Multiple Adenosine-Dopamine (A2A-D2 Like) Heteroreceptor Complexes in the Brain and Their Role in Schizophrenia

Dasiel O. Borroto-Escuela 1,2,*, Luca Ferraro 3*, Manuel Narvaez 4, Sergio Tanganelli 5, Sarah Beggiato 3, Fang Liu 6, Alicia Rivera 7 and Kjell Fuxe 1,6*

1 Department of Neuroscience, Karolinska Institutet, 17170 Stockholm, Sweden
2 Observatorio Cubano de Neurociencias, Grupo Bohío-Estudio, 62100 Yaguajay, Cuba
3 Department of Life sciences and Biotechnology, University of Ferrara, 44121 Ferrara, Italy; frl@unife.it (L.F.); bggsrh@unife.it (S.B.)
4 Facultad de Medicina, Instituto de Investigacion de Málaga, Universidad de Málaga, 29016 Málaga, Spain; mnarvaez@uma.es
5 Department of Medical Sciences, University of Ferrara, 44121 Ferrara, Italy; tgs@unife.it
6 Campbell Research, Centre for Addiction and Mental Health Institute, University of Toronto, Toronto, ON MS5 1A1, Canada; Fang.Liu@camh.ca
7 Department of Cell Biology, University of Málaga, Instituto de Investigación Biomédica (IBIMA), 29016 Málaga, Spain; arivera@uma.es
* Correspondence: Dasiel.Borroto.Escuela@ki.se (D.O.B.-E.); kjell.fuxe@ki.se (K.F.); Tel.: +46-760396319 (D.O.B.-E.)

Received: 31 March 2020; Accepted: 20 April 2020; Published: 27 April 2020

Abstract: In the 1980s and 1990s, the concept was introduced that molecular integration in the Central Nervous System could develop through allosteric receptor–receptor interactions in heteroreceptor complexes present in neurons. A number of adenosine–dopamine heteroreceptor complexes were identified that lead to the A2A-D2 heteromer hypothesis of schizophrenia. The hypothesis is based on strong antagonistic A2A-D2 receptor–receptor interactions and their presence in the ventral striato-pallidal GABA anti-reward neurons leading to reduction of positive symptoms. Other types of adenosine A2A heteroreceptor complexes are also discussed in relation to this disease, such as A2A-D3 and A2A-D4 heteroreceptor complexes as well as higher order A2A-D2-mGluR5 and A2A-D2-Sigma1R heteroreceptor complexes. The A2A receptor protomer can likely modulate the function of the D4 receptors of relevance for understanding cognitive dysfunction in schizophrenia. A2A-D2-mGluR5 complex is of interest since upon A2A/mGluR5 coactivation they appear to synergize in producing strong inhibition of the D2 receptor protomer. For understanding the future of the schizophrenia treatment, the vulnerability of the current A2A-D2 like receptor complexes will be tested in animal models of schizophrenia. A2A-D2-Sigma1R complexes hold the highest promise through Sigma1R enhancement of inhibition of D2R function. In line with this work, Lara proposed a highly relevant role of adenosine for neurobiology of schizophrenia.

Keywords: adenosine receptors; A2A-D2 heteroreceptor complexes; schizophrenia; brain; novel pharmacology; heterobivalent drugs; sigma 1 receptor

1. Introduction

The concept was developed in the 1990s that the communication in the central dopamine neurons could become integrated with adenosine communication through the formation of heteroreceptor complexes built up of adenosine and dopamine receptors in a receptor subtype specific way located...
in the plasma membrane, specially extrasynaptic regions [1]. There exist two main receptors for the modulator of adenosine in the brain, namely adenosine A₁ and A₂A receptors. The major dopamine receptors in the brain are the D₁ and D₂ receptors but also D₃ and D₄ receptors play an important role in the brain [2]. Over the years a number of adenosine–dopamine heteroreceptor complexes have been found, inter alia A₂A-D₂, A₁-D₁, A₂A-D₃, and A₂A-D₄ heteroreceptor complexes besides the dopamine and adenosine homoreceptor complexes, see [2,3]. They illustrated the diversity and specificity of the former heteroreceptor complexes and their impact on molecular integration in the brain.

Adenosine represents an endogenous modulator of the neuronal and astroglia networks of the Central Nervous System (CNS) [2,4] acting via volume transmission [5,6]. Its concentration is dependent on the synthesis and breakdown of ATP which is metabolized to adenosine monophosphate (AMP) by adenosine kinase. The action of 5′-nucleotididase then removes the monophosphate from AMP and adenosine is formed. The intracellular and extracellular concentrations of adenosine are in equilibrium with each other through transporters. The inhibitory A₁ receptors have a widespread distribution in the brain and are especially enriched in the neocortex, the hippocampus, and cerellum [7,8]. Additionally in the striatum, they are mainly located in the striato-entopeduncular/nigral GABA neurons, called the direct pathway [1]. In contrast, the A₂A receptors are found in highest densities in the dorsal and ventral striato-pallidal GABA neurons [2,3,9,10].

The pharmacological treatment of schizophrenia did not begin with targeting the adenosine receptor but with targeting the dopamine (DA) receptors and the major receptor turned out to be the D₂ receptor [11–16]. The DA receptor in the meso-limbic system was proposed to be a major target [17]. This research represented the introduction of the DA hypothesis of schizophrenia. The increased activity in the meso-limbic DA neurons was explained on the basis of the combined glutamate/DA hypothesis of schizophrenia [17,18]. A reduced NMDA receptor function in the descending glutamate cortical systems to the ventral tegmental area (VTA) was proposed to lead to a reduced activation of their GABA interneurons associated with increased activation of the meso-accumbal DA neurons. As a result, the D₂ receptor induced inhibition of the ventral striato-pallidal anti-reward GABA neurons which were enhanced with a reduced glutamate drive reaching the frontal cortex from the mediodorsal glutamate neurons [19]. The mixed hypothesis of DA/glutamate interactions was also beautifully advanced by Fang Liu and colleagues who demonstrated that NMDA receptors and D₂ receptor can form heteroreceptor complexes in glutamate synapses in which the D₂ receptor protomer, upon activation, can significantly inhibit NMDA receptor signaling [20].

