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Selective serotonin 2A receptor antagonism attenuates the effects of amphetamine on arousal and dopamine overflow in nonhuman primates

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Summary

The objective of the present study was to further elucidate the mechanisms involved in the wake-promoting effects of psychomotor stimulants. Many previous studies have tightly linked the effects of stimulants to dopamine neurotransmission, and some studies indicate that serotonin 2A (5-HT_{2A}) receptors modulate these effects. However, the role of dopamine in arousal is controversial, most notably because dopamine neurons do not change firing rates across arousal states. In the present study, we examined the wake-promoting effects of the dopamine releaser amphetamine using noninvasive telemetric monitoring. These effects were evaluated in rhesus monkeys as a laboratory animal model with high translational relevance for human disorders of sleep and arousal. To evaluate the role of dopamine in the wake-promoting effects of amphetamine, we used *in vivo* microdialysis targeting the caudate nucleus, as this approach provides clearly interpretable measures of presynaptic dopamine release. This is beneficial in the present context because some of the inconsistencies between previous studies examining the role of dopamine in arousal may be related to differences between postsynaptic dopamine receptors. We found that amphetamine significantly and dose-dependently increased arousal at doses that engendered higher extracellular-dopamine levels. Moreover, antagonism of 5-HT_{2A} receptors attenuated the effects of amphetamine on both wakefulness and dopamine overflow. These findings further elucidate the role of dopamine and 5-HT_{2A} receptors in arousal, and they suggest that increased dopamine neurotransmission may be necessary for the wake-promoting effects of amphetamine, and possibly other stimulants.

Keywords

Amphetamine; M100907; rhesus monkeys; 5-HT_{2A}; microdialysis; arousal

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Introduction

The neuropharmacology of the sleep and arousal systems involves a complex interplay between multiple neurotransmitter systems. In this regard, previous work has closely tied the serotonin (5-HT), norepinephrine, acetylcholine, histamine, adenosine, and hypocretin/orexin systems to sleep and arousal (Boutrel and Koob 2004). Less consistent effects have been found in examinations of the role of dopamine in sleep and arousal, and the role of this neurotransmitter has been downplayed. For example, it has been widely cited that most dopamine neurons do not appear to change their firing rates between sleep and arousal (Miller et al. 1983; Steinfels et al. 1983). Nevertheless, lesioning the dopamine system of rats imparts marked reductions in behavioral arousal (Jones et al. 1973), and Parkinsonian humans exhibit severe sleep disorders (Paus et al. 2003; Rye 2002). Furthermore, many D1 and D2 receptor agonists promote wakefulness (Isaac and Berridge 2003) and the hallmark effects of the psychomotor class of compounds include increased arousal and alertness, and increased dopamine neurotransmission (Murnane and Howell 2011; Ritz et al. 1989). Indeed, a recent study in nonhuman primates showed that the behavioral effects of the wake-promoting drug modafinil are related to its effects at the dopamine transporter (Andersen et al. 2010). Consistent with these findings, some of the most frequently used wake-promoting drugs, such as amphetamine, are known to elicit release of dopamine from presynaptic terminals. However, well-known side-effects of some dopamine receptor agonists, such as pramipexole, include sedation and somnolence. Moreover, adding further complexity to this issue, a recent study showed increased bursting in ventral tegmental area dopamine neurons during paradoxical sleep (Dahan et al. 2007). Accordingly, further study of the role of dopamine in arousal is clearly warranted.

To further examine the role of dopamine in arousal, we examined the dopamine releasing and wake-promoting effects of amphetamine following pretreatment with the selective 5-HT_{2A} receptor antagonist M100907. We examined the effects of 5-HT_{2A} receptor antagonism because previous studies in rodents have shown that antagonizing this receptor attenuates amphetamine-induced increases in dopamine neurotransmission (Porras et al. 2002), amphetamine-elicited locomotor activity (Sorensen et al. 1993), suppression of activity in areas A9 and A10 of the midbrain by amphetamine (Sorensen et al. 1993) and the behavioral-stimulant effects of cocaine (McMahon and Cunningham 2001). However, to our knowledge, no previous studies have examined the role of these receptors in the wake-promoting effects of stimulants, or in dopamine neurotransmission in nonhuman primates. To mechanistically evaluate the importance of dopamine in any wake-promoting effects of amphetamine, we used *in vivo* microdialysis in the caudate nucleus. We took this approach because it allowed us to step back from the possible complexities of the post-synaptic effects of dopamine, to initially determine whether increased pre-synaptic release of dopamine increases arousal, and whether attenuating this pre-synaptic release of dopamine blunts arousal. In this regard, we hypothesized that selective antagonism of the 5-HT_{2A} receptor would attenuate both the dopamine-releasing and wake-promoting effects of amphetamine.

