

# Long-term effects of aripiprazole exposure on monoaminergic and glutamatergic receptor subtypes: comparison with cariprazine

Yong Kee Choi,<sup>1</sup> Nika Adham,<sup>2</sup> Béla Kiss,<sup>3</sup> István Gyertyán,<sup>4,5</sup> and Frank I. Tarazi<sup>1\*</sup>

<sup>1</sup> Department of Psychiatry & Neuroscience, Harvard Medical School, and McLean Hospital, Belmont, Massachusetts, USA

<sup>2</sup> Department of Pharmacology, Allergan, Jersey City, New Jersey, USA

<sup>3</sup> Department of Pharmacological and Safety Research, Gedeon Richter Plc, Budapest, Hungary

<sup>4</sup> MTA-SE NAP B Cognitive Translational Behavioral Pharmacology Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

<sup>5</sup> Research Center for Natural Sciences-MTA, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary

**Objective.** This study examined the chronic effects of aripiprazole and cariprazine on serotonin (5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>) and glutamate (NMDA and AMPA) receptor subtypes. In addition, the effects of aripiprazole on D<sub>2</sub> and D<sub>3</sub> receptors were tested and compared with previously reported cariprazine data.

**Methods.** Rats received vehicle, aripiprazole (2, 5, or 15 mg/kg), or cariprazine (0.06, 0.2, or 0.6 mg/kg) for 28 days. Receptor levels were quantified using autoradiographic assays on brain sections from the medial prefrontal cortex (MPC), dorsolateral frontal cortex (DFC), nucleus accumbens (NAc), caudate-putamen medial (CPu-M), caudate-putamen lateral (CPu-L), hippocampal CA1 (HIPP-CA1) and CA3 (HIPP-CA3) regions, and the entorhinal cortex (EC).

**Results.** Similar to previous findings with cariprazine, aripiprazole upregulated D<sub>2</sub> receptor levels in various regions; D<sub>3</sub> receptor changes were less than those reported with cariprazine. All aripiprazole doses and higher cariprazine doses increased 5-HT<sub>1A</sub> receptors in the MPC and DFC. Higher aripiprazole and all cariprazine doses increased 5-HT<sub>1A</sub> receptors in HIPP-CA1 and HIPP-CA3. Aripiprazole decreased 5-HT<sub>2A</sub> receptors in the MPC, DFC, HIPP-CA1, and HIPP-CA3 regions. Both compounds decreased NMDA receptors and increased AMPA receptors in select brain regions.

**Conclusions.** Long-term administration of aripiprazole and cariprazine had similar effects on 5-HT<sub>1A</sub>, NMDA, and AMPA receptors. However, cariprazine more profoundly increased D<sub>3</sub> receptors while aripiprazole selectively reduced 5-HT<sub>2A</sub> receptors. These results suggest that the unique actions of cariprazine on dopamine D<sub>3</sub> receptors, combined with its effects on serotonin and glutamate receptor subtypes, may confer the clinical benefits, safety, and tolerability of this novel compound in schizophrenia and bipolar mania.

Received 13 September 2016; Accepted 23 November 2016; First published online 6 January 2017

**Key words:** Aripiprazole, autoradiography, bipolar mania, cariprazine, dopamine, glutamate, schizophrenia, serotonin.

## Clinical Implications:

■ Dysfunctions of the dopamine, serotonin, and glutamate systems contribute to the development and pathophysiology of schizophrenia and bipolar mania.

- Most clinically effective atypical antipsychotics are known to interact with the serotonin system, modulate the glutamate system, and induce region-specific changes in serotonin and glutamate receptor levels.
- Aripiprazole, a dopamine D<sub>2</sub>/D<sub>3</sub> receptor partial agonist, was evaluated for its long-term effects on dopamine, serotonin, and glutamate receptor levels in comparison to cariprazine, a potent dopamine D<sub>2</sub>/D<sub>3</sub> receptor partial agonist with preferential binding for the D<sub>3</sub> receptors, in rat forebrain regions.
- Aripiprazole produced less-pronounced increases in D<sub>3</sub> receptors as compared to those previously reported

\* Address correspondence to: Frank I. Tarazi, Harvard Medical School, McLean Hospital, 115 Mill Street, Belmont, Massachusetts 02478, USA. (Email: [ftarazi@hms.harvard.edu](mailto:ftarazi@hms.harvard.edu))

This study was funded by Forest Laboratories LLC, an Allergan affiliate, and Gedeon Richter Plc.

Editorial support was provided by Paul Ferguson of the Prescott Medical Communications Group (Chicago), a contractor of Allergan.

for another dopamine D<sub>2</sub>/D<sub>3</sub> receptor partial agonist, cariprazine. Aripiprazole and cariprazine treatment induced similar changes in 5-HT<sub>1A</sub>, NMDA, and AMPA receptors in different rat forebrain regions, which suggests that these receptors constitute common targets that mediate the actions of both drugs.

- Similar to other atypical antipsychotic drugs, aripiprazole treatment induced significant decreases in cortical 5-HT<sub>2A</sub> receptors. In contrast, cariprazine failed to downregulate serotonin 5-HT<sub>2A</sub> receptors, which may suggest a minimal role for this receptor in mediating the actions of cariprazine.
- Cariprazine's unique effects on dopamine and serotonin receptor subtypes, as well as its shared effects on glutamate receptor subtypes, may contribute to the improved clinical benefits of this novel atypical antipsychotic drug in the treatment of schizophrenia and bipolar mania.

## Introduction

Dysfunctions in dopaminergic, serotonergic, and glutamatergic neurotransmissions are believed to play an important role in the pathophysiology and symptomatology of schizophrenia and other idiopathic psychotic disorders.<sup>1-4</sup> In addition to their effects at dopamine receptor subtypes, atypical antipsychotics modulate serotonergic and glutamatergic neurotransmission, which may contribute to their clinical efficacy, safety, and tolerability.<sup>1,5,6</sup>

The atypical antipsychotics cariprazine and aripiprazole are both dopamine D<sub>2</sub>/D<sub>3</sub> receptor partial agonists; however, the drugs differ in that cariprazine is D<sub>3</sub> receptor-preferring and has moderate affinity for serotonin 5-HT<sub>1A</sub> receptors.<sup>7</sup> Moreover, cariprazine, but not aripiprazole, demonstrated high and balanced occupancy of both D<sub>2</sub> and D<sub>3</sub> receptors at pharmacologically effective and antipsychotic doses in both animals<sup>8</sup> and schizophrenia patients.<sup>9</sup> In animal behavioral models, cariprazine showed procognitive<sup>10</sup> and antidepressant-like effects,<sup>11</sup> suggesting the potential benefits of cariprazine for both the cognitive deficits and depressive symptoms of schizophrenia. These effects of cariprazine are likely to be mediated by D<sub>3</sub> receptors, since they were absent in transgenic knockout mice that lack expression of functional D<sub>3</sub> receptors.<sup>12,13</sup> We recently reported that long-term treatment with cariprazine increased D<sub>2</sub> and D<sub>3</sub> receptor levels in selected rat forebrain regions, whereas other typical and atypical antipsychotics upregulated D<sub>2</sub> receptors with no effects on D<sub>3</sub> receptors.<sup>14</sup> In the present study, we quantified the long-term effects of aripiprazole on D<sub>2</sub> and D<sub>3</sub> receptors and compared them to findings previously obtained for cariprazine and other antipsychotics.

