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The molecular basis of dopaminergic brain imaging in Parkinson's disease

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The central role of dopamine neuronal loss in Parkinson's disease provides a clear pathologic framework and rationale for imaging the system both to interrogate dynamic pathophysiologic changes as well as to aid in diagnosis and clinical management. Recent post mortem studies of Parkinson's brain provide a much fuller depiction of the inexorable and progressive topology of pathophysiologic changes, including brain alpha-synuclein deposition. This informs PET and SPECT evaluations for testing hypotheses regarding the course of degeneration in longitudinal studies of Parkinson's disease patients. Recent work has underscored the subtlety of change in the dopaminergic neuronal system and its neural connections as a function of disease status and treatment. The interplay between other neurochemical brain systems and dopamine elucidates potential new targets for therapeutic intervention across the stages of the disease.

KEY WORDS: Positron emission tomography - Tomography, emission-computed, single-photon - Dopamine - Parkinson disease - Neuroimaging - Nerve degeneration.

Although Parkinson's disease has probably existed for thousands of years, it was first described in detail in 1817 by a London physician James Parkinson in his publication, *An Essay on the Shaking Palsy*. In the essay, Parkinson documented his observations of several cases of what he defined as shaking palsy, outlining key features which we associate with Parkinson's including bradykinesia, tremor, and difficulty walking and/or standing. James Parkinson's essay documented some of the more well-known motor or movement symptoms of Parkinson's disease (PD), but these symptoms are not the earliest manifestation of the disease which bears his name. Non-motor symptoms, such as constipation, disturbance of normal sleep architecture, and reduced olfactory acuity may precede clinical motor symptoms in Parkinson's disease by several years.[1, 2] Once motor symptoms do manifest, they start unilaterally. As the disorder progresses, motor symptoms become bilateral, increasing in severity on both sides, with the accompaniment of postural instability. However, asymmetry in motor symptoms may be retained throughout the course of the disorder. Non-motor symptoms progress as well. These may include changes in cognitive behavior, depression, hypologia, and autonomic system dysregulation and represent some of the most troubling symptoms for patients and families.

The pathophysiological basis for motor symptoms was first described nearly a century ago creating the first link between the clinical phenomenonology of Parkinson's disease and neurodegeneration of the dopamine system. These initial histopathological studies demonstrated selective loss of nigral-striatal melanin-containing neurons. Specifically, dopaminergic projections from the substantia nigra pars compacta to the basal ganglia, including the caudate and putamen were shown to be extensively lost in Parkinson's brain. A number of years later

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this key finding became the basis for the mainstay of symptomatic Parkinson's disease therapy. For nearly 50 years dopamine replacement strategies including L-dopa and dopamine agonists have been effective for ameliorating Parkinson's symptoms. Nonetheless, the clinical scenario is more complicated than simply restoring lost dopamine.[3] With the inexorable progression of the disease, including ongoing dopaminergic neuronal loss, side effects of dopamine replacement treatment invariably develop including drug-induced dyskinesia and on-off phenomena reflecting severe dysregulation of motor circuits. The field is further challenged to understand the subtlety of changes in not only dopamine systems, but also the many neurochemical networks which impinge upon, affect, and are affected by dopaminergic neurodegeneration and treatment.[4] While dopamine systems continue to play a front and center place of our understanding of both motor pathophysiology and therapy in Parkinson's disease, this is clearly not the whole story. The purpose of this essay is to review the state of our understanding of dopaminergic mechanisms in PD as it bears on imaging the brain with positron emission tomography (PET) and single photon emission tomography (SPECT).

Dopaminergic and non-dopaminergic pathology in Parkinsons

The rationale for imaging dopaminergic systems in Parkinson's disease is best understood in the context of known pathologic changes in the brain. The first demonstration of nigral-striatal neuronal loss in post mortem studies of Parkinson's patients was in 1919. These studies showed the characteristic loss of melanin containing neurons whose cell bodies reside in the *substantia nigra pars compacta*.

There was some time between the discovery of nigral neuronal loss and an understanding of the role of dopamine in Parkinson's disease. The first suggestion that dopamine was depleted in brains of patients with idiopathic Parkinson's disease was made by Hornykiewicz and colleagues in 1960.[5] He specifically noted that the striatal dopamine was reduced in PD patients, subsequently positing that this represented the pharmacological signature of the disorder.[6] This resulted in therapeutic trials with levodopa, which were initially confounded by prominent side-effects developed by patients.[7] Subsequent trials used slower dose escalation strategies resulting in greater tolerance and a clearer evaluation of the efficacy of levodopa. With the introduction of peripheral inhibitors of dopa decarboxylase therapeutic efficacy improved even more. These studies confirmed the critical role of dopamine in the etiology of motor symptoms, like bradykinesia. It was also quickly understood that with ongoing degeneration and treatment, that dopaminergic systems continue to change. In particular, the new problem of levodopa-induced dyskinesias underscored the complex issue involving therapeutics designed to replace dopamine. More recent strategies to replace dopamine beyond levodopa supplementation have focused on dopamine agonists which have lesser tendency to produce dyskinesias with chronic dosing at the cost of slightly lower efficacy.[8-10] Further, new drug delivery formulations or drugs which prolong the persistence of synaptic dopamine, are under clinical evaluation as a way to attenuate rapid rises and falls of synaptic dopamine which may be responsible for motor fluctuations.

The connection between nigral striatal neuron loss and dopamine serves as the central principle guiding dopaminergic imaging in PD. Dopamine cells comprising the nigral-striatal circuit degenerate in asymmetric fashion. Both left-right asymmetry, as well as anterior-posterior asymmetry is evident; the latter due to relatively greater involvement in those neurons projecting to the medial aspect of the putamen. While projections to the caudate are also lost, these are relatively preserved compared with putamen. This pattern provided the first clues as to the scintigraphic patterns to be expected when imaging with radiotracers that interrogate pre-synaptic dopamine function in the basal ganglia.[11] In addition, asymmetry is consistent with clinical presentations of Parkinson's patients who typically demonstrate unilateral onset of symptoms with subsequent bilateral motor impairment with progression of the disease.

The cause of dopaminergic degeneration is unknown, although advances have been made in the last decade improving understanding molecular mechanisms which might lead to neuronal death. Parkinson's disease may be characterized as a proteinopathy, or more correctly, a synucleinopathy.[12] In 1912 Franz Lewy described eosinophilic cytoplasmic inclusion bodies in human post mortem brain. The Lewy body is composed of aggregations of α-synuclein, neurofilaments, and other proteins and represents an important pathological feature of Par-

kinson's disease. Alpha-synuclein-immunoreactive inclusions may appear as thread-like Lewy neurites (LN) within cellular processes or as more circumscribed Lewy bodies (LB) within the neuronal somata. Lewy bodies and Lewy neurites have been demonstrated in the *substantia nigra* and other regions of PD brain, including the hippocampus, amygdala, and *cingulate gyrus*. Neurons with long axonal projections and poor myelination are more susceptible to developing LN or LB inclusions.[13] As lack of myelination increases the energy demands of the neuron, one hypothesis regarding nigral neural dysfunction highlights the role of mitochondrial impairment. MPTP models of parkinsonism suggest direct toxic effects on cellular energetics may be a result of poisoning oxidative phosphorylation pathways in mitochondria. In addition, mitochondria may be key to removing free radicals and to serve central roles in managing apoptotic pathways. Hence, it is proposed that monoaminergic neurons are more vulnerable to Lewy body formation due to their higher energy requirements. The presence of neuronal alpha-synuclein is not unique to Parkinson's disease and found in other neurodegenerative disorders including Lewy body dementia, multiple system atrophy, Down's syndrome, and other disorders.

