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Cocaine-induced sensitization correlates with testosterone in male Japanese quail but not with estradiol in female Japanese quail

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ABSTRACT

Research has indicated that gonadal hormones may mediate behavioral and biological responses to cocaine. Estrogen, in particular, has been shown to increase behavioral responding to cocaine in female rats relative to male rats. The current study investigated the effect of cocaine on locomotor activity and hormonal correlates in male and female Japanese quail (*Coturnix japonica*). In Japanese quail, circulating hormone levels can be manipulated without surgical alterations via modifying the photoperiod. Male and female quail were housed on either 8L:16D (light:dark) or 16L:8D (light:dark) cycle for 21 days. Blood samples were taken prior to the beginning of the experiment and assays were performed to determine the levels of testosterone (T) and estradiol (E2). Quail were given injections of saline or cocaine (10 or 20 mg/kg) once a day for 10 days. Immediately after each injection, birds were placed in open field arenas and distance traveled was measured for 30 min. Results showed that male quail housed under long-light conditions exhibited cocaine-induced sensitization to 10 mg/kg cocaine which was correlated with the high levels of plasma T. Female quail housed under short-light conditions demonstrated sensitization to 10 mg/kg cocaine, but this was not correlated with the levels of plasma E2. The current findings suggest that cocaine-induced locomotor activity was associated with T in males but not with E2 in females.

Introduction

While the rate of cocaine abuse and dependence has remained relatively stable over the last 15 years, abuse and dependence among women have dramatically increased, such that nearly 40% of users over the age of 26 are females (Evans & Foltin, 2010; Jackson et al., 2006). Men and women are equally likely to use cocaine if given the opportunity, but women are more likely to reach dependence criteria compared to their male counterparts (Kasperski et al., 2011; Van Etten and Anthony, 1999). Women report shorter periods of abstinence (Kosten et al., 1993), enter into treatment at younger ages (Griffin et al., 1989; Mendelson et al., 1991), and once admitted for treatment, their use is more severe compared to men (Kosten et al., 1993). Additionally, drug-related cues induce higher levels of craving in cocaine-dependent women than in cocaine-dependent men (Robbins et al., 1999). Collectively, these studies suggest that women may be more sensitive to the reinforcing properties of psychostimulants and may be more vulnerable to some aspects of drug addiction than men.

Research has indicated that gonadal hormones, particularly estrogen, may be responsible for the heightened behavioral and biological responses to cocaine in females (Evans and Foltin, 2006; Hu and Becker, 2003). Women report greater subjective responses to cocaine when tested during the follicular phase compared to the luteal phase of the menstrual cycle (Collins et al., 2007; Evans et al., 2002; Sofuoglu et al., 1999). In fact, sex differences only emerge in humans when men are compared with women in the luteal phase, as women in the follicular phase have similar responses to cocaine as men (Collins et al., 2007; see Quinones-Jenab and Jenab, 2012 for review). Rodent models have demonstrated that intact female rats acquire cocaine self-administration at faster rates and have higher breaking points than ovariectomized (OVX) female rats and male rats (Jackson et al., 2006; Lynch and Carroll, 1999). Russo et al. (2003) showed that female rats develop associations to environmental cues and to the rewarding properties of cocaine at lower doses and at faster rates than male rats. Additionally, intact and estradiol-treated OVX female rats show significantly greater locomotor activity following chronic cocaine administration compared to intact and castrated male rats and OVX female rats (Hu and Becker, 2003). It should be noted that estradiol has been shown to rapidly downregulate D2 in the striatum (Bazzett and Becker, 1994) and may, in part, account for the increased sensitivity to repeated cocaine observed in female rodents (Hu and Becker, 2003; Becker and Hu, 2008). Taken together, these studies suggest that ovarian hormones

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may play a role in the increased vulnerability to psychostimulants among females.

Research on the role of testosterone in cocaine-induced locomotor effects in male rodents is mixed and inconclusive. Some studies have reported no differences between castrated (CAST) and intact male rats in cocaine-induced locomotor activity (Becker et al., 2001; Forgie and Stewart, 1994; Hu and Becker, 2003; Hu et al., 2004; Robinson et al., 1981; van Haaren and Meyer, 1991). In these studies, cocaine increased locomotor activity in both CAST and intact male rats. Other studies have reported increased cocaine-induced locomotor activity and striatal dopamine in CAST male rats relative to intact rats (Camp and Robinson, 1988a,b; Hernandez et al., 1994; Purvis-Tyson et al., 2014; Robinson, 1984). In contrast, Menendez-Delmestre and Segarra (2011) observed cocaine-induced sensitization at 15 and 30 mg/kg in intact and CAST male rats with testosterone replacement but not in CAST male rats. Thus, it is difficult to decipher the role of testosterone on cocaine-induced locomotor based on the current literature.

The current study proposes to examine the effects of gonadal hormones (estradiol and testosterone) on cocaine-induced locomotor activity using a visually-oriented animal model, Japanese quail. Japanese quail have color vision and high visual acuity (Fidura and Gray, 1966) unlike rodent species. While the current study will not manipulate visual cues, it will serve to inform future studies involving the interaction between hormones, drug effects, and visual cues. There are currently no studies investigating this interaction. However, several clinical studies have shown that environmental visual cues play a role in drug addiction and relapse (e.g., Childress et al., 1988; O'Brien et al., 1992). Environmental stimuli may become associated with interoceptive drug cues and later, in the absence of the drug, trigger drug-seeking and ultimately relapse. Therefore, studying drug–hormone interactions in the context of how visual cues may induce relapse may be of importance to understanding drug addiction mechanisms.

