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Time of day differences in dopamine clearance in the rat medial prefrontal cortex and nucleus accumbens

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Abstract

Circadian rhythms influence cocaine-seeking behavior in rats, and this behavior may be mediated by variability in the rate of extracellular dopamine clearance across the day:night cycle. We used rotating disk electrode voltammetry to examine dopamine clearance and inhibition of clearance by cocaine in the rat medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). Rats were housed under light:dark conditions (LD, 12 hr:12 hr) or in constant darkness (DD), the latter given just prior to the day of sacrifice. Tissue was collected at 4 hr intervals under LD and DD conditions. Under LD, dopamine clearance in both brain regions was greatest at 4 hr after lights on. Under DD, there was a blunted but still rhythmic pattern of dopamine clearance across the 24 hr cycle. Cocaine-induced inhibition of dopamine clearance in the mPFC was not different across the day:night cycle in rats under LD. Paradoxically, under DD, dopamine clearance in the mPFC was *enhanced* by cocaine at ZT16, 4 hr into the subjective night, and only minimally inhibited at other times. In the NAc, cocaine inhibition of dopamine clearance was lowest at ZT4 under LD, and did not vary under DD. We conclude that dopamine clearance varies in both a diurnal and possibly in a circadian manner in the mPFC, and in a diurnal manner in the NAc. These results indicate that light itself may be used to manipulate molecules implicated in drug addiction.

Keywords

circadian; cocaine; diurnal; dopamine transporter; reward

INTRODUCTION

Many drugs of abuse exert feelings of reward and lead to drug-seeking behavior and addiction. Dopamine neurotransmission in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) is recognized as an important mediator of these processes (Anderson et al., 2006; Kalivas and Volkow, 2005; McFarland and Kalivas, 2001; Park et al., 2002; Sanchez et al., 2003; Sun and Rebec, 2005). Psychostimulants such as cocaine and amphetamine act directly on the dopamine transporter (DAT), a NaCl-dependent membrane transporter that catalyzes the removal of dopamine from the synapse, resulting in reversal and/or inhibition of dopamine transport and increases in extracellular dopamine (Schenk, 2002; Sulzer et al., 1995). Predictably, alterations in DAT levels affect various cocaine- or amphetamine-induced behaviors in animals and humans (Dietz et al., 2005; Ritz et al., 1987; Silvia et al., 1997; Tyler-McMahon et al., 2001).

In the present study, we explored the influence of time-of-day on DAT function because of previous studies reporting time-of-day variation in dopamine levels within the caudate, NAc and prefrontal cortex (Castaneda et al., 2004; Feenstra et al., 2000; Paulson and Robinson, 1994; Paulson and Robinson, 1996; Schade et al., 1995) and in cocaine-seeking behaviors in mice, rats, and monkeys (Abarca et al., 2002; Akhisaroglu et al., 2004; Negus et al., 1995; Yuferov et al., 2005). However, no studies have examined the extent to which DAT function fluctuates in the NAc or mPFC across the day:night cycle, which may underlie some of the variation in cocaine-induced behaviors. We have previously shown that DAT protein (Sleipness et al., 2007b), cocaine-induced locomotor activity (Sleipness et al., 2005), and aspects of cocaine-induced CPP behavior (Sleipness et al., 2007a) vary with time of day in rats. In addition, we showed that these differences depend on the presence of a functioning master circadian pacemaker (Sleipness et al., 2007a; Sleipness et al., 2007b) that is located in the suprachiasmatic nucleus of the hypothalamus (SCN) (Moore, 1983; Reppert and Weaver, 2001). The circadian clock in the SCN and clocks known to exist in other brain regions and the periphery utilize clock genes to generate daily oscillation (Reppert and Weaver, 2001). Because clock gene products serve as circadian transcription factors either independently or under the influence of SCN outputs (Harms et al., 2004; Hastings and Herzog, 2004; Reppert and Weaver, 2002), they are positioned to generate rhythmicity in a wide array of biological systems. Interestingly, the promoter region of the DAT gene in mice, rats, and humans contains E-boxes, DNA sequences with an affinity for clock proteins (Kawarai et al., 1997; Sleipness et al., 2007b), which in turn can regulate several genes in a circadian fashion. In support of a temporal regulation, we previously found that expression of protein levels of both DAT and tyrosine hydroxylase (TH), the rate-limiting enzyme responsible for dopamine synthesis, vary with time of day, and this expression is dependent on the SCN (Sleipness et al., 2007b).

