EFFECTS OF 2-HYDROXYESTRADIOL ON BINGE EATING IN RATS

A Dissertation in
Physiology

by

R. Keith Babbs

© 2012 R. Keith Babbs

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2012
The dissertation of R. Keith Babbs was reviewed and approved* by the following:

Rebecca L. Corwin  
Professor of Nutritional Neuroscience  
Dissertation Advisor  
Chair of Committee

John E. Hayes  
Assistant Professor of Food Science  
Director of Sensory Evaluation Center

Barbara J. Rolls  
Professor of Nutritional Sciences  
Helen A. Guthrie Chair

Erica Unger  
Research Scientist in Nutritional Sciences

Donna H. Korzick  
Associate Professor of Physiology and Kinesiology  
Chair, Intercollege Graduate Degree Program in Physiology

*Signatures are on file in the Graduate School
ABSTRACT

One conundrum of binge eating is that women are more likely to suffer from binge-related eating disorders, even though estradiol decreases food intake. 2-hydroxyestradiol (2OHE2), an estrogen metabolite, may account for the contradiction, due to possible interference with dopamine (DA) signaling. Therefore, a series of studies was conducted in order to investigate the effects of 2OHE2 on binge eating in an established rat model.

Research presented in Chapter 2 investigated the acute effects of 2OHE2 on bingeing. Two cohorts (1 male, 1 female) of 34 non-food-deprived rats were each separated into two groups: 1) daily control (D) (received an optional source of dietary fat for 20 min every day) or 2) bingeing (INT) (received fat intermittently, i.e. 20 min on Mon, Weds, Fri). During the 5-week binge induction period, shortening intakes escalated over the 5-week binge induction period significantly faster in females than in males, such that males consumed significantly less fat relative to body mass than did females after 5 weeks. This result is consistent with the idea that biological differences contribute to sex differences in bingeing. Rats of both sexes were then injected with 2OHE2 (1.0, 3.0, and 10.0 μg/kg intraperitoneally), vehicle, or 2-methoxyestradiol (2ME2) immediately prior to fat access. Fat intake was significantly stimulated by 2OHE2 only in the INT rats (p<0.03). Furthermore, this effect seemed to be more subtle in females than in males. The results indicate that 2OHE2 can acutely exacerbate intake in a bingeing rat. Additionally, results suggest that females may escalate intakes faster due to endogenous hormones.
Research presented in Chapter 3 investigated the effects of chronic administration of 2OHE2 on bingeing, and the effect of 2OHE2 on DA efflux in the prefrontal cortex (PFC) of bingeing rats. In study 1, three groups of female rats (n=16 per group) were used. Group one was ovariectomized (OVX) and implanted with an osmotic mini-pump containing 2OHE2 (E); group two underwent OVX surgery and received a vehicle implant (O); group three underwent sham surgery and received a vehicle implant (I). All rats were exposed to the INT protocol described above. After four weeks, shortening intake escalated more quickly in the E rats than in either the O or I rats. Additionally, during week 4, E rats ate significantly more shortening than did I rats, and significantly less chow than either O or I rats. The results indicate that 2OHE2 can exacerbate binge escalation and shift intake from more homeostatically-driven eating to more hedonic eating.

In study 2, male rats (n=6) were exposed to a minimum of 5 weeks of the INT protocol before they were implanted with a microdialysis guide cannula targeting the PFC. DA efflux was recorded before, during, and after presentation of shortening and intraperitoneal injection of either 2OHE2 (3.0 μg/kg) or vehicle. 2OHE2 abolished the shortening-evoked DA efflux that was present after injection with vehicle, and significantly enhanced shortening intake. These results demonstrate that 2OHE2 can alter DA signaling in the PFC of bingeing rats.

The results of the present studies indicate that 2OHE2 can exacerbate intake both acutely and chronically in a rat model of bingeing. These effects may due, in part, to the effects of 2OHE2 on DA signaling in the PFC. Together this suggests a novel mechanism explaining the increased risk for binge-related eating disorders in females.
# TABLE OF CONTENTS

LIST OF TABLES.............................................................................................................vii

LIST OF FIGURES.........................................................................................................viii

CHAPTER 1 LITERATURE REVIEW.................................................................................1

1. Binge Eating and Binge-Related Disorders.........................................................2
2. Sex Differences in Bingeing.................................................................................3
3. Involvement of Dopamine Signaling.....................................................................5
4. Interactions of 2-hydroxyestradiol and DA..........................................................8
5. Aims and Hypotheses...........................................................................................12

CHAPTER 2 ACUTE EFFECTS OF 2-HYDROXYESTRADIOL ON BINGEING........23

1. Introduction..........................................................................................................24
2. Materials and Methods.......................................................................................25
3. Results................................................................................................................27
4. Discussion...........................................................................................................30
5. References...........................................................................................................36

CHAPTER 3 BEHAVIORAL AND NEURONAL EFFECTS OF
2-HYDROXYESTRADIOL ON BINGEING.................................................................41

1. Introduction..........................................................................................................42
2. Materials and Methods.......................................................................................43
3. Results................................................................................................................48
4. Discussion

5. References

CHAPTER 4 SUMMARY AND CONCLUSIONS

1. Goals, Aims, Conclusions

2. Speculation, Limitations, and Future Directions

3. References
LIST OF TABLES

CHAPTER 1

Table 1.1. The Lifetime Prevalence of Binge Eating and Related Disorders 3

CHAPTER 2

No tables

CHAPTER 3

Table 3.1. Weight change and food intake across the four week study. 49

CHAPTER 4

Table 4.1. Possible 2OHE2 mechanisms 70
LIST OF FIGURES

CHAPTER 1

Figure 1.1. The VTA, PFC, and NA .............................. 5
Figure 1.2. The proposed mechanisms by which 2OHE2 may affect DA signaling .............................. 11

CHAPTER 2

Figure 2.1. Escalation of normalized shortening intake across the 5-week binge induction period .............................. 29
Figure 2.2. The effect of 2OHE2 on 20-minute shortening intake in male and female rats .............................. 30
Figure 2.3. The possible mechanism by which 2OHE2 could increase DA availability in the prefrontal cortex .............................. 34

CHAPTER 3

Figure 3.1. Escalation in normalized shortening intake across the 4-week binge induction period .............................. 50
Figure 3.2. Week 4 average shortening and chow intake .............................. 51
Figure 3.3 Week 4 shortening to chow ratio .............................. 51
Figure 3.4. Shortening-evoked DA efflux in the PFC of bingeing rats .............................. 52

CHAPTER 4

Figure 4.1. The proposed mechanism by which 2OHE2 enhances binge eating .............................. 69
CHAPTER 1

LITERATURE REVIEW
1. Binge Eating and Binge-Related Disorders

Binge eating is defined as the consumption of more food in relatively brief periods of time than most individuals would eat under similar circumstances. This consumption is associated with a sense of lack of control during the bingeing episode [1]. Thus, bingeing can be conceptualized as an intermittent loss of control over the homeostatic controls of meal size. In its most severe forms, binge eating characterizes the binge-related eating disorders, such as bulimia nervosa (BN), binge-eating/purging subtype anorexia nervosa (AN), or binge eating disorder (BED). Based on criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), diagnosis with either BN or BED requires that an individual has recurrent episodes of binge eating that occur at least twice a week for three months or six months, respectively [1]. Additionally, BN criteria include some sort of compensatory behavior to prevent weight gain, such as vomiting, fasting, excessive exercise, or use of purgative drugs such as laxatives. This behavior is absent in diagnoses of BED [1]. Even if individuals do not meet DSM-IV criteria for an eating disorder, they may still engage in subthreshold or subclinical bingeing.

A recent report from the National Comorbidity Survey Replication indicates that approximately 4.5% of people in the United States, or nearly 14 million people, will engage in some form of bingeing during their lifetimes. This is concerning, considering the high rate of binge-related comorbidities. Approximately 76% of those who binge suffer additional disorders in the form of anxiety, substance abuse, or mood disorders, and as many as 63% of binge eaters seek treatment for emotional problems [2]. Among obese patients, those who binge eat are significantly more likely to suffer from impulse...
control disorders than are non-bingers [3]. A recent report found that 27.5% of a subpopulation of patients with BED had suicidal thoughts, while 12.5% had actually attempted suicide [4]. Binge eating has also been reported by many to affect the ability to function in home or work environments [2]. In addition, an estimated 64% of those seeking bariatric surgery are binge eaters. These patients have a higher incidence of post-surgical bingeing and poorer weight loss and weight regain outcomes [5]. In summary, binge eating has far-reaching effects with both personal and social consequences. Therefore, research that will enhance our understanding of the risks and causes of binge eating is warranted and is the goal of the research presented in this dissertation.

2. Sex Differences in Bingeing

Both men and women binge eat. In fact, there is no significant difference between the lifetime prevalence of bingeing between men (4.0%) and women (4.9%) [2]. However, sex differences exist in the prevalence of the subtypes of binge eating and its related disorders, as shown in Table 1.1.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulimia Nervosa</td>
<td>0.5%</td>
<td>1.5%*</td>
</tr>
<tr>
<td>Binge Eating Disorder</td>
<td>0.8%</td>
<td>1.6%*</td>
</tr>
<tr>
<td>Subthreshold Binge Eating</td>
<td>1.9%</td>
<td>0.6%*</td>
</tr>
</tbody>
</table>

*Significant sex difference based on a 0.05 level [2].

