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The novel atypical antipsychotic cariprazine demonstrates dopamine D_2 receptor-dependent partial agonist actions on rat mesencephalic dopamine neuronal activity

Sarah Delcourte[1] | Charles R. Ashby Jr[2] | Renaud Rovera[1] | Béla Kiss[3] | Nika Adham[4] | Bence Farkas[3] | Nasser Haddjeri[1]

[1]Univ Lyon, Université Claude Bernard Lyon 1, Inserm, Stem Cell and Brain Research Institute U1208, Bron, France

[2]Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY, USA

[3]Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary

[4]Allergan, Jersey City, NJ, USA

Correspondence
Nasser Haddjeri, INSERM U1208, Stem Cell and Brain Research Institute, Université Lyon 1, Bron Cedex, France.
Email: nasser.haddjeri@inserm.fr

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Abstract

Aim: Cariprazine, a dopamine D_3-preferring D_3/D_2 receptor partial agonist, is FDA approved for the treatment of schizophrenia and acute manic or mixed episodes of bipolar disorder. This study used in vivo electrophysiological techniques in anesthetized rats to determine cariprazine's effect on dopaminergic cell activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc).

Methods: Extracellular recordings of individual dopaminergic neurons were performed after oral or intravenous administration of cariprazine, the D_3 receptor antagonist SB 277011A, the D_2 receptor antagonist L741,626, and/or the D_3 receptor agonist PD 128,907.

Results: Acute oral treatment with cariprazine significantly increased and chronic cariprazine significantly decreased the number of spontaneously firing dopaminergic neurons in the VTA, but not in the SNc. Intravenous administration of cariprazine partially but significantly inhibited dopaminergic neuronal firing in both regions, which was prevented by L741,626 but not SB 277011A. In both VTA and SNc, cariprazine, SB 277011A, and L741,626 significantly antagonized the suppression of dopamine cell firing elicited by PD 128,907.

Conclusions: Cariprazine significantly modulates the number of spontaneously active VTA dopamine neurons and moderately suppresses midbrain dopamine neuronal activity. The contribution of dopamine D_2 receptors to cariprazine's in vivo effects is prevalent and that of D_3 receptors is less apparent.

KEYWORDS

cariprazine, dopamine receptors, electrophysiology, schizophrenia, substantia nigra pars compacta, ventral tegmental area

1 | INTRODUCTION

Schizophrenia is a common psychiatric disorder characterized by positive (eg, delusions and hallucinations) and negative symptoms (eg, anhedonia and asociality).[1] The dysregulation of dopaminergic function in the midbrain is thought to contribute to the symptomatology of schizophrenia.[2-4] Clinical data indicate that mesolimbic and mesocortical dopaminergic pathways are dysregulated in schizophrenia. Overactivity of mesolimbic dopaminergic neurons may induce positive symptoms of schizophrenia, while hypoactivation of

mesocortical dopaminergic transmission may explain why some patients, often with prominent negative symptoms, are not responsive to compounds that block dopamine (DA) transmission.[5-7] It is well accepted that typical antipsychotics such as haloperidol reduce positive symptoms by antagonism of the D_2 receptor in the mesocortical pathway, but the blockade of postsynaptic D_2 receptors results in adverse effects such as cognitive impairment, Parkinsonism, and hyperprolactinemia. Moreover, typical antipsychotics do not ameliorate negative symptoms such as apathy, social withdrawal, and anhedonia. In contrast, atypical antipsychotics such as risperidone are favored because they reduce positive symptoms by antagonism of D_2 and serotonergic 5-HT_{2A} receptors. However, they can be associated with adverse metabolic effects, such as hyperlipidemia and weight gain. Dopaminergic partial agonists, such as aripiprazole, have been shown to fine-tune dopaminergic transmission.[8,9] These compounds may decrease dopaminergic hyperactivity (reducing positive symptoms) and increase dopaminergic hypo-activity (improving negative or cognitive symptoms), while they demonstrate favorable metabolic profile (for review, see reference [10,11]).

Dopaminergic neurons in the mesencephalic ventral tegmental area (VTA) project primarily to the frontal cortex and ventral striatum; those in the laterally adjacent substantia nigra pars compacta (SNc) project primarily to the dorsal striatum.[12-14] The firing rates of these mesocortical and mesostriatal projection neurons are regulated by dopamine autoreceptors and other neurotransmitters, including glutamate, gamma-aminobutyric acid (GABA), and serotonin (5-HT).[15] These projections modulate the responses of target neurons that mediate complex, fundamental brain functions including cognition, reward processes, and locomotion.[16,17]

The atypical antipsychotic cariprazine, a DA D_3/D_2 receptor partial agonist, is FDA approved for the treatment of patients with schizophrenia and manic or mixed episodes associated with bipolar I disorder. Differentiating properties of cariprazine include its higher affinity and selectivity for D_3 vs D_2 receptors,[18] displaying subnanomolar and 5-fold to 8-fold higher in vitro binding affinity for the former, and its occupancy of a high percentage of both receptor subtypes in vivo at pharmacological and antipsychotic effective doses in both animals[19] and humans.[20] Additionally, cariprazine is a partial agonist with nanomolar affinity for human 5-HT_{1A} receptors.[18] It has subnanomolar affinity for human 5-HT_{2B} receptors and nanomolar affinity for human 5-HT_{2A} receptors, exerting antagonist action at these serotonin receptor subtypes.[18] Cariprazine also binds to other receptors in vitro with moderate-to-low affinities; namely, the histamine H_1, 5-HT_{2C}, and α_{1A}-adrenergic receptors.[18] Of note, both 5-HT_{1A} and 5-HT_{2B} receptors have been proposed to play role in the antipsychotic-like response in animal models.[21,22] The efficacy and safety of cariprazine in patients with schizophrenia have been supported by 3 positive, randomized, placebo-controlled, phase II/III clinical studies.[23-25] Cariprazine has also shown procognitive[26,27] and antidepressant-like[28] effects in animal behavioral models, suggesting that it may be efficacious in the treatment of the cognitive deficits and negative symptoms of schizophrenia.[29]

The objective of this study was to investigate the contribution of D_2 versus D_3 receptors in the mechanism of action of cariprazine, using relatively subtype-selective DA receptor ligands as tools. Specifically, this study explored the role of these receptors on acute and chronic region-specific effects of cariprazine on mesencephalic dopaminergic neuron firing activity in vivo using extracellular single-cell recordings in anesthetized rats. The effects of cariprazine were evaluated by determining the number of spontaneously active cells in predefined tracks in the target regions and by measuring changes in the firing frequency and bursting activity of individual dopaminergic neurons in the VTA and SNc. Additionally, the efficacy of cariprazine to prevent the decrease in VTA and SNc dopaminergic cell firing activity induced by PD 128,907, a full and preferential D_3 receptor agonist, was measured, as was the ability of the selective D_3 receptor antagonist SB 277011A and the selective D_2 receptor antagonist L741,626 to antagonize the effects of cariprazine or PD 128,907 on mesencephalic dopaminergic cell firing activity.

