UNIVERSITÀ DEGLI STUDI DI MILANO

SCUOLA DI DOTTORATO IN FISIOLOGIA UMANA

DIPARTIMENTO DI FISIOPATOLOGIA E DEI TRAPIANTI

CORSO DI DOTTORATO XXV

DEGREE THESIS

A role for locus coeruleus in Parkinson tremor – Experimental studies

SETTORE SCIENTIFICO DISCIPLINARE: BIO09

PhD candidate:
Dr. Ioannis Ugo Isaias

Tutor and coordinator:
Prof. Paolo Cavallari

A. A. 2012

My deepest gratitude and thanks to:

Prof. Gianni Pezzoli
Centro per la Malattia di Parkinson e i Disturbi del Movimento, C.T.O., I.C.P., Milano

Prof. Paolo Crenna
Laboratorio per l'Analisi del Movimento nel Bambino P. & L. Mariani, Dipartimento di Fisiopatologia e dei Trapianti, Università degli Studi di Milano, Milano

Dr. Alberto Marzegan
Laboratorio per l'Analisi del Movimento nel Bambino P. & L. Mariani, Dipartimento di Fisiopatologia e dei Trapianti, Università degli Studi di Milano, Milano

Prof. Jens Volkmann
Neurologische Klinik und Poliklinik, Universitätsklinikum Würzburg, Würzburg

Dr. Giorgio Marotta
Dipartimento di Medicina Nucleare, Fondazione I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Milano

Prof. Carlo Albino Frigo
Laboratorio di Tecnologie Biomediche, Dipartimento di Bioingegneria, Politecnico di Milano, Milano

Prof. Gabriele Biella
Istituto di Bioimmagini e Fisiologia Molecolare, Milano

Dr. Antonella Costa and Dr. Paul Summers
Dipartimento di Neuroradiologia, Fondazione I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Milano

Fondazione Grigioni per la malattia di Parkinson

I.U.I.

CUMULATIVE PhD DEGREE THESIS IN HUMAN PHYSIOLOGY

A role for locus coeruleus in Parkinson tremor – Experimental studies

Abstract

Background

Hypothesis statement and aims

Completed research activities

- Isaias IU, Marotta G, Pezzoli G, et al. Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC Neurology 2011;11:88.*
- Isaias IU, Marzegan A, Pezzoli G, et al. A role for locus coeruleus in Parkinson tremor. *Front Hum Neurosci. 2011;5:179.*

On-going research activities

- Evidence of a role of LC-NAergic system in Parkinson tremor: a reserpine rat model study.
- Correlations between iron content in the locus coeruleus and substantia nigra with dopaminergic striatal innervation in subjects with Parkinson disease.

Summary and future directions

References

Appendix – More publications published during PhD years

- Isaias IU, Volkmann J, Marzegan A, et al. The influence of dopaminergic striatal innervation on upper limb locomotor synergies. *PLoS ONE 2012;7:e51464*
- Isaias IU, Marotta G, Osama S and Hesse S. [^{123}I]FP-CIT and SPECT in atypical parkinsonism. *Imaging Med 2012;4:411-421.*
- Isaias IU, Moisello C, Marotta G, et al. Dopaminergic striatal innervation predicts interlimb transfer of a visuomotor skill. *J. Neurosci 2011;31:14458-62.*

Abbreviations

AR – adrenergic receptors
BDNF – brain derived neurotrophic factor
BG – basal ganglia
CB – cerebellum
DA – dopamine
DAergic – dopaminergic
DAT – dopamine reuptake transporters
DBH – dopamine β-hydroxylase
DSP-4 – N-(2-chloroethyl)-N- ethyl-2-bromobenzylamine
FP-CIT – [^{123}I]N-ω- fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane
LC – locus coeruleus
MAO – monoamine oxidase
MC – motor cortex
MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NA – noradrenaline
NET – noradrenaline reuptake transporter
NAergic – noradrenergic
PD – Parkinson disease
SN – substantia nigra
SNc – substantia nigra pars compacta
SPECT – single photon computed tomography (SPECT) imaging
TH – tyrosine hydroxylase
VIM – thalamus vetro-intermedio-lateralis
VMAT – vesicular monoamine transporter
VTA – ventral tegmental area
6-OHDA – 6-hydroxydopamine

ABSTRACT

Although Parkinson disease (PD) is characterized by the degeneration of nigrostriatal dopamine (DA) neurons, historic and more recent anatomopathological studies documented also an involvement of the serotonergic and cholinergic systems as well as a profound loss of neurons from the locus coeruleus (LC), the major noradrenergic (NAergic) nucleus in the brain.
In the following studies, I will provide preliminary evidence of a new provocative hypothesis on the significance of LC in conditioning Parkinson tremor. In particular, I speculate that, early at a disease stage, patients with PD and tremor might have an (hyper-)active LC-NAergic system, which would play a key role in the appearance of tremor itself. Furthermore, given a putative compensatory and possibly neuroprotective mechanism of noradrenaline (NA), an intact or hyper-active NAergic system would be responsible for, and support the clinical observation of, a slower disease progression in PD patients with tremor. When verified, this hypothesis will define, for the first time at a physio-pathological level, two different clinical phenotypes (i.e. tremor dominant and akinetic-rigid PD) and possibly suggest new interventional strategies (targeting the NAergic system) to modify disease progression.
A number of drugs that can modulate the NAergic system already exist, ripe for testing. There is no cure for PD, and understanding the cause and progression of the neurodegenerative process is as challenging as it is necessary.

BACKGROUND

Parkinson disease

Parkinson disease is a chronic, progressive, neurodegenerative disorder characterized by pathologic intraneuronal α-synuclein-positive Lewy bodies and neuronal cell loss. This process has been described as involving the dopaminergic (DAergic) cells of the substantia nigra (SN) pars compacta, later becoming more widespread in the central nervous system (CNS) as disease progresses. Recently, there has been a growing awareness that the disease progression may involve more caudal portions of the CNS and a peripheral nervous system prior to the clinical onset of the disease (Braak et al., 2003).

The prevalence of PD steadily increases with age, affecting about 1% to 2% of the population older than 65 years, and over 3% of those older than 85 years (de Lau et al., 2006). Most age-adjusted prevalence rates are reported to be between 100 and 200 cases per 100,000. Estimates of the incidence of PD are more variable. Age is the strongest risk factor for PD. Interestingly, a recent large prospective study found that incidence rates rise steeply through age 89; then lifetime plateaus after age 90 (Driver et al., 2009). The incidence of PD has been reported to be higher in man than women, but only among patients older than 60 years (Taylor et al., 2007).

The primary physio-pathological substrate of PD involves dopamine depletion in the striatum (Kish et al., 1988). The loss of greater than 50–60% of the DAergic neurons, primarily in the lateral ventral tier of the substantia nigra pars compacta (SNc), results in a marked reduction in dopamine concentrations (70–80%) in the striatum (mainly in the putamen). This disrupts corticostriatal processing and explains clinical symptoms such as rigidity and bradykinesia, but not resting tremor (see later; Pirker et al., 2003; Isaias et al., 2007). Of relevance, it is worth resaying that motor signs do not become apparent until ≈80% of the DA terminals have been lost, which suggests the existence of an impressive compensatory mechanism in the earlier stage (pre-symptomatic) of disease (Morrish et al., 1998; Marek et al., 2001).

At a clinical level, PD is a syndrome characterized by resting tremor, slowness of movements (bradykinesia), rigidity and postural instability. Usually, these features present asymmetrically. The contralateral side is eventually affected, but the asymmetry persists throughout the disease course. By using the term *shaking palsy*, James Parkinson in his *An Essay on the Shaking Palsy* (1817) drew attention to tremor as a characteristic feature of PD. Parkinson tremor is defined as a rhythmic, oscillatory, involuntary movement. It is the initial symptom in 50% to 70% of patients. The resting tremor of PD has typically low frequency (4 Hz to 6 Hz) and it is characterized by a pill-rolling (supination-pronation) movement. It commonly affects the distal upper extremities but may also involve the lower extremities and face, with the chin tremor being particularly specific for PD. Some patients with PD complain of an internal, not visible, tremor, called inner tremor. Parkinson tremor is remarkable for several features: (1) it is neither a consistent nor a homogeneous feature across patients or within an individual patient's disease course; (2) it may diminish or disappear in the end-stage of PD; (3) it occurs predominantly at rest and is reduced or disappears by action; (4) it increases in amplitude or can be triggered by maneuvers such as walking or psychological states as anxiety or stress (specific tasks, like simple arithmetic calculation, may induce stress-related tremor) (Deuschl et al., 1998); (5) it is not present during sleep; (6) it may be the predominant or the only clinical sign for years before the appearance of akinesia (see also Brooks et al., 1992); (7) it is typically refractory to medications. Of relevance, a

significant proportion of patients with PD never develops tremor. There is evidence that Parkinson tremor is associated with a distinct cerebellothalamic circuit (Fukuda et al., 2004; Timmermann et al., 2003) involving the ventral intermediate nucleus of the thalamus (VIM), motor cortex (MC), and cerebellum (CB). Tremor-related responses have been observed both in the basal ganglia (BG), (i.e. pallidus [Hurtado et al., 1999] and subthalamic nucleus [Raz et al., 2000; Levy et al., 2000]) and in the cerebello-thalamic circuit (Lenz et al., 1994). Interference (e.g. by means of deep brain stimulation) with either circuit can effectively suppress resting tremor (Benabid et al., 1991; Krack et al., 1997; Lozano et al., 1995). This suggests that resting tremor results from a pathological interaction between the BG and the CB-VIM-CM circuit. In particular, it has been suggested that the BG have a modulatory role in tremor genesis, whereas the CB-VIM-CM circuit drives the tremor on a cycle- by-cycle basis (Fukuda et al., 2004; Timmermann et al., 2003). However, to date it remains unknown how BG dysfunction in PD can drive the distinct CB-VIM-CM circuit into generating resting tremor (Rodriguez-Oroz et al., 2009). Indeed, despite converging evidence of independent oscillating circuits within a widespread "tremor-generating network" (Mure et al., 2011), there is no conclusive explanation for the onset of tremor in what it is fundamentally a hypokinetic movement disorder. Moreover, the simple interplay between BG and CB-VIM-CM circuit would not explain many of the peculiar features of tremor in patients with PD, in particular its intermittent appearance (see *Hypothesis statement*). Interestingly, the presence of tremor as the initial symptom often confers a favorable prognosis with slower progression of the disease, and some have suggested the term "benign tremulous parkinsonism" for a subset of patients with minimal progression, frequent family history of tremor, and poor response to DAergic drugs (Brooks et al., 1992; Ghaemi et al., 2002; Marshall et al., 2003, Josephs et al., 2006; O'Suilleabhain 2006). Although rest tremor is a well-recognized cardinal feature of PD, many PD patients have a postural tremor that is more prominent and disabling than the classic rest tremor. In addition to the rest and postural tremors, a kinetic tremor, possibly related to enhanced physiologic tremor, may also impair normal reach-to-grasp movement (Wenzelburger et al., 2000).

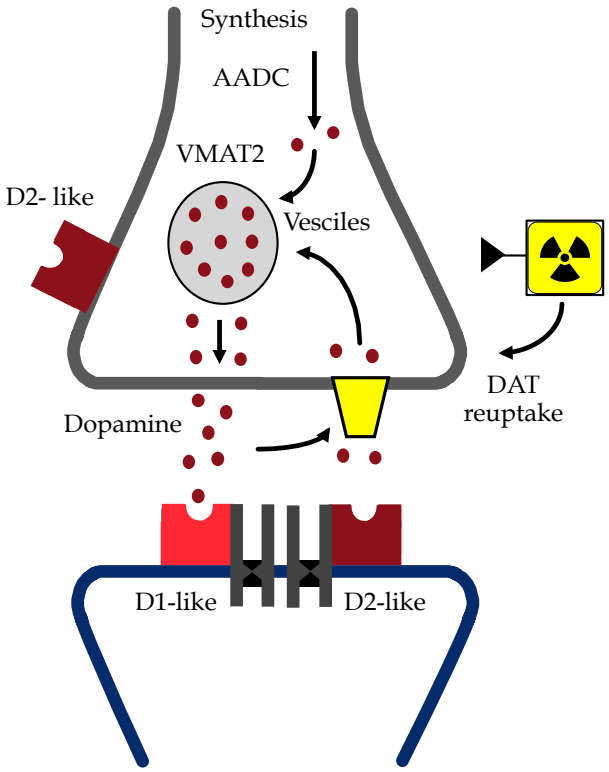
Bradykinesia may be initially manifested by slowness in activities of daily living or lack of movements (Cooper et al., 1994; Touge et al., 1995; Giovannoni et al., 1999; Jankovic et al., 1999). Patients with bradykinesia experience difficulty maintaining the velocity and amplitude of movement. In addition, to whole-body slowness and impairment of fine motor movement, other manifestations of bradykinesia include micrographia (hand writing getting smaller while writing), hypophonia (quite monotone speech), hypomimia (loss of facial expression and decreased blink rate), drooling due to failure to swallow saliva (Bagheri et al., 1999) and reduced armswing when walking (loss of automatic movement) (see also Isaias et al., 2012, in *Appendix*). The pathophysiology of bradykinesia is not well understood, but it is thought to result from failure of BG output to reinforce the cortical mechanisms that prepare and execute the commands to move (from Jankovic, 2003). In recordings from single cortical neurons in free-moving rats, a decrease in firing rate correlated with haloperidol-induced bradykinesia, demonstrating that reduced dopamine action impairs the ability to generate movement and cause bradykinesia (Parr-Brownlie and Hyland, 2005). The pre-movement EEG potential (Bereitschaftspotential) is reduced in patients with PD, probably reflecting inadequate BG activation of the supplementary motor area (Dick et al., 1989). On the basis of electromyographic (EMG) recordings in the antagonistic muscles of PD subjects during a brief ballistic elbow flexion, Hallett and Khoshbin (1980) concluded that the most characteristic feature of bradykinesia was the inability to energize the appropriate muscles to provide a sufficient rate of force required for the initiation and maintenance of a large, fast

(ballistic) movement. Secondary factors that may contribute to bradykinesia include muscle weakness and rigidity.
Rigidity, tested by passively flexing, extending, and rotating the body part, is manifested by increased resistance throughout the range of movement. At physical examination, cogwheeling is often encountered, particularly if there is associated tremor or an underlying, not yet visible, tremor. Rigidity may occur proximally (e.g., neck, shoulders, and hips) and distally (e.g., wrists and ankles). Symptomatically, rigidity is experienced as stiffness and can present as musculoskeletal concerns, such as frozen shoulder. On examination, resistance is consistent throughout the range of movement in all directions and is not velocity dependent.
Postural instability, and gait disturbance, are less prominent in early PD and are rarely presenting symptoms. Indeed, in a recent study aimed to investigate the role of DAergic striatal innervation on upper limb locomotor synergies, we found no difference for lower limbs spatio-temporal gait parameters (i.e. stride length, stride time and stance) between PD patients at an early disease stage and healthy controls when walking at preferred gait speed (Isaias et al., 2012 in *Appendix*). Later in the course of PD, however, gait problems (such as loss of postural reflexes, festination, freezing, and more severe postural changes) can be severe and become the major source of disability. Festination is the feeling of the feet wanting to rush forward, thus the patient experiencing hastening of gait. Freezing of gait is an inability to take effective steps. Patients will describe their feet feeling "stuck to the floor". Freezing of gait typically occurs with gait initiation, turning, and passing through narrow spaces. Gait disturbance is disabling and dangerous problem for patients, commonly leading to falls and injuries.
Several other symptoms (e.g. urinary incontinence, visual hallucinations, psychosis, delusions, dementia, depression, anxiety, impulse control disorders, sleep maintenance, etc.) may accompany the clinical spectrum of patients with PD late at disease stage. Such a clinical heterogeneity in PD patients suggests different clinical-pathologic entities as a possible result of a variable involvement of several neurotransmitter systems (from Fahn and Jankovich, 2007; see also *Hypothesis statement*).
In our studies, we carefully selected patients with PD to represent a putative *in vivo* model of striatal DAergic innervation loss, thus allowing to selectively investigate this pathway and its implication in locomotion (Isaias et al., 2012 in *Appendix*) or motor learning (Isaias et al., 2011 in *Appendix*). To do so, beside a detailed neurological investigation, personally performed, several clinical inclusion criteria were applied to exclude in particular for psychiatric disorders and cognitive decline (see also articles in *Appendix*). The clinical examination remains the standard for the diagnosis of idiopathic PD. Still, the accuracy of diagnosis by general neurologists reaches 70% and by movement disorders specialists approaches 90% (Hughes et al., 2002). In support, brain-imaging studies may be performed (see below).
PD has historically been considered predominantly a sporadic disorder, even though about 10% to 15% of PD patients have familial genetic defects (Lee et al., 2006). Several genes causing familial forms of PD have been discovered in the last decade, providing important insights into the pathogenesis of PD. In 1997, pathogenic point mutations in the α-synuclein gene were found in some PD lineages, and α-synuclein was subsequently identified as a major component in the Lewy bodies that are common in sporadic PD cases (Polymeropoulos et al., 1997; Spillantini et al., 1997). PARK1 and PARK4 (PARK is a nomenclature used for genetic types of PD originally identified by linkage analysis) are caused by mutation (PARK1) or duplication/triplication (PARK4) of the α-synuclein gene (*SNCA*) and are estimated to account for approximately 2% of autosomal dominant PD (Lesage and Brice, 2009). While the normal function of α-synuclein is not well understood, α-

synuclein does have the ability to form insoluble aggregates that may disrupt DAergic transmission, synaptic vesicles, intracellular trafficking, and protein degradation (Lee et al., 2006). Many groups have attempted to investigate the function of normal and mutant α-synuclein by studying genetic mouse models. Recent studies involving both α-synuclein knockout mice and mice expressing the human pathogenic A30P α-synuclein mutation have shown that α-synuclein alters storage and metabolism of NA in addition to DA (Yavich et al., 2006). This suggests that the neuroprotective effect of NA may be reduced in PD patients with α-synuclein mutations (see later), making them less resilient in the event of DAergic neuron degeneration. Indeed, patients with *SNCA* mutations tend to have an earlier age at onset with frequent cognitive decline; additional cortical features not commonly seen in PD, such as aphasia, have been reported. Moreover, these patients have a less-robust response to DAergic therapy, further suggesting at a clinical level an additional, and possibly more prominent, involvement of NAergic system than the DAergic one.
Other familial forms of PD include mutations in genes related to protein degradation. PARK2 is the most common type of autosomal recessive PD and is caused by mutation in the *parkin* gene, encoding for E3 ubiquitin-protein ligase, which normally functions to properly target proteins for proteosomal degradation. PARK2 is a prototypic cause of early-onset PD and may account for up to 50% of familial early-onset PD and about 20% of apparent sporadic early-onset PD (Lücking et al., 2000). These patients tend to have a high incidence of dystonic signs, especially affecting the lower extremities. They have a robust response to levodopa, even though a relatively short delay occurs before they develop motor fluctuations. A role for NA in *parkin*-related PD is supported by preliminary evidence that *parkin* knockout mice experience a distinct loss of LC neurons (von Coelln et al., 2004). Several other genes involved in the onset of PD have been identified but their relevance to the NAergic system has not been investigated yet.

In vivo imaging of dopaminergic striatal innervation

Several radiotracers for Positron emission tomography (PET) and Single photon emission computed tomography (SPECT) have been successfully developed to assess the integrity of presynaptic DAergic neurons end postsynaptic receptors in humans (figure below). Above all, SPECT with FP-CIT ([^{123}I]N-ω- fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane) was shown to be very sensitive to detect loss of striatal DATs (particularly in the putamen) in early PD (for review Isaias and Antonini, 2010), and recent studies even suggest that DAT imaging may be able to detect nigrostriatal DAergic degeneration also in preclinical cases (Ponsen et al., 2004). Registration studies for FP-CIT were started in 1996. In 1998 and 1999, the results of phase-I and -II studies were published, respectively (from Booij et al., 1998; Booij et al., 1999), followed by multicenter phase-III and -IV studies in 2000 and 2004 (Benamer et al., 2000; Catafau et al., 2004). In 2000, FP-CIT was licensed as DaTSCAN™ in Europe to differentiate patients with a parkinsonian syndrome (i.e. PD, multiple system atrophy, or progressive supranuclear palsy) from essential tremor (ET). In clinical practice, DAT imaging by means of SPECT and FP-CIT has the capability to discriminate PD patients with DAergic cell loss from those with other forms of Parkinsonism not characterized by loss of presynaptic DAergic cells (e.g., psychogenic parkinsonism and drug-induced postsynaptic parkinsonism) (Isaias and Antonini, 2010). On the contrary, SPECT and FP-CIT is not a valid tool to discriminate among neurodegenerative parkinsonism (Isaias et al., 2012 *in Appendix*). In our studies, besides supporting the clinical diagnosis, DAT imaging provided a measurement of DAergic striatal loss thus allowing correlations with behavioral and biomechanical measurements (see Isaias et al., 2011 and 2012, in *Appendix*). In these studies, SPECT imaging was performed in collaboration with Dr. Giorgio Marotta and the Department of Nuclear Medicine of the Ospedale Maggiore Policlinico (Milano). For imaging acquisition and reconstruction we have always applied similar methods. Briefly, intravenous administration of 110–140 MBq of FP-CIT was performed 30–40 minutes after thyroid blockade (10–15 mg of Lugol oral solution) in all patients. Brain SPECT was performed 3 hours later by means of a dedicated triple detector gamma-camera (Prism 3000, Philips, Eindhoven, the Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (4 subsets of acquisitions, matrix size 128 × 128, radius of rotation 12.9–13.9 cm, continuous rotation, angular sampling: 3 degree, duration: 28 minutes). Brain sections were reconstructed with an iterative algorithm (OSEM, 4 iterations and 15 subsets), followed by 3D filtering of sections obtained (Butterworth, order 5, cut-off 0.31 pixel-1) and attenuation correction (Chang method, factor 0.12). Data analysis differed according to the aim of the study (see *Appendix*).



Pre-synaptic PET ligands
F-18 DOPA
C-11 DTBZ
C-11 Cocaine
C-11 FE-CIT

Pre-synaptic SPECT ligands
I-123 ß-CIT
I-123 FP-CIT
I-123 IPT
I-123 Altropane
Tc-99m TRODAT-1

PET ligands for D_1
C-11 Sch 23390
C-11 NNC112

PET ligands for D_2
C-11 Raclopride
F-18 Fallypride

SPET ligands for D_2
I-123 IBZM
I-123 Lisuride
I-123 IBF

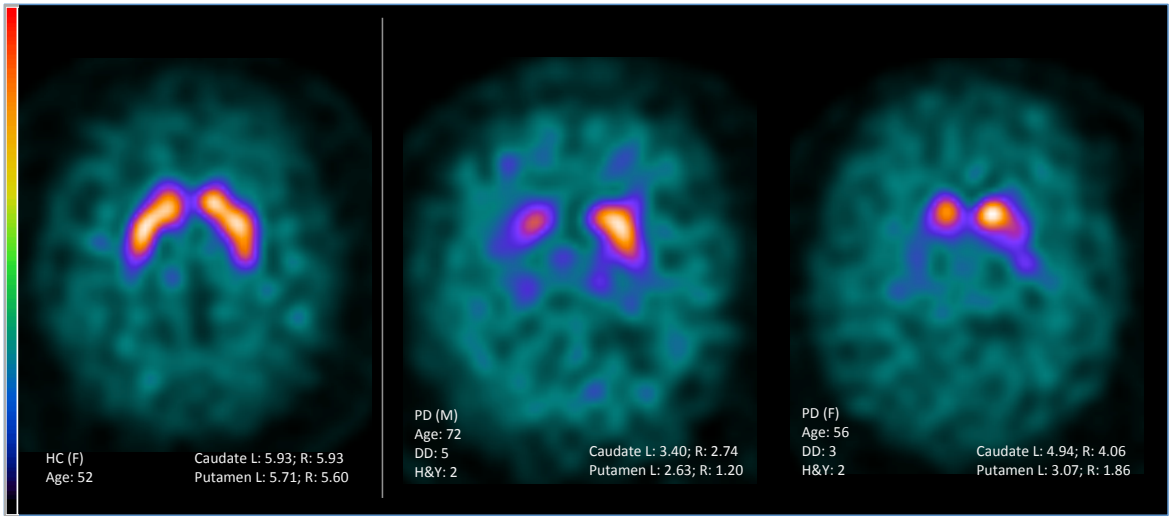


Figure as in Isaias et al., Future Neurology 2012 *(in appendix)*. FP-CIT SPECT images and binding values of one healthy subjects (HC, left in the picture) and two patients with PD. Please note the great asymmetry of the two hemispheres with regards to binding values and the greater involvement of the putamen than the caudate nucleus in both patients.

The locus coeruleus and its relevance to Parkinson disease

The first description of the LC by Reil dates from 1809 (Reil, 1809). Wenzel and Wenzel (1812) first used the term "locus coeruleus". In 1909, Jacobsohn described the presence in the cell bodies of the LC of melanin granules (Jacobsohn, 1909). In 1959, the highest activity of monoamine oxidase (MAO) in the brain was demonstrated in neurons of the rodent LC using enzyme histochemistry (Shimizu et al., 1959). In 1970s, NAergic projections from the LC were identified (Fuxe et al., 1970; Ungerstedt et al., 1971).

The neurons of the LC form a distinct, compact cell group largely contained within the central gray of the isthmus, medial to the mesencephalic nucleus of the trigeminal nerve (Russel, 1955). Due to the very small size of the LC, biochemical studies are unable to differentiate LC nucleus vs. surrounding areas. In humans, the LC has a rostrocaudal extension of approximately 16 mm (Broadal, 1981; German et al., 1988); it begins slightly rostral to the main trigeminal nucleus and extends rostrally as far as the level of the mesencephalic trigeminal nucleus. The nucleus is a tube-like shape, and it consists of two kinds of neurons. The major cell type in the LC is the medium-sized neuron which possess a multipolar arborization and contains neuromelanin (NM) granules; intermingled with these neurons; a second group of cells consist of smaller non-catecholamine neurons with various shape and dendritic arborization (Olszewski et al., 1954; Patt et al., 1993). The cell density is highest in the caudal portion of the nucleus and decreases rostrally. The LC cells have been quantified by different researchers in different animal species both by measuring the NM content and by using immunocytochemistry for the enzyme tyrosine hydroxylase (TH). These studies measured about 19,000 NA neurons in the LC nucleus *sensu stricto* (Vijayashankar et al., 1979) and 60,000 NA neurons in the LC (LC *sensu stricto* with pericoerulear NA nuclei) of young adults, which decrease to 40,000 in normal elderly subjects (Iversen et al., 1983; Baker et al., 1989).

Many retrograde and anterograde tract tracing studies over the years, that have been well recapitulated by Aston-Jones (2004), Samuels and Szabadi (2008a,b) and Benarroch (2009), have demonstrated the numerous afferent and efferent connections of LC neurons.

The LC is the major source of NA in the brain, with projections throughout most central nervous system (CNS) regions, including the cerebral cortex, hippocampus, thalamus, midbrain, brainstem, cerebellum, and spinal cord (reviewed by Aston-Jones et al., 1995 and Samuels and Szabadi 2008a,b). Using a histofluorescence method, Falck and coll. (Falk et al., 1962) identified two main ascending pathways from the LC. A dorsal one (Levitt et al., 1949), innervates the entire cerebral cortex, especially motor and premotor areas, the olfactory tubercle, the septum, the bed nucleus of the stria terminalis, the hippocampal formation and the amygdala (Fuxe et al., 1970; Maeda et al. 1972). A ventral or intermediate pathway supplies the hypothalamus, overlapping with NA projections coming from the A1 and A2 regions. In addition, fibers have been described from the LC to the subthalamic nucleus (Rinvik et al., 1979), the SN (Collingridge et al. 1979), and the ventral tegmental area (VTA) (Jones et al., 1977) and others pass via the superior cerebellar peduncle to the cerebellum (Olson et al., 1971), while a caudal projection has been traced to the reticular formation (Olson et al., 1972).

Synaptic inputs from several sources influence the activity of LC neurons (reviewed by Aston-Jones et al., 1995). This nucleus is densely innervated by fibers that contain opiates, glutamate, gamma-aminobutyric acid (GABA), serotonin, epinephrine, and orexin/hypocretin. The sources of these various inputs have not been fully elucidated, though some major inputs have been identified. The nucleus paragigantocellularis in the ventrolateral rostral medulla, a major input that strongly excites LC neurons, is a source for glutamate, GABA, enkephalin, corticotropin-releasing

hormone (CRH), and adrenaline. The orbitofrontal and anterior cingulate cortices also provides a strong glutamatergic afferent drive to the LC. Inhibitory GABA and enkephalin input originates from the dorsomedial rostral medulla. Orexin/hypocretin inputs originate in the hypothalamus, (Horvath et al., 1999) as do histaminergic inputs (Pollard et al., 1978). The extensive shell of dendrites that surrounds the LC nucleus offers additional extensive targets for afferent termination, and indeed it appears that several areas target these extranuclear dendrites that do not innervate the LC nucleus proper. Thus, projections from the periaqueductal gray matter, (Ennis et al., 1991; Bajic et al., 2000) parabrachial region, (Luppi et al., 1995) preoptic area, (Rizvi et al., 1994; Steininger et al., 2001) amygdala, (Van Bockstaele et al., 1998), medial prefrontal cortex, suprachiasmatic nucleus using the dorsomedial hypothalamus as a relay, among other sites, project to the peri-LC region.

LC neurons fire in two distinct modes, tonic and phasic (Aston-Jones and Cohen, 2005a,b). Tonic activity is characterized by a sustained and highly regular pattern of discharge that is highest during wakefulness and decreases during slow-wave sleep. This tonic activity plays a central role in the sleep-waking cycle anticipating the fluctuations of electroencephalographic activity and promoting a state of vigilance. It is indeed well known that the stimulation of central NAergic receptors leads to changes in the state of vigilance. There is also a sustained increase in tonic discharge rate in response to environmental stimuli that elicit behavioral arousal and exploratory behavior. During focused attention and accurate task performance, LC neurons reduce their tonic firing to a moderate rate and respond phasically to task-relevant stimuli. The phasic bursts of LC activity are closely associated with highly accurate behavioral responses (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005a).

Two subtypes of adrenergic receptors (ARs) have been described: alpha ARs (α1and α2) and beta ARs (β1, β2, and β3). These ARs are found throughout the brain including the striatum and SN. Different subtypes are coupled to different G proteins. In general, excitatory effects are mediated by α1 and β postsynaptic ARs (McCormick and Wang, 1991; McCormick et al., 1991; Arcos et al., 2003) and inhibitory effects by α2 presynaptic ARs (Belujon et al., 2007; Benarroch, 2009). Once released into the extracellular space, reuptake of NA is performed by the noradrenaline reuptake transporter (NET) (Rascol et al., 2001), while extracellular NA also limits its own release through the stimulation of auto-inhibitory α2 ARs (reviewed by Delaville et al., 2011).

To elucidate the way NA could modulate the DAergic system function and play a role in the progression and expression of PD, it is critical to summarize the intimate molecular, functional, and anatomical relationships between NA and DA. First, NA and DA share a biosynthetic pathway and DA is in fact the direct precursor of NA (Molinof et al., 1971). In NAergic neurons dopamine β-hydroxylase (DBH) acts within the synaptic vesicles to convert DA to NA. Second, NAergic neurons directly innervate midbrain DA neurons and the striatum. Stimulation of the LC facilitates burst firing of SNc neurons, while administration of the α1 ARs antagonist prazosin attenuates firing (Grenhoff et al., 1993), and either lesion of the LC or chronic NA depletion decrease striatal DA release (Lategan et al., 1990; Lategan et al., 1992). Furthermore, the peri-LC region receives also DAergic inhibitory control from the VTA (Javoy-Agid et al., 1980; Guiard et al., 2008). Although some of these projections have been shown to contact LC dendrites, additional ultrastructural studies are needed. Finally, DBH, NA, and NET can be detected in the midbrain and striatum (Udenfried et al. 1959, Glowinski et al. 1966, Ross et al. 1974, Liprando et al., 2004) and, as in other regions of the brain, striatal NA release is controlled by both NET and α2 ARs (Gobert et al., 2004, Yavich et al., 1997 and 2003).

Only a few studies investigated the role of DA in the modulation of NAergic pathways in subjects with PD. In general, a decrease in DAergic neuron function seems to enhance NAergic system activity. Rats with a selective DAergic neuron lesion by 6-hydroxydopamine (6-OHDA) show an increase in firing rate of LC neurons (Wang et al., 2009) as well as an upregulation of β ARs in the cerebral cortex, the forebrain, thalamic nuclei, the midbrain, the hippocampus, and the CB (Johnson et al., 1989). Ponzio et al. (1981) demonstrated that NAergic nerve terminals originating from the LC might be involved in regulating the functional activity of the DAergic nerve terminals both in the cerebral cortex and the striatum. This regulation appears to be excitatory in nature and is present early in development. These data are confirmed by pharmacological studies showing that αl ARs antagonism may reduce the sensitivity of the mesolimbic DAergic system to pharmacological or environmental challenge (Davis et al., 1985; Snoddy and Tessel, 1985; Auclair et al., 2002).

The first hints that NA promotes DA neuron survival came from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) studies in nonhuman primates and mice. Both Mavridis and coll. (Mavridis et al., 1991) and Fornai and coll. (Fornai et al., 1994) described that the MPTP-induced damage to nigrostriatal DA neurons was enhanced by pre-treatment with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), a selective LC neurotoxin (see also *On-going research activities*). Furthermore, the tottering mouse, which has NAergic hyper-innervation and increased levels of NA throughout the forebrain, was protected from MPTP toxicity (Kilbourn et al., 1998; Hein et al., 1999). Rommelfanger and coll. also showed that either pharmacological or genetic blockade of NET protects DA neurons from MPTP damage in mice (from Rommelfanger et al., 2004). Despite preliminary evidence of a neuroprotective activity of NAergic system over DA neurons, it has not been well elucidated, at a molecular level, how this can happen. A possible explanation might relate to the release by LC neurons of co-transmitters galanin and brain derived neurotrophic factor (BDNF), two potential molecules for neuroprotection (Rommelfanger and Weinshenker, 2007). Indeed, the central NA system, apart from target neurons, includes effects on glia and brain vessels (Harik et al., 1984; Stone et al., 1989). This is in line with the morphology of NA axons that possess varicosities *(bouttons en passage)* rather than classic *(bouttons terminaux)* typical of non-monoaminergic axon terminals. Finer morphological studies of NA axons arising from the LC have shown that axon terminals are thin, with small (0.5 μm) beaded varicosities. This contrasts with NA axons arising from the medullary A1 and A2 cell groups which branch out with terminals featuring large (1±3 μm) beaded varicosities. It is worth mentioning that monoaminergic axons with smaller beaded varicosities possess a lower threshold to various neurotoxic insults and at the same time are more affected in degenerative diseases. These combined findings call for more in depth studies aimed at relating the cell biology of synaptic varicosities with the selective cell death occurring both in neurotoxic insults and in neurodegenerative disorders (from Gesi et al., 2000).

Apart from its importance for the survival of DA neurons, NA could also play an independent role in the symptoms of PD. Mavridis et al. (1991) have suggested that the activation of α1 ARs, which results in an increase in NAergic tone, facilitates locomotor activity, whereas α2 ARs activation, by decreasing NAergic tone, inhibits locomotor activity. In the reserpine rat (see also *On-going research activities*), yohimbine, a α2 ARs antagonist, blocked tremor and improved rigidity but not hypokinesia (Colpaert, 1987). In the 6-OHDA rat and MPTP monkey models of PD, blockade of α2 ARs by idazoxan improved motor disabilities (Bezard et al., 1999; Belujon et al., 2007) in a manner comparable to that induced by a minimal dose of levodopa (Bezard et al., 1999). Still, idazoxan as a monotherapy in PD patients did not show any anti-parkinsonian effect (Henry et al., 1999; Rascol

et al., 2001; Colosimo and Craus, 2003). Interestingly, the α2 AR agonist clonidine and β ARs blockers (e.g. propranolol) are effective in treating akathisia and tardive dyskinesia (Wilbur et al., 1988). More recently, von Coelln and coll. described that in *parkin* null mice (by targeted deletion of *parkin* exon 7) there is a dramatic reduction of NAergic neurons, while the nigrostriatal DAergic system does not show any impairment. Thus suggesting an earlier involvement of NAergic neurons in *parkin* positive PD patients (von Coelln et al., 2004). In addition to the spectrum of movement abnormalities, many PD patients experience neuropsychiatric symptoms (such as cognitive impairment and depression) that may be also related to LC degeneration (Chaudhuri et al., 2006). Non-motor symptoms also improve by the use of selective α1 AR agonists. For example, naphtoxazine reduced the errors and restored the lateralization of N100 during the shifting reaction time task, suggesting that it may act on the processes underlying the shifting deficit in PD patients (Bedard et al., 1998). PD patients with comorbid depression tend to exhibit more pronounced PD symptoms, and their depression can be alleviated by reboxetine, a specific NA reuptake inhibitor (Lemke, 2002; Papapetropoulos et al., 2006; Pintor et al., 2006). Recently, however, another NET inhibitor atomoxetine failed to reduce depression in PD patients (Weintraub et al., 2010). The use of selective NET inhibitors may be critically dependent on the status of NA neurons.

Finally, the death of NA neurons may modulate the plastic changes and behavioral pathology associated with long-term levodopa therapy (see also *Summary and future directions*). Although DA replacement with levodopa remains one of the most effective treatments for PD, adverse events such as dyskinesia may appear (Rascol et al., 2001). Human and animal studies have shown that α2 ARs antagonists provide relief from levodopa-related dyskinesia (Grondin et al., 2000; Archer et al., 2003). Of relevance, while the mechanism of dyskinesia and the basis of the relief provided by blockade of α2 ARs remains unknown, these data imply that NA continues to function in modulating the plasticity and activity of the basal ganglia during the progression of PD (see also *Hypothesis statement*).

HYPOTHESIS STATEMENT AND AIMS

In my routine movement disorders clinical practice I have often wondered on the great heterogeneity of clinical presentation of patients with PD. In time, I start believing that such a variety of phenotypes may represent different physio-pathological pattern of neuronal degeneration. To some extent, we might call PD different diseases sharing DAergic striatal innervation loss as a solely common feature. My interest was mainly captured by patients with prominent resting tremor that persistently overshadowed other signs of PD throughout the disease course. Remarkably, they also referred no more than mild progression, despite over seven years from motor symptoms onset.

I speculate that the LC is involved in the generation of Parkinson tremor. LC-NAergic activity would play a key role in tremor onset by directly influencing the CB-VIM-CM circuit. Accordingly, some PD patients would have a hyperactive LC-NAergic system, especially early at a disease (pre-motor) stage. Moreover, given a putative compensatory and neuroprotective activity (Srinivasan and Schmidt, 2003; von Coelln et al., 2004), an intact NAergic system would be related to the more benign disease progression of these patients. In addition to Parkinson tremor, an intact and possibly hyperfunctioning LC might possibly contribute to other NA-related signs, such as anxiety and REM sleep behavior disorder. On the contrary, subjects with a DAergic and NAergic parallel degeneration would show depression, hyposmia and a more aggressive disease progression. Preliminary evidence of a role of adrenaline and NA in Parkinson tremor emerged from studies published in the 1960s and 1980s (Constas, 1962; Colpaert, 1987; Wilbur et al., 1988). In time, several independent investigators described that experimental lesions of the LC exacerbate PD pathology and symptomology (e.g. depression, dementia, etc.) (see *Background*). All these studies, however, were carried out under the assumption that PD presents a wider spectrum of motor and non-motor signs related to combined aminergic deficiencies. Although it is relatively common to describe a variety of significant biochemical alterations in PD patients, it is more difficult to demonstrate, for each neurotransmitter, a specific role in the pathophysiology of the disease. This point has been addressed solely by post-mortem studies that correlated a specific biochemical pattern observed in a given patient with his clinical history (McMillan et al., 2011). This approach bares several limitations. In particular, it describes the end-stage of the disease and cannot provide any useful information on the pathophysiological changes along with disease progression. In particular, compensatory mechanisms along the disease course will not be revealed by these studies.

In time we achieved comprehensive information on LC, at least at an anatomical and physiological level (for review: Samuels and Szabadi, 2008a,b). It is therefore possible, within a reasonable timeframe, to apply such knowledge to investigate LC-NAergic activity in PD patients. In addition, new NA-specific tracers for PET will be available soon for human studies thus allowing a direct *in vivo* investigation of LC activity. Two possible outcomes make the research line proposed of significant value. First, many drugs targeting the NAergic system (e.g. NA reuptake inhibitor; L-Threo-Dops; α2 receptors antagonist, etc.) (for review Delaville et al., 2011) are already available and could be used to possibly ameliorate quality of life of PD patients. A better understanding of LC-NAergic activity in PD sub-groups of patients is therefore mandatory. Second, if a compensatory and neuroprotective role of NAergic system over DA cell loss will be proven, we envision new therapeutic options to slow disease progression.

Introduction to Research activities and Methods

In the following sections I will present and discuss published and on-going research activities investigating the LC-NAergic activity in patients with Parkinson tremor.

