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Functional changes of the basal ganglia circuitry in Parkinson's disease

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Abstract

The basal ganglia circuitry processes the signals that flow from the cortex, allowing the correct execution of voluntary movements. In Parkinson's disease, the degeneration of dopaminergic neurons of the substantia nigra pars compacta triggers a cascade of functional changes affecting the whole basal ganglia network. The most relevant alterations affect the output nuclei of the circuit, the medial globus pallidus and substantia nigra pars reticulata, which become hyperactive. Such hyperactivity is sustained by the enhanced glutamatergic inputs that the output nuclei receive from the subthalamic nucleus. The mechanisms leading to the subthalamic disinhibition are still poorly understood. According to the current model of basal ganglia organization, the phenomenon is due to a decrease in the inhibitory control exerted over the subthalamic nucleus by the lateral globus pallidus. Recent data, however, suggest that additional if not alternative mechanisms may underlie subthalamic hyperactivity. In particular, given the reciprocal innervation of the substantia nigra pars compacta and the subthalamic nucleus, the dopaminergic deficit might influence the subthalamic activity, directly. In addition, the increased excitatory drive to the dopaminergic nigral neurons originating from the hyperactive subthalamic nucleus might sustain the progression of the degenerative process. The identification of the role of the subthalamic nucleus and, more in general, of the glutamatergic mechanisms in the pathophysiology of Parkinson's disease might lead to a new approach in the pharmacological treatment of the disease. Current therapeutic strategies rely on the use of L-DOPA and/or dopamine agonists to correct the dopaminergic deficit. Drugs capable of antagonizing the effects of glutamate might represent, in the next future, a valuable tool for the development of new symptomatic and neuroprotective strategies for therapy of Parkinson's disease.

Contents

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Nomenclature

6-OHDA	6-hydroxydopamine
AMPA	α-amino-3-hydroxy-5-methylisoxazole propionic acid
CPP	(±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
GABA	γ-amino-butyric acid
KA	kainic acid
MPP^+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl(f)-quinoxaline
NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
ROS	reactive oxygen species

1. Introduction

Proper execution of voluntary movements results from the correct processing of sensory-motor information in the brain. This task is carried out by a complex neural network, which includes the cerebral cortex, the motor thalamus and the basal ganglia nuclei.

The basal ganglia circuit is functionally interposed between the cortex and the thalamus. The main task of the circuit is to process the signals that flow from the cortex, to produce an output signal that returns to the cortex, through the thalamus, to modulate movement execution.

The anatomical and functional organization of the basal ganglia circuitry has received considerable attention in the last two decades. This has led to a better understanding of the pathophysiological aspects of a dramatic neurological disorder such as Parkinson's disease. Parkinson's disease is a progressive neurodegenerative disorder in which the ability to control voluntary movements is lost as a consequence of profound changes in the functional organization of the basal ganglia nuclei. The deeper understanding of Par-

kinson's disease pathophysiology has also provided the rationale for the development of new therapeutic strategies.

2. The basal ganglia circuitry: anatomical connectivity and neurotransmitters

2.1. Overview

The basal ganglia are located in the basal telencephalon and consist of five interconnected nuclei: the caudate nucleus, putamen, globus pallidus, substantia nigra and subthalamic nucleus. Although the caudate nucleus and putamen are partly separated by the internal capsule, several bridges of cells connect the two nuclei, which are similar in terms of anatomical and functional characteristics. Therefore, the two structures together are generally referred to as *corpus striatum*. or *striatum*.

2.2. Striatum

As mentioned above, in primates, caudate and putamen are partly separated by corticofugal and corticopetal fibers. Conversely, in rodents the striatum presents as an anatomically homogeneous structure. The nucleus accumbens, which represents the other major component of the striatum, is located rostroventrally to the nucleus.

The major neuronal population in the striatum is represented by spiny projection neurons, accounting for almost 95% of total striatal cells (Kemp and Powell, 1971) and using γ-amino-butyric acid (GABA) as a neurotransmitter (Kita and Kitai, 1988). Within the projection neurons, GABA can be co-localized, alternatively, with enkephalin or substance P/dynorphin (Beckstead, 1985). The remaining 5% of striatal cells consists of aspiny interneurons containing, alternatively, acetylcholine, somatostatin, NADPH-diaphorase or GABA associated with parvalbumin or calretin (Kawaguchi et al., 1995). Recently, the presence of dopaminergic neurons intrinsic to the striatum has also been suggested (Betarbet et al., 1997).

The main targets of striatal projections are the medial and lateral segments of the globus pallidus and the substantia nigra pars reticulata (see Parent and Hazrati, 1995a for review). It has been suggested that neurons containing enkephalin project to the lateral globus pallidus, while neurons containing substance P/dynorphin project to the medial globus pallidus and substantia nigra pars reticulata. This functional segregation of the striatal output has represented the basis for the *direct and indirect* pathway model of basal ganglia functional organization, which is discussed below.

The striatum is the main input structure of the basal ganglia circuit. The major neural input to the striatum is excitatory in nature. Glutamatergic projections from virtually all cortical areas (McGeorge and Faull, 1989) converge onto striatal neurons. Other important excitatory inputs to the striatum arise from the midline and intralaminar nuclei of the thalamus (Berendse and Groenewegen, 1990), and from limbic structures, particularly the amygdala (Kelley, 1982). Another important input to the striatum originates from dopaminergic neurons located in the pars compacta of the substantia nigra and in the ventral tegmental area (Nieuwenhuys, 1985). The striatum also receives serotonergic afferent projections from the dorsal nucleus of the raphe and caudal linear nucleus (see Halliday et al., 1995 for review) and a sparse noradrenergic innervation from the locus coeruleus (Aston-Jones et al., 1995).

Because of its input nucleus nature, the striatum exhibits a variety of neurotransmitter receptors, which also show a considerably higher density at the striatal level, compared to the other basal ganglia nuclei.

Glutamate plays a pivotal role in the regulation of striatal activity. The nucleus shows the highest density of glutamate receptors in the basal ganglia circuitry (Albin et al., 1992). This reflects the abundance of glutamatergic projections that reach the striatum, particularly from the cortex (McGeorge and Faull, 1989). Various components of both ionotropic and metabotropic classes of glutamate receptor are present in the striatum. Earlier studies have suggested that *N*-methyl-D-aspartate (NMDA) receptors have a higher density than α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors in the striatum, while the opposite seems to occur in the other basal ganglia nuclei (Tallaksen-Greene et al., 1992; Albin et al., 1992). Subsequent studies, using in situ hybridization and immunocytochemistry techniques for the investigation of the single subunits that compose NMDA and AMPA receptors, have provided further insights and partially modified the picture. Bernard and Bolam (1998), for example, have shown that 80% of spiny projection neurons in the striatum express both the NMDA NR1 and the AMPA GluR2/3 subunits. It has also been shown that projection neurons differ from interneurons in terms of the specific NR subunit mRNAs expressed (Landwehrmeyer et al., 1995). AMPA receptor subunits also show a differential expression in projection neurons and interneurons; in particular, the GluR1 subunit does not appear to be expressed on projection neurons (Tallaksen-Greene and Albin, 1994). Furthermore, Kosinski et al. (1998a) have recently shown a preferential expression of NR1, NR2B and NR2C subunits NMDA receptor in the human striatum, compared to the globus pallidus.

The striatum possesses a high density of binding sites for metabotropic glutamate receptors, as well

(Albin et al., 1992; Fotuhi et al., 1993). Various mRNAs encoding for different members of the three groups of metabotropic receptors are expressed by striatal neurons (Testa et al., 1994; Fotuhi et al., 1993; Romano et al., 1995; Ohishi et al., 1993).

Striatal neurons express both D_1 and D_2 dopamine receptors, which mediate the modulatory effect of dopamine released from nigrostriatal terminal. It has been suggested that D_1 and D_2 receptors be functionally segregated to different subsets of striatal neurons. According to this view, D_1 receptors are expressed by neurons projecting to the substantia nigra pars reticulata and medial globus pallidus, while D_2 receptors are expressed by neurons projecting to the lateral globus pallidus (Gerfen, 1992; Gerfen et al., 1995). A small population of projection neurons expresses both D_1 and D_2 receptors (Surmeier et al., 1996).

The role played by dopamine at the striatal level has been extensively studied, but many aspects are still poorly understood. Electrophysiological studies suggest that dopaminergic transmission modulate the striatal responses to incoming inputs, particularly those mediated by glutamate (Calabresi et al., 1997; Grenhoff and Johnson, 1997; Cepeda and Levine, 1998). Release of glutamate in the striatum seems to be modulated, in part, by nigrostriatal dopaminergic projections. Chronic blockade of D_2 dopamine receptors causes an increase in the levels of both basal extracellular and potassium-releasable glutamate in striatum (Yamamoto and Cooperman, 1994). There is also electrophysiological evidence that striatal dopamine depletion increases spontaneous glutamate release in striatum (Calabresi et al., 1993). In keeping with this view, behavioral studies conducted in freely moving animals show that intra-striatal administration of dopamine attenuates neuronal excitation elicited by cortical activation in both rats (Kiyatkin and Rebec, 1996) and monkeys (Rolls et al., 1984). Studies carried out in anaesthetized animals (Ohno et al., 1987; Johnson et al., 1986; Hu and Wang, 1988) or using in vitro preparations (Mercuri et al., 1985; Nicola et al., 1996; O'Donnell and Grace, 1996) have suggested that the inhibitory modulation might be mediated, primarily, by D_1 receptors. Recently, Kiyatkin and Rebec (1999) have showed that systemic administration of the D_1 antagonist SCH-23390, but not of the D_2 antagonist eticlopride, elevates basal activity and attenuates neuronal responses to dopamine in the striatum of freely moving rats. D_1 blockade also enhances glutamate-mediated activation of striatal neurons.

Another neuromodulator that influences the functional responses of dopamine receptors in the striatum is adenosine. Adenosine acts on specific receptors (A_1, A_{2A}, A_{2B} and A_3) that appear to be co-localized with dopamine receptors. In particular, A_1 receptors are co-localized with D_1 receptors (Ferrè et al., 1996a), while A_{2A} receptors are co-localized with D_2 receptors (Schiffman et al., 1991). In both cases, adenosine antagonizes the effects mediated by dopamine on striatal neurons. For example, activation of A_1 striatal receptors prevents the release of GABA by the entopeduncular nucleus (the rodent homologue of the medial globus pallidus in primates) elicited by stimulation of striatal D_1 receptors. Analogously, stimulation of A_{2A} receptors inhibits the release of GABA from the globus pallidus (the rodent homologue of the lateral globus pallidus in primates) secondary to activation of striatal D_2 receptors (Ferrè et al., 1993, 1996b).

$GABA_A$ receptors are present in the striatum and have been described in detail in both rats (Wisden et al., 1992) and primates (Huntsman et al., 1996; Kultas-Ilinsky et al., 1998). Striatal GABAergic synapses derive primarily from local collaterals of the axons of GABAergic projection neurons and from the axons of GABAergic interneurons (Kultas-Ilinsky et al., 1998). Various subunits contribute to the composition of the $GABA_A$ receptor. Based of the specific cDNAs cloned, 13 subunits (six α subunits, three β subunits, three γ subunits and one δ subunit) have been identified so far (McKernan and Whiting, 1996). In-situ hybridization data show that, in monkeys, the mRNAs expressed at the highest levels in the striatum are those for the α_2, α_3, α_5 and β_3 subunits (Huntsman et al., 1996; Kultas-Ilinsky et al., 1998). Slightly different results have been obtained in the rat striatum, where the α_2, α_4 and β_3 subunits seems to predominate (Wisden et al., 1992).

Striatal neurons also express receptors for serotonin, particularly the 5-HT_{2A}, 5-HT_{2C} and 5-HT_6 subtypes (Wright et al., 1995). Serotonergic receptors seem to be located, preferentially, on projection neurons containing enkephalin, substance P and dynorphin (Ward and Dorsa, 1996).

