The 2017 Barshop Symposium on Aging was held at the Mayan Ranch in Bandera, Texas, Texas Hill Country, October 12-15. The theme was Sex differences in aging: Mechanisms and responses to interventions. The conference organizers were Veronica Galvan, PhD, and James Nelson, PhD from the UT Health Sciences Center at San Antonio, TX. Abstracts from the meeting are presented in this issue, and represent a variety of aging and age-related studies. A large focus was on aging and neurodegeneration including a number of presentations on Alzheimer’s disease. Other topics included cardiomyopathy, frailty, metabolism, pharmaceutical intervention of aging, age-related epigenetic modification, and cancer and aging. This annual meeting is well-attended and provides a seclusive and rustic environment in the Texas Hill Country for small group presentations and extensive informal interactions and discussions on the very latest findings and current thinking in the causes and prevention of aging and age-related diseases.

**LDLR-related protein 1 increases cytokine sensitivity. Implications for recovery after brain damage**

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Patients that express the Apolipoprotein E4 are predisposed to a poor long-term outcome after stroke. Explanations for this increased risk are not yet elucidated. This study aims to test one possible mechanism by which ApoE4 contributes to cognitive decline after stroke. Here, we examine the effect of a major ApoE4 receptor, low-density lipoprotein receptor related protein 1 (LRP1) on sensitivity to stress in astrocytes. LRP1 can promiscuously bind and move several extracellular ligands and plasma membrane proteins into the endocytic system. Notably, LRP1 was previously found to remove the TNF receptor (TNFR1) from the plasma membrane, although this has not been shown in astrocytes. We propose that a similar mechanism occurs in the central nervous system to attenuate inflammatory response after stroke. LRP1 binds and clears ApoE4 from the extracellular space via receptor-mediated endocytosis. However, previous studies have shown that the ApoE4, compared to other ApoE isoforms, slows the trafficking and recycling of endocytic LDL receptors. We propose that ApoE4 similarly inhibits LRP1 trafficking, and hypothesize that ApoE4 inhibits the ability of LRP1 to remove TNFR1 from the plasma membrane. This is expected to increase cytokine sensitivity, which would result in worse outcome after stroke and with aging. We investigated the effect of LRP1 on astrocyte TNFα signaling and response in immortalized ApoE null mouse astrocytes subjected to lentiviral-mediated knockdown of LRP1. The astrocyte response to TNFα stimulation was tested in a concentration dependent manner using Western blotting of NFκB pathway components, which are the downstream mediators of TNFα signaling. We also tested astrocyte viability after prolonged TNFα stimulation using Alamar Blue reagent. We found that LRP1 deficient cells have increased phosphorylation of NFκB upon TNFα stimulation, and that loss of LRP1 resulted in significant loss of astrocyte viability after prolonged stimulation. Altogether, our results indicate that loss of LRP1 renders astrocytes more sensitive to TNFα. Future experiments will focus on treating astrocytes ApoE4 to determine if detrimental effects are exerted through LRP1, as well as testing the influence of LRP1 on recovery after middle cerebral artery occlusion in mice.

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**Elucidating the role of ALCAT-1 in dilated cardiomyopathy**

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Dilated cardiomyopathy (DCM) is a disease characterized by an abnormally large and weakened left ventricle which impairs the heart’s ability to pump blood. Due to its high mortality rate and high prevalence, DCM has been extensively researched and studied with a variety...
of treatment and symptom management options available. However, despite the many advances in the field, most cases are classified as idiopathic and most individuals die within two years of diagnosis. What has yet to be elucidated as a possible contributor to DCM is pathological cardiolipin remodeling. A major indicator that points to pathological cardiolipin remodeling being a primary contributor to DCM is the rare, x-linked genetic disorder Barth Syndrome. In this disease, individuals have a mutated tafazzin gene which causes a decrease in the predominant healthy species of cardiolipin known as tetralinoleoyl cardiolipin. Tetralinoleoyl cardiolipin has been shown to be drastically decreased in rodent models of heart disease as well as in humans with cardiomyopathy. Through investigating the role of cardiolipin remodeling on DCM and altering which cardiolipin species are present, it may open up novel treatment options for the disease and help provide greater insight into the cause of DCM.

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Evaluation of Long-Term Hippocampal NF-κB Suppression on Murine Behavior and Tau Protein Expression

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Tau proteins are most well known for their role in neurodegenerative diseases such as Alzheimer’s disease. Physiologic tau regulates and stabilizes microtubules within neuronal axons. Abnormal tau hyperphosphorylation leads to disruption of microtubule stabilization and subsequent tau aggregation, which can lead to neurodegeneration and dementia seen in Alzheimer’s disease. Although inflammation is known to be a major contributor to Alzheimer’s disease progression, its association with tau protein expression and regulation has not been significantly examined. Prior work by our research group has shown that inhibition of the transcription factor NF-κB, which directly regulates the expression of numerous inflammatory cytokines, increases tau protein expression in neuronal cells. In our ongoing studies, we are examining whether NF-κB regulates tau in vivo using stereotoxic delivery of AAV-IkBαDN super repressor or AAV-GFP targeted to young mouse hippocampal neurons. After long-term suppression of hippocampal NF-κB, cognition and behavior were evaluated in a blinded manner using assays including open field, novel object recognition, Morris water maze, and contextual fear. Significant differences were observed between NF-κB suppressed and control groups in behavioral analysis. Whole brain and hippocampal tissue were collected for immunofluorescence, protein and RNA evaluation of NF-κB suppression, tau protein expression and regulation, and other associated markers of neuro inflammation. Future experiments will investigate hippocampal-specific upregulation of NF-κB-associated inflammation. The results of this work will help elucidate novel mechanisms of tau protein regulation and provide understanding of the role of inflammation and tau in the initial pathogenesis of Alzheimer’s disease.

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Previous midlife estradiol treatment results in increased nuclear erα expression in the hippocampus of aging ovariectomized rats

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Work from our lab has demonstrated that previous midlife estradiol treatment improves memory in ovariectomized female rats months after hormone exposure has ended. Furthermore, midlife estradiol exposure results in lasting increases in levels of estrogen receptor alpha (ERα) in the hippocampus, an effect that mediates the memory enhancements. Traditionally, ERα acts as a nuclear receptor, initiating genomic effects including increased transcription of certain genes. More recently, ERs have been localized to the membrane. Activation of membrane ERα could result in non-genomic, rapid acting effects. The goal of the current work is to determine where ERα is localized following midlife estradiol treatment. Middle-aged rats were ovariectomized and implanted with hormone capsules containing either estradiol or vehicle. Forty days later, capsules were removed. One month after hormone treatment ended, rats were killed and hippocampi were dissected and processed for subcellular fractionation. Hippocampal lysate was homogenized and separated into cytosolic, membrane, and nuclear compartments using the protocol included with a commercially available kit. All samples (cytosolic, membrane, and nuclear) were further processed for western blotting for ERα. Previous estradiol treatment resulted in lasting increases of nuclear protein expression of ERα compared to vehicle-treated rats. There were only trace amounts of cytosolic ERα, regardless of hormone treatment. We found no differences in membrane ERα protein expression. Results demonstrate that in the aging female hippocampus, lasting increases in ERα protein expression following midlife estradiol treatment are due to increases in nuclear-localized ERα.
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Investigating loss of terminal neuronal differentiation in Alzheimer’s disease and related tauopathies

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Tauopathies are progressive neurodegenerative disorders that are defined histologically by deposits of insoluble, hyperphosphorylated tau protein in the brain. Alzheimer’s disease is the most common tauopathy, with reported cases approaching nearly 50 million, globally. Current FDA-approved treatments delay the breakdown of acetylcholine with modest, temporary improvements in cognitive function. Alternative strategies target amyloid-beta (Aβ) and neuro-inflammation, but have yet to arrest, alter, or reverse the neurodegenerative process. Recently, tau has manifested as a promising avenue for therapeutic intervention for many reasons. First, pathological tau correlates more closely with cognitive dysfunction than Aβ in Alzheimer’s disease. Second, deposits of Aβ plaques in an Alzheimer’s disease mouse model fail to induce neuronal loss in the absence of tau. Third, multiple mouse models demonstrate that tau acts downstream of Aβ to mediate neurotoxicity. Recently, our laboratory has reported pathological tau can over stabilize filamentous actin, which disrupts the lamin nucleoskeleton. Further, nucleoskeletal disruptions lead to relaxation of heterochromatin, which can drive abnormal cell cycle entry and gene expression. Preliminary data suggest that transcription factors that maintain a terminally differentiated state are downregulated in adult tau-transgenic Drosophila. Thus far we have identified three critical terminal selectors known for promoting neuronal differentiation such as prospero, midlife crisis, and nerfin-1 that are differentially expressed in our Drosophila model. We are currently testing the hypothesis that neurons harboring pathological tau fail to maintain a terminally differentiated state and lead to neuronal death. If correct, therapeutic strategies aimed towards maintaining terminal differentiation in neurons may ameliorate tau-induced neurotoxicity.

