Army xTechHBCU
Part 2: Draft Proposal

Instructions

- **Due by November 15, 2021 at 5:00 PM ET.**
- **Entries that do not comply with these instructions will not be accepted. Please read carefully!**
- Complete this entire template using an **11-point font size in the Calibri font**. **Do not change this document's margins or alter its format.** Additional reference section is allowed but must be contained within the 3 page limit. **Footnotes are not allowed.**
- **Your completed document must be no more than four total pages:** this company profile page, plus **three pages of text**. Use the embedded **purple prompts** to guide you. The prompts precisely track the evaluation criteria used by the reviewer, so it is in your interest to organize your document using these prompts.
- The main body text on page 2-4 must be contained on a **maximum of three pages**. You **may change the relative lengths of the four suggested sections**: Each proposal's story is different and you may need more space than others for certain sections below.
- **Save this file as a PDF document** and in the file name **replace "Template" with your University Name**. Please DO NOT add any other annotations such as v1, DDMMYYYY, etc.
- **To submit:** Go to this URL https://usg.valideval.com/teams/HBCU_2021/signup, complete the form and upload your PDF.

Basic Information	
University Name	Queens College City University of New York
Proposal Title	Assaying dynamic states of mental health from bloodborne DNA methylation in a model of social adversity using a highly social African cichlid fish
Contact Name	Sebastian Alvarado
Contact's Title	Assistant Professor
Email	Sebastian.alvarado@qc.cuny.edu
Phone number	408-797-5248
University Website	www.alvaradolab.com

Abstract

DNA methylation is a reversible biochemical mark that can regulate gene function and cell differentiation. In the past 20 years, studies of DNA methylation in human populations have revealed signatures associated with various disease states. Despite this breadth of work, such studies report these signatures as a static outcome and not a dynamic state. We propose the adoption of a highly social cichlid fish with reversible social status to describe the tissue-specific (blood + brain) contributions of DNA methylation to individual behavior and social network. The Integration of these layers will allow our group to create a predictive framework for behavioral state transitions that will inform the selection of blood borne biomarkers that can infer socio-behavioral states and other "lived experiences". Given that this system includes robust displays of threat and aggression as part of its natural life history, this proposal will identify molecular signatures that associate with social resilience and coping. We plan to carry out a high throughput unbiased screen of tissue specific methylomes and transcriptomes with the Oxford Nanopore sequencing platform. Within our datasets, we will focus on genes related to the hypothalamic pituitary adrenal stress axis since they are highly conserved in higher vertebrates such as humans. We anticipate this approach will reveal how behavioral states transition in the face of adversity and physical conflict in humans.

Part 1: INTRODUCTION

Traumatic events leave indelible marks on our biology in profound ways that affect mental and physical health. In the field, these marks can predispose a soldier to chronic co-morbidities and steer interventions before or after deployment. Robust signatures of DNA methylation can discriminate biological age, socioeconomic status, and exposure to natural disasters. However, little research has shown how social adversity can shape these patterns or REVERSE them. Here, we adopt a highly social African cichlid fish, *Astatotilapia burtoni*, that shows REVERSIBLE dominant and subordinate phenotypes that result from threat and direct physical conflict from other males. We will use this system to interrogate how DNA methylation can program health outcomes as a factor of social adversity. Furthermore, we plan to use signatures of DNA methylation in both brain and blood to identify DNA methylation overlap and/or associations. The long-term value proposition of this research is to use blood as a surrogate biomarker for changes in mental health state. **Study Design:** Tissues will be collected from males reared in a dyad paradigm (Figure 1). Briefly, two size-matched males will be housed in a 10-gallon tank with three females. Over the course of 1 month, males will develop into subordinate-dominant pairs that possess varying degrees threat (Lateral/Frontal displays) and full-on aggressive behaviors (Bites/ramming). These behaviors will be recorded for 1 hour before sacrifice and scored by undergraduate researchers. Following video recording, males from each dyad pair will undergo tail bleeds to collect blood in heparinized capillaries and have brains collected. Density gradient separation will be used to separate platelets, peripheral blood monocytes and lymphocytes whereas whole brains will be gross dissected and flash frozen. All tissues will be processed for high molecular weight genomic DNA extraction and subsequent loading on a nanopore flow cell (performed by graduate student researchers). Base calling (guppy) and alignment to genome (Minimap2) will be carried out followed by methylation calling (Megalodon + Nanopolish). Bioinformatic workflows and data curation will be carried out by the lead PIs graduate students with support from co-PIs. Top differentially methylated regions of the genome will be further validated using bisulfite PCR and pyrosequencing.

