The Role of Endothelial Pannexin-1 on Memory Subtypes and Related Alzheimer's Disease Implications

A thesis submitted by

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Abstract

Alzheimer's disease (AD), the most prevalent dementia, is characterized by the accumulation of amyloid beta (Aβ) plaques, neurofibrillary tangles, neuroinflammation, and reduced memory and cognitive function. Although several medical treatments are available, they only address systemic symptoms and not AD progression. Therefore, there is a crucial necessity for novel mechanistic discoveries.

AD is associated with reduced cerebral perfusion and impaired cerebrovascular reactivity, which contributes to further accumulation of Aβ plaques. Pannexin1 (Panx1) is a membrane channel that regulates ATP release, activating purinergic signaling cascades, influencing vascular reactivity and inflammation. Our previous studies suggest Panx1 expression is increased in the brain of an AD mouse model, and endothelial cell (EC) Panx1 regulates cerebral vascular tone. We hypothesize that EC Panx1 plays a role in AD neurovascular dysfunction, memory deficits, and neuroinflammation.

To test this hypothesis, we crossed the APP/PS1 mouse model of AD with our EC Panx1 deletion mice and aged them to 9 to 12 months of age. In novel object recognition (NOR) studies, AD mice failed to recognize the novel object, and EC Panx1 deletion did not rescue this memory recognition deficit. Barnes maze (BMz) data illustrates no differences in spatial memory in mice with intact or deleted EC Panx1 at 9-months and 12-months of age. However, Object Location Test (OLT), an alternative spatial memory test, revealed a lack of spatial memory in our mouse model of AD that is presented with EC Panx1 deletion 12 months of age. 9-month-old APP/PS1 mice have reduced cerebral blood flow, which is prevented with EC Panx1 deletion. Lastly, flow cytometry data suggests a trend of decreased infiltrating leukocytes into the brain

with Panx1 deletion in our APP/PS1 mice, primarily T-cells, in male but not female 9-month-old mice.

We conclude that the Panx1 channel mediates cerebral vascular reactivity yet has minimal effects on behavior and AD mediated leukocyte cerebral inflation. These findings are critical in understanding mechanistic progression of AD and has important clinical implications, as drugs which inhibit Panx1 in AD could improve global cerebral perfusion to minimize tissue death and increase spatial memory in late-stage AD. The Panx1 pathway – with further testing and larger sample sizes -- can pave the way for a new therapeutic target to slow-down or reverse AD progression.

Acknowledgements

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Lastly, but certainly not least I would like to thank my parents Filiberto J Ruiz Maroto and Noemi Soler de Ruiz whose encouragement fostered a support system I can always turn to in times of frustration or stress. I would also like to thank my sister Andrea Ruiz Soler, who always cheers me on in my endeavors, my partner Diego Burga who has been a tremendously grounding anchor, and my friends who always want to hear about my experimental developments.

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Table of Contents

List of Figures:

List of Abbreviations

Aβ	Amyloid-beta
AD	Alzheimer's disease
APOE	Apolipoprotein E
APOE4	Apolipoprotein E polymorphism ε4
APP	Amyloid-β precursor protein
APP_{swe}	APP K595N/M596L (Swedish) mutation mouse model of AD
APP/PS1 Tg^{+}	APP_{swe} and PSEN1 deltaE9 mutation mouse model of AD
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BMz	Branes Maze
CBF	Cerebral blood flow
EC	Endothelial cell
EC Panx1	Endothelial cell pannexin1
LSCI	Laser speckle contrast imaging
MWM	Morris water maze
NOR	Novel object recognition
OLT	Object location test
Panx1	Pannexin1
$Panx1^{fl/fl}$	Pannexin1 floxed
PSEN1	Presenilin-1 protein
PSEN2	Presenilin-2 protein
Tam	Tamoxifen
WT	Wild-type mice

Chapter 1: Introduction

1.1 Alzheimer's Disease

Alzheimer's Disease (AD) is a progressive neurodegenerative disease that is the leading cause of dementia in the elderly. By 2050 the elderly population – 65 years and older – is projected to increase from 63 million in 2025 to over 82 million.[1] This projection alone, predicts 2.1 million new cases within the next 25 years, which affects African American and Hispanic individuals at higher rates.[2] In turn, this will proportionately increase caregiver burden for these populations.[3]

AD is characterized by memory loss, behavioral abnormalities, cerebral atrophy, neuronal loss, inflammation, and accumulation of amyloid plaques and tau tangles. Taken together, these pathological and behavioral changes contribute to loss in quality of life. Genetic risk factors predict and help diagnose early to late onset AD. Rare familial inheritance of mutations in the amyloid-β precursor protein (APP), presenilin-1 protein (PSEN1), and presenilin-2 protein (PSEN2) genes are the main causal risk factors for early-onset AD.[4,6] In recent years, the apolipoprotein E (APOE) polymorphism ε4 (APOE4) has been correlated with increased risk of late-onset AD.[5,6] These genetic abnormalities further contribute to mechanistic progression of AD in the brain, yet, with the vastly low prevalence of patients with early-onset genetics compared to the high penetrance late-onset AD, it is paramount to further investigate novel mechanism for AD therapeutics.

Although numerous hypotheses on AD progression are proposed, the amyloid hypothesis is the most widely accepted. The hypothesis proposes that APP metabolism by β-secretase and γ-secretase creates the amyloid-β (Aβ) 40 and 42 monomers. [6,47] The Aβ monomers, with decreased solubility and clearance, form oligomers and create Aβ plaques within the brain that

contribute to neurotoxicity.[7] More recently, the Vascular Hypothesis has been proposed suggesting that impaired cerebral vascular function and cardiovascular diseases, such as hypertension, diabetes, and hyperhomocysteinemia, heighten the risk of developing AD; emphasizing a need to explore vascular-mediated mechanismism contributing to AD.[50] A few FDA approved treatments are available for AD including: 1) anticholinesterase inhibitors and anti-glutaminergic treatments, which temporarily treat symptoms[8,9]; and 2) anti-amyloid antibodies that aim to remove Aβ from the brain, but are only available for patients with mild symptoms and may only temporarily slow the progression of AD symptoms.[48.49] New pharmacologically targetable mechanisms contributing to AD progression are necessary to improve patient health.

1.2 Behavioral Alterations in Animal Models of Alzheimer's Disease

Memory loss is the earliest symptom that is reported in AD. An effect attributed to left ventricular hippocampal atrophy, among other cerebral morphological changes.[10,11,17] However, presentation of this symptom suggests that the patient has already reached a mild progressive AD phase. The difficulty in achieving pre-clinical AD diagnosis has motivated researchers to discover novel mechanisms and biomarkers. The low availability of human tissue – only available post-mortem – makes animal models a crucial outlet for research.

Transgenic mouse models have been developed to study AD progression, including the APP_{swe} (Swedish K595N/M596L mutations) and PSEN1-dE9 (APP/PS1) mouse model of AD. [12,13] Research elucidates that 12 to 14 week old APP/PS1 mice had elevated Aβ protein levels in the brain and at 9 to 12-months have acceleated Aβ plaque deposition coupled with AD phenotypic spatial-working memory alterations in a Y-maze assay.[14] As AD progresses it is

known to alter recognition and spatial memory, where patients lose ability to recognize familiar faces, people, and their environment. 7- to-17-month-old APP/PS1 mice have shown similar deficits in recognition memory using Novel Object Recognition (NOR) assays, and spatial memory alterations in assays such as Morris Water Maze (MWM).[15,16] Overall, animal models remain an effective way to research AD progression and using APP/PS1 models offer an accelerated outlet to research novel treatments.

1.3 Cerebral Blood Flow in Alzheimer's Disease

The brain is one of the most vascularized organs in the body. Alterations in baseline cerebral vascular function can negatively impact cognitive function and AD patients with late-stage AD have decreased cerebral blood flow (CBF) compared to healthy controls.[17] This hypoperfusion has been correlated with Aβ deposition in the brain implicating worsening blood delivery to tissue in patients with higher Aβ plaque load.[18] This effect extends to animal models including the APOE4 knock-in, APP knock-in, and APP/PS1 strains, which show CBF attenuation as early as 4 months and as late as 9 months with an average decrease in 21% CBF across various AD animal models.[19] Pathology can even be accelerated through induced hypoperfusion in four-month-old APP/PS1 mice by way of carotid artery occlusion or stenosis, eliciting earlier behavioral deficits in working or spatial learning at 5 months.[20,21] Chronic reduction in CBF also increases Aβ deposition in mice, which contributes to worsening pathology in these predisposed subjects.[21] It's clear that cerebral hypoperfusion plays a role in AD progression and brain pathology, so further coupling of neuroscience and cerebrovascular research is crucial to identify mechanisms contributing to impaired CBF that affect cognitive function in AD progression.

1.4 Neuroinflammation in Alzheimer's Disease

In addition to cerebral hypoperfusion, neuroinflammation is also an important hallmark of AD. Although acute inflammation serves as a beneficial recovery mechanism, when inflammation becomes chronic the beneficial effects can shift to detrimental side effects.[52] The brain is largely immune privileged with minimal infiltration of peripheral, circulating leukocytes.[52] Astrocytes and microglia are the CNS resident glial cells that monitor the neuronal environment and provide protection against disease and infection.[23,52] It is known that neuroinflammation, including glia cell activation and infiltration of circulating leukocytes, increases as Aβ plaque forms in the brain of AD patients compared to healthy controls.[22] However, the mechanisms contributing to activation and detrimental propagation of neuroinflammation is unknown.

