Published in final edited form as:
Prog Neurobiol. 2007 December ; 83(5): 277–292.

Adenosine A_{2A} receptors and basal ganglia physiology

S.N. Schiffmann[a,*], **G. Fisone**[b], **R. Moresco**[c], **R.A. Cunha**[d], and **S. Ferré**[e]

a*Laboratory of Neurophysiology, Université Libre de Bruxelles, Campus Erasme, 808 route de Lennik CP601, 1070 Brussels, Belgium*

b*Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 17177 Stockholm, Sweden*

c*IBFM-CNR, University of Milan-Bicocca, Scientific Institute H. San Raffaele, Milan, Italy*

d*Center for Neuroscience of Coimbra, Institute of Biochemistry, Faculty of Medicine, University of Coimbra, 3004−504, Coimbra, Portugal*

e*Behavioral Neuroscience Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224, USA*

Abstract

Adenosine A_{2A} receptors are highly enriched in the basal ganglia system. They are predominantly expressed in enkephalin-expressing GABAergic striatopallidal neurons and therefore are highly relevant to the function of the indirect efferent pathway of the basal ganglia system. In these GABAergic enkephalinergic neurons, the A_{2A} receptor tightly interacts structurally and functionally with the dopamine D_2 receptor. Both by forming receptor heteromers and by targeting common intracellular signaling cascades, A_{2A} and D_2 receptors exhibit reciprocal antagonistic interactions that are central to the function of the indirect pathway and hence to basal ganglia control of movement, motor learning, motivation and reward. Consequently, this A_{2A} / D_2 receptors antagonistic interaction is also central to basal ganglia dysfunction in Parkinson's disease. However, recent evidence demonstrates that, in addition to this postsynaptic site of action, striatal A_{2A} receptors are also expressed and have physiological relevance on presynaptic glutamatergic terminals of the cortico-limbic-striatal and thalamo-striatal pathways, where they form heteromeric receptor complexes with adenosine A_1 receptors. Therefore, A_{2A} receptors play an important fine-tuning role, boosting the efficiency of glutamatergic information flow in the indirect pathway by exerting control, either pre- and/or post-synaptically, over other key modulators of glutamatergic synapses, including D_2 receptors, group I metabotropic $mGlu_5$ glutamate receptors and cannabinoid CB_1 receptors, and by triggering the cAMP-protein kinase A signaling cascade.

Keywords

Adenosine A_{2A} receptor; dopamine receptors; metabotropic glutamate receptors; GABAergic enkephalinergic neuron; heteromeric receptor complexes; striatum

1. Introduction: the basal ganglia system

The basal ganglia are a richly interconnected neural network involved in adaptive control of behavior through interactions with sensorimotor, motivational and cognitive brain areas

* Corresponding author: Tel: +32/2/555.42.30; fax: +32/2/555.41.21, E-mail: sschiffm@ulb.ac.be.

(Graybiel et al., 1994). The striatum is the major input structure of the basal ganglia (Fig. 1). 90% of striatal neurons are *medium-size spiny neurons*, named for their high density of dendritic spines. This population of GABAergic striatal efferent neurons directly receives most striatal inputs from various intrinsic and extrinsic sources. Of these, the two predominant inputs are glutamatergic afferents from cortical, limbic and thalamic areas and dopaminergic afferents from the mesencephalon, either the substantia nigra pars compacta or the ventral tegmental area. Both inputs converge in the dendritic spine but have different roles: whereas the glutamatergic input serves as a trigger of striatal circuits, the dopaminergic input subserves a crucial modulatory role, since it is unable to trigger electrical responses in medium spiny neurons in the absence of glutamatergic inputs. The morphological organization of these inputs is consistent with these functional roles: the glutamatergic terminal makes synaptic contact with the head of the dendritic spine, while the dopaminergic terminal makes synaptic contact with the neck of the dendritic spine. In this way, dopaminergic neurotransmission is in a position to regulate glutamatergic neurotransmission (Gerfen, 2004).

The striatum, and hence GABAergic striatal efferent neurons, is critical for the control of movement. Indeed, it is essential both for the selection and initiation of actions (Graybiel et al., 1994) and for the learning of habits and skills (Graybiel 1995; White, 1997), a task mainly controlled by the dorsal striatum. GABAergic striatal efferent neurons also have a prominent role in motivation and reward, in which the ventral striatum plays a central role. Both motor control and motivation/reward are highly dependent on modulation by dopamine. A leading hypothesis for striatal function is that it contributes to the formation of stimulus–response associations through reinforcement learning (Schultz et al. 2003). It is therefore proposed that the striatum processes reward signals that are encoded by the association of dopaminergic input with sensory cues from the cortico-limbic-thalamic striatal projections, to generate appropriate behavioral responses to given contextual situations. In addition, circuits in the striatum generate learning and memory about behavioral responses and reward attainment through use-dependent long-term changes in synaptic efficacy (Wickens et al., 2003).

2. Adenosine as a major modulator of striatal functions

Adenosine is another very important modulator of striatal glutamatergic neurotransmission through its actions on adenosine receptors (Fredholm et al., 2001), which are highly abundant in the striatum (see below). However, the aim and mechanisms of adenosinergic modulation are fundamentally different from dopaminergic modulation. In fact, whereas dopamine is an extrinsic signal (depending on the firing of neurons whose cell bodies are localized in the substantia nigra pars compacta), adenosine is an intrinsic signal since it is locally produced as a function of the activity of striatal circuits. There seem to be two main sources of extracellular adenosine. First, there is the hormonal-like formation of adenosine whereby extracellular levels of adenosine increase as a function of the general workload of the circuit. Thus, with increasing workload (i.e. increased firing per unit of time), greater consumption of ATP in neurons and astrocytes is required to maintain ionic homeostasis and membrane potential. Dephosphorylation of ATP (present intracellularly in the millimolar range) generates adenosine, which levels increase substantially over basal intracellular levels, which are in the nanomolar range. This build-up of intracellular adenosine is translated into increased levels of extracellular adenosine since virtually all cell types are equipped with bidirectional and non-concentrative nucleoside transporters (Geiger and Fyda, 1991). This mechanism ensures that there is a local fluctuation of extracellular adenosine levels as a function of local activity in the striatum.

In parallel, there is a second mechanism that generates extracellular adenosine, which might be particularly related to the control of synaptic activity since it specifically generates a synaptic pool of adenosine. Several studies have provided evidence for the formation of extracellular

adenosine as a result of the action of ecto-nucleotidases (see Zimmermann, 2000) on ATP released upon nerve stimulation (reviewed in Cunha, 2001). ATP can be released with most neurotransmitters since it is present in synaptic vesicles together with different neurotransmitters, including glutamate (reviewed in Cunha and Ribeiro, 2000). In this manner, the synaptic pool of adenosine is replenished as a function of neuronal firing (Cunha, 2001). Extracellular ATP can also be originated from glia (Zhang et al., 2003), since ATP is stored and released from synaptic vesicles in astrocytes (Koizumi et al., 2003; Newman, 2003). The relative contribution of the different cellular (astrocytes or neurons, presynaptically or non-synaptically) and metabolic sources of extracellular adenosine (released as such or formed from released ATP) is still not well-defined. However, irrespective of its source, under physiological conditions extracellular levels of adenosine are always expected to build up locally as a function of the workload of the system.

3. GABAergic striatal efferent neuron subpopulations

GABAergic striatal efferent neurons display particular passive and active membrane properties that shape their intrinsic excitability and their responsiveness to synaptic inputs. Electrophysiological recordings performed *in vivo* showed that these neurons present a unique type of spontaneous electrical behavior, consisting of fluctuations of the membrane potential between two preferred potentials, the “up-state” close to firing threshold and a “down-state” near the potassium equilibrium potential (Wilson and Kawaguchi, 1996; Stern et al., 1997, 1998). It has been proposed that the down-state is due to an inwardly rectifying potassium conductance, the major membrane conductance of these neurons at rest, and that transitions from down- to up-state are mainly triggered by the powerful excitatory glutamatergic synaptic input. This means that these neurons are not intrinsically bistable. However, even if these transitions are triggered by excitatory input, they are also strongly influenced by intrinsic conductances and their regulation. Although such transitions do not spontaneously occur in brain slice preparations, mimicking the synaptic input either by stimulation of the cortico-limbic-striatal fibers or by application of NMDA in the bath can restore this specific behavior *in vitro* (Vergara et al., 2003; Olson et al., 2005)

3.1 GABAergic striatal neuron subpopulations: peptide expression

GABAergic striatal efferent neurons can be classified into two major classes according to their peptide expression: GABAergic enkephalinergic and GABAergic dynorphinergic neurons (Gerfen, 2004) (Fig. 1). In addition, there are different types of GABAergic interneurons (parvalbumin, calretinin or somatostatin internerons) and large cholinergic interneurons (Tepper and Bolam, 2004). In the dorsal striatum the GABAergic dynorphinergic and enkephalinergic neurons give rise to two striatal efferent pathways, which connect the dorsal striatum with the output structures of the basal ganglia, the substantia nigra pars reticulata and the internal segment of the globus pallidus (GPi; entopeduncular nucleus in rodents) (Gerfen, 2004) (see Fig. 1 for a schematic representation of basal ganglia circuitry). These are called “direct” and “indirect” pathways. In the direct pathway, GABAergic dynorphinergic neurons directly connect the striatum with the output structures. The indirect pathway consists of GABAergic enkephalinergic neurons, which connect the striatum with GABAergic neurons in the external segment of the globus pallidus (GPe; globus pallidus in rodents), which project to glutamatergic neurons in the subthalamic nucleus (STN), which in turn connect the STN with the output structures. GPe GABAergic neurons also project directly to the output structures without using the STN relay (Gerfen, 2004). Because of these differences in connectivity, stimulation of the direct pathway results in motor activation and stimulation of the indirect pathway produces motor inhibition. Dopamine, or dopamine agonists, will induce motor activation by activating the direct pathway (via D_1 receptors localized in GABAergic

dynorphinergic neurons) and by depressing the indirect pathway (acting on inhibitory D_2 receptors localized in GABAergic enkephalinergic neurons) (Gerfen, 2004) (Fig. 1).

Different from the dorsal striatum, the ventral striatum (mostly represented by the nucleus accumbens) receives glutamatergic input from limbic and paralimbic cortices, the amygdala and hipoccampus, and dopaminergic input from the ventral tegmental area (Parent and Hazrati, 1995; Gerfen, 2004). The same two classes of GABAergic efferent neurons found in the dorsal striatum exist in the ventral striatum: here, GABAergic enkephalinergic cells project to the ventral pallidum, which is the equivalent of the GPe in the dorsal striatopallidal complex. However, unlike the dorsal striatum, a high proportion of ventral GABAergic enkephalinergic neurons also express substance P and ventral GABAergic dynorphinergic neurons also project to the ventral pallidum (Robertson and Jian, 1995; Lu et al., 1998; Zhou et al., 2003). Furthermore, the ventral pallidum has characteristics of both the GPe and the GPi in its afferent and efferent systems and, therefore, it can also be considered an output structure of the basal ganglia (reviewed in Ferré, 1997).

3.2 Segregation of dopamine and adenosine receptor subtypes

One of the most notable features of these two subtypes of GABAergic striatal efferent neurons is the segregation of dopamine and adenosine receptors subtypes (Fig. 1). Thus, GABAergic enkephalinergic neurons express predominantly facilitatory A_{2A} adenosine receptors and inhibitory D_2 dopamine receptors, while GABAergic dynorphinergic neurons express predominantly inhibitory A_1 adenosine and facilitatory D_1 dopamine receptors (Schiffmann et al., 1991a, 1993; Fink et al., 1992;Ferré et al., 1991,1997,2005;Svenningsson et al., 1999;Gerfen, 2004). Furthermore, there is a tight interplay between the adenosine and dopamine receptors in each of the two striatal pathways. Previous studies have shown that antagonistic interactions between A_1 and D_1 receptors modulate the function of the GABAergic dynorphinergic neuron and antagonistic interactions between A_{2A} and D_2 receptors modulate the function of the GABAergic enkephalinergic neuron (Ferré et al., 1997,2005). It has been hypothesized that these interactions provide a mechanism of action for the depressant motor effects of adenosine agonists and for the motor stimulant effects of adenosine antagonists, like caffeine. Indeed, the adenosine system is the major target for methylxanthines, a class of substances that includes popular psychostimulants such as caffeine and theophylline. Caffeine is a competitive antagonist at both A_1 and A_{2A} receptors, which under normal conditions are activated by endogenous adenosine (Fredholm et al., 1999).

