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Cocaine modulates pathways for photic and nonphotic entrainment of the mammalian SCN circadian clock

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Glass JD, Brager AJ, Stowie AC, Prosser RA. Cocaine modulates pathways for photic and nonphotic entrainment of the mammalian circadian clock. Am J Physiol Regul Integr Comp Physiol 302: R740–R750, 2012. First published January 4, 2012; doi:10.1152/ajpregu.00602.2011.—Cocaine abuse is highly disruptive to circadian physiological and behavioral rhythms. The present study was undertaken to determine whether such effects are manifest through actions on critical photic and nonphotic regulatory pathways in the master circadian clock of the mouse suprachiasmatic nucleus (SCN). Impairment of SCN photic signaling by systemic (intraperitoneal) cocaine injection was evidenced by strong (60%) attenuation of light-induced phase-delay shifts of circadian locomotor activity during the early night. A nonphotic action of cocaine was apparent from its induction of 1-h circadian phase-advance shifts at midday. The serotonin receptor antagonist, metergoline, blocked shifting by 80%, implicating a serotonergic mechanism. Reverse microdialysis perfusion of the SCN with cocaine at midday induced 3.7 h phase-advance shifts. Control perfusions with lidocaine and artificial cerebrospinal fluid had little shifting effect. In complementary in vitro experiments, photic-like phase-delay shifts of the SCN circadian neuronal activity rhythm induced by glutamate application to the SCN were completely blocked by cocaine. Cocaine treatment of SCN slices alone at subjective midday, but not the subjective night, induced 3-h phase-advance shifts. Lidocaine had no shifting effect. Cocaine-induced phase shifts were completely blocked by metergoline, but not by the dopamine receptor antagonist, fluphenazine. Finally, pretreatment of SCN slices for 2 h with a low concentration of serotonin agonist (to block subsequent serotonergic phase resetting) abolished cocaine-induced phase shifts at subjective midday. These results reveal multiple effects of cocaine on adult circadian clock regulation that are registered within the SCN and involve enhanced serotonergic transmission.

Cocaine's interaction with the circadian system is evidenced by strong (60%) attenuation of light-induced phase-delay shifts of circadian locomotor activity during the early night. A nonphotic action of cocaine was apparent from its induction of 1-h circadian phase-advance shifts at midday. The serotonin receptor antagonist, metergoline, blocked shifting by 80%, implicating a serotonergic mechanism. Reverse microdialysis perfusion of the SCN with cocaine at midday induced 3.7 h phase-advance shifts. Control perfusions with lidocaine and artificial cerebrospinal fluid had little shifting effect. In complementary in vitro experiments, photic-like phase-delay shifts of the SCN circadian neuronal activity rhythm induced by glutamate application to the SCN were completely blocked by cocaine. Cocaine treatment of SCN slices alone at subjective midday, but not the subjective night, induced 3-h phase-advance shifts. Lidocaine had no shifting effect. Cocaine-induced phase shifts were completely blocked by metergoline, but not by the dopamine receptor antagonist, fluphenazine. Finally, pretreatment of SCN slices for 2 h with a low concentration of serotonin agonist (to block subsequent serotonergic phase resetting) abolished cocaine-induced phase shifts at subjective midday. These results reveal multiple effects of cocaine on adult circadian clock regulation that are registered within the SCN and involve enhanced serotonergic transmission.

circadian rhythm; suprachiasmatic nucleus; light; serotonin; glutamate; dopamine
PA). The data were collected in 1-min bins, and activity onset was associated with lights-off (designated as zeitgeber time [ZT] 12) as defined by the initial 6-min period that 1) coincided with an intensity of activity that exceeded 10% of the maximum rate for the day; 2) was preceded by at least 4 h of activity quiescence; and 3) was followed by at least 60 min of sustained activity. Under constant darkness (DD), activity onset is designated as circadian time (CT) 12 and is the phase reference point for the onset of the subjective night. Phase shifts were calculated as the difference between the projected times of activity onset of baseline entrainment and days following the cocaine/photic treatment as determined by 1) back extrapolation of the least-squares line through activity onsets on days 3–7 after cocaine/photic treatment and 2) extrapolation of the least-squares line calculated from activity onset data collected the last 5 days of baseline entrainment. Assessments of changes in activity (duration and intensity) after cocaine or saline injection were undertaken using data exported from the Clocklab data acquisition system. An activity count represented an individual event registered by an overhead infrared sensor. An activity bout was defined as the sum of activity counts collected in a 1-min bin. Activity duration represented the length of increased activity bouts (relative to pretreatment level) immediately following cocaine or saline treatment at midday. Activity intensity was the number of bouts integrated over the duration of response.

