Title Page

Activation of adenosine$_1$ (A$_1$) receptors induces antidepressant-like, anti-impulsive effects on differential-reinforcement-of-low rate 72-s behavior in rats

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Running Title Page

Running Title: Adenosine A1 receptor ligands and DRL behavior

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Non-standard abbreviations:
5-choice serial reaction time test (5-CSRTT); 8-cyclopentyl-1,3-dipropylxanthine (DCPCX); differential-reinforcement-of low rate (DRL); forced swim test (FST);
generalized anxiety disorder (GAD); medial prefrontal cortex (mPFC); metabotropic glutamate 2 (mGlu2); N6-cyclohexyladenosine (CHA); phosphodiesterase-4 (PDE4);
positive allosteric modulators (PAMS); tricyclic antidepressants (TCAs);
Abstract

Stress and psychiatric illness has been associated with a dysregulation of glutamatergic neurotransmission. Recently, positive allosteric modulators (PAMs) of the metabotropic glutamate 2 (mGlu2) receptor have been found to exert antidepressant-like activity in rats performing under a differential-reinforcement-of low rate (DRL) 72-s schedule. An autoreceptor role at glutamatergic synapses is the most salient physiological role played by the mGlu2 receptor. Adenosine A1 receptors play a heteroreceptor role at many of the same forebrain synapses where mGlu2 autoreceptors are found. Agonists and/or PAMs of mGlu2 receptors act similar to adenosine A1 receptor agonists with respect to a wide range of electrophysiological, biochemical, and behavioral responses mediated by limbic circuitry thought to play a role in the pathophysiology of neuropsychiatric disease and to mediate therapeutic drug effects. Therefore, the role of adenosine A1 receptor activation on rat DRL 72-s behavior was explored to provide preclinical evidence consistent or inconsistent with potential antidepressant effects. The adenosine A1 receptor agonist N6-cyclohexyladenosine (CHA) increased the reinforcement rate, decreased the response rate and induced a rightward shift in interresponse time distributions in a dose-dependent fashion similar to most known antidepressant drugs. The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DCPCX) blocked these antidepressant-like effects. These novel observations with CHA and DPCPX suggest that activation of adenosine A1 receptors could contribute to antidepressant effects, in addition to previous preclinical reports of anxiolytic and antipsychotic effects. By implication, targeting a dysregulated
glutamatergic system may be an important principle in discovering novel antidepressant agents that may also possess anti-impulsive activity.
Introduction

Stress is known to play a major role in psychiatric illness. Major depression and bipolar depression are associated with increased or decreased blood flow in the medial orbital prefrontal cortex (PFC) and a number of other limbic-related brain regions suggesting dysregulation of glutamatergic neurotransmission. Furthermore, antidepressant treatments normalized the increased cortical blood flow (Price and Drevets, 2010). Metabotropic glutamate2/3 (mGlu2/3) receptor agonism is an effective therapeutic approach for neuropsychiatric illness with divergent etiology/pathophysiology such as generalized anxiety disorder (GAD) and schizophrenia (Dunayevich et al., 2008; Patil et al., 2007), disorders where stress and/or dysregulated glutamate release at key forebrain regions plays a key pathophysiological role. Activation of mGlu2 receptors plays a role in preclinical anxiety and psychosis “efficacy” models extrapolating from experiments with transgenic mice lacking mGlu2 and/or mGlu3 receptors or from comparisons of mGlu2 receptor positive allosteric modulators (PAMs) with orthosteric mGlu2/3 receptor agonists. An glutamatergic autoreceptor function is the most prominent physiological role played by mGlu2 receptors; mGlu2 receptor activation suppresses glutamate release at many synapses throughout the limbic forebrain (Schoepp, 2001). Recent preclinical studies with mGlu2 receptor positive allosteric modulators have suggested that this novel class of drugs may possess antidepressant properties (Fell et al., 2011; Nikiforuk et al., 2010).

