ERP-based detection of brain pathology in rat models for preclinical Alzheimer’s disease

by

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University of Toronto

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Abstract

Early pathological features of Alzheimer’s disease (AD) include the accumulation of hyperphosphorylated tau protein (HP-tau) in the entorhinal cortex and progressive loss of basal forebrain (BF) cholinergic neurons. These pathologies are known to remain asymptomatic for many years before AD is clinically diagnosed; however, they may induce aberrant brain processing which can be captured as an abnormality in event-related potentials (ERPs). Here, we examined cortical ERPs while a differential associative learning paradigm was applied to adult male rats with entorhinal HP-tau, pharmacological blockade of muscarinic acetylcholine receptors, or both conditions. Despite no impairment in differential associative and reversal learning, each pathological feature induced distinct abnormality in cortical ERPs to an extent that was sufficient for machine classifiers to accurately detect a specific type of pathology based on these ERP features. These results highlight a potential use of ERPs during differential associative learning as a biomarker for asymptomatic AD pathology.
Acknowledgments

“If I have seen further it is only by standing on the shoulders of giants.”
-Issac Newton (1642-1727)

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Chapter 1
Introduction

1.1 The history and background

In 1910, Emil Kraepelin published the 8th edition of the book *Psychiatrie* in which he included a description of work done by his student, Alois Alzheimer (1). The name Alzheimer’s disease (AD) was coined by Kraepelin for description of symptoms and pathology of the disease. In his work Alzheimer observed memory impairment and cognitive worsening in his 55 year old patient, Auguste Deter, and described the presence of neurofibrillary tangles (NFTs) and senile plaques (SPs) in a postmortem sample of her brain (2).

Today, AD is the most common and well-known form of dementia affecting the increasingly elderly population. Included in AD symptoms are deterioration of memory and cognitive functions that progress over 10 to 15 years and are associated with the formation of SPs comprised of amyloid beta (Aβ), accumulation of NFTs consisting of hyperphosphorylated tau (HP-tau), and progressive atrophy of cholinergic neurons in the basal forebrain (BF) (Figure 1) (3-8).

Currently, over 750,000 Canadians 65 and older are suffering from AD and the direct and indirect costs of AD reaches a total of $10.4 billion per year (9). Despite recent advancements in AD therapeutics, it remains as one of the most challenging diseases to tackle. This is due to the long asymptomatic course of progression of AD which cannot be detected by current primary screening and diagnostic criteria largely rely on conventional tests on memory and executive function in at-risk individuals.

To put things into perspective, we can compare AD to an uncontrollable train that is speeding towards the end of a railway track. The onset of the first pathological process in the brain starts when the motionless train begins to move. At first, it goes unnoticed that the train is gaining speed until it approaches the end of the track. The time that has elapsed up until this moment represents the preclinical stage of the disease where cognitive symptoms are limited in spite of extensive brain pathology (10). As pathology becomes more severe, it ultimately reach a threshold where cognitive symptoms become evident. Returning to the train journey, we manage to board the
speeding train in hopes of preventing the imminent crash by pulling the emergency break, only to
discover that the break has been damaged and the crash is inevitable. Similarly, once AD is
diagnosed it is too late for it to be cured or slowed down, since the pathology has already caused
severe damage to the brain. The remaining options for patients after AD diagnosis are to make
arrangements for assisted living and use treatments that may ease a subset of disease symptoms
(11).

To change the series of event surrounding the train we must notice the train as soon as it begins to
move and apply the emergency break before it becomes broken. In AD these actions respectively
translate into preclinical diagnosis prior to development of extensive and irreversible brain
damage, and development of disease-modifying or arresting treatments. Hence, it is imperative to
detect AD in its preclinical stage where there might still be a chance to halt or significantly delay
the advancement of the disease.
Figure 1. Pathological hallmarks of Alzheimer's disease

Figure 1. A) A neuron displaying early pathological hallmarks of Alzheimer’s disease. Accumulation of intracellular neurofibrillary tangles (NFTs; red) comprised of hyperphosphorylated tau protein, and formation of extracellular senile plaques (SPs; purple) of amyloid beta aggregates.

Figure 1. B) Projections of basal forebrain cholinergic neurons from the basal nucleus (Bas) and medial septal nucleus (MS) to several different cortical and subcortical regions in a rat brain (Blue; Entorhinal cortex, EC; Basolateral amygdala, BLA). Through the course of Alzheimer’s disease, degeneration of basal forebrain cholinergic neurons results in significant reduction in cholinergic input and disruption of cholinergic modulation at the efferent brain region. Anatomical regions named according to the rat brain atlas (Paxinos G, Watson C., 2007).
1.2 Theories on etiology of Alzheimer’s disease

There has been several theories about the causes of AD since the first time it was described by Alois Alzheimer in 1907 (12). Once a correlation was found between the numbers of SPs, NFTs, and cognitive decline in 1960’s, researchers began probing the underlying mechanisms involved in formation of these pathologies (13). Moreover, AD attracted immense amount of research when it was officially listed as a neurological disorder in 1970’s and was no longer considered a part of normal aging (13).

Majority of current theories on AD pathogenesis emphasize the presence of excessive deposition of abnormal proteins found in SPs and NFTs, such as Aβ peptide and HP-tau. However, imbalance of brain’s homeostatic environment in addition to bacterial and viral agents that are able to cross the blood-brain-barrier have also been proposed as possible causes of AD (14-16) Here, the foremost theories on the causes of AD pathogenesis are presented.

1.2.1 Amyloid theory

One of the theories for the cause of AD involves accumulation of Aβ protein. Although much is not known about the function of Aβ protein, mutation in the gene of its precursor protein, amyloid precursor protein (APP), has been implicated in familial AD. APP is suggested to be important for neural growth (17-19) neural signaling, and may also function as a metalloprotein to mediate copper transport and metabolism (20, 21).

In most cell types APP undergoes the non-amyloidogenic pathway resulting in the production of the P3 peptide fragment, which consists of 16 amino acids and involves α-secretase cleavage followed by a γ-secretase cut within the Aβ domain of the APP protein (28, 29). The amyloidogenic pathway involves cleavage by β-secretase followed by the γ-secretase, releasing the 40-43 amino acid Aβ peptides which are secreted extracellularly and are the main component of amyloid plaques in AD (28-30). This longer form (Aβ42) is more hydrophobic and fibrillates more easily, and is also thought to be more neurotoxic than the Aβ40 peptide (28, 29). These peptides can be found as monomers, oligomers, and fibrils and in the latter stages form the SPs seen in the brains of AD patients.
It is speculated that aggregation of SPs as well as the soluble form of Aβ oligomers can have detrimental effects (31-33). This is supported by studies indicating the neurotoxicity of Aβ oligomers against neurons using transgenic mice that over-expressed APP (33). It has also been suggested that SPs may be brain’s defense mechanism that store and curb neurotoxic oligomers (34). Nonetheless, age-dependent elevation in cortical Aβ peptide level is attributed to decreased effectiveness of mechanisms responsible for its removal, such as phagocytosis by microglia and astrocytes, and enzymatic degradation by neprilysin and insulin-degrading enzyme (35).

Moreover, studies in rats have reported that Aβ oligomers inhibits long term potentiation (LTP) in the dentate gyrus of hippocampus (36, 37), and that they attenuate the neuronal excitability in mouse dentate gyrus by reducing the number of action potentials in response to current stimulation (38). Findings from other studies also suggest that accumulation of Aβ oligomers decreases the level of excitatory transmission at the level of synapse, and induces epileptiform discharges and abnormal pattern of neuronal circuit activity at the network level (39, 40).

The distribution of SP deposits in the brain initially starts in the neocortex and subsequently expands to the diencephalic nuclei, striatum and cholinergic nuclei in the BF, brainstem, and ultimately the cerebellum. Secondary to SP accumulations, NFT formation and neuronal dysfunction occur which provokes clinical symptoms of AD (43). Recently, the validity of the amyloid theory has been challenged as newly developed vaccines successfully removed SP deposits but failed to remove NFT or enhance patients’ cognition (44). The common occurrence of SP lesions in non-demented elderlies also sheds doubt upon their involvement in causing AD (45).

### 1.2.2 Tau theory

Due to the weak correlation between SPs and neuronal death in AD, researches begin to argue that maybe the other hallmark of AD pathology, HP-tau is responsible for the observed neuronal death in AD patients. Tau protein supports tubulin assembly into microtubules which provide structure and flexibility to the cell (46-48). Tau is ubiquitously found in the central nervous system and particularly in neurons. Alternative splicing and phosphorylation of tau messenger ribonucleic acid (mRNA) produces six different isoforms of the protein which are all expressed from MAPT gene.
on chromosome 17 (48, 49). These isoforms range from 441-452 amino acids and contain different number of binding domains, but all are present in AD (47-49). Tau phosphorylation and de-phosphorylation is a normal homeostatic process, however this mechanism is shifted towards tau hyper-phosphorylation in AD (48).

Disequilibrium in tau phosphorylation results in detachment of HP-tau from the microtubules and its aggregation into compact paired helical filaments (PHF) inside the cell. This results in disruption of microtubule assembly and ultimately leads to formation of NFTs and cell death observed in postmortem AD brains (47). After the death of the cell, NFTs remain as ghost tangles and can be observed by silver staining or immunohistochemical (IHC) techniques (50).

In addition to HP-tau as the main constituent of NFTs, heparin sulphate, fibroblast growth factor, casein kinase II, proteoglycan, ubiquitin, and microtubule association protein are also present in NFTs (22-25). It has been suggested that these proteins may act as a secondary mechanism for formation of brain lesions in AD (22-25, 50).

Braak and Braak classified AD progression into six stages (Braak I-VI) based on the site and density of NFT (3). In stages I-II (transentorhinal) NFTs are mostly confined to the entorhinal and perirhinal regions. Transentorhinal stage corresponds to the asymptomatic preclinical stage of AD (54). Stages III-IV (limbic) present with elevated incidence of NFTs in limbic structures, where the numbers of NFTs in hippocampus correlate with the severity of cognitive symptoms. Lastly, spread of NFTs into the isocortical regions completes AD progression in stages V and VI (54).

HP-tau and NFTs are also observed in other neurodegenerative disorders collectively known as tauopathies, including Frontotemporal dementia, Pick’s disease, sporadic corticobasal degeneration, argyrophilic grain disease, and supranuclear palsy (48, 64, 65). Studies have linked hereditary frontotemporal dementia and Parkinsonism to mutations in tau MAPT gene on chromosome 17 in absence of SP (66). Occurrence of neurodegenerative disorders such as hereditary frontotemporal dementia in patients with mutated tau and in absence of SP indicates that mutation in tau protein alone is sufficient to create a toxic form of tau that can cause damage and neuronal death.

Imbalance of activity between kinases and phosphatases responsible for tau modulation has also been proposed to contribute to NFT formation (31). It is an accepted theory that formation of HP-
tau occurs after Aβ accumulation has already begun (47, 48). However, due to its well-observed neurotoxicity and predictive ability of dementia severity, some suggest abnormal tau to be the initiator of neurodegeneration (47, 48, 67). Nonetheless, the exact mechanisms behind these lesions are still to be determined, including whether they are causative in AD, or a secondary events to some other underlying and as yet unknown pathology.

1.2.3 Cholinergic hypothesis

The cholinergic hypothesis was theorized based on the compelling evidence that showed BF cholinergic neurons, which send numerous projections to cortical and limbic structures, degenerate and result in a lower acetylcholine (ACh) level in AD patients compared to non-demented elderlies (68-70). It is suggested that degeneration may be a result of NFTs which were observed inside the cell body of the BF cholinergic neurons (71-77).

Implication of cholinergic system in AD was substantiated by post-mortem neurochemical analysis of brain tissue obtained from AD patients. Labelling of the enzyme, choline acetyltransferase (ChAT), responsible for synthesis of acetylcholine and specific to cholinergic neurons, showed a significant reduction (60-90 %) in ChAT activity in the cerebral cortex and hippocampal formation of patients who died of AD compared to age-matched non-demented elderlies. Conversely, analysis of muscarinic acetylcholine receptor (mAChR) distribution showed no difference in the density of the receptors between AD brains compared to healthy controls. Therefore, the cholinergic deficiency in AD is thought to be largely due to lack of presynaptic input rather than loss of postsynaptic receptors (73, 78).

Post-mortem studies provided the initial evidence for involvement of cholinergic system in AD neuropathology. Further evidence was provided by studies which demonstrated that suppression of cholinergic activity in normal individuals results in impairment of complex behavioral tasks (79, 80). It has been long known that a transient amnesic state could be induced by central mAChRs blockers (83). Administration of low doses of scopolamine, a non-competitive mAChRs blocker, to young adult volunteers resulted in impairment of recent memory but did not affect working or long term memories (84). Moreover, it is known that administration of central cholinergic potentiators improves formation of recent memory and negates the effect of mAChR blockers (85).
Together, these findings suggested that the impairment of short-term memory in early-stage AD is caused by disruption in cholinergic input and HP-tau induced hippocampal neural death (86-90).

Thus, AD treatments (donepezil, rivastigmine, and tacrine etc.) focused on increasing the levels of ACh in the brain by inhibiting cholinesterase, the enzyme responsible for ACh hydrolysis. However, long-term clinical assessments showed that cholinesterase inhibitors provide limited symptomatic treatment without any disease modifying effects (91). Limited effects of anticholinesterase drugs on AD symptoms plus the distressing side effects demonstrate the need for development of more efficacious therapies for patients with AD (92).

1.3 Alzheimer’s disease types

Dominant familial AD accounts for less than 1% of diagnosed patients (93). Familial AD is caused by genetic mutations and has an early manifestation of symptoms before the age of 65 (1, 94). Sporadic AD has a much higher prevalence, however the causes of its pathogenesis and the risks pertaining to the disease are still unclear.

1.3.1 Familial AD & genetic mutations

Much information has been gathered from familial AD about the pathogenesis of the disease in both familial and sporadic forms. Studies on inflicted individuals with familial AD revealed that mutations in either APP gene (APP, chromosome 21) or the genes of the proteins responsible for APP cleavage, Presenilin 1 (PSEN1, chromosome 14) and Presenilin 2 (PSEN2, chromosome 1) can cause elevated production of the longer isoform of Aβ peptide (95-97). This isoform may then aggregate to form SP which is proposed to disrupt neuronal signaling and ultimately result in neuronal death (99).
1.3.2 Sporadic AD

Sporadic AD accounts for the majority AD diagnosis. Currently, there are no definitive causes for sporadic AD, however it is speculated that multiple factors may be involved in instigating the dysfunction that leads into the manifestation of symptoms recognized as AD (100, 101). Recently, differentiation between AD subtypes has been proposed to be important for predicting disease progression and assigning corresponding treatments (102). It has been observed that different cases of the AD may present with variable proportions of AD pathological hallmarks, suggesting the existence of a SP-dominant and NFT-dominant AD subtypes (103-105). By incorporating different variables such as, cerebrospinal fluid (CSF) levels of tau and Aβ peptide, time of disease onset, and the number of apolipoprotein 4 (APOε4) alleles on chromosome 19, it has been suggested that AD may have up to five subgroups (102). As in many complex disorder, several factors such as genetic, dietary, and environmental may trigger AD pathogenesis and incite its progression.

1.4 Stages of Alzheimer’s disease

By incorporating recent scientific and technological advances, the new guideline for diagnostic criteria divides AD into three stages, including 1) dementia due to Alzheimer’s, 2) mild cognitive impairment (MCI), and 3) preclinical Alzheimer’s. Dementia due to AD is characterized by dysfunction of memory, thinking, and behavior which significantly interferes with individual’s functioning in everyday life (106). In MCI stage, deficits in memory and behavior are not severe enough to interfere with individual’s daily functions in life, however these deficits are observable and can be measured with mental status tests (107). Current findings suggest that in the preclinical stage detectable changes in brain may occur many years before manifestation of any impairment in memory, thinking, or behavior (108).

1.4.1 Dementia stage

Patients in this stage show apparent memory deficits, detected as disruption in episodic memory which allows an individual to consciously retrieve a previously experienced item or episode of life (109). In early AD, NFTs accumulate in the EC and hippocampus whereas Aβ plaques are first
observed in the neocortex (110-118). It is in these areas that the subsequent synapse dysfunction, neuronal loss and brain atrophy is first observed. (119-126). At the time of the clinical AD diagnosis, there is a substantial level of neuronal loss in the medial temporal lobes (MTL), and the location of damage appears to reflect the type cognitive impairments present (122-25) The damage spreads to the parietal and frontal lobes sequentially as the disease progresses (112, 127).

Cognitive disturbances in AD are required for clinical diagnosis and can be classified in several groups. (119, 128, 129). The degree of cognitive impairment is an important indicator of disease severity and can be utilized as a predictor and a risk factor for advancement of MCI to AD. Difficulties with delayed recall as well as episodic memory are two most common symptoms of AD at the time of diagnosis (76, 119, 130-132).

