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Cardiac arrest induces cognitive decline and CA1 neuronal death in aged rats

C. H. COHAN[1,2], [1], [1], K. R. DAVE[1],
[1] & M. A. PEREZ-PINZON[1,2]

Cerebral Vascular Disease Research Center, Department of Neurology[1], Neuroscience Program[2]

Cardiopulmonary arrest is a leading cause of death and disability in the United States, which primarily occurs in the aged population (51% over the age of 65). However, a majority of the current cardiac arrest (CA) research is performed in 2-3 month old animals, which is the equivalent of 13-18 humans' years. A total of 2% of CA cases each year occur within this age group. Data from humans suggests that as the age of CA in the individual increases, the severity of damage has a correlative increase. Here, we use an model of 6 minutes of asphyxia cardiac arrest in 9 month old Fischer 344 rats (30 human years) to determine if this increased damage correlates with an increased age in the rat model 7 days after CA. Overall, the survival rate was not dependent on the time to loss of pulse or time to resuscitate, but was significantly dependent on post-CA oxygen levels. Behavior tests were performed (Barnes maze) 3 days after CA or sham procedure and tested for a total of 4 days to determine spatial learning and memory abilities. Preliminary data suggests that animals undergoing CA displayed an increase in the number of errors and path length to the escape tunnel. The total number of normal neurons in the hippocampus cornu ammonis 1 (CA1) region was decreased after CA compared to sham animals. Paired pulse facilitation was increased after CA compared to sham animals, whereas long term potentiation was unchanged from shams. Understanding age dependent dysfunction after CA could help direct novel therapies for treating cognitive decline in aged CA survivors.

Recurrent hypoglycemia exacerbates cerebral ischemic damage in diabetic rats via enhanced post-ischemic mitochondrial dysfunction

K. R. DAVE[1], S. DASANI[1], & A. PILEGGI[2]

[1]Department of Neurology, [2]Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL

Recurrent hypoglycemia (RH) in a rat model of insulin-dependent diabetes exacerbates cerebral ischemic damage. Since post-ischemic mitochondrial dysfunction plays an important role in cerebral ischemic damage, we tested the hypothesis that post-ischemic mitochondrial dysfunction is severe in RH-exposed diabetic rats. Streptozotocin-induced diabetic rats were used as an animal model. Four experimental groups were examined: 1) naïve (non-diabetics), 2) insulin-treated diabetics (ITD), 3) ITD + RH (diabetics on insulin therapy experiencing RH), and 4) ITD + RH + Glucose (control for additional insulin injected to induce hypoglycemia). RH was induced once a day for five days. Global cerebral ischemia was induced the day after the last hypoglycemia treatment by tightening carotid ligatures bilaterally following hypotension (50 mmHg) for eight minutes. Hippocampal mitochondrial function was assessed on the day after induction of global cerebral ischemia by measuring the rate of mitochondrial oxygen consumption in the presence of different substrates of mitochondrial electron transport chain. We observed that the rate of oxygen consumption in the presence of pyruvate and malate was lower in ITD + RH group by 25% ($P<0.05$), 46% ($P<0.001$), and 33% ($P<0.001$) compared to naïve, ITD, and ITD + RH + Glucose groups, respectively. No statistically significant differences were observed between ITD + RH and the other three control groups when oxygen consumption was measured in presence of succinate + glycerol – 3 – phosphate and ascorbate + TMPD as substrates. We are in the process of further characterizing mitochondrial function under these conditions. The results of present study demonstrate that RH-induced exacerbation of cerebral ischemic damage in diabetic rats is associated with a lowered rate of respiration in the presence of substrates of complex I of the mitochondrial electron transport chain. These results suggest that enhanced post-ischemic mitochondrial dysfunction may be responsible for exacerbated cerebral ischemic damage in in RH exposed diabetic rats.

17β-estradiol treatment protects the hippocampal CA1 region against cerebral ischemia via estrogen receptor-β

A. P. RAVAL[1], R. BORGES-GARCIA[1], W. J. MORENO[3], H. BRAMLETT[2, 3,] & M. A. PEREZ-PINZON[1,2]

[1]Cerebral Vascular Disease Research laboratories, Department of Neurology, [2]Neuroscience Program, [3]Department of Neurological Surgery, Leonard M. Miller School of Medicine, University of Miami, Miami, FL - 33101, USA

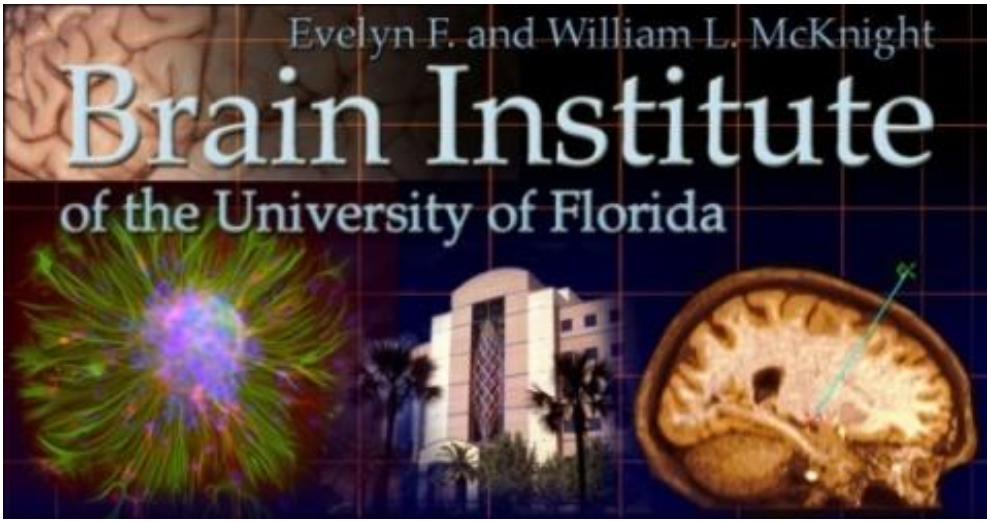
The failure of the Women's Estrogen for Stroke Trial raised concerns regarding the safety of chronic estrogen treatment in women. In contrast to chronic 17β-estradiol treatment, we demonstrated that a single 17beta-estradiol (E_2) bolus protects the hippocampal CA1 region from ischemic damage via phosphorylation of cyclic-AMP response element binding protein (pCREB). Phosphorylation of CREB requires activation of estrogen receptor subtype beta (ER-β) and regulates hippocampus- dependent learning and memory. Therefore, we hypothesized that intermittent E_2-treatment improves cognition and protects hippocampus against ischemia via pCREB-ER-β pathway in female rats. Female rats were ovariectomized (OvX) and 7 days later divided into two groups. One group was treated with E_2 (5 µg/Kg; i.p.) or vehicle-oil at an interval of every 48h for 21 days. The second group was treated with ER-β-antisense (AS) or missense (MS) by bilateral cerebroventricular infusion every 24 h for 4 days and E_2/oil was administered on the second day of antisense treatment. Rats belonging to both groups were exposed to cerebral ischemia 48h after the last E_2 treatment and 7 days later brains were examined for histopathology. In parallel experiments, OvX rats were treated with either E_2/oil or ER-β agonist/vehicle every 48 h for 21 days. These rats were either use to monitor hippocampal-dependent learning and memory capabilities using Morris the water maze or hippocampal tissue was collected to investigate protein levels of pCREB. The normal neuronal count was higher in OvX rats treated with E_2 (52%) as compared to the oil-treated (17%; $p<0.001$). Silencing of hippocampal ER-β followed by E_2 treatment decreased normal neuronal count (13%) as compared to MS-E_2-treated group (67%; $p<0.001$). Results of the water maze study demonstrated that E_2/ER-β agonist treatment significantly improved spatial learning and working memory performance as compared to the respective vehicle groups. Intermittent E_2/ER-β agonist treatment protects hippocampus from ischemic damage and improves hippocampal-dependent cognition in OvX rats, suggesting a role for ER-β in intermittent E_2-mediated ischemic neuroprotection. This study emphasizes the need to investigate an intermittent estrogen hormone replacement regimen to promote cardio- and cerebro-vascular health and reduce stroke/cerebral ischemia incidents in post-menopausal women while avoiding the known side effects of chronic E_2-treatment.

Chronic nicotine hinders hippocampus-dependent learning and memory in female rats

R. BORGES-GARCIA[1], M. BEHAIRY[1], M. A. PEREZ-PINZON[1,2], H. BRAMLETT[2, 3] & A. P. RAVAL[1]

[1]Cerebral Vascular Disease Research laboratories, Department of Neurology, [2]Neuroscience Program, [3]Department of Neurological Surgery, Leonard M. Miller School of Medicine, University of Miami, Miami, FL - 33101, USA

The rise in the number of female smokers is a major public health concern in the United States. Currently 22 million (22 %) American women smoke cigarettes and even though the detrimental effects of smoking-derived nicotine on health are well-established, giving up a smoking habit is more difficult for women than for men. Women metabolize nicotine faster than men and that influences smoking behavior, causing more dependence and increasing the associated risks. Most prior studies to understand the mechanisms of nicotine dependence were carried out on male experimental animals and were focused on identifying the effects of nicotine on its receptors; however, effects of nicotine unique to females warrant investigation. Recently, we demonstrated that chronic nicotine exposure attenuates short-term synaptic plasticity in the hippocampus of female rats. Hippocampus plays important roles in long-term memory and spatial navigation; therefore, we further investigated effects of chronic nicotine on hippocampus-dependent learning and memory. Twenty female rats were randomly assigned to either a nicotine (4.5 mg/kg/day) or saline treatment. Starting on day 16 of nicotine/saline treatment, rats were tested for their hippocampus-dependent cognitive capabilities using the Morris water maze, using a 7-day testing paradigm. The first 4 days measured learning capabilities (learning the position of a platform), the 5^{th} day measured memory (memory of platform location when platform is removed), and the 6^{th} and 7^{th} days measured working memory (learning a new platform location). The results are presented as Mean ± SEM of latency time in seconds. During four days of learning paradigm, nicotine exposed rats took longer time to find platform (40±3s, 37±4s, 39±3s and 41±2s) as compared to saline (36±4s, 31±4s, 31±5s and 26±2s). The results of this test demonstrated significant difference in latency period of nicotine and saline-treated female rats on the 4^{th} day ($p< 0.001$). The outcome of the long-term memory testing performed on fifth day demonstrated that there was a distinct difference between the saline and the nicotine-exposed groups. Similarly, the working memory task demonstrated that saline-exposed rats performed better the nicotine-exposed rats. The nicotine-exposed rats took 38 and 36 seconds to find the platform on the first and second trial respectively, as compared to 36 and 29 seconds in saline group. Overall, the significant increase in latency period in the nicotine-exposed group suggested deficits in spatial learning and memory performance.



Director: Tetsuo Ashizawa, M.D.

Chair: Thomas C. Foster, Ph.D.

Differentiation of developing neocortical neurons is linked to primary cilia structure

S. M. GUADIANA[1], S. SEMPLE- ROWLAND[1], I. MADORSKY[1], D. DAROSZEWSKI[1], K. MYKYTYN[2], & M. SARKISIAN[1]

[1]Neurosci., Univ. of Florida Col. of Med., Gainesville, FL; [2]Pharmacol. and Intrnl. Med., Ohio State Univ., Columbus, OH

The plasma membranes of primary neuronal cilia are selectively enriched with various neuromodulatory G protein-coupled receptors (GPCRs) during development. Excess trafficking of these receptors to cilia can disrupt cilia length homeostasis. Here, we analyzed whether overexpressing the ciliary GPCRs, 5HT6 and SSTR3, disrupts cilia structure and/or alters differentiation of developing neocortical neurons. Neuronal overexpression of 5HT6 and SSTR3 was achieved by electroporating mouse embryo cortex in utero with vectors encoding these receptors. We found that overexpression of ciliary GPCRs, especially 5HT6, in cortical neurons induced the formation of long, swollen, and oftentimes forked cilia with elongated axonemes. These structural changes were accompanied by increases in the levels of molecules involved in intraflagellar transport (IFT), a process that is critical for maintaining normal cilia protein trafficking, growth, and function. In this regard, it was notable that levels of SSTR3 and of type III adenylyl cyclase (ACIII), a signal transduction protein that is normally enriched in neuronal cilia, were significantly reduced in neurons overexpressing 5HT6. These changes in the cilia were accompanied by changes in neuronal differentiation. Specifically, disruption of neuronal cilia length homeostasis, either through overexpression of cilia GPCRs or by interrupting IFT using a dominant negative Kif3a, significantly impaired neurite outgrowth from developing neocortical neurons. Together, our data suggest that factors that impair cilia length homeostasis may be accompanied by deficits in neuronal differentiation and shed potential etiological insight on the numerous neurodevelopmental disorders known to manifest in patients with ciliopathies.

