EXPLORING ALZHEIMER'S DISEASE: THE INTERACTION BETWEEN INCREASED Aß AND LARGE ARTERY STIFFNESS ON COGNITIVE AND CEREBROVASCULAR FUNCTION AND STRUCTURE

by

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Primary Thesis Advisor

Alzheimer's disease (AD) is a type of dementia characterized by loss of cognitive function and build-up of amyloid-ß (Aß) plaques. Aging and cardiovascular decline, indicated by increased arterial stiffness, are primary AD risk factors. I aimed to identify if modeling large artery stiffness (LAS) in the context of AD at young age mimics the impairments in cognitive and cerebrovascular function seen in old age. I hypothesized that LAS in conjunction with AD exacerbates cerebral microvascular and cognitive impairment compared to AD alone. By crossing elastin haploinsufficient (Eln HET) and amyloid precursor protein knock-in (NLGF) animals, we generated a mouse model of LAS and AD. We used young (6 m) male and female mice. Cognition was assessed by novel object recognition, nest building, and rotarod tests. Cerebrovascular function was measured in posterior cerebral arteries using pressure myography. To assess LAS, aortas were cryosectioned and stained to measure elastin, collagen, and wall thickness. Our model of LAS and AD shows no effect on cognition or cerebrovascular function. There were no main or interaction effects of Aß and LAS on nest building, rotarod, or novel object recognition tests, nor for wall thickness, collagen composition, or vasoreactivity to acetylcholine. NLGF x Eln HET animals displayed lower vasoreactivity to sodium nitroprusside, an endothelium-independent vasodilator ($p < 0.05$), suggesting endothelium-independent dilation

may be related to arterial stiffness. Data suggest that at a young age, LAS and Aß do not significantly impact cognition or cerebrovascular function, and that age is a primary contributor to these issues in AD. The insights from this study will aid in understanding how the cardiovascular system contributes to the development of AD.

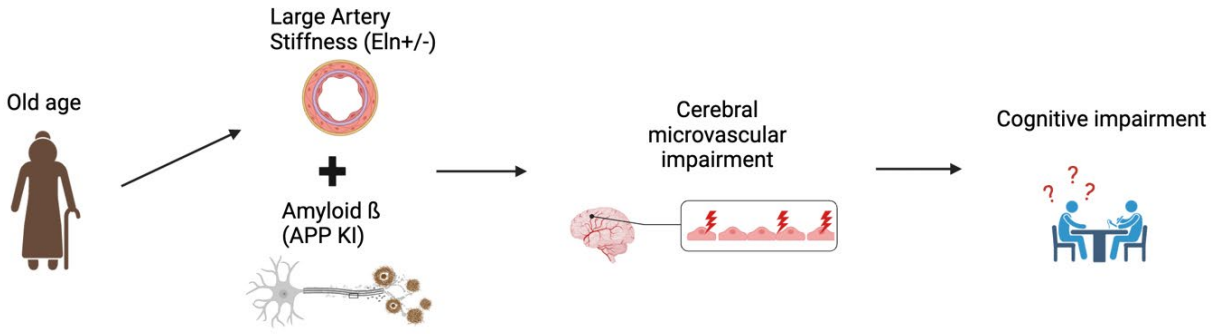

Figure 1. Hypothesized mechanism of large artery stiffness & Aß leading to cerebrovascular and cognitive impairment

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Several funding sources helped me complete this project: the CHC Mentored Research Program, The Human Physiology Research Stipend, and Hui Research Scholars Program, and I am grateful for all of them.

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Introduction

Cognitive Function

Alzheimer's Disease (AD) is associated with cognitive impairments, most often affecting one's memory. In mild AD, signs and symptoms include minor memory loss, poor judgment, misplacing objects, mood changes such as anxiety and aggression, and difficulty with "normal" or daily tasks. As the disease progresses, memory loss worsens, along with organizational skills, behavior, and sleep. Severe AD patients have great difficulties with communication, motor skills, physical activities, and memory loss.[1] Cognitive function declines with age, and large artery stiffness is related to cognitive impairment among both elders and AD patients.[2] It is predicted that cognitive decline due to aging generally begins in all individuals between the ages of 50 to 70 years. Symptoms of cognitive decline due to AD generally begin between the ages of 60 to 65 years, and gradually deteriorate due to the neurodegenerative nature of the disease. [3]

While AD and dementia are often mentioned in the same context, there are important distinctions between the two. Dementia refers to a general term for symptoms that include memory decline and reasoning skills due to damage to brain cells. AD is a specific disease with degenerative dementia symptoms due to marked and complicated brain changes from cell damage. Age is the greatest risk factor for both conditions.[4]

Large Artery Stiffness & Aging

Aging is closely tied to cardiovascular dysfunction, which is a risk factor for the development of AD.[5]

Younger and healthier arteries are compliant and elastic. This elasticity allows them to accommodate the heart's pumping action and maintain a constant pressure gradient.[6] With aging, there is an increase in stiffness of the large arteries such as the aorta, which carries blood from the heart.[7] Large artery stiffness (LAS) refers to the resistance to deformation of a normally elastic arterial wall. Large arteries can grow stiff due to several diseases, such as hypertension and diabetes mellitus. As one ages, the elastin protein, an integral component of walls of large arteries diminishes and fragments. Furthermore, collagen crosslinking increases, which increases large artery stiffness.[8]

Stiffer large arteries are unable to reduce pulse pressure, which is the difference between systolic and diastolic blood pressure. Increased pulse pressure can change arterial structure due to increased mechanical strain on the smooth muscle and endothelial cells that comprise an artery. To combat this mechanical strain, smooth muscle cells stimulate collagen production to protect the arterial wall, which in turn further elevates arterial stiffness.[9] To reach the brain, blood flows from larger arteries to smaller vessels, also known as the microvasculature. Due to arterial stiffness, the microvasculature, which consists of arterioles and capillaries, thus receives a high pulsatile pressure, which they are ill-equipped to accommodate. Thus, the onset of LAS elevates the risk of damage to the microvasculature.[10]

