Wake Forest Graduate School
Neuroscience Program
&
Society for Neuroscience
Western North Carolina Chapter

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Abstracts

WAKE FOREST UNIVERSITY
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PROGRAM IN NEUROSCIENCE

Western North Carolina
SOCIETY FOR NEUROSCIENCE
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1. **Variations in Response Gain in Frontal Cortex Linked to Variability in Saccadic Reaction Time**

CK Hauser, D Zhu, TR Stanford, and E Salinas

We revisited a fundamental question in oculomotor neuroscience: how does single-neuron activity in the frontal eye field (FEF) relate to the timing of eye movements? To investigate the neural correlates of choice, reward availability, reaction time (RT), and movement metrics, monkeys performed a RT variant of the one-direction-rewarded (1DR) task. In each trial, animals maintained fixation at a central spot and made a saccade when an eccentric stimulus appeared at one of 4 possible locations, but crucially, only one location was associated with the primary reinforcer. Behavioral effects were clear: saccades to rewarded locations were precise and of short latency, whereas those to unrewarded locations had longer latencies and highly variable metrics. We exploited the large spread in RT and spatially distinct reward conditions in the 1DR task to study how individual FEF neurons contribute to saccade production. This exposed a novel, strong dependency: for most neurons, the maximum firing level either increased or decreased monotonically as a function of RT. This was true for all neuronal classes in FEF regardless of their visuomotor properties. Furthermore, modeling results suggest that the two complementary populations with similar response fields but opposite temporal selectivities serve a distinct purpose, to control, according to their relative gain, whether the ensuing RT is short or long. These findings are significant for two reasons. First, saccades are thought to be triggered when the firing level in FEF reaches a fixed threshold, but according to our results, this is true only in an average sense; for individual cells, the presaccadic firing rate attained may vary substantially with RT, either positively or negatively. Second, the results pinpoint a fundamental source of variability in RTs — fluctuations in the gain of fast- and slow-preferring complementary populations — that may signal a specific mechanism for regulating the timing of motor commands.

2. **Positive emotions enhance protective effects of distraction coping on repeated stress**

Elaine Z. Shing and Christian E. Waugh

Although distraction has traditionally been categorized as a maladaptive coping strategy, recent experimental evidence suggests otherwise. Many of these studies, however, focus on neutral distraction strategies (e.g. thinking of geographical locations), despite the fact that people tend to employ positive distraction strategies (i.e. engaging in enjoyable activities to distract oneself) in everyday life. The present study examined the effects of positive distraction on individuals' affect levels during a repeated social stressor (mental arithmetic). Participants completed two stressors with a positive or neutral distraction task in between. Although both types of distraction led to decreases in negative affect during the distraction period, only those in the positive distraction group adapted to the second stressor - reporting significant reductions in negative affect from stressor 1 to stressor 2. These results provide support for the protective effects of positive distraction on repeated stressors.

3. **Interactions Between the eIF2α and mTOR Signalling Pathways**

Zimmermann, HR, Yang WJ, Zhou X, and Ma T.

Maintenance of long-lasting synaptic plasticity and long-term memory requires de novo protein synthesis. Two signalling pathways have been shown to play important roles in synaptic plasticity, learning, and memory: the eukaryotic initiation factor 2α (eIF2α), and the mammalian target of rapamycin complex1 (mTORC1) pathways. The mTORC1 pathway is regulated by Akt and AMP-Activated Protein Kinase (AMPK), Tuberous Sclerosis 2 (TSC2), among others, and directly regulates 4E-Binding Protein 1 (4E-BP1) , p70S6 Kinase (p70S6K), and regulates eukaryotic elongation factor 1A (eEF1A) levels. Whether there exists interactions between these two pathways in neuronal systems, is unknown. Taking advantage of genetic and pharmacological approaches, here we investigated in mouse the effects of repressing eIF2α kinases, GCN2 and PERK, effects on mTORC1 signaling. We found (1): Genetic deletion of eIF2α kinase PERK in mice yielded increased phosphorylation of 4E-BP1, and genetic deletion of GCN2 yielded increased phosphorylation of p70S6K. (2) Genetic deletion of eIF2α kinase PERK in mice yielded decreased phosphorylation of Akt and AMPK, and genetic deletion of GCN2 yielded increased phosphorylation in the same upstream regulators of mTORC1. (3-4) Both genetic models yielded decreased phosphorylation of TSC2, and increased levels eEF1A. (5) In contrast, incubation of hippocampal slices with PERK Inhibitor, GSK 2606414, did not impact the components of the mTORC1 pathway, but did increase eEF1A levels. (6) Further, hippocampal long-term potentiation (LTP) failure induced by selective mTORC1 inhibitor rapamycin is reversed in the GCN2 knockout mouse. Our results indicate the mTORC1 and eIF2α pathways clearly interact in neurons and are more closely interwoven than previously thought. Our study provides evidence of an additional layer of complexity to the mechanisms of synaptic plasticity and learning in memory formation.

