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Hilton San Diego Bayfront
Sunday, November 13th, 2016
6:30pm-8:30pm

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MBRF Poster Session Author and Title List

University of Miami

Poster #1) N. D'ADESKY, M. SCHATZ, M. A. PEREZ-PINZON, H. BRAMLETT, J. DE RIVERO VACCARI, & A. P. RAVAL. "Inflammasome activation exhibits sex difference and estrogen receptor beta agonist treatment reduces its activation and protects the brain from ischemic damage"

Poster #2) N. KHOURY, K. KORONOWSKI, I. SAUL, K. DAVE, J. YOUNG, & M. PEREZ-PINZON. "Elucidating the molecular mechanism behind the long-term cerebral ischemic tolerance mediated by resveratrol preconditioning"

Poster #3) C. LI, C. BELLO, J. BRAZILL, S. LIU, Y. ZHU, M. C. V. MALICDAN, R. PAULY, H. WANG, C. E. SCHWARTZ, W. A. GAHL, C. F. BOERKOEL, & R. G. ZHAI. "Polyamine imbalance causes neuronal dysfunctions in a Drosophila model for Snyder-Robinson Syndrome"

Poster #4) M. SCHATZ, N. D'ADESKY, P. BHATTACHARYA, A. P. RAVAL, W. D. DIETRICH, & H. M. BRAMLETT. "Post-ischemic whole body vibration reduces inflammation in the brain of middle-aged female rats"

Poster #5) V. SHUKLA, A. K. REHNI, & K. R. DAVE. "Increased intra-ischemic acidosis in recurrent hypoglycemia exposed rats may activate acid-sensing ion channels in vascular smooth muscle cells"

Poster #6) A. K. REHNI, V. SHUKLA, K. R. DAVE. "Inhibition of glucose transporters attenuate recurrent hypoglycemia-induced increase in intra-ischemic acidosis in insulin-treated diabetic rats"

University of Florida

Poster #7) J. BARTER, A. KUMAR, A. RANI, & T. C. FOSTER. "Systemic inflammation contributes to the onset of cognitive impairment associated with senescence"

Poster #8) T. C. FOSTER, L. IANOV, & A. RIVA. "Epigenetic regulation of medial prefrontal cortex transcription associated with aging and impaired executive function"

Poster #9) L. S. GAYNOR, J. MIZELL, K. T. CAMPOS, L. SANTACROCE, C. MCEWEN, D. K. CHETRAM, A. P. MAURER, R. M. BAUER, & S. N. BURKE. "Stimulus modality affects recognition behavior during spontaneous object recognition and crossmodal object recognition tasks"

Poster #10) A. R. HERNANDEZ, K. CAMPOS, L. TRUCKENBROD, L. SANTACROCE, C. M. HERNANDEZ, Y. SAKARYA, J. A, MCQUAIL, C. S. CARTER, A. P. MAURER, J. L. BIZON, & S. N. BURKE. "The ketogenic diet as therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence"

Poster #11) C. M. HERNANDEZ, M. R. SCHWABE, J. A. MCQUAIL, B. SETLOW, & J. L. BIZON. "Glutamate dysregulation in aged rat prefrontal cortex may contribute to decline of working memory"

Poster #12) L. IANOV, M. DE BOTH, M. K. CHAWLA, A. RANI, A. J. KENNEDY, I. PIRAS, J. J. DAY, A. SINIARD, A. KUMAR, J. D. SWEATT, C. A. BARNES, M. HUENTELMAN, T. C. FOSTER. "Transcriptomic profile for determining regional vulnerability to age and cognitive impairment"

Poster #13) S. A. JOHNSON, W. M. YODER, K. N. LUBKE, J. P. LISTER, A. P. MAURER, J. L. BIZON, & S. N. BURKE. "Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations"

Poster #14) K. B. KELLY, J. L. BIZON, & C. J. FRAZIER. "Functional effects of age on NR2A and NR2B containing NMDA receptors in interneurons and pyramidal cells of the rat medial prefrontal cortex"

Poster #15) A. KUMAR, L. A. BEAN, A. RANI, & T. C. FOSTER. "The long-term estrogen-induced facilitation of NMDA receptor synaptic function is mediated through altered redox state"

Poster #16) C. KYRITSOPOULOS, A. KUMAR, & T. C. FOSTER. "Up regulation of GluN2B type NMDA receptor in CA1 region of hippocampus and its influence on cognitive and synaptic function"

Poster #17) D. G. LAMB, L. N. CORREA, T. R. SEIDER, D. M. MOSQUERA, J. A. RODRIGUEZ, JR, L. SALAZAR, Z. J. SCHWARTZ, R. A. COHEN, A. D. FALCHOOK, & K. M. HEILMAN. "Aging and motor control: movement speed and spatial control tradeoffs with age"

Poster #18) T. LIN, M. MURADOGLU, D. WEIR, T. LILLARD, J. CONNELLY, S. CARTER, & N. C. EBNER. "*OXTR* methylation as a predictor of variations in attachment in young and older adults"

Poster #19) J. MCGUINESS, R. B. SCHEINERT, V. S. SCHWINGEL, A. RANI, A. KUMAR, T. C. FOSTER, & B. K. ORMEROD. "NSAID treatment reverses age-related changes in hippocampal neurogenesis"

Poster #20) M. E. MCLAREN, S. M. SZYMKOWICZ, A. O'SHEA, A. J. WOODS, S. D. ANTON, & V. M. DOTSON. "Symptom dimensions of depression and age impact subfield hippocampal volume"

Poster #21) J. A. MCQUAIL, M. M. BRUNER, E. G. KRAUSE, B. SETLOW, D. A. SCHEUER, & J. L. BIZON. "Stress reactivity predicts impaired working memory in aging: vulnerability of GABAergic synapses"

Poster #22) E. C. PORGES, A. J. WOODS, R. A. E. EDDEN, N. A. J. PUTS, A. D. HARRIS, H. CHEN, A. M. GARCIA, T. R. SEIDER, Y. CRUS-ALMEIDA, N. C. EBNER, D. G. LAMB, J. B. WILLIAMSON & R. A. COHEN. "Recent findings and ongoing explorations of GABA MRS in Aging"

Poster #23) A. RANI, S. KERIC, L. A. BEAN, J. BARTER, T. C. FOSTER, & A. KUMAR. "Expression of G-protein estrogen receptor 1 (GPER1) in the hippocampus and prefrontal cortex over the oestrous cycle: Influence of ovariectomy and aging"

Poster #24) L. A. RICHARDS, V. E. BRYANT, R. COHEN, & A. J. WOODS. "Brain-Derived Neurotrophic Factor (BDNF) and learning & memory in an HIV population"

Poster #25) T. R. SEIDER, A. M. GARCIA, A. J. WOODS, & R. A. COHEN. "Default mode network control, social engagement, and memory in older adults"

Poster #26) S. M. SZYMKOWICZ, M. E. MCLAREN, L. DE WIT, A. O'SHEA, A. J. WOODS, S. D. ANTON, & V. M. DOTSON. "Structural abnormalities in cortical thickness, surface area, and volume of the precuneus in older adults with depressive symptoms"

Poster #27) S. M. TURNER, L. A. SANTACROCE, S. A. JOHNSON, A. P. MAURER, & S. N. BURKE. "A rodent model of medial temporal lobe-dependent discrimination deficits in the elderly"

Poster #28) L. M. VETERE, C. A. ORSINI, J. A. MCQUAIL, S. N. BURKE, B. SETLOW, & J. L. BIZON. "Age-related alterations in working memory and intertemporal choice in Fischer 344 x Brown Norway hybrid rats"

Poster #29) A. J. WOODS, R. COHEN, M. MARSISKE, G. ALEXANDER, S. CZAJA, & C. WRIGHT. "Augmenting cognitive training in older adults: the ACT study"

University of Alabama at Birmingham

Poster #30) A. E. ARRANT, V. ONYILO, D. E. UNGER, & E. R. ROBERSON. "AAV-progranulin improves pathology in *Grn*$^{-/-}$ mice, an animal model of CLN11-Neuronal Ceroid Lipofuscinosis (NCL)"

Poster #31) A. BOHANNON & J. J. HABLITZ. "Selective activation of somatostatin or parvalbumin expressing interneurons triggers GABA-mediated LLDs in rat neocortex"

Poster #32) L. J. BRADY, A. F. BARTLEY, Q. LI, & L. E. DOBRUNZ. "Altered dopamine D4 receptor dependent regulation of synaptic transmission and hippocampal circuit function in PGC-1α-/- mice"

Poster #33) A. A. BUTLER, A.W. CHANG, & F. D. LUBIN. "Context fear memory formation is regulated by Neat1 long non-coding RNA mediated histone lysine methylation changes in the hippocampus"

Poster #34) J. N. COCHRAN, K. L. ENGEL, A. A. HARDIGAN, R. C. RAMAKER, M. D. AMARAL, K. M. BOWLING, C. R. FINNILA, S. M. HIATT, M. L. THOMPSON, D. E. GRAY, J. S. WHITTLE, W. V. KELLEY, M. E. COCHRAN, K. M. EAST, N. E. LAMB, C. A. RICH, K. B. BROTHERS, E. J. LOSE, S. B. SIMMONS, E. M. BEBIN, G. S. BARSH, G. M. COOPER, & R. M. MYERS. "A pipeline for rapid *in vitro* evaluation of variants of uncertain significance from patients with developmental delay and/or intellectual disability exhibiting seizures"

Poster #35) R. B. CREED & M. S. GOLDBERG. "Analysis of alpha-synuclein pathology in PINK1 knockout rats"

Poster #36) C. G. DUKE, A. J. KENNEDY, J. D. SWEATT, & J. J. DAY. "Experience-dependent epigenomic reorganization"

Poster #37) N. V. N. GALLUS, K. E. SAVELL, F. A. SULTAN, R. C. SIMON, J. S. REVANNA, & J. J. DAY. "Extra-coding RNAs regulate dynamic DNA methylation and gene expression"

Poster #38) J. C. GRIFFIS & J. P. SZAFLARSKI. "Damage to white matter bottlenecks contributes to chronic language impairments and disrupts semantic network function in patients with left middle cerebral artery stroke"

Poster #39) J. M. HICKS, R. NENERT, L. A. ROSS, & K. M. VISSCHER. "Training the aging brain: a multi-level network analysis"

Poster #40) B. W. HENDERSON, E. G. GENTRY, T. RUSH, J. C. TRONCOSO, M. THAMBISETTY, T. J. MONTINE, & J. H. HERSKOWITZ. "Rho-associated protein kinase 1

(ROCK1) is increased in Alzheimer's disease and ROCK1 depletion reduces amyloid-β levels in brain"

Poster #41) A. M. LASZCZYK & G. D. KING. "Shed klotho regulates neural stem cell proliferation and differentiation"

Poster #42) D. LEE, W. K. BURGE, A. ELKHETALI, & K. VISSCHER. "Retinotopically-specific variation in cortical thickness in V1 relates to performance on a central visual discrimination task"

Poster #43) M. PHILLIPS, W. LI. & L. POZZO-MILLER. "Stronger contribution and impaired LTP of hippocampal inputs to the medial prefrontal cortex in the *Mecp2* mouse model of Rett syndrome"

Poster #44) T. RUSH, S. J. THOMPSON, J. N. COCHRAN, P. V. DIGGS, & E. D. ROBERSON. "Targeting the tau-fyn interaction for ameliorating Aβ-induced neurotoxicity"

Poster #45) K. E. SCHOONOVER & R. C. ROBERTS. "Abnormalities in copper transporters ATP7A and CTR1 in postmortem substantia nigra in schizophrenia"

Poster #46) R. C. SIMON, N. V. N. GALLUS, K. E. SAVELL, F. A. SULTAN, & J. J. DAY. "Functional modulation of activity-dependent gene expression by non-coding enhancer RNAs"

Poster #47) L. T. STEWART, A. U. KHAN, M. L. OLSEN, J. C. CHATHAM, & L. L. MCMAHON. "Increasing O-GlcNAcylation induces AMPAR internalization at CA3-CA1 synapses"

Poster #48) Y. VOSKOBIYNYK, J. N. COCHRAN, & E. D. ROBERSON. "Effects of tau reduction in excitatory and inhibitory neurons on seizure susceptibility"

Poster #49) X. XU & L. POZZO-MILLER. "EEA1 overexpression reduces synaptic strength and restores homeostatic synaptic plasticity in cultured hippocampal neurons from *Mecp2* knockout mice"

University of Arizona

Poster #50) K. M. ANDERSH, D. T. GRAY, A. C. SMITH, S. N. BURKE, A. GAZZALEY, & C. A. BARNES. "Age-related attentional control and set shifting impairments arise independently in macaque monkeys"

Poster #51) E. BACON, M. K. DESAI, A. MISHRA, Y. WANG, F. YIN, & R. D. BRINTON. "Transcriptional and epigenomic changes across the perimenopause transition"

Poster #52) P. K. BHARADWAJ, L. A. NGUYEN, K. A. HAWS, G. A. HISHAW, T. P. TROUARD, & G. E. ALEXANDER. "Differential regional alterations of white matter integrity in healthy cognitive aging"

Poster #53) R. D. BRINTON, F. YIN, J. YAO, A. MISHRA, Q. DENG, Z. MAO, & E. CADENAS. "Mechanistic role of brain hypometabolism and mitochondrial uncoupling in perimenopausal hot flash"

Poster #54) A. COMRIE, J. P. LISTER, M. K. CHAWLA, & C. A. BARNES. "Activation of neuronal populations in young and aged rat Lateral Entorhinal Cortex during track-running behavior with odors"

Poster #55) M. J. CORENBLUM, S. RAY, D. D. ZHANG, C. A. BARNES, & L. MADHAVAN. "An analysis of nrf2 expression and its effect on aging hippocampal neural stem cell function"

Poster #56) M. DE BOTH, L. IANOV, M. K. CHAWLA, A. RANI, A. J. KENNEDY, I. PIRAS, J. J. DAY, A. L. SINARD, A. KUMAR, J. D. SWEATT, T. C. FOSTER, C. A. BARNES, & M. J. HUENTELMAN. "Transcriptional differences among hippocampal subregions"

Poster #57) D. T. GRAY, J.-P. WIEGAND, L. A. SCHIMANSKA, S. L. COWEN, & C. A. BARNES. "Age-related reduction in signal-to-noise ratio of sharp-wave ripple oscillations following behavior in aged rats"

Poster #58) S. L. GRELLA, J. NEIL, V. D. STRONG, I. V. ODINTSOVA, G. MARTIN, C. W. HARLEY, & D. F. MARRONE. "Norepinephrine as a memory reset signal: Phasic activation of the locus coeruleus drives global remapping in hippocampus"

Poster #59) S. V. KOEBELE, S. E. MENNENGA, S. PATEL, R. HIROI, L. T. HEWITT, A. M. QUIHUIS, L. P. MAYER, C. A. DYER, L. M. DEMERS, & H. A. BIMONTE-NELSON. "Menopause and the aging brain: Relationships among ovarian hormone levels, memory, and choline acetyltransferase-containing neurons in the basal forebrain"

Poster #60) C. KYLE, J. L. BENNETT, J. D. STOKES, M. R. PERMENTER, J. A. VOGT, A. D. EKSTROM, & C. A BARNES. "Histology informed probabilistic hippocampal atlases of young and old rhesus macaques"

Poster #61) A. LAWRENCE & L. RYAN. "Familiar context effects on pattern separation in aging"

Poster #62) A. W. LESTER, A. J. KAPELLUSCH, R. T. SCREEN, & C. A. BARNES. "Aged rats failed to integrate conflicting spatial reference frames"

Poster #63) M. B. MEMEL & L. RYAN. "An fMRI analysis of visual unitization and the age-related associative memory deficit"

Poster #64) W. PYON, D. T. GRAY, K. M. ANDERSH, M. R. PERMENTER, J. A. VOGT, & C. A. BARNES. "Cell counts of midbrain dopamine neurons in memory-impaired aged non-human primates"

Poster #65) R. D. SAMSON, L. DUARTE, & C. A. BARNES. "Expectation of large reward elicits bursts of beta-band oscillations in the aged rat amygdala"

Poster #66) L. UMAPATHY, D. T. GRAY, S. N. BURKE, T. P. TROUARD, & C. A. BARNES. "Uncinate fasciculus integrity assessed in young and aged bonnet macaques"

MBRF Sponsored Abstracts

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- **Evelyn F. McKnight Center for Age-Related Memory Loss, University of Miami . . . . . Pgs. 12-18**

- **Evelyn F. and William L. McKnight Brain Institute University of Florida . . . . . Pgs. 19-43**

- **Evelyn F. McKnight Brain Institute University of Alabama at Birmingham . . . . . Pgs. 44-65**

- **Evelyn F. McKnight Brain Institute University of Arizona . . . . . Pgs. 66-83**

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Inflammasome activation exhibits sex difference and estrogen receptor beta agonist treatment reduces its activation and protects the brain from ischemic damage

N. D'ADESKY, M. SCHATZ, M. A. PEREZ-PINZON, H. BRAMLETT, J. DE RIVERO VACCARI, & A. P. RAVAL

University of Miami, Miami, FL

Cerebral ischemia is known to activate the innate immune response, and the inflammasome is a key component of the innate immune response. The inflammasome is comprised of caspase-1, the adaptor protein apoptosis associated speck-like protein containing a CARD (ASC), and a pattern recognition receptor such as a NOD-like receptor. Prior literature suggested that the inflammasome proteins are regulated by sex steroids. In a published study, we reported that the silencing of estrogen receptor subtype beta (ER-β) attenuated 17β-estradiol mediated decreases in caspase-1, ASC and interleukin-1β (IL-1β). Based on this information, the goal of the current study was to investigate whether inflammasome proteins alter with age and/or sex in the hippocampus and if these differences could contribute to increases in damage due to cerebral ischemia. We hypothesize that inflammasome activation is significantly higher in the hippocampus of reproductively senescent (RS) females as compared to their young counterparts and age-matched males. We also hypothesize that periodic pretreatment of ER-β agonist will reduce both inflammasome activation and cerebral ischemic damage in RS female rats. We tested our hypothesis by investigating inflammasome protein levels in the hippocampus of young (6-7 month old) and reproductively senescent (11-13 month old) female Sprague-Dawley rats and age matched males. To test the efficacy of ER-β agonist on reducing inflammasome proteins and ischemic brain damage, ER-β agonist (1 mg/kg; every 48 h for 21 days) or vehicle (DMSO) treated RS females were sacrificed for collection of brain tissue or exposed to global cerebral ischemia. Results of western blot analysis demonstrated a significant increase in the inflammasome proteins caspase-1 ($p < 0.05$) and ASC ($p < 0.01$) in the hippocampus of RS females as compared to RS matched male rats. In the RS female periodic ER-β agonist pretreated group, we observed a significant decrease of the inflammasome proteins caspase-1 ($p<0.05$), ASC ($p<0.05$) and IL-1β ($p<0.05$) as compared to vehicle treated RS females. Furthermore, histological analysis of the hippocampus 15 days after global cerebral ischemia demonstrated a significantly higher number of live neurons ($p< 0.05$) in the ER-β agonist group as compared to vehicle treated RS female rats. Our findings suggest the role of sex hormones in the regulation of the inflammasomes in the hippocampus and that activation of ER-β could be useful in the prevention of post-ischemic inflammasome activation and reduction in ischemic brain damage.

