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Investigating Synergistic Effects of Mild Traumatic Brain Injury and Reduced Cholinergic Tone on Attentional Deficits and Alzheimer's-Like Pathology in hAβ and hTau mice

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Abstract

Mild traumatic brain injury (mTBI) increases the risk of Alzheimer's disease. The early cholinergic decline in Alzheimer's disease and cholinergic damage observed after TBI suggest a distinct role of this neural system in vulnerability to Alzheimer's disease following TBI. This thesis evaluated the role of repetitive mTBI and cholinergic dysfunction in the development of cognitive deficits, specifically attentional deficits, and Alzheimer's-related pathology in mice expressing humanized amyloid-beta and tau and a vesicular acetylcholine transporter knockdown to induce a mild cholinergic deficit. Using the rodent continuous performance test, it was shown that repetitive mTBI in the presence of an already vulnerable cholinergic system induced chronic, sex-specific attentional impairments. However, repetitive mTBI and cholinergic dysfunction alone did not induce significant deficits. Injured cholinergic deficient mice also exhibited an increase in degenerative hippocampal granules. These findings indicate the synergistic role of cholinergic dysfunction and mTBI in the development of behavioural deficits with aging.

Keywords

mild traumatic brain injury, Alzheimer's disease, cholinergic dysfunction, VAChT, concussion, continuous performance test, touchscreen testing, PAS granules

Summary for Lay Audience

Alzheimer's disease is a progressive age-related brain disease which is the leading cause of dementia – a disorder marked by gradual cognitive decline in memory, attention and thinking. A history of mild traumatic brain injury or concussions is known to increase the likelihood of developing Alzheimer's disease and results in earlier disease onset. Key proteins and mechanisms dysregulated in Alzheimer's disease, particularly amyloid-beta and tau proteins, are also abnormally accumulated following traumatic brain injury. The cholinergic system, one of the brain's neurotransmitter systems involved in cognition, including attention, is particularly damaged in Alzheimer's disease and following traumatic brain injury. My thesis evaluated how mild traumatic brain injury and reduced cholinergic function may interact in the later development of cognitive deficits and Alzheimer's related pathology in mice. I used mice carrying human versions of the amyloid-beta and tau proteins to mimic key aspects of Alzheimer's disease without artificially inducing Alzheimer's disease. To induce a mild reduction in cholinergic signalling, mice with reduced levels of a protein crucial for cholinergic signalling were used. I specifically assessed attention which is the first non-memory domain affected in Alzheimer's disease and critical in maintaining normal daily functioning. Attention was assessed using a rodent touchscreen behavioural system to administer an attention-based task, the continuous performance test, to the mice. Following repetitive mild traumatic brain injury mice with or without reduced cholinergic signalling were evaluated on the attention-based task at chronic timepoints to evaluate attentional performance as the mice age. Mild traumatic brain injury in the presence of reduced cholinergic signalling resulted in attentional deficits that worsened with aging, particularly in females. Repetitive mild traumatic brain injury and reduced cholinergic signalling had minimal effects on their own, suggesting that the combination of traumatic brain injury and cholinergic dysfunction is particularly detrimental to development of deficits. My thesis project shows that reduced cholinergic signalling following repetitive mild traumatic brain injury increases vulnerability to attention impairments and disease related pathology. These findings suggest that individuals with reduced cholinergic signalling, through use of anti-cholinergic drugs, for example, may be at greater risk for developing attentional deficits following repetitive traumatic brain injury.

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List of Abbreviations

α7-nAChR	nicotinic acetylcholine receptor
Aβ	amyloid beta protein
ACh	acetylcholine
AD	Alzheimer's disease
ANOVA	analysis of variance
APP	amyloid precursor protein
ApoE4	apolipoprotein E-ε4
C	response criterion
CCI	controlled cortical impact
CHI	closed head injury
CPT	continuous performance test
CSF	cerebral spinal fluid
d'	discrimination sensitivity
DAI	diffuse axonal injury
FAR	false alarm rate
GFAP	glial fibrillar acidic protein
HR	hit rate
hAβ	humanized amyloid beta
hTau	humanized microtubule associated protein tau

Iba-1	Ionized calcium-binding adaptor molecule 1
KD	knockdown
LOAD	late onset Alzheimer's disease
MAPT	microtubule associated protein tau
MCI	mild cognitive impairment
mTBI	mild traumatic brain injury
mTOR	mammalian target of rapamycin
NFT	neurofibrillary tangles
OC+	conformational specific amyloid fibril antibody
PAS	periodic acidic Schiff
PFC	prefrontal cortex
PSEN	presenilin
p-tau	tau phosphorylation
SAT	sustained attention task
VAChT	vesicular acetylcholine transporter
VAChTKD	vesicular acetylcholine transporter knockdown
VSD	variable stimulus duration
TBI	traumatic brain injury
TBS	Tris-buffer saline
TNFα	tumour necrosis factor α

rCHI	repetitive closed head injury
rCPT	rodent continuous performance test
rmTBI	repetitive mild traumatic brain injury
WT	wildtype
5-CSRTT	5-choice serial reaction time test

Chapter 1

1 Introduction

1.1 Alzheimer's disease

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease arising from a combination of genetic and environmental factors. As the most common form of dementia, AD affects over 7% of the growing population of elderly Canadians (Public Health Agency of Canada, 2017). Age is the most influential risk factor of disease development (Hebert et al., 2013; Zhao et al., 2020), as the incidence triples from those 75-79 to those 80-84 (Kukull et al., 2002; Corrada et al., 2010). Women make up two-thirds of those with AD. However, at least before 80 years old, the incidence does not differ between men and women (Snyder et al., 2016). Unfortunately, recent drug development for AD has been largely unsuccessful and all the current AD treatments are reactive, targeting a subset of the symptoms and not the disease itself (Schneider et al., 2014). If measures can be taken to slow down disease progression, it may protect people at increased risk for developing AD.

AD neuropathology

From studies based in older adults and at-risk groups, it is clear that the processes underlying the pathology of AD begin decades prior to a diagnosis of clinical dementia (Sperling et al., 2011). The long timescale over which pathology develops indicates an extended preclinical window in which intervention to target pathophysiological processes may work to delay disease onset. Late-onset AD, representing over 90% of AD cases, is multifactorial, resulting from abnormal interaction of multiple molecular pathways (Reitz et al., 2020). AD is characterized by amyloid-beta aggregation and hyper-phosphorylated tau protein, leading to synaptic dysfunction, cell death and inflammation (Selkoe, 1991). Neurodegeneration begins in entorhinal and basal forebrain regions and progresses along fiber pathways to temporal, parietal and prefrontal cortices (Raj et al., 2015). Neuroinflammation, accumulation of pathological proteins, oxidative stress, proteolytic stress and mitochondrial dysfunction all contribute to the progressive neurodegeneration.

A chronic immune response in the brain and the sustained alterations in microglia and astrocyte phenotypes, including upregulation of genes involved in proteostasis, phagocytosis and protein clearance, further contribute to neurodegeneration (Kinney et al., 2018; Smith et al., 2022).

The AD field has begun to move towards a biological model of AD classified through core disease biomarkers: amyloid, tau and neurodegeneration, which encourages the view that AD signs, symptoms and pathology are best described as lying on a continuum (Hampel et al., 2021). This framework helps recognize preclinical disease stages that may hold the key to start effective disease treatment before neurodegeneration has progressed to a stage where symptomatic treatment is likely the only option.

AD symptoms: cognitive and behavioural changes

AD is marked by a progressive cognitive and functional decline (Lane et al., 2018). In the early stages, individuals often present with mild cognitive impairment (MCI). MCI is a clinical at-risk stage of AD; patients exhibit cognitive impairments and AD-related pathology to some degree (Hanseeuw et al., 2016; Keller et al., 2005; Scheff et al., 2006; Tramutola et al., 2015) but do not have impairments in activities of daily living. Patients may progress onward to AD with increasing functional deficits presenting either as amnestic (with a prominent deficit in learning and memory) or non-amnestic (with a prominent deficit in language, visuospatial or executive function) (McKhann et al., 2011). Declining cognitive ability occurs in diverse cognitive domains including: spatial navigation, memory (Coughlan et al., 2018; Hort et al., 2007), including episodic, semantic and working memory (Butters et al., 1987; Russo et al., 2017; Van Geldorp et al., 2015), language and executive function, including attention (Huntley et al., 2017). Attention is the first non-memory function to be affected in AD before language and spatial cognition and likely underlies early difficulties in activities of daily living (Perry & Hodges, 1999). AD patients also experience higher neuropsychiatric symptoms beginning with depression, apathy, agitation and, in later disease stages, aggression and delusions (Lyketsos et al., 2011).

AD risk factors

Genetic factors heavily influence AD, with an estimated heritability of 58-79% (Gatz et al., 2006). This heritability is further explained by genome-wide association studies which have identified over 75 risk loci for AD, including genes involved in amyloid/tau processing, endocytosis, immunity and microglial activation (Bellenguez et al., 2022; Park et al., 2021). Apolipoprotein E-ε4 (*ApoE4*) is the strongest genetic risk factor for late-onset AD and is known to be involved in several disease pathways: Aβ processing, tau pathology and neuroinflammation (Fernández-Cabello et al., 2020). As one cannot change their genetics, it is also pertinent to focus on environmental factors to prevent or delay disease onset. In addition to age-related brain changes and genetics, environmental and lifestyle factors contribute to AD development. Modifiable risk factors for AD include traumatic brain injury, cardiometabolic risk factors, including stroke and diabetes on their own and increasing risk with the addition of hypertension, hypercholesterolemia, obesity and heart disease (Honig et al., 2003). Lifestyle factors contributing to AD progression include exercise, education and diet (Larson et al., 2006; H. Li et al., 2022).

1.2 Traumatic brain injury

Traumatic brain injury (TBI) is now recognized as having long-lasting neurological, cognitive and physiological impacts and increases the risk of several neurodegenerative diseases, AD among them (Bigler, 2013; Bray et al., 2022; Faden & Loane, 2015; Mahoney et al., 2022; Mortimer et al., 1991; Plassman & Grafman, 2015). Prevalence of TBIs in the population depends on several factors. By young adulthood, it is estimated that 5% to upwards of 30% of individuals have had a TBI (Langlois et al., 2006), with a higher incidence in teenagers and young adults (Cassidy et al., 2004). The most common causes include falls, motor vehicle accidents and violence (Langlois et al., 2006; Peeters et al., 2015). The incidence of TBI tends to be higher in males; however, the ratio of males to females differs significantly between studies (Peeters et al., 2015). Mild TBIs (mTBI) constitute 70-90% of all TBIs.

Mild TBI

Based on diagnostic criteria from the Mild Traumatic Brain Injury Committee of the American Congress of Rehabilitation Medicine, mild TBI is an injury to the head (arising from blunt trauma or acceleration or deceleration forces) that has a Glasgow Coma Scale between 13-15 30 minutes after injury and one or more of the following: loss of consciousness lasting less than 30 minutes; any period of impaired mental state at time of injury (i.e., confusion, disorientation); any dysfunction of memory around the time of injury (post-traumatic amnesia less than 24hrs); or transient neurological deficit (Lefevre-Dognin et al., 2021). Mild TBIs include concussions which tend to be a milder form marked by short-term impairment in neurological function and clinical symptoms (McCrory et al., 2013). Head acceleration is typically the primary mechanism in mTBI. The brain experiences linear and angular accelerations or decelerations, leading to widespread neuronal, including diffuse axonal injury, and vascular damage (Johnson et al., 2013; Smith et al., 2003). It is worth noting that the angular or rotational component accounts for 90% of total brain strain, whereas linear acceleration results in comparatively less damage (Zhang et al., 2006).

Acute pathophysiology of mTBI

Damage to the brain resulting from TBI encompasses primary injury, initiated when external force is applied to the brain, including both contact and acceleration or deceleration forces (Naumenko et al., 2023). In addition to primary injury, there is secondary injury which is delayed. Acceleration and deceleration forces common to concussions typically result in diffuse injury rather than focal injury, and in particular damage to axons due to their long length. This damage is focused on the corpus callosum, fornix, subcortical U-fibers and cerebellum and mainly affects unmyelinated fine fibers (Johnson et al., 2013). Initial damage to plasma membranes of the axons or sodium influx through mechanical disruption leads to influx of ions that activate proteases resulting in disruption of the cytoskeleton. This can impede axonal transport leading to axonal swelling, detachment and in severe damage downstream deafferentation of the axon (Povlishock & Katz, 2005). Mechanical injury results in tau hyperphosphorylation and

tau mislocalization to dendritic spines mediating synaptic dysfunction (Braun et al., 2020). Widespread cellular membrane disruption also releases pro-inflammatory cytokines and damage-associated molecular patterns, which are recognized by pattern recognition receptors(Simon et al., 2017). Metabolic changes also occur following injury with a phase of neuroexcitation, specifically elevated glutamate, that initially leads to hyperglycolysis and later transforms to reduced glucose metabolism (Jalloh et al., 2015).

TBI in at-risk populations

Particular groups are at higher risk for TBI based on the nature of activities and exposure to situations where head injuries are likely to occur; these include military personnel, athletes and victims of domestic violence (Iverson et al., 2017; McKee & Robinson, 2014). TBI experienced by military personnel is generally mTBI, either sub-concussive, concussive or blast injury, but tends to be rather variable due to its heterogeneous causes. It is estimated that 15-20% of returning military members have experienced a TBI (McKee & Robinson, 2014). In athletes, concussion and sub-concussive impacts are common in many sports and are known to be associated with microstructural changes within the brain, even if impacts do not meet the criteria for concussion (Lipton et al., 2013; Manning et al., 2020). According to a study of head injuries requiring hospital evaluation in the United States, cycling and football were among the sports with the highest risk of head injuries (Centers for Disease Control and Prevention, 2011). TBI is also common among victims of domestic violence, often occurring over a sustained period and resulting in multiple brain injuries. Reports indicate that anywhere from 30% to upwards of 75% of victims of domestic violence, particularly intimate partner violence, experience head or neck injuries, leading to probable TBI (Campbell et al., 2018; Hunnicutt et al., 2017; Iverson et al., 2017). Injury from domestic violence is a severely underreported area of TBI, especially in adult women.

TBI risk factors for poorer outcomes

There exists wide variation in recovery following concussion that cannot be accounted for by injury severity or baseline neurological measures. Post-concussive syndrome, a persistence of at least one physical, cognitive or emotional symptom beyond 30 days

post-concussion, occurs in an estimated 10-15% of individuals (King et al., 2013) with some studies reporting an even higher percentage (close to 50%) (Theadom et al., 2016). Females and individuals with multiple comorbidities or multiple TBIs tend to have poorer outcomes one year out. Compared to a single concussion, repetitive concussions result in worse symptoms post-injury (Oyegbile et al., 2018). Women with mTBI tend to have poorer outcomes and more severe post-concussive symptoms (Mikolić et al., 2021). Genetic factors also contribute to outcomes, with those who carry at least one *ApoE4* allele having a higher likelihood of experiencing enduring post-concussive symptoms and poorer outcomes (McFayden et al., 2021; Merritt et al., 2019). Likely, a combination of prior TBI history, coinciding neuropsychiatric conditions, stress and genetic factors all contribute to the outcome following injury.

TBI cognitive symptoms

Individuals with persisting post-concussive complaints often exhibit cognitive deficits, including selective and sustained attention deficits (Chan, 2002; Chan et al., 2003). In general, concussions often result in mild cognitive behavioural symptoms, including variability in responding (Makdissi et al., 2001; Parks et al., 2015) and attention and memory impairments (Collie et al., 2006; Oyegbile et al., 2018). Differences in functional activity during cognitive tasks have been observed in concussed individuals in the months following injury in the absence of behavioural deficits, suggesting individuals may be able to maintain performance even with mild brain changes (Wu et al., 2012).

1.3 Traumatic brain injury as a risk factor for Alzheimer's disease

The influence of TBI on dementia risk is dependent on injury severity and age. Although, the focus of this thesis is the role of TBI in the risk of developing AD, it should be noted that moderate to severe TBIs increase the onset and risk for all-cause dementia, including Lewy body dementia, vascular dementia, frontotemporal dementia and Parkison's disease (Brett et al., 2022). This increased risk does not appear to be more particular to one neurodegenerative disease over another suggesting that TBI may accelerate or instigate

neurodegenerative processes which can lead to a variety of heterogenous diseases. The only neurodegenerative disease that TBI is thought to cause directly is chronic traumatic encephalopathy, which is often observed in individuals who have experienced repetitive TBI (e.g., athletes who play contact sports). Chronic traumatic encephalopathy is a progressive tauopathy characterized by tau deposits in sulci depths of superficial cortical layers and cerebral atrophy (McKee et al., 2009, 2015).

A number of large studies in veterans report increased dementia risk ranging from 2-4-fold for individuals with a history of moderate to severe injury (Barnes et al., 2018; Fleminger et al., 2003; Jellinger et al., 2001; Plassman et al., 2000). TBI also results in an earlier age of onset by approximately 2-3 years for all case dementia diagnoses, including AD, frontotemporal dementia and others (Iacono et al., 2021; LoBue et al., 2016, 2017). Additionally, those with clinical dementia with a history of TBI appear to have worse cognitive decline than those without a TBI history (Mohamed, Nestor, et al., 2022). This risk increases further when both TBI and genetic risk, particularly *ApoE4*, are present (Logue et al., 2023).

Despite numerous reports indicating a higher risk of dementia development, earlier onset or worse cognitive decline following TBI, the finding of a robust association between brain injury and increased AD risk is not without some controversy (Fleminger et al., 2003). For example, Fleminger & colleagues found increased risk for only males with TBI, but not females (Fleminger et al., 2003). Additionally, other groups have found no relationship between self-reported TBI and AD neuropathology in individuals from the National Alzheimer's Coordinating Center (Sugarman et al., 2019). Similarly, several studies reported no differences in AD biomarkers, including amyloid and tau pathology and brain volume, in those with TBI history (Cummins et al., 2023; Hicks et al., 2022; Weiner et al., 2023). The discrepancy in the finding of these studies may reflect variations in age, either at time of injury or when assessing dementia, time since injury and reported number and severity of TBI which all contribute to the associated risk. In addition, the heterogeneity of injury mechanisms and pathological outcomes following injury also influence disease risk (Lipton et al., 2012).

mTBI likewise confers a higher risk (around 2.5-fold) for developing dementia, although to a lesser degree than moderate to severe injury (Barnes et al., 2018). Those with a history of mTBI also have an earlier onset of AD by 2.5 years which corresponds with the age of onset in *ApoE4* carriers (Lobue et al., 2022). Interestingly, in the instances of both mTBI and *ApoE4* the age of onset is even earlier, by about five years, than those without. Hayes and colleagues (2017) assessed the combined effects of polygenic risk for AD and mTBI on cortical thickness in AD-vulnerable brain regions (Hayes et al., 2017). They found that reduced cortical thickness in AD susceptible regions occurred only in individuals with both a high genetic risk and a history of mTBI, and this reduction increased over time since the injury. Age is also a critical moderating factor in the contribution of mTBI to dementia risk. Gardner and colleagues (2014) report that increased risk conferred by mTBI, unlike moderate to severe injury, depends more on age, with mTBI only beginning to have an effect around 75 (Gardner et al., 2014). When taken together, these findings suggest that mTBI alone has a moderate effect on AD susceptibility; however, when combined with other risk factors, such as genetic predisposition, it plays a more substantial role in AD-related neurodegeneration, particularly as individuals age.

1.4 Neuropathology in Alzheimer's disease and TBI

With the apparent contribution of TBI to the development of AD, it is not surprising that AD-associated pathology is greater in those with a history of TBI and is even found acutely after injury in young individuals (Tagge et al., 2018). Molecularly, AD is characterized by the accumulation of phosphorylated microtubule associated protein tau, forming neurofibrillary tangles (NFT), and the presence of amyloid plaques composed of amyloid-beta fibrils (Selkoe, 1991). The NFT burden predicts cognitive decline while amyloid plaque burden does not; however, the presence of dense amyloid plaques is found exclusively in AD brains (Nelson et al., 2012). AD is also characterized by a sustained elevated immune response with long-lasting reactive glial phenotypes, both in response to amyloid and tau pathology and further exacerbating these pathologies (Kinney et al., 2018).

Amyloid pathology in AD

Plaques are formed from various-sized aggregates of amyloid-beta (Aβ) protein. Aβ monomers form Aβ aggregates, oligomers, protofibrils and fibrils that are recognized for having varying levels of neurotoxicity (Selkoe, 1994). Aβ oligomers are potently synaptotoxic impairing synaptic plasticity, inducing synaptic loss and impairing cognition (Cleary et al., 2005; Shankar et al., 2007; Walsh et al., 2002). Amyloid precursor protein (APP) is cleaved sequentially by β-secretase and γ-secretase to produce Aβ protein, commonly 40 or 42 amino acids long (Lane et al., 2018; Selkoe, 1998). The Aβ pathway has long been considered foundational to AD because familial AD is caused by mutations in *APP* or proteins involved in the processing of APP (Hardy & Allsop, 1991). Based on these familial cases, a model for disease progression posits that Aβ deposition is followed by tau phosphorylation, and NFT formation leading to neuronal and synaptic death (Lane et al., 2018).

Tau pathology in AD

Tau is a microtubule-associated protein alternatively spliced from the microtubule-associated protein tau (*MAPT*) gene to produce six different isoforms in adult humans (Goedert et al., 1989). These isoforms consist of the different combination of three exons from the *MAPT* and the inclusion of either three or four repeat regions located in the microtubule binding domain (3R and 4R) (Buée et al., 2000). Tau plays a role in microtubule stabilization, axonal transport, including mitochondrial axonal transport, and potentially cell signalling and DNA protection (Conde & Cáceres, 2009; Noble et al., 2013). Under normal physiological conditions, tau is functionally phosphorylated for regulation of microtubule binding and axonal transport.

In AD, however, the number of residues that are phosphorylated can increase 4-fold (Hanger et al., 2007), causing tau to detach from microtubules, form aggregates and destabilize microtubules. This increase in phosphorylation is likely due to an imbalance in kinase and phosphatase activity. Several tau phosphorylation (p-tau) sites are abnormally phosphorylated in AD when compared to healthy controls, specifically phosphorylation at Tyr18, Ser199 and Thr231 (Neddens et al., 2018).

Hyperphosphorylated tau in its soluble form is thought to cause synaptic dysfunction and neurodegeneration (Noble et al., 2013). A recent study has put forth several master phosphorylation sites critical to interdependence where initial phosphorylation led to subsequent phosphorylation at other sites along the protein. Phosphorylation of threonine residues at sites 50, 69 and 181 meditates phosphorylation of a host of other sites (Stefanoska et al., 2022). Elevated levels of cerebral spinal fluid (CSF) and plasma p-tau 181 and 271 have recently been established as accurate biomarkers to predict future progression to AD (Palmqvist et al., 2021).

Neuroinflammation in AD

Neuroinflammation, unlike other risk factors and genetics associated with AD, is not inherently causative. Instead, it emerges as a consequence of AD pathologies or existing risk factors, exacerbating disease severity (Kinney et al., 2018). Microglia, the resident immune cells of the central nervous system, respond to Aβ and tau accumulation, leading to microglia activation and phagocytosis of these proteins, promoting clearance in early disease stages (Bamberger et al., 2003). However, after a time microglia become unable to clear Aβ but continue to produce proinflammatory cytokines. This results in further microglial dysfunction and a prolonged inflammatory state causing neuronal death (Hickman et al., 2008). Proinflammatory cytokines, such as TNF-α and IL-1β, are elevated early in AD and play important roles in AD-related pathways, including amyloid processing and hyperphosphorylation of tau (Forlenza et al., 2009; Kinney et al., 2018).

Amyloid pathology after TBI

Elevated levels of amyloid and phospho-tau proteins are observed acutely and chronically following TBI. Studies indicate that individuals with a history of TBI, even decades prior, exhibit heightened amyloid deposition (Mohamed, Nestor, et al., 2022) and an acceleration in amyloid accumulation (Asken et al., 2021). Higher amyloid load is accompanied by decreased cortical thickness and an earlier onset of cognitive impairments in these individuals, indicating a more aggressive progression of AD (Mohamed, Nestor, et al., 2022). This increase in amyloid deposition also correlates with traumatic axonal injury and poorer neuropsychological performance in attention and

other cognitive tests (Scott et al., 2016). Of interest is the rapid formation of Aβ oligomers and protofibrils (Abu Hamdeh et al., 2018), along with increased aggregated amyloid in grey matter and striatum in cases of severe TBI (Hong et al., 2014). This may indicate that TBI, if severe, is directly or indirectly upregulating amyloidogenic pathways. Increases in amyloid deposition and its relation to cognitive decline (Yang et al., 2015) in individuals with TBI history underscores the significant role of amyloid pathology in determining susceptibility to cognitive impairments and the progression of dementia.

Tau pathology in TBI

Tau pathology is likewise elevated in those with a history of TBI, irrespective of the presence or absence of clinical dementia, particularly in the temporal pole, hippocampus and inferior and middle temporal gyri (Mohamed, Cumming, et al., 2022). Johnson and colleagues (2021) report that 60% of those with TBI history exhibited moderate to extensive tau pathology compared to only 20% of non-injured controls. Advanced tau pathology indicated by NFTs is found in young individuals within a year following a single TBI, suggesting that TBI is sufficient to induce this pathology even without aging (Johnson et al., 2012). After TBI, pathologic tau typically accumulates in superficial layers of the cortex, at the base of sulci and at regions of geometric inflection, regions vulnerable to strain with injury (Tagge et al., 2018). Increases in tau, both in the brain and CSF, correlate with the degree of cognitive decline and with deficits in executive functioning (Clark et al., 2021; Mohamed, Cumming et al., 2022). These findings further indicate the role of TBI in the development of tau pathology and the associated cognitive decline.

Amyloid and tau pathology in normal aging

As discussed, characteristic AD pathology, specifically inclusions of diffuse Aβ and NFTs, are found elevated to varying extents in those with TBI; however, this pathology can also be observed in normal aging without the presence of dementia (see Nelson et al., 2012 for a review). Notably, a study involving non-demented older adults revealed that 20-40% of individuals met neuropathological criteria for AD without exhibiting cognitive

impairments (Price et al., 2009). However, the extent of plaques and NFTs correlates with mild deficits in episodic memory, visuospatial ability and executive function (Bennett et al., 2006; Price et al., 2009). This likely indicates some degree of prodromal AD with plaques and tangles occurring years before noticeable cognitive impairments, a state that is increased in those who have had a TBI. It should be noted that other soluble forms of Aβ and phospho-tau play a critical role in disease and their levels correlate with cognitive decline (Lue et al., 1996; Mclean et al., 1999). Nevertheless, as the presence of these amyloid and tau pathologies does not necessarily cause AD, additional mechanisms, such as synaptic dysfunction (Selkoe et al., 2002), must determine neurodegeneration in the presence of these proteins.

Neuroinflammation in TBI

Along with the accumulation of toxic proteins, inflammation increases in the brain immediately following and chronically after injury. Chronic elevation in immunoreactive microglia has been reported years after moderate to severe TBI, suggesting an ongoing neuroinflammatory state (Ramlackhansingh et al., 2011; Gentleman et al., 2004). Preclinical studies report long-lasting inflammation as evidenced by increased astrocyte and microglia activation after repetitive TBI, with localization to white matter tracts (Mouzon et al., 2014; 2018).

1.5 The Cholinergic system

The cholinergic system comprises numerous nuclei primarily situated in the basal forebrain, diencephalon, pontomesencephalic regions, and their cholinergic projecting regions, along with striatal cholinergic interneurons (Woolf, 1991). Notably, the basal forebrain delivers extensive acetylcholine (ACh) innervation to the cortical mantle and hippocampus, playing a vital role in cognition (Li et al., 2017). Choline acetyltransferase synthesizes acetylcholine, and the vesicular acetylcholine transporter (VAChT) loads it into synaptic vesicles (Bravo & Parsons, 2002; Houser, 1990). Cholinergic innervation in these areas is crucial for cognitive functions, including executive functioning and memory, and contributes to regulating inflammatory responses (Hampel et al., 2019;

Schmitz et al., 2020). The cholinergic system modulates an anti-inflammatory pathway mainly through activation of the α7 nicotinic ACh receptor (α7-nAChR), which is highly expressed in microglia and astrocytes (Han et al., 2017). Activation of pathways downstream of microglial α7-nAChRs may have anti-inflammatory/ neuroprotection effects by maintaining appropriate microglia activity and proliferation (Egea et al., 2015).

1.6 Cholinergic dysfunction in AD and TBI

Cholinergic dysfunction in AD

Cholinergic system dysfunction and degeneration occurs early in the development of AD (Grothe et al., 2012, 2013; Peter et al., 2021; Whitwell et al., 2007). Cholinergic dysfunction is initially driven by structural and functional alterations of cholinergic neurons rather than direct neuron loss. These changes include decreased choline uptake, trophic factor receptor expression, ACh release and ACh receptor expression as well as neuronal shrinkage, synaptic loss and axonal degeneration (Gamage et al., 2020; Mufson et al., 2007). Volume loss in the basal forebrain (several nuclei within Nucleus basalis Meynert and Diagonal band of Broca) occurs with normal aging but is accelerated in MCI and worsens in AD (Grothe et al., 2012; Schmitz & Spreng, 2016; Teipel et al., 2011; Whitwell et al., 2007). Not only is the cholinergic system vulnerable to age-related degeneration and degeneration in preclinical AD stages, but lower basal forebrain volume predicts a higher risk of MCI and AD diagnosis (Kerbler et al., 2015). In addition to cholinergic volume loss, reduced levels and activity of proteins involved in cholinergic signaling, such as VAChT, nAChR and ChAT, are observed in MCI and AD (Kolisnyk et al., 2017; Mazère et al., 2008; Sultzer et al., 2022). Selective vulnerability of forebrain cholinergic neurons is thought to be related to their reliance on trophic factor support for survival (Mufson et al., 1999; Sarter & Bruno, 2004). Additionally, these neurons show early development of tau pathology (Grudzien et al., 2007; Mesulam et al., 2004; Tiernan, Ginsberg, et al., 2018; Tiernan, Mufson, et al., 2018), a distinct high level of intraneuronal Aβ in adulthood and accumulation of Aβ oligomeric species with aging (Baker-Nigh et al., 2015).

In the presence of abnormally elevated levels of CSF amyloid and tau, decreases in basal forebrain cholinergic volumes predict volume loss within the entorhinal cortex, a brain region that undergoes degeneration early in AD (Fernández-Cabello et al., 2020; Schmitz & Spreng, 2016). Entorhinal cortex volume likewise predicts temporal cortices degeneration. This suggests that cholinergic forebrain degeneration precedes cortical degeneration. Topographic cortical degeneration aligns with topography of basal forebrain cortical projections which may indicate that loss of cholinergic innervation contributes to the development of cortical degeneration (Schmitz et al., 2018). Loss of basal forebrain cholinergic tone is also associated with decreased intracortical fibre tract integrity (Teipel et al., 2011), indicating that loss of cholinergic tone extensively impacts cortical health. In addition to cholinergic loss advancing cortical degeneration, loss of cholinergic innervation also increases inflammation. As the cholinergic system plays a critical role in the regulation of microglia reactivity in the removal of amyloid and tau accumulations, loss of cholinergic innervation releases these regulations on microglia reactivity, leaving inflammation unchecked (Egea et al., 2015a; Schmitz et al., 2020). Basal forebrain volume loss is also associated with higher neocortical amyloid levels in nondemented older adults and AD patients (Grothe et al., 2014; Kerbler et al., 2015; Seidu et al., 2024; Teipel et al., 2012). Similarly, basal forebrain atrophy is correlated with higher plasma tau levels in older adults (Cavedo et al., 2020). Basal forebrain degeneration is thus linked to all of the main pathologies in AD, including further cortical neurodegeneration, inflammation and amyloid and tau pathology.

Cholinergic dysfunction after TBI

Long-term cholinergic deficits are also well documented after moderate-severe TBI (Dixon et al., 1996, 1997; Murdoch et al., 1998; Shao et al., 1999). For example, reductions in acetylcholinesterase (AChE) activity, the enzyme that breaks down ACh in cholinergic synapses, has been reported in the posterior cingulate and occipital cortex and, to a lesser extent, frontal cortex, lateral temporal cortex and inferior parietal cortex following TBI (Östberg et al., 2018). Volume loss and reduced grey matter density in cholinergic regions (basal forebrain) and cholinergic projection regions, such as the hippocampus and neocortex, has also been observed after TBI (Salmond et al., 2005).

Furthermore, volume loss within several cholinergic innervated cortical structures has been associated with performance deficits (Östberg et al., 2020). Despite some evidence of cholinergic dysfunction following moderate-severe TBI, the long-term impact of mTBI on cholinergic function has not been extensively investigated.

1.7 The cholinergic system in dementia and neurodegeneration

Anticholinergic and cholinergic effects on cognitive decline

The chronic use of anticholinergic drugs, which act to reduce cholinergic signalling, results in a higher risk of developing dementia (Fox et al., 2014; Gray et al., 2015; Jessen et al., 2010). This risk increases with the degree of anticholinergic drug usage. Common examples of anticholinergics include drugs to treat gastrointestinal dysfunction, over-active bladder, asthma, dizziness and motion sickness. It is well documented that cognitive impairments can occur while a patient is taking these drugs (Tannenbaum et al., 2012), however these drugs also produce cognitive impairments long after individuals have stopped taking the drug (Carrière et al., 2009). Additionally, anticholinergic use in those with AD genetic risk factors further predicts progression to MCI and cognitive decline (Weigand et al., 2020). Not only is anticholinergic use linked to cognitive decline, but those with chronic anticholinergic usage have greater levels of amyloid plaque and tau pathology (Perry et al., 2003). In animal models, the delivery of anticholinergic drugs increases neurodegeneration and inflammation (Yoshiyama et al., 2012). The long-term adverse effects of anticholinergic drug use further demonstrate the role of the cholinergic system in neurodegeneration and the development of dementia (Jessen et al., 2010).