Microglia serve as the vacuum of the brain, phagocytosing debris, regulating synaptic pruning, and playing a crucial role in cerebral homeostasis.[23] AD mouse models have shown increased levels of activated microglia in response to Aβ plaques formation likely due to their attempt to remove the amyloid plaque buildup.[24,25] Astrocytes have structural and homeostatic functions in the brain. Their end-feet help form the selectively permeable blood-brain-barrier (BBB) which protects the brain from peripheral circulating factors. As AD progresses, studies have shown that astrocytes are activated in response to Aβ plaque formation and that the BBB integrity declines.[16,26] In turn, the degradation of the vascular barrier allows $CD8^+$ T-cells to infiltrate the brain and increase microglia activation.[21,27] Astrocytes have also been shown to aid in the degradation of Aβ oligomers and can provide a protective mechanisms against AD progression, suggesting a dual conflicting role for astrocytes in AD.[28] Further research on

cerebral inflammation is paramount for understanding the underlying causes that protect from or contribute to AD progression.

1.5 Endothelial Pannexin 1 in Alzheimer's Disease

Pannexin-1 (Panx1) channels mediate adenosine triphosphate (ATP) release, activating purinergic signaling, and may play a possible role in calcium wave propagation contributing further to cell-to-cell communication.[29,30] Panx1 is expressed throughout the body, including endothelial cells (EC) and neuons.[31,32] Moreover, Panx1 is implicated in AD-related pathological changes. Age-dependent increases in Panx1 levels in the hippocampus are correlated with hippocampal Aβ plaque formation in an APP/PS1 mouse model; however, no clear pathophysiological effects or cell-specific functions of Panx1 in AD pathology have been investigated.[33]

We have previously identified EC Panx1 as an important regulator of cerebral vascular function. EC Panx1 is implicated in regulating leukocyte infiltration in the brain and myogenic tone, which contributes to control of CBF, and CBF recovery post-ischemic stroke.[36,51] Although these studies indicate a role for EC Panx1 in acute regulation of CBF and inflammation, the role of EC Panx1 in chronic neuroinflammation or CBF regulation is unknown.

We hypothesize that EC Panx1 mediates AD progression through its effects on cerebral artery vasoconstriction, resulting in reduced CBF, and facilitating leukocyte infiltration, which together worsen memory and contribute to altered behavior. To test this hypothesis, we generated Cdh5-Cre^{ERT2+} Panx1-floxed (Panx1$^{fl/fl}$) mice crossed with APP/PS1 transgenic mice and treated them with tamoxifen (Tam) to activate the cre recombinase to specifically delete Panx1 in EC (EC Panx1 Deleted). We then tested memory deficits, age-related CBF changes, and leukocyte infiltration in APP/PS1 Tg+ EC Panx1 Deleted mice and littermate controls. We

predict that APP/PS1 EC Panx1 Deleted mice will exhibit less prominent behavioral and memory deficits, preserved global CBF, and reduced infiltration of circulating leukocytes into the brain. This study will contribute to the foundational work in understaind cerebral vascular dysfunction in AD and will offer novel treatments to halt progression of AD and minimize disease-associated burdens.

Chapter 2: Methods and Materials

2.1 Mice

Mice (*Mus musculus*) were fed a normal chow diet and housed under a 12-hour light/dark cycle. APPswe/PSEN1dE9 mice were crossed with our Cdh5-Cre^{ERT2+} Panx1$^{fl/fl}$ mice to create four strains (APP/PS1 Tg+ Cdh5-Cre^{-}/Panx1$^{fl/fl}$, APP/PS1 Tg+ Cdh5-Cre^{ERT2+}/Panx1$^{fl/fl}$, APP/PS1 Tg- Cdh5-Cre^{-}/Panx1$^{fl/fl}$, and APP/PS1 Tg- Cdh5-Cre^{ERT2+}/Panx1$^{fl/fl}$) which delete endothelial cell (EC) Pannexin1. At 8 weeks of age, standard food was replaced with a tamoxifen-containing diet for 14 days to induce expression of the Cre protein and resulted in the following genotype sub-groups: APP/PS1Tg+ Panx1 intact (AD model), APP/PS1Tg+ Panx1 deleted, APP/PS1Tg- Panx1 Intact (wild-type controls), APP/PS1Tg- Panx1 deleted mice. Male and female mice were then aged to 9-month or 12-month groups before initiating behavioral testing.

2.2 Novel Object Recognition (NOR)

For all behavioral tests, mice were placed in the testing room for more than 30 minutes prior to starting tests to equilibrate to the environment.

A white opaque open-top cube (20”x 20”x20”) was utilized as the arena to conduct tests and to minimize visual distractions. Two identical rectangular plastic objects (familiar object) and an unrelated rectangular glass object (novel object) were used to measure mice’s recognition memory. Testing constituted of one habituation day and a test day. During day 1 – habituation – a mouse was placed in the empty arena for a 10-minute trial to normalize the mouse to the environment. Day 2 – test day – consisted of two trials. In trial 1 the two identical objects were placed in opposite corners of the arena and the mouse was allowed to explore the arena for a 10-

minute interval. The mouse was then removed back to a clean holding cage and the area and objects cleaned. 30 min after the inititation of trial 1, trail 2 began with one of the identical objects switched with the novel contrasting object. The same object was switched for all inter-group testing. The mouse was allowed to explore for another 10-minute interval at which point the experiment ended.

All experiments were recorded using Ethno-Vision® software, and upon completion, the recordings were analyzed to confirm no nose-tail point switching occurred and corrected accordingly if needed. Data was then analyzed using the following discrimination index ratio equation using time spent with objects (T) or frequency of visits to objects (F): $\frac{(T\ or\ f)_{novel} - (T\ or\ f)_{familiar}}{(T\ or\ f)_{total}}$. Distance traveled (cm) and total frequency of visits to objects were recorded to determine inter-trial variations. Mice were excluded if they traveled < 10 cm or if DI < -0.5 or > 0.5 on training trial.

2.3 Object Location Test (OLT)

On the day after NOR test completion, OLT testing – composed of two trials -- was conducted. The same NOR arena was utilized for this protocol; however, two spatial markers were attached to contralateral sides of the cube to aid in spatial awareness. Two identical plastic objects, distinct from the objects used for NOR testing, were placed in opposite corners of the arena. In trial 1, the mouse was placed in the arena to explore for a 10-minute interval. The mouse was removed, the arena cleaned, and trial 2 began 30-minutes after the initiation of trial 1. In trial 2, one of the objects was moved to the opposite side of the cube - ipsilaterally to the visual markers. The moved object was kept consistent for inter-group trials to minimize

variability. After trial 2 completion, the experiment ended, and Ethno-Vision® recordings were analyzed as previously described in NOR.

2.4 Barnes Maze (BMz)

Mice were tested on their spatial awareness one week after the completion of NOR using a 5-day BMz assay. The arena consisted of a flat circular platform (120 cm diameter) with 40 equal-sized holes in its outer circumference. The arena was arranged to align with the four cardinal directions. The northernmost point was labeled "hole 4," and a rectangular grey "escape box" was placed under the southernmost hole – "hole 24" – to allow for escape away from open field exposure. The arena was also split into four equal quadrants associated with each cardinal direction (i.e. Northern Quadrant, Eastern Quadrant, etc.). Day 1 consisted of one 5-minute habituation trial, where the mouse was placed in the escape box. If the mouse left the escape box during the trial, they were allowed to explored the arena and were guided back to the box upon completion of the trial. Training days 2-4 consisted of four 4.5-minute trials. the mouse was randomly assigned a cardinal direction to which they would face during their first trial. The mouse subsequentially initiated the next three trails facing a different cardinal direction, rotating clockwise. By the fourth trial all four cardinal directions had been faced. This was repeated Day 3 and 4. If the mouse did not find and enter the escape box by the end of each training trial, the mouse was guided to the escape box and habituated for 30-seconds. Day 5 – test day – consisted of one 5-minute trial in which the escape box was removed and the total time spent within each quadrant was measured. Ethno-Vision® recordings were analyzed for latency to escape box (sec), number of errors, and pathlength (cm) for training days and compared between genotype groups.

Test day analysis focused on time spend withing the desired quadrant – Southern Quadrant – where the escapes box had been placed during training trials.

2.5 Laser Speckle Contrast Imaging (LSCI)

Upon completion of behavioral studies, cerebral blood flow was imaged using LSCI (Perimed, 0.2 um resolution). Mice were anesthetized with isoflurane and an incision was made in the skin to expose the skull. The isoflurane was maintained at a low dose (< 2%) to minimize anesthesia-mediated CBF changes. Imaging was then conducted within 5-minutes of anesthetizing the animals. Perfusion was measured for each cerebral hemisphere using PIMSoft® software.

2.6 Flow Cytometry for Infiltrated Leukocytes

Infiltration of leukocytes into the brain was analyzed as previously performed.[36] Briefly, mice were perfused, brains dissected and digested into a single cell suspension using Liberase TL (Sigma), and myelin removed using a Percoll (Sigma) gradient separation. Cells were then stained with an indicator dye for live versus dead cells followed by incubation with a panel of antibodies to identify microglia and infiltrated leukocytes (CD45), neutrophils (Ly6G), myeloid cells (CD11b), and T cells (CD3). Samples were then analyzed using a Cytek Aurora benchtop analyzer. Appropriate gating strategies were obtained using single stained and fluorescence minus one (FMO) controls.