The new criteria revision of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA), suggests isolated disturbances in episodic memory a sufficient symptom for AD diagnosis (94). Moreover, based on the findings of large community cohort studies, early stage AD can be precisely differentiated from healthy state by task that examine episodic memory, perceptual speed, semantic memory, executive and visuospatial functions. (133-136).

Besides memory impairment at the initial stage of the disease, other classical cognitive symptoms include, agnosia, apraxia, and aphasia (119, 130). In patients with advanced AD, there is an evident worsening of initial symptoms and emergence of frontal lobe dysfunction which manifests as change in personality, mood fluctuation, and apathy (130). In late AD, majority of cognitive abilities are deteriorated and the patient is unable to communicate with the surrounding environment (130). In addition to cognitive deficits, depression is also commonly present in individuals with AD at the time of diagnosis (130). Overall, the sequence in which AD neuropathology advances in the brain dictates the order of the manifested cognitive deficits (112, 137).

1.4.2 Mild cognitive impairment stage

Mild cognitive impairment (MCI) is a heterogeneous condition that has been linked to an increased risk of developing AD. Neuropathological features of MCI resemble AD neuropathology with a
substantial amount of NFT and Aβ plaques (10, 104, 117, 121, 138-141). The prevalence of AD neuropathology in individuals with MCI is between 75-95%, with amnesic MCI having the highest prevalence (141, 142).

Consistent with previous studies, the number of NFTs in the MTL and the degree of neuronal death, particularly in the layer II of the EC, is substantially observed and is closely relate to memory dysfunction in MCI (115,140). Additionally, it has been reported that the intensity of neuropathology in MCI, including the degree of neuronal loss, the number of Aβ plaques in the neocortex, and NFTs in the MTL is at an intermediate level between age-matched controls and early-stage AD. (122, 125, 140). Overall, the pathological features of AD, as observed in MCI, precede clinical AD diagnosis and are almost fully developed before or concurrent with the first objective cognitive impairment.

Moreover, MCI is defined by combination of impairment in cognitive abilities (143). Due to heterogeneous nature of MCI, the subset of affected cognitive functions are different among individuals; however problems with episodic memory appears to be a consistent trend in MCI and have a strong predictive value for conversion to AD (131, 137, 128, 134-137). Disturbance in perceptual speed and learning are another indicators of MCI with progressive type and strong predictors for AD development (131, 135, 137, 143, 146, 148).

Compared to individuals who display impairment only in one cognitive function, subjects with deficits in multiple cognitive domain appear be at an increased risk of MCI conversion to AD (135, 148-151). Further, as several cognitive deficits are found in both stable and progressive type of MCI, additional biomarkers are needed to reliably distinguish individuals with stable MCI from subjects with progressive MCI (107).

1.4.3 Preclinical stage

Numerous studies have reported the presence of NFT and Aβ plaques in the brain of elderlies who did not exhibit any cognitive impairment (10, 104,, 113-116, 138, 139, 141, 152-161). Although neuronal loss in asymptomatic elderlies is minimal in contrast to individuals with MCI and AD, pathological changes in the brain follow the progression of AD neuropathology and first affect the MTL and neocortex with NFT and Aβ plaques respectively  (10, 111, 113-117, 121).
The prevalence of AD neuropathy reported in asymptomatic elderlies varies between studies from 10% to 40%. This variability is mostly likely due to the differences in classification criteria or difficulties establishing a standardized definition for non-demented elderlies. (88, 104, 108, 109, 116). Nonetheless, the current consensus regards elderlies with neuropathological hallmarks of AD in absence of cognitive symptoms as preclinical AD (10, 113, 104, 139, 140). The different types AD neuropathological features and their proposed levels in preclinical, MCI, and AD are depicted in figure 2 (162).

Overall, although neuropathological findings allow for differentiating between healthy versus AD state, as well as determining the stage of the disease, they have limited applications in clinical settings since obtaining a brain autopsy of live individuals for neuropathological assessment is not possible. Therefore, biomarkers other than histological features are needed to detect neuropathological changes associated with AD and possibly identify individuals at the preclinical stage.
Figure 2. Progression of pathological features in Alzheimer's disease.

Figure 2. The proposed advancement of different pathological features during the course of Alzheimer's disease (AD) progression (Jack et al., 2010). In the preclinical stage, accumulation of Amyloid-β (purple) is observed at the neocortical regions. Synaptic dysfunction (green), hyperphosphorylated (HP)-tau-mediated neuronal injury (blue), and atrophy of basal forebrain cholinergic neurons (pink), and atrophy of cortical and subcortical structures (brown) appear to begin later during the preclinical stage. In the mild cognitive impairment stage (MCI), different pathological features of AD continue to progress and first signs of cognitive dysfunction are detected (red). In early-stage AD the severity of pathologies and the amount of damage is beyond repair.
Understanding of the preclinical phase of AD has important theoretical and clinical implications. From a theoretical perspective, it may elucidate the mechanisms involved in disease progression by expanding the knowledge on how normal aging may transition to dementia. Clinically, it may allow for early identification of at-risk individuals and maximizing the efficacy of AD treatments (163). The cognitive traits of preclinical AD have been investigated in several large longitudinal community cohort of healthy elderlies, with longer than 20 years follow-up period in the longest study (164). The findings indicated that multiple cognitive features, such as abstract reasoning, attention, cognitive speed, and executive functions are affected in preclinical stage of AD, and effective traits at identifying at-risk individuals (131, 135, 149, 164-172).

Deficits of multiple cognitive functions validate the observation that multiple brain regions are affected long prior to AD diagnosis. In particular, these results are in line with the knowledge that disturbance in episodic memory is a persistent feature of AD, and a result of damage to regions of MTL prior to manifestation of observable memory impairment clinical diagnosis (147, 164, 172-174). Nonetheless, it is important to note that deficits in these cognitive domains are not unique to AD, and that cognitive speed, executive functioning, and episodic memory also decline through the course of adult lifespan (175-177).

Therefore, it is important to differentiate between cognitive disturbances associated with preclinical AD and non-progressive cognitive deficits observed in normal aging. This differentiation may be possible by identifying precipitating risk factors including but not limited to, unrecognized hypertension, proneness to distress, head trauma, and stroke (178-181).

Furthermore, simultaneous examination of other functioning parameters may be useful for detection of preclinical AD in at-risk elderlies. These include volumetric and functional measurement of brain structures, deposition of Aβ plaques and HP-tau, glucose metabolism and cerebral blood flow, subjective memory complaints and depressive symptoms, and genetic risk factors such as apolipoprotein-4 allele (117, 174, 181-193).
1.5 Current biomarkers of Alzheimer’s disease

A biomarker is generally defined as a characteristic that can be measured accurately and reproducibly, and that detects a fundamental and preferably unique feature of a neuropathology in a specific disorder (8, 194). The purpose of a biomarker may be for diagnosis, monitoring disease development, or prognosis and prediction the course of the disease (195). Moreover, a biomarker can be categorized as a marker of trait, state, or rate (194). A trait biomarker indicates the probability or susceptibility of developing a specific disorder. A state biomarker is used for diagnosis, and a rate biomarker indexes the progression or the stage of the pathology (8, 194).

In the past decade neuropathological changes in AD have been regarded as the best standard clinical criteria for diagnosis of probable AD, while the other AD biomarkers in molecular biology and brain imaging have been mainly used within the research field (119). Recently, a working group from the National Institute on Aging proposed that, an ideal biomarker for AD should have sensitivity and specificity of at least 80% for detecting AD and differentiating it from other dementias, it should be reliable, reproducible, non-invasive, inexpensive, and simple to perform (8). However, based on a recent systemic review from the Alzheimer's disease and Related Disorders Association (ADRSA), the best validated biomarkers that are currently being used for early diagnosis of AD are genetic biomarkers, cerebrospinal fluid (CSF) biomarkers, structural imaging with magnetic resonance imaging (MRI), and functional imaging with positron emission tomography (PET) (196).

1.5.1 Genetic biomarkers

Thus far, most successful AD diagnostic exams rely on advanced techniques in molecular genetics, and are limited to diagnosing familial AD with early onset. As discussed previously, mutations in \textit{PS1}, \textit{PS2}, and \textit{APP} genes cause dominant familial AD. Overall, these mutations have only been identified in a few hundred families, in which \textit{PS1} mutation accounts for the majority of early-onset AD below the age of 50, while \textit{APP} mutations are rare, and \textit{PS2} mutations have only been observed in two pedigrees (197-200). It is thus reasonable to look for mutations of \textit{PS1} gene in early-onset familial AD; however, it is impractical to search for mutations in individuals with no history of AD in the family.
Further, in contrast to deterministic genetic causes of AD, a specific apolipoprotein E (apoE) allele is considered a risk factor for AD and has been shown to have 3 times more prevalence in late onset sporadic AD compared to healthy controls. (197, 199). The apoE protein is a part of intermediate-density lipoproteins that transports cholesterol to neurons via the apoE receptors (200). There are three variants of apoE alleles on chromosome 19 found in general population, including ε2, ε3, and ε4, which create apoE proteins that differ by a single amino acid residue substitution, ε2 (Cysteine112, Cysteine158), ε3 (Cysteine112, Arginine158), and ε4 (Arginine112, Arginine158) (201). Among these allele variants, the ε4 subtype has been associated with increased probability of both early-onset and late-onset AD of sporadic and familial type (202-204).

However, since apoE ε4 allele is only considered a risk factor, it is insufficient to provide a diagnosis for AD solely based on genotyping of the apoE gene, as this measurement alone has a low levels of sensitivity and specificity. Further, the majority of the individuals with inherited apoE ε4 allele exhibit no sign of AD even at an old age. Nonetheless, a slight increase in diagnostic confidence is obtained when apoE ε4 allele is found in parallel to conventional diagnostic markers during clinical assessment. (200, 205).

1.5.2 Cerebrospinal fluid biomarkers

Much focus has been given to three proteins in the cerebrospinal fluid (CSF) as the potential biomarkers for AD, including Amyloid beta 42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau). There is a general consensus that low levels of Aβ42 and high levels of tau in the CSF indicate the presence of AD neuropathology as observed in post-mortem brains (208).

A comparison between AD patients and age-matched controls showed high levels of sensitivity and specificity for t-tau and p-tau (81% sensitivity, 91% specificity) and Aβ42 (86% sensitivity, 89% specificity) (209). In addition, the specificity of an AD diagnosis can be improved by using CSF biomarkers in conjunction with other diagnostic techniques, such as computed tomography (CT) or MRI (210, 211).

Reports have shown that CSF biomarkers may also be able to predict MCI conversion to AD. Convergent findings of five studies showed that MCI progression to AD was predicted by high levels of t-tau and p-tau and low levels of Aβ42 in the CSF (212). However, a consensus is yet to
be reached over which of these three proteins or their combination is the strongest predictor for MCI conversion to AD (213-216).

Overall, although CSF biomarkers may provide an accurate diagnosis for AD, obtaining CSF via lumbar puncture is a procedure that is practiced only in specialized centers and requires tremendous changes to services and healthcare systems in order to be implemented universally (217). Further, sampling of CSF suffers from a negative public reputation, and is accompanied by high rates of reservation among patients mainly due to its highly invasive nature and some associated side-effects such as headaches and nausea (218).

### 1.5.3 Blood biomarkers

In contrast to CSF, detection of biomarkers through venipuncture provides a less invasive test. The efforts to identify a reliable blood biomarkers for AD have not been very successful. One of the challenges for finding a suitable blood biomarker is the low expression level of protein of interest, such as Aβ, in the blood and the peripheral tissue which can challenge the protein quantification assays (219). Moreover, compared to CSF, plasma and serum contain a much higher level of proteins which can further hinder the detection of target proteins (219, 220).

There has been incongruent findings in regards to plasma levels of Aβ42. A few studies have reported slight increase of Aβ42 in AD patients compared to healthy controls, while the majority of studies did not show any change (221). Further, a few studies have suggested that high levels of plasma Aβ42 indicates elevated risk of AD development, while findings from other studies did not find evidence supporting this claim (221).

Unlike the negative correlation between CSF Aβ42 levels and cortical Aβ-plaques, as supported by imaging studies, the correlation between plasma Aβ42 levels and Aβ-plaque is yet to be determined (222). Findings in regard to plasma tau levels are also conflicting. Several studies have reported an increase of tau levels in AD patients, while others observed a decrease of plasma tau levels in the inflicted individuals (223, 192). The contributing factor to conflicting observations is likely the extremely low levels of plasma tau which can result in variability between measurements. Currently, ultrasensitive assays for quantification of plasma tau are being developed to address this issue and examine the usefulness of plasma tau as a biomarker for AD (224).
Recently, advanced molecular approaches have characterized a few novel plasma biomarkers. These novel biomarkers include C-reactive protein, selective anti-peptoid antibodies, and other plasma proteins including IL 1alpha, IL-3, and TNF-alpha which can distinguish AD from healthy controls (225-228). Nonetheless, these novel blood biomarkers require to be further studied before being implemented as fully reliable biomarkers for AD diagnosis.

1.5.4 Structural MRI

Studies have shown that progression of AD neuropathology and the severity of cognitive deficits have a strong correlation with the level of brain atrophy measured with structural MRI biomarkers (228, 229). At the early stages of AD, degeneration begins in the structures of the MTL, including the hippocampus and EC, and continues with an average annual rate of 4.7% compared to 1.4% in age-matched controls (230).

Wide implementation of structural MRI analysis in clinical settings has been hindered mainly due to its expensive setup and requirement of an on-site technical expert for measuring the volume of the structure of interest based on the analyzed volumetric and visual tracing data (231). However, recently the latter issues has been addressed by using automated data acquisition and analysis technique, known as tensor-based morphometry, which measures global brain atrophy and calculates group differences between the patients and controls (231).

To further enhance the accuracy of region-of-interest and global brain atrophy measurements, supervised learning algorithms, such as support vector machine (SVM), are trained to automatically classify MRI scans as AD or control, and produce a Structural Abnormality Index (STAND) based on the pattern and level of atrophy observed in each voxel (232). Based on the results of a multi-center study, STAND index can differentiate 98 AD patients from 109 controls with an accuracy of 84% (232). In another study the classification accuracy was increased to 94% for AD versus controls, when MRI and CSF biomarkers were combined (233).

Additionally, structural MRI biomarkers have also been able to predict MCI progression to AD (205). It has been shown that MTL atrophy, measured using automated morphometry, combined with cognitive performance scores can predict MCI progression to AD with 85.2% sensitivity and 90% specificity (234, 235).
Currently, structural MRI biomarkers provide an accurate non-invasive methods for diagnosing AD patients. Nevertheless, this method of diagnosis is unusable for individuals with claustrophobia, metal implants, or pacemakers. In addition, this method is not ideal for detection of preclinical AD, as neuronal loss and structural atrophy during this stage is insufficient to differentiate between preclinical AD and age-matched controls.

1.5.5 Cerebral blood flow and metabolism

Examination of AD with functional imaging techniques became rapidly explored after the development of radio tracers for PET, such as fluorodeoxyglucose (¹⁸F-FDG), and tracer ligands for single-photon emission computed tomography (SPECT), including hexamethyl-propyleneamine oxime (⁹⁹mTc-HMPAO), N-isopropyl-(iodine-123)p-iodoamphetamine (¹²³I-IMP), and ethylene cysteinate dimer (⁹⁹mTc-ECD) (236, 237).

As a glucose analog, FDG captures spatial information about the neural metabolism of cortical and subcortical structures by accumulating in tissues in proportion to their metabolic activity 30 minutes after injection. SPECT radio tracers provide information about cerebral blood flow by binding to tissues with higher level of perfusion (238-240).

In early stages of AD, reduction of cortical metabolism and perfusion first occurs in the MTL, the posterior cingulate, and the bilateral posterior temporo-parietal junction. As the disease progresses, reduced metabolic activity and hypoperfusion are observed in the prefrontal cortex which are thought to be associated with emotional disturbance and personality changes observed in later stages of AD (241-253).

There appears to be an increased likelihood of conversion to AD in MCI patient who show a decreased level of cortical metabolism in temporo-parietal association areas, posterior cingulate, and hippocampus (254-258). Indeed, decreased metabolic activity in the EC and hippocampus measure with PET have been used for discriminating control subjects from individuals with MCI and AD with 81% and 100 % accuracy respectively (259).

A post-mortem analysis on the brains of AD patients who have been evaluated by SPECT revealed a 84% sensitivity and a 92% specificity for this technique (260). Findings of a meta-analysis on
27 studies with HMPAO have indicated sensitivity of 77% and a specificity of 89% for this SPECT ligand in discriminating AD patients from the age-matched controls (261).

The evidence suggest that both PET and SPCET are able to differentiate AD from other types of dementia (262), and that a diagnostic index for AD can be provided from the ratio of association cortex activity to the activity of primary sensory-motor cortex (263). However, the accuracy of both techniques for diagnosis of AD can be further enhanced by analyzing imaging results in combination with information gathered from clinical assessment (264).