SCN Reverse Microdialysis

The reverse-microdialysis procedures are similar to those described in our previous studies on SCN neurotransmitter release (21). Centrally designed microdialysis probes were constructed from a 26-gauge stainless-steel outer cannula, into which was inserted a beveled 32-gauge fused silica tube (Polymeric Technologies, Phoenix, AZ). Hemicellulose dialysis tubing (12 kDa MW cutoff; 230 μm OD; Spectra/Por; Fisher Scientific, Pittsburgh, PA) was inserted 1.0 mm to a length of 1.0 mm, and the tip was sealed with epoxy. Animals received a probe implant with the tip aimed at the lateral margin of the SCN (coordinates: AP: −0.46 mm from bregma, L: +0.2 mm from midline, H: −5.5 mm from dura; head level). Following 48 h of recovery, animals were connected to the inflow and outflow tubings. Artificial cerebrospinal fluid (ACSF) with or without cocaine was perfused through the probe at a rate of 1.0 μL/min using a calibrated syringe pump (CMA/100; Bioanalytical Systems West Lafayette, IN). Probe tip placement was verified histologically at the end of the experiment by staining 20-μm SCN cryosections with cresyl violet. To obtain an in vitro estimate of probe efficiency of cocaine delivery via our probes, a labeled structural analog of cocaine (125I-labeled RTI-55; PerkinElmer, North Billerica, MA) was measured in dialysate samples collected from probes (n = 3) submerged in a known 125I-labeled RTI-55 standard at a perfusion flow rate of 1 μL/min at 37°C. Under these conditions, probe efficiency was calculated as 20%, and this value was used to estimate cocaine dosage delivered from the probes in the reverse-dialysis trials. It should be noted, however, that this estimate may overestimate release of cocaine from the probe in vivo, owing to tissue elements affecting drug diffusion into the brain. In this regard, the in vivo release of another lipophilic compound, ethanol, is <20% less than that occurring in vitro (Glass JD and Brager AJ, unpublished observations).

Brain Slice Preparation and Single-Unit Recording

Coronal brain slices (500 μm) containing the SCN were prepared during the daytime from adult mice (2–5 mo old), housed in 12:12 LD conditions, as reported previously (60, 61, 63). Slices were prepared between ZT 0 and 4. Slices were maintained at the interface of a Hatton-style brain slice chamber, where they were perfused continuously with warm (37°C), oxygenated (95% O2–5% CO2), glucose/bicarbonate-supplemented Earle’s balanced salt solution (EBSS; MP Biomedical, Solon, OH), at pH 7.4–7.5. Gentamicin (0.05%) was also added to the perfusion medium. All drugs were prepared in warm, oxygenated EBSS and were bath-applied to the brain slices. At the onset of the drug treatments, perfusion of the standard medium was stopped, the medium was completely removed from the chamber, and fresh medium containing the drugs was applied. Previous experiments have demonstrated that changing the perfusion medium by itself does not affect the phase of the circadian clock. The procedure for neuronal recordings has been described previously (60, 63). Briefly, the spontaneous activity of single SCN neurons was recorded extracellularly using glass capillary microelectrodes filled with 3 M NaCl. Each neuron was recorded for 5 min, and the data were stored for later determination of firing rate using a DataWave system (Berthoud, CO). Typically, 4–7 cells were recorded during each hour. These individual firing rates were then used to calculate 2-h running averages, lagged by 1 h (± SE) to obtain a measure of the population’s neuronal activity. As in previous studies (56, 60), the time of peak neuronal activity was assessed visually by estimating, to the nearest quarter hour, the time of symmetrically highest activity. For example, if the two highest 2-h means are equal, then the time of peak is estimated to be halfway between them. Phase shifts were calculated as the difference in time-of-peak of untreated slices vs. drug-treated slices. When using these methods, the consistency of the results obtained for each experimental manipulation is such that differences in phase of as little as 1 h are often statistically significant with few (n = 2 to 3) replicates (e.g., 60, 64).

