Published in final edited form as:

Exp Clin Psychopharmacol. 2012 April ; 20(2): 77–83. doi:10.1037/a0026034.

Influence of chronic dopamine transporter inhibition by RTI-336 on motor behavior, sleep and hormone levels in rhesus monkeys

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Abstract

Rationale—Dopamine transporter (DAT) inhibitors have been developed as a promising treatment approach for cocaine dependence. However, the stimulant effects of DAT inhibitors have the potential to disrupt sleep patterns, and the influence of long-term treatment on dopamine neurochemistry is still unknown.

Objectives—The objectives of this study were to (1) explore the stimulant-related effects of chronic DAT inhibitor (RTI-336) treatment on motor activity and sleep-like measures in male rhesus monkeys (*Macaca mulatta*; n=4) and (2) to determine the effect of drug treatment on prolactin and cortisol levels.

Methods—The effects of chronic (21 day) administration of the selective DAT inhibitor RTI-336 (1mg/kg/day; i.m.) were evaluated on locomotor activity, inactivity, and hormone levels. Subjects were fitted with a collar-mounted activity monitor to evaluate their motor activity, with 4 days of baseline recording preceding 3 weeks of daily saline or RTI-336 injections. Blood samples were collected immediately prior to and following chronic treatment.

Results—RTI-336 produced a significant increase in locomotor activity at the end of the daytime period compared to saline administration. During the 3-week treatment period, sleep efficiency was decreased and the fragmentation index and latency to sleep onset were significantly increased. Hormone levels were not changed throughout the study.

Conclusions—Chronic treatment with RTI-336 has a mild but significant stimulant effect, as evidenced by the significant increase in activity during the evening period which may cause minor disruptions in sleep measures.

Introduction

Cocaine is one of the most powerfully addictive drugs of abuse and remains as a major worldwide public health concern. However, no successful pharmacotherapies for cocaine abuse have been reported (see Volkow et al., 2004). It is well established that the actions of

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Conflict of interest: The authors declared no conflict of interest.

drugs of abuse, including cocaine, converge in the mesolimbic dopamine (DA) system and that this action is responsible for their reinforcing effects. Dopaminergic signaling in the brain is primarily modulated by dopamine transporters (DAT), which remove DA from the synapse by transporting it back into the presynaptic neuron. DAT activity can be regulated both acutely (minutes to hours) and longer-term (days) (Mortensen and Amara, 2003; Zahniser and Sorkin, 2004).

Given the direct importance of the DAT in the addictive properties of cocaine (Ritz et al., 1987; Wise et al., 1995), it is a logical target for developing pharmacological treatments for cocaine dependence. Several preclinical studies demonstrate that DAT inhibitors can effectively attenuate cocaine self-administration (Nader et al., 1997; Lindsey et al., 2004; Howell et al., 2000, 2007; Czoty et al., 2010) and drug effectiveness is correlated with DAT occupancy (Wilcox et al., 2002; Lindsey et al., 2004). RTI-336 is the most selective DAT inhibitor among several cocaine analogs examined by Kimmel et al. (2008). Moreover, RTI-336 was effective in producing dose-dependent reductions in cocaine self-administration even when the dose unit of cocaine was increased (Howell et al., 2007) and did not exhibit robust reinforcing effects comparable to cocaine (Kimmel et al., 2007, 2008; Czoty et al., 2010). Furthermore, as a candidate medication, RTI-336 has a favorable profile by virtue of its high potency, slow onset, and long duration of action (Carroll et al., 2006a; Kimmel et al., 2007). Currently, RTI-336 is in clinical trials for treatment of cocaine dependence and has just completed Phase 1 (clinicaltrials.gov).

