Neuropharmacology 56 (2009) 226–234

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Review

Looking for the role of cannabinoid receptor heteromers in striatal function

Sergi Ferré [a,*], Steven R. Goldberg [a], Carme Lluis [b], Rafael Franco [b]

[a] National Institute on Drug Abuse, Intramural Research Program, Biomedical Research Center, National Institutes of Health, Department of Health and Human Services, 251 Bayview Boulevard, Baltimore, MD 21224, USA

[b] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 11 April 2008
Received in revised form 16 June 2008
Accepted 29 June 2008

Keywords:
Local module
Receptor heteromers
Cannabinoid CB_1 receptor
Dopamine D_2 receptor
Adenosine A_{2A} receptor
μ Opioid receptor
Striatum

ABSTRACT

The introduction of two concepts, "local module" and "receptor heteromer", facilitates the understanding of the role of interactions between different neurotransmitters in the brain. In artificial cell systems, cannabinoid CB_1 receptors form receptor heteromers with dopamine D_2, adenosine A_{2A} and μ opioid receptors. There is indirect but compelling evidence for the existence of the same CB_1 receptor heteromers in striatal local modules centered in the dendritic spines of striatal GABAergic efferent neurons, particularly at a postsynaptic location. Their analysis provides new clues for the role of endocannabinoids in striatal function, which cannot only be considered as retrograde signals that inhibit neurotransmitter release. Recent studies using a new method to detect heteromerization of more than two proteins, which consists of sequential BRET–FRET (SRET) analysis, has demonstrated that CB_1, D_2 and A_{2A} receptors can form heterotrimers in transfected cells. It is likely that functional CB_1–A_{2A}–D_2 receptor heteromers can be found where they are highly co-expressed, in the dendritic spines of GABAergic enkephalinergic neurons. The functional properties of these multiple receptor heteromers and their role in striatal function need to be determined.

Published by Elsevier Ltd.

1. Striatal spine modules and their diversity

The minimal portion of one or more neurons and/or one or more glial cells that operates as an independent integrative unit has been termed a "local module" (Ferré et al., 2007a). Conceptually, local module allows a better understanding of the functional relevance of extrasynaptic receptors, which are activated by volume transmission. Furthermore, the concept of local module provides a rationale for the functional relevance of neurotransmitter receptor heteromers, which can integrate signals that arise from the same or different elements that constitute the local module.

We recently analyzed the role of receptor heteromers in the "striatal spine module", a common striatal local module which is centered in the dendritic spine of the GABAergic striatal efferent neurons (also called medium spiny neurons) (Ferré et al., 2007a). This type of neuron makes up more than 95% of the striatal neuronal population and receives two main extrinsic inputs that converge in the dendritic spine: mesencephalic dopaminergic inputs from the substantia nigra pars compacta and the ventral tegmental area and cortical, limbic and thalamic glutamatergic inputs (Gerfen, 2004). The main elements of the "striatal spine module" include the dendritic spine, glutamatergic and dopaminergic terminals that make synaptic contact with the head and neck of the dendritic spine, respectively, and astroglial processes that wrap the glutamatergic synapse (Ferré et al., 2007a). This arrangement allows dopaminergic neurotransmission to control glutamatergic neurotransmission. In our previous functional analysis of the striatal spine modules we also considered the role of acetylcholine, mostly released by volume transmission from asynaptic varicosities of cholinergic interneurons, and adenosine, mostly derived from ATP released from astroglia and glutamatergic terminals (Ferré et al., 2007a). We then analyzed the role of heteromers of dopamine, glutamate, adenosine and acetylcholine receptors in the processing of information by striatal spine modules (Ferré et al., 2007a,b). In our previous analysis we did not consider the roles endocannabinoid and endogenous opioid neurotransmission might play in striatal spines modules. We also did not consider the role of extensive intrinsic GABAergic input to striatal spine modules.

GABAergic innervation in the striatum predominantly originates from collaterals of projecting neurons and from interneurons (Yung et al., 1996; Kawaguchi et al., 1995; Kubota and Kawaguchi, 2000). Nerve terminals from pallidal neurons, conversely, represent only a minority of GABAergic synapses (Bevan et al., 1998). GABAergic interneurons, rather than axon collaterals of GABAergic striatal efferent neurons, provide the predominant inhibitory control of striatal neuron excitability (Koos and Tepper, 1999; Kubota and Kawaguchi, 2000). Like dopaminergic afferents, some GABAergic

* Corresponding author. Tel.: +1 443 740 2647.
E-mail address: sferre@intra.nida.nih.gov (S. Ferré).

0028-3908/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2008.06.076

interneurons make synaptic contact with the neck of the dendritic spines at the same time that glutamatergic terminals make synaptic contact with the head of dendritic spines. In addition, many synapses of GABAergic interneurons make contact with the dendritic shafts but close to the base of the spine (Kubota and Kawaguchi, 2000). It is quite well established that there is a preponderance of dopaminergic inputs on distal portions of the dendrites, which also contain the greatest density of dopaminergic receptors in the dendritic tree (Smith and Bolam, 1990; Sesack et al., 1994). On the other hand, in the proximal portion of the dendrites there is a preponderance of GABAergic intrinsic inputs (and also cholinergic, although these mostly are found in asynaptic varicosities) (Smith and Bolam, 1990; Gerfen, 2004). Therefore, the previously described type of striatal spine module, which includes a glutamatergic and a dopaminergic synapse and we will call striatal spine module I (Fig. 1), includes a glutamatergic and a dopaminergic synapse and is mostly localized in the distal portion of the dendritic tree. There are at least two additional types of modules localized in the middle and proximal portions of the dendritic field, which receive glutamatergic inputs. One type of

