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# **The Role of Dopamine D<sub>3</sub> Receptor Partial Agonism in Cariprazine-Induced Neurotransmitter Efflux in Rat Hippocampus and Nucleus Accumbens**

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**Running title:** Cariprazine increases dopamine and serotonin efflux

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**List of abbreviations:**

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; AAPD, atypical antipsychotic drug; ACh, acetylcholine; ANOVA, analysis of variance; AUC, area under the curve; CIAS, cognitive impairment associated with schizophrenia; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; dSTR, dorsal striatum; GABA, gamma--aminobutyric acid; Glu, glutamate; Gly, glycine;

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HIP, hippocampus; HVA, homovanillic acid; LSD, least significant difference; MK-801, dizocilpine; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; NE, norepinephrine; NMDAR, N-methyl-D-aspartate receptor; NOR, novel object recognition; PCP, phencyclidine; (+)-PD-128907 ([[(4aR, 10bR)-3, 4a, 4, 10b-tetrahydro-4-propyl-2H, 5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol hydrochloride]); PFC, prefrontal cortex; SB-277011A (trans-N-[4-[2-(6-cyano-1, 2, 3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide hydrochloride; SE, standard error; Ser, serine; STR, striatum; UPLC-MS/MS, ultra-performance liquid chromatography with tandem mass spectrometry.

**Recommended section:** Neuropharmacology

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## Abstract

Cariprazine is an approved antipsychotic and antidepressant which is a dopamine (DA) D<sub>3</sub>-preferring D<sub>3</sub>/D<sub>2</sub> receptor partial agonist, serotonin (5-HT) 5-HT<sub>1A</sub> receptor partial agonist, and 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> receptor antagonist, a profile unique for atypical antipsychotic drugs. The purpose of this study was to clarify the effects of cariprazine and selective D<sub>3</sub> receptor ligands on neurotransmitter efflux in the rat nucleus accumbens (NAC) and ventral hippocampus (HIP), brain regions important for reality testing, rewarded behavior, and cognition. *In vivo* microdialysis was performed in awake, freely moving rats after administration of cariprazine, (+)-PD-128907, a D<sub>3</sub> receptor-preferring agonist, and SB-277011A, a selective D<sub>3</sub> receptor antagonist, alone or combined, and extracellular levels of multiple neurotransmitters and metabolites were measured in the NAC and HIP by UPLC-MS/MS. Cariprazine increased DA, norepinephrine (NE), and 5-HT efflux in both regions, while it increased glycine (Gly) and glutamate (Glu) efflux only in the NAC, and DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) efflux, only in the HIP. Similarly, SB-277011A increased DA, NE, DOPAC, and HVA, but not 5-HT, efflux in the NAC and HIP, and ACh efflux in the HIP. Most of these effects of cariprazine and SB-277011A were fully or partially attenuated by the D<sub>3</sub> receptor agonist (+)-PD-128907, suggesting these effects of cariprazine, are related to its D<sub>3</sub> receptor partial agonism and that this mechanism, leading to diminished stimulation of D<sub>3</sub> receptors may contribute to its efficacy in both schizophrenia and bipolar disorder. The possible role of Gly in the action of cariprazine is discussed.

**Key words:** cariprazine, D<sub>3</sub> receptor partial agonism, neurotransmitter, antipsychotic, schizophrenia, bipolar disorder.

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### **Significance statement**

The novel atypical antipsychotic drug, cariprazine, increased nucleus accumbens and hippocampal neurotransmitter efflux, similar to the actions of the D<sub>3</sub> receptor antagonist, SB-277011A. The D<sub>3</sub> receptor-preferring agonist, (+)-PD-128907, diminished the effects of both compounds on neurotransmitter efflux in both regions. These results suggested D<sub>3</sub> receptor partial agonist activity of cariprazine, producing functional antagonism, may contribute to its efficacy in schizophrenia and bipolar disorder.

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## Introduction

Cariprazine (US: Vraylar<sup>®</sup>; Europe: Reagila<sup>®</sup>), an orally active and potent dopamine (DA) D<sub>3</sub>-preferring D<sub>3</sub>/D<sub>2</sub> receptor partial agonist, is a novel atypical antipsychotic drug (AAPD) approved to treat adults with schizophrenia and bipolar I disorder (De Deurwaerdère, 2016; Garnock-Jones, 2017; Stahl, 2016). The DA D<sub>3</sub> receptor, through pre- and postsynaptic action in brain areas, has been suggested to play a key role in depression and negative symptoms (Leggio et al., 2013). Most of the approved AAPDs are more potent serotonin 5-HT<sub>2A</sub> receptor antagonists than DA D<sub>2</sub> receptor antagonists at clinically-effective doses, with additional direct or indirect actions on 5-HT<sub>1A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>, D<sub>1</sub>, D<sub>3</sub>, D<sub>4</sub>, adrenergic, histaminergic, glutamatergic, and muscarinic or nicotinic receptors (Meltzer and Huang, 2008). These, and other indirect effects, may result in increased 5-HT, DA, norepinephrine (NE), acetylcholine (ACh) or glutamate (Glu) efflux (Huang et al., 2014; Kuroki et al., 1999; Meltzer and Huang, 2008), as well as neurotrophin, e.g., brain-derived neurotrophic factor, efflux (Angelucci et al. 2004; Leggio et al., 2013), at multiple sites. The combined effects of these actions may be the basis for their ability to treat psychosis, negative symptoms, cognitive dysfunction, and mood disturbances (Meltzer and Huang, 2008; Meltzer, 2015; Nucifora et al., 2017). The main actions of cariprazine are DA D<sub>3</sub> and D<sub>2</sub> receptor partial agonism, with approximately 10-fold greater binding affinity for the D<sub>3</sub> receptor (pK<sub>i</sub> 10.07 and 9.16-9.31 for human D<sub>3</sub> and D<sub>2L-S</sub> receptors, respectively; Kiss et al., 2010).

Cariprazine is also a potent partial agonist at the 5-HT<sub>1A</sub> receptor (pK<sub>i</sub> 8.59 and 8.34 for human and rat 5-HT<sub>1A</sub> receptors, respectively), an antagonist at the 5-HT<sub>2B</sub> receptor (pK<sub>i</sub> 9.24 for human 5-HT<sub>2B</sub> receptor), and 5-HT<sub>2A</sub> receptor with a moderate binding affinity (pK<sub>i</sub> 7.73 for human 5-

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HT<sub>2A</sub> receptor) (Kiss et al., 2010). 5-HT<sub>2A</sub> receptor antagonism may contribute to its low burden of extrapyramidal side effects and ability to improve cognition (Meltzer, 2015).

The pharmacologic action of cariprazine receiving the greatest attention is its potent partial agonism activity at the D<sub>3</sub> receptor (Kiss et al., 2010; Tadori et al., 2011). D<sub>3</sub> receptors are predominantly located in the nucleus accumbens (NAC), with lower expression in the thalamus, hippocampus (HIP) and cortex, areas important to the development of psychotic, and negative symptoms and the cognitive deficit associated with schizophrenia (CIAS). They are both pre- and post-synaptical (Centonze et al., 2003; Maramai et al., 2016; Sokoloff et al., 2013). In preclinical studies, DA D<sub>3</sub> receptor blockade enhanced, while D<sub>3</sub> receptor agonism impaired learning, memory, attention, speed of processing, social recognition, and executive function (Leggio et al., 2013; 2016; Magnard et al., 2016; Maramai et al., 2016; Pich and Collo, 2015; Zimnisky et al., 2013).

