ORIGINAL RESEARCH

# Not all partial dopamine D<sub>2</sub> receptor agonists are the same in treating schizophrenia. Exploring the effects of bifeprunox and aripiprazole using a computer model of a primate striatal dopaminergic synapse

Athan Spiros[1]
Robert Carr[1]
Hugo Geerts[1,2]

[1]In Silico Biosciences, Berwyn, PA, USA; [2]School of Medicine, University of Pennsylvania, PA, USA

**Abstract:** Species differences in physiology and unique active human metabolites contribute to the limited predictive value of preclinical rodent models for many central nervous system (CNS) drugs. In order to explore possible drivers for this translational disconnect, we developed a computer model of a dopaminergic synapse that simulates the competition among three agents and their binding to pre- and postsynaptic receptors, based on the affinities for their targets and their actual concentrations. The model includes presynaptic autoreceptor effects on neurotransmitter release and modulation by presynaptic firing frequency and is calibrated with actual experimental data on free dopamine levels in the striatum of the rodent and the primate. Using this model, we simulated the postsynaptic dopamine D<sub>2</sub> receptor activation levels of bifeprunox and aripiprazole, two relatively similar dopamine D<sub>2</sub> receptor agonists. The results indicate a substantial difference in dose–response for the two compounds when applying primate calibration parameters as opposed to rodent calibration parameters. In addition, when introducing the major human and rodent metabolites of aripiprazole with their specific pharmacological activities, the model predicts that while bifeprunox would result in a higher postsynaptic D<sub>2</sub> receptor antagonism in the rodent, aripiprazole would result in a higher D<sub>2</sub> receptor antagonism in the primate model. Furthermore, only the highest dose of aripiprazole, but not bifeprunox, reaches postsynaptic functional D<sub>2</sub> receptor antagonism similar to 4 mg haloperidol in the primate model. The model further identifies a limited optimal window of functionality for dopamine D<sub>2</sub> receptor partial agonists. These results suggest that computer modeling of key CNS processes, using well-validated calibration paradigms, can increase the predictive value in the clinical setting of preclinical animal model outcomes.

**Keywords:** dopamine antagonism, partial agonist, antipsychotics, computer simulation

Correspondence: Hugo Geerts
In Silico Biosciences, Inc,
686 Westwind, Berwyn,
PA 19312, USA
Tel +1 267 679 8090
Fax +1 781 862 7690
Email hugo-geerts@in-silico-biosciences.com

## Introduction

All currently marketed drugs in schizophrenia reduce the effects of the dopaminergic striatal pathological hyperactivity by either directly or indirectly reducing the activity of the postsynaptic D<sub>2</sub> receptor.[1] Recently, partial D<sub>2</sub> receptor agonists have been proposed as a therapeutic approach. Their clinical efficacy is hypothesized to be dependent both on the stimulation of presynaptic D<sub>2</sub> autoreceptors that reduce

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synaptic dopamine (DA) release and on the substitution of a full agonist (dopamine) with a partial agonist, with both effects being functionally equivalent to a postsynaptic $D_2$ receptor block. This is an interesting approach as recent imaging studies in an at-risk for mental state cohorts suggest a pre-synaptic pathology in schizophrenia patients.[2] Therefore, drugs acting on presynaptic $D_2$ autoreceptors are supposed to act more closely to the actual pathology. Despite a substantial amount of research, only one partial agonist, aripiprazole, has shown successful clinical efficacy,[3] while the clinical effect of bifeprunox,[4] a partial agonist very similar to aripiprazole, is much more limited, leading to its premature clinical development halt. This suggests that it is difficult to identify the correct range of pharmacology parameters for partial agonism.

Because partial agonists at the postsynaptic receptor tend to counterbalance somewhat the effect at the presynaptic receptor, it is crucial to obtain an optimal balance between binding affinity and potency. In addition, increasing evidence suggests a substantial difference in quantitative biological parameters between rodents and primates in key brain areas important for psychiatric diseases. For instance, the coupling of presynaptic dopamine $D_2$ autoreceptor to DA release in striatal synapses is lower in primates[5] than that in rodents.[6] This difference can have important consequences for the effect of antipsychotics in humans in the treatment of schizophrenia.

Detailed *in vitro* studies[7] show a small difference between aripiprazole and bifeprunox with regard to binding affinity and maximal partial agonist effect. The question arises whether this small difference could actually lead to substantially different functional antagonism at the postsynaptic $D_2$ receptor, which drives a large part of the clinical response.[8] Because bifeprunox has been shown to be equivalent to, if not better than, aripiprazole in preclinical animal models,[9] the ability to estimate more quantitatively the global effect in a humanized situation becomes mandatory.

In order to explore these important questions, we developed a computer model of the striatal dopaminergic synapse, which includes the physiology of time-dependent presynaptic autoreceptor activation and its subsequent modulation of DA release. The model allows for different presynaptic firing regimens, the effect of facilitation and depression on DA release, and simulates the competition between DA and up to three different agents for the same binding site, dependent on their affinity and functionality. Our intention was to develop a model that focuses on the competition between endogenous DA and other agents and takes into account the modulation of free DA by firing frequencies and presynaptic mechanisms that is based on a large population of molecules. This article illustrates how calibration of this model with experimental data from rodents can lead to different conclusions, as compared to the calibration with experimental primate data, and the possible consequences with regard to clinical predictions.

## Methods
### Receptor competition model

The receptor competition model (see Figure 1) consists of a set of ordinary differential equations, describing the time-dependent competition of neurotransmitter and up to three different agents for a presynaptic and postsynaptic receptor, based on the relative affinities and concentrations of each of the different agents. The model runs for a maximum run time of 10,000 msec (10 sec). A detailed mathematical description of the model is given in the Appendix.

The activation of postsynaptic dopamine $D_2$ receptor can be calculated based on the competition between DA and the different agents for both pre- and postsynaptic receptors over time. DA levels are determined by firing-related presynaptic DA release, its half-life in the synaptic cleft, and the activation of presynaptic $D_2$ receptor. In addition, the model considers both high and low DA affinity receptor populations. All outcomes are averages over a 10-sec timescale in which realistic firing patterns of burst and tonic firing are presented to the system (see Appendix).