Elucidating the molecular mechanism behind the long-term cerebral ischemic tolerance mediated by resveratrol preconditioning

N. KHOURY, K. KORONOWSKI, I. SAUL, K. DAVE, J. YOUNG, & M. PEREZ-PINZON

Miller School of Medicine, University of Miami, Miami, FL

In the absence of effective neuroprotective agents in the clinic, ischemic and pharmacological preconditioning are gaining increased interest in the field of cerebral ischemia. Our lab has recently demonstrated that resveratrol preconditioning (RPC) affords tolerance against a cerebral ischemic insult that lasts for at least 2 weeks *in vivo*, making it the longest window of ischemic tolerance discovered to date by pharmacological preconditioning. The goal of this study is to identify the transcriptomic and epigenetic alterations which are induced in the brain after RPC in order to reveal pathways and adaptations that mediate this tolerance. Thus we injected 10 week old C57Bl6 male mice with Vehicle or Resveratrol (10mg/kg) (n=3 per group). Two weeks after the injection we collected the cortex of these mice and performed an RNA-seq experiment using the Illumina HiSeq 2500. Using a cutoff value of 0.1 for the false discovery rate we identified 155 differentially expressed genes. Interestingly, among these genes, 126 were downregulated after RPC and only 29 genes were upregulated. Using the Functional Annotation Clustering tool from DAVID, these differentially expressed genes clustered into several biological processes including: transcription, gene expression, neurotransmitter secretion, regulation of membrane potential, and respiratory electron transport chain among others. The downregulation in these cellular activities is reminiscent of the phenomenon of metabolic depression, an adaptive mechanism observed in hibernating animals that allows them to tolerate extreme hypoxic and ischemic conditions during torpor. To measure the metabolic activity, we used our *in vitro* model of cerebral ischemia consisting of rat neuronal cultures exposed to an oxygen and glucose deprivation (OGD). RPC *in vitro* (100uM) was able to promote tolerance in these cultures lasting for at least 6 days against an OGD as revealed by the LDH assay, PI:DAPI staining, and bright-field images. To measure the metabolic activity of these cells, we followed the indirect method that consists of measuring the rate of oxygen consumption and lactic acid production using the Seahorse Biosciences Technology at the 6-day time-point post-RPC. Our results revealed that the maximal respiration rate of these cells, which is an indication of their cellular activity, is reduced after RPC during the long-term window. In conclusion this study reveals that RPC in the long-term window induces a global downregulation in gene expression in the brain which also correlates with a reduction in metabolic activity in preconditioned neuronal cultures. Ongoing studies in the lab will further test this conclusion and determine whether this mechanism is behind the protection observed during the long-term window of ischemic tolerance.

Polyamine imbalance causes neuronal dysfunctions in a Drosophila model for Snyder-Robinson Syndrome

C. LI[1], C. BELLO[1], J. BRAZILL[1], S. LIU[2], Y. ZHU[1], M. C. V. MALICDAN[3], R. PAULY[4], H. WANG[2], C. E. SCHWARTZ[5], W. A. GAHL[3], C. F. BOERKOEL[3], & R. G. ZHAI[1]

[1]Mol. and Cell. Pharmacol., Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Key Lab. of Mol. Pharmacol. and Drug Evaluation (Ministry of Educ. of China), Yantai Univ. Sch. of Pharm., Yantai, China; [3]Natl. Human Genome Res. Inst., Bethesda, MD; [4]Greenwood Genet. Ctr., JC Self Res. Inst., Greenwood, SC; [5]JC Self Res. Institute, Greenwood Genet. Ctr., Greenwood, SC

Intracellular polyamines, including putrescine, spermidine, and spermine, are tightly regulated polycationic molecules that are essential for cell growth and differentiation. Loss-of-function mutations in human spermine synthase (SMS), an aminopropyl transferase that converts spermidine to spermine, were identified to cause Snyder-Robinson Syndrome (SRS). SRS is an X-linked recessive disease characterized by intellectual disability and developmental delay. The underlying pathogenesis, especially of the neurological phenotypes, is largely unknown. We used Drosophila as a model to study the neuronal function of SMS and found that loss of Drosophila Sms (dSms) recapitulates the polyamine imbalance of SRS patients and causes developmental and survival defects in Drosophila. We showed that abnormal polyamine oxidation resulted in oxidative stress, mitochondria dysfunction, and altered endosomal and autophagic membrane trafficking in both Drosophila nervous system and SRS patient fibroblasts. Importantly, we found that the elevated reactive oxygen species in the Drosophila model can by suppressed through enhanced antioxidant activity either genetically or pharmacologically. Our findings provide significant insights into possible therapeutic strategies for SRS and polyamine-associated neurological disorders.

Post-ischemic whole body vibration reduces inflammation in the brain of middle-aged female rats

M. SCHATZ[1], N. D'ADESKY[1], P. BHATTACHARYA[1], A. P. RAVAL[1], W. D. DIETRICH[2], & H. M. BRAMLETT[2,3,4]

[1]Cerebral Vascular Disease Research Laboratories, Department of Neurology, [2]Department of Neurological Surgery, [3]Neuroscience Program, Leonard M. Miller School of Medicine, University of Miami and [4]Bruce W. Carter Department of Veterans Affairs Medical Center, Miami, and Florida, USA

A woman's risk of stroke increases exponentially after menopause, and even a mild ischemic episode can result in increased disability. Studies performed in laboratory animals and humans support that whole body vibration (WBV) reduces or reverses pathological remodeling of bone and lessens frailty-related physiological deterioration. Using a rodent model of stroke, we have examined whether WBV reduces inflammation and post-ischemic damage and improves motor function in middle-aged female rats. Middle-aged Sprague–Dawley female (9–11 months) rats were exposed to transient middle cerebral artery occlusion (tMCAo; 60 min) and randomly assigned to either WBV or control groups. Animals placed in the WBV (40 Hz) group underwent 30 days of WBV treatment performed twice daily for 15 min each session for 5 days each week. During the treatment period, we tested motor function using a rotarod test at the 7^{th}, 15^{th} and 30^{th} day after tMCAo. Animals were sacrificed on 30^{th} day of WBV treatment and brain tissue was harvested for histopathological and inflammasome protein analysis performed by western blotting. Western blot results demonstrated a two-fold decrease in the inflammasome proteins caspase-1, caspase recruitment domain (ASC), and interleukin-1β. The rotarod test scores from the WBV treatment group were significantly higher than the control group on day 30 ($p < 0.05$) at 10, 30 and 40 RPM speeds, suggesting a significant improvement in functional activity of the WBV group. Overall, WBV has shown promising results in decreasing inflammation and increasing functional activity after stroke in middle-aged female rats.

Increased intra-ischemic acidosis in recurrent hypoglycemia exposed rats may activate acid-sensing ion channels in vascular smooth muscle cells

V. SHUKLA, A. K. REHNI, & K. R. DAVE

CVDRL, Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL

More than 29 million Americans suffer from diabetes. Recurrent hypoglycemia (RH) is common in treated diabetics. Earlier we observed that prior exposure to RH increases cerebral ischemic damage in insulin treated diabetic (ITD) rats. We also observed pronounced intra-ischemic acidosis and post-ischemia hypoperfusion in RH-exposed ITD rats compared to the control group. The goal of the present study was to determine if increased intra-ischemic acidosis contributes to severe hypoperfusion in RH-exposed ITD rats. We hypothesized that increased intra-ischemic acidosis leads to activation of acid-sensing ion channels (ASICs) in vascular smooth muscle cells (VSMCs) contributing to pronounced hypoperfusion in RH-exposed ITD rats. This hypothesis was tested using VSMCs cell line A7r5. We determined contribution of ASICs in Store Operated Calcium Entry (SOCE) at different pH, and in presence and absence of inhibitors of ASIC-1 (PcTX-1) and ASIC-3 (APETx2). Freshly grown A7r5 cells were loaded with Ca^{2+} sensitive fluorescent indicator Fluo-4 AM in presence of pluronic acid in calcium medium (135 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11.5 mM glucose and 11.6 mM Hepes, pH=7.3) and incubated for 40 minutes at 37^{o}C and then for an additional 20 minutes at room temperature. We measured SOCE at three different pH (7.4, 6.5 and 6.0). Fluo-4 AM loaded cells were super fused with Ca^{2+} free medium with respective pH (135 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 11.5 mM glucose and 11.6 mM Hepes, 50 µM diltiazem, and 10 µM cyclopiazonic acid). The changes in Fluo-4 AM florescence from the baseline, as an indicator of SOCE induced increase in $[Ca^{2+}]_i$, was determined upon repletion of extracellular Ca^{2+} (1.5 mM) in presence of diltiazem and cyclopiazonic acid for 30 min. The experiment was performed in presence of PcTX-1, APETx2, or respective vehicle controls. As expected, we observed pH dependent SOCE in A7r5 cells. We also observed that lower pH-induced increase in intracellular Ca^{2+}: an indicator of SOCE was inhibited by APETx2. The intracellular Ca^{2+} influx was reduced significantly ($p<0.05$) to 40 % at pH 6.0 in presence of APETx2 but no significant difference was observed at pH 7.0 and pH 6.5 compared to respective vehicle control. Also, no significant difference in intracellular Ca^{2+} influx was observed at any pH (7.4, 6.5 and 6.0) in presence of PcTX-1 when compared to respective vehicle control. We conclude that activation of ASIC-3 *via* increased intra-ischemic acidosis may contribute to increased cerebral ischemic damage in RH-exposed ITD rats.

Inhibition of glucose transporters attenuate recurrent hypoglycemia-induced increase in intra-ischemic acidosis in insulin-treated diabetic rats

A. K. REHNI[1], V. SHUKLA[1], & K. R. DAVE[1,2]

[1]Cerebral Vascular Disease Research Laboratories, Department of Neurology and [2]Neuroscience Program, University of Miami School of Medicine, 1420 NW 9th Ave, TSL / 203E, Miami, Florida 33136, USA

Stroke is a serious manifestation of diabetes. Glucose lowering therapies available for correcting diabetic hyperglycemia causes recurrent hypoglycemia (RH). In an earlier study we observed that RH exacerbates ischemic brain damage in insulin-treated diabetic (ITD) rats. We have previously observed that ischemia causes enhanced increase in hippocampal lactate levels and a profound drop in pH in ITD + RH rats compared to controls. We tested hypothesis if administration of alkalizing agent (Tris-(hydroxymethyl)-aminomethane: THAM) modulates intra-ischemic acidosis in ITD + RH rats. Also, since RH is shown to increase levels of glucose transporters (GLUTs), we also determined if glucose transporters are responsible for observed drop in intra-ischemic acidosis in ITD + RH rats. Rats were made diabetic by streptozotocin injection and 2-3 weeks later, received insulin pellets for treating diabetes. After 2-3 weeks, moderate hypoglycemia was induced by insulin injection for 3 hours per day for 5 consecutive days. Animals were divided into following groups: (I) THAM treatment: A. ITD + RH (representing treated diabetic population experiencing RH) (n = 7) and, B. ITD + RH + THAM (0.3 M, 3 ml / kg / hr, i.v.) (n = 6); II: 4,6-O-Ethylidene-α-D-glucose (OEDG; GLUT inhibitor) treatment: A. ITD + RH + Vehicle (n = 6), B. ITD + RH + OEDG (2 mmol / kg, i.v. bolus dose followed by 0.2 mmol / kg / min, i.v. maintenance infusion) (n = 6). On the day after last hypoglycemia exposure, global cerebral ischemia was induced by bilateral carotid artery occlusion with hypotension for eight minutes. Rats were treated with THAM or OEDG from 15 minutes prior to ischemia to 80 minutes of reperfusion. CA1 hippocampal pH and lactate (in microdialysate) were measured using microfiber optic pH meter and lactate plus meter, respectively. The pH drop in THAM-treated rats was significantly lower than in the controls from 4 minutes of ischemia to 4 minutes of reperfusion and from 11 to 18 minutes of reperfusion compared to the control group (change in Δ pH in THAM group vs control group was 37 % to 57 %). The fall in pH in OEDG-treated group was significantly lower than in control group from the onset of ischemia to 80 min of reperfusion (change in Δ pH of OEDG group vs control group was 17 % to 64 %). Changes in lactate level in OEDG treatment group was significantly lower than the control group, when measured 4 min after ischemia and from 20 to 60 minutes of reperfusion. Our results demonstrate that ischemia causes pronounced acidosis via a GLUTs mediated increase in acidosis in ITD + RH rats. Studying the mechanism of acidosis in RH exposed ITD rats may help lower ischemic brain damage during diabetes.

Chair: Thomas C. Foster, Ph.D.
Interim Director: Steven T. DeKosky, M.D.

Systemic inflammation contributes to the onset of cognitive impairment associated with senescence

J. BARTER, A. KUMAR, A. RANI, & T. C. FOSTER

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL

Systemic inflammation and associated serum cytokines are thought to induce a latent neuroinflammatory response in specific brain regions. Furthermore, pro-inflammatory cytokines increase in the serum during normal aging and the level of several cytokines is correlated with age-associated cognitive impairments. Episodic memory impairments begin to emerge in middle-age and we hypothesize that systemic cytokines contributes to the onset of impaired hippocampal-dependent spatial learning and memory. For all studies, a low dose (0.25 μg/kg, ip) of lipopolysaccharide (LPS) or vehicle was injected into young (4-6 months, n=18) and middle aged (12-14 months, n=16) Fischer 344 male rats. We examined 11 cytokines by using Multiplex technology at two different time points following LPS injection, 4 hours following one injection (acute effects: young, n =8, middle aged, n = 8) or 48 hours after seven injections delivered on alternated days (chronic effects: young, n =8, middle aged, n = 8). For the chronic LPS study, learning and memory was assessed 48 hr after the fourth injection using the water maze task as described previously. The results indicate that four hours after a single LPS treatment, IL-1α, IL-1β, IL-6, 1P-10, MCP-1, GRO/KC, and RANTES levels were significantly ($p < 0.05$) elevated in blood plasma. MCP-1 and Eotaxin displayed a significant ($p<0.05$) age and treatment effect with higher concentrations in middle aged LPS injected animals. In contrast, IL-12p70 levels exhibited an age effect where young animals had significantly ($p=0.018$) elevated IL-12p70 compared to older animals. For chronic LPS treatment, the results revealed no effect of treatment on cytokine levels 48 hours after the last LPS injection, indicating that cytokine elevation was not long lasting. The behavioral results indicate a trend ($p=.16$) for spatial memory decline 48 hours after the seventh LPS injection in middle aged animals compared to middle aged rats injected with vehicle only. This trend was not reflected in the younger animals. These results demonstrate that systemic inflammation-induced a transient increase in cytokine levels. Further, while cytokine levels decline back to baseline over a 48 hours period, these cytokines may have longer-lasting influence on the brain function and contribute to the onset of cognitive decline during aging.

Epigenetic regulation of medial prefrontal cortex transcription associated with aging and impaired executive function

T. C. FOSTER[1], L. IANOV[1], & A. RIVA[2]

[1]Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL, [2]Bioinformatics Core, Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida Gainesville, FL

The medial prefrontal cortex (mPFC) of rodents is involved in executive function and the mPFC transcriptional profile is altered over the course of aging. Further, transcriptional changes are associated with impaired performance of attentional set-shifting behavior. While it is understood that the transcriptional profile of the mPFC differs with aging and cognitive impairment, studies are needed to identify the mechanism of transcriptional regulation of these target genes. To address this question, the current study investigates DNA methylation of cytosines in guanine-cytosine dinucleotides (CpG) as a possible epigenetic regulator of mRNA. Young (5-6 months) and aged (17-22 months) male Fischer 344 rats were behaviorally characterized on a set-shifting task, a mPFC dependent behavior. The mPFC was isolated bilaterally and, for one hemisphere, RNA expression was determined using next generation sequencing (RNA-seq: Ion Proton). For the other hemisphere, whole genome bisulfite sequencing (WGBS) was implemented. Multiplex sequencing of WGBS libraries was performed in an Illumina NextSeq 500, and a high-performance pipeline for differential methylation analysis was employed. The pipeline includes quality trimming, alignment, DNA methylation base calling and statistical comparison across aging and cognitive impairment in hypo and hypermethylated sites and regions. The results for RNA expression (Ianov et al., Frontiers in Aging Neuroscience) indicated a correspondence of transcription with age-related changes identified in the dorsolateral PFC of aging humans, including expression of genes linked to synaptic function. Moreover, cluster analysis indicated increased expression of genes linked to inflammation. In contrast, impaired set shift behavior was associated with increased expression of genes linked to transcription and synaptic activity/plasticity. Here we will report on the DNA methylation status of these target genes. Currently, we are focused on CpG methylation state of genes identified by RNA-seq as differentially expressed due to age or cognitive status.

Stimulus modality affects recognition behavior during spontaneous object recognition and crossmodal object recognition tasks

L. S. GAYNOR[1,2], J. MIZELL[1], K. T. CAMPOS[1], L. SANTACROCE[1], C. MCEWEN[1], D. K. CHETRAM[1], A. P. MAURER[1], R. M. BAUER[2], & S. N. BURKE[1]

[1]Dept. of Neurosci., [2]Dept. of Clin. and Hlth. Psychology, Univ. of Florida, Gainesville, FL

The time a rodent spends freely exploring a novel object compared to a previously encountered object has been used to measure recognition abilities (Ennaceur & Delacour, 1988). More recently, differences in behavior on these spontaneous object recognition (SOR) tasks have been used to quantify age-related deficits in object recognition and discrimination (Burke et al., 2010). In rodents, this cognitive process likely relies on sensory information obtained from both visual and tactile modalities and declines with age. Rodents, however, have an aversion to lighted areas, exhibiting reduced exploration under these conditions (Crawley & Goodwin, 1980; Belzung et al., 1987). Moreover, the spatial resolution of the rat somatosensory system, which is less than 100 um (Morita et al., 2011; PMID: 21673811), is well suited to support object recognition. Therefore, SOR behavior, as detected by a rat's preference for novelty, may be more readily observed when animals use tactile information relative to visual. We tested young and aged rats' preferences for novel compared to familiar objects in a visual and a tactile SOR task with a 5 min delay between sample and test phases. Furthermore, to examine interactions between these modalities we used the Crossmodal Object Recognition task (CMOR) with and without multimodal pre-exposure to the sample object (Winters & Reid, 2010). This task tests the ability of rodents to identify objects based on visual features after familiarizing themselves with the object based on tactile cues only. In all 4 tasks, there was not a significant effect of age with a 5 min delay ($p > 0.1$ for all comparisons). Together, the young and aged rats showed a significant novelty preference in the tactile SOR task ($t[26] = 6.23$, $p < 0.001$), but not in the visual SOR task ($t[29] = 1.73$, $p = 0.1$). Additionally, both young and aged rats showed a significant novelty preference in the CMOR task with multimodal pre-exposure ($t[29] = 4.36$, $p < 0.001$), but not in the CMOR task without pre-exposure ($t[29] = 1.37$, $p = 0.2$). Together these data suggest that the superiority of the rat somatosensory system compared to the visual system results in preferential use of tactile over visual cues to guide crossmodal object recognition. In the CMOR with pre-exposure condition, rats are given the chance to encounter the test object's visual and tactile cues simultaneously, conceivably allowing the rat to incorporate both into a single, polymodal object representation in advance of the sample and test phase.

The ketogenic diet as therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence

A. R. HERNANDEZ, K. CAMPOS, L. TRUCKENBROD, L. SANTACROCE, C. M. HERNANDEZ, Y. SAKARYA, J. A, MCQUAIL, C. S. CARTER, A. P. MAURER, J. L. BIZON, & S. N. BURKE

Department of Neuroscience, Institute on Aging, University of Florida College of Medicine, Gainesville, FL

The loss of independence in the elderly can result from both physical and cognitive decline. These declines may be a consequence of body weight fluctuations, decreased glucose utilization, and increases in damage from reactive oxygen species. Currently, limited treatment options are available to prevent the myriad of age-related deficits many individuals experience. We propose the use of a ketogenic diet as a metabolic intervention for alleviating the symptoms of several aspects of aging. This high fat, low carbohydrate diet elicits a shift in the body's main energy source away from glucose towards the use of ketone bodies. Previous studies have shown that the ketogenic diet can reduce inflammation, increase oxidative capacity, and reverse aberrant neural activity. The ability of aged animals to enter and maintain ketosis, and the impact of this diet on body composition, however, has not been systematically examined. In this study, young and aged Fisher 344 x Brown Norway Hybrid rats were given 51 kcal/day of a nutrient matched ketogenic or control diet for a duration of 12 weeks. Young and aged rats in the ketogenic group maintained significantly higher levels of β-hydroxybutyrate and lower levels of glucose relative to the controls. While all rats were modestly calorically restricted (by ~15%), rats on a ketogenic diet had significantly less visceral fat than control rats ($p < 0.007$). Moreover, both young ($p < 0.003$) and aged ($p < 0.001$) ketogenic rats had significantly less brown adipose tissue compared to age matched controls. Additionally, TD-NMR was used to determine body composition, revealing that there was a significant increase in the ratio of lean to fat mass in the aged rats on the ketogenic diet ($p < 0.003$) but not in aged controls ($p = 0.119$). Finally, aged rats on the ketogenic diet maintained grip strength over a 10-week period, while aged rats on the control diet showed a significant decline ($p < 0.05$). Future studies will determine the extent that the ketogenic diet can improve performance on a motor-cognitive "dual-task" that requires walking while using working memory, which is particularly sensitive to dysfunction in advanced age.

