REVIEW

G-Protein-Coupled Receptor Heteromers as Key Players in the Molecular Architecture of the Central Nervous System

Marc Brugarolas,^{1,2} Gemma Navarro,^{1,2} Eva Martínez-Pinilla,³ Edgar Angelats,¹ Vicent Casadó,^{1,2} José L. Lanciego^{2,3} & Rafael Franco^{1,3}

¹ Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona (UB), Barcelona, Spain

² Centro investigación biomédica en red enfermedades neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain

³ Department of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain

Keywords

Adenosine receptor; Cannabinoid receptor; Dopamine receptor; Glutamate receptor; GPCR heteromer; Heteromer.

Correspondence

Rafael Franco, Department of Biochemistry and Molecular Biology, Biology School, University of Barcelona, Diagonal 645, 08028 Barcelona, Spain.

Tel.: +34 934021208;

Fax: +34 934021215;

E-mail: rfranco@ub.edu

Received 14 February 2014; revision 2 April 2014; accepted 2 April 2014

Present Address

Marc Brugarolas, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology of Lausanne (EPFL), Lausanne, Switzerland

SUMMARY

The overall architecture of the nervous system, especially the CNS, is remarkable. The anatomy of the nervous system is constituted not only by macroscopic and microscopy identifiable regions and neuronal cell types, but also by protein complexes whose identification and localization require sophisticated techniques. G-protein-coupled receptors (GPCRs) constitute an example of proteins that are the key factors in the framework needed to sustain brain and nerve structure and function. The versatility underlying nervous system anatomy takes advantage of a recently discovered feature of GPCRs, the possibility to form heteromers that, placed at specific neuronal subsets and at specific locations (pre-, post-, or peri-synaptic), contribute to attain unique neural functions.

doi: 10.1111/cns.12277

Introduction

G-protein-coupled receptors (GPCR) constitute a very populated protein family and the most versatile group of cell surface proteins involved in signal transduction. In humans, more than 1% of the genome codes for about 500 of these type of proteins, 90% of which are expressed in the central nervous system (CNS) [1,2]. GPCRs have seven transmembrane domains where the N-terminus is extracellular, whereas the C-terminus is facing the cytosolic side. GPCRs can be activated by a variety of endogenously active substances (first messenger and/or neurotransmitter) including, but not limited to, amino acids, peptides, proteins, lipids, and purine nucleosides and nucleotides. The perception of environmental stimuli (light, taste, and smell) is also mediated by GPCRs. Activation of the receptors leads to signal transduction mediated by four

main heterotrimeric G-protein subclasses (G_s , G_i , G_q , and $G_{12/13}$) and results in the regulation of all kinds of cellular events. Receptor engagement leads to changes in the levels of intracellular second messengers (mainly cAMP and Ca^{2+}) and, often, involves Ser/Thr protein kinases and phosphatases [3].

Although it was for long suspected that GPCRs could form homodimers, heterodimers, or higher-order oligomers, technical problems prevented demonstration of such oligomerization until the late 90s. In fact, an ever increasing number of reports using a variety of techniques have shown that GPCRs do not usually behave as independent functional units but forming oligomers between themselves (homomers) or with other receptors (heteromers) [1,4–14]. Whereas homomers do not introduce new physiological concepts or challenges, heteromerization results in novel functional units and not a mere addition of the signals of the

receptors composing the heteromer. Roles for GPCR heteromers have been proposed starting with the quality control of receptor folding and cell surface targeting of newly synthesized receptors [15–17]. Heteromers also underlie pharmacological diversity as the ligand binding to one receptor may influence the binding of a hormone/neurotransmitter to the second receptor; the affinity of the second binding event may be higher or lower [18,19]. From a pharmacological point of view, the signaling properties of a given ligand may also affect the selectivity of interaction between the GPCR and its G-protein resulting in various possibilities for G-protein coupling [20]. Internalization is another event that may be modified by heterodimerization [21,22].

Different GPCR Heteromers in Different Neural and Neuronal Locations

One of the most expressed GPCRs in the CNS is the cannabinoid receptor 1 (CB₁ receptor). CB₁ receptor has been extensively studied, and from the very beginning, it was considered a presynaptic receptor [23,24]. The physiological substrate is unique as the endocannabinoid-producing enzymes are located in the postsynaptic neuron, whereas endocannabinoids act on presynaptic CB₁ receptors [25]. Recent data show, however, that exceptions may occur. On the one hand, a postsynaptic location for the second cannabinoid receptor subtype (CB₂) has been reported in hippocampus [26]. Concerning this second cannabinoid receptor, it is interesting to note that until pretty recent work in a few laboratories [27,28], it was thought that the CB₂ receptor was not expressed in neurons. On the other hand, Dr. Lanciego's laboratory has just identified postsynaptic CB₁ receptors forming heteromers with CB₂ receptors in neurons of the primate *globus pallidus* (data submitted). Following identification of postsynaptic CB₁–CB₂ receptor heteromers, one may wonder why these receptors are in a small subset of neurons in such location. It is anticipated that CB₁–CB₂ receptor heteromers are performing there a specific task that neither CB₁ nor CB₂ receptors could do. More importantly, the neuronal mechanisms involved in placing CB₁ and CB₂ receptors and CB₁–CB₂ receptor heteromers pre- or postsynaptically may be instrumental to know the intricacies that underlie the anatomy of the nervous system at the molecular level.