In addition to their abuse-related behavioral effects, DAT inhibitors induce motor-stimulant effects (Kimmell et al., 2001) and have been reported to affect sleep-wake cycles in both nonhuman primates (Andersen et al., 2010) and in humans (Bodenmann and Landolt, 2010). These data suggest that long-term use of DAT inhibitors may also produce overall consequences on sleep measures. Moreover, it is well documented that DA also regulates pituitary hormones such as prolactin (Fitzgerald and Dinan, 2008; van der Pol, 2010) and cortisol (Durham et al., 1997; Zorick et al., 2011) and that these hormones are disrupted during cocaine dependence and early abstinence (Contoreggi et al., 2003). Given the role of DA in regulating endocrine response, and that most hormonal functions are carried out in concert (van der Pol, 2010), long term alterations in DA may affect multiple aspects of the hormonal profile. As treatment for cocaine abuse may require long-term pharmacological intervention, and DAT inhibitors are stimulants, it is important to determine the physiological effects of long-term exposure to DAT inhibitors. We examined the effects of RTI-336 on motor activity, sleep measures, and hormone levels. We hypothesized that the wake-promoting effects of chronic treatment with RTI-336 would stimulate motor activity and disrupt sleep-like behavior. Secondly, as DA plays an important role in the regulation of prolactin and cortisol, we expected that chronic DAT inhibitor treatment would decrease prolactin levels while increasing cortisol levels as a consequence of increasing DA levels.

Methods

Subjects

4 male adult rhesus monkeys (*Macaca mulatta*) weighing 15–17kg served as subjects. All subjects had a history of exposure to psychomotor stimulants, including cocaine. Each subject was individually housed in stainless steel home cages and fed with Purina monkey chow (Ralston Purina, St. Louis, MO), fruit, and vegetables. Water was continuously available in the home cage and food restriction protocols were not used. The colony was maintained at an ambient temperature of 22±2°C at 45–50% humidity, and lights set to a 12-h light/dark cycle (lights on at hour 7; lights off at hour 19). Environmental enrichment devices were provided on a regular rotating basis. All procedures and studies strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory

Animals (Publication No. 85–23, revised 1985), and were approved by the Institutional Animal Care and Use Committee of Emory University.

Drugs

RTI-336 HCl (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 100% ethanol in a volume equal to 5% of the total desired volume. The solution was sonicated for 20–30 min, after which sterile water was added to bring the solution to the desired concentration (5 mg/mL). The drug dose was determined as the salt. The dose of 1.0 mg/kg RTI-336 was chosen because it produced peak motor stimulant effects in squirrel monkeys (Kimmel et al., 2007). Furthermore, 1.0 mg/kg resulted in about 90% occupancy of DAT in rhesus monkey, and was roughly the average ED50 dose for reducing cocaine self-administration (Howell et al., 2007).

Locomotor activity and sleep-wake pattern

In order to quantify locomotor activity and sleep-like behavior patterns following RTI-336 administration, subjects were outfitted with Actiwatch (Mini Mitter, Bend, OR, USA) activity monitors for a period of 25 days. Actigraphy is one of the most commonly used methods for assessing sleep-wake cycles (Terrill et al., 2010) and has emerged over the past several decades as a critical method for estimating sleep duration, sleep efficiency, and sleep timing (Weiss et al., 2010). Many studies have shown excellent epoch by epoch agreement rates (i.e. greater than 85%) when compared to polysomnography (see Terril et al., 2010 for review), although it is important to note that real sleep time can only be inferred from the Actiwatch device since the subjects may be at rest and not sleeping when scored “sleep” by the associated Actiwatch software.

The Actiwatch contains an omni-directional sensor that is sensitive to motion (recorded as activity counts) in all directions and the movement data are stored in the Actiwatch’s 64kB of memory. The Actiwatchs-Mini has been shown to generate retrospective, data-logged activity counts recorded from multiple animals in a single arena by means of non-invasive monitoring (Mann et al., 2005). Actiwatch has been previously shown to be a reliable, non-invasive method for activity monitoring (Mann et al., 2005). The devices record intensity, amount and duration of movement in all 3 planes by producing a voltage that is subsequently converted to an arbitrary count and stored (for review see Mann et al., 2005). The monitors were programmed to record the total piezo-electric voltage generated over the preceding 15 sec (i.e. epoch length=15 sec).