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The inverse relationship between body weight and longevity is stronger in males and weakens temporally until it reverses in senescence: Results from a large multi-site study in genetically heterogeneous mice

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The female survival advantage is one of the most robust characteristics of human longevity, but the underlying biological mechanisms are unknown. The National Institute on Aging’s Interventions Testing Program (ITP) was designed to overcome the limitations of inbred strains by evaluating lifespan-extending compounds in genetically heterogeneous Het3 mice. Our detailed analysis of the survival characteristics of ~1700 mice of each sex in this population has revealed a female survival advantage that parallels that of human populations. The female survival advantage is greatest in early adulthood and diminishes progressively thereafter. This finding is observed at the 3 ITP study sites and in 6 separate cohorts over a10-yr span. Here we explore the potential role of the commonly observed inverse relationship between body weight and lifespan in the sexual dimorphism in survival of Het3 mice. The inverse relationship between body weight and longevity, although present in both sexes, is much stronger in males. While the heavier weights of males accounts for at least part of their increased mortality, the effect of bodyweight on lifespan is larger in males than in females. At 24 months, nearing the median lifespan, the relationship between body weight and longevity shifts from negative to positive in both sexes, similar to the human condition.

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The adapted endoplasmic reticulum (ER)-associated protein quality control (ERQC) is critical for the long-lived Caenorhabditis elegans rpn-10 proteasome mutant

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The ubiquitin-proteasome system (UPS) protein degradation mechanism is integral for the optimal preservation of the proteome. Its reduced efficiency during aging results in protein misfolded and aggregation which potentiate several proteotoxic disorders. Paradoxically, our lab reported that the Caenorhabditis elegans proteasomal rpn-10(ok1865) mutant exhibits enhanced proteostasis and extended longevity. The RPN-10/PSMD4 subunit is a 19S regulatory particle (RP) ubiquitin receptor of the 26S proteasome that targets polyubiquitinated substrates to the 20S core for degradation. The rpn-10 mutant, which shows reduced proteasome-mediated ubiquitin-fusion degradation (UFD), is remarkably resistant to many proteomic challenges. While this may be attributed to its distinct proteasomal proteolysis activity, we also sought to elucidate its suite of protective mechanisms. Analyzing our RNA-sequencing data and genome-wide RNAi screen for UFD players, we found that several endoplasmic reticulum (ER) protein quality control (ERQC) genes were upregulated in the rpn-10 mutant. Therefore, we hypothesized that the rpn-10 mutant might exhibit enhanced ERQC or ER-associated protein degradation (ERAD). Indeed, I found that the rpn-10 mutant cytoplasmic proteostasis and longevity benefits are highly contingent on the ER master chaperone grp-3/4 (Bip/grp78) and ERAD ATPase cdc-48.2 (p97/VCP/CDC48). Additionally, the attenuated accumulation of the aggregation-prone ER substrate α-1 antitrypsin proves that ER proteostasis is ameliorated in the rpn-10 mutant. Moreover, the rpn-10 mutant exhibits higher ER stress resistance but lower ER stress induction than the wild-type, which suggests its optimized ER homeostasis. Therefore, I postulate that the rpn-10 mutant possesses adapted ERQC which critically links graded proteasomal function with improved proteostasis and increased lifespan.

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### Consequences of nucleoplasmic reticulum expansion in tauopathy: a possible role for aberrant nuclear RNA export in tau pathogenesis

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Laminopathies are a group of rare hereditary degenerative disorders characterized by aberrant nuclear architecture and genetic dysregulation that lead to progeroid or “advanced aging” phenotypes. Using a Drosophila melanogaster model of tauopathy and postmortem brain tissue from patients with Alzheimer’s disease, we have recently established a novel mechanism of acquired neuronal lamin dysfunction by which pathological tau triggers the formation of invaginations in the nuclear lamina of neurons. Interestingly, increased nuclear invagination has also been reported to occur with normal physiological aging, suggesting that exacerbation of this process by tau may underlie the association between aging and an increased risk of developing Alzheimer’s disease and related tauopathies. These invaginations, referred to as the “nucleoplasmic reticulum,” are thought to bring functions of the nuclear periphery, such as the nucleocytoplasmic transport of macromolecules, into the nuclear interior. We have found that the nuclear invaginations observed in patients with Alzheimer’s disease are lined with nuclear pores and previous studies have reported that nuclear invaginations often terminate at nucleoli, areas of high transcriptional activity. Based on these observations, together with our previous findings of heterochromatin relaxation in tauopathies, we hypothesize that increased transcription and nucleocytoplasmic export of RNA through these invaginations may underlie tau-mediated neuronal death. Preliminary studies support this hypothesis, as both genetic knockdown and pharmacological inhibition of nuclear RNA-export machinery ameliorate tau-induced neurodegeneration in vivo. Additionally, FISH/IF experiments have revealed a strong association of poly(A) RNAs co-localized with lamin invaginations in a Drosophila model of tauopathy.

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### Impact of pre-pubertal and adult gonadectomy on sex differences in impulsive behavior in adult rats

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The goal of the current work was to examine the influence of pubertal and adult gonadal hormones on sex differences on measures of impulsivity. Three sets of male and female rats were used in the study. The first set was gonadectomized prior to puberty (at 28 d of age), the second set was gonadectomized in adulthood (at 90 d of age), and the third set underwent sham surgeries and served as gonadally intact controls. Beginning at ~100 d of age, all rats were
trained on the 5-choice serial-reaction time task (5-CSRTT), a test for impulsive action. The task requires rats to identify (via nose poke) the location of a brief light stimulus among five possible locations. When training was completed, impulsive action, as measured by premature responding, was assessed during sessions in which the onset of the stimulus was unpredictably lengthened. After testing on the 5-CSRTT was completed, rats were trained on a delayed-based reward task that measures impulsive choice, demonstrated as aversion to delayed reward. The task requires rats to choose between a delayed large food reward and an immediate small food reward. On the 5CSRTT, adult males made significantly more impulsive (i.e. premature) responses across all hormone treatment conditions indicating that neither gonadal hormone actions during puberty nor during adulthood mediates the observed sex difference in impulsive action. On the delay-based reward task, no sex difference in impulsive choice was observed across all hormone treatment conditions. Future studies will examine neonatal organizational effects of gonadal hormone exposure on adult sex differences in impulsive action.

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**Second X chromosome decreases motor impairments in aging mice**

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Motor impairment is emblematic of frailty in aging. To combat this biomedical problem, it is crucial that we understand major factors involved. Sex is one such factor – females (XX) live longer than males (XY) and have reduced frailty in aging. We investigated the role of sex chromosomes in aging and frailty using XY* model mice. Progeny of XY* males crossed with XX females include four sex genotypes roughly equivalent to: XX and XY mice with ovaries, and XY and XXY mice with testes. A sexual dimorphism that varies by the presence or absence of a Y is Y-chromosome-mediated; one that varies by the presence of 1 versus 2 X’s, is X chromosome-mediated. We gonadectomized mice to decrease activational effects of gonadal hormones. We then assessed motor learning, memory, coordination, and strength using the rotarod task and grip measures. We found that all genotypes of aging mice were impaired across measures compared to young mice. The presence of a second X chromosome improved motor learning and memory in aging males and females – without altering grip strength or maximal motor performance. This indicates that the second X chromosome probably enhances neural mechanisms of motor learning, memory and coordination. Effects of the second X chromosome extended to young mice, suggesting that it may confer ‘functional reserve’ to counter impairments in aging. Our results indicate a role for the second X chromosome in countering effects of aging. Understanding how an additional X confers neural resilience could lead to novel therapies for women and men.

