Brexpiprazole Alters Monoaminergic Systems following Repeated Administration: an in Vivo Electrophysiological Study

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Abstract

Background: Brexpiprazole was recently approved as adjunctive therapy for depression and treatment of schizophrenia in adults. To complement results from a previous study in which its acute effects were characterized, the present study assessed the effect of repeated brexpiprazole administration on monoaminergic systems.

Methods: Brexpiprazole (1 mg/kg, subcutaneous) or vehicle was administered once daily for 2 and 14 days. Single-unit electrophysiological recordings from noradrenaline neurons in the locus coeruleus, serotonin neurons in the dorsal raphe nucleus, dopaminergic neurons in the ventral tegmental area, and pyramidal neurons in the hippocampus CA3 region were obtained in adult male Sprague-Dawley rats under chloral hydrate anesthesia within 4 hours after final dosing.

Results: Brexpiprazole blunted D2 autoreceptor responsiveness, while firing activity of ventral tegmental area dopaminergic neurons remained unaltered. Brexpiprazole increased the firing rate of locus coeruleus noradrenaline neurons and increased noradrenaline tone on α2-adrenergic receptors in the hippocampus. Administration of brexpiprazole for 2 but not 14 days increased the firing rate of serotonin neurons in the dorsal raphe nucleus. In the hippocampus, serotonin1A receptor blockade significantly disinhibited pyramidal neurons after 2- and 14-day brexpiprazole administration. In contrast, no significant disinhibition occurred after 24-hour washout or acute brexpiprazole.

Conclusions: Repeated brexpiprazole administration resulted in a marked occupancy of D2 autoreceptors, while discharge activity of ventral tegmental area dopaminergic neurons remained unaltered. Brexpiprazole enhanced serotonergic and noradrenergic tone in the hippocampus, effects common to antidepressant agents. Together, these results provide further insight in the neural mechanisms by which brexpiprazole exerts antidepressant and antipsychotic effects.

Keywords: brexpiprazole, single unit electrophysiological recordings, serotonin, norepinephrine, dopamine

Introduction

Brexpiprazole (Rexulti) was recently shown to be clinically efficacious in the treatment of schizophrenia (Kane et al., 2015) and as an augmentation strategy in treatment of depression (Thase et al., 2015a, 2015b). Similarly to aripiprazole, brexpiprazole is a partial dopamine (DA) D2 receptor agonist, a pharmacological feature that distinguishes these agents from other...
antipsychotics, which are D₂ receptor antagonists (Stark et al., 2007). Although D₂ receptor antagonism effectively reduces positive symptoms in schizophrenia (Seeman and Lee, 1975; Rao and Remington, 2013), blockade of the D₂-receptor-mediated signal might be undesirable for management of negative and/or cognitive symptoms. Indeed, aripiprazole improves negative and cognitive symptoms in schizophrenia and schizoaffective disorder (Stip and Tourjman, 2010), effects partly explained by its combined D₂ receptor partial agonism, 5-HT₁A receptor agonism, and D₂ receptor antagonism (Hirose et al., 2004).

The degree of D₂ receptor activation could be of crucial importance to the antipsychotic properties of partial D₂ receptor agonists. Indeed, the clinical failure of bifeprunox has, in part, been ascribed to its excessive agonism at D₂ receptors and hence limited effects on positive symptoms (Stahl, 2008). Compared with aripiprazole, brexpiprazole has a higher in vitro affinity for D₂ receptors (Maeda et al., 2014b). Indeed, unlike aripiprazole, acute in vivo administration of brexpiprazole did not attenuate the firing activity of ventral tegmental (VTA) DA neurons (Oosterhof et al., 2014). Furthermore, it reversed the inhibitory effect of D₂ autoreceptor agonism on these neurons more potently than aripiprazole (Dahan et al., 2009; Oosterhof et al., 2014). In line with functional D₂ receptor antagonism in animal models (Maeda et al., 2014a), brexpiprazole was recently shown to be clinically effective in the treatment of acute schizophrenia (Kane et al., 2015).