2.7 Statistics

All statistical analysis was conducted using Prism® software version 10.4.1. Mean ± SEM. T-tests and two-way ANOVA (with repeated measures) were used as appropriate.
* $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

2.8 Contributions

All genotyping of mice was performed by Transnetyx. All behavioral experiments including NOR, BMz, and OLT were done by Mauricio Ruiz Soler. Surgical procedures and LSCI CBF imaging were done by Mauricio Ruiz Soler, and when absent, by Dr. Amanda Mauro. Initial perfusion and tissue processing for leukocyte infiltration was done as a collaborative effort by all Good Lab members. Flow cytometry was performed by Mauricio Ruiz Soler, with Dr. Miranda Good conducting preliminary data experiments.

Chapter 3: Results

3.1 EC Panx1 deletion does not improve recognition memory in a mouse model of AD.

To determine if EC Panx1 deletion restores AD-associated recognition memory, we tested 9-month mice in a NOR assay. Although most groups showed no differences in the time spent with the novel object (DI_{time}), 9-month male APP/PS1 neg Panx1 Deleted mice trended to stronger preference for the novel object compared to controls and AD mice, although the frequency of visits between objects ($DI_{frequency}$) remained the same (**Figure 1A, 1B**). APP/PS1 Tg+ Panx1 Intact female mice have impaired recognition memory, with reduced time spent with and reduce frequency of visiting the novel object compared to WT control female mice (**Figure 1C, D**). Deletion of EC Panx1 in APP/PS1 Tg mice did not significantly change novel object recognition in both male and female 9-month-old mice (**Figure 1C, D)**.

To test if EC Panx1 deletion affects recognition memory with progression of AD pathology, we examined 12-month old mice. Male mice showed no differences in discrimination between objects (**Figure 2A, 2B**). Our small sample size of mice demonstrate that AD mice trend towards reduced recognition memory with reduced discrimition indexes for time and frequency of visiting the novel object, although not significantly (**Figure 2C, D**). However, unlike at 9-months of age, EC Panx1 deletion did not alter NOR results in either control APP/PS1 neg mice or APP/PS1 Tg+ mice (**Figure 2C, D**).

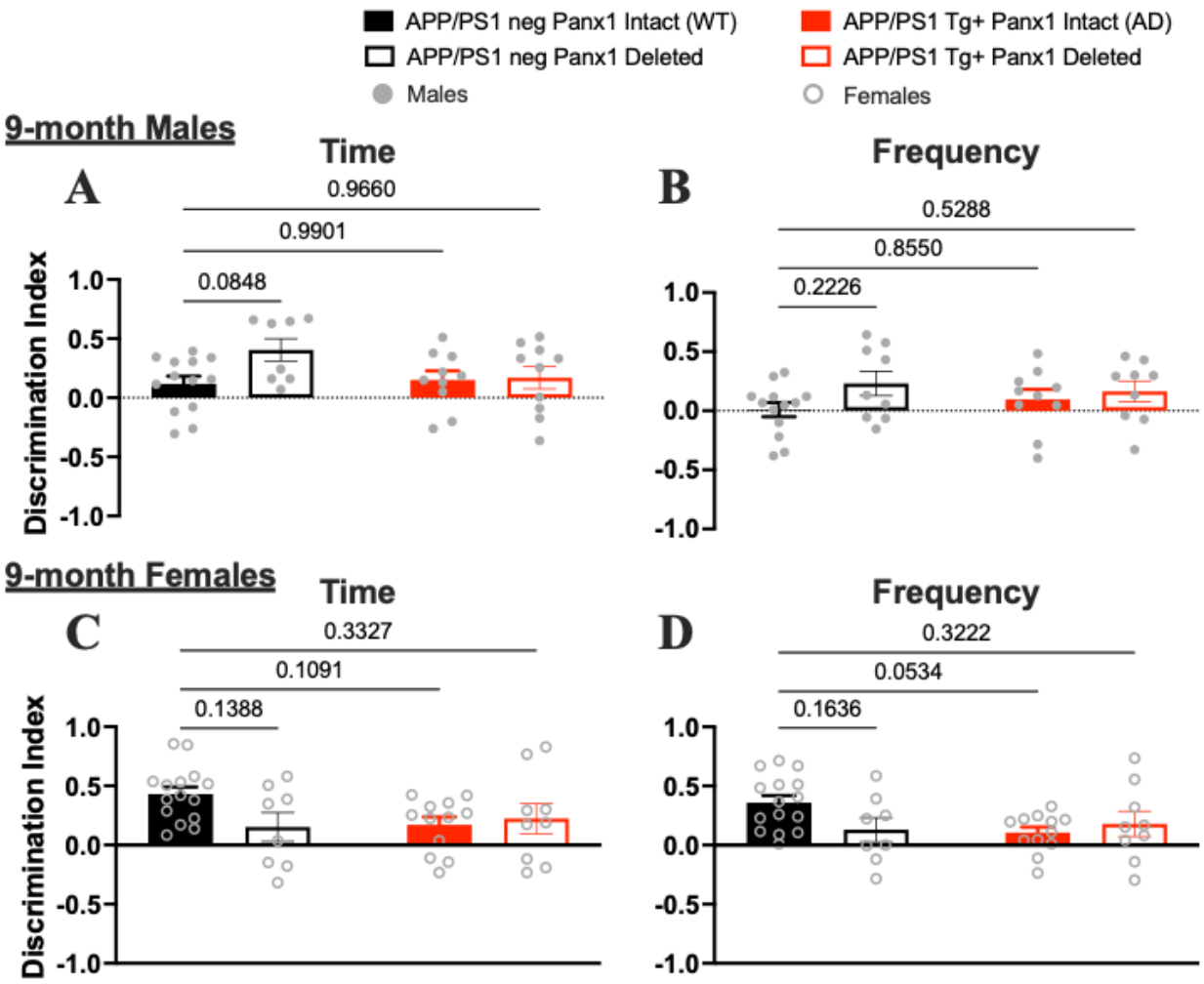

Figure 3.1 APP/PS1 Tg mice have reduced recognition memory at 9-months of age. Recognition memory evaluated by novel object recognition and a discrimination index using time or frequency of object visits as the ratio's metric (**A**) Male DI_{time} (**B**) male $DI_{frequency}$ (**C**) female DI_{time} (**D**) female $DI_{frequency}$. 2-way ANOVA with Tukey multiple comparison post-hoc test (*$p<0.05$). APP/PS1 neg Panx1 intact (N=13 males, 15 females); APP/PS1 neg Panx1 deleted (N=8 males, 8 females); APP/PS1 Tg+ Panx1 intact (N=10 males, 12 females); APP/PS1 Tg+ Panx1 deleted (N=10 males, 9 females).

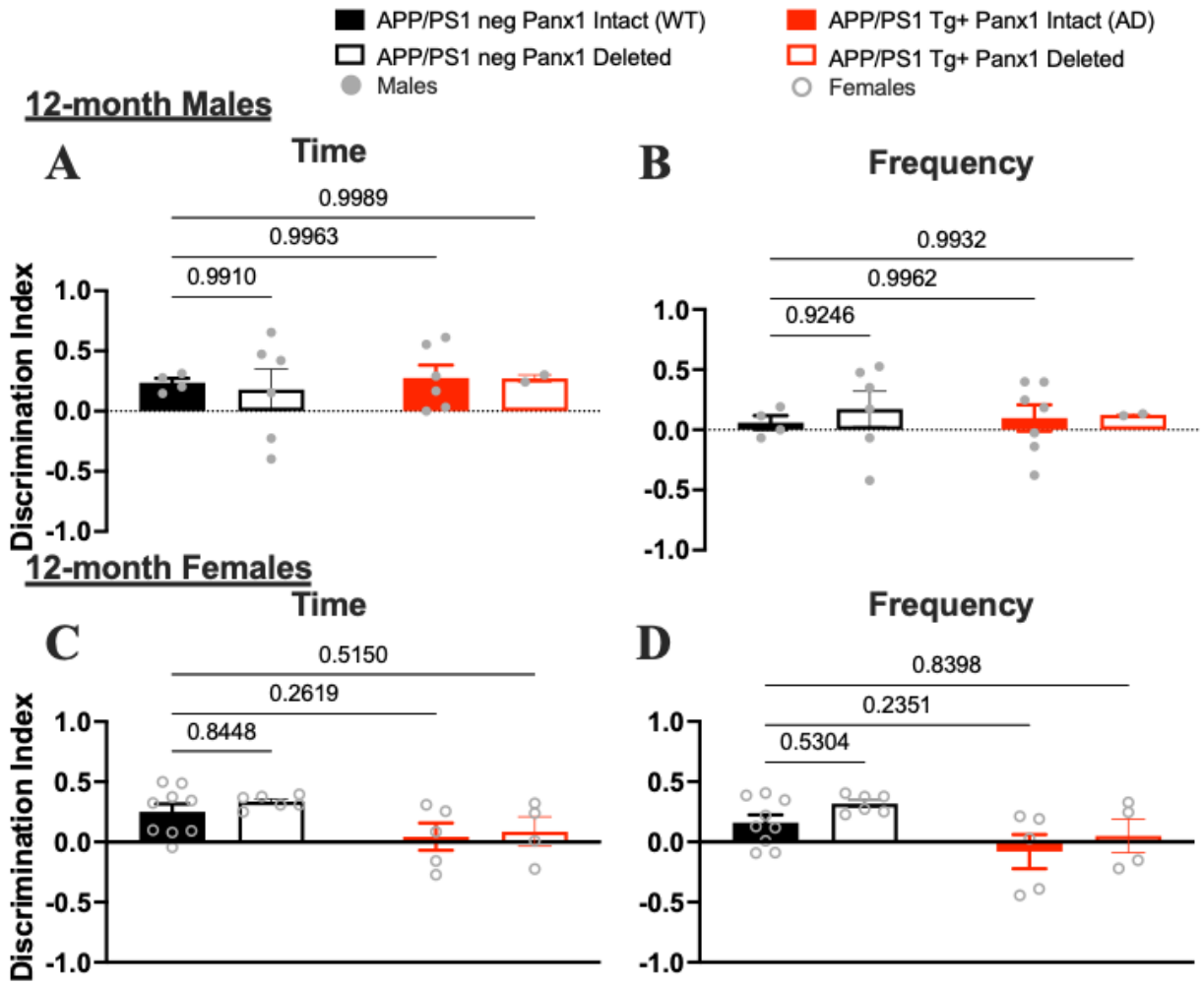

Figure 3.2 EC Panx1 deletion does not improve recognition memory in 12-month mice. Recognition memory evaluated by novel object recognition and a discrimination index using time or frequency of object visits as the ratio's metric (**A**) Male DI_{time} (**B**) male $DI_{frequency}$ (**C**) female DI_{time} using time as our metric. (**D**) Female $DI_{frequency}$. 2-way ANOVA with Tukey multiple comparison post-hoc test (*$p<0.05$). APP/PS1 neg Panx1 intact (N=4 males, 9 females); APP/PS1 neg Panx1 deleted (N=6 males, 6 females); APP/PS1 Tg+ Panx1 intact (N=6 males, 5 females); APP/PS1 Tg+ Panx1 deleted (N=2 males, 4 females).

