

# Medical Student Summer Research Experience of 2023

## Key Dates

January 6, 2023 .....Application cycle opens  
March 3, 2023 .....Application cycle closes  
May 30, 2023 .....Start of SRE and Orientation Day  
July 7, 2023 .....SRE ends  
TBD .....Medical Student Research Day

## Preliminary List of SRE 2023 Research Projects

Mentor: Dr. Thomas Eiting  
Title: Detecting SARS-CoV-2 in Bats of New Mexico using Immunohistochemistry on Formalin-Fixed, Paraffin-embedded Tissues

Mentor: Dr. Thomas Eiting  
Title: Screening Publicly Available CT Datasets of the Head for Potential Use in Studying Normal and Pathological Issues Related to Nasal Airflow

Mentor: Dr. Kristin Gosselink  
Title: Improving cancer prevention by facilitating HPV vaccine uptake: Focus on healthcare provider behavior

Mentor: Dr. Kristin Gosselink  
Title: Neurological and behavioral mechanisms involved in the processing of stress: stress responses, resilience, and coping

Mentor: Dr. Adrienne Kania  
Title: Searching for the Presence of Oscillating Innominates

Mentor: Dr. Harald M. Stauss  
Title: Establishing a Fluorimetric Assay for Plasma Levels of Free 11-Hydroxycorticoids

Mentor: Dr. Harald M. Stauss  
Title: Effects of Occipito-Atlantal Decompression, Transcutaneous Auricular Vagus Nerve Stimulation, and the Splenic Pump on Autonomic Nervous System Activity

Mentor: Dr. Harald M. Stauss  
Title: Effect of Stimulation of Cervical (C2-C3) Cutaneous Sensory Nerve Fibers On Cardiac Autonomic Tone and Salivary Cytokine and Cortisol Concentrations

Mentor: Dr. Michael Woods  
Title: The Spore in the Desert: Investigating the Distribution of Coccidioides in Southern New Mexico

Mentor: Dr. Debra Bramblett  
Title: Development of RT-LAMP Assay for Detection of Dengue viruses

## **Detecting SARS-CoV-2 in Bats of New Mexico using Immunohistochemistry on Formalin-Fixed, Paraffin-embedded Tissues**

### **Significance**

The ongoing COVID-19 pandemic is caused by the novel beta-coronavirus SARS-CoV-2, which is thought to have spread from bats to humans in Wuhan, China in late 2019 (Latinne et al., 2020; Zhou et al., 2020). Bats are thought to serve as hosts for coronaviruses such as SARS-CoV-2 for long periods of time, including during periods of hibernation that last for several months (Subudhi et al., 2017). The outbreak of SARS-CoV-2 has raised the question of whether bats native to North America can also serve as long-term reservoirs of the virus, thereby serving as potential hosts for continued spillover to humans.

This project will be the first known attempt to identify antigens of SARS-CoV-2 in tissues of bat species local to southern New Mexico using immunohistochemistry (IHC) of formalin-fixed, paraffin-embedded tissues. We will also utilize PCR and RNA sequence comparisons to probe for other coronaviruses in these bats, allowing us to better understand the diversity of this family of viruses in our local bat populations. Ultimately, this work will help set the stage for further investigations into whether and how coronaviruses are maintained in local bat populations and the utility of detecting signatures of these viruses using simple immunohistochemical and genetic techniques.

### **Specific Aims**

This study has 2 Aims:

*Aim 1. Detection of SARS-CoV-2 antigens in tissues of local bat populations.* We will utilize a recently-described protocol (Best Rocha et al., 2020; Liu et al., 2020) to screen for antigens of SARS-CoV-2 in bat species native to the desert Southwest. Specifically, we will use tissue from wild-caught pallid bats (*Antrozous pallidus*) in the laboratory of Dr. Teri Orr, NMSU, who originally collected these bats as part of NSF- and NIH-funded projects (together with Dr. Kathryn Hanley, NMSU) to study issues related to SARS-CoV-2 in these local bat species.

*Aim 2. Molecular screening for coronaviruses in local bats.* For this aim, we will perform PCR to detect a coronavirus-specific gene in tissues from the same sample of local bat species used in Aim 1. The goal of this Aim is to determine the relative abundance of coronaviruses more generally in local bats, in part based on the assumption that antigens specific to SARS-CoV-2 might be difficult to detect.

### **Innovation**

A major innovation arising from this work is that we will be the first to adapt techniques for detecting SARS-CoV-2 that have been published in other model species to bats specifically. This is an important step, because bats are thought to be the primary host of this coronavirus, and they likely represent the species responsible for spillover into humans (Ruiz-Aravena et al., 2022). In turn, this preliminary work will set the stage for future research that more fully investigates the prevalence and diversity of coronaviruses in bats, for which funding may be secured through NIH.

### **Research Strategy**

This project will focus on detecting coronaviruses in lung and nasal tissue of locally caught pallid bats (*Antrozous pallidus*) that are permanently housed in the lab of Dr. Teri Orr, Dept. Biology, NMSU (IACUC

available upon request). No animals will be sacrificed as part of this project; instead, we will use tissues from animals that have died of natural causes or were sacrificed for other reasons. Importantly, all tissues will be formalin-fixed prior to transport into Burrell laboratories, substantially minimizing any risk of infectivity or zoonotic transmission. The two aims of this project will be carried out as follows.

*Aim 1. Detection of SARS-CoV-2 antigens in tissues of local bat populations.*

We will utilize a recently-described protocol (Best Rocha et al., 2020; Liu et al., 2020) to screen for antigens of SARS-CoV-2 in bat species native to the desert Southwest. Specifically, immediately after death, whole bats or tissue specimens will be immersion-fixed in 10% formalin for a period of at least one week at room temperature. Subsequently, they will be transferred to 70% ethanol for longer-term storage as needed. If whole bats are preserved, tissues of interest will first be extracted. Both lung and nasal epithelial tissue will be sampled. Lung tissue will be sampled because it is the primary site of infection for SARS-CoV-2, so it may be expected to express antigens for COVID-19 in bat specimens. Nasal epithelial tissue will be sampled because it is hypothesized to be the earliest anatomical location at which SARS-CoV-2 enters the body. Both tissue types have been shown to contain coronavirus antigens in mammalian tissues (Best Rocha et al., 2020; Lean et al., 2020). A previous study (Subudhi et al., 2017) estimated that about a third of bats of a closely related species, *Myotis lucifugus* (little brown bats), are infected with various coronaviruses. Based in part on this finding, we aim to collect tissue from 12-20 bats of both sexes, to ensure a sufficient sample for detecting SARS-CoV-2 antigens. Four bats have already been sacrificed and preserved in formalin, and subsequent specimens will be collected in the coming months.

Tissue sections (3-5  $\mu\text{m}$ ) will be generated according to standard procedures. at NMSU or at Burrell, depending upon availability of equipment (see (Eiting, Smith, & Dumont, 2014) for complete details). Afterwards, IHC will be carried out in the BSRL of the Burrell COM according to a new protocol developed for detecting antigens of SARS-CoV-2 in human tissue (Best Rocha et al., 2020). This protocol uses commercially available probes to detect IHC antigens, coupled with hematoxylin counterstaining.

Light microscopy will be used to screen for the presence of SARS-CoV-2 positive tissue, which is easily identifiable in stained sections. A digital camera will be used to collect photomicrographs of sections that contain positive staining, as well as sections that are negative.

*Aim 2. Molecular screening for coronaviruses in local bats.*

In this aim we will supplement the detection of antigens specific to SARS-CoV-2 with a broader, “shotgun” approach to detect the coronavirus RNA-dependent RNA polymerase (RdRp) gene in tissues from the same specimens used in Aim 1. In addition to nasal and lung tissue, we will also sample from the small intestine, which has been shown to contain coronaviruses in previous studies on bats (Subudhi et al., 2017). In addition to serving as a sort of “control” in case we do not detect SARS-CoV-2 antigens in Aim 1, this aim will also provide preliminary data on the abundance and diversity of coronaviruses in local bat populations.

Formalin-fixed tissues will be collected from bats as in Aim 1. From there, RNA extraction, cDNA preparation, PCR, and high-throughput sequencing will all be carried out as in a recent paper (Subudhi et al., 2017). Amplified products will be sequenced, and then we will then perform sequence alignments and phylogenetic analysis incorporating known coronavirus sequences to determine the families of

coronaviruses present in our specimens. High-throughput screening will be carried out at Burrell and NMSU, depending upon equipment and computer availability. We will also perform PCR amplification of bat RNA as an internal control.

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## **Screening Publicly Available CT Datasets of the Head for Potential Use in Studying Normal and Pathological Issues Related to Nasal Airflow**

### **Significance**

The nasal cavity is responsible for the effectively delivering air to the rest of the respiratory system, as well as to the olfactory mucosa located out of the reach of respiratory airflow. To perform these functions effectively, the internal anatomy of the nose has evolved a series of intricate structures (nasal conchae, or turbinates) to divide and transport air efficiently. However, we still lack a thorough understanding of the diversity of normal nasal airflow, as well as the morphologies that contribute to well-performing airflow, and those that lead to undesirable outcomes.

