RESEARCH ARTICLE

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 (1mg/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 D_2 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 α_2-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, serotonin$_{1A}$ 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 D_2 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) D_2 receptor agonist, a pharmacological feature that distinguishes these agents from other

antipsychotics, which are D_2 receptor antagonists (Stark et al., 2007). Although D_2 receptor antagonism effectively reduces positive symptoms in schizophrenia (Seeman and Lee, 1975; Rao and Remington, 2013), blockade of the D_2-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 D_2 receptor partial agonism, 5-HT_{1A} receptor agonism, and 5-HT_{2A} receptor antagonism (Hirose et al., 2004).

The degree of D_2 receptor activation could be of crucial importance to the antipsychotic properties of partial D_2 receptor agonists. Indeed, the clinical failure of bifeprunox has, in part, been ascribed to its excessive agonism at D_2 receptors and hence limited effects on positive symptoms (Stahl, 2008). Compared with aripiprazole, brexpiprazole has a higher in vitro affinity yet lower intrinsic activity at D_2 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 D_2 autoreceptor agonism on these neurons more potently than aripiprazole (Dahan et al., 2009; Oosterhof et al., 2014). In line with functional D_2 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).

Brexpiprazole had potent antagonistic action on 5-HT_{2A} 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-HT_{2A} 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 α_2-adrenergic heteroceptor activation was potently reduced by brexpiprazole (Oosterhof et al., 2014), an effect potentially preventing attenuated 5-HT release when NE neurotransmission is elevated (Mongeau et al., 1993; Chernoloz et al., 2012).

Both in vitro and in vivo studies demonstrated more potent 5-HT_{1A} receptor agonism by brexpiprazole compared with aripiprazole (Maeda et al., 2014b; Oosterhof et al., 2014). Interestingly, acute brexpiprazole–but not aripiprazole–improved phencyclidine-induced cognitive impairments in the novel object recognition test (Maeda et al., 2014b). Coadministration of the selective 5-HT_{1A} receptor antagonist WAY 100.635 prevented this behavioral effect of brexpiprazole, suggesting that 5-HT_{1A} receptor activation was involved in the restorative effects of brexpiprazole on cognitive-like measures (Maeda et al., 2014b; Yoshimi et al., 2014). Furthermore, since chronic administration of all antidepressants enhances tonic activation of 5-HT_{1A} receptors (Haddjeri et al., 1998), the potent in vivo agonism of brexpiprazole on these receptors could be of therapeutic relevance in the treatment of mood disorders (Blier and Ward, 2003; Blier and El Mansari, 2013).

Antidepressant and antipsychotic medications gradually induce neural adaptations, which presumably underlie their delayed onset of action. To characterize such adaptations within monoamine systems, the present study aimed to determine the effects of 2- and 14-day brexpiprazole administration (1 mg/kg/d, s.c., a dose resulting in clinically relevant blood plasma levels) on the discharge activity of VTA DA, locus coeruleus (LC) NE, and dorsal raphe nucleus (DRN) 5-HT neuron populations. In these brain regions, the functional status of D_2, α_2-adrenergic, 5-HT_{1A}, and 5-HT_{2A} receptors was assessed. In the CA3 region of the hippocampus, the effect of sustained brexpiprazole administration on the activity of the 5-HT and NE transporters (SERT and NET, respectively), the status of terminal 5-HT_{1B} auto- and α_2-adrenergic heteroceptors, sensitivity of postsynaptic 5-HT_{1A} and α_2-adrenergic receptors, and degree of tonic activation of 5-HT_{1A}, α_1- and α_2-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

Brexpiprazole (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 D_2 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-HT_{2A} receptor antagonist M100907 was dissolved in Tween 80 (0.2%) in distilled water. The 5-HT_{1A} receptor agonist flesinoxan (100 µg/kg), the 5-HT_{1A} receptor antagonist WAY 100.635 (100 µg/kg), the preferential 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI, 100 µg/kg), the α_1-adrenoreceptor antagonist prazosin (100 µg/kg), the α_2-adrenoreceptor agonist clonidine (5 µg/kg), and the α_2-adrenoreceptor antagonist idazoxan (1000 µg/kg) were dissolved in distilled water. Brexpiprazole was provided by Lundbeck A/S (Valby, Denmark); all other compounds were purchased from Sigma Aldrich (Oakville, ON, Canada).

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-HT$_{1A}$ autoreceptors on DRN 5-HT neurons, α_2-adrenergic autoreceptors and 5-HT$_{2A}$ receptors on LC NE neurons, and D$_2$ 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 creatinine 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 NE and 5-HT, respectively, expressed as spikes inhibited/nA, was used to determine the status of 5-HT$_{1A}$ and α_2-adrenerigic receptors on these neurons (de Montigny and Aghajanian, 1978; Curet and de Montigny, 1988). The recovery time to 50% of baseline firing (RT$_{50}$) following iontophoretic application of 5-HT and NE was used as an index of SERT and NET activity (de Montigny et al., 1980; Piñeyro et al., 1994) To determine the degree of tonic activation of postsynaptic 5-HT$_{1A}$ receptors, firing activity of CA3 pyramidal neurons was decreased to ~4 Hz. A physiological saline injection was administered followed by 4 consecutive injections of the 5-HT$_{1A}$ receptor antagonist WAY 100.635 (25 μg/kg, 2-minute intervals). The average firing rate (spikes/s) of neurons during the second half of the 120-second period following each WAY 100.635 injection was quantified and expressed as percentage change from baseline. Using the same paradigm, degree of tonic activation of postsynaptic α_1- and α_2-adrenoceptors was quantified as the disinhibitory effect of prazosin (100 μg/kg, i.v.) and idazoxan (1000 μg/kg, i.v.), respectively, on CA3 pyramidal neurons.

To assess the status of 5-HT$_{1B}$ autoreceptors and α_2-adrenergic heteroceptors on 5-HT neurons, 5-HT afferents were electrical stimulated (0.5 ms, 300 μA, 1 and 5 Hz) with a bipolar electrode (NE-110, David Kopf, Tujanga, CA) connected to a stimulator (S8800, Grass instruments, Quincey, MA), causing endogenous release of 5-HT, which activation of postsynaptic 5-HT$_{1A}$ receptors briefly suppresses firing of CA3 pyramidal neurons (Chaput et al., 1986). The inhibitory effect of 200 pulses delivered at 1 and 5 Hz was plotted in a peristimulus time histogram. Duration of silence (DOS, in milliseconds) was defined as the period from the first bin showing a 50% reduction relative to baseline to the first subsequent bin attaining a 90% recovery (Chaput et al., 1986). Increasing the stimulation frequency from 1 to 5 Hz results in greater activation of terminal 5-HT$_{1B}$ receptors and, consequently, decreased 5-HT release. Therefore, a greater DOS following 1-Hz compared with 5-Hz stimulations is indicative for intact function of terminal 5-HT$_{1B}$ autoreceptors (Chaput et al., 1986; Blier et al., 1989). The activity of α_2-adrenergic heteroceptors on 5-HT terminals is known to be frequency independent (Blier et al., 1989). Therefore, a reduced DOS at 1 Hz indicates enhanced activation of α_2-adrenergic receptors (Mongeau et al., 1993), while a normal or DOS in presence of enhanced NE neurotransmission demonstrates α_2-adrenergic heteroceptor blockade (Chernoloz et al., 2012).

