Spring 5-11-2023

All the RAGE: Assessing the AGE/RAGE Signaling Pathway's Effects on Healthspan and the Physiological Processes of Aging

Brandon Ashmore

All the RAGE: Assessing the AGE/RAGE Signaling Pathway's Effects on Healthspan and the Physiological Processes of Aging

By

Brandon Ashmore

A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2023

Approved by

_____

Advisor: Dr. Nicole Ashpole

_____

Reader: Dr. James Stewart

_____

Reader: Dr. Gregg Roman

Acknowledgements

I would like to thank Dr. Ashpole for her continuous guidance and support as my advisor for these last several years and for giving me the opportunity to engage in this work. I would also like to thank Dr. Cellas Hayes for getting me started in research and for his mentorship, and Miguel De Leon for his guidance and support as well. Finally, thank you to all my friends and family for encouraging me all along the way and to the entire Honors College community.

Abstract

Advanced glycation end products (AGEs) are protein, lipid, or nucleotide molecules that have been combined with sugars through nonenzymatic, irreversible glycation and oxidation reactions. Their accumulation in the body has been associated with the natural aging process and a wide range of pathologies, including chronic inflammation, sustained oxidative stress, diabetes, neurodegenerative diseases, atherosclerosis, and cancer. Their interaction with the receptor for advanced glycation end products (RAGE) has been linked to several proinflammatory signaling pathways associated with neurotoxicity and vascular lesions. While some research has been done on the possible health benefits of RAGE inhibition to extend lifespan, our study hopes to further explain the role of AGE accumulation and AGE/RAGE signaling in directly contributing to the aging process in the hopes of extending healthspan.

Table of Contents

List of Figures

Background

The aging process is a major risk factor for the development of various pathologies, including neurodegenerative diseases, metabolic diseases, and cancer, which can significantly impact the quality of life of individuals as they approach advanced age.[1–3] Due to advances in modern medicine and technology, the average lifespan has increased. However, the same cannot be said for age-related comorbidities (e.g., cognitive and motor impairments), as current therapeutic interventions to combat these risks have proven unsuccessful in treatment or prevention.[4] This significant imbalance between our lifespan and healthspan (i.e., period of healthy life) warrants the application of research to attain equilibrium between the two[5,6], subsequently reducing the age-related risks that haunt our lifespan and improving our overall way of life. In order to understand how to achieve this equilibrium, it is important to gain a deeper understanding of the biological processes that drive aging.

Aging can be distinguished by either biological or chronological age.[7] Biological age is the differences in function or physiology of an individual that could result in the increased prevalence of age-related pathologies, and chronological age is the passage of time that an individual is living.[7] These two concepts are not mutually exclusive as some individuals could have a more intense rate of aging due to differences in cellular and molecular processes and genetic and environmental factors that could make an individual more susceptible to age-related pathologies.[1,4,6]

Of the cellular and molecular processes, chronic inflammation, sustained oxidative stress, and cellular senescence are evident in the normal aging process and in chronic diseases like diabetes and Alzheimer's disease.[8] While these pathologies are multifactorial in nature, a key pathway that plays a role in the chronic, low-grade inflammation (i.e., inflammaging) that propagates the severity of these diseases is the advanced glycation end products (AGEs) and its respective receptor (RAGE) pathway.[9,10]

Advanced glycation end products, or AGEs, are proteins, lipids, and polynucleotides modified by nonenzymatic glycation and oxidation due to a prolonged imbalance of glucose and metabolic stress.[11] This will often result in an accumulation of AGEs that will significantly increase with advanced age and is concurrent with a decrease in the enzyme responsible for metabolizing AGEs.[11] Additionally, AGEs can be introduced to the body exogenously through foods high in fat and protein cooked at high heat and through environmental pollution, which also results in the accumulation of AGEs in advanced age.[11,12] The association between AGE accumulation and advanced age highlights the implications of AGEs on overall healthspan.

It is believed that AGEs play a regulatory role in the natural immune response by promoting inflammation.[13] AGEs exert their effects on the body by interacting with the receptor for advanced glycation end products, termed RAGE. RAGE is a

member of the immunoglobulin family of proteins. Its activation is linked with several proinflammatory signaling pathways, including NF-kB, protein kinase B, and mitogen-activated protein kinases.[11] Overexpression of RAGE has been implicated in several pathologies, including sustained inflammation and oxidative stress, neuroinflammation, amyloid-beta protein accumulation, impaired learning and memory, and neurotoxicity.[14] RAGE is also present at higher levels near sites where AGEs have accumulated.[15] AGE formation is also enhanced at sites of oxidative stress, inflammation, and vascular lesions, and creates a positive feedback loop that further exacerbates these physiological responses.[15]

Of the associated pathologies that AGE accumulation has been found in, cognitive impairments (i.e., in learning and memory), behavioral deficits, and issues with sleep are prevalent symptomologies.[16] Additionally, physical impairments such as reduced muscle mass, increased frailty and pain sensitivity are seen through the aging process.[17] Given the prevalence of these associated deficits, there is a growing interest in conducting research to attenuate and prevent the negative effects of AGE accumulation on healthspan. Prior studies have shown through the use of pharmacological approaches that blocking the interaction between AGE/RAGE using a modified form of RAGE (sRAGE) that binds to the same ligands has been shown to decrease AGE formation, subsequently leading to less oxidative stress and inflammation.[15] Further studies have shown similar effects; however, they have done so by blocking AGE production and inhibiting the binding of RAGE.[18] Although literature suggests that

modifying AGE/RAGE signaling can increase healthspan, the consequences of inhibiting RAGE on the global aging process, in the absence of evident associated pathologies, are not well understood and requires further elucidation. The current study will work to further explain the apparent role of AGE accumulation as a direct contributor to the aging process and its associated onset of diseases. Through the use of behavioral techniques, we will examine the effects of the systematic knock-out of RAGE on healthspan, cognition, and overall physical well-being. We hypothesize that 1) as AGE accumulation and RAGE activation increases, there will be a subsequent decline in healthspan, and 2) the systemic knock-out of RAGE will delay the onset of cognitive and physical impairments in advanced age.

Methods

Animals

Wild-type and RAGE knockout (RKO) mice (male and female) ages 4-6 months, 12 months, and 24 months were used for this study. Mice were group housed 4-5 per cage (7.5 x 11.5 x 4.75 in) under standard environmental conditions with a 12hr/12hr light/dark cycle with enriched bedding and food and water ad libitum.As reported previously, the generation of homozygous RKO mice was achieved by flanking exons 2-7 with two loxP sites in the same orientation and exposure to Cre recombinase via breeding with Cre deleter mice[1–4]. This deletion resulted in a constitutive, global loss of RAGE mRNA expression and turn,

knocking out RAGE signaling within these mice. Furthermore, a reverse-orientated transcriptional enhanced green fluorescent protein (eGFP) reporter gene was inserted into intron 7 to confirm RAGE exons 2-7 deletion. Wild-type C57Bl/6 controls were procured from Jackson laboratories at 4-6 weeks of age and housed in tandem with the RKO mice.

