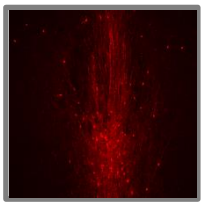
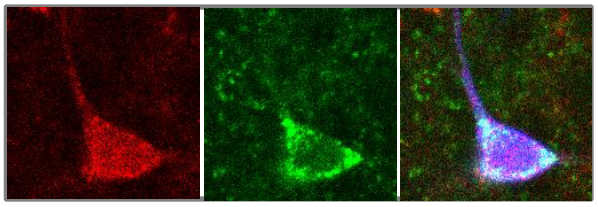
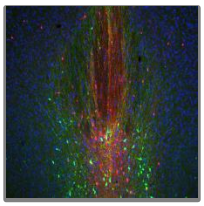




TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER™

Department of Pharmacology & Neuroscience

Research Retreat

Fourth Annual Translational Neuroscience Meeting

Expanding frontiers in translational neuroscience

October 18th and 19th 2018

Marfa, Texas

TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER™
School *of* Medicine
Department of Pharmacology and Neuroscience

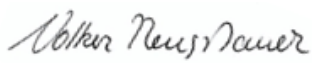
October 10th, 2018

Welcome to the fourth annual Research Retreat of the Department of Pharmacology and Neuroscience in Marfa, TX. Our departmental research retreat continues to provide a unique opportunity for professional development through collegial interactions to inform about research accomplishments and ongoing scholarly activities of our department members, discuss and develop new collaborative research projects and grant applications, and reflect on our academic mission in the generation and dissemination of knowledge.

We are excited to welcome two distinguished guests to our retreat. Dr. Kathryn Cunningham, Professor and Vice Chair in the Department of Pharmacology & Toxicology and Director of the Center for Addiction Research at UTMB Galveston, is a world leader in translational research and education on substance use disorders and related neuropsychiatric conditions. Dr. Quentin Smith, Professor and Dean of the School of Pharmacy at TTUHSC, has been named Senior Vice President of Research and is overseeing many exciting new initiatives and continued growth of the TTUHSC research enterprise. We look very much forward to their presentations and valuable feedback.

This has been a very successful year for our department with several new federal and foundation grant awards and important contributions to medical and graduate education. We are very grateful for the continued support from the offices of the Dean of the School of Medicine and the Sr. VP of Research. I would also like to take this opportunity to thank our staff for their invaluable help and support. A special thank you goes to Dr. Josée Guindon and Tiffany Denton for the organization of this event and the program booklet that contains abstracts of ongoing research projects by our departmental faculty.

Please share your thoughts, ideas, plans and suggestions. Your active participation is essential for the success of our departmental research retreat and for the continued progress of our department. Thank you for all your valuable contributions.



Volker Neugebauer, M.D., Ph.D.
Professor and Chair, Department of Pharmacology and Neuroscience
Giles C. McCrary Endowed Chair in Addiction Medicine
Director, Center of Excellence for Translational Neuroscience and Therapeutics

Thursday Oct 18th 2018

Morning	Individual travel to Hotel Paisano in Marfa
5:00	Welcome and Introduction - Drs. Neugebauer and Guindon
5:15 – 6:15	Distinguished Guest Lecture - Dr. Kathryn A. Cunningham: "Translating Neuropharmacology to Novel Therapeutics for Addiction"
6:30 – 8:30	*Dinner together*

Friday Oct 19th 2018

8:00 – 9:00	*Breakfast*
9:00 - 10:25	Research Presentations - Ongoing Projects
9:00 - 9:10	Departmental Perspective - Dr. Neugebauer
9:10 - 9:25	Dr. Guangchen Ji (Faculty)
9:25 - 9:40	Dr. Takaki Kiritoshi (Postdoc, Neugebauer lab)
9:40 - 9:55	Mariacristina Mazzitelli (Ph.D. Student, Neugebauer lab)
9:55 - 10:10	Henry Blanton (Ph.D. Student, Guindon lab)
10:10 - 10:25	Dr. Khalid Benamar (Faculty)
10:25 - 11:00	*Break*
11:00 - 11:15	Dr. Madhu Narasimhan (Faculty)
11:15 - 11:30	Dr. Sambantham Shanmugam (Postdoc, Henderson lab)
11:30- 11:45	Dr. Susan E. Bergeson (Faculty)
11:45 - 12:00	Dr. Rui Wang (Postdoc, Lawrence lab)
12:00 - 2:30	*Lunch Break*
2:30 - 4:30	Research Presentations - Grant Proposals
2:30 - 3:10	Dr. Khalid Benamar (Faculty)
3:10 - 3:50	Dr. Josée Guindon (Faculty)
3:50 - 4:30	Dr. J. Josh Lawrence (Faculty)
4:30 - 5:30	*Break*
5:30 - 6:30	*Dinner together*
6:30 - 7:30	Distinguished Guest Lecture - Dr. Quentin Smith (SVPR)

Saturday Oct 20th 2018

Morning	Check out from Hotel Paisano (before noon)

Distinguished Guest Speakers

Dr. Kathryn A. Cunningham, Ph.D.

