The Second Annual Meeting of the Upstate New York Pharmacology Society (UNYPS) of ASPET

Monday, May 13, 2013
University at Buffalo Center for the Arts
Buffalo, New York

Report from the 2nd Annual Scientific Meeting

The Upstate New York Pharmacology Society (UNYPS) Chapter of ASPET held its 2nd Annual Scientific Meeting on May 13, 2013 at the University at Buffalo Center for the Performing Arts in Buffalo, NY. The theme of the meeting was entitled Frontiers in Neuropharmacology with principal addresses by the Keynote Speaker David R. Sibley PhD, and by special invited investigators under the Frontiers of Neuropharmacology theme Lynn Wecker PhD, Margaret Gnegy PhD, and Steve Traynelis PhD.

Over 100 pharmacologists attended including principal investigators, principal scientists, postdoctoral fellows, graduate and undergraduate students from the University of Toronto, Albany College of Pharmacy and Health Sciences, the University of Rochester, Roswell Park Cancer Institute, D’Youville College, Albany Molecular Research Institute, EMD Millipore, Invitrogen Life Technologies, Bio-Rad, and the University at Buffalo Schools of Pharmacy, Arts and Sciences, Medicine and Biomedical Sciences, and Office of Research and Economic Development.

Previously selected graduate students delivered oral presentations of their research in the opening Presidential Graduate Student Symposium. The meeting ended with distribution of awards to student presenters and the best poster presenters as judged by a diverse panel of judges and with the announcement of the future president-elect, Gregory G. Tall PhD of the University of Rochester.

Frontiers in Neuropharmacology UNYPS 2013 Program:

8:00 – 9:00AM Registration and Continental Breakfast – Center for the Arts Atrium
8:00 – 1:00PM Poster Sessions (3) – Center for the Arts Atrium. Thirty posters were presented, including 18 doctoral students, 7 master’s students, and 5 undergraduate students.
8:00 – 1:00PM Vendor Exhibits – Center for the Arts Atrium
9:00 – 9:15AM Welcome and Opening Remarks, Peter Bradford, University at Buffalo, Meeting Organizer and UNYPS Secretary-Treasurer – Center for the Arts Screening Room
9:15 – 10:15AM Graduate Student Symposium – Moderator Kim Bernosky-Smith, D’Youville College
9:15 – 9:30AM Pieter Beerepoot, University of Toronto, Identification and characterization of pharmacological chaperones of the dopamine transporter
9:30 – 9:45AM Bharti Patel, University of Rochester, Ric-8A deletion as tumor suppressor of oncogenic G-protein alpha subunit alleles
9:45 – 10:00AM  **David Thorn,** University at Buffalo, *Effects of imidazoline I2 receptor agonist 2-BFI on the development of tolerance and physiological/behavioral dependence to morphine in rats*

10:00 – 10:15AM  **Catharine Mielnik,** University of Toronto, *Characterization of neuronal activation responses to social stimulation in a genetic model of reduced NMDA receptor function*

10:15 – 11:00AM  Poster Review, Coffee break, fruits and muffins, Center for the Arts Atrium

11:00 – Noon  **Keynote Address, David R. Sibley,** National Institute of Neurological Disorders and Stroke NIH, *High throughput screening approaches for identifying novel dopamine receptor modulator*

Noon – 1:00PM  Lunch, Poster Review, and Vendor Exhibits – Center for the Arts Atrium

1:00 – 3:00PM  **Frontiers in Neuropharmacology** Symposium – Moderator **Suzanne Laychock,** University at Buffalo – Center for the Arts Screening Room

1:00 – 1:40PM  **Lynn Wecker,** University of South Florida  *Regulation of neuronal nicotinic receptors and their role in neurological disorders*

1:40 – 2:20PM  **Margaret Gnegy,** University of Michigan  *How protein kinase C beta inhibitors slow “speed” and regulate extracellular dopamine*

2:20 – 3:00PM  **Steve Traynelis,** Emory University  *Effects of potential disease-causing mutations on NMDA receptor function.*

3:00 – 3:30PM  Concluding Remarks, Business Meeting, and Awards Presentation, **Peter Bradford**  UNYPS Secretary-Treasurer - Election Results (**Gregory Tall,**) University of Rochester, elected President-Elect; Awards (Peter Bradford, Judging Coordinator)

A panel of twelve judges previewed all abstracts and posters and teams of judges interviewed all poster presenters. Awards and cash prizes were given to the following top-ranked posters:

**PhD Graduate Students**

**Hannah Stoveken,** Pharmacology and Physiology, University of Rochester  
*Biochemical reconstitution of adhesion GPCR GPR56 activation of heterotrimeric G proteins*

**Meaghan Paganelli,** Neuroscience Program, University at Buffalo  
*Molecular mechanisms of local anesthetic inhibition of NMDA receptors*

**Vincent Lam,** Pharmacology and Toxicology, University of Toronto  
*Development of a new homogenous assay for quantitative measurement of surface expression of membrane proteins*

**Shannon Clough,** Neuroscience Program, University at Buffalo  
*Methamphetamine-induced conditioned place preference in C3H/HeN mice is observed during the day (ZT 6-8) but not at night (ZT 19-21)*

**Master’s Students**

**Taylor Warren,** Pharmacology and Toxicology, University at Buffalo  
*Pre-exposure of the urotensin II receptor to ligand differentially reduces the response to subsequent additions of urotensin II or urotensin II-related peptide*

**Katie Evely,** Pharmacology and Toxicology, University at Buffalo  
*Characterization of MT1 melatonin receptor-expressing neurons in the medial habenula, habenula commissure and periaqueductal grey of the C3H/HeN mouse brain*

**Undergraduate Students**

**Danielle Precourt,** Pharmacology and Toxicology, University at Buffalo  
*Melatonin modulation of novel object recognition*

**Jason Ma,** Pharmacology and Toxicology, University at Buffalo  
*MT1 melatonin receptor role in methamphetamine-induced locomotor sensitization in C57BL/6 mice*
Photo Gallery from the ASPET Upstate New York Pharmacology Society meeting – 13 May 2013

UNYPS ASPET 2013 Welcome Poster

Danielle Precourt of UB - one of the Undergraduate Poster Award Winners

Shannon Clough of UB - Doctoral Student Poster Award Winner

UNYPS ASPET Symposium organizer Peter Bradford welcomes participants

Margaret Folaron of the Roswell Park Cancer Institute

David R. Sibley of NINDS UNYPS ASPET Keynote Speaker

University at Buffalo Center for the Arts hosted the 2nd annual UNYPS Meeting

Taylor Warren with UB Associate VP for Research Ken Tramposch

Suzanne Laychock - Current UNYPS President-Elect
Research Abstracts from the 2013 ASPET Upstate New York Pharmacology Society meeting

Frontiers of Neuropharmacology Keynote Address

A1. High Throughput Screening Approaches for Identifying Novel Dopamine Receptor Modulators

David R. Sibley
Molecular Neuropharmacology Section, National Institute of Neurological Disorders and Stroke, NIH

We have recently used high throughput screening approaches to identify novel modulators of various dopamine receptor (DAR) subtypes. We are particularly interested in identifying allosteric ligands, as these have the potential to be more highly selective than currently available therapeutic agents, which are orthosteric in nature. There are various ways in which G protein coupled receptors can be modulated by allosteric ligands, which include affinity and efficacy modulation as well as the possibility for allosteric agonism. Allosteric agents also offer the possibility for large therapeutic windows, the potential for partial antagonism and as well as less or no desensitization for allosteric agonists. As part of our probe discovery program, we screened ~400,000 compounds using an assay that can detect ligands with agonist (allosteric or orthosteric), potentiator (allosteric), or antagonist (allosteric or orthosteric) activities. A wide range of compounds were detected and evaluated and one scaffold with a unique pharmacological profile at the D2 and D3 receptors is reported here. Using a beta-arrestin recruitment assay to compare activity at all DAR subtypes, we identified a ligand, MLS3508 (compound 3508), that selectively activates the D2R but not other DAR subtypes. Compound 3508 is an antagonist at the D3R for beta-arrestin recruitment and has no activity at the D4R or D1-like DARs (D1R and D5R). Compound MLS3508 exhibits full agonist activity with EC50 values ranging from 100 nM – 1 μM in three different functional assays for the D2R: beta-arrestin recruitment, Ca2+ mobilization, and inhibition of cAMP accumulation. Using a Go BRET activation assay, we found that MLS3508 is a full agonist at the
D₂R but displays weak partial (<10%) agonist activity at the D₃R. Interestingly, MLS3508 is a full antagonist with no agonist activity on D₂R-linked or D₃R-linked GIRQ channel activation, indicating that it is a biased agonist. This is most striking for the D₂R, at which MLS3508 is a full agonist at all the other pathways evaluated. Consistent with our studies in heterologous cells, application of MLS3508 elicited no response in D₂R-activated, whole cell GIRK-mediated currents measured in dopaminergic neurons in mouse midbrain slices, while it effectively blocked the response elicited by the full agonist quinpirole. Molecular modeling studies suggest subtle differences in MLS3508 binding poses to the D₂R and D₃R that may underlie its functional properties. In summary, MLS3508 is a full and selective agonist at both G-protein-linked and beta-arrestin-mediated D₂R signaling pathways; however, it is an antagonist for D₃R GIRK activation, indicating biased agonism. In contrast, because of its lack of agonist efficacy, MLS3508 functions as a potent D₂R antagonist. This is the first compound identified that can selectively stimulate the D₂ DAR, with no D₃ DAR stimulation, or can selectively block the D₃ DAR, with no D₂ DAR blockade.

