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Imaging the high-affinity state of the dopamine D_2 receptor in vivo: Fact or fiction?

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Abstract

Positron Emission Tomography (PET) has been used for more than three decades to image and quantify dopamine D_2 receptors (D_2R) in vivo with antagonist radioligands but in the recent years agonist radioligands have also been employed. In vitro competition studies have demonstrated that agonists bind to both a high and a low-affinity state of the D_2Rs, of which the high affinity state reflects receptors that are coupled to G-proteins and the low-affinity state reflects receptors uncoupled from G-proteins. In contrast, antagonists bind with uniform affinity to the total pool of receptors. Results of these studies led to the proposal that D_2Rs exist in high and low-affinity states for agonists in vivo and sparked the development and use of agonist radioligands for PET imaging with the primary purpose of measuring the proportion of receptors in the high-affinity (activating) state. Although several lines of research support the presence of high and low-affinity states of D_2Rs and their detection by in vivo imaging paradigms, a growing body of controversial data has now called this into question. These include both in vivo and ex vivo studies of anesthesia effects, rodent models with increased proportions of high-affinity state D_2Rs as well as the molecular evidence for stable receptor–G-protein complexes. In this commentary we review these data and discuss the evidence for the in vivo existence of D_2Rs configured in high and low-affinity states and whether or not the high-affinity state of the D_2R can, in fact, be imaged in vivo with agonist radioligands.

Keywords

D_2 receptor; High-affinity state; Agonist binding; PET imaging

1. Introduction

Neuroreceptor imaging techniques such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) have been used for more than three decades to image and quantify dopamine D_2 receptors (D_2R) in the primate brain.

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Neuroreceptor imaging has also been used to assess endogenous dopamine release indirectly by measuring the dopamine displacement of D_2R radioligand binding after a pharmacological challenge with psychostimulants (e.g., amphetamines or methylphenidate). The theoretical justification for this approach to measuring dopamine release in vivo was provided by the classical occupancy model. Briefly, amphetamine-induced release of endogenous dopamine will increase occupancy of the D_2Rs by dopamine, thereby decreasing the binding potential (BP_{ND}) of the radiotracer, a parameter that is measured in PET imaging and is proportional to the product of receptor density (B_{max}) and the affinity (1/Kd) of the radiotracer [1]. Several imaging studies have provided support for the occupancy model using benzamide antagonist radioligands and amphetamine challenge. However, even at high doses of amphetamine, D_2R radioligand binding is not reduced beyond ~50%, a phenomenon referred to as the ceiling effect [1–4]. Several explanations have been proposed for this ceiling effect: (1) receptors located extrasynaptically are less accessible to competition from synaptically released dopamine, or maybe there is not enough dopamine to fully displace the radioligand, (2) internalized receptors are inaccessible to dopamine competition but still accessible to the relatively lipophilic radioligand, and/or (3) since D_2Rs are configured in high and low-affinity state for agonist binding, dopamine competes primarily at the high-affinity sites of D_2R but spares the low-affinity sites [1]. Although several lines of research support the presence of high and low-affinity state D_2Rs and their detection by in vivo imaging paradigms, a growing body of evidence has now called this into question. The purpose of this commentary is to review these data and promote discussion about the existence in vivo of two populations of the D_2R configured in high and low-affinity states for agonist binding, and to address whether a high-affinity state of the D_2R can in fact be imaged with agonist radioligands.

2. The two-state occupancy model

The experimental basis for imaging high-affinity state D_2Rs with agonist radioligands was provided by competition binding assays in washed brain membrane homogenates. These assays demonstrated that agonists bind with both high and low-affinity to the D_2R in the absence of guanine nucleotide triphosphate (GTP), but with low-affinity in the presence of GTP [5–7]. GTP binding to Gα subunit promotes G-protein activation and dissociation from the receptor, resulting in a loss of high-affinity agonist binding. The ternary complex model provided the first description of the mechanistic interactions of agonist-receptor–G-protein (Fig. 1). In this model the receptors exist as G-protein coupled and uncoupled: the G-protein coupled state has a high-affinity for agonist binding whereas the uncoupled form has a low-affinity for agonist [8]. The ternary complex model provided the theoretical framework for the two-state occupancy model for neuroreceptor imaging. The two-state occupancy model predicts that amphetamine challenge would show greater displacement of agonist than of antagonist radioligands by dopamine, and the ceiling effect could be conceptualized as displacement by dopamine only of the fraction of receptors configured in the high-affinity state. A confounding issue with this conceptualization is that cells are not GTP depleted and the ternary complex does not accumulate in vivo as it does in steady state measurements in in vitro membrane preparations. This issue will be discussed further in the section describing the molecular mechanism of agonist binding.