Adenosine is a regulatory molecule present in every cell and tissue. In the course of evolution, it was probably one of the first hormones and of the first neuromodulators. Its role in the nervous system is very important and is mediated by the three adenosine receptors that are present in the CNS (A₁, A_{2A}, and A_{2B}); the fourth receptor does not seem to be highly expressed in neurons. The adenosine A_{2A} receptor is found in a variety of locations (central and peripheral), but it is heavily expressed in the striatum [29]. Whereas the A₁ receptor when expressed in neurons was considered to be presynaptic, A_{2A} has been mainly considered a postsynaptic receptor. It has been demonstrated that A₁ may regulate neurotransmitter release, whereas it has been also shown that the A_{2A} receptor may regulate the action of a given neurotransmitter in the postsynaptic neuron. However, it turns out that both A₁ and A_{2A} may be pre- or postsynaptic depending on the neural region and neuronal subtype. In the case of the A_{2A} receptor, there is a careful immunohistochemical study by Rosin et al. [29],

which shows that—in the rat—“at least some of these central adenosine A_{2A} receptors are found presynaptically.” This finding was further substantiated at the ultrastructural level [30]. What determines the specific location of an adenosine receptor in a specific neuron and for what reason? The answer, which is to achieve a differential effect by a given neuromodulator, requires further players that seem to be required for a correct architecture within the final—and complex—nervous system anatomical scenario. It then seems that neurons place the A₁ receptor presynaptically and the A_{2A} receptor postsynaptically by default, but that there are neuronal mechanisms that may interfere with the default program to attain certain goals. As detailed below, the two receptors are brought together to the nerve terminal to build up a very unique module of neurotransmitter release control.

Differential GPCR Distribution in the Cortico-Striatal Pathways

GPCR heterocomplexes play a crucial role in the integration of information in the striatum, where medium spiny neurons (MSNs) constitute the 95% of the population. These neurons are inhibitory as they use GABA as neurotransmitter. The two main inputs are as follows: glutamatergic afferents from cortical neurons and thalamus and dopaminergic afferents from *substantia nigra pars compacta* (SNc) [31].

MSNs may be divided into two subtypes depending on the region to which they project and according to the expression of peptide and neurotransmitter receptors. Direct pathway MSNs express dynorphin, substance P, dopamine D₁ receptors (coupled to G_s), and adenosine A₁ receptor (coupled to G_i). G_s leads to increases in cAMP levels, whereas G_i leads to the opposite. These striatonigral neurons project directly to the *globus pallidus (pars interna)* and to the *substantia nigra*. Additionally, cortical glutamatergic boutons arriving to striatonigral MSNs express adenosine A_{2A} receptors presynaptically forming, at least in part, heteromers with adenosine A₁ receptors (see section Heteromers located presynaptically). Stimulation of the direct pathway results in motor activation. Indirect pathway MSNs express enkephalin, dopamine G_i-coupled D₂ receptors, and G_s-coupled adenosine A_{2A} receptors; they are also known as striatopallidal MSNs and project to *globus pallidus (pars externa)*. Stimulation of indirect pathway MSNs results in motor inhibition [32–36]. A balance between the direct and the indirect pathway is required for proper motor control; GPCRs and GPCR heteromers directly participate in achieving this balance.

Neurodegenerative Diseases Impacting on Basal Ganglia Function

A main input of basal ganglia in terms of motor control is the *globus pallidus* where direct and indirect pathways converge. These GABAergic inhibitory neurons project to the thalamus which projects back to the cortex via glutamatergic efferents. An adequate equilibrium between both pathways produces normal/controlled movement. Dopamine, which is mainly produced by neurons from SNc, is a key regulator for correct functioning of basal ganglia; it induces motor activation via activation of dopamine D₁

receptor in the direct pathway and inhibition of neurotransmission via activation of dopamine D₂ receptors in the indirect pathway. Parkinson's disease patients, whose nigral neurons degenerate, experience movement depression or hypokinesia due to depletion of nigral dopamine. On the other hand, in Huntington's disease patients, hyperkinetic chorea movements are due to a gradual depression of the indirect inhibitory pathway [37].