Bioanalysis of Brexpiprazole in Plasma

Brexpiprazole concentrations were determined in plasma using ultraperformance liquid chromatography followed by tandem mass spectrometry detection. 150 μL acetonitrile 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 Sciex 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.

Data Analysis / Statistics

Electrophysiological recordings were filtered from artifacts by wavemark analysis in Spike2 software version 6.17 (Cambridge Electronic Design, Cambridge, UK). Firing and burst analysis of DRN 5-HT, LC NE, and VTA DA neurons was performed using burstiDAtor software (Oosterhof and Oosterhof, 2013).

For dose-response effects, data from 2- and 14-day vehicle-administered animals were pooled. The dose-response effects of flesinoxan and clonidine were analyzed with linear regression analysis. Effect of acute brexpiprazole administration on LC NE neurons was analyzed with 1-way ANOVA. Effects on the firing and burst activity of DRN 5-HT, LC NE and VTA DA, dose-response effects of apomorphine and DOI, degree of tonic activation on α-adrenergic receptors, and 5-HT afferent stimulation were analyzed with 2-way ANOVA and Bonferroni posthoc analysis; alphas were corrected for multiple comparisons when appropriate. Body weight gain and degree of 5-HT$_{1A}$ receptor activation was assessed using repeated measures (RM) ANOVA and Bonferroni posthoc analysis. All data were analyzed with Graphpad Prism (Version 5.01, Graphpad software, La Jolla, CA). Data are presented as mean, error bars represent SEM, and $P < .05$ was considered statistically significant.

Results

Brexpiprazole 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 ($F_{13,43} = 1.8$, $P > .05$; Figure 1).
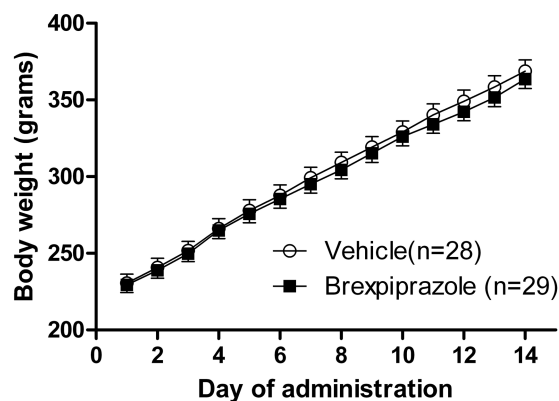
Figure 1. Brexpiprazole administration had no significant effect on body weight. Data are presented as mean; error bars represent SEM.

VTA DA Neurons

Brexpiprazole administration for 2 and 14 days did not modify the firing activity of VTA DA neurons (Figure 2A). Brexpiprazole did not alter VTA DA neuron population or burst activity (Table 1).

The firing rate of VTA DA neurons used for D_2 receptor status assessment did not differ between vehicle, 2-day and 14-day brexpiprazole-administered animals (4.3 ± 0.5, $4.3.0 \pm 0.5$, and 4.3 ± 0.5 Hz, respectively, $P > .05$). Saline administration had no effect on their firing rate ($P > .05$). In vehicle-administered animals, apomorphine (40 µg/kg, i.v.) completely inhibited the firing activity of all VTA DA neurons tested (Figure 2B,E). Following 2- and 14-day brexpiprazole administration, 40 µg/kg of apomorphine had a significantly smaller inhibitory effect compared with vehicle-administered animals (1-way ANOVA, $F_{2,15} = 77$, $P > .001$, data from 7, 6, and 5 neurons, respectively). Up to a dose of 80 µg/kg, apomorphine inhibited the firing of VTA DA neurons by approximately 30%, and not a single neuron was inhibited completely (Figure 2C-E).

Results: NE System

Both acute ($F_{1,96} = 9.9$, $P > .002$; Figure 3A) and repeated brexpiprazole administration ($F_{1,402} = 40.6$, $P > .001$; Figure 3B) significantly increased the firing activity of LC NE neurons. Burst parameters of LC NE neurons were unchanged by brexpiprazole administration ($P > .05$, data not shown).

Baseline firing rate of LC NE neurons used for 5-HT_{2A} receptor status assessment did not differ between vehicle and 2- and 14-day brexpiprazole-administered animals (1.5 ± 0.2, 2.0 ± 0.9, and 2.2 ± 0.6 Hz, respectively, $P > .05$). Saline administration had no effect in either group (ANOVA, $F_{2,21} = 0.6$, $P > .05$). DOI at a dose of 50 or 100 µg/kg had a significantly greater inhibitory effect in vehicle-administered animals compared with 2- and 14-day brexpiprazole administered animals (1-way ANOVA, 50 µg/kg: $F_{2,16} = 16.5$, $P < .001$ data from 5, 7, and 7 neurons, respectively; 100 µg/kg: $F_{2,14} = 26.5$, $P < .001$, data from 3, 7, and 7 neurons, respectively) (Figure 3C). After DOI administration, the α_2-adrenoceptor agonist clonidine (5 µg/kg, i.v.) inhibited all neurons tested (vehicle, n = 3; 2-day brexpiprazole, n = 7; 14-day brexpiprazole, n = 4) (Figure 3C-E). The inhibitory effects of clonidine after DOI administration were not used for statistical analysis and serve illustrative purposes only.

Baseline firing rate of LC NE neurons used for α_2-adrenergic autoreceptor status assessment did not differ between vehicle, 2-day, and 14-day brexpiprazole-administered animals (1.7 ± 0.3,

1.7 ± 0.2, and 1.8 ± 0.1 Hz, respectively, $P > .05$). Clonidine administration (5 µg/kg, i.v.) completely inhibited the firing activity of all neurons tested (n ≥ 3/group, data not shown), and brexpiprazole (500 µg/kg, i.v.) did not reverse the inhibitory effect of clonidine in 2 vehicle-administered animals (Figure 3F).