Glutamate dysregulation in aged rat prefrontal cortex may contribute to decline of working memory

C. M. HERNANDEZ[1*], M. R. SCHWABE[1*], J. A. MCQUAIL[1], B. SETLOW[2], & J. L. BIZON[1]

[1]Neurosci., [2]Psychiatry, Univ. of Florida, Gainesville, FL

Working memory refers to the short-term maintenance of information and this process is thought to require the persistent activity of excitatory pyramidal neurons within the prefrontal cortex (PFC). Metabotropic glutamate receptors (mGluRs) are localized to pre- (Group II and III) and post- (Group 1) synaptic sites in the PFC, where they modulate synaptic transmission and neuronal excitability. Excitatory amino acid transporters (EAATs) are located both presynaptically and on astrocytes, where they clear excess glutamate from the synapse. As the role of PFC mGluRs and EAATs in working memory are still not well-defined, separate cohorts of rats were used to evaluate the effects of antagonists targeting receptors and transporters within each of the three major classes of mGluRs and EAATs: Group 1 (MTEP, mGluR5 antagonist), Group 2 (LY341495, mGluR2/3 antagonist), Group 3 (MMPIP, mGluR7 antagonist), non-selective EAAT antagonist (DL-TBOA), and selective EAAT1 antagonist (UCPH-101). Following surgical implantation of guide cannula targeting the medial PFC (mPFC, the rat homologue of primate dorsolateral PFC), rats were trained to baseline performance on an mPFC-dependent delayed response working memory task. Drugs were then administered acutely into mPFC using a within-subjects, Latin square design, such that all subjects within a cohort received vehicle and three doses of drug, with a 48-hour washout period between successive doses. Using this design,both the mGluR2/3 antagonist (LY341495), the mGluR7 antagonist (MMPIP) and the EAAT antagonist (DL-TBOA) impaired working memory relative to vehicle, whereas the mGluR5 antagonist (MTEP) and selective EAAT1 antagonist (UCPH-101) had no effect. A second series of experiments then used Western blotting to evaluate expression of mGluR2/3, mGluR7 and glutamate transporters (including vesicular transporters) in the mPFC of young (6 mo, n=6) and aged (24 mo., n=12) rats to explore the hypothesis that age-associated changes in PFC mGluRs contribute to the well-described working memory impairments that accompany the aging process. Expression of mGluR2/3, mGluR7, mGluR5, EAAT1, EAAT3, vGLuT1 and vGluT2 proteins was significantly reduced in aged mPFC relative to young adults. Collectively, these findings show that presynaptic mGluRs and glutamate transporters are critical for normal working memory, and further suggest that loss of presynaptic mGluRs and EAATs in the mPFC could contribute to attenuated working memory abilities in aging.

Transcriptomic profile for determining regional vulnerability to age and cognitive impairment

L. IANOV[1], M. DE BOTH[2], M. K. CHAWLA[2], A. RANI[1], A. J. KENNEDY[3], I. PIRAS[2], J. J. DAY[3], A. SINIARD[2], A. KUMAR[1], J. D. SWEATT[3], C. A. BARNES[2], M. HUENTELMAN[2], T. C. FOSTER[1]

[1]Evelyn F. and William L. McKnight Brain Institute, Univ of Florida, Gainesville, FL 32611, [2]Evelyn F. McKnight Brain Institute, Univ of Arizona, Tucson, AZ, 85724, [3]Evelyn F. McKnight Brain Institute, Univ of Alabama, Birmingham, AL, 35294

Hippocampal-dependent episodic memory declines with advancing age in a number of species, including humans and rodents. This collaborative effort across McKnight Brain Institutes examines region-specific hippocampal transcription profiles (i.e., CA1, CA3 and the dentate gyrus, DG) that may help explain differential susceptibility to impairment in specific cognitive domains over the lifespan of the rat. Young (5-6 months) and aged (17-22 months) male Fischer 344 rats were trained on a spatial episodic memory task, and hippocampal regions CA1, CA3 and DG were collected for transcriptional profiling ~2 weeks after behavioral testing. In this regard, next-generation sequencing technology is a powerful tool for examining complex processes, such as aging, by monitoring the parallel expression of thousands of genes. However, the technique requires verification of differentially expressed genes. In many cases, a subset of genes is confirmed using RT-PCR. We have taken advantage of two different next-generation platforms to confirm differential expression associated with aging and cognitive decline. RNA-seq was implemented using the Illumina HiSeq2500 and the Ion Proton. Illumina was used to generate seed lists of genes that were differentially expressed across age or cognitive function in each hippocampal subregion. The gene lists were then retested using the Ion Proton platform for validation of the results. Age effects: Across regions, aging was associated with an increase in expression of genes for gene ontology clusters linked to immune responses (FDR $p<0.05$). The DG region showed the highest number of gene changes related to the age of the animal, including the greatest number of distinct genes, consistent with the idea that the DG may be more vulnerable or responsive to aging. Cognition-related genes: The results suggest that the CA1 region was the most sensitive to cognitive impairment, with 45 up-regulated and 49 down-regulated genes in impaired animals. Clustering was observed for genes linked to Ca^{2+} signaling (FDR $p<0.05$) with decreased expression of *Hpca, Dclk2, Prkca*. Likewise, a decrease was observed for genes associated with Ca^{2+} entry (*Neto1, Prickle2*) and release of Ca^{2+} from intracellular Ca^{2+} stores (*Adra1d, Homer3, Itpr1*). An increase was observed for the Ca^{2+} pump (*Atp2b4*) in aging. In addition, altered expression was observed in genes linked to synaptic function (e.g. *Gabra5, Nptxr)* and K^+ channels (*Hcn4, Kcnk1, Kcnab2*). Together, these results suggest that impaired performance of aged animals is linked to the regulation of Ca^{2+} and synaptic function in region CA1.

Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations

S. A. JOHNSON[1], W. M. YODER[2], K. N. LUBKE[3], J. P. LISTER[5], A. P. MAURER[1,3], J. L. BIZON[1], & S. N. BURKE[1,4]

[1]McKnight Brain Institute and Department of Neuroscience, University of Florida, [2]Department of Psychology, University of Florida, [3]Department of Biomedical Engineering, University of Florida, [4]Institute on Aging, University of Florida, [5]Department of Pathology and Laboratory Medicine, UCLA

The ability to discriminate between similar stimuli is fundamental to accurate encoding and retrieval of episodic memories. For visual and spatial domains, this ability has been linked to activity within hippocampal circuits. However, it is not yet known whether the hippocampus and its cortical inputs perform this role across all sensory modalities. The current study sought to determine the extent to which neuronal populations in perirhinal cortex (PRC) and CA3 are engaged by olfactory discrimination, relative to piriform and lateral entorhinal cortical regions associated with olfactory processing (Chapuis & Wilson 2013). Male F344 rats (N=6) were trained to perform a go/no-go olfactory discrimination task using aliphatic alcohols and aldehydes (C3-C8) as stimuli. We have previously shown performance on this task is inversely related to structural similarity of odorant molecules, such that difficulty of discriminations increases as difference in carbon chain length between odorants decreases (Yoder et al. 2014). Neuronal populations active during easy versus difficult discriminations were identified with cellular compartmental analysis of temporal activity by fluorescence *in situ* hybridization (catFISH), from which the activity history of neuronal ensembles can be inferred based on subcellular distribution of Arc mRNA (Guzowski et al. 2005). After initial training, rats performed similarly on easy (85.7% correct, SD=7.7%) and difficult trials (83.3%, SD=9%). Overall, more neurons were active during olfactory discrimination in the PRC (17.5%, SD=2%; $P < 0.006$) and CA3 region (21.8%, SD=5.8%; $P < 0.007$) relative to piriform cortex (11.3%, SD=2.7%). In the piriform cortex and PRC, the difference in proportion of neurons active during difficult relative to easy trials was not significantly greater than zero (piriform: $P = 0.08$, PRC: $P = 0.30$). Conversely, in CA3 there were more neurons active during difficult trials relative to easy trials (Difference in % Arc+ neurons=6.24, SD=2.7; $P < 0.05$). Our results demonstrate that the relative size of neuronal ensembles recruited across the piriform, PRC, and CA3 increases when rats are accurately discriminating between similar, but not distinct, odorants. These findings are consistent with prior implications of hippocampal activity in olfactory working memory (Kesner et al. 2011; Weeden et al. 2014), and support the hypothesis that the hippocampus is critical for the encoding of distinct representations of similar stimuli across multiple sensory modalities.

Functional effects of age on NR2A and NR2B containing NMDA receptors in interneurons and pyramidal cells of the rat medial prefrontal cortex

K. B. KELLY[1], J. L. BIZON[2], & C. J. FRAZIER[1,2]

[1]Dept. of Pharmacodynamics, [2]Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Biochemical studies from our group and others have shown that NMDA receptor subunit expression is decreased in the aged prefrontal cortex and suggested these changes are associated with specific impairments in cognitive function. This study was designed to carefully evaluate age related changes in NMDA receptor mediated currents in both interneurons and pyramidal cells found in layer 2/3 of the rat medial prefrontal cortex (mPFC). Whole cell patch clamp recordings were used, in combination with selective antagonists, to isolate NMDAR-mediated currents carried by either NR2A or NR2B containing NMDA receptors. We found that the majority of NMDA current was carried by NR2A containing receptors in both interneurons and pyramidal cells from young (4-6 month old) animals, we carefully quantified the percentage of cells that exhibited detectable NR2B mediated currents, we calculated the ratio of NR2A:NR2B current in cells that displayed both components, and we evaluated the extent to which these values are altered by age. We found that ~40% of cells layer 2/3 interneurons tested in young animals had detectable NMDA current carried by NR2B containing NMDARs, which represented ~40% of total NMDA receptor mediated current in those cells. Interestingly, neither the percentage of NR2B positive interneurons, nor the NR2B:NR2A current ratio in those interneurons was altered in aged (20-24 month old) rats. In contrast, we found that the vast majority of young layer 2/3 pyramidal cells exhibited detectable current carried by NR2B containing NMDA receptors (representing ~34% of the total NMDAR mediated current), and that the percentage of NR2B positive pyramidal cells was significantly reduced by age. Interestingly, in aged cells that retained detectable NR2B current, the overall NR2A:NR2B ratio was not altered by age ($p=0.79$). Overall these observations indicate a prominent role for NR2A containing NMDA receptors in cortical function, and are consistent with an age related loss of NR2B containing receptors in layer 2/3 of the rat mPFC that is largely restricted to a subset of cortical pyramidal cells.

The long-term estrogen-induced facilitation of NMDA receptor synaptic function is mediated through altered redox state

A. KUMAR, L. A. BEAN, A. RANI, & T. C. FOSTER

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL 32610

A decline in estradiol (E2)-mediated cognitive benefits denotes a critical window for the therapeutic effects of E2. Our recent results demonstrate that the window for E2-mediated benefits on cognition and hippocampal E2 responsiveness can be reinstated by upregulation of estrogen receptor alpha. Further, the beneficial effects on cognition are associated with an increase in N-methyl-D-aspartate receptor (NMDA) receptor mediated synaptic function; however the mechanisms for the increase in NMDA receptor function is unknown. Here, we hypothesized that a NMDA receptor hypofunction, starting in middle-age, will be ameliorated by an E2-induced reduction in redox state. Furthermore, the ability of E2 to influence the redox mediated NMDA receptor hypofunction will decline in the oldest animals (i.e. following closing of the therapeutic window). We employed female Fisher 344 rats at ages that are on both sides of the therapeutic window (middle aged (MA): 12 months; aged: 20 months) whose ovaries had been removed ~ a week earlier and were injected with cyclic injections of 17β-estradiol-3-benzoate (EB, 10 μg, sc) or oil vehicle, for six to twelve weeks. Starting 48 hr after the final injection, we performed *in vitro* electrophysiological recording from CA3-CA1 hippocampal synapses, and measured total field excitatory postsynaptic potentials and NMDA receptor mediated synaptic responses. After isolating NMDA receptor mediated synaptic responses, we analyzed effect of a reducing agent, dithiotreitol (DTT) on NMDA receptor mediated synaptic transmission. Input/output (I/O) curve confirmed an EB-induced increase in NMDA receptor responses limited to MA animals ($p = 0.046$, n = 8/8 slices). The lack of an effect in aged animals is indicative that the therapeutic window was closed. Following collection of I/O curves, baseline NMDA receptor mediated synaptic responses were collected and DTT (0.5 mM) was bath applied and responses were followed for 60 minutes. An ANOVA across treatment groups indicated a trend for a treatment effect on the DTT-mediated growth of the response (EB vs Oil $p = 0.068$; 21/17 slices), which was largely due to MA animals (EB vs Oil; $p = 0.053$; n = 8/7). In fact, one group *t*-test on the percent increase in the NMDA receptor synaptic response relative to the baseline indicated that all groups (Aged-EB: 127.1 ± 8.9% of baseline, n = 13; Aged-Oil: 133.8 ± 6.8, n = 9; MA-Oil: 139.9 ± 11.8, n = 7) except MA-EB injected (109.5 ± 6.8, n = 7) exhibited a DTT-mediated facilitation of NMDA receptor response. These results suggest that, prior to closing of the therapeutic window, the E2-induced increase in NMDA receptor mediated synaptic function is due to a shift in redox state.

Up regulation of GluN2B type NMDA receptor in CA1 region of hippocampus and its influence on cognitive and synaptic function

C. KYRITSOPOULOS, A. KUMAR, & T. C. FOSTER

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL 32610

The N-methyl D-aspartate (NMDA) receptor is a critical mediator of the changes in synaptic strength that underlie learning and memory. Mounting evidence indicates NMDA receptor function declines with advanced age, and that this decrease is associated with cognitive deficits. Our previous work demonstrates that age-related impairments in episodic spatial memory are related to NMDA receptor hypofunction localized in region CA1 of the hippocampus, and aged animals are more susceptible to cognitive impairment due to a low dose of the activity-dependent NMDA receptor antagonist, MK-801. On the other hand, the idea that cell death in Alzheimer's disease is associated with over activity of NMDA receptor, provides part of the basis for the use of the low affinity activity-dependent NMDA receptor antagonists. Thus, it is important to understand how NMDA receptor function interacts with normal aging and in age-related neurodegenerative diseases. Upregulation of the GluN2B subunit of NMDA receptor can protect against cognitive decline in mice; however, it is unclear if the effects result from increased activity during development, resulting in a "biological reserve" against aging. To determine whether enhancing NMDA receptor function in older animals can protect against memory deficits and enhance NMDA receptor mediated synaptic transmission, we are employing a viral vector-based approach to upregulate GluN2B targeted to the CA1 region of the hippocampus. Lentivirus containing a synapsin promoter to drive green fluorescent protein (GFP) followed by a self-cleaving 2A peptide and GluN2B was injected into the hippocampus of male Fischer 344 rats. Hippocampi were collected at 2 and 4 weeks post-surgery, and expression was verified for GFP and GluN2B via fluorescent microscopy and quantitative Western blotting. Examination of the *in vivo* time course indicates that expression continues to increase from 2 to 4 weeks post vector injection. The extent of expression is estimated at ~1 mm^2. Currently, young (5 mo) and older (15 mo) F344 male animals are receiving bilateral injection of lentivirus containing the GluN2B+GFP vectors or GFP alone, and 4-5 weeks following vector injection, behavior and NMDA receptor-mediated synaptic function will be assessed over the life span.

Aging and motor control: movement speed and spatial control tradeoffs with age

D. G. LAMB[1,2,3,4], L. N. CORREA[2,3], T. R. SEIDER[4,7], D. M. MOSQUERA[2,6], J. A. RODRIGUEZ, JR[2,5], L. SALAZAR[1,2], Z. J. SCHWARTZ[3], R. A. COHEN[4], A. D. FALCHOOK[1,2,4], & K. M. HEILMAN[1,2,3,4]

[1]Malcom Randall Veterans Affairs Medical Center, Gainesville, Florida, [2]Department of Neurology, University of Florida, Gainesville, Florida, [3]Center for Neuropsychological Studies, College of Medicine, University of Florida, Gainesville, Florida, [4]Cognitive Aging and Memory –Clinical Translational Research Program, University of Florida, Gainesville, Florida, [5]Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico, [6]Florida State University College of Medicine, Tallahassee, Florida, [7]Department of Clinical and Health Psychology, University of Florida, Gainesville, Florida

With aging, people commonly develop motor slowing (bradykinesia). Although this slowness with aging may be entirely related to degradation of the cerebral networks important in motor programing, it is possible that, at least in part, it may be a learned procedure for enhancing accuracy. We evaluated this hypothesis with a timed motor control task with varied spatial precision demands. Participants were instructed to complete the task as quickly as possible while not sacrificing precision for speed. The target sizes and hand used varied by trial but were consistent within trial. We found that older adults performed the task more slowly for all target sizes. As the targets decreased in size, the younger adults performed the task more rapidly than did the older participants, but the younger participants also had a greater decline in precision. During this aiming task, healthy older adults were less likely than younger adults to sacrifice precision for speed, following the explicit instruction to preserve precision despite changes in spatial performance demands. Thus, at least in part, their slowing may be a learned adaptive strategy.

OXTR methylation as a predictor of variations in attachment in young and older adults

T. LIN[1], M. MURADOGLU[1], D. WEIR[1], T. LILLARD[2], J. CONNELLY[2], S. CARTER[3], & N. C. EBNER[1,4]

[1]Department of Psychology, University of Florida; [2]Department of Psychology, [3]University of Virginia; Kinsey Institute, Indiana University; [4]Department of Aging and Geriatric Research Cognitive Aging and Memory Program, Clinical Translational Research Program (CAM-CTRP), Institute on Aging, University of Florida

The neuropeptide oxytocin (OT) has been implicated in a wide range of affiliative processes. OT exerts its functions via oxytocin receptors, which are encoded by the oxytocin receptor gene (*OXTR*). Epigenetic modification of *OXTR* through the process of DNA methylation has been associated with individual differences in behavioral phenotypes. Specifically, lower levels of *OXTR* methylation, that is, increased *OXTR* expression, has been linked to enhanced social and affective functioning. However, research on epigenetic mechanisms of *OXTR* is scarce in non-clinical populations and even less is known about changes across adulthood. The present study assessed *OXTR* methylation levels at site -934 in 22 young (20-31 years, *M* = 23.6) and 34 older (63-80 years, *M* = 71.4) participants. Lower levels of *OXTR* methylation were associated with less self-reported attachment anxiety but higher self-reported attachment avoidance. These effects of *OXTR* methylation on adult attachment were more pronounced in young than older participants. Plasma OT levels did not mediate the effects of *OXTR* methylation on adult attachment. These findings suggest that epigenetic properties of *OXTR* are related to adult attachment, with variations across adulthood.

NSAID treatment reverses age-related changes in hippocampal neurogenesis

J. MCGUINESS[1], R. B. SCHEINERT[2], V. S. SCHWINGEL[2], A. RANI[1], A. KUMAR[1], T. C. FOSTER[1], & B. K. ORMEROD[1.2]

[1]McKnight Brain Institute and Neuroscience Department, Univ. of Florida, Gainesville, Fl, [2]J. Crayton Pruitt Family Department of Biomedical Engineering, Univ. of Florida, Gainesville, Fl

Dysregulated inflammatory signaling could contribute to age-related cognitive decline through effects on hippocampal and olfactory bulb neurogenesis. We tested whether non-steroidal anti-inflammatory (NSAID) drug treatment could reverse age-related declines in neurogenesis and spatial behavior in male Fischer 344 rats. Baseline cognitive ability was assessed in young (4-6 mo; n=32), middle-aged (10-12 mo; n=32), and aged (18-20 mo; n=34) rats using a rapid water maze task. In this task, the rats were trained to locate a visible platform in a single session and then a hidden platform in a single session 3 days later. Strength of learning and memory were tested in probe trials administered immediately and 24h after hidden platform trials, respectively. After the first water maze session, the rats were assigned randomly to vehicle (200µl of frozen strawberry milk; n=9-11 per age group), rosiglitazone (10mg/kg, BID; n=11 per age group), or indomethacin (2.5mg/kg, BID; n=11 per age group) treatment groups. Beginning a week after treatment, rats were injected once daily over 3 days with bromodeoxyuridine (BrdU, 50mg/kg; i.p.) to label dividing cells and were then trained and tested again using the same rapid water maze protocol (without visible platform trials). After the final probe session, a subset of the rats from each NSAID treatment group was perfused to quantify BrdU+ new and IBA-1+ microglial cell densities and their phenotypes through the hippocampus, rostral migratory stream (RMS) and olfactory bulb. Because neurogenesis measures were similar between middle-aged and aged rats, we combined these groups into an 'aging group' for subsequent analyses. In young rats, indomethacin ($p < 0.05$) but not rosiglitazone increased new cell number relative to vehicle. In aged rats, both indomethacin ($p < 0.05$) and rosiglitazone ($p < 0.05$) increased new cell number relative to vehicle. New cells in the hippocampus correlated positively with new cells in the olfactory bulb GCL ($p<0.05$). Young rats outperformed aging rats on hidden platform trials (blocks 2-4; p values < 0.05). Both groups performed similarly on the post-treatment immediate probe trial and aging rats actually outperformed young rats on the 24h probe trial ($p < 0.05$). Our results suggest that short-term NSAID treatment can reverse age-related decreases in neurogenesis.