Indicative of its antipsychotic-like activity, cariprazine inhibited the locomotor-stimulating effects of the noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonists, including dizocilpine (MK-801) and phencyclidine (PCP) (Gyertyán et al., 2011). Cariprazine also ameliorated the deficits in animal models of CIAS (Barnes et al., 2018; Neill et al., 2016; Rajagopal et al., 2014; Watson et al., 2016). The ability of cariprazine to attenuate PCP-induced disruption in memory was significantly diminished in DA D<sub>3</sub> receptor knockout mice (Zimnisky et al., 2013).

In microdialysis studies, AAPDs that are more potent 5-HT<sub>2A</sub> than DA D<sub>2</sub> receptor antagonists, e.g., clozapine, olanzapine, risperidone, and lurasidone, increase prefrontal cortical (PFC) and HIP ACh and DA efflux and striatal (STR) DA efflux, and have variable effects on Glu efflux

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(Huang et al., 2014; López-Gil et al., 2010). These AAPDs suppress MK-801- or PCP-induced Glu or 5-HT efflux (Gobert et al., 1996; Huang et al., 2014; 2015; López-Gil et al., 2007; 2010). DA D<sub>3</sub> receptor full antagonists or partial agonists with low intrinsic activity would be expected to function as antagonists when synaptic DA concentrations are elevated. This effect has been suggested to enhance cognition via promoting PFC ACh release, enhancing the release of DA in the STR and PFC, or activating cAMP-response-element binding protein signaling in the HIP, whereas D<sub>3</sub> receptor agonists would have the opposite effect (Huang et al., 2015; Nakajima et al., 2013). In support of this, we reported that the selective D<sub>3</sub> receptor antagonist NGB-2904 increased PFC DA and ACh and STR DA efflux (Huang et al., 2015). This study aimed to compare the effects of cariprazine and the selective DA D<sub>3</sub> receptor antagonist SB-277011A, in the absence and presence of the DA D<sub>3</sub> receptor-preferring agonist (+)-PD-128907 on neurotransmitter efflux in the ventral HIP and NAC.

## **Experimental procedures**

### **Animals and Drugs**

Male Sprague-Dawley rats (250-300 g; Harlan Laboratories, Indianapolis, IN) were used throughout the study. They were housed 3-4 per cage in a controlled 14:10-hour light-dark cycle with free access to food and water. Cariprazine (provided by Allergan, NJ, USA) was dissolved in 0.5% methylcellulose and 0.2% Tween 80 solution and was administered p.o., whereas (+)-PD-128907 ([4aR, 10bR)-3, 4a, 4, 10b-tetrahydro-4-propyl-2H, 5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol hydrochloride]) and SB-277011A (trans-N-[4-[2-(6-cyano-1, 2, 3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide hydrochloride; Tocris, Ellisville, MO) was prepared in aqueous solution and administered i.p. The vehicle (data for

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Vehicle in Fig. 1 and Vehicle+Vehicle in Fig. 2 and Fig. 3 are from the same group of animals: vehicle for (+)-PD-128907 i.p. + vehicle for cariprazine p.o.) or drugs were administered in a volume of 1.0 mL/kg to randomly assigned rats (N=8 per group).

### **Surgery and microdialysis**

The rats were anesthetized with 2% isoflurane (Isothesia, Butler Schein, Dublin, OH). A stainless-steel guide cannula (21 G) with dummy probes was placed and fixed by cranioplastic cement to the NAC and HIP. Relative to the bregma, the stereotaxic coordinate of the implanted probe was A +2.0, L -1.4, V -8.0 mm for the NAC, and A -5.6, L + 5.0, V -7.5 mm for the HIP with an incision bar level of -3.0 mm, according to the atlas (Paxinos and Watson, 1998).

Approximately 2-3 days after cannulation, anesthetized animals were implanted with concentric-shaped dual dialysis probes having 2.0 mm of membrane surface (Synaptech Co., Marquette, MI). Rats were then housed individually in dialysis cages with overnight perfusion (0.2  $\mu$ L/min) of the probes with Dulbecco's phosphate-buffered saline (Sigma, St. Louis, MO), including Ca<sup>2+</sup> (138 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 0.5 mM MgCl<sub>2</sub>, 1.2 mM CaCl<sub>2</sub>, pH=7.4). In the morning on the day of microdialysis, the perfusion rate was increased to 1.2  $\mu$ L/min, and samples were collected every 30 min after a 1-hour stabilization period to achieve stable baseline values. The rats were then administered vehicle, cariprazine (0.03, 0.1, 0.3 mg/kg, p.o.), or SB-277011A (10 mg/kg, i.p.), and/or (+)-PD-128907 (0.03 mg/kg, i.p.). The doses of SB-277011A and (+)-PD-128907 were selected based on previous reports (Lacroix et al., 2006; Huang et al., 2015; Zapata et al., 2001). A 30-min interval was maintained for the co-administration. The effects of the drug(s) on extracellular neurotransmitter and metabolite levels were monitored for an additional 180 min after the second injection. Extracellular neurotransmitter levels of ACh; DA; 5-HT; NE; metabolites of DA,

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namely, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA); metabolite of 5-HT, namely, 5-hydroxy-indole acetic acid (5-HIAA); glycine (Gly); serine (Ser); Glu; and gamma-aminobutyric acid (GABA) were measured by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) (Huang et al., 2014). The procedures applied in this experiment were approved by the Institutional Animal Care and Use Committee of Northwestern University, Chicago, IL, USA.

### **Data analysis**

Only results derived from healthy rats with correctly positioned dialysis probes were used in the data analysis. One rat in the (+)-PD-128907+Cariprazine group was eliminated. Baseline levels, measured for 90 min prior to any drug administration, were averaged and normalized to 100%. Effect of the drugs on extracellular neurotransmitter and metabolite levels are expressed relative to baseline in time–response curves. Area under the curve (AUC) from 0 to 180 min was calculated and analyzed by analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) post hoc tests. Two-way ANOVA was used for the pretreatment and treatment interaction on neurotransmitter efflux. Repeated-measure ANOVA was used for the time-point difference in the time-response curve. Results are presented as mean  $\pm$  standard error (SE), and  $P < 0.05$  was considered significant.

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## Results:

Table 1 summarizes the mean basal neurotransmitter levels in study rats (N=63). There were no significant differences among the treatment groups. N=8 per group, with the exception of N=7 for (+)-PD-128907+Cariprazine group.

Cariprazine enhanced DA, 5-HT, and NE efflux in both the NAC (Fig. 1A) and HIP (Fig. 1B). Cariprazine, 0.1 mg/kg, but not 0.03 mg/kg (p.o.), significantly increased DA ( $P=0.001$ ; Fig. 1C), NE ( $P=0.040$ ), 5-HT ( $P=0.035$ ; Fig. 1D), and Gly ( $P=0.021$ ) efflux in the NAC. Cariprazine also increased DA ( $P=0.001$ ; Fig. 1E), 5-HT ( $P=0.006$ ; Fig. 1F), DOPAC ( $P=0.027$ ), and HVA ( $P=0.025$ ) efflux in the HIP. Cariprazine 0.3 mg/kg increased DA ( $***P<0.001$ ; Fig. 1C), NE ( $P=0.003$ ), Gly ( $P=0.026$ ), and Glu ( $P=0.021$ ) efflux in the NAC, and HIP DA ( $P=0.004$ ; Fig. 1E) and NE ( $P=0.025$ ), as well as DOPAC ( $P=0.002$ ) efflux. Cariprazine had no effect on ACh, Ser, or GABA efflux in either region.

