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Neurotransmitter receptor heteromers and their integrative role in 'local modules': The striatal spine module

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Abstract

'Local module' is a fundamental functional unit of the central nervous system that can be defined as the minimal portion of one or more neurons and-or one or more glial cells that operates as an independent integrative unit. This review focuses on the importance of neurotransmitter receptor heteromers for the operation of local modules. To illustrate this, we use the striatal spine module (SSM), comprised of the dendritic spine of the medium spiny neuron (MSN), its glutamatergic and dopaminergic terminals and astroglial processes. The SSM is found in the striatum, and although aspects such as neurotransmitters and receptors will be specific to the SSM, some general principles should apply to any local module in the brain. The analysis of some of the receptor heteromers in the SSM shows that receptor heteromerization is associated with particular elaborated functions in this local module. Adenosine A_{2A} receptor-dopamine D₂ receptor-glutamate metabotropic mGlu₅ receptor heteromers are located adjacent to the glutamatergic synapse of the dendritic spine of the enkephalin MSN, and their cross-talk within the receptor heteromers helps to modulate postsynaptic plastic changes at the glutamatergic synapse. A₁ receptor-A_{2A} receptor heteromers are found in the glutamatergic terminals and the molecular cross-talk between the two receptors in the heteromer helps to modulate glutamate release. Finally, dopamine D₂ receptor-non- α_7 nicotinic acetylcholine receptor heteromers, which are located in dopaminergic terminals, introduce the new concept of autoreceptor heteromer.

Keywords

Local module; receptor heteromer; volume transmission; dopamine; glutamate; acetylcholine; adenosine; striatum

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1. Introduction

Computation in the central nervous system (CNS) can be performed at various levels. From higher to lower magnification these levels have been identified as ‘neuronal networks’, ‘neurons’, ‘local circuits’ and ‘molecular networks’. Neuronal networks are made of assemblies of neurons that carry out specific functions (Laughlin and Sejnowski, 2003; Buzsaki and Draguhn, 2004; Tsodyks and Gilbert, 2004). The neuronal level of computation has been classically viewed as the summing up of synaptic inputs and the initiation of an action potential when a threshold is reached. However, linear and nonlinear mechanisms in the dendritic tree also play a role in the overall computation performed by the neuron (London and Häusser, 2005). Local circuit has been defined as “any portion of the neuron (or neurons) that, under given conditions, functions as an independent integrative unit” (Goldman-Rakic, 1975). However, this definition is too broad, as a particular local circuit could overlap or be part of another local circuit. It is also too restrictive, as it does not include glial cells, which participate in neuronal processing (Fields and Stevens-Graham, 2002). Furthermore, the word ‘circuit’ implies direct ‘wired pathways’ and extrasynaptic neurotransmission (‘volume transmission’) plays an important role at this level of computation (see below). Therefore, we use the term ‘local module’ to define **the minimal portion of one or more neurons and-or one or more glial cells that operates as an independent integrative unit.**

Molecular networks are made of biomolecules (especially proteins) that are functionally interconnected and can elaborate and transmit information (Xia et al., 2004). Especially relevant for this review are molecular networks that contain protein complexes localized in the plane of the membrane, the so-called ‘horizontal molecular networks’, which include ‘neurotransmitter receptor heteromers’ (Agnati et al., 2003, 2005; Franco et al., 2003). In this review the term neurotransmitter is used as defined by Snyder and Ferris (2000), i.e. a molecule, released by neurons or glia, which physiologically influences the electrochemical state of adjacent cells. This definition includes previous ill-defined terms, such as neuromodulator, neuropeptide, and also gaseous messenger, such as nitric oxide and carbon monoxide. In order to understand the integrative capabilities of the CNS, we must consider both the spatial-temporal relationships among informational elements within a certain computational level and the spatial-temporal relationships among computations carried out at the different levels. This functional hierarchical organization of the computational devices in the CNS raises the question of how information circulates among the different levels. As it will be shown in this review, neurotransmitter receptor heteromers are functional units of special relevance since they act as a ‘bridge’ between the molecular networks and the local module levels of computation.

It is often assumed that communication between neurons just takes place in the synapse, where only one type of neurotransmitter is released to stimulate receptors for that neurotransmitter localized in the synapse. Several assumptions of those classical ideas are wrong. Neurons can release more than one type of biochemical signal (Hokfelt et al., 2000) and neurotransmitters can spill over from the synaptic cleft or be released from asynaptic varicosities and stimulate extrasynaptic receptors. This implies a diffuse mode of extrasynaptic neurotransmission, also called ‘volume transmission’ (Fuxe and Agnati, 1991; Nicholson and Sykova, 1998; Agnati and Fuxe, 2000; Vizi et al., 2004; Bach-y-Rita, 2005). While we would expect to find receptor heteromers in the synaptic space, it is in the extrasynaptic space where we should find most receptor heteromers that are targeted by different neurotransmitters. Although the distance that a neurotransmitter or neuromodulator can cover in the extrasynaptic space is a matter of debate (Fuxe and Agnati, 1991; Nicholson and Sykova, 1998; Agnati and Fuxe, 2000; Vizi et al., 2004; Bach-y-Rita, 2005), volume transmission must have its maximal expression in the perisynaptic space (both at the pre- and post-synaptic sites), and in the vicinity of the asynaptic varicosities. Therefore, volume transmission and neurotransmitter receptor heteromers are important variables for the computation of local modules. This review focuses on the

importance of neurotransmitter receptor heteromers for the operation of local modules. To illustrate this, we use the striatal spine module (SSM). This module is found in the striatum, and although aspects such as neurotransmitters and receptors will be specific to the SSM, some general principles should apply to any local module in the brain.