Genotyping

The genotype of mice was determined by removing a ~2-3 mm tail section and extracting DNA using Sigma Aldrich Extract-N-Amp Tissue PCR Kit (XNAT2-1KT) per manufacturer's protocol. PCR was conducted using Dream Taq Hot Start and predesigned RAGE primers (mRAGE e3-Forward: 5´-CACAGGAAGAACTGAAGCTTGGAAGG-3', e5-Reverse: 5´-CACCTTTGCCATCGGGAATCAGAAG-3´). Thermocycling followed previous reports for these primers; the resulting reaction was separated with gel electrophoresis (100V) in a 1% agarose gel in TBE, and visualized using the iBRIGHT system. A 606bp band indicated RAGE WT, while no band was detected with RAGE KO. KO was verified with subsequent PCR with predesigned eGFP primers (EGFP36-54 primer: 5´-GGTGCCCATCCTGGTCGAG -3´, EGFP346-328 primer: 5´-CGAACTTCACCTCGGCGCG -3´) that detect the Cre deleter GFP sequence (resulting 311 bp band).

Radial Arm Water Maze

Spatial learning and memory were assessed in a radial arm water maze that consisted of 8 submerged arms with an escape platform and spatial cues. Water was placed up to 5-7 cm in a maze and mixed with white food coloring to create an opaque environment to hide the escape platform. Mice were acclimated to the escape platform before beginning the acquisition phase of the maze, which consisted of 24 training trials (6 trials per day for three consecutive days). On day 10, memory was assessed in a 60-second probe trial. The escape platform was moved following the probe trial in a reversal phase for eight additional trials to observe memory extinction and re-learning. Total pathlength to escape platform, latency to escape, the number of errors made, and velocity were calculated using Ethovision Software.

Rotorod

The Rotorod (San Diego Instruments, Inc., San Diego, California) consists of 4 animal lanes with a 1.25” diameter rod that passes through each lane. Mice received one acclimation trial with a constant rotating speed (4rpm) for 5 minutes, an accelerating training trial with increasing rotations in increments of 6 every 100 seconds for 5 minutes until 40rpm (0-40rpm for 5 minutes), and a final testing accelerating trial. All mice received an interval of 5 minutes between trials and were returned to their home cage. Latency to fall and distance traveled were recorded and compared across groups.

Mechanical Sensitivity

MouseMet electronic von Frey systems (Topcat Metrology Ltd.) was used to assess mechanical sensitivity. Mice were acclimated to rectangular pexi-glass containers (3.81 x 11.43 x 11.43 cm) lined with metal rods at the base for 15 minutes. Subsequently, a 0.3mm von Frey plastic filament (probe tip) is brought into contact with the plantar surface of the mice, and withdrawal thresholds are recorded. Thresholds will be recorded twice on each hind paw (i.e., alternating between left and right) with intervals of three minutes in between each probe.

Open Field

A Photobeam Activity System (PAS) – Open Field (San Diego Instruments, San Diego, California) was utilized to assess anxiety-like behavior. This open field is surrounded by a stainless steel frame (20 x 20 in) that encompasses a clear plastic animal enclosure (18 x 18 in). Sixteen photobeams are spaced one inch apart along all sides of the frame's interior to provide reliable and accurate data output. Mice were placed in the center of the open field facing the rear of the field. They were then allowed to explore the field for 10 minutes. Real-time reports of the time spent in the periphery, center, total number of entries into the center and periphery, and velocity were recorded utilizing the PAS software.

Circadian Rhythm

Mice were under a reverse light cycle [insert cycle times] to mimic normal sleep/wake behavior. Free running wheels were used to evaluate circadian rhythm. Before placing animals in cages with running wheels, it was confirmed

that the wheels were registered by the ClockLab software (ActiMetrics, Wilmette, IL) and properly detected. Additionally, each wheel was checked for resistance to ensure proper rotation. Once the software properly detected the running wheels, animals were individually caged [dimensions] and allowed to access the running wheel freely. Daily patterns of mouse activity and abnormalities in the animals' behavior, activity, and appearance were recorded by the Clocklab software.

Y-maze

Working memory was evaluated using a rodent three-arm y-maze. Mice were placed onto the end of one of the arms and allowed to explore for five minutes. Subsequent interactions with the arms consisted of blocking off certain arms of the maze and introducing the mice to the openly available arms. The number of times the animal enters each arm is recorded and calculated using Ethovision Software (Noldus Information Technology Inc., Leesburg, VA, USA).

Necropsy Report

A necropsy report was recorded for each mouse to determine the physiological effects of experiments ex vivo. The methods of euthanasia utilized are consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association, as well as the IACUC guidelines provided by the IACUC committee at the University of Mississippi. Mice were anestesized with vaporized Isoflurane (Covetrus, Batch No.: G139K21B) and subsequently perfused with phosphate buffered saline (Gibco, lot: 2472416). A pair of surgical scissors and

hemostats were used to remove the heart, lungs, liver, spleen, and kidneys from each animal. The weight and identification of any tumor growth were recorded. Mice that were not used for the necropsy report were euthanized with carbon dioxide (CO_2) exposure and cervical dislocation as a secondary method of euthanasia.

Statistics

Statistics were analyzed using Excel and Sigma Plot v14. Two-way ANOVA was applied to assess the impact of genotype and sex on response variables (such as total distance, speed, errors, etc.) and followed by a Tukey post-hoc test. For variables measured over multiple days, a two-way repeated measures ANOVA was used, with genotype, sex and trial as independent variables, and response variable as dependent variable. All tests used an alpha level of 0.05 to determine significance.

Results

Cognitive Assessments in Early and Mid-adulthood

To investigate the response of AGE signaling on learning and memory in the aging process, mice in early (3-4 months) and middle (8-12 months) adulthood were subjected to a series of cognitive behavioral tasks. Specifically, we utilized a radial arm water maze (RAWM) to evaluate learning and memory. During the acquisition phase, mice were trained to locate the escape platform in which we

could make assessments based on the total distance traveled and the total number of errors made. Male and female RAGE KO and WT mice can successfully learn where the escape platform is as seen in a reduced distance traveled over time (Fig. **1A**). Notably, female RAGE KO mice travel significantly more when compared to male RAGE KO and male and female WT ($F_{1, 729}$ = 6.753, p = 0.010). Additionally, we see that there is an overall decrease in the total errors made during the acquisition phase ($F_{1,729}$ = 4.176, p = 0.041) with female RAGE KO mice having significantly more errors than the other groups, respectively (Fig. **1C**; M = 8.835 +/- 0.409, M = 10.001 +/- 0.397). When examining the total distance traveled across all the trials, we see that the female RAGE KO mice have a greater pathlength when compared to the other groups. (Fig. **1A,1C**; MWT: M = 289.620 +/- 19.595, MKO: M = 303.628 +/- 18.792, FWT: M = 257.794 +/- 20.163, FKO: M = 349.848 +/- 20.605). In mid-adulthood, male and female RAGE KO mice show greater pathlengths (Fig. **2A,2B**) and total errors (Fig. **2C**) than WT.

In accordance with investigating learning and memory, we sought to evaluate to what extent memory would be affected in mid-adulthood. Therefore, we continued to utilize the RAWM and analyzed the % success of finding the escape platform after subjecting the mice to an extinction memory phase (i.e., memory probe). We found a lower success rate in male and female RAGE KO mice when compared to WT (Fig. **3**; $F_{1,2}$ = 40.44, p = 0.024). Additionally, working memory

was assessed in a Y-maze. Male and female RAGE KO mice appeared to make more errors when compared to WT mice (Fig. **4**).