2 | METHODS

2.1 | Animals

The acute and chronic oral (p.o.) cariprazine studies were performed using male Sprague-Dawley rats (150-175 g at the start of the study; Taconic Farms, NY). As always used by Univ. of Lyon's group, studies examining the effects of intravenous (i.v.) administration of cariprazine on VTA and SNc neurons were performed using male Sprague-Dawley rats (250-350 g; Charles River, L'Abresle, France) in accordance with the European Communities Council (86/609 ECC) for the care and use of laboratory animals and with the approval of the Regional Animal Care Committee (University—Lyon 1). As always used by Gedeon Richter's group, male Wistar rats (280-350 g; Toxicoop, Budapest, Hungary) were used to determine the effects of intravenous cariprazine on SNc neurons, in accordance with guidelines of the Animal Ethics Committee of Gedeon Richter Plc and experimental procedures approved under registration number 201511. Animals were kept under standard laboratory conditions with food and water available ad libitum.

2.2 | Surgery

For all experiments, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.; Sigma Chemical Co., St. Louis, MO). In the VTA and SNc DA cells/track experiments, supplementary doses of chloral hydrate were given as needed (40 mg/kg/dose). Rats in the SNc experiments received supplementary i.p. doses (60 mg/kg) every 30 minutes. Using standard stereotaxic techniques, electrodes were repeatedly lowered to the VTA and SNc in DA neuron count studies using the following coordinates: VTA, AP: −6.0 to −5.4, L: 0.5 to 0.9 (mm from bregma), DV: −6.5 to −8.5 (mm from overlying dura); SNc, AP: −6.0 to −5.4, L: 2.0 to 2.4, DV: 6.5 to −8.5. The number of spontaneously active DA neurons in the SNc and VTA was determined across 10 stereotaxic descents. The electrode tracks were separated

from each other by 200 μm, and the sequence was constant between animals. For single unit activity measurements, the recording site coordinates, based on a flat-skull position, were as follows: VTA, AP: −4.8 to −5.2, L: 0.5 to 0.8 [mm from bregma], DV: −7.5 to −8.5 [mm from overlying dura]; SNc, AP: −4.8 to −6.1, L: 1.8 to 2.6, DV: 7.0 to 8.5, based on the atlas of Paxinos and Watson.[30] In some cases, electrode placements were verified histologically.

2.3 | Electrophysiological recording

For the VTA and SNc DA cells/track experiments, single-barrel glass borosilicate microelectrodes with a typical impedance of 0.8-1.2 MΩ measured at 135 Hz in vitro and 1.5-2.0 MΩ in vivo were used. For intravenous cariprazine studies, VTA DA neuronal activity was evaluated using single-barrel glass microelectrodes with a tip diameter of 2-4 μm; SNc DA neuronal activity was evaluated using glass-coated tungsten electrodes (Tunglass-1, Kation Scientific, Minneapolis, MN) with a typical impedance of 0.8-2.8 MΩ measured in vitro at 1 kHz in saline. Electrophysiological recordings were performed on single DA neurons in each animal using Spike2 software (Cambridge Electronic Design, Cambridge, UK). The detection of bursts within spontaneously active neurons was performed using a Spike 2 script based on the criteria of Grace and Bunney.[31] The beginning of a burst was defined with the occurrence of two spikes within 80 milliseconds, and the ending of a burst was determined as an interspike interval of longer than 160 milliseconds. The firing and bursting activity were quantified relative to baseline, and drug-induced changes in neuronal activities were defined as percent changes from this baseline after the injection of a pharmacologic compound. Dose-response curves were constructed by plotting the firing rates normalized to the mean firing rate of the vehicle over a period of at least 5 minutes against doses. The ED_{50} values were calculated for each animal using a sigmoid curve fitting method for firing activity; for bursting activity, the ED_{50} values were obtained from pooled, averaged data of each dose.

VTA or SNc neurons were considered dopaminergic if they exhibited the following predetermined characteristics: (i) slow (2.0-9.0 Hz) firing rate, with or without burst firing; (ii) action potentials having biphasic or triphasic waveform with a duration of at least 1.1 ms (measured from spike initiation to the maximal negative phase of the action potential); and (iii) a characteristic, low-pitch sound when monitored through an audio amplifier.[2,32]

At the end of the experiments, animals were deeply anesthetized with a pentobarbital overdose (100 mg/kg), and their brains were removed, snap-frozen in isopentane (Sigma-Aldrich), and stored at −40°C. The location of electrode in the targeted region was determined histologically on serial coronal sections (60 μm) and, as exemplified in Figure 5, only data obtained from rats with correctly implanted electrodes were included in the results.

2.4 | Drugs

Cariprazine was dissolved in 0.9% saline (i.v. studies) or distilled water (p.o. studies). (+)-PD 128,907 (Tocris, Bristol, UK), SB 277011A (Tocris, Bristol, UK), and L741,626 (Tocris, Bristol, UK) were dissolved in a solution of 10% hydroxypropyl-β-cyclodextrin in distilled water.

2.5 | Assays

2.5.1 | Spontaneously active DA neuron counts

Animals (n = 10 per treatment group) received vehicle or cariprazine (0.1, 0.3, or 1.0 mg/kg in a volume of 1 mL/kg, p.o.) once (acute) or once daily for 21 consecutive days (chronic). The number of spontaneously active VTA and SNc dopaminergic neurons was determined 2 hours after the final drug administration for both acute and chronic studies. The electrode was passed through the VTA and SNc in a preset sequence of 10 electrode tracks separated from each other by 200 μm. Each electrode descent was made at a slow (1-3 μm/second), uniform speed using a hydraulic microdrive. Only cells whose electrophysiological profiles matched those previously established for mesencephalic dopaminergic cells were counted. For half of the rats in each group, the order of recording was VTA-SNc, and the order was reversed for the other rats.

