UNDERSTANDING DIFFERENTIAL ROLES OF STRESS AND ENRICHMENT IN PATHOGENESIS OF ALZHEIMER'S DISEASE IN A NOVEL RAT TAU MODEL

by © Sarah Torraville A Thesis submitted

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Abstract

Alzheimer's Disease (AD) is the leading cause of dementia. A major hallmark of AD is the presence of neurofibrillary tangles (NFTs) formed from abnormally phosphorylated tau. Abnormal tau appears early in life in the locus coeruleus (LC), progressing through pre-tangle stages, before forming NFTs, creating an intervention window. Here, we use a novel pre-tangle rat model to examine the roles of stress and enrichment on pre-tangle AD development. Animals underwent stress or enrichment paradigms, either as neonates or adults, LC viral infusion surgeries at 2-3 months old (htauE14 or control), and a series of behavioural tests. htauE14, or pre-tangle, rats showed increased anxiety in an elevated plus maze (EPM), but late enrichment reversed this effect. htauE14 rats were impaired in spatial and olfactory discrimination tests, with males performing better than females in the Y-maze and olfactometer testing. Both early and late enrichment improved pre-tangle rat performance in spontaneous location recall. In olfactory discrimination training, late stress improved control rat performance but hindered htauE14 rats. Early stress, however, improved htauE14 olfactory discrimination. These results indicate increased anxiety and decreased spatial and olfactory acuity as early symptoms associated with pre-tangle pathology but confirm that environmental enrichment may provide rescuing effects.

General Summary

The leading cause of dementia is Alzheimer's Disease (AD), with 564,000 Canadians living with the disease, and expectations for that number to continue rising. A key component of AD development is a protein called tau. Abnormal tau proteins accumulate over decades to form neurofibrillary tangles (NFTs), a major hallmark of AD. Because this occurs over such an extended period, there is time to intervene and either slow or stop disease development. This study was conducted to look at how life circumstances, whether stressful or enriching, can change AD development. To do this, we used a rat model of pre-clinical AD and tested their symptom development. We found that while our AD model rats did show increased anxiety and reduced memory, enrichment, particularly during adulthood, was able to reverse some of the symptoms. These results indicate that there is hope to reduce the incidence of AD through environmental changes during adulthood.

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List of Abbreviations

AD	Alzheimer's Disease
AC3	active caspase 3
AGL	anogenital licking
aMCI	amnesic mild cognitively impaired
ANOVA	analysis of variance
APP	amyloid precursor protein
BMI	body mass index
C99	C-terminal APP fragment
C-E14	Control E14
C-GFP	Cage Control
CRF	corticotropin releasing factor
CUSP	chronic unpredictable stress paradigm
DAB	3,3'-Diaminobenzidine
DBH	dopamine beta hydroxylase
DIO	double floxed inverted open
DOD	difficult odor discrimination
E.ER-E14	Early Enrichment E14
E.ER-GFP	Early Enrichment Control
E.STR-E14	Early Stress E14
E.STR-GFP	Early Stress Control
EPM	elevated plus maze
htauE14	pseudo-phosphorylated human tau

IHC	immunohistochemistry
KK	Kirtan Kriya
L.ER-E14	Late Enrichment E14
L.ER-GFP	Late Enrichment Control
L.STR-E14	Late Stress E14
L.STR-GFP	Late Stress Control
LC	locus coeruleus
LLPS	liquid-liquid phase separation
LTP	long-term potentiation
MBT	marble burying test
mGluR5	metabotropic glutamate receptor subtype 5
MRI	magnetic resonance imaging
NCI	non-cognitively impaired
NE	norepinephrine
NFT	neurofibrillary tangle
NGS	normal goat serum
ODAD	odor detection and discrimination
OFM	open field maze
PD	post-natal day
PFA	paraformaldehyde
PVP	polyvinylpyrrolidone
SLR	spontaneous location recall
SOD	simple odor discrimination
SPT	sucrose preference test

TH	tyrosine hydroxylase
TH-CRE	tyrosine hydroxylase-Cre

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1. Introduction

1.1 Overview

A major hallmark of Alzheimer's Disease (AD) is neurofibrillary tangles (NFTs), formed from abnormally, or persistently phosphorylated tau proteins (Alzheimer Society, 2020; Vaz & Silvestre, 2020; Weller & Budson, 2018). The progression of disease from abnormal phosphorylation of tau to symptomatic and clinical AD occurs over decades, creating a large window for potential intervention. The present study examines environmental stress and enrichment, both early and late in life, as factors that influence AD development and progression in a pre-tangle rat model.

1.2 Alzheimer's Disease

Alzheimer's Disease is the leading form of dementia, causing problems with memory, thought, and everyday behaviours (Alzheimer Society, 2020). The onset of AD is usually gradual, with symptoms worsening as the disease progresses, eventually diminishing the lives of those it affects. As of 2020, roughly 564,000 Canadians are living with dementia, and that number is only expected to rise, given that the actual cause is unknown and the available treatment options solely help to manage symptoms. There are two hallmarks of AD that research is focused on to better understand the disease and search for a potential cure.

1.2.1 Hallmarks of Alzheimer's Disease

The two hallmarks of AD are beta-amyloid plaques and neurofibrillary tangles (NFTs) (Alzheimer Society, 2020; Vaz & Silvestre, 2020; Weller & Budson, 2018). Beta-amyloid plaques form within the brain between neurons preventing communication, ultimately causing neuronal death. Neurofibrillary tangles are formed within neurons from tau proteins which clump together and prevent transport, eventually resulting in neuronal death.

1.2.2 Beta Amyloid Pathology

The amyloid precursor protein (APP) was first discovered in 1987 (Kang et al., 1987). APP is cleaved by β-secretase to form a C-terminal APP fragment (C99) and then by γ-secretase to form and release beta-amyloid into the extracellular space (De Strooper et al., 1998; Vassar et al., 1999). The isoform of beta-amyloid that accumulates early and contributes to plaque formation is Aβ42 (Younkin, 1998).

By examining over 2,600 brains post-mortem, it was determined that beta-amyloid protein deposits typically first appear in cortical layers III and V, having non-distinct boundaries (Braak & Braak, 1997). This appearance happens roughly symmetrically and gradually the deposits transform into distinct globular plaques. Layers containing myelinated neurons tend to have fewer deposits, while layers with less myelin have denser amyloid deposits.

The first deposits are found in the perirhinal and ectorhinal cortices, occurring as early as young adulthood in some cases (Braak & Braak, 1997). From these areas, plaque deposits increase in number and spread to nearby areas, such as the neocortex and hippocampal formation. Spread continues gradually until all areas of the cortex are affected, including myelinated areas, in later stages. More advanced stages are observed more predominantly in an older age group, with very few cases presenting as such before subjects were roughly 65 years old.

Hardy and Higgins (1992) proposed that the deposition of the amyloid plaques was the cause of AD, with NFTs, other brain damage, and dementia following that deposition. They described “The Amyloid Cascade Hypothesis”, in which they posit that when APP is cleaved in a lysosomal pathway, as opposed to by secretases, neurotoxic amyloid proteins cause NFT formation and cell death, though the mechanisms resulting in those changes were unclear. They further suggest that aside from APP gene mutation, other cases could be caused by some other

factors, such as head trauma, that initiate amyloid deposition. However, evidence has suggested that this hypothesis is not correct. For example, in a postmortem study of nursing home residents, they found that a subset of subjects who showed high performance in cognitive examination had high levels of senile plaques, roughly 80% of the amount found in AD patients (Katzman et al., 1988). In another study, Holmes and colleagues (2008) immunized AD patients with a plaque-clearing peptide and monitored for long-term changes in cognitive abilities. They found that there were varying degrees of plaque removal despite successful loading of the plaque-clearing peptide, and most of the subjects with almost-complete clearing who were examined post-mortem had died with severe dementia. Overall, they concluded that clearing of plaques did not improve the survival rate or delay the rate of development of severe dementia. This would suggest that there are other factors contributing to the cause of AD. Further, in a study examining the presence of abnormal tau and amyloid plaque deposits in brains of patients aged 1-100 years old, they found that abnormal tau preceded amyloid deposits, with tau pathology beginning as early as the first decade of life, and amyloid pathology primarily occurring from at least the fourth decade of life (Braak et al., 2011).

1.2.3 Tau Pathology

Tau proteins are found inside cells and help assemble microtubules to facilitate transport within cells (Cleveland et al., 1977). In its purified form, tau is composed of four closely-related polypeptides with molecular weights between 55,000 and 62,000, is asymmetric, and can be phosphorylated by a protein kinase. Tau is typically more heavily concentrated in white matter neurons than grey matter neurons, restricted to the axons, and absent from glial cells (Binder et al., 1985). More recently, it has been found that tau becomes condensed into liquid droplets, acting as a non-membrane bound organelle, sensitive to physiological changes including temperature and salt concentrations (Zhang et al., 2017). Boyko and colleagues (2019)

demonstrated that the liquid-liquid phase separation (LLPS) that occurs to form these droplets occurs due to electrostatic interactions of tau's negatively charged N-terminal and positively charged C-terminal. These droplets have been suggested as a stepping stone on the way to tau aggregation, with similarities to tau retrieved from AD brains and rapid development of positive thioflavin-S aggregates (Wegmann et al., 2018).

Tau loses functionality and becomes pathological when it has been abnormally, or persistently, phosphorylated and eventually forms NFTs, which have long-time been associated with AD (Ball, 1977; Delacourte & Defossez, 1986; Grundke-Iqbal et al., 1986). There are 17 serine/threonine-proline sites in the longest isoform of tau (Goedert et al., 1989). Goedert (1993) discusses how typically in adult brains only two to three of these sites are phosphorylated, whereas abnormal tau is phosphorylated at more of these sites, reducing its microtubule binding affinity. Ercan-Herbst and colleagues (2019) conducted an analysis of tau oligomers and post-translational modification sites and found five specific phosphorylation sites that are consistently phosphorylated in the entorhinal cortex, hippocampus, and temporal cortex. Of these sites, three correlate with multimerization of tau, but are inconsistent with sites typically targeted by antibodies identifying NFTs. They conclude that these specific sites are involved in early tau modifications in AD pathology. Braak and Del Tredici (2011) used antibodies to examine persistently phosphorylated tau accumulation and ß-amyloid deposits from people under 30 years old. They concluded that pretangle material (abnormal tau), which was found in the noradrenergic locus coeruleus in 19 cases, and in the transentorhinal region in 16 cases, likely appears for the first time in these subcortical areas very early in life.

Abnormal tau has been correlated with AD severity. In one study, 70 AD patients of various stages and severity of dementia had their brains examined postmortem for NFTs, senile plaques, and plaques with amyloid cores (Bierer et al., 1995). Those markers were then correlated

with cognitive scores, with NFTs being significantly correlated with more severe clinical dementia, but not senile plaques or plaques with amyloid cores. Similarly, in a multivariate analysis of 22 elderly individuals, NFT counts were strongly associated with the number of affected neurons and cognitive status, with greater amounts of NFTs associated with lower cognitive scores, while amyloid deposits were not well-correlated with the same changes (Giannakopoulos et al., 2003).

Another study, conducted by Braak and colleagues (2011) characterized stages of AD by using stains to examine hyperphosphorylated tau and ß-amyloid in brains of 2332 individuals and categorizing them by age. Pretangle stages a-c, in which abnormal pretangle tau appeared in the locus coeruleus (LC) area and the transentorhinal region with no cortical projections, was seen as early as the first decade and present in most samples by the second decade. Next, pretangle stages 1a and 1b, in which some cortical lesions began to be seen in the temporal lobe in addition to the subcortical lesions from stages a-c, appeared as early as the second decade and were present in most samples by the fourth decade. Once the abnormal tau became insoluble and began to form NFTs, the samples were categorized as neurofibrillary stages I-VI. For these stages, the NFTs were first observed in the transentorhinal region, then into the entorhinal region and hippocampal formation, then into cortical areas beginning in the temporal lobe, onto the insular and frontal areas, the prefrontal cortex and association cortices, and finally onto premotor and primary motor areas. The latest stages V and VI became more prominent in the eighth to tenth decades. ß-amyloid deposits began to appear in the third and fourth decades, when those individuals were in pretangle stages 1a and 1b. This would suggest an important role of the LC in the development of AD, given the first appearance of abnormal tau occurs in this area.

1.2.3.1 Locus Coeruleus

The locus coeruleus is a small pontine nucleus that is the main provider of norepinephrine (NE) to the brain (España & Berridge, 2006; Swanson et al., 1976). It is situated bilaterally, with projections widespread throughout the brain (Gatter & Powell, 1977). The LC is involved in regulating arousal levels, arousal, stress response, and learning and memory (Aston-Jones & Bloom, 1981a; Atucha et al., 2017; Díaz-Mataix et al., 2017; Mei et al., 2015; Saavedra & Torda, 1980; Shakhawat et al., 2015). The LC has been shown to fire in different patterns under different circumstances. For example, high-frequency tonic firing of LC occurs during the stress response, whereas phasic firing of LC is indicative of increased attention and vigilance (Aston-Jones & Bloom, 1981a; Aston-Jones & Bloom, 1981b). Further, cell loss within the LC has been linked to cognitive decline. In a study with 165 participants aged 55 years old or more, post-mortem brain autopsies revealed that greater neuronal density within the LC was associated with slower cognitive decline (Wilson et al., 2013). There are also links between LC cell density and dementia, specifically AD. Bondareff and colleagues (1981) compared neuronal densities amongst controls, and AD patients, which were then classified into two groups based on AD severity. They found that mild AD patients, who lived longer and had lower dementia scores, had 81% of the neuronal density of controls, and severe AD patients had just 20% of the control density. Those results in combination with a 40% decrease in dopamine-beta-hydroxylase activity, lead to the conclusion that noradrenergic cell loss was significant in AD patients. Another study used computer visualization to re-construct the LC post-mortem from controls, and patients with Parkinson's disease, AD, and Down syndrome (German et al., 1992). Across all three conditions, there was a 60% decrease in LC cells compared to controls, with greater LC cell loss with a longer duration of AD. Finally, a study comparing LC cell counts using immunohistochemical analysis between non-cognitively impaired (NCI), amnesic mild

cognitively impaired (aMCI), and mild-moderate AD found a 30% decrease in LC cells from NCI to aMCI, and an additional 25% LC cell loss in AD patients.