Studying the LC can be challenging to say the least. In particular, (1) directly recording in humans the activity of LC is not applicable given its anatomical structure and location; (2) the size of LC is at the spatial resolution limits of currently available imaging techniques; and (3) even receptor binding (or displacement) studies and PET might not catch its phasic electrical activity; (4) last, there are no PET tracer available at the moment to target specifically the NAergic system and the LC in particular (Logan et al., 2007). Despite being an evident clinical sign, Parkinson tremor is also difficult to describe especially given its unpredictable and intermittent appearance (see *Background*). A great effort was therefore required to investigate a putative correlation between LC-NAergic and Parkinson tremor. To do so, we applied a multidisciplinary approach including brain-imaging techniques (e.g. SPECT and MRI), EMG recordings of tremor during behavioral tasks, as well as studies in animal models of PD.

COMPLETED RESEARCH ACTIVITIES

Alles sollte so einfach wie möglich gemacht werden, aber nicht einfacher.
[A. Einstein]

Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease

Isaias IU, Marotta G, Pezzoli G, Sabri O, Schwarz J, Crenna P, Classen J, Cavallari P.
BMC Neurol. 2011;11:88.

This first study aimed to demonstrate a putative functional integrity of LC-NA system in PD patients early at a disease stage.

We retrospectively reviewed clinical and imaging data of 94 PD patients and 15 healthy subjects who underwent SPECT imaging with FP-CIT. Although FP-CIT is mainly used to measure striatal DAT (see *Background*)., it has shown sensitivity, albeit lower, to NET (Booij et al., 2008), thus allowing to possibly investigating also the LC. To do so, besides a voxel-based whole brain analysis, we also applied a volume of interest (VOI) analysis of a priori defined brain regions, focusing on the LC. PD patients were selected only if at early clinical stage (Hoehn and Yahr stage 1 or 2) and according to several other inclusion criteria (see article *Methods-Subjects*).

Average FP-CIT binding in the putamen and caudate nucleus was significantly reduced in PD patients (43% and 57% on average, respectively; $p < 0.001$ Student's t-test), thus confirming the clinical diagnosis of PD. In contrast, subjects with PD showed an increased FP-CIT binding in the LC (166% on average; $p < 0.001$ Student's t-test) (see article *Figure 1* [also below]). More interestingly, LC-binding correlated negatively with striatal FP-CIT binding values (caudate: contralateral, $r = -0.28$, $p < 0.01$ and ipsilateral $r = -0.26$, $p < 0.01$; putamen: contralateral, $r = -0.29$, $p < 0.01$ and ipsilateral $r = -0.29$, $p < 0.01$) (see article *Figure 2*).

These preliminary data are consistent with higher baseline catecholamine transporter binding in the LC region of patients with PD at an early stage, which is well compatible with enhanced NA release (Metzger et al., 2002; Zahniser et al., 2009).

The relevance of this study relies on the fact that we were able to show, for the first *in vivo,* an integrity and possibly hyperactivity of LC-NAergic system in subjects with PD. These findings must be carefully interpreted due to several limitations. In particular, our results derived from the analysis of the binding of FP-CIT, a ^{123}I-labeled cocaine derivative with high affinity for DAT (KD = 2nM) and a lesser affinity towards NET (KD = 140 nM) (Booij et al., 2008). Still, we believe it is unlikely that the higher binding observed in the LC area is due to an enhanced DAergic, rather than NAergic, transporter counts. Indeed, DAT are poorly represented in the LC area (Javoy-Agid et al., 1980). On the contrary, a major NET component is synthesized in the cell body of LC pigmented neurons and exposed on their membrane to be transferred toward axonal terminals (Zahniser and Sorkin, 2009), with a less consistent NET component localized on terminal projections arising from more caudal NAergic cell groups (Ordway et al., 1997). Ideally, the LC-NA system and NET should be investigated in vivo by dedicated, highly specific radiotracers displaying low background non-NET binding, high sensitivity to variations in NET density and fast kinetics (Logan et al., 2007). We hope that such radiotracers will be available soon for more accurate studies.

Our results might be at odd with neuropathological findings where frank neuronal degeneration has been recognized within LC. Indeed, morphologic hallmarks of sporadic PD (i.e. Lewy bodies and dystrophic neurites containing pathologic a-synuclein) may appear initially in the lower brainstem (Braak et al., 2003). Still, this information was derived from anatomopathological studies of patients at the end-stage of the disease and therefore is poorly comparable with our findings,

based on subjects with mild clinical signs (see article *Methods-Subjects*). In addition, such previous studies report data only on a limited number of patients poorly described at a clinical level (e.g. the presence of depression or cognitive impairment) (Hoogendijk et al., 1995; McMillan et al., 2011). Last, Lewy pathology can correlate poorly with neuronal loss in specific areas, thus its validity in predicting neuronal disintegration is questionable (Jellinger, 2009). Indeed, NAergic neurons in the LC are relatively preserved in early PD and do not exhibit the same intracellular changes as in the SN (Halliday et al., 2005). Accordingly, neuromelanin-sensitive imaging methods in vivo (Sasaki et al., 2006) suggests [see also *On-going research activities*] that the loss of NA neurons in PD may be confined to the larger, pigmented cells localized in the caudal part of the nucleus, whereas small unpigmented cells are increased in number, as if derived from shrinkage of larger neurons (Hoogendijk et al., 1995).

Last, it is worth mentioning that we are currently not able to identify non-symptomatic subjects with (at high risk for) PD. Therefore, it was not possible to investigate subjects at a pre-clinical (pre-motor) PD stage. Still, we could have investigated subjects with a genetic mutation (e.g. LRRK2) but yet no clinical signs evocative for PD. Several difficulties prevented such a study. First of all, the ethical issue in offering a study aimed to define, at this stage, only the risk to develop the disease itself. Second, physio-pathological PD-related pathways are not yet clear in genetically defined Parkinsonism and may not necessarily correspond to idiopathic PD.

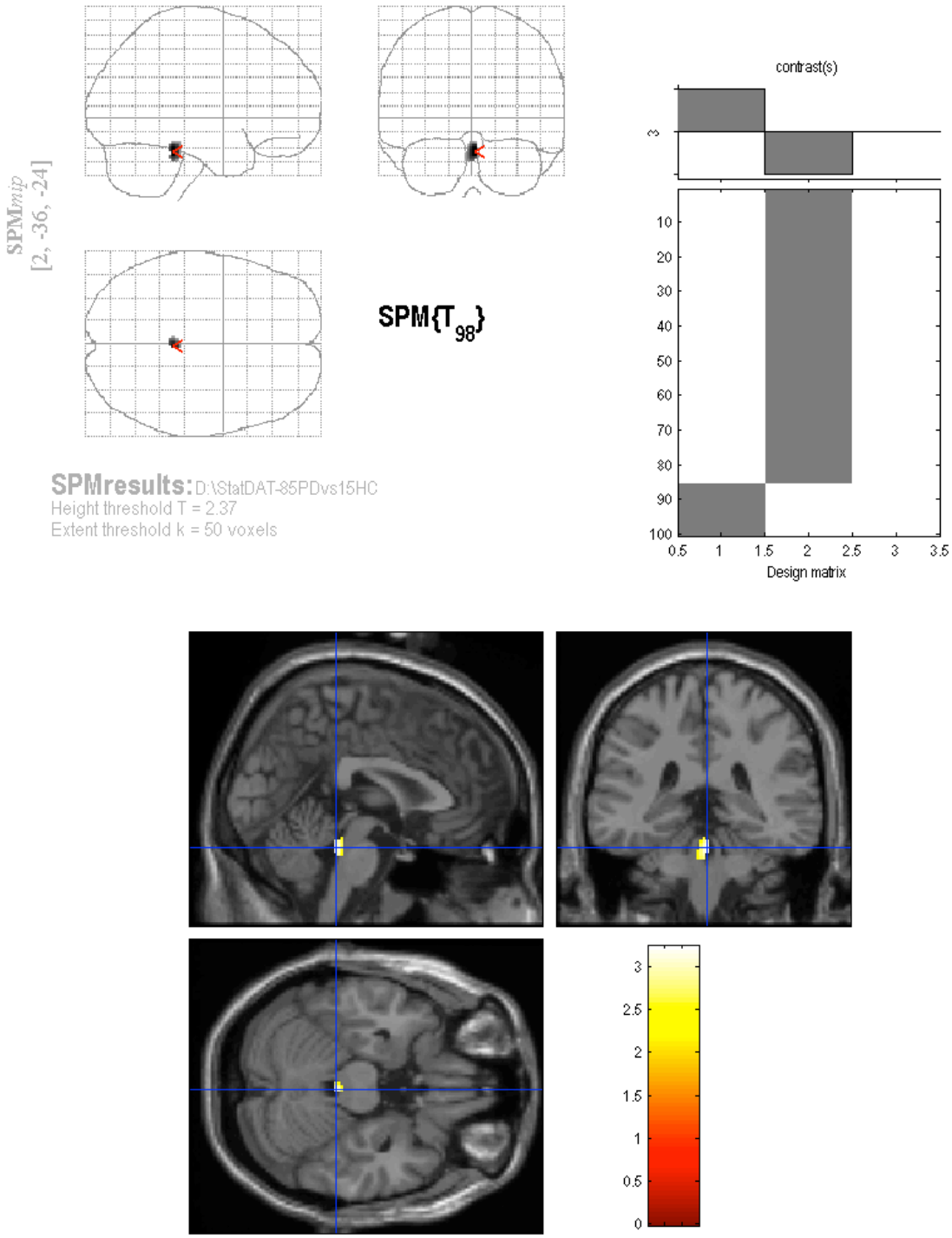


Figure 1 in Isaias et al., BMC Neurol. 2011;11:88 here with detailed statistical analysis [SPM] (see article for description).

BMC Neurology

RESEARCH ARTICLE Open Access

Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease

Ioannis U Isaias[1,2,3*], Giorgio Marotta[4], Gianni Pezzoli[2], Osama Sabri[5], Johannes Schwarz[3], Paolo Crenna[1], Joseph Classen[3] and Paolo Cavallari[1]

Abstract

Background: Studies in animals suggest that the noradrenergic system arising from the locus coeruleus (LC) and dopaminergic pathways mutually influence each other. Little is known however, about the functional state of the LC in patients with Parkinson disease (PD).

Methods: We retrospectively reviewed clinical and imaging data of 94 subjects with PD at an early clinical stage (Hoehn and Yahr stage 1-2) who underwent single photon computed tomography imaging with FP-CIT ([^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane). FP-CIT binding values from the patients were compared with 15 healthy subjects: using both a voxel-based whole brain analysis and a volume of interest analysis of *a priori* defined brain regions.

Results: Average FP-CIT binding in the putamen and caudate nucleus was significantly reduced in PD subjects (43% and 57% on average, respectively; $p < 0.001$). In contrast, subjects with PD showed an increased binding in the LC (166% on average; $p < 0.001$) in both analyses. LC-binding correlated negatively with striatal FP-CIT binding values (caudate: contralateral, $\rho = -0.28$, $p < 0.01$ and ipsilateral $\rho = -0.26$, $p < 0.01$; putamen: contralateral, $\rho = -0.29$, $p < 0.01$ and ipsilateral $\rho = -0.29$, $p < 0.01$).

Conclusions: These findings are consistent with an up-regulation of noradrenaline reuptake in the LC area of patients with early stage PD, compatible with enhanced noradrenaline release, and a compensating activity for degeneration of dopaminergic nigrostriatal projections.

Background

The pontine nucleus locus coeruleus (LC) is the major site of noradrenaline (NA) neurons in the central nervous system, hosting almost half of the NA-producing neurons in the brain [1].

The LC may play an important role in the pathophysiology of Parkinson disease (PD) for several reasons: (i) as a site of neuronal degeneration as part of PD pathology; [2] (ii) as the anatomical origin of projections modulating dopaminergic action of the substantia nigra; [3] (iii) as a structure under putative dopaminergic inhibitory control from the ventral tegmental area (VTA) which is known to degenerate in PD [4,5]. Based on physiological functions ascribed to the noradrenergic system, impaired functioning of LC in PD has been associated primarily to affective disorders, [6] cognitive disturbances, [7] sleep disorders, [8] sensory impairment [2] and autonomic dysfunction [9]. Through its interactions with the dopaminergic system however, the LC may also have a less direct role in the pathogenesis of PD via (i) an interplay of catecholamine systems with one amine cross-talking with receptors belonging to the other system [10,11] or (ii) extra-synaptic neuro-modulatory, metabotroic and trophic activities of noradrenaline itself [12].

Information on the LC in PD is mainly based on post-mortem examination of histopathological specimens, while information on its *in vivo* function is largely

* Correspondence: ioannis.isaias@unimi.it
[1]Università degli Studi di Milano, Dipartimento di Fisiologia Umana, Milano, Italy
Full list of author information is available at the end of the article

absent. Ideally, the LC-NA system and noradrenaline molecular transporters (NET) should be investigated *in vivo* by dedicated, highly specific radiotracers displaying low background non-NET binding, high sensitivity to variations in NET density and fast kinetics. As such a radiotracer is not available for large clinical studies, [13] we employed single photon computed tomography (SPECT) with FP-CIT ([^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane) in a large, homogeneous cohort of early stage PD patients. Although FP-CIT is mainly used for assessing striatal dopamine reuptake transporters, it has shown sensitivity, albeit lower, to NET [14]. Therefore, when applied to an anatomical region with known low dopamine reuptake transporter capacity such as the LC, it allows investigation of the NA-dependent synaptic activity.

Methods

Subjects

We retrospectively reviewed clinical and imaging data of 94 subjects with idiopathic PD in whom FP-CIT SPECT was performed at the "Ospedale Maggiore Policlinico" in Milano within five years of the onset of motor symptoms. Fifteen healthy subjects (healthy controls, HC) were prospectively enrolled for comparisons of FP-CIT binding. At the time of SPECT, HC did not suffer from any disease and were not taking any medications. Clinical inclusion criteria for subjects with PD were: (a) diagnosis according to the UK Parkinson Disease Brain Bank criteria; (b) absence of any signs indicative for atypical parkinsonism (e.g. gaze abnormalities, autonomic dysfunction, significant psychiatric disturbances, etc.) over a follow-up period of at least three years after symptoms onset; (c) Hoehn and Yahr (H&Y) stage 1 or 2 in drugs-off state (i.e. after overnight withdrawal of specific drugs for PD; no patients were taking long-acting dopaminergic drugs) at the time of SPECT; (d) positive clinical improvement at Unified Parkinson Disease Rating Scale (UPDRS) after L-Dopa intake (i.e. > 30% from drug-off state) at some point during the three years of follow up; (e) a normal Magnetic Resonance Imaging (MRI) (no sign of white matter lesion or atrophy). Finally, given a putative role of LC and noradrenaline in cognition and mood (including depression) we excluded from this study patients with a positive score at UPDRS part I.

A quantitative profile of each patient' motor impairment was obtained from clinical assessment performed before SPECT by means of the UPDRS motor part (part III). L-Dopa daily dose and L-Dopa Equivalent Daily Doses (LEDDs) were also recorded, with the latter expressed as follows: 100 mg levodopa = 1.5 mg pramipexole = 6 mg ropinirole. None of the subjects (both PD and HC) were taking or stated to have ever been treated with antipsychotics or drugs known to affect the noradrenergic system (e.g., noradrenaline reuptake inhibitors). Drug naïve patients at the time of SPECT were not included in the study. The Ethics Committee of the Department of Human Physiology approved the study and all subjects gave informed consent.

SPECT data acquisition and processing

Intravenous administration of 110-140 MBq of FP-CIT (DaTSCAN, GE-Healthcare, UK) was performed 30-40 minutes after thyroid blockade (10-15 mg of Lugol oral solution) in the control subjects and in patients after overnight withdrawal of dopaminergic therapy [15]. Brain SPECT was performed 3 hours later by means of a dedicated triple detector gamma-camera (Prism 3000, Philips, Eindhoven, the Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (4 subsets of acquisitions, matrix size 128x128, radius of rotation 12.9-13.9 cm, continuous rotation, angular sampling: 3 degree, duration: 28 minutes). Brain sections were reconstructed with an iterative algorithm (OSEM, 4 iterations and 15 subsets) and then processed by 3D filtering (Butterworth, 5th order, cut-off 0.31 pixel^{-1}) and attenuation correction (Chang method, factor 0.12).

Imaging data analysis

Two different and complementary image analyses were performed: a voxel-based whole brain analysis using Statistical Parametric Mapping SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB R2007a (The Mathworks Inc, USA), and a volume of interest (VOI) analysis of *a priori* defined brain regions.

SPM analysis

A group-specific FP-CIT template was created by (i) spatially normalizing the FP-CIT images of 15 healthy subjects onto a FP-CIT MNI-based template, [16] (ii) subsequent averaging of the normalized images and their symmetric (mirror) images resulting in a mean image, and finally (iii) a smoothing of the mean image using a 3-dimensional Gaussian kernel with 8-mm full width at half maximum (FWHM). To increase the signal-to-noise ratio and account for subtle variations in anatomic structures, the individual subject's FP-CIT images were spatially normalized to this group-specific template and smoothed with a FWHM 10-mm Gaussian kernel. A reference region in the occipital cortex was defined as the union of the superior, middle and inferior occipital gyri along with the calcarine gyri VOIs defined by automated anatomical labelling (AAL), using the Wake Forest University (WFU) PickAtlas 2.4 software. Binding values for each subject's FP-CIT image were then computed in a voxel-by-voxel manner (voxel - occipital reference)/(occipital reference). Using the

General Linear Model in voxel-based statistical analysis of SPM2, a two-sample t-test contrast was used to elucidate group difference between PD and HC. No global normalization, or grand mean scaling, were applied, and the masking threshold was set to zero. Clusters of at least 35 voxels with the height threshold set at $p < 0.001$, were considered significant.

VOI analysis

The LC FP-CIT binding values were for two VOIs (for left and right part of LC) created, using WFU Pick Atlas Tool, through the union of six distinct, contiguous Boxes (of 3 mm on the *z* axis for each side), centered in the mean values on the *x* and *y* axis and dimensioned according to the standard deviation as proposed in Table 1 of Keren and coll., 2009 [17]. FP-CIT binding values for the caudate nucleus (CN) and putamen (PT) were calculated on the basis of VOIs defined with the Basal Ganglia Matching Tool [18]. Student's *t*-test was then applied. We defined as *contralateral*, those brain regions opposite to that of PD most severe sign presentation. For HC, we referred to the right side as *ipsilateral* [15].

General statistical analysis

Unless otherwise stated, data are reported as median and range. Normality of data distribution was tested by means of Shapiro-Wilks test. Chi-Square was used to test gender distribution among groups. Demographic data were compared by means of Wilcoxon two-group test. The Spearman correlation coefficient was calculated to investigate statistical dependence among average binding values, demographic and clinical variables.

Table 1 Demographics and clinical data

	PD	HC
Subjects N. (male/female)	94 (67/27)	15 (4/11)*
Age at SPECT	60 (38 - 75)	63 (51 - 74)
Age at motor symptoms onset	57 (37 - 72)	
Disease duration	3 (1 - 5)	
UPDRS motor score (part-III) [range 0 - 108]	19 (8 - 56)	
Hoehn and Yahr stage [range 1 - 5]	2 (1 - 2)	
L-Dopa in mg/day	400 (0 - 850)	
LEDDs in mg/day	250 (70 - 1200)	

Data are reported as median and range (brackets). Age at SPECT, age at motor symptoms onset and disease duration are in years. All patients were evaluated with the Unified Parkinson Disease Rating Scale motor part (UPDRS part III) in drugs-off state (i.e. after overnight withdrawal of specific drugs for PD, no patients was taking long-acting dopaminergic drugs). LEDDs were calculated as follow: 100 mg levodopa = 1.5 mg pramipexole = 6 mg ropinirole. * $p = 0.0005$.

Statistical analyses were performed with the JMP statistical package, version 8.0 (SAS Institute, Inc., Cary, NC, USA).

Results

Table 1 shows the demographic and clinical characteristics of the study cohorts.

SPM analysis detected one large cluster of 6819 voxels (peaks at coordinates: 28 -8 -4 and at -31 -8 -4) of significantly reduced FP-CIT binding involving the PT and the CN bilaterally (Figure 1, left). A cluster of 37 voxels (peak at coordinates: 2 -36 -26) with higher FP-CIT binding values was found in the LC of PD subjects (Figure 1, right).

Volumes of interest analysis revealed reduced average binding values in the striatum and increased average binding value in LC area, bilaterally (Table 2).

FP-CIT binding in the striatum showed a weak, but significant, negative correlation with binding values of the corresponding LC (caudate: contralateral, $\rho = -0.28$, $p = 0.004$ and ipsilateral $\rho = -0.26$, $p = 0.008$; putamen: contralateral, $\rho = -0.29$, $p = 0.004$ and ipsilateral $\rho = -0.29$, $p = 0.003$) (Figure 2). LC binding did not show other significant correlations. Finally, results for the FP-CIT binding value in the LC area proved to be statistically independent when weighted for demographic (age at SPECT, age at onset, gender) and clinical characteristics (disease duration, disease severity and L-Dopa daily dose and LEDDs).

Discussion

Increased FP-CIT binding in the LC area

The present study provides *in vivo* evidence of higher baseline catecholamine transporter binding in the LC region in a large and homogeneous cohort of subjects with early PD. Our findings are consistent with an up-regulation of noradrenaline reuptake in the LC area, which is well compatible with enhanced noradrenaline release [19,20].

Our results are derived from the analysis of the binding of FP-CIT, a ^{123}I-labeled cocaine derivative with high affinity for dopamine (DAT; $K_D = 2$nM) and a lesser affinity towards noradrenaline transporter (NET; $K_D = 140$ nM) [14]. Despite the higher affinity of FP-CIT for DATs, it is unlikely that the higher binding observed in the LC area is due to an enhanced dopaminergic, rather than noradrenergic, transporter for two main reasons: (i) in LC, DAT represent a minor and inconsistent component of the midbrain-derived dopaminergic terminals which degenerates in PD, along with other dopaminergic projections, [4] and (ii) in the LC a major NET component is synthesized in the cell body of pigmented neurons and exposed on their membrane to be transferred toward axonal terminals, [20] with a less

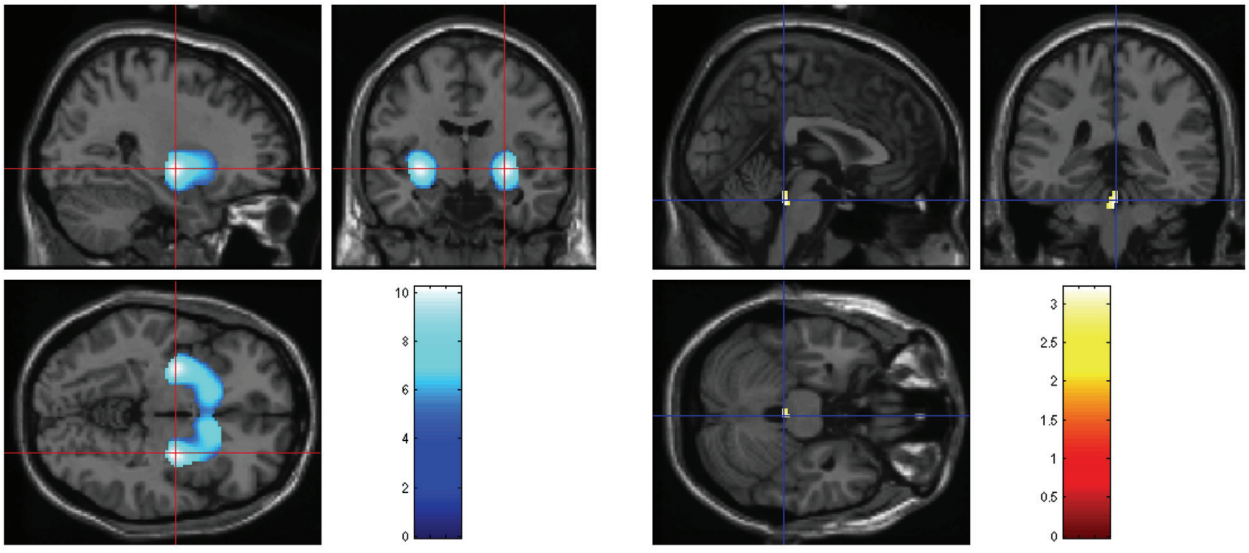


Figure 1 Overlay on a MRI showing the loss of FP-CIT binding bilaterally in the striatum (cluster of 6819 voxels, peaks at coordinates: 28 -8 -4 and at -31 -8 -4) (left in the figure) and increased FP-CIT binding in the locus coeruleus area (cluster of 37 voxels, peak at coordinates: 2 -36 -26) (right in the figure) of the whole group of PD patients compared to controls.

consistent NET component localized on terminal projections arising from more caudal noradrenergic cell groups [21].

In PD, reduced dopamine release from nigro-striatal projections results in loss and adaptive down-regulation of DAT binding sites in the striatal region [22]. In line with this notion, and in agreement with studies in *de novo* and early PD, where 40 to 60% of nigral dopaminergic neurons are lost, [15,23] we found a significantly reduced FP-CIT binding in the caudate and putamen of PD patients. In contrast to the striatal compartment, analysis of FP-CIT labeling in the upper brainstem revealed significantly increased binding in a pontine area adjacent to the floor of the fourth ventricle and extending into the midbrain to the level of the inferior colliculi. This area corresponds topographically to the LC coordinates identified by other studies including those employing neuromelanin-sensitive MRI methods [6,17,24,25]. In addition, the LC is the sole structure in the posterior rostral pons housing monoamine transporters [1], thus further supporting our claim of anatomical targeting of the LC.

Table 2 Binding values obtained with the analysis of volumes of interest

Region of interest	PD	HC	p values
CN contralateral	3.18 (1.2 - 3.84)	5.27 (3.51 - 6.15)	< 0.0001
CN ipsilateral	3.29 (0.98 - 6.26)	5.27 (3.51 - 6.6)	< 0.0001
PT contralateral	1.86 (0.65 - 4.72)	4.83 (3.07 - 6.04)	< 0.0001
PT ipsilateral	1.97 (0.76 - 4.65)	4.83 (3.62 - 6.37)	< 0.0001
LC contralateral	313.5 (81 - 663)	131.43 (59 - 371)	0.001
LC ipsilateral	321.17 (87 - 632)	123.6 (38 - 354)	0.0004

Data are reported as median and range (brackets). Average FP-CIT binding in the caudate nucleus (CN) and putamen (PT) was significantly reduced in subjects with PD subjects compared to HC. On the contrary, PD patients showed a significantly increased binding in the LC area (both right and left regions). We defined *contralateral* brain regions opposite to that of PD signs presentation. For HC, we referred to the right side as *ipsilateral* [15].

Only two prior studies with PET have specifically investigated the LC in PD patients. A first, [24] reported a reduced ^{18}F-dopa intake in patients with advanced PD when compared to patients at an early stage of the disease. Because ^{18}F-dopa intake is more specifically related to dopaminergic neurotransmission, this study does not provide information on noradrenergic functioning of LC. In a second study, [6] PD subjects with depression showed a reduced binding of [^{11}C]RTI-32, a marker of both DAT and NET, when compared to non-depressed patients. Interestingly, the noradrenergic activity of early non-depressed PD patients was within normal range in most patients and enhanced in few of them. In line with these findings, and having enrolled a larger and more selected cohort of subjects, we were able to reveal a significantly higher LC activity at an early stage of PD for the first time.

An acute effect of drugs on FP-CIT binding values appears unlikely since SPECT was performed after overnight withdrawal of anti-parkinsonian drugs. In addition, in animal studies, systemic administration of D_2/D_3 receptor agonists, such as pramipexole or apomorphine, showed little or no effect on the firing rate of LC-NA neurons [26]. Finally, a persistent treatment with

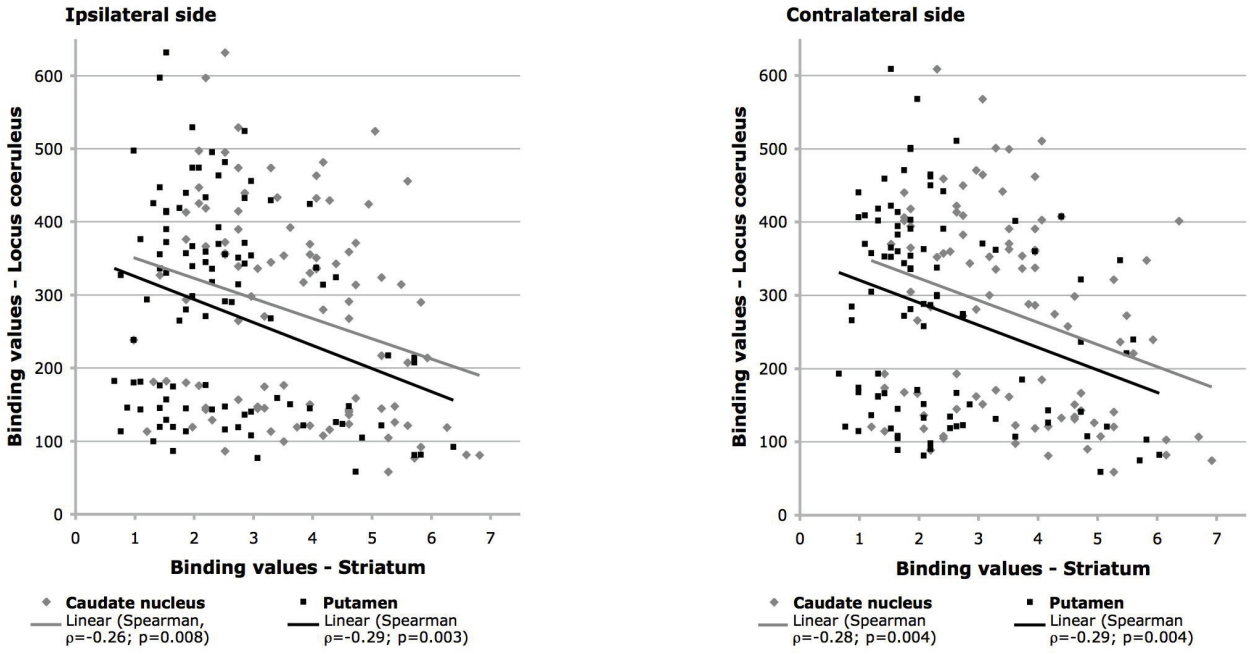


Figure 2 Scatter plots and linear correlations of ipsilateral (left in the figure) and contralateral (right in the figure) FP-CIT binding values of the locus coeruleus and striatum (caudate nucleus and putamen). A statistically significant negative correlation was found between FP-CIT binding values in the locus coeruleus area and the corresponding striatum (both caudate nucleus and putamen).

dopaminergic drugs will eventually down-regulate, rather than up-regulate, the surface expression of DAT and NET through internalization of the transporters [27,28]. Accordingly, the average FP-CIT binding values in the LC remained enhanced when data were L-Dopa weighted for equivalent daily dose and L-Dopa daily dose.

In vivo versus anatomopathological studies

Enhanced noradrenergic binding, and possibly activity, in PD might be considered at odds with neuropathological findings, where frank neuronal degeneration has been recognized within LC, based on detection of specific cellular markers. Indeed, morphologic hallmarks of sporadic PD (Lewy bodies and dystrophic neurites containing pathologic α-synuclein) may appear initially in the lower brainstem [2].

However, Lewy pathology can correlate poorly with neuronal loss in specific areas, thus its validity in predicting neuronal disintegration is questionable [29]. In fact, noradrenergic neurons in the LC are relatively preserved in early PD and do not exhibit the same intracellular changes as in the substantia nigra [30].

Accordingly, neuromelanin-sensitive imaging methods *in vivo*, [25] as well as anatomopathological studies suggested that the loss of NA neurons in PD may be confined to the larger, pigmented cells localized in the caudal part of the nucleus, whereas small unpigmented cells are increased in number, as if derived from shrinkage of larger neurons [31].

However, available information on the LC, so far derived from anatomopathological studies in subjects with PD, is poorly comparable with our findings. In particular, the limited number of PD subjects investigated and the lack of clinical information (e.g. disease duration and the presence of depression or cognitive impairment) of patients in anatomopathological studies prevent a direct comparison between these studies and our results [31,32].

Implications of enhanced LC-NA functioning in PD at an early stage

Based on anatomical and histochemical data, along with neuropharmacological evidence, higher activity of the LC in PD may suggest: (i) in the striatum, noradrenaline plays a compensatory role cross targeting dopaminergic receptors (synaptic action); while (ii) in the substantia nigra, noradrenaline has a neuroprotective bolstering dopaminergic cells (extra-synaptic paracrine action).

As for the compensatory role, there is no absolute specificity for catecholaminergic substrate-receptor interactions, implying that one catecholamine can cross-talk with the pharmacologically defined receptors or transporters belonging to other catecholamines. Indeed, noradrenaline binds to pharmacologically defined dopaminergic receptors [11,33,34]. Therefore, enhanced

noradrenaline release may be able to partially compensate a dopaminergic innervation loss due to degeneration of the substantia nigra.

With reference to a putative neuroprotective activity, noradrenaline suppresses pro-inflammatory and elevates anti-inflammatory molecules [35] and has the ability to scavenge superoxide and reactive oxygen species, which are thought to contribute to cellular damage and dopaminergic cell death [36]. Furthermore, the tottering mouse, which has noradrenergic hyperinnervation and increased levels of noradrenaline throughout the forebrain, appears to be protected from MPTP toxicity [37] while MPTP-induced damage to nigrostriatal dopaminergic neurons was potentiated by pretreatment with DSP-4, a selective LC neurotoxin [38]. Therefore, we speculate that enhanced LC-NA may be regarded as an endogenous paracrine agent promoting dopaminergic neuron survival [39,40]. This hypothesis would predict that degeneration of LC noradrenergic neurons in later stages of the disease might accelerate degeneration of substantia nigra dopaminergic neurons. The negative correlation between FP-CIT binding in the striatum and LC area is consistent with the above considerations of LC-NA compensatory and protective activity.

Conclusions

The present study suggests higher baseline catecholamine transporter binding in the LC area of patients with early stage PD. We propose that enhanced noradrenergic activity may be one factor modulating the severity of motor symptoms and may even influence progression of dopaminergic neurodegeneration.

Acknowledgements

The authors would like to thank Dr. Margherita Canesi, Dr. Swen Hesse, Dr. Philipp Meyer, Dr. Dorothee Saur and Dr. Paul Summers for their critical reading of the manuscript. The authors are grateful to Dr N. Tzourio-Mazoyer and colleagues for providing the AAL volumetric brain template, freely available at http://www.cyceron.fr/freeware.
The study was funded in part by a grant of the Grigioni Foundation for Parkinson disease.

Author details

[1]Università degli Studi di Milano, Dipartimento di Fisiologia Umana, Milano, Italy. [2]Parkinson Institute, Istituti Clinici di Perfezionamento, Milano, Italy. [3]Department of Neurology, University of Leipzig, Leipzig, Germany. [4]Department of Nuclear Medicine, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milano, Italy. [5]Department of Nuclear Medicine, University of Leipzig, Leipzig, Germany.

Authors' contributions

IUI and GM participated in the conception of the study, gathered and analyzed the data. JC, PC, GP, OS, JS and PC contributed to data analysis and participated in the redaction of the paper. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 5 April 2011 Accepted: 21 July 2011 Published: 21 July 2011

References

1. Aston-Jones G, Shipley MT, Chouvet G, Ennis M, van Bockstaele E, Pieribone V: **Afferent regulation of locus coeruleus neurons: anatomy, physiology and pharmacology.** *Prog Brain Res* 1991, **88**:47-75.
2. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E: **Staging of brain pathology related to sporadic Parkinson's disease.** *Neurobiol Aging* 2003, **24**:197-211.
3. Collingridge GL, James TA, MacLeod NK: **Neurochemical and electrophysiological evidence for a projection from the locus coeruleus to the substantia nigra.** 1979, **290**:44.
4. Javoy-Agid F, Agid Y: **Is the mesocortical dopaminergic system involved in Parkinson disease?** *Neurology* 1980, **30**:1326-1330.
5. Guiard BP, El Mansari M, Blier P: **Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus.** *Mol Pharmacol* 2008, **74**:1463-1475.
6. Remy P, Doder M, Lees A, Turjanski N, Brooks D: **Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system.** *Brain* 2005, **128**:1314-1322.
7. Zweig RM, Cardillo JE, Cohen M, Giere S, Hedreen JC: **The locus ceruleus and dementia in Parkinson's disease.** *Neurology* 1993, **43**:986-991.
8. Boeve BF, Silber MH, Saper CB, Ferman TJ, Dickson DW, Parisi J: **Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease.** *Brain* 2007, **130**:2770-2788.
9. Ørskov L, Jakobsen J, Dupont E, de Fine Olivarius B, Christensen NJ: **Autonomic function in Parkinsonian patients relates to duration of disease.** *Neurology* 1987, **37**:1173-1178.
10. Devoto P, Flore G: **On the origin of cortical dopamine. Is it a co-transmitter in noradrenergic neurons?** *Curr Neuropharmacol* 2006, **4**:115-125.
11. Cornil CA, Ball GF: **Interplay among Catecholamine Systems: Dopamine Binds to α_2-Adrenergic Receptors in Birds and Mammals.** *J comp neurol* 2008, **511**:610-627.
12. Waterhouse BD, Devilbiss D, Fleischer D, Sessler FM, Simpson KL: **New perspectives on the functional organization and postsynaptic influences of the locus ceruleus efferent projection system.** *Adv Pharmacol* 1998, **42**:749-754.
13. Logan J, Wang G, Telang F, Fowler JS, Alexoff D, Zabroski J: **Imaging the norepinephrine transporter in humans with *(S,S)*-[^{11}C]*O*-methyl reboxetine and PET: problems and progress.** *Nuc Med Biol* 2007, **34**:667-679.
14. Booij J, Kemp P: **Dopamine transporter imaging with [^{123}I]FP-CIT SPECT: potential effects of drugs.** *Eur J Nucl Med Mol Imaging* 2008, **35**:424-438.
15. Isaias IU, Benti R, Cilia R, Canesi M, Marotta G, Gerundini P, Pezzoli G, Antonini A: **[123I]FP-CIT striatal binding in early Parkinson's disease patients with tremor vs. akinetic-rigid onset.** *Neuroreport* 2007, **14**:1499-1502.
16. Ma Y, Dhawan V, Mentis M, Chaly T, Spetsieris PG, Eidelberg D: **Parametric mapping of [18F]FPCIT binding in early stage Parkinson's disease: a PET study.** *Synapse* 2002, **45**:125-133.
17. Keren NI, Lozar CT, Harris KC, Morgan PS, Eckert MA: **In vivo mapping of the human locus coeruleus.** *NeuroImage* 2009, **47**:1261-1267.
18. Calvini P, Rodriguez G, Inguglia F, Mignone A, Guerra UP, Nobili F: **The basal ganglia matching tools package for striatal uptake semi-quantification: description and validation.** *Eur J Nuc Med Mol Imaging* 2007, **34**:1240-1253.
19. Metzger RR, Brown JM, Sandoval V, Rau KS, Elwan MA, Miller GW: **Inhibitory effect of reserpine on dopamine transporter function.** *Eur J Pharmacol* 2002, **456**:39-43.
20. Zahniser NR, Sorkin A: **Trafficking of dopamine transporters in psychostimulant actions.** *Semin Cell Dev Biol* 2009, **20**:411-417.
21. Ordway GA, Stockmeier CA, Cason GW, Klimek V: **Pharmacology and Distribution of Norepinephrine Transporters in the Human Locus Coeruleus and Raphe Nuclei.** *Journal Neurosci* 1997, **17**:1710-1719.
22. Lee CS, Samii A, Sossi V, Ruth TJ, Schulzer M, Holden JE: **In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease.** *Ann Neurol* 2000, **47**:493-503.
23. Brooks DJ: **The early diagnosis of Parkinson's disease.** *Ann Neurol* 1998, **44(Suppl 1)**:10-18.