Brexiprazole had potent antagonistic action on 5-HT₁A receptors both in vitro and acute in vivo (Maeda et al., 2014b; Oosterhof et al., 2014), a defining pharmacological quality of atypical antipsychotics thought to underlie a lower incidence of side effects on motor function relative to typical antipsychotics (Kuroki et al., 2008). Blockade of 5-HT₁A receptors is also known to prevent the dampening effect on the noradrenaline (NE) system of sustained selective serotonin reuptake inhibitors (Dremencov et al., 2007a; 2007b; Chernoloz et al., 2009), providing a neural mechanism by which coadministration of low-dose atypical antipsychotics improves the therapeutic efficacy of antidepressants in treatment-resistant patients (Blier and Szabo, 2005). In addition to blocking inhibitory input of the 5-HT system on NE, the inhibitory effect on 5-HT release mediated by terminal α₂-adrenergic heteroceptors, sensitivity of postsynaptic 5-HT₁A receptors, and α₂-adrenergic receptors, and degree of tonic activation of 5-HT₁A, α₁- and α₂-adrenergic receptors was assessed using electrophysiological and pharmacological strategies.

**Methods**

**Animals**

Experiments were carried out in chloral hydrate anesthetized male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) weighing 275 to 450 g housed under standard laboratory conditions. All experiments were carried out in accordance with local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, Ontario, Canada).

**Compounds and Dosing**

Brexiprazole (Maeda et al., 2014b) was dissolved in a 0.5% lactic acid solution in distilled water; pH was adjusted to 4.8 by addition of NaOH. Brexpiprazole (1 mg/kg, s.c.) or vehicle was administered acutely for 2 or 14 days. Electrophysiological recordings occurred within 30 minutes to 4 hours after the last injection. Within this time frame, a blood sample was obtained after recordings.

The D₂ receptor antagonist haloperidol (200 μg/kg) and the DA receptor agonist apomorphine (40 μg/kg) were dissolved in a 0.5% lactic acid solution in distilled water; pH of the solution was adjusted to 4.5 by addition of NaOH. The 5-HT₂A receptor antagonist WAY 100635 was dissolved in Tween 80 (0.2%) in distilled water. Brexpiprazole (1 mg/kg, s.c.) was administered acutely for 2 or 14 days. Electrophysiological recordings occurred within 30 minutes to 4 hours after the last injection. Within this time frame, a blood sample was obtained after recordings.

In Vivo Electrophysiological Recordings

Electrophysiological recordings were performed as described previously (Oosterhof et al., 2015). Briefly, chloral hydrate-anesthetized animals were mounted in a stereotaxic apparatus; body temperature was maintained at 37°C utilizing a thermistor-controlled heating pad. A catheter was inserted in a lateral tail vein for systemic i.v. injection of agents.

Single-barrel glass micropipettes (Stoelting, Spencerville, MD) preloaded with 2 M NaCl (impedance 2–6 MΩ) were used to record the electrical activity of VTA DA, DRN 5-HT, and LC NE neurons, as described previously (Vandermaelen and Aghajanian, 1983; Grace and Bunney, 1984). For VTA DA and LC NE neurons, the start of a burst was defined as the occurrence of an interspike interval (ISI) <80 ms; end of a burst was defined as an ISI >160 ms (Grace and Bunney, 1984; Chenu et al., 2013).
Bursts in 5-HT neurons were defined as an ISI <20 ms (Hajós et al., 2007). The dose-response effects of systemic flesinoxan, clonidine, DOI, and apomorphine injections were quantified to determine the status of 5-HT1A autoreceptors on DRN 5-HT neurons, α2-adrenergic autoreceptors and 5-HT2A receptors on LC NE neurons, and D2 autoreceptors on VTA DA neurons, respectively.