2.5.2 | VTA/SNc DA neuron firing and bursting activity

To determine the effect of cariprazine on DA neuron firing and bursting in the VTA, 10 rats received i.v. doses of 0, 5, 5, 10, 10, 20, and 20 μg/kg cariprazine (corresponding to cumulative doses of 0, 5, 10, 20, 30, 50, and 70 μg/kg) at 2-minute intervals. In the SNc experiment, 9 rats received i.v. doses of 0, 2.5, 2.5, 5, 10, 20, and 40 μg/kg cariprazine (corresponding to cumulative doses of 0, 2.5, 5, 10, 20, 40, and 80 μg/kg) at 2-minute intervals.

To determine the effect of cariprazine on DA neuron firing and bursting over time, rats received a single i.v. dose of cariprazine (VTA: 10 μg/kg, n = 6 per treatment group; SNc: 10 μg/kg, n = 6). The firing rate was recorded after vehicle injection and at 2 minutes and 30 minutes after cariprazine administration.

To assess the contribution of DA D_2 and D_3 receptors to the effects of cariprazine in midbrain dopaminergic neurons, rats received either vehicle, SB 277011A (500 μg/kg), or L741,626 (500 μg/kg) before cumulative cariprazine dosing of 5, 10, or 20 μg/kg (VTA: n = 5, 10, and 8, respectively; SNc: n = 5 for each).

To determine the contribution of DA D_2 and D_3 receptors to the partial agonist effect of cariprazine in the VTA and SNc on PD 128,907-induced firing activity changes, rats received: (i) an injection of vehicle and PD 128,907 (10 μg/kg) followed by 20 μg/kg of cariprazine (20 μg/kg), or (ii) received an injection of vehicle, cariprazine (20 μg/kg), SB 277011A (500 μg/kg), or L741,626 (500 μg/kg) followed by 10 μg/kg of PD 128,907. The firing and bursting activity were recorded from baseline to 5 minutes after PD 128,907 administration.

2.6 | Data analysis

The oral and i.v. cariprazine data were analyzed using analysis of variance (ANOVA) followed by an appropriate post hoc test that

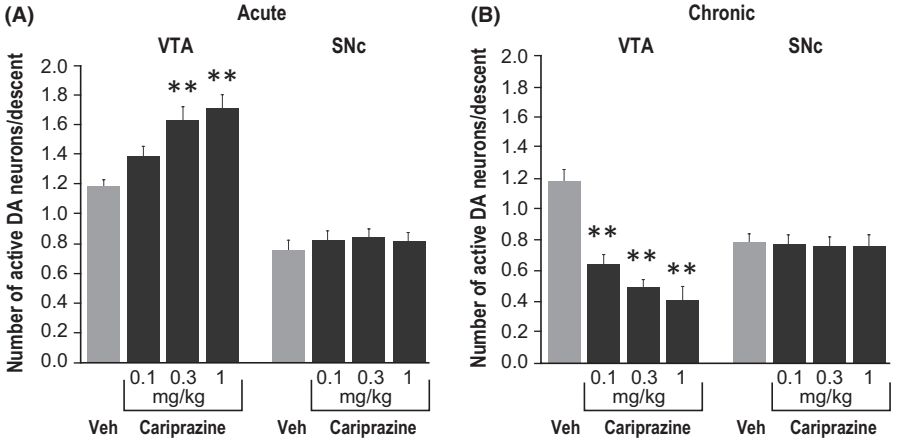

FIGURE 1 The effect of acute and chronic cariprazine administration on the number of spontaneously active dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) in anesthetized male rats. The effect of (A) acute and (B) chronic oral cariprazine administration (once daily for 21 consecutive days) on the number of active dopaminergic neurons in the VTA and SNc. $^{**}P < .01$ vs vehicle using one-way ANOVA followed by Student-Newman-Keuls post hoc test. Values are means ± SEM

was Student-Newman-Keuls (p.o. cariprazine studies) or Fisher's least significance difference (FLSD) (most of i.v. cariprazine studies). As dose-response curves for the effects of cariprazine on firing frequency and bursting activity of SNc dopaminergic neurons were constructed separately for each animal tested, experiments investigating the dose-response relationship of i.v. cariprazine in the SNc were analyzed using a repeated measures ANOVA followed by Dunnett's post hoc test. The a priori significance level was $P < .05$. All summary data are presented as the means ± SEM.

3 | RESULTS

3.1 | Effects of cariprazine on the number of spontaneously active VTA and SNc dopaminergic neurons

The acute oral administration of 0.3 and 1 mg/kg cariprazine, but not 0.1 mg/kg, significantly increased the number of spontaneously active VTA dopaminergic neurons by 38% and 44% compared with vehicle, respectively ($P < .01$; Figure 1A). In contrast, cariprazine did not significantly alter the number of spontaneously active SNc dopaminergic neurons compared with vehicle (Figure 1A). Chronic oral cariprazine administration produced a significant, dose-dependent decrease (45%, 59%, and 66% for 0.1, 0.3, and 1 mg/kg, respectively) in the number of spontaneously active VTA dopaminergic neurons ($P < .01$; Figure 1B). In contrast, oral cariprazine did not significantly alter the number of spontaneously active SNc dopaminergic neurons compared with vehicle (Figure 1B).

3.2 | Dose-response relationship of the effects of cariprazine on firing frequency and bursting activity of VTA and SNc dopaminergic neurons

The i.v. administration of vehicle did not significantly affect firing activity when administered prior to cariprazine (Figure 2A,B); the mean firing levels after vehicle injection were 96.6 ± 1.2% and 102.2 ± 1.5% of baseline in the VTA and SNc, respectively. Cariprazine significantly decreased the firing rate of VTA DA neurons ($F_{(7,56)} = 55.1$, $P < .0001$; Figure 2C), plateauing at approximately 38% inhibition ($ED_{50} = 7.5$ μg/kg). Similarly, cariprazine significantly suppressed the firing rate of SNc DA neurons ($F_{(6,48)} = 23.1$, $P < .0001$; Figure 2D); inhibition plateaued at <50% ($ED_{50} = 5.8$ μg/kg).

