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THE EFFECT OF EARLY LIFE PHOTOPERIOD MANIPULATION ON COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN MALE AND FEMALE JAPANESE QUAIL

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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Arts and Sciences at the University of Kentucky

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ABSTRACT OF THESIS

THE EFFECT OF EARLY LIFE PHOTOPERIOD MANIPULATION ON COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN MALE AND FEMALE JAPANESE QUAIL

Estrogens seem to play a role in the locomotor activating effects of cocaine. Japanese quail provide a good model for hormonal manipulation as alterations of their photoperiod controls hormone levels. The current study aims to examine the role of early life photoperiod manipulation in cocaine-induced behavioral sensitization in quail. It was expected that if quail were raised on a short photoperiod, they would have a reduction in gonadal hormones and this reduction in hormones would affect the acquisition of cocaine-induced behavioral sensitization. Quail were raised on an 8L:16D or a 16L:8D light cycle. Following 2 days of habituation, quail were administered saline, 5 mg/kg, or 10 mg/kg cocaine for 10 days. Restricted photoperiods in early life were correlated to lower gonadal hormone levels in females and males. Male quail raised on the short-light cycle developed a sensitized response to 10 mg/kg cocaine. Female quail raised on the short- or long-photoperiod developed behavioral sensitization to 5 mg/kg cocaine. Furthermore, early life reduction in estradiol in females modulated the amount of activity on day 10 of cocaine treatment. The current research extends previous research by finding a possible early life gonadal hormone control of behavioral sensitization in the quail.

KEYWORDS: Cocaine, Japanese quail, behavioral sensitization, addiction, hormones

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July 20, 2018

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Dedicated to my family

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Model of Psychostimulant Use Disorder

Psychostimulants are highly addictive and the illicit use of stimulants has increased recently after a decade of stable use (Hughes, Williams, Lipari, Van Horn, 2016). Nonmedical use of stimulant medication and illicit stimulant use may cause adverse effects, such as loss of appetite, sleep disturbances, anxiety, pulmonary disease, and premature death (Degenhardt et al., 2011; Williamson et al., 1997). There were more cocaine deaths in 2015 than any of the years in the previous decade (Hughes et al., 2016).

Behavioral sensitization is a behavioral phenomenon that results from frequent stimulant use. As opposed to drug tolerance, behavioral sensitization is an increase in responding following repeated administration of psychostimulants (Akins & Geary, 2008; Post, Lockfeld, Squillace, & Contel, 1981; Strakowski & Sax, 1998). Behavioral sensitization is also thought to be an associative learning process, when animals receive an injection of a psychostimulant in a novel environment, they do not always exhibit an increase in responding following repeated treatment (Post et al., 1981). Studying behavioral sensitization may help researchers understand the behavioral and underlying neurobiological changes associated with drug dependence.

Although the number of studies examining behavioral sensitization in humans is limited, there is some evidence implicating the role of behavioral

sensitization in drug-seeking behaviors. In one study, individuals had increased mood and eyeblink rates following treatment with amphetamine (i.e., 0.25 mg/kg), twice daily for four days (Strakowski, Sax, Setters, & Keck, 1996). Similarly, when amphetamine was administered once daily for six days, participants had increased motor activity and eye-blink rates at the end of the six days (Strakowski & Sax, 1998). Taken together these data demonstrate the development of psychostimulant-induced behavioral sensitization in human subjects.

In animals, behavioral sensitization can be assessed by comparing increased activity from the first to the last treatment (Hu & Becker, 2003). Behavioral sensitization can also be assessed by administration of a challenge dose and comparing the resulting activity of animals that received a stimulant repeatedly to those that only received the acute challenge. Following repeated stimulant treatment, animals display increased locomotor activity and stereotypy when administered a challenge dose of a psychostimulant (Nishikawa, Mataga, Takashima, & Toru, 1983). The increase in behavioral responsiveness is related to changes in the reward pathway of the brain. These changes occur in both rodents and drug users (Henry, & White, 1991; Lecca, Cacciapaglia, Valentini, Acquas, & Di Chiara, 2007; Nishikawa et al., 1983; Volkow et al., 1997).

Biochemistry of Addiction

The biological basis of use disorders has been considered and studied for the better part of the last century. Many theories of stimulant use disorder focus on the monoaminergic systems. Monoamines such as dopamine (DA), serotonin

(5-HT), and norepinephrine (NE) are the primary neurotransmitters (NT) of interest in drug use disorders (for review, see Howell & Kimmel, 2008; Kalivas & Volkow, 2005). Of the monoamines, DA is the primary NT typically focused on in psychostimulant use disorder. DA, like NE, is a catecholamine and is the precursor to NE. Catecholamines are monoamines that have a catechol and single amine. DA has many functions in the brain such as the regulation of movement, hormone secretion, emotion, and reward (for reviews see, Berridge, 2007; Nieoullon & Coquerel, 2003; Puig, Rose, Schmidt, & Freund, 2014; Wickens, Reynolds, & Hyland, 2003; Wise, 2004). The addictive properties of psychostimulants have been linked to an increase of activity in the DAerigic system. When DA receptors are blocked, stimulant-induced behavioral sensitization can be reduced (Kuribara & Uchihashi, 1993).

Dopamine Synthesis. Dopamine production mainly occurs in two areas of the brain stem, the ventral tegmental area (VTA) and the substantia nigra (see Kalivas, 1993 for review). Dopamine is synthesized in several steps. First, L-tyrosine, a non-essential amino acid, is converted to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase, the rate-limiting step in DA production (Elsworth, & Roth, 1997). L-DOPA is then catalyzed into DA by aromatic amino acid decarboxylase. DA is transported and stored in synaptic vesicles and is released via calcium dependent exocytosis (Elsworth, & Roth, 1997). Following release, DA is cleared away by the dopamine reuptake transporter (DAT; Iversen et al., 2009).

Dopaminergic Pathways. DA is localized to four DAergic pathways, including the nigrostriatal, mesolimbic, mesocortical, and the tuberoinfundibular (Andén et al., 1964; Björklund, Moore, Nobin, & Stenevi, 1973; Swanson, 1982). The tuberoinfundibular pathway has projections from the hypothalamus to the posterior pituitary and is primarily involved in regulating the secretion of hormones (Björklund et al., 1973). The nigrostriatal pathway originates in the substantia nigra and terminates in the striatum. The nigrostriatal pathway is mainly involved in the regulation of motor movement. Both the mesocortical pathway and mesolimbic pathways include cells that originate in the VTA (Swanson, 1982). However, the mesocortical pathway has projections to the prefrontal cortex and is involved in the regulation of motivation, whereas the mesolimbic pathway is involved in reward and projects to the nucleus accumbens (see Koob, 1992 for review).

Dopamine receptors. Dopamine exerts its effect by binding to, and activating, dopamine receptors. There are a total of five different dopamine receptors that are all G-protein coupled receptors (GPCR) (see Vallone, Pircetti, & Borrelli, 2000 for review). These five different receptors are categorized into two families based on the type of G-protein attached to them. D1-like receptors are coupled to Gs and D2-like are coupled to Gi. D1-like includes both the D1 and the D5 receptor, whereas D2-like includes D2, D3, and D4 receptors. When activated, the D1 receptors increase adenylyl cyclase which promotes the production of cyclic adenosine monophosphate (cAMP) and has downstream effects on calcium channels, CREB, and other intercellular signaling pathways

(Monsma, Mahan, McVittie, Gerfen, & Sibley, 1990). D2 receptors, on the other hand, inhibit adenylyl cyclase (Yan, Feng, Fienberg, & Greengard, 1999; see Beaulieu & Gainetdinov, 2011 for review).

Addiction research has mainly focused on the roles of D1-like and D2-like receptors in the striatum and nucleus accumbens (NAcc) of the mesocorticolimbic pathway. Both D1-like and D2-like receptors have been associated with addiction (Khroyan, Barrett-Larimore, Rowlett & Spealman, 2000). Rats with fewer striatal D2 receptors were shown to be highly impulsive and self- administer more cocaine (Dalley et al., 2007). Agonists of both receptor families have reinforcing effects (Ikemoto, Glazier, Murphy, & McBride, 1997; White, Packard, & Hiroi, 1991). However, D1-like and D2-like receptors may have differing roles in the brain and in stimulant addiction. For example, elevated levels of c-Fos were discovered in rodents that were administered cocaine, but this effect was dependent on D1 receptors and not D2 receptors (Drago, Gerfen, Westphal, & Steiner, 1996; Young, Porrino, & Iadarola, 1991). Rodents treated with either D1 or D2 antagonists, in addition to cocaine, did not acquire behavioral sensitization (Fontana, Post, Weiss, & Pert, 1993). However, other research indicated administration of D1 and D2 antagonists attenuated locomotion but not behavioral sensitization (Mattingly, Hart, Lim, & Perkins, 1994). Following the development of cocaine-induced behavioral sensitization, only D1 antagonists have been shown to attenuate the expression of behavioral sensitization while D2 antagonists did not (Cabib, Castellano, Cestari, Filibeck, & Puglisi-Allegra, 1991; Sorg, Li, & Wu, 2001).

Dopaminergic sex differences. Sex differences in DAergic activity in the striatum appear to underlie many differences in substance use between males and females. To measure DAergic differences, researchers administered a DA radioligand to visualize DA activity using positron emission tomography [PET; (Murno et al., 2006)]. At baseline, there were no gender differences in striatal DA binding. However, there were baseline differences observed in women at different points in their menstrual cycle; women in the follicular phase had greater binding than those in the luteal phase. Despite these differences in DA binding, there were no differences in DA release in women at any point in their menstrual cycle. There was, however, a gender difference in DA release following administration of a 0.3 mg/kg dose of amphetamine. Men had greater DA release in the striatum than women following the amphetamine treatment (Murno et al., 2006). In addition to less DA released in women, neuroimaging research revealed women had a lower D2 affinity compared to men, perhaps due to higher endogenous levels of DA (Pohjalainen, Rinne, Någren, Syvälahti, & Hietala, 1998). This gender difference in endogenous DA levels was supported by other imaging studies that showed both higher DA synthesis and dopamine transporter (DAT) in the striatum of women compared to men (Laakso et al., 2002; Lavalaye, Booij, Reneman, Habraken, Royen, 2000; Mozley, Gur, Moxley, & Gur, 2001; Staley et al., 2001).