Conversely, cholinesterase inhibitors elevate cholinergic activity by inhibiting cholinesterase, the enzyme that breaks down ACh in the synaptic cleft. Cholinesterase inhibitors, such as donepezil, galantamine and rivastigmine, are used to treat AD, particularly in the mild to moderate stage, and slow the progression of cognitive decline (Howard et al., 2012; Li et al., 2019). For example, treatment with donepezil improves

performance on the mini mental state examination and assessments of daily living (Howard et al., 2012; Takeda et al., 2006). These drugs often have additional cholinergic activity above cholinesterase inhibition which may contribute to their clinical success (Ago et al., 2011). Increased ACh availability by these drugs, particularly donepezil, also attenuates neuroinflammation as treatment reduces pro-inflammatory cytokine release, and microglia and astrocyte density and activation (Kim et al., 2021).

Decreased cholinergic function is sufficient to induce AD pathology

Cholinergic deficient mice similarly exhibit cognitive deficits, neurodegeneration and AD-related pathology, suggesting decreased cholinergic tone is sufficient on its own to produce a disease phenotype. Knockout of VAChT in the basal forebrain of mice significantly reduces cortical and hippocampal ACh levels and leads to abnormal increase in Aβ and phospho-tau, synaptic loss and neuronal degeneration in these cholinergic projection regions (Kolisnyk et al., 2013, 2017). Changes in protein expression in the cholinergic projection regions of these mice match alterations in protein expression observed in the brains of AD patients. For example, VAChT knockout mice have lower levels of heterogenous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1), which causes transcriptional alterations accompanied by changes in APP processing, increases in soluble Aβ, age-dependent tau pathology, pro-inflammatory cytokine levels and apoptotic markers. Similarly, in AD patients decreased VAChT levels correlate with lower levels of hnRNP A2/B1 and tau hyperphosphorylation (Kolisnyk et al., 2017). Basal forebrain VAChT knockout mice also have deficits in executive function on an attention-based touchscreen task (5-choice serial reaction time test) where mice learn slower and exhibit impairments with increased attentional demand compared to controls (Kolisnyk et al., 2013). These deficits reflect the attentional impairments observed in AD patients (Berardi et al., 2005).

Cholinergic dysfunction and cognitive deficits

The degree of cholinergic dysfunction correlates with cognitive decline both in AD patients and in cholinergic deficient mice. For example, decreased nicotinic ACh receptor binding was associated with attention in most brain regions in MCI and AD (Sultzer et

al., 2022). Similarly, levels of VAChT in the hippocampus correspond with performance on a memory task in mice (Al-Onaizi et al., 2017). Cholinergic projections also play a crucial role in cognitive functioning, with the integrity of cholinergic white matter projections exerting a greater impact on cognitive decline than cholinergic volume and age alone (Nemy et al., 2020). This implies that damage in cholinergic projection systems is particularly sensitive to cognitive outcomes. Once surpassing a certain threshold, increasing functional and structural alterations in the basal forebrain cholinergic system are believed to play a role in cognitive deficits, particularly in memory and attention (Schliebs & Arendt, 2011).

1.8 Cholinergic regulation of attention

The cholinergic system, among other neurotransmitter systems, is known to facilitate attentional control (Berry et al., 2015; Gill et al., 2000; Parikh & Bangasser, 2020; Sarter et al., 2005; Sarter & Bruno, 1999). The cortical cholinergic system plays a role in modulating signal detection, the attentional-based process of supporting a state of readiness to improve stimulus detection (Sarter et al., 2005). Deafferentation of cholinergic neurons leads to impairments in a sustained attention task, particularly affecting the ability to detect signal trials (Parikh et al., 2007; Sarter et al., 2005). In tasks of sustained visual attention, cortical cholinergic projections modulate medial prefrontal cortex neuronal activity. Under normal task performance, the prefrontal cortex (PFC) is not needed, however when task conditions change or in the presence of distractors, that is in instances of high attentional demand, cortical cholinergic innervation of the PFC is required to maintain performance (Berry et al., 2015; Gill et al., 2000). Transient increases in cholinergic activity are observed in the medial PFC during correct signal detection (Parikh et al., 2007). Basal forebrain cholinergic neurons modulate cortical cholinergic inputs to control various aspects of attention, including detecting and selecting relevant stimuli and discriminating important stimuli from distractors (Sarter & Bruno, 1999). Cholinergic deficiency in modulating cortical activity is directly responsible for attentional impairments in AD and can be recovered to some degree with drugs that increase acetylcholine levels (Bentley et al., 2008; Sultzer et al., 2022).

1.9 Attention deficits in AD and TBI

Attentional functioning, particularly in AD cases, can be distinguished into domains: sustained, selective and divided attention (Parasuraman & Haxby, 1993). Sustained attention is the basic capacity to maintain focus over a period of time as evidenced by reliable responding throughout a continuous task; deficits in this system result in attentional lapses (Posner & Petersen, 1990). Selective attention requires more executive functioning as it is the capacity to focus specifically on relevant stimuli while disregarding irrelevant stimuli (Perry & Hodges, 1999). Divided attention is the ability to allocate cognitive resources simultaneously between two or more targets or processes (Perry & Hodges, 1999).

Attentional deficits in AD

Early AD patients have deficits in multiple attention-related activities, including selective, divided and sustained attention (Berardi et al., 2005; Huntley et al., 2017; Perry & Hodges, 1999). Attentional deficits likely precede deficits in semantic memory but follow deficits in episodic memory, which is considered the first of the cognitive domains to exhibit impairments (Perry et al., 2000). Compared to healthy elderly controls, early AD patients show deficits in both sustained and selective attention, making more errors on a sustained attention response task (SART), which requires withholding a typical target response to a rare non-target stimulus (Huntley et al., 2017). They also exhibit general deficits in vigilance and attending to target stimuli when the difficultly of the task is increased and requires more effortful processing (Berardi et al., 2005). Similarly, they exhibit slower reaction times (Levinoff et al., 2005).

Additionally, deficits in sustained attention correlate with poorer performance on a standard measure of general cognitive function, mini-mental state examination, as well as impairments in activities of daily living (Huntley et al., 2017). Attentional control, but not episodic memory, is correlated with Aβ levels in CSF, while elevated tau levels predict decline in both attention and episodic memory (Aschenbrenner et al., 2015). Attention deficits in AD, therefore, appear to correlate with cognitive decline across

various domains and are associated with AD biomarkers. This suggests that attentional impairments may be an important aspect of disease progression.

Attention deficits following TBI

Executive functioning, including attentional impairments have also been reported after varying severity of TBI. For example, distinct sustained attention deficits corresponding with attentional lapses in everyday activities are observed in severe TBI patients and correlate with the severity of TBI (Dockree et al., 2004; Robertson et al., 1997). Individuals with mTBI also have reduced attentional control in the months following injury with functional activity changes during attention tasks (Shah-Basak et al., 2018).

1.10 Measuring attention clinically and preclinically

5-choice serial reaction time test (5-CSRTT) and sustained attention task (SAT) have been used extensively in preclinical testing to assess attention; however, they are not comparable on multiple levels to the attention tasks used in the clinic (Kim et al., 2015). In the 5-CSRTT a brief light is flashed in one of five spatial locations on the touchscreen in random order and the subject must respond to the correct location when the light flashes (Robbins, 2002). Typically, clinical tasks require discrimination of complex visual stimuli and selective responding to a target stimulus out of non-target stimuli, unlike 5-CSRTT, which only requires detection and response to the presented stimuli. One commonly used clinical task is the continuous performance test (CPT), originally developed by Rosvold (Rosvold et al., 1956). In the CPT the subject is required to attend to a rapidly presented complex visual stimulus in a single location as to detect and respond to an infrequent ‘target’ stimulus while withholding responses to more frequent ‘non- target’ stimuli. Since its development, many variations of the CPT have been used (Kim et al., 2015). Deficits on the CPT have been reported in AD where patients show impaired accuracy and lose track of the task, but show normal reaction times and do not have decreased distractibility (Stopford et al., 2012). Following moderate to severe TBI, deficits on the CPT are also observed (Zane et al., 2016).

It is this discrimination feature that was lacking in many previous preclinical attention tests that Kim and colleagues (2015) incorporated into a rodent version of the CPT (rCPT) administered on operant touchscreen systems. Cognitive testing on the touchscreen systems allows large-scale standardized, automated and consistent assessment with reduced stress associated with testing (Bussey et al., 2008). Compared to conventional cognitive testing, touchscreen tasks like the CPT are highly translatable to human cognitive assessments (Kim et al., 2015; Mar et al., 2013). Until recently, cognitive phenotyping in preclinical AD models has focused on learning and memory, leaving other cognitive domains out of the picture. Executive function and attention are important contributors to cognitive and functional deficits in AD. Cognitive enhancing effects of cholinesterase drugs typically improve attentional functions rather than memory (Sahakian & Coull, 1993). Thus, assessment of attention, especially in relation to the role of the cholinergic system in disease development, is crucial to provide a well-rounded cognitive assessment in preclinical studies (Romberg et al., 2013).

1.11 Mouse models of AD-related pathology

Accurate preclinical AD models are crucial to understanding the mechanisms underlying disease susceptibility, progression and the clinical success of treatments. Despite decades of preclinical drug trials targeting various AD pathologies, there has been an overwhelming failure of drugs to translate successfully to the clinic (Schneider et al., 2014; Veening-Griffioen et al., 2019). One of the challenges is that mouse amyloid and tau proteins do not form pathological aggregates like those observed in humans (Saito et al., 2019). Aβ in particular has some important disease-relevant differences between the mouse and human as mouse Aβ is less apt to form β sheets and fibrils and is less neurotoxic, producing less reactive oxygen species (Lv et al., 2013). Many original AD models induced mutations in disease-related genes, such as APP and tau, causing the formation of amyloid plaques and NFTs. However, these models often express these proteins and related products at supraphysiological levels, which is thought to produce artificial phenotypes (Saito et al., 2014).

Mouse model of humanized amyloid

To improve preclinical models and correct issues of supraphysiological levels of mutant proteins, the field has turned to several next-generation human Aβ knock-in models. The more recently produced humanized APP mice harbor various AD-related mutations modeling plaque pathology in an age-dependent manner (Saito et al., 2014). Although these models help to recapitulate AD pathogenesis better, they still express early-onset AD-associated *APP* mutations and may therefore not reflect AD in the majority of cases which are not due to mutations in APP. Autosomal dominant inheritance involving *APP*, *PSEN1* and *PSEN2* mutations typically results in early-onset AD, accounting for only 5-10% of all AD patients. Of those patients with early-onset AD cases, only 10% of cases are autosomal dominant inheritance, with the rest of the cases being sporadic and likely polygenic (Wingo et al., 2012). These *APP* mutations, therefore, account for a very small percentage of AD cases, with the remainder associated with various genetic and environmental modifying factors (Reitz et al., 2020).

In order to improve preclinical modeling of LOAD and provide a platform for which additional genetic and environmental risk factors could be added, Baglietto & colleagues (2021) developed mice expressing a humanized version of the Aβ containing region within the mouse *App* (humanized Aβ, hAβ) (Baglietto-Vargas, et al., 2021). Through homologous recombination three amino acids were introduced into the murine *App* locus to humanize the Aβ peptide sequence resulting in mice that express non-mutated human Aβ at murine physiological levels. Replacing the mouse Aβ with the wild-type human Aβ produces alterations in synaptic plasticity, inflammation, gene expression, astrocyte associated granule formation and cognition (Baglietto-Vargas, et al., 2021). Aged, 18–22-month-old humanized Aβ mice show increases in insoluble Aβ in the absence of extracellular deposits or plaques. They also exhibit age-associated hippocampal Periodic Acid Schiff (PAS) positive granules that increase compared to wildtype mice around ten months of age. Indeed, these hippocampal granules appear dependent on the presence of humanized Aβ and AD mutations because cre-mediated deletion (with UBC-CRE$^{ERT2+/-}$ mice) of Aβ decreases these granules. By 10 months of age, the humanized Aβ mice show hippocampal-related cognitive deficits and cortical-

related deficits on novel object recognition tasks at 14 months. Decreases in synaptic puncta measured by synaptophysin staining were observed slightly later at 18 months (Baglietto-Vargas, et al., 2021).

Mouse model of tau

As tau pathology plays such a key role in AD pathogenesis and in predicting clinical symptoms, it is also important that it be included in preclinical models (Montine et al., 2012). Previous preclinical models of tau pathology induce tau mutations; however, these are mutations do not occur in AD but rather frontotemporal dementia (Saito et al., 2019). Despite the lack of tau mutations in AD, several important differences exist between the profile of expression of human tau compared to mouse tau, as adult mice do not express all six tau isoforms. Saito and colleagues (2019) humanized the entire tau gene to more faithfully reproduce the tau pathology from humans that was lacking from the knockin amyloid models (Saito et al., 2019). The mouse *Mapt* gene was replaced with the human *MAPT* gene using homologous recombination in embryonic stem cells and then stem cells were injected in C57BL/6 mice (Hashimoto et al., 2019). While humanized *MAPT* mice express all six isoforms of tau, unlike wildtype mice, the humanized tau is physiologically and structurally normal within the mice. These mice display no specific tau pathology and the humanized tau appears to respond similarly to murine tau in response to amyloid deposition caused by APP mutations (Saito et al., 2019). Thus, even at an old age, the humanization of tau, unlike that of Aβ, does not appear to drive pathological tau pathology, neuroinflammation or cognitive deficits.

Mouse model of cholinergic dysfunction: VAChT knockdown

Vesicular acetylcholine transporter (VAChT) has been the target protein of several models of cholinergic dysfunction (Kolisnyk et al., 2013, 2017; Prado et al., 2006). The sole protein responsible for transporting ACh into synaptic vesicles for release, VAChT is the rate limiting step in ACh release into the synapse (Prado et al., 2013; Song et al., 1997). VAChT levels are reduced in the cortex and hippocampus of AD patients (Kolisnyk et al., 2017; Schmitz et al., 2018). To produce a mild reduction in global

VAChT levels in the mouse, Prado & colleagues generated a hypomorphic allele of the VAChT gene (*Slc18A3*) by inserting a neo cassette before the VAChT gene through homologous recombination (Prado et al., 2006). These mice are referred to as VAChT knockdown (VAChT KD) mice. Mice homozygous for VAChT KD display alterations in neuromuscular function accompanied by extensive motor deficits. Conversely, heterozygous VAChT KD mice (VAChT KD^{HET}) exhibit relatively intact peripheral ACh function, but a larger deficiency in central ACh tone. VAChT KD^{HET} mice express approximately 45% of control levels of VAChT which corresponds with around 30% reductions of extracellular ACh in the frontal cortex and striatum but an overall increase in intracellular ACh (Prado et al., 2006). The mild cholinergic deficit in the VAChT KD^{HET} mice results in impaired social memory and novel object recognition.

1.12 Mouse models of TBI

Classically, TBI has been researched in animal models using several rather severe models, including controlled cortical impact (CCI), lateral fluid percussion injury and weight drop. These models typically display gross morphological changes, including extensive cortical tissue loss, reactive gliosis, neuronal loss, edema and blood-brain barrier disruption (Osier & Dixon, 2016; Smith et al., 1995). CCI and lateral fluid percussion injury also require craniectomy, which itself can induce considerable swelling and inflammation. The foremost limitation for clinical relevance is that these models induce extensive focal contusion but limited diffuse axonal injury, which is common in many human TBIs, especially mild to moderate injuries. In fact, the main factor of human acceleration impacts, like the majority of mTBIs, is not a focal injury but the resulting diffuse axonal injury (Zhang et al., 2006).

Models of mTBI using closed head injuries

Of particular relevance for preclinically modelling mTBI and concussion-like injuries are injuries that are closed head and allow unrestricted motion of the head directly following impact. These injuries typically produce no morphological changes or overt neuropathology post-injury; however, they show microstructural changes including axonal injury, neuroinflammation and some tau pathology (Hoogenboom et al., 2019).

Angular or rotational acceleration of the brain is particular important in these models as it is the main contributor to axonal injury, with linear acceleration contributing to a lesser degree (Smith et al., 2003).

Towards a translatable model of TBI

Based on measurements from football players, rotational accelerations of 5,260, 5,281, 6,383, 6,945 and 7,483 rad/s2 correspond to concussion risks of 10%, 25%, 50%, 75% and 90%, respectively (Rowson et al., 2012). Peak rotational accelerations around 5 krad/s^2 and angular velocities of 22 rad/s occur in the majority of concussive cases, although impact magnitude cannot be used to predict clinical outcomes (Guskiewicz & Mihalik, 2011). Our lab has developed a mouse model of concussion to reflect real-world concussion impact forces that aims to deliver an injury that produces peak rotational and angular velocities that when scaled to humans are in the range of 5krad/s^2 and 22 rad/s. This model induces a closed head injury and allows free rotation of the head upon impact. Analysis of kinematics during and after the impact show that this model produces a reasonably reproducible impact between mice. Histological analysis at 1 week post-injury suggests this model produces diffuse axonal injury and gliosis, mainly in the plane of the principal strain induced by the rotational forces at impact.

1.13 Rationale

Mild TBI, in combination with aging and genetic factors, increases the risk for later development of AD and accelerates disease onset (Asken et al., 2021; Gardner et al., 2014; Hayes et al., 2017). AD pathology begins to develop decades before distinct symptom onset, leaving a long period of molecular pathway changes before cognitive deficits are manifested (Sperling et al., 2011). Thus, the intersection of TBI with potential disease-related pathways during this time is of particular interest.

Reduced cholinergic signalling and volume loss in cholinergic regions are observed in individuals with a history of TBI (Östberg et al., 2018, 2020; Salmond et al., 2005). Cholinergic system dysfunction and degeneration occur early in the continuum of AD (Grothe et al., 2012; Teipel et al., 2011; Whitwell et al., 2007). Cholinergic deficits

predict progression to AD and can increase AD neuropathology (Fernández-Cabello et al., 2020; Kolisnyk et al., 2017). The cholinergic system regulates multiple cognitive processes, including attention (Sarter & Parikh, 2005); deficits in cholinergic signalling are directly linked to attentional impairments (Bentley et al., 2008). Given the cholinergic dysfunction early in AD, it is unsurprising that AD patients have deficits in executive function, including attentional control (Perry & Hodges, 1999).

To understand the role of cholinergic dysfunction in the progression of AD-related pathology and cognitive deficits following mild TBI, this study utilized humanized Aβ (Baglietto-Vargas et al., 2021) and tau mice (Saito et al., 2019) with reduced levels of the vesicular acetylcholine transporter (VAChTKD; Prado et al., 2006). This mouse model was used to recapitulate more accurately the phenotypic features in human disease without directly inducing AD through mutations. As murine Aβ does not aggregate to the same extent as human Aβ and lacks the same neurotoxicity, humanization of Aβ was necessary to facilitate fibril formation and disease related neurotoxicity (Lv et al., 2013; Baglietto-Vargas et al., 2021). Similarly, murine tau has some fundamental differences from human tau, one of which is that only 4R tau is expressed in adult mice while all six tau isoforms are expressed in humans (Saito et al., 2019). Humanized tau within the mouse is functionally and physiologically normal (Saito et al., 2019). As amyloid and tau are thought to play such a crucial role in AD pathophysiology it is important that these proteins have structure that could develop pathological aggregates. To model a mild cholinergic dysfunction VAChTKD mice were used as they exhibit minimal behavioural deficits despite about a 40% reduction in VAChT levels (Prado et al., 2006). This mouse model allowed for a mild cholinergic dysfunction on a background of humanized amyloid and tau without directly causing AD.

To model a clinically relevant mild TBI a repetitive closed head injury with rotational force (rCHI-rf) that mimics the angular velocities that are experienced during concussive impacts. This model induces diffuse axonal injury mainly associated with the rotational forces and not the impact itself. As the goal of this experiment was to investigate the combined role of rmTBI and cholinergic dysfunction in neurodegeneration and cognitive deficits it was necessary to use both a model of mTBI and cholinergic

dysfunction that on their own did not induce considerable deficits. The combination of a humanized mouse model without disease causing mutations and a mTBI allowed for the assessment of cognitive function and degenerative pathology over the natural aging process.

1.14 Hypothesis and aims

Hypothesis: Mild cholinergic dysfunction may increase the risk of developing Alzheimer's-related pathology and cognitive impairments following repetitive mild traumatic brain injury.

Aim 1

The first aim of this study was to assess attention using the touchscreen rCPT in hAβ;hTau;VAChTKD^{HET} (VACHTKD) and hAβ;hTau (WT) mice following repetitive mTBI, specifically looking at the effect of VAChTKD, rmTBI and aging. Executive functioning, specifically attention, is one of the cognitive domains affected in AD and following TBI. Sustained attention and selective attention are affected early in the progression of AD and these attentional deficits are associated with other cognitive functioning and ability with activities of daily living (Huntley et al., 2017; Levinoff et al., 2005). The basal forebrain cholinergic system is affected very early in AD before cognitive impairments appear, and loss of cholinergic tone likely contributes to disease pathology and cortical degeneration (Grothe et al., 2014; Hall et al., 2008; Schmitz & Spreng, 2016). Cholinergic innervation of cortical areas also plays an integral role in modulating attentional processing, especially when attentional demand in high (Berry et al., 2015; Gill et al., 2000; Sarter & Bruno, 1999). The rCPT in operant touchscreen system allows systematic, reproducible testing of sustained and selective attention comparable to the clinical assessment of attention in humans (Kim et al., 2015; Stopford et al., 2012).

Prediction: The combination of rmTBI and VAChTKD will exacerbate cognitive impairments, namely attentional impairments, in comparison to VAChTKD or rmTBI alone.

Aim 2

The second aim of this study was to assess degenerative AD-related pathology in hAβ;hTau;VAChTKD^{HET} (VAChTKD) and hAβ;hTau (WT) mice following repetitive mTBI. Cholinergic dysfunction results in increased amyloid and phospho-tau, inflammation and neurodegeneration (Kolisnyk et al., 2017; Schmitz et al., 2020a). TBI also instigates amyloid, phospho-tau accumulation and inflammation leading to chronic elevations of these pathologies (Mohamed, Cumming, et al., 2022; Mohamed, Nestor, et al., 2022; Ramlackhansingh et al., 2011; Tagge et al., 2018). Amyloid and tau pathology were not directly assessed within the context of this thesis (see section 4.8.4 for further discussion). One pathology extensively characterized in the hAβ mice and known to be increased with aging in AD mouse models and associated with amyloid and tau pathology is PAS hippocampal granules (Baglietto et al., 2021; Manich et al., 2016; Oddo et al., 2003; Robertson et al., 1997; Wander et al., 2022). PAS hippocampal granules were assessed at 6 months post-injury.

Prediction: The combination of rmTBI and VAChTKD will exacerbate degenerative AD-related pathology, in comparison to VAChTKD or rmTBI alone.

Chapter 2

2 Methods

To investigate the role of cholinergic dysfunction in the progression of cognitive deficits and degenerative pathology following repetitive mild traumatic brain injury (rmTBI) this study uses a mouse model of cholinergic dysfunction (VAChTKD) with humanized amyloid and tau. A model of mTBI was used that recapitulates the rotational forces in the range experienced during human concussive impacts. To assess attentional functioning with aging after rmTBI mice were tested on the CPT at baseline and 6- and 12-months post-injury. Pathology was assessed acutely at 1-week post-injury to characterize diffuse axonal injury and at 6 months post-injury to assess degenerative pathology.

2.1 Animals

For the acute behaviour study, 36 three-month-old male C57BL/6 J wildtype mice were obtained from Jackson laboratories. For all other experiments homozygous humanized Aβ (Baglietto-Vargas et al., 2021) and humanized tau (hTau; Saito et al., 2019) mice and heterozygous VAChTKD mice (VAChTKD; Prado et al., 2006) were used. The full nomenclature for this strain is B6N;129S-App$^{tm1.1Aduci}$ Mapt$^{tm1(MAPT)Tcs}$ Slc18a3^{tm1Mca}. All mice were bred in house through crossing male hAβ.hTau with female hAβ.hTau.VAChT KD mice. Animals used for the acute and chronic pathology and behavioural experiments were littermates with carrying hAβ and hTau and either VAChT KD or wildtype VAChT levels. Throughout this thesis they will be referred to as hAβ.hTau.VAChTKD (VAChTKD) and hAβ.hTau (wildtypes, WT), respectively. All mice were group housed and kept on a 12hr:12hr light: dark cycle. Mice had free access to food and water unless under food restriction for touchscreen experiments described below. Access to food was also restricted in the case of obese mice to maintain mice at a healthy weight for their age based on weight curves from Charles River. Ten mice were lost during surgeries due to the anesthetic. Four mice were euthanized before reaching the endpoint due to issues such as a retrobulbar abscess, cancer, and one was found dead. Two mice developed paralysis in their hind limbs at around 20 months of age.

Table 1. Summary of animal numbers for experiments

Group	Number of animals			
	Sham		rmTBI	
	hAβ.hTau	hAβ.hTau.VAChTKD	hAβ.hTau	hAβ.hTau.VAChTKD
Behaviour cohort 1	4(8)	6(8)	5(8)	5(7)
Behaviour cohort 2	6(4)	5(6)	7(4)	5(6)
Behaviour cohort 3	8(5)	5(2)	4(3)	5(3)
1-week post-injury pathology (half for histology half for biochemistry)	9(10)	9(10)	10(10)	10(10)
6-month post-injury pathology (half for histology half for biochemistry)	11(11)	11(11)	11(11)	11(11)

Note. Males (Female)

2.2 TBI model

Mice were anaesthetized with 80mg/kg ketamine and 10 mg/kg xylazine. A controlled cortical impactor (TBI 0310, Precision Systems and Instrumentation) was used to deliver closed head impact between the ears of the mouse positioned to be over the bregma suture. The impact was calibrated to 3.5m/s at a depth of 8mm and the final speed at impact was recorded. At impactor contact with the head, the platform on which the mouse is placed breaks allowing free rotation of the head following impact (Figure 1 B-D). The entirety of the mouse rotates following impact landing on a cushion 8’’ below.

High-speed videos were taken at 5,000 frames per second, allowing 5 frames per ms. The kinematic analysis demonstrated that 3.5 m/s impacts induced peak angular velocities of 277 ± 91 rad/s in males and 246 ± 66 rad/s in females. This is comparable to the peak angular velocities measured in sub-concussive impacts in football players (Rowson et al., 2012). Angular velocities of this degree are associated with a 10% nominal injury risk. Mice received 3 closed head injuries (CHI) with rotational force (one a day for 3 days), while sham mice only received anesthetic injections. The model of injury was intended to maintain a mTBI which typically does not exhibit gross pathological changes or skull fractures. Increasing to 5 hits in a row increased the chance of skull fracture. The velocity used in this experiment was 3.5m/s as 5m/s resulted in frequent skull fractures when tested.

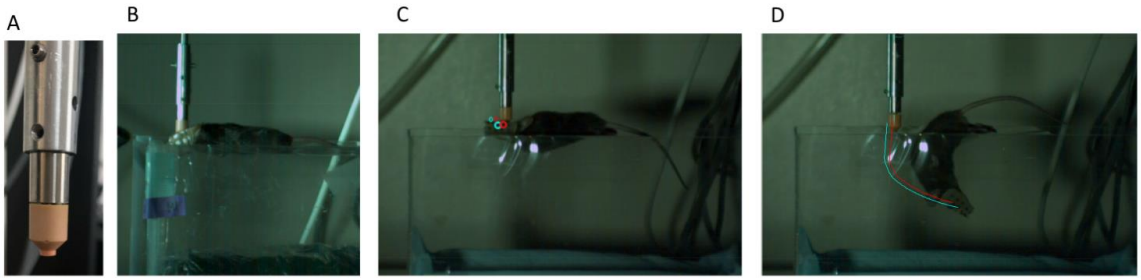


Figure 1. Set up for TBI procedures. Mice were placed on platform (B) and received impact centered on Bregma suture with a silicone tip (A). Upon impactor contact with the head there is rapid rotation of the head through the platform and the mouse falls to the pillow below (C-D).

2.3 Touchscreen Behavioural Testing

Touchscreen behavioural testing was administered in Campen Instruments 81430 touchscreen systems. Training and performance in the task were motivated by strawberry milkshake reward (Neilson Strawberry milkshake). Therefore, to provide sufficient motivation, animals were subject to mild food restriction before task training at 3 months of age, i.e. ≈2 g food pellets (Bio-Serv Product #F0173)/day and free access to water. Milkshake was provided in home cage 2-3 days prior to the start of training. Touchscreen

training and testing was administered between 1-6pm in the light cycle. Mice underwent pretraining to habituate to the touchscreen chamber and food magazine and then were trained to touch the screen for reward.

2.4 Rodent continuous performance test

Attention was assessed using the rodent continuous performance test (rCPT) which is an operant touchscreen task developed based on the CPT previously used in clinical assessments (Kim et al., 2015). CPT requires detecting and responding to target stimuli while withholding responses to non-target stimuli. Thus, a subject can make errors of omission and errors of commission which can be used to measure two facets of performance using signal detection theory. These aspects are: the subject's ability to discriminate stimuli (discrimination sensitivity (d')) and the subject's decision to respond corresponding to their response tendencies, motivation or task strategy (response criterion/bias (C) (Macmillan & Creelman, 2004). Discrimination sensitivity and response bias are parametric measures calculated under assumptions of normal distributions and equal variances in discriminability of target and non-targets. For these experiments, horizontal lines were used for the target stimulus and four non-target stimuli- vertical lines, diagonal lines both left and right and circular lines- were used (see Figure 2 for stimuli used). Correct responses to the screen were rewarded with strawberry milkshake (Neilson) accompanied by the magazine light coming on and a tone (duration 1000ms).

Training stages Stimulus set	Stimulus duration	Criterion
Stage 1 White square 	10s	Achieving 60 rewards Minimum 1 day
Stage 2 S+ introduction 	2s	Achieving 60 rewards Minimum 1 day
Stage 3 S+ (50%) One S- (50%) 	2s	d' > 1.5, HR > 0.6, FAR< 0.2, for two consecutive days Minimum 7 days
Stage 4 S+ (33%) Four S- (66%) 	2s	d' > 1.5, HR > 0.6, FAR< 0.2, for two consecutive days Minimum 10 days

Figure 2. CPT training stimulus and criterion for progressing onto next stage.

Touchscreen parameters

Hit rate (HR): correct responses to target stimuli, indicates attentiveness

$$HR = \frac{Hit}{Hit+Miss}$$

False alarm rate (FAR): incorrect responses to non-target stimuli, indicates impulsivity

$$FAR = \frac{Mistake}{Mistake+Correct\ rejection}$$

Discrimination sensitivity (d'): indicates the ability to discriminate between target and non-target, higher score indicates increased ability to respond selectively to target stimuli.

$$d' = z(HR) - z(FAR)$$

Response criterion/ response bias (c): decision to respond corresponding to subjects' response tendencies, motivation or task strategy, higher score indicates more conservative responding while lower score indicates more liberal responding.

$$C = \frac{z(HR) - z(FAR)}{2}$$

Correct choice latency (CCL): time from target stimulus onset until mouse makes correct response, comparable to reaction time, it can be considered a measure of cognitive processing speed

Centre ITI touches: number of touches to centre screen during the inter-trial interval

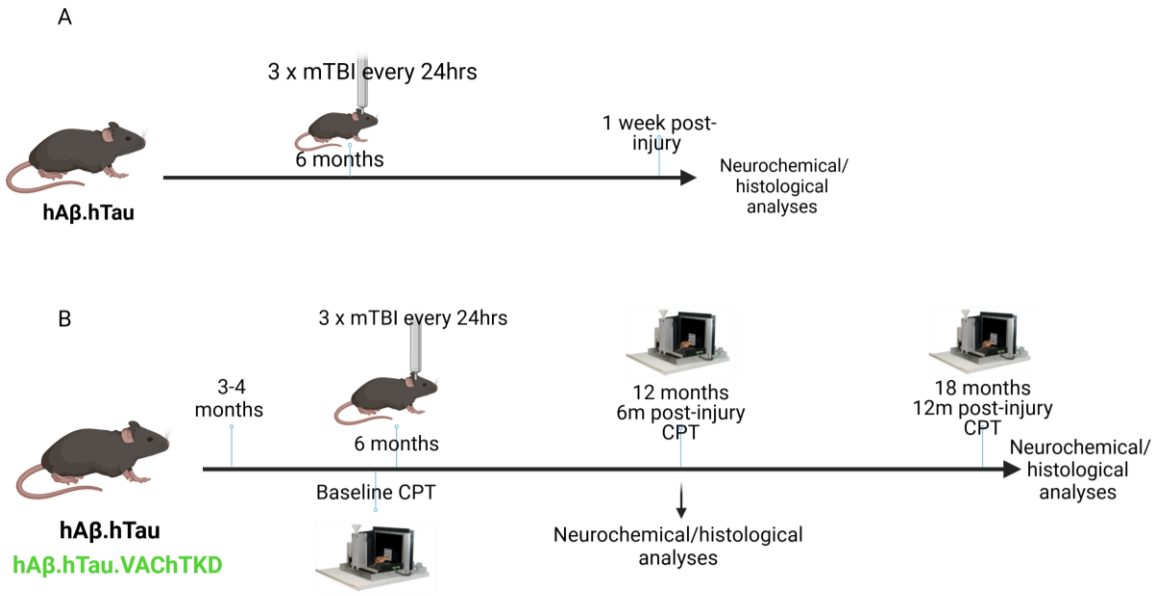


Figure 3. Experimental timeline. (A) For acute characterization of the rmTBI model male and female hAβ.hTau mice received either sham or 3 mTBI 24hrs apart and were collected for histology 1-week post-injury. (B) For the chronic behavioural experiment, hAβ.hTau and hAβ.hTau.VAChTKD mice underwent CPT training at 3-4 months old. Baseline CPT probe testing was performed before injuries at 6 months old and again at 6- and 12-months post-injury. Mice were taken for neurochemical and histological analyses at 6- and 12-months post-injury.