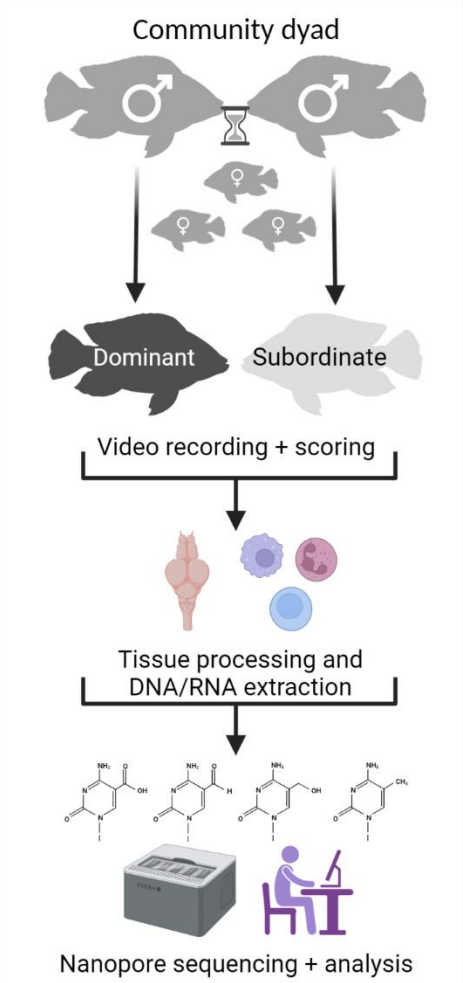

Figure 1: Experimental overview.

Part 2: SCIENTIFIC/TECHNICAL MERIT

Degree of Innovation: Our innovation is driven by three factors: (I) Our model system choice, (II) our technical approach, and (III) a focus on a brain-blood association. (I) *A. burtoni* is a tractable model for sociobiology since changes in its social hierarchy are a natural part of their life history and their social rank is sensitive to perturbations to DNA methylation. Males in our model system become highly aggressive dominants or timid subordinates depending on their easy to manipulate social interactions. These social manipulations generate variation in threat-aggression strategies allowing for the detection of nuanced changes behavior and DNA methylation within dominant-subordinate dyads. High threat/Low Aggression males will subordinate conspecifics without physical violence whereas Low threat/High Aggression leads to subordination because of direct physical violence. (II) Our choice of the Nanopore sequencing platform affords us the ability to assay multiple DNA modifications *in situ* without library preparation, chemical conversion, or processing samples post DNA extraction. (III) The fact that we can study blood and brain along with its constituent cell types allows us to integrate tissue-specific molecular changes, whole organism behavior, and subsequent effects on their surrounding social network. This puts us in a unique position to integrate layers of information that can model changes in mental state using surrogate biomarkers of DNA methylation in blood. We will be able to predict nuanced changes in social behavior from blood. Since this is a dynamic state, we can iteratively repeat

