary function of HDACs is to remove acetyl groups added by

HATs (Day & Sweatt, 2011b), and the effectiveness of HDAC inhibition can be dependent on the function of specific HATs (Chen et al., 2010; Vecsey et al., 2007), targeting specific HATs may be a better therapeutic approach.

CREB-binding protein (CBP), E1A-binding protein (p300), and p300/CBP associated factor (PCAF) are three prominent HATs implicated in both memory and AD. CBP and p300 are highly homologous HATs with apparent roles in AD-like pathology. CBP overexpression was found to attenuate memory impairments in 6 month old triple transgenic (3xTG) mice, by increasing brain-derived neurotrophic factor (BDNF) expression (Caccamo et al., 2010). Similarly, activation of CBP and p300, with CSP-TTK21, restored long-term depression, dendritic spine density, and MWM performance in THY-Tau22 transgenic mice (Chatterjee et al., 2018). However, others have shown that CBP and p300 levels are decreased during amyloid precursor protein (APP) signaling -induced apoptosis (Rouaux et al., 2003), and presenilin (PS)1/PS2 dysfunction reduces expression of CBP (Saura et al., 2004). In contrast, AD pathology can also drive activity of CBP and p300. For example, familial mutations in PS1 upregulate CBP and p300 activity (Marambaud et al., 2003), and familial APP mutations increase p300 expression (Lu et al., 2014). In AD brains, acetylation of p300 substrates is increased and phosphorylated p300 has also been shown to co-localize with phospho-tau (Aubry et al., 2015).

PCAF has also been shown to interact with elements of AD pathology and modulate cognition in mouse models of AD. The PCAF inhibitor, C-30-27, decreased Aβ-

induced inflammation and cell death, inhibited the non-histone acetylation of nuclear factor kappa B (NF-κB), as well as improved MWM performance (Park et al., 2015, 2013). Comparably, Duclot et al. (2010) demonstrated that PCAF KO mice were protected against Aβ-induced toxicity and Aβ-induced increases in PS1 and neprilysin activity; had increased somatostatin expression, which may promote Aβ degradation; and lacked Aβ-induced impairments in spontaneous alteration and passive avoidance, following intra-ventricular Aβ treatment. Interestingly, these findings conflict with what is seen in healthy rodents and suggests that PCAF may function atypically in AD. In healthy rodents, PCAF has been shown to regulate cognition positively. For example, PCAF inhibition or KO impairs performance on several mnemonic tasks (Duclot et al., 2010; Maurice et al., 2008; Mitchnick et al., 2016). PCAF activation has also been shown to enhance memory in wildtype mice (Wei et al., 2012); specifically, selective PCAF activation, via SPV106 (Sbardella et al., 2008), enhanced memory for fear extinction (Wei et al., 2012). Therefore, more research is necessary to clarify the function of PCAF in AD-relevant cognition.

We hypothesize that PCAF functions bidirectionally in AD, initially benefitting memory in young/cognitively unimpaired mice but is detrimental as neuropathology/cognitive impairments become more severe. Here, we longitudinally explored the effects of PCAF activation and inhibition on object recognition (OR) memory in male and female 3xTG mice at 3,6,9 and 12-months-of-age using the PCAF activator SPV106 (Sbardella et al., 2008) and the selective PCAF inhibitor embelin (Modak et al., 2013). We demonstrate that PCAF bidirectionally regulates cognition in male and female

3xTG AD mice.Prior to the development of OR deficits, the PCAF activator, SPV106, enhanced short- (5min) and long-term (3h) OR, whereas the PCAF inhibitor, embelin, impaired. When OR impairment was first observed, SPV106 ameliorated OR deficit. However, eventually SPV106 induced a more severe OR impairment, while embelin ameliorated the OR deficit. These experiments reveal a complex role for PCAF throughout AD progression, initially benefitting memory but detrimental as neuropathology becomes more severe.

4.2 Methods

Object recognition was longitudinally evaluated every 3 months from 3- to 12-months-of-age in male and female wild-type (Wt) and 3xTG mice. Methodological details for behavioural testing and data analysis are described in chapter 2.

4.2.1 Experiments

Experiment 1 was designed to evaluate the memory enhancing effects of PCAF activation on OR memory at 3-months-of-age. Three days prior to behavioural testing, mice were treated acutely with the PCAF activator, SPV106 (25mg/kg intraperitoneal (i.p); Wei et al., 2012), or vehicle (1% DMSO in saline i.p.). Using the Y-apparatus, we ran sub-optimal OR, where mice did not receive sufficient exposure to objects during the sample phase to learn and expression OR memory in the choice phase. The sample phase was either 2min or 4min for the 5min and 3h retention delays, respectively. At the end of the retention delay, mice underwent a 3-min choice phase, in which mice were presented with one object from the sample phase and a novel object.

Experiment 2 was designed to evaluate the memory enhancing effect of PCAF activation on OR memory at 6-months-of-age. Experiment 2 was run identically to experiment 1.

As we observed a selective OR impairment at the 3h retention delay in 9-month-old 3xTG mice, Experiment 3 was conducted to determine if acute PCAF activation could attenuate OR impairments in 3xTG mice. Three days prior to behavioural testing, mice were treated acutely with the PCAF activator, SPV106 (25mg/kg i.p.), or vehicle (1% DMSO in saline i.p.). Using the Y-apparatus, during the sample phase, mice were presented two identical objects to explore for 10-min. Following the sample phase, there was a 5min or 3h retention delay, to assess short- and long-term memory, respectively. At the end of the retention delay, mice underwent a 3min choice phase, in which mice were presented with one object from the sample phase and a novel object.

As we also observed a selective OR impairment at the 3h retention delay in 12-month-old 3xTG mice, Experiment 4 was conducted to determine if acute PCAF activation could still attenuate OR impairments in 3xTG mice at 12-months-of-age. Experiment 4 was run identically to experiment 3.

Experiment 5 was designed to evaluate the memory enhancing effects of PCAF activation on OR memory in 12-month-old Wt mice using the sub-optimal OR task. Experiment 5 was run identically to experiment 1 and 2.

Experiment 6 was conducted to evaluate the effects of PCAF inhibition on OR memory at 3-months-of-age, prior to the development of OR deficits. Using the Y-

apparatus, during the sample phase, mice were presented two identical objects to explore for 10-min. Immediately following the sample phase, mice were treated acutely with the PCAF inhibitor embelin (20mg/kg) or vehicle (5% DMSO, 5% Tween in PBS i.p.). At the end of either a 5min or 3h retention delay, mice underwent a 3min choice phase, in which mice were presented with one object from the sample phase and a novel object.

Experiment 7 was conducted to evaluate the effects of PCAF inhibition on OR memory at 12-months-of-age. Experiment 7 was run identically to experiment 6.

4.3 Results

4.3.1 Longitudinal Effects of Acute PCAF Activation on OR

By longitudinally evaluating the effects of acute PCAF activation on OR at 3, 6, 9, and 12-months-of-age, we showed that PCAF activation bidirectionally regulates object memory in male and female 3xTG mice.

At 3-months-of-age the PCAF activator, SPV106, had an enhancing effect on sub-optimal OR, a 2×2×2×2 split-plot analysis of variance (ANOVA) revealed a significant main effect of drug ($F_{1,44}$ = 62.606, p < .001); Figure 4.1a. Paired samples t-tests between sample and choice discrimination ratio (DR) indicate that vehicle-treated mice did not show OR (Wt males, 5min: t_{11} = –.625, p = .544; Wt males, 3h: t_{11} = –.526, p = .609; Wt females, 5min: t_{11} = –.034, p = .974; Wt females, 3h: t_{11} = 1.697, p = .118; 3xTG males, 5min: t_{111} = 2.181, p = .052; 3xTG males, 3h: t_{11} = –.383, p = .709; 3xTG females, 5min: t_{11} = .974, p = .351; 3xTG females, 3h: t_{11} = .449, p = .662). Conversely, all SPV106-treated mice displayed enhanced OR (Wt males, 5min: t_{11} = –5.460, p < .001; Wt males,

3h: t_{11} = –2.642, p = .023; Wt females, 5min: t_{11} = –4.440, p = .001; Wt females, 3h: t_{11} = –2.958, p = .013; 3xTG males, 5min: t_{11} = –3.883, p = .003; 3xTG males, 3h: t_{11} = –5.642, p < .001; 3xTG females, 5min: t_{11} = –6.623, p < .001; 3xTG females, 3h: t_{11} = –5.795, p < .001).

Similarly, at 6-months-of-age SPV106 had an enhancing effect on sub-optimal OR, a 2×2×2×2 split-plot ANOVA revealed a main effect of drug ($F_{1,43}$ = 196.042, p < .001) and a main effect of genotype ($F_{1,43}$ = 6.892, p = .012); Figure 4.1b. Paired samples t-tests between sample and choice DR indicate that vehicle-treated mice do not demonstrate OR (Wt males, 5min: t_{11} = –1.844, p = .092; Wt males, 3h: t_{11} = .381, p = .711; Wt females, 5min: t_{11} = –.768, p = .459; Wt females, 3h: t_{11} = –.980, p = .348; 3xTG males, 5min: t_{11} = .976, p = .350; 3xTG males, 3h: t_{11} = 1.305, p = .320; 3xTG females, 5min: t_{10} = –1.261, p = .236; 3xTG females; 3h: t_{10} = 1.819, p = .099), whereas all SPV106-treated mice displayed enhanced OR (Wt males, 5min: t_{11} = –6.183, p < .001; Wt males, 3h: t_{11} = –3.986, p = .002; Wt females, 5min: t_{11} = –9.156, p < .001; Wt females, 3h: t_{11} = –5.098, p < .001; 3xTG males, 5min: t_{11} = –4.127, p = .002; 3xTG males, 3h: t_{11} = –6.103, p < .001; 3xTG females, 5min: t_{10} = –4.906, p = .001; 3xTG females, 3h: t_{10} = –9.538, p < .001).

At 9-months-of-age male and female 3xTG mice were delay-dependently impaired on OR, and SPV106 ameliorated this impairment, a 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay × drug interaction ($F_{1,42}$ = 10.054, p = .003), a sex × delay × drug interaction ($F_{1,42}$ = 5.177, p = .028), a main effect of genotype ($F_{1,42}$ = 20.128, p <

.001), and a main effect of delay ($F_{1,42}$ = 52.543, p < .001); Figure 4.1c. *Post hoc t*-tests revealed a significant difference between vehicle-treated 3xTG mice at 3h and all other groups (vehicle-treated Wt mice, 5min: t_{44} = 7.670, p < .001; SPV106-treated Wt mice, 5min t_{44} = 8.390, p < .001, vehicle-treated 3xTG mice, 5min: t_{21} = 5.849, p < .001; SPV106-treated 3xTG mice, 5min: t_{21} = 5.904, p < .001; vehicle-treated Wt mice, 3h: t_{44} = 5.378, p < .001; SPV106-treated Wt mice, 3h: t_{44} = 3.156, p = .048; and SPV106-treated 3xTG mice, 3h: t_{21} = –3.246, p = .032). Paired samples *t*-tests between sample and choice DR suggest intact memory in Wt mice (vehicle-treated males, 5min: t_{11} = –16.132, p < .001; SPV106-treated males, 5min: t_{11} = –6.240, p < .001; vehicle-treated males, 3h: t_{11} = –4.422, p = .001; SPV106-treated males, 3h: t_{11} = –6.361, p < .001; vehicle-treated females, 5min: t_{11} = –6.230, p < .001; SPV106-treated females, 5min: t_{11} = –8.319, p < .001; vehicle-treated females, 3h: t_{11} = –6.345, p < .001; SPV106-treated females, 3h: t_{11} = –3.703, p = .003), and 3xTG mice at 5min (vehicle-treated males: t_{10} = –5.999, p < .001; SPV106-treated males: t_{10} = –7.812, p < .001; vehicle-treated females: t_{10} = –5.311, p < .001; SPV106-treated females: t_{10} = –3.898, p = .003). At the 3h retention delay, vehicle-treated 3xTG mice failed to distinguish between novel and sample objects (males: t_{10} = –1.107, p = .333; females: t_{10} = .696, p = .502), replicating the impairment we observed previously at 9-months-of-age (see chapter 3); treatment with SPV106 ameliorated this impairment (males: t_{10} = –5.360, p < .001; females: t_{10} = –4.002; p = .003).

Conversely, at 12-months-of-age SPV106 exacerbated the OR impairment in 3xTG mice, a 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay × drug

interaction ($F_{1,39}$ = 9.179, p = .004), a genotype × delay interaction ($F_{1,39}$ = 6.354, p < .001), a genotype × drug interaction ($F_{1,39}$ = 8.169, p = .07), a delay × drug interaction ($F_{1,39}$ = 22.620, p < .001), a main effect of genotype ($F_{1,39}$ = 116.367, p < .001), a main effect of delay ($F_{1,39}$ = 57.158, p < .001), and a main effect of drug ($F_{1,39}$ = 12.173, p = .001); Figure 4.1d. *Post hoc* *t*-tests demonstrated significant differences between SPV106-treated 3xTG mice at 5min and all Wt mice (vehicle-treated Wt, 5min: t_{42} = 6.600, p < .001; SPV106-treated Wt, 5min: t_{42} = 5.224, p < .001; vehicle-treated Wt, 3h: t_{42} = 4.091, p < .001; SPV106-treated Wt, 3h: t_{42} = 5.184, p < .001), as well as vehicle-treated 3xTG mice at 5min (t_{19} = 6.031, p < .001). There were also significant differences between vehicle-treated 3xTG mice at 3h and all Wt mice (vehicle-treated Wt, 5min: t_{42} = 9.950, p < .001; SPV106-treated Wt, 5min: t_{42} = 7.504, p < .001; vehicle-treated Wt, 3h: t_{42} = 6.357, p < .001; SPV106-treated Wt, 3h: t_{42} = 8.491, p < .001), and vehicle-treated 3xTG mice at 5min (t_{19} = 7.610, p < .001). In addition, SPV106-treated 3xTG mice at 3h also significantly differed from Wt mice (vehicle-treated Wt, 5min: t_{42} = 9.052, p < .001; SPV106-treated Wt, 5min: t_{42} = 8.180, p < .001; vehicle-treated Wt, 3h: $t_{37.812}$ = 7.059, p < .001; SPV106-treated Wt, 3h: t_{42} = 9.262, p < .001), and vehicle-treated 3xTG mice at 5min and SPV106-treated 3xTG mice at 3h (t_{19} = 4.579, p < .001). Paired samples *t*-tests between sample and choice DR suggest intact memory in Wt mice (vehicle-treated Wt males, 5min: t_{11} = –5.064, p < .000; SPV106-treated Wt males, 5min: t_{11} = –4.398, p = .001; vehicle-treated Wt males, 3h: t_{11} = –4.333, p = .001; SPV106-treated males, 3h: t_{11} = –4.850, p = .001; vehicle-treated Wt females, 5min: t_{11} = –6.854, p < .001; SPV106-treated females, 5min: t_{11} = –7.568, p < .001; vehicle-treated Wt females, 3h: t_{11} = –4.086,

p = .002; SPV106-treated Wt females, 3h: t_{11} = −7.418, p < .001). Interestingly, SPV106-treated 3xTG mice were impaired at 5min (males: t_9 = −1.196, p = .262; females: t_9 = −.260, p = .801), whereas vehicle-treated 3xTG mice were not (males: t_9 = −6.223, p < .001; females: t_9 = −12.476, p < .001). At the 3h retention delay, vehicle and SPV106-treated 3xTG mice were impaired (vehicle-treated males: t_9 = −.722, p = .489; SPV106-treated males: t_9 = 1.389, p = .198; vehicle-treated females: t_9 = 1.024, p = .332; SPV106-treated females: t_9 = −.904, p = .389), replicating the phenotype we observed at 12-months-of-age (see Chapter 3).

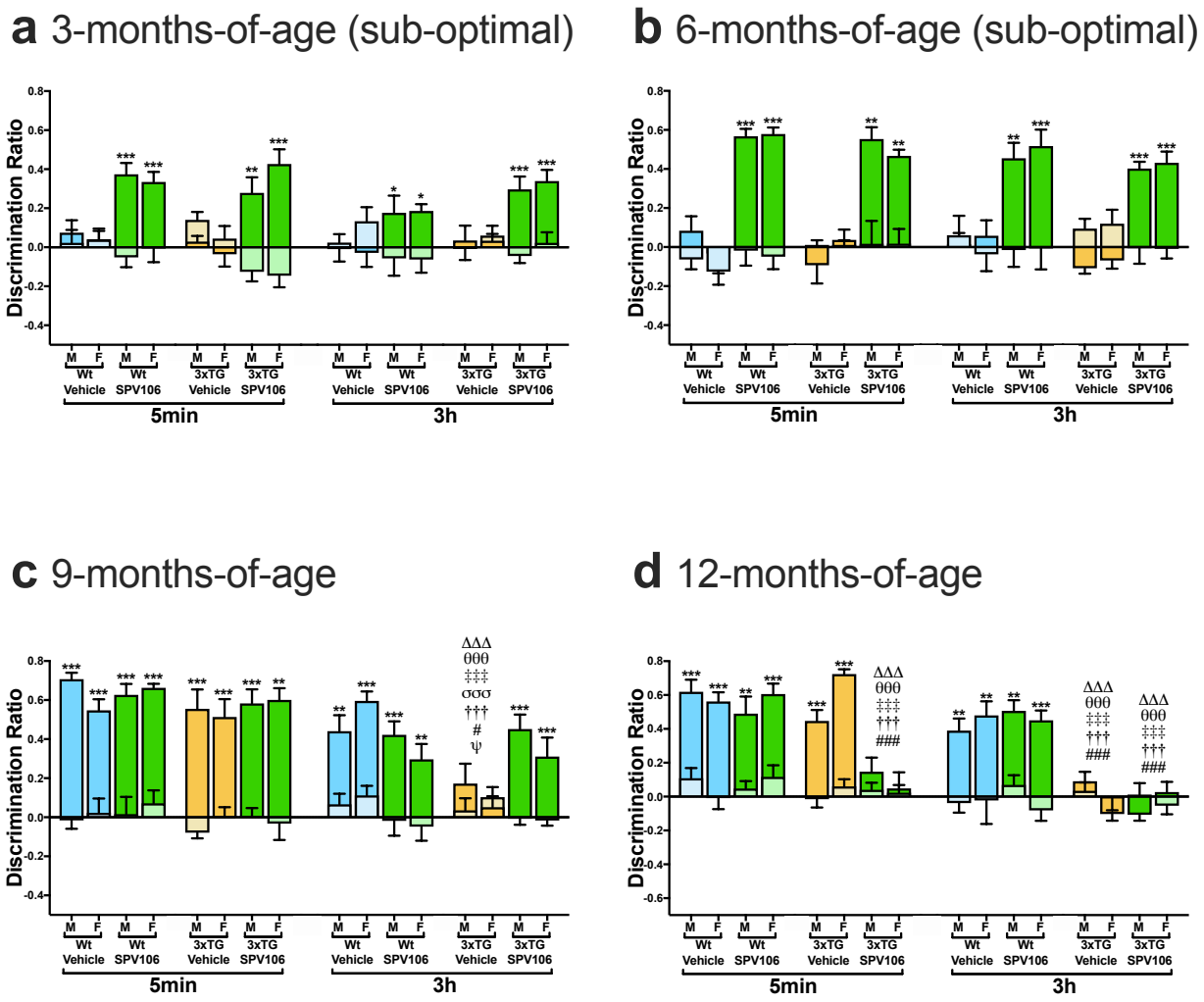

Figure 4.1 Age dependent bidirectional regulation of object memory by PCAF activation (SPV106 25mg/kg) in 3xTG mice. a) At 3-months-of-age, prior to the development of OR deficits, treatment with SPV106 enhanced sub-optimal short- (5min) and long-term (3h) OR in both Wt and 3xTG mice. b) At 6-months-of-age, prior to the development of OR deficits, treatment with SPV106 enhanced sub-optimal OR in Wt and 3xTG mice. c) At 9-months-of-age, vehicle-treated 3xTG mice were delay-dependently impaired at the 3h delay, treatment with SPV106 ameliorated this impairment. d) At 12-months-of-age, vehicle-treated 3xTG mice were delay-dependently impaired at the 3h delay, SPV106 failed to attenuate this impairment and induced an impairment at the 5min delay. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR. ΔΔΔ p < .001 significantly different from vehicle-treated Wt mice at 5min, θθθ p < .001 significantly different from SPV106-treated Wt mice at 5min, ‡‡‡ p < .001 significantly different from vehicle-treated 3xTG mice at 5min, σσσ p < .001 significantly different from SPV106-treated 3xTG mice at 5min, ††† p < .001 significantly different from vehicle-treated Wt mice at 3h, # p < .05, ### p < .001 significantly different from SPV106-treated Wt mice at 3h, Ψ p < .05 significantly different from SPV106-treated 3xTG mice at 3h.