CPT training

Mice were first habituated to touchscreen chambers and reward magazine. Mice were then trained to touch the screen when the white square appeared to receive reward (stage 1) and then they were trained to touch the screen when the target stimulus (horizontal lines) appeared (stage 2). Stage three training added one-non-target stimuli (white snowflake) and mice were rewarded only when they responded to the target stimuli; incorrect responding to the non-target resulted in correction trial where non-target stimuli would appear until mouse correctly withheld response. The final stage of training contained all four non-targets presented at a target probability of 25% (i.e., 3 non-targets: 1 target). Mice were trained to d' > 1.5, HR > 0.6 and FAR < 0.2 at both stage three and stage four with a minimum of 7 and 10 days at each stage, respectively. See Figure 2 for visual. Upon completing training mice were place on maintenance, stage four training, and run once a week until all animals had passed.

CPT probe testing

For acute CPT experiment in C57BL/6 male mice, animals were tested on the CPT probe at baseline prior to injuries at 5 months old and again at 2- and 6-weeks post-injury. For long-term study with hAβ.hTau and hAβ.hTau.VAChTKD male and female mice, animals were tested at baseline prior to injuries at 6 months old and again at 6- and 12-months post-injury (see Figure 3 for experimental timeline). For each testing period performance was established at the 2s stimulus duration - identical to stage four training - over 3-4 days. This was to ensure performance was stable (had reached a plateau) before moving onto probe testing. Reducing stimulus duration is a robust method to tax attention (Kim et al., 2015). Variable stimulus duration (VSD) probe was performed over four consecutive days where the stimulus duration was decreased to increase attentional demand. Performance was averaged over the four days of probe testing. Stimulus lengths of 2s, 1s, 0.5s and 0.2s were randomly presented over the course of the session. After the baseline probe session animals were randomly assigned to receive three mTBIs or three sham procedures. Performance on the VSD probe was assessed within each group to ensure that there were no differences in performance at baseline between animals

assigned to sham and rmTBI groups. Following the procedure mice were maintained once every 1-2 weeks until the final probe testing.

2.5 Tissue preparation

For biochemical analysis, after deep anesthesia with 130mg/kg ketamine and 13mg/kg xylazine mice were transcardially perfused with ice-cold saline. Brains were dissected for prefrontal cortex, hippocampus, midbrain, cortex and brainstem, frozen on liquid nitrogen and stored at -80 until use. For immunohistochemical analysis, saline was followed 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were post-fixed for 24hr in 4% paraformaldehyde, transferred to 20% sucrose for 1-2 days and embedded in O.C.T Tissue Tek (Somagen Diagnostics, SAK4583).

2.6 Silver staining

For silver staining, coronal sections of 50 µm were cryosectioned at 0.345 mm anterior to -0.08 mm posterior to Bregma, -1.15 mm to -1.55 mm posterior to Bregma and -2.55 mm to -3.38 mm posterior to Bregma (according to the Allen Mouse Brain Atlas, Reference Atlas version P56 Coronal) to encompass the corpus callosum, optic tracts and cerebral peduncles. Sections were transferred from 8% sucrose containing 0.05% sodium azide to 4% paraformaldehyde one week prior to silver staining. Then silver staining was performed using the FD NeuroSilver Kit II (FD NeuroTechnologies, Ellicott City, MD) according to the manufacturer's instructions.

2.7 Silver staining acquisition and quantification

2-3 sections from regions containing the optic tracts and corpus callosum were imaged at 20x on an Olympus BX50. Silver staining from the images was quantified separately by two blinded observers. Degree of staining in each tract was rated on a qualitative scale of 0 to 3. A score of 0 being no silver-stained fibers, a scale of 1 was given for a few sparse fibers, a scale of 2 for a moderate degree of fibers and a scale of 3 for densely silver stained fibers. Ratings were averaged across images from each tract to obtain an average qualitative measure of silver staining in the corpus callosum and optic tracts.

2.8 Immunohistochemistry

Cryosections, 20 µm thick, were collected in phosphate buffer saline with 0.01% sodium azide. For staining, sections were washed in 0.1M Tris buffer saline (TBS), followed by 0.3% TBS-Triton X-100 and blocked in 5% goat serum with 0.3% Triton X-100 for 2 h at room temperature (RT) and 2 h at 4 °C (only for OC+ staining). The sections were then incubated with primary antibodies: rabbit polyclonal anti-amyloid fibrils OC (1:100, Millipore Sigma, AB2286), mouse anti-GFAP (1:1000, Millipore Sigma, MAB360), rabbit s100β (1:200, Abcam, ab52642), rabbit anti-Iba1 (1:1000, Wako, 019-19741) (diluted in blocking buffer) for 1h at room temperature and overnight at 4 °C. Brain sections were washed three times with TBS and then incubated with the appropriate secondary antibodies: donkey anti-mouse IgG, conjugated with Alexa Fluor 594 (1:1000; Invitrogen, A21203), donkey-rabbit IgG, conjugated with Alexa Fluro 488 (1:800-1000; Invitrogen, A21206), donkey anti-rabbit IgG, conjugated with Alexa Fluor 594 (1:1000; Invitrogen, A21207) and donkey-mouse IgG, conjugated with Alexa Fluro 488 (1:1000; Invitrogen, A21202) for 1h at room temperature. Sections were washed three times with TBS and then mounted with Prolong Diamond antifade with DAPI (Invitrogen, P36971). Images were acquired on a DM6 B THUNDER imager at 20x or Leica SP8 confocal microscope at 63x.

2.9 Granule quantification

Images (n=6 per genotype, injury and sex) of coronal sections of mouse hippocampal regions taken from between -1.15 mm and -3 mm posterior to the Bregma according to the Allen Mouse Brain Atlas, Reference Atlas version P56 Coronal were taken with Leica DM6 B THUNDER imager microscope. Four-five sections per animal were immune-fluorescently labeled for OC and GFAP antibodies. Two-three images were taken at 20x from each section using the same acquisition parameters for all images. Images were quantified automatically using particle count function and an in-house macro on ImagePro software. Image acquisition and quantification were completed by an observer blinded to genotype and injury. Individual OC+ granule size (μm^2), count and total area

were calculated for each image. The sum of the total area of granules was divided by the total area of all the images to determine an average area for each animal.

2.10 Statistical analysis

All statistics were performed using Graphpad Prism 10.1 software. Comparisons between groups were analysed by one-way or two-way ANOVA with repeated measures. CPT behaviour was analysed by two-way ANOVA followed by Šidák's multiple comparison test when warranted. Statistics are given for the column factor (typically main effect of genotype, injury, or time) unless otherwise stated. MWM was analysed with two-way ANOVA followed by Tukey's multiple comparison test. Pathology was analyzed with one-way ANOVA followed by Tukey's multiple comparison test or unpaired t-test. Values are considered statistically significant with alpha value of 0.05. Data are presented as mean ± SEM.

Chapter 3

3 Results

3.1 rmTBI induces axonal injury specific to the axis of rotation

One of the initial and substantial pathologies associated with mTBI is diffuse axonal injury. Diffuse axonal injury often results in disruption of axonal transport leading to accumulation of degenerating cellular material (Johnson et al., 2013). These degenerating components of axons and neurons can be detected by silver staining (Koliatsos et al., 2011; Xu et al., 2021). To confirm this model of rmTBI induced diffuse axonal injury, tissue was collected from sham and injured male and female hAβ.hTau mice at 1-week post-injury and silver staining was performed. Black silver-stained fibers were visible in optic tracts of injured animals while few if any silver-stained fibers were seen in sham mice (**Figure 4**; $t=5.63$, $df=15$, $p<0.0001$). The optic tracts of injured mice contained silver stained axons with the appearance of end bulbs and punctate beading typically observed with diffuse axonal injury (**Figure 4D'**) (Xu et al., 2021). Silver staining in the optic tracts was accompanied by gliosis as indicated by increased microglia and astrocytes and differing glial morphology in the injured mice (**Figure 5**).

Conversely, strong silver staining of fibers was not observed in corpus callosum following rmTBI (**Figure 6;** $t=1.11$, $df=16$, $p=0.28$). Silver staining indicating axonal damage in the optic tracts, but not the corpus callosum suggests orientation specific axonal damage. The optic tracts run rostral to caudal, perpendicular to the axis of rotation of the injury. The corpus callosum runs medial to lateral, parallel to the axis of rotation of the injury. During injury, axonal fibers that run perpendicular to the axis of rotation experience strain, leading to increased axonal damage. Axonal fibers that run parallel to the axis of rotation are less affected and incur less strain, resulting in comparatively lower levels of axonal damage.

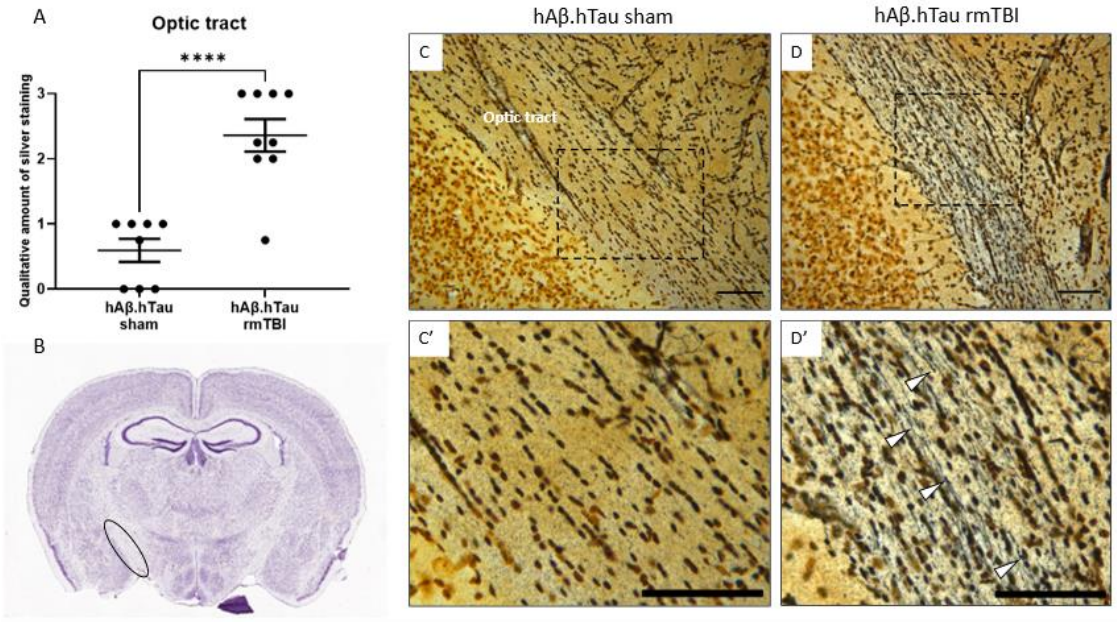


Figure 4. Diffuse axonal injury in the optic tracts 1 week after rmTBI. Average qualitative amount of silver staining in optic tracts of male and female hAβ.hTau sham and injured mice scored by two blinded observers (A). Representative picture of optic tract location- shaded in purple from Allen Mouse Brain Atlas, P56 coronal (B). Background silver staining of nuclei were seen in all mice, but silver stained fibers were not observed in sham mice (C,C'). Silver-stained fibers were visible throughout the optic tracts of injured mice (D). Some axons in injured mice (indicated with arrowheads) had the appearance of end bulbs and the typically beaded appearance seen with diffuse axonal injury (D'). White arrowheads indicate silver-stained fibers. Insets are high magnifications of black-boxed areas in the panel. Scale bar = 50μm. Animal numbers per group are as follows, half male/ half female: sham n = 9, rmTBI n = 9. Unpaired t-test, **** $p < 0.0001$.

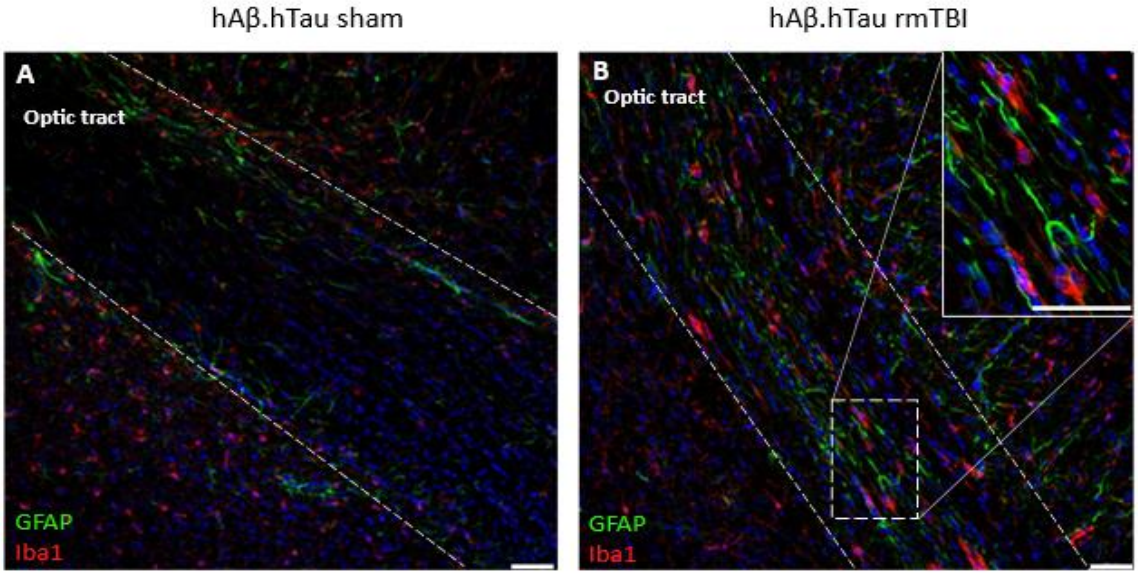


Figure 5. Astrocytes and microglia in the optic tracts 1 week after rmTBI. Astrocyte and microglial staining in optic tract of sham (A) and injured (B) animal at 1-week post-injury. Scale bar = 50μm. DAPI (blue), GFAP- glial fibrillary acidic protein (green), Iba-1 – ionized calcium-binding adapter molecule 1 (red).

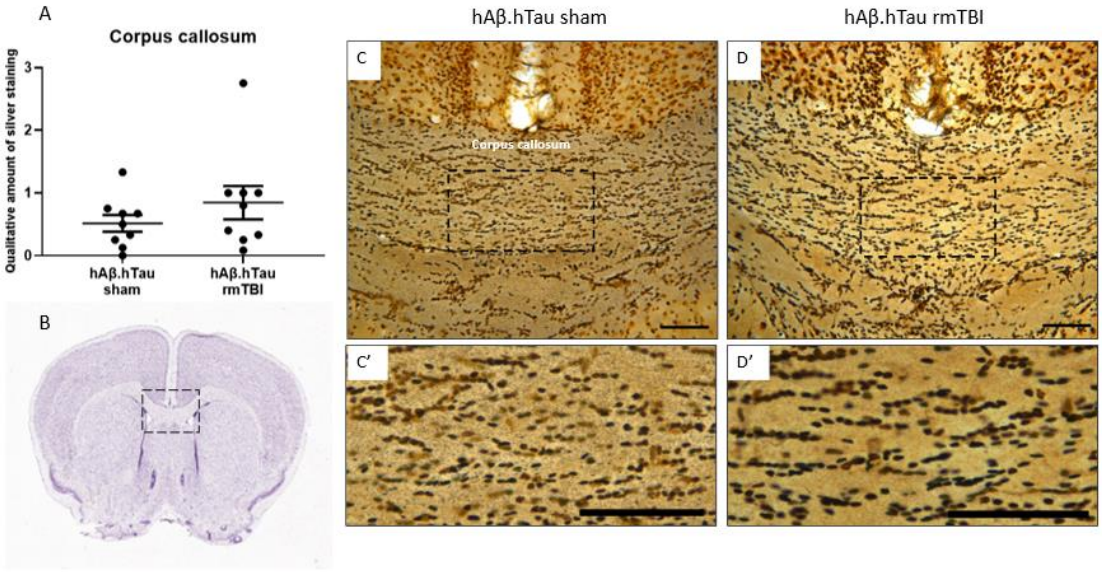


Figure 6. No evidence of diffuse axonal injury in the corpus callosum 1 week after rmTBI. Average qualitative amount of silver staining in corpus callosum of male and female hAβ.hTau sham and injured mice, scored by two blind observers (A). Representative picture of corpus callosum location in Allen Mouse Brain Atlas, P56 coronal (B). Few if any silver stained fibers were observed throughout the corpus callosum of sham (C,C') and injured mice (D,D'). Insets are high magnifications of black-boxed areas in the panel. Scale bar = 50μm. Animal numbers per group are as follows, half male/ half female: sham n = 9, rmTBI n = 9.

3.2 No acute performance deficits on the CPT in male C57BL/6 mice at 2- or 6-weeks post-injury

To evaluate if there were acute attentional deficits following rmTBI as have been observed with other mTBI models (Xu et al., 2021), sham and injured male C57BL/6 mice were assessed on the variable stimulus duration probe of the CPT. In this probe performance is assessed under baseline conditions and behaviourally challenging conditions (i.e., by shortening the stimulus duration from 2s to 1s, 0.5s and 0.2s). No differences were observed between sham and injured mice on CPT measures at 2- or 6-

weeks post-injury (**Figure 7**, 2wk: d': $F_{(1,31)}$=0.435, p =0.514; HR: $F_{(1,31)}$=0.436, p=0.514; FAR: $F_{(1,31)}$=3.45, 0.073; C: $F_{(1,31)}$=1.36, p=0.252; 6wk: d': $F_{(1,31)}$=0.005, p=0.945; HR: $F_{(1,31)}$=0.031, p=0.861; FAR: $F_{(1,31)}$=0.094, p=0.761; RB: $F_{(1,31)}$=0.002, p=0.965).

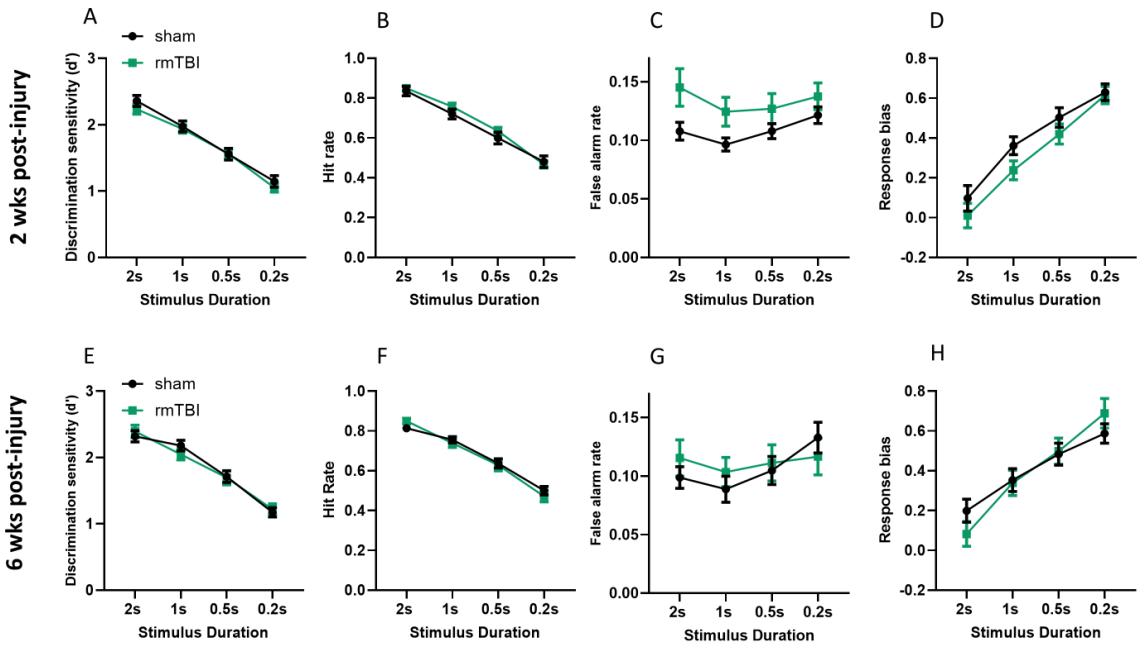


Figure 7. No acute performance deficits on the CPT in male C57BL/6 mice at 2 or 6 weeks post-rmTBI. Male C57BL/6 mice were assessed on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s at 2 and 6 weeks post-rmTBI; performance measures were averaged over four sessions. 2 weeks post-injury (A-D) and 6 weeks post-injury (E-H) discrimination sensitivity (A,E), hit rate (B,F), false alarm rate (C,G), and response bias (D,H). Animal numbers per group are as follows: sham n = 15, rmTBI n = 17. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparison test).

3.3 Reduced VAChT levels do not affect training or learning on the CPT

hAβ.hTau and hAβ.hTau.VAChTKD mice were trained on the CPT. All mice of both genotypes successfully completed stage three and stage four training of the CPT. During stage three training, with one target and one non-target, both hAβ.hTau (WT) and

hAβ.hTau.VAChTKD (VAChTKD) mice learned the task with no differences between genotypes over training days (**Figure 8A,B**; no main effect of genotype, male: $F_{(6,172)}$ = 0.871, p = 0.517; female: $F_{(6,360)}$ = 0.774, p = 0.591). Similarly, WT and VAChTKD mice showed no differences in stage four training in either males or females (**Figure 8C,D**; no main effect of genotype, male: $F_{(8,489)}$ = 1.405, p = 192; female: $F_{(8,475)}$ = 1.363, p = 0.210).

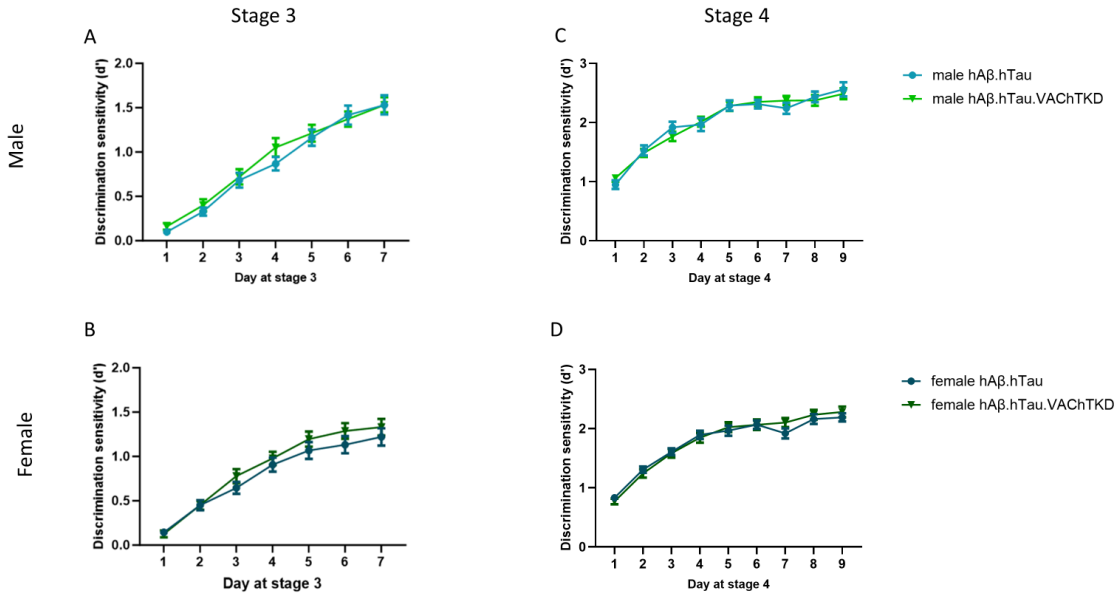


Figure 8. Reduced VAChT levels do not affect training on the CPT. CPT stage 3 and 4 discrimination sensitivity (d') over training days in hAβ.hTau (WT) and hAβ.hTau.VAChTKD (VAChTKD) mice. CPT training on stage 3 in males (A) and females (C). CPT training on stage 3 in males (A) and females (C). CPT training on stage 4 in males (B) and females (D). Animal numbers per group are as follows: male WT n= 34, male VAChTKD n=32, female WT n=30, female VAChTKD n = 33. All data are ± SEM, *p<0.05. (Two-way ANOVA)

Table 2. Summary of CPT results from long-term behavioural study

Time	Comparison	d'	Hit rate	False alarm rate	C	CCL
Baseline						
Male	WT vs VAChTKD	↓*	—	—	—	—
Female	WT vs VAChTKD	—	—	—	—	↑*
Male						
What is the effect of rmTBI in WT males?						
6-m pi	WT sham vs rmTBI	—	—	↓*	↑*	—
12-m pi	WT sham vs rmTBI	—	—	—	—	↑*I
What is the effect of rmTBI in VAChTKD males?						
6-m pi	VAChTKD sham vs rmTBI	—	—	—	—	—
12-m pi	VAChTKD sham vs rmTBI	—	—	—	—	—
What is the effect of low VAChT in uninjured males?						
6-m pi	WT vs VAChTKD sham	—	—	—	—	—
12-m pi	WT vs VAChTKD sham	—	—	—	—	—
What is the effect of low VAChT in injured males?						
6-m pi	WT vs VAChTKD rmTBI	↓*	—	↑**	↓a	—
12-m pi	WT vs VAChTKD rmTBI	—	—	—	—	↓*I
Female						
What is the effect of rmTBI in WT females?						
6-m pi	WT sham vs rmTBI	—	—	—	—	—
12-m pi	WT sham vs rmTBI	—	—	—	—	—
What is the effect of rmTBI in VAChTKD females?						
6-m pi	VAChTKD sham vs rmTBI	↓*0.2s	—	—	—	↑a
12-m pi	VAChTKD sham vs rmTBI	—	—	—	—	—
What is the effect of low VAChT in uninjured females?						
6-m pi	WT vs VAChTKD sham	—	—	—	—	—
12-m pi	WT vs VAChTKD sham	—	—	—	—	—
What is the effect of low VAChT in injured females?						
6-m pi	WT vs VAChTKD rmTBI	↓*I,0.2s	↓*I	—	—	↑a
12-m pi	WT vs VAChTKD rmTBI	↓**,0.5s	↓*,0.5s	—	—	—

Note. The table displays results of two-way ANOVA comparisons followed by Šidák's multiple comparison test where warranted. ↑ = increase; ↓ = decrease; — = no effect, comparisons are shown relative to first group in comparison. d' = discrimination sensitivity; C = response criterion; CCL = correct choice latency; WT = hAβ.hTau; VAChTKD = hAβ.hTau.VAChTKD, vesicular acetylcholine transporter knockdown; rmTBI = repetitive mild traumatic brain injury.

Statistics are given for main effect of genotype or injury unless otherwise noted.

* $p < 0.05$, ** $p < 0.01$, $*^{I}$ interaction is $p<0.05$, $*^{0.2s}$ post-hoc at stimulus duration is $p<0.05$, **a** $p >0.05$ and <0.1.

3.4 Reduced VAChT levels result in mild performance deficits at baseline

Prior to the first concussive injury at 6 months of age all mice underwent pre-injury testing on variable stimulus duration probe (VSD) of CPT. In this probe, performance is assessed under baseline conditions and behaviourally challenging conditions (i.e., by shortening the stimulus duration from 2s to 1s, 0.5s and 0.2s). VAChTKD mice showed mild performance deficits at baseline, particularly when attentional demand was high. Male VAChTKD mice exhibited poorer discrimination sensitivity (d'), the main measure of attentional function in the CPT, compared to WT males (**Figure 9A**; $F_{(1,62)} = 6.424$, $p = 0.0138$). This was driven by a tendency for higher false alarm rates (FAR) in the VAChTKD males that did not reach statistical significance (**Figure 9C**; $F_{(1,63)} = 2.27$, $p = 0.137$), while hit rates (HR) were the same between VAChTKD and WT mice (**Figure 9B**; $F_{(1,63)} = 0.865$, $p = 0.355$). Although there were no differences in response bias (C) (**Figure 9D**; $F_{(1,63)} = 0.067$, $p = 0.797$), VAChTKD males tended to make more touches to the screen during inter-trial interval perhaps indicating higher non-specific responding in general (data not shown).

WT and VAChTKD females did not differ on d', HR, FAR or C at baseline (**Figure 9F-I;** d': $F_{(1,62)} = 0.633$, $p = 0.429$; HR: $F_{(1,62)} = 0.552$, $p = 0.46$; FAR: $F_{(1,62)} = 0.065$, $p = 0.799$; $F_{(1,62)} = 0.0078$, $p = 0.929$). VAChTKD females did, however, have longer latencies to make a correct choice (correct choice latency: CCL) taking on average 0.6s more time to respond to target stimulus than control mice (**Figure 9J**; $_{(1,62)} = 6.001$, $p = 0.0171$). No differences were present between groups on mistake latency or reward retrieval latency (data not shown).

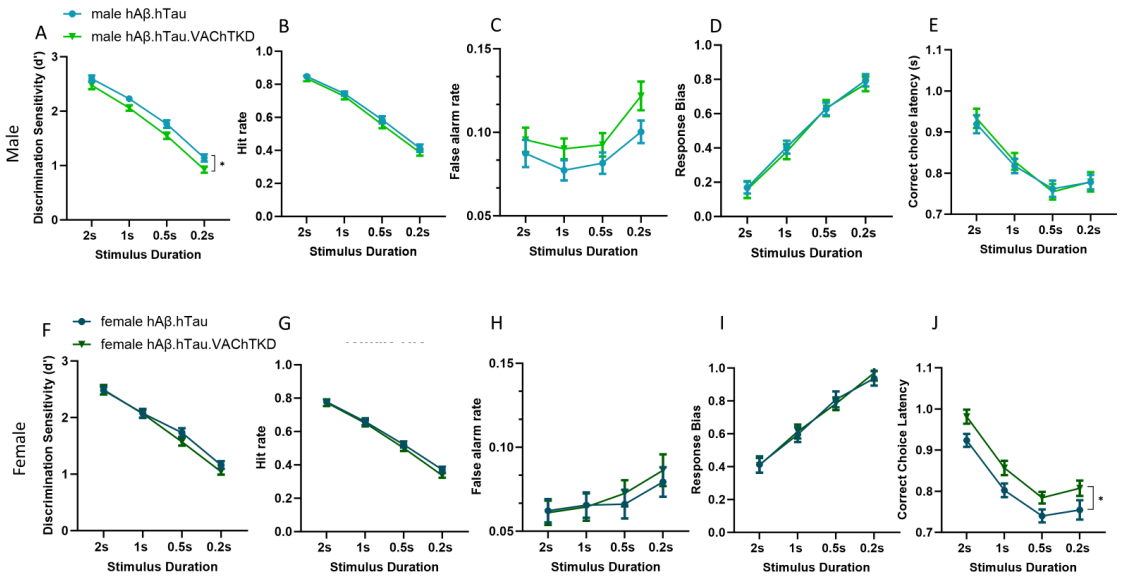


Figure 9. Mild deficits in VAChTKDs at baseline: Baseline CPT VSD probe performance at 6-month-old. Mice were assessed on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. Males (A-E) and females (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). hAβ.hTau (WT), hAβ.hTau.VAChTKD (VAChTKD). Animal numbers per group are as follows: male WT n = 34, male VAChTKD n = 31, female WT n = 31, male VAChTKD n = 33. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparison test).

3.5 rmTBI does not induce performance deficits in WT or VAChTKD male mice

To assess the long-term effect of rmTBI in WT and VAChTKD male mice, CPT performance was assessed between sham and injured males of both genotypes at 6- and 12- months post-injury. In WT males at 6 months post-injury, no differences were obeserved in the main measure of attentional functioning, d', or HR between sham and injured mice (**Figure 10A,B**; d': $F_{(1,28)} = 2.661$, p = 0.114; HR: $F_{(1,28)} = 0.074$, p = 0.787).

Compared to shams, WT injured males had lower FAR and more liberal responding, indicated by lower response bias (**Figure 10C,D**; FAR: $F_{(1,27)} = 4.944$, $p = 0.0347$; C: $F_{(1,28)} = 4.68$, $p = 0.0392$). At 12 months post-injury, no differences were observed between WT sham and WT injured males (**Figure 10F-I**, d': $F_{(1,28)} = 0.188$, $p = 0.668$; HR: $F_{(1,28)} = 0.197$, $p = 0.660$; FAR: $F_{(1,28)} = 0.490$, $p = 0.489$; C: $F_{(1,28)} = 0.939$, $p = 0.341$). The differences in FAR and C between WT sham and WT injured males observed at 6 months post-injury (**Figure 10C-D**) did not persist at the later timepoint.