The clinical work on schizophrenia gives evidence that it is mainly the positive symptoms that are blocked after treatment with D₂ receptor antagonists, like hallucinations and delusions. Instead, cognitive and negative symptoms with lack of social interactions are more difficult to counteract.

The next step regarding the development of novel strategies for treatment of schizophrenia was the demonstration that A₂A receptor agonists can ex vivo strongly reduce the affinity of the D₂ receptor agonist binding sites in the high affinity state through antagonistic allosteric receptor–receptor interactions [19,21,22]. Of importance was also the demonstration using FRET and BRET, coimmunoprecipitation and in situ Proximity Ligation Assay (in situ PLA) that A₂A-D₂ heteroreceptor complexes can be formed [23–27]. It was also demonstrated that coaggregation and cointernalization can develop [28,29].

It was of high interest that the A₂A receptor agonist could be established to be an atypical antipsychotic drug in phencyclidine and amphetamine models of schizophrenia [30]. These results lead to the hypothesis that A₂A receptor agonists can be novel antipsychotic drugs by activating the antagonistic allosteric receptor–receptor interactions in the A₂A-D₂ heteroreceptor complexes located mainly in the ventral striato-pallidal GABA pathway. It represents an anti-reward pathway which is overactivated in schizophrenia due to increased activation of its D₂ receptors. The inhibition of the D₂ receptor function by A₂A receptor activation in this receptor complex can restore the glutamate drive to the frontal cortex from the medial dorsal thalamic nucleus [19,31].
In the current review, the $A_{2A}$-$D_2$ like heteroreceptor complexes will be discussed in detail in relation to schizophrenia. In addition, the potential role in schizophrenia of the adenosine $A_{2A}$ isoreceptor complexes, specially the $A_1$-$A_{2A}$ and $A_{2A}$-$A_{2B}$ complexes, will be covered [32–34]. Additionally, other types of adenosine $A_{2A}$R heteroreceptor complexes will be covered in relation to this disease such as $A_{2A}$-$D_3$ receptor [35] and $A_{2A}$-$D_4$ receptor complexes [36] as well as higher order $A_{2A}$R-$D_2$R-mGluR5 [37], and $A_{2A}$R-$D_2$R-Sigma1R [22,38] heteroreceptor complexes (see Figure 1). Can other types of $A_{2A}$ receptor complexes be involved in schizophrenia? Other adenosine hypotheses will also be explored besides the first one on antagonistic $A_{2A}$-$D_2$ interactions in heteroreceptor complexes in the nucleus accumbens bringing down activity in the overactive $D_2$ receptor protomer in schizophrenia [2,19,31,39,40].

**Figure 1.** $A_{2A}$-$D_2$ heteroreceptor complexes, $A_{2A}$ isoreceptor complexes, and higher order $A_{2A}$-$D_2$ heteroreceptor complexes are illustrated and exist mainly in the ventral and dorsal striatum. Their balance with the $A_{2A}$ and $D_2$ homoreceptor complexes are indicated as well as their allosteric receptor–receptor interactions. The nature of the allosteric receptor–receptor interactions in each complex is provided in the top part of the receptor complex. Antagonistic allosteric modulation is indicated as (-) and facilitatory allosteric modulation as (+).
2. The A2A-D2 Receptor Heteromer Hypothesis and the LARA et al. Adenosine Hypothesis of Schizophrenia

The A2A-D2 heteromer hypothesis of schizophrenia has been introduced and further advanced in a substantial number of papers from our group since the 1990s (see e.g., [1,2,19,31,41]). The most relevant location is its presence in the ventral striato-pallidal GABA neurons of the nucleus accumbens, representing anti-reward neurons, and their glutamate synapses originating mainly from the cerebral cortex. The accumbal pathway regulates the brain circuit to the prefrontal cortex from the ventral pallidum, via the medial dorsal thalamic glutamate system. There is a subnucleus specific loss of these glutamate neurons to the frontal cortex in schizophrenia [42]. It is of interest that D2 like receptor agonists microinjected into the nucleus accumbens significantly reduces extracellular glutamate levels in the prefrontal cortex as determined with dual probe microdialysis [1,43,44]. The glutamate drive from the medial dorsal thalamus to the prefrontal cortex is significantly reduced. This is in line with the evidence that D2 receptor agonists can enhance psychosis [14]. Of high interest is the evidence that microinjections of quinpirole (D2 receptor like agonist) together with the A2A receptor agonist CGS21680 into the nucleus accumbens block the reduction of extracellular glutamate levels in the prefrontal cortex by quinpirole and even lead to a significant A2A receptor agonist induced increase of extracellular glutamate levels in this region [19]. These results were strengthened by the demonstration that the A2A receptor agonist microinjected into the nucleus accumbens counteracted the reduction of extracellular GABA levels induced by the D2-like receptor agonist coinjected with the A2A-like receptor agonist. In a similar way, the D2-like agonist induced increase in the extracellular GABA levels in the medial dorsal thalamic nucleus was counteracted by the A2A receptor agonist [19,45].

There also exists a rat model of schizophrenia based on the amphetamine-induced sensitized state [46]. After an acute amphetamine challenge in the amphetamine sensitized state it was therefore tested if changes developed in the antagonistic A2A-D2 receptor–receptor interactions in the D2 receptor binding affinity of the ventral striatum [47]. Compared with the saline sensitized state, the A2A receptor agonist CGS21680 induced a reinstatement of the antagonistic A2A-D2 receptor interaction in the ventral striatum but not in the dorsal striatum. These results can help explain the atypical antipsychotic profile of the A2A receptor agonist since the motor effects are produced through actions in the dorsal striatum. In line with this view, the D2 receptor homomers dominate in the dorsal striatum in this model of schizophrenia [46]. The major action of amphetamine-induced dopamine release in the dorsal striatum appears to be an enhanced affinity of the D2 receptors for each other, leading to the increased formation of D2 receptor homomers.