The exact relationship between abnormal synuclein aggregation, Lewy body formation, and cell death is unclear.[14] One theory suggests that the Lewy body is an attempt by the neuron to sequester toxic free alpha-synuclein proteins, effectively keeping them from doing harm. Regardless of the underlying insult resulting in cell death, careful cross sectional studies of post mortem Parkinson brain by Braak *et al.* provide insight into the nature and time course of pathologic change. These studies describe an orderly topographic pattern of selective regions involved in LB and LN inclusions from postmortem studies of 110 incidental and clinically diagnosed PD cases and 58 age and gender matched controls.[14]

Briefly, the schema describes six stages of predictable and progressively evolving regional accumulation of LB inclusions (Table I).[15] Broad clinical symptom clusters have been associated with different Braak stages. Braak stages 1-2 are associated with autonomic/olfactory disturbances, stages 3-4 sleep and motor disturbances, and stages 5-6 with emotional and cognitive changes corresponding to pathologic inclusions in olfactory bulb, medulla, and pontine tegmentum (stages 1-2), substantia nigra, midbrain tegmentum, and limbic structures (stages 3-4), and association and primary neocortex (stages 5-6).

Braak staging provides a heuristic approach to understanding the pathophysiology of PD, but several observations are germane;

1) staging of LB/LN inclusions does not reflect neuron loss or impaired connectivity and circuit disruption,

2) the model predicts well the timing of some early PD symptoms like olfactory loss and REM sleep behavior disorder, but not other symptoms like dysautonomias,

3) Braak staging suggests that motor impairment, despite being the clinical criteria upon which a diagnosis of PD is made, is not an early feature of the disease,

4) the schema underscores the fact that PD is more complex than loss of midbrain dopamine neurons in association with Lewy bodies, creating specific and directly testable hypotheses for neuroimaging studies.

Regarding the latter point, there is currently no adequate imaging biomarker of alpha-synuclein. The effects of Lewy body inclusions can only be measured with indirect scintigraphic techniques, some of which are quite relevant to therapy like presynaptic dopaminergic imaging, while other techniques may provide pathophysiologic information concerning CNS responses to regional synucleinopathy. An example of this might be imaging neuroinflammation or glucose metabolism. Further, the molecular im-

TABLE I.—*Braak staging and expected clinical results.*

BRAAK Staging	Expected Clinical
Stage 1: Dorsal motor nucleus of the vagal nerve; anterior olfactory structures	Olfactory loss, autonomic dysfunction
Stage 2: Lower raphe nuclei; locus coeruleus	Affective impairment
Stage 3: Substantia nigra; amygdala; nucleus basilis of Meynert (clinical diagnosis made at this stage)	Motor symptoms
Stage 4: Temporal mesocortex	
Stage 5: Temporal neocortex; sensory association and premotor areas	Emotional/cognitive disturbances
Stage 6: Neocortex; primary sensory and motor areas	Severe disability

aging tool box is now expansive enough to interrogate other monoaminergic targets in Parkinson's disease as well as other relevant neurochemical systems which influence dopamine pathways relevant to motor disease or non-dopamine pathways which are responsible for other symptom clusters.[16]

Returning to the dopamine system, dopamine neurons project from the lateral substantia nigra pars compacta to the basal ganglia in a topographic fashion. All Parkinsonian syndromes (PD, progressive supranuclear palsy, multiple system atrophy, etc) have been associated with a marked loss of dopamine neurons in the substantia nigra resulting in reduction of target sites for imaging tracers. This loss is most evident in the basal ganglia were terminal projections of these nigral neurons exist in putamen and caudate. Characteristic findings when imaging measures are largely correlated across the different pre-synaptic dopaminergic targets, and as expected closely track what is known from limited histopathologic studies of dopamine loss, dopamine neuronal number, and dopamine transporter density (Figure 1).[17]

Moreover, disturbances in normal striatal circuitry with loss of dopaminergic projections result in a series of compensatory changes as described below.

Neuroimaging of dopaminergic function in PD

With much of the focus of imaging in Parkinson's disease on the presynaptic dopamine neuron terminating in the basal ganglia, there are a wealth of PET and SPECT radiopharmaceuticals for interrogating these degenerating neurons, targeting dopamine metabolism, the dopamine transporter, or the vesicular transporter (Table II). These imaging tools are relatively well-established and have been incorporated into the diagnostic assessment of parkinsonism in routine clinical neurology practice.[18]