1.2.3.2 Animal Model of LC Pretangle Tau

Ghosh and colleagues (2019) created an animal model of AD based upon Braak's pretangle stages (Braak et al., 2011) previously described, by infusing the genetic construct of pseudo-phosphorylated human tau (htauE14) in a viral vector bilaterally into the LC of tyrosine hydroxylase-CRE (TH-CRE) rats. They concluded that when infused at a young age (2-3 months old), AD animals performed worse on a difficult olfactory discrimination task 7-8 months post-infusion than controls, but not 4 months post-infusion, and this was accompanied by reduced NE fiber density seen in the piriform cortex. Further, when infused at an old age (14-16 months old), by just 5-6 months post-infusion the AD animals were unable to complete a simple olfactory discrimination task, they had LC cell loss, and a more robust spread of tau. This model, which was created to provide a live model that can be used to better understand tau pathology, was used in this study.

1.3 Role of Stress in Learning and Memory

The effects of stress on learning can vary, sometimes helping and other times hindering memory, depending on the timing and duration (chronic vs. acute) of stressors and type of memory. Chaby and colleagues (2015) exposed rats to a chronic unpredictable stress paradigm (CUSP) during adolescence and performed a series of learning and memory assessments beginning approximately four months later. While there was no effect of stress on associative learning in a radial arm maze, stressed rats performed better at reversal learning, but, by contrast, they were more susceptible to disturbance of working memory.

A recent study examined the effect of early life stress in rats, induced by maternal separation, on later adulthood behaviours (Kambali et al., 2019). They tested anxiety in a light-

dark box, social interaction, and spatial memory in a radial arm maze. Stressed animals displayed increased anxiety, no change in social behaviours, and accelerated spatial learning but not long-term recall. Ultimately, the effects of stress on learning and memory can vary greatly under different contexts.

Another study induced stress in humans before exposing them to stressor-related and -unrelated words to be freely recalled immediately after and 24 hours later (Smeets et al., 2009). Stressed individuals were able to better recall highly-arousing stressor-related words than low-arousal stressor-related words or stressor-unrelated words both immediately after learning and after 24 hours. They concluded that stress can enhance learning when the information is arousing and related to the stressor.

Contrarily, Schwabe and Wolf (2010) induced stress by submerging a hand into ice water during the presentation of words to be remembered 24 hours later. The words presented were either context-related, neutral, positive, or negative. Participants who learned while under cold water stress performed significantly worse than controls during free recall regardless of the word category. Further, recognition memory was also impaired in the stressed group compared to controls. These results suggest that learning during stress impairs memory.

Stress has been associated with AD in that those who scored higher on a neuroticism survey and were, therefore, more prone to stress, were more likely to have accelerated cognitive decline and develop AD (Wilson et al., 2006). One study used chronic restraint stress in transgenic mouse models of AD (both beta-amyloid and tau models) and found that it accelerated the accumulation of beta-amyloid and hyperphosphorylated tau and worsened fear-related memory (Carroll et al., 2011). They also suggested that the mechanism that is responsible for these effects works through corticotropin releasing factor receptor type 1 (CRF_1), as blocking it pre-stress prevented such effects from being seen. Further, overexpression of CRF_1 increased the

amount of hyperphosphorylated tau present as compared to control mice. Overall, it was concluded that stress is a possible mediator of AD development that works through the CRF mechanism.

1.4 Role of Environmental Enrichment in Learning and Memory

Hebb (2002) first showed that environmental enrichment during upbringing improved problem-solving skills in adulthood by raising rats in one of two groups, cage-raised or as pets, and testing their ability to find a food reward with few errors in a maze. Animals raised as pets made fewer errors in the maze and improved more over repeated trials than cage-raised animals. It was concluded that early experiences are important in the shaping of adult intelligence levels.

More recently, a study compared the influence of environmental enrichment, social enrichment, and standard conditions on novel object recognition, spatial memory, and metabotropic glutamate receptor subtype 5 (mGluR5) dependent long-term potentiation (LTP) during young adulthood (Hullinger et al., 2015). Both environmental and social enrichment improved memory compared to standard controls after one month, however, after four months the environmentally enriched animals performed better than socially enriched and control animals. Further, only environmental enrichment was able to induce a long-lasting hippocampal LTP mediated by mGluR5. This study shows a possible mechanism responsible for the improved cognitive abilities following enrichment.

Cortese and colleagues (2018) expanded upon the findings of Hullinger and colleagues (2015) by conducting a similar experiment in 23- to 24-month-old rats. Environmentally enriched rats performed better in spatial memory and novel object recognition than socially enriched and standard control animals and had enhanced hippocampal LTP mediated by mGluR5. They concluded that in aged animals, environmental enrichment for one month is sufficient to effect changes in hippocampal function. Overall, these studies show that environmental enrichment

produces longer-lasting improvements in learning and memory than social enrichment during young adulthood and it is capable of improving learning and memory in aged rats.

Environmental enrichment has also been shown to reduce memory deficits seen in an AD amyloid beta model (Prado Lima et al., 2018). In this study, animals were assigned to either a control, environmental enrichment, social enrichment, or anaerobic exercise group, in which those conditions were applied over 2 months. Animals then received an amyloid beta infusion or sham surgery and were given time to recover before being subjected to behavioural testing. While AD rats had impaired short-term and long-term object recognition memory compared to controls, these effects were not seen in AD animals that had environmental enrichment or anaerobic exercise. Social enrichment did not have the same protective effect. Further, environmental enrichment, anaerobic exercise, and social enrichment all prevented the social recognition deficit seen in AD animals when compared to controls. It was concluded that environmental enrichment and anaerobic exercise are most protective against the effects of amyloid beta neurotoxicity.

1.5 Sex Differences

1.5.1 Sex Differences in Alzheimer's Disease

AD is more commonly diagnosed in females than males (Alzheimer Society, 2020). In a study examining sex differences in dementia, it was found that women had a higher rate of AD (81.7%) than men (24.0%) at 90 years old, and at the age of 65, women had a higher risk of developing AD, but not vascular dementia, than men (Anderson et al., 1999). Not only is AD more common in women, but they are also more likely to have neuropsychiatric comorbidities, including delusions, anxiety, and irritability, than men (Tao et al., 2018). Furthermore, women have been found to have higher levels of pathological tau and further progression in Braak's stages of AD than men (Hu et al., 2021; Sundermann et al., 2020).

1.5.2 Sex Differences in Stress

There are sex differences in the prevalence of certain disorders, with females being more likely to have affective disorders and anxiety disorders than males, whereas males are more likely to have substance use disorders (Kessler et al., 1994). However, there are conflicting results regarding female responses to stressors. Hodes and colleagues (2015) found that following a subchronic variable stress paradigm in mice, females were more susceptible to stress than males, as demonstrated by reduced grooming, increased latency to eat, reduced sucrose preference, and increased cortisol in several tests. Contrarily, Lotan and colleagues (2018) conducted a study using female mice and found that following a chronic unpredictable stress paradigm (CUSP), young adults showed reduced anxiety-like behaviour and improved cognition, while aged animals had more anxiety and poorer cognition.

1.6 Hypotheses

In this study, stress and enrichment paradigms will be applied either in early life or adulthood in the aforementioned LC pretangle tau model rats. The objective is to study how environmental factors influence tau pathophysiology and cognitive functioning. We hypothesize that environmental enrichment reduces the severity of symptoms developed and htau spread throughout the brain, while stress worsens the symptoms and spread. Additionally, it is hypothesized that the stress and enrichment interventions have more pronounced effects when applied during early life than when applied during adulthood. Finally, it is hypothesized that there are differential effects of environmental manipulation and tau pathology between the sexes, with females being more greatly impacted by pre-tangle pathology and more likely to demonstrate increased anxiety-like symptoms.

2. Materials and Methods

2.1 Viral Infusion

2.1.1 Subjects

Subjects for this study were 140 TH-Cre Sprague Dawley rats. Animals were bred using homozygous TH-Cre breeder males (obtained from Charles River Laboratories) and Sprague Dawley females (obtained from Charles River Laboratories). Animals were randomly assigned to one of ten groups to determine in which experiments they would be used. Subjects were housed individually and given *ad libidum* access to dry food pellets and water. They were housed with standard enrichment in standard cages in a reverse 12-hour light-dark cycle except for in the cases specified below. All animal housing and experimental procedures were approved by Memorial University of Newfoundland's Institutional Animal Care Committee and followed the Canadian Council on Animal Care guidelines.

2.1.2 Viral Vectors

Rats were infused with either a virus containing the genetic construct for pseudo-phosphorylated human tau (htauE14) or a control virus. The htauE14 plasmid was chosen because it has been shown that pseudo-phosphorylated tau replicates the effects of persistently phosphorylated tau (Hoover et al., 2010) and this plasmid, provided by Karen Ashe Lab Materials on Addgene, has 14 of 17 possible serine/threonine-proline sites pseudo-phosphorylated, consistent with sites commonly affected in abnormal tau associated with AD. Both viral vectors contained double floxed inverted open (DIO) reading frames, making them Cre-inducible to ensure cell-specific targeting and they had a green fluorescent protein tag. This means the sequence containing the construct is inverted in the vector and Cre-recombinase inverts the sequence into the correct orientation in tyrosine hydroxylase (TH) positive cells (Figure 1). The viral vector for

experimental animals was AAV9-rEF1a-DIO-htauE14-EGFP (1.3e13 vg/ml, MIT) and the control virus was AAV9-rEF1a-DIO-EGFP (2.35e13 vg/ml).

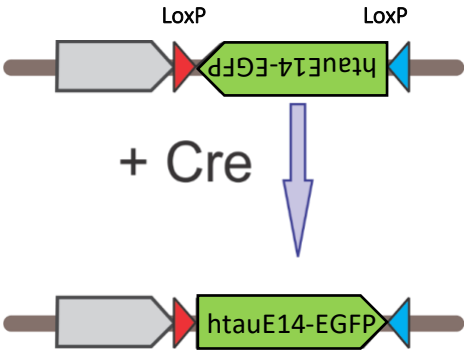

Figure 1. Cre-recombinase mechanism.

2.1.3 Infusion Surgery

As similarly described by Ghosh and colleagues (2019), viral infusion surgeries occurred when the animals were two to three months old. Animals were anesthetized using 3% isoflurane and placed in a stereotaxic apparatus in skull flat position. The animals were placed over a heating pad covered with a puppy pad and cling wrap was used to cover the animal and keep heat surrounding the animal to prevent hypothermia. Meloxicam Slow Release (10mg/ml) was injected subcutaneously as an analgesic, and Sensorcaine Epinephrine (0.25%, 0.25ml) was injected along the incision line for a local anesthetic and vasoconstrictor to reduce bleeding. Holes were drilled into the skull 11.6-12.9 mm posterior and 1.3 mm bilateral to Bregma using a microdrill. An infusion of 1µl of the virus mixed with 0.4µl of blue, fluorescent beads was completed at two sites within LC on both sides (four infusion sites in total) using an infusion pump and guide cannula placed in a parasagittal plane at a 20° angle caudal to the coronal plane. The LC coordinates are 12.0-13.7mm posterior and 1.2-1.4mm bilateral to Bregma, and 6.3mm ventral from the brain surface. Following surgery animals were placed in a clean cage on a

heating pad overnight. Animals were allowed to recover post-surgery for a minimum of 6 weeks before beginning any behavioural experiments.

2.2 Behavioural Experiments

2.2.1 Subjects

Animals were randomly assigned to one of ten experimental groups: early-life stress, early-life enrichment, adulthood stress, adulthood enrichment, cage control, or each of those groups with a control virus variation. These groups, described in Table 1, determined what experiments were done and when they were done as well as which virus would be infused during surgery.

Table 1

Experimental Groups Used in the Present Study

Group	Intervention	Viral Infusion
Cage Control (C-GFP)	None	GFP
Early Stress Control (E.STR-GFP)	Early stress	GFP
Early Enrichment Control (E.ER-GFP)	Early enrichment	GFP
Late Stress Control (L.STR-GFP)	Late stress	GFP
Late Enrichment Control (L.ER-GFP)	Late enrichment	GFP
Control E14 (C-E14)	None	htauE14
Early Stress E14 (E.STR-E14)	Early stress	htauE14
Early Enrichment E14 (E.ER-E14)	Early enrichment	htauE14
Late Stress E14 (L.STR-E14)	Late stress	htauE14
Late Enrichment E14 (L.ER-E14)	Late enrichment	htauE14

2.2.2 Stress Experiments

2.2.2.1 Early-Life

Early-life stress was implemented through maternal separation from post-natal days (PD) 2-10. From PD 2-10, pups were removed from the mother's home cage and placed in a small cage with clean bedding that was kept warm using a heating pad. Pups were separated from each other using barriers in the cage for the duration of the separation from the mother. The separation lasted five hours per day. Mother and pups were moved to a different room to be re-introduced and the first 15 minutes immediately following introduction was video recorded on PD 2, 6, and 10. Videos were later analyzed to by an experimenter blind to the study to assess the mother's attention towards the pups.

2.2.2.2 Adulthood

Stress during adulthood was implemented using a CUSP for six weeks when the animals were five to seven months old, three to five months post-infusion following a protocol similar to those previously described (Yalcin et al., 2005; Zhou et al., 2019). Stressors were applied for 2 hours per day for the duration of the CUSP, including restraint, wetted bedding, tilted cage, and an irregular light cycle.

2.2.3 Enrichment Experiments

2.2.3.1 Early-Life

Early-life enrichment was implemented by brief maternal separation from PD 2-10. Brief maternal separation has been found to enhance maternal care towards pups, thus providing early life enrichment (Lesuis et al., 2017). Pups were separated from their mother for 15 minutes per day from PD 2-10. Pups were placed in a small cage with clean bedding which was kept warm using a heating pad. Following the separation, the mother and pups were re-introduced in a separate room and video recorded for the first 15 minutes upon introduction on PD 2, 6, and 10.

Videos were later analyzed by an experimenter blind to the study to assess the mother's attention towards the pups.

2.2.3.2 Adulthood

Enrichment during adulthood was implemented by placing 4-5 animals together in a 60x60x50cm Plexiglas play arena for 2 hours per day for six weeks. Enrichment occurred when the animals were five to seven months old, three to five months post-infusion. The play arena contained toys, chews, exercise equipment, and treats. Males and females had separate play arenas.

2.2.3.3 Early Life Dam-Pup Interaction Analysis

Early-life stress and enrichment were assessed by examining videos of the mother-pup interactions immediately following re-introduction. This analysis was conducted by an experimenter blind to the conditions. Latency to and duration of contact (sniffing and licking) with pups, retrieval (carrying pups to nest area), anogenital licking (AGL) (visibly holding pups with forepaws and licking genital region), and nesting (hovering over pups while in contact with them) were recorded. Less attention from the mother was an indication of stress for the pups, while more maternal attention was an indication of enrichment for the pups.