Drugs

Cocaine hydrochloride, metergoline (5-HT1A receptor antagonist), fluphenazine dihydrochloride (dopamine D1/D2 receptor antagonist), 8-hydroxy-2-(di-N-propylamino)tetralin (DPAT; 5-HT2A agonist), glutamate, and lidocaine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO).

Experimental Protocols

Effects of acute systemic administration of cocaine on photic phase resetting. This experiment was undertaken to determine whether acute cocaine perturbs photic phase resetting in vivo. Mice under LD were individually caged, and their daily activity rhythms were measured for a 2-wk period prior to experimentation. On the day of experimentation, mice received an intraperitoneal injection of cocaine (20 mg/kg; n = 7) dissolved in physiological saline or saline alone (n = 7) 15 min preceding a 30-min phase-delaying light pulse (25 lux) delivered from ZT 16 to 16.5. Immediately following the light pulse, the animals were released into DD for 2 wk to assess the extent of phase-delaying using an Aschoff Type II procedure (12). Release of animals into DD is used to reveal the extent to which a phase-resetting treatment shifts clock time in the absence of an entraining photocycle that would otherwise mask a phase-resetting effect. An additional control, in which mice received the same cocaine treatment, but no light pulse (n = 4), was undertaken to determine whether cocaine alone has a phase-resetting response at this time.

Behavioral phase-resetting effects of acute systemic cocaine administration. The phase-resetting effect of cocaine was explored to determine whether systemically administered cocaine can act as a nonphotic shifting stimulus. Mice under LD were caged individually, and their circadian locomotor activity rhythms were measured over a 2-wk period prior to experimentation. On the day of experimentation, animals received an injection of cocaine (20 mg/kg; n = 7) dissolved in physiological saline or saline alone (n = 7) at ZT 6, coinciding with the phase-advancing portion of the nonphotic phase-response curve. Immediately following drug injection, the animals were released into DD for 2 wk to assess phase-advancing responses, according to the Aschoff Type II procedure. In a separate trial, mice received an injection of the 5-HT receptor antagonist, metergoline, (10 mg/kg ip) 15 min prior to cocaine (n = 9) or saline (n = 6) injection to explore a serotonergic pathway for cocaine phase-shifting actions on circadian timing.
Phase resetting through microdialysis administration of cocaine into the SCN. In this experiment, cocaine was administered directly to the SCN region using reverse microdialysis perfusion to determine whether the SCN is a direct target of cocaine’s nonphotic phase-resetting effect. Mice under LD were singly caged, and their circadian activity rhythms were measured over a 2-wk period prior to experimentation. Two days prior to experimentation, the animals were surgically outfitted with a microdialysis probe stereotaxically aimed at the lateral margin of the SCN. On the day of experimentation, microdialysis probes were perfused with ACSF alone (n = 4) or ACSF containing cocaine (250 μM or 500 μM; n = 4/dose) from a syringe pump. On the basis of in vitro probe efficiency of ~20%, this provided theoretical cocaine tissue concentrations of 50 μM or 100 μM outside the probe. These dosages were based on those from our in vitro trials, which had a phase-resetting action. Continuous 80-min perfusion of ACSF alone or ACSF+cocaine began at ZT 6. The animals were released into DD at the onset of perfusion to assess phase-shifting responses, according to the Aschoff Type II procedure. Following experimentation, probe placement was verified histologically from fixed frozen sections stained with cresyl violet. An additional group of mice (n = 4) received SCN reverse dialysis of lidocaine (50 μM) to control for a possible anesthetic action of cocaine on clock phase.

