**UW–Madison Undergraduate Research Symposium Participants (WNPRC)**
**April 28-29, 2022**

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HYPOTHALAMIC ESR1 KNOCKDOWN CORRELATION WITH METABOLIC MEASURES IN ADULT FEMALE RHESUS MONKEYS

**Siti Hydara, Molly Willging (Mentor)**

During the menopause transition, reduced serum estradiol levels heighten the risk for metabolic dysfunction. Estrogenic effects on female rodent metabolism are primarily mediated by estrogen receptor alpha (ESR1) activation within the mediobasal hypothalamus (MBH). The role of MBH ESR1 in female primate metabolic homeostasis is unknown. We utilized RNAi technology to assess ESR1 gene knockdown (ESR1KD) in the MBH of adult, female rhesus macaques. ESR1KD presented with increased body weight % change, reduced post-prandial energy expenditure, intermittent reduced morning activity, and reduced caloric intake. Thus far in correlation analyses, the extent of individual animal ESR1 knockdown and these metabolic measures are nonsignificant. Overall, these findings highlight MBH ESR1 role in regulating energy homeostasis but do not yet show a range of knockdown inducing such effects

GENOMIC EDITING BY CRISPR/CAS9 AS A THERAPEUTIC NEURAL-NETWORK STRATEGY FOR PARKINSON'S DISEASE

**Samuel Neuman, Marina Emborg (Mentor)**

The neurons in the substantia nigra pars compacta (SNpc) project into the striatum, while the striatum projects into the substantia nigra pars reticulata (SNpr). Retrograde and anterograde transport between these structures can be exploited as a therapy for Parkinson's Disease. In this project, we evaluated whether genomic editing in the striatum affected protein expression in the SNpr and SNpc. Ai14 reporter mice received intrastriatal injections of NC-containing RNPs targeting the stop codon to permit expression of TdTomato. TdTomato+ fibers were detected in the SNpr when the edited striatal area was >5 mm2 but not <2 mm2. These results suggest that a biological threshold of expression is required for substantial axonal transport of the protein to be used as a neural-network therapy.

GENES, NEONATAL NURSERY AND BIOBEHAVIORAL DEVELOPMENT

**Mili Meredith, YuFan Ye, Allyson Bennett (Mentor)**

Early life stress (ELS) has significant impacts on biobehavioral development and health. In human and nonhuman primates (NHP), neonatal intensive care unit (NICU) placement may present ELS. Understanding of the consequences and risk factors for adverse NICU-associated outcomes can help refine neonatal care practices. We used hair cortisol, clinical health data, and pedigree-based sibling relationships to identify the long-term physiological and health effects of ELS and evaluate potential genetic influences associated with vulnerability to persistent NICU-NR effects. Subjects were mother-reared (MR) and NICU nursery-reared (NICU-NR) adolescent-adult rhesus macaques born at the Wisconsin National Primate Research Center (WNPRC) from 2012-2019. I predict that NICU-NR animals will have higher cortisol and more health issues than the MR group and that adverse NICU-associated outcomes will be heritable.

ESTROGEN-RECEPTOR ALPHA NEURONS IN THE ARCUATE NUCLEUS MEDIATE FEMALE NEURAL INHIBITION OF NEUROENDOCRINE PUBERTY

**Lukas Henjum, David Abbott (Mentor)**

Puberty's onset is characterized by gonadotropin-releasing hormone (GnRH) release from the hypothalamus, and its stimulation of gonadotropin release from the anterior pituitary, initiating menstrual cycles. Non-primate GnRH release is restrained through an ovarian estradiol (E2)-mediated negative feedback mechanism regulating neurons expressing estrogen receptor alpha (ESR1) within the hypothalamus arcuate nucleus (ARC) and preventing ovarian function until adolescence. In prepubertal girls and nonhuman primates, however, absence of ovarian E2 does not trigger puberty prematurely, indicating ovarian independent restraint. We hypothesize that prepubertal restraint in female primates involves neuro-E2 synthesized in the ARC. Following bilateral ovariectomy of prepubertal female monkeys, we anticipate that infusion into the ARC of viral vector containing silencing RNA (shRNA) targeting ESR1 will eliminate GnRH restraint, implicating neuro-E2 in female primate prepubertal restraint.

