

Mothers, Infants, Microbiome and Mental Health (MIMMs): A social neuroscience perspective of maternal-child health to unlock the potential of biobanked data

An Application to the Presidential Fellowship in Data Science

The University of Virginia

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“On my honor, we have neither given nor received aid on this application.”

BACKGROUND AND SIGNIFICANCE: Over 90% of the human body is composed of microbes that are not uniquely ours in nature though integral to our daily functioning (Bailey, et al., 2010). Several studies, in both animal models and humans, have implicated the intestinal microbiome as an important modulator of the central nervous system through the gut-brain axis (Luna & Foster, 2015; Evrensel & Ceylan, 2015; Bravo, Julio-Peiper, et al., 2012). The rapid colonization and the influence of the microbiome begins at birth and continues during infancy.

Microbiome samples can be quantified by relative diversity of microbes but also by specific strains such as *Lactobacillus*, *Bifidobacterium*, *Clostridium* (Luna & Foster, 2015). Intestinal dysregulation, including the excessive presence or absence of particular microbial communities, has been linked to internalizing symptoms such as anxiety and depression (Evrensel & Ceylan, 2015). Anxiety and depression are very common in pregnancy affecting 59.5% and 12% of women respectively (Faisal-Cury, Menezes, 2007; Shakeel, Eberhard-Gran, et al 2015). These internalizing symptoms have been linked to heightened attentional biases towards threatening stimuli, including angry and fearful facial expressions (Cisler & Koster, 2010; Fox, Riccardo, & Dutton, 2008). In pregnancy, theories suspect that mothers develop stronger attentional processing to help identify infant emotion and distress (Pearson, Lightman, Evans, 2011). Such attentional biases develop during pregnancy and are believed to be associated with more successful outcomes concerning maternal-child attachment (Pearson, Lightman, Evans, 2011). Critically, mothers with clinical levels of depression or anxiety during pregnancy exhibit disrupted attentional biases to infant cues (Pearson, Cooper, et al, 2010). Moreover, mothers with high anxiety levels show difficulties in disengaging from threatening emotional stimuli (Fox, Russo, & Dutton, 2002). Given existing evidence indicating an influence of the microbiome on internalizing symptoms and the link between symptoms to an enhanced threat bias, extending this line of research into how the microbiome impacts threat biases in infants and their mothers appears an important next step.

The current proposal will bring together researchers from the UVA Babylab at the Department of Psychology and the School of Nursing to examine how the intestinal microbiome predicts levels of maternal internalizing symptoms and threat bias in mothers and their infants (See Supplementary Figure 1). **Aim 1:** Identify maternal bacterium which are predictive of maternal internalizing symptoms. *Hypothesis:* We hypothesize there will be an increase in certain bacteria (e.g., *Lactobacillus*) and decrease in other bacteria for mothers classified as depressed or anxious (Luna & Foster, 2015). **Aim 2:** Using the identified bacterium strains from Aim 1, we will examine how the composition of these specific maternal bacterium are associated with maternal threat bias. *Hypothesis:* Given that high levels of trait anxiety are linked to a threat bias in facial emotion processing (quicker orientation/heightened attention to angry and fearful facial expressions), we hypothesize that the bacteria strains which occur at a higher frequency in more anxious mothers will also be associated with higher threat bias (Cisler & Koster, 2010). **Aim 3:** Using the identified bacterium strains from Aim 2, we will examine how the composition of these specific strains of bacterium from the infants' sample are associated with infants' behavioral and brain responses to threatening faces. *Hypothesis:* We hypothesize that maternal and infant threat biases are highly correlated. More specifically, we plan to test whether strains of bacterium which predict maternal threat bias will also be highly associated with infant behavioral and brain responses to threat (Pearson, Lightman, Evans, 2011). In summary, the proposed interdisciplinary approach will generate a first-of-its-kind data set allowing for a comprehensive and novel analysis of factors impacting maternal and child mental health.

