20th ANNIVERSARY
1985 – 2005

The Ohio Physiological Society

A Chapter of the American Physiological Society

October 27-28, 2005

THE OHIO PHYSIOLOGICAL SOCIETY

20th ANNIVERSARY MEETING

OCTOBER 27 – 28, 2005

PROGRAM

THE OHIO PHYSIOLOGICAL SOCIETY

20th ANNIVERSARY MEETING

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"The society is organized as a professional association of Ohio physiologists whose purpose is to enhance and advance the field of physiology including all of molecular, cellular, organ and organismal basic and applied disciplines of research and to unite the physiologists for this purpose within the state of Ohio."

(*from* Article II of the Bylaws enacted at the Founders Meeting on May 7, 1986)

The annual Ohio Physiological Society meeting is a forum for faculty, students and post-doctoral fellows to present their results from work in progress. Undergraduate students in particular are encouraged to attend with their faculty mentor. Attendance early in the course of a research project is an important educational opportunity. Topics to be presented come from a broad range of biological research with a physiologic perspective including comparative, biomedical, molecular and biophysical approaches. Presentations on innovative physiology teaching methods also are encouraged. Oral and poster presentations are conducted in an informal atmosphere with opportunities for open discussion.

This year celebrates the 20th anniversary of the founding of The Ohio Physiological Society in the fall of 1985. The meeting is also being held on the site of the Society's inception, the campus of Wright State University in Dayton, Ohio.

The OPS Executive Committee would like to thank the following for their support:

Department of Neuroscience Cell Biology and Physiology – Timothy C. Cope, Chair

Wright State University Boonshoft School of Medicine – Howard M. Part, Dean

Wright State University College of Science and Mathematics – Michele G. Wheatly, Dean

Wright State University Administration – David R. Hopkins, Provost

OPS 2005 Executive Committee:

Dan R. Halm, President
Chris M. Gillen, President-Elect
Roger T. Worrell, President Emeritus
Norma C. Adragna, Secretary/Treasurer

The OPS Executive Committee also extends its appreciation to Donna Maas, Administrative Support Specialist, for her assistance in the planning and organization of this year's program.

Original Founding Members of OPS
May 7, 1986

Norma C. Adragna	Wright State University
Bruce Biagi	Ohio State University, President-Elect
Julie Breay-Pilcher	Wright State University, Student
Lisa Carney	Wright State University, Student
Martin Frank	American Physiological Society
Roger M. Glaser	Wright State University
M.D. Goldfinger	Wright State University
Robert W. Gotshall	Wright State University
John K. Hageman	Ohio State University, Zoology Graduate Student
Peter K. Lauf	OPS Founder and First President
Daniel S. Miles	Wright State University
Noel Nussbaum	Wright State University, OPS Secretary-Treasurer
Marc Post	Ohio State University, Zoology Graduate Student
Robert W. Putnam	Wright State University
Kyoo Hai Ryu	Wright State University
Thomas J. Sernka	Wright State University
David L. Stetson	Ohio State University, Zoology
Kevin Strange	Wright State University
John S. Striffler	Wright State University
Richard D. Tallman	Ohio State University
Dietmar V. Trulzsch	Wright State University
R. Wiley	Miami University, Oxford

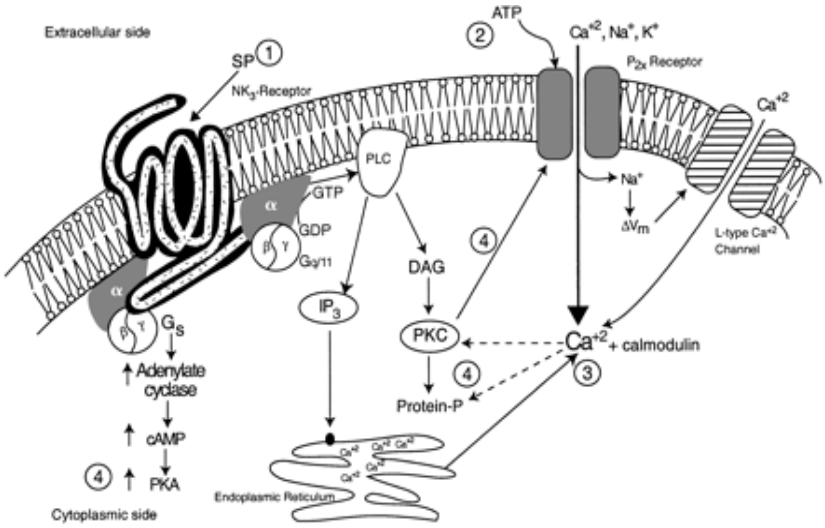

WRIGHT STATE
UNIVERSITY

THE OHIO PHYSIOLOGICAL SOCIETY
20th ANNIVERSARY MEETING

Featured Lecturer

Celia D. Sladek, Ph.D.

Department of Physiology and Biophysics
University of Colorado Health Science Center



Cardiovascular Regulation of Vasopressin Secretion:

Impact of Co-released Neurotransmitters and Unique Signaling Cascades

WRIGHT STATE UNIVERSITY

THE OHIO PHYSIOLOGICAL SOCIETY
20th ANNIVERSARY MEETING

THURSDAY, OCTOBER 27, 2005

WSU Student Union – Discovery Room (E163)

5:00 p.m. Registration

WSU Student Union – Pathfinder Lounge

6:00 p.m. Reception

WSU Student Union – Discovery Room (E163)

8:00 p.m. WSU Welcome
Founding President's Remarks
Featured Lecture

Cardiovascular Regulation of Vasopressin Secretion: Impact of Co-released Neurotransmitters and Unique Signaling Cascades

Celia D. Sladek, Ph.D.
Department of Physiology and Biophysics
University of Colorado Health Science Center

WRIGHT STATE UNIVERSITY

THE OHIO PHYSIOLOGICAL SOCIETY
20th ANNIVERSARY MEETING

Friday, October 28, 2005

WSU Student Union - Discovery Room (E163)

8:30 a.m.	Opening
8:45 a.m.	Phyllis A. Callahan, Miami University *Orphanin FQ Regulation of Prolactin Secretion*
9:10 a.m.	David D. Kline, Case Western Reserve University *Homeostatic Plasticity in the Nucleus Tractus Solitarius Following Intermittent Hypoxia*
9:35 a.m.	Kathrin L. Engisch, Wright State University *Get Your Adrenaline Going: Measuring Adrenaline Release from Single Adrenal Chromaffin Cells*
10:00 a.m.	Coffee Break
10:25 a.m.	ZiJian Xie, Medical University of Ohio at Toledo *Na/K-ATPase Signalosome: The Organization and Its Membership*
10:50 a.m.	J. Gary Meszaros, NEOUCOM *Cellular Determinants of Cardiac Remodeling: Focus on Fibroblasts and Myofibroblasts*
11:15 a.m.	Bryan Mackenzie, University of Cincinnati *The Divalent Metal-Ion Transporter*
11:40 a.m.	Candice C. Askwith, The Ohio State University *Acid-Sensing Ion Channels: The Connection between pH, Peptides and Stroke*

WSU Student Union - Endeavour Room (E156)

12:30 p.m.	Lunch
1:30 p.m.	OPS Business Meeting

WSU Student Union Skylight (E156)

2:00-5:00 p.m. Poster Presentations

WRIGHT STATE UNIVERSITY

THE OHIO PHYSIOLOGICAL SOCIETY

20th ANNIVERSARY MEETING

OCTOBER 27 – 28, 2005

ABSTRACTS

1. Molecular and Immunochemical Evidence for the Presence of KCC Isoforms in a Primary Human Epithelial Cell Line (FHL 124)

2. A Comparison of SERCA Expression during the Development of Freshwater Crayfish and Marine Lobster

3. Ammonium Secretion in Distal Colonic Epithelium

4. Glycerol Permeability in Erythrocytes from an Amphibian that Accumulates Glycerol in the Cold

5. MIP Family Members in Cope's Gray Tree Frog *Hyla Chrysoscelis* Exhibit Tissue-specific and Thermal-sensitive Gene Regulation

6. Serotonin: Effects on Temperature Sensitive and Insensitive Hypothalamic Neurons

7. Homeostatic Plasticity in the Nucleus Tractus Solitarius Following Intermittent Hypoxia

8. The Effect of Chronic Hypercapnia (CH) on Ventilatory Control and Neuronal Intracellular pH (pH_i) Responses in Neonatal Rats

9. The Effects of Hypercapnia on Membrane Potential (V_m) and Intracellular pH (pH_i) in Neurons and Astrocytes from the Retrotrapezoid Nucleus (RTN)

10. Prior Hyposmotic Challenge Sensitizes the ATP-activated Anion Conductance in Cultured Hippocampal Astrocytes

11. Characterization of the Role of OFQ/N in the Stress Response

12. Dopaminergic Mechanisms Involved in Estrogen Modulation of the Prolactin Response to OFQ/N

13. Neural and Endocrine Impact of Orphanin FQ/Nociceptin on Maternal Behavior

14. Forming a Memory of Social Rank: Brain Pathways and the Implications of Neurogenesis

ABSTRACTS *(continued)*

15. Promoting Neuroscience Education with Science Olympiad™

16. Resting Potential Dependent Regulation of the Voltage Sensitivity of Sodium Channel Gating in Rat Skeletal Muscle *in vivo*