1.5.6 Molecular imaging

Development of radio tracers for Aβ-plaques and tau aggregates allowed for in-vivo localization of pathology and advanced the use of positron emission tomography (PET) in the study of AD. Currently, 2-(1-(6-[(2-[F-18] fluoroethyl) (methyl) amino]-2-naphthyl) ethylidene) malononitrile (18FFDDNP) and 11C-labelled Pittsburgh Compound B (PiB) have been most extensively studied (207, 208). In comparison to conventional PET tracers such as, 18F-FDG, that indirectly measured tissue metabolism, direct binding of 18FFDDNP to Aβ-plaques and NFTs provides an index which predicts MCI conversion to AD (267, 268).

Consistent with the presence of NFTs in MTL in early-stage AD, the strongest binding of 18FFDDNP is also observed in this region, and performance on memory tests was positively correlated with the rate of 18FFDDNP clearance from the brain (269). However, detection of NFT pathology with this molecular tracers has proven challenging as the difference in signal intensity of 18FFDDNP between AD patients and age-matched controls is not more than 10% (231).

In contrast, PiB has a higher sensitivity than 18FFDDNP for binding to Aβ-plaques and provides close to 100% change in signal intensity between AD patients and controls (270, 271). However, results from a study have shown patterns of PiB binding in 21% of controls that were not distinguishable from the binding patterns in AD patients (272).

It remains unknown whether comparable PiB binding pattern in controls and AD patients was a reflection of preclinical AD in the control subjects, or simply due to insufficient specificity of PiB
binding. Nonetheless, the PiB binding pattern in approximately 50% of MCI patients is similar to AD and predicts MCI conversion to AD within a two-year period (273).

Unfortunately, PiB radio tracer has short half-life of 20 minutes, rendering PiB-PET-based study of AD invasive and expensive. As such, wide implementation of this diagnostic technology in the clinical setting for large-scale screening of at-risk individuals is yet unattainable (274, 275).

Currently, other Aβ-specific ligands, such as $^{18}$F-BAY94-9172 and $^{11}$C-BF-227, are being investigated to establish their clinical relevance in AD (95, 96, 231). The advantages of these radio tracers include longer half-life of $^{18}$F-BAY94-9172 (i.e. 110 minutes), and higher specificity of $^{11}$C-BF-227 than PiB to dense Aβ plaques (96, 96).

In addition, recent studies with a new radio tracer specific for tau, known as T807 or $^{18}$F-AV-1451, have shown that there is a correlation between the level of tau radio tracer binding in the MTL, the level of tau CSF biomarkers (i.e. t-tau and p-tau), and cognitive performance in AD patients (164, 276).

1.5.7 Electroencephalogram

Electroencephalogram (EEG) is the recording of the brain’s spontaneous electrical activity that occurs because of synchronous firing of a large number of neurons. In a clinical setting EEG recording is often quantified (qEEG) by decomposing it into different frequency bands and each band is assessed for its rhythmic activity (304). The commonly used frequency bands are the delta (0-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (12-30 Hz), and gamma (> 30 Hz) (304).

The characteristic EEG abnormalities in AD include shift of the power spectrum to lower frequency bands and attenuation of coherence in high frequency bands (278). These abnormalities are correlated with disease severity and are thought to be due to functional disconnections among different cortical areas that result from synaptic dysfunction, axonal pathology, cholinergic deficits, and neuronal death (279-281).

The shift of power spectrum to lower frequencies, or the slowing of EEG, has been extensively observed in patients with MCI and AD (282-285). This change includes attenuation of power in
high frequency alpha and beta bands and increase of power in low frequency delta and theta band. However, elevated activity in the gamma range has also been reported in some AD patients (286).

In early stages of AD, it is believed that the first changes in awake resting EEG include an increase in theta power and decrease in beta power, and in later stages of the disease a decrease in alpha power and an increase in delta power is observed (287-290).

Moreover, it has been reported that theta power in in early-stage AD patients is significantly different compared to age-matched controls, but not compared to patients with MCI (287, 291, 292). Studies have also suggested that an increase in theta power is predictive of cognitive decline in individuals with subjective memory complaint (291).

Pharmacological studies have also reported that administration of scopolamine to subjects induced changes in EEG similar to what is observed in AD patients, and that these changes were eliminated by administration of acetylcholinesterase inhibitor (293-296). Also, it has been shown that there is a correlation between the activity of low-frequency EEG bands in AD and several other biomarkers such as hippocampal atrophy in structural MRI (324), elevated CSF tau levels (298), and cortical hypo-metabolism and hypo-perfusion (298-304).

### 1.5.8 Event-related potentials

Another way to analyze EEG data is event-related potentials (ERPs). ERPs are a series of negative and positive voltage deflections of the EEG that are time-locked to sensory or cognitive events (Figure 3) and have been validated as markers of AD in an outpatient setting clinical trial (305).

The first component of ERPs is a positive voltage deflection known as P1. P1 occurs approximately 50 ms after the onset of an auditory stimulus or 100 ms after a visual stimulus. Auditory P1 component has been associated with auditory inhibition and sensory gating (306, 307). Further, studies using magnetoencephalography (MEG) have suggested the sources of auditory P1 component to be located in the superior temporal gyrus and medial prefrontal cortex (mPFC) (308, 309).
Figure 3. To obtain event-related potentials (ERPs) from electroencephalogram (EEG), a time-window on the EEG is specified before and after the stimulus presentation (red), and EEG in that specific time-window is averaged across all trials. ERP waveform displays positive (P) and negative (N) deflections known as P1, N1, P2, N2, and P3 ERP components arising in hundreds of milliseconds (ms) after the onset of stimulus at zero (Luck et al., 2000).
Visual P1 has been proposed to reflect several sensory processing, such as noise suppression, encoding of form and color, and the arousal level (299, 310, 311). Based on the data gathered from PET, brain electrical signal analysis (BETA), and low-resolution brain electromagnetic tomography (LORETA) analysis, multiple brain regions have been proposed to generate visual P1, including ventrolateral occipital cortex, striate, and posterior fusiform gyrus (300-302).

The second ERP component, N1, is a negative voltage deflection that occurs approximately 70-100 ms after the onset of an auditory stimulus and 100-120 ms after the onset of a visual stimulus (303, 304). Studies have associated auditory N1 component with encoding of sensory and physical properties of the stimulus, such as location and intensity, and have shown that the N1 amplitude is increased by elevated levels of attention to the stimulus (312-316).

Results from MEG, lesion studies, and BESA analysis have indicated the primary auditory cortex in superior temporal gyrus and additional structures in the frontal cortex as the likely generators of auditory N1 component (317-321). It has been suggested that visually-induced N1 component reflects preparation of response, as this component was attenuated in tasks that a motor response was not needed (299). Further, it was shown that the amplitude of visual N1 is generally larger during stimulus discrimination, likely due to elevated processing of the location and spatial properties of the stimulus (299, 310, 321, 322). Using MEG, MRI, and LORETA, the sources of visual N1 component have been located in the inferior occipital lobe, occipito-temporal junction, as well as the inferior temporal lobe (323, 324).

The positive deflection after N1 ERP component is known as P2. The amplitude of auditory P2 can be observed as a single or a double peak 150-275 ms after the stimulus onset, and is suggested to be sensitive to physical features of the stimulus, such as pitch and loudness (325-327). According to MEG studies and intracranial recordings, it has been proposed that auditory P2 is generated from sources in the primary and secondary auditory cortices (328-330). The amplitude of visual P2 appears 150-200 ms after the stimulus onset over the frontal region and around 200 ms over the occipital areas (331-333). Further, more complex visual stimuli result in a larger P2 amplitude.

Overall, both auditory and visual P2 are thought to reflect cognitive tasks, including feature detection, stimulus classification, and selective attention (334-339). The N2 ERP component is a negative deflection that peaks approximately 200 ms after stimulus onset, and has been attributed
to multiple cognitive processes including stimulus discrimination, target selection, and mismatch detection (340-343).

The results of scalp current density analysis suggest that bilateral sources in the auditory cortex are responsible for generation of auditory N2 component, with the largest amplitude over the central parietal area (263, 264). Intracranial recordings have localized the source of visual N2 at the fourth occipital gyrus, and the largest visual N2 amplitude was recorded bilaterally close to occipito-temporal sulci (344, 347).

The P3 component is the most extensively researched ERP component since its discovery in 1965 (353). It is believed that P3 reflects the activity of brain during higher cognitive processes, such as attention and memory (354). This finding is based on the work of earlier studies in which stimulus information such as, probability and task relevance, were manipulated in an “oddball” paradigm to examine the effect on the pattern of brain electrophysiological activity (355-358). The results from these studies showed that P3 is elicited in response to an unexpected auditory or visual stimulus approximately 300 ms after stimulus onset (357).

It has been theorized that P3 elicitation to an unexpected stimulus is an index of brain’s attention-dependent reassessment of the mental representation that was created by the stimulus in the working memory (358-361). If no change in stimulus characteristic is detected, the mental representation remains unchanged and only the earlier ERP components are observed (i.e. P1, N1, P2). Upon detection of a change in stimulus attributes, the mental representation of the stimulus is updated via attention-dependent mechanisms and a P3 deflection is observed (362). This context-updating theory of P3 was based on the observations that discrimination of a rare target stimulus from a frequent non-target stimulus elicits a prominent P3 deflection, and that the amplitude of P3 increases as the probability of the target stimulus presentation is lowered (363-365).

The two subcomponents of P3 are P3a and P3b potentials. P3a is observed when a non-task-related rare stimulus, also known as a distractor, is detected among a series of frequent stimuli, whereas P3b is produced upon detection of a task-related target stimulus (366, 367). The P3a displays a rapid habituation and reduction of amplitude with repeated presentation of the distractor stimulus, and therefore it is thought to act as an orienting response that is generated by communication between the hippocampus and the frontal cortex (368-370).
The precise neural origin of the P3 component is yet unknown. In patients with a frontal lobe lesions a reduction in P3a but not P3b amplitude was observed (369). Similarly, patients with focal hippocampal lesions demonstrated an attenuated P3a but an unchanged P3b, indicating the necessity of hippocampus and frontal cortex for generation of P3a potential (371). Further, results from human studies using in-depth electrodes in hippocampus, along with assessment of patients with severe damage to medial temporal lobe demonstrated only a partial involvement of the medial temporal lobe in generation of the P3b potential (372, 373).

1.6 Limitations of current ERP biomarkers

Among biomarkers reviewed above, ERP biomarker holds a number of advantage over other biomarkers, such as non-invasiveness, low cost, portability, and availability. Unlike blood, CSF, and PET biomarkers, obtaining ERPs does not require the use of invasive procedures or exposure to radiation. Also, the cost of required hardware for acquisition and recording is substantially lower than majority of other biomarkers (375, 376). Compared to imaging biomarkers which have temporal resolution of seconds, the millisecond temporal resolution of ERPs allows for examination of processes underlying cognitive function and dysfunction (374). Additionally, ERPs are more tolerant to movement than neuroimaging techniques, and there are methods that can reduce or completely eliminate the movement artifact prior to analysis of the ERP data (377, 378). Therefore ERPs are suitable for studies where subjects are required to perform a task, as well as in behavioral studies with unrestraint animals.

In parallel, current ERP biomarkers also have some limitations. Many studies have reported abnormalities in the amplitude and latency of P3 in MCI, AD patients (379-382) and elderlies who are likely to be in preclinical stages (381, 383). However, observations have been conflicting, and there is no general consensus over which P3 property is the the best candidate for detection of AD at an early stage.

One study reported that an increase in frontal and parietal P3 latency, induced during an auditory oddball paradigm, was correlated with a 3.7 fold increase in probability of AD in individuals with subjective memory complaints (381). In another study, marginal reduction of frontal and parietal
P3 amplitude, induced during an auditory oddball paradigm, was observed in subjects at risk of developing MCI without any abnormality in P3 latency (383).

Although these findings suggest a potential use of P3 as a biomarker for AD (384-387), there appears to be large variability among subjects which undermines the use of P3 as the method of assessment for clinical diagnosis or evaluation of at risk individuals (389).

Moreover, the abnormality of P3 is not always detectable on an individual basis, or between subjects with distinct neurological disorders (384). A study that investigated P3 using an oddball auditory paradigm in patients with AD and individuals suffering from depression, reported that although P3 latency was sufficient to detect the group difference between demented, depressed, and age-matched controls, it was unable to differentiate a demented patient from the control due to lack of sensitivity (389). In a similar study, P3 potential was examined using a two-tone auditory oddball paradigm in individuals with AD of mild to moderate severity, and in patients with depression (390). It was shown that, although AD group had the longest averaged P3 latency in all the scalp regions analyzed, no significant group difference was found between the AD, depressed, and control group. Further, when P3 latency was used for distinguishing individual AD patients from control the classification accuracy was less than 25% (390).

The early ERP components, including P1, N1, and P2 are known as the obligatory cortical potentials due to their strong within-subject and inter-individual consistency (348-350). As seen in above examples, P3 component lacks in consistency and reliability (351, 352). The limitations of this component may go beyond its inter and intra-individual variability. It has been suggested that different stimulus modalities elicit P3 potentials with dissimilar characteristics. Several studies in normal healthy adults reported that auditory P3 potentials display a shorter peak latency, whereas visual P3 potentials show larger peak amplitude (391-393). Further, in patients with mild to moderate AD, auditory and visual P3 components induced using an oddball paradigm display a significant modality-specific alterations, including prolongation of averaged auditory P3 latency and attenuation of averaged visual P3 amplitude (394).

The contradictory findings about P3 may be, in parts, due to discrepancy in recording procedures and differences in experimental criteria across studies (84). More importantly, a sole focus on P3 during oddball paradigm undermines the extensive amount of information on brain dynamic that can be retrieved from ERPs at millisecond resolution. Particularly, different pathologies in
preclinical AD may manifest as abnormalities in specific ERP patterns which have remained unexplored. Thus, efforts must be focused towards further improvement and characterization of ERP patterns for establishing ERP as a low-cost, potentially portable, and noninvasive biomarker for preclinical AD.

1.7 Strategies to improve ERP biomarkers

One strategy to achieve this is to use a behavioral paradigm that depends on brain regions vulnerable to AD pathology. One such paradigm is trace eyeblink conditioning (TEBC) paradigm (395). The paradigm consists of a neutral conditioned stimulus (CS) that is paired with an eyeblink-eliciting stimulation (US) to the eyelid following a stimulus-free interval of 500 ms, and the conditioned response (CR) is defined as preemptive eyeblink occurring after the onset of the CS and prior to onset of the US. Indeed, studies have reported that patients with AD perform significantly worse than age-matched controls in TEBC (396, 397).

The contributing factors to TEBC impairment in AD have been elucidated from studies using knock-out mice or systemic pharmacological blockade of mAChRs in rabbits and humans, which showed that cholinergic input mediated through mAChRs is necessary for acquisition in TEBC (398, 399). Further, a recent study elucidated the role of EC as the key requirement for acquisition in TEBC, by demonstrating that inhibition of EC with local infusion of γ-aminobutyric acid (GABA) receptor agonist in rats prior to conditioning prevented them from acquiring a CR (400). The same study also showed that EC-dependent memory acquisition in TEBC is modulated by local mAChRs, as infusion of scopolamine into the EC before conditioning slowed acquisition in rats (400). Consistent with the cholinergic hypothesis of AD, these findings indicate that impairment of TEBC in AD patients is, in parts, likely due to disruption of BF muscarinic cholinergic input to the EC and hippocampus (401, 402).

Moreover, a recent study in rats showed that pattern of cortical oscillations elicited by TEBC are sensitive for accumulation of HP-tau in the entorhinal cortex (403). By using viral vector approach, a human mutated tau gene was transduced to a subset of cells in the EC of adult rats. Comparable to the control rats, the HP-tau-expressing rats acquired associative memory in TEBC, but exhibited an abnormal pattern of local field potentials (LFPs) in the hippocampus and mPFC
This abnormality, which was absent in control rats transduced with green fluorescent protein (GFP) gene, included an attenuation of hippocampal and prefrontal theta power and elevation of prefrontal beta power. Together, these findings indicted that one of the earliest pathological features in AD brains, accumulation of HP-tau in the EC, induced abnormal LFP pattern without memory impairments (403).

In a modified version of TEBC, known as differential trace eyeblink condition (DTEBC), one CS (CS+) is paired with the US while the other CS remains neutral (CS-). After initial acquisition phase, CS-US contingency can be switched to study reversal learning. Findings of a study on rabbits which received bilateral hippocampal lesion before undergoing two-tone DTEBC, showed that damage to hippocampus greatly impaired reversal learning, but produced a mild effect on acquisition (404). Severe impairment of reversal learning was also reported by a similar study in which rabbits with bilateral hippocampal lesion underwent tone-light DTEBC (405). Since the mPFC receives projections from the hippocampus, it was speculated that damage to the mPFC may produce similar deficits in reversal learning as observed in rabbits with hippocampal lesions. This was tested in a study where rabbits received bilateral lesions to the mPFC prior to undergoing DTEBC (406). Lesions to mPFC caused a significant impairment of reversal learning in rabbits, but similar to previous findings, performance during acquisition was not significantly affected (406). Moreover, impairment of reversal learning has also been reported in amnesic patients with selective hippocampal damage who showed intact learning during the acquisition phase of a visual discrimination task (407). Together, these findings support a role for hippocampus and mPFC in reversal learning, and indicate that having additional paradigm to a simple acquisition phase may enhance the ability to detect subtle signs of memory impairments.

