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INVITED REVIEW

Volume transmission and receptor-receptor interactions in heteroreceptor complexes: understanding the role of new concepts for brain communication

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Abstract

The discovery of the central monoamine neurons not only demonstrated novel types of brain stem neurons forming global terminal networks all over the brain and the spinal cord, but also to a novel type of communication called volume transmission. It is a major mode of communication in the central nervous system that takes places in the extracellular fluid and the cerebral spinal fluid through diffusion and flow of molecules, like neurotransmitters and extracellular vesicles. The integration of synaptic and volume transmission takes place through allosteric receptor-receptor interactions in heteroreceptor complexes. These heterocomplexes represent major integrator centres in the plasma membrane and their protomers act as moonlighting proteins undergoing dynamic changes and their structure and function. In fact, we propose that the molecular bases of learning and memory can be based on the reorganization of multiples homo and heteroreceptor complexes into novel assembles in the post-junctional membranes of synapses.

Key Words: *volume transmission; receptor-receptor interaction; heteroreceptor complexes; oligomerization; brain communication; learning; memory; central monoamine neurons*

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Central Monoamine Neurons

The central dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) neurons in the central nervous system (CNS) were discovered using the Falck-Hillarp technique and immunohistochemistry (Dahlstroem and Fuxe, 1964; Fuxe, 1965). Amazingly, these brainstem monoamine neurons were found to have extensive monosynaptic projections and collaterals forming global terminal networks all over the brain and the spinal cord. The parcellation of the monoamine cell groups in the brainstem was found to be phylogenetically highly conserved (Fuxe et al., 2009). They represent the foundation for our modern understanding of cellular communication in the brain, how drugs act on the brain and how this communication is altered in CNS disorders like Parkinson's disease, depression, schizophrenia, addiction, *etc.* It gave the beginning of a cellular basis for neuropsychopharmacology.

Volume Transmission

The discovery of the central monomine neurons also gave volume transmission (VT) to the wired brain (Fuxe et al., 2009). It was the discovery of the DA, NA and 5-HT neurons in the mammalian CNS in the 1960s and especially their pharmacological characterization that gave the essence of the experimental support for the introduction of the volume transmission theory by Fuxe and Agnati (1986). Since the first international symposium on "Volume transmission in the brain" organized in 1990 in Stockholm (Fuxe and Agnati, 1991), VT began to be considered a major mode of communication in the brain, which also is used by neuropsychoactive drugs to reach their targets (Nicholson and Sykova, 1998). VT represents a widespread mode of intercellular communication that occurs in the extracellular fluid of the brain and in the cerebrospinal fluid. VT signals are molecules like neurotransmitters, trophic factors, ions and gases (*e.g.*, NO) and move from the source cells to the target cells as a consequence of energy gradients leading to diffusion and flow (Descarries and Mechawar, 2000; Trueta and De-Miguel, 2012).

DA, NA and 5-HT neurons were shown to operate mainly *via* short distance (extrasynaptic) VT in the micrometers range in the local circuits of the CNS where the monoamine receptors are mainly extrasynaptically located. Long-distance VT over millimeters mainly involve peptide/protein

transmission like beta-endorphin, oxytocin, prolactin-like and interleukin-1beta transmission (Fuxe et al., 2009). The release of neuropeptides may allow the monoamine neurons to send VT signals to cellular networks further away from the monoamine terminal networks.

Recently, we proposed that extracellular vesicles (exosomes and shedding vesicles) mediate a special form of VT in the CNS (Borroto-Escuela et al., 2015) based on the fundamental work of Simons and Raposo (2009) in peripheral tissue. In this novel type of VT extracellular vesicles are safe vesicular carriers for targeted intercellular communication of proteins, including receptors and homeoproteins, mtDNA and different forms of RNA in the CNS migrating in the extracellular fluid along energy gradients to reach adjacent target cells.