As shown in Table 1.1, males are approximately three times more likely to experience subthreshold binge eating than are females. In contrast, females are approximately three times more likely to suffer from BN, and are twice as likely to suffer from BED than are males. These trends indicate that females are at a higher risk for the
more severe forms of bingeing, and therefore are more likely to meet the frequency and length-of-time requirements of the clinical diagnoses. Additionally, although men are more likely to report overeating than women (26% to 18%, respectively), women are more likely to report loss of control during eating episodes than men (29% to 20%) [6].

The increased risk for the more severe forms of bingeing in females seems to contradict evidence that estrogen is protective. In fact, replacement of estradiol in a cyclic manner decreases meal size, overall food intake, and/or body weight gain in ovariectomized (OVX) rats under non-binge [7, 8] and binge [9] conditions. Furthermore, effects of estradiol appear to be mediated by the satiating gut hormone, cholecystokinin (CCK), which could contribute, in part, to its ability to reduce meal size [10, 11]. Evidence from research in humans also suggests a protective role against bingeing for estrogen. Decreases in estradiol during the menstrual cycle have been associated with increases in food intake [12, 13] and binge frequency [14-16].

Given the fact that women have higher endogenous levels of circulating estrogens, it seems intuitive that women should have decreased risk for severe forms of bingeing, relative to males. Since this is not the case (and in fact, the opposite is true), it raises the question: “How can women have higher rates of binge-related disorders given their higher levels of estrogen?” The answer may involve dopamine (DA) signaling and its interaction with a metabolite of estrogen. This idea is the focus of the research in this dissertation.
3. Involvement of Dopamine Signaling

For many years, DA signaling in various brain regions has been implicated as a key contributor to neural mechanisms of reward [17-19], including food reward [20, 21]. Among these brain areas are the prefrontal cortex (PFC) and the nucleus accumbens (NA), which are terminal regions of dopaminergic projections from the ventral tegmental area (VTA), the major source of DA involved reward signaling (Figure 1.1).

![Figure 1.1. The VTA, PFC, and NA. The ventral tegmental area (VTA) has dopaminergic projections to the prefrontal cortex (PFC), and the nucleus accumbens shell (NAs), and core (NAc). The PFC has glutamatergic projections to the NAc and NAs.](image)

The VTA contains the cell bodies of dopamine neurons that project to the ventral striatum (which contains the NA and other brain regions) and to the PFC [22]. In response to novel stimuli or reward-associated cues, VTA neurons fire and release DA in the terminal regions [21]. Release of DA from the VTA in the PFC is thought to be a marker for reward-related learning and memory [18, 23], and has been linked to changes associated with addiction [19, 24]. DA release in the NA occurs in response to numerous
stimuli, including feeding, drinking, direct brain stimulation, and drugs of abuse [25].

Because of its anatomical and functional links to brain regions responsible for reward and to those responsible for movement, the NA has been described as translating “motivation to action” [26], and is therefore critically associated with motivated behavior [27]. Additionally, the NA, which is subdivided into the shell (NAs) and core (NAc), receives glutamatergic input from the PFC [22]. Pharmacological blockade of glutamate transmission in the NA, specifically in the shell, results in robust feeding [28]. In summary, conditions that reduce activation of glutamatergic cell bodies in the PFC could reduce glutamatergic transmission in the NA, and contribute to binge-type eating. Furthermore, altered DA signaling in the PFC could contribute to binge-type eating by reducing glutamatergic transmission in the NA.

Alterations in DA signaling have been associated with changes in energy balance. Many studies have shown the importance of DA signaling in diet-induced weight gain [29, 30]. Specifically, blockade of dopamine 2-like (D2) receptors in the NAc results in increased feeding duration in rats [31], and decreased DA levels caused larger meal size in DA knock-down mice [32]. Additionally, increased D2 sensitivity by long-term use of antipsychotics has been associated with weight gain [33, 34]. Others have shown that obesity in humans is associated with decreased D2 availability in the striatum [35]. This effect is reversed after gastric bypass-induced weight loss [36], suggesting that obesity, or overeating, may have been a cause, rather than the result, of the D2 reduction. Although it is currently unclear if this relationship is causal or correlational, the available evidence indicates involvement of D2 in obesity.
In addition to its involvement in weight gain, several studies have shown a link between alterations in DA signaling and binge-related eating disorders. For instance, the major DA metabolite, homovanillic acid (HVA), is lower in the cerebrospinal fluid of bulimics [37, 38], suggesting a decrease in DA in these individuals. Raclopride, a D2 antagonist, increases shortening intake in bingeing rats, but not in control rats [39], and increases intake of fat/sugar emulsions in bingeing rats, while decreasing intake in controls [40]. Intermittent (binge) access to sucrose for 21 days repeatedly increases sucrose-induced DA efflux in the NA [41]. This effect is not seen in rats that have ad libitum access to sucrose, or those that receive sucrose on days 1 and 21 only. DA has also been shown to increase in the NA in response to eating and drinking after food deprivation [42]. This is relevant to eating disorders because binge eating in humans often occurs after periods of food restriction [43]. These studies indicate a difference in DA signaling between normal intake of palatable food, and intake under binge-type conditions.

DA release in the PFC and NAc, but not the NAs in response to non-binge-type consumption of palatable food has been found to occur without habituation [44]. This is an important finding because it suggests that rewarding stimuli, such as ingestion of palatable food would repeatedly stimulate dopaminergic firing in the PFC and NAc. Importantly, research suggests that repeated stimulation of striatal regions in response to palatable foods can decrease dopamine 2-like (D2) receptor binding [45], thereby altering the physiology associated with reward. Furthermore, decreased D2 receptor density in the NA caused by binge administration of cocaine is coupled with an increase of G-protein activity induced by D2 receptor activation [46]. Taken together, it is possible that
repeatedly bingeing on highly palatable foods may alter D2 signaling in other reward-related areas, such as the PFC.

4. Interactions of 2-hydroxyestradiol and DA

$17\beta$-estradiol (E2), the major active estrogen, undergoes a series of enzymatic conversions during its metabolism. The first step in this process involves one of the cytochrome P450 enzymes, which can convert the parent compound (E2) to 2-hydroxyestradiol (2OHE2) [47]. Available evidence suggests that 2OHE2 may have effects that alter DA signaling and can therefore affect binge behavior. Figure 1.2 summarizes these possible mechanisms. In the PFC, dopamine 1-like (D1) and D2 receptors modulate the activity of glutamatergic pyramidal cells in response to DA binding. D1 receptors are activated by prolonged, moderate levels of DA, whereas D2 receptors are activated by transient, high levels as when presented with a highly palatable food, such as during a binge episode [48]. There is evidence to support the idea that 2OHE2 may influence D2 receptor signaling via direct, as well as indirect, actions. 2OHE2 has been shown to bind directly to D2 receptors in the pituitary with almost as much affinity as DA [49], but whether this binding is activational or inhibitory remains controversial. Nonetheless, it is reasonable to speculate that 2OHE2 may bind to D2 receptors in other brain regions, such as the PFC and NA, as well. If this binding is activational, then 2OHE2 could either activate postsynaptic D2 receptors, which would decrease cAMP and attenuate glutamatergic activity (Fig 1.2A), or activate presynaptic autoreceptors, which would inhibit DA release (Fig 1.2B) [50].

2OHE2 could also influence DA signaling via inhibition of COMT, the enzyme responsible for synaptic degradation of DA. Because 2OHE2 is also degraded by COMT
to form the metabolite 2-methoxyestradiol (2ME2), and because its affinity for COMT is higher than that of DA [53], 2OHE2 may competitively inhibit the enzyme and therefore increase DA availability (Fig 1.2C). Supporting this idea, research has shown that pharmacological inhibition of COMT with tolcapone increases DA in the PFC in response to food anticipation [54]. The PFC would be particularly sensitive to the effects of COMT inhibition, because elimination of DA from the synapse via DA reuptake is limited by the relatively low number of dopamine transporters (DAT) in the PFC, compared to other regions [51, 52]. Therefore, degradation of DA by the enzyme COMT is especially important in the PFC. Others have shown that activation of D2 receptors at high concentrations of DA in the PFC inhibits the firing of glutamatergic pyramidal cells [55], which are known to project to the nucleus accumbens [56, 57]. Because inhibition of glutamate receptors in the nucleus accumbens has been shown to stimulate feeding in sated rats [28], it is possible that post-synaptic D2 activation in the PFC, as a result of COMT inhibition and the resulting increased DA concentration, exacerbates binge intake.

Another way that 2OHE2 could modulate DA signaling is by its effect on adenylyl cyclase. Activation of D1 receptors causes increased adenylyl cyclase activity and therefore, increased levels of the second messenger cAMP. Conversely, D2 receptor activation inhibits adenylyl cyclase, lowering cAMP and acting in opposition to D1 receptors. In this way, D1 receptor activation is thought to be activational, while D2 receptor activation is thought to be inhibitory. 2OHE2, like D2 receptor activation, has been shown to inhibit adenylyl cyclase activity and lower intracellular cAMP (Fig 1.2D). Importantly, this effect was markedly less potent or absent altogether with the parent compound, E2, or the metabolite 2ME2 [58], and occurs independently of D1 or D2
receptors [59]. Taken together, this suggests that 2OHE2 may either enhance DA’s effect at D2 receptors, or may act independently of DA to mimic D2 receptor-mediated signaling. In this way, 2OHE2 may exacerbate the signaling cascade that is thought to occur during bingeing.