Nevertheless, an important unanswered question is whether the changes in DAT protein levels are reflected by changes in DAT *function*. To expand on our earlier work revealing time-of-day differences in DAT protein concentrations, the present study was conducted to test the hypothesis that DAT function, as measured by dopamine clearance, varies with time of day in the mPFC and NAc. Experiments were performed in tissue collected under different phases of the light:dark (LD) cycle to identify diurnal variation, and from tissue collected in animals housed in constant darkness (DD) to reveal circadian variation.

MATERIALS AND METHODS

Animals and Drugs

Male Sprague-Dawley rats (n = 30; Harlan, Indianapolis, IN) weighing 280–300 g were used. Animals were kept on a 12hr light:12hr dark (LD) light cycle (photoperiod) with *ad libitum* access to food and water. To examine dopamine clearance at different times of day in the absence of external light:dark cues, a separate group of rats (n = 30) was housed in LD as above, but on the day of tissue collection, the lights remained off (DD). For both groups of rats, nighttime sampling was facilitated by the use of a 15W red light in each room producing <5 lux (Mannix model DLM-1337 light meter, Lynbrook, NY) at the level of the rats' heads. This level of illumination does not produce a phase shift in locomotor activity in rats (Bauer, 1992). Testing animals in conditions of constant darkness (DD) allowed for the expression of endogenous rhythmicity, a hallmark of circadian functions (Dunlap et al., 2004). The experiments conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised 1996, and were approved by the Washington State University Institutional Animal Care and Use Committee. Cocaine hydrochloride and dopamine hydrochloride were obtained from Sigma (St. Louis, MO), and doses are expressed as the weight of the salt.

Tissue collection and rotating disk electrode voltammetry

Rotating disk electrode (RDE) voltammetry relies on oxidation or reduction of a dissolved analyte at the surface of an electrode rotating at a constant angular velocity, resulting in the production of a current whose magnitude corresponds directly to the amount of the analyte present (McElvain and Schenk, 1992). Furthermore, the current produced is independent of analyte diffusion, and RDE has therefore been useful for observing release and reuptake of neurotransmitters such as dopamine and norepinephrine in synaptosomal preparations, as well as for the investigation of mechanisms of neurotransmitter transporter function (Schenk et al., 2002).

Rats were decapitated in LD at Zeitgeber times (ZT): ZT0 (lights on), ZT4, ZT8, ZT12 (lights off), ZT16, or ZT20 or at the corresponding circadian times (CT) when housed under DD conditions (CT0, CT4, CT8, CT12, CT16, CT20; where CT0 is the time of subjective light onset). These times of day were chosen to provide a complete profile of dopamine clearance across the 24 hr cycle. The procedure for tissue collection is similar to that previously described (Sleipness et al., 2007b; Wayment et al., 2001). Individual animals were sacrificed at each ZT or CT and the tissue was collected on ice within 5 min of death. Dissections of mPFC and tissue punches containing the NAc (1.5 mm diameter, approximately 2 mm thickness) were collected and weighed. Synaptosome suspensions were then prepared by finely mincing the tissue by hand on an ice-cold Petri dish using an ice-cold razor blade. Tissue was then transferred into a 500 μ L incubation/RDE voltammetric cell kept at 37°C. After allowing the suspension to settle for 10 min, the preparation was washed 8 times with fresh 95% O₂/5% CO₂-saturated physiological buffer (51.3 mmol/L NaCl, 1.7 mmol/L KCl, 4.1 mmol/L CaCl₂, 1.5 mmol/L MgCl₂, 5.0 mmol/L HEPES, adjusted to pH 7.9).

