DMU MENTORED STUDENT RESEARCH PROGRAM

July 17, 2015

Des Moines University
Olsen Center
Des Moines, IA 50312

RESEARCH IS VITAL AND AT DMU STUDENTS ARE VITAL TO RESEARCH.

DES MOINES UNIVERSITY

DMU Mentored Student Research Program
July 17, 2015
Des Moines University, Des Moines, IA

Dear Mentored Students

On behalf of the Research and Grants Committee, welcome to the Mentored Student Research Program closing program. This event represents and celebrates the culmination of your summer research experiences. We know you have worked hard over the last seven weeks; tackling challenges in the laboratory; learning new skills; and building new professional relationships. I was able to see firsthand the engagement, inquisitive minds, and commitment that each of you brought to this year's program and I am thoroughly impressed. Many of you will continue to work on your respective research projects over the coming academic year, and we encourage you to do so.

The goal of the Mentored Student Research Program is to provide opportunities for students to develop their skills as researchers by receiving coaching and mentorship from faculty. Dissemination of new knowledge, by faculty and student researchers, supports Des Moines University's mission. You have contributed to the fulfillment of this mission by your participation in the Mentored Student Research Program. We encourage you to continue to develop as a researcher, and thank you for your hard work this summer.

This program would not be possible without the support of many individuals and departments across campus. The University has invested in this program financially, researchers across campus have taken the time to deliver research talks, and your mentors have invested time in your professional growth and education. Please take the opportunity to thank your mentors for their investment this past summer.

I wish you the best of luck in your future endeavors. Keep asking questions and searching for answers!

Sincerely,
Dr. Feilmeier

Mindi J. Feilmeier, DPM, FACFAS
Assistant Professor, College of Podiatric Medicine and Surgery, Des Moines University
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Elizabeth Kunjummen, DO'18

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- Identify risk factors for ulceration
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- Understand the impact of diabetic foot complications on the health care system

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1:30 pm **Adjourn**

Keynote Speaker

David G. Armstrong, DPM, MD, PhD

Director, Southern Arizona Limb Salvage Alliance (SALSA),
Professor of Surgery, The University of Arizona

Dr. Armstrong is a Professor of Surgery at The University of Arizona, as well as the Director and Co-Founder of the Southern Arizona Limb Salvage Alliance (SALSA). He received his DPM degree from California College of Podiatric Medicine, his Master of Science degree in Tissue Repair and Wound Healing from the University Of Wales College Of Medicine, and his PhD and MD from the University Of Manchester College Of Medicine, where he was appointed Visiting Professor of Medicine.

Dr. Armstrong has produced more than 400 peer-reviewed research papers in more than two dozen scholarly medical journals as well as over five dozen book chapters and is co-editor of the American Diabetes Association's(ADA) Clinical Care of the Diabetic Foot. Dr. Armstrong was selected as one of the first six International Wound Care Ambassadors and is the recipient of numerous awards by national and international medical organizations including the inaugural Georgetown Distinguished Award for Diabetic Limb Salvage. In 2008, he was the 25th and youngest-ever member elected into the Podiatric Medicine Hall of Fame. He is the 2010 and youngest-ever recipient of the ADA's Roger Pecoraro Award, the highest award given in the field.

Dr. Armstrong sits on the Infectious Disease Society of America's Diabetic Foot Infection Advisory Committee. In 2011, he was appointed Chair of the World Diabetic Foot Commission of the FIP, representing clinicians from more than 30 nations. Dr. Armstrong is also the founder and co-chair of the International Diabetic Foot Conference (DF-Con), the largest annual international symposium on the diabetic foot.

Student Keynote Abstracts

The Role of Patient Expectations in Plantar Heel Pain Treatment Outcomes

Ellen Barton, DPM '18[1] and Shane McClinton, DPT, OCS, FAAOMPT[2]

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[2]*Doctor of Physical Therapy Program, Des Moines University, Des Moines, IA*

Patient treatment expectations impact clinical treatment outcomes, but this has not been studied in individuals with plantar heel pain (PHP). The purpose of this study was to assess the effect of patient expectations on PHP treatment outcomes. Patient expectations were recorded as part of randomized clinical trial comparing usual podiatric care (uPOD) and early physical therapy (ePT) intervention for PHP. Expectations for PHP outcomes at 6 weeks and 6 months were measured at baseline using a modified global rating of change (GRC) scale. Changes in levels of pain and function at 6 weeks and 6 months following initial treatment were compared between patients with high versus low expectations in addition to patients who met and did not meet baseline expectations. Analysis revealed no difference in treatment outcome between patients with high and low expectations ($p = .07 - .64$) and both groups demonstrated clinically meaningful improvements in pain and function. Individuals who met their 6 week and 6 month expectations were 5.8 times (95%CI 1.4, 23.6; $p = .01$) and 9 times (95% CI 0.8, 101; $p = .04$) more likely to achieve treatment success, respectively. No significant correlations were found between baseline global expectation and any of the 6 week or 6 month pain, function, and GRC scores. Patients who met their expectations were more likely to achieve treatment success which suggests that clinical practices should discuss reasonable expectations with patients to increase chances of success.

Comparing State Rankings of Health: An Analysis of Agreement between Measures of Population Health and Wellbeing

Elizabeth Kunjummen, DO'18[1]**,** Pamela A. Duffy, PhD[2], Simon Geletta, PhD[2]

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[2]*Department of Public Health, Des Moines University, Des Moines, IA*

Measures of health and wellbeing are increasingly valuable for public health. However, variance in these measures presents a challenge to consumers and policymakers on how to interpret such rankings. The purpose of this study was to examine the construct validity of Gallup-Healthway's Well-Being Index® (G-H WBI) state rankings, based on subjective survey data responses, as compared to America's Health Rankings® (AHR) state rankings, based on data from multiple established measures of health outcomes, environment, and wellness. Pearson correlations, trend line slope analyses, and binomial testing were done to determine correspondence between the two indices.

We found a statistically significant high degree of correlation between the two ranking systems on a national level. However, when divided into regions, or examined on an individual state level, the ranking correlations were no longer statistically significant. Furthermore, our binomial test showed the probability of two states landing in the same quintile for each ranking was significantly higher for the lowest quintile, and was significantly lower for the middle quintiles. This suggests that regionally, the two methodologies do not measure health to the same degree, and do not equally measure population health across the board. These findings support the conclusion that Gallup's wellbeing methodology may be useful in some applications, but is an insufficient measure of community health, as it does not correspond with accepted population health indicators, except at the lowest level of health. Policymakers must therefore determine which index is best suited to the community health issues of their state or region.

