Department of Pharmacology & Neuroscience

Research Retreat

*Third Annual Translational Neuroscience Meeting*

Focus on Pain, Alcohol, and Neuroplasticity

October 17\textsuperscript{th} and 18\textsuperscript{th} 2017

Lubbock, Texas
Texas Tech University Health Sciences Center
and The Spirit Ranch

Supported by the Department of Pharmacology & Neuroscience and
the Center of Excellence for Translational Neuroscience and Therapeutics
Welcome to the third annual Research Retreat of the Department of Pharmacology and Neuroscience. The location of this year's meeting in close proximity to TTUHSC Lubbock allows for a two day event and participation of trainees and students. The Research Retreat continues to provide a unique opportunity for professional development through collegial interactions to inform about latest research accomplishments in our department, create new collaborative research projects, and shape goals and strategies for our department to succeed in our academic mission.

We are excited to welcome Dr. Mary M. Heinricher as our keynote speaker. Dr. Heinricher, a world leader in the neurobiology of pain modulation, comes to us from the Oregon Health Sciences University, where she holds the positions of Professor and Vice Chair for Research, Neurological Surgery, and Associate Dean, Basic Research, in the School of Medicine. Dr. Heinricher has kindly agreed to talk about "Linking pain transmission to pain modulation", and to participate in discussions of research development in our department.

As in previous years, a special thank you goes to Dr. Josée Guindon and Tiffany Denton for their invaluable help with the organization of this event and for creating the Research Retreat program booklet.

Your active participation will guarantee a successful research retreat. Please share your thoughts and insights, expectations and suggestions in our continued efforts to ensure this Department is a great place to work and succeed. Enjoy the meeting!

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
## Research Retreat Itinerary

### Tuesday Oct 17th 2017
*Events on this day will take place at Texas Tech University Health Sciences Center*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>12:00</td>
<td>Lecture from key note speaker, Dr. Mary Heinricher</td>
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<tr>
<td>1:00</td>
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<tr>
<td>1:30</td>
<td>Discussion for students and trainees with Dr. Mary Heinricher</td>
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<tr>
<td>3:00</td>
<td></td>
</tr>
<tr>
<td>3:30-5:30</td>
<td>Pharmacology and Neuroscience Lab Tours</td>
</tr>
</tbody>
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### Wednesday, Oct 18 2017
*Events on this day will take place at The Spirit Ranch*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:30</td>
<td>A lite breakfast will be available for attendees</td>
</tr>
<tr>
<td>9:00</td>
<td><strong>SWOT Analysis</strong></td>
</tr>
<tr>
<td>9:30</td>
<td><em>(Strength <em>Weakness</em> Opportunities _Threats)</em></td>
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<tr>
<td>11:00</td>
<td>Break</td>
</tr>
<tr>
<td>11:30</td>
<td>Lunch Together</td>
</tr>
<tr>
<td>12:30</td>
<td>Break and prepare for Research Proposal Presentations</td>
</tr>
<tr>
<td>1:00</td>
<td>Research Proposal Presentations (30 minutes each)</td>
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<tr>
<td>1:00-1:30</td>
<td>Dr. Susan Bergeson</td>
</tr>
<tr>
<td>1:30-2:00</td>
<td>Dr. Khalid Benamar</td>
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<tr>
<td>2:00-2:30</td>
<td>Dr. Josee Guindon</td>
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<tr>
<td>2:30-3:00</td>
<td>Break and prepare for Individual labs/research groups</td>
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<tr>
<td>3:00-5:00</td>
<td>Presentations by Individual Labs/Research Groups</td>
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<tr>
<td>3:00-3:20</td>
<td>Dr. Khalid Benamar (15 min. talk + 5 min. discussion)</td>
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<tr>
<td>3:20-3:40</td>
<td>Dr. Josee Guindon (15 min. talk + 5 min. discussion)</td>
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<tr>
<td>3:40-4:00</td>
<td>Dr. J. Josh Lawrence (15 min. talk + 5 min. discussion)</td>
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<tr>
<td>4:00-4:25</td>
<td>Drs. Guangchen Ji/Vadim Yakhnitsa (20min. talk + 5 min. discussion)</td>
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<tr>
<td>4:25-4:55</td>
<td>Drs. George Henderson/lenin Mahimainathan/Madhu Narasimhan (25 min. talk + 5 min. discussion)</td>
</tr>
<tr>
<td>5:00</td>
<td>Break for dinner setup</td>
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<tr>
<td>5:30-7:30</td>
<td>Dinner together</td>
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</tbody>
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Dr. Mary M. Heinricher obtained her Ph.D. in Behavioral Neuroscience at Northwestern University. She then moved to UCSF as a postdoctoral fellow, staying as a faculty member in the Department of Neurology until 1995, when she was recruited to OHSU. She is a basic scientist who studies how the brain controls pain.