Intravenous administration of cariprazine also significantly decreased the bursting activity of VTA ($F_{(7,56)} = 36.2$, $P < .001$; Figure 2E) and SNc ($F_{(6,18)} = 31.6$, $P < .0001$; Figure 2F) DA neurons. Importantly, while cariprazine-induced inhibition of the mean firing rate plateaued in both regions, bursting activity was almost completely suppressed by cariprazine (VTA: 91%, $ED_{50} = 5.4$ μg/kg; SNc: 99%, $ED_{50} = 3.0$ μg/kg), indicating that cariprazine preferentially affects bursting activity. These findings suggest that cariprazine acts as a partial agonist on the firing rate and as a full agonist on bursting activity at dopamine receptors on VTA and SNc dopaminergic neurons.

3.3 | Time course of the effect of a single intravenous dose of cariprazine on the firing rate of VTA and SNc dopaminergic neurons

In the VTA, acute cariprazine administration (10 μg/kg, i.v.) inhibited dopaminergic neuron firing frequency within 2 minutes (18%), and there was no significant recovery 30 minutes after administration (27%) ($F_{(2,10)} = 15.90$, $P < .001$; Figure 3A). In the SNc, acute cariprazine (10 μg/kg, i.v.) suppressed the firing rate of SNc dopaminergic neurons within 2 minutes (35%), and there was a partial recovery 30 minutes after administration (18%) ($F_{(2,14)} = 8.27$, $P < .01$; Figure 3B). Full reversal of the inhibitory action of cariprazine on the firing rate occurred after 60 minutes in the SNc but not in the VTA (data not shown). There was no significant reversal of the inhibitory effect of cariprazine on bursting activity in either VTA or SNc DA neurons (data not shown).

3.4 | Dose-response relationship of the effect of cariprazine on the firing rate of VTA and SNc dopaminergic neurons in the presence of selective dopamine D_2 and D_3 receptor antagonists

Cariprazine (cumulative i.v. doses of 5-20 μg/kg) dose-dependently inhibited firing in VTA dopaminergic neurons by approximately 15%-35%.

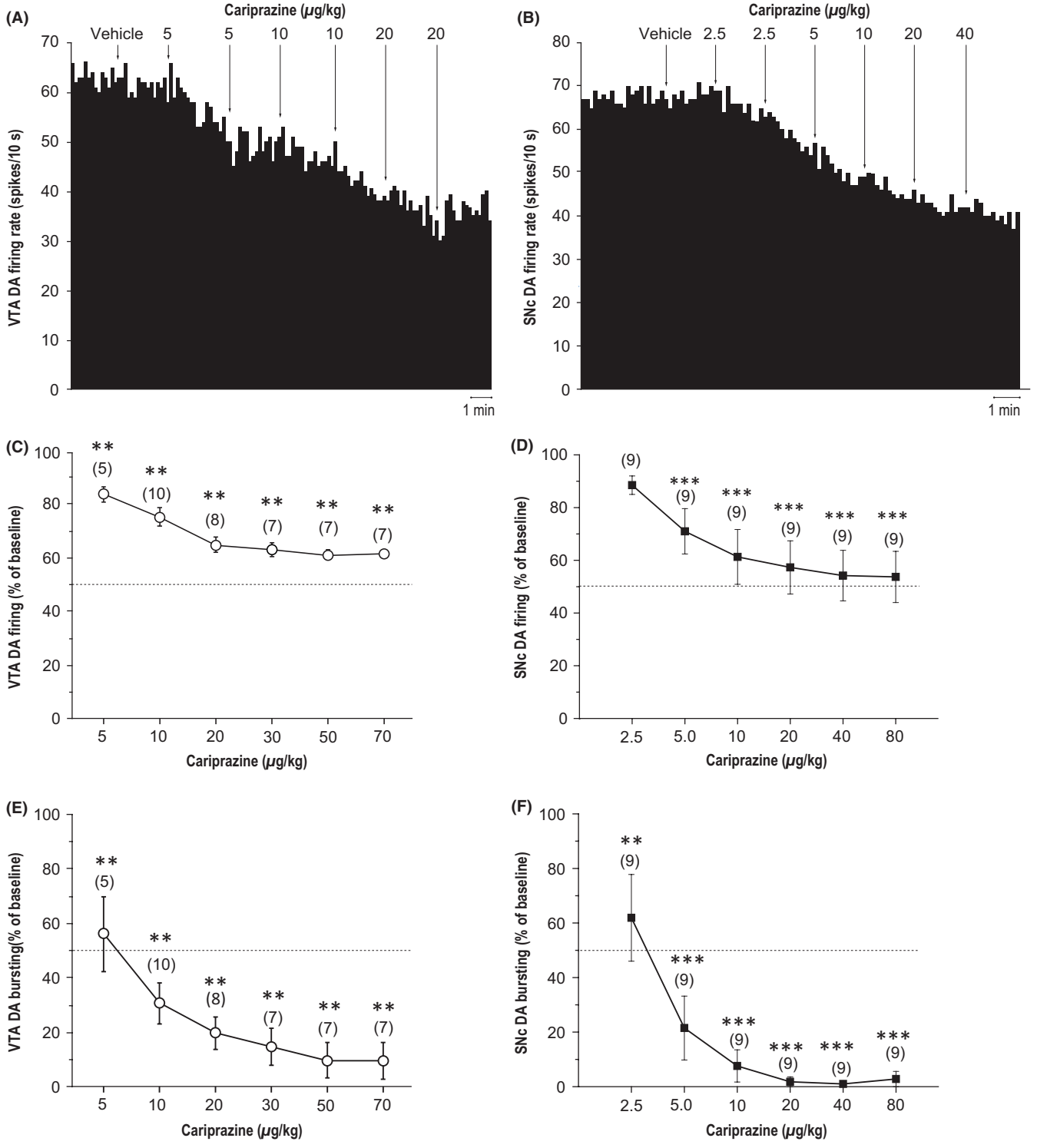

FIGURE 2 The effect of cariprazine on dopaminergic neuronal firing activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Integrated firing rate histograms depicting the response of single dopaminergic neurons in the (A) VTA and (B) SNc to cumulative i.v. doses of cariprazine (VTA: 5.0-70 μg/kg; SNc: 2.5-80 μg/kg). C, VTA DA neuron firing rates (% of baseline) and cumulative cariprazine doses (μg/kg, i.v). D, SNc DA neuron (n = 9) firing rates (% of baseline) and cumulative cariprazine doses (μg/kg, i.v). E, VTA DA neuron bursting activity (% of baseline) and cumulative cariprazine doses (μg/kg, i.v). F, SNc DA neuron (n = 9) bursting activity (% of baseline) and cumulative cariprazine doses (μg/kg, i.v.). The plotted values represent the mean ± SEM decrease in dopaminergic cell firing or bursting activity; parenthetical numbers indicate the number of neurons recorded. $^{**}P < .01$, $^{***}P < .001$ vs vehicle using one-way ANOVA followed by Fisher's LSD post hoc test (VTA) and repeated measures ANOVA followed by Dunnett's post hoc test (SNc)