Rapamycin Inhibits the Protein Expression of the Cytosolic Branched Chain Aminotransferase (BCATc) in Lymphoma Cells

Ashley Torres and Elitsa Ananieva-Stoyanova, PhD

Department of Biochemistry and Nutrition, Des Moines University, Des Moines, IA

The cytosolic branched chain aminotransferase (BCATc) is overexpressed in many cancer types including lymphoma. BCATc catalyzes the first step in the degradation of leucine, isoleucine, and valine. Leucine is a known activator of complex 1 of the mammalian target of rapamycin pathway (mTORC1). Rapamycin specifically inhibits mTORC1 pathway.

The purpose of this study was to test the effect of rapamycin on BCATc protein expression and compare with the effect of other compounds such as cyclosporine A (CsA), which inhibits the Nuclear Factor of Activated T cells (NFAT) and 10058-F4, which inhibits the oncogene c-Myc. c-Myc controls the expression of more than 15% of human genes, BCATc included. NFAT regulates BCATc expression in immune cells.

We used a mouse lymphoma cell line (EL-4) treated with rapamycin, CsA, and 10058-F4 for 24 hours. Western Blotting was used to look at the impact of these inhibitors on BCATc protein expression compared to the expression of other branched chain amino acid (BCAA) enzymes. Rapamycin inhibited BCATc protein expression by 46%, but increased the expression of another BCAA metabolic enzyme, E1α. CsA did not inhibit any of the enzymes studied, while 10058-F4 inhibited BCATc and E1α.

Suppression of BCATc by rapamycin demonstrates that mTORC1 pathway plays a role in the regulation of BCATc protein expression. Lack of response to CsA indicates that BCATc is regulated differently in cancer cells than in immune cells. Understanding the regulation of BCATc expression in cancer cells will help define BCATc function in cancer and potentially find new treatment options.

Mycobacterium avium Utilizes C1q for Engulfment into Macrophages and for Down Regulation of TNF-α Production

Blair Tilkens, DO'18[1] and Suzanne Bohlson, PhD[2]

[1]*Osteopathic Medical Student, Des Moines University, Des Moines, IA*
[2]*Department of Microbiology and Immunology, Des Moines University, Des Moines, IA*

The intracellular pathogen mycobacterium evades the immune system by surviving within the innate macrophage immune cells of the host. Defense collagens such as the protein C1q enhance phagocytosis by macrophages while inhibiting the production of proinflammatory cytokines that are crucial for the host defense in eliminating the bacteria. This study tested the hypothesis that C1q facilitates macrophage engulfment of mycobacteria while dampening macrophage proinflammatory cytokine production. This mechanism may contribute to successful pathogenesis of mycobacteria within the host. To test this hypothesis, mouse bone marrow derived macrophages (BMDM) were harvested and activated with either a control protein (human serum albumin) or C1q. *M. avium* A5 was added to the macrophages and the percentage of macrophages associated with mycobacteria was quantified via fluorescence microscopy. Macrophages stimulated by C1q associated with 2.4 times as many mycobacteria compared to control macrophages ($n=4$, $p<0.05$). The production of the proinflammatory cytokines was quantified by measuring TNF-α from supernatants of macrophages associated with *M. avium* A5 using an ELISA. Activation by C1q resulted in a decrease in proinflammatory TNF-α production in response to *M. avium* A5 at multiple different macrophage to mycobacteria ratios. These results suggest that *M. avium* utilizes C1q for engulfment into macrophages while down regulating the proinflammatory response of TNF-α production allowing for successful pathogenesis within the human host.

Poster Abstracts

♦ 1 ♦

Information Avoidance and Social Support

Brenden Khounlo, **Megan Simpson, DO'18[1],** Rachel Reimer, PhD[2]

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[2] *Master of Public Health Program, Des Moines University, Des Moines, IA*

When given the opportunity to learn about health (e.g., STD tests) or risk for disease (e.g., genetic testing), many individuals will either decline the test, or avoid learning the results. This leads to delays in healthcare that can increase morbidity and mortality. Studies show that fear, anticipated negative emotions or actions, and stigma are key factors influencing the decision to avoid health information. Social support is a key coping mechanism and may influence patients' willingness to learn or avoid personally relevant health information.

The purpose of this study is to examine the effect of perceived available social support on obtaining personally relevant, yet potentially undesirable, health information.

Perceived social support will be experimentally manipulated by asking participants to write about a time of need during which they did or did not receive social support. Participants will be randomly assigned into a high support, low support, or control condition prior to filling out a risk assessment for a fictitious disease (B1AT deficiency). The dependent variable will be whether participants elect to learn their risk for this fictitious disease. We hypothesize that participants exposed to the high social support manipulations will be least likely to decline learning their risk for B1AT deficiency. It is hypothesized that participants in the control condition will avoid learning their risk at a rate between the two experimental groups. The results of this study have the potential to influence patient care and public health interventions for health screening behaviors.

♦ 2 ♦

Characteristics of Surgical Studies and their Relationship to Successful Completion

Iaswarya Ganapathiraju, DO'18 and Simon Geletta, PhD

Des Moines University, Des Moines, IA

BACKGROUND: Although clinical trials are initiated with extensive planning and deliberation, not all trials are completed with results. For example in drug trials, 1 in 12 molecules that make it to the clinical trial stage actually make it to discovery (Girard, 2004). Therefore, further study into what makes a clinical trial successful or not is required in order to help streamline the planning process and save valuable research money.

PURPOSE: Through this study, we sought to investigate the pattern of failure in surgery related trials with the goal of identifying key variables/characteristics that may be indicative of success or failure of such studies.

STUDY DESIGN: Registry based study of clinical trial summaries.

METHODS: We first obtained all randomized clinical trials that were initiated on or after May 28, 2015 from the clinicaltrials.gov database. We then used SAS to identify individual variables that were defined in each study. One such variable was "Overall Status", which helped identify whether the study was successful, whether it failed, or whether it is still in progress. We also divided the clinical studies into categories based on their characteristics such as surgical vs. non-surgical, and type of disease addressed. Finally, we analyzed trends of success versus failure among the various categories to detect any identifiable pattern correlating characteristics of the study and its likelihood of success/failure.

RESULTS: In progress.

CONCLUSION: In progress.

♦ 3 ♦

Tai-Chi Review/Intervention Analysis

Leonard Simon and Kathy Mercuris, PT, DHS

Background: Tai-Chi is a complementary alternative medicine exercise gaining popularity. Studies found improvement in strength, balance, flexibility, and decrease in stress and pain. Numerous variations in protocols rendered current data difficult to apply clinically. The current study measures the effects of Tai Chi for Arthritis (TCA) on stability over a 6-month period with healthy elderly.

Purpose: 1. Review the literature on tai-chi's effects on postural stability in well-elderly. 2. Understand reliability and validity of balance measurements: Four-Square Step Test, Fullerton Advanced Balance scale, Activities-specific Balance Confidence, Figure 8, and tri-axial accelerometry.

Methods: Primary search engines were EBSCO HOST and Superscope. A meta-analysis was completed to understand various protocols. The focus was narrowed to randomized controlled studies (RCT) similar to our study's protocol.

