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GABA_A receptor positive allosteric modulators modify the abuse-related behavioral and neurochemical effects of methamphetamine in rhesus monkeys

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Abstract

GABA_A receptor positive allosteric modulators (GABA_A receptor modulators) are commonly used for the treatment of insomnia. Nevertheless, the effects of these compounds on psychostimulant-induced sleep impairment are poorly understood. Because GABA_A receptor modulators have been shown to decrease the abuse-related effects of psychostimulants, the aim of the present study was to evaluate the effects of temazepam (0.3, 1.0 or 3.0 mg/kg) and eszopiclone (0.3, 1.0 or 3.0 mg/kg), two GABA_A receptor modulators, on the behavioral neuropharmacology of methamphetamine in adult rhesus macaques (n=5). Sleep-like measures and general daytime activity were evaluated with Actiwatch monitors. Methamphetamine self-administration (0.03 mg/kg/inf) was evaluated during morning sessions. Methamphetamine-induced dopamine overflow was assessed through *in vivo* microdialysis targeting the nucleus accumbens. Nighttime treatment with either temazepam or eszopiclone was ineffective in improving sleep-like measures disrupted by methamphetamine self-administration. Acute pretreatment with a low dose of temazepam before self-administration sessions increased methamphetamine self-administration without affecting normal daytime home-cage activity. At a high dose, acute temazepam pretreatment decreased methamphetamine self-administration and attenuated methamphetamine-induced increases in dopamine in the nucleus accumbens, without decreasing general daytime activity. Acute eszopiclone treatment exerted no effects on methamphetamine intake or drug-induced increases in dopamine. Our study suggests that treatments based on GABA_A receptor modulators are not effective for the treatment of sleep disruption in the context of psychostimulant

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Conflict of Interest

The authors declare no conflict of interest.

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use. In addition, distinct GABA_A receptor modulators differentially modulated the abuse-related effects of methamphetamine, with acute treatment with the high efficacy GABA_A receptor modulator temazepam decreasing the behavioral and neurochemical effects of methamphetamine.

Keywords

methamphetamine; temazepam; eszopiclone; self-administration; sleep; microdialysis; nonhuman primates

1. Introduction

Methamphetamine use is a serious public health concern as it is one of the most widely abused illicit drugs (United Nations Office on Drugs and Crime, 2016). Its use is associated with numerous adverse physical, behavioral, and mental health outcomes (Herbeck et al., 2015; Rommel et al., 2015). Disturbed sleep is one of the most prominent symptoms of the use of amphetamines (Cruickshank and Dyer, 2009). Previous studies from our group have shown that methamphetamine self-administration markedly disrupts sleep in rhesus monkeys (Andersen et al., 2013; Berro et al., 2016). It is now known that psychostimulant abuse and sleep impairment show a bidirectional relationship, with a high prevalence of sleep problems in substance-dependent individuals (Mahfoud et al., 2009; Conroy and Arnedt, 2014) and individuals with history of sleep problems showing a higher risk of relapse and for the development of drug abuse (Ford and Kamerow, 1989; Brower and Perron, 2010; Wong et al., 2010). Thus, sleep patterns should be considered in treatment and abuse prevention strategies.

For several decades, the traditional sleep promoting pharmacotherapy for impaired sleep was predominantly based on the use of benzodiazepine drugs. Currently, the FDA approves the use of temazepam – a classic benzodiazepine that binds to GABA_A receptors with a lack of α subunit specificity – for the treatment of transient insomnia (Heel et al., 1981). Afterwards, the so-called “z-drugs” have emerged as hypnotic GABAergic drugs with fewer reported next-day side effects compared to benzodiazepines (Gunja, 2013). Eszopiclone is a z-drug which seems to exert functional selectivity for GABA_A receptors containing $\alpha 3$ subunits ($\alpha 3$ GABA_A receptors) (Dixon et al., 2015; Nutt and Stahl, 2010), with studies consistently showing enhancement in physiological sleep (Nutt and Stahl, 2010).

In addition to sleep-promoting properties, the effects of GABAergic compounds in drug abuse have been studied for years due to the strong interaction between GABAergic and dopaminergic neurotransmissions (Barrot et al., 2012; Borisovska et al., 2013). Indirect GABA receptor agonists attenuate the reinforcing effects of drugs of abuse in both rodents and nonhuman primates (Dewey et al., 1998, 1999; Kushner et al., 1990), indicating that indirect stimulation of both GABA_A and GABA_B receptors can reduce the reinforcing effects of drugs. In fact, treatment with diazepam has been shown to attenuate the development of amphetamine-induced behavioral sensitization in mice (Panhelainen et al., 2011), while the benzodiazepine drug alprazolam attenuates the effects of psychostimulants in humans (Rush et al., 2004). Studies in nonhuman primates further demonstrated that GABA_A receptor positive allosteric modulators (GABA_A receptor modulators) seem to be

more effective in blocking the abuse-related effects of stimulants compared to other GABA_A receptor agents, such as GABA agonists (Negus et al., 2000; Weerts et al., 2005).

Therefore, the above cited considerations indicate a broad and promising action of GABAergic compounds on drug addiction, with a possible role for GABA_A receptor modulators. Together with the fact that sleep promotion might contribute to the treatment of drug abuse, the aim of the present study was to evaluate the acute effects of two GABA_A receptor modulators on the behavioral neuropharmacology of methamphetamine in rhesus macaques. The effects of temazepam and eszopiclone were evaluated on the dopamine releasing and sleep disrupting effects of methamphetamine as well as on methamphetamine self-administration.