Frontiers of Neuropharmacology Invited Speakers

A2. Regulation of Neuronal Nicotinic Receptors and their Role in Neurological Disorders

Lynn Wecker

Psychiatry and Behavioral Neuroscience, U.South Florida Morsani College of Medicine, Tampa, FL 33613

Nearly 15 years ago, studies indicated that the surface expression of α4β2 neuronal nicotinic receptors and their recovery from inactivation was altered by inhibitors of both cyclic AMP-dependent protein kinase (PKA) and protein kinase C (PKC). However, it was unknown whether these effects were mediated by direct phosphorylation of the receptor itself or post-translational modification of another protein involved in receptor expression or function. Thus, studies determined whether α4 subunits isolated from α4β2 receptors were phosphorylated, and if so, which kinases and amino acids were involved. Using a multitude of approaches, results indicated that: both rat and human α4 subunits are phosphorylated by PKA and PKC; multiple phosphorylation sites reside on both serine and threonine residues within the major M3/M4 cytoplasmic domain of the protein; PKA and PKC phosphorylate both common and unique sites within the major (M3/M4) intracellular domain of the subunit; and PKA phosphorylates serines 362 and 467, whereas PKC phosphorylates serine 550 and to a lesser extent serine 362. Further, in the basal state, α4 subunits from mature pentamers (in the plasma membrane) are phosphorylated to a greater extent than immature forms of the subunit, and stimulation of PKA enhances the phosphorylation of both free and immature subunits on serine residues 467 and 362, but does not affect phosphorylation in the mature state, whereas stimulation of PKC enhances phosphorylation of subunits at all stages of maturation on several amino acid residues with serine 550 phosphorylated in the immature and mature, but not the free state.

Sustained exposure to nicotine appears to activate PKC, leading to the phosphorylation of immature α4 subunits, enhancing receptor subunit assembly and receptor maturation, resulting in an up regulation of α4β2 receptors. Because nicotine-induced up regulation of these receptors appears to mediate tolerance and addiction to nicotine, PKC may represent a potential target for modulating the effects of nicotine on the brain. Further, considering that α4β2 neuronal nicotinic receptors have been implicated to have a functional role in several neuropsychiatric disorders including addiction, depression, epilepsy, schizophrenia and Parkinson's disease, understanding the role of PKC in modulating receptor function is critical. (These studies were supported in part by a grant from the National Institute of Drug Abuse of the National Institutes of Health under award number R01DA14010.)

A3. How Protein Kinase C beta Inhibitors Slow “Speed” and Regulate Extracellular Dopamine

Margaret Gnegy

Departments of Neuroscience and Pharmacology, University of Michigan, Ann Arbor, MI 48109
Reinforcing properties of amphetamines depend on the level of extracellular dopamine (DA), which is regulated by DA release, the dopamine transporter (DAT) and dopamine D2-like autoreceptors. We find that protein kinase C (PKC), especially PKCβ, significantly affects both DAT and DA autoreceptor activity in ways that would enhance extracellular DA. Inhibition or deletion of PKCβ, which complexes with DAT, reduces amphetamine-stimulated DA efflux and amphetamine-stimulated locomotor and rewarded behaviors. In addition to enhancing DA efflux through the DAT, PKCβ regulates communication between DAT and D2 receptors. It is well known that D2 receptor activation will increase surface DAT, but we find that DAT itself, acting through PKCβ, regulates D2 trafficking. In heterologous N2A neuroblastoma cells, we found that DAT and PKCβ suppress D2R surface localization as compared to D2R-vector cells. This regulation depends on PKCβ substrate sites in both DAT and D2Rs. Further, we find that PKCβ regulates DA autoreceptor function by reducing dopamine autoreceptor-mediated inhibition of exocytosis. We posit that inhibition of PKCβ would reduce the concentration of extracellular DA in response to amphetamine by reducing outward transport and by enhancing dopamine autoreceptor function. Inhibition of PKCβ could serve a therapeutic function.

A4. Effects of Potential Disease-Causing Mutations on NMDA Receptor Function
Stephen F. Traynelis and Hongjie Yuan
Department of Pharmacology, Emory University, Atlanta, GA 30322
NMDA receptors are ligand-gated ion channels that mediate a slow, Ca²⁺-permeable component of excitatory synaptic transmission. These receptors are involved in many normal brain functions, including development, learning, and memory. In addition, aberrant NMDA receptor activation has been proposed to be involved in numerous neuropathological conditions such as Alzheimer’s disease, Parkinson’s disease, schizophrenia, treatment-resistant depression, stroke-induced damage, and epilepsy. We have begun to investigate whether mutations within the various NMDA receptor subunits from patients with neurological conditions can alter channel function in ways that could be meaningful for the clinical symptoms of patients. In collaboration with the Undiagnosed Disease Program at NIH, we have studied a number of NMDA receptor mutations identified in patients with neurological complications that included developmental delay and seizures. We describe here one GluN2A mutation that enhances NMDA receptor function in a young patient showing signs of neurodegeneration as well as intractable seizures. The effects of this mutation are consistent with it contributing to the clinical symptoms observed for this patient.

Frontiers in Neuropharmacology Presidential Graduate Student Symposium Oral Presentations
A5. Identification and characterization of pharmacological chaperones of the dopamine transporter
Pieter Beerepoot, A. Ramsey, and A. Salahpour; Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON, MSS 1A8
Hereditary DAT deficiency syndrome is a recently discovered rare pediatric condition that is caused by loss-of-function mutations in the DAT. The disorder is characterized by parkinsonism-dystonia and raised CSF dopamine metabolites. When expressed in vitro, the DAT missense mutations reduce or eliminate dopamine uptake as well as preventing DAT protein maturation. We propose that the mutations result in ER retention of an otherwise functional DAT, which could potentially be rescued by using pharmacological chaperones. Compounds that increased surface expression of WT DAT and mutant DAT (G585A and D600A) HEK-293 cells were identified using a β-lactamase-reporter assay, after which effects on DAT protein and function were assessed using western blotting and a dopamine uptake assay respectively. Heterozygous DAT-knockout (DAT-HET, basal DAT levels 50% of DAT in WT mice) mice were treated daily with a putative pharmacological chaperone for a period of two weeks followed by a 1-day washout. Locomotor response to an amphetamine challenge was measured after which animals were
sacrificed. DAT protein levels were assessed by performing western blotting on striatal tissue lysates. We tested a number of known DAT ligands and have identified compounds that can promote maturation of both WT and mutant DAT in vitro, although DAT deficiency syndrome relevant mutations have so far not been tested. Subsequently, we examined the effect of a putative pharmacological chaperone in vivo and our data show that sub-chronic (2-week) treatment can increase striatal DAT protein in DAT-HET mice. Our data suggest that it is possible to increase DAT protein and function using a pharmacological chaperoning approach. Pharmacological chaperones for DAT could be used as a potential treatment to rescue DAT function in DAT deficiency syndrome.

A6. Ric-BA deletion as tumor suppressor of oncogenic G-protein alpha subunit alleles

Bharti Patel and Gregory G. Tall; Department of Pharmacology and Physiology, University of Rochester, Rochester, NY 14642

Constitutively-active, GTPase defective G protein alpha (Gα) subunit mutants are implicated to cause a variety of disease. For instance, GNAQ/11-Q209L mutants were recently found in 83% of human ocular melanomas (OM). There are no therapeutics that specifically target disease-driving mutant G proteins. Ric-8A and Ric-8B proteins are the molecular chaperones specifically required for Gα folding during protein biosynthesis. We hypothesize that blocking Ric-8 function will be a useful therapy to attenuate the abundance of mutant, disease-causing G proteins. To address this, we generated a Ric-8A conditional knockout mouse to study the effect of Ric-8A gene deletion on suppression of GNAQ/11-Q209L-driven established mouse model of the disease. Ric-8A-targeted embryonic stem cell lines from KOMP were used to generate a Ric-8A chimeric mouse with a knockout-first Ric-8A Neo allele. Flp-recombinase breeders were used to convert the null-Neo allele to a conditional Flxed-allele. Homofloxed Ric-8A mouse embryonic fibroblasts (MEFs) were isolated and stably transduced with a lentivirus that express Cre recombinase. Cre-mediated recombination excised Ric-8A exon-5, as expected and resulted in production of a Ric-8A truncated protein that does not function to fold G-proteins. Using MEFs isolated from Ric-8A homofloxed embryos we demonstrate that Cre-mediated recombination successfully induced a Ric-8A knockout at the genetic and protein levels. Significant decreases in levels of endogenous Gai/o and Gaq/11 family of Gα subunits were observed in Ric-8A knockout MEFs. These results confirm the expected phenotype of reduced functional Gα abundance in Ric-8A absence. We generated a Ric-8A conditional knockout mouse and showed Ric-8A-knockout dependent decrease in Gα subunit abundance. Our Ric-8A conditional knockout mouse will be used to investigate the efficacy of Ric-8A-gene deletion suppression of oncogenic G protein allele-driven disease in established mouse models. The proposed work will establish the tenability of Ric-8A as a drug target for diseases caused by mutant G proteins.