In rodents there are sex differences in DAT density, DA release, and production of DA receptors. Striatal DAT density was higher in gonadally intact female rodents compared to male rats and ovariectomized females, and DAT

density appeared to peak during the proestrus phase of the estrus cycle (Morissette & Di Paolo, 1993). Additionally, amphetamine treatment caused a more robust release of DA in females than in males (Becker, 1990b). This increase in DA released by females appeared to be modulated by estradiol (E2), but E2 treatment did not affect DA in males (Becker, 1990; Peris, Decambre, Coleman-Hardee, & Simpkins, 1991). In rodents, E2 appears to exert its effects both genomic (i.e., transcription) and nongenomic mechanisms (i.e., surface receptors, phosphorylation, and intercellular signalling). Specifically E2 is thought to increase tyrosine hydroxylase protein by classical genomic changes resulting in increased protein production (Ivanova & Beyer, 2002). However, increased DA release is thought to result from E2 interacting with surface receptors on GABAeric neurons producing an inhibition of GABAeric signalling within the striatum (for review see, Yoest, Quigley, & Becker, 2018). Furthermore, castration had no effect on DA levels in the striatum or VTA of male rodents (Castner, Xiao, & Becker, 1993; Russo et al., 2003a). Therefore, gonadal steroid hormones seem to have sexually dimorphic roles in DA release.

Similar to adult DAerigic sex differences, rodents also exhibit a sex differences throughout development. According to Andersen & Teicher (2000), adolescent male rats had a four-fold increase in DA receptors in the striatum and NAcc during development, whereas females did not overproduce DA receptors to the same extent. Although developing rodents had a difference in DA receptor density, male and female adult rodents appeared to have similar overall levels of D2 receptors and similar DA receptor levels in the striatum. However, once rats

reached adulthood, males had higher levels of D1 receptors in the NAcc than females (Andersen & Teicher, 2000). Sex differences in rodents emerged in functional receptor variation between the two sexes. Both male and female rodents treated with 0.10 and 0.25 mg/kg of SCH 23390, a D1 antagonist, prior to cocaine administration did not develop a preference for a drug-paired chamber (Nazarian, Russo, Festa, Kraish, & Quinones-Jenab, 2004). However, when treated with a 0.50 mg/kg dose, the conditioned place preference (CPP) was only blocked in males (Nazarian et al., 2004). When male and female rodents were treated with D1 and D2 agonists, females exhibited more locomotion than males (Schindler & Carmona, 2002). Additionally, females were more sensitive to a low dose (i.e., 0.03 mg/kg) of SCH 23390 than males. Treatment with this low dose resulted in a reduction in cocaine-induced locomotion in females only (Schindler & Carmona, 2002). Taken together, there are sex differences in DA receptors and receptor function across ontogeny

Although there are some DAergic sex differences in rodents, there have been few experiments that directly compare male and female DAergic systems in Japanese quail. Ball and colleagues (1995) observed no sex differences in D1-like receptors in any area of the quail brain. However, male quail had higher DA turnover compared to female quail in the POA (Balthazart et al., 1990). Furthermore, there were differences in DA content in the right telencephalon of male and female quail (Ottinger at al., 1986). Specifically, adult males had more DA in the right telencephalon than female quail (Ottinger at al., 1986). Other sex

differences in related DAerigic activity differences remain largely unexplored in quail.

Overall quail have a similar DAergic system compared to the rodents DAergic system, however, there are some slight differences in receptor ratios. Quail have a similar neural distribution of DA and have similar D1-like, and D2-like receptors compared to rodents (Ball, Casto, & Balthazart, 1995; Levens, Green, Akins, & Bardo, 2000). Kubíková, Výboh, and Košťál, (2009) examined DA affinity, association, and dissociation rates using D1 and D2 antagonists in the quail brain and observed DA receptors to be pharmacologically homologous to other species, including rodents and humans. However, there are some species differences in DA receptors in mammals and birds. Quail have a higher ratio of D2:D1 receptors in the striatum than rats (Kleitz, Cornil, Balthazart, & Ball, 2009). Additionally, unlike mammals, sauropsids, including quail, do not have a DAT (Lovell, Kasimi, Carleton, Velho, & Mello, 2015). It is theorized that the norepinephrine transporter (NET) removes most of the DA from the synapse in birds. Despite these receptor and transporter differences between rodents and aves, there have been many similarities between behaviors exhibited following psychostimulant administration, including behavioral sensitization and a preference for a drug-paired environment (Levens & Akins, 2001; Gill, Reynolds, Prendergast, & Akins, 2016; Rosine, Bolin, & Akins, 2009).

Cocaine

Cocaine is derived from the leaves of the coca plant. It is a popular CNS stimulant that is often smoked, insufflated, or injected; and it is the second most

widely used illicit drug following cannabis (Cone, Tsadik, Oyler, & Darwin, 1998; Karila et al., 2014). Frequent use of cocaine is associated with many health problems including greater susceptibility to HIV, psychotic disorders, cardiovascular disease, and premature death compared to the general population (Baum et al., 2009; Darke, Kaye, & Duflou, 2006; Degenhardt et al., 2011; Karch & Billingham, 1988; Satel, Southwick, & Gawin, 1991).

Cocaine exerts its stimulating effects by inhibiting the reuptake of 5-HT, NE, and DA. Cocaine binds to the reuptake transporter on the presynaptic neuron forming a complex that cannot remove neurotransmitter from the synapse (Iversen, Iversen, Bloom & Roth, 2010). As a result of the reuptake inhibition, synaptic levels of these monoamines accumulate in the synaptic cleft. Cocaine has an equal affinity for DAT, NET, and SERT in both humans and rodents (Han & Gu, 2006). Increasing DA levels in the mesolimbic pathway and the nucleus accumbens is thought to lead to the rewarding effects of cocaine (Thomas, Kalivas, & Shaham, 2008).

In mammals, cocaine has a short half-life of about 1.5 hours and is mainly metabolized by cholinesterases in the liver (Jufer, Wstadik, Walsh, Levine, & Cone, 2000). Hepatic enzymes metabolize cocaine into norcocaine, benzoylecgonine, and methyl ester (Mofenson & Caraccio, 1987). Most metabolites and some unchanged cocaine are excreted in the urine (Hamilton et al., 1977; Jufer et al., 2000). Although the chemical metabolism has not been assessed in quail, the behavioral effects appear to peak within the first hour after administration (Geary & Akins, 2007).

Sex differences of the effects of cocaine. Research indicates that men and women experience differences in subjective effects following cocaine use. In women, estrus cycle altered subjective effects of smoked cocaine (Sofuoglu et al., 1999). Women in the luteal phase reported lower ratings of how high they felt compared to females in their follicular phase and men. Overall, women rated lower on a self-reported measure of how "high" they felt, had lower ratings of heart racing, and rated themselves as feeling less stimulated compared to men (Sofuoglu et al., 1999). Similarly, Evans, Haney, and Foltin (2010) found that sex differences only started to emerge when males were compared to females in their luteal phase.

Adult females tend to be more sensitive to the rewarding and locomotor activating effects of cocaine than their male counterparts (McDougall, Eaton, Mohd-Yusof, & Crawford, 2015; Lynch, & Carroll, 1999; Van Haaren & Meyer, 1991). Female rodents have a greater propensity to self-administer cocaine and they develop a preference for a cocaine-paired environment faster and at lower doses than males (Lynch, & Carroll, 1999; Russo et al., 2003b). These enhanced responses in females do not appear to be due to a sex difference in the metabolism rate between males and females, but instead are related to gonadal hormone levels (Bowman et al., 1999). Specifically, gonadal hormones, such as E2, enhance the rewarding effects of cocaine. Ovariectomized rodents treated with E2 spend more time in a drug-paired environment compared to ovariectomized rats without E2 treatment (Segarra et al., 2010). Similarly,

female quail with elevated E2 levels spent more time in a drug-paired environment than female quail with low E2 levels (Gill et al., 2016).

Cocaine-induced behavioral sensitization in animal models

As with other stimulants, repeated use of cocaine results in behavioral sensitization (Hu & Becker, 2003; Zavala, Nazarian, Crawford, & McDougall, 2000). Rodents that received cocaine repeatedly have increased locomotion as measured by distance traveled, rotations, and increased stereotypy (Hu & Becker, 2003; Post et al., 1981). However, many factors can affect the rate of acquisition and expression of cocaine-induced behavioral sensitization, including environmental cues, age, and sex (Bowman & Kuhn, 1996; Hu & Becker, 2003; Laviola, Wood, Kuhn, Francis, & Spear, 1995; Post et al., 1981; Ujike, Tsuchida, Akiyama, Fujiwara, & Kuroda, 1995). Older animals and female rodents tend to be more sensitive to the enhanced behavioral effects of cocaine following repeated administration (Laviola et al. 1995; Zakharova, Wade, & Izenwasser, 2009). However, locomotor changes across ontogeny may be due to physical development as adults move more than preweanlings or adolescent animals since adolescent animals have an overproduction of DA receptors (Andersen & Teicher, 2000; McDougall et al., 2015).

Increased DA release is not necessary for cocaine-induced behavioral sensitization. Instead, sensitization has been related to changes in G-proteins and intracellular signaling that occurs following an increase in activation of D1 receptors and some of these cascades are involved in synaptic plasticity (for review see; Calabresi, Picconi, Tozzi, & Di Filippo, 2007). The sensitizing effect

of cocaine can be altered by administration of haloperidol, a DA receptor antagonist (Mattingly, Rowlett, Ellison, & Rase, 1996). Additionally, D1 deficient mice did not develop a sensitized response to repeated injections of amphetamines (Crawford, Drago, Watson, & Levine, 1997). Furthermore, treatment with glutamatergic NMDA receptor antagonists blocked the development of sensitization in rodents.

Female rodents display more behavioral responsiveness to repeated treatments with cocaine than males (Cailhol, & Mormede, 1999; Glick & Hinds, 1984; Hu & Becker, 2003). This increased sensitivity to cocaine seems to be in part due to female gonadal hormones (Hu & Becker, 2003; Peris, et al., 1991). In one study, an ovariectomy caused a reduction in gonadal hormones, which in turn, caused a blunted response to daily cocaine treatments compared to gonadally intact female rats. The blunted response in ovariectomized female rats was similar to the response observed in male rodents. However, when ovariectomized rats were treated with E2, they responded similarly to non-ovariectomized rats. Conversely, castration had no effect on sensitization compared to sham-operated males (Hu & Becker, 2003). Although others have demonstrated castration and administration of T did alter cocaine- induced behavioral sensitization (Menéndez-Delmestre & Segarra, 2011). In rodents it appears that female gonadal hormones modulate cocaine-induced behavioral sensitization, whereas, it is unclear the role male gonadal hormones may play in sensitization.