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**β-Guanidinopropionic acid as an intervention targeting health-span**

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The AMP-Activated Protein Kinase (AMPK) signaling pathway plays a key role in lifespan and health-span. In Drosophila, genetic manipulation of AMPK expression has been shown to extend lifespan. Similarly, the anti-hyperglycemic drug metformin, an AMPK activator, has been reported to increase lifespan in mice. Beta-Guanidinopropionic acid (β-GPA) is a naturally occurring compound similar to creatine that acts as a competitive inhibitor of creatine phosphokinase, and is thought to activate AMPK signaling by reducing the effective ATP concentration in the cell. Based on previous studies, we investigate both the mechanism and efficacy of β-GPA as a potential intervention to improve health-span across models of aging. In the first model, Drosophila are treated chronically with β-GPA starting at a young age to test whether this drug delays age-related declines in voluntary motility or intestinal barrier function. Using the powerful genetics of this model, we will also identify whether the effects of β-GPA require AMPK signaling and what specific downstream mediators are involved. In the second model, we test whether late-life intervention with β-GPA can delay declines in health span in genetically heterogeneous mice. HET3 mice at 20 months of age are treated with β-GPA in the diet. In contrast to previous studies in young rodents, we report that intervention with 1% β-GPA protects both lean and fat mass in males but not females when delivered late in life. Together these models will allow us to more precisely
define the mechanism and effectiveness of β-GPA in mediating health-span in multiple aging models.

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Early and sustained microglia activation contributes to age-associated reductions in neurogenesis

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The ventricular-subventricular zone (V-SVZ) is the largest neural stem cell (NSC) reservoir of the mammalian forebrain. However, NSC proliferation and neurogenesis is sharply reduced at mid-age through unknown mechanisms. Our studies establish microglia, the resident immune cells in the brain, as integral V-SVZ niche cells closely associated with NSCs, germinal pinwheels and the microvasculature. During aging, microglia undergo substantial positional changes within the niche, losing their close association to the vasculature while becoming increasingly associated with the ependyma and germinal pinwheels. We observed an early and chronic activation of V-SVZ microglia not seen in microglia outside of the niche during aging. This activation was accompanied by increased inflammatory mediators within the NSC compartment. A substantial increase of monocyte infiltration was observed within the aged V-SVZ niche, suggesting the peripheral immune system may also mediate V-SVZ inflammation during aging. Induction of sustained inflammation in young mice results in increased microglia activation accompanied by reduced proliferation in the V-SVZ and in vitro studies revealed secreted factors from activated microglia reduced proliferation and neuron production compared to secreted factors from resting microglia. Furthermore, minocycline treatment in aged mice reduces microglia activation, niche inflammation and partially restores proliferation in the aged niche. Interestingly, microglia depletion in the young V-SVZ results in a reduction of proliferation that is restored after microglia numbers are allowed to normalize. Our results suggest that age-associated chronic inflammation contributes to declines in NSC function within the aging neurogenic niche and microglia may sustain or negatively affect neurogenesis depending on age.

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Sexually divergent DNA methylation patterns with hippocampal aging

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DNA methylation is a central regulator of genome function and altered methylation patterns are indicative of biological aging and mortality. Age-related cellular, biochemical, and molecular changes in the hippocampus lead to cognitive impairments and greater vulnerability to neurodegenerative disease that varies between the sexes. The role of hippocampal epigenomic changes with aging in these processes is unknown as no genome-wide analyses of age-related methylation changes have considered the factor of sex in a controlled animal model. High-depth, genome-wide bisulfite sequencing of young (3 month) and old (24 month) male and female mouse hippocampus revealed that while total genomic methylation amounts did not change with aging, specific sites in CG and non-CG (CH) contexts demonstrated age-related increases or decreases in methylation that were predominantly sexually divergent. Differential methylation with age for both CG and CH sites was enriched in intergenic and intronic regions and underrepresented in promoters, CG islands and specific enhancer regions in both sexes suggesting that certain genomic elements are especially labile with aging, even if the exact genomic loci altered are predominantly sex-specific. Life-long sex differences in autosomal methylation at CG and CH sites were also observed. The lack of genome-wide hypomethylation, sexually divergent aging response, and autosomal sex differences at CG sites were confirmed in human data.
These data reveal sex as a previously unappreciated central factor of hippocampal epigenomic changes with aging. In total, these data demonstrate an intricate regulation of DNA methylation with aging by sex, cytosine context, genomic location, and methylation level.

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**Developmental prozac exposure sex-dependently reduces preference for sociability in female C57BL/6J mice**

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Autism is a developmental disorder that affects an alarming number of individuals (1 in 68 children in the US). Autism is persistent, so it impairs an individual throughout their lifespan, and often requires antipsychotic treatments, which increase incidence of age related diseases such as obesity, diabetes and heart disease with long-term use. This cycle imposes a great financial burden, as intensive health care is needed earlier in individuals with autism than in the general population. Our research goal is to reduce autism incidence by studying a potential environmental etiology of autism: developmental exposure to a selective serotonin reuptake inhibitor (SSRI) such as Prozac (fluoxetine). Our research hypothesis is that exposure to maternally administered fluoxetine (10 mg/kg/day) through gestation and lactation will increase autism-like behaviors in mice. Because serotonin plays many critical roles in early brain development, we also hypothesized that a dietary supplementation of the essential amino acid tryptophan(TRP) could prevent social and repetitive behavioral deficits from occurring in fluoxetine exposed offspring. So far, we have found female offspring from dams receiving this clinically relevant dose of Prozac throughout pregnancy and lactation displayed decreased social interaction preference, which was not rescued by the tryptophan supplement. Pilot measures of offspring serotonin and its metabolite Hydroxyindoleacetic acid show there may be reduced serotonin turnover in the brains of fluoxetine-exposed offspring. Also fluoxetine treated dams took longer to get pregnant, and weaned fewer pups than vehicle controls. This work lays the foundation for understanding how serotonin may be an autism risk factor.

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**Sex-differences in lifespan-extension with acarbose and 17-α estradiol: gonadal hormones underlie male-specific improvements in glucose tolerance, motor function and muscle aging**

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Interventions that extend lifespan in mice can show substantial sexual dimorphism. We find that male-specific lifespan extension with two pharmacological treatments, acarbose (ACA) and 17-α estradiol (17aE2), is associated, in males only, with a range of metabolic benefits in adulthood and anti-aging effects on neuromuscular and cardiovascular function later in life. Females, which show either smaller (ACA) or no lifespan extension (17aE2), do not derive these benefits from drug treatment. We find that male-specific metabolic improvements in adulthood are associated with enhanced hepatic mTORC2 signaling and increased Akt activity – changes that might promote metabolic health and survival in males. By manipulating sex-hormone levels through gonadectomy, we show that sex-specific changes in these metabolic pathways are modulated, in opposite directions, by both male and female gonadal hormones. Male castration, in particular, inhibits males from showing metabolic improvements with either drug treatment. Castrated males also do not gain the anti-aging benefits for neuromuscular function that these drugs provide in intact males. Our results demonstrate that drugs generating sex-specific lifespan extension can also improve mouse metabolism and slow age-related declines in functional traits in a sex-specific way. These sex-specific effects are influenced by the presence of gonadal hormones produced in adulthood, suggesting that gonadally-derived hormones contribute to sexual dimorphism in response to interventions that extend mouse lifespan.