Knowing the integration of synaptic transmission and VT will be fundamental for the understanding of brain function and the concept is introduced that a major site of this integration is represented by heteroreceptor complexes in which inter alia ion channel receptors / G protein coupled receptors (GPCRs) and GPCR/GPCR integrate their activity by direct receptor–receptor interactions through allosteric mechanisms (Fuxe et al., 2014b; **Figure 1**). The same neural-glial network can give a different balance of its outputs. This happens when the diffusing VT-signals (glially and/or neuronally formed) by up-regulating or down-regulating synaptic contacts mainly through receptor-receptor interactions which can change the integrative action of the network and differences in their balance of the outputs are achieved (Fuxe et al., 2013; Borroto-Escuela et al., 2015). This will have a major impact on the flow of information through the brain circuits regulated by this cellular network and thus on brain function and behaviors. Within the neural-glial network we postulated the existence of the CNS trophic unit. It consists of: neurons; glial cells; pericytes; blood vessels with endothelial cells; extracellular matrix (Borroto-Escuela et al., 2015). This term was used to indicate the smallest set of cells within the neural-glial networks which act in a complementary way to support the trophism of one another and includes the neurovascular unit. The neural network is postulated to survive and function through integration of volume transmission and wiring transmission in the trophic unit (Borroto-Escuela et al., 2015). This trophism gives the energy necessary for the neuronal network and its brain circuits to operate.

Receptor-Receptor Interactions in Heteroreceptor Complexes

In 1980–1983, we obtained, for the first time, indications for the existence of direct interactions between different types of GPCRs potentially forming heteroreceptor complexes with allosteric receptor-receptor interactions based on the ability of neuropeptides to modulate the binding characteristics of subtypes of monoamine receptors in membrane preparations (Fuxe et al., 1983). It is now concluded that GPCR heteroreceptor complexes with allosteric receptor-receptor interactions operating through the receptor interface have become major integrative centers at the molecular level and their receptor protomers act as moonlighting proteins (Fuxe et al., 2014a, b). The GPCR heteroreceptor complexes in the CNS have become exciting new targets for neurotherapeutics in Parkinson's disease, schizophrenia, drug addiction, anxiety and depression opening a new field in neuropsychopharmacology (Fuxe et al., 2014b).

Based on a mathematical approach, Tarakanov and Fuxe in 2010 deduced a set of triplet amino acid homologies that may importantly participate in the receptor interface of heteromers. They show how such protriplets of amino acid residues and their 'teams' may be utilized to construct a kind of code that determines (and/or predicts) which receptors should or should not form heterodimers (Borroto-Escuela et al., 2012). Based on the obtained results, they proposed a 'guide-and-clasp' manner for receptor-receptor interactions where 'adhesive guides' might be the triplet homologies.

Over many years evidence for the existence of antagonistic receptor-receptor interactions in A2A-D2 heteroreceptor complexes was obtained in the ventral and dorsal striatum and was validated with proximity ligation assay. In 2011, indications were obtained for the existence of a possible A2A-D2-β-Arrestin2 complex. The results suggested that the antagonistic A2AR-D2R allosteric receptor-receptor interaction in A2AR-D2R heteromers favors β-arrestin2 recruitment *versus* Gi/o coupling to the D2R protomer with subsequent cointernalization associated with a reduced time onset of Akt phosphorylation followed by a rapid dephosphorylation. Thus, a moonlighting phenomenon with a change of D2 receptor function takes place (Fuxe et al., 2014b; George et al., 2014).

Increased support was obtained for the view that A2A agonists at the A2A protomer in the A2A-D2 heteroreceptor complex are novel atypical antipsychotic drugs for treatment of schizophrenia as well as drugs for treatment of cocaine use disorder (Fuxe et al., 2014b; **Figure 1**). A2A receptor antagonists can instead act as blockers of the A2A protomer in the A2A-D2 heteroreceptor complex are antiparkinson drugs for the treatment of Parkinson's disease. It represents a continuation of our previous work starting already in 1974 on effects of methylxanthines in hemiparkinson rats. This is a rational continuation of our search for novel antiantiparkisson drugs since the late 1960s and 1970s, which resulted in the development of a number of DA receptor agonists like apomorphine, ET495 and pravidel (bromocriptine) for treatment of Parkinson's disease.

