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Involvement of 5-HT_{1A} and 5-HT_{2A} Receptors but Not α_2-Adrenoceptors in the Acute Electrophysiological Effects of Cariprazine in the Rat Brain In Vivo

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ABSTRACT

Cariprazine, an orally active and potent dopamine D_3–preferring D_3/D_2 receptor partial agonist, is approved to treat adults with schizophrenia (in the United States and Europe) and manic or mixed episodes associated with bipolar I disorder (in the United States). Cariprazine also displays partial agonism at serotonin [5-hydroxytryptamine (5-HT)] 5-HT_{1A} receptors and antagonism at 5-HT_{2A} and 5-HT_{2B} receptors in vitro. The study objective was to determine whether cariprazine leads to functional alterations of monoamine systems in vivo via electrophysiological recordings from anesthetized rats. Dorsal raphe nucleus (DRN), locus coeruleus (LC), and hippocampus pyramidal neurons were recorded, and cariprazine was administered systemically or locally through iontophoresis. In the DRN, cariprazine completely inhibited the firing activity of 5-HT neurons, which was fully reversed by the 5-HT_{1A} receptor antagonist, WAY100635. In the LC, cariprazine reversed the inhibitory effect of the preferential 5-HT_{2A} receptor agonist, 2,5-dimethoxy-4-iodoamphetamine, on norepinephrine (NE) neurons (ED_{50} = 66 μg/kg) but did not block the inhibitory effect of the α_2-adrenergic receptor agonist, clonidine. Cariprazine, iontophorized into the hippocampus, diminished pyramidal neuronal firing through activation of 5-HT_{1A} receptors, while its concomitant administration did not dampen the suppressant effect of 5-HT. These results indicate that, in vivo, cariprazine acted as a 5-HT_{1A} autoreceptor agonist in the DRN, a 5-HT_{2A} receptor antagonist in modulating the firing activity of LC NE neurons, and a full agonist at 5-HT_{1A} receptors mediating the electrophysiological effect of 5-HT on pyramidal neurons. The modulatory actions of cariprazine on these monoaminergic systems may contribute to its therapeutic effectiveness in patients with depressive episodes.

Introduction

Cariprazine (in the United States: Vraylar; in Europe: Reagila) is a novel dopamine (DA) D_3–preferring D_3/D_2 receptor and serotonin [5-hydroxytryptamine (5-HT)] 5-HT_{1A} receptor partial agonist that has been approved to treat schizophrenia (in the United States and Europe) and manic or mixed episodes associated with bipolar I disorder (in the United States). It was recently reported that adjunctive cariprazine was efficacious in patients who had an inadequate response to their medications used to treat major depressive disorder (Durgam et al., 2016a). Cariprazine has also shown efficacy in improving symptoms of depressive episodes in patients with bipolar I disorder (Durgam et al., 2016b). Unlike aripiprazole, another DA receptor partial agonist indicated for the treatment of schizophrenia and bipolar disorder, cariprazine acts as a D_3/D_2 receptor partial agonist with higher binding affinity and selectivity (5- to 8-fold) for D_3 versus D_2 receptors and as a more potent antagonist at 5-HT_{2A} receptors in vitro (Lawler et al., 1999; Kiss et al., 2010; Maeda et al., 2014). In addition to these properties, cariprazine was shown in vitro to be a partial agonist at 5-HT_{1A} receptors in hippocampal tissue, a high-affinity antagonist at 5-HT_{2B} receptors, and to have moderate affinity for histamine type 1 receptors (Kiss et al., 2010).

The role of 5-HT_{1A} receptors in depression has been demonstrated by findings that the 5-HT_{1A} receptor agonists buspirone and gepirone are effective antidepressants, either as a monotherapy or in combination with selective serotonin reuptake inhibitors (SSRIs) for acute treatment and relapse prevention (Trivedi et al., 2006; Bielski et al., 2008; Fabre et al., 2011). In addition, activation of 5-HT_{1A} receptors by selective agonists such as 8-OH-DPAT increases DA release in the prefrontal cortex (Arborelius et al., 1993; Li et al., 2004; Assié et al., 2005).

Several lines of evidence suggest that blockade of 5-HT_{2A} receptors in combination with SSRI treatment may contribute to substantial therapeutic benefits in major depressive disorder (Blier and Szabo, 2005). Indeed, medications that block 5-HT_{2A} receptors, such as aripiprazole, quetiapine, risperidone, and olanzapine, as well as mirtazapine and mianserin, are effective augmentation strategies in combination with SSRIs (Nelson and Papakostas, 2009; Kennedy et al., 2016). The only property that the aforementioned drugs have in common is their capacity to block 5-HT_{2A} receptors. Thus, it is

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); CA3, cornu ammonis 3; DA, dopamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; DRN, dorsal raphe nucleus; LC, locus coeruleus; NE, norepinephrine; SSRI, selective serotonin reuptake inhibitor.

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likely that the 5-HT_{2A} receptor antagonistic property of these agents acts by removing the inhibitory effects of SSRIs on norepinephrine (NE) systems (Szabo and Blier, 2002; Seager et al., 2004; Dremencov et al., 2007; Chernoloz et al., 2009, 2012).

In vitro studies are necessary to identify potential therapeutic compounds, but it is also important to study the activity of a drug in vivo to have a thorough mechanistic understanding. Prior experiments of this nature have been conducted to characterize the effects of cariprazine on DA neurons (Delcourte et al., 2018) but not on 5-HT or NE systems. To this end, the objectives of the present study were to determine the in vivo effects of acute cariprazine administration at 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN), postsynaptic 5-HT_{1A} receptors in the hippocampus, 5-HT_{2A} receptors controlling NE neuron firing in the locus coeruleus (LC), and α_2-adrenergic autoreceptors within the LC using electrophysiological techniques.

Materials and Methods

Animals. The experiments were carried out on male Sprague-Dawley rats (Charles River Laboratories, St. Constant, QC, Canada) weighing 250–350 g and housed in groups of two per cage, under standard laboratory conditions (12-hour light/dark cycle with food and water ad libitum). In vivo extracellular recordings were carried out in chloral hydrate–anesthetized rats (400 mg/kg, intraperitoneal [i.p.]) that were mounted in a stereotaxic apparatus. Supplemental doses of the anesthetic (100 mg/kg, i.p.) were given to maintain constant anesthesia and prevent nociceptive reaction to pinching of the hind paws. Body temperature was maintained at 37°C throughout the experiment via a thermistor-controlled heating pad. Prior to electrophysiological recordings, a catheter was inserted in a lateral tail vein for systemic intravenous injection of pharmacologic agents. Recordings were generally carried out within 30–60 minutes after achieving complete anesthesia. All experiments were carried out in accordance with the Canadian Council on Animal Care and the local Animal Care Committee (Royal Ottawa Institute of Mental Health Research, Ottawa, Canada).