2. Material and Methods

2.1 Subjects

All behavioral studies were conducted in a group of 3 adult male and 2 adult female rhesus monkeys (*Macaca mulatta*) weighing 9–15 kg. Three adult female rhesus monkeys weighing 7–9 kg served as the subjects in the *in vivo* microdialysis experiments. Animals were fitted with collars (Primate Products) prior to the initiation of the studies. Each subject was individually housed in stainless steel home cages and fed Purina monkey chow (Ralston Purina, St. Louis, MO), supplemented with fruit and vegetables daily. Water was continuously available in the colony. Environmental enrichment was provided on a regular basis. The colony was maintained at an ambient temperature of 22±2°C at 45–50% humidity, and the lights were set to a 12-h light/dark cycle (lights on at 7h; lights off at 19h). All subjects had a history of exposure to methamphetamine. All protocols and animal care and handling strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th Edition, revised 2011) and the recommendations of the American Association for Accreditation of Laboratory Animal Care, and were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2 Drugs

(+/-) Methamphetamine hydrochloride (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% saline and administered intravenously. Temazepam (Sigma-Aldrich®, St. Louis, MO, USA) and eszopiclone (provided as a generous gift from Sunovion®, Marlborough, MA, USA) were dissolved in a 20:25:55 mixture of 95% ethanol, propylene glycol (Sigma-Aldrich®, St. Louis, MO, USA) and 0.9% saline to the proper concentration (3, 1 or 15 mg/ml). Temazepam and eszopiclone were chosen based on their individual pharmacokinetic profiles (~6–7.5h half-life; ~30 min time to maximum plasma concentration) (Curry et al., 1977; Nutt and Stahl, 2010). The highest dose used for the GABA_A receptor modulators was chosen based on previous studies indicating that 3 mg/kg temazepam (Curry et al., 1977) and 3 mg/kg eszopiclone (Uslaner et al., 2013; Gotter et al., 2014) had been proven safe and effective in inducing sleep-promoting effects in rhesus monkeys.

The doses of each drug were calculated and are expressed as the salt form. The order of treatments or doses was randomized across subjects within an experiment.

2.3 Surgery

All surgeries were conducted under aseptic conditions. Animals were initially anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0 mg i.m.) and ketamine HCl (20 mg i.m.), and anesthesia was maintained throughout the procedure with inhaled isoflurane (0.5–1.5%). A major vein (femoral or jugular) was implanted with a chronic indwelling catheter attached to a subcutaneous vascular access port, as previously described (Howell and Wilcox, 2001). The subjects of the microdialysis experiments were also implanted with bilateral CMA/11 guide cannulae (CMA Microdialysis, Holliston, MA, USA) that were stereotaxically targeted for the striatum, as previously described (Murnane et al., 2010). To target the area directly above the nucleus accumbens (NAc), the guide cannulae were positioned 23mm anterior to the interaural midpoint in each subject and placed bilaterally at 4mm off of the midline. Guide cannulae placement was later verified by visual inspection of well-defined anatomical landmarks observed with magnetic resonance imaging.

2.4 Methamphetamine self-administration

The apparatus and self-administration (SA) procedure were previously described by Howell and Wilcox (2001). Animals were trained to respond under a fixed ratio (FR) 20 schedule of drug delivery. Subjects had the opportunity to self-administer methamphetamine during 60-min sessions once a day, 5–7 days/week in the morning (starting between 8–9am). The animals were positioned in a primate chair (Primate Products) and placed in a sound-attenuating experimental chamber for the duration of the session and maintained in their home-cages for the remainder of the day. During the test session, the behavioral chamber was illuminated with a white light which served as a discriminative stimulus. Completion of the FR 20 resulted in a change in the stimulus light from white to red for 15s and a methamphetamine infusion (0.03 mg/kg in 0.5 ml infused over 3s). This infusion was followed by a 60-s timeout. At the end of the timeout, the white light was presented again to signal the opportunity to complete another FR. Methamphetamine intake was determined as the number of infusions received on a given session times 0.03 (drug dose in mg/kg/infusion). The unit dose of methamphetamine chosen for our studies was based on previous studies showing that 0.03 mg/kg/infusion methamphetamine induces marked sleep-disrupting effects in rhesus monkeys (Andersen et al., 2013; Berro et al., 2016). This is a dose on the descending limb of the methamphetamine dose-effect curve for all 5 subjects that took part in the behavioral studies.

2.5 Daytime and nighttime activity

Actiwatch sensors (Mini Mitter, Bend, OR, USA) were used to assess daytime and nighttime activity, as previously described (Andersen *et al*, 2010, 2013). Subjects had been adapted to wearing the activity monitors and trained to cooperate with the attachment of the sensor in their collars. Daytime activity data are generated as activity counts/hour. Nighttime activity data generate the following sleep-like behavior parameters: sleep efficiency (i.e., the percentage of the dark phase spent sleeping); sleep latency (i.e., the time between the lights-off time and the first sleep bout); fragmentation index (i.e., the number of immobile bouts

during the dark phase that lasted less than 1 min during the sleep recording period). All parameters were calculated using the Actiware Sleep 3.4 software program (Mini-Mitter, Bend, OR, USA).

2.6 Protocol design

2.6.1 Nighttime activity studies—Before drug SA, Actiwatches were attached to the monkeys' collars and baseline sleep-like behavior was measured for 1 week. Each subject underwent the methamphetamine SA protocol 5 times, such that each subject received vehicle and 2 different doses (1.0 and 3.0 mg/kg, i.m.) of temazepam and eszopiclone night treatments. SA and recording continued for a total of 3 weeks (5 days a week) for each block of experiments, with: no night treatment on the 1st week; vehicle, temazepam or eszopiclone night treatment at 18h30 (30 min prior to lights off) in the home-cage on the 2nd week; no night treatment on the 3rd week. SA was discontinued after each 3-week block of experiments and sleep-like measures continued for an additional baseline week between experimental blocks. Temazepam and eszopiclone were also administered at the highest (3.0 mg/kg) dose for a 5-day period at 18h30 (30 min prior to lights off) on baseline conditions (no drug SA, subjects in their home-cages). A 1-week interval was given between drug treatments for the baseline sleep studies.

2.6.2 Daytime activity studies—Before daytime treatments began, Actiwatches were attached to the monkeys' collars and baseline daytime activity was measured for 1 week. Vehicle, temazepam (0.3 and 3.0 mg/kg, i.m.) and eszopiclone (3.0 mg/kg, i.m.) were then administered at 9am under the same conditions (no drug SA, subjects in their home-cages) with a 3-day interval between treatments. Following daytime treatments, motor behavior was evaluated in a similar timeframe to the methamphetamine sessions (i.e., between 9am and 12pm).