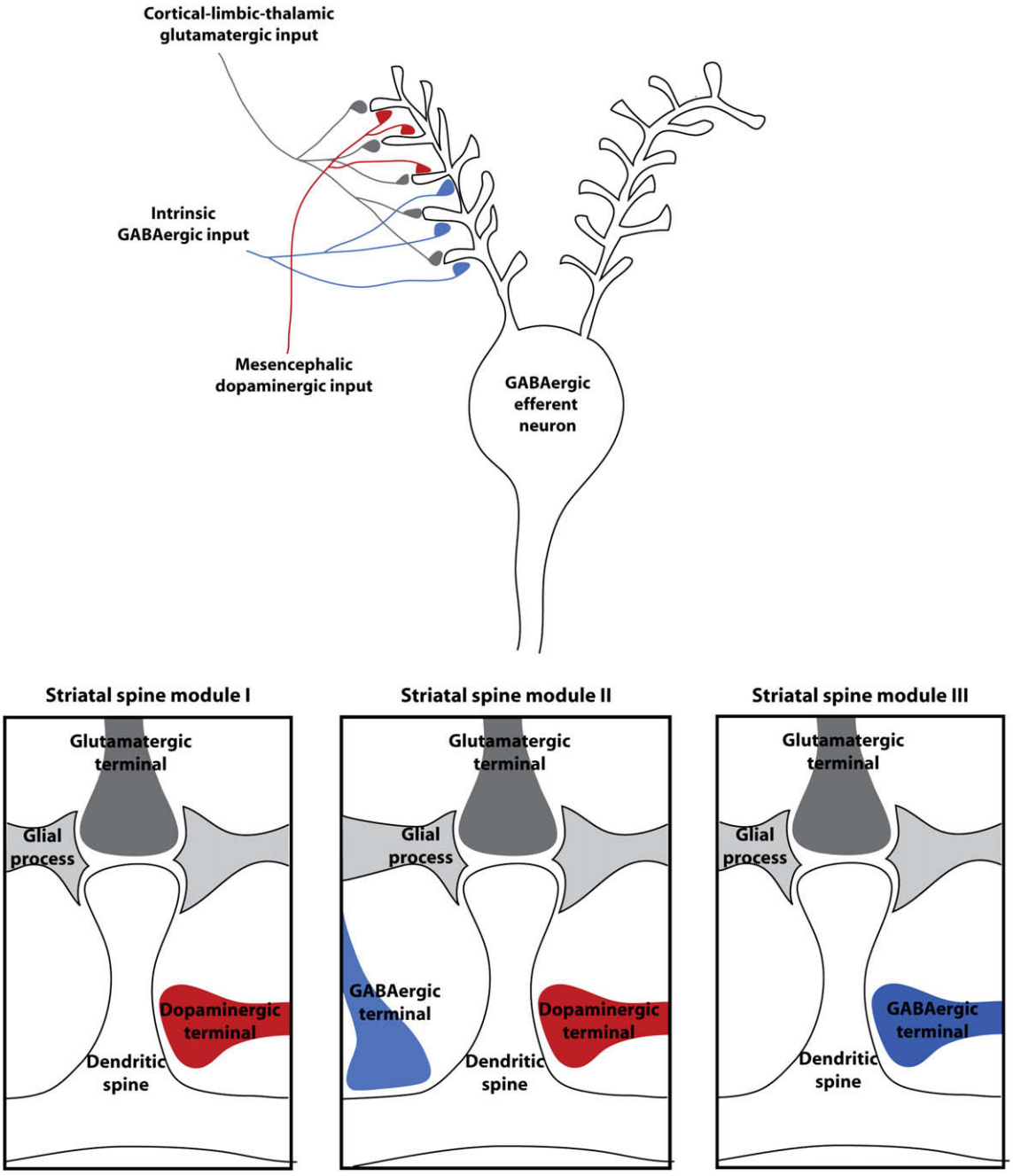

Fig. 1. Scheme for the striatal spine modules of the GABAergic striatal efferent neuron. This is the most common neuron in the striatum, which receives two main extrinsic inputs: glutamatergic afferents from cortical, limbic and thalamic areas and dopaminergic afferents from the mesencephalon. Furthermore, it receives intrinsic inputs from GABAergic interneurons and from collaterals of other GABAergic efferent neurons. The glutamatergic terminals make synaptic contact with the head of the dendritic spine and glial processes wrap the glutamatergic synapse. The dopaminergic and GABAergic inputs contact preferentially the distal and proximal portions of the dendritic tree, respectively, and they make contact with the neck of the dendritic spine or the dendritic shaft close to the base of the dendritic spine. Three main types of striatal modules can be differentiated according to their localization in the dendritic tree (I–III; see text). The scheme is a simplification. The striatal GABAergic efferent neuron contains from 7 to 10 moderately branched dendrites that extend over an area of approximately 200 μm with a spine density of about 20–40 spines/μm (Wilson et al., 1983).

striatal module receives both dopamine and GABAergic inputs (striatal spine module II; Fig. 1), and the other type receives only GABAergic input (striatal spine module III; Fig. 1).

In addition to the differential localization of striatal spine modules in the dendritic tree, there are two other sources of variation that further subdivide the modules. First, two types of GABAergic efferent neurons, which are homogeneously distributed in the striatum, can be differentiated by their output connectivity and their expression of dopamine and adenosine receptors and neuropeptides. GABAergic enkephalinergic neurons connect the striatum with the globus pallidus (lateral globus pallidus) and express the peptide enkephalin and dopamine D_2 and adenosine A_{2A} receptors (Ferré et al., 1997; Gerfen, 2004). GABAergic dynorphinergic neurons connect the striatum with the substantia nigra and the entopeduncular nucleus (medial globus pallidus) and express the peptides dynorphin and substance P and dopamine D_1 and adenosine A_1 receptors (Ferré et al., 1997; Gerfen, 2004). Thus, there are at least two different types of striatal spine modules that should be considered, depending on these two types of GABAergic efferent neurons. Furthermore, a small percentage of GABAergic efferent neurons have a mixed phenotype and express both D_1 and D_2 receptors (Surmeier et al., 1996).

A third source of variation of the striatal spine modules depends on the subdivision of the striatum into patch and matrix compartments. There are two different phenotypes of GABAergic efferent neurons (for both enkephalinergic and dynorphinergic neurons) that are heterogeneously distributed in the patch (striosomes) or the matrix compartments. One of the main characteristics of the patch compartment is that it has a much higher expression of μ opioid receptors than the matrix compartment (Mansour et al., 1987; Gerfen, 2004). In addition to differential expression of other markers, there are clear differences in input and output connectivities, with patch-GABAergic efferent neurons receiving cortical input predominantly from periallocortical areas (such as the infralimbic and prelimbic cortices) and matrix-GABAergic efferent neurons receiving input from neocortical areas (Gerfen, 2004). Also, GABAergic dynorphinergic neurons that originate in the patch project predominantly to the substantia nigra pars compacta and those that originate in the matrix project predominantly to the pars reticulate (Gerfen, 2004). In summary, there are at least 12 different types of striatal spine modules, based on variations in localization on the dendritic tree (I–III), enkephalinergic *versus* dynorphinergic phenotype, and localization in the patch or matrix compartment, and this should be considered when analyzing the role of a given neurotransmitter in the modulation of striatal function (Fig. 2).

2. Endocannabinoid neurotransmission in the striatal spine modules

Endocannabinoids are membrane-derived signaling lipids which stimulate GPCRs receptors that are targeted by Δ^9-tetrahydrocannabinol (THC), the addictive principle of marijuana. Two major endocannabinoids, anandamide and 2-arachidonylglycerol (2-AG), have been discovered. Like classical neurotransmitters they are released from neurons following neuronal depolarization and Ca^{2+} influx into the cell. Unlike classical neurotransmitters, they are not stored in vesicles, but are produced "on demand" from endocannabinoid precursors by the action of enzymes localized in the plasma membrane (Di Marzo et al., 1998; Freund et al., 2003; Piomelli, 2003). The enzymes necessary for the biosynthesis of anandamide are the Ca^{2+}-dependent *N*-acyltransferase and *N*-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD). For the biosynthesis of 2-AG, the main enzymes involved are the Ca^{2+}-dependent and $G_{q\text{-}11}$-coupled receptor-activated phospholipase C and diacylglycerol lipase (DGL). The action of endocannabinoids is terminated by uptake or diffusion into cells followed by intracellular metabolism. Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) are the two primary enzymes involved in intracellular metabolism of anandamide and 2-AG, respectively (Di Marzo et al., 1998; Freund et al., 2003; Piomelli,

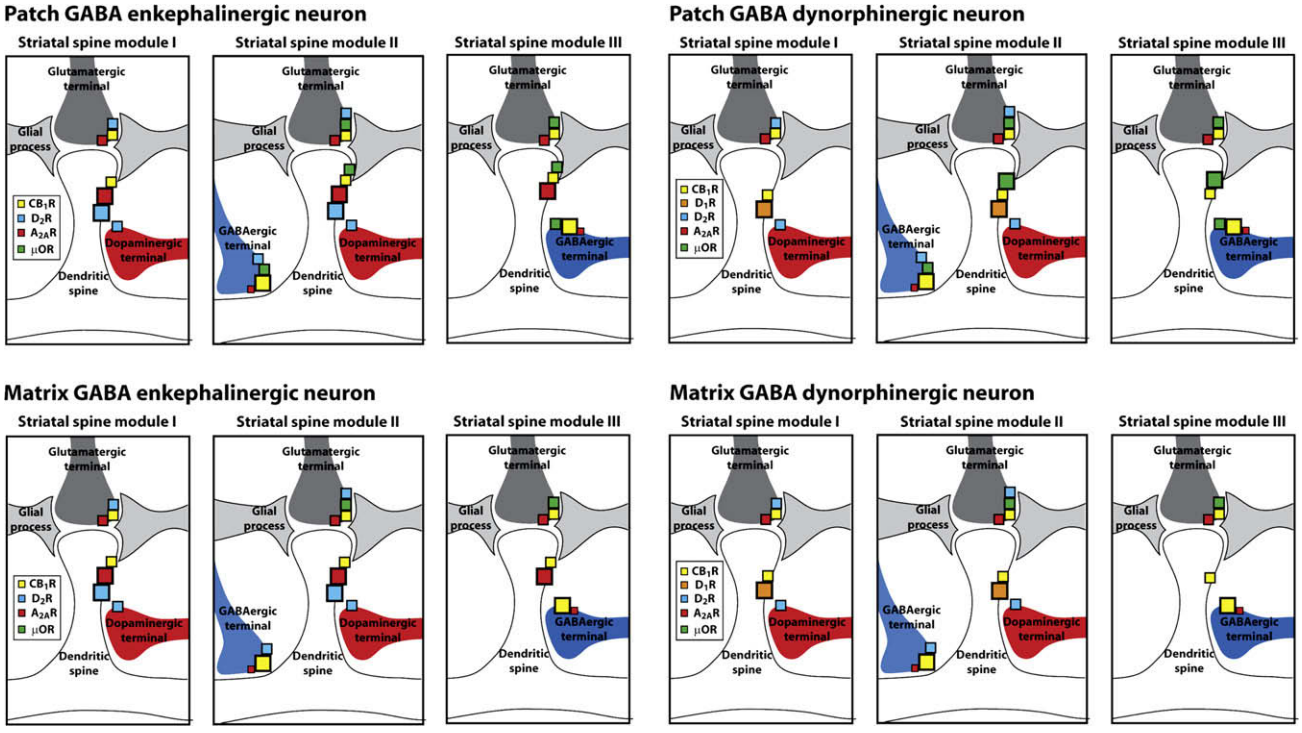

Fig. 2. Relative expression of cannabinoid CB_1, dopamine D_2, adenosine A_{2A} and μ opioid receptors in the different elements of the 12 subtypes of striatal spine modules that depend on their localization in the dendritic tree (I–III; see Fig. 1) and on the phenotype of the striatal GABAergic efferent neuron (enkephalinergic *versus* dynorphynergic and patch *versus* matrix). The squares sizes indicate the approximate relative level of expression in each element of the striatal module.