2. Neurotransmission in the SSM

2.1. Structural elements of the SSM

The striatal efferent neuron, also called the medium spiny neuron (MSN), constitutes more than 95% of the striatal neuronal population (Smith and Bolam, 1990; Gerfen, 2004). It is an inhibitory neuron that uses γ -aminobutyric acid (GABA) as its main neurotransmitter. In addition, there are different types of inhibitory interneurons which also use GABA, and large cholinergic interneurons (Smith and Bolam, 1990; Gerfen, 2004). There are two subtypes of MSN, which selectively express one of two peptides — enkephalin or dynorphin. Enkephalin MSNs predominantly express D₂Rs (D_{2L}R isoform) and A_{2A}Rs, while dynorphin MSNs predominantly express dopamine D₁ receptors (D₁Rs) and adenosine receptors of the A₁ subtype (Ferré et al., 1997; Agnati et al., 2003; Gerfen et al., 2004). The MSN receives two main afferents: glutamatergic afferents from cortical, thalamic and limbic areas and dopaminergic afferents from the mesencephalon (substantia nigra and ventral tegmental area). Both sets of afferents converge on the dendritic spine of the MSN (Fig. 1). Glutamatergic and dopaminergic terminals make preferential synaptic contacts with the head and the neck of the dendritic spine, respectively (Smith and Bolam, 1990; Gerfen, 2004). The dendritic spine, the glutamatergic terminal, the dopaminergic terminal and astroglial processes form part of the most common SSM (Fig. 1). We must, however, point out that the segregation of dopamine and adenosine receptors in the two subtypes of MSNs implies the existence of at least two subtypes of SSMs, which we could call enkephalin SSM and dynorphin SSM.

2.2. Dopaminergic neurotransmission in the SSM

The arrangement of the elements of the SSM allows mesencephalic dopaminergic inputs to modulate glutamatergic excitatory inputs from the cortex, limbic system and thalamus. The MSN moves between a 'down-state' and an 'up-state'. The neuron can generate action potentials only during the up-state, which depends on it receiving temporally convergent excitatory inputs. In dynorphin MSNs, stimulation of postsynaptic dopamine D₁Rs has different effects depending on the state of the neuron, because it is coupled to different ion channels (Nicola et al., 2000). In the down-state, D₁R activation facilitates the function of inward rectifier K⁺ channels (Kirs) of the Kir2 subfamily, which suppresses the response to weak excitatory inputs, making it more difficult for the neuron to switch to the up-state. In the up-state, D₁R activation facilitates the function of L-type voltage-dependent Ca²⁺ channels (VDCCs), which enhances evoked activity. Thus, in the dynorphin MSN, dopamine enhances the effect of strong input signals but dampens the effect of weak signals. In enkephalin MSNs, dopamine decreases neuronal excitability because stimulated D₂Rs inactivate the conductance of L-type VDCCs (Hernandez-Lopez et al., 2000). Thus, in the enkephalin MSN, dopamine does not enhance strong input signals, but still decreases the effect of weak glutamate signals.

As well as filtering glutamatergic inputs through synaptic transmission to postsynaptic dopamine receptors, dopamine also acts in the SSM through volume transmission and stimulation of extrasynaptic receptors. About 60% of dopaminergic axon terminals (varicosities) do not contain synaptic specializations (Descarries et al., 1996). The dopamine transporter is crucial for preventing the extracellular accumulation of dopamine. It is localized outside extrasynaptically, so it acts in competition with extrasynaptic dopamine receptors (Hersch et al., 1997). Dopamine can spill over from synaptic sources or diffuse away from asynaptic dopaminergic varicosities (Venton et al., 2003) and stimulate extrasynaptic receptors

in different locations in the SSM. Extrasynaptic dopamine receptors are commonly found in the perisynaptic ring of the glutamatergic synapse on MSNs (Yung et al., 1995). Extrasynaptic D_{2/4}Rs are also found presynaptically at glutamatergic (Tarazi et al., 1998a; Bamford et al., 2004; Brady and O'Donnell, 2004) and dopaminergic terminals on enkephalin MSNs, where they act as autoreceptors (Sesack et al., 1994; Usiello et al., 2000). There is good evidence that dopamine provides inhibitory modulation of the corticostriatal glutamatergic input through presynaptic D_{2/4}Rs (Bamford et al., 2004; Brady and O'Donnell, 2004). This effect is selective for the least active glutamatergic terminals, (Bamford et al., 2004), thereby contributing to the filter that selects the most active corticostriatal inputs.

2.3. Glutamatergic neurotransmission in the SSM

The neurotransmitter glutamate is the main responsible for fast excitatory neurotransmission in the CNS, which depends on stimulation of ionotropic receptors (*N*-methyl-D-aspartate receptors (NMDARs), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) and kainate-type receptors), which are mostly localized in the postsynaptic density (Bernard and Bolam, 1998). But glutamate can also spill over from the glutamatergic synapse and stimulate extrasynaptic receptors at glutamatergic and dopaminergic synapses (Gracy and Pickel, 1996; Tarazi et al., 1998b; Paquet et al., 2003; Galvan et al., 2006). As for dopamine, glutamate transporters outside the synaptic zone are vital for preventing extracellular accumulation of glutamate (Huang and Bergles, 2004). The two most abundant glutamate transporters (excitatory amino-acid transporter 1 (EAAT1) and EAAT2) are found in astrocyte membranes which are closely apposed to glutamatergic synapses (Huang and Bergles, 2004) (Fig. 1). Extrasynaptic NMDARs seem to be particularly localized both pre- and postsynaptically at dopaminergic synapses in the ventral aspect of the striatum (Gracy and Pickel, 1996). Their localization in dopaminergic terminals could explain the striatal dopamine release induced by NMDAR stimulation observed in different *in vivo* and *in vitro* studies (Morari et al., 1998). The postsynaptic and perisynaptic localization of NMDARs provides a frame for functional interactions between NMDARs and D₁Rs. Stimulation of D₁Rs potentiates NMDAR-mediated currents (Levine et al., 1996) and there is evidence that the signaling pathways of the two receptors interact closely (Dudman et al., 2003). These NMDAR-D₁R interactions depend on the existence of physical contact and heteromerization between D₁Rs and specific subunits of NMDARs (Lee et al., 2002; Woods and Ferré, 2005).

