EFFECTS OF GONADAL HORMONES ON SENSORY AND COGNITIVE TASKS

A dissertation submitted to
Marshall University
in partial fulfillment of
the requirements for the degree of
Doctor of Psychology
by
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Approved by
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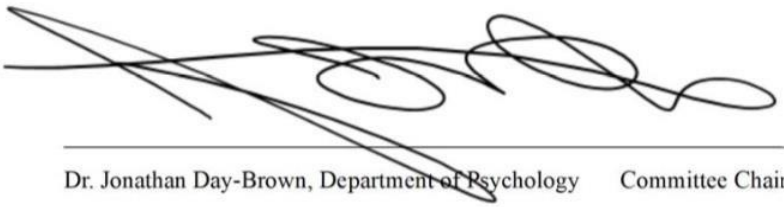
Marshall University
August 2024

Approval of Thesis/Dissertation

We, the faculty supervising the work of John Prentice, affirm that the dissertation, *Effects of Gonadal Hormones on Sensory and Cognitive Tasks,* meets the high academic standards for original scholarship and creative work established by the Department of Psychology and the College of Liberal Arts. The work also conforms to the requirements and formatting guidelines of Marshall University. With our signatures, we approve the manuscript for publication.



6-28-2024

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Dedication

To my family, friends, and mentors; you have my heart, for always. This work is also dedicated to the teacher and professor, who upon learning of my disabilities informed me and my family that “he wouldn’t amount to much.” Thank you for the motivation to prove you wrong.

Acknowledgments

This work would neither have form nor substance without the dedication, mentorship, and collaboration of the following

- Dr. Jonathan Day-Brown
- Dr. Thomas Linz
- Dr. Dawn Goel
- Mr. Eli Asbury
- Ms. Emily Shively
- Ms. Darby McClou

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Abstract

Participants were recruited from the local community, specifically through attendance at professional conferences and LGBTQ+ events in the surrounding area. This sample received a brief battery of cognitive and sensory tasks. Participants also provided a biological sample in the form of saliva collected by the free drool method and completed a short demographic survey regarding their gender identity, medication, and health history. One aim of the presented study and the data collected, is to shed light on the much-neglected area of transgender neurological research.

Neuroendocrinology

When examining the interplay of neuroendocrinology, cognition, and the diversity of behavior across the species and sexual dimorphism, one looks over the body of literature and finds it wanting. In this void an emergent pattern quickly appears. That is, the majority of our understanding of hormones, which interact with physical structures of our brain and, by extension cognition and behavior, is based almost exclusively on homogeneous samples, i.e., straight, white, right-handed, and Cisgender males. Thus, the ignorance of functional differences in minorities is a natural outflow from these biased samples. A gap in understanding has grown and excluded many minorities from appropriate scientific investment. Perhaps most affected by this deficit are those of gender minorities. We endeavor to reduce the aforementioned blind spots by examining the relationship between sex hormone levels collected via free drool and tasks that have been shown to demonstrate sexually dimorphic performance between Cisgender groups. Where the present research differs from its contemporaries is the lens of gender minority, which we use to both collect and interpret our data.

Little work has been done to understand the experience of transgender individuals, especially from a neuropsychological perspective. Existing neuroscience research with sexual minorities has primarily focused on the experiences of white, cisgender, gay men. When these studies have included detailed examination of structural differences between heterosexual and homosexual individuals, anatomical differences in the third interstitial nucleus of the anterior hypothalamus, the dimorphic region of the pre-optic area underneath the frontal lobe, the central subdivision bed nucleus stria terminalis, and the vasoactive intestinal polypeptide containing region of the superchiasmatic nucleus have been discovered (Swaab, 2004).

The current understanding of the Transgender experience reaches little farther than the phenomenological acceptance that the Trans individual exists. It is known that the Transgender community faces some of the largest deficits in the social determinations of health. Recent investigations into these disparities found that Transgender women die at about twice the rate of Cisgender women (*The Lancet Neurology*, 2021). It should be noted that this study exclusively looked at Transgender women as they compare in health outcomes to Cisgender women. However, these staggering statistics are compounded when you learn that the reported average life expectancy, according to the Inter-American Commission on Human Rights, is between 30 and 32 years of age for Trans women of color (IACHR, 2017). The Williamson Institute of Law at UCLA recently found that Trans individuals are four times more likely to be victimized, and that one in four trans individuals experience a violent hate crime (UCLA, 2020). Though Trans individuals only account for 0.39% of the population (Meerwijk & Sevelius, 17), they account for between 5-11% of all hate crimes. Even though there are higher rates of incidents in other groups, such as race-based and religion-based crimes, this statistic is significant due to the higher proportionality within the Trans community. In short, Trans people have a higher chance of being a victim of a hate crime (*FBI*, 2020). However, even these figures likely fail to grasp the totality of the situation because many individuals face intersectional discrimination, which may not be reflected in crime statistics.

Additionally, many Trans individuals do not receive access to the same level of health care afforded to their Cisgender peers and they face extreme societal pressure leading to a heightened risk of suicidality (UCLA, 2020). This pressure makes up a portion of what is known as the social determinants of health, overt and covert systemic and environmental factors that affect a person's ability to live a healthy life. According to the American Association of Pediatrics 50.8% of Trans individuals report a prior suicidal ideation, while the average rate for

Cisgender teens is 14% (2018). Recognizing the trans individual as worthy of equal scientific consideration and medical support as their Cisgender counterparts and adapting our models to include more than just the aggregate human is rapidly becoming a matter of public health and safety.

Our understanding of neurological processes for those in gender minorities is limited since most of the existing research focuses on Cisgender ontogeny. The purpose of this study is to expand the scope of research to individuals of Transgender and gender non-conforming identities in two critical areas, olfaction acuity and mental rotation tasks. These tasks were chosen for their historical ability to demonstrate sexual cognitive differences between Cisgender individuals. Levels of free gonadal hormones were also collected and used as a predictor for task performance. This aids in assessing the role of neuroendocrine factors and variation, as they relate to our cognitive battery. Given that many Transgender and gender non-conforming individuals use exogenous substances to better align their internal hormonal environment with their gender identity, , a practice known as gender-affirming hormone replacement therapy (GAHRT) and commonly abbreviated as hormone replacement therapy (HRT), data on duration and modality of GAHRT has been collected to help explain cognitive task performance variation, as well as aid in comparisons with hormone level variations amongst Cisgender individuals.