Since TEBC and DTEBC paradigms in animals can be applied to human subjects without any major modification, these results hold a significant translational potential. However, there remains the need to investigate how the observed aberrant LFPs translate to ERP measures, which are known to be comparable between rats and humans (408-410). To this end, the proposed research will examine the effects of pathological features of early-stage AD on ERPs while rats learn a modified version of TEBC paradigm.
1.8 Hypothesis and Approach

The main hypothesis of this study is that different pathological features of preclinical stage AD induce distinct abnormal ERP patterns during trace eyeblink conditioning to a degree which is sufficient for an accurate prediction of the type of brain pathology based on the ERP patterns.

To address this hypothesis, we measured cortical EEG while rats with entorhinal HP-tau over-expression, cholinergic deficiency, or both conditions, and their associated controls received differential trace eyeblink conditioning in two phases of acquisition and reversal. In doing so, first we examined the effect of early pathological features of AD on memory acquisition, stimulus discrimination, and reversal learning.

Next, we examined ERP patterns in the frontal, parietal, and temporal cortices by looking at peak amplitude and peak latency of all the visible components, including P1, N1, P2, and P3 potentials. Subsequently, we submitted all the collected ERP data to a machine learning algorithm and identified distinct ERP abnormality sensitive for entorhinal HP-tau over-expression, cholinergic deficiency, and both conditions in conjunction.
Chapter 2
Methods

2.1 Subjects
Forty experimentally naïve male Long-Evans rats (Charles River Laboratories, St.-Constant, QC, Canada), 70 days old upon arrival, were individually housed in transparent plastic cages (45 cm long x 25 cm wide) in a home colony room and maintained on a reversed 12-hour light – 12-hour dark cycle (dark from 10:00 to 22:00) with free access to food and water and behavioral testing occurring during the dark phase, typically between 12-4 pm. This study was approved by University of Toronto’s Institutional Animal Care Committee.

2.2 Design
The study consisted of four groups of animals. Two groups of animals had overexpression of hyperphosphorylated human tau (HP-tau) protein within the entorhinal cortex and the other two groups expressed green fluorescent protein (GFP) in the same region as control. Twenty minutes before conditioning sessions one HP-tau-expressing group (n=10) and one GFP-expressing group (n=11) received cholinergic receptor blocker, and the other HP-tau-expressing group (n=8) and GFP-expressing group (n=11) received saline. All animals underwent the same behavioral protocol described below.

2.3 Surgeries
Surgeries were performed under aseptic conditions using isoflurane anesthesia (4% isoflurane with 1 L/min oxygen for induction; approximately 2% isoflurane with 0.6 L/min for maintenance of anesthesia; Holocarbon Laboratories, River Edge, NJ, USA). Immediately after induction, rats received subcutaneous injections of saline (1 ml/100 g body weight) and carprofen (0.5 ml/kg, 50 mg/L) to prevent dehydration and to treat postoperative pain and inflammation. A small amount of topical analgesic EMLA was applied to the scalp.
2.4 Viral vector infusion

To mimic entorhinal tau pathology observed in preclinical stages of AD (44), a recombinant adeno-associated viral serotype 9 (rAAV9) vector with mutated human tau gene was bi-laterally micro-infused in the entorhinal cortex of adult rats to over-express mutated human tau protein. The form of tau transgene contained the P301L mutation associated with frontotemporal dementia, and four microtubule-binding domains (exons 2−/3−/10+). The cytomegalovirus (CMV) enhancer/chicken β-actin promoter combination and the woodchuck hepatitis post transcriptional regulatory element were used to drive gene expression. The viral vectors were provided by Dr. Klein who validated the vector in rodents (411). The vector was bilaterally infused using a micro infusion pump (Harvard Apparatus, Holliston, MA) in four anteroposterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates (Figure 5; Table 1; 3 μL/site at 0.375 μL/min). The coordinates were based on our previous study to maximally transduct cells in the entorhinal region (379). For control, rAAV9-GFP vector was infused into the same coordinates in another set of animals. Prior to infusion, the titer of vector (rAAV9-tau-P301L or rAAV9-GFP) was prepared to be ~3.7×10^{13} vector genome particles/ml. Following infusion, the incision was closed using silk sutures. The viral vector was incubate for six weeks before initiation of behavioral testing (379).

2.5 EMG-EEG electrode Implantation

Two weeks after recovery from viral vector surgeries, animals were subjected to implantation of electromyogram (EMG) and electroencephalogram (EEG) electrodes to record muscle and brain activities respectively during the behavioral paradigm. Following the incision, each rat was implanted with 3 stainless steel screw electrodes on the skull surface above right frontal (3.2 mm AP, 0.7 mm ML), parietal (-3.5 mm AP, 2.0 mm ML), and temporal (-6.2 mm AP, 7.0 mm ML) cortices, and a reference screw was placed on the skull above the right cerebellar cortex (Figure 6 A). Five additional screws were added that served as anchors for the dental acrylic. Next, four Teflon-coated stainless steel wire (outer diameter = 0.0055”; A-M Systems, Carlsberg, WA) was inserted in orbicularis oculi muscle of the left eyelid to serve as an EMG electrode pair and a stimulus electrode pair (Figure 6 B). The recording and reference electrode wires were then connected to a 9-pin ABS plug (Ginder Scientific, Nepean, Canada) which was fixed to the skull
with dental acrylic. Following the implantation of electrodes, the incision was closed up to the head cap by silk sutures and the EMG electrode wire was uncoated and trimmed at the level of the eyelid.

### 2.6 Drug treatment
Scopolamine hydrobromide (Sigma-Aldrich, Oakville, Canada) was dissolved in 0.9% saline. Half of rats in HP-tau group (n= 8) and GFP group (n=11) received a subcutaneous injection of (0.05 mg/kg body weight) of scopolamine hydrobromide, while the remaining animals in HP-tau group (n=10) and GFP group (n=11) received subcutaneous 0.9% saline injection 20 minutes before the conditioning started. The drug treatment began on the second adaptation session and continued until the last session. To recapitulate the level of cholinergic deficiency in preclinical, asymptomatic AD stage, the dose of scopolamine was chosen to thwart but not completely block memory function or neural activity at the level of the brainstem (398, 412).

### 2.7 Apparatus and Behavioral paradigm
Prior to behavioral testing animals were assigned into four groups of HP-tau and GFP with saline treatment (tau-sal; GFP-sal) and HP-tau and GFP with scopolamine treatment (tau-scop; GFP-scop). The groups underwent a variant of classical conditioning paradigm, known as differential trace eyeblink conditioning (Figure 7; DTEBC). In this paradigm a neutral conditioned stimulus (CS) was paired with an aversive, eyeblink-eliciting stimulus (US) with an inter-stimulus interval of 500 millisecond (ms). This paradigm included an auditory stimulus (ACS, 100-ms, 2.5-kHz, 85-dB pure tone) and a visual stimulus (VCS, 100-ms, 50-Hz, LED light). LED was located at the font of the chamber and a speaker was mounted on the box ceiling directly above the cylinder. The US was a 100 ms periorbital shock (100 Hz square pulses) delivered to the eyelid with a stimulus isolator (ISO-Flex, A.M.P.I., Jerusalem, Israel). The shock level was initiated at 0.3 mA and was adjusted individually and daily for each rat to induce an optimal unconditioned response (UR; an eyeblink/ head-turn response) which was monitored through web cameras mounted inside the chambers.
During habituation (Days 1-2), animals were placed into a transparent, cylindrical, plastic container and put into a sound and light-attenuating chamber for 50 minutes without any stimulus presentation to adapt to the environment, while baseline EMG was recorded. On the second day, animals received their respective drug treatments to observe whether there would be an effect on baseline eyelid activity. During the acquisition phase (Days 3-12), rats received 50 pairings of one CS (CS1+, either the auditory or visual counterbalanced across rats) and the US in addition to 50 presentations of the other CS alone (CS2-). After rats formed the association between the CS1+ and US, reversal sessions began (Days 13-19), in which the CS1 was presented alone (CS1-) while the CS2 was reinforced with the US (CS2+). EEG and EMG were recorded during all session. Eyelid EMG was used to detect conditioned responses (CR, defined as eyeblink responses elicited during a 200 ms window immediately before US onset). Rats’ memory performance was quantified with the ratio of trials with CR expression to the total number of trials in a session (CR %).

2.8 Behavioral testing coupled with EEG recording

During conditioning, EMG from the left upper orbicularis oculi and EEG from the frontal, parietal, and temporal epidural electrodes were continuously recorded. The signals were amplified, band-pass filtered (300–3000 Hz for EMG; 0.1–400 Hz for LFP), and acquired at 6,102 Hz (EMG) or 2,034 Hz (EEG) with a RZ5 recording system (Tucker-Davis Technology, Alachua, FL). The OpenEx software (Tucker-Davis Technology, Alachua, FL) was used to monitor the trial events and insert the time stamps and corresponding comments (CS1, CS2, US, trial number) to the EEG and EMG data. Arduino Mega 2560 was to initiate the paradigm and administer trial events (CS and US presentation).

2.9 EMG Analysis

All of the analyses were performed using custom codes written in Matlab (Mathworks, Natick, MA, USA). The method of analysis of the EMG data was the same as in our previous study (376, 413). The instantaneous EMG amplitude was defined as the difference between the minimum and maximum EMG signal in a 1-ms window. Noise was removed from the EMG amplitude by
subtracting the across-trial mean, plus one SD, of the amplitude during a 300-ms period before the CS onset. All trials in which the pre-CS EMG amplitude exceeded the noise level by an additional 30% of this noise level were excluded from further analyses. A trial was defined as containing a CR if the EMG amplitude during a 200-ms window prior to the US (CR amplitude) exceeded five times the pre-CS amplitude (in the case that the pre-CS amplitude was zero, CR amplitude had to be 10% greater than the noise level). The CR percent was defined as the ratio of the number of trials containing the CR to the total number of valid trials in each session.

2.10 EEG analysis
EEG data from each session were then processed by using custom codes written in Matlab (MathWorks, Novi, USA). EEG data from individual rats were first checked for the contamination of power line noise by examining the EEG power spectrum. EEG data was excluded from future analysis if the size of the peak amplitude at the 60 Hz frequency range was more than half the size of the highest peak amplitude between the 5-10 Hz frequency band. To calculate event-related potentials (ERPs), we extracted EEG activity during 6 second around the onset of CS and averaged the EEG data across all trials in one session. Then, ERPs were averaged across the last three acquisition and reversal sessions separately for two CS types (tone and light) and three brain regions (frontal, parietal, and temporal) in each rat. Next, from the averaged ERPs, we then calculated the amplitude and latency (2 features) of P1, N1, P2, and P3 components. These components were detected as the peak of positive (P1-3) or negative (N1) deflection occurred during a set time window after CS onset (Figure 8). For auditory ERPs, the time window for P1 was 35-80 ms, for P2 was 120-240 ms, for P3 was 240-360 ms, and for N1 was 80-120 ms. For visual ERPs, the time window for P1 was 90-130 ms, for P2 was 180-260 ms, for P3 was 260-360 ms, and for N1 130-180 ms. These time windows were set based on the previously reported ERP patterns induced by auditory (381, 382) and visual stimuli (380) in humans and rats. The detected amplitude and latency were averaged across rats in each of four groups.
2.11 Support Vector Machine

To examine whether each type of pathology induces a distinct pattern of ERP abnormalities, we applied a Support Vector Machine (SVM) classifier on ERP features by using LIBSVM package (414). The SVM is a supervised machine learning algorithm which are used for data classification (415-419). With given set of data containing observations whose categories are known, SVM’s training algorithm builds a model that identifies to which set of categories a new unlabeled observation belongs. The SVM model creates a representation of the labeled observations as points in space, in a way that observations belonging to different categories are separated by a decision surface with maximum margin. The location of the decision surface is determined by the data points (support vectors) that lie closest to it. The margin between the decision surface and the closest data points from each category is maximized to reduce the number of misclassifications. New sets of unlabeled observations are then mapped into the same space and are predicted to belong to a category based on the side of the decision surface they fall on.

The classification procedure involved four steps. From each ERP data, we extracted the amplitude and latency of P1, P2, P3, and N1 components (defined the previous section). We repeated this procedure for three brain regions (frontal, parietal, and temporal), for two CS modality (tone, light), and for two association type (CS-, CS+). This results in total 96 features for each rat (Figure 9). The extracted ERP features were first transformed to the format of an SVM package. All rats within a single group were organized in rows and were assigned the same class labels (1 to 4 for GFP-saline, tau-saline, GFP-scopolamine, and tau-scopolamine respectively). The corresponding features for each rat were placed on the same row level and organized in columns 1 to 96 (Figure 10). Subsequently, the data was linearly scaled to the range [0, 1] to prevent features in greater numeric ranges (i.e. amplitudes) dominate those in smaller numeric ranges (i.e. latencies). As such, the values of each feature across all rats were divided by the maximum value of that feature. Next, a radial basis function (RBF) kernel was used to train the classifier and classify the data (389). RBF kernel was chosen because of its ability to nonlinearly map samples into a higher dimensional space if class labels and features follow a nonlinear relation, and has been used in other studies for classification of neural data (393, 394).

The performance of a given SVM with an RBF kernel depends on the two parameters of the RBF kernel $C$ and $\gamma$, however it is not known beforehand which $C$ and $\gamma$ pair optimize SVM’s
classification of the data. To find the best pair of $C$ and $\gamma$, a grid-search with a five-fold cross-validation rate was executed on a sequence of $(C, \gamma)$ pair values that grew exponentially (i.e. $C = 2^{-5}, 2^{-3}, \ldots, 2^{-5}, \gamma = 2^{-15}, 2^{-13}, \ldots, 2^3$). The parameter $(C, \gamma)$ pair that produced the highest cross-validation accuracy was picked. The classifier was then trained using the best parameter $(C, \gamma)$ pair with the data from all rats except one, and was tested on the remaining rat. The classification accuracy was defined as the probability of correctly classifying a test rat, and computed as the percentage of correctly classified instances. Lastly, the 95% confidence intervals for SVM classification accuracy was calculated using the binofit function in Matlab, and served as a decision boundary for testing whether the observed classification accuracy was significantly better than chance.

2.12 Statistical analysis
The effects of scopolamine, entorhinal HP-tau over-expression, and both conditions together, on the expression of a conditioned blinking response were assessed using a three-way repeated measures analysis of variance (three-way RM-ANOVA), with groups (i.e. GFP-saline, GFP-scopolamine, tau-saline, and tau-scopolamine) as the between-subjects factor and sessions (i.e. acquisition or reversal) and CS types (CS+, CS-) as the within-subjects factor. Data are presented as the mean ± the standard error of the mean (SEM). Differences were considered statistically significant if $p < 0.05$. Next, the effects of scopolamine, entorhinal HP-tau overexpression, and both conditions together, were examined on amplitude and the latency of brain’s ERPs. Due to the known difference in ERPs to auditory and visual stimuli (420, 421), ERPs to the auditory (or visual) CS during the acquisition phase were compared against those during the reversal phase. The ERPs for the last three conditioning sessions of acquisition and reversal were first averaged in each rat, and subsequently averaged across all the rats in each treatment group. The amplitude and latency values of each ERP component were compared between treatment groups by three-way RM-ANOVA with virus (i.e. GFP, tau) and drug (i.e. saline, scopolamine) as the between-subjects factors and CS type (CS+, CS-) as the within-subjects factor. Data are presented as the mean ± the standard error of the mean (SEM Bonferroni corrected p-values were used for multiple comparisons and differences were considered statistically significant if $p < 0.00625$. All analyses were performed using IBM SPSS Statistics software (SPSS, San Jose, USA).
2.13 Immunohistochemistry
Once behavioral testing was finished, viral vector expression and spread were verified by immunohistochemistry. Each rat was given a lethal intraperitoneal injection of sodium pentobarbital (102 mg/kg; Euthansol, Merck, Kirkland, QC, Canada) approximately after 10 weeks of incubation and perfused intercardially with approximately 150 mL of 0.9% saline and 150 mL of 4% paraformaldehyde. The brains were then extracted, post-fixed for 2 hours in 4% paraformaldehyde solution at 4 °C, and transferred to a solution of 30% sucrose in PBS (0.1 M; pH 7.4) for 1 week. Subsequently, brains were sliced to sections 40 µm thick using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada). The presence of HP-tau associated with pretangle material and neurofibrillary changes in AD was assessed immunohistochemically using monoclonal antibody, AT8 (mouse; 1:1000; #MN1020, ThermoFisher Scientific) which detected human tau protein with phosphorylated serine 202 and threonine 205 amino acid residues (pSer202/Thr205) (422). Cell nucleus was visualized with the DAPI stain (EMD Millipore).