It is improbable that endogenous glutamate spilling over from the glutamatergic synapse is able to stimulate extrasynaptic ionotropic receptors, in view of the key role of astrocytes on the modulation of the relatively high extracellular levels of glutamate (Baker et al., 2002; Del Arco et al., 2003). A more plausible hypothesis is that extrasynaptic ionotropic glutamate receptors are under control of astrocytic glutamate release, which depends on neuronal glutamate release (Fields and Stevens-Graham, 2002; Del Arco et al., 2003), justifying the inclusion of astroglial processes in the local module (Fig. 1). It must be pointed out that a different pattern of involvement of ionotropic glutamate receptors in the modulation of dopamine release has been described in the dorsal and ventral striatum (Segovia and Mora, 2001), which indicates the existence of differences in the computation of the SSMs from different striatal compartments.

Glutamate also stimulates metabotropic glutamate receptors (mGluRs), which are G-protein-coupled receptors (GPCRs) mostly localized extrasynaptically. They are classified into three groups. Groups II and III are G_{i/o}-coupled receptors that are mainly found in glutamatergic terminals, where they function as autoreceptors. Their stimulation inhibits glutamate release (Schoepp, 2001). Group I mGluRs (with two subtypes, mGlu₁R and mGlu₅R) are coupled to G_q proteins and they are usually found postsynaptically, preferentially in the perisynaptic ring, adjacent to the postsynaptic density (PSD) (Smith et al., 2000). This localization depends

mainly on the existence of protein–protein interactions involving a series of PSD scaffold proteins (PSD-95, guanylate kinase-associated protein, Shank and Homer), which physically connect group I mGluRs with NMDARs (Kennedy, 2000). This gives a frame for functional interactions between group I mGluRs and NMDARs (Pisani et al., 2001). Furthermore, group I mGluRs are found at dopaminergic synapses, with mGlu₅Rs being localized peri- and postsynaptically and mGlu₁Rs being localized presynaptically (Paquet and Smith, 2003). Here, mGlu₁Rs seem to be involved in negative control of impulse-dependent dopamine release (Zhang and Sulzer, 2003).

There are two modes of impulse-dependent striatal dopamine release, which depend on two main firing modes of the mesencephalic dopaminergic cells: a low-frequency tonic mode (0.5–8 Hz) and bursts of phasic activity (>20 Hz and <1 s). Burst discharges carry phasic information, which is associated with transient and spatially restricted (phasic) increases in extrasynaptic dopamine in the striatum (Grace, 1991; Venton et al., 2003; Floresco et al., 2003). Phasic dopamine release occurs in response to salient and reward-related events (Schultz, 2002) and depends on specific stimulatory inputs to the mesencephalon (Floresco et al., 2003). The tonic firing mode depends on the number of spontaneously active dopaminergic cells, which is increased by disinhibition of inputs to the mesencephalon (Floresco et al., 2003). Furthermore, at the level of the SSM, extracellular dopamine is also tonically modulated by an impulse-independent mechanism that depends on the extracellular concentration of glutamate, which facilitates the reverse transport of dopamine upon stimulation of ionotropic glutamate receptors at dopaminergic nerve terminals (Leviel, 2001; Segovia and Mora, 2001; Borland and Michael, 2004). Therefore, the variables that control the extracellular concentration of glutamate help to modulate the tonic mode of dopamine release. These variables include the neurotransmitter acetylcholine and the neuromodulator adenosine (see below), as well as glutamate transporters (see above) and the glial cysteine-glutamate antiporter (Baker et al., 2002). It can be assumed that the tonic mode modulates in an opposite way the phasic mode of cell firing and dopamine release, probably by stimulating D₂ autoreceptors. Thus, a tonic increase in the partially impulse-independent extracellular dopamine would increase D₂ autoreceptor stimulation and thereby decrease impulse-dependent phasic dopamine release (Grace, 1991; Borland and Michael, 2004). In a similar way to the dopamine-mediated filtering of glutamatergic input, glutamate (by increasing tonic, impulse-independent dopamine release) also filters incoming (phasic) dopaminergic signals. The outcome of this reciprocal dopamine-glutamate modulation seems obvious: selection at the level of the SSM of concomitant strong glutamatergic and phasic dopaminergic signals.

2.3. Cholinergic neurotransmission in the SSM

Large aspiny neurons are cholinergic interneurons that constitute less than 2% of the striatal neuronal population (Smith and Bolam, 1990; Gerfen, 2004). Nevertheless, the high concentration of cholinergic markers in the striatum indicates that acetylcholine (ACh) has an important function in this brain region. The striatal concentrations of ACh and of the enzymes involved in its synthesis and metabolism (acetylcholinesterase and choline acetyltransferase) are among the highest in the brain (Hoover et al., 1978). In addition, the striatum contains the highest densities of vesicular ACh transport sites, high-affinity choline uptake sites and muscarinic and nicotinic ACh receptors (Aubert et al., 1996). Although there are some cholinergic synaptic specializations (targeting some dendritic spines and shafts on MSNs), they are found in less than 10% of the cholinergic nerve terminals (Contant et al., 1996). According to Contant *et al.* (1996), the synaptic incidence of cholinergic nerve terminals in the striatum is among the lowest for any transmitter-defined system in any region of the brain. In fact, volume transmission is the predominant communication mode used by the cholinergic system in the striatum and other brain regions (Descarries et al., 1997).