In addition to affecting the signaling cascade following D2 receptor activation, 2OHE2 may also affect D2 receptor density. 2OHE2 can shift in the ratio of high affinity to low affinity dopamine receptors in the striatum by increasing D2 receptor density [60]. Additional research has demonstrated that E2 causes decreased (short-term), followed by increased (long-term) numbers of D2 receptors in the striatum [61]. This effect was not seen with acute injections of 2OHE2, though it is possible that the very short half-life (<1 min) [62] of the metabolite accounts for this difference. It is currently unknown whether chronic exposure to 2OHE2, which occurs in vivo due to degradation of circulating E2, can alter D2 receptor density. One goal of the research in this dissertation was to address this gap in the current knowledge. This is important, because alterations in the number of dopamine receptors would support the idea that 2OHE2 can cause lasting changes that exacerbate risk for bingeing.

The overarching goal of the research described in this dissertation was to better understand the contributions of the estrogen metabolite 2-hydroxyestradiol to binge eating, using both behavioral and neurochemical approaches. In particular, the ability of 2OHE2 to increase binge size, and its effects on dopamine efflux and D2 receptor number were investigated.
Figure 1.2. The proposed mechanisms by which 2OHE2 may affect DA signaling. (A) 2OHE2 may bind to postsynaptic D2 receptors and activate them to decrease cAMP. (B) 2OHE2 may inhibit DA release by binding to autoreceptors. (C) 2OHE2 may increase DA availability by inhibition of COMT. (D) 2OHE2 may inhibit cAMP production (through inhibition of adenylyl cyclase) independently of DA receptors. (E) 2OHE2 may increase D2 receptor density, enhancing the effect of DA signaling.
5. Aims and Hypotheses

Aim 1. To determine the effect of acute 2-hydroxyestradiol (2OHE2) on shortening intake in bingeing rats.

Hypotheses:

1) Acute intraperitoneal injections of 2OHE2 will increase shortening intake in bingeing animals.
2) 2OHE2 will have no effect on the shortening intakes of non-bingeing, control rats.

Interpretation of Outcomes: An increase in binge intake after acute injection of 2OHE2 will support the idea that the metabolite can contribute to increased binge risk in females. Lack of effect in non-bingeing rats will suggest that 2OHE2 does not acutely cause bingeing, but instead exacerbates intake once bingeing has been established.

Aim 2. To determine if chronic exposure to 2OHE2 can increase binge risk.

Hypotheses:

1) Rats receiving implants of 2OHE2 will have higher and faster escalations in shortening intake relative to rats receiving vehicle implants.
2) Rats with 2OHE2 implants will eat more shortening at the end of the binge induction period than rats with vehicle implants.
Interpretation of Outcomes: Increased escalation and greater shortening intakes in rats treated with 2OHE2 will support the idea that the metabolite can enhance progression of bingeing when environmental conditions support bingeing.

Aim 3. To determine effects of 2OHE2 on dopamine (DA) signaling in the prefrontal cortex (PFC).

Study 1: To determine the effect of acute 2OHE2 injections on DA efflux in the PFC using brain microdialysis in awake behaving rats.
Hypotheses:

1) Presentation of shortening to bingeing rats will stimulate DA efflux in the PFC.

2) Acute injection of 2OHE2 will significantly enhance shortening-evoked DA efflux in the PFC of bingeing rats.

Study 2: To determine the effect of chronic exposure to 2OHE2 on D2 receptor density in the PFC.
Hypothesis:

Rats receiving implants containing 2OHE2 will have higher levels of D2 receptor protein in the PFC, relative to rats receiving vehicle implants.

Interpretation of outcomes: Increased PFC DA efflux in response to shortening will support the idea that the phenomenon of bingeing is related to reward circuitry. Enhanced DA efflux after acute administration of 2OHE2 will indicate that the metabolite can exacerbate bingeing by altering DA signaling. Higher D2
density in rats exposed to chronic 2OHE2 will indicate that the metabolite has long-term effects on DA signaling which may increase risk for bingeing.
References


CHAPTER 2

ACUTE EFFECTS OF 2-HYDROXYESTRADIOL ON BINGEING

INTRODUCTION

As described above in the literature review, binge eating is defined as the consumption of more food in relatively brief periods of time than most individuals would eat under similar circumstances accompanied by a sense of loss of control. Bingeing characterizes eating disorders such as bulimia nervosa (BN) and binge eating disorder (BED) [1]. Additionally, even if individuals do not meet DSM-IV criteria for these disorders, they may still engage in subthreshold bingeing. The total lifetime risk of any form of bingeing is almost 1 in 20 [2], meaning that approximately 14 million Americans will suffer the emotional, personal, and social consequences associated with binge eating.

An important consideration in the study of binge eating is the presence of sex differences. While it is true that both men and women binge, women are more than twice as likely to develop binge-related eating disorders such as BN and BED [2]. A conundrum exists, however, in that women have higher levels of estradiol (E2), which is known to decrease meal size in rats [3, 4] and binge frequency in humans [5], and is therefore thought to be protective. The explanation for this apparent contradiction may involve one of the metabolites of E2, 2-hydroxyestradiol (2OHE2) and its effects on and interactions with dopamine (DA). Specifically, 2OHE2 may competitively inhibit degradation of DA [6] and/or mimic or enhance D2 receptor actions [7-10]. Previous literature has suggested that disruptions in DA signaling are present in bingeing-related eating disorders such as bulimia [11, 12].

The mechanism accounting for increased risk of developing binge-related eating disorders in females is not presently understood. The goal of the current study was to investigate possible causes of sex differences in binge-type eating by administration of
the estrogen metabolite 2OHE2 using a non-food-deprived rat bingeing model that demonstrates face, construct, and predictive validity [13-15].

MATERIALS AND METHODS

Animals

Male and female rats were used in separate cohorts. For each cohort, 34 Sprague Dawley rats (Harlan, Indianapolis, IN), 60 days of age, were individually housed in hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. All rats had ad libitum access to a nutritionally complete commercial laboratory rodent diet at all times during the study (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging metal food hoppers at the front of the cage. Tap water also was freely available. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

After seven days of adaptation to the vivarium, body weights were recorded and solid vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH) was provided during a single overnight period, in addition to the continuously available chow. In each cohort, two groups of 17 rats each were then matched by body weight and the amount of shortening consumed [t-test; \(p>0.05\) for both measures]. All bingeing and drug administration procedures were consistent across the two cohorts.

Bingeing Procedure

After grouping, rats were given limited access to shortening in a glass jar clipped to the front of the cage 2-3 hours prior to the start of the dark cycle. Chow was available
during the shortening access period and at all other times. One group was given shortening every day (D) and the other group was given shortening intermittently, i.e. only on Mondays, Wednesdays, and Fridays (INT). To maximize exposure early in the experiment and to decrease the effects of neophobia, fat was available for longer periods in the first week and availability was decreased in following weeks. That is, in week 1, shortening was available to the rats for one hour; in week 2 it was available for 40 minutes, and for all subsequent weeks it was available for 20 minutes. The shortening availability was limited to 20 minutes in this study due to the relatively short half-life of 2OHE2 ($t_{1/2(1)}=0.54$ min, $t_{1/2(2)}=10$ min) [16].

**Drug Administration**

After 5 weeks, rats were injected intraperitoneally with vehicle (saline with 0.25% ethanol and 2-3 drops of TWEEN-80), 2-methoxyestradiol (2ME2), or 2OHE2 (Sigma Aldrich, St. Louis, MO, USA) with doses assigned to each rat using a Latin Square. Injections were given on Mondays and Fridays. 2OHE2 doses were 1.0, 3.0, and 10.0 µg/kg body weight, and were selected based on preliminary studies. Since 2OHE2 is considered a pro-drug for 2ME2, a 3.0 µg/kg body weight (the effective dose for 2OHE2 in preliminary studies) injection of 2ME2 was included in the Latin Square to rule out its possible effects. Rats were given 20 minutes of access to shortening in their home cages immediately after injection.

**Assessment of Chow Intake**

In order to determine if 2OHE2 would stimulate intake of chow as well as shortening, all rats were injected with either the highest effective 2OHE2 dose (3.0 µg/kg body weight) or vehicle in a crossover design on the Monday and Friday of the week.
immediately following the full dose effect study. Rats were then given 20-minute access to shortening, and both shortening and chow intake were assessed.

Statistics

Data were analyzed using SAS v.9.1 (SAS Institute, Cary, NC). Intakes were assessed in several ways. Terminal normalized intake in week five was analyzed via 2-way ANOVA (sex X access schedule). To determine differences in intakes between INT and D groups within the male and female cohorts, between-group t-tests (INT vs. D) were used. Linear regression analyses equivalent to analysis of covariance were conducted using GraphPad Prism 4 (GraphPad Software, Inc.) to compare escalations in normalized shortening intakes between males and females. Drug effects were assessed using a 3-way repeated measures analysis of variance (ANOVA; drug X sex X group) with drug dose as the repeated measure. Follow-up analyses were conducted using 1-way repeated measures ANOVAs. Tukey’s HSD post-hoc test was used for comparisons among more than two doses (full dose effect function assessment); within-group t-tests were used for comparisons between 3ug/kg 2OHE2 and vehicle (chow intake assessment). Shortening intakes were normalized to body weight using the formula: kcal/body mass^{2/3} [17] to account for sex differences.

RESULTS

Escalation and Onset of Bingeing

At the end of the bingeing procedure (week 5), normalized shortening intake was significantly greater in the females than in the males [main effect of sex: F(1, 64) = 12.65, p 0.0007], and also significantly greater in INT than in D rats [main effect of access schedule: F(1, 64) = 10.38, p 0.0020]. There was no interaction between sex and
access schedule for the terminal week 5 intakes. Shortening intake in the INT rats was significantly higher during the 20-minute access period than in the D rats in both the male (0.82 ± 0.07 v. 0.53 ± 0.06 kcal/g body mass^{2/3}; t-test p<0.01) and female (1.20 ± 0.12 v. 0.85 ± 0.08 kcal/g body mass^{2/3}; t-test p<0.02) cohorts. Therefore, the INT rats in both cohorts were bingeing as operationally defined by previous studies [18-20], which also is consistent with the DSM-IV [1].