Images were acquired using an upright fluorescent microscope (Leica Microsystems, Canada) and Volocity image analysis software (PerkinElmer, Waltham, USA), and were mapped with the stereotaxic atlas of the rat brain (423). The behavioral and EEG data from a rat were included in the subsequent analyses if imaging results showed 1) an accurate placement of the infusion cannula within the entorhinal cortex in all the injection sites, and 2) HP-tau was detected in minimum 6 out of 8 of total correctly-placed injection sites. The volume of entorhinal cortex inflicted with HP-tau was obtained by estimating the volume of expression using the mediolateral, dorsoventral, and anteroposterior lengths of spread in a series of sections collected every 80 µm.
Figure 4. A cartoon representation of rAAV9 vector.

**Model rats: rAAV2/9 Tau**

- CMV
- Chicken β-actin promoter
- Human tau P301L gene

Translation starts  Translation stops

**Control rats: rAAV2/9 GFP**

- CMV
- Chicken β-actin promoter
- Green fluorescent protein gene

Translation starts  Translation stops

Figure 4. A recombinant adeno-associated viral serotype 9 (rAAV9) with the cytomegalovirus (CMV) enhancer and chicken β-actin promoter were used to drive the expression of human tau with P301 mutation in the model rats, or green fluorescent protein (GFP) in the control rats. The vectors were obtained from Dr. R. Klein at the Louisiana State University, Baton Rouge, United States.

Figure 5. Bi-lateral infusion of the viral vector into the entorhinal cortex.

Table 1. Coordinates of the viral vector infusion.

<table>
<thead>
<tr>
<th>Coordinates from bregma (mm)</th>
<th>AP</th>
<th>ML</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>6.0</td>
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<td>-8.8</td>
</tr>
<tr>
<td>Site 2</td>
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</tr>
<tr>
<td>Site 3</td>
<td>7.6</td>
<td>5.6</td>
<td>-7.2</td>
</tr>
<tr>
<td>Site 4</td>
<td>8.5</td>
<td>5.4</td>
<td>-6.8</td>
</tr>
</tbody>
</table>

Table 1. Coordinates of four injection sites where viral vector containing either human mutated tau or GFP is infused into the entorhinal cortex (Anteroposterior, AP; Mediolateral, ML; Dorsoventral, DV).

Figure 5. A depiction of a histological section from a single rat illustrating the first of the four locations of the micro-injection within the entorhinal cortex.
Figure 6. EEG and EMG electrode placements.

A) stainless steel screw electrodes were implanted on rat’s skull surface and penetrated the dura mater to record the cortical activity over the frontal (3.2 mm anterior and 0.7 mm lateral from bregma; yellow), parietal (-3.5 mm AP, 2.0 mm ML; red), and temporal (-6.2 mm AP, 7.0 mm ML; blue) cortices as shown in the sample extracted brain. B) A depiction of EEG and EGM acquisition screen. Four Teflon-coated stainless steel wire (outer diameter = 0.0055”) were inserted in orbicularis oculi muscle of the left eyelid to serve as an EMG electrode pair and a stimulus electrode pair.

(Adapted with few modifications, Madronal et al., 2009.)
Figure 7. A depiction of differential trace paradigm. In this paradigm two stimuli (tone and light) are presented at random trials with equal probability. In phase 1 (acquisition) rats received 50 pairings of the neutral conditioned stimulus 1 (CS1) with an aversive, eyeblink-eliciting stimulus (US) with an inter-stimulus interval of 500 ms, and 50 presentations of the other CS alone (CS2-). In phase 2 (reversal) CS1 was presented alone (CS1-) while the CS2 was reinforced with the US (CS2+).
Figure 8. Time windows for capturing ERP peaks after CS onset.

**Auditory ERPs**

- **N1**
- **P1**
- **P2**
- **P3**

Time windows: 35-80 ms for P1, 80-120 ms for N1, 120-240 ms for P2, 240-360 ms for P3 after the CS onset.

**Visual ERPs**

- **N1**
- **P1**
- **P2**
- **P3**

Time windows: 90-130 ms for P1, 130-180 ms for N1, 180-260 ms for P2, 260-360 ms for P3 component after the onset of the CS.
Figure 9. ERP feature extraction and data set combinations for SVM

A) A total of 96 ERP features were extracted from the combination of 2 stimulus modalities, 2 stimulus association with the US, 2 features of ERP peaks, 3 brain regions, and 4 ERP components from each animal. B) The extracted features were used as the input data for support-vector machine (SVM) classifier in 14 different combinations to examine which feature allows SVM to classify different treatment groups with the highest level of accuracy.
Figure 10. Summary of steps in SVM classification.

A) Data set in SVM format

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Class Label</th>
<th>Feature 1</th>
<th>Feature 2</th>
<th>...Feature 96th</th>
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<td>66</td>
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<tr>
<td></td>
<td>Tau-Saline</td>
<td>2</td>
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<td></td>
<td>GFP-Scopol</td>
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<td>68</td>
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<td>66</td>
<td>64</td>
</tr>
<tr>
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<td>66</td>
</tr>
<tr>
<td></td>
<td>Tau-Saline</td>
<td>2</td>
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<td>GFP-Scopol</td>
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<tr>
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B) Data set scaled 0-1

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<td>Test</td>
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</tbody>
</table>

143 (C & γ) pairs x Complete scaled data set

D) Leave-one-out cross validation with the best (C & γ) pair

Round 1:
Take rat 1 for test,
Train with rats 2-40

Round 2:
Take rat 2 for test,
Train with rats 1, 3-40

Round 3-40:
Take next rat for test,
Train on rest plus rats 1&2

Overall test result

Classification Accuracy

Figure 10. A) Extracted ERP features were transformed into the format of an SVM package before classification of the data. All rats within a single group were organized in rows and were assigned the same class labels (1 to 4 for GFP-saline, tau-saline, GFP-scopolamine, and tau-scopolamine respectively). The corresponding features for each rat were placed on the same row level and organized in columns 1 to 96. B) The data for each feature was then scaled from 0-1 across all rats. C) Using a five-fold cross validation, 143 pairs of C & γ were tested with the data set to obtain the pair with highest cross-validation accuracy D) Using leave-one-out cross validation (LOOCV) one rat is left out while the remaining data set was trained with the best C & γ pair and the model was tested with the sample which was left out. This procedure was repeated until the model was tested once with all the samples (Round 1-40; rat 1-40). The classification accuracy was defined as the probability of correctly classifying a test rat, and computed as the percentage of correctly classified instances.
Chapter 3
Results

3.1 Entorhinal hyperphosphorylated tau over-expression

Histological analyses was conducted at the end of behavioral testing and approximately 10 weeks after incubation of the viral vector with the antibody AT8 targeted to human tau phosphorylated serine residues 202 and 205. From 24 rats that received the rAAV-tau infusion, 4 rat did not show an expression of HP-tau protein and 4 rats had misplaced infusion cannula resulting in expression of HP-tau in ventral hippocampus or perirhinal cortex. Also, 2 of the 24 control rats did not show any GFP expression. The data from these animals were removed from subsequent analyses.

Examination of HP-tau expression in the entorhinal cortex (EC) revealed that a sizable fraction of cells in in the EC expressed HP-tau protein (Figure 11 A). Higher magnification images further revealed the presence of HP-tau inside the dendrites, soma, and axons of entorhinal cells (Figure 11 B).

To estimate the spread of cells expressing HP-tau, we examined the location of tau-expressing cells in a series of 40 um sections from a single rat (Figure 11 C). HP-tau was observed most intensely in areas adjacent to injection sites, but it also spread into areas between the injection sites (Figure 11 D).

In parallel, when examining the cortical areas dorsal to the entorhinal cortex (Figure 11 E), we did not observe any expression of HP-tau in any of these cortical regions at or between the injection sites, except for some minor expression along the injection tract and a small mediolateral (ML) spread of approximately 0.13 ± 0.02 mm into the perirhinal cortex for the last two injection coordinates (AP -7.6 and -8.5).

Based on the location of the entorhinal HP-tau-expressing cells (Figure 11 D), we measured the ML spread of the HP-tau to be approximately 0.69 ± 0.11 mm at and between the injection sites and the anteroposterior (AP) spread to be 3.5 mm. Subsequently, by using ML and AP spread values we estimated the volume of expressed HP-tau to be 47.1% of total entorhinal volume.
Hyperphosphorylated tau is known to spread transynaptically (424, 425). To test whether HP-tau spread to regions that receive entorhinal axonal projections, we examined the expression of HP-tau in two of entorhinal efferent regions, the dorsal hippocampus (424) and prelimbic region of medial prefrontal cortex (424, 426). Our examination did not reveal any expression of HP-tau in the dorsal hippocampus or the prelimbic region of the prefrontal cortex (Figure 11 F).

Together, these results suggest that we successfully induced expression of HP-tau in rat brain. Because tau aggregation is limited to the entorhinal cortex, it corresponds to transentorhinal stage in which the pathology is mostly restricted in the entorhinal and perirhinal regions (1).
Figure 11. Expression of hyperphosphorylated tau in the entorhinal cortex.

A) Sizeable fraction of cells in the entorhinal cortex expressed hyperphosphorylated tau

B) Higher magnification images of entorhinal cells expressing hyperphosphorylated tau

Figure 11. A) Examination of HP-tau expression in the entorhinal cortex (EC) revealed that a sizable fraction of cells in the EC expressed HP-tau protein (red). B) Magnified images of AT8-immunolabelled EC sections (red) of tau-expressing rats showed extensive expression of hyperphosphorylated tau within the cell body (20X, 40X) as well as dendrites and axons of entorhinal cells (40X). Cell nucleus was visualized by DAPI stain (blue).
C) Coronal sections of areas before and at the injection sites

D) Expression and spread of mutated tau protein at the entorhinal cortex

Figure 11. C) Coronal sections from the rat brain atlas corresponding to sections of entorhinal cortex (red) at the site of bilateral viral infusion (red square) as well as the area in between (pink square). D) AT8-immunolabelled images of entorhinal sections from a single tau-expressing rat exhibit a consistent expression of tau throughout the dorsolateral EC, as well as expressions in the medial and central EC from -8.00 to -8.50 anteroposterior coordinate from bregma.
Figure 11. E) Images were taken from cortical areas dorsal to the entorhinal cortex at and between the injection sites, including the ectorhinal cortex, temporal association cortex, and the primary and secondary auditory cortices to examine the spread of HP-tau into the neighboring regions. No expression of mutated tau (red) was observed in these cortical regions at or between the injection sites, except for some minor expression along the injection tract and a small mediolateral ML spread of approximately $0.13 \pm 0.02$ mm into the perirhinal cortex for the last two injection coordinates (AP -7.6 and -8.5).
F) Expression of the viral vector confined to the entorhinal cortex

Figure 11. F) Expression of viral vector carrying GFP (green) or mutated tau (red). Immunolabeling of brain sections from the prelimbic (PrL) region, dorsal hippocampus (dHPC), and entorhinal cortex (EC) of GPF and hyperphosphorylated tau-expressing rats with AT8 antibody targeting phosphorylated tau protein revealed expression of GFP and tau only at the site of viral infusion within the entorhinal cortex, and not in PrL or dHPC.
3.2 Behavior

As expected, there was no significant difference between the groups during the two habituation sessions (three-way repeated measures ANOVA, Sessions × Virus × Drug interaction, $F_{(1, 36)} =0.075, p = 0.786$; Sessions × Virus, $F_{(1, 36)}=0.160, p = 0.692$; Sessions × Drug, $F_{(1, 36)}=0.119, p = 0.732$).

3.2.1 Acquisition

The performance of animals in all groups were comparable through the 10 days of acquisition in DTEBC (Figure 12 A-E; three-way repeated measures ANOVA, CStype × Session × Group interactions, $F_{(27, 324)} = 0.704, p = 0.863$; CStype × Group, $F_{(3, 36)} = 2.273, p = 0.097$; Session × Group, $F_{(27, 324)} = 1.070, p = 0.375$; Group, $F_{(3, 36)} = 0.219, p = 0.882$). Through the 10 days of acquisition, all groups significantly increased the frequency of their expression of the conditioned response (CR %) to the reinforced conditioned stimulus (CS1+), but not in unreinforced CS (CS2-) (CStype × Session, $F_{(9, 324)}=22.153, p < 0.001$; follow-up one-way RM-ANOVA for GFP-saline, Session, $F_{(9,90)} = 8.162, p < 0.001$; one-way RM-ANOVA for GFP-scopolamine, Session, $F_{(9,117)} = 8.788, p < 0.001$; one-way RM-ANOVA for tau-sal, Session, $F_{(4,109, 41.087)} = 8.535, p < 0.001$; one-way RM-ANOVA for tau-scopolamine, Session, $F_{(3,422, 30.801)} = 7.616, p < 0.001$). Further, all groups learned the task at a similar rate. There was no significant difference in number of sessions required for rats in different groups to show a difference of 50% CR between the CS1+ and CS2- (Virus × Drug, $F_{(1, 36)}=0.188, p=0.667$; Virus, $F_{(1, 36)}=1.282, p=0.265$; Drug, $F_{(1, 36)}=1.994, p = 0.166$). Overall, rats with entorhinal HP-tau over-expression, scopolamine treatment, or both in conjunction, were able to acquire an associative memory with similar rates to the control rats.
3.2.2 Reversal

The performance of animals in all groups were comparable through the 7 days of reversal in DTEBC (Figure 13 A-E; three-way repeated measures ANOVA, CStype × Session × Group interactions, $F_{(18, 216)} = 0.921, p = 0.554$; CStype × Group, $F_{(3, 36)} = 10.46, p = 0.384$; Session × Group, $F_{(18, 216)} = 0.852, p = 0.637$; Group, $F_{(3, 36)} = 0.096, p = 0.962$). Through the 7 days of reversal phase, all groups were able to significantly increase their CR % to the newly-reinforced conditioned stimulus (CS2+), but not in unreinforced CS (CS1-) (Figure 13 A-E; CStype × Session, $F_{(6, 216)} = 25.66, p < 0.001$; one-way RM-ANOVA for GFP-saline, Session, $F_{(6, 33.347)} = 8.939, p < 0.001$; one-way RM-ANOVA for GFP-scopolamine, Session, $F_{(3, 43.352)} = 10.633, p < 0.001$; one-way RM-ANOVA for tau-sal, Session, $F_{(3.294, 32.936)} = 5.922, p = 0.002$; one-way RM-ANOVA for tau-scopolamine, Session, $F_{(1.921, 17.289)} = 6.218, p = 0.010$). Further, animals in all groups learned the task at a similar rate. There was no significant difference in number of sessions required for rats in different groups to show a difference of 50% CR between the CS2+ and CS2- (Virus × Drug, $F_{(1, 36)} = 0.040, p = 0.843$; Virus, $F_{(1, 36)} = 0.303, p = 0.586$; Drug, $F_{(1, 36)} = 1.692, p = 0.202$). Together, the results of rats’ performance during acquisition and reversal phase indicate that associative learning and reversal learning are not impaired by entorhinal HP-tau overexpression, scopolamine treatment, or both conditions together.
Figure 12. Differential trace eyeblink conditioning in GFP-saline (A, red), GFP-scopolamine (B, pink), tau-saline (C, dark blue), and tau-scopolamine (D, light blue) groups. All groups underwent 2 days of habituation (H1-2) and 10 days of acquisition (A1-10). All groups were able to acquire a conditioned response (CR) to the reinforced stimulus (CS1-US; solid line) with comparable rates, and did not develop a CR to the non-reinforced stimulus (CS2-alone; dashed line).
Figure 13. DTEBC Reversal (7 Days).

A) GFP-Saline

B) GFP-Scopolamine

C) Tau-Saline

D) Tau-Scopolamine

E) All groups

Figure 13. Differential trace eyeblink conditioning in GFP-saline (A, red), GFP-scopolamine (B, pink), tau-saline (C, dark blue), and tau-scopolamine (D, light blue) groups. All groups underwent 7 days of reversal (R1-7), where the contingency of the stimuli were switched (CS2-US, CS1-alone). All groups acquired conditioned response (CR) to the reinforced stimulus (CS2-US; solid line) through the 7 days of reversal phase. GFP-saline (A) and tau-saline (B) differentiated between the CS2-US and CA1-alone by day 4 of the reversal phase, while GFP-scopolamine (C) and tau-scopolamine (D) became able to differentiate between the stimuli by day 5 and 7 of the reversal respectively.
3.3 Event-related potentials

Examination of EEG power spectrum of each individual rat recorded in the last three days of acquisition or reversal phase did not reveal any power line noise artifact. As such, all the EEG data for each individual rat recorded during these sessions were used to calculate the ERPs.

