THE IMPACT OF A WESTERN-PATTERN DIET ON THE INTERACTION OF PRENATAL STRESS, MATERNAL BEHAVIOR AND OFFSPRING PHENOTYPE

By

NIKKI JO CLAUSS

Bachelor of Science in Psychology
University of Wisconsin
Green Bay, Wisconsin
2012

Master of Arts in Psychological Science
Northern Arizona University
Flagstaff, Arizona
2015

Master of Science in Psychology
Oklahoma State University
Stillwater, Oklahoma
2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirement for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2019

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Dissertation Approved:

Jennifer Byrd-Craven, Ph.D.

Dissertation Adviser
Misty Hawkins, Ph.D.

DeMond Grant, Ph.D.

Mary Towner, Ph.D.

.

Name: NIKKI JO CLAUSS

Date of Degree: MAY 2020

Title of Study: THE IMPACT OF A WESTERN-PATTERN DIET ON THE INTERACTION OF PRENATAL STRESS, MATERNAL BEHAVIOR AND OFFSPRING PHENOTYPE

Major Field: PSYCHOLOGY

Abstract: Maternal prenatal stress is a significant source of developmental stress that can leave an epigenetic signature on offspring, leading to stress-related and anxious behavior. A limited amount of work has been accomplished demonstrating that a Western-pattern diet (WPD) during lactation leads to anxiety reduction in juvenile rodents. However, the impact of early developmental experience on the potential neurobiological pathways that contribute to the association between diet and behavior have not yet been elucidated. It is also unclear whether the apparent diet-induced reduction in anxiety-like behavior extends into adulthood, whether it requires a consistent highly palatable diet, or if there are sex differences. To address this gap in the literature, the focus of the current study was to determine whether the impact of prenatal stress could be mitigated by a diet that stimulates the same neuroendocrine systems influenced by early stress. I hypothesized that the lasting developmental effects of stress on anxious behavior would be mitigated by a highly palatable WPD through the upregulation of Dopamine *D1 (Drd1)* and *D2 (Drd2)* receptor genes in mesolimbic pathways that have downstream effects on the expression of hippocampal glucocorticoid receptor genes (*Gr*). Through the use of behavioral and genetic approaches, three aims were addressed: **Aim 1** assessed whether offspring lactation diet and prenatal stress interact with the expression of *Drd1*, *Drd2*, and *Gr* to predict offspring anxious-like behavior. **Aim 2** determined whether this behavioral phenotype persisted into adulthood and whether it required an ongoing WPD. **Aim 3** assessed whether there were sex differences in the models tested. Results demonstrated that offspring lactation diet and prenatal stress (measured via maternal condition) interacted with the expression of Drd1, Drd2, and Gr to predict juvenile anxious-like behavior. As expected, the chow-fed offspring of stressed dams displayed significantly more anxious-like behavior in the open field test than WPD-fed offspring of stressed dams and their non-stressed counterparts. Indeed, the WPD-fed offspring of prenatally stressed dams exhibited a gene expression and behavioral pattern more similar to the control group than to the chow-fed stressed group, and this effect was driven largely by female offspring. For female offspring, this behavior pattern continued into adulthood if there was no change to their post-weaning diet. However, those offspring whose diet was switched at weaning displayed increased anxious-like behavior, whether their mothers experienced prenatal stress or not. Post-weaning diet did not have a significant impact on male offspring behavior. Ultimately, the results of this research help to elucidate the relationship between social environment, underlying genetics of behavior, and shifts in behavior that lead to long term health effects

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CHAPTER I

INTRODUCTION

The Western-Pattern Diet (WPD), characterized by foods high in fat and simple carbohydrates and low in protein, appears to be responsible for more global deaths than any other health risk (Ashkan et al., 2019). The consumption of these types of foods is pervasive, in part due to their highly rewarding and stress blunting properties (Adam & Epel, 2007; Macht, 2008; Dallman, Pecorano, & la Fleur , 2005). As such, understanding the neurobiological correlates of the interaction between stress and diet is a major goal in the development of interventions to reduce or prevent obesity-related disease.

CHAPTER II

REVIEW OF LITERATURE

Developmental Plasticity

Critical periods in an organism's development may play a particularly important role in the establishment of obesity-related eating behaviors and other behavioral phenotypes. During such critical periods, the biological settings of a developing system are especially responsive to social and biological cues and are therefore open to modification in response to environmental experience (Kuzawa & Quinn, 2009). Importantly, many critical periods overlap with periods of placental and lactational maternal provisioning, which indicates that these periods are windows of opportunity for the transfer of phenotypic information from one generation to the next. Maternal pre- and post-natal stress information is expressed to the developing offspring through several mechanisms, including hormones and nutrients, during placental and lactational maternal provisioning. The developing offspring can subsequently use this information for systemic calibration to an expected future environment. For instance, high levels of maternal glucocorticoids and inadequate maternal nutrition may signal to the offspring that the outside environment is harsh and unpredictable (Kuzawa & Quinn, 2009). Alternatively, lower levels of maternal glucocorticoids and adequate nutrition (or over-nutrition) may signal to the developing offspring that the environment is safe and

resource rich. This dynamic process can benefit the offspring by preparing central and peripheral systems to respond effectively to the environment (Bateson, Gluckman, & Hanson, 2014).

Early Environmental Adversity and Maternal Behavior

Environmental adversity occurring early in development is a crucial factor contributing to disease susceptibility in later life (see Sinclair, Lea, & Rees, 2007). Maternal antenatal stress and postnatal behavior is a significant source of early environmental adversity. In humans, antenatal stressors are associated with adverse perinatal and developmental outcomes, including a range of cognitive, behavioral, and emotional processes (Van Den Bergh, Mulder, Mennes, & Glover, 2005; Wadha, Entringer, Buss, & Lu, 2011). Further, children who experience adversity during early development are more likely to develop adult diabetes and cardiovascular disease (Batten, Aslan, Maciejewski, & Mazure, 2004; Goodwin and Stein, 2004), addiction (Dube, Felitti, Dong, Chapman, Giles, Anda, 2003; Anda et al., 2006), depression (Batten et al., 2004), and anxiety-related disorders (Phillips et al., 2005). In rodents, antenatal stressors induce anxiety- and depression-like behaviors in pregnant dams, which leads to a reduction in sensitive maternal behaviors such as licking and grooming (LG) and arched back nursing (ABN). This is important because these maternal behaviors have been found to modulate offspring physiology, behavior, and gene expression via epigenetic mechanisms (Meaney, 2001).

The Epigenome

Phenotypes that are passed to other cells and sometimes future generations that are not the result of differences in the DNA base sequence are epigenetic effects (Pierce, 2017). As such, epigenetic changes to DNA may facilitate developmental plasticity by encoding information from an organism's early life, including the prenatal environment, to coordinate future gene activity and phenotypes in later-life environments (Bird, 2007).

Many epigenetic phenotypes are the result of alterations in the chromatin structure (Pierce, 2017). Three major molecular processes mediate these alterations: DNA methylation, histone modification, and RNA molecules. Perhaps the most widely studied of these processes is DNA methylation. Methylation occurs when DNA methyltransferases (DNMT) add methyl groups to cytosine bases to create 5-methylcytosine, which subsequently bind to CpG dinucleotides or CpG islands (Champagne, 2010). The methyl group sits within the major groove of the DNA molecule and is recognized by several DNA-binding proteins that have a high affinity for the methyl group and directly repress transcription. Thus, the presence of the methyl group inhibits the binding of transcription factors and other proteins that are required for transcription to occur. Another way in which DNA methylation inhibits gene expression is by altering chromatin structure by attracting enzymes, aptly called histone deacetylase, that removes acetyl groups from the tails of histone proteins (e.g., Turner, 2002).

Modifications to the positively charged tails of histone proteins can also interact with the DNA and impact chromatin structure and subsequent gene transcription

(Champagne, 2010). Among the most studied modifications to histone proteins relative to environmentally induced changes in transcriptional activity include the addition of methyl and acetyl groups. Histone acetylation destabilizes chromatin structure and leads to or more open chromatin configuration, with DNA less tightly wrapped around the histone proteins. This change in chromatin configuration provides more space for the binding of transcription factors and thus is associated with increased transcription.

The results of histone methylation are more nuanced than those of histone acetylation. When histone methylation occurs, the specific effect on chromatin structure depends on which amino acid is methylated, and the number of methyl groups added; as some histone methylation leads to increases in transcription while other types lead to decreased transcription (Champagne, 2010). For example, if three methyl groups are added to histone H3 at lysine 4 (H3K4me3), transcription is upregulated (Pierce, 2017). However, if three methyl groups are added to histone H3 at lysine 27 (H3K27me3), transcription is repressed. Indeed, it is the combined presence of multiple epigenetic marks that determine the activity level of a gene, not single histone modifications (Szyf, 2011). Additionally, histone modifications attract enzymes and proteins that modify other histones.

Importantly, many of the enzymes and proteins that produce epigenetic marks are not able to bind to specific DNA sequences by themselves (Szyf, 2011). Instead, they must be recruited to specific targets on the chromosome through several means including sequence-specific transcription factors, preexisting chromatin marks, and noncoding

RNA factors. Moreover, histone modifications can attract additional enzymes and proteins that modify other histones.

Behavioral Epigenetics

Where an individual's genome is determined by inheritance and is identical in all tissues, epigenetic patterns vary from cell to cell and are potentially dynamic for the life of the organism (McGowan & Szyf, 2010). Indeed, there is a bidirectional relationship between an organism's environmental context and its epigenome. As such, epigenetic mechanisms may serve as an interface between the environment and the genome. Evidence suggests that changes in gene expression within the brain and in peripheral tissues are associated with differences in the quality of early environment and that these developmental effects are maintained by epigenetic mechanisms that control the activity of genes involved in disease risk and behavioral variation (Champagne, 2010).

One remarkable example of epigenetic modulated developmental plasticity is the density-dependent polyphenism of swarming locusts (Simpson, Despland, Hagele, & Dodgson, 2001). The desert and migratory locusts are reared in crowded or non-crowded environments, respectively. The adult desert locust displays gregarious behavior and a colorful appearance; whereas the adult migratory locust displays solitary behavior and a drab appearance. Though the desert and migratory locusts are genetically identical, they exhibit differential expression of genes that encode DNA methyltransferases (DMNT), which suggests that the distinct rearing experiences of the two locusts play a significant role in the development of epigenetic patterns. Indeed, the desert locust nymphs' crowded

environment provides it with abundant tactile stimulation of the legs, triggering the development of the gregarious adult form (Simpson et al., 2001). These dramatic differences in behavior and morphology of swarming locusts provide an example of how epigenetic signals encoded early in life may alter adult phenotypes.