In vitro phase-resetting experiments using the SCN brain slice preparation. Drugs were bath-applied for 10 min to SCN slices at either ZT 6 or ZT 16 during the initial day in culture. In blocking experiments, metergoline (10 μM) or fluphenazine (50 μM) was applied alone for 5 min prior to, and continued 5 min after, the 10-min coapplication with cocaine at ZT 6. As a control for possible anesthetic effects of cocaine, slices were treated with lidocaine (50 μM) at ZT 6. In the desensitization experiments, 0.01 μM DPAT was applied for 2 h starting at ZT 4. At ZT 6, this was replaced with 50 μM cocaine for 10 min. Previous experiments have demonstrated that this concentration of DPAT does not induce phase shifts by itself (64). In the glutamate experiments, cocaine was applied 5 min prior to and extended 5 min after its 10-min coapplication with glutamate (1 μM) at ZT 16. Extracellular single-unit activity recordings commenced near the beginning of the second day in vitro.

Statistics

The in vivo effects of cocaine on photic and nonphotic phase-resetting and locomotor activity were assessed by two-way ANOVA. A Student Neuman-Keuls post hoc comparison test was utilized when the ANOVA revealed significant treatment effects. The in vitro results were assessed using ANOVA. In all cases, the level of significance was set at P < 0.05.

RESULTS

Cocaine Attenuates Circadian Photic Phase Resetting In Vivo

There was a significant attenuating effect of cocaine on the phase-delaying response to photic stimulation at ZT 16. Vehicle controls receiving intraperitoneal saline injection had light-induced phase-delay shifts averaging 1.50 ± 0.10 h. Mice pretreated with 20 mg/kg cocaine had significantly smaller phase-delay shifts that averaged only 0.60 ± 0.20 h (F1,6 = 15.9; P < 0.01; Fig. 1). Cocaine in the absence of a light pulse had no phase-shifting effect (0.00 ± 0.00 h; P = 1.00). Representative actograms of these results are shown in Fig. 2.

Cocaine Induces Circadian In Vivo Phase-Advance Shifts at Midday

Acute intraperitoneal injection of cocaine at midday (ZT 6) induced phase-advance shifts averaging 1.0 ± 0.3 h vs. 0.3 ± 0.1 h and 0.1 ± 0.1 h for saline and uninjected controls, respectively (F2,7 = 9.8; P < 0.01; Fig. 3). Pretreatment with the 5-HT1A,2,7 receptor antagonist, metergoline (10 mg/kg ip), significantly attenuated the phase-advancing action of cocaine, while metergoline alone had no shifting effect vs. saline controls (0.2 ± 0.3 h and 0.2 ± 0.1 h, respectively; F3,9 = 1.2; P > 0.05). Representative actograms of the different treatment groups are presented in Fig. 4.

Cocaine and Locomotor Activity

Quantitative assessments of the duration and intensity of locomotor response immediately following acute cocaine and control treatments were undertaken, because behavioral arousal per se could be causally associated with the circadian phase-resetting responses. These analyses revealed that activity duration following cocaine was equivalent to saline, but 1.4 times greater than uninjected controls (F2,12 = 45.7; P < 0.05). Activity intensity was ~2 times greater following cocaine compared with saline or to no injection (F2,12 = 6.1; P < 0.05).

The SCN is a target for the phase-shifting effects of cocaine. Localized reverse-dialysis perfusion of the SCN region with cocaine at midday in C57 albino mice significantly advanced circadian phase. Perfusion of the SCN with ACSF caused small phase-advance shifts that averaged 0.3 ± 0.2 h. In contrast, perfusions of the SCN with 50 μM and 100 μM cocaine in ACSF (estimated tissue concentrations) induced larger phase-advance shifts of 2.2 ± 0.7 h and 3.7 ± 0.9 h, respectively (F1,12 = 16.4; P < 0.01 vs. ACSF; Fig. 5). Control perfusion with 50 μM lidocaine had no phase-resetting effect (0.05 ± 0.17 h). Representative actograms of the different treatment groups are shown in Fig. 6.