3.3.1 Auditory stimulus-evoked ERPs in the frontal cortex

In GFP-saline group, the frontal auditory ERPs displayed a pronounced P1 and N1 deflection, and a smaller P2 deflection (Figure 14 A). Compared to the GFP-saline group, the amplitude of P1 was larger in GFP-scopolamine and tau-scopolamine group, but not in tau-saline group (Figure 15 A; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1,36)}=27.532, p < 0.001$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline, $t=2.370, p=0.030$; GFP-saline vs. tau-scopolamine, $t=3.140, p=0.005$; GFP-saline vs. GFP-scopolamine, $t=-0.312, p=0.759$). Moreover, the P1 amplitude in tau-scopolamine was significantly larger than that in the tau-saline group ($t=-5.2, p<0.001$). The latency of P1 was comparable across the groups (Figure 16 A; all $p>0.05$).

Compared to the GFP-saline group, the amplitude of N1 was larger in tau-scopolamine and GFP-scopolamine group, but not in tau-saline group (Figure 14 D; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1,36)}=8.615, p=0.006$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline, $t=-2.374, p=0.030$; GFP-saline vs. tau-scopolamine, $t=1.896, p=0.759$; GFP-saline vs. GFP-scopolamine, $t=-0.071, p=0.944$). Moreover, the N1 amplitude in tau-scopolamine was significantly larger than that in the tau-saline group ($t=-5.2, p<0.001$). The latency of N1 was comparable across the groups (Figure 16 D; all $p>0.05$).

Further, compared to the GFP-saline group, the amplitude of P2 was smaller in tau-saline group, but not in GFP-scopolamine group and tau-scopolamine group (Figure 14 G; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1,36)}=6.614, p=0.014$; Virus × Drug interaction, $F_{(1,36)}=11.698, p=0.001$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline, $t=6.923, p<0.001$; GFP-saline vs. tau-scopolamine, $t=-1.393, p=0.741$; GFP-saline vs. GFP-scopolamine, $t=0.042, p=0.967$). Moreover, the P2 amplitude in tau-scopolamine was significantly larger than
that in the tau-saline group \((t_{(13.78)} = -6.254, p < 0.001)\). The latency of P2 was comparable across the groups (Figure 16 G; all P > 0.05).

Overall, the pattern suggests that while entorhinal HP-tau over-expression results in an attenuation of the auditory ERP amplitudes in the frontal cortex, scopolamine alone seems to elevate the level of ERP amplitudes, and even more so in conjunction with HP-tau.

### 3.3.2 Auditory stimulus-evoked ERPs in the parietal cortex

In GFP-saline group, the parietal auditory ERPs displayed a pronounced P1 and N1 deflection, and a smaller P2 and P3 deflection (Figure 14 B). Compared to the GFP-saline group, the amplitude of P1 was smaller in GFP-scopolamine, tau-saline, and tau-scopolamine groups respectively. (Figure 15 B; three-way RM-ANOVA, CStype × Virus × Drug interaction, \(F_{(1, 36)} = 0.112, p = 0.739\); main effect of scopolamine, \(F_{(1, 36)} = 11.344, p = 0.002\); main effect of virus, \(F_{(1, 36)} = 36.789, p < 0.001\)). The latency of P1 was comparable across the groups (Figure 16 B; all P > 0.05).

Compared to the GFP-saline group, the amplitude of N1 was smaller in GFP-scopolamine, tau-saline, and tau-scopolamine groups respectively (Figure 15 E; three-way RM-ANOVA, CStype × Virus × Drug interaction, \(F_{(1, 36)} = 1.504, p = 0.228\); main effect of scopolamine, \(F_{(1, 36)} = 11.268, p = 0.002\); main effect of virus, \(F_{(1, 36)} = 66.726, p < 0.001\)). Further, compared to GFP-saline group, the latency of N1 component was shorter in GFP-scopolamine and tau-scopolamine groups (Figure 16 E; three-way RM-ANOVA, CStype × Virus × Drug interaction, \(F_{(1, 36)} = 0.003, p = 0.514\); main effect of scopolamine, \(F_{(1, 36)} = 17.072, p < 0.001\)).

Additionally, compared to the GFP-saline group, the amplitude of P2 was smaller in tau-saline group, but not in GFP-scopolamine group and tau-scopolamine group (Figure 15 H; three-way RM-ANOVA, CStype × Virus × Drug interaction, \(F_{(1, 36)} = 18.1069, p < 0.001\); follow-up independent sample t-test, GFP-saline vs. tau-saline, \(t = -2.783, p = 0.022\); GFP-saline vs. tau-scopolamine, \(t = 0.307, p = 0.762\); GFP-saline vs. GFP-scopolamine, \(t = -2.632, p = 0.016\)). Moreover, the P2 amplitude in tau-scopolamine was not significantly larger than that in the tau-saline group \((t_{(13.78)} = -2.784, p = 0.013\)). The latency of P2 was comparable across the groups (Figure 16 H; all P > 0.05).
Compared to the GFP-saline group, the amplitude of P3 component was smaller in all the remaining groups (Figure 15 K; three-way RM ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=1.888, p = 0.178$; Virus × Drug interaction, $F_{(1, 36)}=13.068, p < 0.001$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline $t_{(15.193)}=5.37, p < 0.001$; GFP-saline vs. tau-scopolamine, $t= -3.597, p= 0.002$; GFP-saline vs. GFP-scopolamine, $t=3.649, p = 0.001$; tau-saline vs. tau-scopolamine $t_{(20)}= -1.437, p = 0.177$). The latency of P3 was comparable across the groups (Figure 16 K; all $p > 0.05$).

Overall, the pattern suggests that entorhinal HP-tau over-expression as well as scopolamine attenuates of all the parietal auditory ERP amplitudes. The effect on ERP amplitudes is further potentiated when both conditions are present in conjunction.

### 3.3.3 Auditory stimulus-evoked ERPs in the temporal cortex

In GFP-saline group, auditory temporal ERP displayed a large P1 and N1 deflection, and small P2 and P3 deflection (Figure 14 C). Compared to GFP-saline group, the amplitude of P1 component was smaller in GFP-scopolamine, tau-saline, and tau-scopolamine respectively (Figure 15 C; three-way RM ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=1.60, p = 0.214$; Virus × Drug interaction, $F_{(1, 36)}=9.288, p < 0.004$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline $t=6.610, p < 0.001$; GFP-saline vs. tau-scopolamine, $t= -3.597, p= 0.002$; GFP-saline vs. GFP-scopolamine, $t= -8.146, p < 0.001$) Further, the P1 amplitude in tau-saline group was significantly larger than that in the tau-scopolamine group ($t=4.883, p < 0.001$). The latency of P1 was comparable across the groups (Figure 16 C; all $p > 0.05$).

Compared to GFP-saline group, the amplitude of N1 component was comparable in tau-scopolamine groups, but smaller in GFP-scopolamine and tau-saline groups (Figure 15 F; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=1.764, p = 0.192$; Virus × Drug interaction, $F_{(1, 36)}=132.336, p < 0.001$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline $t=15.36, p < 0.001$; GFP-saline vs. tau-scopolamine, $t= -1.961, p=0.077$; GFP-saline vs. GFP-scopolamine, $t=12.93, p < 0.001$) Further, the N1 amplitude in tau-scopolamine group was significantly larger than that in the tau-saline group ($t=8.299, p < 0.001$). The latency of N1 was comparable across the groups (Figure 16 F; all $p > 0.05$).
Compared to GFP-saline group, the largest auditory temporal P2 amplitude was observed in tau-scopolamine group, followed by GFP-scopolamine and tau-saline groups (Figure 15 I; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=0.522, p = 0.474$; main effect of scopolamine, $F_{(1, 36)}=59.897, p < 0.001$; main effect of virus, $F_{(1, 36)}=14.293, p < 0.001$). The latency of P2 was comparable across the groups (Figure 16 I; all $p > 0.05$).

Further, compared to GFP-saline group, the P3 amplitude was larger in in tau-scopolamine and GFP-scopolamine groups, but not in tau-saline group (Figure 15 L; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=0.328, p = 0.570$; main effect of scopolamine, $F_{(1, 36)}=15.813, p < 0.001$). The latency of P3 was comparable across the groups (Figure 16 L; all $p > 0.05$).

Overall, the pattern suggests that while entorhinal HP-tau over-expression and scopolamine can separately attenuate temporal auditory ERP amplitudes, simultaneous presentation of the two conditions increases the level of ERP amplitudes higher than that of controls.

### 3.3.4 Visual stimulus-evoked ERPs in the frontal cortex

In GFP-saline group, the frontal visual ERPs displayed a pronounced P1 and N1 deflection, and a smaller P2 deflection (Figure 17 A). The amplitude of P1 component in GFP-saline group was comparable to tau-saline and GFP-scopolamine groups, but smaller than tau-scopolamine group (Figure 18 A; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=0.774, p=0.385$; Virus × Drug interaction, $F_{(1, 36)}=10.854, p=0.002$; follow-up independent sample $t$-test, GFP-saline vs. tau-saline, $t=-0.173, p=0.932$; GFP-saline vs. tau-scopolamine, $t=4.262, p < 0.001$; GFP-saline vs. GFP-scopolamine, $t=-0.71, p =0.944$). Moreover, the P1 amplitude in tau-scopolamine was significantly larger than that in the tau-saline group (t_{(13.78)} = 3.818, p = 0.002). The latency of P1 was comparable across the groups (Figure 19 A; all $p > 0.05$).

Compared to GFP-saline group, the amplitude of N1 component was larger in tau-saline, GFP-scopolamine, and tau-scopolamine groups respectively (Figure 18 D; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)}=1.395, p = 0.245$; main effect of scopolamine, $F_{(1, 36)}=28.250, p < 0.001$). The latency of N1 was comparable across the groups (Figure 19 D; all $p > 0.05$).
Compared to GFP-saline, the amplitude of P2 component was larger in GFP-scopolamine and tau-scopolamine groups, but similar in tau-saline group (Figure 18 G; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)} = 0.694, p = 0.160$; main effect of scopolamine, $F_{(1, 36)} = 244.9, p < 0.001$; main effect of Virus, $F_{(1, 36)} = 53.403, p < 0.001$). The latency of P2 was comparable across the groups (Figure 19 G; all $p > 0.05$).

Overall, the pattern suggests that while entorhinal HP-tau over-expression does not alter the levels of frontal visual ERP amplitudes, scopolamine can increase frontal visual ERP amplitude levels higher than that of controls. The effect of scopolamine is further potentiated in presence of entorhinal HP-tau.

### 3.3.5 Visual stimulus-evoked ERPs in the parietal cortex

In GFP-saline group, the parietal auditory ERPs displayed a pronounced P1, N1, and P2 deflection (Figure 17 B). The amplitude of parietal visual P1 component was comparable between the GFP-saline group and tau-saline group, but was smaller in GFP-scopolamine and tau-scopolamine groups compared to the GFP-saline group (Figure 18 B; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)} = 0.110, p = 0.742$; main effect of scopolamine, $F_{(1, 36)} = 24.431, p < 0.001$). Further, compared to GFP-saline group, the latency of P1 component was shorter in GFP-scopolamine and tau-scopolamine groups (Figure 19 B; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)} = 2.599, p = 0.116$; main effect of scopolamine, $F_{(1, 36)} = 9.638, p < 0.004$).

Compared to GFP-saline group, the amplitude of N1 component was larger in GFP-scopolamine and tau-saline group, but not in tau-scopolamine group. No effect of treatment was observed on N1 amplitude or latency across all groups (Figure 18 E; all $p > 0.00625$; Figure 19 E; all $p > 0.05$).

Compared to GFP-saline group, the amplitude of P2 component was similar in tau-saline, but was larger in GFP-scopolamine and tau-scopolamine groups (Figure 18 H; three-way RM-ANOVA, CStype × Virus × Drug interaction, $F_{(1, 36)} = 0.987, p = 0.327$; main effect of scopolamine, $F_{(1, 36)} = 17.598, p < 0.001$). The latency of P2 was comparable across the groups (Figure 19 H; all $p > 0.05$).
Overall, the pattern suggests that scopolamine results in an increase of the amplitude levels of parietal visual ERPs, while entorhinal HP-tau over-expression seems not to have an effect.

### 3.3.6 Visual stimulus-evoked ERPs in the temporal cortex

The temporal visual ERP waveforms displayed a prominent P1, and a smaller N1 and P2 deflections (Figure 17 C). Statistical analyses did not reveal any significant differences between the P1 amplitude of different groups (Figure 18 C; all \( p > 0.00625 \)), nor any significant difference were observed between the P1 latency of different groups (Figure 18 C; all \( p > 0.05 \)).

Compared to GFP-saline group, the amplitude of visual N1 component was larger in tau-saline, GFP-scopolamine, and tau-scopolamine groups (Figure 18 F; three-way RM-ANOVA, CStype × Virus × Drug interaction, \( F_{(1,36)}=0.630, p=0.433 \); main effect of scopolamine, \( F_{(1,36)}=9.898, p = 0.003 \)). The latency of N1 was comparable across the groups (Figure 19 F; all \( p > 0.05 \)).

Next, compared to GFP-saline group, the amplitude of visual P2 component was larger in tau-saline, GFP-scopolamine, and tau-scopolamine groups (Figure 18 I; three-way RM-ANOVA, CStype × Virus × Drug interaction, \( F_{(1,36)}=15.301, p<0.001 \); follow-up independent sample \( t \)-test, GFP-saline vs. tau-saline, \( t=-9.005, p < 0.001 \); GFP-saline vs. tau-scopolamine, \( t=1.138, p=0.269 \); GFP-saline vs. GFP-scopolamine, \( t=0.643, p=0.527 \)). Moreover, the P2 amplitude in tau-saline was significantly larger than that in the tau-scopolamine group (\( t_{(13.78)}= 9.147, p < 0.001 \)). The latency of P2 component was comparable across the groups (Figure 19 I; all \( p > 0.05 \)).

Overall, the pattern suggests that scopolamine as well as entorhinal HP-tau over-expression increase the level of temporal visual N1 and P2 amplitudes respectively higher than that of controls.
Figure 14. A depiction of grand average frontal, parietal, and temporal ERPs across treatment groups GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue) and tau-scopolamine (light-blue), during the paring of the auditory CS with the US (ACS+; A-C) and during solo presentation of the auditory CS (ACS-; D-F). Statistical analysis of the frontal ERP (A and D) revealed a significant effect of a three-way interaction between CStype (ACS+/ACS-), Virus (entorhinal tau pathology), and Drug (cholinergic deficiency) on P1 and N1 components (CStype × Virus × Drug; dark red), and a significant effect of a 2-way interaction on the P2 component (Virus × Drug; purple). Examination of the parietal ERP (B and E) found a significant main effect of Drug and a significant main effect of Virus on the P1 component and similarly on the N1 component (gray), a significant effect of a three-way interaction on the P2 component (CStype × Virus × Drug; dark red), and a significant effect of a 2-way interaction on the P3 component (Virus × Drug; purple). Over the temporal cortex, the analyses found a significant effect of a 2-way interaction on the P1 and N1 components (Virus × Drug; purple), a significant main effect of Drug and a significant main effect of Virus on the P2 component, and a significant main effect of Drug on the P3 component (green).
Figure 15. Interaction graphs of auditory ERP amplitude.

Figure 15. Line graphs display the effect of treatments on the mean amplitude of the auditory P1 (A–C), N1 (D–E), P2 (G–I), and P3 (J–K) ERP components across frontal, parietal, and temporal cortices of GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue), and tau-scopolamine (light blue), during CS-US pairing (CS+) and solo presentation of the CS (CS−). Statistical examination of the P1 mean amplitude (A–C) revealed a significant effect of a three-way interaction between CStype (ACS+/ACS−), Virus (entorhinal tau pathology), and Drug (cholinergic deficiency) on the frontal P1 (A; CStype × Virus × Drug; dark red), a significant main effect of Drug and a significant main effect of Virus on the parietal P1 (B; gray), and a significant effect of a 2-way interaction on the temporal P1 (C; Virus × Drug; purple). Similarly, analysis of N1 mean amplitude (D–F) revealed a significant effect of a three-way interaction on the frontal N1 (D; CStype × Virus × Drug; dark red), and a significant main effect of Virus on the parietal N1 (B; gray), and a significant effect of a 2-way interaction on the temporal N1 (F; Virus × Drug; purple). Examination of P2 mean amplitude (G–I) found a significant effect of a 2-way interaction on the frontal P2 (G; Virus × Drug; purple), a significant effect of a three-way interaction on the parietal P2 (H; CStype × Virus × Drug; dark red), and a significant main effect of Drug and a significant main effect of Virus on the temporal P2 (I; gray). Statistical analysis of P3 mean amplitude (J–L) found a significant effect of a 2-way interaction on the parietal P3 (K; Virus × Drug; purple), and a significant main effect of Drug on the temporal P3 (L; green). Error bars represent the standard error of the mean.
Figure 16. Line graphs display the effect of treatments on the mean latency of the auditory P1 (A-C), N1 (D-E), P2 (G-I), and P3 (J-K) ERP components across frontal, parietal, and temporal cortices of GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue), and tau-scopolamine (light blue), during CS-US pairing (CS+) and solo presentation of the CS (CS−). Statistical analysis did not reveal any significant effect of treatment in the latency of P1 component across different brain regions (A-C; white). Examination of N1 mean latency revealed a significant main effect of Drug (cholinergic deficiency) on the mean latency of parietal N1 (E; green). No treatment effects were observed on the N1 mean latency over the frontal and temporal cortices (D and F; white), as well as the mean latency of P2 and P3 components over the frontal, parietal, and temporal cortices (G-L; white). Error bars represent the standard error of the mean.
Figure 17. Visual ERPs during DTEBC paradigm.