Taken together, the results suggest that the antagonistic allosteric receptor–receptor interactions in A2A-R-D2 heteroreceptor complexes in the ventral striato-pallidal GABA neurons are the major target for the atypical antipsychotic actions of the A2A receptor agonist CGS21680. It leads to a marked reduction of D2 receptor protomer recognition and signaling. The dual microdialysis findings provide strong indications that the brake on D2 receptor protomer signaling induced by the A2A receptor agonist leads at the brain circuit level to increased activity in the medial dorsal thalamic glutamate system with increased glutamate drive reaching the prefrontal cortex [19,31]. In support of our A2A-R-D2 heteromer hypothesis of schizophrenia [2,19,31,41], an increase in A2A receptor agonist binding sites was found in the striatum of postmortem brains of chronic schizophrenics [48]. Furthermore, the salience dysregulation found in schizophrenia is likely produced by pathological increases in the activity of the meso-limbic DA neurons [49]. This overactivity can be blocked by restoring the brake on the D2R protomer signaling by agonist activation of the A2A receptor protomer signaling in several types of A2A-D2 heteroreceptor complexes on the ventral striato-pallidal GABA anti-reward neurons. In this way, the salience regulation can become normalized.

Another type of purinergic hypothesis of schizophrenia was introduced by Lara and colleagues in 2000 and is of high interest [50–53]. This model aimed to bring together many aspects of schizophrenia including the role of purines in the immune system. Allopurinol, which reduces purine degradation and increases brain levels of adenosine and inosine, was tested in poorly responsive patients with
schizophrenia [51]. The two patients with schizophrenia, tested with allopurinol, were clinically improved by this treatment which gives clinical support for their hypothesis. The same year they made the interesting observation that chronic treatment with clozapine, but not with haloperidol increased striatal ectonucleotidase activity. Based on their findings this likely leads to increases in adenosine levels and activation of inter alia adenosine A2A receptors [52], which can bring down the function of overactive D2 receptors through antagonistic receptor–receptor interactions. In their 2006 paper, Lara and colleagues proposed the highly relevant role of adenosine for the neurobiology of schizophrenia and its treatment [50]. Pharmacological treatment to increase the formation of adenosine in the brain is proposed by Lara and colleagues to be a relevant strategy using, for example, allopurinol which is well tolerated in patients with schizophrenia. In the future it will be of high interest to compare the effects of, for example, allopurinol and increased ectonucleotidase activity with effects of adenosine A1 receptor and/or A2A receptor agonists in the treatment of schizophrenia.

In 2011, one more adenosine hypothesis of schizophrenia was introduced [54]. Multiple adenosine targets were emphasized which involved also adenosine A1 and A2A receptor agonists and the enzyme adenosine kinase.

3. A1-A2A Isoreceptor Complexes

A major finding was the location of this receptor complex in the striatal glutamate nerve terminals [32]. At high concentrations adenosine activated the A2AR protomers, which through antagonistic A2A-A1 receptor–receptor interaction could reduce the inhibitory action of A1 receptor protomer on glutamate release through reduction of its affinity for A1 receptor agonists. Instead the facilitatory actions of the A2A receptor became dominant and enhancement of glutamate release was observed [32,34]. The available information indicates that the A2A receptor protomer has its major role in reducing symptoms of schizophrenia mainly through enhancing activity of the ventral anti-reward striato-pallidal GABA pathway (see above). It therefore seems possible that such actions can also develop through the activation of the A2A receptor protomer in the prejunctional A1-A2A receptor complex as described in this paragraph. It seems to develop through enhanced release of glutamate, enhancing the effects of the postjunctional antagonistic A2A-D2 receptor interaction also leading to increased activity GABA anti-reward neurons.

4. A2A-A2B Isoreceptor Complexes

These receptor complexes were demonstrated with a number of techniques such as FRET, BRET, bimolecular complemetation, and in situ Proximity Ligation Assay (in situ PLA) [33,55]. The most exciting finding was the observations that the A2B receptor protomer blocked the A2A receptor protomer ligand recognition and signaling [33]. The pharmacology of the A2A receptor was markedly lost since there was no high affinity for A2A-R ligands at the A2A receptor protomer. The potency of A2A receptor ligands to increase cAMP levels was also dramatically reduced.

These results also lead to new understanding for how schizophrenia can develop based on an excessive formation of A2A-A2B heteroreceptor complexes reducing the formation of the A2A-D2 heteroreceptor complexes in the ventral striato-pallidal GABA neurons. We assume the existence of a specific balance between different types of A2A isoreceptor and/or A2A heteroreceptor complexes in cortical and striatal subregions of the brain, involving both neuronal and/or glial cells [31,55,56]. Changes in the balance may in part have a genetic origin due to changes in the expression pattern of the A2A vs. the A2B and the D2 receptor, especially in the ventral striato-pallidal GABA anti-reward neurons [55,56]. Turning off the activity of the A2A receptor by increased formation of A2A-A2B receptor complexes should certainly increase the D2 receptor signaling with increased affinity for DA in the high affinity state [3,57], especially if higher order A2A-A2B-D2 receptor complexes may exist. The development of D2 receptor supersensitivity [58] in this way should result in a block of anti-reward information of the ventral striato-pallidal GABA neurons from reaching the frontal cortex due to inhibition of the medial dorsal thalamic nucleus [22]. As a result, due to the lack of this filter
mechanism, all anti-reward stimuli from this brain circuit are blocked from reaching the frontal cortex. Such a failure can contribute to development of psychotic symptoms in schizophrenia, since even irrelevant stimuli become regarded as salient.

