Receptor–Receptor Interactions and Glial Cell

Subjects: Cell Biology | Allergy

Contributor: Diego Guidolin

The discovery that receptors from all families can establish allosteric receptor–receptor interactions and variably associate to form receptor complexes operating as integrative input units endowed with a high functional and structural plasticity has expanded our understanding of intercellular communication. Regarding the nervous system, most research in the field has focused on neuronal populations and has led to the identification of many receptor complexes representing an important mechanism to fine-tune synaptic efficiency. Receptor–receptor interactions, however, also modulate glia–neuron and glia–glia intercellular communication, with significant consequences on synaptic activity and brain network plasticity.

Keywords: glial cells ; receptor–receptor interactions ; oligomerization ; allostery ; GPCR

1. Introduction

In the early 1980s, however, in vitro and in vivo experiments [1][2][3] provided indirect evidence that GPCRs may also establish structural receptor–receptor interactions (RRI), leading to the formation at the cell membrane of multimeric receptor complexes (see [4][5][6] for reviews) that operate as integrative input units [7]. In the years that followed, direct evidence for the existence of this structural organization was provided by several groups [8][9][10][11][12][13][14][15][16][17][18], and the amount of data supporting the existence of GPCR oligomers further increased when biophysical techniques capable of detecting the spatial proximity of protein molecules became available [19][20].

These findings demonstrated that GPCRs can signal both as monomers and as part of receptor complexes and indicated that oligomeric organization represents a quite common feature in the different receptor families, with the ion channel receptors (where multimerization is needed) lying at one end of the spectrum and GPCRs at the other [21]. Receptor channels, indeed, are constitutively multimeric [22], the majority of nuclear hormone receptors operate as homo- or hetero-dimers [23] and, with few exceptions [24], receptor tyrosine kinases need dimerization for their activation [25]. Thus, as pointed out by Changeux and Christopoulos in a detailed review [26], oligomerization emerges as an efficient mechanism for tuning the functionality of receptor proteins, including those able to signal as monomers, such as GPCRs. In this respect, recently reported evidence for receptor complexes involving protomers from different families [21][27] is also of significant interest.

RRI at the cell membrane have expanded our understanding of intercellular communication and they appeared to play a major role in the physiology and pathology of many districts of the body (see [21] for a review). Examples include the regulation of vascular homeostasis through the angiotensin II AT 1 receptor and its heterodimers [28], the chemoreceptor function of the carotid body [29] and the endocrine system, where a growing number of reports suggested receptor oligomerization as a significant aspect of endocrine regulation [30]. The possibility of pharmacological strategies targeting receptor heteromers has also been proposed in oncology [31]. However, the largest body of available data concerns the central nervous system (CNS). The formation of receptor complexes, indeed, is considered of key importance in neurophysiology (see [32][33][34][35] for more specific reviews), since the integration of input signals already at the level of the plasma membrane significantly helps to tune synaptic efficiency. Furthermore, increasing evidence indicates receptor complexes as potential targets for the treatment of serious diseases of the CNS [36][37][38].

In this context, glial cells, the non-action potential generating cells in the CNS, received less attention. More recently, however, the increased evidence that glial cells are not merely a support to neuronal life, but are actively involved in neuronal development, function and synaptic plasticity [39], generated an intense research interest focused on the mechanisms of glia–neural communication with significant new findings on the role played by receptor–receptor interactions in this process. Thus, after a brief recapitulation of the basic aspects concerning the structural biology of receptor complexes and their signaling, the available data on the role RRI play in the intercellular communication involving glial cells will be the focus of the present review article.

2. Structural Biology and Signaling of Receptor Complexes

It is well known that receptors can interact in a functional sense by sharing signaling patterns or by mechanisms of transactivation, even without coming into physical contact with each other [40]. The term RRI, on the contrary, indicates a type of interaction requiring direct physical contact between the receptors involved, leading to the formation of receptor complexes at the cell membrane. In this respect, a more detailed definition was provided in 2010 by a specific international consensus workshop [41]: “Receptor-receptor interactions: when the binding of a ligand to the orthosteric or allosteric sites of one receptor causes, via direct allosteric interactions, a change in the ligand recognition, decoding and trafficking processes of another receptor”. On this basis, it is also possible to provide an operational definition, in which the term RRI is translated into a set of experimental procedures leading to unambiguous numerical descriptions of the phenomenon [4]. In this respect, it is possible to maintain that two receptors are involved in an RRI process when the binding of one receptor causes detectable changes in the biochemical characteristics of the partner and the two receptor molecules are located in close proximity (<10 nm). In the last few decades, several biophysical techniques have been developed to detect the spatial proximity of protein molecules (see [6][19][20][36] for more details). They include energy transfer-based methods, bimolecular luminescence or fluorescence complementation, total internal reflection fluorescence microscopy, fluorescence correlation spectroscopy, coimmunoprecipitation, assays based on bivalent ligands and in situ proximity ligation assays.

Structural plasticity, however, is important not only to allow intra-receptor interactions and conformational fluctuations, but also to enable the formation of receptor complexes and their dynamics. When protomers, indeed, establish direct RRI leading to a quaternary structure, energy perturbations occurring at some site of one protomer can propagate over the interface between receptors into the nearby protomers, changing their conformational and functional properties, thus allowing the cooperative behavior of the complex [42].