ROLE OF HYPOTHALAMIC ESR1 IN ADULT FEMALE RHESUS MONKEY ENERGY HOMEOSTASIS

**Alexis Woida, Molly Willging (Mentor)**

Declining serum estradiol levels during menopause are associated with heightened risk for metabolic disease. Estrogenic effects on female rodent metabolism are primarily mediated by estrogen receptor alpha (ESR1) activation within the mediobasal hypothalamus (MBH). The role of MBH ESR1 in female primate metabolic regulation, however, remains unclear. We therefore employed RNAi technology to assess ESR1 gene knockdown (ESR1KD) in the MBH of adult, female rhesus macaques. ESR1KD females exhibited a ~22% increase in body weight after ~16 months, versus ~12% increase in controls. Via metabolic cage analysis, postprandial energy expenditure (EE) was inconsistently diminished in ESR1KD versus controls. Overall, these findings highlight MBH ESR1 role in regulating body weight and energy expenditure, and suggest a discrete MBH location for therapeutic development to combat female obesity.

ROLE OF HYPOTHALAMIC ESR1 IN ADULT FEMALE RHESUS MONKEY WHITE ADIPOSE MORPHOLOGY

**Andrew Neilson, Molly Willging (Mentor)**

During the menopause transition, serum estradiol levels decrease and are associated with heightened risk for obesity and increased abdominal fat mass. Estrogenic effects on female rodent metabolism are primarily mediated by estrogen receptor alpha (ESR1) activation within the mediobasal hypothalamus (MBH). However, the role of MBH ESR1 in female primate metabolic homeostasis and fat morphology is unknown. We employed RNAi technology to assess ESR1 gene knockdown (ESR1KD) in the MBH of adult, female rhesus macaques. ESR1KD females exhibited a ~22% increase in body weight after ~16 months, versus ~12% increase in controls. Via microscopy, so far, there have been no differences in subcutaneous or visceral white adipocyte size or number. Overall, these findings highlight MBH ESR1 role in regulating body weight without adipose morphological changes.

HEALTHY AND ATRETIC OVARIAN FOLLICLE POPULATION IN RELATION TO ENDOCRINE PARAMETERS IN NONHUMAN PRIMATE MODEL OF POLYCYSTIC OVARY SYNDROME

**Justine Hill, David Abbott (Mentor)**

This study hypothesized that knockdown of estrogen receptor alpha (ERa) in the mediobasal hypothalamus (MBH) of adult female rhesus macaque monkeys will induce a polycystic ovary syndrome (PCOS) like phenotype, including an abnormal abundance of growing ovarian follicles. The knockdown and control were achieved by infusion of viral vector shRNA against ERa and scrambled shRNA,

respectively. We quantified and classified ovarian follicle population density of knockdown (n=6) control (n=5) and normal (n=7) animals. To shed light on the neuroendocrine mechanisms at play in ovarian pathophysiology, we analyzed relationships between ovarian follicle populations and endocrine parameters.

OVARIAN FOLLICLE POPULATION IN A NON-HUMAN PRIMATE MODEL OF POLYCYSTIC OVARY SYNDROME IN RELATION TO SOMATIC PARAMETERS

**Danielle Bellino, David Abbott (Mentor)**

In this study, we hypothesized that diminished expression of estrogen receptor alpha (ERalpha) in the mediobasal hypothalamus (MBH) in adult female rhesus macaques would produce an ovarian phenotype similar to that in polycystic ovary syndrome (PCOS). We characterized ovarian morphology with respect to follicular development and atresia in order to provide insight into ovarian changes after ERalpha knockdown. We aim to determine how the ovarian follicle population was impacted by ERalpha knockdown (n=6) by comparing the proportion and density of healthy and atretic follicles with a control group (n=5) and a normal group (n=7) that under-went no neural manipulation. To shed further light on the ovarian changes, the ovarian follicle populations were analyzed with respect to somatic parameters such as weight and body mass index.

EVALUATION OF ERÎ± KNOCKDOWN IN HYPOTHALAMUS INVESTIGATES IMPACT ON METABOLIC HOMEOSTASIS IN FEMALE NONHUMAN PRIMATES

**Andi Pieczynski, Lauren Allegretti, Lillian Marrah, David Abbott (Mentor)**

This study aimed to evaluate the arcuate nucleus (ARC) and ventromedial nucleus (VMN) of female adult rhesus macaques and the presence of estrogen receptor alpha (ERÎ±) gene knockdown. Viral vector administration was monitored via MRI for ERÎ± nuclei targeting. Control monkeys (n=4) were injected with scrambled RNA (shRNA), with no known gene targets, while the experimental monkeys (n=4) received shRNA encapsulated in adeno-associated virus 8 (AAV8). Retrospective expression quantification was approximately 11 months after silencing. This is one of the first studies examining ERÎ± in female primates and our findings suggest it is important for metabolic homeostasis. ERÎ±-specific neuroregulation in women is a promising therapeutic target for infertility disorders.