3. PET studies of D_2Rs in the high-affinity state

Since the initial proposal of the two-state occupancy model, multiple studies have aimed at imaging the D_2R in the high-affinity state with agonist radioligands [9–12]. A review of 13 PET studies reveals inconclusive evidence for the two-state occupancy model (Table 1). Seven studies (all used anesthesia) supported the ability to image high-affinity state D_2Rs with agonist radioligands while six studies (four used anesthesia) failed to support this

hypothesis. Consistent with the two-state occupancy model, three novel agonist radioligands [^{11}C]PHNO (4-propyl-9-hydroxynaphthoxazine), [^{11}C]NPA ((−)-N-propyl-norapomorphine) and [^{11}C]MNPA ((R)-2-CH3O-N-n-propyl-norapomorphine) showed almost two-fold greater displacement after amphetamine challenge than antagonist radioligands in anesthetized animals [13–18]. In contrast, amphetamine challenge did not produce greater displacement of [^{11}C]MNPA or [^{11}C]NPA than of [^{11}C]raclopride in conscious monkey or human, suggesting a confounding effect of anesthesia [17,20]. In line with this, PET studies of anesthetized vs conscious monkeys showed increased [^{11}C]MNPA and decreased [^{11}C]raclopride baseline BP_{ND} under anesthetized conditions. Moreover, methamphetamine (1 mg/kg iv) produced a ~44% decrease of [^{11}C]MNPA BP_{ND} in anesthetized monkeys but only ~17% decrease in conscious monkeys, an effect that appeared to be primarily due to the increased baseline BP_{ND} of [^{11}C]MNPA [17,21]. The exact mechanism for the effect of anesthesia on BP_{ND} of agonists and antagonists is unclear but thermodynamic properties of the ligands, shift of high/low-affinity ratio as well as the type of anesthesia have been proposed [17,22–24]. Two other challenge studies used increasing doses of exogenous D_2R agonist to establish an inhibition curve in vivo similar to those obtained with competition assays in vitro [25,26]. Neither study was able to demonstrate the bi-phasic displacement of [^{11}C]raclopride characteristic of the high and low-affinity states observed in in vitro studies. The agonist apomorphine showed similar IC_{50} value for [^{11}C]MNPA and [^{11}C]raclopride, suggesting that the PET radioligands bind to an indistinguishable population of the D_2Rs in vivo [25,26]. In agreement with this, a PET study of a mouse model reported to exhibit increased proportions of high-affinity state D_2Rs failed to find increased baseline BP_{ND} of [^{11}C]MNPA compared to wildtype mice [27–29].

Thus, PET studies have not consistently supported the possibility of imaging high-affinity state D_2Rs with agonists; therefore, the evidence for this is inconclusive.

4. Ex vivo studies of D_2Rs in the high-affinity state

The possibility of measuring high-affinity state D_2Rs with agonist radioligands has also been investigated in a number of ex vivo studies. Briefly, these studies were performed by intravenous administration of radioligands in either conscious or anesthetized rodents. The rodents were euthanized by decapitation at various time points and the radioactivity was measured in the brain tissue. As was the case for the PET investigations, a review of 12 ex vivo studies revealed inconclusive evidence for the two-state occupancy model (Table 2). Seven studies, using striatal tissue from rats that had not received anesthesia, found no difference in the displacement of [^{11}C] or [^{3}H]PHNO, [^{3}H]MNPA [^{3}H]NPA and [^{3}H]raclopride by amphetamine challenge or exogenously administered agonists [30–33]. Notably, one of these studies was performed using rat models previously reported to have an increased proportion of D_2R in the high-affinity state measured with competition binding in striatal homogenates [30,34,35]. In contrast, four studies, including one model of increased high-affinity D_2R, did report greater displacement of agonist than antagonist radioligands by amphetamine challenge or exogenously administered agonists in tissue from conscious rodents [36–38]. A recent ex vivo study by McCormick and colleagues investigated the effect of anesthesia on radioligand binding [33]. As was seen for in vivo PET imaging, anesthesia produced a marked increase in baseline binding of agonist radioligands accompanied by a significantly larger displacement by amphetamine than [^{3}H]raclopride. In contrast, amphetamine challenge produced a similar magnitude of displacement of both agonists and [^{3}H]raclopride in tissue from conscious rats [33].