Similarly, there was no effect of rmTBI in cholinergic deficient male mice. At 6 months post-injury, no differences were observed between VAChTKD sham and injured males on d', HR, FAR, C or CCL (**Figure 11A-E**; d': $F_{(1, 27)} = 0.00038$, $p = 0.951$; HR: $F_{(1, 27)} = 0.615$, $p = 0.439$; FAR: $F_{(1, 26)} = 0.025$, $p = 0.882$; C: $F_{(1, 27)} = 0.749$, $p = 0.394$). No changes were observed with aging at 12 months post-injury either (**Figure 11F-J**, d': $F_{(1,27)} = 0.624$, $p = 0.436$; HR: $F_{(1,27)} = 0.005$, $p = 0.944$; FAR: $F_{(1,27)} = 0.540$, $p = 0.469$; C: $F_{(1, 27)} = 0.263$, $p = 0.612$).

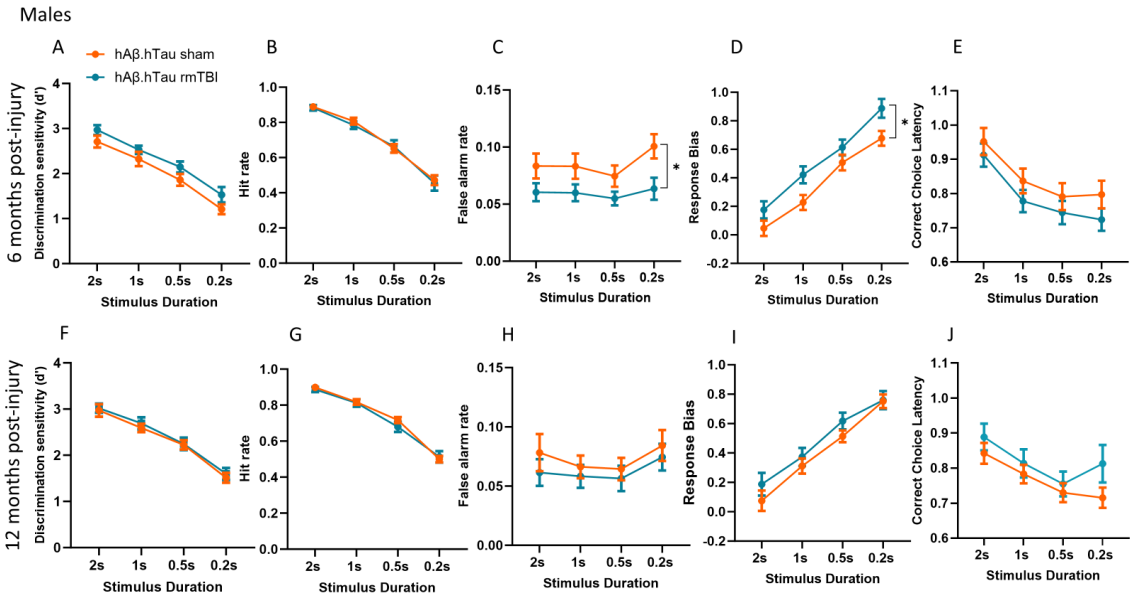


Figure 10. No CPT deficits in injured hAβ.hTau (WT) males: Effect of rmTBI on CPT in hAβ.hTau male mice. hAβ.hTau sham and hAβ.hTau rmTBI males were compared at 6- and 12- months post-injury on VSD probe with random presentation of

2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau sham n = 15, hAβ.hTau rmTBI n = 15. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test)

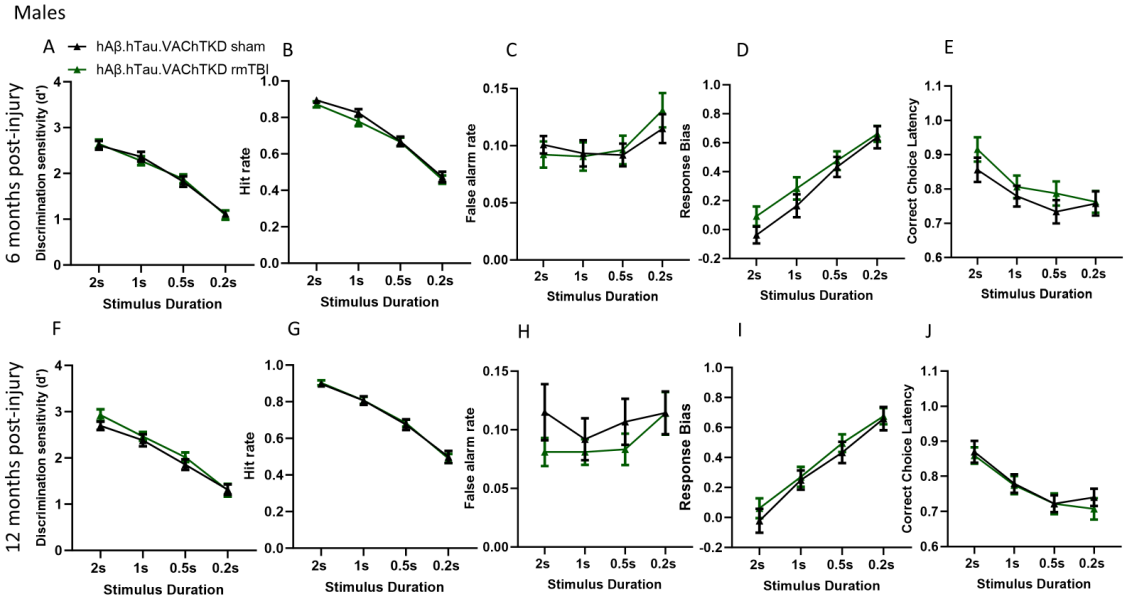


Figure 11. No effect of rmTBI on the CPT in cholinergic deficient mice: Effect of rmTBI in hAβ.hTau.VAChTKD male mice. hAβ.hTau.VAChTKD sham and hAβ.hTau.VAChTKD rmTBI males were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau.VAChTKD sham n = 15, hAβ.hTau.VAChTKD rmTBI n = 14. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test).

3.6 Reduced VAChT levels drive transient CPT deficits in injured males

To evaluate the impact of reduced VAChT levels on attentional functioning after rmTBI, CPT performance was compared assessed in sham and injured males of both genotypes at 6 months post-injury. There was no effect of reduced VAChT levels on CPT outcomes in the absence of rmTBI. At 6 months post-injury, no differences were observed between WT sham and VAChTKD sham males in d', HR, FAR or C (**Figure 12A-D,** d': $F_{(1,28)}$ = 0.086, p = 0.771; HR: $F_{(1,28)}$ = 0.150, p = 0.701, FAR: $F_{(1,26)}$ = 1.184, p = 0.286, C: $F_{(1,28)}$ = 0.606, p = 0.443). No differences were observed with aging at 12 months post-injury either (**Figure 12F-I**, d': $F_{(1,28)}$ = 3.535, p = 0.0705; HR: $F_{(1,28)}$ = 0.311, p = 0.581; FAR: $F_{(1,28)}$ = 2.351, p = 0.136; C: $F_{(1,28)}$ = 1.019, p = 0.321).

Conversely, in injured males, lower VAChT levels resulted in transient attentional impairments. Compared to injured WT males, injured VAChTKD males exhibited significantly lower d' at 6 months post-injury that recovered slightly by 12 months post-injury (**Figure 13A,F**, d': $F_{(1,27)}$ = 5.356, p = 0.0285; d': $F_{(1,27)}$ = 1.842, p = 0.186, respectively). This poorer discrimination was not due changes in response to target stimuli as there were no differences in HR (**Figure 13B**, $F_{(1,27)}$ = 0.0076, p = 0.931). Rather, FAR was the main driver of the lower d' observed in the injured VAChTKD males. With short stimulus duration injured VAChTKD males made significantly more mistakes (**Figure 13C**, main effect of genotype, $F_{(1,27)}$ = 9.81, p = 0.0041; interaction: $F_{(3,81)}$ = 6.64, p = 0.0005). *Post-hoc* analysis revealed a significant difference at 0.5 and 0.2s stimulus duration (95%Cl(-0.079,-0.0034)= -0.041, p = 0.029; 95%Cl(-0.116,-0.019) = -0.067, p = 0.0039, respectively). The higher FAR in the injured VAChTKD males but comparable HR suggests more liberal responding; indeed these males trended towards lower response bias (**Figure 13D**, $F_{(1,27)}$ = 3.139, p = 0.087). At 12 months post-injury these differences in FAR were diminished and there was no difference in HR or C (**Figure 13G-I**, HR: $F_{(1,27)}$ = 0.0057, p = 0.940; $F_{(1,27)}$ = 2.723, p = 0.110; C: $F_{(1,27)}$ = 1.782, p = 0.193). It should be noted that the injured VAChTKD males resembled their sham counterparts with essentially equivalent performance over both timepoints (Figure

10). The differences observed in the injured VAChTKD males are, therefore, likely solely an effect of reduced VAChT levels.

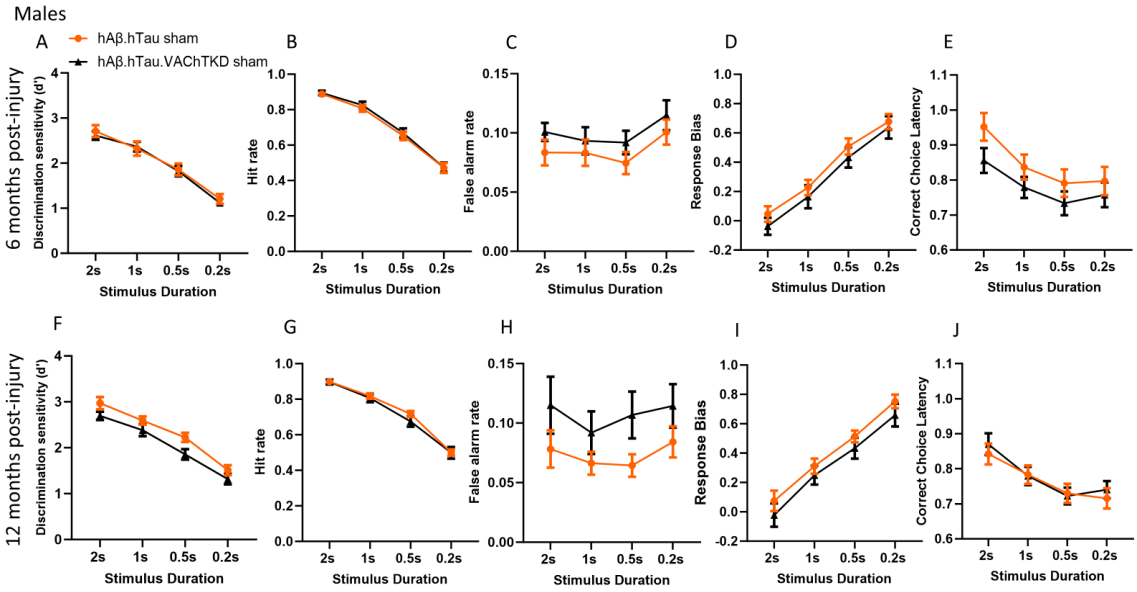


Figure 12. No effect of reduced VAChT levels with aging on the CPT in the absence of rmTBI: Effect of low VAChT in sham male mice. hAβ.hTau sham and hAβ.hTau.VAChTKD sham males were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau sham n = 15, hAβ.hTau.VAChTKD sham n = 15. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test).

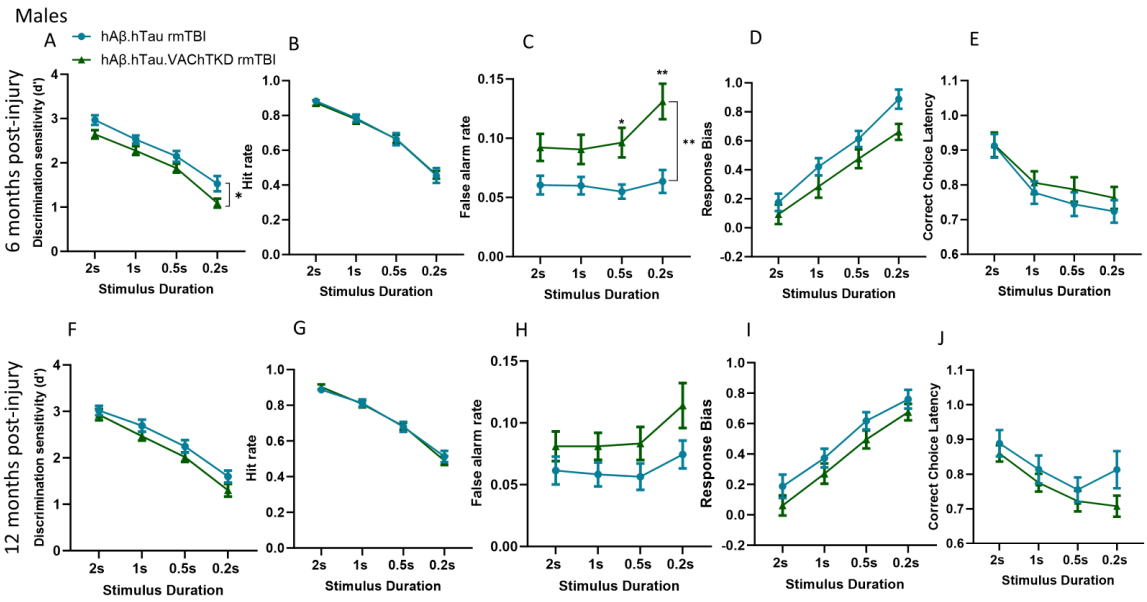


Figure 13. Reduced VAChT levels induce transient performance deficits on the CPT in injured male mice: Effect of low VAChT in injured male mice. hAβ.hTau rmTBI and hAβ.hTau.VAChTKD rmTBI males were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau rmTBI n = 15, hAβ.hTau.VAChTKD rmTBI n = 14. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test).

3.7 Mild deficits with rmTBI only in VAChTKD females

In females, to evaluate the effect of injury on the CPT sham and injured mice were compared in both WT and VAChTKD mice. In WT females, there was no effect of rmTBI. No differences were observed in d', HR, FAR, C or CCL at 6 months post-injury (**Figure 14A-E**; d': $F_{(1,25)}$ = 0.0294, p = 0.865; HR: $F_{(1,25)}$ = 0.452, p = 0.507; FAR: $F_{(1,25)}$ = 0.215, p = 0.647; C: $F_{(1,25)}$ = 0.550, p = 0.465). No deficits emerged with aging either

(**Figure 14F-J**, d': $F_{(1,24)} = 0.921$, p = 0.347; HR: $F_{(1,24)} = 2.397$, p = 0.135; FAR: $F_{(1,24)} = 0.0009$; p = 0.975; C: $F_{(1,24)} = 1.089$, p = 0.307).

In females with reduced VAChT levels, there was a small effect of rmTBI at 6 months post-injury. Female injured VAChTKD mice had lower d' at the shortest stimulus durations, although there was no main effect of injury (**Figure 15A**; $F_{(1, 28)} = 1.387$, p = 0.248), *post-hoc* analysis revealed significantly a difference at 0.2s compared to VAChTKD shams (95%Cl(0.012,0.424)= 0.218, p = 0.034). No differences were observed in HR, FAR or C (**Figure 15B-D**, HR: $F_{(1, 28)} = 0.011$, p = 0.916; FAR: $F_{(1, 28)} = 2.040$, p = 0.164; $F_{(1, 27)} = 0.700$, p = 0.409). These females tended to take longer to make correct responses, however it did not reach statistical significance ($F_{(1, 28)} = 3.848$, p = 0.059). With aging, the lower d' in injured VAChTKD mice remained but was no longer statistically significant (**Figure 15F**, d': $F_{(1,27)} = 1.028$, p = 0.319). Again there were no differences in HR, FAR or C at 12 months post-injury (HR: $F_{(1,27)} = 0.151$, p = 0.701; FAR: $F_{(1,27)} = 0.259$, p = 0.614; C: $F_{(1,27)} = 0.307$, p = 0.584).

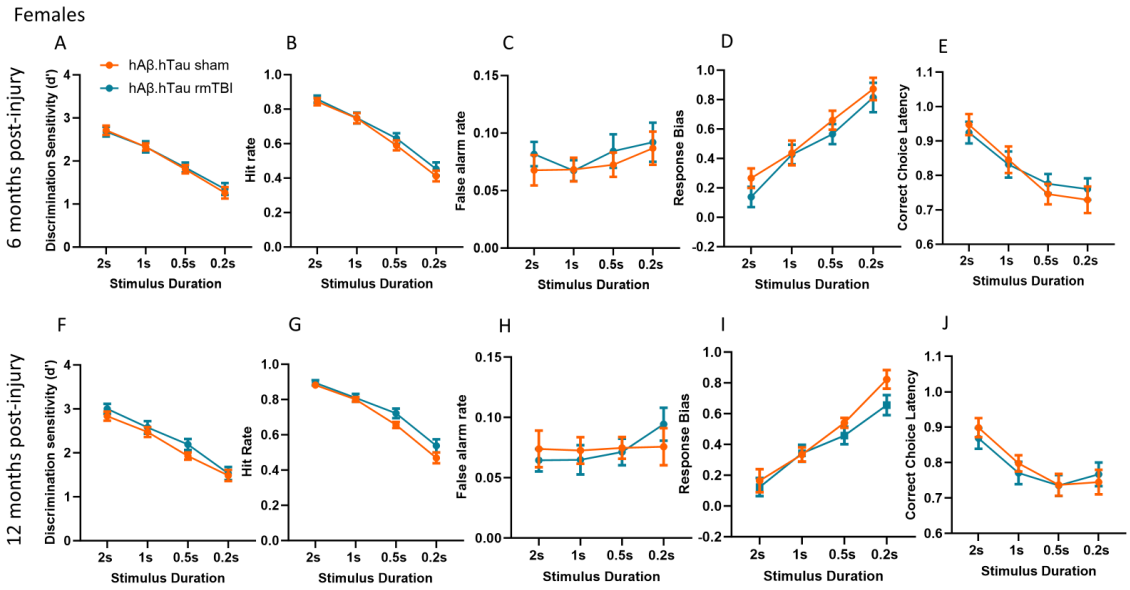


Figure 14. No effect of rmTBI on the CPT in hAβ.hTau female mice: Effect of rmTBI in hAβ.hTau female mice. hAβ.hTau sham and hAβ.hTau rmTBI females were compared at 6- and 12- months post-injury on VSD probe with random presentation of

2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau sham n = 15, hAβ.hTau rmTBI n = 12. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test)

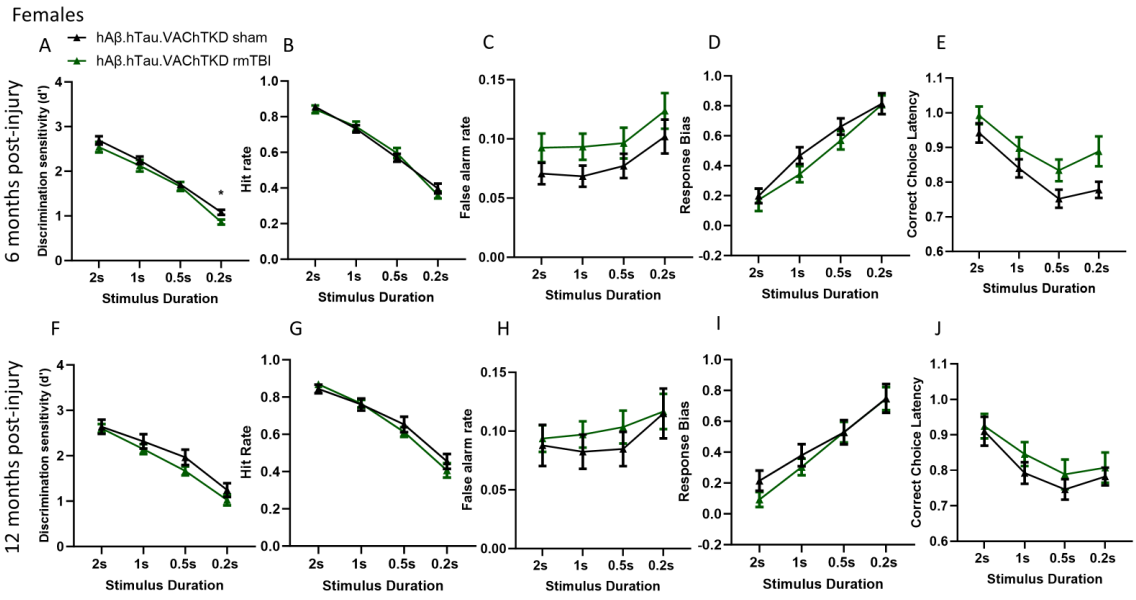


Figure 15. Mild deficits on the CPT in injured cholinergic deficient female mice: Effect of rmTBI in hAβ.hTau.VAChTKD female mice. hAβ.hTau.VAChTKD sham and hAβ.hTau.VAChTKD rmTBI females were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau.VAChTKD sham n = 15, hAβ.hTau.VAChTKD rmTBI n = 14. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test).

3.8 Reduced VAChT levels in females drive chronic CPT deficits in injured, but not sham mice

In females, the role of reduced VAChT levels in CPT performance in the presence and absence of rmTBI was assessed. In sham females, there was no effect of reduced VAChT levels. At 6 months post-injury, no differences in d', HR, FAR or C were observed between WT sham and VAChTKD sham females (**Figure 16A-D**, d': $F_{(1,28)} = 0.776$, p = 0.386; HR: $F_{(1,28)} = 0.108$, p = 0.745, FAR: $F_{(1,28)} = 0.152$, p = 0.699; RB: $F_{(1,28)} = 0.0795$, p = 0.780). No differences emerged with aging, at 12 months post-injury either (**Figure 16F-I**, d': $F_{(1,27)} = 0.623$, p = 0.437: HR, $F_{(1,27)} = 0.453$, p = 0.507; FAR: $F_{(1,27)} = 0.841$, p = 0.367; C: $F_{(1,27)} = 0.00067$, p = 0.979).

In contrast, in injured females' lower levels of VAChT drove persistent deficits that worsened from 6 to 12 months post-injury. At 6 months post-injury, compared to injured WT females, injured VAChTKD females had significantly lower d' (**Figure 17A**, main effect of genotype, $F_{(1,25)} = 3.09$, p = 0.091; interaction, $F_{(3,75)} = 3.714$, p = 0.0151*).* *Post-hoc* analysis revealed a significant difference at 0.2s stimulus duration (95%Cl(0.0655,0.908)= 0.487, p = 0.021). This difference was driven in part by lower HRs in the injured VAChTKD females compared to WT with reduced stimulus duration (**Figure 17B**, interaction: $F_{(3,75)} = 3.239$, p = 0.0268; no main effect of genotype: $F_{(1,25)} = 1.172$, p = 0.289). There was no differences between injured WT or injured VAChTKD females in FAR or C (**Figure 17C-D**, FAR: $F_{(1,25)} = 1.414$, p = 0.246; C: $F_{(1,25)} = 0.0217$, p = 0.874).

With aging the performance deficits worsened, with lower d' in injured VAChTKD females at 12 months post-injury (**Figure 17F**, interaction: $F_{(3,72)} = 0.534$, p = 0.660; main effect of genotype: $F_{(1,24)} = 9.21$, p = 0.0057), specifically at 0.5s (95%Cl(0.0939,0.9548) = 0.524, p = 0.0129). The lower d' in these mice was again driven mainly by lower HR at reduced stimulus durations (**Figure 17G**, interaction: $F_{(3,72)} = 3.675$, p = 0.016; main effect of genotype: $F_{(1,24)} = 7.136$, p = 0.0134). Again, the *post-hoc* analysis revealed a significant difference at 0.5s stimulus duration (95%Cl(0.0098,0.210) = 0.110, p = 0.0274). There was no statistically significant

difference in FAR between the injured WT and injured VAChTKD females (**Figure 17H**, $F_{(1,24)} = 3.249$, $p = 0.0840$). Similarly, response bias was the same between genotypes in the injured females (**Figure 17I**, $F_{(1,24)} = 0.107$, $p = 0.747$). Since reduced cholinergic activity drives attentional impairments in the injured females, but not the sham females, this suggests that reduced cholinergic activity amplifies the impact of injury, which does not have a discernable effect on its own.

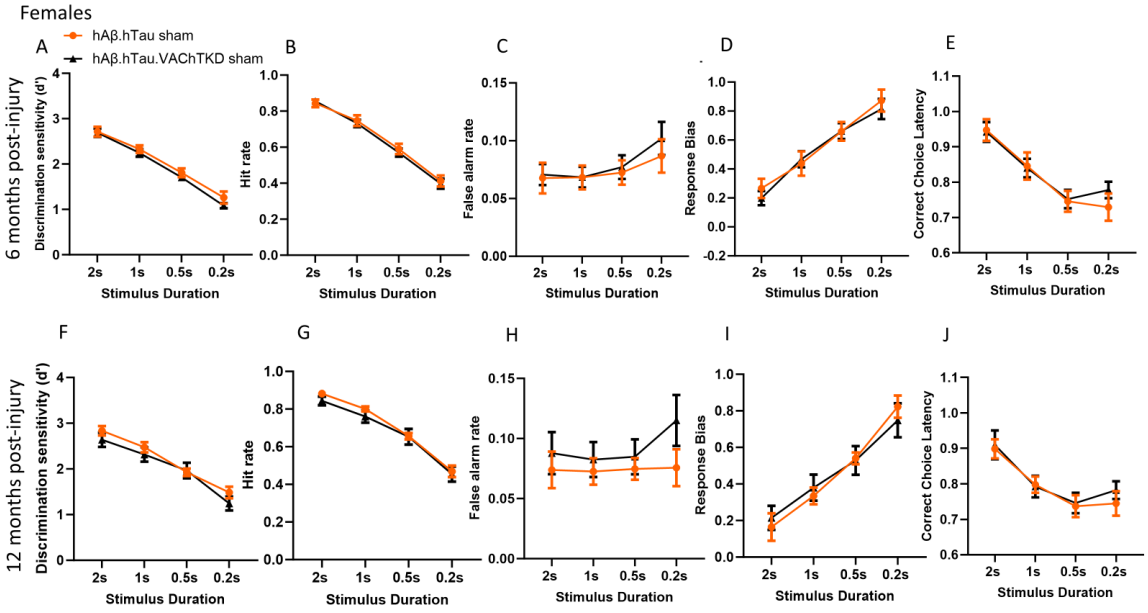


Figure 16. No effect of reduced VAChT levels with aging on the CPT in the absence of rmTBI: Effect of low VAChT in sham female mice. hAβ.hTau sham and hAβ.hTau.VAChTKD sham females were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau sham n = 14, hAβ.hTau.VAChTKD sham n = 15. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test).

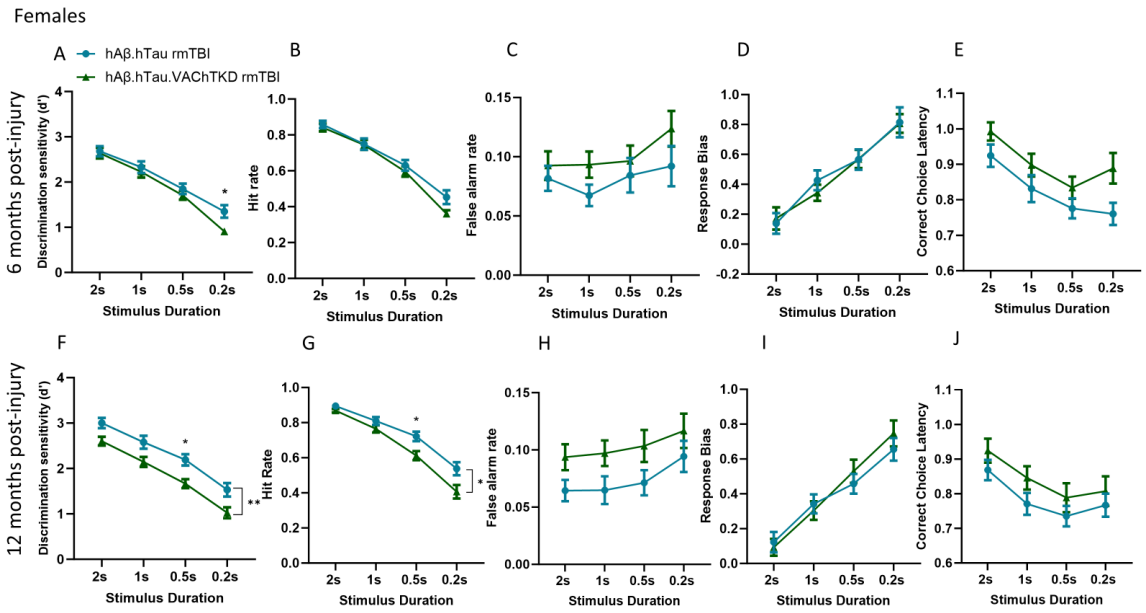


Figure 17. Reduced VAChT levels produce persistent attention deficits in injured females: Effect of low VAChT in injured female mice. Synergistic effect of reduced VAChT levels and rmTBI on d' and hit rate (E,F), but not reduced VAChT levels alone as seen in shams (A,B). hAβ.hTau rmTBI and hAβ.hTau.VAChTKD rmTBI females were compared at 6- and 12- months post-injury on VSD probe with random presentation of 2s, 1s, 0.5s and 0.2s; performance measures were averaged over four sessions. 6 months post-injury (A-E). 12 months post-injury (F-J). Discrimination sensitivity (A,F), hit rate (B,G), false alarm rate (C,H), response bias (D,I) and correct choice latency (E,J). Animal numbers per group are as follows: hAβ.hTau rmTBI n = 15, hAβ.hTau.VAChTKD rmTBI n = 14. All data are mean ± SEM, *p<0.05. (Two-way ANOVA, Šidák's multiple comparisons test)

3.9 Training effect over time is present in all groups except injured VAChTKD females

To assess attentional functioning with aging d' was compared from baseline testing to 6- and 12-months post-injury. In general, mice exhibited increases in d' over time, improving performance from baseline to 6 months post-injury and again from 6 months

post-injury to 12 months post-injury. This is consistent with a training effect on the specific task and probe (Shepherd, et al., 2021a). All male groups improved performance (increasing d') over time (**Figure 18A-D**, WT sham: $F_{(1.95,27.36)} = 11.34$, $p = 0.0003$; WT rmTBI: $F_{(1.81,25.31)} = 10.53$, $p = 0.0007$; KD sham: $F_{(1.618, 22.66)} = 3.864$, $p = 0.0438$; KD rmTBI: $F_{(1.508,19.61)} = 16.69$, $p = 0.0002$). WT females also improved over time (**Figure 18E-F**, WT sham: $F_{(1.89,24.6)} = 5.08$, $p = 0.015$; WT rmTBI: $F_{(1.44,15.81)} = 6.735$, $p = 0.0125$), while VAChTKD females in general showed less substantial improvements. VAChTKD sham females showed an attenuated increase in d' only showing improvements from baseline to 12 months post-injury at 0.5s (**Figure 18G**, no main effect of time, $F_{(1.29,18.0)} = 2.52$, $p = 0.124$, *post-hoc* analysis at 0.5s baseline – 12 month: 95%Cl(-0.810,-0.0189) = -0.415, $p = 0.0396$). Injured VAChTKD females made no improvements from baseline to 6- or 12-months post-injury testing (**Figure 18H**, $F_{(1.72,22.54)} = 1.84$, $p = 0.185$).

The general overall increases in d' score across all stimulus durations suggest that through repeated training and testing mice continue to make small improvements in task performance. It should be noted that these improvements occur despite animals reaching a plateau in performance prior to testing. The lack of improvements in the injured VAChTKD females specifically may indicate a failure to learn from testing. More likely it indicates a deficit in performance that counteracts any possible training effects and that the attention system in these mice is operating at its capacity.