Chauncey Leake Distinguished Professor of Pharmacology
Director, Center for Addiction Research
Vice-Chair for Research, Department of Pharmacology and Toxicology
University of Texas Medical Branch (UTMB)

Dr. Cunningham directs research and educational efforts embedded in a strong collaborative effort with chemists, cell biologists, preclinical and clinical scientists to discover key neuromolecular targets that may be exploited toward the goal of improved diagnostics and therapeutics for substance use disorders (**SUDs**). She has established strengths in neuropharmacology, the biology and pharmacology of G protein-coupled receptors, SUD neurocircuitry, drug discovery, and *in vivo* models of SUDs and related neural disorders. Dr. Cunningham has established a multitude of new cellular, behavioral and molecular tools to study SUD systems and to explore the biology of novel neuroprobes developed by our chemistry collaborators, with the promise as new SUD therapeutics. The National Institutes of Health has funded her research progress for over 25 years. Dr. Cunningham has generated seminal observations, new technologies and patents which are described in 150+ peer-reviewed publications and 30+ reviews and book chapters. She has cultivated and sustained a life-long commitment to fostering the career development of new scientists with over 45 mentees who have crafted successful careers in academia, industry and government. Her research and educational contributions have been recognized by the *American Society for Pharmacology and Experimental Therapeutics-Astellas Award for Translational Pharmacology* as well as the Marian Fischman Memorial Award and the Mentorship Award from the College on Problems of Drug Dependence. Dr. Cunningham is Associate Editor of *Nature Neuropsychopharmacology*, AMSPC Councilor, ASPET Councilor, and an active educator, mentor and board member for community programs in the region.

Dr. Quentin Smith, Ph.D.

Senior Vice President for Research
Professor and Dean, School of Pharmacy
Texas Tech University Health Sciences Center

After working more than 17 years in the National Institutes of Aging of the National Institutes of Health (NIH), where he served as a tenured research investigator and section chief, Smith began at TTUHSC in 1997 as professor and chair of the newly formed Department of Pharmaceutical Sciences in the School of Pharmacy. He worked with the Graduate School of Biomedical Sciences in 1999 to extend their training to the Amarillo campus with creation of the Pharmaceutical Sciences Graduate Program. In 2009, he became the senior associate dean for sciences of the School of Pharmacy, and in 2012 was appointed dean of the School of Pharmacy. Since joining TTUHSC, Smith has worked to build research and education in the School of Pharmacy. Though the school is only 20 years old, it has placed in the top third of Schools of Pharmacy nationally, ranked 16th in the nation by Graduate Programs.com, and 36 of 129 in U.S News and World Report. TTUHSC President Tedd L. Mitchell said in the last five years, the School of Pharmacy has more than doubled the number of graduates going into postgraduate residency training. Smith is an internationally distinguished researcher who, since leaving the NIH, has been awarded substantial extramural grant funding and led several international scientific conferences, including the Gordon Research Conference on Barriers of the Nervous System in 2012. He is a Fellow of the Association of Pharmaceutical Scientists and received both the Award of Merit (1995) from the NIH and the meritorious manuscript award (2014) from the American Association of Pharmaceutical Sciences. At TTUHSC, he received the Chancellor's Council Distinguished Research Award, the Grover E. Murray Professorship, University Distinguished Professor and the President's Excellence in Teaching Award. The graduating class of the School of Pharmacy recognized him twice as the Most Influential Professor.

Fear extinction learning ability predicts pain behaviors

***Guangchen Ji*[1,2], *Peyton Presto*[1], *Vadim Yakhnitsa*[1], *Takaki Kiritoshi*[1], *Volker Neugebauer*[1,2]**

[1]Department of Pharmacology and Neuroscience, [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX

Pain and fear may share neurobiological mechanisms such as plasticity in emotional networks that include the amygdala. The amygdala plays a key role in fear conditioning and has emerged as an important node of emotional-affective aspects of pain modulation. Impaired fear extinction learning, which involves prefrontal cortical control of amygdala processing, has been linked to conditions such as posttraumatic stress disorder (PTSD). Sex differences in pain and disorders such as depression and anxiety are now being recognized. Here we tested the hypothesis that fear extinction learning ability can predict certain aspects of pain-related behaviors of rats and that these may be different in female and male rats.