While activation of mGlu2 receptors may play a therapeutic role in the treatment of schizophrenia and GAD, preclinical evidence also suggests that activation of mGlu2 and/or mGlu3 receptors could play a role in anti-nociceptive effects (including anti-
migraine effects), attenuation of opioid withdrawal effects, and general neuroprotective and antiepileptic effects. Preclinical evidence suggests that activation of adenosine A₁ receptors plays a similar role for this wide range of neuropsychiatric indications (Kaster et al., 2004; Marek, 2009; Ribeiro et al., 2003). The similar distribution of mGlu₂ and adenosine A₁ receptors throughout the limbic forebrain likely is an important underpinning to similar effects of activating these receptors across a wide range of neuropsychiatric preclinical models (Bauer et al., 2003; Richards et al., 2005). Hallucinogen-induced head shakes and hallucinogen/MK-801-induced IEG expression are modulated by mGlu₂ or adenosine A₁ receptors in the PFC or on thalamocortical pathways (Benneyworth et al., 2007; Gotoh et al., 2002; Marek, 2009; Pei et al., 2004; Scruggs et al., 2000; Wischhof and Koch, 2011). While adenosine A₁ receptors are located at both postsynaptic and presynaptic sites, this receptor is known to play a prominent role as a heteroreceptor, decreasing excitatory amino acid release, similar to the mGlu₂ receptor role as an autoreceptor.

While mGlu₂/₃ receptor agonists prodrugs have been demonstrated in double-blind, placebo-controlled trials to exert antipsychotic or anxiolytic action, adenosine A₁ receptor agonists act similarly to many of mGlu₂/₃ receptor agonists in a wide variety of the same preclinical screens detecting antipsychotic (reviewed by Marek (2009)) or anxiolytic drugs. Adenosine A₁ receptor agonists possess antidepressant-like tests in mice with the forced swim test (FST) and the tail suspension test (Kaster et al., 2004). An additional reason for testing potential antidepressant activity of adenosine A₁ receptor agonists is that these compounds presynaptically suppress glutamate release induced by 5-HT₂A receptor activation in the PFC, similarly to activation of mGlu₂ autoreceptors.
(Marek et al., 2000; Stutzman et al., 2001). The DRL 72-s schedule is an antidepressant screen where 5-HT$_{2A}$ receptor antagonists exert frank antidepressant effects and also enhance the antidepressant-like effects of tricyclic antidepressants, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (Ardayfio et al., 2008; Marek et al., 2005). From a clinical standpoint, blockade of 5-HT$_{2A}$ receptors may contribute to the antidepressant effects of tricyclic antidepressants, heterocyclic antidepressants (e.g., mianserin, mirtazapine, trazodone, nefazodone), and atypical antipsychotics (e.g., quetiapine, risperidone, olanzapine and aripiprazole).

Since activation of mGlu$_2$ receptors (Fell et al., 2011; Nikiforuk et al., 2010) and blockade of 5-HT$_{2A}$ receptors results in antidepressant activity in rats performing under a DRL 72-s schedule potentially by attenuating motoric impulsivity, the effects of a selective adenosine A$_1$ receptor agonist N$^6$-cyclohexyladenosine (CHA) both alone and with a selective adenosine A$_1$ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX or PD116,948) was tested in rats trained to perform on a DRL 72-s schedule. The results with CHA are consistent with previous literature suggesting antidepressant-like activity with activation of adenosine A$_1$ receptors (Kaster et al., 2004). The present results on DRL 72-s behavior also are consistent with the working hypothesis that the mechanism underlying these antidepressant-like effects involves an attenuation of motoric impulsivity. These results add to a growing literature suggesting that modulation of glutamatergic activity may lead to the development of truly novel antidepressant and/or mood stabilizing drugs.
Methods

Animals

For the DRL experiments, twenty-one male Sprague-Dawley rats weighing between 300 and 350 g at the beginning of the behavioral experiments (Holtzman, Madison, WI) were housed in suspended stainless steel wire cages (18 x 36 x 20 cm) with two rats occupying each cage. The colony room was maintained at 20 °C and relative humidity (60%). The room was illuminated 12 hr/day (07:00-19:00). All rats had free access to laboratory chow (Teklad 4% Rat Diet) except during experimental sessions. Water was available for only a 20 min period following the daily behavioral session except for Friday after the DRL session when water was available ad libitum until approximately noon Sunday. All animals were treated in accord with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. In addition, all protocols were approved by the Yale University Animal Care and Use Committee.

Apparatus

Eight MED Associates operant-conditioning chambers (30.5 x 24.1 x 29.2 cm; St. Albans, VT; www.med-associates.com) were used for the DRL experiments. The lever in these chambers was mounted on one wall with the water access next to the lever in the middle of the wall. A reinforced response caused the dipper (0.02 cc cup) to be lifted from a water trough to an opening in the floor of the access port for 4 sec. The houselight (which was mounted on the opposite wall) was turned on when the session began, remained on throughout the entire session and turned off at the end of the session. Each
experimental chamber was enclosed in a melamine sound attenuating cubicle and equipped with a white noise generator to provide masking noise.