4. **Anatomical plasticity in spinal terminals and functional release of oxytocin after peripheral nerve injury.**

AL Severino, CA Aschenbrenner, and JC Eisenach

Recovery from injury-induced pain involves many mechanisms, including increased inhibition or decreased sensitization. This study focuses on spinal oxytocin (OT) signaling after peripheral nerve injury. Spinal OT fibers originating in the paraventricular nucleus of the hypothalamus terminate in the superficial laminae of the spinal dorsal horn (SDH), where OT excites inhibitory interneurons that modulate the ascending nociceptive drive.

Preliminary work suggests that the density of OT fibers in the SDH increases in sub-acute pain states, but whether this occurs after a chronic peripheral nerve injury has not be investigated. To test this, we performed partial L5 spinal nerve ligation (pSNL), and quantified OT immunoreactivity (IR) in spinal regions ipsilateral (IPSI) and contralateral (CONTRA) to nerve injury. We randomly assigned male, Sprague-Dawley rats at 7W age to pSNL surgery or not and quantitatively assessed spinal OT IR in animals at 9W and 19W (n=16/group). OT fiber density was greater in the IPSI lumbar SDH in the pSNL compared to normal groups ($P=0.012$), but not in the CONTRA SDH ($P=0.343$). This effect was regionally constrained to the lumbar region of the spinal cord. These data suggest an increased capacity for OT release in the lumbar spinal level, where afferents injured with pSNL terminate.

We further hypothesized that since spinal OT IR is increased after injury, the capacity for basal and noxious-stimulus evoked OT release would also increase. We performed spinal microdialysis in the IPSI lumbar SDH of 64 male rats in 4 groups to assess the effect of injury and noxious-stimulation (n=16/group). Baseline OT concentration did not differ among groups ($P>0.05$), and peripheral noxious stimulation did not evoke spinal release of OT in any group ($P>0.05$). These data suggest that OT anatomical plasticity occurs after injury, but there is no significant increase in spontaneous or evoked OT release in the paradigm tested.

5. **STRESS AND ALCOHOL ABSTINENCE IN DAILY SOCIAL DRINKERS: EFFECTS ON DEFAULT MODE NETWORK COMMUNITY STRUCTURE**

Mayhugh RE, Petrie MR, Rejeski WJ Lyday RG, Burdette JH, Laurienti PJ

Social alcohol consumption is generally considered non-problematic and is commonly observed in everyday life. This study evaluated the effect of alcohol abstinence and stress on functional connectivity during guided mental imagery tasks in social drinkers. The focus was put on community structure and identifying brain regions that exhibited dense connectivity with each other and sparse connectivity with other brain regions. Emphasis was on community structure surrounding brain regions previously shown to be part of the Default Mode Network (DMN), which have been linked to internally driven, self-generated thought. Examining DMN community structure in daily moderate-heavy social drinkers will help in understanding how alcohol abstinence and stress affect brain function in non-dependent drinkers.

Fourteen daily non-binge drinkers between the ages of 24–60 years old participated in this study. Moderate to heavy alcohol consumption was defined as 1-3 drinks per day (females) or 2-4 drinks per day (males). Using an established mental imagery technique, interviews were conducted to prepare personalized scripts based on emotionally neutral and stressful past experiences. These scripts were then recorded and played back to each participant while they underwent a functional magnetic resonance imaging (fMRI) scan after (a) 3 days of social drinking and (b) 3 days of abstinence. This study used a randomized within-subject design in which each individual participated in four conditions (1) Abstained Neutral (no alcohol, emotionally neutral memory), (2) Normal Neutral (typical pattern drinking, emotionally neutral memory), (3) Abstained Stress (no alcohol, emotionally stressful memory) and (4) Normal Stress (typical pattern drinking, emotionally stressful memory). fMRI data were then modeled as a voxel-wise network using the principles of graph theory. Community structure surrounding DMN brain regions was measured using modularity and scaled inclusivity (SI) was used to determine the consistency of the DMN community across individuals in each condition.

SI analyses revealed varying levels of consistency in areas commonly associated with the DMN (ex: Prefrontal Cortex, Precuneus/Posterior Cingulate) in each condition. There was a trend of decreased average SI values during stress compared to neutral and higher average SI values in abstinence compared to normal within the same emotion state (Neutral vs Stress). Average SI values were significantly ($p < 0.05$) higher in the Abstained Neutral group than in the Abstained Stress and Normal Stress groups.