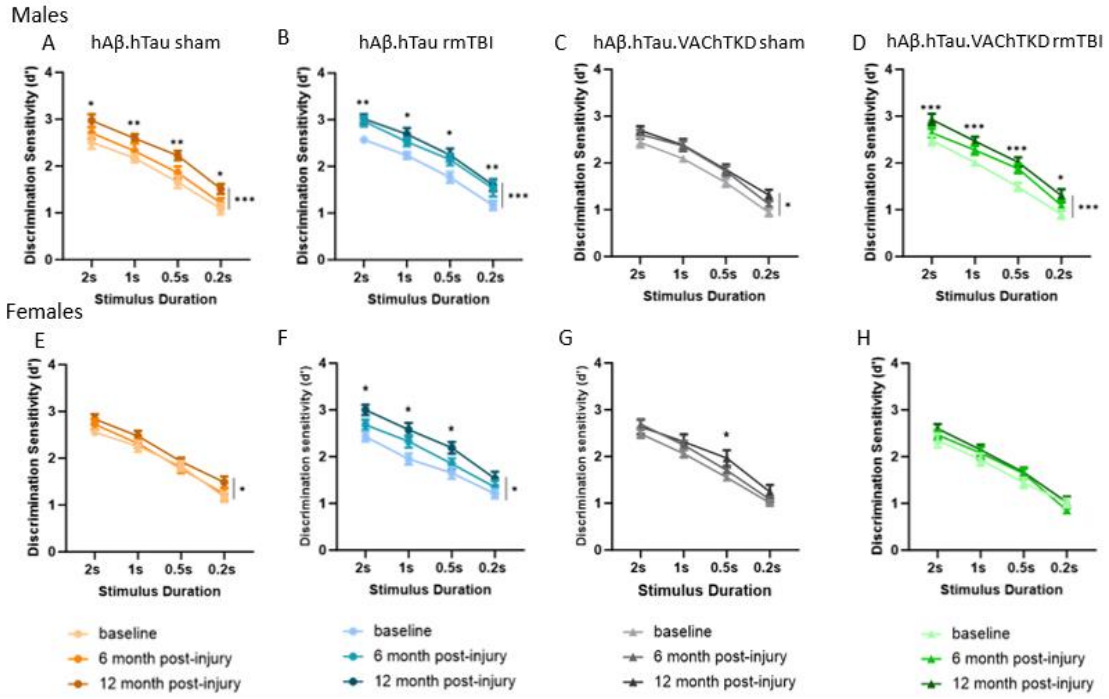


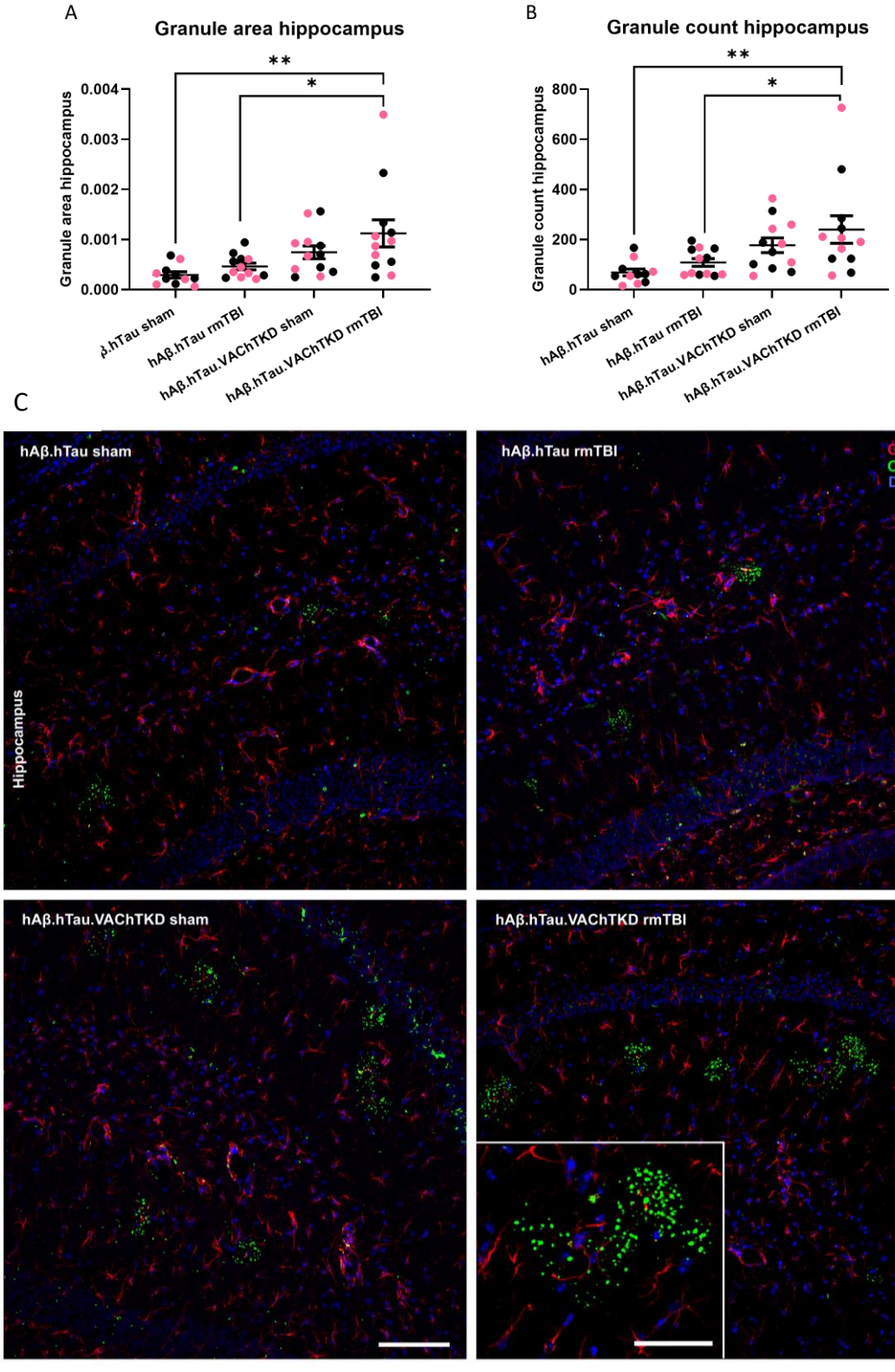
Figure 18. Training effect over time is present in all groups except injured VAChTKD females: CPT d' over time from baseline to 12-month post-injury in males and females. Males (A-D). Females (E-H). hAβ.hTau (WT) sham (A,E), hAβ.hTau rmTBI (B,F), hAβ.hTau.VAChTKD (VAChTKD) sham (C,G), and hAβ.hTau.VAChTKD rmTBI (D,H). Animal numbers per group are as follows: Males: WT sham n = 15, WT rmTBI n = 15, VAChTKD sham n =15, VAChTKD rmTBI n = 14., Females: WT sham n = 14, WT rmTBI n = 12, VAChTKD sham n =15, VAChTKD rmTBI n = 14. All data are mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

3.10 Injured VAChTKD mice have increased OC+ hippocampal granules at 6 months post-injury

Baglietto & colleagues (2021) report accelerated appearance of degenerative hippocampal OC+ granules with aging in the humanized Aβ mice which is further increased with the addition of AD mutations (Baglietto-Vargas et al., 2021). In 6-month post-injury mice these OC+ granules were assessed in the hippocampus and piriform cortex. Clusters of these OC+ granules increased in a step-wise manner from WT shams

to WT injured to VAChTKD shams to VAChTKD injured mice (**Figure 19C**). Granules were quantified throughout the hippocampus and total area and count were evaluated. VAChTKD injured mice exhibited significantly higher total area of granules in the hippocampus compared to WT sham and WT injured mice (**Figure 19A**, area: WT sham: 95%Cl(-0.0014,-0.0002)= -0.00008, p = 0.004; WT rmTBI: 95%Cl(-0.0012,-6.23^{-5})=-0.0006, p = 0.025). Similarly, count of granules was also increased in VAChTKD injured mice (**Figure 19B**, count: WT sham: 95%Cl(-298.8,-44.11) = -171.4, p = 0.004; WT rmTBI: 95%Cl(-256.1,-7.013) = -131.6, p = 0.035).

Granules were selectively found in hippocampus, piriform cortex and cortical amygdala regions as previously reported (Baglietto-Vargas et al., 2021; Manich et al., 2011) and appeared to be closely associated with astrocytic processes. Lee & colleagues (2022) recently reported a novel subtype of astrocytes that increase with age and are found elevated with aging in AD model mice. Found selectively in hippocampal regions, these astrocytes exhibit distinct morphology with swollen processes and loss of fine processes (Lee et al., 2022). Staining with s100β revealed astrocytes matching this morphology in the hippocampi of VAChTKD sham and VAChTKD injured mice (**Figure 20**C,D). Vacuolated processes were reminiscent of OC+ granules (**Figure 20**).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Granule count hippocampus
Granule count hippocampus
800
600
400
200
0
**
*
hAβ.hTau sham
hAβ.hTau rmTBI
hAβ.hTau.VAChTKD sham
hAβ.hTau.VAChTKD rmTBI
C
hAβ.hTau sham
hAβ.hTau rmTBI
GFAP
OC+
DAPI
Hippocampus
hAβ.hTau.VAChTKD sham
hAβ.hTau.VAChTKD rmTBI

Figure 19. Elevated OC+ hippocampal granules in injured VAChTKD mice at 6 months post-injury. Four-five coronal sections were taken from hippocampus regions through Bregma -1.15 to 3 for each mouse. Area and count were analyzed and averaged over images. Males and females are combined; females are represented by pink markers. Granule area in hippocampus (A). Granule count in hippocampus (B). Representative images from hippocampus for each group. OC+ granules (green), GFAP (red), DAPI (blue). Scale bar = 100 µm, scale bar in inset = 50 µm (C). Each group has n=6. One outlier excluded from female WT sham. All data are mean ± SEM, *p<0.05, **p<0.01. (One-way ANOVA, Tukey's multiple comparison test).

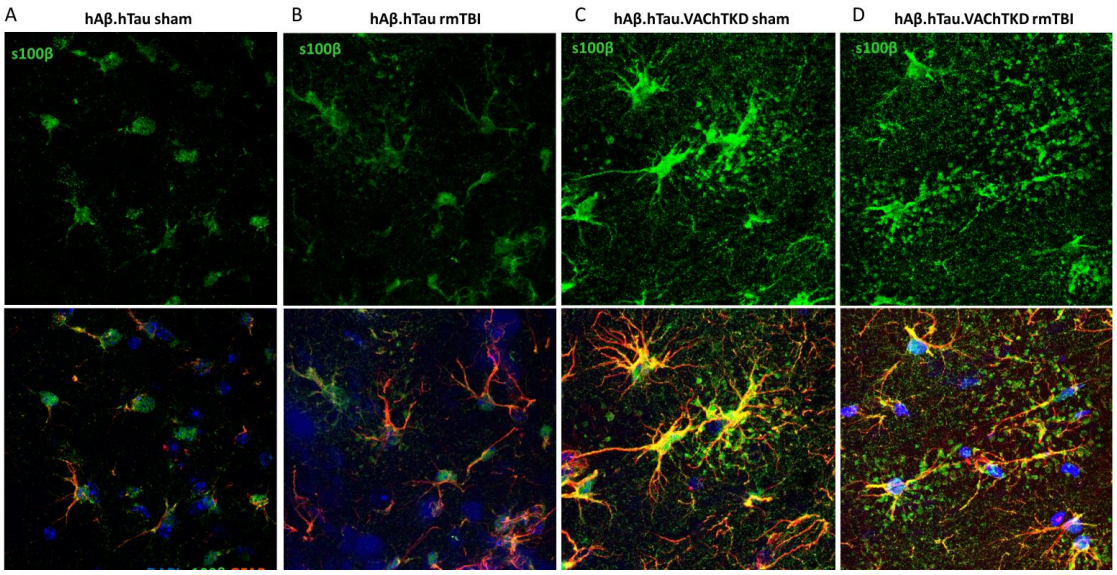

Figure 20. Vacuolated astrocytes in hippocampus of VAChTKD mice at 6 months post-injury. Representative immunofluorescent staining with s100β (green), GFAP (red) and DAPI (blue) at 6 months post-injury in the hippocampus. WT sham (A). WT rmTBI (B). VAChTKD sham (C), VAChTKD rmTBI (D). Scale bar = 50µm.

Chapter 4

4 Discussion

Mild traumatic brain injury (mTBI) increases the risk of later development of Alzheimer's disease (AD). The early and predictive nature of cholinergic dysfunction in AD (Kerbler et al., 2015; Schmitz & Spreng, 2016) and distinct cholinergic damage that occurs after TBI (Östberg et al., 2018, 2020; Salmond et al., 2005) led us to predict that this neural system plays a distinct role in vulnerability to AD development after TBI. The present study longitudinally examined the contribution of rmTBI and cholinergic dysfunction to cognitive deficits, mainly attention, and neurodegenerative associated pathology. Specifically, we were interested in the chronic consequences of mTBI and how it may trigger long-lasting consequences in the presence or absence of reduced cholinergic activity with aging. Following rmTBI reduced cholinergic tone drove attentional impairments and neurodegenerative associated pathology depending on time post-injury and sex.

This study used a newly developed model of rmTBI designed to recapitulate the forces experienced during human impacts with a moderate risk of concussion (Rowson et al., 2012). This model delivers a closed head injury with rotational force producing acute diffuse axonal injury and gliosis specific to the plane of principal strain induced by the rotational forces. The greatest axonal damage occurs in the anterior to posterior direction, encompassing tracts such as the optic tract. Cognition and neurodegenerative pathology were assessed at chronic time points, 6 and 12 months, following rmTBI.

Repetitive mTBI alone had no chronic effect on CPT performance even in the presence of increasing attentional demand. Reduced VAChT levels, and consequently ACh release, in injured mice resulted in specific attentional impairments. At 6 months post-injury, both males and female injured animals with reduced VAChT levels showed impaired performance during challenges to attention. Performance deficits were specific to sex, with female mice exhibiting deficits in hit rate (HR) and males exhibiting increased false alarm rate (FAR). With aging attentional deficits worsened in the females,

but not males. The performance of injured VAChTKD females did not improve from baseline testing to subsequent evaluations at 6- and 12-months post-injury, unlike the improvement observed in other groups. The finding that there is no effect of either rmTBI or reduced cholinergic activity alone on CPT performance while their combination results in impaired performance suggests that rmTBI either directly or indirectly compromises the cholinergic pathways required for CPT performance.

Only one other study has experimentally assessed whether TBI causes more severe cognitive decline in those with reduced cholinergic capacity (Cherian et al., 2019). Cherian & colleagues found that mice with reduced cholinergic capacity (heterozygous for choline transporter) receiving mild concussion injuries had more severe acute sustained attention impairments compared to mice with normal cholinergic system capacity. However, they only assessed attentional deficits during and immediately following injury and did not assess if there were chronic attentional deficits. The present study is the first to demonstrate the combined role of rmTBI and long-term cholinergic dysfunction in the development of chronic attention deficits and neurodegenerative related pathology with age.

4.1 Reduced VAChT levels result in mild deficits but not after extensive training

VAChTKD^{HET} mice have about 45% reduction in VAChT which corresponds with approximately one third reduction in acetylcholine release in projection regions, including the frontal cortex (Prado et al., 2006). An approximate 80% reduction in VAChT protein in the PFC results in distinct deficits in 5-CSRT task with higher omissions at reduced stimulus durations (Kolisnyk et al., 2013). It is unknown if a lesser degree of reduced cholinergic tone would similarly impair attention. On the VSD probe trial of the CPT, VAChTKD mice were able to maintain relatively normal performance at 6 months old. However, with attentionally challenging conditions they males exhibited subtle but detectable deficits. Males had lower d' primarily due to higher FARs. The VAChTKD females exhibited increased correct choice latencies, despite intact responding, suggesting they may have slower cognitive processing speeds (Romberg et

al., 2011). The relatively normal performance of the VAChTKD mice even under increasing attentional demand indicates that circuits involved in attention are sufficiently robust to withstand the challenge of reduced cholinergic activity (around 30%) and may indicate compensation within these circuits (Berry et al., 2015).

Additionally, aging did not further these mild impairments in the VAChTKD mice. In later testing at 12- and 18-months there were no differences between wildtypes and VAChTKD uninjured animals. Aging has been found to interact detrimentally with pre-existing damage to the cholinergic system (with about 40-60% decrease in cortical cholinergic fibers) in sustained attentional performance (Burk et al., 2002). Attentional performance on the SAT was not impaired in the rats with cortical cholinergic deafferentation until very old age. The lack of attentional deficits with aging in the VAChTKD mice may be due to maintenance training on the task which may have led to over-training in the mice, potentially mediating the age-related changes in neural systems involved in attention. This would diminish our capacity to detect differences in performance between the control group and VAChTKDs over time. In summary, the decrease in cholinergic tone achieved with a VAChTKD is not sufficient alone to produce pronounced attentional impairments even with aging.

Humans with reduced endogenous cholinergic capacity, such as those with variants in high-affinity choline transporter, exhibit equivalent performance to controls on sustained attention tasks and with attentional challenges (Berry et al., 2015b). These individuals are, however, more susceptible to distractors during attention tasks (Berry et al., 2014). During instances of high attentional demand, cholinergic activation of the right PFC is increased (St Peters et al., 2011). Interestingly, despite their similar performance on the distractor sustained attention task, individuals with the variant in choline transporter do not exhibit increased right PFC activity during attentional challenges unlike controls. To maintain performance during challenges to attention other brain regions may be more active, such as orbitofrontal and parahippocampal gyrus; additionally, these individuals may rely on different strategies during the task (Berry et al., 2015b). Thus, it is possible that VAChTKD mice relied more heavily on other regions to maintain performance despite reduced cholinergic signalling. These mice were also

highly trained and experienced on the CPT and therefore likely able to develop individual strategies for task performance.

4.2 Repetitive mTBI alone does not lead to attentional deficits with aging

In general, repetitive mTBI alone did not result in substantial chronic changes in CPT performance at 6- or 12-months post-injury in WT or VAChTKD mice. At 6 months post-injury injured WT males had transiently lower FAR and more conservative responding, however this difference did not persist at 12 months post-injury. A small effect of rmTBI on d' in VAChTKD females was also observed at 6 months post-injury. Attention has not been chronically assessed after mTBI in animal studies, thus it is unknown if other models of rmTBI lead to long-lasting attentional impairments. Chronic behavioural and cognitive symptoms, such as spatial learning and memory, locomotion and anxiety have been reported with several rmTBI models. Several of these injuries lead to chronic spatial learning and memory deficits at 6 months extending to 18 months post-injury (Gangolli et al., 2019; Mouzon et al., 2014, 2018). It should be noted that many of these studies only assessed males, however, missing potential important sex differences, which we clearly documented here.

The overall lack of attentional deficits from rmTBI alone support the notion that the injury is sub-threshold and does not cause enough functional damage to induce attentional deficits or that damage is not long-lasting. In a pilot study conducted in C57BL/6 male mice, injured mice did not exhibit any deficits on CPT acute timepoints of 2- and 6-weeks post-injury. The absence of deficits on the CPT at this short-time period after injury suggests that damage produced by this injury may not initially interfere with neural systems involved in performing the CPT, at least in males. Our lab has previously reported attentional deficits on the 5-CSRTT at 6 weeks after a fixed head injury with five mTBIs that induced particular axonal injury and inflammation in the corpus callosum and PFC (Xu et al., 2021). The model of mTBI used in the present study produces quite a different profile of damage, which may explain the lack of attentional deficits observed with this injury at acute timepoints. Further investigation is required to determine if this

injury results in long-lasting changes in white matter tracts or neuroinflammation as has been observed with other injury models (Chen et al., 2017; Mouzon et al., 2019; Xu et al., 2021).

4.3 Reduced cholinergic activity drives attentional deficits following rmTBI

Reduced VAChT levels induced selective CPT deficits after rmTBI that were not observed in uninjured mice. Impairments were observed at 6 months post-injury in males (lower d', higher FAR) and females (lower d', lower HR). Performance deficits persisted at 12 months post-injury in females (lower d' and HR). It should be noted that in the males the VAChTKD shams performed almost identical to injured VAChTKD mice, thus the difference observed in these males at 6 months post-injury can likely be primarily attributed to reduced VAChT levels and not rmTBI. The lower d' in the injured VAChTKD mice specifically at short stimulus durations, which are attentionally more challenging (Kim et al., 2015), provides a strong basis that the performance change is indeed an impairment in attention and not a general impairment in task performance. These findings indicate the synergistic effect of injury and reduced cholinergic capacity on the attention-related neural systems. Two possibilities could explain this combined effect: 1) injury directly affects the cholinergic attention system, or 2) injury impairs one of the compensatory circuits involved in attention.

The mild attention deficits in the VAChTKD mice at baseline indicate that the cholinergic system in these mice is taxed and likely close to the threshold where increased damage would be sufficient to produce deficits. It is possible that the preexisting cholinergic dysfunction in the VAChTKD mice impaired recovery following TBI, as has been reported following more severe TBI (Conners et al., 2005). Although the mTBI in this study is more consistent with triggering slowly developing pathology rather than considerable initial structural damage that cholinergic projections might facilitate with recovery.

Another potential mechanism underlying attentional impairments in the injured VAChTKD mice is dysfunction in the prelimbic cortex. The prelimbic cortex within the

medial PFC is engaged in attentional control processes that involve detecting and discriminating complex visual stimuli among multiple non-target stimuli (Fisher et al., 2020). This is supported by evidence from rats with lesions in the prelimbic cortex, showing reduced d' and HR on the CPT across various challenging conditions (Fisher et al., 2020). Frontoparietal cortical networks between prefrontal cortex and posterior parietal cortex have been implemented in top-down attentional control (Corbetta & Shulman, 2002). The cholinergic system modulates activity in frontoparietal cortical networks in the control of attention, facilitating a state of readiness to prepare networks for target detection (Ljubojevic et al., 2018). Reduction of cortical ACh impairs target detection and reduces readiness-associated increases in activity in the parietal cortex and synchrony between prelimbic cortex and posterior parietal cortex (Ljubojevic et al., 2018). Therefore, the attention deficits observed in VAChTKD injured mice may be related to decreased cholinergic modulation of frontoparietal networks during task performance. The model of rmTBI used in this study induced particular axonal damage and dysconnectivity in the anterior to posterior direction. Given that connectivity between the prelimbic frontal cortex and posterior parietal cortex in frontoparietal cortical networks is predominantly anterior to posterior (see Allen mouse Brain atlas), it is plausible that the injury affected connections between these regions. The synergy of reduced cortical ACh levels and possible damage in frontoparietal cortical networks, crucial for signal detection, may have contributed to the observed attention deficits.

4.4 Sex specific deficits in attentional processes under attentionally challenging conditions

At 6 months post-injury injured VAChTKD mice exhibited sex-dependent deficits in attentionally challenging conditions. Specifically, females had lower d’ scores mainly due to lower HR while males had lower d’ due to higher FAR, but no differences in HR. This suggests that in attentionally challenging conditions females may exhibit decreased vigilance, indicated by lower HR, while males may have specific deficits in response inhibition, exhibited by higher FAR. This is consistent with previous literature suggesting differences in how the sexes perform attentional tasks. For example, in challenging conditions females are more likely to make errors of vigilance while males are more

likely to make errors of inhibitory control (Bayless et al., 2012; Jentsch & Taylor, 2003). Sex differences in attentional performance are known to disappear with extended training (Bayless et al., 2012) which is consistent with the relative lack of sex differences observed at baseline and 1 year testing. However, it seems these differences may become apparent with attentional impairments, with deficits emerging in line with tendencies in how each sex responds to attention-demanding tasks.

Females exhibited persistent deficits at 12 months post-injury while males did not. Males exhibited a lack of deficits, despite some evidence of increased neurodegenerative pathology. In AD, considerable evidence suggests that females progress more quickly to more severe cognitive deficits than males (Filon et al., 2016; Irvine et al., 2012). Attentional processes in cholinergic deficit females may be particularly vulnerable following injury, while males may be more resilient to damage with injury.

4.5 Absence of training effect on the CPT in injured VAChTKD females

The performance of WT and injured VAChTKD females was comparable at baseline before injury; however, subtle differences began to manifest at 6 months and became more pronounced at 12 months post-injury. Persisting deficits indicate enduring damage associated with injury in these mice, which does not ameliorate over time. These differences can be primarily ascribed to the divergence in training effects between the two groups, as WT mice exhibited performance improvement over time, whereas injured VAChTKD mice did not. Presuming that all mice experience some level of training enhancements, as observed in the other groups and other studies (Kent et al., 2018; Shepherd et al., 2021a), the lack of improvement in VAChTKD females implies that the attention system's rate of decline may offset any positive effects from training. Mehla & colleagues found that cognitive training, specifically in multiple domains, preserved cognitive performance and cholinergic function in AD model mice (Mehla et al., 2023). Cognitive reserve- the brain’s resilience or ability to cope with increasing damage while still maintaining normal cognitive functioning - is similarly known to slow the development and progression of cognitive deficits in humans, thus individuals with high

cognitive activity are less susceptible to age-related cognitive decline (Moretti et al., 2012).

CPT training and maintenance is a robust form of cognitive training. Thus, touchscreen training may have preserved cholinergic function and prevented other degenerative pathology in the injured VAChTKD mice allowing them to maintain task performance over time. Repeated training and testing may also have facilitated compensatory changes in neural networks or alternated task strategy masking phenotypes (Kent et al., 2018). Unlike the injured VAChTKD females, the male injured VAChTKD mice did improve d' performance over time. This improvement suggests the cholinergic attentional system in males may be more resilient to damage with injury.

4.6 Neurodegenerative-associated pathology

With aging, humanized Aβ mice exhibit increased degenerative hippocampal OC+ granules (Baglietto-Vargas et al., 2021). These granules are also found to a lesser degree in piriform and entorhinal cortex and cerebellum (Jucker et al., 1994). These clusters of hippocampal granules correspond with the age-related periodic-acid-Schiff (PAS) granules originally described by Lamar et al., (1976) and have been further identified in a wide range of aged animals (Manich et al., 2016). Ultrastructural analysis of PAS granules has revealed degenerating fragments of organelles, other cellular material and breakdown products from neurons and astrocytes (Manich, Cabezón, et al., 2014). Age-related accumulation of granules is thought to induce degeneration of cholinergic and GABAergic basal forebrain projecting neurons to granule containing regions-hippocampus, entorhinal and piriform cortex (Madhusudan et al., 2009).

In the present study, compared to mice with normal levels of VAChT, both male and female VAChTKD mice demonstrated elevated levels of these hippocampal granules at 12-months of age. Further, injured female and male VAChTKD mice had elevated hippocampal granules and clusters at 6 months post-injury, while no significant changes were observed in WT sham or injured mice. Reduced VAChT levels and rmTBI appear to have an additive effect on granule formation as injury induces a slight increase in the granules, while reduced VAChT levels induce a more pronounced increase.

Consequently, injured VAChTKD mice exhibit the combined effect of both. Accelerated accumulation of PAS granules with aging has also been reported in mouse models of neurodegeneration and AD, including in 3xTg-AD, ApoE-deficient, and PS19 tau transgenic mice (Manich et al., 2016; Oddo et al., 2003; Robertson et al., 1997; Wander et al., 2022). ApoE4 the strongest risk factor for AD also increases granules in the hippocampus (Wander et al., 2022). PAS granules have been proposed as the murine analogue of corpora amylacea, the carbohydrate rich waste deposits that accumulate in early stages of AD (Wander et al., 2020, 2022).

Although the accelerated formation of PAS granules is not specific to AD model mice, the close association with AD-related proteins, such as amyloid, tau and ApoE suggests that these granules may be sensitive markers of neurodegenerative processes in the aging mouse brain. The increase in these granules, specifically in injured VAChTKD mice, further contributes to the notion that PAS granules represent a marker of brain vulnerability. Additionally, the particular vulnerability of injured VAChTKD mice to accumulation of these hippocampal granules parallels the findings from behavioural testing that rmTBI and VAChTKD alone are insufficient to induce considerable effects. However, their combination accelerates the progression of age-related pathology.

While previous studies have reported the presence of various proteins within these granules, such as Aβ, tau, reelin and alpha-synuclein, it is now recognized that they contain neo-epitopes with natural IgM auto-antibodies. This can lead to false positive staining with antibodies containing trace amounts of IgM antibodies (Manich, Del Valle, et al., 2014; Manich et al., 2015). Given that many commercial antibodies contain small amounts of IgM antibodies, particular care is necessary to confirm the presence of particular proteins within these granules. As a result, the protein constituents of these granules remain poorly characterized (Wander et al., 2020). Further, the exact function and origin of these granules is still unclear. However, the granules are closely associated with astrocytes and have been postulated to originate from astrocytes (Manich, Cabezón, et al., 2014).

Lee and colleagues (2022) have recently identified a novel subtype of vacuolated astrocytes localized specifically within the hippocampal layers, stratum radiatum and stratum lacunosum. This distinctive astrocytic subtype, referred to as autophagy-dysregulated astrocytes, is notably associated with aging and neurodegenerative diseases. These autophagy-dysregulated astrocytes have impaired protein trafficking and secretion, accumulation of autophagosomes in vacuolated swollen processes and failure to regulate nearby synapse formation and elimination. Decreased mammalian target of rapamycin (mTOR) or proteasome and lysosomal activities contribute to the generation of these astrocytes (E. Lee et al., 2022). Vacuolated astrocytes exhibiting the same characteristic swollen processes were observed specifically in hippocampi of VAChTKD mice. These swollen processes exhibit the same profile of staining as hippocampal granules suggesting OC+ granules are astrocytic vacuoles containing accumulated autophagosomes. Further experiments are necessary to confirm this theory and quantify the levels of these autophagy dysregulated astrocytes in the VAChTKD mice. If the OC+ granules are indeed astrocytic vacuoles, their increase in injured VAChTKD mice may indicate impaired autophagy and accelerated neurodegenerative processes within these mice.

Dysregulation of autophagy, through hyperactivation of mTOR, occurs early in the progression of AD, at the stage of MCI (Perluigi et al., 2021; Tramutola et al., 2015). Dysregulation of autophagy has also been reported after TBI, where increase in autophagic proteins occurs due to failure to clear autophagosomes (Sarkar et al., 2014). Cholinergic α7nAChR activation is known to increase autophagy and reduce proinflammatory cytokine release (Su et al., 2022). As VAChTKD mice have reduced cholinergic signalling and thus reduced α7nAChR activation, they may have impaired autophagy pathways.

4.7 Corresponding neurodegenerative pathology and attention deficits

The present study unveils intriguing insights into the interplay between rmTBI and cholinergic deficits, particularly in the context of neurodegenerative pathology and

attentional deficits. Injured VAChTKD mice exhibited the most substantial and persisting attentional deficits and degenerative-associated pathology. Reduced VAChT levels appears to be the primary driver of these effects while rmTBI may exacerbate vulnerability already present in the VAChTKD mice. The increases in PAS hippocampal granules and attentional deficits likely involve distinct pathways. Therefore, the similar findings in both these areas may indicate widespread alterations in pathways and systems in the injured VAChTKD mice, perhaps beyond what was observed with touchscreen testing.

4.8 Limitations and future directions

4.8.1 Longitudinal touchscreen training and food restriction

This study used longitudinal touchscreen testing which is ideal for comparison of the animal's performance over time and it also ensures equivalent baseline performance prior to injury. However, repeated touchscreen testing necessarily requires continued cognitive training and food restriction which may affect development or progression of cognitive deficits and neuropathology (Kent et al., 2018; Shepherd, et al., 2021a). Although all mice receive training it is possible that training or environmental enrichment through the touchscreens does not have equal affect in all the groups. For example, environmental enrichment reduces neurodegenerative processes following TBI and improves spatial learning and memory (Tapias et al., 2022). Touchscreen training can also act as a cognitive enhancer promoting plasticity and neurogenesis which could improve cognition or mask impairments (Shepherd et al., 2021b; Zeleznikow-Johnston et al., 2023).

Mice were also maintained on food restriction to motivate performance on the touchscreen testing; thus, they experienced caloric restriction from the beginning of training at 3 months of age. Caloric restriction increases rodent longevity and has multiple neuroprotective effects in the brain, including increased neurotrophin expression, enhanced neurogenesis and reduced AD pathology (J. Lee et al., 2002; Patel et al., 2005). Similarly, the caloric restriction mimic, resveratrol, has been shown to have neuroprotective effects, including reducing phospho-tau and PAS hippocampal granules (Porquet et al., 2013). If caloric restriction ameliorated pathology in high-risk groups,

such as VAChTKD injured animals who exhibit increases in PAS granules at 6-months post-injury, it may have prevented more advanced pathology or brain dysfunction. To clarify the role of cognitive training and caloric restriction in mediating cognitive decline and neurodegeneration it would especially interesting to examine mice of the same age but that had not received touchscreen training over their life.

4.8.2 Preclinical modeling of mTBI

This study found no chronic direct effects of rmTBI, but only effects when combined with genetic risk, in this case low cholinergic capacity. Modeling mTBI in rodents continues to be challenging for several reasons including the vast heterogeneity of human mTBIs that make symptoms and resulting pathology widely variable (Lipton et al., 2012). To produce a repetitive injury a rotational CHI was delivered three times 24 hours apart. The number of injuries may not compare to someone who has, for example, experienced several concussions and many sub-concussive injuries over their lifetime. Additionally, all the injuries were administered in exact same way which is likely not comparable to a human who likely experiences several injuries with differing mechanisms and directions of impact (Forbes et al., 2012). Both quantity, distance apart and variation in mechanism and directional forces of the injuries are important considerations in understanding the implications of this study and designing injury models in the future.

4.8.3 Considerations for the use of animal models in AD research

This study was designed with the aim of evaluating the progression of AD-related cognitive deficits in a model that presented several risk factors of late-onset AD without genetic manipulation directly causing AD. One of the challenges with modeling late-onset AD in mice is the lack of development of amyloid-plaques and tau tangles (Baglietto-Vargas et al., 2021; Saito et al., 2019). Despite increases in soluble and insoluble Aβ and phospho-tau the humanized versions of these proteins without mutations do not develop late-stage pathological hallmarks of AD. It is possible that mice either lack certain physiological factors that are critical in the development of these proteinopathies or there still remains insufficient aging in the mice to produce these pathologies.

NIA-funded consortia established with the goal of providing late-onset AD preclinical models are targeting AD modelling with three fundamental aspects, 1) aging timeline that models human onset and progression, 2) genetic appropriate variants and, 3) environmental factors that result in neuropathological and functional changes in humans (Reagan et al., 2022). This study took into consideration many of these aspects, however, as with all neurodegenerative rodent models it was not able to recapitulate all aspects of the disease. Combining important genetic risk variants such as ApoE4 and Trem2, implementing vascular dysfunction either through genetics or comorbidities and introducing other environmental factors, such as high-fat high-sugar diet may be helpful in future disease models to drive AD-phenotypes (Reagan et al., 2022).