2.2.4 General Behaviour Experiments

2.2.4.1 Sucrose Preference Test

Animals were water deprived for 24 hours prior to beginning the sucrose preference test (SPT). Each animal was given two identical water bottles, one containing regular tap water, and the other containing a 0.1% sucrose solution made in tap water. Each of the bottles were weighed before being presented to the animals. The bottles were removed from the cages and weighed 24 hours after beginning the SPT. The animals had *ad libidum* access to both bottles while they were available. The ratio of sucrose consumed to total fluid consumed during the test was recorded. A lower sucrose preference is an indication of anhedonia (Liu et al., 2018). A 3-way Analysis of

Variance (ANOVA) was conducted to compare the effects of environmental conditions, virus, and sex on sucrose preference.

2.2.4.2 Marble Burying Test

For the marble burying test (MBT), sixteen identical dark-blue marbles were placed in a 4x4 grid on clean extra bedding in a clean cage. Animals were placed in the cage for 30 minutes and were video recorded. The number of marbles buried at least 75% in bedding was recorded. A higher number of buried marbles is an indication of increased stress-like symptoms (Archer et al., 1987). A 3-way ANOVA was conducted to compare the effects of environmental conditions, virus, and sex on stress-like symptoms as measured by marbles buried.

2.2.4.3 Elevated Plus Maze

Animals were placed on an elevated plus maze (EPM) for a 5-minute trial. The EPM arm dimensions are 50 x 10 cm with a center platform 11 x 11 cm, wall height 38 cm (on closed arms, opposite each other), and it is raised 52 cm above the ground (Figure 2). The animals were placed facing away from the experimenter and towards an open arm and the trials were video recorded. Amount of time spent in closed and open arms was recorded (when all four paws are within the boundary of the arm), as well as the number of head dips (dipping head over the edge of the maze from the open arm). More time spent in closed arms and fewer head dips were indications of increased stress-like symptoms (Kraeuter et al., 2019). Three-way ANOVAs were conducted to compare the effects of environmental conditions, virus, and sex on stress-like symptoms as measured by time spent in closed and open arms, and number of head dips.

Figure 2. Elevated Plus Maze apparatus. Arms are 50 x 10 cm, walls on closed arms are 38 cm high, center platform is 11 x 11 cm, and it is raised 52 cm off the ground.

2.2.4.4 Open Field Test

Animals were placed into an open field maze (OFM) with dimensions 60x60x50cm (Figure 3) for a 10-minute trial. Animals were allowed to freely explore the maze for the duration of the trial and the trial was video recorded. The average speed and distance travelled were recorded using ANYMaze software, and amount of time spent freezing and rearing were recorded manually. Less exploration of the maze was an indication of increased stress-like symptoms (Seibenhener & Wooten, 2015). Three-way ANOVAs were conducted to compare the effects of environmental conditions, virus, and sex on stress-like symptoms as measured by exploratory behaviour.

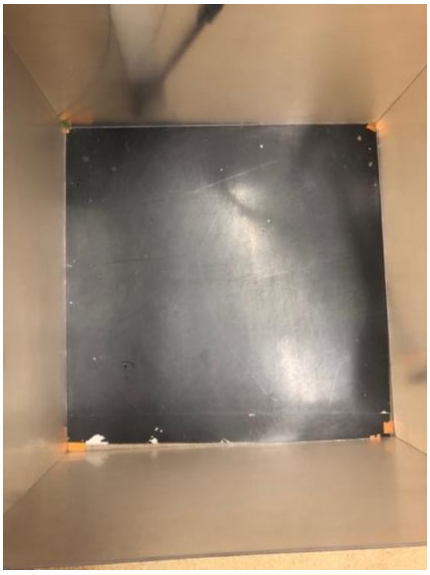

Figure 3. Open Field Maze apparatus. Dimensions are 60 x 60 x 50 cm.

2.2.5 Learning Experiments

2.2.5.1 Spontaneous Location Recall

The spontaneous location recall (SLR) test requires a training and testing phase as described by Bekinschtein and colleagues (2013). For the training phase, a soda can was placed against one wall of the OFM apparatus, 30 cm from each corner, with the logo facing towards the center of the box. Two more identical soda cans were placed against the opposite wall, 10 cm apart and 10 cm from either corner, with the logos facing the center of the box. The animals were placed in the box and allowed to freely explore the box and cans for 10 minutes. The testing took place 24 hours after the training phase. For the testing phase, an identical soda can to the others was placed in the same location as the first can from the training phase, this was known as the familiar location object. Another new identical can was placed on the opposite wall, in the center of where the other two cans from the training phase were placed, with the logo facing the center of the box, becoming the novel location object. The animals were allowed to freely explore the new layout of the box for 10 minutes. Time spent exploring each object was recorded in both training and

testing phases. During the training phase, animals should have explored each of the three objects for roughly the same amount of time. During the testing phase, spending more time exploring the novel object was an indication that the animal recalled the training phase. A 3-way ANOVA was conducted to compare the effects of environmental conditions, virus, and sex on spatial memory.

2.2.5.2 Y-Maze

The Y-maze test required a training and testing phase. The apparatus used was a black opaque Plexiglas Y-shaped maze with three arms 120° apart (50 cm long, 16 cm wide, and 32 cm tall) with a neutral center zone connecting the three arms. Removable barriers were located just inside the arms from the center zone, allowing for maze customization. Infrared sensors were located 3 cm and 25 cm into each arm from the center zone permitting automatic tracking of the animal throughout the maze. A detailed blueprint of the maze is found below as Figure 4. For the training phase, animals were placed facing away from the experimenter at the end of the "start" arm for a 15-minute trial in which they were allowed to freely explore two of three arms. Which of the arms was closed for this training phase was counterbalanced between groups and animals. Four hours after the training phase, animals were re-placed into the same "start" arm for another 15-minute trial and allowed to explore the full maze with all three arms open, with the previously closed arm considered to be the "novel" arm. Number of entries into the arms and duration in each arm was recorded for analysis. Increased entries and time spent in the novel arm is an indication that the animal can recognize it's novelty, suggesting an intact spatial memory (Dellu et al., 1992).

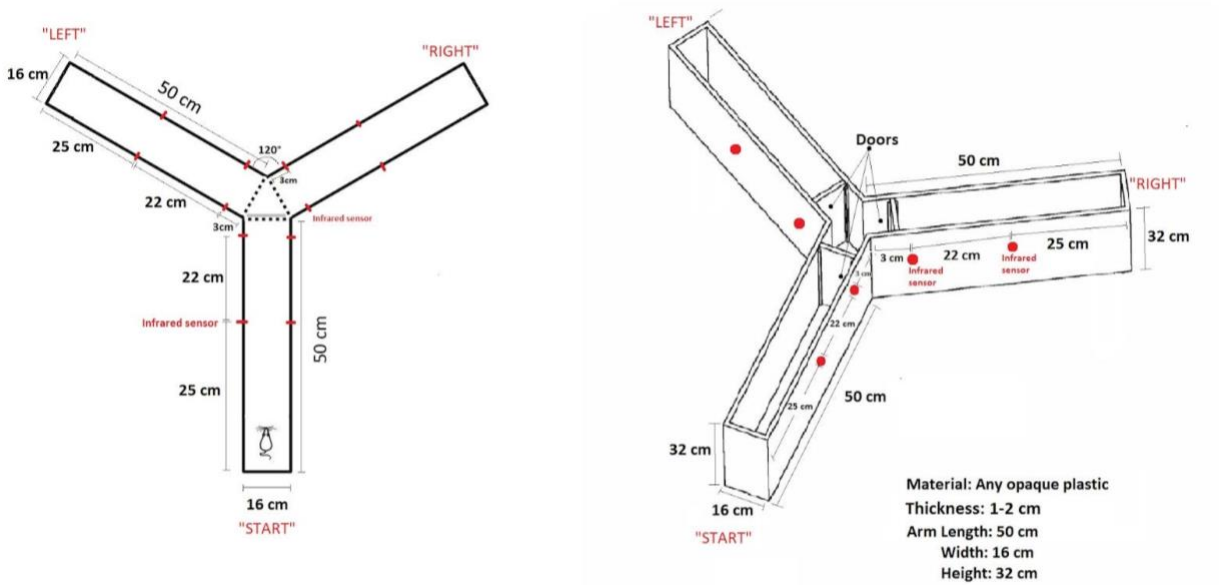

Figure 4. Detailed blueprint for Y-Maze apparatus.

2.2.5.3 Odor Detection and Discrimination

Odor detection and discrimination (ODAD) was completed to ensure animals' ability to detect and discriminate between similar odors to be used later during olfactometer training. For this, animals were placed in a clean mouse cage and presented three times with mineral oil, three times with odor one (0.001% heptanol in mineral oil) and finally with odor two (0.001% heptanol and octanol in a 1:1 ratio in mineral oil). Odors were soaked into filter paper (60ul) and presented in small, perforated tubes through the water bottle opening in the cage for 50 seconds with 5-minute intertrial intervals. The 50-second presentation began once animals first approached the presented tube, and it was promptly removed once the 50-seconds had passed. Amount of time spent sniffing the presented odor tube was recorded for each trial. If an animal was able to identify and distinguish between different odors, an increase in time spent sniffing would be predicted for the first presentation of each novel odor and a gradual decrease over subsequent presentations as the animal acclimates to that odor (Escanilla et al., 2010).

2.2.5.4 Olfactometer

Animals went through a go/no-go odor discrimination learning task in a Knosys olfactometer (Shakhawat et al., 2014, Shakhawat et al., 2015, & Ghosh et al., 2019). Ten days before beginning training, animals were water deprived, receiving 30ml on the first day, decreasing by 5ml per day over the next two days until reaching 20ml, which then remained constant throughout the training period. Initially, animals were trained to lick a water delivery tube for a 30µl water reward following at least 6 licks for a total of 30 rewards. Next, a 1% orange odor (S+), dissolved in mineral oil, was delivered upon trial initiation triggered by the animal, with a gradually increasing time increment from 0.1 seconds to 1.1 seconds before water was delivered. Once animals were able to complete all trials of training with fewer than three mistakes, they were able to move on to simple odor discrimination (SOD) learning.

For SOD, animals were placed in the olfactometer chamber and 1% orange odor (S+) and 1% peppermint odor (S-), dissolved in mineral oil, were delivered in random order ten times each, for a total of 20 trials per block. Animals needed to learn to lick for a 30µl water reward when S+ was presented and not to lick when S- was presented. Responses to each trial were recorded automatically by Knosys computer software and a percentage of correct responses per block was displayed. Each animal was allowed to complete as many blocks it could during a 30-minute period or up to ten blocks per day. Once animals reached the learning criteria of at least 75% correct responses for three consecutive blocks, they were allowed to move on to difficult odor discrimination (DOD) learning.

For DOD, the procedure was identical to that of SOD, except the odors used were very similar. A 0.001% heptanol odor dissolved in mineral oil was the S+, and 0.001% heptanol and octanol in a 1:1 ratio dissolved in mineral oil was the S-. Again, once the animals were able to

reach learning criteria of at least 75% correct responses for three consecutive blocks, they were considered as having learned the rule and the training was complete.

The number of blocks required to reach learning criteria was recorded for both SOD and DOD. A greater number of blocks required to reach criteria was an indication of poorer learning and memory abilities. A 3-way ANOVA was conducted to compare the effects of environmental conditions, virus, and sex on cognitive abilities as measured by number of blocks required to reach criteria for both SOD and DOD.

2.3 Immunohistochemistry (IHC) and Histology

Following all behavioural experiments, animals were sacrificed by transcardial perfusion using ice-cold 0.9% saline and ice-cold 4% paraformaldehyde (PFA) in 0.1M phosphate buffer, pH = 7.4, solution. Brains were collected and stored in 4% PFA at 4°C for 24 hours. Brains were then placed in a 20% sucrose solution for 24-48 hours at 4°C, until brains sunk into the solution, to prepare for cryosectioning. Unless otherwise specified, brains and tissue were kept in minimum light to prevent photobleaching of fluorescence within the tissue.

Brains were blocked using clean razor blades and flash frozen in approximately -80°C 2-methylbutane. Before cutting, brains were stored at -20°C for at least two hours to prevent breaking during sectioning. The brains were collected on chrome-gelatin coated slides at 25µm and stored at -20°C, and free-floating sections at 50µm in a polyvinylpyrrolidone (PVP) solution were stored at 4°C until used for IHC.

Free-floating IHC was completed using a Tris Buffer system and sections were placed in solution on a rotator for each step, as described previously (Walling et al., 2007). Sections were removed from PVP and washed in Tris buffer (0.1M, pH 7.6) three times for 5 minutes. Next, there was a 30-minute wash in 10% hydrogen peroxide in Tris buffer followed by an additional 5-minute wash in Tris buffer. Sections were then washed in Tris A (0.1% Triton-X in Tris

buffer), then Tris B (0.1% Triton-X and 0.005% bovine serum albumin in Tris buffer) for 10 minutes each before being washed in 10% normal goat serum (NGS) in Tris B for 1 hour. Sections were then washed in Tris A and Tris B for 10 minutes each before moving into IBa-1 primary antibody (1:2000; Wako, 019-19741) in Tris B and left to incubate at 4°C overnight and up to 48 hours. Sections were removed from primary antibodies and washed in Tris A and Tris B for 10 minutes each, then placed in biotinylated secondary antibody (1:1000, Vector Laboratories, BA-1000) in Tris B for 45 minutes. Sections were washed in Tris A then Tris D (0.1% Triton-X and 0.005% bovine serum albumin in 0.5M Tris buffer) for 10 minutes each, followed by a 90-minute incubation in 1% A+B (Avidin-Biotin complex, Vector Laboratories, PK-6101) in Tris D. Sections were then washed with Tris buffer three times for 5 minutes before being placed in a 3,3'-Diaminobenzidone (DAB)-tetrachloride solution (50ml Tris buffer, 50ml water, 50mg DAB, 30ul hydrogen peroxide) for 30 minutes or until colour develops. Sections were then washed in Tris buffer before mounting on chrome-gelatin coated slides. Slides were left to dry overnight and then counter-stained with Nissl staining.

The Nissl staining protocol consisted of a series of washes, first with decreasing concentrations of ethanol to re-hydrate the tissue (100%, 95%, and 70%) for 2 minutes each, then they were dipped into distilled water. Next, slides were submerged in 0.1% cresyl violet for up to 5 minutes or until colour developed. After rinsing again in distilled water, slides were dehydrated in increasing ethanol concentrations (70%, 95%, and 100%) for 2 minutes each, and finally in xylene for 2 minutes and until cover slipped with Permount.

