Ovarian Hormones Regulate Dopamine Release and Adaptive Motivation in Female Rats

by

Katie E. Yoest

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Doctoral Committee:

Professor Jill B. Becker, Chair
Associate Professor Brandon J. Aragona
Professor Kent C. Berridge,
Assistant Professor Carrie R. Ferrario

Katie E. Yoest

ktyoest@umich.edu

ORCID iD: 0000-0003-3943-460X

Dedication

This dissertation is dedicated to the memory of my grandfather, Henry Santiago, for teaching me the value of hard work and of laughter, and my grandmother Margaret Yoest, for showing me what it means to be a lifelong learner.

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Abstract

Sex differences in motivated behaviors have evolved due to sex-specific adaptive pressures. Importantly, the neural circuitry underlying motivation is regulated by release of gonadal hormones in females but not in males. This likely allows for the coordination of motivated behaviors which changes in reproductive state in females, which increases the likelihood that females will engage in behaviors that are most likely to enhance their reproductive success. Here, I propose that these changes in levels of circulating ovarian hormones, which regulate dopamine signaling within striatal circuitry, act to direct adaptive choice and motivation for food vs sexual reward.

In Chapter 2, I first establish that acute treatment with estradiol benzoate enhances the effect of cocaine on phasic dopamine release within the nucleus accumbens of in females, but not males. I extend these findings to demonstrate the effects of estradiol seen here are regulated by activation of the selective estradiol receptor (ER) subtype, ERβ. This work further clarifies the role of estradiol in regulating dopamine release but does not indicate the behavioral significance of ovarian hormones acting within this circuitry. Accordingly, I next sought to determine whether administration of estradiol benzoate and progesterone in a regimen that induces sexual receptivity and increases sexual motivation could also reduce motivation for food. I found that ovarian hormones do attenuate motivated responding for a palatable food reward, but only after administration of both estradiol benzoate and progesterone, indicating the effects of ovarian hormones on motivation for food are dissociable from their effects on

consummatory feeding behavior and drug-induced dopamine release. In Chapter 4, I then tested whether ovarian hormones act to increase motivation for sex but decrease motivation for food in order to facilitate adaptive choice when females are sexually receptive. I found that administration of estradiol benzoate and progesterone not only decreased motivation for food and enhanced motivation for a mate when both rewards were available, but also biased a female's choice for food vs access to a sexually experienced male conspecific.

Taken together, these findings propose a potential explanation for why dopaminergic circuitry underlying motivated behaviors is responsive to ovarian hormones, providing an adaptive interpretation of a mechanism that is most commonly investigated within the context of maladaptive and disordered behaviors.

Chapter 1:

Introduction

General Overview

In female rodents, cyclic release of ovarian hormones has widespread effects on both central and peripheral neural systems. One of these effects that has been particularly well studied is the ability of estradiol, and to a lesser extent progesterone, to regulate dopamine (DA) release in response to both natural and drug rewards. Estradiol enhances DA release in response to psychomotor stimulants in females but not males, a phenomenon that has been strongly implicated in sex differences in the susceptibility to develop substance use disorders (Hu and Becker, 2003; Becker, 2016; Yoest et al., 2018).

However, female mammals did not evolve to have a DA system that is sensitive to changes in ovarian hormones in order to render females more susceptible to addiction. Rather, estradiol and progesterone likely mediate changes in DA release in females in order to coordinate changes in motivation across various reproductive states. Here, I will attempt to link the mechanisms that enhance DA release in response to drugs of abuse with adaptive regulation of motivated behaviors in female rodents. I will address the following questions: 1) are the mechanisms that regulate the effect of estradiol on responses to drugs, food, and sex different or similar? And 2) do changes in motivation in response to ovarian hormones mediate adaptive choice across different reproductive states?

As an introduction to the dissertation, I will first introduce how sex differences arise within the central nervous system. I will then provide an overview of the neural circuitry underlying motivated behaviors, before reviewing the literature on sex differences within this circuitry and how estradiol is crucial for mediating sex differences in DA release. I will then discuss how the ability of ovarian hormones to modulate DA release relates to the control of adaptive behaviors, specifically feeding and sexual behavior. Finally, I will introduce the chapters of this dissertation, and how they relate to the overall goal of understanding how and why ovarian hormones regulate DA signaling in females but not males.

Sex differences in brain and behavior

Sex is an important biological variable in the study of neuroscience and behavior. The presence of the SRY gene on the Y chromosome of males leads to the development of the testes from the bipotential gonads early in the development. Subsequent release of testosterone from the testes not only induces development of the male genitalia, but also acts within the brain where it is aromatized to estradiol and facilitates the organization of sexually differentiated neural circuits (Phoenix et al., 1959; Arnold, 2009). Females, who do not produce meaningful amounts of gonadal hormones during perinatal development, retain the default (feminized) organization of the brain (Jost et al., 1973). Additionally, differences in hormone release during puberty results in further differentiation of the brains of males and females (Schulz and Sisk, 2016). Largely due to these sex differences in the organization of the nervous system during development, females and males also show sex specific patterns of hormone release during adulthood (Beatty, 1979; Arnold and Gorski, 1984). Sex differences in adulthood then arise from differences in the release

of hormones that act on differentially organized brain structures to produce sex-specific behaviors.

Neural basis of motivation

Broadly defined, motivation refers to the internal drive to engage in a specific behavior, typically in pursuit of a reward or reinforcer. This can refer to natural reward seeking behaviors, such as those directed towards obtaining food and water, sex, or social interaction, as well as learned behaviors, including those involved in drug seeking and taking. However, this broad conceptualization of motivation does not fully reflect the complexity of motivated behaviors or the underlying neural systems. A full appreciation of the diverse constellation of behaviors that fall under the umbrella of motivation requires recognition not only of the differences between the behaviors themselves and various aspects of a behavior (i.e. appetitive vs. consummatory), but also the conditions under which those behaviors are expressed.

While the specific neural pathways controlling a particular behavior may be distinct, they also overlap in several important ways. One neural system that plays a crucial role in the expression of all motivated behaviors is the striatum. The striatum is commonly divided into two separate but interacting pathways: the ventral striatum, which is largely innervated by the ventral tegmental area (VTA) and includes the nucleus accumbens (NAc) and ventromedial caudate-putamen, and the dorsal striatum, which receives dopaminergic projections from the substantia nigra and incorporates the remaining majority of the caudate-putamen (Haber et al., 2000).

The NAc may be uniquely poised to evaluate and predict rewards, as it receives and integrates DA inputs from the VTA with glutamatergic inputs from the hippocampus,

cortex, and amygdala, as well as GABAergic connections that participate in action selection and coordination of motor outputs (Kalivas et al., 2006; Lammel et al., 2014; Bamford et al., 2018). The NAc integrates these signals in order to select and execute appropriate motivated behaviors, based on the organism's physiological state and environmental stimuli integrated with prior learning about rewards and reward predictive cues (Yun et al., 2004; Flagel et al., 2011; Saddoris et al., 2015; Cone et al., 2016). The NAc has been shown to play a crucial role in generating rewarding or reinforcing aspects of motivation, as well as learning about associations between primary reinforcers and the cues that predict them (Flagel et al., 2008; Aragona et al., 2009; Steinberg et al., 2013; Hamid et al., 2016). DA acting in the NAc is implicated in the attribution of motivational or reinforcing properties – often referred to as "incentive salience" – to cues that predict reward (Robinson and Berridge, 1993). Additionally, NAc dopamine systems may act to energize responding for rewards, as Pavlovian approach behavior is significantly correlated with the magnitude of DA release in the NAc core (Flagel et al., 2011).

In contrast to the NAc, the dorsal striatum has largely been studied in relation to its role in the control of motor output (Kravitz et al., 2010; Yttri and Dudman, 2016). However, the dorsal striatum and NAc are similar in their organizational structure; like the NAc the dorsal striatum receives both cortical and subcortical inputs and contains GABAergic projection neurons that are important for action selection and goal-directed behaviors (Ikemoto et al., 2015). Also like the NAc, DA release within the dorsal striatum increases during instrumental reward seeking, and tracks shifts in the motivational value of rewards (Ostlund et al., 2011). However, while the NAc is thought to act in the control of goal-directed motivated behaviors, the striatum is more strongly

implicated in stimulus-response learning required for establishing and expressing specific patterns of learned behaviors (Faure et al., 2005; Vanderschuren et al., 2005; Everitt and Robbins, 2016). The dorsal striatum may also be particular important for sensorimotor aspects of motivation, as DA projections to the dorsomedial striatum show weak encoding of reward processing, but strong encoding of reward-directed movements (Parker et al., 2016).

Although these brain areas have distinct functions within the context of motivated behavior, they do not function in isolation from one another. Reciprocal projections between midbrain DA neurons and both the ventral and dorsal striatum produce a feed-forward spiraling circuit that allows for coordination of firing in both regions (Haber et al., 2000). Motivational circuitry, then, requires not only the sensorimotor systems that are crucial for the evaluation of stimuli and subsequent expression of targeted behaviors, but also relies on an energizing component that modulates motivation for various rewards as a function of the organism's adaptive needs.

Sex differences in striatal DA release

Much of what is known about the DA systems, in general, has come from studies in male subjects. In fact, some of the first studies that identified sex differences within the striatal DA circuitry were actually investigating sex differences in DA release within the hypothalamus, and only used the striatum as a control (Becker and Ramirez, 1980). The expectation was that sex differences in DA release would exist only within the hypothalamus, a brain region that showed strong sexual dimorphisms and was known to coordinate many sexually differentiated functions and behaviors. Surprisingly, there was no sex difference in K^+-stimulated DA release from medial basal hypothalamic tissue

fragments (Becker and Ramirez, 1980). On the other hand, amphetamine-(AMPH) stimulated DA release from striatal tissue was significantly greater for females compared to males (Becker and Ramirez, 1981). These studies were some of the first to have demonstrated that females showed differential regulation of neural activity outside of structures primarily implicated in reproduction.

Gonadal hormones and estrous cycle mediate sex differences in dopamine release

Understanding how DA release within the striatum is different in males and females also requires understanding how general differences in the physiology of males and females may influence differences in neural functioning. One of the most striking sex differences in animal physiology is the regulation of gonadal hormone release. While release of testosterone in males is under tonic, homeostatic control, showing slight circadian rhythms, release of female gonadal hormones is much more complex. Adult female rodents show cyclic release of estradiol and progesterone over a four or five-day ovulatory cycle. During diestrus and metestrus, levels of estradiol and progesterone are low. As levels of estradiol and progesterone rise, females enter proestrus, until finally levels of ovarian hormones peak and then begin to decline during estrus.

Sex differences in DA release from striatal tissue were only observed in estrous females, while proestrous females showed similar levels of DA release to males. Further investigation demonstrated the role of the gonadal hormones, estradiol and progesterone, in this sex difference; ovariectomized (OVX) females showed significantly reduced AMPH-stimulated DA release from striatal tissue, and treatment with estradiol benzoate (EB) for four days followed by progesterone 4 hours prior to obtaining brain tissue, to

mimic endogenous hormone release on estrus, restored the sex difference seen in intact animals (Becker and Ramirez, 1981).

Interestingly, treatment with EB for 4 days (last treatment 24 hours prior to test), or progesterone 4 hours prior to test (without EB priming), only slightly increased the release of DA from striatal tissue. This demonstrated that the pattern of release of both hormones over the course of the estrous cycle contributes to the sex difference in AMPH-stimulated DA release. Importantly, this effect was not unique to AMPH induced DA release, progesterone treatment in estradiol primed females also enhanced K^+-stimulated DA release (Becker et al., 1984). These early studies not only verified that sex differences exist within the striatal DA response to synaptic activity, but also that in females, striatal DA release is modulated by circulating gonadal hormones over the course of the estrous cycle. In addition, the ability of estradiol to enhance both AMPH- and K^+ -stimulated DA release indicates that rather than specifically enhancing vesicular release, as induced by K^+, or non-vesicular release, as induced by AMPH, gonadal hormones induce a general enhancement of excitability of DA terminals.

Sex differences in drug-induced behaviors

One way to assess functional activity in DA systems was to use rotational behavior as an index of unilateral DA activity. Unilateral electrical stimulation of the ascending mesostriatal pathway induces turning away from the side that is stimulated. Following unilateral dorsal striatum DA denervation with a monoamine-selective neurotoxin, treatment with AMPH induces DA release only from the remaining intact DA terminals, and animals turn in circles away from the intact side and towards the side of the lesion. The intensity of the rotational behavior was related to greater DA release from

the intact DA terminals, and was used as a proxy for the magnitude of DA release (Robinson et al., 1980).

Females showed greater AMPH-induced rotational behavior, compared with males, both at baseline and after unilateral lesions of dopaminergic cells within the substantia nigra (Robinson et al., 1980; Becker and Beer, 1986). Importantly, sex differences in rotational behavior were also dependent on gonadal hormones. Rotational behavior induced by unilateral electrical stimulation of the ascending mesostriatal pathway was greatest in estrous females compared to those in diestrus, and similar results were seen after AMPH administration, even when controlling for sex differences in pharmacokinetic factors (Becker et al., 1982; Robinson et al., 1982). OVX reduced rotational behavior elicited by both AMPH and electrical stimulation, and treatment with estradiol for four consecutive days increased the number of rotations made after AMPH administration to levels seen in intact females (Robinson et al., 1982; Becker and Beer, 1986). This increase in rotational behavior was seen both four hours and four days after cessation of estradiol treatment, but not 24 hours after, suggesting that multiple mechanisms contributed to the effect of estradiol on DA mediated behaviors. In fact, increases in DA release after estradiol treatment were only seen four hours after cessation of estradiol treatment, but not four days later, indicating that while increased DA release may account for changes in rotational behavior four hours following estradiol administration, later increases in rotational behavior are mediated by a different mechanism (Becker and Beer, 1986).

Rapid effects of estradiol on striatum

Further clues to one potential mechanism of estradiol enhancement of DA release came from observations that estradiol significantly altered DA activity within minutes; not on the timescale that would be expected of a typical genomic mechanism (Becker, 1990a, 1990b). Treatment with estradiol, 30 minutes prior to AMPH administration, significantly increased rotational behavior in OVX females with unilateral striatal DA lesions and this was correlated with an increase in AMPH- stimulated DA release within the intact striatum (Becker, 1990b). In addition, rapid potentiation of stimulated DA release was observed after direct application of estradiol to striatal tissue of OVX rats in vitro, indicating that estradiol acts directly on striatal tissue to rapidly enhance stimulated DA release (Becker, 1990a). In these experiments, 17β-estradiol, the most physiologically active estrogen, and the non-steroidal estradiol analogue diethylstilbesterol both significantly increased AMPH-stimulated DA release from superfused striatal tissue from OVX female, but not castrated (CAST) male rats (Becker, 1990a). The less potent 17α-estradiol also slightly increased AMPH induced DA release in females, but this effect did not reach significance (Becker, 1990a).

Similar findings were reported when measuring K^+-induced DA release. In a series of experiments, superfused striatal tissue from OVX females treated with 100 pg/ml 17β-estradiol at a rate of 100 μl/min showed increased K^+-stimulated DA release, while 1000 pg/ml 17β-estradiol reduced K^+-induced DA release. This rapid and direct effect of estradiol was only seen in females, and also only observed after pulsatile administration of estradiol; continuous administration of estradiol over the same period of time had no effect (Becker, 1990a). In these experiments, there was no effect of estradiol

on basal DA release, indicating that estradiol selectively enhanced the excitability of DA terminals without directly inducing DA release.

Membrane receptors mediate rapid effects in striatum

At this time, it was well established that estradiol was able to modulate DA functional activity, but the specific mechanism involved was still unclear. The acute timescale by which estradiol enhanced DA release, combined with the lack of evidence for nuclear receptors within the striatum (Pfaff and Keiner, 1973), led to the development of the hypothesis that estradiol enhances DA activity by acting on membrane receptors within the dorsal striatum (Becker, 1990a).

The prediction that estradiol was acting via membrane associated receptors on dorsal striatal neurons was verified in a series of experiments using whole-cell clamp electrophysiology. These experiments demonstrated that estradiol rapidly and dose-dependently reduced Ca^{2+} currents via L-type Ca^{2+} channels in medium spiny striatal neurons (MSNs). This effect was replicated using estradiol conjugated to bovine serum albumin (BSA), a conformation that cannot penetrate the cellular membrane (Mermelstein et al., 1996). Furthermore, delivering 100 pM estradiol intracellularly, to saturate intracellular receptors, did not disrupt the ability of 1 pM estradiol, applied extracellularly, to decrease Ca^{2+} current, demonstrating that estradiol was acting extracellularly at the membrane. These studies also established that estradiol exerts these effects through a G-protein-coupled-receptor (GPCR), as application of GTPγS (a drug that prevents inactivation of G-protein-mediated events) prevented the reversal of estradiol attenuation of Ca^{2+} currents. This study was carried out on GABAergic MSNs, leading to the conclusion that estradiol inhibits Ca^{2+} currents on GABAergic MSNs via a

membrane GPCR (Mermelstein et al., 1996). This lead to the hypothesis that estradiol enhances DA release in dorsal striatum by inhibition of GABA release.

Experiments using in vivo microdialysis went on to show that estradiol inhibited K^+-stimulated GABA release within the dorsal striatum (Hu et al., 2006). Additionally, overexpression of estradiol receptor alpha (ERα) in dorsal lateral striatum enhanced the effects of estradiol to inhibit K^+-stimulated GABA release (Schultz et al., 2009). In vivo experiments also verified that a membrane receptor was responsible for the effect of estradiol on AMPH-stimulated DA release, demonstrating that this mechanism likely played a role in the behavioral effects of estradiol on DA mediated behaviors (Xiao and Becker, 1998). More recent work has established that application of estradiol directly to the striatum, but not the medial prefrontal cortex (mPFC) or substantia nigra (SN) enhanced DA release induced by both AMPH administration as well as direct electrical stimulation, in OVX female rats (Shams et al., 2016; Shams et al., 2018). Taken together, these findings demonstrate that one mechanism by which estradiol enhances DA release within the dorsal striatum is through direct inhibition of GABAergic MSNs, which tonically inhibit the release of DA from nigrostriatal terminals, leading to disinhibition of DA release and elevated stimulated extracellular DA concentrations.

Identification of estradiol receptors α and β, & GPER-1

At the time that these hypotheses were initially being developed, there was speculation that membrane estradiol receptors could modulate a number of cellular functions, but definitive evidence had not been found. Soon after, the existence of membrane estradiol receptors within the central nervous system were validated (Zheng and Ramirez, 1997). At the same time, a novel isoform of estradiol receptor was

characterized (Mosselman et al., 1996). These two receptor isoforms, ERα and the newly identified, ERβ, were then cloned and transfected into Chinese hamster ovary cells, which did not normally express estradiol receptors, to allow for the characterization of membrane vs. nuclear mediated effects (Razandi et al., 1999). These transfected cells were responsive to estradiol conjugated to BSA, and activation of both ERα and ERβ by 17β-estradiol stimulated production of inositol 1,4,5-triphosphate (IP_3) and cAMP, indicating that at the membrane, estradiol was acting through G-protein coupled pathways (Razandi et al., 1999).

The localization of classical ERs to the plasma membrane is dependent on their association with caveolin proteins, scaffolding proteins that mediate the association of various membrane bound signaling molecules (Evinger and Levin, 2005). Modification of ERs by palmitoylation, or the addition of covalent fatty acid groups, of specific C terminus amino acid residues, results in association of ERs with caveolin proteins, which then facilitate their trafficking to the plasma membrane (Razandi et al., 2003; Luoma et al., 2008).

Within the striatum, caveolin proteins mediated the coupling of ERα and ERβ to membrane bound metabotropic glutamate receptors (mGluRs), allowing for rapid modulation of cellular activity (Micevych and Mermelstein, 2008). Caveolin 1 (CAV1) is associated with mGluR1, while caveolin 3 (CAV3) is associated with mGluR2/3 (Luoma et al., 2008). On striatal neurons, ERα was functionally coupled to both group I and group II mGluRs, leading to activation of Gq or Gi/o subunits and activation or inhibition of CREB phosphorylation, respectively (Boulware et al., 2005; Grove-Strawser et al., 2010) . Specifically, ERα enhancement of CREB phosphorylation was dependent on

mGluR5 activation of MAPK signaling, while ERα coupled to mGluR2/3 attenuates L-type Ca^{2+} mediated CREB phosphorylation (Grove-Strawser et al., 2010). Alternatively, ERβ is coupled only to group II mGluRs, and therefore only acted to decrease CREB phosphorylation (Grove-Strawser et al., 2010).

In addition, the estradiol responsive G-protein receptor-30 (GPR-30, now known as G-protein estradiol receptor-1; GPER-1), is also found on both GABAergic MSNs as well as cholinergic interneurons in dorsal striatum (Almey et al., 2012, 2016). GPER-1 is coupled to a Gαs subunit, and activates both the protein Kinase A (PKA) and extracellular signal-regulated kinase (ERK) signaling pathways (Hadjimarkou and Vasudevan, 2017). Finally, activation of GPER-1 also rapidly induces Ca^{2+} influx, and interactions between GPER-1 and classical estradiol receptors are also reported (Brailoiu et al., 2007; Hadjimarkou and Vasudevan, 2017).

Multiple estradiol receptor subtypes contribute to effects of estradiol on DA activity

A full understanding of the relative contributions of each estradiol receptor subtype to the effects of estradiol on DA systems is still under investigation. Estradiol enhancement of AMPH stimulated DA release was prevented by ICI 182,780 (Xiao et al., 2003) an antagonist for ERα and ERβ. It has recently been found that ICI 182,780 is also an agonist for GPER-1, and this suggests that GPER-1 is not directly responsible for the effects of estradiol on DA release in dorsal striatum (Xiao et al., 2003). ERβ has been linked to the facilitation of AMPH-induced place preference (Boulware et al., 2005; Silverman and Koenig, 2007; Schultz et al., 2009). Selective activation of ERβ, but not ERα, also prevents the OVX induced decrease in both D2 DA receptor and DAT expression. (Le Saux et al., 2006; Morissette et al., 2008). However, estradiol facilitation

of AMPH sensitization is dependent on mGluR5, a group I mGluR that only couples to ERα, and overexpression of ERα within the dorsal striatum enhances the effect of estradiol on K^+ stimulated GABA release (Schultz et al., 2009; Martinez et al., 2014). These results indicate that both ERα and ERβ are involved in estradiol regulation of DA signaling, although the specific mechanisms involved have not been fully elucidated.

Effects of progesterone on DA circuitry

The majority of research has focused on the effects of estradiol on DA systems. Nevertheless, the induction of many adaptive behaviors associated with gonadal hormones in intact animals require concurrent changes in levels of circulating progesterone (Tennent et al., 1980). As mentioned previously, while treatment with estradiol alone resulted in increases in stimulated DA release, hormone priming with repeated estradiol or estradiol and progesterone resulted in even greater stimulated DA release (Becker and Ramirez, 1981; Becker et al., 1984). Treatment of estradiol primed animals with progesterone enhanced stimulated striatal DA release above the effects of estradiol alone (Dluzen and Ramirez, 1984; Becker and Rudick, 1999a). This effect was biphasic, within initial increases in DA release occurring within 30 minutes of progesterone treatment and peak DA release seen at 4 hours after treatment, followed by subsequent inhibition of DA release 24 hours after progesterone administration (Dluzen and Ramirez, 1984). Modulation of DA release by progesterone was also apparent after in vitro application of progesterone to striatal tissue from estradiol primed females, and progesterone conjugated to BSA replicates the effects of unbound progesterone, demonstrating that progesterone acts directly on membrane receptors within the striatum to enhance DA release (Dluzen and Ramirez, 1989, 1990).

Progesterone also modulates expression of DA receptors within the striatum. Animals pre-treated with estradiol showed an increase in D2 DA receptor binding four hours after progesterone administration (Fernández-Ruiz et al., 1989). Somewhat surprisingly, administration of progesterone to OVX females not treated with estradiol reduced D2 DA receptor binding, providing evidence that progesterone can modulate DA functional activity independent of the effects of estradiol (Fernández-Ruiz et al., 1989).

The timing of progesterone's effects on DA activity may indicate its relevance for adaptive motivated behaviors. Progesterone enhancement of DA release in estradiol-primed OVX rats was greatest four hours following hormone treatment, coinciding with the maximal induction of sexual receptivity after hormone priming (Glaser et al., 1983). The inhibition of DA release 24 hours following progesterone, after estradiol priming, also corresponds with the end of behavioral estrus, and it is possible that these effects of progesterone on DA release may coordinate sexually relevant motivated behaviors across the estrous cycle.

Adaptive function of ovarian hormone regulation of DA release

Sex differences in the neural control of motivated behaviors likely reflect differences in the environmental pressures imposed on males and females over the course of evolution. While successful reproduction for males, particularly males who do not contribute to parental care, depends on the ability to mate with and inseminate as many females as possible, female reproductive success requires considerably more effort (Buss and Schmitt, 1993). Like males, females should be motivated to find a mate; however, they must also dedicate considerable resources to gestation, parturition, and maternal care (Klug et al., 2013). Additionally, the inherent dangers associated with reproduction, due

not only to the risk of disease but also predation and general injury from the rigorous copulatory act, prevent females from being highly motivated for sex at times when conception is unlikely to occur, at least in species where males do not offer continued protection (Daly, 1978). Therefore, female sexual motivation is closely timed with ovulation in most species, and gonadal hormones that indicate whether the female is fertile or not can serve as physiological signals that modulate the neural systems responsible for changes in motivation (Wallen, 1990). With this in mind, it is possible that estradiol and progesterone may have evolved the ability to modulate DA release in order to drive adaptive changes in motivation for rewards across various reproductive contexts.

Estradiol and motivation for sex

As mentioned previously, peak enhancement of DA release after treatment with estradiol and progesterone coincides with maximal induction of sexual receptivity, and increased sexual motivation (Cummings and Becker, 2012). Modulation of striatal DA release around the time of ovulation is likely important for specific components of sexual motivation. Aspects of motivated behaviors can be described as being either appetitive or consummatory (Craig, 1917; Ball and Balthazart, 2008). Consummatory behaviors include those involved with the actual consumption of a reward, the actual act of copulation in the case of sexual behavior. Appetitive behaviors, on the other hand, are more variable, and serve to prepare the animal to engage in consummatory behavior. This can include reward seeking, approach, and other behaviors targeted at locating and procuring rewards.

