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Paternal Cocaine Disrupts Offspring Circadian Clock Function in a Sex-Dependent Manner in Mice

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Abstract—The present study is the first to explore the multigenerational effects of mammalian paternal cocaine intake on offspring (F1) circadian clock regulation. Parental cocaine use poses significant health risks to the offspring, through both maternal and paternal drug influences. With respect to the latter, recent evidence suggests that a paternal mode of cocaine inheritance involves epigenetic germ line actions that can ultimately disrupt offspring behavior. Based on our previous report in mice that free-running circadian period (tau) is chronically lengthened following withdrawal from long-term cocaine treatment, the present study was undertaken to explore potential epigenetic effects of paternal exposure to cocaine over the ~40-day murine spermatogenic cycle on F1 circadian regulatory functions. Here we show that, although withdrawal of sires from the cocaine treatment lengthened their tau, such an effect did not persist in adult F1 male or female offspring born from drug-naïve dams. Notably, however, there was a distinct deficit in the ability of F1 cocaine-sired males, but not females, to undergo light-induced phase delay shifts of the circadian clock. In contrast, F1 cocaine-sired females, but not males, had suppressed circadian phase advance shifting responses to two non-photic stimuli: acute i.p. injections of cocaine and the serotonin agonist ([+]8-OH-DPAT). The reduced cocaine shifting in females was not due to suppressed cocaine-induced behavioral arousal. Collectively, these results reveal that a father's cocaine use can disrupt major circadian entrainment mechanisms in his adult progeny in a sex-dependent manner.

Key words: multigenerational inheritance, oral cocaine, photic entrainment, non-photic entrainment.

INTRODUCTION

Cocaine abuse remains a major health concern where reports indicate that cocaine can dramatically alter both long-term brain physiology and behavior (Nestler, 2005). In addition to its direct neurologic effects on users, cocaine also has been found to change brain function and behavior in offspring through various multigenerational mechanisms. For example, it is well documented that maternal cocaine use during pregnancy produces deficits in higher cognitive function, language, developmental delays, and emotional reactivity (reviewed in Martin et al., 2016). There is also emerging evidence for paternal inheritance of cocaine action (Yohn et al., 2015). Paternal cocaine use is linked to epigenetic alterations in biochemical and structural changes in first generation offspring (F1) brains as well as behavioral changes. In rats, cocaine-sired (CocSire) offspring have sex-dependent alterations in reward responses to cocaine self-administration (Vassoler et al., 2013), as well as increased anxiety-like behaviors (Abel et al., 1989; White et al., 2016). CocSire mice also exhibit increases in behavioral depression, (Killinger et al., 2012), increases in anxiety-like behaviors (Fischer et al., 2017), learning deficits (He et al., 2006; Wimmer et al., 2017), and altered reward sensitivity (Finegersh and Homanics, 2014). It is thought that the primary mode of paternal transmission is via spermatogenic alterations in the testes, where cocaine exerts possible epigenetic effects on specific germ line elements (He et al., 2006; Vassoler et al., 2013).

The mammalian circadian timing system, which maintains ~24-h rhythmicity of all molecular and physiological processes, is an important coordinator of motivated behaviors, including those related to drug seeking and reward (Antle and Silver, 2015). It is now clear that drugs of abuse can significantly impair circadian clock timing leading to a downward spiral of increasing

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Abbreviations: 8-OH-DPAT, 8-hydroxy-n,n-dipropylaminotetralin; ANOVA, analysis of variance; BDNF, brain-derived neurotropic factor; CocSire, cocaine-sired offspring; CtlSire, control-sired offspring; DD, constant darkness; F1, first generation offspring; i.p, intraperitoneal; LD, lightdark period; NPY, neuropeptide Y; PCE, prenatal cocaine exposure; SCN, suprachiasmatic nucleus; ZT, zeitgeber time.

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drug abuse and ultimate addiction (Honma and Honma, 1986, 2009; Honma et al., 1986, 1987; Tataroglu et al., 2006; Seggio et al., 2007; Prosser et al., 2008; Li et al., 2009; Seggio et al., 2009; Ruby et al., 2009a,b; Brager et al., 2010, 2011; Hasler et al., 2012). Included in the list of drugs strongly affecting circadian function is cocaine. For example, cocaine causes alterations to circadian behaviors in mice, including long-term lengthening of circadian period (Stowie et al., 2015a) and blocking photic and non-photic phase-resetting responses (Glass et al., 2012; Brager et al., 2013; Prosser et al., 2014). Cocaine also alters circadian clock gene expression (Abarca et al., 2002; Uz et al., 2005) and conversely, clock gene mutations affect cocaine self-administration and other behavioral responses to cocaine (Akhisaroglu et al., 2004; McClung et al., 2005; Sleipness et al., 2005; Ozburn et al., 2012; Brager et al., 2013). Notably, cocaine reward is also regulated by the circadian clock, such that there is a strong circadian influence on the timing of drug seeking and self-administration (Roberts et al., 2002, 2007; Kurtuncu et al., 2004; Ozburn et al., 2012; Stowie et al., 2015a).