3.2 EC Panx1 deletion elicits minimal spatial memory and learning improvement

To test the mechanistic effects of EC Panx1 deletion on spatial memory, we used a BMz assay to minimize stress induced effects associated with MWM. 9- and 12-month-old male and female mice showed no significant differences between groups in latency to reach the goal hole, number of errors, and total movement within the maze suggesting a steady improvement in spatial learning and unaltered spatial memory across all groups (**Figure 3.3A-C**). To test spatial memory retention after training, we removed the goal hole on test day 5 and examined percent-time spent within target and non-target quadrants. No significant differences were observed between genoytpes for both male and female mice at 9-months of age (**Figure 3.3D**). The small cohort of 12-month mice prompted us to combine the male and female data points and no conclusions can be currently made from the data (**Figure 3.3E-G**).

To test spatial memory in an assay similar to NOR, we tested 9- and 12-month-old mice in an OLT assay. This assay was initiated after the BMz did not demonstrate AD-related differences resulting in smaller cohorts for testing. Therefore, male and female mice were combined within their appropriate age and genotypic groups. No significant differences were observed between genoytpes for both male and female mice at 9-months of age (**Figure 3.4A, 4B**). 12-month-old APP/PS1 Tg+ Panx1 Deleted mice had a trend of increased preference for the displaced object compared to AD mice, while all other groups failed to successfully discriminate between objects (**Figure 3.4C, D**).

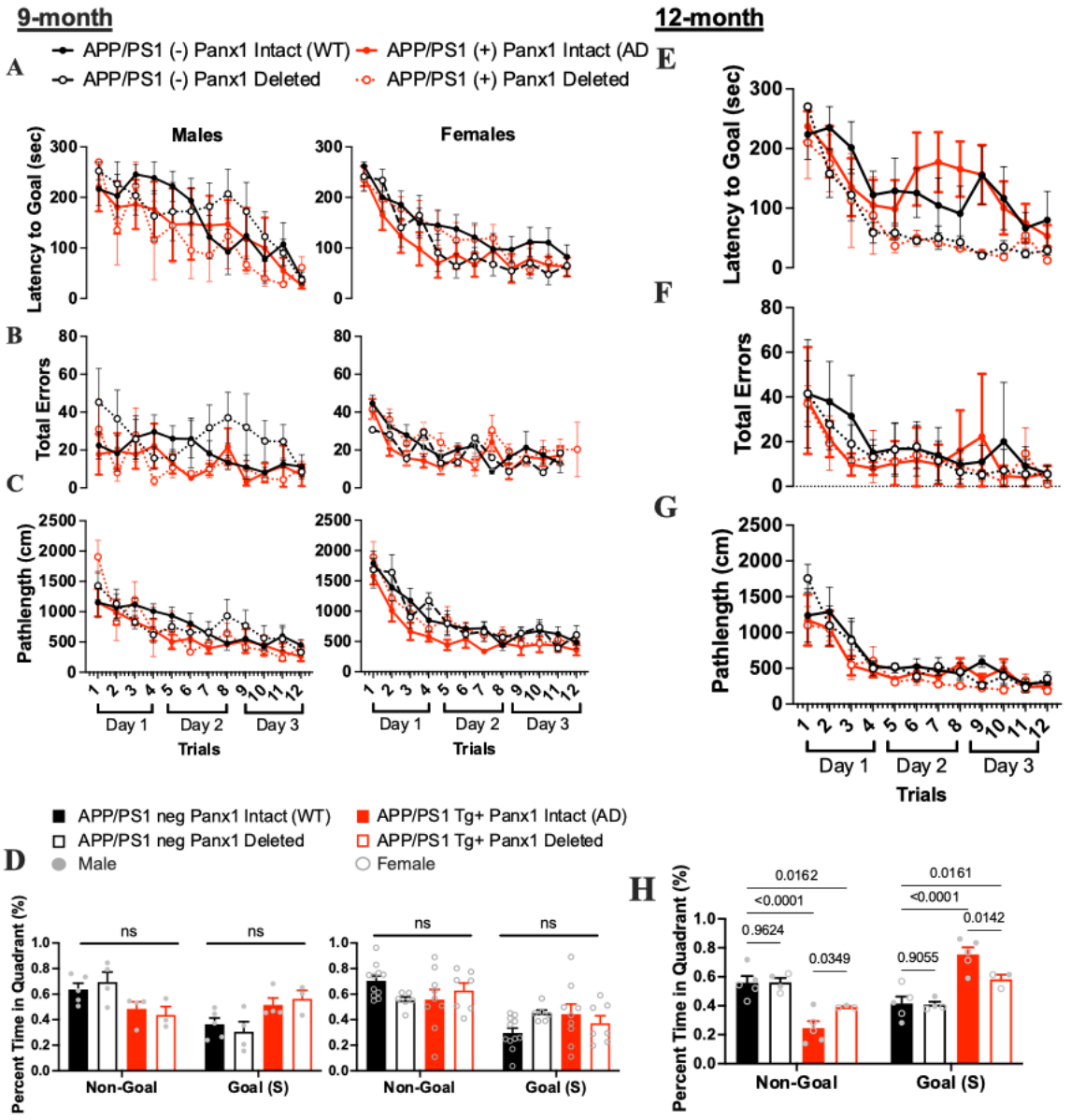

Figure 3.3 EC Panx1 deletion does not alter spatial memory via the Barnes Maze and learning in 9- and 12-month APP/PS1 Tg mice. Spatial memory and spatial learning was measured using the Barnes Maze with (**A**) Latency to escape box, (**B**) total errors (non-goal hole visits), (**C**) total pathlength per trial, and (**D**) percentage of time spent within non-goal (north, east, west) and goal (south) quadrants on test day measured in 9-month old male and female mice. 12-month old male and female mice were combined for (**E**) latency to escape box, (**F**) total errors, (**G**) total pathlength, and (**H**) percent-time spent in non-goal and goal quadrants. 2-way ANOVA with Tukey multiple comparison post-hoc test (*p<0.05). APP/PS1 neg Panx1 intact (N=5 males, 11 females); APP/PS1 neg Panx1 deleted (N=4 males, 6 females); APP/PS1 Tg+ Panx1 intact (N=5 males, 9 females); APP/PS1 Tg+ Panx1 deleted (N=3 males, 7 females).