Adolescent risk taking, dopamine signaling, and cocaine self-administration: A vicious cycle

M. R. MITCHELL, V. C. WEISS, D. MORGAN, J. L. BIZON, & B. SETLOW

Departments of Psychiatry & Neuroscience, University of Florida, College of Medicine, Gainesville, FL

Elevations in risk-taking are characteristic of both adolescence and drug abuse, but the relationships among risk-taking, adolescence, and drug abuse are difficult to disentangle in humans. Here we used a rat model of risky decision making (the Risky Decision-making Task, RDT) to assess relationships between adolescent risk-taking and cocaine self-administration. In addition, we used analyses of mRNA expression and behavioral pharmacology to characterize the involvement of dopamine signaling in risk-taking.In Experiment 1, adolescent male Long-Evans rats (P25) were trained in the RDT, in which they were given choices between two response levers, the first which delivered a small (1 pellet), "safe" food reward and the second which delivered a large (3 pellets), "risky" food reward accompanied by the risk of a mild footshock, the probability of which increased over the course of the test session in consecutive blocks of discrete trials (0, 25, 50, 75, 100%). Upon completion, half of the rats were implanted with i.v. jugular catheters and after recovery, were allowed to self-administer 0.5 mg/kg/infusion cocaine for 2h/day for 5 days, followed by 1.0 mg/kg/infusion for 6h/day for 14 days. The other half of the rats self-administered an oral sucrose solution to control for instrumental learning experience. Upon completion of self-administration, rats remained abstinent from cocaine (or sucrose) for 3 weeks before being retested in the RDT for 4 weeks. In Experiment 2, adolescent rats were characterized in the RDT, followed by sacrifice for *in situ* hybridization analyses of D1 and D2 dopamine receptor expression in striatal subregions. In Experiment 3, adolescent rats were characterized in the RDT, followed by assessment of the effects of microinjections of the D2-like agonist quinpirole directly into dorsal or ventral striatum.

In Experiment 1, there were substantial individual differences in adolescent rat performance in the RDT, such that some rats preferred the large, risky reward whereas others preferred the small, safe reward. This individual variability predicted cocaine intake during acquisition of cocaine self-administration, such that greater preference for the large, risky reward (greater risk-taking) was associated with greater cocaine intake. In addition, following self-administration and 7 weeks of abstinence, rats that self-administered cocaine showed significantly elevated risk-taking compared to both sucrose controls and their performance during adolescence. In Experiment 2, there were significant inverse correlations between risk-taking in adolescence and D1 mRNA in dorsomedial striatum and D2 mRNA in dorsolateral striatum and nucleus accumbens shell, such that greater choice of the large, risky reward (more risk-taking) was associated with lower levels of dopamine receptor mRNA expression. In Experiment 3,

microinjection of quinpirole into ventral (but not dorsal) striatum caused a dose-dependent decrease in preference for the large, risky reward (less risk-taking).

Data from these experiments indicate that elevated risk-taking in adolescence is predictive of future acquisition of cocaine self-administration, and that cocaine self-administration, in turn, causes elevations in risk-taking that last well into abstinence. Elevated risk-taking in adolescence is also associated with low levels of striatal dopamine (particularly D2) receptor mRNA, consistent with previous work across species which has linked low levels of striatal D2 receptor availability with cocaine self-administration. Considered together, these data suggest that attenuation of striatal dopamine receptor activity, particularly during early development, may be a feature of several forms of maladaptive behavior, and furthermore that targeting this attenuation may hold promise for reducing such behaviors.

High Cocaine Intake Causes Long-Lasting Elevations in Impulsive Choice in a Delay Discounting Task

V. G. WEISS, M. R. MITCHELL, D. OUIMET, & B. SETLOW

Department of Psychiatry, University of Florida, College of Medicine, Gainesville, FL

Cocaine use is associated with elevated impulsive choice but the cause and effect relationships between cocaine use and impulsive choice are not entirely clear. In previous work, we found that experimenter- and self-administered cocaine causes long-lasting increases in impulsive choice in rats. The purpose of this study was to extend these original findings, both by taking into account rats' pre-existing level of impulsive choice prior to self-administration, and by allowing rats to self-administer as much cocaine as they wished.

Male Long-Evans rats were trained in standard operant chambers in a delay discounting task in which they made discrete-trial choices between 2 response levers. A press on one lever delivered a small food reward with no delay, and a press on the other delivered a large food reward after a delay period, which increased in consecutive blocks of trials in each session (0-32 s). Once stable performance was achieved, half of the rats were implanted with intravenous jugular catheters and, following recovery, were allowed to self-administer a high dose of cocaine HCl (1.0 mg/kg/infusion) long exposure (6h/d) sessions for 14 d. Control rats were allowed to self-administer a sucrose solution, under conditions in which the number of reinforcers earned was paired to that of a cocaine rat with comparable performance in the delay discounting task. Upon completion of self-administration, rats remained abstinent from cocaine for 3 weeks before retesting in the delay discounting task.

There were no differences between cocaine and sucrose groups prior to self-administration, but afterwards, the cocaine group showed significantly greater impulsive choice than the sucrose group. Additional analyses revealed that long-term shifts in impulsive choice from pre- to post-self-administration sessions were dependent upon the amount of cocaine consumed. Rats that were classified as "low self-administering" (<100 mg/kg over 14 d) did not differ in impulsive choice from paired low self-administering sucrose controls. In contrast, rats that were classified as "high self-administering" (>100 mg/kg over 14 d) were significantly more impulsive than their high self-administering sucrose controls and significantly more impulsive than their pre-self-administration baseline. These data suggest that long-term elevations in impulsivity can be caused by cocaine itself, and that these effects may be dose dependent.

GABA(B) receptor blockade enhances delayed match-to-sample working memory performance in aged but not young rats

C. BAÑUELOS, B. S. BEAS, J.A. MCQUAIL, R. J. GILBERT., B. SETLOW, & J. L. BIZON

Department of Neuroscience and Psychiatry, University of Florida, College of Medicine, Gainesville, FL

A substantial literature demonstrates cognitively enhancing effects of GABA(B) receptor antagonists in young rodents. Moreover, previous work from our laboratory demonstrated that administration of the GABA(B) receptor antagonist CGP55845 reversed age-related impairments in a hippocampal-mediated olfactory learning task (LaSarge et al., 2009). While both age-related hippocampal and prefrontal cortical (PFC) systems are vulnerable to age-related decline, it is becoming evident that these neural systems age orthogonally, and that multiple, potentially divergent mechanisms underlie different aspects of cognitive impairment. As GABA(B) receptor expression and signaling are differentially affected in the aged PFC and hippocampus (McQuail et al., 2012), an important outstanding question is whether GABA(B) receptor antagonists can improve PFC-supported cognition which declines in aging. In the current study, the effects of systemic GABA(B) receptor blockade on delayed match-to-sample working memory performance was examined, using an operant task which is sensitive to medial PFC damage. On each trial in this task, adult (7 mo) and aged (25 mo) male F344 rats were first presented with a "sample" lever (either left or right). A press on this lever caused it to retract and initiated a delay period, which ranged pseudorandomly from 0-24 seconds. Following the delay, both levers were presented, and a press on the same lever presented in the same phase (i.e., a "match") delivered a single food pellet. Performance was assessed as the percentage of correct choices of the sample lever at each delay. Rats received i.p. injections of CGP55845 (0.01 or 0.1 mg/kg) or saline vehicle 40 minutes before test sessions, using a randomized, within-subjects design, with a 48 hour washout period between injections. Under vehicle conditions, aged rats showed impaired performance relative to adult cohorts, an effect that was attenuated with the 0.1 mg/kg dose of CGP55845. In contrast, performance in adult rats was not enhanced and, indeed, in adult rats with highly proficient performance under vehicle conditions, CGP55845 impaired performance. These data are consistent with a well-established inverted "U"-shaped curve associated with neurochemical modulation of PFC-supported cognition, and suggest that GABA(B) receptor antagonists may have utility for improving PFC function in aged subjects with cognitive deficits. Ongoing research in our laboratory is determining the brain loci through which CGP55845 acts to enhance cognitive performance in the working memory task.

Attentional set-shifting and working memory are inversely related in aged rats

B. S. BEAS, B. SETLOW, & J. L. BIZON

Departments of Neuroscience and Psychiatry, University of Florida, College of Medicine, Gainesville, FL

Executive functions supported by prefrontal cortical systems provide essential control and planning mechanisms which guide and optimize goal-directed behavior. As such, alterations in executive functions can have profound and widespread effects on a diverse array of neurocognitive processes. The goal of these experiments was to determine the effects of normal aging on two components of executive function: cognitive flexibility and working memory.

Young adult (6 mo) and aged (22 mo) male F344 rats were serially trained on an attentional set-shifting task and a delayed match-to-sample task to assess cognitive flexibility and working memory abilities, respectively. In the attentional set shifting task, rats first learned to discriminate between two levers based on the presence of a visual cue, then were required to "shift" their behavior and discriminate between the levers on the basis of their spatial position (e.g., always press the left lever). In the delayed match-to-sample (working memory) task, rats had to maintain information concerning the position of the "sample" lever over a delay period which ranged from 0-24 seconds, to recall later for a correct response. A subset of these rats was also tested in the Morris water maze to assess mnemonic function dependent on the hippocampus (but not the prefrontal cortex).

Aged rats were impaired relative to young in both the set-shifting and working memory tasks. Among aged rats, however, comparisons of performance across both tasks revealed a strong inverse relationship, such that better set-shifting performance was associated with worse working memory and vice-versa. In contrast, there was no relationship among aged rats between set-shifting task performance and long-term spatial memory as assessed in the Morris water maze, which does not depend on intact prefrontal cortical function.

These data indicate dissociable effects of aging on subcomponents of executive function and suggest an imbalance in aging between the ability to form and maintain stable representations of information no longer present in the environment and the ability to alter these representations when environmental contingencies are changed. Ongoing research in our laboratory is investigating the role of age-related changes in the mesocortical dopaminergic system in these cognitive alterations.

Memory encoding as a function of age in an elderly sample: An fMRI study

M. L. GREEN[1], C. N. SOZDA[1], J. W. KIRTON[1], T. MANINI[2], S. ANTON[2], W. M. PERLSTEIN[1,3], & V. M. DOTSON

Departments of [1]Clinical & Health Psychology, [2]Aging and Geriatric Research, and [3]Psychiatry, the [4]McKnight Brain Institute, and the Department of [5]Neuroscience, University of Florida, Gainesville, FL

Background: Different memory domains are differentially vulnerable to decline with aging. One memory domain that is particularly susceptible to decline is declarative episodic memory, with greatest age differences seen in tests of recall. Although declines in episodic memory performance and changes in memory-related brain activity have been clearly demonstrated in older adults compared to young adults, the present study aimed to examine differences between young-old and middle-old adults which have not previously been examined.

Methods: Functional magnetic resonance imaging (fMRI; 3Tesla) was employed to probe the neural correlates of memory encoding as a function of age in 22 healthy older adults (8 male/14 female: mean age of 73.0 ± 8.0 years; range 65 to 81). Participants were asked to memorize 15 words in 60 s and then write down words recalled on a sheet of paper for the baseline trial. Participants were then placed in an MRI scanner and asked to complete 3 subsequent free-recall memory trials in which a 42-item categorized shopping list was presented in 7 blocks of 6 words. A 16-s baseline fixation period began each trial followed by a single word presented for 2 s with 2 s interblock interval. fMRI data were analyzed using random-effects whole-brain voxel-wise general linear modeling; subsequent planned bivariate correlations examined relationships between fMRI activation during word presentation/encoding with participant age.

Results: fMRI data revealed a number of regions activated during memory item encoding significantly activated a number of regions, including hippocampal and temporal regions. Word-encoding BOLD activity within the left hipppocampus negatively correlated with participants age ($r(20)= -.57$, $p<.006$). The greatest encoding-related signal change from baseline was seen in young-old adults. Total word recall did not correlate with age ($r(20) =.24$, $p=.27$).

Conclusions: These results are consistent with previous studies suggesting that encoding-related brain activity in hippocampal and other temporal areas differs as a function of age. Moreover, findings suggest that this age effect can also be observed within an elderly sample, as middle-old participants showed the greatest reduction in hippocampal activity. Results have implications for memory decline and risk for Alzheimer's disease in late life.