In addition to gonadal hormones, a variety of different genes affect cocaine responding in rodents. Among possible genes that affect cocaine-induced sensitization are genes responsible for cellular proliferation, androgen receptor genes, circadian rhythm genes, and x-chromosome linked genes that code for α3 subunit -3 (Phillips, Huson, & McKinnon, 1998). Genes that code for proteins responsible for cellular proliferation have been implicated in the acquisition of cocaine-induced behavioral sensitization (Hiroi et al., 1997; Ujike, Takaki, Kodama, & Kuroda, 2006). One such gene examined is the FosB gene. Researchers discovered that FosB mutant mice (-/-) did not develop cocaine-induced behavioral sensitization (Hiroi et al., 1997). Similarly, Kelz et al. (1999) measured FosB expression in the NAcc of mice and found that mice with increased FosB had an increase in behavioral responding and more locomotion. Additionally, circadian rhythm genes may play a role in the development of cocaine-induced sensitization (Abarca, Albrecht, & Spanagel, 2002). Previous research has shown that mice without the circadian rhythm gene mPer1 had increased behavioral responding following acute cocaine administration but did not develop cocaine-induced behavioral sensitization following repeated cocaine administration. In contrast, mice lacking the mPer2 gene had an increased sensitized response following repeated cocaine administration (Abarca et al., 2002).

Japanese quail as an animal model. One of the main issues for an individual recovering from a drug use disorder is the propensity to relapse. One theory of relapse is environmental cues that have been previously associated

with drug taking behaviors cause craving (Kosten et al., 2006). In people these cues are often visual, therefore, it would be appropriate to use a highly visual animal model. Rodents acquire most of their information about their environment from olfaction. Lab rodents tend to be nocturnal and have poor vision. Rodents only have two cones in their retina and in albino lab rodents only 1% of their retina is made up of cones (Szél & Röhlich, 1992). However, quail are a highly visual species and are tetrachromatic, meaning they have four cones (Bennett & Théry, 2007; Bowmaker, Kovach, Whitmore, & Loew, 1993). Therefore, quail make a suitable model for studying drug use disorders.

Aves are unique in their expression of sex chromosomes. In birds, the male is the homogametic sex possessing two Z chromosomes (ZZ) whereas the females are heterogametic (ZW). Further, unlike female mammals, which undergo an x-inactivation in order to maintain similar levels of X-linked gene expression between male (XY) and female (XX) mammals, male aves do not undergo a Z chromosome inactivation (Kuroiwa et al., 2002). Because male birds do not inactivate one of their Z chromosomes, males have a biallelic expression of Z-linked genes (Itoh et al., 2007; Kuroiwa et al., 2002). This unique expression makes birds one of the most sexually dimorphic animals, and therefore makes them a good model to study sex differences.

Quail may also be a good model for examining the role of endocrinology in drug use disorders. Changing the amount of light exposure a quail receives has been shown to increase (i.e., with longer light exposure) or decrease (i.e., with shorter light exposure) gonadal hormones, luteinizing hormone, and follicle

stimulating hormone (Adkins & Nock, 1976). As previous research has shown, gonadal hormones influence drug seeking and the rewarding value of drugs (for review see, Becker & Hu, 2008). Thus when examining the role of gonadal hormones in drug use disorders, quail may be an ideal model to study.

Similar to rodents, Japanese quail develop an enhanced behavioral response to repeated administration of cocaine (Akins & Geary, 2008; Geary & Akins, 2007; Gill et al., 2015; Levens & Akins, 2001). Following 10 days of repeated cocaine administration, quail had increased locomotion, and more photobeam beam breaks compared to saline treated quail (Akins & Geary, 2008; Gill et al., 2015). Similar to rodents, cocaine-induced behavioral sensitization persisted for weeks in quail (Akins & Geary, 2008). Quail exposed to a challenge dose of cocaine 14 days after their last treatment continued to exhibit the locomotor enhancing effects of cocaine (Akins & Geary, 2008).

Unlike female rodents, female quail do not show a greater sensitivity to the effects of cocaine. In contrast, female quail fail to develop cocaine-induced behavioral sensitization, whereas male quail have been shown to display a robust cocaine-induced sensitization response (Gill et al., 2015). The cocaine-induced locomotion observed in male quail was positively related to testosterone (T) levels, but E2 was not related to cocaine-induced locomotion in female quail. Although female quail with high gonadal hormones did not develop a cocaine-induced behavioral sensitization, they did develop a place preference for a cocaine-paired chamber (Gill et al., 2016). Taken together, E2 may be related to the rewarding effects of cocaine, but in adult female quail.

Chapter 2

The effect of early life photoperiod manipulation on cocaine-induced behavioral sensitization in male and female Japanese quail

Introduction

Cocaine use and deaths from overdose have been on the rise in recent years. According to the SAMHSA, cocaine use may be on the rise after about ten years of steady rates of cocaine use (Hughes, Williams, Lipari, Van Horn, 2016; Johnston et al., 2018; Staff et al., 2010). In 2011, about ½ a million emergency room visits involved the use of cocaine (Crane, 2013). In 2015, there was a 26% increase in new cocaine users and, along with the rise in use, the number of cocaine deaths in the US was at the highest it had been since 2006 (Hughes et al., 2016; NIDA, 2016).

Males and females differ in their subjective responses, their sensitivity to cocaine, how much they self-administer cocaine, as well as the rate and extent to which they develop behavioral sensitization to cocaine (Hu & Becker, 2003; Jackson, Robinson & Becker, 2006; Lynch & Carroll, 1999; McDougall, Eaton, & Mohd-Yusof, 2015). Previous research has indicated that gonadal hormones may play a role in the development of these sex differences. Ovariectomized (OVX) female rats developed a cocaine-induced behavioral sensitization at a slower rate, and to a lesser extent, than OVX animals who received estradiol (E2) injections (Hu & Becker, 2003). In humans, men and women had similar subjective responses to cocaine when women were in the luteal phase of their menstrual cycle when E2 levels are low, and progesterone levels are high

(Evans, Haney, & Foltin, 2002; Sofuoglu, Dudish-Poulsen, Nelson, Pentel, & Hatsukami, 1999). However, when women used cocaine in the follicular phase of their menstrual cycle, they had an increased subjective response compared cocaine use during their luteal phase, when E2 levels are high, and progesterone levels are low (Evans et al., 2002).

Japanese quail are a beneficial animal model to examine the role of hormones as manipulating their gonadal hormones does not require castration or ovariectomy. Gonadal hormone levels can be altered by modifying the photoperiod, or how long the quail are exposed to light in the laboratory. Longer photoperiods have been shown to produce increased levels of gonadal hormone and shortened photoperiods resulted in reduced hormone levels, essentially a photocastration of the animals (Adkins & Nock, 1976; Balthazart, Massa, & Negri-Cesi, 1979; Brain, Onagbesan, Peddie, & Taylor, 1988). Quail that were photoregressed for three weeks had similar T and E2 levels as quail that underwent a surgical gonadectomy (Adkins & Nock, 1976).

Cocaine-induced behavioral sensitization has been observed in quail (Akins & Geary, 2008; Geary & Akins, 2007; Gill et al., 2015; Levens & Akins, 2001). Male quail developed a sensitized response to repeated administration of 10 and 20 mg/kg doses of cocaine (Akins & Geary, 2008; Gill et al., 2015). However, female quail failed to develop cocaine-induced behavioral sensitization unless they had been previously photoregressed (Gill et al., 2015). Female quail housed on a short-light cycle developed a sensitized behavioral response to 10 mg/kg but not 20 mg/kg dose of cocaine. Gill and colleagues (2015) did not find

a relationship between cocaine-induced activity in female quail and their E2 levels despite the animals being photoregressed. However, in another experiment, high E2 levels were related to an increase in cocaine-induced conditioned place preference (Gill, Reynolds, Prendergast, & Akins, 2016). Therefore, female quail with high E2 levels found the effects of cocaine rewarding but failed to display the locomotor enhancing effects of cocaine.

In a previous study, 8 month old quail were raised on a long-light cycle for 7 months prior to photoregression (Gill et al., 2015). Starting three weeks before conditioning, quail were randomly selected for long-light and short-light cycles. Because Gill et al. (2015) reduced hormonal levels post-puberty, the organizational effects of E2 may have already occurred, and therefore reduced E2 in adulthood may have no direct effect on the quail and the development of cocaine-induced behavioral sensitization. In the current study, it was theorized that the relationship between E2 and sensitization would emerge when early gonadal hormones were controlled. Therefore, quail were photo-stimulated or regressed from hatch. The current research aimed to extend previous research to younger animals raised from hatch on altered light-cycles. Additionally, the research aims to correlate the degree of cocaine-induced behavioral sensitization with gonadal hormones. Overall, it was expected that photoregression beginning in early life might reduce the neural rewiring occurring at puberty, which may have an effect on cocaine-induced behavioral sensitization. In line with previous research, it was expected that males raised on the photostimulated condition would develop cocaine-induced behavioral sensitization (Akins & Geary, 2008;

Geary & Akins, 2007). Similar to Gill et al. (2015), I expected females raised on the short-light photoperiod to display cocaine-induced behavioral sensitization, whereas females raised on the long-light photoperiod were not expected to show any behavioral sensitization. It was also expected that estradiol would be correlated to cocaine-induced behavioral sensitization because of the early life photoperiod manipulation.

Methods

Subjects

Subjects were male (n=48) and female (n=41) Japanese quail, *Coturnix japonica.* Fertilized eggs were purchased from GQF Breeding Technology (Savannah, GA) and hatched at the University of Kentucky. Immediately following hatch, chicks were randomly selected and separated into long and short photoperiod conditions. Those housed in the long-light conditions were raised on a 16:8 L:D cycle whereas those housed in the short-light cycle were exposed to 8:16 L:D. These hours were chosen as previous research has concluded that an 8:16 L:D cycle reduced gonadal hormone levels to gonadectomy levels (Adkins & Nock, 1976). Animals were group housed in mixed sex brooders until post-hatch day (PHD) 28. They were then separated, and females were group housed in groups of 3-5 and males were single housed in wire mesh cages (GQF Manufacturing, Savannah, GA). Food and water were available *ad lib*. Behavioral testing took place in a separate experimental room during the light phase of the animals light cycle. All animals were cared for according to the “Guide for the Care and Use of Laboratory Animals” under a research protocol approved by the

Institutional Animal Care and Use Committee at the University of Kentucky (National Research Council, 2010).

Drugs

Cocaine hydrochloride was dissolved in saline (0.9%). Drugs were injected intraperitoneally (ip) at a volume of 1 ml/kg. Quail were administered saline, 5 mg/kg, or 10 mg/kg throughout conditioning trials.

Apparatus

Behavioral testing was performed in activity monitoring chambers (28.6 x 21.2 x 21.2 cm) that consisted of white acrylic walls, a transparent ceiling, and white textured paper covering the wire mesh flooring (ENV-013; Med Associates Inc., St. Albans, VT). The chambers had six photobeams approximately 6.4 cm apart and 3.2 cm from the floor. White noise was played throughout each phase of the experiment to reduce extraneous noise. A Med Associates program was used to collect photobeam breaks.

