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Cardiovascular and Subjective Effects of the Novel Adenosine A_{2A} Receptor Antagonist SYN115 in Cocaine Dependent Individuals

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Abstract

A_{2A} receptor antagonists have been proposed as therapeutic tools for dopaminergically-relevant diseases, including Parkinson's disease and substance dependence. The acute subjective and cardiovascular effects of a novel, selective adenosine A_{2A} receptor antagonist (SYN115) were examined. Across an 8-hour experimental testing day, 22 non-treatment seeking cocaine-dependent subjects received either placebo capsules (PO) at both the AM and PM dosing times (Plc/Plc, N = 9), or placebo in the AM and 100 mg SYN115 in the PM (Plc/SYN115, N = 13). Cardiovascular measures (HR, BP) were obtained across the test day, and subjective effects (ARCI, VAS) were obtained once before and once after the AM and PM doses (four time points total). There were no between-group effects on cardiovascular function, however subjective effects consistent with stimulation were observed on the VAS scales in the SYN115 group. In cocaine-dependent subjects, SYN115 may produce stimulant-like effects through a unique mechanism of action. Due to known monoamine dysfunction related to chronic cocaine use, these effects may be specific to this population relative to healthy control or other patient populations.

Keywords

Cocaine-dependence; Adenosine A_{2A} receptor; Subjective effects; Cardiovascular effects; SYN115

Introduction

Chronic stimulant use results in dysregulation of monoamine systems, including a deficiency in dopamine availability and transmission in the striatum and prefrontal cortex

[1,2]. Accordingly, treatment strategies have included supplementation via dopamine-enhancing medications [3–5]. However, compounds that directly alter dopamine receptors (d-amphetamine, bromocriptine) may have both practical and clinical limitations [6]. Recently, there has been interest in compounds that modulate dopamine systems indirectly via receptor heteromers. One such class of compounds acts on adenosine receptors. Adenosine-modulating drugs have been shown to alter dopamine function and, accordingly, have been recommended as potential targets for the treatment of substance abuse [7,8] and Parkinson's disease [9,10].

In the brain, adenosine A_{2A} receptors are located primarily in the dorsal and ventral striatum [11]. Importantly, A_{2A} receptors form functional units (receptor heteromers) with dopamine D₂ receptors [7,8,12], and thereby alter the ligand binding and signaling properties of D₂ receptors [13]. Specifically, A_{2A} receptor antagonists increase the effect of dopamine at these receptors, which are tonically inhibited by endogenous adenosine [7,8,12]. It follows that acute dosing with A_{2A} receptor antagonists would be expected to produce stimulant-like effects. However, because the action on DA is indirect, A_{2A} receptor antagonists may reveal a different magnitude or pattern of stimulant effects than direct DA modulators. Because few specific A_{2A} receptor antagonists are available for human administration, characterization of the psychopharmacological effects of such compounds is of interest. For example, the effects of the non-specific mixed A₁/A_{2A} receptor antagonist caffeine have been well characterized, and produce modest increases in cardiovascular and psychological stimulation relative to more potent stimulants like cocaine and amphetamine [14–17].

The present study was part of a larger brain-imaging project examining pharmaco-magnetic resonance imaging (phMRI) and functional-MRI (fMRI) in cocaine dependent subjects. Here, we report on the subjective and cardiovascular effects of the novel A_{2A} receptor antagonist, SYN115 [9,18]. While it might be expected to produce effects resembling those of caffeine, SYN115 has no affinity for the A₁ receptor, and the characterization of its psychopharmacological effects has yet to be determined. Additionally, due to dopamine regulation, SYN115 may hold potential as a therapeutic agent in the treatment stimulant dependence. To these ends, the present study examined the subjective and cardiovascular effects of SYN115 in cocaine dependent individuals.

Methods

This study was approved by the Committee for the Protection of Human Subjects (the Institutional Review Board for the University of Texas Health Science Center at Houston), and the US Food and Drug Administration under an IND from one of the authors (FGM).

Subjects

Twenty-two non-treatment seeking cocaine dependent subjects were recruited using advertisements in local newspapers for research in a brain imaging study of cocaine use. After reading the consent document and providing written informed consent (which included a layperson's description of SYN115), all subjects underwent a screening consisting of a physical examination and complete blood count, urine pregnancy test (females), serum chemistries, and HIV test. All subjects also underwent a structured psychiatric interview using the Structured Clinical Interview for DSM-IV [19]. Exclusion criteria included any current psychiatric disorder other than cocaine dependence and abuse of cannabis and/or nicotine. Subjects were excluded for any past psychiatric disorder other than substance abuse or dependence or substance induced mood disorder. Non-psychiatric medical exclusion criteria included any clinically significant medical disorder or a disorder requiring medication that could affect the central nervous system, such as hypertension, diabetes, and HIV. Subjects were excluded if they had a positive breath alcohol screen or a positive urine

drug screen for drugs of abuse other than cocaine or marijuana on the day of the scan. Subjects were also excluded if they were pregnant, had anemia, claustrophobia, or had any history of metal fragments in eyes or other soft tissue, or had any clinically significant abnormalities on structural MRI scans as read by a board certified radiologist. A psychiatrist screened all subjects for symptoms of cocaine and marijuana intoxication if they had a positive urine drug screen for cocaine or marijuana on the day of testing. Inclusion criteria included age 18–50 years, meeting current Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition (DSM-IV) [20] criteria for cocaine dependence, and not treatment seeking. All subjects had an ECG prior to testing / dosing, and on the day of testing a repeat ECG was obtained after completion of the test protocol. Subjects were admitted overnight to the Clinical Research Unit of the Center for Clinical and Translational Sciences of the University of Texas Health Science Center at Houston for observation after the scan. Vital signs and repeat ECGs were performed during the observation period until discharge from monitoring at 4:30 the following day (approximately 24 hours after the end of testing).

