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NeuroToxicology 23 (2002) 469–478

NeuroToxicology

Functional Imaging of the Dopamine System: In Vivo Evaluation of Dopamine Deficiency and Restoration

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Received 18 December 2001; accepted 8 May 2002

Abstract

Dopamine deficiency causes a severe impairment in motor function in patients with Parkinson's disease (PD) and in experimental animal models. Recent developments in neuroimaging techniques provide a means to assess in vivo the state of the dopamine system. From a functional perspective, four levels need to be operative and integrated in the system: the dopamine cell (pre-synaptic), the striatal dopamine receptors (post-synaptic), adequate release of dopamine (intra-synaptic), and the cortico-subcortical motor projections. Neuroimaging functional methods can be used to estimate, at these four levels, dopamine cell degeneration, adaptive responses to injury and, importantly, the effect of therapeutic interventions. In this respect, data from functional imaging studies at clinical and pre-clinical stages, support the idea that cell replacement therapy might achieve a more physiological restoration of the dopamine motor system than other therapies (such as ablative surgery, administration of precursor, deep brain stimulation) that currently are equally or more effective in relieving motor symptoms.

Keywords: Dopamine; Parkinson's disease; PET

INTRODUCTION

The nigrostriatal dopamine (DA) neurons that are essential for the control of movement are critically affected in Parkinson's disease (PD). This DA deficiency is significantly correlated with the severity of the main symptoms of PD (Hornykiewicz, 1975). DA modulation of striatal function is implicated in movement preparation, initiation and sequencing (Marsden and Obeso, 1994); consequently, loss of DA in the striatum results in an inability to initiate movements, bradykinesia (slowness), loss of automatic movements and postural adjustments, rigidity and tremor (although the pathophysiological basis of tremor is poorly understood). The capacity of certain substances to damage catecholaminergic neurons has been utilized extensively to produce DA deficiency in animals (Annett et al., 1992; Burns et al., 1983; Ungerstedt and Arbuthnott, 1970). In rodents and in non-human primates, the neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) and 6-hydroxy DA (6-OHDA) produce reliable degeneration of the DA nigrostriatal system. Neuroimaging techniques and behavioral analyses make it possible to assess the in vivo the state of the DA system in patients and animal models. Recent developments of imaging paradigms allow measurements of in vivo changes in DA terminals, receptors, and release of DA. Moreover, metabolic studies provide assessment of activation of neurons downstream of the DA synapse. As these

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0161-813X/02/$ – see front matter
PII: S0161-813X(02)00064-5

Table 1
Radiopharmaceutical tracers are compounds that target specific proteins in the dopamine system by labeling them with positron emitting isotopes (such as ^{18}F or ^{11}C)

Tracer	Target	Evaluation
F-DOPA	AADC/vesicles	Decarboxylation and storage of dopamine
F-*meta*-tyrosine (non-catechol DOPA analogs)	AADC	Decarboxylation
CFT (and other tropane analogs), nomifensine, methylfenidate	DA transporter	Synaptic re-uptake sites
Tetrabenazine	VMAT-2 (vesicles)	Pre-synaptic terminals
Raclopride, methyl-spiperone (D2 antagonists)	D2 receptor	Dopamine receptors
SCH 23390 (D1 antagonist)	D1 receptor	Dopamine receptors

techniques are non- (or only minimally) invasive, it is now possible to perform longitudinal studies in experimental models of PD. Functional studies provide valuable information about the structure and function of DA neurons both in health and disease, such as adaptive changes to injury and degeneration and about the effects of therapeutic approaches. Here we briefly review functional studies using positron emission tomography (PET), but some of the studies discussed can also be performed using single photon emission computed tomography (SPECT). Using positron emitting radioactive tracers it is possible to accurately map the distribution of the tracer in the brain. Ligands are chosen by their capacity to bind to specific receptors or transporters, or are substrate for a specific enzymatic transformation and are then radioactively labeled. The type of ligand utilized will determine the information we can obtain about a particular system. For the dopamine system the most frequently used radiotracers are listed in Table 1. Using glucose or oxygen labeled compounds it is also possible to evaluate the relative changes in regional metabolism related to neuronal activity. However, with recent developments in imaging algorithms functional magnetic resonance imaging (fMRI) offers a better temporal (and to a some extent also spatial) resolution than PET for some metabolic studies (Dale and Halgren, 2001). We address how these techniques contribute to our understanding of PD and the consequences of DA deficiency as well as the functional effect of restorative approaches.