A7. Effects of imidazoline I2 receptor agonist 2-BFI on the development of tolerance and physiological and behavioral dependence to morphine in rats

David A Thorn and Jun-Xu Li; Department of Pharmacology and Toxicology, University at Buffalo, Buffalo NY 14214-3000

Pain is a significant health care challenge and current pharmacotherapy cannot meet increasing clinical needs. Opioids are the drugs of choice for many painful conditions, particularly moderate to severe pain. Accumulating evidence indicates that imidazoline I2 receptor agonists enhance the antinociceptive effects of opioids and therefore may be suitable for combination therapy with opioids for pain treatment. However, little is known of the effects of I2 receptor agonists on the untoward effects of opioids, such as the development of tolerance and physical dependence. In this study, two groups of rats (n=9/group) were trained to lever press for sucrose (10%) under a FR10 schedule. Using a cumulative dosing procedure, the rate-suppressing effects of the μ opioid receptor agonist morphine,
the imidazoline I2 receptor agonist 2-BFI and the μ opioid receptor antagonist naltrexone were examined each week in rats treated with either (20 mg/kg, s.c.) morphine or (10 mg/kg) 2-BFI plus (20 mg/kg) morphine per day for 3 weeks. Chronic morphine administration induced significant tolerance to the rate-suppressing effects of morphine as demonstrated by a greater than 6-fold increase in the ED50 value, while the chronic administration of 2-BFI plus morphine resulted in a less than 4-fold shift of the morphine ED50 value. In addition, chronic administration of morphine resulted in the development of physical dependence, as evidenced by a marked increase in the sensitivity to the rate-suppressing effects of naltrexone as well as significant body weight loss following the naltrexone test session. Rats treated with daily 2-BFI plus morphine exhibited significantly less naltrexone-induced body weight loss and sensitization to the rate-suppressing effects. Taken together, these results indicate that 2-BFI attenuated the development of tolerance and physical dependence to morphine and further support the therapeutic potential of combining I2 receptor agonists and opioids of pain treatment.

A8. Characterization of neuronal activation responses to social stimulation in a genetic model of reduced NMDA receptor function
Catharine A. Mielnik1*, Marija Milenkovic1, Caroline Kim2, Amy J. Ramsey1; 1Pharmacology and Toxicology, University of Toronto, Toronto, ON M5S 1A8; 2Cell Biology, Duke University, Durham, NC
One of the most devastating and consistently reported symptoms in schizophrenia is the loss of social cognitive skills and there remains a lack of effective treatment for social dysfunction. Therefore, it is imperative to gain a more comprehensive understanding of the neurobiological substrates of social behavior to allow for suitable treatment. The NR1-KD mouse model expresses reduced levels of the NR1-subunit of the NMDA receptor and show deficits in social behavior. The NR1-KD mouse model can have heuristic value in understanding the underlying neurobiology of social interaction deficits that are present in those who suffer from schizophrenia, which can be observed as deficits in species-specific social behavior. We aim to determine which brain regions are selectively activated in response to social stimulation and to determine whether differences in neuronal activation could be observed in mice that display reduced sociability. Sociability was measured with a modified “three-chamber sociability test” where the test mouse was exposed to a novel mouse as social stimulus. The amount of time spent in social investigation over a ten-minute period was determined using videotracking software. Neuronal activation was subsequently quantified by c-fos immunoreactivity one hour after exposure to social stimulus. Clozapine was administered one hour before measurement of sociability in wildtype and NR1-KD mice. Several brain regions showed an increase in activation that was selective for exposure to social stimulus: cingulate cortex, lateral septal nuclei, hypothalamus, and amygdala. NR1-KD mice displayed a reduction in social behavior and activation in the two brain regions quantified, the cingulate cortex and septal nuclei. Reduced sociability was more pronounced in adult NR1-KD mice than in younger mutants. Low dose of clozapine did not significantly alter sociability in wild-type or mutant mice. Our studies highlight the role of the cingulate cortex and septal nuclei in affiliative social behavior. The decrease in neuronal excitability in adult NR1-KD mice likely indicates that activation of these regions is important for the expression of affiliative interaction.

UNYPS 2013 – Poster Presentations
A9. Molecular mechanisms of local anesthetic inhibition on NMDA receptors
Meaghan A. Paganelli and Gabriela K. Popescu; Department of Biochemistry, Neuroscience Program, University at Buffalo, Buffalo, NY 14214
Local anesthetics are widely used in clinical practice to prevent and alleviate pain during surgery. Recently, it has been demonstrated that aside from impeding the generation of action potentials by blocking sodium channels, local anesthetics may also affect N-methyl-D-aspartate (NMDA) receptor
currents, which are critical mediators of synaptic plasticity. Importantly, local anesthetics inhibited NMDA receptor-mediated synaptic transmission in the dorsal horn, a spinal cord region involved in central sensitization. To evaluate local anesthetics’ effects on NMDA receptor responses, we recorded single-channel activity form HEK 293 cells transiently transfected with GluN1/GluN2A receptors. Records were obtained in the absence of divalent cations (1 mM EDTA). In these conditions, we observed that bupivacaine, an amide-class local anesthetic, decreased channel open probability in a concentration dependent manner, in which increasing concentrations caused both an increase in the duration of closed events and a subsequent decrease in the duration of open events. Similar potency was observed for both GluN2A and 2B isoforms. Further, we found that in the presence of bupivacaine, but not in its absence, open durations increased with depolarization, an indication of possible voltage-dependent block. However, a mutation that eliminates NMDA receptor voltage-dependent sensitivity to magnesium and zinc, maintained wild type-like sensitivity to bupivacaine. Based on these results we suggest that local anesthetics may act at a different site than divalent cationic pore blockers.

A10. Inhibition of GluN2A-containing NMDA receptors by 2-naphthoic acid
Han Yu and Gabriela K. Popescu; Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214
NMDA receptors (NRs) mediate excitatory synaptic transmission in central nervous system and play important roles in development and synaptic plasticity, but also mediate glutamate neurotoxicity. Recently, 2-naphthoic acid (NPA) and its derivatives have been identified as allosteric, noncompetitive NR inhibitors. The selectivity of NPA derivatives among NR subtypes was mapped structurally to the ligand-binding domain (LBD), and was proposed to be mediated by residues on the S1 segment. The wide range of its selectivity among NRs gives its derivatives great potential in both experimental and clinical applications, so it is important to delineate the kinetic mechanism by which NPA inhibits NR activity. We used whole-cell and cell-attached single-channel patch clamp on HEK293 cells expressing recombinant GluN1/GluN2A. Kinetic modeling was used to investigate the effects of NPA on the channel gating. We found that NPA has 50% inhibitory effect at 1.9 mM. Further, from one-channel current recordings, we found that 4 mM NPA caused a 62% decrease in open probability by decreasing mean open time 2.5-fold and by increasing mean closed time 2-fold. Kinetic modeling suggested that NPA binding stabilizes NR closed states and increases the energy barriers toward open states, causing NRs to dwell longer in pre-open states along the activation pathway. The reaction mechanisms we derived provide quantitative insight into the inhibitory mechanism of NPA, and help anticipate its effects on GluN1/GluN2A receptors during both physiological and pathological activation modalities.