Hypotheses

The present research seeks to investigate the link between gonadal hormones and sensory tasks, expanding it to the transgender population. This exploration will provide pivotal incite into the often-unexplored aspects of psycho-neuroendocrinology. this study predicts (H.1) Self-identified gender performance will be in the same range on cognitive and sensory tasks as those assigned to the similar sex category at birth (i.e., Transgender men and Cisgender men will display similar performance on tasks). For the purposes of analysis (H.1) has been separated into

its main effects and interaction.(H.1.1) Self-identified gender will be predictive of the dimorphic affect on cognitive task performance. (H.1.2) Natal sex assignment will likewise be a secondary predictor of the dimorphic affect cognitive task performance. (H.1.3) Self-identified gender performance will be in the same range on cognitive and sensory tasks as those assigned to the similar sex category at birth. (H.2) Increased levels of free androgen hormones will be related to increased rotation task acuity. (H.3) Increased levels of estrogen and progesterone will be related to increased olfaction acuity.

Olfaction

One may ask, "why use an olfaction acuity measure to assess gender differences?" This perspective was selected chose this perceptive sense because of a small, but significant and persistent variation in the level of acuity noted between Cisgender males and females for very specific odorants. neurological analysis reveals that this may be due to increased density of cells in the olfactory centers of Cisgender female brains, with the average increase in density of 38.7% when normative variations in mass are accounted for. (Oliveira-Pinto et al., 2014)

The etiology of this variation is unknown; however, several evolutionary and psycho-evolutionary theories attempt explain this difference. First, mate selection, while olfaction and chemoreceptors play some role in mate selection across nearly the entire vertebrate taxa, we still have a long way to go in understanding the actual mechanics and selection processes that make one stimulus more desired than another, especially in complex vertebrates like humans. It is suggested, in large rodent field experiments females, with high rates of olfaction acuity are generally selected for, and use this heightened sense to detect signs of genetic diversity and health markers in mate selection (Pablo et al., 2018). However, this has not been replicated in humans. While there may not be a clear reproductive advantage in modern humans, this

difference may have assisted our evolutionary progenitors. Leaving us with this difference in performance as a sort of genetic fossil of who we once were.

Other theories suggest the evolutionary advantages of olfaction as a supplementary source of conditioning stimuli. The proximity of the olfactory bulb to the amygdala may account for the strong association between emotion and olfaction. This is suggested to be especially beneficial for avoiding dangers (sensory-cortex-based threat perception). Thus, acrid smell Would require very few trials of exposure before learned avoidance. (Wen, 2014) in essence, Olfaction operates as tabs in the binders of our memories and highlights important learned Stimuli, especially those that may be dangerous. This is what is referred to as a Mnemonic Theory of Odor Perception (Stevenson & Boakes, 2003). Additionally, the Mnemonic advantage may assist with nutrition and resource mapping, which would be advantageous due to the increased caloric intake necessary for child rearing. Female mammals having a greater obligatory investment in the rearing of offspring than male mammals, would be sufficient to drive the development of this olfactory processing difference across mammalian sexes.

It was also demonstrated that many other factors could affect olfaction acuity, such as age, environmental factors like the use of combustion tobacco, and even genetic predisposition. However, the strongest consistent noted variation in acuity exists among age difference and gender. (Hempstead et al., 2012) It should also be noted that given the damaging effect that Covid-19 has on the sustentacular cell in the epithelial tissue near the chemoreceptors required for olfaction, there has been a large population of individuals with acquired anosmia (TenOver, 2022). Many individuals face long term loss of olfaction even after the infection has resolved. It is unclear how this will affect odorant-based tasks in this and future work.

Specific odorant sensitivity

Increased acuity is not a general advantage documented in Cisgender females; their increased rate of correct corresponding only applies to specific odorants (Oleszkiewicz, 2018). These will be the primary focus of the odorant identification task. For the presented study, we have chosen to use the standardized "Sniffin' sticks" task, which has shown to be a valid measure of olfaction from ages 5-71 across sex, race, and cultural background, and has been used successfully to demonstrate sexual dimorphic olfaction. Battery is divided into three sub-tasks threshold, discrimination, and identification. We plan to use the identification exclusively, as the other facets of this battery are beyond the scope of this current investigation. It was selected as it contains an increased number of odorants sensitive to the dimorphic effect (Oleszkiewicz, 2018).

It should be noted that there has been an initial investigation into the area on the effect of gender-affirming hormone therapy and olfaction conducted, and no significant effect was found (Kranz et al., 2020). However, this investigation focused on only the first 4 months of hormone therapy, and an appreciable difference may not have occurred yet. Many physiological changes related to gender affirming hormones take between six months in a year to begin. (WPATH (World Professional Association for Transgender Health), 17) Therefore, it is the purpose of this study to expand upon this research by providing a larger sample with greater variation in treatment durations and investigating other tasks.

Rotation Task

Rotation tasks are another classic battery used to demonstrate sexual dimorphism, specifically with two-dimensional object manipulation and rotation (Collins & Kimura, 1997). Cisgender male traditionally have a small but repeatable advantage little is known regarding the reason this increased rate of acuity exists (Koscik et al., 2009), but it is believed to be related to increased levels of testosterone we will be using a publicly available rotation task from Psytoolkit.com. Psytoolkit is an online repository of open access psychological surveys and

tools. The online rotation task is a variation on the classic Vandenberg & Kuse Mental Rotation Test (VMRT). The VMRT is a 24 instrument item instrument that requires the participant to select the image that is capable of being rotated into matching the target stimulus. The participant must discriminate between five potential matches, only selecting the one match. The VMRT was developed in 1978 to examine rotational capabilities as they developed. It was at this point the difference in performance based on sex was initially appreciated (Caissie et al.,2009).

Four regions of the brain primarily are correlated to rotational capacity, the inferior parietal region, the temporal parietal substrates, and its peripheral innervations of the primary and secondary at somatosensory cortex is involved. Dimorphic neuronal density has been appreciated in the following regions: The posterior parietal cortex (PPC), perio-occipital cortex, and inferior prefrontal cortex. While variation in gray matter density was relatively small it does appear to be consistent across ontogeny; and this may account for a small but repeatable variation in rotation performance. This has been observed on functional magnetic resonance imaging (FMRI) and positron emission tomography (PET) scans. The evolutionary function of this variation in rotational ability is largely unknown, there are some hypotheses that suggests that *Australopithecus* and other early hominid species engaged in polyandry, polygynous, intermittent monogamous relationships (Fidelis et al, 2016). Thus, it is suggested that increased spatial acuity may improve successfully mating via increased ability to avoid predators. However, this theory has several flaws, as both *Australopithecus* and other early hominids, while dioecious and dimorphic in taxonomy, engaged in both polyandry and polygyny so it is unlikely that one sex would face significantly deferent selection pressure for this reason. Others have proposed that this difference may be due to role pressure (Wynn et al., 1996). Likely those who are phenotypically feminine may need a more detailed visual map of their immediate surroundings as they may be reticent to move during child birthing and rearing. Others have

suggested phenotypically male have a more direct pressure given the scanning and rapid visual data processing needed for hunting excursions. However, as only 14-19% of early hominid's caloric intake came from hunting (Kious, 2002), it is unclear if this would be a more salient pressure then childbirth and rearing responsibility. It is more likely that all the factors listed above contribute to this dimorphism.