The establishment of these supramolecular assemblies is considered of particular importance because it allows the emergence of integrative functions performed by a receptor complex as a whole [6]. In fact, owing to allosteric RRI, a configuration change of a given protomer will change the probability of changing the configuration for the adjacent receptors in the complex and the effect will propagate throughout the cluster, leading to complex collective behavior and to an integrated regulation of multiple effectors. These concepts have been well illustrated by mathematical models of cooperativity in receptor assemblies [43], based on discrete dynamics [44] or on thermodynamics-based approaches [45]. In the former case, receptors are supposed to assume a limited number of configurations (e.g., only two: “active” or “inactive”) changing in the time according to a “switching rule” based on the pattern of interactions each receptor establishes with the partners in the complex. In the latter case, the transition is stochastic and depends on the estimated energy of each protomer in the complex (see [43] for more details). Mathematical models indicated that receptor complexes can be described as possessing “emergent properties”, i.e., biochemical and functional features that cannot be fully anticipated on the basis of the characteristics of the single receptor partners [46].

In a variety of receptor complexes, the modulation of the binding sites has been reported as a consequence of allosteric RRI. Examples include the heterodimer between adenosine A 2A and dopamine D 2 GPCRs [36], where reciprocal antagonism occurs, and the human insulin RTK [47], a glycoprotein existing in two dimeric isoforms that exhibit significant differences in affinity for insulin-like growth factors. Changes in the decoding of signals reaching protomers represent a second mechanism induced by allosteric RRI. This aspect seems of particular importance in GPCRs, as illustrated by the heterodimer formed by dopamine D 1 and histamine H 3 receptors [48], in which the D 1 receptor changes its coupling from the G s to the G i protein, or by the switch from G protein to β-arrestin signaling [49] documented after κ-μ and κ-δ opioid receptor oligomerization. A final relevant aspect of receptor complex formation is the possibility that novel specific allosteric sites suitable for the binding of some modulators could appear in the quaternary structure resulting from the assemblage of protomers [50]. Thus, ligands specific to the receptor complex as such may also exist.

3. Receptor–Receptor Interactions in Glial Cells

Depression or enhancement of synaptic plasticity may also result from cannabinoid receptor-mediated astrocyte activation and the release of gliotransmitter ATP/adenosine, as suggested by studies on the basolateral amygdala [51]. In this respect, the identification by proximity ligation assay of cannabinoid CB 2 and GPR55 receptor complexes in the astrocytes of the dorsolateral prefrontal cortex of the human brain [52] is of potential interest. From the functional point of view, the results revealed an association between the expression levels of this heteromer and mood disorders, but no data are still available on the signaling features specific to this receptor complex.

Of interest in the study of neurological disorders with cognitive decline is the recent demonstration by proximity ligation assay of receptor complexes involving fibroblast growth factor receptor 1 (FGFR1) and serotonin 5HT 1A receptor in hippocampal astrocytes [53]. The FGFR1–5HT 1A heteroreceptor complex may allow astroglial modulation of the hippocampal neurons' gamma oscillations, a pattern of electrical activity (30–80 Hz) playing an important role in cognitive processes, such as memory storage and recall.

A quite large set of receptors allows them to detect molecular patterns associated with tissue damage, to modulate the release of cytokines and to facilitate phagocytosis. In this context, of potential interest for the present discussion are P2X (ligand-gated cationic channels) purinergic receptors. As a matter of fact, P2X 4 and P2X 7 are the dominant forms of P2X receptors expressed in microglia [54]. Although still a matter of debate (see [55]), the possible occurrence of P2X 4–P2X 7 heteromers has been reported in these cells [56], probably allowing a more sophisticated regulation of cytokine production and early inflammatory gene expression [56][54].

In the peripheral nervous system, Schwann cells are the myelinating cells and neuregulins represent an example of axonally derived ligands interacting with cognate receptors in Schwann cells to regulate their development and proliferation [57]. The receptors involved are erb2 and erb3, which become tyrosine phosphorylated and form erb2–erb3 heterodimers upon ligand binding [58].

4. Concluding Remarks and Perspectives

Intercellular communication represents a key feature of living organisms, and in the nervous system it determines virtually all aspects of its function. The main mechanism of communication in biological tissues involves the interaction of chemicals and/or energy forms released from a source with specific receptors expressed by the target cells. In the last few decades, the emerging evidence that receptors from all families can establish allosteric RRI and variably associate to form receptor complexes [21] indicated RRI as a basic mechanism modulating and tuning intercellular communication [6]. In a receptor complex, indeed, the configuration of each single receptor is shaped by a network of electrostatic interactions (hydrogen bonds and Van der Waals forces) defined by the presence of receptor partners, thereby enabling the complex to operate as an integrative input unit [7][42].