In summary, the evidence for high-affinity state D_2Rs in vivo and ex vivo has so far been inconclusive. To explore and discuss potential explanations for this conflicting literature, we will review the molecular basis for high-affinity agonist binding.

5. Molecular mechanisms of agonist binding at the dopamine D_2 receptor

The high and low-affinity states of the D_2R are often referred to as G-protein coupled and uncoupled, respectively. While this makes sense in terms of the ternary complex model and under GTP depleted and stabilized conditions in membrane binding assays, evidence for the existence of pre-coupled complexes in living cells is conflicting. Two different theories have been proposed to explain receptor–G-protein interaction in the absence and presence of agonist (Fig. 2):

1. The pre-coupled theory proposes that some receptors form stable complexes with G-proteins. These complexes induce a state of the receptor with high-affinity for agonist binding. Agonist binding induces a conformational change of the receptor that activates the G-protein [39,40].
2. The collision theory proposes that receptors and G-proteins diffuse freely within the plasma membrane in the absence of agonist. Upon agonist binding, the receptor adopts a conformation with a higher affinity for G-protein, which enhances interaction with and subsequent activation of the G-protein [39,40].

These hypotheses have been investigated using resonance energy transfer (RET) techniques, such as förster/fluorescence (FRET) and bioluminescence (BRET) resonance energy transfer, which allow direct assessment of the interactions between receptor and G-protein. The RET techniques makes use of recombinant receptor and G-protein constructs fused with donor and acceptor molecules. By measuring the energy transfer from donor to acceptor, which takes place only when the molecules are in close proximity (≤10 nm), the RET techniques can study receptor–G-protein interactions [41,42]. One study interpreted basal FRET signal from receptor and G-protein as evidence for pre-coupling but did not further investigate the interactions [43]. Despite the existence of a basal BRET signal that could be consistent with some pre-coupling of receptor and G-protein, a recent study observed a large enhancement of receptor–G-protein BRET upon addition of agonist, consistent with either recruitment of G-protein to the receptor and/or with an optimized interaction with pre-coupled G-protein [44]. Lambert and colleagues argued against pre-coupling of D_2R and G-protein and showed a marked increase in BRET signal after agonist stimulation, suggesting that agonist stimulation of D_2R promoted G-protein coupling [45]. Moreover, the agonist stimulated BRET signal was significantly enhanced by depletion of guanine nucleotides, which stabilizes the ternary complex [45]. A recent study of D_1R even suggested that receptor internalization promotes signaling, thus proposing a delayed onset of G-protein activation [46]. Similar studies of other GPCRs have reported conflicting evidence for the pre-coupling vs collision theory, consistent with the idea that the propensity for G-protein pre-coupling may vary for different receptors [43,45,47–50].

It is important to realize that even the absence of pre-coupled D_2R does not exclude high-affinity agonist binding. The affinity of a ligand is the reciprocal of the equilibrium dissociation constant Kd and dependent on the association and dissociation rate constants (k_{on} and k_{off}):

$$\mathrm{Kd}=\frac{k_{\mathrm{off}}\mathrm{min}^{-1}}{k_{\mathrm{on}}\mathrm{M}^{-1}\mathrm{min}^{-1}}$$