Her work focuses on the physiological and pharmacological properties of brainstem neurons that modulate pain by enhancing or suppressing transmission of nociceptive information from the spinal cord up to the brain. Using a combination of electrophysiology, behavioral testing, and pharmacological and optogenetic/chemogenetic manipulation, her group showed that the neural basis for bi-directional control of pain from the brainstem is two physiologically and pharmacologically distinct classes of neurons: "OFF-cells," which exert a net inhibitory effect on nociception, and “ON-cells”, which have a facilitating action. Her laboratory is now starting to ask how these brainstem pain-modulating systems are engaged by higher centers, and how they are brought into play under physiological and pathophysiological conditions, e.g., during stress or in headache. She is also interested in how pain modulation is coordinated with other adaptive behavioral and physiological responses in order to match pain sensitivity to other priorities. Her work provides insights into the roles of positive feedback and top-down control mechanisms in clinically significant pain, as well as into mechanisms of analgesic drug action.

Supported by the Department of Pharmacology & Neuroscience and the Center of Excellence for Translational Neuroscience and Therapeutics
Pain/analgesia, drugs of abuse and/or HIV-1

Khalid Benamar

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Center of Excellence for Translational Neuroscience and Therapeutics
Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX

The focus of my research is on 1) the neurobehavioral effects (e.g. dependence, tolerance and analgesia) of drugs abuse (e.g. opioids, cannabinoids), 2) functional interaction between HIV-1 viral protein and drugs of abuse, and 3) development of new analgesic drugs. Neurobehavioral effects are evaluated in rat and mouse models using different endpoints such as physical dependence, withdrawal, psychological dependence, hyperactivity, analgesia tolerance, and body temperature regulation. Neurotransmitters (dopamine, serotonin, glutamate), nitric oxide, cyclooxygenase (COX-1 and 2), cytokines, chemokines, chemokine receptors and epigenetic modulators are targets of interest. Glial and neuron mechanisms are studied in brain areas relevant to dependence, addiction and/or pain, e.g., ventral tegmental area (VTA), nucleus accumbens (NAc), periaqueductal gray (PAG), and locus coeruleus (LC). Drugs are applied stereotaxically into selected brain areas or intracerebroventricular in vivo. In vivo microdialysis is used to determine neurochemical changes in the brain. Inflammatory (formalin and carrageenan) and neuropathic (e.g. HIV-1-related neuropathic pain, chronic constriction injury) pain models are established in my group.

Research projects.

Periaqueductal grey area (PAG), immune system and opioid analgesia. PAG is a key element of the descending analgesic system that modulates spinal nociceptive neurotransmission via the rostrum ventromedial medulla (RVM). My recent work provided new insights into how components of the immune system alter the capacity of the opioids to produce analgesia. We found that activation of selected chemokines in the PAG alters descending pain control and reduced the analgesic effectiveness of opioids.

The development of new analgesic drugs. The development of effective, non-addictive pain medication is a public health priority. The broad distribution of GPR55 in the CNS suggests its involvement in central physiology and pathology. In collaboration with Dr. Abood, we identified GPR55 we provided first evidence for analgesic activity of a GPR55 antagonist in the PAG.