Vision, Movement, and Circadian Activity

To further assess healthspan and determine whether the impairments seen in the learning and memory tasks were affected by physical factors, several physiological experiments were conducted. A visual acuity test was conducted in early and mid-adulthood to determine if learning and memory impairments were due to visual impairments (Fig. **5**). In mid-adulthood, there is a significant difference in vision between RAGE KO and WT mice ($F_{1,35} = 7.185$, $p = 0.011$) with male and female RAGE KO mice showing significant impairment (RAGE KO: M = 0.258 +/- 0.69, WT: M = 0.321 +/- 0.067).

When observing motor function in early and mid-adulthood, we see no alteration in motor coordination when subjecting mice to a rotarod task (Fig. **6A,6B**; $F_{1,37} = 0.270$, $p = 0.606$), suggesting that the impairments observed in the aforementioned cognitive tasks were not mediated by motor function as seen in the RAWM data presented above. However, RAGE KO mice reduce circadian activity in early adulthood.

Anxiety and Mechanical Sensitivity

As mentioned in our supplementary data, early-adulthood RAGE KO mice exhibited anxiety-like phenotypes when evaluated for impairments in learning and memory. Mid-adulthood mice were subjected to an open-field task in which

similar anxiety-like phenotypes could be recorded to follow up on this observation. When observing total distance traveled, there is an overall difference seen in genotype, with male and female KO mice traveling less than WT (Fig. **7A,7B**, $F_{1,349} = 54.705$, $p < 0.05$). This overall difference between genotypes is also seen when assessing the total resting time within the area ($F_{1,349} = 48.530$, $p < 0.05$). KO mice are still two times more than WT mice (Fig. **7C**, KO: M = 24.778 +/- 1.417; WT: 12.038 +/- 1.157).

Mice were evaluated for mechanical sensitivity utilizing the electronic von Frey (eVF). No overall differences were observed in withdrawal threshold for either Sex ($F_{1, 38} = 0.547$, $p = 0.465$) or genotype ($F_{1,38} = 1.712$, $p = 0.199$). However, RAGE KO males showed an increase in mechanical sensitivity when compared to WT males (Fig. **8**, KO: M = 4.464 +/- 0.242; WT: M = 5.435 +/- 0.230).

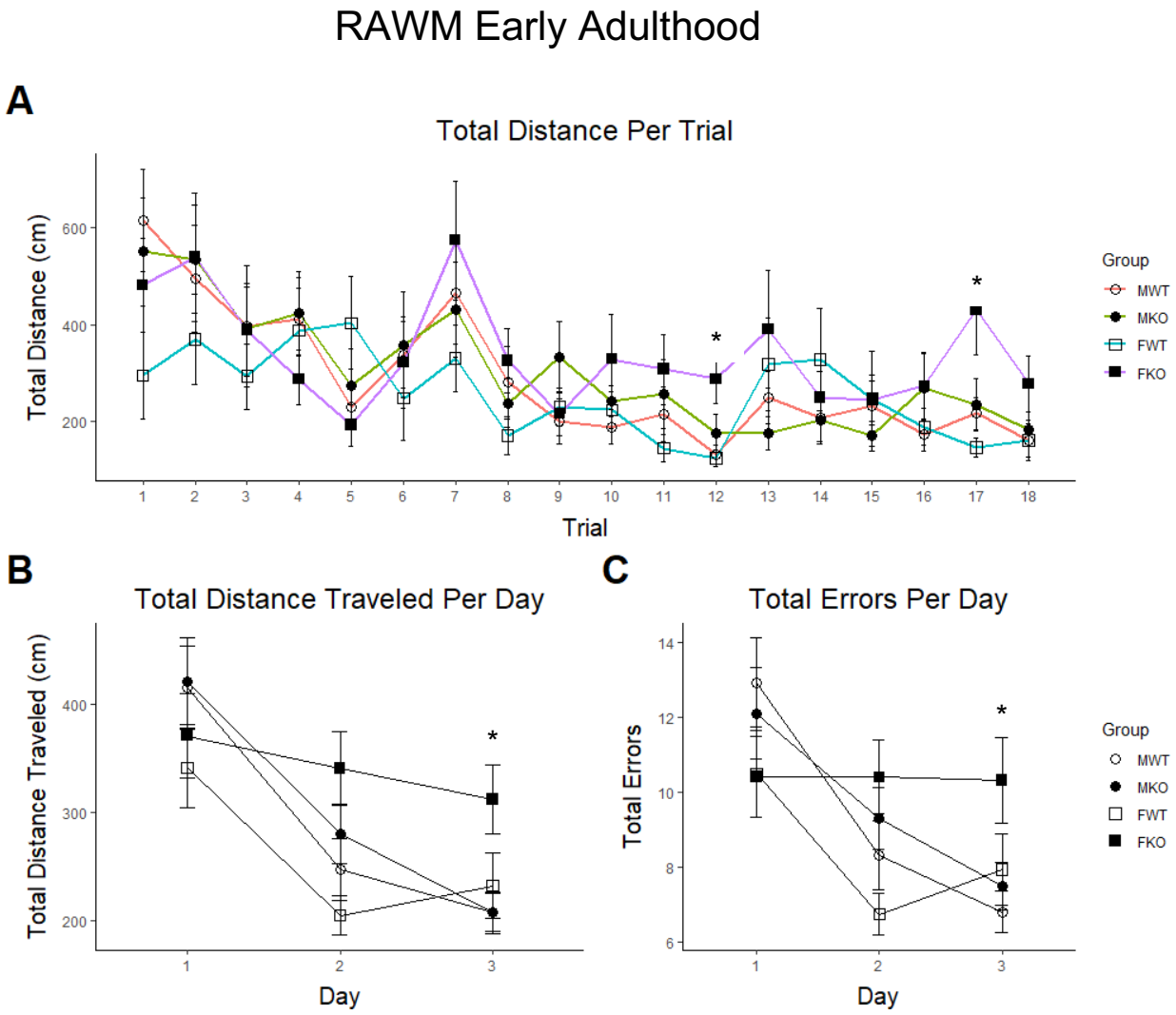

Figure 1: Assessment of spatial learning and memory using the radial arm water maze in early adulthood. **A**) Total distance traveled per trial across all trials (MWT: *M* = 289.620 +/- 19.595, MKO: *M* = 303.628 +/- 18.792, FWT: *M* = 257.794 +/- 20.163, FKO: *M* = 349.848 +/- 20.605). **B**) Total distance traveled per day ($F_{1, 729}$ = 6.753, ***p* = 0.010**). **C**) Total errors assessed per day ($F_{1,729}$ = 4.176, ***p* = 0.04**, M = 8.835 +/- 0.409, M = 10.001 +/- 0.397)