2003). Finally, the existence of an endocannabinoid membrane equilibrative transporter has been postulated to explain cellular uptake of both anandamide and 2-AG (Piomelli, 2003). Some studies suggest that this transporter, which has not yet been identified, plays a role in endocannabinoid release (Ronesi et al., 2004; Adermark and Lovinger, 2007).

Of the two subtypes of cannabinoid receptors so far identified and characterized (CB_1 and CB_2 receptors) the CB_1 subtype is the one predominantly expressed in the adult central nervous system. In fact, the CB_1 receptor is considered the most abundant GPCR in the brain (Herkenham et al., 1990, 1991). CB_1 receptors activate different signaling pathways through coupling to G_i proteins (Demuth and Molleman, 2006) and are often localized presynaptically where their stimulation usually inhibits neurotransmitter release (Di Marzo et al., 1998; Piomelli, 2003). There has been some debate about the cellular and subcellular localization of CB_1 receptors in the striatum. Most studies would agree that CB_1 receptors are localized presynaptically, predominantly in GABAergic terminals of interneurons or collaterals from GABAergic efferent neurons, and also in glutamatergic but not in dopaminergic terminals (Pickel et al., 2004, 2006; Köfalvi et al., 2005; Mátyás et al., 2006; Uchigashima et al., 2007). There is also general agreement that GABAergic enkephalinergic and dynorphynergic neurons express CB_1 receptor mRNA and that CB_1 receptors are highly expressed in the terminals of these neurons, in the globus pallidus (medial and lateral portions) and in the substantia nigra pars reticulata (Herkenham et al., 1991; Julian et al., 2003; Martín et al., 2008). However, there is disagreement about the existence of CB_1 receptors in the somatodendritic area of the GABAergic striatal efferent neurons, with some electron microscopy studies being able (Rodriguez et al., 2001; Pickel et al., 2004, 2006) and others being unable (Mátyás et al., 2006; Uchigashima et al., 2007) to recognize a postsynaptic localization of striatal CB_1 receptors. These discrepancies may be due to the low resolution of electron microscopy (unable to detect low density receptors) and to problems of accessibility of antibodies to epitopes localized in the synapse (accessibility may be hindered by the dense protein matrix that keeps the synapse together). The application of fractionation methods that allow separation of presynaptic and postsynaptic components of striatal synapses has clearly shown that, in fact, CB_1 receptors are significantly localized in a fraction enriched in the postsynaptic density (the postsynaptic side of the glutamatergic synapse in the dendritic spine) (Köfalvi et al., 2005).

One of the main functions of endocannabinoids is retrograde signaling with stimulation of presynaptic CB_1 receptors and the consequent inhibition of neurotransmitter release. 2-AG, rather than anandamide, seems to be mainly responsible for endocannabinoid-mediated retrograde signaling in the striatum and, probably in most brain areas (Hashimotodani et al., 2007). A recent study has demonstrated that in the striatum DGL is mostly localized in the plasma membrane of the dendritic spines of GABAergic striatal efferent neurons (Uchigashima et al., 2007). The same study showed that, in GABAergic striatal efferent neurons, inhibition of DGL abolishes depolarization-induced suppression of inhibition (DSI) and the depolarization-induced suppression of excitation (DSE) (Uchigashima et al., 2007), which depend on activation of postsynaptic $G_{q\text{-}11}$-coupled receptors and on activation of presynaptic CB_1 receptors with inhibition of GABA and glutamate release, respectively (Hashimotodani et al., 2007). The metabotropic glutamate receptor $mGlu_5$ is a $G_{q\text{-}11}$-coupled receptor that is co-expressed with DGL in the dendritic spines of GABAergic striatal efferent neurons and it has been found to be particularly involved in endocannabinoid-dependent retrograde signaling (Uchigashima et al., 2007). Studies performed in the hippocampus suggest that, unlike 2-AG, anandamide may be preferentially involved in anterograde signaling, since NAPE-PLD was found to be concentrated presynaptically, where it was associated with intracellular calcium stores in several types of excitatory axon terminals (Nyilas et al., 2008). Furthermore, FAAH is mostly localized postsynaptically (Freund et al., 2003).

In addition to short-term plasticity mediated by endocannabinoids, which is represented by the short-term inhibition of neurotransmitter release induced by postsynaptic depolarization and activation of postsynaptic $G_{q\text{-}11}$-coupled receptors, endocannabinoid release is involved in long-term synaptic plasticity in several brain regions (for a recent review, see Hashimotodani et al., 2007). In the dorsal and ventral striata (nucleus accumbens), stimulation of cortico-striatal glutamatergic inputs induces endocannabinoid-mediated long-term depression (LTD) (Gerdeman et al., 2002; Robbe et al., 2002). Although LTD induction requires postsynaptic depolarization and an increase in postsynaptic Ca^{2+} influx, LTD expression is associated with a decrease in the probability of glutamate release (Choi and Lovinger, 1997). It is believed that retrograde signaling is mostly involved in endocannabinoid-mediated LTD (Piomelli, 2003; Hashimotodani et al., 2007). Striatal LTD mediated by endocannabinoids also requires activation of D_2 receptors (Kreitzer and Malenka, 2005). In fact, D_2 receptor activation has been shown to enhance striatal endocannabinoid release by enhancing synthesis and inhibiting metabolism of endocannabinoids (Giuffrida et al., 1999; Centonze et al., 2004). Indeed, recent experiments by Kreitzer and Malenka (2007) indicate that only excitatory synapses of the GABAergic enkephalinergic neurons (which express D_2 receptors), but not GABAergic dynorphinergic neurons (which do not express D_2 receptors), show endocannabinoid-mediated LTD.

In summary, the whole machinery of endocannabinoid neurotransmission is highly represented in striatal spine modules and it seems to be designed to mostly play an inhibitory role on GABAergic and glutamatergic neurotransmission by means of both pre- and postsynaptic mechanisms. However, as we will see below, a more relevant fine-tuning of neurotransmission can potentially be achieved by the ability of CB_1 receptors to heteromerize with other GPCRs, particularly, dopamine, adenosine and opioid receptors, which are differentially expressed in different types of striatal spine modules.

3. Oligomerization of GPCR

Oligomerization of GPCRs is a phenomenon that is becoming broadly accepted. When it comes to homomerization, at least two molecules of GPCR seem to be needed to interact with one heterotrimeric G protein complex (Baneres and Parello, 2003; Liang et al., 2003; Herrick-Davis et al., 2005). Strong support for homodimerization also comes from morphological evidence obtained with atomic force microscopy for rhodopsin (Fotiadis et al., 2003), protein crystallography for metabotropic glutamate receptors (Kunishima et al., 2000) and biophysical techniques for many transfected GPCRs (Bouvier, 2001; Gandia et al., 2008). Homomerization of CB_1 receptors in the brain was demonstrated in immunoprecipitates from rat brain membrane preparations using an antibody that preferentially recognizes the dimerized form of the receptor (Wager-Miller et al., 2002; Mackie, 2005).