Chemokines in the brain and neurobehavioral effects of cannabinoids. Chemokines and their receptors are found in several brain areas and may play a role in brain development but also in a number of disorders. We were the first to show a new function of chemokine systems in the brain, i.e., regulation of the neurobehavioral effect of cannabinoids.

HIV-1 and drugs of abuse. Our work pioneered the field of drugs of abuse in HIV-1. We discovered that HIV-1 viral protein (gp120) is a key player in the loss of analgesic effectiveness of methadone and morphine, and interacts with the opioid system in the PAG at cellular level.

Supported by NIH grants DA035926, DA031605, DA029414 and # 121034 from TTUHSC.
Translational Studies on Alcohol Use Disorder

Susan E. Bergeson

Department of Pharmacology and Neuroscience
Center of Excellence for Translational Neuroscience and Therapeutics
Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX

A large body of research, including our own genetical genomic studies, has shown that traits related to Alcohol Use Disorder are mediated, at least in part, through inflammation and innate immune pathways. We were the first to test minocycline, a tetracycline analog with known immune inhibitory action, for efficacy to reduce high alcohol consumption. A structure-function screen of several tetracycline analogs indicated that the C6'-H was necessary for therapeutic action. Additionally, tigecycline reduced binge and chronic alcohol consumption, alcohol withdrawal symptoms, as well as alcohol-elicited emotional and physical pain sensitization, although the latter in a sex-specific manner. Follow-up experiments of immune pathway component neutralization, gastrointestinal sterilization, and intracerebroventricular drug delivery showed that drug action occurred in the CNS rather than through PNS signaling. As a consequence, we chemically modified minocycline and showed that a loss of anti-biotic properties did not change efficacy to reduce high alcohol consumption. These studies were completed in mice and as rodents generally show a poor translational outcomes, we wished to use an improved animal model with a superior physiological match to humans. Therefore, we developed and tested a porcine model of AUD using the standard farm pig. Importantly, we showed that swine have better matched free-choice, excessive drinking and pharmacokinetic elimination of alcohol, as well as a strong response for minocycline treatment to specifically reduce alcohol consumption, but not water intake.

Supported by the Center of Excellence for Translational Neuroscience and Therapeutics – TTUHSC, NIH grant AA021142, TTU/TTUHSC Presidential Initiative, the Laura W. Bush Institute for Women’s Health and The Byran C. Miller, Jr. and Martha H. Miller Foundation.
Changes In The Estrous Cycle Following Cannabinoid Agonists Chronic Administration In An Optimized Chemotherapy-Induced Neuropathic Pain Model

Henry Blanton¹, Kelsey Donckels¹, Seth Brauman¹, Isabel Castro², Kevin Pruitt², and Josée Guindon¹,³

¹Department of Pharmacology and Neuroscience, ²Department of Immunology and Molecular Microbiology, and ³Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX USA.