Normalized intake data were also analyzed and compared as a percentage of the mean shortening intake on Day 1 of the bingeing procedure (Figure 2.1). Regression analyses revealed a slope of 0.048 for the male INT rats and 0.101 for the female INT rats indicating that shortening intake in the INT females escalated significantly faster than it did in the INT males [F(1,506)=6.79, p<0.01] (Figure 2.1B). The slopes of the D rats’ intakes did not differ from zero for either cohort (Figure 2.1A).

**Effect of 2OHE2**

Normalized shortening intake during the 20-minute access period was evaluated after administration of 2OHE2, 2ME2, or vehicle. There was a main effect of sex [F(1,64)=12.54, p<0.0008], in that the female rats had significantly higher normalized intakes than did males across all doses. This is consistent with the differences in escalation between males and females for the first 5 weeks. There was also a main effect of access protocol [F(1,64)=13.18, p<0.006] due to higher intakes in INT rats of both sexes compared to D rats (i.e. bingeing in INT rats).

There was a main effect of drug [F(4,256)=2.86, p<0.024]. Follow-up analyses revealed that the 3.0 µg/kg dose of 2OHE2 significantly stimulated shortening intake relative to vehicle in the INT rats [F(4,33)=5.29, p 0.0006 (Figure 2.2)]; there was no
effect in the D rats. Although there was no sex X schedule X drug interaction 
[F(4,256)=1.61, p =0.171], it is important to note that we were not able to find a significant effect at any dose relative to vehicle in the female rats when assessed with 1-way repeated measures ANOVA. The male INT rats, however, did show significant stimulation at the 3.0 µg/kg dose when assessed in this manner. Additionally, there was a drug by schedule interaction [F(4,256)=4.21, p<0.003], since 2OHE2 only significantly affected intake in the bingeing rats.

![Figure 2.1](image)

**Figure 2.1.** Escalation of normalized shortening intake across the 5-week binge induction period. (A) The intakes of both male D and female D groups did not escalate across time, i.e. neither slope was different from zero. (B) The female INT rats (slope=0.101) escalated significantly faster than the male INT rats (slope=0.048; n=17, p<0.01).
Assessment of Chow Intake

The effect of 2OHE2 on fat intake was reproduced, i.e. 3 µg/kg 2OHE2 stimulated shortening intake relative to vehicle [main effect of dose F(1,64) = 7.42, p < 0.0083]. In contrast, 2OHE2 had no effect on chow consumption (not shown).

DISCUSSION

As with previous studies where rats were given 1-hour access to shortening [18-20], both male and female rats on the INT schedule of access consumed significantly more shortening than rats given daily access. Therefore, both INT males and INT females met our operational criterion for bingeing, i.e. both groups consumed more than their respective D controls during week 5 of the study. Importantly, when consumption was normalized to body weight, shortening intakes in both the male and female INT groups escalated across time. In contrast, neither D group showed an escalation in intake.
during the 5-week period, i.e. the slopes of the two D groups were not different from zero. Thus, the INT schedule caused 20-min shortening intake of male and female INT rats to escalate over time, a finding consistent with other studies from our group when longer shortening access periods were used [21-23].

Although intakes of the male and female INT rats both escalated across the initial 5 weeks of the study, there were differences in the rates of escalation between the two groups. Specifically, the intakes of the INT females escalated faster than did the intakes of the INT males. Furthermore, normalized shortening intakes were not different between male and female INT groups on the first binge day (Student’s t-test, p NS), indicating that a baseline effect cannot explain the differences in escalation. This finding is consistent with previous reports that bingeing in female humans is more likely to progress to binge-related eating disorders than is bingeing in males [2]. It is currently unclear why this sex difference exists, though it is possible that the effect is the result of hormonal differences between sexes.

There were subtle differences present between male and female INT rats during the administration of acute injections of 2OHE2. For instance, intakes in the males after the effective dose (3.0 µg/kg) were comparable to those of females after administration of vehicle (Student’s t-test, p NS). In addition, while the female INT rats seemed to have similar trends in intake as a result of 2OHE2 (Figure 2.2), there were no significant differences at any dose relative to vehicle when we assessed with a 1-way repeated measures ANOVA (Figure 2.2) or paired t-test, however effects were significant in the INT males. This seems to suggest that the stimulation effect of 2OHE2 is much more subtle in female rats, and the overall schedule X drug interaction is driven predominantly
by the male rats. This idea is further supported by the fact that the male INT rats had a much larger overall stimulation at the effective dose than did the female INT rats (42.3% compared to 23.2%, respectively).

These results cannot be explained by order of dose administration, since all doses of drug and vehicle were assigned using a Latin Square. Furthermore, effects of 2OHE2 cannot be explained by generalized increases in food intake, since chow intake was unaffected by doses that stimulated fat intake in the male rats. Effects cannot be explained by the metabolite 2ME2, since 2ME2 at the same dose (3.0 µg/kg or ~1 x 10^-5 M) had no effect on fat intake. The decision to test the same dose of both compounds was based upon reports that 2OHE2 is considered to be a pro-drug of 2ME2 [16]. Finally, the more subtle stimulation in female rats was not due to ceiling effects, since all rats were observed to have consumed larger quantities of shortening during the escalation period than they ate after drug administration. That is, they could have eaten more than they did on drug administration days.

Since females already have higher circulating levels of 2OHE2 and other estrogens than males, it is plausible that the females were under greater influence of these hormones than the males prior to drug administration, and were therefore less sensitive to the acute injections of 2OHE2. This chronic exposure to 2OHE2 could also help to explain the elevated intakes and greater degree of escalation that occurred during the 5-week bingeing period in female INT rats compared to males. Acute exogenous administration of 2OHE2 to the females would result in higher overall levels, essentially shifting the females toward the descending limb of the concentration effect function. Conversely, exogenous administration of 2OHE2 to the males likely produced serum
concentrations comparable to those of the females at baseline [16], and stimulated intake to that seen in the females after administration of vehicle. Importantly, there were no differences in either D group, which supports the idea that acute exposure to 2OHE2 exacerbated intake in bingeing rats, but did not affect intake in the D rats that is presumably driven primarily by palatability.

The mechanism by which 2OHE2 enhances binge intake is unknown, though there is evidence that the metabolite may be affecting neuronal signaling by DA. DA signaling in various brain regions has been heavily implicated in neural mechanisms of reward, including food reward [24, 25]. Among these regions are two terminal areas of ventral tegmental area (VTA) dopamine projections, the prefrontal cortex (PFC) and the nucleus accumbens (NA). Others have shown that PFC DA increases in response to food anticipation [26], as well as to repeated presentation of sugary palatable foods [27]. In addition, accumbens DA is repeatedly stimulated by sucrose consumption in sucrose bingeing rats, but not in sucrose controls [28], suggesting that binge behavior affects DA signaling in a manner that is different from intake induced simply by palatability. Finally, others have reported increases in accumbens DA in rats sham-feeding corn oil [29]. Though differences are known to exist, it is possible that bingeing on fatty foods may affect DA signaling in a manner similar to that of sucrose [30].

2OHE2 may affect reward-associated DA signaling, specifically in cortical regions. In the PFC, where DA transporter (DAT) expression is relatively low [31, 32], degradation by the enzyme catechol-O-methyltransferase (COMT) accounts for approximately 60% of DA removal [33]. Because 2OHE2 is also degraded by COMT to form the metabolite 2-methoxyestradiol (2ME2), and because its affinity for COMT is
higher than that of DA [34], 2OHE2 may competitively inhibit the enzyme and therefore increase DA availability (Figure 2.3). Supporting this idea, inhibition of COMT by pharmacological means with the antagonist tolcapone increases DA in the PFC in response to food anticipation [26]. It is possible that administration of 2OHE2 increased DA availability in these regions by inhibiting COMT activity. Effects may have been specific to the INT rats due to repeated release of DA in binge rats relative to controls, as reported by others for sucrose in the accumbens [28].

Figure 2.3. The possible mechanism by which 2OHE2 could increase DA availability in the prefrontal cortex. (A) Normal degradation of DA to form the DA metabolite 3-methoxytyramine (3MT). (B) Competitive inhibition of COMT by 2OHE2 to form the metabolite 2-methoxyestradiol (2ME) may cause increased DA levels in the prefrontal cortex.

2OHE2 may also affect DA signaling via its effect on adenylyl cyclase. D2 receptor activation is known to inhibit adenylyl cyclase, lowering cAMP and acting in opposition to D1 receptors. 2OHE2, like D2 receptor activation, has been shown to inhibit adenylyl cyclase activity and lower intracellular cAMP. Importantly, this effect
was markedly less potent or absent altogether with the parent compound, E2, or the metabolite 2ME2 [8], and occurs independently of D1 or D2 receptors [9]. Additional evidence suggests that 2OHE2 is able to bind directly to D2 receptors in pituitary with an affinity that is similar to DA [7]. 2OHE2 may, therefore, mimic or enhance the effects of D2 receptor activation. The inverted U-shaped function obtained in the male INT rats may reflect differential presynaptic vs. post-synaptic D2 effects.

