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The dual orexin receptor antagonist almorexant blocks the sleep-disrupting and daytime stimulant effects of methamphetamine in rhesus monkeys

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Abstract

Background: The present study investigated the effects of the dual orexin receptor antagonist (DORA) almorexant, a sleep-modulating drug, on the sleep-disrupting effects of methamphetamine in adult rhesus monkeys.

Methods: Monkeys were fitted with primate collars to which actigraphy monitors were attached. To determine the effects of methamphetamine on daytime activity and sleep-like parameters, monkeys were given acute injections of vehicle or methamphetamine (0.03, 0.1 or 0.3 mg/kg, i.m.) in the morning (9:00h) (n=4 males). We then determined the ability of almorexant to alter the daytime and/or sleep-like effects of the largest (effective) dose of methamphetamine. Vehicle or almorexant (1, 3 or 10 mg/kg, i.m.) were administered in the evening (16:30h, 1.5h before "lights off") following morning (9:00h) administration of methamphetamine (0.3 mg/kg, i.m.), or as a pretreatment (8:30h) before methamphetamine injections (9:00h) (n=4 males). The ability of almorexant (10 mg/kg) to improve sleep-like behaviors also was assessed in a group of monkeys quantitatively identified with short-duration sleep (n=2 males, 2 females).

Results: Morning methamphetamine administration dose-dependently impaired sleep in rhesus monkeys (0.3 mg/kg significantly increased sleep latency and decreased sleep efficiency). Administration of almorexant, both as a pretreatment or as an evening treatment, improved methamphetamine-induced sleep impairment in a dose dependent manner. Morning pretreatment with almorexant also blocked the daytime stimulant effects of methamphetamine. Evening, but not morning, treatment with almorexant in a group of monkeys with baseline short-duration sleep improved sleep measures.

Authors Contribution

LFB and JKR were responsible for the study concept and design. LFB and ECMJ contributed to the acquisition of data. All authors assisted with data analysis and interpretation of findings. LFB and ECMJ drafted the manuscript. All authors provided critical revision of the manuscript for important intellectual content and approved the final version for publication.

Conflict of Interest

No conflict declared.

Conclusions: Our findings indicate that orexin receptor systems are involved in methamphetamine-induced hyperarousal and sleep disruption.

Keywords

actigraphy; DORA; methamphetamine; orexin; rhesus monkeys; sleep

Introduction

Stimulants are important therapeutic tools in the management of psychiatric disorders, such as hypersomnia, narcolepsy and attention deficit/hyperactivity disorders (ADHD) (Arnsten, 2006; Arnulf et al., 2019; Kornum et al., 2017). However, they also induce several unwanted side effects, from sleep-wake disturbances to addiction and dependence (Greenhill, 2006; Ogeil and Phillips, 2015).

Data from the National Surveys on Drug Use and Health (2015–2016) show that approximately 6.6% of U.S. adults in the past year (annual average) used prescription stimulants (Compton et al., 2019). Stimulant drugs are frequently prescribed in the context of sleep-wake disorders (Arnulf et al., 2019; Kornum et al., 2017). In addition, individuals using psychostimulant substances for wakefulness-promoting purposes show a progressively growing nocturnal pattern of drug use, particularly for shift workers or individuals doing extended shifts, as in the medical field (Deng et al., 2018; Fallah et al., 2018). Over the past decades, it has become relatively common to skip one night's sleep because of job or academic demands (National Sleep Foundation, 2019), and psychostimulants are used frequently as alertness-enhancing drugs when irregular work/rest patterns cause excessive sleepiness (Deng et al., 2018; Fallah et al., 2018). In addition, among young adults, late-night parties are often associated with the recreational use of stimulant drugs, which continues to expand markedly in this population (Schepis et al., 2020). Yet, the mechanisms underlying the relationship between stimulant use and sleep impairment remain poorly understood.

Several neurotransmitter systems are involved in the regulation of sleep-wake cycles and could be mediating the effects of stimulant drugs on sleep. One in particular is positioned at the interface of sleep and reward: the orexin/hypocretin system (Tsujino and Sakurai, 2009). Orexin neuropeptides play important roles in multiple physiological functions, particularly in sleep-wake regulation (Peyron and Kilduff, 2017) and in reward processes (Aston-Jones et al., 2010; Baimel et al., 2015). Moreover, of the two orexin receptor subtypes identified, orexin-1 (OX1) receptors appear to be involved in the abuse-related effects of stimulants (Brodnik et al., 2020; Foltin and Evans, 2018), whereas orexin-2 (OX2) receptors have been associated with the sleep-promoting properties of orexin antagonists (Jacobson et al., 2017). However, although OX1 receptor antagonists do not exert robust sleep-inducing effects (Morairty et al., 2012), non-selective orexin antagonists, referred to as "dual orexin receptor antagonists" (DORAs) have shown characteristics of an ideal sleep aid in primates (Winrow et al., 2011), and also are more effective in inducing sleep in rodents than selective OX2 receptor antagonists alone (Morairty et al., 2012).

Together, these findings suggest the important possibility that orexin receptor systems could be involved in sleep disruption induced by the administration of stimulant drugs. We have previously demonstrated that methamphetamine self-administration impairs actigraphy-based nighttime sleep in rhesus monkeys even when self-administration sessions were conducted in the morning (Berro et al., 2017a,b), an effect that also has been recently reported in long-term recreational methamphetamine users (Hermann et al., 2017). However, the extent to which experimenter-administered methamphetamine induces sleep impairment in naïve rhesus monkeys remained unknown. Therefore, the aim of the present study was to investigate the effects of acute non-contingent administration of methamphetamine on sleep-like parameters in adult naïve rhesus monkeys, and the effects of the DORA almorexant on the daytime stimulant and sleep-disrupting effects of methamphetamine. Almorexant was administered as a pretreatment before morning methamphetamine injections, or as an evening treatment following morning methamphetamine injections. Sleep-like parameters and daytime activity were measured using actigraphy. The effects of almorexant on sleep in the absence of methamphetamine were also investigated using a novel approach in which the ability of almorexant to alter sleep-like behaviors was assessed in a group of monkeys quantitatively identified with short-duration sleep (increased sleep latency, decreased sleep efficiency compared to normal sleepers).