5. **A2A-D3 Heteroreceptor Complexes**

The first evidence for their existence was obtained using FRET analysis \[35\] with a significant FRET efficiency demonstrated in the A2AR-D3R complex in the plasma membrane. Like in the A2A-D2 receptor complex, positively charged arginin epitopes were found in the third intracellular loop of the D3R interacting with negatively charged epitopes in the A2A located in its C-terminus \[2,25\]. Again, like in the case of the A2AR-D2, the A2A receptor agonist reduced the affinity of the high affinity binding site of the D3 receptor. The A2A receptor agonist CGS21680 also blocked the DA induced inhibition of the cAMP accumulation produced by activation of the D3 receptor \[35\]. Thus, it appears clear that there exists an allosteric antagonistic receptor–receptor interaction also in the A2A-D3 heteroreceptor complex. The D3 receptors are shown to exist in the brain especially in the frontal cortex, the nucleus accumbens, and the ventral midbrain, and are involved in motivation, emotions, and reward and anti-reward, but the A2A-D3 receptor complexes have not yet been analyzed in the brain.

Through the careful and excellent work of Pierre Sokoloff and Bernard Le Foll \[59\], we know that D3 receptor selective compounds are available for clinical trials in schizophrenia. By use of PET, these D3 selective compounds were shown to occupy D3 receptors in vivo. As to mechanisms involved in the D3R modulation of brain function, it can involve, for example, modulation of the glutamate projections from the prefrontal cortex to the nucleus accumbens and of dopamine nerve cell-dendritic networks in the ventral tegmental area rich in DA nerve cells projecting inter alia into the nucleus accumbens, the prefrontal cortex and the cingulate cortex. It will therefore be of substantial interest to demonstrate and understand the role of the A2A-D3 receptor complexes in these brain circuits.

6. **A2A-D4 Heteroreceptor Complexes**

It is of high interest that very recently the formation was demonstrated of A2A-D4 heteroreceptor complexes in different regions of the rat forebrain using in situ PLA \[36\]. Thus, A2A receptors can likely modulate the function also of the D4 receptors. It should be underlined that D4 receptor specific antagonists have not able to reduce antipsychotic effects in treatment of schizophrenia \[60,61\]. Instead the D4 receptors have a role in cognitive function and in modulating gamma oscillations \[62\]. In fact, the D4 receptor has a potential for reducing the cognitive deficits in schizophrenia. There are also positive interactions found in the ability of D4 receptors to interact with neuregulin/ErbB4 in the modulation of the activity of GABA parvalbumin positive interneurons in the prefrontal cortex and the hippocampus that regulate gamma oscillations and cognitive function \[62\]. Thus, there is the possibility that D4 agonists/antagonists, which alter D4 receptor function, can improve cognition deficits in schizophrenia. It will be highly interesting to see how A2A receptor protomers in the A2A-D4 complexes can modulate the cognition effects of the D4 receptor protomer in schizophrenia, especially in regions with high densities of the A2A-D4 receptor complexes.

7. **A2AR-D2R-mGlu5R Heteroreceptor Complexes**

Several groups have been involved in finding and working on these heteroreceptor complexes located especially in the soma-dendritic regions of the ventral striato-pallidal anti-reward GABA neurons \[19,31,37,40,63,64\]. The early work in 2001 \[65\] indicated the existence of A2AR-mGluR5 heteroreceptor complexes which demonstrated enhanced inhibition of D2 receptor function in the GABA anti-reward neurons. In 2009, evidence was obtained in cellular models that higher order A2A-D2-mGluR5 heteroreceptor complexes exist based on BRET and bimolecular fluorescence complementation \[37\]. The trimeric heteroreceptor complex appeared to be located mainly in perisynaptic locations in the ventral cortico-striatal glutamate synapses. The A2A and mGluR5 receptor protomer upon coactivation appeared to synergize to produce enhanced inhibition of the D2R protomer
recognition and signaling [37]. Such a strong inhibition of D₂ signaling [66] may be necessary when the D₂ shows a high increase in its activity in schizophrenia. Heterobivalent drugs can here be useful in the treatment of schizophrenia by using one A₂A receptor agonist pharmacophore in combination with a mGluR5 positive allosteric modulator pharmacophore to specifically target this receptor complex and inhibit the overactive D₂ receptor protomer signaling.

8. A₂A-D₂-Sigma1 Heteroreceptor Complexes

This trimeric complex is proposed to exist based on many findings, including the existence of A₂A-D₂-Sigma1 heteroreceptor complexes [38,40,67–70], and are of high interest. Thus, the participation of the chaperone protein Sigma1R in this receptor complex leads to a strong increase in the ability of the A₂A receptor protomer to inhibit the recognition and signaling of the D₂ receptor protomer [22]. The cocaine-induced increase in the density of the Sigma1R plays a relevant role as well as its ability to recruit the Sigma1R to the plasma membrane [71]. It remains to be demonstrated if the inhibition of the D₂ receptor protomer function by the A₂A R becomes too strong in the presence of Sigma1R to be used in treatment of schizophrenia (see Figure 2). However, it may be that in the absence of cocaine, the enhanced allosteric A₂A R mediated inhibition of the D₂R protomer function produced by the Sigma1R may be sufficient without leading to a pathological silence of the D₂R function (see Figure 2, panel B). If this is true, this research can lead to a new strategy for treatment of schizophrenia.

9. Conclusions and Future Work

As a new strategy of understanding schizophrenia and its treatment, the vulnerability of the current A₂A-D₂-like receptor complexes will be tested in models of schizophrenia in terms of changes in their density, composition, and stoichiometry as well as in their allosteric receptor-receptor interactions. The current results open up the possibility that the neurochemical results may be linked to schizophrenia-like behavior. In future work it will be of special interest to see which of the changes found in the various A₂A-D₂-like heteroreceptor complexes can correlate to the positive and/or negative symptoms found in models of schizophrenia. The A₂A-D₂-Sigma1 receptor complex appears to hold the highest promise. This is based on our hypothesis that A₂A receptor agonists may strengthen their inhibitory effects on overactive D₂ functions in the GABA anti-reward neurons in the presence of sigma1R in the heteroreceptor complex. Thus, A₂A receptor agonist treatment with or without

