The antipsychotic drug brexpiprazole reverses phencyclidine-induced disruptions of thalamocortical networks

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Short title: Brexpiprazole actions on thalamo-cortical networks

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Abstract

Brexiprazole (Brex), a recently approved antipsychotic drug in US and Canada, improves cognitive dysfunction in animal models, by still largely unknown mechanisms. Brex is a partial agonist at 5-HT_{1A} and D₂ receptors and antagonist at α_{1B} - and α_{2C} -adrenergic and 5-HT_{2A} receptors all with a similar potency. The NMDA receptor antagonist phencyclidine (PCP), used as pharmacological model of schizophrenia, activates thalamocortical networks and decreases low frequency oscillations (LFO; <4 Hz). These effects are reversed by antipsychotics. Here we assessed the ability of Brex to reverse PCP-induced hyperactivity of thalamocortical circuits, and the involvement of 5-HT_{1A} receptors in its therapeutic action. Brex reversed PCP-induced neuronal activation at a lower dose in centromedial/mediodorsal thalamic nuclei (CM/MD; 0.5 mg/kg) than in pyramidal medial prefrontal cortex neurons (mPFC, 2 mg/kg), perhaps due to antagonism at α_{1B} -adrenoceptors, abundantly expressed in the thalamus. Conversely, a cumulative 0.5 mg/kg dose reversed a PCP-induced LFO decrease in mPFC but not in CM/MD. Brex reduced LFO in both areas, yet with a different dose-response, and moderately excited mPFC neurons. The latter effect was reversed by the 5-HT_{1A} receptor antagonist WAY-100635. Thus, Brex partly antagonizes PCP-induced thalamocortical hyperactivity, differentially in mPFC and CM/MD. This regional selectivity may be related to the differential expression of α_{1B} -, α_{2C} -adrenergic and 5-HT_{2A} receptors in both regions and/or different neuronal types. Furthermore, the pro-cognitive properties of Brex may be related to the 5-HT_{1A} receptor-mediated increase in mPFC pyramidal neuron activity. Overall, the present data provide new insight on the brain elements involved in Brex's therapeutic actions.

Key words: antipsychotic drugs, NMDA receptor antagonists, oscillations, phencyclidine, prefrontal cortex, schizophrenia, thalamocortical circuit

1. Introduction

Schizophrenia is a highly disruptive and chronic psychiatric disorder. Its symptoms vary greatly among patients, which complicates finding suitable treatments. Available antipsychotic drugs are partly effective for positive symptoms whereas they fail to alleviate negative symptoms and cognitive deficits, and they induce undesirable side-effects (Leucht et al., 2013). In addition to first (classical, dopamine D₂ receptor (D₂-R) antagonists) and second generation (atypical, serotonin 2A receptor (5-HT_{2A}-R) antagonists) drugs, a third generation of antipsychotic drugs has been developed in the recent years. These new agents, including brexpiprazole (Brex), possess partial agonism at the D₂-R and 5-HT_{1A}-R (Mailman and Murthy, 2010; Newman-Tancredi and Kleven, 2011), thereby stabilizing dopamine and serotonin neurotransmission (Oosterhof et al., 2014), and also possess considerable affinity for other monoamine receptors, including the 5-HT_{2A}-R. Brex was approved in 2015, showing therapeutic efficacy for schizophrenia (Correll et al., 2016; Garnock-Jones, 2016; Kane et al., 2015; McEvoy and Citrome, 2016), and as adjunctive treatment for treatment-resistant major depression (Yoon et al., 2017). Brex also shows efficacy in animal models of cognitive deficits (Maeda et al., 2014a; Yoshimi et al., 2014). Compared with aripiprazole, Brex possesses superior affinity for the 5-HT_{1A}-R (K_i 0.12 nM), α_{1B} -adrenoregic-R (0.17 nM) and α_{2C} -adrenoregic-R (0.59 nM) (Maeda et al., 2014b), and less intrinsic activity at the dopamine D₂-R, thus having a unique receptor binding and functional profile. However, the mechanisms underlying its therapeutic activity are largely unknown, in particular with regard to the brain areas and circuits involved.