In the hippocampus, spikes inhibited/nA NE, and RT_{50} did not differ between 14-day vehicle- and brexpiprazole-administered animals (39 ± 3 vs 34 ± 3 spikes inhibited/nA and 77 ± 7 vs 73 ± 5 seconds, respectively, data from 30 and 23 neurons in 14 and 13 animals, $P > .05$). Baseline firing rate of CA 3 pyramidal neurons used for assessment of degree of α-adrenergic receptor activation did not differ between vehicle and 14-day brexpiprazole-administered animals (3.5 ± 0.3 vs 3.2 ± 0.3 Hz, respectively, $P > .05$). Administration of saline and blockade of α_1-adrenergic receptors with prazosin did not alter the firing activity of CA3 pyramidal neurons ($F_{1,10} = 1.1$ and $F_{1,10} = 3.6$, respectively, $P > .05$). Blockade of α_2-adrenergic receptors with idazoxan had a significant disinhibitory effect on CA3 pyramidal neurons in 14-day brexpiprazole-administered animals ($F_{1,10} = 10.3$, $P = .009$; Figure 4).

Results: 5-HT System

Brexpiprazole administration for 2 but not 14 days increased the firing activity of 5-HT neurons in the DRN ($F_{1,346} = 9.8$, $P = .002$) (Figure 5A). No effect of brexpiprazole was detected on burst parameters ($P > .05$, data not shown). At baseline, the firing rate of 5-HT neurons used for determining 5-HT_{1A} autoreceptor sensitivity did not differ between vehicle, 2-day, and 14-day brexpiprazole-administered animals (1.2 ± 0.3, 1.4 ± 0.4, and 1.2 ± 0.3 Hz, respectively). Administration of saline did not alter firing activity relative to baseline ($P > .05$), and responsiveness to flesinoxan was unaltered after 2- and 14-day brexpiprazole administration ($F_{2,16} = 0.18$, $P > .05$; Figure 5B).

In hippocampus, spikes inhibited/nA 5-HT, and RT_{50} did not differ between vehicle- and 14-day brexpiprazole-administered animals (189 ± 11 vs 181 ± 14 spikes inhibited/nA and 33 ± 3 vs 38 ± 3 seconds, respectively, data from 24 and 19 neurons in groups of 8 and 7 animals, $P > .05$). Baseline firing activity of CA3 pyramidal neurons before assessment of degree of tonic 5-HT_{1A} receptor activation did not differ between 14-day vehicle, acute, 2-day brexpiprazole + 24-hour washout, 2-day brexpiprazole, and 14-day brexpiprazole-administered animals (3.6 ± 0.3, 3.6 ± 1.2, 4.0 ± 0.5, 3.9 ± 0.2, and 3.3 ± 0.2 Hz, respectively, $F_{4,25} = 0.7$, $P > .05$). Blockade of 5-HT_{1A} receptors by WAY 100.635 at a dose of 25 µg/kg had a significant overall disinhibiting effect only in 14-day brexpiprazole-administered animals (RM ANOVA with Bonferroni posthoc, $P < .05$; Figure 6D). At doses of 50, 75, and 100 µg/kg, WAY 100.635 caused a significant disinhibition in 2- and 14-day brexpiprazole-administered animals (RM ANOVA with Bonferroni posthoc, $P < .001$ for 50, 75, and 100 µg/kg, Figure 6D; for illustrative firing histograms of a neuron in a vehicle, 2- and 14-day brexpiprazole-administered animals, see Figure 6A-C, respectively).

Electrical stimulation of 5-HT afferents caused a shorter DOS at 5 compared with 1 Hz in 14-day vehicle- and brexpiprazole-administered animals ($F_{1,44} = 62.0$, $P < .001$) (Figure 6E). The DOS at 1 and 5 Hz did not differ between these groups ($P > .05$).

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 D_2 receptor occupancies ranging between 60% and 75% (Maeda et al., 2014b).
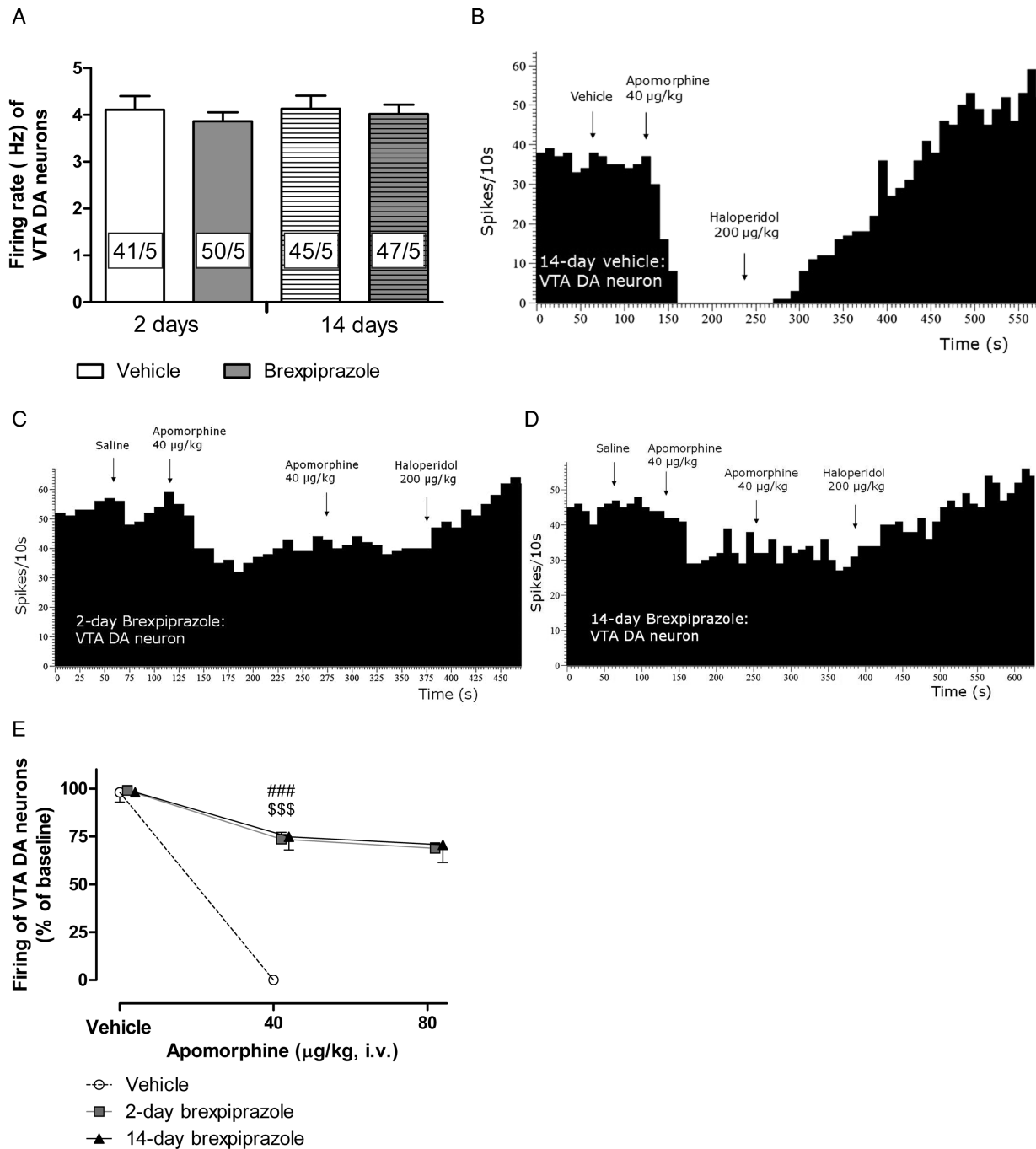
Figure 2. Effect of 2- and 14-day brexpiprazole administration on ventral tegmental area (VTA) DA neurons. (A) Firing activity was unaltered by brexpiprazole administration. (B-D) Illustrative firing histograms of the inhibitory effect of apomorphine in a vehicle- (B), 2-day brexpiprazole- (C), and 14-day 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