these assays to objectively assess how social environments can shape behavioral states. **Scientific Feasibility:** DNA methylation assays have evolved from whole genomic analyses in the field of cancer (50 years ago) to single cell and single base pair resolution (in past 5 years). Furthermore, this technology has been heavily implemented in demographic and epidemiological studies in basic research for the past 10 years. Our model system has a sequenced and well annotated genome and heavily conserved DNA methylation system that is homologous to humans. **Alternative Technical Approaches**: Conventional approaches have several caveats. First, human studies often lack interventions that shape physiological outcomes or use appropriate model systems where changes in social structure are a part of their natural life history. Here, we anticipate our model choice and questions will be highly tractable and translatable to stress responses in vertebrates with novel insights in reversible states of stress. Second, current approaches rely on chemical conversion, methylated DNA immunoprecipitation and/or array hybridization or older generation high throughput sequencing (Illumina). While these technologies have been steppingstones in advancing the state of the art, they have shortcomings. Array hybridization is qualitative compared to sequencing and nearly all high throughput sequencing platforms require sodium bisulfite conversion and whole genome amplification steps to study DNA methylation. These approaches introduce amplification and chemical conversion biases that increase the cost of sequencing coverage on short reads while increasing background "noise" in downstream bioinformatic analyses. These approaches immediately limit the study of other modifications made to DNA such as hydroxymethylcytosine, 5-formylcytosine, 5-carboxycytosine, and N6 methyladenine. We will use a nanopore platform that allows for in situ DNA base modification calls without any additional steps in a range of tissues. The Oxford nanopore is the state of the art and is not limited by chemical degradation or library amplification artifacts in sequencing workflows. Given the physical parameters from which data is collected, our approach will allow for base calls for DNA methylation in our samples along with modifications. Collectively, this approach will provide a single base pair resolution of diverse modifications adding depth of data to discriminate subtle differences in biology. **Research Maturation Plan:** Following initial data generation outlined above, we would have a rich dataset to pursue SEVERAL more tissue-specific directions. For example, blood monocytes, B-cells, and T-cells will likely have different signatures that may regulate immune function and could be amenable to functional studies using (epi)genetic editing *in vitro* or *in vivo*. Changes in neovascular niches within the brain may feedback into systemic changes in hematopoietic cells and steer us towards areas of the brain that are more "in tune" with hematopoietic cell types (co-PI Tajerian). Even integrating and modelling molecular/behavioral/social data will require deep learning and an ability to extract features of these data to narrow in on underlying mechanisms (co-PI Suderman). Outside of our model system, we can build out our research in human populations (co-PI Suderman) with back translational potential in our cichlid fish or murine systems (co-PI Tajerian).

Part 3: CONTRIBUTION TO ARMY MISSION

Problem Alignment: This research fits under two alignments; (I) the Neurophysiology of cognition: Multi-sensory synthesis (Topic 3) and modalities of (II) Human Dimension: Cognitive social interactions (Topic 4). The core idea behind this proposal involves identifying robust bloodborne signatures that are tied to mental health and social behavior. Under (I), we are mapping molecular correlates of physiology tied to the brain as a function of social adversity to better understand the nature of transitions between dominant and subordinate social states in the brain and its associated signatures in blood. Under (II), we are modeling aspects of social behavior within a community where we can quantify behavior across 5 animals. In addition to assaying blood and brain specific molecular signatures, we will profile community structure and social network as defined by the number of agonistic interactions between conspecifics. This approach will allow us to measure deferred aggression amongst subordinated males and females and/or how tradeoffs in aggression and threat in dominant males can affect social structure. **Spin-off and Extension Opportunities:** We will lay the groundwork for translation into human systems by focusing on DNA methylation and other DNA base modifications provided by our technical platform. Comparative translation of this research will identify a DNA methylation universal stress response that may predict resilience and a range of other behavioral outcomes (addiction, psychosis, etc.). DNA methylation as a biomarker has already been demonstrated to change with various degrees of stress and space travel suggesting it can serve as a robust biomarker for "lived experiences" broadly defined. Whether this can provide objective measures of prescreening tier 1 candidates for special forces training or implementing training regimes that can be accompanied with objective measures such as DNA methylation, there will be thousands of possibilities.