The DA  $D_3$  receptor antagonist SB-277011A (10 mg/kg, i.p.) significantly increased DA ( $P<0.001$ ; Fig. 2D), NE ( $P=0.003$ ), DOPAC ( $P=0.003$ ), HVA ( $P=0.001$ ), 5-HIAA ( $P=0.018$ ), Gly ( $P=0.039$ ), and Glu ( $P=0.016$ ) efflux in the NAC (Fig. 2A), as well as ACh ( $P=0.007$ ; Fig. 2E), DA ( $P<0.001$ ; Fig. 2F), NE ( $P<0.001$ ), DOPAC ( $P<0.001$ ), HVA ( $P=0.026$ ), and 5-HIAA ( $P=0.005$ ) efflux in the HIP (Fig. 2B).

The DA  $D_3$  receptor-preferring agonist (+)-PD-128907 (0.03 mg/kg, i.p.) slightly decreased HIP NE ( $P=0.022$ ) efflux, with no effect on other neurotransmitters. (+)-PD-128907 partially, but significantly, attenuated the SB-277011A-induced increase on DA efflux in the NAC ( $P<0.001$ ; Fig. 2A) and HIP ( $P=0.004$ ; Fig. 2B), and NE efflux in the NAC ( $P=0.022$ ; Fig. 2A) and HIP ( $P=0.001$ ; Fig. 2B). Two-way ANOVA demonstrated significant interactions between (+)-PD-

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128907 and SB-277011A in NAC DA ( $P=0.003$ ), DOPAC ( $P=0.011$ ), and 5-HIAA ( $P=0.037$ ) efflux and HIP DOPAC efflux ( $P=0.050$ ). Based on these studies with selective ligands, it was observed that DA D<sub>3</sub> receptor antagonism stimulated both NAC and HIP DA and NE, but not 5-HT, efflux. (+)-PD-128907 partially but significantly attenuated the effects of cariprazine (0.1 mg/kg) on DA efflux ( $P=0.033$ ) in the NAC (Fig. 3A), and NE efflux ( $P=0.009$ ) in the HIP (Fig. 3B), consistent with the conclusion that the actions of cariprazine on DA and NE efflux were due, at least in part, to its D<sub>3</sub> receptor partial agonist activity. Table 2 summarizes the results for the neurotransmitter efflux in the NAC and HIP.

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## Discussion

The major finding of this study is that cariprazine increased DA, NE, 5-HT, Glu, and Gly efflux in the rat NAC or HIP, or both, as did the DA D<sub>3</sub> receptor antagonist, SB-277011A. These increases were attenuated by the D<sub>3</sub> receptor-preferring agonist (+)-PD-128907, providing further support that the antipsychotic and antidepressant effects of cariprazine, which may be based on its action to modulate dopaminergic, noradrenergic, serotonergic and glutamatergic activity, are significantly dependent on DA D<sub>3</sub> receptor blockade. No effects on ACh, Ser, or GABA efflux were detected after either cariprazine, SB-277011A, or (+)-PD-128907.

The present data showed that cariprazine, like other AAPDs, increased NAC and HIP DA efflux (Huang et al., 2014; Meltzer and Huang, 2008). Cariprazine enhanced the turnover of DA and slightly reduced 5-HT turnover in the mouse STR, and frontal cortex, after oral administration (Kiss et al., 2010). Acute oral (0.05, 0.2, or 0.8 mg/kg) treatment with cariprazine dose-dependently attenuated the PCP-induced Glu, DA, NE, and 5-HT efflux in the medial PFC (mPFC), while by itself (only 0.2 mg/kg tested), had no effect on efflux of any of these neurotransmitters (Kehr et al., 2018). Other AAPDs normalize the activity of principal cortical neurons following their activation by the NMDAR antagonists, PCP or MK-801, which enhance the activity of parvalbumin-positive GABAergic interneurons in the mPFC. The AAPDs prevent excessive activation of these neurons (Amargós-Bosch et al., 2006; Carli et al., 2011; López-Gil et al., 2007; 2010).

Aripiprazole, a widely used AAPD, like cariprazine, is also a DA D<sub>2</sub>/D<sub>3</sub> receptor partial agonist, which increases DA efflux in the HIP, but not the NAC (Li et al., 2004). However, cariprazine has much greater affinity and selectivity for D<sub>3</sub> versus D<sub>2</sub> receptors compared to other AAPDs, including aripiprazole (3- to 10-fold greater D<sub>3</sub> versus D<sub>2</sub> selectivity; Kiss et al. 2010). Further, at

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antipsychotic-like effective doses (around 0.1 to 1 mg/kg, orally in rats), cariprazine occupies both DA D<sub>3</sub> and D<sub>2</sub> receptors, whereas aripiprazole occupies D<sub>2</sub> but not D<sub>3</sub> receptors to a significant level (Gyertyán et al., 2011). The DA D<sub>3</sub> receptor-preferring agonist (+)-PD-128907 attenuated the effect of the D<sub>3</sub> receptor antagonist, SB-277011A, on rat HIP ACh efflux. We previously reported that the D<sub>3</sub> receptor antagonist, NGB-2904, increased DA and ACh efflux in mouse mPFC and dorsal STR (dSTR; Huang et al., 2015). SB-277011A and U99194, both D<sub>3</sub> receptor antagonists, increase rat cortical ACh efflux (Barth et al., 2013; Lacroix et al., 2006). On the other hand, (+)-PD-128907 has been reported to decrease DA efflux in the NAC and PFC (Gobert et al., 1996; Pugsley et al., 1995; Zapata and Shippenberg, 2005). Typical antipsychotic drugs, including S(-)-sulpiride and haloperidol, which are D<sub>2</sub> receptor antagonists, increase DA efflux in the NAC (Huang et al., 2014; Kuroki et al., 1999). Selective DA D<sub>3</sub> receptor antagonists, such as SB-277011A studied here, also increased DA efflux in the NAC, as well as the HIP. Therefore, the D<sub>2</sub>, as well as the D<sub>3</sub> receptor, actions of cariprazine may contribute to increased DA efflux in the regions studied (Huang et al., 2014; Kuroki et al., 1999). However, at the dose (0.03 mg/kg, i.p.) in the present study, (+)-PD-128907 is more likely to be selective for D<sub>3</sub> compared to D<sub>2</sub> receptors. This dose of (+)-PD-128907 was reported to slightly decrease (about 20%) NAC DA release in wild-type but not D<sub>3</sub> receptor knockout mice (Pugsley et al., 1995; Zapata et al., 2001). Moreover, the effect in rats of (+)-PD-128907, at a dose of 0.2 mg/kg, s.c., on prepulse inhibition, was blocked by the selective D<sub>3</sub> receptor antagonists, SB-277011 and A-691990, but not by the D<sub>2</sub> receptor antagonist, haloperidol (Zhang et al., 2007). AAPDs, including olanzapine and lurasidone, which are more potent 5-HT<sub>2A</sub> receptor antagonists than D<sub>2</sub> receptor antagonists, also increased cortical ACh efflux in mice (Huang et al., 2014). Blonanserin, is another potent D<sub>2</sub> and D<sub>3</sub> receptor antagonist AAPD (Baba et al., 2015). Neither

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blonanserin, in mice, nor cariprazine, in rats, enhanced HIP ACh efflux (Huang et al., 2015; and present data), which may result from their limited efficacy as direct or indirect 5-HT<sub>1A</sub> receptor partial agonists (Huang et al., 2014; Ichikawa et al., 2002). However, inability to enhance ACh efflux in the HIP did not preclude the efficacy of cariprazine to restore novel object recognition (NOR) and other cognitive deficits in rodents treated with subchronic PCP or MK-801 (Neill et al., 2016; Watson et al., 2016).