In complementary in vitro experiments, treatment of SCN-containing brain slices with cocaine (1–100 μM) at ZT 6 dose-dependently phase-advanced the circadian rhythm of spontaneous single-unit neuronal activity. Maximal cocaine-shifting effect (3.07 ± 0.2 h; P < 0.01 vs. untreated controls) was attained at 50 μM (Fig. 7). Coapplication of metergoline...
completely blocked the phase-resetting action of 50 μM cocaine (0.3 ± 0.2 h phase advance; P < 0.01 vs. cocaine), while metergoline alone had little effect (0.2 ± 0.2 h phase shift). Coapplication of the dopamine antagonist, fluphenazine had no attenuating effect on cocaine shifting (mean phase shift = 3.15 ± 0.4 h). The phase-resetting action of cocaine was not replicated by application of 50 μM lidocaine to SCN brain slices at ZT 6 (mean phase shift = −0.47 ± 0.18 h).

An additional set of experiments further explored the involvement of 5-HT receptors in cocaine’s phase-resetting actions. Previous research has demonstrated that in vitro pretreatments that generate a low level of serotonergic signaling in the SCN brain slice block subsequent phase shifts induced by 5-HT agonists applied at ZT 6 (64), consistent with a down-regulation of serotonin receptors. We tested whether cocaine-induced phase shifts show a similar sensitivity. SCN-containing brain slices were initially treated for 2 h with 0.01 μM DPAT, starting at ZT 4, and then they were treated for 10 min with 50 μM cocaine at ZT 6. As shown in Fig. 8, cocaine treatment under these conditions failed to phase-advance the rhythm in neuronal activity (mean phase shift = −0.27 ± 0.14 h, n = 3).

Consistent with its in vivo effects on photic phase resetting, cocaine also attenuated in vitro phase delays induced by glutamate application. As shown in Fig. 9, treating SCN slices with 1 mM glutamate at ZT 16 induced a 2.26 ± 0.2-h phase delay that was completely blocked by coapplication of 50 μM cocaine (0.02 ± 0.27 h phase delay; P < 0.01 vs. glutamate alone). Cocaine treatment alone at ZT 16 had no effect (0.0 ± 0 h phase shift). This effect of cocaine was also dose dependent, with 10 μM cocaine having little inhibitory effect on glutamate-induced phase delays.

**DISCUSSION**

Cocaine administration and withdrawal produce major disturbances of the daily patterns of circadian-timed homeostatic functions, including endocrine, autonomic, and immune processes, as well as sleep and feeding (16, 28, 29, 32, 53, 90). These actions indicate that cocaine may directly and/or indirectly influence the integrity of circadian clock timekeeping, which could increase susceptibility to drug abuse and addiction. At present, however, relatively little is known concerning what direct effects, if any, cocaine has on the adult circadian timing system. Here, we confirm that cocaine’s effects include direct actions on the SCN clock itself. These drug effects are pervasive, as they extend to functions critical to photic, as well as nonphotic, regulation of clock timing. Notably, acute cocaine markedly attenuates the circadian phase-resetting response to photic input, the primary entrainment stimulus for timing the 24-h sleep-activity cycle. Moreover, cocaine acts in a phase-dependent manner to produce serotonin-like circadian phase shifts directly within the SCN clock at midday, but not at night (characteristic of nonphotic phase-response curves). These results indicate that the circadian clock is vulnerable to cocaine’s actions, which could underlie the pathological effects of cocaine abuse on behavioral, physiological, and endocrine functions associated with drug abuse and addiction.
Cocaine Modulates Circadian SCN Clock Activity

Literature on the effects of cocaine on the adult circadian system is relatively sparse, and in humans, limited primarily to clinical and anecdotal reports, suggestive of a link between cocaine abuse and circadian clock disruption. For example, cocaine dampens circadian fluctuations in immune and autonomic functions and can impair sleep (36). In more numerous laboratory animal studies, cocaine has been shown to affect the daily timing and/or pattern of multiple rhythmic functions. In rats, cocaine self-administration blunts diurnal corticosterone and prolactin rhythms up to several days postadministration (40). Also, cocaine self-binging dampens diurnal fluctuations in body temperature and heart rate (82). With respect to behavioral interactions, repeated systemic administration (subcutaneous) of cocaine over several days disrupts the normal 24-h pattern of feeding behavior, with food intake increased during the light phase and decreased during the dark phase of the LD cycle (29). Chronic systemic cocaine treatment (intraperitoneal) also disrupts the diurnal rhythm of wheel-running by increasing running during the light phase, even on days without cocaine treatment (11).