In addition to GPCR homomers, there is clear evidence for the existence of GPCR heteromers and we are beginning to understand their functional significance (Ferré et al., 2007b; Franco et al., 2007). Receptor heteromerization provides functional entities which have biochemical properties that are different than those of the individual components of the heteromer. A receptor unit in the heteromer can display several biochemical properties, which can be simply dependent on the presence of the other unit or on co-stimulation of the two (or more) receptor units in the heteromer. For instance, in the recently described D_1–D_2 receptor heteromer, ligands lose their D_1 receptor

selectivity and they can also activate the D_2 receptor in the heteromer (Rashid et al., 2007). Another example is the significant decrease in affinity of the A_{2A} receptor for caffeine when it heteromerizes with A_1 receptor (Ciruela et al., 2006). This opens up the possibility that drugs can be developed that target a specific receptor heteromer (George et al., 2002; Franco et al., 2007). Very often, activation of one of the receptor units in the heteromer induces changes in the binding properties of the other unit. This implies a processing of information at the membrane level of signals impinging on the heteromer (Ferré et al., 2007b). This intermolecular cross-talk is also known as "intramembrane receptor–receptor interaction" (Agnati et al., 2003) and is a common property of GPCR heteromers and constitutes a "biochemical fingerprint" that allows their identification in brain tissue (Ferré et al., 2007b; Franco et al., 2007). As an example, stimulation of the A_{2A} receptor decreases the affinity of the D_2 receptor in the A_{2A}–D_2 receptor heteromer, which provides the basis for the successful clinical application of A_{2A} receptor antagonists with L-DOPA in the treatment of Parkinson's disease (Muller and Ferré, 2007). Finally, another common property of GPCR heteromers is their switch to a new type of G protein coupling. For instance, D_2 normally couples to $G_{i\text{-}o}$ proteins, while D_1 and A_{2A} receptors are prototypical $G_{s\text{–}olf}$ protein-coupled receptors. However, in D_1–D_2 receptor heteromers, and probably in A_{2A}–D_2 receptor heteromers, the D_2 receptor switches its coupling to $G_{q\text{-}11}$ protein, resulting in an ability to activate PLC signaling (Ferré et al., 2007c; Rashid et al., 2007). In artificial cell systems, CB_1 receptors have recently been shown to form receptor heteromers with D_2, A_{2A} and μ receptors (Kearn et al., 2005; Rios et al., 2006; Carriba et al., 2007; Marcellino et al., 2008). We will now analyze the possible functional significance of CB_1 receptor heteromers in striatal function.

4. Cannabinoid CB_1–dopamine D_2 receptor heteromers

CB_1–D_2 receptor heteromerization has been demonstrated in co-transfected cells by co-immunoprecipitation and Fluorescence Resonance Energy Transfer (FRET) techniques (Kearn et al., 2005; Marcellino et al., 2008). The CB_1–D_2 receptor heteromer provides a nice example of G protein switching in the heteromer. Thus, both CB_1 and D_2 receptors are usually coupled to $G_{i\text{-}o}$ proteins and their individual activation in co-transfected cells or primary striatal neurons in culture leads to inhibition of forskolin-induced adenylyl-cyclase activation. On the other hand, in these cellular models, co-stimulation, but not individual receptor stimulation of CB_1 and D_2 receptors, results in a G_s protein-dependent (pertussis toxin-insensitive) adenylyl-cyclase activation (Glass and Felder, 1997; Kearn et al., 2005). Another study suggested that simply co-expressing CB_1 and D_2 receptors is sufficient to induce stimulation of adenylyl cyclase in response to CB_1 receptor activation (Jarrahian et al., 2004). The reasons for differences between these studies remain to be resolved, but all of these studies demonstrate that activation of CB_1–D_2 receptor heteromer can have completely opposite effects than activation of the individual receptors.

CB_1 and D_2 receptors are co-localized in different elements of striatal spine modules. As mentioned above, D_2 receptors are highly expressed in the dendritic spines of GABAergic enkephalinergic neurons, particularly those striatal spine modules localized in the distal portion of the dendritic tree (striatal modules I and II in the GABAergic enkephalinergic neurons; Fig. 2). Furthermore, in the striatal spine modules, D_2 receptors are also known to be localized in dopaminergic, glutamatergic and GABAergic terminals, where their stimulation inhibits neurotransmitter release (Usiello et al., 2000; Bamford et al., 2004; Centonze et al., 2004). There are two isoforms of D_2 receptors, D_{2L} and D_{2S} (long and short isoforms, respectively), which differ by the presence of 29 additional amino acids within the third intracellular loop of D_{2L} (Giros et al., 1989). Studies using specific antibodies indicate that the predominant isoform for the postsynaptic D_2 receptor (in the dendritic spine) is D_{2L} and the predominant isoform for the D_2 autoreceptor (localized in dopaminergic terminals) is D_{2S} (Khan et al., 1998). More recent studies using gene inactivation technology suggest that both isoforms participate in presynaptic inhibition of GABA transmission in the striatum, while modulation of glutamate release depends mostly on the D_{2S} isoform. In summary, when considering the above mentioned striatal distribution of CB_1 receptors and CB_1–D_2 colocalization the localization of CB_1–D_2 receptor heteromers can take place both postsynaptically in the dendritic spines of GABAergic enkephalinergic neuron and presynaptically, in GABAergic and glutamatergic terminals of both enkephalinergic and dynorphinergic neurons. In fact, a recent electron microscopy analysis with double labeling in the ventral striatum has confirmed the existence of overlapping subcellular distributions of CB_1 and D_2 receptor immunoreactivities both at the pre- and postsynaptic levels (Pickel et al., 2006), providing important support for the existence of CB_1–D_2 receptor heteromers in the striatum. Additional compelling evidence has recently been provided by experiments in rat striatal membrane preparations, with the demonstration of an intramembrane receptor–receptor interaction. In these preparations, stimulation of CB_1 receptors decreased the affinity of D_2 receptors for dopamine (Marcellino et al., 2008). As mentioned above, intramembrane receptor–receptor interactions constitute a common property of GPCR heteromers (Agnati et al., 2003; Ferré et al., 2007b; Franco et al., 2007).

Both properties of the CB_1–D_2 receptor heteromer, G protein switching and the antagonistic intramembrane interaction, would predict that in functional experiments CB_1 receptor activation should counteract the effects of striatal D_2 receptor-mediated neurotransmission. In fact, it is well known that CB_1 receptor agonists and antagonists counteract and potentiate, respectively, D_2 receptor agonist-mediated motor effects (Maneuf et al., 1997; Rodríguez de Fonseca et al., 1998; Giuffrida et al., 1999; Andersson et al., 2005; Martín et al., 2008; Marcellino et al., 2008). It has, therefore, been suggested that CB_1 receptor agonist-mediated motor depressant effects depend on their ability to counteract dopamine neurotransmission (Rodríguez de Fonseca et al., 1998). Since motor activation induced by D_2 receptor agonists is mostly dependent on postsynaptic D_2 receptors, these behavioral studies strongly suggest that striatal CB_1–D_2 heteromers are localized in the dendritic spines of GABAergic enkephalinergic neurons. Also, as indicated by Maneuf et al. (1997), another locus where CB_1–D_2 heteromerization could partially explain CB_1–D_2 receptor interactions at the behavioral level are the terminals of the GABAergic enkephalinergic neurons in the lateral globus pallidus (known to have a very high expression of CB_1 receptors and a moderate expression of D_2 receptors; see above). In fact, the same authors also reported that CB_1 receptor activation in slices of rat lateral globus pallidus produces a paradoxical increase in cAMP (Maneuf and Brotchie, 1997). Indirectly this supports the existence of CB_1–D_2 receptor heteromers in the striatal collaterals of GABAergic enkephalinergic neurons. On the other hand, although CB_1 and D_2 receptors are co-localized in striatal glutamatergic terminals, recent studies by Kreitzer and Malenka (2007) do not support that they form functional heteromers in this location. As mentioned before, D_2 receptor stimulation favors CB_1 receptor-mediated inhibition of presynaptic glutamatergic neurotransmission, most probably by a D_2 receptor-mediated stimulation of endocannabinoid release (Kreitzer and Malenka, 2007). In view of the differential distribution of D_2 receptor isoforms, it is tempting to speculate that in the striatum CB_1 receptors form heteromers with D_{2L} but not D_{2S} receptors.

5. Cannabinoid CB_1–adenosine A_{2A} receptor heteromers

CB_1–A_{2A} receptor heteromerization has been demonstrated by Bioluminescence Resonance Energy Transfer (BRET) in co-transfected cells (Carriba et al., 2007). In a human neuroblastoma cell line, G_i-dependent CB_1 receptor signaling (inhibition of

adenylyl-cyclase activity) was found to be completely dependent on A_{2A} receptor co-activation. Thus, A_{2A} receptor blockade or incubation with adenosine deaminase counteracted the ability of a CB_1 agonist to inhibit forskolin-induced cAMP accumulation (Carriba et al., 2007). Consistent with these results in artificial cell lines, some biochemical effects of CB_1 receptor agonists in primary striatal cell cultures and striatal slices have been reported to depend on A_{2A} receptor function. Those include cAMP–PKA signaling involving AGS3, an activator of G protein signaling, as well as βγ subunits and types II–IV adenylyl cyclase (Yao et al., 2003, 2006) and DARPP-32 phosphorylation (Andersson et al., 2005).