2.6.3 Methamphetamine self-administration studies—Stable methamphetamine SA was defined as response rates that varied by <30% over a 3-day period. Once responding stabilized, a single injection of vehicle, temazepam (0.3, 1.0, and 3.0 mg/kg) or eszopiclone (0.3, 1.0 mg/kg) were administered i.m. in the home-cage 30 min before the initiation of the SA session (0.03 mg/kg/inf, i.v.). Between different pretreatments, animals were returned to normal methamphetamine SA conditions until stability criteria were met again, when a new pretreatment was administered on the following day.

2.7 In vivo microdialysis

Microdialysis samples were collected and analyzed as previously described (Murnane et al., 2010, 2012). Briefly, all procedures were performed in fully conscious subjects while they sat in primate chairs (Primate Products) within sound-attenuated testing chambers. After the subject was placed in the chamber, 28mm stainless steel microdialysis probes with 4mm membranes (CMA Microdialysis, Holliston, MA, USA) were inserted into the subjects' surgically implanted guide cannulae. All 3 subjects underwent the microdialysis protocol 6 times, such that each subject received vehicle, temazepam (3.0 mg/kg, i.m.) and eszopiclone (3.0 mg/kg, i.m.) pretreatments combined with subsequent saline or methamphetamine (1.0 mg/kg, i.v.) treatments. Based on the outcome of behavioral experiments, additional

preliminary experiments were conducted in 2 subjects during which animals received vehicle or 0.3 mg/kg (i.m.) temazepam pretreatments with subsequent methamphetamine (1.0 mg/kg, i.v.) treatment. Experiments consisted of a 1h equilibrium period after which samples were collected every 10 min. Vehicle, temazepam or eszopiclone were administered 30 min after the sampling began and 30 min before saline or methamphetamine administration. Following saline or methamphetamine administration, samples were collected over the next 2h. The viability of the sampling site was verified through retrodialysis of a potassium-enriched (100mm) solution otherwise ionically matched to artificial cerebrospinal fluid (aCSF). Dopamine concentrations within the dialysate were quantified using high-pressure liquid chromatography with electrochemical detection, as previously described (Murnane et al., 2010, 2012). The data were analyzed by comparison with standard concentration curves using Chromeleon 6.8 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA). *In vivo* microdialysis sessions were performed no more frequently than every 2 weeks for each subject.

2.8 Data analysis

Data for the behavioral experiments were analyzed by 1-way repeated-measures (RM) analysis of variance (ANOVA). Correlational analyses were conducted using linear-regression analysis. Microdialysis data were analyzed by 1- or 2-way RM ANOVA. The 3 data points immediately preceding the pretreatment administration were averaged to create the baseline. The main factors in 2-way RM ANOVA microdialysis analyses were time and pretreatment. All post hoc comparisons were performed using Dunnett's test or paired t tests. All graphical data presentations were created using Prism 5 (GraphPad Software), all statistical tests were performed using PASW Statistics 18 (SPSS Statistics Software), and significance was accepted at an alpha of 0.05.

3. Results

3.1 Nighttime activity studies

Sleep-like measures are presented as normalized data (percentage of baseline). Individual-subject baseline sleep parameters are expressed as mean \pm SEM; individual subject codes followed by corresponding values were: 1) Sleep efficiency (%): ROF8: 85.7 \pm 0.81; RVm8: 68.96 \pm 3.11; RLk4: 74.08 \pm 1.66; RJ18: 88.88 \pm 0.75; RZs9: 25.18 \pm 5.65; 2) Sleep latency (min): ROF8 10.8 \pm 3.42; RVm8: 66.4 \pm 19.23; RLk4: 19.4 \pm 1.63; RJ18: 7.8 \pm 4.02; RZs9: 471.8 \pm 62.64; 3) Fragmentation index: ROF8: 26.66 \pm 2.08; RVm8: 57.9 \pm 4.41; RLk4: 46.9 \pm 2.34; RJ18: 24.36 \pm 2.70; RZs9: 117.28 \pm 6.72.

For temazepam experiments, 1-way RM ANOVA showed a main effect of methamphetamine treatment (baseline vs methamphetamine SA) for sleep efficiency [F(3,12)=4.96, p<0.05] (Fig. 1A), sleep latency [F(3,12)=4.88, p<0.05] (Fig. 1B), and sleep fragmentation [F(3,12)=4.106, p<0.05] (Fig. 1C). Methamphetamine SA significantly decreased sleep efficiency and increased sleep latency and sleep fragmentation compared to baseline, an effect that was not reversed by night treatment with temazepam. Night treatment with 3 mg/kg temazepam exerted no effects on baseline sleep efficiency [F(2,8)=0.623,

$p > 0.05$], sleep latency [$F(2,8)=0.345$, $p > 0.05$], or sleep fragmentation [$F(2,8)=0.941$, $p > 0.05$] (data not shown).

For eszopiclone experiments, 1-way RM ANOVA showed a main effect of methamphetamine treatment (baseline vs methamphetamine SA) for sleep efficiency [$F(3,12)=5.9$, $p < 0.05$] (Fig. 2A), sleep latency [$F(3,12)=5.51$, $p < 0.05$] (Fig. 2B), and sleep fragmentation [$F(3,12)=8.716$, $p < 0.05$] (Fig. 2C). Methamphetamine SA induced sleep disruption, an effect that was not reversed by night treatment with eszopiclone. Night treatment with 3 mg/kg eszopiclone exerted no effects on baseline sleep efficiency [$F(2,8)=1.21$, $p > 0.05$], sleep latency [$F(2,8)=0.088$, $p > 0.05$], or sleep fragmentation [$F(2,8)=1.556$, $p > 0.05$] (data not shown).

Analysis of the weeks preceding and following the week with night treatments showed methamphetamine-induced sleep disruption, with temazepam and eszopiclone having no effects on sleep-like measures in the week following night treatment (data not shown). Importantly, following-day methamphetamine intake was not affected by night treatments with either temazepam or eszopiclone, and analysis of the weeks preceding and following night treatments also showed no differences between treatments for methamphetamine intake (data not shown).

3.2 Daytime activity studies

Daytime activity data were combined across the first three hours after drug treatment, a period in which methamphetamine SA sessions normally were carried out. For temazepam, 1-way RM ANOVA showed no significant differences between treatments [$F(2,8)=0.266$, $p > 0.05$] (Fig. 3A). For eszopiclone, 1-way RM ANOVA [$F(2,8)=8.04$, $p < 0.05$] showed that daytime treatment with 3.0 mg/kg significantly decreased general activity compared to vehicle (Fig. 3B).