A glassy carbon RDE (Pine Instruments, Grove City, PA), rotating at 2000 RPM, was inserted in the cell and a 450-mV potential relative to a Ag/AgCl reference electrode was applied using a Bioanalytical Systems Model LC-4B potentiostat (West Lafayette, IN; time constant = 200 ms). The current produced was measured using a Nicolet model 2090 Digital Oscilloscope (Madison, WI). After a stable baseline was acquired (10 min), dopamine was added to produce a final concentration of 0.9 μ M. The velocity of dopamine clearance, expressed as an initial rate (McElvain and Schenk, 1992), is reported as pmol/(sec $\times$ g wet tissue mass). The electrode was calibrated with a dopamine standard prepared each day to relate current to the mass of dopamine cleared.

After measuring the initial dopamine clearance, cocaine (dissolved in water as vehicle) was added to the suspension to a final concentration of 100 μ M and the synaptosomal preparation was allowed to incubate at 37°C for another 30 sec. Dopamine was then added (final concentration = 0.9 μ M) and the velocity of dopamine clearance in the presence of cocaine was determined once more. To ensure that the order of addition of dopamine and cocaine did not impact the results, a control experiment was performed (at ZT16 and CT16) in which only cocaine vehicle (water) was added rather than cocaine. Thus, for this experiment, 0.9 μ M dopamine was added as described above, and this was followed by cocaine vehicle and then another 0.9 μ M dopamine addition, exactly as described for the cocaine experiment.

Data analysis and statistics

To compare the effects of photoperiod (LD vs. DD) and time of day, a two-way analysis of variance (ANOVA) was conducted, with photoperiod as one factor and time-of-day as the second factor. In the case of a significant interaction, a post-hoc Bonferroni test was performed. To compare within photoperiod time-of-day effects, a one-way ANOVA was

conducted followed by a Tukey's test to compare the different times of day with ZT0 or CT0. To calculate cocaine-induced inhibition of dopamine clearance, the velocity of dopamine clearance in the presence of cocaine was divided by the velocity of dopamine clearance in the absence of cocaine, and multiplied by one hundred. Analysis for the cocaine inhibition experiments was identical to that described above. All ANOVA and post-hoc analyses were performed using Prism software for the Macintosh (V.4.0c; GraphPad Software, Inc.). To determine if the daily pattern of DAT function under LD and DD exhibited a cyclical trend, a one-cycle Kolmogorov-Smirnov (K-S) test was performed (Refinetti, 2006). The K-S test was performed on DAT velocity and percent inhibition by cocaine using onecycle software (v. 2.1) for the PC (Refinetti, 2006). Differences were considered statistically significant if $p < 0.05$.

RESULTS

Velocity of dopamine clearance

Figure 1 illustrates the effects of time of day and photoperiod on the velocity of dopamine clearance in mPFC and NAc suspensions. In the mPFC, under LD conditions, we found a significant effect of time of day on velocity ($F_{5,32} = 3.5$, $p = 0.01$), with dopamine being cleared faster at ZT4 (4 hrs after lights-on) compared with ZT0 (lights-on). In animals housed in both LD and DD conditions, the velocity of dopamine clearance was significantly different across the day:night cycle (K-S test, $p < 0.01$), although the effect was blunted in animals held in DD compared with animals held in LD. When we tested the effect of photoperiod and time of day on dopamine clearance velocity using a two-way ANOVA, we discovered both a main effect of photoperiod ($F_{1,66} = 14.66$, $p = 0.0003$) and an interaction between time of day and photoperiod ($F_{6,66} = 3.63$, $p = 0.004$). The effect of time of day approached significance ($F_{6,66} = 2.17$, $p = 0.057$). *Post hoc* analysis revealed that in the mPFC at ZT4, the rate of dopamine clearance was significantly greater than at CT4 (4 hrs after subjective light onset).