24. Moore RY, Whone AL, Brooks DJ: **Extrastriatal monoamine neuron function in Parkinson's disease: An ^{18}F-dopa PET study.** *Neurobiol of Dis* 2008, **29**:381-390.
25. Sasaki M, Shibata E, Tohyama K, Takahashi J, Otsuka K, Tsuchiya K: **Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease.** *Neuroreport* 2006, **17**:1215-1218.
26. Chernoloz O, Mansari ME, Blier P: **Sustained administration of pramipexole modifies the spontaneous firing rate of dopamine, norepinephrine, and serotonin neurons in the rat brain.** *Neuropsychopharmacology* 2009, **34**:651-661.
27. Saunders C, Ferrer JV, Shi L, Chen J, Merrill G, Lamb ME: **Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism.** *Proc Natl Acad Sci USA* 2000, **97**:6850-6855.
28. Kahlig KM, Javitch JA, Galli A: **Amphetamine regulation of dopamine transport. Combined measurements of transporter currents and transporter imaging suport the endocytosis of an active carrier.** *J Biol Chem* 2004, **279**:8966-8975.
29. Jellinger KA: **Formation and development of Lewy pathology: a critical update.** *J Neurol* 2009, **256(Suppl 3)**:270-279.
30. Halliday GM, Ophof A, Broe M, Jensen PH, Kettle E, Fedorow H: **α-Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease.** *Brain* 2005, **128**:2654-2664.
31. Hoogendijk WJ, Pool CW, Troost D, van Zwieten E, Swaab DF: **Image analyser-assisted morphometry of the locus coeruleus in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.** *Brain* 1995, **118**:131-43.
32. McMillan PJ, White SS, Franklin A, Greenupa JL, Leverenz JB, Raskind MA, Szot P: **Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease.** *Brain Res* 2011, **1373**:240-252.
33. Cornil CA, Balthazart J, Motte P, Massotte L, Seutin V: **Dopamine activates noradrenergic receptors in the preoptic area.** *J Neurosci* 2002, **22**:9320-9330.
34. Newman-Tancredi A, Audinot-Bouchez V, Gobert A, Millan MJ: **Noradrenaline and adrenaline are high affinity agonists at dopamine D_4 receptors.** *Eur J Pharmacol* 1997, **319**:379-383.
35. Feinstein DL, Heneka MT, Gavrilyuk V, Dello Russo C, Weinberg G, Galea E: **Noradrenergic regulation of inflammatory gene expression in brain.** *Neurochem Int* 2002, **41**:357-365.
36. Traver S, Salthun-Lassalle B, Marien M, Hirsch EC, Colpaert F, Michel PP: **The neurotransmitter noradrenaline rescues septal cholinergic neurons in culture from degeneration caused by low-level oxidative stress.** *Mol Pharmacol* 2005, **67**:1882-91.
37. Frey K, Kilbourn M, Robinson T: **Reduced striatal vesicular monoamine transporters after neurotoxic but not after behavioral-sensitizing doses of methamphetamine.** *Eur J Pharmacol* 1997, **334**:273-279.
38. Mavridis M, Degryse AD, Lategan AJ, Marien MR, Colpaert FC: **Effects of locus coeruleus on parkinsonian signs, striatal dopamine and substantia nigra cel loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys: a possible role for the locus coeruleus in progression of Parkinson's disease.** *Neuroscience* 1991, **41**:507-523.
39. Rommelfanger KS, Weinshenker D: **Norepinephrine: the redheaded stepchild of Parkinson disease.** *Biochem Pharmacol* 2007, **74**:177-190.
40. Berglöf E, Strömberg I: **Locus coeruleus promotes survival of dopamine neurons in ventral mesencephalon. An in oculo grafting study.** *Exp Neurol* 2009, **216**:158-65.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2377/11/88/prepub

doi:10.1186/1471-2377-11-88
Cite this article as: Isaias *et al.*: Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC Neurology* 2011 **11**:88.

A role for locus coeruleus in Parkinson tremor

Isaias IU, Marzegan A, Pezzoli G, Marotta G, Canesi M, Biella GE, Volkmann J, Cavallari P. Front Hum Neurosci. 2011;5:179.

In this second study we attempted to investigate the correlation between LC-NAergic activity and Parkinson tremor.

Tremor activity was recorded in 12 consecutive PD subjects during a specific task (A-V200, see below) activating the LC. The intensity of tremor (i.e., the power of EMG signals), but not its frequency, significantly increased during the task in all patients. In six subjects, tremor appeared selectively during the task (see article *Figure 1* [also below] and *Table 3*).

In a second part of the study, we compared DAT binding values (investigated by means of SPECT and FP-CIT) of PD subjects with bilateral tremor (n = 27), unilateral tremor (n = 22), and no tremor (n = 33) (see article *Table 2*) and confirmed in an ample homogeneous study population the lack of a direct correlation between DAergic innervation loss and Parkinson tremor (Isaias et al., 2007).

The present study provides preliminary evidence of a LC-related activity in the generation of tremor in PD.

Relatively few neuroimaging studies in human (and none in PD patients) described an activation of LC (Coull et al., 1999; Sturm et al., 1999; Tracy et al., 2000; Knutson et al., 2000; Dunckley et al., 2005; Liddell et al., 2005; Sterpenich et al., 2006). Amongst these studies, several activated the LC using stress of various sorts, such as aversive loud noises (Tracy et al. 2000), monetary punishment (Knutson et al. 2000), pain stimuli (Dunckley et al. 2005), facial expressions of fear (Liddell et al., 2005) and emotionally aversive images such as snakes (Sterpenich et al. 2006). Sturm et al. (1999) used a non-specific but also non-aversive alertness task, in which subjects had to respond rapidly with a key-press every time a light was flashed. Coull et al., (1999) used a drug test showing that the α2-receptor agonist clonidine impaired subjects' performance of an attentional task (rapid serial visual presentation, or RSVP) and suppressed resting state LC activation, probably via stimulating inhibitory α2-autoreceptors in LC neurons. Regarding this last study it is worth mentioning that the effects of drugs on the LC depend both on dosage and on the subjects' arousal level (Arnsten, 2006). Moreover, they affect the activation not only of NA's source (the LC), but also of the transmitter's targets, including many cortical areas such as prefrontal and parietal, and also different transmitter systems such as that of dopamine (from Pan et al., 2004). Accordingly, the use of a NAergic drug guarantees neither that the LC will be activated, nor that observed brainstem activation must have the LC as its source. Among the many tasks activating the LC, the most easily applicable in a laboratory setting to obtain preliminary data for further brain imaging studies (e.g. fMRI or PET) was the challenge-driven attention task proposed by Raizada and Poldrack (Raizada and Poldrack, 2008). This task consist of an audio-visual simultaneity detection (A-V, see Raizada and Poldrack, 2008) in which subjects are presented with a flashed white disc and a burst of noise, their task being to decide whether the visual and the auditory stimuli were perceived, simultaneously or apart. In our study, a total of 50 visual and auditory stimuli were presented, 10–15 of them, in random order, delivered apart. The stimulus onset asynchrony between the visual and the auditory stimuli was set at 200 ms (A-V200), 100 ms (A-V100) or 0 ms (A-Vnull) in three separate trials. The A-V200 task demonstrated to specifically activate the brainstem and possibly the LC-NA system. Whereas, the A-V100 and the A-Vnull task did not appear to elicit a brainstem response and served as control tasks (see *Figure 2* in Raizada and Poldrack, 2008). Inter-stimuli

time (i.e., the time between a paired audio-visual stimulus and the following one) ranged randomly between 2.5 and 7 s. Each task was interpolated between a pre- (90 s) and a post-task period (up to epoch completion), with no audio-visual input. Trials were randomized for all patients. At the beginning of the experimental session, tremor activity was recorded for 60 s at rest and with no audio-visual input.

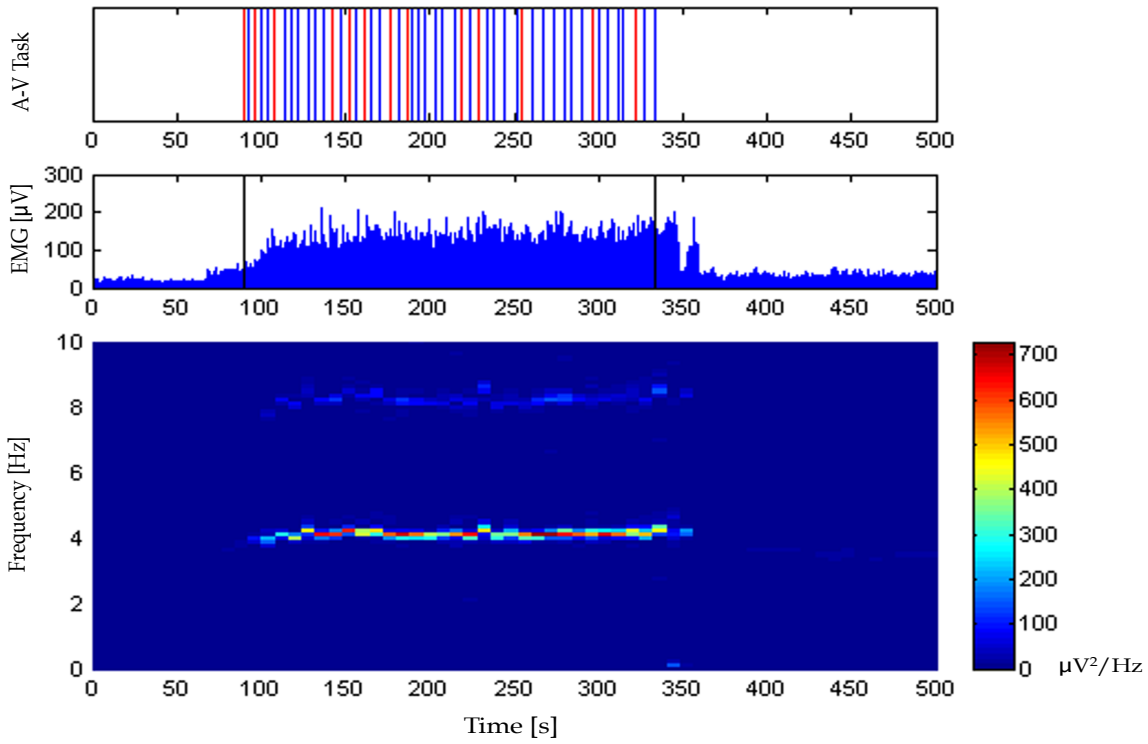


Figure 1 in Isaias et al., Front Hum Neurosci. 2011;5:179 (see article for description).

frontiers in
HUMAN NEUROSCIENCE

HYPOTHESIS AND THEORY ARTICLE
published: 03 January 2012
doi: 10.3389/fnhum.2011.00179

A role for locus coeruleus in Parkinson tremor

Ioannis U. Isaias[1,2,3]*, Alberto Marzegan[1], Gianni Pezzoli[2], Giorgio Marotta[4], Margherita Canesi[2], Gabriele E. M. Biella[5], Jens Volkmann[3] and Paolo Cavallari[1]

[1] Dipartimento di Fisiologia Umana, Laboratorio Analisi del Movimento L.A.M.B., Università degli Studi di Milano, Milano, Italy
[2] Centro per la Malattia di Parkinson e i Disturbi del Movimento, Istituti Clinici di Perfezionamento, Milano, Italy
[3] Neurologische Klinik und Poliklinik, Universitätsklinik Würzburg, Würzburg, Germany
[4] Dipartimento di Medicina Nucleare, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy
[5] Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Segrate (MI), Italy

Edited by:
Hans-Jochen Heinze, University of Magdeburg, Germany

Reviewed by:
Hidenao Fukuyama, Kyoto University, Japan
Alfons Schnitzler, Heinrich-Heine-University, Germany

***Correspondence:**
Ioannis U. Isaias, Dipartimento di Fisiologia Umana, Laboratorio Analisi del Movimento L.A.M.B., Università degli Studi di Milano, Via Mangiagalli, 32, I-20133 Milano, Italy.
e-mail: ioannis.isaias@unimi.it

We analyzed rest tremor, one of the etiologically most elusive *hallmarks* of Parkinson disease (PD), in 12 consecutive PD patients during a specific task activating the locus coeruleus (LC) to investigate a putative role of noradrenaline (NA) in tremor generation and suppression. Clinical diagnosis was confirmed in all subjects by reduced dopamine reuptake transporter (DAT) binding values investigated by single photon computed tomography imaging (SPECT) with [^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane (FP-CIT). The intensity of tremor (i.e., the power of Electromyography [EMG] signals), but not its frequency, significantly increased during the task. In six subjects, tremor appeared selectively during the task. In a second part of the study, we retrospectively reviewed SPECT with FP-CIT data and confirmed the lack of correlation between dopaminergic loss and tremor by comparing DAT binding values of 82 PD subjects with bilateral tremor ($n = 27$), unilateral tremor ($n = 22$), and no tremor ($n = 33$). This study suggests a role of the LC in Parkinson tremor.

Keywords: Parkinson disease, tremor, locus coeruleus, noradrenaline

INTRODUCTION

Tremor in Parkinson disease (PD) is characterized by 4–6 Hz activity at rest in the limbs with distal predominance. Despite its clinical importance and the burden of morbidity associated with it, the etiology of tremor is unknown, and its medical treatment commonly ineffective.

The tremor in PD is remarkable for several features: (1) it is neither a consistent nor a homogeneous feature across patients or within an individual patient's disease course; (2) it may diminish in the end-stage of PD; (3) it occurs predominantly at rest and is reduced or disappears by action; (4) it increases in amplitude or can be triggered by maneuvers such as walking or psychological states as anxiety or stress (specific tasks, like simple arithmetic calculation, may induce stress-related tremor) (Deuschl et al., 1998); (5) it is not present during sleep; (6) it may be the predominant or the only clinical sign for years before the appearance of akinesia (Brooks et al., 1992); and (7) it poorly correlates with the nigrostriatal dopaminergic deficits (Isaias et al., 2007).

Although there is converging evidence of independent oscillating circuits within a widespread "tremor-generating network" (Mure et al., 2011), there is no conclusive explanation for the onset of tremor in what it is fundamentally a hypokinetic movement disorder.

Our hypothesis is that the locus coeruleus (LC) is involved in the generation of tremor in PD. Preliminary evidence of a role of adrenaline and noradrenaline (NA) in parkinsonian tremor emerged from studies published in the 1960s and 1980s (Constas, 1962; Colpaert, 1987; Wilbur et al., 1988).

In this study we applied a specific and selective task activating the LC (Raizada and Poldrack, 2008), the main source of NA in the brain, while recording tremor in 12 consecutive subjects with PD.

In a second part of the study, we confirmed retrospectively, in a large and homogeneous cohort of PD subjects with and without tremor, the lack of correlation between tremor and dopaminergic innervation loss, investigated by means of single photon computed tomography imaging (SPECT) with [^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane (FP-CIT) (Isaias et al., 2007).

MATERIALS AND METHODS

PATIENTS AND CLINICAL ASSESSMENT

The diagnosis of PD was made in all subjects according to the UK Parkinson Disease Brain Bank criteria and patients were evaluated by means of the Unified Parkinson Disease Rating Scale motor part (UPDRS-III). Two additional UPDRS subscores (i.e., a tremor subscore and an akinetic-rigid (AK) subscore) were also calculated (Isaias et al., 2007). L-Dopa Equivalent Daily Doses (LEDDs) were recorded as follows: 100 mg levodopa = 1.5 mg pramipexole = 6 mg ropinirole.

Clinical inclusion criteria were: (1) UPDRS part I and IV of 0; (2) Hoehn and Yahr (H&Y) stage of 1 or 2 in drugs-off state at the time of SPECT (i.e., after overnight withdrawal of specific drugs for PD; no patient was taking long-acting dopaminergic drugs); (3) absence of any sign indicative for atypical parkinsonism (e.g., gaze abnormalities, autonomic dysfunction, and significant psychiatric disturbances) over a follow-up time of at least three years after symptoms onset; (4) positive clinical improvement at UPDRS after L-Dopa intake (i.e., >30% from drug-off state) at some point during the three years of follow-up; (5) a normal

Magnetic Resonance Imaging (MRI) (no sign of white matter lesion or atrophy).

Additional inclusion criteria for the 12 consecutive patients who took part to the prospective study were: (1) no other disease other than PD; (2) no therapy change for the past six months prior to the study; only patients with L-Dopa or dopamine agonist were recruited and the uptake of any drugs affecting the noradrenergic system (including, β-blockers, MAO-B inhibitors, etc.,) was ruled out; (3) no cognitive decline as well as no deficit in visual attention. All subjects had normal scores at the Mini-Mental State examination, the Clock Drawing Test, the Frontal Assessment Battery, the Rey Auditory Verbal Learning Test; the Digit Span Test, and the Attentive Matrices Test. Depression was also ruled out by means of the Beck Depression Inventory.

Fifteen healthy controls (four males, 11 female; 63 years on average ±9 SD; range: 51–74 years) were prospectively enrolled for comparisons of FP-CIT uptake. Healthy controls were recruited from the general population (relatives of patients were excluded) and, at the time of the study, did not suffer from any disease and were not taking any medication.

The Local Ethics Committee approved the study and all subjects gave their informed consent.

IMAGING

Dopamine-transporter (DAT) values were measured by means of SPECT with [^{123}I] FP-CIT.

SPECT data acquisition and reconstruction has been described in details elsewhere (Isaias et al., 2010). In brief, intravenous administration of 110–140 MBq of FP-CIT (DaTSCAN, GE-Healthcare, UK) was performed 30–40 min after thyroid blockade (10–15 mg of Lugol oral solution) in subjects with PD subsequently overnight withdrawal of dopaminergic therapy and in healthy controls. Brain SPECT was performed three hours later by means of a dedicated triple detector gamma-camera (Prism 3000, Philips, Eindhoven, The Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (four subsets of acquisitions, matrix size 128 × 128, radius of rotation 12.9–13.9 cm, continuous rotation, angular sampling: 3 degree, duration: 28 min). Brain sections were reconstructed with an iterative algorithm Ordered Subsets Expectation Maximization (OSEM, four iterations and 15 subsets) and then processed by 3D filtering (Butterworth, order 5, cut-off 0.31 pixel-1) and attenuation correction (Chang method, factor 0.12).

FP-CIT uptake values for the caudate nucleus and putamen of both PD patients and healthy controls were calculated according to Basal Ganglia Matching Tool (Calvini et al., 2007). The FP-CIT uptake values in other brain regions were obtained with the creation, in the shapes menu of WFU Pick Atlas Tool, of specific volumes of interest (VOIs).

Contralateral refers to the side opposite to tremor or to the most affected side. For healthy controls we referred to the right side as *ipsilateral* (Isaias et al., 2007).

AUDIO-VISUAL TASK AND EXPERIMENTAL DESIGN

We used the task of audio-visual simultaneity detection (A-V, Raizada and Poldrack, 2008) in which subjects were presented with a flashed white disc and a burst of noise, their task being to decide whether the visual and the auditory stimuli were perceived, simultaneously or apart. A total of 50 visual and auditory stimuli were presented, 10–15 of them, in random order, delivered apart. The stimulus onset asynchrony between the visual and the auditory stimuli was set at 200 ms (A-V_{200}), 100 ms (A-V_{100}) or 0 ms (A-V_{null}) in three separate trials. The A-V_{200} task demonstrated to specifically activate the LC-NA system. The A-V_{100} and the A-V_{null} task did not appear to elicit a brainstem response and served as control tasks (Raizada and Poldrack, 2008). Inter-stimuli time (i.e., the time between a paired audio-visual stimulus and the following one) ranged randomly between 2.5 and 7 s. Each task was interpolated between a *pre-* (90 s) and a *post-task* period (up to epoch completion), with no audio-visual input. Trials were randomized for all patients. At the beginning of the experimental session, tremor activity was recorded for 60 s at rest and with no audio-visual input.

RECORDINGS

Muscle activity was registered with surface Electromyography (EMG) electrodes placed on the extensor digitorum communis (EDC), the flexor digitorum communis (FDC), and the opponens pollicis (OP) muscles, bilaterally. To include only EMG bursting activity, the EMG was high-pass filtered off-line at 60 Hz and rectified (Timmermann et al., 2003). Recordings were performed during rest and during the audio-visual task while the subjects relaxed their arm and hand muscles. The examination was performed at the same day-time (10:00 h) for all patients. Patients were asked not to drink alcoholic, caffeine, or tea from the evening before the examination and they were off any medication in the last three days before the task. The examination was performed in a quiet room after allowing enough time for the patients to become familiar with the surroundings.

GENERAL STATISTICAL ANALYSIS

Tremor frequency and intensity were compared by use of the Wilcoxon signed-rank test for matched pairs. A pair was the same individual but at a different time during the task (e.g., pre-task vs. task). Student's *t*-test was applied to compare DAT binding values among patient sub-groups and healthy controls. Statistical analyses were performed with the JMP statistical package, version 8.0.2 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Demographic and clinical features are listed in **Table 1A** for all subjects with PD and further detailed in **Table 1B** for the 12 patients who performed the audio-visual tasks.

Table 2 lists DAT binding values. All 12 subjects enrolled for the EMG recording showed a reduced DAT binding value in the striatum (12% and 22% in the ipsilateral and contralateral caudate nucleus; 41% and 53% in the ipsilateral and contralateral putamen), thus further confirming the clinical diagnosis. DAT binding values did not differ between PD subjects with and without tremor in all regions investigated, also when weighted for demographic (age at SPECT, age at onset, gender) and clinical data (disease duration, disease severity, and LEDDs). Of interest, DAT binding values in the pons (where the LC is located) resulted

Table 1 | Clinical and demographic characteristics of the 82 subjects with PD (A) and of the 12 patients enrolled for the audio-visual task (B).

Table 1A

	Bilateral tremor ($n = 27$; 21 M)	On-side tremor ($n = 22$; 16 M)	No tremor ($n = 33$; 22 M)
Age at onset (years)	54 ± 11	58 ± 8	55 ± 8
Disease duration (years)	8 ± 5	5 ± 3	7 ± 5
UPDRS-III (0–108) score	23 ± 10	17 ± 7.4	18.7 ± 9.7
UPDRS-tremor (0–28) score	5.2 ± 2.5	2 ± 0.8	0
UPDRS-AK (0–48) score	9.3 ± 5	6.6 ± 3.5	10.2 ± 6.6
LEDDs (mg)	424.6 ± 270	356.5 ± 256.1	506.0 ± 282.1

Table 1B

Patient	Sex	Age	Disease duration	H&Y	UPDRS III—tremor score left/right	UPDRS III—item 22 left/right (AK score)	UPDRS III total score	LEDDs (mg)
1	M	64	18	2	3/2	2/2 (13)	28	560
2	M	73	10	2	3/0	2/2 (11)	27	510
3	M	56	10	2	2/0	1/0 (7)	20	710
4	M	70	5	2	0/2	1/2 (5)	12	580
5	F	71	4	2	0/2	2/1 (5)	13	610
6	M	73	4	2	3/0	2/0 (5)	19	300
7	M	58	12	2	3/1	2/2 (8)	16	210
8	M	70	4	2	3/0	2/0 (8)	24	480
9	M	69	9	2	0/3	2/2 (11)	23	400
10	F	76	3	2	3/1	2/2 (11)	22	300
11	M	69	6	2	2/0	2/2 (5)	12	255
12	M	65	7	2	0/1	2/2 (9)	18	450

*Each patient's disease severity was assessed using the Hoehn and Yahr (H&Y maximum is 5) stages and the Unified Parkinson Disease Rating Scale part III (UPDRS-III; maximum score is 108). UPDRS item 20 and 21 refer to rest and action or postural tremor; item 22 refers to rigidity. UPDRS akinetic-rigid (AK) score was the sum of items: 18 (speech), 19 (facial expression), 22 (rigidity), 27 (arising from chair), 28 (posture), 29 (gait), 30 (postural stability), 31 (body bradykinesia); tremor score was the sum of items: 20 (tremor at rest), and 21 (action or postural tremor of hands). L-Dopa Equivalent Daily Doses (LEDDs) were recorded as follows: 100 mg levodopa = 1.5 mg pramipexole = 6 mg ropinirole. In **Table 1A**, values are means ± SD. See text for inclusion criteria.*

significantly higher in PD patients with tremor when compared to healthy controls. Still, no difference was found between PD with and without tremor within this region.

All 12 patients showed the typical parkinsonian rest tremor in the frequency range of 4–6 Hz. No changes in tremor frequency or intensity were found during A-V_{100} and A-V_{null}. On the contrary, tremor intensity greatly and consistently increased during the A-V_{200} task in all patients ($p < 0.001$) (**Table 3**). Interestingly, tremor frequency during the same task did not significantly change (**Table 3**, first column). The figure illustrates tremor behavior in one patient during one trial with the A-V_{200} task (*n*. 4 in **Table 3**). Note that EMG activity of left EDC (**Figure 1**, mid box) appears only during the audio-visual task. Interestingly, the power spectral analysis (**Figure 1**, lower box) clearly describes, during the audio-visual task, an enhancement of tremor intensity and a sustained 4.2 Hz tremor frequency. Similar results were found in all other PD subjects (**Table 3**).

DISCUSSION

The present study suggests a LC-related activity in the generation of tremor in PD. We also confirmed in an ample homogeneous study population the lack of a correlation between dopaminergic innervation loss and parkinsonian tremor (Isaias et al., 2007).

There is increasing evidence that resting tremor in PD is associated with a distinct cerebello-thalamic circuit involving the ventral intermediate nucleus of the thalamus (Vim), the cerebellum, and the motor cortex. Indeed, a distinct ponto-thalamo-cortical pattern, possibly involving the cerebellum/dentate nucleus, the primary motor cortex, and the caudate/putamen have been identified in tremor-predominant PD patients (Mure et al., 2011). More recently, a selective dopaminergic depletion of the globus pallidus (but not striatum) has been reported to correlate with clinical tremor severity and suggested to link the basal ganglia with the cerebello-thalamic circuit in the onset of rest tremor in PD (Helmich et al., 2011). We failed to find any difference of dopamine innervation loss (including the globus pallidus) between PD subjects with bilateral, unilateral, or without tremor. Indeed, structural lesions of the substantia nigra produce akinetic syndromes but not tremor (Jenner and Marsden, 1984). The role of the cerebellum has been also questioned by a multi-tracer PET study showing that at least part of the cerebellar hyperactivation seen in tremulous PD might be related to akinesia and rigidity (Ghaemi et al., 2002). The development of rest tremor associated with PD in a patient who had prior cerebellectomy further suggested that the cerebellum is not the primary origin of tremor (Deuschl et al., 1999).

Table 2 | FP-CIT SPECT binding values.

	Bilateral tremor ($n = 27$)	One-side tremor (right $n = 11$; left $n = 11$)	No tremor ($n = 33$)	Healthy controls ($n = 15$)
Frontal lobe	0.11 ± 0.08	0.07 ± 0.11	0.11 ± 0.09	0.06 ± 0.08
Cerebellum	0.12 ± 0.07**	0.12 ± 0.06**	0.11 ± 0.09**	0.05 ± 0.04
Pons	0.54 ± 0.13*	0.53 ± 0.11*	0.49 ± 0.21	0.42 ± 0.14
Brainstem	1.52 ± 1.32	1.97 ± 2.14**	1.85 ± 1.48**	0.54 ± 0.13
Midbrain	0.67 ± 0.16	0.69 ± 0.13	0.63 ± 0.21	0.69 ± 0.15
Thalamus contralateral	0.55 ± 0.14	0.57 ± 0.19	0.52 ± 0.19	0.6 ± 0.13
Thalamus ipsilateral	0.57 ± 0.14	0.6 ± 0.15	0.55 ± 0.16	0.59 ± 0.17
GPe contralateral	1.32 ± 0.33**	1.31 ± 0.26**	1.22 ± 0.42**	2 ± 0.32
GPe ipsilateral	1.32 ± 0.39**	1.49 ± 0.28**	1.3 ± 0.42**	2.2 ± 0.32
GPi contralateral	1.22 ± 0.28**	1.21 ± 0.24**	1.13 ± 0.34**	1.6 ± 0.33
GPi ipsilateral	1.21 ± 0.32**	1.32 ± 0.23**	1.19 ± 0.33**	1.76 ± 0.28
Caudate contralateral	3.14 ± 1.02**	3.17 ± 0.81**	3 ± 0.94**	4.95 ± 0.98
Caudate ipsilateral	3 ± 0.89**	3.65 ± 0.85**	3 ± 0.97**	4.94 ± 0.96
Putamen contralateral	2 ± 0.73**	2 ± 0.59**	2 ± 0.86**	4.6 ± 0.83
Putamen ipsilateral	2.17 ± 0.75**	2.5 ± 0.64**	2.22 ± 0.87**	4.6 ± 0.83

Data are shown as means ± SD (with the bilateral occipital cortex as reference region). Contralateral refers to the side opposite to tremor or to the most affected side for patients without tremor. For healthy subjects, we conventionally referred to the right side as ipsilateral. For the one-side tremor group, the ipsilateral brain regions refer to the hemisoma without rest or postural tremor. The absence of tremor (bilaterally or unilaterally) derived by a UPDRS-III score of 0 at the previous visit (within two months) before SPECT and by the anamnestic statement by the patient of having not experienced tremor (bilaterally or at the non-tremor side).

FP-CIT uptake values for the caudate nucleus and putamen were calculated according to Basal Ganglia Matching Tool. The FP-CIT uptake values in other brain regions were obtained with the creation, in the shapes menu of WFU Pick Atlas Tool, of specific volumes of interest (VOIs).

DAT binding values did not differ among PD sub-groups (with or without tremor), also when weighted for demographic (age at SPECT, age at onset, gender) and clinical data (disease duration, disease severity, and LEDDs). Therefore, statistical significance is marked only vs. healthy controls.

$^*p = 0.01$; $^{**}p < 0.001$.

GPe = globus pallidus pars externa; GPi = globus pallidus pars interna.

Table 3 | Changes of tremor intensity (i.e., power of EMG signals) during the audio-visual task.

Patient	Muscle	f_{PEAK} (f_{AVG})	Pre-task (0–90 s)	A-V_{200} task (90 s–EoT)	Post-task early (EoT–400 s)	Post-task late (400–500 s)
			P_{PEAK} (P_{AVG})	P_{PEAK} (P_{AVG})	P_{PEAK} (P_{AVG})	P_{PEAK} (P_{AVG})
1	R-flex	4.8 *(4.6)*	83 *(50)*	4250 *(1118)*	1975 *(296)*	–
2	R-flex	4.9 *(4.9)*	–	14,130 *(1405)*	9834 *(2704)*	1703 *(376)*
3	L-ext	5.8 *(5.8)*	380 *(181)*	1755 *(912)*	349 *(260)*	265 *(70)*
4	L-ext	4.2 *(4.2)*	–	800 *(296)*	266 *(100)*	–
5	R-ext	4.9 *(5.1)*	2686 *(819)*	8022 *(2240)*	3686 *(501)*	–
6	L-opp	3.9 *(3.9)*	–	14,759 *(4286)*	–	–
7	L-flex	4.9 *(4.9)*	–	9563 *(2937)*	4314 *(1552)*	–
8	L-flex	4.2 *(4.2)*	–	7393 *(838)*	–	–
9	R-opp	4.3 *(4.2)*	1981 *(719)*	8806 *(2963)*	1222 *(231)*	–
10	L-ext	5.5 *(5.3)*	–	1244 *(86)*	145 *(35)*	109 *(41)*
11	L-opp	3.9 *(3.9)*	–	2907 *(267)*	–	–
12	R-opp	3.5 *(3.5)*	–	135 *(56)*	–	–

Pre-task refers to a period of 90 s without stimuli preceding the audio-visual task (A-V_{200}). Post-task early refers to the first time period without stimuli after the task end (from End-of-Task (EoT) to 400 s counting from the start of the EMG recording); post-task late refers to the last part of the period without stimuli (from 400 to the end of EMG recording). We listed only the activity of the most-affected muscle. Tremor frequency, when tremor was present, did not change throughout the task. The intensity of tremor significantly increased during the task in all subjects.

f_{PEAK} = peak frequency; f_{AVG} = average frequency; P_{PEAK} = tremor power peak; P_{AVG} = tremor power average; "–" refers to absence of tremor activity at EMG. Note that given the randomized presentation of the visual and auditory stimuli, the EoT time differed among subjects (ranging from 127 to 198 s).

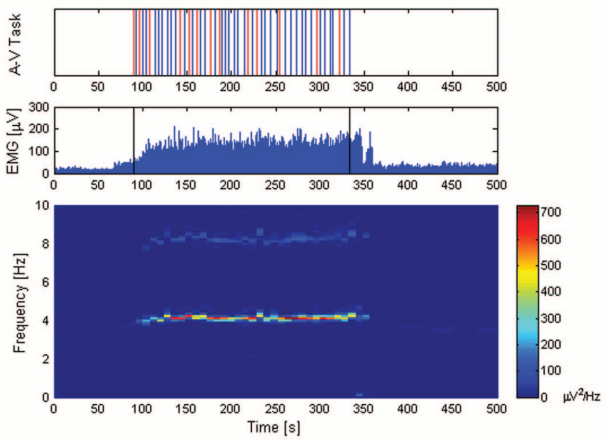


FIGURE 1 | Tremor behavior during the audio-visual (A-V_{200}) task in one patient. *Upper box:* Audio-visual task (A-V_{200} task). Red lines indicate when flashed white disk and noise were not simultaneous, blue lines when they were simultaneous. The stimulus onset asynchrony between the visual and the auditory stimuli was set at 200 ms (visual preceding auditory). A total of 50 visual and auditory stimuli were presented. Space between lines indicate time interval (ranging between 2.5 and 7 s). *Mid box:* EMG recordings (in blue), high-pass filtered off-line at 60 Hz and rectified. Single vertical lines (in black) show the start and end of the A-V_{200} task, respectively. *Lower box:* Spectral analysis of tremor activity showing a sustained tremor frequency at 4.2 Hz. The harmonic double tremor frequency is also clearly represented. The power of EMG signals (intensity of tremor) is shown in colors (right column scale).

The LC is a cluster of NA-containing neurons located in the upper dorsolateral pontine tegmentum. LC neurons fire in a tonic or phasic mode (Aston-Jones and Cohen, 2005). Tonic activity is characterized by sustained and highly regular discharge pattern that is highest during wakefulness, decreases during slow-wave sleep, and virtually ceases during Rapid Eye Movement (REM) sleep. LC phasic mode involves activation of LC neurons following task-relevant processes in response to environmental stimuli that elicit behavioral arousal and exploratory behavior (Aston-Jones and Cohen, 2005). Animal studies reported that neurons in the LC can show also a synchronous activity. Synchronous oscillations would rely on electronic coupling and are seemingly independent of synaptic transmission. Electrotonic coupling between LC neurons could serve to facilitate synchrony in the event of a large afferent input (Ishimatsu and Williams, 1996).

LC neurons have extensively branched axons that project throughout the neuraxis and provide the sole source of NA to the neocortex, cerebellum, and most of the thalamus (Aston-Jones and Cohen, 2005), all possibly involved in tremor onset. In particular, the release of NA from LC axons causes inhibition of ongoing purkinje cell activity, thus facilitating one or more functions of regions under purkinje inhibitory control. Noradrenergic input to the thalamic cells promotes a single spike-firing mode of activity in the thalamus that has been related to increased cortical activity and responsiveness. The LC also innervates extensively and it is the sole source of cortical NA. It is likely that this noradrenergic input to the neocortex is excitatory and contributes to the generally recognized role of the LC as a major wakefulness-promoting nucleus (Samuels and Szabadi, 2008a,b).

The biochemistry of NA neurons has great relevance to PD (Delaville et al., 2011; Isaias et al., 2011). Of note, in the normal condition, dopamine β-hydroxylase synthesizes NA from dopamine. The LC-NA system modulates the survival of its target neuronal populations, which include dopaminergic neurons in the substantia nigra (Mavridis et al., 1991; Frey et al., 1997; Rommelfanger and Weinshenker, 2007). The LC may also directly influence the activity of the nigrostriatal dopaminergic system. Single-cell recording studies demonstrated a noradrenergic modulation of midbrain dopamine neuronal activity consistent with a direct pathway from the LC to the substantia nigra. In particular, injections of the adrenergic neurotoxin *N*-(2-Chloroethyl)-*N*-ethyl-2 bromo benzylamine hydrochloride (DSP-4) in the ventral tegmental area or direct lesioning of the LC by local injection of 6-hydroxy-dopamine (6-OHDA) resulted in a significant decrease in the basal release of dopamine in the caudate nucleus (Collingridge et al., 1979; Lategan et al., 1992), and result in the compensatory up-regulation of striatal D2 receptors (Harro et al., 2003). NA facilitates burst firing of substantia nigra pars compacta, while administration of the α1-adrenoceptor antagonist prazosin attenuates firing (Grenhoff and Swensson, 1993).

The LC is thought to degenerate in PD as part of the disease pathology (Braak et al., 2003). Still, Lewy pathology poorly correlates with neuronal loss in specific areas, thus its predictive validity for neuronal disintegration is questionable (Jellinger, 2009). Moreover, noradrenergic neurons in the LC are relatively preserved in early PD (Isaias et al., 2011; Pavese et al., 2011) and do not exhibit the same intracellular changes as in the substantia nigra (Halliday et al., 2005). Finally, the LC may degenerate only in specific PD patients (Cash et al., 1987). Of interest, animal studies showed a significantly increased activity of noradrenergic neurons in LC after unilateral lesion of the nigrostriatal pathway (Wang et al., 2009).

Mevawalla and colleagues (Mevawalla et al., 2009) reported a case of unilateral rest tremor in a patient with vascular parkinsonism and contralateral lesion of the LC. The Authors suggest that the unilateral LC lesion contributed to the pathogenesis of contralateral tremor. There are several concerns related to this clinical case that prevent any firm conclusion. The patient suffered from vascular parkinsonism and macroscopically presented vascular lesions in the globus pallidus bilaterally, right putamen and internal capsule. The presence of tongue tremor supports a cerebellar involvement, which was not investigated by the Authors. Moreover, no functional studies have been carried out *in vivo*, nor functional staining (e.g., TH-staining) in *ex vivo* specimina. Last but not least, the LC projects almost exclusively ipsilaterally and, therefore, the tremor would relate to the intact LC rather than the lesioned one. This would eventually support, rather than confute our hypothesis. Still, based on our preliminary results, we cannot exclude that tremor in PD derives from a paradoxical effect of NA and dopamine depletion (Dzirasa et al., 2010).

The main limitation of this study relates to poor specificity of the A-V_{200} task. Still, this task proved to selectively activate

the brainstem in an *in vivo* imaging study (Raizada and Poldrack, 2008). We also did not find changes in tremor activity during A-V_{100} and A-V_{null} trials, both tasks being not able to elicit an activation of brainstem but of other brain areas (i.e., frontal region) (Raizada and Poldrack, 2008).

Secondly, the LC-NA system and the noradrenaline molecular transporters (NET) should be ideally investigated *in vivo* by dedicated, highly specific radiotracers displaying low background non-NET binding, high sensitivity to variations in NET density, and fast kinetics. As such, a radiotracer is not available for large clinical studies, we retrospectively collected data of SPECT with FP-CIT in a large, homogeneous cohort of PD patients out of our database (Centro per la Malattia di Parkinson e i Disturbi del Movimento, Milano). Although FP-CIT is mainly used for assessing striatal dopamine reuptake transporters (DATs), it has shown some, albeit lower, sensitivity, to NET (Booij and Kemp, 2008). Hence, when applied to an anatomical region with known low DAT capacity such as the LC, it allows investigation on the NA-dependent synaptic activity (Isaias et al., 2011). In the brainstem, no other anatomical region, beside the LC, show clearly detectable density of NET (nor DAT).

Studying the LC can be challenging to say the least. In particular, (1) directly recording in humans the activity of LC is not applicable given its anatomical structure and location; (2) the size of LC is at the spatial resolution limits of currently available imaging techniques; and (3) even receptor binding (or displacement) studies and PET might not catch its phasic electrical activity. Anyhow, along our results, it is tempting to hypothesize an active role of the LC-NA system in triggering tremor onset in PD. While the frequency of parkinsonian tremor might be related to the intrinsic oscillating properties of dopamine depleted circuits, the onset and intensity of tremor might relate to the activity of the LC and of its noradrenergic output.

The introduction of the LC in the neural network dynamics of parkinsonian tremor might well explain many of its remarkable features. In particular, (1) tremor would appear only in PD subject with no functional damages of the LC; (2) eventually, along with PD progression and a consequent degeneration of LC-NA system, tremor will diminish; (3) tremor will appear during maneuvers that trigger LC activity, above all stress; and (4) PD patients do not manifest tremor during sleep being the LC silent. Finally, given a putative neuroprotective and compensatory activity of LC-NA on its target cells (including the substantia nigra and the striatum), it is tempting to speculate that an intact, or hyperactive, LC-NA system might be responsible for a more benign progression of PD in patients with tremor.

In conclusion, parkinsonian tremor would be the outcome of subtle derangements in the finely tuned NA-DA interplay, where the dopaminergic failure would involve complex noradrenergic adjustments leading to motor anomalies in the presence of an intact LC, which might be only a necessary state but not the direct cause of rest tremor in PD.

All these pending questions obviously require further studies in the future both by imaging and laboratory techniques. On this track, we hypothesize that parkinsonian tremor might represent the clinical sign of an enhanced LC activity as possible compensatory process over dopaminergic loss.

ACKNOWLEDGMENTS

The study was funded by a grant of the Grigioni Foundation for Parkinson disease.

REFERENCES

Aston-Jones, G., and Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.* 28, 403–450.

Booij, J., and Kemp, P. (2008). Dopamine transporter imaging with [123I]FP-CIT SPECT: potential effects of drugs. *Eur. J. Nucl. Med. Mol. Imaging* 35, 424–438.

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A., Jansen Steur, E. N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211.

Brooks, D. J., Playford, E. D., Ibanez, V., Sawle, G. V., Thompson, P. D., Findley, L. J., and Marsden, C. D. (1992). Isolated tremor and disruption of the nigrostriatal dopaminergic system: an 18F-dopa PET study. *Neurology* 42, 1554–1560.

Calvini, P., Rodriguez, G., Inguglia, F., Mignone, A., Guerra, U. P., and Nobili, F. (2007). The basal ganglia matching tools package for striatal uptake semi-quantification: description and validation. *Eur. J. Nucl. Med. Mol. Imaging* 34, 1240–1253.

Cash, R., Dennis, T., L'Heureux, R., Raisman, R., Javoy-Agid, F., and Scatton, B. (1987). Parkinson's disease and dementia: norepinephrine and dopamine in locus ceruleus. *Neurology* 37, 42–46.