Previous studies in rats have shown that long-term treatments with the atypical antipsychotics olanzapine,

risperidone, quetiapine, and asenapine increased serotonin 5-HT<sub>1A</sub> receptor levels in the medial prefrontal cortex (MPC) and dorsolateral frontal cortex (DFC) while decreasing 5-HT<sub>2A</sub> receptor levels in the same regions.<sup>15,16</sup> Additionally, antipsychotic exposure altered the levels of ionotropic glutamate *N*-methyl-D-aspartic acid (NMDA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the caudate putamen, nucleus accumbens, and hippocampus.<sup>17-19</sup> However, all of the tested atypical antipsychotics displayed combined dopamine D<sub>2</sub>/serotonin 5-HT<sub>2A</sub> receptor antagonism, which is a common feature of atypical antipsychotic drugs.<sup>1,5</sup> The chronic effects of D<sub>2</sub>/D<sub>3</sub> receptor partial agonists on serotonin and glutamate receptors require further investigation. Accordingly, we investigated the effects of long-term aripiprazole and cariprazine treatment on serotonin (5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>) and glutamate (NMDA and AMPA) receptor subtypes in the rat forebrain.

## Methods

### Materials

Tritium autoradiography standards and [2,3-<sup>3</sup>H]R(+)-8-hydroxy-*N,N*-di-*n*-propyl-2-amino-1,2,3,4-tetrahydronaphthalene ([<sup>3</sup>H]8-OH-DPAT; 135 Ci/mmol), [<sup>3</sup>H]3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]quinazolin-2,4(1H,3H)-dione ([<sup>3</sup>H]ketanserin; 50 Ci/mmol), [<sup>3</sup>H](+)-5-methyl-10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine ([<sup>3</sup>H]MK801; 23 Ci/mmol), and [<sup>3</sup>H]2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid ([<sup>3</sup>H]AMPA; 58 Ci/mmol) were obtained from Amersham Life Science (Arlington Heights, IL, USA). Kodak Biomax MR film was purchased from Thermo Fisher Scientific (Waltham, MA, USA). D-19 photographic developer and fixative were obtained from Eastman Kodak (Rochester, NY, USA).

Aripiprazole HCl and cariprazine HCl were donated by Forest Laboratories, Inc. (New York, NY, USA). Pargyline HCl, ketamine, 7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile (CNQX), ketanserin tartrate, prazosin, tetrabenzaine, serotonin (5-HT), methysergide, L-glutamate, glycine, and spermine were obtained from Sigma Research Biochemicals International (Natick, MA, USA). Cation hydrochlorides, guanosine-5'-triphosphate sodium (GTP), and tris-(hydroxymethyl)aminomethane-HCl (Tris) were obtained from Sigma Chemicals (St. Louis, MO, USA).

### Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA), initially weighing 200–225 g, were maintained under controlled artificial daylight (7:00 AM–7:00 PM), temperature, and humidity, with free access

to standard food and tap water in a USDA-inspected, veterinarian-supervised, small-animal research facility of the Mailman Research Center, with approval by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital.

### Treatment and tissue preparation

For 28 days, 7 groups of rats (8 animals/group) received a daily intraperitoneal injection of vehicle control (distilled water, 1 ml/kg) or active treatment of aripiprazole (2, 5, or 15 mg/kg) or cariprazine (0.06, 0.20, or 0.60 mg/kg). The selected doses were active in different behavioral paradigms.<sup>8,20</sup> No gross effects on motor behaviors or significant changes in body weight were observed after repeated treatment with the different doses of aripiprazole or cariprazine compared with vehicle-treated animals.

At the end of the treatment period, rats were decapitated 24 hours after the last injection; their brains were quickly removed, frozen in chilled isopentane, and stored at  $-80^{\circ}\text{C}$ . Brain regions assessed included the medial prefrontal cerebral cortex (mPFC), dorsolateral frontal cortex (DFC) (3.2–4.2 mm anterior to bregma), nucleus accumbens (NAc) (1.7–2.2 mm anterior to bregma), medial caudate putamen (CP-M) and lateral caudate putamen (CP-L) (0.7–1.2 mm anterior to bregma), hippocampus (HIP), and entorhinal cortex (0.2–0.7 mm anterior to bregma).<sup>21</sup> The selected extrapyramidal, limbic, and cortical regions of interest mediate the cognitive, emotional, and motor behaviors that are typically disturbed in patients with schizophrenia and other psychotic disorders.<sup>1</sup>

### Receptor autoradiography and image analysis

Coronal sections (10  $\mu\text{m}$ ) of these samples were cut in a cryostat at  $-20^{\circ}\text{C}$ , mounted on gelatin-coated microscope slides, and stored at  $-80^{\circ}\text{C}$ . To minimize experimental variability, brain sections from all vehicle- and drug-treated animals used for a given receptor subtype assay were evaluated at the same time.

### Dopamine receptor autoradiography

For the  $D_2$  and  $D_3$  assays, sections were first preincubated for 1 h at room temperature (RT). We have previously shown that this preincubation step is effective in minimizing the effects of endogenous ligands and the potential interference of residual drugs.<sup>22</sup>

#### $D_2$ receptor binding

Sections were first preincubated for 1 h at RT in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM  $\text{CaCl}_2$ , and 1 mM  $\text{MgCl}_2$ . Sections were

incubated for 1 h at RT in the same buffer containing 1.0 nM [ $^3\text{H}$ ]nemonapride with 0.5  $\mu\text{M}$  DTG and 0.1  $\mu\text{M}$  pindolol to mask sigma ( $\sigma_{1,2}$ ) and 5-HT<sub>1A</sub> sites, respectively. Nonspecific binding (NSB) was determined with 10  $\mu\text{M}$  S(-)-sulpiride. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in cold water, and air-dried. Radiolabeled slides and [ $^3\text{H}$ ] standards were exposed to Hyperfilm for 4 weeks at  $4^{\circ}\text{C}$ .<sup>23,24</sup>

#### $D_3$ receptor binding

Sections were preincubated for 1 h in 50 mM Tris-HCl buffer (pH 7.4) containing 0.3 mM GTP and 40 mM NaCl (no  $\text{MgCl}_2$  added) to minimize labeling of the high-affinity agonist binding state of  $D_2$  receptors, and then incubated for 1 h in the same buffer containing 3 nM [ $^3\text{H}$ ]7-OH-DPAT, with 5  $\mu\text{M}$  DTG to mask sigma sites. NSB was determined with 1  $\mu\text{M}$  S(-)-eticlopride. After incubation, slides were washed (2  $\times$  3 min) in ice-cold buffer and dried. Radiolabeled slides and [ $^3\text{H}$ ] standards were exposed to Hyperfilm for 6 weeks at  $4^{\circ}\text{C}$ .<sup>23,24</sup>

### Serotonin receptor autoradiography

#### 5-HT<sub>1A</sub> receptor binding

Sections were preincubated for 1 h at RT in 50 mM Tris-HCl buffer (pH 7.6) containing ascorbic acid (0.1%, w/v),  $\text{CaCl}_2$  (4 mM), and the monoamine oxidase inhibitor pargyline-HCl (10  $\mu\text{M}$ ). Sections were then incubated for another 60 min at RT in fresh buffer containing 2.0 nM [ $^3\text{H}$ ]8-OH-DPAT. NSB was determined with 1  $\mu\text{M}$  5-HT. After incubation, slides were washed (2  $\times$  5 min) in ice-cold buffer and air-dried. Radiolabeled slides and [ $^3\text{H}$ ] standards were exposed to Hyperfilm for 5 weeks at  $4^{\circ}\text{C}$ .<sup>15</sup>