Hormones

The endocrine system exerts a subtle but powerful control over the human body and ontology. Hormones do this through their ability to bind to cell receptors and excite or inhibit cell behavior. This binding, in essence, transmits information across the body. Of course, this is a gross simplification of the complexity of the endocrine system. However, it is one that is necessary to give the scope of this study. Far More than amorous pubescent teenagers and aggressive muscular males, the quiet workings of hormones go unnoticed for many of us. In contrast, others experience a different reality, one where some of these silent messengers likely feel traitorous. Herein will be discussed the specific gonadal hormones of interest to the presented study estrogen, progesterone, and testosterone. Each of these will be examined in terms of function as well as their role in gender affirming hormone therapy.

Estrogen

The role of estrogen extends to nearly every bodily system regardless of phenotypical sex expression. Some of the more prominent functions include aiding in the development of the vaginal-ovarian system, regulations of menstruation, bone health, energy regulation through glucose metabolic control, and food intake regulation through its high density and action in the hypothalamic nuclei. Low estrogen levels in Cisgender adults have been linked to metabolic disorders and type 2 diabetes (Mauvais-Jarvis et al, 2013).

In gender-affirming hormone replacement therapy, estrogen is commonly administered orally in tablet form or through a Transdermal patch. This is possible due to estrogens' high level of bioavailability. According to the World Professional Association of Transgender Health's (WPATH) Standards of Care 7th edition, hormone treatment timeline, most Trans women begin to experience some of the effects of hormone therapy such as fat redistribution, skin texture changes, and breast tissues growth, with in the first three months, with maximal effects not being reached for 3-5 years. In addition, there is often rapid cessation of spontaneous erections, decreased sperm, and ejaculate, reduced testicular volume within the first months of HRT use. Feminizing Hormones have several associated risks that are known to WPATH including, Venous thromboembolic disease, Gallstone's disease, elevated liver enzymes, weight gain, and hypertriglyceridemia. Trans individuals who have not had an orchidectomy can also use an androgen absorption suppressant which prevents high levels of testosterone from being absorbed in the body.(WPATH, 2017)

Progesterone

As our understanding of Trans healthcare increases, so does our understanding of the importance of progesterone in Transgender HRT. Initial much focus was placed on estrogen, estradiol, and anti-androgens, and given its traditional conception being tied to fertility and menstruation, progesterone was largely ignored. However, we now know that including progesterone as part of hormone therapy improves outcomes and speeds up the acquisition of changes compared to estrogen and anti-androgens alone. When incorporated into the HRT course of treatment, progesterone aids in breast maturation, decreases levels of testosterone production, and mediates some risk related to cardiovascular health. (Prior, 2018)

Testosterone

Just as with estrogen prior, the roles of testosterone in the body are numerous. In Cisgender males, high testosterone levels are correlated with libido, sperm count, muscle mass, red blood cell count, and bone density. Several studies have liked testosterone aggressive behavior (Dabbs et al., 1995). Alliteratively, some theorists suggest that testosterone encourages makes the individual engage in the behavior more socially likely to result in successful mating. In many western cultures, this may look Aggressive; however, individuals with similar levels of testosterone in cultures where aggression has not been praised may engage in other forms of social conformity. (Sapolsky, 2017).

When using testosterone for hormone replacement therapy (HRT), individuals often use an intramuscular injection, transdermal foam, or patch. Much like estrogen, the expected onset of physical changes will likely occur within the first three months. These changes include acne, body/facial hair, vaginal atrophy, clitoral enlargement, body fat redistribution, and vocal changes. (WPATH, 2017) Most changes take 2-5 years for full effect. While increased levels of testosterone may satiate menstruation, many Trans masculine individuals may choose to have an oophorectomy and/or hysterectomy. The known risks for HRT on masculinizing hormones include polycythemia, hyperlipidemia, sleep apnea, acne, weight gain, and androgenic alopecia. Depending on lifestyle and genetic vulnerability, individuals may also experience an increased risk for type-2 diabetes, hypertension, cardiovascular disease, and possible destabilization of otherwise homeostatic psychiatric disorders, such as schizoaffective disorder and bipolar I disorder. Supraphysiological levels of testosterone have been linked to episodes of both mania and psychosis. Thus, frequent initial monitoring of blood serum is vital, especially if the patient has a prior history of psychiatric illness (World Professional Association for Transgender Health [WPATH], 2017).

Table 1: Timeline of the effects of gender affirming hormones.

Effect of Masculinizing Hormones	Expected onset	Effect of feminizing hormones and anti-androgens	Expected onset
Skin oiliness/acne	1-6 months	Softening of skin/decreased oiliness	3-6 months
Facial/body hair growth	3-6 months	Decreased libido	1-3 months
Scalp hair loss	>12 months	Decreased spontaneous erections	1-3 months
Increased muscle mass/strength	6-12 months	Erectile dysfunction	Variable
Body fat redistribution	3-6 months	Body fat redistribution	3-6 months
Cessation of menses	2-6 months	Breast growth	3-6 months
Clitoral enlargement	3-6 months	Decreased testicular volume	3-6 months
Vaginal atrophy	3-6 months	Decreased sperm production	Variable
Deepened voice	3–12 months	Thinning and slowed growth of body and facial hair	6–12 months

Adapted from the WPATH standards of care.

Environmental Effects on Gene Expression

A reasonable question is how gene expression can be modified in already developed organisms; the answer leads to an avant-garde branch of social and genetic research, epigenetics, and gene methylation. We once believed that genes were a road map for someone's life, and apart from accidents or divine intervention, would carry somebody from womb to tomb. We thought these road maps were indelibly inscribed on every cell without variation and were, crucially, resistant to change. We now know that this could not be further from the truth. Our genetic life contains much more flexibility and dynamic coaction with the environment than many of us could have ever believed. Methylation and epimers function as an on/off switch, allowing

different genetic avenues to be taken and expressed depending on the environmental pressure (Nora & Connor, 2021). An example of this seen in nature, and the subject of some initial epigenetic investigations, were ants. Genetically identical ants, when exposed to two different sets of hormones produced by the queen, will develop drastically differently. One sister may grow into an agile worker and another into a hulking warrior, all dependent on the environmental pressures placed on them in their larval state. (Simola et al., 2016) Given the relatively small size and rapid reproduction of many species in the family *Arthropoda*, they have made convenient windows to the mechanics of epigenetics. This has prompted the continued investigation into how the gene-environment interaction and epigenetics effect expression in larger vertebrates. A classic example of this in humans is the interaction that occurs between MAOA, FKBP5, and adverse childhood experiences (ACEs)

The chromatin binding for both MAOA and FKBP5 are susceptible to glucocorticoid hormones such as cortisol. Once activated, these genes can increase neuronal density in the amygdala and positively correlate with a psychiatric condition such as major depressive disorder (MDD), post-traumatic stress disorder (PTSD), and Generalized anxiety disorder (GAD). If the above conditions are met, and there is a mutation in the stop codon for the MAOA gene, this often leads to increased rates of aggression (Shui et al., 2019).