OVARIAN AND EXTRA-OVARIAN ESTRADIOL REGULATION OF CALORIC INTAKE AND LOCOMOTION IN ADULT FEMALE RHESUS MONKEYS

**Samantha Williams, Molly Willging (Mentor)**

Declining serum estradiol (E2) levels during the menopausal transition are associated with heightened risk for metabolic disease. Ovarian estradiol, E2, supports female metabolic function. While ovariectomy (OVX) in rodents enables obesity, OVX in nonhuman primates (NHPs) inconsistently alters weight gain. We therefore hypothesized that in female NHPs, extra-ovarian E2 provides key support for metabolic homeostasis, such as caloric intake and locomotion. To test this, we employed aromatase inhibition to eliminate extra-ovarian E2 biosynthesis. Twenty adult female rhesus monkeys were OVX and received: (1) E2-containing capsules and letrozole treatment (n=6); empty capsules and either (2) vehicle (n=6), or (3) letrozole (n=7) treatment. Six months into the study, no differences in caloric intake or locomotion were observed, suggesting estradiol-mediated energy imbalance may manifest after more prolonged estradiol depletion.

EXTRA- OVARIAN ESTRADIOL REGULATION OF BROWN FAT THERMOGENESIS IN ADULT FEMALE RHESUS MONKEYS

**Sindhu Shankar, Molly Willging (Mentor)**

During menopause, decreasing levels of estradiol are associated with elevated risk for metabolic dysfunction, like obesity and reduced energy expenditure. Rodent studies identify ovarian sources of estradiol as the major regulator of female metabolism. In nonhuman primates (NHPs) ovarian estradiol depletion via ovariectomy (OVX) does not induce metabolic dysfunction. We therefore hypothesized extra-ovarian E2 regulates metabolic homeostasis, specifically, brown fat thermogenesis in female NHPs. To test this, we employed aromatase inhibition to eliminate extra-ovarian E2 biosynthesis. Twenty adult female rhesus monkeys were OVX and received: E2-containing capsules and letrozole treatment (n=6) or empty capsules and either vehicle (n=6), or letrozole (n=7.) Our findings highlight the location of estradiol synthesis regulating brown fat thermogenesis and suggest novel therapeutic approaches to combat menopause-associated metabolic disease.

CHARACTERIZING TRAITS IN FEMALE RHESUS MACAQUES WITH NATURALLY OCCURRING POLYCYSTIC OVARY SYNDROME

**Yuhan Sun, Elizabeth Laning, David Abbott (Mentor)**

Polycystic ovary syndrome (PCOS) is a gynecological hormonal condition present in female-bodied individuals characterized by two of three Rotterdam criteria: high testosterone levels, polycystic ovaries, and absent or irregular menstrual cycles. Furthermore, PCOS is correlated with increased body fat and abdominal circumference, insulin resistance, infertility, among other virilized traits. Though occurring at an increased rate among families, mechanisms causing PCOS are still unknown. Based on genetic and physiological homology, non-human primate models likely provide the most similar insights of PCOS etiology and symptoms for people with PCOS. We are attempting to better understand the heritability and pathogenesis of PCOS by monitoring the somatometric measures, ovarian morphologies, and LCMS (liquid chromatography- mass spectrometry) hormone levels from pedigrees of naturally occurring PCOS-like female rhesus macaques.

CHARACTERIZING PCOS-LIKE TRAITS IN PREGNANT FEMALE RHESUS MACAQUE MONKEYS

**Cheyenne Michelsen, David H. Abbott (Mentor)**

Polycystic ovary syndrome (PCOS) is a hormonal condition that occurs most commonly in women of reproductive age. To be considered a true PCOS candidate a woman must have two of the three common symptoms, otherwise referred to as the Rotterdam criteria. These symptoms consist of oligo/anovulation, hyperandrogenism, and polycystic ovaries. PCOS can naturally occur in female adult rhesus macaque monkeys and can impact phenotypic traits. It is most common to see an increase in body mass index, clitoral volume, and other increased body measurements. Using nonhuman primate models such as pregnant female rhesus macaques provides us with the best insight into PCOS. We are currently analyzing fetal programming and its correlation to gestational weight gain over the course of a pregnancy.