2.3. Pallidal complex

In primates, the pallidal complex comprises two segments, medial globus pallidus and lateral globus pallidus. In rodents, medial and lateral segments correspond to the entopeduncular nucleus and globus pallidus, respectively. Both pallidal segments are populated by GABAergic neurons (Oertel and Mugnaini, 1984).

2.3.1. Entopeduncular nucleus (medial globus pallidus)

The entopeduncular nucleus is the smallest nucleus of the basal ganglia circuit. It plays a central role in the transmission of the basal ganglia output to the thalamus and, ultimately, to the motor cortex. Along with the substantia nigra pars reticulata — which shares many histologic and functional properties with the medial globus pallidus — this area is considered the main *output* nucleus of the basal ganglia circuitry.

The entopeduncular nucleus projects primarily to the motor thalamus. In particular, to the ventral anterior and ventral lateral thalamic nuclei that, in turn, project diffusely to the motor cortex (Parent and Hazrati, 1995a). Other entopeduncular projections reach the parafasicular thalamic nucleus, lateral habenula (Carter and Fibiger, 1978) and pedunculopontine nucleus (Nauta, 1979).

Entopeduncular neurons receive a combination of inhibitory (GABAergic) and excitatory (glutamatergic) projections. The balance between the two opposite systems determines the functional activity of the nucleus. The main source of GABAergic fibers is the striatum (Parent and Hazrati, 1995a). Additional GABAergic projections to the nucleus arise from the adjacent globus pallidus (Parent and Hazrati, 1995b). The excitatory innervation of the entopeduncular neurons is provided by the subthalamic nucleus (Parent and Hazrati, 1995b), with a small contribution from the frontal cortex (Naito and Kita, 1994a).

The entopeduncular nucleus is particularly enriched with $GABA_A$ receptors — mostly α_1 and β_2 subunits — as a consequence of the massive GABAergic input from the striatum (Wisden et al., 1992; Huntsman et al., 1996; Kultas-Ilinsky et al., 1998). Glutamate receptors are also present in the entopeduncular nucleus. It has been initially proposed that — at variance with the striatum — in the entopeduncular nucleus AMPA receptors are more abundantly expressed than NMDA receptors (Tallaksen-Greene et al., 1992; Albin et al., 1992). However, recent evidence shows that, at the entopeduncular level, GluR 1, 2/3, 4 (AMPA) and NR1 (NMDA) subunits are evenly distributed (Clarke and Bolam, 1998).

D_1 dopamine receptors have also been described (Yung et al., 1995), although a dopamine release within the entopeduncular nucleus has not been demonstrated (Ferrè et al., 1996a).

2.3.2. Globus pallidus (lateral globus pallidus)

Pallidal neurons use mainly GABA (co-localized with enkephalin) as neurotransmitter (Fonnum et al., 1978) and project to a number of structures, mostly localized within the basal ganglia circuit. The main targets of pallidal projections are the subthalamic nucleus, substantia nigra pars compacta, entopeduncular nucleus, pedunculopontine nucleus and reticular thalamic nucleus (Parent and Hazrati, 1995b). It has been reported the existence of pallidal, GABAergic projections to the subtantia nigra pars reticulata (for review, see Smith et al., 1998). Cholinergic and noncholinergic projections to the cortex have also been described (Heimer et al., 1995).

The main sources of afferent projections to the globus pallidus are the striatum — sending GABAergic fibers — and the subthalamic nucleus — sending glutamatergic fibers (Parent and Hazrati, 1995b). Fèger (1997) have recently proposed the existence of another excitatory input to the globus pallidus, originating from the thalamus. In addition, the nucleus seems to receive a dopaminergic innervation from collateral fibers originating from the nigrostriatal pathway (Lindvall and Björklund, 1979). This finding has been recently confirmed by Cossette et al. (1999), who showed that, in the human brain, nigrostriatal axons provide collaterals that reach the pallidal complex.

Like the entopeduncular nucleus, the globus pallidus is particularly enriched with $GABA_A$ receptors. Also in this case, the most represented mRNAs are those for the α_1 and β_2 subunits (Wisden et al., 1992; Huntsman et al., 1996; Kultas-Ilinsky et al., 1998).

Glutamate receptors, both of the ionotropic and the metabotropic families, are also present at the pallidal level (Albin et al., 1992). In contrast with the initial hypothesis that AMPA receptors are predominant at this level (Tallaksen-Greene et al., 1992; Albin et al., 1992), recent data show that in the rat globus pallidus most NR1-positive synapses are also positive for the GluR2/3 subunit (Clarke and Bolam, 1998).

D_1 and D_2 dopamine receptors are present in the globus pallidus (Richfield et al., 1987; Yung et al., 1995), which further supports the potential role of dopamine at this level.

2.4. Substantia nigra

Two distinct structures can be recognized within the substantia nigra: a densely populated, pigmented area called *substantia nigra pars compacta* and an adjacent cell-sparse portion, located ventrally, called *substantia nigra pars reticulata*.

2.4.1. Substantia nigra pars compacta

Neurons in the substantia nigra pars compacta contain neuromelanin and use dopamine as neurotransmitter. The main recipients of nigral projections are the striatum, subthalamic nucleus and globus pallidus. Another group of dopaminergic neurons, homogeneous to nigral neurons, is located more medially, in the *ventral tegmental area*. These neurons project to the ventral striatum, amygdala and cerebral cortex (for review, see Hauber, 1998). Recently, electrophysiological and morphological evidence has suggested the existence of a small percentage (5–8%) of nigrostriatal neurons containing GABA instead of dopamine (Rodriguez and Gonzalez-Hernandez, 1999).

Afferent projections to the substantia nigra pars compacta originate from various structures. Both the striatum (Ribak et al., 1980) and the globus pallidus (Smith and Bolam, 1989) send GABAergic projections to nigral dopaminergic neurons. The existence of GABAergic afferent projections from the adjacent sub-

stantia nigra pars reticulata has also been suggested, on the basis of electrophysiological evidence (Tepper et al., 1995). The substantia nigra pars compacta receives glutamatergic projections from the medial prefrontal cortex, subthalamic nucleus and pedunculopontine tegmental nucleus (which also sends cholinergic projections) (Bezard and Gross, 1998; Naito and Kita, 1994b; Reese et al., 1995; Smith et al., 1996). Nigral dopaminergic neurons also receive serotonergic projections from the medial and dorsal raphe nuclei (Hauber, 1998).

Among the diverse neurotransmitters that affect nigral activity, the importance of the glutamatergic input has been recently pointed out. Both NMDA and AMPA receptors are located on the soma and dendrites of dopaminergic neurons and regulate their electrical activity (Chergui et al., 1993; Christoffersen and Meltzer, 1995). Iontophoretic administration of NMDA, AMPA and glutamate increase the firing rate of nigral dopaminergic neurons. These effects are prevented by previous administration of selective antagonists, such as the NMDA antagonists (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) or the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl(f)-quinoxaline (NBQX) (Christoffersen and Meltzer, 1995). Recent evidence show that nigral dopaminergic neurons express primarily GluR1, GluR2/3 and NR1 subunits (Albers et al., 1999). The presence of metabotropic receptors, particularly of the mGluR1 sub-type, has also been demonstrated within the cell body, axons and dendrites of substantia nigra pars compacta neurons (Kosinski et al., 1998b).

2.4.2. Substantia nigra pars reticulata

The substantia nigra pars reticulata is located ventral and adjacent to the substantia nigra pars compacta, and is populated by GABAergic neurons (Oertel and Mugnaini, 1984). Like the entopeduncular nucleus, the substantia nigra pars reticulata sends its inhibitory projections mainly to the ventral anterior and ventral lateral nuclei of the thalamus. Other targets of nigral projection include the superior colliculus and the pedunculopontine nucleus (Parent and Hazrati, 1995a).

The similarity with the entopeduncular nucleus extends to the afferent projections, in that neurons of the substantia nigra pars reticulata receive a combination of inhibitory (GABAergic) and excitatory (glutamatergic) inputs from diverse structures. The main source of inhibitory projections is the striatum (Chevalier and Deniau, 1990; Parent and Hazrati, 1995a). Other sources of GABAergic afferents include the globus pallidus (Smith and Bolam, 1989), nucleus accumbens (Deniau et al., 1994) and ventral pallidum (Groenewegen et al., 1993). Glutamatergic projections to the substantia nigra pars reticulata originate in the subthalamic nucleus (Kita and Kitai, 1987), which plays a very important role in the regulation of nigral activity. Indeed, selective subthalamic lesion reduces the activity of mitochondrial enzymes complex I, II and IV in the substantia nigra pars reticulata. This reflects the reduced activity of nigral neurons resulting from the abolition of the subthalamic excitatory input (Blandini and Greenamyre, 1995a; Blandini et al., 1995).

The expression of $GABA_A$ receptor subunits is also similar to that of the entopeduncular nucleus, with the α_1 and β_2 subunits showing the highest density compared to the other subunits (Wisden et al., 1992; Huntsman et al., 1996; Kultas-Ilinsky et al., 1998).

As described above, the pars reticulata shares many histologic and functional properties — including the input/output connections — with the entopeduncular nucleus. The two nuclei are considered the major output structures of the basal ganglia circuitry. Therefore, they are often referred to as a functional unit termed *basal ganglia output nuclei*.

2.5. Subthalamic nucleus

The subthalamic nucleus is the only glutamatergic nucleus of the basal ganglia circuit (Smith and Parent, 1988). It sends excitatory projections primarily to the basal ganglia output nuclei — substantia nigra pars reticulata and medial globus pallidus (entopeduncular nucleus) — and to the lateral globus pallidus (globus pallidus). Additional targets of subthalamic projections include the striatum, substantia nigra pars compacta and motor cortex (Kita and Kitai, 1987; Parent and Hazrati, 1995b).

Subthalamic neurons receive an important inhibitory innervation from GABAergic neurons of the lateral globus pallidus. Other inhibitory projections arise from the ventral pallidum and ventral striatum (Groenewegen and Berendse, 1990; Kita et al., 1983). The subthalamic nucleus also receives excitatory projections from a number of structures, including the sensory-motor cortex (Fujimoto and Kita, 1993), thalamic parafascicular nucleus, and pedunculopontine nucleus (Canteras et al., 1990).

A dopaminergic innervation of the subthalamic nucleus, originating from the substantia nigra pars compacta, has also been described in rats (Canteras et al., 1990; Hassani et al., 1997). Recently, such innervation has been demonstrated in the human subthalamic nucleus, as well (Cossette et al., 1999; Hedreen, 1999).

Both GABA and glutamate receptors are abundantly expressed in the subthalamic nucleus. As for $GABA_A$ receptors, the α_1, β_2 and γ_1 subunits are the most represented (Wisden et al., 1992; Huntsman et al., 1996; Kultas-Ilinsky et al., 1998). As for the gluta-

mate receptors, components of both the ionotropic and the metabotropic families have been described (Tallaksen-Greene et al., 1992; Albin et al., 1992).

Dopamine receptors are present at the subthalamic level, where they play an important role in the context of the basal ganglia functional organization (see below). The exact representation of the receptor subtypes has not been fully depicted, yet. Both D_1 and D_2 receptors have been repeatedly described in the nucleus (Bouthenet et al., 1987; Dawson et al., 1988; Johnson et al., 1994; Martes et al., 1985). Recently, Flores et al. (1999) reported the presence of mRNAs and binding sites for D_1, D_2 and D_3 receptors.