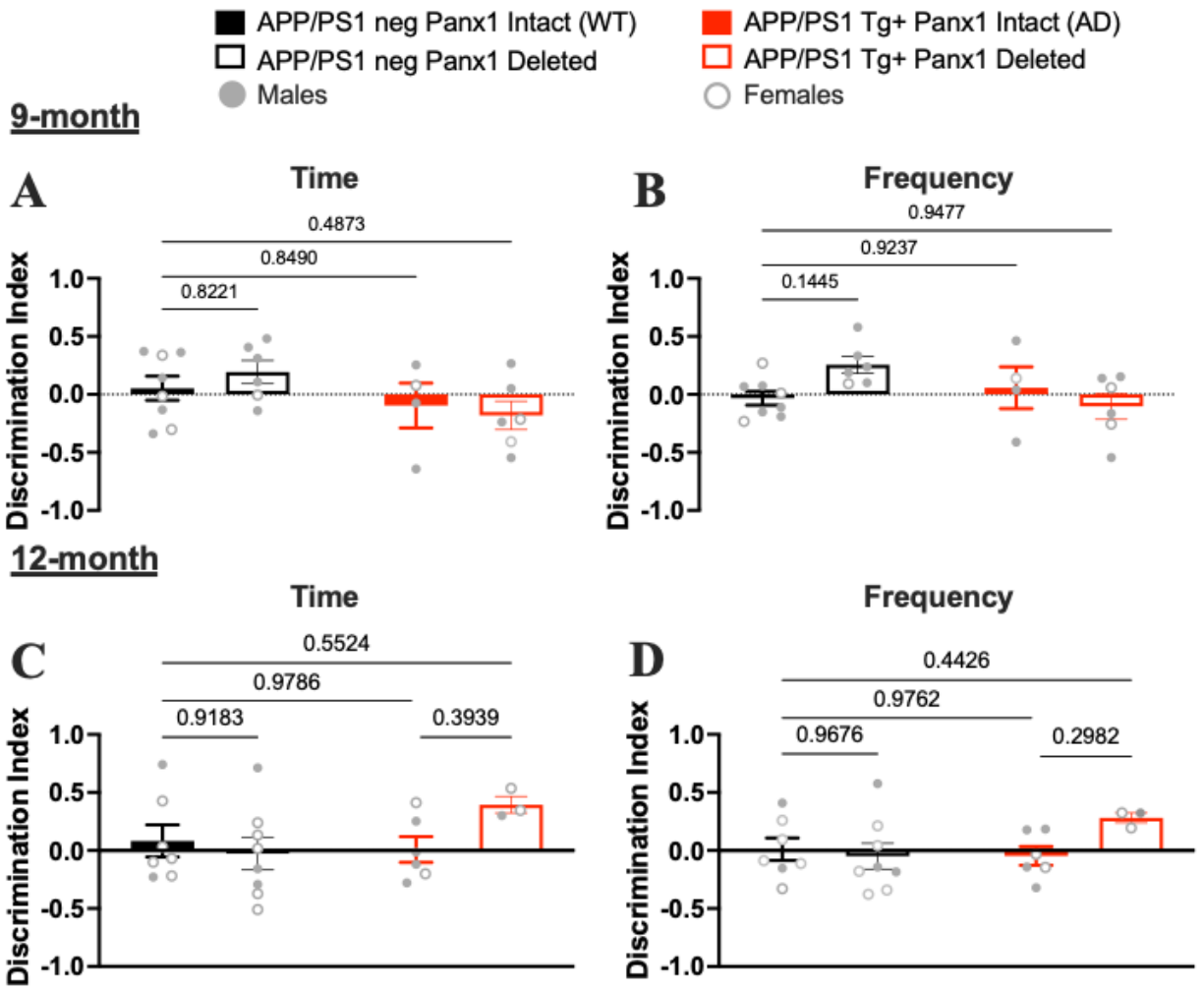

Figure 3.4 EC Panx1 deletion trends to improved spatial memory via the OLT in 12-month APP/PS1 Tg+ mice. Spatial memory was evaluated through the object location test. 9-month-old combined male and female (**A**) DI_{time} and (**B**) $DI_{frequency}$ and 12-month-old combined male and female (**C**) DI_{time} and (**D**) $DI_{frequency}$. were analyzed. 2-way ANOVA with Tukey multiple comparison post-hoc test (*$p<0.05$). Nine-month-old mice: APP/PS1 neg Panx1 intact (N=5 males, 3 females); APP/PS1 neg Panx1 deleted (N=5 males, 1 females); APP/PS1 Tg+ Panx1 intact (N=3 males, 1 females); APP/PS1 Tg+ Panx1 deleted (N=4 males, 2 females). Twelve-month-old mice: APP/PS1 neg Panx1 intact (N=2 males, 5 females); APP/PS1 neg Panx1 deleted (N=3 males, 5 females); APP/PS1 Tg+ Panx1 intact (N=4 males, 2 females); APP/PS1 Tg+ Panx1 deleted (N=1 males, 2 females).

3.3 EC Panx1 deletion rescues cerebral blood perfusion deficits in 9-month AD mice.

To test whether EC Panx1 deletion rescues decreased cerebral blood flow associated with AD, we imaged cerebral perfusion in 9- and 12-month cohorts using laser speckle contrast imaging. Both 9-month male and female AD mice exhibited decreased cerebral perfusion

compared to controls which was rescued to baseline with deletion of EC Panx12 in female mice and trending to significance in male mice (**Figure 3.5A, B**). Preliminary data from a small cohort of mice at 12-months shows no significant differences in CBF thus far (**Figure 3.5C, D**).

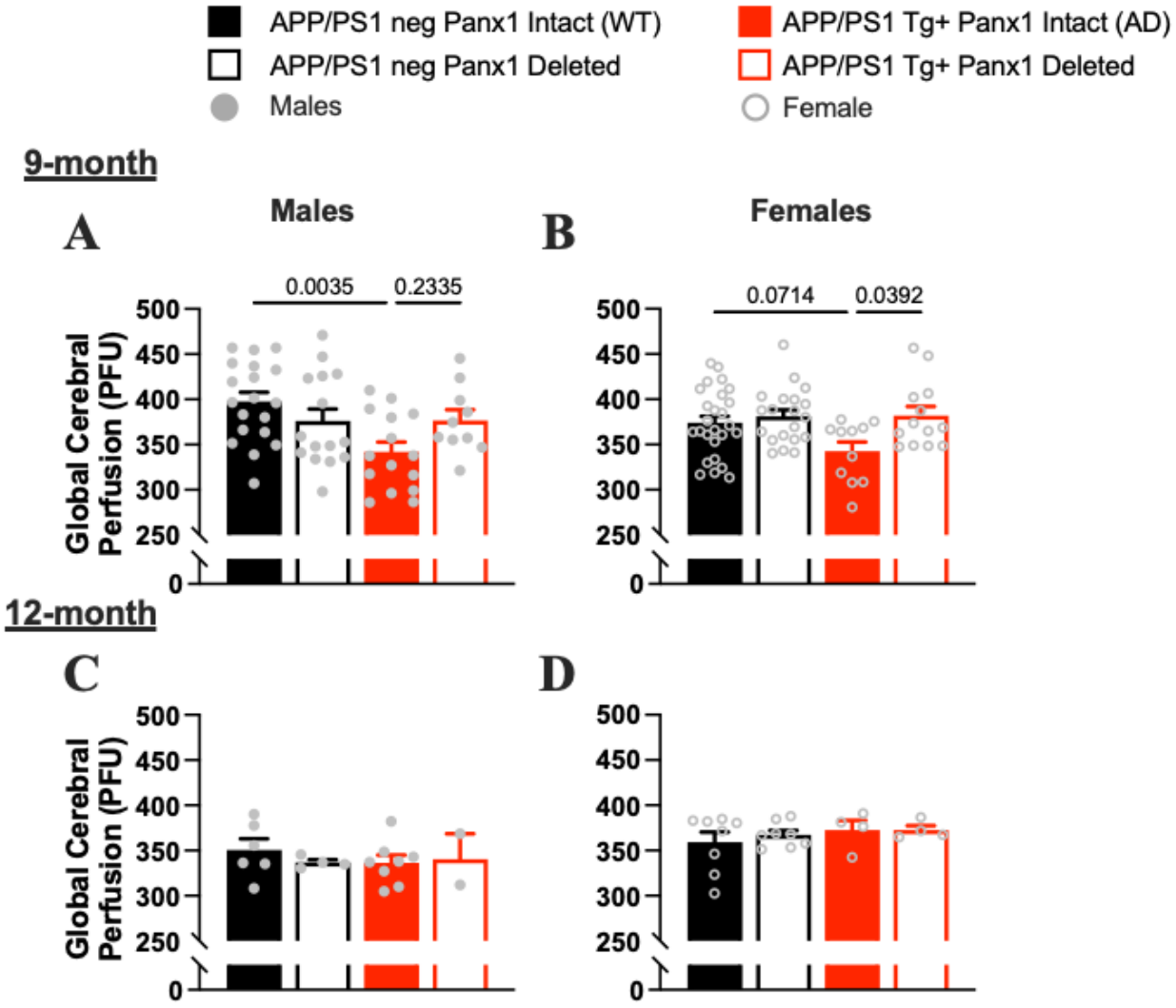

Figure 3.5 EC Panx1 deletion prevents decreased cerebral blood flow in 9-month-old AD mice. Global cerebral vascular perfusion measured by LSCI 9-month (**A**) male and (**B**) female and 12-month (**C**) male and (**D**) female total cerebral perfusion. 2-way ANOVA with Tukey multiple comparison post-hoc test (*p<0.05). 9-month-old mice: APP/PS1 neg Panx1 intact (N= 19 males, 26 females); APP/PS1 neg Panx1 deleted (N=15 males, 20 females); APP/PS1 Tg+ Panx1 intact (N=15 males, 11 females); APP/PS1 Tg+ Panx1 deleted (N=10 males, 19 females). 12-month-old mice: APP/PS1 neg Panx1 intact (N= 6 males, 8 females); APP/PS1 neg Panx1 deleted (N=4 males, 8 females); APP/PS1 Tg+ Panx1 intact (N=8 males, 4 females); APP/PS1 Tg+ Panx1 deleted (N=2 males, 4 females).

3.4 APP/PS1 Tg mice may have increased infiltration of circulating leukocytes.

To examine the effects of EC Panx1 deletion on AD-associated peripheral leukocyte infiltration into the brain, we conducted flow cytometry experiments on isolated leukocytes from harvested brain tissue in 9-month-old mice. Total leukocyte infiltration ($CD45^{high}$ expressing cells) was increased, although not significantly in AD mice compared to control WT mice only in males (**Figure 3.6A**). AD mice exhibited a trend of higher myeloid cell infiltration ($CD11b^{+}$ cells) compared to controls in both sexes (**Figure 3.6B**). No significant differences were found in infiltrated neutrophils ($Ly6G^{+}$ cells; **Figure 3.6C**) or T cells ($CD3^{+}$ cells; **Figure 3.6D**).