	Bursts/ min	% spikes in burst	Spikes/ burst	ISI (ms)	% neurons bursting	Neurons per tract
2-day vehicle	26±3	31±4	3.1±0.2	70±2	90	0.9±0.2
2-day brexpiprazole	23±3	30±4	3.0±0.1	67±2	86	1.1±0.4
14-day vehicle	28±3	35±4	3.2±0.2	71±2	80	1.0±0.1
14-day brexpiprazole	25±3	33±4	3.0±0.2	72±2	92	1.0±0.2

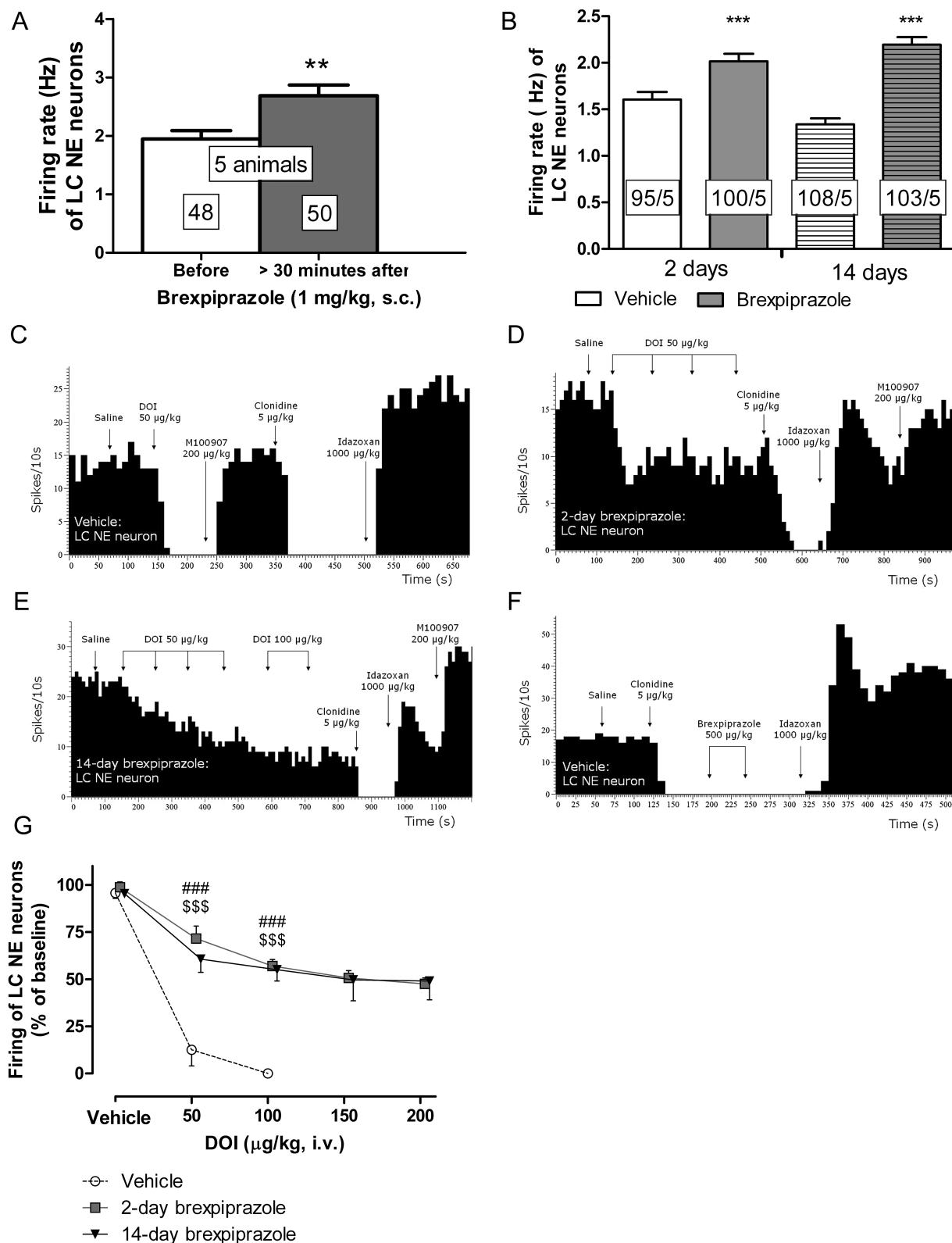No significant effect on any parameter was detected