In this project we will utilize large, publicly available computed tomography (CT) datasets to examine the anatomy of the internal nose and nasal cavity. The goal of this work is to develop a diverse set of 3D airway models that can be used to study both normal nasal airflow and pathologies related to disruption in normal airflow. There have been very few population-based studies of nasal airflow, such that to date we do not have a firm understanding of variability in “normal” airflow, nor in what constitutes abnormal flow. The proposed project will help bridge this gap in our understanding, and importantly, it will help us to develop predictive models of how nasal airway anatomy contributes to clinical disorders, such as chronic rhinosinusitis (CRS), an often debilitating disease that affects about 15% of the population (Halawi, Shintani Smith, & Chandra, 2013).

### **Specific Aims**

*Aim 1. Using publicly available CT datasets to create 3D models of the nasal cavity.* Several anonymized datasets of cranial CT scans are available for us to download and investigate for usefulness in building digital models of the nasal airway. Such datasets include the CQ500 repository (<http://headctstudy.qure.ai/dataset>; (Chilamkurthy et al., 2018), the Medical Imaging and Data Resource Center (<https://data.midrc.org/>), and others. We will access these large datasets, download promising scans, and then examine scans slice-by-slice to determine if the anatomical detail is sufficient for building 3D models. Datasets will be stored on local drives for ease of use going forward. CT scans considered to be good candidates for further study will next be imported to software that can fully render 3D models out of “stacks” of CT slices. These virtual models will also, in some cases, be 3D-printed to allow us to further characterize and inspect the models.

*Aim 2. Perform Computational Fluid Dynamics (CFD) modeling of airflow in the digital models.* Ultimately the purpose of this project is to find CT scans that can be used to build patient-specific airflow models and to perform CFD analyses with them. Such models can be used to study diversity in airflow patterns and rates, microparticle and odorant deposition, and the contributions of anatomical variations to differences in airflow. It can be time consuming and difficult to generate 3D models that can undergo these types of simulations, so in this Aim we will work as a team to iteratively refine models and use them to perform simple computational analyses. Such models will then be a springboard for many future investigations that will be carried out by our group.

### **Innovation**

The major innovation in this project will be in developing methods to efficiently evaluate CT datasets and create 3D models that can be used in computational modeling to study a host of airflow-related

issues of potential clinical significance, such as chronic rhinosinusitis and inflamed concha bullosa. While our initial work will contribute towards gaining a better understanding of population-wide “normal” nasal airflow, the techniques that will be developed as a result of this project may help researchers and clinical practitioners develop detailed, patient-specific models that improve nasal airflow and associated functionality. Emerging evidence has shown that CFD can be a valuable surgical planning tool, including in nasal airflow, as well as in congenital heart defects, liver transplants, and aneurysms, to name a few (Chung & Cebal, 2015; Rutkowski, Reeder, Fernandez, & Roldán-Alzate, 2018; Sundareswaran et al., 2007; Zhao, Malhotra, Rosen, Dalton, & Pribitkin, 2014). The proposed project will help us better understand the diversity in normal nasal airflow and how anatomical variation contributes to predicted deficiencies in function.

## **Research Strategy**

The nasal airway contributes to many fundamental functions of human physiology, including respiration, olfaction, and speech (resonance). These functions rely on effective airflow. For example, a recent study in a small cohort of adults found, using multivariate techniques, that a significant proportion of reduction in olfactory function could be attributed to deficiencies in air reaching the olfactory cleft (Zhao, Jiang, et al., 2014). Such studies are very few, and to date we still do not understand how nasal airway anatomy provides for effective nasal airflow. With the widespread adoption of CT scanning and the accessibility to CT data provided by various online repositories, we can now develop patient-specific models of nasal anatomy and its associated airflow to uncover the links between anatomy and physiology that provide for normal nasal airflow, as well as those that may contribute to airflow deficits. In this project we will begin to address these issues by developing a series of protocols that will generate patient specific CFD models from publicly available CT scans. The procedures here will generally follow those that I have specified previously (Eiting, Perot, & Dumont, 2015; Eiting, Smith, Perot, & Dumont, 2014). The specific aims will be carried out as follows.

*Aim 1. Using publicly available CT datasets to create 3D models of the nasal cavity.*

The initial stages of this work involve download massive CT datasets and evaluating them for potential use in subsequent CFD studies. Many large datasets that are available have been collected and compiled for reasons *other than* evaluating nasal airflow; therefore, a significant amount of effort is needed to determine whether these datasets are even suitable for further analysis. We will initially focus our efforts on two repositories of CT data, the CQ500 repository (<http://headctstudy.ure.ac.uk/dataset>; (Chilamkurthy et al., 2018) and the Medical Imaging and Data Resource Center (<https://data.midrc.org/>), while others may be consulted as necessary or as time and interest allow.

Briefly, the procedures for initial “triage” of the data will be as follows. CT scans will be downloaded to local hard drives and examined slice-by-slice using Irfanview software ([www.irfanview.com](http://www.irfanview.com)). Then, any datasets that do not contain the full nasal cavity and at least some of the nasopharynx will be eliminated. We will also check for enough resolution in the slices to be able to ultimately create a 3D model, as well as a host of other issues related to data quality (for example, the data must have been generated with appropriate parameters to see the nasal anatomy effectively).

Datasets that meet these initial standards will then be brought into the software program 3DSlicer ([www.slicer.org](http://www.slicer.org)), which we will use to create 3D renderings of the CT datasets. This powerful program requires substantial training to use effectively and efficiently, but ultimately it can generate high quality

models that can be 3D printed and moved on to CFD analyses. Very often a second piece of software, Autodesk Meshmixer (<https://meshmixer.com>), is needed to perform fine modifications and repairs to 3D models generated by 3DSlicer.

Once models have been refined, we will 3D print them either in-house or through a 3<sup>rd</sup> party website (e.g., <https://i.materialise.com/en/3dprint> or similar). These 3D models, together with the CT datasets themselves, will then form the basis for discussions of anatomical similarities and differences, and ultimately will help us to determine which 3D models to use in CFD analyses.

*Aim 2. Perform Computational Fluid Dynamics (CFD) modeling of airflow in the digital models.*

Digital 3D models that have made it to this stage will undergo the process of iterative refinement to create so-called “solid models” or “solid meshes” on which CFD analyses can be performed. In essence, CFD calculations require anything that is being modeled (in this case, the nasal airway) to be populated by thousands or millions of tiny “bricks” over which the equations of fluid flow can be calculated. Such solid models are readily created from the “hollow models” that are created from Aim 1, and which themselves are sufficient for submitting to a 3D printer (most commonly these are STL files). Creating solid models from hollow models can be a time-intensive process, because only solid models of a certain quality can be used in CFD. For example, if any of the bricks in the solid model have a very high aspect ratio, such as an incredibly long but very thin “spine,” then the calculations of fluid flow cannot be completed. Significant effort is required to ensure that the final solid mesh is compatible with CFD.

Once a suitable solid mesh has been created, we can then carry out CFD analyses. We will use the open-source platform OpenFOAM ([www.openfoam.com](http://www.openfoam.com)) to carry out all fluid flow simulations. Such simulations are carried out on supercomputers, and Dr. Eiting is a registered user of the Discovery Computer cluster at NMSU, on which these simulations will take place. At first, we will carry out a very basic simulation of inhalation that demonstrates the efficacy of the fluid flow calculations. After this simulation has run, we will examine resulting patterns and rates of airflow in the software package Paraview ([www.paraview.org](http://www.paraview.org)).

After repeating the above steps for each 3D model of interest, we can begin addressing questions that are of basic research interest as well as those that may have clinical relevance. CFD has been used to developed population-based anatomical differences in the nasal vestibule that may hamper delivery of odorants to the olfactory cleft, to study surgical procedures to help patients suffering from Empty Nose Syndrome and olfactory deficits, to understanding effective droplet sizes in drug delivery (Malik et al., 2021; Sicard, Shah, & Frank-Ito, 2022; Zhao, Jiang, et al., 2014). We will follow up on these and similar studies to broaden the sample of nasal airways that correlate both with normal as well as abnormal airflow, based on physiological metrics predicted from the simulated airflow and the data that have been collected from patients in a lab setting. Ultimately, this work will amplify and diversify the basic and clinical topics that relate to effective nasal airflow, and it will provide robust datasets that will be used to investigate such topics by our group in the future.

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## Research Plan (1) – 2023 SRE – Gosselink

### Title:

Improving cancer prevention by facilitating HPV vaccine uptake: Focus on healthcare provider behavior

### Significance:

The human papillomavirus (HPV) is the most common sexually transmitted infection in the U.S., and persistent infection with some HPV strains elevates the risk of developing multiple types of cancer. The HPV vaccine protects against infection and is a **critical factor in cancer prevention**, yet national vaccination rates remain well below designated targets. Adolescents in El Paso County, Texas have the highest rate of first-dose HPV vaccine uptake in Texas and one of the highest in the country (1). This suggests a complex interplay of barriers and facilitators that may impact HPV vaccination in this predominately Mexican-origin Hispanic community, and supports the idea that El Paso is an important ecosystem for studying these factors. Opportunities remain for increasing the numbers of individuals in this region who are fully “up-to-date” on the vaccine series, and little is known about vaccination rates up to 45 years of age, or HPV awareness in older adults. Thus, **HPV continues to be a major health concern** and there is an urgent need to improve community understanding and education in this area. Recent changes in vaccine eligibility, along with rising rates of infection and associated cancers, highlight the importance and timeliness of the proposed work.