In the striatal spine modules, A_{2A} receptors are preferentially expressed in dendritic spines of GABAergic enkephalinergic neurons (Rosin et al., 2003), where they are co-localized and form functional heteromers with D_2 receptors (reviewed in Ferré et al., 2007a,b,c). Furthermore, A_{2A} receptors are localized in a high proportion of glutamatergic terminals, where they form heteromers with A_1 receptors that control glutamate release (Ciruela et al., 2006; Ferré et al., 2007a,b,c). On the other hand, A_{2A} receptors show very little expression in the dopaminergic and GABAergic terminals (Rosin et al., 2003). However, there is clear functional evidence for the existence of A_{2A} receptors localized in the lateral globus pallidus and their stimulation facilitates GABA release (Mayfield et al., 1993; Shindou et al., 2002; Simola et al., 2006). Recent studies suggest A_{2A} receptors are also present in the striatal collaterals of the GABAergic enkephalinergic neurons (Shindou et al., 2008). Less clear is the significance of some results that suggest the existence of striatal A_{2A} receptors localized in GABA terminals, which inhibit GABA release when activated (Mori and Shindou, 2003). Striatal A_{2A} receptors are, therefore, mostly co-localized with CB_1 receptors in the dendritic spines of GABAergic enkephalinergic neuron and in glutamatergic nerve terminals (Fig. 2). A recent double immunohistochemical confocal analysis in rat striatal sections demonstrated a strong colocalization of CB_1 and A_{2A} receptors in fibrilar structures, compatible with dendritic processes or nerve terminals (Carriba et al., 2007). Furthermore striatal CB_1–A_{2A} receptor complexes were obtained by co-immunoprecipitation (Carriba et al., 2007).

The biochemical properties of the CB_1–A_{2A} receptor heteromer, with its dependence on A_{2A} receptor activation, would predict that A_{2A} receptor antagonists would produce effects similar to CB_1 receptor antagonists. Consistent with this prediction, motor depression induced by the bilateral striatal infusion of a CB_1 receptor agonist in rats was completely counteracted by systemic administration of either CB_1 or A_{2A} receptor antagonists (Carriba et al., 2007). Similarly, genetic inactivation of A_{2A} receptors in mice significantly decreased the cataleptic and rewarding effects (in a conditioned place preference paradigm) of systemically administered CB_1 receptor agonists (Andersson et al., 2005; Soria et al., 2006). As mentioned before, the motor depressant effects of CB_1 receptor agonists seem to depend on postsynaptic D_2 receptor-mediated dopaminergic neurotransmission and some of the biochemical CB_1–A_{2A} receptor interactions as described above also seem to depend on D_2 receptor function (Yao et al., 2003; Andersson et al., 2005). Since strong physical and functional interdependence of striatal A_{2A} and D_2 receptors has also been demonstrated (Ferré et al., 2007a,b,c), it becomes an obvious possibility that CB_1, A_{2A} and D_2 receptors form part of the same macromolecular complexes and that the three receptors can heteromerize in dendritic spines of GABAergic enkephalinergic neurons.

We recently introduced a new method to detect heteromerization of more than two proteins in transfected cells, which consists of a Sequential BRET–FRET (SRET) analysis (Carriba et al., 2008). In SRET experiments, emission of light by a bioluminescent donor triggers excitation of an acceptor fluorophor by BRET and this fluorophor emits light on a wavelength that excites a second acceptor fluorophor by FRET. Thus, SRET requires co-expression of three fusion proteins: one fused to the BRET donor (*Renilla luciferase*; *Rluc*), another fused to the BRET acceptor, which is also a FRET donor (p.e., GFP^2), and the third coupled to the FRET acceptor (p.e., YFP). If the proteins under study form heteromers, the addition of an Rluc substrate should elicit a sequence with the final emission of light by the FRET acceptor (Carriba et al., 2008). A very significant SRET was demonstrated when mammalian cells were co-transfected with Rluc–A_{2A}, D_2–GFP^2 and CB_1–YFP receptors (Carriba et al., 2008). Although it has not yet been demonstrated, it is likely that functional CB_1–A_{2A}–D_2 receptor heteromers can be found where they are highly co-expressed in the striatum, particularly in the dendritic spines of GABAergic enkephalinergic neurons, the preferential localization of CB_1–D_2, CB_1–A_{2A} and A_{2A}–D_2 receptor heteromers. The functional properties of these multiple heteromers and their role in striatal function needs to be determined.

6. Cannabinoid CB_1–μ opioid receptor heteromers

Heteromerization of CB_1 and μ receptors was recently demonstrated in co-transfected cells with BRET (Rios et al., 2006), but direct functional interactions between these receptors were demonstrated much earlier by investigators looking for endogenous targets of THC, well before the discovery of endocannabinoids and cannabinoid receptors. It was found that THC allosterically modulates the binding of μ and δ (but not κ) opioid receptor ligands in membrane preparations from rat brain (Vaysse et al., 1987). These results were recently confirmed and extended by another research group (Kathmann et al., 2006) that found a specific increase in the dissociation of μ and δ receptor ligands with THC in rat cortical membrane preparations (although a similar effect was observed with cannabidiol, another constituent of marijuana that has low affinity for CB_1 receptors; Kathmann et al., 2006). That this allosteric modulation involves a CB_1–μ intramembrane receptor interaction and receptor heteromerization is supported by a recent study that shows the absence of direct effects of CB_1 receptor ligands on μ receptors expressed in *Xenopus* oocytes (Kracke et al., 2007). Biochemical experiments in co-transfected cells suggest that occupancy of CB_1 receptors in CB_1–μ receptor heteromer has an antagonistic effect on μ receptor-mediated G protein activation (Rios et al., 2006), which would agree with the antagonistic intramembrane interaction.

The μ receptor, the main target for morphine, is heavily expressed in the striatum, particularly in the patch compartment (Mansour et al., 1987; Gerfen, 2004), where it seems to be preferentially expressed in GABAergic dynorphinergic neurons (Guttenberg et al., 1996). Ultrastructural analysis with electron microscopy has shown that μ receptors in the patch compartment are preferentially localized in the same elements of striatal spine modules as CB_1 receptors, i.e., in the dendritic spines and in the glutamatergic and GABAergic nerve terminals (Pickel et al., 2004; Rodriguez et al., 2001) (Fig. 2), where they exert an inhibitory role on neurotransmitter release (Schoffelmeer et al., 2006; Miura et al., 2007). Recent electrophysiological experiments indicate that there is a similar μ receptor-dependent modulation of glutamate release in both patch and matrix compartments, while μ receptor-dependent modulation of GABA release only takes place in the patch compartment (Miura et al., 2007).

The pentapeptide enkephalins are the main endogenous opioids involved in stimulation of striatal μ receptors. Enkephalins (Met-enkephalin and Leu-enkephalin, from the precursor proenkephalin) are released by GABAergic enkephalinergic neurons and are also endogenous ligands for δ, but not κ receptors (Mansour et al., 1995; Akil et al., 1998). On the other hand, dynorphins (A and B) are released by GABAergic dynorphinergic neurons and are the main endogenous ligands for κ receptors, with very low affinities for μ and δ receptors (Mansour et al., 1995; Akil et al., 1998). Nevertheless, the precursor of dynorphins, prodynorphin, can potentially generate

Leu-enkephalin (as well as Leu-enkephalin-Arg, which has similar affinities for the three opioid receptors; Mansour et al., 1995). In fact, the GABAergic dynorphinergic neurons have been shown to be an important source of Leu-enkephalin in the substantia nigra (Zamir et al., 1984). In the striatum, however, total and extracellular levels of Met-enkephalin are significantly higher than those of Leu-enkephalin (Nylander et al., 1995; Baseski et al., 2005). Endomorphins (1 and 2) are endogenous tetrapeptides which also bind to μ receptors with high affinity and have a low selectivity for δ and κ receptors (Fichna et al., 2007). However, the density of endomorphin-like immunoreactivity is low in the striatum (Martin-Schild et al., 1999) and it is not yet known if they are functional significance in striatal spine modules. Also, the precursor molecules for endomorphins, as well as the striatal neurons that release endomorphins, still need to be determined (Fichna et al., 2007; Martin-Schild et al., 1999). In summary, the main source of endogenous agonist for μ receptors seems to be the collaterals of GABAergic enkephalinergic neurons, which establish synaptic contact with the dendritic shafts and spines of GABAergic striatal efferent neurons (Yung et al., 1996).