Symptom dimensions of depression and age impact subfield hippocampal volume

M. E. MCLAREN[1], S. M. SZYMKOWICZ[1], A. O'SHEA[2,3], A. J. WOODS[2, 3, 4], S. D. ANTON[2], & V. M. DOTSON[1, 4]

[1]Department of Clinical & Health Psychology, University of Florida, [2]Department of Aging & Geriatric Research, University of Florida, [3]Center for Cognitive Aging & Memory, Department of Aging & Geriatric Research, University of Florida; [4]Department of Neuroscience, University of Florida

Depressive symptoms have been associated with volumetric differences in the hippocampus, particularly in the CA1, CA2-3, and subiculum subfields. We recently found an interaction between depressive symptoms and age such that higher depressive symptoms were associated with less age-related volumetric decline in the right subiculum and right CA1 regions. However, it is unclear whether any specific symptom dimensions of depression are responsible for this interaction between age and depressive symptoms. This study was conducted as a follow-up to address this question. Forty-two community-dwelling older adults completed the Center for Epidemiologic Studies Depression Scale (CES-D) and underwent magnetic resonance imaging at 3T. Depressed mood, lack of well-being, and somatic subscale scores were calculated for each participant. Left and right volumes of CA1, CA2-3 and subiculum subfields were obtained via an automated FreeSurfer procedure. Multiple hierarchical regressions were conducted to assess the effects of age, CES-D subscale scores, and their interaction on hippocampal subfield volumes, with sex and estimated total intracranial volume as covariates. A significant interaction between somatic symptoms and age was found in the bilateral subiculum (right p= 0.002, left p= 0.012) and right CA1 (p = 0.003), such that higher levels of somatic symptoms were associated with less age-related volume loss. A depressed mood by age interaction in the right subiculum (p= 0.042) showed a similar pattern. Results suggest somatic symptoms of depression, and to a lesser extent depressed mood, may drive the interrelationship between age, depressive symptoms and hippocampal subfield volume. Findings highlight the importance of examining unique correlates of the heterogeneous symptoms that comprise depressive disorders. More research is needed to better understand the neurobiological underpinnings of depressive symptoms, and how this interacts with age-related brain changes.

Stress reactivity predicts impaired working memory in aging: vulnerability of GABAergic synapses

J. A. MCQUAIL[a], M. M. BRUNER[a], E. G. KRAUSE[b], B. SETLOW[c], D. A. SCHEUER[d], & J. L. BIZON[a]

[a]Department of Neuroscience, [b]Department of Pharmacodynamics, [c]Department of Psychiatry, [d]Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL

Normal aging is associated with impaired cognition, including working memory supported by the prefrontal cortex (PFC). Our prior work determined altered glutamatergic and GABAergic signaling contributes to age-related working memory impairment. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis also accompanies the aging process and it is proposed that the cumulative effects of stress and concomitant glucocorticoid exposure over the lifespan exacerbate neural changes that mediate the emergence of cognitive deficits. As the PFC is enriched in glucocorticoid receptors, the present studies test the hypothesis that individual differences in HPA axis function predict working memory ability and that psychogenic stress recapitulates adverse effects of aging on PFC glutamatergic and GABAergic signaling protein expression. First, we evaluated the relationship between working memory and circulating corticosterone (CORT) in aging rats. When tested on a delayed response task of working memory, aged (22-24 mo) rats were significantly less accurate than young adults (4-6 mo), although aged performance spanned a broad range with some performing similar to young (unimpaired) and others performing worse than young (impaired). In this same cohort, diurnal CORT was elevated in aged compared to young, consistent with impaired ability to attenuate HPA tone during rest. When challenged with 1 hour of restraint-stress, peak CORT release was greater in aged-unimpaired rats and lower in aged-impaired rats. A direct link between working memory performance and acute elevation of CORT was tested in a separate cohort of young rats where acute, systemic injection of CORT was found to enhance delayed response task accuracy. Next we determined the extent to which chronic variable stress alters expression of excitatory and inhibitory synaptic proteins in the PFC. Young adult rats were subjected to a 14-day randomized schedule of twice-daily stressors including insulin-induced hypoglycemia (Day 1 only), forced swim, novel environment, restraint stress and exposure to predator urine. While excitatory signaling proteins (NMDARs, VGluT1) were not reliably changed by stress, expression of GABA(B)R1a, a presynaptic GABA autoreceptor, and VGAT, the presynaptic vesicular GABA transporter, were significantly reduced in the PFC of stressed rats. Collectively, our findings reveal important interrelationships between aging, stress and PFC function and, further, identify a causal role for stress in PFC GABA signaling alterations that could contribute to impaired working memory.

Recent findings and ongoing explorations of GABA MRS in Aging

E. C. PORGES[1], A. J. WOODS[1,2], R. A. E. EDDEN[3,4], N. A. J. PUTS[3,4], A. D. HARRIS[5,6,7], H. CHEN[1,8], A. M. GARCIA[1], T. R. SEIDER[1], Y. CRUS-ALMEIDA[1], N. C. EBNER[1], D. G. LAMB[1,9], J. B. WILLIAMSON[1,9,10] & R. A. COHEN[1]

[1]Center for Cognitive Aging and Memory (CAM), McKnight Brain Institute, University of Florida, Gainesville, FL, 32608, USA
[2]Department of Neuroscience, University of Florida, Gainesville, FL, 32608, USA
[3]FM Kirby Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA
[4]Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[5]Department of Radiology, University of Calgary; Calgary AB, Canada
[6]CAIR Program, Alberta Children's Hospital Research Institute, University of Calgary, Calgary AB, Canada
[7]Hotchkiss Brain Institute, University of Calgary, Calgary AB, Canada
[8]Department of Biostatistics, University of Florida, Gainesville, FL, 32608, USA
[9]Brain Rehabilitation and Research Center, Malcom Randall Veterans Affairs Medical Center, Gainesville, FL, USA
[10]Center for Neuropsychological Studies, Department of Neurology, University of Florida College of Medicine, Gainesville, FL, USA

Gamma-aminobutyric acid (GABA), the brain's principal inhibitory neurotransmitter, has been associated with perceptual and attentional functioning. Recent application of magnetic resonance spectroscopy (MRS) provides in vivo evidence for decreasing GABA concentrations during adulthood. Findings from our group demonstrate that GABA concentrations in both frontal and posterior regions decreased as a function of age. GABA concentrations controlling for age, education, and brain atrophy are positively associated with cognitive performance. Our ongoing, collaborative work is currently extending this aging focused line of research into multiple domains. Summaries and preliminary results from these ongoing research projects will be presented, including social cognition, intranasal oxytocin, chronic pain, motor control and cognitive training augmented with non-invasive brain stimulation.

Expression of G-protein estrogen receptor 1 (GPER1) in the hippocampus and prefrontal cortex over the oestrous cycle: Influence of ovariectomy and aging

A. RANI, S. KERIC, L. A. BEAN, J. BARTER, T. C. FOSTER, & A. KUMAR

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL 32610

Many of the rapid effects of estradiol are diametrically opposite to changes observed in aged memory impaired animals. Estradiol rapidly increases cell excitability and the strength of synaptic transmission. However, the effect of estradiol may decline with advanced age or prolonged estradiol deprivation, contributing to the closing of the therapeutic window. The decline in estradiol responsiveness is associated with altered expression of estrogen receptors. Evidence suggests that the orphan G protein-coupled estrogen receptor 1 (GPER1) mediates the estradiol-induced rapid increase in synaptic strength at CA3-CA1 synapses in females. The current study was designed to analyze GPER1 expression during the estrous cycle, hormonal deprivation, and senescence. We employed Western blotting to investigate expression of GPER1 in the dorsal hippocampal areas CA1, CA3, DG, and prefrontal cortex (PFC) throughout the estrous cycle in young: 3-4 months female Fischer 344 rats. Vaginal lavage was performed each day for 2-3 weeks to confirm an estrous cycle (diestrus, estrus, metestrus, proestrus). Following confirmation of estrous cycle, animals were euthanized and tissues were collected and stored at -80 for Western blot analysis. Results demonstrate that there was not a significant change in GPER1 expression in the hippocampus or the PFC across the estrus cycle (n = 5-6/cycle) of young animals. A second study examined GPER1 expression during aging in intact animals and 3-4 weeks following ovariectomy (OVX) for aged (21-24 months, n = 12) and young animals (2-4 months, n = 24-30 (all estrus cycles included as non OVX)). Western blot analyses demonstrate a significant decrease ($p<0.0001$) in GPER1 expression in the area CA1 regardless of OVX/non OVX state. A tendency for an age-related decline in the DG ($P=0.063$) was due to the fact that OVX decreased expression in young relative to non OVX young ($P<0.05$). No age-associated difference in GPER1 expression was observed in area CA3 and PFC. However, there was a tendency for expression to decrease in the PFC of young OVX vs young non OVX ($p=0.053$). These results indicate that prolonged hormone deprivation in young or advanced age may cause a decrease in GPER1 expression in different areas of the brain. Future studies will determine if these alterations in GPER1 expression contribute to impaired cognitive and synaptic function over the life span.

Brain-Derived Neurotrophic Factor (BDNF) and learning & memory in an HIV population

L. A. RICHARDS, V. E. BRYANT, R. COHEN, & A. J. WOODS

Center for Cognitive Aging and Memory, Institute on Aging, Department of Aging and Geriatric Research, University of Florida/ McKnight Brain Research Foundation

Brain-derived neurotropic factor (BDNF) is an important biomarker related to neural integrity and cognitive performance. Previous literature suggests that BDNF is associated with a variety of different cognitive domains including learning, memory, executive function, processing speed, and global deficits. These facets have mostly been studied in human immunodeficiency virus (HIV) negative populations. Given the rates of HIV-associated neurocognitive disorders (HAND)(approximately 50%), it is important to study BDNF as a potential biomarker in detecting neurocognitive impairment within this population. Additionally, this biomarker may grant some insight into the underlying pathophysiology of neurocognitive impairment. The current analysis aimed to evaluate the relationship between BDNF and cognitive functioning in an HIV population.

Default mode network control, social engagement, and memory in older adults

T. R. SEIDER, A. M. GARCIA, A. J. WOODS, & R. A. COHEN

Center for Cognitive Aging and Memory, Department of Aging and Geriatric Research, University of Florida

We have previously reported social engagement to be positively associated with verbal memory in older adults. Additionally, we found off-task activation of the posterior cingulate (PCC) during a continuous memory task that elicits ongoing rehearsal demands to be negatively associated with performance. The PCC is part of the default mode network, which is typically more active at rest than on task. Given that PCC activation was associated with visual memory, we hypothesized that it would mediate the relationship between social engagement and verbal memory. Fifty-seven older adults received a questionnaire of everyday social activities, a cognitive assessment, and fMRI. Social engagement was moderately negatively associated with off-task PCC activation (p=.06). Off-task PCC activation was negatively related to verbal memory (p=.019), and it partially mediated the relationship between social engagement and verbal memory ($\Delta\beta$=.06, p>.05). Results indicate that greater social engagement is associated with less disengagement from a visual memory task, suggesting that more social individuals employ memory strategies during rest, thus improving performance. While PCC activation was associated with verbal memory, it only partially mediated the link between social engagement and this domain. As such, there may be additional biomarkers to explain the relationship between social engagement and memory.

Structural abnormalities in cortical thickness, surface area, and volume of the precuneus in older adults with depressive symptoms

S. M. SZYMKOWICZ[1], M. E. MCLAREN[1], L. DE WIT[1], A. O'SHEA[2,3], A. J. WOODS[2,3,4], S. D. ANTON[3], & V. M. DOTSON[1,4]

Departments of [1]Clinical & Health Psychology, [2]Center for Cognitive Aging & Memory, [3]Aging & Geriatric Research, and [4]Neuroscience, University of Florida, Gainesville, FL, 32610, USA

Objective: The precuneus has been implicated in many structural and functional studies of depression due to its involvement in self-referential processing and visual imagery. We recently reported that older adults with higher depressive symptoms had greater cortical thickness in the left precuneus. However, it is unclear whether abnormalities in surface area or volume of this region are also evident. The current study addressed these questions.

Participants and Methods: Forty-three community-dwelling adults (mean age = 68.79 ± 7.00 years) completed the Center for Epidemiologic Studies Depression Scale and underwent structural magnetic resonance imaging scanning at 3T. Measures of cortical thickness, surface area, and volume were extracted from the right and left precuneus. Age-related differences in these measurements, as well as their association with total depressive symptoms, were assessed. Exploratory analyses investigating depressive symptom dimensions (depressed mood, lack of well-being, somatic) for any significant relationships were also conducted.

Results: Main effects of age were seen for left precuneus surface area (p = .015) and volume (p = .011), such that higher age was associated with less surface area and volume. An age by depressive symptoms interaction was found for cortical thickness in the left precuneus (p = .037), such that higher depressive symptoms were associated with less age-related cortical thinning. Follow-up analyses found a significant age by somatic symptoms interaction (p < .001), suggesting that somatic symptoms, rather than depressed mood or lack of well-being, drove this effect.

Conclusions: We found that cortical thickness, rather than surface area or volume, of the precuneus is associated with elevated depressive symptoms in older adults, and that this association may be driven by somatic symptoms. Further research is needed to better understand possible mechanisms through which subthreshold depressive symptoms are associated with the precuneus during aging.

A rodent model of medial temporal lobe-dependent discrimination deficits in the elderly

S. M. TURNER[1], L. A. SANTACROCE[1], S. A. JOHNSON[1], A. P. MAURER[1,3], & S. N. BURKE[1,2]

[1]McKnight Brain Institute and Department of Neuroscience, University of Florida, 1149 Newell Drive, Gainesville, FL 32610; [2]Institute on Aging, University of Florida; [3]Department of Biomedical Engineering, University of Florida

Advanced age is associated with cognitive deficits that include impairments in object recognition and discrimination. In humans, the magnitude of these deficits is proportional to the difficulty of the discrimination. That is, elderly subjects show greater impairments when the stimuli to be discriminated have more features in common, relative to when they are more distinct (Yassa et al. 2011). The neurobiological underpinnings of these declines, however, have not yet been elucidated. This is due, in part, to the lack of rodent model for age-related discrimination deficits. The goal of these studies was to determine whether age selectively impairs the ability to distinguish between similar stimuli in a rat model of old age, and to test the role of the hippocampus. In order to systematically vary the difficulty of object discriminations, stimuli were created from LEGO© blocks. In experiment 1, young (6-10 m) and aged (26-30m) male Fischer 344 x Brown Norway hybrid rats were trained on an object discrimination task in which one pair of objects was visually distinct (easy), while the other pair shared features (difficult). Although young [$p<.05$] and aged [$p<.05$] rats took significantly longer to learn the difficult compared to the easy discrimination, the aged rats were selectively impaired relative to young on the difficult discrimination only [$p<0.01$]. In order to more closely replicate the experimental design of human studies, in experiment 2, rats were tested for their ability to correctly discriminate between a well-learned target object and 3 novel foil objects, each with increasing similarity to the target. Similar to the first experiment, aged animals were impaired relative to young on the difficult discriminations [$p<0.05$]. Finally, in experiment 3, a reversible inactivation in young rats was used to test the requirement of a functional hippocampus in performing the difficult discriminations. Inhibition of hippocampal neural activity resulted in a difficulty-dependent deficit similar to that observed in aged rats. These data suggest that advanced age impairs the ability to accurately discriminate between objects that share overlapping features, and this deficit may emerge from hippocampal dysfunction occurring in advanced age.

Age-related alterations in working memory and intertemporal choice in Fischer 344 x Brown Norway hybrid rats

L. M. VETERE[1], C. A. ORSINI[2], J. A. MCQUAIL[3], S. N. BURKE[3], B. SETLOW[2], & J. L. BIZON[3]

[1]University of Florida, Gainesville, FL, [2]Department of Psychiatry, University of Florida, Gainesville, FL, [3]Department of Neuroscience, University of Florida, Gainesville, FL

The ability to evaluate the benefits and potential consequences associated with a choice and to make effective decisions is critical for maintained independence across the lifespan. Poor decision making is associated with impaired prefrontal cortical-dependent cognition across a variety of clinical conditions. However, despite the fact that prefrontal cortical function declines in aging, aged individuals generally show an enhanced ability to delay gratification as is evident by less discounting of delayed rewards in intertemporal choice tasks. The current study was designed to evaluate relationships between two aspects of prefrontal cortical-dependent cognition (working memory and cognitive flexibility) and intertemporal choice in young (6 mo.) and aged (24-28 mo.) Fischer 344 X Brown Norway hybrid rats. First, young and aged rats were tested on a food-motivated intertemporal choice task in which they chose between a small reward available immediately and a large reward available following variable delays ranging from 0-60 seconds. Overall, aged rats showed attenuated discounting of delayed rewards in this task compared to young (i.e., enhanced preference for large, delayed over small, immediate rewards). These same rats were then tested on a delayed response working memory task in which they had to remember the location of a response lever over delays ranging from 0-24 seconds. Aged rats were significantly impaired on the delayed response task compared to young, although among aged rats, those with better working memory tended to show the greatest ability to delay gratification on the intertemporal choice task. Finally, the rats were tested on a set-shifting task in which they had to shift between two response rules in order to receive food rewards. Performance on the set- shifting task did not predict intertemporal choice among aged rats. These data indicate that impairments in working memory and cognitive flexibility cannot account for the robustly attenuated discounting of delayed rewards observed in aged subjects.

Augmenting cognitive training in older adults: the ACT study

A. J. WOODS[1], R. COHEN[1], M. MARSISKE[1], G. ALEXANDER[2], S. CZAJA[3], & C. WRIGHT[3]

[1]Center for Cognitive Aging and Memory, McKnight Brain Institute, University of Florida, [2]McKnight Brain Institute, University of Arizona, [3]McKnight Brain Institute, University of Miami

This recently funded NIA randomized clinical trial will test whether transcranial direct current stimulation (tDCS) of frontal cortices enhances neurocognitive and functional outcomes achieved from cognitive training (CT) in older adults experiencing age-related cognitive decline. Change in well-validated measures of neurocognitive function and everyday abilities will serve as outcome measures. Functional and structural neuroimaging biomarkers of neural plasticity and learning (fMRI, GABA MRS, etc.) will measure intervention-associated alterations in specific brain regions impacted by cognitive aging. tDCS is a noninvasive brain stimulation method that facilitates neural plasticity and learning. Accordingly, when used as an adjunctive intervention, tDCS may augment cognitive training effects. This study leverages existing multisite clinical trial infrastructure at McKnight Brain Institutes located in two of the states with the largest representation of older adults in the United States: University of Florida, University of Miami, and University of Arizona. Adults over the age of 65 represent the fastest growing group in the US population. As such, age-related cognitive decline represents a major concern for public health. Recent research suggests that cognitive training in older adults can improve cognitive performance, with effects lasting up to 10 years. However, effects are typically limited to the tasks trained, with little transfer to other cognitive abilities or everyday skills. Effects may also be reduced in people with Alzheimer's disease risk factors. A two-phase multisite randomized clinical trial will examine the individual and combined impact of pairing cognitive training with transcranial direct current stimulation (tDCS) in older adults experiencing age-related cognitive decline (n = 360; 120 per site). Participants will consist of elderly men and women 65-90 years of age with evidence of age-related cognitive decline, but not MCI or Alzheimer's disease (MoCA≥25). We will compare changes in cognitive and brain function resulting from CT and CT combined with tDCS using a comprehensive neurocognitive, clinical, and multimodal neuroimaging assessment of brain structure, function, and metabolic state. Functional magnetic resonance imaging (fMRI) will be used to assess brain response during working memory, attention, and memory encoding; the active cognitive abilities trained by CT. Proton magnetic resonance spectroscopy (MRS) will assess markers of neural plasticity, GABA concentrations, and cerebral metabolism. We hypothesize that: 1) tDCS will enhance neurocognitive function, brain function, and functional outcomes from CT; 2) Effects of tDCS on CT will be maintained up to 12 months following training, and 3) Neuroimaging biomarkers of cerebral metabolism, neural plasticity (GABA concentrations) and functional brain response (fMRI) during resting vs.

active cognitive tasks will predict individual response to tDCS, with certain Alzheimer's risk factors (e.g., APOE4 genotype, family history of Alzheimer's disease) predicting poorer cognitive and functional outcome. To date, no studies have comprehensively examined combined CT and tDCS intervention in the elderly. This study will provide definitive insight into the value of combating cognitive decline in a rapidly aging US population using tDCS with cognitive training.