A11. Glycine gating of NR1/NR2A NMDA receptors
Kirstie A.Cummings and Gabriela K. Popescu; Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214
N-Methyl-D-Aspartate receptors (NMDARs) are ligand-gated ion channels that mediate excitatory neurotransmission in the mammalian central nervous system. They are required for normal neuronal function and are a factor in several neuropathies including Alzheimer’s disease and schizophrenia. Classical NMDARs require both glycine and glutamate bound for receptor activation. Reaction mechanisms have been developed for several receptor isoforms, however these models assume saturation of glycine sites and a quantitative understanding of glycine-dependent gating kinetics is currently inadequate. We used several patch-clamp configurations including cell-attached, whole-cell, and fast agonist application on outside-out patches to study the mechanism by which glycine gates NMDARs in transiently transfected HEK293 cells. For single channel records, data were idealized with the SKM algorithm in QuB, after filtering digitally at 12 kHz. Modeling with the MIL algorithm (QuB) was done by fitting state models to idealized data. Macroscopic simulations were done in QuB using models
acquired following ligand concentration-dependent global fits across several concentrations. We developed ranked kinetic state models with association and dissociation rate constants under several subsaturating glycine concentrations. To test these schemes, we also developed models for two lower-affinity glycine-site agonists, L-serine and 3,3,3-trifluoro-DL-alanine. In all cases, log likelihood calculations indicate that binding occurs at kinetic state C2. We then measured macroscopic responses using fast application of glycine onto outside-out patches and whole-cell perfusion of glutamate under different concentrations of background glycine. Finally, we generated glycine dose-response curves and calculated EC50 values. For each condition, experimentally-recorded traces were compared to simulated responses for each candidate model. These data taken together support a model in which glycine and glutamate bind and activate NMDARs in a sequential manner. Knowledge about how glycine gates NMDARs will contribute to a more comprehensive understanding of the activation of these physiologically and pathologically relevant receptors. (Supported by RO1NS052669 to GKP)

A12. SNARE proteins are essential in the potentiation of NMDA receptors by group II metabotropic glutamate receptors
Jia Cheng, Wenhua Liu, Zhen Yan: Department of Physiology and Biophysics, University at Buffalo, Buffalo, NY 14214
The group II metabotropic glutamate receptor (mGluRII) has emerged as a new drug target for schizophrenia treatment. To understand the potential molecular mechanisms underlying the antipsychotic effects of mGluRII, we examined its impact on NMDA receptors, since NMDAR hypofunction has been implicated in schizophrenia. We previously found that application of APDC, a highly selective mGluRII agonist, caused a potent enhancement of NMDAR-mediated currents in cortical pyramidal neurons. Here we examined whether this effect of mGluRII involves the exocytosis of NMDA receptors mediated by SNARE proteins, such as SNAP-25 (synaptosomal-associated protein of 25 kDa) and Syntaxin 4. We found that the enhancing effect of APDC on NMDAR currents was abolished when SNARE complex was disrupted by delivering Botulinum toxin or SNAP-25 C-terminal blocking peptide into the neurons. Moreover, knockdown of Syntaxin 4 blocked mGluRII potentiation of NMDAR currents. Syntaxin 4 is a postsynaptic component interacting with Rab4, a small Rab GTPase mediating fast recycling from early endosome to the plasma membrane. The effect of APDC on NMDAR currents was abolished by dominant negative Rab4, and occluded by constitutively active Rab4, suggesting the involvement of Rab4-mediated NMDAR exocytosis to the cell membrane. Taken together, these results have revealed the key molecules involved in mGluRII enhancement of NMDA receptor trafficking and function. (Supported by NIH MH84233 and MH85774 to Z.Y.)

A13. Biochemical reconstitution of adhesion GPCR GPR56 activation of heterotrimeric G proteins
Hannah M. Stoveken1, Brigitta Gehl2, Lei Xu2, and Gregory G.Tall1; 1Pharmacology and Physiology, 2Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642
The adhesion G protein-coupled receptor GPR56, regulates cancer progression and cortical neuron migration during brain development. The proximal signaling events downstream of GPR56 and the pharmacological mechanisms of action of its putative ligands remain largely unknown. The extracellular matrix proteins, transglutaminase 2 and collagen III are proposed natural ligands that may regulate GPR56-dependent melanoma progression and cell migration. GPR56 is auto-proteolyzed during biosynthesis to produce an N-terminal fragment (NTF) that remains non-covalently associated with the 7-transmembrane-spanning C-terminal fragment (7TM-CTF). Previous work showed that the NTF inhibited 7TM-CTF signaling outputs in cells. The mechanism of ligand engagement by the NTF and how this may influence GPR56 7TM-CTF G protein coupling is not understood. We have biochemically
reconstituted GPR56 activation of G protein heterotrimers. Purified, recombinant G protein heterotrimers (Gi/o, Gq, G13, Gs) were pre-coupled to membranes prepared from GPR56-expressing Sf9 insect cells. The GPR56-influenced \(^{35}\)S-GTP\(\gamma\)S binding kinetics of each G protein heterotrimer were measured. The auto-inhibition of the GPR56 7-TM-CTF by its NTF was investigated by extracting the NTF from isolated membranes with urea prior to measurement of G protein \(^{35}\)S-GTP\(\gamma\)S binding. GPR56 robustly stimulated G13 GTP\(\gamma\)S binding. GPR56 modestly enhanced the kinetics of Gi/Go GTP\(\gamma\)S binding, and did not couple to Gq or Gs. Urea-treated GPR56 membranes were substantially more efficacious than untreated membranes towards G13 activation, supporting the inhibitory function of the NTF. Chemically stripping the GPR56 NTF from prepared membranes with urea stabilizes the active conformation of the GPR56 CTF. To prove that the GPR56 NTF suppresses GPR56 signaling during brain development and cancer progression, urea-stripped GPR56 membranes will be reconstituted with purified NTF and a series of designed NTF truncations. GPR56 activation of G13 will be measured to identify the portion(s) of the GPR56 NTF that are sufficient to suppress receptor signaling. (Supported by NIH grant RGM088242A to G.G.T. and NIH grant R01GM098591 to L.X.)

Pu-Yee Chan and Gregory G. Tall; Pharmacology and Physiology, University of Rochester Medical Center at Rochester, NY 14642
We recently demonstrated that Ric-8 guanine nucleotide exchange factors regulate an early event during heterotrimeric G protein \(\alpha\) subunit biosynthesis. Newly made \(\mathrm{G}_{\alpha}\) subunits are defective in initial association with an endomembrane in cells that are \(\text{Ric-8A}^{-}\). To define the precise molecular events by which Ric-8 mediates \(\mathrm{G}_{\alpha}\) biosynthesis, we utilized cell-free translation systems to study potential Ric-8A influence of \(\mathrm{G}_{\alpha}\) subunit translation kinetics and protein folding. The kinetics of \(\mathrm{G}_{\alpha}\) subunit translation and production of functional, folded protein from mock- and Ric-8A-(immuno)depleted RRL were compared. \(\mathrm{G}_{\alpha}\) proteins were examined using a trypsin protection assay of the activated conformation. Resolution of translated \(\mathrm{G}_{\alpha}\) proteins by gel filtration chromatography enabled evaluation of intermediate complexes of chaperones and \(\mathrm{G}_{\alpha}\) subunits during biosynthesis and folding.
Endogenous Ric-8A was immunodepleted from RRL. \(\mathrm{G}_{\alpha}\) subunit translation rates and overall produced protein amounts were equivalent in Ric-8A- and mock-depleted lysates. The function of Ric-8A in \(\mathrm{G}_{\alpha}\) biosynthesis was revealed when folded \(\mathrm{G}_{\alpha}\) protein levels were quantified. Properly folded \(\mathrm{G}_{\alpha}\) subunits can adopt the active GTP-bound conformation, a form resistant to limited trypsinolysis. GDP-AlF\(_4\)\(-\)bound \(\mathrm{G}_{\alpha}\) subunits produced in mock-depleted RRL had characteristic resistance to trypsinolysis. However, \(\mathrm{G}_{\alpha}\) subunits produced from Ric-8A-depleted RRL were not protected. Add back of recombinant Ric-8A protein to the Ric-8A-depleted RRL markedly enhanced trypsin protection of GDP-AlF\(_4\)\(-\)bound \(\mathrm{G}_{\alpha}\) subunits. Similar results were obtained in WGE that has no endogenous Ric-8 component. WGE-translated Gaq was resolved by gel filtration and was found to be a high molecular weight aggregate. Ric-8A addition to WGE made Gaq elute as a dimeric complex with Ric-8A that was dissociable with GTP\(\alpha\)S, producing functional Gaq-GTP\(\alpha\)S monomer.
This is the first report that Ric-8A serves a necessary function as a folding chaperone during biosynthetic folding of \(\mathrm{G}_{\alpha}\) subunits. This work has prompted us to identify additional cellular chaperones that may work with Ric-8A during G protein biosynthesis/folding. A Ric-8A-dependent fluorescence-based \(\mathrm{G}_{\alpha}\) folding assay has been established and will be presented. (Supported by NIDA Grant T32 DA07232 and RGM 088242A).