In the NAc of rats housed under LD, as in the mPFC, we found a significant effect of time of day on the velocity of dopamine clearance ($F_{5,32} = 5.8$, $p = 0.0009$). Similar to what we observed in the mPFC, dopamine was cleared from solution more rapidly at ZT4 than at ZT0. Under DD, there was no significant effect of time of day on the rate of dopamine clearance in the NAc. In animals housed in LD but not in DD conditions, the velocity of dopamine clearance was significantly different across the day:night cycle (KS test, $p < 0.01$). When we examined the effect of photoperiod and time of day on clearance velocity in the NAc using a two-way ANOVA, we found a main effect of photoperiod ($F_{1,68} = 26.41$, $p < 0.0001$), time of day ($F_{6,68} = 2.36$, $p = 0.04$), and an interaction between photoperiod and time of day ($F_{6,68} = 5.17$, $p = 0.0002$). However, opposite to the direction of changes we found in the mPFC, in the NAc, rats housed under DD displayed *greater* rates of dopamine clearance at CT0, 12, and 16 compared with ZT0, 12 or 16 in rats housed under LD.

In general, we found that the rate of dopamine clearance was higher in the NAc than in the mPFC, consistent with previous reports describing a relatively slow clearance rate of dopamine in the mPFC (Garris et al., 1993; Mundorf et al., 2001).

Cocaine inhibition of dopamine clearance

Figure 2 illustrates the effects of 100 μ M cocaine on dopamine clearance in the mPFC and NAc at different times of day. In general, the approximately 40% inhibition of dopamine clearance by cocaine in the mPFC under LD conditions (see Fig. 3) is consistent with previous work (Wayment et al., 2001). A one-way ANOVA revealed no significant effect of time of day on cocaine-induced inhibition of dopamine clearance velocity in the mPFC of

animals held in LD conditions. In contrast, a significant effect of clearance velocity in rats held in DD conditions ($F_{6,41} = 3.82$, $p = 0.005$) was observed, with the highest clearance rate occurring at CT16 compared to CT0. Rhythmicity was not evident in LD, but was present in DD conditions (K-S test, $p < 0.01$). A two-way ANOVA revealed a significant main effect of photoperiod on cocaine inhibition of dopamine clearance velocity ($F_{1,64} = 8.1$, $p = 0.0006$) and a significant interaction between photoperiod and time of day ($F_{6,64} = 3.0$, $p = 0.01$). No effect of time of day was observed. Dopamine clearance velocity in the presence of cocaine was significantly greater at CT16 and CT20 (DD conditions) than at ZT16 or ZT20 (LD conditions), respectively.

In the NAc, a one-way ANOVA revealed a significant effect of time of day on cocaine-induced inhibition of dopamine clearance velocity in animals held in LD conditions ($F_{6,33} = 7.69$, $p < 0.0001$), with a higher clearance velocity at ZT4 compared with ZT0. However, no significant time-of-day changes were found in rats held in DD conditions. Rhythmicity was evident in LD, but not in DD conditions (K-S test, $p < 0.01$). A two-way ANOVA revealed a main effect of time of day ($F_{6,60} = 3.8$, $p = 0.003$) but not photoperiod; however, there was an interaction between the two ($F_{6,60} = 4.4$, $p = 0.0009$). Specifically, clearance velocity was greater at ZT4 (LD condition) than at CT4 (DD condition) in the NAc.

Figure 3 is a plot of the same data from Figure 2 but reported as the percent inhibition of dopamine clearance by cocaine, and the insets to the graphs are representative dopamine clearance profiles. In the mPFC, one-way ANOVA produced results similar to the findings shown in Figure 2. Thus, a significant effect of ZT was observed only in DD ($F_{6,39} = 2.59$, $p = 0.04$) with significantly reduced inhibition by cocaine (enhanced uptake) occurring at CT16 compared with CT0. We also found that this uptake at CT16 was significantly greater than a theoretical mean of zero percent inhibition (one-sampled t-test). Rhythmicity was not evident in LD, but was present in DD conditions (K-S test, $p < 0.01$). A two-way ANOVA revealed no main effect of time of day; however, there was a significant effect of photoperiod ($F_{1,60} = 22.62$, $p < 0.0001$) and a significant interaction between the two ($F_{6,60} = 2.30$, $p = 0.05$). Specifically, the percent inhibition by cocaine was less at CT16 than at ZT16 in the mPFC.