One may reasonably say "what does this have to do with gender?" as illustrated above; endocrine factors can impact both structural and behavioral changes across the life span. This is a fundamental question of Gender diversity. We now know that the process of sex determination is a complicated interplay of multiple genes, endocrine factors, and environmental pressures. as our understanding of this complexity grows, the idea of both binary sexes and genders seems to dwindle. The phenomenology of the Trans experience is irrefutable. These individuals do exist; what is up for question, is the structural mechanics of this identity and being.

Method

A sample was gathered by attending professional conferences and "Pride" events in the local and surrounding communities. Given the small subsection of the population that the transgender community represents, attending targeted events such as "Pride" increased exposure of this study to the population of interest and aided recruitment efforts. General advertisement, as well as targeted events, allowed participants to self-select from the study. A power analysis was performed with the perimeters of $\beta = 0.2$, and the historical and normative data from the dimorphic tasks were selected as analogs (Oleszkiewicz, 2018), (Collins & Kimura, 1997). These results were examined, and the higher sample demanded was selected. It was determined that a sample of approximately n=160, with roughly proportional sub-groups of Cisgender and Transgender n=80, is necessary for the needed levels of power.

Procedure

After self-selection, de-identified biographical data was collected prior to hormone level testing and cognitive task battery. This included, natal sex, gender identity, sexual orientation, and current status of hormone-altering medication and dosage. Gonadal hormone levels were assessed via the collection of a free drool sample. All samples are stored in a locked deep freeze at – 20 and incinerated upon the conclusion of the experiment and analysis. Samples were then processed in accordance with the protocol located in appendix B. Processed plates were then placed within a microplate reader (Multiskan SkyHigh, Thermo Scientific) capable of exposing and measuring each sample at 450 nanometer wavelenth light. Levels of opacity and absorption were measured, and a four-point linear regression was conducted to convert optical densityto hormone concentration levels. The full Salimetric enzyme immunoassay lab protocols are in Appendix B.

Materials

At the time of sample collection each participant completed two cognitive tasks. For the twelve item Sniff'n sticks odorant identification task, participants were exposed to an olfactory stimulus for 3-5 seconds and then asked to make a selection on what scent that they were exposed to. Participants then completed the psytoolkit mental rotation task.

Given the vulnerable population we will examine, significant data safety measures were taken:

1. All data collected is de-identified but correlated to salivary samples through a packet/sample numbering system.
2. All samples are stored in a locked lab and deep freeze when not being analyzed.
3. All biological samples are incinerated after analysis.

It was determined that these additional measures were necessary given the intrinsically identifiable nature of salivary samples and the potential harm of accidental disclosure of a participant's gender identity and medical history.

Results

An initial sample was gathered of n = 117; after accounting for error, insufficient biological sample, and attrition, the final sample assessed was n= 97. This consisted of 24 Cisgender males, 41 cisgender females, 13 transgender males, 9 transgender women, and 21 non-binary individuals, with a mean age of 27 years old (Figure 1.). Given the un-equivalent demographic grouping, the presented study forgoes inferential testing and relies on descriptive statisctics.Future studies with additional participants will increase statistical power and allow for other analyses. An exception to this is that we were able to analyze the relationship between sexual orientation and gender identity using a Chi-Square test. This yielded a statistically significant relationship of p= 0.000274

and an Effect Size (Cramér's V) of 0.335. Similarly, a significant relationship was determined between age and gender identity with a P-Value = 0.0414 and Effect Size (Cramér's V) of 0.621.

Regarding olfactory data, an average composite score of 8.8 was established. This is lower than what would be expected for the average age of our sample. Placing our average participant within the hyposmic range of olfactory ability. Analysis of visual-spatial rotation tasks is retained until additional statistical power is garnered. We also identified certain trends within gender-affirming hormone therapy, specifically the majority of individuals that are using gender-affirming hormone therapy had beren doing so for more than three years.

Discussion

Throughout the course of this endeavor, we set out to explore an area of psycho-neuroendocrinology that has been largely unexamined. This study provides an exploratory examination into the lives of rural queer individuals. Most importantly, it demonstrated the feasibility of this style of research with this population. Though there were several significant challenges, such as delays in gathering the necessary size of sample or the difficulties of field sample collection in a rural context, the presented studies efforts up to this point have demonstrated its feasibility. Despite these challenges, we have gained critical insight into many aspects of rural transgender life that were previously unknown. These descriptives include age, education level, health condition, levels of endogenous and exogenous hormones, duration of gender-affirming, and hormone treatment.

Some of the unique challenges for this study were anticipated, for example, the challenge of statistical power. We knew from the outset that gathering a significant sample would be one of our most significant challenges. As indicated by our power analysis, we fell short of our goal of an n=160 sample, and most significantly, we were unable to reach our goal of n = 80 transgender participants. This also led to significant difficulty in gathering equivalent groups. Given the

inherent difficulty of gathering gender-diverse samples and the lab's geographical and rural nature, it was deemed a large success to gather the number of participants we gathered during our first round of collection. One of the unforeseen aspects of our sample collection process was the greater-than-expected proportion of non-binary participants. This was an exciting opportunity from the lab as even less is known about non-binary endocrine factors than binary-trans individuals. Future studies will account for a greater proportion of individuals that endorse the non-binary gender identity option by adjusting our needed sample size and comparison groups.