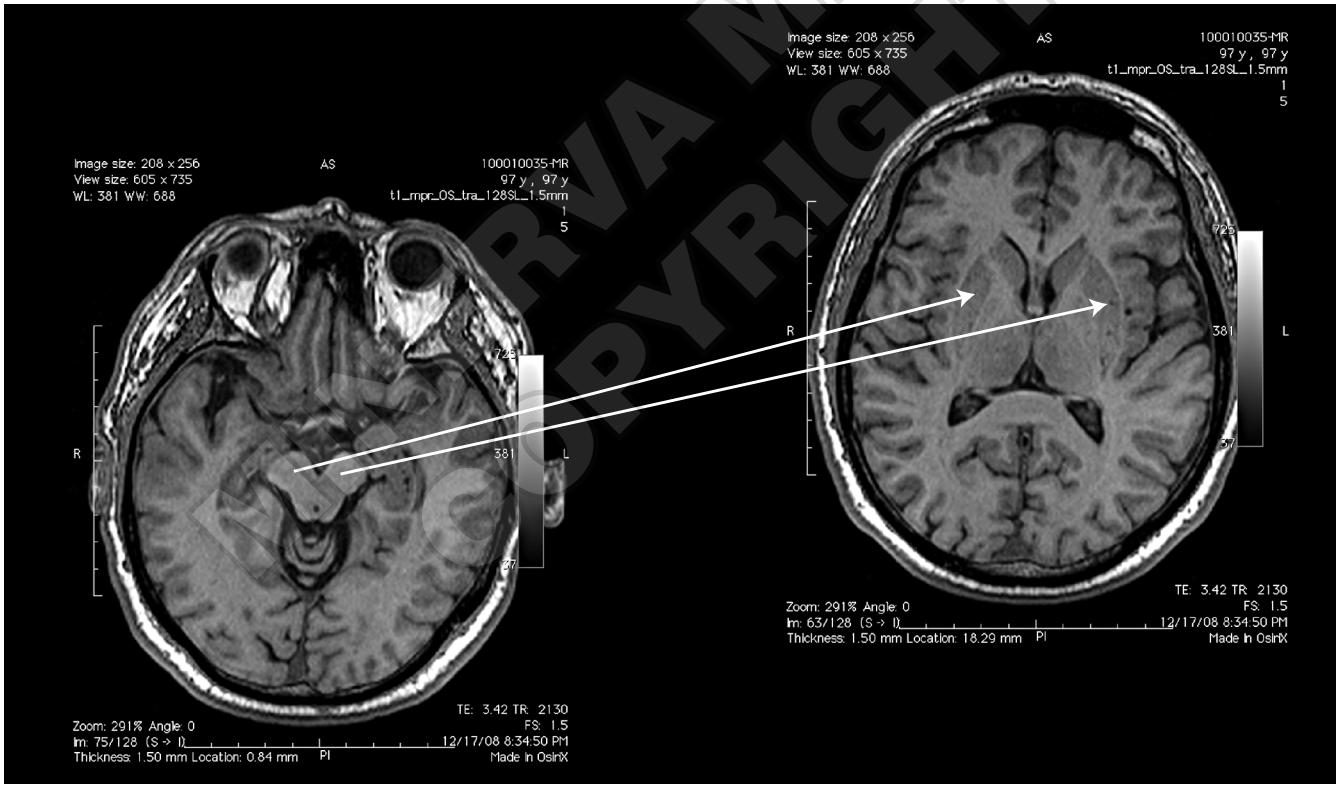

Figure 1.—Loss of nigral striatal dopamine neuronal projections to the basal ganglia represent a key pathologic event in the production of Parkinson's motor symptoms. There are topographical patterns to degeneration of these neurons with relative greater involvement of the lateral and posterior putamen.

TABLE II.—*Dopaminergic imaging biomarkers.*

	Target	Examples
Presynaptic	Dopamine transporter	FP-CIT, PE2I, b-CIT
	Dopamine metabolism	F-dopa
	VMAT2	DTBZ, AV-133
Intrasynaptic	D2/D3	Raclopride, IBZM
Post-synaptic	D2/D3	Raclopride, IBZM, Fallypride, IBF, desmoxyfallypride

A decade after dopamine transporter imaging has been commercially available as a PD diagnostic in Europe, the relationship between dopamine neuron number and quantitative imaging outcome measures for presynaptic dopaminergic imaging agents is still unclear. In particular, a within subject study of Parkinson's patients undergoing imaging with F-dopa, VMAT2, and dopamine transporter (DAT) imaging suggest there may be slight differences amongst these agents. Relative to healthy controls, greatest differences in the PD cohort occurred with DAT. In addition, there has been nagging concern that in the clinical scenario where patients may be treated with dopamine replacement therapies, there may be regulation of the target site resulting in altered imaging outcomes.[19]

The original concern that there may be a large-scale regulatory effects of therapy on F- dopa uptake or dopamine transporter imaging is largely unfounded.[20] A careful within-subject study of the effect of high dose levodopa or the dopamine agonist, pramipexole demonstrated no impact on quantitative measures of dopamine transporters. In this study, *de novo* Parkinson's patients underwent DAT SPECT scanning with [^{123}I] beta CIT at baseline, after treatment with eight weeks of levodopa, pramipexole, or placebo, and after two months washout of the medications. The fairly large cohort (>25 subjects per group) demonstrated no within-subject effects of treatment. The variance on striatal binding ratios between baseline, on medication, or after washout was within the test-retest reproducibility of the outcome measure (Jennings, 2010 personal communication).

Keying off what is known about the pathology of dopamine nerve cell loss from postmortem data to inform the expected findings with selected imaging targets has been a rather straightforward exercise in Parkinson's. In particular, the expected topographical gradients of dopaminergic cell loss in the basal ganglia, with relative preservation of the caudate and greater involvement in the lateral and posterior putamen has been confirmed with *in vivo* imaging biomarkers.[21] Further, presynaptic dopaminergic imaging findings are consistent with the clinical presentation of early Parkinson's disease insofar as there is left right asymmetry with greater reductions on imaging on the side contralateral to symptom expression. These early studies also consistently demonstrated the tantalizing finding that in very early Parkinson's patients with asymmetric clinical motor symptoms, both contralateral and, to a lesser extent, ipsilateral sides show changes. This speaks to the sensitivity of imaging for picking up changes occurring in the brain prior to motor symptom manifestations (Table III).[22]

This has important implications regarding the role of imaging in helping to identify pre-motor Parkinson's disease. In this scenario, alterations in presynaptic dopamine function based on imaging biomarkers could allow the very early identification of patients at risk for developing subsequent motor symptoms.[23] The value of this rests on the anticipation that disease modifying therapies will become available, and the onus for good clinical assessment requires the identification of those patients at the very earliest stages of disease with the most potential for salvage.

There have been a few studies which have attempted to develop a feasible clinical scenario with which the use of dopaminergic imaging biomarkers could provide identification of these very early stage, premotor, or at-risk Parkinson's patients. In particu-

TABLE III.—*Comparison of pathology and expected DAT imaging findings.*

	Imaging findings
Reduction in early PD	50% in putamen
Reduction Putamen>Caudate	Yes
Reduction asymmetric	Yes
Correlation with severity (UPDRS)	Yes
Reduction in Presymtomatic (Hemi-PD)	Yes
Monitor PD progression	Yes

TABLE IV.—*Parkinsons at Risk Study (PARS).*

	DAT imaging by olfactory status				
	HYPOSMIC (<15%) N=203		NORMOSMIC (>15%) N=100		
Age expected Putamen DAT binding ratio	N	% of cohort	N	% of cohort	
<65% (DAT deficit)	23	11.3%	1	1.0%	p<.01
65% - <80% (Indeterminate)	35	17.2%	7	7.0%	p<.05
<80% (DAT deficit + Indeterminate	58	28.5%	8	8.0%	p<.001
>80% (NO DAT deficit)	145	71.5%	92	92.0%	

lar, the combination of high sensitivity screening biomarkers like olfactory acuity, sleep disturbance, or gastrointestinal symptoms may allow adequate prescreening for subsequent confirmation of diagnosis with an imaging measure. The largest such study, the Parkinson's At Risk Study (PARS) is an example of how these screening algorithms might be employed in clinical practice. In this ongoing research study, the major objective is to estimate the frequency of olfactory loss in asymptomatic research subjects, to compare striatal data density in hyposmic, neurologically normal subjects compared with age-matched, normosmic subjects, to compare imaging biological and genetic biomarkers of the defined at-risk cohorts, and to determine if reductions in DAT density in asymptomatic subjects at baseline predicts a subsequent development of clinical PD at two year follow-up.[22]

Subjects were invited via the mail to do a self-evaluation of olfactory acuity using the University of Pennsylvania Smell Identification Test (UPSIT). Approximately 10,000 smell tests were sent to potential subjects with about a 50% return rate. Those subjects scoring in the lowest 15% on the smell test (N.=300) were invited for evaluation by movement disorder specialists and neuropsychologist. In addition, a control group of normosmic controls (N.=100) were invited for similar assessment. Evaluations included dopamine transporter SPECT imaging, non-motor symptom assessment, neuropsychological evaluation, blood, and CSF measures with yearly follow-up.