In the NAc, there were no significant time-of-day or photoperiod effects in rats housed in DD or LD conditions using any of our three analyses.

The inset of Figure 3A shows representative dopamine clearance profiles from the mPFC of a single animal taken from the results shown in Figure 3A at CT16 in the absence and presence of cocaine. The velocity profile from the mPFC indicates that cocaine *enhanced* the rate of dopamine clearance in the presence of cocaine (clearance slope is *greater* than in the absence of cocaine), a result in contrast to a multitude of findings reporting cocaine-mediated inhibition of dopamine clearance in other brain tissue such as the striatum (see Introduction) or NAc (Figure 3B inset). The inset of Figure 3B shows representative dopamine clearance profiles from the NAc of a single animal taken from the findings shown in Figure 3B at CT16 in the absence and presence of cocaine. This is a typical profile of cocaine inhibition, which stands in contrast to that shown for the mPFC at this same time point under DD conditions (see above).

In both the mPFC and NAc, when vehicle was substituted for cocaine as a control for the second addition of $0.9 \mu\text{M}$ dopamine, there were no differences ($p > 0.05$) in dopamine clearance velocity when comparing pre- to post- cocaine vehicle addition at CT16 (data not shown; $F_{1,16} = 1.44$ and $F_{1,16} = 0.24$, respectively, $p > 0.05$).

DISCUSSION

While extracellular dopamine concentrations have been shown to fluctuate in a circadian fashion and to be sensitive to light (Castaneda et al., 2004), the present study provides a potential mechanism for this time-of-day profile *via* modulation of dopamine clearance in the NAc and mPFC. This is the first report to our knowledge that describes time-of-day changes in dopamine clearance rate in these two brain regions, which have been heavily implicated in drug addiction. We found that: 1) in the mPFC, dopamine clearance exhibited diurnal variation (LD condition) and possibly a circadian (DD condition) variation, although the latter was not verified by following multiple daily cycles, and in the NAc, diurnal but not circadian variation was exhibited, because this daily variation was lost in the absence of light on the day of sacrifice (DD condition); 2) In the mPFC, cocaine inhibited dopamine clearance at all times of day when rats were housed under LD. However, under DD conditions, cocaine inhibition was blunted, and at CT16, cocaine actually *enhanced* the rate of dopamine clearance in the mPFC; 3) in the NAc, cocaine inhibited dopamine clearance velocity similarly at all times of day under both LD and DD conditions with exception of a decreased inhibition 4 hr after light onset under LD conditions. Previous studies in the striatum have demonstrated time-of-day effects under both basal and stimulated conditions. Dopamine uptake in the striatum was reported as higher at ZT2 compared with ZT14, similar to what we found in the NAc (Wirz-Justice, 1974). In contrast, dopamine release and/or inhibition of uptake by amphetamine or fencamphamine in the striatum were greater in the light than in the dark phase (Becker et al., 1984; DeLucia et al., 1997). The disparity between our findings with cocaine in the NAc and the mPFC and these latter two studies may be related to the different psychostimulants used and hence, differences in drug mechanism of action, or to the different brain areas examined. Nevertheless, the results from our lab and others suggest that light itself can powerfully influence dopamine clearance rates in brain regions important for the expression of rewarding behaviors.

Medial prefrontal cortex

In the absence of cocaine, we observed the greatest velocity of dopamine clearance in the mPFC at ZT4 under conditions of a fixed (12L:12D) light:dark cycle. This is consistent with our earlier finding of elevated DAT protein expression in the mPFC at ZT4 compared with ZT20 (Sleipness et al., 2007b). Animals housed in complete darkness (DD) on the day of sacrifice displayed a blunted profile, but still maintained significant rhythmicity in dopamine clearance rate across the 24 hr cycle. This suggests that the diurnal variation in dopamine clearance in the mPFC is: 1) entrained by the ambient light:dark cycle (photoperiod), and 2) has the ability to oscillate (at least for one cycle) in the absence of light:dark transitions. It should be cautioned however, that additional studies need to be performed to determine the extent to which this oscillation is self-sustaining and to what extent it is entrainable to the light:dark cycle. As most extra-SCN oscillators in the brain usually dampen after several cycles in the absence of photic stimuli (Abe et al., 2002), the mPFC likely relies on the daily light:dark cycle to maintain its rhythmicity.