METHODOLOGY: The proposed study is a longitudinal examination of maternal microbiome collected during pregnancy, maternal internalizing symptoms, and infant threat bias. Existing samples from the Obstetric and Neonatal Outcome Study (ONOS) will be used for maternal microbiome assessment. ONOS includes serum blood, fecal, placental and cord blood samples from pregnant women, at 3 time points during pregnancy, receiving care at the Battle Building Ob/Gyn clinic. There are approximately 100 participants currently enrolled with ongoing recruitment averaging 30 new participants each month. Additionally, medical record data including history of depression and anxiety as well as scoring from the Edinburgh Postpartum Depression Screening, collected during pregnancy, will be sourced from the UVA Clinical Data Repository and will be matched to the biospecimens via a study identification number. We will analyze the maternal fecal sample, collected between 28-32 weeks gestation, to characterize the intestinal microbiome. To characterize the vast microbiome, the Illumina MiSeq® sequencing system will be used to conduct triplicate 16S community microbiome analysis. Using primers designed to target specific variable regions of the 16S ribosomal RNA gene, this method allows for quantification and diversity measurements of microorganisms within the sample.

Eligibility criteria for ONOS includes plans to remain in the Charlottesville area for at least one year after delivery to allow for potential infant evaluation. Recruitment for the infant neuroimaging and behavioral aspect of the study will be conducted using the ONOS cohort. When study infants reach 7 months of age (the age in which infants are known to discriminate a series of negative and positive emotions; Krol et al., 2015) approximately 60 mother-infant dyads will be contacted to participate. At the one-time, one-hour appointment, mothers will be asked to complete a DEER-T task and infants will participate in an fNIRS and eye tracking study; in addition, participating mothers will bring an infant fecal sample in order to characterize the infant microbiome.

Mothers will complete a dynamic emotional expression recognition task (DEER-T), which is a behavioral measure of maternal threat bias (Krol, Kamboj, Curran, Grossmann, 2014). This is a widely used measure and has previously been used with mothers. Twelve actors will be presented showing a dynamic progression from a neutral to one of six emotion expressions (anger, happiness, fear, sadness, disgust, and neutrality) over 3 seconds. The participants are given 6 response keys to identify the emotions and reaction times are collected.

In order to examine how the infant microbiome influences infant threat bias we will obtain brain and behavioral measures during emotion processing tasks. Infant brain function will be assessed using functional Near-Infrared Spectroscopy (fNIRS). fNIRS measures hemodynamic brain responses by using the reflective and absorption properties of near-infrared light to quantify changes in oxygenated and deoxygenated hemoglobin in the cerebral cortex (NirX, 2016). For the fNIRS paradigm, infants will view up to 36 videos of dynamic emotional expressions (e.g., actor going from a neutral to fearful expression) with each trial spanning approximately 7 seconds in length. Given the experimental paradigm and that the NirX fNIRS system has a sampling rate of 3.9 Hz, this provides us with over 980 moment to moment data points. Infant looking behavior will be examined using eye-tracking technology. Infants will be presented with two faces, one emotion (e.g., sadness, fear, pain) and one neutral face. For this paradigm, the infant will view up to 12 trials of 10 seconds each. Given the experimental paradigm and that the Tobii eye tracker has a sampling rate of 60 Hz, this provides over 7,200 moment to moment data points. Therefore, for infant data alone we will have over 480 million data points in addition to the vast ecosystem of more than 3,000 reference microbial genomes.

ANALYSIS: With the collaborative effort of both Caitlin and Caroline, we will create a highly unique and rich dataset for 60 mother-child dyads of maternal and infant microbiome colonies, maternal-child health and mental health data, mother behavioral threat bias and infant neurological and behavioral threat bias data. First, in order to classify maternal microbiome, and identify strains that are most predictive of maternal internalizing symptoms we will utilize elastic net analysis (using the “elasticnet” package in R; Zou & Hastie, 2005). Next, traditional ways to approach statistics in the neuroscience and medical field is to take a grand average across all points across a construct. However, by doing this you lose the real intricacies and great majority of the predictive power of the data collected. Given the complex nature of the dataset we plan to utilize machine learning algorithms which allow us to appropriately capture and model the dynamics and structure of the DEER-T, fNIRS, and eye-tracking data. More specifically, we plan to use classification algorithms, such as the recurrent neural networking modeling (using the “rnn” package in R analysis software). Artificial neural networks are an array of small, highly interconnected units, that model structure and functions of the nervous system (Hochreiter & Schmidhuber, 1997). The neural network modelling approach we intend to use is designed to recognize patterns in complex sequential data that enable prediction (Hochreiter & Schmidhuber, 1997). This will allow us to: 1) use the bacterium strains with the highest discriminatory value for maternal internalizing symptoms, and examine how these bacterium strains predict maternal threat bias (DEER-T), and 2) use the strains with the highest discriminatory value for maternal threat bias, and examine how these bacterium strains in the infants’ samples predict the dynamics and structure of infant neural and behavioral threat bias.