At 9 and 12-months-of-age we did not observe any significant differences between vehicle and SPV106-treated Wt mice (when tested on OR using a 10min sample phase), likely because all Wt groups were at ceiling performance. We subsequently ran 12+-month-old Wt mice on the sub-optimal OR task and demonstrated that SPV106 still enhances OR in aged mice, a 2×2×2 split-plot ANOVA revealed a delay × drug × sex interaction ($F_{1,22}$ = 5.880, p = .024) and a significant main effect of drug ($F_{1,22}$ = 69.893, p < .001); Figure 4.2. *Post-hoc* independent samples *t*-tests revealed a significant difference between SPV-treated male and female Wt mice at 5min (t_{11} = 3.982, p = .024). In addition, significant differences were found between vehicle and SPV106-treated mice (males, 5min: t_{11} = –3.715, p =.048; males, 3h: t_{11} = –4.606, p = .016; females, 5min: t_{11} = –10.462, p < .001). Paired samples *t*-tests between sample and choice DR suggest impaired OR in vehicle-treated mice (males, 5min: t_{11} = –1.322, p = .213, males, 3h: t_{11} = 1.358, p = .202; females, 5min: t_{11} = .555, p = .590; females, 3h: t_{11} = 1.217, p = .249), but intact OR in SPV106-treated mice (males, 5min: t_{11} = –3.528, p = .005; males, 3h: t_{11} = –2.793, p = .017; females, 5min: t_{11} = –3.011, p = .012; females, 3h: t_{11} = –3.094, p = .010).

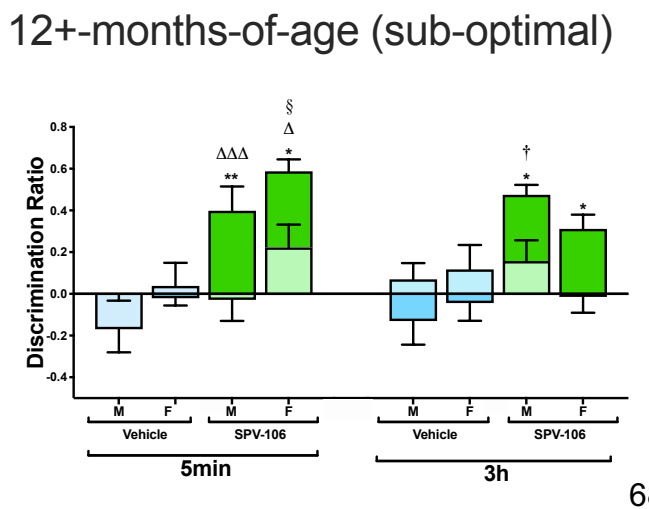

Figure 4.2 PCAF activation (SPV106 25mg/kg) enhances sub-optimal OR in 12+-month-old wt mice. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice. ΔΔΔ p < .001 significantly different from vehicle-treated male Wt mice at 5min, Δ p < .05 significantly different from vehicle-treated female Wt mice at 5min, § p < .05 significantly different from SPV106-treated Wt males at 5min, † p < .05, significantly different from vehicle-treated Wt males at 3h.

4.3.1.1 Control Measure: OR Exploratory Behaviour

At 3-months-of-age a 2×2×2×2 split-plot ANOVA revealed a significant main effect of delay ($F_{1,44}$ = 56.590, p < .001) on exploration during the sample phase; Table 4.1. A 2×2×2×2 split-plot ANOVA revealed a significant sex × delay interaction ($F_{1,44}$ = 22.695, p < .001), a main effect of genotype ($F_{1,44}$ = 4.686, p = .036), and a main effect of delay ($F_{1,44}$ = 4.889, p = .032) on exploration during the choice phase.

At 6-months-of-age a 2×2×2×2 split-plot ANOVA revealed a significant main effect of genotype ($F_{1,43}$ = 5.420, p = .025) and drug ($F_{1,43}$ = 28.225, p < .001). A 2×2×2×2 split-plot ANOVA revealed a significant sex × delay interaction ($F_{1,43}$ = 9.301, p = .004) and a main effect of delay ($F_{1,43}$ = 4.618, p = .037) on exploration during the choice phase.

At 9-months-of-age a 2×2×2×2 split-plot ANOVA demonstrated a significant genotype × sex × delay × drug interaction ($F_{1,41}$ = 4.567, p = .039) and a genotype × sex interaction ($F_{1,41}$ = 7.847, p = .008) on exploration during the sample phase. A 2×2×2×2 split-plot ANOVA revealed a significant main effect of sex on exploration during the choice phase ($F_{1,41}$ = 6.014, p = .018).

At 12-months-of-age a 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay × drug interaction ($F_{1,40}$ = 5.519, p = .024) on exploration during the sample phase. A 2×2×2×2 split-plot ANOVA revealed a significant main effect of drug ($F_{1,40}$ = 4.568, p = .039) on exploration during the choice phase. Finally, for Wt mice on the sub-optimal OR task a 2×2×2 split-plot ANOVA revealed a significant main effect of delay ($F_{1,22}$ = 26.104, p < .001) on exploration during the sample phase for Wt mice on the sub-optimal OR task

and a delay × drug interaction ($F_{1,22}$ = 5.126, p = .034) on exploration during the choice phase.

Table 4.1 Longitudinal OR Exploratory Behaviour in SPV106 Experiments

			Mean Sample Exploration				Mean Choice Exploration			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3 (Sub-optimal)	5min	Vehicle	19.80 (2.46)	20.01 (3.87)	17.11 (2.43)	14.08 (1.54)	23.12 (3.81)	16.30 (2.47)	18.87 (2.18)	13.96 (2.26)
		SPV106	18.17 (2.79)	17.50 (2.87)	13.16 (2.28)	14.24 (1.64)	24.15 (3.38)	17.69 (2.13)	19.40 (2.87)	13.12 (1.41)
	3h	Vehicle	32.84 (3.55)	28.96 (3.54)	25.05 (3.87)	22.59 (2.11)	18.21 (2.52)	19.78 (3.11)	13.33 (1.73)	15.69 (1.13)
		SPV106	37.63 (6.22)	24.49 (4.67)	27.13 (4.91)	24.64 (2.73)	15.74 (2.78)	18.88 (1.69)	12.99 (2.60)	15.96 (2.01)
6 (Sub-optimal)	5min	Vehicle	7.04 (1.52)	7.88 (1.35)	9.67 (0.96)	9.20 (1.24)	9.40 (1.76)	5.92 (1.21)	9.95 (1.75)	8.76 (1.60)
		SPV106	7.81 (0.90)	8.70 (1.76)	9.69 (0.85)	7.14 (0.51)	10.90 (1.60)	7.68 (1.81)	12.27 (0.90)	8.53 (0.85)
	3h	Vehicle	10.28 (1.46)	10.19 (0.99)	12.71 (2.52)	16.81 (2.31)	6.51 (0.74)	6.38 (1.26)	7.16 (1.61)	9.45 (1.24)
		SPV106	12.48 (2.45)	11.23 (1.59)	14.09 (1.83)	15.24 (1.78)	6.29 (1.60)	7.77 (1.23)	7.52 (1.01)	9.88 (1.16)
9	5min	Vehicle	30.02 (3.30)	31.92 (4.34)	28.60 (3.13)	36.80 (3.73)	8.60 (1.80)	10.32 (1.72)	9.62 (1.04)	10.36 (1.89)
		SPV106	36.85 (5.14)	24.80 (3.25)	31.98 (3.82)	40.68 (5.25)	7.48 (1.38)	8.89 (1.43)	9.04 (1.14)	13.19 (1.89)
	3h	Vehicle	40.42 (6.54)	19.88 (2.62)	29.18 (4.79)	30.75 (4.30)	10.39 (2.45)	10.81 (1.68)	5.56 (1.22)	14.63 (2.55)
		SPV106	34.19 (5.52)	26.10 (3.51)	26.13 (3.73)	32.60 (5.15)	8.98 (1.59)	11.49 (2.24)	8.69 (1.57)	12.73 (2.56)
12	5min	Vehicle	26.59 (3.77)	27.14 (3.10)	34.91 (5.26)	32.92 (4.21)	11.63 (2.22)	13.49 (1.67)	11.04 (2.13)	9.42 (1.57)
		SPV106	34.79 (6.87)	30.89 (3.54)	27.42 (4.18)	35.73 (4.24)	11.65 (2.53)	11.64 (2.33)	7.45 (0.95)	10.87 (1.27)
	3h	Vehicle	29.96 (4.81)	24.72 (7.89)	17.81 (2.85)	33.78 (4.49)	14.81 (3.26)	13.61 (2.48)	13.04 (2.21)	14.24 (2.20)
		SPV106	29.52 (4.78)	19.76 (3.63)	23.16 (4.43)	31.15 (4.39)	12.86 (2.63)	13.36 (2.16)	7.74 (2.12)	9.76 (1.32)
12+ (Sub-optimal)	5min	Vehicle	7.39 (1.81)	6.43 (1.14)			12.05 (1.71)	13.69 (1.88)		
		SPV106	8.58 (1.63)	6.39 (0.91)			8.69 (1.27)	9.87 (1.61)		
	3h	Vehicle	14.71 (1.71)	16.01 (2.76)			6.12 (0.80)	10.30 (1.47)		
		SPV106	12.84 (2.85)	13.08 (2.17)			9.86 (2.22)	9.88 (2.15)		

Note. Data are mean exploration (s) ± SEM.

4.3.1.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Despite the differences in exploratory behaviour that were observed at each timepoint, exploration did not consistently correlate with OR performance (Choice DR; Table 4.2). Though, there was a significant negative correlation between total sample exploratory behaviour and choice DR for SPV106-treated 3xTG males tested at the 3h delay ($r_{11} = -.799$, $p = .048$).

Table 4.2 Correlations Between Exploration and Longitudinal OR in SPV106 Experiments

			Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3 (Sub-optimal)	5min	Vehicle	.190	.005	−.026	−.289	.309	.166	−.018	−.367
		SPV106	−.183	.371	−.038	.357	−.079	.388	−.145	−.010
	3h	Vehicle	−.228	−.180	.326	.123	.042	−.399	.238	.056
		SPV106	.078	−.304	.000	−.621	.160	.195	.346	−.232
6 (Sub-optimal)	5min	Vehicle	.037	−.189	.165	−.463	−.196	.273	−.079	.010
		SPV106	−.334	.604	−.187	−.559	−.292	−.042	.174	.471
	3h	Vehicle	.361	−.559	.182	.506	.362	−.025	−.375	.163
		SPV106	.121	−.108	.065	.406	−.177	.349	−.332	.480
9	5min	Vehicle	.201	−.567	−.505	.091	.326	−.303	−.376	.678
		SPV106	.013	.090	−.287	−.353	.018	−.010	−.463	−.289
	3h	Vehicle	−.140	.228	.391	.076	.201	−.245	−.221	.059
		SPV106	−.124	.034	−.799*	.215	−.126	.291	−.594	−.034
12	5min	Vehicle	−.298	−.188	−.038	.370	.010	−.423	.399	−.051
		SPV106	−.067	−.026	−.514	.026	.367	−.179	−.008	.058
	3h	Vehicle	−.148	−.018	−.269	.502	.517	.169	−.643	−.317
		SPV106	.181	−.003	−.425	.545	.005	.091	.204	.007
12+ (Sub-optimal)	5min	Vehicle	.007	.154			−.110	−.035		
		SPV106	.030	.388			.004	−.407		
	3h	Vehicle	.072	.121			.527	.415		
		SPV106	−.073	−.132			.055	.244		

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR).

4.3.2 Effects of Acute PCAF Inhibition on OR

We also evaluated the effects of acute PCAF inhibition on OR in young 3xTG mice (3-months-of-age) and at the critical timepoint identified in our SPV106 experiments, 12+-months-of-age. PCAF inhibition impaired OR in 3xTG mice, prior to the development of OR deficits, whereas PCAF inhibition ameliorated OR impairments in aged 3xTG mice.

At 3-months-of-age the PCAF inhibitor, embelin, had an impairing effect on OR in both Wt and 3xTG mice, a 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay × drug interaction ($F_{1,42}$ = 4.981, p = .031), a significant genotype × sex × delay interaction ($F_{1,42}$ = 8.608, p = .05, a significant genotype × sex interaction ($F_{1,42}$ = 12.293, p < .001), a significant genotype × delay interaction ($F_{1,42}$ = 4.463, p = .041), and a main effect of drug ($F_{1,42}$ = 211.747, p < .001); Figure 4.3a. *Post hoc t*-tests revealed significant differences between vehicle and embelin-treated mice: embelin-treated Wt mice at 5min significantly differed from vehicle-treated Wt mice (5min: t_{21} = 8.099, p < .001; 3h: t_{21} = –6.462, p < .001), and vehicle-treated 3xTG mice (5min: t_{44} = –7.447, p < .001; 3h: t_{44} = –6.501, p < .001); embelin-treated 3xTG mice at 5min differed from vehicle-treated Wt mice (5min: t_{44} = 6.618, p < .001; 3h: $t_{36.226}$ = 6.175, p < .001), and vehicle-treated 3xTG mice (5min: 3xTG, 5min: t_{23} = 5.818; 3h: t_{23} = –5.862, p < .001); embelin-treated Wt mice at 3h differed from vehicle-treated Wt mice (5min: t_{21} = 14.839, p < .001; 3h: t_{21} = 10.686, p < .001), and vehicle-treated 3xTG mice (5min: t_{44} = –9.749, p < .001; 3h: t_{44} = –8.790, p < .001); and embelin-treated 3xTG mice differed from vehicle-treated Wt mice (5min: $t_{34.216}$ = 5.222, p < .001; 3h: $t_{37.129}$ = 4.646, p < .001), and vehicle-treated 3xTG mice (5min: t_{23} = 4.779, p < .001; 3h: t_{23} = 5.254, p < .001). Paired samples *t*-tests between

sample and choice DR suggest intact OR in vehicle-treated Wt and 3xTG mice (Wt males, 5min: $t_{11} = -10.651$, $p < .001$; Wt males, 3h: $t_{11} = -4.861$, $p = .001$; Wt females, 5min: $t_9 = -3.043$, $p = .014$; Wt females, 3h: $t_9 = -6.149$, $p < .001$; 3xTG males, 5min: $t_{11} = -7.600$, $p < .001$; 3xTG males, 3h: $t_{11} = -6.956$, $p < .001$; 3xTG females, 5min: $t_{11} = -5.317$, $p < .001$; 3xTG female, 3h: $t_{11} = -3.808$, $p = .003$). Embelin-treated mice, however, had impaired OR (Wt males, 5min: $t_{11} = 1.004$, $p = .337$; Wt females, 5min: $t_9 = -1.449$, $p = .181$; Wt females, 3h: $t_9 = .042$, $p = .968$; 3xTG males, 5min: $t_{11} = .572$, $p = .337$; 3xTG males, 3h: $t_{11} = -2.109$, $p = .059$; 3xTG females, 5min: $t_{11} = .980$, $p = .348$; 3xTG females, 3h: $t_{11} = 1.198$, $p = .256$), except Wt males at 3h, that showed a significant familiarity preference ($t_{11} = 5.381$, $p < .001$).

At 12+-months-of-age embelin had an impairing effect on Wt mice, but ameliorated OR deficits in 3xTG mice, a 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay × drug interaction ($F_{1,37} = 9.561$, $p = .004$), a genotype × drug interaction ($F_{1,37} = 100.974$, $p < .001$), delay × drug interaction ($F_{1,37} = 9.163$, $p < .001$), a main effect of genotype ($F_{1,37} = 18.260$, $p < .001$), main effect of delay ($F_{1,37} = 9.442$, $p = .004$), and a main effect of drug ($F_{1,37} = 6.795$, $p = .013$); Figure 4.3b. *Post hoc t*-tests revealed a significant difference between embelin-treated Wt mice at 5min and vehicle-treated Wt mice (5min: $t_{23} = 8.089$, $p < .001$; 3h: $t_{23} = -7.224$, $p < .001$), 3xTG mice at 5min (vehicle-treated: $t_{39} = -6.225$, $p < .001$; embelin-treated: $t_{39} = -9.021$, $p < .001$), and embelin-treated 3xTG mice at 3h ($t_{39} = -9.657$, $p < .001$). embelin-treated Wt mice at 3h differed from vehicle-treated Wt mice (5min: $t_{23} = 7.472$, $p < .001$; 3h: $t_{23} = 6.149$, $p < .001$), 3xTG

mice at 5min (vehicle-treated: $t_{39} = -5.394$, $p < .001$; embelin-treated: $t_{39} = -7.114$, $p < .001$), and embelin-treated 3xTG mice at 3h ($t_{39} = -7.572$, $p < .001$). Finally, vehicle-treated 3xTG mice at 3h significantly differed from vehicle-treated Wt mice (5min: $t_{39} = 5.829$, $p < .001$; 3h: $t_{39} = 5.216$, $p < .001$), 3xTG mice at 5min (vehicle-treated: $t_{16} = 4.384$, $p < .001$; embelin-treated: $t_{16} = 5.908$. $p < .001$), and embelin-treated 3xTG mice at 3h ($t_{16} = -7.185$, $p < .001$). Paired samples t-tests between sample and choice DR suggest intact OR in vehicle-treated Wt mice (males, 5min: $t_{11} = -5.020$, $p < .001$; males, 3h: $t_{11} = -5.348$, $p < .001$; females, 5min: $t_{11} = -6.500$, $p < .001$; females, 3h: $t_{11} = -6.958$, $p < .001$), whereas embelin-treated Wt mice were impaired (males, 5min: $t_{11} = 1.027$, $p = .326$; males, 3h: $t_{11} = .632$, $p = .540$; females, 5min: $t_{11} = -1.961$, $p = .076$; females, 3h: $t_{11} = -.759$, $p = .464$). Vehicle-treated 3xTG mice had intact OR at 5min (males: $t_8 = -6.764$, $p < .001$; females: $t_7 = -2.502$, $p = .041$), but were impaired at 3h (males: $t_8 = .319$, $p = .758$; females: $t_7 = -1.251$, $p = .251$); treatment with embelin ameliorated the impairment at 3h (males: $t_8 = -8.391$, $p < .001$; females: $t_7 = -9.599$, $p < .001$) and had no effect at 5min (males: $t_8 = -11.925$, $p < .001$; females: $t_7 = -8.233$, $p < .001$).