We correlated fear extinction learning in adult male and female rats with behavioral outcome measures (sensory thresholds, vocalizations, and anxiety-like behaviors) in models of acute arthritis pain (kaolin/carrageenan-induced knee joint arthritis) and chronic neuropathic pain model (spinal nerve ligation, SNL). Cued (auditory) fear conditioning, extinction, and extinction retention tests were conducted using two chambers. On Day 1 rats were habituated to context A followed by fear conditioning (2 US-CS pairs). On Day 2, rats were habituated to context B followed by extinction training (30 CSs). On Day 3, rats were habituated to context B followed by extinction retention measurement (5 CSs). There was no difference in fear learning between male and female rats. The majority of rats showed a quick decline of freezing level during extinction training and retention (FE+) whereas a smaller percentage of rats maintained a high freezing level (FE-). Female, but not male, FE- rats spent less time exploring the center area in the open field test (OFT) than FE+ rats, reflecting anxiety-like behavior, but there but were no significant differences in sensory thresholds and vocalizations between FE+ and FE- types (male and female) under control conditions. In the arthritis pain model, male and female FE- rats developed higher levels of vocalizations and anxiety-like behavior than FE+ rats, but there were no differences in sensory thresholds. In the neuropathic pain model, male and female FE- rats developed higher levels of vocalizations and anxiety-like behavior than FE+ rats, but there were no differences in sensory thresholds. Both FE+ and FE- female rats showed higher levels of vocalizations than male FE+ and FE- male rats respectively.

The data may suggest a predictive value of fear extinction ability for emotional-affective pain aspects in male and female rats, and greater vulnerability of female than male rats with lower extinction ability.

Supported by NIH grants NS038261, NS081121, and NS106902.

Synaptic transmission in amygdala CRF and non-CRF neurons under normal conditions and in a neuropathic pain model

***Takaki Kiritoshi*[1], *Vadim Yakhnitsa*[1], *and Volker Neugebauer*[1,2]**

[1]Department of Pharmacology and Neuroscience, [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX.

Work in our group is focused on the role of the amygdala in pain. A limbic brain region the amygdala has emerged as an important contributor to the emotional-affective aspects of pain and pain modulation. The central nucleus (CeA) serves major output functions and receives purely nociceptive information via parabrachial nucleus (PB) and polymodal sensory information via the basolateral nucleus (BLA). Pain-related changes at these synapses have been shown in different pain models by our group and others. The CeA contains neurochemically distinct populations of neurons. Corticotropin releasing factor (CRF) neurons in the CeA can form long range projections and CRF plays an important role in pain-related synaptic plasticity. However, it remains to be determined if CRF neurons and non-CRF neurons receive differential synaptic inputs and which type of CeA neurons actually develops pain-related synaptic changes.

Using brain slice physiology, we analyzed synaptic inputs from the PB and the BLA to CRF and non-CRF CeA neurons under control conditions and in a neuropathic pain model. Transgenic Crh-Cre rats were used to visually identify CRF and non-CRF neurons in the lateral CeA of the right hemisphere. Whole-cell patch clamp recordings were obtained in brain slices from sham rats and neuropathic rats (4 weeks after L5 spinal nerve ligation, SNL model). In current clamp mode, action potential firing patterns (regular spiking, late firing, and low-threshold bursting) were measured by injecting depolarizing currents. In voltage clamp mode, amplitudes of excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) and IPSC/EPSC ratio were measured at the PB-CeA and BLA-CeA synapses. For selective optogenetic activation of PB inputs in amygdala brain slices, a viral vector (AAV) encoding channelrhodopsin 2 (ChR2) under the control of the CaMKII promoter (AAV5-ChR2-CaMKII-eYFP) was injected stereotaxically into the right PB. BLA inputs were activated by electrical stimulation in the BLA. We found different proportions of neuronal firing type in CRF versus non-CRF neurons under control conditions but not in the pain model where there seemed to be an increase in low-threshold bursting CRF neurons. PB-driven EPSCs in CRF neurons, but not in non-CRF neurons, were increased in slices from SNL rats. BLA-driven IPSCs (feedforward inhibition) in non-CRF neurons, but not in CRF neurons, were decreased in the SNL model.

These results suggest cell-type specific differential changes in synaptic transmission in neuropathic pain. The data are consistent with pain-related synaptic plasticity of excitatory inputs in CRF neurons.

Supported by NIH grants NS038261, NS081121, and NS106902.

Dr. Neugebauer Research Team

SK-channel function in different amygdala neuronal cell types under normal conditions and in a neuropathic pain model

***Vadim Yakhnitsa*[1], *Takaki Kiritoshi*[1], *and Volker Neugebauer*[1,2]**

[1]Department of Pharmacology and Neuroscience, [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX.

In our continued efforts to better understand the role of the amygdala in pain we are performing a cell type specific analysis of pain-related functional changes in the amygdala output region, the central nucleus (CeA). Work by our group and others has shown synaptic changes in the CeA in different pain models but how these translate into neuronal excitability and output remains to be determined. Furthermore, neurochemically distinct neuronal populations of CeA neurons have emerged. While CeA neurons are GABAergic they express different neuropeptides, most notably corticotropin releasing factor (CRF) and somatostatin (SOM), and/or PKC delta. Important regulators of neuronal excitability are small-conductance calcium-activated potassium (SK) channels that mediate a so-called medium afterhyperpolarization (mAHP). Our previous in vivo studies showed increased activity (action potential firing) of CeA in pain models. We are now testing the novel hypothesis that dysfunction of SK channels contributes to increased excitability of CeA neurons in a rat model of chronic neuropathic pain. Specifically, we are analyzing if there is a decrease or loss of mAHP in CRF and/or SOM CeA neurons.