While the mechanisms by which 2OHE2 stimulated binge intake remain to be elucidated, the findings of the current study contribute to the understanding of sex differences in the risk of binge eating. Though the involvement of an estrogen metabolite, specifically 2OHE2, seems contradictory, the results of this study along with previous literature provide evidence for a possible novel mechanism for this increased risk in females.
REFERENCES


CHAPTER 3

BEHAVIORAL AND NEURONAL EFFECTS OF
2-HYDROXYESTRADIOL ON BINGEING

Manuscript in preparation
INTRODUCTION

Approximately 4.5% of people in the United States, or 14 million people, will binge at some point during their lifetime [1]. While it is true that both men and women binge, sex differences exist. Specifically, women are nearly three times more likely to suffer from a bingeing-related eating disorder such as bulimia nervosa and binge eating disorder. This trend is especially surprising, considering that estradiol (E2) has been shown to reduce meal size [2] and is associated with reduced binge frequency [3, 4]. The explanation for this apparent contradiction may involve the E2 metabolite, 2-hydroxyestradiol (2OHE2).

We have previously shown that acute administration of 2OHE2 can increase shortening intake in bingeing rats [5]. 2OHE2 did not change shortening intake in non-bingeing control rats, indicating that 2OHE2 does not cause bingeing, but can exacerbate its severity in animals that have already learned to binge. We also found that 2OHE2 had a greater effect in male rats than in female rats, and that female rats had a faster escalation of binge intake over a 5-week period than did males. This suggests that sex differences in 2OHE2 effects may exist because of higher endogenous levels of the metabolite in females. One goal of the present study was to determine if chronic administration of exogenous 2OHE2 can cause faster escalation in binge intake in ovariectomized female rats, in a manner similar to that previously reported for intact females.

2OHE2 has been shown to possess characteristics that may enable it to alter or interfere with dopamine (DA) signaling in certain brain regions such as the prefrontal cortex (PFC). This is important, since PFC DA signaling is associated with reward-based
learning and memory [6, 7], including food reward [8]. Furthermore, DA signaling has been heavily implicated in binge-related eating disorders [9], which have also been associated with altered activity in the medial PFC [10]. 2OHE2 has been shown to bind to D2 receptors in the pituitary [11]. Though it is not known if this binding is activating or inhibitory, it is possible that this could occur in the PFC. 2OHE2 has also been shown to have a higher affinity for the catechol-O-methyltransferase (COMT), an enzyme responsible for degradation of both DA and 2OHE2 [12]. This is especially important in the PFC, where expression of the dopamine transporter (DAT) is low [13, 14]. This suggests that 2OHE2 may competitively inhibit COMT, and increase DA availability in the PFC.

2OHE2 is also known to inhibit adenylyl cyclase [15], the protein activated by D1 activation and inhibited by D2 activation. Together, this evidence suggests that 2OHE2 may affect DA signaling, and exacerbate the signaling cascade that is thought to occur during bingeing. Therefore, we sought to elucidate possible mechanisms by which 2OHE2 can affect DA signaling, and to better understand the links between acute and possible long-term effects of the metabolite. Specifically, we investigated the effect of chronic administration of 2OHE2 on food intake, and the effect of acute administration of 2OHE2 on bingeing-evoked DA efflux.

METHODS

Study 1. Chronic Administration of 2OHE2: Food Intake and Binge Escalation

Animals

A total of 56 female Sprague-Dawley rats (Harlan, Indianapolis, IN), 60 days of age, were individually housed in hanging stainless steel wire cages in a temperature- and
humidity-controlled environment placed on a 12:12 light:dark cycle. All rats had *ad libitum* access to a nutritionally complete commercial laboratory rodent diet at all times during the study (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging metal food hoppers at the front of the cage. Tap water also was freely available. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

After seven days of adaptation to the vivarium, body weights were recorded and solid vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH) was provided during a single overnight period, in addition to the continuously available chow. Rats weighing at least 200g were then divided into three groups (n=16 for each group) matched for body weight and amount of shortening consumed during the overnight access period. Eight animals weighing less than 200g (the minimum weight required for minipump implantation) were assigned to a fourth, non-surgical group.

**Ovariectomy (OVX)**

Bilateral OVX surgeries were performed on two of the groups of rats, using the following procedure: Rats were anesthetized using 3-5% inhaled isoflurane. The surgical areas were shaved and then cleaned with iodine and 70% ethanol. An incision (~3 cm) was made on each flank of the rat, perpendicular to the spine, and adjacent to the hind limb. Parallel incisions were made in the underlying musculature. The ovaries were removed, and the uterine horns were clamped and tied with a 4-knot ligature. The ovaries were then excised and the clamp was released. The muscle incisions were closed.
with 2-3 sutures per side. The skin was closed with 2-3 surgical staples per side. The third group of rats was subjected to a sham ovariectomy that included cleaning, incision, and closure techniques performed during the OVX procedure, but left the ovaries and uterine horns intact.

**Osmotic Minipump Implantation**

After suturing the muscle wall, and prior to closure of the skin with staples, an osmotic minipump (model 2ML4, Alzet, Palo Alto, CA) was inserted on the left side through the skin incision and positioned in the scapular region of the animal. The minipumps inserted into the two groups of OVX rats contained either a high physiological dose of 2OHE2 (10 µg/kg/hr; \( E \), Steraloids, Newport, RI) or polyethylene glycol (PEG-400, 2.5 µL/h; \( O \)). The rats that underwent the sham ovariectomy were implanted with minipumps containing PEG-400 (2.5 µL/h; \( I \)).

**Binge Procedure**

After surgery, rats were given limited access to shortening in a glass jar clipped to the front of the cage 2-3 hours prior to the start of the dark cycle. Chow was available during the shortening access period and at all other times. In order to maintain normal colony conditions established in the Corwin model of bingeing [16], the non-surgical group was given shortening every day (D). Because this group was only used to maintain established conditions, the data were not collected. The other 3 groups of rats were given shortening intermittently, i.e. only on Mondays, Wednesdays, and Fridays. As previously described shortening was available for 1 hour in week 1, 40 minutes in week 2, and 20 minutes in weeks 3 and 4 [5]. During this 4-week binge induction period, body weights, shortening intakes, and 24-hour chow intakes were recorded.
Immunoblot Analysis

After the 4-week binge induction period, whole brains were harvested and bilateral PFC tissues were immediately removed. Proteins were extracted from PFC in E (n = 11), I (n=13), and O (n = 11) rats; 100 µg of protein was separated by SDS-PAGE (7.5-8%) and then transferred to a PVDF membrane. The membrane was blocked in 5% non-fat milk for 1 hour at room temperature, followed by an 1 hour incubation at room temperature with primary antibodies against: actin (1:800; Santa Cruz) and D2R (1:200; Santa Cruz). Membranes were then washed and incubated with secondary antibodies (1:4000) for 1 hour at room temperature. Protein bands were visualized using a chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ, USA). Densitometry measurements were made using NIH Image software.

Study 2. Acute Administration of 2OHE2: Food Intake and DA Efflux

Animals, Binge Procedure and Microdialysis Methods

Because our previous research indicated that male rats were more sensitive to acute injections of 2OHE2 [5] and to eliminate confounds caused by hormonal cycling in females, male rats (n=6) were used for this study. Rats were housed as described and were subjected to the binge protocol. After at least 5 weeks on this protocol, the rats were anesthetized with 4% isoflurane, and the top of the head was shaved and sterilized with betadine and 70% ethanol. After placement on a stereotaxic frame (Stoeltig, Wood Dale, IL), the skull was exposed and bregma identified. A hole was drilled through the skull, and a CMA 11 guide cannula was implanted into the PFC (coordinates: Anterior = +2.5 mm, Lateral = +0.5 mm, Depth = -1.5 mm). After 5 days recovery time, rats were moved to the dialysis chamber and a CMA 11 (2 mm) probe was then inserted into the
guide cannula. The rats were allowed to recover overnight with a slow perfusion of artificial cerebrospinal fluid (aCSF; 1.3 µL/min) into the microdialysis probe. The following day, baseline dialysate samples were collected for one hour before intraperitoneal injection with either vehicle (saline with <1% Tween) or 2OHE2 (3µg/kg body weight). Rats were immediately given one hour of access to a jar of shortening after injection. Collections continued throughout the shortening access period, and for at least 90 minutes after the shortening was removed from the chamber. All rats were given both injections in a crossover design.

**High Performance Liquid Chromatography**

Dialysate samples (10 µl) were injected every 15 min onto an ESA MD-150 narrow-bore HPLC column 150 x 2 mm (ESA Inc., Chelmsford, MA) for separation followed by detection by an ESA 5014B microdialysis cell (+300 mV; ESA Coulochem III, ESA, Inc., Chelmsford, MA). A guard cell (ESA 5020) placed in line before the injection loop was set at a potential of +350 mV. The mobile phase consisted of 75 mM sodium phosphate monobasic (EMD Chemical, Gibbstown, NJ), 1.7 mM 1-octanesulfonic acid (EMD Chemical), 25 µM EDTA (Acros, Morris Plains, NJ), 10% acetonitrile (EMD), and 0.01% triethylamine (Sigma Aldrich, St. Louis, MO) in a volume of 1L (pH 3.0). The neurotransmitter and metabolite peak areas were integrated using EZ Chrom Elite software (Scientific Software Inc, Pleasanton, CA) and quantified against known standards of dopamine (ESA Inc.), 3,4-dihydroxyphenylacetic acid (DOPAC; Sigma Aldrich), and homovanillic acid (HVA; Sigma Aldrich).
Statistics

For all analyses, values were considered significantly different at p<0.05. In order to compensate for the effect of weight gain on shortening intake, the intakes were normalized to body weight as previously described [17]. To compare escalation in normalized shortening intake and body weight among the groups, linear regression analyses equivalent to analysis of covariance were conducted using GraphPad Prism 4 (GraphPad Software, Inc.). All other measures of weight and intakes were compared among groups using 1-way ANOVA with Tukey’s HSD post hoc tests.