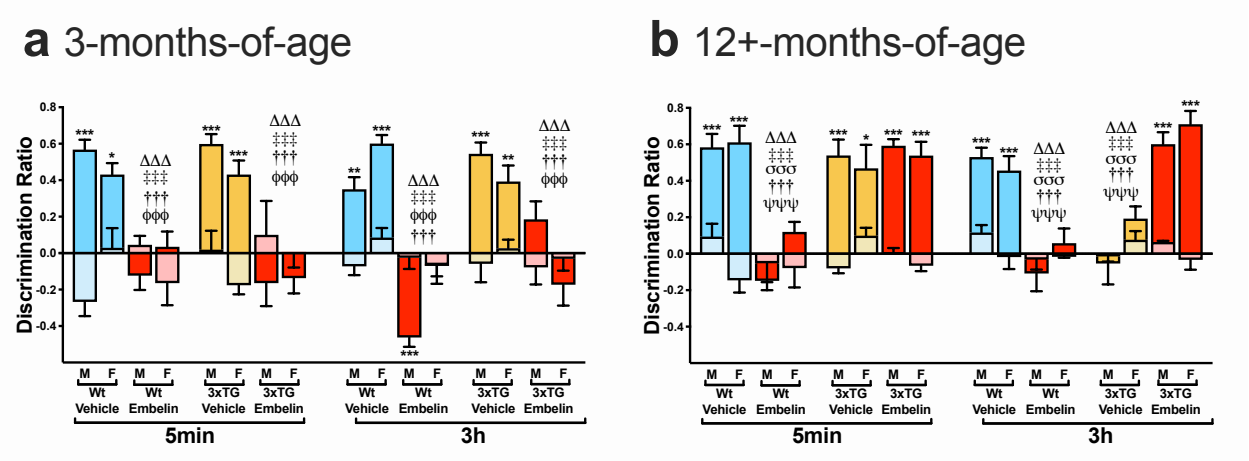

Figure 4.3 Age-dependent bidirectional regulation of OR by PCAF inhibition (embelin 10 or 20mg/kg) in 3xTG mice. a) At 3-months-of-age, prior to the development of OR deficits, treatment with embelin impaired short- (5min) and long-term (3h) OR in both Wt and 3xTG mice. b) At 12+-months-of-age, embelin still impaired Wt mice at both delays. Vehicle-treated 3xTG mice were delay-dependently impaired at the 3h delay, embelin ameliorated this impairment. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR. ΔΔΔ p < .001 significantly different from vehicle-treated Wt mice at 5min, ‡‡‡ p < .001 significantly different from vehicle-treated 3xTG mice at 5min, σσσ p < .001 significantly different from embelin-treated 3xTG mice at 5min, ††† p < .001 significantly different from vehicle-treated Wt mice at 3h, φφφ significantly different from vehicle-treated 3xTG mice at 3h, and Ψ Ψ Ψ p < .001 significantly different from embelin-treated 3xTG mice at 3h.

4.3.2.1 Control Measure: OR Exploratory Behaviour

At 3-months-of-age a 2×2×2×2 split-plot ANOVA did not demonstrate any significant interactions or main effects on exploration during the sample phase; Table 4.3. However, a 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,42}$ = 4.145, p = .048), and a main effect of drug ($F_{1,42}$ = 26.955, p < .001) on exploration during the choice phase.

At 12+-months-of-age a 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex × drug interaction ($F_{1,37}$ = 4.749, p = .036), a main effect of genotype ($F_{1,37}$ = 4.534, p = .040), and a main effect of drug ($F_{1,37}$ = 18.024, p < .000) on exploration during the sample phase. 2×2×2×2 split-plot ANOVA revealed a significant sex × delay ×

drug interaction ($F_{1,37}$ = .480, p = .041), a t genotype × drug interaction ($F_{1,37}$ = 8.542, p = .006), drug × delay interaction ($F_{1,37}$ = 7.127, p = .011), and a main effect of genotype ($F_{1,37}$ = 22.971, p < .001) on exploration during the choice phase.

Table 4.3 Longitudinal OR Exploratory Behaviour in Embelin Experiments

			Mean Sample Exploration				Mean Choice Exploration			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	Vehicle	25.01 (6.29)	16.78 (5.10)	25.44 (4.69)	19.44 (4.60)	25.01 (6.29)	5.63 (1.23)	25.44 (4.69)	7.76 (0.86)
		Embelin	23.34 (3.99)	25.27 (6.53)	20.25 (23.34)	15.46 (3.66)	23.34 (3.99)	4.68 (1.87)	20.25 (23.34)	3.72 (0.85)
	3h	Vehicle	27.56 (4.53)	16.64 (4.23)	25.91 (5.81)	18.31 (3.84)	27.56 (4.53)	8.58 (1.70)	25.91 (5.81)	9.02 (1.20)
		Embelin	28.16 (5.56)	19.61 (6.79)	23.75 (4.63)	22.83 (2.40)	28.16 (5.56)	5.94 (1.80)	23.75 (4.63)	4.66 (1.11)
12+	5min	Vehicle	33.35 (4.67)	25.38 (3.82)	41.87 (4.39)	36.35 (4.94)	10.96 (2.32)	11.12 (1.88)	15.40 (1.08)	13.11 (2.26)
		Embelin	45.81 (7.32)	27.69 (3.48)	39.14 (4.34)	39.34 (7.61)	4.78 (1.05)	6.73 (1.14)	15.08 (1.36)	14.51 (2.74)
	3h	Vehicle	31.29 (4.59)	26.12 (2.45)	37.46 (6.64)	34.99 (5.05)	8.00 (1.37)	9.59 (2.03)	8.77 (1.37)	10.44 (1.42)
		Embelin	38.06 (5.03)	36.21 (10.45)	42.53 (6.32)	58.60 (10.21)	9.55 (1.96)	6.79 (1.14)	15.07 (1.76)	11.90 (0.99)

Note. Data are mean exploration (s) ± SEM.

4.3.2.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Despite the differences in exploratory behaviour that were observed at each timepoint, exploration did not significantly correlate with OR performance; Table 4.4.

Table 4.4 Correlations Between Exploration and Longitudinal OR in Embelin Experiments

			Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	Vehicle	−.038	−.387	−.351	−.626	−.169	−.446	.175	−.112
		Embelin	−.341	.432	.731	.085	−.175	−.249	.039	−.183
	3h	Vehicle	−.413	−.026	−.181	.004	−.420	−.357	−.547	.267
		Embelin	.502	.219	−.419	.160	.524	.709	−.324	.237
12+	5min	Vehicle	.140	−.203	.050	.147	.226	.072	.102	.582
		Embelin	.397	.605	.170	.296	.741	.488	−.170	.000
	3h	Vehicle	−.270	.379	.497	.049	−.600	.768	.203	−.208
		Embelin	−.391	.071	−.317	−.082	.480	.194	.144	−.273

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR).

4.4 Discussion

By longitudinally evaluating the effects of acute PCAF activation and inhibition on OR memory at 3, 6, 9, and 12 months of age, these experiments show that PCAF bidirectionally regulates cognition in male and female 3xTG mice. At 3 and 6-months-of-age, prior to the development of OR deficits, the PCAF activator, SPV106, enhanced short- (5min) and long-term (3h) OR, whereas the PCAF inhibitor, embelin, impaired. At 9 months of age, when OR impairment was first observed, SPV106 ameliorated the long-term OR deficit. At 12 months of age, however, SPV106 induced a short-term OR impairment, while embelin ameliorated the long-term OR deficit. Conversely, in Wt mice, SPV106 solely enhanced sub-optimal OR, whereas embelin exclusively impaired OR.

This work revealed a complex role of PCAF in AD-relevant cognition, where PCAF is initially beneficial for OR memory but becomes detrimental. Our findings clarify the discrepant results demonstrating that while PCAF activation enhances memory in normal rodents memory (Duclot et al., 2010; Maurice et al., 2008; Mitchnick et al., 2016; Mitchnick & Winters, 2018; Wei et al., 2012), in Aβ-treated rodents PCAF inhibition or KO attenuates AD-like cognitive deficits (Duclot et al., 2010; Park et al., 2015, 2013). It is still unclear, however, in what way both the down and upregulation of PCAF activity contributes to cognition.

It is likely that the beneficial effects of PCAF activation on long-term OR memory (3h) in Wt mice and 3xTG mice at 3, 6, and 9-months-of-age results from the addition of acetyl groups to various lysine residues on histone tails, which promotes gene expression by allowing transcriptional machinery to access the DNA, or by acting as a transcription coactivator and recruiting other transcription factors (Peixoto & Abel, 2013). Specifically, SPV106 likely amplifies levels of histone acetylation and expression of memory enhancing genes (e.g. *CREB, reelin, BDNF, CFOS and/or Zif268/Erg1*; Bredy et al., 2007; Koshibu et al., 2009; Lubin et al., 2008; Peixoto & Abel, 2013). Indeed, SPV106 has been shown to increase global levels of H3 and H4 acetylation (Milite et al., 2011; Sbardella et al., 2008), which can correlate with transcriptional activity and long-term memory enhancement (e.g. Bousiges et al., 2010; Chatterjee et al., 2018; Levenson et al., 2004; Peleg et al., 2010; Pokholok et al., 2005). It is also possible that PCAF-induced acetylation of non-histone proteins facilitates OR memory, especially given the enhancing effects of SPV106 on short-term OR memory (5min). Previous work from our group, and others,

has demonstrated that PCAF (Duclot et al., 2010; Maurice et al., 2008; Mitchnick et al., 2016), unlike other HATs (Mitchnick et al., 2016), is necessary for short-term memory. Given the relatively short retention delays (< 20min) used in these experiments, it is more likely that acetylation of cytosolic proteins which initiate non-genomic effects (e.g. intracellular signaling cascades, protein stability or protein degradation) support short-term memory. Indeed, Mitchnick & Winters (2018) show that PCAF interacts with estrogen receptor alpha to facilitate short-term object memory. Intra-HPC administration of SPV106 facilitated both short- and long-term object memory. The effect of SPV106 on short-term, but not long-term, object memory was abolished by co-administration of an ER alpha/beta antagonist. The PCAF inhibitor, embelin, may impair object memory in Wt mice and young/unimpaired 3xTG mice (3-months-of-age) by decreasing histone acetylation and gene expression (Modak et al., 2013) and/or blocking the beneficial effects of estrogen receptor activity (Mitchnick & Winters, 2018).

By 12-months-of-age, it is possible that the memory promoting effects of PCAF activity (e.g. regulation of the expression of memory-related genes) still occur but are insufficient to overcome behavioural impairments induced by AD-related pathological changes. The impairing effects of SPV106 at 12-months-of-age on cognition in 3xTG mice also strongly suggests that PCAF interacts with elements of AD pathology. Indeed, histone acetylation and PCAF activity have been shown to regulate genes upstream from Aβ degradation (e.g. *APP, β-site APP cleavage enzyme (BACE)1, PS1, somatostatin*) and inflammation (e.g. interleukin (IL)- 1β, IL-6, and tumor necrosis factor-α (TNF-α); Duclot et al., 2010; Guo et al., 2011; Ito, Barnes, & Adcock, 2000). Furthermore, PCAF

activity has been shown to increased the non-histone acetylation of NF-κB, which has been linked to increased inflammation and levels of Aβ (Park et al., 2015, 2013). The enhancing effects of embelin treatment on cognition in 12+-month-old 3xTG mice may results from the acute inhibition of these pathological cascades. Another possibility is that embelin attenuates cognitive deficits by decreasing acetylation of the histone variant H2A.Z (see Chapter 7), since H2A.Z is a negative regulator of memory (Zovkic et al., 2014) that accumulates in mouse models of AD (Gjoneska, Pfenning, Mathys, Quon, Kundaje, Tsai, & Kellis, 2015).

It remains to be seen whether other HATs, such as CBP or P300, also function bidirectionally in AD. Although increasing CBP and P300 activity has been shown to strictly attenuate cognitive deficits in transgenic AD mice (Caccamo et al., 2010; Chatterjee et al., 2018), these studies have not employed a systematic longitudinal design.

In conclusion, these experiments demonstrate a remarkable amelioration of cognitive deficits in 3xTG mice, even at advanced ages, following either acute activation or inhibition of PCAF. Therefore, pharmacological manipulation of PCAF, although complex, appears to be a promising therapeutic strategy in AD.

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5 Longitudinal Spatial Memory Phenotype

5.1 Introduction

The hippocampus (HPC) is one of the brain regions affected by neuropathology at relatively early stages of Alzheimer's disease (AD; Braak & Braak, 1991, 1995). Consequently, AD patients frequently suffer from impairments in various aspects of spatial processing including spatial navigation and spatial memory (e.g. Delpolyi, Rankin, Mucke, Miller, & Gorno-Tempini, 2007; Moodley et al., 2015; Toledo-Morrell & Dickerson, 2000).

In mouse models of AD, spatial memory is typically evaluated using the Morris water maze (MWM; Morris, Garrud, Rawlins, & O'Keefe, 1982) or contextual fear conditioning (CFC; Fanselow, 1980). Generally, impairments on these tasks are detected between 4- and 6months-of-age (examples in triple transgenic (3xTG) mice: Billings, Oddo, Green, McGaugh, & LaFerla, 2005; Caccamo et al., 2010; Clinton et al., 2007; Stover et al., 2015). These tasks involve extensive training and aversive stimuli. Since we wanted to evaluate spatial memory in a manner that is comparable to human episodic memory and procedurally similar to object recognition (OR), we used to object location (OL) task. Object location deficits have been previously been reported in 3xTG mice, and generally appear prior to OR deficits (Davis et al., 2013; Frye & Walf, 2008; Gulinello et al., 2009; Middei et al., 2006). However, OL has not be longitudinally evaluated in 3xTG mice. Here, we systematically evaluated spatial memory, using the OL task, in male and female 3xTG mice at 3 ,6, and 9-months-of-age. We report a progressive impairment in OL memory. At 3-months-of-age, 3xTG mice had a selective impairment in long-term spatial memory.

By 6- and 9-months-of-age, 3xTG mice had impaired short- and long-term spatial memory.

5.2 Methods

Object location was longitudinally evaluated every 3 months from 3- to 9-months-of-age in male and female wild-type (Wt) and 3xTG mice. Methodological details for behavioural testing and data analysis are described in Chapter 2.

5.2.1 Experiments

Experiment 1 was designed to evaluate OL memory at 3-months-of-age. During the sample phase, mice were presented two identical objects to explore for 10min. Following the sample phase, there was a 5min or 3h retention delay, to assess short- and long-term memory, respectively. At the end of the retention delay, mice underwent a 3min choice phase, in which mice were presented with one object in the same position as the sample phase, while the other object was positioned in a novel location.

Experiments 2 and 3 were designed to evaluate OL memory at 6- and 9-months-of-age, respectively; using the same mice and identical conditions to Experiment 1.

5.3 Results

5.3.1 Longitudinal OL Phenotype

Object location was delay-dependently impaired in both male and female 3xTG mice at 3-months-of-age, as a 2×2×2 split-plot analysis of variance (ANOVA) revealed a significant genotype × delay interaction ($F_{1,43}$ = 10.062, p = .003, η_p^2 = .190), a significant main effect of genotype ($F_{1,43}$ = 9.362, p = .004, η_p^2 = .179), and a significant main effect

of delay ($F_{1,43}$ = 12.835, p = .001, η_p^2 = .230); Figure 5.1a. *Post hoc t*-tests revealed a significant difference between 3xTG mice at 3h and all other groups (Wt, 5min: t_{45} = 5.916, p < .001; Wt, 3h: t_{45} = 5.095, p < .001; 3xTG, 5min: t_{22} = 4.300, p < .001). Paired samples t-tests between sample and choice discrimination ratios (DRs) indicated intact memory in Wt mice (males, 5min: t_{11} = –5.833, p < .001; males, 3h: t_{11} = –3.655, p = .004; females, 5min: t_{11} = –3.795, p = .003; females, 3h: t_{11} = –3.436, p = .006), and 3xTG mice at 5min (males: t_{11} = –2.207, p < .001; females: t_{10} = –3.267, p = .008), but not 3h (males: t_{11} = –5.833, p < .001; females: t_{10} = –.285, p = .781).

At 6-months-of-age OL was delay-independently impaired in 3xTG mice, as a 2×2×2 split-plot ANOVA revealed a significant genotype × sex interaction ($F_{1,43}$ = 27.902, p < .001, η_p^2 = .394), a significant main effect of genotype ($F_{1,43}$ = 4.152, p < .048, η_p^2 = .088), and a significant main effect of sex ($F_{1,\ 43}$ = 17.738, p < .001, η_p^2 = .292); Figure 5.1b. *Post hoc* tests revealed a significant difference between Wt and 3xTG mice (males: t_{46} = 4.442, p < .001; females: t_{44} = 7.349, p < .001). Paired samples *t*-tests between sample and choice DRs indicated intact memory in Wt mice (males, 5min: t_{11} = –11.463, p < .001; males, 3h: t_{11} = –2.769, p = .018; females, 5min: t_{11} = –7.512, p < .001; females, 3h: t_{11} = –6.486, p < .001), but impaired memory in 3xTG mice (males, 5min: t_{11} = – .688, p = .506; males, 3h: t_{11} = –.783, p = 450; females, 5min: t_{10} = .196, p = .849; females, 3h: t_{10} = 1.795, p = .103).

At 9-months-of-age OL was still delay-independently impaired in 3xTG mice, as a 2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,42}$ =

8.325, $p = .006$, $\eta_p^2 = .165$) and a significant main effect of genotype ($F_{1, 42} = 60.142$, $p < .001$, $\eta_p^2 = .589$); Figure 5.1c. *Post hoc* tests revealed a significant difference between Wt and 3xTG males (5min: $t_{13.674} = 7.490$, $p < .001$; 3h: $t_{22} = 3.353$, $p = .036$) and Wt and 3xTG females at 3h ($t_{13.415} = 5.418$, $p < .001$). Paired samples *t*-tests between sample and choice DRs suggested intact memory in Wt mice (males, 5min: $t_{11} = -12.426$, $p < .001$; females, 5min: $t_{11} = -5.604$, $p < .001$; females, 5min: $t_{10} = -3.073$, $p = .012$; females, 3h: $t_{10} = -8.410$, $p < .001$), but impaired memory in 3xTG mice (males, 5min: $t_{11} = 1.125$, $p = .285$; males, 3h: $t_{11} = -6.96$, $p = .501$; females, 5min: $t_{10} = -.363$, $p = .724$; females, 3h: $t_{10} = 1.388$, $p = .195$).

We did not evaluate OL in 12-month-old mice due to the effects of PCAF activation and inhibition observed at 9-months-of-age (see Chapter 6).

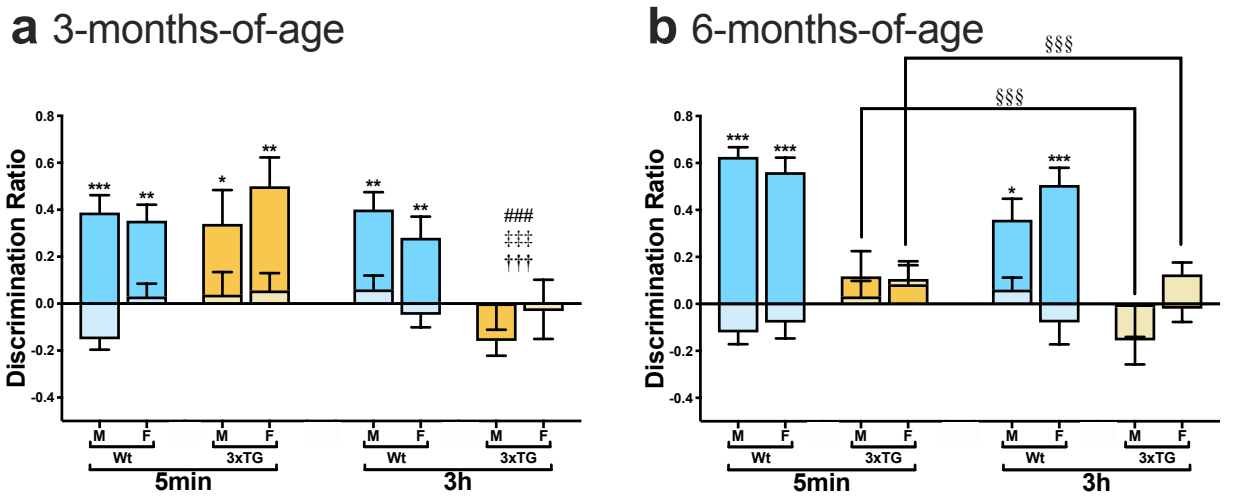

Figure 5.1 Longitudinal OL phenotype. a) 3xTG mice are delay-dependently in the OL task, with deficits observed at the 3h, but not 5min, delay at 3-months-of-age. ### p< .001 significantly different from Wt mice at 5min, ‡‡‡ p < .001 significantly different from 3xTG mice at 5min, and ††† p< .001 significantly different from Wt mice at 3h. b) 3xTG mice are delay-independently impaired on OL at 6-months-of-age. §§§ p < .001 indicates a significant difference between Wt and 3xTG males or females. c) 3xTG mice are delay-independently impaired on OL at 9-months-of-age. ### p< .001 significantly different from male Wt mice at 5min, ††† p < .001 significantly different from male Wt mice at 3h, †† p < .01 significantly different from female Wt mice at 3h. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR.