Results: From the initial 4 meta-analysis articles, 16 RCT and intervention studies were selected for detailed review. Varied definitions of community dwelling elderly were identified. Most studies used Yang or Sun styles with duration ranging from 15 to 52 weeks. A total of 41 balance measures were reported. Internal and external validity and test-retest reliability for our selected measures had an average ICC rating of 0.98

Conclusions: Tai-Chi intervention did not appear to significantly reduce the number of falls. Our current study uses clinical and accelerometer measurements for an assessment of postural stability rather than falls. Participants reported improved stability; supporting our use of questionnaires. Intervention duration of less than 16 weeks did not find significant changes and our study is over 24 weeks.

♦ 4 ♦

The Effects of Walking Velocity on the Function of the Transverse Arch of the Forefoot

Allen Kempf, MS, DPM'18[1], David Stapleton, BS2, Jim Mahoney, DPM[1], Vassilios Vardaxis, PhD[2,3]

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[2] *Human Performance Lab, Des Moines University, Des Moines, IA*
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Forefoot pathology is a common cause of concern for patients, and leads to challenges in their activities of daily living. The standard during diagnosis and evaluation is focused on foot structure; however, understanding foot function may allow physicians to modify potential treatment protocols. We compared the walking speed effect on the transverse arch function during the stance phase rockers (foot-ground interface) in gait.

Methodology: Twenty healthy males were fitted with retro-reflective markers identifying the first, second, and fifth metatarsal heads and bases. Subjects performed five walking trials at both self-selected and fast speeds, and bilateral measurements were recorded. Proximal, distal transverse arch (PTA and DTA, respectively) angles, and Euclidean distances were measured. Results considered significant at $p < 0.05$.

Results/Discussion: No bilateral differences were found during any of the rockers. The bilateral data were averaged for the heel, ankle, and toe rockers. There were no significant differences in the

forefoot function during the heel rocker. There was a significant increase in the Euclidean distance between the 1st and 5th metatarsal heads in the ankle rocker at the fast speed (p = .017). There was a significant decrease in the Euclidean distance between the 1st and 5th metatarsal heads at the fast speed (p = .007) during the toe rocker indicative of the forefoot stiffening with increased walking speed.

Conclusion: These data show for the first time the forefoot function and further our understanding of the role of the transverse arch of the foot during the stance phase of gait.

♦ 5 ♦

The Energy Cost of Walking While Thinking: Methodology for a Pilot Study

KayLynn Bland, DPT'18[1], Taylor Denkinger, DPT'17[1], Anna Hope, DPT'17[1], Jason Pedersen, DPT'17[1], Kaylee Spencer, DPT'17[1], James Lang, PhD[1], Jessie VanSwearingen, PT, PhD[2], Kristin Lowry, PT, PhD[1]

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Background: The brain requires energy to think.[1] Research has shown that engaging in thinking while walking results in changes in cardiovascular and metabolic measures.[2,3] The energy cost of walking is a measure of the work of walking, [5] and is influenced by biomechanical and neuromuscular factors,[4] and possibly brain function.[5] The purpose of this study is to determine if the energy cost of walking is sensitive to the cognitive load of thinking while walking.

Planned Methods: Participants will complete a series of cognitive tasks while walking on a treadmill. The tasks are easy and hard versions of updating, recall, and phenome monitoring. Participants will walk at their usual speed for 4 minutes followed by 2 minutes of engagement in the cognitive task, during which the treadmill speed will be held constant at their usual speed. Indirect calorimetry will be used to derive the energy cost of walking (mean oxygen consumption/gait speed at steady state, ml/kg m) in each condition.

Pre-pilot results (n=1): Without an added cognitive task load, the energy cost of walking ranged from .15-.16 ml/kg m. With the addition of the cognitive task load to walking, the mean energy cost of walking across the cognitive tasks ranged from .16-.17ml./kg-m.

Discussion: As demonstrated in this subject, the energy cost of walking was greater during walking while thinking than walking alone. This preliminary data supports further examination of the sensitivity of the energy cost of walking to the added cognitive load of walking while thinking in the larger sample (n=25).

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♦ 6 ♦

Comparison of Transverse Plane Stability Achieved with Multiple Screw Orientations after First Tarsal Metatarsal Arthrodesis

Andrea Okas, DPM'18[1], Mindi Feilmeier, DPM, FACFAS[2], Paul Dayton, DPM, MS, FACFAS[3], Britney Roberts, DPM'17[4,] Merrel Kauwe, DPM[5]

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Despite intercuneiform instability being recognized as a potential cause of hallux valgus recurrence following surgical intervention, little research has been done to determine how to best prevent this instability. The placement of a screw in the midfoot has been used to provide additional stability, however the anatomic placement of this screw varies widely among surgeons. The purpose of the present study is to evaluate the degree of instability in the first ray following fixation of the first TMTJ and the change in stability with the addition of a supplementary screw in three different anatomic orientations. In this study, locking plates will be secured across the first TMTJ of 12 fresh frozen below-the-knee amputated cadaveric specimens. A device for testing intercuneiform instability will then be constructed by fixating the cadaveric specimen to a testing block, inserting a testing screw into the first metatarsal neck and applying both a direct transverse and a rotational controlled force to this screw. Intercuneiform instability will be tested using this setup for each of the following screw placements: first to second cuneiform, first to second metatarsal and first cuneiform to second metatarsal. Fluoroscopy will be used to evaluate the change in IM angle resulting from the force applied to the first metatarsal. We hypothesize that additional fixation within the midfoot will reduce instability, however the most effective screw placement will be determined by our data collection. This research will contribute to a body of knowledge which has the potential to decrease the recurrence of hallux valgus.

♦ 7 ♦

Role of Labelled 3-D Leg Sections in Helping Students Identify Radiographic Structures

James Mahoney, DPM and **Dana Gustafson, DPM'18**

College of Podiatric Medicine and Surgery, Des Moines University, Des Moines, IA

The literature has shown that medical students have difficulty identifying anatomical structures on 3-D images, often needing extensive remediation during residency to interpret radiographic studies such as MRI. This study explores an additional learning tool to strengthen podiatric medical students' visual identification of MRI anatomy. Plastinated lower leg and foot sections were prepared in the transverse, coronal, and sagittal sections to determine their value in assisting students. These sections were then labelled and inserted into a binder for use by the students in the Lower Limb Anatomy course. Once the study is completed, the file will be stored in the DMU Learning Management System and accessible to all students.