3.3 A_{2A} and D_2 receptors on GABAergic efferent neurons: physical and functional interactions

It was first suggested in the early nineties that the A_{2A}-D_2 receptor interactions could provide a new therapeutic strategy for Parkinson's disease, mostly based on the positive results seen following co-administration of A_{2A} receptor antagonists with L-DOPA or preferential D_2 receptor agonists (Ferré et al., 1997). More recently, it has been demonstrated that, when co-transfected into mammalian cell lines, A_{2A} receptors form heteromeric complexes with D_2 receptors and A_1 receptors form heteromeric complexes with D_1 receptors (Ginès et al., 2000; Hillion et al., 2002; Canals et al., 2003; Ciruela et al., 2004). It is believed that these heteromeric receptor complexes are mainly localized in the perisynaptic ring adjacent to the “postsynaptic density” (PSD) of the glutamatergic synapses on dendritic spines of GABAergic dynorphinergic and enkephalinergic neurons (Ferré et al., 2005).

4. *In vitro*, *ex vivo* and *in vivo* localization of A_{2A} receptors in the basal ganglia

Since the early 1990's, imaging techniques including *in situ* hybridization, immunocytochemistry, and binding autoradiography have all demonstrated high enrichment

of A_{2A} receptors in the different parts of the striatum (caudate-putamen, nucleus accumbens and olfactory tubercle) in all mammalian species studied (from mouse to human) (Fig. 2) (Schiffmann et al., 1991a;1991b, 1993; Svenningsson et al., 1999;Rosin et al., 1998,2003), strongly suggesting their involvement in basal ganglia functions. As stated above, *in situ* hybridization analyzed at the cellular level revealed the specific expression of A_{2A} receptor in GABAergic enkephalinergic striatopallidal neurons (Schiffmann et al., 1991a, 1993, Svenningsson et al., 1999).

The development of positron emission tomography (PET) techniques and selected radiopharmaceuticals has allowed imaging and quantification of defined molecular targets located in the brains of living subjects. These *in vivo* PET methods allow correlation of molecular data with defined clinical or behavioral presentations of human and animal subjects. In this context, the basal ganglia have received great attention in PET research. The development of analytical techniques based on the combined use of morphological and functional imaging (magnetic resonance imaging or MRI, and PET, respectively) has allowed improved analysis of both the dorsal and ventral aspects of basal ganglia. For instance, striatal dopaminergic afferents may be successfully visualized in normal or pathological conditions using tracers that selectively interact with biological targets expressed on dopaminergic nerve endings, such as the amino acid decarboxylase, the dopamine transporter, or the vesicular dopamine transporter. Dopaminergic receptors present on GABAergic efferent neurons have also been visualized in pathological conditions using a number of PET ligands (Fig. 3A). The existence of tracers selective for D_1- and D_2-like receptors has permitted the separate investigation of the direct and indirect pathways. Finally, the sensitivity of tracers such as [^{11}C]raclopride (Fig. 3A) to extracellular levels of dopamine has extended the use of PET to the *in vivo* measurement of basal and stimulated dopamine levels.

Both adenosine A_1 and A_{2A} receptors are present at high density in the basal ganglia and have been visualized *in vivo*. A_1 receptors may be efficiently visualized using selective PET ligands like [^{11}C]FR194921 and [^{18}F]CPFPX (Matsuya et al., 2005; Meyer et al., 2005). The first *in vivo* imaging of A_{2A} receptors in animals and humans was obtained using the xanthine derivative [^{11}C]KF18446 (Ishiwata et al., 2000; 2005). However, its binding profile did not completely overlap with the distribution of A_{2A} receptors, showing a similar accumulation in thalamus and striatum. The kinetic behaviour in rodents and monkeys of the non-xanthine antagonist and [4,3-e]1,2,4-triazolo[1,5-c]pyrimidine derivative [^{11}C]SCH442416 indicated its potential use for *in vivo* imaging of A_{2A} adenosine receptors (Todde et al., 2000). Indeed, *ex vivo* experiments in rats (Fig. 3B, 3C) and *in vivo* experiments in monkeys (Fig. 3E) showed a high abundance of [^{11}C]SCH442416 labelling in the striatum, both in its ventral and dorsal parts (Moresco et al., 2005). The sensitivity of [^{11}C]SCH442416 to changes in A_{2A} receptor density is demonstrated by the significant reduction of striatal [^{11}C]SCH442416 binding observed in rats pre-treated with quinolinic acid (QA, Fig. 3D), a neurotoxin that selectively destroys striatal neurons (Ishiwata et al., 2002).

5. Dopamine D_1 receptors in the GABAergic dynorphinergic neuron

The D_1 receptor is a Gs-Golf-coupled receptor, whose main signaling pathway is the cAMP-protein kinase A (PKA) cascade. D_1 receptor stimulation activates adenylyl-cyclase, which increases the formation of cAMP, leading to the activation of PKA. In the GABAergic dynorphinergic neuron, the A_1 receptor, which is a G_i-coupled receptor, antagonistically modulates D_1 receptor function by inhibiting adenylyl cyclase activation and by means of direct interaction in the A_1-D_1 receptor heteromer, by which stimulation of A_1 receptors decreases the binding of dopamine to the D_1 receptor (Ferré et al., 1998; Ginès et al., 2000). The D_1 receptor modulates neuronal excitability and glutamatergic neurotransmission by inducing PKA-mediated phosphorylation of different substrates such as ion channels, the dopamine and

cAMP-regulated phophoprotein, 32 kDa (DARPP-32), NMDA and AMPA glutamate receptors, and the transcription factor cAMP response element binding protein (CREB).

5.1 D_1 receptor stimulation of PKA: effects on neuronal excitability

D_1 receptor activation also results in PKA-mediated phosphorylation of voltage-dependent Na^+ channels (Surmeier et al., 1992; Schiffmann et al., 1995), leading to decreased Na^+ current and, hence, to reduced neuronal excitability (Schiffmann et al., 1995). On the other hand, D_1 receptor-induced phosphorylation of L-type voltage-dependent Ca^{2+} channels increases their conductance (Surmeier et al., 1995) resulting in facilitation of neuronal excitability (Hernandez-Lopez et al., 1997). These modulations may explain the functional consequences of D_1 receptor activation on the transitions between down- and up-state. At the down-state level, D_1 receptor activation will mostly decrease the probability of shifting to the up-state and of evoking spikes whereas when the striatal neuron succeeds in making a transition to the up-state, D_1 receptor activation will promote increased spiking.

PKA-mediated phosphorylation of NMDA receptors (in the C terminus of the NR1 subunit) potentiates NMDA receptor-mediated currents (Greengard et al., 1999) and there is also evidence for a tight cross-talk between the second messenger pathways of D_1 and NMDA receptors (Dudman et al., 2003). These functional interactions depend on heteromerization between the D_1 receptor and specific subunits of the NMDA receptor (Lee et al., 2002).

5.2 D_1 receptor stimulation of PKA: DARPP-32 and the positive feedback loop

Another important D_1 receptor-dependent PKA substrate is DARPP-32 (Greengard et al., 1999). DARPP-32 is highly expressed in both striatonigral dynorphinergic and striatopallidal enkephalinergic neurons (Ouimet et al., 1998), where it acts as a modulator of the cAMP-PKA pathway (Fienberg et al., 1998). Phosphorylation catalyzed by PKA at Thr34 converts DARPP-32 into an inhibitor of protein phosphatase-1 (PP-1) (Hemmings et al., 1984), whereas phosphorylation catalyzed by cyclin dependent kinase-5 (Cdk-5) at Thr75 converts DARPP-32 into an inhibitor of PKA (Bibb et al., 1999). In this way, regulation of DARPP-32 produces opposite biochemical effects depending on the site of phosphorylation. Activation of D_1 receptors results in PKA-catalyzed phosphorylation of DARPP-32 at Thr34 (Nishi et al., 1997; Svenningsson et al., 1998). Phospho[Thr34]DARPP-32 amplifies the effects of PKA by inhibiting PP-1 and reducing dephosphorylation of downstream target proteins, such as voltage-dependent Ca^{2+} and Na^+ channels, and NMDA, AMPA, and $GABA_A$ receptors (Nairn et al., 2004) with a series of functional consequences (Schiffmann et al., 1998). This positive feedback on protein phosphorylation provided by DARPP-32 appears to be necessary to elicit full behavioral responses produced by activation of the cAMP-PKA cascade in striatal efferent neurons. For example, the hyperlocomotor effect of cocaine, a drug which increases Thr34 and decreases Thr75 phosphorylation via stimulation of D_1 receptors (Bibb et al., 2001; Svenningsson et al., 2000), is strongly attenuated in DARPP-32 knockout mice (Fienberg et al., 1998).

5.3 D_1 receptor stimulation of PKA: other substrates and neuronal adaptation

In addition, D_1 receptor-mediated PKA activation leads to changes that have implications for synaptic plasticity, such as phosphorylation of AMPA receptors and increase in gene transcription. Phosphorylation of AMPA receptors plays a crucial role in the initial plastic changes in glutamatergic synapses seen in long term potentiation (LTP) and depression (LTD). Phosphorylation of AMPA receptors increases their channel conductance and is associated with their recruitment to the postsynaptic density (PSD) (Song and Huganir, 2002) as demonstrated following D_1 receptor-mediated phosphorylation of striatal AMPA receptors (Wolf et al., 2003). These early changes involved in synaptic plasticity are transitory and quickly reversible, but could be followed by gene transcription and protein synthesis, inducing

more permanent phenotypic changes, as formation of new spines or spine pruning (Segal, 2005). PKA activated by D_1 receptor stimulation can translocate to the nucleus and phosphorylate the nuclear constitutive transcription factor CREB (Greengard et al., 1999). Activation of CREB is involved in the D_1 receptor-mediated increase in expression of immediate-early genes, such as *c-fos*, *NGFI-A* and *jun-B* and in the increased expression of the *preprodynorphin* gene, which encodes the precursor of dynorphin (Xu et al., 1994).

6. Adenosine A_{2A} receptor: the dopamine D_1–like receptor in the GABAergic enkephalinergic neuron

The adenosine A_{2A} receptor is functionally very similar to the D_1 receptor. We can say that the A_{2A} receptor is the equivalent of the D_1 receptor in the enkephalinergic neuron. Thus, the main signaling pathway of the A_{2A} receptor, which is also a Gs-Golf-coupled receptor, is the stimulation of the cAMP-PKA cascade (Fig. 4). A_{2A} receptors form heteromers with the D_2 receptor by means of an electrostatic epitope-epitope interaction between an Arg-rich domain of the D_2 receptor (localized in its long third intracellular loop) and a pSer localized in the C-terminus of the A_{2A} receptor (Ciruela et al., 2004). This interaction is very similar to that involved in D_1-NMDA receptor heteromerization. The D_2 receptor is coupled to Gi proteins and antagonizes A_{2A} receptor function by inhibiting adenylyl cyclase activation (Kull et al., 1999;Hillion et al., 2002). In addition, there is a reciprocal intramembrane A_{2A}-D_2 receptor interaction, by which stimulation of the A_{2A} receptor decreases binding of dopamine to the D_2 receptor (Ferré et al., 1991).

6.1 Modulation of membrane potential by A_{2A} and D_2 receptors

The A_{2A} receptor can also modulate neuronal excitability and synaptic transmission. By modulating intrinsic properties as well as the balance between excitatory and inhibitory inputs, it can control the activity of these striatopallidal enkephalinergic neurons over both short and long term. As mentioned before, the membrane potential of striatal neuron undergoes state transitions *in vivo* (Wilson and Kagawashi, 1996) that can be mimicked in brain slice preparations by application of NMDA (Vergara et al., 2003; Olson et al., 2005). These transitions require ion channels that may be regulated by striatal transmitters acting through G protein-coupled receptors such as D_2 and A_{2A} receptors. Preliminary experiments indicate that D_2 receptor activation inhibits the down- to up-state transition in a subpopulation of striatal neurons and abolishes the firing of these neurons in the up-state. A_{2A} receptor activation fully counteracts these effects of D_2 receptor, whereas application of an A_{2A} receptor agonist alone has no effect (Azdad et al., 2006). These observations suggest that, unlike the D_1 receptor, A_{2A} receptor activation triggers a PKA-independent mechanism which remains to be fully determined, but which could involve the antagonistic A_{2A}-D_2 receptor intramembrane interaction. A similar mechanism was proposed for the interaction of these receptors in a human neuroblastoma cell line, in which D_2 receptor stimulation counteracted depolarization-induced Ca^{2+} influx through voltage-dependent Ca^{2+} channels and A_{2A} receptor stimulation antagonized the D_2 effect (Salim et al., 2000).