Several additional lines of future research, however, can be identified. In the CNS, indeed, chemical transmitters are released in two distinct transmission modes: wiring transmission and volume transmission (see [32][59] for reviews). Wiring transmission (WT) is intercellular communication mediated via physically defined connection structures. Synapses and related glial processes represent the typical example. Volume transmission (VT) occurs by the release and diffusion of chemical signals in the extracellular space defined by the intricate morphological organization of neurons, glial cells and extracellular matrix [60]. It is primarily mediated by simple diffusion, but also by pressure waves due to the arterial pulses, thermal gradients and local electric fields [61]. VT signals can be released from any type of brain cells and can be sensed by a relatively large number of cells, including microglia [62] and astrocytes [63][64][65]. VT mainly employs the same set of signals (transmitters, peptides, ions, gases) as WT (see [32] for a summary table). An important finding, however, was that non-synaptic receptors are usually characterized by high affinity for the signal [66]. Thus, an interesting topic for future research in the field could be a differential analysis of the glial and neural receptor complexes involved in the two forms of intercellular communication to assess possible differences in their signaling features. The analysis of such an issue could likely require a more detailed description of the cellular localization of the receptor complexes. As summarized before (see **Table 1**), this aspect has been addressed only to a limited extent, with the majority of available studies being aimed only at demonstrating the presence of receptor complexes in the cells. In some cell populations, however, this issue could be of significant physiological importance, as indicated by the increasing number of studies revealing the existence of functional microdomains in astrocytes (see [67] for a recent review). The term "microdomains" describes Ca 2+ events that are restricted to small portions of individual astrocyte territories and can either remain restricted locally or eventually propagate to the main processes and to the soma of the cells. The characterization of the panel of receptors and receptor complexes associated with these sub-cellular functional domains could, therefore, represent a key step to increase our understanding of the astrocyte role in brain function. This issue, however, poses some methodological challenges. Important techniques currently used to demonstrate receptor complexes, such as proximity ligation assays, also provide morphological information on their location. However, the obtainable resolution at light microscopy may be a limitation and the development of more suitable imaging techniques would be beneficial. In this respect, procedures based on 3D super-resolution microscopy [68], electron microscopy [69], and atomic force microscopy [42] supported by specific image analysis methods [42] have been suggested and may represent topics for further methodological development.

Table 1. Receptor complexes identified in glial cell populations.

Glial Cell Population	Receptor Complex	Cellular Localization	Reference
Astrocytes	A2A–D2	Striatal astrocyte processes	[70][71][72]
	CB2–GPR55	Plasma membrane	[52]
	FGFR1–5HT1A	Plasma membrane	[53]
	GABAB–SSTR4 (probable)	Cortical astrocyte processes	[69]
	A1–A2A	Plasma membrane	[73]
	5HT1A–D2	Mainly cell soma	[74]
	mGluR3–mGluR5 (putative)	Not reported	[75]
	A1–P2Y1	Plasma membrane	[76]
Microglia	P2X4–P2X7	Plasma membrane	[56]
	CB1–CB2	Plasma membrane	[77][78]
	A2A–CB2	Plasma membrane	[79]
	GPR18–CB2	Plasma membrane	[80]
Myelinating cells	GABAB1–GABAB2	OPC–neuron contacts	[81]
	erb2–erb3	Not reported	[58]

Receptor complexes are also of interest from a pharmacological standpoint, and their pharmacology certainly represents a significant line of future research. RRI, indeed, may provide new opportunities to optimize existing pharmacological treatments or to develop completely new pharmacological strategies. In this respect, the use of agonists/antagonists of single protomers in the receptor complexes has been, to some extent, successfully explored [36]. However, the search for receptor heteromers' selective compounds would be of key importance to fully exploit their properties. At least three approaches could be followed to achieve this goal. The first is based on the fact that, due to a different pattern of allosteric RRI, the conformational state of a given protomer may change according to the type of complex in which it is involved [82]. Thus, the pharmacology of some agonists/antagonists of a given protomer in terms of affinity and efficacy may show substantial differences among various types of receptor complexes. A second approach to identify receptor complex selective compounds is based on the possibility that, when the complex forms, the quaternary structure could display novel specific allosteric sites suitable for the binding of some modulators. The abovementioned effect of homocysteine on astrocytic A 2A –D 2 receptor complexes [72] provides an example. The use of bivalent ligands constitutes a third possible approach for targeting receptor heteromers (see [83] for a review). A bivalent ligand consists of two pharmacophoric entities linked by an appropriate spacer. In this way, it should be possible to target GPCR heteromers by adequate, potent, and receptor-selective pharmacophores. The work of Portoghese and collaborators on opioid receptor complexes (see [84]) provided a proof of principle. In this research effort, bioinformatics can be of help and MD simulations appear of particular importance in the field, since they allow the analysis of the conformational dynamics of receptors and receptor complexes in a realistic model of their biological environment, including the lipid bilayer and the extra- and intra-cellular water spaces [85]. MD methods, however, are in general computationally demanding and require specific software and expertise. On-line resources, however, are becoming available to facilitate MD data acquisition and analysis, and some of them are specifically designed to support studies on receptor proteins [86].

When applied to glial receptor complexes, these pharmacological research lines can represent a topic of particular interest from a therapeutical standpoint. Indeed, as suggested by some of the available studies discussed here, they open the possibility to explore novel, glia-mediated strategies to address neurodegenerative [78][79] and functional [52][53][87] CNS disorders.