The effect of cariprazine, 0.1 and 0.3 mg/kg, on neurotransmitter efflux in the HIP did not significantly differ from each other, although the higher dose would be expected to occupy a significantly higher proportion of HIP DA D<sub>2</sub> receptors. Bilateral microinjection of the D<sub>3</sub> receptor antagonist, S33084, into the rat PFC caused a dose-related improvement in NOR; on the other hand, bilateral microinjection of the D<sub>2</sub> receptor antagonist, L741626, caused a dose-related impairment of NOR, suggesting that blockade of D<sub>3</sub> receptors enhances NOR, whereas antagonism of D<sub>2</sub> receptor impairs cognition (Watson et al., 2012).

Extensive evidence supports role of glutamatergic and GABAergic neurotransmission to the development of CIAS. Cariprazine, 0.3 but not 0.1 mg/kg, slightly but significantly increased Glu efflux in the NAC. Because we only showed the effect of (+)-PD-128907 pretreatment with cariprazine, 0.1 mg/kg, we were unable to determine if the Glu efflux could be attenuated by (+)-PD-128907. However, the D<sub>3</sub> receptor antagonist SB-277011A significantly increased Glu efflux in the NAC, and this effect was significantly attenuated by (+)-PD-128907. It seems likely that the increase in Glu in the NAC induced by cariprazine and SB-277011A resulted from functional antagonist activity at the DA D<sub>3</sub> receptor. However, S33138, a preferential D<sub>3</sub> versus D<sub>2</sub> receptor antagonist, failed to affect cortical Glu, Gly, or GABA efflux (Millan et al., 2008). The small effects of cariprazine and SB-277011A on NAC Glu efflux contrasts with those of lurasidone,

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which produces greater increase in the NAC and mPFC (Huang et al., 2014). Other AAPDs have shown variable effects on Glu efflux (Carli et al., 2011; Huang et al., 2014; López-Gil et al., 2007; 2010). Moreover, selective D<sub>3</sub> receptor partial agonists and antagonists have been reported to prevent the locomotor-stimulating effects of MK-801, suggesting that the antipsychotic-like effect of DA D<sub>3</sub> receptor blockers might result from normalizing Glu function, by modulating the release of Glu (Leriché et al., 2003; Sokoloff et al., 2013). The D<sub>3</sub>/D<sub>2</sub> receptor agonist, quinolorane, was reported to selectively decrease dialysate GABA levels in NAC core (Hemmati et al., 2001). Activation of DA D<sub>3</sub> receptors can modulate GABA<sub>A</sub> receptor endocytosis and suppress synaptic GABAergic transmission in the HIP and NAC (Chen et al., 2006; Kohnomi and Konishi, 2015; Swant et al., 2008). We noted no effect of (+)-PD-128907 on GABA efflux in either the NAC or HIP. Further study is indicated to clarify effect of cariprazine on synaptic GABAergic transmission, as there is evidence that decreased GABAergic activity may contribute to the development of CIAS (Tse et al., 2015).

An interesting finding of the present study was augmented Gly efflux in the NAC in response to cariprazine 0.1 and 0.3 mg/kg, and SB-277011A 10 mg/kg. (+)-PD-128907 alone was without effect on Gly. The increases in Gly efflux following cariprazine and SB-277011A, in the NAC, were reversed by (+)-PD-128907, indicating the elevation was resulting from D<sub>3</sub> receptor inhibition. Gly or D-serine, are necessary co-agonists, with Glu, to activate the NMDAR (Benveniste and Mayer, 1991). Activation of NMDAR in the NAC contributes to the induction of long-term potentiation (Schotanus and Chergui, 2008), which may be the basis for some of the beneficial effects of cariprazine in both psychosis and mood disorders. There is evidence that additional Gly in the NAC may be needed for optimization of rewarded behaviors (Saul'skava and Solov'eva 2003). Administration of oral Gly as an adjunct to antipsychotic drug treatment,

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may be effective to improve negative symptoms (Coyle and Tsai, 2004; Heresco-Levy et al. 2004). Further study of the importance of Gly release to the action of cariprazine is indicated.

In summary, the effects of the DA D<sub>3</sub> receptor-preferring partial agonist, cariprazine, parallel to those produced by the selective D<sub>3</sub> receptor antagonist SB-277011A, with regard to DA and NE efflux in the NAC and HIP. Further, significant smaller increases by both cariprazine and SB-277011A, in Gly and Glu efflux were found in the NAC. These effects of cariprazine and SB-277011A were partially or fully reversed by the D<sub>3</sub> receptor-preferring agonist (+)-PD-128907, tested at a dose that predominantly impacts D<sub>3</sub> receptors. These data indicate that D<sub>3</sub> receptor mechanism contributes to the action of cariprazine in modulating the release of key neurotransmitters in the NAC and HIP, which are implicated in cognitive and reward functions. The increased DA, NE, Gly, and Glu efflux may be mediated by disinhibition of GABA interneurons produced by DA D<sub>3</sub> receptor blockade (Diaz et al., 2011). These findings support the evidence that modulatory actions of cariprazine on monoaminergic transmission mediated by D<sub>3</sub> receptor antagonism may contribute to its pro-cognitive, antipsychotic, and antidepressive effects.

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### **Conflicts of interest**

H.Y. Meltzer has been consulting for and receives research funding from Allergan. B. Kiss and B. Farkas are employees of Gedeon Richter Plc. N. Adham is an employee of Allergan. M. Huang and W. He have no conflicts of interest to declare.

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**Contributions:**

Participated in research design: H.Y. Meltzer, M. Huang, N. Adham, B. Kiss, B. Farkas.

Conducted experiments: M. Huang and W. He

Performed data analysis: M. Huang and W. He

Wrote or contributed to the writing of the manuscript: All authors

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## Footnotes

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**Meeting abstracts/Previous presentations:** Meltzer HY, Huang M, He W, Kiss B, Farkas B, Adham N. Cariprazine enhances monoaminergic activity in the hippocampus and ventral striatum of rats: a possible basis for its antipsychotic effect. SOBP (Society of Biological Psychiatry) 73<sup>rd</sup> Annual Meeting, May 10-12, 2018, Hilton Midtown, New York, NY, USA.

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## Figure legends

**Fig. 1.** Effects of cariprazine on the extracellular levels of neurotransmitters and metabolites in the rat NAC and HIP. AUC values in A and B. N=8 per group. Cariprazine was administered at the 0 min time point. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  vs vehicle; one-way ANOVA-LSD.