The consistency of the DMN community across subjects varies by condition during internal self-generated thought states (imagery) in these drinkers. Overall, DMN community consistency was lower when individuals were stressed and higher when individuals had been abstinent. These results may point to an abstinence effect that influences the way in which the brain processes internal thoughts. An influence on whole brain functioning is apparent as DMN connectivity may allow for the emergence of different, possibly more adaptive, sub networks. Additional data is needed to further investigate this trend.

These results help to expand our understanding of the influence of stress and the importance of alcohol pattern maintenance to this population. This work will extend research focused on identifying brain phenotypes for those using alcohol to cope with stress or who are prone to future dependence.

6. **Saccade metrics reflect decision-making dynamics during urgent choices**

Joshua Seideman, Emilio Salinas, and Terrence R. Stanford

In our everyday lives we are constantly moving and because of this we are frequently faced with a choice: where next? While often quite trivial, there are certainly times when the choice is not so clear and we wish we could be in (or move toward) multiple locations at once. In situations such as these, we typically seek additional information until either we are certain of our choice, or, under urgent circumstances, we feel forced to decide anyway in the midst of conflict. Do subtle aspects of the movements that ensue reflect the level of internal conflict present during these decisions?

Previous investigations have shown that competition between target and distractor impacts where a saccadic eye movement is directed and the trajectory it takes. Here, we investigated the temporal impact of perceptual information not on where a saccade is made, but how it is executed. Once a saccade is initiated in a particular direction, does its kinematics reflect the level of information from which the movement decision was based? We investigated these questions by titrating the temporal availability of visual information critical for success above chance on a unique two alternative forced choice task. Surprisingly, although saccadic eye movements are typically believed to be stereotyped and ballistic in their nature, we find that saccade velocity, amplitude and precision are heavily dependent on the availability of the sensory information that is relevant for guiding a perceptual decision.

7. **Sex Specific Differences In Key Signaling Pathways Involved In Sympathetic Nervous System Control Within The Dorsal Medulla Of Adult Sheep With Fetal Betamethasone Exposure**

Alexa S. Hendricks, Hossam A. Shaltout, Mark C. Chappell, Debra I. Diz

Glucocorticoids including betamethasone (BM) are a widely accepted and routine therapy administered to women at risk of early preterm labor to facilitate fetal lung development and reduce infant mortality rates. However, fetal steroid exposure may lead to negative long term consequences for autonomic regulation. In a sheep model of fetal programming, BM-exposed (BMX) adult offspring exhibit elevated mean arterial pressure (MAP), decreased baroreflex sensitivity (BRS) for control of heart rate and insulin resistance accompanied by dysregulation of the brain, renal and circulating renin-angiotensin system (RAS). In the brain solitary tract nucleus of the dorsal medulla, BMX dysregulation there is a shift towards the sympathetic activator angiotensin II (Ang II) actions through the AT1 receptor that oppose the beneficial actions of angiotensin-(1-7) [Ang-(1-7)] at the Mas receptor for BRS regulation. Therefore, we examined RAS and insulin/leptin signaling a pathways to understand further the molecular disturbances that lead to the observed alterations in sympathetic outflow in male and female adult sheep (n = 16 sheep; 4 each control or BMX, male or female). Using a protein array for kinases (PathScan® Intracellular Signaling Array Kit, Cell Signaling) and subsequent verification by Western blot hybridization, female BMX sheep exhibit lower expression of proteins in the PI3 kinase pathway (pGSK3β –33% ±.1023, pAkt –22% ±.333; p = 0.03) related to regulation of metabolic function and blood pressure. In contrast, in male BMX sheep the PI3 kinase pathway components are not different from control sheep, but there is a trend (p = 0.11) for higher expression of the phosphorylated MAP kinase pathway protein ERK (37%) and p38 (68%), known to increase sympathetic activation and facilitate insulin actions. Insulin Growth Factor I (IGF-I) is reported to be low in insulin resistance. Estradiol and IGF-I interact synergistically in the brain to activate (phosphorylate) Akt. The results observed prompt us to investigate the role estrogen plays, since female sheep exhibit greater insulin resistance than males. We will explore whether lower IGF I in dorsal medulla of female sheep explains the observed dramatic decrease in the PI3 kinase pathway. We conclude that in utero BMX promotes an imbalance in adult brain medullary RAS and insulin signaling pathways known to influence sympathetic regulation with different patterns observed in male and female sheep.