A15. Withdrawal from cocaine self-administration alters activin/Smad3-signaling
Amy Gancarz\(^{1,2}\), Gabrielle Schroeder\(^2\), Clarisse Panganiban\(^2\), Danielle Adank\(^2\), Michael Kausch\(^2\), Stewart D.Clark\(^{1,2}\), David M.Dietz\(^{1,2}\); \(^1\) Research Institute on Addictions,\(^2\)Pharmacology and Toxicology, University at Buffalo, Buffalo, NY
The addicted phenotype is characterized as a long-lasting, chronically relapsing disorder that persists following long periods of abstinence leading the hypothesis that the addicted brain has been functionally “re-wired”. Repeated exposure to psychomotor stimulants results in an increase in dendritic spine density in the brain including the nucleus accumbens (NAc) a critical area of the mesolimbic dopamine circuitry mediating drug addiction. These changes are thought to represent alterations in synaptic connectivity that may underlie the life long battle with addiction. Activin receptor signaling is known to regulate the actin cytoskeleton through both direct regulation of actin dynamics, and more indirectly through changes in gene transcription. Here, we examined the role of activin receptor signaling following withdrawal from cocaine-self administration. Following a seven-day withdrawal period from cocaine self-administration, there was a marked increase in the activin receptor II (ActRII) expression at both the mRNA and protein levels in the NAc. Activin receptor activation leads to the phosphorylation of Smad3, which transduce extracellular signals to the nucleus regulating gene transcription. Consistent with the increased expression of activin receptors, we find an increase in phosphorylated Smad3 (p-Smad3), an effect observed seven days but not one day following cocaine self-administration. These data strongly suggests that withdrawal from cocaine self-administration leads to an induction of the transcription factor Smad3 and subsequent activation of Smad-dependent gene expression in the NAc. Taken together, these data indicate that activin/Smad3 signaling is regulated in a time-dependent manner following cocaine self-administration, and may be the molecular bridge between actin dynamics and long-term transcriptional events that have been associated with drug addiction. Grant support: NIAAA training grant T32-AA007583-11

A16. Characterization of a cannabinoid CB1 receptor negative modulator ORG27569 in rats
Yanyan Qiu and Jun-Xu Li; Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, NY 14214

Blockade of the cannabinoid CB1 receptor signaling is implicated in energy homeostasis and the CB1 receptor antagonist Rimonabant® was used clinically for treating obesity. However, its serious side effects (e.g., depression) led to forced withdrawal from the clinic. Recently, a new CB1 receptor modulating site has been described which may achieve functional CB1 receptor antagonism without directly inhibiting CB1 receptor signaling. Such a strategy might be able to retain similar therapeutic potential as orthosteric CB1 receptor antagonists such as Rimonabant® but with better safety profile. However, no in vivo functional studies exist to characterize the pharmacological effects of CB1 receptor modulators. This study examined the effects of a purported CB1 receptor negative modulator ORG27569 on CB1 receptor agonists, CP55940 and anandamide, induced hypothermia in rats. Different groups of rats were used to evaluate the hypothermic effects induced by ORG27569, CP55940 and anandamide, alone or in combination. Rectal temperature was measured using a Physotemp® rat rectal thermometer. CP55940 (0.1-1 mg·kg(-1)) and anandamide (3.2-32 mg·kg(-1)) dose-dependently and markedly decreased the rectal temperature in rats, with varied duration of action. When studied alone, ORG27569 had no effect on the rectal temperature. However, ORG27569 (3.2 and 10mg·kg(-1) ) markedly antagonized CP55940- and anandamide-induced hypothemic effects. ORG27569 attenuates the hypothemic effects induced by CB1 receptor agonists. This effect was likely achieved through negative allosteric modulation of CB1 receptors because ORG27569 does not bind to the orthosteric binding site but has high affinity at a recently described CB1 receptor allosteric modulating site and has been shown to decrease the maximal effects of CB1 receptor agonists in vitro binding assay. These data extend the preliminary observations by confirming that ORG27569 is a CB1 receptor negative modulator and can function as a CB1 receptor antagonist in vivo.

A17. The vitamin D3 metabolite 25-hydroxyvitamin D3 (25(OH)D3) inhibits a subset of lung cancer cells independent of CYP27B1 activity
Ketoconazole, with lung Vincenzo solitary IRAK1 was IU/kg injury. vitamin be Paul Alissa IRAK1 was induced vitamin and Paul IRAK1 was induced as well as labeled transwells, induced as well as 5mg/kg) vitamin minimal. VDRlowCYP24A1high. Those VDRlowCYP24A1high and labeled decrease vitamin with VDRhighCYP24A1low and VDRlowCYP24A1low. Those mice fed the 10,000 IU/kg diet displayed significantly lower overall tumor volumes, with no toxicity and no change in circulating 1α,25(OH)2D3 in the blood. Therefore, VDRhighCYP24A1low are a subset of NSCLC cells that display increase sensitivity towards vitamin D3 treatment, and implies that vitamin D3 may be useful in an adjuvant therapy setting of NSCLC patients with a VDRhighCYP24A1low tumor phenotype.

A18. Central nervous system mediates lung inflammation during septic shock

Vincenzo Russo1, Jianya Peng1,2, Janey James1, Hai Duong Phan1, Hunter MacDonald1, Nancy Gertzberg1, Paul Neumann1, Arnold Johnson1, and Carlos Feleder1,2;1Albany College of Pharmacy, Department of Pharmaceutical Sciences, Albany, NY 12208. 2China Pharmaceutical University, Nanjing, 211198. China.

Septic shock is a systemic inflammatory response due to severe infection, resulting in multiple organ injury. Recently, α7-nicotinic acetylcholine receptors (α7nAChR) have been shown to modulate LPS-induced septic shock, suggesting autonomic nervous system involvement. Additionally, endocannabinoid type 1 (CB1) receptors in the brain are implicated in response to endotoxemia and modulation of the autonomic cholinergic pathway. Our data suggests septic shock is modulated through mechanisms controlled by the brain. Hence, this study tested the hypothesis that brain endocannabinoids and the cholinergic system regulate LPS-induced lung inflammation. Male Sprague-Dawley rats received an intracerebroventricular (ICV) injection of either the CB1 receptor antagonist Rimonabant (250 or 500ng) or vehicle, or a preoptic-anterior hypothalamic area (POA) or nucleus of the solitary tract (NTS) injection of Lidocaine (2%; 1 microL), 5 minutes prior to IV injection of either LPS (1 or 5mg/kg) or saline. Lungs were removed 0.5 h after IV injection of LPS and isolated for assessment of hemodynamics and inflammatory signal biomarkers. In separate studies, permeability to Evan’s-blue-labeled BSA was assessed in rat pulmonary microvessel endothelial monolayers (PMEM) grown on transwells, treated with vehicle or LPS (100ng/ml) with or without the α7nAChR-specific agonist PNU-282,987 (100nM) for 4 h. There were increases in (Wet-Dry/Dry) weight ratios and (Wet-Dry/Dry)/pulmonary capillary pressures in the lungs of vehicle/LPS-treated rats, with decreases in both IRAK1 and IkBα levels in lung homogenate. ICV injection of Rimonabant prevented the LPS-induced increased lung weight ratios and decreases in IRAK1 and IkBα. Lidocaine blockade of the POA or NTS prevented the initial lung hemodynamic response to LPS. Lastly, PNU-282,987 prevented the LPS-induced increase in permeability of PMEM. The data indicate that the brain’s central endocannabinoids, as well as the cholinergic system, participate in the regulation of the lung response to LPS. (Support: NIH R01 HL059901 to A.J. and NIH R15A1072744 to C.F.)
A19. The pathogenesis of chronic pain associated with STZ-induced diabetic neuropathy is associated with increased levels of tumor necrosis factor in the brain
Ashley Re¹, Abdel-Rahman Alnaji², Paul R. Knight¹,⁴, Bruce Davidson¹,³, Tracey A. Ignatowski¹,⁵; ¹Pathology and Anatomical Sciences, ²Pharmacology and Toxicology, ³Anesthesiology, ⁴Microbiology and Immunology, ⁵Neuroscience Program, University at Buffalo, Buffalo, NY 14214
Increases in pro-inflammatory cytokine levels, including tumor necrosis factor-α (TNF), are implicated in neuropathic pain pathogenesis. Inhibition of TNF in the CNS dramatically reduces neuropathic pain, possibly through alteration of autonomic nervous activity. The present goal was to investigate whether an association exists between TNF levels and development of chronic pain during streptozotocin (STZ)-induced diabetes. Male Sprague-Dawley rats were administered STZ (45 mg/kg) to induce diabetes. Rats were tested for pain (thermal hyperalgesia; mechanical allodynia) prior to (baseline) and every other day post-STZ for 60 days. Rat weights were monitored and blood glucose was tested prior to, day-4 post-STZ, and once/week thereafter. On day-61, blood, brain regions, sciatic nerves, and peritoneal macrophages were analyzed for TNF. Adrenergic regulation of lipopolysaccharide-stimulated TNF production by macrophages from control rats (saline), rats with STZ-induced diabetic neuropathy (STZ-DN), and rats injected with STZ, which failed to develop hyperglycemia (non-responders, STZ-NR) was examined. TNF levels were assessed via WEHI bioassay. TNF levels increased in specific brain regions (p<0.05) from STZ-DN rats, while no TNF increase occurred in STZ-NRs. Conversely, there was no increase in TNF in serum from STZ-DN animals (day-60 post-STZ), whereas TNF increased in serum from STZ-NR animals (p<0.05) confirming NRs received STZ. Macrophages from STZ-DN rats produce more TNF, whereas those from STZ-NRs produce less TNF. We have previously demonstrated that increased brain TNF levels play a critical role in central pain generation. Decreased TNF production by lipopolysaccharide-stimulated macrophages and lack of increased brain TNF in STZ-NR rats may explain lack of neuropathy. Systemic antidepressant and siRNA inhibition of hippocampal TNF studies are ongoing that may identify interactions between adrenergic responses and pro-inflammatory TNF offering a novel approach to treat chronic pain associated with diabetic neuropathy.(Supported by Dept. PAS; UB MDRF Award - A. Re)