Student volunteers of the Podiatry classes of 2017 and 2018 will take the Vandenburg and Kuse mental rotation test before beginning the traditional lower limb dissection course. This will provide a baseline for potential differences in spatial identification between students. At the same time, these volunteers will also take a 10-structure MRI identification quiz of transverse sections only. One group of volunteers will be randomly selected to have access to the labelled plastinated structures, and will

have once weekly, one-hour sessions with Dr. Matz to review the 3-D anatomy for 3 consecutive weeks (at 3 weeks the students will have completed the lower leg and foot portion of the course). The same 10-structure identification quiz will then be distributed to all volunteers again 4 weeks after the start of the course, as well as three months after the completion of the lower limb anatomy course. Results of the structure identification quiz between the participant groups before and after exposure to this additional learning material will then be compared. The goal of this project is to determine whether or not having access to the 3-D models will assist students in their interpretation of normal MRI anatomy.

♦ 8 ♦

Using Duplex Ultrasound to Assess Flow-Mediated Dilation and Shear Stress in the Brachial Artery

Kimball C. Kaufman, DO'18 and James A. Lang, PhD

Department of Physical Therapy, Des Moines University, Des Moines, IA

Endothelial dysfunction is an important mediator in the pathogenesis of cardiovascular disease and can be assessed by measuring the vascular responsiveness to an increase in luminal blood flow. The resulting increase in shear stress on a vessel promotes nitric oxide release and vasodilation. This mechanistic response is known as flow-mediated vasodilation (FMD). An image of the brachial artery was obtained utilizing duplex ultrasound, which allowed for simultaneous visualization of arterial waveform. A ten minute recording was performed on a young healthy subject which consisted of a two minute baseline, five minute arterial occlusion, and a three minute post-deflation period. This data was analyzed with dedicated FMD software that allows for automated wall tracking and velocity changes. FMD is presented as maximum diameter during hyperemia as percentage of baseline. In addition, shear rate can be calculated from time averaged values of velocity and vessel diameter. This value is presented as area under the curve. The FMD technique has been utilized in an effort to elucidate the mechanisms that alter vascular function. It has been shown that subjects with FMD values of <2% have significantly more cardiovascular events than those with normal FMD values (>6.3%). Through the use of this high-frequency ultrasound technique, in-vivo, non-invasive assessment of FMD and shear rate allows for reliable characterization of endothelial function.

♦ 9 ♦

Oral Tyrosine Supplementation and the Adrenergic Response to Cold and Exercise Stress

Kevin Smaller[1] and James Lang, PhD[2]

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Availability of L-tyrosine is necessary for the production of norepinephrine, which is released during cold and exercise stress. Focusing on the involvement of L-tyrosine in peripheral vasculature, we hypothesize that supplementation will 1) increase sympathetic-mediated vasoconstriction during gradual whole-body cooling (T_{sk}=30.5^{0}C) and 2) augment the pressor response to static handgrip exercise. Young (18-30 yrs; n=5) and older (60-85 yrs; n=5) subjects ingested L-tyrosine (150 mg/kg) or placebo an hour prior to the experiment. The vasoconstriction response was assessed using laser Doppler flowmetry and venous occlusion plethysmography over the course of a 45 minute cooling ramp and represented as a change in cutaneous and forearm vascular conductance from baseline (%ΔCVC and %ΔFVC), respectively. The pressor response was monitored during 2 minutes of static forearm contraction followed by 3 minutes of brachial occlusion. Preliminary data has demonstrated an increase in cutaneous and forearm reflex vasoconstriction to whole-body cold stress in older

adults, as well as a decrease in the recovery period following handgrip exercise. The beneficence of L-tyrosine was also seen to increase with age. This is particularly important as older adults exhibit a reduction in the sympathetic-mediated vasoconstriction (VC) and pressor response, which decreases their ability to maintain core temperature during cold exposure and regulate blood pressure during exercise. These results suggest oral L-tyrosine supplementation may offer a new, non-pharmacological approach to treating this lost VC response in older individuals.

♦ 10 ♦

Dogs Exhibit Increased Brain Gyrification Relative to Wild Canids

Steven Rose, DO'18[1], Clare Rusbridge[2], Cheuk Tang[3], Patrick Hof[4], Chet C. Sherwood[5], Paul R. Manger[6], Muhammad A. Spocter[1,6]

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Over the last 10 years research on dog cognition has revealed that dogs possess a surprising array of complex socio-cognitive skills, in many cases paralleling the behavioral abilities seen in relatively large brained mammals like great apes. Despite this, evidence in support of neuroanatomical restructuring of the dog brain has lagged behind and has left many wondering how dogs are able to perform these complex tasks with such diminutive brain sizes. One area not explored within the framework of canid domestication has been the degree of cortical folding (i.e., gyrification), a commonly used proxy for the functional complexity of the cerebral cortex and its information processing capacity. In the current study we used a quantitative magnetic resonance imaging (qMRI) approach to measure the gyrification index (whole brain, frontal and parietal-occipital) and associated white and grey matter volumes in the cerebral cortex of 17 carnivore species (including six wild canid varieties and five domestic dogs). Using allometric analyses we demonstrate that the cerebral cortex of the domestic dog is significantly ($P<0.05$) more folded than as predicted based on brain size and that dogs differ most markedly from wild canids in the degree of cortical folding observed in the parietal-occipital lobe, a region known to be involved in sensation, perception and the integration of visual information. These results provide the first evidence that the process of domestication may have inadvertently increased the complexity of the canine brain through expansion of the cortical surface area relative to brain size.

♦ 11 ♦

MRI Meets Video Gaming: An Interactive 3D Model

Steven Rose, DO'18[1], Marton Balogh[2], Clare Rusbridge[3], Cheuk Tang[4], Chris Trace[3], Muhammad A. Spocter[1,5]

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Traditional medical and veterinary education has used the cadaver as an integral part of first year anatomical instruction. However as demand for cadavers and teachers increases, amidst the ever-shrinking space for curricular innovation there is an advantage to using alternative approaches which could help students to engage with anatomical material outside of the dissection halls. In light of this, virtual 3-dimensional (3D) training offers students an opportunity to engage with spatially accurate representations of anatomical structures in a way which is not only less intimidating than dissection but also more immersive than text. Here, we use high resolution magnetic resonance imaging scans (acquired at 7 Tesla) to create a 3D model of the canid brain and identify individual structures including basal ganglia, limbic system, brainstem, cerebellum, and cortical white and gray matter, which often prove difficult for students to conceptualize. Using the image processing, visualization and analysis toolkit Analyze 10.0, we mapped the cortical and subcortical surface and created 3D reconstructions of the brain, with all parts accurately and volumetrically preserved within 3D space. Using Unity, a gaming and development platform, we created a first pass virtual brain learning environment which allows students to examine the brain and interactively peel away the cortical surface. This gaming inspired approach to neuroanatomical instruction allows students to engage and interact with the brain in a meaningful way from any location and with only a computer as a tool.