Figure 2. Proposed alterations of A₂A-D₂-Sigma1 heteroreceptor complexes in nucleus accumbens in schizophrenia. (A) A₂A-D₂-Sigma1 higher-order heteroreceptor complexes given in a control state. The adaptor protein Sigma1 receptor is given in red. (B) In schizophrenia there may develop an increased drive in the accumbal D₂ receptor protomer signaling as discussed. This hyperactivity may be countered by Sigma1R activation (e.g., increased density and/or treatment with Sigma1R agonists) due to its ability to enhance the allosteric A₂A receptor inhibition of the D₂ receptor protomer signaling and recognition in this heterotrimeric receptor complex. This increased inhibition may be brought about through an increased number of hotspot amino acids formed in the A₂A-D₂ receptor interface, outlined as red filled spaces.
co-treatment with Sigma1 receptor agonists should strongly enhance their antagonistic allosteric A2A-D2 receptor interactions. As a consequence, the positive symptoms of schizophrenia should be significantly reduced.

Author Contributions: This is a review article. We declare and confirm that all the authors meet the criteria for authorship according to ICMJE. This includes taking public responsibility for the work done as well as giving approval of the final manuscript. They have also full confidence regarding the integrity and accuracy of the work of other group authors and contributed to the concept or design of the work. They have taken part in the acquisition, analysis and interpretation of the data for the current review version. They have helped revise it in a critical way for important intellectual content and given approval to the version to be published. In this last version they have contributed in terms of writing assistance, technical editing and language editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from Swedish Medical Research Council (Vetenskapsrådet; 04X-715), from Parkinson Fonden to KF and from Hjärnfonden (2018-286 and 2019-296) to DOBE.

Acknowledgments: DOB-E belongs to Academia de Biologos Cubanos.

Conflicts of Interest: The authors declare no conflicts of interest.

References
1. Fuxe, K.; Ferre, 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. Brain Res. Rev. 1998, 26, 258–273. [CrossRef]
2. Fuxe, K.; Marcellino, D.; Borroto-Escuela, D.O.; Guesscini, M.; Fernandez-Duenas, V.; Tanganelli, S.; Rivera, A.; Ciruela, F.; Agnati, L.F. Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci. Ther. 2010, 16, e18–e42. [CrossRef] [PubMed]
3. Borroto-Escuela, D.O.; Pintsuk, J.; Schafer, T.; Friedland, K.; Ferraro, L.; Tanganelli, S.; Liu, F.; Fuxe, K. Multiple d2 heteroreceptor complexes: New targets for treatment of schizophrenia. Ther. Adv. Psychopharmacol. 2016, 6, 77–94. [CrossRef] [PubMed]
4. Fredholm, B.B. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol. Toxicol. 1995, 76, 93–101. [CrossRef] [PubMed]
5. Ferré, S.; Fuxe, K. Adenosine as a volume transmission signal. A feedback detector of neuronal activation. Prog. Brain Res. 2000, 125, 353–361. [PubMed]
6. Borroto-Escuela, D.O.; Agnati, L.F.; Bechter, K.; Jansson, A.; Tarakanov, A.O.; Fuxe, K. The role of transmitter diffusion and flow versus extracellular vesicles in volume transmission in the brain neural-glial networks. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2015, 370, 20140183. [CrossRef] [PubMed]
7. Ciruela, F.; Casado, V.; Malleol, J.; Canela, E.I.; Lluis, C.; Franco, R. Immunological identification of a1 adenosine receptors in brain cortex. J. Neurosci. Res. 1995, 42, 818–828. [CrossRef]
8. Zeraati, M.; Mirnajafi-Zadeh, J.; Fatollahi, Y.; Namvar, S.; Rezvani, M.E. Adenosine a1 and a2a receptors of hippocampal CA1 region have opposite effects on piriform cortex kindled seizures in rats. Seizure 2006, 15, 41–48. [CrossRef]
9. Schiffrmann, S.N.; Fisone, G.; Morescu, R.; Cunha, R.A.; Ferre, S. Adenosine a2a receptors and basal ganglia physiology. Prog. Neurobiol. 2007, 83, 277–292. [CrossRef]
10. Schiffrmann, S.N.; Vanderhaeghen, J.J. Adenosine a2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J. Neurosci. 1993, 13, 1080–1087. [CrossRef]
11. Carlsson, A.; Lindqvist, M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. (Copenh) 1963, 20, 140–144. [CrossRef] [PubMed]
12. Andén, N.E.; Butcher, S.G.; Corrodi, H.; Fuxe, K.; Ungerstedt, U. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Experientia 1970, 11, 303–314. [CrossRef]
13. Seeman, P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987, 1, 133–152. [CrossRef] [PubMed]
14. Seeman, P. Targeting the dopamine d2 receptor in schizophrenia. Expert Opin. Ther. Targets 2006, 10, 515–531. [CrossRef] [PubMed]
15. Seeman, P.; Kapur, S. Schizophrenia: More dopamine, more d2 receptors. Proc. Natl. Acad. Sci. USA 2000, 97, 7673–7675. [CrossRef] [PubMed]
16. Seeman, P.; Tallerico, T.; Ko, F.; Tenn, C.; Kapur, S. Amphetamine-sensitized animals show a marked increase in dopamine d2 high receptors occupied by endogenous dopamine, even in the absence of acute challenges. *Synapse* 2002, 46, 235–239. [CrossRef]

17. Fuxe, K. Biological and pharmacological theories. Discussion. In *The neuroleptics*; Bobon, D.P., Janssen, P.A.J., Bobon, J., Eds.; Karger: Basel, Switzerland, 1970; pp. 121–122.