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 < .001. $Significant effect of 14-day brexpiprazole administration compared with vehicle; $$$$P$ < .001.
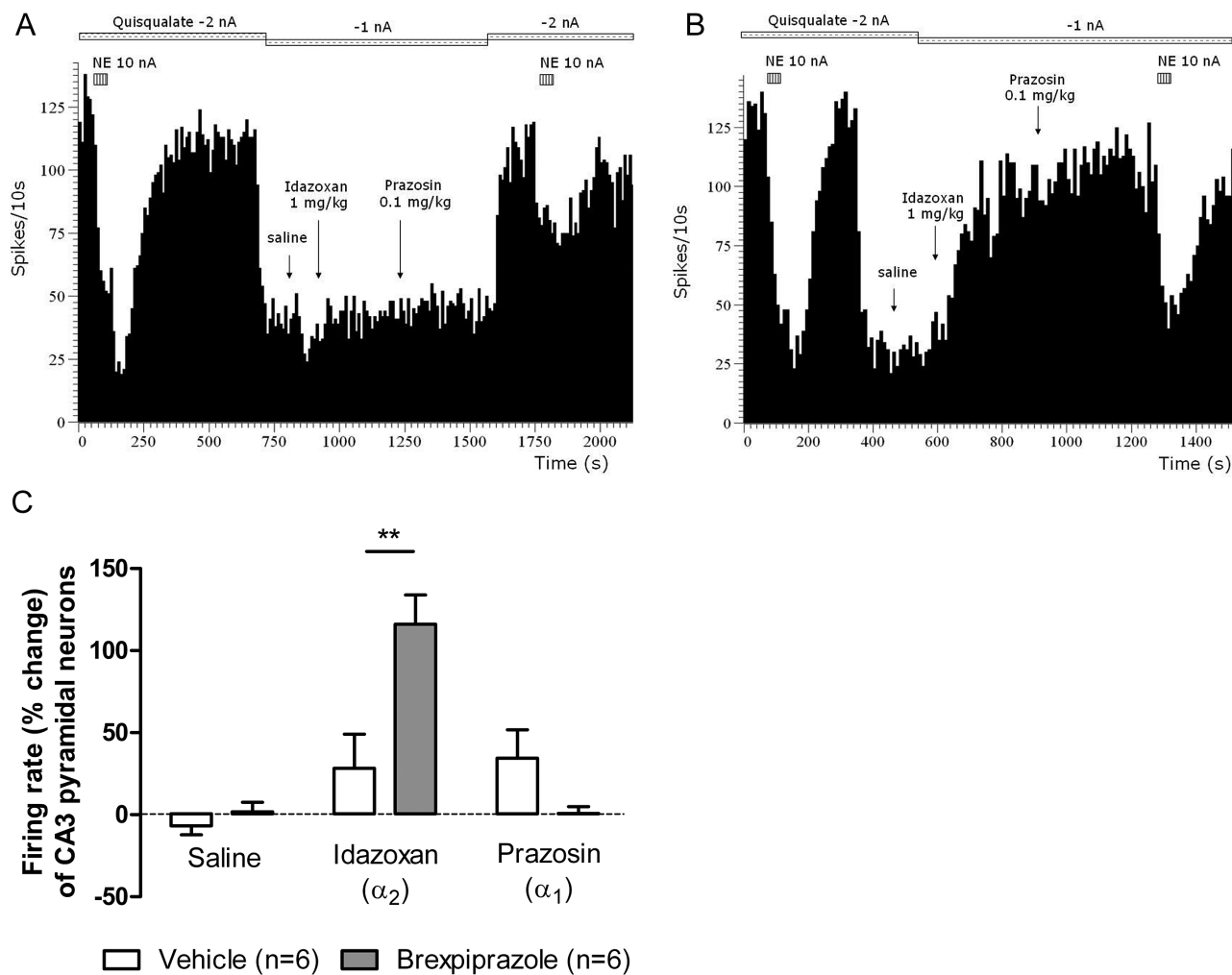
Figure 4. Effect of 14-day brexpiprazole administration on the status of α-adrenoceptors in the hippocampus CA3 region. (A-B) Firing histograms illustrating the effect of acute administration of the α_2-adrenoceptor antagonist idazoxan and the α_1-adrenoceptor antagonist prazosin administration on the firing activity of a CA3 pyramidal neuron in a 14-day vehicle- (A) and 14-day brexpiprazole-administered animal (B). (C) Effect of the α_2-adrenoceptor antagonist idazoxan (1 mg/kg, i.v.) and prazosin (0.1 mg/kg, i.v.) on firing rate of CA3 pyramidal neurons after 14-day vehicle or brexpiprazole administration. Error bars represent SEM. *Significant effect of brexpiprazole administration compared with vehicle group; **$P < .01$.

DA System

Administration of the DA agonist apomorphine (40 µg/kg, i.v.; corresponding to the ED_{100} 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 D_2 receptor 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 D_2 receptors on the activity of VTA DA neurons. Acutely, D_2 receptor antagonists robustly increase the firing activity of VTA DA neurons by blocking the D_2 receptor-mediated autoinhibitory signal of DA (Chiodo and Bunney, 1983; Ghanbari et al., 2009). Depending on their degree of intrinsic activity, partial D_2 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 D_2 receptor antagonism sensitizes D_2 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 D_2 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 D_2 receptor sensitivity following these drug regimens (Figure 2C-E).

NE 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 of 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
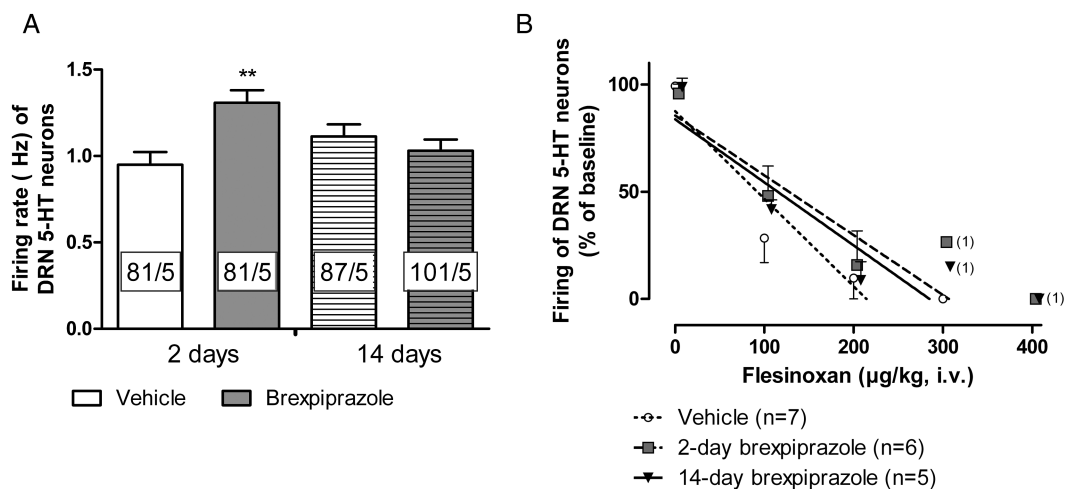
Figure 5. Effect of 2- and 14-day brexpiprazole administration on dorsal raphe nucleus (DRN) 5-HT neurons firing activity and 5-HT$_{1A}$ autoreceptor status. (A) Firing activity was increased after 2, but not 14 days of brexpiprazole administration. (B) The dose-response relationship between DRN 5-HT neuron firing activity and administrations of flesinoxan in vehicle-, 2-day brexpiprazole-, and 14-day brexpiprazole-administered animals. Data were analyzed with 2-way ANOVA (A) or linear regression (B), are presented as mean, error bars represent SEM, numbers in histograms represent neurons recorded/animals used in (A) or neurons per group in (B). In (B), data points were nudged to prevent overlap. *Significant effect of brexpiprazole administration compared with corresponding vehicle group, **$P < .01$.

on α_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 α_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 α_2-adrenergic autoreceptors.