3.3 Methamphetamine self-administration studies

3.3.1 Methamphetamine intake—Our subjects self-administered methamphetamine under a FR20 schedule of reinforcement. An important feature of FR schedules is the direct relationship between rate of responding and frequency of drug injection. Thus, changes in methamphetamine intake were directly accompanied by changes in methamphetamine-maintained response rates.

One-way RM ANOVA [$F(4, 16)=19.5$, $p < 0.0001$] showed that pretreatment with temazepam dose-dependently modified drug intake during methamphetamine SA (Fig. 4A). The lowest dose of temazepam significantly increased methamphetamine intake, whereas the highest dose significantly decreased methamphetamine intake.

One-way RM ANOVA [$F(4, 16)=1.923$, $p > 0.05$] showed no significant differences on methamphetamine intake following vehicle or eszopiclone pretreatments (Fig. 4B). Because of the sedative effects observed with 3.0 mg/kg eszopiclone during the daytime activity experiments, the highest dose of eszopiclone was not used in the SA studies due to safety concerns. The only subject (RZs9) who tolerated the dose and was able to complete a methamphetamine SA session after 3 mg/kg eszopiclone pretreatment showed no differences

in drug intake (methamphetamine intake ~108% of that observed after vehicle pretreatment; data not shown).

3.3.2 Sleep-like parameters—Sleep-like parameters were assessed on the night following treatment preceding SA sessions. For temazepam, 1-way RM ANOVA showed significant differences between treatments for sleep efficiency [F(5,20)=6.119, $p<0.01$] (Fig. 5A), sleep latency [F(5,20)=3.75, $p<0.05$] (Fig. 5B), and sleep fragmentation [F(5,20)=3.918, $p<0.05$] (Fig. 5C). Methamphetamine SA induced sleep disruption. Corroborating the dose-dependent decrease in drug intake, measures of sleep efficiency, sleep latency and sleep fragmentation were improved in the night following methamphetamine SA sessions conducted after a pretreatment with 3.0 mg/kg temazepam. Significant correlations were found between the values for all of the sleep measures (efficiency: $R^2=-0.93$; latency: $R^2=0.91$; fragmentation: $R^2=0.90$) and methamphetamine intake (total amount average intake/session) ($p<0.01$), suggesting that this improvement seems to be due to a lower methamphetamine intake.

For eszopiclone, 1-way RM ANOVA showed significant differences between treatments for sleep efficiency [F(5,20)=4.828, $p<0.01$] (Fig. 5D), sleep latency [F(5,20)=5.718, $p<0.01$] (Fig. 5E), and sleep fragmentation [F(5,20)=7.394, $p<0.01$] (Fig. 5F). Methamphetamine SA induced sleep disruption, an effect that was not changed by pretreatment with eszopiclone prior to SA sessions.

3.4 In vivo microdialysis

Grouped mean extracellular baseline dopamine levels are expressed as mean \pm SEM: vehicle + 1 mg/kg methamphetamine = 1.76 \pm 0.05; 3 mg/kg temazepam + methamphetamine = 1.27 \pm 0.02; 3 mg/kg eszopiclone + 1 mg/kg methamphetamine = 1.69 \pm 0.11.

For temazepam, 2-way RM ANOVA showed significant main effects of time [F(17,68)=10.822, $p<0.0001$] and pretreatment (vehicle vs 3 mg/kg temazepam) [F(1,4)=20.329, $p=0.01$], and a significant interaction [F(17,68)=2.187, $p=0.01$] (Fig. 6A). Bonferroni *post hoc* test showed that, following vehicle pretreatment, methamphetamine significantly elevated extracellular dopamine levels in the NAc from min 20 through 80 after injection compared with baseline, while after temazepam pretreatment this increase was only observed at min 20, 30 and 40 after injection. Methamphetamine was significantly less effective in increasing dopamine overflow in the NAc following temazepam at min 20 through 120 compared to vehicle.

Preliminary data collected with 2 subjects indicated that pretreatment with 0.3 mg/kg temazepam 30min prior to methamphetamine had no effects on methamphetamine-induced dopamine overflow compared to vehicle. Mean \pm SEM dopamine concentration 20min (peak) after methamphetamine administration was 17.39 \pm 2.5 nM for vehicle pretreatment and 18.89 \pm 4.9 nM for 0.3 mg/kg temazepam pretreatment (data not shown).

For eszopiclone, 2-way RM ANOVA showed a significant main effect of time [F(17,68)=6.534, $p<0.0001$], but not pretreatment [F(1,4)=0.34, $p>0.05$] or interaction

[F(17,68)= 0.152, $p>0.05$] (Fig. 6B). No differences were observed on *post hoc* comparisons following vehicle or eszopiclone pretreatments.

Both temazepam and eszopiclone had no effects on baseline dopamine levels (data not shown).

4. Discussion

In the present study, we investigated the effects of temazepam and eszopiclone, two GABA_A receptor modulators, on methamphetamine-induced sleep disruption. Both temazepam and eszopiclone have been shown to improve sleep in insomniac patients (Rosenberg, 2006; Owen, 2011), being FDA-approved and commonly used as hypnotic drugs for the treatment of insomnia (Bertisch et al., 2014). Although our data show that neither temazepam nor eszopiclone were effective in improving baseline sleep-like measures, this effect was most probably due to the high quality of sleep observed in most of our subjects under baseline conditions.