♦ 12 ♦

Cross-Sectional Area of the Corpus Callosum in Wild and Domestic Canids

Ashraf Uddin, DO'18[1], Clare Rusbridge[2,3], Jelena Jovanovik[3],Cheuk Tang[4], Patrick Hof[5], Chet C. Sherwood[6], Paul R. Manger[7], Muhammad A. Spocter[1,7]

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Domestication marked a major turning point in human prehistory, enabling humans to artificially select for animal behaviors that favored the interests of early human communities, and dramatically affecting the behavior and morphology of these target species. While all domesticated varieties exhibit marked reductions in overall brain size, it is unknown whether the corpus callosum, an integral white matter fiber pathway for interhemispheric and intracortical communication, is affected by domestication in an allometric or a mosaic pattern. To answer this question we used a quantitative magnetic resonance imaging approach (qMRI) to compare the mid-saggital cross-sectional areas of the corpus callosum, in 35 carnivore species, including six wild canids and 12 domestic dogs. Using a standardized sectioning approach we also extracted Wittelson regions for the corpus callosum and evaluated potential correlations with cortical white matter volume. The results of this study indicate that under the influence of domestication, cortical white matter undergoes allometric reductions relative to brain size, emphasizing the role of architectural and energetic constraints on the evolution of fiber pathways. These results represent an early step towards a thorough investigation of the canid corpus callosum using complimentary tractography and electron microscopy to evaluate potential axonal caliber and fiber density differences across species.

♦ 13 ♦

Hyperpneumatization of the Maxillary Sinus and its Relationship to Suture Morphology in Four Populations

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Morphological variation of the maxillary sinus and zygomaticomaxillary suture has been used in ancestral classification of human crania. The extent of pneumatization past the suture into the zygoma has been documented in isolated cases, but its relation to suture morphology and population frequency is unknown. The current study investigates the hypothesis that sinuses will more likely cross the simplest Type 1, versus more complex Type 2-3, sutures. CT scans of crania (n=82) from Buriat, Malaysian, West African, and European populations were first visually analyzed for pneumatization extent. Photographs and 3D-models of crania were then used to classify one of three suture types. Results show that while uncommon, each population equally demonstrates sinuses passing the suture into the zygoma. Chi-square analyses further demonstrate that while there is no association between population and frequency of sinus crossing, there is a relationship between population and suture type, as well as the specific suture type crossed by the sinus. While all suture types were observed as being crossed by the sinus, most populations (Malay, West African, and European) exhibit crossing of the Type 1 suture, partially supporting our hypothesis. Still, sinuses cross all three suture types, regardless of ancestry; additional studies are needed to determine other factors influencing hyper-pneumatization. Due to the subjective nature of visual analysis, further studies utilizing micro-CT scans and objective quantification of suture type are recommended. Understanding how the sinus crosses into the zygoma is clinically relevant, particularly in relation to sinus trauma, primary malignancies, and corresponding surgical considerations.

♦ 14 ♦

Analysis of the Sphenoid Sinus and Arrested Pneumatization in Relation to Cranial Base Angle

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Among the paranasal sinuses, the sphenoid is one of the most variable during development and is highly prone to arrested pneumatization (AP). AP, or early termination of sinus development, is recognizable by the presence of fatty bone marrow. High levels of variation and AP in the sphenoid sinus may be related to multiple factors, including cranial base angle (CBA). The project's purpose was to specifically test this relationship using MRIs provided by the PING database. We determined sphenoid size, skull size, and CBA by placing 3D landmarks on the MRIs using the program 3D-Slicer 4.4; our sample includes patients of both sexes, with ages ranging from 12 to 20. Results of t-tests show only craniofacial size being significantly different between male and females, while sphenoid size and CBA do not differ between sexes. Sphenoid height is the only sinus dimension that correlates with CBA. As related to AP, correlation and regression analyses show that there is a significant relationship between AP and cranial breadth. This relationship is primarily found in females, which might be explained by the differences between male and female cranial size. This information may provide some clinical application, such as the prevention of misdiagnosis of tumors in patients with unusual sphenoid sinuses or presence of AP. Additional studies of cranial breadth in relation to AP, especially in females, will be important to further recognize variation between humans, understand the elusive growth patterns and possible sinus functions, and improve clinical diagnosis and risk management of patients.

♦ 15 ♦

55 and 90 High Fructose Corn Syrup Suppression on THP-1 Cell Growth in High Concentrations after 14 Days

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Fructose consumption has dramatically increased in the last 30 years. The principal form has been in the form of high-fructose corn syrup (HFCS) found in soft drinks and processed food. We are only beginning to understand the effect of excessive fructose consumption on human health.

Fructose has been confirmed to induce several obesity related complications leading to the development of metabolic syndrome, production of fatty liver, and hyperuricemia. The elevated risks of atherosclerosis and cardiovascular disease are major causes of morbidity/mortality in patients with metabolic syndrome and have been associated with macrophage activation and formation of the atherosclerotic plaque.

This study evaluates the role high concentrations of HFCS have on the proliferation of THP-1 cells. The different forms of HFCS contained different ratios of fructose to glucose as follows – 42 HFCS: 42% fructose and 58% glucose, 55 HFCS: 55% fructose and 45% glucose, and 90 HFCS: 90% fructose and 10% glucose. THP-1 cells were grown in the presence of these different forms of HFCS and counted at days 7, 10, and 14. There was no difference in cell counts at days 7 and 10 for the three forms of HFCS; however, on day 14 there was suppression of THP-1 cells grown in the presence of high concentrations of 55 HFCS and 90 HFCS. This was compared to the suppressed growth from lipopolysaccharide activated THP-1 cells. These experiments were performed in duplicate, and repeat experiments are required to confirm our results.

♦ 16 ♦

Blueberry as a Promising Radiosensitizer for Treating Cervical Cancer

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Background: Cervical cancer is a leading cause of death in women worldwide. Radiation therapy (RT) for cervical cancer is an effective alternative, but its toxicity remains challenging. Blueberry is amongst the most commonly consumed berries in the United States. We previously showed that resveratrol, a compound in red grapes, can be used as a radiosensitizer for prostate cancer. This study was performed to investigate if blueberry extract (BE) could be used as a radiosensitizer to treat cervical cancer and its possible molecular mechanisms.

Methods: Clonogenic survival assay, immunohistochemistry (IHC), TUNEL staining, proliferation and caspase-3 activity kits were used to evaluate the effects of RT in combination with BE on cell survival, proliferation and apoptosis of a widely used cervical cancer cell line, Siha. We further investigated the possible molecular mechanisms by using RT-PCR, IHC.

Results: We found that the percentage of colonies, PCNA expression level and the OD value of Siha cells were all decreased in RT/BE group compared to those in RT alone group. TUNEL+ cells and the

relative caspase-3 activity in cancer cells were increased in RT/BE group compared to those in RT alone group. The anti-proliferative effect of BE on cancer cells correlated with downregulation of pro-proliferative molecule cyclin D and cyclin E. The pro-apoptotic effect of BE correlated with upregulation of pro-apoptotic molecule TRAIL.