Using whole-cell voltage- and current-clamp recordings, we measured neuronal excitability (frequency-current F-I function) and afterhyperpolarization of neurons in the lateral CeA in brain slices from behaviorally tested sham control and neuropathic rats (4 weeks after L5 spinal nerve ligation, SNL model). Different types of neurons were identified using transgenic Crh-Cre rats that were injected with rAAV5/Ef1a DIO-YFP or mCherry stereotaxically into the CeA to label CRF somata. CRF-CeA neurons and non-CRF-CeA neurons were recorded, filled with biocytin through the patch pipette, and stained immunohistochemically for co-localization with SOM. We found smaller mAHP amplitudes and a smaller proportion of CRF neurons with mAHP in the neuropathic condition. These changes were more pronounced in CRF than SOM amygdala neurons.

Our data suggest that SK-channel dysfunction (loss of mAHP) may be more pronounced in CRF than SOM amygdala neurons in a neuropathic pain model. These findings will provide more detailed understanding of molecular mechanisms that regulate the development and maintenance of chronic pain.

Supported by NIH grants NS038261, NS081121, and NS106902.

Dr. Neugebauer Research Team

Kappa opioid receptors (KOR) in the amygdala: Neuronal and behavioral effects

***Matthew A. Hein*[1], *Vadim Yakhnitsa*[1], *Guangchen Ji*[1,2], *and Volker Neugebauer*[1,2]**

[1]Department of Pharmacology and Neuroscience, [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX.

Neuroplastic changes in the central nervous system have been implicated not only in pain conditions associated with an identifiable injury, but also in functional pain syndrome (FPS), in which the pain cannot be attributed to tissue pathology. Mechanisms of FPS remain to be determined, but these conditions are typically triggered by stress, which can advance the pain condition from episodic to chronic. Corticotropin releasing factor and its CRF1 receptor in the amygdala have been linked to emotional-affective behaviors and pain modulation. The amygdala is also a major site of opioid receptors, including $G_{i/o}$-coupled kappa opioid receptors (KOR). KOR activation by its endogenous ligand dynorphin or agonists can have aversive effects. Preliminary data suggest that KOR is expressed on a certain type of inhibitory interneurons in the central nucleus (CeA). The CeA and CRF neurons serve major amygdala output functions. Here we tested the hypothesis that KOR activation disinhibits CRF neurons in the CeA in naive rats to generate pain-like behaviors in the absence of tissue injury.

Brain slice physiology and behavioral assays were used to determine the effects of a KOR agonist (U-69,593) on CRF-CeA neurons and on pain-like behaviors. To visualize CRF neurons in brain slices, AAV-EF1a-DIO-mCherry was injected into the right CeA of transgenic CRF-Cre rats. For optogenetic activation of glutamatergic afferent input to CRF-CeA neurons from the lateral parabrachial area (PB), AAV5-ChR2-CaMKII-eYFP was injected into the lateral PB. Animals were allowed to recover for four weeks for sufficient expression of ChR2. Using whole-cell patch-clamp recordings of CRF-CeA neurons we measured neuronal excitability (frequency-current F-I relationship), excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) evoked by optical activation of PB terminals in the CeA or by electrical stimulation in the basolateral amygdala, and synaptically-evoked spiking (E-S coupling). Bath application of U-69,593 decreased glutamate driven IPSCs (feedforward inhibition) but had no effect on EPSCs or on F-I function. In behavioral experiments, stereotaxic application of U-69,593 by microdialysis into the CeA facilitated audible and ultrasonic vocalizations in response to brief (10s) noxious stimuli (compression of the knee joint) and decreased mechanical withdrawal thresholds (suggesting increased mechanosensitivity).

The data suggest that KOR activation in the CeA under normal conditions leads to synaptic disinhibition of CRF-CeA neurons, which results in increased pain responses and sensitivity, consistent with a role in FPS.

Supported by NIH grants NS038261, NS081121, and NS106902.

Role of group II metabotropic glutamate receptors in the amygdala in pain modulation

***Mariacristina Mazzitelli*[1] *and Volker Neugebauer*[1,2]**

[1]Department of Pharmacology and Neuroscience, [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX.