Medication and dosing

SYN115 (Synosia Therapeutics, 4-Hydroxy-4-methyl-piperidine-1-carboxylic acid-(4-methoxy-7-morpholin-4-yl-benzothiazol-2-yl)-amide) is a selective adenosine A_{2A} receptor antagonist with 110–260 fold selectivity for the human A_{2A} receptor compared to the other adenosine receptor subtypes, and a 1900 fold selectivity for the A_{2A} receptor over 67 other receptors (including A₁ and A₃), neurotransmitter transporters and ion channels [18]. From pharmacology studies in healthy controls, the half-life of a single dose of 100 mg of SYN115 was found to be 15.9 hours, with a time to peak blood levels of 4.1 hours [18]. SYN115 was administered under IND# 102990 (Moeller). At the time of the experiment, SYN115 for human consumption was available only in 20 mg capsules. Accordingly, subjects received five placebo capsules in the AM dose (at ≈ 9:00 AM), and either five 20 mg capsules of SYN115 (N = 13) or five placebo capsules (N = 9) in the PM dose ≈ 3 hours later. SYN115 and placebo capsules were standardized and provided by Synosia Therapeutics.

Cardiovascular and subjective effects measures

Blood pressure and heart rate were obtained at approximately 60 and 30 min pre-dose and 60 and 120 minutes post-dose for the AM (placebo) dose, and approximately 30 min pre-dose and 60, 120, 180, and 240 min post-dose for the PM dose (placebo/SYN115). All measures were acquired via sphygmomanometer (BpTru Medical Devices, Coquitlam, CA).

Subjective effects were assessed by the Addiction Research Center Inventory (ARCI) and the Visual Analogue Scale (VAS) of drug effects. The 49-item ARCI short form was used [21]. It has been empirically derived to assess five different factors, including stimulation, euphoria, sedation, and dysphoria [22]. The VAS [23,24] for drug effects presents horizontal lines (here 200 mm in length) labeled with an adjective or phrase, including, “any effect”, “good effect”, “bad effect”, “like effect”, “friendly”, “confused”, “able to concentrate”, “excited”, “alert”, and “relaxed”. Each line was anchored by “not at all” on the left (0 mm), and “extremely” on the right (200 mm). Both the ARCI and VAS have well-established sensitivity to stimulant drugs effects [25,26].

Subjective and cardiovascular effects were collected during time periods surrounding an fMRI of working-memory scanning protocol (Philips 3T whole-body scanner, scan protocol details reported in Moeller et al. under review). Cardiovascular effects were recorded by sphygmomanometer with subjects seated in a quiet, unoccupied room. Subjects completed a different number of subjective effects reports (either 5 or 6), before and after two scanning sessions that were conducted over an 8-hour testing day. Number and time of test varied

according to the time required for preparation and execution of the fMRI protocol. However, we were able to select four time points (one pre-dose and one post-dose) surrounding each of the AM and PM doses/scans, in which subjective-effects data were obtained at approximately the same time points across subjects (8:30 AM, 11:00 AM, 12:00 PM and 4:30 PM). The AM subjective effects data were obtained 30 minutes pre-dose (time point #1; placebo for all subjects) and 120 minutes post-dose (time point #2, post-placebo). The PM subjective effects data were obtained 30 minutes pre-dose (time point #3; SYN115 for 13 subjects, placebo for 9 subjects) and 240 minutes post-dose (time point #4). The 240-min post dose test was based on reported pharmacodynamic (peak concentration) effects of SYN115 [9,18].

Both subjects and staff administering the rating scales were blinded to medication condition. Subjects received five placebo capsules in the AM dose, and either five 20 mg capsules of SYN115 (N = 13) or five placebo capsules (N = 9) in the PM dose.

Data analyses

Statistical analysis of the cardiovascular data was performed using a mixed-model analysis of variance (ANOVA) after inspection of the residuals via Normal-Quantile Plot and the Shapiro-Wilk test indicated that they were distributed normally. Systolic blood pressure, diastolic blood pressure and heart rate were examined across pre- and post-dose time points (repeated measures), between the placebo and SYN115 dose conditions (between groups), and for time $\times$ dose condition interaction effects.

Inspection of subjective-effects data revealed violations of the normality assumption that could not be normalized via data transformations (distributions were in some cases bimodal and in other cases skewed at the tails). Accordingly, subjective effects data were analyzed using non-parametric models with a between-group factor of dose condition, a repeated-measures factor of time, and the time $\times$ dose condition interaction. This approach utilizes ranks and produces ANOVA-type scores (B, or Box-approximation for small samples) and p values permitting statistical testing that parallels traditional general linear models [27]. Due to scanning logistics, one subject missed the subjective effects scales at time #1 (AM pre-dose), and one subject missed at time point #3 (PM pre-dose). Data for these two time points were imputed using regression-based imputation methods with relevant predictor variables. All data were analyzed using SAS v 9.2 (Cary, NC).