Since the affinity of Brex is highest for the 5-HT_{1A}-R and α_{1B} -adrenoregic-R, abundantly expressed in the prefrontal cortex (PFC) (both receptors) and in thalamic nuclei (α_{1B} -adrenoceptors) (Pieribone et al., 1994; Pompeiano et al., 1992; Santana et al., 2004; 2013), we hypothesized that Brex may act by normalizing a putative hyperactivity of cortico-thalamocortical loops. Indeed, both brain areas are involved in schizophrenia (Pergola et al., 2015; Sakurai et al., 2015), likely associated to a glutamatergic dysfunction (Bustillo et al., 2010; Goff and Coyle, 2001). On the other hand, the non-competitive NMDA receptor antagonist phencyclidine (PCP), widely used as pharmacological model of schizophrenia, markedly disrupt thalamocortical activity (Celada et al., 2013; Hakami et al., 2009; Kargieman et al., 2007; Kiss et al., 2011; Santana et al., 2011; Troyano-Rodriguez et al., 2015). Specifically, PCP increases excitatory neuron discharge and reduces low frequency oscillations (LFO; 0.15-4 Hz) in both the medial prefrontal cortex (mPFC) and the centromedial (CM) and mediodorsal (MD) thalamic nuclei of rats, reciprocally connected with the mPFC (Kargieman et al., 2007; Santana et al., 2011). Interestingly, first and second generation antipsychotic drugs can reverse PCP effects in mPFC while clozapine

reverse PCP effects in the CM/MD thalamic nuclei (Kargieman et al., 2007; Lladó-Pelfort et al., 2016; Santana et al., 2011; Troyano-Rodriguez et al., 2014).

On account of the above observations, and given the high affinity of BREX for the 5-HT_{1A}-R and α_{1B} -adrenergic-R, here we examined the ability of BREX to reverse a disruption of thalamocortical function induced by PCP, and we examined the potential involvement of the 5-HT_{1A}-R in the effects of BREX.

2. Experimental procedures

2.1. Animals and stereotaxic surgery

Male Wistar rats (Charles River) weighing 250-350 g were used. Animal care followed European Union regulations (directive 2010/63 of 22 September 2010) and was approved by the Institutional Animal Care and Use Committee (Generalitat de Catalunya). Rats were deeply anesthetized with chloral hydrate (400 mg/kg intraperitoneally; i. p.), and a constant level of anesthesia was maintained using a perfusion pump (50–70 mg/kg·h i.p.). The femoral vein was cannulated for intravenous (i.v.) drug administration and the rats were placed in a David Kopf stereotaxic apparatus. Electrodes for extracellular electrophysiological recordings were descended in either the CM and MD thalamic nuclei or the mPFC. Glutamatergic neurons of the CM and MD nuclei were identified by action potential characteristics and burst firing (Santana et al., 2011). Glutamatergic neurons of the mPFC were identified by antidromic stimulation from the dorsal raphe or ventral tegmental area (1-2 mA, 0.2 ms) (Kargieman et al., 2007; Puig et al., 2005) and collision extinction with spontaneously occurring spikes (Fuller and Schlag, 1976). Both stimulation sites identify the same population of midbrain-projecting PFC neurons in cortical layer V (Gabbott et al., 2005) which simultaneously project to both monoaminergic nuclei (Vazquez-Borsetti et al., 2011). Stereotaxic coordinates of recorded and stimulated locations are as follows (in mm, relative to bregma and brain surface): AP -2.5, L -0.6 to -0.8, DV -4.7 to -5.8 for the CM and MD thalamic nuclei; AP +3.2 to +3.4, L -0.5 to -1.0, DV -1.6 to -3.6 for the mPFC; AP -7.8, L -3.1 with a 30° angle, DV -6.8 for the DR; AP -5.8, L -0.4, DV -6.9 for the VTA (Paxinos and Watson, 2005). Single units and local field potentials (LFP) were recorded extracellularly as described previously (Kargieman et al., 2007; Santana et al., 2011). At the end of the experiment, the recording site was marked iontophoretically with pontamine (20 μ A cathodal current, 20 min) for histological verification of the recording site. Rats were euthanized with an anesthetic overdose (chloral hydrate), brains were rapidly removed, frozen in dry ice and stored at -80 °C. Forty μ m-thick sections were obtained with a cryostat and stained with neutral red.