In the SSM, metabotropic muscarinic receptors are mainly found in dendritic spines, particularly in the perisynaptic ring of the PSD (Hersch et al., 1994). These findings led Hersch *et al.* (1994) to point out that, “individual postsynaptic elements containing receptors for multiple neurotransmitters may be a general feature of CNS synapses”. The main subtypes of muscarinic receptor in the SSM are the M_1 and M_4 receptors (M_1R and M_4R), and there is evidence they show some anatomical and functional segregation in the two subtypes of MSN. The G_q -coupled M_1R and the $G_{i/o}$ -coupled M_4R seem to preferentially modulate the function of enkephalin MSNs and dynorphin MSNs, respectively (Di Chiara et al., 1997; Kaneko et al., 2000). This modulation seems to involve antagonistic interactions with dopaminergic receptors: stimulation of M_1Rs and M_4Rs counteracts the effects of stimulation of D_2Rs and D_1Rs , respectively (Harsing and Zigmond, 1998; Gomeza et al., 1999). These ACh-dopamine receptor interactions might be involved in the ability of ACh to oppose the effects of striatal dopamine, which is the basis of the use of muscarinic receptor antagonists to treat patients with Parkinson's disease (Kaneko et al., 2000).

The ACh-mediated modulation of dopamine transmission in the SSM also involves presynaptic mechanisms. Neuronal nicotinic ACh receptors (nAChRs) are ionotropic receptors that are mainly found presynaptically, where they control neurotransmitter release (Vizi and Lendvai, 1999). These receptors are heteromeric pentamers made of a heterogeneous family of eight subunits, which can be subdivided into α -bungarotoxin-sensitive or homomeric α_7 nAChRs and α -bungarotoxin-insensitive or heteromeric non- α_7 nAChRs. In the striatum, these heteromeric nAChRs are mainly located at dopaminergic terminals; stimulation of the receptors increases tonic dopamine release, whereas blocking them decreases it (Chamtioux et al., 2003; Rice and Cragg, 2004; Quarta et al., 2007). Furthermore, homomeric α_7 nAChRs in glutamatergic terminals exert a strong tonic enhancement of glutamate release. Blockade of these α_7 nAChRs with either exogenous or endogenous antagonists induces a significant reduction in striatal extracellular concentrations of glutamate, followed by a reduction in extracellular dopamine (Rassoulpour et al., 2005). Importantly, the astrocyte-derived factor kynurenic acid is a potent α_7 nAChR antagonist at physiological concentrations, which underscores the role of glial cells in the SSM (Rassoulpour et al., 2005). This is, therefore, an example of computation of the SSM, which involves glial cells (kynurenic acid), ACh, glutamate and dopamine.

In summary, ACh facilitates tonic dopamine release by direct and indirect (glutamate-mediated) mechanisms, with a consequent decrease in phasic dopamine release. Taking into account the antagonistic effects of M_1R and M_4R on D_2R and D_1R signalling, respectively, the global effect of ACh release in the SSM seems to be to reduce the filtering effect of dopamine on glutamatergic neurotransmission.

2.4. Adenosinic neurotransmission in the SSM

In addition to dopamine, glutamate and ACh, the neuromodulator adenosine has an important function in the SSM. Adenosine is probably released from both extrasynaptic and intrasynaptic sites to reach extrasynaptic and intrasynaptic adenosine receptors in and around the glutamatergic synapse (Ferré et al., 2005). Adenosine comes from intracellular adenosine, the concentration of which depends on the breakdown and synthesis of ATP. ATP is metabolized to AMP and then converted to adenosine by 5'-nucleotidases. Adenosine is then transported to the extracellular space by equilibrative nucleoside transporters. Thus, adenosine can be produced by MSNs when they are metabolically active (high ATP consumption). Furthermore, adenosine is a signal of increased glutamatergic neurotransmission. Postsynaptically, adenosine is released by stimulation of NMDARs (Delaney and Geiger, 1998). Presynaptically, an increase in the frequency of impulses arriving at the glutamatergic nerve terminal induces a sudden increase in the concentration of adenosine at the glutamatergic synapse (Cunha,

2001). This is probably related to an increase in the release, with glutamate, of synaptic ATP, which is metabolized to adenosine by ecto-nucleotidases (Cunha, 2001). Most of the effects of adenosine in the CNS are mediated by adenosine A₁ receptors (A₁Rs) and A_{2A} receptors (A_{2A}Rs) (Ferré et al., 1997,2005;Cunha, 2001). Postsynaptic A_{2A}R is preferentially found at the perisynaptic ring of the glutamatergic synapse in the enkephalin SSM (Ciruela et al., 2006), where it can interact with D₂Rs and mGlu₅Rs (see below). Both A₁Rs and A_{2A}Rs can also be found in the neck of dendritic spines (Ciruela et al., 2006), where they can interact with extrasynaptic dopamine and metabotropic glutamate receptors. Furthermore, both A₁Rs and A_{2A}Rs are localized presynaptically at glutamatergic terminals, where they modulate glutamate release (see below). Finally, adenosine receptors are also localized at dopaminergic synapses, with A₁Rs being localized presynaptically, where they inhibit dopamine release (Borycz et al., 2007). There is a complex differential A₁R-mediated modulation of dopamine release in different striatal compartments (Borycz et al., 2007), underlying again the existence of differences in the computation of the SSMs from different striatal compartments. To understand the role of adenosine in the SSM, we have to analyze the role of adenosine receptor heteromers in SSM computation.

3. Receptor heteromers in the SSM

3.1. General Considerations

Neurotransmitters and neuromodulators at the synaptic cleft, and those that spill over from the synaptic cleft or asynaptic varicosities, encounter a variety of receptors located synaptically and extrasynaptically. It is now accepted that receptors can occur as homo-oligomers or hetero-oligomers (Bouvier et al., 2001;Agnati et al., 2003,2005;Franco et al., 2003;Prinster et al., 2005). Whereas homo-oligomerization does not change much our concept of neuroregulation, receptor heteromers constitute molecular switches that are crucial for local module operation. In fact, the signal transmitted by a combination of neurotransmitters depends on whether the receptors in the heteromer are activated simultaneously or not. To be more precise, the nature and strength of the signal depends on the composition of the heteromer, on the concentration of active compounds and on the temporal course of the activation of each receptor in the heteromer. Receptor heteromerization has been shown to provide new functional entities which possess new biochemical characteristics with respect to the individual components of the heteromer (Agnati et al., 2003;Prinster et al., 2005). These new functional properties include gain of function and changes in pharmacological properties, which include changes in ligand binding characteristics, signaling and trafficking (Agnati et al., 2003;Prinster et al., 2005). As it will become obvious from the examples given below, the formation of heteromeric receptor complexes also allows elaborate tuning of the regulation of both presynaptic and postsynaptic neuronal responses in the local module.