Results

Demographics

On average, subjects were 39.63 (SD = 5.78) years of age and had completed an average of 12.91 (SD = 1.97) years of education. Twenty-one were male and one was female. Nineteen of 22 were positive for benzoylecgonine (cocaine) on the morning of testing. Three were positive for both benzoylecgonine and THC (marijuana). The average years of cocaine use was 16.9 (SD = 7.24, range = 2 to 28 years). Last use of cocaine was 4.7 days (SD = 6.97, range 1 to 19) prior to testing. Only three subjects reported not being cigarette users, with 19 of 22 reporting daily smoking (mean cigarettes per day = 9.10, SD = 7.24). Comparison of the 13 subjects in the AM placebo/PM SYN115 (PLC/SYN) condition and 9 subjects the AM placebo/PM placebo (PLC/PLC) condition revealed no statistically significant differences in age, education, urine drug screen status on test day, cigarette use, time since last cocaine use, or lifetime history of cocaine use. Appendix A provides demographic information for each group.

Cardiovascular data

Table 1 provides results for the systolic and diastolic blood pressure, and heart rate data. For systolic blood pressure, mixed model ANOVA revealed no significant main effect of drug condition, $F(1, 160) = 0.05$, *ns*. There was a significant main effect of time, $F(8, 160) = 5.47$, $p < .0001$, but no drug condition $\times$ time interaction $F(8, 160) = 1.91$, *ns*. A similar pattern was found for diastolic blood pressure: drug condition $F = 0.42$, *ns*; time $F = 2.25$, $p < .03$; drug condition $\times$ time $F = 1.58$, *ns*; and for heart rate: drug condition $F = 0.78$, *ns*, time $F = 6.95$, $p < .0001$, drug condition $\times$ time $F = 0.68$, *ns*. For all three variables (HR, systolic and diastolic BP), the significant time effects were examined with Tukey HSD post-hoc tests ($\alpha = .05$). The majority of significant differences were between the two earliest AM time points (-60 and -30 pre-dose) and subsequent time points later in the day. Since there was not a significant interaction, these effects can be attributed to diurnal variation in cardiovascular patterns and/or the extended testing protocol, rather than effects of SYN115.

Subjective effects data

Table 2 provides results for the ARCI and VAS data. For the ARCI data, there was a significant main effect of dose condition on the A (amphetamine) scale, Box's approximation $B = 3.83$, $df = 1$, $p = .05$. Table 2 shows a modest increase in the A scales rating in the PLC/SYN115 group at time point 4 (post-dose) and no change in the PLC/PLC group. The relevant examination of the effect of SYN115 is the pre-dose (time point 3) to post-dose (time point 4) change for the PM dose. Thus, within-group post-hoc analysis of the difference between time point 3 (PM pre-dose) and time point 4 (PM post-dose) was conducted using the nonparametric signed-rank test and failed to show statistical significance for either group: PLC/PLC $S = -0.5$, *ns*; PLC/SYN115 $S = 2$, *ns*. Since neither group had a significant pre-post change, the ARCI-A result remains equivocal with regard to the SYN115 dose effect. There was also a statistically significant outcome of dose condition for the PCAG (pentobarbital and chlorpromazine group) scale, $B = 5.43$, $df = 1.0$, $p < .02$. Within-group post-hoc analysis of the difference between from time point 3 (PM pre-dose) and time point 4 (PM post-dose) conducted using the signed-rank test also failed to show statistical significance for either group: PLC/PLC $S = 1.5$, *ns*; PLC/SYN115 $S = 3.5$, *ns*. Thus, the influence of the SYN115 dose on the ARCI-PCAG result is also unclear. There were no other significant dose conditions or dose condition $\times$ time interactions on the ARCI scales.

For the VAS data, there were statistically significant effects of dose condition for the following five variables (all $df = 1$): "good effects" $B = 5.25$, $p < .022$; "bad effects" $B = 4.16$, $p < .007$; "like effect" $B = 5.88$, $p < .016$; "excited" $B = 6.25$, $p < .013$; and "alert" $B = 4.57$, $p < .033$. Follow-up signed-rank tests on the difference within each group in change from time point 3 (PM pre-dose) and time point 4 (PM post-dose) were conducted to examine the relative effects of SYN115 vs. placebo. For the PLC/PLC group, no significant differences were found for any of the VAS scales. For the PLC/SYN115 group the following outcomes were obtained: for "good effects" $S = -26.5$, $p < .036$; for "bad effects" $S = -28.5$, $p < .022$; for "like effect" $S = -25$, $p < .025$; for "excited" $S = -19$, $p < .043$; and for "alert" $S = 3$, *ns*.

There were also effects of time point for the following variables: "any effect" $B = 5.13$, $df = 2.84$, $p < .002$; "good effects" $B = 5.33$, $df = 2.46$, $p < .003$; "bad effects" $B = 4.17$, $df = 2.85$, $p < .007$; and "confused" $B = 5.12$, $df = 2.78$, $p < .003$. Inspection of Table 2 shows these changes were related to time of day rather than dose condition, and thus were not of interest for follow-up testing. There was no time $\times$ dose condition interactions on the VAS scales.

Discussion

The novel A_{2A} receptor antagonist SYN115 [9] was examined in a sample of non-treatment seeking cocaine-dependent subjects. All subjects received placebo capsules in the morning. One group was administered 100 mg SYN115 in the afternoon, the other group was administered a second placebo dose. There were no main effects of dose or dose × time interactions on cardiovascular measures (HR, BP). However, significant effects of dose were found on scales traditionally associated with stimulant effects, e.g. VAS “good effect”, “like effect”, “excited” and “alert”. The distinction between subjective and cardiovascular outcomes stand in contrast to other well known stimulant drugs, including amphetamine, nicotine, methylphenidate, methamphetamine, and caffeine, all of which produce reliable changes in both cardiovascular endpoints and subjective effects [15,16,24,28,29]. Below we discuss these outcomes with regard to SYN115 mechanism of action, the possible specificity of the outcomes within cocaine-dependent individuals, alternative sources of variability that may have contributed to the results, and study limitations.