2.2. Drugs and experimental design

BREX (0.06–2.0 mg/kg) was provided by H. Lundbeck A/S. (Copenhagen, Denmark) and dissolved in a 0.5% lactic acid solution in distilled water. Phencyclidine (PCP) hydrochloride (0.25–0.5 mg/kg; free base) was from Sigma-Aldrich (St. Louis, MO, USA). WAY-100635 maleate (0.1–0.4 mg/kg) was from Tocris Bioscience (Bristol, UK). PCP and WAY-100635 were dissolved in 0.9% saline. All drugs were administered intravenously (i.v.) and the injected volume was 0.02–0.5 ml/kg. Doses are given as free bases and were based on previous studies (Kargieman et al., 2007; Lladó-Pelfort et al., 2012a; Maeda et al., 2014b; Santana et al., 2011).

In all experiments, a 5 min basal period was first recorded and drugs were administered with 2 to 5-min intervals. In experiments where the effect of BREX alone was investigated, four (thalamus; cumulatively 0.125–1.0 mg/kg) or five (mPFC; cumulatively 0.06–1.0 mg/kg) consecutive doses were administered. In separate mPFC recordings, one to three doses of WAY-100635 (cumulatively 0.1–0.4 mg/kg, typically 0.3 mg/kg) were administered after low doses of BREX (cumulatively 0.125–0.25 mg/kg) to study the involvement of 5-HT_{1A} receptors. Where the potential reversal of the effects of PCP by BREX was investigated, two to four BREX doses (cumulatively 0.5 and 2.0 mg/kg, in separate experiments) were administered after one or two doses of PCP (cumulatively 0.25–0.5 mg/kg). BREX was only administered if PCP induced a $\pm 30\%$ change of the firing rate or a $\pm 30\%$ change of the LFO power. A second PCP dose (0.25 mg/kg) was administered when a single dose had no effect. Recordings were kept for at least 10 min after the last dose.

2.3. Data analysis and statistics

Neuronal firing rate was calculated as mean of the last 2-min period before the (next) dose and as mean of the last 2-min period of the 5-min time window after the last dose. Firing rate after WAY-100635 was calculated as mean of the last 1-min period before the next dose and as mean of the last 1-min period of the 10-min time window after the last BREX dose because administration intervals were shorter (2 min). Basal firing activity was recorded for at least 5 min. Neurons were considered to be excited or inhibited when drugs induced a $\pm 30\%$ change of the discharge rate (Lladó-Pelfort et al., 2012). In mPFC pyramidal neuron recordings, burst episodes were defined as periods of consecutive firing within 0.045 ms and analyses were carried out using previously described procedures (Kargieman et al., 2007; Laviolette et al., 2005). The power of low frequency oscillations (LFO; 0.15–4 Hz) was calculated for the last 1-min period before administration of the (next) drug, for min 5 after the last dose or for the last 1-min period of the time window (WAY-100635), as described in Results.

Recordings were analyzed off-line with Matlab 2010 (MathWorks Inc., Natick, MA, USA; firing rate) and Spike2 (LFO power) software using built-in and self-developed scripts. Mean LFO power was calculated over six consecutive ten-second periods and then averaged, using Fast Fourier Transformation with a resolution of 0.15 Hz. To assess differences between two conditions, two-tailed paired Student's t-tests were used. To assess differences between more conditions, as after accumulative drug doses and over time, one-way repeated measures ANOVA were used, followed by Newman-Keuls post-hoc tests (GraphPad Prism version 4). Firing rate is expressed as spikes/s and LFO power in μV^2 . Data are given in mean $\pm$ SEM and significance level was established at $p < 0.05$.