Cariprazine 0.1 mg/kg, but not 0.03 mg/kg (p.o.), significantly increased DA ( $P=0.001$ ; C), NE ( $P=0.040$ ), 5-HT ( $P=0.035$ ; D), and Gly ( $P=0.021$ ) efflux in the NAC; cariprazine also increased DA ( $P=0.001$ ; E), 5-HT ( $P=0.006$ ; F), DOPAC ( $P=0.027$ ), and HVA ( $P=0.025$ ) efflux in the HIP. Cariprazine 0.3 mg/kg increased DA ( $P<0.001$ ; C), NE ( $P=0.003$ ), Gly ( $P=0.026$ ), and Glu ( $P=0.021$ ) efflux in the NAC. It also increased HIP DA ( $P=0.004$ ; E) and NE ( $P=0.025$ ) as well as DOPAC ( $P=0.002$ ) efflux. Cariprazine had no effect on ACh, Ser, or GABA efflux in either region.

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; ANOVA, analysis of variance; AUC, area under the curve; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HIP, hippocampus; HVA, homovanillic acid; LSD, least significant difference; NAC, nucleus accumbens; NE, norepinephrine; p.o., oral administration; Ser, serine.

**Fig. 2.** Effect of SB-277011A and (+)-PD-128907 on neurotransmitter levels in the rat NAC and HIP. AUC values in A and B. N=8 per group. SB-277011A was administered at the 0 min time point, and (+)-PD-128907 was administered at the -30 min time point. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  vs vehicle; + $P<0.05$ , ++ $P<0.01$ , +++ $P<0.001$  vs SB-277011A in one-way ANOVA-LSD. The DA D3 receptor antagonist SB-277011A (10 mg/kg, i.p.) significantly increased DA ( $P<0.001$ ; D), NE ( $P=0.003$ ), DOPAC ( $P=0.003$ ), HVA ( $P=0.001$ ), 5-HIAA ( $P=0.018$ ), Gly ( $P=0.039$ ), and Glu ( $P=0.016$ ) efflux in the NAC (A), as well as ACh ( $P=0.007$ ; E), DA

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( $P < 0.001$ ; F), NE ( $P < 0.001$ ), DOPAC ( $P < 0.001$ ), HVA ( $P = 0.026$ ), and 5-HIAA ( $P = 0.005$ ) efflux in the HIP (B). The DA D3 receptor agonist (+)-PD-128907 (0.03 mg/kg, i.p.) slightly decreased HIP NE ( $P = 0.022$ ) efflux, with no effect on other neurotransmitters. (+)-PD-128907 partially, but significantly, blocked the SB-277011A-induced increase on DA efflux in the NAC ( $P < 0.001$ ; A) and HIP ( $P = 0.004$ ; B), and NE efflux in the NAC ( $P = 0.022$ ; A) and HIP ( $P = 0.001$ ; B).

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; ANOVA, analysis of variance; AUC, area under the curve; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HIP, hippocampus; HVA, homovanillic acid; i.p., intraperitoneal; LSD, least significant difference; NAC, nucleus accumbens; NE, norepinephrine; Ser, serine.

**Fig. 3.** Effect of cariprazine and (+)-PD-128907 on neurotransmitter levels in the rat NAC and HIP. AUC values in A and B.  $N = 8$  per group, except  $N = 7$  for (+)-PD-128907+Cariprazine group. Cariprazine was administered at the 0 min time point, and (+)-PD-128907 was administered at the -30 min time point. \* $P < 0.05$ , \*\* $P < 0.01$  vs vehicle; + $P < 0.05$ , ++ $P < 0.01$  vs cariprazine in one-way ANOVA-LSD. (+)-PD-128907 partially but significantly blocked the effects of cariprazine (0.1 mg/kg) on DA efflux ( $P = 0.033$ ) in the NAC (A), and NE efflux ( $P = 0.009$ ) in the HIP (B).

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; ANOVA, analysis of variance; AUC, area under the curve; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HIP, hippocampus; HVA, homovanillic acid; i.p., intraperitoneal; LSD, least significant difference; NAC, nucleus accumbens; NE, norepinephrine; Ser, serine.

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**Table 1. Basal levels of neurotransmitters in dialysates (nM, Mean  $\pm$  SE; N=63)**

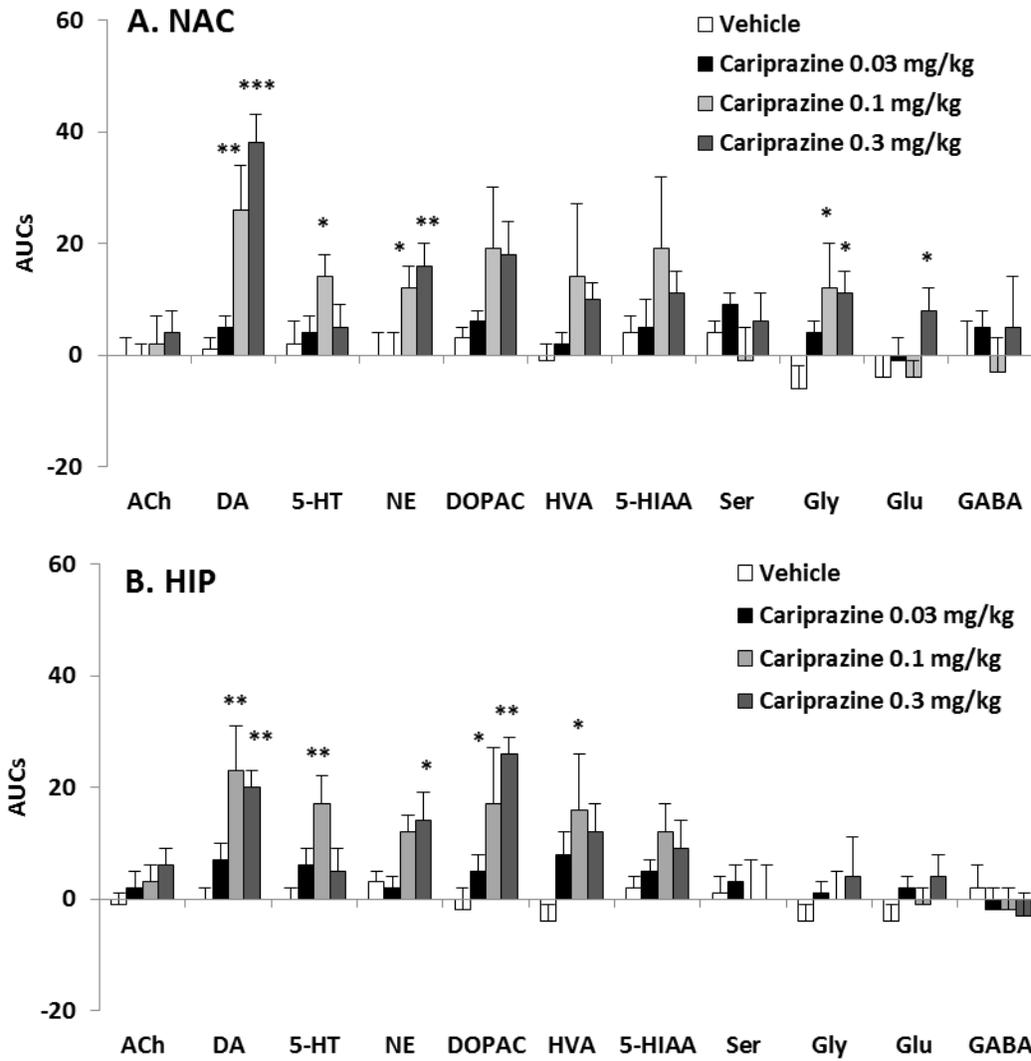
	NAC	HIP
ACh	1.37 $\pm$ 0.05	1.36 $\pm$ 0.06
DA	1.65 $\pm$ 0.06	1.11 $\pm$ 0.07
5-HT	1.22 $\pm$ 0.05	1.23 $\pm$ 0.05
NE	2.15 $\pm$ 0.10	2.08 $\pm$ 0.08
DOPAC	3467.55 $\pm$ 136.54	293.64 $\pm$ 15.32
HVA	2814.42 $\pm$ 82.48	261.45 $\pm$ 10.41
5-HIAA	3026.48 $\pm$ 131.14	2776.67 $\pm$ 132.42
Ser	5362.21 $\pm$ 198.10	5577.61 $\pm$ 244.77
Gly	1308.44 $\pm$ 56.22	2564.08 $\pm$ 118.66
Glu	1357.62 $\pm$ 61.57	1197.97 $\pm$ 51.43
GABA	131.77 $\pm$ 4.68	158.77 $\pm$ 8.06