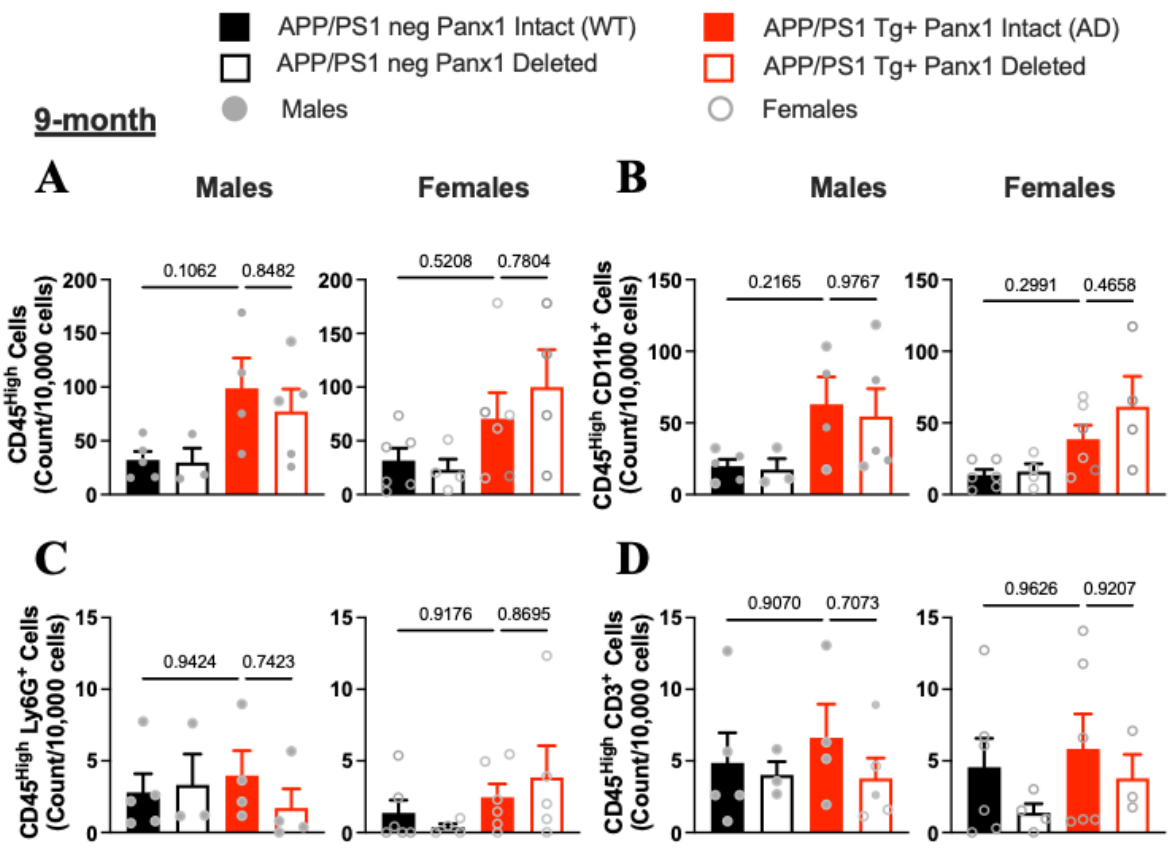

Figure 3.6. 9-month-old AD mice trend to higher infiltration of leukocytes. Infiltrated leukocytes subsets were evaluated, including: (**A**) $CD45^{High}$ for total leukocytes, (**B**) $CD11b^{+}$ for myeloid cells, (**C**) $Ly6G^{+}$ for neutrophils, and (**D**) $CD3^{+}$ for all T-cells. 1-way ANOVA with Tukey multiple comparison post-hoc test (*$p<0.05$). APP/PS1 neg Panx1 intact (N=5 males, 6 females); APP/PS1 neg Panx1 deleted (N=3 males, 4 females); APP/PS1 Tg+ Panx1 intact (N=4 males, 5 females); APP/PS1 Tg+ Panx1 deleted (N=5 males, 4 females).

Chapter 4: Discussion

The present study tested the hypothesis that EC Panx1 mediates AD progression through decreased cerebral perfusion and increased infiltration of leukocytes, which together impairs cognitive function. We evaluated this hypothesis by deleting EC Panx1 in our APP/PS1 Tg^{+} mouse model of AD and studied the effects on behavioral memory, cerebral vascular physiology, and leukocyte cerebral infiltration. We observed: 1) no differences in male memory behavior, but a trend to worse recognition and spatial memory in 9-month-old AD female mice; 2) only spatial memory is improved with deletion of EC Panx1 in APP/PS1 Tg+ mice; 3) decreased cerebrovascular perfusion at 9 months in AD mice is rescued with Panx1 deletion; and 3) no differences in leukocyte infiltration; but a trend of increased leukocytes is present in AD mice. These findings confirm EC Panx1's role in vascular tone and cerebral-vascular perfusion; however, these CBF benefits may not significantly impact AD-associated neuroinflammation or cognitive deficits.

4.1 EC Panx1 deletion does not rescue recognition cognitive function

We observed overall lower discrimination index between 9-month-old male groups, expect in APP/PS1 neg EC Panx1 deleted mice that have a significant increase in the time spent with the novel object in the NOR test and frequency of visiting the moved object in the OLT. (**Figure 3.1, 3.2, 3.4**). Female baseline data in control WT mice demonstrated appropriate discrimination between novel and familiar objects; however, 9-month APP/PS1 neg EC Panx1 deleted female mice exhibited reduced novel object recognition, an effect that was not present in 12-month female mice (**Figure 3.1, 3.2**). These findings suggest an impaired recognition memory in younger healthy female mice with EC Panx1 deletion. These findings were

unexpected since baseline 9-month males showed minimal discrimination between the novel and familiar objects, when a previous NOR study indicates male mice distinguish novelty better than female mice, especially when the object is dissimilar.[37]

A study, measuring different objects with varying size, material, and shape, suggest that mice prefer shiny – metal or glass – material compared to plastic, and male mice prefer larger objects compared to smaller ones.[38] The present study utilized two dissimilar rectangular objects -- a small glass object (novel) and a larger plastic object (familiar). Since the smaller object was used as the novel object, this could have influenced male mice exploration behavior and disinterest in the novel object skewing the results so that baseline mice did not properly discriminate.

The small 12-month-old cohort produced vastly different results than the younger mice. The older group, which has further progression of AD pathology, shows the expected trend of reduced recognition memory in AD mice, suggesting further progression of disease is necessary to observe differences in this mouse model in our hands. This conclusion is further justified by a study conducted in 2022 where experimenters found the 9-month APP/PS1 strain did not exhibit differences in NOR; however, it's important to note they used a 24-hour interval before introducing the novel object whereas the present study used a 20-minute interval.[39] In conclusion, future studies should take into consideration, material and size of objects, age of subject cohorts, and time interveals to minimize innate confounders and maximize disease phenotypic effects.

4.2 EC Panx1 deletion has minimal effects on spatial memory and spatial learning

We observed no differences in latency to goal hole, errors made, and pathlength traveled during training days in 9-month mice, and we only observed that male and female WT mice spent less time in the goal quadrant with no effect of EC Panx1 expression on functional outcome (**Figure 3.3**). 12-month-old mice exhibited similar lack of differences in learning during training trails of the BMz (**Figure 3.3**). Given the small cohort of mice, conclusions cannot be drawn from the 12-month-old BMz data. These results suggest no differences in spatial learning and improved spatial memory in AD mice. The results conflict with previous literature that indicate 9-month APP/PS1 mice exhibit behavioral differences in BMz; however, our data may be under powered to identify difference at this young age.[39]

A review written in 2018 highlights various considerations -- such as maze platform material and proper weak-aversive stimuli use – which are needed for proper function of this assay.[40] In the present study, the surface of the maze was made of a porous absorbent material which could have influenced odor cues aiding AD mice to perform comparably, if not better, to healthy mice. However, in the present study we rotated the platform 90 degrees and sterilized it between trials so this might not be the sole confound. Although the review argues the open-space of the BMz functions as a type of "weak-aversive stimuli" they also urge readers to consider more motivative cues such as noise, because if the environment around the open-space is not aversive enough to elicit a innate hiding response, it will encourage exploration – behavior which would skew healthy mice data to AD-like behavior.[40] Other BMz studies take into consideration a scoring scheme on test day to determine the subject's search strategy -- direct, correctness, serial, or random.[41] Therefore, it is likely non-AD mice utilized a serial search strategy– where they search each hole for the box when it is not found – whereas AD-mice do not search and

remain in the area in a confused state creating the illusion that AD mice had better spatial memory than healthy mice.

OLT data suggest no differences in discrimination between the stationary and displaced object in 9-month-old mice. However, an intriguing effect occurs in the 12-month age group (**Figure 3.4**). These results mimic the previous NOR data in which younger mice displayed no differences in both DI metrics and older mice showed a trend of improved spatial memory in APP/PS1 Tg mice with EC Panx1 deleted. It is important to take into consideration that our OLT data combines male and female data points because of low sample sizes. Therefore, since previous data suggest a clear behavioral sex difference, further testing is necessary before any meaningful conclusions are made. However, if the suggested trend continues with increased sample size, EC Panx1 inhibition in later-stages of AD could have enhancing effects in spatial memory.

4.3 AD associated deficits in global cerebral perfusion are rescued with EC Panx1 deletion

We conclude that AD mice have cerebrovascular hypoperfusion at 9-months of age, a physiological effect that is rescued upon EC Panx1 deletion; however, no differences were found in our preliminary data in 12-month-old mice (**Figure 3.5**). These results underscore previous cerebrovascular dysfunction in AD patients and mice, which affect cognitive function and Aβ plaque deposition.[17,18]

In recent decades, new data illustrates cerebrovascular dysfunction is one of the earliest detectable events in AD progression and has begun to be considered as an underlying factor in disease development; however, no clear mechanistic causes have been presented.[42] Our data, now offers a novel mechanism for such physiological deficits. Since EC Panx1 is a crucial player

in purinergic signaling and vascular function;[31,32] increased Panx1 expression as AD progresses may alter vascular reactivity and decrease global cerebral perfusion. Our 12 month old AD cohort currently shows no differences from baseline; however, its readily accepted that global perfusion decreases with age, so it is unsurprising that the 12-month healthy cohort dropped to AD-like perfusion.[43]

Taken together our data highlights the need for future studies to examine the mechanism by which EC Panx1 regulates CBF and if this has effects on long-term cognitive function.