Materials and Methods

Subjects

Five adult male and 2 adult female rhesus monkeys (*Macaca mulatta*) aged 9–25 and weighing 8–16 kg served as subjects for the studies. All monkeys were experimentally naïve prior to the beginning of the studies. Monkeys were housed individually, but had visual, auditory and olfactory contact with other monkeys throughout the study, as well as access to chew toys and a mirror in their cage. Monkeys were maintained on a 12h light/12h dark cycle (lights on at 6h), at a temperature of 21±2°C, with water available *ad libitum* and monkey diet available once/day, supplemented by fresh fruit and forage (seeds and dry fruit), and were weighed monthly during physical examinations. Monkeys were fitted with collars (Primate Products) prior to the initiation of the studies. All of the procedures and animal maintenance were in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), with review and approval via the Institutional Animal Care and Use Committees of the University of Mississippi Medical Center.

Drugs

Methamphetamine (±) HCl was supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC, USA). Methamphetamine was dissolved in 0.9% sterile physiological saline. Almorexant (MedKoo, NC, USA) was dissolved in 25% (2-Hydroxypropyl)-β-cyclodextrin (Sigma, MO, USA) w/v in saline. All drugs were administered intramuscularly (i.m.) at a volume of 0.1 ml/kg. Doses are based on the salt forms of the drugs.

Actigraphy-based daytime activity and sleep parameters

Actiwatch sensors (Mini Mitter, Bend, OR, USA) were used to assess daytime and nighttime activity, as previously described (Andersen et al., 2010, 2013). Briefly, the Actiwatch device consisted of an omni-directional sensor that is sensitive to motion (recorded as activity counts). The monitors were programmed to record the intensity, amount, and duration of movement over the preceding 60 s (i.e. epoch length=60 s). Monkeys had been adapted to wearing the activity monitors attached to primate collars (Primate Products), and collars were placed on monkeys while under ketamine (10 mg/kg) during physical examinations. Daytime activity data generated activity counts/hour. Nighttime activity data generated the following sleep-like behavior parameters: sleep efficiency (i.e., the percentage of the 12h dark phase – 6pm to 6am – spent sleeping) and sleep latency (i.e., the time between "lights off" at 18h and the first 5min with no detected movement). All parameters were calculated using the Actiware Sleep 3.4 software program (Mini-Mitter, 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. The Actiware analysis software automatically inferred sleep-like parameters from activity counts during each 60-s epoch by comparing the sum of the activity counts in that epoch and neighboring epochs to a predefined criterion.

Study design

Before the beginning of drug treatments, Actiwatches were attached to the monkeys' collars and baseline sleep-like behavior and daytime activity were measured for 1 week. Actigraphy recording continued for the duration of the treatments. Four male monkeys completed Experiments 1 through 3, and 4 different monkeys (2 females and 2 males) completed Experiment 4 (see Table 1 for Individual subject data). During a first set of treatments, each monkey was administered an acute intramuscular injection of saline (vehicle) or methamphetamine (0.03 – 0.3 mg/kg) at 9:00h (Experiment 1). Doses were chosen based on previous studies investigating the pharmacokinetic profile of methamphetamine in rhesus monkeys (Banks et al., 2016). During a second set of treatments, each monkey was administered an acute morning (9:00h) injection of methamphetamine (0.3 mg/kg, dose determined to have significant sleep-disrupting effects during the first treatments). Vehicle or almorexant (1, 3 or 10 mg/kg, i.m.) were then administered as a pretreatment (8:30h) before morning (9:00h) administration of methamphetamine (Experiment 2), or in the evening (16:30h, 1.5h before "lights off") following morning (9:00h) methamphetamine injections (Experiment 3).

In order to determine the sleep-promoting effects of morning vs evening almorexant in the absence of methamphetamine (Experiment 4), vehicle or almorexant (10 mg/kg, i.m.) were administered at 8:30h and at 16:30h following morning (9:00h) administration of vehicle (saline) in a group of monkeys in which sleep latency was longer and sleep efficiency values were less than typical values from our colony, respectively (see Table 1 and Figure 5).

A 2-week baseline period was given between each Experiment. During each set of treatments, at least 2 days were given between each treatment. For Experiment 1, each treatment (vehicle or one of the 3 doses of methamphetamine) was determined twice

and averaged together for analysis. For all other Experiments, each data-point was singly determined for all monkeys. All monkeys were housed in the same colony room. All treatments within an Experiment were carried out contemporaneously and baseline sleep data were collected during the same week for all monkeys. During the treatment phase, the order of drug treatments, including vehicle treatments, and doses was randomized and counterbalanced across monkeys, minimizing any potential influences of one monkey's sleep alterations over others.

Data analysis

Baseline data were combined across a 7-day period for a period immediately preceding each Experiment (1–4). The same monkeys were the subjects for Experiments 1–3, and no significant changes in baseline sleep parameters were observed over the course of the experiments for these monkeys. Sleep and daytime activity data were analyzed using one-way repeated-measures (RM) analysis of variance (ANOVA) with treatment as the factor. Multiple comparisons were conducted using Bonferroni t-test. Sleep-like measures and daytime activity data are presented as normalized data (percentage of baseline). In order to confirm that monkeys used in Experiment 4 (almorexant alone tests), referred to as a "short-duration sleep" cohort, differed in their sleep parameters compared with the larger colony, actigraphy data first were analyzed for monkeys from the housing rooms from which the short-duration sleep monkeys were chosen (N=26). These data were analyzed for skewness/kurtosis (i.e., confirming to a normal distribution) by computing d'Agostino and Pearson tests of normality (K^2). Because both sleep latency and sleep efficiency data sets passed normality tests (see Results), the mean values for the short-duration sleep cohort during baseline assessments were compared with the colony statistics using a one-sample t-test. This comparison was also conducted with the cohort of monkeys from the first three experiments. All graphical data presentations were created and all statistical tests were performed using Prism 8 (GraphPad Software, vers. 8.4.3). Significance was accepted at an alpha of 0.05.