**Operant training**

Rats were water deprived for ~ 22.5 h before each session. Each rat was initially trained under an alternating fixed ratio 1, fixed-time 1 min schedule for water reinforcement. Thus, each response was reinforced and water was also provided every min if a response did not occur. The few rats that did not acquire lever-pressing behavior after three daily 1 h sessions under this schedule were trained by the experimenter using the method of successive approximation. After the rats had acquired lever-pressing behavior they were trained during daily DRL 18-s sessions for ~ 2 weeks before moving directly to DRL 72-s sessions. The responding on these sessions became stable after ~ 8 weeks. Experimental sessions lasted for 1 h and were conducted 5 days/week during light hours.

**Schedule control and statistical analyses**

The experimental chambers were connected to a PC via a MED-SYS-8 interface and software package. All behavioral data are expressed as the mean ± SEM, in some cases normalized to the vehicle or vehicle-vehicle condition. The DRL data analysis was performed on non-transformed data. The effects of CHA (15.6-125 μg/kg) and DPCPX (0.313 mg/kg) alone were analyzed with a one-factor repeated measures analysis of variance (ANOVA). The effects of the combined treatment of CHA and DPCPX were analyzed using a two-factor repeated measures ANOVA with CHA and DPCPX as within-subject factors. Significant main effects or interactions were analyzed by the
Newman-Keuls or Dunnett test where appropriate. The level of significance was set for p<0.05 for all analyses. The cumulative interresponse time (IRT) distribution for the entire group of rats treated with a within subject design were analyzed with the non-parametric Kolmogorov-Smirnov (K-S) test to make comparisons between different treatment conditions. The level of significance was set for p<0.05.

Drugs

Doses were calculated on the basis of the salt forms. The drugs were dissolved in saline, neutralized to a pH ~ 7.4, and injected using a volume of 1 ml/kg body weight. The adenosine A1 receptor agonist CHA (N6-cyclohexyladenosine) and the adenosine A1 receptor antagonist DPCPX (8-cyclopentyl-1,3-dimethylxanthine) were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris (Ballwin, MO), respectively. Drugs were administered to the animals only once weekly by an i.p. route of administration 1 hr prior to the behavioral session (Thursday). All drug treatments were carried out only once weekly to minimize possible carryover effects.
Results

The behavior of each rat appeared to return to baseline the day following drug administration.

Antidepressant-like actions of CHA on DRL 72-s behavior

The adenosine A1 receptor agonist CHA (0.0156-0.125 mg/kg, ip) increased the number of reinforcers earned for rats performing under a DRL 72-s operant schedule in a dose-dependent manner (F(4,28)=3.91, p<0.05; Fig. 1). Post-hoc testing revealed significant increases at the 0.0625 and 0.125 mg/kg dose conditions (Dunnett t-test, p<0.05) compared to the vehicle condition. CHA also decreased the total lever press responses in a dose-dependent manner (F(4,28)=8.27, p<0.001), with significant decreases at the 0.0625 and 0.125 mg/kg dose conditions (Dunnett t-test, p<0.01).

An apparent dose-dependent rightward shift in the IRT distributions was also observed following CHA administration (Fig. 2). This change was suggested by a significantly different IRT distribution for the 0.0625 and 0.125 mg/kg CHA doses compared to the vehicle condition when examining the cumulative IRT distributions for the entire group of 8 rats (p<0.001, Kolmogorov-Smirnov (K-S) test). The changes in the temporal pattern of responding observed for the cumulative IRT distributions of the entire group was also seen when examining IRT distributions for the individual rats. Significantly different IRT distributions from the vehicle condition were observed for zero, four, and six of eight rats at the 0.0156, 0.0313, and 0.0625 mg/kg CHA doses, respectively (p<0.001, K-S test). One rat at the 0.125 mg/kg CHA dose did not respond; five of the remaining seven rats had IRT distributions significantly different from the vehicle condition (p<0.001). A numerical trend for a rightward shift in the mean IRT...
value approaching the 72 sec schedule time requirement occurred following administration of the adenosine A1 receptor agonist CHA (mean IRTs (SD): vehicle, 57.5 (42.6) s; 0.0313 mg/kg, 56.0 (40.2) s; 0.0625 mg/kg, 81.9 (93.8) s; 0.125 mg/kg, 77.6 (62.8) s).