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**HP1γ in sexual dimorphism and telomere maintenance**

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**Pathobiology of aging & age-related diseases**
Epigenetic modifications of chromatin play many roles in chromatin structure and gene expression. It has been suggested that abnormalities in the epigenetic code might lead to sexual dimorphic diseases. Heterochromatin protein 1 (HP1)-alpha, beta and gamma are epigenetic modifiers of chromatin important for regulating gene expression, centromere stability, and telomere capping. Unlike the other two HP1 isoforms, HP1 gamma is present in both euchromatin and heterochromatin, and previous data shows that it plays a role in both the repression and expression of genes in a sexually dimorphic fashion. We have preliminary data suggesting that HP1 gamma binds preferentially to female telomeres. In this preliminary study, wild type and HP1γ knock-out primary mouse embryo fibroblasts were assayed for telomere instability, fusion and loss. Our results obtained from HP1γ−/−female cells showed significantly higher telomere instability compared with cells from wildtype littersm. These results implicate HP1γ in preventing telomere fragility, which, due to its preferential binding to female telomeres, may provide a novel mechanism for sexual dimorphism.

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Reversing age-related decline using non-cytotoxic transplantation of young hematopoietic stem cells

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Aging of the hematopoietic system is associated with various blood disorders in the elderly. Treatment options for these patients are limited to transplantation of compatible donor hematopoietic stem cells (HSCs). Despite over 40 years of progress and innovation, hematopoietic stem cell transplantation (HSCT) is reserved for patients diagnosed with life-threatening diseases due to cytotoxic pre-conditioning such as irradiation or chemotherapy and graft versus host complications. The long term objectives for the proposed research is to develop a non-cytotoxic stem cell therapy tailored to reverse aging, with the ultimate goal of treating humans. Rejuvenation of aged HSCs has been achieved through pharmacological, genetic manipulative, and caloric restrictive methods. However, permanent damage to bone architecture from cytotoxic transplantation limits health-associated benefits from rejuvenated HSCs. In this application, we will employ our novel (patent pending) transplantation regime, free from the deleterious effects of current HSCT methods, to investigate the health-associated benefits of transplanting young HSCs into aged recipients. During preliminary studies, we have achieved 90% donor engraftment and a 17% increase in median lifespan in aged recipients compared to non-transplanted controls using our non-cytotoxic pre-conditioning regime. Based on these findings, I hypothesize that transplantation of young hematopoietic stem cells overcome extrinsic influences from the aged microenvironment to reverse the age-associated phenotype of the hematopoietic system. This hypothesis will be tested by pursuing the following three aims: 1) to establish age-associated phenotype in young stem cells within the aged niche; 2) to determine the overall lifespan of older recipient mice transplanted with young donor stem cells; and 3) to determine the effects of young stem cell transplantation on age-related frailty. The first aim examines the extent of which extrinsic factors from the aged microenvironment influences well established age-associated phenotypes of HSCs. This will be accomplished by investigating these phenotypes in young HSCs after prolonged exposure to the aged microenvironment via our non-cytotoxic HSCT method. The second aim includes a longevity study to distinguish the health-associated benefits of transplanting young HSCs into an older recipient. Since young HSCs exhibit young phenotypic features, they would represent rejuvenated HSCs as the “ideal” model to examine rejuvenation of the hematopoietic system. The third aim will investigate if young donor HSCs prevent or delay the onset of frailty by reducing chronic inflammation. The proposed research addresses essential questions before this technology can be translated into a clinical setting. This approach is particularly attractive because, in addition to eliminating the need for irradiation or chemotherapy, each of the essential reagents have previously been approved for human use through the FDA. Thus, the proposed research has the potential to improve current transplantation regimes by replacing traditional HSCT methods with our novel approach and may lead to the development of the first cell-based anti-aging therapy.

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**mTOR promotes BBB breakdown and dysregulation of tight junction proteins in an Alzheimer’s disease model**

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Cerebral amyloid angiopathy (CAA) is characterized by fibrillar amyloid β (Aβ) association with cerebrovascularity, which leads to impaired brain vascular function, and is present in 87% of people with Alzheimer’s disease (AD). Previously, it has been shown that inhibition of mTOR by rapamycin prevented vascular leakage in 18-19 month old Tg2576 mice – a mouse model that mimics AD-associated CAA. This finding suggests that mTOR plays a role in regulating the integrity and permeability of the blood brain barrier (BBB). To further expand on this study, the abundance of tight junction proteins, zonula occludens 1 (ZO-1), occludin, and claudin -5 were examined using immunofluorescent confocal microscopy on frozen brain tissue sections of the same Tg2576 mice from the previous study. Together, these studies demonstrate that attenuation of mTOR by rapamycin preserves BBB integrity by decreasing the amount of vascular Aβ accumulation, which also showed a reduced likelihood of cerebral micro hemorrhages and an increase in tight junction protein abundance in our transgenic Tg2576 mice. Therefore, these data suggest that mTOR is a key component in vascular Aβ accumulation, BBB breakdown, vascular dysfunction, and BBB permeability in the Tg2576 mice. Thus, the data suggests that using mTOR inhibitors such as rapamycin which is an FDA approved drug, may be therapeutic in the pathogenesis of AD and other dementias with related cerebrovascular dysfunction.

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**Prion-like properties of tau oligomers trigger brain vascular endothelial cell dysfunction**

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Extracellular amyloid plaques and intracellular neurofibrillary tau tangles are the two major hallmarks of Alzheimer’s disease. Tau protein is involved in the stabilization of the microtubule cytoskeleton in neurons and other cell types. When tau becomes hyperphosphorylated, it disassociates from microtubules and forms tau oligomers which can then further aggregate into neurofibrillary tangles. It has been proposed that hyperphosphorylated, misfolded tau species propagate between neurons in a prion-like fashion, causing the aggregation of native tau in the target cell. Here we show that tau oligomers can propagate to endothelial cells. Our *in vitro* primary human brain microvascular endothelial cells (HBECS) showed that tau oligomers are taken up by HBECS, and cause increased endogenous tau phosphorylation, followed by a decrease in microtubule density and stability. Activation of endothelial nitric oxide synthase (eNOS), a critical event in the pathway of NO release by vascular endothelial cells, is partly regulated by localization and trafficking to the cell surface via the microtubule cytoskeleton. Consistent with the observed decrease in microtubule cytoskeleton density and stability, we showed that eNOS activation is decreased in HBECS treated with oligomeric tau. Furthermore, our data shows that oligomeric can induce endothelial cell senescence. Expressions of several markers of senescence were increased after exposure to oligomeric tau including p16, p21, and IL-6. Together these data suggest that oligomeric tau can impair endothelial cell function as measured by the activation of eNOS and endothelial senescence.

Taken together with prior studies showing that misfolded tau can propagate in a prion-like fashion between neurons, our data suggest that tau oligomers can be specifically internalized by brain vascular endothelial cells and cause misfolding of endogenous native tau protein, destabilizing microtubules and decreasing the activation of eNOS. Because brain vascular dysfunction mechanistically contributes to the etiology of Alzheimer’s disease, cerebrovascular oligomeric tau may be a novel target for therapeutic development in Alzheimer’s disease.

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**Increased hepatic steatosis and acylcarnitine levels in mice treated with formoterol, a beta2-adrenergic receptor agonist**

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The role of microtubule cytoskeleton in regulating nuclear architecture and gene expression during aging

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Microtubules (MTs) are essential cytoskeleton involved in cell division, shaping the cell and intracellular transport. Mutations in tubulin genes or microtubule-associated proteins have been reported to affect neuronal integrity and function during aging. We have recently found that mutations in microtubule regulating genes can modulate C. elegans lifespan. Our data suggest that the effect of microtubule stability on lifespan is dependent on DAF-16, the sole C. elegans FOXO homolog that function as a transcription factor in the insulin signal pathway. Among the previously identified direct targets of DAF-16, unc-84 encodes an inner nuclear membrane protein that connects microtubule cytoskeleton and nuclear envelope. Interestingly, we found that the lifespan extension phenotype induced by microtubule stabilization could be partially blocked by loss of UNC-84, while loss of UNC-84 alone does not affect lifespan. We also found that mutations in microtubule regulating genes can affect neuronal nuclear size and shape. Together these data suggest that the effect of microtubules on aging might be partially through regulating nuclear architecture and gene expression. We are currently examining the effect of microtubule disruption on nuclear organization and gene expression by confocal imaging, ATAC-seq and RNA-seq.