Electron microscopy studies with double labeling have shown CB_1–μ receptor colocalization in both pre- and postsynaptic elements (Rodriguez et al., 2001; Pickel et al., 2004). Clearer evidence for the existence of functional CB_1–μ receptor heteromers in the striatum comes from biochemical experiments, such as the above mentioned intramembrane interactions, as well as from experiments in rat striatal slices analyzing CB_1–μ receptor interactions in the modulation of neurotransmitter release (Schoffelmeer et al., 2006). In these studies, a μ receptor antagonist prevented a CB_1 receptor antagonist from blocking the inhibition of glutamate and GABA release mediated by a CB_1 receptor agonist. Conversely, a CB_1 receptor antagonist prevented a μ receptor antagonist from blocking the inhibition of glutamate and GABA release mediated by a μ receptor agonist (Schoffelmeer et al., 2006). These results suggest additional intramembrane cross-talk between CB_1 and μ receptors, since ligand binding to one of the receptors changes the binding characteristics of the other receptor. Also, in the same study, evidence was obtained for additive and synergistic CB_1–μ receptor interactions in the modulation of striatal glutamate and GABA release by studying the effect of CB_1 and μ receptor agonists, alone and in combination (Schoffelmeer et al., 2006). This is not consistent with the antagonistic intramembrane CB_1–μ receptor interactions (Vaysse et al., 1987; Kathmann et al., 2006) and with interactions at the signaling level obtained in co-transfected cells (Rios et al., 2006). Nevertheless, there are also results of synergistic CB_1–μ receptor interactions on cAMP–PKA signaling in striatal neurons in culture, which seem to depend on A_{2A} receptor activation (Yao et al., 2006), suggesting that, *in vivo*, we might be dealing with A_{2A}–CB_1–μ receptor heteromerization. Furthermore, an important amount of data from *in vivo* and *ex vivo* biochemical and behavioral experiments indicates that there is a strong dependence of μ receptor co-activation on CB_1 receptor activation and *vice versa* (Gardner et al., 1988; Tanda et al., 1997; Ledent et al., 1999; Manzanares et al., 1999; Berrendero et al., 2003; Justinova et al., 2004; Solinas and Goldberg, 2005). Probably, striatal CB_1–μ receptor heteromers (and maybe A_{2A}–CB_1–μ receptor heteromers) contribute to the behavioral effects of CB_1 and μ receptor ligands. This should not underestimate the contribution of other loci of interactions in the brain and the reported stimulation of the release of endogenous opioids by CB_1 receptor activation (reviewed in Vigano et al., 2005). For instance, THC induces an increase in the extracellular concentration of enkephalin in the striatum (Valverde et al., 2001).

7. Concluding remarks

The introduction of the two concepts, "local module" and "receptor heteromer", facilitates the understanding of the role of interactions between different neurotransmitters in the brain (Ferré et al., 2007a). Local module also provides the best framework for understanding the role of "volume transmission" (Zoli et al., 1999). In the local module, the overflow of synaptically released and non-synaptically (neuronal or glial in origin) released neurotransmitters can converge and activate extrasynaptically located receptor heteromers, which act as processors of computations that modulate cell signaling (Ferré et al., 2007b,c). Furthermore, receptor heteromers can be localized intrasynaptically (pre- or postsynaptically), where they can act as concentration-dependent switches (in case of heteromers of receptors for the same neurotransmitter) or as processors of information of different neurotransmitters released in the synapse (Ferré et al., 2007c).

The study of CB_1 receptor heteromers in the striatal spine modules provides new clues for the role of endocannabinoids in neuronal function, which cannot only be considered as retrograde signals that inhibit neurotransmitter release. There is strong evidence for tight morphological and functional postsynaptic interactions between CB_1 receptors and D_2, A_{2A} and μ receptors which play important roles in striatal function. Furthermore, the differential expression of CB_1, D_2, A_{2A} and μ receptors in the various types of striatal spine modules allows a significant plasticity in the modulatory role of endocannabinoids in the striatum.

Although the field of receptor heteromers is just beginning, we can foresee that it will have a huge impact on the fields of receptor physiology and pharmacology. Many questions remain to be answered and new ways of understanding receptor function will certainly emerge. For instance, there is already overwhelming experimental evidence that homodimers are the minimal functional unit of GPCRs. This suggests that receptor heteromers are probably heteromers of different homodimers. At the pharmacological level, the unique binding characteristics of receptor heteromers open the possibility of selectively targeting not only specific neurons in a specific brain area, but even specific local modules or specific elements of specific local modules.

Acknowledgements

Supported by the National Institute on Drug Abuse (Intramural Research funds), National Institutes of Health, Department of Health and Human Services.

References

Adermark, L., Lovinger, D.M., 2007. Retrograde endocannabinoid signaling at striatal synapses requires a regulated postsynaptic release step. Proceedings of the National Academy of Sciences of the United States of America 104, 20564–20569.

Agnati, L.F., Ferré, S., Lluis, C., Franco, R., Fuxe, K., 2003. Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. Pharmacological Reviews 55, 509–550.

Akil, H., Owens, C., Gutstein, H., Taylor, L., Curran, E., Watson, S., 1998. Endogenous opioids: overview and current issues. Drug and Alcohol Dependence 51, 127–140.

Andersson, M., Usiello, A., Borgkvist, A., Pozzi, L., Dominguez, C., Fienberg, A.A., Svenningsson, P., Fredholm, B.B., Borrelli, E., Greengard, P., Fisone, G., 2005. Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. Journal of Neuroscience 25, 8432–8438.

Bamford, N.S., Robinson, S., Palmiter, R.D., Joyce, J.A., Moore, C., Meshul, C.K., 2004. Dopamine modulates release from corticostriatal terminals. Journal of Neuroscience 24, 9541–9552.

Baneres, J.L., Parello, J., 2003. Structure-based analysis of GPCR function: evidence for a novel pentameric assembly between the dimeric leukotriene B4 receptor BLT1 and the G-protein. Journal of Molecular Biology 329, 815–829.

Baseski, H.M., Watson, C.J., Cellar, N.A., Shackman, J.G., Kennedy, R.T., 2005. Capillary liquid chromatography with MS3 for the determination of enkephalins in microdialysis samples from the striatum of anesthetized and freely-moving rats. Journal of Mass Spectrometry 40, 146–153.

Berrendero, F., Mendizábal, V., Murtra, P., Kieffer, B.L., Maldonado, R., 2003. Cannabinoid receptor and WIN 55 212-2-stimulated [^{35}S]-GTPgammaS binding in the brain of mu-, delta- and kappa-opioid receptor knockout mice. European Journal of Neuroscience 18, 2197–2202.

Bevan, M.D., Booth, P.A., Eaton, S.A., Bolam, J.P., 1998. Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. Journal of Neuroscience 18, 9438–9452.