Interim Director: David Standaert, M.D., Ph.D.
Co-Director: Erik Roberson, M.D., Ph.D.

AAV-progranulin improves pathology in *Grn*$^{-/-}$ mice, an animal model of CLN11-Neuronal Ceroid Lipofuscinosis (NCL)

A. E. ARRANT, V. ONYILO, D. E. UNGER, & E. R. ROBERSON

Departments of Neurology and Neurobiology, Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham

Progranulin (*GRN*) is a secreted glycoprotein that modulates inflammation and is critical for normal lysosomal function. Loss-of-function *GRN* mutations are a major autosomal dominant cause of Frontotemporal Dementia (FTD), and individuals homozygous for loss-of-function *GRN* mutations develop the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis (NCL). NCL is a devastating, early onset neurodegenerative disorder with multiple genetic subtypes that causes retinal degeneration and seizures. NCL due to *GRN* mutations has been designated as CLN11-NCL. *Grn*$^{-/-}$ mice develop retinal degeneration, thalamic hyperexcitability, and NCL-like pathology, thus providing an animal model of NCL due to progranulin deficiency. *Grn*$^{-/-}$ mice exhibit the characteristic NCL lesion, accumulation of autofluorescent lipofuscin granules, as well as astrogliosis and microgliosis throughout the brain. We hypothesized that restoring progranulin to *Grn*$^{-/-}$ mice might improve this NCL-like pathology. To test this hypothesis, we injected an AAV2/1 vector expressing mouse progranulin (AAV-*Grn*) into the medial prefrontal cortex (mPFC) of 10–12-month-old wild-type and *Grn*$^{-/-}$ mice. At this age, *Grn*$^{-/-}$ mice exhibit robust NCL-like pathology. Additional wild-type and *Grn*$^{-/-}$ mice were injected with AAV-GFP as a control, and uninjected *Grn*$^{-/-}$ mice were run to provide a baseline measure of pathology in the absence of AAV. All mice were euthanized 8–10 weeks after injection, and brains were processed for immunohistochemistry. AAV-*Grn* strongly overexpressed progranulin in wild-type and *Grn*$^{-/-}$ mice, such that AAV-*Grn*-treated *Grn*$^{-/-}$ mice had higher progranulin levels in the mPFC than AAV-GFP-treated wild-type mice. In support of our hypothesis, we observed significant reductions in lipofuscinosis as well as in microgliosis (CD68 immunoreactivity, Iba1+ cell morphology), but no change in astrogliosis (GFAP immunoreactivity) in regions distant from the injection site (motor cortex, CA3 of hippocampus, and ventral posterior thalamus). At the injection site in the mPFC of *Grn*$^{-/-}$ mice, we observed an increase in microgliosis with AAV-*Grn* (CD68 immunoreactivity), as well as immune activation (MHCII immunoreactivity). This indicates a local inflammatory reaction that could be driven by a non-self reaction to progranulin or cleavage of progranulin into pro-inflammatory granulin fragments. As this negative side effect is unlikely to occur in CLN11-NCL patients, who are not completely progranulin-deficient, these data support the use of progranulin-boosting therapies for CLN11-NCL.

Selective activation of somatostatin or parvalbumin expressing interneurons triggers GABA-mediated LLDs in rat neocortex

A. BOHANNON & J. J. HABLITZ

University of Alabama – Birmingham

In the presence of the A-type K^+ channel blocker 4-aminopyridine (4-AP), long-lasting depolarizations (LLDs) occur spontaneously and propagate through the neocortex. These LLDs persist when excitatory glutamatergic neurotransmission is blocked with CNQX and D-APV (EAA blockers), suggesting the events arise from synchronous activity of inhibitory interneurons and represent a propagating GABA-mediated LLD. While this phenomenon is well documented, the role of specific interneuron (IN) classes in generating these events is poorly understood. Recently, we have shown that 4-AP alters the action potential and repetitive firing properties of Martinotti cells (MC) and fast-spiking basket cells (FS-BC) in neocortex, making these cells prime candidates for involvement in LLD initiation. In this study, we independently assessed the ability of MCs and FS-BCs to initiate LLDs using light-activation via genetically encoded channelrhodopsin (ChR) driven by the somatostatin (SST) or parvalbumin (PV) promoters, respectively. Spontaneous and light-initiated LLDs were recorded from putative Layer 2/3 MC, FS-BC and pyramidal cells (PC) in normal saline and following application of 4-AP and EAA blockers. In all cell types, wash-on of 4-AP and EAA blockers induced spontaneous LLDs and increased the amplitude and duration of light-triggered GABAergic events. The amplitude, duration, and response area of light-initiated LLDs in both PV:ChR and SST:ChR animals were statistically equivalent to spontaneous and evoked LLDs , suggesting activation of either interneuron class alone is sufficient to initiate cortical GABA LLDs. These results suggest synchronous GABAergic network activity is driven by neuronal ensembles consisting of multiple IN populations rather than being driven by a single cell type, implicating inter-population cooperativity in aberrant GABAergic activity in the neocortex.

Altered dopamine D4 receptor dependent regulation of synaptic transmission and hippocampal circuit function in PGC-1α-/- mice

L. J. BRADY[a,b], A. F. BARTLEY[a,b], Q. LI[a,b], & L. E. DOBRUNZ[a,b]

[a] Department of Neurobiology and Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, 1825 University Blvd., Birmingham, AL, USA, [b] Civitan International Research Center, University of Alabama at Birmingham, 1719 6th Ave. S., Birmingham, AL, USA

GABAergic interneurons control neuronal excitability, integration, and plasticity & have been shown to be altered in various neurological diseases that have cognitive impairment as a core symptom. In schizophrenia for example, post mortem studies have consistently shown transcriptional dysregulation in GABAergic interneurons, in particular fast-spiking interneurons containing the calcium binding protein parvalbumin (PV). To model this, our lab uses mice with genetic deletion of PGC-1α, a transcriptional coactivator that has been shown to be altered in several neurological disorders. Previous data from our lab has shown that the inhibitory/excitatory (I/E) ratio is increased in PGC-1α-/- mice at the Schaffer collateral – CA1 pathway in hippocampus, caused by enhanced inhibition and increased GABA release from interneurons. PGC-1α-/- mice also have enhanced gamma oscillation power and impaired nesting behavior, indicating hippocampal circuit dysfunction. GABAergic inhibitory synaptic transmission has been shown to be modulated by dopamine (DA) in hippocampus. In particular, DA D4 receptors have been observed to be located on PV+ GABAergic interneurons in hippocampus, and have been shown to be important for regulating gamma oscillations. Here we use PGC-1α-/- mice to investigate the interaction between dysfunction of the dopaminergic and GABAergic systems caused by interneuron transcriptional dysregulation. We find that DA has a disinhibitory effect on synaptic responses in WT and PGC-1α-/- slices, although there was no significant difference in the magnitude. We observed that blocking D4 receptors resulted in an increased field potential in PGC-1α-/- mice due to disinhibition. These data suggest that there is a tonic effect of D4 activation to enhance feedforward inhibition in PGC-1α-/- slices, which may contribute to the enhanced I/E ratio in these mice. We also found that specific D4 receptor antagonists rescued the deficit in basal synaptic transmission observed in PGC-1α-/- mice, and normalized the increased power of hippocampal gamma oscillations and the deficit in nesting behavior. This suggests that alterations in the DA system's modulation of inhibition, through changes in D4 receptor effects, are involved in the circuit dysfunction caused by deletion of PGC-1α. These results also provide mechanistic support for atypical antipsychotics that have a higher affinity for D4 receptors, like Clozapine, in the treatment of cognitive symptoms of neurological disorders.

Context fear memory formation is regulated by Neat1 long non-coding RNA mediated histone lysine methylation changes in the hippocampus

A. A. BUTLER**,** A. W. CHANG, & F. D. LUBIN

University of Alabama at Birmingham, Birmingham, AL

Histone lysine methylation is critical for the formation and maintenance of long-term memories. Manipulation of specific histone lysine methyltransferases and demethylases modulate histone methylation and is sufficient to either improve or impair the formation of long-term memories. However, initiation of histone lysine methylation mechanisms during long-term memory formation or "consolidation" is poorly understood. In recent years, long noncoding RNAs (lncRNAs) have been implicated in the targeting of epigenetic modifiers to gene loci. Indeed, a single lncRNA may target hundreds or thousands of genomic loci for epigenetic regulation, resulting in semi-permanent and functionally important changes in gene transcription changes necessary for neuronal activity. New studies have identified expression of a large population of lncRNAs in the rodent hippocampus, a region critical for the conversion of short-term memories to long-term memories. Here, we examined the role of one such lncRNA, Neat1, in the process of long-term memory consolidation. Using RNA immunoprecipitations, we found multiple chromatin modifying enzymes associated with Neat1 in cultured cells, including the repressive histone methyltransferases Ehmt2 and Ezh2, which mediate H3k9me2 and H3K27me3, respectively. Blocking Neat1 expression via siRNA significantly enhanced memory retention in contextual fear conditioning memory task. Inversely, we found that Neat1 overexpression using CRISPR-dCas9 technology significantly interfered with long-term memory formation. These findings suggest for the first time a unique role for Neat1 as a "molecular brake" on the formation of long-term memories. Using an Informatics approach, we identified several memory-related targets for Neat1 and publicly available sequencing data revealed that Neat1 is up regulated in the hippocampus of aged animals relative to young adults, and after chronic stress – both of which are associated with memory deficits. Collectively, these results support the hypothesis that the Neat1 lncRNA is a powerful negative regulator of long-term memory formation, and further indicate that targeting Neat1 activity may have both clinical relevance and therapeutic potential for the treatment of memory deficit disorders associated with normal aging and stress.

A pipeline for rapid *in vitro* evaluation of variants of uncertain significance from patients with developmental delay and/or intellectual disability exhibiting seizures

J. N. COCHRAN[1], K. L. ENGEL[1], A. A. HARDIGAN[1], R. C. RAMAKER[1], M. D. AMARAL[1], K. M. BOWLING[2], C. R. FINNILA[2], S. M. HIATT[2], M. L. THOMPSON[2], D. E. GRAY[2], J. S. WHITTLE[2], W. V. KELLEY[3], M. E. COCHRAN[3], K. M. EAST[3], N. E. LAMB[3], C. A. RICH[5], K. B. BROTHERS[5], E. J. LOSE[6], S. B. SIMMONS[7], E. M. BEBIN[7], G. S. BARSH[4], G. M. COOPER[2], & R. M. MYERS[1]

[1]Myers Lab, [2]Gregory Cooper Lab, [3]Educ. & Outreach, [4]Barsh Lab, HudsonAlpha Inst. For Biotech., Huntsville, AL; [5]Pediatrics, Univ. of Louisville, Louisville, KY; [6]Genet., [7]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Developmental delay and intellectual disabilities (DD/ID) include devastating phenotypes and comprise a large fraction of rare undiagnosed conditions in children. Unfortunately, little is known about the cellular mechanisms that lead to disease, and as a consequence, therapeutic advancements have suffered. Previous studies have indicated a strong genetic component to these phenotypes, and successful identification of causal genetic variants through exome or whole genome sequencing sometimes leads to clinical diagnoses, revision of treatment strategies, community and support network building, and increased quality of life. However, sequencing-based diagnostic efforts typically solve only a subset of cases; while precise numbers vary according to ascertainment and study enrollment criteria, large fractions of children cannot be given a precise genetic diagnosis even after whole genome sequencing. In our own work as part of the Clinical Sequencing Exploratory Research consortium, we have found diagnostic genetic variants in ~27% of children with conditions refractory to standard diagnostic tests. Rigorous experimental evaluation of variants and genes implicated in DD/ID is needed to increase diagnostic rate, particularly for the subset of the undiagnosed cases where a potential genetic cause is identified but not confirmed, termed to be variants of uncertain significance (VUSs). VUSs typically arise as a result of a lack of information about the relevance (or lack thereof) of a given gene to disease, the impact (or lack thereof) of a given variant on gene function, or both. In our studies to date, ~15% of affected children harbor a VUS, and others harbor variants that may be of interest, but are non-returnable even as a VUS. We are developing a pipeline to assess the effect of VUSs and non-returnable variants in human neurons derived from neural precursor cells. We have tailored the design of this pipeline to detect changes in excitability, as ~50% of our patient population exhibits seizures. This pipeline will provide evidence for or against the association of these sequence variants on key molecular and cellular phenotypes including global gene expression, neuronal excitability, and synapse and/or ion channel composition profile. Thus, this pipeline will provide critical insights regarding the biological roles of genes and

variants associated with DD/ID and, more broadly, will establish a framework for future mechanistic interrogation of genetic variation as it relates to other neurologic diseases.

Analysis of alpha-synuclein pathology in PINK1 knockout rats

R. B. CREED & M. S. GOLDBERG

Center for Neurodegenration and Experimental Therapeutics, Department of Neurology, University of Alabama at Birmingham, Birmingham, AL

Mutations in the PTEN induced kinase 1 (PINK1) gene cause autosomal recessive Parkinson's disease (PD). The main pathological hallmarks of PD are loss dopamine neurons in the substantia nigra pars compacta, which are required for normal movement, and the formation of alpha-synuclein rich aggregates termed Lewy body inclusions. Previous studies of PINK1 knockout (KO) rats have reported mitochondrial dysfunction, behavioral deficits, loss of neurons in the substantia nigra and locus coeruleus, and alpha-synuclein aggregates in various brain regions. We sought to characterize PINK1 KO rats specifically with respect to alpha-synuclein pathology because spontaneous formation of alpha-synuclein aggregates (without alpha-synuclein overexpression or injection) is a rare and important feature of PD animal models and because abnormal alpha-synuclein has been implicated both genetically and neuropathologically as a key mechanism of PD pathogenesis. Given PINK1's proposed function in mitochondrial autophagy, we also investigated the abundance of key mitochondrial proteins in the brains of PINK1 KO rats. We observed alpha-synuclein-immunoreactive aggregates in various brain regions of PINK1 KO rats including cortex, thalamus, striatum and ventral midbrain, but nowhere in wild-type (WT) rats. Proteinase K treatment revealed protease-resistant alpha-synuclein in the brains of PINK1 KO rats, however, the inclusions themselves were not proteinase K resistant. Co-imunofluorescence showed that the alpha-synuclein-immunoreactive aggregates are both ubiquitin immunoreactive and thioflavin S positive. We did not find any tau-immunoreactive pathology or any differences between WT and KO rats in markers of neuroinflammation, such as GFAP and Iba1. Western analysis showed similar levels of key mitochondrial proteins in the brains of WT and PINK1 KO rats. Together, this data indicates that PINK1 deficiency can directly lead to abnormal alpha-synuclein aggregation in vivo. This suggests that alpha-synuclein aggregation may be directly involved in PD-related neurodegeneration caused by PINK1 mutations.

Experience-dependent epigenomic reorganization

C. G. DUKE*, A. J. KENNEDY+, J. D. SWEATT++, & J. J. DAY*

*Department of Neurobiology and Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL, +Department of Neuroscience, Bates College, Lewiston, ME, ++Department of Pharmacology, Vanderbilt University, Nashville, TN

The formation and maintenance of new memories requires transcription and translation of genetic material, and epigenetic mechanisms such as DNA methylation and demethylation serve as powerful regulators of this gene expression that is crucial to these processes. Moreover, aberrant DNA methylation has been identified in neurological and psychiatric disease states associated with impaired cognition, such as Alzheimer's disease, autism-spectrum disorders, schizophrenia, and drug addiction. Despite the clear necessity for epigenetic and transcriptional changes in memory formation, the precise nature of these phenomena has not been comprehensively explored. To illuminate this area, we harnessed whole-genome sequencing tools to systematically characterize memory-related changes in gene expression and DNA methylation status following memory acquisition. Using a hippocampus-dependent memory task (contextual threat learning), we report widespread and coordinated DNA methylation changes in the hippocampus (CA1) of Sprague-Dawley rats that are specific to threat learning and target genes involved in synaptic transmission and neuronal communication. We observed thousands of significant gene expression and epigenomic changes that are experience dependent, and these modifications were evident as early as one hour following the learning experience, becoming more marked and pronounced after twenty-four hours. Gene ontology term analysis showed that significantly hypomethylated genes were enriched for functional categories related to synaptic transmission. Additionally, we integrated these datasets with previously characterized epigenetic and transcriptional changes in brain disease states, such as Alzheimer's disease, to provide a comprehensive resource to aid in the identification of memory-relevant therapeutic targets. Our results shed new light on the gene expression and methylation changes involved in memory formation suggesting that this process is dynamic and experience dependent, in addition to providing a roadmap for future work to identify therapeutic targets.

Extra-coding RNAs regulate dynamic DNA methylation and gene expression

N. V. N. GALLUS, K. E. SAVELL, F. A. SULTAN, R. C. SIMON, J. S. REVANNA, & J. J. DAY

Department of Neurobiology and Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL

Epigenetic mechanisms in neurons are central regulators of neuronal function, experience-dependent gene expression, and adaptive behavior. DNA methylation is a well-studied epigenetic mechanism that exerts potent control over transcription and is critical for synaptic plasticity and long-term memory in multiple brain circuits. Although DNA methylation at specific sites in the genome is actively modified by neuronal activity and behavioral experience, it is presently unclear how methylation status at individual genes or even individual cytosine nucleotides can be targeted for modification. Extra-coding RNAs (ecRNAs) are non-coding, nonpolyadenylated RNA species that arise from protein coding genes and regulate DNA methylation via direct interactions with DNA methyltransferases (DNMTs). Here, we used cortical neuronal culture systems to investigate the regulation, binding, and localization of a specific ecRNA transcript from the Fos gene locus. We find that this ecRNA is sensitive to multiple forms of neuronal activity, binds to both de novo and maintenance DNA methyltransferases with high affinity, and blocks DNA methylation at the Fos locus. To investigate the localization of ecRNA on a single cell basis, we employed single-molecule RNA FISH with multiplexed probes to separately identify mRNA and ecRNA transcripts. This technique confirmed activitydependence of ecRNA induction and revealed a correlation between ecRNA and mRNA expression on a single cell level. Consistent with this observation, anti-sense based knockdown of the Fos ecRNA selectively reduced Fos mRNA. Ongoing experiments are investigating the ability of targeted ecRNA delivery to specific gene loci to alter DNA methylation patterns and gene expression. Overall, these results suggest that ecRNAs are fundamental regulators of the establishment and perpetuation of DNA methylation patterns in neuronal systems, and reveal a promising avenue for epigenetic targeting in neurological and cognitive disease states.

Damage to white matter bottlenecks contributes to chronic language impairments and disrupts semantic network function in patients with left middle cerebral artery stroke

J. C. GRIFFIS & J. P. SZAFLARSKI

Departments of Psychology and Neurology, University of Alabama at Birmingham, Birmingham, AL

Neuroimaging evidence indicates that normalized processing in pre-existing language networks underlies language recovery after left hemisphere stroke. Damage to connections among language areas might be expected to impede recovery by disabling long-range communication and preventing the re-integration of surviving areas into coherent networks for language processing. Indeed, chronic language impairments have been linked to white matter damage near regions that may correspond to spatial bottlenecks containing fibers from several long-range tracts. However, the effects of damage to verified white matter bottlenecks on long-term language outcomes and language network function in stroke patients have not been investigated.