Procedure

On PHD 35 quail were injected with saline and allowed to habituate to the chambers for 1 hour, two days prior to conditioning. Once assigned to an activity chamber, that chamber was used for the entire experiment. Following habituation days, birds received an ip injection of saline, 5, or 10 mg/kg of cocaine before being placed into the activity chamber. Activity was recorded as beam breaks in 5 min time bins for the next hour. Trials were conducted once per day for 10 days.

All testing took place during the light cycle for the birds, and male and females were tested separately. Male and females were tested separately to ensure that vocalizations did not affect locomotor activity.

ELISAs

Similar to Gill et al. (2015), blood was collected from select male and female quail during the habituation period as well as the day following day 10 of behavior. Approximately 0.3 ml of blood was taken from the brachial vein and collected into heparinized tubes. Samples were centrifuged at 1500 rpm for 5 min. Plasma was collected and frozen at -80 C until assays were conducted.

Plasma testosterone (T) and estradiol (E2) were measured in duplicate using an enzyme linked immunoassay kit (DRG Diagnostics; testosterone EIA-1559, estradiol EIA-2693) as previously described by Gill et al. (2015). Briefly, 25 µl of the standards and samples were dispensed into individual wells. Enzyme conjugate (200 µl) was then added to all wells, and the plate was incubated at room temperature for 120 (E2) or 60 (T) min. The contents were then removed, and the plate was washed three times with 400 µl of wash solution. The substrate was then added and allowed to incubate again at room temperature for 15 min. The enzymatic reaction was stopped by adding the stop solution and the absorbance of each well was read at A_{450} using a microtiter plate reader (DTX 880 Multimode Detector, Beckman Coulter Inc., Fullerton, CA, USA).

Statistical Analysis

Weight data were analyzed with a 2 x 2 x 3 x 10 (sex x photoperiod x treatment x day) repeated measures analysis of variance (RM ANOVA). Some

previous studies have shown that cocaine dose can affect the weight of animals, but this finding is not consistent across studies as other studies show no effect of cocaine treatment on weight (Catlow & Kirstein, 2007; Torres-Reverón & Dow-Edwards, 2005).

For activity analyses, male and females were analyzed separately. This separation was done because males and females are fundamentally different, and therefore may perform differently under the different photoperiods. Additionally, E2 was only assessed in female subjects and T in male subjects and, therefore, gonadal hormones could not be compared across sexes.

For locomotor activity, the total number of beam breaks was recorded for one hour and a 2 x 3 x 10 (photoperiod x treatment x day) RM ANOVA was used to analyze the change in locomotion across all 10 days. To further probe interactions, independent ANOVAs and post hoc analyses were performed. A separate RM ANOVA 2 x 3 x 2 (photoperiod x treatment x day) was used to analyze behavioral sensitization by comparing whether activity on day 10 was greater than day 1 (Hu & Becker, 2003). When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Greenhouse-Geisser epsilon statistic was used to adjust degrees of freedom, and rounded to the nearest whole number (Geisser & Greenhouse, 1958).

Plasma E2 and T levels were analyzed as moderators in a regression analysis with cocaine dose predicting the total amount of beam breaks on day 10. Day 10 was selected because plasma was collected the day following day 10

and sensitization effects have been most evident on this day (Gill et al., 2015). For all analyses, statistical significance level was set at $p<.05$.

Results

Weight data

Figure 2.1 shows the mean body weights for male and female quail across conditioning days. Male quail and female quail had a progressive increase in body weight across days [main effect of day, $F(3,92)= 228.57$, $p<.05$]. Overall, females ($M= 152.38$ g, $SEM= 1.92$) on average weighed more than males (M= 137.32 g, $SEM= 1.83$) [main effect of sex, $F(1,81)= 58.32$, $p<.05$]. Light cycle similarly affected weight gain as quail raised on the long-light photoperiod ($M=$ 150.64g, $SEM= 1.85$) were heavier than those raised on the short-light photoperiod ($M= 139.06$, $SEM= 1.89$) [main effect of photoperiod, $F(1,81)=$ 36.35, $p<.05$]. Treatment with cocaine did not have an effect on weight, main effect of treatment, $F(2,81)= 1.78$, p=n.s. There were no other main effects. Female quail also gained more weight across days as revealed by a day x sex interaction [$F(3,208)= 66.35$, $p<.05$]. There was a significant interaction between photoperiod and sex on overall weight as females raised on the long-light photoperiod were heavier [$F(1,81)= 14.97$, $p<.05$]. Additionally, a significant interaction of day x photoperiod x sex revealed an effect on weight gain across days [$F(3,208)= 23.25$, $p< 0.05$]. However, there was no significant overall interaction between sex, lightcycle, treatment, and day [$F(5,208)= 0.26$, $p=$ n.s.].

Male locomotor analyses

Figure 2.2 shows the mean daily beam breaks across all 10 days for male quail raised on the long photoperiod (2.2A) and the short photoperiod (2.2B). Male quail had an overall increase in beam breaks across days [main effect of day, $F(6,240)= 3.82$, $p<.05$]. A main effect of treatment followed by a Tukey's analysis revealed male quail treated with 10 mg/kg cocaine ($M= 7124.24$, $SEM= 479.96$) or 5 mg/kg cocaine ($M= 5037.07$, $SEM= 339.39$) had more beam breaks overall than saline ($M= 3477.49$, $SEM= 479.96$) treated male quail, [$F(2,42)= 26.95$, $p<.05$, Tukey $p<.05$]. There were no other main effects detected. A significant day x treatment interaction indicated that cocaine treated male quail had more activity across days than quail treated with saline [$F(12,240)= 2.85$, $p<.05$]. There was no day x photoperiod x treatment interaction for male quail [$F(12,240)= 0.78$, $p=$ n.s.], therefore no further analyses were conducted.

To test for cocaine-induced behavioral sensitization, day 10 was compared to day 1 in male quail (figure 2.3A and 2.3B). An RM ANOVA revealed a day x treatment x photoperiod interaction [$F(2,43)= 3.16$, $p<.05$]. Further analyses were conducted on each photoperiod. An analysis with males raised on the short photoperiod revealed a significant day x treatment interaction [$F(2,22)= 5.41$, $p<.05$]. Within the short-light raised males there was no change in activity between days 1 and 10 in the saline treated quail [$F(1,7)= 4.39$, $p=$n.s.] or in the quail treated with 5 mg/kg cocaine [$F(1,6)= 0.78$, $p=$n.s.]. However, male quail raised on the short photoperiod that were administered 10 mg/kg had a significant increase in beam breaks between days 1 and 10 [$F(1,8)= 7.10$,

p<.05.]. There was not a significant day x treatment interaction for male quail raised on the long photoperiod [*F*(2,21)= 1.12, *p*=n.s.]; therefore, no further analyses were conducted.

Male hormone analysis

Although a linear regression established photoperiod length predicted T levels, [*F*(1,44)= 11.55, *p*< .05], there was no significant moderating effect of T on the relationship between cocaine dose and total beam breaks on the last day [ΔR^2 = .035, *F*(1,41)= 1.38, *p*=n.s.]. T levels increased significantly between the first blood draw day (*M*= 1.67 ng/ml, *SEM*= 0.26) and the last for quail raised on the long photoperiod (*M*= 2.45 ng/ml, *SEM*= 0.39), [*F*(1,9)= 6.75, *p*< .05], and those raised on the short photoperiod, [*F*(1,20)= 6.32, *p*< .05]. Male quail raised on the short-light photoperiod had significantly lower levels of E2 on both PHD 33 [*F*(1,32)= 4.35, *p*< .05] and PHD 47[*F*(1,46)= 12.03, *p*< .05] .

Female locomotor analysis

An RM ANOVA was used to analyze the mean beam breaks across all 10 days for female quail treated with saline, 5 mg/kg, or 10 mg/kg and raised on the long photoperiod and the short photoperiod (see figure 2.2). There was a main effect of day indicating an overall increase in beam breaks across days [*F*(4,138)= 2.61, p<.05]. A main effect of treatment followed by Tukey's revealed increased activity in female quail that received 10 mg/kg cocaine (*M*= 6257.96, *SEM*= 315.80) compared to 5 mg/kg cocaine (*M*= 4831.41, *SEM*= 293.99) and saline (*M*= 2896.10, *SEM*= 293.99) treated female quail, [*F*(2,37)= 39.27, *p*<.05, Tukey *p*<.05]. Female quail raised on the short-light photoperiod (*M*=4234.76,

SEM=193.58) had less activity than female quail raised on the long-light photoperiod (*M*= 5027.91, *SEM*= 209.64) [main effect of photoperiod, $F(1,37)= 7.33$, $p<.05$]. The RM ANOVA revealed an overall day x photoperiod x treatment interaction for female quail, [$F(8,138)= 1.89$, $p<.05$]. Further analyses were then conducted at each photoperiod.

Females raised on the long photoperiod had a significant day x treatment interaction [$F(8,69)= 3.00$, $p<.05$]. A main effect of treatment revealed significant differences between treatments in long-light raised female quail [$F(2,19)= 30.13$, $p<.05$]. Tukey's post hoc analyses revealed that quail treated with saline (*M*= 2738.49, *SEM*= 354.76) had less activity than quail treated with either cocaine dose. Additionally, long-light raised female quail treated with 10 mg/kg cocaine (*M*= 4992.56, *SEM*= 379.26) had more activity than 5 mg/kg cocaine (*M*= 4992.56 *SEM*= 354.76) treated animals (Tukey $p<.05$).

Females raised on the short photoperiod had a significant day x treatment interaction indicating a significant interaction of cocaine-induced activity across days [$F(8,64)= 2.84$, $p<.05$]. A significant effect of treatment was revealed in short-light raised quail [treatment main effect, $F(2,16)= 8.50$, $p<.05$]. Tukey's post hoc analyses revealed that short-photoperiod raised female quail treated with saline had less activity than 5 mg/kg and 10 mg/kg cocaine treated animals. However, there was no significant difference between cocaine doses in this group (Tukey $p<.05$).