From this we can observe that changes in either of the rate constants will be reflected by a change in affinity. That is, a faster on rate would lead to higher affinity, a faster off rate would lead to lower affinity and vice versa. We know from binding assays in membrane homogenates that high-affinity agonist binding reflects the rate constants from a stabilized ternary complex under guanine nucleotide-depleted conditions. A major difference between the membrane binding assays and PET imaging is that living cells, unlike membranes, contain endogenous GTP and allow G-protein activation. By assuming that agonist binds to a high-affinity state in vivo determined solely by pre-coupling to G-proteins, we automatically exclude the impact of G-protein collision, activation and/or dissociation, which can affect both k_{on} and k_{off} in living cells. Competition binding studies in intact cells better reflect the in vivo situation. One such study detected a very small fraction of high-affinity binding at D_2R only when using a certain antagonist ([^{3}H] domperidone) [51]. Two other studies were unable to detect high-affinity binding to D_2R, suggesting that stable ternary complex does not accumulate in live cells [52,53]. In line with this, depletion of endogenous GTP in intact cells expressing μ-opioid receptors decreased the k_{off} of the opioid agonist [^{3}H]DAMGO with no change in the k_{on}, resulting in increased affinity of the agonist [54]. Interestingly, intact cell binding studies of β-adrenergic receptors under non-equilibrium conditions have detected short-term (<1 min) high-affinity agonist binding, however, when the binding assays were performed under equilibrium only low-affinity binding was observed [55–57]. Thus, more sophisticated in vitro methods are needed to determine how coupling and activation of G-proteins affect the affinity of agonist ligands.

It is important to note that practical experience has shown that PET imaging requires radioligands with nanomolar to sub-nanomolar affinity in order to achieve high signal to noise ratio. In membrane homogenates the Ki values of NPA have been reported to be in the range of 0.1–0.4 nM (average 0.2 nM) for the D_2R high-affinity state and 4.6–26 nM (average 15 nM) for the low-affinity state [5,7,53,58,59]. Similar affinity values have been reported for MNPA, while single Kd/Ki values in the range of 0.2– 8.5 nM (average 1.8 nM) have been reported for PHNO, which is more selective for the D_3R receptors in vivo [53,58,60–63]. Given these values, D_2R agonists would be expected to bind exclusively to the high-affinity state in order to achieve a measurable signal under tracer conditions. A recent review of multiple D_2R ligands, reported that their apparent affinity generally was about 10 fold lower in vivo than the in vitro measurements [64]. For example, measurements of the apparent Kd of raclopride in vivo range from 1.6 to 12 nM (average 9.1 nM at baseline) compared to 0.8–2.5 nM (average 1.2 nM) in vitro [64]. However most of the in vivo studies did not correct for factors such as free fraction in brain tissue and concentration of endogenous dopamine, which affect the accuracy of in vivo estimates of apparent affinities [14,64,65]. D_2R radioligands also bind with high affinity to the D_3R, and in particular PHNO has been shown to be D_3-preferring in vivo. Therefore, the contribution of D_3R binding to BP_{ND} differs among the ligands and may interfere with direct comparisons of agonist vs antagonist ligands [63,66]. As the D_3R is expressed at lower density and in more restricted regions (i.e. ventral striatum and globus pallidus) than the D_2R, this confound might be overcome by avoiding D_3R rich regions and/or by development of radioligands with higher D_2R selectivity.

In summary, more studies are needed to establish whether D_2Rs (and other GPCRs) are stably configured in high and low-affinity states in vivo and whether the high-affinity state can be measured with agonist radioligands. Possible approaches include the use of genetically modified mice expressing a D_2R incapable of G-protein coupling. It is possible however, that high and low-affinity agonist binding may be undetectable in vivo, given the dynamic alterations in receptor conformation that are associated with binding and unbinding of both ligand and G-protein [67–71]. Considering that the affinity measure is an average of numerous events per unit time, distinguishing between two populations of high and low-

affinity state receptors is a simplification that perhaps does not capture the subtlety of the actual phenomenon. It is conceivable that imaging approaches to detect GPCR activation in vivo will become available and thereby provide alternative ways to measure the function of GPCRs, G-protein activation and signal transduction. In addition, new approaches using single molecule imaging may ultimately uncover the behavior of receptors at the level of an individual receptor, thereby avoiding the confounds of time and event averaged data.