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Creating a Drosophila melanogaster model of prion-like tau protein spread

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Tauopathies are a class of neurodegenerative diseases characterized by the accumulation of fibrillar tau protein aggregates. In Alzheimer’s disease, tau pathology propagates hierarchically over time and appears to spread along brain circuitry. Recently, mounting evidence has surfaced to support the hypothesis of a prion-like mechanism for tau spread. Tau can spread between cells and form stably transmitted strains in vitro and in vivo; however, the mechanisms underlying this have not been well characterized. We propose to create a Drosophila melanogaster model of the prion-like spread of pathogenic tau based on the Drosophila olfactory circuitry, part of which consists of mushroom body output neurons (MBONs) projecting to five target regions. We will use a two-pronged approach to model the key features of prion-like protein propagation: 1) spreading of tau to other cells and 2) a capacity to recruit native tau to seed aggregation. A split-Gal4 system will direct expression of a seed-competent tau mutant to a subset of MBONs. The spread of tau will then be assessed by immunofluorescence. To detect the capacity of tau to spread to synaptically connected regions and seed aggregation of native tau protein, the same split-Gal4 system will be used to express seed competent tau fused to the N terminal half of luciferase (Luc\textsuperscript{N}) in...
MBONs, while a LexA system will drive tau<sup>WT</sup> fused to the C terminal half of luciferase (Luc<sup>C</sup>) expression in target regions. Luc<sup>N</sup>-tau spreading and recruiting the local Luc<sup>C</sup>-tau to seed aggregation will reconstitute functional luciferase and emit bioluminescence. A <i>Drosophila</i> model of tau propagation will allow us to investigate the mechanisms controlling this phenomenon, with the long-term goal of developing novel treatment strategies. In this context, a bioluminescence-based model of tau propagation is well suited for screening of genetic and pharmacological interventions that interfere with putative transmission mechanisms.

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**Testing the aldehyde hypothesis of Parkinson’s disease: the role of α-synuclein**

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Parkinson’s disease (PD) is the second most common neurodegenerative disorder. The cause of PD remains unclear, but many studies implicate oxidative stress. Biogenic aldehydes are among the most destructive sources of oxidative stress. The aldehydes, 4-hydroxynonenal (4HNE), the end-product of lipid peroxidation, and 3,4-dihydroxyphenylacetaldehyde (DOPAL), an intermediate in dopamine metabolism, have been shown to be elevated in brains of PD patients. Other studies link α-synuclein (αSyn) to PD. Gene mutations in αSyn in mouse models are associated with behavioral and pathological manifestations of Parkinson’s disease, providing evidence that αSyn plays a role in the neuropathology of this disease. Biogenic aldehydes have been reported to promote the formation of neurotoxic αSyn oligomers, while blocking the formation of less toxic fibrils <i>in vitro</i>. Our working hypothesis is that αSyn is mechanistically related to the behavioral, neuropathological and neurochemical manifestations of PD resulting from elevated biogenic aldehydes. To test this hypothesis we crossed mice overexpressing human wildtype αSyn under the control of the Thy1 promoter (Thy) with mice homozygous null for genes coding for the only two aldehyde dehydrogenase isozymes known to be expressed in nigrostriatal dopamine neurons, Aldh1a1 and Aldh2 (DKO). The result is a triple transgenic line (TTG) with overexpression of αSyn in the presence of elevated levels of biogenic aldehydes. In initial studies, the TTG genotype was associated with exacerbated motor deficits on the accelerate rotarod, pole test, adhesive removal test, and impaired gait performance, as compared to wild-type mice and their genotype controls. L-DOPA treatment is used clinically to diagnose PD. Therefore, we tested the effects of L-DOPA on motor performance in our models of PD. The results will be discussed.

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**Phosphatidylglycerol remodeling regulates ER linked mitochondrial fission and programmed cell death**

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Mitochondria undergo excessive fission during the manifestation of programmed cell death but the underlying cause remains unclear. Accumulating evidence shows that phosphatidylglycerol (PG), a phospholipid known for its role in cardiolipin synthesis, increases dramatically prior to cell death. However, how PG may be involved in programmed cell death is unknown. Given that cardiolipin detachment from mitochondria is one of the commitment steps in programmed cell death and PG levels spike during programmed cell death, we decided to investigate a possible mechanism by which PG may regulate programmed cell death. We found that newly remodeled PG induces programmed cell death via a mechanism that links mitochondrial depolarization, excessive fission, calcium overload, with plasma membrane permeabilization. We show that PG induced programmed cell death can be blunted by DIDS, necrostatin-1, and cycloheximide as well as exacerbated by pro-apoptotic TNFα, LCL161, and ABT-737. Furthermore, we report an unexpected role of PG on induction of mitochondrial fragmentation, of which we found to occur independent of Drp1. Taken together, our work puts forward a novel mechanism by which PG remodeling may play a fundamental role in the regulation of the different forms of programmed cell death.

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Can changes to the neuronal proteasome system delay age related declines?

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Disrupted proteostasis, increased damage and aggregated proteins are hallmarks of aging. A concomitant age-associated decline in proteasome activity has been reported across phyla and in multiple tissues in both mice and humans. The Pros5 proteasome subunit has been reported by our group and others to be rate limiting to proteasome synthesis. We have found that overexpressing the Pros5 subunit increases activity and function of both the 20S and 26S proteasome systems. When Pros5 expression is directed to the nervous system we see an extension in median and maximum lifespan. We also observe an amelioration of age-related cognitive deficits. Intriguingly, our data suggests that this effect may be non-cell-autonomous; boosting neuronal proteasome appears to drive whole organismal changes in the proteasome system. We are now attempting to determine whether the lifespan extension and delay in cognitive deficits are driven by changes to the 20S or 26S proteasome system and to develop a mechanistic understanding of the how neuronal proteasome can drive whole organism proteasome induction.

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SAD-A kinase function in pancreatic β-cells

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Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and β-cell failure, which leads to the depletion of islet β-cell mass. Understanding the molecular triggers of postnatal pancreatic β-cell proliferation may facilitate the development of regenerative therapies for diabetes. The key players during β-cell expansion are tuberous sclerosis complex 1 and 2 (TSC1/2) and the mechanistic target of rapamycin complex 1 (mTORC1) cascade, which integrate growth factors and nutrients. However, how TSC/mTOR signaling regulates β-cell proliferation is not completely understood. The Synaptes of Amphids Defective protein kinase A (SAD-A), also called brain serine/threonine kinase (BRSK2), belongs to the AMP-activated protein kinase-related kinase (AMPK) family; it is primarily expressed in the brain and pancreas, and little in the testis. SAD-A kinase is the first tissue-specific mediator of glucose response in islet β-cells, and it controls islet β-cell size by mediating mTORC1 signaling [1-3]. The preliminary studies showed overexpression of SAD-A increased β-cell proliferation from both cultured islets and the MIN6 β-cell line, whereas knockout SAD-A increased β-cell apoptosis during multiple low-dose streptozotocin treatments in pancreas-specific knockout SAD-A mice and controls. Furthermore, the target deletion of SAD-A in the pancreas would inhibit islet proliferation response to a high-fat diet by decreasing the bromodeoxyuridine (BrdU) incorporation rate and cyclin D2 mRNA was elevated in SADA knockout mice. These results led to hypothesize that SAD-A controls postnatal pancreatic β-cell growth by triggering the entry of quiescent β-cells into the cell division cycle.