IMPLICATIONS: This research project will be the first to study the link between the maternal and infant gut microbiome, perinatal maternal anxiety and depression, and maternal and infant threat bias. The findings from this study promise to provide novel insights into factors that will critically contribute to maternal and infant mental health. This study is well-aligned with the National Institute of Health’s Precision Medicine Initiative, the National Institute of Nursing Research and the National Institute for Child Health and Development’s focus on bio-behavioral research and will open up a new avenue for interdisciplinary research at the University of Virginia with the help of the Data Science Institute.

As graduate students in nursing and psychology, our interdisciplinary and interscholastic collaboration uniquely position us to achieve the aims of this research proposal. Our partnership capitalizes on the already consistent recruitment of the ONOS team and the expertise of the University of Virginia faculty. Taken together, we believe that our project has immense potential to influence future clinical decision-making. In terms of professional development, this project will benefit both Caitlin and Caroline as they pursue research-oriented academic careers. This project will serve as a fundamental base for dissertation research, applying for external funding (NRSA research fellowships), and will result in widespread dissemination through presentations and publications. Cross-mentoring of undergraduates between the Departments will be a cornerstone of the project. Further, this partnership would increase the caliber and diversity of recruitment research programs of the medical and nursing campus. Therefore, this project has vast implications for the greater UVA research community and provide a unique support for the professional and research development of Caitlin and Caroline.

REFERENCES

- Bailey, M., Dowd, S., Galley, J., Hufnagle, A., Allen, R., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*, 397-407.
- Cisler, J. M., & Koster, E. H. W. (2010). Mechanisms of Attentional Biases towards Threat in the Anxiety Disorders: An Integrative Review. *Clinical Psychology Review*, *30*(2), 203. doi: 10.1016/j.cpr.2009.11.003
- Evrensel, A., & Ceylan, M. E. (2015). The Gut-Brain Axis: The Missing Link in Depression. *Clinical Psychopharmacology and Neuroscience*, *13*(3), 239–244. doi: 10.9758/cpn.2015.13.3.239
- Faisal-Cury, A., & Menezes, R. (2007). Prevalence of anxiety and depression during pregnancy in a private setting sample. *Arch Womens Ment Health*, *10*(1), 25-32.
- Fox, E., Riccardo, R., & Dutton, K. (2002). Attentional bias for threat: evidence for delayed disengagement from emotional faces. *Cogn Emot*, *16*(3), 355-379.
- Hochreiter, S., & Schmidhuber, J. (1997). Long short-term memory. *Neural computation*, *9*(8), 1735-1780.
- Krol, K. M., Kamboj, S. K., Curran, H. V., & Grossmann, T. (2014). Breastfeeding experience differentially impacts recognition of happiness and anger in mothers. *Scientific reports*, *4*, 7006.
- Krol, K. M., Monakhov, M., San Lai, P., Ebstein, R. P., & Grossmann, T. (2015). Genetic variation in CD38 and breastfeeding experience interact to impact infants' attention to social eye cues. *Proceedings of the National Academy of Sciences*, *112*(39), E5434-E5442.
- NirX. (2016). Functional Near-Infrared Spectroscopy. Retrieved January 24, 2017, from <http://nirx.net/publications/#infants>
- Pearson, R., Cooper, R., Penton-Voak, I., Lightman, S., & Evans, J. (2010). Depressive symptoms in early pregnancy disrupt attentional processing of infant emotion. *Psychol Med*, *40*(4), 621-31.
- Pearson, R., Lightman, S. & Evans, J. (2011). Attentional processing of infant emotion during late pregnancy and mother-infant relations after birth. *Arch Womens Ment Health*, *14*(1), 23-31.
- Shakeel, N., Eberhard-Gran, M., Sletner, L., Slinning, K., Martinsen, E. W., Holme, I., & Jenum, A. K. (2015). A prospective cohort study of depression in pregnancy, prevalence and risk factors in a multi-ethnic population. *BMC Pregnancy and Childbirth*, *15*, 5. <http://doi.org/10.1186/s12884-014-0420-0>
- Zou, H., & Hastie, T. (2005). Regularization and variable selection via the elastic net. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *67*(2), 301-320.