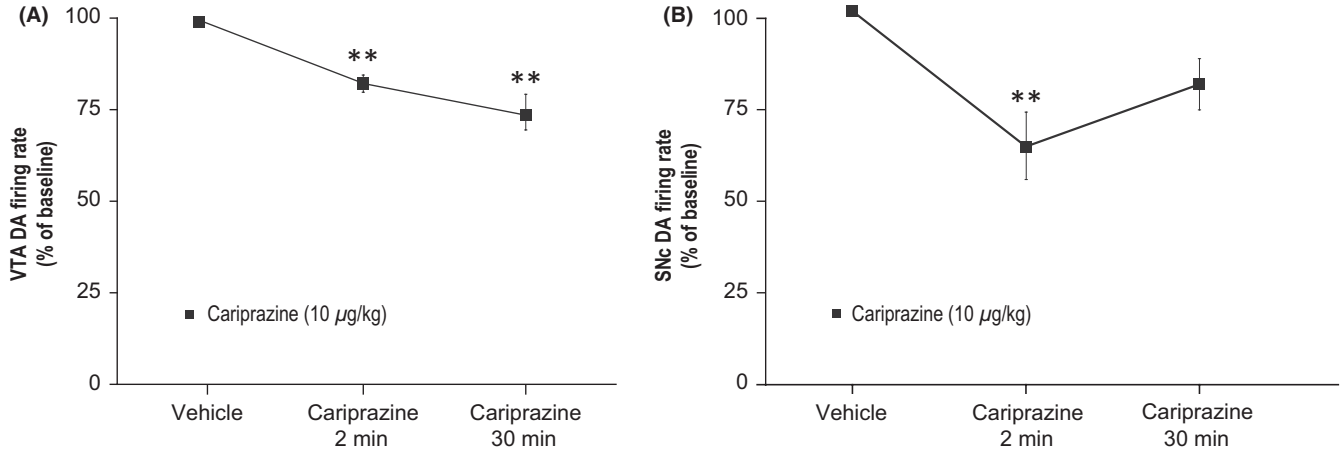

FIGURE 3 Time course effects of cariprazine on ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) DA neuronal firing rates. The effect of a single systemic i.v. dose of cariprazine on dopaminergic neuronal firing in the: (A) VTA (10 μg/kg, n = 6) and (B) SNc (10 μg/kg, n = 6). $^{**}P < .01$ using two-way ANOVA followed by Dunnett's test. Data are presented as mean ± SEM values

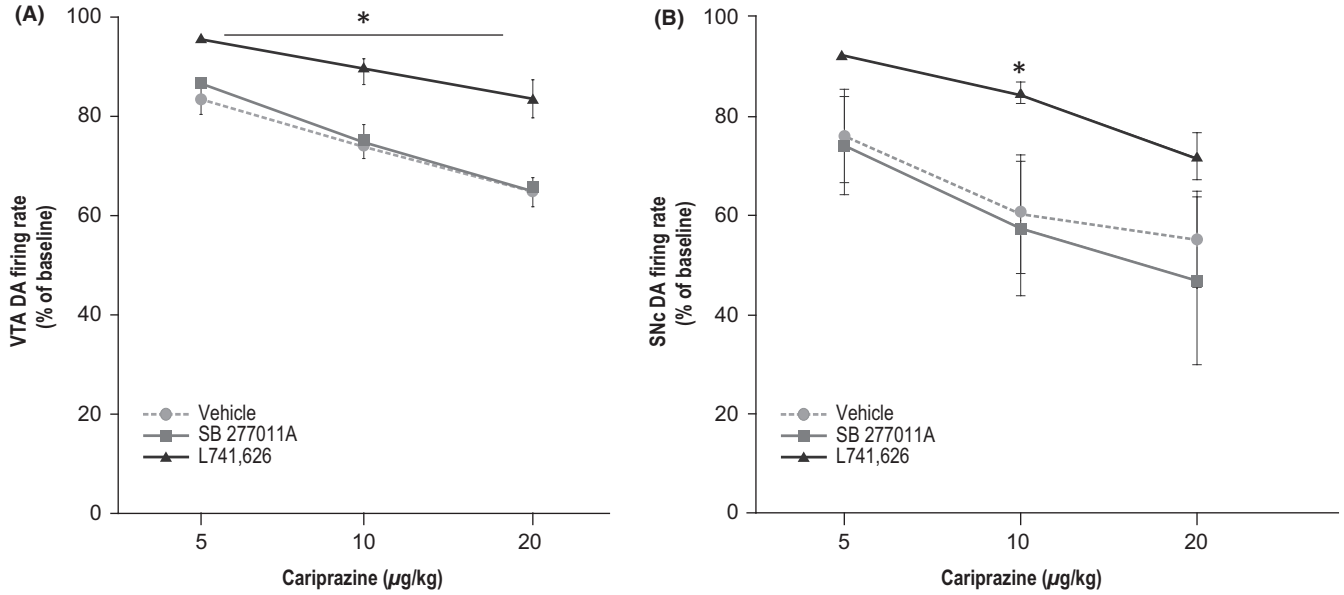

FIGURE 4 Effect of D_3 and D_2 receptor blockade on the suppressant action of intravenous cariprazine on ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) dopaminergic neuron firing rates. The effects of the selective D_3 receptor antagonist SB 277011A (500 μg/kg, i.v.) and the selective D_2 receptor antagonist L741,626 (500 μg/kg, i.v.) on the cariprazine-induced suppression of dopaminergic neuron firing activity in the (A) VTA (vehicle: 5 μg/kg, n = 5; 10 μg/kg, n = 10; 20 μg/kg, n = 8; SB 277011A, n = 6 for each; L741,626, n = 6 for each) and (B) SNc (n = 5 for each). $^{*}P < .05$ using two-way ANOVA followed by Fisher's LSD post hoc test. Data are presented as means ± SEM

The prior administration of the selective D_3 receptor antagonist SB 277011A (500 μg/kg, i.v.) did not significantly alter the suppressant effect of cariprazine on firing activity (Figure 4A). In contrast, the selective D_2 receptor antagonist L741,626 (500 μg/kg, i.v.) significantly reduced the suppressant effect of all doses of cariprazine on the firing rate ($F_{(2,50)}$=23.745, $P < .0001$; Figure 4A) of VTA dopaminergic neurons. In the SNc, the prior administration of L741,626 (500 μg/kg), but not SB 277011A (500 μg/kg), significantly influenced the inhibitory action of cariprazine on DA cell firing after cumulative i.v. administration ($F_{(2,40)}$ = 4.56, $P < .05$), an effect that reached the level of significance for the 10 μg/kg dose of cariprazine ($P < .05$; Figure 4B).