3.2. A_{2A}R-D₂R-mGlu₅R heteromers

As mentioned before, in the enkephalin MSN, A_{2A}R, D₂R and mGlu₅R are colocalized perisynaptically to the PSD of glutamatergic synapses and in the neck of dendritic spines, adjacent to dopaminergic synapses. It is clear that these three receptors interact physically and functionally (Fig. 2). It was initially shown at the biochemical level that, in crude membrane preparations from rat striatum, stimulation of A_{2A}Rs produces a decrease in the affinity of D₂Rs for agonists (Ferré et al., 1991). This intramembrane receptor interaction implied that stimulation of A_{2A}Rs produces a conformational modification of D₂Rs, with a concomitant change in their binding properties. The existence of an A_{2A}R-D₂R intramembrane interaction pointed towards a direct protein-protein interaction between A_{2A}Rs and D₂Rs, to an A_{2A}R-D₂R heteromer. This was shown by FRET and BRET techniques in co-transfected living cells (Canals et al., 2003). Importantly, A_{2A}R agonist-mediated modulation of D₂R binding could also be shown in different co-transfected cell lines (Dasgupta et al., 1996;Kull et al.,

1999;Salim et al., 2000), but not in cells only transfected with D₂R (Dasgupta et al., 1996), implying that the intramembrane interaction between A_{2A}Rs and D₂Rs is a functional property of the A_{2A}R-D₂R heteromer. Also, A_{2A}R has been shown to co-immunoprecipitate with mGlu₅R in experiments with co-transfected cells and striatal tissue (Ferre et al., 2002). In membrane preparations from rat striatum, stimulation of mGlu₅Rs produces a decrease in the affinity of D₂Rs for agonists and co-stimulation of A_{2A}R and mGlu₅R produces a modulation of D₂R binding that is significantly stronger than the reduction induced by stimulation of either receptor alone (Popoli et al., 2001), indicating the existence of striatal A_{2A}R-mGlu₅R and possibly A_{2A}R-D₂R-mGlu₅R heteromers.

In addition to the intramembrane interaction, there is a strong antagonistic interaction between A_{2A}Rs and D₂Rs at the second messenger level. Stimulation of D₂Rs, which are coupled to G_{i/o}, counteracts adenylyl-cyclase activation induced by G_{s/olf}-coupled A_{2A}Rs (Kull et al., 1999;Hillion et al., 2002) (Fig. 2). Stimulation of A_{2A}R might stimulate adenylyl-cyclase, with consequent activation of the protein kinase A (PKA) signalling pathway and induction of the expression of different genes, such as *c-fos* and *preproenkephalin*, by the constitutive transcription factor CREB (Ferre et al., 1997,2005). In addition, A_{2A}R-mediated activation of PKA can induce the phosphorylation of AMPARs (Hakansson et al., 2006), which is important for the development of plastic changes at glutamatergic synapses, including recruitment of AMPARs to the PSD (Song and Haganir, 2002). However, under basal conditions, stimulation of A_{2A}Rs poorly activates cAMP-PKA signalling or increase gene expression, owing to strong tonic inhibition of adenylyl cyclase by endogenous dopamine and D₂R stimulation (Ferre et al., 1997,2005). Thus, systemic administration of selective A_{2A}R agonists does not increase striatal *c-fos* expression (Karcz-Kubicha et al., 2003). Nevertheless, central or local administration of A_{2A}R agonists produces a pronounced decrease in motor activity and catalepsy (reviewed in Ferre et al., 1997). Furthermore, A_{2A}R agonists and antagonists selectively counteract and potentiate, respectively, the motor activation and decrease in neuronal firing and neurotransmitter release that are induced by dopamine D₂R agonists (Ferre et al., 1993, 1997;Stromberg et al., 2000).

A clear picture is emerging about the mechanisms by which reciprocal antagonistic A_{2A}R-D₂R interactions modulate the enkephalin SSM function. Although inhibition of the cAMP-PKA signalling pathway is one of the main biochemical effects of D₂R stimulation in the striatum (Lee et al., 2002), the inhibitory role of D₂Rs in the enkephalin MSN depends mostly on the suppression of Ca²⁺ currents through L-type VDCCs (Nicola et al., 2000) (Fig. 2), which is independent of cAMP-PKA signaling. Adenosine, by acting on A_{2A}Rs, can counteract the effect of D₂Rs on neuronal excitability through the intramembrane A_{2A}R-D₂R interaction. Thus, striatal application of a selective A_{2A}R agonist counteracts the inhibition of neuronal firing and GABA release that are induced by a selective D₂R agonist (Ferre et al., 1993; Stromberg et al., 2000). On the other hand, owing to the strong D₂R-A_{2A}R interaction at the adenylyl-cyclase level, A_{2A}R stimulation alone cannot stimulate cAMP-PKA signaling or induce gene expression and synaptic plasticity. Nevertheless, stimulation of mGlu₅Rs allows A_{2A}Rs to override the D₂R-mediated inhibitory effects. Thus, central co-administration of A_{2A}R and mGlu₅R agonists induces an increase in striatal expression of *c-fos* (Ferre et al., 2002).

In addition to the interactions at the intramembrane level, co-transfected cells revealed a strong synergistic interaction between A_{2A}Rs and mGlu₅Rs at the mitogen-activated protein kinase (MAPK) level (Fig. 2) (Ferre et al., 2002). Studies in striatal slices have also shown that stimulation of mGlu₅Rs potentiates A_{2A}R signalling in a MAPK-dependent manner (Nishi et al., 2003). As both PKA and MAPK activation are involved in recruitment of AMPARs to the PSD (Song et al., 2002;Thomas and Haganir, 2004), A_{2A}R-D₂R, A_{2A}R-mGlu₅R and possibly A_{2A}R-D₂R-mGlu₅R heteromers are in a position to modulate plastic changes in the SSM.