5.3.1.1 Control Measure: OL Exploratory Behaviour

At 3-months-of-age, a 2×2×2 split-plot ANOVA revealed a significant main effect of sex ($F_{1,43}$ = 11.374, p = .002, η_p^2 = .209) on exploratory behaviour during the sample phase; see Table 5.1 for all exploratory data. A 2×2×2 split-plot ANOVA revealed no significant interactions or main effects on exploration during the choice phase.

At 6-months-of-age a 2×2×2 split-plot ANOVA revealed a significant main effect of sex ($F_{1,43}$ = 12.880, p = .001, η_p^2 = .230) on exploration during the sample phase. A 2×2×2 split-plot ANOVA revealed no significant interactions or main effects on exploration during the choice phase.

At 9-months-of-age a 2×2×2 split-plot ANOVA revealed no significant interactions or main effects on exploration during the sample phase. However, 2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,42}$ = 6.837, p = .012, η_p^2 = .140), a genotype × delay interaction ($F_{1,42}$ = 13.024, p = .001, η_p^2 = .237), a sex × delay interaction ($F_{1,42}$ = 5.915, p = .019, η_p^2 = .123), and a significant main effect of sex ($F_{1,42}$ = 22.270, p < .001, η_p^2 = .347) on exploration during the choice phase. *Post hoc* analyses revealed a significant difference between Wt male choice exploration at the 5min and 3h retention delay (t_{11} = 5.122, p < .001), and between Wt males and females at 5min (t_{21} = 6.352, p < .001).

Table 5.1 Longitudinal OL exploratory behaviour

		Mean Sample Exploration				Mean Choice Exploration			
		Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	35.57 (5.67)	40.68 (5.20)	26.48 (4.07)	57.79 (8.72)	9.19 (2.08)	13.05 (2.68)	5.59 (1.48)	13.16 (2.05)
	3h	36.52 (5.56)	49.23 (8.71)	28.59 (4.13)	50.91 (8.31)	8.00 (1.52)	11.99 (2.34)	9.46 (1.64)	16.29 (3.13)
6	5min	45.29 (6.14)	28.02 (3.00)	45.16 (5.04)	27.23 (2.72)	9.34 (1.60)	11.14 (1.70)	9.40 (2.42)	12.74 (1.42)
	3h	44.69 (6.32)	22.62 (4.30)	45.39 (9.25)	43.65 (6.75)	10.54 (2.22)	9.86 (1.33)	7.16 (1.92)	12.08 (2.41)
9	5min	28.61 (4.14)	22.31 (4.29)	28.74 (2.90)	33.07 (4.87)	19.12 (1.81)	5.12 (1.12)	11.33 (2.21)	6.35 (1.10)
	3h	28.76 (3.20)	20.62 (4.97)	30.09 (4.00)	35.89 (8.20)	11.23 (1.57)	6.54 (1.30)	14.69 (1.57)	9.38 (1.57)

Note. Data are mean exploration (s) ± SEM.

5.3.1.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Despite the differences in exploratory behaviour that were observed at 3, 6, and 9-months-of-age, exploration did not significantly correlate with OL performance (choice DR; Table 5.2).

Table 5.2 Correlation between exploration and longitudinal OL performance

		Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
		Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	−.424	.335	−.484	−.501	.180	.693	.162	.549
	3h	−.392	−.199	−.469	−.244	.064	.110	−.011	.076
6	5min	−.336	−.101	−.028	.574	−.196	−.466	−.216	−.004
	3h	.191	−.359	−.468	.036	−.066	.136	.344	.234
9	5min	.208	−.412	.124	−.332	.354	−.229	−.112	−.747
	3h	−.351	.006	−.171	.138	.007	−.040	−.059	.512

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR).

5.4 Discussion

Here we show progressive impairments in spatial memory in male and female 3xTG mice. Specifically, OL was delay-dependently impaired at 3-months-of-age and this impairment advanced to be delay-independent at 6- and 9-months-of-age. These severe impairments in short- and long-term OL memory reaffirm the susceptibility of HPC-dependent processing to AD-related pathology (Assini, Duzzioni, & Takahashi, 2009; Creighton et al., 2019; Oakley et al., 2006; Oddo, Caccamo, Shepherd, et al., 2003).

The deficits reported here are consistent with previous work demonstrating spatial memory impairments that are typically observed between 3- and 6-months-of-age in

mouse models of AD using the OL task (Bollen et al., 2013; Creighton et al., 2019; Davis et al., 2013; Dodel et al., 2011; Frye & Walf, 2008; Good & Hale, 2007; Good et al., 2007; Gulinello et al., 2009; Kornecook, McKinney, Ferguson, & Dodart, 2010; Kroker et al., 2014; Ma et al., 2013; Masciopinto et al., 2012; Middei et al., 2006), as well as other spatial rodent tasks like the MWM (Billings et al., 2007; Billings, Oddo, Green, McGaugh, & LaFerla, 2005; Chen, Chen, Knox, Inglis, Bernard, Martin, Justice, McConlogue, et al., 2000; Clinton et al., 2007; Gulinello et al., 2009; Kotilinek et al., 2002; Lesné et al., 2006; Medeiros et al., 2014; Puoliväli et al., 2002; Schneider, Baldauf, Wetzel, & Reymann, 2014) and CFC (Espana et al., 2010; Jacobsen et al., 2006; Kimura & Ohno, 2009; Medeiros et al., 2014). The observed impairments in OL memory in 3xTG mice also parallel the abnormal spatial processing of objects (Caterini, Sala, Spinnler, Stangalino, & Turnbull, 2002) and general spatial memory deficits (e.g. Amieva et al., 2005; Blackwell et al., 2003; Choi et al., 2011; Lee et al., 2014; Toledo-Morrell & Dickerson, 2000) seen in AD patients.

Object location impairments were first observed at a much earlier age than OR, 3- and 9-months-of-age, respectively, and the impairment was more severe as both short- and long-term OL memory were impaired by 6 months. Similarly, other groups have observed impairments in OL but not OR (Davis, Eacott, Easton, & Gigg, 2013; Frye & Walf, 2008; Gulinello et al., 2009), or impairments in OL prior to OR (Middei et al., 2006). These results suggest the spatial processing of objects is more difficult for 3xTG mice than the processing of object features. It is likely that this differential time course is related to the dependence of these tasks on the HPC, a brain regions that is extensively affected

by AD pathology in 3xTG mice (Mastrangelo & Bowers, 2008; Oddo, Caccamo, Shepherd, et al., 2003). While OL is clearly dependent on the HPC (Assini et al., 2009; Barker & Warburton, 2011; Mumby & Pinel, 1994), there has been debate concerning the contribution of the HPC and perirhinal cortex (PRh) to OR memory. It has been proposed that the HPC is specifically involved in spatial and temporal processing of objects, whereas the PRh is involved in the processing of object features (Barker & Warburton, 2011; Winters et al., 2004). We have suggested that testing OR in a Y-apparatus increases focus on objects and reduces potentially confounding contextual stimuli, thereby decreasing the demand on the HPC (Creighton et al., 2019). Indeed, when we have increased the spatial nature of the OR task, by testing OR in an open-field, impairments in OR in 3xTG mice are observed earlier; 6-months-of-age in the open field (Mendell & MacLusky, 2018) versus 9-months in the Y-apparatus.

Similar to the OR experiments described previously, we did not observe any major differences between male and female 3xTG mice on OL. However, older Wt females (9-months-of-age), generally performed better than males at the 3-h retention delay. This is consistent with our previous findings demonstrating that 12-month-old Wt males, but not females, had difficulty performing OL (Creighton et al., 2019). Similarly, others have demonstrated superior performance by female rodents on OL tasks (Saucier, Shultz, Keller, Cook, & Binsted, 2008) and OL impairments in aged male mice (Wang, Li, Dong, Lv, & Tang, 2009; Wimmer, Hernandez, Blackwell, & Abel, 2012).

In conclusion, these experiments longitudinally characterized OL memory deficits in male and female 3xTG mice. These results suggest that spatial processing is more

difficult for transgenic AD mice than OR, as OL impairments were observed at a much earlier age than OR deficits, 3- and 9-months-of-age, respectively. The distinct time points relevant to spatial memory deficits in 3xTG mice, identified here, will be used to evaluate epigenetic compounds for the attenuation of OL deficits.

6 Age-dependent Bidirectional Attenuation of Spatial Memory Deficits by the Histone Acetyltransferase, p300/CBP Associated Factor (PCAF)

6.1 Introduction

As there is growing evidence that genetic and environmental factors interact during the development and progression of neurogenerative diseases, processes linking these factors are increasingly being investigated. Epigenetic processes that respond to genetic and environmental signals, such as those that modify histones, can regulate the transcription of genes that modulate neuron function and pathology. Specifically, dysregulation of acetylation was reported to play a role in the cognitive deficits and neuropathology that develop in Alzheimer's disease (AD) and increasing histone acetyltransferase (HAT) activity has emerged as a promising therapeutic strategy (Fischer, 2014; Pirooznia & Elefant, 2013; Xu, Dai, Huang, & Jiang, 2011). Given the susceptibility of the hippocampus (HPC) to neuropathology in AD, much of the research evaluating the effectiveness of epigenetic therapies has focused on the attenuation of cognitive deficits in hippocampal-dependent tasks. For example, in rodent models of AD, HDAC inhibition or HAT activation has been shown to restore performance on contextual fear conditioning (CFC) and Morris water maze (MWM) tasks (Caccamo et al., 2010; Chatterjee et al., 2018; Francis et al., 2009; Ricobaraza et al., 2009; Sung et al., 2013).

We recently evaluated the effects of activating one specific HAT, p300/CBP associated factor (PCAF) on object recognition (OR) memory in 3xTG mice, since PCAF activation is known to enhance memory in normal rodents (Mitchnick & Winters, 2018; Wei et al., 2012). Interestingly, we observed an age-related bidirectional regulation of object memory by PCAF (see Chapter 4). Systemic acute administration of the PCAF activator, SPV-106 enhanced OR from 3-6 months of age and ameliorated OR deficits at

9 months of age. At 12 months of age, however, PCAF activation further impaired OR, whereas PCAF inhibition ameliorated the OR impairment. We therefore set out to determine if the bidirectional regulation of cognition by PCAF generalizes to spatial memory. We expected that PCAF will also bidirectionally regulate spatial memory in AD mouse models, since other groups have shown that PCAF inhibition mitigates impairments in spontaneous alternation and MWM in mice or rats that were treated with beta-amyloid (Aβ; (Duclot et al., 2010; Park et al., 2015). We investigated the effects of acute PCAF activation and inhibition on OL memory in male and female triple transgenic (3xTG) mice at 3, 6, and 9-months-of-age using the PCAF activator SPV106 (Sbardella et al., 2008) or the PCAF inhibitor, embelin (Modak et al., 2013). We show that, similar to OR memory, PCAF bidirectionally regulates spatial memory in 3xTG mice. At 3 and 6-months-of-age, SPV106 attenuated OL deficits but by 9-months-of-age, SPV106 was no longer effective and embelin, which inhibited OL in wild-type (Wt) mice, attenuated long-term OL deficits in female 3xTG mice.

6.2 Methods

Object location was longitudinally evaluated every 3 months from 3- to 9-months-of-age in male and female Wt and 3xTG mice. Methodological details for behavioural testing and data analysis are described in Chapter 2.

6.2.1 Experiments

As we observed a OL impairment at the 3h retention delay in 3-month-old 3xTG mice, Experiment 1 was conducted to determine if acute PCAF activation could attenuate OL impairments in 3xTG mice. Three days prior to behavioural testing, mice were treated

acutely with the PCAF activator, SPV106 (25mg/kg i.p.; Wei et al., 2012), or vehicle (1% DMSO in saline i.p.). During the sample phase, mice were presented two identical objects to explore for 10min. Following the sample phase, there was a 5-min or 3-h retention delay, to assess short- and long-term memory, respectively. At the end of the retention delay, mice underwent a 3-min choice phase, in which mice were presented with one object in the same position as the sample phase, while the other object was positioned in a novel location.

As we observed a delay-independent OL impairment in 6- and 9-month-old 3xTG mice, Experiment 2 and 3 were conducted to determine if acute PCAF activation continued to attenuate short- and long-term OL impairments in 3xTG mice. Experiment 2 and 3 were run identically to Experiment 1.

Experiment 4 was conducted to evaluate the effects of PCAF inhibition on OL memory at 9+-months-of-age. During the sample phase, mice were presented two identical objects to explore for 10min. Immediately following the sample phase, mice were treated acutely with the PCAF inhibitor embelin (10mg/kg) or vehicle (5% DMSO, 5% Tween in PBS i.p.). At the end of either a 5min or 3h retention delay, mice underwent a 3min choice phase, in which mice were presented with one object in the same position as the sample phase, while the other object was positioned in a novel location.

6.3 Results

6.3.1 Longitudinal Effects of Acute PCAF Activation on OL

By longitudinally evaluating the effects of acute PCAF activation on OL at 3, 6, and 9-months-of-age, we showed that PCAF activation ameliorates spatial memory deficits in young 3xTG mice, but is ineffective by 9-months-of-age.

In 3-month-olds, the PCAF activator, SPV106, ameliorated OL deficits in 3xTG mice. A 2×2×2×2 split-plot analysis of variance (ANOVA) revealed a significant genotype × sex interaction ($F_{1,43}$ = 4.626, p = .037, η_p^2 = .097), a genotype × drug interaction ($F_{1,43}$ = 13.929, p = .001, η_p^2 = .245), a sex × drug interaction ($F_{1,43}$ = 8.264, p = .005, η_p^2 = .167), and a main effect of drug ($F_{1,43}$ = 20.764, p < .001, η_p^2 = .326); Figure 6.1a. *Post hoc t*-tests revealed a significant difference between vehicle and SPV106-treated 3xTG mice (t_{45} = −5.522, p < .001) and between vehicle-treated 3xTG mice and Wt mice (vehicle: t_{92} = 2.977, p = .032; SPV106: t_{92} = 3.672, p < .001). Paired samples *t*-tests between sample and choice discrimination ratios (DRs) indicated intact spatial memory in all Wt mice (vehicle-treated males, 5min: t_{11} = −6.584, p < .001; SPV106-treated males, 5min: t_{11} = −6.197, p < .001; vehicle-treated males, 3h: t_{11} = −4.567, p = .001; SPV106-treated males, 3h: t_{11} = −7.113, p < .001; vehicle-treated females, 5min: t_{11} = −3.692, p = .004; SPV106-treated females, 5min: t_{11} = −3.283, p = .007; SPV106-treated females, 3h: t_{11} = −4.458, p = .001), except vehicle-treated Wt females at 3h (t_{11} = −1.033, p = .324). Vehicle-treated 3xTG mice were impaired (males, 5min: t_{11} = −1.066, p = .309; males, 3h: t_{11} = −.339, p = .741; females, 5min: t_{11} = 1.050, p = 318; females, 3h: t_{11} = .010, p = .992)

and pre-treatment with SPV106 ameliorated these impairments (males, 5min: t_{11} = –6.197, p < .001; males, 3h: t_{11} = –5.814, p < .001; females, 5min: t_{11} = –4.068, p = .002; females, 3h: t_{11} = –4.020, p = .002).

In 6-month-olds, SPV106 continued to ameliorated OL deficits in 3xTG mice. A 2×2×2×2 split-plot ANOVA revealed a significant genotype × drug interaction ($F_{1,43}$ = 35.906, p < .001, η_p^2 = .455), a main effect of genotype ($F_{1,43}$ = 51.508, p < .001, η_p^2 = .545), and a main effect of drug ($F_{1,43}$ = 33.185, p < .001, η_p^2 = .436); Figure 6.1b. *Post hoc t*-tests indicated a significant difference between vehicle and SPV106-treated 3xTG mice (t_{45} = –6.747, p < .001) and between Wt and vehicle-treated 3xTG mice and Wt mice (vehicle: t_{92} = 8.631, p < .001; SPV106: t_{92} = 8.707, p < .001). Paired samples *t*-tests between sample and choice DRs suggested intact OL in Wt mice (vehicle-treated males, 5min: t_{11} = –6.824, p < .001; SPV106-treated males, 5min: t_{11} = –8.338, p < .001; vehicle-treated males, 3h: t_{11} = –3.953, p = .002; SPV106-treated males, 3h: t_{11} = –12.295, p < .001; vehicle-treated females, 5min: t_{11} = –8.058, p < .001; SPV106-treated females, 5min: t_{11} = –2.443, p = .033; vehicle-treated females, 3h: t_{11} = –3.178, p = .009; SPV106-treated females, 3h: t_{11} = –4.423, p = .001). Vehicle-treated 3xTG mice were impaired (males, 5min: t_{11} = –.122, p = .905; males, 3h: t_{11} = 1.068, p = .308; females, 5min: t_{11} = .021, p = .984; females, 3h: t_{11} = .459, p = .175) and treatment with SPV106 ameliorated these impairments (males, 5min: t_{11} = –3.501, p = .005; males, 3h: t_{11} = –3.595, p = .004; females, 5min: t_{11} = –4.836, p = .001; females, 3h: t_{11} = –2.622, p = .025).

In 9-month-olds, SPV106 failed to attenuate OL deficits in 3xTG mice. A 2×2×2×2 split-plot ANOVA revealed a significant drug × sex interaction ($F_{1,40}$ = 6.154, p = .017, η_p^2 = .133) and a main effect of genotype ($F_{1,40}$ = 99.230, p < .001, η_p^2 = .713); Figure 6.1c. Paired samples *t*-tests between sample and choice DRs indicated intact OL in Wt mice (vehicle-treated males, 5min: t_{11} = –4.303, p = .001; SPV106-treated males, 5min: t_{11} = –7.066, p < .001; vehicle-treated males, 3h: t_{11} = –5.440, p < .001; SPV106-treated males, 3h: t_{11} = –4.236, p = .001; vehicle-treated females, 5min: t_9 = –9.508, p < .001; SPV106-treated females, 5min: t_9 = –3.618, p = .006; vehicle-treated females, 3h: t_9 = –3.249, p = .010; SPV106-treated females, t_9 = –6.215, p < .001), and impaired OL in 3xTG mice (vehicle-treated 3xTG males, 5min: t_{10} = 1.993, p = .074; SPV106-treated males, 5min: t_{10} = .341, p = .740, vehicle-treated males, 3h: t_{10} = 2.161, p = .056; SPV106-treated males, 3h: t_{10} = .698, p = .501; vehicle-treated females, 5min: t_{10} = –.513, p = .619;

SPV106-treated females, 5min: t_{10} = 1.455, p = .176; vehicle-treated females, 3h: t_{10} = 1.695, p = .121; SPV106-treated females, 3h: t_{10} = .645, p = .534).