A20. Suppression of neurodegeneration in Drosophila models of human neurodegenerative disorders
Brittany Casino and Satpal Singh; Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, NY 14214
Our lab has previously identified a mutation (levy) that provides a model of Leigh Syndrome (LS) in Drosophila melanogaster, as well as a second mutation, Su(levy), which suppresses levy induced neurodegeneration (ND). The Su(levy) mutation confers resistance to temperature-induced-paralysis, a phenotypic marker of levy in Drosophila. Experiments are underway to identify and characterize which gene the suppressor mutation resides within. Preliminary experiments suggest that ND in the levy mutant may be caused by oxidative stress, a feature common to many neurodegenerative disorders such as Parkinson’s disease (PD) and Alzheimer’s disease (AD). This fact has broadened the suppressor mutation’s application to possibly include PD and AD. The pesticide rotenone is known to induce Parkinson’s-like symptoms in humans and flies. Using rotenone to create a PD model, our lab is testing whether the suppressor can alleviate the phenotypic symptoms of this model. The suppressor mutation is being genetically combined with AD and PD mutations to further assess its ability to alleviate locomotor symptoms in the fly models of these disorders. Paralysis testing was done in a 38°C water bath. Wild-type and Su(levy) flies were exposed to 0 or 500µM concentrations of rotenone. Crosses and rotenone experiments were done at 25°C. The Su(levy) mutation made the levy flies resistant to paralysis. Su(levy) flies were more resistant to paralysis than wild-type. Su(levy) protected flies from rotenone toxicity. Experiments suggest that the Su(levy) mutation may protect flies from the effect of the pesticide rotenone on locomotion. Suppressor’s effects on PD and AD mutants will be measured.
through longevity, locomotion, and measurement of oxidative stress through ROS assays. If suppressor can alleviate ND in these models, this could provide leads to developing therapeutic approaches toward multiple neurodegenerative disorders. (Supported by NINDS 1R03NS063148-01)

**A21. Pre-exposure of the urotensin II receptor to ligand differentially reduces the response to subsequent additions of urotensin II or urotensin II-related peptide**

Taylor Warren and Stewart D. Clark; Department of Pharmacology and Toxicology, University at Buffalo, SUNY, Buffalo, NY

The urotensin II receptor (UIIR) is a G protein-coupled receptor (GPCR), formerly known as SENR or GPR14. UIIR is activated by two different ligands, urotensin II (UII) and urotensin-related peptide (URP). Urotensin II was originally isolated from the urophysis gland of fish, however, both UII and URP have been found in numerous tetrapod species. Our primary question is “Why is it that there are two ligands for one single receptor?” UIIR activates the Gq coupled pathway, and so we are able to monitor receptor activation via fluorescent calcium chelating dyes. In this assay URP and UII have the same EC50, and previous studies have shown that they have equivalent Kd. In addition, at least in some areas of UIIR expression, URP and UII are expressed by the same neurons and at the same time. Therefore, we hypothesize that URP and UII produce different post-activation events. As a first step to investigate this possibility we have studied how the pre-exposure of the receptor to ligand influences receptor activation by subsequent exposure to ligand. Pre-exposure of UII blocks receptor activation by subsequent additions of UII. However, pre-exposure to URP blunts but does not abolish receptor activation due to subsequent additions of URP. Future studies will focus on the ability of UII and URP to produce receptor desensitization and beta-arrestin recruitment. These studies may help to explain the differential effects of UIIR ligands seen in vivo after repeated exposure.

**A22. Comparative analyses of human estrogen receptor-EF-hand protein complexes: molecular basis for hormone-independent activation**

David H. Lee, Bethany K. Asare, Matthew Hudson, Suchitra Singh, Rajendram V. Rajnarayanan; Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, NY 14214

Estrogen Receptor (ER) belongs to the nuclear receptor super family of ligand-triggered transcription factors. ER is present in 70% of breast cancers. It has been shown that calmodulin (CaM), which control calcium mediated signaling pathways, could bind and activate unliganded ER. CaM has four EF-hand motifs that change conformation upon binding Ca2+ ions. Ca2+-CaM complex binds to the target proteins and initiates various signaling cascades. X-ray and NMR studies show CaM forms a compact globular conformation by bending its central helix upon binding its target peptides, which allows CaM to increase its binding affinity for a number of target proteins. S100, a soluble protein that is recently reported to interact with ER has only two EF-hand motifs. S100 protein is overexpressed in several cancers and shares a high degree of sequence homology with calmodulin.

Protein-protein interactions of CaM, S100 with ER alpha were simulated using HADDOCK and HEX. Docking results were analyzed in UCSF Chimera and integrated with data from in vitro proteomics experiments to determine the conformations of ER bound EF-hand protein complexes. Proteomic experiments consisted of chemical crosslinking of the corresponding protein complexes followed by tryptic digestion, analyzing the resulting peptide peaks using FTICR- Mass Spectrometry. Using the fold information and contact regions obtained from the Mass spectrometry, 3D structures of ER-EF hand protein complexes were reconstructed. These structures of ER best represent the conformational state sensed by the specific interacting partner. ER-S100 complex is distinct from CaM. The lack of the connective peptidic region between the EF-hand pairs in S100 attributed to less interaction coverage indicative of only inducing a partial agonist-like conformation. We believe that the ensemble of ER-EF
hand protein complexes generated by our integrated proteomics-assisted protein interaction profiling will shed light on the lingering issue of hormone independent activation of ER at the molecular level.

A23. Use of cationic polymers to deliver nucleic acid agents
Sangwon Min1, Qiuxia Chen2, Chi-Kuang Chen2, Chong Cheng3, Aiming Yu4
1Department of Pharmaceutical Sciences, SUNY-Buffalo, NY 14214; 2Department of Chemical and Biological Engineering, SUNY-Buffalo, NY 14214
MicroRNAs (miRNA) are small non-coding RNAs which are involved in gene regulation through different pathways of post-transcriptional modification. Thus, their delivery across the cytoplasmic membrane would influence gene regulation. To investigate the relativity in miRNA expression, we implied cationic polylactides (CPLA) and Lipofectamine 2000 as modes of miRNA expression plasmid delivery. CPLAs are positively charged biodegradable polymers possessing “proton sponge” effect that leads to an increase in delivery efficacy. Visual determination of efficacy in CPLA mediated delivery was obtained through transfecting phrGFP-II reporter plasmid with various plasmid to CPLA weight ratios. Cell viability assay was then applied to obtain optimum delivery weight ratio within low cytotoxic range. With the optimized weight ratio, microRNA-1291 (miR-1291) expression plasmid delivery into two pancreatic tumor cell lines, AsPC-1 and BxPC-3 was conducted. Resultant miR-1291 expression level was quantified via qPCR and its altered target protein expression was determined by Western blot analysis. In comparison to Lipofectamine 2000, CPLA mediated delivery exhibits an equivalent to higher expression of miR-1291 in both cell lines with subsequent decrease in target protein expression. Our results imply CPLA54 as a potent vehicle for miRNA delivery.

Vincent Lam1, S. Angers2, and A. Salahpour1; 1Pharmacology and Toxicology, University of Toronto, Toronto, ON, M5S 1A8; 2Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, M5S 1A8
 Trafficking of membrane proteins is a dynamic process that is tightly regulated and sometimes defective in human diseases. It is therefore important to develop new tools that would allow simple and quantitative measurement of surface expression of membrane proteins. The objective of this study is to develop and validate a new assay for quantification of cell surface expression of GPCRs.
We have generated β-lactamase fusion constructs and produced stable cell lines for the following GPCRs: β2-AR and GBR1.Cells seeded in a 48-well plate overnight were washed once and the cell impermeable substrate of β-lactamase, nitrocefin added to the wells. A mouse monoclonal anti-HA antibody was used for all ELISA studies. Using the β-lactamase assay we were able to measure isoproterenol induced internalization of β2-AR in a dose and time dependent manner. The results obtained by the β-lactamase assay are quantitatively and qualitatively similar to classical ELISAs. These conditions also yielded a Z’=0.52 in a 96 well plate for the β-lactamase assay. Using the β-lactamase assay we were also able to show that pre-incubation with antagonists (propranolol or alprenolol) were able to dose dependently block agonist mediated internalization of the β2-AR. Lastly in our stable cell lines for GBR1, transfection of increasing amounts of GBR2 led to increased surface expression of GBR1 in a dose dependent manner in both the β-lactamase assay and ELISA. We have validated a novel assay for the quantification of surface expression of membrane proteins that is qualitatively and quantitatively similar to classical ELISA. However this assay has the advantage of being 5-10 times lower in cost and 4-5 times faster than classical ELISA. Moreover, the measured Z’ Factor indicates that the β-lactamase assay is adequate and amenable to high throughput screening.