The implications of LAS on human health can be quite severe, ranging from increased blood pressure to organ damage to an increased cardiovascular disease mortality rate.[11] In elderly patients, a relationship is seen between increased LAS, dementia, and AD.[12] When the brain lacks proper regulation of blood pressure, the associated damage to the microvasculature inhibits cerebral blood flow and increases the permeability of the blood-brain barrier. This damage can

lead to neuroinflammation, neurodegeneration, and cognitive decline, which are all characteristics associated with AD.[13]

Amyloid-ß

Amyloid-ß (Aß) is a peptide that aggregates to form plaques in the brain and blood vessels during AD. Aß is cleaved from the amyloid precursor protein (APP), which is produced by neurons and endothelial cells.[14] Physiologically, the expression of APP is thought to contribute beneficially towards neuron survival, neuronal repair, and neural stem cell development.[15] APP undergoes successive cleavages by α-, ß-, and γ-secretases, which cut the peptide into fragments of 38 to 43 amino acids until the final forms of Aß are created.[16] Depending on the site of cleavage, a 40 or 42-residue protein is formed; either Aß40 or Aß42. The ratio of Aß40 to Aß42 has been implicated in AD. Furthermore, elevated levels of Aß42 are linked to greater AD risk, and interventions reducing Aß42 have shown a reduction in AD symptoms.[17]

The presence of Aß plaques and neurofibrillary tangles are well-documented and key features of AD.[18] These plaques begin forming in AD individuals around 40-50 years of age, although other cognitive and physiological indicators of AD may not manifest until 15 to 20 years later.[19] Despite substantial research in the area, it is still unknown whether AD causes Aß accumulation, or whether Aß accumulation triggers AD. The interactions between Aß and other proteins, such as tau, are largely unknown. Inadequate clearance, as well as reduced integrity of the blood-brain barrier, contributes to the buildup of Aß in the brain.[20] Aß plaques are primarily found between neurons.

Aß is also present in the cerebral vasculature. The accumulation of Aß creates plaques that coat vascular walls.[21] Aß impairs endothelial cells in the blood vessels and causes

constriction. Specifically, a soluble form of Aß either constricts cerebral arteries or inhibits dilation of endothelium-dependent dilators through a yet unknown mechanism.[22] Some hypotheses include the production of reactive oxygen species, increased calcium activity within cells, or diminished availability of endothelial nitric oxide.[23] Aß's toxicity triggers apoptosis of vascular cells and stimulates inflammatory pathways.[24]

Animal Model

In my research, I worked with a mouse model of arterial stiffness and AD. The study mice were generated by crossing elastin haploinsufficient (Eln HET) mice with APP knock-in mice (NLGF). The Eln HET mouse used to generate the mice used in this study has stiffer large arteries when compared to a wild-type mouse, (Eln WT), as shown by previous studies in the lab.[25] While complete elastin knockout is fatal, elastin haploinsufficiency changes arterial wall development by thinning the elastic laminae and smooth muscle cell layers and leads to arteries containing less elastin, thus contributing to LAS.[26]

The NLGF mouse exhibit Aß accumulation, a condition absent in wild-type (WT) mice. These NLGF mice have an increase in APP due to the insertion of a humanized Aß region on the APP gene construct.[27] This humanized region contains the APP K670_M671delinsNL (Swedish), APP I716F (Iberian), and APP E693G (Arctic) mutations. The Swedish mutation increases total Aß production. The Iberian mutation promotes Aß accumulation via oligomerization. The Arctic mutation reduces Aß degradation. NLGF mice display cognitive and memory impairment and amyloidosis. The mice have Aß deposits beginning at 2 months with maximum saturation at 7 months.[28] Cognitive function in the NLGF model was measured using the Morris Water Maze test and significant memory impairment was observed.[29]

By crossing Eln HET mice with NLGF mice, I can study the impact of LAS on AD-related cognitive and cerebrovascular impairment without needing to age the mice. Examining young mice (aged 6 months) that exhibit AD conditions clarifies the interplay between Aß and large artery stiffness without aging, which can add confounding factors, such as overall physical degeneration, and risk for age-related diseases that are unrelated to AD.

Gap in Knowledge

There is not yet insight into the specific interactions between LAS and Aß, nor their joint effect on cerebrovascular and cognitive dysfunction. Over the next 25 years, the number of Americans aged 65 and older is projected to increase by 24 million, and cardiovascular disease risk is expected to rise by 39%.[30, 31] The insights from this study will aid in understanding how to physiologically target future interventions against AD at a time when the primary risk factors for AD are rapidly increasing.

Methods

To complete these studies, I worked with a mouse model of arterial stiffness and Alzheimer's disease (AD). We must use mice because there is no current alternative to live models for this research. I crossed Eln HET mice with NLGF mice to study the impact of large artery stiffness on AD-related cognitive and cerebrovascular impairment without aging the mice. Below is a description of the tests I conducted on these mice:

Animals & Tissues

Male/female Eln HET x NLGF (NxE HET) and male/female Eln WT x NLGF (NxE WT) were used in this study. Mice were be housed in the University of Oregon animal care facilities and were maintained on a standard chow diet with ad libitum access to food and water. Posterior cerebral arteries were used to conduct ex-vivo analysis. The aortas were saved for cryosectioning and staining to quantify aortal wall thickness, and elastin and collagen composition. All protocols were approved by the University of Oregon IACUC.

Novel Object Recognition Test

The novel object recognition (NOR) task is a commonly used test of memory. During this cognitive test, a mouse is first given two similar objects. Later, in a second session, one of the objects is replaced with a novel object. The time the mouse takes to explore the new object is a measure of recognition memory.[32]

The NOR task is a five-day test. The animals were habituated in the testing room for an hour during the first three days of the test. After each one-hour habituation period, the animals were held for one minute by the person running the experiment to reduce stress. On the fourth day, the mice had a one-hour open field session. Then, the mice were placed in the testing arena

for ten minutes. On the fifth day, the mice were placed in the arena with a randomized combination of either two Lego objects or two Pyrex glass bottles filled with gravel and taped to the bottom of the arena. The mice were released into the arena for ten minutes and recorded via video. Then, using randomization, we decided whether the novel object (the opposite of the object that the mouse was initially placed with) would be placed on the left or right side of the arena. After a two-hour waiting period, the mice were placed in the arena with one novel and one familiar object for ten minutes. This session was also video recorded to assess the amount of time the mouse spent with the familiar and novel objects, respectively.