Hippocampal CA3 neurons were recorded with 5-barrel micropipettes (impedances: central barrel 2–5 MΩ, side barrels 20–30 MΩ). 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 5-HT creatine sulfate (10 mM in 0.2 M NaCl, pH 4), NE bitartrate (10 mM in 0.2 M NaCl, pH 4), or quisqualalic acid (1.5 mM in 0.2 M NaCl, pH 4). 5-HT and NE were ejected as cations (+2 to +20 nA) and retained with a negative current; quisqualate was ejected as an anion (-3 to +1 nA) and retained with a positive current. CA3 neurons were activated within their physiological range (10 to 15 Hz; Ranck 1973) with quisqualate. The inhibitory response to 50-second microiontophoretic application of range (10 to 15 Hz; Ranck 1973) with quisqualate. The inhibitory response to 50-second microiontophoretic application of quisqualate was quantified and expressed as spikes inhibited/nA, was used to determine the status of 5-HT2A and α2-adrenergic receptors on these neurons (de Montigny and Aghajanian, 1978; Curet and de Montigny, 1988). The recovery time to 50% of baseline firing (RT50) following iontophoretic application of 5-HT and NE was used as an index of SERT and NET activity following 1-Hz compared with 5-Hz stimulations is indicative for enhanced activation of postsynaptic 5-HT1A receptors, 5-HT afferents were electrical activation on postsynaptic 5-HT, LC NE and VTA DA, heteroceptors on 5-HT neurons, 5-HT afferents were electrical stimulation for 14 days resulted in a concentration of 131 ± 11 ng/mL (n = 27; data extrapolated to a 2-hour postdose time point). These plasma concentrations reflect the values around Cmax following oral dosing (Maeda et al., 2014b). As the elimination half-life is around 2 hours in rats (Maeda et al., 2014b), drug exposure profiles are thus expected to fluctuate between the 24-hour dosing intervals.

Bioanalysis of Brexpiprazole in Plasma

Brexiprazole concentrations were determined in plasma using ultraperformance liquid chromatography followed by tandem mass spectrometry detection. 150 µL acetanilide containing isotope-labeled internal standard was added to 25 µL of calibration standards and test samples. After centrifugation, 100 µL supernatant from each sample was mixed with 100 µL 0.1% formic acid, centrifuged, and placed in the autosampler. Chromatography was performed on a Waters C18SB HSS column (30 x 2.1 mm, 1.8 µm particles) using a mobile phase gradient of 0.1% formic acid in water and acetonitrile. MS/MS detection was done with an Applied Biosystems Scieon API 4000 instrument in positive-ion electrospray ionization mode. Brexpiprazole was detected at a parent > daughter mass to charge ratio of 434.2 > 273.1. The peak area correlated linearly with the plasma concentration in the range of 0.5 to 1000 ng/mL. All individually measured plasma concentrations of brexpiprazole obtained at varying time points were extrapolated to a 2-hour postdose time point.

Results

Brexiprazole Plasma Levels

Two days of brexpiprazole administration resulted in mean plasma concentrations of 62 ± 8 ng/mL (n = 23), and its administration for 14 days resulted in a concentration of 131 ± 11 ng/mL (n = 27; data extrapolated to a 2-hour postdose time point). These plasma concentrations reflect the values around Cmax following oral dosing (Maeda et al., 2014b). As the elimination half-life is around 2 hours in rats (Maeda et al., 2014b), drug exposure profiles are thus expected to fluctuate between the 24-hour dosing intervals.

Body Weight

Administration of brexpiprazole for 14 days had no effect on body weight (F13,43 = 1.8, P > .05; Figure 1).
Results: 5-HT System

Brexpiprazole administration for 2 but not 14 days increased the firing activity of 5-HT neurons in the DRN (Figure 3A). Brexpiprazole administration had no significant effect on body weight.

Discussion

After 2 and 14 days of administration, brexpiprazole plasma levels were in the clinical range observed in patients taking 1 to 4 mg/d (data on file) and corresponded to striatal D2 receptor occupancies ranging between 60% and 75% (Maeda et al., 2014b).
Figure 2. Effect of 2- and 14-day brexpiprazole administration on ventral tegmental area (VTA) DA neurons. (A) Firing activity was unaltered by brexpiprazole administration. (B-D) Illustrative firing histograms of the inhibitory effect of apomorphine in a vehicle- (B), 2-day brexpiprazole- (C), and 14-day brexpiprazole-administered animal (D). (E) Graphic presentation of the inhibitory effect of apomorphine in vehicle- and 2-day and 14-day brexpiprazole-administered animals. Error bars represent SEM, numbers in histograms of (A) represent neurons recorded/animals used. In (E), data points were nudged to prevent overlap. #Significant effect of 2-day brexpiprazole administration compared with vehicle; $$$ P < .001. $Significant effect of 14-day brexpiprazole administration compared with vehicle; $$$ P < .001.