Results

Experiment 1. Effects of acute morning methamphetamine administration on actigraphy-based sleep parameters

Table 1 shows individual-subject baseline sleep-like parameters. The dose-dependent effects of methamphetamine on sleep-like parameters are illustrated in Figure 1. The ANOVA showed a significant treatment effect for both sleep latency [$F(4,12)=11.36$, $p<0.001$] (Figure 1A) and sleep efficiency [$F(4,12)=14.74$, $p<0.001$] (Figure 1B). For both measures, the Bonferroni tests showed that the 0.3 mg/kg dose of methamphetamine differed significantly from vehicle treatment ($p<0.01$ and $p<0.001$, respectively), with average sleep latencies increased and average sleep efficiency values correspondingly decreased relative to averages observed after vehicle administration. The vehicle averages were not significantly different from average baseline values for any measure.

Experiment 2. Effects of pretreatment with almorexant on methamphetamine-induced sleep impairment and increased daytime activity

The effects of pretreatment (8:30h) with almorexant on sleep impairment induced by morning (9:00h) methamphetamine administration are shown in Figures 2A and 2B. The ANOVA showed a significant treatment effect for both sleep latency [$F(4,12)=7.507$, $p<0.01$] (Figure 2A) and sleep efficiency [$F(4,12)=5.817$, $p<0.01$] (Figure 2B). The Bonferroni tests showed that morning methamphetamine administration (0.3 mg/kg) preceded by vehicle treatment significantly increased average sleep latencies and decreased average sleep efficiency values compared to the corresponding averages under baseline conditions ($p<0.05$). For both sleep measures, the Bonferroni tests also showed that when given as a pretreatment before morning methamphetamine administration, the 10 mg/kg dose of almorexant differed significantly from vehicle treatment ($p<0.05$), with almorexant decreasing average sleep latencies and increasing average sleep efficiency values relative to the averages observed with vehicle.

The effects of pretreatment with almorexant on increased daytime activity induced by morning methamphetamine administration are shown in Figure 3. The ANOVA revealed that a significant treatment effect [$F(4,12)=4.140$, $p<0.05$]. The Bonferroni tests showed that morning methamphetamine administration (0.3 mg/kg) preceded by vehicle treatment significantly increased daytime activity compared to baseline ($p<0.05$). The Bonferroni tests also showed that all doses of almorexant differed significantly from vehicle treatment ($p<0.05$), with almorexant decreasing average daytime activity values relative to vehicle averages when given as a pretreatment before morning methamphetamine administration.

Experiment 3. Effects of evening treatment with almorexant on methamphetamine-induced sleep impairment

The effects of evening (1.5h before “lights off”) treatment with almorexant on sleep impairment induced by morning methamphetamine administration are shown in Figures 2C and 2D. The ANOVA showed a significant treatment effect for both sleep latency [$F(4,12)=4.849$, $p<0.05$] (Figure 2C) and sleep efficiency [$F(4,12)=6.567$, $p<0.01$] (Figure 2D). The Bonferroni tests showed that morning methamphetamine administration (0.3 mg/kg) followed by vehicle treatment significantly increased average sleep latencies and decreased average sleep efficiency values compared to corresponding averages under baseline conditions ($p<0.05$ and $p<0.01$, respectively). For both sleep measures, the Bonferroni tests also showed that the 10 mg/kg dose of almorexant treatment resulted in significantly different values compared with those obtained after vehicle treatment ($p<0.05$), with almorexant decreasing average sleep latencies and increasing average sleep efficiency values relative to corresponding vehicle averages following morning methamphetamine administration.

Experiment 4. Effects of almorexant in monkeys with short-duration sleep

Individual monkey baseline sleep parameters are presented in Table 1. In order to establish whether morning/evening almorexant had sleep-improving effects in the absence of methamphetamine, we selected a group of monkeys with baseline sleep values indicating short-duration sleep compared with the larger pool of available monkeys in our colony

(N=26). Analyses of baseline actigraphy data from the larger pool indicated that for both sleep latency values (mean= 30.1, SEM= 4.85) and sleep efficiency values (mean= 79.2, SEM= 2.36), the scores conformed to a normal distribution (K^2 = 3.82 and 3.52, respectively; p's > 0.10). The group of monkeys in Experiment 4 (2 males, 2 females) showed a significantly lower baseline sleep values compared to the overall pool of monkeys (Figures 4A and 4B, one-sample t-test, sleep latency: [t(3)=3.40, p<0.05]; sleep efficiency: [t(3)=7.65, p<0.05]). For the comparison of baseline sleep parameters from Experiments 1–3 with the overall pool of monkeys, no statistically significant differences were observed (Figures 4A and 4B, one-sample t-test, sleep latency: [t(3)=0.261, p>0.1]; sleep efficiency: [t(3)=0.753, p>0.1).

The effects of morning (8:30h) or evening (16:30h) administration of 10 mg/kg almorexant are shown in Figures 4C and 4D. The ANOVA showed a significant treatment effect for both sleep latency [F(4,12)=8.99, p<0.01] (Figure 4C) and sleep efficiency [F(4,12)=4.932, p<0.05] (Figure 4D). The Bonferroni tests showed that evening (PM), but not morning (AM), treatment with 10 mg/kg almorexant significantly increased average sleep latencies and decreased average sleep efficiency values compared to both baseline values (p<0.05) and values following evening (PM) vehicle administration (p<0.05). Importantly, morning (AM) almorexant administration did not significantly improve sleep measures in the absence of methamphetamine.