On the first day of the study, the Actiwatch sensor was attached to the subject’s collar while the subject was under ketamine (3.0–10mg/kg, i.m.) anesthesia. Activity and sleep measurements for the subsequent 24 h were not included in the analysis in order to allow the subject to recover completely from anesthesia. Spontaneous baseline activity and sleep patterns were measured for the following 4 days (baseline recording). On the 5th day, blood was collected and the first injection of saline or RTI-336 (1 mg/kg) was administered intramuscularly. This first day of injection/treatment was analyzed separately from the subsequent injection days in order to investigate the acute vs. chronic effect of the treatment. Subsequent injections were given once per day at the same time (10 am) in order to reduce variability due to circadian activity patterns. Saline or RTI-336 administration and recording continued for a total of 21 days, after which a second blood sample was collected 24 hours after the last injection. The Actiwatchs were then removed and the data downloaded for analysis. Each subject underwent activity recording and chronic treatment twice, such that each subject received both treatments (saline or RTI-336) in a counter-balanced order across subjects.

The data were downloaded and analyzed with Actiware Sleep 3.4 (Mini-Mitter Co. Inc., Bend, OR, USA). Activity counts were extracted and summed into hourly bins for each time-point. Four days of baseline recordings (2 weekend days and 2 weekdays), were averaged and each animal's post-injection data were normalized to its respective baseline average. Thus, data are presented as a percentage of the baseline activity level. To describe night-time activity, we calculated sleep parameters from the raw actigraphy data using the Actiwatch algorithm. These parameters provide a more detailed description of both the distribution and pattern of night-time activity and are based on activity counts, thus activity counts are not presented for the dark period. "Sleep" is automatically determined from rest intervals (periods of data that contain periods of time when the subject activity is low and the subject is likely to be at rest). Although actual sleep time cannot be confirmed, the following measures will be referred to as "sleep parameters" as per the software terminology. The following sleep parameters were assessed: sleep efficiency (total sleep time percentage scored during the recording time, or total inactive time during the dark period), sleep latency (time elapsed between the lights-off time (hour 19) and the start time of the first immobile period as calculated by the Actiwatch sleep-wake scoring algorithm) and the fragmentation index (calculated by adding the percentage immobility, calculated by the program as number of immobile minutes divided by the lights-off period, and the percentage of minutes spent moving during the lights-off period). The fragmentation index is a measure of the amount of interruption of rest periods by physical movement and it is an indicator of restlessness. These measures (activity counts and sleep parameters) were output directly from the Mini Mitter software for each animal across all days of collection.

Hormones

In order to evaluate the effects of prolonged RTI-336 treatment on the regulation of DA-regulated hormones, prolactin and cortisol levels were assessed both before and after chronic saline or RTI-336 treatment. Blood samples were collected on the first day of the treatment period, immediately prior to the first treatment injection. Post-treatment samples were collected 24 hours after the last treatment injection, to avoid direct effects of RTI-336 on hormone levels. To collect the blood samples, the animals were removed from their home cage and comfortably seated in a primate chair (Primate Products, Florida etc). Blood was then drawn from the saphenous vein. The samples were centrifuged for 15 minutes at 3000g, and the serum collected. Samples were frozen at -80°C for later analysis by the Yerkes National Primate Research Center's Biomarkers Core Laboratory, using a fluorescence-based enzyme-linked immunosorbent assay as described previously (Mook et al., 2005).

Statistical analysis

Sleep and wake statistics are derived from the recorded physical activity values. Data were analyzed using two-way repeated-measures ANOVAs with Bonferroni's post hoc tests to indicate significance (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com; SigmaStat for Windows version 3.00, SPSS Inc). Alpha was set at 0.05. If no significant effect of time was found for a measure, data were combined across the 3 weeks of treatment. Acute effects (first day of treatment) and combined sleep parameter data were compared using Student's t-test.

Results

Locomotor activity and sleep-wake pattern

Locomotor activity—Peak effects for RTI-336 occurred at hour 18 (week 1, $491 \pm 187.8\%$; week 2, $432.3 \pm 90.0\%$; week 3, $400 \pm 75.1\%$). In the next time-point (hour 19), there is a marked decline in the activity (week 1, $280 \pm 96.6\%$, week 2, $272 \pm 52.9\%$; week 3, $304 \pm 36.1\%$), indicating reduction in activity immediately following lights-off (data not

shown). No day-after effects were observed before injection on day 2. No statistically significant differences in daily activity time courses were observed between weeks, thus all activity data were combined. Two-way ANOVA revealed a significant main effects for time of day ($F(3,11)=7.34$; $p<0.0001$), treatment condition ($F(3,1)=26.64$; $p<0.0001$) and a significant interaction effect ($F(3,11)=12.60$; $p<0.0001$) between treatment condition and time of day for locomotor activity. Post-hoc testing revealed that the chronic RTI-336 group had significantly increased activity at 4 pm ($p<0.05$), 5 pm ($p<0.001$) and 6 pm ($p<0.001$), as depicted in Figure 1. The saline group did not show any significant alteration across the time-points of the entire study.