FUNCTIONAL IMAGING OF THE DOPAMINE SYSTEM AND RELATED MOTOR CIRCUITRY

For clarity, we describe here the DA system at four levels: pre-, post-, and intra-synaptic and the basal ganglia-cortical projection (Fig. 1). This involves an oversimplification (i.e. receptors are located both in the pre- and post-synaptic membranes, as well as on the striatal inter-neurons and cortical terminals, enzymes such as aromatic L-aminoacid decarboxylase (AADC) are present in other non-DA cell types, etc.), yet provides a perspective of transmitter dynamics as well as connectivity of DA motor circuitry.

IMAGING THE INTEGRITY OF DA SYSTEM: PRE-SYNAPTIC STUDIES

Development of PET and the use of specific radio labeled ligands allow quantification of pre- and post-synaptic markers of the DA system (Table 1). Different compounds that target proteins localized in DA terminals are currently used to evaluate the pre-synaptic system (Fig. 1). Many of these tracers bind selectively to specific transporters such as the DA transporter (DAT) and the vesicular monoamine transporter 2 (VMAT2). Other tracers are substrate to transformation by specific enzymes located in the nigrostriatal terminals. Using combinations of such different tracers can provide complementary information about both the structural and functional state of DA nigrostriatal terminals.

Fluoro-L-3,4-dihydroxyphenylalanine (F-DOPA) is the most widely used PET ligand to assess the integrity of the DA system. The specific radioactive signal is dependent mainly on decarboxylation of F-DOPA to fluoro-dopamine (F-DA) (by AADC) and to some extent on the packaging of F-DA within intraneuronal storage vesicles. In addition to pharmacological studies (Melega et al., 1990), validation of this tracer is based on the strong correlation between F-DOPA kinetics and nigrostriatal degeneration as demonstrated in non-human primates (Pate et al., 1993) and humans (Snow et al., 1993). The pattern of F-DOPA uptake in PD (earlier and more severe loss in the posterior putamen) is also consistent with neuropathological findings (Fearnley and Lees, 1991; Piggott et al., 1999).