The objective of this behavioral research project is to assess knowledge of HPV, HPV-associated cancer risks, and the HPV vaccine among current (in practice) and emerging (in training) healthcare providers. In addition, we will evaluate actual and expected vaccine recommendation behaviors, and provide an intervention designed to strengthen their communication with patients about HPV and the vaccine. This work is based on multiple indications that provider recommendation is among the most important factors in vaccine uptake (2, 3). We will focus on providers who work or intend to work with the majority-Hispanic population in the Paso del Norte region that includes El Paso. The initial phase of this project, surveying knowledge and behaviors, is complete and data analysis is ongoing. The next phase, the **primary goal of this proposed project**, will be to employ the intervention and evaluate its ability to increase and/or strengthen provider communication and recommendations for the HPV vaccine.

### Specific Aims:

This project seeks to increase and strengthen healthcare provider recommendations for the HPV vaccine, with the **goal of increasing vaccine uptake and improving cancer prevention**. It will utilize tailored training of current (in practice) or emerging (in training) providers to facilitate their understanding of language, cultural, knowledge and other barriers that may exist in their predominantly Hispanic patient population in the Paso del Norte region of West Texas, Southern New Mexico and Northern Mexico. It will also seek to improve provider communication skills in order to facilitate trust and positive interactions with their patients. The **working hypothesis** is that improving provider knowledge and communication strategies about HPV and its vaccine will reduce vaccine hesitancy and increase vaccine completion among eligible individuals.

***The following Specific Aims are proposed:***

1. To assess the knowledge, attitudes and behaviors of current and emerging healthcare providers regarding the HPV vaccine and cancer; and
2. To deploy an educational and professional skills intervention to improve patient-provider communication and strengthen vaccine recommendation behavior.

**Innovation:**

Our plan for studying barriers and facilitators that influence HPV vaccine uptake and knowledge in this region is both novel and innovative as we seek to link factors that affect medical decision-making in patients to the attitudes and practices of their providers. In this way, we hope to strengthen patient-provider communication and increase HPV vaccine uptake, improving cancer prevention in this region. This is especially important for our medically-underserved population who may have low health literacy, value diverse cultural influences, and frequently navigate asymmetrical healthcare standards and costs. In addition, our target population for increasing cancer prevention is relatively homogeneous (Hispanic, of Mexican origin), which increases the power of our investigations and the likelihood of producing impactful findings. These findings may generalize to other Hispanic and potentially other minority, medically underserved, or rural populations that experience similar barriers and facilitators in HPV vaccine uptake and sequence completion.

**Research Strategy:**

We will apply a two-pronged approach to addressing identified gaps in vaccine uptake and socio-cultural and informational barriers that contribute to those gaps. The goal for this project is to better prepare current and emerging providers to discuss vaccination in general, specifically HPV, in an informed and culturally competent manner.

The following activities are proposed: 1. To utilize the data collected from the HPV-VAKS survey (an earlier phase of this project) and determine HPV vaccine acceptability, barriers, and predictors among emerging and current healthcare providers. The specific data for current and emerging healthcare providers along with data about the community have informed a strategic intervention to increase vaccination and cancer prevention 2. To develop and implement this intervention in emerging and current healthcare providers. The intervention will prepare providers to discuss HPV vaccination in an informed and culturally competent manner and strengthen subsequent provider recommendations.

**Activity 1 (completed; data analysis ongoing):** Data derived from the HPV-VAKS survey delivered to a broad community sample (n=600) and a smaller provider sample (n=93) is being analyzed to extract relevant attitudes, knowledge and skills that impact HPV vaccine uptake. Findings from the providers are being compared against those from the community sample, and both data sets have been used to develop the intervention strategy for Activity 2.

**Activity 2 (focus of this proposal):** Current and emerging healthcare providers (n=300) will be recruited from the El Paso and Las Cruces medical communities, with particular emphasis on family practice and pediatric care settings. Emerging providers will be recruited from the Burrell College of Osteopathic Medicine, the University of Texas at El Paso (UTEP) health professions programs (i.e. College of Science,

College of Health Sciences, College of Nursing, School of Pharmacy) and the Texas Tech University Health Science Center.

The educational and professional skills intervention has been developed based on previous data collected in this project. Both control (n=150) and experimental (n=150) participant groups will have their interventions delivered via an online platform. Both interventions include a reading component, a video component, and an interactive component. The control intervention speaks to general strategies for improving communication skills, while the experimental intervention is targeted to improving patient-provider communication. The former draws expertise from the business and leadership literature; the latter includes information on HPV and other matters of sexual health, vaccines and their efficacy and safety, the association of HPV with cancer risk/development, and reasons for vaccine hesitancy among patients or parents. The experimental intervention is built on established models including clinical video vignettes demonstrating role-played conversations with vaccine-hesitant parents (4), curriculum coupling HPV biology, epidemiology and disease morbidity with role-playing exercises and training on communication strategies (5), and fostering “announcement” style communication that work from a presumption of vaccine acceptability in parents and patients (6).

Participants will be administered a 20-minute pre-assessment survey prior to participation in the online intervention. Pre-assessment will include: 1) educational attainment and/or professional role and title, 2) socioeconomic status, 3) geographic information, 4) ethnicity/race, 5) knowledge about HPV vaccine and associated cancers; 6) HPV vaccination status and age/location/timing of vaccine receipt; 7) role of a provider (or other) recommendation in vaccine uptake; and 8) contributing factors in the receipt or denial of first or subsequent vaccine doses. Post-test assessments will occur immediately following and 3- and 6-months after the intervention and will assess actual or intended vaccine recommendation behaviors. Participation will be voluntary, with informed consent before participation. No personal identifiers will be included and participants will be assigned a unique number in order to facilitate follow-up at the post-assessment timelines. Respondents will be randomly selected into control and experimental groups and enter the study as described above. Pre-post test comparisons will be used to evaluate the impact of the intervention(s) and results compared across age and treatment groups; current providers vs. future providers will also be compared.

**Preliminary Results:** Among current or emerging healthcare providers, HPV knowledge was shown to be stronger than that demonstrated in a broad community sample. HPV vaccination rates were also higher than in the general population, but still failed to approach target levels. Moreover, vaccination of female participants was far more frequent than that seen in male participants. Interestingly, sex differences also emerged in actual or anticipated vaccine recommendation behavior, with providers vaccinating their own male children less often and focus more heavily on female children in promoting the vaccine.

**Expected Outcomes and Interpretation:** With continuing analysis of our original data set, increased medical knowledge and trust in healthcare systems are expected to be found among providers, but we anticipate that these participants will express uncertainty about countering anti-vaccination arguments or communicating with vaccine-hesitant patients or parents. The intervention will increase practitioners’ self-assessed ability to communicate effectively with patients and will provide them with tools to conduct these discussions. Outcomes will include more frequent and stronger recommendations of the HPV vaccine, and equal recommendation regardless of the sex of the patient.

**Potential Pitfalls and Alternative Strategies:** Self-reported data, may have errors or gaps. Given the age range of participants, current providers may fall outside the vaccine-eligible window and subsequently

decrease the recorded number of vaccinated individuals in our sample. Separate analyses will be conducted to determine the presence or impact of this potential problem. The degree to which our findings will generalize to workers across healthcare settings, or to communication with other populations, is uncertain, and will be the focus of future investigations. Recruitment of current providers in our study has been somewhat challenging, but we plan to leverage the professional connections of our investigators to increase our likelihood of success.

#### PROTECTION OF HUMAN SUBJECTS

This work will be conducted under an approved IRB protocol, housed at UTEP which serves as the lead institution. A reliance agreement for the participation of Burrell personnel is in place (Dr. Gosselink is an established Co-PI on this project).

The proposed studies will involve adult participants in the age range of 18 years old and older, and will include both males and females. The researchers engaged in this project will be directly involved with individuals in our survey population to alleviate concerns about their participation and to ensure that the instructions for survey completion are clear. The current health status of our participants is not a factor, and no participant under the age of 18 will be surveyed.

**Potential Risks:** There are no physical risks or side effects associated with the study. Participants can withdraw at any time from the research activities without penalty. No significant risks to human participants are anticipated, and all collected data will be in survey form. Care will be taken to ensure participant privacy and data security. All participants will give informed consent prior to completing any survey. Survey responses will be anonymous and “decline to answer” options will be provided for many of the questions to allow for increased participant comfort and security. We do not anticipate any psychological distress associated with the survey questions or the in-depth interviews during the study. Experienced personnel, trained in interviewing and data gathering techniques, will administer all the assessments and will be supervised by the Principal Investigator. In the unlikely event, that a participant expresses distress from the subject matter, the PI will follow up to assess the severity of the distress and refer to the appropriate mental health provider or agency in El Paso, Texas.

**Potential Benefits:** No direct benefits of participation will be realized by our survey respondents. The data have the potential to benefit the community at large by addressing a significant health concern and producing specific approaches to improve health and education. In addition, participants may gain healthy literacy in the area of HPV and vaccination.

**Informed Consent:** Each subject will undergo written and informed consent when they begin the survey process, under the direction of research team members and with the approval of the IRB. Subjects will be given ample time to read the consent forms and ask any questions they may have regarding the study.