Cannabis-like compounds have demonstrated antinociceptive properties in various chronic pain models. Synthetic cannabinoid such as CB1 (ACEA) and CB1/CB2 (CP55,940) agonists can modulate chronic pain perception. Indeed, they have previously been shown to alleviate chemotherapy-induced neuropathic pain. However, the impact of chronic administration of these compounds on the estrous cycle needs to be investigated. The goal of this study is to evaluate the role of these different (ACEA and CP55,940) cannabinoid agonists on the estrous cycle following chronic administration in a chemotherapy-induced neuropathic pain (cisplatin 5 mg/kg intraperitoneal and 4 % sodium bicarbonate subcutaneously weekly) mouse model. We evaluated the effects on the estrous cycle following chronic systemic administration of these different cannabinoid agonists (ACEA and CP55,940) in an optimized chemotherapy-induced neuropathic pain (cisplatin 5 mg/kg intraperitoneal and 4 % sodium bicarbonate subcutaneously weekly) mouse model. We tested the estrous cycle by daily vaginal lavage prior to daily injection with the different synthetic cannabinoids. Further staining of the slides with crystal violet and identification of the estrous cycle under the microscope following cell type identification enable the identification of the stage of the estrus cycle (proestrus, estrus, metestrus and diestrus). Our results suggest compound-specific effects, which may be influenced by hormonal changes and could be mediated by receptor selectivity. The nonselective CB1/2 agonist CP55,940 shifts the cycle towards metestrus - the infertile stage of the cycle. The CB1 selective agonist ACEA is also changing the estrus cycle towards metestrus. Further studies investigating the mechanism behind these compound- or receptor-specific influences on cycle progression is needed to fully appreciate the impact of physiological differences on hormonal responses and potential influence on pain perception. A better understanding of the cannabinoid-specific mechanisms responsible for changes in the estrous cycle and possible hormonal role in pain perception are mandatory to advance the development of long lasting, highly efficacious, and personalized pain therapies. A better understanding of the agonist and sex-specific mechanisms responsible for cannabinoid tolerance are mandatory to advance the development of long lasting, highly efficacious, and personalized pain therapies.

This work is supported by the CPRIT Foundation (Grant RR140008 (KP)), the NCI (Grant CA155223 (KP)) and Texas Tech University Health Sciences Center School of Medicine (Grant 121035 (JG)).
Identification of Positive Allosteric Modulator (PAM) Binding Sites in Neuronal Nicotinic Acetylcholine Receptors

Michael P. Blanton and Ayman K. Hamouda 1,2.

1Department of Pharmacology and Neuroscience, Texas Tech University HSC, Lubbock, TX, USA; 2Department of Pharmaceutical Sciences, Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, Kingsville, TX, USA

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-pyrazol-4-yl) isoxazole (CMPI) are focused on characterizing the binding, functional interaction, and identifying binding site determinants using affinity-purified human α4β2 nAChRs.

This project is supported by TTUHSC Intramural Funds #511763.
Amygdala Contribution to Phenotypic Differences in Fear Extinction and Pain

Guangchen Ji 1 and Volker Neugebauer 1, 2

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

Individual differences in pain and fear are well documented, but underlying mechanisms are largely unknown. Pain and fear may share neurobiological mechanisms such as plasticity in emotional networks. The amygdala plays a key role in fear learning and has emerged as an important node of emotional-affective aspects of pain and pain modulation. Pain is significantly associated with anxiety and depression and can impair prefrontal cortical function through an amygdala-mediated mechanism. Prefrontal cortical control of amygdala processing plays an important role in fear extinction learning, and dysfunction of this control system has been linked to impaired behavioral extinction and related disorders such as posttraumatic stress disorder (PTSD). Here we tested the hypothesis that fear extinction learning ability is an indicator of pain coping ability and could therefore predict the magnitude of neuropathic pain behaviors.

Fear conditioning and fear extinction were measured in adult male rats and were subsequently correlated with behavioral outcome measures (sensory thresholds, vocalizations, anxiety- and depression-like behaviors) before and after induction of neuropathic pain (spinal nerve ligation model). Auditory fear conditioning, cued 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 tests (5 CSs). The majority (80%) of rats showed a quick decline of freezing behavior during extinction training and retention (FE+) whereas 20% of the rats maintained a high level of freezing behavior (FE-). FE- rats showed decreased open-arm preference in the elevated plus maze (EPM), reflecting anxiety-like behavior, but there were no significant differences in sensory thresholds, vocalizations or depression-like behavior (forced swim test, FST) between FE+ and FE- types. After induction of the neuropathic pain model, both phenotypes developed increased mechanosensitivity but FE- rats showed a greater increase in vocalizations and anxiety- and depression-like behaviors than FE+ rats. Extracellular single unit recordings of amygdala neurons in phenotyped anesthetized rats showed greater neuropathic pain-related increases in background activity and in responses to innocuous and noxious mechanical stimuli (compression of the hindpaw with a calibrated forceps) in the FE- than FE+ type.