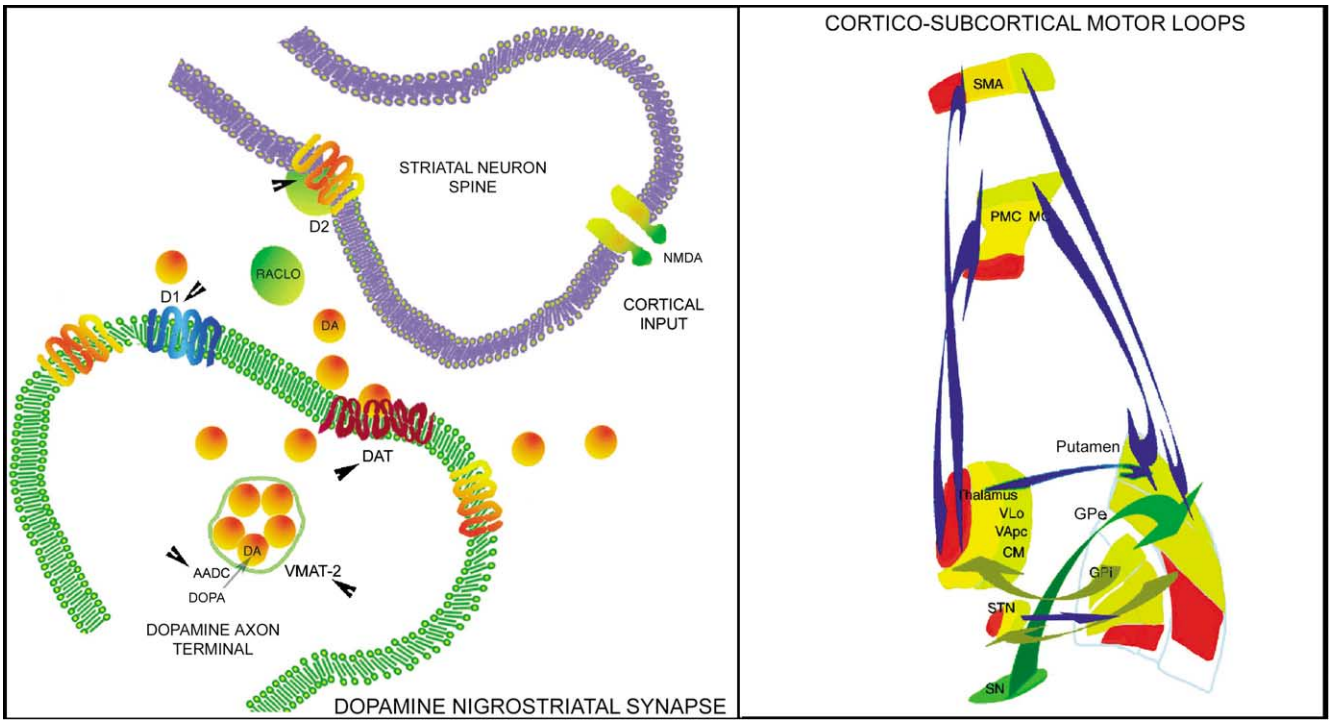

Fig. 1. Dopamine modulates motor activity through the nigrostriatal projection. DA cells located in the pars compacta of the substantia nigra send their axons to the striatum, where DA modulates the response of striatal projection neurons to cortical input. The left panel shows a schematic representation of a DA synapse on the neck of a dendritic spine of a striatal neuron. Arrowheads indicate molecular targets for imaging tracers at the pre-synaptic and post-synaptic levels. In receptor occupancy studies, competition of endogenous DA and a specific ligand (such as raclopride) for the D2 receptor allows measurement of intra-synaptic release of DA. On the right panel the DA nigrostriatal projection is represented, integrated in the cortico-subcortical motor loop (modified from Alexander and Crutcher, 1990). AADC: aromatic aminoacid decarboxylase; DA: dopamine; DAT: dopamine transporter; DOPA: dihydroxyphenylalanine; VMAT-2: vesicular monoamine transporter 2; GPe: globus pallidus pars externa; GPi: globus pallidus pars interna; PMC: premotor cortex; SMA: supplementary motor area; SN: substantia nigra; STN: subthalamic nucleus; Thalamic CM, VApc and Vlo: centromedian, ventral anterior pars parvocellularis and ventral lateral pars oralis thalamic nuclei. Color shades represent the somatotopic organization (yellow: leg; orange: arm; and red: face).

F-DOPA PET has been used in longitudinal studies to measure the progression of PD and the effects of medications and intracerebral transplants (Brooks and Samuel, 2000). It has also been used to estimate the threshold for clinical onset at about 30% loss of DA cells (Morrish et al., 1995) and the duration of pre-clinical stage (Brooks and Samuel, 2000). In humans exposed to MPTP, F-DOPA PET studies demonstrated that short-term exposure to MPTP led to a protracted decline in nigrostriatal DA function, with a similar progression rate to that observed in patients with PD (Vingerhoets et al., 1994)—but with an equivalent loss of F-DOPA uptake in caudate and putamen (Snow et al., 2000).