Methods

Subjects

The sleep studies were carried out in 5 female rhesus monkeys (*Macaca mulatta*), and the microdialysis studies were conducted in a separate group of 4 female rhesus monkeys (*Macaca mulatta*). All subjects weighed between 7.0 and 8.5 kgs and were housed individually within a primate colony with continuous access to water. Their diet consisted of Purina monkey chow (Ralston Purina, St. Louis, MO) supplemented with fresh fruit and vegetables, and food restriction protocols were not used. Ambient conditions within the colony were maintained at a temperature of 22±2 °C and at 45–50% humidity. The room

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lighting was set to a 12-h light/dark cycle with the light period active from 07:00 to 19:00. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Emory University.

Surgery

Prior to this study, the microdialysis subjects were implanted with chronic indwelling venous catheters attached to a subcutaneous vascular access port using aseptic surgical techniques as previously described (Wilcox et al. 2002). These subjects were also implanted with bilateral CMA/11 guide cannulae (CMA, North Chelmsford, MA, USA) that were stereotaxically targeted for the head of the caudate nucleus as previously described (Murnane et al. 2010). During each surgery, subjects were prophylactically administered an antibiotic (Rocephin), an analgesic (Buprenorphine), and a nonsteroidal anti-inflammatory agent (Banamine) to minimize any discomfort from the surgery. The catheters were regularly flushed with heparinized (100 U/ml) saline to maintain patency.

Drugs

S-(+)-amphetamine was commercially purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in 0.9% sterile saline. M100907 was synthesized at the Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism at the National Institutes of Health (Bethesda, MD) and provided as a generous gift from Dr. Kenner C. Rice. M100907 was dissolved in sterile water and 0.1 N HCl. The doses of each drug were calculated and are expressed as salts. All treatments were conducted once in each subject.

Sleep studies

To quantify sleep-wake patterns, each subject was outfitted with an Actiwatch (Mini Mitter, Bend, OR, USA) activity monitor. The Actiwatch consisted is an omni-directional sensor that is sensitive to motion (recorded as activity counts) and is a reliable, non-invasive method for monitoring arousal (Kushida et al. 2001; Sadeh et al. 1995). The monitors were programmed to record the total piezo-electric voltage generated over the preceding 60 s (i.e. epoch length=60 s). The devices record the intensity, amount, and duration of movement in all three planes. The Actiwatch sensor was initially attached to a given subject's collar while the subject was under ketamine (3.0–10mg/kg, i.m.) anesthesia. To allow for recovery, sleep measurements for the subsequent 48 hours were not included in the analysis. Sleep measurements were obtained continuously until the data capacity of the monitor was reached (approximately every 45 days) and were then replaced. Test sessions were conducted in the colony on a Monday or a Wednesday. Based on preliminary observations, each subject was administered an intramuscular injection of amphetamine or saline at 17:30 (90 minutes prior to the beginning of the dark period). To determine whether selective antagonism of the 5-HT_{2A} receptor attenuates the wake-promoting effects of amphetamine, M100907 or its vehicle was administered at increasing pretreatment times relative to the 17:30 administration of amphetamine. The order of the doses or pretreatment times was randomized across subjects within an experiment. The dose of M100907 (0.3 mg/kg) was chosen because it effectively attenuates stimulant self-administration in rhesus monkeys (Fantegrossi et al. 2002), and the behavioral stimulant effects of 3,4-methylenedioxymethamphetamine in squirrel monkeys (Fantegrossi et al. 2009). The data were downloaded and analyzed with Actiware Sleep 3.4 (Mini-Mitter Co. Inc., Bend, OR, USA). Sleep measurements were automatically calculated from the underlying activity counts using a temporal smoothing algorithm on the basis that sleeping or wakefulness are continuous behaviors. In the present study, sleep was assessed in 1-minute bins or "epochs" over the entire recording period. The Actiware analysis software automatically determined