3. Results

3.1 BREX reverses PCP-induced disruptions of cortical function

BREX (2 mg/kg i.v.) significantly reverses a PCP-induced increase in firing rate of mPFC pyramidal neurons: PCP increased neuronal discharge from 1.3 ± 0.4 to 7.4 ± 2.5 spikes/s ($n=5$). Subsequent BREX administration decreased discharge to 1.7 ± 0.6 spikes/s ($F(3,12)=6.0$, $p < 0.01$ $n=5$; figure 1A and B). In parallel, PCP reduced LFO power from 0.28 ± 0.04 to 0.04 ± 0.02 μV^2 after PCP ($n=8$). A dose of 2 mg/kg BREX reversed this LFO decrease ($F(3,21)=20.9$; $p < 0.0001$, $n=8$; figure 1A and C).

In separate experiments we examined the effect of lower BREX doses (0.25-0.5 mg/kg i.v.). These lower doses were unable to reverse PCP-induced excitation of pyramidal neurons ($F(3,36)=8.4$, $p < 0.001$ $n=13$; n.s. post-hoc test; figure 2A). On LFO, 0.25 mg/kg BREX had no effect ($F(3,45)=30.2$, $p < 0.0001$, $n=16$; n.s. post-hoc test) and 0.5 mg/kg partially reversed the LFO fall to 0.21 ± 0.05 μV^2 (figure 2C). Interestingly, BREX affected LFO power by itself at low doses (see below).

3.2 BREX reverses a PCP-induced disruption of thalamic function

PCP (0.25-0.5 mg/kg i.v.) evoked an overall excitation of CM/MD neurons, from 1.6 ± 0.1 to 5.8 ± 0.8 spikes/s ($n=48$). Of the 48 neurons recorded, 65% of the neurons were excited (to 8.5 ± 1.1 spikes/s, $n=31$), 23% were inhibited (to 0.3 ± 0.1 spikes/s, $n=11$) and 12% were unaffected (to 1.4 ± 0.4 spikes/s, $n=6$) by PCP. These PCP effects lasted for at least 15 min after the last dose ($F(3,15)=7.5$, $p < 0.003$, $n=6$; Supplementary Figure S1A-

S1C). Simultaneously, PCP decreased LFO from 0.060 ± 0.004 to $0.017 \pm 0.003 \mu V^2$, at 5 min after administration, an effect lasting for at least 20 min ($F(4,48)=54.1$, $p<0.0001$, $n=13$; Supplementary Figure S2D).

Next we investigated the putative reversal by BREX of PCP actions. PCP excited CM/MD neurons from 1.9 ± 0.3 to 8.8 ± 1.5 spikes/s ($n=13$). BREX (0.25 mg/kg) partly reversed this effect, while a higher dose of BREX (0.5 mg/kg) led to complete reversal of the PCP-induced neuronal excitation ($F(3,36)=11.2$, $p<0.0001$, $n=13$; figure 2B).

PCP decreased LFO power from 0.066 ± 0.009 to $0.017 \pm 0.002 \mu V^2$ ($n=12$). One recording not meeting inclusion criteria (power change of $\pm 30\%$; see Methods) was excluded from calculations.

The subsequent BREX administration (0.25-0.5 mg/kg) did not reverse the fall in LFO ($F(3,33)=35.3$, $p<0.0001$, $n=12$; n.s post hoc test; figure 2D). This might be due to the finding that BREX by itself decreased LFO in the thalamus (see below).

3.3 BREX modulates cortical activity

To study the effects of BREX on PFC pyramidal neuron discharge, five cumulative BREX doses (0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg i.v.,) were administered. This resulted in a bell-shaped dose-response relationship, with maximal effects at 0.125 mg/kg (Supplementary Figure S2). Repeated-measures ANOVA revealed a marginally significant effect of BREX on pyramidal neuron activity ($F(5,50)=2.0$, $p=0.08$, $n=11$; Supplementary Figure S2). Analysis including the three lower BREX doses (0.06, 0.125 and 0.25 mg/kg) revealed a significant effect of BREX ($F(3,21)=5.7$, $p<0.01$, $n=8$) with a significant post-hoc difference of BREX 0.125 versus basal and a marginal ($p=0.06$) difference of BREX 0.25 versus basal (figure 3).