Compounds. The preferential 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), the α_2-adrenergic agonist clonidine, and the selective 5-HT_{1A} receptor antagonist WAY100635 were dissolved in distilled water. Cariprazine [see the chemical structure given in Kiss et al. (2010)] was dissolved in 5% lactic acid and distilled water for intravenous (i.v.) injection. Cariprazine was provided by Allergan (Dublin, Ireland); all other compounds were purchased from Tocris Bioscience (Minneapolis, MN).

In Vivo Electrophysiological Recordings. A burr hole was drilled at the stereotaxic coordinates corresponding to the brain structure of interest (Paxinos and Watson, 2007). Extracellular recordings of neurons in the DRN and LC were carried out using single-barrel glass micropipettes (Stoelting, Spencerville, MD) preloaded with 2 M NaCl and with impedance between 2 and 6 MΩ. Neurons in the cornu ammonis 3 (CA3) region of the hippocampus were recorded with five-barrel micropipettes. The central barrel (used for unitary recordings) and one side barrel (used for automatic current balancing) were filled with 2 M NaCl; the other barrels were filled with cariprazine (10 mM in distilled water and 5% lactic acid, pH 4), 5-HT creatinine sulfate (15 mM in 0.2 M NaCl, pH 4), or quisqualic acid (1.5 mM in 0.2 M NaCl, pH 8). Cariprazine and 5-HT were ejected as cations and retained with a negative current; quisqualate was ejected as an anion and retained with a positive current. Drugs injected intravenously were administered in 0.1 ml aliquots at 60-second intervals to ensure stabilization of the recording and to determine drug effects in incremental doses; these recordings lasted 20 minutes on average.

Recording of DRN 5-HT Neurons. Putative 5-HT neurons were recorded by positioning single-barrel glass micropipettes at the following coordinates (in millimeters from lambda): anterior/posterior, 1.0–1.2; mediolateral, 0; and dorsal/ventral, 5.0–7.0. The following criteria were used to identify 5-HT neurons: a bi- or triphasic extracellular wave form with long-duration (0.8–1.2 milliseconds), positive phase, and regular firing in the range of 0.5–2.5 Hz were recorded (Vandermaelen and Aghajanian, 1983).

Systemic intravenous injections were used to obtain the net effect of cariprazine on the firing of 5-HT neurons since it is one of the main factors controlling 5-HT transmission. Subsequent injection of the selective 5-HT_{1A} receptor antagonist WAY100635 served to determine the 5-HT_{1A} nature of the suppression of firing. Full and partial 5-HT_{1A} receptor agonists should suppress all 5-HT firing and lead to restoration of firing after prolonged administration through desensitization of the 5-HT_{1A} autoreceptor (Blier and El Mansari, 2013); therefore, the intrinsic activity of cariprazine at the 5-HT_{1A} autoreceptor was not determined as for the hippocampus.

Recording of LC NE Neurons. The NE neurons were recorded by positioning single-barrel glass micropipettes at the following coordinates (in millimeters from lambda): anterior/posterior, −1.1 to −1.2; mediolateral, 1.0–1.3; and dorsal/ventral, 5.0–7.0. The following criteria were used to identify NE neurons: regular firing rate (1.0–5.0 Hz), long duration (0.8–1.2 milliseconds) of the action potential, and brisk excitatory response followed by a short period of inhibition in reaction to a nociceptive pinch of the contralateral hind paw (Aghajanian et al., 1977). To test the effect of cariprazine on 5-HT_{2A} receptors, NE neurons were suppressed by the preferential 5-HT_{2A} receptor agonist DOI (Szabo and Blier, 2001). Following an inhibition period, cumulative intravenous doses of cariprazine were administered to antagonize the inhibitory effect of DOI. The reversing effect of cariprazine was quantified relative to the stable baseline firing activity for over at least a 60-second interval preceding the intravenous injection.

Systemic intravenous injections of various drugs were used to obtain their net effect on the firing rate of NE neurons, since it is one of the main determinants of NE transmission. DOI had to be injected intravenously and not locally applied, because the 5-HT_{2A} receptors controlling the NE neuron firing activity are not located in the LC (Szabo and Blier, 2001).

Recording of Pyramidal Neurons in the CA3 Region of the Hippocampus. The CA3 pyramidal neurons were recorded by positioning multibarrel micropipettes at the following coordinates (in millimeters from lambda): anterior/posterior, 3.8–4.2; mediolateral, 4.0–4.2; and dorsal/ventral, 3.5–4.5. Because most CA3 pyramidal neurons are not spontaneously active in chloral hydrate-anesthetized rats, a small ejection current (+2 to −2 nA) was applied to the quisqualate barrel to activate them to be within their physiologic firing range (10–15 Hz) (Ranck, 1975). Partial or full agonism of cariprazine on 5-HT_{1A} receptors cannot be assessed in vivo using systemic injections. Therefore, it was assessed by comparing the inhibitory effect of 5-HT, per se, to the inhibitory effect of concomitant ejection of 5-HT and cariprazine, following restoration of the firing rate to the same level as before by increasing quisqualate ejection. In this paradigm, coapplication of a partial agonist reduces the inhibitory effect of 5-HT, whereas coapplication of a full agonist does not change the inhibitory effect of 5-HT, provided the ensuing concentration of the agent tested against 5-HT (cariprazine here) is initially sufficient to induce inhibition of the firing activity of pyramidal neurons by at least 50% (Blier and de Montigny, 1990; Dong et al., 1998; Ghanbari et al., 2009, 2010; Oosterhof et al., 2014). To ascertain whether the inhibitory effect of 5-HT and cariprazine was mediated by 5-HT_{1A} receptors, the inhibitory effect of iontophoretic 5-HT and cariprazine application was compared before and after administration of the selective 5-HT_{1A} receptor antagonist WAY100635.