3.5 | Effects of cariprazine on VTA and SNc dopaminergic neurons after PD 128,907 administration

The in vitro partial agonistic activity of cariprazine at DA D_3 receptors suggested that its effects on DA neuronal firing activity might result from activation of this receptor in the current in vivo experimental paradigm. The systemic administration of the D_3 receptor full agonist PD 128,907 (10 μg/kg, i.v.; 0 second) robustly, but transiently, suppressed the firing activity and the bursting activity (data not shown) of DA neurons in the VTA (Figure 5A) and SNc

(Figure 5C). The administration of cariprazine (20 μg/kg, i.v.; 30 seconds) partially and significantly reversed the suppressant effect of PD 128,907 on firing activity after administration ($F_{(1,100)}$ = 6.43, P < .05 ; Figure 5A; $F_{(1,80)}$ = 13.84, P < .01; Figure 5C), but had no significant effect on bursting activity (data not shown).

3.6 | Effects of cariprazine, SB 277011A, and L741,626 on the suppressant action of PD 128,907 on VTA and SNc dopaminergic neuron firing activity

PD 128,907 is a DA D_3 receptor full agonist that also has efficacy as a DA D_2 receptor agonist.[33] The different contributions of the D_2 and D_3 receptors to the efficacy of PD 128,907 were investigated using selective DA D_2 and D_3 receptor antagonists. PD 128,907 (10 μg/kg, i.v.) significantly suppressed both VTA dopaminergic neuron firing ($F_{(3,21)}$ = 9.162, P < .01) and SNc dopaminergic neuron firing ($F_{(3,19)}$ = 7.052, P < .01). Cariprazine (20 μg/kg, i.v.), the selective D_3 receptor antagonist SB 277011A (500 μg/kg, i.v.), and the selective D_2 receptor antagonist L741,626 (500 μg/kg, i.v.), significantly prevented this suppression (Figure 6A,B) and the maximum effect occurred 30-60 seconds postinjection (data not shown). In contrast, while the systemic administration of PD 128,907 (10 μg/kg, i.v.) completely suppressed the bursting activity of DA neurons (data not shown), only L741,626 significantly reduced the suppressant effect of PD 128,907 on bursting activity in these cells in the VTA (bursting remaining of 25.8 ± 13.1%, P < .01) and almost significantly in the SNc (bursting remaining of 21.2 ± 14.6%, P = .0619). These findings indicate that the effect of PD 128,907 on dopaminergic neuron