Similar to the swarming locusts, the quality of the postnatal environment can also factor into long term changes in gene expression for rodents. Rodent gestational stress and maternal LG and ABN behavior have been shown to impact offspring epigenetic encoding and alter the adult behavioral phenotype, and are all associated with decreased hippocampal GR mRNA, increased hypothalamic CRH mRNA, and decreased BDNF (Kapoor, Leen, & Matthews, 2008; Lippman, Bress, Nemeroff, Plotsky, & Monteggia, 2007; Francis, Diorio, Liu, & Meaney, 1999; Liu, Diorio, Day, Francis & Meaney, 2000). This stress-related pattern of gene expression leads to increased hypothalamic pituitary adrenal axis (HPAa) responsivity to stress, a distinctly anxious and fearful phenotype, and impairments in cognition and social behavior (e.g. Champagne, Francis, Mar, & Meaney, 2003; Meaney, 2001; Lippman, et al., 2007).

There is evidence that these mechanisms are also relevant to human neurodevelopment. For example, analysis of neonatal cord blood revealed that infants born to mothers with high ratings of depression during the third trimester also exhibited elevated GR promotor methylation (Oberlander, Weinberg, Papsdorf, Grunau, Misri, & Devlin, 2008). The implication is that early environmental conditions can result in long-term silencing of the GR promoter through DNA methylation and that this epigenetic

modification results in changes to HPAa responsivity that persists into adulthood (Liu et al., 1997; Weaver et al., 2004).

Thus, an individual's early social environment has a long-lasting impact on mental and physical health trajectories via the epigenetic marking of specific genes. Moreover, although epigenetic markers are long lasting, evidence suggests that specific social and pharmacological interventions could reverse those deleterious epigenetic markings sculpted by early adverse social exposures (i.e., Weaver, et al.., 2005; Masi et al., 2001).

Interaction Between Diet and Stress

Stress, particularly occurring early in development, is implicated in the development of overweight and obesity. Rodents and humans are likely to change feeding behavior under both acute and chronic stress conditions. Approximately 40% of organisms studied display a marked decrease in food consumption in response to stress, while another 40% demonstrate a marked increase (Block, He, Zaslavsky, Ding, & Ayanian, 2009; Mikolajczyk, Ansari, & Maxwell, 2009). However, whether an organism increases or decreases caloric intake, the type of food they eat changes, with negative emotion driving individuals toward highly palatable foods (Adam & Epel, 2007).

Stressors engage a network of limbic structures that are responsive to both interoceptive and exteroceptive inputs, including the hippocampus (i.e. Sapolsky, Romero, & Much, 2000). The recruitment of this stress network depends on the release and action of glucocorticoids from the adrenal cortex and subsequent release of

corticotrophin releasing factor (CRF) from extrahypothalamic neurons. Acute and chronic stressors both lead to increases in synapses and dendritic bushing in the amygdala and anterior cingulate cortex (areas of the brain involved in the processing of emotional stimuli), and reduce synaptic contacts in the hippocampus and prefrontal cortex (areas of the brain involved in stimuli interpretation, planning, and behavioral control; Holmes & Wellman, 2009; Vyas et al., 2002). This combination of events sets a system up for limbic-biased stress responses and alter eating in a reward-based fashion toward greater comfort food (Groesz et al., 2012).

Dopamine projections to the mPFC also play an essential role in mediating stress responsivity (Weaver et al., 2004). The increased CRF secretion that occurs in response to acute stress acts on dopamine neurons in the ventral tegmental area and increases dopamine secretion to the nucleus accumbens, which is also stimulated by drugs of abuse (Wanat et al., 2008; Lodge & Grace, 2005). Dopamine stimulation promotes approach behavior toward pleasurable stimuli, and food is a widely available and inexpensive substance. This helps explain why the overconsumption of highly palatable foods and drug use relapse are more likely to occur during times of acute stress.

Alternatively, chronic stress in general and low levels of LG/ABN specifically are associated with a reduction in extracellular dopamine in the nucleus accumbens shell and medial prefrontal cortex (mPFC), leading to sensorimotor gating and increased anxious-like behavior (Zhang et al., 2005; Weaver et al., 2004). This stress-related reduction in extracellular dopamine may be mitigated by exogenous substances, such as highly

palatable food, that are associated with increases in dopamine release, as dopamine stimulation leads to downstream increases in the expression of glucocorticoid receptor genes (*Gr*) mRNA and enhances negative feedback following stress (Meaney, 2001). For example, rats that are trained to earn vanilla sugar pellets and then exposed to stress have basal levels of dopamine accumulation in the nucleus accumbens shell comparable to their counterparts who received sugar pellets without subsequent stress (Masi et al., 2001).

Maintenance of Stress Eating Through Negative Feedback

Stress eating may be maintained by negative reinforcement. Evidence demonstrates that highly palatable food consumption triggers an increase in dopamine secretion in the mesolimbic pathway from the ventral tegmental area to the nucleus accumbens, further activating dopamine and opioid secretion from neurons throughout the homeostatic feeding network (Pecorano, Reyes, Gomez, Bhargave, & Dallman, 2004). Thus, eating highly palatable foods after a stressor reduces activity in the central stress response network and serves as feedback to sharpen the activity of the network and reduce the duration of its activity. Indeed, people who eat more comfort food have dampened HPA-axis stress responses (Tomiyama, Dallman, & Epel, 2010).

These strong opioid and dopamine responses in the reward center during stress promote the encoding of habits in the basal ganglia (Wickens, Horvitz, Costa, & Killcross, 2007). Therefore, either acute or chronic stress may augment wanting, pleasure, and memories associated with palatable food intake. Memories of responses to

stimuli are stored both in the cortex, where flexibility of response is engendered by the knowledge of outcome, and in the basal ganglia, where habit is expressed, and a learned response follows the stimulus.

Sex Differences

Sex is a crucial variable that modulates a wide range of neurobiological processes, likely including the relationship between stress and eating behavior, and has clinical relevance. Genes, environment, and hormones all interact during critical periods of development and result in widespread sex differences in mammalian brain, behavior, and physiology (McCarthy & Arnold, 2011).

Sex differences initially emerge during the aforementioned critical periods of development. Organizational effects on sex begins prenatally when fetal testes produce a surge in androgens that gain access to the brain, are converted to estrogens in large amounts, and initiate brain masculinization (i.e. McCarthy, Nugent, & Lenz, 2017). For rodents, the end of this critical period is approximately 10 days after birth. There is no corresponding steroidogenesis in females during this period, but it is nonetheless a sensitive period in which several neuroanatomical end points are differentiated in males versus females in a region-specific manner. For example, there are sex differences in the occurrence of apoptosis in some regions, while in other regions there are sex differences in synaptogenesis.

DNMT3a levels, which are important in de novo methylation induction, are highest in several brain regions of the rodent female beginning at post-natal day (PND) 1

but are eliminated by the second week of life (McCarthy et al., 2017). Therefore, it appears as though there are sex differences in the overarching critical period, such that the period in which an organism is most sensitive to endogenous and exogenous influences is longer in males (beginning prenatally at androgen surge) than in females (beginning at PND 1). This sex difference may contribute to differential epigenetic responses to the environment (Weinstock, 2007; Geary, 2015).

Indeed, epigenetic mechanisms could contribute to sexual differentiation of environmental effects on the brain throughout the lifespan (McCarthy & Arnold, 2011). In the case of maternal licking and grooming, rat dams engage in significantly more anogenital grooming of their male offspring compared to female offspring, which has enduring effects on adult behavior and is mediated, in part, by epigenetic mechanisms (Cavigelli, Ragan, Barrett, & Michael, 2010). Therefore, it is important to assess sex differences when analyzing potential diet-induced behavior and gene expression.

Current Study

The main question the current study is concerned with is: Can the lasting effects of developmental stress be mitigated by a diet that stimulates the same neuroendocrine systems depleted by early stress? I began by conducting an analysis of the association between maternal behavior and maternal condition as a way to check whether the prenatal stress impacted maternal behavior. My objective was to test the following central hypotheses: (**1**) Maternal Condition (stress status and lactation diet) will predict the expression of receptor genes Drd2 in the VTA, Drd1 in the Nacc, and Gr in the

hippocampus. **(2)** Juvenile behavioral phenotype (anxious-like/exploratory behavior; represented by metrics measured from the OFT) will be predicted by sex and the interaction between maternal condition and gene expression. (**3**) Behavioral phenotype will persist into adulthood regardless of adult diet and maternal condition for males and females.

CHAPTER III

METHODOLOGY

All animals were treated per the Policy on Humane Care and Use of Laboratory Animals, and all procedures were approved by the Institutional Animal Care and Use Committee at the university.

Subjects

Subjects were experimentally, and sexually naïve female C57BL/6J *Mus musculus* and their offspring (dams: $n = 37$; offspring: $n = 252$; see Figure 1) obtained from the breeding colony maintained in the Integrative Biology Department at a southern U.S. university. C57BL/6J *Mus musculus* were chosen because they are an inbred strain and are therefore genetically identical. All animals were allowed ad libitum access to food and water and were maintained on a 12- hour light-dark cycle (lights on at 0900 hours). For breeding, females were placed in the home cage of a male for 3 days. After the breeding period, females were removed from the male's cage. Pregnant females were individually housed in clear plastic cages with Sani-chips bedding and were provided with nesting material (cotton nestlets). The 37 females delivered and reared to weaning litters composed of 5-10 pups ($M = 7$). This does not include females that failed to rear 5 or more healthy pups to weaning ($N = 4$) or lost more than two pups that were born alive ($N = 3$). Losses did not vary by condition.