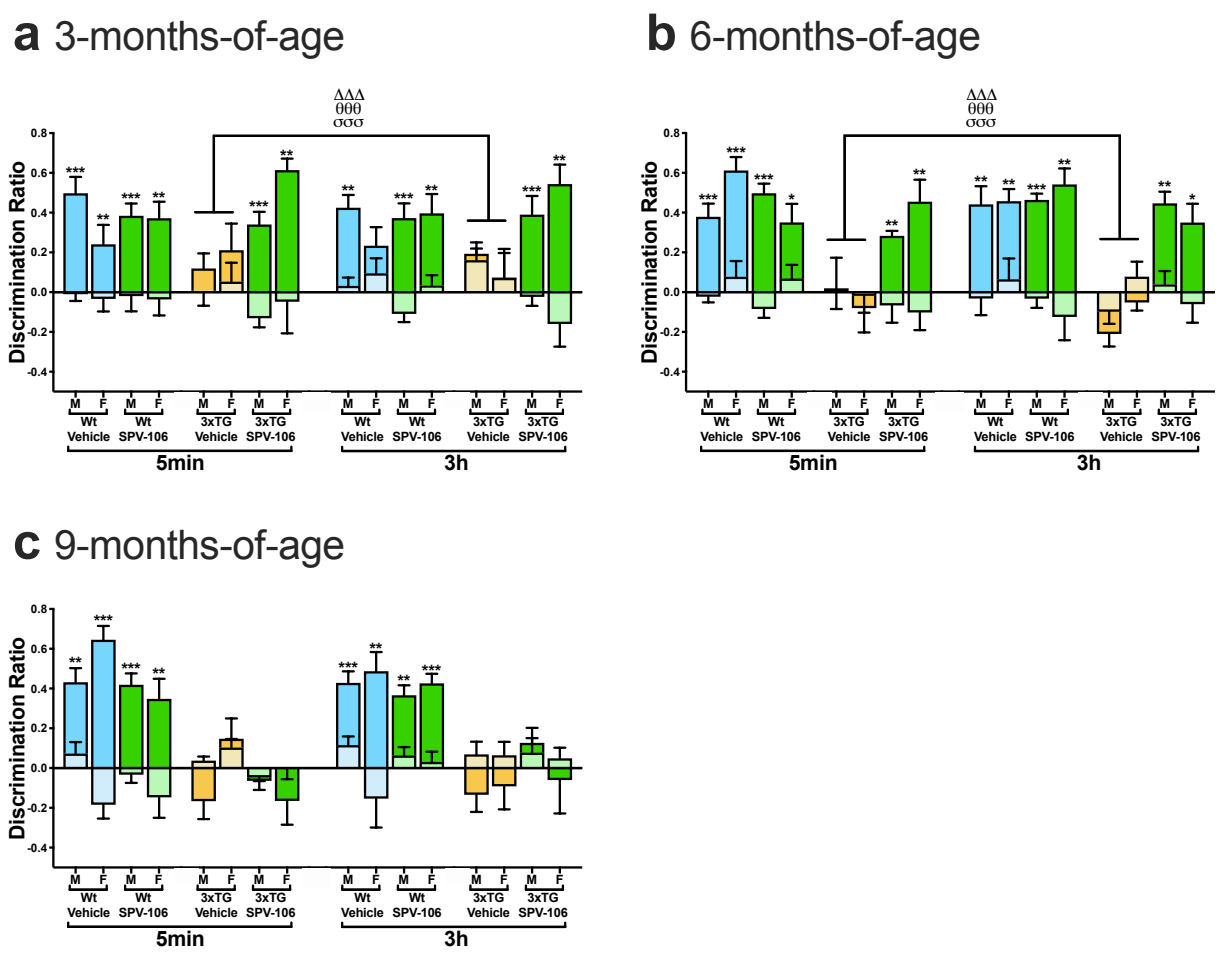

Figure 6.1 Age-dependent amelioration of spatial memory by PCAF activation (SPV106 25mg/kg) in 3xTG mice. a) At 3-months-of-age, vehicle-treated 3xTG mice were delay-independently impaired on OL, treatment with SPV106 ameliorated this impairment. b) At 6-months-of-age, vehicle-treated 3xTG mice were delay-independently impaired on OL, treatment with SPV106 continued to ameliorate this impairment. c) At 9-months-of-age, vehicle-treated 3xTG mice were delay-independently impaired on OL but treatment with SPV106 failed to attenuate this impairment. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR. ΔΔΔ p < .001 significantly different from vehicle-treated Wt mice, θθθ p < .001 significantly different from SPV106-treated Wt mice, σσσ p < .001 significantly different from SPV106-treated 3xTG mice.

6.3.1.1 Control Measure: OL Exploratory Behaviour

A 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,43}$ = 4.167, p = .047, η_p^2 = .144), a significant sex × delay interaction ($F_{1,43}$ = 8.135, p = .007, η_p^2 = .274), and a main effect of sex ($F_{1,43}$ = 17.303, p < .001, η_p^2 = .521) on exploration during the sample phase in 3-month-old mice; see Table 6.1 for all exploratory data. A 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex × delay interaction ($F_{1,43}$ = 5.307, p = .026, η_p^2 = .128), a genotype × sex × drug interaction ($F_{1,43}$ = 4.837, p = .033, η_p^2 = .164), and a main effect of sex ($F_{1,43}$ = 7.097, p < .001, η_p^2 = .287) on exploration during the choice phase.

A 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex interaction ($F_{1,43}$ = 4.427, p = .041, η_p^2 = .093), a main effect of genotype ($F_{1,43}$ = .627, p = .014, η_p^2 = .134), and a main effect of sex ($F_{1,43}$ = 8.153, p < .001, η_p^2 = .297) on exploration during the sample phase in 6-month-old mice. A 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex interaction ($F_{1,43}$ = 4.287, p = .044, η_p^2 = .091), a genotype × delay interaction ($F_{1,43}$ = 6.194, p = .017, η_p^2 = .126), and a main effect of genotype ($F_{1,43}$ = 5.431, p = .025, η_p^2 = .112) on exploration during the choice phase.

A 2×2×2×2 split-plot ANOVA demonstrated a significant genotype × sex interaction ($F_{1,40}$ = 11.623, p = .001, η_p^2 = .225), sex × delay interaction ($F_{1,40}$ = 10.245, p = .003, η_p^2 = .204), a main effect of genotype ($F_{1,40}$ = 8.144, p < .001, η_p^2 = .312), a main effect of sex ($F_{1,40}$ = 7.511, p = .009, η_p^2 = .158), and a main effect of delay ($F_{1,40}$ = 6.311, p = .016, η_p^2 = .136) on exploration during the sample phase in 9-month-old mice. A 2×2×2×2 split-plot ANOVA revealed a significant genotype × delay interaction ($F_{1,40}$ = 5.276, p = .027,

η_p^2 = .117), a significant sex × delay interaction ($F_{1,40}$ = 13.263, p = .001, η_p^2 = .249), and a main effect of sex ($F_{1,40}$ = 29.279, p < .001, η_p^2 = .423) on exploration during the choice phase.

Table 6.1 Longitudinal OL Exploratory Behaviour in SPV106 Experiments

			Mean Sample Exploration				Mean Choice Exploration			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	Vehicle	26.82 (4.25)	28.84 (5.11)	23.27 (5.78)	28.45 (4.96)	10.47 (1.56)	16.96 (2.73)	5.82 (0.87)	15.18 (2.09)
		SPV106	19.62 (1.83)	29.96 (5.74)	22.23 (4.02)	31.99 (4.49)	8.16 (1.37)	15.87 (2.26)	12.03 (2.41)	13.21 (1.96)
	3h	Vehicle	21.53 (2.18)	32.60 (4.22)	18.67 (4.29)	47.90 (7.68)	12.08 (2.35)	11.27 (1.89)	7.34 (1.00)	14.65 (2.12)
		SPV106	25.69 (4.69)	32.91 (4.93)	15.46 (1.56)	36.93 (5.67)	10.58 (1.75)	13.95 (2.47)	5.45 (0.66)	14.25 (2.23)
6	5min	Vehicle	45.08 (5.26)	14.47 (2.13)	43.70 (7.78)	36.42 (6.03)	13.53 (2.28)	6.28 (1.07)	8.29 (1.53)	7.87 (1.50)
		SPV106	35.90 (3.61)	20.41 (2.81)	37.64 (4.85)	26.74 (3.48)	6.89 (1.18)	5.99 (0.81)	8.33 (1.56)	10.53 (2.66)
	3h	Vehicle	36.00 (6.90)	20.18 (3.40)	44.21 (5.51)	40.86 (6.19)	8.95 (2.69)	5.39 (1.38)	10.21 (1.61)	8.15 (0.80)
		SPV106	44.71 (6.91)	16.78 (3.15)	42.78 (8.33)	33.86 (6.64)	7.52 (1.22)	4.02 (0.56)	11.37 (1.80)	12.10 (1.30)
9	5min	Vehicle	41.14 (5.28)	9.52 (1.91)	43.43 (7.83)	36.95 (5.72)	15.86 (2.17)	8.02 (0.69)	15.40 (2.38)	4.88 (1.49)
		SPV106	26.56 (1.64)	20.72 (2.48)	37.64 (4.11)	29.00 (2.61)	14.76 (1.84)	7.45 (1.20)	13.34 (1.84)	5.13 (0.60)
	3h	Vehicle	39.81 (4.06)	25.72 (4.26)	41.77 (5.16)	45.51 (5.12)	11.44 (1.71)	7.73 (1.79)	12.80 (1.77)	8.95 (1.01)
		SPV106	34.04 (3.54)	25.37 (6.97)	29.55 (3.26)	47.72 (7.46)	9.94 (1.62)	5.90 (1.68)	9.91 (1.61)	10.38 (1.43)

Note. Data are mean exploration (s) ± SEM.

6.3.1.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Despite the differences in exploratory behaviour that were observed at each timepoint, exploration did not consistently significantly correlate with OL performance (choice DR; Table 6.2).

Table 6.2 Correlations Between Exploration and Longitudinal OR in SPV106 Experiments

			Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
3	5min	Vehicle	−.076	.208	.082	−.314	−.356	.454	−.254	.064
		SPV106	−.110	.666*	.579	−.404	−.254	.292	−.131	−.389
	3h	Vehicle	.064	−.207	.003	−.130	.060	.527	−.072	−.285
		SPV106	.533	−.432	−.472	−.084	.052	.143	−.545	.256
6	5min	Vehicle	−.310	−.454	.349	−.301	−.383	.121	−.162	.269
		SPV106	−.236	.063	.665	−.039	−.143	.348	−.333	.481
	3h	Vehicle	.215	.494	−.023	−.310	.379	.035	.086	.443
		SPV106	.056	−.095	−.450	−.038	−.249	.294	−.519	.167
9	5min	Vehicle	.199	.701	−.105	.124	.054	.194	−.550	.045
		SPV106	−.373	−.454	−.630	−.169	.330	−.242	−.533	−.082
	3h	Vehicle	−.023	.457	−.126	.245	−.278	.074	.214	.172
		SPV106	.048	−.057	−.487	−.280	.296	.095	−.103	.182

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR). * $p < .05$.

6.3.2 Effects of Acute PCAF Inhibition on OL

We also evaluated the effects of acute PCAF inhibition on OL after the timepoint identified in our SPV106 experiments at which impairments were no longer ameliorated, 9+-months-of-age. We show that PCAF inhibition attenuated spatial memory deficits in aged female 3xTG mice. A 2×2×2×2 split-plot ANOVA revealed a significant genotype × sex × drug interaction ($F_{1,38} = 6.330$, $p = .016$, $\eta_p^2 = .143$), a significant genotype × drug interaction ($F_{1,38} = 110.642$, $p < .001$, $\eta_p^2 = .744$), and a significant main effect of drug ($F_{1,38} = 28.441$, $p < .001$, $\eta_p^2 = .428$); Figure 6.2. *Post hoc t*-tests revealed significant

differences between vehicle-treated Wt males and embelin-treated Wt mice (male: t_{21} = 8.442, p < .001; female: t_{40} = 1.074, p < .001), as well as vehicle-treated 3xTG mice (males: t_{40} = 6.572, p < .001; females: $t_{28.822}$ = 3.863, p = .014). In addition, there were also significant differences between vehicle-treated Wt females and embelin-treated Wt mice (males: t_{40} = –9.520, p < .001; females t_{19} = 11.730, p < .001), vehicle-treated 3xTG mice (males: $t_{29.873}$ = –7.766, p < .001, females: $t_{31.274}$ = 4.700, p < .001), and embelin-treated 3xTG males (t_{38} = –3.688, p = .014). Paired samples t-tests between sample and choice DR suggest intact OL in vehicle-treated Wt mice (males, 5min: t_{10} = –4.958, p = .001; males, 3h: t_{10} = –5.137, p < .001; females, 5min: t_9 = –2.819, p = .020; females, 3h: t_9 = –4.239, p = .002), whereas embelin-treated Wt mice were impaired (males, 5min: t_{10} = 2.193, p = .053; males, 3h: t_{10} = .998, p = .342; females, 5min: t_9 = 1.283, p = .232; females, 3h: t_9 = .1.919, p = .087). Vehicle-treated 3xTG mice were impaired on OL at both delays (males, 5min: t_9 = .528, p = .610; males, 3h: t_9 = –.457, p = .658; females, 5min: t_{10} = .437, p = .671; females, 3h: t_{10} = .606, p = .58); treatment with embelin attenuated the impairment in females at the 3h delay (t_{10} = –2.803, p = 019), but not in other groups (males, 5min: t_9 = –1.453, p = .610; males, 3h: t_9 = –1.961, p = .082; females, 5min: t_{10} = –1.626, p = .135).

9+-months-of-age

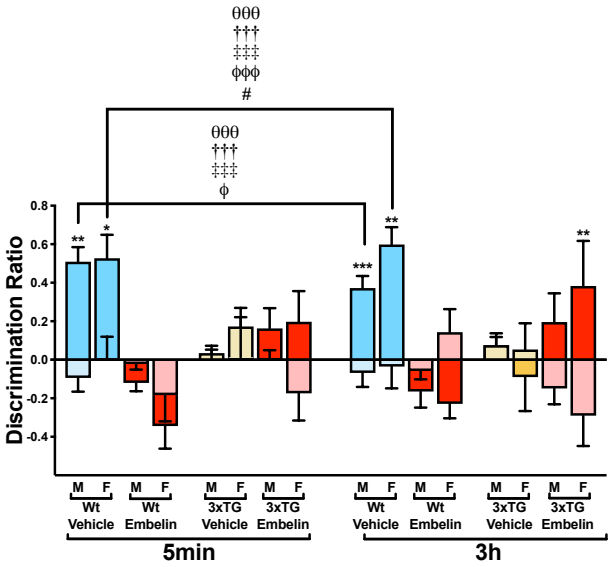

Figure 6.2 Regulation of spatial memory by PCAF inhibition (embelin 10mg/kg). At 9+ months of age, embelin impaired Wt mice at both delays. Vehicle-treated 3xTG mice were impaired at the 5min and 3h delay, embelin may attenuate this impairment, particularly in 3xTG females at 3h. Data are mean ± SEM. Sample DR's are represented with lightly shaded bars and choice DR's are represented with brightly coloured bars. * p< .05, ** p < .01, ***p < .001 indicates significant differences between sample and choice DR. θθθ p<.001 significantly different from embelin-treated Wt males, ††† p<.001 significantly different from embelin-treated Wt females, ‡‡‡ p<.001 significantly different from vehicle-treated 3xTG males, φ p<.01, φφφ p<.001 significantly different from vehicle-treated 3xTG females, and # p<.05 significantly different from embelin-treated 3xTG males.

6.3.2.1 Control Measure: OL Exploratory Behaviour

A 2×2×2×2 split-plot ANOVA demonstrated a significant sex × delay interaction ($F_{1,38}$ = 4.568, p = .039, η_p^2 = .107) and a significant main effect of delay ($F_{1,38}$ = 5.736, p = .022, η_p^2 = .131) on exploration during the sample phase in 9+-month-old mice; see Table 6.3 for all exploratory data. A 2×2×2×2 split-plot ANOVA demonstrated a significant genotype × delay interaction ($F_{1,38}$ = 14.814, p < .001, η_p^2 = .280), a sex × delay interaction ($F_{1,38}$ = 16.403, p < .001, η_p^2 = .302), a delay × drug interaction ($F_{1,38}$ = 16.263, p < .001, η_p^2 = .300), a main effect of genotype ($F_{1,38}$ = 5.890, p < .001, η_p^2 = .295), sex ($F_{1,38}$ = 4.939, p = .032, η_p^2 = .115), and drug ($F_{1,38}$ = 9.442, p = .004, η_p^2 = .199) on exploration during the choice phase.

Table 6.3 Longitudinal OL Exploratory Behaviour in Embelin Experiments

			Mean Sample Exploration				Mean Choice Exploration			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
9+	5min	Vehicle	35.57 (5.98)	22.28 (5.44)	30.47 (3.16)	34.85 (6.10)	11.49 (1.23)	8.25 (2.18)	8.63 (1.64)	6.81 (1.25)
		Embelin	34.32 (3.10)	24.14 (4.82)	35.32 (3.29)	24.96 (3.80)	2.97 (0.48)	1.85 (0.43)	12.15 (1.70)	4.93 (1.18)
	3h	Vehicle	31.62 (3.92)	38.77 (9.50)	32.50 (6.22)	42.59 (7.39)	4.15 (0.69)	6.41 (1.64)	8.63 (1.64)	9.30 (1.52)
		Embelin	40.91 (.96)	44.97 (11.56)	33.78 (5.76)	35.06 (6.58)	2.97 (0.48)	4.31 (0.75)	12.15 (1.70)	11.01 (2.00)

Note. Data are mean exploration (s) ± SEM.

6.3.2.2 Correlation Between Exploratory Behaviour and Choice Discrimination Ratio

Although there were differences in exploratory behaviour, exploration did not significantly correlate with OL performance (choice DR); Table 6.4.

Table 6.4 Correlations Between Exploration and Longitudinal OR in Embelin Experiments

			Correlation Between Sample Exploration and Choice DR (r)				Correlation Between Choice Exploration and Choice DR (r)			
			Wt		3xTG		Wt		3xTG	
Age (Months)	Delay	Drug	Male	Female	Male	Female	Male	Female	Male	Female
9+	5min	Vehicle	−.452	−.297	−.135	.121	−.122	.138	.119	−.206
		Embelin	.140	−.340	.209	−.228	.325	.620	.205	−.121
	3h	Vehicle	.453	.344	−.162	−.208	.072	−.091	−.610	−.157
		Embelin	.044	−.584	.306	.058	.014	−.160	.447	.069

Note. Pearson correlations between total sample or choice exploration (s) and task performance index (choice DR).

6.4 Discussion

By longitudinally evaluating the effects of PCAF activation on OL memory, these experiments shows that PCAF also bidirectionally regulates spatial memory in 3xTG mice. At both 3 and 6-months-of-age, the PCAF activator, SPV106, ameliorated short- and long-term OL deficits. At 9-months-of-age, SPV106 no longer modulated the OL impairment in 3xTG mice and in female 3xTG mice the PCAF inhibitor, embelin, attenuated the long-term OL deficit. Conversely, in Wt mice, embelin impaired OL at 9-months-of-age. Consistent with our previous OR data, we reveal a complex role for PCAF in AD-relevant cognition.

As we suggested previously, it is probable that the beneficial effects of PCAF activation on short- and long-term spatial memory at 3 and 6-months-of-age are the result of increased of histone acetylation (Milite et al., 2011; Sbardella et al., 2008) and expression of memory enhancing genes (Peixoto & Abel, 2013) or the acetylation of non-histone proteins involved in cell signaling cascades that support memory (e.g. Mitchnick & Winters, 2018). By 9-months-of-age, SPV106 failed to attenuate OL deficits, suggesting that the memory promoting effects of PCAF activation are insufficient to overcome OL impairments induced by AD neuropathology at this age. At this age, the PCAF inhibitor, embelin, appeared to attenuated OL deficits, particularly in female 3xTG mice at the 3h delay. In these mice. embelin may improve cognition by reducing PCAF-mediated activation of NF-κB-dependent inflammation (Park et al., 2015, 2013), or increasing expression of genes involved in Aβ degradation (Duclot et al., 2010), or by decreasing acetylation of the histone variant H2A.Z that is a negative regulator of memory (Zovkic et

al., 2014). Based on our previous OR data, it was anticipated that embelin would have similar effects in all 3xTG mice. It is not known why the OL impairments were not reversed in 3xTG males at the 3h delay.