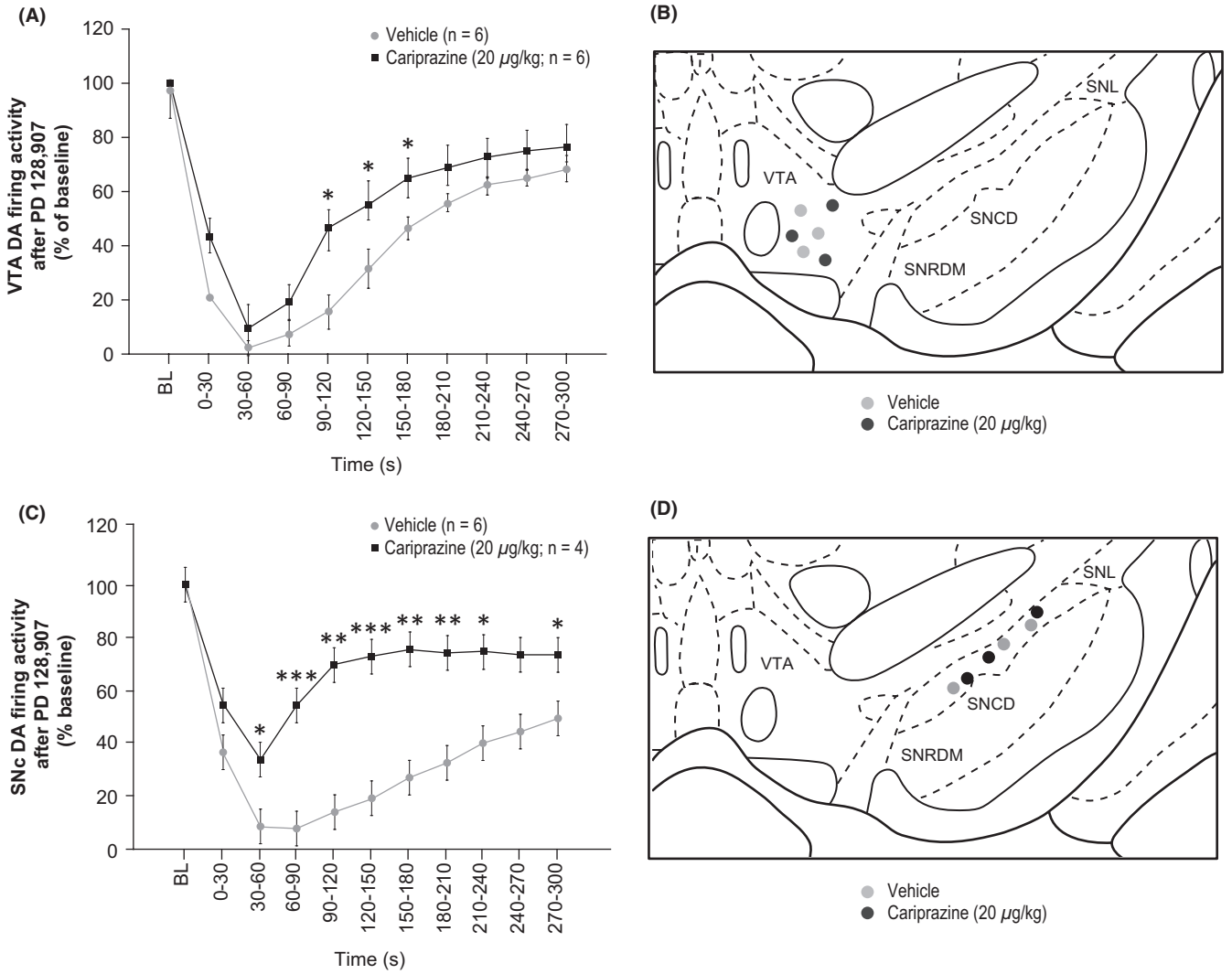

FIGURE 5 The effects of cariprazine on PD 128,907-induced suppression of ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) dopaminergic neuronal firing and bursting activity. The effect of cariprazine on the suppressant actions of PD 128,907 on the firing rate activity of VTA (A) and SNc (C) dopaminergic neurons. B, D, Schematic diagrams, taken from Paxinos and Watson,[30] show (black points) the anatomical localization of DA neurons recorded in the VTA (B, 5.2 mm posterior to the bregma) and the SNc (D, 5.0 mm posterior to the bregma). BL: baseline; SNCD: substantia nigra, compacta part, dorsal tier; SNL: substantia nigra, lateral part; SNRDM: substantia nigra, reticular part, dorsomedial tier. *P < .05, **P < .01, ***P < .001, compared to PD 128,907 alone, using one-way ANOVA followed by FLSD post hoc test. Plotted values are means ± SEM

firing is mediated via both the DA D_2 and D_3 receptors, whereas its effect on bursting is due to D_2 receptor activation.

4 | DISCUSSION

This electrophysiological study analyzed the following: (i) the in vivo effects of acute and chronic administration of cariprazine on the number of spontaneously active dopaminergic neurons in the VTA and SNc regions of the midbrain, and (ii) the electrophysiological effects of acute cariprazine on those neurons, with a special emphasis on the relative contribution of DA D_2 and D_3 receptors to these effects.

As mentioned previously, schizophrenia symptoms are suggested to be associated with a hyperactivity of mesolimbic dopaminergic pathways and a hypofunction of the dopaminergic mesocortical circuits.[34,35] In vivo, DA neurons exhibit tonic, irregular single spike firing interrupted by bursts of spikes often with decreasing spike amplitude followed by brief periods of quiescence.[31,36] Furthermore, it has been shown that the mean firing rate and the bursting pattern of DA neurons can be modulated independently.[37,38] A state-dependent shift in the discharge pattern might be crucial as the burst firing of DA neurons results in a much larger synaptic DA release than single spike firing[37,39] because positive symptoms of schizophrenia are supposed to rely on a dysregulation of phasic—that is, burst—firing.[40] As previously shown with aripiprazole or bifeprunox,[41,42] the present in vivo electrophysiological study reveals that acute administrations of cariprazine partially inhibited the firing activity and potently suppressed the bursting activity of both VTA and SNc DA neurons. Both dopamine D_2 and D_3 receptors are expressed in all mesencephalic dopamine neurons.[43,44] It has been suggested that D_3 receptors function as autoreceptors and control the phasic, but not tonic, activity of dopamine neurons.[45] Importantly, cariprazine potently suppressed the bursting activity confirming that this new antipsychotic preferentially suppresses the phasic activity.

The acute oral administration of 0.3 or 1 mg/kg of cariprazine significantly increased the number of spontaneously active VTA, but not SNc, dopaminergic neurons. These results are consistent with those observed after acute administration of other atypical antipsychotics (eg, clozapine), which also produce a significant increase in the number of VTA dopaminergic neurons.[46] In contrast, the acute administration of typical antipsychotics (eg, haloperidol and chlorpromazine) significantly increases the number of spontaneously active dopaminergic neurons in both the VTA and SNc.[46]

Following chronic exposure, cariprazine significantly decreased the number of spontaneously active VTA dopaminergic neurons, but not the number of SNc dopaminergic neurons. Thus, chronic cariprazine administration appears to induce adaptive changes in spontaneously active VTA dopaminergic neurons consistent with depolarization block, which may be related to the delay in the onset of therapeutic efficacy for antipsychotic drugs.[47] The chronic administration of atypical antipsychotics[2,3,46,48-50] significantly decreases the number of spontaneously active VTA dopaminergic neurons, while either: (i) increasing the number of spontaneously active SNc dopaminergic neurons,[3,46,49] or (ii) having no effect on the number of spontaneously active SNc DA neuron.[2,48] In contrast, the chronic administration of typical antipsychotics, such as haloperidol or chlorpromazine, significantly decreases the number of spontaneously active dopaminergic neurons in both the VTA and SNc compared with vehicle treatment.[2,46,49] Overall, findings of the present work indicate that the in vivo electrophysiological profile of cariprazine