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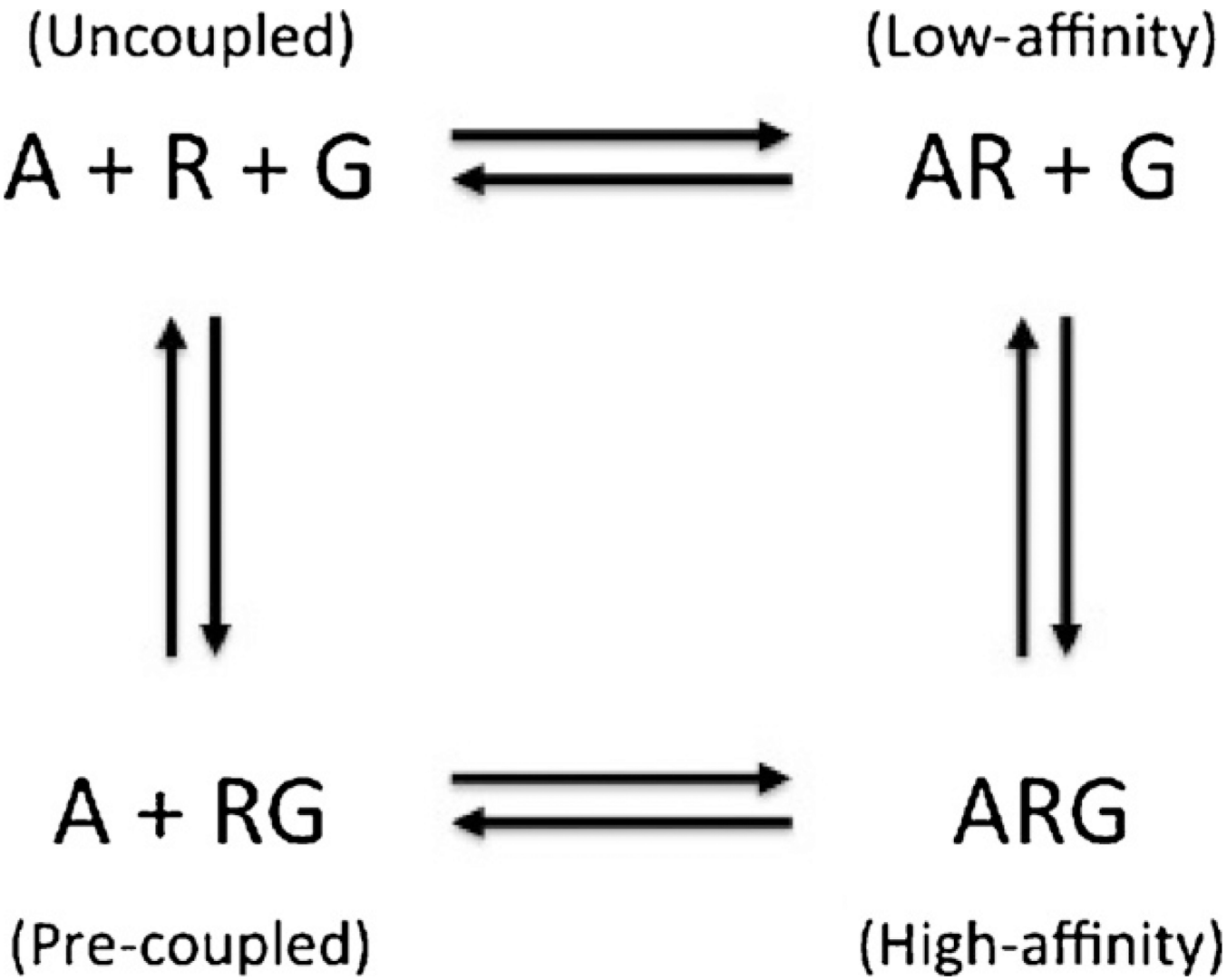

Fig. 1.

The ternary complex model proposes that D_2Rs exist as G-protein coupled and uncoupled, of which the G-protein coupled form reflects a state with high-affinity for agonist binding and the uncoupled form reflects a low affinity state [8].