The data may suggest a positive correlation between extinction learning ability and neuropathic pain control through a mechanism that involves the amygdala.

Work in the authors’ laboratory is supported by NIH Grants NS038261 and NS081121 and by Pain Research Challenge Award - Virginia Kaufman Endowment Fund and Clinical & Translational Science Institute, University of Pittsburgh.
The Interneuron Energy Hypothesis in SLC13A5 Deficiency-Induced Epileptic Encephalopathy: Differential Roles of Low Intracellular and Excess Extracellular Citrate

Betty Zhang¹², Jani Manring³, Rui Wang¹, Jonathan Kopel³, Sabarish Ramachandran⁴, Vadim Yakhnitsa⁵, Volker Neugebauer³⁵, Vadivel Ganapathy⁴, and J. Josh Lawrence³⁵

¹Methodist Ladies’ College, Kew, Australia, ²Clark Scholars Program, Honors College, Texas Tech University, Lubbock, USA, ³Department of Pharmacology and Neuroscience, ⁴Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Centre-School of Medicine, Lubbock, USA and ⁵Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX USA.

SLC13A5 is a sodium-coupled citrate transporter that is expressed in the plasma membrane of cells in the liver, testes, and brain. Loss-of-function mutations of SLC13A5 cause severe early onset epileptic encephalopathy that is accompanied by characteristic teeth hypoplasia and global developmental delay. Human SLC13A5 has low affinity for citrate transport, whereas the mouse Slc13a5 has high affinity. Slc13a5-knockout mice possess teeth hypoplasia but it is not clear whether these mice are susceptible to seizures induced by loss-of-function of SLC13A5. To better understand neuronal consequences of different SLC13A5 isoforms, transgenic C57Bl/6 mice carrying the human SLC13A5 gene (hSLC13A5 mice) were generated. We first demonstrated that SLC13A5 protein was present in the mouse hippocampus via Western Blot analysis. Immunohistochemical staining using 3’3’-diaminobenzidine tetrahydrochloride (DAB) in hSLC13A5 and control mice detected SLC13A5 expression for human and mouse SLC13A5 isoforms, respectively. Similar staining patterns were observed for both isoforms, suggesting no variations in the localisation of SLC13A5 expression. SLC13A5 was highly expressed in the principal cell layers of the Cornu Ammonis (CA) and Dentate Gyrus (DG) regions which correlates with mRNA expression (Inoue et al.2002). For the first time, we demonstrate expression of SLC13A5 in inhibitory neurons, specifically GABAergic interneurons in the stratum oriens and stratum radiatum layers of CA1 and Hilus of DG. Surprisingly, we also discovered populations of SLC13A5-negative inhibitory neurons, suggesting that SLC13A5 expression was cell type-specific. In kinetic simulation experiments, we show that high extracellular citrate induces NMDA receptor hyperfunction through zinc chelation. Our results suggest that expression of SLC13A5 contributes to neuronal function and its loss of function in SLC13A5 deficiency results in excitation-inhibition imbalance from reduced inhibition and increased excitation due to low intracellular and high extracellular citrate, respectively. The differential expression of SLC13A5 in hippocampal inhibitory neuron subpopulations suggests vulnerability of specific interneuron types, implying a complex relationship between SLC13A5 function, excitation-inhibition balance and seizure susceptibility.