17. Signal Transduction Mechanisms of K-Cl Cotransport Regulation and its Relationship to Disease

18. The Calcium/Calmodulin Dependent Protein Phosphatase PP2B Has a Role in Regulating Exocytosis in *Paramecium*

19. Leukocyte Chemoattractants Induce Gene Expression in Promyelocytic Leukemia HL-60 Cells: Participation of PLD1 and PLD2 Isoforms on mTOR and S6K Cell Signaling

20. Lipid Signaling Pathways Stimulate and Inhibit Cl^- and K^+ Secretion across Guinea Pig Distal Colonic Mucosa

21. Estrogen Receptor Activation Protects against Pro-inflammatory Cytokine-induced Apoptosis

22. A Peripheral CIRCADIAN Oscillator in the Gastrointestinal Tract of Zebra Finches (*Taeniopygia guttata*)

23. Rapid Changes in Corticosteroid Binding Globulin in Response to Acute Stress in the Zebra Finch

24. Role of Endothelin Receptors in α_1-Adrenoceptor-mediated Coronary Vasoconstriction and Modulation by Obesity in Isolated Mouse Hearts

25. Cholinergic Input Is Critical in the Regulation of Heart Rate Variability and Stress Reactivity in Mice

26. Development of a Computerized Method for Analysis of Circadian Blood Pressure Rhythms in Mice

27. Angiotensin-Converting Enzyme2 (ACE2) Activity in Mouse Brain: Use of Mass Spectrometry in Cardiovascular Research

28. Evaluation of Plasma Angiotensin Converting Enzyme I (ACE1) Activity in Streptozotocin-diabetic Mice Using Mass Spectrometry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1. Molecular and Immunochemical Evidence for the Presence of KCC Isoforms in a Primary Human Epithelial Cell Line (FHL 124)

Sandeep Misri, Ameet Chimote, Norma C. Adragna and Peter K. Lauf
Cell Biophysics Group, Boonshoft School of Medicine, Wright State University, Dayton, OH (sandeep.misri@wright.edu)

We have functional (ion fluxes, Exp. Eye Res. 2005) and molecular (RT-PCR, Western blot and immunocytochemistry) evidence for the presence of Na-K-2Cl (NKCC1) and K-Cl (three KCC1, 3 and 4 isoforms) cotransporters in SV40-immortalized human lens epithelial B3 (HLE-B3) cells. We have extended this work to the primary (non-transformed) human lens epithelial cell line FHL124 (kindly provided by Dr. John Reddan, Oakland University). RT-PCR revealed the expected sizes for the KCC1 (233bp), KCC3a (250bp), KCC3b (409bp) and KCC4 (556bp) isoforms. Expression of the KCC1, 3 and 4 isoforms was verified by Western blot and immunocytochemical labeling of FHL124 with primary rabbit anti-rtKCC1, anti-hmKCC3 and anti-msKCC4 antibodies, and secondary horseradish peroxidase and Cy3-labeled donkey anti-rabbit IgG, respectively. We conclude that, as in HLE-B3 cells, FHL124 cells also display three KCC isoform mRNAs and express their respective proteins.

2. A Comparison of SERCA Expression during the Development of Freshwater Crayfish and Marine Lobster

Susan Bibby, Yongping Gao and Michele G. Wheatly
Biological Sciences, Wright State University, Dayton, OH 45435

Of the different calcium transport proteins in the cell, the Sarco/Endoplasmic Reticulum Ca_2^+ adenosine triphosphatase (SERCA) is the most important in regulating intracellular calcium concentrations. The SERCA protein moves calcium into the lumen of the sarco/endoplasmic reticulum to reduce calcium levels in the cell's cytosol. In muscle tissue, it takes in calcium to the SR/ER from the cytosol after muscle contraction. In non-muscle tissue, it regulates intracellular Ca_2^+ concentration during routine signaling events or to effect mass transit of Ca_2^+ during periods of transepithelial flux.

The availability of ambient calcium is vastly different in saltwater as compared to freshwater crustaceans. In saltwater, calcium remains around 9.5mM, while the concentration of calcium in a freshwater environment is >1mM (Wheatly, 1996 *Physiological Zoology* 69(2):351-382). This inequality leads to the development of vastly different methods of calcium conservation in crustaceans: one of high conservation (freshwater) and one of low conservation (saltwater). These two methods of calcium conservation reflect the availability of calcium in the surrounding environment. Regulation of calcium for ecdysis (molting) is important for adult crayfish and lobster, but critical for a developing egg or larva.

In this study, SERCA protein expression was compared in four developmental stages: fertilized egg, larva/hatchling, juvenile and adult, using the axial muscle or whole egg of freshwater crayfish and saltwater lobster. Proteins were isolated by grinding frozen tissue in a lysis buffer. Western blot analysis was performed on the samples to compare the relative expression of SERCA at each of these life stages. Antibodies for SERCA were manufactured in this lab using procedure described in Wheatly et al., 2001, *J. of Exp. Biol*. 204: 959-966. Data was then normalized using an actin antibody on a duplicate membrane, and protein concentration was controlled using Ponceau stains.

Results indicate that the SERCA expression in the crayfish is greatest in the egg, decreases in the hatchling as well as in the juvenile until it levels out in the adult stage. The lobster shows no detectable SERCA expression in the egg or larval stages, increased expression in the juvenile, and a greater increase in the adult. SERCA expression appears to be visible by Western blot only when the exoskeleton of the crayfish or lobster is calcified and more greatly expressed during stages of rapid molting.
Supported by NSF grant IBN 0076035 to MGW.

3. Ammonium Secretion in Distal Colonic Epithelium

Roger T. Worrell and Jeffrey B. Matthews
Depts. of Surgery and Molecular & Cellular Physiology, Univ Cincinnati School of Medicine (Roger.Worrell@uc.edu)

Elevated portal vein NH_4^+ concentration compared to systemic levels suggests that NH_4^+ production or absorption occurs in the colon. The possibility that colonic mucosa might also possess the capacity for NH_3/NH_4^+ excretion or that transepithelial secretion and absorption might occur in different cell types has never been explicitly addressed. It is intuitively appealing to

consider the possibility that colonic epithelial cells would possess the capability to actively extrude NH_4^+ so as to limit the interstitial accumulation of this toxic substance, particularly in the proliferative crypt compartment. Indeed, there are a number of examples in nature where epithelia are faced with a high apical NH_4^+ concentration yet are able to actively excrete NH_4^+. Substantial circumstantial evidence suggests the colon may also be an active NH_4^+-extruding organ. In particular, recent studies have indicated that colonic crypt cells have a low apical NH_3/NH_4^+ permeability, and these cells contain the key transport proteins used in other species and tissues to actively excrete NH_4^+. Using the colonic crypt cell line T84, we sought to determine the ability of these cells to secrete NH_4^+. All models of NH_4^+ excretion described to date involve basolateral loading of NH_4^+ by NH_4^+ substituting for K^+ on Na/K-ATPase and/or NKCC1. NH_4^+ was transported on NKCC1 in T84 cells nearly as well as K^+, as determined by bumetanide-sensitive ^{86}Rb-uptake. pH_i measurements also indicated bumetanide-sensitve NH_4^+ entry. ^{86}Rb-uptake and ouabain-sensitive current measurements indicated that NH_4^+ is transported by Na/K-ATPase in these cells to a greater extent than K^+. Basolateral NH_4^+ application also induced an increase in transepithelial resistance. The basolateral loading of NH_4^+ by active transport systems and increased epithelial barrier function supports the likelihood of NH_4^+ secretion. Using T84 cells mounted in Ussing chambers, we determined the unidirectional transepithelial flux of NH_4^+ under asymmetrical conditions. With 10 mM NH_4^+ added to the apical side, the apical to basolateral flux (J_{ab}) was 6.9±2.0 μg/cm^2/hr. Basolateral NH_4^+ addition produced an NH_4^+ J_{ba} of 39.7±8.4 μg/cm^2/hr. Taken together, these data result in a net NH_4^+ flux in the secretory direction of 32.8±8.1 μg/cm^2/hr. Similar flux results were also obtained using native mouse distal colon, with the net secretory flux of NH_4^+ having a somewhat higher value (~350±50 μg/cm^2/hr). *Supported by NIH DK051630.*

4. Glycerol Permeability in Erythrocytes from an Amphibian that Accumulates Glycerol in the Cold

David L. Goldstein,[1] James Frisbie,[1] Andrew Diller,[1] Julie Carroll,[2] and Carissa Krane[2]
[1]Dept of Biological Sciences, Wright State University (david.goldstein@wright.edu) and [2]Dept of Biology, University of Dayton (Carissa.Krane@notes.udayton.edu)

Cope's gray treefrogs *Hyla chrysoscelis* tolerate freezing. As part of the strategy for cold acclimation in anticipation of the possibility of freezing, these amphibians accumulate high concentrations of glycerol, up to 50 – 100 mM in the extracellular fluid. It is likely that glycerol is synthesized in the liver and then delivered to other tissues where it acts as an intracellular cryoprotectant and osmotic agent. We therefore hypothesized that the permeability of cells to glycerol would be enhanced in cold acclimation. We tested this hypothesis on erythrocytes using two protocols: a lysis assay in which glycerol uptake induces osmotic water uptake and lysis, and measures of uptake of radio-labeled glycerol. We addressed four questions: a) What is the effect of temperature on glycerol permeability? [Glycerol permeability is markedly reduced in cold cells compared with warm.] b) What is the effect of cold acclimation on the permeability properties? [In our assays, we were unable to detect a change in permeability in erythrocytes from cold-acclimated frogs.] c) Is there geographic variation in these parameters? [Frogs from populations in Alabama had similar properties as those from Dayton.] and d) Are aquaporins involved in the uptake of glycerol? [Both lysis induced by glycerol uptake and uptake of radio-labeled glycerol were essentially abolished by mercurial compounds that inhibit aquaporins. We have been able to detect one aquaporin from treefrog erythrocytes, identified using primers designed from an orthologue we suspect of belonging to the glyceroporin family of aquaporin proteins.] Thus, we suggest that treefrog red blood cells have glyceroporin-mediated uptake of glycerol. Those glyceroporins may be constitutive, but they allow intracellular accumulation of glycerol as that solute is added to the circulating plasma during cold acclimation.