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whether the subject was awake or asleep during each epoch by comparing the sum of the activity counts in that epoch and neighboring epochs to a predefined criterion. For 1-minute epochs, the specific formula used is the sum of the activity in the epoch under evaluation times 1, plus the activity in the epochs immediately before and after the epoch under evaluation times 1/5, plus the activity in the epochs that occurred two epochs before and after the epoch under evaluation times 1/25. The software-supplied criteria provide a low, medium, or high threshold for determining that a subject was asleep. In the present study, to provide the most unbiased assessments possible, we choose to use the medium threshold. We assessed both sleep latencies (time elapsed from the beginning of the dark period to the first period in which the subject was determined to be asleep) and sleep durations (total time the subject was determined to be asleep over the entire 12 hour dark period).

In vivo microdialysis

Microdialysis measurements were collected and samples analyzed similar to previously described procedures (Banks et al. 2009). Briefly, all procedures were carried out in fully conscious subjects while they sat in commercially available primate chairs (Primate Products, Woodside, CA), within sound attenuated testing chambers. After the subject was placed in the chamber, 24 mm stainless steel microdialysis probes with a 4 mm membrane (CMA/Microdialysis) were inserted into the subject's surgically implanted guide cannulae. Drugs were administered through the subcutaneous vascular access port. Experiments consisted of a 1 h equilibrium period after which samples were collected every 10 min for 3 h. Adequate probe recovery was verified for each experimental session, both pre- and post-session. The viability of the sampling site was verified through retrodialysis of a potassium-enriched (100 mM) solution ionically matched to cerebrospinal fluid. Dopamine concentrations within the dialysate were quantified via electrochemical detection utilizing high pressure liquid chromatography (HPLC) as previously described (Banks et al. 2009). In these experiments, to take advantage its faster kinetics, and limit the duration of each dialysis experiment, all treatments were administered intravenously. As the kinetics of intravenous administration are faster than those of intramuscular administration, M100907 was injected 30 minutes before amphetamine. The vehicle and M100907 pretreatments were counterbalanced across the subjects.

Data Analysis

Graphical presentation of all data depicts the mean ± the standard error of the mean (S.E.M.). All graphical data presentations were created using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA), all statistical tests were performed using SigmaStat 3 (Systat Software, Inc., San Jose, CA), and significance was accepted at $P < 0.05$. The primary dependent variables tested in the sleep studies were the latency from the time the colony lights turned off to the first sleep bout and the total duration of sleep over the 12-hour dark epoch. The data were analyzed via a one-way repeated measures (RM) analysis of variance (ANOVA) with correction for multiple comparisons using Dunnett's method. The primary dependent variable tested in the microdialysis experiments was the striatal extracellular concentration of dopamine. Dopamine levels were quantified in comparison to known concentration curves with the EZChrom Elite software package (version 3.1, Scientific Software, Pleasanton, CA). For dopamine, differences in basal levels across days were assessed via a one-way RM ANOVA, and the data were then normalized to the baseline levels in each experimental session. Pretreatment effects of M100907 were analyzed via a two-way RM ANOVA with the main factors of pretreatment and time. For time, individual comparisons were made at each time point with correction for multiple comparisons using Dunnett's method versus baseline. For pretreatment, individual comparisons were conducted with a paired t-test.

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Results

In initial experiments, we determined the diurnal pattern of activity in rhesus monkeys, under the conditions employed (Figure 1). Using this approach, we found that a clear pattern of diurnal activity in these subjects, with substantially higher activity during the light phase. The subjects exhibited marked consistency in their daily activity patterns both within subject and across the subjects. Moreover, there were clear and dose-dependent increases in nighttime activity following administration of amphetamine compared to saline or baseline days. Two weeks of activity in a single representative subject are plotted in Figure 1, including days showing the effects of each of the three doses of amphetamine and saline.

Based on the activity data, the Actiware Sleep software calculated the sleep parameters using a temporal smoothing algorithm. Using this procedure, we determined the effects of amphetamine on sleep (Figure 2). A one-way RM ANOVA revealed a significant main effect of amphetamine on both sleep latencies ($F_{4,3} = 15.280$; $p < 0.001$) and sleep durations ($F_{4,3} = 138.587$; $p < 0.001$).