IDENTIFICATION OF SARS-COV-2 RECEPTOR IN THE TESITS OF THE RHESUS MACAQUE (MACACA MULATTA) AND ITS IMPLICATIONS ON REPRODUCTIVE HEALTH AND FERTILITY

**Sierra Block, Thaddeus Golos (Mentor)**

Severe respiratory complications from severe acute Coronavirus 2 (SARS-CoV-2) have prompted an array of research to understand its pathophysiology in the COVID-19 pandemic. Angiotensin-converting enzyme 2 (ACE2) has been identified as the receptor for SARS-CoV-2 with widespread expression across organ systems, including unexpectedly high expression in the testis. SARS-CoV-2 has also been identified in semen samples of men recovering from COVID-19. This study investigated localization of ACE2 expression in the testis and the presence of ACE2 in rhesus macaque semen. Immunohistochemistry and western blot methods performed on testis tissues and semen confirmed the expression of ACE2 in the

testis and sperm cells, and absence in seminal fluid. The presence of ACE2 in the male reproductive tract has implications regarding reproductive health, fertility, and sexual transmission.

CHARACTERIZING TRAITS IN JUVENILE FEMALE RHESUS MACAQUES FROM PEDIGREES WITH NATURALLY OCCURRING POLYCYSTIC OVARY SYNDROME

**Ava Grotting, David Abbott (Mentor)**

Polycystic ovary syndrome (PCOS) is a gynecological condition presenting in reproductive-aged women characterized by two of three characteristics: high testosterone levels, polycystic ovaries, and absent or irregular menses. Additionally, PCOS is associated with increased body fat and virilized genital measures, among other traits. Though present at an increased rate among families, the mechanisms causing PCOS are unknown. Increased testosterone exposure in utero has been thought to program PCOS-like traits in offspring. Based on genetic and physiological homology, nonhuman primate models provide insight into PCOS origins and its accompanying traits. Using somatometric measures and testosterone levels determined by liquid chromatography-mass spectrometry, we will phenotype juvenile females from pedigrees of naturally occurring PCOS-like adult female rhesus macaques to understand the heritability and pathogenesis of PCOS.

EFFECTS OF ESTRADIOL DEPRIVATION ON PANCREATIC ISLET MORPHOLOGY OF OVARIECTOMIZED FEMALE MARMOSETS

**Mihika Sathe, Ashley McQuiston-Keil, Alison Gregorian, David Abbott (Mentor)**

Estradiol (E2) is a female sex hormone that regulates the reproductive cycle. E2 depletion is a common side effect of taking letrozole, a drug that prevents the conversion of androgens to estrogen and is used to treat breast cancer. Previous studies in rodent models suggest that E2 deprivation leads to the accumulation of extracellular plaque in the pancreatic islets due to oxidative stress and apoptosis. This study aims to determine whether the same effects occur in non-human primates. We hypothesize that ovariectomized female marmosets undergoing total estradiol deprivation will have increased extracellular plaque in their pancreatic islets as compared to controls.

EFFECTS OF KISSPEPTIN-10 (KP10) ON THE TIMING OF PUBERTAL INITIATION

**Veronica Goveas, Ei Terasawa Grilley (Mentor)**

Gonadotropin-releasing hormone (GnRH) neurons regulate gonadotropin release, which is critical for pubertal initiation. While the hypothalamic kisspeptin neuron is an upstream regulator of GnRH release, whether kisspeptin determines pubertal initiation is unknown. Two prepubertal male rhesus macaques at 16 months (mo) of age continuously received hourly pulsatile infusion of either KP10 or saline and the signs of puberty were assessed. The preliminary results at 21 mo indicate that the KP10-treated male exhibited accelerated increase in testicular volume and body weight as compared to that in the saline-treated male. Because an increase in the testicular volume does not characteristically start until ~30 mo, KP10 infusion appears to accelerate the timing of puberty. Whether KP10 accelerates the pubertal changes in gonadotropins and testosterone remains to be assessed.

ROLE OF NEUROESTRADIOL IN THE BRAIN: SEX DIFFERENCE

**Stephanie Li, Ei Terasawa (Mentor)**

Gonadotropin releasing hormone neurons in the hypothalamus controls reproductive function in both males and females. Despite the clear sex difference in reproductive function, the hypothalamus in primates is not sexually differentiated. Because neuroestradiol, synthesized in the brain, also plays a role in regulation of GnRH release in female macaques, this study examines whether neuroestradiol also plays a similar role in male macaques. To test the neuroestradiol's role, castrated males will be treated with high dose of estradiol benzoate along with the aromatase inhibitor letrozole, which blocks the

synthesis of estradiol from testosterone, while periodical blood samples for hormone analysis are obtained. Controls will receive estradiol benzoate and vehicle. Findings from this study will clarify the sex difference in the role of neuroestrodiol in the hypothalamus.