The IBa-1 antibody was used as a marker of microglia, and therefore inflammation, in the LC region. Neuroinflammation and microglial mechanisms are being considered more prominently in AD research and it is possible that it contributes to disease development and progression (Kinney et al., 2018).

2.4 Statistical Analysis

Statistical analysis was conducted via 3-way ANOVAs with post-hoc Bonferroni corrections to compare effects of environmental manipulations, virus, and sex on measures of AD symptoms and underlying pathological changes.

3. Results

3.1 IBa-1 Cell Counts

Early enriched, htauE14 infused animals had lower IBa-1 cell counts per mm^2 in the LC than control animals and early stressed, htauE14 infused animals, indicating less inflammation. A 2-way ANOVA revealed a significant main effect of environmental condition on IBa-1 cell counts ($F(5,62) = 3.189$, $p = 0.013$). There was no significant main effect of sex ($F(1,62) = 0.558$, $p = 0.458$) and no interaction between environmental condition and sex ($F(5,62) = 1.803$, $p = 0.125$). These results are depicted in Figure 5 below.

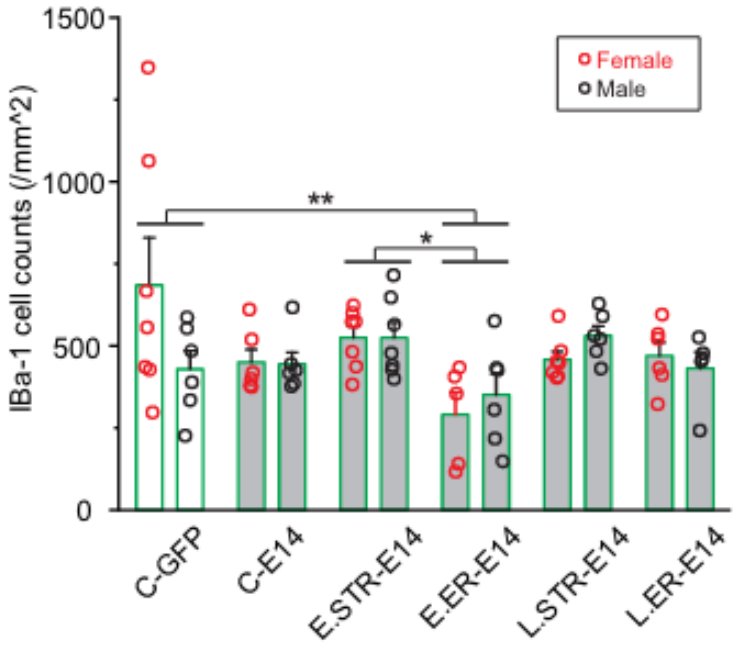

Figure 5. Summary of IBa-1 cell counts per mm^2 in the LC. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. Early enriched, htauE14 infused animals had less IBa-1 positive cells than controls and early stressed animals.

3.2 General Behaviour

3.2.1 Sucrose Preference Test

Pre-tangle animals had a greater sucrose preference than control animals, though all animals showed a preference for sucrose solution over water. Sucrose preference indicates lack of anhedonia. A 3-way ANOVA revealed a significant main effect of virus on sucrose preference ($F(1,92) = 5.219$, $p = 0.024$), no main effect of environmental condition ($F(4,92) = 1.077$, $p = 0.372$), and a significant main effect of sex ($F(1,92) = 4.048$, $p = 0.047$). There were no significant interactions between virus and environmental condition, virus and sex, or environmental condition and sex ($F(4,92) = 2.404$, $p = 0.055$, $F(1,92) = 0.004$, and $F(4,92) =$

2.048, $p = 0.094$, respectively). Finally, there was no significant three-way interaction ($F(4,92) = 2.217$, $p = 0.073$). These results are depicted in Figure 6 below.

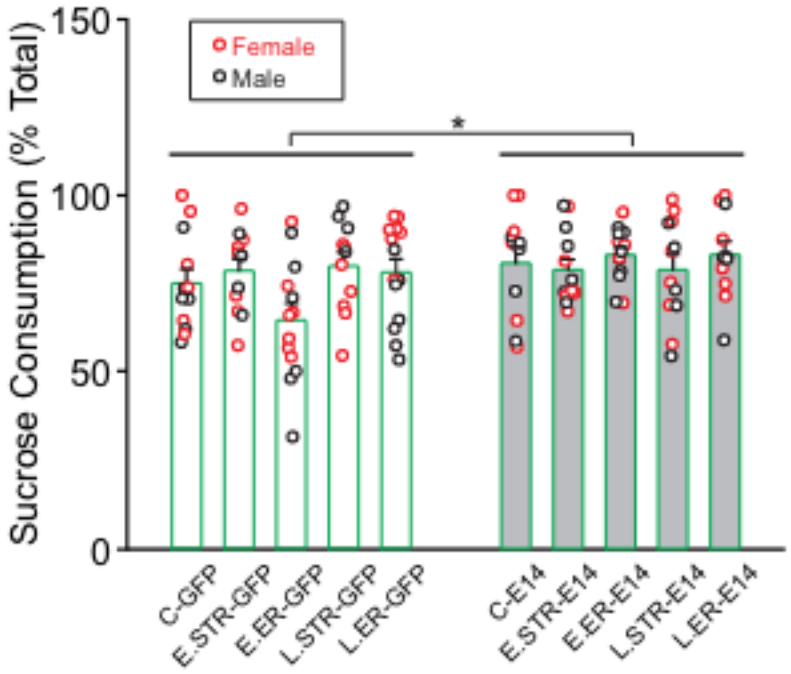

Figure 6. Summary of percentage of total sucrose consumed in 24 hours during the sucrose preference test. * indicates min. $p < 0.05$. htauE14 infused animals had a greater sucrose preference than control virus infused animals.

3.2.2 Marble Burying Test

htauE14 infused animals buried more marbles than control animals, with pre-tangle males burying more than females, indicating increased stress. Early stressed, control infused animals buried fewer marbles than controls, while both early and adulthood stressed, htauE14 infused animals buried more than controls. A 3-way ANOVA revealed a significant main effect of virus on number of marbles buried ($F(1,100) = 40.556$, $p < 0.001$), no significant main effect of environmental condition ($F(4,100) = 0.799$, $p = 0.529$), and no significant main effect of sex

($F(1,100) = 0.608$, $p = 0.437$). There was a significant interaction between virus and environmental condition ($F(4,100) = 8.269$, $p < 0.001$) and between virus and sex ($F(1,100) = 5.684$, $p = 0.019$). There was no interaction between environmental condition and sex ($F(4,100) = 2.222$, $p = 0.072$) or a three-way interaction ($F(4,100) = 0.605$, $p = 0.660$). The results are depicted in Figure 7 below.

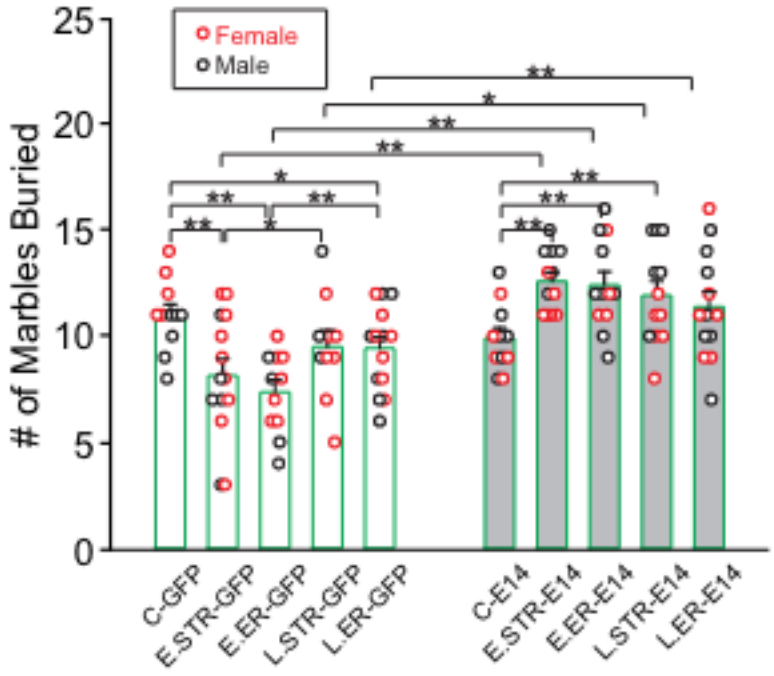

Figure 7. Summary of number of marbles buried during the marble burying test. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. htauE14 infused animals buried more marbles than control virus infused animals. htauE14 males buried more marbles than females. Early-life stress animals buried fewer marbles in control infused groups, while both stress condition animals buried more marbles than controls in htauE14 infused groups.

3.2.3 Elevated Plus Maze

Pre-tangle animals spent more time in closed arms and performed fewer head dips than controls, indicating increased stress. Late, or adulthood, enriched, htauE14 infused animals spent less time

in closed arms and performed more head dips than other pre-tangle animals. A 3-way ANOVA revealed significant main effects of virus, environmental condition, and sex on the percentage of time spent in closed arms during the EPM trial ($F(1,93) = 27.884$, $p < 0.001$, $F(4,93) = 8.241$, $p < 0.001$, and $F(1,93) = 8.287$, $p = 0.005$, respectively). There were no significant interactions between virus and environmental condition, virus and sex, environmental condition and sex, or a three-way interaction ($F(4,93) = 0.347$, $p = 0.845$, $F(1,93) = 0.713$, $p = 0.401$, $F(4,93) = 0.333$, $p = 0.855$, and $F(4,93) = 0.211$, $p = 0.932$, respectively). A 3-way ANOVA revealed significant main effects of virus, environmental condition, and sex on number of head dips performed during the EPM test ($F(1,90) = 12.257$, $p = 0.001$, $F(4,90) = 4.444$, $p = 0.003$, and $F(1,90) = 9.661$, $p = 0.001$, respectively). There was a significant interaction between virus and environmental condition ($F(4,90) = 5.088$, $p = 0.001$). There were no significant interactions between virus and sex or environmental condition and sex ($F(1,90) = 0.031$, $p = 0.861$ and $F(4,90) = 0.328$, $p = 0.858$, respectively). Finally, there was no significant three-way interaction ($F(4,90) = 0.221$, $p = 0.926$). These results are depicted in Figure 8 below.

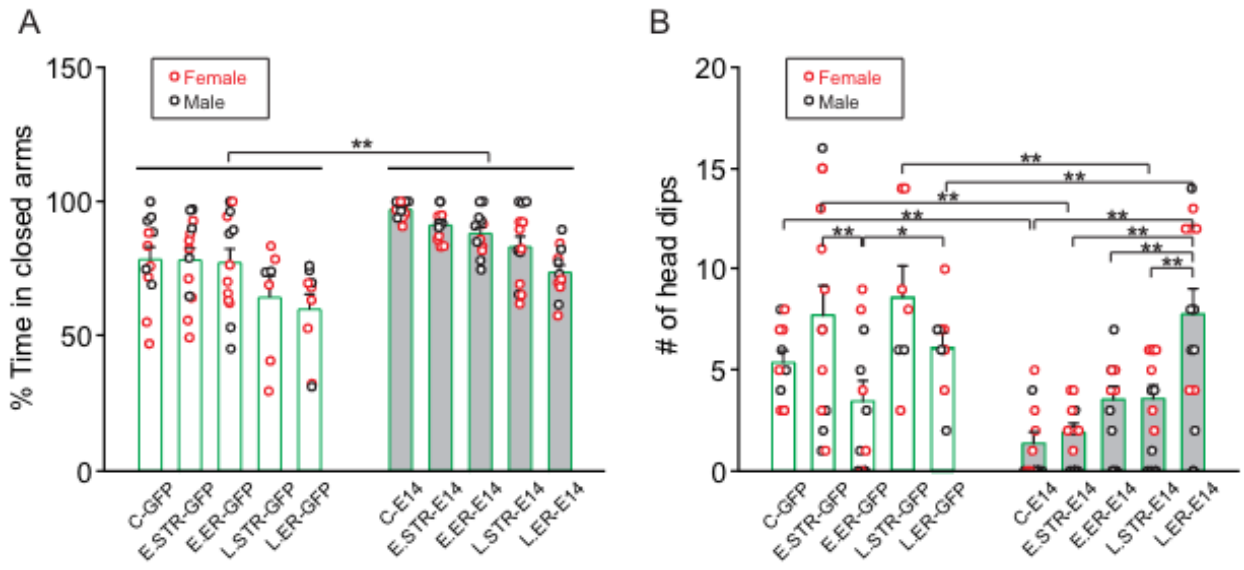

Figure 8. Summary of EPM results. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. A) Percent of time spent in closed arms during the EPM trial. Control infused animals spent less time in closed arms than htauE14 animals. Adulthood enriched animals spent less time in closed arms than other environmental condition animals. Females spent less time in closed arms than males. B) Number of head dips performed during the EPM trial. Control infused animals performed more head dips than htauE14 infused animals. Females performed more head dips than males. Adulthood enriched animals performed more head dips than htauE14 infused groups, while stress condition animals performed more head dips than other control infused groups.

3.2.4 Open Field Maze

Pre-tangle animals demonstrated reduced activity and exploration in the OFM than controls through distance traveled, time spent rearing, and time spent freezing. Adulthood enriched animals were more active than other groups and females were more active than males. A 3-way ANOVA indicated a significant main effect of virus, environmental condition, and sex on distance traveled in the OFM ($F(1,95) = 12.226$, $p = 0.001$, $F(4,95) = 7.517$, $p < 0.001$, and

$F(1,95) = 25.240$, $p < 0.001$, respectively). There were no significant interactions between virus and environmental condition, virus and sex, environmental condition and sex, or a three-way interaction ($F(4,95) = 1.015$, $p = 0.404$, $F(1,95) = 0.788$, $p = 0.377$, $F(4,95) = 1.243$, $p = 0.298$, and $F(4,95) = 1.230$, $p = 0.303$, respectively). A 3-way ANOVA revealed significant main effects of virus, environmental condition, and sex on time spent rearing during the OFM ($F(1,93) = 38.164$, $p < 0.001$, $F(4,93) = 12.270$, $p < 0.001$, and $F(1,93) = 20.501$, $p < 0.001$, respectively). There were also significant interactions between virus and environmental condition, virus and sex, and environmental condition and sex ($F(4,93) = 5.428$, $p = 0.001$, $F(1,93) = 10.596$, $p = 0.002$, and $F(4,93) = 5.631$, $p < 0.001$, respectively). There was no significant three-way interaction ($F(4,93) = 1.857$, $p = 0.125$). A 3-way ANOVA revealed no significant main effect of virus on amount of time spent freezing during the OFM trial ($F(1,82) = 2.556$, $p = 0.114$). There were significant main effects of environmental condition and sex on freezing ($F(4,82) = 7.099$, $p < 0.001$ and $F(1,82) = 4.365$, $p = 0.040$, respectively). There was an interaction between virus and environmental condition ($F(4,82) = 3.031$, $p = 0.022$). There were no interactions between virus and sex or environmental condition and sex ($F(1,82) = 0.047$, $p = 0.828$ and $F(4,82) = 2.321$, $p = 0.064$, respectively). There was no significant three-way interaction ($F(4,82) = 0.717$, $p = 0.583$). These results are shown below in Figure 9.