Next, the effects of 5-HT_{2A} receptor antagonism on sleep disruption by amphetamine were determined via pretreatments with the selective antagonist M100907 (Figure 3). Although the dose of M100907 was chosen on the basis that it had been previously shown to antagonize stimulant self-administration in rhesus monkeys(Fantegrossi et al. 2002), initial experiments showed that M100907 was marginally effective at antagonizing the effects of amphetamine on sleep. Therefore, the pretreatment time of M100907 was systematically increased to determine whether there are conditions under which it is more effective. A one-way RM ANOVA revealed a significant main effect of pretreatment time on the effectiveness of M100907 to antagonize amphetamine's effects on both sleep latencies ($F_{4,3} = 16.358$; $p < 0.001$) and sleep durations ($F_{4,3} = 15.514$; $p < 0.001$). Importantly, in separate experiments (data not shown), we verified that this dose of M100907 did not influence sleep latencies or durations as compared to vehicle administration.

Additional experiments were undertaken to determine the effects of antagonism of the 5-HT_{2A} receptor on amphetamine-elicited dopamine release. To ensure that any effects on dopamine release were not a result of altered basal dopamine levels, the effects of 5-HT_{2A} antagonism by 0.3mg/kg of M100907 on basal levels of dopamine were evaluated (Figure 4). A two-way RM ANOVA revealed no significant main effect of either pretreatment ($F_{3,1} = 2.205$; $p = 0.234$) or time ($F_{3,4} = 1.147$; $p = 0.379$) and no significant interaction ($F_8 = 1.809$; $p = 0.171$).

The effects of antagonism of the 5-HT_{2A} receptor antagonism by 0.3mg/kg of M100907 on dopamine release elicited by amphetamine (0.3 mg/kg or 1.0 mg/kg) were then evaluated (Figure 5). A two-way RM ANOVA revealed a significant main effect of treatment with amphetamine at 0.3 mg/kg ($F_{3,12} = 3.309$; $p = 0.003$) but no main effect of pretreatment with M100907 ($F_{3,1} = 3.200$; $p = 0.172$) and no significant interaction ($F_{12} = 1.635$; $p = 0.125$). Following vehicle pretreatment, Dunnett's test showed that 0.3 mg/kg of amphetamine significantly increased dopamine levels compared to baseline at 30 and 40 minutes. Following pretreatment with M100907, there was no effect of treatment with amphetamine. Furthermore, a two-way RM ANOVA revealed a significant main effect of treatment with amphetamine at 1.0 mg/kg ($F_{3,12} = 6.924$; $p < 0.001$), pretreatment with M100907 ($F_{3,1} = 20.013$; $p = 0.021$), and a significant interaction ($F_{12} = 2.870$; $p = 0.007$). Following vehicle pretreatment, Dunnett's test showed that 1.0 mg/kg of amphetamine significantly increased dopamine levels compared to baseline at 20, 30, and 50 minutes. Following pretreatment with M100907, there was only a significant effect of amphetamine at 20 minutes. Moreover, M100907 significantly decreased dopamine overflow at 30 minutes.

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Discussion

In the present study, we examined the diurnal pattern of activity in rhesus monkeys. We found that the subjects consistently exhibited markedly higher activity during the light phase of each day and consistent activity levels from day to day. These data suggest that the activity patterns in these subjects were highly entrained to the light and dark phases. This may be a result of the highly controlled environment in which they were housed. As such, it would be interesting to determine whether rhesus monkeys housed in outdoor enclosures that allow for more natural environmental conditions would exhibit similar diurnal activity patterns.