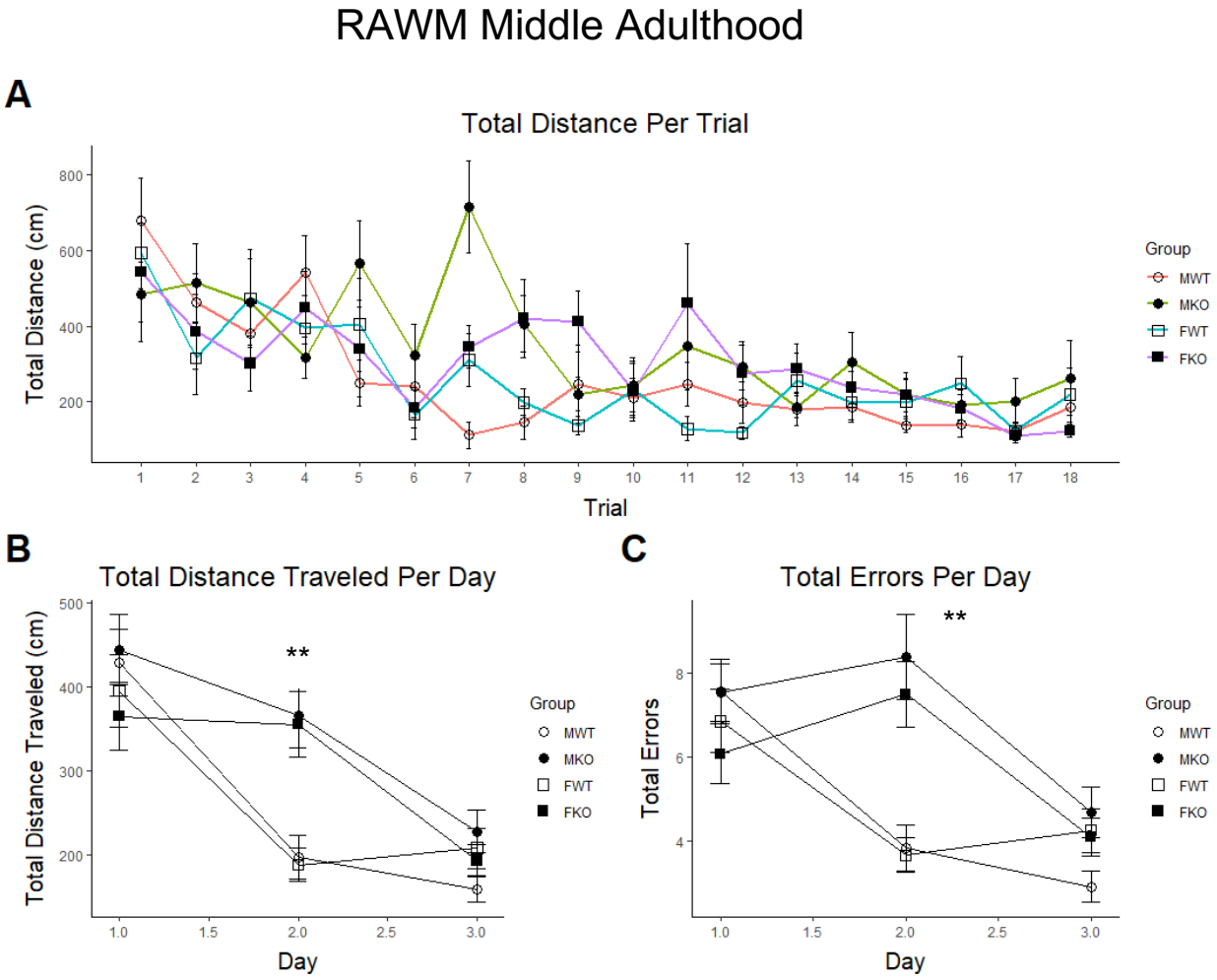

Figure 2: Assessment of spatial learning and memory using the radial arm water maze in mid adulthood. **A**) Total distance traveled per trial across all trials. **B**) Total distance traveled per day ($F_{1, 712} = 7.43$, $\boldsymbol{p = 0.006}$). **C**) Total errors assessed per day ($F_{1, 712} = 4.515$, $\boldsymbol{p = 0.033}$)

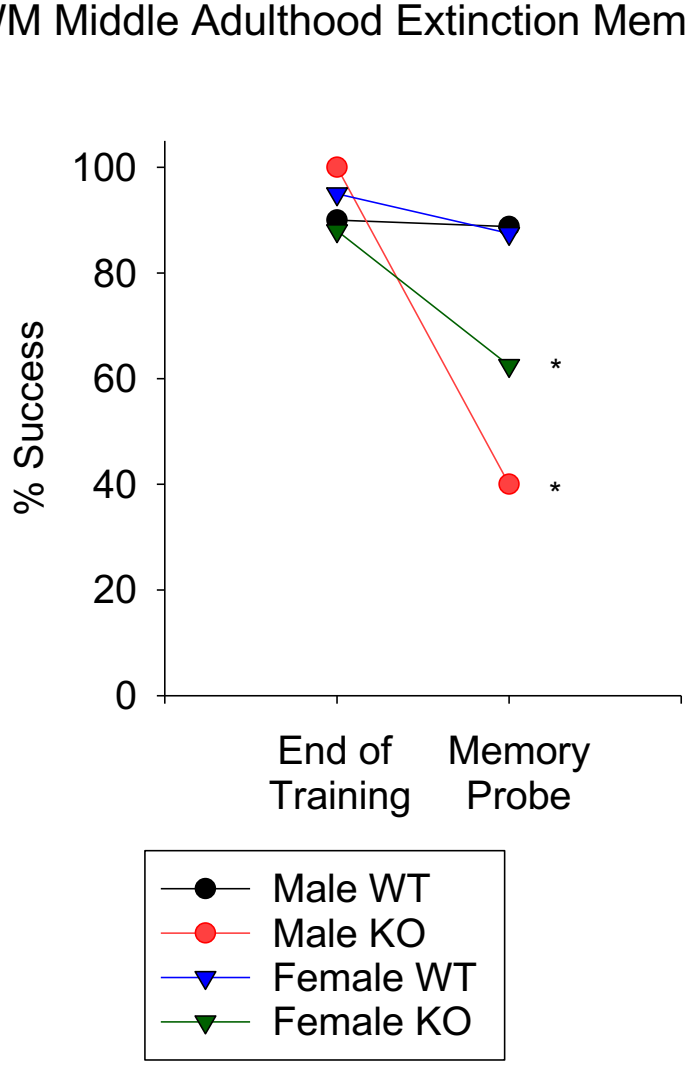

Figure 3: Percent success rate of mice when subjected to extinction memory phase one week after the end of the training period ($F_{1,2}$ = 40.44, ***p* = 0.024**).