Nowadays, the basal ganglia circuitry is being revisited to consider it not as a simple anatomical substrate where the connectivity and functional interactions occur unidirectionally, but as a complex structure with microcircuits sustained and reinforced by reciprocal innervations [38]. In those circuits GPCR and GPCR heteromers play a crucial role; it should be noted that two adjacent but morphologically indistinguishable neurons might respond differently to dopamine as each of them may contain a different dopamine-containing heteromer (A₁–D₁ in the direct or A_{2A}–D₂ in the indirect pathway, shown in Figure 1). It has been recently identified the occurrence of heteromers in models of Parkinson's disease [39], meaning that current therapies for the disease (L-DOPA mainly) are targeting dopamine receptor-containing heteromers. This fact opens new concepts in the way pharmaceutical companies select targets to combat neurological/neurodegenerative/neuropsychiatric diseases. Interestingly, L-DOPA treatment leads to disruption of A_{2A}–D₂ receptor heteromers and this correlates with dyskinesia [39,40]. Whether disruption of heteromers may be in full or in part cause of dyskinesias merits further experimental effort.

Heteromers Located Presynaptically

To our knowledge, O'Kane and Stone [41] were among the first to report a functional interaction—in hippocampus *in vitro*—between adenosine A₁ and A_{2A} receptors. The interaction was deciphered using synthetic compounds due to the fact that the natural agonist for the two receptors is adenosine. In other words, it is strange that two receptors for the same agonist are establish-

ing a kind of competition. Even more strange is to find cells expressing two receptors, one coupled to G_s and another to G_i, for the same hormone/neurotransmitter. Expression of two different receptors for the same neurotransmitter is not exclusive to adenosine but occur for a variety of neurotransmitters (a detailed description is out of the scope of the present article).

As it was not expected that two receptors for adenosine were physically interacting, the A₁–A_{2A} receptor heteromer was first assayed as a negative control. The results were, however, contrary to the expectations, and therefore, the A₁ and A_{2A} receptor heteromer was detected—in transfected cells—by Ciruela et al. [42]. The existence of an intramembrane (inter-receptor) interaction in the A₁–A_{2A} receptor heteromer results in various actions that require the heteromer and cannot be obtained by individual receptors. For instance, stimulation of adenosine A_{2A} receptor decreases adenosine A₁ receptor affinity for agonist binding. But this would not explain why the two receptors (and the A₁–A_{2A} receptor heteromer) are expressed in glutamatergic terminals coming from the cortex to the striatum. The explanation for this conundrum lies in the fact that these specific heteromers behave as sensors of the adenosine concentration and make the neuron respond accordingly (Figure 2). On the one hand, weak extracellular adenosine concentrations preferentially stimulate adenosine A₁ receptors, which would inhibit glutamatergic transmission. On the other hand, under a strong adenosine release to the extracellular space, adenosine A_{2A} receptor is activated and this activation blocks adenosine A₁ receptor-mediated effects and results in potentiation of glutamate release [42,43]. Overall this GPCR heteromer system works as a switch that produces opposite outcomes depending on the adenosine concentration.

In the striatal MSNs, cannabinoid CB₁ receptors may participate in different heteromeric configurations and their activation leads to inhibition of glutamate release by blocking the calcium channels that participate in neurotransmitter release. At this presynaptic level, cannabinoid CB₁ receptors in the direct pathway may heteromerize with adenosine A_{2A} receptors to give rise to A_{2A}–CB₁

Figure 1 Differential receptor distribution in striatal synapses. Reported localization of different receptor homodimers and heteromers and their inter-receptor interactions, negative modulation in red lines, and positive modulation in blue arrows. Receptor oligomers localized presynaptically control glutamate release inhibiting (in red) or enhancing it (in blue). Postsynaptic oligomers modulate adenylate cyclase inhibiting cAMP production (in red) or enhancing it (in blue). Further studies are required to elucidate the localization of other receptor homodimers or heteromers and their specific signaling pathways.

Figure 2 Adenosine concentration switching device in glutamatergic nerve terminals expressing A_1 – A_{2A} receptor heteromers. Concerning regulation of glutamate release, completely opposite outputs result at low or high adenosine concentrations. At low concentrations, the A_1 receptors become activated and G_i -mediated signalling occurs. At high concentrations, the A_{2A} receptors become activated and both A_1 receptors lose affinity for adenosine and G_s -mediated signalling occurs. The mechanism underlying this phenomenon, that is, G_i -blockade when the A_{2A} receptor is activated is not known, but it is originated within the receptor heteromer and likely impacts on the coupling to G_s and G_i proteins.

receptor heteromers (Figure 1) [44,45]. It is not known whether adenosine A_{2A} receptor-mediated inhibition of cannabinoids modulation of synaptic events [46] is dependent on a specific action of the heteromer or on a cross talk occurring at the level of intracellular signalling cascades. It should be noted that in neuroblastoma cells endogenously expressing CB_1 receptor and A_{2A} receptors (and therefore A_{2A} – CB_1 receptor heteromers), CB_1 receptor signalling via G_i is dependent on A_{2A} receptor activation [47].