Preliminary findings are described in Table IV. From a cohort of over 200 hyposmic, otherwise healthy individuals compared with 100 healthy, age and gender-matched controls, there is a significantly higher proportion of abnormal DAT SPECT scans defined as lower than 65% of age-expected putamenal DAT binding ratios in hyposmics compared with normosmics (11.3% *vs.* 1%, P<0.01). Considering those additional subjects with 65-80% age expected putamenal binding ratios includes 28.5% of the hyposmics, but only 8.0% of normosmic subjects (P<0.001). The next question to be addressed, how many of the hyposmics *vs.* normosmics develop PD, awaits longitudinal follow-up. Whether algorithms can be created to identify and manage patients at risk for PD based on high sensitivity screening followed by confirmatory imaging studies remains to be seen.

Other aspects of dopamine imaging are of interest as well and include postsynaptic dopamine receptors and intrasynaptic dopamine function (Figure 2).

It is possible using reversibly-bound D2/D3 imaging agents like [^{11}C] raclopride or [^{123}I] IBZM to measure the effects of drugs which influence synaptic dopamine levels. There is some suggestion that fallypride may also be sensitive to changes in

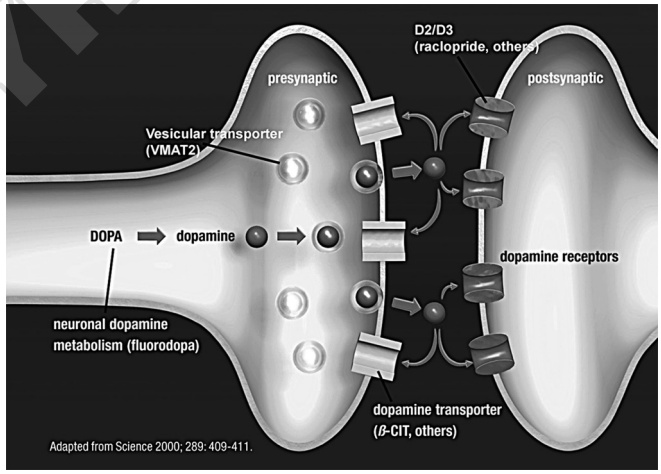

Figure 2.—A schematic model of the dopamine synapse in the striatum demonstrates presynaptic targets for imaging dopaminergic neuron loss. Different targets in the pre-synaptic neuron may demonstrate the loss of presynaptic terminals. Postsynaptic medium spiny neurons are inhibited by dopaminergic projections from the midbrain.

endogenous dopamine, even if perhaps not to the same extent as raclopride or IBZM. Measuring endogenous dopamine is germane to PD where newer putative treatments aim to reduce marked fluctuations in dopamine release associated with oral levodopa dosing regimens.[24, 25] Imaging studies show lower post-synaptic binding of the D2/D3 radiotracer following administration of agents that enhance endogenous synaptic dopamine levels. This effect occurs as dopamine competes with the radiotracer for binding to the receptor, reducing specific binding to D2/D3 visualized in striatum. Fuente-Fernández *et al.* used this paradigm to describe levodopa-induced changes in synaptic dopamine levels, noting increase flux with progression of Parkinson's disease. This work suggests development of dyskinesias reflect hypersensitivity of dopamine responses with chronic treatment.[26] Postsynaptic dopaminergic imaging with agents interrogating D1 and D2 dopamine receptors have also been evaluated in PD subjects with dyskinesias.[27] These studies show reductions of both D1 and D2 in relative to normal controls but no differences between dyskinetic and non-dyskinetic patients.

Consquences of dopaminergic degeneration neurochemical networks on motor circuits

With the degeneration of nigrostrial dopamine inputs onto striatal medium spiny neurons, come a host of compensatory changes Involving a complex interplay of different neurochemical systems which have relevance to treatment. The results of these changes produce motor manifestations of PD but also underpin the development of complications from dopamine replacement therapy. Early in the course of PD dopaminergic medications generally provide a steady and consistent therapeutic response. However, as the disease progresses the combination of dopaminergic terminal loss and cumulative exposure to levodopa ultimately leads to a series of erratic treatment responses collectively called motor fluctuations. Most prominent among these are dyskinesia, on/off phenomena, and significant wearing off. The onset of prominent motor fluctuations correlates with the onset of significant functional disability. Furthermore, it is the medium spiny neurons which express receptor targets for new classes of antiparkinsonian medication for both improving primary motor symptoms and ameliorating untoward side effects from chronic levodopa treatment. These are schematized in Figures 3, 4 indicating the points of interplay between the degenerating nigral dopamine neurons and its downstream connections at the medium spiny neuron in striatum and beyond.

Medium spiny neurons are classified into two groups based on the neuropeptides and receptors they express: Substance P, dynorphin and dopamine D1 receptors comprise one group, and the other group has enkephalin and dopamine D2 receptors. The former group is designated the direct pathway and project to the internal globus pallidus and *substantia nigra pars reticulata* while the latter is the indirect pathway with projections to the external *globus pallidus*. The direct and indirect pathways may best be thought of having opposing effects on thalamic motor pathways influencing cortical motor regions; somewhat simplistically, the direct pathway results in disinhibition of cortical motor neurons, while the indirect pathway is inhibitory.

At the level of the basal ganglia the key gatekeeper of the motor circuit is the medium spiny neuron. These neurons are GABAergic, having inhibitory effects on their targets. The direct pathway neurons cause excitation of cortical motor neurons via a complex circuit involving projections to the internal globus pallidus (GPi) and substantia nigra pars reticulata (SNpr) inhibiting output from these structures to the ventroanterior and ventrolateral thalamus, which in turn has excitatory effects on cortical motor regions. Since GPi and SNPr have inhibitory effects, direct pathway medium spiny neuron firing results in a cumulative disinhibition of motor cortical pathways. Thalamic neurons are tonically quiescent without cortical neuron stimulation, preventing involuntary motor movements.

The indirect pathway, again mediated by gatekeeping medium spiny neurons, has opposing effects to the direct pathway. In this instance, projections extend from the striatum to the globus pallidus externa and then the subthalamic nuclei before synapsing with the GPi and SNpr through excitatory imputs.[28] The remaining pathways to the ventroanterior and ventrolateral thalamus and motor cortex are identical to the direct pathway. Stimulation through the indirect pathway results in an overall enhancement of inhibition to the motor cortex.

In both pathways, cortical glutaminergic projections have excitatory effects on medium spiny neurons. Figure 4 describes some of the influences on

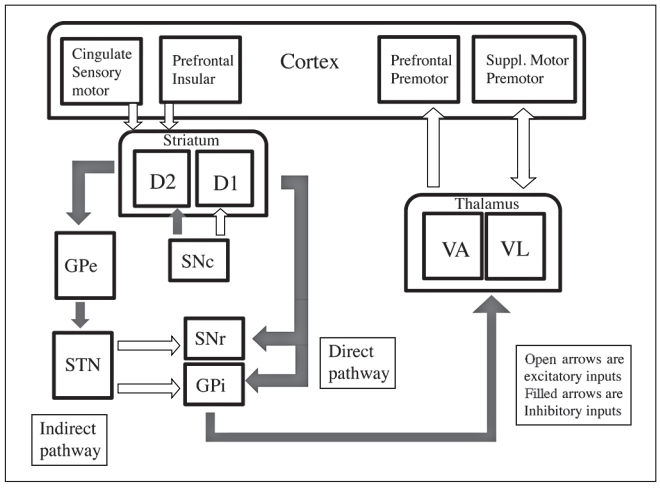

Figure 3.—Simplified circuit shows motor pathways in the striatum and the two pathways for controlling movements. The indirect pathway and direct pathway serve opposite roles in regulating involuntary movements. Red arrows indicate inhibitory inputs and green arrows indicate excitatory inputs. In Parkinson's disease loss of substantia nigra pars compacta input results in a relative greater influence of indirect pathway inputs to globus pallidus interna producing bradykinesia.
GPe: globus pallidus externa; SNc: *substantia nigra pars compacta*; STN: subthalamic nucleus; SNr: *substantia nigra pars reticulate*; VA: venteroanterior thalamus; VL: venterolateral thalamus