Discussion

The present study shows for the first time that acute morning administration of methamphetamine in naïve rhesus monkeys dose-dependently impaired sleep parameters in rhesus monkeys, with the dose of 0.3 mg/kg significantly increasing sleep latency and decreasing sleep efficiency. Administration of the DORA almorexant, both as a morning pretreatment or as an evening treatment, improved methamphetamine-induced sleep impairment in a dose-dependent manner. Morning pretreatment with almorexant also blocked the daytime stimulant effects of methamphetamine at all doses tested. Evening treatment with almorexant (10 mg/kg) in a group of monkeys with baseline short-duration sleep (i.e., in the absence of methamphetamine) "normalized" sleep measures, i.e., decreased sleep latency and improved sleep efficiency. However, morning administration of almorexant in this same group of monkeys did not have sleep-improving effects, contrary to that observed when animals were given methamphetamine following almorexant pretreatment.

Our findings showing that morning administration of methamphetamine induces sleep impairment in rhesus monkeys are in agreement with our previous studies. We have demonstrated previously that methamphetamine self-administration impairs actigraphy-based nighttime sleep in rhesus monkeys even when self-administration sessions were conducted in the morning (Berro et al., 2017a,b). The present study establishes that methamphetamine-induced disruption of sleep is not dependent on the drug being available in a self-administration context. Of note, the experimental design used in the present study has some unique advantages for investigating stimulant-induced sleep impairment. By administering methamphetamine response-independently, we were able to standardize the methamphetamine dosing, which is a challenge in self-administration studies, as the animals

control drug intake with contingent drug delivery. Also, by giving methamphetamine as a single injection, we controlled for the potential effect that a pretreatment with almorexant might have had on methamphetamine intake, which would ultimately affect sleep outcomes due to decreasing methamphetamine exposure, as seen in previous studies from our group (Berro et al., 2017b). Finally, the present study also shows for the first time that we can replicate our previous findings showing that methamphetamine disrupts sleep, even in the absence of drug-associated cues, which we also have shown to contribute to methamphetamine-induced sleep impairment in the context of drug self-administration (Berro et al., 2016).

Accumulating evidence suggests that the orexin system is a potential therapeutic target in psychostimulant use disorders (James et al., 2017) and insomnia (Janto et al., 2018). Consistent with the role of orexin in regulating reward processes in general, selective OX1 receptor antagonists and DORAs, but not selective OX2 antagonists, have been shown to block the abuse-related effects of stimulants in rodents (Gentile et al., 2018; James et al., 2017; Steiner et al., 2013). While OX1 receptor antagonists do not exert robust sleep-inducing effects, selective OX2 antagonists promote sleep in rodents (Morairty et al., 2012). Although these initial findings suggest that the two subtypes represent distinct targets for stimulant abuse vs. sleep disorders, studies suggest that a synergistic effect may exist between antagonism of OX1 and OX2 receptors, particularly regarding their sleep-promoting effects. In that regard, DORAs are more effective in inducing sleep in rodents than selective OX2 receptor antagonists (Morairty et al., 2017). In addition, anatomical localization of orexin receptors in the rodent brain suggests that both receptors may be involved in the promotion of wakefulness (Lindemann et al., 2008; Marcus et al., 2001). Therefore, in the present study we investigated the effects of a DORA on sleep disruption induced by acute methamphetamine administration. While we were not able to directly address the contribution of OX1 vs OX2 receptors on stimulant-induced sleep impairment, we hoped to add to the literature indicating that DORAs are potential candidates for the treatment of insomnia and/or sleep disruption.

DORAs have shown characteristics of an ideal sleep aid in primates (Winrow et al., 2011), and seem to facilitate sleep by reducing wake drive without altering normal sleep architecture (Coleman et al., 2017; Morairty et al., 2012). On the other hand, conventional sleep aids (e.g., benzodiazepine-type drugs) induce sleep via generalized inhibition, markedly changing sleep architecture (suppressing slow wave sleep and rapid eye movement – REM – sleep; Arbon et al., 2015). Although the effects of DORAs on sleep quality and sleep architecture have been well established in several species (Gotter et al., 2013; Winrow et al., 2011), only recently have studies started to investigate the potential of DORAs for treatment of sleep disruption in the context of psychostimulant use and abuse. In the only preliminary study published on the topic to date, Suchting and colleagues (2020) investigated the effects of the DORA suvorexant on stress, sleep, cue reactivity, and inhibitory control as factors related to relapse in 20 non-treatment seeking individuals with cocaine use disorder. The study found improvements in all these domains with administration of suvorexant as a sleep aid before bedtime (22h), including improvements in actigraphy-based sleep, compared to placebo (Suchting et al., 2020). The authors emphasize that given the study design and sample size, they were unable to test

causation (Suchting et al., 2020). Although drug accumulation may be expected given the chronic drug regimen and the 12-hour half-life of oral suvorexant in humans (Bennett et al., 2014), research suggesting that sleep impairment may be a risk factor for relapse to drug use (Brower and Perron, 2010) indicate that suvorexant may have exerted a beneficial effect on craving simply by improving sleep.

The present study demonstrates that the DORA almorexant blocked the sleep-disrupting effects induced by acute methamphetamine injections. Although a different protocol compared to the study by Suchting and colleagues (2020), in which volunteers were chronic cocaine users, our study further indicates the potential of DORAs for the treatment of stimulant-induced sleep impairment. Our findings also show that morning administration of almorexant blocks the sleep-disrupting effects of a subsequent methamphetamine injection. In order to rule out the possibility that pretreatment with almorexant blocked the nighttime effects of methamphetamine because of a long duration of action, we also investigated the effects of almorexant on sleep in the absence of methamphetamine in a group of monkeys quantitatively identified with short-duration sleep. Our results show that morning administration of almorexant in this group of monkeys was not effective in inducing significant sleep-promoting effects. While 2 monkeys showed improved sleep latency after morning almorexant administration, no significant differences were observed in the group analysis for both sleep latency and sleep efficiency. On the other hand, evening administration of almorexant significantly improved sleep in monkeys with short-duration sleep. Therefore, the effects of morning almorexant on methamphetamine-induced sleep impairment likely are not due to almorexant having significant exposure levels during the nighttime. Instead, antagonism of orexin receptors seems to block the downstream effects of methamphetamine that are responsible for its sleep-disrupting effects.