Heteromers Located Postsynaptically

Postsynaptic A_{2A} receptor is found in enkephalin MSNs of the indirect pathway. It is mainly found perisynaptically and adjacent to dopaminergic synapses [48]. Three different populations of heteromers may be found: the A_{2A} – A_{2A} receptor homodimer, the A_{2A} – D_2 receptor heteromer [49,50], the A_{2A} – D_2 –mGlu5 receptor heterotrimer [51] and the A_{2A} – CB_1 – D_2 receptor heterotrimer [52,53] (Figure 1).

The antagonistic interactions between A_{2A} receptors and D_2 receptors have been demonstrated at biochemical, functional, and behavioral levels. The first indication about an antagonist relationship was obtained from behavioral analysis of Parkinson's disease animal models [54]. More information to understand the consequences of the interaction of A_{2A} and D_2 receptors was obtained in membrane preparations from rat striatum, where stimulation of A_{2A} receptors produced a decrease in the affinity of D_2 receptors for agonists due to conformational modification in the D_2 receptor-binding site [55]. After this and other more recent

studies, the use of A_{2A} receptor antagonists in Parkinson's disease treatment was proposed. Nowadays, the A_{2A} receptor antagonist KW-6002, which preferentially blocks A_{2A} receptor-forming heteromers with D_2 receptors [56], has been approved in Japan for Parkinson's disease treatment [57,58]. As indicated above, L-DOPA is targeting D_2 -containing receptor heteromers, whereas KW-6002 is targeting A_{2A} -containing receptor heteromers.

Heterotrimers formed by A_{2A} , D_2 , and mGlu5 receptors are also present in striatum. A_{2A} receptors co-immunoprecipitate with mGlu5 receptors in co-transfected cells and they colocalize in striatal tissue [59]. Radioligand binding assays in rat striatum membranes showed that stimulation of mGlu5 receptors also produced a decrease in the affinity that D_2 receptors display for agonists. Moreover, when A_{2A} receptors and mGlu5 receptors are costimulated, the inhibitory effect on D_2 receptors is stronger than the reductions induced by stimulation of either receptor alone [60]; due to trimer formation (or otherwise to cross talk at the intracellular level), these data suggest tight inter-receptor interactions. Upon the D_2 receptor-mediated motor control, mGlu5 receptor agonists and antagonists produced similar effects as, respectively, A_{2A} receptor agonists and antagonists. A selective mGlu5 receptor agonist may preferentially inhibit motor activation induced by D_2 receptor agonists [60], whereas mGlu5 receptor antagonists may reduce the effects of D_2 receptor antagonists [61]. Furthermore, A_{2A} and mGlu5 receptor agonists and antagonists can result in synergistic effects at the behavioral level [59,60,62]. A_{2A} – D_2 –mGlu5 receptor interactions provide the rationale for the possible application of mGlu5 receptor antagonists or combined A_{2A} and mGlu5 receptor antagonists in Parkinson's disease treatment [62–64].

Tebano et al. [65] described that A_{2A} receptor activation potentiates the synaptic effects of the CB_1 receptor. Furthermore, CB_1 receptor-induced depression of synaptic transmission may be prevented by inactivation of A_{2A} receptors [66,67]. A postsynaptic mechanism would depend on the interaction between A_{2A} receptors and CB_1 receptors in the encephalergic MSNs and probably also on the interaction with dopamine receptors as some of the effects of A_{2A} – CB_1 interactions depend on D_2 receptor function [68]. A_{2A} – CB_1 – D_2 receptor heterotrimers have been detected in HEK cells [53], rat striatum [40], and primate caudate nucleus [39]. Thus, it seems that distinct CB_1 receptor-containing heteromers differentially located at pre- or postsynaptic membranes can account for the described diverse relationship between adenosine and cannabinoid receptors.

Relevance of Heteromers in CNS Disorders

Evidence in recent years points to heteromers as therapeutic targets in neurological or neuropsychiatric disorders. A heteromer deeply characterized in the CNS is the $G_{q/11}$ -coupled dopamine D_1 – D_2 receptor heteromer, which has been identified in rat striatal neurons. Whereas individual receptors, D_1 and D_2 , couple to, respectively, G_s and G_i and modify cAMP levels, the heteromer switches coupling to $G_{q/11}$, calcium mobilization and a cascade of events that may include accelerated neuronal growth and increased expression of BDNF. It has been proposed that this specific dopaminergic signaling may have an important role in drug addiction, Parkinson's disease, and schizophrenia [69,70].