Peripheral metabolism and distribution of labeled metabolites (mainly *O*-methylated) complicate analysis and interpretation of F-DOPA studies (Isacson, 1994) and have prompted the development of other fluorinated aminoacid analogs that are substrate to AADC (but not to catechol-*O*-methyl transferase, COMT) such as fluoro-*meta*-tyrosine (FMT) (Barrio et al., 1996; Nahmias et al., 1995). These compounds allow a simpler analysis and provide a superior image contrast, making it possible to assess the activity also in extrastriatal regions.

Nomifensine, methylphenidate (Lee et al., 2000) and the tropane-based tracers bind to DA uptake sites on nigrostriatal terminals and also provide specific information about the integrity of nigrostriatal DA projections. We have extensively used 2-β-carbomethoxy-3-β-(4-fluorophenyl) tropane, CFT, a selective ligand for DAT which provides a validated measure of DA pre-synaptic terminals in monkeys (Hantraye et al., 1992) (Fig. 2). In MPTP-treated primates severe reduction in CFT binding is correlated with development of motor symptoms (Brownell et al., 1998a) (Fig. 2). In rodents we have shown reduction of carbon 11-CFT binding following 6-OHDA lesion and restoration of the signal by fetal DA grafts (Brownell et al., 1998b) and by stem cells (Bjorklund et al., 2002) (Fig. 3)