The neurotransmitter is released following a user-defined set of firing patterns. Dopaminergic neurons tend to switch between low-frequency tonic firing frequencies and high-frequency burst firing patterns.[10,11]

The presynaptic autoreceptor modulates the release of the neurotransmitter based on actual physiological processes and can be calibrated using experimental data on free DA. Because the $D_2$ receptor uses a G-coupled protein pathway, a short time delay is introduced by basing the effect on how many receptors were bound 150 msec before release (see Appendix, Eq. 6). Fast cyclic voltammetry experiments in rats indeed suggest that the effect of autoreceptor activity on DA release is complete within a few hundred milliseconds.[6]

The release dynamics can be described by a depression or facilitation mechanism[12] (see Appendix, Eq. 7). Instead of using a detailed model of internal $Ca^{++}$ levels to determine DA release, we consider the facilitation and depression of DA release based on the amount of time elapsed since the previous firing using a phenomenological equation.
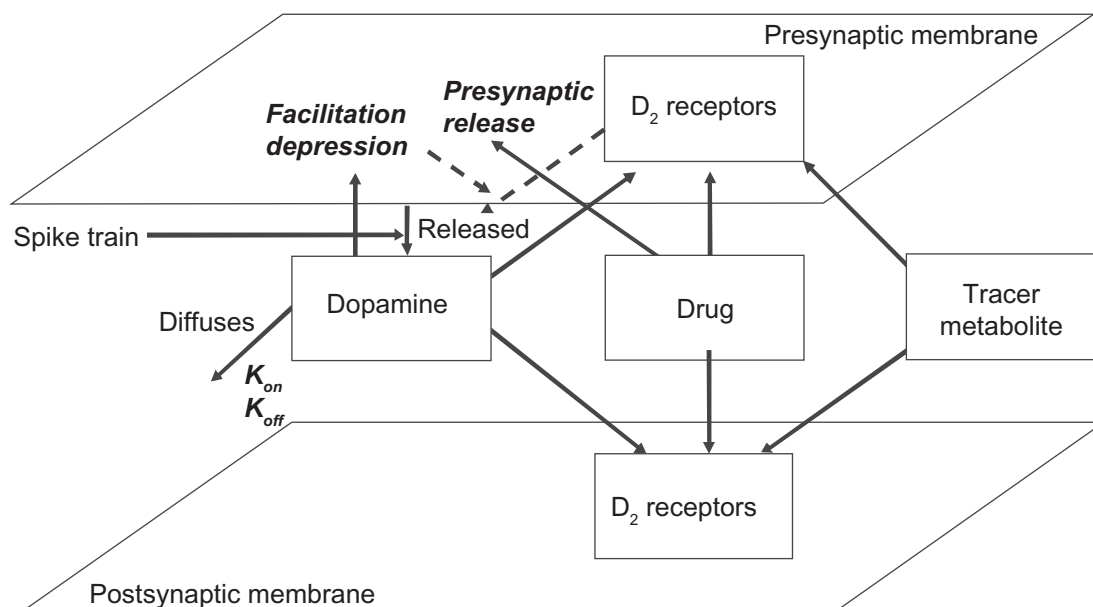
**Figure 1** General description of the striatal dopaminergic synapse and receptor competition model. The model allows user-defined presynaptic firing patterns for neurotransmitter release and simulates the effect of presynaptic $D_2$ autoreceptor negative feedback on presynaptic neurotransmitter release, facilitation and depression of synaptic release, the decay of DA in the cleft due to diffusion, transporters and enzymes, the competition between four agents (the neurotransmitter, up to two drugs, and a tracer), and the dynamics of $k_{on}/k_{off}$ binding of each of these agents to their respective receptors using ordinary differential equations (see Appendix) at millisecond time resolution. The output is the time-dependent activation level of pre- and postsynaptic dopamine $D_2$ receptors, the fraction of each agent bound to these receptors in the low- and high-affinity state, as well as the concentration of free DA in the cleft.

The parameters that govern presynaptic release in the rodent are then calibrated by correlating the effects of high doses of haloperidol with real-time striatal DA voltammetry data obtained *in vivo*[6] and by microdialysis using $D_2$ agonists[13] in the rat. We use previously published data on sulpiride in the marmoset to calibrate a more primatized dopaminergic synapse[5] (see Results).

Free DA removal from the cleft is modeled by an exponential decay. The decay rate is adjusted so as to correspond to the specific DA kinetics in various brain regions and to take into account the removal of DA from the cleft, not only via diffusion but also via transporter and/or enzyme mechanisms. In the striatum, DA is mostly taken up by the dopamine transporter (DAT) and is much less degraded by Catechol-$O$-methyl Transferase (COMT). The half-life of free DA from rodent striatal areas such as n. accumbens[14,15] is in the range of 30–50 msec. We further assume that the binding *on* rate for DA and the compounds is diffusion limited and that this depends on the size and molecular weight of the molecules using the Stokes–Einstein equation. The fraction of receptors with bound DA, tracer, drug, or metabolite can be calculated by solving ordinary differential equations that describe the binding and unbinding processes (see Appendix, Eqs. 1–4). We use a value of 10 nM for the affinity of DA for the high-affinity signal-transducing $D_2$ receptor.[16]

The simulation is initiated by running for a period of 5 sec at the tonic firing rate of 4 Hz. The simulation then runs with a firing regime of 4 Hz for 2 sec, 40 Hz for 0.5 sec, 1 Hz for 5 sec, 4 Hz for 1 sec, and 80 Hz for 0.125 sec, all together for 8.625 sec, based on the fact that the subcortical areas are usually silent and only fire in bursts when salient stimuli are presented.[10] For calibration purposes, we apply the specific (artificial) firing frequencies, as outlined in the experimental papers.

## Calibration of the striatal DA receptor in rodents and marmosets

We calibrated the presynaptic effect on striatal dopaminergic release using previously published, experimental, fast-cyclic voltametry *in vivo* rodent data with high doses of the $D_2$ receptor antagonist, haloperidol.[6] These parameter settings were further verified with experimental data in wild-type and $D_3$ KO mice from PD128907, a $D_2/D_3$ agonist.[13] For a calibration on the primate striatal dopaminergic synapse, we used *in vitro* fast-cyclic voltametry data from experiments on marmosets.[5]

Rapid cyclic voltammetry was used in rats[6] that were pretreated with RTI-76, an irreversible DAT inhibitor, to monitor the free caudate putamen and n. accumbens DA after a high dose of haloperidol (0.5 mg/kg, i.p.) and at different stimulation frequencies in the ventral tegmentum area

(10–50 Hz for 2 sec). This haloperidol concentration corresponds to an extremely high $D_2$ receptor occupancy well above 80%; for instance, tenfold lower concentrations (0.04–0.08 mg/kg) correspond to clinically relevant $D_2$ receptor occupancies of 70%–80% in rodents.[17] Haloperidol increased free DA with a maximum of ninefold increase over the no-haloperidol condition at 30-Hz frequency. Blocking DA uptake with RTI76 ensured that only the effect on presynaptic $D_2$ autoreceptors was measured. The forced VTA firing paradigm ensured that any feedback effect of $D_2$ receptor modulation at the midbrain dopaminergic neurons

was overruled and that the resulting change in free DA in the striatum was almost exclusively driven by the effect of haloperidol on presynaptic autoreceptors.