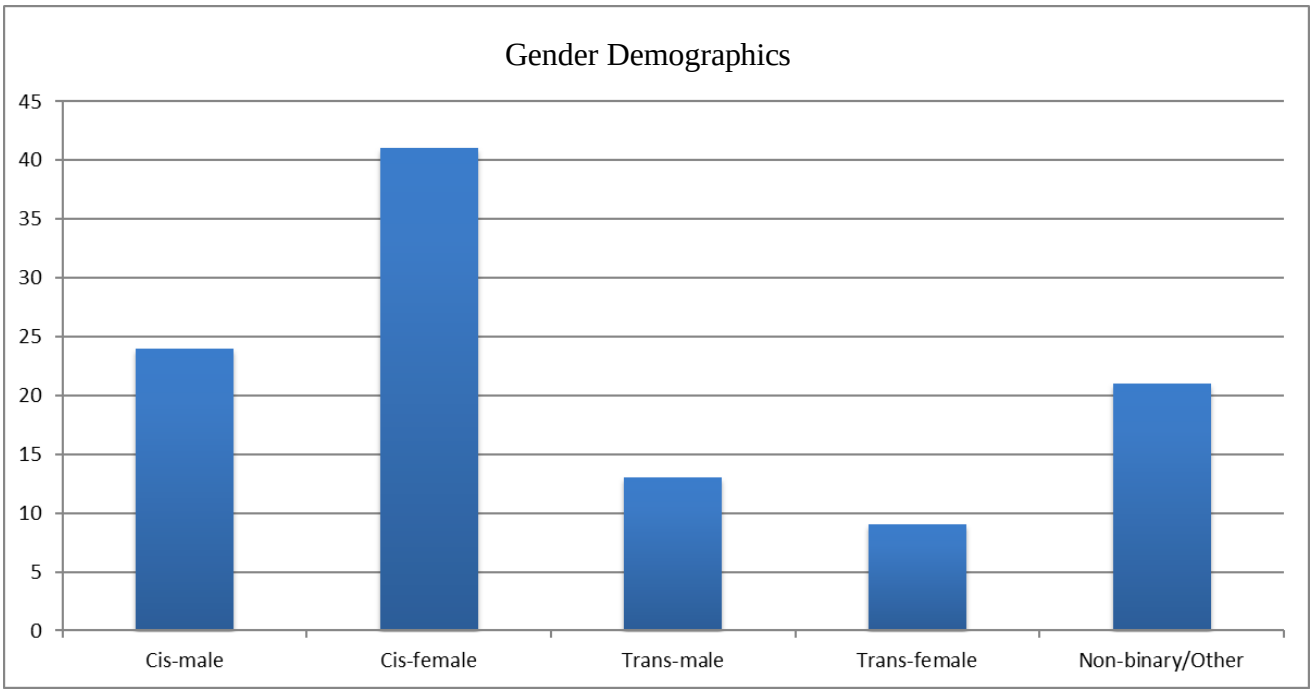
The disproportionality of the sample leads to an inability to rely on inferential statistics, thus our reliance on descriptives. Additionally, due to logistical constraints, we were unable to analyze estradiol samples tat the time of this writing. Thus, we were unable to include this crucial aspect in several of our hypotheses.

Apart from these challenges, The Marshall Hormone Olfaction and Rotation Lab made significant strides. Within our first year of active collection, the lab established significant relationships with several queer organizations within the surrounding five counties and, more importantly, established a new level of trust between the LGBTQ+ community and the Marshall research community. Moving forward, the lab's goal is to continue our collection of participants and samples to increase our statistical power and establish equivalent groups. Additionally, we are expanding the scope of gathered information to include additional elements previously demonstrated to exhibit dimorphic scoring, such as examining the speed-accuracy tradeoff and visual perception. Additionally, we plan to add measures assessing mental health factors that may be uniquely salient for the queer community. At present, the lab plans to attend several pride events this June, as well as continue to collect from the local population.

Future analyses may include age, recent COVID diagnosis, and acquired anosmia due to smoking as potential confounding variables affecting olfactory acuity measures, such as

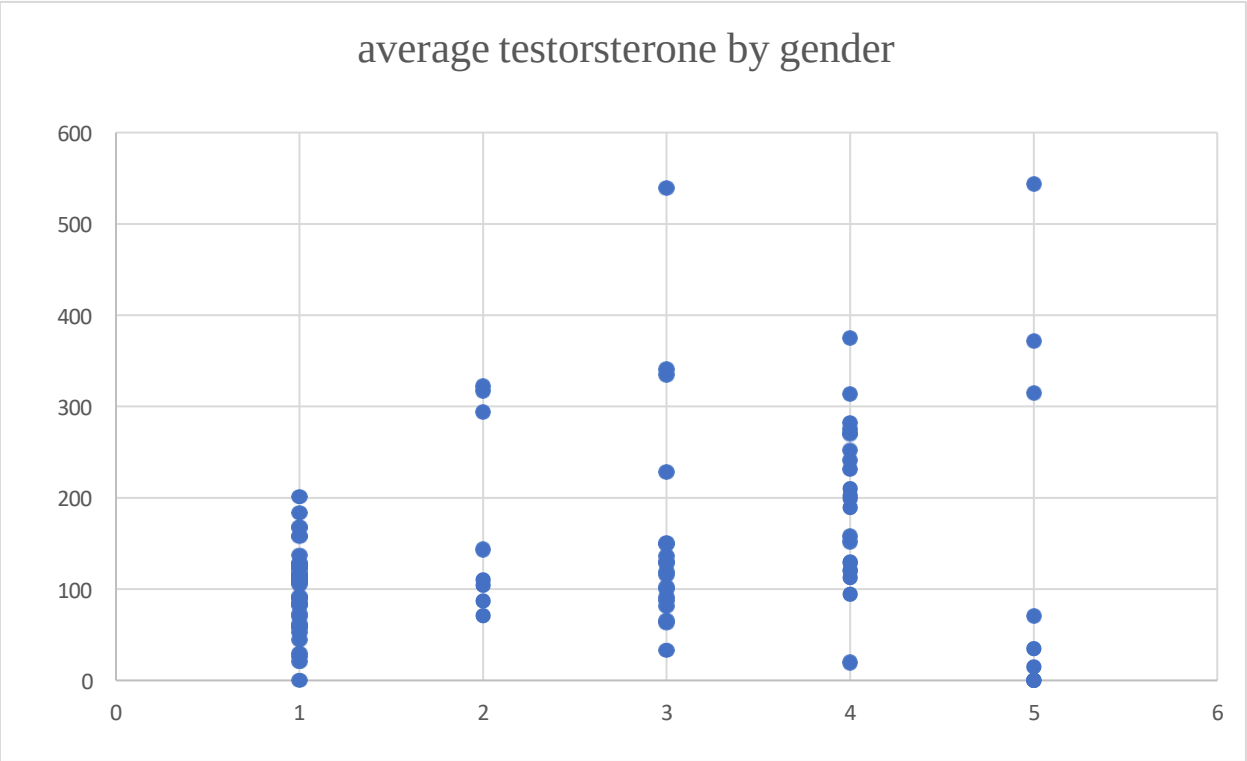
difficulty with open-air testing environments and competing stimuli. Additionally, future studies may want to control for the things that limit the ability of participants to concentrate adequately on tasks such as mental rotation. Given the inherent difficulty of field collection, the competing stimuli at pride events may have impacted participants ability to concentrate on tasks. Finally, this study plans to continue collection. Once a sample of sufficient size is gathered, the presenting study proposes to implement the following analytical techniques. Given the nature of studying diverse populations, difficulty with sampling is nearly ubiquitous. This has posed a unique challenge for prospective inferential analysis. Thus, we will be operating under a set of assumptions as we collect data; these assumptions may be proven false, and alterations to the analysis may be required. Thus, a tiered approach will be taken. To this end, the assumptions we will be operating under are as follows: 1. A large enough sample of Trans and Cis individuals are gathered, and 2. Both groups are approximately equivalent 3. Hormone levels and task performance have a parametric relationship. If each of these assumptions proves true, then we will move forward with analysis under the General Linear Model (GLM) and the Hierarchical Linear Model (HLM). Given this, Hypothesis H.1: Self-identified gender performance will be in the same range on cognitive and sensory tasks as those assigned to the similar sex category at birth have been separated into its main effects and interaction.