Interestingly, at 9-months-of-age, systematic SPV106 ameliorated OR impairments (Chapter 4). It is likely that this differential time course for the bidirectional regulation of object and spatial memory is related to the severity of cognitive deficits and pathology in task-relevant brain regions. Object location is dependent on the HPC (Assini et al., 2009; Barker & Warburton, 2011; Mumby & Pinel, 1994), as well as the fornix and anterior cingulate cortex (Abdelkader Ennaceur et al., 1997). Although the HPC may be involved in OR in mice (Cohen et al., 2013; Hammond et al., 2004), it has been suggested that the HPC is specifically involved in spatial and temporal processing of objects, whereas the perirhinal cortex (PRh) is involved in the processing of object features (Barker & Warburton, 2011; Winters et al., 2004). When testing OR under conditions where spatial cues are minimized by using a Y-apparatus (Forwood, Winters, & Bussey, 2005; Winters et al., 2004), or with shorter retention delays (< 24h; Hammond et al., 2004), as we have, the HPC may be dispensable. In 3xTG mice, the HPC develops an earlier and more aggressive AD pathology and seems to be more susceptible do cognitive deficits than the PRh (Creighton et al., 2019; Davis et al., 2013; Mastrangelo & Bowers, 2008; Oddo, Caccamo, Kitazawa, et al., 2003; Oddo, Caccamo, Shepherd, et al., 2003).

In conclusion, these experiments demonstrate that PCAF also bidirectionally attenuates spatial memory deficits in 3xTG mice. Interestingly, the bidirectional effects of systemic PCAF activation and the impact of PCAF inhibition on spatial memory follows a

differential time course compared with OR, which is likely related to differences in the severity of AD-pathology in task-relevant brain regions.

7 Regulation of Histone Acetylation by Pharmacological Activation and Inhibition of the Lysine Acetyltransferase CBP/p300 Associated Factor (PCAF)

The experiments presented in this chapter were performed in collaboration with Dr. Iva Zovkic at the University of Toronto Mississauga with the help of Dr. Gilda Stefanelli

7.1 Introduction

Histone acetylation, catalyzed by histone acetyltransferases (HATs), involves the addition of acetyl groups (Ac; $-C_2H^3O$) from acetyl coenzyme A (acetyl CoA) to lysine (K) residues on histone protein tails (Kuo & Allis, 1998). There are a number of lysine residues that are known to be acetylated on both canonical histones and histone variants (Corujo & Buschbeck, 2018; Gräff & Tsai, 2013; McKittrick, Gafken, Ahmad, & Henikoff, 2004; Tweedie-Cullen et al., 2012). This process is reversed by histone deacetylases (HDACs).

Increasing HAT or decreasing HDAC activity increases histone acetylation, which enhances gene transcription and many facets of learning and memory (Peixoto & Abel, 2013). Although both pharmacological HAT activators and HDAC inhibitors are available, research into the memory-enhancing enhancing effects of increasing histone acetylation for cognitive remediation in models of neurodegenerative diseases has predominantly focused on HDAC inhibition (Fischer, 2014; Fischer et al., 2010). Global HDAC inhibition has been shown to attenuate cognitive deficits, increase histone acetylation, and can have beneficial effects on AD neuropathology in rodent AD models (Fischer, 2014). However, broad HDAC inhibition provides limited mechanistic insight and can be toxic (Salminen et al., 1998). Since the primary function of HDACs is to remove acetyl groups added by HATs (Day & Sweatt, 2011), and the effectiveness of HDAC inhibition can be dependent on the function of specific HATs (Chen et al., 2010; Vecsey et al., 2007), targeting specific HATs may be a better therapeutic approach. Indeed, activation of the HAT, cAMP response element-binding protein (CREB) binding protein (CBP), has been shown to increase histone acetylation, restore spatial memory deficits, and improve

spatial memory in mouse models of Alzheimer's disease (AD; Caccamo et al., 2010; Chatterjee et al., 2018). In this thesis, we evaluated the behavioural effects of targeting another specific HAT, p300/CBP associated factor (PCAF), in the triple transgenic (3xTG) mouse model of AD. Specifically, systemic acute administration of the PCAF activator, SPV106 enhanced object recognition (OR) from 3-6 months of age and ameliorated OR deficits at 9-months-of-age. At 12-months-of-age, however, PCAF activation further impaired OR, whereas the PCAF inhibitor, embelin, ameliorated the OR impairment. A similar, albeit accelerated, pattern of results was observed for spatial memory using the object location task. Changes in histone acetylation likely mediate some of our effects of SPV106 and embelin on cognition. Indeed, SPV106 has been shown to increase global acetylation of H3 and H4 in human kidney cells, U937 leukemia cells, and MCF-7 breast cancer cells (Milite et al., 2011; Sbardella et al., 2008), whereas embelin decreases H3K9 acetylation in mouse liver cells, and parasites (Modak et al., 2013; Srivastava et al., 2014). Despite studies demonstrating behavioural effects of SPV106 and embelin (Mitchnick et al., 2016; Mitchnick & Winters, 2018; Wei et al., 2012), changes in acetylation have not been demonstrated in neurons or brain tissue. Additionally, the effects of PCAF activation and inhibition on the acetylation of histone variants, like H2A.Z, has not been examined.

To begin to probe the effects of PCAF activation and inhibition on histone acetylation in neurons, we evaluated the effects of embelin and SPV106 on acetylation of H3, H4, and/or H2A.Z in primary hippocampal neurons and cortices isolated from drug-treated Wt mice. We predicted that SPV106 administration would increase histone acetylation, whereas embelin would decrease histone acetylation.

7.2 Methods

7.2.1 Drugs

7.2.1.1 Embelin

Embelin (2,5-Dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione; Abcam) is a non-competitive specific PCAF antagonist (Modak et al., 2013). For cell culture experiments, primary hippocampal neuron cultures were treated with embelin (20μM final concentration) in 10% dimethylsulfoxide (DMSO) for 6 hours.

7.2.1.2 SPV106

SPV106 (2-pentadecylidene-propanedioic acid 1,3-diethyl ester, pentadecylidenemalonate 1b; donated by Gianluca Sbardella, University of Salerno, Italy) is a selective PCAF activator and CBP inhibitor (Sbardella et al., 2008). For cell culture experiments, primary hippocampal neurons were treated with vehicle or various SPV106 concentrations, beginning with 50 μM for 6 hours (Sbardella et al., 2008), 100μM for 6 hours, or 200 μM for 6 hours, 200μM for 24h, and 200μM for 36 hours. Only data from the highest dose and longest treatment time (200μM for 36 hours) is shown. For experiments in Wt mice, acute SPV106 (25mg/kg) or vehicle (1% DMSO) treatments were administered three days prior to sacrifice. This dosing regimen follows the procedure used in our behavioural experiments (Chapters 4 and 6).

7.2.2 Primary Hippocampal Neuron Cultures

Primary hippocampal neuron cultures were prepared from embryonic day 17 C57bl/6J mice. All procedures were approved by the UTM animal care committee and complied with institutional guidelines and the Canadian Council on Animal Care.

Hippocampi were dissected and washed in ice-cold Hank's Buffered Salt Solution (HBSS), and digested with Trypsin (Life Technologies). The reaction was stopped using 10% fetal bovine serum (FBS) in Dulbecco's Modified Eagle's Medium (DMEM). Hippocampi were then dissociated by mechanical pipetting. Dissociated cells were plated on 6-well poly-L-lysine (0.1mg/mL; Sigma) at a density of 250000/well. Neurons were maintained in Neurobasal media containing 25 μM glutamine (Gibco), B27 supplement (50x; Life Technologies), in an incubator at 37 °C and 5 % CO2 for 13 days *in vitro*.

Following treatment with embelin or SPV106, media was removed and cells were washed with HBSS and lysed with 200ul/well of 6X sample buffer (Tris·HCl, pH 6.8, SDS, Glycerol, DTT, Bromophenol Blue). Cell lysates were scraped and collected in 1.5 mL Eppendorf tubes, stored at -20 °C or immediately run on (SDS)-polyacrylamide gel electrophoresis.

7.2.3 Animals used for Experiments in Wt Mice

3-month-old wild-type (B6129SF2/J male n=6, female n=2) mice were used for molecular experiments. Following treatment with SPV106 (25mg/kg i.p. 3 days prior to sacrifice), unilateral cortices and hippocampi were dissected and placed on dry ice prior to being immediately stored at -80°C.

Both salt and acid extraction of proteins were performed; two common biochemical techniques used to isolate histone proteins (Shechter, Dormann, Allis, & Hake, 2007).

For salt extraction of histones, samples were homogenized in cytosol buffer (10 mM hepes pH7.5, 1.5 mM MgCl2, 10mM KCL, 0.2 % NP40, 0.1,mM EDTA, 10% glycerol), centrifuged at 2000 x g for 5min, and the supernatant was collected as the soluble fraction (proteins minimally bound to chromatin). The pellet was resuspended in 10mM Tris (pH 7.4) with 600mM NaCl, incubated on ice for 10min, centrifuged at 10 000 x g for 10min, and the supernatant was collected as the salt extractable fraction (proteins moderately associated bound to chromatin). The final pellet (proteins tightly bound to chromatin) was resuspended in 10mM Tris (pH 7.4).

For acid extraction of histones, nuclei were isolated by homogenizing in buffer A (0.25M sucrose, 60mM KCl, 15mM NaCl, 10mM MES/Pipes pH 6.5, 5mM MgCl2, 0.5% triton), centrifuged at 7000 x g for 5min, the supernatant was discarded and the pellet (nuclei) was resuspended in buffer B (50mM NaCl, 10mM Pipes pH 6.8, 5MM MgCl2, 1mM CaCl2). Using 100ul of resuspended nuclei, HCl (1.2N) was added prior to centrifugation at 13.3 x g for 10 min at 4°C and the supernatant was collected and mixed with cold acetone (6 volumes) and precipitated overnight at -20°C. Following precipitation, samples were centrifuged at 13.3 x g for 10min at 4°C, washed with warm acetone and centrifuged at 13.3 x g for 10min at room temperature. The supernatant was discarded and the pellet was resuspended in dH_2O.

7.2.4 Western Blotting

Protein extracts were separated by 15% sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) using a Mini-PROTEAN Tetra cell system.

Samples were transferred to polyvinylidene difluoride (PVDF(membranes, blocked in 5% milk in tris-buffered saline containing 0.1% Tween20 (TBS-T) for 1h, and incubated in primary antibodies (see table 7.1) in TBS-T containing 5 % milk overnight at 4°C. Blots were subsequently rinsed with TBS-T, incubated in either rabbit or mouse secondary antibody (see table 7.1) in TBS-T containing 5 % milk for 1h at room temperature, rinsed with TBS-T, developed using enhanced chemiluminescence (Pierce) and visualized using the using GE ImageQuant LAS 500 imaging system.

For primary hippocampal neuron experiments, samples probed for H3, acetylated H3, H2A.Z, and acetylated H2A.Z were run on separate gels. Densitometry was performed using ImageJ (NIH). Band densities were compared to corresponding beta actin on each membrane. For salt and acid extraction experiments, acetylated H3, acetylated H4, and acetylated H2A.Z were compared to total levels of H3, H4, or H2A.Z, respectively, that were run on separate gels.

Table 7.1 Antibody Table

Target	Species and Clonality	Dilution used for Western Blotting	Manufacturer and Product Number
H3	Mouse monoclonal	1: 1000	Millipore, 05-499
Acetylated H3	Rabbit polyclonal	1: 1000	Millipore, 06-599
H4	Rabbit polyclonal	1: 1000	Abcam, ab10158
Acetylated H4 (Lys 5, 8, 12, 16)	Rabbit monoclonal	1: 1000	Abcam, ab177790
H2A.Z	Rabbit polyclonal	1: 1000	Millipore, ABE1348
Acetylated H2A.Z (Lys 5, 7, 11)	Rabbit polyclonal	1: 1000	Millipore, ABE1363
Beta Actin	Rabbit polyclonal	1: 2000	Cell Signaling, 4967S
Anti-Rabbit IGG, HRP-linked antibody	Goat	1: 10 000	Cell Signaling, 7074S
Anti-Mouse IGG, HRP-linked antibody	Horse	1: 10 000	Cell Signaling, 707P2

7.2.5 Statistical Analyses

The relative densities are presented as mean ± SEM and statistical analyses were conducted with a significance level of $\alpha = .05$, using IBM SPSS statistics. Independent samples *t*-tests were used to compare vehicle and drug-treated groups.

7.2.6 Experiments

Experiment 1 an 2 were designed to assess the effect of embelin and SPV106 on histone acetylation in primary hippocampal neuron cultures. In experiment 1, primary hippocampal neurons were treated with embelin (20μM) or vehicle for 6 hours and acetylation of H3 and H2A.Z were evaluated using western blotting. In experiment 2, primary hippocampal neurons were treated with SPV106 (200μM) or vehicle for 36 hours and acetylation of H3 and H2A.Z were evaluated using western blotting.

Experiment 3 and 4 were designed to assess histone acetylation in Wt mice treated with SPV106. In experiment 3, cortices from Wt female mice treated with SPV106 (25mg/kg i.p. 3 days prior to sacrifice) or vehicle were processed using salt extraction and acetylation of H2A.Z was evaluated using western blotting, In experiment 4, cortices from Wt male mice treated with SPV106 (25mg/kg i.p. 3 days prior to sacrifice) or vehicle were processing using acid extraction and acetylation of H3, H4, and H2A.Z were evaluated using western blotting.

7.3 Results

7.3.1 Effects of the PCAF inhibitor, Embelin, on Histone Acetylation

7.3.1.1 Hippocampal Primary Neuron Cultures Treated with Embelin

As a first pass at exploring the effects of PCAF inhibition on histone acetylation, we evaluated the effects of embelin on acetylation of H3 and H2A.Z in primary hippocampal neurons. We show that embelin decreases acetylation of H2A.Z, but not H3, acetylation in primary hippocampal neurons; Figure 7.1. Representative immunoblots in Figure 7.1a depict the levels of total and acetylated H3 and H2A.Z. Total H3 and H2AZ

protein levels (top and middle panels, respectively) and acetylated H3 (second panel) appear relatively equal in all vehicle and embelin treated samples, while acetylated H2A.Z (fourth panel) is substantially reduced in samples obtained from embelin-treated neurons.

Densitometric analysis followed by an independent samples t-test demonstrated that acetylation of H3 did not decrease following treatment with embelin ($t_4 = -.055$, $p = .959$; Figure 7.1b), in primary hippocampal neurons. Furthermore, embelin did not affect total levels of H3, as there was no effect of drug treatment on H3 expression relative to beta actin ($t_4 = 2.169$, $p = .096$; Figure 7.1c). In contrast, independent samples t-tests revealed a significant decrease in acetylation of H2A.Z, in primary hippocampal neurons, following treatment with embelin ($t_4 = 5.017$, $p = .007$; Figure 7.1d) that cannot be explained by changes in the level of H2A.Z, as there was no effect on the expression of H2A.Z relative to beta actin ($t_4 = -.193$, $p = .857$; Figure 7.1e).

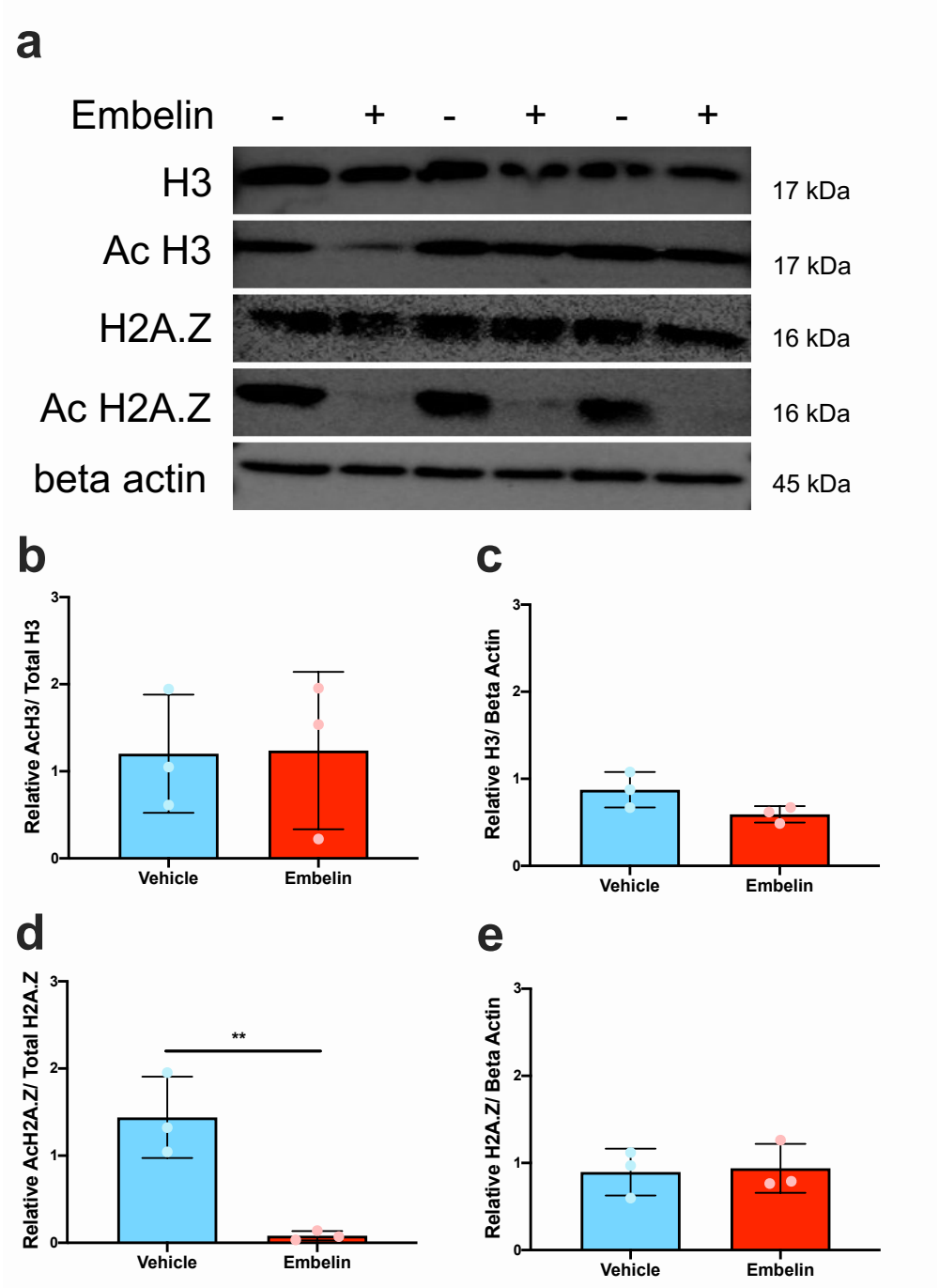

Figure 7.1 Decreased acetylation of H2A.Z, but not H3, by the PCAF inhibitor, embelin, in primary hippocampal neurons. a) Representative western blot images showing levels of H3, acetylated H3, H2A.Z, acetylated H2A.Z, and a representative beta actin in samples obtained from primary hippocampal neurons treated with vehicle (DMSO) embelin (20μM) for 6 hours. b) Corresponding quantitative densitometric analysis for b) acetylated H3 relative to total H3; c) total H3 relative to beta actin; d) acetylated H2A.Z relative to total H2A.Z; d) total H2A.Z relative to beta actin. Data are mean ± SEM, presented as fold changes. **p < .01.

7.3.2 Effects of the PCAF activator, SPV106, on Histone Acetylation

7.3.2.1 Hippocampal Primary Neuron Cultures Treated with SPV106

To being to probe the effects of PCAF activation on histone acetylation, we evaluated the effects of SPV106 treatment on the acetylation of H3 and H2A.Z in primary hippocampal neurons. We show that SPV106 had no effects on acetylation of H3 or H2A.Z in primary hippocampal neurons; Figure 7.2. Representative immunoblots in Figure 7.2a depict the levels of total H3, acetylated H3, and H2A.Z. Total H3 (top panel), acetylated H3 (second panel), H2A.Z (third panel), and acetylated H2A.Z (fourth panel) appear relatively equal in all vehicle and SPV106 treated samples.