We reproduced the outcomes of this experiment by using 50 nM of haloperidol in the computer model (leading to over 90% block at the $D_2$ receptor) at different firing frequencies and increasing the half-life of free DA threefold to mimic the block of DAT. This allowed us to identify the best settings for the presynaptic autoreceptor-related parameters. Figure 2A shows the outcome of the model and the experiment as a function of the firing frequency for the best parameters (Table 1).
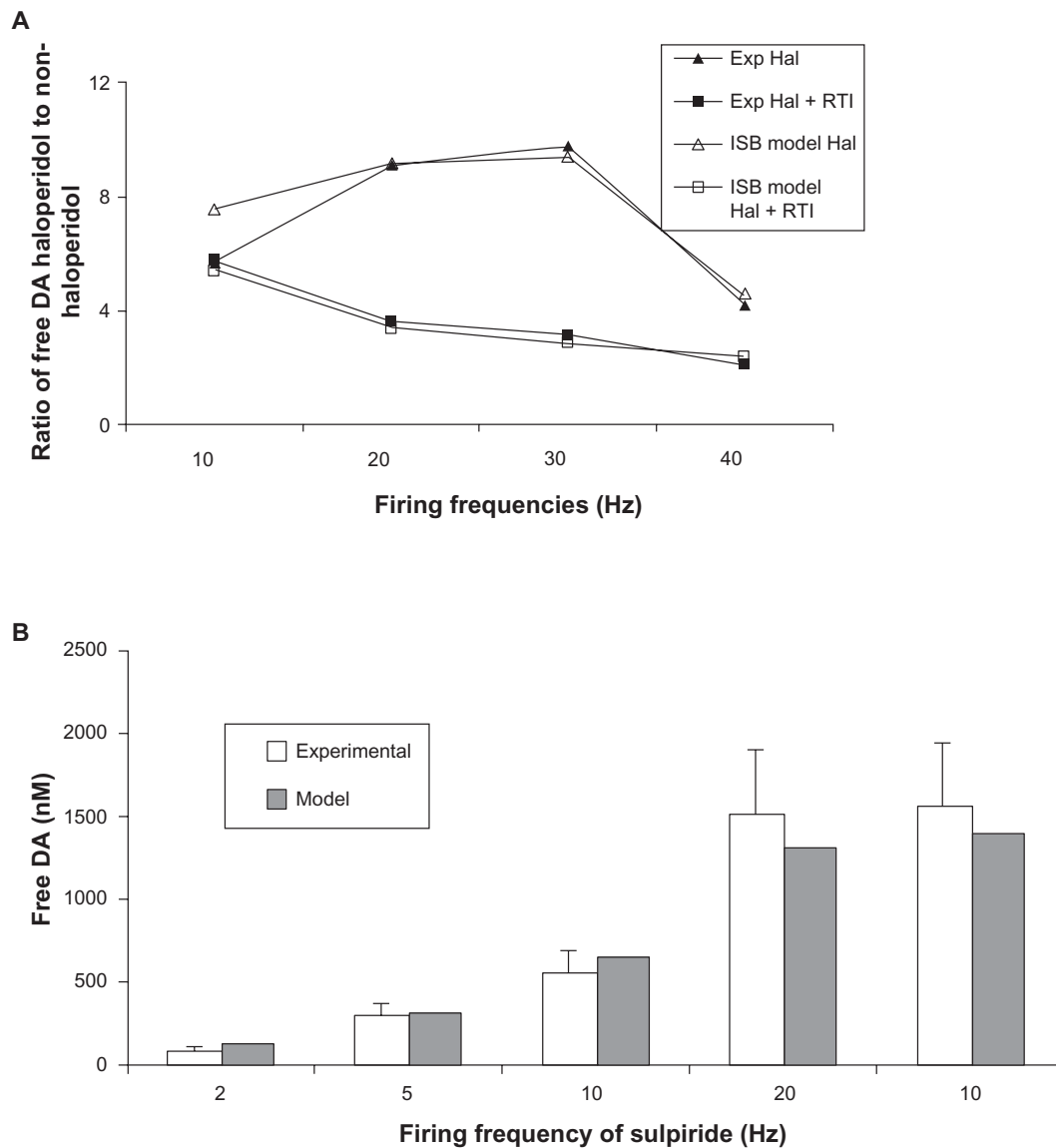


**Figure 2 A**) Calibration of striatal dopaminergic synapse model in rodents (open shapes) using the experimental data (closed shapes) on the ratio of free DA levels with fast cyclic voltametry *in vivo* in the presence and the absence of high haloperidol treatment and forced dopaminergic firing and in the absence or presence of RTI-76 a potent DAT inhibitor.[6] Using these experimental data, we calibrated the appropriate values in Appendix Eq. 6 that led to the observed ratios of free DA in haloperidol treated versus nontreated situations in all four conditions. **B**) Calibration of striatal dopaminergic synapse model using the experimental data in marmosets on free DA levels with fast cyclic voltametry *in vitro* and forced dopaminergic firing.[5] There is an additional data point with 10-Hz firing with 1 μM of sulpiride, a specific $D_2$ receptor antagonist. Using these experimental data, we calibrated the appropriate values in Appendix Eq. 6 that led to the model outcomes similar to experimentally observed free DA levels.

**Table 1** Different parameters for fitting the striatal dopaminergic synapse to experimental rodent and primate data suggest that presynaptic autoreceptor coupling to dopaminergic release is weaker in the primate case, as well in absolute size as in the extent of temporal relationship

| Parameter | Calibration rodent | Calibration primate | Note |
|---|---|---|---|
| relScale | 1 | 0.95 | Maximum increase due to presynaptic $D_2$ autoreceptor |
| relSens | 1.95 | 0.85 | Amplification factor for presynaptic $D_2$ autoreceptor effect |
| normBound | 950 | 370 | Amount of normal presynaptic $D_2$ binding before release |
| Facilitation size | 2 | 0.9 | Size of the coupled facilitation |
| Facilitation half-life | 400 msec | 90 msec | Half-life of the facilitation effect |

A different experiment in rodents documented the decrease of free DA in n. accumbens, measured by microdialysis, after application of a $D_2/D_3$ receptor agonist, PD128907.[13] These authors reported a maximal effect of 65% decrease in free DA, and this effect was similar in $D_3$ KO mice, suggesting a predominant $D_2$ receptor mechanism. Because free DA was quantified in this study using the slow detection process of microdialysis, we used a more normal *in vivo* firing pattern of tonic low-frequency firing (1–4 Hz) interspersed with rare high-frequency bursts of 40–80 Hz (see Methods). The reported affinity of PD128907 for the human $D_2$ receptor is 340 nM,[18] but for the rodent $D_2$ receptor it is 18 nM,[19] and the compound has a partial agonist effect of 25% relative to DA. Using the parameters derived from the haloperidol experiment, for a partial agonist effect of 0.25, the model results in a 70% decrease of free DA, comparable to the observed decrease of 65%.

In contrast, in marmosets, *in vitro* preparations of caudate putamen[5] and application of high concentrations of sulpiride, a specific presynaptic $D_2$ autoreceptor antagonist, lead to only a 285% increase in free DA for ventromedial striatum when stimulated at 10 Hz, rather than the sixfold increase seen in rodents at the same firing frequencies. Although not directly comparable, these data already suggest that the coupling between presynaptic $D_2$ receptor activation and DA release in nonhuman primates might be lower than that in the rodent case. The available data on free DA after different firing frequencies were used to determine the best-fit parameters for the primate striatal dopaminergic synapse. This leads to a different set of calibration parameters (see Figure 2B and Table 1).

For a realistic set of burst and tonic firing frequencies, the average postsynaptic $D_2$ receptor occupancy by DA is in the range of 30%–45% for the rodent calibration set and 35%–50% for the primate calibration set. The results of the simulations are therefore mechanistically determined.

In the following sections, we will illustrate the consequences of these differences.