3. Functional organization of the basal ganglia circuitry

3.1. The direct and indirect pathway model

The functional architecture of basal ganglia has attracted the interest of numerous researchers in the last decade. This has led to the formulation of a model of basal ganglia functioning that has become very popular (Albin et al., 1989; Alexander and Crutcher, 1990; Gerfen, 1992; Graybiel, 1990). According to the model, the striatum — the main input nucleus of the circuit — transmits the flow of information received from the cortex to the basal ganglia *output nuclei*, substantia nigra pars reticulata and medial globus pallidus, via a *direct* and an *indirect* pathway. The two pathways originate from different subsets of striatal neurons and, in the model, remain functionally segregated. In the direct pathway, striatal GABAergic neurons, containing dynorphin as a co-transmitter and expressing D_1 dopamine receptors, project monosynaptically to the substantia nigra pars reticulata and medial globus pallidus. In the indirect pathway, the striatal output reaches the target nuclei via a more complicated route. In fact, a different subset of GABAergic neurons — containing enkephaline and expressing D_2 receptors — project to the lateral globus pallidus, which sends GABAergic projections to the subthalamic nucleus. The subthalamic nucleus, in turn, sends its glutamatergic efferents to the output nuclei and to the lateral globus pallidus. From the output nuclei, inhibitory, GABAergic projections reach the ventral lateral and ventral anterior nuclei of the motor thalamus. Thalamic nuclei then send glutamatergic projections to the motor cortex, thus closing the loop (Fig. 1).

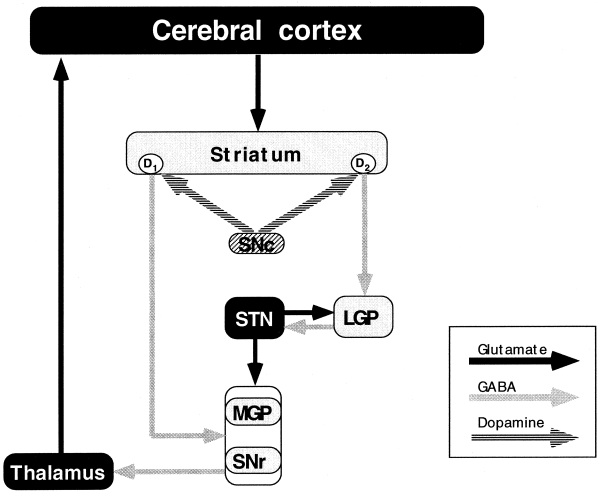

Fig. 1. Schematic representation of the functional organization of basal ganglia circuitry, according to the classical direct and indirect pathway model (modified from Alexander and Crutcher, 1990). MGP: medial globus pallidus, LGP: lateral globus pallidus; SNc: substantia nigra pars compacta; SNr: substantia pars reticulata; STN: subthalamic nucleus. Different colors of boxes and arrows indicate the neurotransmitters (glutamate, GABA and dopamine) implicated at the various levels.

The functional consequence of such organization is that, according to this scheme, activation of the direct or the indirect pathway leads to opposite changes in the net output of the basal ganglia circuitry. In fact, activation of the striatal GABAergic neurons that give rise to the direct pathway causes inhibition of GABAergic neurons of the output nuclei. This leads to disinhibition of thalamic nuclei, which are under the inhibitory control of the output nuclei projections. Conversely, activation of the striatal neurons that project to the lateral globus pallidus, in the indirect pathway, causes inhibition of the lateral globus pallidus and subsequent disinhibition of the subthalamic nucleus. The activation of the subthalamic nucleus — which is glutamatergic — increases the activity of the output nuclei. Consequently, their inhibitory control over the motor thalamus results enhanced (Alexander and Crutcher, 1990; DeLong, 1990).

From such scheme, it emerges that the subthalamic nucleus holds a strategic position in the circuitry. Subthalamic excitatory projections modulate the neuronal activity in the substantia nigra pars reticulata and medial globus pallidus. This has been demonstrated both directly and indirectly. Electrical subthalamic stimulation increases metabolic rates for glucose in these nuclei (Tzagournissakis et al., 1994). Analogously, unilateral ablation of the subthalamic nucleus causes an ipsilateral reduction in the activity of mitochondrial enzymes complex I, complex II and complex IV in the recipients of subthalamic projections (Blandini and Greenamyre, 1995b; Blandini et al., 1995). Thus, the final output of the basal ganglia processing is under the direct control of the subthalamic nucleus.

3.2. Is the model still valid?

This model of basal ganglia functional organization

has the undeniable merit of having provided a framework for anatomical, physiological and clinical studies. However, the model has been recently object of criticisms and is being revised and integrated with data provided by the latest studies (Albin et al., 1995; Chesselet and Delfs, 1996; Parent and Cicchetti, 1998).

One of the major criticisms regards the hypothesized segregation of the striatal output pathways. Striatal spiny neurons give rise to extensive local axon collaterals. As a consequence, the two populations of neurons that give rise to the direct and indirect pathways are, in fact, synaptically interconnected (Yung et al., 1996). Striatofugal axons are highly collateralized, as well. It has been shown, for example, that a single striatofugal axon can arborize in both pallidal segments and in the substantia nigra (Parent and Hazrati, 1995a). Recently, Reiner et al. (1999) have confirmed that striatal neurons projecting to the medial globus pallidus contain both substance P and dynorphin, but not enkephalin, and that striatal neurons projecting to the lateral globus pallidus contain only enkephalin. However, within the neuronal population giving rise to the direct pathway, the authors described cells containing substance P, but not dynorphin. Moreover, some of these neurons contained enkephaline — alone or co-localized with substance P. In the rat, intrastriatal administration of D_1 agonist SKF 38393 or D_2 agonist quinpirole elicits a similar pattern of metabolic changes in the three main targets of striatal projections, subtantia nigra pars reticulata, entopeduncular nucleus and globus pallidus (Blandini and Orzi, unpublished data). Therefore, these notions seem to contradict the assumption that the striatal output reaches the output nuclei through two completely separated channels.

Other two elements have emerged recently, which are to be taken into account for a re-modeling of the current scheme of basal ganglia organization. Firstly, various studies have described the existence of direct projections connecting the lateral globus pallidus to the medial globus pallidus and to the substantia nigra pars reticulata (see Parent and Hazrati, 1995b and Smith et al., 1998 for review). Therefore, in contrast with the model outlines, the lateral globus pallidus may interfere directly with the basal ganglia output, without the interposition of the subthalamic nucleus. Secondly, the subthalamic nucleus receives direct excitatory projections from the primary motor cortex (see Smith et al., 1998 for review). More importantly, the substantia nigra pars compacta and the subthalamic nucleus send each other reciprocal projections (Canteras et al., 1990; Cossette et al., 1999; Hassani et al., 1997; Hedreen, 1999; Smith et al., 1996).

These recent data on basal ganglia connectivity have been therefore incorporated in the current model of basal ganglia functional organization. The result is a more articulate organization of the circuit, from which a central and, to a certain extent, independent role for the subthalamic nucleus seems to emerge (Fig. 2).

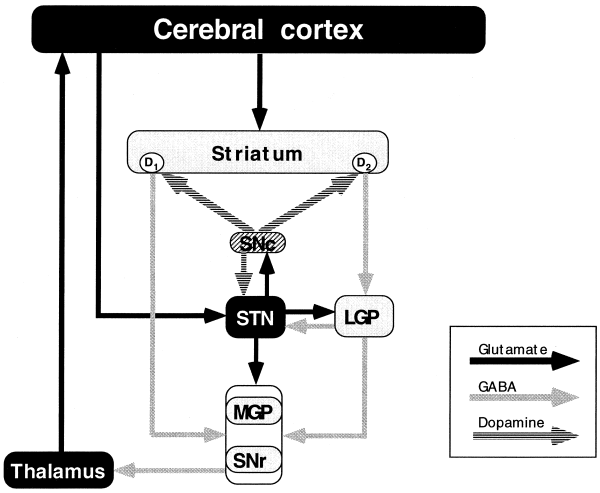

Fig. 2. Updated scheme of basal ganglia circuitry functional organization (modified from Alexander and Crutcher, 1990). MGP: medial globus pallidus, LGP: lateral globus pallidus; SNc: substantia nigra pars compacta; SNr: substantia pars reticulata; STN: subthalamic nucleus. Different colors of boxes and arrows indicate the neurotransmitters (glutamate, GABA and dopamine) implicated at the various levels. Note that, compared to the scheme depicted in Fig. 1, new connections have been added. These adjuncts include the cortico-subthalamic pathway, the reciprocal innervation of subtantia nigra pars compacta and subthalamic nucleus and the pathway connecting the LGP to the output nuclei, MGP and SNr.

4. Parkinson's disease

Parkinson's disease (PD) is a progressive, neurodegenerative disorder, in which the capacity of executing voluntary movements is lost gradually. The clinical picture of PD includes tremor, rigidity and bradykinesia (slowness of movement). The pathologic hallmark of the disease is the degeneration of melanine-containing, dopaminergic neurons of the substantia nigra pars compacta. Residual nigral neurons show characteristic, eosinophilic, inclusions called *Lewy bodies*, that are made up of neurofilaments and show ubiquitin immunoreactivity (Jellinger, 1987). The loss of dopamine-containing neurons leads to severe dopaminergic denervation of the striatum. The loss of nigral neurons causes a cascade of functional modifications that involves all components of the basal ganglia circuitry. These changes represent the neural substrate for the expression of PD motor symptoms (see below).

Despite the progress made since the original description of PD-related pathological and biochemical changes (Ehringer and Hornykiewicz, 1960), the *primum movens* of the degenerative process affecting the substantia nigra pars compacta remains unknown. So

far, no single causative factor has been identified. Instead, various mechanisms — including mitochondrial defects, oxidative stress, glutamate toxicity, genetic factors and apoptosis — seem to play a role. This suggests that the etiopathogenesis of PD is most likely multi-factorial

4.1. Ethiopathogenetic hypotheses: an overview

4.1.1. Mitochondrial defects

An accidental contribution to the understanding of PD pathogenesis came in the early 1980s, when numerous young adults presented with a PD-like syndrome. The phenomenon was due to the unintentional self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP), a product of the illicit synthesis of meperidine analogs (Langston et al., 1983). MPTP, which has since been widely used to reproduce a clinical and neuropathological picture of PD in primates (DeLong, 1990), freely crosses the blood–brain barrier. Once in the brain, MPTP is oxidized to 1-methyl-4-phenylpyridinium (MPP^+), the active toxin, by monoamine oxidase type B (Markey et al., 1984). MPP^+ is then taken up selectively by nigral neurons, via the dopamine transporter, and further accumulated in mitochondria, where it blocks mitochondrial respiration, by binding to complex I (NADH:ubiquinone oxidoreductase), the proximal enzyme of the mitochondrial electron transport chain (Nicklas et al., 1985). Interestingly, MPP^+ is also actively transported into blood platelets (Cesura et al., 1987), where it inhibits complex I with a similar potency as in the brain (Greenamyre et al., 1992; Blandini and Greenamyre, 1995a). These findings have prompted extensive research on the efficiency of mitochondrial enzymes in PD patients. This has led to the identification of a selective deficiency of complex I activity in the substantia nigra of parkinsonian patients (Parker et al., 1989; Schapira et al., 1990). A less marked deficit of complex I has been found also in the platelets of PD patients (Krige et al., 1992; Yoshino et al., 1992).

4.1.2. Oxidative stress

Reactive oxygen species (ROS) form continuously in the body, as byproducts of numerous biochemical reactions. Due to the presence of an unpaired electron, ROS are extremely unstable and tend to stabilize by oxidizing cell constitutive elements, particularly, membrane lipids and nuclei acids. Such oxidative reaction accounts for the potential toxicity of these molecules (Ebadi et al., 1996). Compared to the rest of the brain, the substantia nigra pars compacta is exposed to higher levels of oxidative stress. Catabolism of dopamine by MAO-B, auto-oxidation of melanin and presence of high levels of iron are all factors that induce a high rate of ROS formation (Coyle and Puttfarcken, 1993). This process is likely to be enhanced in PD. Various markers of lipid peroxidation and oxidative damage to DNA are increased in the substantia nigra of parkinsonian patients (Dexter et al., 1989; Sanchez-Ramos et al., 1994; Yoritaka et al., 1996). These phenomena may be the consequence of altered efficiency of endogenous anti-oxidants — such as glutathione or the enzyme superoxide dismutase — which may render PD patients more vulnerable to oxidative stress. Indeed, glutathione is deficient in the substantia nigra of these patients (Perry and Yong, 1986; Sian et al., 1994), while the activity of superoxide dismutase is increased (Poirier et al., 1994).