18. Svensson, T.H. Dysfunctional brain dopamine systems induced by psychotomimetic nmda-receptor antagonists and the effects of antipsychotic drugs. *Brain Res. Rev.* 2000, 31, 320–329. [CrossRef]

19. Fuxe, K.; Marcellino, D.; Rivera, A.; Diaz-Cabiale, Z.; Filip, M.; Gago, B.; Roberts, D.C.; Langel, U.; Genedani, S.; Ferraro, L.; et al. Receptor-receptor interactions within receptor mosaics. Impact on neuropsychopharmacology. *Brain Res. Rev.* 2008, 58, 415–452. [CrossRef]

20. Liu, X.Y.; Chu, X.P.; Mao, L.M.; Wang, M.; Lan, H.X.; Li, M.H.; Zhang, G.C.; Parelkar, N.K.; Fibuch, E.E.; Haines, M.; et al. Modulation of d2r-nr2b interactions in response to cocaine. *Neuron* 2006, 52, 897–909. [CrossRef]

21. Ferre, S.; Von Euler, G.; Johansson, B.; Fredholm, B.B.; Fuxe, K. Stimulation of high-affinity adenosine a2 receptors decreases the affinity of dopamine d2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. USA* 1991, 88, 7238–7241. [CrossRef]

22. Borroto-Escuela, D.O.; Wydra, K.; Filip, M.; Fuxe, K. A2ar-d2r heteroreceptor complexes in cocaine reward and addiction. *Trends Pharmacol. Sci.* 2018, 39, 1008–1020. [CrossRef] [PubMed]

23. Canals, M.; Marcellino, D.; Fanelli, F.; Ciruela, F.; De Benedetti, P.; Goldberg, S.R.; Neve, K.; Fuxe, K.; Agnati, L.F.; Woods, A.S.; et al. Adenosine a2a-dopamine d2 receptor-receptor heteromerization: Qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. *J. Biol. Chem.* 2003, 278, 46741–46749. [CrossRef] [PubMed]

24. Fuxe, K.; Ferre, S.; Canals, M.; Torvinen, M.; Terasmaa, A.; Marcellino, D.; Goldberg, S.R.; Staines, W.; Jacobsen, K.X.; Lluis, C.; et al. Adenosine a2a and dopamine d2 heteromorphic receptor complexes and their function. *J. Mol. Neurosci.* 2005, 26, 209–220. [CrossRef]

25. Borroto-Escuela, D.O.; Marcellino, D.; Narvaez, M.; Flajolet, M.; Heintz, N.; Agnati, L.; Ciruela, F.; Fuxe, K. A serine point mutation in the adenosine a2ar c-terminal tail reduces receptor heteromerization and allosteric modulation of the dopamine d2r. *Biochem. Biophys. Res. Commun.* 2010, 394, 222–227. [CrossRef] [PubMed]

26. Borroto-Escuela, D.O.; Romero-Fernandez, W.; Tarakanov, A.O.; Gomez-Soler, M.; Corrales, F.; Marcellino, D.; Narvaez, M.; Frankowska, M.; Flajolet, M.; Heintz, N.; et al. Characterization of the a2ar-d2r interface: Focus on the role of the c-terminal tail and the transmembrane helices. *Biochem. Biophys. Res. Commun.* 2010, 402, 801–807. [CrossRef] [PubMed]

27. Kamiya, T.; Saitoh, O.; Yoshioka, K.; Nakata, H. Oligomerization of adenosine a2a and dopamine d2 receptors in living cells. *Biochem. Biophys. Res. Commun.* 2003, 306, 544–549. [CrossRef]

28. Hillion, J.; Canals, M.; Torvinen, M.; Casado, V.; Scott, R.; Terasmaa, A.; Hansson, A.; Watson, S.; Olah, M.E.; Mallol, J.; et al. Coaggregation, cointernalization, and codesensitization of adenosine a2ar receptors and dopamine d2 receptors. *J. Biol. Chem.* 2002, 277, 18091–18097. [CrossRef] [PubMed]

29. Borroto-Escuela, D.O.; Romero-Fernandez, W.; Tarakanov, A.O.; Ciruela, F.; Agnati, L.F.; Fuxe, K. On the existence of a possible a2a-d2-beta-arrestin2 complex: A2a agonist modulation of d2 agonist-induced beta-arrestin2 recruitment. *J. Mol. Biol.* 2011, 406, 687–699. [CrossRef]

30. Rimondini, R.; Ferre, S.; Ogren, S.O.; Fuxe, K. Adenosine a2a agonists: A potential new type of atypical antipsychotic. *Neuropsychopharmacology* 1997, 17, 82–91. [CrossRef]

31. Borroto-Escuela, D.O.; Carlsson, J.; Ambrogini, P.; Narvaez, M.; Wydra, K.; Tarakanov, A.O.; Li, X.; Millon, 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. [CrossRef]

32. Ciruela, F.; Casado, V.; Rodrigues, R.J.; Lujan, R.; Burgueno, J.; Canals, M.; Borycz, J.; Rebola, N.; Goldberg, S.R.; Mallol, J.; et al. Presynaptic control of striatal glutamatergic neurotransmission by adenosine a1-a2a receptor heteromers. *J. Neurosci.* 2006, 26, 2080–2087. [CrossRef] [PubMed]

33. Hinz, S.; Navarro, G.; Borroto-Escuela, D.; Seibt, B.F.; Ammon, Y.C.; De Filippo, E.; Danish, A.; Lacher, S.K.; Cervinkova, B.; Rafehi, M.; et al. Adenosine a2a receptor ligand recognition and signaling is blocked by a2b receptors. *Oncotarget* 2018, 9, 13593–13611. [CrossRef] [PubMed]
34. Cristovao-Ferreira, S.; Navarro, G.; Brugarolas, M.; Perez-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 g i/o proteins modulate gaba transport into astrocytes. *Purinergic Signal*. 2013, 9, 433–449. [CrossRef] [PubMed]

35. Torvinen, M.; Marcellino, D.; Canals, M.; Agnati, L.F.; Lluis, C.; Franco, R.; Fuxe, K. Adenosine a2a receptor and dopamine d3 receptor interactions: Evidence of functional a2a/d3 heteromeric complexes. *Mol. Pharmacol.* 2005, 67, 400–407. [CrossRef] [PubMed]

36. Fuxe, K.; Borroto-Escuela, D.O. Receptor-Receptor Interactions in the Central Nervous System; Humana Press: New York, NY, USA, 2018; Volume 140, p. 346.