Figure 1



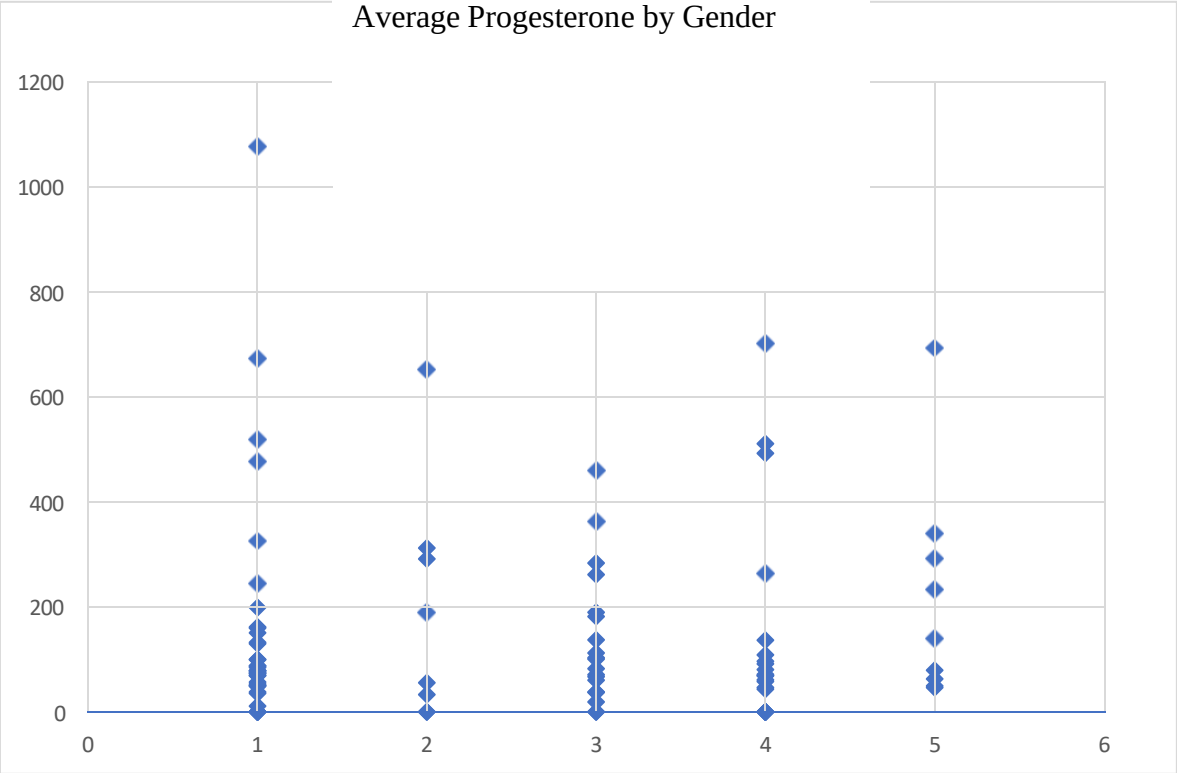
Note. Distribution of gender identity in sample.

Figure 2



Note. Distribution of salivary testosterone concentrations clustered by gender. Category labels: 1 = Cisgender female; 2 = Transgender female; 3 = Non-binary; 4 = Cisgender Male; 5 = Transgender Male

Figure 3.



Note. Distribution of salivary progesterone concentrations clustered by gender. . Category labels: 1 = Cisgender female; 2 = Transgender female; 3 = Non-binary; 4 = Cisgender Male; 5 = Transgender Male

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Appendix A: IRB Approval Letter

MARSHALL UNIVERSITY
www.marshall.edu

Office of Research Integrity
Institutional Review Board
One John Marshall Drive
Huntington, WV 25755

FWA 00002704

IRB1 #00002205
IRB2 #00003206

February 3, 2023

Jonathan Day-Brown, PhD
Psychology Department

RE: IRBNet ID# 1572372-1
At: Marshall University Institutional Review Board #2 (Social/Behavioral)

Dear Dr. Day-Brown:

Protocol Title:	[1572372-1] Effects of Gonadal Hormones on Neurocognitive Tasks	
Site Location:	MU	
Submission Type:	New Project	APPROVED
Review Type:	Expedited Review	

In accordance with 45CFR46.110(a)(3)&(7), the above study was granted Expedited approval today by the Marshall University Institutional Review Board #2 (Social/Behavioral) Chair. An annual update will be required on February 3, 2024 for administrative review and approval. The update must include the Annual Update Form and current educational certificates for all investigators involved in the study. All amendments must be submitted for approval by the IRB Chair prior to implementation and a closure request is required upon completion of the study.