Data was collected using EthoVison XT (Noldus, Leesburg,VA), which tracks the nose point of a mouse from a video file. Mouse exploration was defined as an animal having its nose point within 2 centimeters of the object. If the mouse spent 50% or more of their time with the novel object, its cognition was considered to be unimpaired. If the mouse spent equal time with both objects, or less than 50% of the time with the novel object, then the animal was considered to be cognitively impaired. The latter outcome indicates that the mice do not remember which of the objects is novel, as mice are expected to spend more time with a novel object.

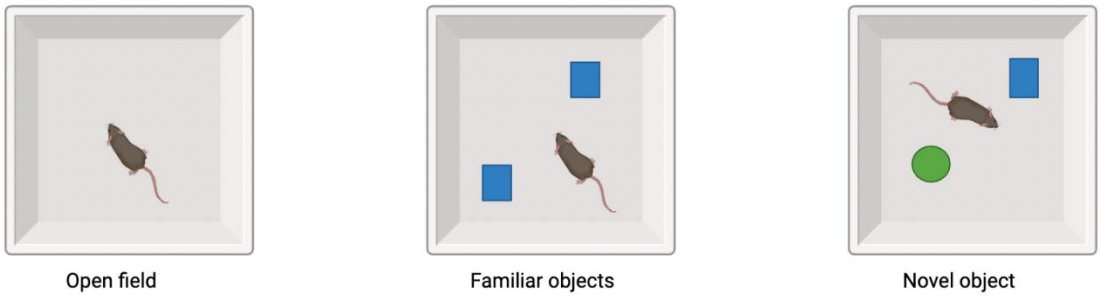

Figure 1. Novel object recognition test setup schematic. Figure created with BioRender.

Nest Building

Nest building is a test of instinctual behavior, and was used to assess cognitive function in the mice. The animals were housed overnight in a new cage, with condensed cotton nestlet. The next day, I scored the nests on a scale of 0 to 5 based on structural characteristics.

The scoring guidelines were:

0: nestlet was untouched.

1: nestlet had been moved, but without significant tearing;

2: significant tearing without formation of a nest;

3: significant tearing, partial nest, or nest was in the corner of the cage;

4: significant tearing, with a nest in the corner of the cage;

5: significant tearing, a nest in the cage corner, and a high bowl-like nest[33]

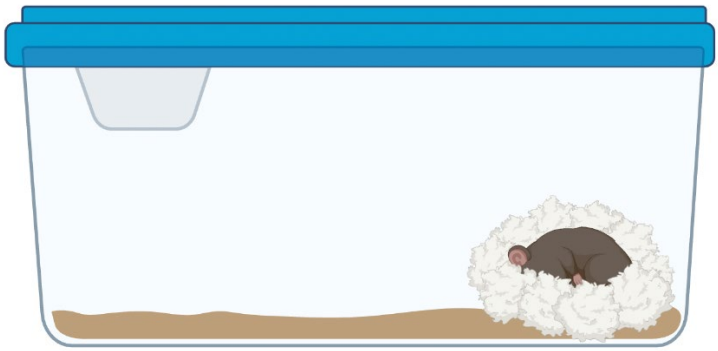

Figure 2. Fully formed nest in the corner of the cage. Figure created with BioRender.

Accelerated Rotarod Test

The rotarod test was used to assess motor coordination. The rotarod machine (47650 Rotarod NG, Ugo Basile, Gemonio, Italy) consists of a rotating rod, which gradually accelerates. The mice's ability to stay on the rotarod was measured. To reduce stress, mice were habituated to the testing room in individual, fresh cages with a white noise background 1 hour before the experiment. The experiment took place over two days.

On the first day, mice were placed on the rotarod machine, rotating at 4 revolutions per minute (rpm) for 90 seconds. If the mouse fell off of the rod, it was placed onto the rod until it could complete the 90-second trial. On the second day, the mice were placed on the rotarod as it accelerated from 4 rpm to 40 rpm over a 5-minute window. Mice ran on the rod until they fell, or until 5 minutes had passed. The time during which the mice stayed on the rod was measured. Three trials, spaced 10 minutes apart, were conducted.

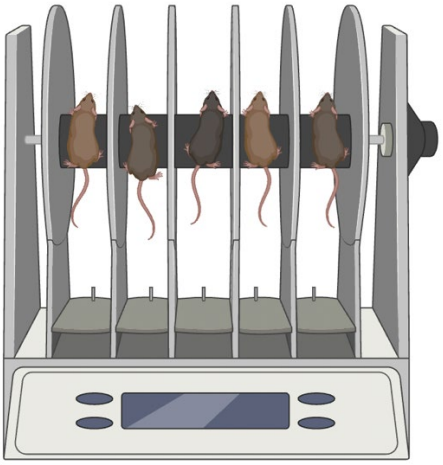

Figure 3. Rotarod apparatus. Figure created with BioRender.

Pulse Wave Velocity

Aortic stiffness was measured using pulse wave velocity (PWV). Mice were anesthetized with 3% isoflurane in 100% oxygen, then placed in a supine position on a temperature-controlled surgical pad at 37°C, with limbs restrained by tape. The paws were coated in electrode gel (IndusIndustries, Webster, TX, USA), and placed on electrode pads to record the ECG during the procedure. Ultrasound gel was applied to the thorax and abdomen along with two Doppler Transducers positioned to record the aortic arch and abdominal aorta.

After recording 3 ~10 s segments of the Doppler signal, the distance between the two probes was measured. To analyze the PWV, the Doppler Signal Processing Workstation program (Indus Industries, Webster, TX, USA), was used. Two blinded researchers analyzed each file independently and inter-observation confidence intervals were measured. Those over 15% were reanalyzed by a third researcher. The PWV was calculated by dividing the distance between the two probes by ejection time, which is the time taken for blood to travel between the aortic arch and abdominal aorta. [34]

Cerebral Vascular Function

Posterior cerebral arteries (PCAs) were collected for pressure myography studies to assess cerebral vascular function. Dose responses to acetylcholine (ACh) and sodium nitroprusside (SNP) were used to determine endothelium-dependent and endothelium-independent dilation, respectively. Before the dose responses, PCAs were excised and placed in myograph chambers (DMT Inc., Denmark) with physiological salt solution 145 mM NaCl, 4.7 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 mL BSA, pH 7.4 at 37°C, cannulated onto glass micropipettes, and secured with nylon (11-0) sutures.[35] The arteries were warmed to 37°C, pressurized to 68 mmHg, and allowed to equilibrate for 20 minutes before responses began.