Oxidative stress may also account for the nigral defect of complex I activity, in that complex I is highly vulnerable to oxidative damage (Allen et al., 1995). On the other hand, inhibition of complex I leads to increased ROS formation (Cleeter et al., 1992). Thus, whether it is oxidative stress that causes complex I deficiency, or vice versa, remains unclear.

4.1.3. Glutamate toxicity

Amino acid glutamate is the predominant fast excitatory transmitter in the central nervous system. Glutamate activates several classes of receptors, which have been initially divided into ''ionotropic'' and ''metabotropic''. Activation of ionotropic receptors leads to opening of associated ion channels, whereas activation of metabotropic receptors, which are linked to G proteins, produces changes in cyclic nucleotides or phosphoinositol metabolism (Greenamyre and Porter, 1994; Nicoletti et al., 1996). Ionotropic receptors have been further classified into NMDA, AMPA or KA (kainic acid) receptors, according to the preferred agonist (McBain and Mayer, 1994; Bettler and Mulle, 1995). The metabotropic receptor family comprises eight subtypes ($mGluR_1$–$mGluR_8$), subdivided into three groups (Nicoletti et al., 1996). Further studies of molecular biology have demonstrated that NMDA, AMPA and KA receptors are composed of sub-units encoded by different gene families. Conductance to ions of ionotropic receptors depends on their sub-unit composition. Two families of sub-units, $NMDAR_1$ and $NMDAR_2$, have been identified for the NMDA receptor (McBain and Mayer, 1994). As for the AMPA receptor, four subunits, $GluR_1$–$GluR_4$, have been cloned, while five subunits, $GluR_5$, $GluR_6$, $GluR_7$, KA_1 and KA_2, have been identified for the KA receptor (Bettler and Mulle, 1995).

Beside its central role in excitatory neurotransmission, glutamate can also act as a neurotoxin. Glutamate neurotoxicity or *excitotoxicity*, as it was termed by Olney (1971), is caused by a massive influx of extracellular Ca^{2+}, secondary to activation of the NMDA receptor (Meldrum and Garthwaite, 1990). The increase in cytoplasmic Ca^{2+} activates a number of

Ca^{2+}-dependent enzymes involved in the catabolism of proteins, phospholipids and nucleic acid, as well as in the synthesis of nitric oxide. This leads to necrotic cell death through different pathways, including membrane breakdown, cytoskeletal alterations and nitric oxide derived free radicals (Coyle and Puttfarcken, 1993). Glutamate can also trigger apoptosis (Kure et al., 1991), a gradual process of cell elimination resulting from the activation of cell death programs, characterized by membrane blebbing, compacting of organelles and chromatin condensation. An alternative form of toxicity is based on the ability of glutamate to induce ROS formation in neuronal cells, with a mechanisms that does not involve the activation of glutamate receptors. In this case, glutamate inhibits the uptake of cystine, which is required for the intra-cellular synthesis of glutathione. Reduced availability of cystine causes reduction of glutathione levels, increased formation of ROS and, ultimately, cell death (Murphy et al., 1989). Interestingly, cell death induced by glutamate with this mechanism shows the morphological characteristics of both apoptosis and necrosis (Tan et al., 1998).

Excitotoxicity can be the *direct* result of excessive stimulation of the NMDA receptor, due to increased levels, or decreased removal from the synaptic cleft, of glutamate. Direct excitotoxicity was initially proposed as a causative factor in the pathogenesis of neurodegenerative disorders. However, it soon became evident that a direct action of glutamate is likely to play a role in acute neurological disorders, such as hypoxic/ischemic brain damage, but not in a chronic disorder, such as PD. In fact, large increases in extracellular glutamate occur in the hypoxic/ischemic (Rothman and Olney, 1986) damage, but not in PD.

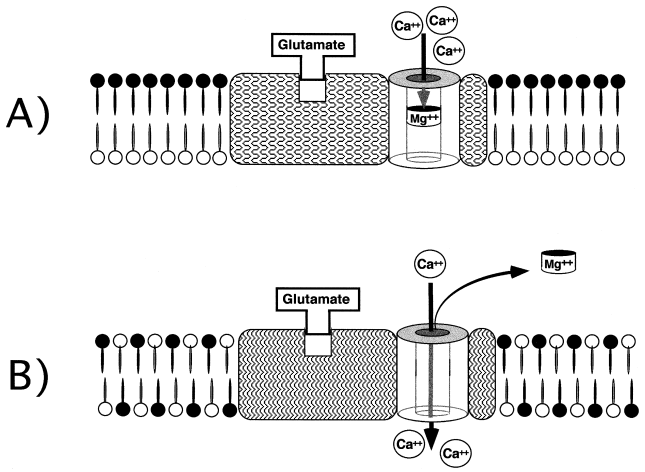

Fig. 3. Schematic representation of the NMDA receptor (modified from Blandini et al., 1996). The NMDA receptor is a calcium-permeable, ligand-gated ion channel which opens in response to the binding of glutamate. (A) At resting membrane potential, ambient extracellular magnesium blocks the channel. This prevents calcium flux. (B) Because the magnesium blockade of the channel is voltage-dependent, depolarization causes extrusion of the magnesium and allows calcium to flow inward. The ability to maintain membrane polarity depends on functional ion pumps, in particular, the Na^+/K^+ ATPase. The Na^+/K^+ ATPase, in turn, depends on an adequate supply of ATP, more than 90% of which is derived from mitochondrial oxidative metabolism. Impaired mitochondrial function depletes ATP, disrupts Na^+/K^+ ATPase activity, and causes depolarization. This results in exaggerated responsiveness of the receptor to glutamate, leading to excessive flow of calcium into the cell.

Glutamate can also be toxic indirectly. In normal conditions, the ion channel associated with the NMDA receptor is blocked by extracellular Mg^{2+}. This blockade, that prevents influx of Ca^{2+} in the presence of physiological concentrations of glutamate, is voltage-dependent. Therefore, if a neuron is depolarized the Mg^{2+} blockade is relieved and binding of glutamate leads to a large Ca^{2+} influx (Fig. 3). Maintenance of membrane polarity is a process that requires continuous energy supplementation. Consequently, impaired mitochondrial function causes depolarization (Erecinska and Dagani, 1990). The final result is that, if the cell is depolarized for a bioenergetic deficit, even non-toxic levels of glutamate become lethal (Novelli et al., 1988).

This has led to the formulation of the "indirect excitotoxic hypothesis" (Albin and Greenamyre, 1992; Beal et al., 1993). According to such hypothesis, any process that impairs a neurons ability to maintain normal membrane potential enhances its vulnerability to the toxic effects of glutamate. Substantial experimental evidence has supported this hypothesis, showing that the inhibition of mitochondrial respiration — both in vitro and in vivo — causes excitotoxic lesions. The excitotoxic nature of these lesions is confirmed by the fact that they are prevented by NMDA antagonists (see Greene and Greenamyre, 1996 for review).

As mentioned above, mitochondrial abnormalities have been reported repeatedly in the substantia nigra pars compacta of PD patients. Therefore, a synergistic interaction between bioenergetic defects and glutamatergic stimulation is likely to play a role in the degeneration of nigral neurons.

4.1.4. Genetic factors

In past years, the search for a genetic marker for PD has failed to provide substantial evidence of the existence of genetic abnormalities in PD patients. Only recently, this search has led to the identification of mutations in the α-synuclein gene, on chromosome 4, in a restricted number of families in Italy, Greece and Germany (Polymeropoulos et al., 1997; Krüger et al., 1998). The product of the gene, the protein α-synuclein, is normally expressed in all brain regions, including the substantia nigra pars compacta. The protein is also found abundantly in *Lewy bodies*. However, other studies have failed to find such mutation in other cases

of familial PD or in any of the sporadic PD patients tested. Thus, it appears that the α-synuclein mutation is responsible for a small percentage of familial cases of PD and for none of the sporadic cases, which represent the majority of PD cases (Gasser et al., 1997).

4.1.5. Apoptosis

Recent evidence indicates that apoptosis, rather than necrosis, mediates cell death in neurodegenerative disorders, including PD (Tatton et al., 1997). As mentioned above, apoptosis differs from necrosis in many ways. Necrosis is a rapid process that induces organelle disruption, cell swelling and membrane rupture, and occurs passively, without the active participation of the cell. Conversely, apoptosis is a gradual process of cell death, triggered by the activation of specific cellular programs, which evolves through a series of defined steps. Apoptosis can be initiated by a variety of conditions, some of which are certainly implicated in PD pathogenesis. For example, MPTP-treated mice show apoptotic nuclei in the substantia nigra pars compacta (Tatton and Kish, 1997); as already mentioned, glutamate can induce apoptosis (Kure et al., 1991). In addition, complex I inhibitors can induce apoptosis in cell cultures (Hartley et al., 1994). This finding is of particular interest, in the light that complex I is deficient in PD patients. A decrease in the mitochondrial membrane potential seems to be a critical effector of apoptosis (Scorrano et al., 1997). Therefore, Tatton and Chalmers-Redman (1998) have hypothesized that the decrease in complex I activity that affects nigral neurons, in PD patients, might render the substantia nigra pars compacta more vulnerable to apoptosis. Indeed, evidence of apoptotic cell death has been shown in the substantia nigra pars compacta of PD patients (Anglade et al., 1997).

All this considered, a unified hypothesis of PD pathogenesis can be envisaged, in which all the factors mentioned above can potentially contribute to the degenerative process (Fig. 4).

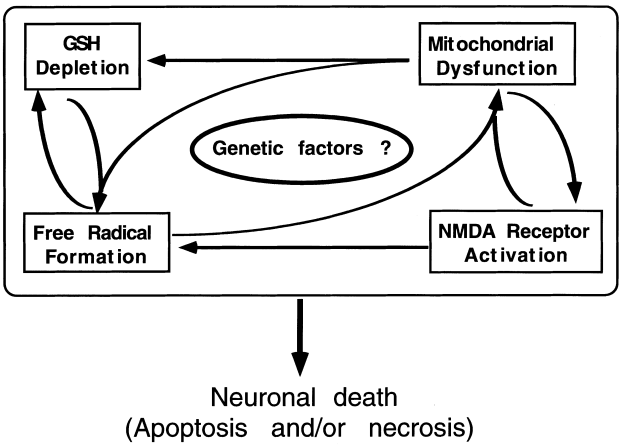

Fig. 4. The diagram illustrates the relationship that may link the various mechanisms potentially involved in the pathogenesis of PD (modified from Blandini et al., 1996). Such mechanisms, which include mitochondrial (bioenergetic) defects, excitotoxicity, excessive free radical formation and depletion of endogenous anti-oxidants, can be viewed as parts of a cycle, in which any one of these processes might lead to all of the others. For example, mitochondrial defects can lead indirectly to NMDA receptor-mediated excitotoxicity, and activation of NMDA receptors can lead to mitochondrial dysfunction. Thus, a feed-forward cycle of NMDA receptor activation and mitochondrial dysfunction can be envisaged. The initiating event could be at the level of the mitochondrion, or the NMDA receptor. Either way, once the cycle is set in motion, an escalating pattern of neuronal damage and death may ensue. A consequence of both NMDA receptor activation and mitochondrial dysfunction is generation of free radicals which, in turn, may lead to depletion of GSH. It is also possible that excessive levels of free radicals, possibly secondary to a loss of GSH, could initiate this cascade. Free radicals damage the components of the mitochondrial electron transport chain and cause both decreased GSH and increased free radical production. The final result of this cycle is neuronal death, which can be of both apoptotic and necrotic type. Whether genetic factors interact with any of the pathogenetic mechanisms included in this cycle is presently unclear.