This research was supported by NIH grant NS069689 and TTUHSC start-up funding grant #121041.
In Utero Ethanol (E) Suppresses Excitatory Amino-Acid Carrier 1 (EAAC1)-Mediated Cysteine Transport in a Rat Fetal Alcohol Syndrome Model

Lenin Mahimainathan, Madhusudhanan Narasimhan, Marylatha Rathinam, Dhyanesh Patel, George Henderson

Department of Pharmacology and Neuroscience
Center of Excellence for Translational Neuroscience and Therapeutics
Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX

Background
Redox-shift related alterations in glutathione (GSH) homeostasis and apoptotic death of neurons in developing brain are connected events central to fetotoxic responses to in utero E exposure. Although this is accompanied by an up-regulation of Nrf2, a cytoprotective transcription factor, a complete protection is not achieved and fetal neurons still succumb to E-induced apoptotic death. New studies illustrate that an underlying mechanism is limited availability of cysteine (Cys), a control point for GSH synthesis, which reduces intracellular GSH levels. This is likely due at least in part, to an E-related decrease in Cys inward transport by the excitatory amino acid transporter EAAT3/EAAC1.

Methods
Two models were utilized. The In utero binge model consists of administration of isocaloric dextrose control or 20% E (3.5 g/kg) by gastric gavage at 12 hour intervals to pregnant SD rats, starting gestation day (gd) 17 with a final dose on gd19, 2 hours prior to sacrifice. The second model utilized cultured cerebral cortical neurons (PCNs) prepared from E16-E17 fetal SD rats. The primary neurons were treated with E (4 mg/ml), with appropriate controls.

Results
E reduced both PCN and cerebral cortical GSH and Cys up to 50% and the abridged GSH could be blocked by augmentation of available Cys by administration of N-acetylcysteine. E reduced EAAC1 protein expression in utero and in PCNs significantly (p<0.05). This was accompanied by a 60-70% decrease of neuron surface EAAC1 expression and significant reductions of EAAC1/Slc1a1 mRNA (27% within 6 hours of E and 49% by 24 hours, p<0.05). In PCNs, knockdown of EAAC1 caused significant decreases in GSH but not GSSG illustrating while not the sole provider of cys, the EAAC1 transporter plays an important role in neuron GSH homeostasis.

Conclusions
These studies strongly support the concept that in both E exposed intact brain and cultured PCNs a mechanism underlying E impairment of GSH homeostasis (and resulting apoptotic neuron death) is reduction of import of external Cys which is mediated by perturbations of EAAC1 expression/function.

This research was supported by NIAAA, RO1 (5RO1 AA010114-20) (GIH).
Prenatal Ethanol (E) Alters Cysteine Synthesis with GSH Defects in Rat Fetal Brain: A Role for Cystathionase (CSE)

Madhusudhan Narasimhan, Lenin Mahimainathan, Marylatha Rathinam, Dhyanesh Patel, George Henderson

Department of Pharmacology and Neuroscience
Center of Excellence for Translational Neuroscience and Therapeutics
Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX

Background
Previously, our laboratory showed that Nrf2, an important regulator of glutathione (GSH), is upregulated by ethanol (E) in the developing brain. Despite the increase in Nrf2, E-induced altered GSH homeostasis and oxidative stress-induced death of fetal neurons are incompletely protected. We have found that this is due to an impaired supply of Cysteine (Cys), a control point for GSH synthesis. While E inhibits transport of Cys via EAAC1 (unpublished), it also inhibits the synthesis of Cys by the transsulfuration pathway which is shown below to be a relevant factor in E damaged GSH homeostasis.

Methods
We used an in utero E binge model where the pregnant dams received a 20% E solution (3.5 g/kg) by gastric intubation every 12 hour beginning gestation day (gd) 17 with a final dose on gd19, 2 hours prior to sacrifice. The in vitro model was culture of cerebral cortical neurons prepared from E16-E17 fetuses (SD rats). E concentrations were 4 mg/ml. Methodologies used were (i) Enzyme activity assay for CSE (ii) GSH/GSSH determinations by GSH/glo assay (iii) CSE expression by Rt qPCR and immunoblotting (iv) Cystathionine levels by HPLC analysis.