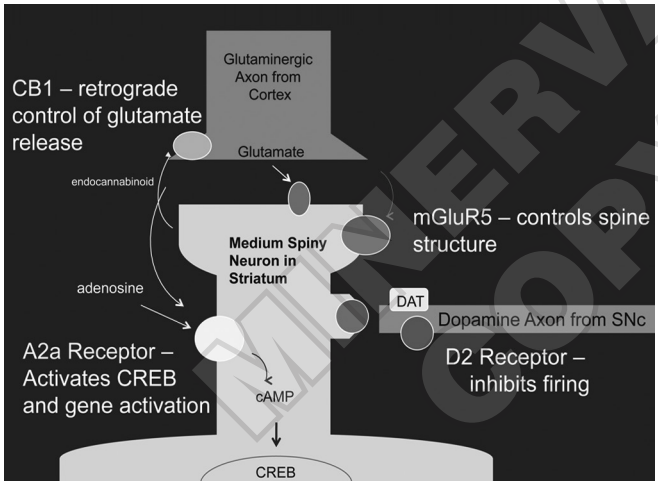

Figure 4.—In this coned down view of striatum, the medium spiny neuron receives glutamate projections from the cortex and dopamine projections from substantia nigra. The medium spiny neuron is regulated by a number of mechanisms including adenosine receptors which activate CREB for gene regulation, CB1 receptors located on presynaptic glutamate neurons control glutamate release, and postsynaptic mGluR5 receptors which modulate spine structure. All these mechanisms serve to regulate the system threshold for firing and are important targets for both therapeutics and imaging in Parkinson's disease.

indirect pathway medium spiny neurons firing.[29] Spiny neurons receive a significant number of projections not only from cortical regions but also a number of interneurons that serve to regulate excitability. In essence the latter adjust the gain on the system to make a neuron more or less responsive to cortical stimulation by adjusting the threshold for firing. Glutaminargic cortical inputs to the medium spiny neurons contain presynaptic endocannabinoids receptors which regulate glutamate release. Furthermore, structural modification of the medium spiny neuron can occur causing different functional characteristics. For example, glutamate binding to mGluR5 receptors alter the structure of dendritic spines on medium spiny neurons. In addition, stimulation of adenosine2a sites on medium spiny neurons activates CREB (cAMP response element-binding), a cellular transcription factor, which binds to DNA sequences to increase or decrease gene transcription.

In Parkinson's disease the loss of inhibitory dopamine neurons produces an overall imbalance with relative increased weighting on indirect pathways resulting in bradykinesia. The development of disabling on-off phenomenon following chronic levodopa administration may be due in part to structural changes of medium spiny neurons. These circuit alterations pose a significant therapeutic challenge in mid-to-late stages of the disease.

Relevance of new targets to clinical imaging and treatment of PD

Although the basal ganglia is best understood in terms of the functions of dopamine, numerous studies indicate that interrogation of other neurotransmitter systems provides complementary and additional relevant information.[30] These other neurotransmitter systems provide insights to distinct brain functions and also shed light on the functional state of the dopamine system through their dynamic interactions with it. Keying off understanding of the neurochemcial influences in normal and Parkinson's basal ganglia circuitry described above has resulted in a number of new therapeutic trials targeting glutamate, adenosine, and other relevant receptors to address issues of levodopa induced dyskinesia, on-off phenomenon, and wearing off. In addition to offering targets for therapy, developing imaging biomarkers for the non-dopamine aspects of basal ganglia

pathology is now possible. PET or SPECT imaging biomarkers can not only aid in clinical trials by helping to identify optimal dosing, but can also serve to provide additional insights into the nature of alterations in these systems. Specifically, it might be possible to directly assess hypothesized alterations based on current understanding of neural circuitry. The role of some of these targets is discussed below.

Glutamate

Glutamate is the primary excitatory neurotransmitter in brain and is, thus, is critically involved in many brain disorders, particularly those involving predominantly forebrain structures, such as cortex and striatum. Therefore, glutaminergic signaling is an attractive target of investigation of potential pharmaceuticals for disorders of cognition, mood, and movement. In Parkinson's disease in particular, cortical inputs onto medium spiny neurons express glutamate, hence, agents influencing glutaminergic function may have beneficial effects on both motor symptoms and cognition. However, direct interaction of drugs with the ionotropic glutamate receptors ion channels can lead to unacceptable side-effects, including hallucinations or other alterations in consciousness or possibly neurotoxicity. Therefore, interest has been focused on exploiting other mechanisms to modulate glutaminergic signaling. Examples include the development of drugs targeting metabotropic glutamate receptors (*e.g.* mGluR5) or altering endogenous small molecule modulators of glutamate signaling, such as glycine.[31]

Adenosine

Adenosine plays a prominent role throughout the basal ganglia, with high density expression of various adenosine receptors in regionally specific patterns.[32-35] Specifically, the adenosine receptor type A2a (A2aR) should be a particularly useful marker of striatal degeneration, *e.g.* in Huntington disease (HD), or of striatal denervation-related plasticity, *e.g.* in Parkinson disease (PD). The A2aR may best be known as a high affinity receptor for caffeine and a major site of its action.[36-39] In addition to its diagnostic and therapeutic potential for PD and HD, A2aR agents have neuroprotective effects in several neurodegenerative disease models.[40-45] Modulatory receptor systems, such as the A2aR, may detect functional changes in dopaminergic signalling and, thus, may be up- or down-regulated prior to frank loss of dopaminergic terminals.[46-48]

A2aR is of interest in PD research due its strong striatal localization and the prominent role the neurotransmitter adenosine plays in striatal function. Adenosine arises in the striatum from several different sources including vesicular neurotransmitter release and diffusion and efflux of end-products of cellular metabolism. Notably, A2aR antagonists have shown benefit in human PD clinical trials.[49-53] Furthermore, studies indicate that alterations in A2aR may underlie the pathophysiology of the development of motor fluctuations such as dyskinesia.[52, 54-57] Therefore, alteration in A2aR expression may signal early or impending motor dysfunction or vulnerability to motor complications.

Thus, quantitative in vivo measurement of striatal A2aR expression may detect very early PD or predict risk for development of later complications. Additionally, assessing two or more neuronal systems together may provide a more accurate and/or predictive measure of the PD disease process than assessing dopaminergic markers only.