Another consideration is the aging timeline in this study. CPT probes were run at baseline prior to injury when mice were around 6 months old and then at 12 and 18 months old. As an increase in the accumulation of insoluble Aβ and decrease in synaptic puncta is just beginning around 18 months of age in the humanized Aβ mice (Baglietto-Vargas, et al., 2021) it may have been valuable to age the mice past this point before running the final probe. AD requires decades to develop and there is evidence to suggest that the contribution of mTBI to AD development is mainly in those over the age of 75 (Gardner et al., 2014). Testing at a very old age in the mice would allow confirmation of whether wildtype mTBI or VAChTKD sham mice would develop deficits or if cognitive deficits would expand in injured VAChTKD mice.

4.8.4 Amyloid and tau pathology within the context of this model

Amyloid and tau pathology were not directly assessed within the context of this thesis, but would be important next steps considering the inclusion of humanized amyloid and tau genes in these mice. Although amyloid plaque pathology has not been observed in hAβ mice without the addition of AD-related mutations (i.e., in *PSEN1*), the hAβ mice do exhibit age-dependent increases in insoluble Aβ (Baglietto et al., 2021). Cholinergic deficient mice, such as mice with VAChT knockout in the basal forebrain, have alterations in amyloid processing and increased levels of soluble Aβ despite no changes in insoluble Aβ (Kolisnyk et al., 2017). The lack of any insoluble Aβ may be due to the

reduced ability of wildtype murine Aβ to aggregate like human Aβ. Thus, with the background of hAβ in our model it would be predicted that cholinergic dysfunction and rmTBI in combination would increase insoluble Aβ. Similarly, cholinergic deficient mice also have increases in phospho-tau, specifically ptau Thr231 (Kolisnyk et al., 2017). Assessing the role of rmTBI in combination with mild cholinergic dysfunction in the development of tau pathology will be an important next step.

4.8.5 Implications for humans with genetically or drug-induced reduced cholinergic capacity

Variants in cholinergic proteins can lead to reduced endogenous cholinergic capacity in humans. For example, the Ile89Val variant of choline transporter gene *SLC5A7*, around a 6% prevalence in Caucasian groups, results in approximately 40-60% decreased rate of choline transport (English et al., 2009; Okuda et al., 2002). These individuals exhibit mild deficits in attention, particularly with high attentional demand and in the presence of distractors (Berry et al., 2014). Variations also occur in the VAChT gene *SLC18A3* that in rare homozygotes result in neuromuscular dysfunction, however heterozygotes for these variants appear largely unaffected (O'Grady et al., 2016; Stankiewicz et al., 2012). Although the frequency of such variants, including copy number variants, in the population is unknown these individuals may be at higher risk for developing attentional deficits. Additionally, anticholinergic drugs which typically act as nicotinic or muscarinic cholinergic receptor antagonists, similarly reduce cholinergic activity (Chew et al., 2008; Gosens & Gross, 2018). Considering the increased risk observed with mTBI in combination with reduced cholinergic tone in the present study particular care should be taken in those with history of mTBI to minimize cholinergic dysfunction. Indeed, many of the drugs used in older adults to treat various conditions exhibit anticholinergic activity, however alternative drugs are often available that do not have anticholinergic activity (Chew et al., 2008). Studies investigating dementia risk in individuals with the Ile89Val variant or long-term anti-cholinergic users accounting for history of TBI would be a next step.

Chapter 5

5 Conclusion and relevance

In conclusion, this study used a closed head injury that induces diffuse axonal injury largely attributable to the strain produced by the rotational forces in the injury. This study demonstrates that rmTBI in the presence of reduced cholinergic tone leads to attentional deficits on the CPT. Sex-specific deficits in VAChTKD injured mice were observed, with females displaying reduced attentiveness and signal detection, while males exhibited deficits in response inhibition. Concurrently, there was an increase in degenerative PAS granules in the hippocampus at 6 months post-injury, indicating chronic effects on executive function and neurodegenerative-related pathology in cholinergic deficient mice following rmTBI. The lack of cognitive and pathological differences between VAChTKD sham and injured animals highlights that the relative importance of cholinergic dysfunction is greater than rmTBI in inducing cognitive and molecular dysfunction. These findings underscore the potential risks for individuals with endogenous low-capacity cholinergic systems in developing attentional impairments post-mTBI. Additionally, caution is warranted for individuals with a history of TBI when considering the use of anti-cholinergic drugs, as they may exacerbate neurodegenerative processes (Fox et al., 2014; Gray et al., 2015).

References

Abu Hamdeh, S., Waara, E. R., Möller, C., Söderberg, L., Basun, H., Alafuzoff, I., Hillered, L., Lannfelt, L., Ingelsson, M., & Marklund, N. (2018). Rapid amyloid-β oligomer and protofibril accumulation in traumatic brain injury. *Brain Pathology*, *28*(4), 451–462. https://doi.org/10.1111/bpa.12532

Ago, Y., Koda, K., Takuma, K., & Matsuda, T. (2011). Pharmacological aspects of the acetylcholinesterase inhibitor galantamine. In *Journal of Pharmacological Sciences* (Vol. 116, Issue 1). https://doi.org/10.1254/jphs.11R01CR

Al-Onaizi, M. A., Parfitt, G. M., Kolisnyk, B., Law, C. S. H., Guzman, M. S., Barros, D. M., Stan Leung, L., Prado, M. A. M., & Prado, V. F. (2017). Regulation of cognitive processing by hippocampal cholinergic tone. *Cerebral Cortex*, *27*(2), 1615–1628. https://doi.org/10.1093/CERCOR/BHV349

Apolipoprotein E4 Polymorphism and Outcomes from Traumatic Brain Injury: A Living Systematic Review and Meta-Analysis, 38 Journal of Neurotrauma 1124 (2021). /pmc/articles/PMC8054520/

Aschenbrenner, A. J., Balota, D. A., Fagan, A. M., Duchek, J. M., Benzinger, T. L. S., & Morris, J. C. (2015). Alzheimer Disease Cerebrospinal Fluid Biomarkers Moderate Baseline Differences and Predict Longitudinal Change in Attentional Control and Episodic Memory Composites in the Adult Children Study. *Journal of the International Neuropsychological Society*, *21*(8). https://doi.org/10.1017/S1355617715000776

Asken, B. M., Mantyh, W. G., La Joie, R., Strom, A., Casaletto, K. B., Staffaroni, A. M., Apple, A. C., Lindbergh, C. A., Iaccarino, L., You, M., Grant, H., Fonseca, C., Windon, C., Younes, K., Tanner, J., Rabinovici, G. D., Kramer, J. H., & Gardner, R. C. (2021). Association of remote mild traumatic brain injury with cortical amyloid burden in clinically normal older adults. *Brain Imaging and Behavior*, *15*(5). https://doi.org/10.1007/s11682-020-00440-1

Baglietto-Vargas, D., Forner, S., Cai, L., Martini, A. C., Trujillo-Estrada, L., Swarup, V., Minh Thu Nguyen, M., Do Huynh, K., Javonillo, D. I., Minh Tran, K., Phan, J., Jiang, S., Kramár, E. A., Nuñez-Diaz, C., Balderrama-Gutierrez, G., Garcia, F., Childs, J., Rodriguez-Ortiz, C. J., Antonio Garcia-Leon, J., … LaFerla, F. M. (2021). Generation of a humanized Aβ expressing mouse demonstrating aspects of Alzheimer's disease-like pathology. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-22624-z

Baker-Nigh, A., Vahedi, S., Davis, E. G., Weintraub, S., Bigio, E. H., Klein, W. L., & Geula, C. (2015). Neuronal amyloid-β accumulation within cholinergic basal forebrain in ageing and Alzheimer's disease. *Brain*, *138*(6), 1722–1737. https://doi.org/10.1093/brain/awv024

Bamberger, M. E., Harris, M. E., McDonald, D. R., Husemann, J., & Landreth, G. E. (2003). A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation. *Journal of Neuroscience*, *23*(7). https://doi.org/10.1523/jneurosci.23-07-02665.2003

Barnes, D. E., Byers, A. L., Gardner, R. C., Seal, K. H., Boscardin, W. J., & Yaffe, K. (2018). Association of mild traumatic brain injury with and without loss of consciousness with dementia in US military veterans. *JAMA Neurology*, *75*(9), 1055–1061. https://doi.org/10.1001/jamaneurol.2018.0815

Bayless, D. W., Darling, J. S., Stout, W. J., & Daniel, J. M. (2012). Sex differences in attentional processes in adult rats as measured by performance on the 5-choice serial reaction time task. *Behavioural Brain Research*, *235*(1). https://doi.org/10.1016/j.bbr.2012.07.028

Bellenguez, C., Küçükali, F., Jansen, I. E., Kleineidam, L., Moreno-Grau, S., Amin, N., Naj, A. C., Campos-Martin, R., Grenier-Boley, B., Andrade, V., Holmans, P. A., Boland, A., Damotte, V., van der Lee, S. J., Costa, M. R., Kuulasmaa, T., Yang, Q., de Rojas, I., Bis, J. C., … Lambert, J. C. (2022). New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nature Genetics*, *54*(4). https://doi.org/10.1038/s41588-022-01024-z

Bennett, D. A., Schneider, J. A., Arvanitakis, Z., Kelly, J. F., Aggarwal, N. T., Shah, R. C., & Wilson, R. S. (2006). Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*, *66*(12). https://doi.org/10.1212/01.wnl.0000219668.47116.e6

Bentley, P., Driver, J., & Dolan, R. J. (2008). Cholinesterase inhibition modulates visual and attentional brain responses in Alzheimer's disease and health. *Brain*, *131*(2). https://doi.org/10.1093/brain/awm299

Berardi, A. M., Parasuraman, R., & Haxby, J. V. (2005). Sustained attention in mild Alzheimer's disease. *Developmental Neuropsychology*, *28*(1). https://doi.org/10.1207/s15326942dn2801_4

Berry, A. S., Blakely, R. D., Sarter, M., & Lustig, C. (2015a). Cholinergic capacity mediates prefrontal engagement during challenges to attention: Evidence from imaging genetics. *NeuroImage*, *108*, 386–395. https://doi.org/10.1016/j.neuroimage.2014.12.036

Berry, A. S., Blakely, R. D., Sarter, M., & Lustig, C. (2015b). Cholinergic capacity mediates prefrontal engagement during challenges to attention: Evidence from imaging genetics. *NeuroImage*, *108*, 386–395. https://doi.org/10.1016/j.neuroimage.2014.12.036

Berry, A. S., Demeter, E., Sabhapathy, S., English, B. A., Blakely, R. D., Sarter, M., & Lustig, C. (2014). Disposed to Distraction: Genetic Variation in the Cholinergic System Influences Distractibility But Not Time-on-Task Effects. *Journal of Cognitive Neuroscience*, *26*(9), 1981–1991. https://doi.org/10.1162/jocn_a_00607

Bigler, E. D. (2013). Traumatic brain injury, neuroimaging, and neurodegeneration. In *Frontiers in Human Neuroscience* (Issue JUL). https://doi.org/10.3389/fnhum.2013.00395

Braun, N. J., Yao, K. R., Alford, P. W., & Liao, D. (2020). Mechanical injuries of neurons induce tau mislocalization to dendritic spines and tau-dependent synaptic dysfunction. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(46). https://doi.org/10.1073/pnas.2008306117

Bravo, D., & Parsons, S. M. (2002). Microscopic kinetics and structure-function analysis in the vesicular acetylcholine transporter. *Neurochemistry International*, *41*(5), 285–289. https://doi.org/10.1016/S0197-0186(02)00058-X

Bray, M. J. C., Bryant, B. R., Esagoff, A. I., Richey, L. N., Rodriguez, C., Krieg, A., McCullough, G., Tsai, J., Tobolowsky, W., Jahed, S., Cullum, C. M., LoBue, C., Ismail, Z., Yan, H., Lyketsos, C. G., & Peters, M. E. (2022). Effect of traumatic brain injury on mild behavioral impairment domains prior to all-cause dementia diagnosis and throughout disease progression. *Alzheimer's and Dementia: Translational Research and Clinical Interventions*, *8*(1). https://doi.org/10.1002/trc2.12364

Brett, B. L., Gardner, R. C., Godbout, J., Dams-O'Connor, K., & Keene, C. D. (2022). Traumatic Brain Injury and Risk of Neurodegenerative Disorder. *Biological psychiatry*, *91*(5), 498–507. https://doi.org/10.1016/j.biopsych.2021.05.025

Buée, L., Bussière, T., Buée-Scherrer, V., Delacourte, A., & Hof, P. R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. In *Brain Research Reviews* (Vol. 33, Issue 1). https://doi.org/10.1016/S0165-0173(00)00019-9

Burk, J. A., Herzog, C. D., Porter, M. C., & Sarter, M. (2002). Interactions between aging and cortical cholinergic deafferentation on attention. *Neurobiology of Aging*, *23*(3). https://doi.org/10.1016/S0197-4580(01)00315-3

Bussey, T. J., Padain, T. L., Skillings, E. A., Winters, B. D., Morton, A. J., & Saksida, L. M. (2008). The touchscreen cognitive testing method for rodents: How to get the best out of your rat. *Learning and Memory*, *15*(7). https://doi.org/10.1101/lm.987808

Butters, N., Granholm, E., Salmon, D. P., Grant, I., & Wolfe, J. (1987). Episodic and semantic memory: a comparison of amnesic and demented patients. *Journal of Clinical and Experimental Neuropsychology : Official Journal of the International Neuropsychological Society*, *9*(5). https://doi.org/10.1080/01688638708410764

Campbell, J. C., Anderson, J. C., McFadgion, A., Gill, J., Zink, E., Patch, M., Callwood, G., & Campbell, D. (2018). The Effects of Intimate Partner Violence and Probable Traumatic

Brain Injury on Central Nervous System Symptoms. *Journal of Women's Health*, *27*(6), 761–767. https://doi.org/10.1089/jwh.2016.6311

Carrière, I., Fourrier-Reglat, A., Dartigues, J. F., Rouaud, O., Pasquier, F., Ritchie, K., & Ancelin, M. L. (2009). Drugs with anticholinergic properties, cognitive decline, and dementia in an elderly general population: The 3-city study. *Archives of Internal Medicine*, *169*(14), 1317–1324. https://doi.org/10.1001/ARCHINTERNMED.2009.229

Cassidy, J. D., Carroll, L. J., Peloso, P. M., Borg, J., von Holst, H., Holm, L., Kraus, J., & Coronado, V. G. (2004). Incidence, risk factors and prevention of mild traumatic brain injury: Results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. In *Journal of Rehabilitation Medicine, Supplement* (Issue 43). https://doi.org/10.1080/16501960410023732

Cavedo, E., Lista, S., Houot, M., Vergallo, A., Grothe, M. J., Teipel, S., Zetterberg, H., Blennow, K., Habert, M. O., Potier, M. C., Dubois, B., & Hampel, H. (2020). Plasma tau correlates with basal forebrain atrophy rates in people at risk for alzheimer disease. *Neurology*, *94*(1). https://doi.org/10.1212/WNL.0000000000008696

Centers for Disease Control and Prevention. (2011). Nonfatal traumatic brain injuries related to sports and recreation activities among persons aged ≤19 years--United States, 2001-2009. *MMWR. Morbidity and Mortality Weekly Report*, *60*(39).

Chan, R. C. K. (2002). Attentional deficits in patients with persisting postconcussive complaints: A general deficit or specific component deficit? *Journal of Clinical and Experimental Neuropsychology*, *24*(8). https://doi.org/10.1076/jcen.24.8.1081.8371

Chan, R. C. K., Hoosain, R., Lee, T. M. C., Fan, Y. W., & Fong, D. (2003). Are there sub-types of attentional deficits in patients with persisting post-concussive symptoms? A cluster analytical study. *Brain Injury*, *17*(2). https://doi.org/10.1080/0269905021000010168

Chen, H., Desai, A., & Kim, H. Y. (2017). Repetitive Closed-Head Impact Model of Engineered Rotational Acceleration Induces Long-Term Cognitive Impairments with

Persistent Astrogliosis and Microgliosis in Mice. *Journal of Neurotrauma*, *34*(14), 2291–2302. https://doi.org/10.1089/neu.2016.4870

Cherian, A. K., Tronson, N. C., Parikh, V., Kucinski, A., Blakely, R. D., & Sarter, M. (2019). Repetitive mild concussion in subjects with a vulnerable cholinergic system: Lasting cholinergic-attentional impairments in CHT+/- mice. *Behavioral Neuroscience*, *133*(4), 448–459. https://doi.org/10.1037/bne0000310

Chew, M. L., Mulsant, B. H., Pollock, B. G., Lehman, M. E., Greenspan, A., Mahmoud, R. A., Kirshner, M. A., Sorisio, D. A., Bies, R. R., & Gharabawi, G. (2008). Anticholinergic activity of 107 medications commonly used by older adults. *Journal of the American Geriatrics Society*, *56*(7). https://doi.org/10.1111/j.1532-5415.2008.01737.x

Clark, A. L., Weigand, A. J., Bangen, K. J., Thomas, K. R., Eglit, G. M. L., Bondi, M. W., & Delano-Wood, L. (2021). Higher cerebrospinal fluid tau is associated with history of traumatic brain injury and reduced processing speed in Vietnam-era veterans: A Department of Defense Alzheimer's Disease Neuroimaging Initiative (DOD-ADNI) study. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*, *13*(1). https://doi.org/10.1002/dad2.12239

Cleary, J. P., Walsh, D. M., Hofmeister, J. J., Shankar, G. M., Kuskowski, M. A., Selkoe, D. J., & Ashe, K. H. (2005). Natural oligomers of the amyloid-β protein specifically disrupt cognitive function. *Nature Neuroscience*, *8*(1). https://doi.org/10.1038/nn1372

Collie, A., Makdissi, M., Maruff, P., Bennell, K., & McCrory, P. (2006). Cognition in the days following concussion: Comparison of symptomatic versus asymptomatic athletes. *Journal of Neurology, Neurosurgery and Psychiatry*, *77*(2). https://doi.org/10.1136/jnnp.2005.073155

Conde, C., & Cáceres, A. (2009). Microtubule assembly, organization and dynamics in axons and dendrites. In *Nature Reviews Neuroscience* (Vol. 10, Issue 5). https://doi.org/10.1038/nrn2631

Conner, J. M., Chiba, A. A., & Tuszynski, M. H. (2005). The Basal Forebrain Cholinergic System Is Essential for Cortical Plasticity and Functional Recovery following Brain Injury. *Neuron*, *46*(2), 173–179. https://doi.org/10.1016/J.NEURON.2005.03.003

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, *3*(3). https://doi.org/10.1038/nrn755

Corrada, M. M., Brookmeyer, R., Paganini-Hill, A., Berlau, D., & Kawas, C. H. (2010). Dementia incidence continues to increase with age in the oldest old the 90+ study. *Annals of Neurology*, *67*(1), 114–121. https://doi.org/10.1002/ana.21915

Coughlan, G., Laczó, J., Hort, J., Minihane, A. M., & Hornberger, M. (2018). Spatial navigation deficits — Overlooked cognitive marker for preclinical Alzheimer disease? In *Nature Reviews Neurology* (Vol. 14, Issue 8). https://doi.org/10.1038/s41582-018-0031-x

Cummins, T. L., Doré, V., Feizpour, A., Krishnadas, N., Bourgeat, P., Elias, A., Lamb, F., Williams, R., Hopwood, M., Landau, S., Villemagne, V. L., Weiner, M., & Rowe, C. C. (2023). Tau, β-Amyloid, and Glucose Metabolism Following Service-Related Traumatic Brain Injury in Vietnam War Veterans: The Australian Imaging Biomarkers and Lifestyle Study of Aging-Veterans Study (AIBL-VETS). *Journal of Neurotrauma*, *40*(11–12). https://doi.org/10.1089/neu.2022.0172

Dixon, C. E., Bao, J., Long, D. A., & Hayes, R. L. (1996). Reduced evoked release of acetylcholine in the rodent hippocampus following traumatic brain injury. *Pharmacology Biochemistry and Behavior*, *53*(3), 679–686. https://doi.org/10.1016/0091-3057(95)02069-1

Dixon, C. E., Xiecheng, M., & Marion, D. W. (1997). Reduced evoked release of acetylcholine in the rodent neocortex following traumatic brain injury. *Brain Research*, *749*(1), 127–130. https://doi.org/10.1016/S0006-8993(96)01310-8

Dockree, P. M., Kelly, S. P., Roche, R. A. P., Hogan, M. J., Reilly, R. B., & Robertson, I. H. (2004). Behavioural and physiological impairments of sustained attention after traumatic

brain injury. *Cognitive Brain Research*, *20*(3), 403–414. https://doi.org/10.1016/j.cogbrainres.2004.03.019

Egea, J., Buendia, I., Parada, E., Navarro, E., León, R., & Lopez, M. G. (2015). Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. In *Biochemical Pharmacology* (Vol. 97, Issue 4, pp. 463–472). Biochem Pharmacol. https://doi.org/10.1016/j.bcp.2015.07.032

English, B. A., Hahn, M. K., Gizer, I. R., Mazei-Robison, M., Steele, A., Kurnik, D. M., Stein, M. A., Waldman, I. D., & Blakely, R. D. (2009). Choline transporter gene variation is associated with attention-deficit hyperactivity disorder. *Journal of Neurodevelopmental Disorders*, *1*(4). https://doi.org/10.1007/s11689-009-9033-8

Faden, A. I., & Loane, D. J. (2015). Chronic Neurodegeneration After Traumatic Brain Injury: Alzheimer Disease, Chronic Traumatic Encephalopathy, or Persistent Neuroinflammation? *Neurotherapeutics*, *12*(1). https://doi.org/10.1007/s13311-014-0319-5

Fernández-Cabello, S., Kronbichler, M., van Dijk, K. R. A., Goodman, J. A., Nathan Spreng, R., & Schmitz, T. W. (2020). Basal forebrain volume reliably predicts the cortical spread of Alzheimer's degeneration. *Brain*, *143*(3), 993–1009. https://doi.org/10.1093/brain/awaa012

Filon, J. R., Intorcia, A. J., Sue, L. I., Vazquez Arreola, E., Wilson, J., Davis, K. J., Sabbagh, M. N., Belden, C. M., Caselli, R. J., Adler, C. H., Woodruff, B. K., Rapscak, S. Z., Ahern, G. L., Burke, A. D., Jacobson, S., Shill, H. A., Driver-Dunckley, E., Chen, K., Reiman, E. M., … Serrano, G. E. (2016). Gender differences in Alzheimer disease: Brain atrophy, histopathology burden, and cognition. *Journal of Neuropathology and Experimental Neurology*, *75*(8). https://doi.org/10.1093/jnen/nlw047

Fisher, B. M., Saksida, L. M., Robbins, T. W., & Bussey, T. J. (2020). Functional dissociations between subregions of the medial prefrontal cortex on the rodent touchscreen continuous performance test (rCPT) of attention. *Behavioral Neuroscience*, *134*(1). https://doi.org/10.1037/bne0000338

Fleminger, S., Oliver, D. L., Lovestone, S., Rabe-Hesketh, S., & Giora, A. (2003). Head injury as a risk factor for Alzheimer's disease: The evidence 10 years on; a partial replication. *Journal of Neurology Neurosurgery and Psychiatry*, *74*(7). https://doi.org/10.1136/jnnp.74.7.857

Forbes, J. A., Awad, A. J., Zuckerman, S., Carr, K., & Cheng, J. S. (2012). Association between biomechanical parameters and concussion in helmeted collisions in American football: A review of the literature. *Neurosurgical Focus*, *33*(6). https://doi.org/10.3171/2012.9.FOCUS12288

Forlenza, O. V., Diniz, B. S., Talib, L. L., Mendonça, V. A., Ojopi, E. B., Gattaz, W. F., & Teixeira, A. L. (2009). Increased serum IL-1beta level in Alzheimer's disease and mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders*, *28*(6). https://doi.org/10.1159/000255051

Fox, C., Smith, T., Maidment, I., Chan, W. Y., Bua, N., Myint, P. K., Boustani, M., Kwok, C. S., Glover, M., Koopmans, I., & Campbell, N. (2014). Effect of medications with anti-cholinergic properties on cognitive function, delirium, physical function and mortality: A systematic review. In *Age and Ageing* (Vol. 43, Issue 5). https://doi.org/10.1093/ageing/afu096

Gamage, R., Wagnon, I., Rossetti, I., Childs, R., Niedermayer, G., Chesworth, R., & Gyengesi, E. (2020). Cholinergic Modulation of Glial Function During Aging and Chronic Neuroinflammation. In *Frontiers in Cellular Neuroscience* (Vol. 14). https://doi.org/10.3389/fncel.2020.577912

Gangolli, M., Benetatos, J., Esparza, T. J., Fountain, E. M., Seneviratne, S., & Brody, D. L. (2019). Repetitive Concussive and Subconcussive Injury in a Human Tau Mouse Model Results in Chronic Cognitive Dysfunction and Disruption of White Matter Tracts, but Not Tau Pathology. *Journal of Neurotrauma*, *36*(5). https://doi.org/10.1089/neu.2018.5700

Gardner, R. C., Burke, J. F., Nettiksimmons, J., Kaup, A., Barnes, D. E., & Yaffe, K. (2014). Dementia risk after traumatic brain injury vs nonbrain trauma: The role of age and severity. *JAMA Neurology*, *71*(12). https://doi.org/10.1001/jamaneurol.2014.2668

Gatz, M., Reynolds, C. A., Fratiglioni, L., Johansson, B., Mortimer, J. A., Berg, S., Fiske, A., & Pedersen, N. L. (2006). Role of genes and environments for explaining Alzheimer disease. *Archives of General Psychiatry*, *63*(2). https://doi.org/10.1001/archpsyc.63.2.168

Gill, T. M., Sarter, M., & Givens, B. (2000). Sustained visual attention performance-associated prefrontal neuronal activity: Evidence for cholinergic modulation. *Journal of Neuroscience*, *20*(12). https://doi.org/10.1523/jneurosci.20-12-04745.2000

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., & Crowther, R. A. (1989). Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*, *3*(4). https://doi.org/10.1016/0896-6273(89)90210-9

Gosens, R., & Gross, N. (2018). The mode of action of anticholinergics in asthma. *European Respiratory Journal*, *52*(4). https://doi.org/10.1183/13993003.01247-2017

Gray, S. L., Anderson, M. L., Dublin, S., Hanlon, J. T., Hubbard, R., Walker, R., Yu, O., Crane, P. K., & Larson, E. B. (2015). Cumulative use of strong anticholinergics and incident dementia: A prospective cohort study. *JAMA Internal Medicine*, *175*(3). https://doi.org/10.1001/jamainternmed.2014.7663

Grothe, M., Heinsen, H., & Teipel, S. (2013). Longitudinal measures of cholinergic forebrain atrophy in the transition from healthy aging to Alzheimer's disease. *Neurobiology of Aging*, *34*(4), 1210–1220. https://doi.org/10.1016/J.NEUROBIOLAGING.2012.10.018

Grothe, M., Heinsen, H., & Teipel, S. J. (2012). Atrophy of the Cholinergic Basal Forebrain Over the Adult Age Range and in Early Stages of Alzheimer's Disease. *Biological Psychiatry*, *71*(9), 805–813. https://doi.org/10.1016/J.BIOPSYCH.2011.06.019

Grothe, M. J., Ewers, M., Krause, B., Heinsen, H., & Teipel, S. J. (2014). Basal forebrain atrophy and cortical amyloid deposition in nondemented elderly subjects. *Alzheimer's & Dementia*, *10*(5), S344–S353. https://doi.org/10.1016/J.JALZ.2013.09.011

Grudzien, A., Shaw, P., Weintraub, S., Bigio, E., Mash, D. C., & Mesulam, M. M. (2007). Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. *Neurobiology of Aging*, *28*(3), 327–335. https://doi.org/10.1016/j.neurobiolaging.2006.02.007

Guskiewicz, K. M., & Mihalik, J. P. (2011). Biomechanics of sport concussion: Quest for the elusive injury threshold. *Exercise and Sport Sciences Reviews*, *39*(1). https://doi.org/10.1097/JES.0b013e318201f53e

Hall, A. M., Moore, R. Y., Lopez, O. L., Kuller, L., & Becker, J. T. (2008). Basal forebrain atrophy is a presymptomatic marker for Alzheimer's disease. *Alzheimer's & Dementia*, *4*(4), 271–279. https://doi.org/10.1016/J.JALZ.2008.04.005

Hampel, H., Cummings, J., Blennow, K., Gao, P., Jack, C. R., & Vergallo, A. (2021). Developing the ATX(N) classification for use across the Alzheimer disease continuum. In *Nature Reviews Neurology* (Vol. 17, Issue 9). https://doi.org/10.1038/s41582-021-00520-w

Hampel, H., Mesulam, M. M., Cuello, A. C., Khachaturian, A. S., Vergallo, A., Farlow, M. R., Snyder, P. J., Giacobini, E., & Khachaturian, Z. S. (2019). Revisiting the Cholinergic Hypothesis in Alzheimer's Disease: Emerging Evidence from Translational and Clinical Research. In *The journal of prevention of Alzheimer's disease* (Vol. 6, Issue 1, pp. 2–15). Springer. https://doi.org/10.14283/jpad.2018.43

Han, B., Li, X., & Hao, J. (2017). The cholinergic anti-inflammatory pathway: An innovative treatment strategy for neurological diseases. *Neuroscience and Biobehavioral Reviews*, *77*, 358–368. https://doi.org/10.1016/j.neubiorev.2017.04.002

Hanger, D. P., Byers, H. L., Wray, S., Leung, K. Y., Saxton, M. J., Seereeram, A., Reynolds, C. H., Ward, M. A., & Anderton, B. H. (2007). Novel phosphorylation sites in Tau from

Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *Journal of Biological Chemistry*, *282*(32). https://doi.org/10.1074/jbc.M703269200

Hanseeuw, B. J., Schultz, A. P., Betensky, R. A., Sperling, R. A., & Johnson, K. A. (2016). Decreased hippocampal metabolism in high-amyloid mild cognitive impairment. *Alzheimer's and Dementia*, *12*(12), 1288–1296. https://doi.org/10.1016/J.JALZ.2016.06.2357

Hardy, J., & Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. In *Trends in Pharmacological Sciences* (Vol. 12, Issue C). https://doi.org/10.1016/0165-6147(91)90609-V

Hashimoto, S., Matsuba, Y., Kamano, N., Mihira, N., Sahara, N., Takano, J., Muramatsu, S. ichi, Saido, T. C., & Saito, T. (2019). Tau binding protein CAPON induces tau aggregation and neurodegeneration. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10278-x

Hayes, J. P., Logue, M. W., Sadeh, N., Spielberg, J. M., Verfaellie, M., Hayes, S. M., Reagan, A., Salat, D. H., Wolf, E. J., McGlinchey, R. E., Milberg, W. P., Stone, A., Schichman, S. A., & Miller, M. W. (2017). Mild traumatic brain injury is associated with reduced cortical thickness in those at risk for Alzheimer's disease. *Brain*, *140*(3), 813–825. https://doi.org/10.1093/BRAIN/AWW344

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology*, *80*(19). https://doi.org/10.1212/WNL.0b013e31828726f5

Hickman, S. E., Allison, E. K., & El Khoury, J. (2008). Microglial dysfunction and defective β-amyloid clearance pathways in aging alzheimer's disease mice. *Journal of Neuroscience*, *28*(33), 8354–8360. https://doi.org/10.1523/JNEUROSCI.0616-08.2008

Hicks, A. J., Ponsford, J. L., Spitz, G., Dore, V., Krishnadas, N., Roberts, C., & Rowe, C. C. (2022). β-Amyloid and Tau Imaging in Chronic Traumatic Brain Injury: A Cross-

sectional Study. *Neurology*, *99*(11), e1131–e1141. https://doi.org/10.1212/WNL.0000000000200857

Hong, Y. T., Veenith, T., Dewar, D., Outtrim, J. G., Mani, V., Williams, C., Pimlott, S., Hutchinson, P. J. A., Tavares, A., Canales, R., Mathis, C. A., Klunk, W. E., Aigbirhio, F. I., Coles, J. P., Baron, J. C., Pickard, J. D., Fryer, T. D., Stewart, W., & Menon, D. K. (2014). Amyloid Imaging With Carbon 11–Labeled Pittsburgh Compound B for Traumatic Brain Injury. *JAMA Neurology*, *71*(1), 23–31. https://doi.org/10.1001/JAMANEUROL.2013.4847

Honig, L. S., Tang, M. X., Albert, S., Costa, R., Luchsinger, J., Manly, J., Stern, Y., & Mayeux, R. (2003). Stroke and the Risk of Alzheimer Disease. *Archives of Neurology*, *60*(12). https://doi.org/10.1001/archneur.60.12.1707

Hoogenboom, W. S., Branch, C. A., & Lipton, M. L. (2019). Animal models of closed-skull, repetitive mild traumatic brain injury. *Pharmacology and Therapeutics*, *198*, 109–122. https://doi.org/10.1016/j.pharmthera.2019.02.016

Hort, J., Laczó, J., Vyhnálek, M., Bojar, M., Bureš, J., & Vlček, K. (2007). Spatial navigation deficit in amnestic mild cognitive impairment. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(10). https://doi.org/10.1073/pnas.0611314104