Conclusions: BE sensitizes Siha cells to RT by inhibition of proliferation and promotion of apoptosis, suggesting that blueberry might be uses as a promising radiosensitizer to treat cervical cancer.

♦ 17 ♦

N-acetyl-leucine-amide (NALA), a Leucine Antagonist, Impacts the Energy Status and Growth of Osteosarcoma Cells

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Bone sarcomas have proven themselves a challenge to doctors and researchers with their unresponsiveness to traditional treatments and therapies. One novel solution to bone sarcoma resistance is to explore the role of branched chain amino acid metabolism of leucine, isoleucine, and valine. Leucine is a nutrient signal that regulates protein synthesis, and we hypothesized that the leucine antagonist, N-acetyl-leucine-amide (NALA), would produce an inhibitory effect on cell growth and protein synthesis in bone sarcoma cells.

For that purpose, we treated a human osteosarcoma cell line (143B) with NALA (25 mM and 50 mM) for a twenty-four hour period. The impact of NALA on major signaling pathways (mammalian target of rapamycin [mTOR] and AMP-activated protein kinase [AMPK]) in these cells was examined by Western Blotting.

The lower concentration of NALA [25 mM] caused cell growth inhibition along with increased phosphorylation of AMPK. The higher concentration of NALA [50 mM] had a severe inhibitory effect on cell growth and protein expression of all proteins examined.

The activation of AMPK in leucine-compromised cells indicates that leucine and/or leucine metabolism may impact the energy status of osteosarcoma cells, and cause a reduction in growth. Thus, limited leucine availability may be one novel solution to treat bone cancer patients.

♦ 18 ♦

EGCG and Telomerase Inhibitor MST-312 Inhibit HSV Infection in Vero Cells

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Approximately 90% of the world population has been exposed to herpes simplex virus (HSV). HSV infection often results in painful ulcerative lesions, with lifelong recurrent outbreaks. Antivirals for HSV exist, but drug resistance is an increasing concern, prompting the search for novel antivirals. Epigallocatechin gallate (EGCG) is a natural compound derived from green tea that can inhibit telomerase and has been investigated for anti-tumor properties. MST-312 is a synthetic analogue of EGCG. Here, antiviral properties of these compounds are assessed in Vero cells using microscopy and immunoblot assays. MST-312 and EGCG treatments failed to increase cell toxicity or decrease cell numbers compared to vehicle controls, demonstrating that they are neither cytotoxic nor cytostatic in this system. The presence of MST-312 during HSV infection reduced viral cytopathic effect (CPE)

and caused a dose-dependent reduction in the accumulation of late viral proteins. Drug addition after viral attachment also decreased viral protein levels. Although EGCG treatment did not dramatically alter HSV CPE, treatment with 70 and 100μM EGCG inhibited the accumulation of the immediate early viral protein ICP4, and all late proteins tested. Subsequently, the virucidal activity of EGCG and MST-312 were assessed by quantifying the plaque forming ability of pre-treated virus. For MST-312, the minimum concentration required to significantly decrease plaques was 20 μM. For EGCG, 20 μM was a sufficient concentration to yield undetectable plaques. Thus, both compounds exhibited direct virucidal properties. Together, these results provide further insight into the antiviral properties of EGCG and its chemical analogue MST-312.

♦ 19 ♦

Assessing the Conservation of a Functionally Analogous *Acinetobacter* Type VI Secretion System Toxin and Antitoxin Pair

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Acinetobacter spp., are known to be important causes of nosocomial infections, mainly in patients who are immunocompromised or on ventilators. Mortality rates among the infected are relatively high, which appears to be less dependent on virulence attributes and more dependent on *Acinetobacter* spp.'s multi-drug resistance.

We have previously shown that *Acinetobacter* nosocomialis, strain M2, possesses a type VI secretion system (T6SS), which is used to kill bacterial competitors. Like other gram-negative bacteria, the *Acinetobacter* T6SS, impales targets with a "dagger" and injects a toxic effector protein that elicits cell death. Many such bacteria produce an antitoxin, which inhibits the action of this toxin, preventing the intoxication of self and kin. Recently, we identified two adjacent genes in the M2 genome that appear to be a T6SS toxin and antitoxin, respectively.

These proteins could serve as targets for novel therapies used to treat infections caused by *Acinetobacter* spp. if the aforementioned M2 proteins have homologs in other *Acinetobacter* spp. We suspect the latter is true and hypothesize that the adjacent *A. baumannii* 19606 genes, 3110 and 3111, code for the toxin and corresponding antitoxin, respectively. These putative functional analogs in 19606 were identified using conserved domain prediction and conserved genes adjacent to the M2 toxin and antitoxin genes as markers.

We are currently generating unmarked, in-frame mutations of 3110 or both 3110 and 3111 in 19606. We will assess whether the mutations in these genes will result in an inability to kill other bacteria and survive competition with wild-type.

♦ 20 ♦

Initial Exploration of a Direct Interaction Between the Beta-1 Adrenergic Receptor and the G Protein-Coupled Estrogen Receptor

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The G protein-coupled estrogen receptor (GPER) is a novel receptor with many important roles in the cardiovascular system. Reductions in estrogen levels following menopause are associated with increased incidence of cardiovascular disease. Abnormal adrenergic responses are common in post-menopausal subjects and contribute to the development of cardiovascular disorders. The underlying mechanisms, however, are unknown. Our overall hypothesis is that GPER plays a significant role in these responses by directly interacting with adrenergic receptors. This project aimed specifically to explore a physical interaction between GPER and the β-1 adrenergic receptor (β_1AR). Initial efforts were made to verify a commercial β_1AR antibody. Immunoblotting with increased loads of porcine ventricular tissue demonstrated bands with increasing density, whereas a blocking peptide developed based on the antibody's epitope sequence from β_1AR completely prevented its recognition of the same loads. In addition, lysate from HEK293 cells overexpressing β_1AR showed increases in the band detected by the antibody, further verifying its specificity. Coimmunoprecipitation was subsequently performed in porcine ventricular tissue using this β_1AR antibody and a previously verified anti-GPER antibody. SDS-PAGE analysis of β_1AR-immunoprecipitated fractions clearly demonstrated the presence of GPER. These results strongly suggest direct interaction between β_1AR and GPER, and pave the way for further investigation on the mechanisms and functional impact of this association. Currently, we are testing the role of C-terminal binding motifs using wild-type and truncated versions of the two receptors. Moving forward, we hope to achieve a mechanistic understanding of how estrogen controls adrenergic functions and a therapeutic benefit thereof.

♦ 21 ♦

Determination of Diffusion Kinetics of Ketamine in Brain Tissue: Implications for Studies of Drug Mechanisms in vitro

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Background: Ketamine (2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone) has long been used as a general anesthetic acting through blockade of N-methyl-D-aspartate (NMDA) receptors in the brain. Ketamine, at sub-anesthetic doses, has shown promise as a potent and fast-acting antidepressant, especially in patients with treatment-resistant depression. The spectrum of action of ketamine is concentration dependent, so it is thus critical for *in vitro* mechanistic studies to use ketamine concentrations linked to specific behavior effects. We examined the diffusion kinetics of ketamine to determine the concentration of ketamine applied to brain tissue *in vitro* which appropriately represents *in vivo* conditions.