We first validated predictions of the current model of language recovery after stroke by showing that task-driven fMRI activity in the pre-existing semantic network (identified in 43 healthy individuals) during semantic decisions predicts naming, fluency, and comprehension abilities in 43 patients with chronic post-stroke aphasia. Diffusion MRI tractography of the healthy individuals verified the presence of 2 white matter bottlenecks involving (1) callosal, arcuate, inferior fronto-occipital, and inferior longitudinal fibers in the posterior temporal white matter, and (2) anterior thalamo-cortical, uncinate, and inferior fronto-occipital fibers in the anterior prefrontal white matter. Using multiple regression to control for lesion volume effects, we found that while damage to the posterior bottleneck predicts chronic deficits in fluency, naming, and comprehension, damage to the anterior bottleneck only predicts deficits in fluency. Results from a multivariate lesion-behavior analysis corroborated these findings. Additional fMRI analyses revealed that damage to the posterior bottleneck was associated with reduced task-driven activity throughout the bilateral semantic network.

Our results provide support for the current model of language recovery after stroke. They also show that damage to white matter bottlenecks, specifically in the posterior temporal lobe, contribute to chronic language impairments in multiple domains and disrupt function in bilateral portions of the pre-existing semantic network. Damage to white matter bottlenecks may represent an under-recognized source of chronic language deficits after stroke, and may underlie previous associations between posterior temporal white matter damage and poor prognosis in patients with post-stroke aphasia.

Training the aging brain: a multi-level network analysis

J. M. HICKS[1,2], R. NENERT[3], L. A. ROSS[4], & K. M. VISSCHER[2]

[1]Department of Mathematics, University of Alabama at Birmingham, [2]Department of Neurobiology, University of Alabama at Birmingham, [3]Department of Neurology, University of Alabama at Birmingham, [4]Department of Human Development and Family Studies, The Pennsylvania State University

One strategy shown to be effective in improving cognitive performance of older adults is known as Speed of Processing (SOP) training. SOP training shows reliable effects that lead to improvements in performance on tasks similar to the training paradigm as well as important life activities. While SOP training yields reproducible behavioral effects, the neural mechanisms underlying these effects are largely unknown. We aim to determine how SOP training affects the resting-state brain networks of older adults. Yet because the brain is a vastly complex structure with many interconnected units, understanding the organization of these connections and how they change through training is a challenging endeavor. A further challenge is how to accurately define these interconnected units of the brain. Current analytical methods require the experimenter to make assumptions about how the brain is subdivided into parts within a network, but clearly, these assumptions can influence the outcomes of analyses. Thus, we use resting-state functional connectivity data along with a k-means clustering algorithm to create parcellations of cortical and subcortical gray matter across multiple spatial scales. The resulting parcellations demonstrate validity as they identify previously defined brain regions, group regions that are known to be connected, and exhibit a high degree of bilateral symmetry. We found a significant correlation between the degree of behavioral improvement with training and a change in global network characteristics, suggesting SOP training may improve cognitive performance by altering network structure. Further, the relationship to behavior exists primarily at a limited set of spatial scales, suggesting that behaviorally relevant network changes occur at a favored range of spatial scales. This work shows that the spatial scale of parcellations influences the sensitivity of analyses of network structure and that improvements in speed of processing may be a result of subtle changes in network structure.

Rho-associated protein kinase 1 (ROCK1) is increased in Alzheimer's disease and ROCK1 depletion reduces amyloid-β levels in brain

B. W. HENDERSON[a,b], E. G. GENTRY[a,b], T. RUSH[a,b], J. C. TRONCOSO[d], M. THAMBISETTY[e], T. J. MONTINE[f], & J. H. HERSKOWITZ[a,b,c]

[a]Center for Neurodegeneration and Experimental Therapeutics, Departments of [b]Neurology, and [c]Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama 35294, [d]Departments of Pathology and Neurology, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, [e]Unit of Clinical and Translational Neuroscience, Laboratory of Behavioral Neuroscience, National Institute on Aging, Baltimore, Maryland 21224, [f]Department of Pathology, University of Washington, Seattle, Washington 98104

Alzheimer's disease (AD) is the leading cause of dementia and mitigating amyloid-β (Aβ) levels may serve as a rational therapeutic avenue to slow AD progression. Pharmacologic inhibition of the Rho-associated protein kinases (ROCK1 and ROCK2) is proposed to curb Aβ levels, and mechanisms that underlie ROCK2's effects on Aβ production are defined. How ROCK1 affects Aβ generation remains a critical barrier. Here, we report that ROCK1 protein levels were elevated in mild cognitive impairment due to AD (MCI) and AD brains compared to controls. Aβ42 oligomers marginally increased ROCK1 and ROCK2 protein levels in neurons but strongly induced phosphorylation of Lim kinase 1 (LIMK1), suggesting that Aβ42 activates ROCKs. RNAi depletion of ROCK1 or ROCK2 suppressed endogenous Aβ40 production in neurons, and Aβ40 levels were reduced in brains of ROCK1 heterozygous knock-out mice compared to wild-type littermate controls. ROCK1 knockdown decreased amyloid precursor protein (APP) in neurons, and treatment with bafilomycin accumulated APP levels in neurons depleted of ROCK1. These observations suggest that reduction of ROCK1 diminishes Aβ levels by enhancing APP protein degradation. Collectively, these findings support the hypothesis that both ROCK1 and ROCK2 are therapeutic targets to combat Aβ production in AD.

Shed klotho regulates neural stem cell proliferation and differentiation

A. M. LASZCZYK[1] & G. D. KING[1]

[1]Department of Neurobiology, University of Alabama at Birmingham, 1825 University Blvd Shelby 913, Birmingham, AL, 35294

While klotho overexpression was first recognized for its ability to extend both mean and overall lifespan, recent excitement around klotho function has turned to the brain where overexpression in mice enhanced cognition. The cognitive effects of klotho extend to humans carrying a polymorphism that increases klotho expression as they show enhanced cognitive function across lifespan. Inversely, klotho knockout mice display rapid onset of cognitive deficits. While it is clear that klotho expression impacts brain function we do not understand how the protein mediates these effects. Our previous work identified a potential mechanism in defining a role for klotho in adult neurogenesis. Klotho overexpressing mice produce more neurons with higher dendritic complexity, while klotho knockouts display decreased production of new neurons that are stalled in early phases of neuronal maturation. Additionally our studies suggested changes to neural stem cells. We set out to understand how klotho affects stem/progenitor cells (NSP) using primary culture to isolate the small population of hippocampal stem cells in knockout and overexpressor adult mouse brains. We confirmed proliferation changes observed *in vivo*, and through both qPCR for klotho expression and the addition of recombinant klotho to NSP media, identified the form of klotho that mediates effects. Adding shed klotho to the media was sufficient to alter NSP properties, including proliferation and maintenance of stem cell frequency, and provides evidence of a powerful, direct effect of klotho on neural stem cells. Together, our work suggests that shed klotho regulation of neural stem cells and thus adult neurogenesis is one direct mechanism through which klotho impacts cognition.

Retinotopically-specific variation in cortical thickness in V1 relates to performance on a central visual discrimination task

D. LEE[1,2], W. K. BURGE[2], A. ELKHETALI[2], & K. VISSCHER[1]

[1]Department of Neurobiology, University of Alabama at Birmingham, [2]Department of Psychology, University of Alabama at Birmingham

The anatomy of primary visual cortex (V1) has been hypothesized to relate to visual behavior. Indeed, recent studies have examined the relationship of surface size of V1 to visual performance. However, these studies have not typically addressed the fact that different portions of V1 correspond to different locations within visual space. Anatomical variations across the visual cortex as a whole could give rise to a generalized visual improvement; conversely it could be the case that anatomical variation in V1 influences behavior in a retinotopically-specific manner. In this study, we examine how cortical thickness in segments of V1 corresponding to more central and more peripheral regions of space relate to visual performance in a central vision task.

Anatomical T1 weighted scans of participants (n=22) were collected and reconstructed to create regions of interest (ROI) corresponding to segments of V1 with varying visual eccentricity. For the visual discrimination task, participants were presented with two sequential 500ms Gabor patches of different spatial frequencies and asked to determine which had the higher spatial frequency. Just noticeable difference (JND) thresholds were calculated as the difference in spatial frequency needed for participants to correctly perform the task 70% of the time. Thresholds were correlated with cortical thickness from ROIs for each participant.

We found that thicker V1 correlated to lower JND thresholds ($p < 0.05$), indicating that thicker cortex related to better visual discrimination ability. However, this relationship was specific to ROIs corresponding to central vision. Moving from centrally-representing to peripherally representing cortex, the correlation to behavior decreased. These results show that the thickness of V1 relates to visual behavior in a retinotopically specific way, and are an important step in better understanding experience-driven shifts in cortical thickness.

Stronger contribution and impaired LTP of hippocampal inputs to the medial prefrontal cortex in the *Mecp2* mouse model of Rett syndrome

M. PHILLIPS, W. LI. & L. POZZO-MILLER

Department of Neurobiology, Civitan International Research Center, University of Alabama at Birmingham

The balance between excitation and inhibition (E/I) in the CNS is crucial for proper brain function. This balance is altered in several brain regions of mouse models of Rett syndrome, a neurodevelopmental autism spectrum disorder caused by mutations in the methyl-CpG binding protein-2 (*MECP2*) gene. E/I imbalance results in altered levels of neuronal activity, causing neural networks to be either hyper or hypoactive. We quantified network activity by imaging voltage-sensitive dye (VSD) signals in slices from symptomatic male *Mecp2* knockout (KO) mice and wildtype (WT) littermates. Evoked VSD signals are larger, longer lasting, and wider spreading in slices of the ventral hippocampus (vHipp) from *Mecp2* KO mice, compared to WT slices. In contrast, VSD signals in slices from the medial prefrontal cortex (mPFC) of *Mecp2* KO mice are shorter lasting and less spreading. These data are confirmed by the number of neurons expressing the immediate early gene c-Fos: the *Mecp2* KO vHipp has more c-Fos positive neurons, while the *Mecp2* KO mPFC has fewer c-Fos neurons. Intriguingly, stimulation of identified vHipp fibers in mPFC slices evoked larger and wider spreading VSD signals in *Mecp2* KO slices. Normalized to intra-cortical stimulation in the same slices, there is a stronger contribution of vHipp inputs to the *Mecp2* KO mPFC (93% of intra-cortical responses *vs*. 71% in WT). Altogether, these data suggest that vHipp fibers drive hyperactivation of the mPFC network in *Mecp2* KO mice, in contrast to the hypoactivity evoked by stimulation of intra-cortical fibers. In addition, high-frequency stimulation of vHipp fibers triggers an enduring enhancement of the duration and spread of VSD signals in mPFC slices from WT mice, which is reminiscent of long-term potentiation (LTP) of synaptic potentials. This LTP of VSD signals at vHipp-mPFC synapses is absent in *Mecp2* KO mice, which may contribute to behavioral deficits during their social interaction with known and unknown mice. Current experiments are designed to identify the postsynaptic receptors responsible for VSD signals evoked by either stimulation of vHipp inputs or intra-cortical fibers, and whether LTP of VSD signals at vHipp-mPFC synapses in *Mecp2* KO slices is saturated by already potentiated synapses, as occurs in the hippocampus.

Targeting the tau-fyn interaction for ameliorating Aβ-induced neurotoxicity

T. RUSH[1], S. J. THOMPSON[2], J. N. COCHRAN[3], P. V. DIGGS[2], & E. D. ROBERSON[2]

[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Hudson-Alpha Inst., Huntsville, AL

Tau is widely considered a prime therapeutic target for Alzheimer's disease (AD), in part because tau reduction is protective in cell and mouse models of AD. Despite wide support, strategies to target tau have proven difficult to develop. Much like tau, the Src-family tyrosine kinase, fyn, is also a requisite mediator of Aβ-driven neuropathology. Indeed, reducing fyn levels is protective in AD mouse models and overexpressing fyn results in behavioral abnormalities not otherwise present in a mouse line expressing low levels of APP. Inhibiting fyn kinase activity has been reported to be protective, but this approach also leads to learning and memory deficits and impaired synaptic plasticity in non-transgenic mice. Importantly, tau and fyn interact via their respective proline-rich and SH3 domains. In addition, recent evidence suggests that Aβ-driven behavioral abnormalities and neuropathology involve the tau-fyn interaction. We recently completed a high-throughput screen to identify small molecule inhibitors of the tau-fyn interaction. We also developed a cell-permeable TAT-peptide that spans full-length tau's 5th and 6th proline-rich, PxxP motifs and competitively inhibits the tau-fyn interaction. We used rat primary neuron cultures to test whether this tau-fyn interaction peptide inhibitor could ameliorate Aβ-induced neurotoxicity. Exposing primary neurons to Aβ1-42 oligomers (Aβo) for 48 hours resulted in neurotoxicity that was precluded by tau knock-down (using antisense oligonucleotides) and was blocked by pre-treatment with the TAT-PxxP peptide. These data suggest that the tau-fyn interaction is an important mediator of Aβ-driven neuropathology and represents a promising therapeutic approach to translate the mechanisms of tau-reduction for the treatment of AD.

Abnormalities in copper transporters ATP7A and CTR1 in postmortem substantia nigra in schizophrenia

K. E. SCHOONOVER & R. C. ROBERTS

University of Alabama at Birmingham, Birmingham, AL

Dysbindin, encoded by risk factor gene *DTNBP1*, is downregulated in cortex and hippocampus of schizophrenia subjects and modulates copper (required for monoamine metabolism and myelination). The current study used Western blot analysis to compare protein levels of copper transporters ATP7A and CTR1, and dysbindin in postmortem substantia nigra in schizophrenia subjects (n=15) and matched controls (n=12). As a preliminary analysis, the schizophrenia group was subdivided by 1) treatment status: off- (n=4) or on-medication (n=11); or 2) treatment response: treatment resistant (n=6) or treatment responsive (n=4). The combined schizophrenia group exhibited decreased CTR1 levels (a decrease of 42.6%, p=0.0003) versus controls. When subdivided by medication status, this decrease was observed in medicated (a decrease of 39.5%, p=0.007) and unmedicated subjects (a decrease of 52.9%, p=0.001) versus controls. ATP7A levels were decreased in unmedicated subjects versus medicated subjects (a decrease of 38.3%, p=0.007) and controls (a decrease of 35.3%, p=0.017). Decreased CTR1 levels were also observed in responsive schizophrenia subjects versus controls (a decrease of 47.1%, p=0.007) when subdivided by treatment response. Protein levels of dysbindin were not significantly different for any of the analyses. Correlational analyses and comparison of correlation coefficients revealed no significant relationships between CTR1 and dysbindin in any group. However, a positive relationship between ATP7A and dysbindin was observed in unmedicated subjects (r=0.997, p=0.003) that was significantly different from medicated subjects (r=-0.190, p=0.575, CC: p=0.007) and controls (r=0.180, p=0.557, CC: p=0.003). These results indicate disrupted nigral copper homeostasis in schizophrenia subjects, potentially related to genetic variation of *DTNBP1*.

Functional modulation of activity-dependent gene expression by non-coding enhancer RNAs

R. C. SIMON, N. V. N. GALLUS, K. E. SAVELL, F. A. SULTAN, & J. J. DAY

Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL

Enhancers are DNA regulatory elements that contribute to establishment of cell identity during development by modulation of gene expression both *in cis* and *in trans*. Arising from enhancers are recently-discovered noncoding RNAs, coined enhancer RNAs (eRNAs). While evidence suggests eRNAs are involved in transcriptional pausing, their potential role in activity-dependent neuronal function and development remains unclear. Here, to investigate eRNA function *in vitro*, we selected an eRNA site upstream of *Fos*, an immediate early gene that codes for a transcription factor implicated in neuronal plasticity and cognitive processes. Consistent with previous studies, we show that neuronal activation leads to recruitment of RNA Polymerase II (PolII) to the *Fos* enhancer, resulting in bidirectional RNA PolII-dependent eRNA synthesis. In addition, neuronal silencing with tetrodotoxin decreases *Fos* eRNA expression and blocks the ability of glutamate receptor agonists to upregulate *Fos* eRNA. To determine whether eRNAs have a functional role in expression of the associated protein-coding *Fos* mRNA, we employed a selective eRNA knockdown approach using stable anti-sense oligonucleotides. Remarkably, whereas *Fos* mRNA knockdown had no effect on eRNA levels, eRNA knockdown resulted in a concomitant downregulation of protein-coding mRNA, suggesting a functional role for this non-coding transcript. Moreover, single-molecule RNA FISH revealed that *Fos* eRNAs are restricted to the nuclei of neurons. Finally, we employed mobility shift assays to show that *Fos* eRNAs bind directly to epigenetic modifiers conventionally believed to bind DNA. These findings indicate that eRNAs directly modulate gene expression and suggest that activity-driven induction of eRNAs could be an important regulatory mechanism in the central nervous system.

Increasing O-GlcNAcylation induces AMPAR internalization at CA3-CA1 synapses

L. T. STEWART[1], A. U. KHAN[2], M. L. OLSEN[2], J. C. CHATHAM[3], & L. L. MCMAHON[2]

[2]Cell, Developmental and Integrative Biol., [3]Dept. of Pathology, [1]Univ. of Alabama at Birmingham, Birmingham, AL

Regulated trafficking of AMPARs at mammalian excitatory synapses is a fundamental mechanism required for acquisition and storage of new information. Evidence suggests that AMPARs containing GluA2/3 subunits undergo constitutive recycling at hippocampal synapses, while those containing GluA1/2 subunits undergo activity dependent insertion or removal during many forms of long-term plasticity required for learning and memory. This activity dependent trafficking is modulated by serine/threonine phosphorylation of AMPAR subunits. We recently reported that another dynamic post-translational modification at serine/threonine residues involving the O-linkage of β-*N*-acetylglucosamine, termed O-GlcNAcylation, induces a novel form of LTD at CA3-CA1 synapses. In recent studies, we found that acute increases in O-GlcNAcylation also dampen hyperexcitability in vitro and in vivo. We previously showed that GluA2, but not GluA1 subunits are O-GlcNAc modified, that O-GlcNAc transferase co-immunoprecipitates with GluA2 subunits, and O-GlcNAc LTD is absent in GluA2 KO mice. Because serine phosphorylation of GluA2 subunits drives AMPAR endocytosis during expression of NMDAR-dependent LTD, we investigated whether expression of O-GlcNAC LTD is due to AMPAR endocytosis and used the endocytosis blocking peptide, TatGluR23Y. In preliminary experiments we find that O-GlcNAc LTD at CA3-CA1 synapses is inhibited in the presence of the peptide but is normal in the presence of the scrambled peptide, suggesting O-GlcNAcylation of GluA2 subunits drives endocytosis similar to phosphorylation. Additional experiments will test the requirement of AMPAR GluA2 subunit in O-GlcNAc mediated dampening of neuronal hyperexcitability. Based on our current and previously published work, the hexosamine biosynthetic pathway is an important mechanism in the modification of synaptic efficacy at hippocampal synapses, via targeted O-GlcNAcylation of synaptic protein substrates.