Acute treatment with eszopiclone, but not temazepam, significantly decreased general daytime activity at the highest dose used in the present study. The sedative effects of GABA_A receptor modulators have been predominantly associated with the action of benzodiazepine receptor ligands on GABA_A receptors containing $\alpha 1$ subunits ($\alpha 1$ GABA_A receptors) (Nutt and Stahl, 2010). Because eszopiclone, but not temazepam, exerted sedative effects on daytime activity, our findings corroborate the hypothesis that newer GABA_A receptor modulators such as “z-drugs” exert their effects predominantly through $\alpha 1$ GABA_A receptors (Nutt and Stahl, 2010). However, there is lacking evidence that eszopiclone has selective affinity or efficacy for $\alpha 1$ GABA_A receptors. Instead, studies indicate that eszopiclone appears to have functional selectivity for $\alpha 3$ GABA_A receptors and low intrinsic efficacy at GABA_A receptors containing $\alpha 5$ subunits ($\alpha 5$ GABA_A receptors) (Nutt and Stahl, 2010; Dixon et al., 2015). Thus, although further studies are warranted for a conclusive determination of the receptor selectivity of eszopiclone, a major difference in the binding profile of temazepam and eszopiclone seems to be their efficacy at $\alpha 5$ GABA_A receptors. Temazepam shows high efficacy and eszopiclone shows low efficacy at $\alpha 5$ GABA_A receptors. Regarding their distribution in the brain, $\alpha 5$ GABA_A receptors seem to be located at pyramidal cells in the hippocampus and neocortex in mice, being strategically located to modulate excitatory glutamatergic input into these cells (Rudolph and Möhler, 2014). Importantly, both the hippocampus (Keleta and Martinez, 2012; Galinato et al., 2015) and the neocortex (Fujiyama et al., 2003; Kuczenski et al., 2007) have been implicated in the reinforcing properties and behavioral effects of methamphetamine.

Our data also show that both temazepam and eszopiclone were ineffective in improving methamphetamine-induced sleep impairment. These results suggest that GABAergic mechanisms related to the GABA_A benzodiazepine receptor are not primarily involved in the sleep-disrupting effects of methamphetamine. We have previously demonstrated that nighttime treatment with a selective serotonin 5HT_{2A} receptor antagonist or a selective 5HT_{2C} receptor agonist was effective in improving sleep-like measures disrupted by methamphetamine SA in rhesus monkeys (Perez Diaz et al., 2017). Although the dopamine-

modulating properties of serotonergic drugs (Howell and Cunningham, 2015) suggest that increases in dopamine levels in the striatum are necessary for the wake-promoting effects of methamphetamine, in the present study temazepam had no effects on methamphetamine-induced sleep disruption despite effectively attenuating the increase in extracellular dopamine levels induced by methamphetamine in the NAc. Thus, although the mesolimbic dopaminergic neurotransmission seems to be important for the wake-promoting effects of methamphetamine, it is not sufficient to explain the neurochemical mechanisms underlying methamphetamine-induced disruption of sleep.

GABA_A receptor modulators, such as temazepam and eszopiclone, act by enhancing the effects of GABA when binding at the benzodiazepine site on the GABA_A receptor (Bergman et al., 2000). The high efficacy with which classic benzodiazepine drugs act at GABA_A receptors seems to be related to their nonselective action at GABA_A receptors containing α 1, 2, 3, and 5 subunits (Smith et al., 2001). In fact, despite being FDA-approved for the treatment of insomnia, temazepam has been shown to exert several side effects in humans (Hansen et al., 2015; Schroeck et al., 2016), which is consistent with it being a high efficacy positive modulator at GABA_A receptors with lack of α subunit specificity (Heel et al., 1981). Nevertheless, newer GABAergic modulators, such as z-drugs, seem to have lesser efficacy at the GABA_A receptor compared to classic benzodiazepines. Consistent with it being a low efficacy GABA_A positive modulator, eszopiclone has been reported to induce fewer next-day side effects compared to benzodiazepines (Gunja, 2013) and to have distinct binding profiles at different GABA_A receptor subtypes (Nutt and Stahl, 2010; Dixon et al., 2015).

According to our results, acute treatment with a high dose of temazepam decreased methamphetamine SA, while eszopiclone had no effects on methamphetamine intake. Our findings are consistent with previous studies suggesting that high efficacy GABA_A receptor modulators are more effective than low efficacy GABA_A modulators and other GABAergic compounds in attenuating the abuse-related effects of stimulants (Negus et al., 2000; Barrett et al., 2005; Weerts et al., 2005). Negus and colleagues (2000) showed that pretreatments with the high efficacy GABA_A receptor modulator triazolam, but not the low efficacy benzodiazepine imidazenil, attenuated the discriminative-stimulus effects of cocaine in a food-reinforced drug discrimination task in rhesus monkeys. The same has been found for the GABA_A receptor modulators midazolam (high efficacy) and enazenil (low efficacy), with midazolam only altering the discriminative-stimulus effects of cocaine and decreasing cocaine SA in rats (Barrett et al., 2005). Importantly, high efficacy GABA_A modulators were more potent in decreasing cocaine- than food-maintained behavior (Negus et al., 2000; Barrett et al., 2005; Weerts et al., 2005), suggesting selectivity to the abuse-related effects of stimulants. Studies have also shown that midazolam attenuated the stimulus properties of *d*-amphetamine in rats (Druhan et al., 1991) while triazolam decreased the discriminative-stimulus effects of methamphetamine in humans (Sevak et al., 2009). Acutely administered alprazolam attenuated the discriminative stimulus and self-reported effects of D-amphetamine in humans (Rush et al., 2004). However, chronic treatment with extended-release alprazolam had no effects on methamphetamine self-administration and subject-rated effects, suggesting that tolerance can develop to the effects of benzodiazepines on the reinforcing effects of methamphetamine (Marks et al., 2016).

At a dose that decreased methamphetamine intake, temazepam had no effects on general daytime activity. Thus, our findings also indicate that temazepam-induced decreases in response maintained by methamphetamine was not due to a decrease in motor activity, suggesting that these effects are due to changes in the modulatory effects of temazepam on methamphetamine-induced dopamine release. However, our study did not evaluate the effects of temazepam on operant food-maintained responding, preventing us from conclusively inferring the behavioral selectivity of temazepam in our results. The effects of high efficacy GABA_A receptor modulators in attenuating the abuse-related effects of stimulant drugs are proposed to be mediated by a decrease in stimulant-induced dopamine overflow in the mesolimbic dopaminergic pathway. Our study supports this proposed mechanism by showing that acute treatment with temazepam decreased methamphetamine SA at the same dose that attenuated methamphetamine-induced increases in extracellular dopamine levels in the NAc. Importantly, acute treatment with eszopiclone was ineffective in decreasing methamphetamine SA, and it did not influence the dopaminergic effects of methamphetamine.