Significant subjective effects consistent with stimulation were observed (some, such as “good effects” and “excited” were robust). In experimental non-human data, some of the behavioral effects of A_{2A} receptor antagonists have been established to modulate dopamine function by acting on striatal postsynaptic A_{2A} receptors forming heteromers with D₂ receptors [8,13], while other stimulants such as reuptake inhibitors (cocaine, methylphenidate) and releasing agents (amphetamine) modulate dopamine in a broader fashion.

Caffeine, the closest compound pharmacologically to SYN115 that is regularly used by humans, is a mixed A₁ and A_{2A} receptor antagonist that appears to modulate dopamine function at both presynaptic and postsynaptic sites [30]. The ability of caffeine to produce cardiovascular effects in the experimental animal seems to depend on its mixed antagonistic A₁ and A_{2A} receptor profile. Thus, administration of maximal locomotor-activating doses of selective A₁ or A_{2A} receptor antagonists in rats did not produce significant cardiovascular effects, while equipotent doses of caffeine significantly increased mean blood pressure [31]. Notably, postsynaptic striatal A_{2A} receptors are closely tied to reinforcing, attention-enhancing, and psychomotor effects [13], evidenced by several studies that administered experimental A_{2A} receptor antagonists [10,32–39]. These findings are consistent with the present data to the extent that, while cardiovascular effects were not observed, the subjective effects that were found are related to the rewarding properties of stimulant drugs, e.g., “good effects”, “like effects”, “excited” – although, in general, the effects were clinically modest in relation to stimulants with high abuse liability [24,29]. Indeed, the conclusion the SYN115 produces stimulant-like effects should be considered a preliminary observation in light of (a) the modest number and range of significant subjective effects outcomes and (b) several limitations in the study design, described below. Additionally, while stimulant-like subjective effects have traditionally been associated with abuse potential, it appears premature to speculate on the abuse liability profile of SYN115 based on the present results. Further studies featuring drug discrimination and relative reinforcement (choice) models, and assessing full dose-response functions will be needed to examine this issue.

It is important to note that the results are necessarily limited to individuals with cocaine dependence. Cocaine abuse is associated with several cardiovascular sequelae, including compensatory mechanisms in response to tachycardia, which rapidly follows administration [40]. Stimulant abuse may induce sensitization to the psychomotor or subjective effects of stimulants, including caffeine [41,42]. Therefore, the present results may be specific to cocaine users, and may not generalize to healthy controls subjects or other patient groups. For example, the present cardiovascular results are not consistent with those from Phase 1

studies of SYN115 in healthy volunteers, where small but consistent increases in standing and supine systolic and diastolic BP were observed at 5–6 hours following the first administration of doses ranging from 60 mg to 480 mg. On continued dosing, rapid tachyphylaxis was observed, with reduced BP increases at 7 days and no change following 14 days administration. The magnitude of the maximum changes following the first dose of 480 mg were comparable to the acute changes seen with caffeine ([18], S. Bandak, personal communication). The procedures accompanying the fMRI, shorter 240-minute time window, and single 100 mg dose (vs. 60–480 mg) may have affected the study's capacity to detect to detect maximum changes in BP. Thus, while no significant cardiovascular effects were observed out to 240 minutes, it is quite possible that cardiovascular changes (e.g., possible rebound effects) would be observed at extended time periods as far out as 7–8 hours.

The present subjective effects outcomes also differ from those observed in healthy volunteers in a Phase 1 study. In this study the Bond-Lader scale was used to assess subjective effects following single doses of SYN115 up to 100 mg, and no differences from placebo were observed ([18], S. Bandak, personal communication). Additionally, there are no published preclinical data evaluating pretreatment or simultaneous administration of SYN115 and cocaine on cardiovascular or behavioral effects. However, Ferre and colleagues have shown that (1) both selective A₁ and A_{2A} antagonists produce acute cardiovascular effects, and (2) A_{2A} antagonists can substitute for stimulants under drug discrimination protocols [13,30,43]. This raises the interesting question of how cocaine and SYN115 might influence cardiovascular and behavioral systems when given simultaneously. Future preclinical studies evaluating SYN115 in this domain should prove informative.

Currently, no selective adenosine A_{2A} antagonist is approved clinically for human administration. Other than the present report, none have been examined in stimulant abusers. Thus, it is also not possible to draw comparisons with previous studies involving cocaine-dependent subjects, a population with known monoamine dysregulation. However, selective A_{2A} compounds have been administered to both Parkinsons patients, which share some neurobiological characteristics with stimulant dependent individuals, e.g. [4,44], and healthy human control subjects. While several clinical trials have been conducted in Parkinson's patients with the selective A_{2A} antagonist KW-6002 (Istradefylline), and some have reported reductions in motor dysfunction [45,46], data are not readily available on subjective, stimulant, or cardiovascular effects of this compound. In a previous brain imaging study of SYN115, Black et al. [9] reported changes in rCBF in brain regions that may reflect arousal and attention (i.e., decreased CBF in thalamus and increased CBF in anterior cingulate and precuneus). Cardiovascular and subjective effects were not reported. However, Campbell et al. [47] administered SYN115 60 mg BID in Parkinsons patients and reported significant reductions in self-reported sleepiness, which may be cautiously interpreted as the converse, increased arousal.