#### 5-HT<sub>2A</sub> receptor binding

Sections were preincubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.7), and then incubated for 1 h at RT in fresh buffer containing 3.0 nM [ $^3\text{H}$ ]ketanserin, 1  $\mu\text{M}$  prazosin (to block  $\alpha_1$ -adrenoceptors), and 100 nM tetrabenazine (to block monoaminergic nerve terminal sites). NSB was determined with 1  $\mu\text{M}$  methysergide. After incubation, slides were washed (2  $\times$  30 min) in ice-cold buffer, dipped in cold water, and air-dried. Radiolabeled slides and calibrated [ $^3\text{H}$ ] standards were exposed to Hyperfilm for 5 weeks at  $4^{\circ}\text{C}$ .<sup>15</sup>

### Glutamate receptor autoradiography

#### NMDA receptor binding

Sections were preincubated for 60 min in 50 mM Tris-HCl buffer (pH 7.4) to remove endogenous ligand and then incubated for 150 min at RT in buffer containing

10 nM [<sup>3</sup>H]MK-801, 100 μM L-glutamate, 100 μM glycine, 1 mM EDTA, and 75 μM spermine to enhance the binding of [<sup>3</sup>H]MK-801 to NMDA receptors. NSB was determined with 20 μM ketamine. After incubation, slides were washed in ice-cold buffer (2 × 20 min) and then air-dried. Radiolabeled slides and calibrated [<sup>3</sup>H] standards were exposed to Hyperfilm for 4 weeks at 4 °C.<sup>17,18</sup>

#### *AMPA receptor binding*

Sections were preincubated for 60 min in 50 mM Tris-HCl buffer (pH 7.3) at 4 °C and then incubated in fresh buffer containing 20 nM [<sup>3</sup>H]AMPA for 60 min at 4 °C.<sup>18</sup> NSB was determined with 20 μM unlabeled CNQX. After incubation, slides were washed (3 × 15 sec) in ice-cold buffer and air-dried. Radiolabeled slides and [<sup>3</sup>H] standards were exposed to Hyperfilm for 3 weeks at 4 °C.<sup>17,18</sup>

#### **Image analysis**

Biomax MR films were developed and fixed in Kodak D-19 for 5 min at RT; images were quantified using an MCID image analyzer and MCID-M4 Imaging Research software (St. Catharines, Ontario, Canada). A calibration curve was generated from [<sup>3</sup>H]-microscale standards that were exposed along with tissue sections. Regions of interest were outlined and their optical density measured, with the left and right sides of two contiguous sections representing total binding and matching samples representing nonspecific binding. Four determinations were averaged for each subject sample. Optical density of sampled regions was measured, and the amount of ligand bound was calculated as nCi/mg tissue and expressed as fmol bound/mg tissue.

#### **Statistical analysis**

Receptor binding data were analyzed first for overall effects of drug versus vehicle for all receptor types and brain regions using multiple regression modeling methods. Density measures were logarithmically transformed to achieve more Gaussian-like distributions before regression modeling. Model goodness-of-fit was checked using partial residual plot methods. Since individual brain specimens provided receptor-binding data for several brain regions, resulting in incomplete independence across observations, we used robust standard error estimates to adjust for this clustering effect. This estimation method permits relaxation of the assumption of independence of all observations and requires only that the observations be independent across specimens.<sup>25</sup> Estimates of interaction effects were employed for post-hoc tests of drug effects for specific receptors and brain regions, with adjustment of *p* values obtained from the regression analyses estimating these multiple comparisons by the standard method of Sidák.<sup>26</sup> Data are presented as means ± standard error of the mean

(SEM). Comparisons were considered significant at *p* < 0.05 (two-tailed test).

## **Results**

### *Long-term effects of aripiprazole on dopamine receptors*

Aripiprazole increased D<sub>2</sub> receptor levels in mPFC (29–48%). However, only the high dose of aripiprazole (15 mg/kg) increased D<sub>2</sub> receptors in NAc (40%), medial (35%), and lateral (50%) CPu, and HIPP (57%) (Table 1). Chronic aripiprazole treatment (5 and 15 mg/kg) significantly increased D<sub>3</sub> receptors in the olfactory tubercle (OT) (15–37%) and Islands of Calleja (IC) (18–41%) (Table 1). However, aripiprazole, unlike cariprazine, did not alter the levels of D<sub>3</sub> receptors in the shell of the NAc at any of the doses tested (Table 1).

### *Long-term effects of aripiprazole and cariprazine on 5-HT<sub>1A</sub> receptors*

Aripiprazole (2, 5, or 15 mg/kg) treatment dose-dependently increased 5-HT<sub>1A</sub> receptors in the MPC (24–59%) and DFC (20–39%). Higher doses of aripiprazole (5 and 15 mg/kg) also increased 5-HT<sub>1A</sub> receptors in the HIPP-CA1 (31 and 56%) and HIPP-CA3 (30 and 50%) regions (Table 1, Figure 1A). Similar to aripiprazole, cariprazine (0.06, 0.2, or 0.6 mg/kg) treatment dose-dependently increased 5-HT<sub>1A</sub> receptor binding in the hippocampal CA1 (33, 42, and 50%, respectively) and CA3 (29, 39, and 52%, respectively) regions. In addition, repeated treatment with higher doses of cariprazine (0.2 and 0.6 mg/kg) increased 5-HT<sub>1A</sub> receptors in the MPC (41 and 61%) and DFC (42 and 61%) (Table 1, Figure 1B).

### *Long-term effects of aripiprazole and cariprazine on 5-HT<sub>2A</sub> receptors*

Aripiprazole (2, 5, or 15 mg/kg) decreased 5-HT<sub>2A</sub> receptors in the MPC (33, 36, and 42%, respectively) and DFC (32, 39, and 43%, respectively). Aripiprazole (5 and 15 mg/kg) also decreased 5-HT<sub>2A</sub> receptors in the HIPP-CA1 (28 and 32%) and HIPP-CA3 (26 and 30%) regions (Table 1, Figure 2A). In contrast, long-term administration of cariprazine (0.06, 0.2, and 0.6 mg/kg) failed to alter 5-HT<sub>2A</sub> receptor levels in all forebrain regions examined (Table 1, Figure 2B).

### *Long-term effects of aripiprazole and cariprazine on NMDA receptors*

Long-term administration of higher doses of aripiprazole (5 and 15 mg/kg) decreased NMDA receptors in the NAc (26 and 30%), CP-M (29 and 31%), and CP-L (30 and 32%) regions. Aripiprazole also dose-dependently

TABLE 1. Changes in forebrain receptors following long-term exposure to cariprazine and aripiprazole