Microdialysis samples for DA were converted to a percentage of the mean of the first two baseline measurements (timepoints -60 minutes and -45 minutes). Area under the curve (AUC) calculations were conducted using Graphpad Prism 4 and were compared with a paired t-test. Additionally, a 2-way ANOVA was conducted on the data from the curves, with time and treatment as repeated measures. A Bonferroni correction was applied to this analysis by the software, and planned repeated-measures t-tests were used at each time point between treatments.

RESULTS

Study 1. Weight Change and Food Intake During the Binge Induction Period

Table 3.1 summarizes the starting and ending body weight of the rats, as well as the intakes of both shortening and chow over the entire course of Study 1. There was no significant difference in body weight among groups at the beginning of the study [F(2,47)=0.9394, p NS]. However, the O group had a significantly higher mean body weight than the I and E groups [F(2,47)=78.8, p<0.0001] at the end of the study. The
total amount of food consumed during the study reflected this finding, in that the O group had a significantly higher overall energy intake than either of the other groups [F(2,47)=58.89, p<0.0001]. This trend was not present, however, for the shortening intake data. The O group ate significantly more shortening than did the I group [F(2,47)=4.678, p<0.02], but the E group’s shortening intake did not differ from either of the other groups’. Chow intake did not differ between the I and either O or E, but the E group ate significantly less chow than did the O group [F(2,47)=10.64, p<0.002].

Normalized shortening intake data were analyzed as a percentage of the shortening intake on Day 1 of the study (Figure 3.1). Regression analyses revealed slopes of 0.277 ± 0.038, 0.160 ± 0.035, and 0.128 ± 0.043 for E, O and I groups, respectively. These data indicate that the E group had significantly faster shortening intake escalation than either the I or O groups [F(2,522)=4.06, p<0.02]. This supports the idea that 2OHE2, in the absence of E2, exacerbated binge escalation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Start Weight</th>
<th>End Weight</th>
<th>Total Intake Shortening</th>
<th>Total Intake Chow</th>
<th>Total Intake All Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>219.3 (2.1)</td>
<td>252.9 (4.0) a</td>
<td>259.1 (26.9) a</td>
<td>1235.0 (37.6) ab</td>
<td>1494.2 (21.5) d</td>
</tr>
<tr>
<td>E</td>
<td>216.3 (1.8)</td>
<td>251.7 (2.2) a</td>
<td>358.4 (26.7) ab</td>
<td>1099.8 (24.3) a</td>
<td>1458.2 (12.3) a</td>
</tr>
<tr>
<td>O</td>
<td>220.1 (2.4)</td>
<td>307.8 (4.2) b</td>
<td>391.2 (40.0) b</td>
<td>1374.1 (57.4) b</td>
<td>1765.3 (28.7) b</td>
</tr>
</tbody>
</table>

Significant differences (at alpha <0.05) are represented by different lower case letters. All data presented as mean and (SEM).
Mean shortening and chow intakes during the terminal week (week 4) were assessed in order to determine the maximal effects of the treatments (Figure 3.2). During the final week of the study, the E and O groups ate significantly more shortening than did the I group, but did not differ from one another [F(2,47)=5.97, p<0.005]. This indicates that the rats in the E group ate the same amount of shortening as rats in the O group, even though the O rats weighed greater than 20% more than the E group. The average daily chow intake in the final week was lower in the E group than in either of the other two groups [F(2,47)=13.43, p<0.0001], suggesting compensatory behavior in the E group for the relatively high shortening intake.

Figure 3.1. Escalation in normalized shortening intake across the 4-week binge induction period. The E group’s shortening intake escalated significantly faster than did either the O or I groups. *p<0.02.
Figure 3.2. Week 4 average shortening and chow intake. (A) The E and O groups ate significantly more shortening during the 20-minute binge period than did the I group. (B) The E group ate significantly less chow, on average, than did the I group or O group during the final week of study 1.

Figure 3.3. Week 4 shortening to chow ratio. The E group had a significantly higher shortening to chow ratio than the I group. Significant differences are indicated by different letters.

Study 2. Microdialysis for DA release in the PFC During Bingeing

During the microdialysis procedure, rats consumed significantly more shortening after injections of 2OHE2 (5.3 ± 1.4g) than after vehicle (1.7 ± 0.3g, p<0.03, n=6). It is likely that the stress of the environment during the microdialysis procedure decreased the intakes relative to intakes in the home cage (5.5 ± 1.2g). Regardless, 2OHE2 still enhanced intake. The DA collected from the PFC relative to baseline is shown in Figure
3.4. AUC calculations, analyzed with a paired t-test, revealed a larger AUC in the vehicle treatment than in the 2OHE2 treatment (p<0.05, n=6). This indicates that 2OHE2 decreased DA efflux in the PFC in bingeing rats. Additionally, the 2-way ANOVA revealed a main effect of time [F(8,80)=2.30, p<0.03], a main effect of treatment (that is, 2OHE2 or vehicle) [F(1,80)=5.17, p<0.05], and a time by treatment interaction [F(8,80)=2.305, p<0.03]. Planned comparisons revealed a significant 2OHE2-induced reduction in DA at the 30-minute timepoint (p<0.05, n=6).

Figure 3.4. Shortening-evoked DA efflux in the PFC of bingeing rats. After injection with vehicle, presentation of shortening at time 0 minutes caused an increase in DA efflux that reached maximal levels at 30 minutes. This effect was significantly inhibited after injection with 2OHE2. *p<0.05 vs. 2OHE2.

DISCUSSION

Three new findings are reported: 1) chronic administration of 2OHE2 enhanced escalation of binge intake over time, 2) OVX rats exposed to chronic 2OHE2 ate significantly more shortening and significantly less chow than intact vehicle controls, and 3) acute injections of 2OHE2 abolished binge food-evoked DA efflux in the PFC of bingeing rats.
At the end of Study 1, the O rats weighed significantly more than either the E or I rats. Body weights of rats in the E group, which had also undergone OVX surgery, did not differ from the body weights of rats in the I group. This finding confirms the metabolic activity of the 2OHE2 implants and is consistent with the findings of others [18, 19]. The weight differences are reflected in the total food intakes across the study. Specifically, O rats ate significantly more total energy than either of the other two groups. Therefore, the 2OHE2 implants prevented OVX-induced weight gain by decreasing overall food intake to levels expected in non-OVX rats. This finding also is consistent with findings of others [19]. Previous work has shown a similar suppression of food intake in OVX bingeing rats by supplementation with the parent compound, E2 [20]. However, the present findings suggest that this effect may be mediated by the presence of 2OHE2, and not by the parent compound directly.

Although the overall energy intake of the rats in the E group did not differ from that of rats in the I group, the pattern of intakes from shortening and chow individually were not the same. In week 4 of the study, rats in the E groups consumed significantly more shortening than rats in the I group (Figure 3.2). The E rats’ shortening intakes did not differ from the O rats’ intakes, even though the O rats weighed approximately 60 g more than the E rats. Importantly, the E rats compensated for the relatively high shortening intake by under-eating chow. In fact, the E rats ate significantly less chow than either of the other two groups. Additionally, the E rats had a significantly higher ratio of shortening to chow energy than the I group [0.45 and 0.23 respectively; F(2,47)=5.241, p<0.01, Figure 3.3]. These data indicate that 2OHE2 can shift food
intake from more homeostatic eating to binge-type eating, even though total energy intake remains unchanged.

In addition to causing a shift in food intake, chronic administration of 2OHE2 altered the rate of increase of binge intake. Regression analyses indicate a faster binge escalation (i.e. steeper slope) in the E group than in the other groups relative to body weight. This finding is similar to our previous study which showed faster escalation of shortening intake in female bingeing rats than in male bingeing rats [5], and suggests that 2OHE2, and not anatomical differences or E2 itself, can account for the faster escalation in females. Together, these studies support the idea that 2OHE2 may expedite progression towards more severe forms of bingeing.

Although no studies have investigated the effect of 2OHE2 on food intake in humans, previous studies have found important effects of the parent compound on food intake. E2 is known to reduce food intake in both rats and humans, and others have shown that binge frequency is lowest on days when E2 is high, and increases several days later when E2 levels are low [3, 4]. However, during pregnancy, when E2 is chronically high, women are at much higher risk for bingeing onset and are more likely to continue bingeing behavior than they are to continue other forms of disordered eating [21]. Furthermore, E2 is known to alter D2 receptor number in a biphasic manner, first decreasing (after 10 hrs), and then increasing them (after 24 hrs) [22]. Together this suggests that although E2 acutely decreases meal size and binge risk, it may increase bingeing in a delayed fashion, or when at constantly high levels. Based on our current data, 2OHE2, which would be present at high levels when E2 is high, may be the mediator of these effects due to its effects on DA signaling.
Due to technical complications, immunoblot assays conducted here were not able to be interpreted. However, previous studies have shown that OVX reduces D2 receptor number in the striatum, an effect that is prevented with E2 supplementation [23, 24]. Others have shown that D2 receptor number assessed by PET is higher in the frontal cortices of women compared to men [25]. Together this suggests that E2 increases D2 receptors in the prefrontal cortex. It is possible that this effect is mediated by the metabolite 2OHE2. This is especially important in the phenomenon of binge eating, since DA signaling has been closely linked to food reward [8], and DA release in the PFC is considered a marker for reward-related learning and memory [6, 7].