Burst firing analyses revealed that BREX significantly increased the number of burst episodes in mPFC pyramidal neurons ($F(5,50)=13.9$, $p<0.0001$, $n=11$; table 1), with a maximal effect at a dose of 0.125 mg/kg BREX (from 39 ± 21 to 124 ± 42 ; $p<0.05$) and attenuation of this effect at higher doses.

Analyses of LFO power in the mPFC showed that BREX (0.06-0.5 mg/kg) modulates LFO in a U-shaped dose-effect mode ($F(5,50)=8.8$, $p<0.0001$, $n=11$; figure 4A).

3.4 Excitation of mPFC pyramidal neurons by BREX is 5-HT_{1A}-R dependent

Low BREX doses (0.125-0.5 mg/kg, n=8) significantly increased the firing rate of mPFC pyramidal neurons from 1.2 ± 0.2 to 3.1 ± 0.6 spikes/s (265%) at 5 min after the last BREX dose ($p < 0.01$), and to 2.6 ± 0.5 (223%) spikes/s at 10 min after the last BREX dose ($p < 0.05$) ($F(2,14)=6.6$, $p < 0.01$, $n=8$; figure 5C and D).

Administration of WAY-100635 significantly reversed this BREX-induced excitation ($F(2,12)=33.4$, $p < 0.0001$, $n=7$; figure 5A and B). Firing rate increased from 1.4 ± 0.4 to 3.1 ± 0.4 spikes/s (225% of basal) after BREX (0.125-0.25 mg/kg; $p < 0.001$). The subsequent administration of WAY-100635 (0.1-0.4 mg/kg i.v., cumulative doses) reduced pyramidal discharge to 1.6 ± 0.3 (121% of basal; $p < 0.001$ vs BREX). In addition, WAY-100635 reversed a LFO decrease induced by BREX (0.125-0.25 mg/kg; $F(2,12)=5.4$, $p < 0.05$, $n=7$; Supplementary Figure S3): LFO power was significantly reduced from 0.29 ± 0.04 to $0.24 \pm 0.04 \mu V^2$ after BREX ($p < 0.05$) and reversed to $0.32 \pm 0.04 \mu V^2$ ($p > 0.05$ vs. basal) after the last WAY-100635 dose.

3.5 BREX modulates thalamic activity

To evaluate the effects of BREX on thalamic activity, four consecutive BREX doses (0.125, 0.125, 0.25, 0.5 mg/kg i.v.) were administered. There was no significant effect of BREX on thalamic neuron discharge ($F(4,52)=0.7$, $p > 0.6$, $n=14$; SM figure 2B). In contrast, BREX (0.12-0.5 mg/kg) significantly decreased thalamic LFO independent of dose in this same group, excluding two recordings with unstable baseline ($F(4,44)= 8.4$, $p < 0.0001$, $n=12$; figure 4B).

4. Discussion

Here we show that the antipsychotic drug brexpiprazole (BREX) normalizes the hyperactivity of thalamocortical circuits induced by phencyclidine (PCP), yet with differential effects in cortex versus thalamus, possibly reflecting the differential expression of BREX's receptor targets in these brain areas. Likewise, we show that BREX moderately increases pyramidal neuron activity in PFC through 5-HT_{1A}-R activation, an effect possibly related to its pro-cognitive properties.

We confirm and extend previous observations (Kargieman et al., 2007; Santana et al., 2011) showing that PCP increases excitatory neuron activity in the mPFC and CM/MD thalamic nuclei, while simultaneously reducing the power of low frequency oscillations (LFO). During sleep and under anesthesia, cortical and thalamic neurons exhibit synchronous low frequency alternations of their membrane potential between depolarized ("up") and hyperpolarized ("down") states which temporally encode neuronal discharge (occurring only during

“up” states) (Steriade et al., 1993; Steriade, 2006). These individual neuronal changes are reflected at population level as LFO in LFP and EEG recordings. The maintenance of thalamocortical LFO is essential for sensory and information processing (Engel and Singer, 2001; Schroeder and Lakatos, 2009) and its enhancement improves memory acquisition and consolidation in rodents and humans (Binder et al., 2014; Kirov et al., 2009; Marshall et al., 2006). PCP markedly reduces the power of LFO and alters neuronal discharge in PFC and CM/MD thalamic nuclei in both directions, with an overall enhancing effect. This results in the uncoupling of neuronal discharge with “up” states (Kargieman et al., 2007) which markedly disrupts the function of thalamocortical networks. Given the top-down control of most brain areas by the PFC (Miller and Cohen, 2001), these alterations likely translate into downstream activity changes in other cortical and subcortical areas, that contribute to the overall effects of PCP on perception, mood and cognition.