To our surprise, in the presence of cocaine, we saw no diurnal variation in dopamine clearance in the mPFC under LD conditions. In stark contrast, under DD conditions, dopamine clearance was either marginally inhibited or was even enhanced. Specifically, dopamine clearance at CT16 was significantly enhanced, rather than blocked, after cocaine addition. These observations further support the hypothesis that light is important for mediating dopamine clearance in the mPFC, presumably via modulation of DAT function. To our knowledge, this is the first demonstration of a light-dependent reversal of cocaine-mediated inhibition of dopamine clearance.

In addition to DAT, other monoamine transporters influence dopamine clearance. For example, the mPFC is extensively innervated by noradrenergic inputs arising from the brainstem (Devoto et al., 2005). Both DAT and norepinephrine transporters (NET) are expressed in the mPFC, and NET has a higher affinity for dopamine than does DAT (Eshleman et al., 1994; Giros and Caron, 1993; Gu et al., 1994), effectively removing dopamine from the extracellular space (Moron et al., 2002). Taken together, it is likely that both transporter systems modulate the minute-to-minute and daily patterns of extracellular dopamine in the mPFC.

Although the mechanism responsible for the cocaine-induced increase in dopamine clearance in the mPFC at CT16 remains unknown, one possibility is a contribution from either catechol-O-methyl transferase or monoamine oxidase (MAO). Indeed, we have previously shown that dopamine clearance in the mPFC is dependent on both a transporter process (DAT and NET) and a second process that is inhibited by MAO inhibitors (Wayment et al., 2001). Variation in MAO activity with time of day has been reported for other brain regions (Chevallard et al., 1981; Kamase, 1980). Therefore, it is possible that an MAO-dependent process to remove extracellular dopamine is upregulated in the presence of cocaine, compensating for DAT antagonism at certain times of day or under DD conditions. This possibility remains to be confirmed.

Nucleus accumbens

In the NAc, as in the PFC, we observed the greatest velocity of dopamine clearance at ZT4. This is somewhat unexpected based on our previous observation of greater DAT protein expression in the NAc at ZT20 compared with ZT4 (Sleipness et al., 2007b). Clearly, in this brain region there is not a simple, direct relationship between total DAT protein levels and the velocity of dopamine clearance (DAT function). Although the reason for this discrepancy is not known, one possibility is that there exists extensive modulation of DAT surface expression, or as described above, extensive NET activity. From the present data, we cannot determine whether a change in K_m and/or V_{max} of the transporter occurs over the day:night cycle, and further exploration of these mechanisms is needed.

Unlike in the mPFC, a rhythm of dopamine clearance in the NAc was lost when animals were housed in DD, suggesting that diurnal variation in dopamine clearance in the NAc depends on external light:dark cues. Intriguingly, the velocity of DA clearance at three of the six time points examined was elevated under DD compared to LD conditions, suggesting that the light:dark cycle has a tonic (and phasic) inhibitory influence on DAT function. The significance of this remains to be determined but represents yet another difference between the NAc and mPFC.

Cocaine inhibited dopamine clearance the least at ZT4 under LD conditions, and this may be a reflection of the high dopamine clearance rate in these same animals in the absence of cocaine. Under DD, no time-of-day changes in cocaine-mediated inhibition were observed, supporting the notion that the extent of inhibition of uptake by this drug is dependent on light:dark cues.