In female quail, day 1 was compared to day 10 to test for cocaine-induced behavioral sensitization (see figure 2.3C and 2.3D). An RM ANOVA revealed a

day x treatment x photoperiod interaction [$F(2,37)= 5.23$, $p<.05$]. Further analyses were then conducted on each photoperiod. An analysis with females raised on the short photoperiod revealed a significant day x treatment interaction [$F(2,16)= 4.10$, $p<.05$]. Saline treated female quail raised on the short photoperiod had a significant decrease in beam breaks from day 1 ($M= 3408.86$, $SEM= 838.81$) to day 10 ($M= 2275.57$, $SEM= 1060.54$), [$F(1,6)= 6.45$, $p<.05$]. Female quail raised on the short-light cycle treated with 5 mg/kg cocaine had a significant increase in beam breaks from day 1 ($M= 3594.57$, $SEM= 906.02$) to day 10 ($M= 6351.28$, $SEM= 1145.52$), [$F(1,6)= 7.64$, $p<.05$]. However, analysis of short-light females administered 10 mg/kg revealed that they did not have an increase in beam breaks between days [$F(1,6)= 0.51$, p=n.s.]. An analysis with females raised on the long photoperiod revealed a significant day x treatment interaction [$F(2,21)= 14.53$, $p<.05$]. Saline treated female quail raised on the long-light cycle did not have any change in beam breaks from day 1 to 10 [$F(1,7)= 2.12$, p=n.s.]. Long-light female quail treated with 5 mg/kg had a significant increase from day 1 ($M = 4093.00$, $SEM = 784.64$) to day 10 ($M= 5518.25$, $SEM= 992.05$), [$F(1,7)= 12.29$, $p<.05$]. Conversely, long-light raised female quail treated with 10 mg/kg had a significant decrease from day 1 ($M= 6656.25$, $SEM= 346.72$) to day 10 ($M= 4189.88$, $SEM= 233.07$), [$F(1,7)= 10.95$, $p<.05$].

Female hormone analysis

Figure 2.6 shows the relationship between gonadal hormones and number of beam breaks on the last day of cocaine administration for female quail (figure

2.6A). A linear regression established that photoperiod length significantly predicted plasma estradiol levels on PHD 47, [$F(1,42)= 28.805$, $p<.05$]. An ANOVA revealed female quail raised on the short-light photoperiod had significantly lower levels of E2 on PHD 33 [$F(1,40)= 9.74$, $p< .05$] and PHD 47 [$F(1,43)= 28.81$, $p< .05$] compared to long-light raised females. By the end of conditioning, average E2 for quail raised on the long-light cycle (M= 171.23 pg/ml, SEM= 5.19) was significantly higher than quail raised on the short-light period (M= 90.65 pg/ml, SEM= 10.21). E2 was examined as a moderator of the relationship between cocaine dose and total beam breaks on day 10. A hierarchical multiple regression revealed plasma estradiol in females on PHD 47 was a significant moderator of the relationship between cocaine dose and total amount of beam breaks on day 10, [R^2 =.192, $F(3,36)= 7.26$, $p<.05$], the addition of the interaction term explaining an additional 5.7% of the total variance, [ΔR^2 = .057, $F(1,36)=5.05$, $p< .05$]. Thus, E2 levels were a significant moderator of the relationship between cocaine dose and total beam breaks on day 10. However, there was an increase in E2 levels between the blood draw days (i.e., PHD 33 and PHD 47) for female quail raised on the long photoperiod, [$F(1,21)= 47.10$, $p< .05$], but not for quail raised on the short photoperiod, [$F(1,17)= 4.22$, p = n.s.]. Specifically, female quail raised on the long-light cycle had lower levels of E2 on PHD 33 (M= 67.86 pg/ml, SEM= 39.94) which increased to PHD 47 (M= 171.23 pg/ml, SEM= 5.19). The short-light females did not have the same change in E2 from PHD 33 (M= 67.86 pg/ml, SEM= 7.53) to PHD 47 (M= 90.65 pg/ml, SEM= 10.21).

Discussion

Quail progressively gained weight across all 10 days. Similar to previous findings (for review see, Mills, Crawford, Domjan, & Faure, 1997), females were heavier than males. Quail raised on long-light photoperiod weighed more than quail raised on short-light photoperiod. On average, female quail raised on the long-light photoperiod were the heaviest across all days. The long-light raised female gained the most weight over the 10 days of conditioning. This could possibly be due to the development of the photostimulated quail. Photostimulated female quail began producing eggs during the experiment. Egg production demands a considerable amount of resources and therefore quail beginning oviparity gained more weight to meet these resource needs (Monaghan & Nager, 1997). In addition, the presence of an egg would affect body weight considerably. Surprisingly, cocaine treatment did not have any effect on weight gain. Although previous research examining the impact of a similar psychostimulant found no change in weights or weight gain in preweanlings, adolescent, or adult rats following administration of methylphenidate (Torres-Reverón & Dow-Edwards, 2005). Catlow & Kirstein (2007) found adult rats pretreated with cocaine weighed more than saline pretreated animals, adolescent rats pretreated with cocaine did not have any treatment effect on weight. Although the literature is mixed, younger animals treated with cocaine or similar psychostimulants did not have much change in weight following treatment similar to the current study.

Male quail raised on the short photoperiod developed a cocaine-induced behavioral sensitization to the 10 mg/kg dose only. These findings were contrary to the hypothesis and previous findings. Gill et al. (2015) did not observe an increase in behavioral sensitization in short-light housed male quail. Unlike previous findings, behavioral sensitization was not observed in males raised on the long photoperiod (Akins & Geary, 2008; Geary & Akins, 2007; Gill et al., 2015). Similar to Geary and Akins (2007), males treated with 5 mg/kg did not acquire cocaine-induced behavioral sensitization. However, these differences could be due to age related differences between previous studies and the current study. The current study used animals that were younger than 47 days old, whereas Gill et al. (2015) used animals that were 8 months old. Previous research indicates that male quail reach adulthood between 28 and 35 days old (Ottinger & Brinkley, 1979). Although both studies used animals that might be considered adults there could have been differences in the hormonal changes the animals were experiencing at the time of testing.

Similar to previous research, male quail raised on the long-light cycle had higher T levels compared to short-light raised male quail (Balthazart et al., 1979; Gill et al., 2015). However, unlike the aged male quail examined previously (Gill et al., 2015), the current research did not find a positive relationship between T levels and the amount of activity on the last day of treatment. Additionally, although Gill et al. (2015) demonstrated a relationship between cocaine-induced locomotion and T, other research has not revealed such a relationship (Hu & Becker, 2003). Previous research from the Becker lab indicated there was no

relationship between T and cocaine-induced locomotion (Becker et al., 2001; Hu & Becker, 2003). Therefore, the current findings may be more in line with rodent literature. Although speculative, hormone levels increased throughout the experiment, which may have affected the activity on the last day. Furthermore, because the quail used were younger than quail in previous research (Gill et al., 2015), it is possible that T has different locomotor activating effects in young males than in adult male quail.

Female quail raised on both the short and long-light cycles developed cocaine-induced behavioral sensitization to a 5 mg/kg dose of cocaine after 10 pairings. Behavioral sensitization had not previously been observed in long-light cycle housed females, however, only higher doses of cocaine (i.e., 10 & 20 mg/kg) had been administered (Gill et al., 2015). Neither short nor long-light cycle raised females acquired behavioral sensitization to the 10 mg/kg dose. Photoregressed female quail treated with 10 mg/kg cocaine did not develop a sensitized response contrary to Gill and colleagues (2015). Perhaps a surge of gonadal hormones occurring around puberty and the low dose of cocaine interact to facilitate the acquisition of cocaine-induced behavioral sensitization. Although the quail were photoregressed, there was still an increase in E2 across conditioning days. In mammalian tissue co-treatment with E2 and amphetamine has been shown to increase DA release (Becker, 1990a; Becker, 1990b). Although no such effect has been observed in quail, it is possible that cocaine and pubertal increases in E2 interact resulting in an inverse U activity response. Specifically lower levels of E2 may interact with increased DA resulting from

cocaine administration causing an increase in activity, but high levels of cocaine in combination with increasing E2 result in a decrease in activity.

Previous research in rodents has provided evidence for the enhancing role of E2 in behavioral sensitization (for review see, Becker & Hu, 2008). However, previous quail research did not find support for enhanced behavioral sensitization in photostimulated female quail (Gill et al., 2015). In the current study, female quail raised on the long-light photoperiod and treated with the 10 mg/kg cocaine dose started to display an increase in activity midway through conditioning. However, following day 7 this was no longer the case, perhaps due to a surge in pubertal hormonal levels which occurs just prior to oviparity in birds (Brain et al., 1988; Peterson & Webster, 1974). This behavioral pattern of an increase in activity followed by the decrease seen in the long-light raised quail could be due to an increase in stereotypy-like behaviors resulting from repeated treatment of cocaine. However, it seems more likely that these changes may be due to fluctuations in E2 over conditioning days, similar to the photoregressed quail. On the first day of conditioning, none of the female quail were laying eggs, however, by day 10, all long-light raised females were laying eggs and had an overall reduction in activity compared to day 1. E2 levels increased significantly for long-light raised quail from PHD 33 and 47. Although speculative, perhaps the lower E2 levels before oviparity allowed for some activity enhancing effects of cocaine, but the surge of hormones just prior to oviparity (i.e., the start of egg laying) resulted in a reduction in cocaine-induced activity.

As predicted, quail raised on the long-light cycle had higher E2 levels compared to those raised on the short-light cycle. Although previous studies did not find a relationship between E2 and cocaine-induced activity in adult female quail (Gill et al., 2015), the current study revealed a moderating relationship between E2 levels in developing female quail and activity on the last day. Specifically, high levels of E2 appeared to increase activity in saline treated animals, but reduced activity in cocaine treated quail. These results may support previous findings from an ethological point. The long-light cycle mimics the breeding season for quail (for review see, Mills et al., 1997). Elevated E2 levels may inhibit movement and encourage squatting for mating, nest building, and laying eggs in breeding females (for review see, Mills et al., 1997). The current research found that high levels of E2 caused a reduction in cocaine-induced activity on day 10. This could be due to the interaction between DA and E2 on the breeding behaviors.

One possible limitation of this study was that blood samples were not collected daily and, therefore, it was not clear when the pubertal shift in hormones occurred during the study. Additionally, in order to properly examine the effect of age on cocaine-induced behavioral sensitization, prepubertal, pubertal, and postpubertal groups are necessary to examine any age associated changes that may occur. However, the current research extends on the literature exploring the effect of photoperiod manipulation on cocaine-induced behavioral sensitization in the quail.