6.2 Regulation of inhibitory transmission by adenosine A_{2A} receptors

A_{2A} receptor activation also regulates striatal and pallidal inhibitory transmission. A_{2A} receptor stimulation enhances the $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) evoked in principal neurons of the globus pallidus (Shindou et al., 2001, 2002, 2003). This effect involved the cAMP-PKA pathway since it could be mimicked by cAMP analogs and occurred presynaptically in terminals of GABAergic enkephalinergic efferent neurons. This effect confirms that the A_{2A} receptor positively regulates GABA release in the globus pallidus (Ferré et al., 1993; Mayfield et al., 1996). On the other hand, A_{2A} receptor agonists decreased $GABA_A$-mediated IPSCs evoked in the striatal efferent neurons by intra-striatal stimulation

(Mori et al., 1996). This effect was also mimicked by cAMP analogs and was proposed to be mediated at a presynaptic site either on recurrent terminals of the GABAergic enkephalinergic efferent neurons, on terminals of GABAergic interneurons or via a disynaptic pathway involving both terminals. Reports showing that A_{2A} receptor antagonists blocked the depressant effects of kainate receptors on large-sized striatal IPSCs favour the latter hypothesis (Chergui et al. 2000).

6.3 Regulation of excitatory transmission by adenosine A_{2A} receptors

Modulation of excitatory glutamatergic neurotransmission at cortico-limbic-striatal and thalamo-striatal synapses is a main target for A_{2A} receptor regulation of striatal function. This regulation seems to occur both at the post-and pre-synaptic levels (see below). At the post-synaptic side, A_{2A} receptor activation reduced the amplitude of NMDA-mediated inward currents (Norenberg et al., 1997, 1998) and of the NMDA component of the excitatory postsynaptic currents (EPSCs) (Gerevich et al., 2002; Wirkner et al., 2004) in a subpopulation of striatal efferent neurons. One report suggests that the postsynaptic effect is independent of the cAMP-PKA pathway and appears to involve phospholipase C-induced stimulation of the Ca^{2+}-calmodulin kinase II pathway (Wirkner et al., 2000). The same group showed that A_{2A} receptor activation did not affect the AMPA-mediated inward current or the AMPA component of excitatory postsynaptic potentials (EPSCs; Norenberg et al., 1997; 1998; Gerevich et al., 2002) despite its ability to lead to PKA-induced phosphorylation of $GluR_1$ subunit of the AMPA receptor (see below). Reports of stimulation of the Ca^{2+}-calmodulin kinase II pathway by A_{2A} receptors are rare. Instead, other groups have shown that striatal NMDA currents are increased upon activation of the cAMP-PKA pathway (Colwell and Lewine, 1995), the prototypal cascade induced by A_{2A} receptors. Clearly, additional research is required to clarify which pathways are activated by A_{2A} receptor stimulation.

6.4 Adenosine A_{2A} receptors signaling in enkephalinergic striatal neurons: cAMP pathways, DARPP-32 and interaction with D_2 receptors

Considerable evidence indicates the importance of DARPP-32 in the interaction between A_{2A} and D_2 receptors in GABAergic enkephalinergic neurons. Activation of A_{2A} receptors results in increased PKA-dependent phosphorylation of DARPP-32 at Thr34 (Svenningsson et al., 2000) and decreased phosphorylation at Thr75 (Lindskog et al., 2002). The opposite regulation of the cAMP-PKA cascade exerted by A_{2A} and D_2 receptors is reflected by the observation that the increase in phosphorylation of DARPP-32 at Thr34 produced by the A_{2A} receptor agonist CGS21680 is counteracted by quinpirole, a D_2-like receptor agonist (Lindskog et al., 1999). The negative regulation exerted by D_2 receptors on the phosphorylation of DARPP-32 at Thr34 is further demonstrated by studies showing that, in intact animals, administration of D_2 receptor antagonists, such as eticlopride and haloperidol, increases phosphoThr34-DARPP-32 levels (Svenningsson et al., 2000; Håkansson et al., 2006). This effect depends on tonic dopamine D_2 receptor activation and some maintenance of basal cAMP production and PKA activity by endogenous adenosine via A_{2A} receptors. In line with this interpretation it has been shown that the increase in phosphoThr34-DARPP-32 produced by eticlopride is prevented by administration of KW-6002, an A_{2A} receptor antagonist (Svenningsson et al., 2000), and is strongly reduced in A_{2A} receptor knock out mice (Svenningsson et al., 2000). In summary, the state of Thr34 phosphorylation of DARPP-32 in GABAergic enkephalinergic neurons is in large part controlled by the opposing actions of adenosine at A_{2A} receptors, and dopamine at D_2 receptors. These competing effects on the cAMP-PKA-DARPP-32 cascade account for the opposite regulation of protein phosphorylation (Håkansson et al. 2006) and gene expression by A_{2A} and D_2 receptors (for review, see Fisone et al., 2004).

The functional significance of changes in the state of phosphorylation of DARPP-32 is demonstrated by studies using adenosine receptor antagonists such as caffeine. Blockade of A_{2A} receptors reduces basal cAMP production, suppressing phosphorylation of DARPP-32 at Thr34 (Andersson et al., 2005) and increasing phosphorylation at Thr75 (Lindskog et al., 2002). In addition, the motor stimulant effect typically exerted by caffeine, or by selective A_{2A} receptor antagonists, is attenuated in DARPP-32-deficient mice (Lindskog et al., 2002). Therefore, DARPP-32 not only promotes responses to drugs that activate cAMP signalling in GABAergic efferent neurons, but also amplifies the behavioural effects produced by inhibition of the cAMP-PKA cascade. This latter action is most likely exerted via a parallel pathway involving increased phosphorylation of Thr75 and further inhibition of PKA activity.

Recent work has led to the identification of downstream target proteins regulated by the cAMP-PKA-DARPP-32 pathway and involved in the control of GABAergic enkephalinergic neuron excitability. It is well established that activation of D_2 receptors negatively modulates striatal glutamatergic function via inhibition of AMPA receptor transmission. Thus, AMPA current amplitude is reduced by quinpirole (Hernández-Echeagaray et al., 2004), and glutamatergic transmission is potentiated in D_2 receptor knockout mice (Cepeda et al., 2001). Recently, it has been demonstrated that, in the dorsal striatum, activation of D_2 receptors decreases phosphorylation of $GluR_1$ at the PKA site, Ser845 (Håkansson et al., 2006). This finding suggests that the inhibition exerted by D_2 receptors on glutamate transmission is mediated, at least in part, via suppression of PKA-catalyzed phosphorylation of AMPA receptors. Blockade of D_2 receptors results in increased phosphorylation of $GluR_1$ at Ser845 (Håkansson et al., 2006). This effect is dependent on tonic PKA activation in GABAergic enkephalinergic neurons, as it is blocked by an adenosine A_{2A} receptor antagonist, but not by a dopamine D_1 receptor antagonist. Furthermore, eticlopride does not induce phosphorylation of GluR1 in DARPP-32-deficient mice, or in mice in which the phosphorylation site for PKA in $GluR_1$ is replaced by a non-phosphorylable Ala (Håkansson et al., 2006). It therefore appears that, in GABAergic enkephalinergic neurons, D_2 receptor antagonist-dependent phosphorylation of AMPA receptors occurs via disinhibition of the cAMP-PKA-DARPP-32 cascade. Hypolocomotion induced by D_2 receptor blockade is thought to occur following activation of GABAergic enkephalinergic neurons, inhibition of thalamocortical neurons and reduction of motor cortex activity (Parr-Brownlie and Hyland, 2005). In this regard, it is conceivable that the enhancement in glutamate transmission produced in GABAergic enkephalinergic neurons by eticlopride and haloperidol, via DARPP-32-dependent $GluR_1$ phosphorylation, is involved in the motor depressant effect produced by these drugs. This possibility is supported by the observation that catalepsy produced by raclopride, a potent D_2 receptor antagonist, is attenuated in DARPP-32 deficient mice (Fienberg et al., 1998).

6.5 Adenosine A_{2A} receptors in enkephalinergic striatal neurons: interaction with $mGlu_5$ receptors

As with the D_1 receptor, A_{2A} receptor-mediated PKA activation can potentially phosphorylate several substrates involved in synaptic plasticity. However, to our knowledge, A_{2A} receptor-mediated phosphorylation of L-type voltage dependent Ca^{2+} channels or NMDA receptors has not been reported. Furthermore, A_{2A} receptor blockade does not produce a decrease in the basal PKA-mediated phosphorylation of $GluR_1$, although it counteracts $GluR_1$ phosphorylation induced by D_2 receptor blockade (Hakansson et al., 2006). This seems to be related to the fact that, under basal conditions, there is a strong tonic activation of D_2 receptors in the striatum that impairs the ability of A_{2A} receptor to signal through the cAMP-PKA cascade. Given these observations, what are the conditions that allow A_{2A} receptors to fully activate PKA? It has been shown that co-stimulation of the group I metabotropic glutamate receptor $mGlu_5$ allows A_{2A} receptor stimulation to override the inhibitory tone imposed by endogenous dopamine via D_2 receptors (Ferré et al., 2002).

The $mGlu_5$ receptor is a Gq-coupled receptor located primarily in the perisynaptic ring (Kennedy, 2000), although some of our recent studies suggest that they can also be located at the PSD together with A_{2A} receptors (Rodrigues et al., 2005) (Fig. 4). $mGlu_5$ functionally interacts with the NMDA receptor and is anatomically linked to the NMDA receptor by means of intermediate scaffolding proteins of the PSD. A_{2A} and $mGlu_5$ receptors form heteromeric receptor complexes in transfected cells and in the rat striatum. $mGlu_5$ receptor stimulation potentiates the effects of A_{2A} receptors both at the intramembrane level by increasing its ability to inhibit dopamine D_2 receptor binding (Popoli et al., 2001; Domenici et al., 2004), and at the mitogen-activated protein kinase (MAPK) level (Ferré et al., 2002; Nishi et al., 2003). In fact, central co-administration (in the lateral ventricle) of a selective A_{2A} receptor agonist and a selective $mGlu_5$ agonist induces an increase in the striatal expression of *c-fos*, while no significant effect is obtained when each is administered alone (Ferré et al., 2002). Furthermore, the synergistic interaction between A_{2A} and $mGlu_5$ receptors is in notable agreement with the recently reported synergistic effects of A_{2A} and $mGlu_5$ receptor antagonists in animal models of Parkinson's disease (Coccurello et al., 2004; Kachroo et al., 2005).

7. Presynaptic control of glutamatergic neurotransmission by adenosine A_{2A} receptors

Changes in the efficiency of glutamatergic synapses (i.e. plasticity) do not only depend on postsynaptic changes, but also on presynaptic changes. Specifically, there is clear evidence for increases in the probability of vesicular neurotransmitter release associated with LTP and decreases associated with LTD (Schulz, 1997; Sola et al., 2004; Ronesi and Lovinger, 2005). Vesicular neurotransmitter release follows a cycle, with filling, docking, fusion and recycling. The protein machinery involved in vesicular fusion seems to play a key role in presynaptic plasticity. Stimulation of G protein-coupled receptors localized in the glutamatergic terminals can significantly modify the probability of neurotransmitter release by acting on the mechanisms involved in vesicular fusion. There are two major reported mechanisms involved in the modulation of neurotransmitter release by G protein-coupled receptors. Stimulation of Gi protein-coupled receptors, such as A_1 receptors (Fig. 4), induces a decrease in the probability of neurotransmitter release (Lovinger and Choi, 1995; Flagmeyer et al., 1997) by direct inhibition of N- and P-Q-type voltage dependent Ca^{2+} channels by beta-gamma subunits of Gi proteins (Wu and Saggau, 1997; Jarvis and Zamponi, 2001) as well as direct actions on the release machinery (reviewed in Silinsky et al., 1999). Stimulation of Gs protein coupled receptors, such as A_{2A} receptors (Fig. 4), on the other hand, increases the probability of neurotransmitter release by a cAMP-PKA-dependent mechanism (Evans and Morgan, 2003; Leenders and Sheng, 2005) or alternatively, through PKC-mediated facilitation of neurotransmitter release (reviewed in Fredholm et al., 2005). In the striatum, A_{2A} receptors are located in glutamatergic nerve terminals and their activation enhances the evoked release of glutamate (Rodrigues et al., 2005; Ciruela et al., 2006). This raises the question of whether the modulatory effects operated by A_{2A} receptors in the striatum are only due to post-synaptic A_{2A} receptors or if the presynaptic A_{2A} receptors located in glutamatergic terminals may also play a relevant role.