Activation of 5-HT$_{2A}$ 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$_{2A}$ 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$_{2A}$ 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$_{2A}$ receptors (Figure 3D,E,G). Using the same methodology, antagonistic effects on 5-HT$_{2A}$ 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$_{2A}$ 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 [Kroeze 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$_{2A}$ receptors and consequently, decreased NE firing. Under these conditions, 5-HT$_{2A}$ receptor blockade normalizes NE firing (Dremencov et al., 2007a, 2007b; Chernoloz et al., 2009,

2012), providing a rationale for the augmenting effects of coadministrating low-dose atypical antipsychotics to an antidepressant regimen (Blier and Szabo, 2005). In accordance, the present demonstration of 5-HT$_{2A}$ 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 α_2- but not α_1-adrenergic receptors on CA3 pyramidal neurons (Figure 4C). Since the activity of the NET and the sensitivity of postsynaptic α_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 α_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 (Westerink et al., 1998), suggesting that its enhancing effect on NE neurotransmission requires delayed neural adaptations.

5-HT System

Brexpiprazole 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
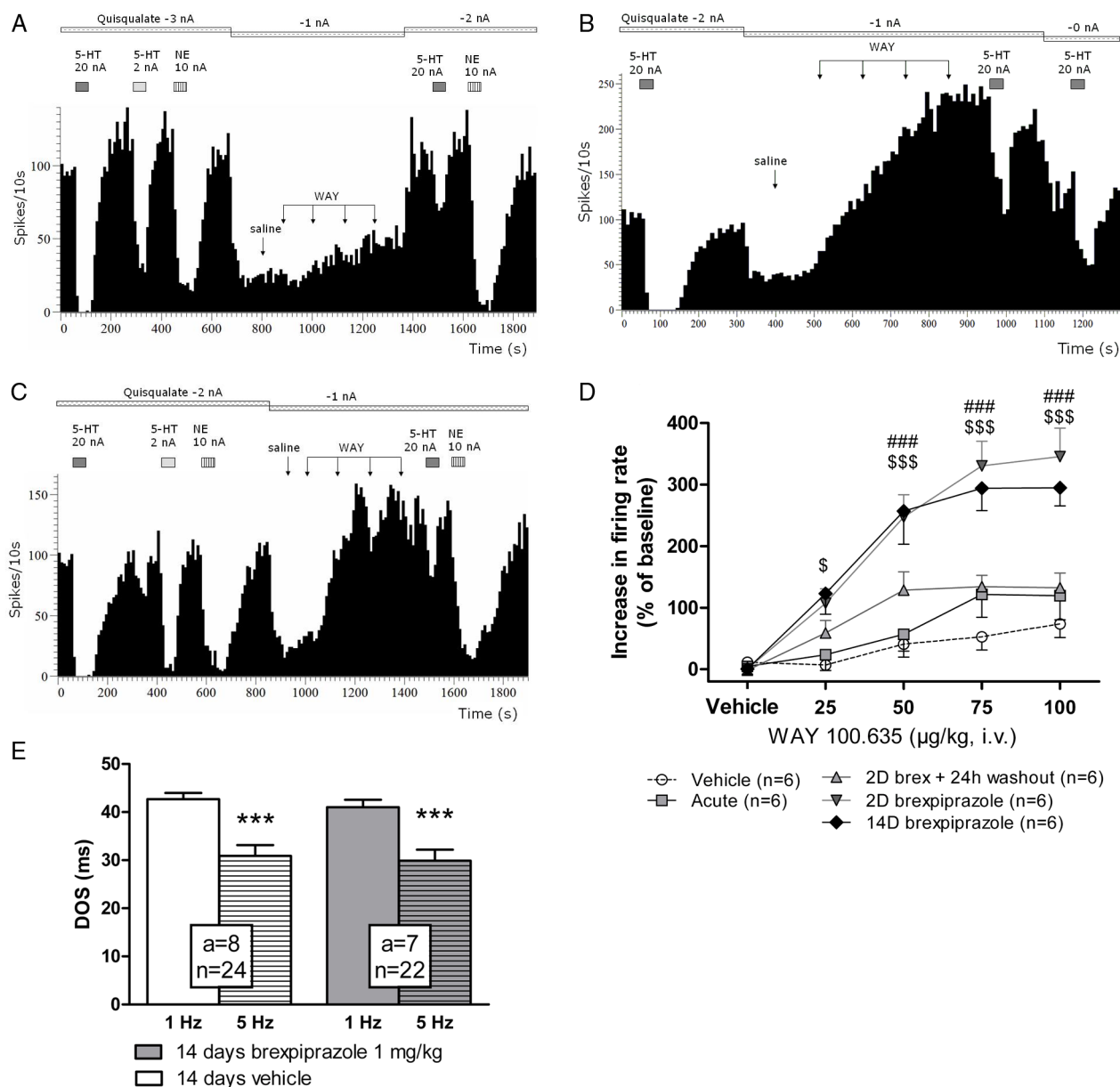
Figure 6. Effect of 14-day brexpiprazole administration on the status of 5-HT$_{1A}$ receptors in the CA3 region of the hippocampus. (A-C) Illustrative firing histograms on the effect of cumulative WAY 100.635 (WAY) administrations on the firing activity of a CA3 pyramidal neuron in a 14-day vehicle- (A), a 2-day brexpiprazole- (B), and 14-day brexpiprazole-administered animal (C). (D) Quantification of the effect of WAY 100.635 on basal firing rate in rats administered with vehicle or brexpiprazole days. (E) Comparison of DOS on CA3 pyramidal neurons produced by 5-HT fiber bundle stimulation in vehicle- and 14-day brexpiprazole-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 (a) and neurons (n) is provided within the histograms (D); error bars represent SEM. #Significant effect of 2-day brexpiprazole administration compared with controls; ###P < .001. $Significant effect of 14-day brexpiprazole administration compared with controls; $$P$ < .05, $$$$P$ < .001. *Significant effect of stimulation frequency; ***P < .001.