5. MIP Family Members in Cope's Gray Tree Frog *Hyla Chrysoscelis* Exhibit Tissue-specific and Thermal-sensitive Gene Regulation

Sarah L. Zimmerman,[1] David L. Goldstein,[2] James Frisbie,[2] Andrew Diller,[2] and Carissa M. Krane[1]
[1]Dept of Biology, University of Dayton (zimmersl@notes.udayton.edu, Carissa.Krane @notes.udayton.edu) and [2]Dept of Biological Sciences, Wright State University (david.goldstein@wright.edu)

Aquaporins belong to a family of membrane integral proteins (MIP) present in all organisms. Vertebrates express 2 main functional classes of these proteins. Aquaporins (AQPs) are water selective channels, whereas glyceroporins (GLPs) allow passage of both water and organic compounds such as glycerol. Cope's gray treefrog *Hyla chrysoscelis* accumulates glycerol as part of its strategy for cold acclimation in anticipation of possible freezing. Given the ubiquitous occurrence of AQPs and GLPs and the substantial movements of water and glycerol in cold and freezing frogs, we hypothesized that AQPs and GLPs are

involved in amphibian osmoregulation. Since thermal acclimation in *H. chrysoscelis* is dependent upon rapid and regulated glycerol transport, we sought to identify members of the MIP family that facilitate glycerol transport in order to examine their potential role in this process. We identified mRNA for 3 proteins from the MIP family. HC-1 is predicted to encode a 272 amino acid protein. HC-1 exhibits 98% amino acid identity and 97% nucleotide similarity with the water channel AQP1 and exhibits ubiquitous expression in all tissues tested. HC-2 is predicted to encode a 280 amino acid protein. Interestingly, no apparent similarities between HC-2 and other known aquaporin family members were observed at the nucleotide level. Though divergent at the nucleotide level, HC-2 exhibits strong amino acid identity and similarity identified with AQP2 orthologs, the ADH-regulated aquaporin. HC-2 expression is highly tissue-specific, found primarily in organs of osmoregulation and varies with thermal acclimation. Whereas HC-2 expression is present in cold skin and warm fat body, it is absent in warm skin and cold fat body. HC-2 is also expressed in both warm and cold kidney, and warm and cold bladder. HC-3, a third aquaporin, was identified using primers designed against conserved regions of several GLP-type proteins. Motif analysis of HC-3 identified GLP-distinctive amino acids indicating that HC-3 is a likely member of the AQGLP branch of the MIP family with a potential glycerol transporting role. HC-3 is most similar to anuran and mammalian AQP3 and subject to both tissue-specific and thermal-selective regulation. HC-3 cDNA is present in warm brain but not cold brain, cold liver but not warm liver, and is present in both warm and cold skin, kidney, fat body, eye, bladder, and gut. In conclusion, we have identified three new members of the MIP family present in *H. chrysoscelis*, two of which are subject to tissue-specific and thermal-responsive gene regulation. Our data strongly implicate the participation of the AQP/GLP molecules in mediating the physiological events of fluid and glycerol transport that occur during thermal acclimation in Cope's gray tree frog.

6. Serotonin: Effects on Temperature Sensitive and Insensitive Hypothalamic Neurons

Nicholas T. Unger,[1] Catherine Gabarée-Boulant, Ph.D.,[1] Jack A. Boulant, Ph.D.[2]
[1]Biology Department, Capital University, Columbus,Ohio; [2]Department of Physiology & Cell Biology, The Ohio State University, Columbus, Ohio

Neurons in the rostral hypothalamus sense body temperature and control thermoregulatory responses. Changes in hypothalamic serotonin (5-HT) are associated with alterations in body temperature. In particular, activation of 5-HT(7) receptors are linked with hypothermia due to enhanced heat loss or suppressed heat production. To understand the role of serotonin in hypothalamic synaptic networks, the present study characterized the effects of 5-HT on different types of hypothalamic neurons. Intracellular recordings were made from warm sensitive and temperature insensitive neurons in rat hypothalamic tissue slices. These 350 μm thick slices were constantly perfused with either normal artificial cerebral spinal fluid (ACSF) or ACSF containing 10 μmol 5-HT. To date, the recordings of action potential firing rates show a general trend in which 5-HT often excites warm sensitive neurons (linked with heat loss responses) and inhibits temperature insensitive neurons (linked with heat production responses). These results suggest that serotonin has specific effects on neuronal networks controlling different thermoregulatory responses. *Supported by NIH grants NS14644 and NS045758.*

7. Homeostatic Plasticity in the Nucleus Tractus Solitarius following Intermittent Hypoxia

David Kline, Angelina Ramirez-Navarro and Diana Kunze
Dept of Neurosciences, Case Western Reserve University and Dept of Research, MetroHealth Medical Systems, Cleveland OH 44106 (david.kline@case.edu)

Changes in peripheral arterial chemoreflexes and cardiorespiratory parameters have been recognized in humans with OSA and animal models following intermittent hypoxia (IH). However, the neural mechanisms and brainstem circuits that mediate these physiological changes under IH remain elusive. In the present study, we examined neuronal and synaptic activity in the nucleus of the solitary tract (NTS), the primary integration site of cardiorespiratory sensory afferents, by electrophysiological methods in rats exposed to IH. Sham IH rats served as controls. Ten days of IH (i.e., a continuous cycle of 6% O_2 (50 sec) and 21% O_2 (3 min), 8 hrs/day) led to adaptation and plasticity in the afferent limb of the arterial chemoreflexes, in particular, in the synaptic transmission between chemosensory afferents and NTS relay cells. IH significantly increased NTS action potential (AP) discharge and promoted AP short-term facilitation following episodic stimulation, while decreasing evoked synaptic transmission between chemosensory afferents and their NTS relay cell. An increase in asynchronous and miniature neurotransmitter release counter-balanced this decrease in evoked transmission. Changes in neurotransmitter release were due to modification of calcium-dependence of release probability rather than the number of vesicles available, as determined by monitoring exocytosis under several release conditions. An increase in the phosphorylation state of alpha calcium-dependent

protein kinase II (CaMKII) occurred in the NTS and sensory afferents after IH. Blockade of CaMKII reversed EPSC depression in IH rats. Altogether, results demonstrate a novel form of homeostatic plasticity in the NTS brainstem circuit induced by events commonly observed in many disease states such as obstructive sleep apnea. These changes produce an increase in transmission at this synapse that would lead to improved cardiorespiratory responses.

8. The Effect of Chronic Hypercapnia (CH) on Ventilatory Control and Neuronal Intracellular pH (pH_i) Responses in Neonatal Rats

Nicole Nichols,[1] Amit S. Rattan,[2] Nick A. Ritucci,[1] Jay B. Dean,[1] Robert W. Putnam[1]
[1]Dept of Anatomy & Physiology, Wright State University School of Medicine, Dayton, Ohio, [2]Miami University, Oxford, Ohio

We studied the effect of CH on ventilatory control by exposing neonatal rats to CH (7.5%) starting either before birth (Protocol 1) or after birth (Protocol 3). Neither protocol resulted in changes of body weight, brain weight, or hematocrit, but both protocols resulted in increased heart weight. We studied the ventilatory response to inspired CO_2. The normal biphasic developmental response (Stunden et al. *Resp. Physiol.* 127:135-155, 2001) was shifted to the right with Protocol 1, but Protocol 3 resulted in no ventilatory response to inspired CO_2 (P6-P16). We also studied the pH_i response to hypercapnia in medullary neurons from chemosensitive and non-chemosensitive regions. Hypercapnic acidosis (HA) caused decreased pH_i with no recovery in neurons from chemosensitive regions in control and CH rats (both protocols). The normal pH_i recovery seen in response to HA with neurons from non-chemosensitive regions was not present in neurons from CH rats (both protocols). In summary, CH alters the ventilatory response of neonatal rats to acute hypercapnia. The basis for altered ventilatory response to hypercapnia is unclear, but it does not appear to involve altered pH_i responses of chemosensitive neurons. This CH model will be useful for studying altered ventilatory control and the pathophysiological effects of diseases such as heart or lung failure which involve CH. *Supported by NIH grants R01 HL56683 and T35 HL07805.*

9. The Effects of Hypercapnia on Membrane Potential (V_m) and Intracellular pH (pH_i) in Neurons and Astrocytes from the Retrotrapezoid Nucleus (RTN)

Robert W. Putnam,[1] Nick A. Ritucci,[1] Joseph S. Erlichman,[2] J.C. Leiter[3]
[1]Dept of Anatomy and Physiology, Wright State Univ School of Medicine, Dayton, OH 45435, [2]Dept of Biology, St. Lawrence Univ, Canton, NY 13617, [3]Dept of Physiology, Dartmouth Medical School, Lebanon,NH 03756

The RTN has a glutamatergic region thought to be involved in respiratory control. We studied neurons and astrocytes from this and other RTN regions. Whole cell pipettes containing pyranine, a pH-sensitive dye, were used to measure V_m and pH_i in response to hypercapnia (10% CO_2). All RTN neurons had a maintained acidification of about 0.15 pH unit (n=21). Of 8 neurons studied within the respiratory control region, 3 (38%) increased firing in response to hypercapnia. Of 13 neurons from other RTN regions, only 2 (18%) increased firing in response to hypercapnia. Whole cell pipettes can washout cellular responses, therefore, we repeated the V_m measurements using perforated patch pipettes. In the respiratory control region, 4 of 8 neurons (50%) increased firing in response to hypercapnia while 4 of 10 (40%) neurons from other RTN regions increased firing in response to hypercapnia. Astrocytes from all RTN regions responded similarly, using either whole cell or perforated patch pipettes. Hypercapnia resulted in a maintained acidification of about 0.15 pH unit (n=8) and depolarization of about 5 mV (n=13) in astrocytes. These data show there is a partial washout of the V_m, but not the pH_i, response of RTN neurons to hypercapnia. About 40-50% of all RTN neurons increase their firing rate in response to hypercapnia and are highly chemosensitive (chemosensitivity index of about 200). Supported by NIH grants HL56683 and HL71001.