Cannabinoid

The endocannabinoids are endogenous lipid signalling molecules that act through the cannabinoid receptors. These receptors are perhaps most widely known for mediating the diverse physiological effects of the exogenous cannabinoid, tetrahydrocannabinol or THC, found in marijuana.[58, 59] The endocannabinoids are arachidonate-based lipids, including anandamide (N-arachidonoylethanolamine or AEA) and 2-arachidonoylglycerol (2-AG). These act through two related receptors, CB1 and CB2, and perhaps additional 'novel cannabinoid receptors'. CB1 is expressed predominantly in many brain and spinal cord regions.[60-64] CB2 is expressed predominantly in immune cells, but also at a low level in brain.[65-69] THC is a partial agonist at both these receptors. Unlike classical neurotransmitters, endocannabinoids are not stored in neurons, but released 'on demand' after stimulation. The best known effect of the endocannadinoids relates to their prominent retrograde signalling capacity through CB1 in cortical and other grey matters areas. While widespread, the expression of CB1 is highest in cerebellum.[59, 70]

CB1 is a G protein-coupled receptor (GPCR) linked largely to Gi/o,[71-74] which inhibits the pro-

duction of cAMP, while also activating several MAP kinase signalling pathways, the PI-3-kinase pathways, and stimulating membrane vesicle translocation. Since CB1 is largely presynaptic, this receptor provides direct retrograde modulatory activity at the synapse. CB1 is the most abundant GPCR in the brain.[74-76] Similarly, CB2 appears to play a prominent modulatory role in immune and inflammatory functions.[65-67] In these capacities, both appear to play roles in modulating pain, particularly chronic pain adaptations. Due to its impact on numerous brain regions and signaling systems, CB1 has been proposed or investigated as a therapeutic target for may disease states and conditions, including pain, nausea, stress and anxiety, depression, metabolic syndrome, diabetes, hypoxia and stroke, obesity and appetite, memory enhancement, tinnitus, and others. Abnormalities in CB1 signalling have been found in degenerative diseases such as Parkinson's disease, Huntington's disease, and Alzheimer's disease. CB1 has even been found to influence neuronal survival and neurogenesis. Alteration on CB1 expression may be an early indicator of disease complications or progression in several neurodegenerative diseases or of underlying genetic predispositions to psychiatric dysfunction).[31, 77-81] However, the complexity and heterogeneity of the cannabinoid receptor system also probably underlies the failures thus far in CB1 and CB2 drug development, due to side effects and complex pharmacological relationships.[82]

Serotonin

Other monoaminergic systems contribute to the development and expression of PD motor fluctuations. Among these, serotonin (a.k.a. 5HT) and striatal serotonergic nerve terminals have been implicated in the development of PD motor fluctuations, and 5HT receptors are under investigation as targets for potential motor fluctuation therapies. There are seven classes of 5-HT receptors (5HT1-7), of which the 5HT1 receptor family serves as both presynaptic autoreceptors and post-synaptic inhibitory receptors. Abundant evidence implicates the 5HT1A receptor in the development and expression of levodopa-induced motor fluctuation in PD.

The 5HT1A receptor is widely expressed throughout many brain regions, with particularly dense expression in the raphe nucleus, hippocampus, and amygdala with lower but significant receptor binding activity also in cerebral cortex, thalamus and the striatum.[83, 84] White matter and the cerebellum are essentially devoid of 5HT1A. Very little 5HT1A mRNA is detected in striatum, suggesting that expression in these basal ganglia nuclei originates largely from neuron terminals.[85, 86] Primate studies demonstrate that dopaminergic denervation increases striatal 5HT1A.[87] Furthermore, 5HT1A is thought to contribute to the expression of dyskinesia after prolonged levodopa response, and drugs targeting 5HT1A can modulate dyskinesia.[88-98] Studies regarding the role of 5HT1A specifically in human PD are, however, limited.

Serotonin systems are also involved in non-motor symptoms in PD, such as depression. In particular, imaging with markers for the 5HT transporter have demonstrated changes in PD relevant to depressive symptomatology. Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures) in PD patients both with and without frank clinical depression using 11C-DASB PET.[99] Increases in these regions may be due to lower endogenous 5HT. Further, 5HT transporter binding in other brain regions were decreased relative to healthy controls in both depressed and non-depressed PD patients.

Conclusions

Dopaminergic degeneration provides a critical central focus to understanding the pathophysiology of PD and rationale for treatment. As our understanding of both the molecular mechanisms of alpha-synuclein deposition and its relation to neuronal cell loss, as well as the alterations in motor circuitry responsible for untoward dyskinesias, opportunities are available for novel treatments. New imaging targets represent a fresh way to assess potential alterations in these circuits as well as aid in the development of drugs binding to these targets.

References

1. Chaudhuri KR, Odin P, Antonini A, Martinez-Martin P. Parkinson's disease: The non-motor issues. Parkinsonism Relat Disord 2011;17:717-23.
2. Zoccolella S, Savarese M, Lamberti P, Manni R, Pacchetti C, Logroscino G. Sleep disorders and the natural history of Parkinson's disease: the contribution of epidemiological studies. Sleep Med Rev 2011;15:41-50.
3. Hornykiewicz O. Dopamine (3-hydroxytyramine) and brain function. Pharmacol Rev 1966;18:925-64.