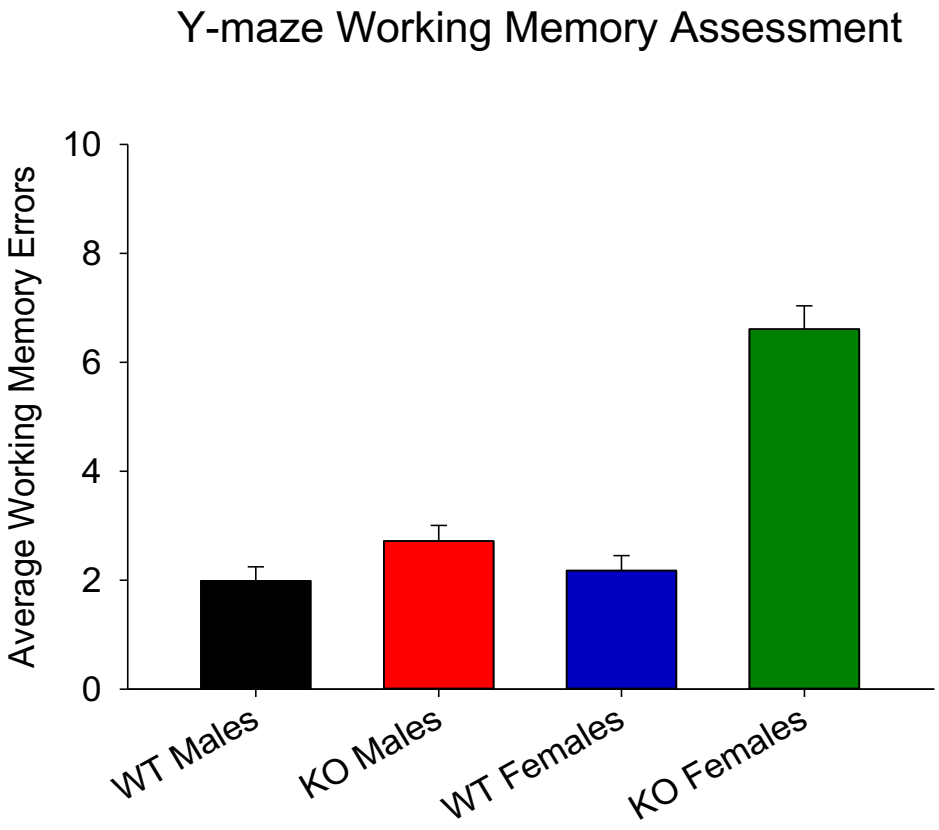

Figure 4: Working memory assessment in Y-maze scored by number of errors

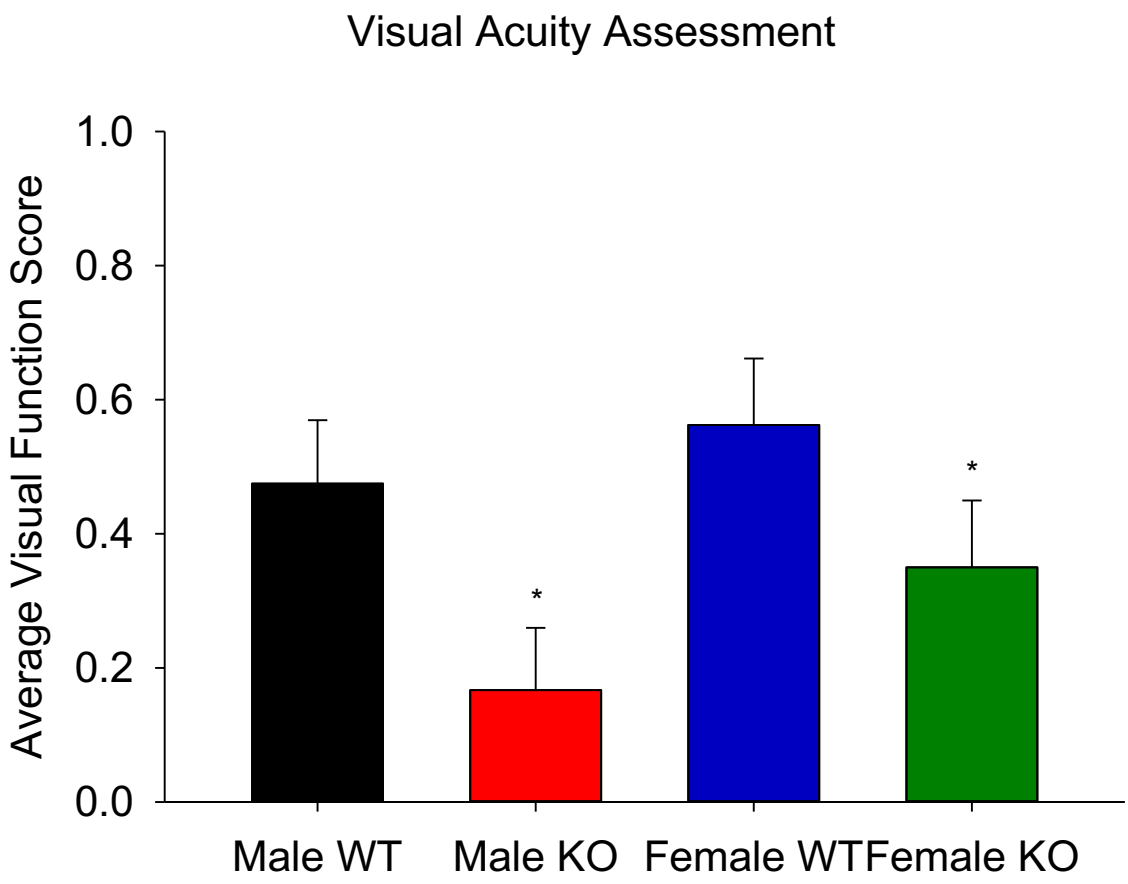

Figure 5: Visual acuity assessment measuring average visual function score ($F_{1,35}$ = 7.185, **p = 0.011**, RAGE KO: M = 0.258 +/- 0.69, WT: M = 0.321 +/- 0.067).