Disruption of signaling from NMDARs to gene transcription contributes to age-related learning impairments

T. C. FOSTER, A. RANI, O. TCHIGRINOVA, & A. KUMAR

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

Memory depends on hippocampal gene transcription occurring around the time of training. The initiation of this early phase of transcription likely results from Ca^{2+} entry through NMDARs and voltage-dependent Ca^{2+} channels, activating critical kinases including the extracellular signal-regulated kinase (ERK), resulting in epigenetic modification of chromatin structure, including histone acetylation. Age-related cognitive decline is associated with impaired NMDAR function and a decrease in the expression of synaptic plasticity genes suggesting that memory impairments are related to a decrease in signaling from NMDARs to gene transcription. Male F344 rats (5-8, 12-14, and 20-22 mo) were trained on the Morris water maze (cue followed by spatial training) and classified as learning impaired or unimpaired following a single day of spatial training. Ten days later animals were removed directly from the home cage (CON) or after inhibitory avoidance foot shock training and hippocampi dissected for analysis of pERK and histone acetylation (H3 and H4) expression. Basal (i.e. CON) pERK and H3-acetylation was elevated in the dentate gyrus (DG) relative to CA1 across age groups. Basal pERK, H3-acetylation, and H4- acetylation increased during aging; however, basal levels were not related to impaired learning. Expression of pERK and H3-acetylation increased at 10 min following foot shock and declined over the next 1 hour. H4- acetylation was decrease at 1 hour relative to 10 min. For the 1 hour time point, pERK, particularly in region CA1, and H3-acetylation in regions CA3 and DG were increased in unimpaired relative to impaired older rats. The results support the idea that decreased signaling from NMDARs to gene transcription during learning contributes to age-related impairment of rapidly acquired and flexible spatial information

Ca^{2+} from VGCCs and NMDA receptors contributes to LTD induced by either synaptic activity or mGluR activation at CA3-CA1 hippocampal synapses during senescence

A. KUMAR & T. C. FOSTER

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

The mechanisms for induction of synaptic modifications change during the course of aging. In the hippocampus of aged animals, there is an increase in the susceptibility to long-term depression (LTD) induced either by pattern synaptic activity (synaptic-LTD) or activation of metabotropic glutamate receptors (mGluR-LTD). The present study examines the role of NMDA receptors (NMDARs), voltage-gated Ca^{2+} channels VGCCs), and mGluRs in synaptic depression induced by either pattern synaptic activity (1 Hz paired-pulse) or the group I mGluR by selective agonist, (RS)-3,5-dihydroxyphenylglycine (DHPG, 100 µM) at CA3-CA1 hippocampal synapses in slices obtained from aged (22-24 mo) male Fischer 344 rats. Induction of synaptic-LTD did not occlude mGluR-LTD; however, prior mGluR-LTD occluded synaptic-LTD. Pre-incubation of slices with the L-type VGCC blocker, nifedipine, or mGluR antagonist, AIDA, significantly attenuated mGluR-LTD, but not synaptic-LTD. Blockade of NMDARs with AP-5 did not attenuate either synaptic-LTD or mGluR-LTD. Finally, combined bath application of AP-5 and nifedipine attenuated both forms of synaptic depression. Results indicate that DHPG induces an overwhelming synaptic depression, occluding synaptic-LTD, which involves activation of L-type VGCCs and NMDARs. In contrast, synaptic stimulation to induce LTD appears to involve Ca^{2+} from VGCCs or NMDARs. Finally, the fact that blockade of VGCCs and NMDARs only attenuated LTD suggests that other mechanisms or Ca^{2+} sources are involved. The results emphasized that <u>all</u> Ca^{2+} sources normally contribute to LTD induction in the absence of specific antagonists.

Age related changes in central and circulating cytokines and their relationship to learning and memory

R.B. SPEISMAN[1], A. KUMAR[2], A. RANI[2], A. ASOKAN[1], T.C. FOSTER[2], & B.K. ORMEROD[1]

[1] J. Crayton Pruitt Family Department of Biomedical Engineering and [2] Neuroscience Department and McKnight Brain Institute
University of Florida, Gainesville, FL, 32611

Neuroinflammatory genes are upregulated with age, most robustly in rats that exhibit impaired memory across spatial tasks. Here we quantified immunomodulatory cytokines across age in the serum and brains of behaviorally characterized rats using BioPlex technology. Specifically, IL-12, eotaxin, GM-CSF, G-CSF, IL-1α, MCP-1, leptin, MIP-1α, IL-4, IL-1β, IL-2, IL-6, IL-9, IL-13, IL-10, IL-5, IFN-γ, IL-17, IL-18, IP-10, GRO-KC, RANTES, TNF-α and VEGF were quantified in the serum and hippocampal protein harvested from young (8mo; n=14), middle-aged (14mo; n=39), and aged (20mo; n=27) male Fisher 344 rats that were characterized as memory-unimpaired or -impaired using a rapid acquisition spatial water maze task. Relative to young rats, aged rats (p=0.031) exhibited significantly lower probe trial discrimination index scores. Although middle aged rats performed as well as young rats, their performances were notably variable. Serum eotaxin, GRO-KC, RANTES, IFN-γ and IL18 concentrations were all elevated in aged relative to young rats (p's<0.05). Within the hippocampus, immunomodulatory cytokine profiles varied with age. Some cytokine concentrations increased with age (ex. MIP-1α, IL-9, IL-18 and RANTES, p's<0.04) while others decreased (ex. IL-5, p's <0.03 in middle-aged and aged rats). Pathway analyses on Spearman rank correlations between cytokine concentrations and discrimination index scores revealed clusters of circulating and central cytokines that increased and decreased with age and that related to memory. We are currently investigating the effects of non-steroidal anti-inflammatory drug treatment on both memory and circulating and central cytokine concentrations across age. We anticipate that our data may reveal immunomodulatory mechanisms behind age-related cognitive decline and could lead to the development of a biomarker assay that predicts age-related cognitive decline.

Synaptic dysfunction and early cognitive impairment in a mouse model of AD

K. S. MONTGOMERY, A. KUMAR, K. DEMARS, T. FOSTER, & J. BIZON

Department of Neuroscience, University of Florida, College of Medicine, Gainesville, FL

A wealth of genetic, neurohistopathological and biochemical data implicate the accumulation of neurofibrillary tangles, Aβ plaques and extensive synaptic and neuronal loss in Alzheimer's Disease (AD). Of these, synaptic changes have been most widely linked to early cognitive deficits in humans; however, such relationships have been difficult to establish in rodent models of AD as cognitive deficits in mouse models are generally subtle and emerge relatively late in their lifespan. Previous work from our laboratory has shown that APPswe(PSEN1dE9)85Dbo/o) mice exhibit robust and early performance deficits in a hippocampal-dependent transfer learning task. The current study was conducted to investigate the hypothesis that synaptic alterations in the hippocampal formation contribute to this early decline in cognitive function. To investigate synaptic integrity, Western blotting was used to evaluate the expression of pre- and post-synaptic proteins in the hippocampus of PS1/APP mice of different ages, which were first characterized on the transfer learning task. Parallel electrophysiology experiments were performed to evaluate paired-pulse facilitation (PPF) and long-term potentiation (LTP) in PS1/APP mice. Initially, APPswePS1 (3, 6, 12 mo) mice and age-matched NTg control mice were trained in a transfer learning task which assessed their ability to apply previously learned information to a novel context (Montgomery et al., 2011). While 3 mo old APPswePS1 mice were not impaired, both 6 and 12 mo old APP/PS1 mice exhibited significant deficits in performance relative to age-matched NTg controls. Semi-quantitative analyses of hippocampal synaptophysin, synapsin I, and PSD95 provided evidence of changes in both pre- and post-synaptic proteins that were specific to the APPswePS1 mice and that paralleled the learning deficit. Extracellular synaptic field potential recordings at CA3-CA1 hippocampal synapses in slices obtained from 6 month old APPswePS1 and NTg mice indicated a significant decrease in baseline synaptic transmission in APPswePS1 mice. Induction of LTP in slices indicated a tendency for a decrease in the amplitude of LTP in APPswePS1 mice. No significant difference was observed in PPF ratio. Together, these results suggest that amyloid β deposition is associated with a decrease in synaptic transmission, possibly due to the loss of synaptic contacts, and that these deficits in synaptic integrity may contribute to the emergence of hippocampal cognitive dysfunction.

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GABAergic depolarization promotes excitatory synaptogenesis on adult-generated neurons

J. CHANCEY, E. ADLAF, M. SAPP, P. PUGH, J. WADICHE, & L. OVERSTREET-WADICHE

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

Adult-generated dentate granule cells (GCs) must survive and integrate into the existing network in order to contribute to hippocampal function. Only a fraction of newly born GCs survive, while most undergo apoptosis within the first few weeks of maturation. Experiences, such as environmental enrichment (EE), increase the survival of adult-generated GCs, but only during an early maturation stage termed the "critical period." Both glutamatergic and GABAergic synaptic input have also been shown to be important for survival and maturation of newborn GCs, but little is known about how experiences like EE affect synaptic connectivity, nor how glutamatergic and GABAergic mechanisms interact to promote synaptic integration. We used proopiomelanocortin enhanced-green fluorescent protein (POMC-GFP) reporter mice to identify a population of adult-generated GCs in the critical period. Cells at this early developmental stage have depolarizing GABAergic inputs, and here we show that they also have glutamatergic transmission mediated exclusively by NMDARs or "silent synapses". Using *in vitro* paradigms, we show that GABA synaptic activity provides the depolarization necessary for rapid AMPAR incorporation into silent synapses (synapse usilencing). Furthermore, we show that EE promotes synapse unsilencing *in vivo*, in a manner correlated with enhanced GABAergic input. Together these results reveal a mechanism by which GABAergic activity controls integration of adult generated neurons and suggest that newborn GCs in the critical period are primed to respond to network activity by initiating functional glutamatergic transmission.

Spillover activation of inhibition functionally segregates interneurons in the cerebellar cortex

L. T. CODDINGTON, S. RUDOLPH, P. V. LUNE, L. OVERSTREET-WADICHE, & J. WADICHE

Dept of Neurobiology and Evelyn McKnight Brain Institute, University of Alabama at Birmingham

Neurotransmitter spillover represents a form of neural transmission not restricted to morphologically defined synaptic connections. Communication between climbing fibers (CFs) and molecular layer interneurons (MLIs) in the cerebellum is mediated exclusively by glutamate spillover. Here, we show how CF stimulation functionally segregates MLIs based on their location relative to glutamate release. Excitation of MLIs that reside within the domain of spillover diffusion coordinates inhibition of MLIs outside the diffusion limit. CF excitation of MLIs is dependent on extrasynaptic NMDA receptors that enhance the spatial and temporal spread of CF signaling. Inhibition mediated by functionally segregated MLIs converges onto neighboring Purkinje cells (PCs) to generate a long-lasting biphasic change in excitability. These data demonstrate how glutamate release from single CFs modulates excitability of neighboring PCs, thus expanding their influence on cerebellar cortical activity not predicted solely by anatomical connectivity.

Why is speed of processing training effective?: Developing and testing tools to examine the neural mechanisms of training

C. R. DENNING[1], R. NENERT[2], W. BURGE[2], L. A. ROSS[3], & K. M. VISSCHER[2]

[2]Neurobiology, [3]Psychology, [1]University of Alabama at Birmingham, Birmingham, AL

Speed of processing is one of the first cognitive domains to decline with aging. Speed of processing is defined as the ability to perceive and interpret complex sensory information and is measured with the Useful Field of View test (UFOV). UFOV scores have been shown to improve with a paradigm called speed of processing training. This training also has been shown to transfer to other domains such as cognition, everyday functioning, mental health, driving, and physical health. However, the neural changes associated with this training are not well understood.

In order to effectively study the neural underpinnings of training, we must first develop a task that 1) can be performed in the fMRI scanner, 2) requires similar cognitive demands to the UFOV test, and 3) allows examination of neural responses to cues independent from performance of the task. This study aims to develop and test a task that fits these criteria.
Five different tasks were examined. For the task that correlated with the above criteria most closely ($r = -0.781$, $p = 0.002$), participants were presented with a stimulus screen with a central identification stimulus and a peripheral localization stimulus for 450ms. They then responded to two different screens, noting: (1) whether the central object on the response screen was the same as or different from the previous stimulus screen and (2) whether the presented peripheral object in the response screen was in the same location or a different location as it was on the stimulus screen. In order to assess participants' responses to cues, the cues are infrequently presented in the absence of a stimulus.

Participants at-risk for age-related cognitive decline were able to perform the task with a level of accuracy above 70%. Performance on this fMRI UFOV task negatively correlated with performance on the standard UFOV task and was able to differentiate between high-risk and low-risk participants. This indicates that the UFOV and the task developed have similar cognitive demands.

This fMRI UFOV task was performed in the scanner by a group of older adults at-risk for cognitive decline, evoking visual and auditory neural responses.
We show that the fMRI UFOV task can be performed in the scanner, requires similar cognitive demands as the UFOV task, and allows examination of neural responses to visual stimuli. Future work will employ this task to examine the mechanisms behind speed of processing training.