Data Analysis. Electrophysiological recordings were made using Spike2 software version 6.17 (Cambridge Electronic Design, Cambridge, UK). Quantification of firing rates was performed using Spike2. When

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appropriate, groups were analyzed with a paired t test or with repeated-measures analysis of variance followed by a Holm-Sidak method. All data were analyzed with GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA). Data are presented as mean ± S.E.M.; $P < 0.05$ was considered significant.

Results

Effect of Cariprazine on the Firing Activity of DRN 5-HT Neurons. The role of cariprazine in inhibiting the firing activity of 5-HT neurons via 5-HT_{1A} autoreceptors was investigated in the DRN. Cumulative intravenous injections of 50 μg/kg of cariprazine decreased the firing activity of 5-HT neurons ($n = 15$ rats; one neuron per animal). This effect was subsequently reversed by the selective 5-HT_{1A} receptor antagonist WAY100635, indicating that cariprazine was acting as an agonist at the 5-HT_{1A} autoreceptors in vivo (Fig. 1A).

Although the response was dose dependent for each neuron tested, the degree of firing inhibition for a given dose varied considerably. Indeed, the dose required to completely inhibit the firing rate ranged from a minimum of 150 μg/kg to a maximum of 850 μg/kg (Fig. 1B). However, 80% of the tested neurons ($n = 12$ of 15) were completely inhibited by cumulative intravenous doses of cariprazine within a range of 150–350 μg/kg. Upon detailed analysis, there was no correlation between the initial baseline firing rate of an individual 5-HT neuron and the cariprazine dose required to completely inhibit the firing (Fig. 1C).

Effect of Cariprazine on Postsynaptic 5-HT_{1A} Receptors on Pyramidal Neurons in the Hippocampus. Microiontophoretic application of cariprazine significantly inhibited the firing activity of pyramidal neurons, as did 5-HT (Fig. 2A). There was no statistically significant difference between the degree of inhibition induced by 5-HT alone compared to when it was concomitantly applied with cariprazine ($P > 0.05$; Fig. 2, A and B), indicating that cariprazine acted as a full agonist at 5-HT_{1A} receptors *in vivo* in the hippocampus.

The effects of ejecting cariprazine and 5-HT in the CA3 pyramidal neurons were assessed after an intravenous injection of the selective 5-HT_{1A} receptor antagonist WAY100635 (50 μg/kg) to confirm that these two substances were acting through the 5-HT_{1A} receptor. Indeed, the degree of inhibition induced by both cariprazine and 5-HT was significantly reduced by the antagonist (**$P < 0.01$, ***$P < 0.001$, respectively; Fig. 2, C and D).

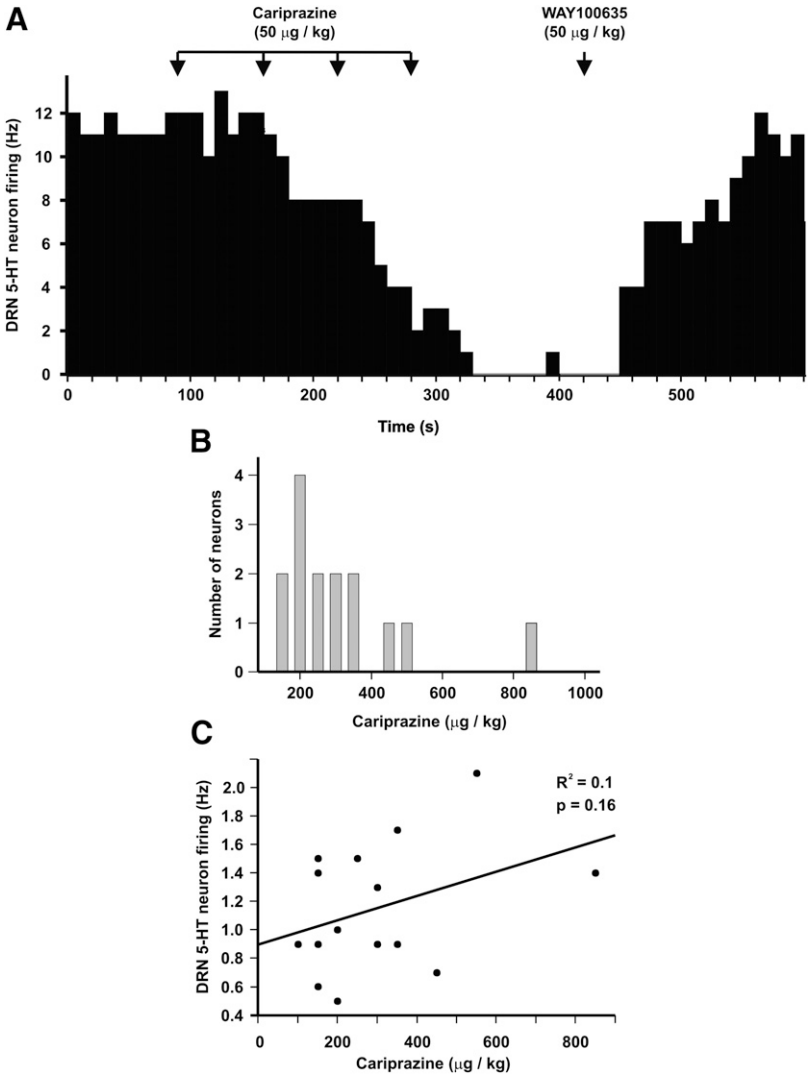

Fig. 1. (A) Integrated firing rate histogram of a single 5-HT neuron showing its response to four cumulative intravenous injections of cariprazine and the subsequent reversal with an intravenous dose of the selective 5-HT_{1A} antagonist WAY100635. (B) Relationship between the number of 5-HT neurons showing 100% inhibition of firing and the dose of cariprazine necessary to achieve the complete suppression of firing. One neuron was recorded per rat. (C) No significant correlation between the degree of firing activity of 5-HT neurons and the dose of cariprazine necessary to achieve complete inhibition.