4. Szabo N, Kincses ZT, Vecsei L. Novel therapy in Parkinson's disease: adenosine A(2A) receptor antagonists. Expert Opin Drug Metab Toxicol 2011;7:441-55.
5. Hornykiewicz O. Pharmacology and pathophysiology of dopaminergic neurons. Adv Cytopharmacol 1971;1:369-77.
6. Hornykiewicz O, Lisch HJ, Springer A. Homovanillic acid in different regions of the human brain: attempt at localizing central dopamine fibres. Brain Res 1968;11:662-71.
7. Hornykiewicz O. How L-DOPA was discovered as a drug for Parkinson's disease 40 years ago. Wien Klin Wochenschr 2001;113:855-62.
8. Lieberman A, Ranhosky A, Korts D. Clinical evaluation of pramipexole in advanced Parkinson's disease: results of a double-blind, placebo-controlled, parallel-group study. Neurology 1997;49: 162-8.
9. Gottwald MD, Bainbridge JL, Dowling GA, Aminoff MJ, Alldredge BK. New pharmacotherapy for Parkinson's disease. Ann Pharmacother 1997;31:1205-17.
10. Pramipexole vs levodopa as initial treatment for Parkinson disease: A randomized controlled trial. Parkinson Study Group JAMA 2000;284:1931-8.
11. Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B *et al.* [123I] beta-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. Neurology 1996;46:231-7.
12. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 2002;249(Suppl 3):III/1-5.
13. Braak H, Rüb U, Schultz C, Del Tredici K. Vulnerability of cortical neurons to Alzheimer's and Parkinson's diseases. J Alzheimers Dis 2006;9(3 Suppl):35-44.
14. Braak H, Del Tredici K. Invited Article: Nervous system pathology in sporadic Parkinson disease. Neurology 2008;70:1916-25.
15. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 2004;318:121-34.
16. Brooks DJ, Pavese N. Imaging non-motor aspects of Parkinson's disease. Prog Brain Res 2010;184:205-18.
17. Brooks DJ. Imaging dopamine transporters in Parkinson's disease. Biomark Med 2010;4:651-60.
18. Darcourt J, Booij J, Tatsch K, Varrone A, Vander Borght T, Kapucu OL *et al.* EANM procedure guidelines for brain neurotransmission SPECT using (123)I-labelled dopamine transporter ligands, version 2. Eur J Nucl Med Mol Imaging 2010;37:443-50.
19. Sossi V, Dinelle K, Schulzer M, Mak E, Doudet DJ, de la Fuente-Fernández R. Levodopa and pramipexole effects on presynaptic dopamine PET markers and estimated dopamine release. Eur J Nucl Med Mol Imaging 2010;37:2364-70.
20. Booij J, Kemp A, Dopamine transporter imaging with [(123)I] FP-CIT SPECT: potential effects of drugs. Eur J Nucl Med Mol Imaging 2008;35:424-38.
21. Booij J, Habraken JB, Bergmans P, Tissingh G, Winogrodzka A, Wolters EC *et al.* Imaging of dopamine transporters with iodine-123-FP-CIT SPECT in healthy controls and patients with Parkinson's disease. J Nucl Med 1998;39:1879-84.
22. Marek K, Jennings D. Can we image premotor Parkinson disease? Neurology 2009;72(7 Suppl):S21-6.
23. Booij J, Knol RJ. SPECT imaging of the dopaminergic system in (premotor) Parkinson's disease. Parkinsonism Relat Disord 2007;13(Suppl 3):S425-8.
24. Ishibashi K, Ishii K, Oda K, Mizusawa H, Ishiwata K. Competition between 11C-raclopride and endogenous dopamine in Parkinson's disease. Nucl Med Commun 2010;31:159-66.
25. Goerendt IK, Messa C, Lawrence AD, Grasby PM, Piccini P, Brooks DJ. Dopamine release during sequential finger movements in health and Parkinson's disease: a PET study. Brain 2003;126(Pt 2):312-25.
26. de la Fuente-Fernández R, Sossi V, Huang Z, Furtado S, Lu JQ, Calne DB *et al.* Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. Brain 2004;127(Pt 12):2747-54.
27. Turjanski N, Lees AJ, Brooks DJ. In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. Neurology 1997;49:717-23.
28. Loucif KC, Wilson CL, Baig R, Lacey MG, Stanford IM. Functional interconnectivity between the globus pallidus and the subthalamic nucleus in the mouse brain slice. J Physiol 2005;567(Pt 3):977-87.
29. Wilson CJ, Bevan MD. Intrinsic dynamics and synaptic inputs control the activity patterns of subthalamic nucleus neurons in health and in Parkinson's disease. Neuroscience 2011 [Epub ahead of print].
30. Hauber W. Involvement of basal ganglia transmitter systems in movement initiation. Prog Neurobiol 1998;56:507-40.
31. Benarroch EE. Metabotropic glutamate receptors: Synaptic modulators and therapeutic targets for neurologic disease. Neurology 2008;70:964-8.
32. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998;50:413-92.
33. Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 1999;59:355-96.
34. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001;24:31-55.
35. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S. Adenosine A(2A) receptors and basal ganglia physiology. Prog Neurobiol 2007;83:277-92
36. Jacobson KA, Nikodijević O, Padgett WL, Gallo-Rodriguez C, Maillard M, Daly JW. 8-(3-Chlorostyryl)caffeine (CSC) is a selective A2-adenosine antagonist in vitro and in vivo. FEBS Lett 1993;323:141-4.
37. Svenningsson P, Nomikos GG, Ongini E, Fredholm BB. Antagonism of adenosine A2A receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate-putamen and nucleus accumbens. Neuroscience 1997;79:753-64.
38. Cauli O, Morelli M. Caffeine and the dopaminergic system. Behav Pharmacol 2005;16:63-77.
39. Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. Journal of Neurochemistry 2008;105:1067-79.
40. Chen JF, Sonsalla PK, Pedata F, Melani A, Domenici MR, Popoli P *et al.* Adenosine A2A receptors and brain injury: Broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. Prog Neurobiol 2007;83:310-31.
41. Popoli P, Blum D, Domenici MR, Burnouf S, Chern Y. A critical evaluation of adenosine A2A receptors as potentially "druggable" targets in Huntington's disease. Curr Pharm Des 2008;14:1500-11.
42. Blum D, Hourez R, Galas MC, Popoli P, Schiffmann SN. Adenosine receptors and Huntington's disease: implications for pathogenesis and therapeutics. Lancet Neurol 2003;2:366-74.
43. Alfinito PD, Wang SP, Manzino L, Rijhsinghani S, Zeevalk GD, Sonsalla PK. Adenosinergic protection of dopaminergic and GABAergic neurons against mitochondrial inhibition through receptors located in the substantia nigra and striatum, respectively. J Neurosci 2003;23:10982-7.
44. Scattoni ML, Valanzano A, Pezzola A, March ZD, Fusco FR, Popoli P *et al.* Adenosine A2A receptor blockade before striatal excitotoxic lesions prevents long term behavioural disturbances in the quinolinic rat model of Huntington's disease. Behav Brain Res 2007;176:216-21.
45. Chou SY, Lee YC, Chen HM, Chiang MC, Lai HL, Chang HH *et al.* CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. J Neurochem 2005;93:310-20.
46. Pinna A, Corsi C, Carta AR, Valentini V, Pedata F, Morelli M. Modification of adenosine extracellular levels and adenosine