**Adverse Events:** None anticipated.

#### STATISTICAL DESIGN AND POWER

A 2 (treatment condition, control condition) x 2 (preassessment, post-assessment) mixed factorial design will be used to examine the effectiveness of the Education and Professional Skills Intervention that will be delivered to current and emerging healthcare providers. Approximately 150 providers will be randomly assigned to each of: 1) an experimental condition in which the targeted intervention is received, and 2) a control condition in which they receive non-targeted materials. A review of the literature did not reveal the use of a similar intervention, thus, the sample size of 150 per treatment was a reasonable but conservative estimate considering our approach to analysis, Central Limit Theorem, and anticipated attrition for a 2x2 group design. These decisions were made in consultation with the Statistical Analysis Core of the Border Biomedical Research Center at UTEP.

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## **Research Plan – 2023 SRE – Gosselink**

### **Title:**

Neurological and behavioral mechanisms involved in the processing of stress: stress responses, resilience, and coping

### **Significance:**

It is well-established that significant exposure to stress can precipitate, worsen, or predispose for a wide array of disease states and health challenges. Whether through repeated or extended activation of the sympatho-adrenal medullary system or the hypothalamic-pituitary-adrenocortical axis, stress can impact multiple body systems and has been implicated in cardiovascular, neurological, metabolic, and immune dysfunction. Responses to stress at the level of the brain have been heavily studied, but vary by stressor type, severity, and duration as well as the age at which stress exposure occurs. Connections between stress-sensitive brain regions and the mechanisms that ultimately lead to poor(er) health conditions or outcomes are more difficult to draw, but gaining knowledge in this area is critical to the development of treatment strategies for stress-related illness.

The hypothalamus and, specifically, the paraventricular nucleus of the hypothalamus (PVN), is the main site in the brain from which systemic stress responses are generated. As such, in animal models, PVN activation can serve as a proxy for stress, and the timing and patterns of neuronal activation in this nucleus can provide important information about the stressor in lieu of repeated blood sampling to test for glucocorticoid levels. Extensive mapping has identified neuronal subpopulations within the PVN that respond to acute versus repeated restraint stress (1), and numerous other brain regions have been evaluated for their role in mediating or modulating the stress response. Few studies, however, have provided a more comprehensive neuroanatomical assessment, and almost none have done so in female animals despite the fact that sex differences in stress responses and stress-related illness manifestations clearly exist. One recent article (2) looked at neuronal activation in 18 brain regions in acutely stressed female and male mice, and showed sexually dimorphic responses in some areas but similarities in what they refer to as the generalized “core” response machinery. This study did not examine responses to repeated or chronic stress.

### **Specific Aims:**

This project will approach stress responses, resilience, and coping from different angles, with the goal of understanding how the brain is impacted by acute versus chronic stress. The focus of this work will be on female subjects – both animal and human – as affective disorders such as depression are more common in females, can result from chronic stress exposure, and have been shown to decrease after menopause when estrogen levels are low.

Animal studies have shown that anxiety-like behaviors were decreased in female rats during estrus; these animals also had reduced levels of glucocorticoid receptor expression in their hippocampal tissue (3). Circulating estrogen levels can also influence the neurological and behavioral response to stress (4), highlighting the importance of understanding how sex and sex hormones contribute to stress-related health issues. Previous work in the PI’s laboratory has shown that sex-specific stress responses are evident at the level of neuronal activation, which may contribute to female-male differences in the consequences of chronic stress exposure (5). It was further suggested that females may be more

sensitive to negative feedback from secreted glucocorticoid hormones, possibly impacting how stress responses are initiated or terminated.

The goal of this project is to identify stress-sensitive regions in the adult female rat brain, and to evaluate stress levels, perceptions, resilience, and coping in adult women. Through these analyses, we hope to better understand brain-behavior links that could be utilized in the treatment or prevention of stress-related illness.

***The following Specific Aims are proposed:***

Aim 1. To determine the distribution of activated neuronal populations in the female rat brain under conditions of acute and repeated restraint stress.

Aim 2. To assess the impact of a surf therapy program to build resilience and facilitate stress coping in women.

**Innovation:**

The proposed animal studies are not particularly novel or innovative by themselves, but it is important to continue to foster a greater understanding of neuroscience and behavior in female subjects and how they differ from males. The human studies we propose herein are quite innovative, and will extend and more deeply analyze an existing therapeutic framework that has been shown to be effective in improving mental health in women. This will be our first attempt at a project of this type, and we anticipate that our findings will allow us to propose future studies and funding applications in which imaging of the human amygdala or other structures may inform stress coping and resilience strategies in individuals at risk for stress-related disorders.

**Research Strategy:**

1. ANIMAL STUDIES

*Note: the animal studies described herein were conducted at another institution, under an approved animal care and use protocol. The tissue to be stained and analyzed in this project, coronal sections of rat brain, have been transferred to the laboratory at Burrell and are available for study. This project will not involve the further use of vertebrate animals.*

Experimental animals

Adult male or female Sprague/Dawley Rats were housed individually in standard cages and maintained on a 12:12 h cycle with food and water ad libitum. Rats were allowed to acclimate for one week before being used for experiments. All animals used in this study were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the UTEP Institutional Animal Care and Use Committee (IACUC protocol A-200601-1, A-201006-1).

Restraint stress

Rats were randomly assigned to one of three stress conditions: Control (Con), Acute restraint (Acu), or Repeated restraint (Rep). Physical restraint was used as an emotional stressor, with the rats placed inside an acrylic restraining device (Kent Scientific) for 30 min. All restraint and exposure was done near the beginning of the light cycle, between 0900 and 1100 h. Acu rats were exposed to open restraining

devices in their home cages for 30 min/d for 13 consecutive days, and then restrained for 30 min on the 14<sup>th</sup> day only, while Rep rats were restrained for 30 min on each of the 14 days. Con rats were exposed to the restraining device daily for 30 min but never restrained.

#### Perfusion and tissue collection

At the end of the restraining treatments, the animals were deeply anesthetized with 100 mg/kg of sodium pentobarbital, i.p. (Nembutal®; McKesson), followed by perfusion through the ascending aorta with ~100 mL of 0.9% saline, and 400–500 mL of 4% paraformaldehyde (JT Baker) at pH 9.5 in 0.1 M borate buffer. Brains were dissected, post-fixed for 5 h at 4 °C, and cryoprotected overnight at 4°C in KPBS with 10% sucrose, then serially sectioned in 30 µm sections on a freezing microtome (Model SM 2000R; Leica) and stored in antifreeze (30% ethylene glycol, 20% glycerol) at –20°C until used for immunohistochemical analysis.

*Note: Methods prior to this point have already been completed. Subsequently-described methods will be carried out as part of the current proposed project.*

#### Immunohistochemistry and cell counting

Brain sections will be immunohistochemically stained for Fos, the protein product of the immediate early gene *c-fos*, as an indicator of neuronal activation in response to stress. Fos expression will be localized using an avidin-biotin-immunoperoxidase technique. Tissue sections will be incubated overnight at room temperature in primary antiserum against Fos (1:10,000; Abcam), and incubated on the following day for 1 h at room temperature in secondary antibody (biotinylated goat anti-rabbit IgG, 1:200; Vector). An avidin-biotin-complexing solution (Vectastain Elite kit; Vector) will be applied for 1 h, and a peroxidase method using diaminobenzidine (DAB) as a chromogen will be used to visualize specific binding. Stained sections will be mounted on gelatin-coated slides, dehydrated through a graded series of ethanol and xylene, and coverslipped with DPX mountant (Electron Microscopy Sciences).

#### Quantification of Staining

The neurological response to stress will be assessed by counting the number of Fos-positive cells in the paraventricular hypothalamic nucleus (PVN). A light microscope coupled to a digital camera will be used to capture images of this brain region, taken unilaterally from 4-5 sections throughout its rostrocaudal extent, and staining will be quantified using ImageJ software. Additional brain regions associated with stress may also be quantified for Fos expression. Counts from all sections within a region will be summed by animal; group averages will be calculated and compared statistically using two-tailed t-tests with a p value of ≤0.05 considered significant.

## 2. HUMAN STUDIES

We propose to work in partnership with Ms. Natalie Small and the Groundswell Community Project (<https://www.groundswellcommunity.org/>), specifically the Groundswell Surf Therapy program, to evaluate the relationship between chronic stress and mental health in women. Their current research examines the ability of surf therapy to build resilience and initiate healing from chronic stress and trauma from diverse sources. They employ a survey that includes demographic information and voluntary disclosure of current mental health challenges, along with:

- an Emotional Regulation Affective Style Questionnaire;
- a Brief Body Acceptance Scale (BAS-2);
- the Connor-Davidson Resiliency Scale (CD-RISC); and
- a Gratitude Questionnaire-Six-Item Form (GQ-6).