Densitometric analysis followed an independent samples t-test demonstrated that acetylation of H3 did not increase following treatment with SPV106 ($t_4 = -.056$, $p = .958$; Figure 7.2b), in primary hippocampal neurons. Furthermore, SPV106 did not alter total levels of H3, as there was no effect of drug treatment on H3 expression relative to beta actin ($t_4 = -.375$, $p = .727$; Figure 7.2c). An independent samples t-test demonstrated that acetylation of H2A.Z did not increase following treatment with SPV106 ($t_4 = -.281$, $p = .792$; Figure 7.2d), in primary hippocampal neurons. Furthermore, SPV106 did not alter total levels of H2A.Z, as there was no effect of drug treatment on H2A.Z expression relative to beta actin ($t_4 = 1.145$, $p = .317$; Figure 7.2e).

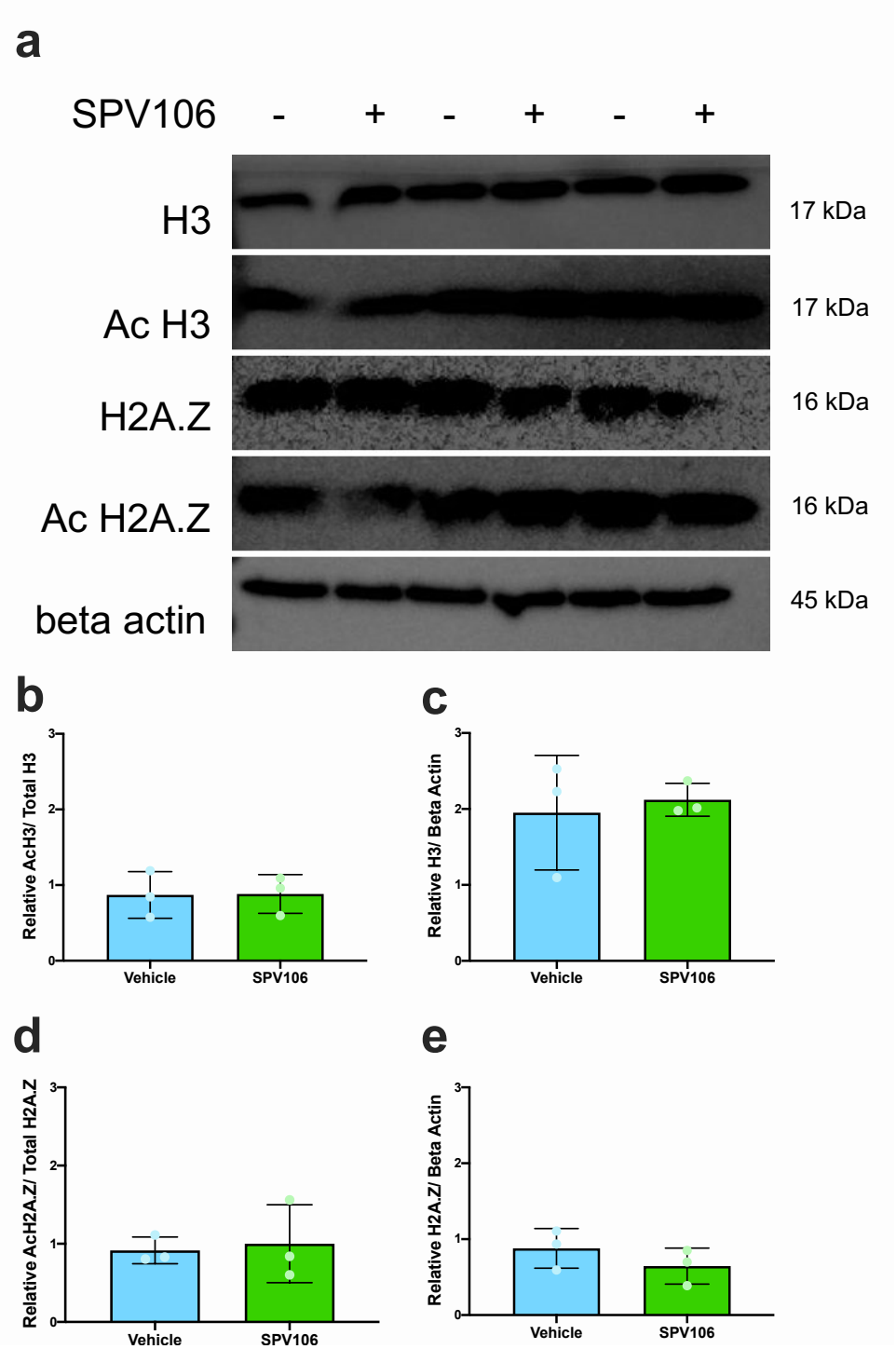

Figure 7.2 The PCAF activator, SPV106, did not alter histone acetylation in primary hippocampal neurons. a) Representative western blot images depicting levels of H3, acetylated H3, H2A.Z, acetylated H2A.Z, and a representative beta actin in samples obtained from primary hippocampal neurons treated with vehicle (DMSO) or SPV106 (200μM) for 36 hours. b) Corresponding quantitative densitometric analysis for b) acetylated H3 relative to total H3; c) total H3 relative to beta actin; d) acetylated H2A.Z relative to total H2A.Z; d) total H2A.Z relative to beta actin. Data are mean ± SEM, presented as fold changes.

7.3.2.2 Salt Extraction of Histones from Wt Mice Treated with SPV106

Since we failed to observe an effect of SPV106 on histone acetylation in primary hippocampal neurons, we looked for changes in acetylation in Wt mice treated with SPV106 using the dosing regimen that was effective in our behavioural studies (25mg/kg 3 days prior to sacrifice).

We used salt extraction to fractionate cellular proteins into those that are minimally (soluble), moderately (salt extractable), and strongly (pellet) bound to chromatin. Following salt extraction, we evaluated the effects of SPV106 on acetylation of H2A.Z in the cortex of female Wt mice; Figure 7.3. Qualitatively, this data demonstrates that all of the acetylated H2A.Z (bottom panel) is located in the pellet fraction and there is no difference in the level of H2A.Z (top panel) or acetylated H2A.Z in vehicle and SPV106-treated mice.

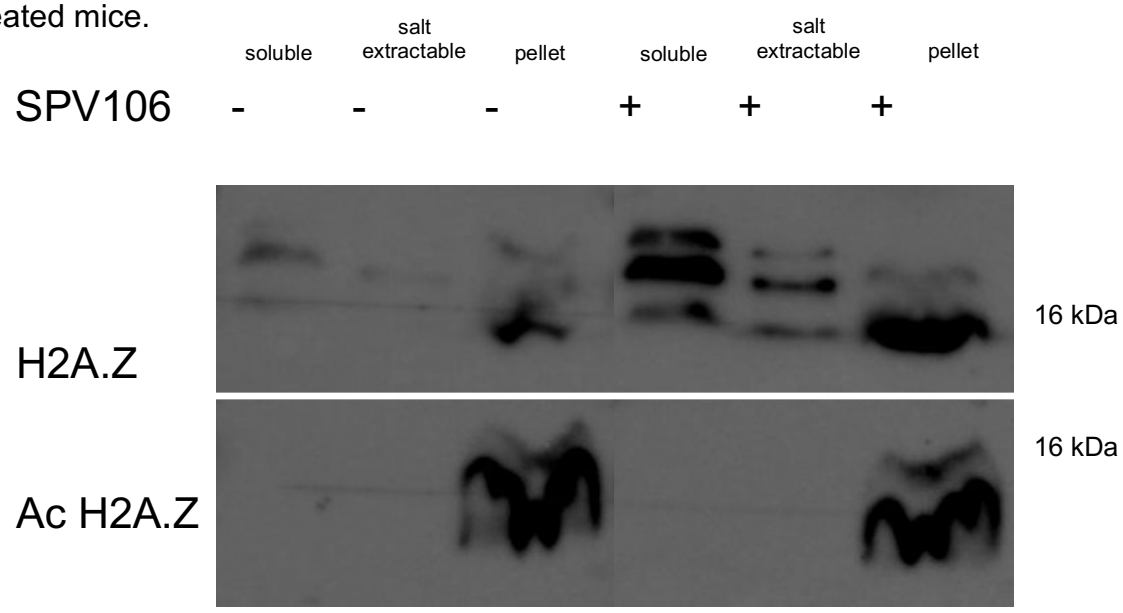

Figure 7.3 Representative western blot of H2A.Z and acetylated H2A.Z following chromatin fractionation in the cortex of female Wt mice treated with SPV106 (25mg/kg). Histones were fractionated using a salt extraction. H2A.Z is detectable in the soluble (proteins loosely bound to chromatin), salt extractable (moderately bound to chromatin), and pellet (tightly bound to chromatin) fractions. Acetylated H2A.Z, however is only detectable in the pellet.

7.3.2.3 Acid Extraction of Histones from Wt Mice Treated with SPV106

Following acid extraction of basic proteins, which are predominantly histones, we evaluated the effects of SPV106 on acetylation of H3, H4, and H2A.Z in the cortex of male Wt mice. Representative immunoblots in Figure 7.5a depict the levels of total H3, acetylated H3, total H4, acetylated H4, total H2A.Z, and acetylated H2A.Z. Total H3 (top panel), acetylated H3 (second panel), total H4 (third panel), acetylated H4 (fourth panel), total H2A.Z (fifth panel), and acetylated H4 (bottom panel) appear relatively equal in all vehicle and SPV106 treated samples.

Still, we did not observe any effects of SPV106 on histone acetylation; Figure 7.4. Densitometric analysis followed an independent samples t-tests revealed no significant differences between acetylated H3 relative to total H3 ($t_{10} = -.051$, $p = .960$; Figure 7.4b), acetylated H4 relative to total H4 ($t_{10} = -.881$, $p = .399$; Figure 7.4c), or acetylated H2A.Z relative to total H2A.Z ($t_{10} = .508$. $p = .622$; Figure 7.4d).

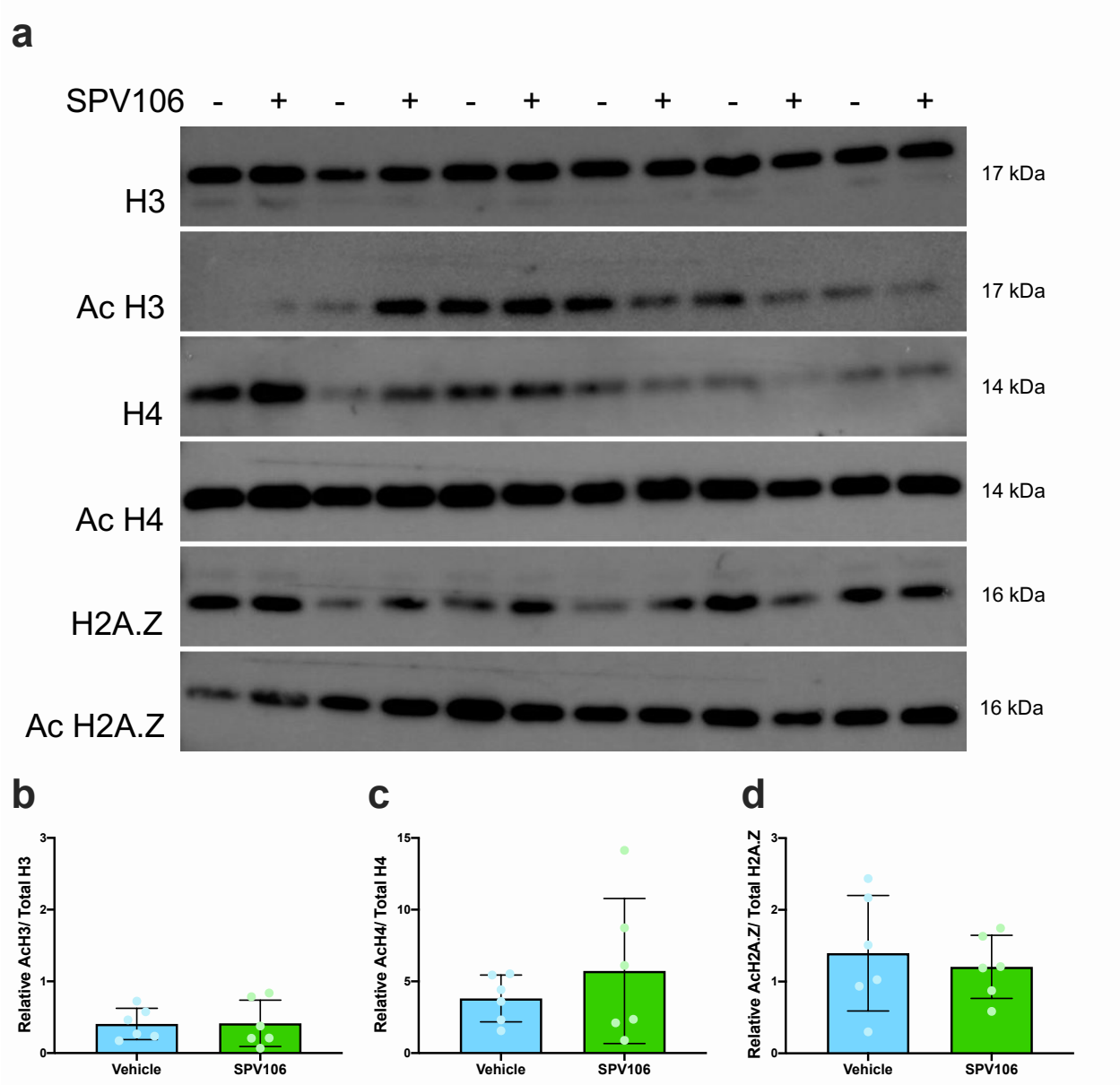

Figure 7.4 The PCAF activator, SPV106, did not alter histone acetylation in Wt mice. a) Representative western blot image of H3, acetylated H3, H4, acetylated H4, H2A.Z, and acetylated H2A.Z in the cortex of Wt male mice treated with vehicle (DMSO) or SPV106 (25mg/kg) following acid extraction of histones. b) Corresponding quantitative densitometric analysis for b) acetylated H3 relative to total H3; c) acetylated H4 relative to total H4; d) acetylated H2A.Z relative to total H2A.Z. Data are mean ± SEM, presented as fold changes.

7.4 Discussion

By assessing acetylation of H3, H4, and H2A.Z following pharmacological activation or inhibition of PCAF, these experiments demonstrate that the PCAF inhibitor, embelin, selectively decreases acetylation of the histone variant H2A.Z in primary hippocampal neurons. Conversely, we were unable to detect any effects of the PCAF activator, SPV106, on the acetylation of H3, H4, or H2A.Z.

Consistent with previous work in other cells types, we demonstrated that embelin decreases histone acetylation in primary neurons. We observed a decrease in H2A.Z acetylation, but not H3. To our knowledge, this is the first demonstration of a changes in H2A.Z acetylation following pharmacological manipulation of HATs, although Narkaj et al. (2018) demonstrated that the Tip60 inhibitor, Nu9056, decreased total levels of H2A.Z. The significant reduction in H2A.Z acetylation following embelin treatment is particularly interesting because H2A.Z negatively regulates memory (Zovkic et al., 2014), and accumulates with age and in AD (Gjoneska, Pfenning, Mathys, Quon, Kundaje, Tsai, Kellis, et al., 2015; Stefanelli et al., 2018). It is likely that H2A.Z also accumulates in aged 3xTG mice and could contribute to cognitive impairment, the amelioration of cognitive impairments by embelin could be related to decreased acetylation of H2A.Z.

Although we did not observe any significant changes in H3 acetylation following embelin treatment, Modak et al. (2013) found a selective decrease in H3K9 acetylation. This apparent discrepancy in H3 acetylation may result from a lack of change in pan H3 acetylation, as Modak et al. (2013) also did not observe significant changes in H3K14,

H3K8, H3K12, or total H3 acetylation. It is also possible that a different dosing regime is necessary to see an effect of embelin on total H3 acetylation.

Despite reporting clear effects of SPV106 on cognition, we did not observe any changes in H3, H4, or H2A.Z acetylation with SPV106 treatment. In contrast to findings reported by Sbardella et al. (2008) and Milite et al. (2011) SPV106 had no effect on histone acetylation in primary hippocampal neuron cultures with doses up to 200 μM. Since we failed to observe effects of SPV106 in neurons *in vitro*, we subsequently assessed histone acetylation in the cortex of SPV106-treated Wt mice using the dosing regimen that produced behavioural effects. Under these conditions no changes in pan H3, H4, or H2A.Z acetylation were observed. It is possible that we failed to detect an effect of SPV106 because subtle changes at individual lysine residues are not robust enough to detect at the global acetylation level. A lack of change in global acetylation could be explained by the inhibitory effect of SPV106 on the acetyltransferase activity of the HATs CBP and p300 (Sbardella et al., 2008). Another explanation for our null results could be that the effects of SPV106 on histone acetylation, in neurons, is activity dependent. The theory of epigenetic priming postulates that the effects are greater when administered around the time of learning (Gräff & Tsai, 2013). For example, Peleg et al. (2010) demonstrated increases in histone acetylation following HDAC inhibition in the hippocampus of fear conditioned mice, but not control mice that did not receive a foot shock. Alternatively, it is possible that we failed to detect a change in acetylation because PCAF-mediated acetylation of non-histone proteins, such as nuclear factor kappa B (NFκB) or estrogen receptors (Mitchnick & Winters, 2018; Park et al., 2015, 2013), is

driving our behavioural effects. Specifically, SPV106 may enhance cognition though acetylation of estrogen receptors. Mitchnick & Winters (2018) have shown that PCAF interacts with the estrogen receptor alpha to facilitate short-term object memory, an effect that was abolished by co-administration of an ER alpha/beta antagonist. Conversely, SPV106 may impair cognition in 3xTG mice by acetylating NFκB and inducing inflammation and neurotoxicity. Park et al. (2013; 2015) demonstrated that the PCAF inhibitor, C-30-27, blocked beta-amyloid (Aβ)-induced cell death in BV-1 cells, as well as inhibited acetylation of NF-κB and improved Morris water maze (MWM) performance in Aβ-treated rats.

8 General Discussion

8.1 Results Summary

Histone acetylation, catalyzed by histone acetyltransferases (HATs), plays a critical role in the transcriptional regulation that supports mnemonic processes (Peixoto & Abel, 2013). Since, dysregulated histone acetylation in Alzheimer's disease (AD) is associated with cognitive deficits and neuropathology, there is growing evidence that restoring histone acetylation has therapeutic potential (Fischer, 2014; Selvi et al., 2010). By longitudinally evaluating the effects of p300/CBP associated factor (PCAF) on object and spatial memory in the triple transgenic (3xTG) mouse model of AD, this thesis revealed a complex role of this specific HAT throughout the progression of AD-like pathology, where PCAF activation is initially beneficial for memory but becomes detrimental (see Table 8.1 for a summary of all behavioural results).

First, we evaluated object recognition (OR) memory at 3, 6, 9, and 12-months-of-age (Chapter 3). We found a progressive impairment in OR memory, where both male and female 3xTG mice had intact OR at 3 and 6-months-of-age but were selectively impaired on long-term OR memory (3h delay) at 9 and 12-months-of-age. These experiments define relevant time points for evaluating the effects of epigenetic therapies on cognition. We subsequently evaluated the effects of modulating one specific HAT, PCAF, on OR memory in 3xTG mice (Chapter 4). Interestingly, we demonstrated an age-related bidirectional regulation of OR memory by PCAF in 3xTG mice. Systemic administration of the PCAF activator, SPV106 enhanced OR from 3-6 months of age and ameliorated OR deficits at 9-months-of-age. At 12-months-of-age, however, SPV106 further impaired OR, whereas the PCAF inhibitor, embelin, ameliorated the OR

impairment. In wild-type (Wt) mice, however, SPV106 strictly enhanced OR in mice exposed to a subthreshold training duration that did not result in long-term memory, whereas embelin strictly impaired OR. These findings clarify previous results demonstrating that, while PCAF activation improves a cognitively normal rodents' memory (Duclot et al., 2010; Maurice et al., 2008; Mitchnick et al., 2016; Mitchnick & Winters, 2018; Wei et al., 2012), in beta-amyloid (Aβ)-treated rodents PCAF inhibition or KO attenuates AD-like cognitive deficits (Duclot et al., 2010; Park et al., 2015, 2013).