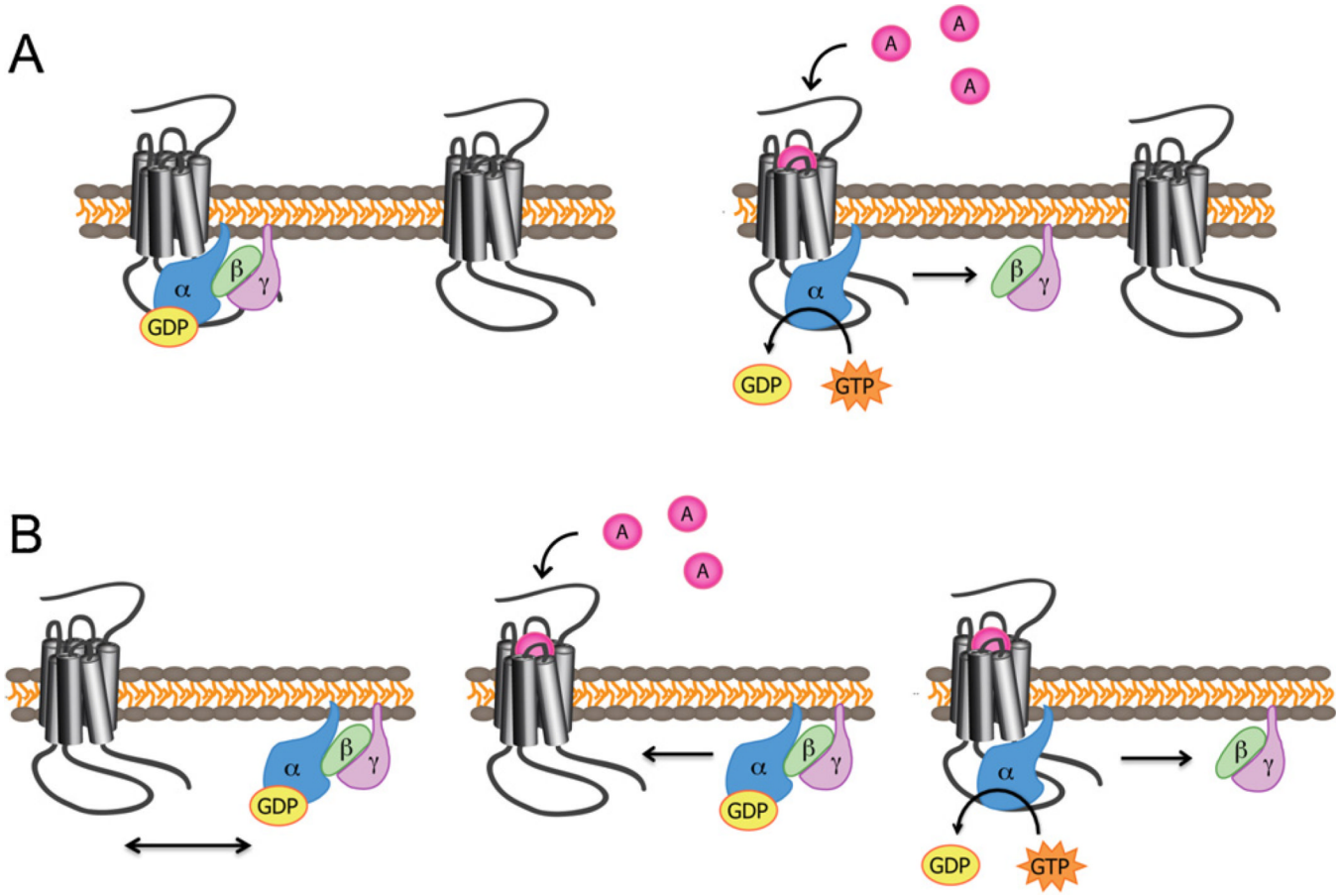

Fig. 2.

(A) The pre-coupled theory proposes that receptors form stable complexes with G-proteins with high-affinity for agonist. (B) The collision theory proposes the receptors and G-proteins diffuse freely within the plasma membrane in the absence of agonist. Upon agonist binding the receptors will couple to and activate the G-proteins [39,40

Table 1

PET studies.