Although these results are suggestive of cocaine-mediated effects on circadian timing, a direct action of cocaine on clock functioning has never been explored. It is possible that the blunting effects of cocaine on hormonal and physiological rhythms are due to effects on central and/or peripheral systems modulating these functions, instead of effects on circadian timing per se. Also, the daily anticipatory reactions to cocaine that persist after withdrawal could reflect involvement of an extra-SCN oscillator system, similar to that proposed for methamphetamine (the methamphetamine-sensitive circadian oscillator) (50), rather than SCN circadian clock entrainment.

The present results reveal that cocaine exerts effects directly within the SCN to affect circadian phase regulation. Notably,
cocaine’s daytime phase-resetting action could cause inappropriate adjustments in rhythms of sleep, feeding, and other related behaviors. Interestingly this effect of cocaine is phase-dependent, in that cocaine did not induce phase shifts when administered alone at ZT 16. The daytime phase-shifting effect of cocaine is likely manifest through enhanced SCN serotonergic activity, because SCN treatment with the 5-HT antagonist, metergoline, completely blocked this effect of cocaine. Like-

Fig. 6. Representative double-plotted actograms of general locomotor activity showing the phase-advancing effect of direct reverse microdialysis perfusion of the SCN with cocaine (50 μM) or ACSF at midday (ZT 6). The blank days on the actograms represent the postsurgical recuperative period, where activity was not measured. Asterisks designate time of perfusion and release into constant darkness. Shaded area represents exposure to DD.

Fig. 7. Cocaine phase-advances the SCN clock at midday (ZT 6) in vitro. A: 2-h means ± SE of SCN neuronal activity in individual experiments. Top: neuronal activity peaks near ZT 6 on the second day in vitro in a control (no drug) experiment. Middle: neuronal activity peaks 3 h earlier after treatment with cocaine (50 μM) applied alone at ZT 6, indicating the SCN clock had been phase-advanced by 3 h. Bottom: co-application of the 5-HT antagonist, metergoline (Meterg; 10 μM) with cocaine completely abolishes the phase-resetting effect of cocaine. Co-application of the dopamine antagonist fluphenazine (Fluphen; 50 μM) with cocaine had no effect on cocaine phase-resetting. Lidocaine alone (50 μM) had no phase-resetting action. Horizontal solid bars denote time of lights-off in the animal colony; vertical bars denote time of drug treatment; and dotted line denotes mean time-of-peak in control experiments. B: histogram plot of mean phase shifts induced by the different in vitro treatments at ZT 6. C: dose-response curve for cocaine-induced phase advances. a,b,c For each graph, bars or closed circles (representing means ± SE) with different letters show significant difference (P < 0.05). n = 3 mice for each group.
wise, in vitro phase shifts induced by cocaine were blocked by cotreatment with metergoline, as well as by a serotonin-desensitizing pretreatment with DPAT. The basis of this action could be cocaine’s impairment of the serotonin transporter, resulting in increased extracellular levels of 5-HT in the SCN. Within the hypothalamus, the ability of cocaine to bind to serotonin transporters is evidenced by the total displacement of the cocaine analog (H25I-labeled RTI-55) by the selective 5-HT transport blocker, citalopram (79). Moreover, microdialysis measurements have shown that systemic administration of cocaine causes large (300–500%) increases in extracellular levels of 5-HT in various brain regions, including the nucleus accumbens (18, 27, 56), and localized perfusion of cocaine into this structure causes a striking (1,300%) elevation in 5-HT levels (9, 18). The less robust effect of systemic cocaine administration on 5-HT is thought to reflect a limiting effect of raphe 5-HT1A autoreceptor stimulation that suppresses neuronal discharge and 5-HT release in terminal fields (10, 19, 20, 26).

One consideration basic to our data supporting serotonergic mediation of cocaine’s effects in the SCN relates to the patency of serotonergic terminals in the slice preparation, subsequent to deafferentation. In particular, the blocking action of metergoline on cocaine phase resetting assumes continued release of 5-HT in the slice. Evidence for such release comes from previous research demonstrating serotonergic phase resetting in response to 5-HT reuptake (fluoxetine) and/or precursor (l-tryptophan) treatments to SCN brain slices. These observations support continued serotonin synthesis and output from nerve terminals under these in vitro conditions (64, 76, 77).