Table 1 Examples of receptor heteromers, their potential effects, and diseases for which they may become therapeutic targets

Heteromer	Effect	Disease
CB ₁ -CB ₂	Stress, pain, memory, learning, reproductive function, development	Mental illness, cancer, metabolic disorders
A ₁ -A _{2A} [41–43]	Sleep, arousal, cognition, learning, motor activity	Neurodegenerative diseases: Alzheimer's, Parkinson's
A _{2A} -D ₂ [50,54–58]	Motor activation, memory	Huntington's disease, dyskinesia, Parkinson's
A _{2A} -CB ₁ [44–47,65–67]	Psychomotor activation	Neuropsychiatric disorders, drug abuse
D ₁ -D ₂ [69,70]	Motor activation, neuronal growth	Parkinson's, schizophrenia, drug addiction
5HT _{2A} -D ₂ [71,72]	Cognitive control, weight gain	Drug abuse, psychotic drugs, schizophrenia
5HT _{2A} -mGlu2[73–76]	Impulsive behavior, stress	Psychiatric disorders: schizophrenia, depression
A _{2A} -CB ₁ -D ₂ [39,40,53,68]	Motor activation	Parkinson's, drug abuse, schizophrenia
A _{2A} -D ₂ -mGlu5[59–64]	Motor activation	Parkinson's

Heteromers containing the serotonin receptor 5-HT_{2A} seem to play a key role in the psychotic actions of the hallucinogenic actions of the diethylamide of the lysergic acid (LSD) and of 2,5-dimethoxy-4-iodoamphetamine (DOI), which behave as 5-HT_{2A} agonists. In fact, oligomerization of serotonin 5-HT_{2A} and dopamine D₂ receptors leads to cross talk by which 5-HT_{2A} receptor-mediated phospholipase C (PLC) activation is synergistically enhanced by the concomitant activation of the D₂ receptor. Moreover, the D₂ receptor-mediated adenylate cyclase inhibition is decreased by the costimulation with 5-HT_{2A} receptor agonists. This allosteric cross-modulation provides a new framework to understand the psychotic actions of the hallucinogens acting via 5-HT_{2A} receptors [71,72]. Interestingly, another player has entered into scene to explain the actions of these drugs. Some of the neuropsychological responses induced by hallucinogens seem to involve another heteromer formed by 5-HT_{2A} and mGlu2 receptors. By competition binding studies in mouse sensory cortex membranes, it has been shown that the binding affinity of hallucinogenic 5-HT_{2A} receptor agonists is increased by an mGlu2/3 receptor agonist, while DOI decreases the binding affinity of mGlu2/3 agonists. Again, there is an allosteric cross-modulation that supports the view of multiple signaling possibilities due to the occurrence of multiple 5-HT_{2A} receptor-containing heteromers. It should be noted that complementary data obtained in different model systems and in mGlu2 and 5-HT_{2A} knockout mice prove that antipsychotic effects of mGlu2 activation or 5-HT_{2A} inhibition require the presence of the two receptors and thus suggest a key role of this heteromer in higher cerebral functions [73–76].

Conclusions

GPCRs are the key factors in the molecular architecture of the CNS. The stoichiometry of GPCR complexes, which is still under study in *in vitro* cell models, and the exact composition of the pre- or postsynaptic complexes, that is, whether they contain 1, 2, 3... different receptors, are important details in the anatomical ultra-

structure of the CNS. Examples of heteromers relevant for CNS function are indicated in Table 1.

The exact pre- or postlocalization of receptors in the neuron is fundamental to obtain a very precise action. At first, a given GPCR in the CNS had just a given effect, proximally modifying the level of second messengers, and overall regulating either neurotransmission (if postsynaptic) or neurotransmitter release (if presynaptic). The picture now is that a given GPCR may be both pre- and postsynaptic and play very different roles depending on its localization and on the exact structure of the GPCR complex in which it participates.

GPCR heteromers are formed by two or more receptors and their action is unique (and not a mere addition of the action of individual receptors within the heteromer). This explains, for instance, why two opposite-coupled GPCRs, one leading to activation and the other leading to inhibition of adenylate cyclase, may be found in the same cell. The heteromer can achieve something that two individual receptors cannot achieve (the above-described A₁-A_{2A} receptor heteromer, which is postsynaptically expressed in glutamatergic neurons arriving to the striatum, is a paradigmatic example).

The CNS uses heteromers to achieve different outcomes for a given neurotransmitter input. In fact, individual receptors being activated by a given neurotransmitter can only provide one response. In contrast, the occurrence of heteromers implies that a given neurotransmitter may lead to differential signaling depending on the heteromer context of the targeted receptor. Indeed, the heteromer context may vary from cell to cell and from place to place within a given neuron.