Moreover, it is also worth mentioning that activation of A_{2A} receptors has been repeatedly suggested to result in a stimulation of striatal dopamine as well as acetylcholine release (Brown et al., 1990; Zetterstrom and Fillenz, 1990; Kurokawa et al., 1994; Okada et al., 1996; 1997; but see Jin and Fredholm, 1997). Although still a subject of debate, this was supported by a decreased basal dopamine level in the striatum of A_{2A} receptor-deficient mice (Dassesse et al., 2001). A_{2A} receptor activation also leads to the stimulation of GABA release by striatopallidal neurons as detected in the globus pallidus (Ferré et al., 1993; Shindou et al., 2001; 2002; 2003).

7.1 Presynaptic control: interaction of A_{2A} receptors with other receptors

Most of the receptor-receptor interactions first described post-synaptically in GABAergic enkephalinergic neurons apparently also exist presynaptically to control glutamate release from cortico-limbic-thalamic nerve terminals. In fact, there is also a presynaptic co-localization of A_{2A} and $mGlu_5$ receptors in striatal glutamatergic terminals (Fig. 4) and there is a synergistic effect between A_{2A} and $mGlu_5$ receptors in the facilitation of glutamate release (Rodrigues et al., 2005). Furthermore, dopamine D_2 receptors also control glutamate release in the striatum (Bamford et al., 2004) and preliminary experiments also indicate a tight inter-dependence between presynaptic A_{2A} and D_2 receptors (Tscherter et al., 2006). Likewise, other receptor subtypes located presynaptically in glutamatergic terminals, such as cannabinoid CB_1 receptors (Kofalvi et al., 2005), may control striatal function by modulating neurotransmitter release and may do so by interacting with adenosine A_{2A} receptors (Carriba et al., 2007). Particularly surprising is the observation that there are functional antagonistic interactions between A_1 and A_{2A} (Fig. 4) receptors that modulate glutamate release in the striatum (Ciruela et al., 2006a; 2006b;Quarta et al., 2004). The use of synaptosomes allowed us to conclude a robust facilitation of glutamate release by activation of presynaptic A_{2A} receptors (Rodrigues et al., 2005) that co-exists with an ability of presynaptic A_1 receptors to inhibit striatal glutamate release (Ciruela et al., 2006a;2006b). It was found, using the *in vivo* microdialysis technique in freely moving rats, that intrastriatal perfusion with either an A_{2A} receptor agonist or an A_1 receptor antagonist induces glutamate outflow (Quarta et al., 2004).

7.2 Presynaptic A_1-A_{2A} receptors interaction: fact or artifact?

Two questions arise: are A_1 and A_{2A} receptors co-localized in the same glutamatergic terminals? If that is the case, what is the functional significance of a co-localization of two receptors operated by the same ligand that have opposite effects? Electron microscopy studies have demonstrated the co-localization of A_1 and A_{2A} receptors in the same striatal glutamatergic terminal (Ciruela et al., 2006). Furthermore, studies with isolated striatal nerve terminal preparations indicated that most striatal glutamatergic terminals contain both A_1 and A_{2A} receptors (Ciruela et al., 2006a). Experiments in co-transfected cells using BRET (Bioluminescence Resonance Energy Transfer) techniques demonstrated the existence A_1-A_{2A} receptor heteromers. Furthermore, radioligand-binding experiments showed the existence of a strong intramembrane A_1-A_{2A} receptor interaction, by which stimulation of A_{2A} receptors decreases the affinity of A_1 receptors for their agonists (Ciruela et al., 2006a). The intramembrane A_1-A_{2A} receptor interaction was used as a biochemical fingerprint of the A_1-A_{2A} receptor heteromer, which allowed demonstrating the existence of A_1-A_{2A} receptor heteromers in the striatum (Ciruela et al., 2006a). Finally, functional experiments in striatal glutamatergic terminals demonstrated that the A_1-A_{2A} receptor heteromer provides a “switch mechanism” by which low and high concentrations of adenosine produce opposite effects on glutamate release (Ciruela et al., 2006a; 2006b).

However, in electrophysiological experiments, in most conditions of patch clamp or extracellular field recordings, activation of A_{2A} receptors does not modify striatal evoked EPSCs or EPSPs which are fully dependent on AMPA receptors (Lovinger and Choi, 1995; Flagmeyer et al., 1997; D'Alcantara et al., 2001; Gerevich et al., 2002; Schiffmann et al., 2003). This lack of A_{2A} receptor effect was demonstrated not only by using pharmacological tools in wild type animals (Lovinger and Choi, 1995; Flagmeyer et al., 1997; D'Alcantara et al., 2001; Gerevich et al., 2002) but also by the observation that there was no difference between wild-type and A_{2A} knockout mice (D'Alcantara et al., 2001; Schiffmann et al., 2003). In contrast with these negative results, it was recently shown that presynaptic A_{2A} receptors are able to modulate excitatory synaptic cortico-striatal transmission but only in the presence of 4-aminopyridine (Tebano et al., 2004) or by controlling miniature events upon activation of

D_2 receptors (Tscherter et al., 2006), demonstrating that in certain conditions of presynaptic activity, A_{2A} receptor activation may exhibit a facilitatory effect.

8. Integration: pre- and postsynaptic adenosine A_{2A} receptors

This brief description of the known effects mediated by adenosine A_{2A} receptors in the striatum makes it evident that this receptor is engaged in tight interactions with other metabotropic receptors to fine-tune glutamatergic transmission at cortico-limbic-striatal and thalamo-striatal synapses. Interactions between postsynaptic A_{2A}, D_2 and $mGlu_5$ receptors (and also CB_1 receptors) are well-documented in the control of medium size efferent neuron responsiveness. However, presynaptic interactions also appear to control the release of glutamate, which actually triggers the functioning of striatal circuits. For most of these interactions between metabotropic receptors and A_{2A} receptors there is strong evidence for the formation of heterodimers (Ferré et al., 2005 for review). However, from the functional point of view, the most relevant question is to understand how presynaptic and postsynaptic heteromers containing A_{2A} receptors modulate glutamatergic neurotransmission in the striatal spines of enkephalinergic neurons.

8.1 Effect of adenosine concentration on adenosine receptor function in the basal ganglia

At the presynaptic side, low concentrations of adenosine, probably obtained during weak cortico-limbic-thalamic input (Fig. 4A), bind preferentially to A_1 receptors, which decreases the probabilility of glutamate release. On the other hand, high concentrations of synaptic adenosine, probably obtained by strong cortico-limbic-thalamic input (Fig. 4B) with high co-release of glutamate and ATP, will also induce occupation of A_{2A} receptors, counteracting the effects of A_1 receptor stimulation and increasing the probability of neurotransmitter release. This shift of function of adenosine from inhibition into facilitation is probably aided by $mGlu_5$ receptors. Thus, the frequency-dependent increase in extracellular levels of adenosine as well as glutamate will increase activation of $mGlu_5$ receptors in parallel with increased activation of A_{2A} receptors and these two receptor systems synergize to facilitate glutamate release (Rodrigues et al., 2005). Finally, at the presynaptic site, it remains to be seen whether increased firing frequencies in the cortico-limbic-thalamic-striatal projection bring presynaptic A_{2A} receptors into play and if this is able to overcome the tonic D_2 receptor inhibition of glutamate release (Bamford et al., 2004;Tscherter et al., 2006).

At the postsynaptic side, under weak cortico-limbic-thalamic input (Fig. 4A) there is a preferential D_2 receptor-mediated modulation of the A_{2A}-D_2-$mGlu_5$ heteromeric receptor complex, which is associated with low glutamatergic neurotransmission, low neuronal excitability and low gene expression. Under strong cortico-limbic-thalamic input (fig. 4B), there is a strong release of glutamate and formation of synaptic adenosine, which activates postsynaptic A_{2A} receptors, probably concentrated in the perisynaptic ring adjacent to the PSD. Furthermore, glutamate is able to overflow from the synaptic cleft and stimulate the $mGlu_5$ receptors of the perisynaptic ring. Thus, under these conditions there is strong activation of the A_{2A} and $mGlu_5$ receptors in the A_{2A}-D_2-$mGlu_5$ receptor heteromer, which allows gene expression, protein synthesis and plastic changes. In conclusion, the A_{2A} receptor, which is localized pre- and postsnaptically forming part of different heteromeric receptor complexes, plays a key role in the functional changes of glutamatergic synapses of the enkephalinergic striatal neurons during conditions of strong cortico-limbic-thalamic input. In agreement with this hypothesis, we found that administration of a selective A_{2A} receptor antagonist or caffeine counteracted both MAPK activation and AMPA receptor phosphorylation in GABAergic enkephalinergic neurons following cortical electrical stimulation (Quiroz et al., 2006).

8.2 Relative functional impact of pre- vs. postsynaptic adenosine A_{2A} receptors

A key question that remains to be clarified is the relative importance of presynaptic and postsynaptic A_{2A} receptors in the control of glutamatergic synapses between cortico- and thalamo-striatal projections and medium size efferent neurons. Electron microscopy, immunohistochemical studies, and *in situ* hybridization studies suggest that striatal A_{2A} receptors are mainly localized postsynaptically (Schiffmann et al., 1993; Rosin et al., 2003). In fact, the available evidence makes it clear that postsynaptic A_{2A} receptors have a strong impact on several key biochemical features that are accepted as determinants of striatal efferent neuron function, such as cAMP levels, DARPP-32 phosphorylation and regulation of L-type voltage-dependent Ca^{2+} channel and AMPA receptor activities. Less evidence is available on the contribution of presynaptic A_{2A} receptors. From a teleological point of view, the strong impact of A_{2A} receptors on glutamate release would be expected to play a crucial role. In fact, it is the release of glutamate that triggers postsynaptic activity and minor changes in the probability of release of glutamate are expected to have a significant impact on the function of the striatal efferent neurons. For instance, this should affect the down- to up-state transitions of striatal medium size spiny neurons with a higher proportion of time in the up-state. However, there are still no electrophysiological studies evaluating the relative importance of presynaptic and postsynaptic A_{2A} receptors in glutamatergic synapses of the dorsal striatum. It should be pointed out that in order for presynaptic A_{2A} receptors to play a relevant role in the control of the indirect pathway, A_{2A} receptors would need to be selectively localized in the glutamatergic nerve terminals that impinge on the GABAergic enkephalinergic neurons of the indirect pathway. This possibility awaits experimental support, although it is known that there is a selective tagging of cortico-striatal synapses to different medium size spiny neuron populations (Parthasarathy and Gaybriel, 1997; Lei et al., 2004) and previous studies in different brain areas have documented presynaptic modulatory systems that are confined to particular synapses contacting particular targets within the same neuron (Khakh et al., 2003).

8.3 Other factors affecting adenosine A_{2A} receptor function in striatum

Finally, it is essential to keep in mind the fact that A_{2A} receptors may come into play only under particular physiological conditions. There is evidence indicating that the activation of A_{2A} receptors might be achieved by a particular pool of adenosine, i.e., the adenosine derived from the extracellular catabolism of released ATP (for reviews see Cunha, 2001; Ferré et al., 2005). Thus, one could anticipate that A_{2A} receptors might play a prominent role in the control of glutamatergic striatal synapses under conditions of enhanced release of ATP, which occurs upon increased firing rates of glutamatergic neurons (Cunha et al., 1996; Wieraszko et al., 1989). This leads to long-term modifications of synaptic strength such as LTP and LTD at the cortico-striatal or cortico-accumbal synapses. In accordance with the anticipated particular role of A_{2A} receptors at higher firing frequencies, LTP of the AMPA receptor-mediated EPSP could be elicited in the nucleus accumbens of both wild-type and A_{2A} receptor-deficient mice but it was quantitatively reduced in the mutant animals as it was in slices from wild-type mice treated with an A_{2A} receptor antagonist (D'Alcantara et al., 2001; Schiffmann et al., 2003). The involvement of PKA was supported by a reduced level of LTP in wild-type slices treated with an inhibitor of this enzyme. As previously discussed, it is unclear whether this A_{2A} receptor-mediated regulation of cortico-striatal synaptic plasticity occurs pre- or postsynaptically or both. Whatever the mechanism, the regulation of LTP induction by A_{2A} receptors should have important consequences for motor learning and reward processes as suggested during chronic cocaine adaptation (Baldo et al., 1999) or behavioral sensitization to repeated amphetamine (Bastia et al., 2005) or L-DOPA treatment (Freduzzi et al., 2002). It should also be pointed out that this global (pre- and post-synaptic) facilitation of glutamatergic transmission by adenosine A_{2A} receptors may not only contribute to implement changes in synaptic efficiency but may also be responsible for excitotoxic effects once over-stimulated, in a manner analogous to the double-edge sword role played by NMDA receptors. This is re-enforced by the observation

that the pharmacological or genetic blockade of A_{2A} receptors indeed confers robust neuroprotection against different chronic noxious stimuli (Popoli et al., 2002; Blum et al., 2003; Cunha, 2005).