Collingridge, G. L., James, T. A., and MacLeod, N. K. (1979). Neurochemical and electrophysiological evidence for a projection from the locus coeruleus to the substantia nigra. *J. Physiol.* 290, 44.

Colpaert, F. C. (1987). Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. *Neuropharmacology* 26, 1431–1440.

Constas, C. (1962). The effects of adrenaline, noradrenaline, and isoprenaline on parkinsonian tremor. *J. Neurol. Neurosurg. Psychiatry* 25, 116.

Delaville, C., Deurwaerdère, P. D., and Benazzouz, A. (2011). Noradrenaline and Parkinson's disease. *Front. Syst. Neurosci.* 5, 31. doi: 10.3389/fnsys.2011.00031

Deuschl, G., Bain, P., and Brin, M. (1998). Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov. Disord.* 13, 2–23.

Deuschl, G., Wilms, H., Krack, P., Würker, M., and Heiss, W. D. (1999). Function of the cerebellum in Parkinsonian rest tremor and Holmes' tremor. *Ann. Neurol.* 46, 126–128.

Dzirasa, K., Phillips, H. W., Sotnikova, T. D., Salahpour, A., Kumar, S., Gainetdinov, R. R., Caron, M. G., and Nicolelis, M. A. (2010). Noradrenergic control of cortico-striato-thalamic and mesolimbic cross-structural synchrony. *J. Neurosci.* 30, 6387–6397.

Frey, K., Kilbourn, M., and Robinson, T. (1997). Reduced striatal vesicular monoamine transporters after neurotoxic but not after behavioral-sensitizing doses of methamphetamine. *Eur. J. Pharmacol.* 334, 273–279.

Ghaemi, M., Raethjen, J., Hilker, R., Rudolf, J., Sobesky, J., Deuschl, G., and Heiss, W. D. (2002). Monosymptomatic resting tremor and Parkinson's disease: a multitracer Positron emission tomographic study. *Mov. Disord.* 17, 782–788.

Grenhoff, J., and Swensson, T. H. (1993). Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *Eur. J. Pharmacol.* 233, 79–84.

Halliday, G. M., Ophof, A., Broe, M., Jensen, P. H., Kettle, E., and Fedorow, H. (2005). α-Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease. *Brain* 128, 2654–2664.

Harro, J., Terasmaa, A., Eller, M., and Rinken, A. (2003). Effect of denervation of the locus coeruleus projections by DSP-4 treatment on [3H]-raclopride binding to dopamine D2 receptors and D2 receptor-G protein interaction in the rat striatum. *Brain Res.* 976, 209–216.

Helmich, R. C., Janssen, M. J. R., Oyen, W. J. G., Bloem, B. R., and Toni, I. (2011). Pallidal dysfunction

drives a cerebellothalamic circuit into Parkinson tremor. *Ann. Neurol.* 69, 269–281.

Isaias, I. U., Benti, R., Cilia, R., Canesi, M., Marotta, G., Gerundini, P., Pezzoli, G., and Antonini, A. (2007). [^{123}I]FP-CIT striatal binding in early Parkinson's disease patients with tremor vs. akinetic-rigid onset. *Neuroreport* 14, 1499–1502.

Isaias, I. U., Marotta, G., Pezzoli, G., Sabri, O., Schwarz, J., Crenna, P., Classen, J., and Cavallari, P. (2011). Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC Neurol.* 11, 88.

Isaias, I. U., Marotta, M., Hirano, S., Canesi, M., Benti, R., Righini, A., Tang, C., Cilia, R., Pezzoli, G., Eidelberg, D., and Antonini, A. (2010). Imaging essential tremor. *Mov. Disord.* 25, 679–686.

Ishimatsu, M., and Williams, J. T. (1996). Synchronous activity in locus coeruleus results from dendritic interactions in peri-coerulear regions. *J. Neurosci.* 16, 5196–5204.

Jellinger, K. A. (2009). Formation and development of Lewy pathology: a critical update. *J. Neurol.* 256, 270–279.

Jenner, P., and Marsden, C. D. (1984). "Neurochemical basis of parkinsonian tremor," in *Movement Disorders: Tremor,* eds L. J. Findley and R. Capildeo (Oxford, England: Oxford University Press), 305–319.

Lategan, A. J., Marien, M. R., and Colpaert, F. C. (1992). Suppression of nigrostriatal and mesolimbic dopamine release *in vivo* following noradrenaline depletion by DSP-4: a microdialysis study. *Life Sci.* 50, 995–999.

Mavridis, M., Degryse, A. D., Lategan, A. J., Marien, M. R., and Colpaert, F. C. (1991). Effects of locus coeruleus on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys: a possible role for the locus coeruleus in progression of Parkinson's disease. *Neuroscience* 41, 507–523.

Mevawalla, N., Fung, V., Morris, J., and Halliday, G. M. (2009). Unilateral rest tremor in vascular parkinsonism associated with a contralateral lesion of the locus coeruleus. *Mov. Disord.* 24, 1242–1244.

Mure, H., Hirano, S., Tang, C. C., Isaias, I. U., Antonini, A., Ma, Y., Dhawan, V., and Eidelberg, D. (2011). Parkinson's disease tremor-related metabolic network: characterization, progression, and treatment effects. *Neuroimage* 54, 1244–1253.

Pavese, N., Rivero-Bosch, M., Lewis, S. J., Whone, A. L., and Brooks, D. J. (2011). Progression of monoaminergic dysfunction in Parkinson's disease: a longitudinal 18F-dopa PET study. *Neuroimage* 56, 1463–1468.

Raizada, R. D. S., and Poldrack, R. A. (2008). Challenge-driven attention: interacting frontal and brainstem systems. *Front. Hum. Neurosci.* 1, 3. doi: 10.3389/neuro.09.003.2007

Rommelfanger, K. S., and Weinshenker, D. (2007). Norepinephrine: the redheaded stepchild of Parkinson disease. *Biochem. Pharmacol.* 74, 177–190.

Samuels, E. R., and Szabadi, E. (2008a). Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. *Curr. Neuropharmacol.* 6, 235–253.

Samuels, E. R., and Szabadi, E. (2008b). Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. *Curr. Neuropharmacol.* 6, 254–285.

Timmermann, L., Gross, J., Dirks, M., Volkmann, J., Freund, H.-J., and Schnitzler, A. (2003). The cerebral oscillatory network of parkinsonian resting tremor. *Brain* 126, 199–212.

Wang, T., Zhang, Q., Liu, J., Wu, A., and Wang, S. (2009). Firing activity of locus coeruleus noradrenergic neurons increases in a rodent model of Parkinsonism *Neurosci. Bull.* 25, 15–20.

Wilbur, R., Kulik, F. A., and Kulik, A. V. (1988). Noradrenergic effects in tardive dyskinesia, akathisia and pseudoparkinsonism via the limbic system and basal ganglia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 12, 849–864.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 September 2011; accepted: 16 December 2011; published online: 03 January 2012.

Citation: Isaias IU, Marzegan A, Pezzoli G, Marotta G, Canesi M, Biella GEM, Volkmann J and Cavallari P (2012) A role for locus coeruleus in Parkinson tremor. Front. Hum. Neurosci. ***5****:179. doi: 10.3389/fnhum.2011.00179*

ON-GOING RESEARCH ACTIVITIES

The game's afoot; Follow your spirit: and upon this charge, Cry — God for Harry! England and Saint George!
[W. Shakespeare, Henry V (scene i)]

Evidence of a role of LC-NAergic system in Parkinson tremor: a reserpine rat model study

In this study we were interested in describing reserpine-induced tremor in rats pre-treated with DSP-4, a selective LC-NAergic neurotoxin. This is a rather simple and straightforward study but has the great value to possibly disentangle the LC-NAergic effect on tremor onset from a concomitant role of other amines (e.g. DA and serotonin). Moreover, this study could provide direct evidence of the involvement of the LC in the onset of Parkinson tremor. Indeed, if the NAergic system does play a role in Parkinson tremor onset, a selective damaging of LC, by means of DSP-4, would prevent its appearance after reserpine injections.

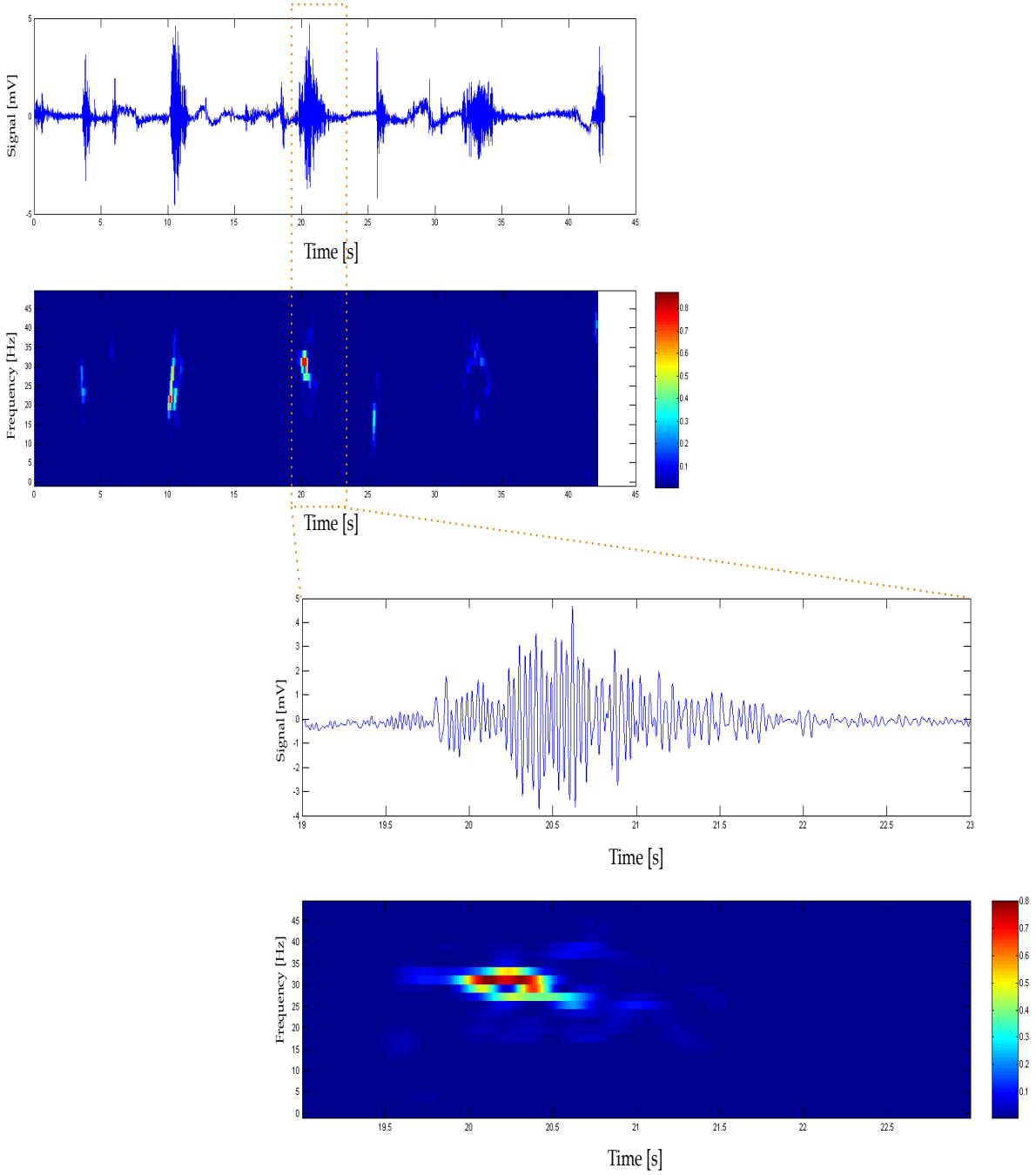
We recorded tremor in 12 male Sprague Dawley rats (200-220g) treated with reserpine (10 mg/Kg i.p.) and in 12 rats (matched for gender, age and weight) treated two weeks ahead with DSP-4 (50 mg/Kg i.p.) before receiving reserpine (also 10 mg/Kg i.p.). The presence, frequency and amplitude of tremor was documented by means of an accelerometer placed on the lower left limbs of the rat for 2 consecutive minutes before and at 20, 40, 60, 80, 100, 120, 180 minutes after reserpine injection.

The reserpinized rat has been more widely recognized as an animal model for PD. Reserpine binds to vesicular monoamine transporter (VMAT) with high affinity (Ki at subnanomolar concentration) (Henry et al., 1998), blocking neurotransmitter uptake into the vesicle and ultimately depleting catecholamines from storage vesicles (Henry et al., 1987; Kirshner, 1962a; Kirshner, 1962b). The actions of reserpine on the VMAT result in an acute catecholamine release, followed by chronic inhibition of catecholamine secretion, as a result of diminished releasable stores of vesicular catecholamine (Carmichael et al., 1980; Slotkin and Edwards, 1973). Reserpine induces symptoms resembling those of PD in humans (Flach, 1955; Kline and Stanley, 1955) and laboratory animals (Glow, 1959; Windle and Cammermayer, 1958). In the rat, reserpine induces rigidity of skeletal muscles, postural flexion, hypokinesia and tremor (Colpaert, 1987). Among rodent models of tremor, the reserpinized rat possibly represent the only model of (Parkinson) tremor with a clear (and maybe solely) involvement of the BG, or at least of catecholamine pathways (Miwa, 2007). No other rodent model of PD (e.g. 6-OHDA or MPTP induced) shows tremor. On the contrary to rigidity, tremor is present also at a low dose of reserpine (2.5 mg/Kg) and does not enhance in frequency or amplitude at higher doses. Tremor reaches its maximal amplitude within 40 min after reserpine injections, while other symptoms (i.e. bradykinesia and rigidity) reached a peak at 60-100 min after injection (see also *Figure 1* in Colpaert, 1987). The main issue of reserpine-induced motor disturbances is the great variability within and between each rat (Goldstein et al., 1975), thus making it difficult to define and quantify possible effects of drugs on symptoms. Anyhow, in our study we were more interested in the presence/absence of tremor. Besides blind scoring of video recordings, tremor was described by means of an accelerometer thus allowing an objective measurement of tremor appearance, frequency and amplitude (see Figure below). Our study follows preliminary evidence of tremor amelioration in rats with tremorine-induced tremor by means of a unilateral electrolesion of LC (Dickinson and Slater, 1982). It is worth mentioning that the primary mechanism responsible for tremorine-induced motor phenomena almost certainly involves central cholinergic neurons rather than aminergic ones.

DSP-4 can be used for the temporary selective degradation of the central and peripheral NAergic neurons, mainly those from the LC (for review Hormigo et al., 2012). It has been suggested that this

selectivity of DSP-4 toward LC neurons may be related to the significant difference found in the affinity of DSP-4 for the NA uptake carrier in different brain structures (Zaczek et al., 1990). In rodents, a systemic injection of DSP-4 causes depletion in the levels of NA, in the release capacity and in the activity of DBH (Ross et al., 1973 and 1985). The effects of DSP-4 administration on the behavior of rats (open-field tests) can include neophobia (distorted reactions to new things or experiences), increased emotionality (more grooming activities and number of stools), defensive or submissive behavior, an altered resident/intruder paradigm, increased aggressiveness and a lack of fear to environmental factors (Spyraki et al., 1982; Delini-Stula et al., 1984; Cornwell-Jones et al., 1992; Harro et al., 1995; van den Buuse et al., 2001). More recent studies in non-human primates and genetic mice models of PD also described severe motor deficits similar to those reported after 6-OHDA lesion (Pifl et al., 1991; Rommelfanger and Weinshenker, 2007, Delaville et al., 2011). Of relevance, in contrast to a 6-OHDA lesion (Deumens et al., 2002), the motor impairments consequent to NA depletion were not related to DAergic cell loss and the motor deficits described in 6-OHDA-lesioned rats is aggravated by the additional depletion of NA (Delaville et al., 2011). Another study has reported that denervation of LC NAergic terminals potentiated the 6-OHDA-induced partial DAergic neurodegeneration and akinesia only in rats treated with a D2 receptor antagonist, haloperidol (Srinivasan and Schmidt, 2003).

So far, we were able to validate the reserpinized rat as a model for Parkinson tremor (see Figure below). Pending activity are still the behavior and tremor evaluation of DSP-4 pre-treated rats after reserpine injection and the tyrosine hydroxylase (TH)-staining to document LC lesioning by DSP-4.



Tremor behavior in one rat induced by 10 mg/Kg reserpine at 40 minutes after injection. Upper box: continuous recordings (first 45 sec.). Lower box: close up at about 20 sec. showing tremor presence. Tremor was recorded at lower left limb while the rat was laying on the right side (lower left limb was elevated). The power of EMG signals (intensity of tremor) is shown in colors (right column scale).

Correlations between iron content in the LC and SN with dopaminergic striatal innervation in subjects with PD

Aim of this study was to measure by means of 3 Tesla Magnetic resonance imaging (MRI) iron content in the LC, as a putative indirect measurement of cell damage, and to correlate it with striatal DAergic innervation loss, measured by SPECT and FP-CIT. We expect PD patients with predominant tremor to show less iron content in the LC in comparison to PD patients with mainly akinetic-rigid signs, regardless of DAergic striatal innervation loss. We also predict that iron deposition in the LC would negatively correlate with iron deposit in the SN and possibly also with striatal DAT binding loss. Such findings would further support the hypothesis of a neuroprotective and compensatory role of LC-NAergic system on DAergic one (see *Background* and *Hypothesis statement*).

Of relevance, despite its great potential value, this study should be considered a preliminary anatomical study aiming to improve targeting of LC for future activation studies (by means of functional MRI). Indeed, in the future, we aim to investigate PD patients with tremor by means of EMG recording, brain imaging (e.g. functional MRI) and possibly coherence analysis (i.e. EEG).

The presence of iron deposition in the BG appears to play a pivotal role in the manifestation of parkinsonian-like features or Parkinsonism caused by DA deficiency. Increased iron levels in PD have been demonstrated using both quantitative (biochemical analysis using postmortem brain tissue) and semi-quantitative methods (i.e. histochemical methods, transcranial sonography, MRI imaging techniques) (Dexter et al. 1991; Sian-Hülsmann J et al., 2011). In brief, the sources of augmented iron in subjects with PD could be related to (1) a dysregulation of iron homeostasis caused by pathological variation in iron tissue distribution or (2) a malfunction in molecules involved in sequestrating excess iron, above all ferritin and NM (Bazelom et al., 1967; Sian-Hülsmann J et al., 2011). Regardless whether iron accumulation represents the cause or the result of neuronal destruction, an increase in iron content may exacerbate the neuronal damage and indirectly contribute to the average duration of the disease. Accordingly, MRI studies described a positive correlation between degree of nigral iron elevation and severity of the motor symptoms (Bartzokis et al. 1999). Still, no nigral iron changes (Dexter et al. 1992) have been found in the SN of pre-symptomatic PD or incidental Lewy body disease (although this has been disputed) and mild PD (Riederer et al. 1989) and excessive iron deposits in the SN do not correlate with disease duration (Wallis et al. 2008). Iron deposits in the LC have not been consistently studied in subjects with PD.

The contrast underlying structural MRI results from T1 and T2 relaxation times, which characterize how fast water magnetization returns to equilibrium after perturbation by a radiofrequency (RF) pulse. During an MR sequence, an RF pulse results in changes to longitudinal (T1) and transversal (T2) components of the MR signal, after which the T1 and T2 components return to equilibrium via exponential increase (for T1 relaxation) or decrease (for T2 relaxation). T1 and T2 relaxation times correspond to the time constant of this exponential function, that is, a return to two thirds of baseline value (in milliseconds). R1 and R2 relaxation rates correspond to 1/T1 and 1/T2, respectively. T1 and T2 relaxation times are a characteristic of tissue composition and therefore vary depending on its molecular structure. T2 is particularly sensitive to local magnetic field inhomogeneities and therefore is modified in the presence of metallic particles such as iron. The apparent transverse relaxation time, T2* (T2 star), takes into account the contribution of magnetic

field inhomogeneities. The corresponding relaxation rate is denoted by R2* 1⁄4 1/T2*. A gradient-echo image is T2*-weighted, whereas a spin-echo image is T2-weighted. Structures that accumulate iron appear hypointense on T2-weighted and T2*-weighted MR images, and many experiments have shown that the relaxation rate is a noninvasive estimate of iron content (from Hardy et al., 2005 and Lehéricy et al., 2012). These results are promising to improve the targeting of the LC in structural scans, but attempts to ascribe functional MRI activity to the LC still face the problem of determining whether activation observed in the lower resolution BOLD images truly corresponds to the LC tissue identified in a higher resolution anatomical scan. NM has paramagnetic T1-shortening effects when combined with metals such as iron and copper (Enochs et al., 1997; Zecca et al., 2003). This means that on T1-weighted images with high spatial resolution at 3 Tesla, LC and SN (in particular SNc) appear as areas of high signal intensity. Indeed, recent anatomical MRI studies have used NM as an in vivo marker of the LC in humans (Sasaki et al., 2006).
We first aimed to validate what described by Sasaki and coll. (Sasaki et al., 2006) and confirmed a slight hyperintensity of LC (Image 1), and possibly SN (Image 2), on T1 weighted images. We then tried to quantify the T1 values in these areas for possible correlations. On the contrary to Sasaki and coll. (Sasaki et al., 2006) we found little distinction of both the LC and SN in these maps suggesting it is not characterized by difference T1 values (Image 3 and 4). Given these doubtful results when looking at NM via T1 imaging, we preferred investigating iron deposition via another MRI modality. Preliminary results (Image 5 and 6) were encouraging.
We are currently studying best MRI sequences to measure iron content in the LC area and SN. At the moment, preliminary data were obtained in five PD patients (two with tremor) and four healthy controls.

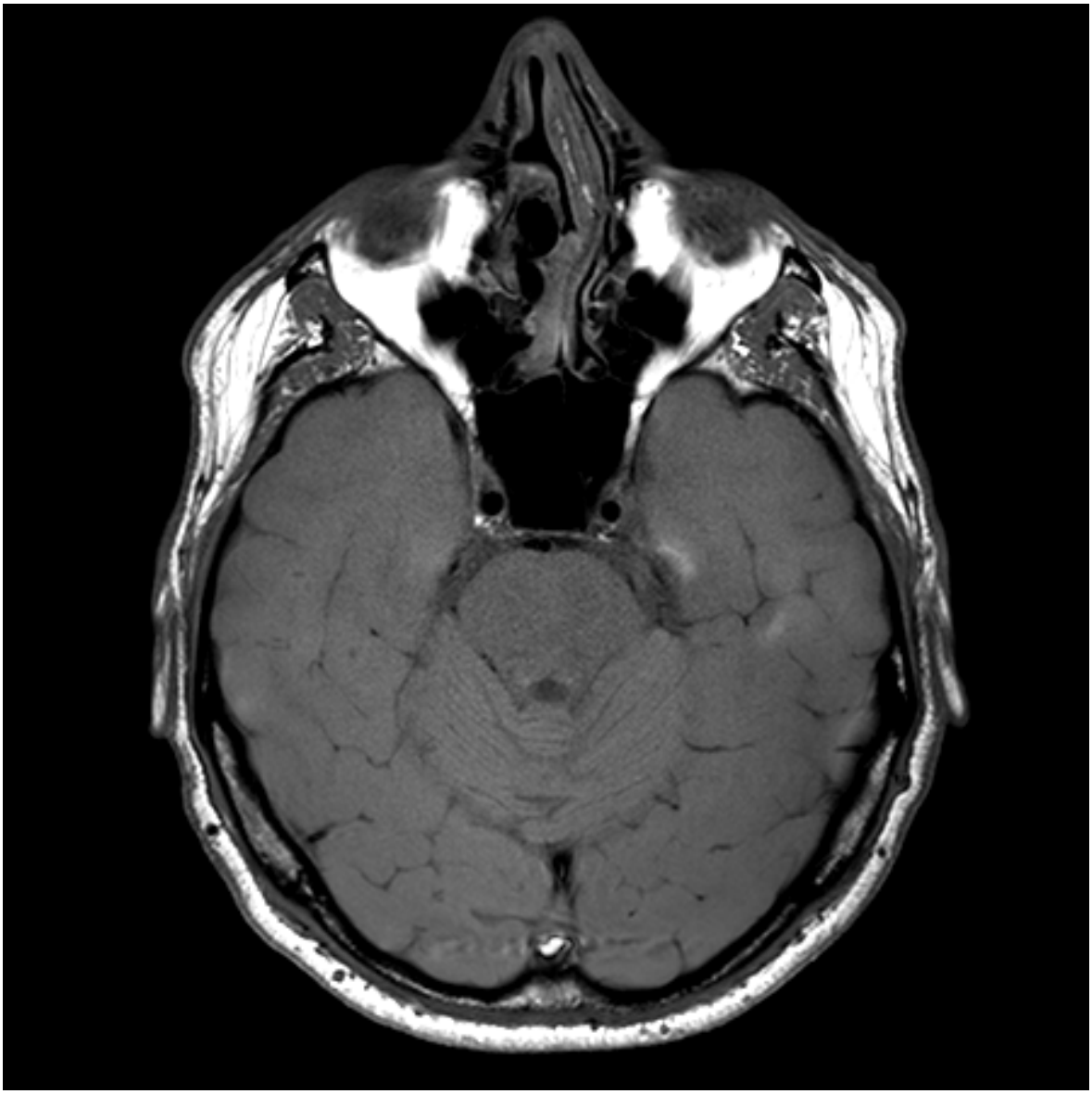


Image 1

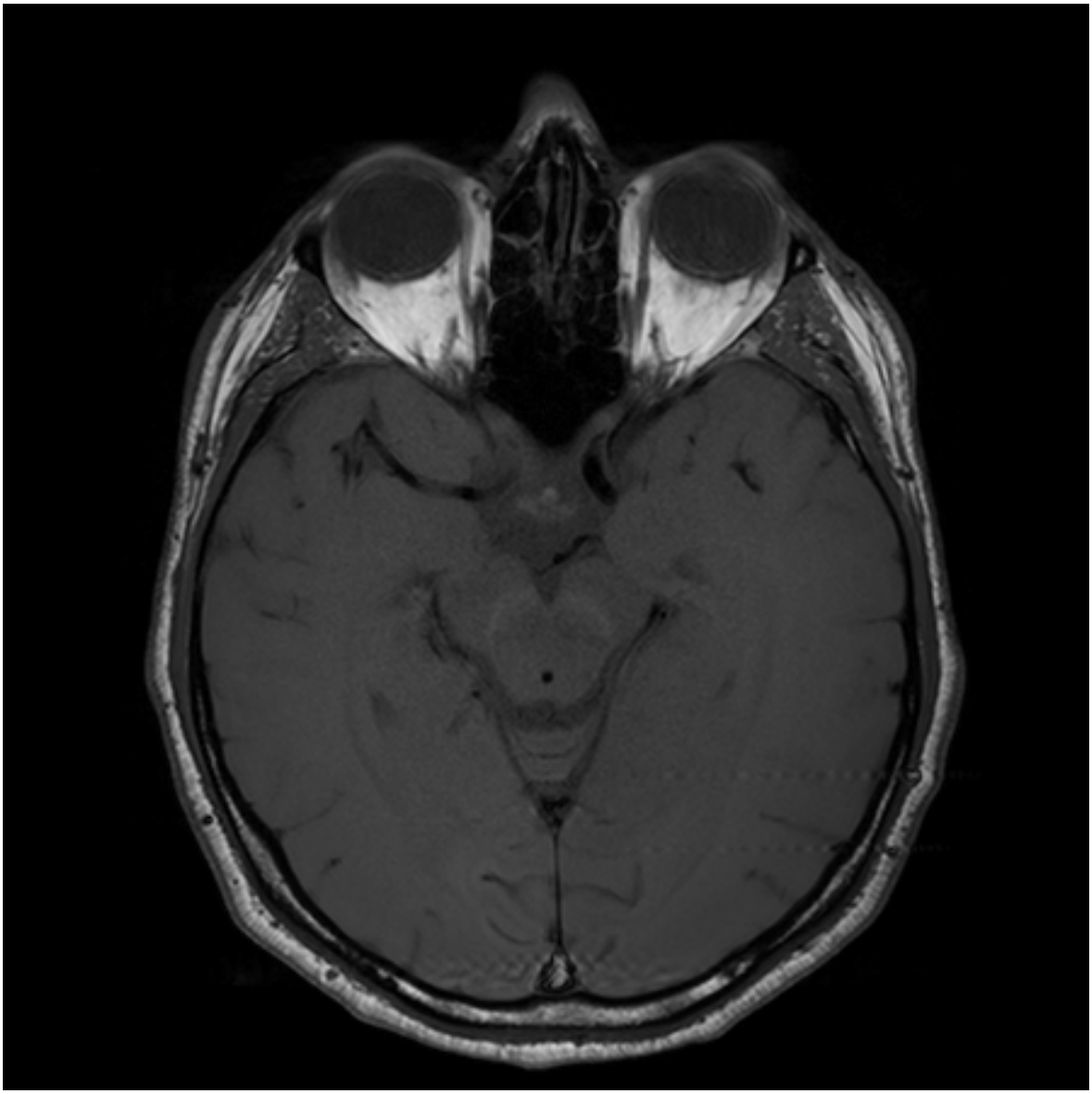


Image 2

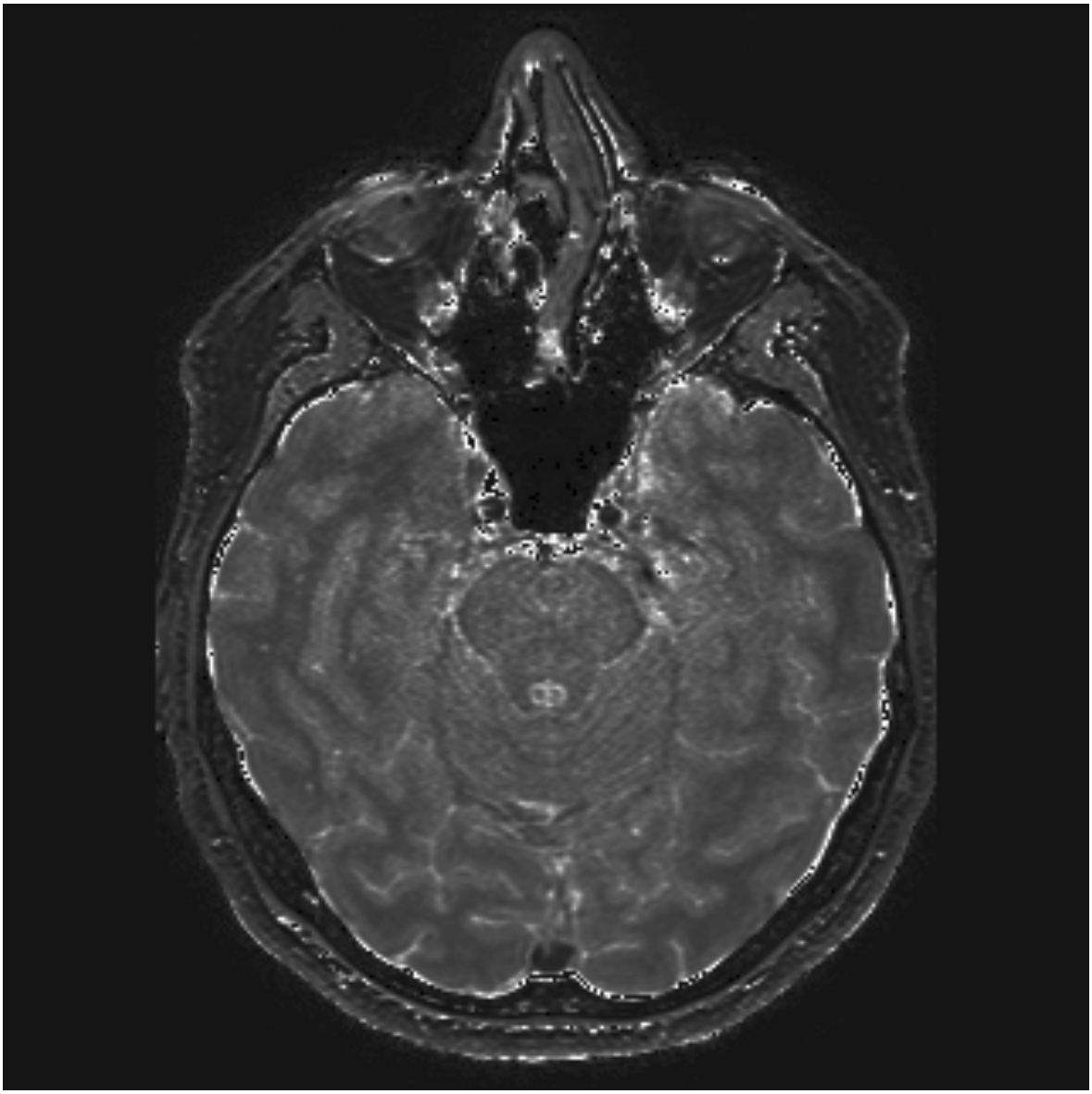


Image 3 (and 4). T1map – Quantitative estimates of T1 based on gradient echo images (not shown).

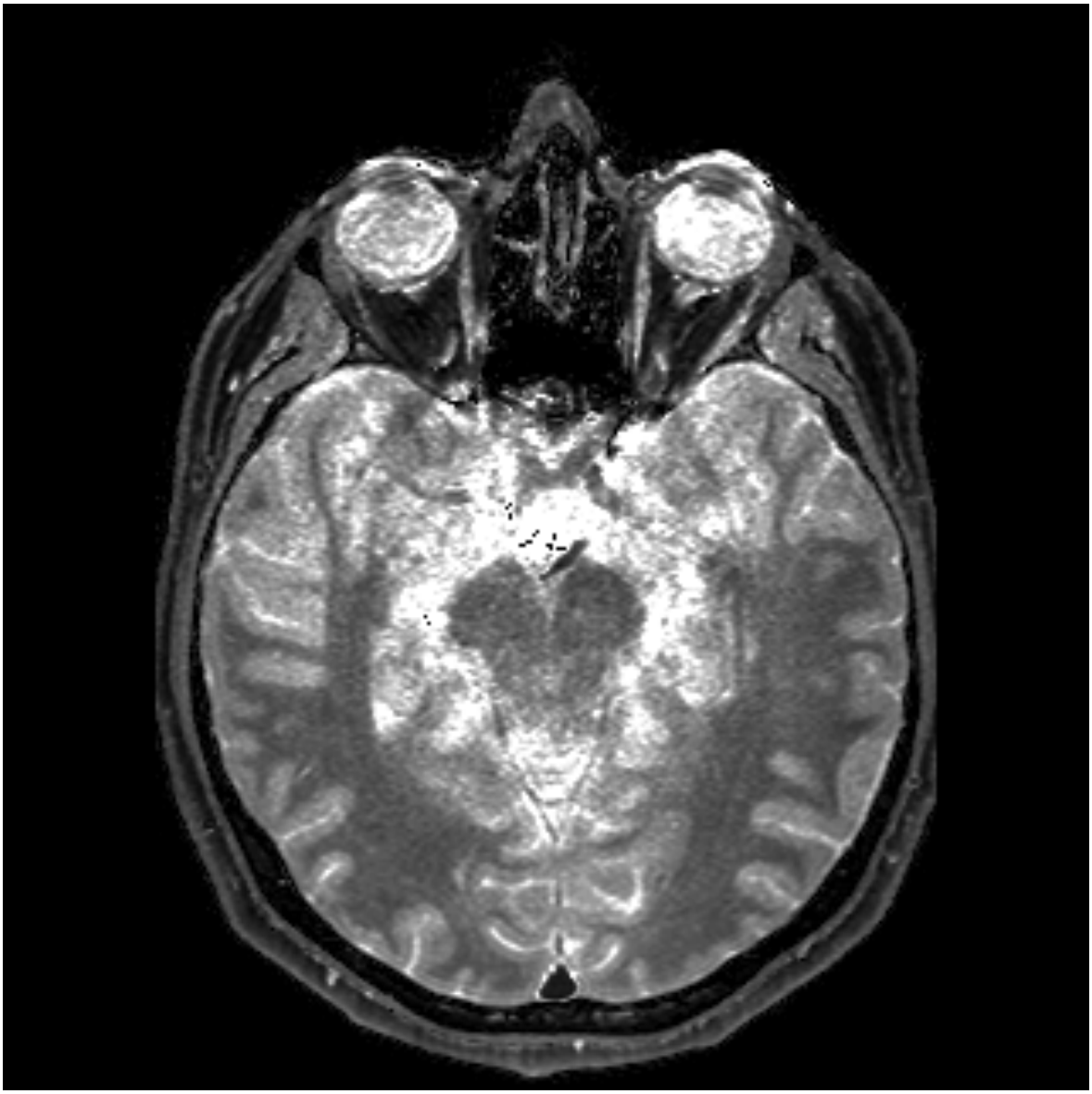


Image 4

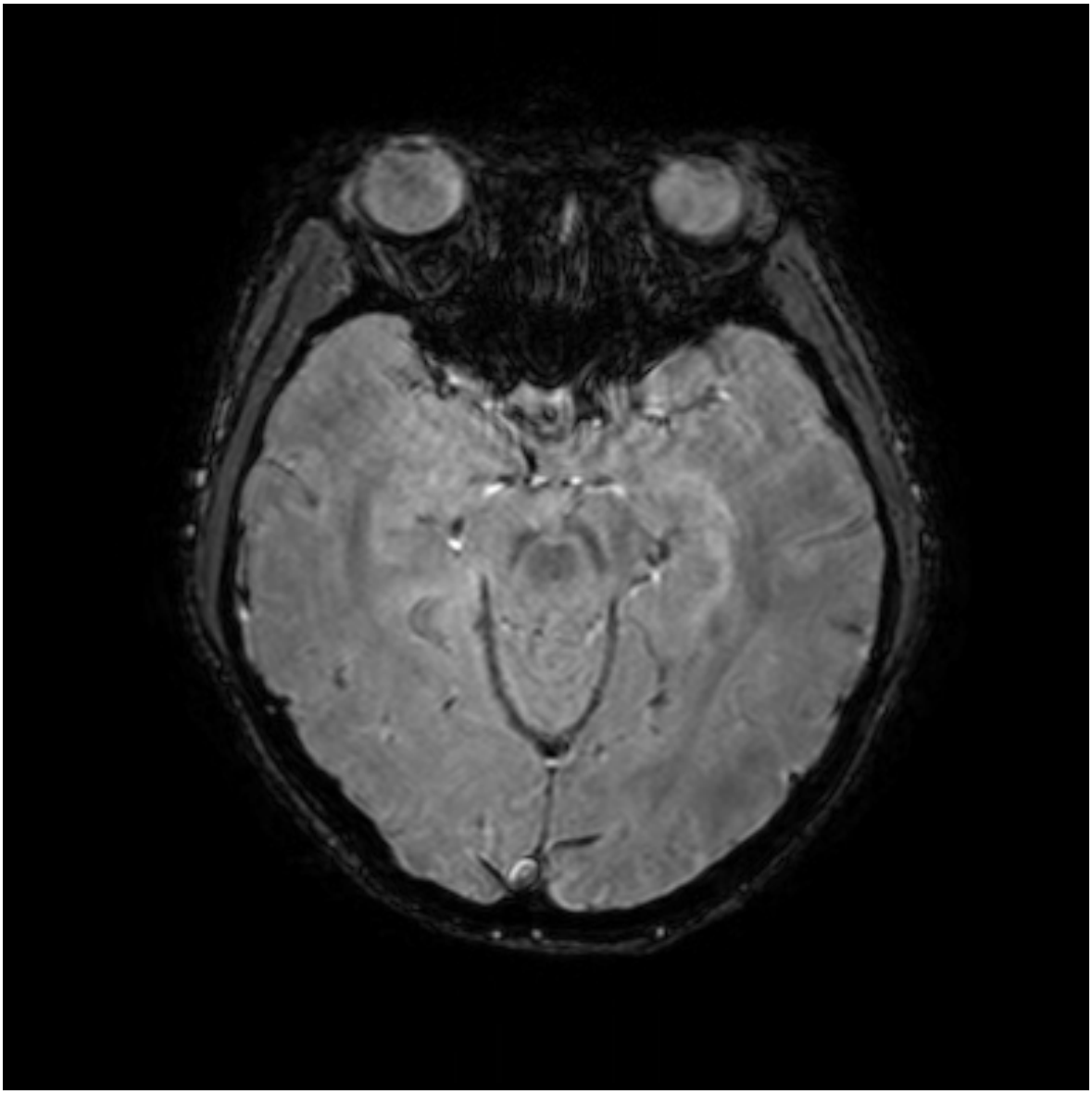


Image 5 – T2* weighted image showing strong hypointensity in the brainstem nuclei.