BAY x 3702 and trazodone desensitized 5-HT$_{1A}$ 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 brexpiprazole (Figure 5A). The DRN receives excitatory NE projections from the LC (Svensson et al., 1975), and excitatory D$_2$ 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$_2$ receptor agonism (Chernoloz et al., 2009). In addition, since

brexpiprazole plasma concentrations were significantly higher after 14 compared with 2 days, it is conceivable that this caused a greater activation of 5-HT$_{1A}$ autoreceptors, preventing firing of DRN 5-HT neurons to remain elevated after the longer regimen.

In the hippocampus CA3 region, brexpiprazole increased the tonic activation of 5-HT$_{1A}$ 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 brexpiprazole did not alter the firing activity of 5-HT neurons, activity of the SERT, and status of 5-HT$_{1B}$ terminal autoreceptors, it was deemed critical to assess whether this enhanced tonic activation was due to adaptive

changes or attributable to the full agonistic action of brexpiprazole on postsynaptic 5-HT$_{1A}$ receptors (Oosterhof et al., 2014). Interestingly, 2-day brexpiprazole 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 postsynaptic 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 brexpiprazole 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 brexpiprazole on 5-HT$_{1A}$ receptors. Clearly, adaptive changes after 2-day brexpiprazole 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 brexpiprazole 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 brexpiprazole levels and less tonic activation of 5-HT$_{1A}$ receptors compared with the 2- and 14-day regimens, where brexpiprazole accumulated with repeated injections. Alternatively, the combination of adaptive changes together with higher levels of brexpiprazole resulted in the marked enhancement of tonic activation on postsynaptic 5-HT$_{1A}$ receptors after 2- and 14-day brexpiprazole administration.

Conclusion

Repeated administration of brexpiprazole 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. Brexpiprazole produced a significant occupation of D$_2$ autoreceptors while the firing activity of VTA DA neurons remained unchanged, supporting a stabilizing effect of brexpiprazole 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; Slifstein 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 brexpiprazole on the DA system (Kane et al., 2015). Repeated brexpiprazole administration caused a blockade of 2 receptor populations mediating inhibitory crosstalk between the 5-HT and NE systems. First, it blocked 5-HT$_{2A}$ 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 heteroceptors on 5-HT terminals, thereby not attenuating 5-HT release despite enhanced NE neurotransmission. Finally, accumulation of brexpiprazole 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 naïve rats, these data provide insight in the effects of repeated brexpiprazole administration on monoamine systems that might be relevant to symptom domains.

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, Forest, Labopharm, Lundbeck/Takeda, Merck, Pfizer, Servier, sunovion, and Valeant.

References

Besson A, Haddjeri N, Blier P, de Montigny C (2000) Effects of the co-administration of mirtazapine and paroxetine on serotonergic neurotransmission in the rat brain. Eur Neuropsychopharmacol 10:177–188.

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

Blier P, Ramdine R, Galzin AM, Langer SZ (1989) Frequency-dependence of serotonin autoreceptor but not alpha 2-adrenoceptor inhibition of [3H]-serotonin release in rat hypothalamic slices. Naunyn Schmiedebergs Arch Pharmacol 339:60–64.

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

Blier P, Ward NM (2003) Is there a role for 5-HT$_{1A}$ agonists in the treatment of depression? Biol Psychiatry 53:193–203.

Chaput Y, Blier P, de Montigny C (1986) In vivo electrophysiological evidence for the regulatory role of autoreceptors on serotonergic terminals. J Neurosci 6:2796–2801.

Chenu F, El Mansari M, Blier P (2013) Electrophysiological effects of repeated administration of agomelatine on the dopamine, norepinephrine, and serotonin systems in the rat brain. Neuropsychopharmacology 38:275–284.

Chernoloz O, El Mansari M, 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, 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.

Chiodo LA, Bunney BS (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J Neurosci 3:1607–1619.

Curet O, de Montigny C (1988) Electrophysiological characterization of adrenoceptors in the rat dorsal hippocampus. I. Receptors mediating the effect of microiontophoretically applied norepinephrine. Brain Res 475:35–46.

Dahan L, Husum H, Mnie-Filali O, Arnt J, Hertel P, Haddjeri N (2009) Effects of bifeprunox and aripiprazole on rat serotonin

and dopamine neuronal activity and anxiolytic behaviour. J Psychopharmacol 23:177–189.

De Montigny C, Aghajanian GK (1978) Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. Science 202:1303–1306.

De Montigny C, Wang RY, Reader TA, Aghajanian GK (1980) mono-aminergic denervation of the rat hippocampus: microionto-phoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin. Brain Res 200:363–376.

Dong J, de Montigny C, 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, Blier P (2007a) Noradrenergic augmentation of escitalopram response by risperidone: electrophysiologic studies in the rat brain. Biol Psychiatry 61:671–678.

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

Ghanbari R, El Mansari M, 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, Blier P (2011) Enhancement of serotonergic and noradrenergic neurotransmission in the rat hippocampus by sustained administration of bupropion. Psychopharmacology (Berl) 217:61–73.

Ghanbari R, El Mansari M, Shahid M, Blier P (2009) Electrophysiological characterization of the effects of asenapine at 5-HT$_{1A}$, 5-HT$_{2A}$, alpha 2-adrenergic, and D$_2$ receptors in the rat brain. Eur Neuropsychopharmacol 19:177–187.

Ghanbari R, Mansari M El, Blier P (2012) Electrophysiological impact of trazodone on the dopamine and norepinephrine systems in the rat brain. Eur Neuropsychopharmacol 22:518–526.

Grace AA, Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877–2890.

Haddjeri N, Blier P, de Montigny C (1998) Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT$_{1A}$ receptors. J Neurosci 18:10150–10156.

Haj-Dahmane S (2001) D$_2$-like dopamine receptor activation excites rat dorsal raphe 5-HT neurons in vitro. Eur J Neurosci 14:125–134.

Hajós M, Allers KA, Jennings K, Sharp T, Charette G, Sík A, Kocsis B (2007) Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. Eur J Neurosci 25:119–126.

Hirose T, Uwahodo Y, Yamada S, Miwa T, Kikuchi T, Kitagawa H, Burris KD, Altar CA, Nabeshima T (2004) Mechanism of action of aripiprazole predicts clinical efficacy and a favourable side effect profile. J Psychopharmacol 18:375–383.

Jensen NH, Rodriguiz RM, Caron MG, Wetsel WC, Rothman RB, Roth BL (2008) N-desalkylquetiapine, a potent norepinephrine reuptake inhibitor and partial 5-HT$_{1A}$ agonist, as a putative mediator of quetiapine's antidepressant activity. Neuropsychopharmacology 33:2303–2312.