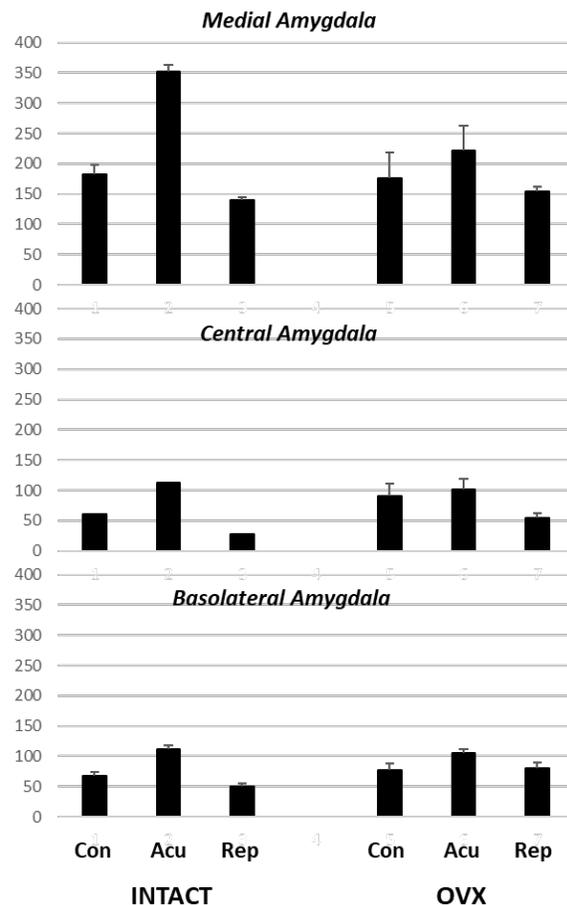
Our project will access and evaluate the data collected by Groundswell and will add two additional survey components: an Adverse Childhood Experiences (ACE) score, and a Perceived Stress Scale (Cohen's; 6) to which a few longitudinal/duration-related questions will be added. Through these instruments, we will examine the persistence or frequency of stress in our participants, their cumulative stress levels, and how they perceive their own relative stress levels. All participants will be at least 18 years of age, and we have the goal of recruiting at least 30 participants for this first phase of the project.

The majority of survey items in this study will have Likert scale-type answers, which will be converted to numeric values for analysis and comparison. Perceived Stress Scale data will be compared by individual respondent against national norm data. Descriptive statistics will be used for demographic variables.

**Preliminary Results:**

Earlier work by the PI identified sex differences in the response to an emotional stressor (5). With acute restraint stress exposure, both male and female rats showed increased Fos expression in the PVN but the increase was greater in males than in females. Females, on the other hand, had a greater proportion of Fos-positive cells that co-localized the glucocorticoid receptor (GR). Repeated restraint stress led to an habituation of the Fos response in both sexes, but Fos-GR co-expression remained more prevalent in the female PVN. These data suggest that females may cope with acute stress more effectively than their male counterparts, and may have greater sensitivity to glucocorticoid negative feedback and an ability to terminate stress responses more efficiently.

More recently, Dr. Gosselink's research team has begun to investigate the effects of acute and repeated restraint stress on neuronal activation in different subregions of the amygdala (see graph). As expected, Fos expression was increased in the medial, central, and basolateral amygdalar nuclei of normal (intact) female rats following acute restraint, and habituated with repeated restraint exposure. Removal of the ovaries (ovariectomy; OVX), however, blunted the acute stress responses, indicating a role for ovarian hormones in facilitating neurological responses to stress. This work is ongoing, with the goal of increasing the n for all treatment groups and assessing OVX females who received estradiol replacement. These studies form the foundation for the current proposal that seeks to evaluate stress responses, resilience, and coping in females.



The Groundswell Surf Therapy program in 2020 served 36 participants in California, 22 of whom identified within the BIPOC and/or LGBTQ+ communities. Among them, 35 disclosures associated with

anxiety, depression or trauma were made, along with 32 additional disclosures of other current challenges to mental health. Four- or eight-weeks of therapy built community and facilitated healing. The goal of this organization is to improve women's health and mental health, increase accessibility to this type of therapy, and facilitate the development of surf therapy as a therapeutic modality and recommended form of treatment. Toward this end, more formal outcomes assessments will be made.

#### **Potential Pitfalls and Alternative Strategies:**

Any connections drawn between animal and human studies in this project will be correlative, at best. In the human participant studies, self-reported data, may have errors or gaps. The human component of this project is under development, and challenges in recruitment are possible. We expect to include a retrospective analysis of previous surveys delivered through the Groundswell Community Project as part of our IRB application, so that we may evaluate earlier data even as we work to collect new data and expand our inquiry. Lastly, the human studies proposed have a significant emotional component and our participants may be at risk of additional stress or emotional harm as they complete the requested surveys. All participants will have joined the surf therapy program voluntarily, and may choose to discontinue our study at any time. The Groundswell team includes experienced and licensed therapists who are available and ready to care for the participants as needed.

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## **Searching for the Presence of Oscillating Innominates**

### **Significance**

Consistency in determining vertebral and non-vertebral somatic dysfunction has been difficult to determine in the osteopathic profession due to poor inter-examiner reliability.<sup>1-5</sup> This difficulty challenges the theory of somatic dysfunction, plagues the profession in its ability to develop research protocols as well as teach palpation and diagnosis to osteopathic medical students. Evaluation of the process in which palpatory skills are acquired indicates that it is a complex multi-factorial process to achieve competency.<sup>6-8</sup> It has been purported that repeated testing may change the position of anatomical landmarks and the range of motion because of the viscoelastic properties of fascial structures and the dynamic state of the human body.<sup>4,5</sup> It is also possible that there is the presence of oscillation or rotation of bones and thereby, anatomical landmarks, that confounds accurate diagnosis of somatic dysfunction. This possibility has not been explored in a research capacity and has the potential to change how Osteopathic Manipulative Treatment (OMT) is taught.

### **Specific Aims**

The aim of this research project is to quantify the presence of oscillating anatomical landmarks in human subjects and determine the rate(s) of oscillation.

### **Innovation**

If this study proves the existence of oscillation of the innominates, it will shift current clinical diagnostic and practice paradigms. The current goal in OMT is to treat the innominate that has a somatic dysfunction based on a lateralizing test. If the innominate is the bone rotating, then it is unlikely to be the somatic dysfunction. Determining this potentially will improve diagnostic accuracy when evaluating a patient for somatic dysfunction.

### **Research Strategy**

The evaluation of the position of the Anterior Superior Iliac Spine (ASIS) will be obtained through serial photographs. The subject will lie on an examination table with their clothing lowered to expose their ASISs on the lower abdomen. The ASIS will be marked with an ink that contrasts with their skin color. Photographs will be obtained every 6 seconds for 60-120 seconds. Using a grid overlying the photographs, variations of ASIS positioning will be determined along with the frequency of oscillation.

### **Challenges:**

1. This project needs students who can build a framework for taking the photographs, control the shutter speed for serial photographs and perform data analysis.

2. It has been suggested the incidence of oscillating bones occurs in <20% of patients. This requires a large sample size to evaluate, a minimum of 100 subjects.
3. There are no hazards with exposure to materials. The subjects will have a cloth draped over their lower abdomen consistent with what would be exposed while wearing a modest bikini bottom.

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## Establishing a Fluorimetric Assay for Plasma Levels of Free 11-Hydroxycorticoids

Mentor: Dr. Stauss

**Significance:** Cortisol has many physiologic functions, including metabolic functions, modulation of immune processes, anti-inflammatory properties, and central nervous system effects. Thus, measuring plasma cortisol levels is a necessity for research studies in a large range of biomedical research areas. Currently, the state-of-the-art laboratory technique to determine cortisol plasma concentration is based on enzyme-linked immunosorbent assays (ELISA). This technique is well established and has high sensitivity and specificity. However, the cost of this assay (approximately \$10 per sample) can be prohibitive if large number of samples need to be analyzed. Thus, a more economic assay to determine cortisol plasma concentrations is needed.

Fluorimetric assays to determine plasma cortisol levels were described as early as 1952 (1). In 1962 (before the introduction of ELISAs), Mattingly described “A simple fluorimetric method for the estimation of free 11-hydroxycorticoids in human plasma” (2) that was based on a method described two years earlier by De Moor and colleagues (3). These historic methods require relatively large volumes of plasma (2.0 mL) and a cuvette-based fluorimeter. The goal of this Summer Research Experience project is to modify the method described by Mattingly to allow plasma cortisol measurements in smaller volumes of plasma (e.g., 100  $\mu$ L) using a modern 96-well plate fluorimeter. This project is potentially significant, because the reagents required for the method described by Mattingly are inexpensive, largely reducing the cost for cortisol assays in research studies requiring large number of plasma samples to be analyzed.

**Specific Aim:** The Aim of this research project is to develop a fluorimetric assay for the determination of cortisol levels in small volumes of plasma (in the range of 100  $\mu$ L) based on the technique described by Mattingly (2).

At the completion of this project, we expect to have established a fluorimetric assay that allows for the determination of cortisol concentrations in small volumes of plasma samples using only inexpensive reagents. This expected outcome has positive impact because it would allow for cortisol measurements in large number of plasma samples, in research studies for which cost of the assay is a limiting factor. This may potentially apply to unfunded pilot studies that are required for external grant applications.

**Innovation:** Currently, the status quo for determination of plasma cortisol levels is based on ELISAs. While this method is highly sensitive and specific, it is also costly. The relatively high cost of cortisol ELISA kits prevents its use for unfunded pilot studies, especially if large number of plasma samples need to be analyzed. We believe that this research project is potentially innovative because our approach deviates from the current status quo by utilizing an inexpensive fluorimetric assay. This will potentially open new horizons by providing the opportunity to determine plasma cortisol levels in large number of plasma samples for unfunded pilot studies.