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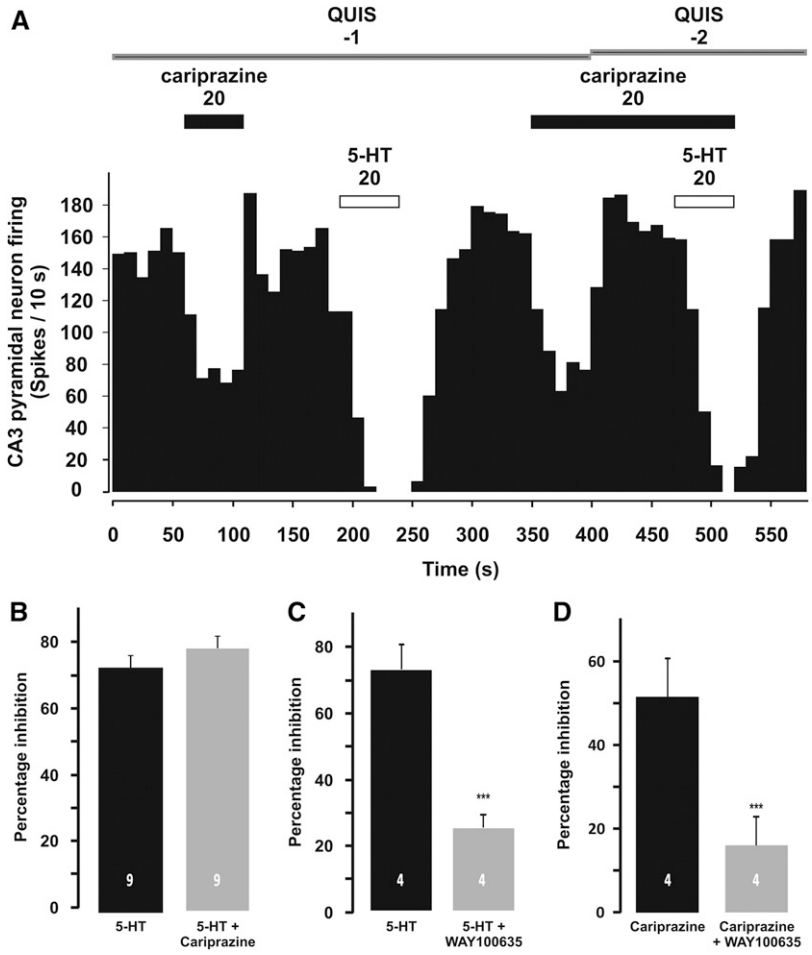

Fig. 2. (A) Integrated firing rate histogram of a single pyramidal neuron in the hippocampus, showing inhibition by cariprazine and 5-HT. Note that one-way analysis of variance with repeated measures showed subsequent full inhibition by 5-HT was not significantly different when concomitantly administered with cariprazine (A and B). (C and D) Both inhibitions induced by cariprazine and 5-HT were antagonized by the intravenous injection of the selective 5-HT_{1A} receptor antagonist WAY100635. The number of neurons and animals are presented in the histograms; data were analyzed with a paired t test and are presented as mean ± S.E.M. $^{**}P < 0.01$; $^{***}P < 0.001$ for effect of WAY100635 administration on the inhibitory effect of cariprazine and 5-HT.

Effect of Cariprazine on the Firing Activity of LC NE Neurons: Role of 5-HT_{2A} Receptors and α_2-Adrenergic Receptors. The preferential 5-HT_{2A} receptor agonist DOI (100 μg/kg, i.v.) induced near complete cessation of NE neuronal firing (Fig. 3A). Cumulative intravenous injections of 50 μg/kg of cariprazine restored the firing activity up to 70% of the baseline level, with an ED_{50} value of 65.5 μg/kg (Fig. 3; $n = 7$).

As shown in Fig. 4, in the presence of cariprazine, the effect of the α_2-adrenoceptor agonist clonidine was compared with its effect under control conditions. After cariprazine pretreatment, clonidine fully inhibited the firing activity of NE neurons upon dosing with two cumulative injections (10 μg/kg, i.v.) as it did in control conditions (Fig. 4). The effects of clonidine on NE neuronal firing were reversed by administration of the α_2-adrenoceptor antagonist idazoxan (1 mg/kg, i.v.), indicating that cariprazine did not block α_2-adrenoceptors (Fig. 4).

Discussion

In the DRN, cariprazine fully inhibited the firing activity of 5-HT neurons. This effect was reversed by the selective 5-HT_{1A} receptor antagonist WAY100635, which indicated that cariprazine acted as an agonist in vivo at the 5-HT_{1A} autoreceptors in this brain structure. Although cumulative doses inducing inhibition varied for each 5-HT neuron tested, the effect of cariprazine was dose dependent for each neuron; nevertheless, the majority of neurons were inhibited by doses ranging between 150 and 350 μg/kg. A potential role of α_1-adrenoceptors in altering the responsiveness of 5-HT neurons to cariprazine, as is the case with olanzapine and clozapine (Sprouse et al., 1999), can be excluded since cariprazine has very weak or negligible affinity for these receptors (Kiss et al., 2010). It could be assumed that the varied neuronal responsiveness of 5-HT_{1A} autoreceptors to cariprazine stems from differences in baseline firing of individual neurons, since a previous study suggested that 5-HT neurons with slow firing activity are more sensitive to 5-HT receptor agonists than neurons with faster discharge (Jacobs et al., 1983). In the present study, however, this variable response was not related to the baseline firing rate of individual neurons. Such variation in the degree of inhibition was unexpected because all selective 5-HT_{1A} receptor agonists and other agents with 5-HT_{1A} receptor partial agonist activity (e.g., aripiprazole and brexpiprazole) tested in this paradigm yielded a tight dose-response relationship upon systemic administration, unlike cariprazine (Blier and de Montigny, 1990; Dong et al., 1998; Rueter and Blier, 1999; Dahan et al., 2009; Oosterhof et al., 2014). Interestingly, both aripiprazole and brexpiprazole display greater in vitro affinity for 5-HT_{1A} receptors than