We also assessed spatial memory using the object location (OL) task in 3xTG mice at 3, 6, and 9-months-of-age (Chapter 5). We found that spatial memory deficits were more severe than OR impairments. Specifically, at 3-months-of-age, 3xTG mice had a selective impairment in long-term spatial memory (3h delay). By 6- and 9-months-of-age, 3xTG mice had impairments in short- and long-term spatial memory (5min and 3h delays, respectively). The time points relevant to spatial memory deficits in 3xTG mice, identified in these experiments, were subsequently used to evaluate the effects of PCAF activation and inhibition for the attenuation of spatial memory deficits (Chapter 6). We found that, similar to OR memory, PCAF bidirectionally regulated spatial memory in 3xTG mice. At 3 and 6-months-of-age, SPV106 ameliorated OL deficits, but by 9-months-of-age, SPV106 was no longer effective and embelin attenuated long-term OL impairment in female 3xTG mice. In Wt mice, embelin impaired OL at 9-months-of-age.

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Table 8.1 Behavioural Results Summary

		3-months-of-age				6-months-of-age				9-months-of-age				12-months-of-age			
		Wt male	Wt female	3xTG male	3xTG female	Wt male	Wt female	3xTG male	3xTG female	Wt male	Wt female	3xTG male	3xTG female	Wt male	Wt female	3xTG male	3xTG female
Object Recognition																	
Phenotype	**5min**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3h	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×	×
PCAF activation (SPV106)	**5min**	⇑	⇑	⇑	⇑	⇑	⇑	⇑	⇑	-	-	-	-	-/⇑ *	-/⇑ *	↓	↓
	3h	⇑	⇑	⇑	⇑	⇑	⇑	⇑	⇑	-	-	↑	↑	-/⇑ *	-/⇑ *	-	-
PCAF inhibition (embelin)	**5min**	↓	↓	↓	↓									↓	↓	-	-
	3h	↓	↓	↓	↓									↓	↓	↑	↑
Object Location																	
Phenotype	**5min**	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×	×				
	3h	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×				
PCAF activation (SPV106)	**5min**	-	-	↑	↑	-	-	↑	↑	-	-	-	-				
	3h	-	-	↑	↑	-	-	↑	↑	-	-	-	-				
PCAF inhibition (embelin)	**5min**									↓	↓	-	-				
	3h									↓	↓	-	↑				

Note. ✓ indicates intact memory, × indicates impaired memory, ⇑ indicates an enhancing effect of drug treatment on sub-optimal OR, ↑ indicates an enhancing effect of drug treatment, ↓ indicates an impairing effect of drug treatment, - indicates no effect of drug treatment. *Starting at 12-months-of-age Wt mice were tested on both the sub-optimal and standard OR task, while no effect of SPV106 was observed on the standard OR task, SPV106 enhanced suboptimal OR.

Finally, we began to probe the epigenetic correlates of our behavioural results by evaluating the effects of PCAF activation and inhibition on histone acetylation (Chapter 7). We demonstrated that the PCAF inhibitor, embelin, selectively decreased acetylation of the histone variant H2A.Z in primary hippocampal neurons, which could contribute to the positive effects of embelin on object and spatial memory in aged 3xTG mice. We failed to detect an effect of the PCAF activator, SPV106, on the acetylation of H3, H4, or H2A.Z in primary hippocampal neurons or cortices isolated from drug-treated Wt mice.

8.2 Object and Spatial Memory Deficits

Spontaneous OR and OL tasks were the behavioural tests used to characterize mnemonic deficits and elucidate the therapeutic effects of PCAF activation and inhibition. These tasks for rodents are both procedurally and neurobiologically similar to human tests of visual recognition in which human AD patients are impaired (Didic et al., 2013, 2010). Therefore, evaluation of memory in transgenic AD mouse models using spontaneous object memory tasks has significant translational potential (Grayson et al., 2015; Winters, Saksida, & Bussey, 2010). Additionally, these tasks are procedurally similar, allowing for comparison between the two facets of memory with minimal task confounds.

While similar patterns of results were observed for both object and spatial memory in 3xTG mice, spatial memory abnormalities followed an accelerated timeline. In our phenotype experiments, we observed progressive impairments in both OR and OL, but impairments in OL were first observed at a much earlier age and the impairment was more severe, as both short- and long-term OL memory became impaired. Similarly, an accelerated time course was observed for the bidirectional regulation of spatial memory,

in comparison to object memory. In fact, at 9-months-of-age when SPV106 ameliorated OR deficits in 3xTG mice, SPV106 was ineffective at restoring OL memory, whereas embelin improved OL in 3xTG females. This is particularly interesting considering that all of our experiments used systemic drug treatments. It is likely that this differential time course observed in these experiments is related to the degree of neuropathology in task relevant brain regions (discussed below). Indeed, OR and OL are dependent on not entirely overlapping brain regions that have different pathological staging in 3xTG mice.

There has been debate regarding the brain regions involved in OR. While the perirhinal cortex (PRh) is unequivocally necessary for OR memory, the hippocampus (HPC) plays a more controversial role (Winters et al., 2004). Experiments in rats have suggested that the PRh is involved in the processing of object features, whereas the HPC becomes involved in OR when spatial and/or temporal information is present during encoding. Specifically, hippocampal lesions or inactivation have occasionally been shown to impair OR when tested in an open-arena (Clark, Zola, & Squire, 2000; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002), however, Winters et al. (2004) and Forwood et al. (2005) demonstrated that when spatial cues are minimized, by testing in a Y-apparatus, the HPC is not necessary for OR memory. Similarly, Barker & Warburton (2011) demonstrated that the HPC is selectively involved in object tasks when spatial or temporal information processing is involved. In mice, OR is also PRh-dependent (Romberg et al., 2013), but the HPC seems to play a more substantial role in OR, even when spatial information is extraneous to task performance, when tested with longer retention delays (>24h; Cohen et al., 2013; Hammond et al., 2004). We believe that testing OR in the Y-

apparatus and using relatively short retention delays (5min or 3h) minimizes the necessity of the HPC for this task in mice (Creighton et al., 2019). Conversely, OL is known to be dependent on the HPC (Assini et al., 2009; Barker & Warburton, 2011; Mumby & Pinel, 1994), fornix, and cingulate cortex (Abdelkader Ennaceur et al., 1997), but not the PRh (Barker & Warburton, 2011).

The behavioural deficits we observed in OR and OL map nicely onto what is known about hallmark pathological features of AD in the PRh and HPC of 3xTG mice. Mastrangelo & Bowers (2008) performed a detailed immunohistochemical analysis of pathology in male 3xTG mice, which established an earlier and more substantial accumulation of intracellular Aβ1-42 in the cornu ammonis (CA)1 region and subiculum of the HPC, than in the entorhinal cortex. Similarly, Belfiore et al. (2019) detected Aβ plaques in the HPC (6-months-of-age) prior to the cortex (12-months-of-age). Davis et al., (2013) also demonstrated more Aβ in the HPC than the PRh in 5-month-old female 3xTG mice, and we have previously observed more Aβ in the HPC, compared to the cortex, of 12-month-old 3xTG male and female mice (Creighton et al., 2019). However, it should be noted that the authors that developed the 3xTG mice originally described intracellular Aβ accumulation and amyloid plaques first in the neocortex beginning at 3 to 4-months-of-age and in the HPC by 6-months-of-age (Oddo, Caccamo, Shepherd, et al., 2003). Changes in the levels of human tau and phospho-tau were reported to occur in the HPC prior to changes in cortical regions including the entorhinal or motor cortex have also been reported (Mastrangelo & Bowers, 2008; Oddo, Caccamo, Shepherd, et al., 2003). Interestingly, in human AD patients, cognitive deficits and the specific brain regions

affected are more strongly correlated with abnormalities in tau than with the severity of Aβ deposition (Giannakopoulos et al., 2003; Guillozet et al., 2003). Given the overlapping brain regions involved in these two tasks, It is possible that a different timecourse for the bidirectional regulation of object memory may have been observed if drugs had been infused directly into the PRh. p

8.3 Sex Differences

There are established sex differences in human patients with AD, where females often exhibit more severe pathological change and cognitive deficits (Rocca et al., 1986; Ruitenberg et al., 2001). However, some of these differences may be confounded by longevity, since females tend to live longer than men and age is a major risk factor for dementia. Similar sex differences in pathological, longevity, and cognition are seen in several transgenic mouse models of AD, including 3xTG mice (Carroll et al., 2010; Clinton et al., 2007; Creighton et al., 2019; Hirata-Fukae et al., 2008; Mendell & MacLusky, 2018; Rae & Brown, 2015). For example, a more aggressive amyloid pathology has been reported in 3xTG females (Carroll et al., 2010; Clinton et al., 2007; Hirata-Fukae et al., 2008). However, both female and male sex hormones have been shown to be neuroprotective in 3xTG mice (Carroll & Pike, 2008; Carroll et al., 2007; Levin-allerhand et al., 2002; Rosario, Carroll, Oddo, LaFerla, & Pike, 2006; Rosario, Carroll, & Pike, 2012, 2010; Zheng et al., 2002). We did not observe major sex differences in object or spatial memory in 3xTG mice. We did, however, find that embelin-treatment was able to attenuate spatial memory deficits in aged 3xTG females, but not males. This may suggest that OL impairments are worse in 3xTG, which is in agreement with our previous findings,

using a similar object task, demonstrating that 3xTG males had more severe object memory impairments when the spatial nature of the task was increased (Creighton et al., 2019).

We also observed subtle sex differences between male and female mice. In OR, both Wt and 3xTG males generally performed better than females (at 3 and 6-months-of-age). This result is consistent with findings from other groups demonstrating better OR performance in males (Frick & Gresack, 2003). Conversely, in OL, older Wt females (9-months-of-age), outperformed males at the 3-h retention delay. Similarly, others have shown superior performance on OL by female rats (Saucier et al., 2008), and OL is often impaired in aged male mice (Creighton et al., 2019; Wang et al., 2009; Wimmer et al., 2012).

8.4 Possible Mechanisms

We propose that effects of PCAF activation and inhibition on object and spatial memory are mediated by alterations in the acetylation of both histone and non-histone proteins. Others have demonstrated that SPV106 increases global acetylation of H3 and H4 in several different cell lines (Milite et al., 2011; Sbardella et al., 2008). In the brain, increased acetylation of H3 and H4 have been shown to increase the expression of genes involved in learning and memory (e.g. *CREB, reelin, BDNF, CFOS and/or Zif268/Erg1*; Bredy et al., 2007; Koshibu et al., 2009; Lubin et al., 2008; Peixoto & Abel, 2013), which is associated with better performance on various cognitive tasks (Peixoto & Abel, 2013). Similar patterns of histone acetylation and gene expression may enhance memory in SPV106-treated Wt mice and young 3xTG mice. Conversely, in aged 3xTG mice, PCAF

activation may increase the expression of genes that increase AD-related pathology. For example, PCAF has also been shown to regulate genes indirectly involved in Aβ degradation. For example, Duclot et al. (2010) demonstrated that PCAF knockout (KO) increased levels of somatostatin as well as neprilysin activity in Aβ-treated mice, which should decrease pathology because neprilysin metabolizes Aβ. Although our behavioural experiments used retention delays (5min or 3h) that are shorter than those commonly used to assess the effects of gene expression on memory consolidation, early changes within 3-hours of learning are critical for memory formation (Bekinschtein et al., 2007). Moreover, epigenetic modifications, including histone acetylation, can occur during the early phases of memory consolidation. For example, Levenson et al. (2004) demonstrated increased H3 acetylation, in the HPC, 1h following contextual fear conditioning (CFC). This increase in acetylation had returned to baseline 24h after CFC. Although it is unlikely that learning-dependent changes in gene expression enhanced object or spatial memory at the 5min retention delay, since SPV106 was administered 3-days prior to learning it is possible that epigenetic changes prior to the learning event primed the system to support short-term memory.

In contrast to previous reports of increased acetylation (Milite et al., 2011; Sbardella et al., 2008), we did not detect an effect of SPV106 on histone acetylation. There are several possible explanations for this, including: timing, dose, inhibition of CBP and p300 acetyltransferase activity, lack of change in global H3, H4, or H2A.Z acetylation, or that the effect of SPV106 on histone acetylation, in neurons, is activity dependent. Alternatively, it is possible that we failed to detect a change in acetylation with SPV106

because acetylation of non-histone proteins mediates our behavioural effects. For example, PCAF has been shown to interact with estrogen receptors to enhance short-term memory. Specifically, Mitchnick & Winters (2018) recently demonstrated that intra-HPC administration of SPV106 facilitates short-term memory in male rats, and this effect is blocked with co-administration of a low dose of an ER alpha/beta antagonist. In aged 3xTG mice, however, SPV106 may impair memory through the acetylation of nuclear factor kappa B (NF-κB), which can increase inflammation and levels of Aβ (Park et al., 2015, 2013). Embelin may impair memory in Wt and young 3xTG mice by blocking the memory enhancing effects of acetylated histone and non-histone proteins but promote memory in aged 3xTG mice by decreasing the pathological cascades regulated by PCAF-mediated histone acetylation. The significant reduction in H2A.Z acetylation by embelin could also improve memory in aged 3xTG mice because H2A.Z is a negative regulator of memory that accumulates in aging and AD (Gjoneska, Pfenning, Mathys, Quon, Kundaje, Tsai, Kellis, et al., 2015; Stefanelli et al., 2018).

8.5 Future Directions

The data presented in this thesis reveal that PCAF bidirectionally regulates cognition in AD but provide limited mechanistic insight. Future experiments are necessary to elucidate the epigenetic and pathological correlates of our behavioural effects. For example, the interactions between PCAF, histone acetylation, non-histone acetylation, gene transcription, and AD pathology, should be explored in 3xTG mice throughout disease progression.

We will continue to look for changes in acetylation following SPV106 treatment using chromatin immunoprecipitation (ChIP) or look for activity dependent changes in acetylation following OR and/or OL learning. Given the effects we observed on short-term memory, the lack of detected changes in histone acetylation following SPV106 administration, and the role of non-histone proteins in learning and memory and AD neuropathology, the effects of SPV106 and embelin on the acetylation of non-histone proteins should also be assessed.

The bidirectional regulation of memory suggests that PCAF may regulate the expression of memory and pathology-related genes in AD, but this has yet to be investigated. Indeed, it is well established that HAT activation or histone acetylation promotes the expression of memory-promoting genes (e.g. *CREB, reelin, BDNF, and Zif268/Erg1*; Bredy et al., 2007; Koshibu et al., 2009; Lubin et al., 2008). Histone acetylation has also been shown to regulate genes upstream from Aβ degradation (e.g. somatostatin; Duclot et al., 2010)) and inflammation (e.g. *interleukin (IL)- 1β, IL-6, and tumor necrosis factor-α* (*TNF-α*); Ito et al., 2000).

The effects of SPV106 and embelin on AD pathology should also be explored, since some histone deacetylase (HDAC) inhibitors have previously been shown to attenuate AD neuropathology. For example, Sung et al. (2013) demonstrated that mercaptoacetamide-based class II and hydroxamide-based class I HDAC inhibitors decreased Aβ40 and/or Aβ42 in 3xTG mice by decreasing the expression of β- and γ-secretase components and increasing expression of genes involved in Aβ degradation (e.g. *Nep, Ece1, Mmp2, Ctsd and Ctsb*). These HDAC inhibitors also decreased tau

phosphorylation (Sung et al., 2013). Similarly, Zhang & Schluesener (2013) observed decreased microglial activation and Aβ deposition in amyloid precursor protein (APP)/presenilin (PS)1 mice following treated with the benzamide histone deacetylase inhibitor, MS-275.

The possibility that other HATs function bidirectionally in AD should also be investigated. Thus far, enhancing the activity of other HATs, such as CBP or p300, has only been shown to improve cognition in mouse models of AD. For example, Caccamo et al. (2010) demonstrated that impairments in the Morris water maze (MWM) were restored in 6-month-old 3xTG mice following intra-ventricular treatment with a CBP-expressing lentivirus. The attenuation of memory impairment was related to increased BDNF expression, but no changes in Aβ or tau pathology were observed (Caccamo et al., 2010). Chatterjee et al. (2018) also demonstrated that pharmacologically activating CBP and p300, using CSP-TTK-21, restored MWM performance in the THY-Tau22 mouse model of tauopathy. These memory enhancing effects likely resulted from corresponding TTK-21-mediated restoration of long-term depression, increased dendritic spines, normalized H2B acetylation, partially restored HPC transcriptome, and increased expression of activity and memory-related genes (Chatterjee et al., 2018). However, these studies did not use a systematic longitudinal approach like we did in this thesis. Moreover, there are clear interactions where AD pathology drives HAT activity. For example, familial mutations in PS1 upregulate CBP and p300 activity (Marambaud et al., 2003), familial APP mutations increase p300 expression (Lu et al., 2014), and p300-mediated acetylation has also been shown to interact with phospho-tau (Aubry et al., 2015).

Dissimilarly, the HAT, Tip60, may have the strongest therapeutic potential as it appears to be strictly beneficial in AD. Tip60 has recently been shown to support memory in rodents through acetylation of K4K12 (Uchida et al., 2017), an epigenetic marker that is associated with age-related memory loss (Peleg et al., 2010). Tip60 has also been implicated in the etiology of AD based on experiments using transgenic APP drosophila. Decreased expression of Tip60 in APP-drosophila increased Aβ-induced cell death and axonal transport, and this effect was blocked by Tip60 overexpression (Johnson, Sarthi, Pirooznia, Reube, & Elefant, 2013; Pirooznia et al., 2012).

8.6 Conclusions

The growing evidence that dysregulation of histone acetylation plays a role in the cognitive deficits and neuropathology in AD suggests that increasing histone acetylation with HDAC inhibitors or HAT activators is a promising therapeutic approach. Most research into the memory-enhancing effects of histone acetylation in neurological disease has predominantly focused on HDAC inhibition. HDAC inhibitors have been very effective at ameliorating cognitive deficits and attenuating neuropathology in rodent models of AD (Fischer, 2014; Stilling & Fischer, 2011; Xu et al., 2011). Thus, in addition to cognitive enhancement, the reinstatement of histone acetylation has the potential to attenuate AD neuropathology. However, there are a few limitations associated with HDAC inhibitors: global HDAC inhibition can be toxic (Salminen et al., 1998), the primary function of HDACs is to remove acetyl groups added by HATs, and the effectiveness of HDAC inhibition can be dependent on the function of specific HATs (Chen et al., 2010; Vecsey et al., 2007); therefore targeting specific HATs may be a better therapeutic approach. In

this thesis we systematically evaluated the effects of activating one specific HAT, PCAF, in 3xTG mice. We observed a robust amelioration of cognitive deficits in 3xTG mice, even at advanced ages, following either acute activation or inhibition of PCAF. Our behavioural findings clarify the conflicting studies demonstrating that although PCAF activation enhances memory (Mitchnick & Winters, 2018; Wei et al., 2012) and PCAF KO or inhibition impairs memory in cognitively normal rodents (Duclot et al., 2010; Maurice et al., 2008; Mitchnick et al., 2016), in Aβ-treated mice PCAF inhibition or KO attenuates AD-like cognitive deficits (Duclot et al., 2010; Park et al., 2015, 2013). Overall, this thesis demonstrates that PCAF appears to be a promising therapeutic target in AD, providing additional support for the use of epigenetic therapies in neurodegenerative diseases, like AD. However, the complex role of PCAF throughout the progression of AD suggests that greater mechanistic insight into the interactions between HATs, AD pathology, and cognition is required for the success of epigenetic therapies.

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