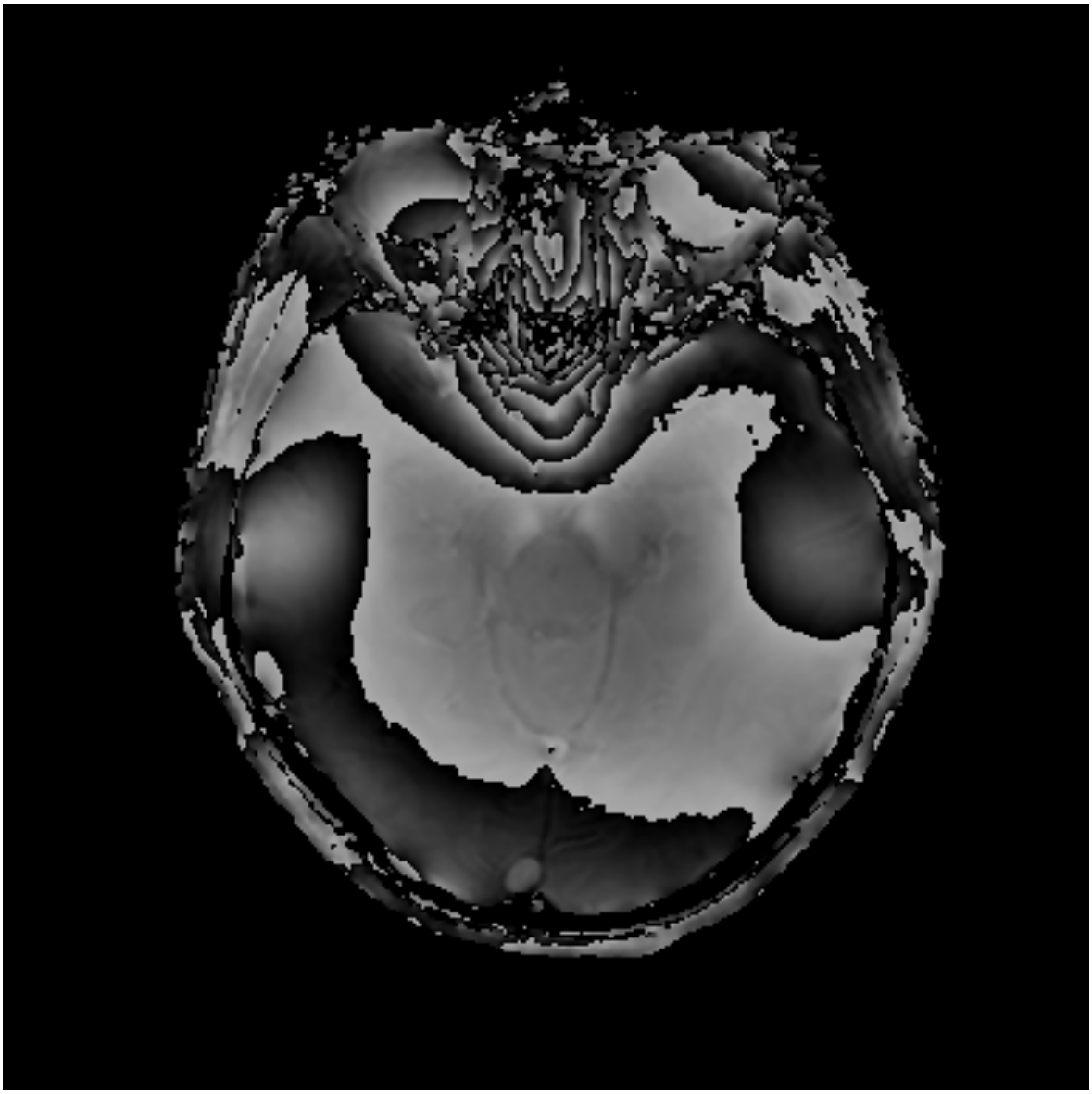


Image 6 – Signal phase image from T2* scan, showing hyperintensity due to susceptibility effect of iron in the nuclei. A more sophisticated data processing is required in order to eliminate the background magnetic field uniformities that give rise to the dark-light bands near the perimeter of the brain. These scans could be used to provide a semi-quantitative assessment of myelin density in white matter and iron (or generically metal) content in grey matter.

SUMMARY AND FUTURE DIRECTIONS

In summary, we first suggested that in PD patients at an early stage the LC-NAergic system might be intact and even hyperfunctioning (Isaias et al., 2011). We then described preliminary evidence of a link between LC-NAergic activity and Parkinson tremor (Isaias et al., 2012). We now aim to confirm, in the reserpinized rat model for PD, a distinct role of LC-NAergic system in Parkinson tremor and to disentangle it from the influence of other amines (e.g. serotonin, Caretti et al., 2008). A second on-going study will serve to enhance accuracy in targeting LC and to obtain preliminary *in vivo* evidence of a putative neuroprotective role of LC-NAergic system in PD patients with tremor. This study will also provide preliminary data for future activation studies (e.g. by means of functional MRI) possibly describing *in vivo* the LC activity (Raizada and Poldrack, 2008).

Understanding the physiological interactions between NA and DA pathology in PD is of great relevance as it could lead to novel therapies with the potential to compensate for, and protect from, DAergic neurons loss. Still, many issues need to be addressed in order to move forward with testing NAergic drugs as potential PD treatments.
It appears that the LC may somehow compensate for DA loss (see *Introduction*), but how and where it does so remains unclear, as NAergic innervation of the nigrostriatal system is sparse (Mavridis et al., 1991; Bing et al., 1994; Fornai et al., 1997). Stated that the LC facilitates SN activity (Grenhoff et al., 1988 and 1993) (see *Background*), it is tempting to speculate that the NAergic system may compensate DAergic activity by a direct up-regulation. PET imaging studies, targeting in vivo the NAergic terminals, will provide important data to support this hypothesis. The LC might also protect DAergic neurons by a NA-related trophic activity. As mentioned above (see *Background*), one consistent finding is that NA suppresses the expression of proinflammatory molecules and elevates the expression of anti-inflammatory molecules (Feinstein et al., 2002). Furthermore, NA and DA have equal antioxidant potential and can reduce oxidative stress and protect mesencephalic cultures from death (Troadec et al., 2001). Healy and coll. have also shown that a polymorphism resulting in low DBH activity in human patients correlates with a lower incidence of PD (Healy et al., 2004). A direct role for DBH in mitochondrial function and the modulation of free radical production deserves further investigation.
A second relevant question regarding neuroprotection mediated by the LC is timing. At what point in the degenerative process is the NAergic system recruited to protect or compensate for dying DAergic neurons? For example, would a NAergic supplements act as a prophylactic treatment for PD, or would NAergic drugs be helpful even after the neurons have begun to die and the disease has manifested into clinical signs? Would treatments need to be chronic or would more acute regimens suffice? More detailed experiments testing different administration paradigms of NA pharmacotherapies in PD animal models could begin to answer these questions.
Above all future lines of research, the effect of LC activity on DAergic drugs should be also investigated. The side effects associated with long-term levodopa treatment, such as abnormal involuntary movements (i.e. dyskinesia), represent an important cause of functional disability and remain difficult to manage. The causes of levodopa-induced dyskinesia are unclear. It probably involves non-physiological pulsatile stimulation of DAergic receptors or non-physiological release of DA (e.g. from serotonergic nerve terminals) in the striatum (Tanaka et al., 1999; Carta et al., 2007; Navailles et al., 2010b). Recently, a dysfunctioning LC has been correlated with the onset of

levodopa-induced dyskinesia (Fornai et al., 2007). Of relevance, NAergic neurons do not release DA synthesized from levodopa (Navailles et al., 2010a,b) but could participate in the clearance of extracellular DA. Because NET is able to transport DA, NAergic fibers could modify the pattern of levodopa-induced DA release depending on the relative innervation of brain regions by NAergic neurons. Indeed, in MPTP-treated monkeys, idazoxan (α2 ARs antagonist) in combination with levodopa did not impair the anti-parkinsonian response but significantly reduced dyskinesia and delayed their onset (Henry et al., 1999; Grondin et al., 2000; Fox et al., 2001). The same antidyskinetic effect was confirmed by other α2 ARs antagonists (i.e. fipamezole, yohimbine and rauwolscine) (Gomez-Mancilla and Bedard, 1993; Henry et al., 1999; Grondin et al., 2000; Savola et al., 2003; Fox and Brotchie, 2010). In humans, however, neither idazoxan nor fipamezole had any effect on duration of drugs-on time (Rascol et al., 1994). It is not clear whether the doses and routes employed of either drug might have impaired their efficacy. Indeed, the appearance of side-effects (i.e. hypertension, facial flushing and headache) prevented using high, and possibly more efficacious, doses (Fox and Brotchie, 2010). The efficacy of these drugs would be also critically dependent on the status of LC-NAergic fibers (from Logan et al., 2007 and Delaville et al., 2011).
There are good theoretical and clinical reasons, and encouraging preliminary results, to consider NAergic compounds for the treatment of different PD symptoms, possibly including tremor. The development of a direct investigation in patients of LC functionality and innervation, by means of functional MRI and PET, is now mandatory.

REFERENCES

Archer T, Fredriksson A. An antihypokinesic action of alpha2-adrenoceptors upon MPTP-induced behaviour deficits in mice. J Neural Transm 2003;110(2):183–200.

Arcos D, Sierra A, Nunez A, et al. Noradrenaline increases the firing rate of a subpopulation of rat subthalamic neurones through the activation of alpha 1-adrenoceptors. Neuropharmacology 2003;45:1070–1079.

Arnsten AFA. Stimulants: therapeutic actions in ADHD. Neuropsychopharmacology, 2006;31:2376–2383.

Aston-Jones G and Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 2005;28:403–450(b).

Aston-Jones G, Shipley MT, et al. The locus coeruleus, A5 and A7 noradrenergic cell groups. In: The Rat Nervous System, ed Paxinos G. New York: Academic Press, 1995. p.p. 183-214.

Aston-Jones G. Locus coeruleus, A5 and A7 noradrenergic cell groups. In: The Rat Nervous System, 3rd Edn, ed Paxinos G. New York: Elsevier, 2004. p.p. 259–284.

Aston-Jones G. and Cohen JD. Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. J Comp Neurol 2005;493:99–110.

Auclair A, Cotecchia S, Glowinski J and Tassin JP. D-amphetamine fails to increase extracellular dopamine levels in mice lacking alpha 1b-adrenergic receptors: relationship between functional and nonfunctional dopamine release. J. Neurosci. 2002;22:9150–9154.

Bagheri H, Damase-Michel C, Lapeyre-Mestre M, et al. A study of salivary secretion in Parkinson's disease. Clin Neuropharm 1999;22:213–215.

Bajic D, Proudfit HK, et al. Periaqueductal gray neurons monosynaptically innervate extranuclear noradrenergic dendrites in the rat pericoerulear region. J Comp Neurol 2000;427:649-662.

Baker KG, Törk I, Hornung JP, Halasz P. The human locus coeru- leus complex: an immunohistochemical and three-dimensional reconstruction study. Exp Brain Res 1989;77:257-70.

Bartzokis G, Cumming JL, Markham CH, et al. MRI evaluation of brain iron earlier and later onset Parkinson's disease and normal subjects. Magn Reson Imaging 1999;17:213–222.

Bazelon M, Fenichel GM, Randall J. Studies on neuromelanin. I. A melanin system in the human adult brainstem. Neurology. 1967; 17:512–519.

Bedard MA, el Massioui F, Malapani C, et al. Attentional deficits in Parkinson's disease: partial reversibility with naphtoxazine (SDZ NVI-085), a selective noradrenergic alpha 1 agonist. Clin Neuropharmacol 1998;21:108–117.

Belujon P, Bezard E, Taupignon A, Bioulac B and Benazzouz A. Noradrenergic modulation of subthalamic nucleus activity: behavioral and electrophysiological evidence in intact and 6-hydroxy- dopamine-lesioned rats. J Neurosci. 2007;27:9595–9606.

Benabid AL, Pollak P, Gervason C, et al. Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. Lancet 1991;337:403–406.

Benamer TS, Patterson J, Grosset DG, et al. Accurate differentiation of parkinsonism and essential tremor using visual assessment of [123I]-FP-CIT SPECT imaging: the [123I]-FP-CIT study group. Mov Disord 2000;15: 503–10.

Benarroch EE. The locus ceruleus norepinephrine system: functional organization and potential clinical significance. Neurology 2009;73:1699–1704.

Berridge CW and Waterhouse BD. The locus coeruleus- noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev 2003;42:33–84.

Bezard E, Brefel C, Tison F, et al. Effect of the alpha 2 adrenoreceptor antagonist, idazoxan, on motor disabilities in MPTP-treated monkey. Prog Neuropsychopharmacol Biol Psychiatry 1999;23:1237–1246.

Bing G, Zhang Y, Watanabe Y, McEwen BS, Stone EA. Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. Brain Res 1994;668:261–5.

Booij J and Kemp P. Dopamine transporter imaging with [123I]FP-CIT SPECT: potential effects of drugs. Eur J Nucl Med Mol Imaging 2008;35:424-438.

Booij J, Busemann Sokole E, Stabin MG, et al. Human biodistribution and dosimetry of [123I] FP-CIT: a potent radioligand for imaging of dopamine transporters. Eur J Nucl Med 1998;25:24–30.

Booij J, Hemelaar TG, Speelman JD, et al. One-day protocol for imaging of the nigrostriatal dopaminergic pathway in Parkinson's disease by [123I]FPCIT SPECT. J Nucl Med 1999;40:753–61.

Braak H, Del Tredici K, Rub U, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211.

Brodal A. Neurological anatomy in relation to clinical medicine. New York: Oxford University Press, 1981 p.p. 416-9.

Brooks DJ, Playford ED, Ibanez V, et al. Isolated tremor and disruption of the nigrostriatal dopaminergic system: an 18F-dopa PET study. Neurology 1992;42:1554-1560.

Caretti V, Stoffers D, Winogrodzka A, Isaias IU, et al. Loss of thalamic serotonin transporters in early drug-naïve Parkinsonian patients is associated with tremor: an [123I]β-CIT SPECT study. J Neural Transm 2008;115:721-9.

Carmichael SW, Weber A, Winkler H. Uptake of nucleotides and catecho- lamines by chromaffin granules from pig and horse adrenal medulla. J. Neurochem 1980;35:270–272.

Carta M, Carlsson T, Kirik D, Bjorklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain 2007;130:1819–1833.

Catafau AM and Tolosa E. [123I]FP-CIT Clinically Uncertain Parkinsonian Syndromes Study Group. Impact of dopamine transporter SPECT using 123I-Ioflupane on diagnosis and management of patients with clinically uncertain Parkinsonian syndromes. Mov Disord 2004;19:1175–82.

Chaudhuri KR, Healy DG, Schapira AH. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol 2006;5(3):235–45.

Collingridge GL, James TA, MacLeod NK. Neurochemical and electrophysiological evidence for a projection from the locus coeruleus to the substantia nigra. J Physiol 1979;290:44.

Colosimo C and Craus A. Noradrenergic drugs for levo- dopa-induced dyskinesia. Clin Neuropharmacol 2003;26:299–305.

Colpaert FC. Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. Neuropharmacology 1987;26:1431-1440.

Constas C. The effects of adrenaline, noradrenaline, and isoprenaline on parkinsonian tremor. J Neurol Neurosurg Psychiatry 1962;25:116.

Cooper JA, Sagar HJ, Tidswell P, et al. Slowed central processing in simple and go/no-go reaction time tasks in Parkinson's disease. Brain 1994;117:517–530.

Cornwell-Jones CA, Palfai T, Krasenbaum D, Byer E Jr, et al. Housing influences exploration and social interaction of control and DSP- 4-treated rats. Physiol Behav 1992;52:271–276.

Coull JT, Buchel C, Friston KJ and Frith CD. Noradrenergically mediated plasticity in a human attentional neuronal network. Neuroimage 1999;10:705–715.

Davis M, Kehne JH and Commissaris RL. Antagonism of apomorphine-enhanced startle by alpha 1-adrenergic antagonists. Eur J Pharmacol 1985;108:233–241.

de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol 2006;5:525–35.

Delaville C, Deurwaerdère PD, Benazzouz A. Noradrenaline and Parkinson's disease. Front Syst Neurosci 2011; doi: 10.3389/fnsys.2011.00031.

Delini-Stula A, Mogilnicka E, Hunn C and Dooley DJ. Novelty-oriented behavior in the rat after selective damage of locus coeruleus projections by DSP-4, a new noradrenergic neurotoxin. Pharmacol Biochem Behav 1984;20:613–618.

Deumens R, Blokland A and Prickaerts J. Modeling Parkinson's dis- ease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol 2002;175:303–317.

Deuschl G, Bain P and Brin M. Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. Mov Disord 1998;13:2–23.

Dexter DT, Carayon A, Javoy-Agid F, et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 1991;114:1953-1975.

Dick JPR, Rothwell JC, Day BL, et al. The Bereitschaftpotential is abnormal in Parkinson's disease. Brain 1989;112:233–244.

Dickinson SL and Slater P. Effect of lesining dopamine, noradrenaline and 5-hydroxytryptmine pathways on tremorine-induced tremor and rigidity. 1982;21:787-794.

Driver JA, Logroscino G, Gaziano JM, Kurth T. Incidence and remaining lifetime risk of Parkinson disease in advance age. Neurology 2009;72:432-438.

Dunckley P, Wise R, Fairhurst M, et al. A comparison of visceral and somatic pain processing in the human brainstem using functional magnetic resonance imaging. J Neurosci 2005;25:7333–7341.

Ennis M, Behbehani MM, et al. Projections from the periaqueductal gray to the rostromedial pericoerulear region and nucleus locus coeruleus: anatomic and physiologic studies. J Comp Neurol 1991;306:480-494.

Enochs WS, Petherick P, Bogdanova A, Mohr U, Weissleder R. Paramagnetic metal scavenging by melanin: MR imaging. Radiology 1997;204:417–423.

Falck H, Hillarp NA, Thieme G, Torp A. Fluorescence of catecholamines and related compounds condensed with formaldehyde. J Histochem Cytochem 1962;10:348-54.

Feinstein DL, Heneka MT, Gavrilyuk V, Dello Russo C, Weinberg G, Galea E. Noradrenergic regulation of inflammatory gene expression in brain. Neurochem Int 2002;41:357–65.

Flach F. Clinical effectiveness of reserpine. Ann N Y Acad Sci 1955;61:161-166.

Fornai F, Bassi L, Bonaccorsi I, Giorgi F, Corsini GU. Noradrenaline loss selectivity exacerbates nigrostriatal toxicity in different species of rodents. Funct Neurol 1997;12:193–8.

Fox SH and Brotchie JM. The MPTP-lesioned non-human primate models of Parkinson's disease. Past, present, and future. Prog Brain Res 2010;184:133–157.

Fox SH, Henry B, Hill MP, Peggs D, Crossman AR, Brotchie JM. Neural mechanisms underlying peak-dose dyskinesia induced by levodopa and apomorphine are distinct: evidence from the effects of the alpha(2) adrenoceptor antagonist idazoxan. Mov Disord 2001;16:642–650.

Fukuda M, Barnes A, Simon ES, et al. Thalamic stimulation for par- kinsonian tremor: correlation between regional cerebral blood flow and physiological tremor characteristics. Neuroimage 2004;21:608–615.

Fuxe K, Hökfelt T, Ungerstedt U. Morphological and functional aspects of central monoamine neurons. In: Pfeifer CC, Smythies JR, edts. Int Rev Neurobiol, 13. New York: Academic Press, 1970. p.p. 93-126.

German DC, Walker BS, Manaye K, Smith WK, Woodward DJ, North AJ. The human locus coeruleus: computer reconstruction of cellular distribution. J Neurosci 1988;8:1776-88.

Gesi M, Soldani P, Giorgi FS, Santimani A, Bonaccorsi I, Fornai F. The role of locus coeruleus in the development of Parkinson's disease. 2000;24:655-668.

Ghaemi M, Raethjen J, Hilke R, et al. Monosymptomatic Resting Tremor and Parkinson's Disease: A Multitracer Positron Emission Tomographic Study. Movement Disorders 2002;17:782–788.

Giovannoni G, Van Schalkwyk J, Fritz VU, Lees A. Bradykinesia aki- nesia incoordination test (BRAIN TEST): An objective comput- erised assessment of upper limb motor function. J Neurol Neurosurg Psychiatry 1999;67:624–629.

Glow P. Some aspects of the effects of acute reserpine treatment on behaviour. J Neural Neurosurg Psychiat 1959;22:11-32.

Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. J Neurochem 1966;13:655–69.

Gobert A, Billiras R, Cistarelli L, Millan MJ. Quantification and pharmacological characterization of dialysate levels of noradrenaline in the striatum of freely-moving rats: release from adrenergic terminals and modulation by alpha2-autoreceptors. J Neurosci Meth 2004;140:141–52.

Goldstein JM, Barnett A and Malick JB. The evaluation of anti-Parkinson drugs on reserpine-induced rigidity in rats. Eur. J. Pharmac. 1975;33:183-188.

Gomez-Mancilla B and Bedard PJ. Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. Clin Neuropharmacol 1993;16:418–427.

Grenhoff J and Svensson TH. Clonidine regularizes substantia nigra dopamine cell firing. Life Sci 1988;42:2003–9.

Grenhoff J, Svensson TH. Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. Eur J Pharmacol 1993;233:79–84.

Grondin R, Hadj Tahar A, Doan VD, Ladure P, Bedard PJ. Noradrenoceptor antagonism with idazoxan improves L- DOPA-induced dyskinesias in MPTP monkeys. Naunyn Schmiedebergs Arch Pharmacol 2000;361:181–6.

Guiard BP, El Mansari M and Blier P. Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. Mol Pharmacol 2008;74:1463-1475.

Hallett M and Khoshbin S. A physiological mechanism of bradykinesia. Brain 1980;103:30l-314.

Halliday GM, Ophof A, Broe M, et al. α-Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease. Brain 2005;128:2654-2664.

Hardy PA, Gash D, Yokel R, Andersen A, Ai Y, Zhang Z. Correlation of R2 with total iron concentration in the brains of rhesus monkeys. J Magn Reson Imaging. 2005;21:118–127.

Harik SI, McGunigal Jr T. The protective influence of the locus coeruleus on the blood-brain barrier. Ann Neurol 1984;15:568-74.

Harro J, Oreland L, Vasar E and Bradwejn J. Impaired exploratory behaviour after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation. Eur. Neuropsychopharmacol 1995;5:447–455.

Healy DG, Abou-Sleiman PM, Ozawa T, et al. A functional polymorphism regulating dopamine beta-hydroxylase influences against Parkinson's disease. Ann Neurol 2004;55:443–6.

Hein L, Limbird LE, Eglen RM, Kobilka BK. Gene substitution/knockout to delineate the role of alpha 2-adrenoceptor subtypes in mediating central effects of catecholamines and imidazolines. Ann N Y Acad Sci 1999;881:265–71.

Henry B, Fox SH, Peggs D, Crossman AR and Brotchie JM. The alpha2-adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian actions of L-dopa in the MPTP-lesioned primate model of Parkinson's disease. Mov Disord 1999;14:744–753.

Henry JP, Gasnier B, Roisin MP, Isambert MF, Scherman D. 1987. Molecular pharmacology of the monoamine transporter of the chromaffin granule mem- brane. Ann. N.Y. Acad. Sci. 1987;493:194–206.

Henry JP, Sagne C, Botton D, Isambert MF, Gasnier B. Molecular phar- macology of the vesicular monoamine transporter. Adv. Pharmacol. 1998;42:236–239.

Hoogendijk WJ, Pool CW, Troost D, et al. Image analyser-assisted morphometry of the locus coeruleus in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Brain 1995;118:131-43.

Hormigo S, de Anchieta de Castro e Horta Jr J, Gómez-Nieto R and López DE. The selective neurotoxin DSP-4 impairs the noradrenergic projections from the locus coeruleus to the inferior colliculus in rats. 2012; doi: 10.3389/fncir.2012.00041.

Horvath TL, Peyron C, et al. Strong hypocretin (orexin) innervation of the locus coeruleus activates noradrenergic cells. J Comp Neurol 1999;415:145-159.

Hurtado JM, Gray CM, Tamas LB, et al. Dynamics of tremor- related oscillations in the human globus pallidus: a single case study. Proc Natl Acad Sci U S A 1999;96:1674–1679.

Isaias IU and Antonini A. SPECT in the diagnosis and differential diagnosis of Parkinson disease. Neurodegener Dis 2010;7(5):319-29.

Isaias IU, Benti R, Cilia R, et al. [123I]FP-CIT striatal binding in early Parkinson's disease patients with tremor vs. akinetic-rigid onset. Neuroreport 2007;18:1499-502.

Isaias IU, Marotta G, Osama S and Hesse S. [123I]FP-CIT and SPECT in atypical parkinsonism. Imaging Med 2012;4:411-421.

Iversen LL, Rossor MN, Reynolds GP, et al. Loss of pigmented dopa- mine-beta-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type. Neurosci Lett 1983;39:95-100.

Jacobsohn L. Uber die Kerne des Menschlichen Hirnstamms. (Meddulla oblongata, Pons und Pedunculus cerebri) Anhang zu den Abhandlungen der Kgl. Preuss, Akad d. Wiss. Phys.-Mathem. Kasse. CP. 1909.

Jankovic J, Ben-Arie L, Schwartz K, et al. Movement and reaction times and fine coordination tasks following pallidotomy. Mov Disord 1999;14:57–62.

Jankovic J. Pathophysiology and clinical assessment of parkinsonian symptoms and signs. In Pahwa R, Lyons K, Koller WC eds: Handbook of Parkinson's Disease. New York: Marcel Dekker. 2003 p.p. 71–107.

Javoy-Agid F and Agid Y. Is the mesocortical dopaminergic system involved in Parkinson disease? Neurology 1980;30:1326-1330.

Jellinger KA. Formation and development of Lewy pathology: a critical update. J Neurol 2009;256:270-279.

Johnson EW, Wolfe BB and Molinoff PB. Regulation of subtypes of beta-adrenergic receptors in rat brain following treatment with 6-hydroxy- dopamine. J Neurosci 1989;9:2297–2305.

Jones BE, Moore RY. Ascending projections of the locus coeruleus in the rat. II Autoradiographic study. Brain Res 1977;127:23-53.

Josephs KA, Matsumoto JY, Ahlskog JE. Benign tremulous parkin- sonism. Arch Neurol 2006;63:354–357.

Kilbourn MR, Sherman P, Abbott LC. Reduced MPTP neurotoxicity in striatum of the mutant mouse tottering. Synapse 1998;30(2):205–10.

Kirshner N 1962. Uptake of catecholamines by a particulate fraction of the adrenal medulla. J Biol Chem 1962(a);237:2311–2317.

Kirshner N. Uptake of catecholamines by a particulate fraction of the adrenal medulla. Science 1962(b);135:107–108.

Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med 1988;318:876–880.

Kline NS and Stanlev AM. Use of reset-nine in a neuropsychiatric hospital. knn. i. Y. Acad. Sci. 1955;61:85-91.

Knutson B, Westdorp A, Kaiser E and Hommer D. fMRI visualization of brain activity during a monetary incentive delay task. Neuroimage 2000;12:20–27.

Krack P, Pollak P, Limousin P, et al. Stimulation of subthalamic nucleus alleviates tremor in Parkinson's disease. Lancet 1997;350:1675.

Lategan AJ, Marien MR, Colpaert FC. Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis. Brain Res 1990;523:134–8.

Lategan AJ, Marien MR, Colpaert FC. Suppression of nigrostriatal and mesolimbic dopamine release in vivo following noradrenaline depletion by DSP-4: a microdialysis study. Life Sci 1992;50:995–9.

Lee VM, Trojanowski JQ. Mechanisms of Parkinson's disease linked to pathological alpha-synuclein: new targets for drug discovery. Neuron 2006;52:33–8.

Lehéricy S, Sharman MA, Longo Dos Santos C, Paquin R, Gallea C. Magnetic Resonance Imaging of the Substantia Nigra in Parkinson's Disease. Mov Disord *In press*

Lemke MR. Effect of reboxetine on depression in Parkinson's disease patients. J Clin Psychiatry 2002;63:300–304.

Lenz FA, Kwan HC, Martin RL, et al. Single unit analysis of the human ventral thalamic nuclear group. Tremor-related activity in functionally identified cells. Brain 1994;117:531–543.

Lesage S and Brice AP. Parkinson's disease : from monogenic forms to genetic susceptibility factors. Hum Mol Genet 2009;18:R48-59.

Levitt P, Moore RY. Origin and organization of brainstem catecholamine innervation in the rat. J Comp Neurol 1979;186:505-28.

Levy R, Hutchison WD, Lozano AM, et al. High-frequency synchro- nization of neuronal activity in the subthalamic nucleus of parkinsonian patients with limb tremor. J Neurosci 2000;20: 7766–7775.

Liddell BJ, Brown KJ, Kemp AH, et al. A direct brainstem-amygdala-cortical alarm system for subliminal signals of fear. Neuroimage 2005;24:235–243.

Liprando LA, Miner LH, Blakely RD, Lewis DA, Sesack SR. Ultrastructural interactions between terminals expressing the norepinephrine transporter and dopamine neurons in the rat and monkey ventral tegmental area. Synapse 2004;52(4):233–44.

Logan J, Wang G, Telang F, Fowler JS, Alexoff D, Zabroski J. Imaging the norepinephrine transporter in humans with (S,S)-[11C]O-methyl reboxetine and PET: problems and progress. Nuc Med Biol 2007;34:667-679.

Lozano AM, Lang AE, Galvez-Jimenez N, et al. Effect of GPi pallidotomy on motor function in Parkinson's disease. Lancet 1995;346:1383–1387.

Lücking CB, Dürr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. N Engl J Med 2000;342:1560-1567.

Luppi PH, Aston-Jones G, et al. Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. Neuroscience 1995;65:119-160.

Maeda T, Shimizu N. Projections ascendantes du locus coeruleus et d'autres neurones aminergiques pontiques au niveau du prosencephale du rat. Brain Res 1972;36:19-35.

Marek K, Innis R, van Dyck C, Fussell B, Early M, Eberly S. et al. [123I]β-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. Neurology 2001;57:2089–2094.

Marien M, Briley M, Colpaert F. Noradrenaline depletion exacerbates MPTP-induced striatal dopamine loss in mice. Eur J Pharmacol 1993;236:487–9.

Marshall V, Grosset DG. Role of dopamine transporter imaging in the diagnosis of atypical tremor disorders. Mov Disord 2003;18:22-27.

Mavridis M, Degryse AD, Lategan AJ, Marien MR, Colpaert FC. Effects of locus coeruleus lesions on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys: a possible role for the locus coeruleus in the progression of Parkinson's disease. Neuroscience 1991;41:507–23.

McCormick DA and Wang Z. Serotonin and noradrenaline excite GABAergic neurones of the guinea pig and cat nucleus reticularis thalami. J Physiol 1991;442:235–255.

McCormick DA, Pape HC and Williamson A. Actions of norepinephrine in the cerebral cortex and thalamus: implications for function of the central noradrenergic system. Prog. Brain Res 1991;88:293–305.

McMillan PJ, White SS, Franklin A, et al. Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease. Brain Res 2011;1373:240-252.

Metzger RR, Brown JM, Sandoval V, et al. Inhibitory effect of reserpine on dopamine transporter function. Eur J Pharmacol 2002;456:39-43.

Miwa H. Rodent models of tremor. Cerebellum 2007;6:66-72.

Molinoff PB, Axelrod J. Biochemistry of catecholamines. Annu Rev Biochem 1971;40:465–500.

Morrish PK, Rakshi JS, Bailey DL, Sawle GW, Brooks DJ. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. J Neurol Neurosurg Psychiatry 1998;64: 314–319.

Mure H, Hirano S, Tang CC, Isaias IU, et al. Parkinson's Disease Tremor-Related Metabolic Network: Characterization, Progression, and Treatment Effects. Neuroimage, 2011;54:1244-53.

Navailles S, Bioulac B, Gross C, De Deurwaerdere P. Chronic L-DOPA therapy alters central serotonergic function and L-DOPA- induced dopamine release in a region- dependent manner in a rat model of Parkinson's disease. Neurobiol Dis 2010;41:585–590.

O'Suilleabhain PE. Parkinson disease with severe tremor but otherwise mild deterioration. Arch Neurol 2006;63:321–322.

Olson L, Fuxe K. Further mapping out of central noradrenaline neuron systems: projections of the subcoeruleus area. Brain Res 1972;43:289-95.

Olson L, Fuxe K. On the projections from the locus coeruleus noradrenaline neurons: the cerebellar innervation. Brain Res 1971;28:165±71.

Olszewski J, Baxter D. Cytoarchitecture of the human brain stem. New York: Karger, 1954.

Ordway GA, Stockmeier CA, Cason GW and Klimek V. Pharmacology and Distribution of Norepinephrine Transporters in the Human Locus Coeruleus and Raphe Nuclei. Journal Neurosci 1997;17:1710-1719.

Pan WHT, Yang SY and Lin SK. Neurochemical interaction between dopaminergic and noradrenergic neurons in the medial prefrontal cortex. Synapse 2004;53:44–52.

Papapetropoulos S, Ellul J, Argyriou AA, Chroni E, Lekka NP. The effect of depression on motor function and disease severity of Parkinson's disease. Clin Neurol Neurosurg 2006;108(5):465–9.

Parkinson J. An Essay on the Shaking Palsy. London, Sherwood, Neely, and Jones, 1817.

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

Patt S, Gerhard L. A Golgi study of human locus coeruleus in normal brains and in Parkinson's disease. Neuropathol Appl Neurobiol 1993;19:519-23.

Pifl C, Schingnitz G and Hornykiewicz O. Effect of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine on the regional distribution of brain monoamines in the rhesus monkey. Neuroscience 1991;44:591–605.

Pintor L, Bailles E, Valldeoriola F, Tolosa E, Marti MJ, de Pablo J. Response to 4-month treatment with reboxetine in Parkinson's disease patients with a major depressive episode. Gen Hosp Psychiatry 2006;28:59–64.

Pirker W. Correlation of dopamine transporter imaging with parkinsonian motor handicap: how close is it? Mov Disord 2003;18:43–S51.

Pollard H, Llorens-Cortes C, et al. Histamine and histidine decarboxylase in brain stem nuclei: distribution and decrease after lesions. Brain Res 1978;157:178-181.

Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–7.

Ponsen MM, Stoffers D, Booij J, et al. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Ann Neurol 2004;56:173–81.

Raizada RDS and Poldrack RA. Challenge-driven attention: interacting frontal and brainstem systems. Front. Hum. Neurosci. 2008:1, 3. doi: 10.3389/neuro. 09.003.2007.

Rascol O, Arnulf I, Peyro-Saint Paul H, et al. Idazoxan, an alpha-2 antagonist, and L-DOPA-induced dyskinesias in patients with Parkinson's disease. Mov Disord 2001;16:708–713.

Rascol O, Fabre N, Blin O, et al. Naltrexone, an opiate antagonist, fails to modify motor symptoms in patients with Parkinson's disease. Mov Disord 1994;9:437–440.

Raz A, Vaadia E, Bergman H. Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine vervet model of parkinsonism. J Neurosci 2000;20:8559–8571.

Reil JC. Untersuchungen über den Bau des grossen Gehirns im Menschen. Arch. Physiol. (Halle) 1809;9:136–524.

Riederer P, Sofic E, Rausch WD, et al. Transition metals, ferritin, glutathione and ascorbic acid in parkinsonian brains. J Neurochem 1989;52:515–520.

Rinvik E, Grofova I, Hammond C, Deniau JM, Feger J. Afferent connections to the subthalamic nucleus in the monkey and the cat studied with the HRP technique. In: Poirier LJ, Sourkes TL, Bedard PJ, editors. The extrapyramidal system and its disorders, New York: Raven Press, 1979. p.p. 53-70.

Rizvi TA, Ennis M, et al. Preoptic projections to Barrington's nucleus and the pericoerulear region: Architecture and terminal organization. J Comp Neurol 1994;347:1-24.

Rodriguez-Oroz MC, Jahanshahi M, Krack P, et al. Initial clinical manifestations of Parkinson's disease: features and pathophysio- logical mechanisms. Lancet Neurol 2009;8:1128–1139.

Rommelfanger KS and Weinshenker D. Norepinephrine: the redheaded stepchild of Parkinson's disease. Biochem Pharmacol 2007;74:177–190.

Rommelfanger KS, Weinshenker D, Miller GW. Reduced MPTP toxicity in noradrenaline transporter knockout mice. J Neurochem 2004;91(5):1116–24.

Ross RA, Reis DJ. Effects of lesions of locus coeruleus on regional distribution of dopamine-beta hydroxylase activity in rat brain. Brain Res 1974;73(1):161–6.

Ross SB, Johansson JG, Lindborg B and Dahlbom R. Cyclizing compounds. I. Tertiary N-(2-bromobenzyl)- N-haloalkylamines with adrenergic blocking action. Acta Pharm Suec 1973;10:29–42.

Ross SB. DSP-4 and behavioural experiments. Pharmacol. Sci. 1985;6:237.

Russell GV. The nucleus locus coeruleus (dorsalis tegmenti). Tex Rep Bioi Med 1955;13:939-88

Samuels ER and Szabadi E. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. Curr Neuropharmacol 2008(a);6:254-85.

Samuels ER, and Szabadi E. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. Curr Neuropharmacol 2008(b);6:235-53.

Sasaki M, Shibata E, Tohyama K, et al. Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. Neuroreport 2006;17:1215-1218.

Savola JM, Hill M, Engstrom M, et al. Fipamezole (JP-1730) is a potent alpha2 adrenergic receptor antagonist that reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Mov Disord 2003;18:872–883.

Shimizu N, Morikawa N, Okada K. Histochemical demon- stration of monoamine oxidase of the brain of rodents. Z. Zell-forsch. 1959;49:39–400.

Sian-Hülsmann J, Mandel S, Youdim MBH and Riederer P. The relevance of iron in the pathogenesis of Parkinson disease. J Neurochem 2011;118:939–957.

Slotkin TA, Edwards K. Effects of reserpine on the content and properties of rat adrenal medullary storage vesicles. Biochem Pharmacol 1973;22:549–560.

Snoddy AM and Tessel RE. Prazosin: effect on psychomotor-stimulant cues and locomotor activity in mice. Eur J Pharmacol 1985;116:221–228.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–40.

Spyraki C, Arbuthnott GW and Fibiger HC. The effect of DSP-4 on some positively rein- forced operant behaviors in the rat. Pharmacol. Biochem. Behav 1982;16:197–202.

Srinivasan J and Schmidt WJ. Potentiation of parkinsonian symptoms by depletion of locus coeruleus noradrenaline in 6-hydroxydopamine-induced partial degeneration of substantia nigra in rats. Eur J Neurosci 2003;17:2586–2592.

Steininger TL, Gong H, et al. Subregional organization of preoptic area/anterior hypothalamic projections to arousal- related monoaminergic cell groups. J Comp Neurol 2001;429:638-653.

Sterpenich V, D'Argembeau, A, Desseilles M, et al. The locus ceruleus is involved in the successful retrieval of emotional memories in humans. J Neurosci 2006;26:7416–7423.

Stone EA, Ariano MA. Are glial cells targets of the central noradrenergic system? A review of the evidence. Brain Res Rev 1989;14:297-309.

Sturm W, de Simone A, Krause BJ, et al. Functional anatomy of intrinsic alertness: evidence for a fronto-parietal-thalamic-brainstem network in the right hemisphere. Neuropsychologia 1999;37:797–805.

Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. Neuroreport 1999;10:631–634.

Taylor KS, Cook JA, Counsell CE. Heterogeneity in male to female risk for Parkinson's disease. J Neurol Neurosurg Psychiatry 2007;78:905-906.

Timmermann L, Gross J, Dirks M, et al. The cerebral oscillatory network of parkinsonian resting tremor. Brain 2003;126: 199–212.

Touge T, Werhalm KJ, Rothwell JC, et al. Movement-related cortical potentials preceding repetitive and random-choice hand movements in Parkinson's disease. Ann Neurol 1995;37: 791–799.

Tracy JI, Mohamed F, Faro S, et al. The effect of autonomic arousal on attentional focus. Neuroreport 2000;11:4037–4042.

Troadec JD, Marien M, Darios F, et al. Noradrenaline provides long-term protection to dopaminergic neurons by reducing oxidative stress. J Neurochem 2001;79:200–10.

Udenfriend S, Creveling CR. Localization of dopamine- beta-oxidase in brain. J Neurochem 1959;4:350–2.

Ungerstedt U. Stereotaxic mapping monoamine pathways in the rat brain. Acta Physiol Scand 1971;367:1–49.

Van Bockstaele EJ, Colago EE, et al. Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: substrate for the co-ordination of emotional and cognitive limbs of the stress response. J Neuroendocrinol 1998;10:743-757.

Van den Buuse M, Lambert G, Fluttert M and Eikelis N. Cardiovascular and behavioural responses to psychological stress in spontaneously hypertensive rats: effect of treatment with DSP-4. Behav Brain Res 2001;119,:131–142.

Vijayashankar N, Brody HA. Quantitative study of the pigmented neurons in the locus coeruleus and sub-coeruleus in man as related to ageing. J Neuropathol Exp Neurol 1979;38:490-4.

Von Coelln R, Thomas B, Savitt JM, et al. Loss of locus coeruleus neurons and reduced startle in parkin null mice. Proc Natl Acad Sci USA 2004;101:10744-9.

Wallis LI, Paley MN, Graham JM, et al. MRI assessment of basal ganglia iron deposition in Parkinson's disease. J Magn Reson Imaging 2008;28:1061–1067.

Wang T, Zhang QJ, Liu J, Wu ZH and Wang, S. Firing activity of locus coeruleus noradrenergic neurons increases in a rodent model of Parkinsonism. Neurosci Bull 2009;25:15–20.