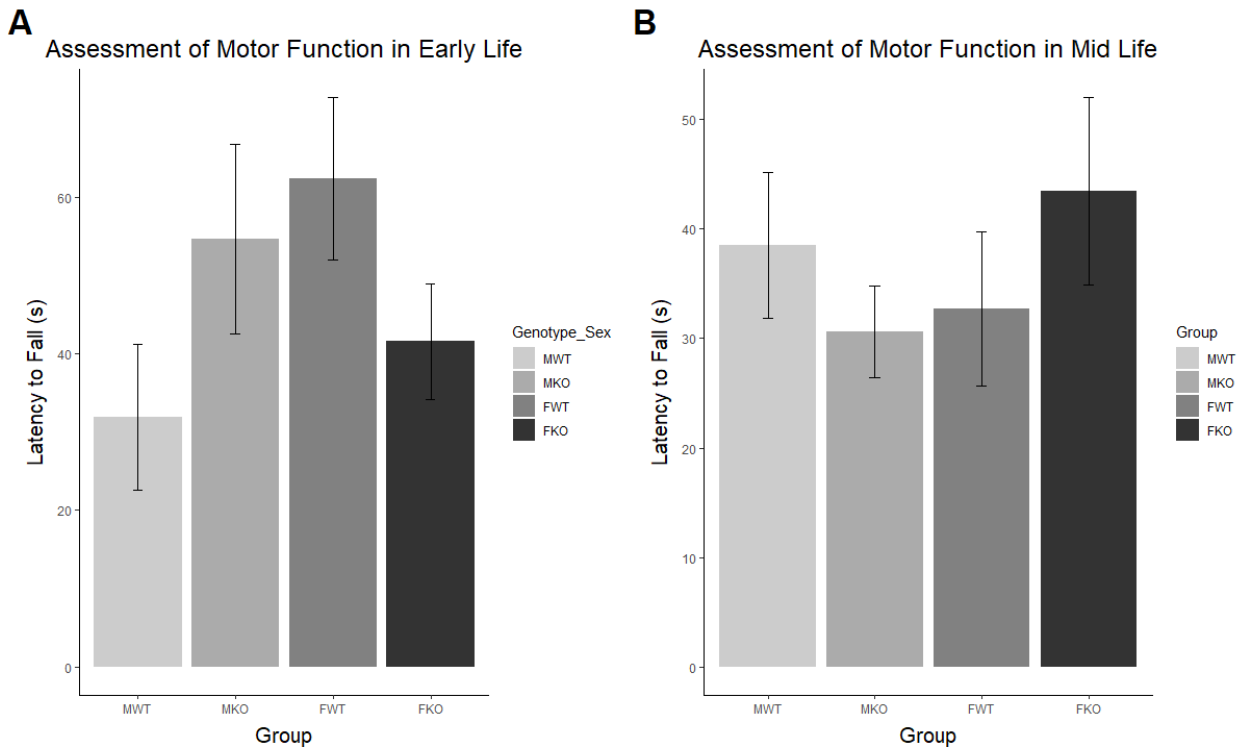

Figure 6: Assessment of motor function using rotarod to measure latency to fall. **A**) Motor function in early adulthood (3-4 months). **B**) Motor function in middle adulthood (8-12 months). ($F_{1,37} = 0.270$, $p = 0.606$)

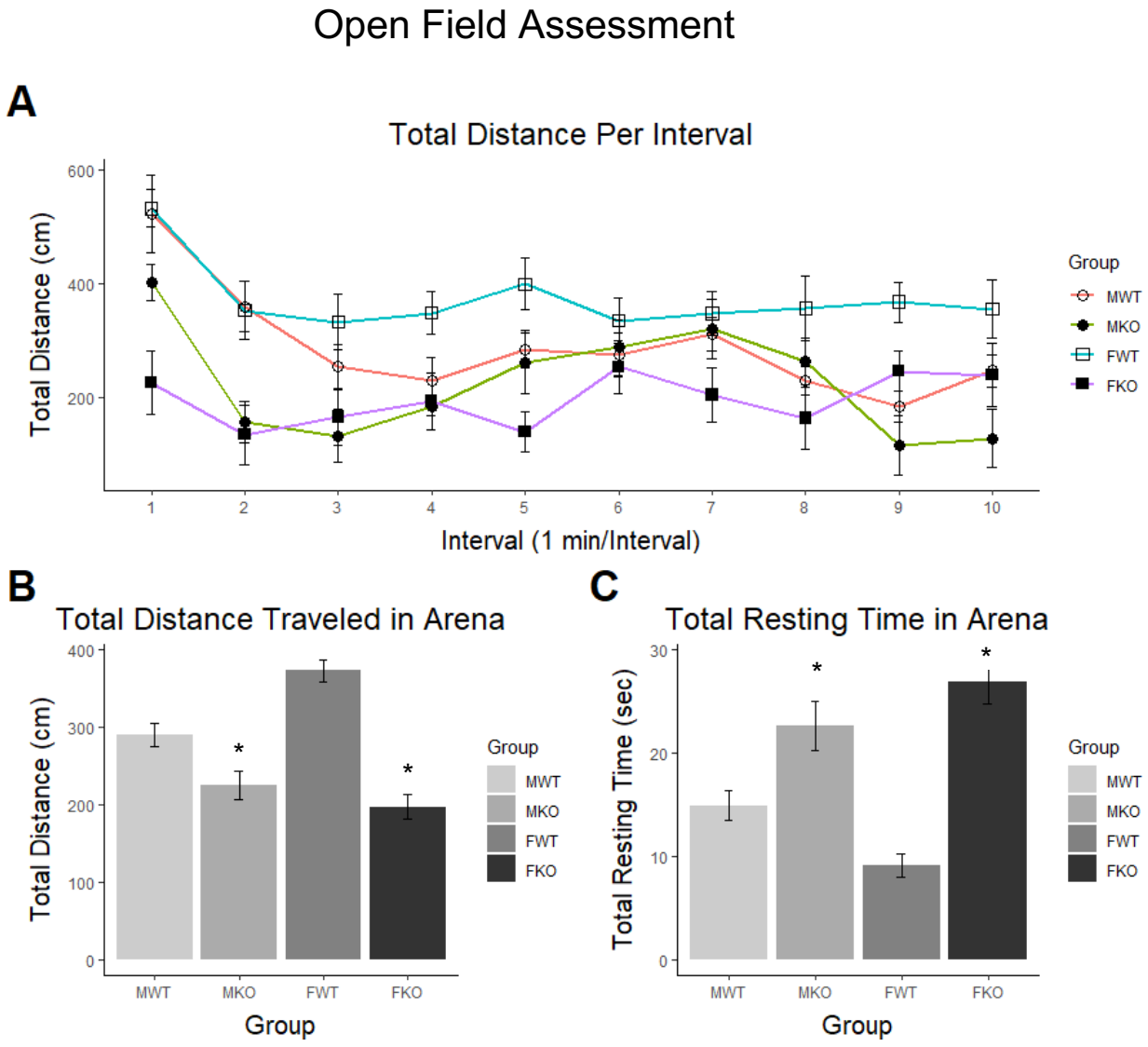

Figure 7: Open field assessment measuring locomotion. **A**) Total distance measured in cm per interval across all intervals. **B**) Total distance traveled in arena for all groups ($F_{1,349} = 54.705$, **p <0.05**). **C**) Total resting time in arena for all groups ($F_{1,349} = 48.530$, **p <0.05**).