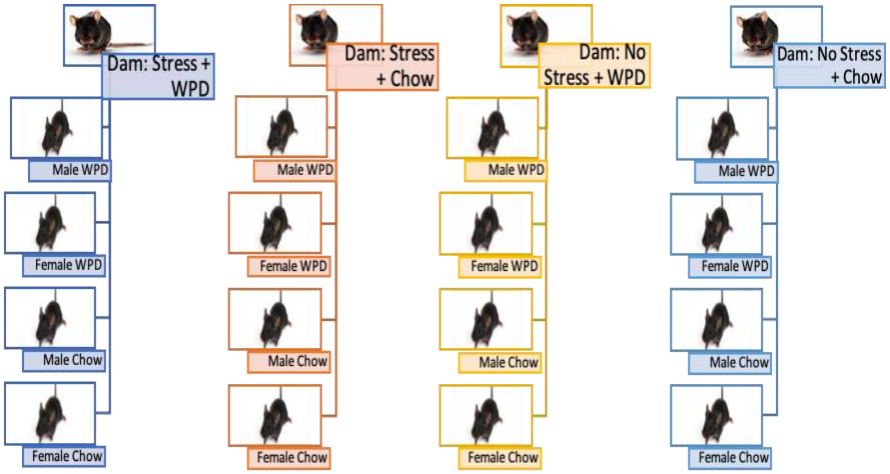


Figure 1. Maternal experimental groups, lactation diet experimental groups, and weaning diet experimental groups.

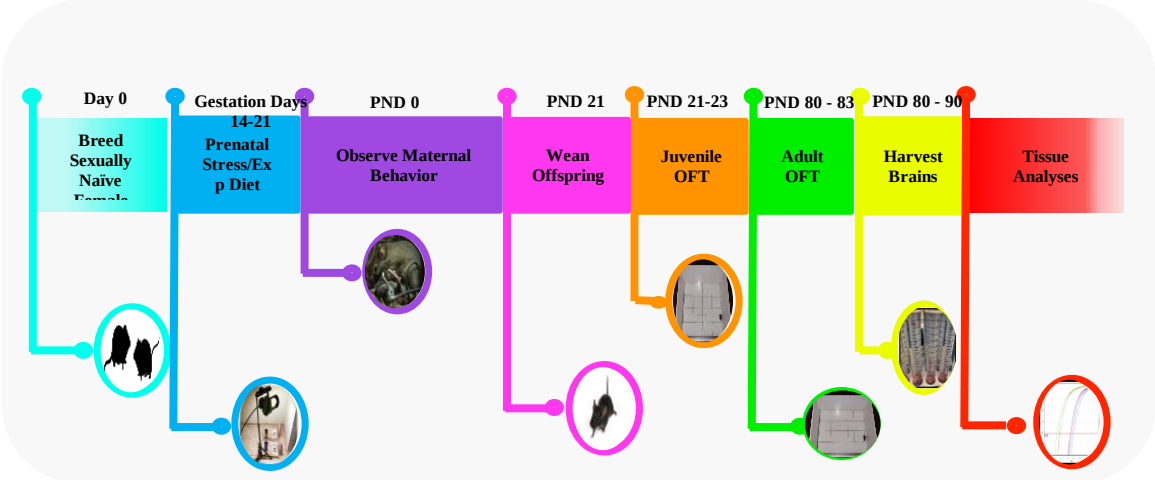


Figure 2. Procedure

Prenatal Stress

A Kaytee Crittertrail Fun-Nel Tube that measures 8.89-cm long and 5.72-cm wide was utilized to restrain pregnant dams for prenatal stress induction. The mice were placed in the tube, which was then capped at each end to prevent escape and placed back in the home cage. A light (36 lux) was pointed directly above the home cage. Mice were left in the restraint apparatus under the bright light for 45 minutes per day between the hours of 11 A.M. and 2 P.M., every day during the last six days of pregnancy.

Postnatal Diet

Dams assigned to the WPD groups had their diet changed from standard chow (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) to WPD (D12079B, Research Diets, New Brunswick, NJ, USA) on gestation day 14. Dams who were assigned to the chow groups had no change in their diet. Composition and physiological fuel values can be found in Figure 3.

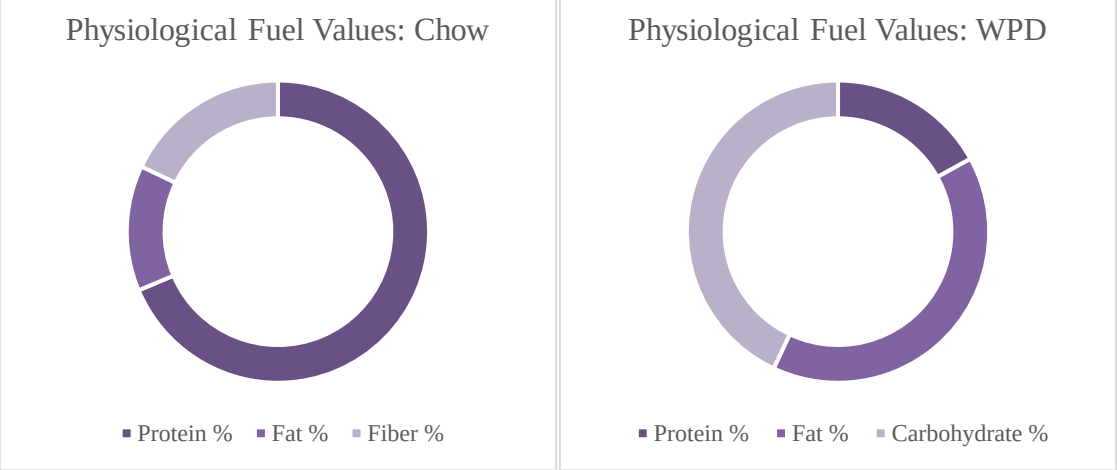


Figure 3. Macronutrient composition of standard chow and Western-pattern diets**.**

Maternal Behavior

Time Spent in Nest. VitalView hardware and software system was used to assess the time a new dam spent in her nest. The VitalView system includes a hardware interface card that plugs into an expansion slot on a computing platform and coordinates software and hardware functions. It also contains a real time clock for time keeping. VitalView measures movement activity passively via an infrared sensor placed over the top of the home cage. VitalView monitors movement without filtering data. Rather, the

activity count is the total number of activity counts that have transpired since the last query was made. In the current study, counts were collected at 1-minute intervals.

Post-Weaning Diet

Pups were weaned on PND 21 and randomly assigned to a chow or WPD diet. Pups were housed with same-sex/same-diet siblings.

Offspring Behavior

Anxious and Exploratory Behavior. Offspring completed an open field test (OFT) for measurement of anxious-like and exploratory behavior once at PND 23 and once at PND 70. To control for offspring weight, they were weighed immediately before the juvenile OFT and again after the adult OFT at the time of sacrifice. A 69.5-cm wide x 69.5-cm long x 69.5-cm high open field arena with clear plexiglass walls and a white plexiglass floor divided into 16 squares by black lines was used. The open field arena was cleaned with 70% ethanol solution after removal of each mouse. Parameters measured included: latency to enter OFT, line crosses, time spent in center of OFT, rearing frequency, and time spent grooming.

Tissue Collection

Between PND 70 and 80, the pups were sacrificed by cervical dislocation and subsequently decapitated. The brain was removed immediately and stored in RNALater at -20° C for subsequent microdissection of the nucleus accumbens, hippocampus, and the ventral tegmental area. Tissue was immediately stored in RNALater at -20° C for

later determination of mRNA expression of Dopamine D1 (*Drd1)* and D2 (*Drd2)* receptor genes, and *Gr*.

RNA extraction and cDNA synthesis. Gene expression was assessed by using reverse transcription followed by quantitative real-time PCR with a CFX Connect Real-Time System (Bio-Rad). RNA extraction was performed according to manufacturer protocol (RNeasy Mini Kit, Qiagen). cDNA was synthesized the same day using iScript RT Supermix (Bio-Rad Laboratories). By using specific primer sets (Table 1), mRNA levels of the following genes were be examined: *Drd1*, *Drd2*, and *Gr*.

Table 1: Primer sequences for qPCR

Gene	Forward Primer (5')	Reverse Primer (3')
Nr3c1	CAAGGGTCTGGAGAGGACAA	CTGGACGGAGGAGAACTCAC
Drd1	GCAAATCCGGCGCATCTCA	AGCCAGCAGCACACGAATA
Drd2	GCCATCAGCATCGACAGGTA	ATGACAGTAACTCGGCGCTT
Actb	CTGTATTCCCCTCCATCGTG	CTTCTCCATGTCGTCCCAGT

Note: *Gr: Glucocorticoid receptor gene; Drd1: Dopamine receptor D1 gene; Drd2: Dopamine receptor D2 gene; Actb: Beta Actin.*

CHAPTER IV

RESULTS

Statistical Analyses

Jamovi version 0.9 was used for all analyses (The jamovi project, 2019). Data were screened and cleaned to ensure no violations of the assumptions for our statistical analyses were present. An overview of all statistical models employed can be seen in Table 2.

Table 2. Overview of Statistical Analyses

Description	**Analysis**	**IV(s)**	**DV(s)**	**Input Variables**	**Random Effect Variable (for LMM)**
Descriptive Statistics	Jamovi Descriptives	NA	NA	Sex, Maternal Condition, Post-Wean Diet, Weight	NA
Juvenile Offspring Behavior	Principle Components Analysis	NA	NA	latency to leave center, line crosses, rearing, stereotypy, time in the center	NA
Adult Offspring Behavior	Principle Components Analysis	NA	NA	latency to leave center, line crosses, rearing, stereotypy, time in the center	NA
Receptor Gene Expression	Principle Components Analysis	NA	NA	Expression of Drd2, Drd1, and Gr	NA
Maternal Behavior	Two-way ANOVA	- Prenatal Stress - Lactation diet	- Nest Time	NA	NA
Hypothesis 1	Two-way MANOVA with planned difference contrasts	- Prenatal stress - Lactation diet	- Drd1 Expression - Drd2 Expression - Gr Expression	NA	NA
Hypothesis 2	Linear Mixed Model	- Prenatal Stress - Lactation Diet - Gene Expression Factor	- Exploratory/anxious-like behavior factor	NA	Litter ID
	Linear Mixed Model	- Prenatal Stress - Lactation Diet - Gene Expression Factor	- Grooming Behavior	NA	Litter ID
Hypothesis 3	Linear Mixed Model	- Sex - Prenatal Stress - Lactation Diet - Post-wean diet	- Change in exploratory/anxious-like behavior from juvenility to adulthood	NA	Litter ID

Descriptive Statistics

Data were collected from 252 C57 offspring from 37 litters. Jamovi (The jamovi project, 2019) Descriptives was used to assess descriptive statistics (see Table 3).