![Fig. 8. Cocaine phase advances are blocked by desensitizing serotonergic pretreatment.](image)

![Fig. 9. Glutamate-induced phase delays are inhibited by cocaine in vitro.](image)
It must also be noted that cocaine enhances extracellular dopamine (DA) levels by inhibiting DA transporters, and this could participate in its circadian phase-resetting action. However, although DA is important for fetal SCN clock entrainment, it is most likely not involved in the intra-SCN cocaine phase resetting demonstrated here, as the adult SCN does not respond to DA agonist stimulation (85). Moreover, our in vitro treatment of the SCN with the dopamine antagonist, fluphenazine, had no attenuating effect on cocaine phase resetting. Nevertheless, a DA-mediated phase-resetting action of cocaine on extra-SCN (reward) pathways is possible in view of our preliminary results showing that in vivo application of cocaine to the ventral tegmental reward area induces phase-advance shifts at midday that are independent of induced locomotor activity (Glass JD and Brager A, unpublished observations).

The putative 5-HT-mediated effect of cocaine in the SCN is highly relevant to the proposed role of 5-HT in regulating SCN nonphotic phase resetting. The SCN contains one of the highest concentrations of 5-HT in the forebrain, and 5-HT can act in the SCN to mediate nonphotic circadian phase resetting. Agonists of 5-HT reset the SCN clock in vitro (46, 60, 63, 73) and in vivo (17, 24, 25, 57, 81). Also, nonphotic phase-resetting stimuli (wheel-running and sleep deprivation) increase 5-HT release in the SCN (21, 31), while depleting central 5-HT inhibits nonphotic phase resetting evoked by wheel running (42, 80). The ability of DPAT to induce phase shifts (37, 49) and the blockade of these shifts by intra-SCN application of 5-HT2,7 and 5-HT7 receptor antagonists, ritanserin and DR4004, respectively (25, 37), strongly implicate SCN 5-HT7 receptors in this response. Conversely, the ability of DPAT to induce phase shifts in SCN brain slices prepared from 5-HT7 knockout mice (77) and the inability of the 5-HT7 antagonist, mesulergine, to prevent 5-HT-induced phase shifts in vitro (78) suggest that 5-HT7 receptors may also participate in intra-SCN serotonergic phase resetting.

The second major modulatory effect of cocaine on circadian timing is its marked attenuation (~60%) of phase resetting by light. This is significant, as the SCN circadian clock synchronizes to the external environment largely through photic information conveyed from the retina to the clock. The principal pathway is a monosynaptic projection from retinal ganglion cells to the SCN, the retinohypothalamic tract (RHT), which is necessary for entrainment of the SCN pacemaker (34, 52, 58). Impairment of this entrainment mechanism by cocaine use could cause marked disturbances in the timing of behavioral and other circadian clock-regulated functions, and such desynchrony could be exacerbated by cocaine’s nonphotic shifting effect outlined above. The mechanism underlying the attenuating effect of cocaine on photic phase resetting may again be due to increased 5-HT signaling, since 5-HT is a potent negative modulator of RHT-mediated signaling in the SCN. This is based on findings that 5-HT receptor agonists attenuate various light-activated SCN responses, including increased neuronal activity (48, 66, 87), immediate-early gene (c-fos) gene expression, and behavioral phase resetting (66, 67, 72). Importantly, our present in vitro results demonstrating cocaine-induced inhibition of glutamate-induced phase shifts in SCN-containing brain slices indicates that cocaine acts postsynaptically rather than by modulating presynaptic glutamate release. For example, it could be mediated by serotonergic activation of 5-HT5A or 5-HT7 receptors expressed on retinorecipient SCN cells (87).