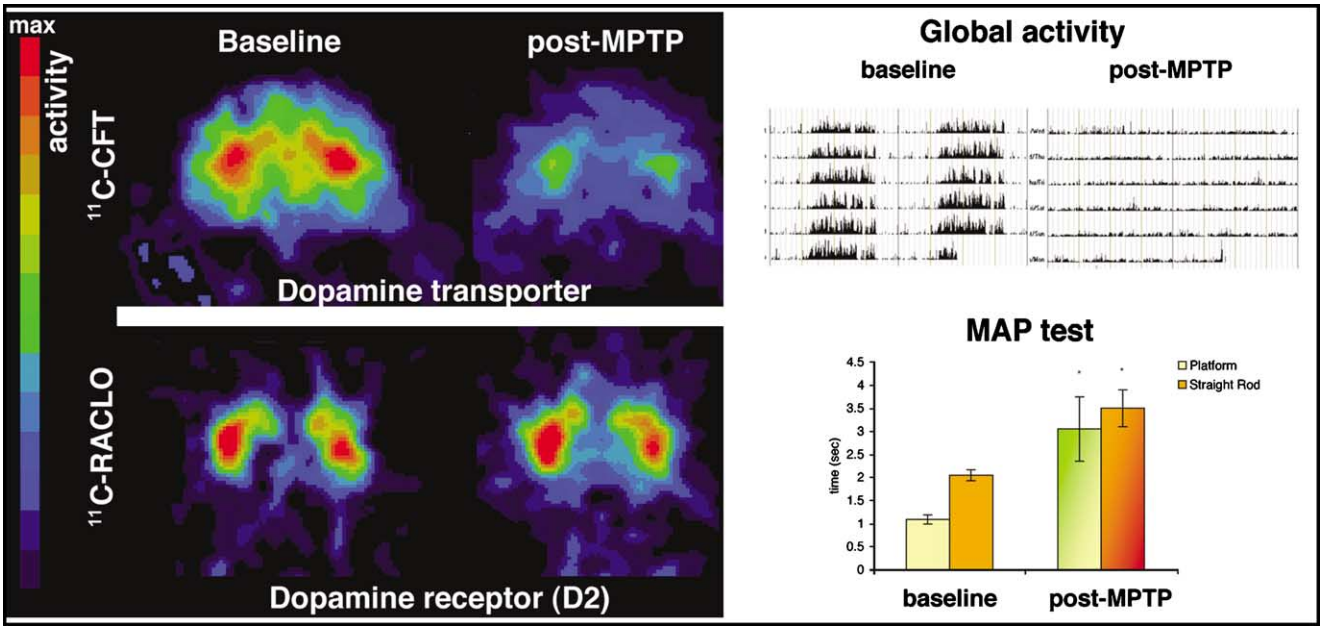

Fig. 2. In vivo evaluation of MPTP toxicity of the DA system in primates using PET and motor tests. Using PET and specific radio labeled ligands for pre and post-synaptic markers we can quantify the extent of damage to the DA nigrostriatal system (left panel). After MPTP administration there is a marked decrease in DA terminals, labeled here with ^{11}C-CFT, a cocaine analog that binds to the dopamine transporter (upper panel) and a moderate up-regulation of post-synaptic receptors as determined by ^{11}C-raclopride, a selective D2 receptor antagonist (lower panel). These imaging findings correlate with objective measures of parkinsonism (right panel): MPTP induces a significant decrease in global activity, a measure of hypokinesia (upper panel) and a significant increase in the time used to perform a reaching task (bradykinesia) (lower panel). We routinely test the performance in two standardized computerized tasks, shown in the graph before and after MPTP, using the motor activity panel (MAP) described by Gash et al. (1999).

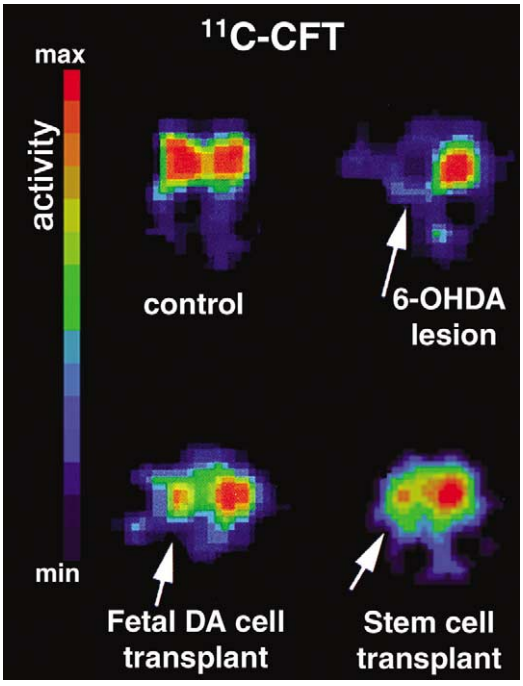

Fig. 3. High resolution PET and the dopamine transporter ligand ^{11}C-CFT allow measuring DA integrity in the rodent brain. Using this technique we have studied in vivo the effect of neurotoxins like 6-OHDA and restorative approaches as illustrated here. Color-coded images of specific activity showing the symmetric signal over the striatum in a normal rat, a complete loss of signal ipsilateral to 6-OHDA administration and partial restoration achieved by transplanting fetal DA cells (Brownell et al., 1998b, with permission) and stem cells (Bjorklund et al., 2002).

but not after grafting non-DA cells (Brownell et al., 1998b). In Parkinson's patients CFT is a sensitive marker for early stages of the disease (Frost et al., 1993) and for the rate of disease progression (Nurmi et al., 2000). Dihydrotetrabenazine can be used to map the vesicular transporter, VMAT-2 (Chan et al., 1999). Studies combining the use of these tracers increase our insight into the progression of the degeneration, pathogenic mechanisms and novel therapies. Predictive models of disease progression are required for testing disease-modifying hypothesis and here PET can play a fundamental role, since PET studies provide an objective measure of the pre-synaptic DA system. However, it is necessary to carefully assess biological (and pharmacological) factors that may lead to under- or overestimation of the extent of DA degeneration, such as enzymatic up or down-regulation, competition for the transporter, etc. (Lee et al., 2000).