In accordance with studies showing altered dopaminergic neuroplasticity following sleep deprivation (Volkow et al., 2012); Wiers et al., 2016), the mesolimbic dopaminergic system has been proposed as the main pathway mediating stimulant-induced sleep impairment and hyperarousal. However, our previous findings suggest that alterations in mesolimbic dopaminergic neurotransmission are not sufficient to explain sleep disruption induced by methamphetamine (Berro et al., 2017b). In this regard, the $GABA_A$ receptor modulator temazepam did not improve sleep-like measures in rhesus monkeys self-administering methamphetamine, yet this drug attenuated methamphetamine-induced dopamine levels in the nucleus accumbens (Berro et al., 2017b). Considering that the half-life of methamphetamine in rhesus monkeys is relatively short (~3 hours; Banks et al., 2016), the fact that sleep disruption is observed more than 9 hours after methamphetamine administration suggests a downstream mechanism that might not be related directly to the drug-induced increases in mesolimbic dopamine levels. Our findings with morning almorexant administration suggest that orexin receptor blockade prevents the downstream effects of methamphetamine that are responsible for its sleep disrupting, or arousal-inducing, effects.

In fact, pretreatment with almorexant also blocked the methamphetamine-induced increase in daytime activity, even at doses that were not effective at improving sleep disruption induced by methamphetamine. Because the present study's activity data were averaged

across the 12h light cycle, it is possible that the effects of methamphetamine on general daytime activity were more prominent during the hours immediately following drug injection. Moreover, almorexant was administered shortly before methamphetamine in the daytime study, raising the possibility that lower doses of almorexant were effective at blocking methamphetamine-induced daytime activity due to pharmacokinetic factors. It is also possible that different mechanisms mediate the daytime stimulant vs sleep disrupting effects of methamphetamine, and that orexin systems mediate both effects. If this is the case, then our results suggest that the mechanisms involved in methamphetamine-induced hyperarousal are more sensitive to methamphetamine than those regulating sleep.

In summary, our findings indicate that orexin receptor systems are involved in methamphetamine-induced hyperarousal and sleep disruption, and that DORAs may block the sleep-disrupting effects of stimulant drugs. While limited by a small sample size, the effects observed in the present study are consistent across experimental protocols. Although further studies are needed to investigate whether DORAs would also block sleep impairment in the context of chronic methamphetamine use and abuse, the present findings provide important insights into clinical practice regarding patients on stimulant prescription. In addition to narcolepsy and hypersomnia (Arnulf et al., 2019; Kornum et al., 2017), stimulant medications are important tools in the management of ADHD, which is prevalent in up to 9% of children (Merikangas et al., 2011) and 8% of college students in the U.S. (Ascherman and Shaftel, 2017). Our findings indicate that DORAs may be useful in the management of insomnia induced by stimulant use and misuse, but further studies to investigate the effects of DORAs on chronic stimulant use are needed.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Highlights

- Morning methamphetamine administration impaired evening sleep in rhesus monkeys
- Almorexant improved methamphetamine-induced sleep impairment
- Almorexant was effective both as a pretreatment or as an evening treatment
- Pretreatment with almorexant blocked methamphetamine's daytime stimulant effects
- Evening treatment with almorexant improved sleep in monkeys with short sleep