Both ACh and SNP are vasodilators. ACh dilates vessels by activating nitric oxide synthase, which produces nitric oxide from the endothelium. Dilation via ACh is used to assess endothelium-dependent dilation (EDD).[36] ACh was administered to the vessel at doses of increasing concentration, from 10^{-9} to 10^{-4} M after preconstriction with 2 μM phenylephrine (PE). Following the ACh dose response, vessels were incubated with N omega-Nitro-L-arginine methyl ester hydrochloride (L-NAME), 0.1 mm for 30 minutes, which inhibited endothelial

nitric oxide synthase (eNOS). ACh was administered at the same dosage as before to the vessels after L-NAME incubation to measure EDD post-eNOS inhibition, thereby gaining insight into the contribution of nitric oxide to dilation. Dilation via SNP was used to assess endothelium-independent dilation, as it acts by directly relaxing vascular smooth muscle.[37] SNP was administered in doses with increasing concentration, from 10^{-10} to 10^{-4} M after preconstriction with 2 μM of PE.

Arterial Remodeling

Upon study days, aortas were dissected and a portion of the descending aorta just under the arch was preserved in OCT. To assess structural changes of the ECM, these preserved aortas were cut to 8 μm by cryosectioning and stained via immunohistochemistry for prominent ECM proteins collagen and elastin. Verhoeff van Gieson (VVG) staining was used to assess the elastin composition of the aortas. Trichrome staining was used to quantify the amount of collagen in the aortas, using ImageJ software. The wall thickness of the arteries was measured, also by using ImageJ.

Statistical Analyses

All data was checked for sex differences using an ANOVA. Since no sex differences were found, genotype groups were combined.

For the nest building and rotarod tests, a two-way ANOVA was used to determine differences between genotype groups. Cognitive function using the NOR test was analyzed by calculating the discrimination index. The discrimination index can be calculated by:

$$\frac{(time\ spent\ with\ the\ novel\ object - \ time\ spent\ with\ the\ familiar\ object)}{time\ spent\ with\ the\ novel\ object + time\ spent\ with\ the\ familiar\ object}$$

A greater discrimination index indicates that the mouse spent more time with the novel object, and implies less memory impairment. A negative discrimination index means that more time was spent with the familiar object, a sign of impaired memory. A two-way ANOVA was used to determine differences in object exploration between genotype groups during the open field and testing portion of the novel object recognition test, as well as the discrimination index between groups.

Differences in PWV between genotype groups were also analyzed using a two-way ANOVA. A repeated measures two-way ANOVA was conducted to analyze the dose responses to ACh and SNP. If ANOVA results were significant, a post-hoc Tukey's multiple comparisons test was used to determine group differences.

Wall thickness and collagen composition were analyzed using an unpaired two-tailed t-test to test for differences in the NxE HET and WT groups, as the group size for Eln HET and WT animals was $n = 1$, disqualifying an ANOVA.

Significance was set to $p < 0.05$.

Results

Nest Building, Rotarod, Pulse Wave Velocity Tests:

Aß and LAS did not impact innate behavior or motor coordination, but did influence PWV.

Nest Building

There was no interaction effect between NLGF and Eln genotypes for nest-building ($p = 0.48$). Moreover, there were no main effects of NLGF and Eln on nest-building activity ($p = 0.12$ and $p = 0.84$, respectively) (**Figure 1A**).

Accelerating Rotarod Test

There was no interaction effect between NLGF and Eln genotypes for the rotarod test (**Figure 1B**, $p = 0.92$). There was no main effect of NLGF ($p = 0.73$) or Eln genotype ($p = 0.85$) on rotarod performance (**Figure 1B**).

Pulse Wave Velocity

A main effect of LAS on PWV was identified ($p = 0.0018$), where Eln HET mice had lower PWV. Further analysis revealed significantly lower PWV in NxE HET mice compared to NxE WT mice (**Figure 1C**, $p = 0.0063$).