Effects of the adenosine A1 receptor antagonist DPCPX on DRL 72-s behavior

The adenosine A1 receptor antagonist DPCPX (0.31 mg/kg, i.p.) alone did not alter the frequency of reinforcers obtained for rats responding on a DRL 72-s schedule compared to vehicle (t(12)=1.46, p>0.1, Fig. 3) in a second cohort of 13 rats. However, DPCPX (0.31 mg/kg) did increase the frequency of responses when compared to vehicle (t(12)=3.64, p<0.01). The cumulative IRT distribution for the entire group of rats was not altered for the DPCPX treatment compared to the vehicle condition (p>0.1, K-S test, not shown). Similarly, the mean (+SD) IRT interval was unchanged for the DPCPX treatment (38.7 ± 26.2 s) compared to vehicle (37.6 ± 26.1 s). In a subset (n=7) of this cohort an extended DPCPX dose range was tested (2.5, 5, 10 mg/kg vs placebo). There was no effect of DPCPX on the number of reinforcers obtained (F(3,18)= 2.22, p=0.121) though there was a trend for an increased number the total number of lever presses (F(3,18)=2.74, p=0.074).

DPCPX blocks the antidepressant-like effects of CHA on DRL 72-s behavior

In a third experiment again using the second cohort of rats, CHA (0.0625 mg/kg, i.p.) increased the frequency of reinforcers obtained for rats performing under a DRL 72-s schedule and this effect of CHA was blocked by the adenosine A1 receptor antagonist
DPCPX (F(4,48)=11.58, p<0.0001; Fig. 4). CHA significantly increased the reinforcement rate by ~100% compared to the vehicle-vehicle condition both times the adenosine receptor agonist was administered (p<0.001, Newman-Keuls test, second vehicle/CHA treatment not shown). In contrast, the reinforcement rate was unchanged compared to the vehicle-vehicle condition when DPCPX was co-administered with CHA at either the 0.31, 1.25 or 5 mg/kg dose. However, the number of reinforcers obtained for each dose of DPCPX administered with CHA was significantly decreased compared to the vehicle-CHA condition (p<0.001, 0.31, 1.25 or 5 mg/kg DPCPX administered with CHA, Newman-Keuls test).

CHA (0.0625 mg/kg, i.p.) decreased the total response frequency; and this CHA effect was blocked by the adenosine A1 receptor antagonist DPCPX (F(4, 48)=11.15, p<0.0001; Fig. 4). CHA significantly decreased the response rate by 24-37% compared to the vehicle-vehicle condition both times the adenosine receptor agonist was administered (p<0.001 and p<0.01 (2nd administration not shown), respectively, Newman-Keuls test). In contrast, the response rate was unchanged from the vehicle-vehicle condition when DPCPX was co-administered with CHA. However, the total number of lever press responses for each dose of DPCPX administered with CHA was significantly increased compared to the vehicle-CHA condition (p<0.001, Newman-Keuls test).

The rightward shift of the IRT distribution induced by CHA (0.0625 mg/kg) was blocked by the adenosine A1 receptor antagonist DPCPX (0.31 mg/kg). CHA induced a significant alteration in the cumulative group IRT distribution (n=13; p<0.001, K-S test; mean ± SD of the IRTs: 52.3 ± 38.5 s) compared to the vehicle condition (37.7 ± 26.1 s, mean IRT ± SD). In contrast, the IRT distribution was not significantly altered by
administration of both CHA and DPCPX (35.7 ± 26.0 s, mean IRT ± SD) compared to the vehicle condition. Rechallenge with CHA (0.0625 mg/kg) again resulted in a significant shift in the cumulative group IRT distribution (p<0.001, K-S test; 47.7 ± 47.0 s, mean IRT ± SD) compared to the vehicle-vehicle condition.
**Discussion**