Lastly, acute administration of high doses of temazepam and eszopiclone alone had no effect on baseline dopamine levels in the NAc. The reinforcing properties of benzodiazepine drugs have been linked to a disinhibition of dopaminergic neurons and increased dopamine levels in the mesolimbic system (Riegel and Kalivas, 2010; Tan et al., 2011). In fact, benzodiazepine-type compounds exhibit reinforcing properties in nonhuman primates, with several studies showing benzodiazepine SA in primates (Licata and Rowlett, 2008; Fischer et al., 2016). Spence and colleagues (2016a, b) recently demonstrated that alprazolam, a high affinity GABA_A receptor modulator, enhanced methamphetamine SA in rats. The authors suggested that alprazolam-induced increases in methamphetamine intake were mediated by increases in extracellular dopamine levels (Spence et al., 2016a, b). However, in the present study, acute treatment with temazepam increased methamphetamine intake at a dose that did not affect methamphetamine-induced dopamine overflow in the NAc or general daytime activity. Hence, alternative mechanisms other than dopamine may contribute to the reinforcing properties of GABAergic drugs.

Together, our data suggest that although GABA_A receptor modulators exert little or no effect on methamphetamine-induced sleep disruption, GABA_A mediated neurotransmission exerts important inhibitory effects on methamphetamine intake and methamphetamine-induced dopamine overflow in the NAc. By showing that temazepam does not improve sleep-like measures despite attenuating methamphetamine-induced dopamine levels, our findings provide opposing evidence to the assumption that the dopamine system directly underlies the sleep-disrupting effects of psychostimulants.

These outcomes may have significant clinical relevance concerning medication-assisted treatment of stimulant abuse. Because sleep loss is considered a risk factor for drug relapse (Brower and Perron, 2010), sleep patterns should be considered in addiction treatment strategies. Currently, the primary treatments for sleep impairment are based on the use of GABA_A receptor modulators, such as temazepam and eszopiclone (Riemann et al., 2015). Our study suggests that treatments based on GABA_A receptor modulators are not effective for the treatment of sleep disruption in the context of psychostimulant use, suggesting that

drugs targeting other brain systems, such as the serotonergic system (Murnane et al., 2013; Perez Diaz et al., 2017), may be better candidates for the management of sleep disturbances in psychostimulant addiction.

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Highlights

- Temazepam and eszopiclone did not improve sleep disrupted by methamphetamine (METH);
- Temazepam at a low dose increased METH intake;
- Temazepam at a high dose decreased METH intake and METH-induced dopamine levels;
- Eszopiclone had no effect on METH intake or METH-induced dopamine levels.

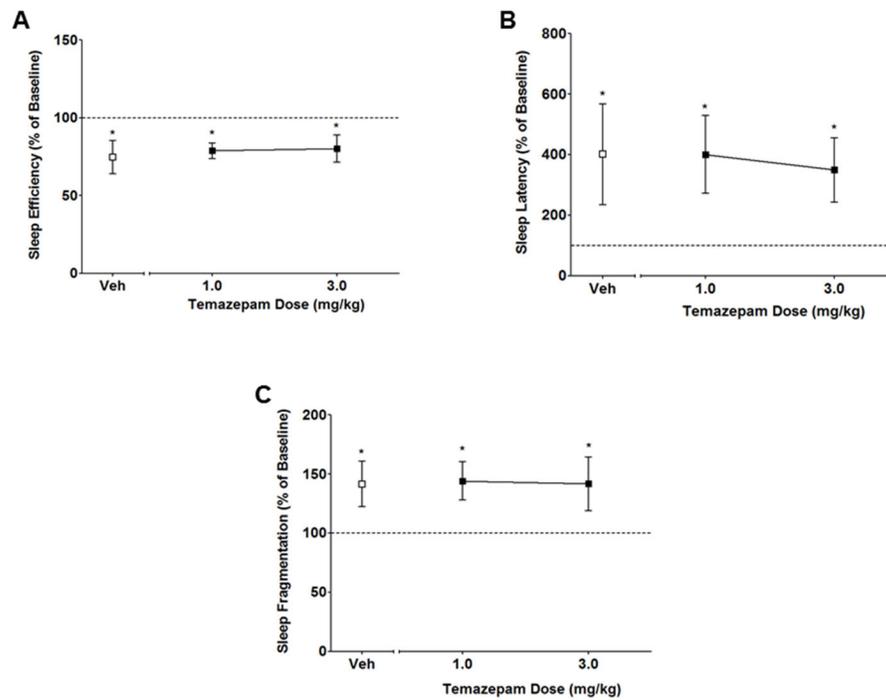


Figure 1. Effects of night treatment with temazepam on methamphetamine-induced sleep disruption (N = 5). (A) Sleep efficiency, (B) sleep latency and (C) sleep fragmentation in the nights after methamphetamine (METH) self-administration sessions (0.03 mg/kg/infusion, i.v.) following night treatments with vehicle (Veh) or temazepam (1.0 and 3.0 mg/kg, i.m.). Sleep-like measures are presented as normalized data (percentage of baseline) and were combined across a 5-day span of time. Data are expressed as mean \pm SEM. Dotted lines represent baseline sleep parameters (100%). *p<0.05 compared with baseline.