Table 3. Descriptive Statistics

Variable		***N***	***%***	***Mean (SD)***
Sex	Male	121	48%	
	Female	131	52%	
Maternal Condition	No Stress + Chow	65	25.80%	
	No Stress + WPD	60	23.81%	
	Stress + Chow	62	24.60%	
	Stress + WPD	65	25.79%	
Post-Wean Diet	WPD	130	51.59%	
	Chow	122	48.41%	
Weight (grams)	No Stress + Chow			23.30 (4.12)
	No Stress + WPD			24.80 (4.55)
	Stress + Chow			23.50 (3.09)
	Stress + WPD			25.40 (4.86)

Note: WPD = Western-Pattern Diet, SD = Standard Deviation.

Offspring Behavior

The factorability of the 5 OFT metrics (latency to leave center of maze, number of line crosses, number of rearing behaviors, time spent grooming, and time spent in the center of the maze) were examined for the juvenile and adult OFT's separately. Several well-recognized criteria for the factorability of a correlation were used. First, it was observed that all metrics correlated at least .30 with at least one other item, suggesting reasonable factorability. Second, the Kaiser-Meyer-Olkin measure of sampling adequacy

was .70 for the juvenile OFT and .65 for the adult OFT, above the commonly suggested value of .60, and Bartlett's test of sphericity was significant for both tests. The diagonals of the anti-image correlation matrix were also all over .50. Finally, the communalities were all above .30, further confirming that each item shared some of the common variance with other items. Given these overall indicators, factor analysis was deemed to be suitable with all metrics.

Principle components extraction with varimax rotation was performed for juvenile and adult OFT metrics separately.

Juvenile PCA. Inspection of the correlation matrix showed that all variables had at least one correlation coefficient greater than 0.30. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.72, with individual KMO measures all greater than 0.70, indicating a classification of "middling" according to Kaiser (1974). Bartlett's Test of Sphericity was statistically significant (χ_2= 480.93, df = 10, p < .001), indicating that the data were likely factorizable.

PCA revealed two components that had eigenvalues greater than one and which explained 41.77% and 21.20% of the total variance, respectively. Visual inspection of the scree plot indicated that two components should be retained (Cattell, 1966). In addition, a two-component solution met the interpretability criterion. As such, two components were retained.

The two-component solution explained 62.97% of the total variance. A Varimax orthogonal rotation was employed to aid interpretability. The rotated solution exhibited simple structure (Thurstone, 1947). The interpretation of the data was consistent with the exploratory and anxious-like behavioral attributes the metrics were designed to measure,

with strong loadings of exploratory behavior on Component 1, and time spent grooming (a measure of anxious-like behavior) loading on Component 2.

Adult PCA. Inspection of the correlation matrix showed that all variables had at least one correlation coefficient greater than 0.30. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.72, with individual KMO measures all greater than 0.70, indicating a classification of "middling" according to Kaiser (1974). Bartlett's Test of Sphericity was statistically significant (χ_2= 375.36, df = 10, p < .001), indicating that the data were likely factorizable.

PCA revealed two components that had eigenvalues greater than one and which explained 40.89% and 22.90% of the total variance, respectively. Visual inspection of the scree plot indicated that two components should be retained (Cattell, 1966). In addition, a two-component solution met the interpretability criterion. As such, two components were retained.

The two-component solution explained 63.80% of the total variance. A Varimax orthogonal rotation was employed to aid interpretability. The rotated solution exhibited simple structure (Thurstone, 1947). The interpretation of the data was consistent with the exploratory and anxious-like behavioral attributes the metrics were designed to measure, with strong loadings of exploratory behavior on Component 1, and time spent grooming (a measure of anxious-like behavior) loading on Component 2.

Maternal Behavior

A two-way ANOVA was conducted to examine the effects of prenatal stress and lactation diet on the amount of time dams spent in the nest with their new pups. Data are mean +/- standard deviation, unless otherwise stated. Residual analysis was performed to

test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot; normality was assessed using Shapiro-Wilk's normality test for each cell of the design and homogeneity of variances was assessed by Levene's test. There were no outliers, residuals were normally distributed ($p > 0.05$, and there was homogeneity of variances ($p = 0.064$).

There was a statistically significant interaction between prenatal stress and lactation diet on nest time, F (1, 248) = 47.33, $p < 0.001$, partial $\eta^2 = 0.20$. Therefore, an analysis for the simple main effects for prenatal stress and lactation diet was performed with statistical significance receiving a Bonferroni adjustment and being accepted at the $p < .025$ level. There was a statistically significant difference in the amount of time dams spent inside their nest based on prenatal stress condition, $F(1, 248) = 24.99$, $p < 0.001$, partial $\eta^2 = 0.10$; but not based on lactation diet alone, $F(1, 248) = 0.12$, $p = 0.73$, $\eta^2 = 0.001$.

Inspection of the means revealed that stressed dams fed chow spent significantly more time moving outside of the nest than dams in the other three groups (see Figure 4).

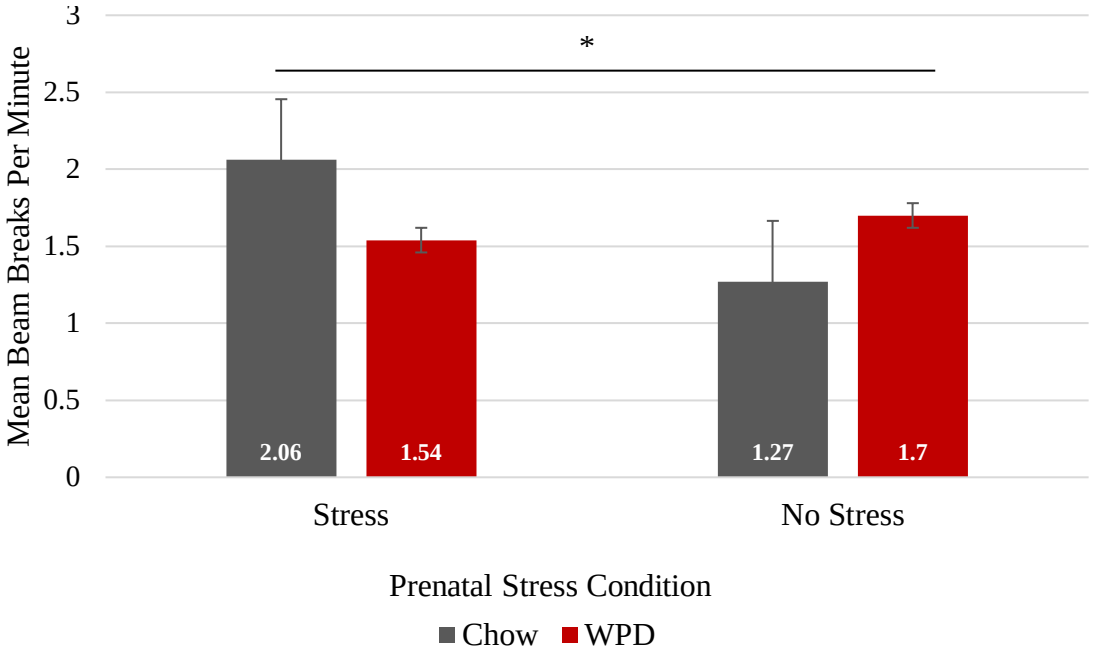


Figure 4. Maternal Movement outside of Nest by Prenatal Stress and Lactation Diet. WPD = Western-Pattern Diet. Error bars are standard errors.

Hypothesis 1: Maternal Condition (stress status and lactation diet) will predict the expression of Drd1 and Drd2 in the VTA and Nacc and Gr in the hippocampus in offspring.

To determine whether there were between-group differences in offspring gene expression of the receptors Drd1, Drd2, and Gr based on lactation diet and prenatal stress (maternal condition), a two-way multivariate analysis of variance with planned difference contrasts was conducted using maternal prenatal stress and lactation diet as the predictors, and Drd1, Drd2, and Gr as the outcome variables. Data were tested to ensure all assumptions of the test were met.

There was a statistically significant interaction effect between lactation diet and prenatal stress on the combined dependent variables, (F (5, 241) = 38.77, $p < .001$, *Wilks' Λ* = 0.55, partial $\eta 2$ = 0.45. Tests of between subjects effects demonstrated a statistically

significant interaction effect between lactation diet and prenatal stress for Drd2 expression, $F(1, 249) = 73.39$, $p < .001$, partial $\eta 2 = 0.23$; Drd1 expression, $F(1, 249) = 74.04$, $p < .001$, partial $\eta 2 = 0.23$; and Gr expression, $F(1, 249) = 60.51$, $p < .001$, *partial* $\eta 2 = 0.20$ As such, simple main effects of gene expression were explored for prenatal stress and lactation diet. There was a statistically significant difference in Drd2 expression ($F(1, 249) = 269.47$, $p < .0001$, *partial* $\eta 2 = 0.52$), Drd1 expression ($F(1, 249) = 261.99$, $p < .0001$, *partial* $\eta 2 = 0.52$), and Gr expression ($F(1, 249) = 159.03$, $p < .0001$, *partial* $\eta 2 = 0.39$) based on prenatal stress condition. Specifically, Drd2 expression scores for prenatally stressed offspring were 2.89 (*SE* = 0.18, *95% CI* [2.54, 3.24], $p < .001$) higher compared to non-prenatally stressed offspring; Drd1 expression scores for prenatally stressed offspring were 3.11 (*SE* = 0.19, *95% CI* [-3.49, -2.73], $p < .001$) lower compared to non-prenatally stressed offspring; and Gr expression scores for prenatally stressed offspring were 3.08 (*SE* = 0.25, *95% CI* [-3.57, -2.60], $p < .001$) lower compared to non-prenatally stressed offspring.

There was also a statistically significant difference in Drd2 expression ($F(1, 249) = 97.89$, $p < .0001$, *partial* $\eta 2 = 0.29$), Drd1 expression ($F(1, 249) = 90.99$, $p < .0001$, *partial* $\eta 2 = 0.27$), and Gr expression ($F(1, 249) = 39.76$, $p < .0001$, *partial* $\eta 2 = 0.14$) based on lactation diet. Specifically, Drd2 expression scores for lactation chow-fed offspring were 1.74 (*SE* = 0.18, *95% CI* [1.39, 2.09], $p < .001$) higher compared to lactation WPD-fed offspring; Drd1 expression scores for lactation chow-fed offspring were 1.83 (*SE* = 0.19, *95% CI* [-2.21, -1.45], $p < .001$) lower compared to lactation WPD-fed offspring; and Gr expression scores for lactation chow-fed offspring were 1.54

(*SE* = 0.25, *95% CI* [-2.02, -1.06], $p < .001$) lower compared to lactation WPD-fed offspring. Results are visualized in Figure 5.