Future studies should examine the role of ontogeny and genetic factors influencing cocaine-induced behavioral sensitization in the quail. In rodents, age has a large impact on cocaine-induced behavioral sensitization as sex differences in cocaine responsiveness occur in adolescence (Parylak, Caster, Walker, & Kuhn, 2008; Spear & Brake, 1983). In this age group, future studies should examine the role of E2 and T in cocaine-induced behavioral sensitization in both sexes, as previous research has shown that it is E2 that is involved in the onset of puberty in male quail (Ball & Balthazart, 2004; Schumacher & Balthazart, 1983). Examining the role of genetic influences on cocaine-induced behavioral sensitization should also be examined as previous research has shown circadian rhythm influences on cocaine-induced behavioral sensitization in rodents and drosophila (Abarca, Albrecht, & Spanagel, 2002; Andretic, Chaney, & Hirsh, 1999; McClung et al., 2005). In quail, photoperiod length alters expression of circadian rhythm genes (Yasuo, Watanabe, Okabayashi, Ebihara, & Yoshimura, 2003). Therefore, with light-cycle manipulation in quail, it is important to understand the relationship between cocaine-induced behavioral sensitization and photoperiodic controlled changes in gene expression that occur in addition to the hormonal changes.

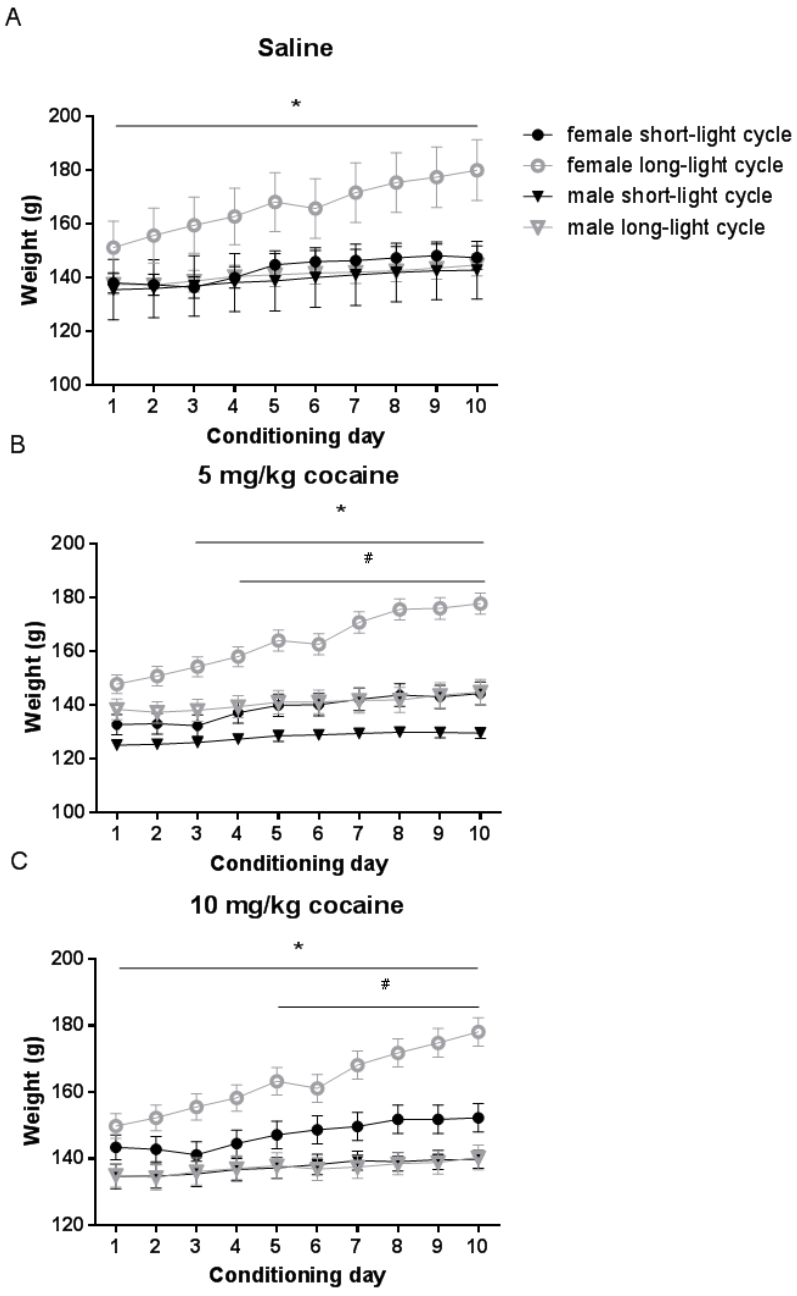

Figure 2. 1. Total weight across 10 conditioning days for saline (A), 5 mg/kg cocaine (B) and 10 mg/kg cocaine (C). Each data point represents mean weight in grams ±SEM. * indicates a sex difference between long-light raised female and male quail, $p<.05$. # indicates a sex difference between short-light raised female and male quail, $p<.05$.

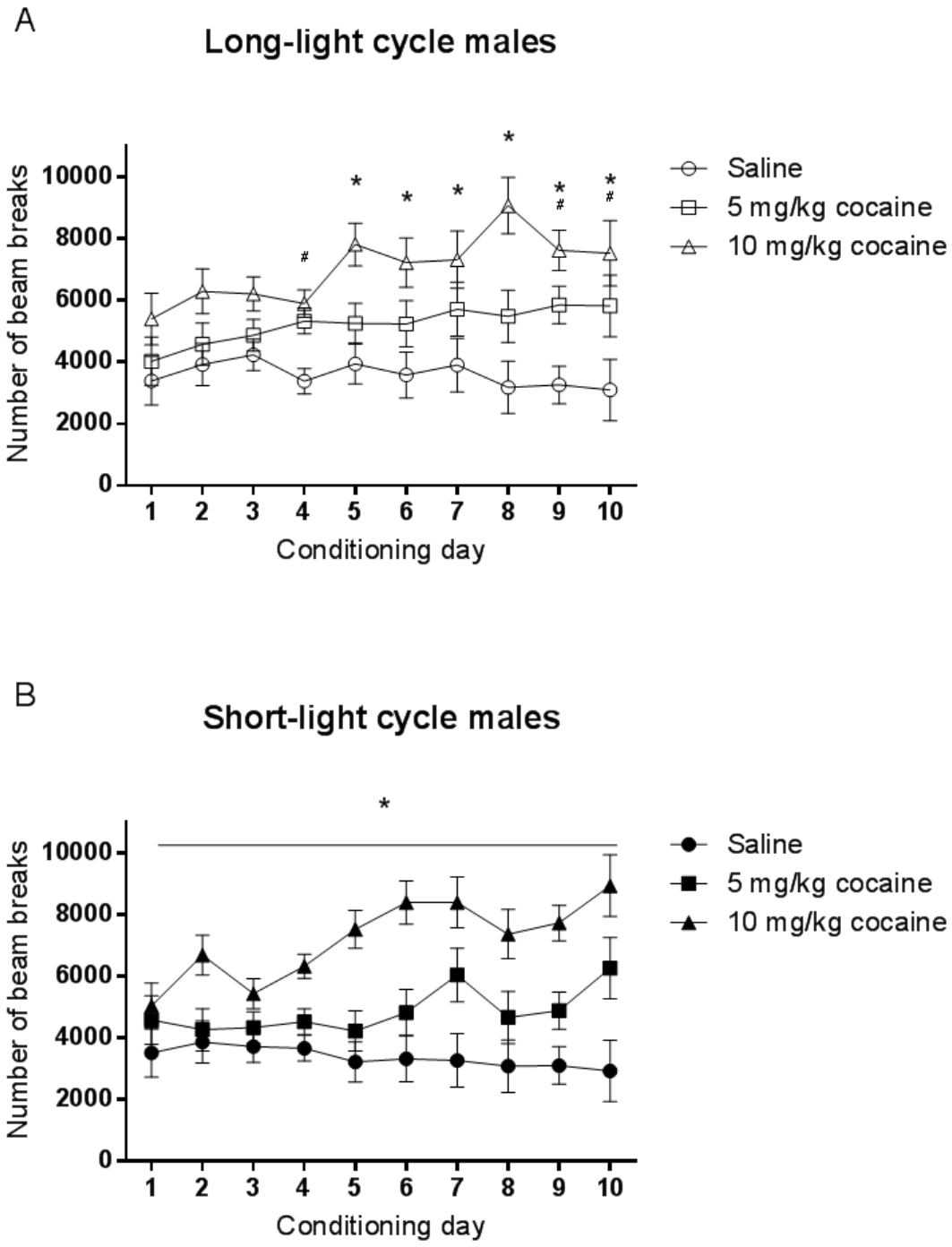

Figure 2. 2.Total number of beam breaks across conditioning days for males raised in a long-light cycle (A) and short-light cycle (B). Each data point represents the average number of beam breaks ±SEM for one hour following the administration of cocaine. * represents differences between 10 mg/kg treated quail and saline treated quail, $p<.05$.