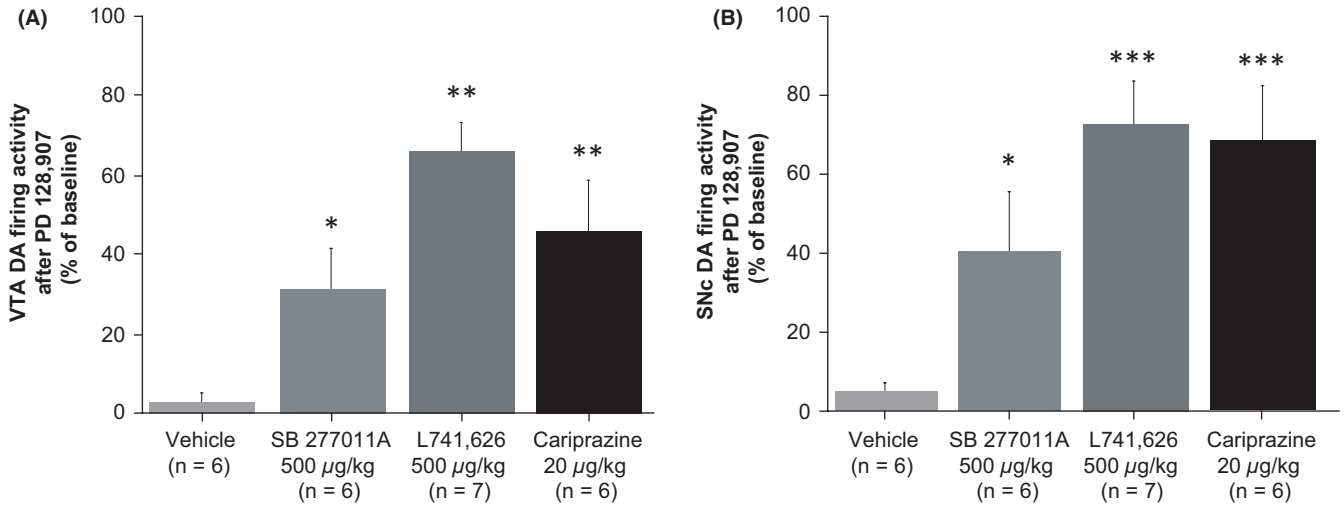

FIGURE 6 The effects of cariprazine, SB 277011A, and L741,626 on the firing and bursting activity of ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) dopaminergic neurons. A, The effects of intravenous SB 277011A, L741,626, and cariprazine on the PD 128,907-induced suppression of the firing activity of VTA dopaminergic neurons. B, The effects of intravenous SB 277011A, L741,626, and cariprazine on the PD 128,907-induced suppression of the firing activity of SNc dopaminergic neurons. *$P < .05$, **$P < .01$, ***$P < .001$ compared to the full inhibition induced by PD 128,907 using one-way ANOVA followed by FLSD post hoc test. Plotted values represent means ± SEM. The numbers of neurons recorded are indicated in parentheses

resembles that of other atypical antipsychotics. The decrease in the number of spontaneously active dopaminergic neurons in the VTA and SNc produced by antipsychotic drugs may be responsible for their therapeutic action and neurological side effects, respectively.[2,3] Thus, the electrophysiological profile of cariprazine on VTA and SNc dopaminergic neurons suggests a potential physiological mechanism underlying its demonstrated efficacy in schizophrenia and bipolar mania.

The intravenous administration of cariprazine dose-dependently decreased firing rates and bursting activity of DA neurons in both the VTA and SNc, although a higher inhibitory tendency in the SNc seems to be shown. Although the maximal decrease in firing rate was <50% in VTA and SNc DA neurons, bursting activity was completely blocked in both regions, indicating that cariprazine acts as a partial agonist for firing rate and as a full agonist for bursting activity. The preferential action on bursts may be therapeutically relevant, as bursts result in increased DA release compared with that resulting from neurons firing with the same frequency, but without burst events.[36,39,51]

Cariprazine rapidly suppresses dopaminergic neuron firing. The maximal or near maximal inhibition occurred 2 minutes after intravenous dosing, and full reversal of inhibition did not occur until after at least 30 minutes. However, the firing activity was completely restored by 60 minutes after cariprazine injection in the SNc. Taken together, these results suggest that the suppressive effect of cariprazine on firing rate may last longer in the VTA, where inhibition remains at near maximal levels at 30 minutes, than in the SNc, where recovery begins within the same time frame. The efficacy of cariprazine to partially suppress firing rate and preferentially suppress bursting activity in VTA and SNc dopaminergic neurons is similar to that reported for other atypical antipsychotics that are D_2 receptor partial agonists, such as aripiprazole, bifeprunox, and brexpiprazole.[36,42,52,53] However, cariprazine's effects are in marked contrast to that of the D_2 receptor antagonist haloperidol, a typical antipsychotic, which increases the firing activity of dopaminergic neurons in the VTA and SNc.[32,54]

The prior administration of the selective D_2 receptor antagonist L741,626,[55] but not the selective D_3 receptor antagonist SB 277011A,[56] reduced the suppressive effects of intravenous cariprazine on dopaminergic neurons in both the VTA and SNc. Thus, in this paradigm, the partial inhibitory effect of cariprazine does not appear to result from its activation of D_3 receptors, but from a D_2 receptor-mediated action. This result is surprising given the high affinity of cariprazine for D_3 receptors[18,27] and the fact that mesencephalic dopaminergic neurons express D_3 autoreceptors in high density.[44,57] Although the systemic nature of the injections makes it difficult to determine exactly where cariprazine functions in the brain, these results suggest a potential role for mesencephalic D_2 autoreceptors[43] or presynaptic D_2 receptors.[58] However, cariprazine has a complex receptor binding profile, including subnanomolar affinity for serotonin 5-HT_{2B} and nanomolar affinity for 5-HT_{1A} and 5-HT_{2A} receptors; thus, its effect on nondopaminergic receptors may also contribute to these results.

PD 128,907 is a D_3 receptor-preferring full agonist that also binds to and activates D_2 receptors.[33,59,60] As demonstrated previously, the administration of PD 128,907 completely inhibited firing and bursting activity in VTA[61] and SNc[62] DA neurons. The administration of intravenous cariprazine partially and significantly reversed the suppressive effect of PD 128,907 on the firing rate in both the VTA and SNc, although a higher inhibitory tendency in SNc DA neurons was observed. A similar, but nonsignificant, trend was observed on bursting activity. In addition to being partially reversed by intravenous cariprazine, the inhibitory effect of PD 128,907 on firing rate was also significantly reduced by prior intravenous administration of either the selective D_3 receptor antagonist SB 277011A or the selective D_2 receptor antagonist L741,626, whereas bursting activity was counteracted only by L741,626. Thus, the effects of PD 128,907 on the firing rate in the VTA and SNc are likely mediated by both D_3 and D_2 receptors, while those on bursting activity are mediated by D_2 receptors.

5 | CONCLUSION

In summary, the acute oral administration of cariprazine increased the number of spontaneously active VTA dopaminergic neurons, whereas chronic administration produced the opposite effect. Neither acute nor chronic oral cariprazine administration alters the number of spontaneously active SNc dopaminergic neurons. Under the current experimental conditions, cariprazine alters the activity of VTA and SNc DA neurons mainly via DA D_2 receptors in vivo. Due to the lack of highly selective dopamine D_2 and D_3 receptor agonist compound tools, future studies using D_3 and/or D_2 receptor knockout mice may be necessary to determine whether D_3 receptors contribute to the cariprazine-mediated effects on dopamine neuronal firing activity in the midbrain. The effects of cariprazine on dopaminergic neuron firing are similar to those of other atypical antipsychotics, with partial agonist properties on D_2 receptors (eg, aripiprazole and brexpiprazole), and strikingly different from those elicited by the typical antipsychotic haloperidol.

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CONFLICTS OF INTEREST

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ORCID

Nasser Haddjeri http://orcid.org/0000-0002-2161-2204

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