Results
E decreased cystathionine-γ-lyase (CSE) mRNA expression in PCNs (32% reduction within 6 hours of exposure, p<0.05) and in fetal brain (33%, p<0.05). Concomitantly, E significantly decreased CSE protein expression in PCN (p<0.05) and in fetal cerebral cortex in utero (70%, p<0.05). In PCN, E reduced CSE activity by 41% within 24 hours of E. In parallel, E induced accumulation of cystathionine suggesting that lesions in CSE cannot break down cystathionine, a required process for Cys supply. Knock down of CSE in PCNs mimicked E-induced decrease in GSH/GSSG ratio (48%, with no effect on GSSG). The mechanism(s) involving CSE on E-induced impairment in GSH pathway are currently being investigated using CSE knockout mice.

Conclusions
In summary, these studies illustrate the importance of the transsulfuration pathway involving CSE-in maintenance of GSH via Cys synthesis in neurons and fetal brain. E dysregulates this process by inhibition of CSE at a transcriptional level. This supports the concept that E-impaired GSH synthesis is partially independent of Nrf2, also reflecting CSE/Cys perturbations.

Our work was supported by NIH/NIAAA grant RO1 (5RO1 AA010114-20).
Amygdala Contribution to Phenotypic Differences in Fear Extinction and Pain

Vadim Yakhnitsa, Takaki Kiritoshi, Jeremy Thompson, Lenin Mahimainathan and Volker Neugebauer

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

Chronic pain is a critical health care problem. Pain has a strong emotional component and is defined by its unpleasantness. A limbic brain region, the amygdala plays a key role in emotions and affective disorders and has emerged as an important node in the emotional-affective aspects of pain and pain modulation. Amygdala hyperactivity in pain conditions drives pain behaviors, anxiety, depression and cognitive deficits. Underlying mechanism include an imbalance between excitatory drive and inhibitory cortical control of amygdala processing. What remains to be determined is how this imbalance generates increased amygdala output. Here we tested the new hypothesis that dysfunction of small-conductance calcium-activated potassium (SK2) channels contributes to neuropathic pain-related maladaptive amygdala plasticity and behaviors. SK channels can inhibit neuronal excitability through actions that include mediating the medium afterhyperpolarization (mAHP), shunting excitatory and enhancing inhibitory transmission.

Neuropathic rats (spinal nerve ligation model, SNL) showed increased audible (nocifensive response) and ultrasonic (affective response) vocalizations and mehanosensitivity (von Frey test) compared to sham controls 4 weeks after surgery. Stereotaxic administration of an SK channel blocker (apamin) into the CeA by microdialysis increased vocalizations in sham but not SNL rats. Patch-clamp recordings of regular firing lateral-capsular CeA neurons in brain slices from SNL rats found reduced mAHPs, increased action potential frequency-current (F-I) relationship, and enhanced excitatory synaptic transmission. Apamin blocked the mAHP and increased excitability and excitatory transmission in brain slices from sham but not SNL rats, indicating that SK channel activation is lost in the pain state. Western blotting and reverse transcription polymerase chain reaction (RT-PCR) detected decreased levels of SK2 subunit protein and mRNA in the amygdala of SNL compared to sham rats, suggesting pretranscriptional SK channel downregulation. To rescue SK2 channel function, an AAV viral vector construct with synapsin promoter-driven expression of the tetracycline transactivator protein and tet-CMV promoter-driven expression of SK2 channels was injected into the CeA. Viral-vector mediated SK channel expression restored the apamin-sensitive mAHP and inhibited excitability (F-I relationship) in CeA neurons; and this intervention also decreased vocalizations, mechanical sensitivity, anxiety-like (elevated plus maze) and depression-like (sucrose preference) behaviors compared to SNL rats without AAV vector treatment.

The data suggest that SK channel dysfunction contributes to maladaptive neuropathic pain-related amygdala plasticity and behavior; restoring functional SK channels is a rescue strategy.

Work in the authors’ laboratory is supported by NIH Grants NS038261 and NS081121 and by Pain Research Challenge Award - Virginia Kaufman Endowment Fund and Clinical & Translational Science Institute, University of Pittsburgh.