Kane JM, Skuban A, Ouyang J, Hobart M, Pfister S, McQuade RD, Nyilas M, Carson WH, Sanchez R, Eriksson H (2015) A multicenter, randomized, double-blind, controlled phase 3 trial of fixed-dose brexpiprazole for the treatment of adults with acute schizophrenia. Schizophr Res 164:127–135.

Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Roth BL (2003) H1-his-tamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacology 28:519–526.

Kuroki T, Nagao N, Nakahara T (2008) Neuropharmacology of second-generation antipsychotic drugs: a validity of the serotonin-dopamine hypothesis. Prog Brain Res 172:199–212.

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS, Innis RB (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci 93:9235–9240.

Maeda K, Lerdrup L, Sugino H, Akazawa H, Amada N, McQuade RD, Stensbøl TB, Bundgaard C, Arnt J, Kikuchi T (2014a) Brexpiprazole II: antipsychotic-like and procognitive effects of a novel serotonin-dopamine activity modulator. J Pharmacol Exp Ther 350:605–614.

Maeda K, Sugino H, Akazawa H, Amada N, Shimada J, Futamura T, Yamashita H, Ito N, McQuade RD, Mørk A, Pehrson AL, Hentzer M, Nielsen V, Bundgaard C, Arnt J, Stensbøl TB, Kikuchi T (2014b) Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. J Pharmacol Exp Ther 350:589–604.

Mongeau R, Blier P, de Montigny C (1993) In vivo electrophysiological evidence for tonic activation by endogenous noradrenaline of alpha 2-adrenoceptors on 5-hydroxytryptamine terminals in the rat hippocampus. Naunyn Schmiedebergs Arch Pharmacol 347:266–272.

Oosterhof CA, El Mansari M, 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.

Oosterhof CA, El Mansari M, Blier P (2015) Asenapine alters the activity of monoaminergic systems following its subacute and long-term administration: an in vivo electrophysiological characterization. Eur Neuropsychopharmacol 25:531–543.

Oosterhof NN, Oosterhof CA (2013) BurstiDAtor: a lightweight discharge analysis program for neural extracellular single unit recordings. https://github.com/nno/burstiDAtor. Retrieved 21 Oct 2014.

Piñeyro G, Blier P, Dennis T, de Montigny C (1994) Desensitization of the neuronal 5-HT carrier following its long-term blockade. J Neurosci 14:3036–3047.

Ramirez OA, Wang RY (1986) Locus coeruleus norepinephrine-containing neurons: effects produced by acute and subchronic treatment with antipsychotic drugs and amphetamine. Brain Res 362:165–170.

Ranck JB (1973) Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. Exp Neurol 41:461–531.

Rao NP, Remington G (2013) Investigational drugs for schizophrenia targeting the dopamine receptor: phase II trials. Expert Opin Investig Drugs 22:881–894.

Rueter LE, De Montigny C, Blier P (1998) Electrophysiological characterization of the effect of long-term duloxetine administration on the rat serotonergic and noradrenergic systems. J Pharmacol Exp Ther 285:404–412.

Seager MA, Barth VN, Phebus LA, Rasmussen K (2005) Chronic coadministration of olanzapine and fluoxetine activates locus coeruleus neurons in rats: implications for bipolar disorder. Psychopharmacology (Berl) 181:126–133.

Seeman P, Lee T (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 188:1217–1219.

Shahid M, Walker GB, Zorn SH, Wong EHF (2009) Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. J Psychopharmacol 23:65–73.

Skarsfeldt T (1995) Differential effects of repeated administration of novel antipsychotic drugs on the activity of midbrain dopamine neurons in the rat. Eur J Pharmacol 281:289–294.

Slifstein M, van de Giessen E, Van Snellenberg J, Thompson JL, Narendran R, Gil R, Hackett E, Girgis R, Ojeil N, Moore H, D'Souza D, Malison RT, Huang Y, Lim K, Nabulsi N, Carson RE, Lieberman JA, Abi-Dargham A (2015) Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging study. JAMA psychiatry 72:316–324.

Stahl SM (2008) Do dopamine partial agonists have partial efficacy as antipsychotics? CNS Spectr 13:279–282.

Stark AD, Jordan S, Allers KA, Bertekap RL, Chen R, Mistry Kannan T, Molski TF, Yocca FD, Sharp T, Kikuchi T, Burris KD (2007) Interaction of the novel antipsychotic aripiprazole with 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors: functional receptor-binding and in vivo electrophysiological studies. Psychopharmacology (Berl) 190:373–382.

Stip E, Tourjman V (2010) Aripiprazole in schizophrenia and schizoaffective disorder: a review. Clin Ther 32:S3–20.

Svensson TH, Bunney BS, Aghajanian GK (1975) Inhibition of both noradrenergic and serotonergic neurons in brain by the α-adrenergic agonist clonidine. Brain Res 92:291–306.

Szabo ST, 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.

Tarazi FI, Stahl SM (2012) Iloperidone, asenapine and lurasidone: a primer on their current status. Expert Opin Pharmacother 13:1911–1922.

Thase ME, Youakim JM, Skuban A, Hobart M, Augustine C, Zhang P, McQuade RD, Carson WH, Nyilas M, Sanchez R, Eriksson H (2015a) Efficacy and safety of adjunctive brexpiprazole 2 mg in major depressive disorder: a phase 3, randomized, placebo-controlled study in patients with inadequate response to antidepressants. J Clin Psychiatry 76(9):1224–1231.

Thase ME, Youakim JM, Skuban A, Hobart M, Zhang P, McQuade RD, Nyilas M, Carson WH, Sanchez R, Eriksson H (2015b) Adjunctive brexpiprazole 1 and 3 mg for patients with major depressive disorder following inadequate response to antidepressants: a phase 3, randomized, double-blind study. J Clin Psychiatry 76(9):1232–1240.

Vandermaelen CP, 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.

Vogelsang GD, Piercey MF (1985) The supersensitivity of dopaminergic neurons to apomorphine in rats following chronic haloperidol. Eur J Pharmacol 110:267–269.

Westerink BHC, De Boer P, De Vries JB, Kruse CG, Long SK (1998) Antipsychotic drugs induce similar effects on the release of dopamine and noradrenaline in the medial prefrontal cortex of the rat brain. Eur J Pharmacol 361:27–33.

Yoshimi N, Fujita Y, Ohgi Y, Futamura T, Kikuchi T, Hashimoto K (2014) Effects of brexpiprazole, a novel serotonin-dopamine activity modulator, on phencyclidine-induced cognitive deficits in mice: a role for serotonin 5-HT$_{1A}$ receptors. Pharmacol Biochem Behav 124C:245–249.