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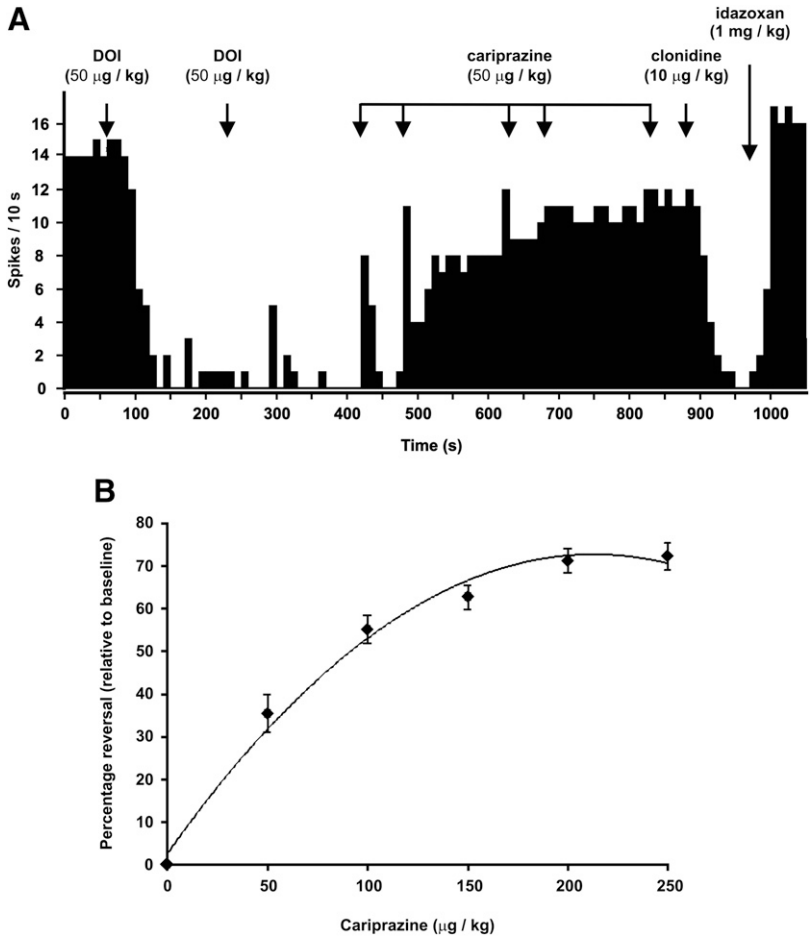

Fig. 3. (A) Integrated firing rate histogram showing DOI-induced inhibition of NE neuronal firing by the preferential 5-HT_{2A} receptor agonist DOI and its reversal by cumulative doses of cariprazine. Note subsequent inhibition by clonidine and reversal by idazoxan. (B) Dose-response curve showing the percentage reversal of DOI-induced inhibition relative to baseline in NE neurons by cumulative doses of cariprazine (n = 7, one neuron was recorded per rat).

cariprazine but were less potent than cariprazine in activating the 5-HT_{1A} autoreceptors (Dahan et al., 2009; Oosterhof et al., 2014; Citrome, 2015). Therefore, a potential explanation for the wide range of cariprazine doses needed to inhibit 5-HT neurons may be due to the balance of the inhibitory effect of 5-HT_{1A} receptor activation versus the excitatory action of D_2-like receptors for different 5-HT neurons (Chernoloz et al., 2009). Indeed, 5-HT neurons are endowed with D_2-like receptors that mediate an excitatory influence on neuronal firing (Aman et al., 2007; Katz et al., 2010). This explanation can be envisioned if it is assumed that the 5- to 8-fold greater binding affinity and selectivity of cariprazine for D_3 versus D_2 receptors, compared with aripiprazole and brexpiprazole (which show a lower affinity for D_3 receptors and a higher selectivity for D_2 vs. D_3 receptors than cariprazine), exert a larger excitatory effect on some 5-HT neurons. Interestingly, D_3 receptor expression has been demonstrated by binding assays in the median and dorsal raphe nuclei of the midbrain (Stanwood et al., 2000), and thus may also influence 5-HT neuronal firing in this region. However, further studies using selective tools are needed to explore the specific role of the D_3 receptor on 5-HT neuronal activity. At this point, it is unclear whether the variability in the response of 5-HT neurons to cariprazine translates into a functional difference compared, for example, to other DA receptor partial agonists such as brexpiprazole and aripiprazole. Nevertheless, although cariprazine and aripiprazole had a superior effect on mood symptoms when compared with placebo, the magnitude of their effect appears to be similar in patients with schizophrenia (Durgam et al., 2015).

In the hippocampus, cariprazine did not reduce the effectiveness of the endogenous ligand 5-HT at postsynaptic 5-HT_{1A} receptors when the two compounds were applied concomitantly. This indicates that cariprazine acted as a full 5-HT_{1A} receptor agonist in this brain region. Similar to cariprazine, brexpiprazole has also been shown to act as a full agonist at the postsynaptic 5-HT_{1A} receptors in the hippocampus (Oosterhof et al., 2014). It is also important to note that agents acting on 5-HT_{1A} receptors can have heterogeneous effects at 5-HT_{1A} receptors in different brain areas. For instance, studies of the 5-HT_{1A} receptor agonist/5-HT_{2A} receptor antagonist flibanserin revealed that it acts as a full agonist at presynaptic 5-HT_{1A} receptors in the DRN and at postsynaptic 5-HT_{1A} receptors in the medial prefrontal cortex, but as a partial agonist at the postsynaptic 5-HT_{1A} receptors in the CA3 region of the hippocampus (Reuter and Blier, 1999). Moreover, it was reported that selective activation of these postsynaptic receptors enhances 5-HT transmission and DA release in the medial prefrontal cortex (Chung et al., 2004). Indeed, activation of 5-HT_{1A} receptors (by 5-HT_{1A} receptor agonists) was shown to increase DA release in the medial prefrontal cortex (Ichikawa et al., 2001; Díaz-Mataix et al., 2005).

A previous study has shown that both aripiprazole and cariprazine decreased 5-HT turnover rate in mouse prefrontal cortex through an action on 5-HT_{1A} receptors (Kiss et al., 2010).