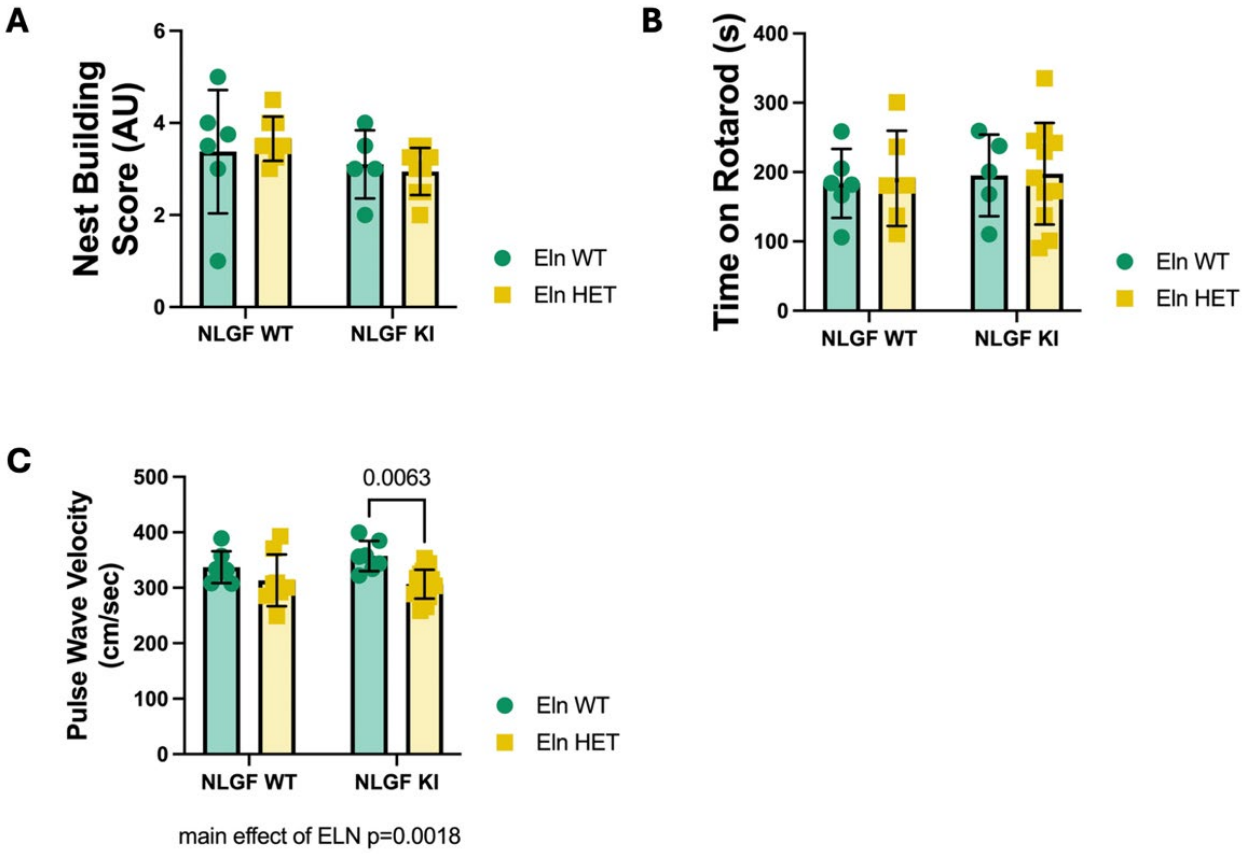

Figure 5**.** Nest Building, Accelerated Rotarod, and Pulse Wave Velocity Results. No significant difference in nest-building scores was found between Eln WT (n = 6), Eln HET (n = 8), NxE WT (n = 5), or NxE HET (n = 9). No significant difference in Rotarod timing was found between Eln WT, Eln HET, NxE WT or NxE HET. Analysis indicated significantly lower pulse wave velocity in the NLGF x Eln HET compared to NLGF x Eln WT) ($p = 0.0063$), and a main effect of Eln ($p = 0.0018$). Values are mean ± SEM.

Novel Object Recognition Test: Discrimination Index, Open Field, Object Recognition

Discrimination Index

There were no main or interaction effects of genotype on the discrimination index (**Figure 6A**, $p > 0.05$).

Open Field

There was no significant interaction or main effect of NLGF and Eln genotypes during the Open Field portion (all $p > 0.05$), indicating that there was no bias for mouse anxiety in the arena (**Figure 6B**).

Object Recognition

During the novel object portion, there was no interaction effect for the genotypes on novel object memory outcomes ($p = 0.62$). Additionally, there were no main effects of LAS ($p = 0.18$) or Aß ($p = 0.31$) (**Figure 6C**).

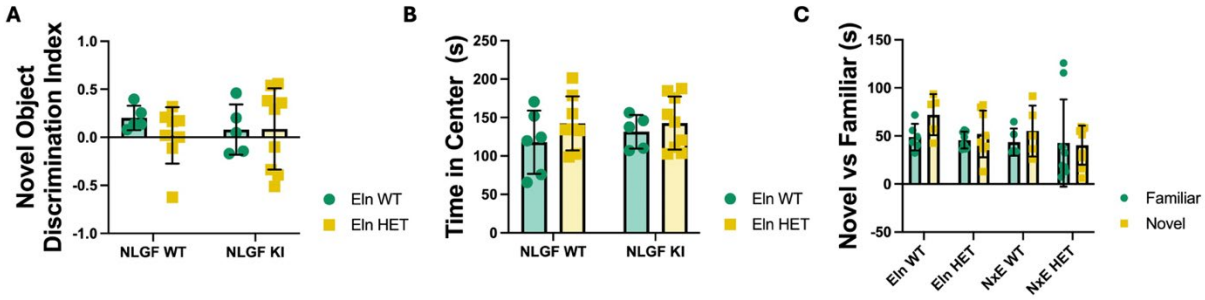

Figure 6. Novel Object Recognition Test: Discrimination Index, Open Field, Object Recognition Results. There were no significant differences in discrimination index, open field, or novel object recognition between Eln WT (n = 6), Eln HET (n = 8), NxE WT (n = 5), or NxE HET (n = 9) (all $p > 0.05$). Values are mean ± SEM.

Arterial Structure: Wall Thickness & Collagen

Wall Thickness

Wall thickness was measured between NxE KI and NxE WT genotypes using a two-tailed unpaired t-test. There was no interaction effect of LAS and Aß on aortic wall thickness (**Figure 7**, p=0.16).

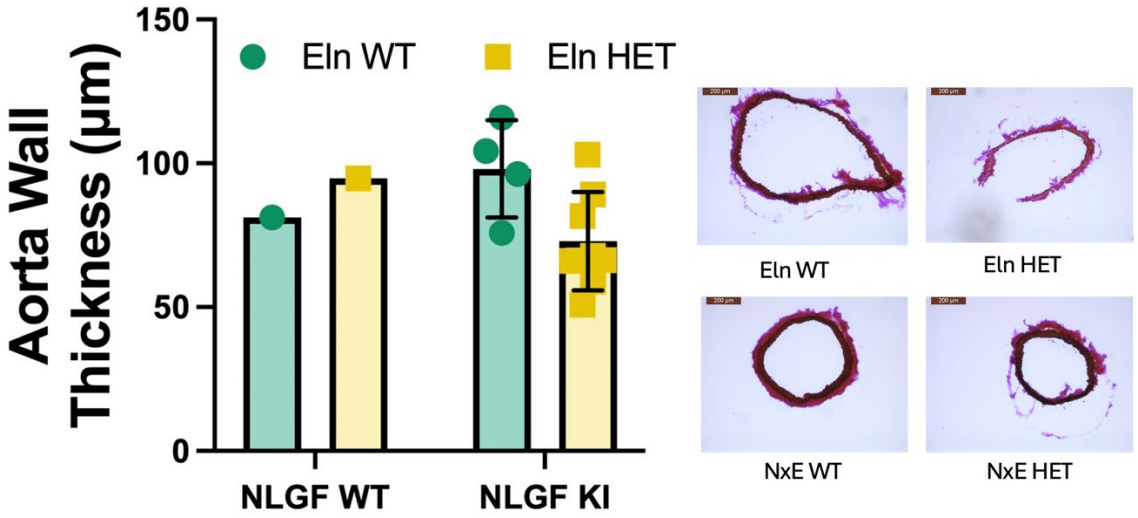

Figure 7. Aortic Wall Thickness. There were no significant differences in aortic wall thickness between Eln WT (n = 1), Eln HET (n = 1), NxE WT (n = 4), or NxE HET (n = 8) (all $p > 0.05$). Values are mean ± SEM.