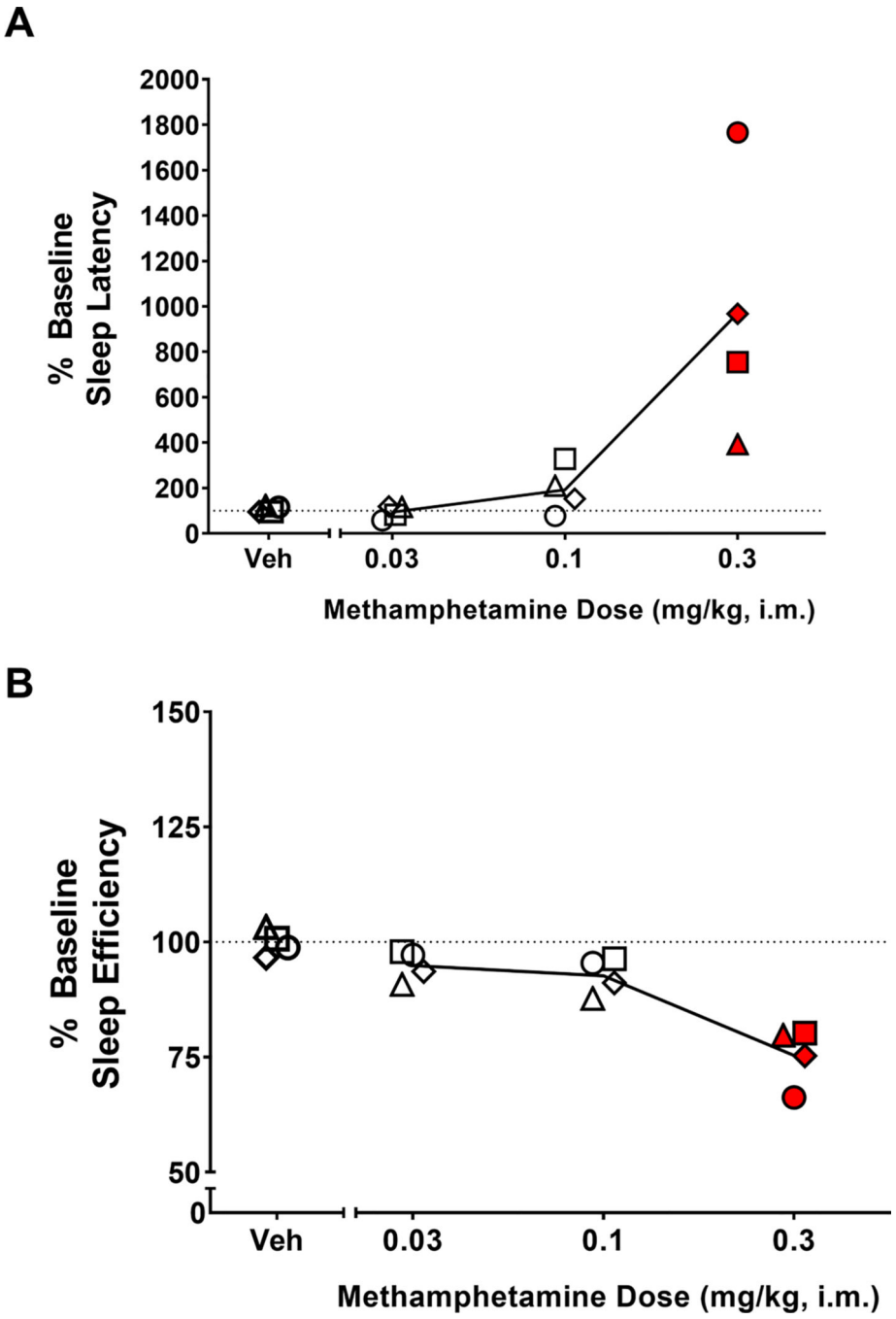

Figure 1.

Effects of acute morning methamphetamine administration on actigraphy-based sleep parameters. Dose-response curve of the effects of methamphetamine on sleep parameters. Sleep latency (A) and sleep efficiency (B) in the nights after morning (9:00h) administration of vehicle (Veh) or methamphetamine (0.03, 0.1 or 0.3 mg/kg, i.m.) in male rhesus monkeys (N=4). Actigraphy-based sleep-like measures are presented as normalized data (percentage of baseline). Data are normalized sleep parameters for individual monkeys (symbols) and the mean for each sleep parameter (lines). Dotted lines represent baseline sleep parameters

(100%). Red-filled symbols represent doses of methamphetamine for which the mean sleep parameters were significantly different from vehicle (Bonferroni t-tests, $p<0.05$).

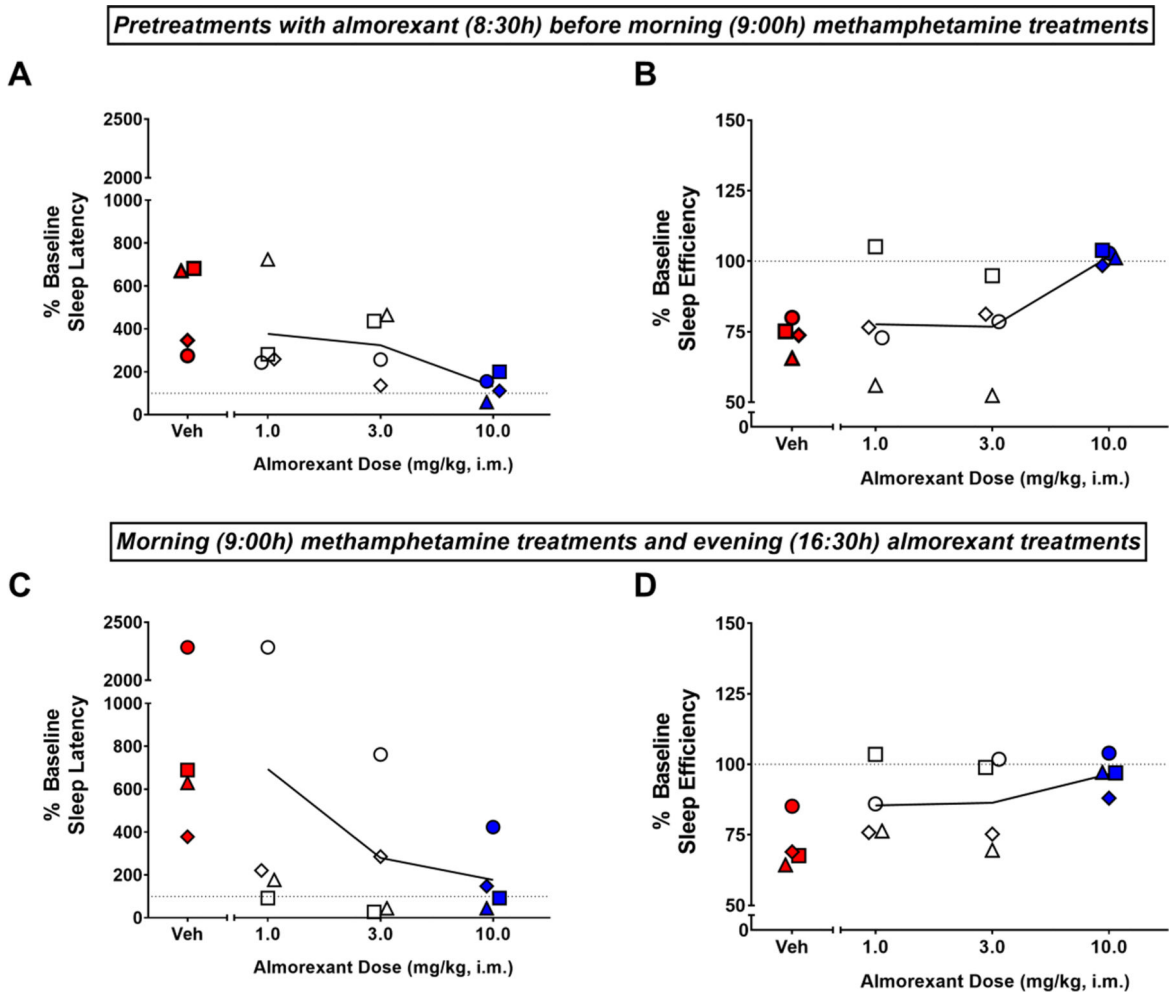

Figure 2.