Light:dark modulation of dopamine clearance

The specific mechanism(s) responsible for producing the diurnal and potentially circadian variation in DAT function remains to be identified. Nevertheless, several candidate molecules for mediating DAT function over the course of the light:dark cycle are considered. One possible candidate is the pineal hormone, melatonin, which serves as an endocrine signal of dark onset (Reiter, 1991). Melatonin rhythms can persist in total darkness, albeit with reduced amplitude (Liu and Borjigin, 2005). Thus, melatonin may

serve as a mediator of diurnal variation in DAT function and possibly endogenous rhythmicity in the mPFC, where DAT function remained rhythmic in DD conditions.

A second candidate to mediate the time-of-day influence on DAT function is protein kinase C (PKC), which modulates DAT expression. Activation of PKC speeds up internalization and degradation of DAT (Daniels and Amara, 1999) while concomitantly decreasing its rate of return to the plasma membrane (Loder and Melikian, 2003). PKC activity also varies with time of day in the retina and SCN (Gabriel et al., 2001; Jansen et al., 2003), two regions containing self-sustaining oscillators. Given the involvement of the PKC pathway in the circadian system (Rivera-Bermudez et al., 2003; Shim et al., 2007), this protein and its auxiliary proteins may contribute to the diurnal variation in dopamine clearance reported here.

A third set of candidate molecules by which DAT function could be influenced by the light:dark cycle is the clock genes and proteins. As mentioned earlier, the promoter for the gene encoding DAT contains E-boxes (Kawarai et al., 1997), thus making it a potential target for clock gene products. The master circadian pacemaker in the SCN is essential for photic entrainment of circadian rhythms (Rea, 1998) and utilizes clock genes to generate rhythmicity (Hastings and Herzog, 2004; Reppert and Weaver, 2001). Indeed, our previous work revealed that daily variation in DAT protein expression in the mPFC was dependent on the SCN (Sleipness et al., 2007b). However, in addition to the SCN, clock genes are expressed in many other brain regions, including the ventral tegmental area dopaminergic neurons (McClung et al., 2005). Clock gene expression in various brain regions can therefore be either dependent or independent of SCN influences. For example, a *Per1* rhythm in the NAc and caudate can be abolished by pinealectomy in animals with an intact SCN (Uz et al., 2003), suggesting a role for the pineal gland in this rhythm. On the other hand, a rhythm of *Per2* in the extended amygdala is rhythmic but is dependent on the SCN (Segall et al., 2006). Because the SCN is necessary for entraining daily rhythms of melatonin secretion (Perreau-Lenz et al., 2003), which mediates the effects of changing photoperiod on the animal's physiology, the SCN can also serve as a transducer of daylength (Schwartz et al., 2001; Sumova et al., 2004). Therefore, it is possible that photoperiods other than the 12:12 LD cycle we used (e.g., longer days vs. shorter days) could also modulate DAT function.

A fourth candidate for time-of-day modulation of DAT function is the glucocorticoid, corticosterone, which modulates DAT binding to the DAT-selective ligand, RTI-121 (Sarnyai et al., 1998). Corticosterone is involved in the acquisition, maintenance and reinstatement phases of cocaine self-administration in rats (Deroche et al., 1997; Goeders and Guerin, 1996; Shalev et al., 2003). Cocaine self-administration disrupts the diurnal changes in this hormone (Mantsch et al., 2000) in rats, and in human cocaine addicts. Intriguingly, dysfunction of the hypothalamic-pituitary-adrenal axis may predict relapse rates (Fox et al., 2008; Sinha et al., 2006). This disruption in and persistence of altered corticosterone levels after repeated cocaine exposure could therefore disrupt the rhythm of clock gene expression in specific brain regions such as the amygdala (Segall et al., 2006), and so may influence DAT function as a downstream effect. Glucocorticoids are also implicated in maintaining the rhythms of extra-SCN clocks (Segall et al., 2006). Taken together, altered glucocorticoid secretion following cocaine administration could impact brain clocks in a variety of locations.