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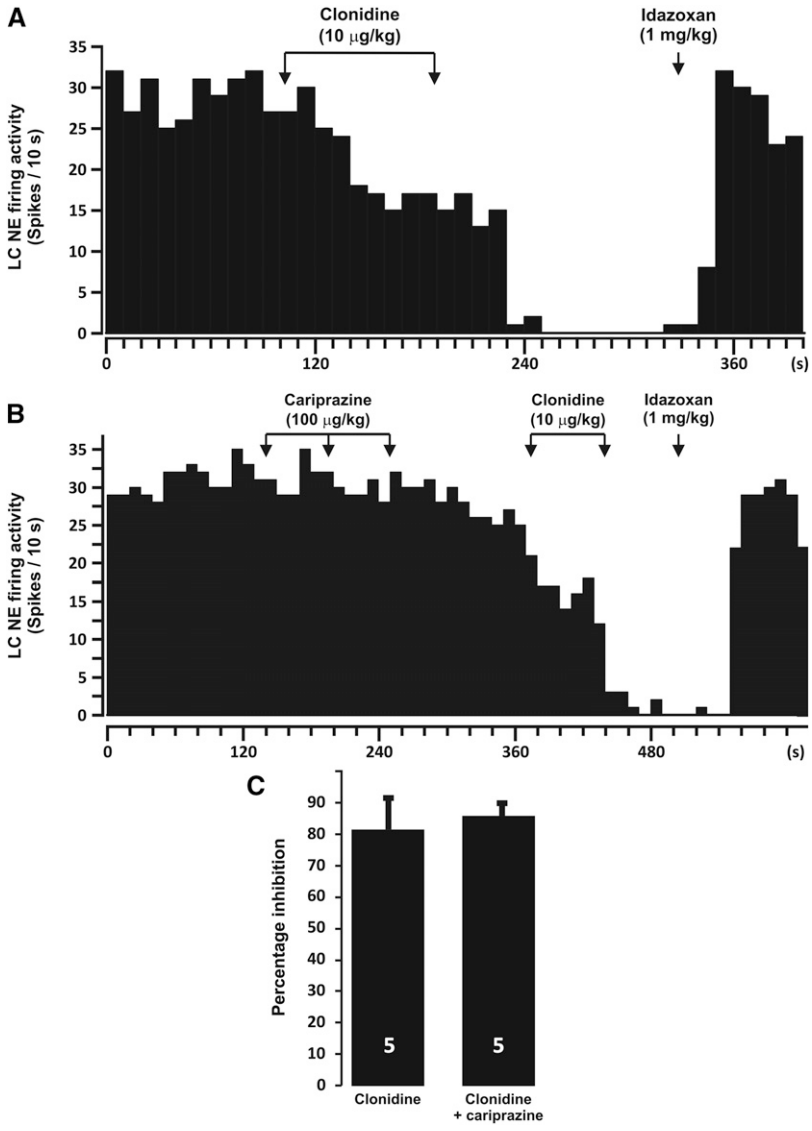

Fig. 4. Representative integrated firing rate histograms illustrating inhibition of NE neuron firing activity by clonidine (A), and the lack of effect of cariprazine on this activity following pretreatment with cariprazine (B and C). Note that this inhibition is reversed by the intravenous injection of the α_2-adrenoceptor idazoxan.

In various in vitro assays, cariprazine was shown to act either as a partial agonist (Kiss et al., 2010) or full agonist depending on the assay system used. The present in vivo study found that cariprazine acted rather as a full agonist at 5-HT_{1A} receptors controlling the firing activity of pyramidal neurons in the hippocampus. It has been suggested that a partial agonist can behave differently depending on the level of receptor reserve; for example, the partial agonist may behave as expected or even as an antagonist in tissue with low or negligible levels of receptor reserve, but in the presence of high levels of receptor reserve that same partial agonist may behave like a full agonist instead (Kenakin, 1987). This is, however, not the case with the in vivo electrophysiological response reported herein because, with the approach used in this study, both partial and full 5-HT_{1A} receptor agonists have been identified in the DRN and hippocampus (Blier and de Montigny 1990; Dong et al., 1998; Ghanbari et al., 2009, 2010; Oosterhof et al., 2014). It is nevertheless possible that these responses are partially dependent on the coupling between specific 5-HT_{1A} receptors and the signal transduction mediating their response (Valdizán et al., 2010). Indeed, in the hippocampus 5-HT_{1A} receptors are coupled to adenylyl cyclase but also to potassium channels, the latter being involved in the electrophysiological responses measured in this study, and both of which have displayed differential properties in previous studies (Yocca et al., 1992; Blier et al., 1993).

Cariprazine acted as a potent antagonist at 5-HT_{2A} receptors on LC NE neurons, since it reversed the inhibitory effect of the preferential 5-HT_{2A} receptor agonist DOI. These receptors are located on GABA neurons that control the activity of NE neurons (Haddjeri et al., 1997; Szabo and Blier, 2002). Interestingly, administration of YM992 (a 5-HT_{2A} receptor antagonist and an inhibitor of 5-HT reuptake) or blockade of 5-HT_{2A} receptors by the selective 5-HT_{2A} receptor antagonist MDL100907 during treatment with the SSRI citalopram was shown to synergistically increase cortical NE levels, which were measured by microdialysis (Hatanaka et al., 2000). Furthermore, blockade of 5-HT_{2A} receptors by various medications, such as risperidone, aripiprazole, and olanzapine, reverses the inhibitory effect of SSRIs on LC NE neurons (Seager et al., 2004; Dremencov et al., 2007; Chernoloz et al., 2009). Despite the lower in vitro binding affinity of cariprazine for 5-HT_{2A} receptors when compared with other drugs commonly used to

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treat psychosis and mania (Ghanbari et al., 2009; Oosterhof et al., 2014), its in vivo 5-HT_{2A} receptor antagonist potency for reversing the inhibitory effect of DOI was similar to those medications.

The α_2-adrenergic autoreceptors were not blocked by cariprazine in the present study. Indeed, cariprazine pretreatment did not result in dampening of the inhibitory action of the α_2-adrenoceptor agonist clonidine on NE neurons; this is consistent with its weak or negligible affinity for these receptors as determined by in vitro binding assays (Kiss et al., 2010). It is interesting to note that this lack of effect on α_2-adrenergic receptors differs from other medications used to treat psychosis and/or mania (Dremencov et al., 2007; Ghanbari et al., 2009; Oosterhof et al., 2014).

It is important to consider whether the doses of cariprazine used in the present experiments produced plasma levels within the range of those achieved in humans. Because 5-HT_{2A} antagonism is an important feature of this class of medication, both in mood disorders and schizophrenia, the cariprazine concentrations that reversed the effect of the 5-HT_{2A} receptor agonist DOI by cariprazine in the LC were used for comparison with human plasma concentrations. Given that a dose of 1 mg/kg of cariprazine (intravenous) results in a peak plasma level of 240 ng/ml in rats (Gyertyán et al., 2011), it can be extrapolated that the 0.2 mg/kg dose of cariprazine necessary to reverse the suppression of firing by DOI should have produced a level of 48 ng/ml in plasma. This is consistent with the 50 ng/ml plasma level of cariprazine active moieties reported in patients receiving cariprazine at 3 mg/d (Nakamura et al., 2016).