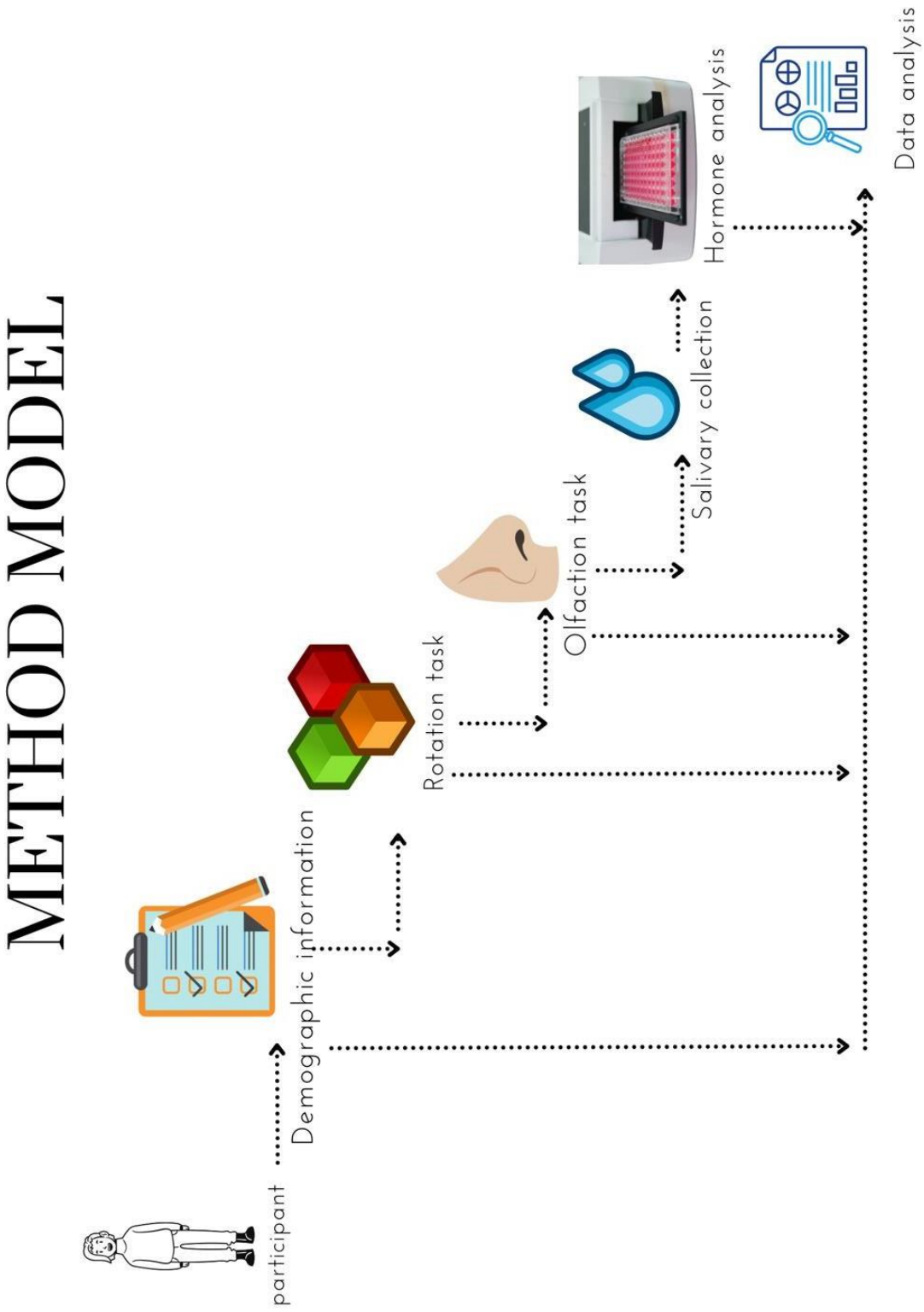
This study is for students John Prentice and Darby McCloud.

If you have any questions, please contact the Marshall University Institutional Review Board #2 (Social/Behavioral) Coordinator Lindsey Taylor at (304) 696-6322 or l.taylor@marshall.edu. Please include your study title and reference number in all correspondence with this office.

Sincerely,

Bruce F. Day, ThD, CIP
Director, Office of Research Integrity

Appendix B: Method Model



Appendix C: Hormone Assay Protocol

Testosterone- Salimetrics assay processing protocol

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSB wells included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Pipette 18 mL of Testosterone Assay Diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards, controls, and saliva samples into appropriate wells.
- Pipette 25 µL of Testosterone Assay Diluent into 2 wells to serve as the zero.
- Pipette 25 µL of Testosterone Assay Diluent into each NSB well.

Step 5: Dilute the Enzyme Conjugate 1:1000 by adding 18 µL of the conjugate to the 18 mL tube of Testosterone Assay Diluent. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube

bottom. Immediately mix the diluted conjugate solution and add 150 μL to each well using a multichannel pipette.

Step 6: Mix plate on a plate rotator for 5 minutes at 500 rpm and incubate at room temperature for a total of 1 hour.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 μL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 μL of TMB Substrate Solution to each well with a multichannel pipette. Step 9: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark (covered) at room temperature for an additional 25 minutes.

Step 10: Add 50 μL of Stop Solution with a multichannel pipette.

Step 11: Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

Caution: Spillage may occur if mixing speed exceeds 600 rpm.

• Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.

• Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 490 to 492 nm is recommended.)

Estradiol- Salimetrics assay processing protocol

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSB wells included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Pipette 12 mL of HS Estradiol Assay Diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 100 µL of standards, controls, and saliva samples into appropriate wells.
- Pipette 100 µL of HS Estradiol Assay Diluent into 2 wells to serve as the zero.
- Pipette 100 µL of HS Estradiol Assay Diluent into each NSB well.

Step 5: Dilute the Enzyme Conjugate 1:800 by adding 15 µL of the conjugate to the 12 mL tube of HS Estradiol Assay Diluent. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and add 100 µL to each well using a multichannel pipette.

Step 6: Place adhesive cover provided over plate. Mix plate on a plate rotator for 5 minutes at 500 rpm and incubate at room temperature for a total of 2 hours.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 µL of TMB Substrate Solution to each well with a multichannel pipette. Step 9: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark (covered) at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of Stop Solution with a multichannel pipette.

Step 11: Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.
Caution: Spillage may occur if mixing speed exceeds 600 rpm.

• Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.

• Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 620 to 630 nm is recommended.)

Progesterone- Salimetrics assay processing protocol

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 empty. Break off 2 NSB wells from the strip of NSB wells included in the foil

pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.

2. Do not insert wells from one plate into a different plate.

Step 3: Pipette 18 mL of Assay Diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

Step 4:

• Pipette 50 µL of standards, controls, and saliva samples into appropriate wells.

• Pipette 50 µL of Assay Diluent into 2 wells to serve as the zero.

• Pipette 50 µL of Assay Diluent into each NSB well.

Step 5: Dilute the Enzyme Conjugate 1:800 by adding 22.5 µL of the conjugate to the 18 mL tube of Assay Diluent. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and add 150 µL to each well using a multichannel pipette.

Step 6: Place adhesive cover provided over plate. Mix plate on a plate rotator continuously at 500 rpm for 1 hour at room temperature.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 μL of TMB Substrate Solution to each well with a multichannel pipette. Step 9: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark (covered) at room temperature for an additional 25 minutes.

Step 10: Add 50 μL of Stop Solution with a multichannel pipette.

Step 11: Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.
Caution: Spillage may occur if mixing speed exceeds 600 rpm.

• Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.

• Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 490 to 492 nm is recommended.

CURRICULUM VITAE
John H. Prentice

Phone: 304-642-3696
E-Mail: Prentice2@marshall.edu

CURRENT EMPLOYER

06/2020 Valley Health Systems- pre-doctoral psych intern

EDUCATION

2024 (in progress) Marshall University- PsyD
Emphasis: Clinical Psychology
(APA accredited program)

2020 Marshall University- M.A
Program: Clinical Psychology

2016 West Virginia Wesleyan- B.A. Cum Laude
Major: Psychology (Track 1 and Track 2)

CLINICAL EXPERIENCE

2021- 2022 HARMONY HOUSE- PRACTICUM

A clinical Practicum where I maintained a therapeutic caseload, provided consultation for the primary care clinic, and assisted in developing a monthly outpatient primary care clinic. I also assisted individuals in accessing substance use disorder recovery services and harm reduction tools. I also became certified as a Smart recovery facilitator to provide an ongoing recovery group.