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mTOR regulates PICALM in brain vascular cells

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Advanced age is the greatest known risk factor for the development of Alzheimer’s disease (AD). A hallmark of AD is the accumulation of amyloid beta (Aβ) in brain, the majority of which is cleared from brain into the bloodstream across the blood-brain barrier (BBB) via transcytosis mediated by the low-density lipoprotein receptor-related protein 1 (LRP1). Several recent genome-wide association studies (GWAS) have implicated the allele rs3851179 near the phosphatidylinositol-binding clathrin assembly protein (PICALM) gene as a protective against the development of AD. This allele is associated with an increase in mRNA expression of PICALM, which is abundant in brain endothelial cells (EC). PICALM binds to LRP1 to initiate transcytosis by recruiting clathrin to form clathrin-coated vesicles, ultimately removing toxic Aβ from the brain.

In the present study, we tested the hypothesis that mTOR may contribute to increased Aβ brain levels by reducing LRP1-mediated transport across BBB via a reduction in PICALM. To test this hypothesis, we measured protein levels and mRNA expression of PICALM and LRP1 in cultured endothelial cells and in brain microvasculature of transgenic AD mice that were treated with control or with the mTOR inhibitor rapamycin. While mTOR attenuation did not affect PICALM nor LRP1 mRNA expression levels, our studies demonstrated significant increases in
PICALM protein levels in rapamycin-treated cultured EC and in microvasculature of rapamycin-treated AD mice, suggesting that mTOR attenuation may increase PICALM in vitro and in vivo. The observed increases in PICALM levels as a result of rapamycin treatment were recapitulated by knock-down of Raptor, the obligatory companion of mTOR complex 1. Our data suggest that mTOR-mediated reduction of PICALM may contribute to increased brain Aβ levels in mice modeling AD, thus revealing a novel mechanism by which mTOR attenuation may reduce brain Aβ levels and significantly delay or stop the progression of AD.

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17α-estradiol inhibits oxidative stress and Aβ release in two in vitro models of Aβ production

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Alzheimer’s disease (AD), a progressive age-related dementia, diagnosed at autopsy by the presence of β-amyloid (Aβ) plaques, neurofibrillary tangles, and severe neuron loss. Although the etiology of AD is currently unknown, oxidative stress from the production of reactive oxygen species (ROS) causes neural apoptosis and stimulates Aβ production. In this study, we tested the hypothesis that using 17α-estradiol (17αE2), the non-feminizing structural analog of 17βestradiol (17βE2), might protect against apoptosis and Aβ deposition in two transgenic neuroblastoma mouse cell lines associated with Aβ production. The N2a-APP695 cell line expresses the Swedish mutation of amyloid precursor protein, APP695; the N2a-APP695/PS1DE9 cell line coexpresses PS1DE9, a presenilin (CTE) and over twenty others. Mechanisms mediating tau toxicity are poorly understood. Tau-containing neurofibrillary tangle (NFT) accumulation is the closest correlate with cognitive decline and cell loss, yet NFT-containing neurons do not die suggesting the involvement of secondary mechanisms. To gain insight into NFT-mediated toxicity, we evaluated gene expression patterns of NFT-containing neurons microdissected from AD patient brains. We find that they display a gene expression profile consistent with cellular senescence. This complex stress response induces permanent cell cycle arrest in dividing cells, apoptosis resistance, cellular remodeling and metabolic dysfunction. Senescent cells induce chronic degeneration of surrounding tissue through the secretion of pro-inflammatory, pro-apoptotic molecules termed the senescence-associated secretory phenotype (SASP). Using transgenic mouse models of tau-associated pathogenesis we find that NFTs induce a senescence-like phenotype in the brain including...
DNA damage, karyomegaly, mitochondrial dysfunction and SAP. Cdkn2a transcript level, a hallmark measure of senescence, directly correlates with brain atrophy and NFT load. We find this relationship extends to humans with PSP suggesting a phenomenon common to tau toxicity. Moreover, like in the human population where women have increased risk of developing AD, we find female mice to be more greatly affected by cellular senescence to suggest its role in sex-dependent pathophysiology. In summary, we identify cellular senescence, the quintessence of latent tissue degeneration, as an anti-apoptotic mechanism of NFT-containing neurons in AD. Our results suggest that NFTs formed in early pathogenic stages may contribute to neurodegeneration through senescence-like mechanisms by altering the bioenergetic state of the brain and inducing the release of toxic SASP. Therapeutically targeting senescent cells, or their secondary pro-apoptotic byproducts, may interrupt perpetual chronic neurodegeneration common to AD and >20 additional tau-associated diseases, and is an area of our ongoing research.

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HSP25 overexpression extends lifespan through protective aggregation in an HSF1 dependent manner

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Long-lived animals show resistance to a broad spectrum of environmental stressors. Our previous work indicated that this is attributed in part to altered changes in molecular chaperone levels that influence the transport and disposal of damaged proteins. Particularly striking were the high levels of the small molecular weight chaperone heat-shock chaperone 25 kDa (HSP25) and its putative transcription factor heat shock factor-1 (HSF-1). These showed as significant correlation with rodent longevity and were 5-fold higher our longest-lived species compared to the shortest. This suggests that HSP25 may play a key role in age-related maintenance of protein homeostasis in long-lived animals. To examine if this correlation was more causal we constructed transgenic C. elegans worms which ubiquitously overexpress HSP25 fused to GFP. Worms overexpressing this construct show resistance to heat stress (20% increase in mean survival) and have a 25% extension of lifespan. This lifespan extension depended on the expression of Hsf-1 but not Daf16 as determined by RNAi experiments. Generally, an increase life span and heat resistance corresponds with decreased aggregation. However, when we crossed our longer-lived, heat-resistant worms with worms containing a repeat of 44 glutamines (PolyQ) targeted to the worm intestine (Q44::YFP) we surprisingly observed that the HSP25 transgenic worm had a higher percentage of animals showing aggregation and more aggregates per worm than the control-crossed worm. Yet the lifespan of the HSP25 transgenic animals was still longer. This could suggest a role for this protein in promoting controlled aggregation which then removes unstable and/or damaged proteins by packaging them into insoluble aggregates. In theory, these now sequestered, aggregated proteins might be removed by autophagy or other protein degradation pathways which we are currently testing. We are also testing whether this phenomenon is specific to PolyQ aggregates or is a general process by examining HSP25 overexpression in worms containing control and mutant human tau. The sequestration of these aggregate-prone structures by HSP25 and other sHSPs could be a protective mechanism within the cell.

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Serum proteins mediate gender’s association with dementia

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Background: Alzheimer’s Disease (AD) is diagnosed in women more often than in men, and female gender is a risk factor for incident dementia and MCI conversion. The latent variable “δ” (for “dementia”) appears to be uniquely responsible for the dementing aspects of cognitive impairment. Age, depression, the apolipoprotein E (APOE) e4 allele and gender are independently associated with δ. Methods: In this analysis, we explore serum proteins as potential mediators of gender’s specific association with δ in a large, ethnically diverse, longitudinal cohort, the Texas Alzheimer’s Research and Care Consortium...
Scores. Models were adjusted for age, APOE, education, ethnicity, depression ratings, homocysteine and hemoglobin A1c. Significance was Bonferroni corrected to $p < 0.001$. Significant effects were replicated in random 50% subsets of TARCC’s sample. Results: 17 proteins were found to be full or partial mediators of gender’s effect on δ. Several sex hormones (i.e., FSH, LH, and testosterone) strongly moderated gender’s effect. Prostatic acid phosphatase (PAP) fully mediated gender’s effect. Other sex hormones (i.e., progesterone and prolactin) were not associated with gender in this (post-menopausal) sample. The remaining 13 proteins had partial mediation effects. Most were weak, but Thyroxine Binding Globulin (TGB) explained a sizable fraction of Gender’s effect (75.5%). Gender’s mediators did not overlap substantially with those we have previously associated with age, or depression. However, C-reactive protein (CRP) mediates both Gender and APOE’s effects, and may explain Gender’s published interaction with APOE’s dementia risk. Similarly, BDNF’s association with dementia is reported to be gender specific, consistent with our findings. Conclusions: This analysis identifies serum protein targets for the modulation of gender effects, and may explain fundamental differences at the cellular level (i.e., XX or XY genetics). Our future studies will address these questions as well as test whether these basic cellular sex differences might contribute to the robust survival advantage of women over men globally.