In Study 2, acute 2OHE2 dramatically reduced DA efflux. In fact, acute administration of 2OHE2 to bingeing rats completely abolished the DA efflux evoked by presentation of shortening. This effect was coupled with a corresponding increase in shortening intake, which confirms the findings the acute injection study described in Chapter 2 [5]. The reduced DA efflux is consistent with a previous study showing reduced basal DA levels in the medial PFC during proestrus, when E2 (and therefore, 2OHE2) levels are highest. Furthermore, that study also showed that ethanol-stimulated DA release (which was present during the estrus phase) was inhibited during proestrus [26]. Considering our current findings, it is possible that these reductions in dopamine were mediated by 2OHE2. Though the mechanism by which 2OHE2 inhibits DA in the PFC is currently unknown, studies done by others may provide clues. 2OHE2 has been shown to bind to D2 receptors in the pituitary [11]. Assuming that this binding is activational, 2OHE2 could inhibit DA release by binding to presynaptic D2 autoreceptors, which are known to “shut off” DA release.
The contribution of decreased DA levels in the PFC to binge eating may not be immediately apparent. However, long-term decreases in DA levels in the nucleus accumbens of monkeys have been associated with postsynaptic D2 supersensitivity [27]. If the 2OHE2-associated decrease in DA in the PFC causes supersensitization of postsynaptic D2 receptors, at the same time that 2OHE2 (or the parent compound, E2) is increasing the number of these receptors, then 2OHE2 could be creating conditions that exacerbate bingeing. Supporting this idea, the phenomenon of simultaneous supersensitivity and increased number of D2 receptors has been shown with brain lesions in adult animals [28, 29]. Importantly, activation of D2 receptors in the PFC inhibits the firing of glutamatergic pyramidal cells [30], which project to the nucleus accumbens [31, 32]. Because inhibition of glutamate receptors in the nucleus accumbens stimulates feeding in sated rats [33], it is possible that D2 activation in the PFC exacerbates binge intake. Therefore, a relatively high density of supersensitized postsynaptic D2 receptors would enhance bingeing by potentially increasing the D2-mediated inhibition of glutamatergic signaling to the nucleus accumbens. In addition, 2OHE2 may bind directly to both presynaptic and postsynaptic D2 receptors in an activating capacity, which also would inhibit glutamatergic firing to the nucleus accumbens. Another possible mechanism involves 2OHE2’s ability to inhibit adenylyl cyclase [15, 34], an effect of D2 receptor binding by DA. In this manner, 2OHE2 may either bypass D2 receptors altogether and mimic the action of D2 binding, or may enhance the activity of D2 receptors once they are bound by endogenous DA. Based on the current research, it is not likely that the effects of 2OHE2 are caused by inhibition of COMT leading to
increased DA availability, since 2OHE2 decreased bingeing-evoked DA levels in the PFC in Study 2.

Here we have shown that chronic administration of 2OHE2 can exacerbate the induction of binge eating. Specifically, we found that 2OHE2 attenuates the increases in body weight and food intake associated with OVX, but shifts food intake away from homeostatically-driven chow intake to reward-related palatable fatty food (shortening) intake. Additionally, we have shown that 2OHE2 increases the escalation of binge size over time, which may reflect increased progression to more severe forms of bingeing. Though the mechanisms by which this occurs are not completely clear, we have additionally provided evidence for mechanisms that could contribute. We have shown that acute 2OHE2 decreases bingeing-evoked DA release in the PFC. These effects point to a role for 2OHE2 in the alteration of glutamatergic firing in the nucleus accumbens, which can stimulate binge eating. These results provide a novel mechanism explaining the increased risk for more severe types of binge eating in females.
REFERENCES


CHAPTER 4

SUMMARY AND CONCLUSIONS
The overall goal of the research presented here was to examine the contribution of the estrogen metabolite 2-hydroxyestradiol (2OHE2) to binge eating. Behavioral and neurochemical approaches were used to assess the ability of 2OHE2 to increase binge size and to affect dopamine efflux and D2 receptor number in the PFC. The following specific aims were addressed.

Summary

Aim 1. To determine the effect of acute 2-hydroxyestradiol (2OHE2) on shortening intake in bingeing rats.

Acute injections of 2OHE2 (3μg/kg body weight) significantly increased shortening intake in bingeing (INT) rats that received shortening intermittently. Furthermore, this effect was greater in male rats than in female rats, and did not alter shortening intake in control non-bingeing rats that received shortening everyday (D). In addition, shortening intake, normalized to body weight, escalated more quickly in female bingeing rats than in male bingeing rats. In contrast, shortening intakes did not escalate in either the male or the female D control rats.

The results of this study indicate that 2OHE2 can acutely exacerbate shortening intake in rats that have already learned to binge, but not in non-bingeing rats. The sex differences in the response to 2OHE2 can be explained by the fact that females have higher endogenous levels of 2OHE2, and may already be acting under the influence of the metabolite. Thus, providing additional 2OHE2 via exogenous administration would not have had any effect. This idea is supported by the finding that shortening intake escalated more quickly in female bingeing rats than in male bingeing rats. Together, this
provides evidence not only for acute effects of 2OHE2 in exacerbating bingeing, but also suggests a possible long-term contribution of 2OHE2 in the progression of bingeing in females.

**Aim 2.** To determine if chronic exposure to 2OHE2 can increase binge risk.

2OHE2 significantly increased escalation of shortening intake in bingeing female rats over time. Specifically, OVX rats with 2OHE2 implants (E) demonstrated significantly faster escalation of shortening intake than did intact rats with vehicle implants (I) or OVX rats with vehicle implants (O). The results also indicated that E rats ate more shortening than did I rats, and as much shortening during week 4 of the study as the O rats, even though the O rats outweighed the E rats by approximately 60 g. Additionally, E rats compensated for this relatively high shortening intake by eating less chow during week 4 than either of the other two groups, and their shortening intake to chow intake ratio was significantly higher.

These results indicate that that chronic administration of 2OHE2 can exacerbate escalation of binge intake over time, and that 2OHE2 causes a shift in food intake from more homeostatic eating (chow) to more hedonic, binge-type eating (shortening). The results provide support for the proposed explanation of findings in Aim 1 that escalation of shortening intake in the females may have been due to higher endogenous levels of 2OHE2. This suggests that 2OHE2 may have a role in the higher prevalence of binge-related eating disorders in women compared to men. Specifically, the higher endogenous levels of 2OHE2 in women may increase their risk for progressing to more severe forms of bingeing.
**Aim 3.** To determine effects of 2OHE2 on dopamine (DA) signaling in the prefrontal cortex (PFC).

Shortening evoked DA efflux in the PFC of bingeing rats treated with vehicle reached maximal levels at a time point of 30 minutes after presentation. Acute injection of 2OHE2 completely abolished this shortening-evoked DA efflux. This effect was coupled with a stimulation in shortening intake during the access period, which confirms the findings obtained in Aim 1. This suggests that 2OHE2 can alter DA signaling in the PFC acutely, which may contribute to the exaggerated intakes that characterize bingeing. Unfortunately, the results from the study of chronic effects of 2OHE2 on PFC D2 receptor number were inconclusive.

**Conclusions**

Several conclusions can be drawn from the results of the studies described herein. First, although the parent compound, E2, has been associated with reduced meal size under non-binge conditions [1] as well as decreased binge frequency [2, 3], one of its metabolites, 2OHE2 can act in opposition to the parent compound. That is, 2OHE2 can acutely increase shortening intake in a rat model of bingeing. Second, chronic administration of 2OHE2 can have long-term effects that cause enhanced escalation of binge size over time, and can cause a shift from homeostatically-driven intake of chow to hedonically-driven intake of shortening. Finally, 2OHE2-induced stimulation of binge size is accompanied by a dramatic change in DA signaling in the PFC of bingeing rats. Specifically, 2OHE2 abolishes shortening-evoked DA efflux in the PFC under binge conditions.
The data suggest that 2OHE2 does not cause bingeing, *per se*, since acute injections of 2OHE2 did not affect the shortening intakes of non-bingeing D rats. Instead, 2OHE2 contributes to binge escalation as the behavior is developing and exacerbates intake once bingeing has been established. In short, 2OHE2 appears to be part of a vicious cycle of positive feedback that serves to promote and maintain binge-type eating. This reflects findings in the human literature. Specifically, there is no difference between men and women in the lifetime prevalence of bingeing, but women are more likely to be diagnosed with the more severe binge-related eating disorders [4].

Although the mechanisms by which 2OHE2 enhances binge intake both acutely and long-term are still not clear, the research presented here, along with studies by others, suggest some possibilities. Acute injections of 2OHE2 to bingeing rats completely abolished the DA efflux evoked by presentation of shortening. This is consistent with a previous study showing reduced basal DA levels in the medial PFC during proestrus, when E2 (and therefore, 2OHE2) levels are highest. Furthermore, that study also showed that ethanol-stimulated DA release (which was present during the estrus phase) was inhibited during proestrus [5]. Importantly, the inhibited DA release in the PFC can be indicative of attenuation in signaling involved with reward pathways. In research done in humans, this has been hypothesized to drive preference for highly palatable food in order to overcome the blunted reward response [6, 7]. In this manner, 2OHE2 may acutely attenuate the reward response by inhibiting DA efflux in the PFC, ultimately resulting in increased intake of palatable food during a binge.