10. Prior Hyposmotic Challenge Sensitizes the ATP-activated Anion Conductance in Cultured Hippocampal Astrocytes

Guangze Li,[1] and James E. Olson[1,2]
[1]Depts of Emergency Medicine, [2]Neuroscience, Cell Biology, and Physiology, Wright State University, Boonshoft School of Medicine, Kettering, OH, USA

Extracellular ATP and osmolality regulate anion channels in brain cells such as neurons and astrocytes. Previous studies indicated that activation of anion channels in cultured hippocampal astrocytes by extracellular ATP is enhanced by con-temporaneous exposure to hyposmotic conditions. We used whole cell patch clamp recording to investigate the relationships between activation of anion channels by ATP and by osmotic challenges. Patch and perfusate solutions contained 100 mM CsCl. Osmolality was adjusted by the addition of sucrose. Cells recorded in voltage clamp mode with a holding potential of 0 mV were stepped from -100 mV to +100 mV in 20 mV steps. Average currents were measured between 70 msec and 90 msec from the start of the voltage command pulse. Membrane conductance was determined by linear regression of the data between -40 mV and +40 mV. Under isosmotic conditions (290 mOsm), a single or repeated exposure to 100 μM ATP for 5 min caused no change in anion conductance. Similarly, exposure to 250 mOsm perfusate solution for 5 min had no effect on anion conductance. However, exposure to 100 μM ATP increased membrane conductance to 157±19% of the initial value (N=8, p=0.04) if cells had previously been exposed for 5 min to 250 mOsm perfusate followed by a 5 min washout with isosmotic perfusate. This sensitizing effect of hyposmotic exposure was completely blocked if 50 μM genistein, a tyrosine kinase inhibitor, was added to the 250 mOsm perfusate solution. The anion channel blocker, 100 μM niflumic acid, also blocked the ATP-activated membrane conductance observed following hyposmotic exposeure. Our results indicate that 100 μM ATP is not sufficient to activate purinergic-activated anion channels in hippocampal astroglial cells. The purinergic signaling pathway that activates these anion channels is sensitized by a protein kinase-dependent mechanism triggered by hyposmotic challenge. *Support contributed by Kettering Medical Center.*

11. Characterization of the Role of OFQ/N in the Stress Response

K. Zullig,[1] E. Murphree,[2] R. Reinscheid,[3] J. Janik[1] and P. Callahan[1]
[1]Center for Neuroscience, Dept of Zoology, zulligkl@muohio.edu, [2]Dept of Mathematics and Statistics, Miami University,Oxford, OH 45056 and [3]Dept of Pharmacology, University of California, Irvine 92697

The purpose of this study was to characterize the role of Orphanin FQ/Nociceptin (OFQ/N) in the stress response. Wild type (wt) and OFQ/N knockout (ko) mice were divided into four treatment groups: control, acute, chronic and chronic + acute. Controls were handled daily for eight days while acutes were handled daily for eight days, then subjected to a 30 min novel acute stress on an orbital shaker on the ninth day. Chronics were subjected to 30 min of immobilization daily for eight days and the chronic + acute group endured the chronic treatment followed by the novel acute stress on the ninth day. Animals were immediately sacrificed following handling or respective stress bouts. Circulating levels of corticosterone (CORT) and prolactin (PRL) were determined by radioimmunoassay. Relative levels of prolactin receptor (PRLR) mRNA in the choroid plexus were determined by real time RT-PCR. In both wt and ko mice, all stressed groups had significantly higher levels of CORT than the control group, showing no adaptation in the CORT response. PRL levels were elevated in one wt chronic animal and three ko chronic animals. All other groups' PRL levels were similar to control levels. This was suspected to be the result of a rapid and transient increase in PRL, so a time course study was conducted (acute stress bouts of 5, 15 and 30 min). In wt and ko females and ko males, PRL levels were increased after 5 min, but returned to basal levels by 15 min. Wt males did not show a PRL response to acute stress. CORT levels in all groups were lowest after 5 min and highest after 30 min. The time course of the PRL response was different from the CORT response, i.e., rapid increase, but returned to basal levels by 15 minutes. PRLR mRNA levels were lower in ko vs. wt under resting conditions and after chronic stress. Following chronic stress, ko animals had lower levels of PRLR when compared to ko controls, but wt animals had higher levels when compared to wt controls. Results indicate that OFQ/N may play a role in PRLR expression in the choroid plexus under resting conditions and in the upregulation of PRLR following stress. *Supported by NIH DK61956-01 to PC.*

12. Dopaminergic Mechanisms Involved in Estrogen Modulation of the Prolactin Response to OFQ/N

B. Johnson, J. Janik and P. Callahan
Center for Neuroscience, Dept of Zoology, Miami University, Oxford, OH 45056, (Johnsobn@muohio.edu)

The goal of this study was to determine the neural mechanisms involved in estrogen modulation of the prolactin (PRL) response to Orphanin FQ/Nociceptin (OFQ/N). We hypothesize that estrogen sensitizes the tuberoinfundibular dopaminergic (TIDA) neurons so the inhibitory effects of OFQ/N on dopamine are greater under estrogen modulation and that this modulation mediates the prolactin secretory response to OFQ/N. Ovariectomized Sprague-Dawley rats received chronic estrogen replacement or a placebo for two weeks before tissues were collected. OFQ/N or saline vehicle was injected into the right lateral ventricle and the rat was sacrificed at 1, 3, or 10 minutes after injection. The neurointermediate lobe, anterior

pituitary,

median eminence, hypothalamus, and trunk blood were collected. Circulating prolactin levels were detected by radioimmunoassay. Prolactin levels increased by 3 minutes after injection of OFQ/N and remained elevated through 10 minutes in estrogen and placebo replaced animals. However, the magnitude of the response was much greater in the estrogen replaced animals. DOPAC/DA, an indicator of dopaminergic activity, was quantified in the median eminence by HPLC/ECD. In estrogen replaced animals, dopaminergic activity in the median eminence was inhibited by 1 minute after injection of OFQ/N. Dopaminergic activity in the median eminence of the placebo replaced animals was inhibited, but the magnitude was less than the estrogen replaced animals. This indicates TIDA neurons are inhibited after OFQ/N administration and that this inhibition mediates the prolactin secretory response to OFQ/N. Also, the TIDA inhibition and prolactin secretory response are greater in the presence of estrogen. *Supported by NIH.*

13. Neural and Endocrine Impact of Orphanin FQ/Nociceptin on Maternal Behavior

K. Fisher,[1] A. Cady,[1] J. Janik[1] E. Murphree,[2] R. Reinscheid[3] and P. Callahan[1]
[1]Center for Neuroscience, Dept of Zoology, [2]Dept of Mathematics and Statistics, Miami University, Oxford, OH 45056 and [3]Dept of Pharmacology, University of California, Irvine 92697

The goal of this study was to test the hypothesis that Orphanin FQ/Nociceptin (OFQ/N) is necessary for normal maternal behavior. Homologous pairs of transgenic mice were bred; those expressing normal levels of OFQ/N (WT) were compared to OFQ/N knock-out (KO) mice. After parturition, the female mice were assigned to one of two groups: behavioral or pup survival. Twenty-four hours after parturition, dams in the behavioral group were observed, scored for maternal behaviors and then sacrificed. The frequency and duration of these behaviors were recorded. In the pup survival group, pup and dam body weights, and the number of live pups were recorded daily until there were no longer any living pups present in the litter. Dams were sacrificed on that day. Upon sacrifice, the pituitary gland was removed and the hypothalamus was microdissected from the rest of the brain tissue; trunk blood was also collected to determine circulating prolactin levels. In addition, the mammary gland was removed and examined for developmental differences between wild type and OFQ/N knock-outs. Twenty-four hours after parturition, behavioral group knock-outs had a significant decline in pup survival (57.5%) compared to the wild type. A greater decline in pup survival over time was also observed. Cross-fostering experiments indicated that the offspring of knock-out mothers could survive if supported by either a heterozygous or wild type mother. Although there were no significant differences in histology in the mammary glands, knock-out mice had significantly lower PRL levels than wild type dams. These results suggest that OFQ/N plays a physiologically significant role in PRL regulation in post-partum females. We hypothesize that either less PRL is synthesized in the pituitary or less PRL is released in response to suckling. Anterior pituitary PRL content is currently being quantified. Additionally, estrogen receptor (ERα) expression levels in the hypothalamus and pituitary are being measured as an indication of pituitary sensitivity to stimulation. These studies will provide insight into the physiological role of OFQ/N role in mediating physiological control of PRL secretion. *Supported by NIH DK061956-01 to PC.*