8. **ALDH1A2 is a candidate biomarker for M2-type macrophages in glioblastoma.**

Amanda Carr, Denise Herpai, Yue Huang, Darren Seals, Ryan Mott, Lance Miller, and Waldemar Debinski

Within the glioblastoma (GBM) microenvironment, tumor-associated macrophages (TAM) are abundant, and they play an active role in tumor progression/maintenance. Thus, TAM represent attractive therapeutic targets. TAM are found across a spectrum of polarization states ranging from proinflammatory M1 type to immunosuppressive/pro-tumor progression M2 type. To address the lack of biomarkers which specifically distinguish these populations within tumors, we analyzed gene expression in a THP1 human monocyte model: naïve THP1 (M), or THP1 activated with phorbol 12-myristate 13-acetate (PMA; M0), PMA/Lipopolysaccharide/Interferon gamma (M1), or PMA/IL-4/IL-13 (M2a) using human 219 Gene Atlas Array Strips (Affymetrix). Gene expression of each condition was filtered against that of G48a GBM cells in order to identify factors that are specific to monocytic cells and are differentially regulated according to polarization state. One of these, the gene encoding retinoic acid metabolizing enzyme ALDH1A2, showed a 4.7/4.6/4.8 log fold increase in polarized M2a compared to M, M0, and M1 conditioned cells, respectively. This was validated by western blot in which ALDH1A2 is up-regulated 3-fold in the M2a cells when compared to M THP1 cells. Next, ALDH1A2 was readily detected in 10 out of 10 human GBM specimens, but in only 2 GBM cell lines out of 10, thus demonstrating the enzyme's weak immunoreactivity in western blots lacking macrophage populations. Protein levels were also increased in GBM vs. nontumor brain tissue. Further, sub-confluent cultures of naïve THP1 cells in conditioned media from primary GBM cell lines showed significant up-regulation of ALDH1A2. Initial studies showed a greater than 4 fold increase in protein levels as compared to naïve THP1 cultures. Importantly, ALDH1A2 expression by immunohistochemistry was found to be primarily in macrophage-like cells within human GBM tumors. These data support ALDH1A2 as a novel biomarker for the M2a phenotype of TAM in GBM.

9. **Attentional modulation of frontal eye field neurons during urgent decisions**

Veronica E. Scerra, M. Gabriela Costello, Emilio Salinas, and Terrence R. Stanford

The study of visual target selection in the frontal eye field (FEF) focuses on the roles of three characteristic cell types: visual (V), which fire when sensory stimuli fall in the response field, motor (M), which fire leading up to a saccade into the response field, and visuomotor (VM), which respond to both stimulus and motor action. In tasks previously used to study the activity of FEF neurons, visually-responsive cells "select" the target, presumably informing the motor choice that is to be carried out by cells with saccade-related activity. However, in a recent study we found that visual cells fail to resolve target locations ahead of correctly informed saccades in a two-choice urgent decision paradigm, the Compelled-Saccade (CS) task. Crucially, the CS task differs from a traditionally-used oddball task in two fundamental ways: (1) an urgency requirement precludes strict seriality of perceptual decision and motor choice, and (2) a target that is not uniquely salient (i.e., it does not "pop-out"). To explore the relative impact of temporal and attentional demands on the expression of FEF target selection activity, monkeys were trained to perform the two-choice CS task and both the standard oddball task and an urgent version, the Compelled-Oddball (CO) task, while we recorded the activity of V, M, and VM cells. Our findings indicate that the expression of both target- and response-selection correlates within V and M cells in FEF is highly dependent on the attentional demands of the task.

10. **Optogenetic Insights into Ethanol Effects on Presynaptic Dopamine Terminal Dynamics**

J.R. Melchior and S.R. Jones

Dopamine (DA) signaling in the nucleus accumbens has been implicated in reward learning and motivated behavior. All drugs of abuse, including ethanol, have been shown to alter DA levels in the nucleus accumbens via various mechanisms, suggesting that effects on DA signaling may underlie addictive behaviors. While ethanol is known to increase DA cell firing and subsequently increase DA levels in the nucleus accumbens, direct ethanol targets on DA cells remains elusive, prompting hypotheses that ethanol actions on DA signaling occur indirectly via modulation of non-DAergic afferent inputs. Previously, we have demonstrated that ethanol decreases stimulated DA release in *ex vivo* nucleus accumbens slices. Specifically, 10 pulse stimulation trains are inhibited by moderate ethanol doses, an effect that is blocked in the presence of the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine. Cholinergic interneurons in the nucleus accumbens provide excitatory input onto presynaptic DA terminals, and 10 pulse stimulations may be sufficient to activate these neurons, making it difficult to determine whether ethanol effects are directly on DA terminals or on local interneurons. We have attempted to address this question using optogenetics. We injected the ventral tegmental area (VTA) of mice with a virus encoding expression of channelrhodopsin-2 (ChR2), and allowed 4 weeks to incubate in order to achieve expression of ChR2 in the terminals of VTA projection neurons, including DA neurons. We made slices of nucleus accumbens and stimulated DA release either electrically or optically. We found that optically stimulated release after 10 pulse stimulation trains resulted in greater DA release, compared to electrically stimulating the tissue. Further, we found that light stimulations were insensitive to the nAChR antagonist mecamylamine, while electrical stimulations were significantly inhibited, suggesting that optical excitation of the tissue does not evoke acetylcholine release. In order to assess whether ethanol effects on DA release were direct or indirect, we measured ethanol effects on DA release using 10 pulse stimulations with each stimulation method. We found that optically stimulated DA release was similarly sensitive to ethanol inhibition compared to electrical stimulation. These results suggest that ethanol has targets directly on DA terminals that are independent of local cholinergic interneuron activity.