In our arousal studies, we found that amphetamine produced substantial and dose-dependent increases in the latencies of these subjects to fall asleep and dose-dependent decreases in their total durations of sleep. As relatively few determinations of acute drug effects on arousal have been carried out in rhesus monkeys, it is important to note that these effects are consistent with what would be predicted based on the known effects of amphetamine (Boutrel and Koob 2004). Furthermore, these data support the use of the currently described procedures in the study of the neuropharmacology of wakefulness. In subsequent experiments, we examined the role of the 5-HT_{2A} receptor in the wake-promoting effects of amphetamine, and found that pretreatment with the selective 5-HT_{2A} receptor antagonist M100907 attenuated the wake-promoting effects of amphetamine in a pretreatment-time-dependent manner. This finding is consistent with previous work showing that antagonizing this receptor attenuates amphetamine-induced increases in dopamine neurotransmission (Porras et al. 2002), amphetamine-elicited locomotor activity (Sorensen et al. 1993), suppression of activity in areas A9 and A10 of the midbrain by amphetamine (Sorensen et al. 1993) and the behavioral-stimulant effects of cocaine (McMahon and Cunningham 2001). However, it extends this work by demonstrating a role of this receptor in modulating the wake-promoting effects of amphetamine. Additionally, this outcome provides strong support for the continued evaluation of the role of this receptor in the behavioral effects of psychomotor-stimulants, with particular relevance for its role in their wake-promoting effects.

Complementing the behavioral experiments, in the present study, *in vivo* microdialysis determinations showed that amphetamine significantly elevated extracellular dopamine levels in the caudate at the doses that were required to increase wakefulness. This suggests that increased dopamine neurotransmission contributed to the wake-promoting effects of amphetamine. Building upon these findings, we also established that pretreatment with M100907 attenuated the elevations in dopamine levels elicited by amphetamine. Taken together, these results strongly suggest that elevations in brain levels of dopamine contribute to the wake-promoting effects of amphetamine. Supporting this interpretation, it is important to note that lesioning the dopamine system of rats imparts marked reductions in behavioral arousal (Jones et al. 1973), Parkinsonian humans exhibit severe sleep disorders (Paus et al. 2003; Rye 2002), and some dopamine receptor agonists promote wakefulness (Isaac and Berridge 2003). Moreover, psychomotor stimulants increase arousal and alertness, and the neuropharmacology of the stimulants has been closely linked to dopamine systems (Murnane and Howell 2011; Ritz et al. 1989; Wilcox et al. 2002). Furthermore, a recent study in nonhuman primates showed that the behavioral effects of the wake-promoting drug modafinil are related to its effects at the dopamine transporter (Andersen et al. 2010). As such, previous studies support our conclusion that that increased dopamine neurotransmission contributes to the wake-promoting effects of amphetamine, and likely other stimulants.

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An important caveat to the present work is that we have not addressed whether the noradrenergic effects of amphetamine contribute to its wake-promoting effects. Although this is possible, we propose that it is unlikely because of five principal reasons. First, it has been established that amphetamine retains its wake-promoting effects in the face of marked depletion of norepinephrine by locus coeruleus lesions in the cat (Jones et al. 1977). Second, dopamine transporter knockout mice are insensitive to the wake-promoting effects of a variety of stimulants (Wisor et al. 2001). Third, in contrast to selective dopamine reuptake inhibitors, selective norepinephrine reuptake inhibitors do not promote wakefulness in normal and narcoleptic dogs (Nishino et al. 1998). Fourth, the potencies of the stereoisomers of amphetamine to promote wakefulness correlate with their potencies to release dopamine but not norepinephrine (Kanbayashi et al. 2000). Fifth, the behavioral stimulant effects of amphetamine and other stimulants in nonhuman primates have been closely linked to increased dopamine neurotransmission (Murnane and Howell 2011).

Another important caveat to the results of these studies is that they were generated using non-invasive actigraphic methods rather than the polysomnographic methods traditionally used in sleep studies. However, it is important to note that this actigraphic approach is a valid proxy for polysomnogrphic techniques as the two approaches exhibit greater than 90% agreement in humans (Kushida et al. 2001; Sadeh et al. 1995). Moreover, there is a strong correlation between actigraphy-based assessments of sleep and video-based assessments of sleep in rhesus monkeys (Papailiou et al. 2008). Nevertheless, small motor movements that do not involve the entire body, such as isolated head or limb movements, may not be accurately recorded by actigraphy (Papailiou et al. 2008). As such, it is possible that actigraphy may overcount sleep durations (Barrett et al. 2009). While recognizing this limitation, we suggest that it did not overly affect the results of the present study because the effects of both amphetamine and M100907 were robust and dependent on either the dose administered or the pretreatment time, parameters that are known to govern pharmacological effects.