Our demonstration of facilitatory allosteric D2R–OTR interactions in the accumbens heteroreceptor complexes may represent at least a significant part of the molecular mechanism for oxytocin-induced changes in social and emotional behavior. D2R–OTR heteroreceptor complexes may become a new target for drug development and treatment of dysfunctions in the emotional networks of the brain taking place *e.g.*, in schizophrenia as seen from the negative symptoms. Our

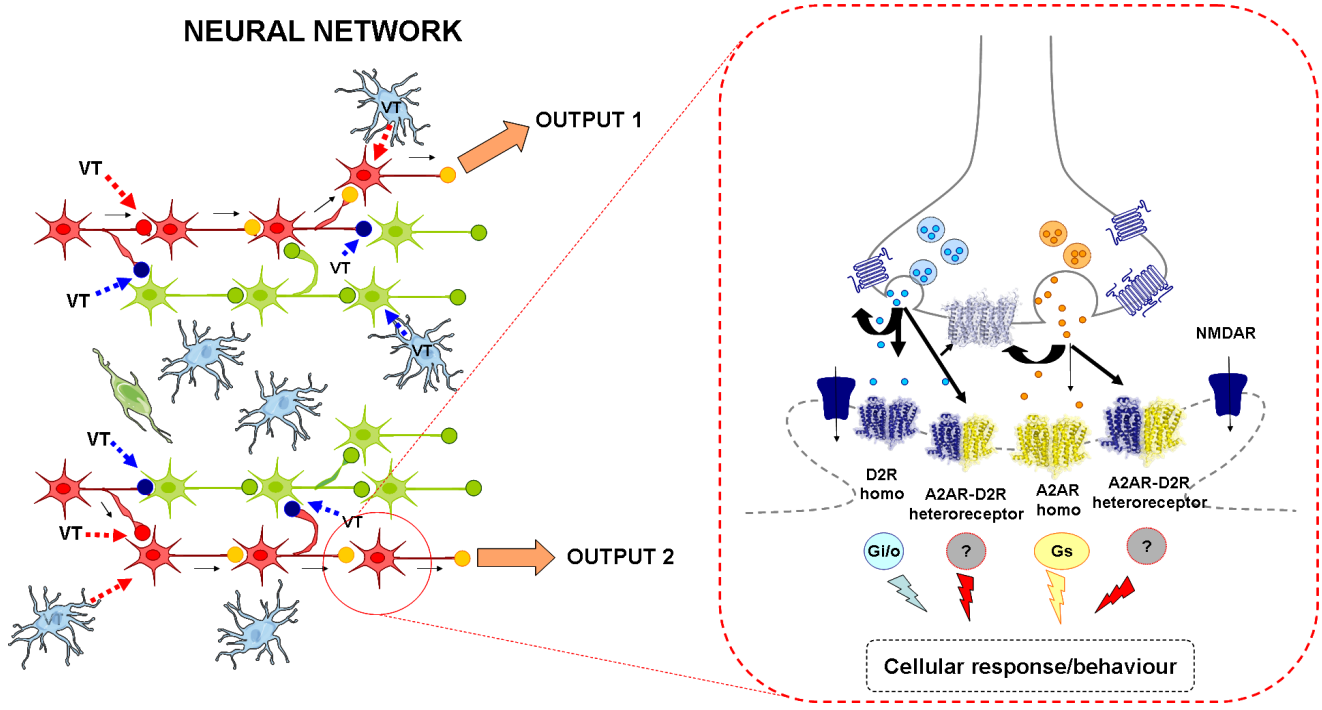

Figure 1 Balancing and integration of volume transmission (VT) and synaptic transmission through receptor-receptor interactions.
(Left panels) Relevance for the control of brain circuits. Changes in the modulation by VT of synaptic transmission have a fundamental role in the control of brain circuits. The same neural network can in this way give different outputs. As shown in this schematic illustration, VT signals *via* up (in red) and down (in blue) regulation of the synaptic strength of discrete synapses of the glial-neural network can give rise to different types of outputs through changes in the integrative interactions of the VT and synaptic signaling of the network (*e.g.*, output 1 and 2). The VT signals may significantly arise from glial cells of the local trophic unit, from extrasynaptic release of transmitters of neural afferents to the trophic unit, and from local collaterals and soma-dendrites of nerve cells of the trophic unit. Red intermittent arrow: VT signal upregulating synaptic strength; blue intermittent arrow: VT signal down regulating synaptic strength; filled red circle: upregulated synapse; filled blue circle: downregulated synapse; filled yellow circle: active synapse: filled green circle: inactive synapse. The blue cells illustrate glial cells. Active glial cells providing VT signals are labelled with VT. (Right panel) The major mechanism for the balance and integration of VT and synaptic transmission is likely the receptor-receptor interactions in synaptic and extrasynaptic heteroreceptor complexes located both at the prejunctional and postjunctional level and their signaling cascades. Illustration of the potential role of the reorganization of adenosine A2AR and dopamine D2R homo and heteroreceptor complexes in the postjunctional part of the striato-pallidal GABA neurons (synaptic and extrasynaptic components (not shown) partly as a consequence of the changes in neurotransmitter synthesis and release. The development of CNS disorders (*e.g.*, Schizophrenia, addiction) can involve an imbalance of the activity between different types of A2AR and D2R homo and heteroreceptor complexes. This may develop through reorganization of the available homo- and heteroreceptor complexes structurally and/or resetting their multiple allosteric receptor-receptor interactions, as well as by formation of novel receptor complexes potentially related to alterations in the pattern of release of synaptic and volume transmission signals.

work demonstrated that D2R is in fact a hub receptor capable of forming heteromers with many other types of GPCRs in cellular models and likely also in the neuronal-glial networks of the brain (Borroto-Escuela et al., 2014).