References

1. Agnati, L.F.; Fuxe, K.; Zini, I.; Lenzi, P.; Hökfelt, T. Aspects on receptor regulation and isoreceptor identification. Med. Biol. 1980, 58, 182–187.
2. Agnati, L.F.; Fuxe, K.; Benfenati, F.; Battistini, N.; Härfstrand, A.; Takemoto, K.; Hökfelt, T.; Mutt, V. Neuropeptide Y in vitro selectivity increases the number of α2-adrenergic binding sites in membranes of the medulla oblongata of the rat. Acta Physiol. Scand. 1983, 118, 293–295.

3. Fuxe, K.; Agnati, L.F.; Benfenati, F.; Celani, M.; Zini, I.; Zoli, M.; Mutt, V. Evidence for the existence of receptor-receptor interactions in the central nervous system. Studies on the regulation of monoamine receptors by neuropeptides. J. Neural Transm. 1983, 18, 165–179.
4. Fuxe, K.; Marcellino, D.; Borroto-Escuela, D.O.; Frankowska, M.; Ferraro, L.; Guidolin, D.; Ciruela, F.; Agnati, L.F. The changing world of G protein-coupled receptors: From monomers to dimers and receptor mosaics with allosteric receptor-receptor interactions. J. Recept. Signal Transduct. Res. 2010, 30, 272–283.
5. Farran, B. An update on the physiological and therapeutic relevance of GPCR oligomers. Pharm. Res. 2017, 117, 303–327.
6. Guidolin, D.; Marcoli, M.; Tortorella, C.; Maura, G.; Agnati, L.F. G protein-coupled receptor-receptor interactions give integrative dynamics to intercellular communication. Rev. Neurosci. 2018, 29, 703–726.
7. Agnati, L.F.; Guidolin, D.; Vilardaga, J.P.; 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.
8. Fuxe, K.; Ferré, S.; Zoli, M.; Agnati, L.F. Integrated events in central dopamine transmission as analyzed at multiple levels. Evidence for intramembrane adenosine A2A/dopamine D2 and adenosine A1/dopamine D1 receptor interactions in the basal ganglia. Brain Res. Rev. 1998, 26, 258–273.
9. Bockaert, J.; Pin, J.P. Molecular tinkering of G proteincoupled receptors: An evolutionary success. EMBO J. 1999, 18, 1723–1729.
10. Marshall, F.H.; White, J.; Main, M.; Green, A.; Wise, A. GABA(B) receptors function as heterodimers. Biochem. Soc. Trans. 1999, 27, 530–535.
11. Xie, Z.; Lee, S.P.; O'Dowd, B.F.; George, S.R. Serotonin 5-HT1B and 5-HT1D receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Lett. 1999, 456, 63–67.
12. Franco, R.; Ferré, S.; Agnati, L.F.; Torvinen, M.; Ginés, S.; Hilion, J.; Casadò, V.; Lledò, P.; Zoli, M.; Lluis, C.; et al. Evidence for adenosine/dopamine receptor interactions: Indications for heteromerization. Neuropsychopharmacology 2000, 23, S50–S59.
13. Overton, M.C.; Blumer, K.J. G protein-coupled receptors function as oligomers in vivo. Curr. Biol. 2000, 10, 341–344.
14. Zeng, F.; Wess, J. Molecular aspects of muscarinic receptor dimerization. Neuropsychopharmacology 2000, 23, S19–S31.
15. Angers, S.; Salahpour, A.; Bouvier, M. Biochemical and biophysical demonstration of GPCR oligomerization in mammalian cells. Life Sci. 2001, 68, 2243–2250.
16. Dean, M.K.; Higgs, C.; Smith, R.E.; Bywater, R.P.; Snell, C.R.; Scott, P.D.; Upton, G.J.; Howe, T.J.; Reynolds, C.A. Dimerization of G protein-coupled receptors. J. Med. Chem. 2001, 44, 4595–4614.
17. Kenakin, T. Drug efficacy at G protein-coupled receptors. Annu. Rev. Pharm. Toxicol. 2002, 42, 349–379.
18. Waldhoer, M.; Fong, J.; Jones, R.M.; Lunzer, M.M.; Sharma, S.K.; Kostenis, E.; Portoghese, P.S.; Whistler, J.L. A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers. Proc. Natl. Acad. Sci. USA 2005, 102, 9050–9055.
19. Trifilieff, P.; Rives, M.L.; Urizar, E.; Piskorowski, R.A.; Vishwasrao, H.D.; Castrillon, J.; Schmauss, C.; Stätmann, M.; Gullberg, M.; Javitch, J.A. Detection of antigen interactions ex vivo by proximity ligation assay: Endogenous dopamine D2-adenosine A2A receptor complexes in the striatum. Biotechniques 2011, 51, 111–118.
20. Petazzi, R.A.; Aji, A.K.; Chiantia, S. Fluorescence microscopy methods for the study of protein oligomerization. In Progress in Molecular Biology and Translational Science; Giraldo, J., Ciruela, F., Eds.; Academic Press: Cambridge, MA, USA, 2020; Volume 169, pp. 1–42.
21. Guidolin, D.; Marcoli, M.; Tortorella, C.; Maura, G.; Agnati, L.F. Receptor-receptor interactions as a widespread phenomenon: Novel targets for drug development? Front. Endocrinol. 2019, 10, 53.
22. Corringer, P.-J.; Baaden, M.; Bocquet, N.; Delarue, M.; Dufresne, V.; Nury, H.; Prevost, M.; Van Renterghem, C. Atomic structure and dynamics of pentameric ligand-gated ion channels: New insights from bacterial homologs. J. Physiol. 2010, 588, 565–572.
23. Burris, T.P.; Solt, L.A.; Wang, Y.; Crumbley, C.; Banerjee, S.; Griffet, K.; Lundasen, T.; Hughes, T.; Kojetin, D.J. Nuclear receptors and their selective pharmacological modulators. Pharm. Rev. 2013, 65, 710–778.
24. Zahavi, E.E.; Steinberg, N.; Altman, T.; Chein, M.; Joshi, Y.; Gradus-Pery, T.; Perlson, E. The receptor tyrosine kinase TrkB signals without dimerization at the plasma membrane. Sci. Signal. 2018, 11, eaao4006.
25. Ulrich, A.; Schlessinger, J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990, 61, 203–212.