Sleep-wake pattern

Sleep efficiency: No significant effect of time was found for sleep efficiency, thus the data were combined across the 3 weeks (saline: 77.1 ± 2.4 , 78.0 ± 2.0 , 77.9 ± 2.5 ; RTI-336: 69.6 ± 3.9 , 63.9 ± 5.3 , 63.6 ± 8.4 ; week 1–3 respectively). A significant decrease in sleep efficiency was found during chronic RTI-336 treatment ($p=0.01$ – Fig. 2A). There was no significant difference between saline and RTI-336 baselines or first day of treatment (acute effects).

Sleep latency: No significant effect of time was found for sleep latency, thus the data were combined across the 3 weeks (saline: 16.5 ± 1.8 , 11.6 ± 2.2 , 14.3 ± 1.9 ; RTI-336: 45.5 ± 9.1 , 44.7 ± 19.3 , 54.55 ± 32.1 ; week 1–3 respectively). Student's t-test found a significant increase in the period of time to sleep induction during chronic administration of RTI-336 as observed in Fig. 2B ($p<0.001$). Latency to sleep onset was increased 3-fold during treatment (14.12 ± 1.42 vs. 48.24 ± 19.58 minutes). No significant differences in sleep latency were found between saline and RTI-336 baselines or the first day of treatment.

Sleep fragmentation: No significant effect of time was found for sleep fragmentation, thus the data were combined across the 3 weeks (saline: 33.0 ± 2.9 , 33.7 ± 2.4 , 34.6 ± 2.2 ; RTI-336: 41.6 ± 4.2 , 53.5 ± 7.8 , 48.8 ± 6.5 ; week 1–3 respectively). The sleep fragmentation index was significantly increased during the long-term treatment ($p<0.01$; Figure 2C). No significant differences were found between saline and RTI-336 baselines or first day of treatment.

Hormone levels

Prolactin—Baseline prolactin levels were not significantly different preceding saline (6.54 ± 0.65 ng/ml) or RTI-336 (4.85 ± 0.32 ng/ml) treatment. No significant changes were observed following RTI-336 treatment (6.73 ± 0.96 ng/ml) as compared to saline (5.58 ± 0.43 ng/ml), and neither condition differed significantly from its respective pre-treatment baseline measure (main effect of treatment $F(1,3) = 0.131$, $p = 0.74$; main effect of time point $F(1,3) = 6.314$, $p = 0.09$; interaction $F(1,3) = 0.383$, $p = 0.58$). To control for individual differences, each animal's data was then normalized to its pre-treatment level. No significant differences were seen ($t(3) = 0.36$, $p = 0.74$; Figure 3). **Cortisol:** Baseline cortisol levels were not significantly different preceding saline (11.71 ± 2.57 ug/dl) or RTI-336 (18.09 ± 7.12 ug/dl) treatment. No significant changes were observed following RTI-336 treatment (23.83 ± 5.9 ug/dl) as compared to saline (21.82 ± 4.72 ug/dl), and neither condition differed significantly from its respective pre-treatment baseline measure (main effect of treatment $F(1,3) = 0.92$, $p = 0.48$; main effect of time point $F(1,3) = 9.48$, $p = 0.054$; interaction $F(1,3) = 0.199$, $p = 0.69$). To control for individual differences, each animal's data were then normalized to its pre-treatment level. No significant differences were seen ($t(3) = 0.71$; $p = 0.53$; Figure 3).

Discussion

Studies on DAT inhibitors have typically focused on acute effects and have not evaluated the possible effects that long-term drug administration could have on behavioral measures. For this purpose, the present study tested the effects of 21 days of RTI-336 administration on both behavior and hormonal levels. To the best of our knowledge, this is the first investigation of the consequences of this drug on sleep measures. Our analyses indicate that chronic RTI-336 treatment promoted changes in activity levels. During treatment, evening activity, sleep latency, and sleep fragmentation were significantly increased, while sleep efficiency was decreased. Prolactin and cortisol levels were unaffected by the long-term treatment of RTI-336. Chronic treatment with RTI-336 was well-tolerated and no health concerns arose.