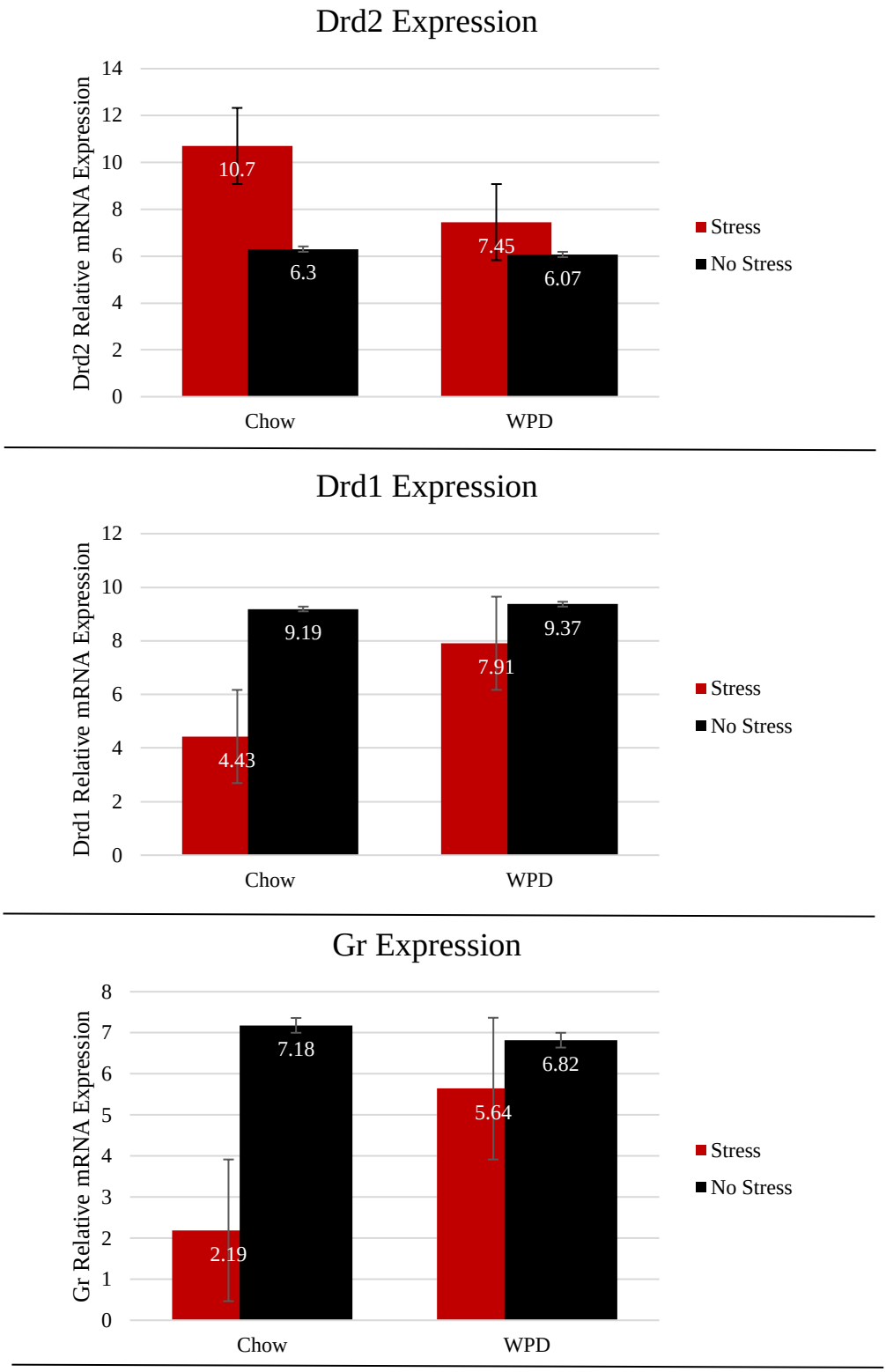


Figure 5. Gene expression comparisons by prenatal stress and lactation diet. Drd2 = VTA dopamine receptor d2, Drd1 = Nacc Dopamine receptor d1, Gr = hippocampal glucocorticoid receptor, WPD = Western-Pattern Diet. Error bars = +/- 2 SE

Hypothesis 2: Juvenile behavioral phenotype (anxious-like/exploratory and grooming behavior; represented by metrics measured from the OFT) will be predicted by sex and the interaction between maternal condition and gene expression.

Gene Expression. First, PCA with varimax rotation was utilized to as a dimension reduction technique for gene expression. Inspection of the correlation matrix showed that all variables had at least one correlation coefficient greater than 0.30. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.74, with individual KMO measures all greater than 0.70, indicating a classification of "middling" according to Kaiser (1974). Bartlett's Test of Sphericity was statistically significant (χ_2= 834.25, df = 3, p < .001), indicating that the data were likely factorizable.

PCA revealed a single component that had eigenvalues greater than one and which explained 91.98% of the total variance. Visual inspection of the scree plot further indicated that one component should be retained (Cattell, 1966). In addition, a one-component solution met the interpretability criterion. As such, one component was retained. Interpretation of the data was consistent with the previous analysis of individual gene expression by maternal condition, such that Drd2 expression was low when Drd1 and Gr expression was high.

Exploratory and Anxious-Like Behavior. Subjects whose diet remained consistent post-weaning (n = 114) were included in the analysis of hypothesis two. A linear mixed model was conducted using prenatal stress condition, lactation diet, and offspring sex as the predictors, the gene expression factor as a covariate, juvenile offspring anxious-like behavior as the outcome, and litter ID as the random effect. Fixed effects included sex, prenatal stress condition, lactation diet, the three-way interaction between prenatal stress condition, lactation diet, and gene expression on juvenile

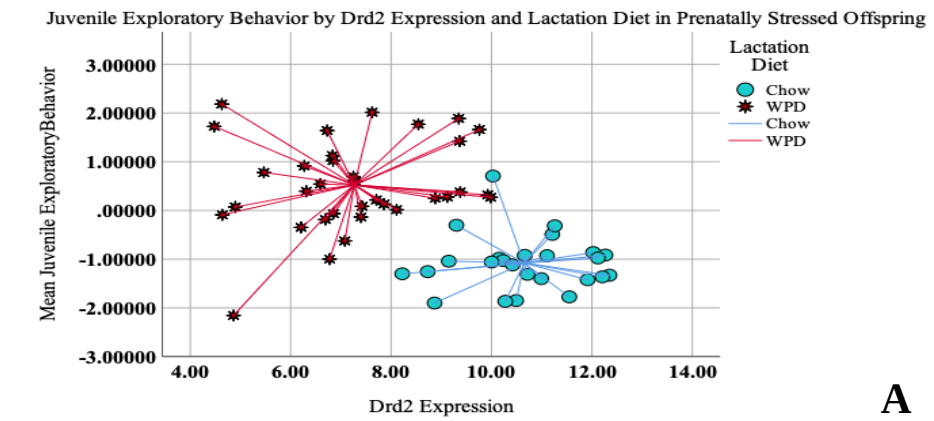
anxious-like behavior. The overall model explained 65% of the variance in offspring juvenile anxious-like behavior (R^2 = .65). Prenatal Stress ($F(3, 56) = 8.02$, $p = .007$) and lactation diet ($F(1, 62.8) = 6.40$, $p = .015$) significantly predicted juvenile anxious-like behavior, but sex did not ($F(1, 103.6) = .08$, $p = 0.79$). Additionally, there was a significant three-way interaction between gene expression, prenatal stress, and lactation diet on juvenile anxious-like behavior ($F(4, 103.5 = 4.59)$, $p = .002$). Specifically, this three-way interaction was a significant predictor of behavioral phenotype among offspring who were prenatally stressed and fed a chow lactation diet (see Table 4 and Figure 6). Post-hoc comparisons indicated that the chow-fed offspring of dams who were stressed displayed significantly more anxious-like behavior in the open field test than their non-stressed counterparts. However, the WPD-fed offspring of stressed dams displayed significantly less anxious-like behavior than their chow-fed and stressed counterparts and were not significantly different than their chow-fed and non-stressed counterparts (see Table 5, Figure 7).

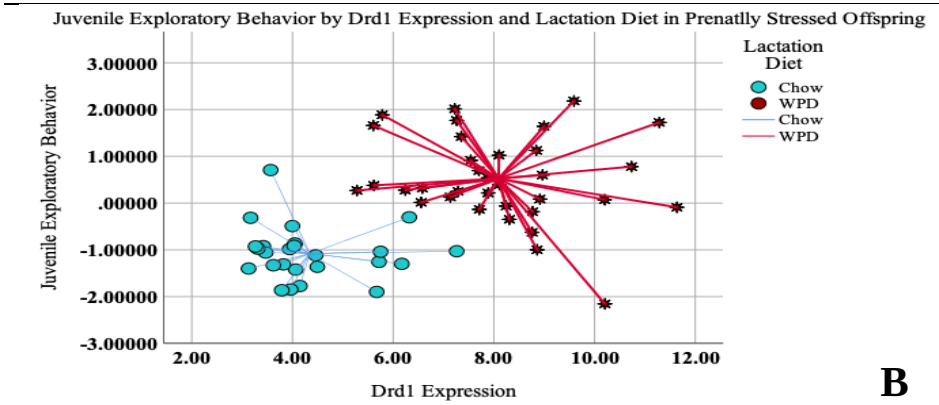
Grooming Behavior. A linear mixed model was conducted using prenatal stress condition, lactation diet, and offspring sex as the predictors, the gene expression factor as a covariate, juvenile offspring grooming behavior as the outcome, and litter ID as the random effect. Analysis of the fixed effects indicated no significant differences in juvenile grooming behavior between subjects (see Table 6).