Weintraub D, Mavandadi S, Mamikonyan E, et al. Atomoxetine for depression and other neuropsychiatric symptoms in Parkinson disease. Neurology 2010;75:448–455.

Wenzel J and Wenzel C. De Penitiori Structura Cerebri Hominis et Brutorum. Tübingen. 1812.

Wenzelburger R, Raethjen J, Loeffler K, et al. Kinetic tremor in a reach-to-grasp movement in Parkinson's disease. Mov Disord 2000;15: 1084–1094.

Wilbur R, Kulik FA and Kulik AV. Noradrenergic effects in tardive dyskinesia, akathisia and pseudoparkinsonism via the limbic system and basal ganglia. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:849–864.

Windle WF and Cammermayer J. Functional and structural observations on chronically reserpinized monkeys. Science 1958;127:1503-1504.
Yavich L, Jakala P, Tanila H. Abnormal compartmentalization of norepinephrine in mouse dentate gyrus in alpha-synuclein knockout and A30P transgenic mice. J Neurochem 2006;99:724–32.

Yavich L, Lappalainen R, Sirvio J, Haapalinna A, MacDonald E. Alpha2-adrenergic control of dopamine overflow and metabolism in mouse striatum. Eur J Pharmacol 1997;339(2–3):113–9.

Yavich L, Sirvio J, Haapalinna A, Ylinen A, Mannisto PT. Atipamezole, an alpha2-adrenoceptor antagonist, augments the effects of L-DOPA on evoked dopamine release in rat striatum. Eur J Pharmacol 2003;462(1–3):83–9.

Zaczek R, Fritschy JM, Culp S, et al. Differential effects of DSP-4 on noradrenaline axons in cerebral cortex and hypothalamus may reflect heterogeneity of noradrenaline uptake sites. Brain Res 1990;522:308–314.

Zahniser NR and Sorkin A. Trafficking of dopamine transporters in psychostimulant actions. Semin Cell Dev Biol 2009;20:411-417.

Zecca L, Zucca FA, Wilms H, Sulzer D. Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic char- acteristics. Trends Neurosci. 2003;26:578–580.

APPENDIX

More publications published during PhD years

OPEN ACCESS Freely available online

PLOS ONE

The Influence of Dopaminergic Striatal Innervation on Upper Limb Locomotor Synergies

Ioannis U. Isaias[1,2,3]*, Jens Volkmann[3], Alberto Marzegan[1], Giorgio Marotta[4], Paolo Cavallari[1], Gianni Pezzoli[2]

1 LAMB P.&L. Mariani, Section of Human Physiology Department, Università degli Studi di Milano, Milano, Italy, **2** Centro per la Malattia di Parkinson e i Disturbi del Movimento, CTO, ICP, Milano, Italy, **3** Neurologische Klinik und Poliklinik, Universitätsklinik Würzburg, Würzburg, Germany, **4** Dipartimento di Medicina Nucleare, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

Abstract

To determine the role of striatal dopaminergic innervation on upper limb synergies during walking, we measured arm kinematics in 13 subjects with Parkinson disease. Patients were recruited according to several inclusion criteria to represent the best possible *in vivo* model of dopaminergic denervation. Of relevance, we included only subjects with normal spatio-temporal parameters of the stride and gait speed to avoid an impairment of upper limbs locomotor synergies as a consequence of gait impairment *per se*. Dopaminergic innervation of the striatum was measured by FP-CIT and SPECT. All patients showed a reduction of gait-associated arms movement. No linear correlation was found between arm ROM reduction and contralateral dopaminergic putaminal innervation loss. Still, a partition analysis revealed a 80% chance of reduced arm ROM when putaminal dopamine content loss was >47%. A significant correlation was described between the asymmetry indices of the swinging of the two arms and dopaminergic striatal innervation. When arm ROM was reduced, we found a positive correlation between upper-lower limb phase shift modulation (at different gait velocities) and striatal dopaminergic innervation. These findings are preliminary evidence that dopaminergic striatal tone plays a modulatory role in upper-limb locomotor synergies and upper-lower limb coupling while walking at different velocities.

Citation: Isaias IU, Volkmann J, Marzegan A, Marotta G, Cavallari P, et al. (2012) The Influence of Dopaminergic Striatal Innervation on Upper Limb Locomotor Synergies. PLoS ONE 7(12): e51464. doi:10.1371/journal.pone.0051464

Editor: Veronique Sgambato-Faure, INSERM/CNRS, France

Received June 22, 2012; **Accepted** November 5, 2012; **Published** December 7, 2012

Funding: The study was supported by the Fondazione Grigioni per la malattia di Parkinson. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: iuisaias@yahoo.it

Introduction

Upper limb locomotor synergies are a basic component of human gait. They represent an active phenomenon driven by locomotor centers within spinal cord and the brainstem and modulated by cortical and subcortical inputs [1,2]. Information on neural control of locomotor automatism and gait-related arm motion in humans is still scanty. Animal studies, as well as preliminary functional imaging studies in humans, indicate that locomotor movements are coordinated by spinal networks referred to as a central pattern generators (CPGs), which are governed by the brainstem locomotor command region that, in turn, is under the control of the basal ganglia and premotor cortex [3–9].

Parkinson disease (PD) is a progressive neurological condition characterized by bradykinesia, rigidity, postural instability and possibly tremor. Patients with PD typically show little or no arm oscillation while walking, and this is often the first clinical motor sign to appear [10,11]. In PD patients, the arm swing is not correlated with clinically tested shoulder rigidity [10] and often disproportional to the degree of upper limb akinesia during voluntary alternating movements, thus pointing to a task-specific motor disturbance associated with walking. At an anatomopathological level, PD is mainly characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, which leads to striatal dopamine depletion [12]. It has been estimated that motor PD symptoms appear when the loss of dopamine neurons reaches the 50% to 60% threshold, [13] which corresponds to a 70% to 80% decrease in putaminal dopamine content [14].

The aim of this study is to investigate a putative role of dopamine and the striatum in locomotor upper limb automatism, taking into account their relationship with walking speed. Patients were carefully selected to represent a clinically homogenous *in vivo* model of dopaminergic striatal innervation loss (see, *Subjects*) which was measured by [^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane (FP-CIT) and single-photon computed tomography (SPECT).

Materials and Methods

Ethics Statement

The local institutional review board (Section of Human Physiology, DePT) approved the study and all patients provided written informed consent.

Subjects

We tested 13 subjects with idiopathic PD (six males; mean age: 64 years; range: 52–73 years; disease duration mean: 5 years; range: 3–6) and a control group (HC) of 10 neurologically healthy adults (seven males; mean age: 64 years; age range: 55–70 years).

The diagnosis of PD was made according to the UK Parkinson Disease Brain Bank criteria and patients were clinically evaluated by means of the Unified Parkinson Disease Rating Scale motor part (UPDRS-III; range: 0–108). Median UPDRS-III score was 21 (range: 11–32). A sub-score for unilateral arm rigidity and bradykinesia (UPDRSrb) was calculated as the sum of UPDRS items 22 (rigidity), 23 (finger taps), 24 (hand movements), 25 (rapid alternating movements of hands). Median UPDRSrb score of the worst arm was 8 (range: 4–11) and of the less affected arm was 4 (range: 1–5). We selected only mildly affected patients for this study (Hoehn & Yahr II) (see later).

At the time of the study, and during a follow-up time of at least six months after enrollment, no patient showed any signs indicative for atypical parkinsonism (e.g. gaze abnormalities, autonomic dysfunction, psychiatric disturbances, etc.). All patients reported a marked improvement (>30% UPDRS-III score reduction) after the intake of L-Dopa or dopamine agonists. L-Dopa daily dose and L-Dopa Equivalent Daily Doses (LEDDs) were also recorded, with the latter calculated according to the following conversion ratio: 100 mg levodopa = 1.5 mg pramipexole = 6 mg ropinirole. Median value of LEDDs per day was 500 mg (range 200–625 mg).

All subjects were screened for cognitive impairment by the Mini-Mental State examination, Clock Drawing Test and Frontal Assessment Battery and excluded if not meeting normal, age-related performance.

Other exclusion criteria for study participation were a history of neurological disorders (other than PD for patients), head trauma with loss of consciousness, orthopedic diseases, systemic illness or previous orthopedic, brain or spinal cord surgery. A MRI was performed within six months from enrollment and only subjects with normal results (i.e., no sign of white matter lesion or atrophy) participated in the study.

Gait disturbance is a key component of motor disability of PD and patients may variably present with reduced gait speed, shortened stride length, prolonged stance and double support phases [15]. At an early disease stage, PD patients may still show normal spatio-temporal gait parameters during steady linear walking [16]. To avoid an impairment of upper limbs locomotor synergies as a consequence of gait impairment *per se,* we enrolled only PD patients with normal spatio-temporal parameters of the stride (Table 1).

Experimental Protocol and Data Processing

After a 3-day washout of antiparkinsonian medication, subjects performed three sets of six walking trials, at a self-chosen "preferred" speed, "slow" and "fast" speed, in random order along a 10 m path, following verbal instruction in the absence of external feedback. Set-up and data processing has been extensively described elsewhere [16,17]. In brief, kinematics of body segments were measured during walking, using an optoelectronic system (SMART, BTS, Milan, Italy, sampling frequency 60 frames/s), which computed the 3D coordinates of spherical markers (15 mm diameter) attached on bony fixed landmarks. The marker coordinates were low-pass filtered (cut-off frequency 3–7 Hz, self-estimated by a linear-phase autoregressive model-based derivative assessment algorithm). Specific sets of parameters for the characterization of each task were automatically extracted by *ad hoc* algorithms and then visually inspected to check for possible errors. In particular, the time course of the angular displacement of the humerus segment of the arm with respect to the vertical (positive forward) [17] were computed in planes perpendicular to the inter-acromion line. These measures allowed to analyze the pendular behavior of the arm segment independently from shoulder and pelvic girdle horizontal rotation associated with trunk torsion. Angular profiles were normalized in time as a percentage of the stride duration, and for each cycle we extracted the range of motion (ROM) of absolute arm angle. Finally, arm swing asymmetry (ASA) was calculated according to Zifchock and coll. [18] as follows: $ASA = (45^\circ\text{-}\arctan(ArmSwing_{LEFT}/ArmSwing_{RIGHT}))*100/90^\circ$.

For arm ROM larger than 3°, the phase-shift (upper-lower limb) was further computed as the temporal delay between the positive peak (antero-posterior swing) of the wrist and the negative peak of malleolus, between 20% and 80% of the stride cycle. When the upper limb produced two oscillations per stride, which may occur at the lower walking speeds, phase-shifts were computed using the first positive peak of arm oscillation.

For each subject all variables (e.g. gait speed, arm ROM, phase-shift, etc.) were averaged over trials.

To characterize the speed-related effects, the slopes of the regression lines of arm ROM, phase-shift as well as of spatio-temporal parameters of the stride were computed for each subject as a function of the gait speed.

SPECT Data Acquisition, Processing and Analysis

Dopamine-transporter (DAT) values were measured by means of Single Photon Computed Tomography (SPECT) with [^{123}I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane (FP-CIT).

SPECT data acquisition and reconstruction has been described in details elsewhere [19]. In brief, intravenous administration of 110–140 MBq of FP-CIT (DaTSCAN, GE-Healthcare, UK) was performed 30–40 minutes after thyroid blockade (10–15 mg of Lugol oral solution) in PD patients subsequently overnight withdrawal of dopaminergic therapy. Data were compared with a group of 15 healthy subjects (four males; mean age, 62; age range: 44–70 years).

Brain SPECT was performed by means of a dedicated triple detector gamma-camera (Prism 3000, Philips, Eindhoven, the Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (4 subsets of acquisitions, matrix size 128×128, radius of rotation 12.9–13.9 cm, continuous rotation, angular sampling: 3 degree, duration: 28 minutes).

Brain sections were reconstructed with an iterative algorithm (OSEM, 4 iterations and 15 subsets) and then processed by 3D filtering (Butterworth, order 5, cut-off 0.31 pixel-1) and attenuation correction (Chang method, factor 0.12).

FP-CIT uptake values for the caudate nucleus and putamen of both PD patients and healthy subjects were calculated according to Basal Ganglia Matching Tool [20].

Striatal uptake values were used to calculate an asymmetry index (AI), as follows: $AI = (VOI_{LEFT} - VOI_{RIGHT})/(VOI_{LEFT} + VOI_{RIGHT})*200$.

General Statistical Analysis

Distribution was non-normal for most of the variables, as assessed by Shapiro-Wilk's test. Accordingly, descriptive statistics and comparisons were always based on median/range values and non-parametric tests, respectively.

ChiSquare was used to test demographic homogeneity among groups regarding gender.

To relate comparisons to DAT binding values, upper and lower limbs were also re-classified into ipsilateral and contralateral according to the putamen with greater dopaminergic innervation loss. For healthy controls, left hemibody refers conventionally to ipsilateral.

Table 1. Spatio-temporal parameters of the stride, arm ROM and phase shift of subjects with Parkinson disease and healthy subjects.

	Parkinson patients		Healthy subjects	
Gait speed (Km/h)	**3.81 (1.86, 7.81) (preferred gait speed range: 2.87, 4.35)**		**3.82 (1.24, 9.94) (preferred gait speed range: 3.35, 4.4)**	
	Contralateral	**Ipsilateral**	**Contralateral (Right)**	**Ipsilateral (Left)**
Arm ROM (°)	7.48 (1.47, 32.06)**	18.19 (2.11, 29.8)**	25.27 (2.29, 31.8)	25.71 (5.21, 38,15)
Phase shift (%stride)	−15.35 (−23.1, −5.6)*	−11.8 (−16.9, −6.1)	−10.17 (−19.1, −5.4)	−9.97 (−18.9, −3.5)
Stride length (mm/BH)	0.68 (0.55, 0.28)	0.68 (0.49, 0.82)	0.7 (0.57, 0.82)	0.69 (0.54, 0.81)
Stride time (sec)	0.89 (0.73, 1.47)	0.92 (0.75, 1.42)	0.86 (0.8, 1,28)	0.87 (0.64, 1.28)
Stance (%stride)	65.2 (58.78, 71.25)	66.56 (62.77, 72.84)	64.42 (61.76, 72.6)	64.28 (61.54, 81.82)
Slope arm ROM [°/(km h^{-1})]	2.08 (0.12, 6.68)	3.47 (0.43, 7.82)	3.96 (2.11−6.34)	3.5 (1.24−6.58)
Slope phase shift [%stride/(km h^{-1})]	4.98 (2.9, 11.6)*	3.85 (2.52, 9.6)*	3.04 (1.55−4.68)	2.88 (1.8−4.16)
Slope stride length [mm/BH/(km h^{-1})]	0.07 (0.05, 0.09)	0.08 (0.05, 0.09)	0.06 (0.05−0.08)	0.07 (0.05−0.08)
Slope stride time [sec/(km h^{-1})]	−0.18 (−0.3, −0.12)	−0.17(−0.3, −0.11)	−0.16 (−0.22, −0.09)	−0.16 (−0.23, −0.09)
Slope stance [%stride/(km h^{-1})]	−0.18 (−0.29, −0.12)	−2.0 (−3.7, −1.4)	−0.15 (−0.22, −0.1)	−1.86 (−2.55, −1.23)

Median values, non-outlier min-max, and levels of statistical difference (Mann-Whitney U-test or Matched pairs) are reported. Data refer at walking at different velocities unless otherwise specified.

*$p<0.05$ (PD vs. HC); ** $p<0.01$ (PD vs. HC). BH = body height (mm). ROM = range of motion; Phase shift = temporal delay (%stride) between the positive peak (antero-posterior swing) of the wrist and the negative peak of malleolus; Stride = the period from initial contact of one foot and following initial contact of the same foot, is one gait cycle. Stance = gait phase when a foot is in contact with the ground, it begins with initial heel contact and ends with toe off.

For Parkinson patients, ipsilateral and contralateral refers to the more dopamine depleted hemisphere. For healthy controls, left hemibody refers conventionally to ipsilateral.

doi:10.1371/journal.pone.0051464.t001

Differences in spatio-temporal gait parameters, arm ROM, phase shifts indexes and slope parameters between control and patient groups were analyzed by means of Wilcoxon two-sample test.

To quantify the (in)consistency of these measures and the stride-to-stride variability, we calculated the coefficient of variation (CV) of these measures when walking at preferred gait speed.

When comparing two hemibodies of the same subject (both for kinematic as well as DAT binding values) we applied a Wilcoxon matched pair test.

Hoeffding's D measure was used to identify correlations among DAT binding values and biomechanic data (including asymmetry indexes) that differed among patients and healthy controls. If no linear correlation was found, we applied partition analyses in search for a DAT binding cut-off value related to abnormal kinematic parameters.

Statistical analyses were performed with the JMP statistical package, version 8.0.2 (SAS Institute, Inc., Cary, NC, USA).

Results

No difference was found among PD patients and HC for gender distribution and age.

Imaging Findings

In comparison to HC, patients showed reduced DAT binding values in the putamen (PD, right median: 2.19; right range: 1.2–3.51; left median: 2.63, left range: 1.41–3.4; HC, right median: 4.94, right range: 3.07–5.71; left median: 4.94, left range: 2.96–5.71; $p<0.01$ all) and caudate nucleus (PD, right median: 4.06; right range: 2.74–5.49; left median: 4.17, left range: 2.3–4.94; HC, right median 5.16, right range: 3.18–6.48; left median: 5.05, left range: 3.07–6.7; $p<0.05$ all) thus further supporting the clinical diagnosis of PD. In HC, no difference was described when comparing DAT binding values of right and left hemisphere. In PD patients, DAT binding values of the most affected putamen (median: 1.97; range: 1.2–3.07) were on average 30% lower than in the opposite hemisphere (median: 2.85; range: 1.86–3.51; $p<0.01$). No statistical difference was instead found when comparing DAT binding values of the caudate nucleus of PD patients (most affected, median: 3.73; range: 2.3–4.83; less affected, median: 4.2; range: 2.74–5.5). Average AI value for the putamen of PD patients was 30 (range 6–59); all HC had a putamen AI score below 5 (putamen AI score average 2.1; range 0–4).

UPDRSrb score was negatively correlated with striatal DAT binding values ($p = 0.01$, RSquare = 0.20), but this relationship explained only 20% of the variance. This finding is in agreement with previous results of SPECT and FP-CIT in subjects with PD and confirms the validity of the methods applied in this study [21].

Walking at Preferred Speed

As expected, no difference was found for lower limbs spatio-temporal gait parameters (i.e. stride length, stride time and stance) between patients and controls at preferred gait speed (see, Subject).

In HC, right and left hemibodies did not show any difference for any gait-related parameters (Table 1).

Consistency of the spatio-temporal measures did not differ significantly between the patient and control group.

When arms were re-classified into ipsilateral and contralateral to the more dopamine depleted hemisphere (see, General statistical analysis), the contralateral arm ROM of PD patients

was reduced when compared to the ipsilateral one (trend towards significance, p = 0.07; Table 1). Contralateral arms also showed a significant anticipation of maximum arm flexion (forward movement) in relation to ipsilateral thigh extension (backward movement), compared to controls (Table 1).

Of relevance, only four subjects with PD showed reduced arm ROM bilaterally. The remaining nine patients had one arm with ROM in the range of normality (ROM>18° [mean ROM_{HC}-1SD ROM_{HC}]). In the latter group of patients, all but two arms with reduced ROM were contralateral to the putamen with lower DAT binding values. Still, the putamen corresponding to the arm with ROM in the range of normality had over 45% DAT binding loss (with respect to the median value of our normal subjects).

No linear correlation was found between arm ROM reduction and dopaminergic innervation loss. A partition analysis revealed a 80% chance of reduced arm ROM when putaminal DAT binding value was below 2.63 (>47% reduction with respect to median value of normal subjects).

Average ASA value was 29 (range: 18–35) for PD and 6 for HC (range: 4–12). ASA and AI indices of both the caudate nucleus (p = 0.005, RSquare = 0.52) and the putamen (Figure 1; p = 0.001; RSquare = 0.62) were strongly correlated. This correlation proved to be statistically significant also when weighted for UPDRSrb and UPDRS-III scores. Last, no correlation was found between UPDRSrb and UPDRS-III scores and arm ROM.

Walking at Different Speeds

When walking at different velocities, the range of speeds was comparable across subjects and PD patients and large enough to reliably compute a slope line.

No difference was found for lower limbs spatio-temporal gait parameters (i.e. stride length, stride time and stance) between patients and controls also when walking at different gait velocities.

Phase shift modulation (slope) was significantly higher in PD patients than controls ($p<0.05$, Table 1). All patients were able to normally modulate all other spatio-temporal gait parameters and arm ROM (both ipsilateral and contralateral) when walking at different velocities (Table 1). Interestingly, patients with reduced arm ROM (<18°) when walking at preferred gait speed showed a significantly higher capability of modulating upper-lower limb phase shift which positively correlated with the corresponding dopaminergic content of the putamen (RSquare = 0.37, p = 0.01) and caudate nucleus (RSquare = 0.38, p = 0.01). This correlation was not present if arm ROM was in the range of normality when walking at preferred gait speed (Figure 2A and B).

Discussion

Some relevant conclusion can be drawn from the present study: (1.) We confirmed that early-stage PD patients may exhibit normal spatio-temporal gait parameters [16]. The presence of normal lower limb locomotor automatisms in subjects with reduced arm ROM supports the notion that both types of movement may be differentially organized [17].

(2.) We did not find a linear correlation between arm ROM reduction and corresponding putaminal dopaminergic depletion. Rather, we were able to define a cut-off value for dopaminergic putaminal innervation loss before arm ROM would decrease. (3.) Inter-limb synergies might be influenced by the imbalance of dopaminergic striatal tone between the two hemispheres as shown by the strong correlation between ASA and AI indexes. These findings question a prominent unihemispheric control of arm swing during walking. Still, the ASA index should be carefully interpreted as possibly related to the cut-off itself. Indeed, in all but two patients with arm ROM unilaterally reduced, the arm with reduced ROM (according to our reference value of 18°) was contralateral to the putamen with

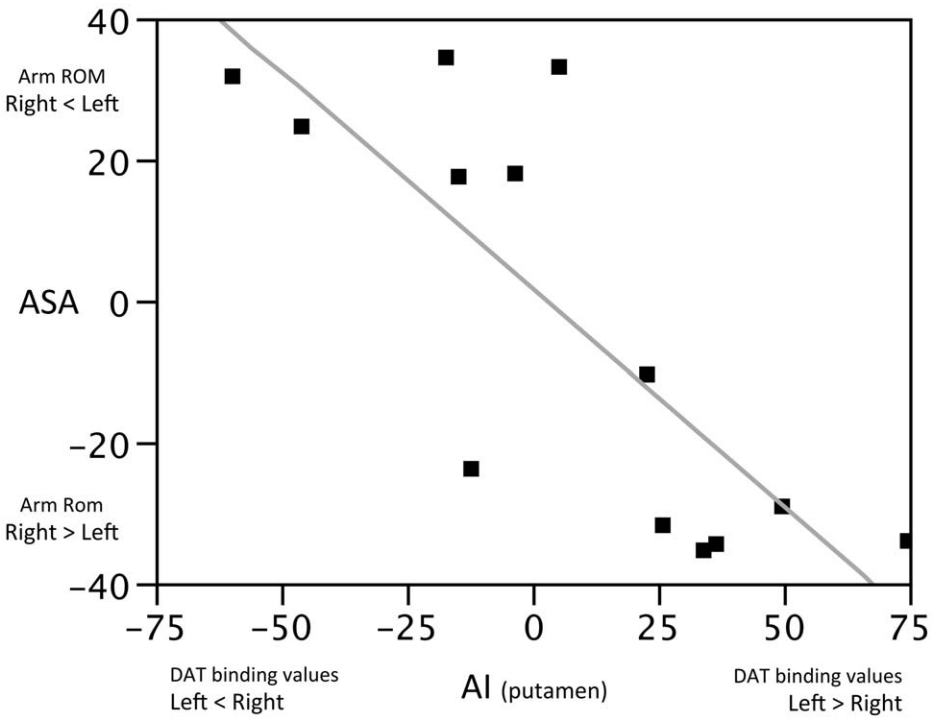


Figure 1. ASA significantly correlated to the AI of DAT binding values of the putamen (see, Methods).
doi:10.1371/journal.pone.0051464.g001

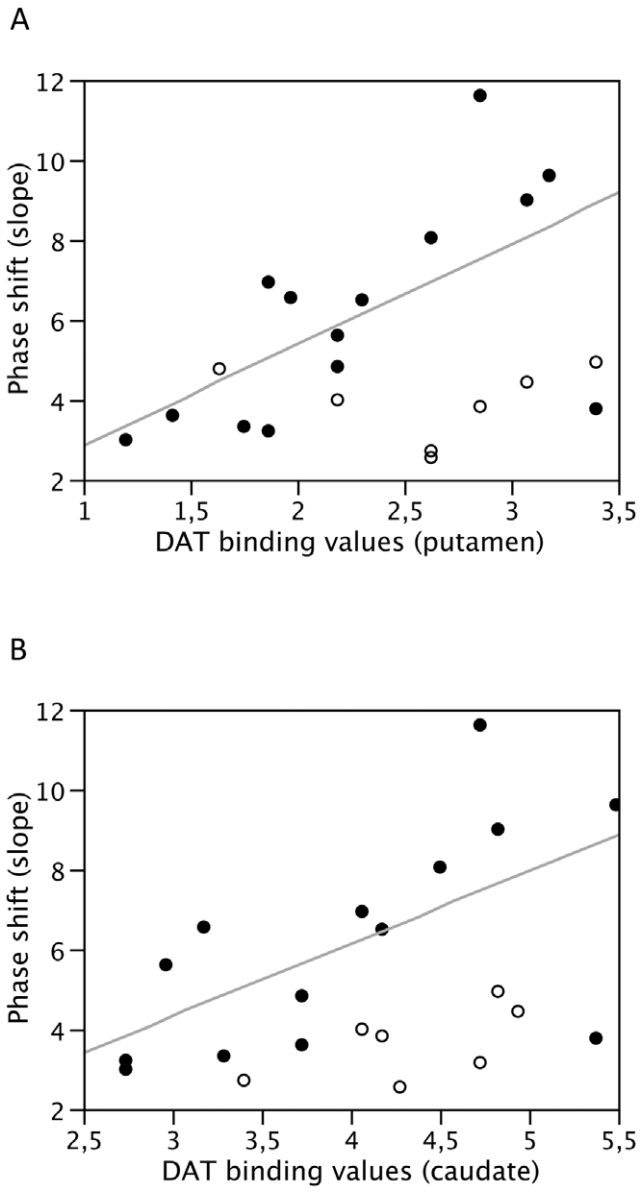


Figure 2. Correlation between phase shift modulation at different gait velocities and DAT binding values of the putamen (A) and caudate nucleus (B). Full dots represent arms with reduced (<18°) ROM when walking at preferred gait speed. Empty dots shows arms with arm ROM within the normal range. When arm ROM was reduced, a positive correlation was found between upper-lower limb phase shift modulation and both DAT binding values of putamen (RSquare = 0.37, p = 0.01) and caudate nucleus (RSquare = 0.38, p = 0.01). Correlation lines for arms with normal ROM (>18°) are not shown.
doi:10.1371/journal.pone.0051464.g002

greater dopaminergic innervation loss. (4.) When walking at different gait velocities, arms with reduced ROM showed an upper-lower limb coupling (phase shift) influenced by putaminal dopaminergic innervation. (5.) Locomotor synergies were independent of the lateralization of akinetic-rigid symptoms. Such a discrepancy may provide preliminary evidence for a different central organization of these entities in PD patients. Indeed, bradykinesia and rigidity are mainly related to the thalamo-cortical-basal ganglia loop with strict lateralized organization. [21,22] Conversely, limbs coordination, especially during automatically performed motor task, may be related to inter-hemispheric projections of basal ganglia and possibly involve also mesencephalic centers such as the pedunculopontine nucleus (PPN) and the reticular system [23].

Limitations in our study must be considered. We arbitrarily excluded patients with abnormal spatio-temporal parameters at lower limbs to possibly avoid upper limbs related changes. By doing this, we limited the patient sample and neglected well known PD-related gait disturbances, including abnormal timing of gait and stride-to-stride variability [24]. Another limit of this study is that PD patients were not drug-naïve. Still, the 3-day wash-out as well as the several inclusion criteria support the assumption that the enrolled PD patients well represent an *in vivo* model of dopaminergic deficit and allowed us to selectively investigate the role of intrinsic dopamine and the striatum in upper limbs locomotor synergies. Last, we cannot exclude in this study a direct role of a dopaminergic spinal innervation originating from the dorsal posterior hypothalamus (A11 region) on locomotor-related movements. Beside local hypothalamic connections, projections to the neocortex and to the serotonergic dorsal raphe, A11 neurons descend as the sole source of spinal dopamine mainly through the dorsolateral funiculus [25] and innervate most heavily the superficial sensory-related dorsal horn and the intermediolateral nucleus [26]. A loss of A11 neurons might eventually alter a possible interplay between dopamine and serotonin at a spinal level and result in loss of modulation during locomotion-like activity [27]. The role of the A11 neurons in the pathophysiology of PD and in locomotion in general, has not been explicitly tested.

From a anatomo-physiological perspective, the gait-related pendular motion of upper extremities is a subconsciously and automatically performed motor task. Inter-limb coordination remains stable despite changes of limb segment mass, suggesting independence from peripheral mechanism [28] and it is maintained across kinematically and kinetically different tasks, thus possibly related to a common neural control [29].

Descending pathways responsible for the control of locomotor limb movements, can be ascribed to direct cortical-motoneuronal input and indirect pathways of the basal ganglia [30,31,32]. Preliminary evidence suggest that dopaminergic neurons play an important role in the execution of self-determined movements [33], in the automatic nature of the rhythmic bilateral movements of the lower-limbs [34] and the persistence of gait execution [9]. This study provides additional information to disentangle a putative role of dopamine and the striatum in locomotor synergies. We suggest an interhemispheric rather than unihemispheric influence on inter-limb coupling. This may be particularly evident when dopaminergic striatal innervation is greatly reduced (>47% dopaminergic putaminal innervation loss). Furthermore, when arm ROM is reduced, the modulation of upper-lower limb coupling (phase shift) is also related to dopaminergic striatal content.

Acknowledgments

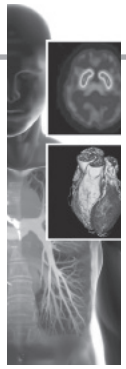
The authors would like to thank Prof. Carlo Albino Frigo (Politecnico di Milano, Milano) for his help in the design of the study; Dr. Margherita Canesi (Centro per la Malattia di Parkinson e i Disturbi del Movimento, CTO, ICP, Milano) for patient referral; Ilaria Trezzi and Andrea Cattaneo (LAMB P.&L. Mariani, DePT, Università degli Studi di Milano) for helping with data collection.

Author Contributions

Conceived and designed the experiments: IUI JV AM PC. Performed the experiments: IUI AM GM. Analyzed the data: IUI AM GM. Contributed reagents/materials/analysis tools: AM GM. Wrote the paper: IUI JV GM PC GP.

References

1. Fernandez Ballestreros ML, Buchthal F, Rosenfalck P. (1965) The pattern of muscular activity during the arm swing of natural walking. Acta Physiol Scand 63: 296–310.
2. Craik R, Herman R, Finley FR (1976) Human solutions for locomotion: interlimb coordination. In: Herman RM, Grillner S, Stein PSG, Stuart DG, editors. Neural control of locomotion. New York: Plenum Press 51–64 p.
3. Grillner S, Hellgren J, Ménard K, Saitoh K, Wikström MA (2005) Mechanism for selection of basic motor programs-roles for the striatum and pallidum. Trends Neurosci 28: 364–370.
4. Thompson RH, Ménard A, Pombal M, Grillner S (2008) Forebrain dopamine depletion impairs motor behavior in lamprey. Eur J Neurosci 27: 1452–1460.
5. Reiner A, Medina L and Veenman CL (1998) Structural and functional evolution of the basal ganglia in vertebrates. Brain Res Rev 28: 235–285.
6. Garcia-Rill E (1986) The basal ganglia and the locomotor regions. Brain Res 396: 47–63.
7. Takakusaki K, Saitoh K, Harada H, Kashiwayanagi M (2004) Role of basal ganglia-brainstem pathways in the control of motor behaviors. Neurosci Res 50: 137–151.
8. Hellstrand E. Brain metabolism and human gait – a PET study (1985) Acta Physiol Scand 125: 555–6.
9. Malouin F, Richards CL, Jackson PL, Dumas F, Dojon J (2003) Brain activations during motor imagery of locomotor-related tasks: a PET study. Hum Brain Mapp 19: 47–62.
10. Buchthal F, Fernandez-Ballestreros ML (1965) Electromyographyc study of the muscles of the upper arm and shoulder during walking in patients with Parkinson's disease. Brain 88: 875–896.
11. Stolze H, Kuhtz-Buschbeck JP, Drücke H, Jönk K, Illert M, et al. (2001) Comparative analysis of the gait disorder of normal pressure hydrocephalus and Parkinson's disease. J Neurol Neurosurg Psychiat 70: 289–297.
12. Hornykiewicz O (1998) Biochemical aspects of Parkinson's disease. Neurology 51: S2–S9.
13. Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114: 2283–2301.
14. Nandhagopal R, Kuramoto L, Schulzer M, Mak E, Cragg J, et al. (2009) Longitudinal progression of sporadic Parkinson's disease: a multi-tracer positron emission tomography study. Brain 132: 2970–2979.
15. Morris ME, McGinley J, Huxham F, Collier J and Iansek R (1999) Constraints on the kinetic, kinematic and spatiotemporal parameters of gait in Parkinson's disease. Human Movement Sci 18: 461–483.
16. Carpinella I, Crenna P, Calabrese E, Rabuffetti M, Mazzoleni P, et al. (2007) Locomotor function in the early stage of Parkinson's disease. IEEE Trans Neural Syst Rehabil Eng 15: 543–551.
17. Crenna P, Carpinella I, Lopiano L, Marzegan A, Rabuffetti M, et al. (2008) Influence of basal ganglia on upper limb locomotor synergies. Evidence from deep brain stimulation and L-DOPA treatment in Parkinson's disease. Brain 131: 3410–3420.
18. Zifchock RA, Davis I, Higginson J, Royer T (2008) The symmetry angle: a novel, robust method of quantifying asymmetry. Gait Posture 27: 622–627.
19. Isaias IU, Marotta G, Hirano S, Canesi M, Benti R, et al. (2010) Imaging essential tremor. Mov Disord 25: 679–686.
20. Calvini P, Rodriguez G, Inguglia F, Mignone A, Guerra UP, et al. (2007) The basal ganglia matching tools package for striatal uptake semi-quantification: description and validation. Eur J Nucl Med Mol Imaging 34: 1240–1253.
21. Hirato M, Ishihara J, Horikoshi S, Shibazaki T, Ohye C (1995) Parkinsonian rigidity, dopa-induced dyskinesia and chorea – dynamic studies on the basal ganglia-thalamocortical motor circuit using PET scan and depth microrecording. Acta Neurochir Suppl 64: 5–8.
22. Benamer HTS, Patterson J, Wyper DJ, Hadley DM, Macphee GJA, et al. (2000) Correlation of Parkinson's Disease Severity and Duration With 123I-FP-CIT SPECT Striatal Uptake. Mov Disord 15: 692–698.
23. Winn P (2008) Experimental studies of pedunculopontine functions: are they motor, sensory or integrative?. Parkinsonism Relat Disord 14: S194–S198.
24. Baltadjeva R, Giladi N, Gruendlinger L, Peretz C and Hausdorff JM (2006) Marked alterations in the gait timing and rhythmicity of patients with *de novo* Parkinson's disease. Europena Journal of Neuroscience 24: 1815–1920.25. Holstege JC, Dijken HV, Buijs RM, Goedkengt H, Gosens T, et al. (1996) Distribution of dopamine immunoreactivity in the rat, cat, and monkey spinal cord. J Comp Neurol 376: 631–652.
25. Lindvall O, Bjorklund A, Skagerberg G (1983) Dopamine-containing neurons in the spinal cord: anatomy and some functional aspects. Ann Neurol 14: 255–260.
26. Madriaga MA, McPhee LC, Chersa T, Christie KJ, Whelan PJ (2004) Modulation of locomotor activity by multiple 5-HT and dopaminergic receptor subtypes in the neonatal mouse spinal cord. J Neurophysiol 92: 1566–1576.
27. Donker SF, Daffertshofer A, Beek PJ (2005) Effects of velocity and limb loading in the coordination between limb movements during walking. J Motor Behav 37: 217–230.
28. Wannier T, Bastiaanse C, Colombo G, Dietz V (2001) Arm leg coordination in humans during walking, creeping and swimming activities. Exp Brain Res 141: 375–9.
29. Zehr EP, Carrol TJ, Chua R, Collins DF, Frigon A, et al. (2004) Possible contribution of CPG activity to the control of rhythmic human arm movement. Can J Physiol Pharmacol 82: 556–568.
30. Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25: 462–467.
31. Takakusaki K, Habaguchi T, Ostinata-Sugimoto J, Saitoh K, Sakamoto T (2003) Basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion: a new concept for understanding motor disorders in basal ganglia dysfunction. Neuroscience 119: 293–308.
32. Ouchi Y, Kanno T, Okada H, Yoshikawa E, Futatsubashi M, et al. (2001) Changes in dopamine availability in the nigrostriatal and mesocortical dopaminergic systems by gait in Parkinson's disease. Brain 124: 784–792.
33. Doyon J, Laforce RJ, Bouchard JP, Gaudreau D, Roy J, et al. (1998) Role of the striatum, cerebellum and frontal lobes in the automatization of a repeated visuomotor sequence of movements. Neuropsychologia 36: 625–641.



[123I]FP-CIT SPECT in atypical degenerative parkinsonism

One of the most widely used techniques to support the clinical diagnosis of Parkinson's disease is the SPECT scan with [123I]FP-CIT. This tracer binds reversibly and visualizes the striatal presynaptic dopamine transporters. Several uncertainties remain on the value of [123I]FP-CIT and SPECT in atypical degenerative parkinsonian syndromes. In this concise review, we discuss the contribution of SPECT and [123I]FP-CIT in supporting the clinical diagnosis of Parkinson's disease and their role in the differential diagnosis of Parkinson's disease and atypical degenerative parkinsonism. The chemistry, pharmacodynamics and pharmacokinetics of [123I]FP-CIT are also discussed.

KEYWORDS: atypical degenerative parkinsonism ■ FP-CIT ■ ioflupane ■ SPECT

Ioannis U Isaias*[1,2,3], Giorgio Marotta[4], Gianni Pezzoli[2], Osama Sabri[5] & Swen Hesse[5,6]

[1]*Università degli Studi di Milano, Dipartimento di Fisiologia Umana, Milano, Italy*
[2]*Centro per la Malattia di Parkinson e i Disturbi del Movimento, Istituti Clinici di Perfezionamento, Milano I-2016, Italy*
[3]*Universitätsklinik Würzburg, Neurologische Klinik und Poliklinik, Würzburg, Germany*
[4]*Dipartimento di Medicina Nucleare, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[5]*Department of Nuclear Medicine, University of Leipzig, Germany*
[6]*IFB Adiposity Diseases, Leipzig University Medical Centre, Leipzig, Germany*
**Author for correspondence: iuisaias@yahoo.it*

Parkinson's disease (PD) is the second most common neurodegenerative disorder [1], yet early accurate diagnosis remains challenging. The estimated prevalence of PD is 0.5–1% in those aged 65–69 years and 1–3% in those aged ≥80 years [1]. Although the clinical diagnosis of PD may be straightforward in cases with a classic presentation [2], accurate distinction between PD and atypical degenerative parkinsonism (ADP) may be difficult, particularly in the early or mild stages of disease [3]. In autopsy series, ADP (multiple system atrophy [MSA], progressive supranuclear palsy [PSP] and corticobasal syndrome [CBS]) accounted for half of PD misdiagnoses at specialized centers [4,5], while in the community Alzheimer's disease and vascular parkinsonism were most common [6,7]. Assessment of the clinical features suggests that an accuracy of 90% for PD may be the highest that can be expected using the currently available clinical diagnostic criteria. Accurate diagnosis of patients with ADP is important to predict the disease course and avoid unnecessary medical examinations and therapies and their associated side effects, safety risks and financial costs. Correct diagnosis is also critically important when patients are being recruited into clinical trials.

Post-mortem studies demonstrate severe reductions in dopamine concentration in the striatum of patients with PD, with greater reductions in the putamen [8,9]. SPECT with [123I]FP-CIT specifically identifies presynaptic dopaminergic deficits within the striatum [10–13]. Accordingly, an abnormal dopamine transporter SPECT image should be regarded as exclusion criteria for essential tremor [14], dystonic tremor [15] and psychogenic parkinsonism [16,17]. In this concise review, we will discuss the role of SPECT and [123I]FP-CIT in supporting the clinical diagnosis of PD and its differential diagnosis with ADP.

[123I]FP-CIT SPECT scans in atypical degenerative parkinsonism

Clinically, MSA is a sporadic, progressive neurodegenerative disease characterized by varying severity of parkinsonian features, cerebellar ataxia, autonomic failure and corticospinal disorders [18].