Figure 17. A depiction of grand average frontal, parietal, and temporal ERPs across treatment groups GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue) and tau-scopolamine (light-blue), during the paring of the visual CS with the US (VCS+; A-C) and during solo presentation of the visual CS (VCS-; D-F). Statistical analysis of the frontal ERP (A and D) revealed a significant effect of a 2-way interaction between Virus (entorhinal tau pathology) and Drug (cholinergic deficiency) on the P1 component, a significant main effect of Drug on the N1 component, and a significant main effect of Virus on the P2 component (gray). Examination of the parietal ERP (B and E) found a significant main effect of Drug on the P1, N1, and P2 components (green). Over the temporal cortex, the analyses found a significant main effect of Drug on the N1 component (green), and a significant effect of a three-way interaction between CStype (VCS+/VCS-), Virus, and Drug on the P2 component (CStype × Virus × Drug; dark red).
Figure 18. Interaction graphs of visual ERP amplitude.

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**Treatment effect**
- CStype × Virus × Drug
- Main effect of Drug
- Virus × Drug
- No effect
- Main effects of Drug and Virus

**Treatment groups**
- GFP-Saline
- GFP-Scopolamine
- Tau-Saline
- Tau-Scopolamine

Figure 18. Line graphs display the effect of treatments on the mean amplitude of the visual P1 (A-C), N1 (D-E), P2 (G-I), and P3 (J-K) ERP components across frontal, parietal, and temporal cortices of GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue), and tau-scopolamine (light blue), during CS-US pairing (CS+) and solo presentation of the CS (CS-). Statistical examination of the P1 mean amplitude (A-C) revealed a significant effect of a 2-way interaction between Virus (entorhinal tau pathology) and Drug (cholinergic deficiency) on the frontal P1 (A; Virus × Drug; purple), a significant main effect of Drug on the parietal P1 (B; green), and no significant effect on the temporal P1 component (C; white). Analysis of N1 mean amplitude (D-F) revealed a significant main effect of Drug on the frontal N1 (D; green), a significant effect a 2-way interaction between CStype (CS+/CS-) and Drug on the parietal N1 (E; CStype × Drug; pink, and a significant main effect of Drug on the temporal N1 (F; green). Examination of P2 mean amplitude (G-I) found a significant main effect of Drug and a significant main effect of Virus on the frontal P2 (G; gray), a significant main effect of Drug on the parietal P2 (H; green), and a significant effect of a three-way interaction on the temporal P2 (I; CStype × Virus × Drug; dark red), No treatment effects were observed on the P3 mean amplitude over the frontal and temporal cortices (J-L; white). Error bars represent the standard error of the mean.
Figure 19. Line graphs display the effect of treatments on the mean latency of the visual P1 (A-C), N1 (D-E), P2 (G-I), and P3 (J-K) ERP components across frontal, parietal, and temporal cortices of GFP-saline (red), GFP-scopolamine (pink), tau-saline (dark blue), and tau-scopolamine (light blue), during CS-US pairing (CS+) and solo presentation of the CS (CS-). Statistical analyses P1 mean latency revealed a significant main effect of Drug (cholinergic deficiency) on the mean latency of parietal P1 component (E; green). No treatment effects were observed on the P1 mean latency over the frontal and temporal cortices (A and C; white), as well as the mean latency of N1, P2, and P3 components over the frontal, parietal, and temporal cortices (D-L; white). Error bars represent the standard error of the mean.
3.4 SVM-based classification of ERPs

Figure 20 (A-F) show the classification accuracy of SVM for GFP-saline, tau-saline, GFP-scopolamine, and tau-scopolamine based on different combination of extracted ERP features. When all of the 96 ERP features were incorporated in the classification procedure, the classification accuracy of the SVM was 100% for GFP-saline, 100% for tau-saline, 100% for GFP-scopolamine, and 90% for tau-scopolamine (Figure 20 A; all features). The accuracy for all groups was significantly better than what was expected by chance (binominal test, \( p < 0.05 \)). To identify which combination of features yield the best classification accuracy, we then repeated the SVM analysis by using a subset of 96 features.

Examination of the effect of stimulus modality on SVM’s classification accuracy revealed no significant difference between the auditory versus visual stimulus. With either 48 auditory or 48 visual ERP features SVM was able to detect all the treatment groups with high accuracy (Figure 20), and the accuracy for all groups was significantly better than what was expected by chance (binominal test, \( p < 0.05 \)).

Next, the effect of CS-US contingency on SVM classification accuracy was investigated. Accuracy of SVM did not differ based on CS-US contingency (Figure 20 C). Specifically, the accuracy obtained with 48 ERP features during CS+ was comparable with the accuracy obtained during the CS-, and the accuracy for all groups was significantly better than what was expected by chance (binominal test, \( p < 0.05 \)).

Furthermore, the discriminatory powers of the two ERP peak properties, amplitude and latency, were compared. By using 48 ERP amplitude features SVM was able to detect all the groups, but with 48 ERP latency features only GFP-scopolamine group was detected. The accuracy was significantly better than what was expected by chance (Figure 20 D; binominal test, \( p < 0.05 \)).

Next, the discriminatory powers of ERP data from frontal, parietal, and temporal cortices were compared. The accuracy of the classifier was comparable across different brain regions, and was significantly better than what was expected by chance (Figure 20 E; binominal test, \( p < 0.05 \)). This suggests that features from one brain regions are sufficient for detecting different groups, among them features from the temporal cortex allowed the classifier to detect the groups with 100% accuracy.
Moreover, extracted features from P1, N1, P2, and P3 ERP components were tested separately to find the component that yielded the highest classification accuracy. Majority of the ERP components allowed the classifier to detect the pathologies accurately and significantly better than what was expected by chance (binominal test, $p < 0.05$), with exception of P2 component for tau-scopolamine group, and P3 component for GFP-scopolamine group (Figure 20 F).

Together, the results indicate that different pathologies such as accumulation of HP-tau protein in the entorhinal cortex, or scopolamine-induced deficiency of the central cholinergic system, induce abnormalities in the pattern of brain’s ERPs that are distinct from each other. These abnormal patterns are observed mainly as alterations in the level of the peak amplitude of the ERP components and can be utilized by machine learning classifiers such as a support vector machine to detect the type of pathology present (Figure 21).
Figure 20. SVM classifier accuracy.

A) All features

\[ 2 \times 2 \times 2 \times 3 \times 4 = 96 \text{ features} \]

<table>
<thead>
<tr>
<th>Tone</th>
<th>CS+</th>
<th>Amplitude</th>
<th>Frontal</th>
<th>P1</th>
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<tbody>
<tr>
<td>Light</td>
<td>CS-</td>
<td>Latency</td>
<td>Parietal</td>
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B) Stimulus modality

\[ 1 \times 2 \times 2 \times 3 \times 4 = 48 \text{ features} \]

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<tr>
<th>Tone</th>
<th>CS+</th>
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<tr>
<td>Light</td>
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C) CS-US contingency

\[ 2 \times 1 \times 2 \times 3 \times 4 = 48 \text{ features} \]

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<th>Tone</th>
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<tr>
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D) ERP peak properties

\[ 2 \times 2 \times 1 \times 3 \times 4 = 48 \text{ features} \]

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<th>CS+</th>
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<td>Light</td>
<td>CS-</td>
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### E) Brain regions

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<tr>
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### F) ERP components

<table>
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<table>
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<th><strong>Classification accuracy (%)</strong></th>
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<td>Frontal</td>
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Figure 20. Bar graphs display SVM’s classification accuracy (CA) for GFP-saline (Gsal; red), tau-saline (Tsal; blue), GFP-scopolamine (Gsco; pink), and tau-scopolamine (Tsco; light blue) with different combinations of extracted ERP features as indicated in the charts (Blue, selected features; Yellow, compared features). A) The CA with all 96 features was 100% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco.

B) Across stimulus modalities, auditory stimulus (tone) yielded a CA of 100% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco, and visual stimulus (light) yielded a CA of 100% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco.

C) Features extracted during CS-US pairing (CS+) resulted a CA of 100% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco, and features extracted during solo CS presentation (CS-) yielded a CA of 90.91% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco.

D) When comparing the properties of the ERP peak, ERP peak amplitude achieved a CA of 100% for Gsal, 100% for Tsal, 100% for Gsco, and 90% for Tsco, while ERP peak latency yielded an accuracy level of 45.4% for Gsal, 0 for Tsal, 81.8% for Gsco, and 30% for Tsco.

E) Among different brain regions, features from frontal ERP yielded a CA of 100% for Gsal, 100% for Tsal, 90.1% for Gsco, and 80% for Tsco, while parietal ERP features achieved a CA of 81.8% for Gsal, 75% for Tsal, 81.9% for Gsco, and 80% for Tsco, and temporal ERP features resulted in a CA of 100% for Gsal, 100% for Tsal, 100% for Gsco, and 100% for Tsco.

F) When comparing separate ERP components, CA with P1 component was 81.2% for Gsal, 100% for Tsal, 81.2% for Gsco, and 90% for Tsco. Features from N1 component also yielded a CA of 100% for Psal and 90% for Tsco, while increasing the CA for Gsal and Gsco to 90.9% and 100% respectively. The CA achieved with features from P2 component was 100% for Gsal, 100% for Tsal, 100% for Gsco, and 50% for Tsco, and with features from P3 component was Gsal, 75% for Tsal, 45.4% for Gsco, and 60% for Tsco.
Figure 21. The heat map summarized the classification accuracy (CA) results (dark blue 0-100% dark red) of the SVM classifier for each treatment groups (GFP-saline, Tau-saline, GFP-scopolamine, and Tau-scopolamine; column) with different combinations of test features (rows). For stimulus modality, tone-CS and light-CS had comparable CAs. For CS contingency, reinforced CS with the US (CS+) and the non-reinforced CS (CS-) had similar CAs. Between the properties of the ERP peak, amplitude displayed an exceedingly better accuracy than latency. Classification results with features from frontal, parietal, and temporal cortices were comparable. Within different ERP components, P2 and P3 failed to detect Tau-scopolamine and GFP-scopolamine respectively.
Chapter 4
Discussion

4.1 Comparison of histological changes with AD staging

Based on our histological analysis we observed that HP-tau was accumulated in the entorhinal cortex, but was absent in two of entorhinal cortex efferent regions, hippocampus and prelimbic area. Together these results suggest that in our model we successfully induced tau pathology that corresponds to the transentorhinal stage of preclinical AD (427).

Although systemic injection of scopolamine at the dose used in this study allowed us to induce cholinergic deficiency without memory impairment, the mechanism that induced cholinergic deficiency in our model was not the same as the mechanism observed in preclinical stage of AD. In preclinical AD, a presynaptic cholinergic deficit is caused by the reduction in choline acetyltransferase activity which results in a lowered level of acetylcholine production (428), whereas in our model, scopolamine induces a postsynaptic cholinergic deficit by blocking muscarinic receptors. Another difference is that the reduction in cholinergic input during preclinical AD occurs progressively over the years, but scopolamine induces an acute deficiency in cholinergic input.

4.2 Effects of preclinical AD pathology on associative learning and reversal learning

Using an associative memory paradigm known as differential trace eyeblink conditioning (DTEBC), we show that associative learning and reversal learning is not impaired in rats with entorhinal HP-tau over-expression, cholinergic deficiency, or both pathologies in conjunction. We tested associative learning during a ten-day acquisition phase, in which rats with brain pathology and the control rats acquired a preemptive eyeblink conditioned response (CR) to the reinforced conditioned stimulus (CS+) but not to the neutral CS (CS-). Next, we assessed reversal learning by switching the contingency between CS and the unconditioned stimulus (US), and observed that
rats with pathology, similar to control rats, were able suppress their CR to the previously-reinforced CS and acquire CR to the new CS+.

The absence of cognitive deficits observed in this study is consistent with other studies that demonstrated that preclinical AD pathologies do not produce cognitive deficits. For example, a study in mice with accumulated HP-tau in EC and hippocampus reported that pathology at this stage is not robust enough to cause deficits in spatial navigation memory (429). Another study with clinically normal adults who exhibited biomarker evidence of Aβ deposition did not find any impairment in associative memory examined with cued recall test. Our observation on the behavioral effects of the preclinical AD pathology in rats is in parallel with previous studies in rodents and humans, which report that AD neuropathology in the preclinical stage of the disease is asymptomatic (429-431).

4.3 Mechanisms underlying ERP abnormality and susceptibility of different cortical regions to features of preclinical AD pathology

During differential trace eyeblink conditioning, we also recorded event related potentials during the trace interval between the CS and US. Our ANOVA-based comparison of ERP peak amplitude and latency showed that entorhinal HP-tau over-expression significantly decreased the amplitude of frontal auditory P2, parietal auditory P1, N1, and P3, and temporal auditory P1, N1, and P2 components, whereas it significantly increased the amplitude of frontal and temporal visual P2 component.

In addition, cholinergic deficiency significantly decreased the amplitude of parietal auditory P1, N1, and P3, parietal visual P1, and the temporal auditory P1 and N1 components. However, cholinergic deficiency significantly increased the amplitude of the temporal auditory P2 and P3, temporal visual N1, and frontal visual N1 and P2 components. Further, the latency of parietal auditory N1 and visual P1 were significantly as a result of cholinergic deficiency.

Interestingly, we observed abnormal parietal auditory P3 component rats with entorhinal HP-tau, cholinergic deficiency, and conjunction of both pathologies (Figure. 14 B). Similar findings have been reported in human oddball paradigm studies with probable AD and MCI patients where the
amplitude of auditory P3 component was reduced compared to age-matched controls (432, 434, 435). Our finding suggests that pathological features of AD appear to affect ERP components similarly in rats and humans.

Further, based on the number of affected ERP components by each pathological condition in separate brain regions, we observed that temporal ERPs were mainly affected by the entorhinal HP-tau over-expression, parietal ERPs were mostly impacted by cholinergic deficiency, and frontal ERPs were largely affected by the conjunction of both conditions. Examination of ERP features from each brain region with the classifier however, revealed that ERPs from one cortical area are capable of accurately detecting different types of pathologies (Figure. 20 E). These findings suggest that, different brain regions may be affected by AD pathologies at different levels, and that the within one cortical region different pathologies affect ERPs with substantially distinct pattern.

4.3.1 HP-tau-induced synaptic dysfunction and cortical hyperexcitability

The temporal ERPs are mostly affected by HP-tau over-expression likely because HP-tau results in loss of dendritic spines, disrupting axonal transport, and synaptic anchoring which ultimately leads to synaptic dysfunction of entorhinal neurons (436-438). Indeed, many studies have implicated HP-tau in synaptic dysfunction and cortical hyperexcitability (439, 440). It has been shown that localization of HP-tau to the spines of hippocampal pyramidal cells in 4-month-old rTg4510 mice can initiate spine loss and dendritic regression by decreasing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated synaptic currents through removal of glutamate receptor 1 (GluR1) AMPA receptors from spines (441, 442). Loss of spine density reduces the dendritic diameter of the presynaptic cortical neuron and attenuates the frequency and the amplitude of the synaptic current moving through the dendrites (443).

Further, HP-tau can reduce synaptic synchrony by impairing axonal transport and synaptic anchoring (444-449). Reduction of synaptic synchrony together with the attenuation of frequency and amplitude of the synaptic current cause insufficiency of synaptic input to the postsynaptic neurons as observed in the hippocampus of transgenic mouse with P301S mutation (450). This
may explain the attenuated peak amplitude of the ERP components in rats with entorhinal HP-tau over-expression (Figure. 14).

As the pathology progress, increasing number of presynaptic cortical neurons are lost, leaving a significant population of postsynaptic neurons deafferented (451). It is speculated that the loss of afferent input is compensated by the surviving neurons in the 9-month-old rTg4510 mice through increased excitability, as observed by elevated frequency of glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) and increased amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs), and sprouting of new axonal buttons and formation of new synapses (452). These compensatory homeostatic mechanisms may also account for the long preclinical stage of the AD, during which maintaining network stability supports cognitive functions until the pathology affects the compensatory mechanisms and results in a severe network dysfunction (453).

### 4.3.2 Cholinergic deficiency and the hippocampus

In addition to the abnormalities in temporal ERPs, we detected that parietal ERPs are particularly sensitive to cholinergic deficiency. The sensitivity of parietal ERPs to cholinergic deficiency in rats may be attributed to disruption of neuronal activity in the underlying hippocampal structures, which receives dense cholinergic projections from the medial septal nucleus (454-456).