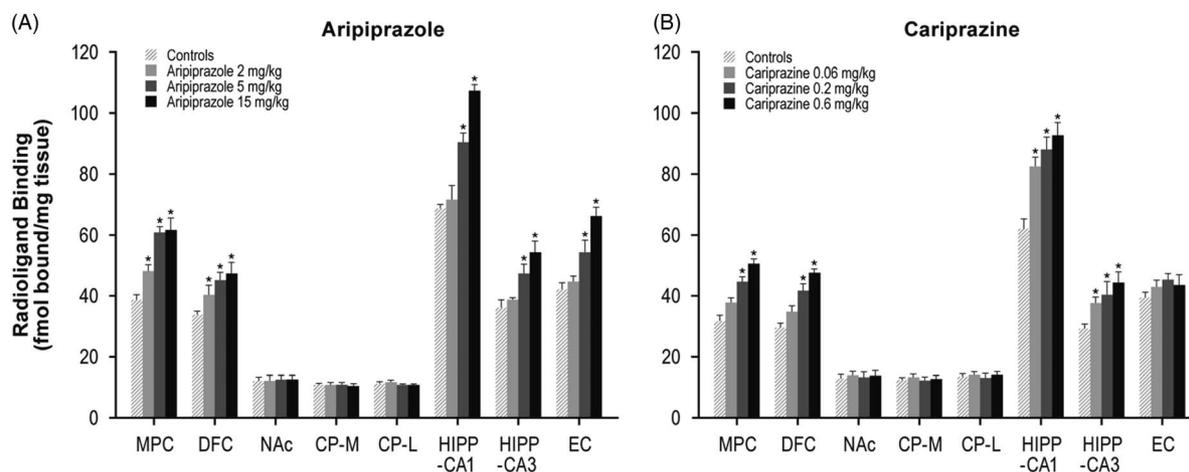
Brain region	% Control					
	Cariprazine			Aripiprazole		
	0.06 mg/kg	0.2 mg/kg	0.6 mg/kg	2 mg/kg	5 mg/kg	15 mg/kg
<b>Serotonin receptor subtypes</b>						
<b>5-HT<sub>1A</sub></b>						
Cerebral cortex						
Medial prefrontal	–	+41*	+61*	+25*	+57*	+59*
Dorsolateral	–	+42*	+61*	+20*	+34*	+40*
Hippocampus						
CA1	+33*	+42*	+50*	–	+32*	+57*
CA3	+29*	+39*	+52*	–	+30*	+50*
Entorhinal cortex	–	–	–	–	+29*	+57*
<b>5-HT<sub>2A</sub></b>						
Cerebral cortex						
Medial prefrontal	–	–	–	–33*	–36*	–42*
Dorsolateral	–	–	–	–32*	–39*	–43*
Hippocampus						
CA1	–	–	–	–	–29*	–32*
CA3	–	–	–	–	–26*	–30*
<b>Glutamate Receptor Subtypes</b>						
<b>NMDA</b>						
Cerebral cortex						
Medial prefrontal	–	–	–	–	–	–
Dorsolateral	–	–	–	–	–	–
Nucleus accumbens	–	–37*	–40*	–	–25*	–30*
Caudate putamen						
Medial	–	–34*	–38*	–	–29*	–30*
Lateral	–	–35*	–38*	–	–30*	–32*
Hippocampus						
CA1	–	–25*	–28*	–18*	–27*	–33*
CA3	–	–23*	–30*	–20*	–26*	–34*
<b>AMPA</b>						
Cerebral cortex						
Medial prefrontal	–	–	–	–	–	–
Dorsolateral	–	–	–	–	–	–
Hippocampus						
CA1	–	+31*	+47*	–	+45*	+75*
CA3	–	+34*	+50*	–	–	+79*
<b>Dopamine Receptor Subtypes</b>						
<b>D<sub>2</sub><sup>a</sup></b>						
Cerebral cortex						
Medial prefrontal	–	+27*	+43*	–	+29*	+48*
Nucleus accumbens	–	+40*	+45*	–	–	+40*
Caudate putamen						
Medial	–	+41*	+53*	–	–	+35*
Lateral	–	+52*	+63*	–	–	+50*
Hippocampus	–	–	+38*	–	–	+57*
<b>D<sub>3</sub><sup>a</sup></b>						
Islands of Calleja	+32*	+41*	+57*	–	+18*	+41*
Olfactory tubercle	+27*	+49*	+67*	–	+15*	+37*
Nucleus accumbens						
Shell	–	+31*	+48*	–	–	–

\* $p < 0.05$  versus respective control; – = not significantly different from respective control.

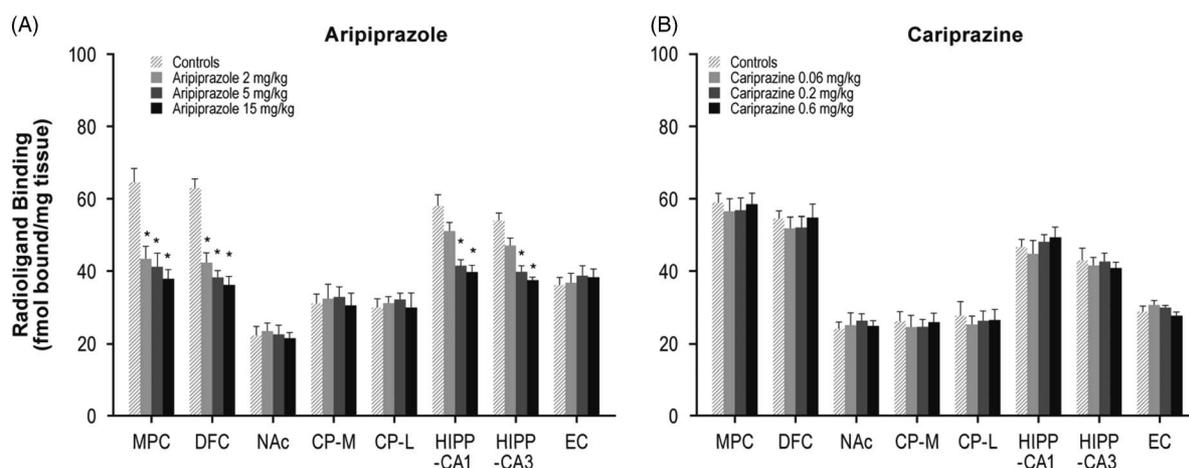
<sup>a</sup> Cariprazine data are reproduced from Choi *et al.*<sup>27</sup>

decreased NMDA receptors in the HIPP-CA1 (18, 27, and 33%) and HIPP-CA3 (20, 25, and 34%) regions (Table 1, Figure 3A). Similarly, long-term administration of higher doses of cariprazine (0.2 and 0.6 mg/kg) significantly reduced NMDA receptor binding in the

NAC (37 and 40%), CP-M (34 and 38%), and CP-L (35 and 38%) regions. In addition, repeated treatment with 0.2 and 0.6 mg/kg cariprazine decreased NMDA receptor binding in hippocampal CA1 (25 and 28%) and CA3 (23 and 30%) (Table 1, Figure 3B).



**FIGURE 1.** Long-term effects of cariprazine and aripiprazole on serotonin 5-HT<sub>1A</sub> receptors. Data are mean  $\pm$  SEM (*n* = 8/group). \* *p* < 0.05 vs. control. CP-L indicates caudate putamen-lateral; CP-M = caudate putamen-medial; DFC = dorsolateral frontal cortex; EC = entorhinal cortex; HIPP = hippocampus; MPC = medial prefrontal cortex; NAc = nucleus accumbens.



**FIGURE 2.** Long-term effects of cariprazine and aripiprazole on serotonin 5-HT<sub>2A</sub> receptors. Data are mean  $\pm$  SEM (*n* = 8/group). \* *p* < 0.05 vs. control. CP-L indicates caudate putamen-lateral; CP-M = caudate putamen-medial; DFC = dorsolateral frontal cortex; EC = entorhinal cortex; HIPP = hippocampus; MPC = medial prefrontal cortex; NAc = nucleus accumbens.