Research in male rats has implicated DA in both appetitive and consummatory aspects of sexual behavior. DA levels in both the NAc and striatum rise during the presentation of a sexually receptive female and subsequent copulation in both experienced and sexually naïve rats (Wenkstern et al., 1993; Pfaus et al., 1995; Robinson et al., 2001). Additionally, pharmacological inactivation of DA receptors in the ventral striatum increases, while administration of a DA agonist decreases, latency to mount and intromit in male rats (Everitt, 1990).

However, reproductive behavior in the female differs from that of males in several important ways. While intact male rats are continuously capable of engaging in sexual behavior, a surge of estradiol at proestrus and the subsequent rise in progesterone is required for the onset of sexual receptivity in females (Beach et al., 1942). In addition, the conditions used to assay male sexual behavior – where the male has free access to the female and the female is not allowed to escape - are not rewarding for female rats, and do not allow for expression of the full complement of female sexual behaviors.

In a semi-natural environment, females will actively pace sexual behavior by running away from the male at a regular interval (Erskine, 1989). This pattern of behavior increases the latency between bouts of intromissions and allows for activation of a neuroendocrine reflex that increases the probability of conception (Erskine et al., 1989). Under these conditions, females will express a complex pattern of behaviors that solicit the male to approach and then mount (Erskine, 1989). These behaviors include ear wiggling, hops and darts, and other general approach behaviors, and serve not only to attract attention from conspecifics, but also to hold the male's attention between intromissions (Adler and McClintock, 1978; Erskine, 1989). Female sexual behaviors are

thus crucial for pacing, particularly in natural group mating settings, and serve as evidence that females play an active role in mating (McClintock and Anisko, 1982; McClintock et al., 1982; McClintock, 1984).

Receptive females will develop a conditioned place preference following paced mating behavior and will readily work for access to a mate when they are able to pace the rate of copulation, indicating that the female will find copulation to be rewarding under her preferred conditions (Jenkins and Becker, 2003b; Cummings and Becker, 2012). Importantly, extracellular DA levels in striatum and NAc rise during sexual behavior only when copulation occurs at the female's preferred interval (Mermelstein and Becker, 1995; Pfaus et al., 1995; Becker et al., 2001b; Jenkins and Becker, 2003a). The greatest increase in DA release is seen prior to the male's intromission, and not due to coital stimulation or general social interaction, indicating that DA is involved in anticipation of sexual behavior, rather than the sensorimotor aspects of sexual behavior (Jenkins and Becker, 2003a).

The majority of research on female sexual behavior has used reflexive behaviors such as lordosis quotient, ear wiggling, and the number of hops and darts as indices of female sexual arousal and proceptivity (Pfaus et al., 1999; Mazzucco et al., 2008). However, these reflexive behaviors may be neuroanatomically dissociable from sexual motivation, as was seen in the dissociation between male sexual performance and rates of operant responding for access to a mate. In support of this idea, SSRI induced sexual dysfunction, which primarily affects precopulatory and appetitive sexual behaviors in women, is detectable using operant tasks that measure sexual motivation, but not using classic tests of female proceptive behaviors (Uphouse et al., 2015). This highlights the

importance of using behavioral paradigms that dissociate these specific aspects of female sexual behavior.

Increased DA release seen in estrous females may also facilitate reward learning during estrous. Females conditioned to associate a specific context with cocaine reward during estrus showed enhanced firing of VTA DA cells when entering the drug paired context even when neural activity was measured when females are no longer in estrus, indicating that ovarian hormones can facilitate learning about rewarding stimuli and contexts (Calipari et al., 2017). In studies of sexual activity, olfactory cues that predict sexual reward elicit neural activation of striatal DA circuitry and anticipatory increases in DA release during paced mating are only seen after the first few intromissions (Jenkins and Becker, 2003a; Coria-Avila and Pfaus, 2007). Therefore, modulation of DA signaling during estrus may not only enhance sexual motivation, but also enhance learning about sex paired cues.

Estradiol and motivation for food

Although increases in estradiol and progesterone around the time of ovulation generally increase motivation for sex and drugs of abuse, this is not true for all rewards. The ability of ovarian hormones to induce sexual receptivity is accompanied by a complementary decrease in feeding behavior (Wade, 1972; Roney and Simmons, 2017). This may seem counter-intuitive, as copulatory behavior is energetically demanding, and one might expect females to increase their food intake in order to compensate for the increased metabolic demands associated with reproduction (Gittleman and Thompson, 1988; Schneider et al., 2013). An alternative variable that may better explain the observed decrease in food consumption is time. Although copulation itself is not necessarily time

consuming, mate seeking requires a significant time investment (Fessler, 2003). Importantly, many of the behavioral changes seen during periods of fertility, such as increased locomotor behavior in rats and expansion of geographic ranges in many primates, increase the probability of coming across a mature male and subsequently copulating (Eckel et al., 2000; Fessler, 2003). Following this logic, females who spend less time eating during periods when probability of conception is high are able to spend comparatively more time looking for a mate and engaging in other reproductive behaviors and are therefore more likely to successfully reproduce. This explanation highlights the ecological significance of the dual role of estradiol and progesterone in inducing receptivity while attenuating feeding.

Although both estradiol and progesterone are released during the periovulatory period, estradiol alone is sufficient to reduce food intake and body weight in female rats. Estradiol is primarily responsible for sexual dimorphism in body fat distribution, and removal of endogenous hormones by ovariectomy (OVX) results in weight gain due to changes in both energy expenditure and food intake (Brown & Clegg, 2010). Importantly, these changes are reversed by peripheral or central administration of estradiol, underscoring the importance of estradiol in the behavioral control of body weight (Eckel et al., 2000; Eckel, 2011; Santollo et al., 2012).

Estradiol interacts with a number of orexigenic and anorexigenic peptides, including neuropeptide Y, ghrelin, glucagon-like peptide-1 (GLP-1), insulin, leptin, cholecystokinin (CCK), and well as endogenous cannabinoids (Clegg et al., 2006; Brown and Clegg, 2010; Mela et al., 2015). The estrogen receptor that is believed to be responsible for mediating estradiol's effect on feeding and energy balance is ERα, while

ERβ is thought to modulate these effects. ERα knock out mice are obese, and selective knock-down of ERα in the ventromedial hypothalamus, a key brain area involved in regulation of food intake and body weight, has the same effect (Musatov et al., 2007). Acute pharmacological activation of ERα, but not ERβ, decreases meal size without an effect on meal frequency (Santollo, Wiley, & Eckel, 2007). Interestingly, activation of ERα is also required for the induction of sexual receptivity in female rats, whereas ERβ again plays a modulatory role (Mazzucco et al., 2008).

Similarly to sexual behavior, research on hormonal regulation of feeding behavior in female rats has focused on changes consummatory aspects of food intake. Few studies have evaluated the direct effect of estradiol on motivation for food, even though many of the signaling molecules regulated by estradiol have been shown to regulate motivated feeding (Cone et al., 2014; Olarte-Sánchez et al., 2015; Stouffer et al., 2015; van der Plasse et al., 2015; Hayes and Schmidt, 2016). The limited work that has been done has shown that motivation for palatable food reward is reduced during proestrus and estrus, an effect that is mediated by estradiol acting directly on the VTA (Richard et al., 2017). Further, repeated treatment with estradiol potentiates the ability of GLP-1 to attenuate motivation for food via ERα (Richard et al., 2016).

Even less work has been done to investigate the role of progesterone in feeding behavior. Progesterone is positively correlated with feeding in women, and others have speculated that progesterone may inhibit the anorexigenic effects of estradiol on feeding (Wade, 1972; Yu et al., 2011; Roney and Simmons, 2017). The effect of progesterone on motivation for food is unknown.

Trade-offs between competing rewards

The majority of research on how ovarian hormones regulate motivated behaviors has evaluated motivation when only one reward is available. This context strongly contradicts the natural environment, where organisms are continuously faced with opportunities for alternate rewards. Adaptive decision making requires information about the amount of reward that the animal will receive by performing the behavior, the odds that the behavior will end up being rewarded, as well as information about the animal's physiological or adaptive needs (Carr, 1996; Niv et al., 2006; Porter-Stransky et al., 2013; Aitken et al., 2016; Bach and Dayan, 2017). Often, pursuit of one reward precludes the ability to earn other available rewards, requiring the animal to not only decide which reward they should pursue, but also which reward they must forgo.

Trade-offs between feeding and reproductive behaviors have been studied within a number of contexts (Lutterschmidt and Maine, 2014; Schneider and Deviche, 2017). Many of the signaling molecules involved in the regulation of feeding have opposing effects on sexual behavior (Babcock et al., 1988; Schneider et al., 2013). Furthermore, changes in energy balance by food restriction results in a shift toward preference for food over a mate in female hamsters (Schneider et al., 2007). Interestingly, mild food restriction that does not alter consummatory sexual behavior or increase overall food intake will still attenuate appetitive sexual behaviors and enhance food hoarding (Klingerman et al., 2011). This indicates that changes in appetitive behaviors may be particularly sensitive to factors that alter the cost/benefit pay off when animals are deciding between food vs sex.

Summary of Present Experiments

The experiments and results described here aim to provide a link between proximate mechanisms of sex differences in the effects of ovarian hormones on DA release and ultimate explanations of the adaptive purpose of ovarian regulation of DA circuitry. First, I establish a sex difference in the effect of estradiol and selective estradiol receptor activation on NAc dopamine release after cocaine administration. I then describe an adaptive motivated behavior that exhibits opposite regulation by ovarian hormones. While estradiol increases DA release and motivation for drugs of abuse in females, administration of estradiol and progesterone reduces motivation for palatable food reward in OVX female rats. Finally, I test the hypothesis that regulation of motivation by ovarian hormones acts to bias female motivation toward food or sex at different points of the estrus cycle.

Chapter 2: Estradiol and dopamine release from the nucleus accumbens shell: sex differences and the role of selective estradiol receptor subtypes

Sex differences in the acute response to psychomotor stimulants is largely due to the ability of estradiol to potentiate drug-induced DA release. While this effect is well established within the dorsal striatum, investigation of the effect of estradiol on NAc dopamine release has produced mixed results. In this chapter, I verify that acute treatment with EB enhances the effect of cocaine on NAc DA release in females but not in males. I build upon these findings to determine that selective activation of ERβ, but not ERα, is sufficient to potentiate the increase in DA release following cocaine administration in females.

Chapter 3: Induction of sexual receptivity in female rodents reduces motivation for food

Cyclic changes in the release and circulation of ovarian hormones allows for the coordination of ovulation with both reproductive and non-reproductive behaviors, including feeding. Here, I sought to determine whether administration of EB and progesterone in a regimen that induces sexual receptivity and increases sexual motivation could also reduce motivation for food. I found that administration of EB and progesterone reduced motivation for food using a fixed interval schedule adapted from previous studies of sexual motivation. Although previous work from other labs has demonstrated that selective activation of ERα is sufficient to reduce food intake, I show that selective activation of ERα, ERβ, or GPER-1 during hormone priming did not attenuate motivation for food in ovariectomized female rats, indicating a dissociation between the effects of estradiol on consummatory and appetitive aspects of feeding. In addition, I found that progesterone is necessary for the effects of hormone priming on motivation for food, and while blockade of progesterone receptors (PR) within the NAc does not prevent the effects of hormone priming, there are hormone independent effects of NAc PR antagonism on food motivation.

Chapter 4: Ovarian hormones mediate changes in adaptive choice and motivation in female rats

Opposing changes of motivation for food and sex at the time of ovulation have been proposed to mediate adaptive decision making in female rodents. To test this hypothesis, I used a concurrent fixed interval choice paradigm to determine how administration of EB + P regulate motivation for food and sex when both rewards are available. I found that females bias their choice for food vs sex depending on their reproductive state: non-

receptive females show a greater preference for food, while receptive females are more likely to choose mate. At the same time, hormone treatment shifts the motivation for food or sex in opposite directions by increasing motivation for sex but decreasing motivation for food. Interestingly, this shift in motivation does not fully reverse differences in motivation for food vs sex. While unprimed females are more motivated for food than sex, hormone primed females show similar levels of responding for both rewards

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Chapter 2:

Estradiol influence on dopamine release from the nucleus accumbens shell: Sex differences and the role of selective estradiol receptor subtypes

Abstract

Females are more sensitive than males to both the acute and prolonged effects of psychomotor stimulants. In females, this is regulated by estradiol, which enhances dopamine (DA) release in the dorsal striatum. In this study we tested the acute effect of estradiol on DA release in the nucleus accumbens shell (NAcSh) before and after cocaine administration and investigated which estradiol receptors (ER) contribute to sex differences in the response to cocaine.

The ability of estradiol benzoate (EB) to acutely modulate the effect of cocaine on phasic DA release in NAcSh was measured by fast-scan cyclic voltammetry (FSCV) in anesthetized male and female rats. In addition, the role of ER subtypes ERα and ERβ was determined through use of subtype selective agonists. EB acutely enhanced the effect of cocaine on stimulated DA release from NAcSh in females but not in male rats. Enhanced DA release after cocaine administration was also observed in females treated with the ERβ selective agonist DPN, but not the ERα selective agonist PPT. EB attenuated the effect of cocaine on NAcSh DA reuptake in males but not females. Estradiol acutely and rapidly regulates DA release in females and DA reuptake in males. In females, estradiol rapidly enhances the effect of cocaine on DA release, likely via activation of ERβ. The

effect of estradiol in males is not seen with selective receptor subtype activation, a topic deserving of further study.

Introduction

Sex differences affect both the initial response to psychomotor stimulants as well as the propensity to develop addiction-like behaviors after prolonged use (Becker and Koob, 2016). In clinical populations, women present for treatment earlier than men, and report taking more drug, even though they have been using for less time (Brady and Randall, 1999). After treatment, women have lower rates of sustained abstinence, which is attributed to a greater susceptibility to both cue and stress induced relapse (Potenza et al., 2012). Similar sex differences have been found in preclinical rodent models. Female rats acquire cocaine self-administration more rapidly, show greater escalation of cocaine self-administration, and maintain levels of cocaine intake at higher levels than males (Becker and Hu, 2008).

Sex differences in sensitivity to psychomotor stimulants are in part due to differences in the activational effects of gonadal hormones on reward systems. Removal of gonadal hormones by ovariectomy (OVX) attenuates sex differences in the behavioral response to cocaine in females, while removal of the testes has no effect on the response in males (Hu et al., 2004; Jackson et al., 2006). Estradiol replacement to OVX females results in enhanced responses to psychomotor stimulants, underscoring the importance of estradiol for mediating sex differences (Becker and Rudick, 1999b; Becker et al., 2001a; Hu et al., 2004; Cummings et al., 2014). Importantly, estradiol treatment in males has no effect, indicating a sex difference in the activational effects of estradiol on neural systems mediating drug taking.

Estradiol enhancement of the behavioral responses to psychomotor stimulants has been linked to changes in striatal dopamine (DA) release. Within the dorsal striatum, estradiol acutely enhances DA release in females but not in males (Cummings et al., 2014; Shams et al., 2016). A substantial body of work has demonstrated that estradiol acts directly on GABAergic striatal tissue to disinhibit DA terminals and promote DA release within the striatum (Yoest et al., 2018). However, while the effects of estradiol in dorsal striatum have been well established, studies on the rapid effects of estradiol on DA release within the nucleus accumbens (NAc) have produced mixed results. Some studies have shown that estradiol is able to act directly on NAc circuitry to rapidly enhance stimulated DA release (Thompson and Moss, 1994), others have demonstrated an effect of estrous cycle but no effect of direct estradiol application (Calipari et al., 2017), and still others have failed to show an acute effect of estradiol at all (Cummings et al., 2014).

Of note, many of the studies that have seen an effect of ovarian hormones, either by direct application of estradiol or measurement of estradiol across the estrous cycle, have measured DA using fast-scan cyclic voltammetry (FSCV), while studies that failed to show an effect used microdialysis. FSCV measures phasic DA release on the scale of seconds within discrete subregions of the NAc, while experiments using microdialysis measures slower changes in tonic release within the NAc as whole. Estradiol may have opposite effects within the NAc core and shell, and decreased regional specificity in experiments using microdialysis likely contributes to the lack of effects in these studies (Peterson et al., 2015).

Additionally, for studies using FSCV, differences in estradiol dose may account for discrepancies in findings. The range at which estradiol modulates cellular function is

very narrow (Becker, 1990a; Tanapat et al., 2005). Doses that are lower than this range fail to have an effect, and higher concentrations either have no effect or the opposite effect, presumably due to disruption of the cell membrane (Whiting et al., 2000). Therefore, we hypothesized that an acute, physiological dose of estradiol would enhance phasic DA release specifically within the NAc shell.

Here, we first sought to characterize the receptor mechanism by which estradiol could modulate DA release within the NAc shell. There are multiple estradiol receptor subtypes, the most well-studied of which are ERα and ERβ (Morissette et al., 2008). These receptors can be expressed either within the cytosol or bound to plasma membrane and are differentially expressed within striatal circuitry. While ERα is expressed on GABAergic interneurons and DAergic terminals within the NAc, ERβ has only be found within the ventral tegmental area (VTA; (Mitra et al., 2003; Almey et al., 2015). Therefore, a secondary goal of the current study was to determine which estradiol receptor subtypes may mediate estradiol's ability to modulate stimulated DA release after systemic cocaine treatment.

Methods

Animals

Male (n=29) and female (n=29) Sprague Dawley rats (Charles River Breeding Laboratory; Portage, MI) 50-55 days of age were maintained on 14:10 L:D cycle (lights on at 9:00 AM) and housed in same-sex pairs in standard laboratory cages with *ad libitum* access to water and phytoestrogen free rat chow (2017 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were carried out in accordance with the National Institutes of Health

guidelines on laboratory animal use and care, using a protocol approved by University of Michigan Institutional Animal Care and Use Committee. Animals were gonadectomized as described previously (Cummings et al., 2014). Absence of estrous cycle in ovariectomized females was verified by daily vaginal lavage, and animals were left otherwise undisturbed in their home cage for two weeks following gonadectomy.

FSCV Surgery

On the day of surgery, animals were anesthetized with urethane (1.5g/kg, IP in 0.9% sterile saline; Sigma Aldrich, MO) and prepared in a stereotaxic frame (Kopf Instruments, CA). A glass-encased cylindrical carbon fiber electrode was lowered into the NAc shell (AP, +1.7; ML, ± 0.8; DV, -6) and an Ag/Cl reference electrode was placed in the contralateral cortex (AP, -2.3; ML ±2.7). DA release was recorded by oxidation and reduction of DA in response to the application of a triangular waveform (oxidative scan, 0.4-1.3 V; reductive scan, 1.3-0.4 V; 400V/s) applied to the carbon fiber electrode. Prior to taking recordings of stimulated release, this waveform was repeatedly cycled at a rate of 60 Hertz (Hz) for 30 minutes, followed by another 10 minutes of cycling at 10 Hz. All recordings of stimulated release were taken at 10 Hz. A bipolar stimulating electrode (AP, -5.2; ML, ±0.8) was lowered into the VTA incrementally starting at -7 DV while periodically checking for DA release using 60Hz 60 pulse (p) biphasic stimulations. Once release was optimized, three recordings were taken during application of a 20Hz 24p stimulation to the VTA in order to verify stability of DA response; recordings were considered stable if peak release during these recordings varied by less than 10%. Stability of the electrode was also verified periodically at the beginning and end of each stage of the experiment. After placement of the recording and

stimulating electrodes, rats then received a subcutaneous intrascapular injection of either estradiol benzoate (EB, Sigma Aldrich, MO; 5μg/0.1ml peanut oil) or vehicle (peanut oil; 0.1ml) for experiment 1. To determine the role of estradiol receptor subtypes, the ERα selective agonist propyl pyrazole triol (PPT, Tocris, MN; 1mg/kg) or the ERβ selective agonist diarylpropionitrile (DPN, Tocris, MN; 1 mg/kg) were used in experiment 2. For both experiments 1 and experiment 2, baseline recordings were taken 30 minutes following treatment. The experimental timeline is outlined in Figure 2.1A.

FSCV Recordings

For recordings of stimulated release, ten recordings were taken for each stimulation parameter. Stimulations of 20Hz 24p, 20Hz 12p, 10Hz 24p, and 10Hz 12p were applied in order to determine whether the effect of estradiol on DA signaling after cocaine administration is dependent on the amount of DA release. The highest stimulation parameter, 20Hz 24p, was selected based on previous work demonstrating that stimulation within this range saturates transporters and leads to steady state overflow kinetics (Wightman et al., 1988; Wightman and Zimmerman, 1990). Representative current traces (Fig 2.1A, B) and color plots (Fig 2.1C, D) are presented in Figure 2.1. Following baseline recordings, animals received an IP injection of Cocaine HCl (10mg/kg, NIDA). After cocaine administration, a second set of recordings were taken using the same stimulation parameters.

Data Processing and Analysis

Analysis of stimulated DA release and uptake was conducted using DEMON Voltammetry software provided courtesy of Dr. Sara R. Jones at Wake Forest University (Yorgason et al., 2011). From each recording, peak DA concentration was extracted as a

measure of DA release, and Tau was calculated as a measure of DA reuptake. This method of quantifying DA release and reuptake is recommended when stimulation does not induce substantial synaptic overflow, and therefore does not fulfill the assumptions required for Michaelis-Menten modeling techniques. At lower levels of stimulation (10Hz 24p and 10Hz 12p), the DA signal was not sufficient to reliably extract release and reuptake values for all animals; such animals (10Hz 24p: n=2, 10Hz 12p: n=6) were excluded from further analysis for these parameters.

Average DA release and reuptake at each stimulation parameter was calculated for each animal, and the % change from baseline was obtained by dividing the post-cocaine values by the pre-cocaine values and multiplying by 100. Data are expressed as % change from baseline in order to control for variability in the sensitivity of electrodes used or individual differences in baseline DA release and reuptake.

Experimental Design

Target group sizes (n=6) were calculated based on effect sizes found in previous studies from our lab (Cummings et al., 2014). Power analysis (α=0.05, 1-β=0.9) using an estimated effect size of 1 indicated a minimum necessary sample size of 6 animals per treatment group. Male and female animals were assigned to receive either EB (females: n=8, males: n=6), the ERα selective agonist PPT (females: n=8, males: n=8), the ERβ selective agonist DPN (females: n=6, males: n=7), or vehicle (females: n=7, males: n=8). Due to the high rate of detection failure in anesthetized voltammetry experiments due to missed probe sites, lack of recording stability, or premature mortality, animals were tested in pseudorandom order until minimally required group size was reached for each condition.

Statistical Analysis

The data and statistical analyses comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). Group comparisons were performed using GraphPad Prism v7.0a (GraphPad, San Diego, CA). Data were analyzed using two-way ANOVA with Holm-Sidak post hoc tests to compare animals treated with hormones or agonists to controls within sex, as well as to determine if there were sex differences in the effect of each treatment. The accepted value for statistical significance was set to $p<0.05$, and post hoc tests were only run when initial F values reached this threshold.

Materials

EB was suspended in peanut oil at a concentration of 5μg per 0.1 ml and left on a stir plate overnight prior to use. EB was stored at room temperature for the duration of use. PPT and DPN were suspended in gelatin at a concentration of 1mg/ml. Agonists were stored at 4^{o}C until the day of use, at which time syringes were prepared and brought to room temperature. All hormones and agonists were used within two weeks of preparation.

Results

Effect of estradiol on stimulated DA release after cocaine administration

The effect of estradiol on stimulated DA release after cocaine administration was assessed at four levels of stimulation. Stimulation of VTA with 20Hz 24p was used to promote maximal occupancy of DA transporters (DAT) without exceeding a balance between release and reuptake. Lower levels of stimulation were used to assess changes in

DA release and reuptake after cocaine when release did not achieve maximal reuptake thresholds.

At the highest stimulation parameter (Fig. 2.2A), there was a significant effect of sex ($F_{1,49}$=5.24, p<0.05). In animals treated with vehicle, there was no sex difference in the effect of cocaine on DA release demonstrating that there were no pre-existing sex differences in the response to cocaine in gonadectomized animals. There was a sex difference, however, in animals treated with estradiol, where EB-treated females showed a significantly greater increase in DA concentration after cocaine compared to EB-treated males (p<0.05; Fig 2.2A). Thus, treatment with estradiol enhanced the effect of cocaine on stimulated DA release in females, but not in males, when stimulation parameters promoted maximal DAT occupancy.

At the 20Hz 12p stimulation level (Fig 2.2B), there was a significant main effect of sex ($F_{1,49}$=4.71, p<0.05) but only a trending effect of treatment (p=0.07) or interaction between sex and treatment (p=0.06). Multiple comparisons revealed no significant differences in any of the treatment groups, though there was a trend toward significance where EB treated females showed slightly enhanced DA release compared to vehicle.

After 10Hz 24p stimulation (Fig 2.2C), there was a significant main effect of treatment on DA release ($F_{3,47}$=5.85, p<0.05) but no effect of sex and no significant interaction. Subsequent analysis showed no significant differences between any of the individual treatment groups. There was also no significant effect of treatment or sex on cocaine enhanced DA release after 10Hz 12p stimulation of the VTA, nor a significant interaction between the two (Fig 2.2D).

Estradiol enhancement of cocaine's effect on DA release is mediated by ERβ

Treatment with the ERβ selective agonist DPN increased the effect of cocaine on stimulated DA release in females ($p<0.05$; Fig 2.3). There was also a sex difference in animals treated with DPN, where DPN-treated females showed greater enhancement of DA release following cocaine compared to DPN-treated males when stimulation parameters promoted maximal DAT occupancy ($p<0.05$). There was no effect of DPN during sub-maximal stimulation of the VTA, although there was a trend toward a significant enhancement of DA release after DPN in females after 20Hz 12p stimulation of the VTA ($p=0.10$).

The ERα selective agonist PPT had no effect on DA release in either sex (Fig. 2.3).