In this study, in agreement with its in vitro affinity, cariprazine showed acute in vivo activity with effective agonism at 5-HT_{1A} receptors and antagonism at 5-HT_{2A} receptors. Agonism at 5-HT_{1A} receptors has been shown to play a key role in the control of mood and cognition (Newman-Tancredi, 2010), while antagonism at 5-HT_{2A} receptors is thought to play a role in modulating the NE system. Hence, it is possible that the activation of 5-HT_{1A} receptors and the blockade of 5-HT_{2A} receptors by cariprazine may contribute to its beneficial therapeutic action seen in major depressive disorder (Durgam et al., 2016a) and bipolar depression (Durgam et al., 2016b).

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Authorship Contributions

Participated in research design: El Mansari, Kiss, Farkas, Blier.

Conducted experiments: Herman, El Mansari.

Wrote or contributed to the writing of the manuscript: Herman, El Mansari, Adham, Kiss, Farkas, Blier.

References

Aghajanian GK, Cedarbaum JM, and Wang RY (1977) Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. *Brain Res* **136**:570–577.

Aman TK, Shen RY, and Haj-Dahmane S (2007) D_2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J Pharmacol Exp Ther* **320**:376–385.

Arborelius L, Nomikos GG, Hacksell U, and Svensson TH (1993) (R)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. *Acta Physiol Scand* **148**:465–466.

Assié MB, Ravailhe V, Faucillon V, and Newman-Tancredi A (2005) Contrasting contribution of 5-hydroxytryptamine 1A receptor activation to neurochemical profile of novel antipsychotics: frontocortical dopamine and hippocampal serotonin release in rat brain. *J Pharmacol Exp Ther* **315**:265–272.

Bielski RJ, Cunningham L, Horrigan JP, Londborg PD, Smith WT, and Weiss K (2008) Gepirone extended-release in the treatment of adult outpatients with major depressive disorder: a double-blind, randomized, placebo-controlled, parallel-group study. *J Clin Psychiatry* **69**:571–577.

Blier P and de Montigny C (1990) Differential effect of gepirone on presynaptic and postsynaptic serotonin receptors: single-cell recording studies. *J Clin Psychopharmacol* **10** (Suppl):13S–20S.

Blier P and El Mansari M (2013) Serotonin and beyond: therapeutics for major depression. *Philos Trans R Soc Lond B Biol Sci* **368**:20120536.

Blier P, Lista A, and De Montigny C (1993) Differential properties of pre- and postsynaptic 5-hydroxytryptamine1A receptors in the dorsal raphe and hippocampus: I. Effect of spiperone. *J Pharmacol Exp Ther* **265**:7–15.

Blier P and Szabo ST (2005) Potential mechanisms of action of atypical antipsychotic medications in treatment-resistant depression and anxiety. *J Clin Psychiatry* **66** (Suppl 8):30–40.

Chernoloz O, El Mansari M, and Blier P (2009) Electrophysiological studies in the rat brain on the basis for aripiprazole augmentation of antidepressants in major depressive disorder. *Psychopharmacology (Berl)* **206**:335–344.

Chernoloz O, El Mansari M, and Blier P (2012) Effects of sustained administration of quetiapine alone and in combination with a serotonin reuptake inhibitor on norepinephrine and serotonin transmission. *Neuropsychopharmacology* **37**: 1717–1728.

Chung YC, Li Z, Dai J, Meltzer HY, and Ichikawa J (2004) Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT_{1A} receptor agonism. *Brain Res* **1023**:54–63.

Citrome L (2015) The ABC's of dopamine receptor partial agonists—aripiprazole, brexpiprazole and cariprazine: the 15-min challenge to sort these agents out. *Int J Clin Pract* **69**:1211–1220.

Dahan L, Husum H, Mnie-Filali O, Arnt J, Hertel P, and Haddjeri N (2009) Effects of bifeprunox and aripiprazole on rat serotonin and dopamine neuronal activity and anxiolytic behaviour. *J Psychopharmacol* **23**:177–189.

Delcourte S, Ashby CR Jr, Rovera R, Kiss B, Adham N, Farkas B, and Haddjeri N (2018) The novel atypical antipsychotic cariprazine demonstrates dopamine D_2 receptor-dependent partial agonist actions on rat mesencephalic dopamine neuronal activity. *CNS Neurosci Ther* DOI: 10.1111/CNS.12867 [published ahead of print].

Díaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, Celada P, and Artigas F (2005) Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J Neurosci* **25**:10831–10843.

Dong J, de Montigny C, and Blier P (1998) Full agonistic properties of BAY x 3702 on presynaptic and postsynaptic 5-HT_{1A} receptors electrophysiological studies in the rat hippocampus and dorsal raphe. *J Pharmacol Exp Ther* **286**: 1239–1247.

Dremencov E, El Mansari M, and Blier P (2007) Distinct electrophysiological effects of paliperidone and risperidone on the firing activity of rat serotonin and norepinephrine neurons. *Psychopharmacology (Berl)* **194**:63–72.

Durgam S, Cutler AJ, Lu K, Migliore R, Ruth A, Laszlovszky I, Németh G, and Meltzer HY (2015) Cariprazine in acute exacerbation of schizophrenia: a fixed-dose, phase 3, randomized, double-blind, placebo- and active-controlled trial. *J Clin Psychiatry* **76**:e1574–e1582.

Durgam S, Earley W, Guo H, Li D, Németh G, Laszlovszky I, Fava M, and Montgomery SA (2016a) Efficacy and safety of adjunctive cariprazine in inadequate responders to antidepressants: a randomized, double-blind, placebo-controlled study in adult patients with major depressive disorder. *J Clin Psychiatry* **77**:371–378.

Durgam S, Earley W, Lipschitz A, Guo H, Laszlovszky I, Németh G, Vieta E, Calabrese JR, and Yatham LN (2016b) An 8-week randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of cariprazine in patients with bipolar I depression. *Am J Psychiatry* **173**:271–281.

Fabre LF, Brown CS, Smith LC, and Derogatis LR (2011) Gepirone-ER treatment of hypoactive sexual desire disorder (HSDD) associated with depression in women. *J Sex Med* **8**:1411–1419.

Ghanbari R, El Mansari M, and Blier P (2010) Sustained administration of trazodone enhances serotonergic neurotransmission: in vivo electrophysiological study in the rat brain. *J Pharmacol Exp Ther* **335**:197–206.