Restricted expression of mutant Huntington to parvalbumin-positive cell populations causes hyperactivity and altered synaptic function in the motor cortex

DOUGHERTY, S.E. [A, C], HOLLIMON, J. F. [C], HABLITZ, J.J. [B], & COWELL, R. M. [C]

[a]Neuroscience Graduate Program, University of Alabama at Birmingham [b]Department of Neurobiology, University of Alabama at Birmingham [c]Department of Psychiatry & Behavioral Neurobiology, University of Alabama at Birmingham

Huntington Disease (HD) is a devastating neurological disorder characterized by motor, psychiatric, and cognitive disturbances. Though the mutant huntingtin (mthtt) protein is ubiquitously expressed, its presence has different consequences for cellular function and viability depending on the affected cell type. GABAergic interneurons are an integral part of the neuronal circuitry throughout the brain. These neurons have been shown to modulate regional output, entrain network activity, and limit hyperexcitability. One specific subtype of interneuron thought to play a fundamental role in modulating regional firing patterns is the parvalbumin-positive (PV+) interneuron. Evidence suggests that there may be physiological dysfunction within these interneurons prior to dysfunction in pyramidal neurons in mouse models of HD. In order to investigate the role of the PV+ subclass of GABAergic interneurons in HD, we utilized a cre/lox system of conditional gene expression. The resultant mice had expression of the mthtt gene only in PV+ cells, including interneurons in the cortex. These mice exhibited a hyperactive motor phenotype between 10 and 12 months of age. To assess physiological consequences of this conditional mthtt expression, we performed whole cell voltage clamp recordings in the motor cortex of 12 and 24 month old mice. These mice showed impaired synaptic physiology, including alterations in both spontaneous and evoked activity, indicating abnormalities in basal GABA release. Additionally, upon repetitive stimulation at 66 Hz there was a reduction in overall GABA release suggesting a reduction in network inhibition and a potential for an increase in cortical excitability. This hyperexcitability in the cortex could influence downstream striatal function and contribute to the altered motor behavior observed in these mice. Taken together, these data suggest that PV+ interneuron dysfunction contributes to hyperactivity and altered neuronal physiology in HD.

Increased expression of Eph receptors and their ligands, ephrins, in anterior cingulate cortex in schizophrenia

J. B. DRUMMOND[1], K. WOOD[1], V. HAROUTUNIAN[2] & J. H. MEADOR-WOODRUFF[1, 3]

[1]University of Alabama at Birmingham, Department of Psychiatry and Behavioral Neurobiology, Birmingham, AL 25294 [2] Mount Sinai School of Medicine, Department of Psychiatry, New York NY 10468 [3] Evelyn F. McKnight Brain Research Foundation

The glutamate hypothesis of schizophrenia suggests abnormal glutamatergic neurotransmission occurs in this illness, and a growing body of literature suggests that Eph receptor tyrosine kinases and Eph receptor ligands, named ephrins (EFN), directly associate with AMPA receptor (AMPAR) auxiliary and trafficking proteins to modulate AMPAR function and localization within the postsynaptic neuron. Our laboratory has previously reported altered expression levels of AMPAR auxiliary proteins including PICK1, GRIPs and TARPs in schizophrenia, suggesting abnormal AMPAR trafficking and tethering within intracellular compartments in this illness. To determine whether Eph receptors/ephrins are also dysregulated in schizophrenia, we measured their transcript expression in the anterior cingulate cortex of patients with schizophrenia (N=41) and a comparison group (N=35) using quantitative real-time PCR (qPCR). We found transcripts encoding EphA3, EphA6, EphA7, EphB3, EphB6, EFNA2, EFNA3, EFNA5, EFNB1, EFNB2 and EFNB3 were all significantly increased in schizophrenia. Additionally, we examined Eph receptor/ephrin gene expression in the prefrontal cortex of rats treated chronically with haloperidol to determine if these changes might be due to antipsychotic treatment. These data contribute to the increasing evidence that AMPAR dysfunction may be associated with the pathophysiology of schizophrenia.

Dissociation of frontotemporal dementia–related deficits and gliosis in progranulin haploinsufficient mice

A. J. FILIANO, L. H. MARTENS, A. H. YOUNG, P. ZHOU, G. DIAZ-RAMIREZ, E. J. HUANG, R. V. FARESE, JR., & E. D. ROBERSON

University of Alabama at Birmingham, Birmingham, AL; Gladstone Institute of Cardiovascular Disease, San Francisco, CA;University of California, San Francisco, CA

Frontotemporal dementia (FTD) is a rapidly progressive neurodegenerative disease that leads to incapacitating changes in social and emotional behavior. It can be caused by mutations in the GRN gene, which lead to haploinsufficiency and decreased levels of the progranulin protein. Progranulin is expressed in neurons and microglia and effects on each cell type can be observed in FTD. Neuronal dysfunction is most evident in neural circuits that include regions in the frontal and insular cortices, amygdala, and striatum. Along with neuronal dysfunction, gliosis is present in the brains of FTD patients; although it is unclear what role inflammation plays in disease progression. Complete deletion of progranulin from mice (Grn–/– mice) results in FTD-related behavioral dysfunction as well as gliosis; however Grn–/– mice do not model progranulin haploinsufficiency in the human disease. In our studies we set out to investigate the effects of progranulin haploinsufficiency on neurons and microglia in Grn+/– mice. We observed a disassociation of FTD-related deficits and gliosis in Grn+/– mice; Grn+/– mice had FTD-related behavioral deficits similar to Grn–/– mice, but without an increase in gliosis. Grn+/– mice, like Grn–/– mice, spent less time investigating a novel mouse versus an inanimate object. Grn+/– mice also displayed an abnormal social phenotype in the tube test for social dominance. Further, Grn+/– and Grn–/– mice had deficits in classical fear conditioning. Unlike the behavioral deficits, Grn+/– mice did not have the gliosis present in Grn–/– mice; we did not observe increased Iba1 or GFAP expression, nor increased TNFα mRNA levels in any brain regions analyzed. However, we found decreased neuronal activation in the frontal cortex and amygdala of Grn+/– and Grn–/– mice. Our data demonstrate that gliosis is not necessary for dysfunction due to progranulin haploinsufficiency. Rather, they suggest that dysfunction of neural circuits in the frontal cortex and amygdala may be a better pathological correlate. Our data also demonstrate the viability of Grn+/– mice as a useful tool in studying the behavioral deficits in FTD.

A double staining strategy to identify noradrenergic sympathetic sprouting in Alzheimer's disease postmortem human hippocampus

A. R. NELSON & L.L. MCMAHON

Cell, Developmental and Integrative Biology, University of Alabama at Birmingham

Objective: Pathological hallmarks of Alzheimer's disease (AD) include accumulation of beta-amyloid (Aβ) and hyperphosphorylated tau together with degeneration of basal forebrain cholinergic neurons. In rats, cholinergic degeneration stimulates sprouting of noradrenergic sympathetic fibers from the superior cervical ganglia into hippocampus and cortex. Sympathetic sprouting is a consequence of NGF accumulation caused by decreased retrograde transport resulting from loss of cholinergic nerve terminals. We previously reported that coincident with adrenergic sprouting is a 14% increase in hippocampal cholinergic innervation which is linked with a recovery of M1 muscarinic receptor dependent plasticity at CA3-CA1 synapses. The benefits of sympathetic sprouting in rats prompted us to investigate the presence of noradrenergic sympathetic sprouting in human AD hippocampus first suggested by Booze et. al.,1993.

Methods: We developed a double immunohistochemical labeling strategy to distinguish between noradrenergic sympathetic axons and central noradrenergic axons from the locus coeruleus in human hippocampus. the colocalization of two antigens in formalin fixed human brain using double immunohistochemistry is challenging due to formalin and lipofuscin induced autofluorescence (AF). We used primary antibodies to the noradrenergic marker, tyrosine hydroxylase (TH), with the low affinity neurotrophin receptor, p75NTR, in hippocampus to identify noradrenergic sympathetic fibers in AD, MCI, and NCI subjects from the Joseph and Kathleen Bryan Alzheimer's Disease Research Center at Duke University . We first attempted to reduce AF using several different strategies: 0.3% sudan black in 70% EtOH; copper sulfate in ammonium acetate ($CuSO_4$); Millipore autofluorescence eliminator; or photobleaching with a custom built light box with bulbs specific to each fluorophore spectra. Next, we attempted different combinations of chromagens (Vector Labs, VIP and DAB). Finally, we tested one chromagen, Vector SG, with one fluorescent secondary (Alexa 647).

Results: Sudan black alleviates some but not all of the AF in formalin fixed, aged human brain tissue without hampering antibody sensitivity or specificity. $CuSO_4$ eliminated AF more thoroughly, but changed the staining pattern for p75NTR significantly. Millipore autofluorescence eliminator was found to be no more effective than 0.3% sudan black. The custom built light box required a 16 hour treatment to eliminate AF which significantly dereased the antigenicity of the tissue. From the chromagen combinations tried, SG with NovaRed was the best for distinguishing each color/antigen individually and with some confidence, to confirm colocalization. SG with Alexa 647 allowed for easy identification of each antigen and

colocalization. Using the aforementioned methods, we have definitively identified sympathetic sprouting in human AD, MCI and NCI hippocampus.

Conclusions: Combining Vector SG with Alexa 647 is a novel method for colocalization that should be applicable to any tissue with AF. Using this method we have definitively identified sympathetic sprouting in human AD, MCI and NCI hippocampus. We found sympathetic sprouting in subjects in the absence of Aβ plaques, suggesting that sympathetic sprouting occurs earlier in the disease process. Based on the benefits of sympathetic sprouting we have observed in rats with medial septal lesion, we propose that enhancing sympathetic sprouting in AD patients could function to slow or reverse disease progression.

Age-related macular degeneration affects local functional connectivity.

R. NENERT[1,4], L. A. ROSS1,[2], D. K. DECARLO1,[3], C. OWSLEY1,[3], M. GRAHAM1,[2], K. M. VISSCHER

[2]Psychology, [3]Opthalmology, [4]Neurobiology, [1]Univ. of Alabama At Birmingham, Birmingham, AL

Age related macular degeneration (AMD) is a pathology that accounts for about half of all vision impairment in the developed world. It results in reduced central vision with loss of visual discrimination necessary for reading, driving and recognizing faces. Here we study how long-term deprivation of central visual input influences visual processing networks.

The representation of the central retina in early visual areas (V1-3) is found at the occipital pole. The consequences of deprivation of visual input to this brain area are still a matter of debate. Some studies suggest that lesioning the retina leaves a section of cortex unused. Other studies suggest that the occipital lobe can undergo plastic changes when its inputs are removed by retinal lesions, reallocating cortical resources once used for processing central vision to processing peripheral vision.

However, certain studies have challenged this idea of functional remapping, providing evidence of an absence of plasticity in animal models and patients suffering from AMD. It is also possible that the deprivation of central visual input causes a second type of plasticity: it could affect the participation of the occipital visual cortex in higher-level cognitive function. The study of functional connectivity using resting state fMRI data may provide an opportunity to detect this aspect of plastic change.

Subjects with AMD were recruited and paired with matched controls (matched in age and level of education). All participants underwent two 6-minute resting-state fMRI scans. Preprocessing steps were optimized to remove artifactual effects from the data, especially subjects' movement in the scanner. Functional connectivity to six anatomically-defined seed regions was assessed: Left and right polar calcarine sulcus (normally representing central vision), left and right deeper calcarine sulcus (normally representing peripheral vision), left and right hand motor cortex. Subjects with AMD showed significantly weaker local functional connectivity within visual cortical regions. Our results reveal that the deprivation of central vision in people suffering from AMD affects the connectivity of brain networks involved in vision.