Effects of morning pretreatment (top panel) or evening treatment (bottom panel) with almorexant on methamphetamine-induced sleep impairment. Sleep latency (A, C) and sleep efficiency (B, D) obtained on the nights after morning (9:00h) administration of methamphetamine (0.3 mg/kg, i.m.) and morning administration (8:30h, top panel) or evening administration (16:30h, bottom panel) of vehicle (Veh) or almorexant (1, 3 or 10 mg/kg, i.m.) in male rhesus monkeys (N=4). Actigraphy-based sleep-like measures are presented as normalized data (percentage of baseline). Data are normalized sleep parameters for individual monkeys (symbols) and the mean for each sleep parameter (lines). Dotted lines represent baseline sleep parameters (100%). Red-filled symbols represent mean sleep parameters significantly different from baseline (Bonferroni t-tests, $p<0.05$). Blue-filled symbols represent mean sleep parameters significantly different from vehicle (Bonferroni t-tests, $p<0.05$).

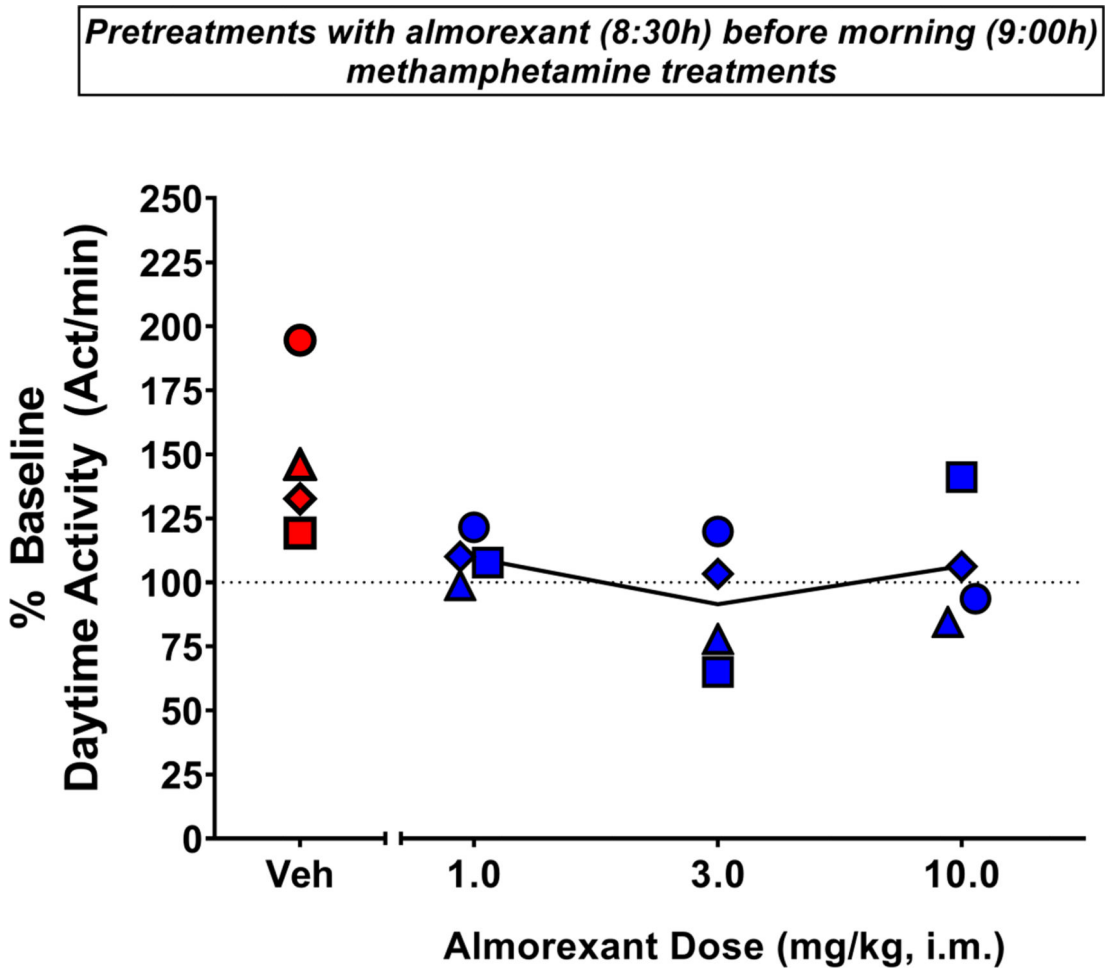

Figure 3.

Effects of pretreatment with almorexant on methamphetamine-induced increased daytime activity. Daytime activity (9:00h-18:00h) after pretreatment (8:30h) with vehicle (Veh) or almorexant (1, 3 or 10 mg/kg, i.m.) before morning (9:00h) administration of methamphetamine (0.3 mg/kg, i.m.) in male rhesus monkeys (N=4). Actigraphy-based activity measures are presented as normalized data (percentage of baseline). Data are daytime activity for individual monkeys (symbols) and the mean for each sleep parameter (lines). Dotted lines represent baseline sleep parameters (100%). Red-filled symbols represent mean daytime activity data significantly different from baseline (Bonferroni t-tests, $p<0.05$). Blue-filled symbols represent mean daytime activity data significantly different from vehicle (Bonferroni t-tests, $p<0.05$).