GESTATIONAL ORIGIN FOR METABOLIC DYSFUNCTION IN PCOS-LIKE FEMALE RHESUS MACAQUES
**Caitlyn McQuiston-Keil, David Abbott (Mentor), with Abbott and Levine lab researchers**
Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder endured by many women from adolescence to menopause. Gestational exposure to testosterone (T) excess generates a PCOS-like phenotype in female rhesus macaques that meets all three of the Rotterdam criterion. Researchers in the Abbott/Levine lab have found that adult female PCOS-like rhesus macaques develop pancreatic islet abnormalities, insulin resistance and experience a progression to type 2 diabetes (T2D). These conditions result from increased oxidative stress in the macaque's beta cells causing apoptosis and, therefore, an accumulation of extracellular fibrin and plaque in the macaques islets. We hypothesize that gestational exposure to testosterone excess negatively impacts female PCOS-like rhesus macaques abilities to create and sustain healthy islet cells, and therefore maintain homeostasis.

SEARCHING FOR CHEMICAL SIGNALS IN ORANG-UTAN URINE USING LIQUID
**Anusha Ray Dey, Graham Banes (Mentor)**
Orang-utans are critically endangered great apes, where morphologically female and socially subordinate male orangutans cannot be easily distinguished. Socially dominant males develop irreversible cheek pads. In this study, a total of 116 samples were analyzed to quantify the chemical compounds excreted. This was done to identify what compounds might differ in presence or quantity between the different classes of orang-utan. Using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique, it is expected to better understand how male bimaturism might manifest in orangutans, and if chemical signals might be involved in their development, maintenance and/or suppression. All samples are going through LC-MS/MS, and results will be analyzed with appropriate statistical methods and will be presented in the spring 2022 at the Undergraduate Symposium.

GENE KNOCKDOWN OF HYPOTHALAMIC ESTROGEN-RECEPTOR ALPHA IN RELATION TO NEUROPEPTIDE S RECEPTOR 1 EXPRESSION IN ADULT FEMALE RHESUS MACAQUES
**Jaclyn Fahey, David Abbott (Mentor)**
Endometriosis is an estrogen-dependent inflammatory condition of the uterus causing pelvic pain and infertility. Its cause is unknown and treatment options are limited. Whole exome sequencing of women and female rhesus monkeys, however, recently associated gene variants in neuropeptide S receptor type 1 (NPSR1) with naturally occurring endometriosis. An unrelated study focusing on estrogen regulation of female weight gain and employing estrogen receptor alpha (ERa) knockdown (KD) in the hypothalamus of adult female rhesus monkeys to block estrogen action in a brain center regulating metabolism, has induced a high incidence of endometriosis compared to controls. To test whether altered hypothalamic NPSR1 expression is associated with ERaKD-induced endometriosis, immunohistochemistry experiments are underway. This investigation will increase our understanding of the role of NPSR1 in endometriosis.

GESTATIONAL TESTOSTERONE ALTERS PANCREATIC ISLET MORPHOLOGY AND PROGRESSION TOWARDS DIABETES IN ADULT MALE RHESUS MONKEYS
**Nicole Byington, David Abbott (Mentor)**
Although polycystic ovary syndrome (PCOS) is a women's reproductive disorder, it is commonly accompanied by metabolism dysfunction of unknown origin. Adult female rhesus macaques exposed to

gestational testosterone (T), a known model for PCOS, manifest PCOS-like metabolic disorders, including insulin resistance and type 2 diabetes (T2D). Male macaques exposed to the same gestational T, exhibit insulin resistance and poor pancreatic beta cell response to glucose in adulthood. The PCOS-like male monkeys exhibit islet morphology indicative of increased oxidative stress and apoptosis: fibrin with plaque accumulation. Reduced functional islet area may accelerate progression to T2D. Gestational T exposure may provide a common origin for diabetic dysfunction in women with PCOS and their close male relatives.

OVARIAN FOLLICLE QUANTIFICATION IN A NON-HUMAN PRIMATE MODEL OF PCOS Jacob Blanchar, **Savannah Knaak, David Abbott (Mentor)**

This study hypothesized that the knockdown of the estrogen receptor alpha (ERalpha) in the mediobasal hypothalamus (MBH) in adult female rhesus macaques would produce an ovarian phenotype similar to that found in polycystic ovary syndrome (PCOS). This phenotype is characterized by an abnormal abundance of small antral follicles. To provide insight into ovarian changes after ERalpha knockdown (n=6), the proportions of healthy and atretic follicles were calculated to compare with controls (n=5) and normals (n=7). We aimed to determine how ovarian morphology was impacted, due to the ERalpha knockdown, through proportional analysis.