Pain is a multidimensional experience with an important averse-affective dimension. The amygdala, a limbic brain area, plays a critical role in the emotional-affective aspects of behaviors and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions, and neuroplasticity in the CeA is mechanistically linked to pain-related behaviors in different pain conditions. Emotional-affective mechanisms of pain, particularly in the brain, are not well understood, and modulation of neurotransmitter function plays an important role in pain-related plasticity. The activation of Gi/o-coupled group II metabotropic glutamate receptors (mGluRs), which consist of mGluR2 and mGluR3 subtypes, can decrease neurotransmitter release and regulate synaptic plasticity. Evidence from preclinical studies suggests that mGluR2/3 may be a target for neuropsychiatric disorders and can inhibit pain-related processing and behaviors. Therefore, we hypothesized that group II mGluRs may be useful tools to regulate amygdala function. The contribution of individual subtypes (mGluR2 and mGluR3) to amygdala-dependent pain behaviors and pain modulation remains to be determined. This knowledge gap was addressed here in a rodent model of arthritis pain.

Audible (nocifensive response) and ultrasonic (averse affective response), mechanical withdrawal thresholds were measured in adult rats before and 5-6 h after the induction of a kaolin/carrageenan-mono-arthritis in the left knee joint. To determine the modulation of spinal nociceptive processing by amygdala output, extracellular single unit recordings were performed from spinal dorsal horn (L2-4) neurons in arthritic rats. Systemic (intraperitoneal) application of a group II mGluR agonist (LY379268 disodium salt) decreased vocalizations and mechanosensitivity and also reduced the activity (action potential firing) of dorsal horn neurons. To determine the contribution of mGluRs in the amygdala, a group II mGluRs antagonist (LY341495 disodium salt), a positive allosteric modulator for mGluR2 (PAM, LY487379 hydrochloride), or a negative allosteric modulator for mGluR2 (NAM, VU6001966) was applied stereotaxically into the right CeA (contralateral to the arthritic knee) by microdialysis. The group II antagonist and the mGluR2 NAM in the CeA reversed the systemic group II mGluR agonist effects in the pain model. Stereotaxic application of the mGluR2 PAM alone in CeA was able to mimic the effect of the systemically applied group II mGluR agonist in arthritic rats.

These results suggest that group II mGluRs, and particularly mGluR2, in the amygdala can regulate pain-related behaviors and spinal nociceptive processing and play a major role in the effects of systemic group II agonists.

Supported by NIH grants NS038261, NS081121, and NS106902.

Mechanisms of cannabinoid tolerance

***Henry Blanton*[1], *Jennifer Brelsfoard*[1], *Diana E. Castro*[3],*Angela Redmond Henderson*[3], *Madhu Narasimhan*[1], *Daniel J. Morgan*[3], *and Josée Guindon*[1,2]**

[1]Department of Pharmacology and Neuroscience and [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX USA; [3]Department of Anesthesiology, Penn State College of Medicine, Hershey, PA.

This study will investigate the mechanisms of cannabinoid tolerance. This objective will be achieved by determining whether cannabinoid tolerance is mediated through agonist-specific mechanisms using a model of chemotherapy-induced neuropathic pain. Our approach will examine tolerance to the anti-allodynic and antinociceptive effects of Δ9-THC, CP55,940, and WIN55,212-2, three cannabinoid agonists with distinct signaling and chemical features. Tolerance to Δ9-THC antinociception in the tail-flick test was eliminated by pre-treatment of S426A/S430A mutants with SP600125, a selective c-Jun N-terminal kinase (JNK) inhibitor suggesting that JNK (SP600125 inhibitor) and GRK/βarrestin2 (S426/S430A mutation) signaling mechanisms coordinate to mediate tolerance to the antinociceptive effect of Δ9-THC. The first objective of this study is to, fully and systematically, test the hypothesis that cannabinoid tolerance is mediated through agonist-specific mechanisms. The second objective is to test the hypothesis that JNK-mediated tolerance for Δ9-THC requires the presence of β−arrestin2. The third objective is to test the hypothesis that β−arrestin2 and JNKs can form protein-protein interactions in vivo. The fourth objective is to test the hypothesis that JNKs can directly phosphorylate CB1 when activated by Δ9-THC using a technologically innovative chemical-genetic approach. The first three hypotheses will be tested in a clinically relevant model of chemotherapy (cisplatin)-induced model of neuropathic pain. The last hypothesis is equally innovative and will provide important information regarding the molecular mechanism of action that is responsible for JNK-mediated Δ9-THC tolerance. The overarching goal of this project is to gain a better understanding of the agonist-specific mechanisms responsible for cannabinoid tolerance that will facilitate the development of long lasting, highly efficacious, and personalized pain therapies.

Supported by NIH/NIDA grant DA044999-01A1.

Non-addictive therapeutic strategy for chronic neuropathic pain

***Khalid Benamar*[1,2], *Steven Muester*[1] *and* *Madhusudhanan Narasimhan*[1]**

[1]Department of Pharmacology and Neuroscience, [2]Department of Immunology and Molecular Microbiology, and [2]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX USA.