Pharmacological or genetic inactivation of A_{2A}Rs or mGlu₅Rs impairs corticostriatal long-term potentiation (d'Alcantara et al., 2001; Gubellini et al., 2003), a well-established form of synaptic plasticity that depends on cAMP–PKA signalling and MAPK activation (Song and Huganir, 2002; Thomas and Huganir, 2004).

In different behavioral models, mGlu₅R agonists and antagonists produce similar effects to A_{2A}R agonists and antagonists, respectively, including selective modulation of D₂R-mediated effects. A selective mGlu₅R agonist preferentially inhibits motor activation induced by D₂R agonists (Popoli et al., 2001), whereas mGlu₅R antagonists counteract the effects of D₂R antagonists (Ossowska et al., 2001). Furthermore, A_{2A}R and mGlu₅R agonists and A_{2A}R and mGlu₅R receptor antagonists also show synergistic effects at the behavioral level (Popoli et al., 2001; Ferré et al., 2002; Kachroo et al., 2005). A_{2A}R–D₂R–mGlu₅R receptor interactions provide the rationale for the application of A_{2A}R antagonists and possible application of mGlu₅R antagonists in Parkinson's disease (Ferre et al., 1992, 1997; Ossowska et al., 2001; Jenner, 2005; Kachroo et al., 2005).

3.3. The A₁R–A_{2A}R heteromer

Time-resolved FRET and BRET experiments in co-transfected cells have shown that A_{2A}Rs and A₁Rs can form receptor heteromers (Ciruela et al., 2006). These heteromers are mainly found in glutamatergic terminals, as shown by immunocytochemical and co-immunoprecipitation experiments in striatal nerve terminal preparations (Ciruela et al., 2006). Like the A_{2A}R–D₂R heteromer, the A_{2A}R–A₁R heteromer shows antagonistic reciprocal interactions (Fig. 3). Through an intramembrane receptor–receptor interaction, stimulation of A_{2A}Rs decreases the affinity of A₁Rs for agonists (Ciruela et al., 2006). The same as with the A_{2A}R–D₂R heteromer, the A_{2A}R–A₁R intramembrane interaction is a functional property of the A_{2A}R–A₁R heteromer, which cannot be demonstrated in cells only expressing one of the receptors (Ciruela et al., 2006). Also, A₁R is a G_{i/o}-coupled receptor, stimulation of which might be able to inhibit A_{2A}R-mediated stimulation of adenylyl-cyclase. Although it seems counterintuitive that receptors with opposite signaling effects to the same neurotransmitter form receptor heteromers, the A_{2A}R–A₁R heteromer allows adenosine to exert detailed modulation of glutamate release (Quarta et al., 2004; Ciruela et al., 2006). In glutamate nerve terminals, stimulation of A_{2A}Rs produces glutamate release and counteracts the inhibition of glutamate release that is induced by A₁R stimulation (Ciruela et al., 2006). The inhibitory effect of A₁R on striatal glutamate release probably involves inhibition of N- and P/Q-type VDCCs by $\beta\gamma$ -G-protein subunits (Fig. 3); this is the most commonly reported mechanism for inhibition of neurotransmitter release by G_{i/o}-coupled receptors, including A₁Rs (Yawo and Chuhma, 1993; Jarvis and Zamponi, 2001). The stimulatory effect of A_{2A}Rs on striatal glutamate release is probably related to their ability to activate cAMP–PKA signalling, as this mechanism has been shown for A_{2A}R-induced ACh release in the striatum, GABA release in the globus pallidus and serotonin release in the hippocampus (Gubitz et al., 1996; Shindou et al., 2002; Okada et al., 2001). This effect is related to the ability of PKA to phosphorylate different elements of the machinery that is involved in vesicular fusion (Leenders and Sheng, 2005) (Fig. 3).

Unlike D₂Rs, A₁Rs do not seem to strongly inhibit A_{2A}R-mediated signaling (Quarta et al., 2004; Ciruela et al., 2006). So what is the role of the A₁R–A_{2A}R heteromer in the regulation of striatal glutamate release? In view of the preferential occupancy of A₁Rs over A_{2A}Rs by endogenous adenosine (Fredholm et al., 2001), under basal conditions, low endogenous adenosine tone favors a decrease in the probability of glutamate release. On the other hand, A_{2A}R seems to play a more important role under conditions of increased adenosine release. Under these conditions, stimulation of A_{2A}R produces a switch to A_{2A}R-mediated signalling through the intramembrane A_{2A}R–A₁R interaction, with a concomitant increase in the

probability of glutamate release. In fact, in striatal glutamatergic nerve terminal preparations, low and high concentrations of adenosine inhibit and stimulate glutamate release, respectively (Ciruela et al., 2006).

The analysis of the properties of the A_{2A}R-D₂R-mGlu₅R and A_{2A}R-A₁R heteromers suggests their involvement in synaptic plasticity in the enkephalin SSM. We have postulated that a strong cortico-limbic-thalamic glutamatergic input produces a sufficient amount of intrasynaptic glutamate and adenosine (most probably derived from ATP co-released with glutamate), with a significant stimulation of A_{2A}Rs in presynaptic A_{2A}R-A₁R heteromers and postsynaptic A_{2A}R-D₂R and A_{2A}R-mGlu₅R heteromers, which can provide a mechanism of facilitation of plastic changes in the excitatory synapse of the enkephalin SSM (Ferre et al., 2005). In fact, blockade of A_{2A}Rs completely counteracts cortical stimulation-induced MAPK activation and PKA-mediated phosphorylation of AMPARs in the enkephalin MSN (Quiroz et al., 2006), which, as mentioned above, are critical initial steps in the establishment of plastic changes in excitatory synapses, involving recruitment of AMPA receptors to the PSD (Song et al., 2002; Thomas and Huganir, 2004).