Research Strategy: In 1960, De Moor and colleagues described a fluorimetric method to determine free plasma 11-hydroxycorticosteroids in man (3). This method was subsequently optimized by Mattingly (2). The method is based on the reaction of 11-hydroxycorticosteroids with sulfuric acid, which forms a fluorochrome that has an absorption peak between 470 nm and 480 nm and an emission peak at 540 nm. Thus, this fluorochrome can be measured using a standard fluorometer equipped with appropriate filters.

The method consists of three sequential steps: (1) extraction of free steroids from plasma using methylene chloride (e.g., 100  $\mu$ L plasma in 750  $\mu$ L methylene chloride); (2) fluorescence reaction (mix methylene chloride extract (e.g., 500  $\mu$ L) with fluorescence reagent (e.g., 250  $\mu$ L) consisting of sulfuric acid and ethyl alcohol); (3) measure fluorescence in plate reader and calculate plasma concentration based on a standard curve.

#### Protocol 1: Establish Sensitivity of Cortisol Assay

We will use standard dilution curves to establish the sensitivity of the cortisol assay. Depending on the time of day and other factors, physiologic cortisol plasma levels are in the range from 60-230 ng/mL. Thus, the standard dilution curve will span a cortisol concentration range from 10 ng/mL to 1000 ng/mL. This experiment will establish the sensitivity of the assay and the linear range of the relationship between cortisol concentration and fluorescence intensity. As standard we will use hydrocortisone (H4001, Sigma-Aldrich).

#### Protocol 2: Establish Validity of Cortisol Assay

Cortisol plasma concentrations will be determined in plasma samples that are already available to Dr. Stauss using the fluorimetric assay and a commercial ELISA kit. Validity of the assay will be assumed if the plasma concentrations determined by the two assays do not differ by more than 20% of the arithmetic average of the two plasma concentrations.

Hazardous material: Methylene Chloride (aka dichloro-methane) is a hazardous substance. Concentrated Sulfuric Acid is a highly corrosive substance. Dr. Stauss has performed a Risk Assessment and performed a trial run of the protocol together with Ms. Kalli Martinez on 09/01/2022. Appropriate safety measures have been established. Students working on the project will receive specific training prior to handling these hazardous substances. Dr. Stauss will be with the students in the laboratory when handling these hazardous substances.

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## Effects of Occipito-Atlantal Decompression, Transcutaneous Auricular Vagus Nerve Stimulation, and the Splenic Pump on Autonomic Nervous System Activity

Mentor: Dr. Stauss

**Significance:** While the sympathetic nervous system has long been a target for treatment of many cardiovascular disorders, including hypertension, the parasympathetic nervous system has been largely neglected as a potential therapeutic target in such conditions. This is surprising, because many cardiovascular conditions are characterized by autonomic dysregulation with increased sympathetic and decreased parasympathetic activity (1-5). **Our long-term goal** is to prevent cardiovascular end-organ damage by restoring parasympathetic and reducing sympathetic activity through non-invasive interventions. Our previous study demonstrated that restoring parasympathetic tone by invasive cervical vagus nerve stimulation prevents vascular end-organ damage in an animal model of fulminant hypertension (6). However, **it is not known** whether parasympathetic nervous system activity can also be restored non-invasively using osteopathic manipulative treatment (OMT) or non-invasive transcutaneous auricular vagus nerve stimulation (taVNS) in humans. **Answering this question is highly significant**, because if it were possible to restore parasympathetic function non-invasively using OMT or taVNS in humans, it would potentially be possible to prevent vascular end-organ in patients with cardiovascular diseases as demonstrated in our previous animal study (6).

**Specific Aim:** The specific aim of the proposed research is to investigate the effects of occipital-atlantal decompression (OA-D), the splenic pump (SP), and taVNS on autonomic nervous system activity assessed by heart rate and blood pressure variability analysis. Based on the literature (7-14), the **hypothesis of this study is** that OA-D and taVNS increases high frequency heart rate variability (cardiac parasympathetic modulation) and decreases low frequency blood pressure variability (sympathetic modulation of vascular tone) and that these effects are augmented by concomitant application of the splenic pump technique.

**Innovation:** The status quo as it pertains to the treatment of many cardiovascular disease is to target the sympathetic nervous system by drugs, such as  $\beta$ -blockers and inhibitors of the renin-angiotensin-system that are known to reduce sympathetic tone. Our research is potentially innovative, because we will deviate from the status quo by targeting the parasympathetic nervous system in addition to the sympathetic nervous system. This approach is likely to open new horizons in the prevention of cardiovascular end-organ damage secondary to cardiovascular conditions, including hypertension which – according to the Center of Disease Control (CDC) - has a prevalence of 48% in the adult US population.

**Research Strategy:** Existing EKG and blood pressure recordings from subjects who underwent three consecutive days of OA-D, taVNS, or a control intervention combined with either the splenic pump or a sham-splenic pump intervention will be analyzed for heart rate and blood pressure variability. Parasympathetic modulation of cardiac function will be assessed high

frequency heart rate variability and root mean square of successive differences between normal heartbeats (RMSSD). Sympathetic modulation of cardiac function will be assessed by low frequency heart rate variability. Sympathetic modulation of vascular tone will be assessed by low frequency blood pressure variability. Statistical data analysis will be performed by multiple linear regression analysis, considering age, gender, and body mass index of the subjects as well as the experimental interventions (OA-D, taVNS, or splenic pump).

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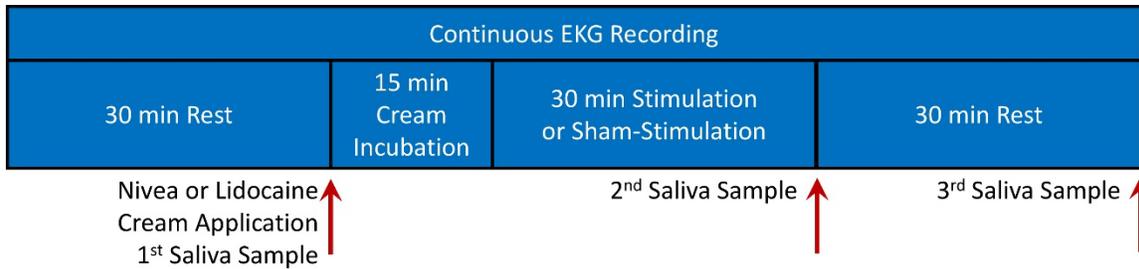
## Effect of Stimulation of Cervical (C2-C3) Cutaneous Sensory Nerve Fibers On Cardiac Autonomic Tone and Salivary Cytokine and Cortisol Concentrations

Mentor: Dr. Stauss

**Significance:** In a previous study, we found that OA-D reduced salivary concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) potentially through activation of the cholinergic anti-inflammatory pathway (1). Likewise, traditional Chinese acupuncture at the Fengchi (风池, GP20) acupuncture point has been demonstrated to reduce serum calcitonin gene-related peptide (CGRP), substance P (SP), interleukin-1  $\beta$  (IL-1  $\beta$ ) and TNF- $\alpha$  plasma levels in a rat model of migraine (2). Interestingly, the Fengchi acupuncture point is located within the same anatomical location of the occiput at which OA-D is performed. This gives rise to the intriguing hypothesis that OA-D and acupuncture at the Fengchi point utilize the same neuronal pathways to activate the cholinergic anti-inflammatory pathway. The skin of this anatomical location receives afferent sensory innervation through C2 and C3 spinal neurons traveling within the N. occipitalis major and N. occipitalis tertius. It has been demonstrated that chemical or electrical stimulation of cardiac vagal and sympathetic afferent fibers activate C1–C3 spinothalamic tract neurons and dorsal horn neurons that also receive somatic inputs from the skin at the location at which OA-D is performed and in which the Fengchi acupuncture point is located (3, 4). Thus, it is possible that convergence of peripheral afferent autonomic nerve fibers and cutaneous sensory nerve fibers to C1-C3 spinal neurons constitute a mechanism by which OA-D and acupuncture at the Fengchi point elicits autonomic effects, including activation of the cholinergic anti-inflammatory pathway. The significance of this idea is that it may provide a mechanistic rationale for using OA-D or acupuncture for the treatment of chronic inflammatory diseases.

**Specific Aim:** The Aim of this research project is to investigate the effect of stimulation of cutaneous sensory nerve fibers at the anatomical location where OA-D is performed and the Fengchi acupuncture point is located on autonomic tone as well as salivary cytokine and cortisol concentrations. The hypothesis of this study is that stimulation of cervical (C2-C3) cutaneous sensory nerve fibers will increase parasympathetic modulation of heart rate variability and decrease salivary cytokine and cortisol concentrations.

**Innovation:** Currently, the use of Osteopathic Manipulative Treatment (OMT) is based on clinical experience rather than systematic research into the mechanisms underlying the effects of OMT. The approach of this research is potentially innovative because it deviates from the status quo by applying a mechanistically driven approach to utilize OMT. Specifically, we hypothesize that OA-D elicits its anti-inflammatory effects through activation of cutaneous sensory nerve fibers. A mechanistically driven approach is expected to improve the clinical effectiveness of OMT.