Table 1. Discharge parameters of VTA DA neurons

<table>
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<tr>
<th></th>
<th>Bursts/ min</th>
<th>% spikes in burst</th>
<th>Spikes/burst</th>
<th>ISI (ms)</th>
<th>% neurons bursting</th>
<th>Neurons per tract</th>
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<tbody>
<tr>
<td>2-day vehicle</td>
<td>26 ± 3</td>
<td>31 ± 4</td>
<td>3.1 ± 0.2</td>
<td>70 ± 2</td>
<td>90</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>2-day brexpiprazole</td>
<td>23 ± 3</td>
<td>30 ± 4</td>
<td>3.0 ± 0.1</td>
<td>67 ± 2</td>
<td>86</td>
<td>1.1 ± 0.4</td>
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<tr>
<td>14-day vehicle</td>
<td>28 ± 3</td>
<td>35 ± 4</td>
<td>3.2 ± 0.2</td>
<td>71 ± 2</td>
<td>80</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>14-day brexpiprazole</td>
<td>25 ± 3</td>
<td>33 ± 4</td>
<td>3.0 ± 0.2</td>
<td>72 ± 2</td>
<td>92</td>
<td>1.0 ± 0.2</td>
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No significant effect on any parameter was detected
Figure 3. Effect of acute, 2-day, and 14-day brexpiprazole administration on locus coeruleus (LC) noradrenaline (NE) neurons and status of 5-HT_2A receptors. (A) Firing activity after acute and (B) after 2 and 14 days of brexpiprazole administration. (C-E) Illustrative firing histograms of the inhibitory effect of the 5-HT_2A receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) after vehicle (C), 2-day brexpiprazole (D), and 14-day brexpiprazole administration (E). Note the unaltered response to acute administration of the α_2-adrenoceptor agonist clonidine after brexpiprazole administration. (F) Firing histogram illustrating no reversal by brexpiprazole of neural inhibition by clonidine in a vehicle-administered animal. (G) Graphic presentation of the inhibitory effect of DOI in control, 2-day, and 14-day brexpiprazole administration. Error bars represent SEM; numbers in histograms of (A–B) represent neurons recorded/animals used. *Significant effect of brexpiprazole administration; **p<0.01, ***p<0.001. #Significant effect of 2-day brexpiprazole administration compared with vehicle; ###p<0.001. $Significant effect of 14-day brexpiprazole administration compared with vehicle; $$$p<0.001.
Administration of the DA agonist apomorphine (40 µg/kg, i.v.; corresponding to the ED₁₀₀ in controls) reduced the firing activity of VTA DA neurons in 2- and 14-day brexpiprazole-administered animals to ~70% of baseline activity, demonstrating appreciable occupancy of D₂ receptors by brexpiprazole (Figures 2C-E). Interestingly, firing, bursting, and population activity of VTA DA neurons remained unaltered by these regimens (Figure 2A, Table 1). These data support and extend insight in different dynamics of agents with antagonistic vs partial agonistic action on D₂ receptors on the activity of VTA DA neurons. Acutely, D₂ receptor antagonists robustly increase the firing activity of VTA DA neurons by blocking the D₂ receptor-mediated autoinhibitory signal of DA (Chiodo and Bunney, 1983; Ghanbari et al., 2009). Depending on their degree of intrinsic activity, partial D₂ receptor agonists acutely either decrease (eg aripiprazole, bifeprunox) or do not alter (brexpiprazole) the firing activity of DA neurons (Dahan et al., 2009; Oosterhof et al., 2014). Chronic D₂ receptor antagonism sensitizes D₂ autoreceptors and decreases population activity of VTA DA neurons (Vogelsang and Piercey, 1985; Skarsfeldt, 1995). Of particular interest, asenapine partially blocked the inhibitory effect of apomorphine on DA neurons after 2 days of administration, similarly to the effect of 2-day brexpiprazole administration. However, 21-day asenapine administration sensitized D₂ autoreceptors (Oosterhof et al., 2015). In contrast, the response of VTA DA neurons to apomorphine was indistinguishably dampened following 2- and 14-day brexpiprazole administration, demonstrating unaltered D₂ receptor sensitivity following these drug regimens (Figure 2C-E).