In summary, the body of available evidence supports the conclusion that A_{2A} receptors play an important fine-tuning role in striatal function by boosting the efficiency of information flow in glutamatergic synapses of the indirect pathway. This is mostly achieved by the organization of A_{2A} receptors in different heteromers allowing control of other key modulators of glutamatergic synapses, such as dopamine (acting through D_2 receptors), glutamate (acting through group I metabotropic receptors) and endocannabinoids (acting through CB_1 receptors) as well as by triggering the cAMP-PKA signaling cascade. However, further neurochemical and electrophysiological studies are clearly required to better understand the relative participation of presynaptic and postsynaptic A_{2A} receptors in modulation of striatal pathways.

Acknowledgments

These works were supported by Fondation Médicale Reine Elisabeth (FMRE 2005−2007), Fonds National de la Recherche Scientifique (FNRS grants 3.4507.02 F and 3.4509.06F), Van Buuren Foundation, Action de Recherche Concertée Communauté Française Wallonie-Bruxelles (ARC N° 02/07−290) to SNS, the National Institute on Drug Abuse Intramural Research Funds to SF and Fundação para a Ciência e Tecnologia (grant POCI/SAU-FCF/59215/2004) to RAC. The authors thank N.Rebola and K. Azdad for helpful discussion and contribution to these studies.

Abbreviatons

AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionate; BRET, Bioluminescence Resonance Energy Transfer; Cdk-5, cyclin dependent kinase-5; CREB, cAMP response element binding protein; DARPP-32, dopamine and cAMP-regulated phosphoprotein, 32 kDa; EPSPs, excitatory postsynaptic potentials; EPSCs, excitatory postsynaptic currents; Gpe, external segment of the globus pallidus; Gpi, internal segment of the globus pallidus; IPSCs, inhibitory postsynaptic currents; L-DOPA, L-3,4-dihydroxyphenylalanine; LTD , long term depression; LTP, long term potentiation; MAPK, mitogen-activated protein kinase; MRI, magnetic resonance imaging; NMDA, N-methyl-D-aspartate; PET, positron emission tomography; PKA, protein kinase A; PP-1, protein phosphatase-1; PSD, postsynaptic density; QA, quinolinic acid; STN, subthalamic nucleus.

References

Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 1990;13:266–271. [PubMed: 1695401]

Andersson M, Usiello A, Borgkvist A, Pozzi L, Dominguez C, Fienberg AA, Svenningsson P, Fredholm BB, Borrelli E, Greengard P, Fisone G. Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. J. Neurosci 2005;25:8432–8438. [PubMed: 16162925]

Azdad, K.; Gall, D.; Schiffmann, SN. Electrophysiological evidence for an antagonistic modulatory role of adenosine A_{2A} receptor on dopamine D_2 receptor regulation of down- and up-state transitions in the nucleus accumbens.; Abstract of the International Symposium “Targeting A2A adenosine receptors in Parkinson's disease and other CNS disorders”; Boston MA, USA. 17−20 May 2006; 2006.

Baldo BA, Koob GF, Markou A. Role of adenosine A2 receptors in brain stimulation reward under baseline conditions and during cocaine withdrawal in rats. J. Neurosci 1999;19:11017–11026. [PubMed: 10594082]

Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, Schmauss C, Zakharenko SS, Zablow L, Sulzer D. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 2004;42:653–663. [PubMed: 15157425]

Bastia E, Xu YH, Scibelli AC, Day YL, Linden J, Chen JF, Schwarzschild MA. A crucial rle for forebrain adenosine A_{2A} receptors in amphetamine sensitization. Neuropsychopharmacol 2005;30:891–900.

Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 2001;410:376–380. [PubMed: 11268215]

Bibb JA, Snyder GL, Nishi A, Yan Z, Meijer L, Fienberg AA, Tsai LH, Kwon YT, Girault JA, Czernik AJ, Huganir R, Hemmings HC, Nairn AC, Greengard P. Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. Nature 1999;402:669–671. [PubMed: 10604473]

Blum D, Galas M-C, Pintor A, Brouillet E, Ledent C, Muller CE, Bantubungi K, Galluzzo M, Gall D, Cuvelier L, Rolland A-S, Popoli P, Schiffmann SN. A dual role of adenosine A2A receptors in 3-nitropropionic acid-induced striatal lesions: implications for the neuroprotective potential of A2A antagonists. J. Neurosci 2003;23:5361–5369. [PubMed: 12832562]

Brown SJ, James S, Reddington M, Richardson PJ. Both A1 and A2a purine receptors regulate striatal acetylcholine release. J. Neurochem 1990;55:31–38. [PubMed: 2355224]

Canals M, Marcellino D, Fanelli F, Ciruela F, de Benedetti P, Goldberg SR, Neve K, Fuxe K, Agnati LF, Woods AS, Ferré S, Lluis C, Bouvier M, Franco R. Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J. Biol. Chem 2003;278:46741–46749. [PubMed: 12933819]

Carriba P, Ortiz O, Patkar K, Justinova S, Stroik J, Themann A, Müller C, Woods AS, Hope BT, Ciruela F, Casadó V, Canela EI, Lluis C, Goldberg SR, Moratalla R, Franco R, Ferré S. Striatal adenosine A_{2A} and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacology. 2007(in press)

Cepeda C, Hurst RS, Altemus KL, Flores-Hernández J, Calvert CR, Jokel ES, Grandy DK, Low MJ, Rubinstein M, Ariano MA, Levine MS. Facilitated glutamatergic transsmission in the striatum of D2 dopamine receptor deficient mice. J. Neurophysiol 2001;85:659–670. [PubMed: 11160501]

Chergui K, Bouron A, Normand E, Mulle C. Functional GluR6 kainate receptors in the striatum: indirect downregulation of synaptic transmission. J. Neurosci 2000;20:2175–2182. [PubMed: 10704492]

Ciruela F, Burgueno J, Casado V, Canals M, Marcellino D, Goldberg SR, Bader M, Fuxe K, Agnati LF, Lluis C, Franco R, Ferre S, Woods AS. Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope-epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors. Anal. Chem 2004;76:5354–5363. [PubMed: 15362892]

Ciruela F, Casado V, Rodrigues RJ, Lujan R, Burgueno J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortes A, Canela EI, Lopez-Gimenez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J. Neurosci 2006a;26:2080–2087. [PubMed: 16481441]

Ciruela F, Ferre S, Casado V, Cortes A, Cunha RA, Lluis C, Franco R. Heterodimeric adenosine receptors: a device to regulate neurotransmitter release. Cell. Mol. Life Sci 2006b;63:2427–2431. [PubMed: 17058035]

Coccurello R, Breysse N, Amalric M. Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology 2004;29:1451–1461. [PubMed: 15039773]

Colwell CS, Levine MS. Excitatory synaptic transmission in neostriatal neurons: regulation by cyclic AMP-dependent mechanisms. J. Neurosci 1995;15:1704–1713. [PubMed: 7891129]

Cunha RA, Vizi ES, Ribeiro JA, Sebastiao AM. Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. J. Neurochem 1996;67:2180–2187. [PubMed: 8863529]

Cunha RA, Ribeiro JA. ATP as a presynaptic modulator. Life Sci 2000;68:119–137. [PubMed: 11191632]

Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem. Int 2001;38:107–125. [PubMed: 11137880]

Cunha RA. Neuroprotection by adenosine in the brain: from A_1 receptor activation to A_{2A} receptor blockade. Purinergic Signalling 2005;1:111–134.

D'Alcantara P, Ledent C, Swillens S, Schiffmann SN. Inactivation of adenosine A2A receptor impairs long term potentiation in the accumbens nucleus without altering basal synaptic transmission. Neuroscience 2001;107:455–464. [PubMed: 11719000]

Dassesse D, Massie A, Ferrarri R, Ledent C, Parmentier M, Arckens L, Zoli M, Schiffmann SN. Functional striatal hypodopaminergic activity in mice lacking adenosine A2A receptors. J. Neurochem 2001;78:183–198. [PubMed: 11432985]2001

Domenici MR, Pepponi R, Martire A, Tebano MT, Potenza RL, Popoli P. Permissive role of adenosine A2A receptors on metabotropic glutamate receptor 5 (mGluR5)-mediated effects in the striatum. J. Neurochem 2004;90:1276–1279. [PubMed: 15312183]

Dudman JT, Eaton ME, Rajadhyaksha A, Macias W, Taher M, Barczak A, Kameyama K, Huganir R, Konradi C. Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. J. Neurochem 2003;87:922–934. [PubMed: 14622123]

Evans GJ, Morgan A. Regulation of the exocytotic machinery by cAMP-dependent protein kinase: implications for presynaptic plasticity. Biochem. Soc. Trans 2003;31:824–827. [PubMed: 12887314]

Ferré S. Adenosine-dopamine interactions in the ventral striatum. Implications for the treatment of schizophrenia. Psychopharmacology 1997;133:107–120. [PubMed: 9342776]

Ferré S, von Euler G, Johansson B, Fredholm BB, 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. [PubMed: 1678519]

Ferré S, O'Connor WT, Fuxe K, Ungerstedt U. The striopallidal neuron: a main locus for adenosine-dopamine interactions in the brain. J. Neurosci 1993;13:5402–5406. [PubMed: 8254382]

Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 1997;20:482–487. [PubMed: 9347617]

Ferré S, Torvinen M, Antoniou K, Irenius E, Civelli O, Arenas E, Fredholm BB, Fuxe K. Adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. J. Biol. Chem 1998;273:4718–4724. [PubMed: 9468534]

Ferré S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, Casado V, Fuxe K, Goldberg SR, Lluis C, Franco R, Ciruela F. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc. Natl. Acad. Sci. USA 2002;99:11940–11945. [PubMed: 12189203]

Ferré S, Borycz J, Goldberg SR, Hope BT, Morales M, Lluis C, Franco R, Ciruela F, Cunha R. Role of adenosine in the control of homosynaptic plasticity in striatal excitatory synapses. J. Integr. Neurosci 2005;4:445–464. [PubMed: 16385640]

Fienberg AA, Hiroi N, Mermelsten P, Song WJ, Snyder GL, Nishi A, Cheramy A, O'Callaghan JP, Miller DB, Cole DG, Corbett R, Haile CN, Cooper DC, Onn SP, Grace AA, Ouimet CC, White FJ, Hyman SE, Surmeier DJ, Girault JA, Nestler EJ, Greengard P. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 1998;281:838–842. [PubMed: 9694658]

Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM, Reppert SM. Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Mol. Brain Res 1992;14:186–195. [PubMed: 1279342]

Fisone G, Borgkvist A, Usiello A. Caffeine as a psychomotor stimulant: mechanism of action. Cell. Mol. Life Sci 2004;61:857–872. [PubMed: 15095008]

Flagmeyer I, Haas HL, Stevens DR. Adenosine A1 receptor-mediated depression of corticostriatal and thalamostriatal glutamatergic synaptic potentials in vitro. Brain Res 1997;778:178–185. [PubMed: 9462890]

Fredduzzi S, Moratalla R, Monopoli A, Cuellar B, Xu K, Ongini E, Impagnatiello F, Schwarzschild MA, Chen JF. Persistent behavioral sensitization to chronic L-DOPA requires A2A adenosine receptors. J. Neurosci 2002;22:1054–1062. [PubMed: 11826134]

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev 1999;51:83–153. [PubMed: 10049999]

Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev 2001;53:527–552. [PubMed: 11734617]

Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int. Rev. Neurobiol 2005;63:191–270. [PubMed: 15797469]

Geiger, JD.; Fyda, DM. Adenosine transport in nervous tissues.. In: Stone, TW., editor. Adenosine in the Central Nervous System. Academic Press; London: 1991. p. 1-23.

Gerevich Z, Wirkner K, Illes P. Adenosine A2A receptors inhibit the N-methyl-D-aspartate component of excitatory synaptic currents in rat striatal neurons. Eur. J. Pharmacol 2002;451:161–164. [PubMed: 12231386]

Gerfen, CR. Basal Ganglia.. In: Paxinos, G., editor. The Rat Nervous System. Elsevier Academic Press; Amsterdam: 2004. p. 445-508.