Epigenetic mechanisms in temporal lobe epilepsy and associated memory deficits

R. R. PARRISH, A. J. ALBERTSON, J. J. HABLITZ, K. L. MASCIA, & F. D. LUBIN

Dept. Neurobiol, Univ. Alabama Birmingham, BIRMINGHAM, AL

Epilepsy is a neurological disorder that involves recurrent unprovoked seizures as its hallmark. In humans, temporal lobe epilepsy (TLE), which often is associated with cognitive deficits, can be triggered by an insult such as brain trauma or status epilepticus (SE). Recently epigenetic mechanisms, including DNA methylation, have been shown to be critical regulators of temporal lobe synaptic plasticity, memory formation, and behavior. However, it is currently unclear whether epigenetic mechanisms such as DNA methylation in the hippocampus influence memory formation with epilepsy. Using the kainic acid (KA) model of TLE, we determined the contribution of DNA methylation mechanisms to gene transcription changes in the hippocampus at the stage at which animals developed spontaneous recurrent seizures (TLE). Mechanistically, we found that KA-induced SE triggered acute and persistent changes in two states of DNA methylation, 5-methylcytosine and 5-hydroxymethylcytosine, in the hippocampus (SE: $p < 0.05$). We also found significant changes in the gene expression pattern of DNA methylating enzymes in the hippocampus following KA-induced SE (SE: $p < 0.05$). In addition, induction of SE resulted in DNA methylation changes at the brain-derived neurotrophic factor (*BDNF*) gene, corresponding to altered mRNA levels in hippocampal regions. Functionally, we found that blockade of *de*methylating enzyme activity (DNMT1) in the hippocampus significantly decreased DNA methylation levels and reduced latency to behavioral seizure onset with increased field excitatory postsynaptic potential in hippocampal slices from post-SE animals. These results suggest that chromatin structure modifications in the form of DNA methylation may serve as a novel candidate mechanism for gene transcription events in the hippocampus after SE. Thus, we next hypothesized that aberrant DNA methylation changes in the hippocampus may contribute to memory deficits associated with epilepsy. Indeed, our data suggest that manipulation of DNA methylation levels can serve to restore proper memory formation in our experimental model of TLE. Together, these studies constitute an initial first-step towards elucidating the role of DNA methylation in aberrant memory formation in the seizure-damaged hippocampus. Unraveling the pathological role of epigenetic mechanisms in TLE will open new strategies for pharmacologic treatment of memory disorders in epilepsies.

Altera**tions of the myristoylated, alanine-rich C kinase substrate (MARCKS) in frontal cortex in Schizophrenia**

A. L. PINNER[1], V. HAROUTUNIAN[2], & J. H. MEADOR-WOODRUFF[1]

[1]Dept. of Psychiatry, Univ. Alabama at Birmingham, BIRMINGHAM, AL; [2]Dept. of Psychiatry, Mount Sinai Sch. of Med., New York, NY

Schizophrenia is a devastating psychiatric illness with an etiology still unknown. Dysfunction at the synaptic membrane resulting in *N*-methyl-D-aspartate receptor (NMDAR) hypofunction has been implicated in the pathophysiology of schizophrenia, but the exact mechanism remains unclear. Protein modifications are essential for neuronal synaptic plasticity and stable neurotransmission; the addition of a hydrophobic group is required for membrane binding. Myristoylation promotes phospholipid membrane binding with the addition of the 14-carbon fatty acid, myristate, onto the N-terminal glycine residue, catalyzed by N-myristoyltransferase (NMT); the reaction can occur both co- and post-translationally. Target proteins of myristoylation are critical components of subcellular localization and protein-protein interactions; the reversible membrane interaction with its hydrophobic myristoyl tail is ideal for signaling, function, and further modifications. One such myristoylated protein with reversible membrane interaction is MARCKS, which has been suggested to have a role in mood disorders. MARCKS is highly expressed in the brain and plays a crucial role in dendritic morphology during neurogenesis by regulating changes to the cortical actin cytoskeleton. Decreased dendritic morphology has been found in several brain illnesses, including schizophrenia. The release of phosphorylated MARCKS into the cytoplasm allows for rearrangement and plasticity of the actin cytoskeleton. Phosphorylation of MARCKS is a biomarker of Protein Kinase C (PKC) activity and evidence suggests that MARCKS may mediate PKC-dependent dendritic spine plasticity. PKC activation promotes postsynaptic trafficking and insertion of functional NMDARs. Disruption of myristoylation may contribute to the pathology of schizophrenia; alterations in MARCKS could help explain the mechanism of NMDAR hypofunction through changes in dendritic morphology and function. In the present study, total protein expression of MARCKS and its phosphorylated form were measured in two cortical regions, dorsolateral prefrontal cortex (DLPFC) and superior temporal gyrus (STG) from postmortem schizophrenia patients (n=16) and a comparison group (n=20). The protein levels of the two isoforms of the enzyme NMT were also measured. Protein levels of both total and phosphorylated MARCKS were found to be significantly decreased in schizophrenia in DLPFC, but not in STG; NMT expression was unchanged. These new findings of decreased MARCKS in schizophrenia DLPFC could have implications related to both decreased dendritic morphology and NMDAR hypofunction associated with this illness.

Mitochondrial impairments in the hippocampus of *Mecp2* mutant mice

K. RICART[1]; G. CALFA[2]; L. POZZO-MILLER[2]; & A. LANDAR[1]

[1]Dept. Pathology, [2]Dept. Neurobio, University of Alabama at Birmingham

Mitochondria are not only necessary for ATP synthesis, but also essential for intracellular Ca^{2+} homeostasis and redox signaling cascades. Mitochondrial morphology is dynamic and controlled by balanced fission and fusion events, which are critical to proper mitochondrial function. Rett syndrome is an X-linked neurological disorder that almost exclusively affects girls, being the leading cause of several intellectual disabilities (1:10,000 births). The symptoms include irregularities in motor activity, including gait and motor imbalance and a stereotypic hand movement, altered breathing patterns, continued cognitive decline, and seizures. Intriguingly, some of these RTT features are also present in various mitochondrial disorders. The perception that mitochondrial abnormalities contribute to RTT predates the discovery of mutations in *MECP2* as its primary causative factor. Previous studies in *Mecp2* mutant mice showed abnormal respiration and impaired activity of mitochondrial complexes of the respiratory chain, further supporting the hypothesis that mitochondrial dysfunction plays a role in RTT. Here, we performed a quantitative analysis of mitochondrial density and morphology at the electron microscopy level in dendrites and axons within hippocampal CA1 *stratum radiatum* of symptomatic *Mecp2* mice (P50-60, "Jaenish" strain) and age-matched wildtype (wt) littermates (3 mice per genotype). The number of mitochondria in a total sampled area of 10,000μm^2 is not significantly different between genotypes (wt 5±0.4 vs. *Mecp2* 4±0.4 mitochondria per 10μm^2; $p>0.05$). On the other hand, the circularity index of individual mitochondria is significantly different, with more circular mitochondria (index=1) in *Mecp2* mutants (wt 0.789±0.0002 n=4,213 vs. *Mecp2* 0.833±0.0019 n=4,555; $p>0.05$, K-S test). Consistently, the maximum dimension (wt 0.392±0.004µm vs. *Mecp2* 0.375±0.003µm; $p<0.0001$) and perimeter of individual mitochondria (wt 1.248±0.011µm vs. *Mecp2* 1.209±0.0009µm; $p<0.00001$) were also significantly different (K-S test). These differences in mitochondrial ultrastructure may be due to changes in mitochondrial fusion and fission, which in turn may affect their motility and bioenergetic function. Experiments under way will determine the consequences of *Mecp2* deletion on all these mitochondrial parameters. The observed differences in mitochondrial ultrastructure may be related to heightened neuronal activity, as observed in the hyperexcitable hippocampal network of symptomatic *Mecp2* mutant mice (Calfa et al. *J Neurophysiol* 2011).

Abnormalities of the Ubiquitin-Proteasome System in the Superior Temporal Gyrus in Schizophrenia

M. D. RUBIO[1], V. HAROUTUNIAN[2], & J. H. MEADOR-WOODRUFF[1]

[1]Department of Psychiatry and Behavioral Neurobiology, Universityof Alabama at Birmingham School of Medicine, Birmingham, Alabama 35294, USA.
[2]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Background: Schizophrenia is a complex psychiatric disorder, whose underlying patophysiology is still unknown. Diverse post- and co-translational modifications, such as palmitoylation, myristoylation, SUMOylation and ubiquitination, are thought to underlie protein expression and trafficking abnormalities seen in this illness. At the mRNA level, several groups have reported abnormalities of the ubiquitin-proteasome system in different areas of the brain. These findings, in addition to the differences in the expression level of specific proteins consistently found schizophrenia, led us to hypothesize that some protein expression abnormalities seen in this illness are a consequence of abnormal protein degradation driven by the ubiquitin-proteasome system.

Methods: We studied protein expression of the ubiquitin-proteasome system by performing Western blot analysis of the superior temporal gyrus (STG) in paired subjects with schizophrenia or a matched control. We measured overall protein ubiquitination, overall K63 and K48 linked ubiquitination and free ubiquitin. Based on previous peripheral blood mRNA findings, we also studied the expression of E1 activases, E2 conjugases and E3 ligases.

Results: Our results show an overall reduction in protein ubiquitination accompanied by a decrease in free ubiquitin in schizophrenia. Although overall K63 and K48 linked ubiquitination was not modified, individual band analysis yielded differences at specific molecular weights in schizophrenia. We also found a decrease in E1 activases UBA3, UBA6, MOCS3, ATG7 and NAE1 and E3 ligases Nedd4 and USP2 in this group.

Discussion: This study of the ubiquitin-proteasome pathway showed abnormalities in ubiquitination in the STG in schizophrenia postmortem brains. These findings are likely to be the result of defective ubiquitin activation and subsequent ligation, given the decrease in the expression of E1 activases and E3 ligases. The defects in the ubiquitin-proteasome pathway we report may underlie of abnormal protein expression in schizophrenia.

Impaired coupling of glycolytic enzymes and characterization of the EAAT2 interactome in schizophrenia

SHAN D., MOUNT D., MOORE S., HAROUTUNIAN V., MEADOR-WOODRUFF J.H., **&** MCCULLUMSMITH R.E.

Dept. of Psychiatry, University of Alabama at Birmingham

Excitatory amino acid transporter 2 (EAAT2) belongs to a family of sodium-dependent glutamate transporters that maintain low synaptic concentration of glutamate by removing glutamate from the synaptic cleft into astroglia and neurons. Efficient reuptake of glutamate by EAAT2 relies on sodium and potassium gradients generated principally by Na^+/K^+ ATPase and energy intermediates (ATP) that drive glial glutamate reuptake. Hexokinase 1(HK1), an initial enzyme of glycolysis, binds to mitochondrial outer membrane where it couples cytosolic glycolysis to mitochondrial oxidative phosphorylation, producing ATP utilized by the EAAT2/ Na^+/K^+ ATPase complex to facilitate glutamate reuptake. In this study, we hypothesized that EAAT2 doesn't work independently but works cooperatively with Na^+/K^+ ATPase and HK1 in a large multiprotein complex; breakdown of this complex may lead to abnormal glutamate transmission, contributing the pathophysiology of schizophrenia. Thus, first, we determined the association of EAAT2 with Na^+/K^+ ATPase and HK1 proteins in human prefrontal cortex. EAAT2 was found to colocalize with Na^+/K^+ ATPase and HK1 by immunofluorescence double-labeling. Colocalization of EAAT2 with Na^+/K^+ ATPase and HK1 was also confirmed by mass spectrometry. Second, we performed western blot analysis to examine expression of EAAT2, Na^+/K^+ ATPase a1, b1, hexokinase 1 and the mitochondrial matrix protein, ubiquinol-cytochrome *c* reductase core protein 2 (UQCRC2) in the dorsolateral prefrontal cortex in subjects with schizophrenia compared to a comparison group. We didn't find changes in regional level expression of these proteins in the dorsolateral prefrontal cortex in schizophrenia. Next, we assessed the localization of EAAT2 isoforms using subcellular fractionization and Western blot analysis in the dorsolateral prefrontal cortex. We found an increase in the EAAT2B isoform of EAAT2 in a fraction containing extrasynaptic membranes in subjects with schizophrenia. Finally, an increased ratio of HK1 protein in the cytosolic fraction / mitochondrial fraction was found in the dorsolateral prefrontal cortex in subjects with schizophrenia. Increased expression of EAAT2B isoform and HK1 detachment from the mitochondrial outer membrane suggest that the integrity of the EAAT2/mitochondrial complex may be disrupted, leading to decreased perisynaptic buffering and reuptake of glutamate and impaired energy metabolism in this illness.

Abnormal gene expression in pyramidal neurons within specific layers of acc in schizophrenia

M. S. SIMMONS[1], S. D. YATES[1], V. HAROUTUNIAN[2], R. MCCULLUMSMITH[1], & J. H. MEADOR-WOODRUFF[1]

[1]PSYCH-BEHAVIORAL NEUROBIOGY, UAB, Birmingham, AL; [2]Psychiatry, The Mt. Sinai Sch. of Med., New York, NY

Schizophrenia (SZ) is a debilitating psychiatric illness with a not well understood pathophysiology. For decades, studies in postmortem brain have been published yet robust and reproducible findings have remained elusive. Recently developed tools permit the study of gene expression in defined cellular subpopulations which may result in more specific studies in this illness. In this study, laser capture microdissection (LCM) was used to harvest pyramidal neurons from superficial (II-III) and deep (V-VI) layers of anterior cingulate cortex (ACC) from 12 pairs of subjects with schizophrenia and a matched control (Ctrl) using Affymetrix GeneChip® microarrays. Gene expression profiles were compared between and within SZ and Ctrl in superficial and deep pyramidal neurons. Genes that were significantly altered in each analysis were functionally categorized using Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7. Receptor signaling, sensory transduction, catabolic process, and cell regulation were among the highest scoring functional categories for genes that were changed between SZ and Ctrl in deep and superficial pyramidal neurons from different layers. In addition to identifying gene expression changes between SZ and Ctrl within layers of ACC, differences between superficial and deep pyramidal neurons in normal subjects were also observed. Genes involved with phosphorylation, acetylation, non-membrane bound organelles, and cytosol were expressed differently between cells of the superficial and deep layers. To our knowledge this is the first study to identify differences in gene expression between pyramidal neurons in superficial and deep layers of ACC. These data also suggest novel pathways and potential targets disrupted in specific neuronal subpopulation in the ACC in SZ.