**Research Strategy:** Healthy adult research participants will be recruited from the local community. Only subjects who provide written informed consent will be enrolled in the study. The experimental protocol is illustrated in the image above. Throughout the experimental protocol, subjects will be in the supine position and the EKG will be recorded continuously. After a 30-minute rest period (to establish stable baseline conditions) a cream (either Nivea moisturizing cream or a 5% lidocaine numbing cream) will be applied to the skin of the target area located at the meeting-place of the base of the skull and top of the neck, just lateral to the tendons of the trapezius muscle (Feng Chi points in image below) and a first saliva sample will be obtained. Fifteen minutes later (lidocaine incubation), the cutaneous receptors in the target area will be stimulated using an EMS Neck Acupoints Lymphvity Massage Device (see image below) for 30 minutes and a second saliva sample will be obtained. This is followed by another rest period of 30 min, after which a third saliva sample will be collected. For each subject, this experimental protocol will be repeated twice on separate days for a total of three study days. On one day the stimulator device will remain off and Nivea cream will be applied (control condition). On the other two days the stimulator device will be turned on and either Nivea cream or lidocaine cream will be applied (experimental conditions).



The EKG will be used to assess cardiac autonomic tone via heart rate variability analysis. Salivary cytokine concentrations (GM-CSF, IL-6, IFN- $\gamma$ , IL-8, IL-2, IL-10, IL-4, and TNF- $\alpha$ ) will be determined using a BioPlex assay (Bio-Plex Pro Human Cytokine 8-plex Assay #M50000007A, Bio-Rad, CA) and salivary cortisol concentration will be measured using ELISA (K003-H1W, Arbor Assays, MI).

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## The Spore in the Desert: Investigating the Distribution of *Coccidioides* in Southern New Mexico

### Significance

*Coccidioides posadasii* is the etiological agent of Valley Fever, a potentially severe disease characterized by chronic pneumonia with extrapulmonary manifestations. Most cases of Valley Fever are diagnosed in southern Arizona and in California's San Joaquin Valley; however, Valley Fever does occur in New Mexico.<sup>1</sup> Unfortunately, no information exists describing the environmental distribution of *C. posadasii* in the region, which is a major knowledge gap given that southern NM soil conditions are predicted to be highly suitable for survival of the organism, as shown in **Figure 1**.<sup>2</sup> While there are currently very few cases of Valley Fever diagnosed each year in NM, continued population expansion, coupled with the predicted effects of climate change, could increase the threat from Valley Fever. For this reason, we seek to develop techniques for detecting *C. posadasii* in soil samples that will allow us to then conduct an environmental survey to determine the local and regional distribution of the agent.

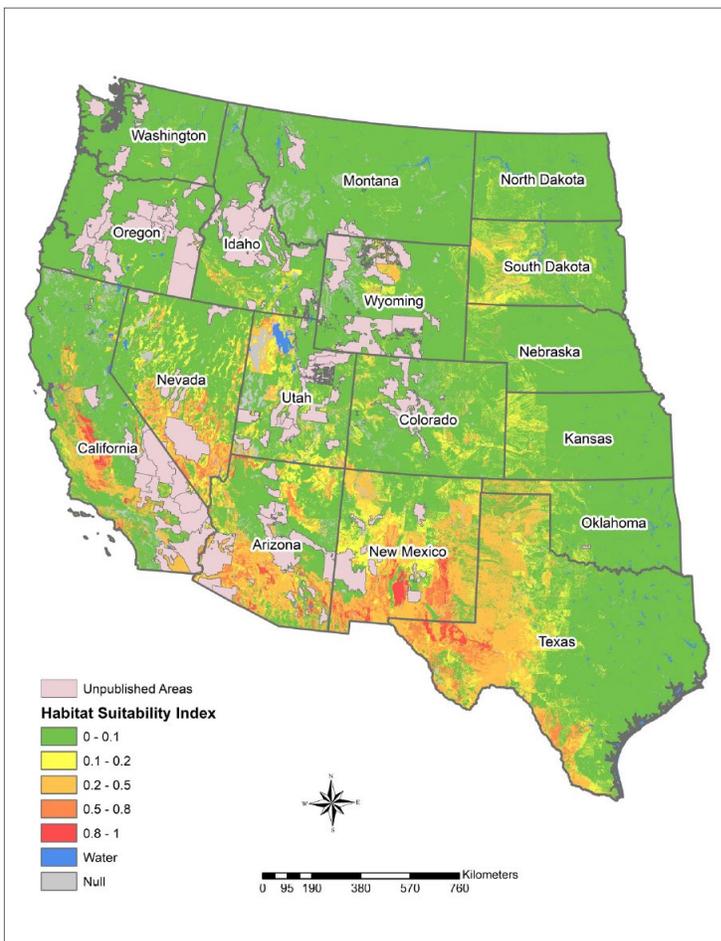


Fig 1. Predicted *Coccidioides* habitat suitability index for the western United States.

<https://doi.org/10.1371/journal.pone.0247263.g001>

## Specific Aims

The primary goal of this summer project is to develop the tools required to conduct a more extensive environmental survey of local and regional soil samples to determine the distribution of *C. posadasii*. We will focus on 2 specific aims:

1. Develop in our laboratory the ability to utilize the CocciEnv and/or CocciDX real-time PCR assays to detect *Coccidioides* DNA.
2. Develop in our laboratory the techniques for processing and isolating DNA from a soil matrix.

## Innovation

Environmental niche modeling predicts that southern NM soil conditions are highly suitable for the survival of *Coccidioides*. Despite this, the New Mexico Department of Health reports relatively few cases of Valley Fever every year. This inconsistency deserves investigation. There is currently a dearth of information describing the environmental distribution of *Coccidioides* in New Mexico soil samples, despite numerous publications describing the presence of the agent in Arizona and elsewhere.<sup>3</sup> This project will establish the techniques and processes required to conduct a more extensive environmental survey to determine the distribution of *Coccidioides* spp. throughout the region.

## Research Strategy

The primary focus for this summer will be to develop the CocciENV and/or CocciDX real-time PCR assays for use in our laboratory, as well as the techniques required to successfully and reliably isolate DNA from soil samples. The CocciEnv and CocciDX assays are published assays that have been utilized to detect *Coccidioides* DNA in soil samples in Arizona; however, we currently have no experience using these assays in our laboratory space. These assays are especially more complex than typical real-time PCR assays, as shown in **Table 1**, because of the high level of genetic heterogeneity in the *Coccidioides* genus. Whereas most real-time PCR assays use a forward primer, reverse primer, and a TaqMan probe, CocciEnv utilizes 11 forward primers, 18 reverse primers, and a TaqMan probe. Therefore, successfully deploying this assay in our hands has yet to be demonstrated, and will be the primary goal for this project.

The student(s) will be tasked with testing these assays against standardized DNA samples to demonstrate reliability, sensitivity and specificity consistent with the published literature. This will include trouble shooting potential issues faced when adapting the techniques to our in-house real-time PCR platforms, including the BioRad CFX 96 and CFX Opus. Given that these are published, validated assays, we expect that the primary obstacles we will face will be related to repeating published results in our laboratory, rather than developing assays *de novo*.

Secondly, we seek to develop the tools to reliably isolate DNA from soil samples. As a complex environmental matrix, PCR detection from soil has special considerations. For example, the high presence of inhibitors of PCR amplification require specialized DNA preparation methods to limit the impacts on test sensitivity.<sup>4</sup> For this stage of assay development and validation, we will work with soil samples spiked with standardized target DNA, and not soil containing *Coccidioides* organisms. This will allow us to assess the impact of soil processing and cleanup techniques on our ability to detect a known quantity of DNA.