Short-Term Effects of Cocaine on Locomotor Activity

Acute treatment with cocaine produces psychomotor stimulation for extended periods following acute injection in mice and rats. Our analyses showed that the intensity, but not duration, of cocaine-induced locomotor activity was greater (approximately twofold) than that induced by saline injection. In a previous study, cocaine-induced activity in mice [measured over a shorter (30 min) postinjection interval] was ~5-fold greater than saline (1), and in rats, cocaine elevates ambulatory activity for 4 h (11). From a circadian perspective, the activity induced by cocaine could be significant, as locomotor activity/arousal can induce nonphotic phase-advance shifts at midday in hamsters (15). Thus, the increased activity rather than a direct chronotypic action of the drug per se could be responsible for the phase shifts observed here. Two lines of evidence argue against this possibility, however. First, as opposed to hamsters and rats, mice do not exhibit the same degree of circadian shifting response to acute activity pulses as do other animal models, such as hamsters (42). Second, cocaine’s phase-shifting action in the isolated SCN brain slice preparation confirms that cocaine can induce phase shifts independent of an increase in locomotor behavior.

SCN is a Direct Target for Cocaine

The present demonstration of a strong phase-shifting response to direct administration of cocaine to the SCN (both in vivo and in vitro) is the first evidence showing that the adult SCN is responsive to cocaine. Our results also demonstrate that cocaine can act directly in the SCN to block photic phase resetting. It is unlikely that these localized effects of cocaine are related to its anesthetic properties, as our in vivo and in vitro control applications of lidocaine to the SCN had no chronotropic effect. Cocaine’s actions in the SCN strongly suggest that it could impair the synchrony of multiple physiological and behavioral rhythms controlled by the SCN master circadian clock. Thus, these results have significant health implications concerning the disruptive effects of cocaine on daily rhythmic activities throughout the brain and periphery, and they place cocaine on the list of abused drugs with direct action in the SCN, including fentanyl (84), cannabinoids (2, 71), and ethanol (45, 62, 69). It is important to note that the present results do not rule out possible phase-resetting actions of cocaine mediated by regulatory sites projecting to the SCN, such as the midbrain raphe nuclei, whose neuronal activities (c-fos expression) are activated by cocaine (55), or the IGL that expresses 5-HT transporters (6) that could be targeted by cocaine. Both of these areas are also target sites where 5-HT has been shown to reset the clock (17, 22, 23).

In the context of circadian substrate(s) for cocaine action, it also must be noted that a reciprocal linkage between cocaine and the circadian timing system exists (41). In addition to the present demonstration of cocaine’s direct interaction with the circadian clock, this drug dramatically affects a variety of other circadian-related processes. For example, cocaine significantly alters the expression of circadian clock genes (including Clock, Perl, and Per2) throughout the CNS (3, 38, 39, 77, 86, 88), which, in turn, influences the activities of multiple neurotrans-
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A.B., A.S., and R.A.P. prepared figures. J.D.G., A.B., A.S., and R.A.P. approved final version of manuscript; A.B.,

AUTHOR CONTRIBUTIONS

future studies to address the effects of chronic and binge
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namely, the photic and nonphotic entrainment pathways. Such
regulatory systems that maintain proper circadian phase:
regulation. Notably, cocaine alters two of the fundamental
Perspectives and Significance

The present study is the first to confirm that acute cocaine
exposure directly affects adult SCN circadian clock phase
regulation. Notably, cocaine alters two of the fundamental
regulatory systems that maintain proper circadian phase: namely, the photic and nonphotic entrainment pathways. Such
effects could impair the ability of the clock to maintain normal
synchrony with the outside environment and to maintain har-
mony among multiple internal daily rhythmic processes. We
also provide evidence that cocaine’s effects in the SCN are
mediated by elevated serotonergic tones, possibly produced by
suppressed 5-HT transporter activity. It will be important in
future studies to address the effects of chronic and binge
cocaine applications on the circadian system.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.D.G. and R.A.P. conception and design of research;
J.D.G. and R.A.P. interpreted results of experiments; J.D.G. and R.A.P. drafted
manuscript; J.D.G., A.B., A.S., and R.A.P. edited and revised manuscript;
J.D.G., A.B., A.S., and R.A.P. approved final version of manuscript; A.B.,
A.S., and R.A.P. performed experiments; A.B., A.S., and R.A.P. analyzed data;
A.B., A.S., and R.A.P. prepared figures.

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