For all the above reasons, a final conclusion would be that dynamics of GPCR and of GPCR heteromers expressed in different regions and/or trafficking to different neuronal localizations are fundamental for neural transmission and plasticity.

Conflict of Interest

The authors declare no conflict of interest.

References

- George SR, O'Dowd BF, Lee SP. G-protein-coupled receptor oligomerization and its potential for drug discovery. *Nat Rev Drug Discov* 2002;1:808–820.
- Gudermann T, Schöneberg T, Schultz G. Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu Rev Neurosci* 1997;20:399–427.
- Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: Emerging paradigms. *Trends Pharmacol Sci* 2001;22:368–376.
- Agnati LF, Guidolin D, Vilardaga JP, Ciruela F, Fuxe K. On the expanding terminology in the GPCR field: The meaning of receptor mosaics and receptor heteromers. *J Recept Signal Transduct Res* 2010;30:287–303.
- Agnati LF, Ferré S, Lluis C, Franco R, Fuxe K. Molecular mechanisms and therapeutic implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the

- striatopallidal GABA neurons. *Pharmacol Rev* 2003;**55**:509–550.
6. Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. *Nat Rev Neurosci* 2001;**2**:274–286.
 7. Carriba P, Navarro G, Ciruela F, et al. Detection of heteromerization of more than two proteins by sequential BRET-FRET. *Nat Methods* 2008;**5**:727–733.
 8. Devi LA. Heterodimerization of G-protein-coupled receptors: Pharmacology, signaling and trafficking. *Trends Pharmacol Sci* 2001;**22**:532–537.
 9. Ferré S, Franco R. Oligomerization of G protein-coupled receptors: a reality. *Curr Opin Pharmacol* 2010;**10**:1–5.
 10. Ferré S, Navarro G, Casadó V, et al. G protein-coupled receptor heteromers as new targets for drug development. *Prog Mol Biol Transl Sci* 2010;**91**:41–52.
 11. Ferré S, Baler R, Bouvier M, et al. Building a new conceptual framework for receptor heteromers. *Nat Chem Biol* 2009;**5**:131–134.
 12. Franco R, Canals M, Marcellino D, et al. Regulation of heptaspanning-membrane-receptor function by dimerization and clustering. *Trends Biochem Sci* 2003;**28**:238–243.
 13. Prinster SC, Hague C, Hall RA. Heterodimerization of G protein-coupled receptors: Specificity and functional significance. *Pharmacol Rev* 2005;**57**:289–298.
 14. Tadagaki K, Jockers R, Kamal M. History and biological significance of GPCR heteromerization in the neuroendocrine system. *Neuroendocrinology* 2012;**95**:223–231.
 15. Breit A, Lagacé M, Bouvier M. Hetero-oligomerization between beta2- and beta3-adrenergic receptors generates a beta-adrenergic signaling unit with distinct functional properties. *J Biol Chem* 2004;**279**:28756–28765.
 16. Bulenger S, Marullo S, Bouvier M. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci* 2005;**26**:131–137.
 17. Law P-Y, Erickson-Herbrandson LJ, Zha QQ, et al. Heterodimerization of mu- and delta-opioid receptors occurs at the cell surface only and requires receptor-G protein interactions. *J Biol Chem* 2005;**280**:11152–11164.
 18. Ferré S, Ciruela F, Woods AS, Lluís C, Franco R. Functional relevance of neurotransmitter receptor heteromers in the central nervous system. *Trends Neurosci* 2007;**30**:440–446.
 19. Franco R, Casadó V, Cortés A, et al. Novel pharmacological targets based on receptor heteromers. *Brain Res Rev* 2008;**58**:475–482.
 20. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. *EMBO Rep* 2004;**5**:30–34.
 21. Ellis C. The State of GPCR Research in 2004. *Nat Rev Drug Discov* 2004;**3**:577–626.
 22. Terrillon S, Bouvier M. Receptor activity-independent recruitment of betaarrestin2 reveals specific signalling modes. *EMBO J* 2004;**23**:3950–3961.
 23. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 2003;**83**:1017–1066.
 24. Mackie K. Cannabinoid receptors: Where they are and what they do. *J Neuroendocrinol* 2008;**20**(Suppl 1):10–14.
 25. Micale V, Di Marzo V, Sulcova A, Wotjak CT, Drago F. Endocannabinoid system and mood disorders: Priming a target for new therapies. *Pharmacol Ther* 2013;**138**:18–37.
 26. Brusco A, Tagliaferro P, Saez T, Onaivi ES. Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse* 2008;**62**:944–949.
 27. Gong J-P, Onaivi ES, Ishiguro H, et al. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain Res* 2006;**1071**:10–23.
 28. Lanciego JL, Barroso-Chinea P, Rico AJ, et al. Expression of the mRNA coding the cannabinoid receptor 2 in the pallidal complex of Macaca fascicularis. *J Psychopharmacol* 2011;**25**:97–104.
 29. Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. *J Comp Neurol* 1998;**401**:163–186.
 30. Hettinger BD, Lee A, Linden J, Rosin DL. Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. *J Comp Neurol* 2001;**431**:331–346.
 31. Kreitzer AC. Physiology and pharmacology of striatal neurons. *Annu Rev Neurosci* 2009;**32**:127–147.
 32. Schiffmann SN, Vanderhaeghen JJ. Does the absence of clinical expression of choreoathetosis, despite severe striatal atrophy, correlate with plasticity of neuropeptide synthesis? *J Neural Transm Suppl* 1991;**33**:99–103.
 33. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S. Adenosine A2A receptors and basal ganglia physiology. *Prog Neurobiol* 2007;**83**:277–292.
 34. Schiffmann SN, Dassel D, d'Alcantara P, Ledent C, Swillens S, Zoli M. A2A receptor and striatal cellular functions: Regulation of gene expression, currents, and synaptic transmission. *Neurology* 2003;**11**(Suppl 6):S24–S29.