Methods: Brain slices of adult mice were prepared at a thickness of 300μm and incubated for 0.5-120 minutes in artificial cerebrospinal fluid containing 17.7 μM ketamine HCl. Concentration of ketamine was measured using HPLC-MS. The diffusion and partition coefficients for ketamine were determined and computational modeling was used to represent the concentration of ketamine within the brain slices as a function of depth and time.

Results: The diffusion coefficient for ketamine into brain tissue was approximately 0.04×10^{-6} cm^2 s^{-1}. The brain:aCSF partition coefficient for ketamine was 5. The uptake of ketamine into brain tissue fit with a mono-exponential function with time constant τ=15.7 minutes.

Conclusions: Ketamine is highly soluble in both water and lipids, quickly equilibrating in brain tissue at a concentration 5 times higher than the surrounding media. Caution should be exercised when interpreting results derived from previous *in vitro* studies where the concentrations of ketamine used greatly exceed those which produce antidepressant effects *in vivo*.

♦ 22 ♦

The Effects of Chronic Stress on the Microbiome: A Metagenomic Analysis

Aaron Shoskes, DO'18, Alexandra Proctor, Kathryn Battani, MBS'16, Vanja Duric, PhD, Gregory Phillips, LiLian Yuan, PhD

Background: Evidence supports communication between the brain, the gastrointestinal tract, and the gut microbiota. Stressors influence both the GI tract and its resident microbiota, and dysbiosis of GI tract colonization is associated with different diseases. We investigated the relationship between chronic stress and the murine gut microbiota by comparing the taxonomic composition before and after chronic stress.

Methods: Mice were subjected to chronic unpredictable stress (CUS) for six weeks. Bacterial DNA was extracted and sequenced from fecal samples before and after CUS. Taxonomic comparisons of the resultant sequences were made between the pre- and post- samples, and PICRUSt was used to predict functional metagenomic profiles based on organism abundance.

Results: We observed phylum level differences; mean abundance of *Bacteroidetes* increased and *Firmicutes* decreased over the chronic stress period. Decreases in *Bacilli* (a class within *Firmicutes*) and *Lactobacillus* (a genus in *Bacilli*) appeared to contribute to the phylum abundance decrease. No significant difference in alpha diversity was noted. PICRUST analysis revealed stress-induced significant differences in predicted amount of genes encoding for synthesis of short chain fatty acids and reuterin, molecules implicated in stress-related microbial signaling.

Conclusions: Our results support the hypothesis that CUS alters the murine gut microbiome resulting in changes in microbial signaling metabolites. Potential mechanisms for changes include stress-induced reductions in gastric acid or altered motility causing an altered environment for gut colonization Manipulation of the gut microbiome with medications could influence mental well-being. We currently await data addressing whether chronic pain results in similar microbiotic changes.

♦ 23 ♦

Comparison of Three Different Fetal Bovine Sera for Macrophage Growth and Function

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To culture macrophages in a laboratory setting, fetal bovine serum (FBS) is often added to culture media. The increase in cost of FBS has compelled our laboratory to explore less expensive alternatives to our current FBS, produced by HyClone. Macrophage growth rate, phagocytosis, and cytokine production after culture in media supplemented with HyClone FBS was compared to results seen with two potential alternatives, Gibco and Atlanta Biologicals FBS. Macrophages used for assays were derived from mouse bone marrow stem cells and cultured in media supplemented with 10% HyClone, Gibco, or Atlanta Biologicals FBS. Macrophages were allowed to mature, then the growth rate of macrophages in each of the sera was recorded for five days. Macrophages in Gibco FBS media showed the highest growth rate, with maximum cell counts twice those of Atlanta Biologicals FBS. For phagocytosis assays, sheep erythrocytes were sub-optimally opsonized with IgG and fed to macrophages cultured in the three different sera. Gibco and Atlanta Biologicals had a higher percentage of phagocytic cells and phagocytic index when compared to HyClone. For cytokine experiments, LPS was added to macrophages cultured in each of the sera. Cell supernatants were collected and the amount of TNF alpha produced was measured by ELISA. LPS-stimulated macrophages produced more TNF alpha under all conditions. These experiments demonstrate that all

three sera were sufficient to support macrophage growth and development, however the decreased growth rate in Atlanta Biologicals serum is prohibitive. Additional experiments will be performed using macrophages derived in Gibco and HyClone sera.

♦ 24 ♦

Renal Inflammatory Response to Pain-Induced Depression

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Mood disorders, such as major depression, are prevalent in patients with chronic kidney disease (CKD), possibly because of chronic stress. Inflammation is a well-characterized cause of CKD progression and has been associated with chronic stress in recent studies. These evidences suggest a bidirectional relationship between stress-related mood disorders and development of systemic/peripheral illnesses, such as CKD, via enhanced, prolonged activation of immune responses. However, a direct association between exposures to chronic stress and anomalies of the kidney function has not been determined thus far. The neutrophil gelatinase-associated lipocalin (NGAL) is a commonly used biomarker of CKD; it accumulates in the kidney nephron and urine in response to inflammation, kidney injury and decreased kidney function. We analyzed the protein level of NGAL by immunocytochemistry in rats subjected to either neuropathic (spared nerve injury, SNI) or inflammatory (injections of complete Freund's adjuvant, CFA) pain, and their respective controls. These pain models were selected because of ongoing studies on interrelation between chronic pain state and development of depressive-like behavioral deficits. Interestingly, NGAL protein level was increased in both rat models, with a prevalent increase in NGAL in the inflammatory pain model (about 300% increase). These observations suggest that chronic pain, hence, chronic stress induces renal inflammation and a reduction in kidney function. In summary, this study might help a mechanistic understanding of a bidirectional pathway between chronic stress processes and peripheral organ illnesses such as CKD.

♦ 25 ♦

Effects of Chronic Inflammatory Pain on Expression of Hippocampal Genes Involved in Development of a Depressive-Like Phenotype

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Comorbidity between prolonged pain conditions and mood disorders is supported by clinical reports estimating that 30-50% of chronic pain patients suffer from clinical depression. Although recent studies have focused on neural events connecting these two conditions, the exact cellular and molecular links between them are still not well understood.

We hypothesize that chronic pain induces negative neuronal effects within specific regions of the brain, through activation of mechanisms that resemble those involved in chronic stress responses and the development of depression. To test this hypothesis we exposed rats to 21 days of chronic inflammatory pain by peripheral injection of complete Freund's adjuvant (CFA). Behavioral analysis revealed this pain model produces robust mechanical and thermal hypersensitivity throughout the 21 day period, accompanied by a depressive-like phenotype. Biochemical analysis was conducted to identify molecular changes within the brain that may underlie behavioral deficits. We used real-time

PCR (qPCR) to quantitate changes in gene expression within the contralateral hippocampus, a limbic brain area involved in mood control, which may also play a role in the regulation of the affective component of pain.