Effects of tau reduction in excitatory and inhibitory neurons on seizure susceptibility

Y. VOSKOBIYNYK[1], J. N. COCHRAN[1], & E. D. ROBERSON[1]

Center for Neurodegeneration and Experimental Therapeutics[1], Alzheimer's Disease Center[1], Evelyn F. McKnight Brain Institute[1], Departments of Neurology and Neurobiology[1], University of Alabama at Birmingham, Birmingham, AL, USA

Considerable evidence indicates that tau is required for the toxic effects of amyloid beta (Aβ). Tau reduction prevents Aβ-induced deficits in multiple AD mouse models and other degenerative diseases. This seems to involve a strong effect of tau reduction decreasing hyperexcitability; global tau reduction ameliorates Aβ-induced epileptiform activity and seizure susceptibility in multiple mouse models of AD. However, it is still unclear how tau reduction manifests its positive effects, and specifically the contributions of excitatory and inhibitory neurons. We hypothesize that tau reduction decreases neuronal excitability in both excitatory and inhibitory neurons, which results in opposite effects on the network level since these neurons have opposite electrical drives. To test this hypothesis, we generated excitatory and inhibitory neuron–specific tau knockout mice by crossing mice with a floxed tau allele with mice expressing Cre recombinase under the CaMKII and Viaat promoters, respectively. Tau was reduced by about 70% in the excitatory knockout line and by about 30% in the inhibitory knockout line. We used a model of pharmacologically induced seizures with pentylenetetrazole (PTZ), a GABAergic antagonist, to assess seizure susceptibility as a direct measure of network excitability. We also characterized behavioral differences in both excitatory and inhibitory tau knockout models. Excitatory tau knockout mice had lower clinical seizure scores, decreased latencies to reach a given seizure stage, and reduced number of deaths due to seizures after PTZ injections. Meanwhile, inhibitory tau knockout mice had the opposite effects. Our findings contribute to understanding the role of tau on cellular and network levels, as well as its contribution to AD pathogenesis, and may facilitate the development of new therapeutic approaches targeting tau-related processes.

EEA1 overexpression reduces synaptic strength and restores homeostatic synaptic plasticity in cultured hippocampal neurons from *Mecp2* knockout mice

X. XU & L. POZZO-MILLER

Department of Neurobiology, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham

Negative-feedback homeostatic synaptic plasticity (HSP), also known as synaptic scaling, maintains the global synaptic strength of individual neurons in response to sustained alterations in neuronal activity. This cell-wide homeostatic balance is critical to allow the potentiation or depression at small subsets of synapses during positive-feedback synaptic plasticity (i.e. Hebbian plasticity). Rett syndrome (RTT) is a progressive autism spectrum disorder caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). Previously, we discovered an imbalance of synaptic excitation and inhibition (E/I) in the hippocampus of symptomatic *Mecp2* knockout (KO) mice (Calfa *et al.* 2015). Since E/I balance is thought to be maintained by homeostatic mechanisms, we examined the role of MeCP2 in HSP at the excitatory synapses. We found that hippocampal neurons obtained from P1 *Mecp2* KO mice (9-11 days *in vitro*) do not show the characteristic homeostatic scaling-up of mEPSC amplitude and of synaptic levels of GluA1 after 48hs silencing with the Na^+ channel blocker TTX. This deficit in HSP is bidirectional because *Mecp2* KO neurons also failed to scale-down mEPSC amplitude and synaptic levels of GluA1 after 48hs of desinhibition with the GABA-A receptor antagonist bicuculline. The best-characterized mechanism of HSP is the regulated trafficking into and out of synapses of the AMPA-type of glutamate receptors (AMPARs). We focused on early-endosome-antigen-1 (EEA1) because it participates in synaptic removal of GluA1 (Selak *et al.* 2006), and was found to be activated by MeCP2 in a microarray study (Chahrour *et al*. 2008); consistently, mRNA and protein EEA1 levels are lower in the *Mecp2* KO hippocampus (Li *et al.* 2016). Here, we tested whether EEA1 overexpression restores mEPCS amplitudes and HSP in hippocampal neurons from *Mecp2* KO mice. EEA1 overexpression in *Mecp2* KO neurons reduced mEPSC amplitudes to levels comparable to those in WT neurons. In addition, *Mecp2* KO neurons overexpressing EEA1 scaled-down mEPSC amplitudes after desinhibition with bicuculline, suggesting that HSP is restored. Our characterization of the role of EEA1 during HSP in *Mecp2* KO neurons provides novel targets for improving hippocampal function in RTT individuals.

Director: Carol A. Barnes, Ph.D.
Associate Director: Lee Ryan, Ph.D.

Age-related attentional control and set shifting impairments arise independently in macaque monkeys

K. M. ANDERSH[1,2], D. T. GRAY[1,2], A. C. SMITH[1], S. N. BURKE[4], A. GAZZALEY[5], & C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, Univ. of Arizona, Tucson, AZ; [4]Evelyn F. McKnight Brain Institute, Department of Neuroscience, University of Florida, Gainesville, FL; [5]Departments of Neurology and Physiology, University of California, San Francisco, San Francisco, CA

Goal-directed behaviors provide the behavioral flexibility necessary for selecting appropriate responses when similar stimuli are encountered in different contexts. The cognitive processes that provide this flexibility have been collectively described under the category of executive functions. Executive function can be segregated into at least 3 separate components: inhibition of prepotent responses, set shifting, and attentional control and monitoring. In humans, partially non-overlapping neural networks in the prefrontal cortex underlie the different components of the executive function network. For example, orbitofrontal and striatal networks have been shown to underlie set shifting, while dorsolateral prefrontal and medial prefrontal networks have been shown to underlie attentional control processes. At the behavioral level, age-related impairments in inhibition, set shifting, and attentional control arise independently of one another, suggesting that the separate neural networks that underlie these behaviors are also altered independently with age. To test whether different executive functions are similarly affected independently in the macaque, young (n = 6) and aged (n = 7) monkeys were tested on a set shifting and attentional control task in a Wisconsin general testing apparatus. The results show that aged monkeys were deficient on both tasks, but the impairment scores between the two paradigms did not correlate, suggesting that set shifting-impaired animals were not necessarily impaired on the attentional control task and *vice versa*. These results suggest that, like in humans, different components of executive function in aged monkeys are impacted by normative aging independently. Furthermore, these data argue against the suggestion that the age-related deficits in attentional control seen in aged humans arise due to differences in exposure to technology, relative to young, which may negatively impact their ability to perform computerized tasks. All monkeys in the current study were exposed equally to all aspects of the task environment, suggesting that the detrimental effects seen in the aged individuals are in fact due to differences in attentional control processes.

Transcriptional and epigenomic changes across the perimenopause transition

E. BACON[1], M. K. DESAI[1], A. MISHRA[1], Y. WANG[1], F. YIN[1], & R. D. BRINTON[2]

[1]University of Southern California, Los Angeles, CA; [2]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Transition states represent critical periods during development when systems under go dramatic and widespread changes. In women, the perimenopause transition spans several years and the resulting loss of estrogen has profound effects in nearly all tissues, including breast, bone, cardiovascular, and brain. Menopause in humans is also marked by an increased risk for stroke, coronary heart disease, and neurological disorders. Although a majority of women have no serious long-term health consequences, many women suffer neurological symptoms during and after the perimenopause transition. Heritability of menopause timing is 44-66% and variability is present in monozygotic twins and inbred rat strains, suggesting that epigenetics and environmental factors could play a large role in orchestrating timing of events involved in reproductive aging. Individual differences in epigenetic regulation, in addition to individual differences of sex hormone levels may help explain some of the differences seen in menopausal age, risk for cognitive impairment, and response to hormone therapy. To better understand the potential underlying mechanisms of neurological symptoms associated with perimenopause, as well as the control of age of onset, the current study aims to characterize the transcriptional and epigenomic changes that occur during this transition using a rat model recapitulating fundamental characteristics of the human perimenopause. Duration of the perimenopause transition in Sprague Dawley rats (time spent cycling irregularly before loss of cyclicity) can be separated into three groups: short, average, long. All animals begin to cycle irregularly around the same time (9-10mo), however animals complete the perimenopause transition at different ages. Older ages are correlated with longer overall durations of transition. Transcriptional changes of genes related to epigenetic regulation were observed across all perimenopause groups (RC, IR, AC). Our analysis suggests that hypothalamic aging and changes in epigenetic regulation begin before the onset of irregular cycling, between 6 and 9 months. Changes in DNA methylation across the perimenopause transition were investigated via global 5mC ELISA and genome-wide bisulfite sequencing (RRBS). Furthermore, IPA upstream analysis of transcriptional data identifies key players of one-carbon metabolism (involved in epigenome maintenance) as likely regulators of endocrine aging. Impaired one-carbon metabolism during perimenopause may contribute to increased risk for neurodegenerative diseases after menopause.

Differential regional alterations of white matter integrity in healthy cognitive aging

P. K. BHARADWAJ[1,2,7], L. A. NGUYEN[1,2,7], K. A. HAWS[1,2,7], G. A. HISHAW[3], T. P. TROUARD[4], & G. E. ALEXANDER[1,2,5,6,7]

[1]Psychology, [2]Evelyn F. McKnight Brain Institute, [3]Department of Neurology, [4]Biomedical Engineering, [5]Physiological Sciences Graduate Interdisciplinary Program, [6]Neuroscience Graduate Interdisciplinary Program, University of Arizona, Tucson, AZ; [7]Arizona Alzheimer's Consortium, Phoenix, AZ

White matter (WM) microstructural integrity assessed with diffusion weighted imaging is diminished in the context of healthy aging. Such age effects have often shown an anterior-posterior gradient and may be influenced by vascular risk factors such as hypertension. The aim of this study was to investigate regional differences in WM integrity measured by fractional anisotropy (FA) and mean diffusivity (MD) in a healthy sample of community dwelling older adults, ranging in age from 50 to 89 years. The study sample comprised 74 healthy older adults divided into four age groups (50-59, 60-69, 70-79 and 80-89 years). Only neurologically healthy participants without a clinical diagnosis of diabetes or hypertension were included. T1-weighted and diffusion weighted images were acquired at 3T and processed using FreeSurfer (Fischl et al., 2004a) and TRACULA (Yendiki et al., 2011), to create individual ROIs, perform probabilistic tractography, and compute regional values of FA and MD for 18 major WM tracts. The diffusion metrics were tested using ANCOVA with age as the between group factor and total cortical WM volume as a covariate. The results showed that FA decreased with age bilaterally in the anterior thalamic radiation (ATR, $0.011 \leq p \leq 0.021$), and the left temporal and parietal branches of the superior longitudinal fasciculus (SLFT, $p = 0.005$; SLFP, $p = 0.05$). MD demonstrated age-related increases bilaterally in the ATR ($0.003 \leq p \leq 0.014$), cingulum angular bundles ($0.002 \leq p \leq 0.042$), inferior longitudinal fasciculus ($0.001 \leq p \leq 0.006$), uncinate fasciculus ($0.0003 \leq p \leq 0.001$), and the right SLFT ($p = 0.043$). Pairwise comparisons of FA and MD in these tracts showed varying patterns of age group differences that differed between the two diffusion metrics. These results demonstrate that measures of WM microstructure assessed by FA and MD differ by age group, for select tracts, as well as by which specific age groups are most affected. Together these findings suggest that measures of diffusivity (MD) and microstructural organization (FA) may show differences in their ability to detect age-related WM integrity changes among healthy older adults.

Mechanistic role of brain hypometabolism and mitochondrial uncoupling in perimenopausal hot flash

R. D. BRINTON[1,2], F. YIN[1], J. YAO[1], A. MISHRA[1], Q. DENG[1], Z. MAO[1], & E. CADENAS[1]

[1]School of Pharmacology, University of Southern California, Los Angeles, CA; [2]Center for Innovation in Brain Science and Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

The goal of the current study is to determine the mechanism of the signature symptom of the menopausal transition, the perimenopausal hot flash. We hypothesized that loss of ovarian hormone regulation of bioenergetics in brain induces a series of adaptive responses in the brain, which are initiated by decline in glucose metabolism, followed by activation of alternative fuel sources, ketone bodies and free fatty acids, which lead to mitochondrial uncoupling and temperature dysregulation. We used the ovariectomy (OVX) rat model as a reliable and predictive inducer of temperature dysregulation. Loss of ovarian hormones in the OVX rats led to decreased uterine weight, increased body weight and a significant increase in peripheral (tail skin) temperature. Further, our analyses in the OVX rat model indicated that peripheral temperature dysregulation coincided with systemic glucose intolerance and decreased cerebral glucose metabolism (FDG-PET). We further tested our hypothesis that loss of ovarian hormones leads to disruption and uncoupling of the proton motive force-dependent energy conservation systems and the consequent dissipation of energy as heat. Results of these analyses indicated that mitochondrial respiratory control ratio (RCR) was decreased in OVX rats accompanied by increased mitochondrial uncoupling, the upregulation of mitochondrial uncoupling proteins (UCPs), and enhancement of mitochondrial fragmentation in multiple brain regions. OVX-induced changes were completely or partially prevented by 17beta-estradiol treatment, suggesting an obligatory role of estrogen signaling in these events. Finally, to investigate the relationship between mitochondrial uncoupling in the brain and the increase in peripheral temperature, mitochondrial uncoupling was induced by 2,4-dinitrophenol (2-DNP), a mitochondrial uncoupler. Our analyses indicate that intracerebroventricular injection of 2-DNP induced sequential fluctuations in brain temperature, core temperature and tail skin temperature. Collectively, we established the physiological and bioenergetic phenotype of a rat model of hot flash, and our findings provide new mechanistic details of hot flash by connecting loss of ovarian hormones, brain hypometabolism, mitochondrial uncoupling and dysfunction, and peripheral temperature dysregulation.

Activation of neuronal populations in young and aged rat Lateral Entorhinal Cortex during track-running behavior with odors

A. COMRIE[1,2], J. P. LISTER[1,4], M. K. CHAWLA[1,2], & C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ; [4]Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA

The hippocampus is known to show biological changes with age that are related to changes in memory processes. For example, in aged rats, the CA1 region of the hippocampus fails to show accurate map retrieval upon revisiting a familiar environment (Barnes et al., 1997). The distal part of CA1 receives major inputs from Lateral Entorhinal Cortex (LEC) layer III. In contrast to the well-studied Medial Entorhinal Cortex (MEC), LEC neurons do not show substantial spatial selectivity in their firing patterns (Deshmukh and Kneirim, 2011). Rather, LEC is thought to be involved in non-spatial memory, such as encoding object and odor information. The role of LEC in odor discrimination and how the corresponding neural activity may change with age remain unknown. In this study we aim to discover if LEC neuronal populations are active in response to distinct odors during track running, and whether age-related changes in activation patterns may provide faulty input to the hippocampus that may explain remapping in older animals. To test this, 24 young (9 months) and 24 aged (24 months) male rats were trained to run in alternating clockwise and counterclockwise laps on a circular track in a constant spatial environment. One behavioral group (A/A) experienced the same set of 6 odors mixed with sand in ramekins in the same order around the track during two run sessions separated by 20 minutes. A second group (A/B) also experienced two run sessions, but the odor stimuli were all distinct between the two time points. A positive control group underwent Maximal Electroconvulsive Shock (MECS), and a negative control group was sacrificed from their home cages (CC). The mRNA of immediate-early gene *Arc* is localized to distinct cellular compartments based on the time since neuron activation. We use cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH; Guzowski et al., 1999) and confocal microscopy to visualize this time-dependent subcellular distribution of *Arc* mRNA. This method enables us to identify neurons activated during the first, second, or both running sessions in LEC. Preliminary data from LEC averaged across both treatments and age groups confirm that the track-running behavior with odors elicits 26% neural activation in comparison to low resting *Arc* expression (2%) for CC animals. With additional animals added to treatment conditions and age groups we will be able to determine if LEC encodes odor information that can discriminate between the A/A and A/B conditions, and if population representation of odor stimuli in LEC changes across the lifespan.

An analysis of nrf2 expression and its effect on aging hippocampal neural stem cell function

M. J. CORENBLUM[1], S. RAY[2], D. D. ZHANG[3], C. A. BARNES[1,4,5], & L. MADHAVAN[1,5]

[1]Department of Neurology, [2]Neuroscience and Cognitive Science Undergraduate Program, [3]Department of Pharmacology and Toxicology, [4]Departments of Psychology and Neuroscience, [5]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Our recent work has examined the dynamics of neural stem progenitor cell (NSPC) function in the subventricular zone (SVZ) of aging animals (Corenblum et al., 2016). These studies have identified a critical time-period during middle-age (13-15 mos), when a marked reduction in NSPC survival and regenerative capacity occurs, and determined the reduced expression of the redox-sensitive transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), as an important mechanism underlying this phenomenon. Given this, in the current study we analyzed the function, and Nrf2 expression, of NSPCs residing within the other major mammalian germinal niche, the dentate gyrus (DG) of the hippocampus. More specifically, using cells from seven groups of aging male Fisher 344 rats (0, 2, 9, 11, 13, 15 and 24 mos) we found that, similar to SVZ NSPCs, the proliferative capacity of cultured DG NSPCs also declined substantially during the 13-15 month critical period. However, the survival of the DG NSPCs was only compromised significantly ($p<0.05$) from the 0 to 2 mos old stage, after which it remained relatively stable throughout adulthood until old age (24 mos). Correlatively, the number of Nrf2 expressing DG NSPCs was also prominently reduced from 0 to 2 mos of age, with no further changes in Nrf2 labeled cell numbers noted across the age groups. Immunohistological assessment of hippocampal tissues from the various groups of aging animals confirmed the results from the *in vitro*analysis. Furthermore, at a behavioral level, these data correlated with a decline in spatial memory when the animals were tested via a Morris water maze task. Here it was observed that increasingly more time was taken by the animals to learn the location of the hidden platform (higher CIPL scores) with advancing age, and that the 15 mos old rats were the first adult age-group exhibiting a significant decrement in spatial memory function. Based on these results, we are currently further examining Nrf2's role in DG NSPC function using Nrf2 knock-out (Nrf2 -/-) mice, as well as tissues from young and old non-human primates. Overall, this work will provide important information on whether and how Nrf2 regulates DG NSPC activity with age.

Transcriptional differences among hippocampal subregions

M. DE BOTH[1], L. IANOV[2], M. K., CHAWLA[1], A. RANI[2], A. J. KENNEDY[3], I. PIRAS[1], J. J. DAY[3], A. L. SINARD[1], A. KUMAR[2], J. D. SWEATT[3], T. C. FOSTER[2], C. A. BARNES[1], & M. J. HUENTELMAN[1]

[1]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ; [2]Evelyn F. and William L. McKnight Brain Institute, University of Florida, Gainesville, FL; [3]Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, Birmingham, AL

Subregions of the hippocampal formation have been suggested to possess varying vulnerabilities to aging and disease-related pathologies, but the underlying molecular mechanisms of these differences are not well understood. RNA-sequencing allows an unbiased comparison of transcriptional differences between hippocampal subregions and can therefore potentially identify the underpinnings of the differential regional susceptibility. Here, we apply RNA-seq to adult rat hippocampal subregions CA1, CA3, and DG to investigate the transcriptional differences among them. Male Fischer 344 rats (n=34) were aged to 5-6 months ("young") or 17-22 months ("old") and tested with the Morris Water Maze to identify "good" and "bad" performing individuals. Two weeks after cognitive testing, hippocampal subregions CA1, CA3, and DG were isolated. RNA-sequencing was conducted in replicate by two different laboratories on two different platforms, the Illumina HiSeq 2500 at the University of Arizona and the Ion Proton at University of Florida. Pair-wise differential expression was conducted among the three regions, between young and old rats, and between rats with good and bad cognitive performance. While the study design allows the investigation of transcriptional changes associated with aging or cognitive decline, here we present subregion-specific expression regardless of age or performance. To identify subregion-specific levels of expression, a gene must be significantly different from both of the other regions using both sequencing platforms. Region-specific level of expression can be up-regulated, down-regulated, or fall between expression in the other two regions. Overall, we identified 1068 genes exhibiting a region-specific level of expression in CA1, 1200 genes in CA3, and 2532 genes in DG. These genes likely play a key role in the development and aging of the distinct hippocampal subregions.