Considerable preclinical research has been directed toward developing 3-phenyltropane analogs as substitute agonist medications for cocaine dependence (Carroll et al., 2006a,b). These analogs, including RTI-336, decrease cocaine self-administration and are hypothesized to minimize craving for cocaine (Carroll et al., 2006b). Previous preclinical studies have characterized the behavioral and neurochemical effects of 3-phenyltropane analogs of cocaine. Kimmel et al. (2007) reported that the faster-onset analogs, including RTI-336, produced behavioral-stimulant effects in squirrel monkeys, while a slower-onset nonselective analog (RTI-112) did not. Furthermore, the authors reported a modest (150%) increase in DA following RTI-336 administration. This increase occurred approximately 20 minutes following injection and did not completely dissipate by the end of the 2-hour sampling period (Kimmel et al., 2007).

Rhesus macaques are diurnal animals, i.e., sleep during the dark phase of the light-dark cycle. It is possible that behavioral-stimulant effects were masked by the higher levels of baseline activity earlier in the light phase. However, the significant increase in activity at the last 3 time-points indicates that RTI-336 clearly has long-acting behavioral-stimulant effects, as reported previously by Kimmel et al. (2007). Additionally, chronic administration of RTI-336 increased sleep latency and fragmentation, thus compromising sleep efficiency, although no significant changes were seen following a single dose. These results are consistent with the profile of stimulant effects observed in the present study as well as in previous studies (Kimmel et al., 2007, 2008), and are typical of the stimulant class as a whole. It is well-documented that the dopaminergic system modulates sleep (Tufik et al., 2009), and many dopaminergic drugs such as amphetamine, cocaine, and bupropion, have acute and chronic effects on sleep architecture (Gruner et al., 2009). Thus, it would be expected that any proposed substitute agonist medication for cocaine addiction may also have disruptive effects on rest or sleep. However, compared to other candidate medications such as amphetamine and sustained-release methamphetamine (Mooney et al., 2009; Czoty et al., 2011), RTI-336 may have less intrusive stimulant and sleep-wake cycle effects as well as lower abuse potential as previously reported (Carroll et al., 2006a; Kimmel et al., 2007). Furthermore, it has been demonstrated that cocaine addicts experience substantial disruption of sleep during the initial stages of abstinence (Morgan & Malison, 2007; Matuskey et al., 2011); in light of these findings, the disruptions caused by RTI-336 may not pose a problem for treatment applications. Moreover, it has recently been reported that a DAT inhibitor with mild wake-promoting effects, modafinil, can actually normalize the disrupted sleep experienced during initial abstinence from cocaine (Morgan et al., 2010); further studies are needed to determine if this effect is specific to modafinil, or if it may also result from other mild wake-promoting drugs such as RTI-336.

While stimulant drugs can induce changes in sleep, changes in sleep have been shown to intensify stimulant-induced behaviors such as stereotypy and locomotion, suggesting that

alterations in sleep can alter the underlying neurobiology of dopamine (see Tufik et al., 2009 for review). However, although the DAT is the key regulator of DA levels in the synaptic cleft and implicated in these effects, recent studies conducted in both animals (Andersen et al., 2005) and humans (Volkow et al., 2008; Martins et al., 2010) show that DAT availability is not affected by sleep deprivation paradigms. Furthermore, the changes in sleep measures observed, while significant, were mild; sleep latency only increased by 34 minutes while sleep efficiency only decreased by 12%. Thus, it is unlikely that the increased activity during chronic RTI-336 treatment would be caused by the changes in sleep patterns.

Dopamine, as well as being important in sleep regulation, also influences prolactin and cortisol release. Dopamine neurons in the arcuate nucleus are thought to exert tonic inhibition on prolactin-secreting cells; thus, a reduction in dopaminergic activity results in an increase in prolactin levels (see van de Pol, 2010 for review). Interestingly, chronic treatment with RTI-336, while producing mild but significant effects on sleep-like behavior, did not cause any significant change in prolactin levels. Additionally, there were no significant changes in cortisol. The lack of alteration in these hormonal levels suggests either that the effect is confined to the time when RTI-336 is actually present in the system, or that there were adaptive changes that compensated for altered DA transmission and normalized hormone regulation. Furthermore, this indicates that compromised sleep is not enough to produce changes in prolactin or cortisol.