26. Changeaux, J.P.; Christopoulos, A. Allosteric modulation as a unifying mechanism for receptor function and regulation. Diabetes Obes. Metab. 2017, 19, 4–21.

27. Di Liberto, V.; Borroto-Escuela, D.O.; Frinchi, M.; Verdi, V.; Fuxe, K.; Belluardo, N.; Mudò, G. Evidence of muscarinic acetylcholine receptor (mAChR) and fibroblast growth factor receptor (FGFR) heteroreceptor complexes and their enhancement of neurite outgrowth in neural hippocampal cultures. Biochim. Biophys. Acta 2017, 1861, 235–245.

28. Tòth, A.D.; Turu, G.; Hunyady, L.; Balla, A. Novel mechanisms of GPCR functions: AT1 angiotensin receptor acts as a signaling hub and focal point of receptor cross-talk. Best Pract. Res. Clin. Endocrinol. Metab. 2018, 32, 69–82.

29. Stocco, E.; Sfriso, M.M.; Borile, G.; Contran, M.; Barbon, S.; Romanato, F.; Macchi, V.; Guidolin, D.; De Caro, R.; Porzionato, A. Experimental Evidence of A2A–D2 Receptor–Receptor Interactions in the Rat and Human Carotid Body. Front. Physiol. 2021, 12, 645723.

30. Kleinau, G.; Miller, A.; Biebermann, H. Oligomerization of GPCRs involved in endocrine regulation. J. Mol. Endocrinol. 2016, 57, 859–880.

31. Moreno, E.; Cavic, M.; Krivocuca, A.; Casadò, V.; Canela, E. The endocannabinoid system as a target in cancer diseases: Are we there yet? Front. Pharm. 2019, 10, 339.

32. Guidolin, D.; Marcoli, M.; Maura, G.; Agnati, L.F. New dimensions of connectomics and network plasticity in the central nervous system. Rev. Neurosci. 2017, 28, 113–132.

33. Borroto-Escuela, D.O.; Carlsson, J.; Ambrogini, P.; Narvàez, M.; Wydra, K.; Tarakanov, A.; Li, X.; Millón, C.; Ferraro, L.; Cuppini, R.; et al. Understanding the role of GPCR heteroreceptor complexes in modulating the brain networks in health and disease. Front. Cell. Neurosci. 2017, 11, 37.

34. Ferré, S.; Casadò, V.; Devi, L.A.; Filizola, M.; Jockers, R.; Lohse, M.J.; Milligan, G.; Pin, J.P.; Guitart, J. G protein-coupled receptor oligomerization revisited: Functional and pharmacological perspectives. Pharm. Rev. 2014, 66, 413–434.

35. Fuxe, K.; Borroto-Escuela, D.O. Heteroreceptor complexes and their allosteric receptor-receptor interactions as a novel biological principle for integration of communication in the CNS: Targets for drug development. Neuropsychopharmacology 2016, 41, 380–382.

36. Guidolin, D.; Marcoli, M.; Tortorella, C.; Maura, G.; Agnati, L.F. Adenosine A2A-Dopamine D2 Receptor-Receptor Interaction in Neurons and Astrocytes: Evidence and Perspectives. Prog. Med. Biol. Transl. Sci. 2020, 169, 247–277.

37. Borroto-Escuela, D.O.; Ambrogini, P.; Cruscicka, B.; Lindskog, M.; Crespo-Ramirez, M.; Hernàndez-Mondragòn, J.C.; Perez de la Mora, M.; Schellekens, H.; Fuxe, K. The role of central serotonin neurons and 5HT heteroreceptor complexes in the pathophysiology of depression: A historical perspective and future prospects. Int. J. Mol. Sci. 2021, 22, 1927.