Chronic pain such as neuropathic pain is a major health issue. Current treatment regimens in neuropathic pain management are limited, results often unsatisfactory, and side effects common (Finnerup et al., 2015). The complexity of pain requires analyses at every level of the nervous system. Brain mechanisms in particular are still less well understood than peripheral and spinal nociceptive processing. Our goal is to identify GPR18 as a novel brain mechanism of pain chronification hence opportunity for rescue strategy. To do so we propose to test the novel hypothesis that loss of GPR18 activation in the periaqueductal gray(PAG) by its endogenous ligand N-arachidonoyl glycine (NAGly) is a critical mechanism of the dysfunction of descending control systems in chronic neuropathic pain, allowing the abnormal persistence of pain behaviors. This deficit can be mitigated with a non-addictive pharmacological rescue strategy (NAGly). Our studies will focus on the PAG because it is a key element of the descending pain control system that modulates spinal nociceptive neurotransmission via the rostral ventromedial medulla (RVM) (Fields et al., 1991 Heinricher et al., 2009). Behavioral, pharmacological and biochemistry assays will be used for mechanistic analyses of loss and rescue of GPR18 function in the well-established chronic constriction injury (CCI) model of neuropathic pain. Behavioral experiments will test the hypothesis that failure to activate GPR18 by its endogenous ligand (NAGly) in the PAG allows the abnormal persistence of pain behaviors but exogenous activation can inhibit neuropathic pain behaviors (pharmacological rescue strategy) independently of side effects commonly seen with opioid and cannabinoids.

Molecular biology studies will test our hypothesis that GPR18 expression in the PAG is not impaired in neuropathic pain, but lack of NAGly availability plays a key role in the loss of GPR18 function. These experiments will also determine if impairment of enzymes involved in the synthesis or degradation of NAGly contributes to the failure to engage GPR18-driven descending pain control in neuropathic pain. This deficit can be mitigated by a pharmacological rescue strategy, because functional GPR18 remains available in the pain state. Therefore, successful completion of these conceptually innovative studies will significantly advance our understanding of brain mechanisms of neuropathic pain and provide a novel non-addictive target for chronic pain management. The novel concept that NAGly-GPR18 dysfunction in the PAG has pain function, that is independent of endocannabinoid and opioid systems, and can opposes the effect of analgesics could explain the relatively limited effect of current therapies in chronic neuropathic pain.

Is there a placental cross-talk between Nrf2/Antioxidant and mTOR/growth signaling network during *in-utero* alcohol exposure?

***Madhusudhanan Narasimhan*[1], *Dhyanesh Patel*[1], *Sambantham Shanmugam*[1], *Marylatha Rathinam*[1], *Lenin Mahimainathan*[1], *Srivatsan Kidambi*[2], *George Henderson*[1]**

[1]Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX
[2]Department of Chemical and Biomolecular Engineering, University of Nebraska, Lincoln, NE

Background
Placenta mediates the transfer of nutrients from the maternal blood to the fetus and provides support to embryonic growth and survival. An exposure to xenobiotics and/or recreational compounds, including ethanol (E) during pregnancy has been shown to affect the placental functions causing fetotoxicity. Increased placental oxidative stress and apoptosis have been evidenced on chronic gestational alcohol exposure in rats. The objective of this study was to investigate the oxidative pathways, related to the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) system in the placenta of E-exposed pregnant rats.

Methods
Pregnant Sprague-Dawley rats were exposed to intermittent ethanol vapor (IEV) daily starting from gestational day 11 (GD) to GD21 with a 6h ON/18h OFF in vapor chambers set to deliver 2.97ml/min. This method overcome stress due to intubation/handling and produced reliable blood alcohol concentration (BAC) between 150 to 200 mg/dl. On GD21 the dams were sacrificed and placentas were excised for real-time and immunoblotting analysis.

Results
E decreased nuclear levels of Nrf2 protein. This was associated with a significant decrease in the expression of Nrf2 target - antioxidant genes (Nqo1, Gclc, Gsr, Ho-1) and aminoacid transporter genes (Slc1a1, xCT) ($p<0.05$). In addition, Nrf2 mRNA expression was also significantly reduced upon E exposure ($p<0.05$). Further, E significantly decreased the protein and phosphorylation levels of mTOR, the master regulator of stress response and growth ($p<0.05$) without a change in its transcript expression.

Conclusions
The deficient Nrf2 and its antioxidant response resulting from ethanol exposure indicate an apparent oxidative stress in the placenta. Further, the associated impairment in mTOR expression and its activity suggests a dysregulation of placental metabolism, growth, and proliferation. Thus, gestational alcohol can interfere with master regulators of antioxidant (Nrf2) and growth (mTOR) signaling thereby can offset the placental function and thus, the fetal development.

Supported by NIH/NIAAA grant AA010114-19.