This is the first known demonstration that activation of adenosine A₁ receptors, potentially by suppressing glutamate release in the medial PFC and associated limbic macrocircuitry, results in effects similar to antidepressant drugs using the rat DRL 72-s schedule of reinforcement. The known pharmacological selectivity of both CHA and DPCPX for adenosine A₁ receptors argues that the effects of these drugs are mediated via activation and blockade of adenosine A₁ receptors. While CHA is only modestly selective for rat adenosine A₁ compared to rat adenosine A₃ receptors (>200-fold), this agonist is nearly 500-fold and over 10,000-fold selective for rat A₂A and human A₂B receptors (Bruns, 1980; Daly et al., 1993; van Galen et al., 1994) While DPCPX is only modestly selective for adenosine A₁ compared to A₂B receptors (~400-fold), this antagonist is nearly 300-1,000-fold and nearly 10,000-fold selective compared to rodent A₂A and A₃ receptors (Auchampach et al., 1997; Bruns, 1980; Weyler et al., 2006). Thus, the potent antagonism exerted by DPCPX against CHA is mediated by their most potent shared site of action, adenosine A₁ receptors.

Furthermore, previous *in vivo* experiments suggest that the doses of CHA and DPCPX chosen for the present experiments do specifically interact with adenosine A₁ receptors. First, complete displacement of the binding for a different adenosine A₁ receptor radiotracer was obtained with a 1 mg/kg ip dose of DPCPX (Paul et al., 2011). Second, CHA and DPCPX (each 0.03-0.30 mg/kg doses) altered locomotor activity through a specific interaction with brain adenosine A₁ receptors (Marston et al., 1998). Third, while adenosine A₁ and adenosine A₂A receptors easily are the most abundant adenosine receptor subtypes found in the brain (Ribeiro et al., 2003), DPCPX (0.375-1.5 mg/kg ip)
mg/kg, ip) failed to attenuate the locomotor effects of the dopamine D2 receptor antagonist eticlopride unlike an adenosine A2A receptor antagonist (Collins et al., 2010). Unfortunately, the 0.3 mg/kg DPCPX dose completely suppressed the antidepressant-like effect of CHA. A more complete understanding of CHA and DPCPX pharmacological potency available after the present studies were conducted suggests that the 0.3 mg/kg DPCPX dose results in maximal effects at blocking adenosine A1 receptors.

Furthermore, similar antidepressant-like effects of CHA alone were observed in this experiment both times the agonist was administered. PDE4 inhibition also results in antidepressant-like effects on DRL behavior that may be mediated by increases in cAMP (reviewed elsewhere, O’Donnell et al., 2005) raising the question as to whether adenosine A1 receptors with transduction pathways through Gs-proteins and increased cAMP (adenosine A2A or A2B receptors) might play a role in the effects of CHA and DPCPX. However, the common in vitro and in vivo pharmacological potency of both CHA and DPCPX only for adenosine A1 receptors suggests that this adenosine receptor subtype mediates the antidepressant-like action of CHA on DRL behavior.

The prominent presynaptic localization of adenosine A1 receptors throughout the limbic forebrain is consistent with the hypothesis that suppression of glutamate release in forebrain circuits may be responsible for the antidepressant-like effects of CHA on DRL behavior. Synapses where adenosine A1 receptors suppress glutamate release include thalamocortical (Fontanez and Porter, 2006), thalamostriatal (Flagmeyer et al., 1997), corticostriatal (Flagmeyer et al., 1997), the hippocampal formation including the perforant pathway (Dragunow et al., 1988), and the subthalamic nucleus (Shen and Johnson, 2003). Adenosine A1 receptors also appear to modulate corpus callosal axon
physiology (Swanson et al., 1998). However, these considerations do not rule out a role for postsynaptic adenosine A₁ receptors.

A significant overlap exists between forebrain distributions of mGlu₂ and adenosine A₁ receptors in thalamocortical pathways (regulated by 5-HT₂A receptor activation) from midline/intralaminar thalamic nuclei (Benneyworth et al., 2007; Marek et al., 2001; Stutzman et al., 2001), the medial perforant pathways in the hippocampal formation (Dragunow et al., 1988; Shigemoto et al., 1997) and corticostriatal pathways (Conn et al., 2005; Flagmeyer et al., 1997; Johnson et al., 2005) where both receptors play autoreceptor or heteroreceptor functions. Given the prominent autoreceptor and heteroreceptor effects of mGlu₂ and adenosine A₁ receptors in the CNS, it is parsimonious to propose the working hypothesis that the antidepressant-like effects observed with mGlu₂ receptor PAMs (Fell et al., 2011; Nikiforuk et al., 2010) and the adenosine A₁ receptor agonist CHA on DRL 72-s behavior is attributable to a suppression of glutamate release at limbic synapses.