A(2A) receptor mRNA by dopamine denervation. Eur J Pharmacol 2002;446:75-82.
47. Hurley MJ, Mash DC, Jenner A. Adenosine A(2A) receptor mRNA expression in Parkinson's disease. Neurosci Lett 2000;291:54-8.
48. Mishina M, Ishiwata K, Kimura Y, Naganawa M, Oda K, Kobayashi S *et al*. Evaluation of distribution of adenosine A(2A) receptors in normal human brain measured with [(11)C]TMSX PET. Synapse 2007;61:778-84.
49. LeWitt PA, Guttman M, Tetrud JW, Tuite PJ, Mori A, Chaikin P *et al*. Adenosine A2A receptor antagonist istradefylline (KW-6002) reduces ldquooffrdquo time in Parkinson's disease: A double-blind, randomized, multicenter clinical trial (6002-US-005). Ann Neurol 2008;63:295-302.
50. Hauser RA, Shulman LM, Trugman JM, Roberts JW, Mori A, Ballerini R *et al*. Study of istradefylline in patients with Parkinson's disease on levodopa with motor fluctuations. Movement Disorders 2008;23:2177-85.
51. Hauser RA. Randomized trial of the adenosine A2A receptor antagonist istradefylline in advanced PD. Neurology 2003;61:297-303.
52. Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M *et al*. Adenosine A2A receptor antagonist treatment of Parkinson's disease. Neurology 2003;61:293-6.
53. Stacy M, Silver D, Mendis T, Sutton J, Mori A, Chaikin P *et al*. A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson disease. Neurology 2008;70:2233-40.
54. Calon F, Dridi M, Hornykiewicz O, Bédard PJ, Rajput AH, Di Paolo T. Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. Brain 2004;127(Pt 5):1075-84.
55. Xiao D, Bastia E, Xu YH, Benn CL, Cha JH, Peterson TS *et al*. Forebrain adenosine A2A receptors contribute to L-3,4-dihydroxyphenylalanine-Induced dyskinesia in hemiparkinsonian mice. J Neurosci 2006;26:13548-55.
56. Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA. Role of adenosine A2A receptors in parkinsonian motor impairment and l-DOPA-induced motor complications. Progress Neurobiol 2007;83:293-309.
57. Chen JF, Fredduzzi S, Bastia E, Yu L, Moratalla R, Ongini E, Schwarzschild MA. Adenosine A2A receptors in neuroadaptation to repeated dopaminergic stimulation: implications for the treatment of dyskinesias in Parkinson's disease. Neurology 2003;61(11 Suppl 6):S74-81.
58. Elkashef A, Vocci F, Huestis M, Haney M, Budney A, Gruber A *et al*. Marijuana neurobiology and treatment. Subst Abus 2008;29:17-29.
59. Herkenham M. Cannabinoid receptor localization in brain. Ann N Y Acad Sci 1990;87:1932-6.
60. Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacology & Therapeutics 1997;74:129-80.
61. Compton DR, Aceto MD, Lowe J, Martin BR. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. Journal of Pharmacology and Experimental Therapeutics 1996;277:586-94.
62. Gérard CM, Mollereau C, Vassart G, Parmentier M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. Biochem J 1991;279(Pt 1):129-34.
63. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561-4.
64. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O *et al*. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Molecular Pharmacology 1995;48:443-50.
65. Cabral GA, Griffin-Thomas L. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. Expert Rev Mol Med 2009;11(-1).
66. Lu D, Vemuri VK, Duclos RI Jr, Makriyannis A. The Cannabinergic System as a Target for Anti-inflammatory Therapies. Curr Top Med Chem 2006;6:1401-26.
67. Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A *et al*. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. Brain Res 2006;1071:10-23.
68. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61-5.
69. Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessègue B, Bonnin-Cabanne O *et al*. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1996;1307:132-6.
70. Glass M, Faull RLM, Dragunow M. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 1997;77:299-318.
71. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 2009;89:309-80.
72. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58:389-462.
73. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 2003;83:1017-66.
74. Hashimotodani Y, Ohno-Shosaku T, Kano M. Endocannabinoids and synaptic function in the CNS. Neuroscientist 2007;13:127-37.
75. Piomelli D, Beltramo M, Giuffrida A, Stella N. Endogenous cannabinoid signaling. Neurobiol Dis 1998;5:462-73.
76. Mackie K, Stella N. Cannabinoid receptors and endocannabinoids: evidence for new players. AAPS J 2006;8:E298-306.
77. Kreitzer AC, Malenka RC. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 2007;445:643-7.
78. Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Hunyady L *et al*. Promoter variants of the cannabinoid receptor 1 gene (CNR1) in interaction with 5-HTTLPR affect the anxious phenotype. Am J Med Genet B Neuropsychiatr Genet 2009;150B:1118-27.
79. Onaivi ES, Hari Shanker S. Cannabinoid receptors in brain: pharmacogenetics, neuropharmacology, neurotoxicology, and potential therapeutic applications, in international review of neurobiology. 2009, Academic Press. p. 335-69.
80. Urigüen L, García-Fuster MJ, Callado LF, Morentin B, La Harpe R, Casadó V *et al*. Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. Psychopharmacology 2009;206:313-24.
81. Fernández-Ruiz J. The endocannabinoid system as a target for the treatment of motor dysfunction. Br J Pharmacol 2009;156:1029-40.
82. Butler H, Korbonits M. Cannabinoids for clinicians: the rise and fall of the cannabinoid antagonists. Eur J Endocrinol 2009;161:655-62.
83. Dillon KA, Gross-Isseroff R, Israeli M, Biegon A. Autoradiographic analysis of serotonin 5-HT1A receptor binding in the human brain postmortem: effects of age and alcohol. Brain Res 1991;554:56-64.
84. Marazziti D, Marracci S, Palego L, Rotondo A, Mazzanti C, Nardi I *et al*. Localization and gene expression of serotonin1A (5HT1A) receptors in human brain postmortem. Brain Res 1994;658:55-9.
85. Pasqualetti M, Nardi I, Ladinsky H, Marazziti D, Cassano GB. Comparative anatomical distribution of serotonin 1A 1D alpha and 2A receptor mRNAs in human brain postmortem. Brain Res Mol Brain Res 1996;39:223-33.
86. Burnet PW, Eastwood SL, Lacey K, Harrison PJ. The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Research 1995;676:157-68.
87. Frechilla D, Cobreros A, Saldise L, Moratalla R, Insausti R, Luquin M *et al*. Serotonin 5-HT1A receptor expression is selectively en-

hanced in the striosomal compartment of chronic parkinsonian monkeys. Synapse 2001;39:288-96.
88. Muñoz A, Li Q, Gardoni F, Marcello E, Qin C, Carlsson T *et al.* Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of l-DOPA-induced dyskinesia. Brain 2008;131:3380-94.
89. Bibbiani F, Oh JD, Chase TN. Serotonin 5-HT1A agonist improves motor complications in rodent and primate parkinsonian models. Neurology 2001;57:1829-34.
90. Kannari K, Yamato H, Shen H, Tomiyama M, Suda T, Matsunaga M. Activation of 5-HT1A but not 5-HT1B receptors attenuates an increase in extracellular dopamine derived from exogenously administered l-DOPA in the striatum with nigrostriatal denervation. J Neurochem 2001;76:1346-53.
91. Lane EL, Cheetham S, Jenner D. Dopamine uptake inhibitor-induced rotation in 6-hydroxydopamine-lesioned rats involves both D1 and D2 receptors but is modulated through 5-hydroxytryptamine and noradrenaline receptors. J Pharmacol Experiment Therapeut 2005;312:1124-31.
92. Politis M, Wu K, Loane C, Quinn NP, Brooks DJ, Rehncrona S *et al.* Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. Sci Transl Med 2010;2:38ra46.
93. Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verrièle L, Carpentier N *et al.* Differential Actions of Antiparkinson Agents at Multiple Classes of Monoaminergic Receptor. III. Agonist and Antagonist Properties at Serotonin, 5-HT1 and 5-HT2, Receptor Subtypes. J Pharmacol Exp Ther 2002;303:815-22.
94. Iravani MM, Tayarani-Binazir K, Chu WB, Jackson MJ, Jenner P. In 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Treated Primates, the Selective 5-Hydroxytryptamine 1a Agonist (R)-(+)-8-OHDPAT Inhibits Levodopa-Induced Dyskinesia but Only with\ Increased Motor Disability. J Pharmacol and Exp Ther 2006;319:1225-34.
95. Durif F, Debilly B, Galitzky M, Morand D, Viallet F, Borg M *et al.* Clozapine improves dyskinesias in Parkinson disease. Neurology 2004;62:381-8.
96. Carta M. Serotonin-dopamine interaction in the induction and maintenance of L-DOPA-induced dyskinesias. Progress in Brain Research. Amsterdam: Elsevier; 2008. p. 465-78.
97. Fabbrini G, Brotchie JM, Grandas F, Nomoto M, Goetz CG. Levodopa-induced dyskinesias. Mov Disord 2007;22:1379-89.
98. Bara-Jimenez W, Bibbiani F, Morris MJ, Dimitrova T, Sherzai A, Mouradian MM *et al.* Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. Mov Disord 2005;20:932-6.
99. Politis M, Wu K, Loane C, Turkheimer FE, Molloy S, Brooks DJ, Piccini P. Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. Neurology 2010;75:1920-7.