Another important limitation of this study is the site-directed nature of the microdialysis technique that we used, as it is possible that 5-HT_{2A} receptor antagonism modulates the dopamine releasing effects of amphetamine in a regionally specific manner. Despite this limitation, *in vivo* microdialysis is a powerful approach to studying neurochemistry because it is one of only a few techniques that provides a direct measure of the neurochemical of interest. Nonetheless, future studies could extend this work using measures of prolactin secretion, as amphetamine reduces circulating prolactin levels (Murnane et al. 2010) and prolactin provides an integrated measure of a neurochemical response across the whole organism (Emiliano and Fudge 2004). Alternatively, PET imaging could be used to assess regional differences in dopamine overflow through displacement of raclopride or another radiotracer that is selective for dopamine receptors.

In conclusion, these studies demonstrate that amphetamine promotes wakefulness in rhesus monkeys, under the conditions employed. Moreover, amphetamine only elicited wakefulness at doses that engendered increases in extracellular levels of dopamine. Perhaps most compellingly, the selective 5-HT_{2A} receptor antagonist M10907 attenuated the effects of amphetamine on both wakefulness and dopamine overflow. These findings are consistent with previous work in rodents showing that M100907 attenuates the effects of amphetamine on locomotor activity, midbrain dopamine cell firing, and dopamine overflow. However, they extend this work by showing that M100907 attenuates the effects of amphetamine on arousal and dopamine overflow in nonhuman primates. These findings contribute to the continuing debate regarding the role of dopamine in arousal and support the contention that the dopamine system and 5-HT_{2A} receptors have important roles in this area. Moreover,

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they suggest that increased dopamine neurotransmission may be necessary for the wake-promoting effects of amphetamine, and possibly other stimulants.

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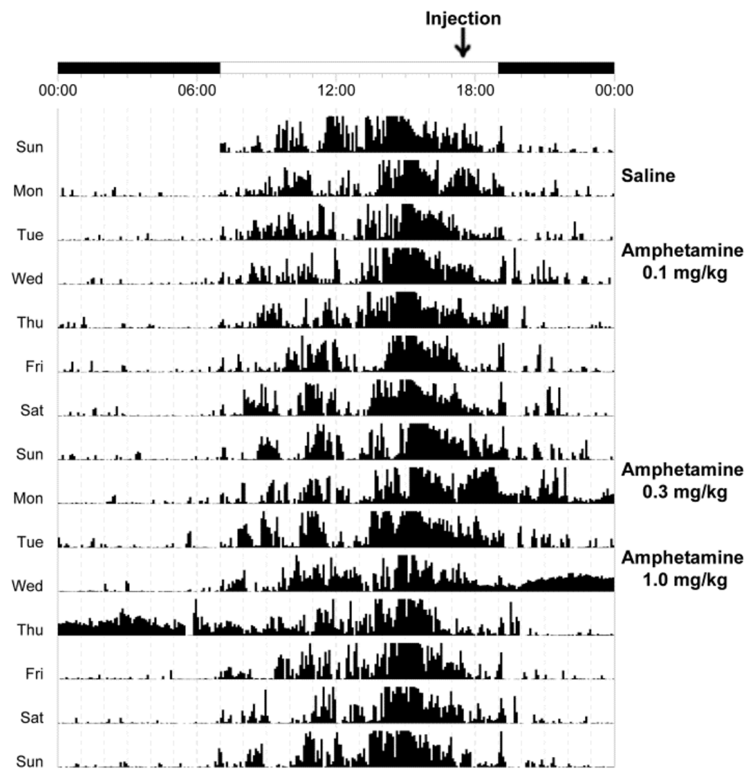

Figure 1.

Diurnal activity pattern over two weeks of recordings in a single representative subject. The days when amphetamine or saline was administered are labeled on the right. The time at which amphetamine or saline was administered and the light and dark phases are labeled on the top. The days of the week are labeled on the left. *Abscissae:* Time bins with each bins representing 5 min of recording. *Ordinates:* Total activity within a time bin with a larger column indicating greater activity.