 35. Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. *Prog Neurobiol* 1999;**59**:355–396.
 36. Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. *Nat Rev Neurosci* 2006;**7**:464–476.
 37. Glass M, Dragunow M, Faull RL. The pattern of neurodegeneration in Huntington's disease: A comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* 2000;**97**:505–519.
 38. Obeso JA, Lanciego JL. Past, present, and future of the pathophysiological model of the Basal Ganglia. *Front Neuroanat* 2011;**5**:39.
 39. Bonaventura J, Rico AJ, Moreno E, et al. L-DOPA-treatment in primates disrupts the expression of A2A adenosine-CB1 cannabinoid-D2 dopamine receptor heteromers in the caudate nucleus. *Neuropharmacology* 2013;**79C**:90–100.
 40. Pinna A, Bonaventura J, Farré D, et al. L-DOPA disrupts adenosine A2A-cannabinoid CB1-dopamine D2 receptor heteromer cross-talk in the striatum of hemiparkinsonian rats: Biochemical and behavioral studies. *Exp Neurol* 2014;**253**:180–191.
 41. O'Kane EM, Stone TW. Interaction between adenosine A1 and A2 receptor-mediated responses in the rat hippocampus in vitro. *Eur J Pharmacol* 1998;**362**:17–25.
 42. Ciruela F, Casadó V, Rodrigues RJ, et al. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. *J Neurosci* 2006;**26**:2080–2087.
 43. Cristóvão-Ferreira S, Navarro G, Brugarolas M, et al. A1R-A2AR heteromers coupled to Gs and Gi/o proteins modulate GABA transport into astrocytes. *Purinergic Signal* 2013;**9**:433–449.
 44. Ferré S, Lluís C, Justinova Z, et al. Adenosine-cannabinoid receptor interactions. Implications for striatal function. *Br J Pharmacol* 2010;**160**:443–453.
 45. Tebano MT, Martire A, Popoli P. Adenosine A (2A)-cannabinoid CB(1) receptor interaction: An integrative mechanism in striatal glutamatergic neurotransmission. *Brain Res* 2012;**1476**:108–118.
 46. Martire A, Tebano MT, Chiodi V, et al. Pre-synaptic adenosine A2A receptors control cannabinoid CB1 receptor-mediated inhibition of striatal glutamatergic neurotransmission. *J Neurochem* 2011;**116**:273–280.
 47. Carriba P, Ortiz O, Patkar K, et al. Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. *Neuropharmacology* 2007;**52**:2249–2259.
 48. Ferré S, Agnati LF, Ciruela F, et al. Neurotransmitter receptor heteromers and their integrative role in "local modules": The striatal spine module. *Brain Res Rev* 2007;**55**:59–67.
 49. Ciruela F, Ferré S, Casadó V, et al. Heterodimeric adenosine receptors: A device to regulate neurotransmitter release. *Cell Mol Life Sci* 2006;**63**:2427–2431.
 50. Hillion J, Canals M, Torvinen M, et al. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. *J Biol Chem* 2002;**277**:18091–18097.
 51. Cabello N, Gandía J, Bertarelli DCG, et al. Metabotropic glutamate type 5, dopamine D2 and adenosine A2a receptors form higher-order oligomers in living cells. *J Neurochem* 2009;**109**:1497–1507.
 52. Navarro G, Ferré S, Cordomi A, et al. Interactions between Intracellular Domains as Key Determinants of the Quaternary Structure and Function of Receptor Heteromers. *J Biol Chem* 2010;**285**:27346–27359.
 53. Navarro G, Carriba P, Gandía J, et al. Detection of heteromers formed by cannabinoid CB1, dopamine D2, and adenosine A2A G-protein-coupled receptors by combining bimolecular fluorescence complementation and bioluminescence energy transfer. *ScientificWorldJournal* 2008;**8**:1088–1097.
 54. Fuxe K, Goldstein M, Hökfelt T, Jonsson G, Lidbrink P. Dopaminergic involvement in hypothalamic function: Extrahypothalamic and hypothalamic control. A neuroanatomical analysis. *Adv Neurol* 1974;**5**:405–419.
 55. Ferré S, von Euler G, Johansson B, Fredholm BB, Fuxe K. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proc Natl Acad Sci U S A* 1991;**88**:7238–7241.
 56. Orru M, Bakesová J, Brugarolas M, et al. Striatal pre- and postsynaptic profile of adenosine A2A receptor antagonists. *PLoS ONE* 2011;**6**:e16088.
 57. Chen W, Wang H, Wei H, Gu S, Wei H. Istradefylline, an adenosine A2A receptor antagonist, for patients with Parkinson's Disease: A meta-analysis. *J Neurol Sci* 2013;**324**:21–28.
 58. Park A, Stacy M. Istradefylline for the treatment of Parkinson's disease. *Expert Opin Pharmacother* 2012;**13**:111–114.
 59. Ferré S, Karcz-Kubicha M, Hope BT, et al. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: Implications for striatal neuronal function. *Proc Natl Acad Sci U S A* 2002;**99**:11940–11945.
 60. Popoli P, Pezzola A, Torvinen M, et al. The selective mGlu(5) receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: Interactions with adenosine A(2a) receptors. *Neuropsychopharmacology* 2001;**25**:505–513.
 61. Ossowska K, Konieczny J, Wollarth S, Wieroniska J, Pilc A. Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. *Neuropharmacology* 2001;**41**:413–420.
 62. Kachroo A, Orlando LR, Grandy DK, Chen J-F, Young AB, Schwarzschild MA. Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. *J Neurosci* 2005;**25**:10414–10419.
 63. Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci* 1997;**20**:482–487.
 64. Jenner P. Istradefylline, a novel adenosine A2A receptor antagonist, for the treatment of Parkinson's disease. *Expert Opin Investig Drugs* 2005;**14**:729–738.
 65. Tebano MT, Martire A, Chiodi V, et al. Adenosine A2A receptors enable the synaptic effects of cannabinoid CB1