Collagen

There was no significant difference in aortic collagen cross sectional area (**Figure 8A**) nor collagen percent area (**Figure 8B**) after comparing NxE KI and NxE WT groups using a two-tailed unpaired t-test ($p > 0.05$).

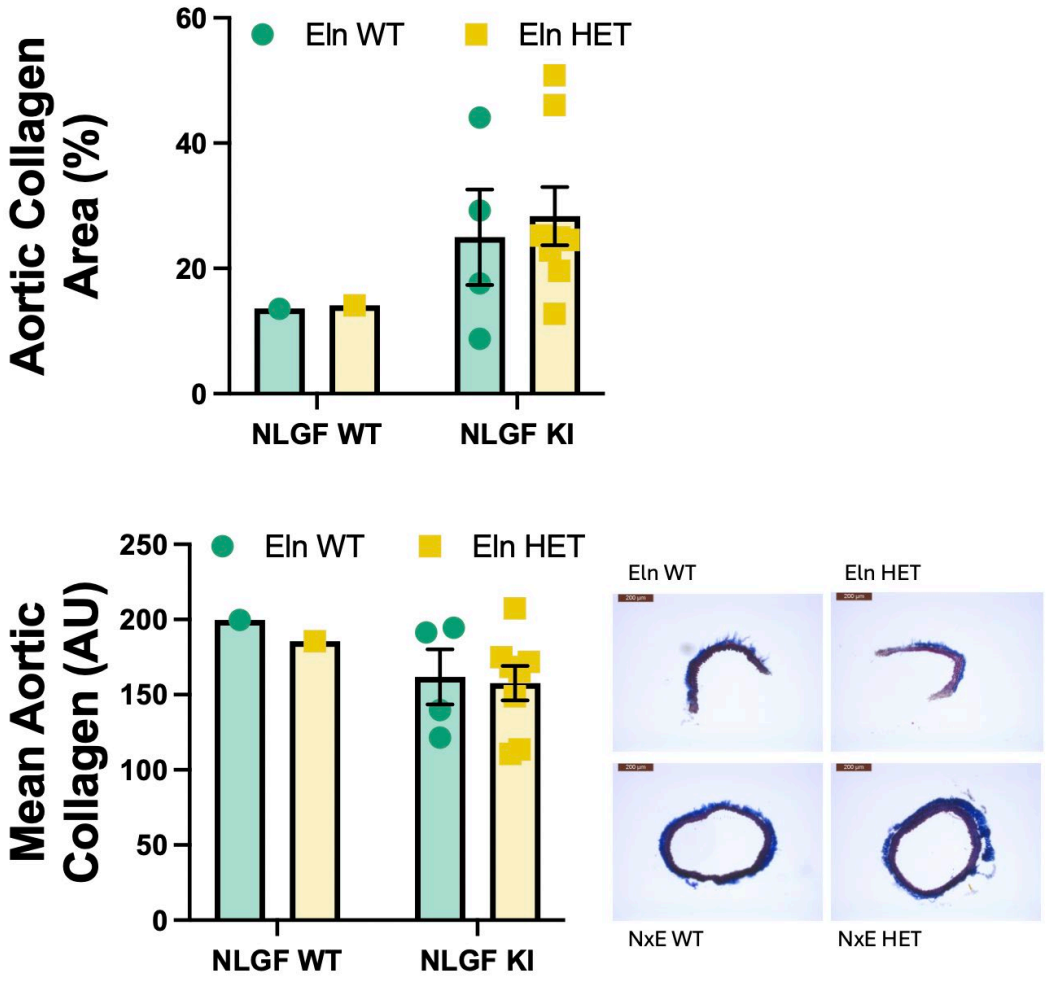

Figure 8. Mean Aortic Collagen Cross-Sectional Area & Collagen Percent Area. There were no significant differences in aortic collagen cross-sectional area and percent area of collagen between Eln WT (n = 1), Eln HET (n = 1), NxE WT (n = 4), or NxE HET (n = 8) (all $p > 0.05$). Values are mean ± SEM.

Cerebrovascular Reactivity: Acetylcholine, Acetylcholine + L-NAME, Sodium Nitroprusside

Acetylcholine Response

In the posterior cerebral arteries (PCAs), maximal vasodilation to the endothelium-dependent dilator ACh was not significantly different between groups (**Figure 9A**, $p > 0.05$).

Acetylcholine + L-NAME Response

In the presence of L-NAME, there was no vasodilation in response to ACh (**Figure 9B**, $p < 0.05$), indicating that endothelium-dependent dilation is dependent upon nitric oxide availability.

Sodium Nitroprusside Response

There were no significant differences in endothelium-independent maximum dilation to SNP for all groups (all $p > 0.05$). However, at two doses of SNP, NxE HET animals displayed significantly lower percent vasodilation compared to NxE WT animals (**Figure 9C**, $p < 0.05$).