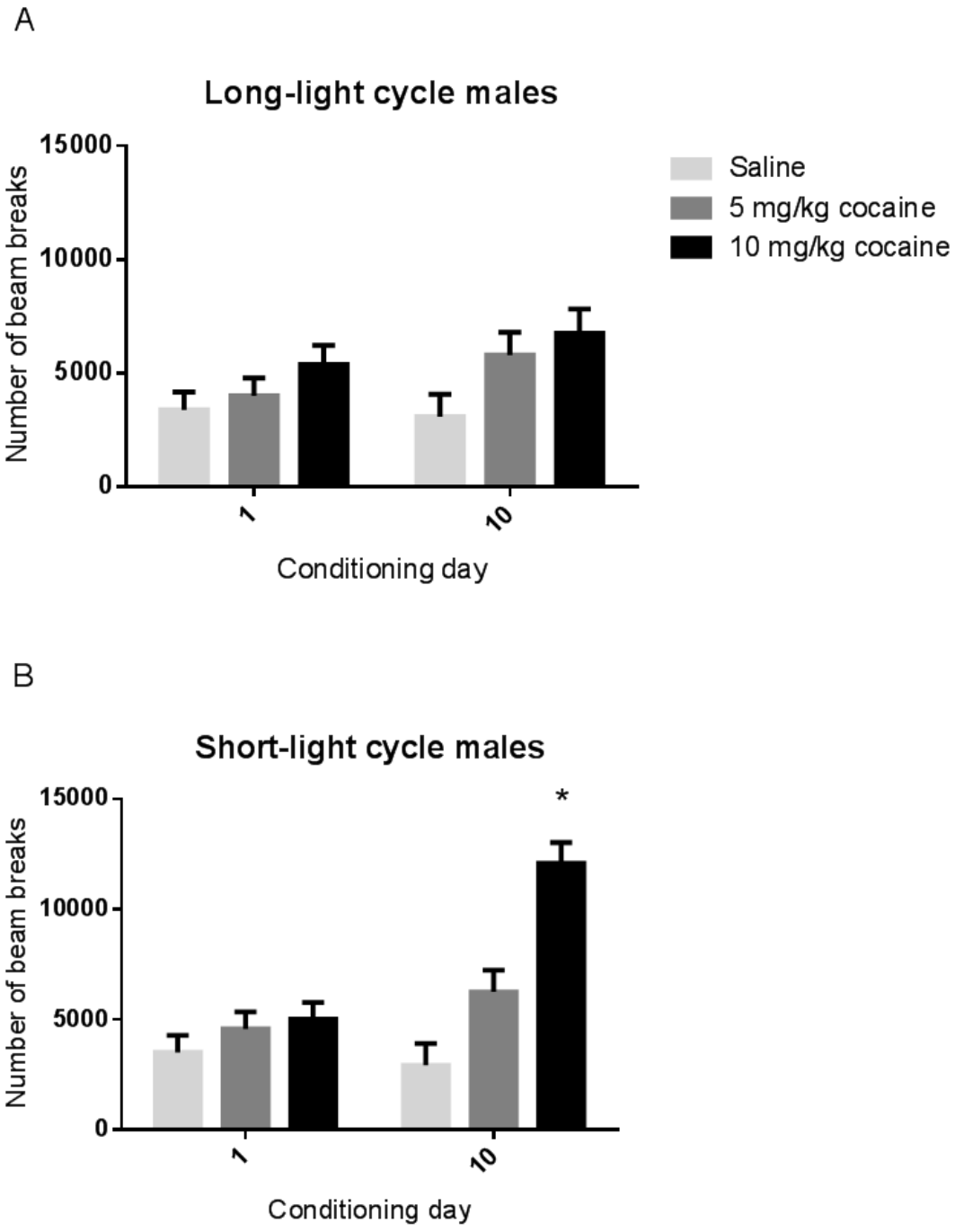

Figure 2. 3. Bars represent the average number of beam breaks ±SEM on the first day and last day of cocaine administration for males. * indicates an increase in beam breaks from day 1, p<.05.

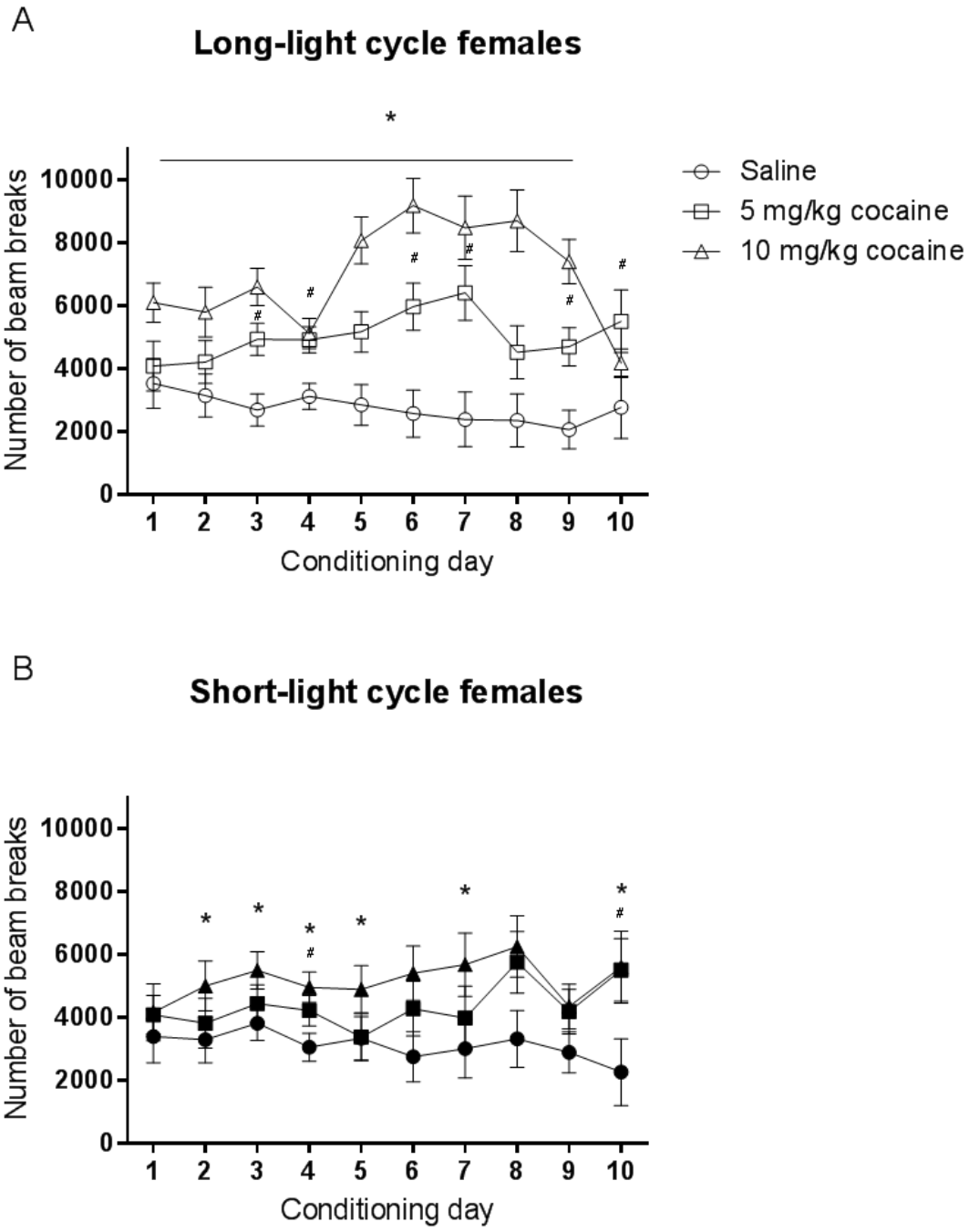

Figure 2. 4. Total number of beam breaks across conditioning days for females raised in a long-light cycle (A) and short-light cycle (B). Each data point represents the average number of beam breaks ±SEM for one hour following the administration of cocaine. * represents differences between 10 mg/kg treated quail and saline treated quail, $p<.05$.

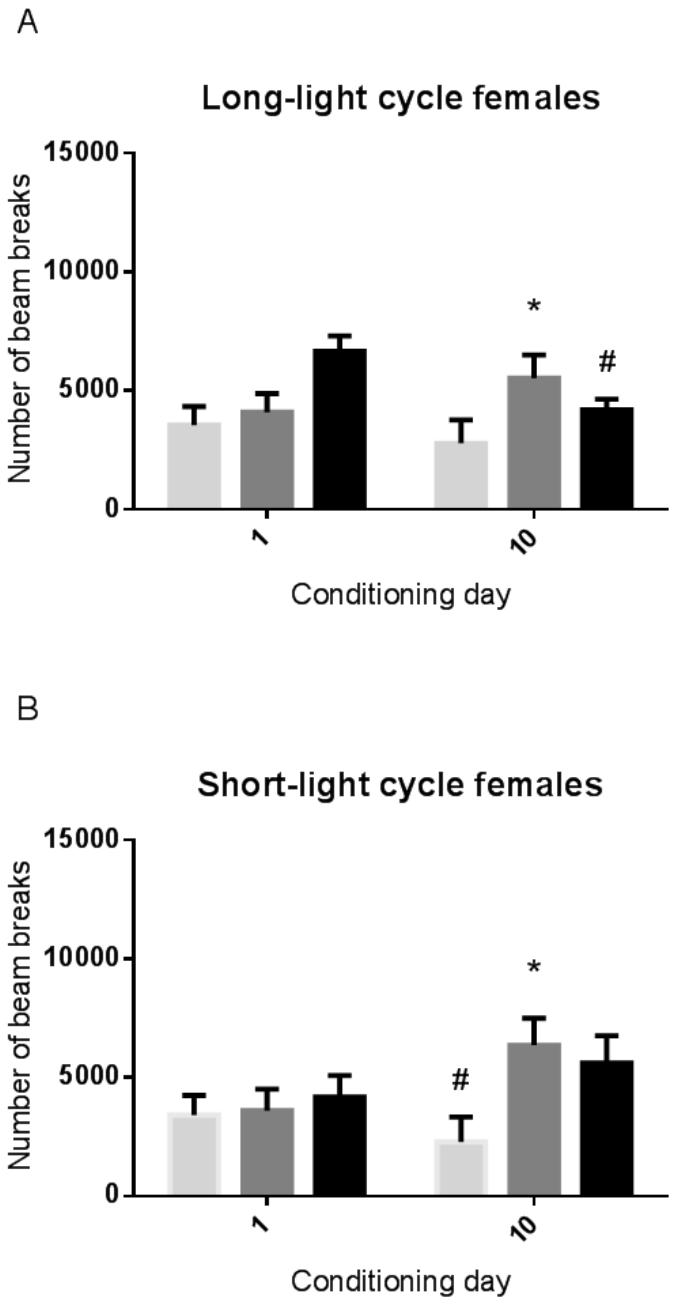

Figure 2. 5. Bars represent the average number of beam breaks ±SEM on the first day and last day of cocaine administration for females. * indicates an increase in beam breaks from day 1, $p<.05$. # indicates a decrease in beam breaks from day 1, $p<.05$.

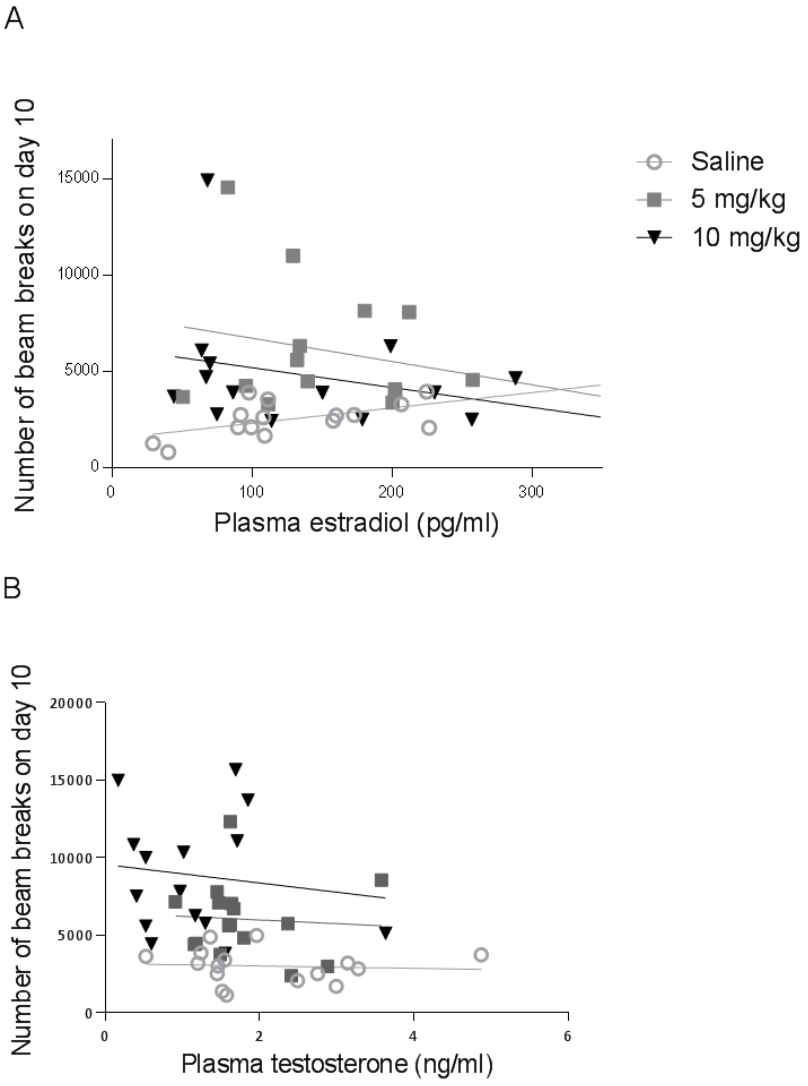

Figure 2. 6. Relationship between the mean distance traveled activity on Trial 10 for females (A) and males (B). Each point represents the hormone level and total beam breaks on day 10 for each bird. Linear regression analysis represented by the solid line on each graph.