What is not clear is if there are inherited effects of paternal cocaine on circadian timing. The present study was thus undertaken to explore the effects of paternal cocaine intake in mice on a suite of circadian measures in F1 in male and female offspring, including free-running circadian period (tau) and light-induced as well as non-photic circadian clock resetting responses. Based on the reports discussed above, impaired clock function could theoretically lead to altered susceptibility to drug reward and abuse in offspring of cocaine using fathers.

EXPERIMENTAL PROCEDURES

Animals

Adult (>6 weeks old) male and female C57BL/6J mice obtained from Jackson Laboratories were used. Except for breeding, mice were singly housed in a polycarbonate cage and maintained under a 12:12 LD photoperiod (LD; lights on at 08:00) or constant darkness (DD) in a temperature-controlled chamber (23 °C). Food and water were provided *ad libitum*. These experiments followed the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Kent State University Animal Care Use Committee.

Circadian activity measurements

Circadian locomotor activity was measured in 5-min bins using overhead infrared motion detectors interfaced with a computerized data acquisition system (ClockLab; Coulbourn Instruments, Whitehall, PA, USA). Free-running circadian period (tau) was analyzed under DD using a least-squares regression line from a minimum of 14 daily activity onsets. Activity onsets were characterized by an initial period of activity that: (1) exceeded 10% of the maximum rate for that day; (2) was preceded by at least 4 h of activity quiescence (inactivity or activity that did not exceed 10% of the daily maximum); and (3) was followed by at least 60 min of sustained activity (activity which exceeded 10% of the daily maximum without longer than 5 min rest periods). Behavioral phase shifting was calculated using the difference between the least squares regression lines through 10-day periods of activity onsets preceding and following the day of experimentation. Prior to a phase shifting experiment, locomotor activity was recorded over a 2-wk period to establish a stable baseline rhythm expression. On the day of experimentation, animals received a phase-resetting stimulus, and were immediately released into DD to assess phase resetting responses using an Aschoff Type II procedure (Aschoff, 1965). The daily pattern of oral cocaine intake was measured using a "drinkometer" system (Coulbourn Instruments) interfaced with the ClockLab data acquisition system. The drinkometer is a passive system that registers insertion of an animal's nose into a port containing the drinking solution by breaking a light beam transmitted across the drinking port.

Experimental protocols

Paternal cocaine treatment and breeding. Sires were treated with forced, chronic cocaine dissolved in drinking water (0.5 mg/ml) over a period spanning one spermatogenic cycle (~40 days). Separate groups of sires were used in two rounds of breeding to generate offspring for all experiments. Stock cocaine solutions were adjusted to pH 3 and stored at 4 °C to prevent hydrolysis (Murray and Al-Shora, 1978); cocaine at this pH can be maintained at room temperature for up to 45 days without significant degradation. To ensure accurate cocaine dosing, the animals' drug solution, administered via graduated 15-ml polyethylene drinking vials, was measured daily, and replaced at 5-day intervals (Stowie et al., 2015a). Control sires received water alone adjusted to pH 3 *ad libitum*. The present oral mode of cocaine treatment was used to avoid the stress of long-term daily systemic injection that could impact offspring behavior (Stuart and Robinson, 2015). Oral cocaine administration provides peak plasma concentrations similar to those produced by trans-nasal cocaine exposure (Javaid et al., 1978; Van Dyke et al., 1978; Wilkinson et al., 1980; Bromley and Hayward, 1988), and oral cocaine has been utilized in studies of maternal prenatal cocaine exposure (PCE) (Lidow, 1998; Markowski et al., 1998, 2000; Zhou et al., 2001; Lidow and Song, 2001a,b), as well as circadian rhythms (Stowie et al., 2015a). Weight specific cocaine consumption was calculated by measuring average cocaine solution intake over a 40-day testing period. One day after drug withdrawal the drug-treated and control males were individually caged with 2 drug-naïve females for 1 wk, then removed and placed under DD for ~2 months to measure free-running tau. Immediately after the sires' removal, the dams were individually housed and visually checked for pregnancy.

Circadian timing of water and oral cocaine consumption. Male C57BL/6J mice were housed under LD.