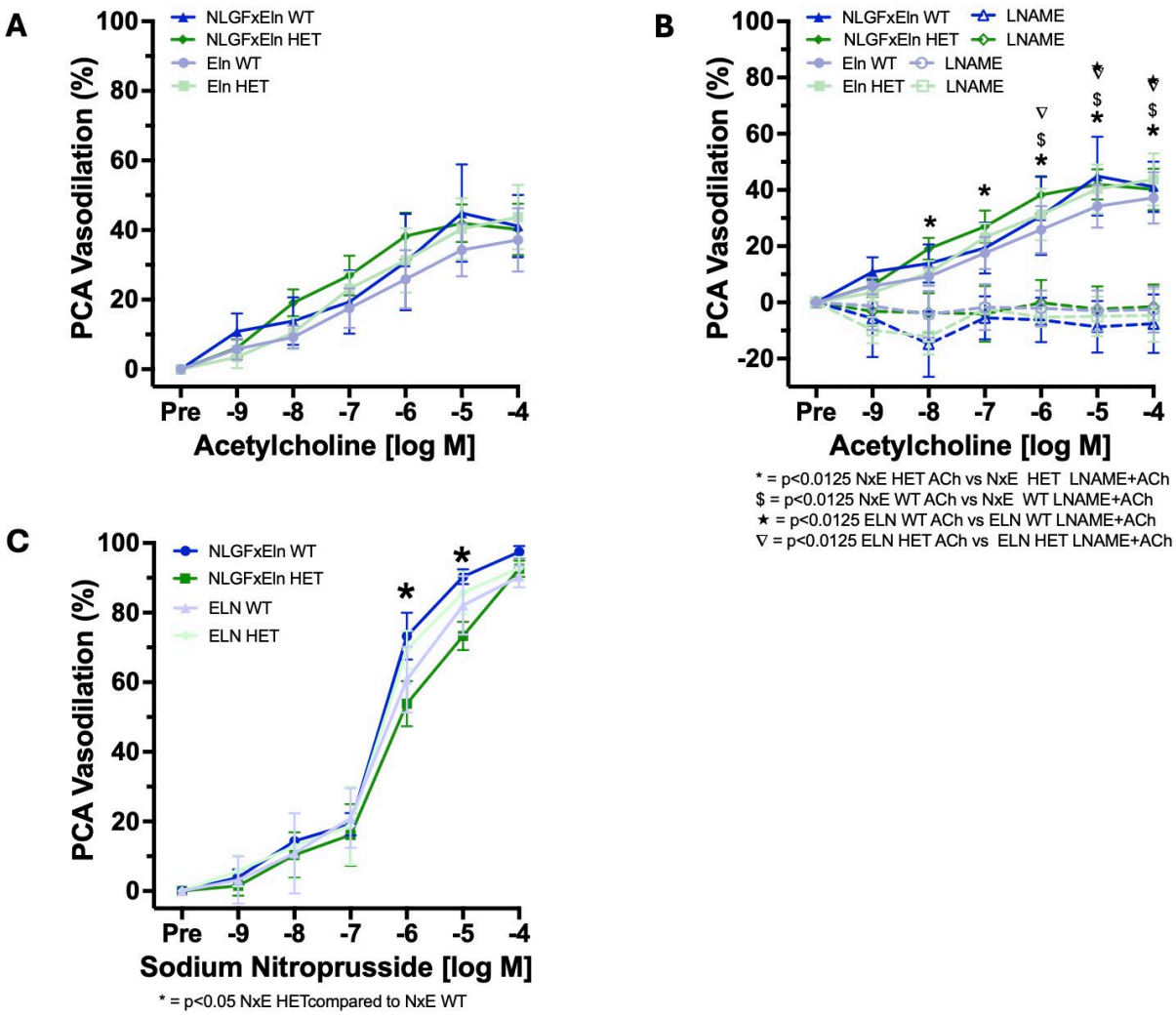

Figure 9. Posterior Cerebral Artery Vasoreactivity to ACh, ACh + L-NAME, and SNP. There were no significant differences in PCA dilation in response to ACh between Eln WT, Eln HET, NxE WT, or NxE HET (n = 4-9 per group) (all $p > 0.05$). In the presence of L-NAME, vasodilation in response to ACh was eliminated (all $p < 0.05$) for Eln WT, Eln HET, NxE WT, or NxE HET. Maximum dilation due to SNP is not significantly different between Eln WT, Eln HET, NxE WT, or NxE HET (all $p > 0.05$). At the 10-6 M and 10^{-5} M doses of SNP, NxE HET animals displayed lower percent vasodilation compared to NxE WT animals ($p < 0.05$). Values are mean ± SEM.

Discussion

In this study, I sought to understand the impact of increased presence of Aß in conjunction with LAS on cognitive function and cerebral microvascular function and structure. Given the documented independent deleterious effects of LAS and Aß on cognitive and cerebrovascular function, I hypothesized that increased LAS alongside Aß (a model for AD) exacerbates cerebral microvascular and cognitive impairment compared to AD alone. I specifically hypothesized that this cerebral microvascular impairment would decrease cognitive function in Eln HET and NLGF mice. To elucidate the relationship between large artery stiffness, Aß, and cognitive function, I conducted the NOR test, collected nest building data, measured motor coordination via rotarod testing, studied cerebral vascular function ex vivo, stained aortas to measure arterial composition, and analyzed pulse wave velocity.

There were no interaction or main effects for Aß and LAS in the nest building, rotarod, vascular reactivity, or NOR tests. Surprisingly, there was a main effect of elastin genotype on pulsewave velocity wherein Eln HET animals had lower pulse wave velocity than Eln WT mice. There was no interaction effect between Eln and NLGF for pulse wave velocity.

Cognitive Function

The NOR task is a commonly used cognitive test of object recognition memory in mice. The amount of time the mouse takes to explore a new object is a measure of recognition memory.[38] In this study, we found no main or interaction effect of genotypes on object recognition. This finding does not provide support for the hypothesis that increased LAS and Aß accumulation negatively impacts cognitive function. The NOR test is widely used as a facile test of cognitive function for mice with AD. Many studies have found that mice expressing high

levels of APP exhibit deficits in cognitive function—specifically, memory impairment— as measured by lack of object memory during the NOR test.[39, 40]

Nest-building activity is a measure of instinctual behavior, which provides insight into cognitive function. Previous studies show that mice with genotypes of AD create less developed nests than wildtype mice.[41, 42] Since the results of our study showed neither a main nor interaction effect of LAS or Aß on nest building behavior, it is possible that nest building behavior is not as influenced by the NLGF genotype.