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HIP, hippocampus; HVA, homovanillic acid; NAC, nucleus accumbens; NE, norepinephrine; SE, standard error; Ser, serine.

**Table 2. Summary of the effects of the tested compounds on neurotransmitter efflux (-: no effect; I: increase; D: decrease)**

	ACh	DA	5-HT	NE	DOPAC	HVA	5-HIAA	Ser	Gly	Glu	GABA
<b>NAC</b>											
Cariprazine 0.03 mg/kg	-	-	-	-	-	-	-	-	-	-	-
Cariprazine 0.1 mg/kg	-	I	I	I	-	-	-	-	I	-	-
Cariprazine 0.3 mg/kg	-	I	-	I	-	-	-	-	I	I	-
(+)-PD-128907 0.03 mg/kg	-	-	-	-	-	-	-	-	-	-	-
SB-277011A 10 mg/kg	-	I	-	I	I	I	I	-	I	I	-
(+)-PD-128907 + SB-277011A 0.03 mg/kg +10 mg/kg	-	I	-	-	-	-	-	-	-	-	-
(+)-PD-128907 + Cariprazine 0.03 mg/kg + 0.1 mg/kg	-	-	-	-	-	-	-	-	-	-	-
<b>HIP</b>											
Cariprazine 0.03 mg/kg	-	-	-	-	-	-	-	-	-	-	-
Cariprazine 0.1 mg/kg	-	I	I	-	I	I	-	-	-	-	-
Cariprazine 0.3 mg/kg	-	I	-	I	I	-	-	-	-	-	-
(+)-PD-128907 0.03 mg/kg	-	-	-	D	-	-	-	-	-	-	-
SB-277011A 10 mg/kg	I	I	-	I	I	I	I	-	-	-	D
(+)-PD-128907 + SB-277011A 0.03 mg/kg + 10 mg/kg	I	I	-	-	-	I	-	-	-	-	-
(+)-PD-128907 + Cariprazine 0.03 mg/kg + 0.1 mg/kg	-	-	-	-	-	-	-	-	-	-	-

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gly, glycine; HIP, hippocampus; HVA, homovanillic acid; NAC, nucleus accumbens; NE, norepinephrine; Ser, serine.



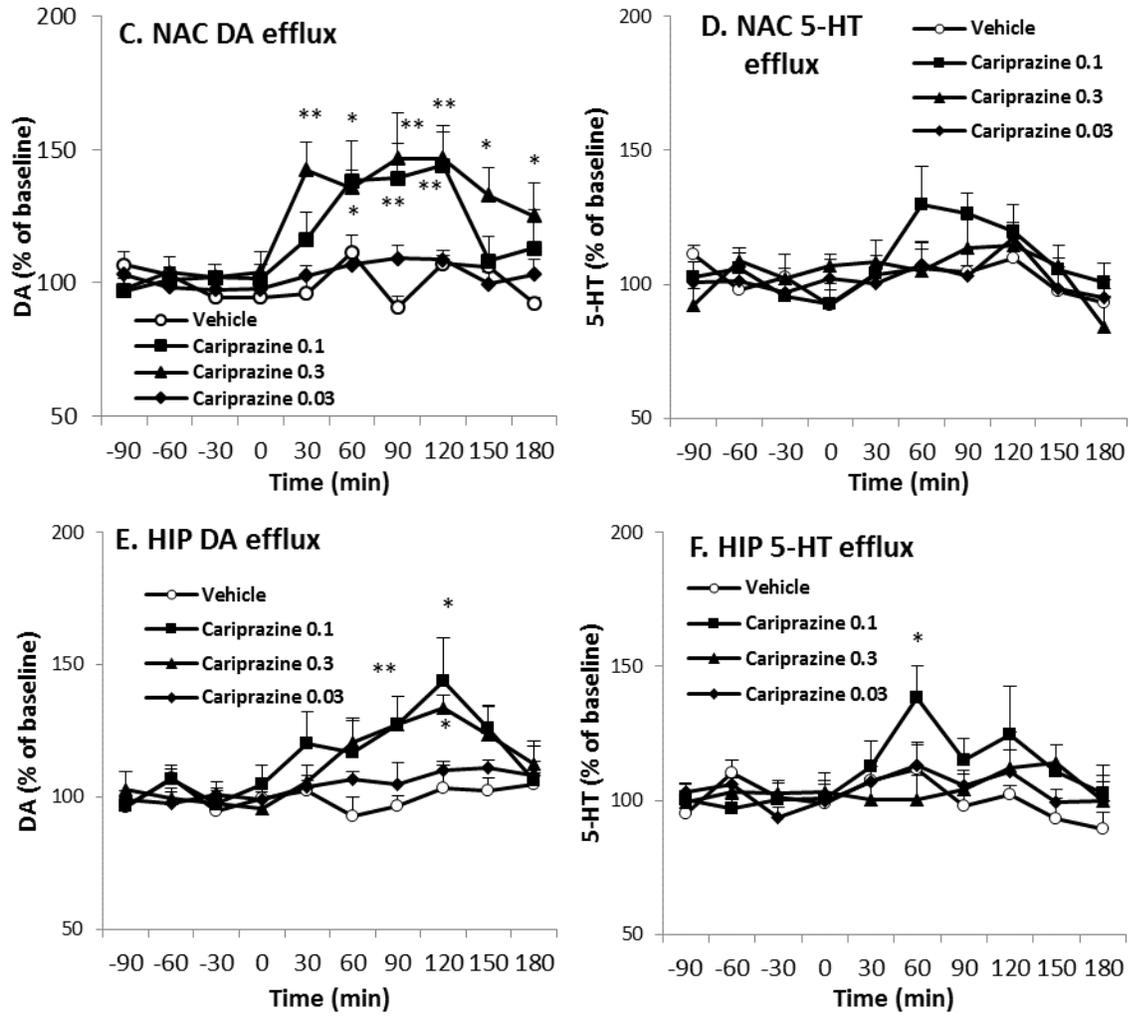
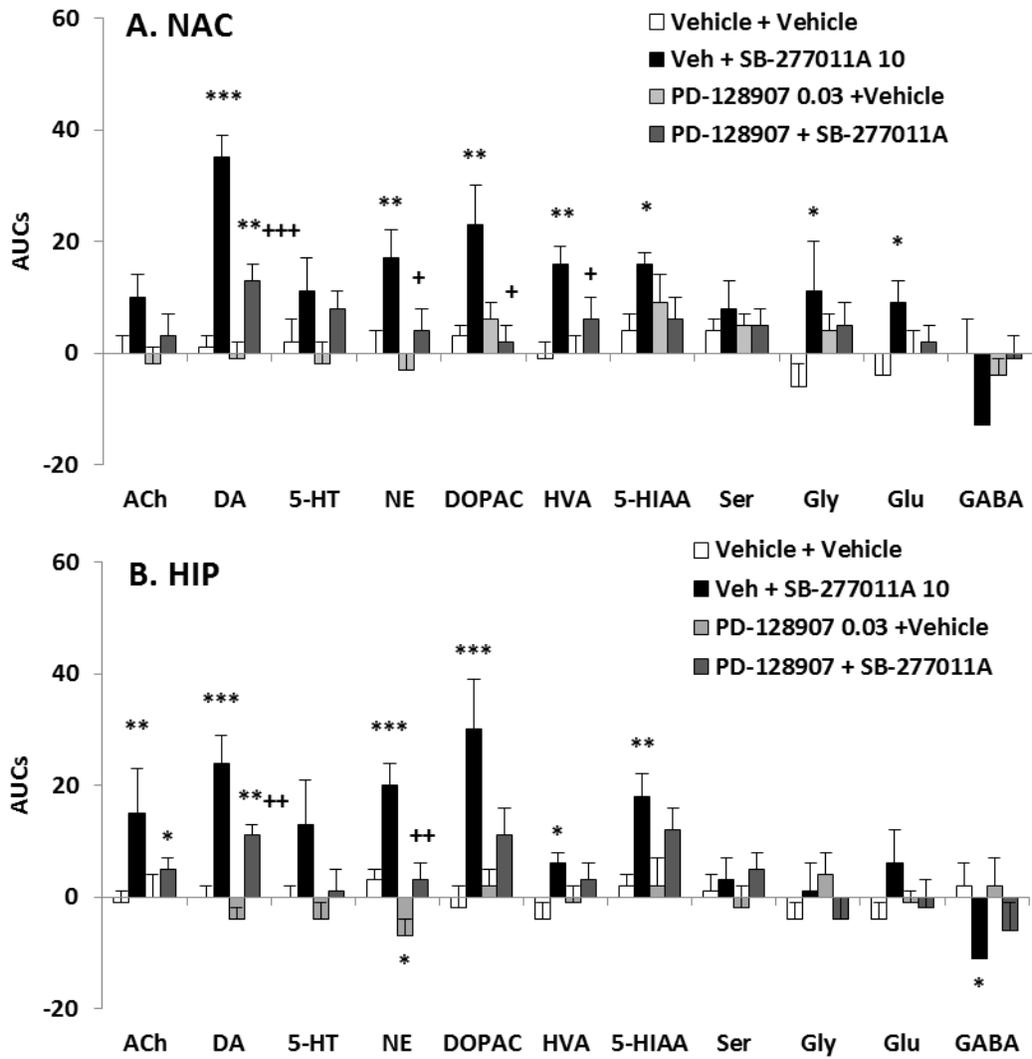