Houser, C. R. (1990). Cholinergic synapses in the central nervous system: Studies of the immunocytochemical localization of choline acetyltransferase. *Journal of Electron Microscopy Technique*, *15*(1), 2–19. https://doi.org/10.1002/jemt.1060150103

Howard, R., McShane, R., Lindesay, J., Ritchie, C., Baldwin, A., Barber, R., Burns, A., Dening, T., Findlay, D., Holmes, C., Hughes, A., Jacoby, R., Jones, R., Jones, R., McKeith, I., Macharouthu, A., O'Brien, J., Passmore, P., Sheehan, B., … Phillips, P. (2012). Donepezil and Memantine for Moderate-to-Severe Alzheimer's Disease. *New England Journal of Medicine*, *366*(10). https://doi.org/10.1056/nejmoa1106668

Hunnicutt, G., Lundgren, K., Murray, C., & Olson, L. (2017). The Intersection of Intimate Partner Violence and Traumatic Brain Injury: A Call for Interdisciplinary Research. *Journal of Family Violence*, *32*(5). https://doi.org/10.1007/s10896-016-9854-7

Huntley, J. D., Hampshire, A., Bor, D., Owen, A. M., & Howard, R. J. (2017). The importance of sustained attention in early Alzheimer's disease. *International Journal of Geriatric Psychiatry*, *32*(8). https://doi.org/10.1002/gps.4537

Iacono, D., Raiciulescu, S., Olsen, C., & Perl, D. P. (2021). Traumatic Brain Injury Exposure Lowers Age of Cognitive Decline in AD and Non-AD Conditions. *Frontiers in Neurology*, *12*. https://doi.org/10.3389/fneur.2021.573401

Irvine, K., Laws, K. R., Gale, T. M., & Kondel, T. K. (2012). Greater cognitive deterioration in women than men with Alzheimer's disease: A meta analysis. *Journal of Clinical and Experimental Neuropsychology*, *34*(9). https://doi.org/10.1080/13803395.2012.712676

Iverson, K. M., Dardis, C. M., & Pogoda, T. K. (2017). Traumatic brain injury and PTSD symptoms as a consequence of intimate partner violence. *Comprehensive Psychiatry*, *74*, 80–87. https://doi.org/10.1016/j.comppsych.2017.01.007

Jalloh, I., Carpenter, K. L. H., Helmy, A., Carpenter, T. A., Menon, D. K., & Hutchinson, P. J. (2015). Glucose metabolism following human traumatic brain injury: methods of assessment and pathophysiological findings. In *Metabolic Brain Disease* (Vol. 30, Issue 3). https://doi.org/10.1007/s11011-014-9628-y

Jellinger, K. A., Paulus, W., Wrocklage, C., & Litvan, I. (2001). Traumatic brain injury as a risk factor for Alzheimer disease. Comparison of two retrospective autopsy cohorts with evaluation of ApoE genotype. *BMC Neurology*, *1*. https://doi.org/10.1186/1471-2377-1-3

Jentsch, J. D., & Taylor, J. R. (2003). Sex-related differences in spatial divided attention and motor impulsivity in rats. *Behavioral Neuroscience*, *117*(1). https://doi.org/10.1037/0735-7044.117.1.76

Jessen, F., Kaduszkiewicz, H., Daerr, M., Bickel, H., Pentzek, M., Riedel-Heller, S., Wagner, M., Weyerer, S., Wiese, B., Van Den Bussche, H., Broich, K., & Maier, W. (2010). Anticholinergic drug use and risk for dementia: Target for dementia prevention. *European Archives of Psychiatry and Clinical Neuroscience*, *260*(SUPPL. 2), 111–115. https://doi.org/10.1007/S00406-010-0156-4/FIGURES/2

Johnson, V. E., Stewart, W., & Smith, D. H. (2012). Widespread tau and amyloid-beta pathology many years after a single traumatic brain injury in humans. *Brain Pathology*, *22*(2), 142–149. https://doi.org/10.1111/j.1750-3639.2011.00513.x

Johnson, V. E., Stewart, W., & Smith, D. H. (2013). Axonal pathology in traumatic brain injury. *Experimental Neurology*, *246*, 35–43. https://doi.org/10.1016/J.EXPNEUROL.2012.01.013

Jucker, M., Walker, L. C., Schwarb, P., Hengemihle, J., Kuo, H., Snow, A. D., Bamert, F., & Ingram, D. K. (1994). Age-related deposition of glia-associated fibrillar material in brains of c57BL/6 mice. *Neuroscience*, *60*(4). https://doi.org/10.1016/0306-4522(94)90269-0

Keller, J. N., Schmitt, F. A., Scheff, S. W., Ding, Q., Chen, Q., Butterfield, D. A., & Markesbery, W. R. (2005). Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology*, *64*(7). https://doi.org/10.1212/01.WNL.0000156156.13641.BA

Kent, B. A., Heath, C. J., Kim, C. H., Ahrens, R., Fraser, P. E., St George-Hyslop, P., Bussey, T. J., & Saksida, L. M. (2018). Longitudinal evaluation of Tau-P301L transgenic mice reveals no cognitive impairments at 17 months of age. *Brain and Behavior*, *8*(1). https://doi.org/10.1002/brb3.896

Kerbler, G. M., Fripp, J., Rowe, C. C., Villemagne, V. L., Salvado, O., Rose, S., & Coulson, E. J. (2015). Basal forebrain atrophy correlates with amyloid β burden in Alzheimer's disease. *NeuroImage: Clinical*, *7*, 105–113. https://doi.org/10.1016/j.nicl.2014.11.015

Kim, C. H., Hvoslef-Eide, M., Nilsson, S. R. O., Johnson, M. R., Herbert, B. R., Robbins, T. W., Saksida, L. M., Bussey, T. J., & Mar, A. C. (2015). The continuous performance test

(rCPT) for mice: A novel operant touchscreen test of attentional function. *Psychopharmacology*, *232*(21–22), 3947–3966. https://doi.org/10.1007/s00213-015-4081-0

Kim, J., Lee, H. J., Park, S. K., Park, J. H., Jeong, H. R., Lee, S., Lee, H., Seol, E., & Hoe, H. S. (2021). Donepezil regulates LPS and aβ-stimulated neuroinflammation through MAPK/NLRP3 inflammasome/STAT3 signaling. *International Journal of Molecular Sciences*, *22*(19). https://doi.org/10.3390/ijms221910637

King, E. G., Kretzmer, T. S., Vanderploeg, R. D., Asmussen, S. B., Clement, V. L., & Belanger, H. G. (2013). Pilot of a novel intervention for postconcussive symptoms in active duty, veterans, and civilians. *Rehabilitation Psychology*, *58*(3). https://doi.org/10.1037/a0033116

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M., & Lamb, B. T. (2018). Inflammation as a central mechanism in Alzheimer's disease. In *Alzheimer's and Dementia: Translational Research and Clinical Interventions* (Vol. 4). https://doi.org/10.1016/j.trci.2018.06.014

Koliatsos, V. E., Cernak, I., Xu, L., Song, Y., Savonenko, A., Crain, B. J., Eberhart, C. G., Frangakis, C. E., Melnikova, T., Kim, H., & Lee, D. (2011). A Mouse Model of Blast Injury to Brain: Initial Pathological, Neuropathological, and Behavioral Characterization. *Journal of Neuropathology and Experimental Neurology*, *70*(5), 399–416. https://doi.org/10.1097/NEN.0b013e3182189f06

Kolisnyk, B., Al-Onaizi, M. A., Hirata, P. H. F., Guzman, M. S., Nikolova, S., Barbash, S., Soreq, H., Bartha, R., Prado, M. A. M., & Prado, V. F. (2013). Forebrain deletion of the vesicular acetylcholine transporter results in deficits in executive function, metabolic, and RNA splicing abnormalities in the prefrontal cortex. *Journal of Neuroscience*, *33*(37), 14908–14920. https://doi.org/10.1523/JNEUROSCI.1933-13.2013

Kolisnyk, B., Al-Onaizi, M., Soreq, L., Barbash, S., Bekenstein, U., Haberman, N., Hanin, G., Kish, M. T., Souza Da Silva, J., Fahnestock, M., Ule, J., Soreq, H., Prado, V. F., & Prado, M. A. M. (2017). Cholinergic Surveillance over Hippocampal RNA Metabolism and

Alzheimer's-Like Pathology. *Cerebral Cortex*, *27*(7), 3553–3567. https://doi.org/10.1093/CERCOR/BHW177

Kukull, W. A., Higdon, R., Bowen, J. D., McCormick, W. C., Teri, L., Schellenberg, G. D., Van Belle, G., Jolley, L., & Larson, E. B. (2002). Dementia and Alzheimer disease incidence: A prospective cohort study. *Archives of Neurology*, *59*(11). https://doi.org/10.1001/archneur.59.11.1737

Lane, C. A., Hardy, J., & Schott, J. M. (2018). Alzheimer's disease. *European Journal of Neurology*, *25*(1), 59–70. https://doi.org/10.1111/ene.13439

Langlois, J., Rutland-Brown, W., & Wald, M. (2006a). The Epidemiology and Impact of Traumatic Brain Injury: A Bri... : The Journal of Head Trauma Rehabilitation. *Journal of Head Trauma Rehabilitation*, *21*(5), 375–378.

Langlois, J., Rutland-Brown, W., & Wald, M. (2006b). The Epidemiology and Impact of Traumatic Brain Injury: A Bri... : The Journal of Head Trauma Rehabilitation. *Journal of Head Trauma Rehabilitation*, *21*(5), 375–378. https://journals.lww.com/headtraumarehab/Fulltext/2006/09000/The_Epidemiology_and_Impact_of_Traumatic_Brain.1.aspx

Larson, E. B., Wang, L., Bowen, J. D., McCormick, W. C., Teri, L., Crane, P., & Kukull, W. (2006). Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Annals of Internal Medicine*, *144*(2). https://doi.org/10.7326/0003-4819-144-2-200601170-00004

Lee, E., Jung, Y. J., Park, Y. R., Lim, S., Choi, Y. J., Lee, S. Y., Kim, C. H., Mun, J. Y., & Chung, W. S. (2022). A distinct astrocyte subtype in the aging mouse brain characterized by impaired protein homeostasis. *Nature Aging*, *2*(8). https://doi.org/10.1038/s43587-022-00257-1

Lee, J., Seroogy, K. B., & Mattson, M. P. (2002). Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *Journal of Neurochemistry*, *80*(3). https://doi.org/10.1046/j.0022-3042.2001.00747.x

Lefevre-Dognin, C., Cogné, M., Perdrieau, V., Granger, A., Heslot, C., & Azouvi, P. (2021). Definition and epidemiology of mild traumatic brain injury. *Neurochirurgie*, *67*(3). https://doi.org/10.1016/j.neuchi.2020.02.002

Levinoff, E. J., Saumier, D., & Chertkow, H. (2005). Focused attention deficits in patients with Alzheimer's disease and mild cognitive impairment. *Brain and Cognition*, *57*(2), 127–130. https://doi.org/10.1016/j.bandc.2004.08.058

Li, D. D., Zhang, Y. H., Zhang, W., & Zhao, P. (2019). Meta-analysis of randomized controlled trials on the efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease. *Frontiers in Neuroscience*, *13*(MAY). https://doi.org/10.3389/fnins.2019.00472

Li, H., Li, S., Yang, H., Zhang, Y., Zhang, S., Ma, Y., Hou, Y., Zhang, X., Niu, K., Borné, Y., & Wang, Y. (2022). Association of Ultraprocessed Food Consumption With Risk of Dementia: A Prospective Cohort Study. *Neurology*, *99*(10). https://doi.org/10.1212/WNL.0000000000200871

Li, X., Yu, B., Sun, Q., Zhang, Y., Ren, M., Zhang, X., Li, A., Yuan, J., Madisen, L., Luo, Q., Zeng, H., Gong, H., & Qiu, Z. (2017). Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(2). https://doi.org/10.1073/pnas.1703601115

Lipton, M. L., Kim, N., Park, Y. K., Hulkower, M. B., Gardin, T. M., Shifteh, K., Kim, M., Zimmerman, M. E., Lipton, R. B., & Branch, C. A. (2012). Robust detection of traumatic axonal injury in individual mild traumatic brain injury patients: Intersubject variation, change over time and bidirectional changes in anisotropy. *Brain Imaging and Behavior*, *6*(2). https://doi.org/10.1007/s11682-012-9175-2

Lipton, M. L., Kim, N., Zimmerman, M. E., Kim, M., Stewart, W. F., Branch, C. A., & Lipton, R. B. (2013). Soccer heading is associated with white matter microstructural and cognitive abnormalities. *Radiology*, *268*(3). https://doi.org/10.1148/radiol.13130545

Ljubojevic, V., Luu, P., Gill, P. R., Beckett, L. A., Takehara-Nishiuchi, K., & De Rosa, E. (2018). Cholinergic modulation of frontoparietal cortical network dynamics supporting supramodal attention. *Journal of Neuroscience*, *38*(16). https://doi.org/10.1523/JNEUROSCI.2350-17.2018

Lobue, C., Kelley, B. J., Hart, J., Helphrey, J., Schaffert, J., Cullum, C. M., Peters, M. E., & Douglas, P. M. (2022). Mild Traumatic Brain Injury Is Related to Elevated Cerebrospinal Fluid Tau in Alzheimer's Disease Dementia. *Journal of Alzheimer's Disease*, *87*(4). https://doi.org/10.3233/JAD-220112

LoBue, C., Wadsworth, H., Wilmoth, K., Clem, M., Hart, J., Womack, K. B., Didehbani, N., Lacritz, L. H., Rossetti, H. C., & Cullum, C. M. (2017). Traumatic brain injury history is associated with earlier age of onset of Alzheimer disease. *Clinical Neuropsychologist*, *31*(1). https://doi.org/10.1080/13854046.2016.1257069

LoBue, C., Wilmoth, K., Cullum, C. M., Rossetti, H. C., Lacritz, L. H., Hynan, L. S., Hart, J., & Womack, K. B. (2016). Traumatic brain injury history is associated with earlier age of onset of frontotemporal dementia. *Journal of Neurology, Neurosurgery and Psychiatry*, *87*(8). https://doi.org/10.1136/jnnp-2015-311438

Logue, M. W., Miller, M. W., Sherva, R., Zhang, R., Harrington, K. M., Fonda, J. R., Merritt, V. C., Panizzon, M. S., Hauger, R. L., Wolf, E. J., Neale, Z., & Gaziano, J. M. (2023). Alzheimer's disease and related dementias among aging veterans: Examining gene-by-environment interactions with post-traumatic stress disorder and traumatic brain injury. *Alzheimer's & Dementia*, *19*(6), 2549–2559. https://doi.org/10.1002/ALZ.12870

Lue, L. F., Brachova, L., Civin, W. H., & Rogers, J. (1996). Inflammation, Aβ deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. *Journal of Neuropathology and Experimental Neurology*, *55*(10). https://doi.org/10.1097/00005072-199655100-00008

Lv, X., Li, W., Luo, Y., Wang, D., Zhu, C., Huang, Z. X., & Tan, X. (2013). Exploring the differences between mouse maβ1–42 and human haβ1–42 for alzheimer's disease related

properties and neuronal cytotoxicity. *Chemical Communications*, *49*(52). https://doi.org/10.1039/c3cc40779a

Lyketsos, C. G., Carrillo, M. C., Ryan, J. M., Khachaturian, A. S., Trzepacz, P., Amatniek, J., Cedarbaum, J., Brashear, R., & Miller, D. S. (2011). Neuropsychiatric symptoms in Alzheimer's disease. In *Alzheimer's and Dementia* (Vol. 7, Issue 5). https://doi.org/10.1016/j.jalz.2011.05.2410

Macmillan, N. A., & Creelman, C. D. (2004). Detection Theory: A User's Guide: 2nd edition. In *Detection Theory: A User's Guide: 2nd edition*. https://doi.org/10.4324/9781410611147

Madhusudan, A., Sidler, C., & Knuesel, I. (2009). Accumulation of reelin-positive plaques is accompanied by a decline in basal forebrain projection neurons during normal aging. *European Journal of Neuroscience*, *30*(6). https://doi.org/10.1111/j.1460-9568.2009.06884.x

Mahoney, S. O., Chowdhury, N. F., Ngo, V., Imms, P., & Irimia, A. (2022). Mild Traumatic Brain Injury Results in Significant and Lasting Cortical Demyelination. *Frontiers in Neurology*, *13*. https://doi.org/10.3389/fneur.2022.854396

Makdissi, M., Collie, A., Maruff, P., Darby, D. G., Bush, A., McCrory, P., & Bennell, K. (2001). Computerised cognitive assessment of concussed Australian rules footballers. *British Journal of Sports Medicine*, *35*(5). https://doi.org/10.1136/bjsm.35.5.354

Manich, G., Augé, E., Cabezón, I., Pallàs, M., Vilaplana, J., & Pelegrí, C. (2015). Neo-epitopes emerging in the degenerative hippocampal granules of aged mice can be recognized by natural IgM auto-antibodies. *Immunity and Ageing*, *12*(1). https://doi.org/10.1186/s12979-015-0050-z

Manich, G., Cabezón, I., Augé, E., Pelegrí, C., & Vilaplana, J. (2016). Periodic acid-Schiff granules in the brain of aged mice: From amyloid aggregates to degenerative structures containing neo-epitopes. In *Ageing Research Reviews* (Vol. 27). https://doi.org/10.1016/j.arr.2016.03.001

Manich, G., Cabezón, I., Camins, A., Pallàs, M., Liberski, P. P., Vilaplana, J., & Pelegrí, C. (2014). Clustered granules present in the hippocampus of aged mice result from a degenerative process affecting astrocytes and their surrounding neuropil. *Age*, *36*(4), 1–14. https://doi.org/10.1007/s11357-014-9690-8

Manich, G., Del Valle, J., Cabezón, I., Camins, A., Pallàs, M., Pelegrí, C., & Vilaplana, J. (2014). Presence of a neo-epitope and absence of amyloid beta and tau protein in degenerative hippocampal granules of aged mice. *Age*, *36*(1). https://doi.org/10.1007/s11357-013-9560-9

Manich, G., Mercader, C., Del Valle, J., Duran-Vilaregut, J., Camins, A., Pallàs, M., & Vilaplana, J. (2011). Characterization of amyloid-β granules in the hippocampus of SAMP8 mice. *Journal of Alzheimer's Disease*, *25*(3). https://doi.org/10.3233/JAD-2011-101713

Manning, K. Y., Brooks, J. S., Dickey, J. P., Harriss, A., Fischer, L., Jevremovic, T., Blackney, K., Barreira, C., Brown, A., Bartha, R., Doherty, T., Fraser, D., Holmes, J., Dekaban, G. A., & Menon, R. S. (2020). Longitudinal changes of brain microstructure and function in nonconcussed female rugby players. *Neurology*, *95*(4), E402–E412. https://doi.org/10.1212/WNL.0000000000009821

Mar, A. C., Horner, A. E., Nilsson, S. R. O., Alsiö, J., Kent, B. A., Kim, C. H., Holmes, A., Saksida, L. M., & Bussey, T. J. (2013). The touchscreen operant platform for assessing executive function in rats and mice. *Nature Protocols*, *8*(10). https://doi.org/10.1038/nprot.2013.123

Mazère, J., Prunier, C., Barret, O., Guyot, M., Hommet, C., Guilloteau, D., Dartigues, J. F., Auriacombe, S., Fabrigoule, C., & Allard, M. (2008). In vivo SPECT imaging of vesicular acetylcholine transporter using [123I]-IBVM in early Alzheimer's disease. *NeuroImage*, *40*(1). https://doi.org/10.1016/j.neuroimage.2007.11.028

McCrory, P., Meeuwisse, W. H., Aubry, M., Cantu, B., Dvořák, J., Echemendia, R. J., Engebretsen, L., Johnston, K., Kutcher, J. S., Raftery, M., Sills, A., Benson, B. W., Davis, G. A., Ellenbogen, R. G., Guskiewicz, K., Herring, S. A., Iverson, G. L., Jordan,

B. D., Kissick, J., … Turner, M. (2013). Consensus statement on concussion in sport: The 4th International Conference on Concussion in Sport held in Zurich, November 2012. *British Journal of Sports Medicine*, *47*(5), 250–258. https://doi.org/10.1136/bjsports-2013-092313

McKee, A. C., Cantu, R. C., Nowinski, C. J., Hedley-Whyte, E. T., Gavett, B. E., Budson, A. E., Santini, V. E., Lee, H. S., Kubilus, C. A., & Stern, R. A. (2009). Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *Journal of neuropathology and experimental neurology*, *68*(7), 709–735. https://doi.org/10.1097/NEN.0b013e3181a9d503

McKee, A. C., & Robinson, M. E. (2014). Military-related traumatic brain injury and neurodegeneration. *Alzheimer's and Dementia*, *10*(3 SUPPL.). https://doi.org/10.1016/j.jalz.2014.04.003

McKee, A. C., Stein, T. D., Kiernan, P. T., & Alvarez, V. E. (2015). The neuropathology of chronic traumatic encephalopathy. *Brain pathology (Zurich, Switzerland)*, *25*(3), 350–364. https://doi.org/10.1111/bpa.12248

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's and Dementia*, *7*(3). https://doi.org/10.1016/j.jalz.2011.03.005

McLean, C. A., Cherny, R. A., Fraser, F. W., Fuller, S. J., Smith, M. J., Beyreuther, K., Bush, A. I., & Masters, C. L. (1999). Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Annals of Neurology*, *46*(6). https://doi.org/10.1002/1531-8249(199912)46:6<860::AID-ANA8>3.0.CO;2-M

Mehla, J., Deibel, S. H., Karem, H., Hong, N. S., Hossain, S. R., Lacoursiere, S. G., Sutherland, R. J., Mohajerani, M. H., & McDonald, R. J. (2023). Repeated multi-domain

cognitive training prevents cognitive decline, anxiety and amyloid pathology found in a mouse model of Alzheimer disease. *Communications Biology*, *6*(1), 1145. https://doi.org/10.1038/s42003-023-05506-6

Merritt, V. C., Lapira, K. M., Clark, A. L., Sorg, S. F., Werhane, M. L., Jak, A. J., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2019). APOE-ϵ4 Genotype is Associated with Elevated Post-Concussion Symptoms in Military Veterans with a Remote History of Mild Traumatic Brain Injury. *Archives of Clinical Neuropsychology*, *34*(5), 706–712. https://doi.org/10.1093/arclin/acy082

Mesulam, M., Shaw, P., Mash, D., & Weintraub, S. (2004). Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. *Annals of Neurology*, *55*(6), 815–828. https://doi.org/10.1002/ana.20100

Mikolić, A., Van Klaveren, D., Groeniger, J. O., Wiegers, E. J. A., Lingsma, H. F., Zeldovich, M., Von Steinbüchel, N., Maas, A. I. R., Roeters Van Lennep, J. E., & Polinder, S. (2021). Differences between Men and Women in Treatment and Outcome after Traumatic Brain Injury. *Journal of Neurotrauma*, *38*(2). https://doi.org/10.1089/neu.2020.7228

Mohamed, A. Z., Cumming, P., & Nasrallah, F. A. (2022). Escalation of Tau Accumulation after a Traumatic Brain Injury: Findings from Positron Emission Tomography. *Brain Sciences*, *12*(7). https://doi.org/10.3390/brainsci12070876

Mohamed, A. Z., Nestor, P. J., Cumming, P., & Nasrallah, F. A. (2022). Traumatic brain injury fast-forwards Alzheimer's pathology: evidence from amyloid positron emission tomorgraphy imaging. *Journal of Neurology*, *269*(2), 873–884. https://doi.org/10.1007/S00415-021-10669-5

Montine, T. J., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Dickson, D. W., Duyckaerts, C., Frosch, M. P., Masliah, E., Mirra, S. S., Nelson, P. T., Schneider, J. A., Thal, D. R., Trojanowski, J. Q., Vinters, H. V., & Hyman, B. T. (2012). National institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of

Alzheimer's disease: A practical approach. *Acta Neuropathologica*, *123*(1). https://doi.org/10.1007/s00401-011-0910-3

Moretti, L., Cristofori, I., Weaver, S. M., Chau, A., Portelli, J. N., & Grafman, J. (2012). Cognitive decline in older adults with a history of traumatic brain injury. In *The Lancet Neurology* (Vol. 11, Issue 12). https://doi.org/10.1016/S1474-4422(12)70226-0

Mortimer, J. A., van Duijn, C. M., Chandra, V., Fratiglioni, L., Graves, A. B., Heyman, A., Jorm, A. F., Kokmen, E., Kondo, K., Rocca, W. A., & . (1991). Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int.J.Epidemiol.*, *20 Suppl 2*(0300–5771).

Mouzon, B., Bachmeier, C., Ojo, J., Acker, C., Ferguson, S., Crynen, G., Davies, P., Mullan, M., Stewart, W., & Crawford, F. (2019). Chronic White Matter Degeneration, but No Tau Pathology at One-Year Post-Repetitive Mild Traumatic Brain Injury in a Tau Transgenic Model. *Journal of Neurotrauma*, *36*(4), 576–588. https://doi.org/10.1089/neu.2018.5720

Mouzon, B. C., Bachmeier, C., Ferro, A., Ojo, J. O., Crynen, G., Acker, C. M., Davies, P., Mullan, M., Stewart, W., & Crawford, F. (2014). Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. *Annals of Neurology*, *75*(2), 241–254. https://doi.org/10.1002/ana.24064

Mouzon, B. C., Bachmeier, C., Ojo, J. O., Acker, C. M., Ferguson, S., Paris, D., Ait-Ghezala, G., Crynen, G., Davies, P., Mullan, M., Stewart, W., & Crawford, F. (2018). Lifelong behavioral and neuropathological consequences of repetitive mild traumatic brain injury. *Annals of Clinical and Translational Neurology*, *5*(1), 64–80. https://doi.org/10.1002/acn3.510

Mufson, E., Counts, S., Fahnestock, M., & Ginsberg, S. (2007). Cholinotrophic Molecular Substrates of Mild Cognitive Impairment in the Elderly. *Current Alzheimer Research*, *4*(4). https://doi.org/10.2174/156720507781788855

Mufson, E. J., Kroin, J. S., Sendera, T. J., & Sobreviela, T. (1999). Distribution and retrograde transport of trophic factors in the central nervous system: Functional implications for the

treatment of neurodegenerative diseases. In *Progress in Neurobiology* (Vol. 57, Issue 4). https://doi.org/10.1016/S0301-0082(98)00059-8

Murdoch, I., Perry, E. K., Court, J. A., Graham, D. I., & Dewar, D. (1998). Cortical cholinergic dysfunction after human head injury. *Journal of Neurotrauma*, *15*(5), 295–305. https://doi.org/10.1089/neu.1998.15.295

Naumenko, Y., Yuryshinetz, I., Zabenko, Y., & Pivneva, T. (2023). Mild traumatic brain injury as a pathological process. In *Heliyon* (Vol. 9, Issue 7). https://doi.org/10.1016/j.heliyon.2023.e18342

Neddens, J., Temmel, M., Flunkert, S., Kerschbaumer, B., Hoeller, C., Loeffler, T., Niederkofler, V., Daum, G., Attems, J., & Hutter-Paier, B. (2018). Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathologica Communications*, *6*(1), 52. https://doi.org/10.1186/s40478-018-0557-6

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., Castellani, R. J., Crain, B. J., Davies, P., Tredici, K. Del, Duyckaerts, C., Frosch, M. P., Haroutunian, V., Hof, P. R., Hulette, C. M., Hyman, B. T., Iwatsubo, T., Jellinger, K. A., Jicha, G. A., … Beach, T. G. (2012). Correlation of alzheimer disease neuropathologic changes with cognitive status: A review of the literature. In *Journal of Neuropathology and Experimental Neurology* (Vol. 71, Issue 5). https://doi.org/10.1097/NEN.0b013e31825018f7

Nemy, M., Cedres, N., Grothe, M. J., Muehlboeck, J. S., Lindberg, O., Nedelska, Z., Stepankova, O., Vyslouzilova, L., Eriksdotter, M., Barroso, J., Teipel, S., Westman, E., & Ferreira, D. (2020). Cholinergic white matter pathways make a stronger contribution to attention and memory in normal aging than cerebrovascular health and nucleus basalis of Meynert. *NeuroImage*, *211*. https://doi.org/10.1016/J.NEUROIMAGE.2020.116607

Noble, W., Hanger, D. P., Miller, C. C. J., & Lovestone, S. (2013). The importance of tau phosphorylation for neurodegenerative diseases. *Frontiers in Neurology*, *4 JUL*. https://doi.org/10.3389/fneur.2013.00083

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., Metherate, R., Mattson, M. P., Akbari, Y., & LaFerla, F. M. (2003). Triple-transgenic model of Alzheimer's Disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. *Neuron*, *39*(3). https://doi.org/10.1016/S0896-6273(03)00434-3

O'Grady, G. L., Verschuuren, C., Yuen, M., Webster, R., Menezes, M., Fock, J. M., Pride, N., Best, H. A., Benavides Damm, T., Turner, C., Lek, M., Engel, A. G., North, K. N., Clarke, N. F., Macarthur, D. G., Kamsteeg, E. J., & Cooper, S. T. (2016). Variants in SLC18A3, vesicular acetylcholine transporter, cause congenital myasthenic syndrome. *Neurology*, *87*(14). https://doi.org/10.1212/WNL.0000000000003179

Okuda, T., Okamura, M., Kaitsuka, C., Haga, T., & Gurwitz, D. (2002). Single nucleotide polymorphism of the human high affinity choline transporter alters transport rate. *Journal of Biological Chemistry*, *277*(47). https://doi.org/10.1074/jbc.M207742200

Osier, N. D., & Dixon, C. E. (2016). The controlled cortical impact model: Applications, considerations for researchers, and future directions. In *Frontiers in Neurology* (Vol. 7, Issue AUG). https://doi.org/10.3389/fneur.2016.00134

Östberg, A., Ledig, C., Katila, A., Maanpää, H. R., Posti, J. P., Takala, R., Tallus, J., Glocker, B., Rueckert, D., & Tenovuo, O. (2020). Volume Change in Frontal Cholinergic Structures After Traumatic Brain Injury and Cognitive Outcome. *Frontiers in Neurology*, *11*, 832. https://doi.org/10.3389/fneur.2020.00832

Östberg, A., Virta, J., Rinne, J. O., Oikonen, V., Luoto, P., Någren, K., Arponen, E., & Tenovuo, O. (2018). Brain Cholinergic Function and Response to Rivastigmine in Patients with Chronic Sequels of Traumatic Brain Injury: A PET Study. *Journal of Head Trauma Rehabilitation*, *33*(1). https://doi.org/10.1097/HTR.0000000000000279

Oyegbile, T. O., Delasobera, B. E., & Zecavati, N. (2018). Postconcussive Symptoms After Single and Repeated Concussions in 10- to 20-Year-Olds: A Cross-Sectional Study. *Journal of Child Neurology*, *33*(6). https://doi.org/10.1177/0883073818759436

Palmqvist, S., Tideman, P., Cullen, N., Zetterberg, H., Blennow, K., Dage, J. L., Stomrud, E., Janelidze, S., Mattsson-Carlgren, N., & Hansson, O. (2021). Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nature Medicine*. https://doi.org/10.1038/s41591-021-01348-z

Parasuraman, R., & Haxby, J. V. (1993). Attention and Brain Function in Alzheimer's Disease: A Review. *Neuropsychology*, *7*(3). https://doi.org/10.1037/0894-4105.7.3.242

Parikh, V., Kozak, R., Martinez, V., & Sarter, M. (2007). Prefrontal Acetylcholine Release Controls Cue Detection on Multiple Timescales. *Neuron*, *56*(1). https://doi.org/10.1016/j.neuron.2007.08.025

Park, J. H., Park, I., Youm, E. M., Lee, S., Park, J. H., Lee, J., Lee, D. Y., Byun, M. S., Lee, J. H., Yi, D., Chung, S. J., Park, K. W., Choi, N., Kim, S. Y., Yoon, W., An, H., Kim, K. woong, Choi, S. H., Jeong, J. H., … Kim, J. W. (2021). Novel Alzheimer's disease risk variants identified based on whole-genome sequencing of APOE ε4 carriers. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/s41398-021-01412-9

Parks, A. C., Moore, R. D., Wu, C. T., Broglio, S. P., Covassin, T., Hillman, C. H., & Pontifex, M. B. (2015). The association between a history of concussion and variability in behavioral and neuroelectric indices of cognition. *International Journal of Psychophysiology*, *98*(3). https://doi.org/10.1016/j.ijpsycho.2015.08.006

Patel, N. V., Gordon, M. N., Connor, K. E., Good, R. A., Engelman, R. W., Mason, J., Morgan, D. G., Morgan, T. E., & Finch, C. E. (2005). Caloric restriction attenuates Aβ-deposition in Alzheimer transgenic models. *Neurobiology of Aging*, *26*(7). https://doi.org/10.1016/j.neurobiolaging.2004.09.014

Peeters, W., van den Brande, R., Polinder, S., Brazinova, A., Steyerberg, E. W., Lingsma, H. F., & Maas, A. I. R. (2015). Epidemiology of traumatic brain injury in Europe. *Acta Neurochirurgica*, *157*(10). https://doi.org/10.1007/s00701-015-2512-7

Perluigi, M., Di Domenico, F., Barone, E., & Butterfield, D. A. (2021). mTOR in Alzheimer disease and its earlier stages: Links to oxidative damage in the progression of this

dementing disorder. In *Free Radical Biology and Medicine* (Vol. 169). https://doi.org/10.1016/j.freeradbiomed.2021.04.025

Perry, E. K., Kilford, L., Lees, A. J., Burn, D. J., & Perry, R. H. (2003). Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. *Annals of Neurology*, *54*(2), 235–238. https://doi.org/10.1002/ANA.10639

Perry, R. J., & Hodges, J. R. (1999). Attention and executive deficits in Alzheimer's disease. A critical review. In *Brain* (Vol. 122, Issue 3). https://doi.org/10.1093/brain/122.3.383

Perry, R. J., Watson, P., & Hodges, J. R. (2000). The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: Relationship to episodic and semantic memory impairment. *Neuropsychologia*, *38*(3). https://doi.org/10.1016/S0028-3932(99)00079-2

Peter, J., Mayer, I., Kammer, T., Minkova, L., Lahr, J., Klöppel, S., Grothe, M. J., & Orth, M. (2021). The relationship between cholinergic system brain structure and function in healthy adults and patients with mild cognitive impairment. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-95573-8

Plassman, B. L., & Grafman, J. (2015). Traumatic brain injury and late-life dementia. In *Handbook of Clinical Neurology* (Vol. 128, pp. 711–722). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63521-1.00044-3

Plassman, B. L., Havlik, R. J., Steffens, D. C., Helms, M. J., Newman, T. N., Drosdick, D., Phillips, C., Gau, B. A., Welsh-Bohmer, K. A., Burke, J. R., Guralnik, J. M., & Breitner, J. C. S. (2000). Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology*, *55*(8), 1158–1166. https://doi.org/10.1212/WNL.55.8.1158

Porquet, D., Casadesús, G., Bayod, S., Vicente, A., Canudas, A. M., Vilaplana, J., Pelegrí, C., Sanfeliu, C., Camins, A., Pallàs, M., & Del Valle, J. (2013). Dietary resveratrol prevents Alzheimer's markers and increases life span in SAMP8. *Age*, *35*(5). https://doi.org/10.1007/s11357-012-9489-4

Posner, M. I., & Petersen, S. E. (1990). The attention system of the human brain. *Annual Review of Neuroscience*, *13*(1). https://doi.org/10.1146/annurev.neuro.13.1.25

Povlishock, J. T., & Katz, D. I. (2005). Update of neuropathology and neurological recovery after traumatic brain injury. In *Journal of Head Trauma Rehabilitation* (Vol. 20, Issue 1). https://doi.org/10.1097/00001199-200501000-00008

Prado, V. F., Martins-Silva, C., de Castro, B. M., Lima, R. F., Barros, D. M., Amaral, E., Ramsey, A. J., Sotnikova, T. D., Ramirez, M. R., Kim, H. G., Rossato, J. I., Koenen, J., Quan, H., Cota, V. R., Moraes, M. F. D., Gomez, M. V., Guatimosim, C., Wetsel, W. C., Kushmerick, C., … Prado, M. A. M. (2006). Mice Deficient for the Vesicular Acetylcholine Transporter Are Myasthenic and Have Deficits in Object and Social Recognition. *Neuron*, *51*(5), 601–612. https://doi.org/10.1016/j.neuron.2006.08.005

Prado, V. F., Roy, A., Kolisnyk, B., Gros, R., & Prado, M. A. M. (2013). Regulation of cholinergic activity by the vesicular acetylcholine transporter. In *Biochemical Journal* (Vol. 450, Issue 2). https://doi.org/10.1042/BJ20121662

Price, J. L., McKeel, D. W., Buckles, V. D., Roe, C. M., Xiong, C., Grundman, M., Hansen, L. A., Petersen, R. C., Parisi, J. E., Dickson, D. W., Smith, C. D., Davis, D. G., Schmitt, F. A., Markesbery, W. R., Kaye, J., Kurlan, R., Hulette, C., Kurland, B. F., Higdon, R., … Morris, J. C. (2009). Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. *Neurobiology of Aging*, *30*(7). https://doi.org/10.1016/j.neurobiolaging.2009.04.002

Public Health Agency of Canada. (2017). *Dementia in Canada, Including Alzheimer's Disease Highlights From the Canadian Chronic How Many Canadians Live With Dementia , Including*. 5–10.