- receptors in the rodent striatum. *J Neurochem* 2009;**110**:1921–1930.
66. Soria G, Castañé A, Berrendero F, et al. Adenosine A2A receptors are involved in physical dependence and place conditioning induced by THC. *Eur J Neurosci* 2004;**20**:2203–2213.
 67. Yao L, McFarland K, Fan P, Jiang Z, Ueda T, Diamond I. Adenosine A2a blockade prevents synergy between mu-opiate and cannabinoid CB1 receptors and eliminates heroin-seeking behavior in addicted rats. *Proc Natl Acad Sci U S A* 2006;**103**: 7877–7882.
 68. Andersson M, Usiello A, Borgkvist A, et al. Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. *J Neurosci* 2005;**25**:8432–8438.
 69. Hasbi A, Fan T, Alijanian M, et al. Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc Natl Acad Sci U S A* 2009;**106**:21377–21382.
 70. Perreault ML, O'Dowd BF, George SR. Dopamine receptor homooligomers and heterooligomers in schizophrenia. *CNS Neurosci Ther* 2011;**17**:52–57.
 71. Borroto-Escuela DO, Romero-Fernandez W, Narvaez M, Ollijan J, Agnati LF, Fuxe K. Hallucinogenic 5-HT2AR agonists LSD and DOI enhance dopamine D2R protomer recognition and signaling of D2-5-HT2A heteroreceptor complexes. *Biochem Biophys Res Commun* 2014;**443**:278–284.
 72. Albizu L, Holloway T, González-Maeso J, Sealfon SC. Functional crosstalk and heteromerization of serotonin 5-HT2A and dopamine D2 receptors. *Neuropharmacology* 2011;**61**:770–777.
 73. Moreno JL, Holloway T, Albizu L, Sealfon SC, González-Maeso J. Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. *Neurosci Lett* 2011;**493**:76–79.
 74. Moreno JL, Muguruza C, Umali A, et al. Identification of three residues essential for 5-hydroxytryptamine 2A-metabotropic glutamate 2 (5-HT2A-mGlu2) receptor heteromerization and its psychoactive behavioral function. *J Biol Chem* 2012;**287**:44301–44319.
 75. González-Maeso J, Ang RL, Yuen T, et al. Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 2008;**452**:93–97.
 76. Fribourg M, Moreno JL, Holloway T, et al. Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell* 2011;**147**:1011–1023.