4.4 EC Panx1 deletion does not alter infiltrated leukocytes in AD

We conclude that leukocytes trend to elevated levels in our AD mouse model at 9 months of age (**Figure 3.6**). The lack of significance in our results were unexpected since inflammation increases as BBB breakdown occurs with AD progression.[17,26] However, EC Panx1 may also regulate activation of resident glial cells to impact neuroinflammation. Overall, larger cohort sizes are necessary to investigate if genotype or sex-specific differences exist in EC Panx1-mediated AD-associated neuroinflammation.

4.5 Clinical Applications

The current study offers a novel mechanism for addressing clinical cerebrovascular deficits in AD patients. A previous study investigating effects of Panx1 deletion in hippocampal brain regions suggests that pyramidal cell have a compensatory effect and more complex arborization allowing for baseline-like excitability.[46] Therefore, EC Panx1 inhibition or CRISPR genetic modification is an intriguing therapy to investigate. If such effects carry over to clinical AD patients it could lighten Aβ deposition, improve blood delivery to tissue, and improve

cognitive function. Effects that could slow the progression of AD and improve patient and family burden.

4.6 Limitations

The present study had various limitations affecting the viability of results. First, the previously explained innate behavioral confounds could have skewed our behavioral data providing unrealistic and non-representative results. Second, various experiments had small sample sizes. This arose from the unpredictability of genotypic outcome when breeding our mice, coupled with the longitudinal ageing process required for appropriate AD progression. These small sample sizes urged us to combine data, and thus produced potentially skewed results or small non-representative sex group data. Future studies will provide additional cohorts to strengthen the power of our studies.

Bibliography

1. Projected Populations and Components of Change. (n.d.). United States Census Bureau. Retrieved January 19, 2025, from https://www.census.gov/data/tables/2023/demo/popproj/2023-summary-tables.html
2. Rajan, K. B., Weuve, J., Barnes, L. L., McAninch, E. A., Wilson, R. S., & Evans, D. A. (2022). Population Estimate of People with Clinical AD and Mild Cognitive Impairment in the United States *(2020–2060)*.
3. Kim, B. (2021). Behavioural and psychological symptoms of dementia in patients with Alzheimer's disease and family caregiver burden: A path analysis.
4. Lanoiselée, H.-M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., Richard, A.-C., Pasquier, F., Rollin-Sillaire, A., Martinaud, O., Quillard-Muraine, M., De La Sayette, V., Boutoleau-Bretonniere, C., Etcharry-Bouyx, F., Chauviré, V., Sarazin, M., Le Ber, I., Epelbaum, S., Jonveaux, T., … collaborators of the CNR-MAJ project. (2017). APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLOS Medicine*, *14*(3), e1002270. https://doi.org/10.1371/journal.pmed.1002270
5. Verghese, P. B., Castellano, J. M., & Holtzman, D. M. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. *The Lancet Neurology*, *10*(3), 241–252. https://doi.org/10.1016/S1474-4422(10)70325-2
6. Paroni, G., Bisceglia, P., & Seripa, D. (2019). Understanding the Amyloid Hypothesis in Alzheimer's Disease. *Journal of Alzheimer's Disease*, *68*(2), 493–510. https://doi.org/10.3233/JAD-180802

7. Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., Krafft, G. A., & Klein, W. L. (1998). Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. USA*.
8. Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules*, *25*(24), 5789. https://doi.org/10.3390/molecules25245789
9. Passeri, E., Elkhoury, K., Morsink, M., Broersen, K., Linder, M., Tamayol, A., Malaplate, C., Yen, F. T., & Arab-Tehrany, E. (2022). Alzheimer's Disease: Treatment Strategies and Their Limitations. *International Journal of Molecular Sciences*, *23*(22), 13954. https://doi.org/10.3390/ijms232213954
10. Jahn, H. (2013). Memory loss in Alzheimer's disease. *Dialogues in Clinical Neuroscience*, *15*(4), 445–454. https://doi.org/10.31887/DCNS.2013.15.4/hjahn
11. Kannappan, B., Gunasekaran, T. I., Te Nijenhuis, J., Gopal, M., Velusami, D., Kothandan, G., Lee, K. H., & for the Alzheimer's Disease Neuroimaging Initiative. (2022). Polygenic score for Alzheimer's disease identifies differential atrophy in hippocampal subfield volumes. *PLOS ONE*, *17*(7), e0270795. https://doi.org/10.1371/journal.pone.0270795
12. Puzzo, D., Gulisano, W., Palmeri, A., & Arancio, O. (2015). Rodent models for Alzheimer's disease drug discovery. *Expert Opinion on Drug Discovery*, *10*(7), 703–711. https://doi.org/10.1517/17460441.2015.1041913
13. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. (1996). Correlative Memory Deficits, Aβ Elevation, and Amyloid Plaques in Transgenic Mice. *Science*, *274*(5284), 99–103. https://doi.org/10.1126/science.274.5284.99

14. Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.-M., Eckman, C., Younkin, S., Hsiao, K., & Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Medicine*, *4*(1), 97–100. https://doi.org/10.1038/nm0198-097

15. Donkin, J. J., Stukas, S., Hirsch-Reinshagen, V., Namjoshi, D., Wilkinson, A., May, S., Chan, J., Fan, J., Collins, J., & Wellington, C. L. (2010). ATP-binding Cassette Transporter A1 Mediates the Beneficial Effects of the Liver X Receptor Agonist GW3965 on Object Recognition Memory and Amyloid Burden in Amyloid Precursor Protein/Presenilin 1 Mice*. *Journal of Biological Chemistry*, *285*(44), 34144–34154. https://doi.org/10.1074/jbc.M110.108100

16. Minkeviciene, R., Ihalainen, J., Malm, T., Matilainen, O., Keksa-Goldsteine, V., Goldsteins, G., Iivonen, H., Leguit, N., Glennon, J., Koistinaho, J., Banerjee, P., & Tanila, H. (2008). Age-related decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice. *Journal of Neurochemistry*, *105*(3), 584–594. https://doi.org/10.1111/j.1471-4159.2007.05147.x

17. Roher, A., Debbins, Malek-Ahmadi, M., Pipe, Maze, Belden, Maarouf, Hunter, Kokjohn, Walker, Kruchowsky, Belohlavek, Beach, Sabbagh, M., Chen, K., Mo, H., & Thiyyagura. (2012). Cerebral blood flow in Alzheimer's disease. *Vascular Health and Risk Management*, 599. https://doi.org/10.2147/VHRM.S34874

18. Mattsson, N., Tosun, D., Insel, P. S., Simonson, A., Jack, C. R., Beckett, L. A., Donohue, M., Jagust, W., Schuff, N., & Weiner, M. W. (2014). Association of brain amyloid-β with cerebral

perfusion and structure in Alzheimer's disease and mild cognitive impairment. *Brain*, *137*(5), 1550–1561. https://doi.org/10.1093/brain/awu043

19. Bracko, O., Cruz Hernández, J. C., Park, L., Nishimura, N., & Schaffer, C. B. (2021). Causes and consequences of baseline cerebral blood flow reductions in Alzheimer's disease. *Journal of Cerebral Blood Flow & Metabolism*, *41*(7), 1501–1516. https://doi.org/10.1177/0271678X20982383
20. Pimentel-Coelho, P. M., Michaud, J.-P., & Rivest, S. (2013). Effects of mild chronic cerebral hypoperfusion and early amyloid pathology on spatial learning and the cellular innate immune response in mice. *Neurobiology of Aging*, *34*(3), 679–693. https://doi.org/10.1016/j.neurobiolaging.2012.06.025
21. Zhai, Y., Yamashita, T., Nakano, Y., Sun, Z., Shang, J., Feng, T., Morihara, R., Fukui, Y., Ohta, Y., Hishikawa, N., & Abe, K. (2016). Chronic Cerebral Hypoperfusion Accelerates Alzheimer's Disease Pathology with Cerebrovascular Remodeling in a Novel Mouse Model. *Journal of Alzheimer's Disease*, *53*(3), 893–905. https://doi.org/10.3233/JAD-160345
22. Parbo, P., Ismail, R., Hansen, K. V., Amidi, A., Mårup, F. H., Gottrup, H., Brændgaard, H., Eriksson, B. O., Eskildsen, S. F., Lund, T. E., Tietze, A., Edison, P., Pavese, N., Stokholm, M. G., Borghammer, P., Hinz, R., Aanerud, J., & Brooks, D. J. (2017). Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. *Brain*, *140*(7), 2002–2011. https://doi.org/10.1093/brain/awx120
23. Salter, M. W., & Stevens, B. (2017). Microglia emerge as central players in brain disease. *Nature Medicine*, *23*(9), 1018–1027. https://doi.org/10.1038/nm.4397
24. Dickson, D. W., Farlo, J., Davies, P., Crystal, H., & Fuld, P. (1988). *A Double-Labeling Immunohistochemical Study OfSenile Plaques*. *132*.