In conclusion, marked advances have been made in the discovery and preclinical development of selective DAT inhibitors as potential pharmacotherapies for treating cocaine addiction. The behavioral and neurochemical profile for RTI-336, particularly its long duration of action and lower abuse potential compared to cocaine, has been deemed most promising (Carroll et al., 2006a,b; Kimmel et al., 2007, 2008; Czoty et al., 2010). To this end, it is currently being evaluated in clinical trials. However, little information is available on possible consequences of long-term use. Here, we present evidence of motor-stimulant effects and potential disruptive effects on sleep; however, these effects, while significant, were modest and may not pose problems for extended therapy. Furthermore, no significant effect was found on prolactin or on cortisol levels. Thus, the present study suggests that RTI-336, a promising candidate medication for cocaine addiction, may have a favorable side-effect profile.

Acknowledgments

The authors express their gratitude to Lisa Neidert, Juliet Brown, Heather Kimmel and Kevin Murnane for capable technical assistance. We also thank Mi Zhou for her excellent aid with preparing the drug solutions. The research was supported by USPHS Grants DA10344 (LLH), DA00517 (LLH), DA05477 (FIC), RR00165 (Yerkes National Primate Research Center), T32-DA015040 (EKS) and Associacao Fundo de Incentivo a Pesquisa (AFIP) and CNPq (MLA).

References

- Andersen ML, Kessler E, Murnane KS, Mcclung JC, Tufik S, Howell LL. Dopamine transporter-related effects of modafinil in Rhesus monkeys. *Psychopharmacology*. 2010; 210:439–448. [PubMed: 20386883]
- Andersen ML, Papale LA, Hipolide DC, Nobrega J, Tufik S. Involvement of dopamine receptors in cocaine-induced genital reflexes after paradoxical sleep deprivation. *Behavioral Brain Research*. 2005; 160:44–50.
- Bodenmann S, Landolt HP. Effects of modafinil on the sleep EEG depend on Val158Met genotype of COMT. *Sleep*. 2010; 3:1027–35. [PubMed: 20815183]
- Carroll FI, Howell LL, Kuhar MJ. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. *J Med Chem*. 1999; 42:2721–2736. [PubMed: 10425082]