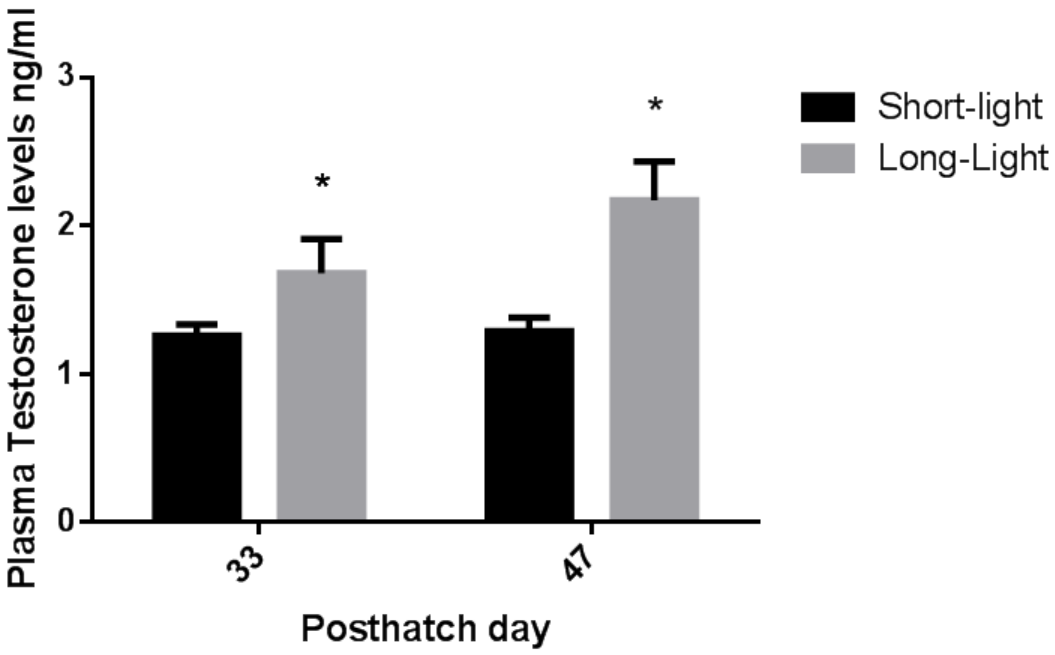

Figure 2. 7. Bars represent the average plasma testosterone for males on posthatch day 33 and posthatch day 47. * represents higher plasma testosterone levels for quail raised on the long-light cycle compared to the same age short-light cycle quail, $p<.05$.

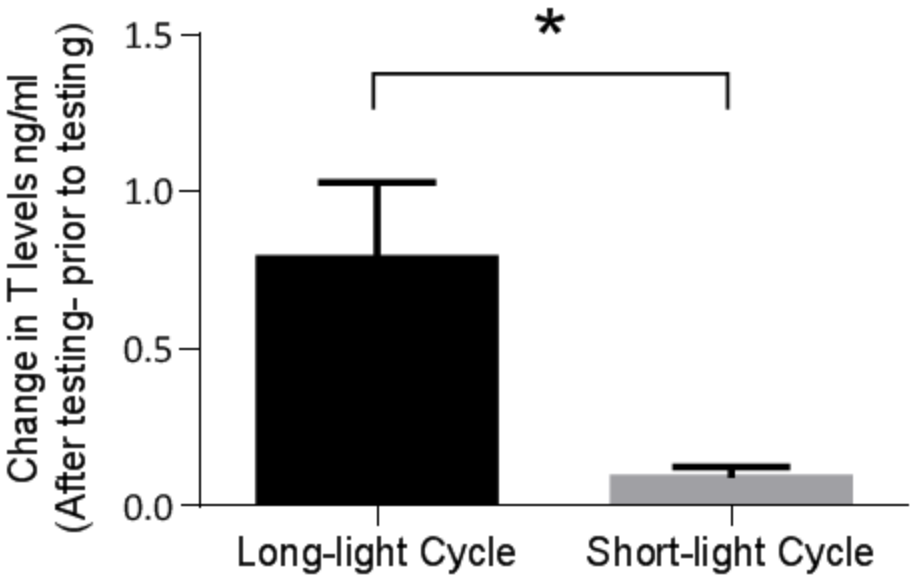

Figure 2. 8. Bars represent the change in plasma testosterone for males between posthatch day 33 and posthatch day 47. * represents a larger change in plasma testosterone levels for quail raised on the long-light cycle compared to the same age short-light cycle quail, $p<.05$.

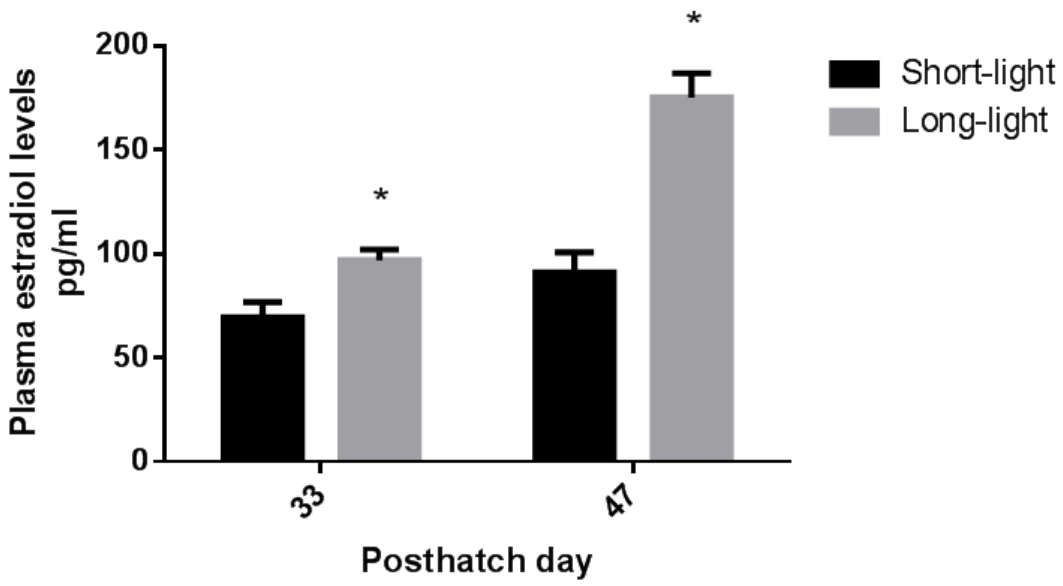

Figure 2. 9. Bars represent the average plasma estradiol for females on posthatch day 33 and posthatch day 47. * represents an higher plasma estradiol levels for quail raised on the long-light cycle compared to the same age short-light cycle quail, $p<.05$.

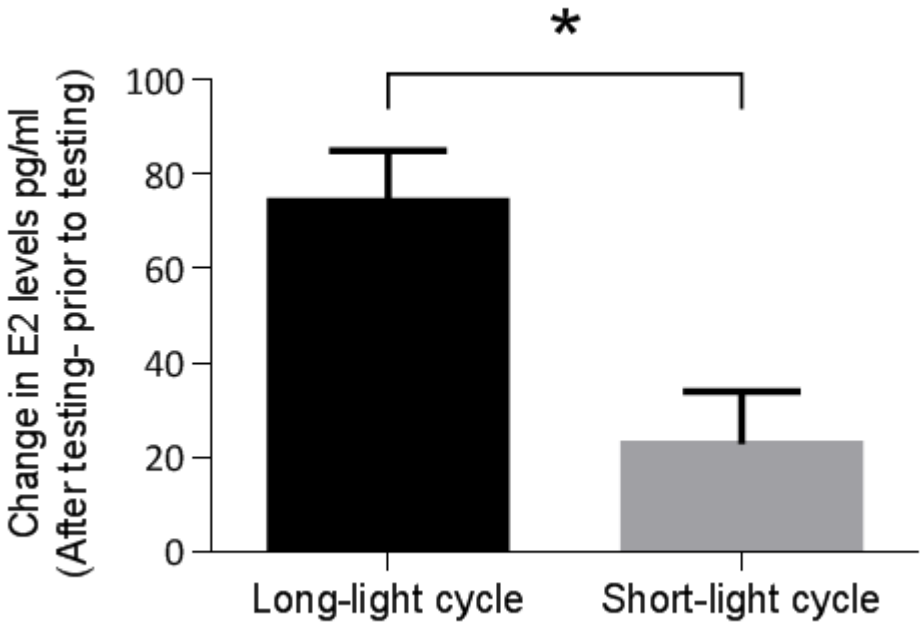

Figure 2. 10. Bars represent the change in plasma estradiol for females between posthatch day 33 and posthatch day 47. * represents a larger change in plasma estradiol levels for quail raised on the long-light cycle compared to the same age short-light cycle quail, $p<.05$.

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PUBLICATIONS

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- Amodeo, L. R., Greenfield, V. Y., Humphrey, D. E., Varela, V., Pipkin, J. A., **Eaton, S. E.**, ... & Crawford, C. A. (2015). Effects of acute or repeated paroxetine and fluoxetine treatment on affective behavior in male and female adolescent rats. *Psychopharmacology*, *232*(19), 3515-3528.
- S. A. McDougall, **S. E. Eaton**, A. Mohd-Yusof, C. A. Crawford (2015). Age-dependent changes in cocaine sensitivity across early ontogeny in male and female rats: Possible role of dorsal striatal $D2^{High}$ receptors. *Psychopharmacology*, 1-15.
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AWARDS AND SCHOLARSHIPS

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