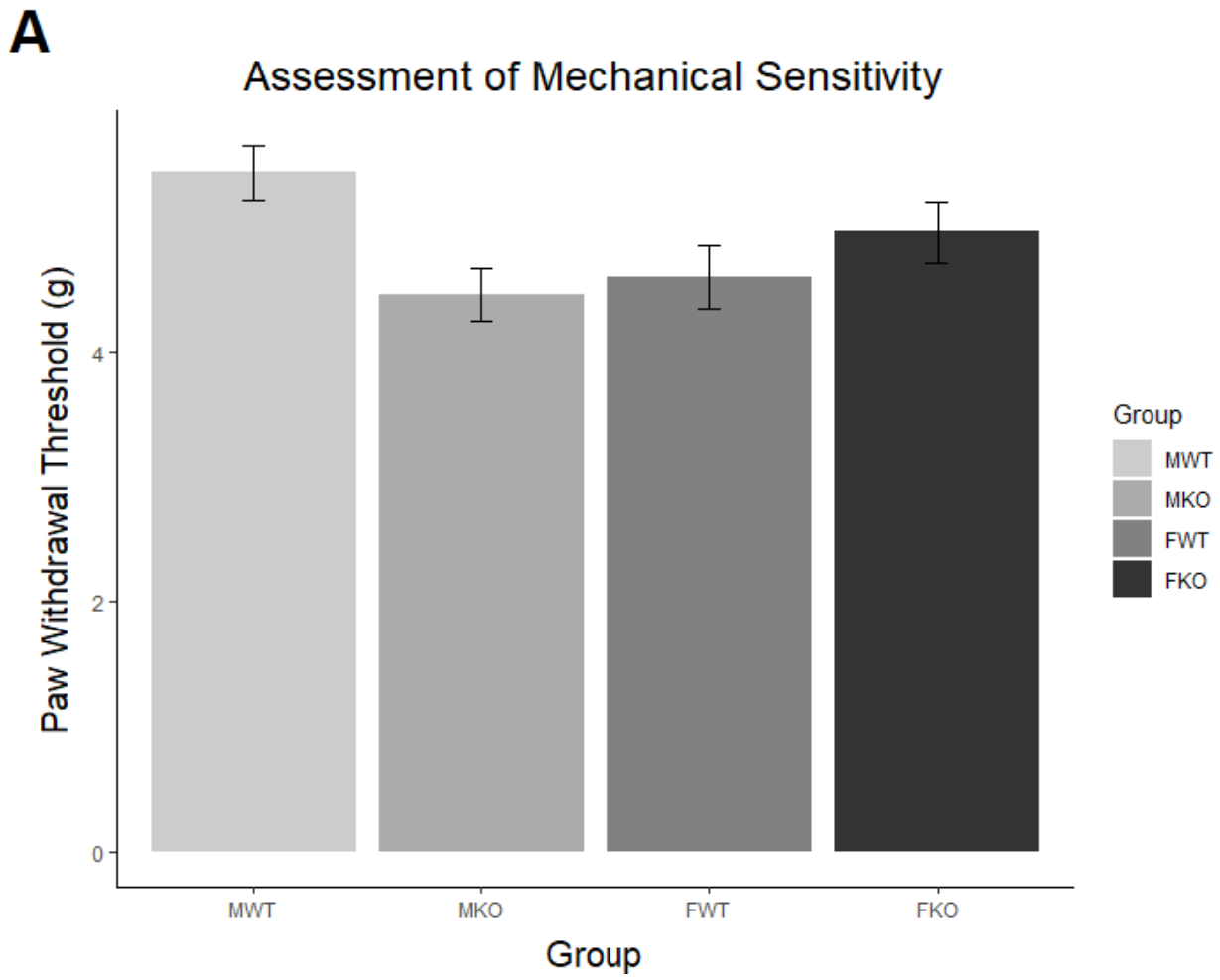

Figure 8: Paw withdrawal threshold measured using electronic Von Frey (eVF) to assess mechanical sensitivity. Sex ($F_{1, 38} = 0.547$, $p = 0.465$), Genotype ($F_{1,38} = 1.712$, $p = 0.199$). (KO: M = 4.464 +/- 0.242; WT: M = 5.435 +/- 0.230).

Discussion

The aim of this study was to define the role of AGE/RAGE signaling in the continuation of the aging process and in the natural physiological deficits that follow longevity. We hypothesized that knocking out RAGE systematically would delay the onset of cognitive and physical impairments seen in natural aging and decrease the molecular markers associated with advanced age. To test our hypothesis, we utilized behavioral tests to assess cognitive function (i.e., spatial learning and memory) as well as conducting assessments of physicality (i.e., vision, motor function, pain sensitivity).

When examining the effects of RAGE KO on cognition in early and mid-life, we observed impairments in spatial learning and memory, contrary to our original hypothesis. We believe this could have occurred due to unregulated AGE accumulation not being taken up by RAGE, leading to an increase of circulating AGEs. Subsequently, exerting negative effects on the central nervous system. As seen in prior studies, just the presence of circulating AGEs leads to mitochondrial dysfunction and cognitive impairment by enhancing the aggregation of glycated proteins, which increase cross-links in various parts of the brain.[19] These cross-links induce oxidative stress, which plays an important role in the development of neurodegenerative diseases.[19] AGEs have also been shown to contribute to amyloid precursor protein (APP) processing, which enhances the cell-death-related pathway and impairs the protective pathway of neuronal cells.[19] Similarly, when we examined the effects of RAGE-KO on

behavior, we observed impairments in circadian rhythm and increased anxiety-like behavior starting in early age. These behaviors could also correlate to oxidative stress induced by circulating AGEs. A longitudinal study (=5 yrs) aimed to investigate the effect of oxidative stress on sleep disorders demonstrated a significant worsening of sleep quality among patients with high levels of AGEs.[20] Another study demonstrated a strong correlation between diabetic patients and clinically diagnosed anxiety.[21]

When examining the effects of RAGE-KO on physicality, we observed impairments in vision and increased pain sensitivity contrary to our hypothesis. This could be explained by AGE interactions with other receptors that we did not account for. While our study focused specifically on the receptor for advanced glycation end products (RAGE), AGEs have a wide range of receptors, including AGE-R1, AGE-R2, AGE-R3, SR-A, SR-BI, cell surface glycoprotein CD36, lactoferrin, and lysozyme[26]. In addition, a novel scavenger receptor, the fasciclin EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1 (FEEL-1), and its paralogous gene, FEEL-2, have been characterized to be important endocytic receptors for AGEs that may play a significant role in the development of diabetic vascular complications, including retinopathy and neuropathy, leading to vision and mechanical sensitivity.[26]

Another possible explanation for the mismatch of our results with our hypothesis is that RAGE binding to non-AGE ligands may have important roles in early

development. One such ligand is high mobility group box 1 (HMGB1). HMGB1 is a damage-associated molecular pattern that is found in high levels in autoimmune diseases, tissue injury, and infection[27]. However, it may also play a regulatory role in the immune system by inducing anti-inflammatory macrophages under certain conditions[27], thus RAGE may play a homeostatic role in immune development. By knocking out RAGE, critical interactions with the HMGB1 ligand and other ligands that may be involved in healthy development are no longer being carried out, possibly contributing to the detrimental cognitive and physical effects that we observed.

Limitations and Future Directions

Our study had some limitations. For one, we only evaluated the systemic knock-out of one receptor, RAGE. Given the diversity of ligands and receptors that interact with AGE and RAGE, there may be more suitable methods than RAGE-KO for reducing AGE-related pathologies, or more selective strategies to block AGE/RAGE interaction without affecting other important pathways. We also did not perform mitochondrial staining to assess if number of mitochondria was affected by RAGE-KO. In addition, we did not follow our mice through all the way to the end of their natural lifespan. Mice were euthanized after midlife to obtain ex vivo cytokine levels.

Further studies should explore cohorts of mice that expand beyond mid-life with the aim of continuing to elucidate the role of AGE signaling during the aging

process and additionally, examining histological changes that could arise due to altered AGE/RAGE signaling and examining other AGE-receptor pathways and RAGE-ligand pathways that could potentially play a role in the prevalence of age-related risks.

List of References

(1) Franceschi, C.; Garagnani, P.; Morsiani, C.; Conte, M.; Santoro, A.; Grignolio, A.; Monti, D.; Capri, M.; Salvioli, S. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. *Front. Med.* **2018**, *5*, 61. https://doi.org/10.3389/fmed.2018.00061.

(2) Niccoli, T.; Partridge, L. Ageing as a Risk Factor for Disease. *Current Biology* **2012**, *22* (17), R741–R752. https://doi.org/10.1016/j.cub.2012.07.024.

(3) Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S. G.; Croteau, D. L.; Bohr, V. A. Ageing as a Risk Factor for Neurodegenerative Disease. *Nat Rev Neurol* **2019**, *15* (10), 565–581. https://doi.org/10.1038/s41582-019-0244-7.