Effect of estradiol on DA reuptake after cocaine administration

Tau is an exponential decay constant that has been recommended for quantifying DA reuptake after stimulated release (Yorgason et al., 2011). Tau is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. Therefore, treatment with cocaine, which blocks DAT and prevents reuptake, leads to an increase in Tau. After the 20Hz 24p stimulation, there was a significant interaction between sex and treatment ($F_{3,48}= 3.34$, $p<0.05$; Fig 2.4). There was no preexisting sex difference in the effect of cocaine on DA reuptake, and no effect of EB on reuptake in males or females when compared to within sex vehicle. However, there was a sex difference in the effect of estradiol, where males showed reduced sensitivity to the effect of cocaine on DA reuptake when compared to estradiol treated females ($p<0.05$; Fig. 2.4). Neither the ERα selective agonist PPT or the ERβ selective agonist DPN had a

significant effect on reuptake in either sex and there was no significant effect of treatment, sex, or interaction between treatment and sex at any of the lower stimulation parameters (Fig. 2.5).

Discussion

Estradiol increases DA release in response to cocaine in females

Estradiol treatment rapidly enhanced the effect of cocaine on stimulated DA release in the NAc shell of female rats, while increasing DA reuptake in males. These data clarify the role of estradiol on ventral striatal DA release and provide a potential mechanism by which estradiol renders females more susceptible to drug abuse.

The effect of estradiol in females was only significant when release was elicited by stimulation of the VTA that was expected to saturate DAT, although the general direction of an effect of estradiol on DA release after cocaine administration was still apparent at lower levels of stimulation in females. These results suggest that the rapid effects of estradiol in the NAc shell of females are affecting the stimulated increase in DA release, since the greatest effects are seen when DAT is fully occupied.

The reinforcing properties of cocaine and other psychomotor stimulants are strongly linked to their ability to enhance striatal DA transmission. Blockade of D1 DA receptors in both the NAc and VTA reduces the reinforcing effects of cocaine and disrupts cocaine induced conditioned place preferences (Maldonado et al., 1993; Ranaldi and Wise, 2001; Nazarian et al., 2004). Sex differences in these behaviors are likely due to sex differences in the underlying DA response. Females show greater acquisition and escalation of drug taking and establish conditioned place preferences for stimulants at lower doses than do males (Russo et al., 2003; Hu et al., 2004). Importantly, these sex

differences are dependent on circulating gonadal hormones, where estradiol enhances the effects of stimulant drugs in ovariectomized females but not in castrated males.

One model of how ovarian hormones enhance the effect of cocaine has proposed that shifts in the excitability of VTA DA neurons during estrus lead to increased expression of the phosphorylated form of DAT, to enhance cocaine binding efficacy (Calipari et al., 2017). It is possible that increased DAT activation could increase DA release. On the other hand, cocaine-induced increases in NAc DA release are still apparent in mice lacking DAT, so other mechanisms may regulate DA signaling within this brain area (Carboni et al., 2001). Importantly, DAT is not required for behavioral expression of cocaine reward. Mice lacking DAT will still acquire cocaine self-administration and show conditioned place preferences for cocaine (Rocha et al., 1998; Sora et al., 1998). Furthermore, although DAT is not required for the effects of cocaine on DA release or cocaine reinforcement, it is still necessary for changes in DA reuptake after cocaine administration (Budygin et al., 2002). This underscores the importance of changes in DA release, and not reuptake, in drug taking behaviors. This is further supported by our findings that estradiol, which enhances drug taking in females but not males, alters DA release without an effect on reuptake in female rats.

Effects of estradiol on cocaine induced DA release are mediated by ERβ

We tested whether selective activation of ER subtypes would also modulate the effects of cocaine on DA release in male and female rats. Activation of ERβ alone was sufficient to enhance the effect of cocaine on stimulated DA release. The ERβ selective agonist, DPN, but not the ERα selective agonist, PPT, duplicated the effects of estradiol on cocaine-induced DA release. This is not the first report that ERβ activation enhances

the response to cocaine in females. ERβ has also been shown to mediate the effects of chronic estradiol on expression of D2 receptors and DAT within the NAc (Morissette et al., 2008). Activation of ERβ within the NAc also mediates estradiol's enhancement of conditioned place preference to both cocaine and amphetamine (Silverman and Koenig, 2007; Satta et al., 2018). The findings presented here confirm the importance of ERβ in mediating sex differences in the response to cocaine, and provide a potential mechanism for how estradiol enhances DA activity in females.

ERβ is not expressed locally within the NAc, but studies in mice have found ERβ within the VTA (Creutz and Kritzer, 2002). This would be consistent with the idea that estradiol enhances excitability of VTA DA neurons, rather than altering release within the NAc (Calipari et al., 2017; McHenry et al., 2017). However, this directly contradicts previous work showing rapid increases in K^+-stimulated DA release after direct application of estradiol to NAc (Thompson and Moss, 1994). Although studies have not established the expression of ERβ within the NAc, there is indirect evidence for ERβ activity in this region. Knockdown of ERβ mRNA within the NAc prevented the effect of estradiol on cocaine-conditioned place preference, as well as estradiol enhancement of cocaine-induced NAc cFos (Satta et al., 2018). Future work, utilizing site-specific microinjections to either the NAc or VTA, could clarify where estradiol is acting to alter the DA response to cocaine. It is also possible that ERs within hypothalamic or amygdala circuitry indirectly modulate DA release, leading to increased DA signaling in response to cocaine.

Estradiol modulates the effect of cocaine on DA reuptake after VTA stimulation in males

Consistent with previous research, we did not see an effect of estradiol on DA release in males. However, males treated with estradiol showed decreased DA reuptake after electrical stimulation of the VTA in the presence of systemic cocaine treatment. To our knowledge, this is the first evidence that estradiol rapidly modulates NAc DA transmission in males. Neither the ERβ or ERα selective agonist reduced changes in DA reuptake after cocaine. There are multiple potential explanations for this inconsistency. The decreased DA reuptake after estradiol administration, in the presence of systemic cocaine treatment, may require an interaction between ERα and ERβ. These receptors are known to interact and have potentially opposing effects to one another. In particular, ERβ often regulates the effects of ERα, which could explain the differences after selective and non-selective ER activation seen here. Alternatively, estradiol may act through another ER to attenuate the effect on DA reuptake seen after cocaine treatment. G-protein estradiol receptor-1 (GPER-1) is expressed in the NAc in female rats, and GPER-1 activation may be responsible for the attenuated changes in DA reuptake seen in EB treated males in this study.

Conclusion

These data add to the growing body of literature on sex differences in the neurochemical and behavioral responses to psychomotor stimulants. DA release within the NAc is integral to the rewarding and addictive properties of cocaine, and sex differences in the cocaine induced DA release likely contributes to the greater vulnerability to addictive behaviors in females. As estradiol is known to regulate the DA

response to drugs of abuse in females, anti-estradiol treatments have been proposed as a possible therapeutic for stimulant abuse (Mikelman et al., 2017). However, the significant side effects associated with these drugs, particularly side effects mediated by actions at ERα, are a barrier to their widespread use. Here we demonstrate that ERβ mediates the effects of estradiol on DA release within the NAc and thus may be a better target for development of pharmacotherapies for stimulant abuse in females. Future work characterizing how estradiol mediates acute responses to drugs of abuse should be prioritized in understanding sex differences in addiction.

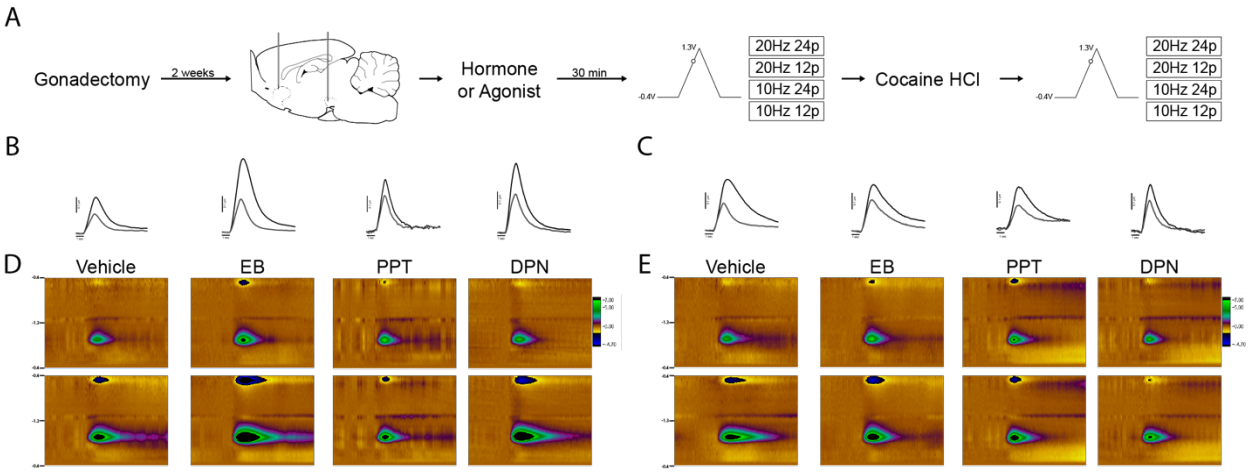

Figure 2.1 Measurement of stimulated DA release by FSCV.
(A) Animals were gonadectomized two weeks prior to surgery. During the FSCV surgery, a glass encased carbon fiber electrode was lowered in the NAc shell and a bipolar stimulating electrode (PlasticsOne, Roanoke, VA) was lowered into the VTA. Hormone, agonist, or vehicle treatment was delivered, and a series of recordings at various levels of stimulated release were taken before and after administration of Cocaine HCl. Representative current traces over time before (gray) and after (black) cocaine administration in females **(B)** and males **(C)**. Representative false-color plots before (top) and after (bottom) cocaine administration in females **(D)** and males **(E)**.

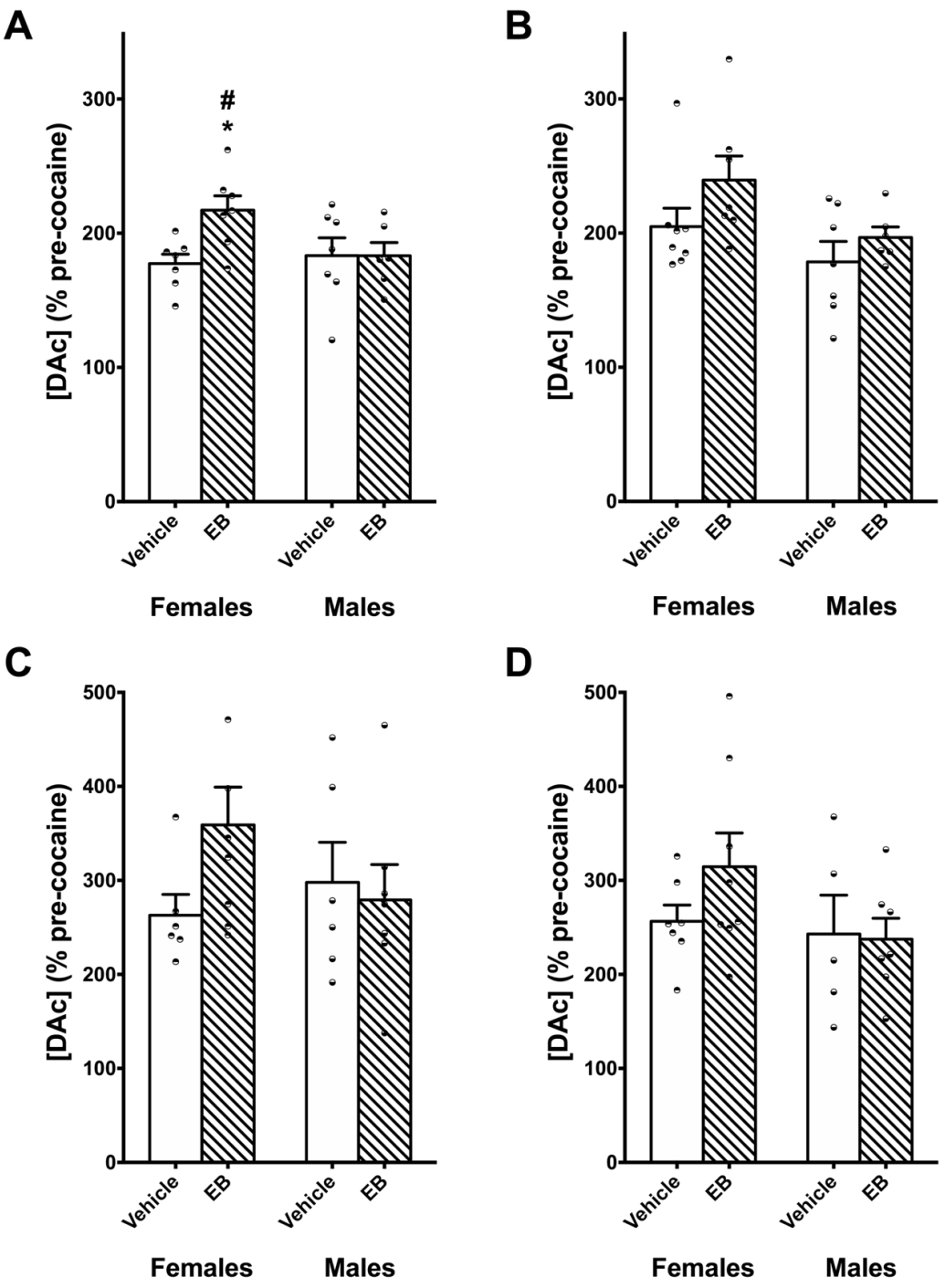

Figure 2.2 Estradiol enhances the effect of cocaine on NAc DA release in females but not in males.

Estradiol treated females show significantly greater DA release after cocaine administration during **(A)** 20Hz 24p stimulation of the VTA but not **(B)** 20Hz 12p, **(C)** 10Hz 24p, or **(D)** 10Hz 12p stimulation. Values represent mean ± SEM. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect.

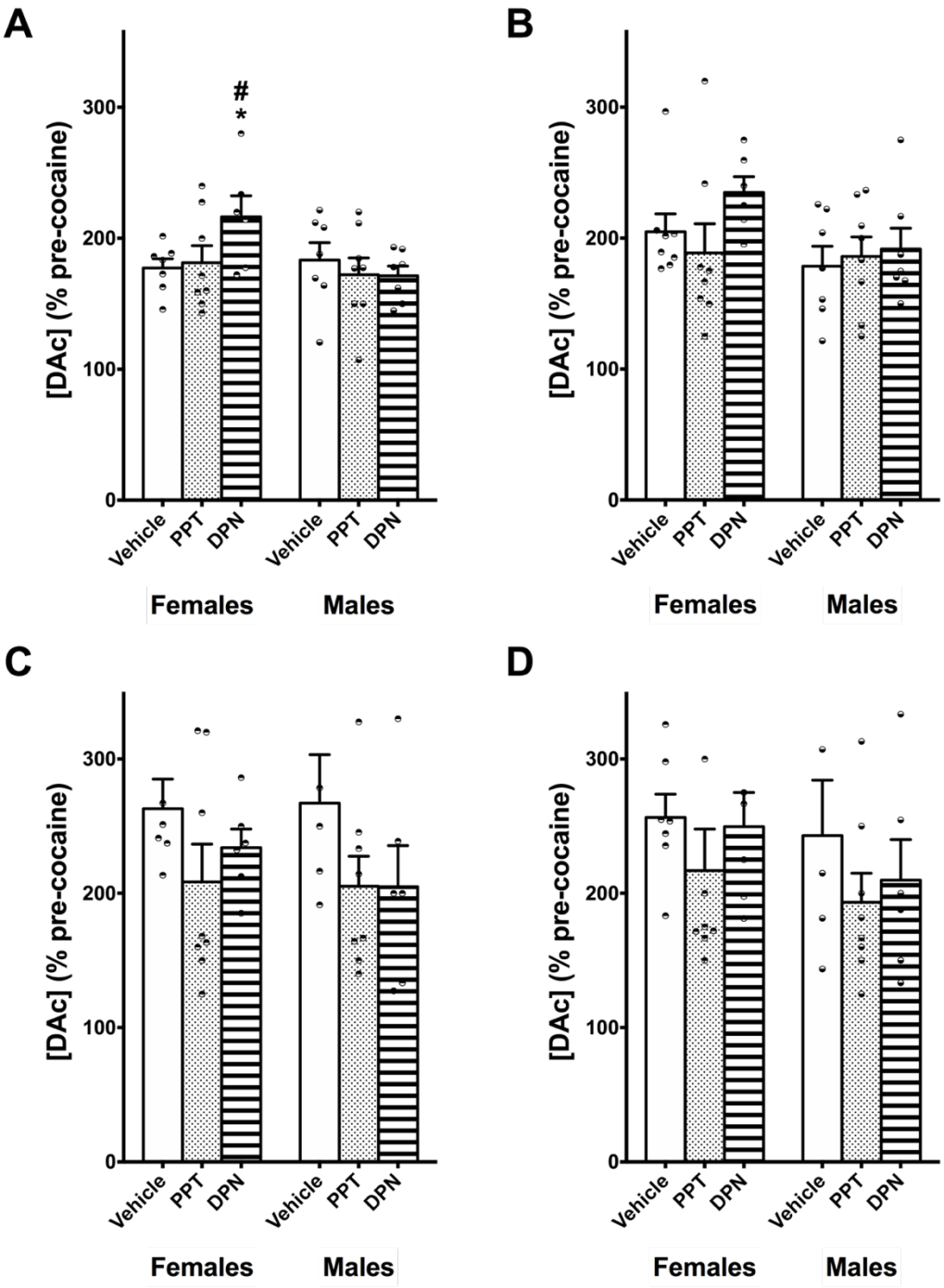

Figure 2.3 The effect of estradiol on DA release after cocaine is mediated by ERβ. DPN treated females show significantly greater DA release after cocaine administration during **(A)** 20Hz 24p stimulation of the VTA but not **(B)** 20Hz 12p, **(C)** 10Hz 24p, or **(D)** 10Hz 12p stimulation. Values represent mean ± SEM. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect.

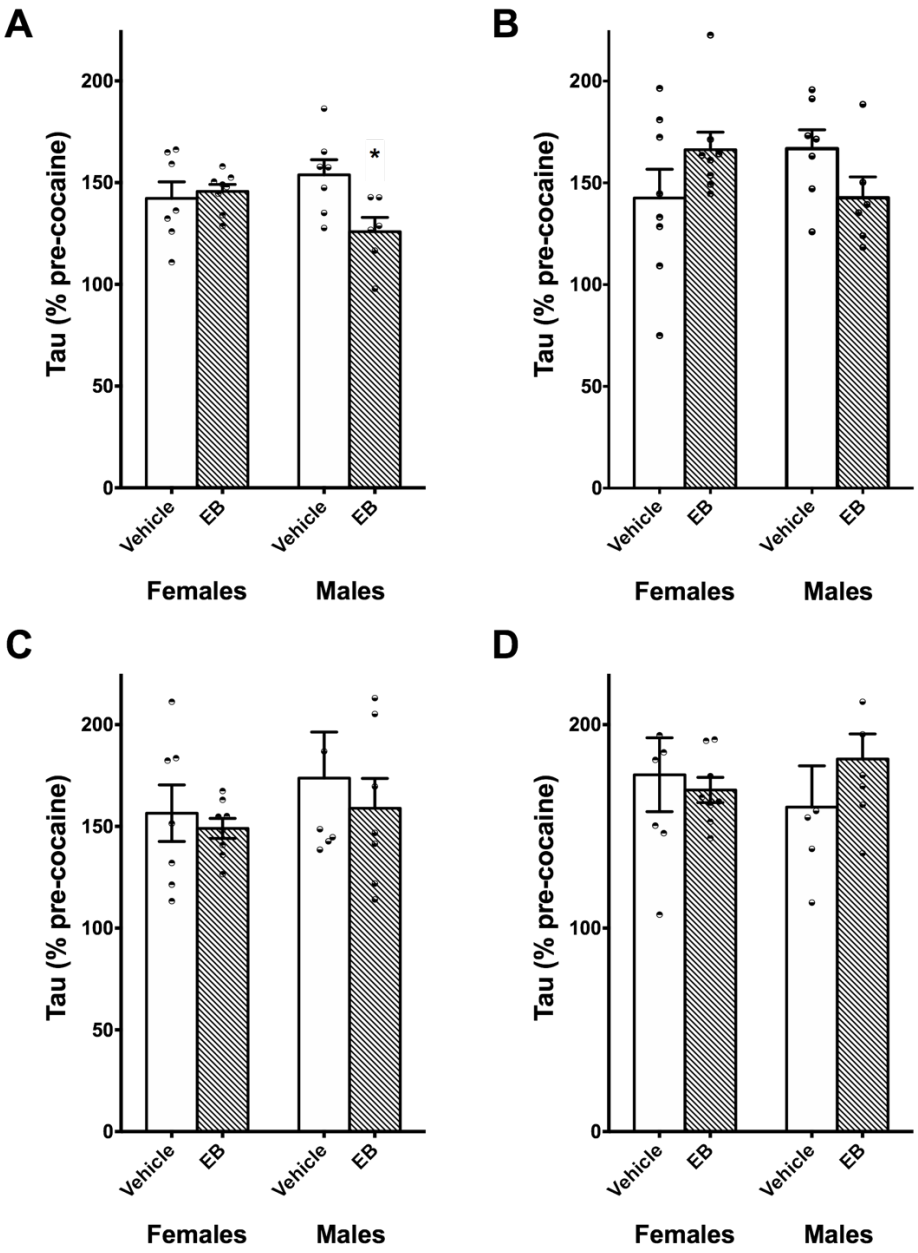

Figure 2.4 Estradiol attenuates the effect of cocaine on NAc DA reuptake in males but not in females.

EB treated males show significantly less of an effect of cocaine on DA reuptake after **(A)** 20Hz 24p stimulation of the VTA. Reuptake is represented by the exponential decay constant Tau. Tau is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. There was no difference in the effect of cocaine on DA reuptake during **(B)** 20Hz 12p, **(C)** 10Hz 24p, or **(D)** 10Hz 12p stimulation of the VTA. Values represent mean ± SEM. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect.

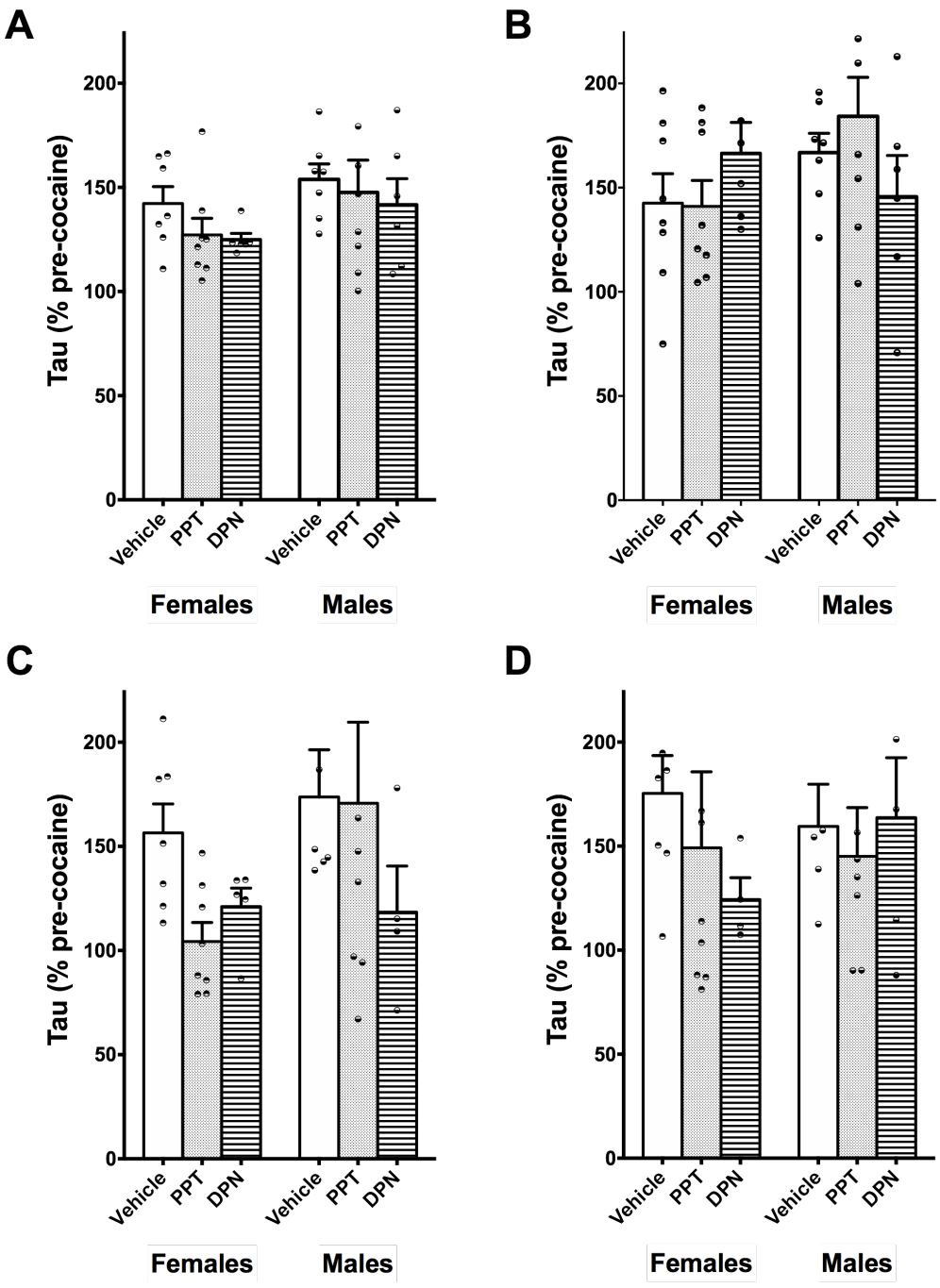

Figure 2.5 There is no effect of selective ER activation on DA reuptake in males or females.

Reuptake is represented by the exponential decay constant Tau, which is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. There was no difference in the effect of cocaine on DA reuptake during **(A)** 20Hz 24p, **(B)** 20Hz 12p, **(C)** 10Hz 24p, or **(D)** 10Hz 12p stimulation of the VTA. Values represent mean ± SEM. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect.