Based on literature review and our previous findings, we primarily examined genes known to be dysregulated in a depressed state. We targeted 16 candidate genes with functional roles in inflammatory processes, intracellular signaling (e.g., MAPK pathway) and neuronal events such as neurogenesis and synaptic remodelling. This project is a part of an ongoing effort to comprehensively study emotional aspects of chronic pain at a molecular level in an established rodent model.

♦ 26 ♦

Cloning of a α-Galactosidase from *Trichomonas Vaginalis*

Mackenzie Thomas, Mikaela Millslagle, Andrew Brittingham, PhD, Wayne A. Wilson, PhD

There are three Trichomonads that colonize humans: *Trichomonas tenax* (oral cavity), *Pentatrichomonas hominis* (intestinal tract), and *Trichomonas vaginalis* (urogenital tract). *T. tenax* and *P. hominis* are regarded as commensal flora while *T. vaginalis* is the causative agent of trichomoniasis, the most common non-viral sexually transmitted disease worldwide.

Previous work by our group found that the three different Trichomonads are able to metabolize different carbon sources. . *T. vaginalis* and *T. tenax* have the ability to grow on glucose, glycogen, and galactose. *P. hominis* has a wider range of carbohydrate utilization, having the ability to also grow on melibiose, sucrose, raffinose, and showing moderate growth on cellobiose.

Here, we further characterize growth of Trichomonads on melibiose. Melibiose is a disaccharide containing galactose and glucose, which can be hydrolyzed by α-galactosidase, an enzyme that cleaves the galactose from the glucose. Despite the fact that *T. vaginalis* is unable to grow on melibiose, we were able to measure α-galactosidase activity in cell extracts derived from both *T. vaginalis* and *P. hominis*, although the activity associated with *P. hominis* was substantially higher. Furthermore, the *T. vaginalis* genome contains a sequence, TVAG_145340, that encodes an α-galactosidase. Despite measurable α-galactosidase activity and the capacity to encode α-galactosidase, the organism is unable to grow on melibiose. To investigate this area further, we amplified the TVAG_145340 α-galactosidase from genomic DNA of *T. vaginalis* and sub-cloned the open reading frame into a bacterial expression vector to allow the production and characterization of recombinant protein in *E. coli*.

♦ 27 ♦

Trichomonads Increase Invertase Activity in Response to Substrate Availability

Mikaela Millslagle, Wayne A. Wilson, PhD, Andrew Brittingham, PhD

Trichomonas vaginalis is one of three trichomonads that are found in humans. *T. vaginalis* is found in the urogenital tract and is responsible for causing trichomoniasis, the most common non-viral sexually transmitted infection world-wide. *Pentatrichomonas hominis* and *Trichomonas tenax* are two other trichomonads found in humans that are non-pathogenic. *P. hominis* is found in the large intestine and *T. tenax* in the mouth. Previously work in our lab has shown that there are differences in carbohydrate metabolism between the three trichomonads, with *P. hominis* using the most diverse array of carbohydrates. While *T. vaginalis* and *T. tenax* are unable to grow on either sucrose or raffinose, *P. hominis* demonstrates robust growth. To further characterize sucrose/raffinose utilization by trichomonads, we aim to measure changes in the expression of invertase activity. Invertases are enzymes responsible for the hydrolysis of the glucose-fructose bond present in both sucrose and

raffinose. Our studies show that there is an increased level of invertase activity found in *P. hominis* relative to *T. vaginalis*. Furthermore, trichomonads grown in media with raffinose have increased levels of activity relative to those grown in glucose. Additional studies suggest that invertase expression in *P. hominis* is enhanced by the presence of raffinose rather than simply by the absence of glucose, suggesting the enzyme activity is induced by the presence of its substrate.

♦ 28 ♦

Boric Acid Inhibits NAD Oxidoreductases in Yeast

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Boric acid (BA) is a ubiquitous element that is beneficial at low concentrations but toxic at higher doses. Neither the beneficial nor the harmful physiological actions of BA have been understood – complicating an assessment of the agent's safety for therapeutic applications. The exposure to sub-inhibitory concentrations of BA significantly increases the toxicity of ethanol for yeast while not affecting the potency of 24 other agents selected to target a wide spectrum of cellular processes. *In situ* assays of permeabilized cells demonstrated that BA in the sub-inhibitory concentration range reduces the activity of alcohol dehydrogenase at concentrations as low as 0.025%. A similar assay on glyceraldehyde 3-phosphate dehydrogenase was inconclusive but showed a similar trend. We conclude that the data show that at concentrations too low to inhibit growth, BA exposure limits the availability of NAD for the oxidation of ethanol and – possibly - glyceraldehyde 3-phosphate. We postulate that the lack of NAD during BA exposure impairs the yeast's ability to metabolize ethanol and explains the low ethanol tolerance of BA-treated cultures.

♦ 29 ♦

Upregulation of Endothelial Calmodulin via the G Protein-Coupled Estrogen Receptor

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Estrogen is strongly linked to cardiovascular health. Calmodulin (CaM) is required for many cellular activities yet is expressed insufficiently for its target proteins. It is unknown if estrogen can affect vascular functions via calmodulin network. We observed that in endothelial cells, chronic 17β-estradiol treatment (CE_2T) upregulates both CaM mRNA and protein levels. GPER/GPR30 agonist G-1 mimics this effect, while ERα and ERβ agonists do not. ICI18270, a known antagonist of both ERα and ERβ and agonist of GPER/GPR30, also upregulates CaM expression. Additionally, SKBr3 cells, which express only GPER/GPR30 and not ERα or ERβ, express increased CaM level in response to CE_2T, further confirming the role of GPER/GPR30 in mediating the effect of CE_2T on CaM. We have recently shown that GPER/GPR30 itself interacts with CaM at all four of its submembrane domains. To test the effect of the upregulation in CaM on GPER/GPR30's function per se, G-1 induced ERK1/2 phosphorylation was compared between wild-type and GPER/GPR30 mutants with substitutions in individual submembrane domains that drastically reduce CaM binding affinity. ERK1/2 phosphorylation was significantly reduced in cells expressing CaM binding-reducing mutations in individual submembrane domains or a combination thereof. Co-immunoprecipitation experiments show that the mutant receptors associate well with $G\alpha_s$, confirming that the reduction in ERK1/2 phosphorylation is due to reduction in CaM binding affinity and not to a reduction in initial receptor-G protein association. These data strongly indicate that GPER/GPR30 mediates the effect of chronic estrogen treatment to upregulate CaM in the endothelium.

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