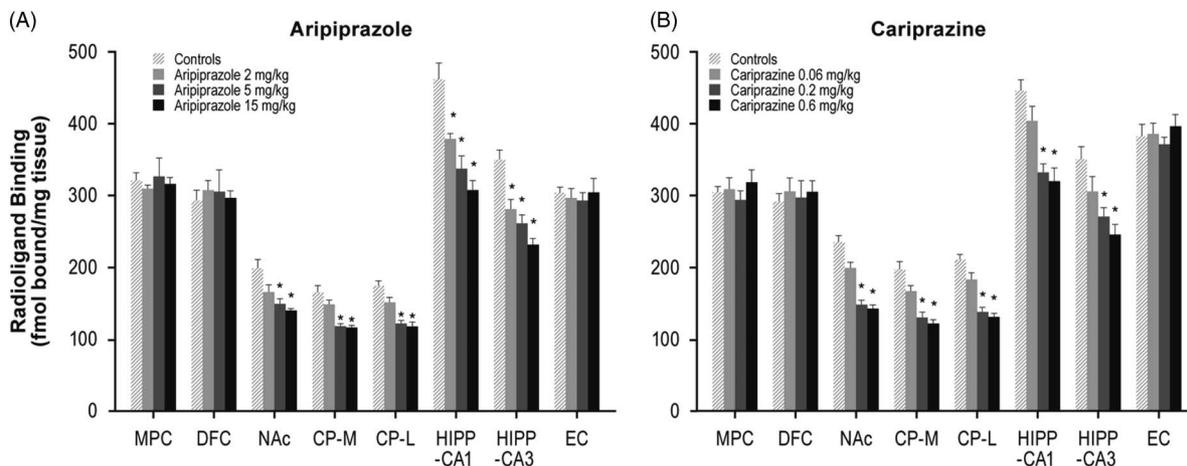
### Long-term effects of aripiprazole and cariprazine on AMPA receptors

Aripiprazole at 5 and 15 mg/kg increased [<sup>3</sup>H]AMPA in the NAc (26 and 30%), CP-M (29 and 31%), and CP-L (30 and 32%) regions. Aripiprazole also dose-dependently increased AMPA receptors in the HIPP-CA1 (18, 27, and 33%) and HIPP-CA3 (20, 25, and 34%) regions (Table 1, Figure 4A). Repeated treatment with the higher doses of cariprazine (0.2 and 0.6 mg/kg) dose-dependently increased [<sup>3</sup>H]AMPA binding in the HIPP-CA1 (31 and 47%) and HIPP-CA3 (34 and 50%) regions. Cariprazine treatment did not alter AMPA receptors in the other brain regions examined (Table 1, Figure 4B).

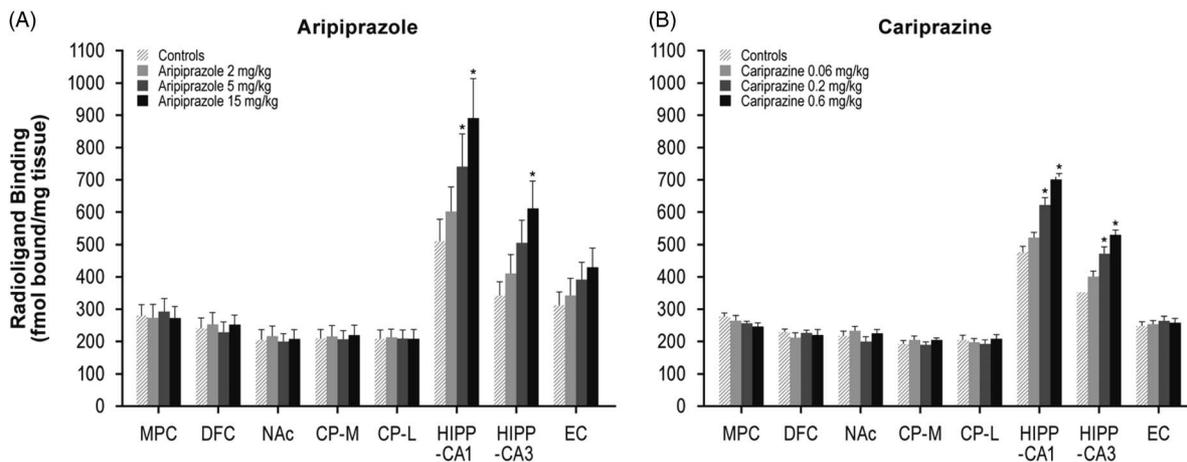
### Discussion

#### Long-term effects of aripiprazole and cariprazine on dopamine D<sub>2</sub>/D<sub>3</sub> receptors

Chronic aripiprazole treatment upregulated D<sub>2</sub> receptor levels in different brain regions, with a maximum increase similar to that previously described for cariprazine and other antipsychotics (Table 1; Choi *et al.*<sup>27</sup>). However, aripiprazole was at least 25-fold less potent than cariprazine (Table 1; Choi *et al.*<sup>27</sup>). Aripiprazole also increased D<sub>3</sub> receptor levels in selective brain regions; however, these effects were less pronounced (cariprazine had ~80-fold or greater potency for D<sub>3</sub> receptors) and were produced in fewer brain regions



**FIGURE 3.** Long-term effects of cariprazine and aripiprazole on NMDA receptors. Data are mean  $\pm$  SEM ( $n = 8$ /group). \*  $p < 0.05$  vs. control. CP-L indicates caudate putamen-lateral; CP-M = caudate putamen-medial; DFC = dorsolateral frontal cortex; EC = entorhinal cortex; HIPP = hippocampus; MPC = medial prefrontal cortex; NAc = nucleus accumbens.



**FIGURE 4.** Long-term effects of cariprazine and aripiprazole on AMPA receptors. Data are mean  $\pm$  SEM ( $n = 8$ /group). \*  $p < 0.05$  vs. control. CP-L indicates caudate putamen-lateral; CP-M = caudate putamen-medial; DFC = dorsolateral frontal cortex; EC = entorhinal cortex; HIPP = hippocampus; MPC = medial prefrontal cortex; NAc = nucleus accumbens.

than previously described for cariprazine (Table 1; Choi *et al.*<sup>27</sup>). In particular, aripiprazole produced no effect at any dose tested on D<sub>3</sub> receptors in the shell of the NAc, whereas cariprazine significantly increased these receptors in the same brain region. Increases in D<sub>3</sub> receptor expression in the shell of the NAc has been proposed to be a common neurobiological mechanism of antidepressant treatments.<sup>28</sup> These findings suggest that cariprazine is more potent than aripiprazole in upregulating D<sub>3</sub> receptor subtypes, and that D<sub>3</sub> receptors play a more prominent role in mediating the actions of cariprazine compared to aripiprazole. Moreover, the unique upregulation of dopamine D<sub>3</sub> receptors in areas related to antidepressant action may offer therapeutic benefits against various mood disorders, including depression and the negative symptoms of schizophrenia.

#### Long-term effects of aripiprazole and cariprazine on serotonin 5-HT<sub>1A</sub> receptors

Repeated treatment with the three doses of aripiprazole (2, 5, and 15 mg/kg) and higher doses of cariprazine (0.2 and 0.6 mg/kg) dose-dependently increased the binding of the 5-HT<sub>1A</sub> radioligand [<sup>3</sup>H]8-OH-DPAT in MPC and DFC (Table 1, Figure 1A). This finding is consistent with the effects of the atypical antipsychotics olanzapine, risperidone, quetiapine, and asenapine on the same receptor in the same brain region.<sup>15,16,29</sup> Both aripiprazole and cariprazine have substantial affinity for 5-HT<sub>1A</sub> receptors ( $K_i = 5.6$  and  $2.6$  nM, respectively) and display partial agonist activity against this receptor subtype.<sup>7,30</sup> Observed increases in cortical 5-HT<sub>1A</sub> receptors by both drugs may reflect direct blockade of 5-HT<sub>1A</sub> receptors or

secondary changes resulting from more potent effects on dopamine D<sub>2</sub> and D<sub>3</sub> receptors.<sup>7,14,30</sup>

Earlier studies reported that 5-HT<sub>1A</sub> receptors are more highly expressed in cortical and limbic brain regions than in the basal ganglia.<sup>31,32</sup> In the frontal cortex, 5-HT<sub>1A</sub> receptors are located on neocortical glutamatergic pyramidal neurons that are typically implicated in different cognitive functions.<sup>33</sup> Additional studies have suggested that 5-HT<sub>1A</sub> partial agonism can stimulate cortical dopamine release<sup>34–36</sup> and minimize the incidence of antipsychotic-induced extrapyramidal side effects.<sup>37,38</sup> 5-HT<sub>1A</sub> receptors have increasingly become attractive targets for novel agents to treat schizophrenia and other psychiatric disorders.<sup>39,40</sup> Aripiprazole- and cariprazine-induced increases in cortical 5-HT<sub>1A</sub> receptors further validate the hypothesis that the 5-HT<sub>1A</sub> receptor may contribute, at least in part, to the beneficial therapeutic effects of these two agents as well as other dissimilar atypical antipsychotic drugs.<sup>15</sup>