Supplementary Materials:

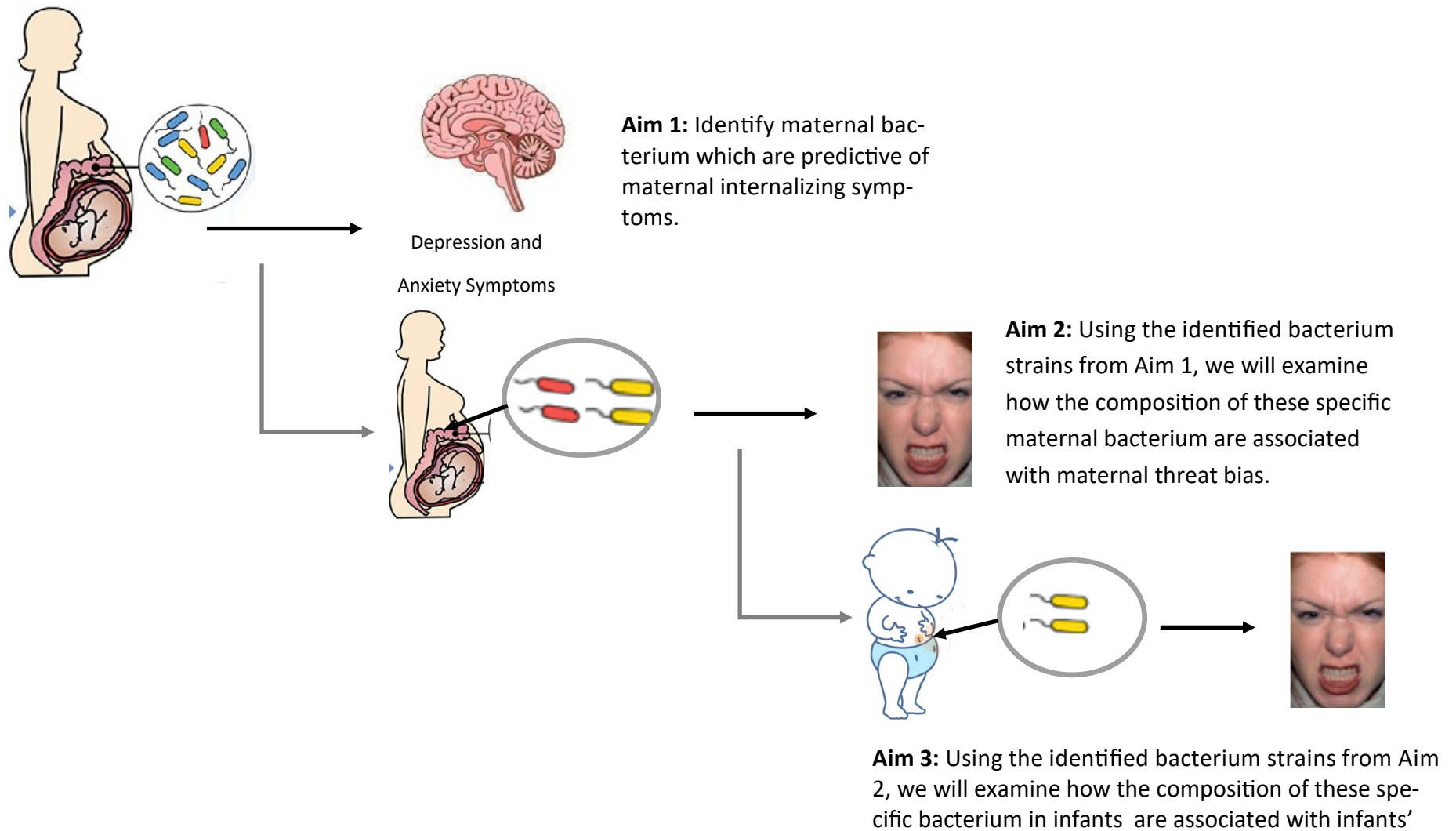


Figure 1. Schematic of research aims.

Note: Pictures are modified and taken from : Advance Lipids (2017); Gohir, Ratcliffe, and Sloboda (2015) , and Krol et al. (2015)