Ghanbari R, El Mansari M, Shahid M, and Blier P (2009) Electrophysiological characterization of the effects of asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2-adrenergic and D_2 receptors in the rat brain. *Eur Neuropsychopharmacol* **19**:177–187.

Gyertyán I, Kiss B, Sághy K, Laszy J, Szabó G, Szabados T, Gémesi LI, Pásztor G, Zájer-Balázs M, Kapás M, et al. (2011) Cariprazine (RGH-188), a potent D_3/D_2 dopamine receptor partial agonist, binds to dopamine D_3 receptors in vivo and shows antipsychotic-like and procognitive effects in rodents. *Neurochem Int* **59**: 925–935.

Haddjeri N, de Montigny C, and Blier P (1997) Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system. *Br J Pharmacol* **120**:865–875.

Hatanaka K, Yatsugi S, and Yamaguchi T (2000) Effect of acute treatment with YM992 on extracellular serotonin levels in the rat frontal cortex. *Eur J Pharmacol* **395**:23–29.

Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, and Meltzer HY (2001) 5-HT_{2A} and D_2 receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem* **76**:1521–1531.

Jacobs BL, Heym J, and Rasmussen K (1983) Raphe neurons: firing rate correlates with size of drug response. *Eur J Pharmacol* **90**:275–278.

Katz NS, Guiard BP, El Mansari M, and Blier P (2010) Effects of acute and sustained administration of the catecholamine reuptake inhibitor nomifensine on the firing activity of monoaminergic neurons. *J Psychopharmacol* **24**:1223–1235.

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Kenakin T (1987) Agonists, partial agonists, antagonists, inverse agonists and agonist/antagonists? *Trends Pharmacol Sci* **8**:423–426.
Kennedy SH, Lam RW, McIntyre RS, Tourjman SV, Bhat V, Blier P, Hasnain M, Jollant F, Levitt AJ, MacQueen GM, et al.; CANMAT Depression Work Group (2016) Canadian network for mood and anxiety treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 3. Pharmacological treatments. *Can J Psychiatry* **61**:540–560.
Kiss B, Horváth A, Némethy Z, Schmidt E, Laszlovszky I, Bugovics G, Fazekas K, Hornok K, Orosz S, Gyertyán I, et al. (2010) Cariprazine (RGH-188), a dopamine D_3 receptor-preferring, D_3/D_2 dopamine receptor antagonist–partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther* **333**: 328–340.
Lawler CP, Prioleau C, Lewis MM, Mak C, Jiang D, Schetz JA, Gonzalez AM, Sibley DR, and Mailman RB (1999) Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology* **20**:612–627.
Li Z, Ichikawa J, Dai J, and Meltzer HY (2004) Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. *Eur J Pharmacol* **493**:75–83.
Maeda K, Sugino H, Akazawa H, Amada N, Shimada J, Futamura T, Yamashita H, Ito N, McQuade RD, Mørk A, et al. (2014) Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. *J Pharmacol Exp Ther* **350**:589–604.
Nakamura T, Kubota T, Iwakaji A, Imada M, Kapás M, and Morio Y (2016) Clinical pharmacology study of cariprazine (MP-214) in patients with schizophrenia (12-week treatment). *Drug Des Devel Ther* **10**:327–338.
Nelson JC and Papakostas GI (2009) Atypical antipsychotic augmentation in major depressive disorder: a meta-analysis of placebo-controlled randomized trials. *Am J Psychiatry* **166**:980–991.
Newman-Tancredi A (2010) The importance of 5-HT1A receptor agonism in antipsychotic drug action: rationale and perspectives. *Curr Opin Investig Drugs* **11**: 802–812.
Oosterhof CA, El Mansari M, and Blier P (2014) Acute effects of brexpiprazole on serotonin, dopamine, and norepinephrine systems: an in vivo electrophysiologic characterization. *J Pharmacol Exp Ther* **351**:585–595.
Paxinos G and Watson C (2007) *The Rat Brain in Stereotaxic Coordinates*, 7th ed, Elsevier Inc, Burlington, MA.
Ranck JB Jr (1975) Behavioral correlates and firing repertoires of neurons in the dorsal hippocampal formation and septum of unrestrained rats, in *The Hippocampus* (Isaacson RL and Pribram KH eds) pp 207–244, Plenum Press, New York.
Rueter LE and Blier P (1999) Electrophysiological examination of the effects of sustained flibanserin administration on serotonin receptors in rat brain. *Br J Pharmacol* **126**:627–638.
Seager MA, Huff KD, Barth VN, Phebus LA, and Rasmussen K (2004) Fluoxetine administration potentiates the effect of olanzapine on locus coeruleus neuronal activity. *Biol Psychiatry* **55**:1103–1109.
Sprouse JS, Reynolds LS, Braselton JP, Rollema H, and Zorn SH (1999) Comparison of the novel antipsychotic ziprasidone with clozapine and olanzapine: inhibition of dorsal raphe cell firing and the role of 5-HT_{1A} receptor activation. *Neuropsychopharmacology* **21**:622–631.
Stanwood GD, Artymyshyn RP, Kung MP, Kung HF, Lucki I, and McGonigle P (2000) Quantitative autoradiographic mapping of rat brain dopamine D3 binding with [^{125}I]7-OH-PIPAT: evidence for the presence of D3 receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther* **295**:1223–1231.
Szabo ST and Blier P (2001) Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. *Brain Res* **922**:9–20.
Szabo ST and Blier P (2002) Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT_{2A} receptor antagonism on the firing activity of norepinephrine neurons. *J Pharmacol Exp Ther* **302**:983–991.
Trivedi MH, Fava M, Wisniewski SR, Thase ME, Quitkin F, Warden D, Ritz L, Nierenberg AA, Lebowitz BD, Biggs MM, et al.; STAR*D Study Team (2006) Medication augmentation after the failure of SSRIs for depression. *N Engl J Med* **354**:1243–1252.
Valdizán EM, Castro E, and Pazos A (2010) Agonist-dependent modulation of G-protein coupling and transduction of 5-HT1A receptors in rat dorsal raphe nucleus. *Int J Neuropsychopharmacol* **13**:835–843.
Vandermaelen CP and Aghajanian GK (1983) Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Res* **289**:109–119.
Yocca FD, Iben L, and Meller E (1992) Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine1A receptors negatively coupled to adenylyl cyclase activity in rat hippocampal membranes. *Mol Pharmacol* **41**:1066–1072.

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