**DA System**

Acute administration of brexpiprazole increased the firing activity of LC NE neurons (Figure 3A). This firing activity remained elevated after repeated administration (Figure 3B), similarly to the effect of sustained asenapine, clozapine, quetiapine, and olanzapine administration (Ramirez and Wang, 1986; Seager et al., 2005; Chernoloz et al., 2012; Oosterhof et al., 2015). Interestingly, 2- and 14-day aripiprazole administration did not alter the firing activity of LC neurons (Chernoloz et al., 2009), demonstrating distinct effects of aripiprazole and brexpiprazole on the NE system. Brexpiprazole did not cause a blockade...
on $\alpha_2$-adrenergic autoreceptors, as an ED$_{100}$ dose of clonidine (5 µg/kg, i.v.) indistinguishably inhibited LC NE neurons in vehicle, 2-day, and 14-day brexpiprazole-administered animals (Figure 3C-F). Although these data do not exclude the possibility that brexpiprazole caused a sensitization of $\alpha_2$-adrenergic autoreceptors, this is highly unlikely; first, since such sensitization would decrease—and not increase—the firing rate of LC NE neurons, and second because intravenous brexpiprazole did not reverse the inhibitory effect of clonidine on NE neurons (in vehicle-administered animals; Figure 3F). These observations support the lack of activity of brexpiprazole on $\alpha_2$-adrenergic autoreceptors.

Activation of 5-HT$_{1A}$ receptors located on terminals arising from the hypoglossal nucleus stimulates GABA release on NE neurons, providing a pharmacological node by which the 5-HT system modulates the activity of LC NE neurons (Szabo and Blier, 2001). Indeed, acute administration of the 5-HT$_{1A}$ receptor agonist DOI inhibited all LC NE neurons in control animals (ED$_{100}$ = 83 µg/kg), an effect fully reversed by the selective 5-HT$_{1A}$ receptor antagonist M100907 (Figure 3C,G). In accordance with its acute behavioral and in vivo electrophysiological effects (Maeda et al., 2014b; Oosterhof et al., 2014), brexpiprazole administration for 2 and 14 days potently reduced the inhibitory effect of DOI, thus demonstrating antagonistic action on 5-HT$_{1A}$ receptors (Figure 3D,E,G). Using the same methodology, antagonistic effects on 5-HT$_{1A}$ receptors were previously demonstrated following sustained asenapine and quetiapine + norquetiapine administration (Chernoloz et al., 2012; Oosterhof et al., 2015). Notably, the in vitro affinity for 5-HT$_{1A}$ receptor of asenapine (Ki = 0.06 nM; Shahid et al., 2009), brexpiprazole (Ki = 0.47 nM; Maeda et al., 2014b), and quetiapine + norquetiapine (quetiapine Ki = 101 nM [Kroese et al., 2003]; norquetiapine Ki = 58 nM [Jensen et al., 2008]) is reflected in their respective complete, partial (Figure 3G), and slight blockade of DOI in vivo (Chernoloz et al., 2012; Oosterhof et al., 2015).

Previous studies showed that sustained antidepressant administration increases 5-HT neurotransmission, causing tonic activation of 5-HT$_{1A}$ receptors and consequently, decreased NE firing. Under these conditions, 5-HT$_{1A}$ receptor blockade normalizes NE firing (Dremencov et al., 2007a, 2007b; Chernoloz et al., 2009, 2012), providing a rationale for the augmenting effects of coadministering low-dose atypical antipsychotics to an antidepressant regimen (Blier and Szabo, 2005). In accordance, the present demonstration of 5-HT$_{1A}$ receptor blockade provides one mechanism for the clinical effectiveness of brexpiprazole when administered in adjunct to antidepressants (Thase et al., 2015a, 2015b).