As many of the aforementioned molecules continue to exhibit rhythmicity in animals housed in constant conditions (e.g., DD), it is reasonable to propose a potential role for these in DAT fluctuations under constant conditions. Endocrine rhythms may be especially suited for this purpose as a means for synchronizing neural activity in multiple brain regions. Indeed,

both corticosterone (Moore and Eichler, 1972) and melatonin (Liu and Borjigin, 2005) exhibit free-running rhythmicity in DD conditions. Similarly, various PKC isoforms continue to exhibit rhythmicity in DD conditions in various brain regions including the caudate nucleus and SCN (Cagampang et al., 1998). It remains to be determined precisely how these factors influence the diurnal and potential circadian variation in DAT function.

Expansion of the Reward Potential model

Previously, we developed a conceptual model for integrating time-of-day information with the variable ability of stimuli to be rewarding (Sleipness et al., 2005). Specifically, we found robust expression of long-term locomotor sensitization to cocaine at ZT12, or dark onset, compared with other times of the day. Relative to the present study, it is possible that the reduction in mPFC dopamine clearance, and hence increased extracellular dopamine, at ZT12 relative to ZT4 would normally increase the reward potential of natural reinforcers such as food, water and sex in a nocturnal species to appropriately time a peak of reward potential early in the dark phase (i.e., at ZT12). Changes in DAT function in other brain regions (e.g., NAc) at other times of the day (i.e., at ZT4), could also critically mediate daily fluctuations in reward potential, although how this is mediated remains to be determined. Taken together, these daily regional and temporal changes could provide a mechanism for gating the expression of rewarding behaviors. Additionally, the dramatic influence of light on dopamine clearance in the mPFC in the presence of cocaine suggests that altering the light:dark ratio could provide a novel method for modulating the reward potential of drugs of abuse.

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Abbreviations

CT	circadian time
DAT	dopamine transporter
DD	constant darkness
LD, light	dark (12 hr:12 hr)
mPFC	medial prefrontal cortex
NAc	nucleus accumbens
NET	norepinephrine transporter
RDE	rotating disk electrode
SCN	suprachiasmatic nucleus
SEM	standard error of the mean, ZT, Zeitgeber time

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Figure 1.

Velocity of dopamine clearance at different times of day in the mPFC (A) and NAc (B) in the *absence* of cocaine. Data are means $\pm$ SEM. $N = 5-6$ per time point, per photoperiod. The data from ZT0 or CT0 are replotted as ZT24 and CT24, respectively, for clarity. ^b $p < 0.05$ within photoperiod, compared with ZT0. * $p < 0.05$ between photoperiods at the same time of day. + $p < 0.01$ Kolmogorov-Smirnov one-cycle test. Bars below graphs show lighting regimen under LD and DD, respectively (black areas indicate periods of darkness).

Figure 2.

Velocity of dopamine clearance at different times of day in the mPFC (A) and NAc (B) in the *presence* of cocaine. Data are means $\pm$ SEM. $N = 5-6$ per time point, per photoperiod. The data from ZT0 or CT0 are replotted as ZT24 and CT24, respectively, for clarity. ^b $p < 0.05$ within photoperiod, compared with ZT0. * $p < 0.05$ between photoperiods at the same time of day. + $p < 0.01$ Kolmogorov-Smirnov one-cycle test. Bars below graphs show lighting regimen under LD and DD, respectively (black areas indicate periods of darkness).

Figure 3.

Percent inhibition of dopamine clearance by 100 μ M cocaine at different times of day in the mPFC (A) and NAc (B). Data are means $\pm$ SEM and are re-plotted from the same animals shown in Figures 2A and B, respectively. $N = 5-6$ per time point, per photoperiod. The data from ZT0 or CT0 are replotted as ZT24 and CT24, respectively, for clarity. * $p < 0.05$ between photoperiods at the same time of day. Insets: representative dopamine clearance profiles before and after cocaine addition at CT16 under DD are shown for the mPFC (A) and the NAc (B). Note the opposite effect of cocaine on dopamine clearance rate in the mPFC vs. the NAc at this time point. + $p < 0.01$ Kolmogorov-Smirnov one-cycle test. Bars below graphs show lighting regimen under LD and DD, respectively (black areas indicate periods of darkness).