Age-related reduction in signal-to-noise ratio of sharp-wave ripple oscillations following behavior in aged rats

D. T. GRAY[1,2], J.-P. WIEGAND[1,2], L. A. SCHIMANSKA[1,2], S. L. COWEN[1,2,3], & C. A. BARNES[1,2,3,4]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Department of Psychology, [4]Departments of Neurology and Neuroscience, University of Arizona, Tucson, AZ

The consolidation of episodic memories relies on the transfer of information from hippocampal networks to cortical networks, and much of this information is thought to be transferred during hippocampal sharp-wave ripple events in periods of rest. During normative aging, we have recently shown that the rate of ripple occurrence decreases, and the mean frequency of ripple events is reduced by roughly 19 Hz prior to and following behavior on a spatial eye-blink conditioning task (Wiegand et al., 2016). To extend these recent findings, here we present an age-comparison of spectral power in the local field potential before and after performance on a spatial eye-blink conditioning task. Specifically, the time periods analyzed were: sharp-wave ripple events, the 50 ms immediately preceding and following ripple events, and quiet inter-ripple periods. In young rats, the spectral power in the 80-200 Hz frequency band during ripple events was greater in the post-behavior rest period compared to the pre-behavior rest period, although in aged rats the ripple power was not different between the rest epochs. During the inter-ripple periods spectral power was significantly lower in young rats during post-behavior rest relative to pre-behavior rest, and again the power in aged rats did not differ between rest epochs. No changes were noted between rest epochs for the 50 ms immediately preceding and following the ripple events for either age group. The observations that ripple power increases and inter-ripple power decreases following behavior only in young rats may suggest a mechanism for increased signal-to-noise in these young animals. The signal-to-noise ratios were examined by computing the ratio of the summed squared magnitudes of the 80-240 Hz spectral power of ripple events relative to inter-ripple periods. This analysis showed an increase in the signal-to-noise ratio of ripple events during post-behavior rest relative to pre-behavior rest in young animals, while aged rats did not show a change in signal-to-noise ratio following behavior. Increases in the signal-to-noise ratio of ripple oscillations in rest periods following behavior may increase the efficacy by which ripple oscillations transfer information during memory consolidation. The absence of this signal to noise ratio increase in older animals suggests less efficiency in consolidation processes carried out in hippocampal networks.

Norepinephrine as a memory reset signal: Phasic activation of the locus coeruleus drives global remapping in hippocampus

S. L. GRELLA[1], J. NEIL[2], V. D. STRONG[2], I. V. ODINTSOVA[1], G. MARTIN[2], C. W. HARLEY[2], & D. F. MARRONE[1,3]

[1]Department of Psychology, Wilfrid Laurier University, Waterloo, ON, Canada; [2]Psychology, Memorial University of Newfoundland, St. John's, NL, Canada; [3]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

The locus coeruleus (LC) responds to novelty in the environment and sends a major noradrenergic projection to the hippocampus (HPC). It is hypothesized that novelty-associated activation of the LC may help to sculpt representations in the HPC, but influence of norepinephrine (NE) over HPC representations remains poorly understood. One possible mechanism is that NE may provide a "reset" signal causing the HPC to recruit distinct populations of neurons (neuronal ensembles). Thus, NE may provide a molecular switch to dictate if hippocampal circuits should generate new representations or update existing representations to incorporate novel information. This hypothesis suggests that agonism of the NE system should cause the hippocampus to recruit a unique population even in the presence of the same stimuli an animal has just experienced. The compartmental expression of *Arc* and*zif268* allows us to test this hypothesis by mapping the activity history of individual HPC neurons as animals engage in spatial processing following manipulation of the NE system. Rats were placed in either the same context twice (A/A) or two different contexts (A/B). Prior to placement in the second context, separate groups of rats were infused bilaterally in the LC with glutamate (phasic LC activation) or clonidine (blockade of LC discharge). Additional groups were infused bilaterally with orexin A, bethanachol, and CRF (tonic LC activation). Remapping was assessed in the dentate gyrus, the CA3 and the CA1. Preliminary data show that phasic, but not tonic, LC activation can drive global remapping in the HPC, consistent with the notion of NE as a novelty "reset signal" for hippocampal mnemonic circuits from retrieval to encoding.

Menopause and the aging brain: Relationships among ovarian hormone levels, memory, and choline acetyltransferase-containing neurons in the basal forebrain

S. V. KOEBELE[1,2], S. E. MENNENGA[1,2], S. PATEL[1,2], R. HIROI[1,2], L. T. HEWITT[1,2], A. M. QUIHUIS[1,2], L. P. MAYER[3], C. A. DYER[3], L. M. DEMERS[4], & H. A. BIMONTE-NELSON[1,2]

[1]Psychology, Arizona State University, Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ; [3]SenesTech Inc., Flagstaff, AZ; [4]The M. S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA

Memory changes during the menopause transition can negatively impact quality of life in women. These alterations in memory function may be related to the characteristic erratic fluctuations followed by the decline in ovarian hormone levels, including estrogens, observed during the perimenopausal period (Sherwin, 2012). Using the 4-vinylcyclohexene diepoxide (VCD) rat transitional menopause model, we investigated menopause- and age- related changes in choline acetyltransferase (ChAT) in the basal forebrain (BF), a primary synthesis site for acetylcholine. The BF is important for cognitive function, as these cholinergic neurons send long-range projections to the hippocampus, a key structure in spatial cognition. It is well established that 17β-estradiol (E2) treatment increases BF ChAT levels (Luine, 1985; Gibbs, 1997, 2000) and BF lesions impair spatial memory and prevent E2-induced memory enhancements (Hagan et al., 1988; Gibbs, 1998, 2002). Thus, fluctuating ovarian hormone levels during the transition to menopause may impact acetylcholine synthesis and the BF-hippocampal cholinergic pathway. Young (6 mo) and Middle-Aged (12 mo) Fischer-344 rats were trained on a water radial-arm maze (WRAM). Following training, rats were administered Vehicle or VCD treatment, which accelerates depletion of ovarian follicle reserve. Rats were then repeatedly tested on the WRAM for four months, across the menopausal transition to a follicle-deplete state. A subset of rats was sacrificed early in the menopausal transition to evaluate physiological changes that occur early in perimenopause. The remaining rats were sacrificed after six months, when VCD-treated rats were post-follicular depletion. The BF was stained for ChAT-immunoreactive (IR) cells, and unbiased stereology was used to estimate ChAT-IR populations in the medial septum and vertical/diagonal bands. Preliminary results suggest that the ovarian hormone fluctuations associated with follicle depletion are related to ChAT-IR BF estimates, particularly during the early menopause transition time point. Further, the relationship between ovarian hormones and ChAT-IR BF estimates changes with both aging and follicular depletion. Dynamic relationships between hormone levels and ChAT-IR estimates with memory performance will be discussed. Understanding the neurobiological changes that occur early in the menopause transition period may help elucidate a critical window for hormone intervention in at-risk women so they can maintain a high quality of life, and the possibility to postpone or prevent the development of cognitive impairment or dementia later in life.

Histology informed probabilistic hippocampal atlases of young and old rhesus macaques

C. KYLE[1,2], J. L. BENNETT[4], J. D. STOKES[5], M. R. PERMENTER[6], J. A. VOGT[6], A. D. EKSTROM[5,7], & C. A BARNES[1,2,3,6]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, Univ. of Arizona, Tucson, AZ; [4]Department of Psychiatry and Behavioral Science and M.I.N.D. Institute, [5]Department of Psychology, [6]California National Primate Research Center, [7]Center for Neuroscience, University of California, Davis, Davis, CA

Identifying primate hippocampal subfields *in vivo* using structural MRI imaging relies on variable anatomical guidelines, signal intensity differences, and heuristics to differentiate between regions, and lack a clear anatomically-driven basis for subfield demarcation (Yushkevich et al., 2015). Recent work, however, has begun to develop methods to use *ex vivo* histology or MRI to better inform subfield demarcations of *in vivo* images (Iglesias et al., 2015, Adler et al., 2014). For optimal results, though, *ex vivo* and *in vivo* images should be matched to the same subjects, with the goal to develop a neuroanatomically-driven basis for *in vivo* structural MRI images. Here, we address this issue in young and aging rhesus macaques (young n=2 and old n=2) using *ex vivo* Nissl-stained sections in which we identified the dentate gyrus, CA3, CA2, CA1, subiculum, presubiculum, and parasubiculum using morphological cell properties (30 μm thick sections spaced at 240μm intervals and imaged at 161 nm/pixel). These were merged with *in vivo* structural MRIs (0.625 x 0.625 x 1 mm) from the same subjects via iterative rigid and diffeomorphic registration resulting in probabilistic atlases of young and old rhesus macaques. These methods will inform subfield differentiation by identifying features of the MRI images that correspond to histological properties in the same animals, useful for work in both young and aging primates. Furthermore, we believe that this approach may be helpful in developing a phylogenetically-driven "ground truth" for more accurate identification of hippocampal subregions in human brains.

Familiar context effects on pattern separation in aging

A. LAWRENCE & L. RYAN

[1]Department of Psychology, [2]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Research suggests that recognition memory performance declines with age (Yassa et al., 2011). However, recent work in our laboratory indicates that although older adults perform more poorly at object recognition, their recognition is boosted to the same degree as younger adults when the object is presented in the same context at study and test. Older adults may be using relatively spared scene recognition processing to boost recognition of objects presented in a scene. However, other studies suggest that older adults are simply biased by familiar background scene information to falsely recognize a similar object. We tested these two alternative hypotheses using a continuous recognition pattern separation paradigm. Young adults (n=32, mean age=19) and older adults (n=30, mean age= 71) were given three continuous recognition tasks consisting of objects, scenes, and objects in scenes. Participants indicated whether each image in the series was either the same as, similar to, or different from an image they had seen previously. Both younger and older adults performed more poorly recognizing similar scenes and objects in a scene than recognizing similar object on a white background ($t = -2.584$, $p < .01$). Interestingly, both younger and older adults demonstrated significantly greater bias to falsely recognize objects presented in scenes than objects presented on a white background ($t=2.785$, $p<.01$). This suggests that contextual information may be biasing individuals to falsely recognize similar lure objects. These findings also suggest that the degree to which an individual is biased to falsely recognitize a similar object is not related to age, as has been previously suggested (Gutchess et al., 2007), but rather to the presence of a familiar background context.

Aged rats failed to integrate conflicting spatial reference frames

A. W. LESTER[1,2], A. J. KAPELLUSCH[1,2], R. T. SCREEN[1,2], & C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ

As with older adults, aged rats show robust impairments on a number of different spatial navigation tasks. There is some evidence that these navigation impairments are accompanied by a bias away from using an allothetic-based (i.e., external cue) navigation strategy towards relying on an idiothetic-based (self-motion) strategy (Rosenzweig et al., 2003). To test the degree and timing with which aged animals utilize these two forms of spatial information, a novel behavioral arena has been developed that allows for complete and immediate control of all visual cues in the environment in order to put idiothetic and allothetic reference frames in direct conflict. The arena is composed of a circular track with a 360 degree panorama of visual cues projected on the walls. Identical feeders are spaced every 10 degrees along the perimeter of the track and animals learn to run to only one of them for food reward. By instantaneously rotating the cues we were able to characterize how quickly and accurately aged animals utilize allothetic feedback to navigate to a new rotated feeder location. Behavioral data collected from six young (9 – 15 mo) and six aged (24 - 30 mo) animals revealed that immediately following cue rotation aged rats were significantly more likely to navigate to either the exact original (idiothetically aligned) or rotated (allothetically aligned) feeder locations. Young rats, by comparison, were more significantly more likely to stop at multiple feeders, particularly those half-way between the original and rotated reward location. These findings suggest that when spatial reference frames are put into conflict young rats settle on a strategy which combines the two sources of spatial information, while aged animals adhere more rigidly to only one spatial reference frame. Previous studies have shown that when spatial reference frames are put into conflict the place cells of the same CA1 network can anchor to entirely different reference frames, while CA3 place cells will align coherently with only one reference frame (Lee et al., 2004). The behavior we observe may be a consequence of the reported hyperexcitability and excessive pattern completion of aged CA3 principle cells driving an all-or-none shift to one or the other reference frame. If this is the case we expect that electrophysiological data from downstream CA1 place cells will match our behavioral findings and that CA1 place fields of aged animals will tend to snap to one or the other reference frames coherently as a population while those of young animals well show more variability in terms of which reference frame they anchor to.

An fMRI analysis of visual unitization and the age-related associative memory deficit

M. B. MEMEL[1] & L. RYAN[1,2]

[1]Department of Psychology, [2]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Healthy aging impairs associative memory -- the ability to remember associations between previously unrelated pieces of information (Naveh-Benjamin, 2000). Unitization, the process of creating one semantic representation that includes both items in an associative pair, attenuates age-related associative deficits (Zheng, et al., 2015; Bastin et al., 2013; Ahmad et al., 2014). Examples of unitized pairs include compound words (Giovanello et al., 2006; Quamme, et al., 2007) and words imagined in a specific color (Diana et al., 2011). Many studies have argued that unitizeable associative pairs rely less on hippocampal function than traditional associative pairs. For example, hypoxic patients, with damage relatively limited to the hippocampus, benefit from word pairs that can be unitized (Quamme et al., 2007). Thus, it is believed that with verbal stimuli, unitization of associative pairs allows for memory based on non-hippocampal medial temporal structures. Less is known about the benefit of unitization with visual stimuli, such as objects and scenes. Objects and scenes are two types of visual stimuli frequently encountered in the world and perceived in relation to one another. In the present study, we manipulated the degree of visual integration between objects and scenes, by presenting objects either next to their paired scene (Separated condition) or embedded within their paired scene (Combined condition) and measured associative memory performance in young and older adults while undergoing fMRI.

Based on previous studies of unitization, we hypothesized that older adults would receive a mnemonic benefit from the integration of visual associative pairs. Further, we predicted greater hippocampal activation during the encoding of Separated associative pairs than Combined pairs, due to a greater need for binding between items. Consistent with our hypotheses, visual integration of objects and scenes improved associative memory performance in older adults. In addition, hippocampal activation was greater during the Separated than Combined condition across age groups. However, the degree of hippocampal activation during the Separated condition was related to performance in young, but not older adults. In older adults, the perirhinal cortex was the only medial temporal region that significantly predicted performance. These findings may suggest that hippocampal activation is less successful in older adults than young. Alternatively, object processing, which relies on the perirhinal cortex and is impaired in some older adults (Ryan et al., 2012) may be a greater predictor of successful associative memory with object-scene pairs.

Cell counts of midbrain dopamine neurons in memory-impaired aged non-human primates

W. PYON[1,2], D. T. GRAY[1,2], K. M. ANDERSH[1,2], M. R. PERMENTER[4], J. A. VOGT[4], & C. A. BARNES[1,2,3,4]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ; [4]California National Primate Research Center, University of California, Davis, Davis, CA

The midbrain dopamine complex is a collection of nuclei found in the ventral mesencephalon. These nuclei contain dopaminergic projection neurons that innervate multiple forebrain regions, including the prefrontal cortex, ventral hippocampal formation, and striatum. Among other functions, dopamine neurons are thought to provide reward prediction error signals critical to reinforcement learning, and modulate frontal cortical networks underlying working memory. Dysfunction in dopaminergic networks has been implicated in several brain pathologies. During normative aging, deficits in mental operations that require dopamine are common. While our understanding of alterations in the midbrain dopamine complex across the primate lifespan is limited, it is evident that degradation of dopaminergic innervation in the rodent prefrontal cortex plays a key role in age-related cognitive decline (Allard et al., 2011). Furthermore, it has previously been shown, by our laboratory and others, that aged macaques require more trials than younger monkeys to learn delayed nonmatching-to-sample (DNMS) and reversal learning (RL) tasks, which assess object recognition and working memory functions, respectively. The aged monkeys included in this experiment have been previously shown to be impaired on both the DNMS and RL tasks (Comrie et al., 2015). To test whether alterations in the mesencephalic dopamine complex relate to learning impairments in the macaque, we have identified coronal sections of tissue containing regions of the midbrain dopamine complex in monkeys ranging in age from 8-32 years. The numbers of tyrosine hydroxylase-positive neurons in A8, A9, A10 nuclei will be quantified using unbiased stereological sampling techniques. Dopaminergic neurons in this region of the midbrain have been shown to project to the prefrontal cortex and hippocampus, both regions understood to play key roles in learning and memory processes. Furthermore, we will quantify the numbers of GAD67-positive neurons and calbindin-positive neurons in the same sections as we assess TH. Immunohistochemically-classified cell numbers will then be correlated with DNMS and RL learning scores to identify if and to what extent changes in dopaminergic circuits underlie age-related impairments on working memory and object recognition tasks.

Expectation of large reward elicits bursts of beta-band oscillations in the aged rat amygdala

R. D. SAMSON[1,2], L. DUARTE[1,2], & C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Institute, [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ

With aging, older adults tend to use strategies that differ from those used by young adults to solve decision making tasks. This is often accompanied by the recruitment of larger brain areas, inter-hemispheric bilateralization or added brain structures, which can be interpreted as compensatory mechanisms for less effective brain networks. It has been suggested that this process is facilitated through synchronized oscillations that occur between distant brain areas, presumably enabling connections that allow more optimal performance. Because the aging process is known to alter circuit properties that may impact brain oscillations, the present study examined how network changes in the basolateral complex of the amygdala (BLA), known to support reward-based decision making, may be altered in aged rats. To examine this problem, we trained young and old rats to perform three different versions of a decision making task. Two of the tasks were versions of discrimination problems in which either the reward magnitude (reward magnitude discrimination) or the probability of receiving a reward (probability discrimination) was manipulated. The third task version was a probability discounting task in which rats had a choice between a small/certain reward and a large/uncertain reward (probability discounting). In the BLA of old, but not young rats, we found task-specific increased oscillatory power in the beta range (15-30Hz) after lever presses as the animals reached the goal location. Periods of high-power beta were minimal at first, but developed over training days in the aged rats. Within a daily session, the incidence of beta epochs was greater for the early trials and less evident by the end of the session. Both the incidence and power of beta epochs were affected by tasks that involved differing reward magnitudes. Indeed, beta power was significantly greater after pressing for the large reward option. Thus, our results suggest that aging impacts BLA networks in a way that promotes the emergence of beta band activity when learning or deciding between differently sized rewards. Furthermore, we found a correlation between beta incidence and how often the small/certain reward was selected in a session, for both the reward magnitude discrimination and probability discounting tasks. Thus, increased beta oscillations in the BLA of aged rats may reflect compensatory mechanisms which promote a more exploratory type strategy to solve certain reward-based decision making tasks.

Uncinate fasciculus integrity assessed in young and aged bonnet macaques

L. UMAPATHY[1], D. T. GRAY[2,3], S. N. BURKE[6], T. P. TROUARD[2,4], & C. A. BARNES[2,3,5]

[1]Department of Electrical and Computer Engineering, [2]Evelyn F. McKnight Brain Institute, [3]Divison of Neural Systems, Memory and Aging, [4]Department of Biomedical Engineering, [5]Departments of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ; [6]Evelyn F. McKnight Brain Institute, Department of Neuroscience, University of Florida, Gainesville, FL

Cognitive aging is known to alter reward-guided behavior, implicating dysfunction of prefrontal cortical circuits, including the orbitofrontal cortex (OFC). It has been reported that there is a decline in OFC volume with age, which correlates with performance on a reward devaluation task (Burke et al., 2014) in bonnet macaques. The basolateral amygdala (BLA) volume, however, does not change with age, nor correlate with performance. In the present study, the integrity of the uncinate fasciculus (UF), the primary white matter tract between the BLA and OFC is examined using high angular resolution diffusion imaging (HARDI) along with anatomical T1- and T2-weighted imaging. The number of tracks between the BLA and OFC and fractional anisotropy (FA) were calculated and performance measures were obtained in a modified Wisconsin General Testing Apparatus in a group of 11 healthy adult female bonnet macaques (10 to 31 years). Specifically, monkeys were tested on a delayed response (DR) working memory task, reversal learning task (RL, affective shifting) and delayed nonmatching-to-sample (DNMS) with interference task. HARDI scans were acquired using a single shot EPI sequence with a diffusion weighting of b = 1000 s/mm^2 in 51 diffusion directions and with an isotropic resolution of 1.4 mm. Data pre-processing involved corrections for distortions due to eddy currents using FSL, field inhomogeneity corrections using TORTOISE followed by local PCA-based denoising. Diffusion data were then registered with anatomical T1 images using a rigid body transformation. Gray matter volumes corresponding to BLA and OFC were identified previously (Burke et al., 2014). Gray matter, white matter and CSF were segmented, and the interface of the white matter and the BLA boundary was used for seeding the streamlines. Mrtrix3 was used for probabilistic tractography to identify tracks between the BLA and the OFC. Exclusion masks were identified to eliminate tracks that cross between the hemispheres or continue posterior to the BLA. Binary masks were created using track density images to extract the mean FA values along the UF for each animal. There were no statistically significant differences between age groups in mean FA values along the UF, nor were there significant relationships between mean FA values and performance on any of the behavioral tasks. The number of tracks (normalized by the seed volume), however, did significantly correlate with the DNMS task with interference, such that animals with a lower number of tracks performed more poorly. These data suggest that disruptions in the interaction between the OFC and the BLA may contribute to certain age-related cognitive deficits.