4.2. Parkinson's disease-related changes in basal ganglia functional organization

The neurodegenerative process of PD causes a functional re-arrangement of the basal ganglia circuitry. The current model (Albin et al., 1989; Gerfen, 1992; Graybiel, 1990) predicts that the dopaminergic denervation of the striatum triggers a cascade of events that leads, ultimately, to the increased activity of basal ganglia output nuclei. Enhanced activity of the output nuclei would be the result of enhanced glutamatergic drive from the subthalamic nucleus. The model also predicts that the enhanced activity of the output nuclei results in an increased inhibitory control over the motor thalamus and subsequent reduction of the thalamic glutamatergic output to the motor cortex. These changes are thought to represent the neural substrate for parkinsonian motor symptoms.

4.2.1. Experimental models of Parkinson's disease

The most valuable contribution to the clarification of the functional changes occurring in PD has been provided by the animal models of the disease, particularly those applied to rodents and non-human primates.

In rodents, the most popular technique to obtain a selective lesion of the nigrostriatal pathway is based on the intracerebral injection of neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968). The toxin is usually injected directly into the substantia nigra pars compacta, unilaterally, where it causes massive cell death (Fig. 5) as a consequence of its pro-oxidant properties (Jonsson, 1980). Alternatively, 6-OHDA can be injected in the striatum. In this case, the toxin

causes an immediate degeneration of nigrostriatal terminals, followed by a retrograde, progressive degeneration of dopaminergic cell bodies of the substantia nigra pars compacta (Sauer and Oertel, 1994). Rats do not develop typical PD symptoms. The toxin causes a unilateral dopaminergic denervation — and resulting supersensitivity — of the striatum, which alters the balance of the basal ganglia outputs in the two cerebral hemispheres (Ungerstedt, 1971). The behavioral consequence of such imbalance is a stereotyped behavior (contralateral rotations) that occurs when the animals receive the systemic administration of the non-selective dopaminergic agonist apomorphine (Ungerstedt and Arbuthnott, 1970). This motor stereotypy represents the more reliable index of nigrostriatal lesion in rodents.

In non-human primates, systemic (intra-carotid) injection of the neurotoxin MPTP causes selective destruction of dopaminergic neurons of the substantia nigra pars compacta, which results in a PD-like syndrome (DeLong, 1990). As mentioned before, after crossing the blood–brain barrier, MPTP is oxidized to MPP^+, which is taken up selectively by nigral neurons, via the dopamine transporter (Markey et al., 1984). Once in the neuron, MPP^+ blocks mitochondrial respiration (Fig. 6), by binding to mitochondrial enzyme complex I (Nicklas et al., 1985). The blockade of mitochondrial respiration causes an energy crisis, which leads to massive cell death.

These experimental models of PD have allowed extensive investigation on the functional consequences of the nigrostriatal lesion. The intra-cerebral administration of 6-OHDA, to rodents, or the systemic administration of MPTP, to primates, causes a number of functional changes in the basal ganglia nuclei. The most significant alterations include increases in the neuronal firing rate (Bergman et al., 1994; Kreiss et al., 1997), glucose metabolism (Mitchell et al., 1989), and mitochondrial enzyme activity (Porter et al., 1994; Vila et al., 1996; Vila et al., 1999) in the subthalamic nucleus or its projection nuclei. In rats, lesion of the nigrostriatal pathway causes down-regulation of glutamate receptors in the projection nuclei of the subthalamic nucleus, which is in keeping with the hypothesized

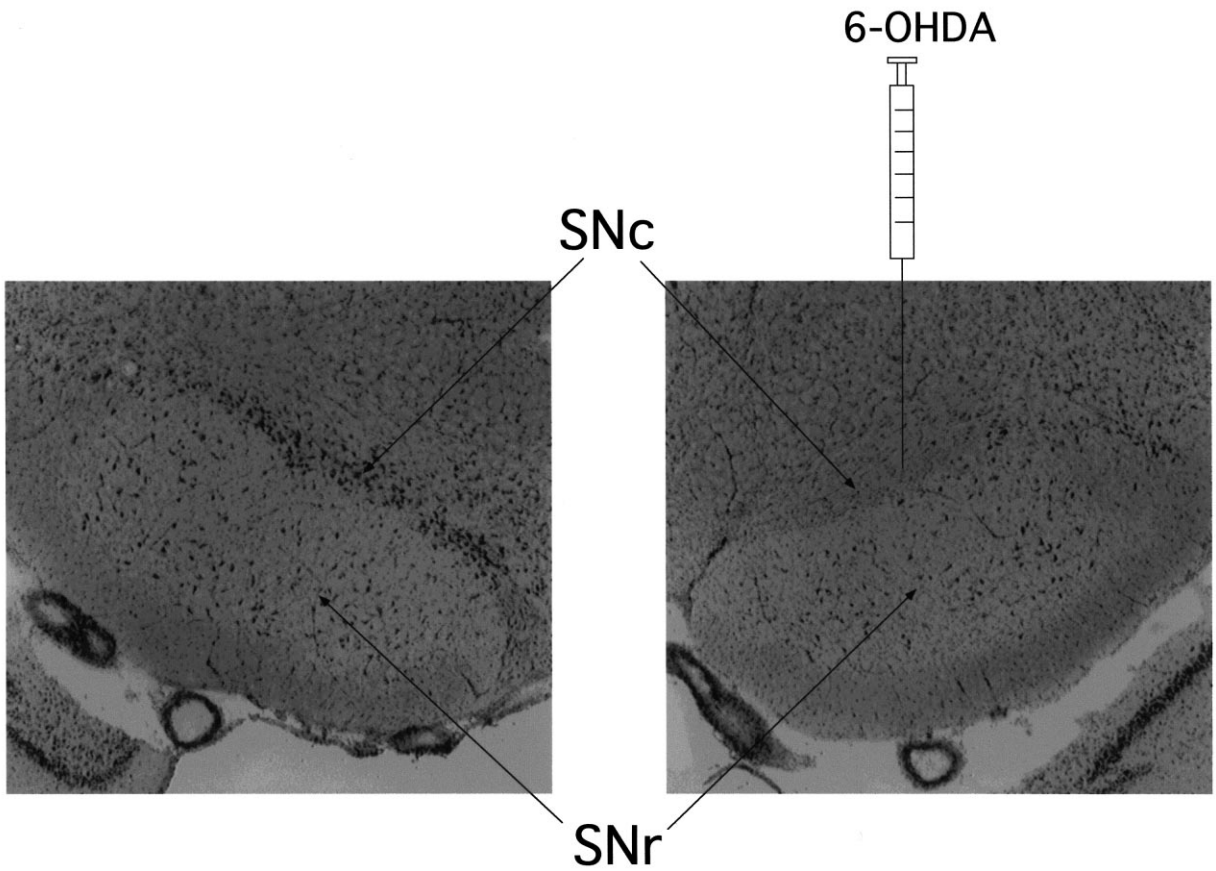

Fig. 5. The figure shows a Nissl staining of a coronal section of rat brain, cut at the level of the substantia nigra (modified from Blandini et al., 1997). The animal received a sterotactical, unilateral injection of 6-OHDA in the right substantia nigra pars compacta (SNc). Note the complete absence of neurons in the SNc of the lesioned side (right panel), compared to the unlesioned side (left panel). Also note the sparing of neurons in the adjacent substantia nigra pars reticulate (SNr). Neurons in the SNr are GABAergic, while neurons in the SNc are dopaminergic. Only these latter are susceptible to 6-OHDA toxicity, which therefore induces a selective lesion of the SNc.

hyperactivity of subthalamic glutamatergic projections (Porter et al., 1994; Wüllner et al., 1994). Subthalamic ablation reverts the increases in mitochondrial enzyme activity found in the entopeduncular nucleus and substantia nigra pars reticulata of 6-OHDA lesioned rats (Blandini et al., 1997) (Fig. 7). Subthalamic lesion also abolishes the rotational response to apomorphine (Burbaud et al., 1995; Blandini et al., 1997). In addition, it normalizes the firing rate and discharge pattern of pars reticulata neurons (Burbaud et al., 1995) and prevents the changes in gene expression in the striatum, entopeduncular nucleus and globus pallidus (Delfs et al., 1995) of rats with unilateral nigrostriatal lesion.

Human studies also support the view that subthalamic hyperactivity plays a central role in PD pathophysiology. For example, disappearance of PD symptoms has been reported in a patient after the occurrence of a subthalamic hematoma (Sellal et al., 1992). Furthermore, Lange et al. (1997) have reported down-regulation of NMDA receptors in the medial globus pallidus of PD patients, which has been interpreted as a consequence of the increased activity of subthalamic projections to the medial globus pallidus. All these observations have provided a solid rationale for the introduction of electrophysiological techniques in the therapy of PD, which aim at relieving the motor symptoms by blocking the pathological hyperactivity of subthalamic neurons (see below).

The subthalamic nucleus seems to play a central role also in the compensatory mechanisms that sustain the dopaminergic function in the pre-symptomatic phase of PD. The gradual cell loss occurring in the substantia nigra pars compacta is accompanied, initially, by an increased efficiency of residual dopaminergic neurons, which results in increased release of dopamine at the striatal level (Zigmond et al., 1990). These compensatory mechanisms mask PD symptoms in an early