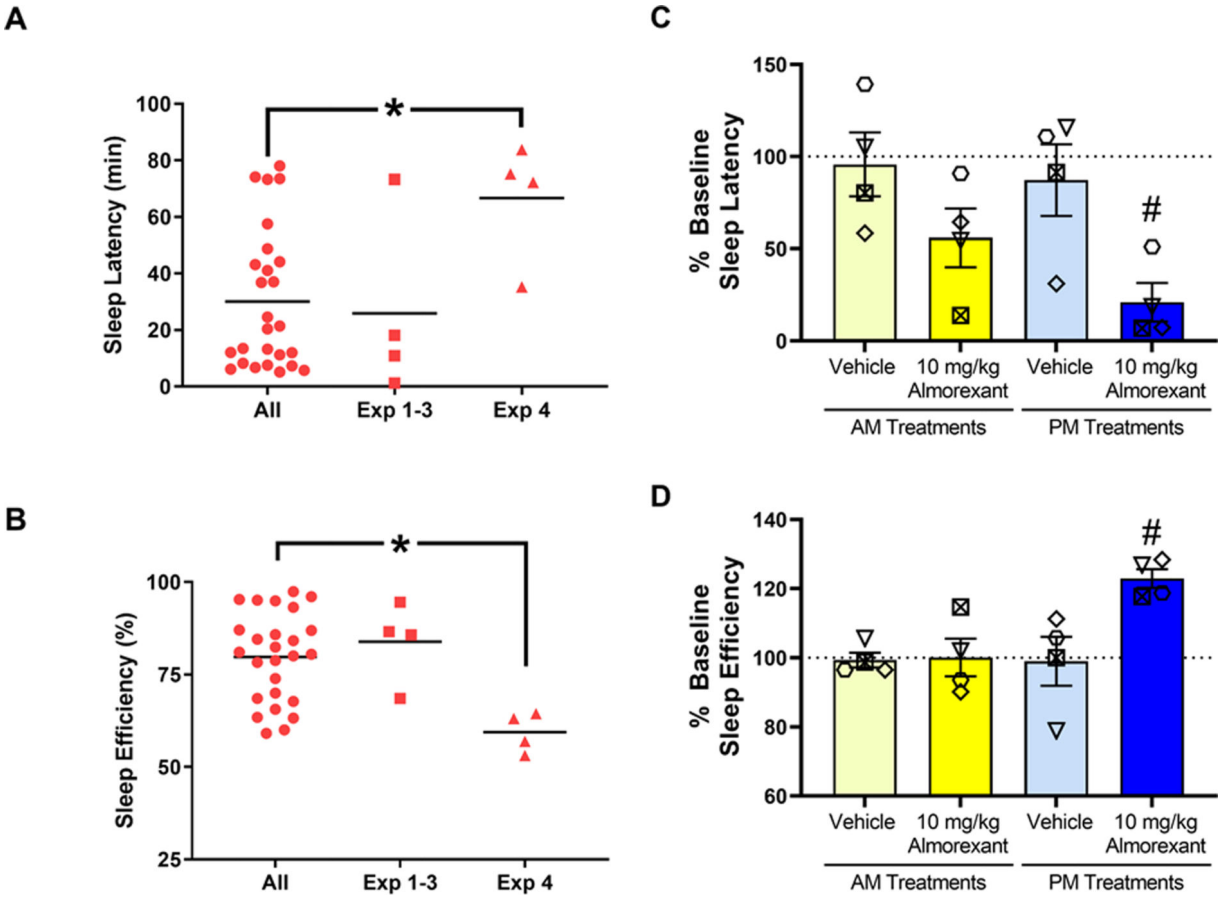

Figure 4.

Effects of almorexant on baseline sleep measures. (A) Sleep latency and (B) sleep efficiency for the group of monkeys with baseline sleep values indicating short-duration sleep (N=4, Exp. 4) compared with the larger pool of available monkeys in our colony (N=26, All) and with monkeys in Experiments 1–3 (N=4, Exp. 1–3). Sleep latency (C) and sleep efficiency (D) in the nights after morning (AM, 8:30h) or evening (PM, 16:30h) treatment with vehicle (Veh) or an effective dose of almorexant (10 mg/kg, i.m.) in male rhesus monkeys (N=4). Actigraphy-based sleep-like measures for (C) and (D) are presented as normalized data (percentage of baseline) for individual monkeys (symbols) and the mean for each sleep parameter (bars). Data in bar charts are expressed as mean ± SEM. Dotted lines represent baseline sleep parameters (100%). * $p<0.05$ compared to All; # $p<0.05$ compared to respective (AM/PM) Vehicle.

Table 1.

Individual-subject baseline sleep-like parameters

Subject	Age	Symbol on Graphs	Sleep Latency (min)	Sleep Efficiency (%)
Experiments 1–3, Figures 1–3				
271–2002 (M)	19	Circle	1.2 (0 – 9)	94.5 (92.4 – 96.2)
205–2002 (M)	19	Triangle	18.1 (8 – 21)	85.7 (80.1 – 89.2)
385–2001 (M)	20	Square	10.9 (8 – 20)	86.6 (83.5 – 89.3)
248–2005 (M)	16	Rhombus	73.3 (71 – 170)	68.5 (62.9 – 72)
		Mean ± SEM=	*25.9 ± 16.2*	*83.8 ± 5.5*
Experiment 4, Figure 4				
248–2005 (M)	16	Rhombus	83.8 (33 – 324)	63.1 (59.8 – 73.3)
98–003 (M)	25	Hexagon	35.2 (13 – 45)	56.9 (54 – 62.2)
BG-93 (F)	10	Inverted triangle	75.2 (18 – 164)	53.1 (49.3 – 56.5)
BH-91 (F)	9	Crossed square	72.2 (12 – 166)	64.4 (56 – 68.3)
		Mean ± SEM=	*66.6 ± 10.7*	*59.4 ± 2.7*

M = male rhesus monkey; F = female rhesus monkey. Individual data are expressed as mean (range) for a 7-day period of baseline sleep recording. Grouped data are expressed as mean ± SEM.