11. **Cerebrospinal Fluid and Plasma Ceramides are Modified by Dietary Intervention in Adults with Mild Cognitive Impairment and Healthy Controls**

B Neth, JL Bayer-Carter, A Hanson, LD Baker, KS Nair, and S Craft

Sphingolipid-derived ceramides play an important role in cell signaling and disease pathology. Serum ceramides are higher in insulin resistance, Type 2 Diabetes and other metabolic conditions, and are modulated by diet in rodent studies (Haus et al., 2009; Turpin et al., 2014). Moreover, recent studies have described elevated blood ceramide concentrations in adults with Alzheimer's disease (AD) and other neurologic conditions (Mielke et al., 2012; Filippov et al., 2012). Interestingly, several cerebrospinal fluid (CSF) ceramides are lower in adults with Mild Cognitive Impairment (MCI) and AD and correlate with AD biomarkers (Fonteh et al., 2015). Modification of ceramide levels through diet and other metabolic manipulations may reveal therapeutic targets to alleviate AD pathology.
Data from 49 participants were used in this analysis. Twenty participants (13F, 7M) were cognitively normal (CN) with a mean [SD] age of 69.3 [7.4] and 29 participants (13F, 16M) were adults with amnestic MCI with a mean [SD] age of 67.6 [6.8]. Participants were randomized to one of two equicaloric dietary interventions (High Saturated Fat/Glycemic Index – "High Diet" & Low Saturated Fat/Glycemic Index – "Low Diet") for 4 weeks. All meals were supplied during the study period. Blood (plasma) and CSF samples were collected prior to starting diet (week 0) and ending diet (week 4). CSF and plasma ceramide concentrations were measured with Liquid Chromatography–Mass Spectrometry, and subjected to analysis of covariance adjusting for age, gender, and APOE-e4 status.
In CSF, adults with MCI showed decreased concentrations of C16:0 ($p<0.05$) and C22:0 ($p<0.05$) on the Low relative to the High diet. The ceramide C18:0 trended toward significance ($p=0.08$). Conversely, concentrations of C16:0 ($p<0.05$) for CN were increased on the Low relative to the High diet. In plasma, concentrations of 5 ceramides (C14:0 ($p<0.0001$), C16:0 ($p<0.0001$), C18:0 ($p<0.0001$), C20:0($p<0.0005$), C24:1($p<0.001$)) decreased significantly after consumption of the Low diet relative to the High, with exception to C24:1, which showed an opposite pattern.
Our results demonstrate both plasma and CSF ceramides are modified after a 4-week dietary intervention in cognitively normal adults, as well as in adults with amnestic MCI. Further study is needed to fully understand the role of ceramides in AD and whether dietary intervention is an effective therapeutic or preventative approach.

12. **Underestimating Superadditivity in Multisensory Integration**

Benjamin A. Rowland, Alexander Dakos, Terrence R. Stanford, and Barry E. Stein

Multisensory neurons in the superior colliculus (SC) integrate concordant signals derived from different sensory modalities to produce enhanced responses. The quantitative framework traditionally used for reckoning the products of such multisensory integration is based on changes in the average number of impulses elicited from a neuron on a given trial. According to this simple quantification, superadditive computations (i.e., a multisensory response greater than the sum of the component unisensory responses) are only found in a minority of samples, most frequently those in which the sum of the averaged unisensory responses is less than 5 impulses. Based on summing activity over a protracted stimulus epoch, this “rule of thumb” implies that superadditive computations are represented only on the lower tail of a distribution of multisensory products, and found only when neurons are responding near their threshold. The present study questions the veracity of this inference. Two important factors must be considered in this context: 1) the moment-by-moment computations yielding the integrated multisensory products change during a neuron’s response window, and 2) interspersed with neurons that are overtly responsive to each sensory input (the typical multisensory neuron used in most studies) are many "covert" neurons which exhibit multisensory integration, but do not respond overtly to one of the component sensory inputs. To examine the impact of these two factors on multisensory integration, we evaluated the moment-by-moment products of integrating visual and auditory signals in both single neurons and multi-neuron recordings in the SC of both awake and anesthetized animals. The data show that superadditive enhancement is an exceedingly common computation in the early response window of both preparations (~100ms after stimulus onset), a period that includes the response onsets and peaks for both sensory inputs. It is rare for it not to be present. This early enhanced multisensory response proved not only to be more potent, but to be statistically more reliable. Furthermore, due to the presence of covert neurons, results from most single neuron analyses significantly underestimate the potency of multisensory enhancement within the local circuit, and do so by a multiplicative factor. Thus the emergent picture of SC multisensory integration is one in which a dynamic process produces a wave of enhancement to the downstream neuron that is dominated by a leading edge of superadditivity.