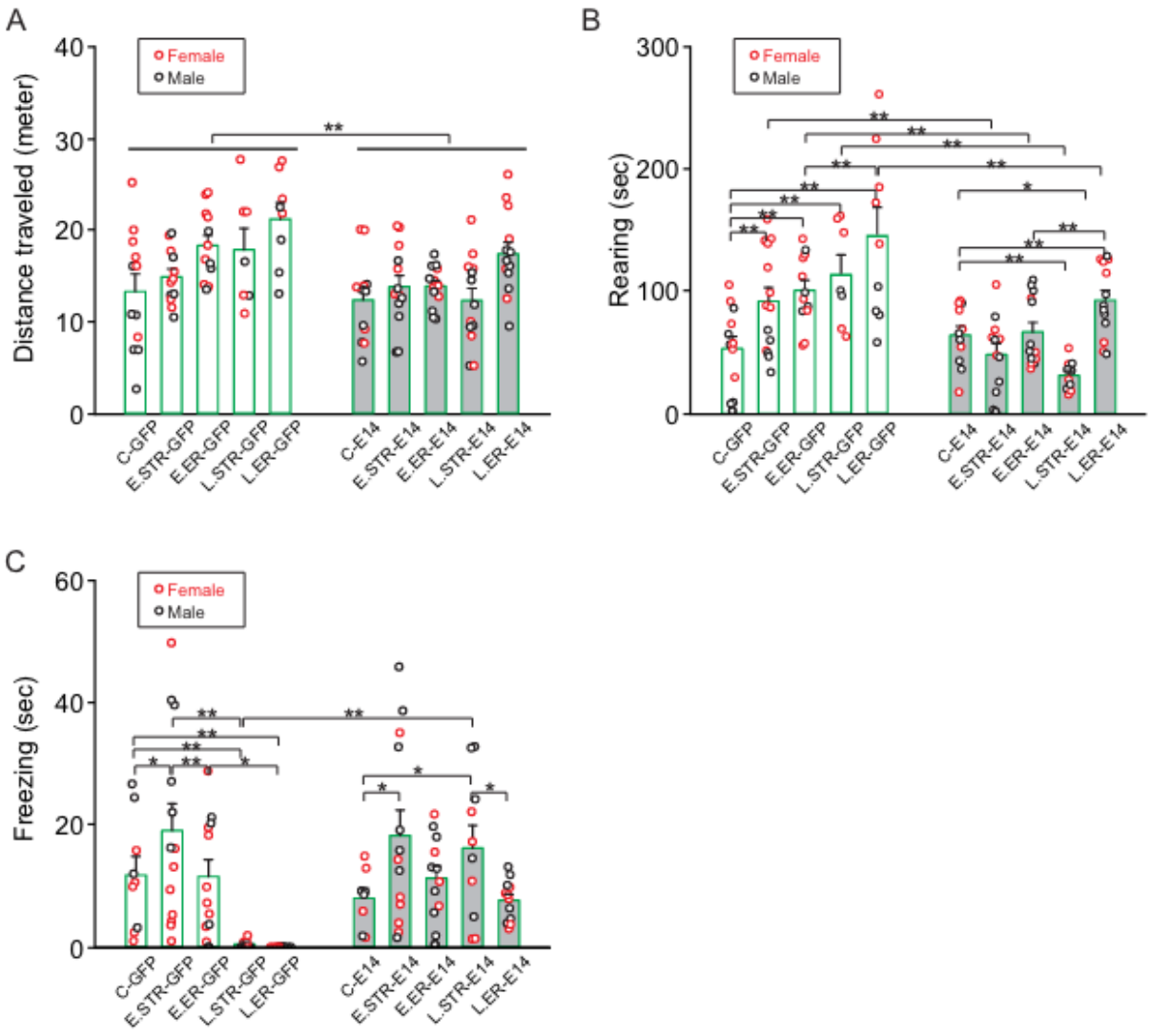

Figure 9. Summary of OFM results. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. A) Distance traveled in the OFM. Control infused animals traveled more than htauE14 infused animals. Enriched group animals were more active than control group animals, with adulthood enriched animals being most active. Females traveled more than males. B) Time spent rearing during the OFM. Control infused animals spent more time rearing than htauE14 infused animals. Adulthood enriched animals spent more time rearing than animals from other conditions. Females spent more time rearing than males, particularly adulthood enriched females. Enriched males spent more time rearing than other males. C) Time spent freezing during OFM trial.

Adulthood enriched animals spent less time freezing than other groups, while early-life stressed animals spent more time freezing. Females spent less time freezing than males.

3.3 Learning

3.3.1 Spontaneous Location Recall

htauE14 infused animals were impaired compared to controls in recognizing the novel location object, though enrichment, both early and late, improved performance. A 3-way ANOVA showed a significant main effect of virus and environmental condition on the discrimination index for the SLR ($F(1,88) = 13.217$, $p < 0.001$ and $F(4,88) = 3.788$, $p = 0.007$, respectively). There was no main effect of sex ($F(1,88) = 0.079$, $p = 0.780$). There were no interactions between virus and environmental condition, virus and sex, environmental condition and sex, or a three-way interaction ($F(4,88) = 0.509$, $p = 0.729$, $F(1,88) = 0.614$, $p = 0.436$, $F(4,88) = 1.213$, $p = 0.311$, and $F(4,88) = 0.279$, $p = 0.891$, respectively). The results are summarized in Figure 10 below.

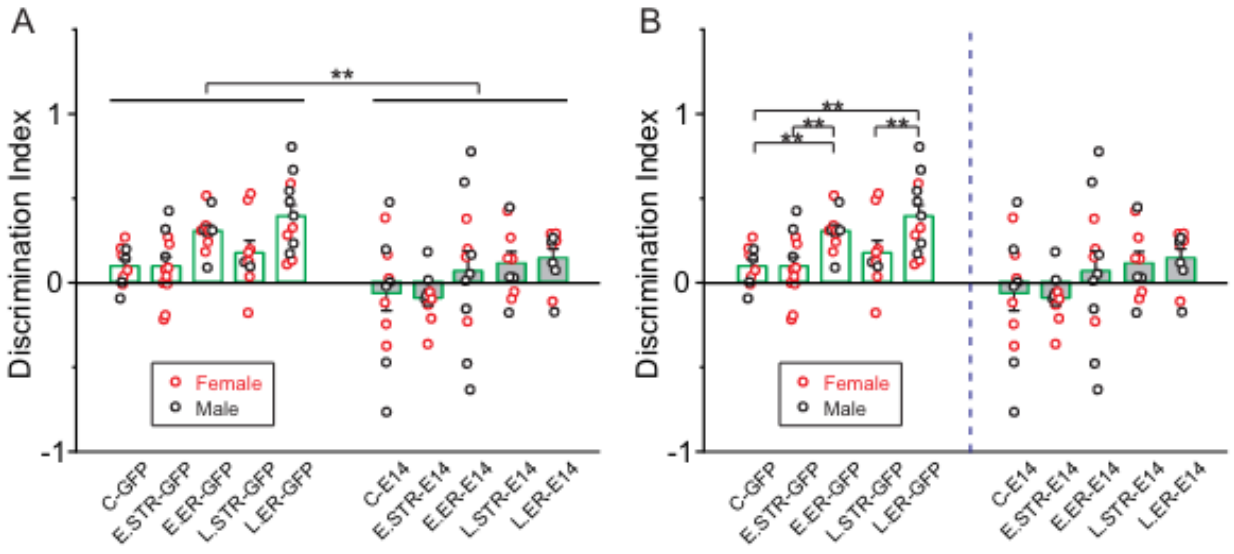

Figure 10. Summary of SLR test results. ** indicates min. $p < 0.01$. A) Discrimination index values from the SLR test. Control infused animals were better able to recognize the novel location object than htauE14 infused animals. B) Discrimination index values from the SLR test indicating environmental condition differences. Enriched animals were better able to distinguish the novel location object compared to other groups, particularly adulthood enriched animals.

3.3.2 Y-Maze

htauE14 infused animals were impaired in recognizing the novel arm in the Y-maze compared to controls, indicated by fewer entries into the arm. Males performed better than females, though early enriched, htauE14 infused animals showed improvement. A 3-way ANOVA indicated a significant main effect of virus on percentage of total entries into the novel arm ($F(1,92) = 5.407$, $p = 0.022$). There were no main effects of environmental condition or sex on percent novel entries ($F(4,92) = 1.733$, $p = 0.150$ and $F(1,92) = 0.679$, $p = 0.412$, respectively). There was no significant interaction between virus and environmental condition ($F(4,92) = 1.779$, $p = 0.140$), but there were significant interactions between virus and sex ($F(1,92) = 5.900$, $p = 0.017$) and

environmental condition and sex ($F(4,92) = 3.196$, $p = 0.017$). There was also a significant three-way interaction between virus, environmental condition, and sex ($F(4,92) = 3.106$, $p = 0.019$). These results are summarized in Figure 11 below.

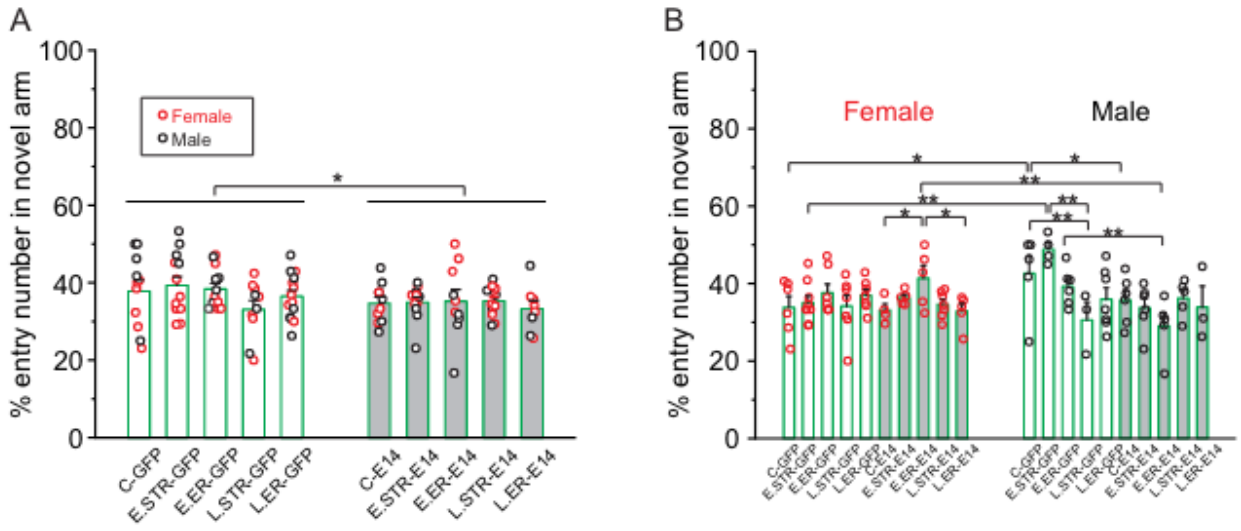

Figure 11. Summary of Y-Maze results. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. A) Percent of total entries into the novel arm during the testing phase of the Y-maze test. The htauE14 infused animals had fewer entries into the novel arm than control virus infused animals. B) Y-maze results with sexes separated. Control infused males performed better than females, with early stressed males performing best, while adulthood stressed males are most impaired. htauE14 infused early enriched females performed better than other groups, males did not show the same improvement.

3.3.3 Odor Detection and Discrimination

htauE14 infused animals were impaired at discriminating between similar odors compared to controls. A 3-way ANOVA revealed a significant main effect of virus on ODAD discrimination index ($F(1,94) = 9.223$, $p = 0.003$). There were no main effects of environmental condition or sex

($F(4,94) = 0.149$, $p = 0.963$ and $F(1,94) = 0.013$, $p = 0.909$, respectively). There were no interactions between virus and environmental condition, virus and sex, environmental condition and sex, or a three-way interaction ($F(4,94) = 1.407$, $p = 0.238$, $F(1,94) = 1.254$, $p = 0.266$, $F(4,94) = 0.733$, $p = 0.572$, and $F(4,94) = 1.999$, $p = 0.101$, respectively). The results are shown in Figure 12 below.

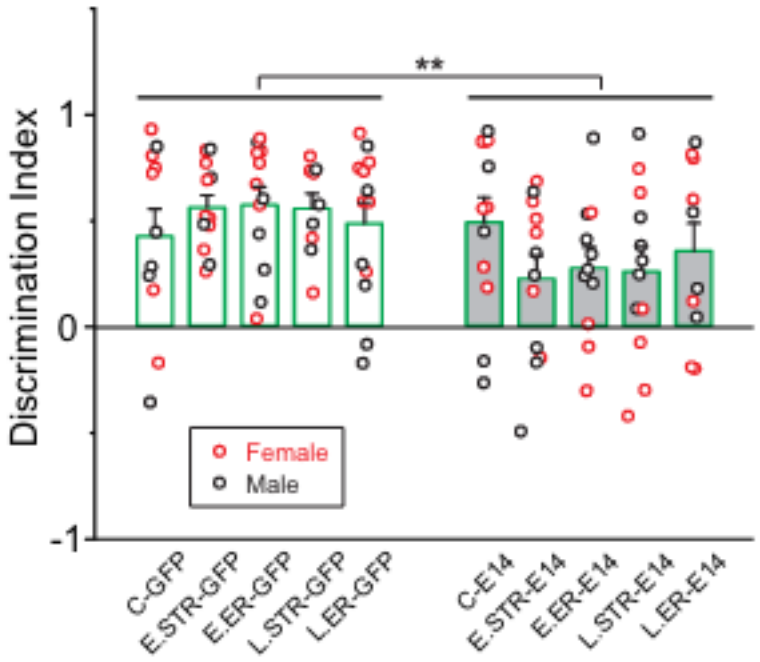

Figure 12. Summary of discrimination index values from the ODAD test. ** indicates min. $p <$ 0.01. The htauE14 infused animals were impaired compared to control infused animals at discriminating between the similar odors.