The pivotal role of cholinergic system in cognitive processes such as working memory and attention has been well established through lesion and pharmacological studies in rodents and nonhuman primates (457-461). It is known that acetylcholine modulates synaptic plasticity in hippocampus and facilitates the firing of hippocampal pyramidal neurons through muscarinic receptors (462-465). Additionally, studies in rats have shown that blocking of muscarinic receptors by scopolamine inhibits long-term potentiation (LTP) in the dentate gyrus and the CA1 regions (466, 467) and that muscarinic agonists enhanced hippocampal LTP (468).

Moreover, cholinergic modulation may influence cognitive functions through the processing of sensory information, in which cholinergic neurons project to many cortical targets to facilitate neural sensory processing by enhancing signal-to-noise ratio at the synaptic level (469-474). Therefore, the effect of cholinergic deficiency on parietal ERP may also be an outcome of
disrupted cholinergic-dependent modulation of mechanisms that take part in processing of inputs to the hippocampus (475-478).

4.3.3 HP-tau, cholinergic deficiency, and the abnormal frontal ERPs

Unlike temporal and parietal ERPs, frontal ERPs are sensitive to the conjunction of entorhinal HP-tau and cholinergic deficiency. This sensitivity may be due to disruption of input from the hippocampus, entorhinal cortex, and the basal forebrain cholinergic projections to the frontal cortex.

It is known that medial prefrontal cortex (mPFC) is involved in a number of cognitive functions such as learning and memory (479-481) and is believed to mediate its function through multiple connections with limbic and sensory cortical structures in the parietal and temporal cortices (482-485).

The membrane potential of the mPFC pyramidal neurons in rats oscillates from an inactive hyperpolarized state to a depolarized state at which action potentials are fired (486, 487). Hippocampal contribution to regulation of mPFC activity is believed to arise from modulation of this bistable state by a combination of excitatory and inhibitory inputs from the hippocampus to the mPFC (486, 489). Further, studies with retrograde tracers have shown that entorhinal cortex sends nonreciprocal projections from its layer III and V that synapse onto infralimbic and prelimbic mPFC respectively (490). In vivo recordings of prelimbic and infralimbic neurons in rats has shown that stimulation of entorhinal cortex results in a major inhibition of mPFC neurons and that this response is not altered by disruption of hippocampal projections to mPFC (491).

Contribution of cholinergic deficiency to aberrant frontal ERPs may be due interruption of cholinergic-dependent modulation of local interneurons in the frontal cortex. It is known that medial and lateral PFC are heavily innervated by projections from the BF cholinergic and GABAergic neurons (492, 493).

The majority of cholinergic neurons project to somatostatin (SOM)-expression interneurons, while the remaining contact vasoactive intestinal polypeptide (VIP)-expressing interneurons (494). The SOM-expressing interneurons are depolarized by activation of muscarinic receptors and inhibit the
dendrites of the pyramidal cells, whereas VIP interneurons provide disinhibitory control by preferentially targeting and inhibiting other interneurons upon depolarization (494, 495).

It appears that the cholinergic projections via the SOM-expressing and VIP interneurons are able to be able to differentially modulate the activity of frontal pyramidal cells. However, as the majority of cholinergic projections are to the SOM-expressing interneurons, blockade of muscarinic receptors may disinhibit pyramidal cells in the frontal cortex and increase the level of observed frontal ERP.

Further, it has been shown that the BF GABAergic neurons contact the pyramidal cells in the frontal cortex by direct projections and also indirectly via the parvalbumin (PV) interneurons (494-496). Recently, it has been shown that a subset of BF neurons fire a phasic burst response that precedes the frontal ERP activity by a few milliseconds every time a behaviorally-relevant stimulus is presented, and that the change in the amplitude of the bursting response to relevant versus irrelevant stimuli is highly comparable to the change in the frontal ERP amplitude (498). This bursting firing pattern has been attributed to the activity of the GABAergic neurons, and it has been suggested that they initiate the activity of the pyramidal cells by disinhibition via the PV interneurons (499-501).

4.4 Use of ERP features as a detection tool for early brain pathology

Next, we asked whether different types of pathologies could be detected from ERPs. We tested all of the extracted ERP features with the support vector machine (SVM) classifier and observed that all three types of pathologies and the control group were detected with high accuracy (Figure. 20 A). SVM-based classification of ERPs have been successfully used in the past to examine the neurophysiological state of the brain in humans (502, 503). Consistent with our finding, application of SVM classifier to extracted ERP features in humans have shown promising results for early diagnosis of AD (504). Similarly, other studies have used the SVM-based classification on magnetic resonance imaging (MRI) results to differentiate between normal and AD patients, and to predict conversion from MCI to AD (505, 506). In addition, SVM classifier has been applied to combined EEG-MRI data for differential diagnosis of AD and dementia with Lewy bodies
Our finding in addition to these evidence indicate that ERPs indeed contain diagnostically useful information.

4.4.1 Effect of stimulus modality and contingency on ERP-based detection of pathology

By using different stimulus modalities, we examined which of auditory or visual stimuli are better for detecting pathology. A comparison between the total number of affected auditory ERPs versus the visual ERPs revealed that a higher number of auditory ERP components than visual ERP components were affected by entorhinal HP-tau over-expression alone, and in conjunction with cholinergic deficiency. This finding suggests that auditory ERPs may be more susceptible to neuropathological changes in the brain than the visual ERPs. The likely reason is that the sources of the auditory ERPs in the temporal lobe are closer to the site of pathology in the entorhinal cortex than the sources of the visual ERPs, which are thought to be located at the inferior occipital area (508-511).

Nonetheless, when the extracted auditory and visual ERP features were tested separately with the SVM classifier the outcome was comparable between different modalities and all pathologies were detected with high accuracy (Figure. 20 B), indicating that the discriminatory power of auditory ERPs is comparable to visual ERPs. Consistent with our observation, it has been reported that auditory ERPs have a comparable sensitivity scores to visual ERPs for diagnosis of individuals with early-stage AD, and for differentiation of progressive MCI from the stable form (512-515).

We also observed that the overall pattern of ERPs in each brain region was comparable between CS+ and CS- conditions for auditory and visual stimulus. (Figure. 14 & 17). Subsequently, we separately tested ERP features from CS+ and CS- conditions with the classifier, and observed that the classification accuracy was comparable between the data sets and that all pathologies were detected with high accuracy (Figure. 20 C). This observation suggests that CS-US contingency does not substantially alter the overall pattern and consequently the discriminatory power of the cortical ERPs.

Although distinct properties of ERPs including, amplitude and latency, are dependent on the specific CS-US contingency implemented, some ERP components can overcome these
contingency-dependent changes (516). Specifically, it appears that CS-US contingency has a stronger effect on ERP components with longer latencies (P2, P3) than the earlier ERP components (P1, N1) in rats, most likely because longer time windows allow for greater level of impact form the neuromodulators, such as the cholinergic projections (517, 518).

Additionally, it is possible that unlike allocortical structures such as the entorhinal cortex and the hippocampus that are involved in associative learning and formation of discrete conditioned (519-521) the neocortical structures, where cortical ERPs are thought to be originating (522) are more involved with perceptual aspect of learning than with stimulus-response adaptations (523).

### 4.4.2 Susceptibility of ERP properties to preclinical AD pathology

We observed that entorhinal HP-tau, cholinergic deficiency, and both mainly affected the peak amplitude and not the peak latency of the ERP components. Using ERP amplitude features, SVM classifier was able to detect all pathologies with high accuracy; however, when the ERP latency features was tested, only rats with cholinergic deficiency were detected (Figure. 20 D). Our findings suggest that among ERP properties, amplitude has stronger discriminatory power and is more accurate than latency for detection of AD pathology.

Currently, due to conflicting reports from different studies there is no consensus over which ERP property is the most sensitive for detection of AD pathology. Recent ERP studies in AD have mainly focused on the P3 component, which is thought to reflect brain’s information processing when attentional and memory systems are activated (524). Some studies have reported a significant reduction of P3 peak amplitude (525, 526) while others observed a significant prolongation of P3 peak latency in AD patients (527-529).

It is believed that amplitude and latency of P3 component respectively reflect the level of attentional resources present (530), and the speed of stimulus detection and classification (531). Therefore, reduction of P3 amplitude can occur when more resources are allocated for processing of tasks with increasing attentional demands, or when fewer resources are available due to neuronal death. Similarly, prolongation of P3 latency can be a consequence of disrupted communication between neural networks that has occurred because of axonal damage and synaptic dysfunction (532, 439, 440). Since synaptic dysfunction, damage to axonal transport, and neuronal death are
all part of AD neuropathology, variability in their combined effect on brain electrophysiology across AD patients may explain the conflicting reports on the observed changes on P3 components.

Many studies in humans have also shown that increased attention and arousal level results higher P1, N1, and P2 peak amplitudes (533-539), while other studies have either observed a reduction in the latency or no effect at all (540-544). While our findings supports the idea that pathology-induced changes in the level of attentional resources may reflect as changes in the ERP amplitude, we did not find any evidence to suggest P3 is more reliable than the earlier components for detecting these alterations. Other factors that could give rise to current conflicting observations are discrepancy in recording procedures and differences in experimental paradigms across studies (545). Additionally, factors such as multiple intra-component peaks and inter-personal variation in topographic distribution can further complicate the measurement and analysis of ERP peak amplitude and peak latency (546).

**4.4.3 Detection of AD pathology with a single ERP component**

Although different pathological features of AD affected several of the same ERP components in one cortical region, there were specific ERP components that were altered only in one of the pathological conditions. We specifically observed that temporal visual P2 component was only affected by entorhinal HP-tau over-expression, while parietal visual P1, frontal and temporal visual N1, and temporal auditory P3 were affected by cholinergic deficiency, and frontal visual and auditory P1 was affected by conjunction of both conditions. In parallel, we observed that combined auditory and visual features from one ERP component were sufficient for detection of all pathologies with the classifier, except for P2 features that did not detect HP-tau over-expression in conjunction with cholinergic deficiency, and P3 features that did not identify rats with cholinergic deficiency (Figure. 20 F).

Together these findings suggest that, although entorhinal HP-tau over-expression and cholinergic deficiency affect the same ERP component in one cortical area, the pattern by which these pathologies affect the component (i.e., increase or decrease of amplitude and the magnitude of change) is distinct. This is consistent with our hypothesis that different pathological features of preclinical stage AD induce distinct abnormal ERP patterns, and that this distinct pattern allows
the classifier to detect and differentiate between pathologies using features from one ERP component.

4.5 Summary and conclusion

Overall, we found that features of preclinical AD pathology cause ERP abnormalities in absence of detectable memory impairment. Consistent with preclinical AD patients, rats with preclinical AD pathology did not show any deficits in associative memories (Figure. 12 & 13) despite showing significant alterations in ERP activity (Figure. 14 & 15).

By testing ERP data with a machine learning algorithm, we show that separate pathologies can be detected from ERP features independent of stimulus modality (Figure. 20 A & B), indicating that different pathological features manifest themselves as distinct ERP abnormalities in the brain. These abnormalities are mainly exhibited as changes in the ERP peak amplitude (Figure. 20 D) and are not influenced by CS-US contingency (Figure. 20 C). ERPs from one brain region are sufficient for detection of all pathological feature, among which temporal ERPs appear to be more accurate than frontal and parietal ERPs (Figure. 20 E). Moreover, all ERP components can detect separate pathological features, except entorhinal HP-tau over-expression in conjunction with cholinergic deficiency for P2 component, and cholinergic deficiency for P3 component.

Together our findings indicate that, ERPs may be a useful non-invasive and inexpensive tool for detection of Alzheimer’s disease pathology in asymptomatic elderlies, and that SVM-based classification may enhance the detection of aberrant ERPs during a differential associative learning paradigm.

4.6 Limitations

It must be noted that the expression of mutated tau was not specific to neurons, because the chicken β-actin promoter in rAAV9 recombinant viral vector is ubiquitously found in neurons as well as glial cells (411). Thus, the observed effects of HP-tau over-expression may not solely be a result
of accumulation of mutated tau in neural population of the entorhinal cortex. Moreover, delivery of the viral vector via the infusion cannula damages the brain tissue as the cannula is lowered into the target coordinate, which may cause inflammation around the injection tract and cause an interference with the activity of neurons unrelated to the effects of HP-tau.

Further, due to limited number of recording channels we were restricted in number of brain regions we could record. Since EEG is reflective of brain’s global activity originating from multiple cortical and subcortical sources, recording from the occipital cortex in addition to frontal, parietal, and temporal cortices could have provided a more complete picture of how the global brain network is affected by different pathological features of AD.

Next, to induce cholinergic deficiency in the brain, we systemically administrated muscarinic receptor blocker, scopolamine hydrobromide (0.05 mg/kg), which inhibited central as well as the peripheral muscarinic receptors. However, inhibition of peripheral muscarinic receptors by scopolamine at doses 0.03 mg/kg and 1.0 mg/kg, have been reported to have no effects on associative learning in rats and rabbits (398). Further, pharmacological blockade of muscarinic receptors in our manipulation induced a reversible acute cholinergic deficiency, which is not consistent with gradual attenuation of cholinergic input though the course of preclinical AD.

Further, each of our experimental models only capture one of many pathologies in preclinical stage. Therefore, it is easier to detect pathology than the reality in which multiple types of pathology may induce a complexed mixture of ERP abnormality.

Lastly, although the conditioning chambers used in this study were sound attenuated, there was always the possibility of environmental noise distracting the animals during the conditioning sessions.

### 4.7 Future Direction

In this study we showed that pathological features of preclinical stage AD, including entorhinal HP-tau over-expression, cholinergic deficiency, or both, induce distinct abnormal ERP patterns during DTEBC to a degree which is sufficient for an accurate prediction of the type of brain pathology based on the ERP patterns.
Next series of experiments need to investigate whether the other pathological feature of preclinical AD, cortical amyloidosis, induces memory dysfunction and aberrant ERP activity. As such, will record EEG activity from rats with cortical amyloidosis, cortical amyloidosis with cholinergic deficiency, and cortical amyloidosis with entorhinal HP-tau overexpression while the animals receive training in tone-light DTEBC paradigm.

In groups of rats examined in this study and the other groups that are proposed for the next series of experiments, we will track ERPs transactionally to observe how ERP abnormality changes as pathology is progressed. Moreover, we will examine the amplitude of specific frequency band of oscillations and synchronization of oscillations between the recording sites in these groups of rats.

Additionally, we will implement the same conditioning paradigm in transgenic rats with multiple pathological features to examine how the complex interaction of multiple pathologies affects ERPs. Specifically, potentially useful models is the TgF344-AD rat model, which expresses mutant human APP and PS1 gene, and exhibits age-dependent cortical amyloidosis followed by tau pathology, gliosis, and neuronal death in the hippocampus and cortex (547). In addition, a rat model with a strong risk factor for sporadic AD, human apolipoprotein e4 would be useful to expand our findings to the majority of patients who do not carry causative mutations for AD (548).

At Last, since the DTEBC paradigm in animals can be applied to human subjects without any major modification, these results hold a significant translational potential. To test our approach in patient population we propose several steps that may help with successful implementation of this strategy.

First, we suggest that all testing centers evaluate patients’ cognitive function using the same test, such as the Mini-Mental State Examination (MMSE), and that they utilize one standardized EEG recording protocol and acquisition setup up to minimize discrepancy in clinical assessment and the obtained ERP data. Next, we suggest tone-light DTEBC with an eyeblink-eliciting air puff to be used as the conditioning paradigm during EEG recording, and that the ERP data form patients be compared with ERPs from healthy individuals who have been matched for age, sex, and education.

To maximize our chances of detecting AD at the preclinical stage, we suggest forming an ERP data repository by compiling the obtained ERP data from all testing centers. The data in this repository will be organized as three main categories of ERPs from healthy controls, individuals
at risk of developing AD, and the diagnosed patients; and within each category the data is indexed based on the MMSE score. Once the number of ERP data samples in the repository reach a threshold that can train a machine learning algorithm which has a high classification accuracy (e.g. 95% <), we can test the ERP data of asymptomatic individuals using the trained machine classifier.
References


workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia, 


dendritic spines mediates synaptic dysfunction independently of neurodegeneration.
*Neuron* 68(6):1067–1081


reflect organelles accumulation at points of microtubule polar mismatching. *Traffic*, 9,
458–471.

traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances

449. Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J.,

450. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T,
Trojanowski JQ, Lee VM (2007). Synapse loss and microglial activation precede tangles

Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse

Homeostatic responses by surviving cortical pyramidal cells in neurodegenerative
tauopathy. *Acta Neuropathol.* 122, 551–564


454. Dudar JD. (1975). The effect of septal nuclei stimulation on the release of acetylcholine
from the rabbit hippocampus. *Brain Res*, 83:123–33.