38. Perez de la Mora, M.; Hernandez-Mondragon, J.C.; Crespo-Ramirez, M.; Regon-Orantes, J.; Borroto-Escuela, D.O.; Fuxe, K. Conventional and novel pharmacological approaches to treat dopamine-related disorders: Focus on Parkinson’s disease and schizophrenia. Neuroscience 2020, 439, 301–318.

39. Allen, N.J.; Lyons, D.A. Glia as arquitects of central nervous system formation an function. Science 2018, 362, 181–185.

40. Prezeau, L.; Rives, M.L.; Comps-Agrar, L.; Maurel, D.; Knlazeff, J.; Pin, J.P. Functional crosstalk between GPCRs: With or without oligomerization. Curr. Opin. Pharm. 2010, 10, 6–13.

41. Kenakin, T.; Agnati, L.F.; Caron, M.; Fredholm, B.; Guidolin, D.; Kobilka, B.; Lefkowitz, R.J.; Lohse, M.; Woods, A.; Fuxe, K. International workshop at the Nobel Forum, Karolinska Institutet on G protein-coupled receptors: Finding the words to describe monomers, oligomers and their molecular mechanisms and defining their meaning. J. Recept. Signal Transduct. Res. 2010, 30, 284–286.

42. Agnati, L.F.; Guidolin, D.; Leo, G.; Carone, C.; Genedani, S.; Fuxe, K. Receptor-receptor interactions: A novel concept in brain integration. Prog. Neurobiol. 2010, 90, 157–175.

43. Guidolin, D.; Ciruela, F.; Genedani, S.; Guescini, M.; Tortorella, C.; Albertin, G.; Fuxe, K.; Agnati, L.F. Bioinformatics and mathematical modeling in the study of receptor-receptor interactions and receptor oligomerization. Focus on adenosine receptors. Biochim. Biophys. Acta 2011, 1808, 1267–1283.

44. Agnati, L.F.; Guidolin, D.; Leo, G.; Fuxe, K. A Boolean network modelling of receptor mosaics: Relevance of topology and cooperativity. J. Neural Transm. 2007, 114, 77–92.

45. Jackson, M.B. Molecular and Cellular Biophysics; Cambridge University Press: Cambridge, UK, 2006; pp. 111–141.

46. Kenakin, T. Cellular assays as portals to seven-transmembrane receptor-based drug discovery. Nat. Rev. Drug Discov. 2009, 8, 617–626.

47. Andersen, M.; Nargaard-Pedersen, D.; Brandt, J.; Pettersson, I.; Slaabi, R. IGF1 and IGF2 specificities to the two insulin receptor isoforms are determined by insulin receptor amino acid 718. PLoS ONE 2017, 12, e0178885.

48. Ferrada, C.; Moreno, E.; Casadò, V.; Bongers, G.; Cortés, A.; Mallol, J.; Canela, E.I.; Leurs, R.; Ferré, S.; Lluís, C.; et al. Marked changes in signal transduction upon heteromerization of dopamine D1 and histamine H3 receptors. Br. J. Pharm. 2009, 157, 64–75.

49. Le Naour, M.; Lunzer, M.M.; Powers, M.D.; Kalyuzhny, A.E.; Benneyworth, M.A.; Thomas, M.J.; Portoghese, P.S. Putative Kappa Opioid Heteromers as Targets for Developing Analgesics Free of Adverse Effects. J. Med. Chem. 2014, 57, 6383–6392.

50. Agnati, L.F.; Leo, G.; Genedani, S.; Andreoli, N.; Marcellino, D.; Woods, A.; Piron, L.; Guidolin, D.; Fuxe, K. Structural plasticity in G-protein coupled receptors as demonstrated by the allosteric actions of homocysteine and computer-assisted analysis of disordered domains. Brain Res. Rev. 2008, 58, 459–474.

51. Martin-Fernandez, M.; Jamison, S.; Robin, L.M.; Zhao, Z.; Martín, E.D.; Aguilar, J.; Benneyworth, M.A.; Marsicano, G.; Araque, A. Synapse-specific astrocyte gating of amygdala-related behavior. Nat. Neurosci. 2017, 20, 1540–1548.

52. García-Gutiérrez, M.S.; Navarrete, F.; Navarro, G.; Reyes-Resina, I.; Franco, R.; Lanciego, J.L.; Giner, S.; Manzanares, J. Alterations in Gene and Protein Expression of Cannabinoid CB2 and GPR55 Receptors in the Dorsolateral Prefrontal Cortex of Suicide Victims. Neurotherapeutics 2018, 15, 796–806.

53. Narváez, M.; Andrade-Talavera, Y.; Valladolid-Acebes, I.; Fredriksson, M.; Siegele, P.; Hernandez-Sosa, A.; Fisahn, A.; Fuxe, K.; Borroto-Escuela, D.O. Existence of FGFR1-5-HT1AR heteroreceptor complexes in hippocampal astrocytes. Putative link to 5-HT and FGF2 modulation of hippocampal gamma oscillations. Neuropharmacology 2020, 170, 108070.

54. Köles, L.; Leichsenring, A.; Rubini, P.; Illes, P. P2 Receptor Signaling in Neurons and Glial Cells of the Central Nervous System. Adv. Pharmacol. 2011, 61, 441–493.