3.3.4 Olfactometer

Adulthood stress improved odor discrimination learning in control infused animals, but impaired performance in pre-tangle animals. A 3-way ANOVA revealed significant main effects of sex, treatment condition (virus and environmental condition), and training duration on odor

discrimination learning ($F(1,512) = 19.963$, $p < 0.001$, $F(9,512) = 3.821$, $p < 0.001$, and $F(7,512) = 40.629$, $p < 0.001$, respectively). There was a significant interaction between sex and treatment condition ($F(9,512) = 2.642$, $p = 0.005$) but no interactions between sex and training duration ($F(7,512) = 0.843$, $p = 0.552$), treatment condition and training duration ($F(63,512) = 0.459$, $p = 0.999$), or a three-way interaction ($F(63,512) = 0.432$, $p = 0.999$). These results are summarized in Figure 13 below.

Post hoc Bonferroni tests revealed significant differences between sexes and treatment conditions, with notable comparisons between control infused, adulthood stressed males and htauE14 infused, adulthood stressed males ($p = 0.004$), htauE14 infused, early stressed males and htauE14 infused, adulthood stressed males ($p = 0.002$), htauE14 infused, early stressed males and htauE14 infused, early stressed females ($p = 0.049$), and htauE14 infused, early enriched males and htauE14 infused, early enriched females ($p < 0.001$).

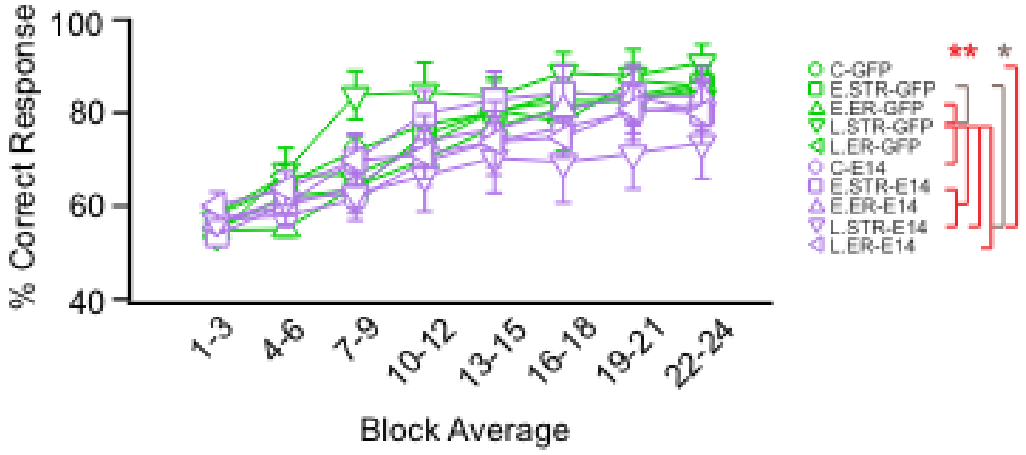

Figure 13. Summary of olfactometer results. * indicates min. $p < 0.05$, ** indicates min. $p < 0.01$. Adulthood stress enhanced learning in control infused animals, but worsened performance in htauE14 infused animals.

4. Discussion

4.1 Summary of Major Findings

This study demonstrates that environmental conditions and sex both differentially influence outcomes in our pre-tangle model. Generally, we see that our pre-tangle animals show increased signs of anxiety and poorer spatial and olfactory learning and memory, though there are some sex differences and some effects of environmental manipulations.

First, under general behaviour, the SPT shows us that the pre-tangle animals do not have less sucrose preference than our control infused animals. There were no groups that did not prefer sucrose over plain water. As previously mentioned, a lack of sucrose preference would be an indication of anhedonia, or in other words, depressive-like symptoms (Liu et al., 2018), therefore our animals do not show signs of depression.

Next, in the MBT the pre-tangle animals buried more marbles, indicating increased anxiety-like symptoms (Archer et al., 1987) compared to controls, particularly in those that experienced early-life stress.

Consistently, the htauE14 infused animals continued to show increased stress symptoms in the EPM (Kraeuter et al., 2019), spending more time in closed arms and performing fewer head dips than control group animals. Importantly, despite being pre-tangle animals, those in the adulthood enrichment group did not demonstrate the same stress-like symptoms, instead spending significantly less time in closed arms and performing significantly more head dips. This is a good indication that environmental factors can still influence symptomology later in life.

Also consistently, the pre-tangle animals showed decreased exploration in the OFM, indicating increased stress (Seibenhener & Wooten, 2015). They spent less time rearing, more time freezing, and traveled less distance within the maze than control group animals. However,

adulthood enriched animals again showed a reversal of those trends, being more active and exploratory than other pre-tangle animals, similarly to the results of the EPM described above.

The first of our learning experiments was the SLR, testing rats' ability to recognize a novel position of an object, with a higher discrimination index being indicative of that ability (Bekinschtein et al., 2013). Our results show that our pre-tangle animals were impaired in this task compared to controls. Despite the difficulty of the task, as determined by object proximity during the training phase, and confirmed by the results showing control groups not performing perfectly, both early-life and adulthood enrichment groups showed increased ability to perform this task, with adulthood enriched animals performing particularly well.

Another spatial memory task, the Y-maze, also showed impairment of the pre-tangle animals compared to controls in their ability to recognize the novel arm. There are also some sex differences demonstrated with these results, with control males performing better than control females and htauE14 males. Further, in control males, early stress improved performance, while adulthood stress worsened performance, while in htauE14 females, early enrichment improved performance. This task highlights the differential effects of both sex and environmental condition on symptom development in our model.

Both tests of spatial memory, the SLR and Y-maze, were used as there are some key differences in their testing criteria. First, the SLR is a relatively difficult task in comparison to the Y-maze, in that rats must be able to recognize a novel location of an object that is in close proximity to the previously located objects, as opposed to an entirely new piece of the environment to explore in the Y-maze test. Additionally, the Y-maze test is more a measure of short-term memory, with testing occurring four hours after training, while the SLR is a measure of long-term memory, with testing occurring 24 hours after training. Finally, the Y-maze test was designed to be a simple and sensitive test of memory, while the SLR was designed to

demonstrate that pattern separation occurs during the encoding of memory (Bekinschtein et al., 2013; Dellu et al., 1992). Impairment of the pre-tangle animals in these tasks suggests that early pathological changes in AD influence working short- and long-term memory and pattern separation.

Next, we examined the animals' ability to discriminate between two very similar mild odors and again found the pre-tangle animals impaired compared to control animals. This is consistent with other testing results showing the general impairment of the pre-tangle animals, in tests of anxiety, activity, and learning.

The results from the olfactometer testing demonstrate differences between sexes and groups in their ability to learn to discriminate between two very similar odors. Generally, males performed better than females and adulthood stress enhanced learning in the control groups but had the opposite effect in the htauE14 animals. Particularly in early intervention groups, the sex difference is highlighted, with males outperforming females, including improvement in performance in the htauE14 groups. Interestingly, while early stress improved the pre-tangle male performance, adulthood stress did not.

Finally, early enriched htauE14 animals had lower IBa-1 cell counts than controls and early stressed htauE14 animals. IBa-1 staining of microglia being sparser in these animals indicates reduced inflammation.

Overall, these results demonstrate differential effects of both sex and environmental factors in symptom development of the pre-tangle tau model.

4.2 Comparison to Existing Studies

An important consideration when comparing this study to others in the field is the unique model being used. Our model mimics human origins of AD by introducing pseudo-phosphorylated human tau directly into the LC and maintains a lack of NFTs and beta-amyloid plaques, meaning

it is a pre-tangle and pre-clinical model (Ghosh et al., 2019). Other tau pathology animal models do not share all those properties. For example, Zimova and colleagues (2016) used a mouse model of tauopathy in which truncated human tau was introduced and passed on in a transgenic line. While they did see pre-tangle pathology and early symptoms associated with tau, their model also developed NFTs and neuropil threads, more consistent with later stages of disease progression. Another transgenic mouse model expressed wild-type human tau, specifically the 1N4R isoform as it is the most abundant in the brain, and created a pre-tangle model (Wheeler et al., 2015). However, this model still differs from the one used in the present study in that it does not mimic the same origin of tau pathology from the LC that is proposed in human AD.

As discussed earlier, there are varying effects reported for stress on learning and memory. Similarly, the results of this study also showed varying effects of stress on several learning and memory assessments. As example, a study found that a CUSP resulted in decreased working memory, but improved reversal learning (Chaby et al., 2015), and in this study we found that our control group adulthood stressed males had decreased performance in the Y-maze, but improved abilities in olfactory discrimination training. Another study found that early life stress resulted in increased anxiety during adulthood, but also improved spatial learning (Kambali et al., 2019). Our results do not show the same increased anxiety for early stressed animals specifically, however, we did see an improvement in their olfactory discrimination training amongst the htauE14 infused animals. Further, both mice and human studies have linked stress with increased risk of AD, with neurotic individuals having increased likelihood of later AD diagnosis (Wilson et al., 2006), and chronically stressed mice having increased biomarkers for AD, as well as poorer fear memory (Carroll et al., 2011). Overall, our results also suggested a link between tau pathology, the precursor to AD, and stress, as we saw across multiple measures that the htauE14 infused animals had increased stress-like symptoms and poorer cognitive functioning. Given that

there are many factors, including timing, duration, and context of stressors that could determine how stress influences outcomes, the present study is consistent with other findings.

Contrarily to effects of stress, the effects of enrichment are generally more consistent, with the consensus being that enrichment improves cognitive functioning. As previously mentioned, Hebb (2002) found that pet-raised rats had greater problem-solving skills compared to cage-raised. Another group compared environmental and social enrichment and found that environmental and social enrichment improved novel object recognition and spatial memory (Hullinger et al., 2015). Later, a similar study conducted in aged rats showed similar results, but that environmental enrichment had more robust effects in a shorter amount of time (Cortese et al., 2018). Finally, using an amyloid beta model of AD, Prado Lima and colleagues (2018) found that both environmental and anaerobic enrichment reversed AD-related deficits in object recognition, and environmental, anaerobic, and social enrichment reversed AD-related deficits in social recognition. Consistently, the results of the present study also indicate that enrichment has positive influence on outcomes. Our results demonstrate that across several measures adulthood enrichment reduces stress. Moreover, early life enriched females in the htauE14 group showed improvement in spatial memory, while their male counterparts showed improvement in olfactory discrimination learning. Finally, both early life and adulthood enrichment, especially adulthood, improved long-term spatial memory.

Another factor considered throughout this study has been sex differences. Andersen and colleagues (1999) found that there was a greater incidence of AD in females compared to males, with females having an 81.7 incident rate compared to just 24.0 in men at 95 years old in a study using patient data across four European countries. A more recent study conducted in the United States consistently found that the AD incidence was higher in women than men from census data and predicted greater increases in AD incidence in women over the next 40 years (Rajan et al.,

2021). It is now widely known that females are at increased likelihood of developing AD compared to males. Additionally, there are differences in symptomology between the sexes, including neuropsychiatric symptoms (Tao et al., 2018). This group recruited patients from eight clinical centers and assessed several psychiatric symptoms, including agitation, depression, anxiety, hallucinations, and elation. They found that women were more likely to exhibit neuropsychiatric comorbidities in general, but specifically delusions, anxiety, and irritability. Moreover, there are sex differences in AD biomarkers, with females having higher levels of pathological tau, and according to Sundermann and colleagues (2020) testosterone provides a protective effect against that tau, providing a possible explanation for the difference between sexes. Another study found that women had higher Braak stages than men, and higher prevalence of pathological tau even in individuals that did not show cognitive impairment (Hu et al., 2021). In the present study, the sex differences varied between tests. In measures of general behaviour, females were more exploratory and showed fewer indications of stress. However, in tests of cognition, males outperformed females in short-term spatial recall and olfactory discrimination learning. There was an exception to that as well, with adulthood stressed females outperforming their male counterparts in spatial recall. Given that females are more greatly affected by AD pathology, it is consistent that the females in this study were impaired in cognitive testing. However, they also demonstrated decreased stress-like symptoms. This may be due to sex differences in the stress response and differences in resiliency to stress, as well as the timing and duration of stressors. For example, one study found that following a CUSP, young female mice showed lower anxiety-like behaviour and increased cognition, while aged female mice following the same CUSP demonstrated irregular locomotive activity and reduced memory (Lotan et al., 2018). This, in combination with variable outcomes due to stress dependent on timing, duration, and circumstances of stressors could explain why the results of this study show female subjects

being less stressed and more active, while simultaneously performing worse in cognitive assessments.

4.3 Limitations

One limitation within this study was that the experimenter conducting behavioural tests was not necessarily blind to the group assignment of the animals during the tests. However, efforts were made to avoid experimenter bias, including following strict protocols during testing, and video recording trials to verify results, and having a blind experimenter conduct the test when possible.

Another limitation to this study was the sample size per group. There were between 12-15 animals per group, and in some cases, there were outliers, meaning fewer were included in analysis. Increasing the sample sizes would increase statistical power of the analyses conducted and potentially provide further insight into the factors being considered and their relationship to AD pathology.

4.4 Conclusions and Future Directions

This study set out to examine the roles of environmental stress and enrichment on Alzheimer's disease progression in our pre-tangle model, as well as whether timing or sex was significant in those roles. As the results show, there are differential effects of stress and enrichment, varying with timing as they were implemented on symptom progression and development. Generally, htauE14 infused animals showed increased stress-like symptoms and reduced cognitive abilities. While there were effects of stress and enrichment paradigms on symptom development, they were more variable than originally hypothesized. It was expected that stress would result in a worsening of symptoms, however, in some cases it was beneficial. Environmental enrichment was expected to improve symptoms, and in some cases, this did hold true, though it was not a robust effect seen across all measures. Further, there were varying influences depending on timing of environmental manipulations, with a notable beneficial effect of adulthood enrichment

on outcomes of the pre-tangle animals. Across multiple tests, we found that adulthood enriched animals within the htauE14 infused group reversed deficits seen in other htauE14 infused animals, confirming that there is potential for intervention and reversal of decline through lifestyle changes during the adult pre-tangle stages of Alzheimer's disease progression.