## Interpreting radiotracer experiments

The effect of different compounds *in vivo* depends on the dose, brain penetration, and target engagement of the compound. Therefore, the best way to compare the effects of compounds is to normalize the concentration of the compounds against the apparent $D_2$ receptor occupancy displacement of specific radiotracers such as ^{11}C-raclopride and ^{125}I-IBZM. Ideally, one would like to quantify the binding of a specific radiotracer before and after neuroleptic treatment to correct for any individual baseline variability of the $D_2$ receptor. Although this is possible with our model, it is usually difficult in the clinical setting; hence, many studies define a binding index (Eq. 1) compared to a normal control population.

$$\text{BindIndex} = 100 \times \left( 1 - \frac{(A_m - \text{Cer}_m)_{\text{patients}}}{(A_m - \text{Cer}_m)_{\text{controls}}} \right), \quad (1)$$

where $A_m$ and $\text{Cer}_m$ are the specific radioactive signals in the region of interest, ie, in the striatum and cerebellum, respectively.

We used a radiotracer at a concentration of 1 pM. The $K_d$ of raclopride, IBZM, and FLB457 for the $D_2$ receptor are 1.3 nM, 0.6 nM, and 0.018 nM, respectively.[20,21] We defined the apparent $D_2$ receptor occupancy as

$$\text{AppOcc} = 100 \times \left( 1 - \frac{R_{\text{drug}}^{\text{tracer}}}{R_{\text{drug}}^{\text{tracer}}} \right), \quad (2)$$

where $R_{\text{drug}}$ and $R_{\text{control}}$ are the receptor tracer occupancies, respectively, in the presence and the absence of the $D_2$ receptor modulator. Because the density of radioactive-sensitive $D_2$ receptor binding sites is about 100-fold lower in the cerebellum than that in the striatum,[22] our simulations indicate that applying Eq. 2 results in between 1% and 1.5% error, compared to the correct use of Eq. 1 (data not shown).

Figure 3 shows the effect of different radiotracers on the calculated receptor occupancy for a range of haloperidol doses. The observation that the calculated $D_2$ receptor occupancy level decreases when higher-affinity tracers are
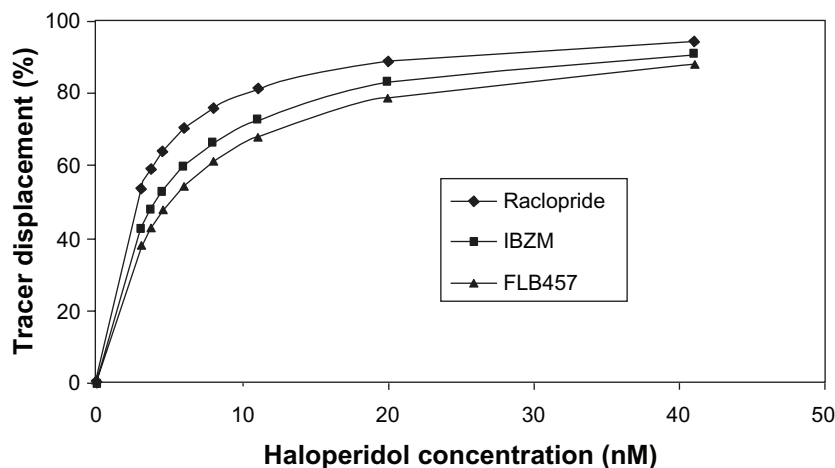
**Figure 3** Effect of tracer molecules with different affinities on the apparent $D_2$ receptor occupancy at fixed haloperidol concentrations (nM). All tracers are applied at 1 pM concentration, and the appropriate affinity constants were used (the $K_d$ of raclopride, IBZM, and FLB457 for the $D_2$ receptor are 1.3 nM, 0.6 nM, and 0.018 nM, respectively). For each haloperidol concentration, the apparent $D_2$ receptor occupancy level, measured by the radioactive tracers, decreases in the order raclopride > IBZM > FLB457, with the same order of their $K_d$ binding affinity constants against $D_2$ receptor.

used is in line with reported differences between apparent receptor occupancy measured with raclopride and IBZM in the same subjects.[23] This can be partially explained by the fact that the greater the affinity a tracer has for a receptor, the more difficult it is for the drug to compete with it and dislodge it.

## Results

## Effect of partial agonists and full antagonists on functional postsynaptic $D_2$ receptor activation

In order to compare the effect of partial agonists with full antagonists on postsynaptic receptor physiology, we introduce the concept of functional $D_2$ receptor antagonism, FA. This is calculated as the reduced postsynaptic $D_2$ receptor activation, normalized to the no-drug situation:

$$FA = 100\left(1 - \frac{Act_{drug}}{Act_{control}}\right), \quad (3)$$

where $Act_{drug}$ and $Act_{control}$ are actual postsynaptic $D_2$ receptor activation levels in the presence and absence of drug, respectively.

These functional antagonist levels reflect the complex consequences of both an indirect effect of the partial agonist on presynaptic $D_2$ receptor-mediated DA release and its direct effect on inhibiting the postsynaptic receptor. The rationale for using partial agonists in schizophrenia is partly based on the experimental observation that stimulating presynaptic $D_2$ autoreceptors reduces free DA release, which is functionally equivalent to a postsynaptic $D_2$ receptor block. In addition, a partial agonist at the postsynaptic receptor can compete

with endogenous DA (a full agonist) to reduce the activation level. As these two processes require vastly different degrees of partial agonism, the ideal profile is a balance of affinity for the pre- and postsynaptic receptor and the degree of partial activation. We will now compare the effect of aripiprazole and bifeprunox on functional postsynaptic $D_2$ receptor antagonism in function of the tracer-reported $D_2$ receptor occupancy.

A recent study[7] reports a low partial agonism for aripiprazole (25%), whereas the affinity of aripiprazole for both pre- and postsynaptic receptors is identical ($K_d$ of 7.1 nM). To further complicate matters, in humans, the major metabolite dehydro-aripiprazole OPC14857 has a lower partial agonist effect (18% maximal effect) and a higher affinity ($K_d = 3.5$ nM); whereas, the major rat metabolite 4-hydrox-phenylpiperazine DM1451 is a full antagonist at the rodent receptor with a $K_d$ of 1.5 nM.[24] In humans, this metabolite contributes to between 25% and 40% of the active moiety.[25] We assume an identical level of the metabolite in rodents.