IMAGING STUDIES OF DA RECEPTORS: POST-SYNAPTIC STUDIES

There is some controversy regarding the post-synaptic changes in DA receptors both in animal models and in patients with PD. For the most part, this controversy originates in the comparison of results obtained with different techniques (mRNA versus autoradiography), different stages of degeneration or pharmacological conditions. There is evidence of specific and significant D2 receptor upregulation in post-mortem analysis of patients (Piggott et al., 1999). PET studies of the D2 receptor have yielded different results and interpretation of these results is sometimes difficult. The majority of studies using raclopride (a selective reversible D2 antagonist) describe a moderate upregulation in L-DOPA naïve patients (Antonini et al., 1994, 1997; Rinne et al., 1993, 1995; Sawle et al., 1993) at least in early stages of the disease. L-DOPA treatment does not seem to change raclopride binding (Antonini et al., 1994) which is consistent with neuropathological findings. Recently, Kaasinen et al. (2000) have documented a similar degree of upregulation of the D2 receptors using methyl-spiperone (a different class of D2 antagonist). However, Dentresangle et al. (1999) reported no upregulation in early L-DOPA naïve patients. Interestingly, D2 upregulation is absent in familial parkinsonism associated with some parkin mutations (Hilker et al., 2001) which also show a symmetric loss of pre-synaptic markers suggesting that the mechanisms of disease progression might be different than in sporadic PD.

Reports in MPTP-induced parkinsonism in humans (Perlmutter et al., 1987) and monkeys (Leenders et al., 1988) have shown upregulation of D2 receptors in response to DA cell loss caused by the neurotoxin. In a long-term study in rhesus monkeys Doudet et al. (2000) have confirmed an acute upregulation of D2 binding following MPTP that decreased, but was still present 10 years after the lesions. In our primate PD model using chronic systemic administration of MPTP (Brownell et al., 1998a) animals often show a moderate upregulation of ^{11}C-raclopride binding (Fig. 2). Restoration of DA striatal release by fetal transplantation has been shown to normalize D2 receptor upregulation in a patient (Piccini et al., 1999) as demonstrated previously in rodents (Cenci et al., 1992) and primate studies with autoradiography (Elsworth et al., 1998). In comparison to D2 receptors, the state of D1 receptors has not raised much interest. No significant changes have been reported in patients using PET and SCH 23,390 (Ouchi et al., 1999; Turjanski et al., 1997) in agreement with neuropathological findings (Piggott et al., 1999).

IMAGING STUDIES OF RECEPTOR OCCUPANCY: A FORM OF NON-INVASIVE MICRODIALYSIS

Another interesting application of receptor studies is quantification of displacement of raclopride receptor binding by DA released in the synaptic cleft either by motor tasks or pharmacologic challenges (Dewey et al., 1993b). This imaging paradigm is based on competition between the tracer and endogenous DA for D2 receptor occupancy (Laruelle et al., 1997a). Manipulation of DA synaptic levels induces changes in binding potential of several D2 radiotracers (Laruelle, 2000) such as catecholamines and benzamides (more questionable in other non-benzamide antagonists like spiperone and pimozide) and thus provides a way to measure synaptic DA release in a non-invasive way. Numerous studies (reviewed in Laruelle, 2000) have shown that the decrease in benzamide specific uptake results from a decrease in binding potential (as opposed to changes in cerebral blood flow, tracer clearance or non-specific binding) and the magnitude of the change is correlated with the magnitude of changes in DA level measured with microdialysis (Laruelle et al., 1997b). However, the measurable displacement effect is limited by the number of D2 receptors susceptible to an additional occupancy by DA, which are those receptors that are in a high affinity state, not occupied by basal levels of DA and that are located in the synaptic cleft (i.e. 10–30% of the total number of receptors present). Most displacement studies use benzamides, such as raclopride, which has a weaker affinity for the D2 receptor than DA. Nonetheless, it is also possible to use high affinity ligands, as in the equilibrium state receptor occupancy by DA depends only upon the concentration and affinity of DA for the receptor (Verhoeff, 1999). Although in some instances the model can be complicated by ligand–receptor complex internalization, phosphorylation or change in affinity, the occupancy model provides a way to measure synaptic transmission in the living brain (Laruelle, 2000). This method is being used in human studies providing a wealth of information such as DA release by mental tasks (Koepp et al., 1998), expectation of reward (placebo effect) in Parkinson patients (de la Fuente-Fernandez et al., 2001), L-DOPA symptomatic effect (Tedroff et al., 1996) and changes in re-uptake associated with motor fluctuations (de la Fuente-Fernandez et al., 2000), and