Going forward, there are a number of factors that could be considered to expand upon these findings. The first of which being expanding upon the histological analysis to correlate underlying pathological changes to behavioural results discussed here. This analysis is planned to include measuring viral uptake and expression, markers of apoptosis, and markers of NE activity in LC projection sites. This would allow for model validation and identification of potential markers of early-stage AD pathology, as well as provide possible explanations to behavioural results.

Secondly, exploring other methods of enrichment within our model may provide a possible explanation as to why we did not see the effect of enrichment across all tests. Our adulthood enrichment took place for 2 hours per day over six weeks, and included environmental, social, and aerobic aspects, as rats were placed in the arena together, with plenty of different objects of different sizes, shapes, colors, and textures, and included a running wheel. In some other enrichment paradigms, animals were housed with enrichment objects, or in larger cages with other animals for social enrichment, or trained to complete a specific exercise for anaerobic enrichment (Hullinger et al., 2015; Prado Lima et al., 2018). In these cases, environmental and social enrichment are more permanent fixtures in the lives of the animals than in the present study. Comparing the present methods of enrichment with the more permanent alternatives in our model may show differing results in symptom development. Regardless, our results do show significant effects of non-permanent enrichment on reducing pre-tangle symptom development.

Another avenue worth exploring would be to examine roles of other factors that could contribute to AD pathology. A meta-analysis examined the relationship between AD and many potential factors and identified risk factors, protective factors, and factors that did not show any significant relationship with AD (Xu et al., 2015). Examples of risk factors that could be tested in our model include a high body mass index (BMI) during midlife and depression. On the other hand, some examples of factors that are said to be protective against AD that could be tested in our model include caffeine consumption, alcohol consumption, high BMI during late-life, and intake of vitamins E and C. The more factors we are able to examine and better understand their relationship with AD pathology, particularly in our pre-tangle model, the better we may be able to use that information to advise lifestyle changes to reduce risk of AD, potentially slow or reverse pathology, or in the development of early-screening tests or treatment options.

Further, and expanding upon the idea of advising lifestyle changes, considering the significant effect of adulthood enrichment seen in this study, it would be beneficial to consider various methods of enrichment that could be incorporated into one's lifestyle in order to reduce risk of AD, or potentially reverse tau pathology. For example, Khalsa (2015) discusses in a review a method of meditation called Kirtan Kriya (KK), which can be done in less time than other meditation techniques, allows for more natural posture and breathing during the session, and uses a simple "song" accompanied by finger movements with each syllable. The benefits described in the review include increased cerebral blood flow, reduced loss of brain volume with age, lower levels of depression, improved memory, and improved sleep. This could be a simple, time-efficient method of enrichment during adulthood that could improve AD symptoms and the underlying pathological process. It would be interesting to observe the effects of meditation on AD patients, or those at risk of developing AD. Another interesting potential intervention is dancing. Rehfeld and colleagues (2017) conducted a study in which elderly people participated in

either a dance program or sport program over 18 months and used magnetic resonance imaging (MRI) to examine hippocampal structure. After program completion, both groups demonstrated increased hippocampal volume, though the dance group had volume increases in additional regions, including the dentate gyrus and right subiculum, and had improvements in balance scores which the sport group did not. This suggests that dance could be used as a method of enrichment. It is not only aerobic enrichment, but often involves social enrichment, and involves memorization, balance, and bodily control, and can be customized to personal taste with different styles and music choices. Like meditation, it would be interesting to see how dance as a source of enrichment influences AD patients or those at risk of developing AD.

Another consideration is whether stress is a causal factor in AD progression, or if the disease makes one more prone to stress. Results of this study indicate that htauE14 infused animals, regardless of environmental grouping, had increased stress-like symptoms, which indicates that AD pathology contributes to experienced stress. However, as discussed earlier, studies across animal models and in humans have indicated that increased stress increases cognitive decline and risk of later AD development (Carroll et al., 2011; Chaby et al., 2015; Kambali et al., 2019; Schwabe & Wolf, 2010; Wilson et al., 2006). As such, further studies will need to be done to tease out the relationship between stress and AD pathology. It is possible that stress is a causal factor leading to AD, but AD simultaneously induces stress. Similarly, this study shows that enrichment can improve AD symptoms. It would be interesting to examine if enrichment elicits these effects by preventing and improving AD pathology or by reducing stress that would otherwise increase the risk of AD. Again, it is possible that enrichment improves AD symptomology through reducing or slowing the pathological process as well as by reducing stress.

References

Andersen, K., Launer, L. J., Dewey, M. E., Letenneur, L., Ott, A., Copeland, J. R. M., Dartigues, J. F., Kragh-Sorensen, P., Baldereschi, M., Brayne, C., Lobo, A., Martinez-Lage, J. M., Stijnen, T., Hofman, A., & the EURODEM Incidence Research Group (1999). Gender differences in the incidence of AD and vascular dementia. *Neurology, 53*(9), 1992-1997. doi: 10.1212/wnl.53.9.1992

Alzheimer Society of Canada (2020). Retrieved April 27, 2020, from https://alzheimer.ca/en/Home

Archer, T., Fredriksson, A., Lewander, T., & Söderberg, U. (1987). Marble burying and spontaneous motor activity in mice: interactions over days and the effect of diazepam. *Scandinavian Journal of Psychology, 28*, 242-249. doi: 10.1111/j.1467-9450.1987.tb00761.x

Aston-Jones, G. & Bloom, F. E. (1981a). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *The Journal of Neuroscience, 1*(8), 876-886. doi: 10.1523/JNEUROSCI.01-08-00876.1981

Aston-Jones, G. & Bloom, F. E. (1981b). Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *The Journal of Neuroscience, 1*(8), 887-900. doi: 10.1523/JNEUROSCI.01-08-00887.1981

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., McGaughm J. L., de Quervain, D. J.-F., & Roozendaal, B. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences, 114*(34), 9176-9181. doi: 10.1073/pnas.1710819114

Ball, M. J. (1977). Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. *Acta Neuropathologica, 37*(2), 111-118. doi: 10.1007/bf00692056

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., & Perl, D. P. (1995). Neocortical Neurofibrillary Tangles Correlate With Dementia Severity in Alzheimer's Disease. *Archives of Neurology, 52*, 81-88. doi: 10.1001/archneur.1995.00540250089017

Bekinschtein, P., Kent, B. A., Oomen, C. A., Clemenson, G. D., Gage, F. H., Saksida, L. M., & Bussey, T. J. (2013). BDNF in the Dentate Gyrus is Required for Consolidation of "Pattern-Separated" Memories. *Cell Reports, 5*(3), 759-768. doi: 10.1016/j.celrep.2013.09.027

Binder, L. I., Frankfurter, A., & Rebhun, L. I. (1985). The Distribution of Tau in the Mammalian Central Nervous System. *Journal of Cell Biology, 101*, 1371-1378. doi: 10.1083/jcb.101.4.1371

Bondareff, W., Mountjoy, C. Q., & Roth, M. (1981). Selective loss of neurones of origin of adrenergic projection to cerebral cortex (nucleus locus coeruleus) in senile dementia. *The Lancet, 317*(8223), 783-784. doi: 10.1016/S0140-6736(81)92657-X

Boyko, S., Qi, X., Chen, T.-H., Surewicz, K., & Surewicz, W. K. (2019). Liquid-liquid phase separation of tau protein: The crucial role of electrostatic interactions. *Journal of Biological Chemistry, 294*(29), 11054-11059. doi: 10.1074/jbc.AC119.009198

Braak, H. & Braak, E. (1997). Frequency of Stages of Alzheimer-Related Lesions in Different Age Categories. *Neurobiology of Aging, 18*(4), 351-357. doi: 10.1016/s0197-4580(97)00056-0

Braak, H. & Del Tredici, K. (2011). The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathologica, 121,* 171-181. doi: 10.1007/s00401-010-0789-4

Braak, H., Thal, D. R., Ghebremedhin, E., & Del Tredici, K. (2011). Stages of the Pathologic Process in Alzheimer Disease: Age Categories From 1 to 100 Years. *Journal of Neuropathology and Experimental Neurology, 70*(11), 960-969. doi: 10.1097/NEN.0b013e318232a379

Carroll, J. C., Iba, M., Bangasser, D. A., Valentino, R. J., James, M. J., Brunden, K. R., Lee, V. M.-Y., & Trojanowski, J. Q. (2011). Chronic Stress Exacerbates Tau Pathologu, Neurodegeneration, and Cognitive Performance through a Corticotropin-Releasing Factor Receptor-Dependent Mechanism in a Transgenic Mouse Model of Tauopathy. *Journal of Neuroscience, 31*(40), 14436-14449. doi: 10.1523/JNEUROSCI.3836-11.2011

Chaby, L. E., Cavigelli, S. A., Hirrlinger, A., Lim, J., Warg, K. M., & Braithwaite, V. A. (2015). Chronic Stress During Adolescence Impairs and Improves Learning and Memory in Adulthood. *Frontiers in Behavioural Neuroscience, 9*. doi: 10.3389/fnbeh.2015.00327

Cleveland, D. W., Hwo, S. Y., Kirschner, M. W. (1977). Physical and Chemical Properties of Purified Tau Factor and the Role of Tau in Microtubule Assembly. *Journal of Molecular Biology, 116,* 227-247. doi: 10.1016/0022-2836(77)90214-5

Cortese, G. P., Olin, A., O'Riordan, K., Hullinger, R., & Burger, C. (2018). Environmental Enrichment Improves Hippocampal Function in Aged Rats by Enhancing Learning and Memory, LTP and mGluR5-Homer1c Activity. *Neurobiology of Aging, 63*, 1-11. doi: 10.1016/j.neurobiolaging.2017.11.004

Delacourte, A. & Defossez, A. (1986). Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments. *Journal of the Neurological Sciences, 76*(2-3), 173-186. doi: 10.1016/0022-510X(86)90167-X

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., & Simon, H. (1992). A two-trial memory task with automated recording: study in young and aged rats. *Brain Research, 588*, 132-139. doi: 10.1016/0006-8993(92)91352-f

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., & Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature, 391*, 387-390. doi: 10.1038/34910

Díaz-Mataix, L., Piper, W. T., Schiff, H. C., Roberts, C. H., Campese, V. D., Sears, R. M., & LeDoux, J. E. (2017). Characterization of the amplificatory effect of norepinephrine in the acquisition of Pavlovian threat associations. *Learning and Memory, 24*, 432-439. doi: 10.1101/lm.044412.116

Ercan-Herbst, E., Ehrig, J., Schöndorf, D. C., Behrendt, A., Klaus, B., Ramos, B. G., Oriol, N. P., Weber, C., & Ehrnhoefer, D. E. (2019). A post-translational modification signature defines changes in soluble tau correlating with oligomerization in early stage Alzheimer's disease brain. *Acta Neuropathologica Communications, 7*(192). doi: 10.1186/s40478-019-0823-2

Escanilla, O., Arrellanos, A., Karnow, A., Ennis, M., & Linster, C. (2010). Noradrenergic modulation of behavioral odor detection and discrimination thresholds in the olfactory bulb. *European Journal of Neuroscience, 32*, 458-468. doi: 10.1111/j.1460-9568.2010.07297.x

España, R. A. & Berridge, C. W. (2006). Organization of Noradrenergic Efferents to Arousal-Related Basal Forebrain Structures. *The Journal of Comparative Neurology, 496*, 668-683. doi: 10.1002/cne.20946

Gatter, K. C. & Powell, T. P. S. (1977). The Projection of the Locus Coeruleus upon the Neocortex in the Macaque Monkey. *Neuroscience, 2*, 441-445. doi: 10.1016/0306-4522(77)90009-4

German, D. C., Manaye, K. F., White, C. L., Woodward, D. J., McIntire, D. D., Smith, W. K., Kalaria, R., N., & Mann, D. M. A. (1992). Disease-specific Patterns of Locus Coeruleus Cell Loss. *American Neurological Association, 32*(5), 667-676. doi: 10.1002/ana.410320510

Ghosh, A., Torraville, S. E., Mukherjee, B., Walling, S. G., Martin, G. M., Harley, C. W., & Yuan, Q. (2019). An experimental model of Braak's pretangle proposal for the origin of Alzheimer's disease: the role of locus coeruleus in early symptom development. *Alzheimer's Research and Therapy, 11*(1). doi: 10.1186/s13195-019-0511-2

Goedert, M. (1993). Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Trends in Neuroscience, 16*(11), 460-465. doi: 10.1016/0166-2236(93)90078-z

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., & Crowther, R. A. (1989). Multiple Isoforms of Human Microtubule-Associated Protein Tau: Sequences and Localization in Neurofibrillary Tangles of Alzheimer's Disease. *Neuron, 3*, 519-526. doi: 10.1016/0896-6273(89)90210-9

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., & Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein τ (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences, 83*, 4913-4917. doi: 10.1073/pnas.83.13.4913

Hardy, J. A. & Higgins, G. A. (1992). Alzheimer's Disease: The Amyloid Cascade Hypothesis. *Science, 256*, 184-185. doi: 10.1126/science.1566067

Hebb, D. O. (2002). The organization of behaviour : A neuropsychological theory. Retrieved from http://ebookcentral.proquest.com

Hodes, G. E., Pfau, M. L., Purushothaman, I., Ahn, H. F., Golden, S. A., Christoffel, D. J., Magida, J., Brancato, A., Takahashi, A., Flanigan, M. E., Ménard, C., Aleyasin, H., Koo, J. W., Lorsch, Z. S., Feng, J., Heshmati, M., Wang, M., Turecki, G., Neve, R., … Russo, S. J. (2015). Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility versus Resilience to Subchronic Variable Stress. *The Journal of Neuroscience, 35*(50), 16362-16376. doi: 10.1523/JNEUROSCI.1392-15.2015

Holmes, C., Boche, D., Wilkinson, D., Ghasem, Y., Hopkins, V., Bayer, A., Jones, R. W., Bullock, R., Love, S., Neal, J. W., Zotava, E., & Nicoll, J. A. R. (2008). Long-term effects of Aß$_{42}$ immunization in Alzheimer's disease: follow-up of a randomized, placebo-controlled phase I trial. *Lancet, 372*, 216-223. doi: 10.1016/S0140-6736(08)61075-2

Hoover, B. R., Reed, M. N., Su, J., Penrod, R. D., Kotilinek, L. A., Grant, M. K., Pitstick, R., Carlson, G. A., Lanier, L. M., Yuan, L.-L., Ashe, K. H., & Liao, D. (2010). Tau Mislocalization to Dendritic Spines Mediates Synaptic Dysfunction Independently of Neurodegeneration. *Neuron, 68*, 1067-1081. doi: 10.1016/j.neuron.2010.11.030