2021-2022 WEST VIRGINIA AUTISM TRAINING CENTER-PRACTICUM

A clinical Practicum where I maintained a therapeutic caseload, provided consultation for academic services, and maintained two therapeutic groups, One of which was focused on LGBTQ+ concerns. I also participated in the interdisciplinary diagnostic clinic that met monthly to provide cross-discipline and integrated diagnostic assessments to children suspected to be on the autism spectrum.

2019-2020 ST. MARY'S MEDICAL CENTER- MENTAL HEALTH INTERN

A clinical internship with responsibilities including cardiac post-operative consultation and outpatient services, oncology supportive care and rounding, infusion center supportive care and outpatient counseling, neurological consultation, assessment, and counseling, therapeutic services for the School of nursing, and pre-operative bariatric evaluation.

2018-2019 MARSHAL PSYCHOLOGY CLINIC- STUDENT CLINICIAN

Maintained an outpatient caseload through the Marshall campus clinic. Providing a variety of services, including psychotherapy, assessment, and an LGBTQ+ support group.

2019 MARSHAL PEDIATRICS- BEHAVIORAL HEALTH SPECIALIST

BHWET Grant placement, providing physician consultation, assessment, and brief intervention to pediatric clinic patients.

RESEARCH

2020- PRESENT The Effects of Gonadal Hormones on Cognitive Tasks (in progress)

2020 Non-Directive Intervention in Rural Homeless Populations - Poster presented at APA National Conference

ACADEMIC EMPLOYMENT

August 2017 -2020

Graduate Assistant, Marshall University worked in an advisory capacity to first-year and sophomore psychology majors, aiding in the creation of Academic schedules and facilitating the planning and completion of the course of study.

2016 Research Assistant, aiding faculty in data collection, manipulation, and report writing. We are examined the salience of motivators and their correlation with personality typography.

Fall 2014-Spring 2016 Teaching Assistant/Assistant Lab Supervisor, West Virginia Wesleyan,

Fall 2014- Spring 2016 work-study, animal lab facility caretaker, maintained a colony of approximately 30 rats on a fixed nutrition schedule—training in biohazard

transmission prevention, pertinent OSHA regulation, and autoclave use.

OTHER RELEVANT EXPERIENCE

Fall 2021 — Co-authored a $1.3 million HRSAgrant. (GPE) This grant was focused on increasing training opportunities for integrated practice in third-year Doctor of Clinical Psychology Students in rural and underserved areas of West Virginia.

SPEAKING ENGAGEMENTS

2022 — Autism Across the Lifespan Conference
-90 minute-Keynote Speaker
I provided a 90-minute informational Keynote regarding the intersection of autism spectrum disorder and LGBTQ identities. Discussing double minority stigma, gender heuristics, and a brief introduction to the evolutionary benefits of gender diversity.

2021 — MidAtlantic AIDS Education and Training Center Conference- 90-minute-Keynote Speaker - "Sexual Orientation, Gender Diversity, and the Modern Ethical Practice." Topics discussed included sexual and gender diversity, evolutionary biology, and neuroendocrinology behind LGBTQ persons. Ethical use of Hormone replacement therapy and the fundamentals of clinical ethics and practice with regards to gender diversity and sexuality. This presentation was reviewed by the Mid-Atlantic aids education and training center, And continuing education credits were provided to those in attendance.

2021 — West Virginia Nursing Association speaker series- Keynote Speaker- "Sexual Orientation, Gender Diversity, and the Modern Ethical Practice." Topics discussed included sexual and gender diversity, ideology, evolutionary biology, and neuroendocrinology behind LGBTQ persons. Ethical use of Hormone replacement therapy and the fundamentals of clinical ethics and practice with regards to gender diversity and sexue West Virginia nursing association reviewed this presentation, And continuing education credits were provided to those in attendance.

2019 — West Virginia Suicide Prevention Conference-

co-paneled a presentation that provided information about the complex trauma of loss related to suicide and how it affects children and adolescents

OTHER EMPLOYMENT

2020- 2021

CABELL COUNTY COALITION FOR THE HOMELESS- BEHAVIOR HEALTH SPECIALIST
A position where I provided behavioral health consultation/referral, mental health screenings, and crisis management/ de-escalation. I provided mental health training to the coalition and sat on both the training and advocacy sub comity. I was later appointed as the LGBTQ+ housing and patient rights advocate

June 2016 - July 2017

Residential counselor- Home base inc.
Detect client care/group and individual supportive counseling, behavior management, strategic content tutoring, and curriculum creation.

Summers 2016-2017

AmeriCorps-Vista /
Child education, community engagement, behavior management planning, rural food access, arts, literacy, and STEM curriculum creation.

VOLUNTEER WORK

2019-PRESENT

THE CENTER-YOUTH OPPORTUNITY HUB
Co-facilitated ongoing arts and mental wellness group for local at-risk young adults between the ages of 16-24, covering such topics as life skills, LGBTQ issues, stress, anger, grief, relaxation, and personal wellness.

2017- PRESENT

HARMONY HOUSE Co-facilitated an ongoing arts and mental wellness group for local homeless and housing unstable populations. Covering such topics as stress, anger, grief, life skills, and personal wellness.

2017-2020-

MARSHALL MEDICAL OUTREACH
Participated in multiple integrated medical outreach days to service local homeless and housing unstable populations. Providing screenings, brief intervention, and referral to treatment.

Outreach

Senior Fest	Provided brief screening and referral at Cabell Hospital senior healthcare. We screened for neurocognitive decline as well as depression and anxiety in senior adults.
Joyful Noise Choir- Director	Met diverse needs of differently-abled singers ages 19-55. Employed Behavior management and adaptive communication to enhance instruction, achieve performance goals, and ensured a positive experience for performers.
International student presentation	Provided a discussion-based activity designed to aid in the matriculation of international students. Addressing such concerns as loneliness and homesickness.
Psi Chi Philanthropy.	Planned and executed multiple meals and activity nights for local homeless and housing unstable population.

AFFILIATIONS

AMERICAN PSYCHOLOGICAL ASSOCIATION- STUDENT AFFILIATE	2014-PRESENT
WEST VIRGINIA PSYCHOLOGICAL ASSOCIATION- STUDENT MEMBER	2020-PRESENT
PSI CHI- PSYCHOLOGY HONOR SOCIETY- GRADUATE MENTOR	2016-2020
Phi mu alpha, national men's music fraternity, -president • committee oversight: executive, finance, nominations & elections. Grew chapter by 35%.	2014-2016
National society for leadership and success	2013-2016
Leadership exploration through applied practice (L.E.A.P)	2013-2016
Appalachian impact youth leadership initiative	2015-2017
Sigma tau delta- English honor society	2016