also DA release by grafted neurons (Piccini et al., 1999). In a different context, these studies have also provided significant information in psychiatric disorders and substance abuse measuring DA release induced by cocaine (Schlaepfer et al., 1997), nicotine, alcohol and morphine in the accumbens (Gerasimov et al., 1999) and pharmacological release of DA induced by methylphenidate (Dewey et al., 1993a; Volkow et al., 1997), tetrabenazine and ketamine (Smith et al., 1998).

Using a similar approach, but measuring CFT binding before and after walking in PD patients, Ouchi et al. (2001) have demonstrated a shift in areas activated by locomotion from putamen (controls) to caudate and orbitofrontal cortex. These findings suggest that motor-triggered DA release has a different distribution in PD and might help identify complex adaptive changes in the DA system. Occupancy studies can be performed for other neurotransmitter systems to examine transmitter interactions by measuring the effect of agonists and antagonists on transmitter release. With a selective nicotine receptor ligand it was shown, using DA receptor agonists and antagonists that striatal DA regulation of acetylcholine is mainly achieved through D2 receptors (Ding et al., 2000). In summary, these studies provide a novel, non-invasive way to assess transmitter function in vivo.

METABOLIC STUDIES OF THE MOTOR CIRCUITRY

Metabolic studies are based on the coupling between neuronal activation and glucose/oxygen consumption that can be measured using PET and radio labeled glucose or oxygen. The increased metabolic demand related to neuronal activation is accompanied by hemodynamic changes and, subsequently, changes in MRI signal which allow to localize and quantify such activity using fMRI that for some studies provides a better temporal and spatial resolution than PET (Dale and Halgren, 2001). DA modulates the output activity of the striatum in response to cortical input (Fig. 1) (Alexander and Crutcher, 1990; DeLong, 1990). Loss of DA alters the activity of striatopallidal projections with a net result of decreased neuronal activation in cortical premotor areas. In the resting state, PD patients show an abnormal metabolic brain network. The characteristic regional covariance pattern (Eidelberg et al., 1994) in PD patients is characterized by relative pallido-thalamic and pontine hypermetabolism associated with relative decrease in cortical motor areas.

Activation of the motor circuitry induced by motor tasks or pharmacological agents allows investigate the effects of DA release on brain metabolic indices. Both PET and fMRI can be used to measure the increased metabolic requirements by neurons firing in response to DA release located several synapses away from the nigrostriatal DA terminal. Significant changes in activation of projection areas in the motor cortex are associated with DA deficiency. Consistently, PD patients fail to activate the rostral supplementary motor cortex (SMA) and dorsolateral prefrontal cortex (DLPFC) during motor performance (Antonini et al., 1994; Jenkins et al., 1992; Playford et al., 1992; Sabatini et al., 2000). Failure to activate these cortical regions is thought to underlie the akinesia and lack of internally generated movements that characterizes PD, since normal subjects activate SMA and DLPC in relation with selection and programming of a new movement (Berardelli et al., 2001). In addition, recruitment of associative cortices and cerebellum seems to be required during movement to compensate for the basal ganglia deficit (Catalan et al., 1999; Rascol et al., 1997; Sabatini et al., 2000; Samuel et al., 1997). Parietal recruitment can explain why initiation of movement in PD patients may be facilitated by external cues. Cerebellar recruitment has been observed with simple hand movements (Rascol et al., 1997) but performance of movements in complex sequences results in an increase in lateral premotor and parietal cortices but in cerebellar hypoactivation (Catalan et al., 1999) suggesting a shift from subcortico-cortical to cortico-cortical circuits. Cerebellar projections to thalamus appear to be implicated in the generation of tremor since thalamic stimulation abolishing tremor is associated with a reduction in cerebellar blood flow (Davis et al., 1997; Deiber et al., 1993; Parker et al., 1992).