In the hippocampus, brexpiprazole increased the tonic activation of $\alpha_1$- but not $\alpha_2$-adrenergic receptors on CA3 pyramidal neurons (Figure 4C). Since the activity of the NET and the sensitivity of postsynaptic $\alpha_2$-adrenergic receptors remained unaltered in this brain region, increased hippocampal NE transmission was likely due to the excitatory effect of brexpiprazole on LC NE neurons (Figure 3A,B), an effect previously observed following sustained trazodone and quetiapine administration (Chernoloz et al., 2012; Ghanbari et al., 2012). In further resemblance to these agents, brexpiprazole has antagonistic action on $\alpha_1$-adrenergic receptors in vivo (Oosterhof et al., 2014), providing a mechanism by which it prevented tonic activation of these receptors. Importantly, brexpiprazole did not increase hippocampal or cortical NE levels following its acute administration (Maeda et al., 2014b), in contrast to other atypical antipsychotics (Westenink et al., 1998), suggesting that its enhancing effect on NE neurotransmission requires delayed neural adaptations.

**5-HT System**

Brexiprazole administration for 2 days significantly increased the firing activity of DRN 5-HT neurons (Figure 5A), similarly to the effect of 2-day aripiprazole administration (Chernoloz et al., 2009). However, the firing activity of 5-HT neurons returned to control levels after 14-day brexpiprazole administration, while this remained elevated after 14-day aripiprazole administration (Chernoloz et al., 2009). In further contrast to aripiprazole, 2- and 14-day brexpiprazole administration did not alter the responsiveness of 5-HT neurons to acute 5-HT$_{1A}$ receptor agonist administration, demonstrating that 5-HT$_{1A}$ autoreceptor desensitization did not occur (Figure 5B). This finding might be surprising, especially since brexpiprazole was previously shown to act as a full 5-HT$_{1A}$ receptor agonist in the hippocampus (Oosterhof et al., 2014). Indeed, the full 5-HT$_{1A}$ receptor agonists...
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BAY x 3702 and trazodone desensitized 5-HT<sub>1A</sub> autoreceptors after long-term administration (Dong et al., 1998; Ghanbari et al., 2010). On the other hand, these agents decreased the firing activity of 5-HT neurons following 2-day administration, in contrast to the effect of brexiprazole (Figure 5A). The DRN receives excitatory NE projections from the LC (Svensson et al., 1975), and excitatory D<sub>2</sub> receptors are also located on DRN 5-HT neurons (Haj-Dahmane, 2001). Possibly, a combination of these excitatory inputs prevented DRN 5-HT neurons to decrease their firing. Indeed, the excitatory effect of aripiprazole on DRN 5-HT neurons was to a significant degree mediated by partial D<sub>2</sub> receptor agonism (Chernoloz et al., 2009). In addition, since brexiprazole plasma concentrations were significantly higher after 14 compared with 2 days, it is conceivable that this caused a greater activation of 5-HT<sub>1A</sub> autoreceptors, preventing firing of DRN 5-HT neurons to remain elevated after the longer regimen.

In the hippocampus CA3 region, brexiprazole increased the tonic activation of 5-HT<sub>1A</sub> receptors on pyramidal neurons after 14 days of administration (Figure 6D), an effect common to all antidepressant agents tested in this paradigm (Blier and El Mansari, 2013). Since 14-day brexiprazole did not alter the firing activity of 5-HT neurons, activity of the SERT, and status of 5-HT<sub>1B</sub> terminal autoreceptors, it was deemed critical to assess whether this enhanced tonic activation was due to adaptive