Imaging paradigm	Ref	Anesthesia	Radioligand		Species	Favor D2 high
			Agonist	Antagonist		
Challenge						
Amphetamines	Narendran et al. [13]	Isoflurane	[^{11}C]NPA	[^{11}C]Raclopride	Monkey	Y
	Hwang et al. [19]	Isoflurane	[^{11}C]NPA	[^{11}C]Raclopride	Monkey	Y
	Ginovart et al. [15]	Isoflurane	[^{11}C]PHNO	[^{11}C]Raclopride	Cat	Y
	Seneca et al. [16]	Ketamine/xylazine	[^{11}C]MNPA	[^{11}C]Raclopride	Monkey	Y
	Ohba et al. [17]	Ketamine/xylazine	[^{11}C]MNPA		Monkey	Y
	Ohba et al. [17]	Conscious	[^{11}C]MNPA		Monkey	N
	Skinbjerg et al. [18]	Isoflurane	[^{11}C]MNPA	[^{18}F]Fallypride	Mouse	Y
	Narendran et al. [20]	Conscious	[^{11}C]NPA	[^{11}C]Raclopride	Human	N
(+)-PD 128907	Kortekaas et al. [26]	Isoflurane		[^{11}C]Raclopride	Monkey	N
Apomorphine	Finnema et al. [25]	Ketamine/xylazine	[^{11}C]MNPA	[^{11}C]Raclopride	Monkey	N
Scatchard						
Variable specific activity	Narendran et al. [14]	Isoflurane	[^{11}C]NPA	[^{11}C]Raclopride	Monkey	Y
	Ginovart et al. [15]	Isoflurane	[^{11}C]PHNO	[^{11}C]Raclopride	Cat	N
Animal models of increased D2 high						
Baseline	Skinbjerg et al. [18]	Isoflurane	[^{11}C]MNPA		Mouse	N
Anesthesia effect						Effect of anesthesia on binding
Baseline	Tsukada et al. [21]	Conscious vs ketamine		[^{11}C]Raclopride	Monkey	Decreased
	Hassoun et al. [24]	Conscious vs ketamine		[^{11}C]Raclopride	Cat	No change
	Hassoun et al. [24]	Conscious vs halothane		[^{11}C]Raclopride	Cat	Increase
	Ohba et al. [17]	Conscious vs ketamine/xylazine	[^{11}C]MNPA		Monkey	Increase

Table 2

Ex vivo studies.

Study design	Ref	Anesthesia	Radioligand		Species	Favor D2 high
			Agonist	Antagonist		
Challenge						
Amphetamines	Cumming et al. [37,38]	Conscious	[^{3}H]NPA	[^{11}C]Raclopride	Mouse	Y
	McCormick et al. [31]	Conscious	[^{11}C]PHNO	[^{3}H]Raclopride	Rat	N
	McCormick et al. [33]	Isoflurane	[^{3}H]PHNO [^{11}C]PHNO [^{11}C]NPA	[^{3}H]Raclopride	Rat	Y
	McCormick et al. [33]	Conscious	[^{3}H]PHNO [^{11}C]PHNO [^{11}C]NPA	[^{3}H]Raclopride	Rat	N
Quinagolide	Cumming et al. [37,38]	Conscious	[^{3}H]NPA	[^{11}C]Raclopride	Mouse	Y (N)[a]
NPA	McCormick et al. [31]	Conscious	[^{11}C]PHNO	[^{3}H]Raclopride	Rat	N
Aripiprazole	McCormick et al. [31]	Conscious	[^{11}C]PHNO	[^{3}H]Raclopride	Rat	N
NPA	Seeman [36]	Conscious	[^{3}H]PHNO	[^{3}H]Raclopride	Rat	Y
Quinpirole	Peng et al. [32]	Conscious	[^{3}H]PHNO [^{3}H]MNPA	[^{3}H]Raclopride	Rat	N
Aripiprazole	Peng et al. [32]	Conscious	[^{3}H]PHNO [^{3}H]MNPA	[^{3}H]Raclopride	Rat	N
Animal models of increased D2 high						
NPA challenge	Seeman [36]	Conscious	[^{3}H]PHNO		Rat	Y
Amphetamine challenge (3 rat models)	McCormick et al. [30]	Conscious	[^{11}C]PHNO	[^{3}H]Raclopride	Rat	N
Anesthesia effect						Effect of anesthesia on binding
Baseline	McCormick et al. [33]	Conscious vs isoflurane	[^{3}H]PHNO [^{11}C]PHNO [^{11}C]NPA		Rat	Increase
	McCormick et al. [33]	Conscious vs isoflurane		[^{3}H]Raclopride	Rat	Unchanged

[a]Baseline was defined as the dopamine depleted condition. When compared to the saline treated group, quinagolide displaced both ligands to the same extent (Cumming, et al. [37,38]; Finnema et al. [25]).