Figure 1.



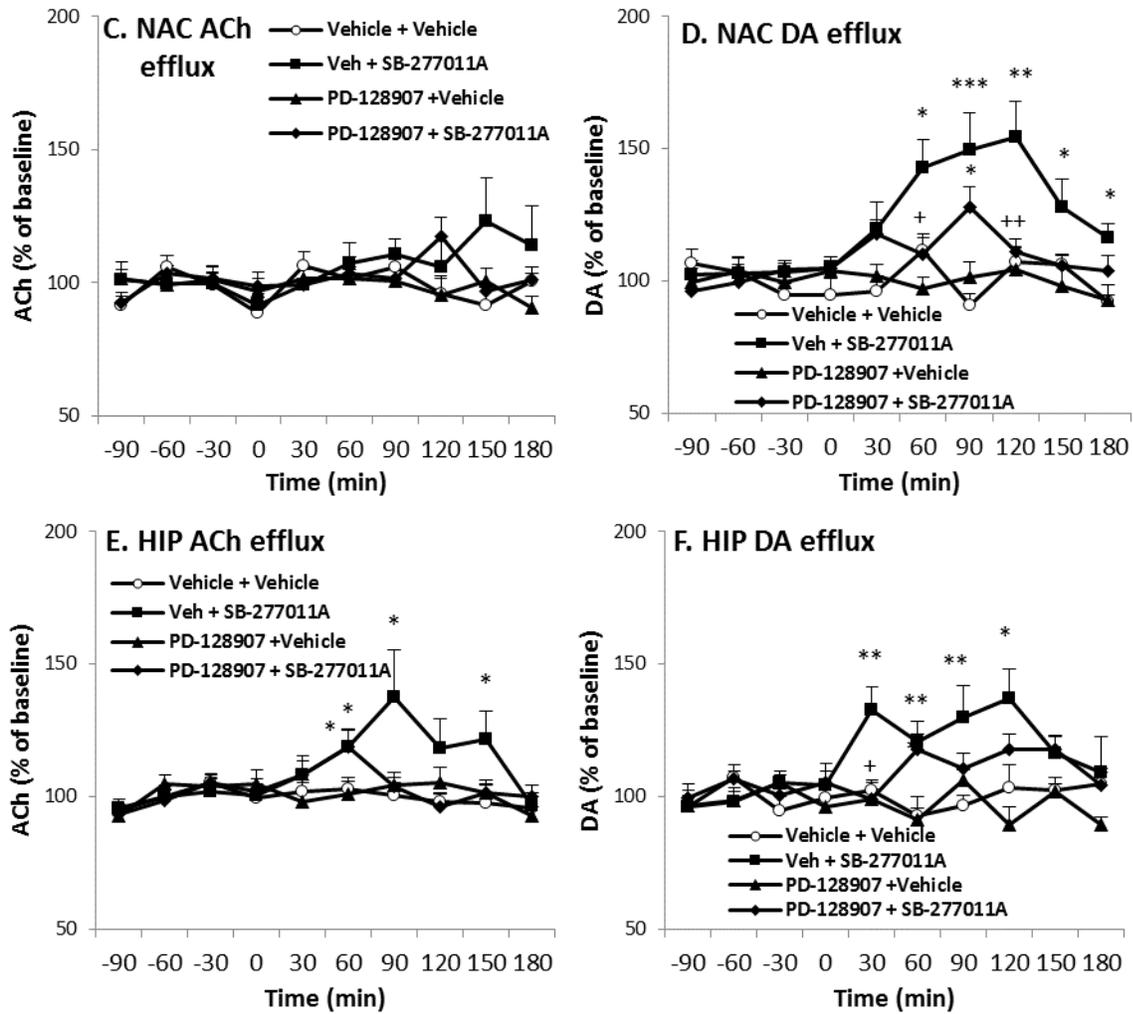
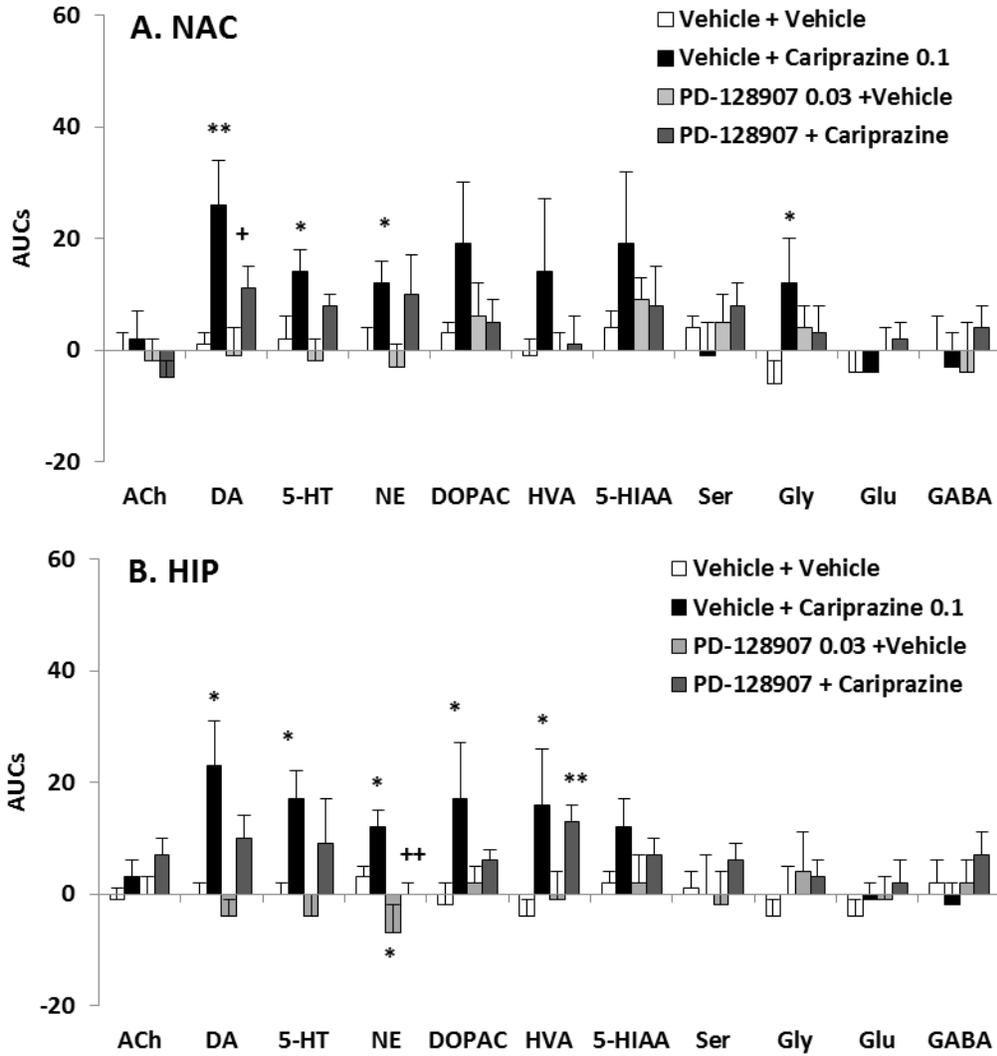