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Chapter 3:

Induction of sexual receptivity in female rodents reduces motivation for food

Abstract

In females, cyclic changes in ovarian hormones regulate both consummatory and appetitive aspects of feeding behavior. While estradiol alone is sufficient to reduce food intake and body weight in ovariectomized female rats, the role of ovarian hormones in mediating changes in motivation for food remains understudied. Here we sought to parse the pharmacological and behavioral mechanism by which changes in circulating gonadal hormones over the course of the rodent estrous cycle influences motivation for food. We administered estradiol benzoate (EB) and progesterone (P) to ovariectomized female rats in a manner that mimics the release of endogenous hormones during ovulation and measured motivation for palatable food reward using a fixed interval operant schedule. Administration of EB + P transiently reduced motivation for food only while animals were sexually receptive. Selective activation of estradiol receptors (ER) during hormone priming were unable to fully replicate the effect of EB + P on motivation for food, indicating that multiple ER subtypes are involved in changes in food reward when animals are sexually receptive. Further, while administration of EB alone still reduced the number of rewards that animals earned over the course of each session, it was not sufficient to attenuate motivation for food. Finally, while blockade of nucleus accumbens progesterone receptors (PR) with RU486 enhanced motivation for food prior to hormone administration, there was no effect of PR antagonism during hormone priming, and this

effect may be mediated by inactivation of glucocorticoid receptors. Taken together, we find that decreases in motivation for food during periods of sexual receptivity in female rodents are transiently induced by progesterone, and may have important significance for understanding changes in adaptive behavior during ovulation.

Introduction

The coupling of ovulation with behaviors that enhance the likelihood that a female will find a mate and reproduce is essential for adaptive fitness. Many mammals, including rodents, coordinate the physiological control of ovulation with behavior through the release of ovarian hormones. This can include behaviors that are directly involved in reproduction, e.g. the induction of the lordosis reflex in female rodents, as well as behaviors that only indirectly enhance reproductive success, including feeding. Food intake and body weight decrease during proestrus and estrus, when levels of the ovarian hormones estradiol and progesterone (P) reach their peak (Beatty, 1979; Asarian and Geary, 2006). A large body of work has demonstrated that these periovulatory changes in feeding are dependent on estradiol, which rapidly attenuates food intake via activation of estradiol receptor (ER) α (Asarian and Geary, 2002; Santollo et al., 2007; Eckel, 2011). While the majority of research has focused on reductions in consummatory feeding behaviors (e.g. food intake) during periods of sexual receptivity, appetitive and motivational components of feeding are also regulated by ovarian hormones. There are sex differences in both the behavioral and neural response to rewarding food and food-paired cues, and motivation for food in females is reduced during proestrus and estrus (Pitchers et al., 2015; Abbott et al., 2016; Reichelt et al., 2016). As seen in studies of consummatory feeding behavior, administration of exogenous estradiol, either systemically or the ventral tegmental area (VTA), to ovariectomized (OVX) females recapitulates the decrease in motivation seen during ovulation (Richard et al., 2017). Taken together, it is clear that ovarian hormones regulate both appetitive and

consummatory aspects of feeding behavior, although the extent to which the mechanisms that control these two behaviors overlap remains to be elucidated.

While previous work on both appetitive and consummatory aspects of feeding has emphasized the role of estradiol in estrous cycle mediated changes in behavior, both estradiol and P are required for the coordination of reproductive behaviors and ovulation. P regulates the appetitive components of female sexual behavior and has been linked to increased motivation for food during the luteal phase in humans (Brandling-Bennett et al., 1999; Pfaus et al., 1999; Roney and Simmons, 2017). However, the role of P in mediating ovarian hormone induced changes in feeding behavior in rodents remains poorly understood, particularly when P is administered following sequential administration of estradiol to induce sexual receptivity. This is particularly important for understanding how changes in ovarian hormones orchestrate adaptive changes in behavior. Researchers have speculated that decreases in feeding behavior during ovulation serve to reduce the amount of time that animals dedicate to food seeking and consumption in order to increase the amount of time available for reproductive behaviors (Fessler, 2003). If this is the case, the mechanism by which ovarian hormones reduce motivation for food should be similar, or integrated with, the mechanism that induces sexual receptivity and enhances sexual motivation.

In this study, we sought to delineate the relative roles of estradiol and P in estrous cycle mediated changes in motivation for food. We first aimed to determine whether administration of a long acting estrogen, estradiol benzoate (EB), and P in a regimen that induces sexual receptivity in OVX females also decreases motivation for food. We then tested the relative contribution of ERα, ERβ, and G-protein coupled ER-1 (GPER-1), in

order to determine whether the effects of estradiol on motivation for food are regulated by the same receptor mechanism implicated in consummatory feeding. Finally, we identified an important contribution of P in motivation for food and provide preliminary evidence to suggest an effect of progesterone receptors (PR) within striatal circuitry on motivated feeding behaviors.

Methods

Animals

Female Long Evans rats (Charles River Breeding Laboratory; Portage, MI) 50-55 days of age were maintained on a 14:10 L:D cycle (lights off 1 hr prior to behavioral testing) and housed in same-sex pairs in standard laboratory cages with ad libitum access to water and phytoestrogen free rat chow (2017 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were carried out in accordance with the National Institutes of Health guidelines on laboratory animal use and care, using a protocol approved by University of Michigan Institutional Animal Care and Use Committee. Animals were ovariectomized as previously described and absence of estrous cycle was verified by daily vaginal lavage for at least one week prior to the beginning of training (Cummings et al., 2014).

Operant Training Paradigm and Schedule

Animals were trained 5 days a week (M-F) at the same time each day. Approximately 2-4 hours prior to training (1-3 hours prior to lights off), food hoppers were removed from the home cages. 1 hour after lights off, animals were transported to standard operant chambers (Med Associates, Inc., Georgia, VT) for operant conditioning. Operant conditioning sessions lasted 40 minutes, and the start of the task was indicated

by illumination of the house light and activation of a white noise speaker within each chamber. Two nose poke holes in the chamber were used to register active or inactive responses during the session, and the active vs. inactive hole was counterbalanced across animals. Responses in either port resulted in illumination of a cue light within the respective nose poke hole. Responses on the active nose poke hole also resulted in the delivery of a single food pellet (45 mg banana flavor, BioServ, Framingham, NJ) and transient inactivation of the house light for 40s once the response requirement had been met. During this 40s period, responses on both nose pokes were counted but did not result in presentation of either cue light. This time out (TO) period was used to approximate the amount of time that a female would spend interacting with the male during similar tests of male sexual motivation.

Animals started training on a fixed-ratio (FR) 1 schedule, during which every active response resulted in reward delivery. After one week on the FR1 schedule, the response requirement was increased to an FR2 for 2 sessions, followed by an FR3 for 3 sessions, and an FR5 for 5 sessions. Fixed interval (FI) training was only initiated once animals were able to stably respond on the FR schedules. Animals were trained on FI schedule with intervals ranging from 5s during early sessions, to 15s during final sessions and testing. The interval was increased for each animal individually once they received no fewer than five less rewards than they received on the FR5 schedule for two consecutive days. The majority of animals were able to reach the FI 15s within one week. Animals were then trained on the FI15s for an additional two weeks, until within-week levels of responding had stabilized.

Operant Testing Paradigm

Motivation for food pellets was measured using a FI 15s operant schedule during which animals were able to make a variable number of responses for each reward. The first nose poke on the active port initiated a 15s interval, during which time all responses were counted and resulted in presentation of the cue light but had no other consequence. The first nose poke within 5s of the conclusion of this interval resulted in pellet delivery and transient inactivation of the house light during a 40s time-out (TO) period. If the animal did not make a response within 5s, the interval was failed, and no rewards were delivered until the animal initiated and completed the next trial.

Progressive Ratio

Motivation for food pellets was also measured by a progressive ratio reinforcement schedule as described previously (Perry et al., 2013). The number of responses required to earn each reward progressively increased in an exponential series: 1, 3, 6, 9, 12, 17, 24, 32, 46, 56, 73, 95, 124, 161, 208, 268, 346, 445, 573, 737, 948… (Richardson and Roberts, 1996). The breaking point (BP) was defined as the largest response ratio that the animal completed. Progressive ratio testing ended when the animal failed to reach the next response requirement within 1 hour, or after 6 hours (no animals reached this 6-hour time limit).

Drug Preparation

Estradiol benzoate (EB; Sigma Aldrich, St Louis, MO; 5 μg/0.1 ml) was suspended in either 1% gelatin solution or emulsified in peanut oil. The ERα selective agonist Propyl pyrazole triol (PPT; Tocris, Minneapolis, MN; 1mg/kg), ERβ selective agonist diarylpropionitrile (DPN; Tocris, Minneapolis, MN; 1mg/kg), or GPER-1

selective agonist G1 (Cayman Chemicals, Ann Arbor, MI; 1mg/kg) were suspended in 1% gelatin. All gelatin solutions were stored at 40o celcius until the day of use, at which point syringes containing 0.1 ml of solution for EB and 1ml/kg for selective agonists were prepared and brought to room temperature. P (Sigma Aldrich, Minneapolis, MO; 500ug/0.1ml) was emulsified in peanut oil (500 μg/0.1 ml) and stored at room temperature for the duration of the experiment.

The anti-progestin RU486 (Sigma Aldrich, Minneapolis, MO) was used to test whether NAc P receptor activation is required for the effect of hormone priming on motivation for food. RU486 was dissolved in a 1:10 ratio with cholesterol in ethanol and dried to a powder. A second cholesterol preparation not containing RU486 was also made and both powders were tamped into stylets. This method of drug delivery provides slow, constant release of the drug for the duration of testing, and has been successfully used in our laboratory in previous experiments (Becker et al., 1987; Xiao and Becker, 1997; Xiao et al., 2003). Following testing, stylets were removed, and the availability of additional drug was verified by visual confirmation.

Hormone Priming

Induction of sexual receptivity in ovariectomized animals was achieved by subcutaneous administration of EB for two days followed by P or EB on the third day (Figure 3.1A). EB was always administered 1 hour prior to testing, and P was administered 4-6 hours prior to testing. PPT, DPN, and G1 were used in the place of estradiol during hormone priming to determine the contribution of specific estradiol receptor subtypes to the effect of hormone priming on motivation for food (Figure 3.2A).

Testing Schedule

Animals were tested either over the course of 5 days and animals were either tested every day (Figures 3.1 & 3.2) or every other day (Figures 3.3 & 3.4). On the first day, animals received vehicle injections one hour prior to testing to establish a weekly baseline. Animals were tested after acute administration of EB or agonists, and 4-6 hours after administration of P to induce sexual receptivity. Animals that were tested every day were also tested following the second EB or agonist injection, as well as 28-30 hrs following P.

Stereotaxic surgery

After initial training up to an FR5 schedule, animals were anesthetized with ketamine (VetOne; 60mg/kg) and dexdormitor (Zoetis; 0.5mg/kg) and placed in a stereotaxic frame (Kopf Instruments, CA). Bilateral cannulae (Plastics One, VA) aimed at the nucleus accumbens (NAc; ±2.0 ML, +1.5 AP, -7.0 DV) were implanted and 26GA solid stylets were placed inside the cannulae during recovery. Following recovery animals were retrained on a FR5 for 1 week, after which time they underwent repeated progressive ratio testing. During surgery and initial postoperative care, animal and animal cages were placed on a heating pad maintained at 34^{o} C. Following surgery, animals were returned to their home cage and received carprofen (5mg/kg for analgesia) for 3 days and continued monitoring for an additional 7 days.

Histology

Following testing, animals were anesthetized with sodium pentobarbitol (FatalPlus) and perfused with PBS and paraformaldehyde. Brains were removed and kept in 4% PFA for 4-6 hrs, after which time they were transferred to 30% sucrose for 7-10

days and sectioned at 40μm on a freezing microtome (Leica, Germany). Sections were stained cresyl violet and placement of the stylets was verified by a blind observer.

Experimental Design and Statistical analysis

All statistical analyses were performed using GraphPad Prism v7.0a (GraphPad, San Diego, CA). Normality was established by Shapiro-Wilk normality test. Raw values were analyzed using repeated measures ANOVA with Holms-Sidak post hoc tests or Mann Whitney tests when data violated the assumption of normality. When there were significant group differences in baseline measures, data were normalized to the individual subjects within week baseline values as described in Results. The threshold for significance was set to $p<0.05$ ($\alpha=0.05$). Data are presented as mean ± s.e.m.

Behavioral tests were performed by an investigator with knowledge of the treatment groups. All behavioral data was acquired using automated computer system and was analyzed without reference to treatment. Only female animals were used and animals that did not complete all testing were excluded from the analysis. The same animals were used for both treatments in Figure 3.1 (n=7) and Figure 3.3 (n=47). For the data presented in Figure 3.2B & D, 7 female rats received PPT, DPN and vehicle, and 6 additional females received G1 and vehicle. The data presented in figure 3.2C was obtained from the same animals used to test the effect of PPT and DPN in Figures 3.2B & D. Behavioral data in Figure 3.3 was obtained from 48 female rats. In Figure 3.3B-E, 47 females completed received each treatment. In Figure 3.3F, animals received either EB+P and vehicle (n=24) or EBx3 and vehicle (n=24). The behavioral data in figure 3.4 was obtained from 44 female rats. Three animals failed to complete the second wave of

testing, resulting in a total of 41 animals receiving both conditions presented in Fig 3.3B (left panel).

Results

Induction of sexual receptivity reduces motivation for palatable food reward

Previous research identifying the importance of ovarian hormones for sex differences in food reward has emphasized the importance of estradiol, but both estradiol and P are released during the estrous cycle in control of ovulation and reproductive behaviors (Eckel et al., 2002; Santollo et al., 2007, 2011; Eckel, 2011; Maske et al., 2017; Richard et al., 2017). Increases in P during the luteal phase are associated with increased motivation for food in humans, and administration of P to rats either has no effect, or reverses the reduction in food intake induced by estradiol (Wade, 1975; Yu et al., 2011; Roney and Simmons, 2017). We have previously shown that induction of sexual receptivity in OVX females enhances motivated responding for access to a mate (Cummings and Becker, 2012). In considering the adaptive purpose of within cycle changes in motivated behaviors, we hypothesized that decreases in feeding behavior act as a compensatory response to increased sexual and reproductive behaviors.

Therefore, we tested whether induction of sexual receptivity using the same paradigm previously used to measure sexual motivation would also reduce motivation for a palatable food reward (Fig 3.1A). In order to account for significant differences in baseline responding on the FI schedule during the weeks when animals were treated with EB + P or vehicle ($p<0.05$), data were normalized to each animal's within week baseline. Repeated administration of EB + P in a regimen known to induce sexual receptivity significantly reduced the number of rewards that animals earned during each session

($F_{4,24}$=3.33, p<0.05; Vehicle vs EB+P, p<0.05, Baseline vs Primed, p<0.01; Fig 3.1B). At the same time, hormone primed animals made less responses on average during each fixed interval ($F_{1,6}$=7.03, p<0.05, Vehicle vs EB+P, p<0.01, Baseline vs Primed, p<0.01; Fig 3.1D). Importantly, both the number of rewards earned (Fig 3.1C) and the average number of responses per FI (Fig 3.1E) were not reduced after initial treatment with estradiol (Rewards – Day 1: Vehicle vs EB+P, p>0.99, Baseline vs Primed: p=0.81 ; Day 2: Vehicle vs EB+P, p=0.18, Baseline vs Primed: p=0.43; Responses/FI – Day 1: Vehicle vs EB+P, p=0.52, Baseline vs Primed, p=0.68; Day 2: Vehicle vs EB+P, p=0.16, Baseline vs Primed, p=0.65) or the day after priming (Rewards – Vehicle vs EB+P, p=0.18, Baseline vs Primed: p=0.12; Responses/FI – Vehicle vs EB+P, p=0.16, Baseline vs Primed, p=0.65). This demonstrates that hormone priming regimens that induce sexual receptivity and increase sexual motivation also transiently reduce motivation for food.

Selective estradiol receptor activation differentially modulates motivation for food

There are multiple ER subtypes expressed in the brain. ERα and ERβ are derived from a single gene transcript and can be localized either within the cytosol or on the cell membrane (Razandi et al., 1999). GPER-1, previously identified as the orphan GCPR GPR30, is only found on the membrane, where it can activate both PKA and ERK transduction pathways via Gαs (Thomas et al., 2005; Hadjimarkou and Vasudevan, 2017). Activation of ERα is required for the anorexigenic effects of estradiol, while GPER-1 activation decreases fluid intake with no effect on feeding (Santollo et al., 2007; Santollo and Daniels, 2015a, 2015b). ERβ has not been implicated in estradiol regulation of feeding, but may still play a role in motivation for food via direct effects on motivational circuitry (Silverman and Koenig, 2007; Morissette et al., 2008). In order to

determine whether the effects of hormone priming on motivation for food are mediated by activation of specific estradiol receptor subtypes, we tested the effect of hormone priming with selective agonists and P on motivated responding on the FI schedule (Fig 3.2A). We again found significant variability in baseline responding over time ($p<0.05$), requiring normalization of behavioral data as described previously.

There was a significant effect of treatment day on the number of rewards that animals earned over the course of each session ($F_{12,92}=2.04$, $p<0.05$; Fig 3.2D). Selective activation of GPER-1 during hormone priming significantly increased the number of rewards earned both on the day of priming (Vehicle vs G1+P, $p<0.05$, Baseline vs Primed, $p=0.06$) and the following day (Vehicle vs G1+P, $p<0.05$, Baseline vs Primed, $p=0.06$). There was no effect of the ERβ selective agonist DPN, but a trend toward a significant decrease in reward consumption after hormone priming with the ERα selective agonist PPT (Vehicle vs PPT+P, $p=0.26$, Baseline vs Primed, $p=0.09$). Thus, selective activation of G1 during hormone priming had the opposite effect of estradiol on task performance, while selective activation of ERα or ERβ alone had no effect.

Motivated responding for food was not significantly affected by hormone priming with selective agonists (Treatment: ($F_{3,23}=1.98$, $p=0.14$; Day: $F_{4,92}=0.36$, $p=0.84$; Interaction: $F_{12,92}=1.08$, $p=0.38$; Fig 3.2B). However, pairwise comparisons revealed selective activation of ERα during hormone priming significant reduced motivation for food, but only on the day after animals were sexually receptive (Vehicle vs PPT+P, $p<0.05$, Baseline vs Primed, $p=0.07$). Selective activation of ERα has consistently replicated the effects of estradiol alone on consummatory feeding behavior (Santollo et al., 2007), and the lack of an effect seen here indicates a dissociation of the mechanisms

by which consummatory and appetitive aspects of feeding behavior are regulated by estradiol.

Interactions between ERα and ERβ in the regulation of food motivation

The effect of hormone priming with PPT and DPN was tested repeatedly within subjects using a Latin Square design. After initial testing, we noticed a difference in the effect of PPT and DPN when animals were treated with a selective agonist during the previous week of testing (data not shown). We therefore completed additional testing to determine if the effect of hormone priming with PPT or DPN was dependent on previous selective ER activation. There was a significant interaction between the current treatment and preceding drug on the average number of responses animals made for each reward (three way repeated measures ANOVA, $F_{1,1}$=4.34, p<0.05), and a trend toward a significant interaction between priming and the preceding drug on the number of rewards earned over the course of each session (three way repeated measures ANOVA, $F_{1,1}$=2.87, p=0.10). Although pairwise comparisons revealed no significant differences between the specific groups, this effect of prior treatment may indicate an important role for repeated hormone priming in the effect of estradiol on motivation for food and may partially explain the lack of effects seen after use of selective agonists in OVX females naïve to hormone treatment.

Progesterone is required to reduce motivation for food during sexual receptivity

While concurrent release of both estradiol and P coordinate ovulation and sexual receptivity in intact female rats, receptive behaviors can also be induced by estradiol alone (Green et al., 1970; Parsons et al., 1981; Micevych and Sinchak, 2018). However, although females treated with estradiol alone show reflexive reproductive behaviors, they

do not demonstrate the same proceptive and appetive sexual behaviors seen in normally cycling females or females treated with EB+P (Edwards et al., 1968; Whalen, 1974; Brandling-Bennett et al., 1999). This indicates that while estradiol may regulate the reflexive, consummatory aspects of female sexual behavior, P is required for the appetitive and motivational components, and may also be critical for mediating the effects of hormone priming on the motivated feeding behaviors described here.

At the same time, studies of hormone effects on feeding behavior have found that P reverses the effects of estradiol on food intake and body weight, and is positively correlated with feeding in human females (Wade, 1975; Yu et al., 2011; Roney and Simmons, 2017). Thus, it may also be possible that the effects of hormone priming on motivation for food would be enhanced during priming with estradiol alone, rather than estradiol and P.

In order to determine whether induction of sexual receptivity by estradiol alone was sufficient to reduce motivation for food, we tested motivation for food when animals were primed with EB+P or EB for three days (EBx3). Animals were tested three times over the course of the week, once after vehicle treatment to establish each animal's unique weekly baseline, once after acute treatment with estradiol, and once when animals were fully primed (Fig 3.3A). After normalizing the data by individual weekly baseline, we again found a significant effect of hormone priming on the number of rewards earned per session (two way repeated measures ANOVA, Day: $F_{2,92}$=10.89, p<0.0001, Treatment, $F_{2,92}$=4.46, p<0.05, Interaction: $F_{4,184}$=3.29, p<0.05). Priming with both EBx3 (Vehicle vs EBx3, p<0.001, Baseline vs Primed, p<0.001) or EB+P (Vehicle vs EB+P, p<0.0001, Baseline vs Primed, p<0.001) both similarly reduced the number of rewards

that animals earned (Fig 3.3B). This demonstrates that P is not required to reduce reward consumption on the FI operant schedule.

We also replicated our previous findings that hormone priming reduced motivation for palatable food reward as measured by the average number of responses made during each interval (two way repeated measures ANOVA, Day: $F_{2,92}$=22.60, p<0.0001, Treatment, $F_{2,92}$=3.29, p<0.05, Interaction: $F_{4,184}$=2.636, p<0.05). Hormone priming with EB+P again reduced motivated responding on the fixed interval schedule (Vehicle vs EB+P, p<0.001, Baseline vs Primed, p<0.0001). However, while hormone priming with EBx3 was sufficient to reduce the number of rewards, there was a significant difference in motivation for food when animals were primed with EBx3 or EB+P (p<0.0001; Fig 3.3C). Animals treated with EB alone still showed a significant decrease in motivation when compared to baseline (p<0.01), but this was decrease not significantly different from the effect of repeated vehicle treatment on motivated responding (p=0.61) and was significantly less robust than the reductions seen after priming with EB+P.

Failure to respond during the five second window immediately following the 15s FI resulted in a failed trail, where animals were required to initiate and complete a new trial in order to be rewarded. We compared the number of trials that animals failed when primed, and found a significant effect of treatment on the number of failed trials (one-way repeated measures ANOVA, $F_{2,92}$=8.46, p<0.001; Fig 3.3D). Animals treated with EB+P failed significantly more trials than animals primed with EBx3 (p<0.01) or vehicle (p<0.001). This increase in the number of trials failed during each session does not explain the observed decrease in the average rate of responding in animals treated with

EB+P. There was no difference in the average number of responses per interval after removing any trials that were not rewarded (three way repeated measures ANOVA, $F_{1,1}$=0.49, p=0.48). When only rewarded trials were analyzed (Fig 3.3E), there was still a significant effect of hormone priming on motivated responding (two way repeated measures ANOVA, Day: $F_{2, 92}$=14.98, p<0.0001), and treatment with EB+P significant reduced the number of responses made per interval (p<0.01), over the effect of EBx3 (p<0.05).

As an additional test of motivated responding, we also tested the effect of EBx3 or EB+P using a PR schedule (Fig 3.3F). Data obtained from PR testing violated the assumption of normality (Shapiro-Wilk test, Z=0.82, p<0.0001) and were analyzed with non-parametric Mann-Whitney rank comparisons. Breakpoint was significantly reduced when animals were treated with EB+P (Vehicle vs EB+P, p<0.05), but not when animals were treated with EBx3 (Vehicle vs EBx3, p=0.66).

Nucleus accumbens progesterone receptor antagonism regulates motivation for food

Motivated behaviors are ultimately under the regulation of striatal dopamine (DA) circuitry (Ikemoto and Panksepp, 1999; Berridge, 2007). However, although administration of EB to OVX females induces expression of PR within dopaminergic cells in the arcuate nucleus and preoptic area, PR have not been found within the major sources of striatal DA, the VTA and substantia nigra (Lonstein and Blaustein, 2004). If the effect of P during hormone priming is mediated by direct actions on striatal circuitry, it is likely that this would occur at the downstream targets of midbrain DA projections, including the NAc. Therefore, to determine whether P reduces motivation for food via acting directly on striatal DA systems, we tested whether local administration of the PR

antagonist RU486 to the NAc would prevent the decrease in motivation for food seen in hormone primed female rats.

Animals were tested on the PR schedule three time over the course of 5 days, as described previously, and received both treatments (RU486 or cholesterol) twice (Fig 3.4A); the order of treatment was randomly assigned and counterbalanced across animals. We first tested the effect of administering RU486 for the entire duration of testing (Fig 3.4B, left). Stylets containing RU486 or cholesterol were inserted 24 hrs prior to baseline testing. There was a significant effect of hormone priming on breakpoint ($F_{2,80}$=12.54, p<0.0001), that was modulated by the RU486 treatment ($F_{2,80}$=3.44, p<0.05). Animals treated with RU486 showed significantly greater motivation for food during baseline testing, prior to administration of any hormones (p<0.0001; Fig 3.4C). However, this effect was not sustained, and there were no differences in breakpoint during the following test (p=0.11). Due to these differences in baseline responding, we calculated the change in breakpoint on the day of priming compared to the day of acute testing, when no group differences were observed (Fig 3.4D). These data violated the assumption of normality and were analyzed using non-parametric Mann-Whitney rank comparisons. Hormone priming similarly decreased breakpoint on a PR schedule whether animals were treated with RU486 or cholesterol (p=0.23).

We then tested whether RU486 could block the effect of P on motivated responding if given just before priming (Fig 3.4B, right). Stylets containing RU486 or cholesterol were inserted following baseline and acute testing, 24 hours before animals were fully primed. Again, animals showed similar decreases in breakpoint when treated with RU486 or cholesterol (p=0.70; Fig 3.4D). However, animals treated with RU486

just prior to the final administration of P showed an attenuated response to hormone priming compared to when RU486 administration started prior to baseline testing ($p<0.05$). Therefore, although it does not appear that NAc PR are responsible for changes in motivation during hormone priming, they may instead provide a separate mechanism by which ovarian hormones can modulate motivated behaviors.