PSP presents with early onset postural instability associated with supranuclear vertical gaze impairment, symmetrical akinetic-rigid syndrome together with prominent bulbar dysfunction, dementia and axial rigidity [19,20].

CBS is characterized by asymmetric akinetic-rigid parkinsonism and limb dystonia, variably associated with cortical signs [21].

The parkinsonian types of MSA (MSA-P) and PSP (PSP-P) can be very difficult to distinguish from PD before disease-specific signs and symptoms occur. This also applies to CBS because of its marked asymmetrical akinetic-rigid syndrome before apraxia, myoclonus and cognitive problems become evident. Correct differentiation is important as PD has a better prognosis than ADP syndromes and responds better to a symptomatic treatment [22].

In PD the decrease in [123I]FP-CIT binding usually occurs in the dorsal putamen contralateral to the side of the neurological symptoms, in time progressing anteriorly and ipsilaterally (Figure 1) [12,14,23].

Future Medicine part of fsg

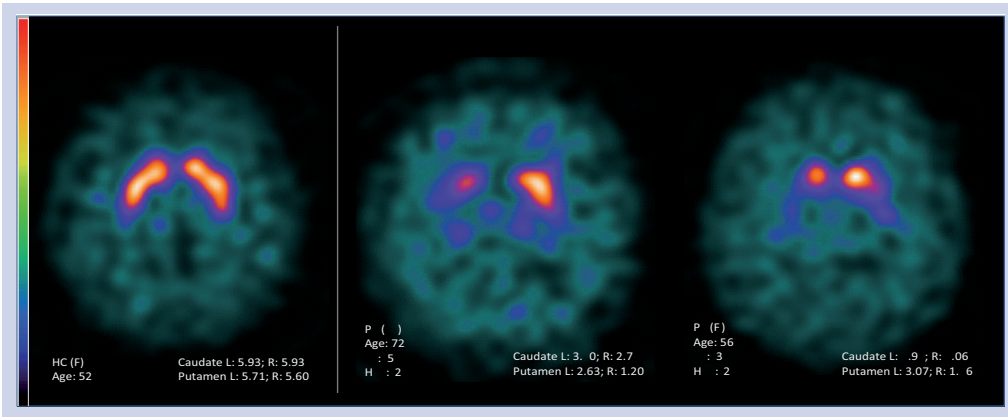


Figure 1. [^{123}I]FP-CIT of healthy controls and Parkinson's disease subjects. [^{123}I]FP-CIT SPECT images and binding values of healthy subjects and patients with Parkinson's disease or atypical degenerative parkinsonism.

Several studies investigated PD and ADP by means of [^{123}I]FP-CIT and SPECT scans. However, only few specifically established the value of [^{123}I]FP-CIT in setting a differential diagnosis (Table 1).

The amount and pattern of reduced striatal DAT binding in MSA have been demonstrated to be within the range of PD. However, asymmetry of DAT binding loss tends to be more pronounced in PD and the progression of dopaminergic innervation loss is faster in MSA compared with PD [24,25]. In a recent study that compares the accuracy of dual-tracer DAT and perfusion SPECT imaging in the differential diagnosis of parkinsonism using template-based discriminant analysis [26], no reduction of MSA versus PD was noted. In contrast to what was reported by Scherfler and colleagues, demonstrating differential [^{123}I]β-CIT binding capacity between PD and MSA [27], the aforementioned study could not detect differences even at the least stringent threshold ($p = 0.05$ uncorrected). The reverse contrast (PD < MSA) demonstrated a decreased binding in the left posterior putamen. However, El Fakhry and colleagues reported lower striatal binding values in PD (55%) and MSA (23%) compared with normal controls ($p < 0.01$) and lower values in PD compared with MSA ($p < 0.05$) [28]. Asymmetry index was greater for PD than for MSA and controls in both the caudate nucleus and the putamen ($p < 0.05$). In addition, there was a significantly decreased perfusion in the left and right nucleus lentiformis in MSA compared with PD and controls ($p < 0.05$).

A reduction of [^{123}I]β-CIT uptake in the midbrain appears to separate patients with clinically fully developed MSA-P or PSP from patients with PD [23,27,29]. Indeed, a reduced midbrain [^{123}I]β-CIT uptake was found in patients with MSA-P and allowed a correct classification of 95% of MSA-P and PD patients [23,30]. Finally, clinically pure forms of MSA-C (cerebellar variant) may show a DAT binding loss but less compared with MSA-P or PD [31].

Antonini and colleagues reported a greater DAT reduction in patients with PSP (0.51 ± 0.39; $p < 0.01$) compared with MSA-P patients (0.70 ± 0.33) or PD patients (0.95 ± 0.38) [32]. No difference was found between patients with MSA and PD. Putamen/caudate ratios were greater in PSP (0.83 ± 0.12; $p < 0.01$) than in PD patients (0.51 ± 0.11), suggesting a more-uniform involvement of dopamine nerve terminals in both caudate nucleus and putamen. Van Laere and colleagues also demonstrated a greater involvement of the caudate heads in PSP patients, although when directly contrasted with PD patients, a difference was found in the left striatum only at a $p = 0.05$ uncorrected level [26].

DAT loss in CBS is in the same range as that in PD patients [33–35]; but more asymmetrical and less pronounced than in MSA and PSP patients [34]. In CBS, unlike PSP or PD, unilateral balanced (caudate/putamen) reduction in tracer uptake has been observed [33]. DAT binding may result occasionally within the normal range in patients with CBS [35–37]. The low sensitivity and specificity of [^{123}I]FP-CIT for the diagnosis of CBS also relies on the pathological and clinical heterogeneity of this syndrome (Figure 2) [37,38] .

Overall, a meta-analysis of diagnostic accuracy on SPECT in parkinsonian syndromes [39] revealed, for presynaptic tracers in general, a moderate to high sensitivity but a low specificity in differentiating PD from MSA and PSP (pooled odds ratio with 95% CI was 2). Similarly, presynaptic tracers showed a very

high sensitivity (78–100%) but a low specificity (0–33%) in discriminating between MSA and PSP (pooled odds ratio with 95% CI was 2).

In conclusion, [123I]FP-CIT and SPECT have little value in discriminating between ADPs in routine clinical practice and they should be used with great care when differentiating ADP from PD, as only hints towards a more accurate clinical diagnosis can be obtained. On the other hand, a normal result is considered inconsistent with a clinical diagnosis of PD or ADP [39,40].

Postsynaptic dopamine receptor imaging, in addition to presynaptic dopamine transporter imaging, may be necessary, together with clinical reassessment and follow-up imaging, to improve diagnostic accuracy in PD and ADP [41]. The

Table 1. Summary of [123I]FP-CIT findings in subjects with atypical degenerative parkinsonism.

Study (year)	Tracer	Clinical diagnosis	Imaging findings	Ref.
Benamer *et al.* (2000)	[123I]FP-CIT	PD (n = 145), MSA (n = 2), PSP (n = 10), ET (n = 33), HC (n = 38)	Clear visual differentiation between ET and HC vs PD and atypical parkinsonism. No differences between atypical parkinsonism and PD	[10]
Antonini *et al.* (2003)	[123I]FP-CIT	PD (n = 70), MSA (n = 10), PSP (n = 10), HC (n = 12)	Striatal binding reduced in PD, MSA and PSP vs HC Greater and more uniform bilateral binding loss in PSP vs PD and MSA	[32]
Plotkin *et al.* (2005)	[123I]FP-CIT [123I]IBZM	PD (n = 25), MSA (n = 13), PSP (n = 8), DLB (n = 6), CBS (n = 9), ET (n = 11)	[123I]FP-CIT: all ET subjects had normal scans No significant differences among atypical parkinsonism CBS patients showed a significantly lower asymmetry index for striatum and putamen [123I]IBZM: no difference between ET and PD. Lower binding values in MSA, PSP and CBD in comparison with PD, ET and DLB but no differences between ADPs	[30]
El Fakhri *et al.* (2006)	[123I]FP-CIT [99Tc]ECD	PD (n = 5), MSA (n = 5), HC n = 9)	[123I]FP-CIT: significant binding loss in PD and MSA vs HC Greater reduction in PD vs MSA [99Tc]ECD-CIT: no significant regional perfusion differences between PD and HC Reduced bilateral perfusion in caudate nucleus and nucleus lentiformis in MSA when compared with PD and HC	[28]
Koch *et al.* (2007)	[123I]FP-CIT [123I]IBZM	PD (n = 69), MSA (n = 18), HC (n = 13)	[123I]FP-CIT: lower binding in PD vs atypical parkinsonism [123I]IBZM: higher D_2 receptor binding in PD vs atypical parkinsonism No differentiation between atypical parkinsonism at imaging analysis	[65]
Vlaar *et al.* (2008)	[123I]FP-CIT [123I]IBZM	PD (n = 127), ET (n = 22), MSA (n = 17), PSP (n = 8), CBD (n = 1)	The differentiation between PD and atypical parkinsonism by both tracers scored relatively low accuracy. The combination of the two only minimally increased accuracy	[40]
Mo *et al.* (2010)	[123I]FP-CIT [123I]IBZM	PD (n = 104), MSA (n = 7), PSP (n = 3)	[123I]FP-CIT: significantly reduced binding in PD, MSA and PSP vs HC. No statistical binding differences between PD and atypical parkinsonism [123I]IBZM: no significant differences in postsynaptic uptake ratios between PD and controls, and PD and atypical parkinsonism	[53]
Cilia *et al.* (2011)	[123I]FP-CIT	PD (n = 37), CBS (n = 36), HC (n = 24)	More uniform reduction throughout the striatum and greater hemispheric asymmetry (for the caudate nucleus) in CBS vs PD On average, CBS patients showed reduced binding values vs HC and higher asymmetry indices in the caudate nucleus and putamen and similar caudate-to-putamen ratio in CBS vs HC Normal binding values in four CBS patients	[37]
Südmeyer *et al.* (2011)	[123I]FP-CIT [123I]IBZM [123I]MIBG	PD (n = 31), MSA (n = 11), PSP (n = 6)	The combined use of tracers reached distinguished atypical parkinsonism from PD with a sensitivity of 94%, specificity of 94% (test accuracy 94%), positive predictive value of 89% and negative predictive value of 97% Differential diagnosis within atypical parkinsonism was not reported	[63]

ADP: Atypical degenerative parkinsonism; CBD: Corticobasal degeneration; CBS: Corticobasal syndrome; DLB: Dementia with Lewy bodies; ET: Essential tremor; HC: Healthy control; MSA: Multiple system atrophy; PD: Parkinson's disease; PSP: Progressive supranuclear palsy.

most widely applied radiotracer for imaging D_2 receptors with SPECT is [^{123}I]iodobenzamide ([^{123}I]IBZM) [42,43]. Postsynaptic receptor density is normal or upregulated in early PD but invariably decreased in ADP [44–47]. Along with PD progression, binding values of PD patients are still in the range of control subjects, possibly due to a decline in the presynaptic dopaminergic drive, which results in dopamine receptor upregulation [48–51]. The preserved D_2/D_3 receptor availability is a prerequisite for the response to L-Dopa therapy [52]. The combination of pre- ([^{123}I]FP-CIT) and post-synaptic ([^{123}I]IBZM) dopamine SPECT can serve as an indicator for excluding ADP with a reasonably high accuracy of 85%, especially in early diagnosed drug-naive PD patients [23,53]. Compared with [^{123}I]FP-CIT [30], [^{123}I]IBZM has a distinctly lower ability to detect alterations of the dopaminergic system, and therefore should only be used as an additional examination to further corroborate a potential sufferer of ADP. [^{123}I] IBZM binding is variably decreased in ADPs and cannot discriminate between them [40]. As a cautious suggestion, [^{123}I]IBZM SPECT may more often result in a normal range in CBS than in MSA or PSP [34]. The diagnostic performance might be substantially improved with the use of a D_2/D_3 receptor radioligand and PET [54].

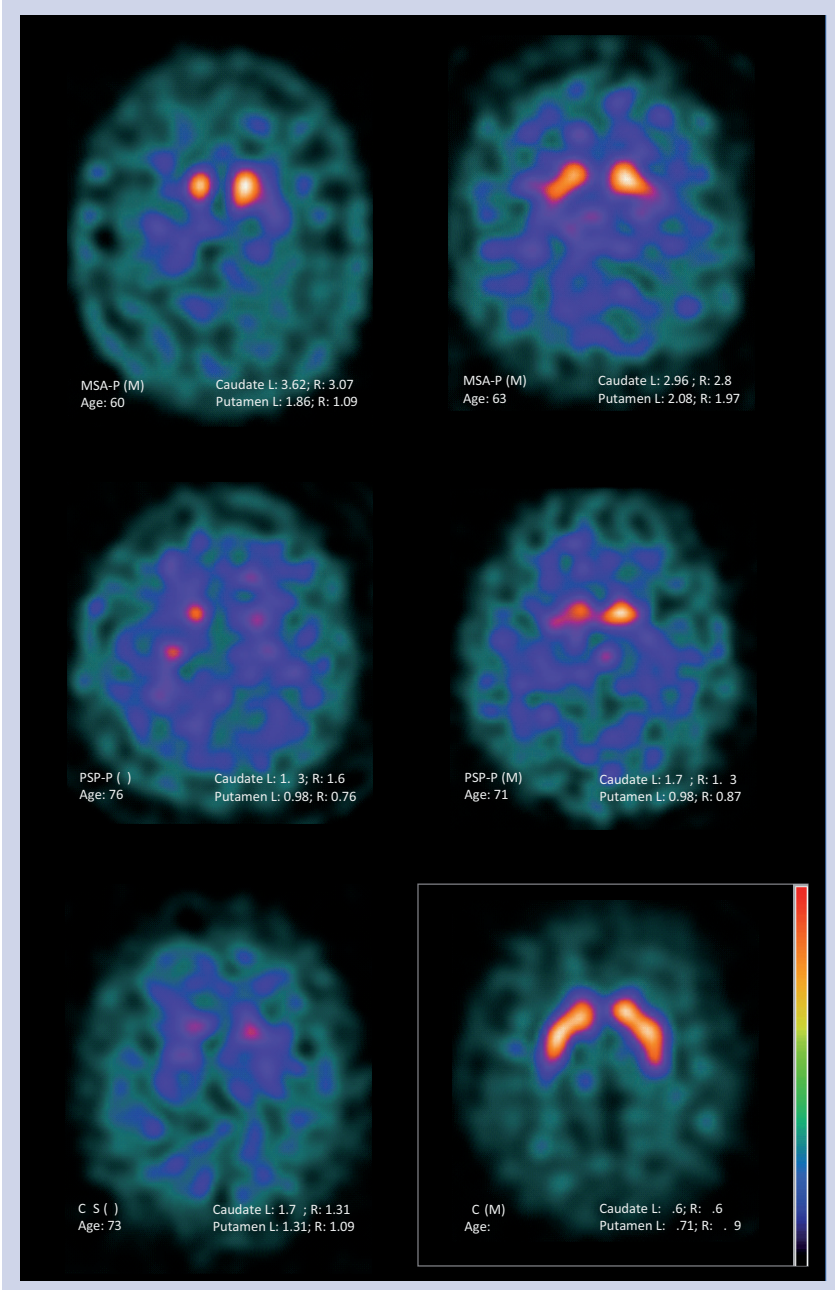


Figure 2. [^{123}I]FP-CIT of healthy controls and atypical degenerative parkinsonisms.

Metabolic and perfusion studies using PET or SPECT have also shown some value in the differential diagnosis of ADP. In particular, disease-related spatial covariance patterns identified a marked bilateral reduction in the lentiform nuclei and the cerebellum in MSA. By contrast, PSP is characterized by the presence of metabolic decrements in midline frontal regions and in the brainstem. The distinguishing feature of the pattern of glucose metabolism in CBS is the asymmetrical distribution of radiotracer uptake with a relative metabolic reduction in many cortical areas, the insula and in the basal ganglia contralateral to the most affected side [55,56].

However, the overall diagnostic accuracy of metabolic and perfusion studies seems rather poor in a single patient. When comparing [^{123}I] IBZM SPECT and [^{18}F]FDG PET in neurodegenerative parkinsonism, it is clear that interrater agreement of visual analysis is substantial in both methods [57]. However, findings of either are discordant in a significant number of cases.

It is an open issue which nuclear medicine examination best relates to clinical course. Clinical tests and follow-up examinations, as well as morphologic information (e.g., MRI), are still necessary as additional diagnostic tools to discriminate within ADP [46,58,59].

As an alternative, cardiac imaging with ([^{123}I] MIBG) has demonstrated changes consistent with heart denervation in patients with PD that are not present in patients with MSA or PSP [60]. However, the use of this tracer is limited because of insufficient sensitivity in patients with short disease duration [61]. Furthermore, by applying a PET radioligand, cardiac sympathetic denervation was found to occur not only in PD but also in other movement disorders,

such as MSA and PSP [62]. This finding implies that scintigraphic detection of cardiac sympathetic denervation cannot be used independently to discriminate idiopathic PD from other movement disorders, such as MSA and PSP. Furthermore, cardiac sympathetic denervation was not correlated with striatal denervation (measured with a PET-compound for vesicular monoamine transporter, which is supposed to be of at least similar diagnostic values as the DAT radiotracers). This suggests that the pathophysiologic processes underlying cardiac denervation and striatal denervation occur independently in patients with parkinsonian syndromes [62].

The combined use of [123I]MIBG and [123I]FP-CIT or [123I]IBZM significantly improved diagnostic accuracy in PD versus ADP reaching a sensitivity of 94%, specificity of 94%, positive predictive value of 89% and negative predictive value of 97%. However, even the combination of these tracers was not able to discriminate between PSP and MSA with more success than clinical follow-up at 2 years [63].

Although disease-related differences in the pattern of nigrostriatal degeneration of PD and ADP are present [64], DAT imaging does not significantly improve diagnostic accuracy in all cases and it is of little help in the differential diagnosis between ADP [25]. The best imaging approach to clarify whether the cause of presynaptic dopaminergic loss is PD or ADP is a combined radiotracer or multimodal approach including dopamine D_2 receptor imaging [30,65], MRI techniques [66,67] and eventually cardiac imaging of the sympathetic nervous system [62].

Finally, in the absence of histopathological material nearly all imaging studies have used clinical diagnoses as the gold standards so far, which may not always be accurate. Given the high rate of clinical misdiagnosis [4,5] this should be regarded as the main bias of all such studies.

Introduction to the compound

■ Chemistry

[123I]FP-CIT (ioflupane) is commercially available as DaTSCAN in many European countries (e.g., in Germany since 2000) and also in the USA since 2011. It is delivered as a pyrogen-free radiopharmaceutical for intravenous injection in single-use vials. It is a cocaine analog substance and tropane derivative (Figure 3).

Iodine-123 is a cyclotron-produced, γ-emitting radionuclide with a main energy level of 159 keV and a physical half-life of 13.2 h. The active drug compound is *N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-[123I]iodophenyl)nortropane. *In vitro,* ioflupane binds reversibly to the human recombinant DAT with IC_{50} ranging from 0.71 to 1.67 nM as examined using rat striatal tissue homogenates by displacement of the radiotracer [3H]-WIN 35,428 [68–70]. Potency of ioflupane was much greater than for cocaine (IC_{50}: 89.1 nM) and similar to β-CIT (IC_{50}: 1.24 nM). High binding affinity of ioflupane in human striatum has been shown autoradiographically using a C-11 labeled compound [71]. Binding was low in the cortex and other brain regions, and in the thalamus it was 10% of binding in the putamen. Approximately 80–90% of the striatal binding was blocked by the DAT inhibitor GBR 12,909; the inhibition constant was K_i = 0.62 nM. High specificity to the presynaptic DAT was demonstrated by competition studies with GBR 12,909, the serotonin reuptake inhibitor citalopram and the norepinephrine reuptake inhibitor desipramine in post-mortem human brain slices exposed to radiolabeled ioflupane. Autoradiographically, binding was at high concentrations in the DAT-rich striatum; (in other words, the caudate nucleus and the putamen) and this binding to the striatum was abolished in the presence of high concentrations of GBR 12,909.

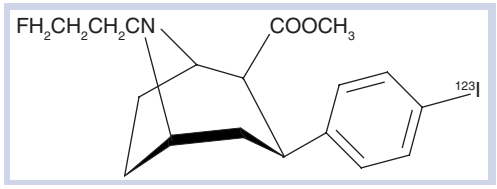


Figure 3. [123I]FP-CIT.

The recommended dosage of [123I]FP-CIT is 111–185 MBq (3–5 mCi), typically 185 MBq (5 Ci). In our own experience, no doses less than 150 MBq (4 mCi) should be applied, since lower amounts of activity may have an impact on scan quality and hence, diagnostic performance.

■ Pharmacodynamics

Overall, imaging agents contain only a small quantity of active compound so that no pharmacologic effects are expected. In general, the DAT-binding tropanes are good markers for the integrity of the nigrostriatal systems. *Ex vivo* studies in an animal lesion model using both an analog of levodopa and a DAT-radiotracer revealed that the uptake of [18F]FDOPA and [18F]CFT correlated well with the density of

dopaminergic fibers [72]. This indicated a high sensitivity of both radiotracers in PD. However, [^{18}F]FDOPA demonstrated a higher unspecific uptake that was probably due to extensive compensatory metabolism. So it seems that this tracer was less sensitive than the DAT-tracer [^{18}F]CFT in detecting nigrostriatal degeneration [72]. Since the uptake of the latter was heavily reduced in degeneration stage two, a downregulation of DAT was hypothesized. Another lesion study in rats using [^{125}I]β-CIT and [^{14}C] L-DOPA provides similar results, indicating that the marker of the decarboxylase underestimated the decrease of dopaminergic neurons and that DAT levels more precisely reflected the decrease [73]. Such different detection sensitivities of radiotracers for DAT, the vesicular monoamine transporters (VMAT2) and [^{18}F] FDOPA in (early) PD [74] were also predicted in humans. Other head-to-head comparisons in humans did not reveal any significant differences in the diagnostic utilities of radiotracers for DAT, the vesicular monoamine transporters (VMAT2) or [^{18}F]FDOPA [13].

In addition, there are no differences among tropane derivatives in detecting early dopaminergic degeneration (FP-CIT; β-CIT; IPT; TRODAT-1 [75–78]). A direct comparison of [^{123}I]FP-CIT versus [^{123}I]β-CIT revealed similar capabilities for the detection of dopaminergic degeneration [79].

■ Pharmacokinetics & metabolism

The pharmacokinetics of [^{123}I]FP-CIT were studied by monitoring radioactivity following intravenous injection and whole-body scintigraphy. Such biodistribution studies revealed that 5% of the administered radioactivity remains in whole blood 5 min after the injection and 7% of injected radioactivity enters the brain 10 min after the injection. Radioactivity in the brain decreases to 3% after 5 h. The striatum-to-background ratio is relatively constant between 3 and 6 h after the injection, meaning that clinical imaging is feasible with comparative results during this time window. This is a major advantage of [^{123}I]FP-CIT over other tropane-based radiotracers, in particular [^{123}I]β-CIT, which reaches an 'equilibrium' after 24 h for DAT-rich regions. For [^{123}I] FP-CIT, approximately 30% of the whole brain radioactivity was attributed to striatal uptake. The biodistribution, metabolism and dosimetry of ioflupane in nonhuman primates and in humans are further described in several papers [71,80–83].

■ Drug interactions

Based on published data, it is likely that several drugs of abuse, including cocaine, amphetamines, modafinil, certain antidepressants (e.g., mazindol, bupropion and radafaxine, among others), adrenergic agents (e.g., phenylephrine and norepinephrine) and the anticholinergic agent benzatropine, influence the visual interpretation and quantification of [^{123}I]FP-CIT SPECT scans in routine clinical studies.

Ideally, such medications should be withdrawn, before the administration of the radiotracer, at a time five-times greater that the drug's biological half-life [84]. The decision to withdraw any medication must always be made by the specialist in charge of the patient's care, balancing the potential risks of such a withdrawal.

Antiparkinsonian medications (i.e., levodopa, dopamine agonists, *N*-methyl-D-aspartate receptor blockers, monoamine oxidase-B inhibitors and catechol-*O*-methyltransferase inhibitors) taken at standard doses do not markedly affect dopamine transporter binding, and therefore they need not be withdrawn before dopaminergic imaging [84,85].

Interestingly, one study showed a significant higher binding in patients with ADP without antiparkinsonian medication in comparison to subjects in drugs-on state [23].

■ Data acquisition & analysis

A prerequisite for fully utilizing the diagnostic potential of DaTSCAN imaging is, however, good quality control and standardization of the entire procedure, from patient preparation through to positioning, γ camera specifications, acquisition, reconstruction parameters and quality control of the acquired data. In addition data should be analyzed and reported according to guidelines (i.e., European Association of Nuclear Medicine [84] and the guidelines of the Society of Nuclear Medicine). Technical issues with regard to data acquisition comprise correct field of view, rotational radius, energy window set at the photopeak, additional scatter windows (if applicable), matrix size and zoom factor, among others. Strict standardizations of acquisition time after radiotracer injection and collection of sufficient numbers of total counts within the acquisition time also have to be considered. After reviewing the projection data (i.e., for motion artifacts), images are processed with distinct reconstruction methods (iterative reconstruction or filtered back-projection) and filtering (e.g., with a low pass filter). Employing the correct filter is mandatory for either visual

or quantitative readings. Attenuation correction is recommended, either with simultaneously or sequentially acquired transmission scans, or calculated, as with a correction matrix, according to Chang [86].

For display, images are reformatted into slices in three planes (axial, coronal and sagittal). Reorientation makes visual interpretation easier and is essential when semi-quantification is used (not least to ensure the right placement of the reference region). The anterior commissure-posterior commissure line represents a good anatomical standard here as it is used for brain MRI. A simultaneously acquired CT scan or, alternatively, coregistration with (individual) MRI by commercial available software (e.g., HERMES MultiModality, Hermes Medical Solutions, Stockholm, Sweden) will allow precise re-alignment of the head.

Visual assessment is robust in detecting presynaptic DAT binding. However, in visually uncertain cases and for intergroup, as well as for interinstitutional comparisons, semiquantitative approaches using regions of interest (ROIs) might help and have been recommended by nuclear medicine associations to be incorporated in the routine work-up of DAT-SPECT. With semi-quantification, striatal binding ratios (specific striatal binding) are calculated by comparing the activity in the target region with the activity in a reference region (with a very low DAT-density) according to listed formula (**Box 1**).

Reference region should conventionally refer to the occipital cortex for [123I]FP-CIT. Other parameters are described in **Box 1**.

ROIs are manually drawn on to one or more slices (usually three or four adjacent slides) with the highest striatal activity. This method is simple and provides a quantitative measure to allow comparisons of healthy reference data (where an age-dependent decline in healthy volunteers always has to be considered) but interobserver variability is considerable (due to variability in ROI placement). Therefore, it is important to standardize realignment (using predefined ROIs). Here, coregistration with individual MRI for delineation of striatal and reference volumes of interest offers the most accurate manual results [87].

Besides these observer-dependent approaches, fully-automated image analysis techniques are under validation in the clinical setting and have the potential as tools to improve the diagnostic accuracy and confidence in DAT-SPECT in patients who have parkinsonian features. Examples of these more advanced automated systems using volumes of interest, and voxel-based mathematical systems are DATQUANT™ (GE Healthcare, WI, USA), EXINI dat™ (EXINI Diagnostics AB, Lund, Sweden), and a modified version of the Brain Analysis Software (BRASS, BRASS-DaT, Hermes Medical Solutions, Stockholm, Sweden). They are all capable of producing more objective, observer-independent results and are faster compared with individual ROI/volume-of-interest-based methods. The voxel-based systems often use statistical parametric mapping (Wellcome Department of Cognitive Neurology, University College London, UK) that runs on a MATLAB® platform (The MathWorks Inc., MA, USA); however, this is for scientific purposes and not in routine clinical practice.

■ Safety & tolerability

No adverse event has been directly correlated with the tracer itself. However, several symptoms (e.g., headache, flu-like symptoms, injection site bleeding, vertigo and parasthesia) were described to be possibly or probably due to [123I]FP-CIT injection [10].

■ Cost–effectiveness

[123I]FP-CIT SPECT scans proved to be economically advantageous in the diagnostic work-up of patients with uncertain parkinsonism (including essential tremor), especially when total indirect treatment costs over time were calculated [88,89]. No studies specifically addressed cost–effectiveness of [123I]FP-CIT SPECT for

Box 1. Binding ratio formulas.

$$\text{Striatal binding ratio} = \frac{\text{Mean counts of striatal ROI} - \text{mean counts of reference (background) ROI}}{\text{Mean counts of reference (background) ROI}}$$

$$\text{Putamen - to - caudate ratio} = \frac{\text{Specific striatal binding}_{\text{putamen}}}{\text{Specific striatal binding}_{\text{caudate}}}$$

$$\text{Asymmetry} = \frac{\text{Specific striatal binding}_{\text{right striatum}} - \text{specific striatal binding}_{\text{left striatum}} \times 2}{\text{Specific striatal binding}_{\text{right striatum}} + \text{specific striatal binding}_{\text{left striatum}}}$$

ROI: Region of interest.

ADP. Cost–effectiveness may also derive from the incorrect screening of suitable candidates for drug trials or complex surgical procedures (e.g., deep brain stimulation) that may not be effective for ADP.

Conclusion & future perspective

Differentiating PD from ADP solely on [123I] FP-CIT imaging is still a challenge. Some suggestions may derive from asymmetry and diffusion of dopaminergic deficit or from targeting the brainstem. No additional value to the clinical experience is added instead in discriminating between ADPs. This result derives also in part from the limited number of patients with ADP investigated and from the uncertainties of clinical diagnosis still used as the reference in the absence of anatomopathological findings. The combination of several available SPECT tracers may not necessarily enhance specificity and sensitivity in discriminating between ADPs. Nowadays, imaging should not be considered as a replacement for a thorough clinical investigation and patients should be referred to movement disorder specialists. However, [123I] FP-CIT and SPECT imaging has been proven to be a safe and effective tool to investigate dopaminergic innervation and help exclude diseases without such an innervation loss.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- [123I]FP-CIT SPECT has little value in discriminating between atypical degenerative parkinsonism (ADPs) in routine clinical practice and it should be used with great care when differentiating between ADP and Parkinson's disease.
- A normal result at [123I]FP-CIT SPECT is considered inconsistent with a clinical diagnosis of Parkinson's disease or ADP.
- The combination of [123I]FP-CIT and other radioligands for SPECT (e.g., [123I]MIBG and [123I]IBZM) may enhance diagnostic accuracy with respect to Parkinson's disease although it may not necessarily enhance specificity and sensitivity in discriminating between ADPs.
- Antiparkinsonian medications (e.g., levodopa and dopamine agonists, among others) taken at standard doses do not markedly affect dopamine transporter binding, and therefore they need not be withdrawn before SPECT with [123I]FP-CIT.
- Semiquantitative approaches and automated systems for data analysis, possibly with individual MRI coregistration, should be used to improve the diagnostic accuracy. Normality values should be based on results obtained in healthy age-matched subjects in each center.
- [123I]FP-CIT SPECT scans proved to be safe and economically advantageous in the diagnostic work-up of patients with uncertain (degenerative vs nondegenerative) parkinsonism.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. *N. Engl. J. Med.* 348, 1356–1364 (2003).

2 Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch. Neurol.* 56, 33–39 (1999).

3 Litvan I, Bhatia KP, Burn DJ *et al.* Movement disorders society scientific issues committee report: SIC task force appraisal of clinical diagnostic criteria for Parkinsonian disorders. *Mov. Disord.* 18, 467–486 (2003).

4 Hughes AJ, Daniel SE, Kilford L *et al.* The accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathological study. *J. Neurol. Neurosurg. Psychiatry* 55, 181–184 (1992).

5 Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology* 57, 1497–1499 (2001).

▪▪ **A detailed clinical–pathological analysis showing the relatively low diagnostic accuracy of a clinical evaluation in Parkinson's disease (PD) and atypical parkinsonism.**

6 Meara J, Bhowmick BK, Hobson P. Accuracy of diagnosis in patients with presumed Parkinson's disease. *Age Ageing* 28, 99–102 (1999).

7 Schrag A, Ben-Shlomo Y, Quinn N. How valid is the clinical diagnosis of Parkinson's disease in the community? *J. Neurol. Neurosurg. Psychiatry* 73, 529–534 (2002).

8 Kish SJ, Shannak K, Hirnykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiological and clinical implications. *N. Engl. J. Med.* 318(14), 876–880 (1988).

9 Piggott MA, Marshall EF, Thomas N *et al.* Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer's and Parkinson's diseases: rostrocaudal distribution. *Brain* 122, 1449–1468 (1999).

10 Benamer TS, Patterson J, Grosset DG *et al.* Accurate differentiation of parkinsonism and essential tremor using visual assessment of [123I]-FP-CIT SPECT imaging: the [123I]-FP-CIT study group. *Mov. Disord.* 15, 503–510 (2000).

▪ **The first multicenter study using a blind consensus reading and showing that a simple visual assessment of [123I]FP-CIT SPECT images is an easily applied diagnostic test for a reliable differentiation of tremor disorders and in confirming a clinical diagnosis of a hypokinetic-rigid syndrome.**

11 Isaias IU, Benti R, Goldwurm S *et al.* Striatal dopamine transporter binding in Parkinson's disease associated with the LRRK2 Gly2019Ser mutation. *Mov. Disord.* 21, 1144–1147 (2006).

12 Isaias IU, Benti R, Cilia R *et al.* [123I]FP-CIT striatal binding in early Parkinson's disease patients with tremor vs. akinetic-rigid onset. *Neuroreport* 18, 1499–1502 (2007).

13 Eshuis SA, Jager PL, Maguire RP, Jonkman S, Dierckx RA, Leenders KL. Direct comparison of FP-CIT SPECT and F DOPA PET in patients with Parkinson's disease and healthy controls. *Eur. J. Nucl. Med. Mol. Imaging* 36, 454–462 (2009).

14 Isaias IU, Marotta G, Hirano S *et al.* Imaging essential tremor. *Mov. Disord.* 25, 679–686 (2010).

15 Schneider SA, Edwards MJ, Mir P *et al.* Patients with adult-onset dystonic tremor resembling Parkinsonism tremor have scans without evidence of dopaminergic deficit (SWEDDs). *Mov. Disord*. 22, 2210–2215 (2007).

16 Benaderette S, Zanotti Fregonara P, Apartis E *et al.* Psychogenic parkinsonism: a combination of clinical, electrophysiological, and [123I]-FP-CIT SPECT scan explorations improves diagnostic accuracy. *Mov. Disord.* 21, 310–317 (2006).

17 Gaig C, Martí MJ, Tolosa E *et al.* 123I-Ioflupane SPECT in the diagnosis of suspected psychogenic parkinsonism. *Mov. Disord.* 21, 1994–1998 (2006).

18 Gilman S, Wenning GK, Low PA *et al.* Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* 71, 670–676 (2008).

19 Brooks DJ. Diagnosis and management of atypical parkinsonian syndromes. *J. Neurol. Neurosurg. Psychiatry* 72, 10–16 (2002).

20 Lubarsky M, Juncos JL. Progressive supranuclear palsy: a current review. *Neurologist* 14, 79–88 (2008).

21 Mahapatra RK, Edwards MJ, Schott JM, Bhatia KP. Corticobasal degeneration. *Lancet Neurol.* 3, 736–743 (2004).

22 Adbo WF, Borm GF, Munneke M, Verbeek MM, Esselink RA, Bloem BR. Ten steps to identify atypical parkinsonism. *J. Neurol. Neurosurg. Psychiatry* 77, 1367–1369 (2006).

23 Hesse S, Oehlwein C, Barthel H *et al.* Possible impact of dopamine SPECT on decision-making for drug treatment in Parkinsonian syndrome. *J. Neural Transm.* 113, 1177–1190 (2006).

24 Pirker W, Djamshidian S, Asenbaum S *et al.* Progression of dopaminergic degeneration in Parkinson's disease and atypical parkinsonism: a longitudinal β-CIT SPECT study. *Mov. Disord.* 17, 45–53 (2002).

25 Varrone A, Marek KL, Jennings D *et al.* [123I] β-CIT SPECT imaging demonstrates reduced density of striatal dopamine transporters in Parkinson's disease and multiple system atrophy. *Mov. Disord.* 16, 1023–1032 (2001).

26 Van Laere K, Casteels C, De Ceuninck L *et al.* Dual-tracer dopamine transporter and perfusion SPECT in differential diagnosis of parkinsonism using template-based discriminant analysis. *J. Nucl. Med.* 47, 384–392 (2006).

27 Scherfler C, Seppi K, Donnemiller E *et al.* Voxel-wise analysis of [123I]-CIT SPECT differentiates the Parkinson variant of multiple system atrophy from idiopathic Parkinson's disease. *Brain* 128, 1605–1612 (2005).

▪▪ **An excellent approach to distinguishing between multiple system atrophy and PD at the voxel level.**

28 El Fakhri G, Habert MO, Maksud P *et al.* Quantitative simultaneous ^{99m}Tc-ECD/^{123}I-FP-CIT SPECT in Parkinson's disease and multiple system atrophy. *Eur. J. Nucl. Med. Mol. Imaging* 33, 87–92 (2006).

29 Seppi K, Scherfler C, Donnemiller E *et al.* Topography of dopamine transporter availability in progressive supranuclear palsy: a voxel wise [123I] βCIT SPECT analysis. *Arch Neurol.* 63, 1154–1160 (2006).

30 Plotkin M, Amthauer H, Klaffke S *et al.* Combined [123I]-FP- CIT and [123I]-IBZM SPECT for the diagnosis of parkinsonian syndromes: study on 72 patients. *J. Neural Transm.* 112, 677–692 (2005).

▪ **Evaluates the accuracy of combined SPECT imaging versus clinical follow-up in a large series of patients with PD.**

31 Rinne JO, Burn DJ, Mathias CJ *et al.* Positron emission tomography studies on the dopaminergic system and striatal opioid binding in the olivopontocerebellar atrophy variant of multiple system atrophy. *Ann. Neurol.* 37, 568–573 (1995).

32 Antonini A, Benti R, De Notaris R *et al.* ^{123}I-Ioflupane/SPECT binding to striatal dopamine transporter (DAT) uptake in patients with Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy. *Neurol. Sci.* 24, 149–150 (2003).

▪ **One of the first attempts to investigate and compare dopamine transporter binding values, as measured by [123I]FP-CIT SPECT, in atypical degenerative parkinsonism.**

33 Sawle GV, Brooks DJ, Marsden CD *et al.* Corticobasal degeneration: a unique pattern of regional cortical oxygen hypometabolism and striatal fluorodopa uptake demonstrated by positron emission tomography. *Brain* 114, 541–556 (1991).

34 Pirker W, Asenbaum S, Bencsits G *et al.* [123I] β-CIT SPECT in multiple system atrophy, progressive supranuclear palsy, and corticobasal degeneration. *Mov. Disord.* 15, 1158–1167 (2000).

35 Klaffke S, Kuhn AA, Plotkin M *et al.* Dopamine transporters, D2 receptors, and glucose metabolism in corticobasal degeneration. *Mov. Disord.* 21, 1724–1727 (2006).

▪ **Addresses distinct SPECT features of corticobasal syndrome. However, without any neuropathological reference the results are preliminary.**

36 O'Sullivan SS, Burn DJ, Holton JL, Lees AJ. Normal dopamine transporter single photon emission CT scan in corticobasal degeneration. *Mov. Disord.* 23, 2424–2426 (2008).

37 Cilia R, Rossi C, Frosini D *et al.* Dopamine transporter SPECT imaging in corticobasal syndrome. *PLoS ONE* 6(5) e18301 (2011).

38 Schneider JA, Watts RL, Gearing M, Brewer RP, Mirra SS. Corticobasal degeneration: neuropathologic and clinical heterogeneity. *Neurology* 48, 959–969 (1997).

39 Vlaar AM, van Kroonenburgh MJ, Kessels AG, Weber WE. Meta-analysis of the literature on diagnostic accuracy of SPECT in parkinsonian syndromes. *BMC Neurol.* 7, 27 (2007).

▪▪ **Meta-analysis of the literature describing the diagnostic accuracy of SPECT in parkinsonian syndromes.**

40 Vlaar AM, de Nijs T, Kessel AG *et al.* Diagnostic value of ^{123}I-ioflupane and ^{123}I-iodobenzamide SPECT scans in 248 patients with parkinsonian syndromes. *Eur. Neurol.* 59, 258–266 (2008).

41 Felicio AC, Shih MC, Godeiro-Junior C, Andrade LA, Bressan RA, Ferraz HB. Molecular imaging studies in Parkinson disease: reducing diagnostic uncertainty. *Neurologist* 15, 6–16 (2009).

42 Tissingh G, Booij J, Winogrodzka A, van Royen EA, Wolters EC. IBZM- and CIT- SPECT of the dopaminergic system in parkinsonism. *J. Neural Transm. Suppl.* 50, 31–37 (1997).

43 Halldin C, Gulyas B, Langer O, Farde L. Brain radioligands state of the art and new trends. *Q. J. Nucl. Med.* 45, 139–152 (2001).

44 Antonini A, Leenders KL, Vontobel P *et al.* Complementary PET studies of striatal neuronal function in the differential diagnosis between multiple system atrophy and Parkinson's disease. *Brain* 120, 2187–2195 (1997).