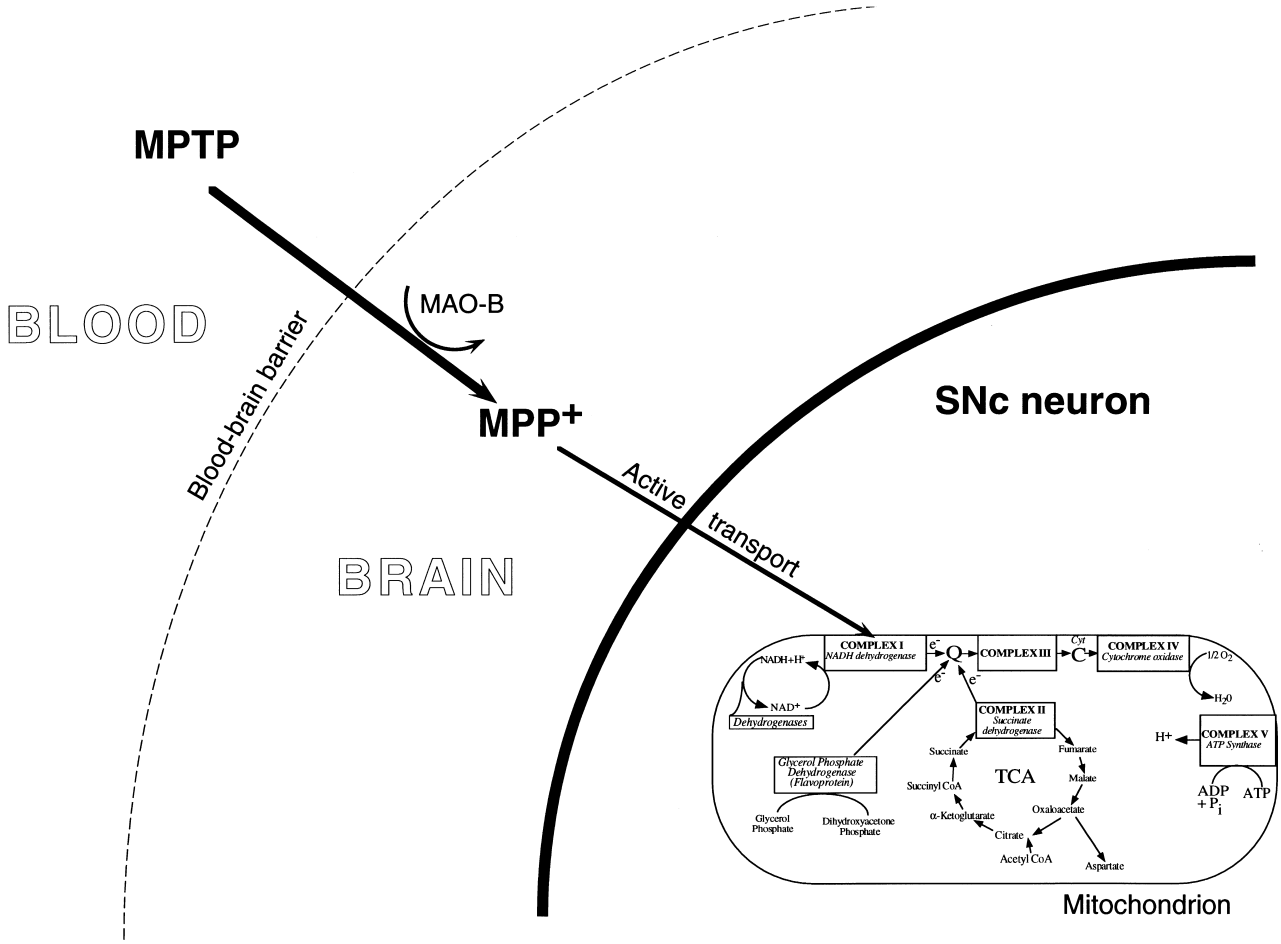

Fig. 6. The figure shows a highly simplified scheme of the mechanism of action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP). When administered systemically to primates, MPTP crosses the blood–brain barrier easily, due to its extremely hydrophobic nature. Once in the brain, MPTP is oxidized to 1-methyl-4-phenylpyridinium (MPP^+), the active toxin, by monoamine oxidase type B (MAO-B). MPP^+ is then taken up selectively by neurons of the substantia nigra pars compacta (SNc), via the dopamine transporter, and further accumulated in mitochondria. At the mitochondrial level, MPP^+ blocks mitochondrial respiration, by binding to the proximal enzyme of the mitochondrial electron transport chain, complex I (NADH:ubiquinone oxidoreductase). The resulting energy crisis leads to cell death.

phase of the disease, until the progression of the degenerative process reaches a threshold after which the functional changes that leads to PD motor symptoms are triggered. It has been demonstrated that, in monkeys rendered parkinsonian by means of MPTP administration, increased glutamatergic inputs to the substantia nigra pars compacta contribute to these mechanisms, considerably. Indeed, temporary blockade of these glutamatergic inputs unveils PD motor abnormalities in otherwise asymptomatic MPTP-treated monkeys (Bezard et al., 1997a, 1997b). Recent investigation has demonstrated that the compensatory increase in the glutamatergic input to the substantia nigra originates mainly from the subthalamic nucleus. There is electrophysiological evidence that, in MPTP-treated monkeys, subthalamic neurons show significantly enhanced activity even before the first appearance of clinical signs (Bezard et al., 1999). Compensatory mechanisms involving glutamatergic transmission occur at the striatal level, as well. It has been repeatedly shown, for example, that the intrastriatal injection of the group I metabotropic glutamate receptor agonist (RS)-3,5-dihydroxyphenylglycine induces vigorous contralateral rotations in intact rats (Kaatz and Albin, 1995; Kearney et al., 1997). The same drug elicits rotational behavior in rats with chronic dopamine depletion (6-OHDA induced nigrostriatal lesion). However, the drug fails to cause rotational behavior in rats with acute dopamine depletion, as that induced by systemic administration of reserpine and α-methyl-para-tyrosine (Kearney et al., 1998). This suggests the existence of compensatory

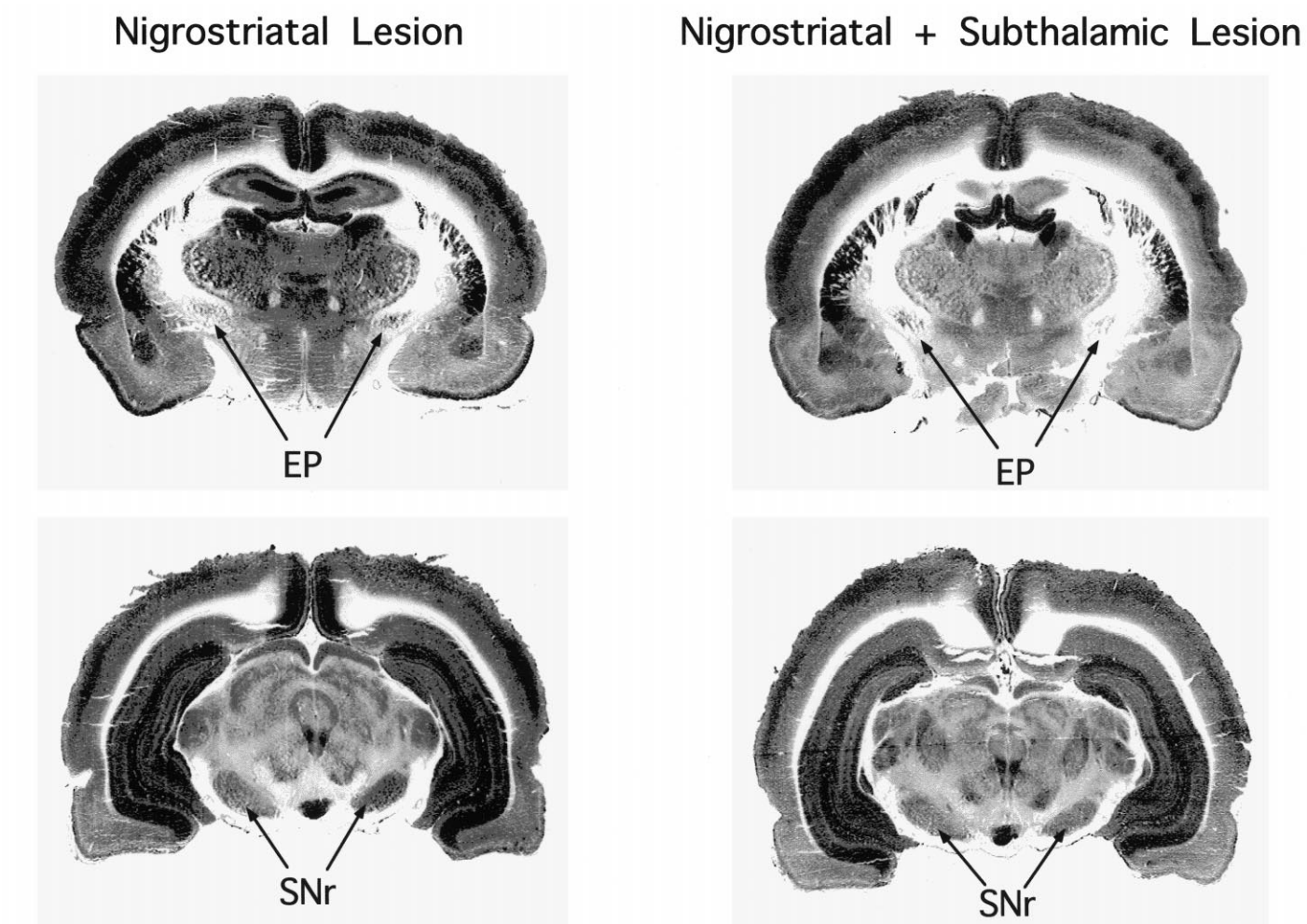

Fig. 7. Histochemical staining for mitochondrial enzyme succinate dehydrogenase in coronal sections of rat brain including the entopeduncular nucleus (EP) and the substantia nigra pars reticulata (SNr). The left panels show succinate dehydrogenase activity in the EP and SNr of a rat bearing a unilateral (right) nigrostriatal lesion. The right panels show succinate dehydrogenase activity in the EP and SNr of a rat with a combined, ipsilateral nigrostriatal and subthalamic lesion. Staining intensity is directly correlated with the activity of the enzyme and, consequently, with the state of neuronal activation (Baker and Santer, 1990; Wong-Riley, 1989). The animal with only the nigrostriatal lesion shows increased activity (darker staining) in the right EP and SNr, compared to the corresponding nuclei of the contralateral hemisphere. In the animal with both lesions, this difference disappears in the SNr and is reversed in the EP.

changes, developing in response to chronic dopamine depletion, which induce changes in the striatal sensitivity of metabotropic glutamate receptors.

4.2.2. Dopaminergic mechanisms in the subthalamic nucleus

A major criticism to the current model of basal ganglia organization regards the mechanism proposed to explain the functional changes of the subthalamic nucleus associated with the striatal dopamine denervation. According to the model, subthalamic hyperactivity results from reduction of the inhibitory control exerted by the lateral globus pallidus, which would be a consequence of the reduced activity of pallidal neurons (Albin et al., 1989; DeLong, 1990). More recent data, however, seem to contradict these hypothesis. Studies conducted in both rodent and primate models of PD have shown either unchanged (Blandini et al., 1997) or increased levels of neuronal metabolic activity in the globus pallidus (Porter et al., 1994; Vila et al., 1996). In addition, Hassani et al. (1996) have demonstrated that complete removal of the pallidal influence on subthalamic neurons — obtained by lesioning the globus pallidus — causes only a slight increase in the firing rate of subthalamic neurons. Such increase appears to be far less pronounced than the increase observed in the subthalamic nucleus of animals bearing a nigrostriatal damage. These data suggest that an additional, if not alternative, explanation for the subthalamic hyperactivity occurring in PD is to be taken into consideration (Fig. 8).

As mentioned above, emerging evidence shows that dopamine plays an important role at the subthalamic level. The subthalamic nucleus receives dopaminergic projections from the pars compacta of the substantia nigra (Hassani et al., 1997). The nucleus also has dopamine receptors (Dawson et al., 1988; Flores et al., 1999), which mediate the functional response of sub-