Discussion

These findings demonstrate that decreases in consummatory feeding behavior in response to increased levels of ovarian hormones are also associated with decreased motivation for palatable food. We show that administration of EB+P in a regimen that will induce sexual receptivity in OVX female rats decreases both the amount of palatable food rewards that animals will work to earn, as well as the amount of work that animals will expend for each reward. Previous studies evaluating the role of ovarian hormones in consummatory food intake demonstrate an acute effect of estradiol on food intake and body weight (Geary and Asarian, 1999; Asarian and Geary, 2002; Santollo et al., 2007). However, the effect of hormone priming on motivation for food seen here was only seen after animals were fully primed. This differs from previous reports that show attenuated motivation for sucrose reward within 1hr of estradiol administration (Richard et al., 2017). However, while previous studies used 17-β-estradiol, which is active immediately and metabolized rapidly, the experiments reported here used EB, a synthetic ester of estradiol with increased absorption and sustained bioavailabilty. Therefore, differences in the immediate availability of EB vs 17-β-estradiol may account for differences in the results of these studies.

Motivation for food is specifically decreased during periods of sexual receptivity

Reduced motivation for food in hormone primed females was transient, and both motivated responding and the number of rewards earned returned to baseline by the following day. This indicates that decreases in motivation are specifically linked to induction of sexual receptivity. Importantly, these changes in motivation for food are similar but opposite to the effects of hormone priming on sexual motivation (Cummings and Becker, 2012). Thus, changes in motivation for both rewards may be linked, and represent a specific mechanism regulating shifts in motivation for various rewards across the estrous cycle.

Selective activation of estradiol receptor subtypes was not sufficient to replicate the effects of EB + P on motivation for food. While selective activation of ERα did significantly reduce motivation for food, this only reached significance the day after animals were sexually receptive, contradictory to the transient, time-locked decrease in both reward consumption and motivation seen after administration of EB + P. Conversely, selective activation of G1 increased the number of rewards that animals earned during each session on both the day of priming as well as the following day, indicating a sustained increase in reward consumption after selective activation of G1 during hormone priming. Again, this differs from studies of consummatory behavior, which have repeatedly implicated ERα as the major regulator of estradiol induced changes in food intake and body weight, and GPER-1 as a mediator of fluid intake (Santollo et al., 2007; Santollo and Eckel, 2009; Santollo and Daniels, 2015b, 2015a). This is not entirely surprising, as mechanisms mediating consummatory and appetitive behaviors are often dissociable (Kupfermann, 1974; Everitt, 1990; Baldo and Kelley,

2007). Taken together with our findings that decreases in motivation are specifically linked to the induction of sexual receptivity, this further supports that the mechanism regulating motivation for food by ovarian hormones is dissociable from the mechanisms regulating overall food intake.

Selective estradiol receptors interact to regulation of food reward

Selective activation of ERβ had no effects during priming, but we saw an interaction between activation of ERα and ERβ, where hormone priming targeting one receptor altered the effect of the other receptor during subsequent testing. It is unlikely that this is due to residual agonists that have not been metabolized, as the half-lives of PPT and DPN are approximately 12 hours. This is not the first report that ERα and ERβ may interact in the control of food intake. Uban and colleagues (2012) found that estradiol reduces, while PPT and DPN increases, choice of a high-effort/high-reward food reward in ovariectomized female rats. However, combined administration of PPT and DPN increased effort-based discounting for food reward, indicating that concurrent activation of both ERα and ERβ is necessary for the effect of estradiol on effort-based food choice (Uban et al., 2012). ERα and ERβ auto-regulate the expression of one another in cells where they are co-expressed, and although ERα and ERβ have not been found on the same cells within brain areas directly involved in motivated behaviors, it remains possible that changes in expression of either ERα or ERβ during selective vs. non-selective ER activation contribute to this effect (Osterlund et al., 1998; Frasor et al., 2003; Mitra et al., 2003; Nomura et al., 2003; Matthews et al., 2006; Almey et al., 2012, 2015, 2016) It is also possible that selective activation of ERα and ERβ promote

differential expression of specific PR subtypes, which may then exert specific effects on motivation for food following P administration (Rickard et al., 2002; Mani et al., 2006).

Progesterone is required to reduce motivation for food during hormone priming

Our findings highlight the importance of progesterone in mediating changes in motivation for food during periods of sexual receptivity. Repeated administration of estradiol alone reduced the number of rewards the animals would work for during each session but did not significantly reduce motivation for food reward on the FI or PR reinforcement schedules. This parallels the effect of ovarian hormones on female sexual behavior. While repeated administration of estradiol alone is sufficient to induce reflexive copulatory behaviors (e.g. lordosis) in OVX females, progesterone is required for the expression of appetitive sexual behaviors, including hops, darts, and ear wiggles (Brandling-Bennett et al., 1999).

NAc PR blockade increases motivation for food independently of hormone treatment

The similarities in the effects of hormone priming on sexual behavior and motivation for food indicate the potential for a single mechanism mediating changes in motivation for both rewards. Due to the underlying role of DA circuitry in motivated behaviors, we hypothesized that the effects of P on motivation for food may be regulated by the NAc. However, while blockade of PR by RU486 within the NAc did enhance motivation for food in animals prior to hormone priming, there was no effect of PR antagonism on motivation for food in hormone primed animals. This effect of RU486 is likely not mediated by PR. Systemic administration of P alone to OVX animals had no effect on PR responding. This is not surprising, as PR is a gene product of ER activation, and PR expression is low in OVX animals not treated with estradiol (McGinnis et al.,

1981). It is more likely that RU486, which is also a glucocorticoid receptor (GR) antagonist increases motivation via NAc GR. However, there was no effect of GR inactivation on food intake, even when DAergic systems were directly targeted (Parnaudeau et al., 2014). We also saw no effect of RU486 72 hrs after initial administration, indicating that if GR are responsible for changes in motivation for food seen here, the effects are not sustained after continuous GR inactivation.

Conclusion

From these results, we understand that induction of sexual receptivity by EB + P transiently reduces motivation for palatable food reward. Unlike the control of consummatory feeding behavior, sequential administration of both EB + P is necessary to attenuate motivation for food, demonstrating a dissociation of the control of consummatory and motivational components of feeding behavior by ovarian hormones. The schedule of hormone delivery used in our studies was designed to mimic the changes in endogenous hormone release around the time of ovulation. It has been speculated that the control of feeding by reproductive processes is important to shift the animal's focus and motivation toward sexual behavior. Parallels between our results and studies of the role of ovarian hormones in sexual behavior would support this interpretation. In particular, induction of sexual receptivity by EB + P increased the number of trials that animals failed to complete. This may indicate that animals are less focused on their pursuit of the food reward, which in a natural setting might increase the potential that they could find a mate and reproduce. Future work investigating the trade-off between motivation for food and sex when both rewards are available is necessary to further define the adaptive function of ovarian hormones in motivated behavior.

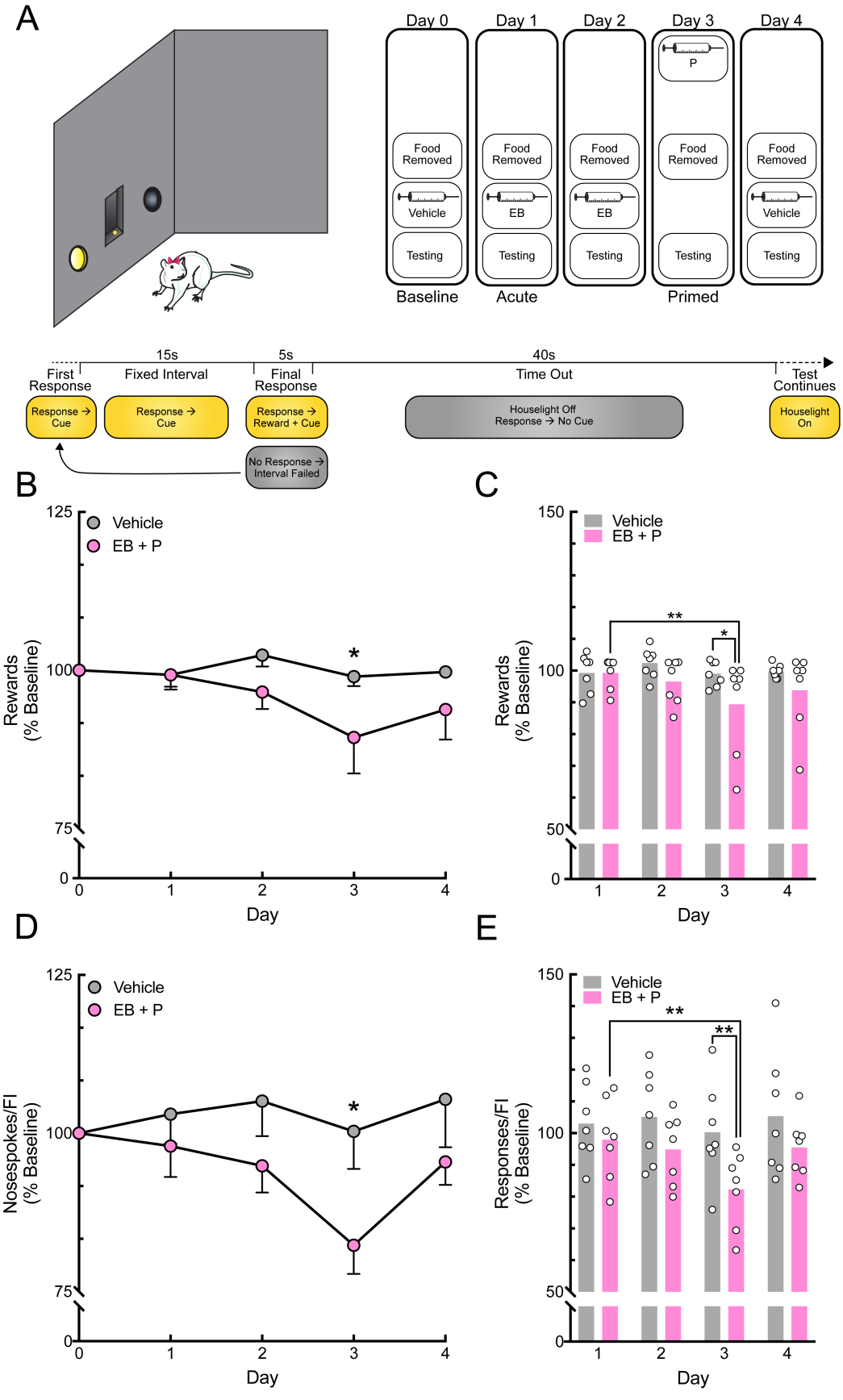

A
Day 0
Day 1
Day 2
Day 3
Day 4
P
Food Removed
Vehicle
EB
Testing
Baseline
Acute
Primed
15s
5s
40s
First Response
Fixed Interval
Final Response
Time Out
Test Continues
Response → Cue
Response → Reward + Cue
Houselight Off
Response → No Cue
Houselight On
No Response → Interval Failed
B
C
Vehicle
EB + P
Rewards (% Baseline)
Day
D
E
Nosespokes/FI (% Baseline)
Responses/FI (% Baseline)

Figure 3.1 Induction of sexual receptivity reduces motivation for palatable food reward on a fixed interval operant task.
(A) Rats instrumentally responded on a 15s FI schedule in which responses into the active nose poke hole initiated a 15s interval during which subsequent responses were counted and resulted in presentation of the reward cue, but had no other consequence. At the conclusion of this 15s interval, responses made during the following 5s resulted in presentation of the cue and delivery of a palatable food reward, while failure to respond during this window resulted in a failed interval and no reward. Animals tested daily during hormone priming over the course of five days (top left). **(B-C)** Hormone priming resulted in a transient decrease in the number of rewards that animals earned over the course of each session. This effect was transient, as the decrease in rewards was only significant when animals were fully sexually receptive (Day 3), and not following initial estradiol treatment (Day 1 &2) or on the day after priming (Day 4). **(D-E)** Hormone priming also reduced motivation for food as measured by the amount of responses that animals made for each reward. Again, reductions in motivation for were transient, and motivation for food was not significantly different from baseline on the days prior to or following priming. n=7, repeated measures design. Data are shown as mean ± SEM. *$p<0.05$, **$p<0.01$.

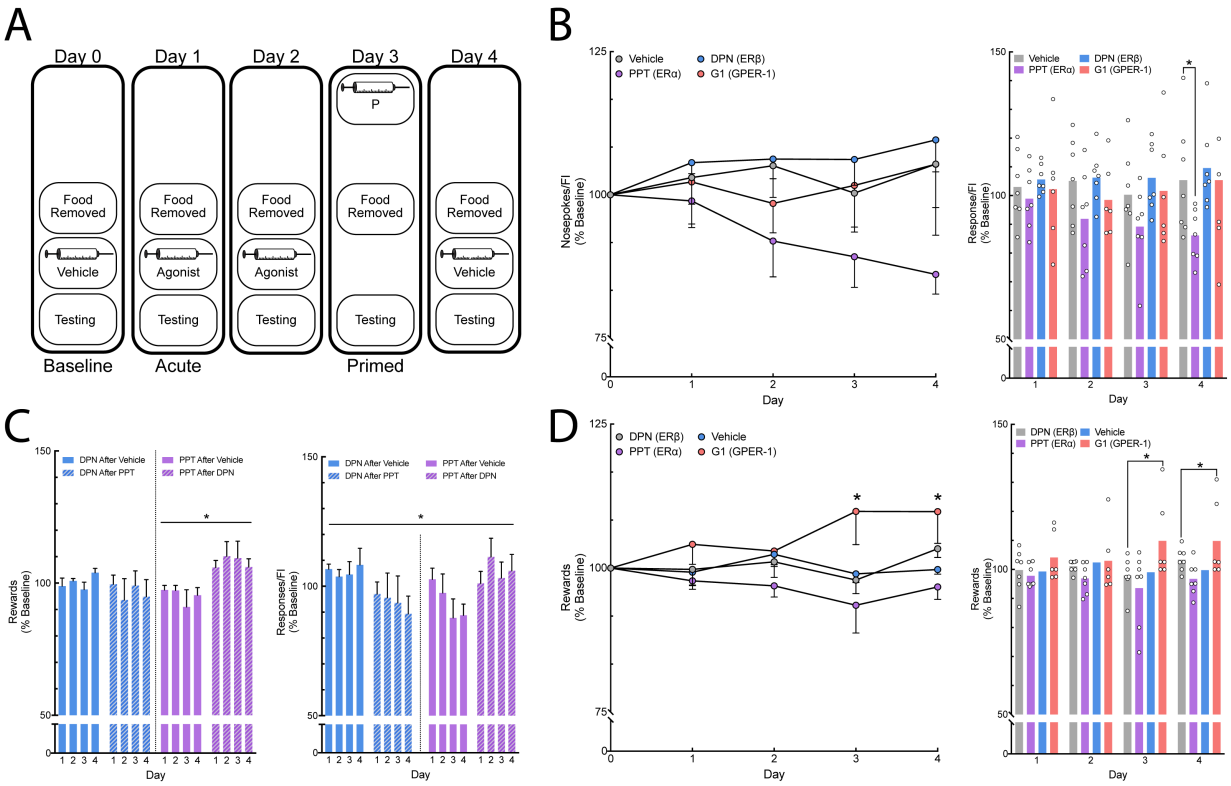


Figure 3.2 Selective activation of estradiol receptor subtypes during priming is not sufficient to reduce motivation for palatable food reward.
(A) Schedule of behavioral testing and hormone priming. Testing occurred daily for 5 days during hormone priming with selective agonists. **(B)** Treatment with selective estradiol agonists did not significantly modulate motivation for food, although pairwise comparisons revealed a significant reduction in motivation in animals treated with the ERα selective agonist PPT, but only on the day after priming (Day 4). **(C)** The effect of selective activation of ERα and ERβ is dependent on prior hormone priming. Animals treated with PPT show opposite effects of hormone priming on the number of rewards earned (left) following treatment with the ERβ selective agonist DPN. The effect of both DPN and PPT on motivation for food is modulated by prior selective agonist treatment (right). **(D)** Only the GPER-1 selective agonist G1 had a significant effect on the number of rewards animals earned over the course of hormone priming. Hormone priming with G1 significantly increased the number of rewards that animals earned (Day 3), and this effect was sustained on the day following priming (Day 4). Vehicle: n=7, PPT: n=7, DPN: n=7, G1: n=7. Data are shown as mean ± SEM. *p<0.05, **p<0.01.

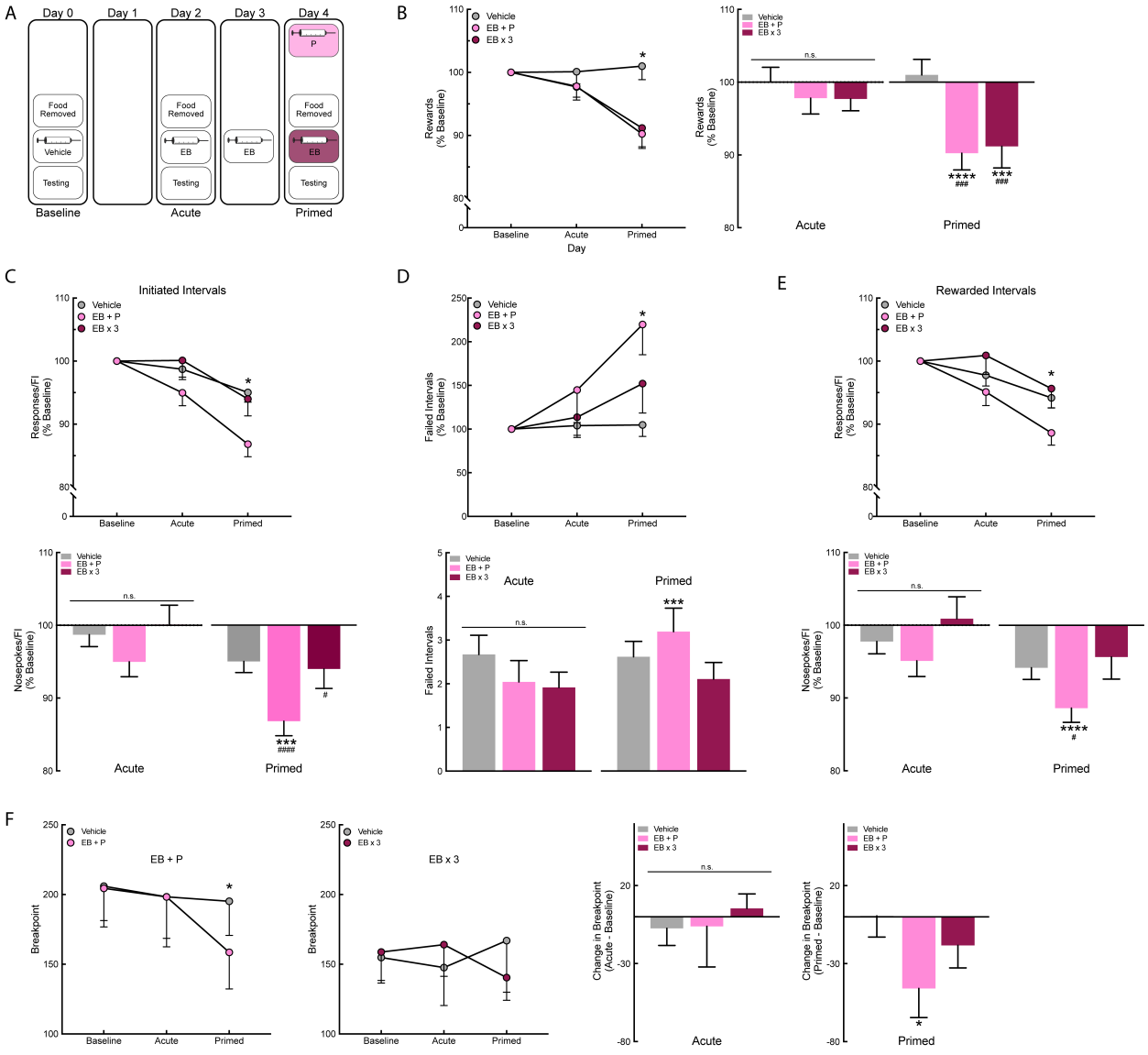
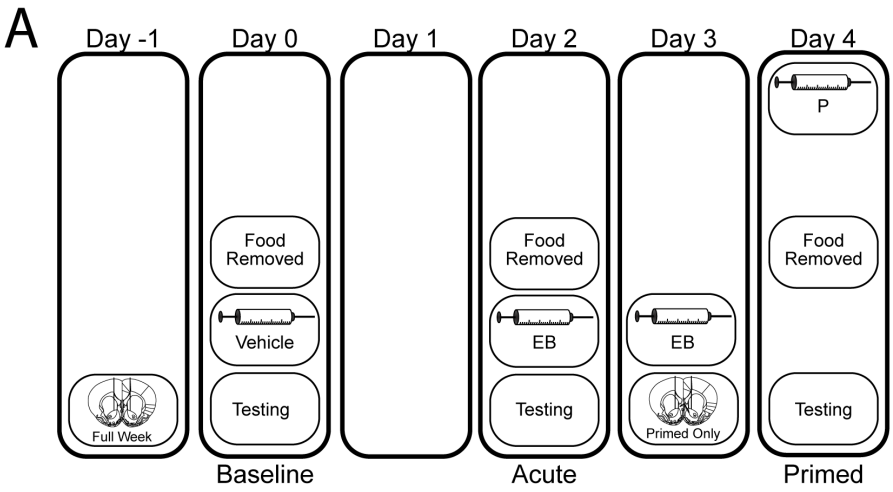
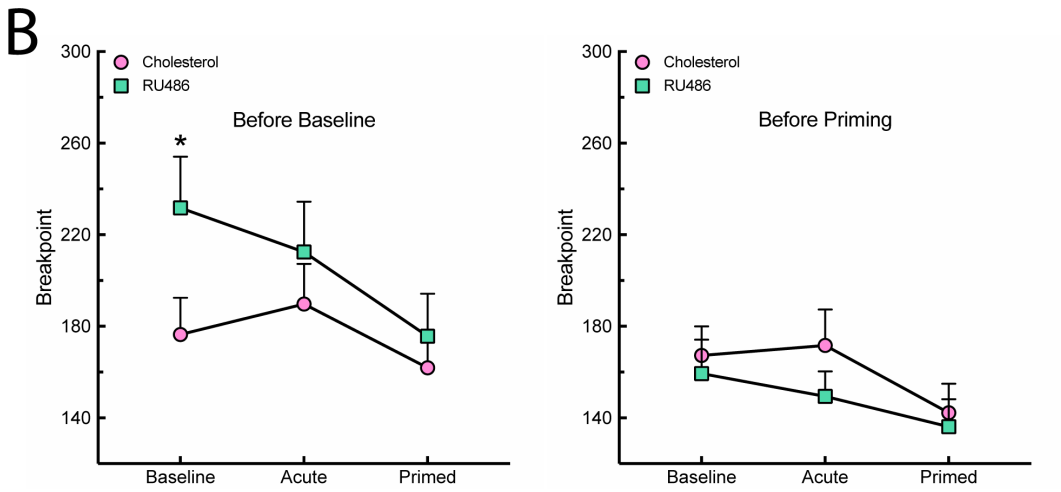
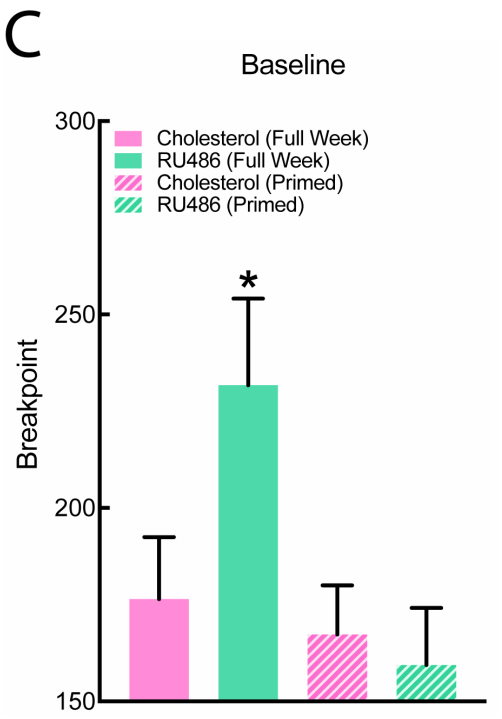

A
Day 0
Day 1
Day 2
Day 3
Day 4
Food Removed
Vehicle
Testing
EB
P
Baseline
Acute
Primed
B
Vehicle
EB + P
EB x 3
Rewards (% Baseline)
Baseline
Acute
Primed
Day
n.s.
Acute
Primed
C
Initiated Intervals
Responses/FI (% Baseline)
Nosepokes/FI (% Baseline)
D
Failed Intervals (% Baseline)
Failed Intervals
E
Rewarded Intervals
F
EB + P
EB x 3
Breakpoint
Change in Breakpoint (Acute - Baseline)
Change in Breakpoint (Primed - Baseline)

Figure 3.3 Progesterone administration is necessary for the effect of hormone priming on specific measures of food reward behavior.
(A) Schedule of behavioral testing and hormone priming. Testing occurred three times over the course of 5 days during hormone priming with either estradiol and progesterone (EB + P) or estradiol alone (EBx3). **(B)** Hormone priming with both EB+P and EBx3 both similarly reduced the number of rewards that animals earned when sexually receptive (Primed). Again, this was only seen after full priming, and not following acute treatment with estradiol (Acute). **(C)** Induction of sexual receptivity by EBx3 was not sufficient to reduce motivation for food during hormone priming. The effect of hormone priming on motivation for food was apparent when looking at responding during all intervals that were initiated (left) as well as only intervals that were completed (right). **(D)** Induction of sexual receptivity with EB+P also reduces motivation for palatable food reward as measured by progressive ratio responding (right). Again, treatment with estradiol alone is not sufficient to reduce motivation for food (left). **(E)** There is no effect of acute estradiol treatment on motivation for food. Motivated responding during all trials (left) or rewarded trials (right) after acute estradiol treatment does not differ between treatment groups. **(F)** Hormone priming with EB+P but not EBx3 reduces breakpoint on the progressive ratio schedule when animals are primed (right). There were no differences in the effect of estradiol alone on either treatment schedule (left). **(G)** Reduced motivation for food in EB+P primed animals is apparent when averaging responding across all intervals (left), as well as only intervals that resulted in reward (right). **(H)** Hormone priming with EB+P increases the number of intervals that animals are not completed (Failed Intervals).