3.4. The D₂R-non- α_7 nAChR heteromer

Several mechanisms have been proposed to be involved in the modulation of dopamine release by D₂ autoreceptors. They include a decrease in dopamine synthesis and packaging (Onali et al., 1988; Pothos et al., 1998), upregulation of the dopamine transporter (Gulley and Zahniser, 2003) and a reduction in membrane excitability by increasing K⁺ conductance through Kir3, which are also called G-protein regulated inward rectifier K⁺ channels (GIRKs) (Congar et al., 2002) (Fig. 4). D₂Rs can form stable complexes with GIRKs (Lavigne et al., 2002). We have recently found a functional mechanism that allows D₂ autoreceptors to decrease dopaminergic neurotransmission which depends on the function of heteromers of D₂ autoreceptors and non- α_7 nAChRs in the striatum (Quarta et al., 2007) (Fig. 4). Stimulation of nACh receptors induces an influx of Ca²⁺, which produces neurotransmitter release by directly acting on the protein machinery involved in vesicular fusion or by activating VDCCs after membrane depolarization (Vizi and Lendvai, 1999). *In vivo* microdialysis experiments in freely moving rats show that local perfusion of nicotine into the ventral striatum produces a marked increase in extracellular levels of dopamine, which can be completely counteracted by co-perfusion with a non- α_7 nAChR antagonist or with a D₂R agonist. Furthermore, a D₂R antagonist produces an increase in extracellular dopamine that is partially, but significantly, counteracted by co-perfusion with a non- α_7 nAChR antagonist (Quarta et al., 2007). This demonstrates potent cross-talk between G-protein-coupled receptors and ligand-gated ion channels in dopaminergic nerve terminals, with D₂ autoreceptors regulating the efficacy of non- α_7 nAChR-mediated modulation of dopamine release. Selective molecular interactions between the β_2 subunits of non- α_7 nAChRs and D₂ autoreceptors were shown in co-immunoprecipitation experiments on membrane preparations from co-transfected mammalian cells and rat striatum (Quarta et al., 2007). Thus, striatal dopaminergic neurotransmission is under the control of receptor heteromers containing D₂ autoreceptor and non- α_7 ACh heteroreceptors, although we do not know if there are differences in the function of the D₂-non- α_7 ACh heteromer in different striatal compartments.

The term autoreceptor was introduced to define receptors in nerve terminals that respond to the neurotransmitter released by the same neuron (Langer, 1974). Functionally, autoreceptors acted as a feedback mechanism to inhibit neurotransmitter release. Later, the term autoreceptor also included receptors localized in the somatodendritic region that respond to somatodendritic neurotransmitter release (Aghajanian and Bunney, 1977) and receptors that enhanced neurotransmitter release, such as nAChRs localized in cholinergic nerve terminals (Wilkie et al., 1996). The term heteroreceptor is used for presynaptic receptors that can regulate (stimulate or inhibit) the release of a neurotransmitter other than their own, which is one of the main

functions of nAChRs in the CNS (Vizi and Lendvai, 1999). The term ‘autoreceptor heteromer’ expands the concepts of autoreceptor and heteroreceptor, to include them in a functional macromolecular complex that contains receptors for other neurotransmitters or neuromodulators.

4. Conclusions and perspectives

The SSM provides an example of the impressive degree of computation that takes place in local modules. This depends on the different modes of communication (synaptic and volume transmission) used by neurotransmitters and neuromodulators (dopamine, glutamate, acetylcholine and adenosine) and on the existence of receptor heteromers. The analysis of some of these receptor heteromers shows that receptor heteromerization is associated with particular elaborated functions in the local module. In the SSM, A_{2A}R–D₂R and A_{2A}R–mGlu₅R heteromers are located adjacent to the glutamatergic synapse of the dendritic spine of enkephalin MSNs and their cross-talk within the receptor heteromers helps to modulate postsynaptic plastic changes at the glutamatergic synapse. The A₁R–A_{2A}R heteromer is found in glutamatergic terminals and the molecular cross-talk between the two receptors in the heteromer helps to modulate glutamate release. Finally, the D₂R–non- α_7 nAChR heteromer, which is located in dopaminergic terminals, introduces the new concept of autoreceptor heteromer.

We believe that local modules, as defined in this review, should be considered as functional units of the CNS. But this is just the beginning of our understanding of the computational abilities of local modules. We have described what it might look as an extensive overview of one type of local module, the SSM. However, in some cases, the SSM corresponded to a particular striatal compartment (ventral *versus* dorsal striatum) or a particular neuron (enkephalin *versus* dynorphin MSN). Although ACh and its muscarinic and nicotinic receptors have been included in our analysis of the striatal local module, we have not reviewed the evidence for dopaminergic, glutamatergic and adenosinic modulation of cholinergic neurotransmission, which depends on the existence of multiple dopamine, glutamate and adenosine receptor subtypes in cholinergic terminals (Ferré et al., 1997; Marti et al., 2001). Also, more work still needs to be done to integrate other neurotransmitter-neuromodulators, such as GABA, serotonin, histamine and endocannabinoids as well as their different receptor subtypes. Furthermore, at present, we have very little knowledge about other local modules, either in the striatum or in other areas of the CNS. This is because no other area has been so intensively studied at the local module level as the striatum. Nevertheless, the present analysis of the SSM gives clues for what it can be a successful approach to the study of local modules. First, the possible main structural elements of the local module, like those including dendritic spines and their main afferent nerve terminals should be defined (p.e., in cortical or hippocampal pyramidal cells or cerebellar Purkinje cells, which contain a high density of dendritic spines). Second, we need to find out which neurochemical signals and which receptor heteromers determine that those elements become a functional integrative unit.