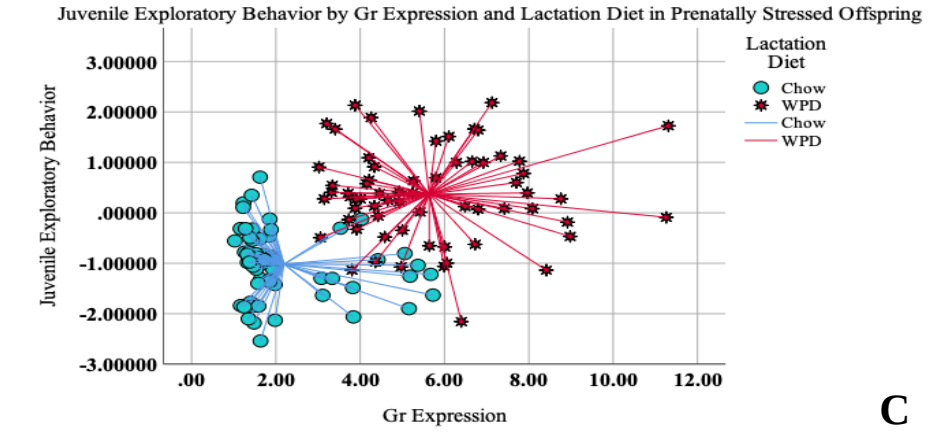
Table 4. Interaction between maternal condition and receptor gene expression on juvenile anxious-like behavior.

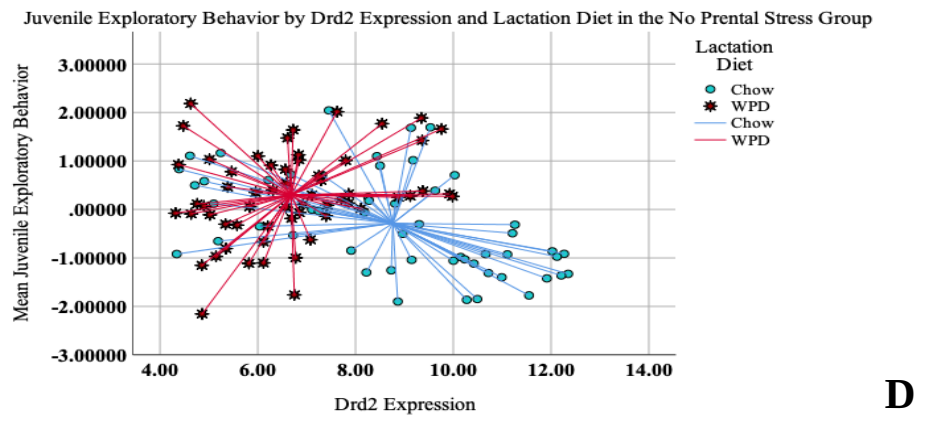
				95% Confidence Intervals				
Variable	**Effect**	***b***	***SE***	**Lower**	**Upper**	***df***	***t***	***p***
Sex	Male-Female	0.05	.17	-0.29	0.38	103.6	.28	0.702
Prenatal Stress	Stress-No Stress	-0.69	.25	-1.17	-0.21	43.7	-2.83	0.007
Lactation Diet	WPD-Chow	0.62	.25	0.14	1.10	43.8	2.52	0.015
PS*LD* GE	No Stress*Chow*Gene Exp Factor	-0.06	.21	-0.46	0.35	100.9	-0.29	0.775
PS*LD* GE	Stress*Chow* Gene Exp Factor	0.94	.16	0.64	1.25	59.8	6.06	<.001
PS*LD* GE	No Stress*WPD* Gene Exp Factor	-0.29	.22	-0.72	0.13	102.4	-1.36	0.176
PS*LD* GE	Stress*WPD*Gene Exp Factor	-0.07	.20	-0.46	0.33	93.5	-0.33	0.739

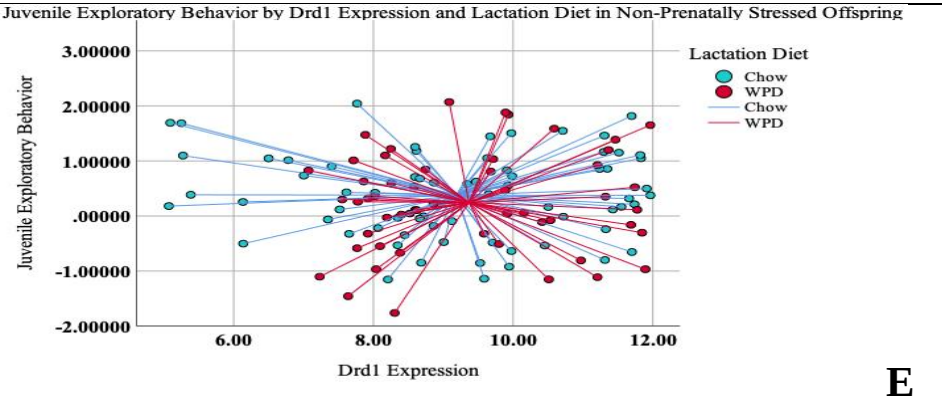
Note: WPD = Western-Pattern Diet; PS = Prenatal Stress, LD = Lactation Diet, GE = Gene Expression Factor.











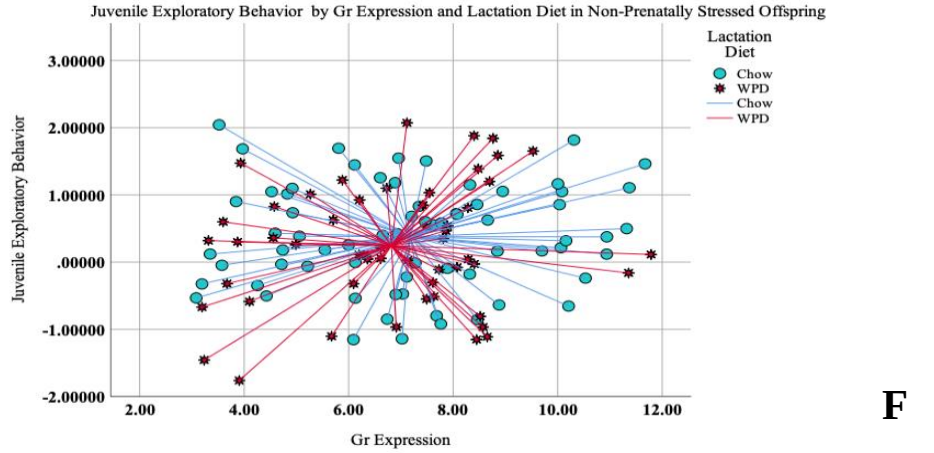


Figure 6. Juvenile Exploratory/Anxious-Like Behavior by Receptor Gene Expression, Lactation Diet, and Prenatal Stress Condition. Figures A-C represent expression in offspring who experienced prenatal stress. Figures D-F represent expression patterns in offspring who did not experience prenatal stress. Lower numbers indicate more anxious-like behavior.

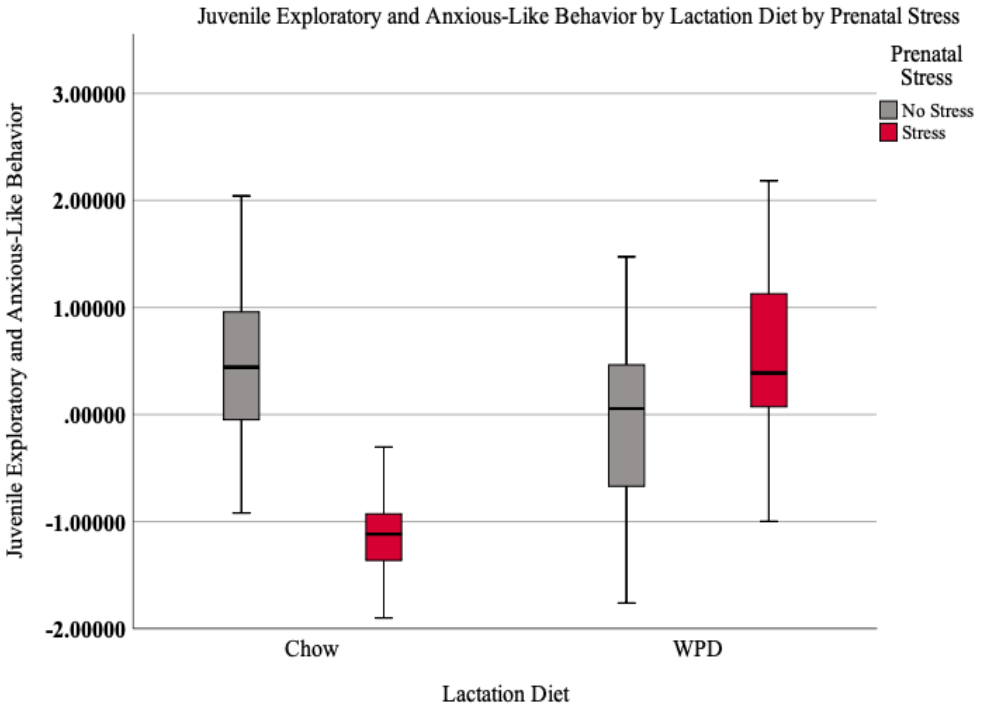


Figure 7. Mean juvenile exploratory/anxious-like behavior by lactation diet and prenatal stress. More negative numbers indicate more anxious-like behavior. **WPD** = Western-Pattern Diet.

Table 5. Post-hoc Comparisons of Juvenile Exploratory/Anxious-like Behavior by Lactation Diet, Prenatal Stress Condition, and Sex

Comparison		-	Comparison		Difference	*SE*	*t*	*df*	$p_{bonferroni}$
Lactation Diet	**Prenatal Stress**		**Lactation Diet**	**Prenatal Stress**					
Chow	No Stress	-	Chow	Stress	1.54	.24	6.39	66.9	<0.001
Chow	No Stress	-	WPD	No Stress	0.20	.26	0.75	55.2	0.457
Chow	No Stress	-	WPD	Stress	0.17	.25	0.59	55.5	0.507
Chow	Stress	-	WPD	Stress	-1.37	.26	-5.28	54.6	< 0.001
WPD	No Stress	-	Chow	Stress	1.34	.27	4.91	54.4	< 0.001
WPD	No Stress	-	Chow	Stress	-0.03	.28	-0.11	51.7	0.913

Note: Subject include only those mice whose diet was not switched at weaning (N = 114). Positive numbers indicate more activity. **WPD** = Western-Pattern Diet

Table 6. Juvenile Grooming Behavior by Sex, Prenatal Stress Condition, Lactation Diet, and Receptor Gene Expression.