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**Metabolic effects of donor sex persist ex vivo in primary cell cultures**

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While there are numerous significant sex differences in physiological characteristics, the basis for these sexual dimorphisms is not completely evident. Sex hormones certainly play a significant role, though evidence is growing that basic genetic differences may also differentiate males from females. To test this idea, we generated lines of primary fibroblasts from young adult HET3 mice that were expanded under standard culturing conditions. That is, native cells derived from mice were exposed to normal in vivo environments, including sex hormones, through 12 weeks of age but after isolation were grown in media containing identical culture media and serum. These cell lines retained metabolic properties dependent on their genetic sex/sex of donor even after more than 3 weeks in culture. Fibroblasts from male HET3 mice display higher basal oxygen consumption rates (+27%), are less resilient to mitochondrial uncoupling due to FCCP (+42%), and devote more of their oxygen consumption to ATP generation (+27%) than do female-derived cells, suggesting an increased need for energy utilization in genetically male cell cultures. For each, the variability among male-derived cells in each of these measures was approximately two-fold that of female-derived cells. It is unclear whether sexual differences in cellular physiology level are due to previous exposure to sex-specific hormone concentrations in vivo or to fundamental differences at the cellular level (i.e., XX or XY genetics). Our future studies will address these questions as well as test whether these basic cellular sex differences might contribute to the robust survival advantage of women over men globally.

Advanced age is the greatest risk factor for most chronic diseases, yet pathogenic mechanisms tend to be highly tissue specific and sex dependent. In brain, intraneuronal inclusions of tau protein are the most common pathology. Collectively referred to as “tauopathies,” these diseases encompass over 15 distinct disorders. Alzheimer’s disease (AD) is the most common tauopathy and disproportionately affects women, but through unknown mechanisms. To investigate tau-associated pathogenesis and sex-dependent effects, we use transgenic mice that model human AD tau pathogenesis, referred to here as tauNFT. Like humans, female tauNFT mice develop more severe pathology, neurodegeneration and cognitive decline than male mice. During tau pathogenesis, neurons accumulate hyperphosphorylated tau, soluble tau oligomers and eventually large insoluble neurofibrillary tangles (NFTs). NFTs are the closest histopathological correlate with neuron loss and cognitive decline in AD, but the
neurons with NFTs do not die. Thus, the contribution of NFTs to AD pathophysiology remains unknown but suggests the contribution of alternative pathways and mechanisms. Using our mouse model of tauopathy, we recently found evidence of the involvement of cellular senescence, a cell stress pathway known to exert toxicity through non-cell autonomous mechanisms. Moreover, we found this cell stress pathway to be significantly higher in female than male mouse brains. However, when the endogenous mouse tau gene, Mapt, is geneti-
cally removed, female and male mice become equally affected indicating that removal of endogenous mouse tau may uniquely benefit female mice. We also find significant, and sex-dependent, changes in body weight with Mapt removal indicating its expression contributes to sexual dimorphic physiological traits. We are now using transgene suppression studies and stereotaxis to identify the brain region(s) responsible for these sex-specific effects. By using both male and female mice, the interplay among sex-specific differences among tau pathology, senescence, cognitive decline and obesity will be determined.

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Impact of sex on thyroid hormone protection against stroke

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Stroke is a leading cause of death and disability in the United States with a disproportionate impact on the elderly, who have a higher stroke risk and are less likely to recover from neurological and physical impairments. Research in our lab has identified acute treatment with thyroid hormone as a protective strategy against a photothermoclastic model of permanent ischemia, but the influence of sex on cerebral photothermoclastic neuroprotection has not previously been reported. After collecting the brains of mice 24 hours post-stroke, we observed no significant difference between vehicle-treated male and female mice (t-test, p=0.39). However, we did detect an impact of sex on the thyroid hormone dose required to effectively reduce lesion size when treatment was delivered 30 minutes after stroke (two-way ANOVA, p=0.0001). Furthermore, we found that while the optimal dose for neuroprotection in female mice remains effective when administered up to 12 hours post-stroke, protection in males conferred by the 30-minute optimal dose rapidly declines as the time-to-treatment period increases (one-way ANOVA, p=0.02). Neuroprotection up to 12 hours post-stroke is restored by increasing T3 concentration, suggesting a time-dependent sensitivity to thyroid hormone treatment in male, but not female, mice (one-way ANOVA, p=0.01). In conclusion, our observations indicate an influence on sex on the thyroid hormone-mediated mechanism that mitigates brain damage following ischemic stroke.

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Loss of TMEM127 in liver protects against insulin resistance

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Inhibition of mTOR complex 1 (mTORC1) by rapamycin increases lifespan in mice and is clinically used to treat cancer. However, long term treatment of rapamycin also inhibits mTOR complex 2 (mTORC2) and promotes insulin resistance, limiting its use as an anti-aging or anti-cancer agent. TMEM127 is a lysosomal protein that impinges on mTORC1 through unknown mechanisms. To better characterize the TMEM127 gene we have generated mice with targeted deletion of Tmem127 under a generic promoter. Chow diet-fed Tmem127 knockout (KO) mice have reduced body weight and fat mass, and are protected from age-related insulin resistance. When fed a high fat diet (HFD) the KO mice gained fat mass but were remarkably protected from hepatosteatosis-and diet-induced insulin resistance. mTORC2 complexes were more abundant in the liver of adult KO mice, both under chow and after HFD, in support of their higher insulin sensitivity. Chronic treatment with rapamycin caused downregulation of mTORC1 and mTORC2 signaling, but unlike WT, KO mice did not develop insulin resistance and their livers retained higher levels of mTORC2 complex. Liver-specific, but not adipose specific ablation of Tmem127 in mice resulted in increased insulin sensitivity suggesting a liver-specific role for Tmem127 in modulation of insulin sensitivity. We have generated HepG2 cells with CRISPR mediated knockdown of TMEM127 to investigate cell autonomous effects of TMEM127 in the liver, and to uncover the molecular mechanisms underlying these changes.
Pathogenic tau drives a toxic activation of transposable elements

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Tauopathies, including Alzheimer’s disease, are age-related progressive neurodegenerative disorders that are pathologically defined by aggregates of tau protein in the brain. We have previously reported that tau-induced heterochromatin relaxation promotes neuronal death in tauopathies, and allows transcription of genes that are normally silenced by heterochromatin. We hypothesized that heterochromatin relaxation also causes transcription and mobilization of transposable elements in tauopathy. In support of this hypothesis, we find increased transposable element transcription and mobilization in a Drosophila model of tauopathy. Mechanistically, we find that transposable element activation is likely due to decreased levels of piRNAs, a class of small RNAs that target transposable element transcripts for silencing. Caloric restriction and treatment with an FDA-approved pharmacological inhibitor of reverse transcriptase, 3TC, decreases transposable element mobilization and neuronal death in tau transgenic Drosophila. We detect increased transposable element activation in a mouse model of tauopathy, and differentially expressed transposable elements in brain tissue from human Alzheimer’s disease and Progressive Supranuclear Palsy (a “primary” tauopathy) patients. Taken together, our findings suggest that pathological tau activates transposable elements, and that tau-induced activation of transposable elements is amenable to environmental and pharmacological inhibition. Our findings may lead to the development of predictive biomarkers and will guide therapeutic strategies.

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evaluate the relationship between cellular senescence and neurodegenerative disease, we use the rTg4510 mouse model of Alzheimer’s disease associated tau pathogenesis and neurodegeneration. We have observed several senescence biomarkers in the brain including p16INK4A expression, senescence associated secretory phenotype (SASP) factors and senescence associated β-galactosidase activity, and karyomegalic in neurons. Moreover, mice possessing tau pathology expressed significantly higher levels of several senescence factors than controls, including p16INK4A, TNFa, IL-1β, Cxcl2, B2m, Nfkb, and Tlr4, while also being karyomegalic. Collectively our data indicate that cellular senescence occurs in post-mitotic tissue, and that Alzheimer’s disease-associated tau pathology exacerbates the cell stress response. Our future studies are aimed at targeting cellular senescence as a new therapeutic approach to ameliorate Alzheimer’s disease pathogenesis and cognitive decline.