The present experiment featured several limitations that temper the results and their interpretation. (1) All subjects received placebo in the AM, 13 received SYN115 the afternoon, 9 received a second placebo dose. A more advantageous design would have featured a counterbalanced dosing sequence. Mitigating this option is the T_{1/2} of SYN115 (almost 16 hours); a counterbalanced design would have introduced active-drug carry over effects in the afternoon for subjects administered SYN115 in the AM. (2) At 8 hours, the experimental protocol was a long and imposing arrangement for subjects, who may well have become fatigued over the course of the multiple tests they were asked to complete across the day. (3) Nineteen of 22 subjects were smokers, and nicotine was not allowed during the 8-hour testing protocol. This introduces the likelihood that some subjects experienced acute nicotine withdrawal during the testing period. Nicotine withdrawal symptoms were not directly measured, and thus the influence of withdrawal is unknown. (4)

The cardiovascular and subjective effects data were obtained during time periods surrounding two 90-minute fMRI sessions, with scans obtained in both the AM and PM corresponding to the placebo and SYN115 dosing. The requirements of lying prostrate and immobile while complying with the fMRI protocol could have influenced the post-scan cardiovascular and subjective effects data. (5) Only a single 100 mg dose was administered, and thus no information on dose-response functions was obtained. Further, this dose is in the upper range relative to the few previously published studies that have administered this compound. (6) Across subjects, there was substantial variance in self-reported hours since last cocaine use. This variance may have been due to the nature of cocaine dependence, in which binge use patterns followed by variable periods of abstinence are common. Additionally, impairments in cognition that accompany chronic cocaine use are well documented and likely contributed to bias and error in recall of past use episodes, thus inflating the variance in self-reported time since last use. Though subjects were kept overnight in the CRU following the dose, they were not maintained overnight prior to dosing, which may have helped stabilize mood and activity patterns prior to the day of testing. The impact of these six limitations on the variability and direction of the obtained data is unknown, but surely introduced unwanted and unaccounted influences on the results. Thus, postsynaptic sites [30]. The ability of caffeine to produce cardiovascular effects in the experimental animal seems to depend on its mixed antagonistic A₁ and A_{2A} receptor profile. Thus, administration of maximal locomotor-activating doses of selective A₁ or A_{2A} receptor antagonists in rats did not produce significant cardiovascular effects, while equipotent doses of caffeine significantly increased mean blood pressure [31]. Notably, postsynaptic striatal A_{2A} receptors are closely tied to reinforcing, attention-enhancing, and psychomotor effects [13], evidenced by several studies that administered experimental A_{2A} receptor antagonists [10,32–39]. These findings are consistent with the present data to the extent that, while cardiovascular effects were not observed, the subjective effects that were found are related to the rewarding properties of stimulant drugs, e.g., “good effects”, “like effects”, “excited” – although, in general, the effects were clinically modest in relation to stimulants with high abuse liability [24,29]. Indeed, the conclusion the SYN115 produces stimulant-like effects should be considered a preliminary observation in light of (a) the modest number and range of significant subjective effects outcomes and (b) several limitations in the study design, described below. Additionally, while stimulant-like subjective effects have traditionally been associated with abuse potential, it appears premature to speculate on the abuse liability profile of SYN115 based on the present results. Further studies featuring drug discrimination and relative reinforcement (choice) models, and assessing full dose-response functions will be needed to examine this issue.

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which binge use patterns followed by variable periods of abstinence are common. Additionally, impairments in cognition that accompany chronic cocaine use are well documented and likely contributed to bias and error in recall of past use episodes, thus inflating the variance in self-reported time since last use. Though subjects were kept overnight in the CRU following the dose, they were not maintained overnight prior to dosing, which may have helped stabilize mood and activity patterns prior to the day of testing. The impact of these six limitations on the variability and direction of the obtained data is unknown, but surely introduced unwanted and unaccounted influences on the results. Thus, the generalizability of the data may be limited until these results can be replicated. On the other hand, the magnitude of change in some VAS subscales in the SYN115 group (Table 2), compared to virtually no change in control group, provides some confidence that drug effect signal was large enough to be observed against a backdrop of noise associated with the study limitations.

Using perfusion MRI to study patients with Parkinson disease, Black et al. [9] found that either 20 or 60 mg of SYN115 twice daily for one week produced significant decreases in CBF in thalamus, midbrain, precuneus, and cingulate cortex. The authors suggest that the result may be due to reduction of “inhibitory output of the basal ganglia indirect pathway” [9]. A_{2A} receptors modulate dopamine neurotransmission via formation of A_{2A}-D₂ receptor heteromers in GABAergic striatopallidal neurons, located in this indirect pathway [7,8]. To the extent that agonist-like, or “replacement,” approaches have shown some efficacy in the treatment of stimulant dependence [4,6], the use of A_{2A} antagonists provide an intriguing pharmacotherapeutic avenue (see [7,12] for neurobiological rationale). This hypothesis will require further examination of subjective and cardiovascular effects using more extensive study designs, including larger sample sizes and characterization of dose-response functions.