(4) Kennedy, B. K.; Berger, S. L.; Brunet, A.; Campisi, J.; Cuervo, A. M.; Epel, E. S.; Franceschi, C.; Lithgow, G. J.; Morimoto, R. I.; Pessin, J. E.; Rando, T. A.; Richardson, A.; Schadt, E. E.; Wyss-Coray, T.; Sierra, F. Geroscience: Linking Aging to Chronic Disease. *Cell* **2014**, *159* (4), 709–713. https://doi.org/10.1016/j.cell.2014.10.039.

(5) Srinivasan, M. When Life Span Exceeds Health Span. *J GEN INTERN MED* **2015**, *30* (3), 267–268. https://doi.org/10.1007/s11606-014-3167-4.

(6) López-Otín, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. Hallmarks of Aging: An Expanding Universe. *Cell* **2023**, S0092867422013770. https://doi.org/10.1016/j.cell.2022.11.001.

(7) Hamczyk, M. R.; Nevado, R. M.; Barettino, A.; Fuster, V.; Andrés, V. Biological Versus Chronological Aging. *Journal of the American College of Cardiology* **2020**, *75* (8), 919–930. https://doi.org/10.1016/j.jacc.2019.11.062.

(8) López-Otín, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. *Cell* **2013**, *153* (6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039.

(9) Sell, D. R.; Lane, M. A.; Johnson, W. A.; Masoro, E. J.; Mock, O. B.; Reiser, K. M.; Fogarty, J. F.; Cutler, R. G.; Ingram, D. K.; Roth, G. S.; Monnier, V. M. Longevity and the Genetic Determination of Collagen Glycoxidation Kinetics in Mammalian Senescence. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (1), 485–490. https://doi.org/10.1073/pnas.93.1.485.

(10) Chaudhuri, J.; Bains, Y.; Guha, S.; Kahn, A.; Hall, D.; Bose, N.; Gugliucci, A.; Kapahi, P. The Role of Advanced Glycation End Products in Aging and Metabolic Diseases: Bridging Association and Causality. *Cell Metabolism* **2018**, *28* (3), 337–352. https://doi.org/10.1016/j.cmet.2018.08.014.

(11) Lin, L. RAGE Signaling in Inflammation and Arterial Aging. *Front Biosci* **2009**, *Volume* (14), 1403. https://doi.org/10.2741/3315.

(12) Luevano-Contreras, C.; Chapman-Novakofski, K. Dietary Advanced Glycation End Products and Aging. *Nutrients* **2010**, *2* (12), 1247–1265. https://doi.org/10.3390/nu2121247.

(13) Ramasamy, R.; Vannucci, S. J.; Yan, S. S. D.; Herold, K.; Yan, S. F.; Schmidt, A. M. Advanced Glycation End Products and RAGE: A Common Thread in Aging, Diabetes, Neurodegeneration, and Inflammation. *Glycobiology* **2005**, *15* (7), 16R-28R. https://doi.org/10.1093/glycob/cwi053.

(14) Fang, F.; Lue, L.-F.; Yan, S.; Xu, H.; Luddy, J. S.; Chen, D.; Walker, D. G.; Stern, D. M.; Yan, S.; Schmidt, A. M.; Chen, J. X.; Yan, S. S. RAGE-Dependent Signaling in Microglia Contributes to Neuroinflammation, Abeta Accumulation, and Impaired Learning/Memory in a Mouse Model of Alzheimer's Disease. *FASEB J* **2010**, *24* (4), 1043–1055. https://doi.org/10.1096/fj.09-139634.

(15) Schmidt, A. M.; Yan, S. D.; Wautier, J.-L.; Stern, D. Activation of Receptor for Advanced Glycation End Products: A Mechanism for Chronic Vascular Dysfunction in Diabetic Vasculopathy and Atherosclerosis. *Circulation Research* **1999**, *84* (5), 489–497. https://doi.org/10.1161/01.RES.84.5.489.

(16) Lane, C. A.; Hardy, J.; Schott, J. M. Alzheimer's Disease. *Eur J Neurol* **2018**, *25* (1), 59–70. https://doi.org/10.1111/ene.13439.

(17) Yeo, D.; Kang, C.; Ji, L. L. Aging Alters Acetylation Status in Skeletal and Cardiac Muscles. *Geroscience* **2020**, *42* (3), 963–976. https://doi.org/10.1007/s11357-020-00171-7.

(18) Senatus, L. M.; Schmidt, A. M. The AGE-RAGE Axis: Implications for Age-Associated Arterial Diseases. *Front Genet* **2017**, *8*, 187. https://doi.org/10.3389/fgene.2017.00187.

(19) Twarda-Clapa, A.; Olczak, A.; Białkowska, A. M.; Koziołkiewicz, M. Advanced Glycation End-Products (AGEs): Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs. *Cells* **2022**, *11* (8), 1312. https://doi.org/10.3390/cells11081312.

(20) Konishi, S.; Hatakeyama, S.; Imai, A.; Okita, K.; Kido, K.; Ozaki, Y.; Uemura, N.; Iwane, T.; Okamoto, T.; Yamamoto, H.; Yoneyama, T.; Hashimoto, Y.; Ohyama, C. Effect of Advanced Glycation End Products on Nocturia or Sleep Disorders: A Longitudinal Study. *BJUI Compass* **2022**, *3* (2), 162–168. https://doi.org/10.1002/bco2.114.

(21) Bickett, A.; Tapp, H. Anxiety and Diabetes: Innovative Approaches to Management in Primary Care. *Exp Biol Med (Maywood)* **2016**, *241* (15), 1724–1731. https://doi.org/10.1177/1535370216657613.

(22) Singh, N.; Baby, D.; Rajguru, J. P.; Patil, P. B.; Thakkannavar, S. S.; Pujari, V. B. Inflammation and Cancer. *Ann Afr Med* **2019**, *18* (3), 121–126. https://doi.org/10.4103/aam.aam_56_18.

(23) Yan, S. D.; Chen, X.; Fu, J.; Chen, M.; Zhu, H.; Roher, A.; Slattery, T.; Zhao, L.; Nagashima, M.; Morser, J.; Migheli, A.; Nawroth, P.; Stern, D.; Schmidt, A. M. RAGE and Amyloid-Beta Peptide Neurotoxicity in Alzheimer's Disease. *Nature* **1996**, *382* (6593), 685–691. https://doi.org/10.1038/382685a0.

(24) Peter-Derex, L.; Yammine, P.; Bastuji, H.; Croisile, B. Sleep and Alzheimer's Disease. *Sleep Med Rev* **2015**, *19*, 29–38. https://doi.org/10.1016/j.smrv.2014.03.007.

(25) American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* **2013**, *36 Suppl 1* (Suppl 1), S67-74. https://doi.org/10.2337/dc13-S067.

(26) Tamura, Y.; Adachi, H.; Osuga, J.; Ohashi, K.; Yahagi, N.; Sekiya, M.; Okazaki, H.; Tomita, S.; Iizuka, Y.; Shimano, H.; Nagai, R.; Kimura, S.; Tsujimoto, M.; Ishibashi,

S. FEEL-1 and FEEL-2 Are Endocytic Receptors for Advanced Glycation End Products. *J Biol Chem* **2003**, *278* (15), 12613–12617. https://doi.org/10.1074/jbc.M210211200.

(27) Watanabe, H.; Son, M. The Immune Tolerance Role of the HMGB1-RAGE Axis. *Cells* **2021**, *10* (3), 564. https://doi.org/10.3390/cells10030564.