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**Methionine restriction: Mechanism and cell culture**

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Methionine is an essential amino acid and is required for many biological processes such as methylation, protein translation, and cysteine production. Restriction of this single amino acid in the diet can provide numerous benefits in rodents including extension of lifespan, reduced body weight, and improved glucose metabolism. The mechanisms that regulate this process are at least partially distinct from the mechanisms by which calorie restriction mediates its effects. It has been proposed that sulfur metabolism and methionine processing are important in understanding the mechanisms of methionine restriction. To help elucidate these mechanisms, our goal is to develop a cell culture model to provide a new tool for studying methionine restriction in different cell types. Further, we address the central role of methionine biochemistry in these potential mechanisms. The sulfur atom in methionine is sensitive to oxidation, though this can be reduced by methionine sulfoxide reductases found in eukaryotic cells. Interestingly, mice lacking one of these reductases (MsrA−/−) do not respond to the metabolic effects of methionine restriction. How aging, or healthy aging, is impacted by the oxidation status of the methionine pool is not known, but these interactions are likely to change during the aging process and under dietary restriction. Moreover, these findings suggest functional expression of MsrA may be a requirement for the longevity benefits of methionine restriction.

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**Sex differences in the human skeletal muscle transcriptome during aging and exercise training**

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Muscle mass and strength decline with age in both men and women, resulting in reduced physical function and quality of life. Endurance exercise training can mitigate and even reverse some of the large-scale changes in skeletal muscle structure and function. To date, few studies in humans have focused on sex differences in mechanisms responsible for sarcopenia and the adaptive response to exercise training during aging. To gain insight into the potential mechanisms driving sarcopenia and the adaptive response to endurance training in older men and women we conducted deep sequencing on vastus lateralis muscle obtained from healthy, younger (n=14, age=27±1 y) and older (n=14, age=71±1 y) adults before and after a 4 month endurance training program. We utilized this sequencing data to determine miRNAs and mRNA expression patterns in our study’s cohorts. In females, 23 miRNAs decreased with age and were reverted back to youthful levels with training. Conversely, in males only one miRNA showed this same expression pattern. Also, in males 4 miRNAs increased with age and were reverted back to youthful levels with training, whereas no miRNAs with these expression patterns were observed in females. Pathway analyses in older male (but not female) subjects revealed enrichment and activation of mTOR, FGF, and VEGF pathways in response to training. These pathways are important for the adaptive response to training by increasing protein synthesis/vascularization in muscle. In contrast, older female subjects showed enrichment and activation of inflammatory pathways such as NFkB, inflammasome, apoptosis, and JNK signaling, while these inflammatory pathways were not enriched in
males. MicroRNAs miR-365-3p, miR-36073p had youthful signatures after training, in older men. These two miRNAs are predicted to target members of the PI3K/AKT signaling pathway and they were downregulated after training with concomitant upregulation of the mRNA transcripts (i.e. PIK3R3, PRKCI, AKT3, and SGK1) in our data set. Surprisingly, no miRNA/mRNA relationships of this kind were observed in older female subjects after training. Therefore, we conclude that the potential mechanisms responsible for sarcopenia and adaption to endurance training in older men and woman may vary between sexes. Furthermore in females, we find that miRNAs that changed with age and were reverted to youthful levels with training do not regulate mRNA expression changes induced by training in the older group. Yet, miRNAs with this expression patterning in older men are capable of regulating transcriptional changes including pathways important for insulin signaling and action.

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**MTOR-dependent neuronal nitric oxide dysfunction underlies neurovascular coupling deficits in a model of Alzheimer's disease**

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Cerebrovascular dysfunction emerges prior to the onset of cognitive dysfunction in patients with Alzheimer’s disease (AD). Specifically, patients with AD exhibit deficits in neurovascular coupling (NVC, increased cerebral blood flow evoked by neuronal activation), which is recapitulated in various mouse models of AD. We have previously identified the mechanistic/mammalian target of rapamycin (mTOR) as a major driver of cerebrovascular dysfunction in AD. Therefore, the aims of the present study were to 1) establish the mTOR-dependent contribution to NVC deficits in AD mice, and 2) define the mechanisms of mTOR mediated neuronal nitric oxide synthase (nNOS) insensitivity using in vivo and in vitro techniques.  

J20 mice at 6 and 12 months old had profound impairments in NVC, particularly in the nNOS-dependent component of NVC, which was preserved by attenuation of mTOR activity with rapamycin treatment for 8 weeks. Further, NVC deficits were present prior to cognitive impairments, as contextual memory was not significantly impaired in the 6MO mice. *Our in vitro* studies indicate that mTOR inhibition with rapamycin increases nNOS activation as measured by phosphorylation of Ser1177. Additionally, preliminary coimmunoprecipitation studies suggest that mTOR attenuation increases the binding of nNOS to its binding partners PSD95 and HSP90, which positively regulate nNOS. These studies establish that mTOR drives the loss of the nNOS component of NVC in an AD mouse model, possibly by inhibiting nNOS phosphorylation at Ser1177.  

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**Immunosenescence drives systemic aging**

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Aging is the primary risk factor for numerous chronic diseases and the impact of aging on the immune system contributes to this. The progressive decline of immune function that occurs with aging, termed immunosenescence, can negatively impact both lifespan and healthspan due to impaired response to immunologic stimuli. Immunosenescence is characterized by a shift in T cell populations, expression of senescence markers, and a decline in both cell-mediated and humoral immunity. These changes lessen the ability of the elderly to mount a productive immune response to pathogenic infection, increase the risk of autoimmune diseases and cancer, and diminish the response to vaccinations. It remains controversial whether immunosenescence is driven primarily by cell autonomous and/or non-autonomous events. We utilized a mouse model (*Vav-iCre+/−;Ercc1−/−*) with increasing burden of endogenous DNA damage in immune cells to determine if this is sufficient to drive senescence and functional decline of the immune compartment. *Vav-iCre+/−;Ercc1−/−* mice exhibited an early and progressive onset of lymphopenia and expansion of the memory T cell population, hallmark signs of immunological aging. By 20 weeks, a diminished response to immunological stimuli
was observed in comparison to controls and increased senescence marker expression in peripheral blood lymphocytes, a biomarker of aging, were increased in Vav-iCre^+/−;Ercc1^−/−/mice, similar to naturally aged wild-type mice. Interestingly, we also found increased senescence in the liver, kidney and brain, in addition to the spleen and bone marrow of Vav-iCre^+/−;Ercc1^−/−/mice, suggesting that immune cells are a key driver of secondary senescence and that immunosenescence helps promote systemic aging.

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The role of small RNAs in a Drosophila model of tauopathy

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The microtubule associated-protein tau is known to play a role in the pathogenesis of Alzheimer’s disease and other related disorders. While differential expression of some small RNAs has been previously correlated with Alzheimer’s disease in humans, it is currently unknown if alterations in small RNA expression are causal for the disease process. Recent small RNA sequencing data from our lab reveal differential expression of microRNAs (miRNAs), endogenous siRNAs (esiRNAs), and piwi-associated RNAs (piRNAs) in a Drosophila model of human tauopathy. miRNA have a well-established role in post-transcriptional regulation of gene expression, while piRNAs and esiRNAs are recently-described, highly conserved classes of small noncoding RNAs that post-transcriptionally silence transposable elements. Interestingly, the majority of differentially expressed miRNAs are increased in tau transgenic Drosophila, while the majority of differentially expressed esiRNAs and piRNAs are decreased in tau transgenic Drosophila compared to control. We are currently determining if differentially expressed small RNAs play a causal role in promoting neurotoxicity, and are investigating the mechanism underlying the expression differences among miRNAs, piRNAs, and esiRNAs. Our studies suggest that tau-induced dysregulation of small RNAs may mediate neuronal death in tauopathies.

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