The rotarod test assesses neuromuscular motor function, specifically balance and coordination. Prior research finds that even at a young age, mouse models with Aß-42 accumulation, similar to our NLGF model, have impairments in rotarod performance.[43] The results from our study contradicted this finding, displaying no main effect of APP and Aß, nor an interaction effect of LAS and Aß on rotarod performance.

Arterial Stiffness

Emerging evidence highlights the interface between arterial stiffness and cerebrovascular outcomes. Large arteries, such as the aorta, can grow stiff due to aging and several diseases. With aging, a reduction in elastin along with an increase in collagen cross-linking can increase large artery stiffness.[44] Stiffer large arteries cannot reduce pulse pressure as effectively as normal large arteries, placing greater pulsatile pressure on the microvasculature (small arteries, arterioles, and capillaries), increasing its risk of damage.[45] Longitudinal epidemiological studies have found associations between arterial stiffness measured by aortic pulse wave velocity, brain volume, and cortical thickness.[46]

Previous studies have found that increased wall stiffness results in greater PWV in the cardiovascular system.[47] The Eln HET genotype has also been found to have greater PWV in

prior studies.[48] In this study, there was a main effect of LAS on PWV ($p = 0.0018$). Surprisingly, Eln HET animals had a lower pulse wave velocity compared to Eln WT animals regardless of APP genotype. The results of this study appear to indicate that LAS from elastin haploinsufficiency reduced pulse wave velocity, while Aß did not influence pulsewave velocity.

Wall thickness correlates with stiffer arteries: prior research suggests that decreased elastin content, decreased smooth muscle content, hypoxia, and accumulation of collagen are all associated with increased wall thickness.[49] Greater wall thickness, and thus stiffer aortas, are implicated as precursors for heart disease. Assessing the wall thickness of aortas of Eln WT and Eln HET animals, there was no main effect of genotype ($p = 0.16$). However, with a very small sample size ($n = 1$ in each group), our understanding of the role of diminished elastin and LAS is limited.

Cerebrovascular Reactivity

Aß forms plaques in the brain and blood vessels during AD. [50] Whether AD causes Aß accumulation, or whether Aß accumulation triggers AD is unknown. A soluble form of Aß constricts cerebral arteries, inhibits EDD and constriction of endothelium-dependent vasoconstrictors through a yet unknown mechanism. Hypotheses for this inhibition include increased reactive oxygen species production, increased calcium activity within cells, or diminished availability of endothelial nitric oxide.[51] Furthermore, Aß's toxicity triggers apoptosis of vascular cells and stimulates inflammatory pathways.[52] Constriction of large arteries due to Aß may pose a greater burden to the microvasculature, similar to larger artery stiffness, leading to negative cerebral and cognitive outcomes.

No main effect of Aß or LAS was found on endothelium-dependent or independent dilation. Given that the dilation response to ACh in the presence of L-NAME was eliminated (p

< 0.05), suggests that EDD is dependent upon the bioavailability of nitric oxide. There was an interaction effect of Aß and LAS on endothelium-independent dilation as measured by vasodilatory responses to SNP, in which the response to SNP was lower in the NxE HET animals compared to NxE WT animals ($p < 0.05$). The response to SNP suggests that differences in dilation were likely endothelium-independent and mediated by smooth muscle. Additionally, this difference could potentially indicate a decreased sensitivity to SNP in the NxE HET animals compared to NxE WT. Furthermore, as a structural component of arteries, elastin may have a role to play in endothelium-independent dilation. Specifically, a lack of elastin (implicating stiffer arteries) coupled with the deleterious effects of Aß, found in the NLGF x Eln HET genotype, may reduce cerebral arteries' ability to dilate in response to endothelium-independent dilators.

Sex Differences

Several murine and human studies call attention to the disparate progression of arterial stiffening and age between males and females. In humans, women show a greater increase in aortic stiffness post-menopause, compared to men.[53] In mice, arterial stiffness begins earlier in males than females but progresses at a more gradual rate.[54] Another recent study found that males, rather than females had a stronger relationship between aortic stiffness and impaired cerebrovascular function.[55] Given that changes in sex hormones in both males and females are observed with aging, it is likely that sex differences may be attributed to sex hormones and their associated signaling networks.[56] In this study, no significant sex differences were found, so we combined groups. While the diversity of findings regarding sex differences and arterial stiffness highlights evidence of an association between these two variables, further research with a greater sample size would help elucidate this relationship.

Future Directions

In the future, it will be constructive to study cerebral perfusion to further understand cerebrovascular reactivity in response to large artery stiffness and Aß accumulation. Using these results, one can determine the interactions between Aß accumulation, large artery stiffness, and cerebral blood flow. Furthermore, studying different mouse models of LAS and Aß accumulation might provide a more comprehensive comparative framework to understand the effects of these variables. Staining and RNA expression analysis of Aβ, MMP, and ECM regulation pathways would provide a way to correlate the structural and gene expression implications of increased APP and LAS, features associated with the mouse model.

Limitations

The results from our study did not support an effect of Aß, a model of AD, on cognitive function at a young age. Potential drawbacks of the NOR test include lack of object exploration due to stress, general disinterest in objects chosen for the test, smell and other external distractions redirecting interest from objects, and too long of a time frame in the testing arena causing the mouse to lose interest in the objects.[57,58] Acknowledging that because we had a small sample size (n=1) of mice for two of four groups in aortic wall thickness and collagen analyses, there is not sufficient data yet to align with other studies' findings. Additionally, the discrepancy between our rotarod results and previous findings may be due to limitations of sample size, or that there is not necessarily an association between Aß and rotarod performance at a young age. Lastly, Aß is known to become progressively more harmful the longer it remains in the body, so the young age of the mice studied may have masked the negative effects of Aß, which worsen over time and accumulation.[59]

Conclusion

Both the presence and lack of relationships can be instructive in understanding the mechanisms of a disease with so many unknowns. These results, taken in conjunction, suggest that aging, rather than large artery stiffness, is a primary risk factor for AD.

Over the next 25 years, the number of Americans aged 65 and older is projected to increase by 24 million.[60] The insights from this study, and follow up experiments, will aid in understanding how to physiologically target future interventions against AD at a time when the primary risk factors are rapidly increasing.

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