**TABLE 1.** CocciDx and CocciEnv real-time PCR assays.

Assay component	Name	Sequence	Final concentration in PCR ( $\mu$ M)
CocciDx Assay			
Forward primer	CocciDx_F1	GTGTTAGGTAGTCCAAGTACACCT	0.6
Forward primer	CocciDx_F2	GTGTTAGGTAATCCAACCAGCACCT	0.6
Forward primer	CocciDx_F3	GTGTTAGGTAATCCAAGTACACCT	0.6
Reverse primer	CocciDx_R1	CTGATGGAGGACTCGTATGCTTGT	0.6
Reverse primer	CocciDx_R2	CTGATGGAGGACTTGTACACTTGT	0.6
Reverse primer	CocciDx_R3	CTGATGGAGGAATTGTATGCTTGT	0.6
Reverse primer	CocciDx_R4	CTGATGGAGGACTTGTATGCTTGT	0.6
Taqman probe	CDxQ-FAM-MGB	6FAM-ACCCACATAGATTAGC-MGBNFQ	0.25
CocciEnv Assay			
Forward primer	CocciEnv_F1d1	CGTTGCACRGGGAGCACCT	0.375
Forward primer	CocciEnv_F2	AAGCTTTGGATCTTGTGGCTCT	0.375
Forward primer	CocciEnv_F3	AATTGATCCATTGCAAGCACCT	0.25
Forward primer	CocciEnv_F4	AATCCAACCTTTGGAACACACCT	0.25
Forward primer	CocciEnv_F5	TTTTCCGGTATGGACTAGCACCT	0.375
Forward primer	CocciEnv_F6d2	TGTTAGGTAATCYAACYAGCACCT	0.125
Forward primer	CocciEnv_F7d2	TRTTAGGTAATYCAACTAGCACCT	0.125
Forward primer	CocciEnv_F8d1	TGTTAGATAATCCAACYAGCACCT	0.125
Forward primer	CocciEnv_F9d2	GKTARGTAATCCAACACTAGCACCT	0.125
Forward primer	CocciEnv_F10d2	TGTTAGGTARTCCAACACTAGCAYCT	0.125
Forward primer	CocciEnv_F11d2	TGTTAGGTAATCCAACACTMGCACYT	0.125
Reverse primer	CocciEnv_R1	GATGGAGGACTCTATATGCTTGT	0.375
Reverse primer	CocciEnv_R2	ATGGAGGACTCGTTATGCCTGT	0.375
Reverse primer	CocciEnv_R3	GGAGGACCCGTATGCTTGTGT	0.375
Reverse primer	CocciEnv_R4	TGCTAAATGATGGAGGGCTTGT	0.375
Reverse primer	CocciEnv_R5	GATGGAGGCTCGTATGCTTGT	0.375
Reverse primer	CocciEnv_R6	AAGGGGTTTGTGGTGAATCCTTA	0.375
Reverse primer	CocciEnv_R7	CAGAAAAATAGCCGTATGCTTGT	0.375
Reverse primer	CocciEnv_R8d2	TRATGGAGRACTTGTATGCTTGT	0.125
Reverse primer	CocciEnv_R9d1	TGATGGAGGACTCGTATGCYTGT	0.125
Reverse primer	CocciEnv_R10d2	TGATGGARRACTCATATGCTTGT	0.125
Reverse primer	CocciEnv_R11d2	TGATAGAGAACTTGTATRCCTRT	0.125
Reverse primer	CocciEnv_R12d2	TGATGAAGAACTTRTATRCCTTGT	0.125
Reverse primer	CocciEnv_R13d2	TGATRRAGGACTTGTATGCTTGT	0.125
Reverse primer	CocciEnv_R14	TGATGGAAAACCTTGTATGCTTGT	0.125
Reverse primer	CocciEnv_R15d2	TGATGGAGGACTTGTAYAYTTGT	0.125
Reverse primer	CocciEnv_R16d2	TGATGGAGGACTTGTAYGCTTRT	0.125
Reverse primer	CocciEnv_R17d2	TGATGGAGGACTYATATGCTTRT	0.125
Reverse primer	CocciEnv_R18d2	GATGGAGGACTCGTWYGGCTTGT	0.125
Taqman probe	CocciEnv_FMGB	6FAM-ACCCACATAGATTAGC-MGBNFQ	0.25

This stage of work will not involve handling any potentially contaminated soil samples. Collecting and processing soil samples containing *Coccidioides* requires specialized biosafety considerations and work practices. Future studies work related to this project will be conducted under enhanced BSL-2 work practices; however, we do not anticipate making it to that stage of the project within the allotted time for the 2023 Summer Research Experience.

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**Mentor(s):**

Debra Bramblett, PhD  
Associate Professor  
Biomedical Sciences

**Project Title:**

DEVELOPMENT OF RT-LAMP ASSAY FOR DETECTION OF DENGUE VIRUSES

**Project Abstract:**

The long-term goal of this research program is to establish an institutional capability to conduct arbovirus surveillance in New Mexico and elsewhere, and to position BCOM to play a central role in responding to emerging infectious diseases that may one day impact the southwestern US. The explosive emergence of Zika, Dengue and Chikungunya into Central America and the Caribbean in recent years has raised concerns that these diseases may one day establish themselves in the United States just as West Nile virus did following its initial introduction into the US in 1999. Furthermore, a warming climate has expanded the range of the primary mosquito vector for these viruses, *Aedes aegypti*, to include most of the southern United States, including New Mexico. The US is no less immune to emerging infectious diseases than any other place in the world, and therefore it is essential that we prepare for the inevitability that these “foreign” diseases will one day be our diseases.

To accomplish our goal, we propose to develop an isothermal nucleic acid amplification technique capable of detecting multiple emerging arboviruses in a rapid, “low-tech” format that can be performed in the field or at the bedside without the need for complex or expensive equipment. This assay will be based on a multiplexed reverse transcription-loop-mediated isothermal amplification (RT-LAMP) reaction targeting West Nile virus (WNV), Zika virus, Dengue virus (DENV) and Chikungunya virus (CHIKV). About five years ago we started by developing the protocol for the West Nile Virus based on the work of Parida et al. Four years ago, we developed novel RT-LAMP primers specific for ZIKA virus. Two years ago, we successfully optimized the RT-LAMP assays specific to West Nile sequences and Zika virus sequences in the context of human body fluids (Urine, whole blood and plasma) using several different readout mechanisms including fluorescence, colorimetric (pH based or hydroxynaphthol blue dye), and lateral flow assay. During the 2022 summer research program, tested the Zika and WNV assay in the context of homogenized Mosquito, successfully demonstrating the potential application of these molecular assays in the field and student successfully developed an RT-LAMP assay for the detection of Chikungunya CHIKV RNA.

During the summer research program 2023, develop a RT-LAMP assays for the detection for **Dengue viruses** and an endogenous mosquito gene CO1 or actin-1 (positive control), using online bioinformatics software for LAMP primer designed called Primer explorer and/or the primer design tool available through New England Biolaboratories. CO1 has been used to differentiate between mosquito species by several researchers ( Chan et al 2014, Morlais 2014) and the invertebrate actin-1 sequence has been used as an internal control to test the quality of RNA from mosquitoes collected in vector surveillance studies by Staley et al.

The purpose of developing RT-LAMP assays for several arboviruses is that we intend to develop a multiplex assay in the future. We are in the process of developing a one tube a

multiplex LT-LAMP assay using a method called QUASR (Ball 2016) that utilizes a fluorescence read out and should there be time, we hope to optimize this assay as well. In the long term, we hope to develop a collaboration with NMSU and/or UTEP faculty to develop a microfluidics chip platform for these the RT-LAMP assays optimized to be used for regional surveillance and/or bedside assays. The hope is to develop a chip that can be used to screen for multiple viruses in one assay. We will need to optimize each LT-LAMP assay for detection of viral sub-types that are either present in our region ( i.e the El Paso WNV strain) now or have the potential to spread to this region based on presence of the natural vector. Once the LT-LAMP assays have been thoroughly validated we will then proceed to developed the microfluidics chip platform for regional surveillance and or bedside assay.

Specific Aims for the 2023 summer student project:

- Specific Aim (Student) #1: Use bioinformatics tools to develop/design Reverse-Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) primers specific to the Dengue 1,2,3 or 4 viruses. context of homogenized mosquito, spiked with synthetic or genomic viral RNA.
- Specific Aim (Student) #2 Optimize RT- LAMP reaction conditions for Dengue viral RNA detection in several contexts including homogenized mosquito, spiked with synthetic or genomic viral RNA.

BCOM Medical students will be intimately involved in all aspects of the larger project. Due to the limited experience and time available to most medical students, we plan to establish student projects that will be meaningful and fruitful in a short period of time.

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We expect that all students who participate in the summer research program will present at the 2023 Burrell Research symposium. Depending upon the outcomes of the summer students may be able to present at the Clinical Virology Symposium. The ASM Clinical Virology Symposium brings together top biomedical scientists, physicians and laboratorians to discuss the latest in diagnostic virology, vaccine development, and molecular diagnostics. Other possibilities to

present locally are the BBRC Symposium at UTEP and The Rio Grande Branch of the American Society for Microbiology depending on the state the pandemic and the openness of the state.

Mentoring plan:

BCOM Medical students will be intimately involved in all aspects of the larger project. Due to the limited experience and time available to most medical students, we plan to foster student projects that will be meaningful and fruitful in a short period. Different students will be involved in the program at different points, from assay design, development, optimization, and application with each student project building on the previous. Students will make small but significant contributions to the overall long-term goal of developing a rapid multiplex assay for WNV, Zika, DENV and CHIKV and using it in a real-world setting to determine the distribution of arthropod-borne infections in New Mexico.

Students will be instructed on how to keep a lab notebook and collect data. Initially student will be trained in laboratory methods and procedures, working side by side with faculty. Once students have been trained how to use specific lab equipment, use particular methods and protocols, they will move on to design their own experiments and work independently.

Students will be expected to participate in group/lab meetings during which time they will share experimental results with the team and provide suggestions and ideas to enhance the experimental outcomes of the group.

Students will participate in a journal club during the summer during which time they will select an article from the primary literature, which is pertinent to the project, and then provide an informal presentation. Each student will have at least one opportunity to lead the Journal club. There will be weekly if not daily meetings to discuss results and progress so that the mentor can help focus or refocus the direction of the project. Students and faculty will be working as a team in the lab and out of the lab.

The faculty members responsibility is to train and guide the student and will make sure that the student is trained in Biohazards, use of recombinant DNA, and laboratory safety. Though students will be encouraged to develop their, own research questions under the guidance of the faculty mentors, their projects are going to be part of a larger ongoing research project. We will oversee the student's work providing critiques and editing of written work and protocols. We consider it part of our role as mentors to challenge our mentees and will do so on occasion to help the student researcher grow and develop their skills. At the same time, we want the experience to be positive and enjoyable and so will incorporate opportunities for fun.