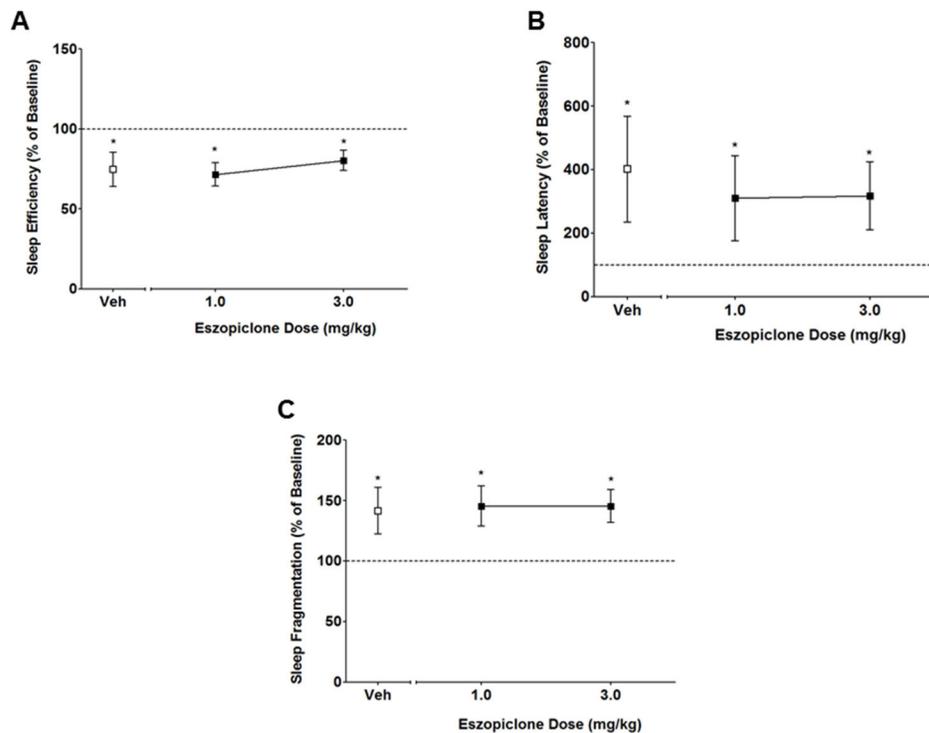


Figure 2. Effects of night treatment with eszopiclone on methamphetamine-induced sleep disruption (N = 5). (A) Sleep efficiency, (B) sleep latency and (C) sleep fragmentation in the nights after methamphetamine (METH) self-administration sessions (0.03 mg/kg/infusion, i.v.) following night treatments with vehicle (Veh) or eszopiclone (1.0 and 3.0 mg/kg, i.m.). Sleep-like measures are presented as normalized data (percentage of baseline) and were combined across a 5-day span of time (n = 5). Data are expressed as mean \pm SEM. Dotted lines represent baseline sleep parameters (100%). *p<0.05 compared with baseline.

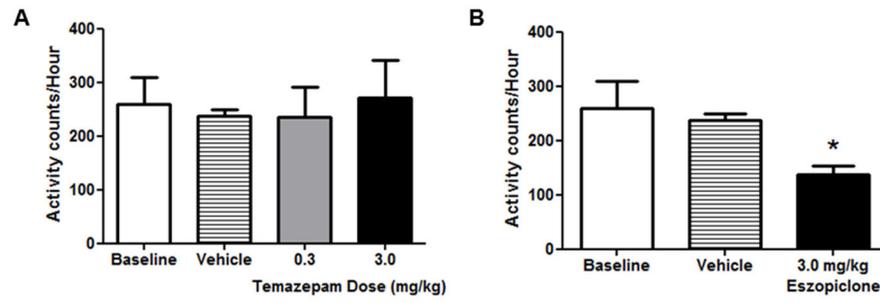


Figure 3. Effects of a daytime treatment with GABA_A receptor modulators on home-cage general activity during the morning (N = 5). Activity counts per hour after a morning treatment with vehicle (Veh), (A) temazepam (0.3 and 3.0 mg/kg, i.m.) or (B) eszopiclone (3.0 mg/kg, i.m.). Data are expressed as mean±SEM. *p<0.05 compared with vehicle daytime treatment.