37. Cabello, N.; Gandia, J.; Bertarelli, D.C.; Watanabe, M.; Lluis, C.; Franco, R.; Ferre, S.; Lujan, R.; Ciruela, F. Metabotropic glutamate type 5, dopamine d2 and adenosine a2a receptors form higher-order oligomers in living cells. *J. Neurochem.* 2009, 109, 1497–1507. [CrossRef] [PubMed]

38. Borroto-Escuela, D.O.; Narvaez, M.; Wydra, K.; Pintsuk, J.; Pinton, L.; Jimenez-Beristain, A.; Di Palma, M.; Jastrzebska, J.; Filip, M.; Fuxe, K. Cocaine self-administration specifically increases a2ar-d2r and d2r-sigma1r heteroreceptor complexes in the rat nucleus accumbens shell. Relevance for cocaine use disorder. *Pharmacol. Biochem. Behav.* 2017, 155, 24–31. [CrossRef]

39. 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. [CrossRef]

40. Borroto-Escuela, D.O.; Fuxe, K. Diversity and bias through dopamine d2r heteroreceptor complexes. *Curr. Opin. Pharmacol.* 2017, 32, 16–22. [CrossRef]

41. Ferre, S.; O’Connor, W.T.; Snaprud, P.; Ungerstedt, U.; Fuxe, K. Antagonistic interaction between adenosine a2a receptors and dopamine d2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. *Neuroscience* 1994, 63, 765–773. [CrossRef]

42. Popken, G.J.; Bunney, W.E.; Potkin, S.G.; Jones, E.G. Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. *Proc. Natl. Acad. Sci. USA* 2000, 97, 9276–9280. [CrossRef]

43. Tanganelli, S.; Sandager Nielsen, K.; Ferraro, L.; Antonelli, T.; Kehr, J.; Franco, R.; Ferre, S.; Agnati, L.F.; Fuxe, K.; Scheel-Kruger, J. Striatal plasticity at the network level. Focus on adenosine a2a and d2 interactions in models of parkinson’s disease. *Parkinsonism Relat Disord.* 2004, 10, 273–280. [CrossRef] [PubMed]

44. Antonelli, T.; Fuxe, K.; Agnati, L.; Mazzoni, E.; Tanganelli, S.; Tomasini, M.C.; Ferraro, L. Experimental studies and theoretical aspects on a2a/d2 receptor interactions in a model of parkinson’s disease. Relevance for l-dopa induced dyskinesias. *J. Neurol. Sci.* 2006, 248, 16–22. [CrossRef] [PubMed]

45. Agnati, L.F.; Ferre, S.; Lluis, C.; Franco, R.; Fuxe, K. Molecular mechanisms and therapeutic implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal gaba neurons. *Purinergic Signal.* 2013, 9, 509–550. [CrossRef] [PubMed]

46. Wang, M.; Pei, L.; Fletcher, P.J.; Kapur, S.; Seeman, P.; Liu, F. Schizophrenia, amphetamine-induced sensitized state and acute amphetamine exposure all show a common alteration: Increased dopamine d2 receptor dimerization. *Mol. Brain* 2010, 3, 25. [CrossRef]

47. Pintsuk, J.; Borroto-Escuela, D.O.; Lai, T.K.; Liu, F.; Fuxe, K. Altered regulation of ventral and dorsal striatal allosteric a2ar-d2r receptor-receptor interactions after amphetamine challenge: Relevance for schizophrenia. *Life Sci.* 2016, 167, 92–97. [CrossRef] [PubMed]

48. Kurumaji, A.; Toru, M. An increase in [3H] cgs21680 binding in the striatum of postmortem brains of chronic schizophrenics. *Brain Res.* 1998, 808, 320–323. [CrossRef]

49. Grace, A.A. Dopamine system dysregulation and the pathophysiology of schizophrenia: Insights from the methyloxazymethanol acetate model. *Biol. Psychiatry* 2015, 81, 5–8. [CrossRef]

50. Lara, D.R.; Dall’Ignia, O.P.; Ghisolfi, E.S.; Brunstein, M.G. Involvement of adenosine in the neurobiology of schizophrenia and its therapeutic implications. *Prog. Neuro Psychopharmacol. Biol. Psychiatry* 2006, 30, 617–629. [CrossRef]

51. Lara, D.R.; Brunstein, M.G.; Ghisolfi, E.S.; Lobato, M.I.; Belmonte-de-Abreu, P.; Souza, D.O. Allopurinol augmentation for poorly responsive schizophrenia. *Int. Clin. Psychopharmacol.* 2001, 16, 235–237. [CrossRef]

52. Lara, D.R.; Vianna, M.R.M.; De Paris, F.; Quevedo, J.; Osse, J.P.; Battastini, A.M.O.; Sarkis, J.J.F.; Souza, D.O. Chronic treatment with clozapine, but not haloperidol, increases striatal ecto-5′-nucleotidase activity in rats. *Neuropsychobiology* 2001, 44, 99–102. [CrossRef]
53. Lara, D.R.; Souza, D.O. Schizophrenia: A purinergic hypothesis. *Med. Hypotheses* **2000**, *54*, 157–166. [CrossRef] [PubMed]

54. Boison, D.; Singer, P.; Shen, H.Y.; Feldon, J.; Yee, B.K. Adenosine hypothesis of schizophrenia—Opportunities for pharmacotherapy. *Neuropsychopharmacology* **2012**, *62*, 1527–1543. [CrossRef] [PubMed]

55. Borroto-Escuela, D.O.; Hagman, B.; Woolfenden, M.; Pinto, L.; Jiménez-Beristain, A.; Olfijan, J.; Narvaez, M.; Di Palma, M.; Feltmann, K.; Sartini, S.; et al. In situ proximity ligation assay to study and understand the distribution and balance of gpcr homo- and heteroreceptor complexes in the brain. In *Receptor and Ion Channel Detection in the Brain*; Lujan, R., Ciruela, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2016; Volume 110, pp. 109–126.