We simulated the effect of bifeprunox versus the active moiety of aripiprazole in comparison to haloperidol on the functional antagonism dose–responses under primate calibration parameters (Figure 4A). Bifeprunox has a slightly higher partial agonist effect (range 27%–35%, average 31%) than aripiprazole (average 25%).[7,26–28] We also indicated the postsynaptic $D_2$ receptor antagonism of 4 mg haloperidol (corresponding to 65% $D_2$ receptor occupancy), a dose which is known to have a minimal antipsychotic effect. The modeling results suggest that the dose–response of haloperidol is steeper than that of both partial agonists. In addition, only
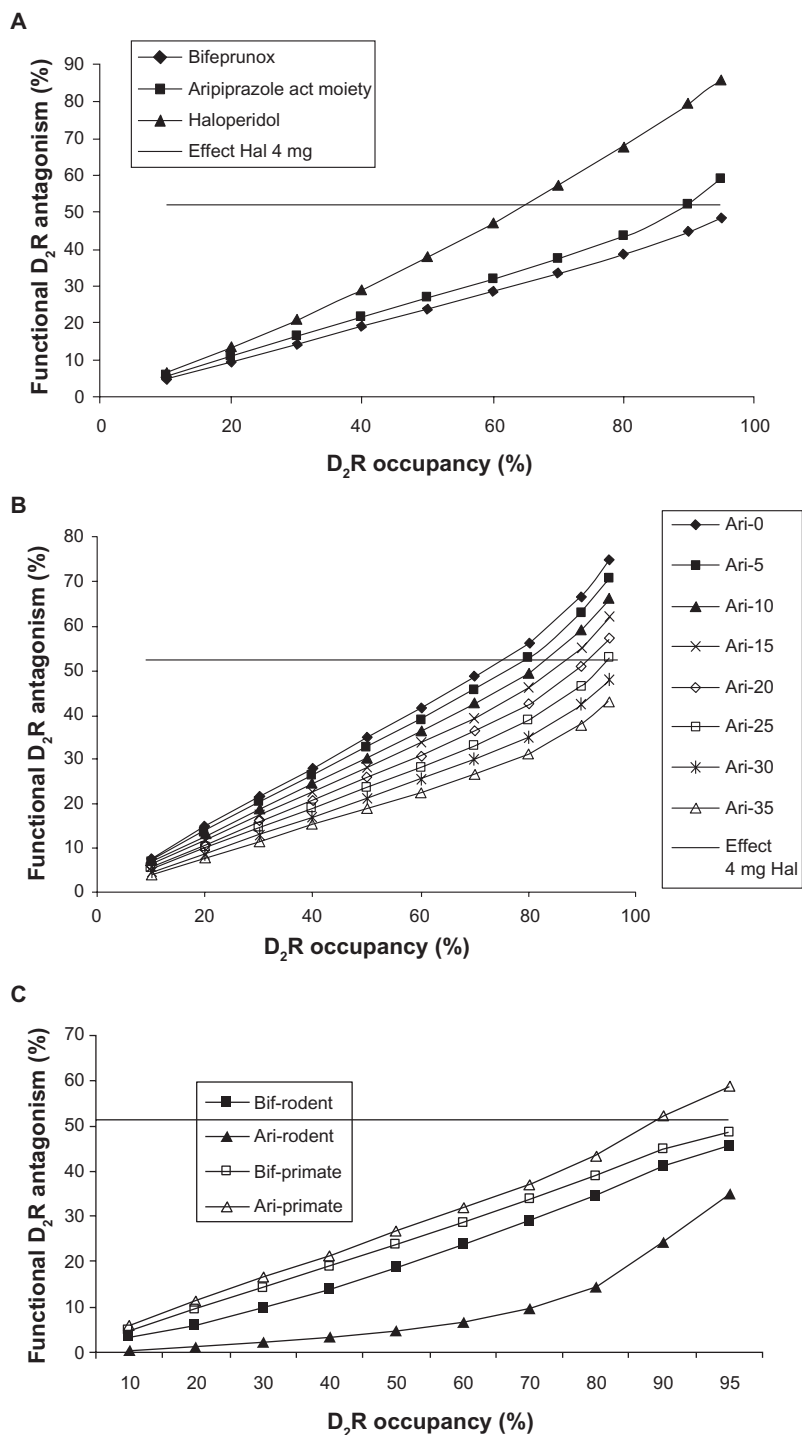
**Figure 4 A**) Functional antagonism (ie, the normalized decrease in actual $D_2$ receptor activation) for different doses of haloperidol, bifeprunox, and the active moiety of aripiprazole and OPC-14857 using primate-derived calibration parameters. The x-axis (dose) is expressed as measured $D_2$ receptor occupancy in a positron emission tomography (PET) tracer imaging experiment with raclopride for each drug. The clinically relevant functional antagonism corresponding to a 4-mg haloperidol dose of about 52% is indicated by the horizontal line. The data suggest that the dose–response of the partial agonists is not as pronounced as haloperidol's dose response and that aripiprazole at the highest dose, but not bifeprunox, can achieve $D_2$ receptor functional antagonist levels comparable to clinically active levels of haloperidol. **B**) Functional antagonism at the postsynaptic $D_2$ receptor of hypothetical partial agonists with the same 7.1 nM $K_d$ for the $D_2$ receptor as aripiprazole but with different maximal agonists effects (0%–35%) in a firing frequency paradigm similar to an *in vivo* situation. The x-axis is expressed as the apparent $D_2$ receptor occupancy measured with raclopride for each hypothetical molecule. A 4-mg haloperidol dose corresponds to a functional $D_2$ receptor antagonism of about 52% (the horizontal line). The figure suggests that there is only a limited degree of partial agonism (0%–25%) that results in sufficient functional $D_2$ receptor antagonism at the highest achievable dose. **C**) Comparison of functional postsynaptic $D_2$ receptor antagonism between the relevant active moiety of aripiprazole and bifeprunox using both rodent and primate calibration parameters. The major human metabolite of aripiprazole is a partial agonist with a $K_d$ of 3.5 nM and a maximal effect of 18%, while the rodent metabolite is a full antagonist with a $K_d$ of 1.9 nM; both of them account for 25% of the active moiety. The functional postsynaptic $D_2$ receptor antagonism is plotted against the apparent $D_2$ receptor occupancy measured with raclopride. The data suggest that for the rodent case, bifeprunox is superior to the active moiety of aripiprazole, while the opposite is true for the primate situation, where bifeprunox does not achieve the same functional $D_2$ receptor antagonism with 4 mg haloperidol.

the active human moiety of aripiprazole at the highest doses achieves a postsynaptic $D_2$ receptor antagonism comparable to haloperidol at 70% $D_2$ receptor occupancy. Bifeprunox fails to reach the minimum level of functional postsynaptic $D_2$ receptor antagonism, corresponding to 4 mg haloperidol, at any dose likely because in the primate dopaminergic synapse there is a smaller effect on presynaptic DA release so that the increased partial agonism of bifeprunox doesn't pay off presynaptically and ends up hurting more postsynaptically.

## Balance of pre- and postsynaptic effect of partial agonists

The previous data suggest that the balance of pre- and postsynaptic effects is critical to achieve sufficient postsynaptic functional $D_2$ receptor antagonism. We simulated the effects of hypothetical analogs of a partial agonist with the same high affinity as that of aripiprazole (7.1 nM) for the $D_2$ receptor but with different degrees of partial agonism (equal at both the pre- and postsynaptic receptors) in a dopaminergic synapse calibrated with the primate parameter set. Figure 4B suggests that there is an optimal window of partial agonism (<25%) that can result in sufficient functional $D_2$ receptor antagonism similar to clinically relevant doses of haloperidol. This can account for the balance between presynaptic autoreceptor activation and postsynaptic competition with endogenous DA. Note that for higher levels of partial agonism, an increasingly higher dose is needed. However, for a partial agonist with a maximal effect >30%, the functional antagonism at the postsynaptic $D_2$ receptor never gets in the range of 4-mg-haloperidol-induced functional postsynaptic $D_2$ receptor antagonism.