14. Forming a Memory of Social Rank: Brain Pathways and the Implications of Neurogenesis

Kaushik Ghosal and Kathleen A. Killian
Center for Neuroscience, Department of Zoology, Miami University, Oxford, OH-45056 (ghosalk@muohio.edu, killiaka@muohio.edu)

We are investigating the neural basis of aggression in the cricket *Acheta domesticus*. Our goals are to: 1) identify neural pathways responsible for establishing social rank; 2) determine whether crickets maintain a memory of their rank, and 3) ask if brain neurogenesis is involved in the formation of this memory. When two male crickets make antennal contact, they begin fighting until establishment of dominance and subordinance. The dominant male sings a rivalry song and chases the subordinate, which runs away. We hypothesized that antennal contact activates rank-specific brain pathways that trigger these behaviors. We used c-Fos, a marker of neuronal activation, to identify these pathways. Male crickets interacted for one hour following establishment of rank. Control males were handled similarly except remained isolated. Brains of dominant, subordinate and control males were removed, fixed, embedded in paraffin and serially sectioned. Two anti c-Fos antibodies mapping to different regions of the Fos peptide were used to immunocytochemically localize Fos. Our results revealed cytoplasmic and/or nuclear Fos-like immunoreactivity in neurons of the pars intercerebralis, an important neurosecretory region and in neurons and processes near the mushroom bodies, brain regions important in sensory integration. To investigate the hypothesis that fought males retained a memory of their social rank, we allowed size-matched males to engage in two fights separated by 24 hrs. During the second fight, 82% of pairs (n=50) maintained their rank established during the first fight. Also,

the level of aggressiveness of the second fights was lower than that of the first ($p<0.001$, ANOVA). To investigate the role of neurogenesis in this memory, we

injected crickets with 15 mg/ml BrdU in saline immediately after the first fight (dominants, subordinates) or period of isolation (controls). All crickets were sacrificed 24 hr later and BrdU, a marker of newly divided cells, localized to cells in the proliferative zone of control and fought crickets. Preliminary counts showed no difference in the number of BrdU positive cells in this proliferative zone for fought and control males (n=8 for each). However, dominants and subordinates do tend to have more BrdU-positive cells near the mushroom bodies. The mushroom bodies are known to be centers for learning and memory in insects, suggesting that these migrated cells may play a role in social memory formation in the cricket. Future experiments will test this idea. *Supported by NIMH, Sigma Xi and the Miami University DUOS Program.*

15. Promoting Neuroscience Education with Science Olympiad™

James E. Olson
Dept of Emergency Medicine, Dept of Neuroscience, Cell Biology, and Physiology, Wright State University, Boonshoft School of Medicine, Kettering, OH (james.olson@ wright.edu)

Science Olympiad™ represents a significant opportunity to promote neuroscience education for students in grades 6-12. Science Olympiad (www.soinc.org) originated in 1985 as an annual interscholastic competition for students in middle and high school. Competitions consist of 23 events that encompass natural, physical, and earth sciences as well as long-term engineering and technology projects. Teams of 15 students compete at regional, state, and national levels. The top-ranked teams at each competition progress to the next level. Approximately 14,000 teams participated in Science Olympiad programs last year from all 50 states and Canada. In 2004, I conceived and developed an event entitled "Neuroscience…This is Your Brain" that was chosen by Science Olympiad Inc. as a pilot event. Separate curricula outlines were designed for middle school (B-division) and high school (C-division) students. Topics for B-division included the gross anatomy of the central and peripheral nervous systems and functional cellular anatomy. Students in C-division were expected to know all B-division topics plus functional gross anatomy, basic electrophysiology and synaptic action. In 2005, Neuroscience…This is Your Brain was held in Ohio at eight invitational and regional competitions and the state tournament with over 150 teams from Ohio and adjacent states taking part in this activity. Human specimens displayed during the examinations had a positive impact on the visibility and interest of the event and of neuroscience in general. Test scores showed a significant trend toward higher values throughout the competition season, suggesting students were preparing for the event and learning the subjects. National Science Olympiad organizers have decided to incorporate the neuroscience event in the national competition for 2006. This would be a valuable opportunity for neuro-scientists interested in neuroscience education to become involved in Science Olympiad teams in their communities and to promote neuroscience awareness and education. *Support contributed by Centerville Science Olympiad Organization.*

16. Resting Potential Dependent Regulation of the Voltage Sensitivity of Sodium Channel Gating in Rat Skeletal Muscle *in vivo*

Gregory N Filatov, Martin J Pinter and Mark M Rich
Dept. of Neuroscience, Cell Biology and Physiology, Wright State University, Dayton OH 45435

Normal muscle has a resting potential of –85 mV but in a number of situations, there is depolarization of the resting potential that alters excitability. To better understand the effect of resting potential on muscle excitability we attempted to accurately simulate excitability at both normal and depolarized resting potentials. To accurately simulate excitability, we found it necessary to include a resting potential-dependent shift in the voltage dependence of sodium channel activation and fast inactivation. We recorded sodium currents from muscle fibers *in vivo* and found that prolonged changes in holding potential cause shifts in the voltage dependence of both activation and fast inactivation of sodium currents. We also found that altering the amplitude of the prepulse or test pulse produced differences in the voltage dependence of activation and inactivation, respectively. Since only the Nav1.4 sodium channel isoform is present in significant quantity in adult skeletal muscle, this suggests there are multiple states of Nav1.4 that differ in their voltage dependence of gating or there is a distribution in the voltage dependence of gating of Nav1.4. Taken together, our data suggest that changes in resting potential towards more positive potentials favor states of Nav1.4 with depolarized voltage dependence of gating, thus shift voltage dependence of the sodium current. We propose that resting potential-induced shifts in the voltage dependence of sodium channel gating are essential to properly regulate muscle excitability *in vivo*.

17. Signal Transduction Mechanisms of K-Cl Cotransport Regulation and Its Relationship to Disease

Norma C. Adragna,[1,2] Christina M. Ferrell,[2] Jing Zhang,[2] Mauricio Di Fulvio,[2] Carlos F. Temprana,[1,2] Alok Sharma,[1,2] Robert W.E. Fyffe,[3] David R. Cool[2] and Peter K. Lauf[1,4]
[1]Cell Biophysics Group, Depts of [2]Pharmacology & Toxicology, [3]Neuroscience, Cell Biology & Physiology, and [4]Pathology, Wright State University School of Medicine, Dayton, OH 45435 (norma.adragna@wright.edu)

The volume- and oxidation-sensitive K-Cl cotransport (COT) is involved in ion homeostasis through modulation by different osmotic, chemical and humoral factors acting independently or synergistically. Lately, three mechanisms of K-Cl COT regulation have been identified in vascular cells: 1) the Li-sensitive pathway, 2) the platelet-derived growth factor (PDGF)-sensitive pathway, and 3) the nitric oxide (NO)-dependent pathway. Li, commonly used in the treatment of *mania depressiva*, induces stimulation of the volume-sensitive K-Cl COT of low K sheep red blood cells at cellular concentrations lower than 1 mM and inhibition above 3 mM. In addition, Li causes cell swelling, as determined by relative cell volume and morphological measurements, and appears to regulate swelling-activated K-Cl COT through a protein kinase C-dependent pathway. PDGF, a potent serum mitogen for vascular smooth muscle cells (VSMCs), plays an important role in membrane transport regulation and in atherosclerosis. PDGF stimulates VSM K-Cl COT in a time- and concentration-dependent manner, both acutely and chronically, and these effects are abolished by AG-1296, a selective inhibitor of the PDGF receptor tyrosine kinase and occur at a posttranslational level. Furthermore, regulation of K-Cl COT by PDGF involves the signaling molecules phosphoinositide 3-kinase and protein phosphatase-1. Finally, the NO/cGMP/protein kinase G pathway, which is involved in vasodilation and when altered can lead to cardiovascular disease, regulates K-Cl COT in VSMCs at the mRNA expression and transport levels. Our recent studies on protein expression will open new avenues to explore the mechanism of regulation of K-Cl COT by different signaling pathways at a protein level.

In conclusion, a complex and diverse array of mechanisms and effectors are involved in the regulation of K-Cl COT, which in turn, enable this transporter to perform its volume regulatory and homeostasis functions.

18. The Calcium/Calmodulin Dependent Protein Phosphatase PP2B Has a Role in Regulating Exocytosis in *Paramecium*

Dean Fraga, Sabrina Barros, and Whit Schofield
Department of Biology, 931 College Mall, The College of Wooster, Wooster OH 44691 (dfraga@wooster.edu)

Paramecium tetraurelia are ciliated protozoa that contain membrane-docked vesicles called trichocysts. The trichocysts fuse with the membrane in a calcium-dependent manner and release proteins that form long crystalline spines which help *Paramecium* escape predators. We used a bacteria-mediated RNA interference (RNAi) protocol to determine if the calcium/calmodulin-dependent protein phosphatase PP2B was involved in trichocyst discharge in *Paramecium*. *Paramecium* cells were fed *Escherichia coli* containing an inducible PP2B dsRNA expressing vector (pL4440), as described previously with minor modifications. After treatment, *Paramecium* cells were selected and exocytosis triggered by the addition of a saturated picric acid solution. Cells were viewed at 20x using a phase contrast objective and images captured digitally. Cells were scored visually to determine exocytosis efficiency. Scores were verified by digital image analysis, and the total area covered by the discharged trichocysts was determined and normalized to the total cell body area in cross section. Normalized values were compared and the results indicated that PP2B RNAi treatment resulted in a reduced efficiency of exocytosis when compared to the controls. The number of cells exhibiting full exocytosis was reduced from 76% in control cells (n=129) to 25% in PP2B treated cells (n=190). Cells exhibiting dramatically reduced exocytosis (<50% full discharge) increased from 2% (control treatment) to 41% (PP2B treatment). Treated and control cells had no visible effects upon endocytosis, cell morphology or cell division. Based on these results, we conclude that PP2B has a role in the regulation of calcium-dependent exocytosis in *Paramecium tetraurelia* similar to its role in other systems.