A25. Disrupting the vasculature for enhanced drug delivery and therapeutic efficacy against gliomas
Margaret Folaron\textsuperscript{1}, Marianne Marcotte\textsuperscript{1}, Steve Turowski\textsuperscript{1}, Michael Ciesielski\textsuperscript{2}, Robert Fenstermaker\textsuperscript{2} and Mukund Seshadri\textsuperscript{1}; \textsuperscript{1}Pharmacology and Therapeutics and \textsuperscript{2}NeuroOncology, Roswell Park Cancer Institute, Buffalo, NY, USA 14263

Angiogenesis, or the formation of new blood vessels, is a major hallmark in the progression of most solid tumors including gliomas. Tumor-vascular disrupting agents (tumor-VDAs) are a distinct class of agents that cause disruption of established tumor vasculature, depriving the tumor of nutrients and oxygen and leading to inhibition of tumor growth. Since microvascular proliferation is a critical component of glioma biology, we hypothesized that targeting glioma vasculature using tumor-VDAs could be of potential benefit against gliomas. To test this hypothesis, we examined the activity of the tumor-VDAs alone and in combination with chemotherapeutic agents in multiple experimental glioma models.

As experimental glioma models, subcutaneous and intracranial GL261 and U87 gliomas were established in C57BL6 and athymic nude mice. Animals were treated with VDA (ASA404 or EPC2407), Temozolomide, Irinotecan, Dexamethasone alone or in combination. Magnetic Resonance Imaging (MRI) was performed to assess both short term and long term response of VDA therapy. Treatment with VDA alone results in an early increase in vascular permeability within a few hours of treatment (detected by MRI). VDA treatment enhances the antitumor activity of multiple classes of chemotherapeutic agents against gliomas, exhibited through enhanced long term survival. In addition, combination therapy was well-tolerated and resulted in enhanced inhibition of tumor growth compared to either monotherapy. These results demonstrate the potential of combining tumor-VDAs with chemotherapy against gliomas. MRI offers a useful, noninvasive method of monitoring changes in the glioma microenvironment following VDA treatment. (Supported by the American Brain Tumor Association Translational Grant award (M.S) – In honor of Michael Baldasaro)

A26. Melatonin modulation of novel object recognition

\textsuperscript{1}Danielle M Precourt, \textsuperscript{1,2}Shannon J Clough, \textsuperscript{1}Iwona Stepien, \textsuperscript{2}Randall L Hudson & \textsuperscript{1}Margarita L Dubocovich \textsuperscript{1}Department of Pharmacology and Toxicology, \textsuperscript{2}Department of Physiology and Biophysics, \textsuperscript{3}Neuroscience Program, School of Medicine and Biomedical Sciences, University at Buffalo SUNY, Buffalo, NY 14214

Melatonin acts on two receptors, termed MT\textsubscript{1} and MT\textsubscript{2}, which are expressed in the central nervous system. Action on the MT\textsubscript{2} receptor is shown to inhibit long-term potentiation, a key component in learning and memory. The goal of this study was to assess whether melatonin has an effect on learning and memory. We used a novel object recognition paradigm (NOR) which is based on the theory that rodents are novelty preferring. Therefore when exposed to novel and familiar objects, mice should show a preference for the novel alternatives. We expect that the mice lacking the MT\textsubscript{1} receptor (MT\textsubscript{1}KO) will not show an increased preference for novelty. Male C57 mice (wild-type [WT] or MT\textsubscript{1}KO) were evaluated in one of two NOR paradigms. Paradigm 1 involved a 10 minute chamber exposure followed by 3 exposures to the familiar object for 6 minutes each. Paradigm 2 involved a 10 minute chamber exposure on day 1 and a 10 minute exposure to the familiar objects on day 2. Both paradigms utilized a 5 minute novel object test one hour after the familiar object exposure. Interaction with objects was recorded utilizing the LocoScan system (CleverSys, Reston VA). The first paradigm resulted in no preference for the novel (53.03±11.00s, n=4) vs. familiar object (24.73±11.00s, n=4). The second paradigm showed a strong preference for the novel object (66.20±2.10, n=5, p<0.01) over the familiar object (33.80±2.10, n=5) in WT mice. When MT\textsubscript{1}KO mice were run in the second paradigm, they showed no preference for the novel object (59.74±18.16, n=7) over the familiar (40.26±8.16, n=7). MT\textsubscript{1}KO displayed a deficit in learning and memory compared to WT mice. The learning deficit observed is potentially due to action on the MT\textsubscript{2} receptor in the absence of the MT\textsubscript{1}, resulting in an inhibition of long-term potentiation.
A27. MT\textsubscript{1} melatonin receptor role in methamphetamine-induced locomotor sensitization in C57BL/6 mice

Jason Ma,\textsuperscript{1} Anthony J. Hutchinson,\textsuperscript{1} Randall L. Hudson\textsuperscript{2} and Margarita L. Dubocovich\textsuperscript{1,1}; Pharmacology & Toxicology,\textsuperscript{2} Physiology and Biophysics, University at Buffalo, Buffalo, NY 14214

Methamphetamine (METH) and other abused drugs induce sensitization, which may underlie drug abuse related symptoms. Clues to molecular mechanisms between METH and melatonin signaling come from melatonin blocking the inhibitory effect of METH on the phosphorylation of the mammalian target of rapamycin (mTOR; Kongsphul et al., 2008). We investigated the MT\textsubscript{1} receptor in locomotor sensitization and regulation of mTOR after a single METH pretreatment in C57BL/6 mice. Wild-type (WT) and MT1KO mice were pretreated with a single vehicle or METH pretreatment (1.2 mg/kg, i.p.) on Day 1, then challenged with METH (1.2 mg/kg, i.p.) on Day 9. Another group of WT and MT1KO mice treated with vehicle or METH on Day 1 were decapitated 2½ hours or 8 days later for brain tissue harvest and Western blot analysis. Locomotor sensitization was expressed in METH pretreated WT mice but not in MT1KO mice. METH treated WT mice expressed total mTOR greater than VEH treated WT mice in caudate putamen and nucleus accumbens (Day 9). MT1KO mice mTOR levels were not altered. METH treated WT mice also exhibited greater mTOR phosphorylation in the caudate putamen (Day 9) but not the MT1KO mice. MT\textsubscript{1} receptors mediated the induction of locomotor sensitization to METH in C57BL/6 mice after a single pretreatment. Also, expression of METH-induced locomotor sensitization may involve MT\textsubscript{1} receptor mediated mTOR expression and phosphorylation.

A28. Characterization of MT\textsubscript{1} melatonin receptor expressing neurons in the medial habenula, habenula commissure and periaqueductal grey of the C3H/HeN mouse brain

Katherine M Evely,\textsuperscript{1} Ekue B Adamah-Biass,\textsuperscript{2} Randall L Hudson,\textsuperscript{1} Margarita L Dubocovich;\textsuperscript{1} Department of Pharmacology and Toxicology,\textsuperscript{2} Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, University at Buffalo, SUNY, Buffalo, New York, 14214

Melatonin (MLT) is rhythmically secreted from the pineal gland and acts on two G protein-coupled receptors, termed MT\textsubscript{1} and MT\textsubscript{2}. Brain tissue from a transgenic mouse line expressing red fluorescence protein (RFP) at the MT\textsubscript{1} receptor promoter provides a method of localizing the receptor. RFP-MT\textsubscript{1} fluorescence and immunoreactivity was localized to the medial habenula (MHB), habenula commissure (HbC) and the midbrain dorsal medial periaqueductal grey (DMPAG) area. The habenula acts as a relay station from forebrain to midbrain. The downstream PAG plays a prominent role in pain transmission (Behbehani, Prog Neurobiol 1995;46:575-605). The goal of our research is to investigate the distribution of the MT\textsubscript{1} receptor in these cholinergic, dopaminergic and glutamatergic neuronal systems. Immunofluorescence co-staining for RFP along with choline acetyl transferase (ChAT), tyrosine hydroxylase (TH) or vesicular glutamate transporter (VGLUT2) is used to investigate the distribution of the MT\textsubscript{1} receptor. Results show RFP-MT\textsubscript{1} expression in the dorsal MHB, clearly separated from ChAT staining in the ventral MHB. TH colocalized with RFP-MT\textsubscript{1} in the HbC, PAG, and the ependymal lining of the aqueduct. VGLUT2 and RFP-MT\textsubscript{1} positive cells are present in dorsal MHB neurons. These results indicate a possible role for the MT\textsubscript{1} receptor in the modulation of glutamatergic and dopaminergic neurotransmission. (Supported by DA 021870 MLD)