Regarding a triangulation of 5-HT₂A, mGlu₂ and adenosine A₁ receptors on operant behavioral, the highly selective 5-HT₂A receptor antagonist M100907 exerts effects similar to most antidepressant drugs with DRL 72-s behavior (Ardayfio et al., 2008; Marek et al., 2005). The present demonstration that CHA exerted effects on DRL 72-s behavior similar to most antidepressant drugs is interesting given the previously documented antidepressant-like effects for several mGlu₂ receptor PAMs (Fell et al., 2011; Nikiforuk et al., 2010). Thus, the hypothesis that both mGlu₂ receptor PAMs and adenosine A₁ receptor agonists induce antidepressant-like effects on DRL 72-s behavior by virtue of attenuating glutamate release in limbic synapses is driven by (1) overlapping
anatomical distribution of mGlu2 and adenosine A1 receptors, (2) activation of both receptors suppress glutamate release, (3) activation of both receptors suppress excitatory synaptic currents induced by 5-HT2A receptor activation, and (4) mGlu2 receptor activation suppresses hallucinogen-induced IEG expression in the mPFC.

How might CHA, mGlu2 receptor PAMs and selective 5-HT2A receptor antagonists exert antidepressant-like effects on DRL behavior? Alteration of sedation is one potential explanation for the effects of CHA and DPCPX on DRL 72-s behavior. While this could contribute to the behavioral effects of CHA, numerous drugs which decrease the response rate and are known to have sedative action in both animals and man such as typical antipsychotic drugs, alcohol, antihistamines, α1-adrenergic receptor antagonists, 5-HT1B/1C receptor agonists, and the peripheral administration of 5-HT generally do not induce an antidepressant-like effect on DRL 72-s as reviewed elsewhere (O'Donnell et al., 2005).

Thus, a different explanation is required to understand how 5-HT2A receptor antagonists, CHA and mGlu2 receptor PAMs induce a constellation of effects similar to most antidepressant drugs on DRL behavior independent of simply a sedative-like decrease in the response rate. Alterations of motoric impulsivity (behavioral inhibition) may be a shared explanatory construct (Navarra et al., 2008; Pattij et al., 2003; Robinson et al., 2008) for these drugs and a range of other drugs including TCAs and norepinephrine transporter inhibitors (Blondeau and Dellu-Hagedorn, 2007; Carli et al., 2006; Greco et al., 2005; Paine et al., 2007; Winstanley et al., 2004; Wischhof and Koch, 2011). This potential involvement of impulsivity in mediating the antidepressant-like effects of NET inhibitors, TCAs, 5-HT2A receptor antagonists, mGlu2 receptor PAMs and
adenosine $A_1$ receptor agonists on DRL behavior highlights an important differential feature compared to the forced swim test (FST), for which adenosine $A_1$ receptor agonists and mGlu2 receptor agonists also test similar to antidepressants. Rather than potentially modulating impulsivity on DRL behavior, the FST measures the ability of drugs to modulate an escape behavior. Learned helplessness provides a well-known theoretical background for the FST.

Caveats regarding the present study include (1) the use of a single adenosine $A_1$ receptor agonist and antagonist to examine DRL behavior and (2) the failure to demonstrate a no effect dose for the adenosine $A_1$ receptor antagonist in blocking the effects of CHA. However, the novel antidepressant-like action reported here for CHA agrees with an earlier study suggesting that activation of adenosine $A_1$ receptors mediates antidepressant-like effects on the FST and the tail suspension test (Kaster et al., 2004). The convergence of these different antidepressant screens is intriguing since they would appear to measure different behavioral constructs. Adenosine $A_1$ receptor agonists also decrease rapid eye movement (REM) sleep, consistent with the preclinical/clinical action of most antidepressants (Schwierin et al., 1996). This confluence of positive results on DRL 72-s behavior, both the FST and tail suspension test, and suppression of REM sleep increase the probability of these divergent behavioral screens correctly predicting effects in patients. Adenosine $A_1$ receptor agonists are not available to test the general hypothesis that decreasing glutamate release in limbic circuitry will result in antidepressant effects in depressed patients due to the peripheral on-target cardiovascular toxicity. However, administration of mGlu2 receptor PAMs could test this hypothesis.

While one mGlu2 receptor PAM has been tested for antipsychotic effects by AstraZeneca
and another Johnson & Johnson phase 2 schizophrenia study is currently recruiting subjects to test JNJ-40411813 (www.clinicaltrials.gov), testing of these novel glutamatergic drugs in depressed patients should be pursued (Fell et al., 2011).