Ginés S, Hillion J, Torvinen M, Le Crom S, Casado V, Canela EI, Rondin S, Lew JY, Watson S, Zoli M, Agnati LF, Verniera P, Lluis C, Ferré S, Fuxe K, Franco R. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. Proc. Natl. Acad. Sci. USA 2000;97:8606–8611. [PubMed: 10890919]

Graybiel AM. Building action repertoires: memory and learning functions of the basal ganglia. Curr. Opin. Neurobiol 1995;5:733–741. [PubMed: 8805417]

Graybiel AM, Aosaki T, Flaherty AW, Kimura M. The basal ganglia and adaptive motor control. Science 1994;265:1826–1831. [PubMed: 8091209]

Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 1999;23:435–447. [PubMed: 10433257]

Håkansson K, Galdi S, Hendrick J, Snyder G, Greengard P, Fisone G. Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. J. Neurochem 2006;96:482–488. [PubMed: 16336634]

Hemmings JHC, Greengard P, Tung HYL, Cohen P. DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. Nature 1984;310:503–505. [PubMed: 6087160]

Hernández-Echeagaray E, Starling AJ, Cepeda C, Levine MS. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? Eur. J. Neurosci 2004;19:2455–2463. [PubMed: 15128399]

Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E. D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca2+ conductance. J. Neurosci 1997;17:3334–3342. [PubMed: 9096166]

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J, Canela EI, Zoli M, Agnati LF, Ibanez CF, Lluis C, Franco R, Ferré S, Fux,e K. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J. Biol. Chem 2002;277:18091–18097. [PubMed: 11872740]

Ishiwata K, Noguchi J, Wakabayashi S, Shimada J, Ogi N, Nariai T, Tanaka A, Endo K, Suzuki F, Senda M. 11C-labeled KF18446: a potential central nervous system adenosine A2a receptor ligand. J. Nucl. Med 2000;41:345–354. [PubMed: 10688121]

Ishiwata K, Ogi N, Hayakawa N, Oda K, Nagaoka T, Toyama H, Suzuki F, Endo K, Tanaka A, Senda M. Adenosine A2A receptor imaging with [11C]KF18446 PET in the rat brain after quinolinic acid lesion: comparison with the dopamine receptor imaging. Ann. Nucl. Med 2002;16(7):467–475. [PubMed: 12508837]

Ishiwata K, Mishina M, Kimura Y, Oda K, Sasaki T, Ishii K. First visualization of adenosine A(2A) receptors in the human brain by positron emission tomography with [11C]TMSX. Synapse 2005;55:133–136. [PubMed: 15543628]

Jarvis SE, Zamponi GW. Interactions between presynaptic Ca2+ channels, cytoplasmic messengers and proteins of the synaptic vesicle release complex. Trends Pharmacol. Sci 2001;22:519–525. [PubMed: 11583809]

Jin S, Fredholm BB. Adenosine A2A receptor stimulation increases release of acetylcholine from rat hippocampus but not striatum, and does not affect catecholamine release. Naunyn Schmiedebergs Arch. Pharmacol 1997;355:48–56. [PubMed: 9007842]

Kachroo A, Orlando LR, Grandy DK, Chen JF, Young AB, Schwarzschild MA. Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. J. Neurosci 2005;25:10414–10419. [PubMed: 16280580]

Kennedy MB. Signal-processing machines at the postsynaptic density. Science 2000;290:750–754. [PubMed: 11052931]

Khakh BS, Gittermann D, Cockayne DA, Jones A. ATP modulation of excitatory synapses onto interneurons. J. Neurosci 2003;23:7426–7437. [PubMed: 12917379]

Kofalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA, Sperlagh B. Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J. Neurosci 2005;25:2874–8284. [PubMed: 15772347]

Koizumi S, Fujishita K, Tsuda M, Shigemoto-Mogami Y, Inoue K. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. Proc. Natl. Acad. Sci. USA 2003;100:11023–11028. [PubMed: 12958212]

Kull B, Ferré S, Arslan G, Svenningsson P, Fuxe K, Owman C, Fredholm BB. Reciprocal interactions between adenosine A2A and dopamine D2 receptors in Chinese hamster ovary cells co-transfected with the two receptors. Biochem. Pharmacol 1999;15:1035–1045. [PubMed: 10509756]

Kurokawa M, Kirk IP, Kirkpatrick KA, Kase H, Richardson PJ. Inhibition by KF17837 of adenosine A2A receptor-mediated modulation of striatal GABA and ACh release. Br. J. Pharmacol 1994;113:43–48. [PubMed: 7812630]

Lee FJ, Xue S, Pei L, Vukusic B, Chery N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111:219–230. [PubMed: 12408866](2002)

Leenders AG, Sheng ZH. Modulation of neurotransmitter release by the second messenger-activated protein kinases: implications for presynaptic plasticity. Pharmacol. Ther 2005;105:69–84. [PubMed: 15626456]

Lei W, Jiao Y, Del Mar N, Reiner A. Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J. Neurosci 2004;24:8289–8299. [PubMed: 15385612]

Lindskog M, Svenningsson P, Fredholm BB, Greengard P, Fisone G. Activation of dopamine D2 receptors decreases DARPP-32 phosphorylation in striatonigral and striatopallidal projection neurons via different mechanisms. Neuroscience 1999;88:1005–1008. [PubMed: 10336115]

Lindskog M, Svenningsson P, Pozzi L, Kim Y, Fienberg AA, Bibb JA, Fredholm BB, Nairn AC, Greengard P, Fisone G. Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. Nature 2002;418:774–778. [PubMed: 12181566]

Lovinger DM, Choi S. Activation of adenosine A1 receptors initiates short-term synaptic depression in rat striatum. Neurosci. Lett 1995;199:9–12. [PubMed: 8584233]

Lu XY, Ghasemzadeh MB, Kalivas PW. Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience 1998;82:767–780. [PubMed: 9483534]

Matsuya T, Takamatsu H, Murakami Y, Noda A, Ichise R, Awaga Y, Nishimura S. Synthesis and evaluation of [11C]FR194921 as a nonxanthine-type PET tracer for adenosine A1 receptors in the brain. Nucl. Med. Biol 2005;32:837–844. [PubMed: 16253808]

Mayfield DR, Larson G, Orona RA, Zahniser NR. Opposing actions of adenosine A2a and dopamine D2 receptor activation on GABA release in the basal ganglia: evidence for an A2a/D2 receptor interaction in globus pallidus. Synapse 1996;22:132–138. [PubMed: 8787129]

Meyer PT, Elmenhorst D, Bier D, Holschbach MH, Matusch A, Coenen HH, Zille,s K, Bauer A. Quantification of cerebral A1 adenosine receptors in humans using [18F]CPFPX and PET: an equilibrium approach. Neuroimag 2005;24:1192–204.

Moresco RM, Todde S, Belloli S, Simonelli P, Panzacchi A, Rigamonti M, Galli-Kienle M, Fazio F. In vivo imaging of adenosine A2A receptors in rat and primate brain using [11C]SCH442416. Eur. J. Nucl. Med. Mol. Imaging 2005;32:405–413. [PubMed: 15549298]

Mori A, Shindou T, Ichimura M, Nonaka H, Kase H. The role of adenosine A2a receptors in regulating GABAergic synaptic transmission in striatal medium spiny neurons. J. Neurosci 1996;16:605–611. [PubMed: 8551344]

Nairn AC, Svenningsson P, Nishi A, Fisone G, Girault JA, Greengard P. The role of DARPP-32 in the actions of drugs of abuse. Neuropharmacology 2004;47(Suppl 1):14–23. [PubMed: 15464122]

Newman EA. Glial cell inhibition of neurons by release of ATP. J. Neurosci 2003;23:1659–1666. [PubMed: 12629170]

Nishi A, Liu F, Matsuyama S, Hamada M, Higashi H, Nairn AC, Greengard P. Metabotropic mGlu5 receptors regulate adenosine A2A receptor signaling. Proc. Natl. Acad. Sci. USA 2003;100:1322–1327. [PubMed: 12538871]

Nishi A, Snyder GL, Greengard P. Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J. Neurosci 1997;17:8147–8155. [PubMed: 9334390]

Norenberg W, Wirkner K, Assmann H, Richter M, Illes P. Adenosine A2A receptors inhibit the conductance of NMDA receptor channels in rat neostriatal neurons. Amino Acids 1998;14:33–39. [PubMed: 9871438]

Norenberg W, Wirkner K, Illes P. Effect of adenosine and some of its structural analogues on the conductance of NMDA receptor channels in a subset of rat neostriatal neurones. Br. J. Pharmacol 1997;122:71–80. [PubMed: 9298530]

Okada M, Mizuno K, Kaneko S. Adenosine A1 and A2 receptors modulate extracellular dopamine levels in rat striatum. Neurosci.Lett 1996;212:53–56. [PubMed: 8823761]

Okada M, Kiryu K, Kawata Y, Mizuno K, Wada K, Tasaki H, Kaneko S. Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. Eur.J.Pharmacol 1997;321:181–188. [PubMed: 9063686]

Olson PA, Tkatch T, Hernandez-Lopez S, Ulrich S, Ilijic E, Mugnaini E, Zhang H, Bezprozvanny I, Surmeier DJ. G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca2+ channels is dependent on a Shank-binding domain. J. Neurosci 2005;25:1050–1062. [PubMed: 15689540]

Ouimet CC, Langley-Guillion KC, Greengard P. Quantitative immunochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. Brain Res 1998;808:8–12. [PubMed: 9795103]

Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res. Rev 1995;20:91–127. [PubMed: 7711769]

Parr-Brownlie LC, Hyland BI. Bradykinesia induced by dopamine D2 receptor blockade is associated with reduced motor cortex activity in the rat. J. Neurosci 2005;25:5700–5709. [PubMed: 15958736]

Parthasarathy HB, Graybiel AM. Cortically driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. J. Neurosci 1997;17:2477–2491. [PubMed: 9065508]

Popoli P, Pezzola A, Torvinen M, Reggio R, Pintor A, Scarchilli L, Fuxe K, Ferré 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. [PubMed: 11557164]

Popoli P, Pintor A, Domenici MR, Frank C, Tebano MT, Pezzola A, Scarchilli L, Quarta D, Reggio R, Malchiodi-Albedi F, Falchi M, Massotti M. Blockade of striatal adenosine A_{2A} receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. J. Neurosci 2002;22:1967–1975. [PubMed: 11880527]

Quarta D, Borycz J, Solinas M, Patkar K, Hockemeyer J, Ciruela F, Lluis C, Franco R, Woods AS, Goldberg SR, Ferré S. Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation. J. Neurochem 2004;91:873–880. [PubMed: 15525341]

Quiroz C, Gomes C, Pak AC, Ribeiro JA, Goldberg SR, Hope BT, Ferre S. Blockade of adenosine A2A receptors prevents protein phosphorylation in the striatum induced by cortical stimulation. J. Neurosci 2006;26:10808–10812. [PubMed: 17050719]

Robertson GS, Jian M. D1 and D2 dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. Neuroscience 1995;64:1019–1034. [PubMed: 7753373]

Rodrigues RJ, Alfaro TM, Rebola N, Oliveira CR, Cunha RA. Co-localization and functional interaction between adenosine A and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. J. Neurochem 2005;92:433–441. [PubMed: 15659214]

Ronesi J, Lovinger DM. Induction of striatal long-term synaptic depression by moderate frequency activation of cortical afferents in rat. J. Physiol 2005;562:245–256. [PubMed: 15498813]

Rosin DL, Hettinger BD, Lee A, Linden J. Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 2003;61:S12–8. [PubMed: 14663003]

Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. J. Comp. Neurol 1998;401:163–186. [PubMed: 9822147]

Salim H, Ferré S, Dalal A, Peterfreund RA, Fuxe K, Vincent JD, Lledo PM. Activation of adenosine A1 and A2A receptors modulates dopamine D2 receptor-induced responses in stably transfected human neuroblastoma cells. J. Neurochem 2000;74:432–439. [PubMed: 10617149]

Schiffmann SN, Jacobs O, Vanderhaeghen JJ. Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. J. Neurochem 1991a;57:1062–1067. [PubMed: 1713612]

Schiffmann SN, Libert F, Vassart G, Vanderhaeghen JJ. Distribution of adenosine A2 receptor mRNA in the human brain. Neurosci. Lett 1991b;130:177–181. [PubMed: 1795877]

Schiffmann SN, Vanderhaeghen JJ. Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J. Neurosci 1993;13(3):1080–1087. [PubMed: 7680065]

Schiffmann SN, Lledo PM, Vincent JD. Dopamine D1 receptor modulates the voltage-gated sodium current in rat striatal neurones through a protein kinase A. J. Physiol. (Lond) 1995;483:95–107. [PubMed: 7776243]

Schiffmann SN, Desdouits F, Menu R, Vincent JD, Greengard P, Vanderhaeghen JJ, Girault JA. Modulation of the voltage-gated sodium current in rat striatal neurons by DARPP-32, an inhibitor of protein phosphatase 1. Eur. J. Neurosci 1998;10:1312–1320. [PubMed: 9749785]

Schiffmann SN, Dassesse D, d'Alcantara P, Ledent C, Swillens S, Zoli M. A_{2A} Receptor and Striatal Cellular Functions: Regulation of Gene Expression, Currents and Synaptic Transmission. Neurology 2003;61:S24–29. [PubMed: 14663005]