				95% Confidence Interval				
Names	**Effect**	**Estimate**	***SE***	**Lower**	**Upper**	***df***	***t***	***p***
SEX Female	Female	0.2277	0.209	-0.182	0.637	97.4	1.0898	0.279
SEX Male	Male	0.0486	0.179	-0.302	0.399	73.5	0.2722	0.786
Prenatal Stress	Stress - No Stress	0.0269	0.363	-0.685	0.738	87.2	0.0740	0.941
Lactation Diet	WPD - Chow	-0.2246	0.367	-0.943	0.494	92.9	-0.6124	0.542
PS✻ LD ✻ GE	No Stress ✻ Chow ✻ Gene Factor	0.0143	0.276	-0.527	0.556	104.2	0.0517	0.959
PS✻ LD ✻ GE	Stress ✻ Chow ✻ Gene Factor	0.0998	0.307	-0.502	0.702	106.0	0.3248	0.746
PS✻ LD ✻ GE	No Stress ✻ WPD ✻ Gene Factor	0.0898	0.335	-0.567	0.746	101.3	0.2681	0.789
PS✻ LD ✻ GE	Stress ✻ WPD ✻ Gene Factor	-0.7131	0.263	-1.228	-0.199	95.3	-2.7163	0.008

Note: WPD = Western-Pattern Diet; PS = Prenatal Stress, LD = Lactation Diet, GE = Gene Expression Factor.

Hypothesis 3: Behavioral phenotype will persist into adulthood regardless of adult diet and maternal condition for males and females

Exploratory and Anxious-Like Behavior. A linear mixed model was conducted to test the effect of prenatal stress and lactation diet, post-weaning diet, and sex on change in behavioral phenotype from juvenility to adulthood. Juvenile behavior scores were subtracted from Adult behavior scores to create a behavior change score. Change in behavior was the outcome variable, prenatal stress and lactation diet condition, post-weaning diet, and sex were the predictor variables, and litter ID was used as the random effect variable. Subject weight was used as a covariate due to the likelihood

of it contributing to locomotion. Scores closer to zero indicate less change in behavior from juvenile to adulthood. Positive numbers indicate an increase in exploratory behavior and negative numbers indicate an increase in anxious-like behavior.

Inspection of the fixed effects indicated that post-weaning food condition by itself did not predict changes in offspring anxious-like behavior from juvenility to adulthood. However, there was a significant three-way interaction between maternal condition, sex, and post-weaning food condition on change in behavior from juvenility to adulthood ($F(3, 252) = 6.18$, $p < .001$). Further, females displayed less change in behavioral phenotype from juvenility to adulthood than males did overall ($M_{diff} = .47$, $SE = .09$, $t(252) = 5.13$, $p < .001$).

Females (see Table 7, Figure 8). Female offspring of stressed dams who were fed a chow lactation diet demonstrated a significant decrease in exploratory behavior (increase in anxious-like behavior) if their post-weaning diet was switched to a WPD compared to their non-stressed, chow-lactation-fed counterparts. Similarly, female offspring reared by stressed dams and fed a lactation WPD displayed a significant increase in anxious-like behavior if their post-weaning diet was switched to chow; alternatively, if there was no change in their post-weaning diet, they displayed a significant increase in exploratory behavior compared to their non-stress, lactation chow-fed counterparts.

Males (see Table 7, Figure 8). Male offspring of non-stressed dams who were fed a chow lactation diet displayed a significant increase in anxious-like behavior if they were switched to a WPD post-weaning compared to their stressed and lactation chow-fed

counterparts. S+WPD offspring displayed significantly less change in behavior (and more exploratory behavior) than the NS+C offspring regardless of post-weaning diet.

Table 7. Comparison of Exploratory Behavior Change by Maternal Condition, Post-Wean Diet, and Sex

Sex	Post-Wean Diet	Contrast		*b*	*SE*	95% CI Lower	95% CI Upper	*t*	*p*
Female	**WPD**	No Stress +WPD	No Stress + Chow	.33	.22	-.10	.76	1.51	.133
		Stress + Chow	No Stress + Chow	-1.72	.25	-2.21	-1.24	-7.02	< .001
		Stress + WPD	No Stress + Chow	.36	.20	-.04	.75	1.74	.079
	Chow	No Stress + WPD	No Stress + Chow	-.99	.23	-1.44	-.533	-4.28	< .001
		Stress + Chow	No Stress + Chow	-1.78	.29	-2.35	-1.21	-6.11	< .001
		Stress + WPD	No Stress + Chow	-1.71	.24	-2.18	-1.23	-7.05	< .001
Male	**WPD**	No Stress + WPD	No Stress + Chow	.13	.21	-.28	.55	.65	.519
		Stress + Chow	No Stress + Chow	.56	.27	.02	1.10	2.05	.041
		Stress + WPD	No Stress + Chow	1.29	.21	.88	1.71	6.14	< .001
	Chow	No Stress + WPD	No Stress + Chow	-.17	.23	-.63	.29	-.72	.472
		Stress + Chow	No Stress + Chow	-.12	.24	-.60	.36	-.49	.628
		Stress + WPD	No Stress + Chow	.99	.24	.52	1.46	4.15	< .001

Note: WPD = Western-Pattern Diet. *N* = 252.

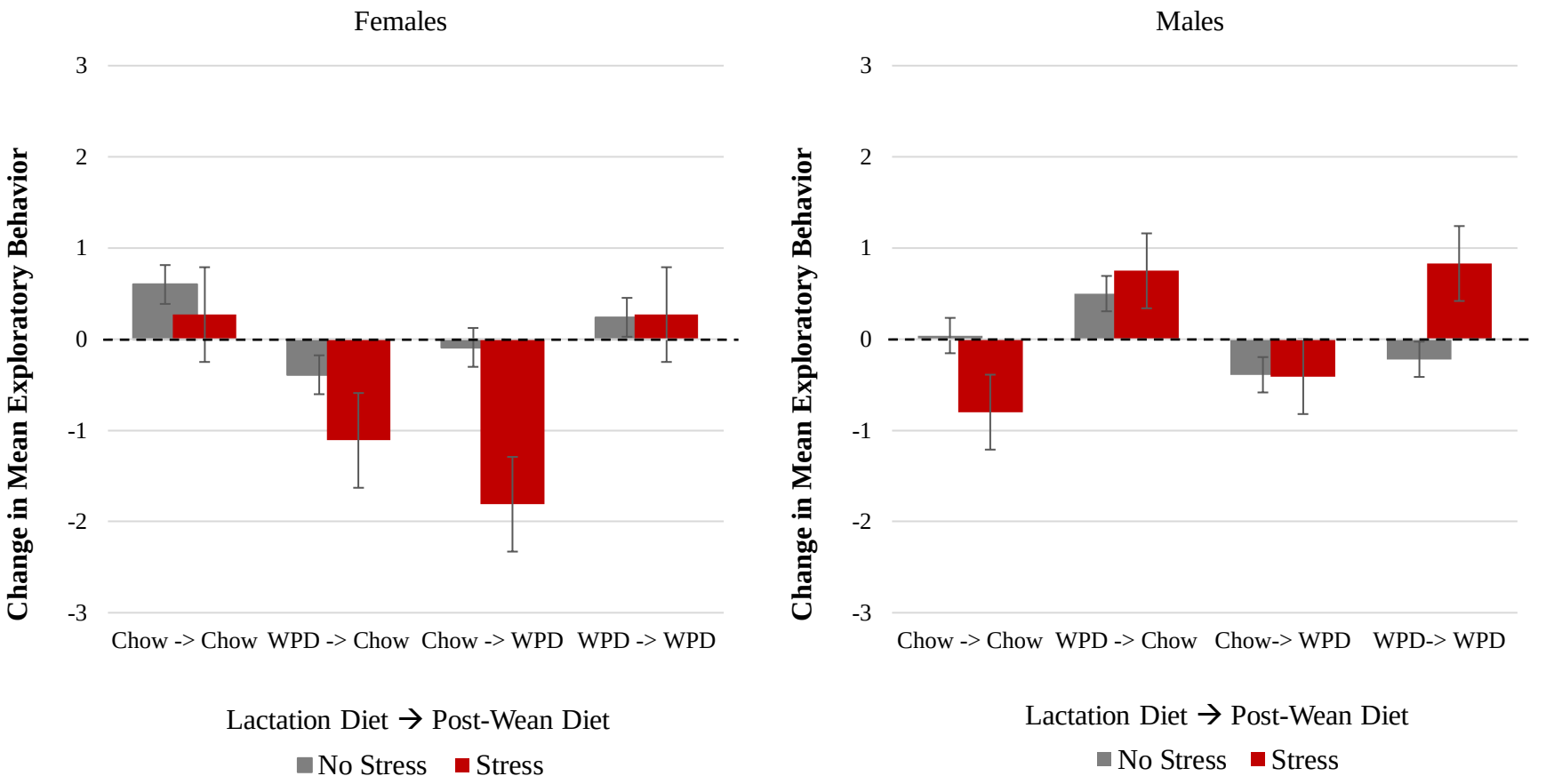


Figure 8. Change in exploratory and anxious-like behavior by prenatal stress condition, lactation diet condition and post-weaning diet condition for females and males. The dotted line indicates no change in behavior from juvenility to adulthood. Negative numbers indicate a decrease in exploratory behavior (or increase in anxious-like behavior). Error bars = +/- 2 SE.

Grooming Behavior. A linear mixed model was conducted to test the effect of maternal condition, post-weaning diet, and sex on change in behavioral phenotype from juvenility to adulthood. Juvenile Behavior scores were subtracted from Adult Behavior scores to create a Behavior Change score. Change in behavior was the outcome variable, maternal condition, post-weaning diet, and sex were the predictor variables, and litter ID was used as the random effect variable. Subject weight was used as a covariate due to the likelihood of it contributing to locomotion. Scores closer to zero indicate less change in behavior from juvenile to adulthood. Positive numbers indicate an increase in exploratory behavior and negative numbers indicate an increase in anxious-like behavior. Inspection of the fixed effects indicate that none of our predictors had an effect on changes in grooming behavior from juvenility to adulthood (See Table 8).