13. **Distinguishing cognitive state with multifractal complexity of hippocampal interspike interval sequences**

D. Fetterhoff, R. A. Kraft, R. A. Sandler, I. Opris, C. A. Sexton, V.Z. Marmarelis, S. A. Deadwyler, R. E. Hampson

Fractality, represented as self-similar repeating patterns, is ubiquitous in nature and the brain. Dynamic patterns of hippocampal spike trains are known to exhibit multifractal properties during working memory processing; however, it is unclear whether the multifractal properties inherent to hippocampal spike trains reflect active cognitive processing. To examine this possibility, hippocampal neuronal ensembles were recorded from rats before, during and after a spatial working memory task following administration of tetrahydrocannabinol (THC), a memory-impairing component of cannabis. Multifractal detrended fluctuation analysis was performed on hippocampal interspike interval sequences to determine characteristics of monofractal long-range temporal correlations (LRTCs), quantified by the Hurst exponent, and the magnitude of multifractal complexity, quantified by the width of the singularity spectrum. Our results demonstrate that multifractal firing patterns of hippocampal spike trains are a marker of functional memory processing, as they are more complex during the working memory task and significantly reduced following administration of memory impairing THC doses. Conversely, LRTCs are largest during resting state recordings, therefore reflecting different information compared to multifractality. In order to deepen conceptual understanding of multifractal complexity and LRTCs, these measures were compared to traditional methods of frequency content and firing variability measures. These results showed that LRTCs, multifractality, and theta rhythm represent independent processes, while delta rhythm correlated with multifractality. To investigate the heightened multifractality during the task, temporal variations in local Hölder exponents were extracted during memory encoding (sample) and memory retrieval (nonmatch) task events. Additionally, we used coefficient of variation and CV2 to explain the relationship between multifractality, variability and delta rhythm. Taken together, these results provide a novel perspective on memory function by demonstrating that the multifractal nature of spike trains reflect hippocampal microcircuit computation that can be used to detect and quantify cognitive, physiological, and pathological states.

14. **^{1}H-Magnetic Resonance Spectroscopy Reveals Elevation of MyoInositol and other Markers of Inflammation in the Dorsal Medulla of Children with Orthostatic Intolerance**

Ashley L. Wagoner, John D. Olson, John E. Fortunato, M.D., Debra I. Diz, PhD., Hossam A. Shaltout, PhD.

Children with orthostatic intolerance (OI) have exaggerated decreases in heart rate variability (HRV) and suppression of baroreflex sensitivity (BRS) with standing. Inflammation is proposed as a possible factor contributing to impaired HRV in cardiometabolic disorders; whether systemic or brain inflammation better predicts impaired HRV is arguable. We used ^{1}H magnetic resonance spectroscopy (MRS) to quantify markers of neuronal and glial integrity in children with OI compared with asymptomatic controls. Fifteen subjects ages 10-18 years were evaluated for blood pressure, HR and autonomic function in supine and upright positions and 7 tested positive for OI. An average of 2 weeks following OI testing all subjects underwent ^{1}H-MRS scans of dorsal medulla on a clinical 3T magnet while supine. OI subjects had higher myoinositol (mIns) as a marker of glial inflammation than asymptomatic controls (7.8 ± 0.4 vs 5.6 ± 0.9 mmol/L, p = 0.03). Trends were observed for higher glycerophosphocholine (higher GPC, reduced myelination and axonal integrity) (2.3 ± 0.2 vs 1.8 ± 0.2 mmol/L, p = 0.08) and lower N-acetyl aspartate (lower NAA, reduced neuronal integrity) (2.8 ± 0.3 vs. 3.7 ± 0.4 mmol/L, p = 0.1) in OI subjects vs controls (mean ± SEM). mIns concentrations did not correlate with indices of autonomic function measured in the supine position. However, supine measures of mIns correlated with autonomic measures taken in the upright position: negatively correlated with spontaneous BRS (R = -0.64, p = 0.01), parasympathetic tone measured by high frequency alpha index (HFα, R= -0.547, p=0.04) and HRV measured by root of mean square of successive differences (rMSSD, R = -0.45, p = 0.09); there was a positive correlation with HR (R = 0.53, p < 0.05). In summary, children with OI have higher mIns in dorsal medulla while supine that is predictive of impairment in BRS, HRV and parasympathetic tone upon upright posture. This first report that OI in children is associated with elevated mIns, a marker of glial inflammation in a variety of neuropathies, raises the intriguing possibility that brain inflammation plays a role in the autonomic dysfunction observed while standing in these subjects.