A
Day -1
Day 0
Day 1
Day 2
Day 3
Day 4
Full Week
Food Removed
Vehicle
Testing
Food Removed
EB
Testing
EB
Primed Only
P
Food Removed
Testing
Baseline
Acute
Primed


B
Cholesterol
RU486
Before Baseline
Before Priming
Breakpoint
300
260
220
180
140
Baseline
Acute
Primed
*


C
Baseline
Cholesterol (Full Week)
RU486 (Full Week)
Cholesterol (Primed)
RU486 (Primed)
Breakpoint
300
250
200
150
*

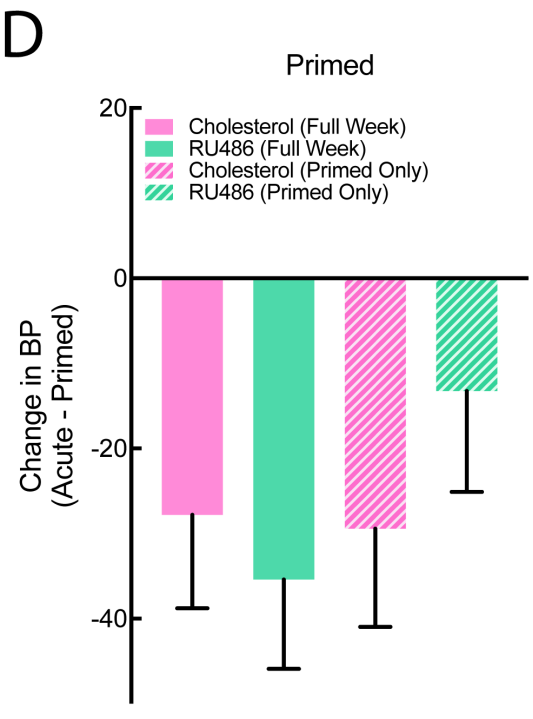

D
Primed
Cholesterol (Full Week)
RU486 (Full Week)
Cholesterol (Primed Only)
RU486 (Primed Only)
Change in BP
(Acute - Primed)
20
0
-20
-40

Figure 3.4 Blockade of PR within NAc increases motivation for food in non-primed animals without effects on motivation after hormone treatment.
(A) Schedule of behavior testing and intra-NAc drug delivery during hormone priming. Testing occurred three times over the course of 6 days of hormone priming with estradiol and progesterone. RU486 or cholesterol were administered either prior to testing began (Day -1) or just prior to priming (Day 3). **(B)** RU486 administration starting prior to baseline testing increased breakpoint during baseline testing, but did not affect responding on the progressive ratio schedule during hormone priming. **(C)** Animals treated with RU486 showed increased motivation compared to when treated with cholesterol, or during treatment with RU486 and cholesterol when drug delivery started after baseline testing. **(D)** There was no significant effect of RU486 on the change in breakpoint during priming. Cholesterol (Full Week): n=44, RU486 (Full Week): n=44, Cholesterol (Primed Only): n=41, RU486 (Primed Only): n=41. Data are shown as mean ± SEM. *$p<0.05$.

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Chapter 4:

Ovarian hormones mediate changes in adaptive choice and motivation in female rats

Abstract

In female rodents, sexual receptivity is coordinated with cyclic changes in the release of gonadal hormones. Increases in estradiol (E) and progesterone (P) during proestrus and estrus not only induce ovulation, but also modulate behaviors that increase the likelihood that the female will find a mate and reproduce. This includes changes in receptive behaviors, such as lordosis, as well as changes in appetitive or proceptive behaviors, including motivation. Interestingly, the direction of these changes in motivation is dependent on the type of reward that is being pursued. While induction of sexual receptivity by E and P increases motivation for access to a male, motivation for a palatable food reward is decreased. These concurrent changes may facilitate adaptive choice across the estrous cycle; females bias their choice for sex when fertilization is most likely to occur, but for food when copulation is unlikely to result in impregnation. In order to test this hypothesis, we developed a novel paradigm to measure motivated choice between a palatable food reward and access to a male conspecific. Ovariectomized, hormone primed females were trained to respond for both food and sex on a fixed interval (FI) schedule. After training, unprimed and primed females were tested in a chamber that allows them to choose between food and sex while still requiring responding on the FI schedule for reach reward. From this we can not only determine the

impact of hormone priming on female choice for food or sex, but also how this is reflected by changes in motivation for each specific reward, as measured by the average number of responses made during each fixed interval. Induction of sexual receptivity by hormone priming biases choice toward sex over food, and this change is accompanied by an increase in motivation for food but a decrease in motivation for sex. This work provides a novel framework for understanding how release of ovarian hormones over the course of the estrous cycle modulates adaptive behavioral choice in females.

Introduction

All behaving organisms are continually faced with alternative and competing demands from which they must direct behavior in order to enhance their adaptive success. Motivation is a key regulator of these goal-directed behaviors and has been proposed to modulate not only decision-making processes, but also the vigor with which these behaviors are executed (Niv et al., 2006). In order for behavior to be appropriately selected based on physiological needs, internal signals for hunger, thirst, and reproductive status have evolved the ability to direct motivation for specific stimuli based on the organism's internal state (Zardetto-Smith et al., 1993; Balleine, 1994; Dickinson and Balleine, 1994; Salamone et al., 2003; Robinson and Berridge, 2013; Cone et al., 2014; Aitken et al., 2016).

One example of how internal signals interact with the neural circuitry underlying motivation is the regulation of motivated behaviors by ovarian hormones. In female rodents, sexual receptivity is coordinated with cyclic changes in the release of estradiol (E) and progesterone (P) (Beach et al., 1942). Increases in these hormones during proestrus and estrus not only induce ovulation, but also modulates behaviors that increase the likelihood that the female will find a mate and reproduce (Fessler, 2003). This includes changes in receptive behaviors, e.g. lordosis, as well as appetitive or proceptive behaviors, including motivation. Importantly, the direction of these changes in motivation is dependent on the type of reward that is being pursued. While increases in E and P lead to enhanced motivation for access to a mate, motivation for food is decreased (Cummings and Becker, 2012; Richard et al., 2017).

The adaptive benefit of coordinating sexual behaviors with ovulation is quite clear. Copulatory behaviors are accompanied by necessary danger: risk of predation during mate seeking, injury due to the copulatory act itself, or infectious disease (Daly, 1978). Therefore, females that are not in estrous are not motivated to find a mate and will actively reject male advances (Hardy, 1972; Cummings and Becker, 2012). Changes in feeding behavior, on the other hand, are less obviously adaptive. Although copulation and reproduction are energetically costly, and would be expected to require greater food intake, females show decreased food intake and motivation for food around the time of ovulation (Asarian and Geary, 2006; Richard et al., 2017). In order to explain this paradoxical change in feeding behavior, researchers have speculated that changes in feeding behavior serve to reduce the amount of time and energy that animals dedicate toward obtaining and consuming food, thus increasing the amount of time available for mate-seeking and reproductive activities (Fessler, 2003; Schneider et al., 2013). Although this hypothesis has powerful explanatory potential as an ultimate explanation of animal behavior, it remains untested within experimental settings.

One reason for this gap may be the difficulties in studying female sexual motivation in the laboratory. During sexual encounters, female rodents will actively pace the rate of copulation through repeated approach and avoidance of the male (Adler and McClintock, 1978). This behavior increases the amount of time between subsequent intromissions, which leads to the optimal induction of the progestational reflex required for pregnancy (McClintock and Anisko, 1982; Erskine et al., 1989). Importantly, sexual behavior is only rewarding for females when intromissions occur at their preferred rate

(Jenkins and Becker, 2003b). Therefore, it is crucial that females be able to pace the rate of copulation during experimental paradigms that measure female sexual motivation.

Our lab has developed an operant paradigm that allows females to actively pace the sexual encounter while also providing a quantitative measure of the females motivation for access to the male (Cummings and Becker, 2012). Using this same paradigm to measure motivation for a palatable food reward, we have seen concurrent changes in motivation for food and a mate on the same operant paradigm. However, these previous studies evaluated motivation for each reward in isolation, when only one reward was available. Therefore, the current experiment aimed to evaluate whether these concurrent changes in motivation for food vs a mate are able to facilitate motivated choice during periods of sexual receptivity. Ovariectomized (OVX) female rats were trained to respond for both food and a mate simultaneously. During testing, animals were able to choose between the two rewards, after which the number of responses animals made for each reward was used as an indicator of their motivation. Thus, the present study demonstrates the how increases in ovarian hormones associated with induction of sexual receptivity directs both choice and motivation for food and a mate in female rats.

Methods

Animals

Ten female Long Evans rats 50-55 days of age and 15 proven breeder stimulus males (Charles River Breeding Laboratory; Portage, MI) were maintained on a 14:10 L:D cycle (lights of at 1300hr) and housed in same-sex pairs in large laboratory cages (Allentown NextGen 1800; Allentown, NJ) with ad libitum access to water and phytoestrogen free rat chow (2017 Teklad Global, 14% protein rodent maintenance diet,

Harlen rat chow; Harlan Teklad, Madison, WI). All procedures were carried out in accordance with the National institutes of Health Guidelines on laboratory animal use and care, using a protocol approved by the University of Michigan Institutional Animal Care and Use Committee. Experimental animals were OVX as previously described (Cummings et al., 2014). Vaginal lavage samples were collected daily starting 10 days after surgery in order to verify absence of estrous cycle.

Drug preparation and hormone priming

Estradiol benzoate (EB; Sigma Aldrich, MO; 5μg/0.1 ml) and P (Sigma Aldrich, MO; 500μg/0.1 ml) were administered subcutaneously in order to induce sexual receptivity in OVX females. Both hormones were emulsified in peanut oil and stored at room temperature for the duration of use. EB was administered at 1300 hr for two days, followed by P at 1000 hr on the third day. Animals were considered fully hormone primed and sexually receptive 4-6 hrs after P administration.

Operant Task

All training and testing took place in custom built operant pacing chambers (Fig 4.1). Control of the apparatus and video recording and analysis was performed using AnyMaze (Stoelting, Wood Dale, IL). Two compartments within the chamber were separated by a horizontal sliding door. The larger compartment contained a tethered stimulus male (Male Side). The smaller of the two compartments (Female Side) was outfitted with four nose poke ports. Two ports were located on the wall adjacent to the sliding door and served as response elements (active and inactive) to open the door. The additional two ports were located on the wall opposite the door spaced around the food tray in which a palatable food pellet (45 mg banana flavor, BioServ, Flemington, NJ)

would be delivered. Responding on the active port for each reward resulted in activation of a discrete light cue located directly above the port and initiation of the FI15. During this interval, all responses were counted and resulted in presentation of the cue light but had no other consequence. The first response made within 5s of the conclusion of this 15s interval resulted in either delivery of a single food pellet or activation of the sliding door to allow access to the second compartment. If the female did not make a response within this 5s window, the interval was failed, and no rewards were delivered until the animal initiated and completed a new trial. Importantly, although both rewards were concurrently available, initiation of the FI15 for one reward precluded responding to earn the other reward until the initial FI15 was either completed or failed. Any responses made for the other reward during this window were counted but did not result in activation of the cue light and could not initiate a new trial or earn the reward. Thus, animals must first choose between the two available rewards, and then sustain responding for this choice until the end of the 15s interval in order to be rewarded.

Training Paradigm and Schedule

Animals were initially trained to respond for each reward separately. All training sessions lasted 30 minutes and animals had no more than one training session per day. Animals started training on a fixed-ratio (FR) 1 schedule, during which every response on the active port resulted in delivery of the respective reward. Once animals made at least 10 active responses during training for sex, and 20 active responses during training for food, the FR requirement was increased to five. The FI schedule was introduced after animals mastered the FR5 (same criterion as FR1). Animals continued training on the FI15 for each reward separately for one week, at which point they started training on the

concurrent FI15 schedule. At this point, animals were trained twice a week, once when unprimed and once when primed. Training on the concurrent FI15 continued for three weeks. 8 out of 10 animals reached stable levels of responding after this point. The two animals that failed to successfully learn the task were excluded from subsequent analyses.

Testing Schedule

During the week of testing, animals were tested once when primed and once when unprimed. The order of testing was counterbalanced across animals and animals were always tested with a novel stimulus male. Four hours prior to testing (1000hr), animals were given a single subcutaneous injection of either P, if they had been primed with EB, or oil, if they were unprimed. At this time, food hoppers were removed from the home cage and animals were lavaged to verify hormonal status. At 0200hr, animals were transported to the testing room. Testing sessions lasted 30 minutes, after which animals were returned to their home cage.

Video Scoring

Behavioral video was scored offline by an observer blind to the animal treatment group. Videos were analyzed to verify the amount of time animals spent in each chamber during testing, as well as to determine which components of the apparatus animals engaged with. All durations were normalized to the total amount of time in the chamber prior to analysis. Finally, sexual behavior was scored in order to account for the effect of the male's behavior on female sexual motivation.

Statistical Analysis

Group comparisons were performed using GraphPad Prism v7.0a (GraphPad, San Diego, CA). Shapiro-Wilk normality tests were used to test for normal distributions. The

effect of hormone priming on discrete variables was analyzed using paired t-tests or Wilcoxon matched-pairs signed rank tests when data violated the assumption of normality. Interactions between hormone priming and other variables, e.g. trial type, were analyzed using two-way repeated measures ANOVA with Holm-Sidak post hoc tests. The effect of ejaculation on motivation for sex in hormone primed animals was analyzed using one-way ANOVA. Data are presented as mean ± SEM.

Results

Induction of sexual receptivity alters preference for food vs sex

There was a significant effect of hormone priming on the number of trials that animals initiated ($F_{1,7}$=19.27, p<0.01), that differed between food and mate trials ($F_{1,7}$=7.30, p<0.05). As shown in Figure 4.2A, hormone primed animals initiated fewer food trials than unprimed animals (p<0.05), but a similar number of mate trials overall (p=0.96). However, unprimed animals also initiated a greater number of trials overall, (p<0.01; Fig 4.2A). Therefore, in order to account for differences in the total number of trials that animals initiated, we normalized the number of mate vs. pellet trials to the total number of trials initiated during each session. After normalizing, there was a still a significant effect of hormone priming on the number of mate vs. food trials that animals initiated relative to the total number of trials initiated that differed between food and mate trials ($F_{1,14}$=22.20, p<0.001; Fig 4.2B). Hormone primed animals initiated a smaller proportion of food trials (p<0.01) but a greater proportion of mate trials (p<0.01) than unprimed animals, indicating that hormone priming does indeed bias choice toward a sexual partner and away from a palatable food reward.

Hormone priming specifically increased the number of completed food ($p<0.001$) or mate ($p<0.001$) trials, without altering the number of failed trials for either reward (food: $p=0.94$, mate: $p=0.88$; Fig 4.2C). After controlling for the total number of trials initiated, we found that hormone priming significantly altered the proportion of failed trials, but this effect was again dependent on the reward that was being pursued ($F_{1,27}=4.88$, $p<0.05$; Fig 4.2D).

There was also a significant effect of hormone priming on the average duration of food and pellet trials ($F_{1,7}=11.43$, $p<0.05$) where all trials were longer when animals were hormone primed (Fig 4.3A). In addition, mate trials were longer than pellet trials overall ($F_{1,7}=7.11$, $p<0.05$) but this effect did not differ by hormone treatment ($F_{1,7}=0.76$, $p=0.41$; Fig 4.3A).

When looking at total duration of time animals spent engaging in either food seeking or mate seeking, a measure that accounts for both differences in the number of trials animals initiated as well as the duration of each trial, there was a significant interaction between trial type and hormone treatment ($F_{1,7}=25.16$, $p<0.01$). As shown in Figure 4.3B, unprimed animals spent a similar amount of time engaged in mate and pellet trials ($p=0.40$), while in unprimed animals the total amount of time spent in mate trials was significantly greater than the amount of time spent in pellet trials ($p<0.001$).

Hormone priming alters motivation for food vs sex

In addition to altering which reward females chose more frequently, hormone priming increased motivation for sex ($p<0.05$), while simultaneously reducing motivated responding for pellets ($p<0.01$; Fig 4.4A). This effect of hormone priming on responding was dependent on whether or not the trial was rewarded, (Pellet trials: $F_{2,14}=8.10$, $p<0.01$;

Mate trials: $F_{2,14}$=19.18, p<0.0001). Hormone priming only reduced motivated responding for sex when the trial resulted in delivery of reward (p<0.001), and not during failed trials (p=0.91; Fig 4.4C). The same was true during pellet trials (Fig 4.4D); where primed animals made less responses than unprimed animals during completed trials (p<0.01), but not during failed trials (p=0.07).

Although hormone treatment did not alter motivated responding for the initially chosen reward during failed trials, there were important differences in responding for the alternate reward during failed trials (Pellet trials: $F_{2,14}$=4.40, p<0.05; Mate trials: $F_{2,14}$=4.67, p<0.05). Animals made significantly more mate responses during failed pellet trials than completed pellet trials (p<0.05), but only when they were hormone primed (Fig 4.4F). Interestingly, hormone primed animals also made more pellet responses during failed mate responses (p<0.05; Fig 4.4E).

Motivation for access to a mate is reduced following ejaculation

Animals were only sexually receptive following hormone treatment (Fig 4.5B). Hormone treated animals received significantly more mounts (p<0.01), intromissions, (p<0.01), and ejaculations (p<0.01). In order to determine the effect of male ejaculation on sexual motivation, we compared the average number of mate responses during the trials leading up to and following ejaculation. As shown in Figure 4.5A, Ejaculation significantly reduced motivation for access to a mate ($F_{5,57}$=3.487, p<0.01). Animals decreased responding during the three trials following ejaculation (1: p<0.01; 2: p<0.05; 3: p<0.05).

Motivation for food increases over the test session

In order to determine if animals altered their motivation for food over the course of the session, we plotted the number of responses females made during pellet trials as a function of trial number. We found that overall the number of responses animals made during the session increased over time ($F_{1,54}$=15.91, p<0.001), indicating that satiety is not influencing motivation for food during the test session. Interestingly, when animals were grouped by hormone treatment, there was a significant increase in the number of active pellet responses unprimed animals (Fig 4.6A) made during pellet trials ($F_{1,54}$=17.96, p<0.0001), but no change in responding over time in hormone treated animals ($F_{1,21}$=0.41, p=0.53; Fig 4.6B). This indicates ovariectomized females increase their motivation for food over the course of the session, but not after hormone priming.

Hormone priming biases where animals are located in the chamber

Females were willing to work for access to a mate regardless of hormone treatment. However, receptive and non-receptive animals differed in their behavior once they gained access to the male chamber (Fig 4.7B). Receptive females spent a greater proportion of time in the male side (p<0.05). Alternatively, when animals were not sexually receptive, they spent more time in the door to the chamber, where they could see the male but he could not physically interact with them (p<0.05). Although females spent comparable about of time on the female side regardless of hormonal status (p=0.22; Fig 4.7A), there were important differences in how they directed their focus within the operant chamber (Fig 4.7C).

Non-primed females spent more time oriented toward and engaging with the pellet nose poke hole, cue, and food tray than primed females (p=0.001; Fig 4.7D, right

panel). This was also true when we measured attention toward the food associated cues (p<0.01) or food tray alone (p<0.01; Fig 4.7F). However, there was no effect of hormone treatment on the amount of time that animals spent engaging with the mate nose poke hole, cue, or door, (p=0.35; Fig 4.7D, left panel). This indicates that hormone primed females, when they are not actively attending to the task, are engaging in some third behavior, presumably waiting for the desired time period between intromissions.

Discussion

Scholars have long speculated that seemingly paradoxical reductions in food intake and body weight during periods where energetic demand is increased and food remains freely available serve to decrease the likelihood that feeding behaviors will disrupt other, more important, activities (Mrosovsky and Sherry, 1980). One such example that has been the subject of much research is the peri-ovulatory decrease in food consumption seen in most female mammals (Tarttelin and Gorski, 1971; Wade, 1972, 1975; Fessler, 2003; Asarian and Geary, 2006). However, while the proximate mechanisms underlying the effects of the ovulatory cycle on food intake are well understood, enquiry into the ultimate or adaptive purpose of these changes remains mostly speculative. Here, we describe experimental evidence that administration of EB + P to induce sexual receptivity in female rats simultaneously biases both choice and motivation for sex vs. food.

Hormone priming biases choice between sex and food

OVX female rats trained to respond on a concurrent FI operant paradigm for both food and sex show a bias toward choice of food reward over access to a sexually experienced male conspecific. This is indicated by both the number of trials that animals

initiate in pursuit of the palatable food reward, as well as a shift in the proportion of pellet vs mate trials. Hormone priming reduces the number of pellet trials that animals initiate, therefore shifting the proportion of pellet vs mate trials toward a preference for pursuit of access to the male, but does not increase the total number of mate trials that animals initiate. This may be due to differences in the average length of each trial, and particularly in the amount of time that animals spend with the male after gaining access to the male compartment. Indeed, hormone treated animals spent more time engaged in mate seeking and copulation compared to food seeking, as well as when compared to the amount of time unprimed animals spent engaged in mate seeking behaviors. Taken together, this suggests that measurement of the raw number of times the female will attempt to gain access to the male is a poor indicator of her sexual motivation.

Parsing sexual vs social motivation

Rats are social animals, and will work to gain access to a same-sex conspecific even over drug reward (Venniro et al., 2018). Thus, it is not surprising that non-receptive females will still engage with the operant task in pursuit of a social reward. However, when non-receptive females do gain access to the male, their behavior differs in several important ways. When hormone treated, females engage in sexual behavior (Fig 4.4B) and spend more time with the male and less time in the doorway between the two sides of the cage (Figure 4.5B). Unprimed females spend comparatively less time with the male, and instead will remain in the doorway where they can see the male but are out his reach and cannot physically interact with him. This further indicates when unprimed females respond for access to the male, they are doing not doing so in pursuit of sexual reward, but perhaps instead for social reward or general novelty seeking.

Induction of sexual receptive has reward specific effects on motivation

The number of responses that a female made on the FI schedule was used as an indicator of her motivation for each reward. Females were more motivated for a palatable food pellet when unprimed, but more motivated for access to a mate when primed. Interestingly, this shift in motivation for food vs sex appears to equalize motivation for the two rewards during periods of sexual receptivity. When females are not sexually receptive, they show greater motivation for food, as opposed to the access to the male. After hormone priming, motivation for food decreases, while motivation for sex increases, leading to a comparable level of responding for both rewards in hormone treated animals.

This is somewhat surprising, as one would expect that hormone primed animals would show greater motivation for sex compared to food. There are a number of potential explanations for this difference. One may be that the design of the apparatus leads to less responding for sex overall. During the fixed interval, animals will often check for the reward after responding. The food trough is located directly adjacent to the active nosepoke hole and this checking behavior only requires the female to shift her head, whereas the door to the male side is diagonal from the active port and shifting her attention toward the door requires a greater movement for the animal to reorient her body away from the nose poke hole. Thus, the ceiling in the number of responses females can make within the 15s interval when responding for access to a mate may be lower than the number of responses animals can make for food. However, this is somewhat unlikely as the total amount of time that animals spend engaging with either the food tray or the door

is similar, and increases in responding for food are associated with increases attention to the food tray.

Another explanation is that motivation for food remains high because it is still adaptive for females to be motivated for food, even when sexually receptive. Even sated animals will respond for palatable food reward, a strategy that is clearly beneficial in unpredictable environments where food availability is sporadic. Indeed, E alters food intake by decreasing meal size without altering the meal frequency by enhancing the effects of satiety hormones (Blaustein and Wade, 1976; Eckel et al., 2002; Santollo et al., 2007; Brown and Clegg, 2010; Maske et al., 2017). This makes sense within the adaptive explanation that has been proposed – specifically enhancing satiety mechanisms ensures that females will not overlook opportunities to eat but instead spend less time eating during each bout in order to return to the important business of mate seeking and reproduction. In support of this, we found that females generally increased their motivation for food over time, but this did not happen when they were hormone primed. This does not indicate satiety specifically, as primed females do not show decreased motivation for food over time but does suggest that there is an effect of hormone treatment on how motivation for food changes over the course of the session, where normal increases in motivation for palatable food reward are blunted in primed females.

Finally, although animals in the current experiment were not food deprived at any point, we did remove their food from the home cage four hours prior to testing, and three hours prior to the start of the dark cycle. Ad lib fed female hamsters show a strong bias toward sex when both food and males are freely available, which is reversed following food deprivation (Schneider et al., 2007). It is possible that even the marginal food

restriction used in the current experiment prevents any further decrease in motivation for food in hormone treated female rats.

Effects of hormone priming on task performance

The effect of hormone treatment on choice between food and sex was specifically driven by an increase in the number of trials that were rewarded, without altering the number of trials that animals failed. This resulted in an overall shift in the proportion failed trials that was specific to which reward was being pursued. Although the number of trials that animals failed was unchanged after hormone treatment, the animal's behavior during failed vs completed trials did differ based on reproductive status.

Increased motivation for each reward was driven by an increase in responding during completed trials. During failed trials, animals show no changes in the number of responses for the active reward as a consequence of hormone treatment. However, during both pellet and mate trials, hormone primed, but not unprimed, failed trials were characterized by an increase in the number of responses that animals made for the alternate reward.

Hormone priming biases attention for food paired cues

In addition to changes in instrumental responding for each reward, the shift in preference for food vs. sex was apparent in what elements of the apparatus animals attended to during the task. There was no effect of hormone priming on the amount of time animals spent interacting with elements of the apparatus associated with access to the mate, including the mate paired light cue and door. However, females spent significantly less time interacting with the food tray and the food paired cue after hormone treatment. Within the female side of the chamber, where all of the response

elements are located, animals have limited options for what to direct their attention toward. The decrease in time spent focused on the food associated elements, without a concurrent increase in time spent focused on the mate paired elements, indicates that hormone primed females are instead increasing the amount of time they spend engaging in some third behavior. One possibility is that the animals are waiting for the desired time period between intromissions to elapse before returning the male side. As mentioned previously, females will actively pace the rate of copulation when given the opportunity. The length of the interval between intromissions is dependent on the intensity of stimulation and is necessary for induction of the progestational reflex required for successful implantation of a fertilized embryo as well as sexual reward (Erskine et al., 1989, 2004; Jenkins and Becker, 2003b). Dopamine release during female sexual behavior rises during the time leading up to, but not during intromissions (Jenkins and Becker, 2003a). This may indicate that this waiting period, rather than being a passive phase in between bouts of sexual behavior, is instead an active behavior that is important for the rewarding aspects of the female sexual experience.