19. Leukocyte Chemoattractants Induce Gene Expression in Promyelocytic Leukemia HL-60 Cells: Participation of PLD1 and PLD2 Isoforms on mTOR and S6K Cell Signaling

Farnaz Tabatabaian, Katheleen Frondorf, Nicholas Lehman and Julian Gomez-Cambronero
Department of Cell Biology and Physiology. Wright State University School of Medicine, Dayton, OH 45435

The cross talk between signaling mechanisms in leukocytes is beginning to be recognized as crucial for their functionality. Our studies concentrate on the cross talk between two major signaling pathways, phospholipase D (PLD) and ribosomal S6K,

and their role on leukocyte cell migration (chemotaxis). We hypothesized that the two known mammalian PLD isoforms, PLD1 and PLD2, play a differential role in regulating S6K/Chemotaxis. Specifically, we postulated that PLD1 is linked to mTOR and PLD2 to S6K at the level of gene expression, in a temporal fashion. To study the effect of PLD overexpression on S6K/mTOR kinases, certain preliminary experiments were conducted to gain a deeper understanding of our system at hand (cells, plasmids enzyme activities and biological functions). We identified the best transfection/differentiation sequence that will result in optimal expression of PLD1 and PLD2 proteins in differentiated HL-60 cells. To this enf=d, we used two constructs with the PLD1 and PLD2 genes: phCMV2-HA-PLD1 and pcDNA3-myc-PLD2. Differentiated HL-60 cells (dHL-60), but not the undifferentiated counterpart (uHL-60), undergo chemotaxis in response to IL-8, FMLP and ENA-78. We have shown feasibility of the immunofluorescence technique with the visualization of myc- and HA-tagged overexpressed proteins in dHL-60 cells. Gene expression of PLD1 and PLD2 was also measured by quantitative RT-PCR using TaqMan Gene Expression assay (PLD1 or PLD2, FAM-labeled). We conducted three kinds of characterization experiments: primers/probe optimization, standard curves of fluorescence *vs.* amount of cDNA to identify absolute cDNA and that multiplexing with two housekeeping genes β-tubulin and glucuronidase (Texas Red-labeled). The importance of our studies is that inflammation, wound repair and angiogenesis have in common an initial event of chemotaxis towards host- or pathogen-derived chemical stimuli (chemoattractants). Understanding the molecular basis of chemotaxis will also provide important insight into cell migration-related pathological processes such as chronic inflammation, atherosclerosis and cancer metastasis.

20. Lipid Signaling Pathways Stimulate and Inhibit Cl^- and K^+ Secretion across Guinea Pig Distal Colonic Mucosa

Susan Halm, Tianjiang Liao, Julian Gomez-Cambronero and Dan Halm
Department of Neuroscience, Cell Biology & Physiology, Boonshoft School of Medicine, Wright State University, Dayton, Ohio (susan.halm@wright.edu)

Secretagogue activation of Cl^- secretion across the colonic epithelium also stimulates electrogenic K^+ secretion. Thus, the cellular mechanism of Cl^- secretion in the colon includes apical membrane K^+ channels. The proportion of K^+ secretion to Cl^- secretion varies with secretagogue type, ranging from primarily K^+ secretion with epinephrine, through equal secretory rates with prostaglandin-E_2, to primarily Cl^- secretion with carbachol+PGE_2. Cyclo-oxygenase inhibitors indomethacin [2μM] and NS-398 [2μM] were used to suppress endogenous production of prostaglandins during secretagogue stimulation. It is likely that secre-tory control of ion transporters involves other membrane lipid derived elements resulting from activities of three major phos-pholipases: PLA_2, PLC and PLD. The primary K^+ secretion of modulatory activation by epinephrine occurred with only increased PLC activity (25%). PGE_2 stimulated PLC (40%) and PLD (25%); CCh stimulated PLD (20%). Inhibition of PLC with ET-18-OCH_3 [100μM] or D609 [100μM] reduced epi-stimulated K^+ secretory current in guinea pig distal colonic mucosa, measured as short-circuit current and transepithelial conductance. Similarly, epi-stimulated K^+ secretion was reduced during inhibition of PKC with staurosporine [0.3μM] or rottlerin [100μM], but not with Gö6850 [3μM]. Inhibiting DAG-lipase with RHC-80267 [50μM] stimulated K^+ secretory current but did not augment the maximal secretory capacity. These results support a signaling pathway for K^+ secretion involving DAG release by PLC followed by PKC-μ activation. PGE_2-stimulated Cl^- secretion was augmented by using the PLA_2-inhibitor aristolochic acid [300μM], the P450-inhibitor ketoconazole [20μM] or the lipoxygenase-inhibitor nordihydroguaiaretic acid [30μM]. These results support the presence of a repressor signaling pathway for Cl^- secretion involving conversion of arachidonate by lipoxygenase and P450. Thus, control of electrogenic Cl^- and K^+ secretion involves release of several membrane lipid components, with PLC/PKC activity leading to K^+ secretion and PLA_2/LOX:P450 activity leading to repression of Cl^- secretory rate. *Supported by NIH grant DK65845.*

21. Estrogen Receptor Activation Protects against Pro-inflammatory Cytokine-induced Apoptosis

Kashmira S. Kulkarni and Thomas L. Brown
Biomedical Sciences Ph.D. Program and Dept of Neurosciences, Cell Biology and Physiology, Wright State University, Dayton, OH

Sjögren syndrome (SS) is an autoimmune disorder characterized by destruction of exocrine tissue such as the salivary and lachrymal glands. In affected individuals, dry mouth (xerostomia) and dry eyes (xeropthalmia) are observed, suggesting this

results from the apoptotic destruction of the glands. In particular, inflammatory cytokines such as Tumor necrosis factor alpha (TNF-alpha) and Interferon gamma (IFN-gamma) have been suggested to induce apoptosis within the salivary gland.

In this study, the cytokines TNF-alpha and IFN-gamma were shown to induce apoptosis in the HSG cells, a human salivary gland cell line. This data was supported by results indicating activation of initiator caspases such as caspase 8 and caspase 9 and of effector caspase 3. Cleavage of caspase substrates such as XIAP, Bid, PARP, and alpha-II-spectrin was also observed. These results suggested the involvement of the receptor-mediated as well as the mitochondrial pathways in the apoptosis of the HSG cells.

Menopause has been associated with the onset of many autoimmune disorders. Decreased levels of estrogen are observed in menopausal women. Estrogen has been shown to inhibit apoptosis in various cell types by upregulating antiapoptotic proteins such as Bcl-2. Therefore, we hypothesize that Estrogen protects the human salivary gland cells from cytokine-mediated death by the upregulation of the antiapoptotic protein Bcl-2.

HSG cells expressing functional estrogen receptor-alpha showed an increased expression of the antiapoptotic protein Bcl-2 both in the presence as well as the absence of estrogen. When treated with cytokines TNF-alpha and IFN-gamma in the presence of estrogen receptor and estrogen, HSG cells show a decrease in apoptosis levels, suggesting a protective effect of estrogen in cytokine-induced apoptosis.

Understanding the mechanisms of cell death and the protective effects of estrogen in salivary gland cells will advance the development of better therapeutic tools.

22. A Peripheral CIRCADIAN Oscillator in the Gastrointestinal Tract of Zebra Finches (*Taeniopygia guttata*)

Thomas J. Van't Hof, Takashi Yoshomura* and Shinobu Yasuo*
*Department of Biological Sciences, Wright State University and Takizaki Corporation, Tokyo, Japan, *Nagoya University, Nagoya, Japan (thomas.vanthof@wright.edu)*

The phenomenon of food-entrainable rhythms and the role of gastrointestinal tract (GIT) melatonin remains unsolved. In previous work, we have shown that melatonin appears to be produced in the GIT of zebra finches and production is coordinated with the absence of food in the gut. Although it is likely the case, it is not known if melatonin synthesis occurs in the gut, if receptors for melatonin are present or if clock genes regulating the output from a circadian oscillator are present. Therefore, we investigated the presence of melatonin receptors, enzymes essential to the synthesis of melatonin and clock genes Per2 and Bmal1 in the GIT of fasted and fed zebra finches.

There is strong expression of mRNA for the committed step enzymes in the synthesis of melatonin, N-Aceyltransferase (NAT) and Hydroxyindol-O-Methyltransferase (HIOMT) in the lumen of proventriculus, duodenum, and ileum of both fasted and fed birds. Two melatonin receptors Mela and Melc were expressed in the same tissues. However, only in the proventriculus were the sense signals not observed. Expression of avian circadian clock genes Per2 and Bmal1 were rhythmically expressed in the SCN, proventriculus, duodenum and ileum. Expression of Per2 and Bmal1 was influenced by fasting in the luminal layer of the proventriculus. In the smooth muscle layer of both the duodenum and the ileum Per2 and Bmal1, expression was also observed and was significantly influenced by fasting in both tissues.