## Consequence of rodent and primate settings for partial agonists

We further illustrate the difference between rodent and primate parameter settings by comparing bifeprunox and the active moiety of aripiprazole in dopaminergic synapses in rodent synapses. As mentioned above, they do have a similar affinity[26–28] for the $D_2$ receptor (a $K_d$ of 1.9 nM for bifeprunox and 7.1 nM for aripiprazole), but the functional activity of bifeprunox is slightly higher than partial agonism (36% versus 25%).[7] In addition, the major rat metabolite of aripiprazole, DM-1457, is a potent full $D_2$ receptor antagonist with a fivefold higher affinity and accounts for about 25% of the active moiety.

*In vivo* preclinical animal studies suggest that bifeprunox is at least as active as, if not more active than, aripiprazole,[9]

whereas the clinical efficacy of bifeprunox in schizophrenia is substantially lower.[6] We used our receptor competition model to address the difference of these compounds in the rodent animal models and the primate setting.

Figure 4C shows the functional antagonism for rodent versus primate calibration parameters. The model outcome suggests that in terms of functional postsynaptic $D_2$ receptor antagonism, bifeprunox outperforms the active moiety of aripiprazole under rodent calibration settings, but that the opposite is true for the primate settings. The major human metabolite of aripiprazole has a somewhat lower maximal partial receptor effect than the parent molecule, ensuring that the active moiety is well within the range of sufficient postsynaptic $D_2$ receptor antagonism for the primate calibration setting (see Figure 4B), whereas the full antagonism of the rodent metabolite tends to raise inadvertently presynaptic DA levels in the rodent calibration setting.

Recently new data suggest that aripiprazole is a full antagonist in cell systems expressing the long isoform, $D_2L$.[29] As $D_2L$ is likely the postsynaptic $D_2$ receptor,[30] this would even increase the level of functional antagonism at the postsynaptic $D_2$ receptor (data not shown). However, in the absence of similar data for bifeprunox, we wanted to limit our analysis to two very similar agents with similar pharmacology and explore the parameter space of a partial agonist that would lead to a robust postsynaptic functional $D_2$ receptor antagonism.

## Correlation with the clinical situation

Because the calibration conditions for rodent and primate were determined in different conditions, we next addressed the question "How will the two calibration sets compare to the clinical situation?" We therefore simulated the four clinical doses of aripiprazole and the three clinical doses of bifeprunox together with the reference drug haloperidol at 4 mg in the computer model with both calibration sets and compared the outcome to the reported clinical effects on the Positive and Negative Syndrome Scale (PANSS) total scale. The actual compound concentrations for all the conditions were determined by simulating the reported positron emission tomography (PET) imaging displacement studies with raclopride.

In stable treatment-responsive patients who were switched from other antipsychotics, it was reported that a dose of 5 mg aripiprazole resulted in a PANSS total improvement of 9.4 points,[31] whereas a dose of 15 mg,[32,33] 20 mg,[34,35] and 30 mg[33,35,36] improved the PANSS total by 15.5 points,

14.5 points, and 12.6 points, respectively. These doses corresponded to receptor occupancies of 83%, 91%, 95%, and 97%, respectively.[37,38] Similarly, the improvements for bifeprunox on the PANSS total in a 6-week study were reported to be 9.7 points, 5 points, and 11.3 points with 5 mg, 10 mg, and 20 mg of bifeprunox, respectively.[6] Unfortunately, no PET imaging data are publicly available for bifeprunox; however, from the reported clinical data and side effects,[6] we inferred that bifeprunox readily crossed the blood–brain barrier and results in receptor occupancies of 80% and above.

The clinical data for 4 mg haloperidol were derived as weighted averages from 4 studies covering 623 patients.[39–42] Actual haloperidol concentration for this dose was simulated to be 70% using the reported PET displacement studies with raclopride.[43–45]

Figure 5 shows the correlation between the clinical outcomes (improvement in PANSS total), the postsynaptic functional $D_2$ receptor antagonism, and the primate calibration sets. The primate calibration sets result in a correlation of $r^2 = 0.601$ or $r = 0.77$ ($P = 0.016$), while a correlation coefficient of $r^2 = 0.023$ or $r = 0.15$ ($P = 0.70$) is obtained using the $D_2$ receptor occupancy as independent variable and a correlation of $r^2 = 0.237$ or $r = -0.45$ ($P = 0.20$) when using the functional postsynaptic $D_2$ receptor antagonism calibrated with rodent data, suggesting that the computer model with the primate calibration set is able to explain much more of the variance. Moreover, the correlation with the rodent calibration set would lead to a negative slope, ie, more clinical efficacy with lower postsynaptic $D_2$ receptor antagonism. This is clearly not in line with the clinical observations.

## Discussion

The actual competition among the neurotransmitter, the tracer, the drug, and the active drug metabolite in a typical CNS synapse for a receptor binding site is complex and hard to understand quantitatively without systematic computer modeling. Neurotransmitters are released in well-defined firing patterns, and their release is modulated by presynaptic autoreceptor activation. Time-dependent facilitation and depression can further alter the quantity of neurotransmitter released, while the dynamics of free neurotransmitter in the synapse are determined by transporters, diffusion, and catabolic enzymes. The ability to quantitatively simulate the interactions between different physicochemical processes is one of the main arguments for this approach of computer modeling.

This report describes a computer model and its calibration and application to the striatal dopaminergic synapse. As our objective was to compare partial agonists with full antago-
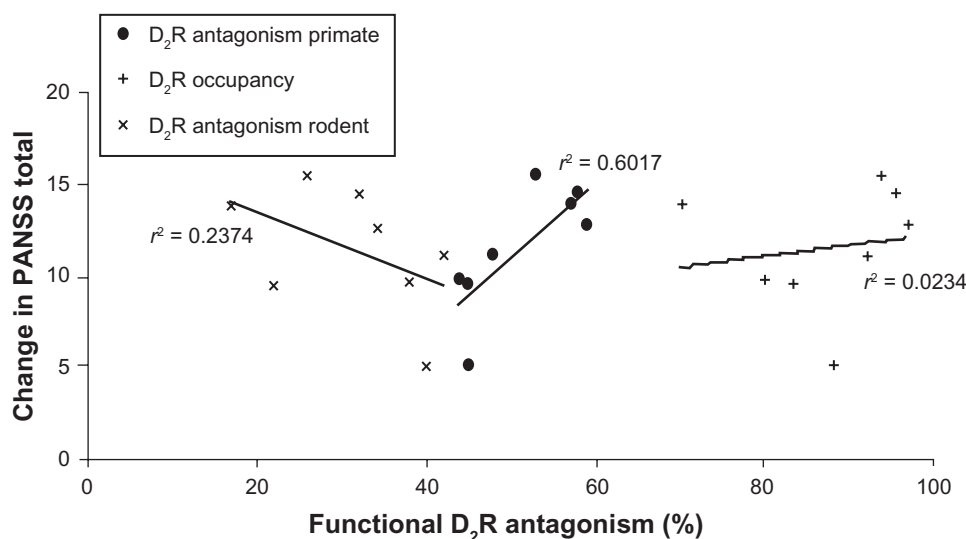