56. Borroto-Escuela, D.O.; Brito, I.; Di Palma, M.; Jiménez-Beristain, A.; Narvaez, M.; Corrales, F.; Pita-Rodriguez, M.; Sartini, S.; Ambrogini, P.; Lattanzi, D.; et al. On the role of the balance of gpcr homo/heteroreceptor complexes in the brain. *J. Adv. Neurosci. Res.* **2015**, *2*, 36–44. [CrossRef]

57. Fuxe, K.; Borroto-Escuela, D.O.; Tarakanov, A.O.; Romero-Fernandez, W.; Ferraro, L.; Tanganelli, S.; Perez-Alea, M.; Di Palma, M.; Agnati, L.F. Dopamine d2 heteroreceptor complexes and their receptor-receptor interactions in ventral striatum: Novel targets for antipsychotic drugs. *Prog. Brain Res.* **2014**, *211*, 113–139.

58. Kostrzewa, R.M.; Wydra, K.; Filip, M.; Crawford, C.A.; McDougall, S.A.; Brown, R.W.; Borroto-Escuela, D.O.; Fuxe, K.; Gainetdinov, R.R. Dopamine d2 receptor supersensitivity as a spectrum of neurotoxicity and status in psychiatric disorders. *J. Pharmacol. Exp. Ther.* **2018**, *366*, 519–526. [CrossRef]

59. Sokoloff, P.; Le Foll, B. The dopamine d3 receptor, a quarter century later. *Eur. J. Neurosci.* **2017**, *45*, 2–19. [CrossRef]

60. Bristow, L.J.; Collinson, N.; Cook, G.P.; Curtis, N.; Freedman, S.B.; Kulagowski, J.J.; Leeson, P.D.; Patel, S.; Ragan, C.I.; Ridgill, M.; et al. L-745,870, a subtype selective dopamine d4 receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. *J. Pharmacol. Exp. Ther.* **2013**, *344*, 109–126. [CrossRef] [PubMed]

61. Tarazi, F.I.; Zhang, K.; Baldessarini, R.J. Dopamine d4 receptors: Beyond schizophrenia. *J. Recept. Signal. Transduct. Res.* **2004**, *24*, 131–147. [CrossRef]

62. Furth, K.E.; Mastwal, S.; Wang, K.H.; Buonanno, A.; Vullhorst, D. Dopamine, cognitive function, and gamma oscillations: Role of d4 receptors. *Front. Cell. Neurosci.* **2013**, *7*. [CrossRef]

63. Ferre, S.; Karcz-Kubicha, M.; Hope, B.T.; Popoli, P.; Burgueno, J.; Gutierrez, M.A.; Casado, V.; Fuxe, K.; Goldberg, S.R.; Lluis, C.; et al. Synergistic interaction between adenosine a2a and glutamate mglu5 receptors: Implications for striatal neuronal function. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11940–11945. [CrossRef]

64. Fuxe, K.; Agnati, L.F.; Jacobsen, K.; Hillion, J.; Canals, M.; Torvainen, M.; Tinner-Staines, B.; Staines, W.; Rosin, D.; Terasmaa, A.; et al. Receptor heteromerization in adenosine a2a receptor signaling: Relevance for striatal function and parkinson’s disease. *Neurology* **2003**, *61*, S19–S23. [CrossRef] [PubMed]

65. Popoli, P.; Pezzola, A.; Torvainen, M.; Reggio, R.; Pintor, A.; Scarchilli, L.; Fuxe, K.; Ferre, S. The selective mglu(5) receptor agonist chpg inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine d(2) receptors in the rat striatum: Interactions with adenosine a(2a) receptors. *Neuropsychopharmacology* **2001**, *25*, 505–513. [CrossRef]

66. Wieronska, J.M.; Zorn, S.H.; Doller, D.; Pilc, A. Metabotropic glutamate receptors as targets for new antipsychotic drugs. *Pharmacol. Ther.* **2016**, *157*, 10–27. [CrossRef] [PubMed]

67. Beggiato, S.; Borelli, A.C.; Borroto-Escuela, D.; Corbucci, I.; Tomasini, M.C.; Marti, M.; Antonelli, T.; Tanganelli, S.; Fuxe, K.; Ferraro, L. Cocaine modulates allosteric d2-sigma1 receptor-receptor interactions on dopamine and glutamate nerve terminals from rat striatum. *Cell. Signal.* **2017**, *40*, 116–124. [CrossRef] [PubMed]

68. Navarro, G.; Moreno, E.; Bonaventura, J.; Brugarolas, M.; Farre, D.; Aguinaga, D.; Mallol, J.; Cortes, A.; Casado, V.; Lluis, C.; et al. Cocaine inhibits dopamine d2 receptor signaling via sigma-1-d2 receptor heteromers. *PloS ONE* **2013**, *8*, e61245. [CrossRef] [PubMed]

69. Pinton, L.; Borroto-Escuela, D.O.; Narváez, M.; Jiménez-Beristain, A.; Olfijan, J.; Ferraro, L.; Agnati, L.F.; Fuxe, K. Dopamine d2 receptor dynamic and modulation in the d2r-sigma1r heteroreceptor complexes: Role in cocaine actions. In *European Neuropsychopharmacology*; Elsevier: Amsterdam, The Netherlands, 2015; Volume 25, pp. S609–S610.
70. Pinton, L.; Borroto-Escuela, D.O.; Narváez, M.; Oflijan, J.; Agnati, L.F.; Fuxe, K. Evidence for the existence of dopamine d2r and sigma 1 allosteric receptor-receptor interaction in the rat brain: Role in brain plasticity and cocaine action. In *European Society of Neurochemistry*; Springer: Berlin/Heidelberg, Germany, 2015; Volume 4, p. P37.
71. Kourrich, S.; Su, T.P.; Fujimoto, M.; Bonci, A. The sigma-1 receptor: Roles in neuronal plasticity and disease. *Trends Neurosci.* **2012**, *35*, 762–771. [CrossRef]