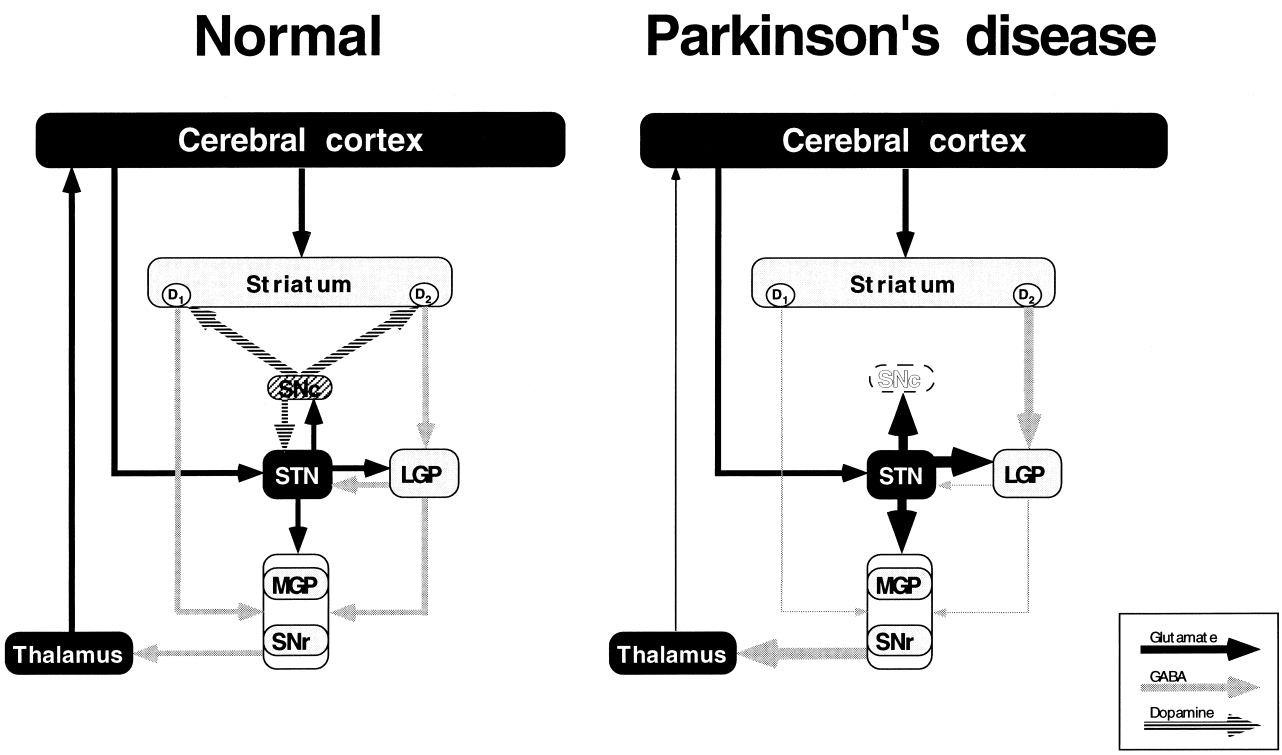

Fig. 8. Schematic diagram illustrating the changes occurring in the basal ganglia functional organization in Parkinson's disease, with respect to normal condition (modified from Alexander and Crutcher, 1990). Relative thickness of arrows indicate the degrees of activation of the transmitter pathways. MGP: medial globus pallidus, LGP: lateral globus pallidus; SNc: substantia nigra pars compacta; SNr: substantia pars reticulata; STN: subthalamic nucleus. In Parkinson's disease, the dopaminergic cell loss of the SNc causes a cascade of alterations affecting all the other components of the circuit. The final result is the increased activity of GABAergic output nuclei, MGP and SNr, which causes increased inhibition of the motor thalamus and, possibly, reduced thalamo-cortical signaling. Hyperactivity of subthalamic, glutamatergic projections to the output nuclei is likely to play the main role in this phenomenon. The classical model predicts that STN hyperactivity results from a decrease in the inhibitory control exerted over the nucleus by the LGP (Albin et al., 1989; DeLong, 1990). However, recent evidence tends to contradicts this hypothesis (see text). In fact, other direct projections to the STN — which are not usually included in the classical scheme — may influence subthalamic activity, in both normal and pathological conditions. These projections include the glutamatergic cortico-subthalamic pathway and the dopaminergic fibers that the SNc sends to the STN. Dopaminergic innervation of the STN, in particular, has proved to play an important role in the modulation of neuronal subthalamic activity. Therefore, it can be hypothesized that the changes occurring in the STN in Parkinson's disease are due, at least in part, to a dopaminergic denervation of the nucleus, resulting from the SNc cell loss.

thalamic neurons to a variety of dopaminergic drugs (Kreiss et al., 1996, 1997; Ruskin and Marshall, 1995). Increasing evidence suggests that subthalamic dopaminergic mechanisms play a pivotal role in the alterations found in animal models of PD. For example, apomorphine increases the firing rate of subthalamic neurons in intact rats, but decreases firing in subthalamic neurons of rats bearing a nigrostriatal lesion (Kreiss et al., 1997). In intact rats, only the combination of the D_1 agonist SKF 38393 and the D_2 agonist quinpirole induce the expression of Fos in the subthalamic nucleus. However, in rats with prior nigrostriatal lesion the administration of SKF 38393 alone is sufficient to induce the subthalamic expression of Fos (Ruskin and Marshall, 1995). More recently, Hassani and Feger (1999) have found that microinjections of dopaminergic agonists in the subthalamic nucleus reduce the discharge rate and do not induce c-Fos expression in subthalamic neurons of normal rats. However, in rats bearing a nigrostriatal lesion, apomorphine (a mixed, D_1 and D_2 agonist) and quinpirole (a selective D_2 agonist) increase the discharge rate and induce massive expression of c-Fos in subthalamic neurons. Conversely, the selective D_1 agonist SKF 82958 decreases the discharge rate and induces only a slight c-Fos expression. It has also been reported that rats with nigrostriatal lesion have increased binding of [^{125}I]sulpiride to D_2 receptors in the subthalamic nucleus. Oral treatment with L-dopa reverts this phenomenon (Murer et al., 1999). Finally, Flores et al. (1999) have shown that density of D_2 binding sites increases in the subthalamic nucleus of rats bearing a nigrostriatal lesion.

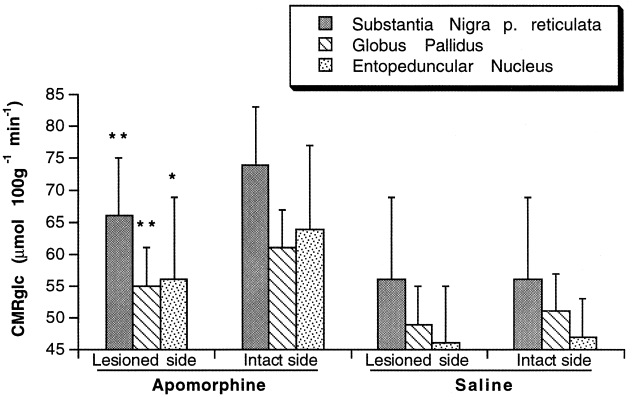

Fig. 9. The graph shows the mean ± SD values of cerebral metabolic rates for glucose (CMRglu) in the subtantia nigra pars reticulata, globus pallidus and entopeduncular nucleus of rats bearing a unilateral lesion of the subthalamic nucleus. Animals that receive systemic apomorphine (1 mg/kg, sub-cutaneously) show significant inter-hemispheric asymmetries, with the nuclei of the lesioned hemisphere showing lower levels of glucose utilization compared to the nuclei of the intact hemisphere. These asymmetries are absent in animals with unilateral subthalamic lesion that received saline solution. The asymmetries seen in the lesioned animals that received apomorphine are likely due to increases in glucose utilization on the intact side rather than to decrease on to the lesioned side. In fact, on the intact side, apomorphine per se increases glucose utilization by 36% in the entopeduncular nucleus, 32% in the substantia nigra pars reticulata and 20% in the globus pallidus. $**p < 0.001$; $*p < 0.01$ vs. Intact side (Student's *t*-test for paired observations).

Subthalamic dopaminergic mechanisms play also a role in the motor stereotipies caused by systemic administration of dopamine agonists. It is known that rats bearing a unilateral lesion of the nigrostriatal pathway respond to the systemic administration of apomorphine with a robust, contralateral turning behavior (Ungerstedt and Arbuthnott, 1970). Animals with unilateral subthalamic lesion show a rotational response to apomorphine, as well. However, in this case, the animals turn ipsilaterally to the lesioned side and considerably higher doses of the drug are required (Kafetzopoulos and Papadopoulos, 1983; Murer et al., 1993; Piallat et al., 1996; Blandini et al., 1999). The rotational response to apomorphine is associated with significant asymmetries in the rate of [^{14}c]deoxyglucose utilization in the projection nuclei of the subthalamic nucleus, i.e. entopeduncular nucleus, substantia nigra pars reticulata and globus pallidus. These asymmetries are due to the increased metabolic activity of these nuclei in the intact hemisphere (Blandini et al., 1999) (Fig. 9). Interestingly, the rotational response to apomorphine in rats with unilateral subthalamic lesion is higher following the second than the first administration of the drug (Piallat et al., 1996; Blandini et al., 1999). This phenomenon is reminiscent of the sensitization process (*priming*) described in animals with unilateral lesion of the nigrostriatal pathway. In such animals, repeated stimulation with apomorphine or D_1 agonists potentiates the dopamine-mediated behavioral responses, probably as a consequence of progressive augmentation of dopamine receptor sensitivity at level of the denervated striatum (Gancher and Mayer, 1995; Morelli et al., 1989, 1993; Paul et al., 1995). Given the presence of dopamine receptors in the subthalamic nucleus and assuming that apomorphine acts directly on this nucleus, it is likely that repeated challenges with dopamine agonists alter the response properties of subthalamic neurons, as well.

Taken together, these data indicate that dopamine plays a central role in the regulation of subthalamic activity, mostly through D_2 receptors. They also show that the degeneration of dopamine neurons of the substantia nigra — that characterizes PD — is likely to affect subthalamic activity directly, inducing a dopaminergic denervation of the nucleus.

4.2.3. Subthalamic hyperactivity and nigral damage

As mentioned before, glutamate indirect toxicity is likely to contribute to the degenerative process underlying PD (Albin and Greenamyre, 1992; Beal et al., 1993). Glutamatergic inputs to the subtantia nigra pars

compacta may synergize with the bioenergetic defects — related to the complex I deficiency — which affect the nigral neurons. This synergistic interaction between glutamate and mitochondrial defects may trigger or, more likely, aggravate the progression of the degenerative process.

In the setting of such an excitotoxic damage to substantia nigra pars compacta, the subthalamic hyperactivity is likely to play a major role. There is anatomic evidence of a glutamatergic pathway connecting the subthalamic nucleus to the substantia nigra pars compacta (Smith et al., 1996). Through this excitatory pathway, the subthalamic nucleus affects the activity of nigral neurons (Smith and Grace, 1992). Therefore, the subthalamic disinhibition that follows the nigrostriatal damage might lead to a glutamatergic overstimulation of residual neurons of the substantia nigra pars compacta. Therefore, a self-sustaining vicious circle may ensue, in which the nigrostriatal damage causes subthalamic hyperactivity, which in turn worsens the nigrostriatal damage (Rodriguez et al., 1998). Indeed, in rats, subthalamic nucleus lesion protects neurons of the substantia nigra pars compacta against the toxicity of 6-OHDA (Piallat et al., 1996) and prevents transneuronal degeneration of the substantia nigra pars reticulata (Saji et al., 1996). More recently, Nakao et al. (1999) have shown that previous ablation of the subthalamic nucleus attenuates the loss of nigral dopaminergic neurons by intrastriatal administration of neurotoxin 3-nitropropionic acid. Thus, in addition to the well established role played in the development of PD motor symptoms, the subthalamic nucleus may also be directly involved in the mechanisms underlying the progression of the neurodegenerative process.

5. Current and future strategies for the pharmacological treatment of Parkinson's disease: the role of glutamate antagonists

5.1. Overview

The current therapeutic approach to PD is of a symptomatic type. The use of L-DOPA, which is still considered the gold standard, aims to relieve PD motor symptoms by replacing the deficient neurotransmitter, dopamine. However, the therapeutic efficacy of L-DOPA tends to fade with time. Concomitantly, motor and/or psychiatric side effects extremely discomforting for the patient emerge. An issue that is currently debated is the hypothesized toxicity of L-DOPA. Experimental data suggest that prolonged use of the drug may contribute to the degenerative process of PD. Various in vivo and in vitro studies have shown that L-DOPA can be toxic, as a result of its pro-oxidant properties (Graham, 1978; Lai and Yu, 1997; Pardo et al., 1995; Ziv et al., 1997). Whether L-DOPA is toxic per se or because it converts to dopamine whose metabolism is associated with formation of reactive oxygen species, as well (Coyle and Puttfarcken, 1993) is unclear. On the other hand, other studies have suggested that, at low doses, L-DOPA may actually be neuroprotective (see Agid, 1998 for review). In fact, two different studies failed to demonstrate any drug-related toxicity in humans (Ahlskog et al., 1996; Shults et al., 1995). We have recently found that isolated platelets of PD patients treated with L-DOPA form higher levels of hydroxyl free radical, compared to both normal subjects and untreated PD patients. The phenomenon is positively correlated with the intracellular levels of the drug (Martignoni et al., 1999). However, the increases observed were modest in terms of absolute values. Thus, whether these intra-cellular alterations are sufficient to trigger toxicity or, on the contrary, they can stimulate anti-oxidant systems, leading to a protective response remains to be established.