Transulfuration pathway involving cystathionine-gamma-lyase (CSE) determines the redox response and growth control to alcohol in rat cortical neuroblasts

***Sambantham Shanmugam*[1], *Dhyanesh Patel*[1], *Marylatha Rathinam*[1], *Lenin Mahimainathan*[1], *Srivatsan Kidambi*[2], *Madhusudhanan Narasimhan*[1], *George Henderson*[1]**

[1]Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX
[2]Department of Chemical and Biomolecular Engineering, University of Nebraska, Lincoln, NE

Background
Earlier, we identified *in utero* ethanol (E) decreased cystathionine γ-lyase (CSE), a gene involved transsulfuration pathway in fetal brain during the period of corticogenesis and in cortical neurons (unpublished). Neocortex development depends on neuroblasts/neural progenitors' proliferation that is shown to be impaired by E. Thus here we investigated whether alteration in CSE-dependent transsulfuration/redox homeostasis is associated with neuroblast lesion involving proliferation and associated cell fate.

Methods
Cells were exposed to 86 mM E for 24 h to mimic the acute consumption pattern in humans. Small interference RNA (siRNA) strategy was used to knockdown CSE. Detection of reactive oxygen species (ROS) in live cells was performed using CellROX based flow cytometry. Redox perturbations were assessed in terms of GSH-NEM and DMPO-nitrone adducts using immunofluorescence. Cell fate was assessed using MTT assay, Annexin-FITC/PI fluorescence, and immunoblotting.

Results
E decreased cystathionine-γ-lyase (CSE) expression in neuroblasts similar to changes observed in fetal brain cortices and primary cortical neurons (PCNs). In parallel, loss of CSE enhanced the E-induced accumulation of ROS and DMPO-nitrone adducts and GSH reduction. E-induced CSE decrement was found to be associated with a concomitant decrease in NFATc4 expression in neuroblasts and PCNs. FK-506, a calcineurin/NFAT signaling inhibitor experiments enhanced E-induced CSE loss. In contrast, NFATc4 overexpression prevented the loss of E-induced CSE and neuroblast cell fate changes.

Conclusions
These studies illustrate that ethanol impairs transsulfuration pathway involving CSE-in maintenance of redox homeostasis and associated cell fate in neuroblasts that may be partly mediated via perturbation in NFAT signaling.

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Development of therapeutic interventions for alcohol use disorder

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Bioinformatics analyses of genomic and epigenetic screens for several mouse models of Alcohol Use Disorder (AUD) uncovered innate immune genes and biological pathways associated with alcohol-related behaviors and responses. Tetracycline class drugs with potential neuroimmune modulatory functions were tested and shown to reduce alcohol consumption and withdrawal-related handling-induced convulsions via CNS, rather than peripheral, action. Importantly, variation between females and males was also identified. Recently, structure function studies allowed the development of improved compounds. Toward that end, patent protection has been filed for several chemically modified tetracyclines, which no longer have antimicrobial properties, yet showed reduction of alcohol consumption in mice and swine (farm pigs) animal models. Current pre-clinical studies are focused on the development of lead compounds and FDA approval for Investigational New Drug (IND) status. ADME and toxicology studies will be completed. Phase I and II clinical trials will follow. A second project involves the development of non-invasive stereotaxic radiomodulation of brain nuclei to reduce AUD traits using a Sinclair mini-pig model. Animals made dependent will undergo the non-invasive 'surgery' and be tested for reduction of alcohol consumption and other behaviors related to DSM-V AUD characteristics.

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Parvalbumin and somatostatin circuitry dysfunction differentially impairs hippocampal DG/CA3 function during Alzheimer's disease pathogenesis

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Hippocampal hyperexcitability during Alzheimer's disease (AD) pathogenesis causes spatial memory deficits and seizure susceptibility. During memory formation, spatial information is routed through the dentate gyrus (DG) and CA3 region. The DG separates patterns by sparse coding of incoming sensory input to the hippocampus, whereas the CA3 region completes patterns by recruiting internally stored memory traces. Intact parvalbumin (PV)-positive feedforward and somatostatin (SOM)-positive feedback circuits are required for both pattern separation and completion operations. Moreover, hippocampal rhythms rely on the integrity of long-range PV and SOM circuits. Despite increased appreciation of the importance of these inhibitory circuits in normal DG/CA3 function, their roles during AD have yet to be determined. In this study, we investigated the differential contributions of specific PV and SOM circuits to the etiology of AD using the J20 mouse transgenic model that overexpresses a mutant form of human amyloid precursor protein (hAPP). We generated novel triple transgenic (3Tg) $J20^{+/-}$ AD mouse models, which enable CRE recombinase-dependent expression of the red fluorescent protein tdTomato in PV or SOM circuits, and we found significant differential expression of tdTomato in PV/SOM cells in a region- and time-specific manner. Particularly, in 7-month-old 3Tg mice, SOM-CRE; tdTomato expression revealed a massive upregulation of SOM circuits, including a *de novo* SOM circuit targeting DG inner molecular layer (IML). The appearance of this *de novo* circuit coincides with published reports of memory deficits and was absent from 2-month-old $J20^{+/-}$ mice and age-matched $J20^{-/-}$ sibling littermate controls. In contrast, PV-CRE; tdTomato expression was reduced in DG and CA3 principal cell layers in $J20^{+/-}$ mice at both 2 and 7 months of age compared to $J20^{-/-}$ littermate controls, suggesting an earlier global impairment of PV signaling. These novel findings indicate differential alterations of PV and SOM circuitry in the etiology of AD, suggesting that disruption of PV and SOM circuits impairs spatial memory formation through divergent synaptic mechanisms. For future directions, we aim to further identifying PV and SOM neuron subpopulations that cause aberrant synaptic connections, determine synaptic consequences and cellular mechanisms of PV/SOM dysfunction in 3Tg AD mouse models, and investigate the behavioral impact of PV and SOM dysfunction on hippocampal memory tasks.