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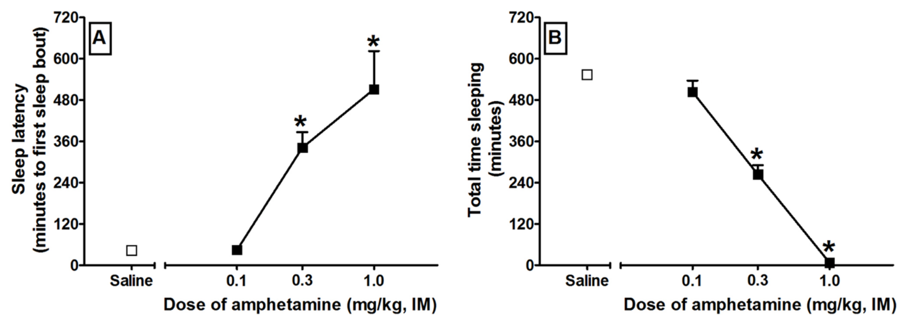

Figure 2.

Effects of amphetamine on sleep in rhesus monkeys. All points represent the mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data point. *Abscissae:* Dose of drug expressed as mg/kg and plotted on a logarithmic scale. The points at saline represent administration of the saline vehicle of amphetamine. *Ordinates:* Latency to the first sleep bout from the commencement of the dark phase (A) or total sleep duration over the entire dark phase (B). A * indicates a significant difference from saline treatment as assessed by Dunnett's method.

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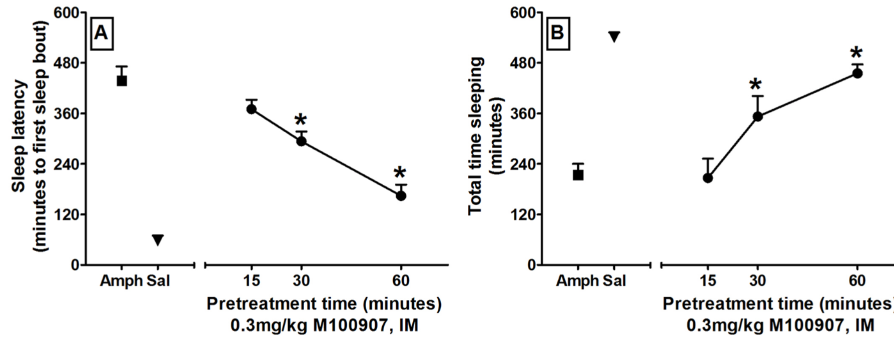

Figure 3.

Influence of pretreatment time on the effects of 5-HT_{2A} receptor antagonism by M100907 (0.3 mg/kg, IM) on wakefulness elicited by amphetamine (0.3 mg/kg, IM). *Abscissae:* Dose of drug expressed as mg/kg and plotted on a logarithmic scale. The points at Amph and Sal represent the administration of amphetamine or saline after the administration of the vehicle of M100907, respectively. *Ordinates:* Latency to the first sleep bout from the commencement of the epoch of darkness (A) or total sleep duration over the entire epoch of darkness (B). A * indicates a significant difference from pretreatment with vehicle as assessed by Dunnett's method.