BREX was able to modulate neuronal activity and to reverse PCP effects in thalamocortical circuits, yet with a differential effect in the PFC versus the thalamus, likely resulting from the following factors: 1) the multi-target activity of BREX, resulting in different receptor occupancies at the various doses used, 2) the differential expression of these receptor targets in the PFC and thalamic nuclei, and 3) the different microcircuit composition in the thalamus -lacking GABAergic interneurons- and the PFC, where both GABAergic interneurons and pyramidal neurons express most BREX targets, such as 5-HT_{1A}-R, 5-HT_{2A}-R, D₂-R and α_{1B} - adrenoceptors (Santana et al., 2004; 2009; 2012).

Hence, like first and second generation antipsychotics (Kargieman et al., 2007; Lladó-Pelfort et al., 2016; Santana et al., 2011), BREX reversed the PCP-induced increase in neuronal discharge in the mPFC and CM/MD thalamic nuclei. The dose required was lower in the thalamus (0.5 mg/kg) than in the mPFC (2 mg/kg), suggesting a greater sensitivity of the thalamus versus the mPFC to the action of BREX, which may be accounted for by the high affinity of BREX for α_{1B} -adrenoceptors, which are abundantly expressed in the MD and CM thalamus (Pieribone et al., 1994). This possibility is also supported by the observation that BREX (1 mg/kg) fully blocked the α_{1B} -adrenoceptor-mediated excitation in another thalamic area, the lateral geniculate nucleus (Oosterhof et al., 2014). Here, this regional selectivity also became apparent through the effects of BREX on the PCP-induced LFO decrease: in the mPFC, BREX evoked a partial reversal of the LFO decrease at 0.5 mg/kg (see figure. 2) and required a higher dose (2 mg/kg) for a full reversal. On the contrary, despite normalizing neuronal discharge at 0.5 mg/kg in the thalamus, BREX could not reverse the loss in LFO power in this area at the same dose. The lower sensitivity of LFO changes to BREX –as compared to neuronal discharge– is likely due to an action of BREX on LFO power by itself. In the thalamus, this effect occurred at low doses and reached a plateau, whereas in mPFC, BREX evoked a U-shaped dose-response curve, with a reduction at low

doses and a return to basal values at 1.0 mg/kg. Again, the dissimilar action of BREX in both areas likely reflects the various factors mentioned above.

The mechanisms involved in LFO maintenance/suppression are still poorly known. Changes in the excitatory/inhibitory balance have been implicated (Compte et al., 2003; Sanchez-Vives et al., 2010). However, much less is known about the role played by monoamines, acting mainly through modulatory mechanisms. The multi-target profile of BREX suggests that one or several of its pharmacological activities may account for its actions on LFO and for the reversal of PCP effects. In particular, BREX's partial agonist activity at 5-HT_{1A}-R may be involved, as also demonstrated recently for its anticataleptic activity (Mombereau et al., 2017). Hence, 1) in common with other 5-HT_{1A}-R agonists (Díaz-Mataix et al., 2006; Lladó-Pelfort et al., 2010; 2012b), BREX evokes a moderate increase of pyramidal neuron activity when administered alone (see below), 2) 5-HT_{1A}-R agonists like 8-OH-DPAT and BAYx3702 fully reverse the fall in LFO induced by PCP (Lladó-Pelfort et al., 2016), and 3) other pharmacological activities common to second generation antipsychotics such as 5-HT_{2A}-R or non-selective α_1 -adrenoceptor blockade fail to reverse this PCP effect on LFO (Lladó-Pelfort et al., 2016). Partial blockade of the D2-R may also be accountable, since the PCP-induced fall in LFO was partly reversed by D2-R antagonists and also by aripiprazole (Lladó-Pelfort et al., 2016).