55. Trang, M.; Schmalzing, G.; Müller, C.E.; Markwardt, F. Dissection of P2X4 and P2X7 receptor current components in BV-2 microglia. Int. J. Mol. Sci. 2020, 21, 8489.

56. Guo, C.; Masin, M.; Qureshi, O.S.; Murrell-Lagnado, R. Evidence for Functional P2X4/P2X7 Heteromeric Receptors. Mol. Pharmacol. 2007, 72, 1447–1456.

57. Morrissey, T.K.; Levi, A.D.; Nuijens, A.; Sliwkowski, M.X.; Bunge, R.P. Axon-induced mitogenesis of human Schwann cells involves heregulin and p185erbB2. Proc. Natl. Acad. Sci. USA 1995, 92, 1431–1435.

58. Vartanian, T.; Goodearl, A.; Viehöver, A.; Fischbach, G. Axonal neuregulin signal cells of the oligodendrocyte lineage throughactivation of HER4 and Schwann cells through HER2 and HER3. J. Cell Biol. 1997, 137, 211–220.

59. Agnati, L.F.; Guidolin, D.; Guescini, M.; Genedani, S.; Fuxe, K. Understanding wiring and volume transmission. Brain Res. Rev. 2010, 64, 137–159.

60. Khelashvili, G.; Dorff, K.; Shan, J.; Camacho-Artacho, M.; Skrabanek, L.; Vroling, B.; Bouvier, M.; Devi, L.A.; George, S.R.; Javitch, J.; et al. GPCR-OKB: The G Protein Coupled Receptor Oligomer Knowledge Base. Bioinformatics 2010, 26, 1804–1805.

61. Syková, E.; Nicholson, C. Diffusion in Brain Extracellular Space. Physiol. Rev. 2008, 88, 1277–1340.

62. Färber, K.; Kettenmann, H. Physiology of microglial cells. Brain Res. Rev. 2005, 48, 133–143.

63. Hirase, H.; Iwai, Y.; Takata, N.; Shinohara, Y.; Mishima, T. Volume transmission signalling via astrocytes. Philos. Trans. R. Soc. B Biol. Sci. 2014, 369, 20130604.

64. Rózsa, M.; Baka, J.; Bordé, S.; Rózsa, B.; Katona, G.; Tamás, G. Unitary GABAergic volume transmission from individual interneurons to astrocytes in the cerebral cortex. Brain Struct. Funct. 2015, 222, 651–659.

65. Fuxe, K.; Agnati, L.F.; Marcoli, M.; Borroto-Escuela, D.O. Volume Transmission in Central Dopamine and Noradrenaline Neurons and Its Astroglial Targets. Neurochem. Res. 2015, 40, 2600–2614.

66. Vizi, E.S. Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the CNS. Pharmacol. Rev. 2000, 52, 63–89.

67. Lia, A.; Henriques, V.J.; Zonta, M.; Chiavegato, A.; Carmignoto, G.; Gómez-Gonzalo, M.; Losi, G. Calcium Signals in Astrocyte Microdomains, a Decade of Great Advances. Front. Cell. Neurosci. 2021, 15, 177.

68. Arizono, M.; Inavalli, V.V.G.K.; Panatier, A.; Pfeiffer, T.; Angibaud, J.; Levet, F.; Ter Veer, M.J.T.; Stobart, J.; Bellocchio, L.; Mikoshiba, K.; et al. Author Correction: Structural basis of astrocytic Ca2+ signals at tripartite synapses. Nat. Commun. 2020, 11, 1906.

69. Mariotti, L.; Losi, G.; Lia, A.; Melone, M.; Chiavegato, A.; Gómez-Gonzalo, M.; Sessolo, M.; Bovetti, S.; Forli, A.; Zonta, M.; et al. Interneuron-specific signaling evokes distinctive somatostatin-mediated responses in adult cortical astrocytes.

Nat. Commun. 2018, 9, 82.