These considerations have led to the introduction of dopamine agonists in PD therapy. This class of drugs, that includes compounds such as pergolide, bromocriptine, lisuride or the more recent ropinirole and pramipexole, acts directly on the dopamine receptors — mostly of the D_2 type — mimicking the effect of dopamine. The use of dopamine agonists is associated with a reduced occurrence of motor complications, in both experimental and clinical studies. Therefore, dopamine agonists have been proposed as an adjunct to L-DOPA in patients with dyskinetic movements and fluctuations in the motor response to the drug (Olanow, 1992). In recent years, there has been increasing interest in the use of dopamine agonists, either in combination with L-DOPA or alone, even in the early stages of PD. Like L-DOPA, dopamine agonists act as *symptomatic* agents, in that they relieve PD symptoms by restoring the normal efficiency of the dopaminergic pathways. However, recent evidence has suggested that dopamine agonists may have also *neuroprotective* properties. Such properties would be related to the ascertained efficacy of dopamine agonists in antagonizing oxidative stress, at various levels (see Olanow et al., 1998 for review)

5.2. Prospects of glutamate antagonists for PD therapy

The progressive clarification of the functional changes affecting the basal ganglia in PD has pointed out the crucial role played by glutamatergic mechanisms in the pathophysiology of the disease (Blandini et al., 1996). As we have seen, glutamate mediates the excitatory transmission from the cortex to the striatum. In addition, glutamatergic projections from the subthalamic nucleus modulate the activity of the output nuclei of the circuit.

Another important characteristic of glutamatergic transmission within the basal ganglia is the functional interaction with dopaminergic transmission, particularly at the striatal level. Indeed, any modification in the dopaminergic tone at the striatal level causes relevant changes in the glutamatergic transmission. For example, it has been shown that release of glutamate in the striatum is enhanced after striatal dopamine depletion or chronic D_2 receptor blockade (Calabresi et al., 1993; Yamamoto and Cooperman, 1994). Moreover, in rats with unilateral nigrostriatal lesion, blockade of the NMDA receptor by MK-801 potentiates turning behavior and striatal expression of c-Fos induced by D_1 receptor stimulation (Morelli et al., 1992, 1994). Stimulation of striatal group I metabotropic receptors, in rats, elicits a contralateral rotational behavior which is most likely due to increased release of striatal dopamine (Kaatz and Albin, 1995; Kearney et al., 1997, 1998). Dopaminergic denervation also causes significant changes in the striatal expression of glutamate receptors. Rats with a unilateral 6-OHDA induced lesion show increased striatal levels of the mRNAs coding for the subtypes 3 and 4 of the metabotropic receptor, ipsilaterally to the lesion. Subsequent administration of D_1 agonist SKF 38393 attenuates such increase. The drug also causes a bilateral decrease in the striatal levels of NR1 mRNA and an increase in the levels of NR2B mRNA (Rodriguez-Puertas et al., 1999).

By virtue of its neurotoxic properties, glutamate might also play a role in the degenerative process affecting neurons of the substantia nigra pars compacta. It ensues that drugs capable of antagonizing the effects of glutamate might have beneficial effects in PD therapy, as both *symptomatic* and *neuroprotective* agents.

5.2.1. Symptomatic treatment

The hyperactivity of substantia nigra pars reticulata and medial globus pallidus, which follows the lesion of the nigrostriatal pathway, is due to the enhanced glutamatergic drive from the subthalamic nucleus. Therefore, reducing or abolishing such excitatory drive to the output nuclei represent a possible approach for the abolition of PD motor symptoms. Indeed, lesion or functional inactivation of the subthalamic nucleus ameliorates parkinsonian symptoms in MPTP-treated monkeys (Aziz et al., 1991; Bergman et al., 1990). In rats, nigrostriatal lesion increases firing rate and metabolic activity of substantia nigra pars reticulata and entopeduncular nucleus (the rodent homologue of medial globus pallidus). These changes are abolished by selective subthalamic lesion (Blandini et al., 1997; Burbaud et al., 1995). These experimental findings have led to the introduction of surgical procedures that improve parkinsonian symptoms by abolishing the activity of the subthalamic nucleus by means of electrophysiological techniques. Such result is obtained by stimulating the subthalamic nucleus at high frequency with local electrodes, implanted stereotactically (Pollak et al., 1997). These procedures are currently applied in a small percentage of patients, particularly those presenting with profound motor impairment, motor fluctuations in the response to L-DOPA and, in general, sub-optimal response to pharmacological treatments.

Pharmacological manipulation of glutamatergic neurotransmission, at the level of either the subthalamic nucleus or its target nuclei, may represent a tool to reduce the pathological hyperactivity of the subthalamic nucleus and its projection nuclei, thus improving PD symptoms. In parkinsonian monkeys, stereotactic administration of the non-selective glutamate antagonist, kynurenate, into the medial globus pallidus reverses parkinsonian signs in a reversible, dose-dependent fashion (Brotchie et al., 1991). Similarly, microinjections of glutamate antagonists into the entopeduncular nucleus, substantia nigra pars reticulata, or subthalamic nucleus have shown anti-parkinsonian effects in dopamine-depleted rats (Klockgether et al., 1991; Klockgether and Turski, 1990).

Systemic administration of glutamate antagonists, particularly in combination with L-DOPA, has also proved highly effective. Co-administration of the AMPA antagonist, NBQX, or the competitive NMDA antagonist, CPP, with threshold doses of L-DOPA ameliorates parkinsonian symptoms in animal models of PD (Löschmann et al., 1991). Similarly, the clinical response to co-administration of L-DOPA and remacemide, a low-affinity NMDA receptor channel blocker with anticonvulsant properties, has been shown to be better than L-DOPA alone (Greenamyre et al., 1994). The NMDA antagonist MK-801, or the AMPA antagonist LY293558, reverses the increases in the levels of mRNA coding for glutamic acid decarboxylase and cytochrome oxidase found in the subthalamic nucleus, and its projection nuclei, of rats with nigrostriatal lesion. Recent experimental data suggest antiparkinsonian activity also for the noncompetitive NMDA antagonist MRZ 2/579, which acts at the glycine site of the NMDA receptor (Karcz-Kubicha et al., 1999).

Glutamate antagonists are also effective in counteracting the motor response complications induced by long-term treatment with L-DOPA. As reported by Papa and Chase (1996), LY 235959, a competitive NMDA antagonist, suppresses oral dyskinesias and attenuates choreic movements in MPTP-lesioned monkeys.

5.2.2. Neuroprotection

As mentioned above, indirect excitotoxicity might be involved in the degenerative process affecting nigral dopaminergic neurons in PD. This view is supported

by the fact that NMDA antagonists protect the substantia nigra against MPP^+ toxicity (Turski et al., 1991). Analogously, the noncompetitive NMDA antagonist MK-801, the competitive antagonist LY274614 and the glycine site antagonist 7-chlorokynurenate block the toxicity of a mitochondrial poison in vivo (Greene and Greenamyre, 1995). In addition, Zhang et al. (1998) have recently shown that the apoptosis induced by dopamine in dopaminergic cell cultures is partially attributable to increased vulnerability to non-toxic levels of glutamate.

As mentioned before, the hyperactivity of the subthalamic, glutamatergic projections to the substantia nigra pars compacta may play a role in sustaining the degenerative process that affects nigral dopaminergic neurons (Rodriguez et al., 1998). Therefore, inhibition of subthalamic hyperactivity — particularly in the early phase of the disease — may protect residual nigral neurons against glutamate-mediated toxicity, slowing the progression of the disease. This seems particularly important in the light of recent data, which show that the subthalamic nucleus becomes hyperactive in an early phase of PD, long before the first clinical signs become apparent (Bezard et al., 1999). A potential problem is that — as suggested by Bezard et al. (1999) — the hyperactivity of subthalamic projections to the substantia nigra pars compacta may play a role in the compensatory mechanisms occurring in the early phases of PD. In particular, the authors suggest that the enhanced glutamatergic input from the subthalamic nucleus might increase the activity of residual nigral neurons, leading to a compensation for the loss of dopaminergic function. This mechanism would mask, initially, the consequences of the nigrostriatal damage (Bezard and Gross, 1998). It ensues that reducing the subthalamic activity in an early phase of the disease may slow the progression of the degenerative process. On the other hand, it may unmask PD motor symptoms before the natural progression of the disease overcomes the compensatory mechanisms that develop in the basal ganglia circuitry.

5.2.3. Human studies

All these experimental data have promoted interest for the potentialities of glutamate antagonists in the clinical practice of PD. It has been recognized, for example, that three drugs used for treatment of PD patients, amantadine, memantine and budipine, are NMDA receptor antagonists (Kornhuber et al., 1989, 1991; Porter and Greenamyre, 1995; Klockgether et al., 1993). Recently, it has been reported that co-administration of L-DOPA and amantadine reduces severity of dyskinesias by 60%, without altering the anti-parkinsonian effect of L-DOPA (Verhagen Metman et al., 1998). Zipp et al. (1995) showed that lamotrigine, a glutamate-release inhibitor, has anti-parkinsonian activity when co-administered with L-DOPA. However, a subsequent, controlled clinical trial has not confirmed the efficacy of lamotrigine (Shinotoh et al., 1997). The clinical use of other NMDA antagonists has also provided controversial results. Ifenprodil, a non-competitive antagonist of the NMDA receptor at the polyamine modulatory site, did not improve PD symptoms when used as an add-on therapy to L-DOPA (Montastruc et al., 1992). Conflicting results have been obtained with dextrometorphan, a dextrorotatory analogue of codeine that modulates the ion channel linked to the NMDA receptor by acting at the phencyclidine site. While Bonuccelli et al. (1992) reported that dextrometorphan can improve, at high doses, some PD features, other authors failed to show any significant amelioration with this drug (Montastruc et al., 1994). Unfortunately, for most of these studies, design and sample size issues make it difficult to interpret the negative results. On the other hand, remacemide seems to be a promising drug that has already shown significant anti-parkinsonian activity in MPTP-treated monkey (Greenamyre et al., 1994). Controlled clinical trials with remacemide are currently ongoing and may provide important novelties in PD therapy.

6. Conclusions

In PD, the degeneration of dopamine-containing neurons of the substantia nigra pars compacta triggers a cascade of functional changes, which leads to a significant re-arrangement of the basal ganglia functional organization. The most relevant changes occur at the level of the output nuclei of the circuit, medial globus pallidus and substantia nigra pars reticulata, which become hyperactive. Such hyperactivity is sustained by the enhanced glutamatergic inputs that the output nuclei receive from the subthalamic nucleus. The mechanisms that lead to the disinhibition of subthalamic neurons are still poorly understood. According to the current model of basal ganglia functional organization, the phenomenon is due to a decrease in the inhibitory control exerted over the subthalamic nucleus by the lateral globus pallidus. Recent investigation, however, suggests that additional if not alternative mechanisms may account for the subthalamic hyperactivity. A reciprocal innervation connects the substantia nigra pars compacta to the subthalamic nucleus. In addition, the response of subthalamic neurons to the administration, both local and systemic, of dopamine agonists changes dramatically after chronic nigrostriatal lesion. This suggests that the dopaminergic deficit might influence the subthalamic activity, directly. In turn, given the potentially toxic effects of glutamate, the increased glutamatergic drive to the dopaminergic

nigral neurons, originating in the hyperactive subthalamic nucleus, might play a role in sustaining the progression of the degenerative process. These data indicate that the subthalamic nucleus does not act as a passive point of passage for the information flowing in the indirect pathway, but is likely to play an active role in the pathophysiology of PD.

The identification of the role of glutamatergic mechanisms might lead to a new therapeutic approach in the pharmacological treatment of PD. Current therapeutic strategies for PD rely, essentially, on the use of L-DOPA and/or dopamine antagonists to correct the dopaminergic deficit. Drugs capable of antagonizing the effects of glutamate might represent, in the next future, a valuable tool for the development of new symptomatic and neuroprotective strategies for therapy of PD.

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