Repeated administration of aripiprazole (5 and 15 mg/kg) and cariprazine (0.06, 0.2 or 0.6 mg/kg) dose-dependently increased binding of the 5-HT<sub>1A</sub> radioligand [<sup>3</sup>H]8-OH-DPAT in hippocampal CA1 and CA3 regions. Asenapine is the only atypical antipsychotic that shares this effect, as other atypical antipsychotics, including olanzapine, risperidone, and quetiapine, did not alter 5-HT<sub>1A</sub> receptors in rat hippocampus.<sup>15,16</sup> These findings suggest that aripiprazole and cariprazine can influence hippocampal 5-HT<sub>1A</sub> receptors and consequently normalize the increased hippocampal activity postulated to occur in schizophrenic patients and ameliorate the psychotic symptoms of the disease.<sup>41</sup>

#### *Long-term effects of aripiprazole and cariprazine on serotonin 5-HT<sub>2A</sub> receptors*

Long-term treatment with all three aripiprazole doses significantly and equipotently decreased 5-HT<sub>2A</sub> receptor binding in the MPC and DFC regions. Aripiprazole has high affinity for 5-HT<sub>2A</sub> receptors (K<sub>i</sub> = 8.7 nM),<sup>30</sup> and the decrease in 5-HT<sub>2A</sub> binding may reflect direct blockade of 5-HT<sub>2A</sub> receptors by the different doses of aripiprazole. We have previously reported that long-term administration of several atypical antipsychotics—including clozapine, olanzapine, risperidone, quetiapine, and asenapine—but not the typical antipsychotic haloperidol, resulted in decreased 5-HT<sub>2A</sub> receptor labeling in rat frontal cerebral cortex.<sup>15,22,42,43</sup> 5-HT<sub>2A</sub> receptor downregulation may minimize the higher incidence of extrapyramidal side effects associated with typical antipsychotic drug treatment.<sup>1,44,45</sup> Aripiprazole (5 and 15 mg/kg) also decreased 5-HT<sub>2A</sub> receptors in the hippocampal CA1 and CA3 regions. Such downregulation may further contribute to the stabilization of dopamine neurotransmission in the limbic system and, consequently, to the improvement of

affective and emotional behaviors typically disturbed in schizophrenia patients.<sup>1</sup>

In contrast, cariprazine failed to alter 5-HT<sub>2A</sub> receptor binding in all the forebrain regions examined. The lack of effect of cariprazine on 5-HT<sub>2A</sub> receptors may result from its lower in vitro affinity to these receptors, resulting in insufficient occupancy of 5-HT<sub>2A</sub> receptors to the level required to induce receptor changes. These findings suggest that unlike several of the atypical antipsychotics, which downregulate cortical 5-HT<sub>2A</sub> receptors,<sup>15,22,42,43</sup> 5-HT<sub>2A</sub> receptors are less likely to contribute to the mechanism of action of cariprazine in vivo.

#### *Long-term effects of aripiprazole and cariprazine on glutamate NMDA receptors*

Continuous treatment with the higher doses of aripiprazole (5 and 15 mg/kg) and cariprazine (0.2 and 0.6 mg/kg) significantly decreased binding of [<sup>3</sup>H]MK-801 to NMDA receptors in the NAc and the medial and lateral CPu. These effects were similar to those previously reported for the atypical antipsychotics clozapine, olanzapine, risperidone, and asenapine, and the experimental atypical antipsychotic JL13, but not the typical agent haloperidol.<sup>17–19,46</sup> Aripiprazole- and cariprazine-induced reductions in striatal NMDA receptors may result from indirect interactions with dopamine and serotonin systems, both of which modulate glutamatergic neurotransmission.<sup>47,48</sup> Such reductions in striatal NMDA receptors may reflect post-transcriptional modifications, since dissimilar atypical antipsychotics did not alter expression of mRNA-encoding NMDA-forming subunits in rat striatum.<sup>49,50</sup>

We have demonstrated previously that acute and subchronic administration of cariprazine, as well as other atypical antipsychotics, reverses both NMDA receptor antagonist-mediated hypermobility<sup>8</sup> and PCP-induced cognitive and social deficits in animal models,<sup>10,12,51</sup> indicating an ability to normalize abnormal glutamatergic transmission; however, the potential neurochemical and behavioral consequences of long-term aripiprazole- and cariprazine-induced reductions in striatal NMDA receptors are not clear. Studies reported that stimulation of NMDA receptors promoted typical antipsychotic-induced catalepsy, an animal model predictive of extrapyramidal side effects.<sup>52</sup> In contrast, blockade of NMDA receptors attenuated the cataleptic effects of typical antipsychotic drugs.<sup>53</sup> Downregulation of striatal NMDA receptors by aripiprazole, cariprazine, and several dissimilar atypical antipsychotic drugs,<sup>18,22</sup> as well as the subsequent modification of glutamate neurotransmission in the basal ganglia, may contribute, at least in part, to the benign extrapyramidal profile of atypical antipsychotic agents.<sup>45</sup>

Repeated treatment with aripiprazole and cariprazine reduced NMDA receptor binding in the hippocampal

CA1 and CA3 regions. These effects are similar to the observed reduction in hippocampal NMDA receptors after repeated administration of the atypical antipsychotics olanzapine and risperidone.<sup>18</sup> Reductions in hippocampal NMDA receptors may normalize the abnormalities in glutamatergic neurotransmission postulated to occur in the hippocampus of schizophrenia patients,<sup>54</sup> and thus subsequently improve their psychotic symptoms.<sup>55</sup>

### Long-term effects of aripiprazole and cariprazine on glutamate AMPA receptors

The three doses of aripiprazole and the higher doses of cariprazine (0.2 and 0.6 mg/kg) dose-dependently increased AMPA receptors in the hippocampal CA1 and CA3 regions. These findings are in agreement with the atypical antipsychotic asenapine, which induced increases in hippocampal AMPA receptors.<sup>19</sup> Such increases in AMPA receptors may result from the actions of both aripiprazole and cariprazine on D<sub>2</sub> receptors, which are coexpressed with AMPA in rat brains.<sup>56</sup> Increases in hippocampal AMPA receptors may further contribute to the improvement of psychotic symptoms in schizophrenia patients. Interestingly, long-term administration of aripiprazole and cariprazine produced opposite effects on NMDA versus AMPA receptors, suggesting that ionotropic glutamate receptors may respond differently to repeated treatment with partial D<sub>2</sub>/D<sub>3</sub> receptor agonists.

### Conclusions

Long-term treatment with aripiprazole induced increases in D<sub>3</sub> receptors in OT and IC. In contrast, repeated treatment with cariprazine produced more profound increases in D<sub>3</sub> receptors in OT and IC, and extended its effect to induce increases in D<sub>3</sub> receptors in the shell of the NAc. These findings are in agreement with the potent affinity of cariprazine at D<sub>3</sub> receptors, and provide a distinction in the action mechanisms of cariprazine versus aripiprazole in selectively targeting D<sub>3</sub> receptors as leading sites that mediate the beneficial actions of cariprazine.