Schultz W, Tremblay L, Hollerman JR. Changes in behavior-related neuronal activity in the striatum during learning. Trends Neurosci 2003;26:321–328. [PubMed: 12798602]

Schulz PE. Long-term potentiation involves increases in the probability of neurotransmitter release. Proc. Natl. Acad. Sci. USA 1997;94:5888–5893. [PubMed: 9159170]

Segal M. Dendritic spines and long-term plasticity. Nat. Rev. Neurosci 2005;6:277–284. [PubMed: 15803159]

Shindou T, Mori A, Kase H, Ichimura M. Adenosine A(2A) receptor enhances GABA(A)-mediated IPSCs in the rat globus pallidus. J. Physiol 2001;532:423–434. [PubMed: 11306661]

Shindou T, Nonaka H, Richardson PJ, Mori A, Kase H, Ichimura M. Presynaptic adenosine A(2A) receptors enhance GABAergic synaptic transmission via a cyclic AMP dependent mechanism in the rat globus pallidus. Br. J. Pharmacol 2002;136:296–302. [PubMed: 12010779]

Shindou T, Richardson PJ, Mori A, Kase H, Ichimura M. Adenosine modulates the striatal GABAergic inputs to the globus pallidus via adenosine A2A receptors in rats. Neurosci. Lett 2003;352:167–170. [PubMed: 14625011]

Silinsky EM, Hirsh JK, Searl TJ, Redman RS, Watanabe M. Quantal ATP release from motor nerve endings and its role in neurally mediated depression. Prog. Brain Res 1999;120:145–58. [PubMed: 10550994]

Sola E, Prestori F, Rossi P, Taglietti V, D'Angelo E. Increased neurotransmitter release during long-term potentiation at mossy fibre-granule cell synapses in rat cerebellum. J. Physiol 2004;557:843–861. [PubMed: 15090602]

Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. Trends Neurosci 2002;25:578–588. [PubMed: 12392933]

Stern EA, Jaeger D, Wilson CJ. Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo. Nature 1998;394:475–478. [PubMed: 9697769]

Stern EA, Kincaid AE, Wilson CJ. Spontaneous subthreshold membrane potential fluctuations and action potential variability of rat corticostriatal and striatal neurons in vivo. J. Neurophysiol 1997;77:1697–1715. [PubMed: 9114230]

Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron 1995;14:385–897. [PubMed: 7531987]

Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST. Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc. Natl. Acad. Sci. U.S.A 1992;89:10178–10182. [PubMed: 1332033]

Svenningsson P, Lindskog M, Rognoni F, Fredholm BB, Greengard P, Fisone G. Activation of adenosine A_{2A} and dopamine D1 receptors stimulates cyclic AMP-dependent phosphorylation of DARPP-32 in distinct populations of striatal projection neurons. Neuroscience 1998;84:223–228. [PubMed: 9522376]

Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog. Neurobiol 1999;59:355–396. [PubMed: 10501634]

Svenningsson P, Lindskog M, Ledent C, Parmentier M, Greengard P, Fredholm B, Fisone G. Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa *in vivo* by dopamine D_1, dopamine D_2, and adenosine A_{2A} receptors. Proc. Natl. Acad. Sci. U.S.A 2000;97:1856–1860. [PubMed: 10677546]

Tebano MT, Pintor A, Frank C, Domenici MR, Martire A, Pepponi R, Potenza RL, Grieco R, Popoli P. Adenosine A2A receptor blockade differentially influences excitotoxic mechanisms at pre- and postsynaptic sites in the rat striatum. J. Neurosci. Res 2004;77:100–107. [PubMed: 15197743]

Tepper JM, Bolam JP. Functional diversity and specificity of neostriatal interneurons. Curr. Opin. Neurobiol 2004;14:685–692. [PubMed: 15582369]

Tscherter A, Rossi S, Centonze D, Borsini F, Calabresi P. Interaction of adenosine A2A and dopamine D2 receptors modulates glutamatergic corticostriatal transmission. FENS Abstr 2006;3:A075.11.

Todde S, Moresco RM, Simonelli P, Baraldi PG, Cacciari B, Spalluto G, Varani K, Monopoli A, Matarrese M, Carpinelli A, Magni F, Kienle MG, Fazio F. Design, radiosynthesis, and biodistribution of a new potent and selective ligand for in vivo imaging of the adenosine A(2A) receptor system using positron emission tomography. J. Med. Chem 2000;43:4359–4362. [PubMed: 11087559]

Vergara R, Rick C, Hernandez-Lopez S, Laville JA, Guzman JN, Galarraga E, Surmeier DJ, Bargas J. Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. J. Physiol 2003;553:169–182. [PubMed: 12963790]

White NM. Mnemonic functions of the basal ganglia. Curr. Opin. Neurobiol 1997;7:164–169. [PubMed: 9142761]

Wickens JR, Reynolds JN, Hyland BI. Neural mechanisms of reward-related motor learning. Curr. Opin. Neurobiol 2003;3:685–690. [PubMed: 14662369]

Wieraszko A, Goldsmith G, Seyfried TN. Stimulation-dependent release of adenosine triphosphate from hippocampal slices. Brain Res 1989;485:244–250. [PubMed: 2566360]

Wilson CJ, Kawaguchi Y. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J. Neurosci 1996;16:2397–2410. [PubMed: 8601819]

Wirkner K, Assmann H, Koles L, Gerevich Z, Franke H, Norenberg W, Boehm R, Illes P. Inhibition by adenosine A(2A) receptors of NMDA but not AMPA currents in rat neostriatal neurons. Br. J. Pharmacol 2000;130:259–269. [PubMed: 10807662]

Wirkner K, Gerevich Z, Krause T, Gunther A, Koles L, Schneider D, Norenberg W, Illes P. Adenosine A2A receptor-induced inhibition of NMDA and GABAA receptor-mediated synaptic currents in a subpopulation of rat striatal neurons. Neuropharmacology 2004;46:994–1007. [PubMed: 15081796]

Wolf ME, Mangiavacchi S, Sun X. Mechanisms by which dopamine receptors may influence synaptic plasticity. An. N.Y. Acad. Sci 2003;1003:241–249.

Wu LG, Saggau P. Presynaptic inhibition of elicited neurotransmitter release. Trends Neurosci 1997;20:204–212. [PubMed: 9141196]

Xu M, Moratalla R, Gold LH, Hiroi N, Koob GF, Graybiel AM, Tonegawa S. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 1994;79:729–742. [PubMed: 7954836]

Zetterstrom T, Fillenz M. Adenosine agonists can both inhibit and enhance in vivo striatal dopamine release. Eur.J.Pharmacol 1990;180:137–143. [PubMed: 2364998]

Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S. ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron 2003;40:971–982. [PubMed: 14659095]

Zhou L, Furuta T, Kaneko T. Chemical organization of projection neurons in the rat accumbens nucleus and olfactory tubercle. Neuroscience 2003;120:783–798. [PubMed: 12895518]

Zimmermann H. Extracellular metabolism of ATP and other nucleotides. Naunyn Schmiedebergs Arch. Pharmacol 2000;362:299–309. [PubMed: 11111825]

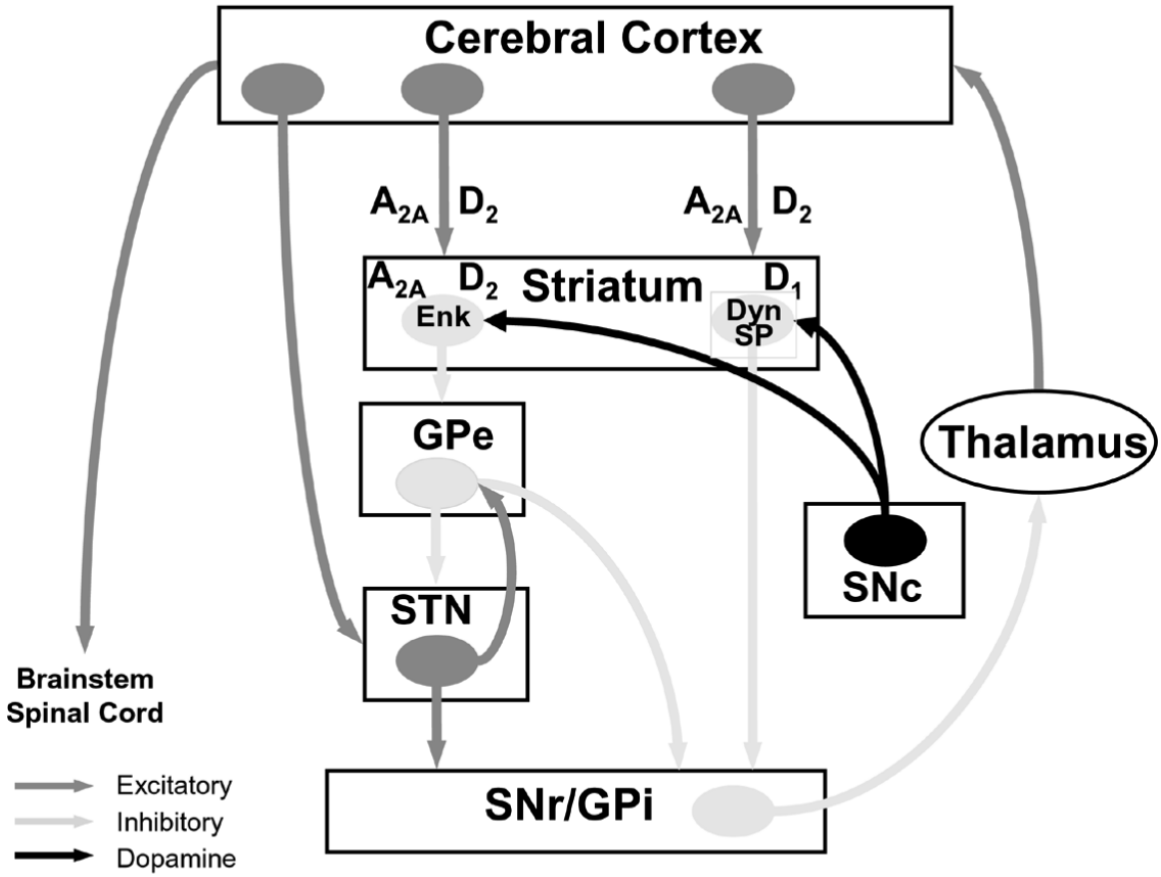

Figure 1.

Schematic representation of the basal ganglia circuitry including the major connections of the system and showing the main neuropeptides used by the different populations of striatal GABAergic efferent neurons, together with the main localization of adenosine A_{2A} and dopamine receptors. Arrows represent the synaptic connections between the different structures; excitatory, inhibitory and dopaminergic connections are represented by dark grey, light grey and black arrows, respectively. Dyn, dynorphin; Enk, enkephalin; Gpe, external segment of the globus pallidus; Gpi, internal segment of the globus pallidus; SNc, substantia nigra *pars compacta;* SNe, substantia nigra *pars reticulata;* SP, substance P; STN, subthalamic nucleus. Adapted from Alexander and Crutcher, 1990.