At low doses, BREX enhanced the discharge of pyramidal neurons in the mPFC, an effect lost at higher doses. This effect appears to involve 5-HT_{1A}-R activation, given its reversal by the selective antagonist WAY-100635. This may seem at variance with the inhibitory character of the 5-HT_{1A}-R, as assessed *in vitro* (Araneda and Andrade, 1991) and *in vivo* (Puig et al., 2005). However, the systemic administration of 5-HT_{1A}-R agonists increases pyramidal neuron discharge in the mPFC (Díaz-Mataix et al., 2006; Hajos et al., 1999; Lladó-Pelfort et al., 2010, 2012a). This effect appears to be mediated via a preferential action on 5-HT_{1A}-R located in GABAergic interneurons by low doses of the agonists (e.g., 8-OH-DPAT), resulting in pyramidal neuron disinhibition. Higher 8-OH-DPAT doses also activate 5-HT_{1A}-R in pyramidal neurons, thus counteracting this initial effect, and resulting in a bell-shaped dose-response curve (Lladó-Pelfort et al., 2012b), similar to that observed for some effects of BREX.

The pyramidal neuron-enhancing effect of BREX alone may seem at variance with the reversal of the PCP-induced elevation of pyramidal discharge. Although we do not have a definite explanation, given the presence of 5-HT_{1A}-R in pyramidal and GABAergic neurons, it is likely that the effect of agonists depends on the state of activation of local microcircuits involving both neuronal types. However, given the multi-target character of BREX, we cannot exclude other possibilities.

The pro-cognitive effects of BREX and the elevation of pyramidal neuron discharge are both mediated via 5-HT_{1A}-R activation (reviewed in Citrome et al., 2015), which suggests an association between both effects. In line with this possibility, primate studies have shown that the neurobiological substrate of short-term -or working-memory (an essential component of executive functions and a necessary step in long-term memory) is the emergence and maintenance of patterns of persistent neuronal activity in the dorsolateral PFC (equivalent to the dorsal mPFC in rodents) (Curtis and Esposito, 2003; Fuster and Alexander, 1971; Miller and Cohen, 2001; Wang et al., 2015). Hence, the moderate elevation of neuronal discharge induced by BREX may facilitate short-term memory and therefore contribute to its pro-cognitive effects. In line with this view, the 5-HT_{1A}-R agonist tandospirone enhanced cognitive function when added to antipsychotic treatments (Sumiyoshi et al., 2001) and newly developed antipsychotic drugs incorporate this pharmacological activity (McCreary and Newman-Tancredi, 2015).

In conclusion, we show that BREX antagonizes thalamocortical hyperactivity associated with schizophrenia, with a more potent effect in the CD/MD thalamus, as compared to mPFC, indicating a primary action in this area which is perhaps mediated by α_{1B} -adrenoceptor antagonism. BREX also induced a 5-HT_{1A}-mediated moderate excitation of pyramidal neurons, which is relevant for its pro-cognitive properties. Overall, these data provide further insight in the brain circuits involved in the therapeutic action of BREX.

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	Basal	BREX 0.06	BREX 0.125	BREX 0.25	BREX 0.5	BREX 1.0
Firing rate (spikes/s)	1.5 ± 0.6	1.9 ± 0.8	3.5 ± 1.0	2.8 ± 0.9	2.4 ± 0.8	2.6 ± 0.9
Burst episodes (2 min)	39 ± 21	69 ± 34	124 ± 42*	108 ± 40	91 ± 33	76 ± 29
% spikes in burst	39 ± 7	48 ± 8	53 ± 8	52 ± 7	52 ± 5	50 ± 6
Spikes/burst	2.17 ± 0.06	2.27 ± 0.07	2.35 ± 0.14	2.32 ± 0.09	2.20 ± 0.04	2.26 ± 0.09
ISI within burst (ms)	14.8 ± 1.6	14.9 ± 2.0	16.0 ± 1.2	15.7 ± 1.6	18.7 ± 1.4	18.1 ± 2.1
Burst duration (ms)	17.3 ± 2.1	18.8 ± 2.4	21.5 ± 2.8	20.7 ± 2.3	22.4 ± 1.6	23.3 ± 3.4
N	11	11	11	11	11	11

Table 1. Effect of BREX (0.06-1 mg/kg) on mPFC firing activity. The number of burst episodes was significantly increased after BREX 0.125. Note that BREX 1.0 was measured 5 min after administration. ISI: interspike interval. *p<0.05 vs basal.