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Authorship Contributions

Participated in research design: Marek

Conducted experiments: Marek

Performed data analysis: Marek

Wrote or contributed to the writing of the manuscript: Marek
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Footnotes

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Legends for figures

Fig. 1. Dose-dependent changes in the frequency of reinforcers and responses induced by the adenosine A<sub>1</sub> receptor agonist CHA (0.0156-0.125 mg/kg, i.p.) for rats performing under a DRL 72-s schedule (n=8). For the vehicle condition, the mean (± SEM) number of reinforcers obtained was 7.9 (± 1.49) while the mean number of total responses was 86.8 (± 11.20). Rats were injected 1 h prior to a 1 h behavioral session. Significant changes from the vehicle control are indicated for p<0.05, * and p<0.01, ** using the Dunnett test.

Fig. 2. Effects of the adenosine A<sub>1</sub> receptor agonist CHA (0.0313-0.125 mg/kg) on inter-response time (IRT) distributions for rats performing on a DRL 72-s schedule. Cumulative IRT for this group of rats by obtaining the relative frequency of all responses for each rat separately for each dose condition. The cumulative IRT distribution for the entire group for each CHA dose was compared to the group cumulative IRT distribution for the vehicle condition using the non-parametric Kolmogorov-Smirnov test (*** p<0.001).

Fig. 3. Effects of the adenosine A<sub>1</sub> receptor antagonist DPCPX (0.31 mg/kg, i.p., n=13, graphs on left; 2.5-10 mg/kg, n=7, graphs on right) on the number of reinforcers obtained (top graphs) and the total responses (bottom graphs) made by rats performing on a DRL 72-s schedule. Open bar: vehicle; hatched bars, DPCPX. ** p<0.01 compared to the vehicle condition.
Fig. 4. Effects of the adenosine A₁ receptor antagonist DPCPX (0.31, 1.25, and 5 mg/kg) on the antidepressant-like effects of the adenosine A₁ receptor agonist CHA (0.0625 mg/kg) in rats performing on a DRL 72-s schedule. Rats were tested using a within-subject design with drugs administered at weekly intervals in the order shown (n=13) via an i.p route of administration. Open bar: vehicle-vehicle; solid black bars: vehicle-CHA (0.0625 mg/kg); blue hatched bar: DPCPX (0.31 mg/kg) – CHA (0.0625 mg/kg); green cross-hatched bar: DPCPX (1.25 mg/kg) – CHA (0.0625 mg/kg); red hatched bar: DPCPX (5 mg/kg) – CHA (0.0625 mg/kg). *** p<0.001 compared to the vehicle-vehicle condition. ### p < 0.001 compared to the vehicle-CHA (0.0625 mg/kg) condition. The significant effects the combination of vehicle-CHA (0.0625 mg/kg) administered a second time compared to the vehicle-vehicle condition are not shown.

Fig. 5. Effects of the adenosine A₁ receptor agonist CHA (0.0625 mg/kg) on inter-response time (IRT) distributions for rats performing on a DRL 72-s schedule both in the absence and the presence of the adenosine A₁ receptor antagonist DPCPX (0.31 mg/kg). Cumulative IRT distributions for the entire group of 13 rats are plotted for each of the respective dose conditions, including a replication of the CHA alone effect. Only the two different CHA alone treatments were significantly different from the vehicle condition (*** p<0.001, K-S test).
Figure 2
Figure 3

- Top left: Graph showing the number of reinforcers per 60 minutes for vehicle and DPCPX 0.31 mg/kg.
- Top right: Graph showing the number of reinforcers per 60 minutes for different doses of DPCPX (mg/kg, ip).
- Bottom left: Graph showing the number of responses per 60 minutes for vehicle and DPCPX 0.31 mg/kg.
- Bottom right: Graph showing the number of responses per 60 minutes for different doses of DPCPX (mg/kg, ip).
Figure 5

The graph shows the relative frequency of IRT (in seconds) over time. Different conditions are represented by distinct lines and markers:
- **veh-veh**
- **CHA 0.0625 mg/kg ***
- **CHA + DCPCX 0.31 mg/kg**
- **CHA 0.0625 mg/kg ***

The x-axis represents IRT (seconds) ranging from 0 to 144, while the y-axis represents relative frequency ranging from 0.00 to 0.25.