Table 8. Comparison of Grooming Behavior Change by Maternal Condition, Post-Wean Diet, and Sex

Variable	***F***	**Num df**	**Den df**	***p***
Maternal Condition	1.907	3	150	0.131
SEX	1.324	1	226	0.251
Food Condition	1.960	1	226	0.163
Maternal Condition ✻ SEX ✻ Food Condition	0.816	10	227	0.613

CHAPTER V

DISCUSSION

The current study provided further evidence that stress-related reductions in dopamine signaling may be mitigated by diet (Masi et al., 2001). Differences in anxious-like and exploratory behavior were reflected in the expression of Drd1 and Drd2 in the Nacc and VTA and Gr in the hippocampus. The impact of prenatal stress is typically associated with lower expression of Drd1 in the Nacc and lower expression of Gr in the hippocampus (e.g. Meaney, 2001; Champagne et al., 2001, 2003; Francis et al., 2000; Weaver et al., 2004; Zhang et al., 2005). In the current study, the offspring of stressed dams who were fed standard chow did, in fact, display this expected pattern of gene expression, along with a highly anxious-like behavioral phenotype. However, the offspring of stressed dams who were fed a WPD displayed *increased* Drd1 and Gr expression compared to their chow-fed counterparts, along with a more exploratory behavioral phenotype. Indeed, the pattern of gene expression and behavior observed among WPD-fed offspring of stressed dams was more similar to what was seen among the control group of chow-fed offspring of non-stressed dams.

These data suggest that diet and stress interact to have an effect on transgenerational plasticity, in which parents process and then transmit cues regarding the environment to their offspring. Offspring can utilize the information in these parental cues, integrate it with other relevant information (such as genes, personal experience,

etc.; Dall et al., 2015) to influence phenotypic development. In the current study, data suggest that maternal prenatal stress and diet served as a cue to dams and offspring regarding the environment. Dams altered their behavior depending on their stress and diet condition. Their resulting behavior and likely hormonal cascade served as transmitted environmental that offspring then interpreted and used to adjust their behavioral phenotype accordingly. It is also possible that while stress tends to serve as a cue for a dangerous or unpredictable environment, the presence of a high-energy WPD contradicted the stress cue and leading offspring to fail to adjust their behavioral phenotype to an unpredictable environment.

Approaching this question from an addiction perspective is useful in bridging the gap between results that demonstrate stress-buffering effects of highly palatable food (Levin, 1996; Buwalda et al., 2001; Pecoraro et al., 2004; Dallman et al., 2005) and that chronic stress leads to a reduction in extracellular dopamine and glucocorticoid receptor gene expression. For example, the idea that highly palatable food may have an impact on mesolimbic dopamine is consistent with evidence from addiction literature, which suggests that drugs of abuse reduce the stress response through their influence in mesolimbic dopamine activity (Bardo, 2013). While highly palatable food is not considered a drug of abuse, over-consumption of highly palatable food leads to similar patterns of neural activity and behavior that is routinely seen among drug users (Bardo, 2013). Rewarding substances, such as highly palatable food and drugs, all activate dopaminergic activity in the VTA to varying degrees. This increase in activity has

downstream effects on several other areas of the brain. Of importance to stress and reward or motivation are the dopamine activity in the Nacc and glucocorticoid activity in the hippocampus. This pattern of neural activity is reflected in our data. The diet-induced changes in dopaminergic signaling were correlated with changes in the expression of Gr, which suggests that a WPD may have the potential to mitigate stress-induced epigenetic changes that occur early in development. This is not to suggest that consuming highly palatable comfort food is a healthy or desirable form of stress management. As previously detailed, the neurobiological processes that occur during stress and feeding contribute to sustained negative reinforcement, leading to a short-term gain in well-being (stress reduction) with a long-term cost to health (i.e. obesity, metabolic disease)(Epel, Tomiyama, & Dallman, 2012). Instead, these results could help explain why certain individuals continue over-consuming highly palatable comfort food to their detriment, as well as why non-surgical weight-loss interventions have such a low success rate in the long-term (Hall & Kahan, 2018).

One unique aspect of the current study was that stress and diet were assessed from a developmental perspective. This approach has facilitated the furthering of knowledge by demonstrating that Drd1 and Drd2 expression in the mesolimbic pathway may impact the expression of hippocampal glucocorticoid receptor genes in the face of early developmental adversity, particularly for females. While behavioral phenotype was more consistent from juvenility to adulthood for females than it was for males, they appeared to be more affected by a change in adult diet than males were. For example, female

offspring of stressed dams displayed a marked increase in anxious-like behavior if their diet was switched at weaning, whereas their behavioral phenotype remained stable (no change from juvenility to adulthood) if their diet remained the same. For males, post-weaning diet did not make a significant difference in their behavioral phenotype. This was in opposition to my hypothesis that there would be no significant change in behavioral phenotype from juvenility to adulthood. One explanation for this could involve the level of maternal care provided to offspring. Previous work has demonstrated that males receive significantly more sensitive maternal care, such as licking and grooming, than do females (Gubernick & Alberts, 1985, Moore et al., 1997). More recently, Champagne and colleagues (2003) found no significant difference in the frequencies at which male and female pups were licked although the average frequency was higher for males. Nonetheless, greater frequency or less variability in the frequency of sensitive maternal behaviors toward male pups could potentially explain why females were more affected than males were.

The WPD-fed offspring of stressed dams displayed a marked imbalance between Drd1 and Drd2 expression in the VTA and the Nacc. Recent research has demonstrated that a Drd1/Drd2 imbalance could indicate sensitization of the mesolimbic system and contribute to addictive-like behavior (Dobbs et al., 2019). Thus, the WPD-fed offspring of stressed dams may be more susceptible than their non-stressed counterparts to engage not only in compulsive eating, but also potentially drug use. Especially due to the consistency between our results and those of de Jong and colleagues (2015), who found

that VTA Drd2 knockout rats demonstrate an increase in incentive motivation toward cocaine and highly palatable food. This further suggests the important role of Drd2 in compulsive eating and addictive behavior.

Potential Limitations and Future Directions

This study had several limitations. Some of the litters had very uneven sex distributions (e.g. a litter with 7 males and no females, or a litter with 6 females and one male). This uneven distribution may impact maternal behavior toward pups, which could subsequently impact offspring behavior and gene expression. Though the quantification of maternal behavior is incredibly laborious and difficult with mice, this study would have benefited from a more precise measure of maternal behavior. For example, Champagne and colleagues (2003) have assessed maternal behavior in C57bl/6 mice by successfully recording time spent nursing and grooming pups in the nest. They were successful at this difficult task, and transgenic mice are the gold standard for genetics work. However, a transgenic rat model may be better suited for developmental questions involving maternal behavior, simply because it is easier to assess maternal behaviors in rats.

We demonstrated that offspring of stressed dams displayed an imbalance between Drd1 and Drd2 expression in the VTA and Nacc, which is indicative mesolimbic sensitization and a potential predisposition to compulsive eating or drug use. Therefore, more work should be done to further examine the parallels between addiction and compulsive eating. Including an incentive learning paradigm to the current method could

provide more information regarding compulsive eating behavior and cross addiction with other substances by allowing us to test the reward value the animal is placing on a particular substance, and whether the animal is judging highly palatable food as rewarding as other drugs of abuse.

In addition to using an incentive learning paradigm to assess addictive-like behavior, it would also be useful to pursue the research questions posed in this project utilizing techniques that can better determine causality. While patterns of gene expression that correlate with behavior are a start, these results do not tell us for certain that the changes in expression observed in one candidate gene is for certain affecting the function of other candidate genes. It also cannot tell us the order in which these processes occur. However, in vivo chronoamperometry is a cutting-edge voltammetry technique that may provide more information in this regard. These voltammetry techniques are the only techniques currently available to measure real-time in-vivo neurotransmitter activity. Voltammetry techniques can be used to quantify release and clearance kinetics for biogenic amines in discrete brain regions (Daws, Owens, & Toney, 2016). It has been used to study the impact of drugs and stress on the function of neurotransmitters in vivo. Thus, it could be used in combination with gene expression to map the specific path of activation that stress-related highly palatable food consumption is inducing.

Conclusion

These results contribute to the mounting neurobiological and behavioral evidence of the similarities between a highly palatable WPD and drugs of abuse, and further

describe how stress and diet interact to have long-term consequences on behavior. There are potentially important implications for humans. For example, these data suggest that at least some forms of obesity originating in early development resemble addictive disorders at the molecular and behavioral level. If replicated in humans, these results could provide insight into developmental correlates of pathological addictive-like eating and inform intervention development. As previously mentioned, these results could also help elucidate why long-term weight loss is so difficult to achieve. Although physiology plays a major role in the difficulties of long-term weight-loss (Hall & Kahan, 2018) , at least part of the story may concern the impact of a WPD on a stressed and developing brain. Obesity and addiction are both heterogenous conditions. However, these data suggest that efforts to reduce pathological eating behavior might benefit from approaches that have been successfully employed with other addictive substances, such as pharmacological intervention. Further, policymakers and researchers could make an impact by shifting their focus to groups of mothers who are particularly prone to stress, such as those mothers of lower socioeconomic status. Individuals susceptible to this stress-eating phenotype will face challenges that addicts of other drugs of abuse so not. A recovering drug addict can completely abstain from drug use and remove themselves from environments laden with their drugs of abuse. An individual with compulsive-like eating tendencies must still eat to survive, and they are surrounded by obesogenic messages. So, while it is possible to target groups of people who would be most

susceptible to developing compulsive-like eating, it is a much more difficult job to change the way an entire society thinks about food.

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VITA

Nikki Jo Clauss

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE IMPACT OF A WESTERN-PATTERN DIET ON THE INTERACTION OF PRENATAL STRESS, MATERNAL BEHAVIOR AND OFFSPRING PHENOTYPE

Major Field: Psychology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Psychology at Oklahoma State University, Stillwater, Oklahoma in December, 2019.

Completed the requirements for the Master of Science in Psychology at Oklahoma State University, Stillwater, OK/USA in 2017.

Completed the requirements for the Bachelor of Science in Psychology at University of Wisconsin, Green Bay, WI/USA in 2012.

Experience:

Research Experience

- Biobehavioral and developmental correlates of the HPA-axis stress response

Skills and Techniques

- Rodent husbandry
- Enzyme-linked immunosorbent assay (ELISA) protocols
- Isolation of RNA and analysis by transcriptional profiling
- Quantitative PCR
- Assessment of heart rate variability and respiratory sinus arrhythmia using BIOPAC hardware
- Behavior Analysis