HCN Channel Modulation of Synchronous GABAergic Network Activity in Rat Neocortex

S. B. WILLIAMS & J. J. HABLITZ

University of Alabama at Birmingham, Neurobiology Department

GABAergic interneurons provide the main source of inhibition in the neocortex and are highly important in regulating neocortical network activity through the modulation of synaptic integration, control spike timing, and synchronization of network activity. In the presence of 4-aminopyridine (4-AP) and the glutamate receptor blockers CNQX and APV, evoked and spontaneous depolarizing responses (GABA waves) are observed in the neocortex. Such responses are thought to be mediated by $GABA_A$ receptors and to arise from synchronized activity in GABAergic interneurons. Variable expression of HCN channel mediated I_h currents has been seen in GABAergic interneurons. The role of HCN channels in modulating synchronized GABAergic interneuron activity has not been determined. We hypothesized that I_h inhibition would enhance depolarizing synchronous GABA responses in neocortical neurons. Whole-cell current clamp recordings were obtained from L5 pyramidal neurons in neocortical slices from PN 20-25 rats. A bipolar stimulating electrode was placed in L5. At the resting membrane potential (– 67.4 ± 0.64 mV, n = 6), stimulation evoked depolarizing responses with superimposed action potentials. Such responses reversed near the expected Cl^- equilibrium potential and were blocked by 10 μM gabazine. Bath application of the HCN channel antagonist ZD-7288 (20 μM) produced an 296% increase in area under the curve (n= 6, $p < 0.01$) and 128% increase in duration ($p < 0.05$) of evoked depolarizing responses, whereas the reversal potential was not significantly changed. Spontaneous depolarizing events were increased in frequency (0.007 ± 0.001 Hz in control vs. 0.013 ± 0.003 Hz in ZD-7288); $p = 0.01$, n=8) in the presence of ZD-7288. Under voltage clamp conditions to prevent HCN channel activation, I_h inhibition still produced a prolongation indicating that there was an increased synaptic input. These results indicate that HCN channels modulate synchronous network activity in neocortical GABAergic interneurons.

Partial Tau Reduction Does Not Induce Neuronal Dysfunction in Aged Mice

Z. LI, A. E. HALL, & E. D. ROBERSON

Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics, UAB

The microtubule-associated protein tau is emerging as an attractive target for Alzheimer disease. Tau reduction prevents Alzheimer-disease-like behavioral deficits and confers resistance to excitotoxin without causing major neuronal dysfunction in young mice. However, tau reduction is recently reported to induce Parkinsonism and dementia in aged mice. While in clinical situation tau targeting agents will only affect tau partially and all tau-related disorders happen in aged population, it is then of paramount importance to know whether partial tau reduction in aged mice causes any abnormalities and whether tau reduction is also effective in aged brain. Here we examined a broad spectrum of behavior, gross brain anatomy, dopamine homeostasis and sensitivity to excitotoxin-induced seizure of aged $Tau^{+/-}$ and $Tau^{-/-}$ mice. Consistent with previous report, $Tau^{-/-}$ mice exhibited some behavior abnormalities and reduced brain size. On the other hand, $Tau^{+/-}$ mice didn't exhibit any abnormalities in all behavior tests we conducted, and also no abnormalities in gross brain anatomy and dopamine homeostasis. Similar to finds in young mice, both aged $Tau^{+/-}$ and $Tau^{-/-}$ mice exhibited greater resistance to excitotoxin-induced seizure. In summary, we conclude that partial tau reduction does not impair behavior functioning of aged mice but retains efficacy against excitotoxin. Tau reduction is a viable and effective strategy for Alzheimer disease treatment.

Evelyn F. McKnight Brain Institute
The University of Arizona

Director: Carol A. Barnes, Ph.D.

Cognitive effects of surgical menopause in the rat: a cross sectional evaluation of the impact of ovariectomy from young adulthood to old age

J. I. ACOSTA, E. B. ENGLER-CHIURAZZI, J. S. TALBOOM, & H. A. BIMONTE-NELSON

Arizona State Univ., TEMPE, AZ

There is evidence in the rodent literature that the cognitive effects of surgical hormone loss vary depending on age. For example, ovariectomy (Ovx) in young female rats was detrimental to spatial working memory performance (Bimonte and Denenberg, 1999), while a separate study found that Ovx in aged female rats facilitated spatial working memory performance (Bimonte-Nelson et al., 2003). However, the effects of Ovx at multiple timepoints during aging have not yet been methodically addressed in one study. Here, we assessed the effects of Ovx at several different ages ranging from young adulthood to old age. The design of the current study was based on our previous findings, which led to the current hypothesis that the cognitive effects of Ovx would transition from beneficial to detrimental with age. We used a cross-sectional, between-subjects design to test spatial working and reference memory on the water radial arm maze (WRAM), and spatial reference memory on the Morris water maze, in ovary-intact Sham or Ovx rats at 5, 12, 18, or 20 months of age. At the end of behavioral testing, levels of LH, FSH, 17β-estradiol, and progesterone were measured in serum. Results demonstrated that for the WRAM, 12 month old Ovx animals made more working memory errors relative to Sham animals as trials progressed and working memory load increased. However, at 18 months of age, the Ovx effect was reversed, with 18 month old Ovx animals making fewer working memory errors on two orthogonal measures relative to 18 month old Sham animals. Collectively, for working memory the data support a transition of Ovx from detrimental at 12 months of age, to beneficial at 18 months of age. There were agerelated decrements in performance on each task, with a distinct pattern of age-related changes for the WRAM versus Morris water maze. The patterns of learning and memory alterations with age, and with surgical menopause, will be discussed. Further, the hormone changes across age for LH, FSH, 17β-estradiol, and progesterone will be discussed in the context of the behavior results.

Functional compensation in response to increasing task difficulty: Comparing semantic and episodic memory tasks in young and older adults

E. BAENA, & L. RYAN

Department of Psychology; Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Previous fMRI studies have suggested that older adults engage bilateral frontal regions to a greater degree than young adults during memory tests. This increase in bilateral activation has been described as a form of "functional compensation" and may reflect an effort to maintain performance in the face of increasing cognitive load or difficulty. Whether or not the compensation response is specific to older adults or represents a more general response of any individual to increasing task difficulty is unclear. The present fMRI study compared patterns of brain activation in young and older adults while performing two memory tasks - episodic and semantic - as task difficulty increased. In the semantic task, participants judged whether pairs of words were either synonyms or antonyms. In the episodic task that followed, participants made yes/no recognition judgments for the word pairs previously presented. Difficulty was manipulated with word frequency. Young (ages 18-24) and older healthy adults (ages 60-83) were scanned on a 3T GE magnet using a single-shot spiral pulse sequence. Behavioral results showed a double dissociation - older adults were adversely affected by word frequency in the episodic but not the semantic task, while young adults were adversely affected by word frequency in the semantic but not the episodic task. fMRI activation showed linear increases in bilateral frontal and parietal regions as a function of increasing task difficulty in the older adults, for both tasks. Increases in left inferior frontal gyri was observed only among the young adults during the semantic task as difficulty increased. The results suggest that both young and older adults may demonstrate functional or strategic compensatory processes with fMRI in the face of increasing task difficulty. In particular, young adults show increased activation only for the task that they have difficulty with -the hard version of the semantic/vocabulary task- paralleling their behavioral results. But older adults show increases in activation for both tasks, regardless of whether or not they show behavioral decreases. This suggests that young adults show a compensation response that is more task-network related, while older adults show a fronto-parietal compensation network that is task-independent.

The effects of age and environmental change on Arc transcription in perirhinal cortical ensembles following object exploration

S. N. BURKE, A. L. HARTZELL, J. P. LISTER, L. T. HOANG, & C. A. BARNES

Evelyn F. McKnight Brain Institute and ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Object recognition memory requires the perirhinal cortex (PRC) and this cognitive function declines during normal aging. Specifically, old animals are able to retain the memory of a familiar object but they are more likely to falsely recognize a novel stimulus (Burke et al., 2010). This 'false memory' for novel stimuli observed in aged rats also occurs when the environmental context is changed between the familiarization and test phase of the spontaneous object recognition task. Recent electrophysiological recordings from awake-behaving rats have shown that neurons in the PRC of young rats are activated by 3-dimensional objects (Burke et al., 2012). Thus, it is possible that age-related object recognition deficits could be due to alterations in how PRC neuron activity is modulated by objects in older animals. The present study used cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) with confocal microscopy to monitor subcellular distributions of activity-induced *Arc* RNA in the PRC. Activity was monitored during two distinct epochs of object exploration. In one group of rats (6 young and 6 aged) an animal was placed in a familiar testing arena and was allowed to explore five different 3-dimensional objects for two 5-min sessions separated by a 20-min rest (AA condition). The second group of animals (6 young and 6 aged) explored the same five 3-dimensional objects for two 5-min sessions but the environment was changed between the first and the second epoch of exploration (AB). Behavioral data showed that both young and aged rats spent less time exploring objects during the second epoch of behavior in both conditions, indicating successful object recognition. Interestingly, the reduction in object exploration during the second epoch relative to the first was not associated with reduction of cells that expressed *Arc* between epochs. This indicates that similar populations of neurons are activated by exploring the same objects during two distinct epochs, even when the objects are familiar and the environment changes. When numbers of *Arc* positive neurons were compared between age groups, the old rats had significantly lower proportions of *Arc*-positive PRC neurons for both the AA and AB behavioral conditions. The agerelated reduction in the population of PRC neurons that express *Arc* during object exploration could reflect reduced glutamate levels in aged compared to young rats that have been reported for this brain region (Rushaidhi et al., 2012). Together these data support the hypothesis that age-associated functional alterations in the PRC contribute to declines in stimulus recognition over the lifespan.

An fMRI study of age-related differences in complex object discrimination

J. A. CARDOZA[1], J. WALLENTIN-FLORES[1], F. E. POWERS[1], W. ARNOLD[1], M. BARENSE[3], L. RYAN[1,2]

[1]Department of Psychology; [2]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ; [3]Rotman Research Institute, University of Toronto, Toronto, ON, Canada

Previous perirhinal cortex lesion studies have shown reduced object discrimination in rats, monkeys and human patients with lesions to perirhinal regions. Recent experiments have shown that aged rats are also impaired on object discrimination when those objects have overlapping features, but no studies to date have addressed this issue in aged humans. To investigate the influence of age on complex object discrimination, we used an object matching paradigm utilizing blob-like objects with varying levels of overlapping features. Older and younger adults were asked to indicate if two blobs simultaneously presented on the screen matched in all features. Difficulty was manipulated by varying the number of overlapping features within the pair. fMRI data was acquired to determine the involvement of the perirhinal cortex in both groups. Behavioral data showed that while some older adults scored similarly to young adults on all tasks, a subgroup of older adults were specifically impaired on the difficult object matching task relative to young adults. fMRI activation was observed in perirhinal cortex during the difficult object matching task for younger adults and high performing older adults, but not the impaired older adults. These results suggest that complex object discrimination is impaired in some, but not all older adults, and may depend upon the degree to which they can functionally engage the perirhinal cortex.

The role of DNA methylation and hydroxymethylation in aging and Alzheimer's disease

D. L. VAN DEN HOVE[1,2], L. CHOULIARAS[1], G. KENIS[1], J. VAN OS[1], H. W. STEINBUSCH[1], D. MASTROENI[3], P. D. COLEMAN[3], & B. P. RUTTEN[1]

[1]Dept. of Psychiatry and Neuropsychology, Maastricht University, Maastricht, Netherlands; [2]Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany; [3]L.J. Roberts Center for Alzheimer's Research, Sun Health. Research Institute, Sun City, AZ

Aging of the brain has been associated with aberrant DNA methylation patterns, while a global loss of methylation has been observed in the entorhinal cortex of Alzheimer's disease (AD) patients. Meanwhile, DNA hydroxymethylation is sparsely investigated in aging and AD. We have recently reported that the levels of the DNA methylation marker 5-methylcytidine (5-mC) and the DNA hydroxymethylation marker 5-hydroxymethylcytosine (5-hmC) were increased in the mouse hippocampus during aging from 12 to 24 months, while caloric restriction was able to prevent these observed age-related changes. The aim of the present study was to further investigate hippocampal 5-mC and 5-hmC immunoreactivity in relation to aging as well as in AD pathology. For that purpose, we analyzed 5-mC and 5-hmC IR in the hippocampus of wild-type and transgenic APPswe/PS1dE9 mice at 3- and 9-months of age as well as in AD patients and carefully-matched controls. While age-related increases in levels of both 5-mC and 5-hmC were found in wildtype mice, APPswe/PS1dE9 mice showed decreased levels of 5-mC and no age-related changes in 5-hmC. On the other hand, both 5-mC and 5-hmC were decreased in the human AD hippocampus while negative correlations between DNA (hydroxy)methylation markers and Aβ plaque and neurofibrillary tangle load were observed.