70. Cervetto, C.; Venturini, A.; Passalacqua, M.; Guidolin, D.; Genedani, S.; Fuxe, K.; Borroto-Esquela, D.O.; Cortelli, P.; Woods, A.; Maura, G.; et al. A2A-D2 receptor-receptor interaction modulates gliotransmitter release from striatal astrocyte processes. J. Neurochem. 2016, 140, 268–279.
71. Pelassa, S.; Guidolin, D.; Venturini, A.; Averna, M.; Frumento, G.; Campanini, L.; Bernardi, R.; Cortelli, P.; Buonaura, G.C.; Maura, G.; et al. A2A-D2 Heteromers on Striatal Astrocytes: Biochemical and Biophysical Evidence. Int. J. Mol. Sci. 2019, 20, 2457.
72. Cervetto, C.; Venturini, A.; Guidolin, D.; Maura, G.; Passalacqua, M.; Tacchetti, C.; Cortelli, P.; Genedani, S.; Candiani, S.; Ramoino, P.; et al. Homocysteine and A2A-D2 Receptor-Receptor Interaction at Striatal Astrocyte Processes. J. Mol. Neurosci. 2018, 65, 456–466.
73. Cristovão-Ferreira, S.; Navarro, G.; Brugarolas, M.; Pérez-Capote, K.; Vaz, S.H.; Fattorini, G.; Conti, F.; Lluis, C.; Ribeiro, J.A.; McCormick, P.J.; et al. A1R-A2AR heteromers coupled to Gs and Gi/0 proteins modulate GABA transport into astrocytes. Purinergic Signal. 2013, 9, 433–449.
74. Kolasa, M.; Solich, J.; Faron-Gòreka, A.; Zurawek, D.; Pabian, P.; Lukasiewicz, S.; Kuśmider, M.; Szafran-Pilch, K.; Szlachta, M.; Dziedzicka-Wasylewska, M. Paroxetine and low-dose risperidone induce serotonin 5-HT1A and Dopamine D2 receptor heteromerization in the mouse prefrontal cortex. Neuroscience 2018, 377, 184–196.
75. Di Menna, L.; Joffe, M.E.; Iacovelli, L.; Orlando, R.; Lindsley, C.W.; Mairesse, J.; Gressèns, P.; Cannella, M.; Caraci, F.; Copani, A.; et al. Functional partnership between mGlu3 and mGlu5 metabotropic glutamate receptors in the central nervous system. Neuropharmacology 2017, 128, 301–313.
76. Tonazzini, I.; Trincavelli, M.L.; Montali, M.; Martini, C. Regulation of A1adenosine receptor functioning induced by P2Y1 purinergic receptor activation in human astroglial cells. J. Neurosci. Res. 2008, 86, 2857–2866.
77. Callén, L.; Moreno, E.; Barroso-Chinea, P.; Moreno-Delgado, D.; Cortés, A.; Mallol, J.; Casadó, V.; Lanciego, J.; Franco, R.; Lluis, C.; et al. Cannabinoid Receptors CB1 and CB2 Form Functional Heteromers in Brain. J. Biol. Chem. 2012, 287, 20851–20865.
78. Navarro, G.; Borroto-Escuela, D.O.; Angelats, E.; Etayo, I.; Reyes-Resina, I.; Pulido-Salgado, M.; Rodrìguez-Peréz, A.I.; Canela, E.I.; Saura, J.; Lanciego, J.L.; et al. Receptor-heteromer mediated regulation of endocannabinoid signaling in activated microglia. Role of CB1 and CB2 receptors and relevance for Alzheimer's disease and levodopa-induced dyskinesia. Brain. Behav. Immun. 2018, 67, 139–151.
79. Franco, R.; Reyes-Resina, I.; Aguinaga, D.; Lillo, A.; Jiménez, J.; Raïch, I.; Borroto-Escuela, D.O.; Ferreiro-Vera, C.; Canela, E.I.; De Medina, V.S.; et al. Potentiation of cannabinoid signaling in microglia by adenosine A2A receptor antagonists. Glia 2019, 67, 2410–2423.
80. Reyes-Resina, I.; Navarro, G.; Aguinaga, D.; Canela, E.I.; Schoeder, C.T.; Załuski, M.; Kieć-Kononowicz, K.; Saura, C.A.; Müller, C.E.; Franco, R. Molecular and functional interaction between GPR18 and cannabinoid CB2 G-protein-coupled receptors. Relevance in neurodegenerative diseases. Biochem. Pharmacol. 2018, 157, 169–179.
81. Bai, X.; Kirchhoff, F.; Scheller, A. Oligodendroglial GABAergic Signaling: More rhan Inhibition! Neurosci. Bull. 2021, 37, 1039–1050.
82. Fuxe, K.; Borroto-Escuela, D.O.; Romero-Fernandez, W.; Palkovits, M.; Tarakanov, A.O.; Ciruela, F.; Agnati, L.F. Moonlighting Proteins and Protein–Protein Interactions as Neurotherapeutic Targets in the G Protein-Coupled Receptor Field. Neuropsychopharmacology 2013, 39, 131–155.
83. Hiller, C.; Kühhorn, J.; Gmeiner, P. Class A G-Protein-Coupled Receptor (GPCR) Dimers and Bivalent Ligands. J. Med. Chem. 2013, 56, 6542–6559.
84. Daniels, D.J.; Lenard, N.R.; Etienne, C.L.; Law, P.-Y.; Roerig, S.C.; Portoghese, P.S. Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. Proc. Natl. Acad. Sci. USA 2005, 102, 19208–19213.
85. Javanainen, M. Universal Method for Embedding Proteins into Complex Lipid Bilayers for Molecular Dynamics Simulations. J. Chem. Theory Comput. 2014, 10, 2577–2582.
86. Rodriguez-Espigares, I.; Torrens-Fontanals, M.; Tiemann, J.K.S.; Aranda-García, D.; Ramírez-Anguita, J.M.; Stepniewski, T.M.; Worp, N.; Varela-Rial, A.; Morales-Pastor, A.; Medel-Lacruz, B.; et al. GPCRmd uncovers the dynamics of the 3D-GPRCome. Nat. Methods 2020, 17, 777–787.
87. Manev, H.; Uz, T.; Manev, R. Glia as a putative target for antidepressant treatments. J. Affect. Disord. 2003, 75, 59–64.