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References

1. Martinez D, Broft A, Foltin RW, Slifstein M, Hwang DR, et al. Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology*. 2004; 29:1190–1202. [PubMed: 15010698]
2. Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry*. 2004; 9:557–569. [PubMed: 15098002]
3. Grabowski J, Shearer J, Merrill J, Negus SS. Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict Behav*. 2004; 29:1439–1464. [PubMed: 15345275]
4. Herin DV, Rush CR, Grabowski J. Agonist-like pharmacotherapy for stimulant dependence: preclinical, human laboratory, and clinical studies. *Ann N Y Acad Sci*. 2010; 1187:76–100. [PubMed: 20201847]
5. Schmitz JM, Mooney ME, Moeller FG, Stotts AL, Green C, et al. Levodopa pharmacotherapy for cocaine dependence: choosing the optimal behavioral therapy platform. *Drug Alcohol Depend*. 2008; 94:142–150. [PubMed: 18164144]
6. Moeller FG, Schmitz JM, Herin D, Kjome KL. Use of stimulants to treat cocaine and methamphetamine abuse. *Curr Psychiatry Rep*. 2008; 10:385–391. [PubMed: 18803911]
7. Ferré S, Ciruela F, Quiroz C, Luján R, Popoli P, et al. Adenosine receptor heteromers and their integrative role in striatal function. *ScientificWorldJournal*. 2007; 7:74–85. [PubMed: 17982579]

8. Orru M, Bakešová J, Brugarolas M, Quiroz C, Beaumont V. Striatal pre- and postsynaptic profile of adenosine A(2A) receptor antagonists. *PLoS One*. 2011; 6:e16088. [PubMed: 21264319]
9. Black KJ, Koller JM, Campbell MC, Gusnard DA, Bandak SI. Quantification of indirect pathway inhibition by the adenosine A_{2A} antagonist SYN115 in Parkinson disease. *J Neurosci*. 2010; 30:16284–16292. [PubMed: 21123574]
10. LeWitt PA, Guttman M, Tetrud JW, Tuite PJ, Mori A, et al. Adenosine A2A receptor antagonist istradefylline (KW-6002) reduces “off” time in Parkinson’s disease: a double-blind, randomized, multicenter clinical trial (6002-US-005). *Ann Neurol*. 2008; 63:295–302. [PubMed: 18306243]
11. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S. Adenosine A2A receptors and basal ganglia physiology. *Prog Neurobiol*. 2007; 83:277–292. [PubMed: 17646043]
12. Ferré S, Diamond I, Goldberg SR, Yao L, Hourani SM, et al. Adenosine A2A receptors in ventral striatum, hypothalamus and nociceptive circuitry implications for drug addiction, sleep and pain. *Prog Neurobiol*. 2007; 83:332–347. [PubMed: 17532111]
13. Ferré S, Quiroz C, Orru M, Guitart X, Navarro G, et al. Adenosine A(2A) Receptors and A(2A) receptor heteromers as key players in striatal function. *Front Neuroanat*. 2011; 5:36. [PubMed: 21731559]
14. Childs E, de Wit H. Subjective, behavioral, and physiological effects of acute caffeine in light, nondependent caffeine users. *Psychopharmacology (Berl)*. 2006; 185:514–523. [PubMed: 16541243]
15. Garrett BE, Griffiths RR. Intravenous nicotine and caffeine: subjective and physiological effects in cocaine abusers. *J Pharmacol Exp Ther*. 2001; 296:486–494. [PubMed: 11160635]
16. Heishman SJ, Henningfield JE. Stimulus functions of caffeine in humans: relation to dependence potential. *Neurosci Biobehav Rev*. 1992; 16:273–287. [PubMed: 1528521]
17. Sigmon SC, Griffiths RR. Caffeine choice prospectively predicts positive subjective effects of caffeine and d-amphetamine. *Drug Alcohol Depend*. 2011; 118:341–348. [PubMed: 21600707]
18. Synosia-Therapeutics. SYN115 Investigator’s Brochure. San Francisco: 2008.
19. First, MB.; Gibbon, M.; Spitzer, RL.; Williams, JB. Patient Edition. New York: Biometrics Research, New York State Psychiatric Institute; 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version.
20. Association, AP. Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR Fourth Edition (Text Revision). Washington, DC: American Psychiatric Publishing, Inc.; 2000.
21. Martin WR, Sloan JW, Sapira JD, Jasinski DR. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clin Pharmacol Ther*. 1971; 12:245–258. [PubMed: 5554941]
22. Haertzen, C.; Hickey, JE. Addiction Research Center Inventory (ARCI): Measurement of euphoria and other drug effects. New York: Springer-Verlag; 1987. p. 489-524.
23. Bond AJ, James DC, Lader MH. Physiological and psychological measures in anxious patients. *Psychol Med*. 1974; 4:364–373. [PubMed: 4456431]
24. Fischman MW. Relationship between self-reported drug effects and their reinforcing effects: studies with stimulant drugs. *NIDA Res Monogr*. 1989; 92:211–230. [PubMed: 2512494]
25. Fischman MW, Foltin RW. Utility of subjective-effects measurements in assessing abuse liability of drugs in humans. *Br J Addict*. 1991; 86:1563–1570. [PubMed: 1786488]
26. Jasinski DR, Henningfield JE. Human abuse liability assessment by measurement of subjective and physiological effects. *NIDA Res Monogr*. 1989; 92:73–100. [PubMed: 2512504]
27. Brunner, E.; Domhof, S.; Langer, F. Nonparametric analysis of longitudinal data in factorial experiments. New York: J. Wiley and Sons; 2002.
28. Heil SH, Holmes HW, Bickel WK, Higgins ST, Badger GJ, et al. Comparison of the subjective, physiological, and psychomotor effects of atomoxetine and methylphenidate in light drug users. *Drug Alcohol Depend*. 2002; 67:149–156. [PubMed: 12095664]
29. Kollins SH, MacDonald EK, Rush CR. Assessing the abuse potential of methylphenidate in nonhuman and human subjects: a review. *Pharmacol Biochem Behav*. 2001; 68:611–627. [PubMed: 11325419]