In addition to possible acute affects that increase intake during a binge, long-term decreases in DA levels have been associated with changes in D2 receptor sensitivity,
which may contribute to binge vulnerability. Specifically, decreased DA levels in the nucleus accumbens (NA) of monkeys have been associated with postsynaptic D2 supersensitivity [8]. This suggests the possibility that 2OHE2 may promote postsynaptic D2 supersensitivity in the PFC by inhibiting DA efflux presynaptically. In addition, research by others indicates that the parent compound itself can alter DA signaling; the present research indicates that these effects may be mediated by 2OHE2. Specifically, it is known that OVX reduces D2 receptor number in the striatum, an effect that is prevented with E2 supplementation [9, 10]. As discussed in Chapter 3, others have shown that D2 receptor number assessed by PET is higher in the frontal cortices of women compared to men [11]. The present research suggests the possibility that the E2-mediated increases in D2 receptor number reported by others for the striatum may be mediated by 2OHE2. It is proposed that 2OHE2 may have similar effects in the prefrontal cortex. Combined with the results presented here, it is possible that these effects could lead to a relatively high number of supersensitized D2 receptors in the PFC. Activation of D2 receptors in the PFC inhibits the firing of glutamatergic pyramidal cells [12], which project to the nucleus accumbens [13, 14]. Because inhibition of glutamate receptors in the nucleus accumbens stimulates feeding in sated rats [15], it is possible that post-synaptic D2 activation in the PFC exacerbates binge intake. Therefore, a relatively high density of supersensitized postsynaptic D2 receptors would enhance bingeing by potentially increasing the D2-mediated inhibition of glutamatergic signaling to the nucleus accumbens. This proposed mechanism is shown in Figure 4.1.

The idea that 2OHE2 is responsible for increased risk for bingeing in females may seem contradictory, given that 2OHE2 is highest when E2 is highest and binge frequency
is lowest in cycling females [3]. However, it is possible that the enhancing effect of 2OHE2 is overshadowed by the suppressing effect of E2, but that 2OHE2 alters the neural physiology in such a way that days later, when E2 is low, bingeing is exacerbated. In this way, 2OHE2 may have a delayed long-term effect on bingeing, i.e. the system is “primed” to respond to a binge episode should one be initiated. This would also explain the contradiction associated with bingeing and pregnancy, which is that binge risk is much higher during this time [16], and E2 levels are at their physiologically highest levels.

In conclusion, the novel mechanism proposed here would account for many of the questions and apparent contradictions about sex differences in bingeing. Although it is supported by both the research presented here, as well as the work of others, there are still many unanswered questions. For instance, it is unclear exactly how 2OHE2 is able to inhibit DA efflux, and in what manner it interacts with DA receptors and their downstream signaling. Nonetheless, the research presented here offers a novel explanation for increased risk for binge-related eating disorders in females.
Figure 4.1. The proposed mechanism by which 2OHE2 enhances binge eating. (A) Others have shown that the PFC receives dopaminergic input from the VTA, and that inhibition of glutamatergic signaling in the NA results in stimulation of feeding (see text for detail and references). The present research demonstrated that DA efflux in the PFC that is stimulated during a binge is abolished by 2OHE2. (B) It is proposed that long-term exposure to 2OHE2 (and possibly E2) results in a high number of supersensitized D2 receptors in the PFC that enhance inhibition of pyramidal cell firing to the NA and exacerbates binge size. The system, therefore, essentially is “primed” to respond to a binge-type episode, due to the long-term effects of chronic exposure to 2OHE2. Non-binge-type eating would not be affected by the “priming”, as the efflux of DA upon binge initiation would not be as great. It is unclear if 2OHE2 interacts directly with D2 receptors or with the signaling cascade downstream of the receptors.

Speculation, Limitations, and Future Directions

Table 4.1 shows the possible mechanisms that were tested here, as well as those that remain to be tested. Though not directly tested here, research by others supports the idea that 2OHE2 may be acting directly at D2 receptors presynaptically and/or postsynaptically [17]. If this is the case, it would explain how 2OHE2 could simultaneously inhibit DA efflux and still allow D2 receptors to inhibit pyramidal cell firing to the NA. Binding in an activating capacity to presynaptic D2 autoreceptors
would inhibit DA release [18], while binding in the same manner postsynaptically would inhibit pyramidal cell activity [12].

Table 4.1. Possible 2OHE2 mechanisms.

<table>
<thead>
<tr>
<th>Possible Mechanism</th>
<th>Tested ?</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Binding to postsynaptic D2 receptors</td>
<td>No</td>
<td>Possible, based on literature</td>
</tr>
<tr>
<td>2) Binding to D2 autoreceptors presynaptically</td>
<td>*Indirectly</td>
<td>Possible, based on findings of inhibited DA efflux in Aim 3</td>
</tr>
<tr>
<td>3) Competitive inhibition of COMT causing increased DA level</td>
<td>Yes</td>
<td>Not likely, based on findings of inhibited DA efflux in Aim 3</td>
</tr>
<tr>
<td>4) Inhibition of adenylyl cyclase</td>
<td>*Indirectly</td>
<td>Possible, based on findings of inhibited DA efflux in Aim 3</td>
</tr>
<tr>
<td>5) Alteration of D2 receptor number</td>
<td>Yes</td>
<td>Possible, inconclusive data, but supported by literature</td>
</tr>
</tbody>
</table>

See text description for summary and explanations of findings and literature. * Though not directly tested, it is likely that 2OHE2 operates through one of these mechanisms.

In addition to directly binding to D2 receptors in an activating capacity, 2OHE2 has been shown to inhibit adenylyl cyclase activity which attenuates production of the 2nd messenger, cAMP [19]. Since D2 activation also inhibits adenylyl cyclase, and therefore cAMP production, inhibition by 2OHE2 would mimic or enhance the effects of D2 binding without interaction with D2 directly.

In order to determine which (if either) of these two mechanisms 2OHE2 acts through, pharmacological testing could be conducted. Specifically, as a proof of concept, adenylyl cyclase inhibitors such as SQ22536 could be administered to bingeing rats under the same conditions as the microdialysis study presented here. Inhibition of DA efflux and stimulation of shortening intake after SQ22536 would be the same as the outcomes
described here with 2OHE2, and would be consistent with the idea that 2OHE2 could be exerting its effect through inhibition of adenylyl cyclase. Previous results indicate that D2 antagonism by raclopride stimulated shortening consumption in bingeing rats [20]. It is possible that this is the result of stimulation of DA efflux presynaptically, which, in turn, would activate post-synaptic D2 receptors, an effect similar to the post-synaptic activation of D2 proposed here for 2OHE2 (see Fig 1.2A of Literature Review).

However, to test the idea that 2OHE2 is inhibiting DA efflux presynaptically, an autoreceptor-specific antagonist, such as UH-232, would need to be tested. If UH-232 restored DA efflux after an injection of 2OHE2, this would provide evidence that 2OHE2 was acting presynaptically in a D2 autoreceptor-dependent manner. However, if 2OHE2 still inhibited DA efflux in the presence of UH-232, then a D2-independent mechanism would be more likely.

It is unlikely that 2OHE2 is acting through inhibition of the enzyme catechol-O-methyltransferase (COMT). Research by others has shown that tolcapone, a potent COMT inhibitor, enhanced food-evoked DA efflux in the PFC [21]. Because results presented here indicate that 2OHE2 inhibited food-evoked DA efflux, inhibition of COMT is likely not responsible for stimulating shortening intake.

An additional possible mechanism that would contribute to the effects of 2OHE2 is an increase in D2 receptor number in the PFC. Although preliminary results testing this hypothesis were inconclusive, the parent compound, estradiol (E2), increased D2 density in OVX rats [10]. Therefore, the mechanism proposed here is supported, but it is not clear if this effect is from the parent compound or from 2OHE2. Additional studies
with chronic 2OHE2 administration in the absence of E2 would need to be conducted to clarify this.

A limitation of the research presented here involves the presence of sex differences in bingeing. Because acute injection of 2OHE2 was more effective in stimulating shortening intake in male bingeing rats than in female bingeing rats, the microdialysis study utilized male rats. However, acute administration of 2OHE2 to OVX females should produce similar results. Therefore, this should be addressed in the future. Also, chronic administration (i.e. implant) experiments were conducted with female rats. Male rats with 2OHE2 implants should respond with similar behavioral outcomes as the female E group, but this should be confirmed with additional research in male bingeing rats.

Another limitation of the research here involves the limitations of the model itself. The rat model of bingeing used for this research demonstrates face, construct, and predictive validity [22-24]. However, the findings reported here involve bingeing on fat exclusively. Chronic bingeing on fat (shortening) can result in behavioral outcomes that differ from those of sugar [25]. Additionally, effects of drugs such as baclofen and raclopride have differential effects on intake, depending on the fat and sugar composition of the food under investigation [26]. Although many of the findings reported here have been attributed to bingeing and reward, such as the shift from chow intake to shortening intake reported in Chapter 3, it is possible that these findings are a result of fat, and not of bingeing per se. Therefore, research should be done with additional binge foods before generalizing the findings reported here to all binge foods.
References


Curriculum Vitae
R. Keith Babbs

110 Chandlee
University Park, PA 16802
Email: rkb145@psu.edu

EDUCATION
The Pennsylvania State University
Ph.D., Physiology, expected 2012
The University of Central Arkansas
M.S., Biology, August 2009
The University of Central Arkansas
B.S.E., Art Education, December 1998

PUBLICATIONS


PRESENTATIONS

Poster – “Exacerbation of binge eating by inactivation of prefrontal cortex"
  Society for Neuroscience, 2011
  Washington D.C.
Poster – “Effect of an estradiol metabolite on binge eating in rats”
  Experimental Biology, 2011
  Washington D.C.
Poster – “Effects of certainty of food-cue associations on binge eating in rats”
  Society for the Study of Ingestive behavior, 2010
  Pittsburg, PA
Poster – “An estrogen metabolite enhances binge eating in rats”
  Society for the Study of Ingestive behavior, 2009
  Portland, OR