Repeated treatment with aripiprazole and cariprazine induced similar changes in 5-HT<sub>1A</sub>, NMDA, and AMPA receptors in different rat forebrain regions. In contrast, aripiprazole downregulated 5-HT<sub>2A</sub> receptors in the frontal cortex, an effect not seen with cariprazine. Accordingly, 5-HT<sub>2A</sub> receptors may contribute more to the molecular actions of aripiprazole and less to the actions of cariprazine. In conclusion, the combined effects of cariprazine on serotonin and glutamate receptors, together with its unique actions on dopamine D<sub>3</sub> receptors, may confer the clinical benefits, safety, and

tolerability of cariprazine in improved treatment of schizophrenia and bipolar mania.

### Disclosures

Yong Kee Choi hereby states that he has no conflicts of interest to declare. Nika Adham is an employee of Allergan. Béla Kiss is an employee of Gedeon Richter Plc. István Gyertyán was an employee of Gedeon Richter Plc at the time of the study. Frank I. Tarazi has received research grants from Forest Laboratories LLC, H. Lundbeck A/S, and Shire Plc.

### REFERENCES:

- Baldessarini RJ, Tarazi FI. Pharmacotherapy of psychosis and mania. In: Brunton LL, Lazo JS, Parker K, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill; 2005: 461–500.
- Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology*. 2012; **37**(1): 4–15; Epub ahead of print Sep 28, 2011. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3238069/pdf/npp2011181a.pdf>.
- Eggers AE. A serotonin hypothesis of schizophrenia. *Med Hypotheses*. 2013; **80**(6): 791–794; Epub ahead of print Apr 1.
- Hu W, MacDonald ML, Elswick DE, Sweet RA. The glutamate hypothesis of schizophrenia: evidence from human brain tissue studies. *Ann N Y Acad Sci*. 2015; **1338**: 38–57; Epub ahead of print Oct 14, 2014. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4363164/pdf/nihms625030.pdf>.
- Meltzer HY, Massey BW. The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr Opin Pharmacol*. 2011; **11**(1): 59–67; Epub ahead of print Mar 21.
- Ellenbroek BA, Cesura AM. Antipsychotics and the dopamine-serotonin connection. In: Celanire S, Poli S, eds. *Small Molecule Therapeutics for Schizophrenia*. Cham: Springer International Publishing; 2015: 1–49.
- Kiss B, Horváth A, Némethy Z, et al. Cariprazine (RGH-188), a dopamine D<sub>3</sub> receptor-preferring, D<sub>3</sub>/D<sub>2</sub> dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther*. 2010; **333**(1): 328–340; Epub ahead of print Jan 21. <http://jpet.aspetjournals.org/content/jpet/333/1/328.full.pdf>.
- Gyertyán I, Kiss B, Ságghy K, et al. Cariprazine (RGH-188), a potent D<sub>3</sub>/D<sub>2</sub> dopamine receptor partial agonist, binds to dopamine D<sub>3</sub> receptors in vivo and shows antipsychotic-like and procognitive effects in rodents. *Neurochem Int*. 2011; **59**(6): 925–935.
- Slifstein M, Abi-Dargham A, D'Souza DC, et al. Cariprazine demonstrates high dopamine D<sub>3</sub> and D<sub>2</sub> receptor occupancy in patients with schizophrenia: a clinical PET study with [<sup>11</sup>C]-(+)-PHNO. *Neuropsychopharmacology*. 2013; **38**(Suppl 2): S520.
- Neill JC, Grayson B, Kiss B, Gyertyán I, Ferguson P, Adham N. Effects of cariprazine, a novel antipsychotic, on cognitive deficit and negative symptoms in a rodent model of schizophrenia symptomatology. *Eur Neuropsychopharmacol*. 2016; **26**(1): 3–14; Epub ahead of print Nov 19, 2015.
- Papp M, Gruca P, Lason-Tyburkiewicz M, Adham N, Kiss B, Gyertyán I. Attenuation of anhedonia by cariprazine in the chronic mild stress model of depression. *Behav Pharmacol*. 2014; **25**(5–6): 567–574.
- Zimnisky R, Chang G, Gyertyán I, Kiss B, Adham N, Schmauss C. Cariprazine, a dopamine D<sub>3</sub>-receptor-preferring partial agonist,