Figure 6. Effect of 14-day brexiprazole administration on the status of 5-HT<sub>1A</sub> receptors in the CA3 region of the hippocampus. (A-C) Illustrative firing histograms on the effect of cumulative WAY 100.635 (WAY) administrations on the firing activity of a CA3 pyramidal neuron in a 14-day vehicle- (A), a 2-day brexiprazole- (B), and 14-day brexiprazole-administered animal (C). (D) Quantification of the effect of WAY 100.635 on basal firing rate in rats administered with vehicle or brexiprazole days. (E) Comparison of DOS on CA3 pyramidal neurons produced by 5-HT fiber bundle stimulation in vehicle- and 14-day brexiprazole-administered animals. Data were analyzed with repeated-measures ANOVA followed by Bonferroni posthoc analysis (D), or a 2-way ANOVA (D). The number of animals (n) and neurons (n) is provided within the histograms (D); error bars represent SEM. #Significant effect of 2-day brexiprazole administration compared with controls; $$$P < .001. $Significant effect of 14-day brexiprazole administration compared with controls; $P < .05, $$$$P < .001. *Significant effect of stimulation frequency; ***P < .001.
changes or attributable to the full agonistic action of brexipiprazole on postsynaptic 5-HT_{1A} receptors (Oosterhof et al., 2014). Interestingly, 2-day brexipiprazole administration caused a tonic activation on 5-HT_{1A} receptors quantitatively comparable to the 14-day regimen. In contrast, 2-day bupropion, mirtazapine, and duloxetine administration all produced a lesser degree of post-synaptic 5-HT_{1A} receptor activation, presumably due to adaptive changes following sustained administration (Rueter et al., 1998; Besson et al., 2000; Ghanbari et al., 2011). Because the strong tonic activation after 2-day brexipiprazole was unexpected, it was decided to assess whether this effect was due to adaptive changes occurring within this short exposure period or full agonistic action of brexipiprazole on 5-HT_{1A} receptors. Clearly, adaptive changes after 2-day brexipiprazole administration alone did not lead to increased tonic activation of 5-HT_{1A} receptors, as a 24-hour washout prevented this effect. Similarly, full 5-HT_{1A} agonism alone was insufficient to produce this effect, as the acute administration of brexipiprazole did not significantly enhance 5-HT_{1A} receptor activation, at least at this dose. Based on these results, it is possible that acute administration resulted in lower brexipiprazole levels and less tonic activation of 5-HT_{1A} receptors compared with the 2- and 14-day regimens, where brexipiprazole accumulated with repeated injections. Alternatively, the combination of adaptive changes together with higher levels of brexipiprazole resulted in the marked enhancement of tonic activation on postsynaptic 5-HT_{1A} receptors after 2- and 14-day brexipiprazole administration.

**Conclusion**

Repeated administration of brexipiprazole had profound effects on the activity of the DA, NE, and 5-HT monoamine systems, both at their cell body level and in projection areas. Brexipiprazole produced a significant occupation of D_{2} autoreceptors while the firing activity of VTA DA neurons remained unchanged, supporting a stabilizing effect of brexipiprazole on DA neurotransmission. This might be particularly relevant in the treatment of schizophrenia, because a striatal hyperdopaminergic state has been commonly associated with positive symptoms, and low DA neurotransmission in prefrontal regions presumably contributes to negative symptoms and/or cognitive impairments (Laruelle et al., 1996; Silfstein et al., 2015). Although the precise effects of antipsychotics with partial D_{2} receptor agonism on DA neurotransmission in patients requires further characterization, their therapeutic effect in schizophrenia is in line with a stabilizing effect of brexipiprazole on the DA system (Kane et al., 2015). Repeated brexipiprazole administration caused a blockade of 2 receptor populations mediating inhibitory cross-talk between the 5-HT and NE systems. First, it blocked 5-HT_{1A} receptors, a pharmacological target important in the lower incidence of motor side effects produced by second-generation antipsychotics in the treatment of schizophrenia (Tarazi and Stahl, 2012) and in adjunct to serotonin reuptake inhibitors in the treatment of depression (Blier and Szabo, 2005). Secondly, it blocked α_{2}-adrenergic heteroreceptors on 5-HT terminals, thereby not attenuating 5-HT release despite enhanced NE neurotransmission. Finally, accumulation of brexipiprazole and/or adaptive changes produced enhanced tonic activation of postsynaptic 5-HT_{1A} receptors, a common effect of all agents with antidepressant action (Blier and El Mansari, 2013). With the limitation that the present findings were obtained in naive rats, these data provide insight in the effects of repeated brexipiprazole administration on monoamine systems that might be relevant to symptom domains.

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**Statement of Interest**

M. El Mansari and C.A. Oosterhof declare no conflict of interest. P. Blier received grants and/or honoraria for giving lectures and/or participating on advisory boards from Astra Zeneca, Bristol-Myers Squibb, Eli Lilly, Janssen, Labopharm, Lundbeck/Takeda, Merck, Pfizer, Servier, sunovion, and Valeant.

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