Our findings indicate that neurotrophic and antidepressant effects of 5-HT in brain may be mediated by activation of the 5-HT1A receptor protomer in the hippocampal FGFR1-5-HT1A receptor complex enhancing the FGFR1 signaling (Borroto-Escuela et al., 2012, 2016). Thus, a molecular neurotrophic mechanism exists in 5-HT nerve cell communication the activation of which may cause a relief from depression. A new strategy for treatment of depression is given. It represents an exciting continuation of the work of our group in this field which started with the introduction by Carlsson, Fuxe and Ungerstedt in 1968 of a novel target for antidepressants, the serotonin reuptake mechanism in the serotonin neurons (Carlsson et al., 1968). It led to the development of the selective serotonin re-uptake inhibitors (SSRIs).

Novel Concept on the Molecular Basis of Learning and Memory (Molecular Engram)

Molecular basis of learning and memory (molecular engram) is proposed to be based on the reorganization of multiple homo- heteroreceptor complexes into novel molecular assemblies in the postjunctional membrane of synapses with changes in the prejunctional receptor complexes to facilitate the pattern of transmitter release to be learned (Fuxe et al., 2014a; Borroto-Escuela et al., 2015).

Learning. A new temporal pattern of release of multiple transmitters in the synapse is learnt through a transient reorganization of sets of homo- and heteroreceptor complexes (receptors, ion channels, adapter proteins) in the postsynaptic and adjacent perisynaptic membranes. This results in novel

allosteric receptor-receptor interactions altering receptor protomer functions. In this way a short-term memory is created from the novel pattern of transmitter release to be learned leading to a novel transient bar-code representing a molecular engram of short term memory (Fuxe et al., 2014a; Borroto-Escuela et al., 2015). The novel pattern of transmitter release to be learned can be stabilized by reorganization of pre-synaptic and associated perisynaptic homo-heteroreceptor complexes. This can involve agonist dependent processes, release of soluble molecules like neuropeptides and trophic factors and exosomes from the post-perisynaptic membranes as a result of the new bar-code.

Long-term memory may be created by the transformation of parts of the heteroreceptor complexes into unique transcription factors which can lead to the formation of specific adapter proteins which can consolidate the heteroreceptor complexes into long-lived complexes with conserved allosteric receptor-receptor interactions (Fuxe et al., 2014a; Borroto-Escuela et al., 2015). Thus, new types of "barcodes" can be formed based on reorganized homo-and heteroreceptor complexes and on novel receptor complexes formed in the post and perisynaptic membranes of synapses through allosteric mechanisms:

1. Production of unique transcription factors from the new barcode.
2. Unique adapter proteins formed through trough these transcription factors. Thus, protein synthesis is needed.
3. Consolidation of the receptor complexes themselves and also to their link to the cytoskeleton.
4. Volume transmission signals from adjacent terminals of emotional pathways reaching the postjunctional membrane leads to life-long memories through exceptional formation of special adapter proteins.

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