Figure 2.



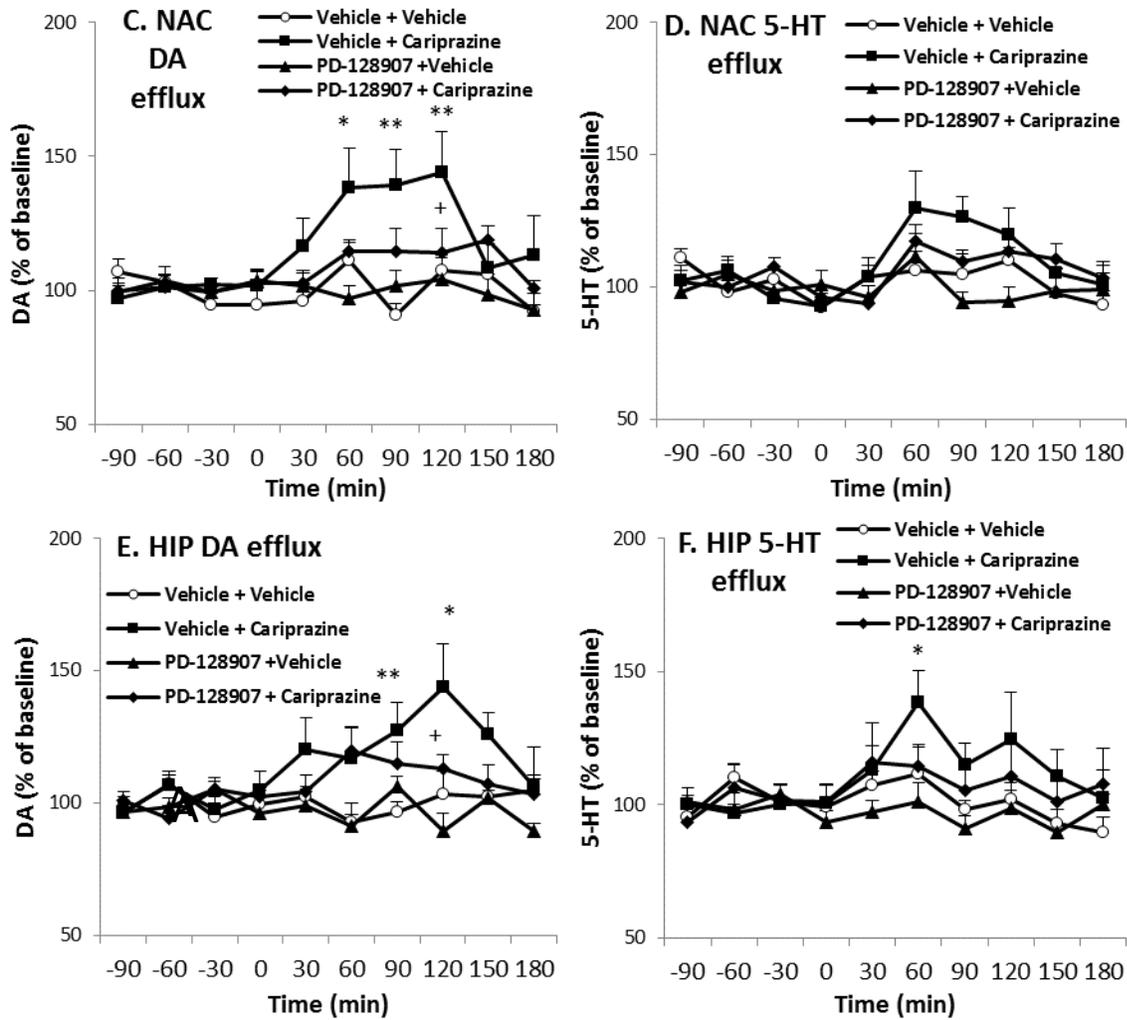


Figure 3.