Operational Impact: We present the possibility to develop an objective diagnostic of subjective experiences (here, defined as social adversity). When considering the warfighter, the US Army is limited to pre and post deployment health assessments (PDHA) which rely only on self-reporting mental and physical health. Even with PDHAs, many soldiers may feel socially pressured to report dishonestly in order avoid the stigmas related to mental health. We are confident that base-pair resolution of DNA modifications in blood can provide an objective and reliable measurement of physical and mental state. Profiling DNA methylation before/after combat can identify risk groups for mental health and predict deployment outcomes. This can allow for interventions of Behavioral Health to increase the effectiveness of warfighters and reduce self-harm and/or suicide. **Scale of Impact:** Molecular profiling of this nature can be incredibly versatile if extensively collected over years and in diverse conditions. In one regard, it can provide iterative diagnostics that can be used to train soldiers to become resilient under certain pre-deployment conditions and better suited for field operations. Given the cost of training highly skilled warfighters such as Tier One soldiers, we would assume these objective measures could increase the retention of candidates in special forces training, thus reducing the cost of training and overall attrition of candidate pools. We also argue the value of this data towards acquiring military intelligence. For example, measuring blood DNA modification profiles from captured enemy forces could provide an opportunity to infer "lived experiences" such as exposure to different weather conditions, variation in physical fitness or diet. This information could provide a tactical advantage in almost any setting with human populations.

Part 4: TEAM

Relevance of Skills and Background: This diverse team is led by Dr. Sebastian Alvarado (lead PI), Dr. Maral Tajerian (co-PI), and Dr. Matthew Suderman (co-PI). Dr. Alvarado (Queens College CUNY) is a leader in neuroepigenomics and functional validation of DNA methylation in diverse tissues and models that describe epigenetic phenomena with robust biological plasticity. Dr. Tajerian (Queens College CUNY) is a neurophysiologist with an interest in brain plasticity and how changes in the periphery can affect the brain and vice versa. Dr. Suderman (University of Bristol United Kingdom) is a bioinformatician that studies associations of early life stress and patterns of blood borne DNA methylation in human populations. These PIs already share authorship across 7 publications pioneering the field of pain epigenetics which also includes one manuscript profiling the overlapping signatures of DNA methylation in blood T-cells and the prefrontal cortex of a murine model of peripheral nerve injury. Our collaboration will involve behavioral profiling, tissue collection/processing, and data generation at Queens College CUNY (Alvarado + Tajerian), followed by remote consulting of bioinformatic datasets between Queens College and the University of Bristol (Alvarado+ Tajerian+ Suderman). The proposed work will be carried out in the Alvarado-Tajerian labs where we have the required sequencing capabilities (Nanopore GridION X5, Bioanalyzer, and Qubit) and staff (5 graduate students + 10 undergraduates) to succeed. Between the Tajerian and Alvarado Labs, graduate and undergraduate researchers have been identified that will be responsible for carrying out behavioral paradigms, blood collection, cell separation, tissue processing and sequencing workflows. **Passion / Motivation:** Our ability to shape our environment/health has been a driving force behind the lead PI and co-PIs research program. This philosophy is represented in the trainees that have joined the PIs research program with a personal interest in stress physiology and/or resilience. **Independent Mindsets**: PIs Tajerian and Alvarado have pioneered the field of pain epigenetics during their graduate training and have collaborated on several projects despite having separate and independent training programs. Together with co-PI Suderman, they possess the training with alternative model systems, neurobiology, and bioinformatics perfectly suited to achieve the goals of this proposal **Past USG Performance:** The PI Alvarado was supported on an NIH diversity supplement during his postdoctoral training and currently holds an Emerging Frontiers Rules of Life NSF grant (1921773) that seeks to show how visual ecology can shape morphological variation in *A. burtoni.* This grant equipped the Alvarado lab with the Nanopore GridIonX5 and sequencing workflow required for this current proposal. Co-PI Tajerian currently holds a SC2 NIH grant (9855716) to develop a research program studying how chronic peripheral pain affects the extracellular matrix of the brain. Additionally, PI Tajerian is a co-PI on two other NIH training grants for underrepresented minorities in science (MARC+BRIDGES). Please note that PIs Alvarado and Tajerian are early career researchers that were awarded their grants 1 year before the pandemic.