30. Ferré S. Role of the central ascending neurotransmitter systems in the psychostimulant effects of caffeine. *J Alzheimers Dis.* 2010; 20:S35–S49. [PubMed: 20182056]
31. Schindler CW, Karcz-Kubicha M, Thorndike EB, Müller CE, Tella SR, et al. Role of central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine A1 and A2A subtype receptor agonists. *Br J Pharmacol.* 2005; 144:642–650. [PubMed: 15678095]
32. Harper LK, Beckett SR, Marsden CA, McCreary AC, Alexander SP. Effects of the A2A adenosine receptor antagonist KW6002 in the nucleus accumbens in vitro and in vivo. *Pharmacol Biochem Behav.* 2006; 83:114–121. [PubMed: 16451807]
33. Higgins GA, Grzelak ME, Pond AJ, Cohen-Williams ME, Hodgson RA, et al. The effect of caffeine to increase reaction time in the rat during a test of attention is mediated through antagonism of adenosine A2A receptors. *Behav Brain Res.* 2007; 185:32–42. [PubMed: 17707919]
34. Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, et al. Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. *Ann Neurol.* 1998; 43:507–513. [PubMed: 9546333]
35. O'Neill M, Brown VJ. The effect of the adenosine A(2A) antagonist KW-6002 on motor and motivational processes in the rat. *Psychopharmacology (Berl).* 2006; 184:46–55. [PubMed: 16344986]
36. Randall PA, Nunes EJ, Janniere SL, Stopper CM, Farrar AM, et al. Stimulant effects of adenosine antagonists on operant behavior: differential actions of selective A2A and A1 antagonists. *Psychopharmacology (Berl).* 2011; 216:173–186. [PubMed: 21347642]
37. Salamone JD, Farrar AM, Font L, Patel V, Schlär DE, et al. Differential actions of adenosine A1 and A2A antagonists on the effort-related effects of dopamine D2 antagonism. *Behav Brain Res.* 2009; 201:216–222. [PubMed: 19428636]
38. Shen HY, Coelho JE, Ohtsuka N, Canas PM, Day YJ, et al. A critical role of the adenosine A2A receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A2A receptor knockouts. *J Neurosci.* 2008; 28:2970–2975. [PubMed: 18354001]
39. Varty GB, Hodgson RA, Pond AJ, Grzelak ME, Parker EM, et al. The effects of adenosine A2A receptor antagonists on haloperidol-induced movement disorders in primates. *Psychopharmacology (Berl).* 2008; 200:393–401. [PubMed: 18594798]
40. Schwartz BG, Rezkalla S, Kloner RA. Cardiovascular effects of cocaine. *Circulation.* 2010; 122:2558–2569. [PubMed: 21156654]
41. Liguori A, Hughes JR, Goldberg K, Callas P. Subjective effects of oral caffeine in formerly cocaine-dependent humans. *Drug Alcohol Depend.* 1997; 49:17–24. [PubMed: 9476695]
42. Rush CR, Sullivan JT, Griffiths RR. Intravenous caffeine in stimulant drug abusers: subjective reports and physiological effects. *J Pharmacol Exp Ther.* 1995; 273:351–358. [PubMed: 7714788]
43. Justinova Z, Ferré S, Segal PN, Antoniou K, Solinas M, et al. Involvement of adenosine A1 and A2A receptors in the adenosinergic modulation of the discriminative-stimulus effects of cocaine and methamphetamine in rats. *J Pharmacol Exp Ther.* 2003; 307:977–986. [PubMed: 14557381]
44. Nikolaus S, Antke C, Kley K, Poeppel TD, Hautzel H, et al. Investigating the dopaminergic synapse in vivo. I. Molecular imaging studies in humans. *Rev Neurosci.* 2007; 18:439–472. [PubMed: 18330212]
45. Jenner P, Mori A, Hauser R, Morelli M, Fredholm BB, et al. Adenosine, adenosine A2A antagonists, and Parkinson's disease. *Parkinsonism Relat Disord.* 2009; 15:406–413. [PubMed: 19446490]
46. Mizuno Y, Hasegawa K, Kondo T, Kuno S, Yamamoto M. Clinical efficacy of istradefylline (KW-6002) in Parkinson's disease: a randomized, controlled study. *Mov Disord.* 2010; 25:1437–1443. [PubMed: 20629136]
47. Campbell MC, Koller JM, Bandak SI, Black KJ. Cognition in Parkinson disease: effects of levodopa and an adenosine A2a antagonist. *J Int Neuropsychol Soc.* 2010:16.

Table 1

Pre- and post-dose measures of heart rate, systolic and diastolic blood pressure across two groups of dosing conditions (Placebo AM / Placebo PM, 9 subjects = Plc/Plc) or (Placebo AM / SYN15 PM, 13 subjects = Plc/SYN15). Data show means ($\pm$ SD). There were no statistically significant differences between dose conditions.