These studies indicate the GIT has the capacity to act as a peripheral oscillator and suggests it may be the first gate in the regulation of both circadian feeding behavior and circadian production of metabolic machinery responding to food in the gut. It also confirms suspicions that the GIT has the metabolic capacity to synthesize melatonin like the pineal gland.

23. Rapid Changes in Corticosteroid Binding Globulin in Response to Acute Stress in the Zebra Finch

Sharon E. Lynn and Creagh W. Breuner
College of Wooster, Dept of Biology (Slynn@wooster.edu); University of Texas, Austin, Integrative Biology (creagh@mail.texas.edu)

Unpredictable events such as severe storms and decreased food supply lead to an increase in circulating levels of glucocorticoids in vertebrates. However, studies of acute stress physiology often assume changes in glucocorticoids represent the primary mediator of an organism's response to stress. Growing evidence indicates that corticosteroid binding globulin (CBG), a steroid-specific binding globulin, may regulate tissue availability of glucocorticoids. The prevailing Free Hormone Hypothesis suggests that only unbound hormone is available to enter target tissues and bind receptors or to be cleared by the liver, suggesting the unbound or "free" fraction of hormone is the likely primary mediator of hormone action. Nonetheless,

CBG levels have not been considered to be a dynamic component of the acute stress response in vertebrates. We investigated changes

in plasma corticosterone (CORT, the primary glucocorticoid in birds) and CBG in captive male Zebra finches (*Taeniopygia guttata*) over a 60-minute standardized acute capture and handling protocol. Plasma CORT increased in a predictable way during the capture and handling paradigm, and CBG capacity declined significantly within 60 minutes of capture. We estimated the change in free CORT over the period of handling using two methods: 1) a baseline CBG level (to estimate what free CORT levels would have been if CBG levels were assumed to be static) and 2) the appropriate CBG level measured at each time point. When CBG binding capacity was assumed to be static, we calculated a 6-fold increase in CORT levels over the period of handling; however, when the appropriate dynamic CBG binding capacity was taken into account, we detected a 20-fold increase in CORT over baseline levels. We suggest this decline in CBG capacity may serve to rapidly increase the level of CORT that reaches tissues and interacts with receptors during periods of acute stress and may have important implications for behavioral and physiological responses to stressors in this species. We explore multiple hypotheses for the adaptive significance of such a change within a broad phylogenetic context.

24. Role of Endothelin Receptors in α_1-Adrenoceptor-mediated Coronary Vasoconstriction and Modulation by Obesity in Isolated Mouse Hearts

S.B. Bender, E.E. Drummond, R.E. Klabunde
Ohio University College of Osteopathic Medicine, Dept of Biomedical Sciences (sb450601@ohio.edu)

Diet-induced obesity and the metabolic syndrome are major risk factors for the development of coronary vascular diseases. The progression of these diseases is characterized by endothelial dysfunction leading to altered responses to vasoactive substances including nitric oxide (NO) and endothelin-1 (ET-1) as well as to α-adrenoceptor (α-AR) activation. Research has shown many vascular regulatory systems do not work independently of each other but rather in concert. The purpose of this study was to analyze the role of endothelin type A (ET_A) and type B (ET_B) receptors in α_1-adrenoceptor-mediated coronary vasoconstriction in isolated mouse hearts from normal and obese mice. We hypothesized that ET_A and ET_B receptor blockade would reduce α-AR-mediated vasoconstriction to a greater extent in obese mice. Obesity was induced in C57BL/6J mice by placing them on a high-fat, high-carbohydrate diet for 15 weeks. Hearts were rapidly excised, cannulated, and perfused under constant flow conditions with a Krebs-Henseleit buffer to which all drugs were added at known concentrations. Coronary perfusion pressure (CPP), which is directly proportional to coronary vascular resistance (CVR) under constant flow conditions, was measured through a side port in the cannula. We have previously shown that α_1-AR activation by phenylephrine (PE) produces pronounced vasoconstriction in both normal and obese hearts only when preceded by pretreatment with the nitric oxide synthase inhibitor L-NAME, although the vasoconstriction was less in obese hearts than in normal hearts. Therefore, all ET-1 receptor blocking experiments were performed in concert with L-NAME treatment. ET_A receptor blockade with BQ-123 enhanced PE vasoconstriction in obese hearts but had no effect in normal hearts. ET_B receptor blockade with BQ-788 did not affect phenylephrine-induced vasoconstriction in either group. These results suggest that ET-1, acting through ET_A receptors, acts to attenuate α_1-adrenoceptor-mediated vasoconstriction in obese hearts. These data support previous work from other labs showing that ET_A receptor activation can phosphorylate and thereby inhibit α_1-ARs. Therefore, with the enhancement in α_1-adrenoceptor-mediated vasoconstriction in obese hearts due to ET_A blockade, these data suggest obesity may lead to an alteration in the normal signaling interaction between ET_A and α_1-AR receptors, such that ET_A activation acts to suppress α_1-AR activity.

25. Cholinergic Input Is Critical in the Regulation of Heart Rate Variability and Stress Reactivity in Mice

Danielle D.B. Senador, Vera Farah, Luis F. Joaquim, Maria Claudia Irigoyen and Mariana Morris
Wright State University, Department of Pharmacology & Toxicology (mariana.morris@ wight.edu)

Stress is a risk factor in cardiovascular disease; however, the mechanisms through which stress mediates its cardiovascular effects are not defined. Using radiotelemetric recording coupled with autoregressive spectral analysis (SA), we studied the cardiovascular effects of stress with and without cholinergic blockade in conscious mice.

Mice (Male C57/BL, n=7) were exposed to shaker stress (5 min, 150 rpm) and muscarinic receptors were selectively blocked (atropine, 4mg/kg, ip). Arterial pressure was recorded at 5 kHz under baseline conditions and after stress, atropine and atropine/stress. Pulse interval (PI) and systolic arterial pressure (SAP) were submitted to SA with variability measurements in the low (LF, 0.1-1.0 Hz) and high (HF, 1-5 Hz) frequency ranges.

Stress increased MAP (97 ± 4 vs.113 ± 2 mmHg, baseline vs. stress) and HR (539 ± 15 vs. 631 ± 15 bpm, baseline vs. stress). PI variability was increased after stress when evaluated in both time (41 ± 6 vs. 75 ± 14 ms^2; baseline vs. stress) and

frequency domains. Similarly, there were stress-induced increases in SAP variance and its LF component.

Cholinergic blockade did not alter MAP but increased resting HR (~ 619 ± 20 bpm). Atropine reduced PI variance from 41 ± 6 to 5 ± 1 ms^2. Both components of PI variance were reduced by cholinergic blockade, the LF component of PI variability from 19±3 to 1.5±0.9 ms^2 and the HF component from 14 ± 4 to 3 ± 0.8 ms^2. SAP variance was not altered by atropine. The stress-induced increase in MAP was not affected by atropine, while the tachycardic response was attenuated (+ 17% vs. + 8%; stress vs. stress/atropine). Atropine blocked the increase in PI variance and in its LF component, while a residual HF component was still evident after stress/atropine. The stress-induced increase in SAP variance and in its LF component were not altered by atropine.

Data verify the role of parasympathetic nervous system in stress-induced changes in HR, BP and indices of variance. This study characterizes the role of the autonomic nervous system in controlling cardiovascular function in mice using acute stress to perturb the system and spectral analysis for quantitative evaluation.

26. Development of a Computerized Method for Analysis of Circadian Blood Pressure Rhythms in Mice

Lopes Leandro, Farah Vera, Senador Danielle and Morris Mariana
Wright State University, Dept of Toxicology & Pharmacology, (lopes.2@wright.edu)

The objective was to establish a computerized method for analysis of blood pressure (BP) rhythms in mice, from digital data acquisition to graphical presentation of rhythms. The method used a computer program called "Clocklab" which is specialized for the analysis of behavioral patterns. This program is interfaced with "Matlab," a mathematical program for graphical analysis. Clocklab uses Matlab functions to build graphics and export results. Since the method was developed specifically for use with behavioral data, it was not directly applicable to blood pressure data. The reason for this is the difference in the type of data. Behavioral data is collected sporadically with a relatively slow rate as compared to blood pressure in which the data is recorded continuously (500Hz). After optimization of the programs, we were able to determine the parameters of circadian cycles in mice. BP was recorded using a telemetric system (model TA11PA-C20, Data Sciences International, St. Paul, MN) which provides for long-term measurements. Male C3H/HeN mice were prepared with carotid arterial catheters. After a recovery period of at least one week, BP was recorded continuously (500Hz) for 5 days. Each day had two periods: light (0501-1700h) and dark (1701-0500h). Digital data was transformed into an Excel file and then transferred to Clocklab. The Clocklab analysis collapsed the 5 days into a one-day graphic (24 hr) and provided the mean of the light and dark periods. MAP was 102.0 +/- 4.0 and 108.0 +/- 3.6 mmHg during the light and dark periods, respectively. To obtain the peaks and troughs, the program fitted (mathematically) the most accurate sine wave over the one-day composite data. This sine function was used to determine the acrophase (peak of a sine wave), bathyphase (trough), and MAP as well as timing. Results showed the lowest level of MAP occurred around 9:45am (4:48 hrs after lights went on). In addition, the highest level of blood pressure occurred around 9:45 pm (4:48 hrs after lights went off). Through this method and BP analysis, the experiment may have established an average time for trough and peak. In this case, in a 24-hour-period mouse, the trough and peak may happen around 4 hours after the light-state (on or off) change.