15. **Neural Responses in Macaque Superior Colliculus During Urgent Decision-Making Tasks**

CK Hauser, EE Rogers, TR Stanford, and E Salinas

The way in which the brain makes decisions is a complex phenomenon. Previous research has attempted to constrain the underlying neural mechanisms involved in making simple decisions by using eye movements as a reliable measure of choice. Numerous brain regions incorporate these oculomotor signals, including the frontal eye fields (FEF) and the superior colliculus (SC). To examine how the SC contributes to visuomotor control, the neural correlates of perceptual judgment and motor selection in SC were evaluated and compared to those previously reported for FEF during the same behavioral tasks.

16. **Dysregulation of Eukaryotic Elongation Factor 1A Expression and Synaptic Plasticity Impairments in Alzheimer's Disease**

Brenna Beckelman & Tao Ma

Alzheimer's disease (AD) is the most common form of dementia in the elderly and is quickly rising to epidemic status. Meanwhile, the molecular mechanisms associated with the disease are still elusive, which hinders our ability to search for therapeutic targets. Previous studies have suggested that eukaryotic elongation factor 1A (eEF1A) upregulation is critical for maintenance of hippocampal long-term potentiation (LTP), a synaptic model for learning and memory. Further, eEF1A synthesis is controlled by the mammalian target of rapamycin complex 1 (mTORC1) pathway, which has been linked to LTP impairments in certain mouse models of AD. Here, we explored the dysregulation of eEF1A in AD pathophysiology using biochemical and electrophysiological methods. First, we observed that basal eEF1A levels are downregulated in hippocampi of APP/PS1 AD model mice and postmortem human AD patients. In contrast, no AD-related change of eEF1A levels was found in cerebellum. Furthermore, eEF1A upregulation in hippocampal slices associated with chemical-LTP induction was blunted in AD model mice. Lastly, brain-specific knock-down of tuberous sclerosis complex 2 (TSC2), a negative regulator of mTORC1, rescued the deleterious effects of amyloid beta (Aβ) on hippocampal LTP. In summary, our studies demonstrated impairments of neuronal plasticity-related eEF1A regulation, thus, revealing a potential novel therapeutic avenue for AD and other aging-related memory deficits.

17. **The Sympathetic Nervous System and the Stability of the Neuromuscular Junction with Aging**

Ping Kwan, Tao Li, Zhong-Min Wang, Maria Laura Messi, and Osvaldo Delbono

The neuromuscular junction (NMJ) is a tripartite synapse composed of the presynaptic motor neuron axon, postsynaptic myofiber specialization, and nonmyelinating perisynaptic or terminal Schwann cells (tSCs). With age, the NMJ becomes unstable in a process characterized by fragmentation, shrinkage, and simplification of the postsynaptic terminal. Detailed studies indicate that the tripartite model includes elements that are crucial for normal skeletal muscle structure and function. However, the mechanisms underlying the gradual destabilization of the NMJ from its normal stable state is not clear yet.

In humans, autonomic innervation and function become impaired with age. As sympathetic axons innervate skeletal muscle fibers (some investigators suggested that they innervate the myofiber at the NMJ), their role in maintaining NMJ integrity over time is unknown. Our pilot data support direct sympathetic innervation of the myofiber at the NMJ, and sympathetic regulation of motor/somatic fiber innervation.

Here, we propose a new, quadripartite model (consisted of the motor neuron axon, postsynaptic myofiber specialization, tSCs, and the sympathetic neuron axon) — by which myofiber sympathetic innervation stabilizes the NMJ. We hypothesize that the sympathetic nervous system (SNS) innervates the skeletal muscle at the NMJ, while age-dependent sympathetic denervation leads to NMJ instability, disorganization, and motor denervation.

18. **ETHANOL GATED DISRUPTION OF GLUTAMATERGIC VESICLE POOL DYNAMICS IN DBA/2J AND C57BL/6J MICE**

D. Gioia, B. McCool

Different strains of mice exhibit unique innate anxiety related phenotypes and are useful for understanding the gene x environment interactions that confer resistance or vulnerability to ethanol induced anxiety. The DBA/2J strain exhibits high levels of innate anxiety and is vulnerable to withdrawal induced anxiety while the C57BL/6J strain exhibits low levels of innate anxiety and is resistant to withdrawal induced anxiety following moderate ethanol exposure. Using whole cell electrophysiology we have found that differences in glutamatergic transmission in the basolateral amygdala between these strains may contribute to differences in innate anxiety like behaviors. Specifically, DBA mice exhibit enhanced spontaneous and electrically evoked presynaptic glutamatergic function compared to C57 mice. Furthermore, differences in presynaptic release can be equalized following repetitive electrical stimulation or through bath application of phorbol esters. Additionally, we have found that acute application of ethanol in brain slice preparations produces unique alterations in synaptic vesicle pool dynamics between strains, which may provide a novel mechanism contributing to the differential vulnerability to withdrawal induced anxiety in these strains.