Bouvier, M., 2001. Oligomerization of G-protein-coupled transmitter receptors. Nature Reviews Neuroscience 2, 274–286.
Carriba, P., Ortiz, O., Patkar, K., Justinova, Z., Stroik, J., Themann, A., Muller, C., Woods, A.S., Hope, B.T., Ciruela, F., Casado, V., Canela, E.I., Lluis, C., Goldberg, S.R., Moratalla, R., Franco, R., Ferré, S., 2007. Striatal adenosine A(2A) and cannabinoid CB(1) receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacology 32, 2249–2259.
Carriba, P., Navarro, G., Ciruela, F., Ferré, S., Casado, V., Agnati, L.F., Cortes, A., Mallol, J., Fuxe, K., Canela, E.I., LluisFranco, R., 2008. Detection of heteromerization of more than two proteins by sequential BRET–FRET. Nature Methods 5, 727–733.
Centonze, D., Battista, N., Rossi, S., Mercuri, N.B., Finazzi-Agrò, A., Bernardi, G., Calabresi, P., Maccarrone, M., 2004. A critical interaction between dopamine D_2 receptors and endocannabinoids mediates the effects of cocaine on striatal GABAergic transmission. Neuropsychopharmacology 29, 1488–1497.
Choi, S., Lovinger, D.M., 1997. Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. Proceedings of the National Academy of Sciences of the United States of America 94, 2665–2670.
Ciruela, F., Casado, V., Rodrigues, R.J., Lujan, R., Burgueno, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S.R., Mallol, J., Cortes, A., Canela, E.I., Lopez-Gimenez, J.F., Milligan, G., Lluis, C., Cunha, R.A., Ferré, S., Franco, R., 2006. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A_1–A_{2A} receptor heteromers. Journal of Neuroscience 26, 2080–2087.
Demuth, D.G., Molleman, A., 2006. Cannabinoid signaling. Life Sciences 78, 549–563.
Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends in Neurosciences 21, 521–528.
Ferré, S., Fredholm, B.B., Morelli, M., Popoli, P., Fuxe, K., 1997. Adenosine–dopamine receptor–receptor interactions as an integrative mechanism in the basal ganglia. Trends in Neurosciences 20, 482–487.
Ferré, S., Agnati, L.F., Ciruela, F., Lluis, C., Woods, A.S., Fuxe, K., Franco, R., 2007a. Neurotransmitter receptor heteromers and their integrative role in 'local modules': the striatal spine module. Brain Research Reviews 55, 55–67.
Ferré, S., Ciruela, F., Woods, A.S., Lluis, C., Franco, R., 2007b. Functional relevance of neurotransmitter receptor heteromers in the central nervous system. Trends in Neurosciences 30, 440–446.
Ferré, S., Ciruela, F., Quiroz, C., Lujan, R., Popoli, P., Cunha, R.A., Agnati, L.F., Fuxe, K., Woods, A.S., Lluis, C., Franco, R., 2007c. Adenosine receptor heteromers and their integrative role in striatal function. TheScientificWorldJOURNAL 7, 74–85.
Fichna, J., Janecka, A., Costentin, J., Do Rego, J.C., 2007. The endomorphin system and its evolving neurophysiological role. Pharmacological Reviews 59, 88–123.
Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D.A., Engel, A., Palczewski, K., 2003. Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature 421, 127–128.
Franco, R., Casado, V., Cortes, A., Ferrada, C., Mallol, J., Woods, A., Lluis, C., Canela, E.I., Ferré, S., 2007. Basic concepts in G-protein-coupled receptor homo- and heteromerization. TheScientificWorldJOURNAL 7, 48–57.
Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiological Reviews 83, 1017–1066.
Gandía, J., Lluís, C., Ferré, S., Franco, R., Ciruela, F., 2008. Light resonance energy transfer-based methods in the study of G protein-coupled receptor oligomerization. Bioessays 30, 82–89.
Gardner, E.L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., Morrison, D., 1988. Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. Psychopharmacology 96, 142–144.
Gerfen, C.R., 2004. Basal ganglia. In: Paxinos, G. (Ed.), The Rat Nervous System. Elsevier Academic Press, Amsterdam, pp. 445–508.
George, S.R., O'Dowd, B.F., Lee, S.P., 2002. G-protein-coupled receptor oligomerization and its potential for drug discovery. Nature Reviews in Drug Discovery 1, 808–820.
Gerdeman, G.L., Ronesi, J., Lovinger, D.M., 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nature Neuroscience 5, 446–451.
Giros, B., Sokoloff, P., Martres, M.P., Riou, J.F., Emorine, L.J., Schwartz, J.C., 1989. Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature 342, 923–926.
Giuffrida, A., Parsons, L.H., Kerr, T.M., Rodríguez de Fonseca, F., Navarro, M., Piomelli, D., 1999. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nature Neuroscience 2, 358–363.
Glass, M., Felder, C.C., 1997. Concurrent stimulation of cannabinoid CB_1 and dopamine D_2 receptors augments cAMP accumulation in striatal neurons: evidence for a G_s linkage to the CB_1 receptor. Journal of Neuroscience 17, 5327–5333.
Guttenberg, N.D., Klop, H., Minami, M., Satoh, M., Voorn, P., 1996. Co-localization of mu opioid receptor is greater with dynorphin than enkephalin in rat striatum. NeuroReport 7, 2119–2124.
Hashimotodani, Y., Ohno-Shosaku, T., Kano, M., 2007. Endocannabinoids and synaptic function in the CNS. Neuroscientist 13, 127–137.
Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proceedings of the National Academy of Sciences of the United States of America 87, 1932–1936.
Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. Journal of Neuroscience 11, 563–583.
Herrick-Davis, K., Grinde, E., Harrigan, T.J., Mazurkiewicz, J.E., 2005. Inhibition of serotonin 5-hydroxytryptamine2c receptor function through heterodimerization: receptor dimers bind two molecules of ligand and one G-protein. Journal of Biological Chemistry 280, 401–451.
Jarrahian, A., Watts, V.J., Barker, E.L., 2004. D_2 dopamine receptors modulate Galpha-subunit coupling of the CB_1 cannabinoid receptor. Journal of Pharmacology and Experimental Therapeutics 308, 880–886.
Julian, M.D., Martin, A.B., Cuellar, B., Rodriguez De Fonseca, F., Navarro, M., Moratalla, R., Garcia-Segura, L.M., 2003. Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. Neuroscience 119, 309–318.
Justinova, Z., Tanda, G., Munzar, P., Goldberg, S.R., 2004. The opioid antagonist naltrexone reduces the reinforcing effects of delta 9 tetrahydrocannabinol (THC) in squirrel monkeys. Psychopharmacology 173, 186–194.
Kathmann, M., Flau, K., Redmer, A., Tränkle, C., Schlicker, E., 2006. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. Naunyn-Schmiedeberg's Archives of Pharmacology 372, 354–361.
Kawaguchi, Y., Wilson, C.J., Augood, S.J., Emson, P.C., 1995. Striatal interneurones: chemical, physiological and morphological characterization. Trends in Neurosciences 18, 527–535.
Kearn, C.S., Blake-Palmer, K., Daniel, E., Mackie, K., Glass, M., 2005. Concurrent stimulation of cannabinoid CB_1 and dopamine D_2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Molecular Pharmacology 67, 1697–1704.
Khan, Z.U., Mrzljak, L., Gutierrez, A., de la Calle, A., Goldman-Rakic, P.S., 1998. Prominence of the dopamine D_2 short isoform in dopaminergic pathways. Proceedings of the National Academy of Sciences of the United States of America 95, 7731–7736.
Köfalvi, A., Rodrigues, R.J., Ledent, C., Mackie, K., Vizi, E.S., Cunha, R.A., Sperlágh, B., 2005. Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. Journal of Neuroscience 25, 2874–2884.
Koos, T., Tepper, J.M., 1999. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nature Neuroscience 2, 467–472.
Kracke, G.R., Stoneking, S.P., Ball, J.M., Tilghman, B.M., Washington, C.C., Hotaling, K.A., Johnson, J.O., Tobias, J.D., 2007. The cannabinoid receptor agonists, anandamide and WIN 55,212-2, do not directly affect mu opioid receptors expressed in *Xenopus* oocytes. Naunyn-Schmiedeberg's Archives of Pharmacology 376, 285–293.
Kreitzer, A.C., Malenka, R.C., 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. Journal of Neuroscience 25, 10537–10545.
Kreitzer, A.C., Malenka, R.C., 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445, 643–647.
Kubota, Y., Kawaguchi, Y., 2000. Dependence of GABAergic synaptic areas on the interneuron type and target size. Journal of Neuroscience 20, 375–386.
Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumasaka, T., Nakanishi, S., Jingami, H., Morikawa, K., 2000. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature 407, 971–977.
Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.F., Beslot, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. Science 283, 401–404.
Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D.A., Palczewski, K., Engel, A., 2003. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. Journal of Biological Chemistry 278, 21655–21662.
Mackie, K., 2005. Cannabinoid receptor homo- and heterodimerization. Life Sciences 77, 1667–1673.
Maneuf, Y.P., Brotchie, J.M., 1997. Paradoxical action of the cannabinoid WIN 55,212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. British Journal of Pharmacology 120, 1397–1398.
Maneuf, Y.P., Crossman, A.R., Brotchie, J.M., 1997. The cannabinoid receptor agonist WIN 55,212-2 reduces D_2, but not D_1, dopamine receptor-mediated alleviation of akinesia in the reserpine-treated rat model of Parkinson's disease. Experimental Neurology 148, 265–270.
Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., Watson, S.J., 1987. Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. Journal of Neuroscience 7, 2445–2464.
Mansour, A., Hoversten, M.T., Taylor, L.P., Watson, S.J., Akil, H., 1995. The cloned mu, delta and kappa receptors and their endogenous ligands: evidence for two opioid peptide recognition cores. Brain Research 700, 89–98.
Manzanares, J., Corchero, J., Romero, J., Fernández-Ruiz, J.J., Ramos, J.A., Fuentes, J.A., 1999. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends in Pharmacological Sciences 20, 287–294.
Marcellino, D., Carriba, P., Filip, M., Borgkvist, A., Frankowska, M., Bellido, I., Tanganelli, S., Müller, C.E., Fisone, G., Lluis, C., Agnati, L.F., Franco, R., Fuxe, K., 2008. Antagonistic cannabinoid CB(1)/dopamine D(2) receptor interactions in striatal CB(1)/D(2) heteromers. A combined neurochemical and behavioral analysis. Neuropharmacology 54, 815–823.
Martín, A.B., Fernandez-Espejo, E., Ferrer, B., Gorriti, M.A., Bilbao, A., Navarro, M., Rodriguez de Fonseca, F., Moratalla, R., 2008. Expression and function of CB(1) receptor in the rat striatum: localization and effects on D(1) and D(2) dopamine receptor-mediated motor behaviors. Neuropsychopharmacology, doi:10.1038/sj.npp.1301558.