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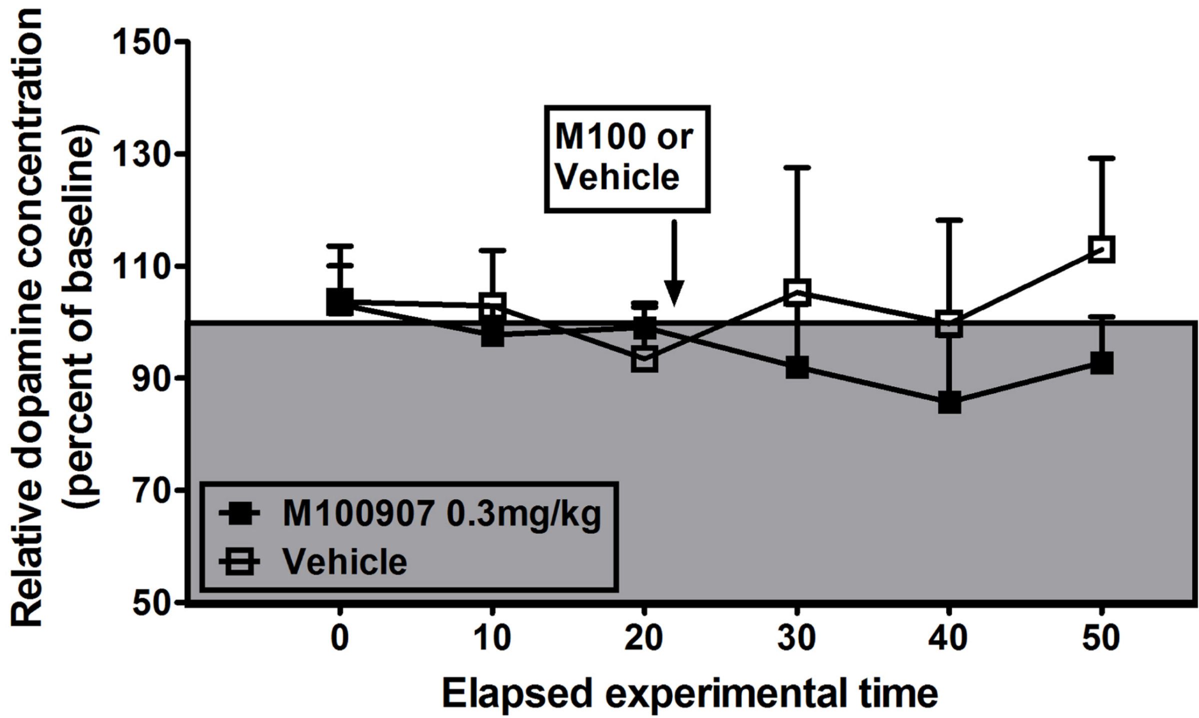

Figure 4.

Determination of the effects of 5-HT_{2A} receptor antagonism by M100907 (0.3 mg/kg, IV) on basal levels of dopamine within the caudate. The shaded area represents the area of the graph that includes or is below the defined baseline area of 100%. *Abscissae:* Time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale. *Ordinates:* Extracellular concentration of dopamine within the caudate expressed as a percent change from baseline.

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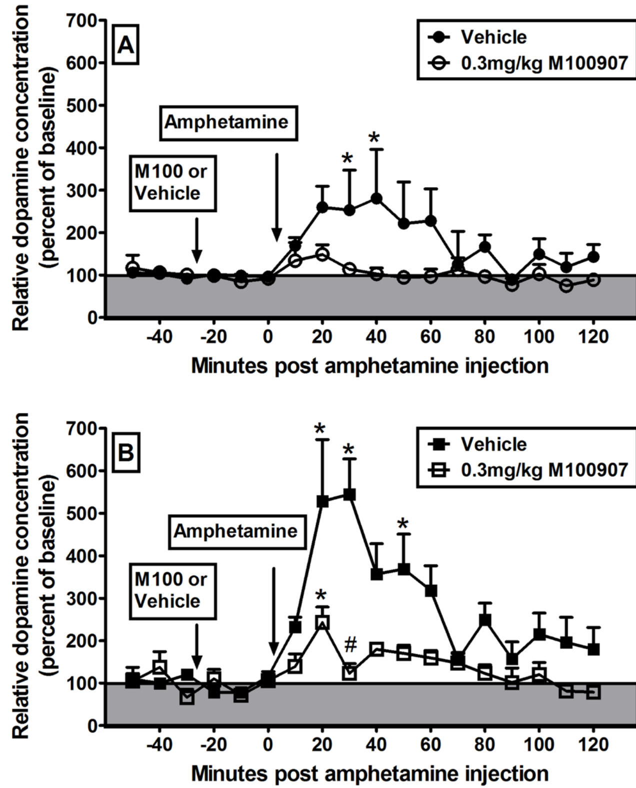

Figure 5.

Determination of the effects of 5-HT_{2A} receptor antagonism by M100907 (0.3 mg/kg, IV) on amphetamine elicited dopamine release within the caudate nucleus. Amphetamine was administered intravenously at 0.3 (A) and 1.0 mg/kg (B) following pretreatment with M100907 (open symbols) or its vehicle (closed symbols). vehicle (closed symbols). The shaded area is the same as in Figure 4. *Abscissae:* Time expressed in minutes in reference to the administration of amphetamine and plotted on a linear scale. *Ordinates:* Extracellular concentration of dopamine within the caudate expressed as a percent change from baseline. An * indicates a significant difference from baseline assessed via Dunnett's test. A # indicates a significant difference in dopamine levels between the pretreatments as assessed by a paired t-test.