Altogether, these findings suggest that ageing and AD-related pathology in the mouse and human hippocampus are associated with epigenetic alterations in DNA methylation and hydroxymethylation.

Mapping the spatial navigation network of young and aged rhesus macaque monkeys: A positron emission tomography study

J. R. ENGLE[1,3], C. J. MACHADO[3], A. P. MAURER[1,2], M. PERMENTER[3], J. VOGT[3], & C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Institute, [2]ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ; [3]California National Primate Research Center, Davis, CA

Activation of the neural substrates devoted to spatial navigation is largely dependent upon behavioral and environmental cues. Much of our current understanding of how such cues engage these neural substrates comes from studies of freely moving rodents, as it is technically more difficult to study this problem in the nonhuman primate brain. To gain a better understanding of nonhuman primate spatial navigation networks and how they change with age, four rhesus monkeys were trained to freely or passively traverse a long enclosure, and to walk on a treadmill. We measured cerebral glucose metabolism with the radiotracer 2-deoxy-2[18F]-fluoro-D-glucose (FDG) and high-resolution positron emission tomography (microPET). Two of the four monkeys have been successfully scanned after the four conditions given in pseudorandom order: 1) freely traversing the enclosure, 2) passive traversals in the same enclosure not excluding optic flow, 3) walking on a treadmill without optic flow, 4) sitting in a cage (control condition). We used ROI analysis to assess the contributions of behavioral state and age on spatial navigation network activity. Our preliminary results from two monkeys (12 and 27 years old) suggest that ROIs in the right hemisphere overall had a significantly greater uptake of FDG than ROIs in the left hemisphere (t-test, $p < .001$). The magnitude of the percent change across the behavior conditions, however, interacted with age. The difference in FDG uptake compared to the control condition was more pronounced when the young monkey was considered separately, although movement behavior was matched across age. This interaction was found in the right medial temporal lobe, area TE, anterior cingulate, retrosplenial and medial occipitoparietal area ROIs. These preliminary results suggest that the right hemisphere of the rhesus monkey may contribute to spatial navigation, and may undergo similar age-related changes in the spatial navigation network that has been reported in aged rodents.

Regional brain network of MRI gray matter with gradual induction of hypertension in the Cyp1a1-Ren2 transgenic rat

M. C. FITZHUGH[1,2], J. W. TOTENHAGEN[2,3], E. S. YOSHIMARU[4], A. RICHARDS[2,5], L. T. HOANG[2,5], A. N. ALLEN[7], M. TURK[7], J. KRATE[7], L. A. BIWER[8], T. M. HALE[8], K. CHEN[9], J. R. MOELLER[10], P. D. COLEMAN[11], K. D. MITCHELL[12], M. J. HUENTELMAN[2,7], C. A. BARNES[2,3,5], T. P. TROUARD[2,4], & G. E. ALEXANDER[1,2,6]

[1]Physiological Sciences Graduate Interdisciplinary Program, [2]Evelyn F. McKnight Brain Institute, [3]Department of Psychology, [4]Biomedical Engineering, [5]ARL Division of Neural Systems, Memory & Aging; [6]Department of Psychology, University of Arizona, Tucson, AZ ; [7]Neurogenomics Division, The Translational Genomics Research Institute, Phoenix, AZ; [8]Department of Basic Medical Science, University of Arizona, Colloge of Medicine, Phoenix, AZ; [9]Samaritan PET Center, Banner Alzheimer's Institute, Phoenix, AZ; [10]Department of Psychiatry, Columbia University, New York, NY; [11]Banner Sun Health Research Institute, Sun City, AZ; [12]Department of Physiology, Tulane University, School of Medicine, New Orleans, LA

It is well established that hypertension (HTN) in humans can lead to regional brain atrophy and cognitive decline. With a high prevalence in the community-dwelling elderly population, HTN may be an important factor influencing the development and progression of cognitive aging. We sought to investigate the effects of HTN on gray matter volume from magnetic resonance imaging (MRI) scans of the brain obtained in a rodent model of HTN. Since the onset and progression of HTN in humans often occurs gradually after middle-age, we used transgenic rats that allow for the gradual induction of HTN in middle-aged animals (Mitchell et al., 2006). The inbred male rats, with a Fischer 344 background, have the cytochrome P450 promoter (Cyp1a1) inserted to up-regulate the expression of the mouse renin (Ren2) gene. Administration of 0.15% indole-3-carbinol (I3C) activates the Cyp1a1 promoter to induce a gradual onset of HTN. We administered an I3C augmented diet at 16 months of age over a 6-week interval to produce a HTN group of Cyp1a1-Ren2 rats (N = 5), whereas the control group of transgenic rats (N = 5) received normal food pellets. Volumetric T2-weighted MRI scans were acquired at 7.0 T with 150-micron isotropic voxel resolution for both the HTN and control groups. Multivariate network analysis with voxel-based morphometry (VBM) and the Scaled Subprofile Model (SSM; Alexander and Moeller, 1994) was used to identify a regional network pattern of MRI gray matter that differed between the groups. The HTN group had higher mean systolic ($p < 0.009$) and diastolic ($p < 0.016$) blood pressures during the 6-week interval than controls. Additionally, when analyzed as a proportion of body weight, heart and kidney weights were increased for the HTN group compared to controls ($p < 0.009$). SSM analysis of MRI VBM with bootstrap re-sampling of the HTN and control groups combined identified two regional network patterns of gray matter that distinguished the hypertensive rats from controls ($p < 0.009$). This linearly combined pattern was characterized by gray matter reductions in the vicinity of the thalamus, basal ganglia, cerebellum, and a region of the hippocampus with relative increases

observed in selective frontal and temporal areas. Together, these findings provide preliminary support for the use of the Cyp1a1-Ren2 rat to help advance translational research on the cerebrovascular effects of HTN during aging. The use of such transgenic rodent models combined with high resolution MRI and network analyses may aid efforts in the evaluation of new treatments and prevention therapies for the brain changes associated with healthy and pathological aging.

The influence of advanced age on noradrenergic-dependent novelty detection

J. FRIEL, J. J. FERNG, A. L. HARTZELL, S. N. BURKE, & C. A. BARNES

Evelyn F. McKnight Brain Institute, ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Previous research has shown that age-associated object recognition deficits are not due to aged rats 'forgetting' a previously experienced stimulus, but because old animals are more likely to identify novel objects as familiar (Burke et al., 2010). The extent that this impairment can be explained by object discrimination problems, or a problem in novelty detection has not been examined. Previous studies have suggested that a functionally intact noradrenergic system is necessary to facilitate novelty detection (Sara et al., 1995). The main source of noradrenaline in the brain, the locus coeruleus (LC), shows several age-associated changes including, altered activity levels (Olphe & Steinmann, 1982; Shirokawa et al., 2000), differences in gene expression (Zhu et al., 2005), and changes in its metabolic pathways (Shirokawa et al., 2003; Matsunaga et la., 2004). The current experiment tested the hypothesis that these alterations in the LC may partially account for the inability of aged rats to correctly respond to novel objects using a task that is known to require noradrenergic activity (Sara et al., 1995). Young (9 months) and old (24 months) rats were placed in a hole board apparatus for a habituation and novel-object detection phase separated by a 3-hour period. The 9 holes of the apparatus were left empty for the habituation phase. In contrast, during the novelty-detection phase the holes were filled with 4 identical objects. Novelty detection was quantified by comparing the number and duration of nose pokes in holes with objects relative to the holes without objects. Because rats were not required to discriminate between novel and familiar objects, this task allows one to measure the ability of animals to detect novelty in the absence of stimulus discrimination requirements. Contrary to the idea that age-related changes in the LC contribute to impairments in object recognition, there was no significant difference in the performance of young and old animals on this task. These data suggest that the decline in the ability of aged rats to appropriately identify novel objects is due to a reduced capacity to discriminate between novel and familiar stimuli, which critically relies on the perirhinal cortex, rather than to an overall decline in LC-dependent novelty detection.

Effect of aging on the activity pattern of Arc expression in the deep layers of lateral entorhinal cortex

J. P. LISTER, S. J. CLASEN, A. L. HARTZELL, S. N. BURKE, & C. A. BARNES

Evelyn F. McKnight Brain Institute, ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Age-related changes in hippocampal function have been studied extensively, but less is known about the effect of aging on input from entorhinal cortex. Neurons of lateral entorhinal cortex (LEC) lack spatial specificity in their firing properties (e.g., Hargreaves at al., 2005) and are thought to provide the hippocampus with information about the contents of the environment. Data from earlier experiments indicate that neither age nor changing environmental features altered the proportion of cells with *Arc* activation in superficial layers of LEC (Lister et al., SfN 2011). These data were not consistent with the suggestion that object identity information was carried by LEC cells in this layer, leaving open the possibility that perhaps cells in the deep layers may do so. Here we report results from deep layers of LEC in 24 young and 24 old F344 rats. There were four groups: 1) caged control, 2) positive control with electroconvulsive shock, inducing maximal expression of the immediate early gene *Arc*, 3) an A/A behavioral group that explored for two 5 min sessions in a box populated with the same set of 5 objects during both sessions, and 4) an A/B group, identical to A/A with entirely dissimilar objects during the second session compared to the first. Behavior rats had a 20 minute rest period between sessions and were sacrificed immediately after the second session. This design tests the hypothesis that the identity of objects will be critical to individual neuron activity and that this function will be affected by aging. The kinetics of *Arc* mRNA within discrete intracellular compartments (nucleus or cytoplasm) allows each *Arc*+ neuron to be identified as active within a specific temporal window, including the ability to identify cells active in both sessions. 3 sections per brain from posterior LEC were stained for *Arc* expression via fluorescence *in situ* hybridization and imaged using confocal microscopy. *Arc*+ neurons were classified as foci-positive (Foci+), cytoplasmicpositive (Cyto+), and double-labeled (Dbl+) using the ImageJ software package. Repeated measures ANOVA showed that there were no significant differences in age or object change treatment (AB) factors. About a third of the cells counted in this layer were active in response to the behavioral treatment overall, and half of these were active in both epochs of behavior, regardless of object identity. The proportion of neurons active in both sessions (Dbl+) was significantly greater than either Foci+ or Cyto+ positive neurons alone. Thus, while half of the responsive cells do show stable activity across behavioral treatment sessions, these cells do not appear to carry information about distinct object identities.

Aging does not affect the proportion of dorsal medial entorhinal cortex cells active during track running behavior

J. LIANG, J. P. LISTER, & C. A. BARNES

Evelyn F. McKnight Brain Institute, ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Previous work has shown that hippocampal place cells in aged rats show changes in their population dynamics: in CA1 there are errors in retrieving the correct map for a known location (e.g., Barnes et al., 1997), and in CA3 they exhibit difficulty in establishing a new map for a novel location (Wilson et al., 2005). The entorhinal cortex provides input to the hippocampus and could contribute to these observed age-related changes in the population code for space. The medial entorhinal cortex (MEC) contains "grid cells" that fire in a highly spatially regular fashion (e.g., Hafting et al., 2005). These cells fire in repeating patterns across an entire environment and are believed to contribute significantly to the spatial signal within downstream hippocampal regions. The current experiment investigated whether aging affects population activity in MEC during track running, by quantifying expression of the immediate early gene *Arc* using the "catFISH" method (Guzowski et al., 1999). The rigid kinetics of *Arc* localization to distinct cellular compartments allows this gene to mark neuronal activity during discrete behavioral epochs. Young (9 months) and old (24 months) Fischer 344 male rats (N = 24 at each age) were divided into three groups: caged control (CC), maximal electroconvulsive shock control (MECS), and track running behavior. Rats learned to run alternating clockwise and counterclockwise laps on a circular track (48 inch diameter) for food reward. For the experiment, CC rats were sacrificed immediately from home cages, and the MECS group was sacrificed following a shock that activates Arc expression in all neurons capable of expressing the gene. Rats in the running group performed two 5 min sessions of track running, separated by a 20 min rest period in their home cage. Immediately after the second session, animals were sacrificed. After sacrifice, brains were flash-frozen to preserve mRNA, sectioned on a cryostat, stained for *Arc* expression with fluorescence *in situ* hybridization, and the dorsal MEC was examined in each rat (3 sections per brain). Neurons were quantified as nuclear positive (Nuc+), cytoplasmic positive (Cyto+), or double-labeled (Dbl+). Repeated measures ANOVA revealed a significant ($p<0.01$) effect of the *Arc*-compartment factor - there was a significantly higher proportion of Dbl+ neurons than Nuc+ or Cyto+ in both age groups. The age factor was not significant, indicating that the ability of MEC to reactivate the same populations during the two sessions of track running in the same environment is not affected by age. This suggests that at least the cellular composition of the path integration input to the hippocampus is preserved during aging.