**Figure 5** Correlation between reported clinical efficacy of 4 mg haloperidol, four doses of aripiprazole (5 mg, 10 mg, 20 mg, and 30 mg), and three doses of bifeprunox (5 mg, 10 mg, and 20 mg) and the functional postsynaptic $D_2$ receptor antagonism in the dopaminergic synapse computer model with primate calibration. The *y*-axis value for each point is the weighted average of PANSS total for a specific dose of the drug; the *x*-axis value is the functional postsynaptic $D_2$ receptor antagonism as simulated in the DA synapse model using the primate calibration set (●), the rodent calibration set (×), and the postsynaptic $D_2$ receptor occupancy (+). The observed correlation coefficient for the primate calibration set (●) of $r^2 = 0.602$ or $r = 0.77$ ($P = 0.015$) contrasts with a correlation coefficient of $r^2 = 0.023$ or $r = 0.15$ ($P = 0.70$) when using the $D_2$ receptor occupancy as independent variable (+) and a correlation of $r^2 = 0.234$ or $r = -0.45$ ($P = 0.20$) when using the functional postsynaptic $D_2$ receptor antagonism calibrated with rodent data (×), suggesting that the computer model with the primate calibration set is able to explain much more of the variance. In addition, the computer model with the rodent calibration set would suggest a negative correlation between postsynaptic $D_2$ receptor antagonism and clinical efficacy.

nists, we introduced the concept of functional $D_2$ receptor antagonism at the postsynaptic $D_2$ receptor. This is calculated as the reduction in receptor activation, normalized to the no-drug situation. Interestingly, for a full antagonist, this functional $D_2$ receptor antagonism, although proportional to the $D_2$ receptor occupancy level calculated from the imaging experiments, is somewhat smaller and reflects the complex interaction between the drug and the endogenous neurotransmitter in such a synapse. For instance, inhibition of the presynaptic receptor by a full antagonist leads to an increase in the release of free DA, thereby countering its effect at the postsynaptic receptor.

The model has been calibrated using published experimental data that quantified the strength of presynaptic DA receptor feedback on DA release with high concentrations of dopamine $D_2$ receptor antagonists and forced firing frequencies of dopaminergic neurons in rodents[6] or in primates.[5] Both calibration sets lead to an average $D_2$ receptor occupancy by endogenous DA in drug-free conditions between 30% and 50%, with the primate data being slightly higher. This is in the experimental range observed in humans,[46] baboons,[22] and rodents.[47] In primates at least, the experimental data suggest that the effect of presynaptic receptor coupling to DA release is substantially lesser, leading to a different dose dependency of a partial agonist on the functional postsynaptic $D_2$ receptor antagonism.

Marmosets are increasingly being used for biomedical research; their usefulness is likely to be limited as they can differ quite substantially from old-world primates.[48] However, they have characteristic anthropoid primate traits. It is of interest to note that similar measurements in striatal slices of squirrel monkeys result in low DA levels that are at least in qualitative agreement with data from the marmoset.[49]

Although we don't have similar data for the human situation, such disconnect between rodent and primate physiology suggests that extrapolations from preclinical rodent models to the human clinical situation need to proceed cautiously.

Interestingly, the model outcome suggests that the calculated $D_2$ receptor occupancy from a PET imaging study can be different when different radiotracers are used in the same patient treated with antipsychotic medication. This is in line with reported clinical data that systematic lower occupancy readout was found with IBZM when compared to raclopride for all subjects and brain regions in the same schizophrenia patients.[23] The authors concluded that the reasons for the radiotracer differences were due to the degradation by high-energy photons in the IBZM images, but that they were not due to the choice of reference region or the assumption of

pseudo-equilibrium. Our results suggest that the differential competition among the tracer, the neurotransmitter, and the drug at the level of the binding site can contribute additionally to this difference. Failure to appreciate this effect can lead to misinterpretation with regard to receptor occupancies when using different radiotracers. Fortunately, the large majority of clinical imaging studies with antipsychotics have been performed with the same radiotracer, raclopride, making comparisons between drugs easier.

Partial $D_2$ receptor agonists are an interesting option to achieve functional $D_2$ receptor inhibition, because they tend to reduce the amount of presynaptic DA released by stimulating the negative-feedback $D_2$ autoreceptor. These drugs are supposed to act more closely to the actual pathology as recent imaging studies in an at-risk for mental state cohorts suggests a presynaptic pathology in schizophrenia patients.[4] The differences in calibration of this presynaptic effect also suggest that partial agonists in the rodent calibration will lead to greater functional postsynaptic antagonism, as compared to the primate calibration setting, because they reduce the levels of released DA more strongly. This might lead to an overestimation of the effect of partial agonists in rodent models.

It is important to note that the model outcome is presented as the level of postsynaptic $D_2$ receptor activation and does not take into account any further physiological intracellular effect or pathway activation. Recently, the concept of functional selectivity has been proposed to account for some of the *in vivo* effects of aripiprazole.[29] The aripiprazole effect can range from partial agonism to full antagonism, depending on the cell system, the latter mostly in cell systems overexpressing human $D_2L$ receptors. These $D_2L$ receptors are most likely located postsynaptically,[30] and an antagonistic effect of aripiprazole can therefore explain the clinical observations of worsening motor symptoms in the treatment of patients with Parkinson's.[50] Substituting the partial agonist effect of aripiprazole at the postsynaptic $D_2$ receptor by a full antagonist effect will increase the functional postsynaptic $D_2$ receptor antagonism, as free DA is now replaced by a compound that, rather than partially activating, completely blocks the receptor, whereas the partial agonist effect at the presynaptic receptor continues to reduce levels of released DA.

The simulation further suggests that $D_2$ receptor functional antagonism of the active moiety of aripiprazole at high $D_2$ receptor occupancy levels ($>90\%$) can achieve similar levels as clinically relevant doses of haloperidol. We compared the effects of the partial agonists on postsynaptic $D_2$ receptor antagonism in the presence of 4 mg haloperidol. Although often higher doses are used in clinical practice, a 4-mg dose

gives the minimal clinical benefit[43] and is therefore a good benchmark. Although the pharmacology of both aripiprazole and bifeprunox is quite complex, including effects on other dopaminergic, serotonergic, and noradrenergic receptors,[29] functional postsynaptic $D_2$ receptor antagonism drives a substantial part of the clinical response.[8] The fact that high doses are needed is in line with the reported proportional dose–response for aripiprazole in clinical trials,[35,36] with clinically efficacious doses that correspond to >95% $D_2$ receptor occupancy.[37]

The model simulations further suggest that there is a limited therapeutic window for partial agonists, because of the delicate balance between pre- and postsynaptic effects. Having too strong an affinity and maximal agonist effect might indeed lead to a lower postsynaptic inhibition that outweighs the benefits of presynaptic $D_2$ receptor stimulation.

Such computer models can help to better extrapolate findings from preclinical rodent models to the clinical situation. Because of the stronger coupling of presynaptic $D_2$ autoreceptor to DA release in the rodent case, bifeprunox outperforms the active moiety of aripiprazole, a result observed experimentally in preclinical rodent models.[9] In contrast, the model predicts a superiority of the active moiety of aripiprazole in primate conditions, which is partially due to the different pharmacological activities of its major metabolite[24] and partially due to the lower coupling of presynaptic $D_2$ autoreceptor activation to subsequent DA release.[5] The results of the model suggest that aripiprazole has a substantial clinical advantage over bifeprunox at least in functional $D_2$ receptor antagonism. Obviously, it is possible that clinical efficacy is also driven by other processes as discussed above, but it is of interest to note that the bifeprunox clinical effect on PANSS total is limited[6] to the point that the clinical development of the compound has been halted.