To fully understand the mechanisms and potentialities of the computation in local modules, some concepts of receptor physiology and pharmacology must be revisited, according to the recent discoveries related to GPCRs. Receptor homo- and hetero-merization is changing our view about GPCR-ligand interactions. New models that take into account homodimers as basic units for the operation of GPCRs are being developed which fit experimental data better than classical models. For instance, the recently introduced ‘two-state dimer receptor model’ allows, for the first time, analyzing binding of radioligands to GPCRs showing cooperativity (Franco et al., 2005,2006). It is possible that, although with different effectiveness, most GPCR can potentially heteromerize in heterologous systems. But the question is what determines the formation of receptor heteromers in the CNS. Most probably, co-expression is a main factor

that determines the presence of heteromers in a given cell and neuronal compartments. We still need to determine the basic units and models of operation of the receptor heteromers, since they can be forming heterodimers, hete-oligomers of different homodimers or random receptor mosaics. Furthermore, we also need to know about the stability of receptor heteromers and which mechanisms determine their formation or disruption. Particularly interesting in this respect are the receptor heteromers that depend on arginine-phosphate electrostatic interactions, such as the NMDAR-D₁R heteromer and the A_{2A}R-D₂R heteromer (Woods and Ferré, 2005). Thus, these interactions seem to be dependent on phosphorylation-dephosphorylation events (Woods and Ferré, 2005).

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Abbreviations

ACh	Acetylcholine
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
CNS	central nervous system
EAAT	excitatory amino-acid transporter
GABA	γ -aminobutyric acid
GIRK	G-protein regulated inward rectifier K ⁺ channel
GPCR	G-protein-coupled receptors
Kir	inward rectifier K ⁺ channel
mGluR	metabotropic glutamate receptor
MAPK	mitogen-activated protein kinase
MSN	medium spiny neuron
nAChR	

	nicotinic ACh receptor
NMDA	<i>N</i> -methyl-D-aspartate
PKA	protein kinase A
PSD	postsynaptic density
SSM	striatal spine module
VDCC	voltage-dependent Ca ²⁺ channel

Figure 1. Striatal medium spiny neuron

This is the most common neuron in the striatum and it receives two main inputs: glutamatergic afferents from cortical, limbic and thalamic areas, and dopaminergic afferents from the mesencephalon. The magnified scheme shows the most common striatal spine module, which includes the dendritic spine of the MSN and glutamatergic and dopaminergic terminals, which make synaptic contact with the head and neck of the dendritic spine, respectively. Astroglial processes, which express a high density of glutamate transporters, are in close apposition with the glutamatergic synapse.

Dendritic spine of the GABAergic enkephalinergic neuron

Figure 2. Heteromers of adenosine A_{2A}, dopamine D₂ and metabotropic mGlu₅ receptors in the dendritic spine of the enkephalin medium spiny neuron

The differential stimulation of the units of the receptor heteromers determines the predominant signalling pathway and the consequent changes in neuronal excitability and gene transcription, with implications for plastic changes in the glutamatergic synapse, such as phosphorylation and recruitment of AMPA receptors (AMPA_Rs) to the postsynaptic density. Under conditions of weak glutamatergic neurotransmission, there is a predominant effect of D₂ receptor (D₂R) signalling, with decreased neuronal excitability and gene transcription. Under conditions of strong glutamatergic neurotransmission, which is associated with adenosine release, there is a potent stimulation of metabotropic glutamate mGlu₅ receptors (mGlu₅R_s) and adenosine A_{2A} receptors (A_{2A}R_s), which dampens D₂R signalling through intramembrane receptor interactions and favors increased neuronal excitability and gene transcription (see text). In black and red, stimulatory and inhibitory effects, respectively. VDCC, voltage-dependent calcium channel; MAPK, mitogen activated protein kinases; PKA, protein kinase A; PLC, phospholipase C; PP-2B, protein phosphatase 2B or calcineurin.

Striatal glutamatergic terminal

Figure 3. Heteromers of adenosine A_{2A} and A₁ receptors in the glutamatergic terminal of the striatal spine module

The adenosine A_{2A}-A₁ receptor heteromer modulates glutamate (Glu) release by acting on the presynaptic protein machinery involved in vesicle exocytosis. Vesicular neurotransmitter release requires the formation of a complex of vesicle- and plasma-membrane proteins (v-prot. and p-prot., respectively). The vesicles dock with the plasma membrane and, after Ca²⁺ influx through N- and P/Q-type voltage-dependent calcium channels (VDCCs), the vesicle and plasma membranes fuse and release the neurotransmitter. The plasma-membrane proteins involved in vesicle fusion interact molecularly with VDCCs. Low concentrations of adenosine predominantly stimulate A₁ receptors (A₁Rs), which decreases the probability of glutamate release by inhibiting the entrance of calcium through VDCCs. High concentrations of adenosine also bind to A_{2A}Rs, which dampens A₁ receptor signalling through an intramembrane interaction and stimulates glutamate release by a cAMP-protein kinase A (PKA)-mediated mechanism, by phosphorylation of vesicle- and plasma-membrane proteins. In black and red, stimulatory and inhibitory effects, respectively. βγ, subunits of the G protein.

Striatal dopaminergic terminal

Figure 4. Heteromers of dopamine D₂ and non- $\alpha 7$ nicotinic acetylcholine receptors in the dopaminergic terminal of the striatal spine module

Heteromers of dopamine D₂ autoreceptor (short isoform of the D₂ receptor or D_{2S}R) and non- α_7 nicotinic acetylcholine receptor (non- α_7 nAChR) represent an important mechanism by which D_{2S}R modulates dopamine (DA) release, in addition to D_{2S}R-mediated modulation of dopamine synthesis and dopamine transport to the vesicles and a D_{2S}R-mediated stimulation of GIRKs. In the D_{2S}R-non- α_7 nAChR heteromeric complex, D_{2S}R stimulation decreases the effects of non- α_7 nAChR activation, which induces dopamine release by directly acting on the protein machinery involved in vesicular fusion (vesicle- and plasma-membrane proteins: v-prot. and p-prot., respectively), through Ca²⁺ influx, or by activating N- and P/Q-type voltage-dependent calcium channels (VDCCs). In black and red, stimulatory and inhibitory effects, respectively. Tyr, tyrosine; VMAT, vesicular monoamine transporter.