Martin-Schild, S., Gerall, A.A., Kastin, A.J., Zadina, J.E., 1999. Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent. Journal of Comparative Neurology 405, 450–471.

Mátyás, F., Yanovsky, Y., Mackie, K., Kelsch, W., Misgeld, U., Freund, T.F., 2006. Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. Neuroscience 137, 337–361.

Mayfield, R.D., Suzuki, F., Zahniser, N.R., 1993. Adenosine A_{2a} receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. Journal of Neurochemistry 60, 2334–2337.

Miura, M., Saino-Saito, S., Masuda, M., Kobayashi, K., Aosaki, T., 2007. Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. Journal of Neuroscience 27, 9721–9728.

Mori, A., Shindou, T., 2003. Modulation of GABAergic transmission in the striato-pallidal system by adenosine A_{2A} receptors: a potential mechanism for the antiparkinsonian effects of A_{2A} antagonists. Neurology 61, S44–S48.

Muller, C.E., Ferré, S., 2007. Blocking striatal adenosine A_{2A} receptors: a new strategy for basal ganglia disorders. Recent Patents on CNS Drug Discovery 2, 1–21.

Nylander, I., Vlaskovska, M., Terenius, L., 1995. Brain dynorphin and enkephalin systems in Fischer and Lewis rats: effects of morphine tolerance and withdrawal. Brain Research 683, 25–35.

Nyilas, R., Dudok, B., Urbán, G.M., Mackie, K., Watanabe, M., Cravatt, B.F., Freund, T.F., Katona, I., 2008. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. Journal of Neuroscience 28, 1058–1063.

Pickel, V.M., Chan, J., Kash, T.L., Rodríguez, J.J., MacKie, K., 2004. Compartment-specific localization of cannabinoid 1 (CB_1) and mu-opioid receptors in rat nucleus accumbens. Neuroscience 127, 101–112.

Pickel, V.M., Chan, J., Kearn, C.S., Mackie, K., 2006. Targeting dopamine D_2 and cannabinoid-1 (CB_1) receptors in rat nucleus accumbens. Journal of Comparative Neurology 495, 299–313.

Piomelli, D., 2003. The molecular logic of endocannabinoid signaling. Nature Reviews in Neurosciences 4, 873–884.

Rashid, A.J., So, C.H., Kong, M.M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B.F., George, S.R., 2007. D_1–D_2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of $G_{q/11}$ in the striatum. Proceedings of the National Academy of Sciences of the United States of America 104, 654–659.

Rios, C., Gomes, I., Devi, L.A., 2006. Mu opioid and CB_1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. British Journal of Pharmacology 148, 385–386.

Robbe, D., Kopf, M., Remaury, A., Bockaert, J., Manzoni, O.J., 2002. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proceedings of the National Academy of Sciences of the United States of America 99, 8384–8388.

Rodriguez, J.J., Mackie, K., Pickel, V.M., 2001. Ultrastructural localization of the CB_1 cannabinoid receptor in mu-opioid receptor patches of the rat caudate putamen nucleus. Journal of Neuroscience 21, 823–833.

Rodríguez de Fonseca, F., Del Arco, I., Martín-Calderón, J.L., Gorriti, M.A., Navarro, M., 1998. Role of the endogenous cannabinoid system in the regulation of motor activity. Neurobiology of Disease 5, 483–501.

Ronesi, J., Gerdeman, G.L., Lovinger, D.M., 2004. Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. Journal of Neuroscience 24, 1673–1679.

Rosin, D.L., Hettinger, B.D., Lee, A., Linden, J., 2003. Anatomy of adenosine A_{2A} receptors in brain: morphological substrates for integration of striatal function. Neurology 61, S12–S18.

Sesack, S.R., Aoki, C., Pickel, V.M., 1994. Ultrastructural localization of D_2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. Journal of Neuroscience 14, 88–106.

Simola, N., Fenu, S., Baraldi, P.G., Tabrizi, M.A., Morelli, M., 2006. Involvement of globus pallidus in the antiparkinsonian effects of adenosine A(2A) receptor antagonists. Experimental Neurology 202, 255–257.

Shindou, T., Nonaka, H., Richardson, P.J., Mori, A., Kase, H., Ichimura, M., 2002. Presynaptic adenosine A(2A) receptors enhance GABAergic synaptic transmission via a cyclic AMP dependent mechanism in the rat globus pallidus. British Journal of Pharmacology 136, 296–302.

Shindou, T., Arbuthnott, G.W., Wickens, J.R., 2008. Actions of adenosine A_{2A} receptors on synaptic connections of spiny projection neurons in the neostriatal inhibitory network. Journal of Neurophysiology, doi:10.1152/jn.01259.2007.

Schoffelmeer, A.N., Hogenboom, F., Wardeh, G., De Vries, T.J., 2006. Interactions between CB_1 cannabinoid and mu opioid receptors mediating inhibition of neurotransmitter release in rat nucleus accumbens core. Neuropharmacology 51, 773–781.

Smith, A.D., Bolam, J.P., 1990. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends in Neurosciences 13, 259–265.

Soria, G., Castañé, A., Ledent, C., Parmentier, M., Maldonado, R., Valverde, O., 2006. The lack of A_{2A} adenosine receptors diminishes the reinforcing efficacy of cocaine. Neuropsychopharmacology 31, 978–987.

Solinas, M., Goldberg, S.R., 2005. Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. Neuropsychopharmacology 30, 2035–2045.

Surmeier, D.J., Song, W.J., Yan, Z., 1996. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. Journal of Neuroscience 16, 6579–6591.

Tanda, G., Pontieri, F.E., Di Chiara, G., 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276, 2048–2050.

Uchigashima, M., Narushima, M., Fukaya, M., Katona, I., Kano, M., Watanabe, M., 2007. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. Journal of Neuroscience 27, 3663–3676.

Usiello, A., Baik, J.H., Rougé-Pont, F., Picetti, R., Dierich, A., LeMeur, M., Piazza, P.V., Borrelli, E., 2000. Distinct functions of the two isoforms of dopamine D_2 receptors. Nature 408, 199–203.

Valverde, O., Noble, F., Beslot, F., Daugé, V., Fournié-Zaluski, M.C., Roques, B.P., 2001. Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. European Journal of Neuroscience 12, 533–539.

Vaysse, P.J., Gardner, E.L., Zukin, R.S., 1987. Modulation of rat brain opioid receptors by cannabinoids. Journal of Pharmacology and Experimental Therapeutics 241, 534–539.

Vigano, D., Rubino, T., Vaccani, A., Bianchessi, S., Marmorato, P., Castiglioni, C., Parolaro, D., 2005. Molecular mechanisms involved in the asymmetric interaction between cannabinoid and opioid systems. Psychopharmacology 182, 527–536.

Wager-Miller, J., Westenbroek, R., Mackie, K., 2002. Dimerization of G protein-coupled receptors: CB_1 cannabinoid receptors as an example. Chemistry and Physics of Lipids 121, 83–89.

Wilson, C.J., Groves, P.M., Kitai, S.T., Linder, J.C., 1983. Three-dimensional structure of dendritic spines in the rat neostriatum. Journal of Neuroscience 3, 383–388.

Yao, L., Fan, P., Jiang, Z., Mailliard, W.S., Gordon, A.S., Diamond, I., 2003. Addicting drugs utilize a synergistic molecular mechanism in common requiring adenosine and G_i-beta gamma dimers. Proceedings of the National Academy of Sciences of the United States of America 100, 14379–14384.

Yao, L., McFarland, K., Fan, P., Jiang, Z., Ueda, T., Diamond, I., 2006. Adenosine A_{2a} blockade prevents synergy between mu-opiate and cannabinoid CB_1 receptors and eliminates heroin-seeking behavior in addicted rats. Proceedings of the National Academy of Sciences of the United States of America 103, 7877–7882.

Yung, K.K., Smith, A.D., Levey, A.I., Bolam, J.P., 1996. Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. European Journal of Neuroscience 8, 861–869.

Zamir, N., Palkovits, M., Weber, E., Mezey, E., Brownstein, M.J., 1984. A dynorphinergic pathway of Leu-enkephalin production in rat substantia nigra. Nature 307, 643–645.

Zoli, M., Jansson, A., Syková, E., Agnati, L.F., Fuxe, K., 1999. Volume transmission in the CNS and its relevance for neuropsychopharmacology. Trends in Pharmacological Sciences 20, 142–150.