- Carroll FI, Fox BS, Kuhar MJ, Howard JL, Pollard GT, Schenk S. Effects of dopamine transporter selective 3-phenyltropane analogs on locomotor activity, drug discrimination, and cocaine self-administration after oral administration. *Eur J Pharmacol.* 2006a; 553:149–56. [PubMed: 17067572]
- Carroll FI, Howard JL, Howell LL, Fox BS, Kuhar MJ. Development of the dopamine transporter selective RTI-336 as a pharmacotherapy for cocaine abuse. *AAPS J.* 2006b; 8:E196–203. [PubMed: 16584128]
- Contoreggi C, Herning RI, Koeppl B, Simpson PM, Negro PJ Jr, Fortner-Burton C, Hess J. Treatment-seeking inpatient cocaine abusers show hypothalamic dysregulation of both basal prolactin and cortisol secretion. *Neuroendocrinology.* 2003; 78:154–62. [PubMed: 14512708]
- Czoty PW, Martelle JL, Carroll FI, Nader MA. Lower reinforcing strength of the phenyltropane cocaine analogs RTI-336 and RTI-177 compared to cocaine in nonhuman primates. *Pharmacol Biochem Behav.* 2010; 96:274–8. [PubMed: 20580733]
- Czoty PW, Gould RW, Martelle JL, Nader MA. Prolonged attenuation of the reinforcing strength of cocaine by chronic d-amphetamine in rhesus monkeys. *Neuropsychopharmacology.* 2011; 36:539–47. [PubMed: 20962765]
- Durham RA, Eaton MJ, Moore KE, Lookingland KJ. Effects of selective activation of dopamine D2 and D3 receptors on prolactin secretion and the activity of tuberoinfundibular dopamine neurons. *Eur J Pharmacol.* 1997; 335:37–42. [PubMed: 9371544]
- Fitzgerald P, Dinan TG. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol.* 2008; 22(2S):12–19. [PubMed: 18477617]
- Gruner JA, Marcy VR, Lin YG, Bozyczko-Coyne D. The roles of dopamine transport inhibition and dopamine release facilitation in wake enhancement and rebound hypersomnolence induced by dopaminergic agents. *Sleep.* 2009; 32:1425–38. [PubMed: 19928382]
- Howell LL, Carroll FI, Votaw JR, Goodman MM, Kimmel HL. Effects of combined dopamine and serotonin transporter inhibitors on cocaine self-administration in rhesus monkeys. *J Pharmacol Exp Ther.* 2007; 320:757–765. [PubMed: 17105829]
- Howell LL, Czoty PW, Kuhar MJ, Carroll FI. Comparative behavioral pharmacology of cocaine and the selective dopamine uptake inhibitor RTI-113 in the squirrel monkey. *J Pharmacol Exp Ther.* 2000; 292:521–529. [PubMed: 10640288]
- Howell LL, Wilcox KM. The dopamine transporter and cocaine medication development: drug self-administration in nonhuman primates. *J Pharmacol Exp Ther.* 2001; 298:1–6. [PubMed: 11408518]
- Kimmel HL, Carroll FI, Kuhar MJ. Locomotor stimulant effects of novel phenyltropanes in the mouse. *Drug Alcohol Depend.* 2001; 65:25–36. [PubMed: 11714587]
- Kimmel HL, O'Connor JA, Carroll FI, Howell LL. Faster onset and dopamine transporter selectivity predict stimulant and reinforcing effects of cocaine analogs in squirrel monkeys. *Pharmacol Biochem Behav.* 2007; 86:45–54. [PubMed: 17258302]
- Kimmel HL, Negus SS, Wilcox KM, Ewing SB, Stehouwer J, Goodman MM, Votaw JR, Mello NK, Carroll FI, Howell LL. Relationship between rate of drug uptake in brain and behavioral pharmacology of monoamine transporter inhibitors in rhesus monkeys. *Pharmacol Biochem Behav.* 2008; 90:453–62. [PubMed: 18468667]
- Lindsey KP, Wilcox KM, Votaw JR, Goodman MM, Plisson C, Carroll FI, et al. Effects of dopamine transporter inhibitors on cocaine self-administration in rhesus monkeys: relationship to transporter occupancy determined by positron emission tomography neuroimaging. *J Pharmacol Exp Ther.* 2004; 309:959–969. [PubMed: 14982963]
- Mann TM, Williams KE, Pearce PC, Scott EA. A novel method for activity monitoring in small non-human primates. *Lab Anim.* 2005; 39:169–77. [PubMed: 15901360]
- Martins RCS, Andersen ML, Garbuio SA, Bittencourt LR, Guindalini CSC, Shih MC, Hoexter MQ, Bressan RA, Castiglioni MLV, Tufik S. Dopamine transporter regulation during four nights of REM sleep deprivation and recovery – *in vivo* molecular imaging study in humans. *Sleep.* 2010; 33:243–251. [PubMed: 20175408]
- Matuskey D, Pittman B, Forselius E, Malison RT, Morgan PT. A multistudy analysis of the effects of early cocaine abstinence on sleep. *Drug Alcohol Depend.* 2011; 115:62–66. [PubMed: 21144676]