Circadian Parameters of Blood Pressure Rhythms in Mice

Bathyphase (Time – 24hrs)	Bathyphase (mmHg)	Acrophase (Time – 24hrs)	Acrophase (mmHg)	Amplitude (mmHg)
4:48	97.7	16:48	113.4	7.9

27. Angiotensin-Converting Enzyme2 (ACE2) Activity in Mouse Brain: Use of Mass Spectrometry in Cardiovascular Research

Tatiana S. Cunha, Khalid M. Elased, Mariana Morris
Wright State University Boonshoft School of Medicine OH/USA, State University of Campinas Faculty of Dentistry of Piracicaba SP/Brazil (mariana.morris@wright.edu)

ACE2 was first discovered in heart and kidney and thought to be associated with cardiovascular disease. Our studies extend these results to show that ACE2 is also present and active in brain. For this, we developed a new mass spectrometric (MS) assay which provides quantitative data on ACE2 activity. The method uses tissue extract incubation with Ang II as the substrate with

measurement of the peptide product, Ang 1-7. Kidney, hypothalamus, hippocampus, brainstem, pituitary and cerebral cortex from C57BL/6 mice were homogenized in Tris HCl (50mM, pH 7.4). Tissue extracts (.06 - 2 ug total protein) were added to MES buffer containing protease inhibitor and spiked with Ang-II (10 uM). The mixture was incubated in the presence or absence of ACE2 inhibitor (MLN-4760) or EDTA. Reaction mixture and generated peptides were spotted onto weak cation exchange ProteinChips and analyzed in the Ciphergen ProteinChips® Reader. Results were expressed as the ratio of the intensity of the formed Ang (1-7) (899, *m/z*) to Ang II (1045, *m/z*). As expected, ACE2 activity was detected in kidney. ACE2 activity was measured in brain with activity dependent on brain region. Activity (expressed as Intensity Units, IU) was highest in hypothala-mus (1.35± 0.22) followed by cortex (0.69±0.05), hippocampus (0.53±0.18), pituitary (0.27±0.16) and brainstem (0.06±0.02), respectively. There was a linear relationship between incubation time, protein concentration and product formation (Ang 1-7). Proteolytic activity was blocked in the presence of EDTA or MLN- 4760, showing the specificity of the reaction. This study shows 1) characterization of a novel, sensitive and specific assay for ACE2 activity and 2) the first evidence for ACE2 activity in brain with differences dependent on brain region. *TSC supported by CAPES/PDEE.*

28. Evaluation of Plasma Angiotensin Converting Enzyme I (ACE1) Activity in Streptozotocin-diabetic Mice Using Mass Spectrometry

Khalid M. Elased and Mariana Morris
Wright State University Boonshoft School of Medicine, Dayton OH

The renin-angiotensin system (RAS) plays a crucial role in regulation of blood pressure, cardiac function and electrolyte balance. Renin acts upon the precursor substrate angiotensinogen to release the inactive decapeptide Angiotensin I (Ang I). Angiotensin converting enzyme (ACE1) cleaves off two amino acids from Ang I to form the vasoconstrictor octapeptide, angiotensin II (Ang II). There is much interest in the use of plasma renin and ACE as markers for cardiovascular disorders. The typical methods of quantifying angiotensin peptides and related enzyme activity are spectrophotometry and radioimmunoassay (RIA). These methods have down sides related to the requirement for specific antisera and a large sample volume. In the present study, a novel method based on Surface Enhanced Laser Desorption/Ionization Mass Spectrometry (SELDI-TOF-MS) was developed and optimized for assay of plasma renin and ACE activity. Plasma aliquots (0.5-2.5 µl) from normal and STZ-diabetic mice were added to 50 µl MES buffer (50 mM, pH 6.7) containing 2mM PMSF spiked with either renin substrate (*1759*, *M/Z*) or Ang I (1297, *M/Z*) and incubated for 2 hours at 37^{o}C. Weak cation exchange ProteinChips were spotted with 1µl of the reaction mixture and analyzed in the Ciphergen ProteinChips® Reader. Results demonstrated the formation of peaks corresponding to Ang I (*1297*, *M/Z*) and Ang II (*1045*, *M/Z*) which indicates renin and ACE enzymatic activity, respectively. There was a significant increase in the activity of ACE in plasma obtained from STZ-diabetics compared to controls. This study shows the potential for using SELDI-TOF-MS to study the processing of angiotensin peptides.

THE OHIO PHYSIOLOGICAL SOCIETY
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PARTICIPANT LIST

NAME	*INSTITUTION*	*E-MAIL*
Norma Adragna	Wright State University	norma.adragna@wright.edu
Javier Alvarez-Leefmans	Wright State University	francisco.alvarez-leefmans@wright.edu
Candice C. Askwith	Ohio State University	askwith.1@osu.edu
Catherine Gabaree Boulant	Capital University	cboulant@capital.edu
Phyllis A. Callahan	Miami University	callahp@muohio.edu
Kerry Cheesman	Capital University	kcheesman@capital.edu
Timothy C. Cope	Wright State University	timothy.cope@wright.edu
Adrian Corbett	Wright State University	adrian.corbett@wright.edu
Khalid M. Elased	Wright State University	khalid.elased@wright.edu
Kathrin Engisch	Wright State University	kathrin.engisch@wright.edu
Cate Fenster	College of Wooster	cfenster@wooster.edu
Dean Fraga	College of Wooster	dfraga@wooster.edu
Robert W. Fyffe	Wright State University	robert.fyffe@wright.edu
Ping Gao	Wright State University	ping.gao@wright.edu
Chris Gillen	Kenyon College	gillenc@kenyon.edu
David L. Goldstein	Wright State University	david.goldstein@wright.edu
Julian Gomez-Cambronero	Wright State University	julian.cambronero@wright.edu
Dan Halm	Wright State University	dan.halm@wright.edu
Susan Halm	Wright State University	susan.halm@wright.edu
Lynn K. Hartzler	Wright State University	lynn.hartzler@wright.edu
Richard Henderson	Wright State University	richard.henderson@wright.edu
Richard Klabunde	Ohio University	klabunde@ohio.edu
David D. Kline	Case Western Reserve University	david.kline@case.edu
Carissa M. Krane	University of Dayton	carissa.krane@notes.udayton.edu
Peter K. Lauf	Wright State University	peter.lauf@wright.edu
Guangze Li	Wright State University	guangze.li@wright.edu
Tianjiang Liao	Wright State University	tianjiang.liao@wright.edu
Sharon E. Lynn	College of Wooster	slynn@wooster.edu
Bryan Mackenzie	University of Cincinnati	mackenb@uc.edu
J. Gary Meszaros	NEOUCOM	jgmeszar@neoucom.edu
Marianna Morris	Wright State University	mariana.morris@wright.edu
James E. Olson	Cox Institute	james.olson@wright.edu
Robert W. Putnam	Wright State University	robert.putnam@wright.edu
Mark Rich	Wright State University	mark.rich@wright.edu
Ramachandran Thiagarajan	Wright State University	ramachandran.thiagarajan@wright.edu

Thomas J. Van't Hof	Wright State University	thomas.vanthof@wright.edu
Michele Wheatly	Wright State University	michele.wheatly@wright.edu
Roger T. Worrell	University of Cincinnati	roger.worrell@uc.edu
ZiJian Xie	Medical University of Ohio	zxie@meduohio.edu

STUDENT PARTICIPANT LIST

NAME)	*INSTITUTION*	*E-MAIL*
Hayley Ashbaugh	Wright State University	hayley.ashbaugh@gmail.com
Shawn B. Bender	Ohio University	sb450601@ohio.edu
Susan Bibby	Wright State University	suzzi_katherine@yahoo.com
Xiaomi Chen	Wright State University	xiaomichen@yahoo.com
Tatiana De Sousa Da Cunha	Wright State University	tatiana.cunha@wright.edu
Aumend Cunningham	Capital University	acunning@capital.edu
Kristin Fisher	Miami University	fisherkj@muohio.edu
James Frisbie	Wright State University	frisbie.2@wright.edu
Kaushik Ghosal	Miami University	ghosalk@muohio.edu
Russell Horton	Capital University	rhorton@capital.edu
Zeenat Jamal	Wright State University	jamal.4@wright.edu
Brandi Johnson	Miami University	johnsonbn@muohio.edu
Nathaniel Kenworthy	Capital University	nkenwort@capital.edu
Kashmira S. Kulkarni	Wright State University	kulkarni.6@wright.edu
Leandro Lopes	Wright State University	lopes2@wright.edu
Casey McDonald	University of Dayton	mcdonaca@notes.udayton.edu
Shihong Mao	Wright State University	mao.5@wright.edu
Nicole L. Nichols	Wright State University	nichols.36@wright
Hector I. Rocha-Gonzalez	Wright State University	hector.issac@gmail.com
Danielle D. B. Senador	Wright State University	danielle.senador@wright.edu
Meera Seshadri	Miami University	seshadm@muohio.edu
Daisy Sigman	Capital University	dsigman@capital.edu
Alison Staton	University of Dayton	statonaa@notes.udayton.edu
Matt Stevens	Capital University	mstevens@capital.edu
Sarah Stith	Capital University	sstith@capital.edu
Farnaz Tabatabaian	Wright State University	tabatabaian.2@wright.edu
Laxminath Tumburu	Wright State University	laxminath@gmail.com
Nicholas T. Unger	Capital University	nunger@capital.edu
Meredith Wylde	Kenyon College	wyldem@kenyon.edu
Sarah L. Zimmerman	University of Dayton	simmersl@notes.udayton.edu
Kelly Zullig	Miami University	sulligkl@muohio.edu

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