In this study, we assumed that, unlike aripiprazole, bifeprunox has no major active metabolite in the human/primate or the rodent situation, as there are no published data on such a metabolite. It could still be the case that human metabolites of bifeprunox, if present, could have a pharmacological activity that is sufficiently different to change dramatically the functional $D_2$ receptor antagonism. Notwithstanding this, the data illustrate the power of the model to assess the effect of unique human metabolites.

As with any model, there are limitations on this specific modeling approach. Because we don't take into account spatial dimensions, we assume that all of the agents have perfect access to each other. This could lead to an overestimation of the amount of bound receptors. However, in terms of understanding the *relative* effects, such as inhibition fraction, we expect the model to behave reasonably well. Because we are dealing with synaptic effects where the distance between the membranes is very small, there is also a greater probability that the different agents will come into contact with a receptor, making it more similar to the well-mixed solution that the equations describe.

Although the model is based on biological properties of the dopaminergic synapse (using realistic values for DA dynamics), some of the relations, for instance those between presynaptic dopamine $D_2$ autoreceptor and DA release after stimulation, are introduced in a phenomenological, rather than an exact biological, way. The biology of presynaptic DA release is quite complex, and there are still many unresolved issues (for a complex computational model see Qi et al[51]). This particular computational model has focused on the biochemistry of processes we have modeled in a phenomenological way but calibrated using actual experimental data with a limited set of parameters. In contrast to rodent data, the primate values are measured from *ex vivo* preparations[5] that obviously might lack a number of physiological processes that are active *in vivo*. However, the relative effect and the dose–response for the active moiety of aripiprazole with primate parameters corresponds to observed clinical effects, again assuming that the $D_2$ receptor block drives most of the clinical outcome.[8,52]

Unlike other models dealing with the interpretation of PET imaging[22] that simulate the binding kinetics of tracers over many minutes, our model takes into account detailed physiological processes on a millisecond timescale.

We anticipate that this method becomes even more valuable in the cases where simple occupancy rules/formulae do not apply in conditions such as (1) in the absence of tracers, (2) with multiple drugs (and metabolites) acting on the same receptor, (3) when dealing with partial agonists, and (4) when the rodent physiology is different from the human physiology. In principle, such a generic receptor competition model could also be applied to different CNS synapses, such as cortical DA synapses or serotonergic synapses, using appropriate affinity and physiology parameters.

## Acknowledgments

## Disclosure

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# Appendix

Here, we present a more detailed description of the receptor competition model.

Dynamical binding and unbinding of the different agents to the receptor sites are calculated as follows. If [dop] is the free DA concentration and $[D_f]$ is the concentration of free receptors, then the change in receptors bound by DA, $[D_n]$, is governed by the following ordinary differential equation (ODE):

$$\frac{\partial [D_n]}{\partial t} = k_{on}^n \times [\text{dop}] \times [D_f] - k_{on}^n \times K_d^n \times [D_n], \quad (1)$$

with the initial condition that all receptors begin in the free state. With sub- and superscripts n, d, m, and t referring to neurotransmitter DA, drug, metabolite, and tracer, respectively, the change in receptors for drug, metabolite, and tracer binding is governed by the following coupled ODEs:

$$\frac{\partial [D_d]}{\partial t} = k_{on}^d \times [\text{drug}] \times [D_f] - k_{on}^d \times K_d^d \times [D_d], \quad (2)$$

$$\frac{\partial [D_m]}{\partial t} = k_{on}^m \times [\text{met}] \times [D_f] - k_{on}^m \times K_d^m \times [D_m], \quad (3)$$

$$\frac{\partial [D_t]}{\partial t} = k_{on}^t \times [\text{tracer}] \times [D_f] - k_{on}^t \times K_d^t \times [D_t]. \quad (4)$$

Alternatively, the metabolite can be substituted by a second drug in simulation of polypharmacy. We assume that the association to the receptor is diffusion limited, giving us a value for the forward binding rate constant $k_{on}$ of 140–150 $\mu M^{-1}$ $sec^{-1}$ for a molecule with MW in the range of 200–400 (as an example for the case of Ach[53]). In general, $K_d = k_{off}/k_{on}$ so that $K_{off} = K_d \times K_{on}$. Furthermore,

$$D_f = D_o - D_n - D_d - D_m - D_t, \quad (5)$$

where $D_o$ is the concentration of receptors. All differential equations are solved with a fourth-order Runge–Kutta method with a time step of 0.01 msec.

The amount of free DA depends on two processes, exponential decay and quantal release. Exponential decay is classically defined as $[\text{dop}](t) = \exp(-t \ln(2)/\text{half-life},$

where half-life is the half-life of the decay process. At times of release, [dop] is immediately updated by adding the release amount.

The amount of presynaptic receptor activation which occurred 150 msec before the current release event then determines the amount of new release as follows

$$\text{release}_{\text{new}} = \text{release}_0 \left[ 1 + \text{relScale} \times \left( 1 - 2 \frac{\text{recAct}^{\text{relSens}}}{\text{recAct}^{\text{relSens}} + \text{normBound}^{\text{relSens}}} \right) \right] \quad (6)$$

where $\text{release}_0$ is the base release amount, relScale is the maximum relative change for release, recAct is the actual presynaptic receptor activation 150 msec earlier, relSens is the sensitivity to the presynaptic receptor (lower values create a shallow response and higher values create a sharp difference between activation levels), and normBound is the amount of normal presynaptic binding that one would expect in the tonic case (ie, when recAct equals normBound, the new release equals the baseline release amount). The dynamical independent variable recAct is calculated from earlier time points and is initiated using a run-in time period of 4 sec. We calibrate the parameters so that the coupling of presynaptic $D_2$ receptor activation to DA release reflects actual experimental data (as seen in Results).

In addition, the release can be modulated by a depression or facilitation mechanism.[12] Instead of using internal $Ca^{++}$ levels to determine DA release, we consider the facilitation and depression of DA release based solely on the amount of time elapsed since the previous firing using a simple transfer equation. Thus, the amount of DA released is based both on the history of firing and the activation level of the presynaptic $D_2$ autoreceptors. If we denote the time of the $n$th firing by $t_n$, then the release amount is modified based on all previous firings as follows

$$\text{release}_{\text{new}} = \text{release} \left( 1 + \sum_{i=1}^{n-1} w_f \exp\left[ -k_f(t_n - t_i) \right] - w_d \exp\left[ -k_d(t_n - t_i) \right] \right), \quad (7)$$

where $w_f$ is the facilitation weight, $w_d$ is the depression weight, $k_f$ is the decay rate of facilitation, and $k_d$ is the decay

rate of depression. These four parameters will be calibrated to experimental data.

The simulation is initiated by first finding the equilibrium, given a constant amount of free DA at 500 nM. The simulation is then run for a transitory time of 5 sec at the tonic firing rate of 4 Hz. Finally, the simulation runs for an additional 2.5 sec during which time average binding levels are determined and then the simulation runs with the pre-defined firing pattern.