Conclusion

These findings demonstrate experimentally that changes in motivation for food after hormone treatment act to enhance motivation for sex. When given the opportunity to choose between sex and food, OVX female rats show a preference for food reward that is reversed following administration of EB and P. We propose that these findings provide experimental evidence for the ultimate or adaptive purpose of periovulatory changes in feeding behavior. Future work exploring the neural mechanisms underlying the effect of ovarian hormones on motivation for both food and sex is necessary for a full

understanding of how ovarian hormones regulate adaptive behaviors during the estrus cycle.

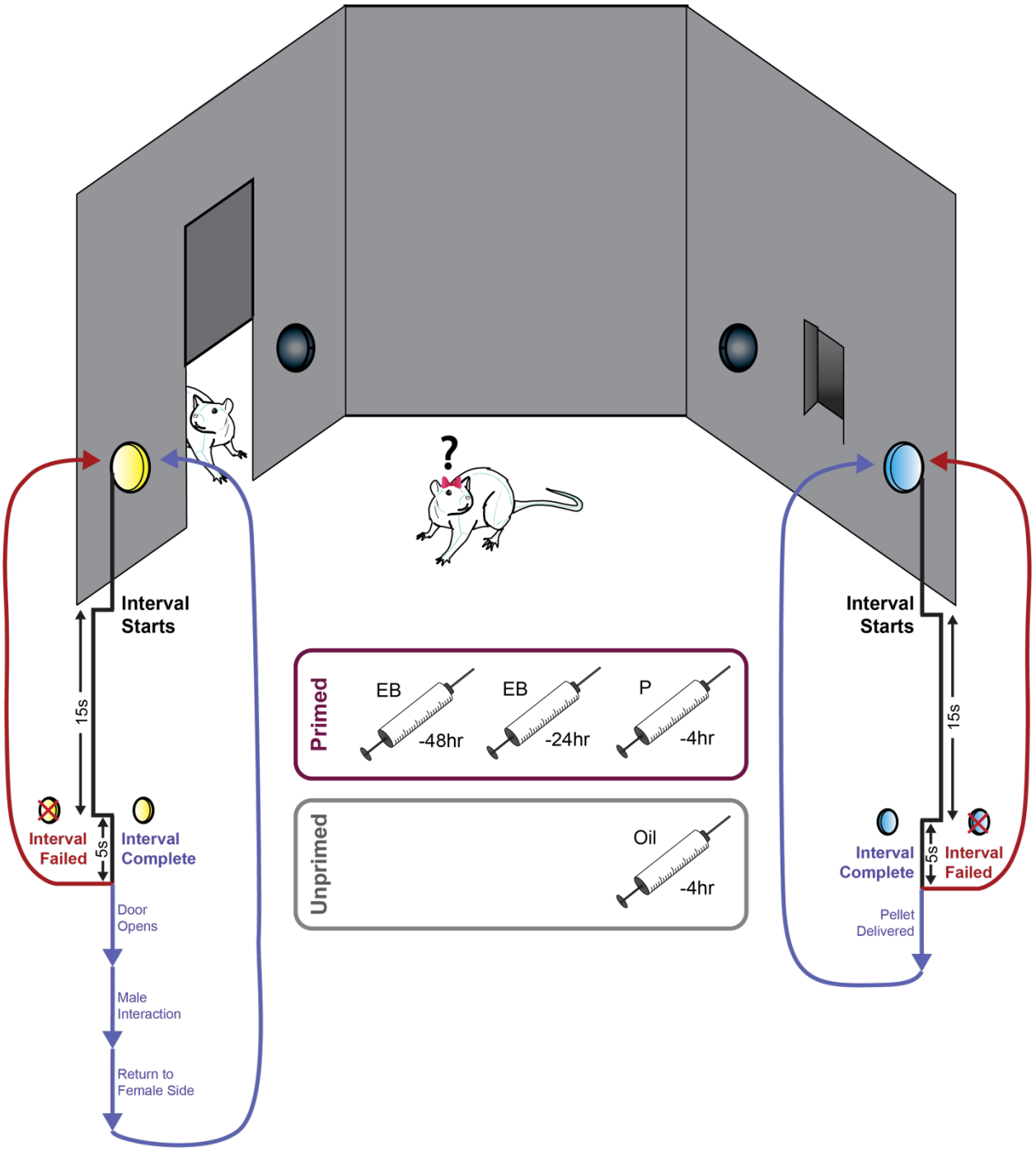
?
Interval Starts
15s
Interval Failed
5s
Interval Complete
Door Opens
Male Interaction
Return to Female Side
Primed
EB
-48hr
EB
-24hr
P
-4hr
Unprimed
Oil
-4hr
Interval Starts
15s
Interval Complete
5s
Interval Failed
Pellet Delivered

Figure 4.1 An operant paradigm for concurrent measurement of motivation for food and sex.
Female rats were trained to respond for both food and sex on a fifteen second fixed-interval (FI15) schedule. Activation of either active port initiated an FI15 for that specific reward (food or sex), and animals could only respond for access to the initially selected reward for the duration of the trial. Completion of the FI15 on the active port located adjacent to the food trough resulted in delivery of a palatable food pellet (45mg banana flavor, BioServ, Flemington, NJ) accompanied by presentation of a discrete light cue for 1s. Completion of the FI15 on the active port located adjacent to the door leading to the other chamber resulted in the door opening and presentation of a different light cue for 1s. The door remained open until the female crossed into the male side to interact with the male, and then closed upon her return to the female side. Failure to complete the FI15 did not result in reward delivery, and the next response made on either port would initiate a new trial.

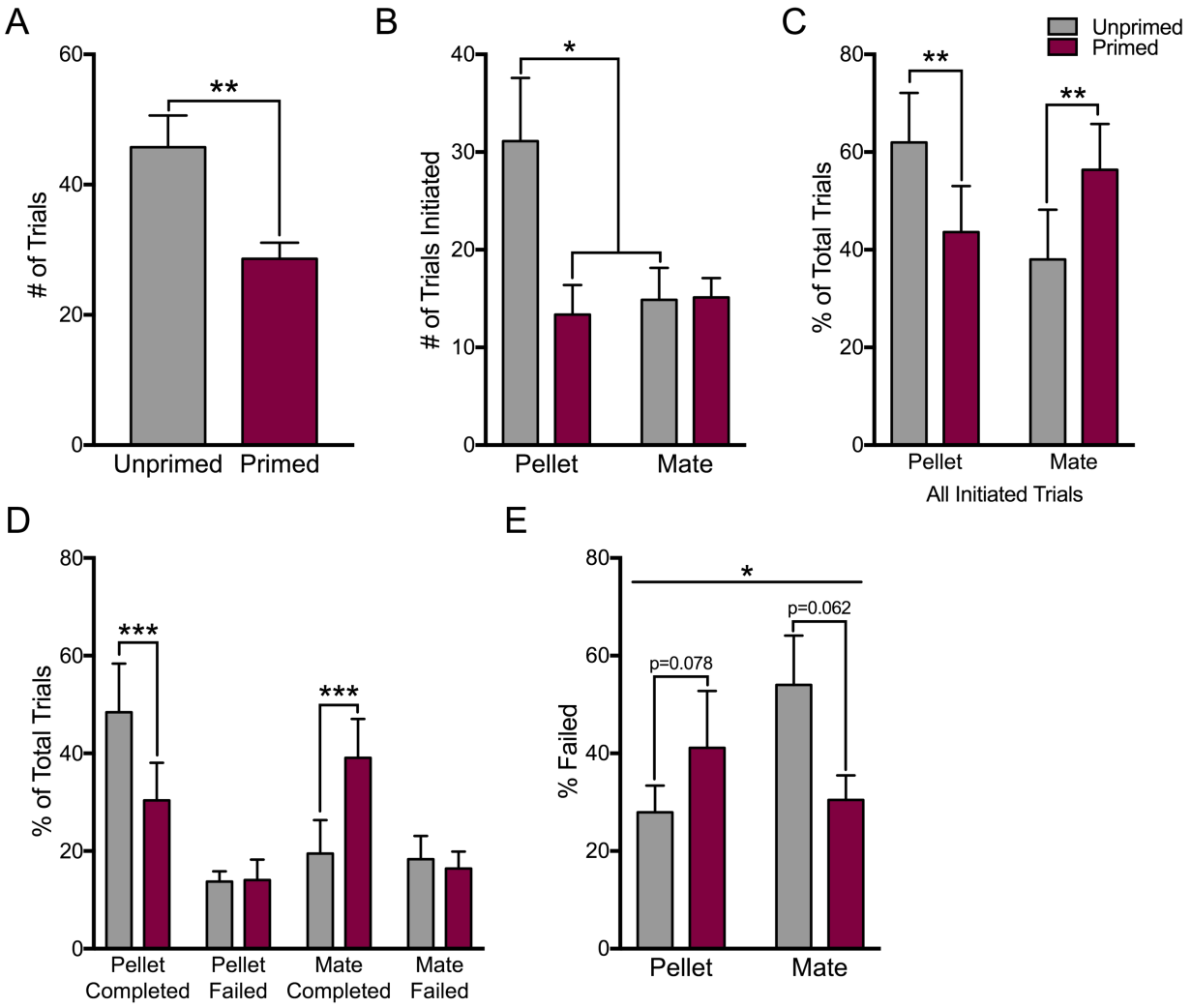

Figure 4.2 Reproductive status biases choice for food vs sex.
Hormone treatment reduced the total number of trials that animals initiated **(A)**, specifically by reducing the number of pellet trials **(B)**. The proportion of mate or pellet trials that animals initiated was altered following hormone treatment **(C)**. Animals initiated more mate trials when hormone primed than when unprimed, and more pellet trials when unprimed than when hormone primed. Changes in the proportion of pellet or mate trials that animals initiated were driven by increases in completed trials, without altering the total number of trials that animals failed **(D)**. Although the total number of failed trials remained unchanged, changes in the corresponding number of completed trials resulted in a significant interaction between hormone treatment and trial type on the proportion of trials that animals failed **(E)**. Data are shown as mean ± S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

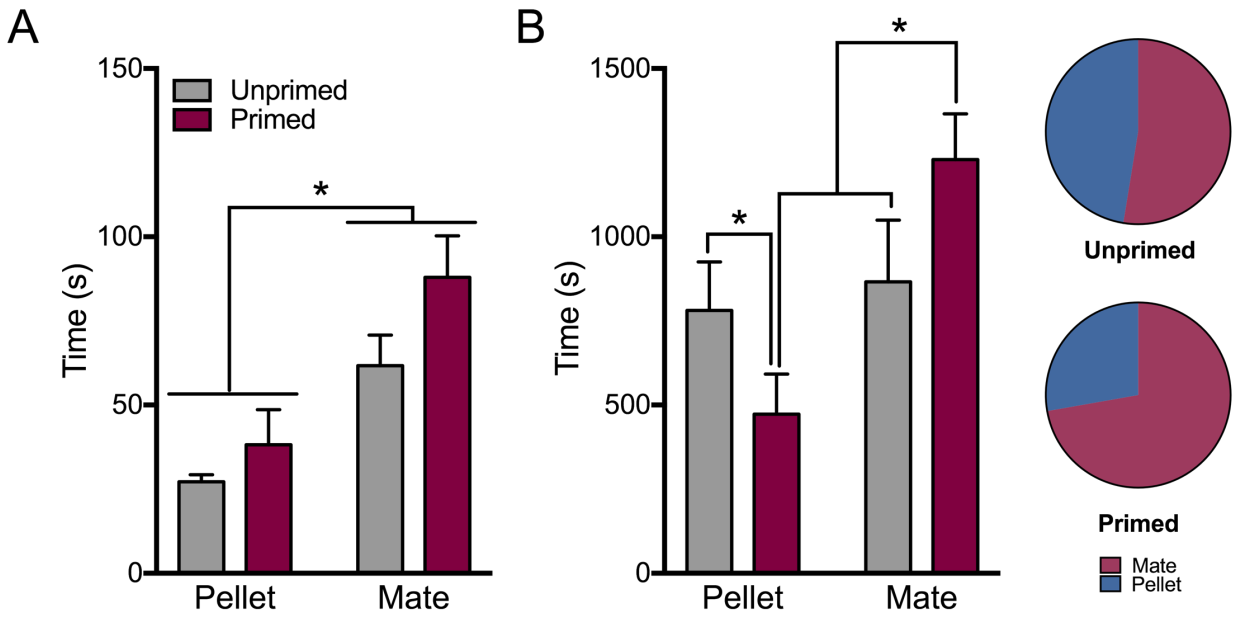

Figure 4.3 Hormone treatment alters the amount of time animals spend in pursuit of each reward.
(A) Mate trials are on average longer than pellet trials, and trial length for both rewards is increased in after hormone priming. **(B)** The total amount of time that animals spent engaged in mate trials was increased following hormone priming, which was accompanied by a decrease in the amount of time spent engaged in pellet trials. Data are shown as mean ± S.E.M. * $p<0.05$.

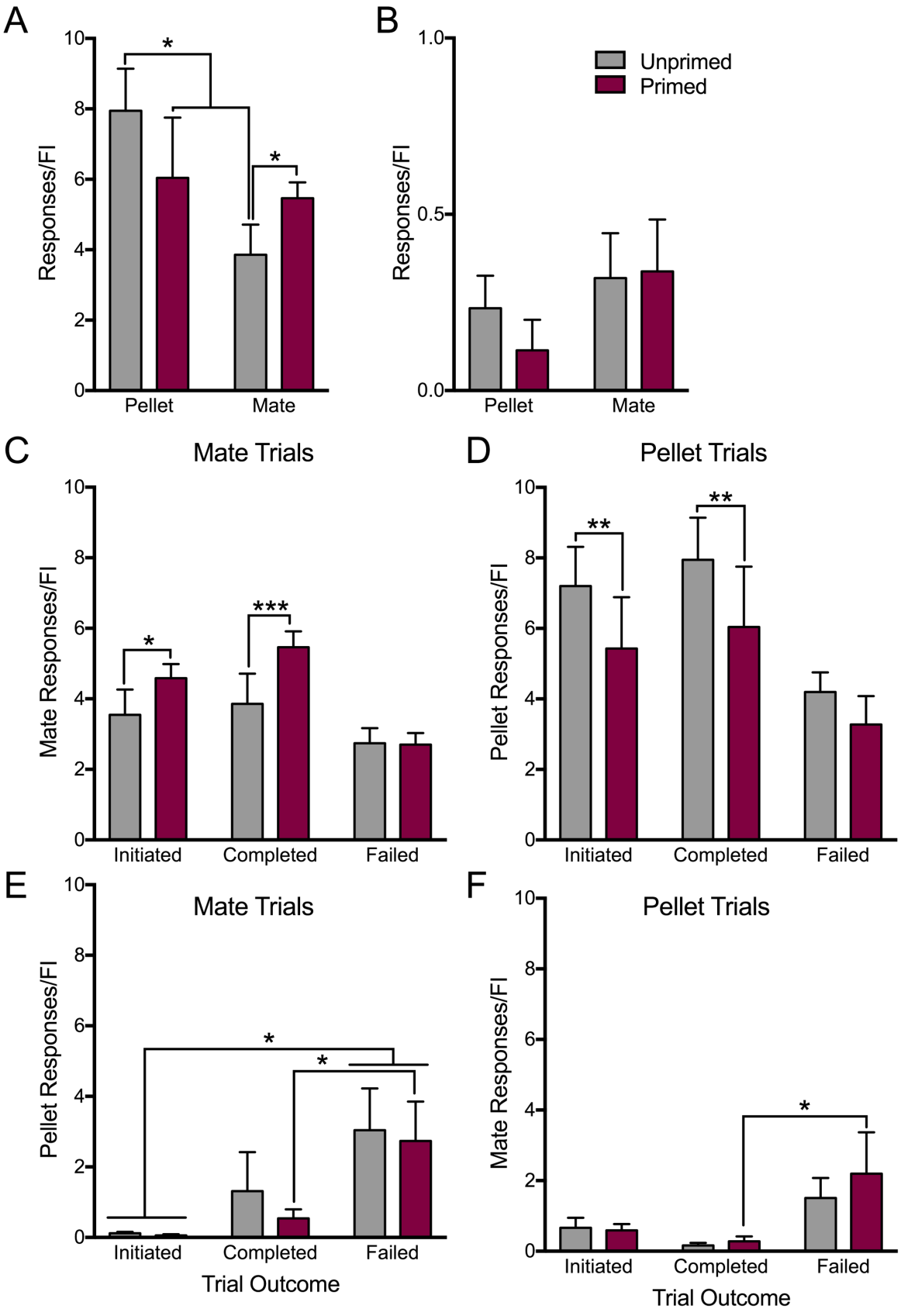
A
Responses/FI
10
8
6
4
2
0
*
*
Pellet
Mate
B
Responses/FI
1.0
0.5
0.0
Unprimed
Primed
Pellet
Mate
C
Mate Trials
Mate Responses/FI
*
***
Initiated
Completed
Failed
D
Pellet Trials
Pellet Responses/FI
**
**
Initiated
Completed
Failed
E
Mate Trials
Pellet Responses/FI
*
*
Initiated
Completed
Failed
Trial Outcome
F
Pellet Trials
Mate Responses/FI
*
Initiated
Completed
Failed
Trial Outcome

Figure 4.4 Hormone priming increases motivation for sex while simultaneously decreasing motivation for palatable food reward.
The effect of hormone priming on motivated responding is dependent on the reward being pursued **(A)**. Unprimed animals show greater motivation for food than for access to a mate. Induction of sexual receptivity by hormone priming reduces motivation for food, but increases motivation for access to a mate, resulting in similar levels of motivated responding for both rewards. The effect of hormone priming on motivated responding is not mediated by changes in overall locomotor behavior, as there was no effect of hormone priming on the number of responses made on the inactive ports **(B)**. Hormone priming specifically increased responding during completed mate trials, without changing the number of active mate responses during failed trials **(C)**. Similarly, hormone priming only reduced responding for pellet during completed pellet trials, but not during failed pellet trials **(D)**. Although responding for the active reward was not altered during failed trials, hormone primed animals made more responses for the inactive reward during failed trials than completed trials during both mate **(E)** and pellet **(F)** trials. Both primed and unprimed animals made more responses for the alternate reward during failed trials when compared to all trials that were initiated. Data are shown as mean ± S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

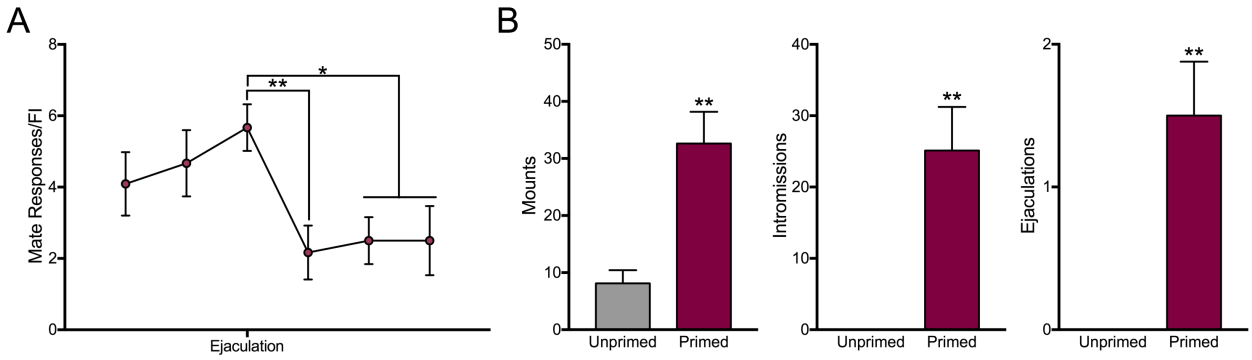

Figure 4.5 Motivation for access to a mate decreases following ejaculation in sexually receptive female rats.
(A) Motivation for access to a mate was attenuated during three trials following ejaculation. **(B)** As expected, only sexually receptive animals engaged in sexual behavior. Data are shown as mean ± S.E.M. * $p<0.05$, ** $p<0.01$.

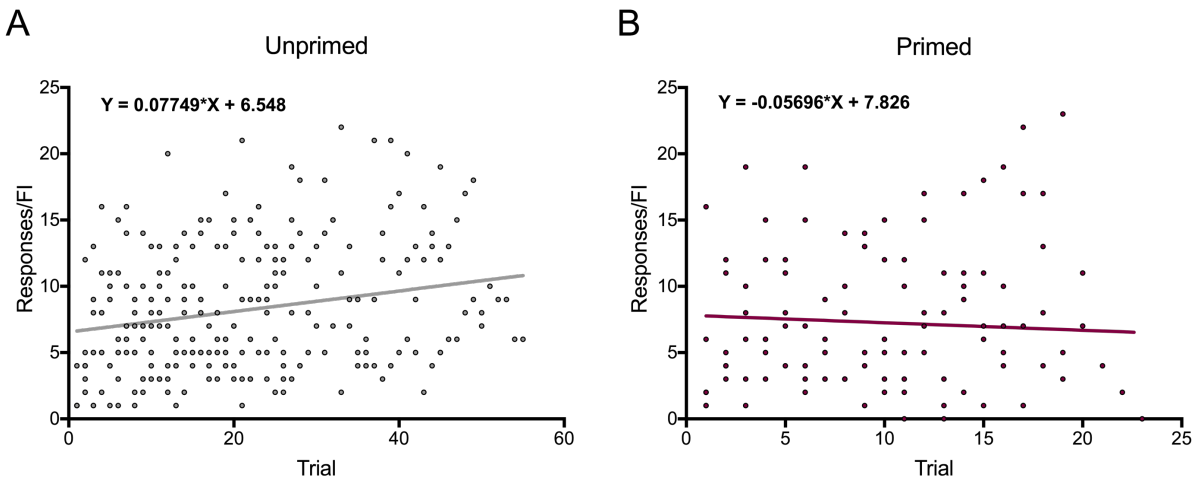

Figure 4.6 Changes in motivation for food over time.
(A) Motivation for food increases significantly in over the course of each session in ovariectomized female rats. **(B)** Primed females do not increase their motivation for food from the beginning to the end of each session.

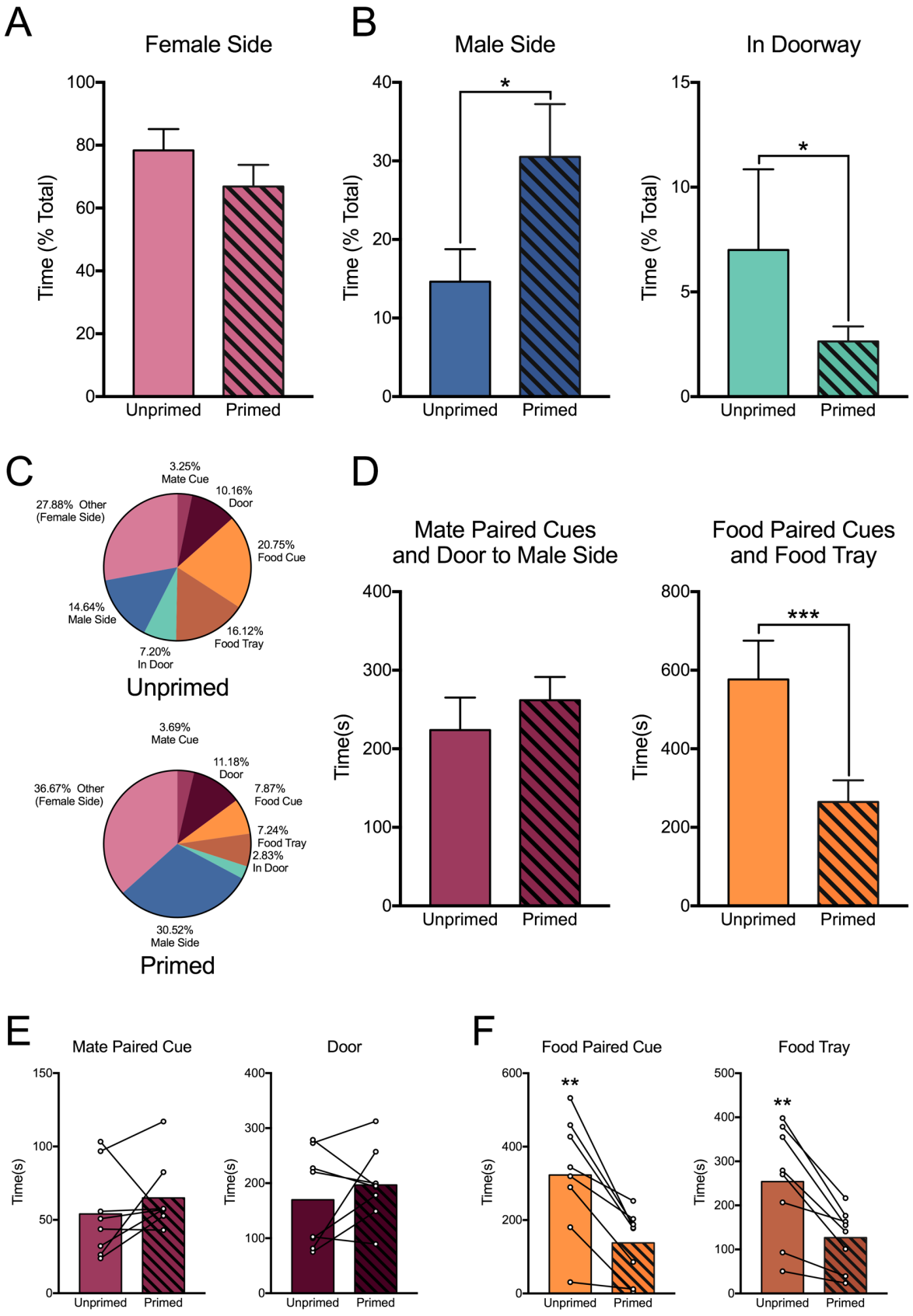
A
Female Side
Time (% Total)
100
80
60
40
20
0
Unprimed
Primed
B
Male Side
Time (% Total)
40
30
20
10
0
*
Unprimed
Primed
In Doorway
Time (% Total)
15
10
5
0
*
Unprimed
Primed
C
3.25%
Mate Cue
10.16%
Door
27.88% Other
(Female Side)
20.75%
Food Cue
14.64%
Male Side
16.12%
Food Tray
7.20%
In Door
Unprimed
3.69%
Mate Cue
11.18%
Door
36.67% Other
(Female Side)
7.87%
Food Cue
7.24%
Food Tray
2.83%
In Door
30.52%
Male Side
Primed
D
Mate Paired Cues
and Door to Male Side
Time(s)
400
300
200
100
0
Unprimed
Primed
Food Paired Cues
and Food Tray
Time(s)
800
600
400
200
0
***
Unprimed
Primed
E
Mate Paired Cue
Time(s)
150
100
50
0
Unprimed
Primed
Door
Time(s)
400
300
200
100
0
Unprimed
Primed
F
Food Paired Cue
Time(s)
600
400
200
0
**
Unprimed
Primed
Food Tray
Time(s)
500
400
300
200
100
0
**
Unprimed
Primed

Figure 4.7 Hormone priming alters the behavior of female rats within the chamber. **(A)** Females spent a similar amount of time in the female (instrumental) compartment regardless of hormone treatment. **(B)** However, when females were given access to the male chamber, hormone primed animals spent more time with the male, while unprimed animals spent more time in the doorway, out of reach of the male. **(C)** Hormone priming altered the distribution of time that animals spent engaging in various aspects of the task. **(D)** Hormone primed animals spent a similar amount of time engaging with the mate paired cue, active mate port, and door, but reduced the amount of time they spent engaging with the food paired cue, active food port, and food trough. This was true when considering the amount of time animals engaged with each aspect of the apparatus individually for both mate **(E)** and food **(F)** paired cues. Data are shown as mean ± S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

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Chapter 5:

Discussion

Sex differences in the effect of psychomotor stimulants on striatal dopamine (DA) release arise due to the ability of ovarian hormones to regulate DA release in females but not in males. The experiments presented within this dissertation not only investigate the mechanism by which estradiol acutely regulates nucleus accumbens (NAc) DA release after cocaine treatment, but also explore potential adaptive explanations for the ability of ovarian hormones to modulate dopamine release and motivated behaviors.