Raj, A., LoCastro, E., Kuceyeski, A., Tosun, D., Relkin, N., & Weiner, M. (2015). Network diffusion model of progression predicts longitudinal patterns of atrophy and metabolism in Alzheimer's disease. *Cell Rep.*, *10*(3), 359–369. https://doi.org/10.1016/j.celrep.2014.12.034

Ramlackhansingh, A. F., Brooks, D. J., Greenwood, R. J., Bose, S. K., Turkheimer, F. E., Kinnunen, K. M., Gentleman, S., Heckemann, R. A., Gunanayagam, K., Gelosa, G., & Sharp, D. J. (2011). Inflammation after trauma: Microglial activation and traumatic brain injury. *Annals of Neurology*, *70*(3), 374–383. https://doi.org/10.1002/ana.22455

Reagan, A. M., Onos, K. D., Heuer, S. E., Sasner, M., & Howell, G. R. (2022). Improving mouse models for the study of Alzheimer's disease. In *Current Topics in Developmental Biology* (Vol. 148). https://doi.org/10.1016/bs.ctdb.2021.12.005

Reitz, C., Rogaeva, E., & Beecham, G. W. (2020). Late-onset vs nonmendelian early-onset Alzheimer disease. *Neurology Genetics*, *6*(5). https://doi.org/10.1212/nxg.0000000000000512

Robbins, T. W. (2002). The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. In *Psychopharmacology* (Vol. 163, Issues 3–4). https://doi.org/10.1007/s00213-002-1154-7

Robertson, I. H., Manly, T., Andrade, J., Baddeley, B. T., & Yiend, J. (1997). "Oops!": Performance correlates of everyday attentional failures in traumatic brain injured and normal subjects. *Neuropsychologia*, *35*(6), 747–758. https://doi.org/10.1016/S0028-3932(97)00015-8

Robertson, T. A., Dutton, N. S., Martins, R. N., Roses, A. D., Kakulas, B. A., & Papadimitriou, J. M. (1997). Age-related congophilic inclusions in the brains of apolipoprotein e- deficient mice. *Neuroscience*, *82*(1). https://doi.org/10.1016/S0306-4522(97)00284-4

Romberg, C., Bussey, T. J., & Saksida, L. M. (2013). Paying more attention to attention: Towards more comprehensive cognitive translation using mouse models of Alzheimer's disease. In *Brain Research Bulletin* (Vol. 92). https://doi.org/10.1016/j.brainresbull.2012.02.007

Romberg, C., Mattson, M. P., Mughal, M. R., Bussey, T. J., & Saksida, L. M. (2011). Impaired Attention in the 3xTgAD Model of Alzheimer's Disease Assessed Using a

Translational Touchscreen Method for Mice: Rescue by Donepezil (Aricept). *National Institute of Health*, *31*(9).

Rosvold, H. E., Mirsky, A. F., Sarason, I., Bransome, E. D., & Beck, L. H. (1956). A continuous performance test of brain damage. *Journal of Consulting Psychology*, *20*(5). https://doi.org/10.1037/h0043220

Rowson, S., Duma, S. M., Beckwith, J. G., Chu, J. J., Greenwald, R. M., Crisco, J. J., Brolinson, P. G., Duhaime, A. C., McAllister, T. W., & Maerlender, A. C. (2012). Rotational head kinematics in football impacts: An injury risk function for concussion. *Annals of Biomedical Engineering*, *40*(1), 1–13. https://doi.org/10.1007/s10439-011-0392-4

Russo, M. J., Cohen, G., Campos, J., Martin, M. E., Clarens, M. F., Sabe, L., Barcelo, E., & Allegri, R. F. (2017). Usefulness of Discriminability and Response Bias Indices for the Evaluation of Recognition Memory in Mild Cognitive Impairment and Alzheimer Disease. *Dementia and Geriatric Cognitive Disorders*, *43*(1–2). https://doi.org/10.1159/000452255

Sahakian, B. J., & Coull, J. T. (1993). Tetrahydroaminoacridine (THA) in Alzheimer's disease: An assessment of attentional and mnemonic function using CANTAB. *Acta Neurologica Scandinavica*, *88*(149 S). https://doi.org/10.1111/j.1600-0404.1993.tb04251.x

Saito, T., Matsuba, Y., Mihira, N., Takano, J., Nilsson, P., Itohara, S., Iwata, N., & Saido, T. C. (2014). Single App knock-in mouse models of Alzheimer's disease. *Nature Neuroscience*, *17*(5). https://doi.org/10.1038/nn.3697

Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., Zhang, B., Murayama, S., Higuchi, M., Lee, V. M. Y., Trojanowski, J. Q., & Saido, T. C. (2019). Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *Journal of Biological Chemistry*, *294*(34), 12754–12765. https://doi.org/10.1074/jbc.RA119.009487

Salmond, C. H., Chatfield, D. A., Menon, D. K., Pickard, J. D., & Sahakian, B. J. (2005). Cognitive sequelae of head injury: Involvement of basal forebrain and associated structures. *Brain*, *128*(1), 189–200. https://doi.org/10.1093/brain/awh352

Sarkar, C., Zhao, Z., Aungst, S., Sabirzhanov, B., Faden, A. I., & Lipinski, M. M. (2014). Impaired autophagy flux is associated with neuronal cell death after traumatic brain injury. *Autophagy*, *10*(12). https://doi.org/10.4161/15548627.2014.981787

Sarter, M., & Bruno, J. P. (1999). Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: Differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience*, *95*(4), 933–952. https://doi.org/10.1016/S0306-4522(99)00487-X

Sarter, M., & Bruno, J. P. (2004). Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities. In *Neurobiology of Aging* (Vol. 25, Issue 9). https://doi.org/10.1016/j.neurobiolaging.2003.11.011

Sarter, M., Hasselmo, M. E., Bruno, J. P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: Interactions between signal-driven and cognitive modulation of signal detection. In *Brain Research Reviews* (Vol. 48, Issue 1, pp. 98–111). Elsevier. https://doi.org/10.1016/j.brainresrev.2004.08.006

Sarter, M., & Parikh, V. (2005). Choline transporters, cholinergic transmission and cognition. *Nature Reviews Neuroscience*, *6*(1), 48–56. https://doi.org/10.1038/nrn1588

Scheff, S. W., Price, D. A., Schmitt, F. A., & Mufson, E. J. (2006). Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiology of Aging*, *27*(10). https://doi.org/10.1016/j.neurobiolaging.2005.09.012

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. In *Behavioural Brain Research* (Vol. 221, Issue 2). https://doi.org/10.1016/j.bbr.2010.11.058

Schmitz, T. W., Mur, M., Aghourian, M., Bedard, M. A., & Spreng, R. N. (2018). Longitudinal Alzheimer's Degeneration Reflects the Spatial Topography of Cholinergic Basal Forebrain Projections. *Cell Reports*, *24*(1), 38–46. https://doi.org/10.1016/j.celrep.2018.06.001

Schmitz, T. W., & Nathan Spreng, R. (2016). Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology. *Nature Communications*, *7*(1), 1–13. https://doi.org/10.1038/ncomms13249

Schmitz, T. W., Soreq, H., Judes Poirier, X., & Nathan Spreng, X. R. (2020). Longitudinal basal forebrain degeneration interacts with TREM2/C3 biomarkers of inflammation in presymptomatic Alzheimer's disease. *Journal of Neuroscience*, *40*(9), 1931–1942. https://doi.org/10.1523/JNEUROSCI.1184-19.2019

Schneider, L. S., Mangialasche, F., Andreasen, N., Feldman, H., Giacobini, E., Jones, R., Mantua, V., Mecocci, P., Pani, L., Winblad, B., & Kivipelto, M. (2014). Clinical trials and late-stage drug development for Alzheimer's disease: An appraisal from 1984 to 2014. *Journal of Internal Medicine*, *275*(3). https://doi.org/10.1111/joim.12191

Scott, G., Ramlackhansingh, A. F., Edison, P., Hellyer, P., Cole, J., Veronese, M., Leech, R., Greenwood, R. J., Turkheimer, F. E., Gentleman, S. M., Heckemann, R. A., Matthews, P. M., Brooks, D. J., & Sharp, D. J. (2016). Amyloid pathology and axonal injury after brain trauma. *Neurology*, *86*(9), 821–828. https://doi.org/10.1212/WNL.0000000000002413

Seidu, N. M., Kern, S., Sacuiu, S., Sterner, T. R., Blennow, K., Zetterberg, H., Lindberg, O., Ferreira, D., Westman, E., Zettergren, A., & Skoog, I. (2024). Association of CSF biomarkers with MRI brain changes in Alzheimer's disease. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, *16*(1). https://doi.org/10.1002/dad2.12556

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. In *Neuron* (Vol. 6, Issue 4, pp. 487–498). https://doi.org/10.1016/0896-6273(91)90052-2

Selkoe, D. J. (1994). Normal and abnormal biology of the β-amyloid precursor protein. In *Annual Review of Neuroscience* (Vol. 17). https://doi.org/10.1146/annurev.ne.17.030194.002421

Selkoe, D. J. (1998). The cell biology β-amyloid precursor protein and presenilin in Alzheimer's disease. In *Trends in Cell Biology* (Vol. 8, Issue 11). https://doi.org/10.1016/S0962-8924(98)01363-4

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. In *Science* (Vol. 298, Issue 5594). https://doi.org/10.1126/science.1074069

Shah-Basak, P. P., Urbain, C., Wong, S., Da Costa, L., Pang, E. W., Dunkley, B. T., & Taylor, M. J. (2018). Concussion Alters the Functional Brain Processes of Visual Attention and Working Memory. *Journal of Neurotrauma*, *35*(2), 267–277. https://doi.org/10.1089/neu.2017.5117

Shankar, G. M., Bloodgood, B. L., Townsend, M., Walsh, D. M., Selkoe, D. J., & Sabatini, B. L. (2007). Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *Journal of Neuroscience*, *27*(11). https://doi.org/10.1523/JNEUROSCI.4970-06.2007

Shao, L., Ciallella, J. R., Yan, H. Q., Ma, X., Wolfson, B. M., Marion, D. W., Dekosky, S. T., & Dixon, C. E. (1999). Differential effects of traumatic brain injury on vesicular acetylcholine transporter and M2 muscarinic receptor mRNA and protein in rat. *Journal of Neurotrauma*, *16*(7), 555–566. https://doi.org/10.1089/neu.1999.16.555

Shepherd, A., May, C., Churilov, L., Adlard, P. A., Hannan, A. J., & Burrows, E. L. (2021). Evaluation of attention in APP/PS1 mice shows impulsive and compulsive behaviours. *Genes, Brain and Behavior*, *20*(1). https://doi.org/10.1111/gbb.12594

Shepherd, A., Zhang, T., Hoffmann, L. B., Zeleznikow-Johnston, A. M., Churilov, L., Hannan, A. J., & Burrows, E. L. (2021). A Preclinical Model of Computerized Cognitive Training: Touchscreen Cognitive Testing Enhances Cognition and Hippocampal Cellular

Plasticity in Wildtype and Alzheimer's Disease Mice. *Frontiers in Behavioral Neuroscience*, *15*. https://doi.org/10.3389/fnbeh.2021.766745

Simon, D. W., McGeachy, M. J., Baylr, H., Clark, R. S. B., Loane, D. J., & Kochanek, P. M. (2017). The far-reaching scope of neuroinflammation after traumatic brain injury. In *Nature Reviews Neurology* (Vol. 13, Issue 3, pp. 171–191). Nature Publishing Group. https://doi.org/10.1038/nrneurol.2017.13

Smith, A. M., Davey, K., Tsartsalis, S., Khozoie, C., Fancy, N., Tang, S. S., Liaptsi, E., Weinert, M., McGarry, A., Muirhead, R. C. J., Gentleman, S., Owen, D. R., & Matthews, P. M. (2022). Diverse human astrocyte and microglial transcriptional responses to Alzheimer's pathology. *Acta Neuropathologica*, *143*(1). https://doi.org/10.1007/s00401-021-02372-6

Smith, D. H., Meaney, D. F., & Shull, W. H. (2003). Diffuse axonal injury in head trauma. In *Journal of Head Trauma Rehabilitation* (Vol. 18, Issue 4). https://doi.org/10.1097/00001199-200307000-00003

Smith, D. H., Soares, H. D., Pierce, J. S., Perlman, K. G., Saatman, K. E., Meaney, D. F., Dixon, C. E., & Mcintosh, T. K. (1995). A Model of Parasagittal Controlled Cortical Impact in the Mouse: Cognitive and Histopathologic Effects. *Journal of Neurotrauma*, *12*(2). https://doi.org/10.1089/neu.1995.12.169

Snyder, H. M., Asthana, S., Bain, L., Brinton, R., Craft, S., Dubal, D. B., Espeland, M. A., Gatz, M., Mielke, M. M., Raber, J., Rapp, P. R., Yaffe, K., & Carrillo, M. C. (2016). Sex biology contributions to vulnerability to Alzheimer's disease: A think tank convened by the Women's Alzheimer's Research Initiative. In *Alzheimer's and Dementia* (Vol. 12, Issue 11). https://doi.org/10.1016/j.jalz.2016.08.004

Song, H. J., Ming, G. L., Fon, E., Bellocchio, E., Edwards, R. H., & Poo, M. M. (1997). Expression of a putative vesicular acetylcholine transporter facilitates quantal transmitter packaging. *Neuron*, *18*(5). https://doi.org/10.1016/S0896-6273(00)80320-7

Sperling, R. A., Jack, C. R., & Aisen, P. S. (2011). Testing the right target and right drug at the right stage. In *Science Translational Medicine*. https://doi.org/10.1126/scitranslmed.3002609

St Peters, M., Demeter, E., Lustig, C., Bruno, J. P., & Sarter, M. (2011). *Enhanced Control of Attention by Stimulating Mesolimbic-Corticopetal Cholinergic Circuitry*. https://doi.org/10.1523/JNEUROSCI.1902-11.2011

Stankiewicz, P., Kulkarni, S., Dharmadhikari, A. V., Sampath, S., Bhatt, S. S., Shaikh, T. H., Xia, Z., Pursley, A. N., Cooper, M. L., Shinawi, M., Paciorkowski, A. R., Grange, D. K., Noetzel, M. J., Saunders, S., Simons, P., Summar, M., Lee, B., Scaglia, F., Fellmann, F., … Shaffer, L. G. (2012). Recurrent deletions and reciprocal duplications of 10q11.21q11.23 including CHAT and SLC18A3 are Likely Mediated by Complex Low-Copy Repeats. *Human Mutation*, *33*(1). https://doi.org/10.1002/humu.21614

Stefanoska, K., Gajwani, M., Tan, A. R. P., Ahel, H. I., Asih, P. R., Volkerling, A., Poljak, A., & Ittner, A. (2022). Alzheimer's disease: Ablating single master site abolishes tau hyperphosphorylation. *Science Advances*, *8*(27). https://doi.org/10.1126/sciadv.abl8809

Stopford, C. L., Thompson, J. C., Neary, D., Richardson, A. M. T., & Snowden, J. S. (2012). Working memory, attention, and executive function in Alzheimer's disease and frontotemporal dementia. *Cortex*, *48*(4), 429–446. https://doi.org/10.1016/j.cortex.2010.12.002

Su, Y., Zhang, W., Zhang, R., Yuan, Q., Wu, R., Liu, X., Wuri, J., Li, R., & Yan, T. (2022). Activation of Cholinergic Anti-Inflammatory Pathway Ameliorates Cerebral and Cardiac Dysfunction After Intracerebral Hemorrhage Through Autophagy. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.870174

Sugarman, M. A., McKee, A. C., Stein, T. D., Tripodis, Y., Besser, L. M., Martin, B., Palmisano, J. N., Steinberg, E. G., O'Connor, M. K., Au, R., McClean, M., Killiany, R., Mez, J., Weiner, M. W., Kowall, N. W., Stern, R. A., & Alosco, M. L. (2019). Failure to detect an association between self-reported traumatic brain injury and Alzheimer's

disease neuropathology and dementia. *Alzheimer's and Dementia*, *15*(5). https://doi.org/10.1016/j.jalz.2018.12.015

Sultzer, D. L., Lim, A. C., Gordon, H. L., Yarns, B. C., & Melrose, R. J. (2022). Cholinergic receptor binding in unimpaired older adults, mild cognitive impairment, and Alzheimer's disease dementia. *Alzheimer's Research & Therapy*, *14*(1). https://doi.org/10.1186/S13195-021-00954-W

Tagge, C. A., Fisher, A. M., Minaeva, O. V., Gaudreau-Balderrama, A., Moncaster, J. A., Zhang, X. L., Wojnarowicz, M. W., Casey, N., Lu, H., Kokiko-Cochran, O. N., Saman, S., Ericsson, M., Onos, K. D., Veksler, R., Senatorov, V. V., Kondo, A., Zhou, X. Z., Miry, O., Vose, L. R., … Goldstein, L. E. (2018). Concussion, microvascular injury, and early tauopathy in young athletes after impact head injury and an impact concussion mouse model. *Brain*, *141*(2), 422–458. https://doi.org/10.1093/brain/awx350

Takeda, A., Loveman, E., Clegg, A., Kirby, J., Picot, J., Payne, E., & Green, C. (2006). A systematic review of the clinical effectiveness of donepezil, rivastigmine and galantamine on cognition, quality of life and adverse events in Alzheimer's disease. In *International Journal of Geriatric Psychiatry* (Vol. 21, Issue 1). https://doi.org/10.1002/gps.1402

Tannenbaum, C., Paquette, A., Hilmer, S., Holroyd-Leduc, J., & Carnahan, R. (2012). A systematic review of amnestic and non-amnestic mild cognitive impairment induced by anticholinergic, antihistamine, GABAergic and opioid drugs. In *Drugs and Aging* (Vol. 29, Issue 8). https://doi.org/10.1007/bf03262280

Tapias, V., Moschonas, E. H., Bondi, C. O., Vozzella, V. J., Cooper, I. N., Cheng, J. P., Lajud, N., & Kline, A. E. (2022). Environmental enrichment improves traumatic brain injury-induced behavioral phenotype and associated neurodegenerative process. *Experimental Neurology*, *357*. https://doi.org/10.1016/j.expneurol.2022.114204

Teipel, S., Ewers, M., Krause, B., Heinsen, H., & Grothe, M. (2012). O1-10-05: Increased cerebral amyloid load is associated with reduced volume of the cholinergic basal forebrain in nondemented elderly. *Alzheimer's & Dementia*, *8*(4S_Part_3). https://doi.org/10.1016/j.jalz.2012.05.261

Teipel, S. J., Meindl, T., Grinberg, L., Grothe, M., Cantero, J. L., Reiser, M. F., Möller, H. J., Heinsen, H., & Hampel, H. (2011). The cholinergic system in mild cognitive impairment and Alzheimer's disease: An in Vivo MRI and DTI study. *Human Brain Mapping*, *32*(9), 1349–1362. https://doi.org/10.1002/HBM.21111

Theadom, A., Parag, V., Dowell, T., McPherson, K., Starkey, N., Barker-Collo, S., Jones, K., Ameratunga, S., & Feigin, V. L. (2016). Persistent problems 1 year after mild traumatic brain injury: A longitudinal population study in New Zealand. *British Journal of General Practice*, *66*(642). https://doi.org/10.3399/bjgp16X683161

Tiernan, C. T., Ginsberg, S. D., He, B., Ward, S. M., Guillozet-Bongaarts, A. L., Kanaan, N. M., Mufson, E. J., & Counts, S. E. (2018). Pretangle pathology within cholinergic nucleus basalis neurons coincides with neurotrophic and neurotransmitter receptor gene dysregulation during the progression of Alzheimer's disease. *Neurobiology of Disease*, *117*, 125–136. https://doi.org/10.1016/j.nbd.2018.05.021

Tiernan, C. T., Mufson, E. J., Kanaan, N. M., & Counts, S. E. (2018). Tau oligomer pathology in nucleus basalis neurons during the progression of Alzheimer Disease. *Journal of Neuropathology and Experimental Neurology*, *77*(3), 246–259. https://doi.org/10.1093/jnen/nlx120

Tramutola, A., Triplett, J. C., Di Domenico, F., Niedowicz, D. M., Murphy, M. P., Coccia, R., Perluigi, M., & Allan Butterfield, D. (2015). Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): Analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. *Journal of Neurochemistry*, *133*(5). https://doi.org/10.1111/jnc.13037

Van Geldorp, B., Heringa, S. M., Van Den Berg, E., Olde Rikkert, M. G. M., Biessels, G. J., & Kessels, R. P. C. (2015). Working memory binding and episodic memory formation in aging, mild cognitive impairment, and Alzheimers dementia. *Journal of Clinical and Experimental Neuropsychology*, *37*(5). https://doi.org/10.1080/13803395.2015.1037722

Veening-Griffioen, D. H., Ferreira, G. S., van Meer, P. J. K., Boon, W. P. C., Gispen-de Wied, C. C., Moors, E. H. M., & Schellekens, H. (2019). Are some animal models more equal

than others? A case study on the translational value of animal models of efficacy for Alzheimer's disease. *European Journal of Pharmacology*, *859*. https://doi.org/10.1016/j.ejphar.2019.172524

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., & Selkoe, D. J. (2002). Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*, *416*(6880). https://doi.org/10.1038/416535a

Wander, C. M., Tseng, J. H., Song, S., Al Housseiny, H. A., Tart, D. S., Ajit, A., Ian Shih, Y. Y., Lobrovich, R., Song, J., Meeker, R. B., Irwin, D. J., & Cohen, T. J. (2020). The Accumulation of Tau-Immunoreactive Hippocampal Granules and Corpora Amylacea Implicates Reactive Glia in Tau Pathogenesis during Aging. *IScience*, *23*(7). https://doi.org/10.1016/j.isci.2020.101255

Wander, C. M., Tsujimoto, T. H. M., Ervin, J. F., Wang, C., Maranto, S. M., Bhat, V., Dallmeier, J. D., Wang, S. H. J., Lin, F. C., Scott, W. K., Holtzman, D. M., & Cohen, T. J. (2022). Corpora amylacea are associated with tau burden and cognitive status in Alzheimer's disease. *Acta Neuropathologica Communications*, *10*(1). https://doi.org/10.1186/s40478-022-01409-5

Weigand, A. J., Bondi, M. W., Thomas, K. R., Campbell, N. L., Galasko, D. R., Salmon, D. P., Sewell, D., Brewer, J. B., Feldman, H. H., & Delano-Wood, L. (2020). Association of anticholinergic medications and AD biomarkers with incidence of MCI among cognitively normal older adults. *Neurology*, *95*(16), e2295–e2304. https://doi.org/10.1212/WNL.0000000000010643

Weiner, M. W., Harvey, D., Landau, S. M., Veitch, D. P., Neylan, T. C., Grafman, J. H., Aisen, P. S., Petersen, R. C., Jack, C. R., Tosun, D., Shaw, L. M., Trojanowski, J. Q., Saykin, A. J., Hayes, J., & De Carli, C. (2023). Traumatic brain injury and post-traumatic stress disorder are not associated with Alzheimer's disease pathology measured with biomarkers. *Alzheimer's and Dementia*, *19*(3), 884–895. https://doi.org/10.1002/ALZ.12712

Whitwell, J. L., Przybelski, S. A., Weigand, S. D., Knopman, D. S., Boeve, B. F., Petersen, R. C., & Jack, C. R. (2007). 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. *Brain*, *130*(7). https://doi.org/10.1093/brain/awm112

Wingo, T. S., Lah, J. J., Levey, A. I., & Cutler, D. J. (2012). Autosomal recessive causes likely in early-onset Alzheimer disease. *Archives of Neurology*, *69*(1). https://doi.org/10.1001/archneurol.2011.221

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. In *Progress in Neurobiology* (Vol. 37, Issue 6). https://doi.org/10.1016/0301-0082(91)90006-M

Xu, X., Cowan, M., Beraldo, F., Schranz, A., McCunn, P., Geremia, N., Brown, Z., Patel, M., Nygard, K. L., Khazaee, R., Lu, L., Liu, X., Strong, M. J., Dekaban, G. A., Menon, R., Bartha, R., Daley, M., Mao, H., Prado, V., … Brown, A. (2021). Repetitive mild traumatic brain injury in mice triggers a slowly developing cascade of long-term and persistent behavioral deficits and pathological changes. *Acta Neuropathologica Communications*, *9*(1). https://doi.org/10.1186/s40478-021-01161-2

Yang, S. T., Hsiao, I. T., Hsieh, C. J., Chiang, Y. H., Yen, T. C., Chiu, W. T., Lin, K. J., & Hu, C. J. (2015). Accumulation of amyloid in cognitive impairment after mild traumatic brain injury. *Journal of the Neurological Sciences*, *349*(1–2). https://doi.org/10.1016/j.jns.2014.12.032

Yoshiyama, Y., Kojima, A., Itoh, K., Uchiyama, T., & Arai, K. (2012). Anticholinergics boost the pathological process of neurodegeneration with increased inflammation in a tauopathy mouse model. *Neurobiology of Disease*, *45*(1), 329–336. https://doi.org/10.1016/J.NBD.2011.08.017

Zane, K. L., Gfeller, J. D., Roskos, P. T., & Bucholz, R. D. (2016). The Clinical Utility of the Conners' Continuous Performance Test-II in Traumatic Brain Injury. *Archives of Clinical Neuropsychology*, *31*(8), 996–1005. https://doi.org/10.1093/arclin/acw078

Zeleznikow-Johnston, A. M., Burrows, E. L., Renoir, T., & Hannan, A. J. (2023). Translatable Models of Brain and Cognitive Reserve. In *Contemporary Clinical Neuroscience: Vol. Part F276*. https://doi.org/10.1007/978-3-031-24930-3_4

Zhang, J., Yoganandan, N., Pintar, F. A., & Gennarelli, T. A. (2006). Role of translational and rotational accelerations on brain strain in lateral head impact. *Technical Papers of ISA*, *464*.

Zhao, N., Ren, Y., Yamazaki, Y., Qiao, W., Li, F., Felton, L. M., Mahmoudiandehkordi, S., Kueider-Paisley, A., Sonoustoun, B., Arnold, M., Shue, F., Zheng, J., Attrebi, O. N., Martens, Y. A., Li, Z., Bastea, L., Meneses, A. D., Chen, K., Thompson, J. W., … Bu, G. (2020). Alzheimer's Risk Factors Age, APOE Genotype, and Sex Drive Distinct Molecular Pathways. *Neuron*, *106*(5). https://doi.org/10.1016/j.neuron.2020.02.034

Appendices

Appendix A: Animal Use Protocol approval.

AUP Number: 2022-174

PI Name: Brown, Arthur

AUP Title: Investigations of CNS Injury and Regenerative Therapies - Mouse

Yearly Renewal Date: 03/01/2025

The **annual renewal** to Animal Use Protocol (AUP) 2022-174 has been approved by the Animal Care Committee (ACC), and will be approved through to the above review date.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP. As per your declaration within this approved AUP, you are obligated to ensure that:

1. This Animal Use Protocol is in compliance with:

   Western's Senate MAPP 7.12 [PDF]; and

   Applicable Animal Care Committee policies and procedures.

2. Prior to initiating any study-related activities—as per institutional OH&S policies—all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have:

   Completed the appropriate institutional OH&S training;

   Completed the appropriate facility-level training; and

   Reviewed related (M)SDS Sheets.

Submitted by: McInnis, Jennifer on behalf of the Animal Care Committee

Curriculum Vitae

Elizabeth Teasell

Academic Background

M.Sc Neuroscience 2021-present
Supervisors: Dr. Arthur Brown and Dr. Marco Prado
Schulich School of Medicine and Dentistry
University of Western Ontario
London, Ontario, Canada

B.Sc (Honours) Neuroscience 2017-2021
Schulich School of Medicine and Dentistry
University of Western Ontario
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Awards and Scholarships:

Bright Focus Diversity travel award
BrightFocus
Valued at $500 for research in discovering cures for Alzheimer's disease, macular degeneration and glaucoma 2023

Undergraduate Student Research Award, 2020
Supervisor: David Purcell at National Centre for Audiology
University of Western University, London, Ontario

Related Work Experience

Animal Care Assistant 2024
Robarts Research Institute
University of Western Ontario

Abstracts and Presentations:

Teasell E, Geremia N, Moradi N, Xu K, Prado V, Prado M, Brown A. May 2023
Traumatic brain injury and cholinergic dysfunction as determinants of Alzheimer's disease susceptibility in a humanized mouse model.
Abstract and Poster presentation, Canadian Association for Neuroscience Conference 2023, Montreal, Quebec, Canada

Teasell E, Geremia N, Xu K, Prado V, Prado M, Brown A. June 2023
Evaluating mild TBI and cholinergic dysfunction as determinants of

Alzheimer's disease susceptibility in hTau and hAPP mice, Presentation, Touchscreen Symposium 2023, University of Western Ontario, London, Ontario, Canada

Teasell E, Geremia N, Xu K, Prado V, Prado M, Brown A. June 2022
Investigating the role of traumatic brain injury and cholinergic dysfunction as determinants of Alzheimer's disease susceptibility in a mouse carrying humanized amyloid and tau, Abstract and poster, Robarts Research Retreat 2022, University of Western Ontario, London, Ontario, Canada