Loss of acetylcholine-GABA interactions in a novel transgenic mouse model of autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting 1-2% of the US population. ASD is characterized by a triad of behavioral abnormalities: impaired social functioning, verbal deficits, and repetitive actions. Accumulating evidence suggests that these abnormalities signify disruption in excitatory and inhibitory balance in the brain. In addition, post-mortem histology and electrographic disturbances in human autism are consistent with a loss of subcortical cholinergic neuromodulation. Because GABAergic interneurons are highly modulated by acetylcholine (ACh), interactions between cholinergic and GABAergic circuits could be important in maintaining excitation-inhibition balance. Recent evidence demonstrating co-transmission of acetylcholine and GABA in the hippocampus suggests that cholinergic and GABAergic dysfunction could further disrupt excitation-inhibition balance. In this study, we will determine how GABAergic and cholinergic neurotransmitter systems interact in an autism mouse model. We generated a triple transgenic mouse (3Tg) in which cholinergic neurons were visualized with green fluorescent protein (GFP) and tdTomato was driven by the parvalbumin promoter to visualize of parvalbumin-positive GABAergic neurons. We have employed this uniquely powerful transgenic technology in the valproic acid (VPA) mouse model of autism. In our initial preliminary studies, we have successfully visualized GFP-positive cholinergic fibers innervating tdTomato-positive inhibitory circuits in the hippocampus. Histological analyses of the medial septum indicated that cholinergic and PV-positive populations were mutually exclusive. VPA treatments are ongoing. We expect to find a decrease in the density of GFP-positive fibers in the VPA-treated mice and reduced interations with tdTomato-positive circuit elements, correlating with deficits in socialization. Because GABA is also released from cholinergic terminals, we would also expect impaired cholinergic signaling in VPA-treated mice to be accompanied by a anxiogenic phenotype, consistent with an impairment in GABAergic inhibition and disrupted excitation-inhibition balance. Finally, we expect that social behavior can be restored by therapeutically enhancing brain ACh and/or GABAergic signaling.

Dr. Blanton Research Team

Identification of positive allosteric modulator (PAM) binding sites in neuronal nicotinic acetylcholine receptors

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The Blanton and Hamouda Labs: For the past twenty-two years a major focus of the Blanton lab has been the heterologous expression, affinity-purification, and structure/function analysis of ligand gated ion channels (LGIC) and in particular human neuronal nicotinic acetylcholine receptors (nAChRs). More recently, the Blanton lab has joined forces with a former graduate student, Dr. Ayman K. Hamouda, who is now a NIH funded tenure track Assistant Professor in the TX A&M College of Pharmacy, to further study these receptors and identify sites of drug binding.

Neuronal Nicotinic Acetylcholine Receptors (nAChRs): Neuronal subtypes of the nicotinic acetylcholine receptor (nAChR) are each members of the Cys-loop family of LGICs and play a critical role in many physiological and pathophysiological conditions. The α3β4 nAChR subtype is the primary mediator of (cholinergic) fast synaptic transmission in both sympathetic and parasympathetic ganglia (autonomic nervous system) that innervate the heart. The α4β2 nAChR subtype, the most abundant and diffuse subtype found in the CNS, is located presynaptically and modulates the release several neurotransmitters. The α4β2 nAChR is implicated in many neurological diseases and conditions including Parkinson's and Alzheimer's disease, epilepsy, mental illness and nicotine dependence. Nicotine dependence (i.e cigarette smoking) is a major risk factor for heart disease, including stroke, heart attack, and aneurysm.

Current focus: The Blanton/Hamouda labs are currently focused on using the combined approaches of photoaffinity labeling, molecular biology, and electrophysiology to identify the binding sites for a promising new class of potential therapeutic agents: positive allosteric modulators (PAMs). PAMs bind to site(s) distinct from the ACh (agonist) binding sites and may therefore provide the required receptor subtype specificity that avoids side-effects associated with current therapeutic agents. However, the molecular pharmacology of this class of compounds is unclear and specific PAM binding sites have not been unambiguously identified. Ongoing studies with radioactive and nonradioactive analogs of two promising PAMs, desformylflustrabromine (dFBr) and 3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrrazol-4-yl) isoxazole (CMPI) are focused on characterizing the binding, functional interaction, and identifying binding site determinants using affinity-purified human α4β2 nAChRs.

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