25. Huang, Y., Happonen, K. E., Burrola, P. G., O'Connor, C., Hah, N., Huang, L., Nimmerjahn, A., & Lemke, G. (2021). Microglia use TAM receptors to detect and engulf amyloid β plaques. *Nature Immunology*, *22*(5), 586–594. https://doi.org/10.1038/s41590-021-00913-5

26. Zlokovic, B. V. (2011). Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nature Reviews Neuroscience*, *12*(12), 723–738. https://doi.org/10.1038/nrn3114

27. Unger, M. S., Li, E., Scharnagl, L., Poupardin, R., Altendorfer, B., Mrowetz, H., Hutter-Paier, B., Weiger, T. M., Heneka, M. T., Attems, J., & Aigner, L. (2020). CD8+ T-cells infiltrate Alzheimer's disease brains and regulate neuronal- and synapse-related gene expression in APP-PS1 transgenic mice. *Brain, Behavior, and Immunity*, *89*, 67–86. https://doi.org/10.1016/j.bbi.2020.05.070

28. Nielsen, H. M., Mulder, S. D., Beliën, J. A. M., Musters, R. J. P., Eikelenboom, P., & Veerhuis, R. (2010). Astrocytic Aβ1-42 uptake is determined by Aβ-aggregation state and the presence of amyloid-associated proteins. *Glia*, *58*(10), 1235–1246. https://doi.org/10.1002/glia.21004

29. Panchin*, Y., Kelmanson, I., Matz, M., Lukyanov, K., Usman, N., & Lukyanov, S. (1997). A ubiquitous family of putative gap junction molecules Yuri Panchin*, Ilya Kelmanson*, Mikhail Matz†, Konstantin Lukyanov†, Natalia Usman† and Sergey † Lukyanov. *Nature*, *387*(6632), 489–493. https://doi.org/10.1038/387489a0

30. Bao, L., Locovei, S., & Dahl, G. (2004). Pannexin membrane channels are mechanosensitive conduits for ATP. *FEBS Letters*, *572*(1–3), 65–68. https://doi.org/10.1016/j.febslet.2004.07.009

31. Vogt, A., Hormuzdi, S. G., & Monyer, H. (2005). Pannexin1 and Pannexin2 expression in the developing and mature rat brain. *Molecular Brain Research*, *141*(1), 113–120. https://doi.org/10.1016/j.molbrainres.2005.08.002

32. Lohman, A. W., Billaud, M., Straub, A. C., Johnstone, S. R., Best, A. K., Lee, M., Barr, K., Penuela, S., Laird, D. W., & Isakson, B. E. (2012). Expression of Pannexin Isoforms in the Systemic Murine Arterial Network. *Journal of Vascular Research*, *49*(5), 405–416. https://doi.org/10.1159/000338758

33. Flores-Muñoz, C., Gómez, B., Mery, E., Mujica, P., Gajardo, I., Córdova, C., Lopez-Espíndola, D., Durán-Aniotz, C., Hetz, C., Muñoz, P., Gonzalez-Jamett, A. M., & Ardiles, Á. O. (2020). Acute Pannexin 1 Blockade Mitigates Early Synaptic Plasticity Defects in a Mouse Model of Alzheimer's Disease. *Frontiers in Cellular Neuroscience*, *14*, 46. https://doi.org/10.3389/fncel.2020.00046

34. Koval, M., Cwiek, A., Carr, T., Good, M. E., Lohman, A. W., & Isakson, B. E. (2021). Pannexin 1 as a driver of inflammation and ischemia–reperfusion injury. *Purinergic Signalling*, *17*(4), 521–531. https://doi.org/10.1007/s11302-021-09804-8

35. Chekeni, F. B., Elliott, M. R., Sandilos, J. K., Walk, S. F., Kinchen, J. M., Lazarowski, E. R., Armstrong, A. J., Penuela, S., Laird, D. W., Salvesen, G. S., Isakson, B. E., Bayliss, D. A., & Ravichandran, K. S. (2010). Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature*, *467*(7317), 863–867. https://doi.org/10.1038/nature09413

36. Good, M. E., Eucker, S. A., Li, J., Bacon, H. M., Lang, S. M., Butcher, J. T., Johnson, T. J., Gaykema, R. P., Patel, M. K., Zuo, Z., & Isakson, B. E. (2018). Endothelial cell Pannexin1

modulates severity of ischemic stroke by regulating cerebral inflammation and myogenic tone. *JCI Insight*, *3*(6), e96272. https://doi.org/10.1172/jci.insight.96272

37. Bettis, T., & Jacobs, L. F. (2012). Sex differences in object recognition are modulated by object similarity. *Behavioural Brain Research*, *233*(2), 288–292. https://doi.org/10.1016/j.bbr.2012.04.028
38. Tropea, M. R., Sanfilippo, G., Giannino, F., Davì, V., Gulisano, W., & Puzzo, D. (2022). Innate Preferences Affect Results of Object Recognition Task in Wild Type and Alzheimer's Disease Mouse Models. *Journal of Alzheimer's Disease*, *85*(3), 1343–1356. https://doi.org/10.3233/JAD-215209
39. Hulshof, L. A., Frajmund, L. A., Van Nuijs, D., Van Der Heijden, D. C. N., Middeldorp, J., & Hol, E. M. (2022). Both male and female APPswe/PSEN1dE9 mice are impaired in spatial memory and cognitive flexibility at 9 months of age. *Neurobiology of Aging*, *113*, 28–38. https://doi.org/10.1016/j.neurobiolaging.2021.12.009
40. Gawel, K., Gibula, E., Marszalek-Grabska, M., Filarowska, J., & Kotlinska, J. H. (2019). Assessment of spatial learning and memory in the Barnes maze task in rodents—Methodological consideration. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *392*(1), 1–18. https://doi.org/10.1007/s00210-018-1589-y
41. Rodríguez Peris, L., Scheuber, M. I., Shan, H., Braun, M., & Schwab, M. E. (2024). Barnes maze test for spatial memory: A new, sensitive scoring system for mouse search strategies. *Behavioural Brain Research*, *458*, 114730. https://doi.org/10.1016/j.bbr.2023.114730
42. Fang, X., Fan, F., & Roman, R. J. (2024). Cerebrovascular Dysfunction in Alzheimer's Disease and Transgenic Rodent Models. *Journal of Experimental Neurology*, *5*(2), 42–64. https://doi.org/10.33696/Neurol.5.087

43. Braz, I. D., & Fisher, J. P. (2016). The impact of age on cerebral perfusion, oxygenation and metabolism during exercise in humans. *The Journal of Physiology*, *594*(16), 4471–4483. https://doi.org/10.1113/JP271081

44. Malech, H. L., DeLeo, F. R., & Quinn, M. T. (2014). The Role of Neutrophils in the Immune System: An Overview. In M. T. Quinn & F. R. DeLeo (Eds.), *Neutrophil Methods and Protocols* (Vol. 1124, pp. 3–10). Humana Press. https://doi.org/10.1007/978-1-62703-845-4_1

45. Sun, L., Su, Y., Jiao, A., Wang, X., & Zhang, B. (2023). T cells in health and disease. *Signal Transduction and Targeted Therapy*, *8*(1), 235. https://doi.org/10.1038/s41392-023-01471-y

46. Flores-Muñoz, C., García-Rojas, F., Pérez, M. A., Santander, O., Mery, E., Ordenes, S., Illanes-González, J., López-Espíndola, D., González-Jamett, A. M., Fuenzalida, M., Martínez, A. D., & Ardiles, Á. O. (2022). The Long-Term Pannexin 1 Ablation Produces Structural and Functional Modifications in Hippocampal Neurons. *Cells*, *11*(22), 3646. https://doi.org/10.3390/cells11223646

47. Pauwels, K., Williams, T. L., & Morris, K. L. (2012). Structural basis for increased toxicity of pathological Aβ42:Aβ40 ratios in Alzheimer disease. *Journal of Biological Chemistry, 287*(8), 5639–5649. https://doi.org/10.1074/jbc.M111.291229

48. Dobson, R., Patterson, K., Malik, R., Mandal, U., Asif, H., Humphreys, R., Payne, M., E, O. C., Huzzey, L., Clare, A., Green, K., Morton, M., Sohrabi, C., Singh, N., Pasupathy, A., et al. (2024). Eligibility for anti-amyloid treatment: Preparing for disease-modifying therapies for Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 95*(9), 796–803. https://doi.org/PMC11347253

49. Pittock, R. R., Aakre, J. A., Castillo, A. M., Ramanan, V. K., Kremers, W. K., Jack, C. R. Jr., Vemuri, P., Lowe, V. J., Knopman, D. S., Petersen, R. C., Graff-Radford, J., & Vassilaki, M. (2023). Eligibility for anti-amyloid treatment in a population-based study of cognitive aging. *Neurology, 101*(19), e1837–e1849. https://doi.org/PMC10663008

50. Scheffer, S., Hermkens, D. M. A., van der Weerd, L., de Vries, H. E., & Daemen, M. J. A. P. (2021). Vascular hypothesis of Alzheimer disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 41*(4), 1265–1283. https://doi.org/10.1161/ATVBAHA.120.311911

51. Tomàs-Gracia, M., Mauro, A. K., Duffy, C. K., Dai, E. Y., Shahab, G., Medina, C. B., Ravichandran, K. S., Isakson, B. E., & Good, M. E. (2025). Endothelial cell pannexin1 overexpression impairs ischemic stroke outcome in a sex-dependent manner. bioRxiv. https://doi.org/10.1101/2025.02.07.636909

52. DiSabato, D. J., Quan, N., & Godbout, J. P. (2016). Neuroinflammation: The devil is in the details. *Journal of Neurochemistry*, *139*(S2), 136–153. https://doi.org/10.1111/jnc.13607