Interestingly, different therapeutic approaches acting through distinct mechanisms and circuitry levels, demonstrate a similar improvement in cortical activation using metabolic studies. An increase in activation in the SMA and usually also in anterior cingulate and dorsolateral prefrontal cortex has been observed in relationship with pharmacological treatment using DA agonists (Jenkins et al., 1992) and L-DOPA (Haslinger et al., 2001), ablative lesions of the pallidum and subthalamic nucleus (STN) (Samuel et al., 1997), deep brain stimulation (Ceballos-Baumann et al., 1999; Fukuda et al., 2001; Limousin et al., 1997) and transplantation (Piccini et al., 2000). Surgical lesions appear also to increase activation in premotor areas (even though these areas not hypoactive but rather overactive

in PD patients). Deactivation of motor cortex has been observed in patients during STN high frequency stimulation and is probably related to a direct effect on the cortico-STN projection (Ceballos-Baumann et al., 1999). Disruption of the circuitry outflow may cause deficits in specific tasks (Jahanshahi et al., 2000; Marsden and Obeso, 1994). In fact, L-DOPA might achieve a more physiological effect by inducing a parallel decrease of the compensatory hyperactivity within the lateral premotor-parietal-primary motor circuitry (Haslinger et al., 2001). However, the effect of L-DOPA depends on remaining DA terminals. Transplantation of fetal DA cells can provide restoration of the DA system at all levels. In patients receiving fetal cell transplants, partial restoration of F-DOPA signal indicates cell survival and correlates with motor improvement (Brundin et al., 2000; Hagell et al., 1999; Hauser et al., 1999; Wenning et al., 1997). However, the presence of DA cells is not enough to restore function since these cells need to establish functional connections. Prior to functional integration there is a dissociation between F-DOPA PET and motor indices, which has been be misinterpreted (Isacson et al., 2001) as lack of therapeutic effect (Freed et al., 2001). Nevertheless, complementary PET studies have examined, in a limited number of patients, the long-term effects of cell transplantation. These functional studies show not only cell survival but also normalization of D2 receptors (Piccini et al., 1999), dopamine release in response to amphetamine using a raclopride displacement paradigm (Piccini et al., 1999) and improved activation of SMA and dorsolateral prefrontal cortex (Piccini et al., 2000). Together with pre-clinical experimental data, these studies provide a rationale for cell replacement therapy in PD, though several technical aspects need to be refined. Recently, the possibility to use embryonic stem (ES) cell derived DA neurons as a source for cell replacement therapy in PD has been explored. We have found that embryonic stem cells transplanted into the 6-OHDA-lesioned rat striatum differentiate in vivo into a DA phenotype and mediate motor recovery (Bjorklund et al., 2002). DA neurons derived from ES cells express all DA markers and restore CFT signal in the same way that fetal DA cells (Fig. 3). Restoration of specific DAT binding was observed only in those animals showing motor recovery (Brownell et al., 1998b; Bjorklund et al., 2002). Using fMRI we have also identified restoration of cortical activation in response to amphetamine (Bjorklund et al., 2002).

In summary, the use of functional imaging techniques allows detecting and characterizing the state of the DA system and the effects of therapeutic interventions in clinical and pre-clinical studies. These studies are likely to help understanding the mechanisms underlying the functional consequences of DA deficiency and the ways to restore the DA system.

ACKNOWLEDGEMENTS

This work is supported by the following federal grant awards to O.I., Udall Parkinson's Disease Research Center of Excellence (P50 NS39793), NS-41263 and DAMD17-98-1-8618. Support from the Parkinson Foundation of the National Capital Area, Century Foundation and Kinetics Foundation is also gratefully acknowledged.

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