Diminished place field density and direction-dependent learning in the aged rat

L. A. SCHIMANSKI, P. LIPA, & C. A. BARNES

Evelyn F. McKnight Brain Institute, ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Remembering the locations of specific stimuli within an environment becomes more difficult during senescence. The changes in neural information processing underlying this age deficit are not yet fully understood. In this study, the simultaneous activity of ensembles of hippocampal CA1 principal neurons ('place cells') was recorded from 6 young adult (9-12 month) and 6 aged (26-28 month) male F344 rats, while they learned the locations of eye-blink stimuli on a circular track. Rats were trained to run alternating clockwise and counterclockwise laps on this circular track with a barrier at 12 o'clock, where they received food reward. Using electrical stimuli applied to the eyelid, the animals were conditioned to blink at two distinct locations on the track: one eye-blink stimulus location was conditioned in only the clockwise running direction while the other one was only administered in the counterclockwise direction. As reported by Schimanski et al. (2011), young and old rats exhibited equivalent accuracy of the blink response at both conditioned locations, but old rats were less successful in restricting the blink response to the conditioned running *direction*. Here we examine place field firing properties of young and old pyramidal cells under these conditions in more detail. When the number of place fields identified per CA1 pyramidal cell was measured in both running directions, the aged rats exhibited fewer place fields per cell than did young rats. These data suggest a subtle difference between age groups in how the CA1 'map' or population code represents the same environmental experience. Moreover, when we applied a random sampling algorithm to the place field data (Monte Carlo method), the outcome showed that the number of bidirectional fields observed in young and old rats was close to what one would expect from 'accidental' overlap of firing in opposite direction trajectories, given the situation that each unidirectional field was randomly positioned to occur in either the clockwise or counterclockwise direction. Taken together, these findings imply that because the number of CA1 place fields per cell is reduced during aging, the number of bidirectionally-active place fields also must decline accordingly. These agedependent alterations of the population code may contribute to the reduced ability of aged rats to discriminate one running direction from another in learning this locationand direction-dependent version of eye-blink conditioning.

Gray matter volume in the orbital prefrontal cortex correlates with reinforce devaluation but not reversal learning performance in bonnet macaques

K. PLANGE[1,2], S. N. BURKE[1,2], A. THOME[1,2], J. R. ENGLE[1,2], T.P. TROUARD[3], K. M. GOTHARD[4], & C. A. BARNES[1,2]

[1]Evelyn F. McKnight Brain Institute, [2]ARL Division of Neural Systems, Memory & Aging, [3]Department of Physics, [4]Department of Physiology and College of Medicine, University of Arizona, Tucson, AZ

Behaviors that rely on the prefrontal cortex are particularly vulnerable to the process of normal aging, but the neurobiological changes that occur over the lifespan in the different subregions of this brain structure are not completely understood. In the current experiment, young (n = 7) and aged (n = 9) bonnet macaques were trained on reversal learning and reinforcer devaluation tasks using a Wisconsin General Testing Apparatus. The reinforcer devaluation task tests the degree to which response selection can be guided by reward value, by pairing two different food rewards (food 1 and food 2) with distinct sets of objects. Monkeys learn this association over weeks and are then satiated on food 1. Successful performance occurs when the animal selects the objects associated with the non-satiated food 2. In contrast, reversal learning tests an animal's ability to learn that a previously rewarded stimulus is no longer associated with a reward on an object discrimination task. Data from lesion studies suggest that the orbital prefrontal cortex (OFC) is necessary for successful performance on both of these tasks (e.g., Gallagher et al., 1999; Bohn et al., 2003). To assess the extent that OFC structural integrity predicts performance on these two tasks, anatomical MRIs were obtained from 7 young and 6 aged monkeys that had completed both behavioral conditions. Boundaries of the OFC were manually determined by three independent observers blind to the age and behavioral performance of the animal (high inter-rater reliability was obtained for all comparisons, $r[12] > 0.7$, $p < 0.01$). Although as a group the aged monkeys performed significantly worse than did young animals on both the reinforce devaluation ($T[14] = 2.07$, $p < 0.05$) and the reversal learning tasks ($T[14] = 3.80$, $p < 0.01$), there was no significant difference in total OFC volume between age groups ($T[12] = 0.88$, $p = 0.15$). While there was no significant correlation between OFC volume and reversal learning performance ($r[12] = -0.22$, $p = 0.46$), a significant correlation was found between OFC volume and performance on the reinforce devaluation task ($R[12] = 0.61$, $p < 0.05$). Together these data suggest that OFC grey matter volume is associated with an animal's ability to guide its behavior based on predicted reward value, but not to reverse a previously learned association. Because reinforcer devaluation performance requires functional connectivity between the amygdala and the OFC (Baxter et al., 2000), these data suggest that alterations in structural integrity of the OFC may be involved in defective communication between these two brain structures, which disrupts an animal's ability to use reward value to guide behavior.

A role for kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the regulation of estrous cycles and the estrogen modulation of body temperature

S. J. KRAJEWSKI-HALL[1], M. A. MITTELMAN-SMITH[1], H. WILLIAMS[1], K. J. LAFRANCE[1], N. T. MCMULLEN[2], & *N. E. RANCE[1,3]

[1]Pathology, [2]Cellular and Molecular Medicine University of Arizona College of Medicine; [3]Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

We have recently described a method to selectively ablate kisspeptin/neurokinin B/dynorphin (KNDy) neurons using stereotaxic injections of NK3-SAP, a neurokinin 3 receptor agonist conjugated to saporin (Mittelman-Smith, Endocrinology, 2012). These studies revealed a critical role for arcuate KNDy neurons in tonic gonadotropin secretion, the rise in serum LH after ovariectomy and estrogen modulation of body weight. Here we determine the effects of KNDy neuron ablation on estrous cycles and the estradiol modulation of body temperature. In the first study, stereotaxic injections of NK3-SAP or Blank-SAP were made in the arcuate nucleus of ovary-intact, adult female rats. Rats with nearly complete KNDy-neuron ablation (verified by NKB immunohistochemistry) exhibited constant diestrus and ovarian atrophy, confirming the importance of these neurons in reproductive regulation. In a second experiment, we evaluated the effects of KNDy neuron ablation on the thermoregulatory axis in rats that were ovariectomized (OVX) and then treated with 17β-estradiol (E2). Tail skin temperatures (TSKIN) and core temperatures (TCORE) were recorded in rats throughout the light/dark cycle and during exposure to different ambient temperatures (TAMBIENT) in an environmental chamber. Notably, the average TSKIN of KNDy-ablated rats was consistently lower than control rats, indicative of lower levels of cutaneous vasodilatation. Moreover, KNDy neuron ablation blocked the reduction of TSKIN by E2 that occurred during the light phase in the environmental chamber, but did not affect the E2 suppression of TSKIN during the dark phase. At a high TAMBIENT of 33 C, the mean TCORE of OVX control rats increased to 39.0 C, and was reduced by E2 replacement. In contrast, at this high TAMBIENT, the average TCORE of OVX, KNDy-ablated rats was lower than OVX control rats, and TCORE was not altered by E2 replacement. Because KNDy neurons exhibit dramatic changes in morphology and gene expression in postmenopausal women, we have hypothesized these neurons contribute to the generation of hot flushes. These studies support this hypothesis by providing the first evidence that KNDy neurons participate in the E2 modulation of body temperature and promote cutaneous vasodilatation, one of the cardinal signs of a hot flush.

Changing characteristics of neural stem cells across the lifespan during aging

K. SMITH[1], C. A. BARNES[1,2], M. J. CORENBLUM[1], & L. MADHAVAN[1,2]

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

In the context of understanding plasticity in the brain during aging, we have begun to characterize the properties of neural stem cells (NSCs) obtained from the subventricular zones of Fisher 344 rats in vitro over an aging continuum of 2 (adolescent), 9 (mature), 15 (middle-aged) and 24 (old) months. Characterization in this manner provides a comprehensive view of the population as it changes throughout a lifetime rather than comparing only the young and old endpoints. To begin with, significantly greater numbers of NSCs were isolated from the more younger (2, 9 months) than the more older (15, 24 months) animals. The isolated adolescent, mature, middle-aged and old NSCs differed in size and morphology although being immunophenotypically similar with no difference in the expression of NSC markers, Nestin or Sox2. The proliferative capacity of the NSCs was next determined by quantifying sphere formation using a serial dilution assay as well as incorporation of the thymidine analog bromodeoxyuridine (BrdU). The dilution assay indicated that adolescent and mature younger cells formed significantly greater number of neurospheres than the middle aged and old cells at every dilution level. Concurrent with this, a significant decline in the fraction of BrdU labeled cells was observed with increasing age. Interestingly, in both the dilution and BrdU assays, NSCs isolated from the middle-aged (15 months) animals showed greater impairment in proliferative ability compared to the cells from old (24 months) animals. In support of the proliferation data, survival of NSCs measured using a live/dead cell assay did not show any significant differences between adolescent, mature and old age groups, but the middle-aged cells displayed a considerably greater fraction of dead cells. Further, adolescent, mature, and old cells were found to be capable of being propagated in culture for a greater number (>15) of passages than middle-aged cells (<10). NSC differentiation was also examined. While the number of RIP+ oligodendrocytes remained fairly constant, TUJ1+ neurons declined, and S100B+ astrocytes increased with age. Overall, the results indicate (1) increased senescence, (2) reduced survival, proliferation, and neuronal differentiation of NSCs as aging progresses, and (3) suggests a particular vulnerability of cells during middle-age which needs to be analyzed further. These ongoing studies will help establish baseline changes in characteristics of NSCs throughout the natural lifespan, and provide a framework for further investigations of underlying factors, as well NSC potential to modify the aged brain.

Senescence modifies the structure of information encoding in the medial temporal lobe

A. THOME[1,2], P. LIPA[1,2], C. A. ERICKSON[1], & C. A. BARNES[1,2]

[1]Evelyn F. McKnight Brain Institute, [2]ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ

Primates are remarkably adept at categorizing and remembering visual objects. The ability for organisms to discriminate and recall previously encountered information is critically dependent on the integrity of the medial temporal lobe (MTL) and inferior temporal cortex (ITC) (e.g., Mahut et al., 1982; Zola; Morgan et al., 1994; Buffalo et al., 2000). Lesions of these structures produce profound deficits on a variety of recognition memory and perceptual discrimination tasks. Data across a number of species indicate that these processes are similarly disrupted in senescence (e.g., Moss et al., 1988; Burke et al., 2011). However, the neural mechanisms underlying age-related deficits are poorly understood.

Information processing in the ventral visual stream is believed to proceed along a hierarchy of association, in which the responses in one region reflect the conjunctions of stimulus features represented discretely in upstream cortical areas (e.g., Barlow, 1961; Mishkin et al., 1983). Single-unit recording studies in primates have demonstrated that neurons in these regions represent information via population codes (e.g., Tanaka et al., 1991; Gross, 1992; Logothetis et al., 1995). However, the stability of a population code rests on the reliability of the responses of individual neurons. One theory of cognitive aging proposes that deficits in perceptual discrimination may be tied to deficits in sensory processing (e.g., Baltes and Lindenberger, 1997; Li and Lindenberger, 2002). Selective degradation of sensory information in lower level sensory areas may result in instability of information representation in higher level association areas. A number of theoretical and empirical findings now indicate that behavioral accuracy may rely on the stability of these responses.

To test this hypothesis we recorded the activity of 1633 units from 2 old and 3 young chronically implanted rhesus macaques (*macaca mulatta*) during two different visual tasks. A variety of analytical techniques were used to examine the tuning characteristics on individual neurons, as well as the underlying signal to noise ratio. These data show that in aged animals there exists a significant decrease in quality of information encoding across the hippocampus (CA3/CA1) as well as upstream cortical structures (EC/PRC/TF). These results provide not only validation of a number of theoretical proposals and behavioral results, but further open new avenues for the development of cognitive and therapeutic approaches.