Effects of ovarian hormones on motivation are reward specific

The overarching goal of my dissertation work was to not only investigate *how* ovarian hormones regulate motivation and dopamine release, but also *why* females evolved a neural circuit for motivation that is responsive to changes in ovarian hormones. We hypothesized that changes in DA release in response to estradiol facilitate adaptive decision making across the estrous cycle by increasing motivation for sex while simultaneously reducing motivation for food.

One of the important aspects of this hypothesis is the specificity of changes in motivation for specific rewards. Increased levels of circulating ovarian hormones during proestrus and estrus do not enhance motivation in general, but instead specifically direct motivation for food or sex at times when each reward is most beneficial to the animal's overall reproductive success. This differs from previous work focusing on the effect of estradiol on motivated behaviors, which has emphasized the ability of estradiol to

enhance motivation for sex and drugs of abuse (Yang et al., 2007; Zhao and Becker, 2010; Cummings and Becker, 2012). However, others have shown that reductions in consummatory aspects of feeding behavior by estradiol are accompanied by decreased motivation for food (Richard et al., 2017). These initial studies make it clear that estradiol does not just increase motivation for all rewards, but do not establish a link between induction of sexual receptivity and changes in motivation for food.

Changes in motivation for food are tied to induction of sexual receptivity

Thus, in Chapter 3 I verified that administration of estradiol benzoate (EB) and progesterone (P) in a regimen that induces sexual receptivity and enhances motivation for access to a mate also attenuates motivation for palatable food reward. Importantly, I used the same instrumental paradigm that was previously used to measure motivation for sex: a 15 second fixed interval (FI15) that measures both reward taking (as measured by the number of rewards the animal earns over the course of each session) as well as motivation or vigor during reward seeking (as measured by the number of responses the animal makes during each fixed interval).

One important advantage of the studies on motivation for food described in Chapter 3 was that I was able to test an animal's motivation repeatedly for the duration of hormone priming, allowing me to measure the effect of acute EB administration as well as repeated EB and full priming. This was useful not only in determining the temporal specificity of the effect of ovarian hormones on motivation for food, but also in linking the effects of ovarian hormones on motivation for food with work from Chapter 2 showing that acute administration of EB enhances the effect of cocaine on DA release.

If reductions in motivation for food are mediated by direct effects of EB on DA release, changes in motivation for should occur at a similar timescale to the effects of EB on DA release in response to drugs of abuse. However, I found that females showed reduced responding for food and earned fewer rewards only after they were fully primed with EB and P, and not on the first or second day of EB priming.

It is important here to point out that the results described in Chapter 2 do not show an effect of hormone treatment on DA release in general, but on the effects of cocaine on DA release, as this is important to understanding why manipulations that enhance DA release in response to drug administration do not also increase motivation for other rewards. Palatable food reward (and presentation of cues that predict reward) increase firing of VTA DA neurons, resulting in increased DA release within the NAc (Schultz et al., 1993; Roitman et al., 2004). This is very different from the effects of cocaine, which primarily increases DA overflow by direct inhibition of DA reuptake (Mateo et al., 2004). Thus, comparisons between these two studies are speculative, and further work in both areas is needed to provide a definitive link between the two.

Alternatively, reductions in motivation for food may be secondary to changes in overall food intake. Many manipulations that alter overall food intake also alter motivation for food, potentially via direct projections that modulate VTA DA activity (Harris et al., 2005; Cone et al., 2016; Hayes and Schmidt, 2016). However, the effects of estradiol on food intake and body weight can also be seen acutely (within 24 hours), and progesterone reverses the effects of estradiol on food intake (Santollo et al., 2007; Yu et al., 2011). Thus, the temporal specificity of the findings in Chapter 3, combined with the observation that progesterone is actually required for reductions in motivated responding,

together indicate that ovarian hormones regulation of motivation for food is dissociable from their effects on consummatory feeding behaviors.

In the control of sexual behavior, initial activation of ERs actually inhibits lordosis expression by activation of μ-opioid receptors within the medial preoptic area (MPOA) (Mills et al., 2004). Progesterone then acts to reverse this activation, leading to inhibition of the MPOA and facilitation of lordosis (Sinchak and Micevych, 2001). The temporal specificity of this effect of hormone priming reinforces the hypothesis that changes in motivation for food have adaptive significance for sexual behavior. Motivation for food is not reduced during initial EB priming, when lordosis would be inhibited, but only after progesterone, when animals are fully sexually receptive. This reinforces the hypothesis that changes in motivation for food specifically tied to changes in sexual receptivity.

Progesterone is required to alter motivation during hormone priming

Repeated administration of estradiol alone, or a single high dose of estradiol, can induce sexual receptivity without progesterone. However, although progesterone is not required to induce sexual receptivity in ovariectomized female rats, it is important for the expression of proceptive solicitation behaviors (Brandling-Bennett et al., 1999; Micevych and Sinchak, 2018). Progesterone was also required to reduce motivation for food on both the FI15 and a progressive ratio schedule of reinforcement. This is particularly interesting because it links the appetitive aspects of sexual receptivity with changes in appetitive food seeking and motivation.

The induction of lordosis by EB and progesterone or EB alone involves recruitment of distinct neuronal populations within the hypothalamus, and specifically the

arcuate nucleus (Micevych and Sinchak, 2018). The arcuate also plays an important role in energy balance and regulation of feeding, and has received attention for its potential to link metabolic and reproductive processes (Kelley et al., 2005; Hill et al., 2008; Zhang and van den Pol, 2016). Importantly, the arcuate is also implicated in the processing of food reward (Kelley et al., 2005). Perhaps the pattern of arcuate activation induced by estradiol and progesterone, but not estradiol alone, modulates projections that also regulate motivation for food. The arcuate may be a promising target for further work investigating the brain circuitry underlying ovarian hormone mediated changes in motivation for food, particularly since direct manipulations of PR within the NAc were unsuccessful in altering the effects of hormone priming on motivation for food.

Progesterone was specifically required to reduce motivation for food, but did not reduce the number of rewards that animals received over the course of the session. This may indicate that the number of rewards is a better marker of overall food intake within the context of this paradigm, rather than specific motivation for each reward. This would make sense within the interpretation that progesterone modulates appetitive, rather than consummatory, aspects of motivated behavior. Perhaps estradiol initially alters general consummatory behaviors, and progesterone then activates the specific behaviors that increase the likelihood that those consummatory behaviors can be expressed. In this context, estradiol would provide direction (specifically reducing general feeding behaviors), while progesterone provides vigor (integrating changes in feeding with changes in motivation).

Role of selective estradiol receptor subtypes in motivation and dopamine release

In both Chapters 2 and 3 I use selective estradiol receptor (ER) agonists to parse the relative contribution of these receptors to the effects of estradiol on DA release after drug administration as well as motivation for natural rewards. While activation ERβ alone was able to recapitulate the effect of the non-selective agonist EB on DA release after cocaine administration, none of the selective agonists used in Chapter 3 were able to completely replicate the effects of EB on motivation for food. Administration of the ERα selective agonist during hormone priming did significantly reduce motivation for food, but this was not temporally specific to the induction of sexual receptivity. It may be that ERα activation, which has been implicated in the effect of estradiol on consummatory and metabolic aspects of food intake, indirectly reduced motivation for food. However, if this is the case it is surprising that PPT only reduced motivated responding after hormone treatment had already been completed, as the ERα activation transiently reduces food intake and body weight within the first 24 hours after administration (Santollo et al., 2007).

Activation of ERα is also important for the control of sexual behavior. Hormone priming with PPT and P induces sexual receptivity as well as the expression of proceptive sexual behaviors (e.g. hops, darts, and ear wiggling), while hormone priming with the ERβ selective agonist diarylpropionitrile (DPN) does not (Mazzucco et al., 2008). Interestingly, expression of proceptive behaviors is attenuated in females that were hormone primed with both PPT and DPN, a finding that has been interpreted as providing evidence that ERα controls the induction of sexual receptivity and proceptivity, while ERβ fine-tunes the degree to which sexual receptivity is expressed.

Although the contribution of specific ER subtypes was emphasized in these studies, it is worth mentioning again that proceptive behaviors are dependent on progesterone receptor activation. However, initial ER activation is still required for inducing later expression of PR, and the specific PR isoforms that are expressed are dependent on which ER subtypes are activated (Rickard et al., 2002). While the PR isoform PR-A is required for induction of lordosis during hormone priming, PR-B may be more important for DA-dependent aspects of sexual receptivity (Mani et al., 2006). To add further complexity, differential activation of ER subtypes has been shown to produce differential localization of PR expression within brain areas important for the regulation of female sexual behavior (Sá et al., 2015). Thus, selective ER activation during hormone priming may lead to aberrant induction of PR expression, altering the ability of progesterone to induce changes in motivation.

Direct vs. indirect effects of ovarian hormones on the circuitry underlying motivation

Our initial hypothesis was that estradiol and progesterone act directly on striatal circuitry to regulate changes in motivation for food vs sex. However, blockade of PR within the NAc was not effective in preventing the effect of hormone priming on motivation for food. This does not fully rule out a direct effect of ovarian hormones on striatal circuitry, particularly since the PR antagonist used in Chapter 3 also acts on glucocorticoid receptors. Still, it is important to consider extra-striatal circuitry, that may induce indirect changes in DA signaling and motivated behaviors.

As discussed previously, the arcuate nucleus is a promising candidate due to its role in the regulation of both feeding and sexual behavior. The arcuate primarily controls

the expression of sexual receptivity via inhibition of the MPOA, and this brain area may also regulate some of the effects of ovarian hormones on motivated behaviors. ER positive cells in the MPOA have been shown to project to the VTA, and inhibit DA release in the NAc (Will et al., 2016). The effect of estradiol on DA release in response to cocaine was diminished after lesions of the MPOA, and estradiol microinjections in the MPOA enhanced cocaine induced DA release in female rats (Tobiansky et al., 2015). GABAergic neurotensin neurons within the MPOA express ERα and are dynamically regulated by changes in reproductive state (McHenry et al., 2017). This circuit, which was linked to hormonal regulation of social reward, inhibited VTA GABA neurons, leading to increases in firing of DA neurons during proestrus and estrus ((McHenry et al., 2017). Importantly, activation of ER within the MPOA is sufficient to reduce food intake in OVX female rats (Mascarenhas, 1986; Santollo et al., 2011). Further investigation to determine similarities and differences in the control of feeding and sexual behavior by the MPOA, as well as whether these mechanisms are important for motivation specifically, is warranted.

The ventromedial hypothalamus (VMH) is another major output nuclei in the control of lordosis (Veening et al., 2014). The VMH is also sensitive to changes in metabolic hormones, including leptin and mice lacking ERα within the VMH show increased body weight (Hill et al., 2008; Xu et al., 2011). However, estradiol interactions with the VMH to induce changes in body weight are not linked to metabolic changes, rather than changes in food intake (Xu et al., 2011). Thus, the VMH may important for peripheral changes during ovulation, but not central coordination of adaptive behaviors.

Brain areas outside of the hypothalamus may also contribute to the effects of estradiol and progesterone on motivation for food. Sensitization of proceptive female sexual behaviors by amphetamine is prevented by DA or PR antagonism within the medial amygdala (Holder et al., 2015). In males, activation of the medial amygdala leads to an increase in DA release in the MPOA, which has been linked to changes in sexual motivation (Dominguez and Hull, 2001; Kleitz-Nelson et al., 2010). The medial amygdala receives inputs from the olfactory bulbs and vomeronasal organ and is likely important for integrating salient olfactory input associated with specific reward (Kevetter and Winans, 1981; Wood, 1997). In support of this, females with lesions of the medial amygdala show impaired recognition of salient sex odors (Petrulis and Johnston, 1999). This circuit, which may integrate chemosensory signals with peripheral information about reproductive and metabolic state, could then coordinate directed changes in motivation for specific rewards and reward-related stimuli.

Ovarian hormones and adaptive decision making

While previous work from our lab established that induction of sexual receptivity increases motivation for sex, and I verified that the same regimen of hormone administration reduces motivation for food, it had not been determined whether these changes in motivation were relevant for adaptive choice when both rewards were available. In chapter 4, I modified the paradigm used in previous studies and Chapter 3, in order to test both motivation and choice for food and sex simultaneously in the same test session. I found that females not only shifted how motivated they were for food vs sex as a function of hormone treatment, but also biased their choice for each reward.

These findings verify that changes in motivation for food vs sex act to guide adaptive choice across reproductive states. This also underscores the importance of specific modulation of motivation for each reward. If ovarian hormones non-selectively enhanced motivation for all rewards, there would be no benefit toward decision making when both rewards are available. It is possible that ovarian hormones direct both motivation and choice by altering the value of each reward and their related cues. DA within the NAc circuitry has been proposed to act as a value signal that simultaneously drives motivated effort (Hamid et al., 2016). Within this context, though, changes in DA release direct the magnitude of effort, but not the target. Understanding how the behavioral changes described here may regulated by changes in DA signaling across the estrous cycle may be helpful in understanding the role of DA in motivated choice in both sexes.

I did not thus far determine whether progesterone is required for changes in motivated responding for sex vs. food. Based on previous work showing that progesterone is required for expression of proceptive sexual behaviors and decreases in motivation for food, I would expect that progesterone is important for mediating changes in motivation for food vs sex. However, it may not be equally necessary for shifts in choice between these two rewards. Perhaps estradiol, via regulation of hypothalamic or amygdala circuitry involved in feeding and sexual behavior, alters the choice between these two rewards, while progesterone specifically alters motivated responding. Future work defining progesterone's role in various aspects of this behavior could greatly increase our understanding of progesterone's importance in the estrus cycle mediated behaviors.

General changes in behavior across the estrous cycle

In both Chapters 3 and 4, I found an effect of hormone priming on non-rewarded trials during responding on the FI15 paradigm. In Chapter 3, when only a food reward was available, hormone priming increased the number of trials that animals failed during each session. In Chapter 4, when both food and sex were available, hormone priming had no effect on the total number of trials that animals failed, but did alter the way that females engaged with the task during failed trials. Unprimed females make more responses in pursuit of pellet during mate trials regardless of whether the trial is rewarded or not – likely another indicator of their preference for food over sex. This differs from hormone primed females, who only make more responses for food during failed mate trials, and show a similar, reverse pattern of responding during failed pellet trials (where they make more responses for access to a mate than during completed pellet trials). Thus, in hormone primed females, increased responding for the alternate reward predicts that they will fail to complete the interval, indicating a sustained shift in reward seeking for food or sex. Unprimed females, on the other hand, may still shift which reward they pursue, but this shift is not sustained, and is specific towards pursuit of food reward.

It is interesting that hormone priming also increased the likelihood that females would stop responding during a trial when only one reward was available, as in this scenario there is no alternate reward for the animal to shift their attention toward. Females may instead have shifted their attention toward non-reward directed pursuits, which would indicate that this is not necessary a switch in focus from one reward to another, but rather a lack of overall focus on reward seeking. Taken together, these

findings may indicate that hormone priming altered performance on the fixed interval by altering how well females are able to sustain their focus on a specific reward.

There are many other changes in non-reproductive behavior that occur during the periovulatory period. In addition to decreasing food intake, females show increased locomotor behavior, reduced anxiety, and a shift toward use of place-based over response-based strategies to solve spatial tasks (Beatty, 1979; Mora et al., 1996; Korol et al., 2004). All of these changes in behavior would be helpful during mate seeking. Increased locomotor behavior would enhance the likelihood of coming across a male conspecific. Reduced anxiety could facilitate migration outside of a familiar home range, at which point it would be advantageous for animals to rely on a place-based spatial learning strategy. Perhaps more frequent shifts in sustained pursuit of a specific reward may also be advantageous during mate seeking, as it would decrease the likelihood that females would waste time and energy on non-rewarded activities.

While this does not completely make sense as a strategy during instrumental responding for reward, when sustained responding would lead to reward, it is important to consider that animals did not evolve these strategies to facilitate responding on operant behavioral tasks. This is especially pertinent for sexual behavior. During copulation, females engage in soliticiation behavior but are chased by the male prior to intromission (Adler and McClintock, 1978). This chasing behavior may facilitate inter-male competition during group mating, which would increase the likelihood that the female will be inseminated by the dominant male (McClintock et al., 1982). Thus, in the case of sexual behavior, it is possible that initial pursuit of the male, followed by shift in

attention to allow the male to chase, is more advantageous than focused pursuit on a single male.

Role of DA in the effects of ovarian hormones on motivated behaviors

The majority of work I have presented in this dissertation focuses either on the effects of ovarian hormones on striatal DA release *or* behaviors that we believe to be regulated by striatal DA signaling. The question remains whether changes in motivation for food or sex are coordinated by the effects of estradiol on DA release specifically. In males, manipulations that disrupt NAc DA signaling selectively prevent expression of sexual motivation without altering the ability for males to copulate (Everitt, 1990). In females, DA agonists dose dependently facilitate sexual receptivity induced by estradiol and progesterone, and sensitization of DA circuitry by repeated amphetamine exposure facilitates the display of proceptive behaviors (Everitt and Fuxe, 1977; Afonso et al., 2009). The paced mating paradigm used in Chapter 4 has been shown to increase DA release and induce a conditioned place preference (Jenkins and Becker, 2003a, 2003b). Moreover, presentation of cues that are paired with paced mating activate the NAcc, VTA, and caudate putamen (Coria-Avila and Pfaus, 2007). Taken together, it is clear that DA signaling plays an important role in the control of rewarding sexual behavior in both males and females.

Food reward and motivation for food is similarly regulated by striatal DA signaling. As mentioned previously, NAc DA is increased in response to food reward and food paired cues, and is particularly enhanced when a behavioral response is required for reward delivery (Roitman et al., 2004; McCutcheon and Roitman, 2017). Responding for food on the FI reinforcement schedule specifically is reduced by blockade of NAc DA

receptors (Cory-Slechta et al., 1997). Importantly, DA regulation of food reward is also sensitive to peripheral signals of energy-balance and physiological state (Cone et al., 2014; Woods et al., 2016). Work in this area can then serve as an example of how to link peripheral mechanisms that signal changes in reproductive state and DAergic regulation of motivated behaviors.

DA signaling is also implicated in adaptive attentional shifts similar to what I observed during failed trials. Shifts in behavior following reward omission on a task developed to model foraging behavior is dependent on changes in occupancy of D2 DA receptors (Porter-Stransky et al., 2013). Importantly, the expression of high vs. low affinity D2 receptors is altered during the estrous cycle, and although there was no effect of estrous cycle on the foraging task described above, this may still provide a link between the effects of ovarian hormones on DA systems and DA dependent shifts in adaptive choice.

While it is clear that the behaviors under investigation in this dissertation are regulated by DA signaling, and that DA circuitry is sensitive to changes the levels of circulating ovarian hormones, I have not established a causal link between hormonal regulation of DA release and the coordination of motivation for sex and food. Research to fill this gap will be crucial to provide a link between literature on sex differences in addiction and sex-specific regulation of adaptive motivated behaviors.

In Chapter 2, I demonstrate an acute effect of estradiol on stimulated DA release after cocaine treatment in females but not males. In order to link this with the findings presented in Chapters 3 and 4, it would be helpful to determine whether hormone priming with estradiol and progesterone similarly alters the effects of cocaine on DA release.

The finding that males treated with EB show an attenuated decrease in reuptake following cocaine administration was perhaps the most surprising result of the experiments presented in Chapter 2. However, perhaps this should be less surprising considering the paucity of work evaluating sex differences in the effects of estradiol and drugs of abuse. Still, these results should be interpreted with caution, as males treated with EB only show a reduced effect of cocaine on reuptake when compared to females treated with EB and not in comparison to same sex control animals.

A further question of the physiological relevance of the effect of estradiol in castrated males must also be considered. In males, testosterone that is aromatized to estradiol is important in activating sex-specific behaviors, and many neural circuits are responsive to estradiol in males (Seredynski et al., 2015; Oberlander and Woolley, 2016; Schulster et al., 2016). However, control of testosterone release is not under the same cyclic regulation as estradiol is in females, but instead is thought to increase in response to specific ethologically relevant stimuli (Wingfield et al., 1990). Whether or not these changes in the effect of cocaine on DA reuptake are relevant for understanding how testosterone and estradiol control motivated behaviors in males is unclear, particularly since the castrated males used in these studies had low basal levels of testosterone that further remove them from physiological comparisons.

It is possible that, while males may not show cyclic regulation of motivation for natural rewards like food or sex, gonadal hormones regulate a shift in motivation across developmental stages. In hamsters, increased testosterone release during the transition to adolescence induces the maturation of sexual reward by facilitating recruitment of mesolimbic DA circuitry (Bell and Sisk, 2013; Bell et al., 2013). Future work evaluating

if this shift in motivation is accompanied by changes in motivation for alternate rewards would be useful in providing a comprehensive understanding of how gonadal hormones regulate adaptive behaviors in both sexes.

Maternal behavior and estradiol regulation of motivation

Although I have proposed here that estradiol regulation of DA release and motivation serves to guide adaptive choice across the estrous cycle, this is not the only possible reason that females evolved a DA system that is sensitive to changes in ovarian hormones. Another important motivated behavior that is sensitive to changes in ovarian hormones is maternal behavior. In female rats, hormone release during pregnancy and parturition induces changes in the neural circuitry underlying maternal behaviors. Release of estradiol and progesterone during pregnancy prime the female to respond to a surge of estradiol and prolactin at the time of birth which, combined with sensory stimulation from the pups, results in rapid initiation of maternal behavior (Rosenblatt et al., 1988). Importantly, striatal DA circuitry is crucial in the expression of these maternal behaviors, to the extent that activation of D1 receptors within either the MPOA or NAc can induce maternal behavior in the absence of estradiol (Stolzenberg et al., 2007; Numan and Stolzenberg, 2009). It is then possible that facilitation of DA activity by gonadal hormones acts to increase motivated behaviors related to offspring care, as well as allow for plasticity within motivational systems to maintain maternal behaviors in the absence of gonadal hormones during later motherhood.

Future directions

As is to be expected, the results presented here raise more questions than provide answers. Where in the brain do estradiol and progesterone act to induce shifts in choice

and motivation for food vs sex? Are DA receptors within the NAc or dorsal striatum important in regulating this shift and if so, how do estradiol mediated changes in DA receptor expression contribute to this? Are their specific neural circuits or projections that are activated in unprimed or primed females? How is this shift in behavior relevant for understanding sex differences in the response to drugs of abuse?

An important first step would be to identify patterns of estradiol and progesterone receptor expression in females across various reproductive states. There is a surprising lack of research in this area, in part due to technical challenges associated with differentiating between ERα and ERβ, which show substantial homology at both DNA and ligand binding domains (Delaunay et al., 2000). However, as these issues begin to be addressed and new technologies are introduced, it will be important to apply them to not only understand sex differences in receptor expression, but also how expression changes over the course of the estrous cycle.

In particular, localization of specific brain areas that show changes in progesterone receptor expression after estradiol priming could identify specific targets for future pharmacological manipulation. It will also be of interest to determine the projection systems involved in regulating both behaviors, which may be differentially recruited in ovariectomized and hormone primed females.

Resolving the similarities and differences in how striatal DA circuitry regulates both innate and learned motivated behaviors will be crucial in developing a comprehensive understanding of this system. Further, in a system that shows substantial sex differences at both behavioral and neurobiological levels of analyses, it is particularly important to understand its significance in both males and females.

Conclusion

A comprehensive understanding of how neural circuits produce complex behaviors requires an appreciation of both the underlying mechanisms as well as the selection pressures that produced the system(s) of interest. Here, I present a series of experiments investigating not only the receptor mechanism by which estradiol alters DA signaling in females, but also a potential explanation for why the DA system is sensitive to changes in levels of circulating ovarian hormones. Taken together, this work underscores the importance of integrating both proximate and ultimate levels of explanation in the study of sexually differentiated behaviors.

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