19. **Mindfulness Meditation-Induced Pain Relief Does Not Require Endogenous Opioidergic Systems**

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Mindfulness meditation, a cognitive practice premised on non-judgmental awareness of arising sensory events, has been repeatedly found to decrease pain. Mindfulness meditation reduces pain by engaging cognitive control processes, improving mood, and reducing expectations of impending stimuli, mechanisms that are potentially mediated by endogenous opioids. Mindfulness-based pain relief is also associated with greater activation in prefrontal, anterior cingulate, and insular cortices, brain regions containing high concentrations of opioid receptors. Taken together, these findings suggest that mindfulness meditation attenuates pain through opioidergic systems. To test this hypothesis, we examined the effect of the opioid antagonist, naloxone, on mindfulness meditation-induced pain relief. In this double-blind, psychophysical study in pain-free participants, subjects were randomly assigned to one of four groups: 1) meditation during naloxone infusion, 2) meditation and saline administration, 3) a non-manipulation control and naloxone, and 4) a non-manipulation control and saline in response to noxious heat stimulation (49°C). After four days of meditation training or a book-listening control regimen, meditation during saline administration significantly reduced pain intensity and unpleasantness ratings when compared to the control groups (ps<.001). However, naloxone did not reverse meditation-related pain reductions. There were no significant differences in pain intensity (p=.69) or pain unpleasantness (p=.75) reductions between the meditation + naloxone and the meditation + saline groups. Furthermore, pain intensity and unpleasantness reductions during meditation + naloxone remained sufficiently large to be significantly different from those of the control groups (ps<.001). These novel findings demonstrate that mindfulness meditation requires non-opioid, supraspinally mediated systems to reduce pain.

20. **Functional specialization of areas along the anterior-posterior axis of the primate prefrontal cortex prior to training in a task**

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The prefrontal cortex (PFC) is critical for working memory and executive function. Functional specialization of areas along the anterior-posterior PFC has been speculated but little evidence exists about the functional properties of neurons in these areas. To address this question we segmented the dorsal PFC into 3 regions: posterior-dorsal (area 8A of Petrides and Pandya), mid-dorsal (area 8B and area 9/46), and anterior-dorsal (area 9 and area 46). We contrasted the responsiveness and selectivity of neurons in these regions with the posterior ventral region (area 45). Three stimulus sets were used to evaluate selectivity: a spatial set consisting of 9 white squares arranged in a 3x3 grid of 10° eccentricity between stimuli; a feature set consisting of 8 white geometric shapes matched for total area; and a color set consisting of 8 equiluminant color squares. A total of 1878 neurons were recorded in 6 monkeys passively viewing these stimuli, prior to training in a working memory task. There was no significant difference in the total percentage of neurons that responded to the stimuli between the anterior, mid and posterior dorsal area (chi-square test, $p>0.5$), though the percentage of neurons responding to stimuli in the ventral posterior area was significantly lower than the dorsal areas (posterior-dorsal: 44%; mid-dorsal: 41%; anterior-dorsal 35%; posterior-ventral 20%; chi-square test, $p<0.05$). On the other hand, selectivity for stimuli was highest for the posterior-dorsal area and generally declined along the anterior-posterior axis. When quantified with a selectivity index expressed as (Max-Min)/(Max+Min), significant differences were present for the spatial, feature, and color selectivity values across areas (spatial: posterior 0.693, mid 0.577, anterior 0.566; feature: posterior 0.492, mid 0.385, anterior 0.411; color: posterior 0.420, mid 0.267, anterior 0.256; 1-way ANOVA, $p<0.05$, for each stimulus set). In addition, firing rates during the presentation of the stimuli were significantly higher for the posterior-dorsal area (posterior 14.5 spikes/s, mid 9.9 spikes/s, anterior 10.3 spikes/s; 1-way ANOVA, $p<0.05$). These differences were only seen during the display of the stimuli; activity during the baseline fixation period did not significantly differ between areas (1-way ANOVA, $p>0.07$). Our results provide neurophysiological evidence for a rostral-caudal gradient of stimulus selectivity through the PFC, suggesting that posterior areas are selective for stimuli even when these are not relevant for execution of a task whereas anterior areas may acquire selectivity as a result of task performance.