- blocks phencyclidine-induced impairments of working memory, attention set-shifting, and recognition memory in the mouse. *Psychopharmacology*. 2013; **226**(1): 91-100; Epub ahead of print Oct 19, 2012. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572273/pdf/nihms416352.pdf>.
13. Duman RS, Duric V, Banasr M, Adham N, Kiss B, Gyertyán I. Cariprazine exhibits dopamine D<sub>3</sub>-receptor-dependent antidepressant-like activity in the chronic unpredictable stress model of anhedonia. *Neuropsychopharmacology*. 2012; **38**(Suppl 1): S84-S85.
  14. Tarazi FI, Kiss B, Choi YK, Adham N, Gyertyán I. Effects of chronic aripiprazole administration on dopamine receptors: comparison with cariprazine. *Neuropsychopharmacology*. 2014; **39**: S138-S139.
  15. Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on serotonin 1A, 2A and 2C receptors in rat forebrain regions. *Psychopharmacology*. 2002; **161**(3): 263-270; Epub ahead of print Apr 4.
  16. Tarazi FI, Moran-Gates T, Wong EH, Henry B, Shahid M. Asenapine induces differential regional effects on serotonin receptor subtypes. *J Psychopharmacol*. 2010; **24**(3): 341-348; Epub ahead of print Aug 21, 2008.
  17. Tarazi FI, Florijn WJ, Creese I. Regulation of ionotropic glutamate receptors following subchronic and chronic treatment with typical and atypical antipsychotics. *Psychopharmacology*. 1996; **128**(4): 371-379.
  18. Tarazi FI, Baldessarini RJ, Kula NS, Zhang K. Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther*. 2003; **306**(3): 1145-1151; Epub ahead of print Jun 26. <http://jpet.aspetjournals.org/content/jpet/306/3/1145.full.pdf>.
  19. Tarazi FI, Choi YK, Gardner M, Wong EH, Henry B, Shahid M. Asenapine exerts distinctive regional effects on ionotropic glutamate receptor subtypes in rat brain. *Synapse*. 2009; **63**(5): 413-420.
  20. Gao J, Qin R, Li M. Repeated administration of aripiprazole produces a sensitization effect in the suppression of avoidance responding and phencyclidine-induced hyperlocomotion and increases D<sub>2</sub> receptor-mediated behavioral function. *J Psychopharmacol*. 2015; **29**(4): 390-400; Epub ahead of print Jan 13. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4757439/pdf/nihms758943.pdf>.
  21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*, 2nd ed. New York: Academic Press; 1986.
  22. Florijn WJ, Tarazi FI, Creese I. Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. *J Pharmacol Exp Ther*. 1997; **280**(2): 561-569 <http://jpet.aspetjournals.org/content/jpet/280/2/561.full.pdf>.
  23. Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther*. 2001; **297**(2): 711-717 <http://jpet.aspetjournals.org/content/jpet/297/2/711.full.pdf>.
  24. Tarazi FI, Yeghiayan SK, Baldessarini RJ, Kula NS, Neumeyer JL. Long-term effects of (+)-N-propylnorapomorphine compared with typical and atypical antipsychotics: differential increases of cerebrocortical D<sub>2</sub>-like and striatolimbic D<sub>4</sub>-like dopamine receptors. *Neuropsychopharmacology*. 1997; **17**(3): 186-196 <http://www.nature.com/npp/journal/v17/n3/full/1395027a.html>.
  25. Greene WH. *Econometric Analysis*. Upper Saddle River, NJ: Prentice Hall; 2000. <http://stat.smmu.edu.cn/DOWNLOAD/ebook/econometric.pdf>.
  26. Winer BJ, Brown DR, Michels KM. *Statistical Principles in Experimental Design*, 3rd ed. New York: McGraw-Hill; 1991.
  27. Choi YK, Adham N, Kiss B, Gyertyán I, Tarazi FI. Long-term effects of cariprazine exposure on dopamine receptor subtypes. *CNS Spectr*. 2014; **19**(3): 268-277; Epub ahead of print Nov 8, 2013.
  28. Lammers CH, Diaz J, Schwartz JC, Sokoloff P. Selective increase of dopamine D<sub>3</sub> receptor gene expression as a common effect of chronic antidepressant treatments. *Mol Psychiatry*. 2000; **5**(4): 378-388.
  29. Newman-Tancredi A, Assie MB, Leduc N, Ormiere AM, Danty N, Cosi C. Novel antipsychotics activate recombinant human and native rat serotonin 5-HT<sub>1A</sub> receptors: affinity, efficacy and potential implications for treatment of schizophrenia. *Int J Neuropsychopharmacol*. 2005; **8**(3): 341-356; Epub ahead of print Feb 11.
  30. Shapiro DA, Renock S, Arrington E, et al. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology*. 2003; **28**(8): 1400-1411; Epub ahead of print May 21.
  31. Pazos A, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain, I: serotonin-1 receptors. *Brain Res*. 1985; **346**(2): 205-230.
  32. Pompeiano M, Palacios JM, Mengod G. Distribution and cellular localization of mRNA coding for 5-HT<sub>1A</sub> receptor in the rat brain: correlation with receptor binding. *J Neurosci*. 1992; **12**(2): 440-453 <http://www.jneurosci.org/content/jneuro/12/2/440.full.pdf>.
  33. Francis PT, Pangalos MN, Pearson RC, Middlemiss DN, Stratmann GC, Bowen DM. 5-Hydroxytryptamine<sub>1A</sub> but not 5-hydroxytryptamine<sub>2</sub> receptors are enriched on neocortical pyramidal neurones destroyed by intrastriatal volkensin. *J Pharmacol Exp Ther*. 1992; **261**(3): 1273-1281.
  34. Rollema H, Lu Y, Schmidt AW, Sprouse JS, Zorn SH. 5-HT<sub>1A</sub> receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biol Psychiatry*. 2000; **48**(3): 229-237.
  35. Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY. 5-HT<sub>2A</sub> and D<sub>2</sub> receptor blockade increases cortical DA release via 5-HT<sub>1A</sub> receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem*. 2001; **76**(5): 1521-1531.
  36. Bantick RA, Deakin JF, Grasby PM. The 5-HT<sub>1A</sub> receptor in schizophrenia: a promising target for novel atypical neuroleptics? *J Psychopharmacol*. 2001; **15**(1): 37-46.
  37. Christoffersen CL, Meltzer LT. Reversal of haloperidol-induced extrapyramidal side effects in Cebus monkeys by 8-hydroxy-2-(di-n-propylamino)tetralin and its enantiomers. *Neuropsychopharmacology*. 1998; **18**(5): 399-402 <http://www.nature.com/npp/journal/v18/n5/full/1395157a.html>.
  38. Daniel DG, Zimbardo DL, Potkin SG, Reeves KR, Harrigan EP, Lakshminarayanan M. Ziprasidone 80 mg/day and 160 mg/day in the acute exacerbation of schizophrenia and schizoaffective disorder: a 6-week placebo-controlled trial. Ziprasidone Study Group. *Neuropsychopharmacology*. 1999; **20**(5): 491-505 <http://www.nature.com/npp/journal/v20/n5/full/1395273a.html>.
  39. Millan MJ. Improving the treatment of schizophrenia: focus on serotonin 5-HT<sub>1A</sub> receptors. *J Pharmacol Exp Ther*. 2000; **295**(3): 853-861 <http://jpet.aspetjournals.org/content/jpet/295/3/853.full.pdf>.
  40. Celada P, Bortolozzi A, Artigas F. Serotonin 5-HT<sub>1A</sub> receptors as targets for agents to treat psychiatric disorders: rationale and current status of research. *CNS Drugs*. 2013; **27**(9): 703-716.
  41. Heckers S, Konradi C. GABAergic mechanisms of hippocampal hyperactivity in schizophrenia. *Schizophr Res*. 2015; **167**(1-3): 4-11; Epub ahead of print Oct 18, 2014. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4402105/pdf/nihms639551.pdf>.
  42. O'Dell SJ, La Hoste GJ, Widmark CB, Shapiro RM, Potkin SG, Marshall JF. Chronic treatment with clozapine or haloperidol

- differentially regulates dopamine and serotonin receptors in rat brain. *Synapse*. 1990; **6**(2): 146-153.
43. Kuoppamaki M, Seppala T, Syvalahti E, Hietala J. Chronic clozapine treatment decreases 5-hydroxytryptamine<sub>1C</sub> receptor density in the rat choroid plexus: comparison with haloperidol. *J Pharmacol Exp Ther*. 1993; **264**(3): 1262-1267.
  44. Meltzer HY. The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*. 1999; **21**(Suppl 2): 106S-115S <http://www.nature.com/npp/journal/v21/n1s/full/1395370a.html/>.
  45. Tarsy D, Baldessarini RJ, Tarazi FI. Effects of newer antipsychotics on extrapyramidal function. *CNS Drugs*. 2002; **16**(1): 23-45.
  46. Spurney CF, Baca SM, Murray AM, Jaskiw GE, Kleinman JE, Hyde TM. Differential effects of haloperidol and clozapine on ionotropic glutamate receptors in rats. *Synapse*. 1999; **34**(4): 266-276.
  47. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res Brain Res Rev*. 2000; **31**(2-3): 302-312.
  48. Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu Rev Pharmacol Toxicol*. 2001; **41**: 237-260.
  49. Tascadda F, Blom JM, Brunello N, et al. Modulation of glutamate receptors in response to the novel antipsychotic olanzapine in rats. *Biol Psychiatry*. 2001; **50**(2): 117-122.
  50. Tascadda F, Lovati E, Blom JM, et al. Regulation of ionotropic glutamate receptors in the rat brain in response to the atypical antipsychotic Seroquel (quetiapine fumarate). *Neuropsychopharmacology*. 1999; **21**(2): 211-217.
  51. Watson DJ, King MV, Gyertyan I, Kiss B, Adham N, Fone KC. The dopamine D<sub>3</sub>-preferring D<sub>2</sub>/D<sub>3</sub> dopamine receptor partial agonist, cariprazine, reverses behavioural changes in a rat neurodevelopmental model for schizophrenia. *Eur Neuropsychopharmacol*. 2016; **26**(2): 208-224.
  52. Schmidt WJ, Bubser M. Anticataleptic effects of the *N*-methyl-d-aspartate antagonist MK-801 in rats. *Pharmacol Biochem Behav*. 1989; **32**(3): 621-623.
  53. Yoshida Y, Ono T, Kizu A, Fukushima R, Miyagishi T. Striatal *N*-methyl-d-aspartate receptors in haloperidol-induced catalepsy. *Eur J Pharmacol*. 1991; **203**(2): 173-180.
  54. Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol*. 2002; **42**: 165-179.
  55. Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, Tamminga CA. Ionotropic glutamate receptors and expression of *N*-methyl-d-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am J Psychiatry*. 2000; **157**(7): 1141-1149 <http://ajp.psychiatryonline.org/doi/pdf/10.1176/appi.ajp.157.7.1141>.
  56. Ariano MA, Larson ER, Noblett KL, Sibley DR, Levine MS. Coexpression of striatal dopamine receptor subtypes and excitatory amino acid subunits. *Synapse*. 1997; **26**(4): 400-414.