Figure legends

Figure 1. BREX (2 mg/kg i.v.) reverses PCP-induced disruptions of mPFC cortical activity. BREX reverses a (A) PCP-induced increase in firing rate as well as a (B) PCP-induced decrease in LFO. (C) Representative recording. Arrows mark drugs injection. Drug doses are given in mg/kg. * $p < 0.05$ vs basal, # $p < 0.05$ vs PCP. (A) $n = 5$; (B) $n = 8$.

Figure 2. Dose-effect of two subsequent low BREX doses (0.25-0.5 mg/kg i.v.) on corticothalamic activity. (A) In the mPFC, BREX did not reverse a PCP-induced excitation of pyramidal neurons. (B) In the thalamus, one BREX dose of 0.25 mg/kg i.v. (BREX 0.25) partially reversed, while a second dose (BREX 0.5) completely reversed excitation by PCP. (C and D) Effect of BREX on a PCP-induced reduction in LFO power. (C) Two subsequent BREX doses (0.25 + 0.25 mg/kg) partially reversed a PCP-reduction of LFO power in the mPFC. (D) Two subsequent BREX doses (0.25 + 0.25 mg/kg) did not reverse the PCP-induced reduction in thalamic LFO power. * $p < 0.05$ vs basal, # $p < 0.01$ vs PCP. (A) $n = 13$; (B) $n = 13$; (C) $n = 16$; (D) $n = 12$. Note the different effect of BREX 0.5 mg/kg on LFO when given as a single dose (Fig. 1B) or as cumulative dose (0.25 + 0.25 mg/kg; Fig. 2C), a difference possibly related to pharmacokinetic effects.

Figure 3. Effect of low BREX doses on the firing rate of mPFC pyramidal neurons. Effect of BREX in a subpopulation of pyramidal neurons that was excited after administration of 0.125 mg/kg ($n = 8$, 73%). There was a significant increase in firing rate after 0.125 mg/kg (* $p < 0.01$) and a trend for increase at 0.25 mg/kg BREX (# $p = 0.06$).

Figure 4. Effect of BREX on LFO in the (A) mPFC and the (B) thalamus. Four or five consecutive BREX doses (cumulatively (0.06,) 0.125, 0.25, 0.5 and 1.0 mg/kg) with 3-min intervals resulted in a decrease in LFO in both regions, although this effect was reversed after the last dose in the mPFC. * $p < 0.05$ vs basal. (A) $n = 11$; (B) $n = 12$.

Figure 5. WAY-100635 reverses a BREX-induced excitation of pyramidal neurons. (A) BREX (0.125-0.25 mg/kg, i.v. 1-2 doses) increased firing rate to 225%, which was reversed by WAY-100635 (WAY) (0.1-0.4 mg/kg, i.v. 1-3 doses) within 10 min after BREX. * $p < 0.001$ vs basal. (B) Representative recording of consecutive BREX and WAY administration. (C) Control experiments showing that neurons remain excited for at least 10 minutes after BREX. * $p < 0.05$ vs basal. (D) Representative recording of low BREX doses. Arrows mark drugs injection. Drug doses are given in mg/kg.

Figure(s)

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Contributors

FA and PC designed experiments, HvdM performed experimental work, JA contributed to experimental design and data evaluation and all authors wrote and revised the manuscript.

Conflict of interest

F.A. has received consulting honoraria on antidepressant drugs from Lundbeck and he has been PI of grants from Lundbeck. P.C. has been co-PI of a grant from Lundbeck. J.A. is consultant to and previous employee of H. Lundbeck A/S. The rest of authors declare no competing financial interest.

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Supplementary Material

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