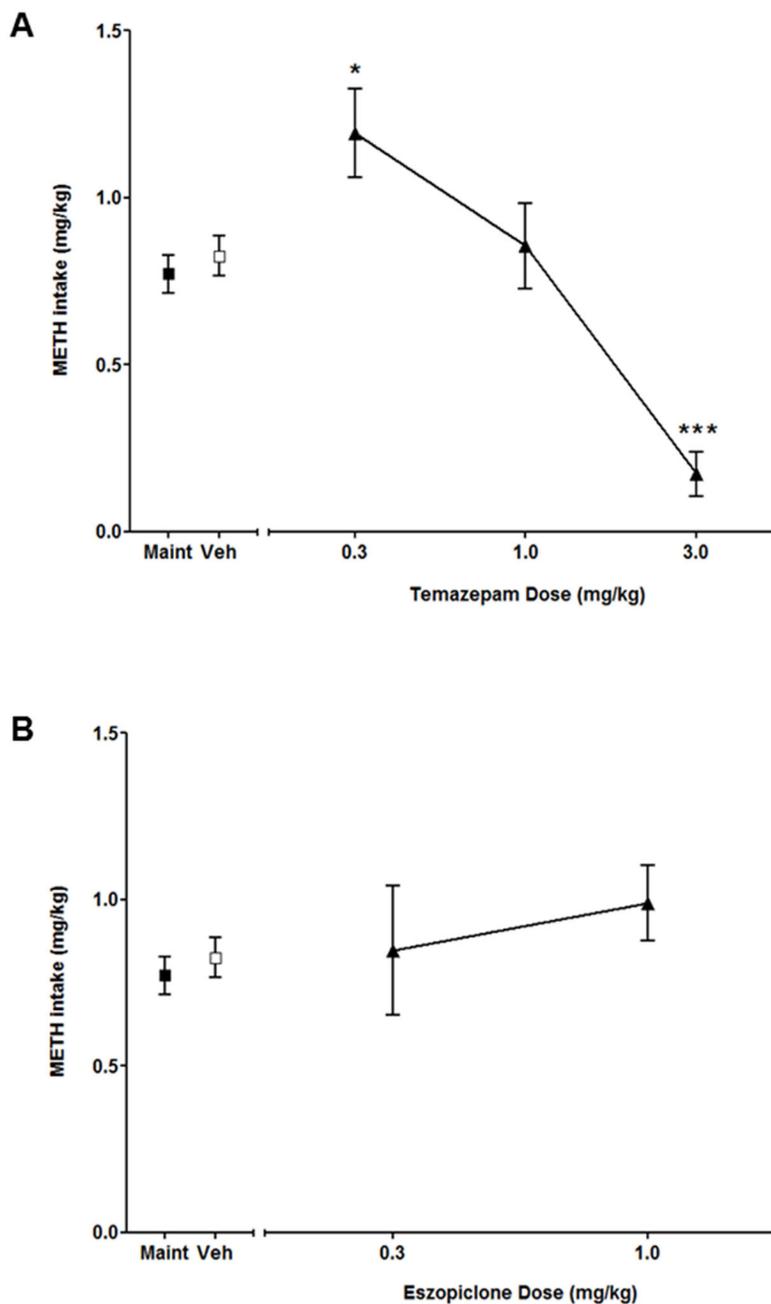


Figure 4. Effects of pretreatment with GABA_A receptor modulators on methamphetamine (METH) intake during drug self-administration (N = 4). Methamphetamine intake (mg/kg) during self-administration maintenance (0.03 mg/kg/infusion, i.v.) and after pretreatment with vehicle (Veh), (A) temazepam (0.3, 1.0 and 3.0 mg/kg, i.m.) or (B) eszopiclone (1.0 and 3.0 mg/kg, i.m.). Data are expressed as mean±SEM. *p<0.05 and ***p<0.001 compared with vehicle pretreatment.

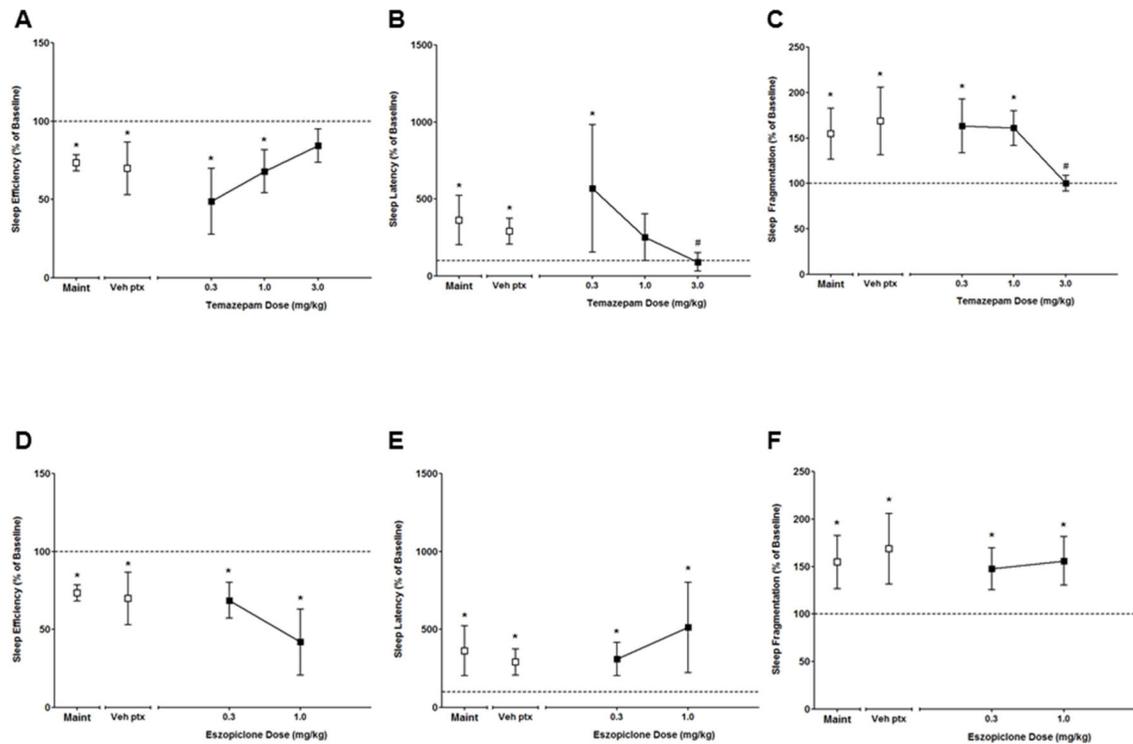


Figure 5.

Effects of pretreatment with GABA_A receptor modulators prior to methamphetamine (METH) self-administration (SA) sessions on methamphetamine-induced sleep disruption (N = 4). (A) Sleep efficiency, (B) sleep latency and (C) sleep fragmentation in the nights following temazepam pretreatment (0.3, 1.0 and 3.0 mg/kg, i.m.) prior to SA sessions (0.03 mg/kg/infusion, i.v.). (D) Sleep efficiency, (E) sleep latency and (F) sleep fragmentation in the nights following eszopiclone pretreatment (0.3 and 1.0 mg/kg, i.m.) prior to SA sessions (0.03 mg/kg/infusion, i.v.). Sleep-like measures are presented as normalized data (percentage of baseline). Data are expressed as mean ± SEM. Dotted lines represent baseline sleep parameters (100%; the variability around normalized baseline was 100 ± 4.72 for sleep efficiency, 100 ± 23.4 for sleep latency and 100 ± 8.36 for sleep fragmentation). *p < 0.05 compared with baseline; #p < 0.05 compared with vehicle pretreatment.

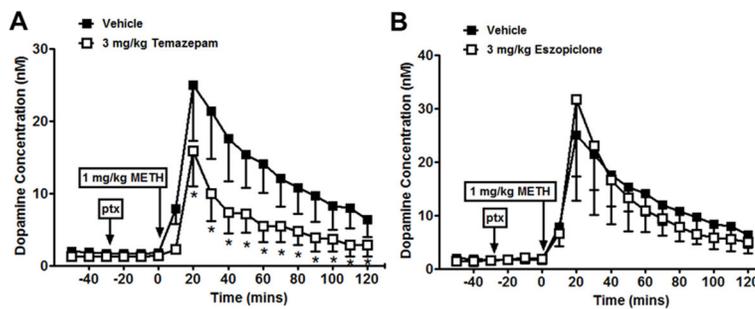


Figure 6.

Effects of pretreatment with GABA_A receptor modulators on methamphetamine-induced dopamine overflow in the nucleus accumbens (NAc, N = 3). Increases in extracellular dopamine levels induced by methamphetamine (1 mg/kg, i.v.) in the nucleus accumbens following pretreatment with vehicle, (A) temazepam (3.0 mg/kg, i.m.) or (B) eszopiclone (3.0 mg/kg, i.m.). All data points represent the mean ± SEM. *p < 0.05 compared with vehicle pretreatment. Analysis of variance showed a significant main effect of methamphetamine.