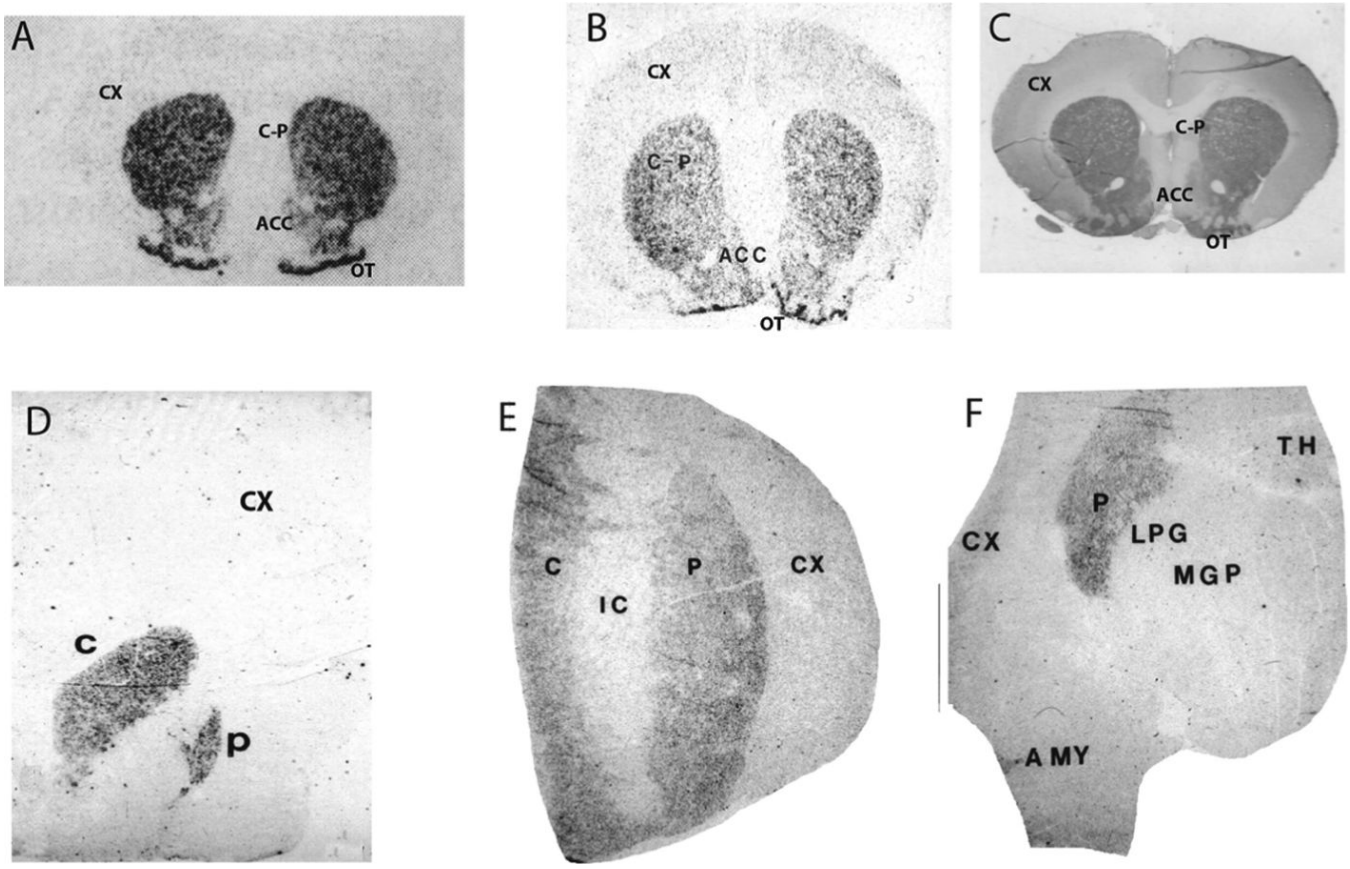

Figure 2.

Distribution of A_{2A} receptor in he basal ganglia of different mammalian species: *in situ* hybridization shows a very high level of A_{2A} receptor mRNA in the different sectors of the striatum including the caudate-putamen, the nucleus accumbens and the olfactory tubercle in mouse (A), rat (B), dog (D) and human (E, F) brain as compared to other brain areas. C. Immunohistochemical detection of A_{2A} receptor confirms its high abundance in the rat striatum. Abbreviations: acc, accumbens ; amy, amygdala; c, caudate; c-p, caudate-putamen; cx, cerebral cortex; ic, internal capsule; lgp, lateral globus pallidus; mgp, medial globus pallidus; p, putamen; ot, olfactory tubercle; th, thalamus. Modified from Schiffmann et al., 1991b.

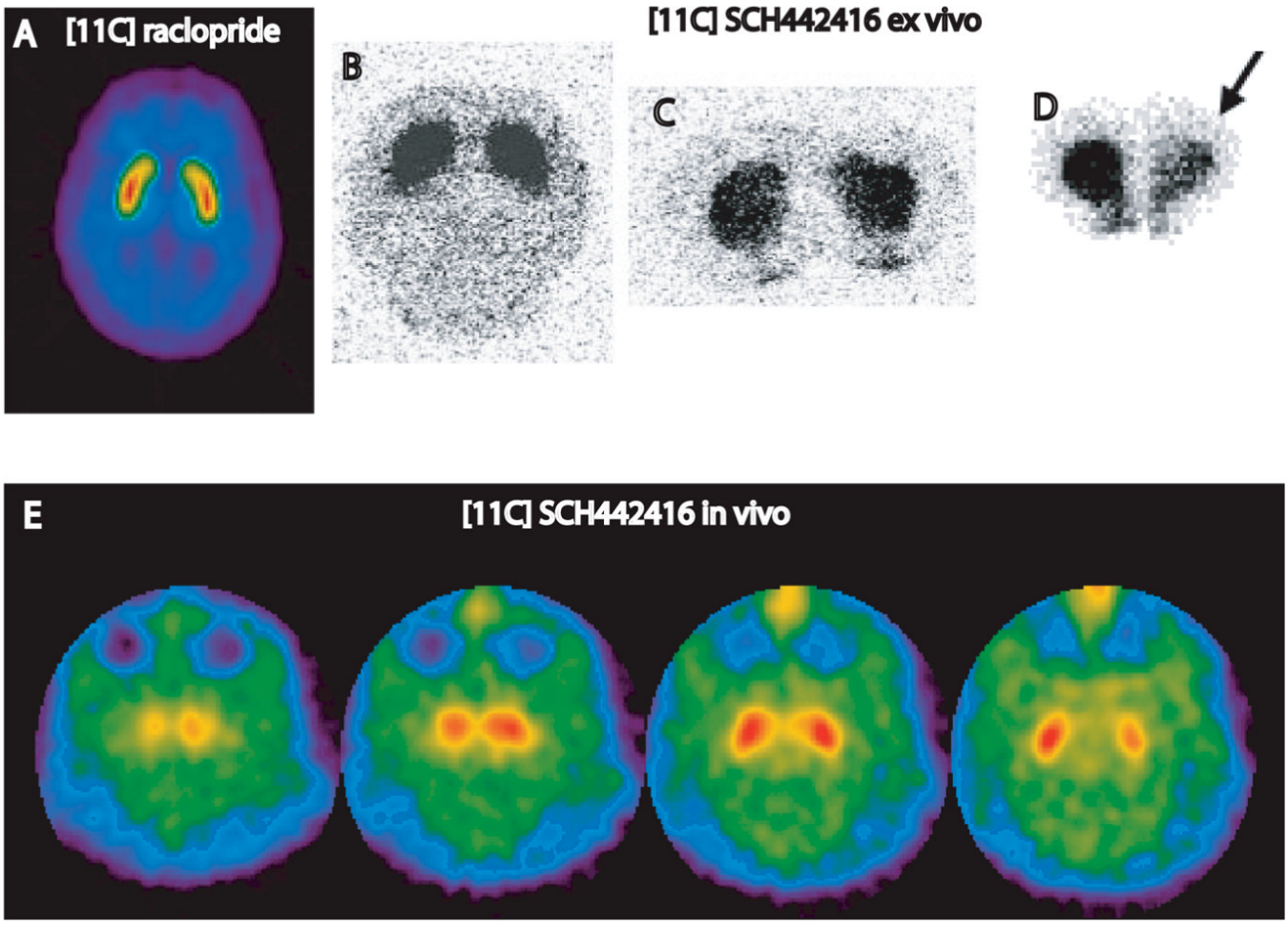

Figure 3.

Ex vivo and *in vivo* distribution of A_{2A} receptor using the new ligand [^{11}C]SCH442416. A. For comparison, D_2 receptor detection in human brain using PET technique and the selective [^{11}C]raclopride ligand shows a high density in striata. B to D: *ex vivo* autoradiography of brain sections on horizontal (B) and coronal (C,D) sections from naive (B,C) or unilaterally quinolinic acid (QA)-treated (D, arrow = injected side) rats obtained 15 min after an intravenous injection of the selective A_{2A} receptor ligand [^{11}C]SCH442416. Autoradiograms show a high binding signal in striatum including caudate-putamen, nucleus accumbens and olfactory tubercles and a decrease in striatal signal due to QA-induced striatal cell death. E. *In vivo* detection of [^{11}C]SCH442416 binding in a *Macaca Nemestrina* brain (horizontal sections) using PET shows a high level of binding in the striatum from ventral to dorsal areas. A and E are pseudo-color images with red and blue corresponding to highest and lowest levels of detected radioactivity, respectively.

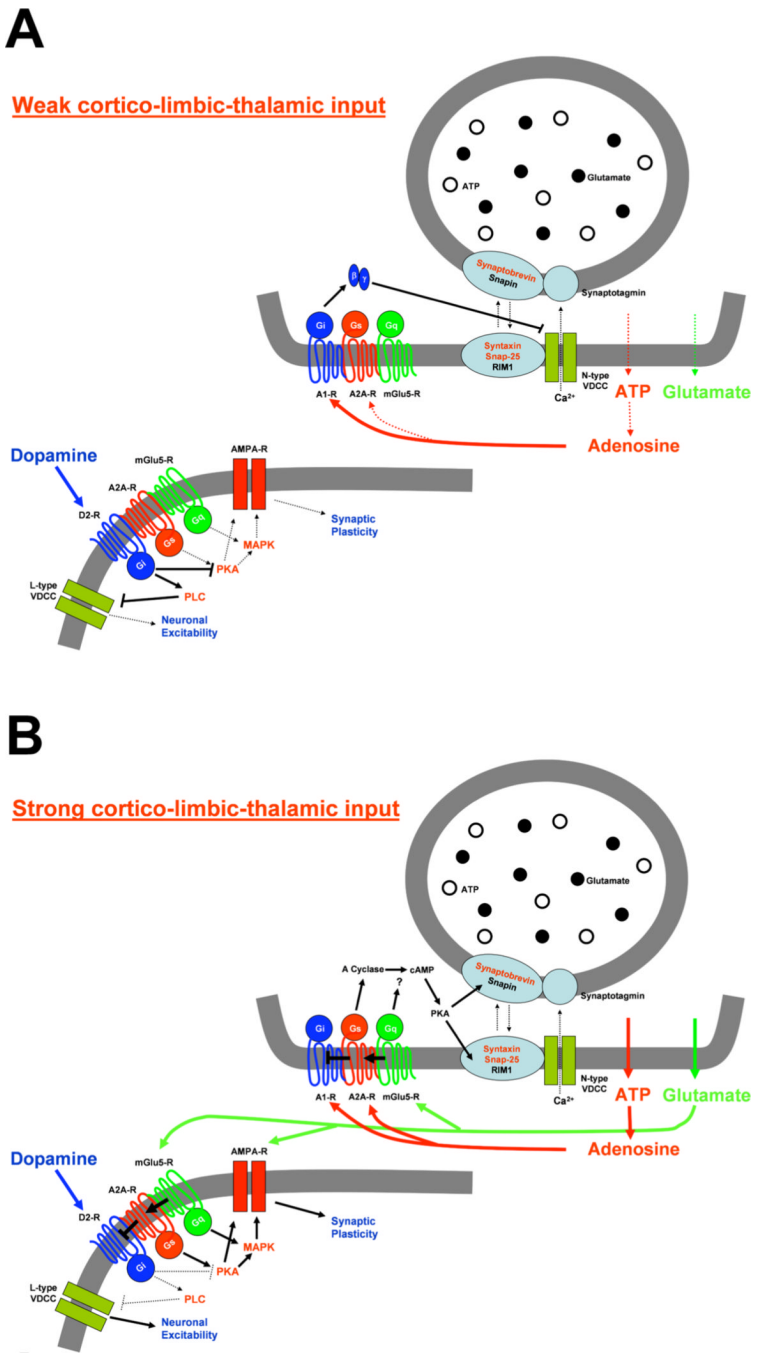

Figure 4.

Schematic representation of the possible role of presynaptic and postsynaptic A_{2A} receptor-containing heteromeric receptor complexes in the modulation of striatal glutamatergic transmission in the GABAergic enkephalinergic neuron. **A.** The low concentrations of adenosine present during weak cortico-limbic-thalamic input favor a decrease in the probability of glutamate release, due to a preferential stimulation of A_1 receptors, which inhibits N- or P-Q-type voltage-dependent Ca^{2+} channels, mediated by the β-γ sububits of Gi proteins. At the postsynaptic site, low concentrations of adenosine favor D_2 receptor signaling, with reduced neuronal excitability and plastic changes. **B.** The high concentrations of glutamate and ATP-derived adenosine obtained during strong cortico-limbic-thalamic input facilitate A_{2A} and

$mGlu_5$ synergistic activation. This results in increases in the probability of glutamate release, due to inhibition of A_1 receptor signaling in the A_1-A_{2A} receptor heteromer by means of an intramembrane interaction and by the ability of A_{2A} receptors to activate PKA, which phosphorylates multiple elements of the presynaptic protein machinery involved in vesicular fusion, such as SNAP-25, RIM1 and snapin (Leenders and Sheng, 2005). At the postsynaptic site, a synergistic A_{2A}-$mGlu_5$ receptor interaction counteracts D_2 receptor signaling by means of intramembrane interactions in the A_{2A}-D_2-$mGlu_5$ heteromeric receptor complexes and favors neuronal excitability and synaptic plasticity.