Cocaine-induced behavioral sensitization (Akins and Geary, 2008; Geary and Akins, 2007; Levens and Akins, 2001) and cocaine reward (Akins et al., 2004; Levens and Akins, 2001) have been demonstrated in our laboratory in male Japanese quail. These studies utilized male quail that were on long-light photoperiods (functionally intact males). The current study proposes to compare cocaine effects on locomotor activity in long-light males and short-light (functionally CAST) males and then to determine whether testosterone is correlated with those effects. Similarly, no studies have examined cocaine effects on locomotor activity in female quail nor whether estradiol levels are correlated with those effects.

Japanese quail allow for the utilization of a practical laboratory technique for manipulating hormone levels. In quail, circulating hormone levels can be manipulated through the alterations of photoperiod without surgical methods (Robinson and Follett, 1982). Male and female quail exposed to long photoperiods exhibit increased plasma levels of testosterone and estradiol, respectively (Adkins and Adler, 1972; Balthazart et al., 1979; Balthazart et al., 1983; Brain et al., 1988; Delville et al., 1986; Delville and Balthazart, 1987; Doi et al., 1980; Domjan, 1987; Guyomarc'h and Guyomarc'h, 1994; Mills et al., 1997; Noble, 1972). Male and female quail exposed to short photoperiods exhibit decreased plasma levels of testosterone and estradiol, respectively (Adkins and Adler, 1972; Balthazart et al., 1979; Brain et al., 1988; Delville et al., 1986; Delville and Balthazart, 1987; Doi et al., 1980; Domjan, 1987; Guyomarc'h and Guyomarc'h, 1994; Mills et al., 1997; Noble, 1972). Furthermore, exposure to short-light conditions has been shown to be comparable to surgical gonadectomy in both male and female quail (Adkins and Nock, 1976).

The present study was designed to investigate the role of gonadal sex hormones in cocaine-induced behavioral sensitization in a visual species. The overarching hypothesis was that increases in plasma sex hormones would correlate with increases in cocaine-induced locomotor activity in Japanese quail and that female quail would be more sensitive to the locomotor-activating effects of cocaine than males. Specifically, it was predicted that photostimulated (long-light) female and male quail would dose-dependently exhibit increases in locomotor activity to repeated administration of cocaine, and that photostimulated female quail would sensitize to a greater degree than male quail. Based on previous cocaine sensitization studies in male quail and the rodent literature, it was predicted that cocaine-induced locomotor effects would be correlated with testosterone in male quail and with estradiol in female quail.

Materials and methods

Subjects

Forty-six male and 45 female Japanese quail (*Coturnix japonica*), approximately 5–8 months old were the subjects in the experiment. Eggs were supplied by Northwest Gamebirds (Kennewick, WA) and quail chicks were hatched and then raised in mixed-sex groups until approximately 28 days of age. At 28 days of age, male quail were housed individually and female quail were group-housed in wire mesh cages (GQF Manufacturing, Savannah, GA). Female quail were housed individually when selected for the experiment. Subjects were raised on a long-light (16L: 8D) cycle with food and water available ad libitum. Approximately 3 weeks (21 days) prior to the start of the experiment, twenty-two male and twenty-one female quail were transferred to a short-light (8L: 16D) cycle with lights on at 0900 (Adkins, 1973; Henare et al., 2011; Robinson and Follett, 1982). In the current experiment, the short-light cycle resulted in plasma T (0.65 ± 0.71 ng/ml) and E2 (43.72 ± 41.62 pg/ml) levels similar to previous reports (Balthazart et al., 1979, 1983; Brain et al., 1988). Thus, male and female steroid hormone levels were adequately low under short-light conditions in the current experiment.

All subjects were drug and sexually naïve prior to experimentation. All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Plasma testosterone and estradiol ELISA

Blood samples were taken two days prior to the start of the experiment. Approximately 0.5 ml of blood was taken from the brachial (wing) vein of the quail and placed into heparinized tubes. To minimize corticosteroid release, the time between the removal of quail from carrier box and collection did not exceed 5 min (Dallman and Bhatnagar, 2001; Mizrahi et al., 2001). Blood was immediately centrifuged at 1500 rpm for 5 min and plasma was stored at −20 °C until assayed.

Plasma testosterone (T) and estradiol (E2) were measured in duplicate via an enzyme-linked immunoassay kit (DRG Diagnostics; testosterone EIA-1559, estradiol EIA-2693) modified from the procedure described by Wilhelms et al. (2005). The kits were validated for use with quail plasma by testing for parallelism and recovery of added mass (standard biochemical validations). To test for parallelism, high and low T and E2 pools were pipetted at five different volumes in quadruplicate to ensure that the dose response curves were parallel to the standards under dilution and to confirm that the sample bound to the antibody had the same affinity. The test for parallelism ensures that the assay maintains linearity under dilution, and recovery of exogenous T or E2 verifies accurate measurement throughout the working range of the assay. To test for recovery of added mass, three standard curve points (from the middle of the standard curve) were added to the high and low pools to ensure that the added mass could be detected, indicating that the sample was not blocking the antibodies' ability to bind with the standard. The intra-assay and inter-assay coefficients of variance (CVs) for the T ELISA were 8% and 10%, respectively. The intra-assay and inter-assay CVs for the E2 ELISA were 9% and 12%, respectively.