Hu, Y., Boonstra, J., McGurran, H., Stormmesand, J., Sluiter, A., Balesar, R., Verwer, R., Swaab, D., & Bao, A. (2021). Sex differences in the neuropathological hallmarks of Alzheimer's disease: focus on cognitively intact elderly individuals. *Neuropathology and Applied Neurobiology, 47*, 958-966. doi: 10.1111/nan.12729

Hullinger, R., O'Riordan, K., & Burger, C. (2015). Environmental enrichment improves learning and memory and long-term potentiation in young adult rats through a mechanism requiring mGluR5 signaling and sustained activation of p70s6k. *Neurobiology of Learning and Memory, 125*, 126-134. doi: 10.1016/j.nlm.2015.08.006

Kambali, M. Y., Anshu, K., Kutty, B. M., Muddashetty, R. S., & Laxmi, T. R. (2019). Effect of early maternal separation stress on attention, spatial learning, and social interaction behaviour. *Experimental Brain Research, 237*, 1993-2010. doi: 10.1007/s00221-019-05567-2

Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K., & Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature, 325*, 733-736. doi: 10.1038/325733a0

Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., Renbing, X., & Peck, A. (1988). Clinical, Pathological, and Neurochemical Changes in Dementia: A Subgroup with Preserved Mental Status and Numerous Neocortical Plaques. *Annals of Neurology, 23*(2), 138-144. doi: 10.1002/ana.410230206

Kelly, S. C., He, B., Perez, S. E., Ginsberg, S. D., Mufson, E. J., & Counts, S. E. (2017). Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathologica Communications, 5*, 8. doi: 10.1186/s40478-017-0411-2

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S., Wittchen, H.-U., & Kendler, K. S. (1994). Lifetime and 12-Month Prevalence of DSM-III-R Psychiatric Disorders in the United States. *Archives of General Psychiatry, 5*, 8-19. doi: 10.1001/archpsyc.1994.03950010008002

Khalsa, D. S. (2015). Stress, Meditation, and Alzheimer's Disease Prevention: Where The Evidence Stands. *Journal of Alzheimer's Disease, 48*, 1-12. doi: 10.3233/JAD-142766

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M., & Lamb, B. T. (2018). Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's and Dementia, 4*, 575-590. doi: 10.1016/j.trci.2018.06.014

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). The Elevated Plus Maze for Measuring Anxiety-Like Behavior in Rodents. *Methods in Molecular Biology, 1916,* 69-74. doi: 10.1007/978-1-4939-8994-2_4

Lesuis, S. L., van Hoek, B. A. C. E., Lucassen, P. J., & Krugers, H. J. (2017). Early postnatal handling reduces hippocampal amyloid plaque formation and enhances cognitive performance in APPswe/PS1dE9 mice at middle age. *Neurobiology of Learning and Memory, 144*, 27-35. doi: 10.1016/j.nlm.2017.05.016

Liu, M.-Y., Yin, C.-Y., Zhu, L.-J., Zhu, X.-H., Xu, C., Luo, C.-X., Chen, H., Zhu, D.-Y., & Zhou, Q.-G. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature Protocols, 13*(7), 1686-1698. doi: 10.1038/s41596-018-0011-z

Lotan, A., Lifschytz, T., Wolf, G., Keller, S., Ben-Ari, H., Tatarsky, P., Pillar, N., Oved, K., Sharabany, J., Merzel, T. K., Matsumoto, T., Yamawaki, Y., Mernick, B., Avidan, E., Yamawaki, S., Weller, A., Shomron, N., & Lerer, B. (2018). Differential effects of chronic stress in young-adult and old female mice: cognitive-behavioral manifestations and neurobiological correlates. *Molecular Psychiatry, 23*, 1432-1445. doi: 10.1038/mp.2017.237

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., Ford, C. P., & Bruchas, M. R. (2015). CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. *Neuron, 87*, 605-620. doi: 10.1016/j.neuron.2015.07.002

Mei, Y., Jiang, C., Wan, Y., Lv, J., Jia, J., Wang, X., Yang, X., & Tong, Z. (2015). Aging-associated formaldehyde-induced norepinephrine deficiency contributes to age-related memory decline. *Aging Cell, 14*, 659-668. doi: 10.1111/acel.12345

Prado Lima, M. G., Schimidt, H. L., Garcia, A., Daré, L. R., Carpes, F. P., Izquierdo, I., & Mello-Carpes, P. B. (2018). Environmental enrichment and exercise are better than social

enrichment to reduce memory deficits in amyloid beta neurotoxicity. *Proceedings of the National Academy of Sciences, 115*(10), 2403-2409. doi: 10.1073/pnas.1718435115

Rajan, K. B., Weuve, J., Barnes, L. L., McAninch, E. A., Wilson, R. S., & Evans, D. A. (2021). Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020-2060). *Alzheimer's & Dementia,* 1-10. doi: 10.1002/alz.12362

Rehfeld, K., Müller, P., Aye, N., Schmicker, M., Dordevic, M., Kaufmann, J., Hökelmann, A., & Müller, N. G. (2017). Dancing or Fitness Sport? The Effects of Two Training Programs on Hippocampal Plasticity and Balance Abilities in Healthy Seniors. *Frontiers in Human Neuroscience, 11*(305). doi: 10.3389/fnhum.2017.00305

Saavedra, J. M. & Torda, T. (1980). Increased Brain Stem and Decreased Hypothalamic Adrenaline-Forming Enzyme after Acute and Repeated Immobilization Stress in the Rat. *Neuroendocrinology, 31,* 142-146. doi: 10.1159/000123065

Seibenhener, M. L. & Wooten, M. C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments, 96,* e52434. doi: 10.3791/52434

Shakhawat, A. M., Harley, C. W., & Yuan, Q. (2014). Arc Visualization of Odor Objects Reveals Experience-Dependent Ensemble Sharpening, Separation, and Merging in Anterior Piriform Cortex in Adult Rat. *The Journal of Neuroscience, 34*(31), 10206-10210. doi: 10.1523/JNEUROSCI.1942-14.2014

Shakhawat, A. M., Gheidi, A., MacIntyre, I. T., Walsh, M. L., Harley, C. W., & Yuan, Q. (2015). Arc-Expressing Neuronal Ensembles Supporting Pattern Separation Require Adrenergic Activity in Anterior Piriform Cortex: An Exploration of Neural Constraints on Learning.

The Journal of Neuroscience, 35(41), 14070-14075. doi: 10.1523/JNEUROSCI.2690-15.2015

Schwabe, L. & Wolf, O. T. (2010). Learning under stress impairs memory formation. *Neurobiology of Learning and Memory, 93*, 183-188. doi: 10.1016/j.nlm.2009.09.009

Smeets, T., Wolf, O. T., Giesbrecht, T., Sijstermans, K., Telgen, S., & Joëls, M. (2009). Stress selectively and lastingly promotes learning of context-related high arousing information. *Psychoneuroendocrinology, 34*, 1152-1161. doi: 10.1016/j.psyneuen.2009.03.001

Sundermann, E. E., Panizzon, M. S., Chen, X., Andrews, M., Galasko, D., Banks, S. J., & the Alzheimer's Disease Neuroimaging Initiative (2020). Sex differences in Alzheimer's-related Tau biomarkers and a mediating effect of testosterone. *Biology of Sex Differences, 11*(33). doi: 10.1186/s13293-020-00310-x

Swanson, L. W. (1976). The locus coeruleus: A cytoarchitectonic, golgi and immunohistochemical study in the albino rat. *Brain Research, 110*, 39-56. doi: 10.1016/0006-8993(76)90207-9

Tao, Y., Peters, M. E., Drye, L. T., Devanand, D. P., Mintzer, J. E., Pollock, B. G., Porsteinsson, A. P., Rosenberg, P. B., Schneider, L. S., Shade, D. M., Weintraub, D., Yesavage, J., Lyketsos, C. G., & Munro, C. A. (2018). Sex Differences in the Neuropsychiatric Symptoms of Patients With Alzheimer's Disease. *Am J Alzheimers Dis Other Demen, 33*(7), 450-457. doi: 10.1177/1533317518783278

Vassar, R., Bennet, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., … Citron, M. (1999). β-Secretase

Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science, 286*, 735-741. doi: 10.1126/science.286.5440.735

Vaz, M. & Silvestre, S. (2020). Alzheimer's disease: Recent treatment strategies. *European Journal of Pharmacology, 887*, 173554. doi: 10.1016/j.ejphar.2020.173554

Walling, S. G., Rigoulot, M.-A., & Scharfman, H. E. (2007). Acute and chronic changes in glycogen phosphorylase in hippocampus and entorhinal cortex after status epilepticus in the adult male rat. *European Journal of Neuroscience, 26*(1), 178-189. doi: 10.1111/j.1460-9568.2007.05657.x

Wegmann, S., Eftekharzadeh, B., Tepper, K., Zoltowska, K. M., Bennett, R. E., Dujardin, S., Laskowski, P. R., MacKenzie, D., Kamath, T., Commins, C., Vanderburg, C., Roe, A. D., Fan, Z., Molliex, A. M., Hernandez-Vega, A., Muller, D., Hyman, A. A., Mandelkow, E., Taylor, J. P., & Hyman, B. T. (2018). Tau protein liquid-liquid phase separation can initiate tau aggregation. *The EMBO Journal, 37*, e98049. doi: 10.15252/embj.201798049

Weller, J. & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research, 7*(F1000 Faculty Rev), 1161. doi: 10.12688/f1000research.14506.1

Wheeler, J. M., McMillan, P. J., Hawk, M., Iba, M., Robinson, L., Xu, G. J., Dombroski, B. A., Jeong, D., Dichter, M. A., Juul, H., Loomis, E., Raskind, M., Leverenz, J. B., Trojanowski, J. Q., Lee, V. M. Y., Schellenberg, G. D., & Kraemer, B. C. (2015). High copy wildtype human 1N4R tau expression promotes early pathological tauopathy accompanied by cognitive deficits without progressive neurofibrillary degeneration. *Acta Neuropathologica Communications, 3*(33). doi: 10.1186/s40478-015-0210-6

Wilson, R. S., Arnold, S. E., Schneider, J. A., Kelly, J. F., Tang, Y., & Bennett, D. A. (2006). Chronic Psychological Distress and Risk of Alzheimer's Disease in Old Age. *Neuroepidemiology, 27*, 143-153. doi: 10.1159/000095761

Wilson, R. S., Nag, S., Boyle, P. A., Hizel, L. P., Yu, L., Buchman, A. S., Schneider, J. A., & Bennet, D. A. (2013). Neural reserve, neuronal density in the locus coeruleus, and cognitive decline. *Neurology, 80*(13), 1202-1208. doi: 10.1212/WNL.0b013e3182897103

Xu, W., Tan, L., Wang, H.-F., Jiang, T., Tan, M.-S., Tan, L., Zhao, Q.-F., Li, J.-Q., Wang, J., & Yu, J.-T. (2015). Meta-analysis of modifiable risk factors for Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 86*, 1299-1306. doi: 10.1136/jnnp-2015-310548

Yalcin, I., Aksu, F., & Belzung, C. (2005). Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *European Journal of Pharmacology, 514*, 165-174. doi: 10.1016/j.ejphar.2005.03.029

Younkin, S. G. (1998). The role of A beta 42 in Alzheimer's disease. *Journal of Physiology Paris, 92*(3-4), 289-292. doi: 10.1016/s0928-4257(98)80035-1

Zhou, X.-D., Shi, D.-D., & Zhang, Z.-J. (2019). Antidepressant and anxiolytic effects of the proprietary Chinese medicine Shexiang Baoxin pill in mice with chronic unpredictable mild stress. *Journal of Food and Drug Analysis, 27*, 221-230. doi: 10.1016/j.jfda.2018.08.001

Zhang, X., Lin, Y., Eschmann, N. A., Zhou, H., Rauch, J. N., Hermandez, I., Guzman, E., Kosik, K. S., & Han, S. (2017). RNA stores tau reversibly in complex coacervates. *PLoS Biology, 15*(7). doi: 10.1371/journal.pbio.2002183

Zimova, I., Brezovakova, V., Hromadka, T., Weisova, P., Cubinkova, V., Valachova, B., Filipcik, P., Jadhav, S., Smolek, T., Novak, M., & Zilka, N. (2016). Human Truncated Tau Induces Mature Neurofibrillary Pathology in a Mouse Model of Human Taupathy. *Journal of Alzheimer's Disease, 54*, 831-843. doi: 10.3233/JAD-160347

Appendix 1. Ethics approval documentation

Dear: Dr. Qi Yuan, Faculty of Medicine\Division of BioMedical Sciences

Researcher Portal File No.: 20220211
Animal Care File:
Entitled: Locus coeruleus norepinephrine modulation in learning and Alzheimer's Disease
Status: Active
Related Awards:

Awards File No	Title	Status	
20190269	Material Transfer Agreement - Not Publishable	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20191784	Locus coeruleus NE modulation in learning and Alzheimer's disease	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20200652	MTA for cis p-tau antibody from Harvard University	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20200817	Understanding what pretangle tau does to neurons and testing potential damage control by an anti-cis-ptau treatment	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses

Ethics Clearance Terminated: May 01, 2024

Your three-year renewal application was reviewed by the ACC on Tuesday June 1, 2021, and the committee approved the renewal, however they did note to please write lay summaries in simpler terms in the future.

This ethics clearance includes the following Team Members: Dr. Qi Yuan (Principal Investigator)
Dr. Xihua Chen (Co-Investigator)
Dr. Susan Walling (Co-Investigator)
Dr. Carolyn Harley (Co-Investigator)

This ethics clearance includes the following Sponsors: [[AllSponsorAgencyNames]]
This ethics clearance includes the following related awards:

Awards File No	Title	Status	
20190269	Material Transfer Agreement - Not Publishable	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20191784	Locus coeruleus NE modulation in learning and Alzheimer's disease	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20200652	MTA for cis p-tau antibody from Harvard University	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses
20200817	Understanding what pretangle tau does to neurons and testing potential damage control by an anti-cis-ptau treatment	Active	1. Research Grant and Contract Services (RGCS) – St. John's and Grenfell Campuses

An Event [Annual Report] will be required following each year of protocol activity.

Should you encounter an unexpected incident that negatively affects animal welfare or the research project relating to animal use, please submit an Event [Incident Report].

Any alterations to the protocol requires prior submission and approval of an Event [Amendment].

NOTE: You can access a copy of this email at any time under the "Shared Communications" section of the Logs tab of your file in the Memorial Researcher Portal.

Sincerely,

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