A29. Melatonin accelerates the re-entrainment rate of multiple spontaneous homecage behavioral rhythms in the C3H/HeN mice

Ekue B Adamah-Biass,\textsuperscript{1} Ivona Stepien,\textsuperscript{2} Randall L Hudson,\textsuperscript{1} Margarita L Dubocovich;\textsuperscript{1} Department of Pharmacology and Toxicology,\textsuperscript{2} Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, University at Buffalo, SUNY, Buffalo, NY 14214
Activation of MT₁ receptors by melatonin (MLT) accelerates the re-entrainment of circadian rhythms of wheel running activity after an abrupt advance of the dark cycle (Dubocovich et al., 2005). Here, we investigated the effects of melatonin on the re-entrainment rate of multiple spontaneous homecage behaviors after a 6hr advance of the dark onset in C3H/HeN mice. The 15 behaviors assessed include activity (i.e. comedown, jump, hang, walk), exploration (i.e. dig, groom, rearup, sniff, stretch), ingestion (i.e. drink, eat) and resting (i.e. awake, remainlow, rest, twitch). Mice (n=8 per group) were treated for three consecutive days with either vehicle (VEH) (3% ethanol in saline) or MLT (3 mg/kg in VEH, s.c.) at the new dark onset. MLT significantly decreased the number of days (accelerate) necessary for re-entrainment of the spontaneous behaviors including activity (e.g. Walk: VEH, 8.3 ± 0.3d & MLT, 6.2 ± 0.2d, p<0.001), exploration (e.g. Groom: VEH, 8.8 ± 0.7d & MLT, 5.80 ± 0.2d, p<0.01), ingestion (e.g. Eat: VEH, 9.0 ± 0.4d & MLT, 6.6 ± 0.4d, p<0.005) and resting (e.g. RemainLow: VEH, 9.167 ± 0.7d & MLT, 6.8 ± 0.5d, p<0.05). We concluded that MLT acting at MT₁ melanotonin receptors accelerates the re-entrainment rate of spontaneous homecage behavioral rhythms. We suggest that MLT or MLT agonists could be useful in the treatment of circadian related disorders including jet lag, circadian sleep and mood disorders. (Supported by NS 061068)

**A30. Methamphetamine-induced conditioned place preference in C5H/HeN mice is observed during the day (ZT 6-8) but not at night (ZT 19-21)**

Shannon J Clough, Anthony J Hutchinson, Iwona Stepien, Randall L Hudson, Margarita L Dubocovich

Department of Pharmacology and Toxicology, Department of Physiology and Biophysics, Neuroscience Program, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214

Methamphetamine produces reward through its action on the mesolimbic system. Melatonin is synthesized following a circadian rhythm, with low levels during the day (Zeitgeber Time [ZT] 6-8; 12h:12h light-dark cycle, ZTO = lights on) and high levels at night (ZT19-21) (Reiter, Mol Cel Endocrinol 1991;79:C153-C158). Melatonin exerts its effects through the MT₁ and MT₂ receptors, which are located in several brain regions including areas of the reward pathway (Uz et al., Mol Brain Res 2005, 136:45-53). At ZT6-8 methamphetamine induces a place preference in C3H/HeN wild-type mice. Deletion of either melatonin receptor blocks this effect, suggesting a role for melatonin in the modulation of methamphetamine-induced reward. The goal of this study was to examine the contributions of endogenous melatonin in the modulation of methamphetamine-induced place preference by comparing preference scores at ZT6-8 and ZT19-21. Male C3H/HeN wild-type mice were subjected to a conditioned place preference test during ZT6-8 and ZT19-21. Animals were conditioned for 6 days with alternating treatments of methamphetamine (1.2mg/kg, ip) and vehicle and tested for place preference 1 day after the last conditioning session. Compartment duration was measured using the LocoScan System (Clever Inc, Reston, VA). A preference score was derived by subtracting the duration spent in the vehicle-paired compartment from the duration spent in the methamphetamine-paired compartment during the post-test. At ZT19-21 mice exhibited a similar preference score regardless of receiving methamphetamine (70.1±37.8s, n=17) or vehicle (23.3±21.5s, n=17). This is in contrast to mice tested at ZT6-8, which displayed a place preference for methamphetamine (226.0±35.5s, n =11, p<0.001) compared to vehicle (-36.3±28.1s, n=12). This time dependent difference suggests the involvement of a mechanism subject to a circadian rhythm, such as melatonin, which may act through inhibition of long-term potentiation (Wang et al., Eur J. Neuroscience 22:22-31, 2005). (Supported by DA021870 to MLD)

**A31. Shank3 deficiency induces NMDA receptor hypofunction via an actin-dependent mechanism**

Lara J. Duffney¹, Jing Wei², Katharine R. Smith², Josef T. Kittler² and Zhen Yan¹; Physiology & Biophysics, University at Buffalo, Neuroscience, Physiology and Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK
Shank3, which encodes a scaffolding protein at glutamatergic synapses, is a genetic risk factor for autism. In this study, we examined the impact of Shank3 deficiency on the NMDA-type glutamate receptor, a key player in cognition and mental illnesses. We found that knockdown of Shank3 with a small interfering RNA (siRNA) caused a significant reduction of NMDAR-mediated ionic or synaptic current, as well as the surface expression of NR1 subunits, in cortical cultures. The effect of Shank3 siRNA on NMDAR currents was blocked by an actin stabilizer, and was occluded by an actin destabilizer, suggesting the involvement of actin cytoskeleton. Since actin dynamics is regulated by the GTPase Rac1 and down-stream effector p21-activated kinase (PAK), we further examined Shank3 regulation of NMDARs when Rac1 or PAK was manipulated. We found that the reducing effect of Shank3 siRNA on NMDAR currents was mimicked and occluded by specific inhibitors for Rac1 or PAK, and was blocked by constitutively active Rac1 or PAK. Immunocytochemical data showed a strong reduction of F-actin clusters after Shank3 knockdown, which was occluded by a PAK inhibitor. Inhibiting cofolin, the primary downstream target of PAK and a major actin depolymerizing factor, prevented Shank3 siRNA from reducing NMDAR currents and F-actin clusters. Taken together, these results suggest that Shank3 deficiency induces NMDAR hypofunction by interfering with the Rac1/PAK/cofilin/actin signaling, leading to the loss of NMDAR membrane delivery or stability. It provides a potential mechanism for the role of Shank3 in cognitive deficit in autism.

A32. Affinity and selectivity of luzindole analogues in human and mouse MT1 and MT2 melatonin receptors transiently expressed in mammalian cells

Marina Popovska-Gorevska, Kathleen A. McGowan and Margarita L. Dubocovich; Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, New York 14214

Melatonin (5-methoxy-N-acetyltryptamine) is released following a circadian rhythm with high levels at night. Melatonin signals through activation of two G-protein coupled receptors, MT1 and MT2, which show distinct molecular structures, different chromosomal localizations and select pharmacological characteristics (Dubocovich et. al. Naunyn Schmiedebergs Arch. Pharmacol., 355: 365-375). The goal of this study was to compare the binding affinity and selectivity of luzindole [(2-benzyl N-acetyltryptamine (NAT)] analogues [5-methoxy-NAT; 2-benzyl-N-propionyl-AT (N-0889); p-methoxy-benzyl-NAT (N-0890); 2-p-methyl-benzyl-NAT (N-0891)], 5-hydroxyluzindole, 6-hydroxyluzindole and 5-methoxyluzindole in the human and mouse MT1 and MT2 melatonin receptors transiently expressed in COS-7 cells. COS-7 cells were transiently transfected with either human or mouse MT1 and MT2 cDNA plasmid. The affinities (Ki) of the various compounds (1 pM to 100 µM) competing for 2-[125I]-iodomelatonin (100 pM) binding for mMT1 and mMT2 melatonin receptors were determined and compared with the Ki values for the human receptors. Melatonin competed for 2-[125I]-iodomelatonin binding for the human [Ki (nM) for hMT1: 0.34 and hMT2: 0.44] and the mouse [Ki (nM) for mMT1: 0.80 and mMT2: 0.33] melatonin receptors with equal affinity. The affinity of luzindole [Ki (nM): 11.23 vs. 368.85], N-0889, N-0890 and N-0891 for hMT2 receptors was higher than for the mMT2 receptor. N-0890, N-0891 and 5-methoxyluzindole showed higher selectivity affinity ratios (Ki MT1/MT2) for hMT2 than hMT1 melatonin receptors, having 56, 91 and 130-fold differences, respectively. In contrast, their selectivity affinity ratios for the mMT1 and mMT2 melatonin receptors were identical. These results show differences between the human and the mouse melatonin receptor in terms of the affinities and selectivity ratios of luzindole and its analogues. We conclude that caution is needed when affinities and selectivity ratios are extrapolated to different species. (Support by NS 061068 to MLD)