	Time (AM Dose)					Time (PM Dose)				
	-60	-30	+60	+120	-30	+60	+120	+180	+240	
HEART RATE										
Plc/Plc										
69.78 (9.27)	67.0 (10.81)	63.0 (9.29)	60.56 (6.56)	62.22 (9.74)	65.22 (8.97)	62.78 (8.21)	64.0 (7.94)	61.44 (4.95)		
Plc/SYN15										
72.85 (11.26)	72.30 (9.97)	65.69 (7.21)	63.38 (4.86)	64.76 (6.49)	63.85 (8.16)	62.76 (6.15)	66.15 (7.82)	65.23 (4.75)		
SYSTOLIC BP (mm Hg)										
Plc/Plc										
111.89 (10.09)	112.56 (10.44)	112.22 (11.19)	111.67 (13.71)	113.0 (11.32)	111.56 (5.48)	107.0 (5.41)	114.11 (13.46)	121.33 (13.9)		
Plc/SYN15										
108.77 (10.84)	108.08 (11.84)	114.69 (12.73)	115.15 (9.05)	112.15 (8.59)	111.92 (9.21)	113.38 (11.38)	116.84 (12.44)	122.0 (17.1)		
DIASTOLIC BP (mm Hg)										
Plc/Plc										
72.89 (10.95)	73.11 (7.08)	69.67 (8.08)	70.56 (11.40)	72.67 (12.35)	77.22 (4.02)	73.56 (5.75)	67.22 (8.30)	73.33 (6.81)		
Plc/SYN15										
71.54 (11.12)	72.15 (10.67)	73.76 (12.28)	73.23 (9.81)	74.54 (6.84)	76.54 (6.41)	75.85 (11.30)	75.76 (9.82)	76.77 (10.83)		

Table 2

Pre- and post-dose subjective effects measures (ARCI, VAS) across two groups of dosing conditions (Placebo AM / SYN115 PM, 13 subjects = Plc/SYN115) or (Placebo AM / Placebo PM, 9 subjects = Plc/Plc). Data show medians and interquartile ranges.

Scale	AM (pre-dose)	AM (post-dose)	PM (pre-dose)	PM (post-dose)
** ARCI-A Plc/Plc1 Plc/SYN115	1(1–2) 2 (1–4)	1 (0–2) 2 (1–4)	1 (0–1) 2 (1–4)	1 (0–1) 3 (2–5)
ARCI-PCAG Plc/Plc1 Plc/SYN115	4 (4–7) 4 (1–4)	7 (4.8–8) 4 (1–4)	5.03 (4–8) 4 (3–7)	5 (4–8) 4 (3–6)
ARCI-MGB Plc/Plc1 Plc/SYN115	1 (0–2) 3 (0–6)	2 (0–3) 4 (0–8)	1 (1–2) 2 (0–8)	1 (0–2) 2 (1–8)
ARCI-BG Plc/Plc1 Plc/SYN115	4 (3–5) 5 (4–6)	4 (3–5) 6 (4–8)	4 (3–5) 5 (4–7)	4 (3–4) 5 (4–7)
ARCI-LSD Plc/Plc1 Plc/SYN115	3 (2–4) 2.8 (2–3)	3.1 (3–4) 3 (2–4)	4 (3–4) 3.3 (3–4)	4 (4–4) 3 (3–5)
* VAS “any effect” Plc/Plc1 Plc/SYN115	2 (0–4) 4 (1–21)	2 (0–8) 6 (0–20)	2 (0–6) 10 (7–32)	7 (2–15) 15 (10–95)
*, ** VAS “good effects” Plc/Plc1 Plc/SYN115	1 (0–5) 5 (2–23)	4 (2–13) 13 (2–30)	4 (0–10) 11 (7–34)	5 (3–19) 88 (16–107)
* VAS “bad effects” Plc/Plc1 Plc/SYN115	0 (0–3) 4 (0–7)	3 (0–3) 0 (0–6)	0 (0–3) 8 (0–11)	3 (0–6) 12 (6–63)
** VAS “like effect” Plc/Plc1 Plc/SYN115	1 (0–3) 6 (2–63)	4 (0–8) 11 (5–69)	5 (1–11) 12 (5–61)	5 (0–6) 77 (13–92)
VAS “friendly” Plc/Plc1 Plc/SYN115	109 (0–114) 128 (5–158)	110 (4–122) 56 (0–171)	112 (0–125) 22 (6–128)	96 (7–118) 119 (47–171)
* VAS “confused” Plc/Plc1 Plc/SYN115	1 (0–3) 4 (0–8)	4 (0–5) 3 (1–5)	3 (0–3) 8 (3–23)	4 (2–11) 7 (4–20)
VAS “able to concentrate” Plc/Plc1 Plc/SYN115	128 (118–151) 175 (134–209)	114 (114–151) 134 (100–145)	114 (55–118) 123 (114–188)	114 (94–134) 150 (118–194)
** VAS “excited” Plc/Plc1 Plc/SYN115	3 (0–13) 109 (7–115)	5 (4–55) 55 (5–122)	4 (0–6) 57 (25–119)	7 (5–114) 108 (23–119)
** VAS “alert” Plc/Plc1 Plc/SYN115	114 (91–117) 137 (106–158)	105 (18–112) 125 (112–156)	59 (2–119) 156 (114–188)	79 (31–119) 139 (111–170)
VAS “relaxed” Plc/Plc1 Plc/SYN115	114 (102–131) 136 (115–159)	108 (26–151) 123 (108–176)	113 (9–133) 130 (114–176)	79 (21–184) 122 (110–165)

* indicates significant within-subject result (time), $p < .05$

** indicates significant between-group result (dose condition), $p < .05$