- Mook D, Felger J, Graves F, Wallen K, Wilson ME. Tamoxifen fails to affect central serotonergic tone but increases indices of anxiety in female rhesus macaques. *Psychoneuroendocrinology*. 2005; 30:273–283. [PubMed: 15511601]
- Mooney ME, Herin DV, Schmitz JM, Moukaddam N, Green CE, Grabowski J. Effects of oral methamphetamine on cocaine use: a randomized, double-blind, placebo-controlled trial. *Drug Alcohol Depend*. 2009; 101:34–41. [PubMed: 19058926]
- Morgan PT, Pace-Schott E, Pittman B, Stickgold R, Malison RT. Normalizing effects of modafinil on sleep in chronic cocaine users. *Am J Psychiatry*. 2010; 167:331–40. [PubMed: 20080983]
- Morgan PT, Malison RT. Cocaine and sleep: early abstinence. *Scientific World Journal*. 2007; 7:223–30. [PubMed: 17982597]
- Mortensen OV, Amara SG. Dynamic regulation of the dopamine transporter. *Eur J Pharmacol*. 2003; 479:159–70. [PubMed: 14612147]
- Nader MA, Grant KA, Davies HM, Mach RH, Childers SR. The reinforcing and discriminative stimulus effects of the novel cocaine analog 2beta-propanoyl-3beta-(4-tolyl)-tropane in rhesus monkeys. *J Pharmacol Exp Ther*. 1997; 280:541–50. [PubMed: 9023262]
- Ritz MC, Kuhar MJ. Relationship between self-administration of amphetamine and monoamine receptors in brain: comparison with cocaine. *J Pharmacol Exp Ther*. 1989; 248:1010–1017. [PubMed: 2703961]
- Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science (Wash DC)*. 1987; 237:1219–1223.
- Terrill PI, Mason DG, Wilson SJ. Development of a continuous multisite accelerometry system for studying movements during sleep. *Conf Proc IEEE Eng Med Biol Soc*. 2010; 2010:6150–3. [PubMed: 21097146]
- Tufik S, Andersen ML, Bittencourt LRA, De Mello MT. Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. *Acad Brasil Ciencias*. 2009; 81:521–538.
- van den Pol AN. Excitatory neuromodulator reduces dopamine release, enhancing prolactin secretion. *Neuron*. 2010; 65:147–9. [PubMed: 20152122]
- Weiss AR, Johnson NL, Berger NA, Redline S. Validity of activity-based devices to estimate sleep. *J Clin Sleep Med*. 2010; 6:336–42. [PubMed: 20726281]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry*. 2004; 9:557–69. [PubMed: 15098002]
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Wong C, Ma J, Pradhan K, Tomasi D, Thanos PK, Ferré S, Jayne M. Sleep deprivation decreases binding of [¹¹C]raclopride to dopamine D₂/D₃ receptors in the human brain. *J Neurosci*. 2008; 28:8454–61. [PubMed: 18716203]
- Wilcox KM, Lindsey KP, Votaw JR, Goodman MM, Martarello L, Carroll FI, Howell LL. Self-administration of cocaine and the cocaine analog RTI-113: relationship to dopamine transporter occupancy determined by PET neuroimaging in rhesus monkeys. *Synapse*. 2002; 43:78–85. [PubMed: 11746736]
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB. Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)*. 1995; 120:10–20. [PubMed: 7480530]
- Zahniser NR, Sorkin A. Rapid regulation of the dopamine transporter: role in stimulant addiction? *Neuropharmacology*. 2004; 47(S1):80–91. [PubMed: 15464127]
- Zorick T, Mandelkern MA, Lee B, Wong ML, Miotto K, Shabazian J, London ED. Elevated plasma prolactin in abstinent methamphetamine-dependent subjects. *Am J Drug Alcohol Abuse*. 2011; 37:62–7. [PubMed: 21142706]

Figure 1.

Effect of chronic saline and RTI-336 injection (at hour 10 daily) on daytime locomotor activity in rhesus monkeys (n=4). Activity counts were extracted and summed into hourly bins, normalized to a pre-treatment baseline, and averaged across the 21 days of treatment. RTI-336 significantly increases activity during the evening hours (hours 16–18). Arrow indicates time of injection. Abscissae: time expressed in hours and plotted on an absolute linear scale. Ordinates: activity counts expressed as a percentage of the baseline activity level. *indicates a significant difference from saline group. Each data point represents the group mean $\pm$ SEM.

Figure 2.

Sleep parameters at baseline, day 1 of injection and during repeated treatment (21 days). No differences between baseline and day 1 effects were seen and there was no effect of time across chronic treatment for any measure. However, RTI-336 significantly decreased sleep efficiency (A) and increased sleep latency (B) and sleep fragmentation (C). Data are expressed as mean $\pm$ SEM.

Figure 3. Prolactin and cortisol levels following chronic saline (open bars) or RTI-336 (closed bars) as a percentage of their respective baseline levels. No significant differences were observed.