45 Kim YJ, Ichise M, Ballinger JR *et al.* Combination of dopamine transporter and D2 receptor SPECT in the diagnostic evaluation of PD, MSA, and PSP. *Mov. Disord.* 17, 303–312 (2002).

46 Ghaemi M, Hilker R, Rudolf J, Sobesky J, Heiss WD. Differentiating multiple system atrophy from Parkinson's disease: contribution of striatal and midbrain MRI

volumetry and multi-tracer PET imaging. *J. Neurol. Neurosurg. Psychiatry* 73, 517–523 (2002).

47 Knudsen GM, Karlsborg M, Thomsen G *et al.* Imaging of dopamine transporters and D2 receptors in patients with Parkinson's disease and multiple system atrophy. *Eur. J. Nucl. Med. Mol. Imaging* 31, 1631–1638 (2004).

48 Schwarz J, Tatsch K, Arnold G *et al.* ^{123}I-iodobenzamide-SPECT predicts dopaminergic responsiveness in patients with *de novo* parkinsonism. *Neurology* 42, 556–561 (1992).

49 Hierholzer J, Cordes M, Schelosky L *et al.* The differential diagnosis of Parkinson diseases 123I-IBZM-SPECT vs. the apomorphine test. *Rofo* 159, 86–90 (1993).

50 Hierholzer J, Cordes M, Venz S *et al.* Loss of dopamine-D2 receptor binding sites in Parkinsonian plus syndromes. *J. Nucl. Med.* 39, 954–960 (1998).

51 Pizzolato G, Cagnin A, Rossato A *et al.* Striatal dopamine D2 receptor alterations and response to L-DOPA in Parkinson's disease. A [^{123}I]IBZM SPET study. *Adv. Neurol.* 69, 467–473 (1996).

52 Schwarz J, Tatsch K, Gasser T, Arnold G, Oertel WH [123].IBZM binding predicts dopaminergic responsiveness in patients with parkinsonism and previous dopaminomimetic therapy. *Mov. Disord.* 12, 898–902 (1997).

53 Mo SJ, Linder J, Forsgren L, Larsson A, Johansson L, Riklund K. Pre- and postsynaptic dopamine SPECT in the early phase of idiopathic parkinsonism: a population-based study. *Eur. J. Nucl. Med. Mol. Imaging* 37, 2154–2164 (2010).

54 la Fougère C, Pöpperl G, Levin J *et al.* The value of the dopamine $D_{2/3}$ receptor ligand ^{18}F-desmethoxyfallypride for the differentiation of idiopathic and nonidiopathic parkinsonian syndromes. *J. Nucl. Med.* 51, 581–587 (2010).

▪ **Interesting paper showing the higher accuracy of dopamine $D_{2/3}$ receptor PET when compared with $D_{2/3}$ receptor SPECT.**

55 Eckert T, Barnes A, Dhawan V *et al.* FDG PET in the differential diagnosis of parkinsonian disorders. *Neuroimage* 26, 912–921 (2005).

▪▪ **A milestone publication demonstrating different patterns of brain glucose metabolism in idiopathic and atypical PD.**

56 Eckert T, Van Laere K, Tang C *et al.* Quantification of Parkinson's disease-related network expression with ECD SPECT. *Eur. J. Nucl. Med. Mol. Imaging* 34, 496–501 (2007).

57 Derlin T, Afzal W, Wilke F *et al.* IBZM SPECT and FDG PET in the differential diagnosis of Parkinsonian syndromes: comparison with respect to inter-rater agreement. *Nuklearmedizin* 49, 139–147 (2010).

58 Morelli M, Arabia G, Novellino F *et al.* MRI measurements predict PSP in unclassifiable parkinsonisms: a cohort study. *Neurology* 77, 1042–1047 (2011).

59 Köllensperger M, Wenning GK. Assessing disease progression with MRI in atypical parkinsonian disorders. *Mov. Disord.* 24, S699–S702 (2009).

60 Spiegel J, Möllers MO, Jost WH *et al.* FP-CIT and MIBG scintigraphy in early Parkinson's disease. *Mov. Disord.* 20, 552–561 (2005).

61 Sawada H, Oeda T, Yamamoto K *et al.* Diagnostic accuracy of cardiac meta-iodobenzylguanidine scintigraphy in Parkinson disease. *Eur. J. Neurol.* 16, 174–182 (2009).

62 Raffel DM, Koeppe RA, Little R *et al.* PET measurement of cardiac and nigrostriatal denervation in Parkinsonian syndromes. *J. Nucl. Med.* 47, 1769–1777 (2006).

▪▪ **Excellent study that questions the accuracy of dedicated heart SPECT.**

63 Südmeyer M, Antke C, Zizek T *et al.* Diagnostic accuracy of combined FP-CIT, IBZM, and MIBG scintigraphy in the differential diagnosis of degenerative parkinsonism: a multidimensional statistical approach. *J. Nucl. Med.* 52, 733–740 (2011).

64 Cummings JL, Henchcliffe C, Schaier S, Simuni T, Waxman A, Kemp P. The role of dopaminergic imaging in patients with symptoms of dopaminergic system neurodegeneration. *Brain* 134, 3146–3166 (2011).

65 Koch W, Hamann C, Radau PE, Tatsch K. Does combined imaging of the pre- and postsynaptic dopaminergic system increase the diagnostic accuracy in the differential diagnosis of parkinsonism? *Eur. J. Nucl. Med. Mol. Imaging* 34, 1265–1273 (2007).

66 Stoessl AJ, Martin WW, McKeown MJ, Sossi V. Advances in imaging in Parkinson's disease. *Lancet Neurol.* 10, 987–1001 (2011).

67 Brooks DJ. Imaging approaches to Parkinson disease. *J. Nucl. Med.* 51, 596–609 (2010).

68 Scheffel U, Lever JR, Abraham P *et al.* N-substituted phenyltropanes as *in vivo* binding ligands for rapid imaging studies of the dopamine transporter. *Synapse* 25, 345–349 (1997).

69 Neumeyer JL, Wang S, Gao Y *et al.* *N*-ω-fluoroalkyl analogs of (1R)-2β- carbomethoxy-3β-(4-iodophenyl)-tropane (β-CIT): radiotracers for positron emission tomography and single photon emission computed tomography imaging of dopamine transporters. *J. Med. Chem.* 37, 1558–1561 (1994).

70 Neumeyer JL, Tamagnan G, Wang S *et al.* *N*-substituted analogs of 2β-carbomethoxy- 3β- (4′-iodophenyl) tropane (β-CIT) with selective affinity to dopamine or serotonin transporters in rat forebrain. *J. Med. Chem.* 39, 543–548 (1996).

71 Lundkvist C, Halldin C, Ginovart N, Swahn CG, Farde L. [^{18}F] β-CIT-FP is superior to [^{11}C] β-CIT-FP for quantitation of the dopamine transporter. *Nucl. Med. Biol.* 24, 621–627 (1997).

72 Forsback S, Niemi R, Marjamäki P *et al.* Uptake of 6-[^{18}F]fluoro-L-dopa and [^{18}F]CFT reflect nigral neuronal loss in a rat model of Parkinson's disease. *Synapse* 51, 119–127 (2004).

73 Ito Y, Fujita M, Shimada S *et al.* Comparison between the decrease of dopamine transporter and that of L-DOPA uptake for detection of early to advanced stage of Parkinson's disease in animal models. *Synapse* 31, 178–185 (1999).

74 Fischman AJ. Role of [^{18}F]-dopa-PET imaging in assessing movement disorders. *Radiol. Clin. North Am.* 43, 93–106 (2005).

75 Tissingh G, Booij J, Bergmans P *et al.* Iodine-123-N-Ω-fluoropropyl-2β- carbomethoxy-3β-(4-iodophenyl)tropane SPECT in healthy controls and early-stage, drug-naive Parkinson's disease. *J. Nucl. Med.* 39, 1143–1148 (1998).

76 Marek KL, Seibyl JP, Zoghbi SS *et al.* [^{123}I] β-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. *Neurology* 46, 231–237 (1996).

77 Schwarz J, Linke R, Kerner M *et al.* Striatal dopamine transporter binding assessed by [I-123]IPT and single photon emission computed tomography in patients with early Parkinson's disease: implications for a preclinical diagnosis. *Arch. Neurol.* 57, 205–208 (2000).

▪ **Demonstrates that an early (preclinical) PD diagnosis is feasible with dopamine transporter SPECT in cases of hemiparkinsonism.**

78 Huang WS, Lin SZ, Lin JC, Wey SP, Ting G, Liu RS. Evaluation of early-stage Parkinson's disease with 99mTc-TRODAT-1 imaging. *J. Nucl. Med.* 42, 1303–1308 (2001).

79 Seibyl JP, Marek K, Sheff K *et al.* Iodine-123-β-CIT and iodine-123-FPCIT SPECT measurement of dopamine transporters in healthy subjects and Parkinson's patients. *J. Nucl. Med.* 39, 1500–1508 (1998).

80 Booij J, Andringa G, Rijks LJ *et al.* [123I] FP-CIT binds to the dopamine transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned monkeys. *Synapse* 27, 183–190 (1997).

81 Booij J, Busemann Sokole E, Stabin MG, Janssen AG, de Bruin K, van Royen EA. Human biodistribution and dosimetry of [123I]FP-CIT: a potent radioligand for imaging of dopamine transporters. *Eur. J. Nucl. Med.* 25, 24–30 (1998).

82 Lundkvist C, Halldin C, Swahn CG *et al.* *O*-methyl-11C] β-CIT-FP, a potential radioligand for quantitation of the dopamine transporter: preparation, autoradiography, metabolite studies, and positron emission tomography examinations. *Nucl. Med. Biol.* 22, 905–913 (1995).

83 Chaly T, Dhawan V, Kazumata K *et al.* Radiosynthesis of [18F] *N*-3-fluoropropyl-2-β-carbomethoxy-3-β-(4-iodophenyl) nortropane and the first human study with positron emission tomography. *Nucl. Med. Biol.* 23, 999–1004 (1996).

84 Darcourt J, Booij J, Tatsch K *et al.* EANM procedure guidelines for brain neurotransmission SPECT using 123I-labelled dopamine transporter ligands, version 2. *Eur. J. Nucl. Med. Mol. Imaging* 37, 443–450 (2010).

85 Schillaci O, Pierantozzi M, Filippi L *et al.* The effect of levodopa therapy on dopamine transporter SPECT imaging with 123I-FP-CIT in patients with Parkinson's disease. *Eur. J. Nucl. Med. Mol. Imaging* 32, 1452–1456 (2005).

86 Chang CC, Liu JS, Chang YY, Chang WN, Chen SS, Lee CH. 99mTc-ethyl cysteinate dimer brain SPECT findings in early stage of dementia with Lewy bodies and Parkinson's disease patients: a correlation with neuropsychological tests. *Eur. J. Neurol.* 15, 61–65 (2008).

87 Barthel H, Müller U, Wächter T *et al.* Multimodal SPECT and MRT imaging data analysis for an improvement in the diagnosis of idiopathic Parkinson's syndrome. *Radiologe* 40, 863–869 (2000).

88 Van Laere K, Everaert L, Annemans L, Gonce M, Vandenberghe W, Vander Borght T. The cost effectiveness of 123I-FP-CIT SPECT imaging in patients with an uncertain clinical diagnosis of parkinsonism. *Eur. J. Nucl. Med. Mol. Imaging* 35, 1367–1376 (2008).

89 Antonini A, Berto P, Lopatriello S *et al.* Cost-effectiveness of 123I-FP-CIT in the differential diagnosis of essential tremor and Parkinson's disease in Italy. *Mov. Disord.* 23(15), 2202–2209 (2008).

Brief Communications

Dopaminergic Striatal Innervation Predicts Interlimb Transfer of a Visuomotor Skill

Ioannis U. Isaias,[1,2,3] Clara Moisello,[4] Giorgio Marotta,[5] Mauro Schiavella,[2] Margherita Canesi,[2] Bernardo Perfetti,[4] Paolo Cavallari,[1] Gianni Pezzoli,[2] and M. Felice Ghilardi[4]

[1]Università degli Studi di Milano, Dipartimento di Fisiologia Umana, 20133 Milano, Italy, [2]Centro per la Malattia di Parkinson e i Disturbi del Movimento, Istituti Clinici di Perfezionamento, 20126 Milano, Italy, [3]Universitätsklinik Würzburg, Neurologische Klinik und Poliklinik, 97080 Würzburg, Germany, [4]CUNY Medical School, Department of Physiology and Pharmacology, New York, New York 10031, and [5]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Dipartimento di Medicina Nucleare, 20122 Milano, Italy

We investigated whether dopamine influences the rate of adaptation to a visuomotor distortion and the transfer of this learning from the right to the left limb in human subjects. We thus studied patients with Parkinson disease as a putative *in vivo* model of dopaminergic denervation. Despite normal adaptation rates, patients showed a reduced transfer compared with age-matched healthy controls. The magnitude of the transfer, but not of the adaptation rate, was positively predicted by the values of dopamine-transporter binding of the right caudate and putamen. We conclude that striatal dopaminergic activity plays an important role in the transfer of visuomotor skills.

Introduction

Motor learning relies on the intact function of dopaminergic transmission (Knowlton et al., 1996; Seidler et al., 2006; Seidler and Noll, 2008; Karabanov et al., 2010). In particular, dopamine neurons play an important role in coding reinforcement prediction errors, a key signal in many learning models (Sutton and Barto, 1998; Maia, 2009). Prediction errors are used to learn the values of states, state-action pairs, or both, which are then used to select optimal actions (Sutton and Barto, 1998). At the cellular and synaptic level, such learning is thought to occur through long-term changes in synaptic strength at striatal synapses, with dopamine release as an essential signaling event starting the process (Montague et al., 2004; Calabresi et al., 2007).

Adaptation of reaching movements to visuomotor rotation is a type of learning that can take place implicitly. During this learning process, a new internal model or memory is gradually formed, while the discrepancy between the desired and the executed trajectory gradually diminished (Krakauer, 2009). Several studies have suggested a right-hemisphere dominance in the acquisition of this motor skill (Ghilardi et al., 2000; Huber et al., 2004), but the role of dopamine and its basal ganglia innervation in visuomotor adaptation has been poorly investigated. Previous studies have shown that adaptation but not retention is normal in patients with Parkinson disease (PD) (Marinelli et al., 2009; Bédard and Sanes, 2011; Venkatakrishnan et al., 2011), but have not addressed the role of dopamine in this task. However, the existing evidence in normal subjects suggests that the striatum plays an important role in the formation and retrieval of this motor skill (Ghilardi et al., 2000; Seidler et al., 2006; Seidler and Noll, 2008).

When learning is accomplished with one limb through task repetition, the ability to perform the same task with the opposite, untrained limb can also improve. This process is referred to interlimb transfer and has been shown to apply to visuomotor adaptation (Sainburg and Wang, 2002). Interlimb transfer implies that the limb-specific motor memory, stored during adaptation, can be retrieved and applied to the other limb with different dynamic characteristics. As reported in several studies, the corpus callosum is likely involved in this process (for review, see Halsband and Lange, 2006). However, given their important role in motor learning, dopamine and the striatum could be also involved in such a skill, but their function has not been directly assessed.

The aim of this study is to determine whether the adaptation to visuomotor rotation and its transfer to the untrained hand depend on dopamine levels. Thus, we investigated the time course of adaptation to a rotated display with the right hand and its transfer to the left hand in drug-naive patients with PD and in age-matched controls. We then correlated the indices of learning and transfer to dopaminergic innervation as measured by [^{123}I] *N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)tropane (FP-CIT) and single-photon computed tomography (SPECT) (Isaias et al., 2007).

Materials and Methods

Subjects. We tested 11 patients with PD (five males; median age, 52 years; range, 38–66 years) and a control group of 10 neurologically intact adults (three males; median age, 52 years; range, 42–79 years). Median age of PD patients at motor symptoms onset was 47 years (33–64 years).

Received July 13, 2011; revised Aug. 16, 2011; accepted Aug. 18, 2011.

Author contributions: I.U.I., C.M., and M.F.G. designed research; I.U.I., G.M., M.S., and M.C. performed research; I.U.I., C.M., G.M., M.S., M.C., B.P., P.C., G.P., and M.F.G. analyzed data; I.U.I., C.M., G.M., B.P., P.C., G.P., and M.F.G. wrote the paper.

This study was supported by the Grigioni Foundation for Parkinson Disease (to I.U.I.), the National Parkinson's Foundation (to G.M.F.), and NIH Grant NS054864 (to G.M.F.). We thank Prof. Jens Volkmann (Department of Neurology, University of Würzburg, Würzburg, Germany) for critically reading this manuscript.

The authors declare no competing financial interests.

Correspondence should be addressed to Dr. Ioannis U. Isaias, Dipartimento di Fisiologia Umana, Università degli Studi, 20133 Milano, Italy. E-mail: iuisaias@yahoo.it.

DOI:10.1523/JNEUROSCI.3583-11.2011

 0270-6474/11/3114458-05$15.00/0

All subjects were right-handed, as assessed by a modified Edinburgh handedness inventory.

The diagnosis of PD was made according to the UK Parkinson Disease Brain Bank criteria and patients evaluated with the Unified Parkinson Disease Rating Scale motor part (UPDRS-III). Two additional UPDRS sub-subscores were calculated (Isaias et al., 2007). Median UPDRS-III score was 12 (range, 6–20). Median akinetic-rigid score (UPDRS-AK) was 5 (range, 2–11) and median UPDRS tremor score (UPDRS-T) was 1 (range, 0–2).

Clinical inclusion criteria for subjects with PD were as follows: (1) UPDRS part I score of 0; (2) UPDRS-T score <3; (3) disease duration <5 years; (4) Hoehn and Yahr scale, stage 2; (5) drug naive (patients had never been treated with any antiparkinsonian drugs); (6) no psychiatric disorders or other neurological diseases other than PD; and (7) absence of any signs indicative for atypical parkinsonism (e.g., gaze abnormalities, autonomic dysfunction, psychiatric disturbances, etc.).

All subjects (patients and controls) had no cognitive decline as well as no deficit in visual attention, task switching, memory, or learning strategies, as assessed by the Mini-Mental State examination, Clock Drawing Test, Frontal Assessment Battery, Corsi block-tapping task, Corsi supraspan learning task, Corsi Recall, and Trail Making Test (A, B, and A-B). An MRI was also performed and only subjects with normal results (i.e., no sign of white matter lesion or atrophy) were enrolled in the study. MRIs were performed within 6 months from subjects' enrollment. None of the controls had a history of neurological disorders, head trauma with loss of consciousness, epilepsy, brain surgery, systemic illness, or excessive drug or alcohol consumption at any time during their life. Participants were instructed not to drink any beverages containing caffeine or alcohol during the 24 h before the experiment. The local institutional review board approved the study.

Task and experimental design. The motor tasks have been extensively described in previous papers (Ghilardi et al., 2000; Huber et al., 2004; Marinelli et al., 2009). Briefly, subjects moved a cursor with their dominant or nondominant hand on a digitizing tablet and performed out-and-back movements toward one of eight targets presented on the computer screen every 1.5 s. Vision of both the hand and arm was prevented by an opaque panel, but the position of the cursor on the screen was always visible. Targets were positioned 4 cm from a common starting point and were presented in blocks of 48 (block duration, 72 s). Hand trajectory was sampled at 200 Hz.

We used two tasks: a baseline motor task, in which the cursor position on the screen and the hand position on the tablet corresponded; and a visuomotor adaptation task (ROT), in which the cursor position on the screen was rotated 30° counterclockwise to the actual hand movement.

In the main experiment, after familiarization with the apparatus, subjects performed two blocks of baseline condition with each hand; no visual distortion was applied. Subsequently, they performed 10 blocks of ROT with the dominant (right) hand, followed by three blocks of ROT with the nondominant (left) hand.

Three weeks later, seven patients (six women; median age, 50 years; range, 39–57 years) and eight normal controls (five women; median age, 58.5 years; range, 42–66 years) were retested, in the same clinical conditions, with one block (48 movements) of the baseline motor task performed with the left hand to assess long-term aftereffects.

Behavioral data analysis. In this study, we were interested in describing the process of adaptation to the imposed cursor distortion. Therefore, we first measured the initial planning of the movement direction as directional error at peak velocity, which is the difference between the target direction and the movement direction at peak velocity. For each hand, directional errors in the ROT blocks were normalized (DirErrVnorm) by subtracting the mean error in the corresponding baseline motor task where no rotation was applied.

We then computed the average percentage adaptation to the applied distortion for each ROT block as: percentage adaptation $= 100 * (1 -$ DirErrVnorm/30). We then measured the movement curvature to investigate the degree of online correction as the absolute difference between the directional error at peak velocity and that at the reversal point.

To measure the degree of total adaptation achieved with the right hand, we computed the difference in percentage adaptation between the first and last block. The same difference was computed for the movement curvature.

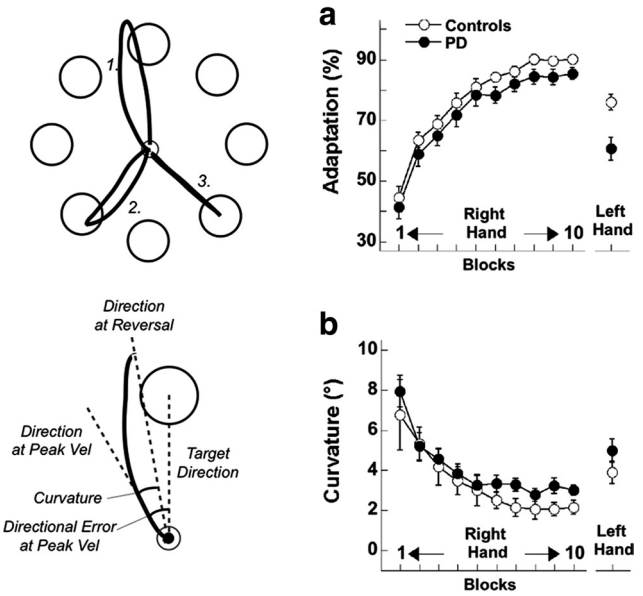


Figure 1. Top left, Target array with three trajectories representative of initial (1), intermediate (2), and complete (3) levels of adaptation. Bottom left, Illustration of directional error and curvature at an initial level of adaptation (see line 1, top left panel). ***a***, ***b***, Time course of adaptation (based on directional errors at the peak velocity; ***a***) and curvature (***b***) as a function of blocks for the right hand and for transfer to the left hand in patient and control groups. Bars represent SEs.

Table 1. Anatomic locations with side, spatial extent of cluster in voxels (Ke), *t* score, and *Z* score of striatal areas showing significant values, MNI coordinates

	Side	Cluster size	*t* value	*Z* score	MNI coordinates (*x*, *y*, *z*)
Two-samples *t* test: PD versus HC					
Putamen	R	1844	13.56	6.95	32, 0, 0
Caudate	R	Subcluster	9.58	5.95	16, 12, 10
Ventral striatum	R	Subcluster	4.95	4.61	18, 20, −6
Putamen	L	1575	11.14	6.39	−28, −6, −2
Caudate	L	Subcluster	6.58	4,84	−14, 20, −8
Covariance analysis of adaption transfer score					
Caudate	R	76	3.7	3.23	16, 8, 14
Putamen	R	219	3.0	2.71	32, 4, −2

L, Left; R, right.

Right-to-left interlimb transfer was measured as the percentage difference between the last adaptation block with the right hand and the first with the left hand for both indices.

We assessed differences in the rate of adaptation with the right hand, as well as changes in curvature, by performing a mixed-model ANOVA ($\alpha = 0.05$) on both variables with Group (PD, controls) as the between-subject factor and Block (1–10) as the within-subject factor.

The between-group differences in the degree of total adaptation achieved and the difference in curvature, as well as for the interlimb transfer of both these variables, were assessed using a one-way ANOVA with Group as main factor.

SPECT data acquisition and reconstruction. SPECT data acquisition and reconstruction has been described in detail previously (Isaias et al., 2010). In brief, dopamine-transporter (DAT) values were measured with SPECT with FP-CIT. Intravenous administration of 110–185 MBq of ^{123}I-FP-CIT (DaTSCAN; GE Healthcare) was performed 30–40 min after thyroid blockade (10–15 mg of Lugol solution per os) in all patients. Data were compared with data from a group of 15 healthy subjects matched for age (mean age, 62 years; ±9 SD; range, 44–68 years) and sex

Table 2. DAT binding values

	CN right	CN left	PT right	PT left
PD	1.21 ± 0.16 (0.99–1.51)*	1.2 ± 0.18 (0.99–1.46)*	1.74 ± 0.26 (1.2–2.1)*	1.92 ± 0.32 (1.47–2.42)*
HC	1.77 ± 0.29 (1.15–2.1)	1.59 ± 0.33 (1.19–2.04)	2.54 ± 0.32 (1.85–3.04)	2.79 ± 0.35 (2.06–3.47)

Subjects with PD showed a significant reduction of DAT binding values in both the caudate nucleus (CN) and putamen (PT) bilaterally when compared to a group of 15 HC. Data are reported as mean ± SD and range (in brackets). *$p < 0.001$.

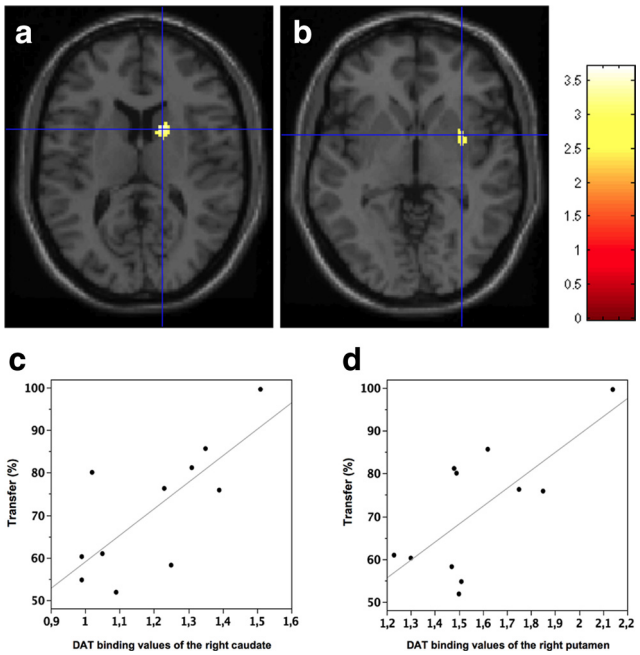


Figure 2. ***a***, ***b***, Whole-brain voxelwise statistical analysis (SPM) showing a positive correlation between transfer of adaptation and DAT density in the right caudate (*x*, *y*, *z*: 16, 8, 14) and right putamen (*x*, *y*, *z*: 32, 4, −2). ***c***, ***d***, A volume of interest analysis confirming a positive correlation for the right caudate nucleus ($\rho = 0.74, p < 0.01$) and right putamen ($\rho = 0.71$, $p = 0.01$).

(four males). Brain SPECT was performed 3–4 h later by means of a dedicated triple detector gamma-camera (Prism 3000; Philips) equipped with low-energy, ultra-high-resolution fan beam collimators (four subsets of acquisitions; matrix size, 128 × 128; radius of rotation, 12.9–13.9 cm; continuous rotation; angular sampling, 3°; duration, 28 min) in patient and control groups. Brain sections were reconstructed with an iterative algorithm (ordered subset expectation maximization, four iterations and 15 subsets), followed by 3D filtering of sections obtained (Butterworth, order 5, cutoff 0.31 Ny) and attenuation correction (Chang method, factor 0.12).

Imaging data processing. The reconstructed images were analyzed for regionally specific FP-CIT binding using Statistical Parametric Mapping (SPM2; Wellcome Department of Imaging Neuroscience, London, UK) in conjunction with MATLAB version R2007a (Mathworks).

First, we created a group-specific ^{123}I-FP-CIT SPECT template with SPM2 by spatially normalizing the FP-CIT images of 15 healthy subjects onto a ^{18}F-FP-CIT PET MNI-based template as previously described (Ma et al., 2002; Isaias et al., 2010), averaging the normalized images and their symmetric (mirror) image and filtering using a 3D Gaussian kernel with 8 mm full width at half maximum (FWHM) (Kas et al., 2007). Then, the FP-CIT images of all subjects were spatially normalized onto this FP-CIT template and smoothed with a FWHM 10 mm Gaussian kernel to increase the signal-to-noise ratio and to account for subtle variations in anatomic structures. For each individual FP-CIT SPECT image, a parametric binding ratio image was calculated using the ImCalc toolbox in SPM. Binding values for each FP-CIT image were computed in a voxel-by-voxel manner [(voxel − occipital)/occipital]. The reference region in the occipital cortex was defined using the volume-of-interest of superior, middle, and inferior occipital gyri and calcarine gyri of the automated anatomical labeling (Tzourio-Mazoyer et al., 2002), using the Wake Forest University PickAtlas 2.4 software.

Voxelwise statistical analysis. A general linear model was used to perform the appropriate voxelwise statistics using SPM2 in whole brain of all subjects. The analysis was applied to SPECT images by means of single-subject: conditions and covariates design. The PD patients and healthy controls were modeled as conditions and the covariates were the scores of acquisition and transfer for both adaptation and curvature. In addition, we performed a covariance analysis between the transfer scores and FP-CIT SPECT images. In every analysis, we used no global normalization, no grand mean scaling, and threshold masking absolute of >1. For *t* test in PD versus healthy controls (HC), $p < 0.001$ with false discovery rate correction was considered significant and for covariance analysis, $p < 0.01$ uncorrected was significant, both at clusters of at least 50 voxels. All coordinates are reported in MNI space.

General statistical analysis. Normality of data distribution was tested by the Shapiro-Wilks test. Gender distribution among groups was tested with χ square. Demographic data were compared by means of Wilcoxon two-group test. A multivariate pairwise correlation analysis was used to investigate statistical dependence among SPECT binding values demographic, clinical, and behavioral data. Statistical analyses were performed with the JMP statistical package, version 8.0.2 (SAS Institute).

Results

In the first 10 blocks, patients and controls gradually decreased their directional error at peak velocity and adapted to the imposed rotation at a similar rate (Block: $F_{(9,171)} = 165.52$, $p < 0.00001$; Group: $F_{(1,19)} = 1.70$, $p = 0.20$; Block × Group: $F_{(9,171)} = 0.25$, $p = 0.99$; Fig. 1*a*). The degree of total adaptation achieved was comparable in patients (44.1% ± 2.7%) and controls (45.7% ± 2.9%; $F_{(1,19)} = 0.14$, $p = 0.71$).

Both groups showed a similar and significant reduction in curvature across blocks (Block: $F_{(9,171)} = 15.77$, $p < 0.00001$; Group: $F_{(1,19)} = 0.59$, $p = 0.45$; Block × Group: $F_{(9,171)} = 0.31$, $p = 0.97$; Fig. 1*b*), further indicating that adaptation indeed resulted from updating the internal model with reduced reliance on online corrections.

The transfer of adaptation to the performance with the left hand was lower in patients compared with controls (PD: 71.4 ± 4.6 vs HC: 85.5 ± 2.4, mean ± SE; $F_{(1,19)} = 5.82$, $p = 0.03$), while the transfer of curvature was similar in the two groups (PD: 2 ± 0.6 vs HC: 1.9 ± 0.6, mean ± SE; $F_{(1,19)} = 0.07$, $p = 0.80$).

In the subgroup of subjects tested 3 weeks later, we found that aftereffects were significantly larger in controls (−7.49 ± 3.40°) than in patients with PD, where such effects were virtually absent (−0.29 ± 3.78°; $F_{(1,13)} = 15.01$; $p = 0.002$).

A significant, although weak, correlation ($\rho = 0.53, p < 0.025$) was found between the 3 week aftereffects and right-to-left hand transfer.

No correlation was found between behavioral and clinical or demographic data. In particular, we found no correlations between transfer of adaptation and UPDRS-III ($\rho = 0.22$; $p = 0.51$), UPDRS-AK ($\rho = -0.18$; $p = 0.58$), and UPDRS-item 22 (rigidity score left: $\rho = -0.45$, $p = 0.15$; rigidity score right: $\rho = 0.15$, $p = 0.64$), suggesting that the learning and transfer indices are not predicted by clinical signs of PD. In addition, transfer of adaptation did not correlate with age ($\rho = -0.33$, $p = 0.31$) or disease duration ($\rho = -0.06$, $p = 0.84$).

Binding values are listed in Tables 1 and 2. On average, PD subjects showed a reduced DAT binding value of 32% in the right caudate, 25% in the left caudate, and 32% in both right and left putamen. Average DAT binding loss was not statistically different between left and right striatum.

Whole-brain SPM analysis revealed a selective positive correlation between the transfer of adaptation, but not of curvature, and DAT binding values of the right caudate and putamen only (Fig. 2, *a* and *b*, respectively). These results were confirmed by a volume of interest analysis (transfer of adaptation and right caudate: $\rho = 0.74$, $p < 0.01$; transfer of adaptation and right putamen: $\rho = 0.71$, $p = 0.01$; Fig. 2, *c* and *d*, respectively).

No correlation was found between the adaptation rate of the right hand and DAT binding of any brain areas.

Discussion

The main result of this study is that the transfer of visuomotor learning correlated with DAT binding of the right striatum, suggesting, for the first time, that the levels of striatal dopamine influence the degree of interlimb transfer of a visuomotor skill.

In agreement with previous studies with similar paradigms (Marinelli et al., 2009; Bédard and Sanes, 2011; Venkatakrishnan et al., 2011) or prism adaptation (Weiner et al., 1983; Stern et al., 1988), patients with PD adapted their movements to an applied visual distortion similarly to healthy subjects. This visuomotor learning occurred by decreasing both initial directional error and hand path curvature. The decrement of both measures suggests that adaptation was achieved by the progressive modification of the motor plan and not by the constant use of online corrections of the trajectory.

When we tested the left hand, PD patients showed a reduced transfer of visuomotor learning compared with controls. The degree of transfer positively correlated with the values of DAT binding of the right striatum, but was not predicted by the UPDRS scores. Therefore, the observed deficit in transfer cannot be ascribed to a disease-related motor impairment, but depends on the dopaminergic innervation itself.

The specific mechanisms underlying interlimb transfer of visuomotor adaptation are still largely unknown. Adapting to a visuomotor rotation is likely to involve both effector-independent and effector-specific processes. The former consist of the progressive creation of new mapping between the direction of the hand trajectory and of the target, expressed in visual coordinates and therefore independent of the characteristics of the actual effector (i.e., the limb). The latter consist of the selection and implementation of the appropriate dynamic motor commands that translate the new motor plan into the actual muscle-activation patterns, which are specific for the effector involved. Training with one hand, therefore, likely promotes the formation of both a visuospatial memory, which can be shared by different effectors, and an effector-specific visuomotor memory. Therefore, we can assume that interlimb transfer is possible as the untrained hand can access the newly formed visual mapping. The fact that transfer is not complete, at least at the beginning of a task, implies that the formation of an effector-specific memory is also essential to complete the adaptation process. The reduced amount of transfer that we found in patients with PD could be due to impairments in both the formation and retrieval of the new visuospatial map, as well as in the efficient conversion of such a map to the untrained arm. Of note, the clinical signs in our patients were bilateral and of comparable severity, as also shown by similar DAT binding loss in both right and left striatum. Therefore, the reduced interlimb transfer should not be ascribed to a differential impairment of the two limbs. In this and previous studies (Marinelli et al., 2009), we also found that PD patients display an impairment of the long-term retention of this visuomotor skill that, in the present patient population, is highly correlated with the degree of transfer. Thus, it is possible that the reduced transfer in PD is part of a general deficit in skill formation, retention, and retrieval. Despite adaptation rates within normal limits, it is possible that the formation of a stable memory trace is not effective in patients with PD, thus impairing short- and long-term memory processes. Accordingly, animal studies suggested a central role of striatal dopaminergic innervation in skill formation (Graybiel, 1998) and strengthening of synaptic plasticity (Calabresi et al., 2000; Lovinger et al., 2003).

The correlation found between the magnitude of the transfer and the dopamine levels was confined to the right striatum. Such lateralization could be explained by the direction of the transfer, from the right to the left hand. However, other theories should consider the right-hemisphere dominancy for this task (Ghilardi et al., 2000; Huber et al., 2004; Seidler et al., 2008) and for visuospatial abilities in general (Heilman et al., 1986). Future studies on the transfer in the opposite direction (left hand toward right hand) could settle the question of hemispheric dominance in this task. So far, studies with our task (Ghilardi et al., 2000; Huber et al., 2004) or similar paradigms (Seidler at al. 2008) showed that activity in the right-hemisphere, and in particular in the right parietal cortex, is essential for the formation and the retention of a new visuospatial mapping. Retrieval of the learned visuospatial skill could thus involve both cortical and striatal structures on the right. It is important to note that this study aimed at specifically examining the role of dopamine in interlimb transfer and not the related anatomical network. Future studies will investigate whether such a transfer relies on transcallosal projections from the cortex (e.g., right parietal cortex) to the ipsilateral and contralateral striatum, or a direct interstriatal transfer through the anterior commissure (Steiner et al., 1985).

References

Bédard P, Sanes JN (2011) Basal ganglia-dependent processes in recalling learned visual-motor adaptations. Exp Brain Res 209:385–393.

Calabresi P, Gubellini P, Centonze D, Picconi B, Bernardi G, Chergui K, Svenningsson P, Fienberg AA, Greengard P (2000) Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. J Neurosci 20:8443–8451.

Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.

Ghilardi MF, Alberoni M, Rossi M, Franceschi M, Mariani C, Fazio F (2000) Visual feedback has differential effects on reaching movements in Parkinson's and Alzheimer's disease. Brain Res 876:112–123.

Graybiel AM (1998) The basal ganglia and chunking of action repertoires. Neurobiol Learn Mem 70:119–136.

Halsband U, Lange RK (2006) Motor learning in man: a review of functional and clinical studies. J Physiol Paris 99:414–424.

Heilman KM, Bowers D, Valenstein E, Watson RT (1986) The right hemisphere: neuropsychological functions. J Neurosurg 64:693–704.

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78–81.

Isaias IU, Benti R, Cilia R, Canesi M, Marotta G, Gerundini P, Pezzoli G, Antonini A (2007) [^{123}I]FP-CIT striatal binding in early Parkinson's disease patients with tremor vs. akinetic-rigid onset. Neuroreport 18:1499–1502.

Isaias IU, Marotta G, Hirano S, Canesi M, Benti R, Righini A, Tang C, Cilia R, Pezzoli G, Eidelberg D, Antonini A (2010) Imaging essential tremor. Mov Disord 25:679–686.

Karabanov A, Cervenka S, de Manzano O, Forssberg H, Farde L, Ullén F (2010) Dopamine D2 receptor density in the limbic striatum is related to implicit but not explicit movement sequence learning. Proc Natl Acad Sci U S A 107:7574–7579.

Kas A, Payoux P, Habert MO, Malek Z, Cointepas Y, El Fakhri G, Chaumet-Riffaud P, Itti E, Remy P (2007) Validation of a standardized normalization template for statistical parametric mapping analysis of ^{123}I-FP-CIT images. J Nucl Med 48:1459–1467.

Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Science 273:1399–1402.

Krakauer JW (2009) Motor learning and consolidation: the case of visuomotor rotation. Adv Exp Med Biol 629:405–421.

Lovinger DM, Partridge JG, Tang KC (2003) Plastic control of striatal glutamatergic transmission by ensemble actions of several neurotransmitters and targets for drugs of abuse. Ann N Y Acad Sci 1003:226–240.

Ma Y, Dhawan V, Mentis M, Chaly T, Spetsieris PG, Eidelberg D (2002) Parametric mapping of [18F]FPCIT binding in early stage Parkinson's disease: a PET study. Synapse 45:125–133.

Maia TV (2009) Reinforcement learning, conditioning, and the brain: successes and challenges. Cogn Affect Behav Neurosci 9:343–364.

Marinelli L, Crupi D, Di Rocco A, Bove M, Eidelberg D, Abbruzzese G, Ghilardi MF (2009) Learning and consolidation of visuomotor adaptation in Parkinson's disease. Parkinsonism Relat Disord 15:6–11.

Montague PR, Hyman SE, Cohen JD (2004) Computational roles for dopamine in behavioural control. Nature 431:760–767.

Sainburg RL, Wang J (2002) Interlimb transfer of visuomotor rotations: independence of direction and final position information. Exp Brain Res 145:437–447.

Seidler RD, Noll DC (2008) Neuroanatomical correlates of motor acquisition and motor transfer. J Neurophysiol 99:1836–1845.

Seidler RD, Noll DC, Chintalapati P (2006) Bilateral basal ganglia activation associated with sensorimotor adaptation. Exp Brain Res 175:544–555.

Steiner H, Morgan S, Huston JP (1985) Effect of forebrain commissurotomy on recovery from unilateral 6-OHDA lesions of the substantia nigra and circling induced by apomorphine. Behav Brain Res 17:245–249.

Stern Y, Mayeux R, Hermann A, Rosen J (1988) Prism adaptation in Parkinson's disease. J Neurol Neurosurg Psychiatry 51:1584–1587.

Sutton RS, Barto AG (1998) Reinforcement learning: an introduction. Cambridge, MA: MIT.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.

Venkatakrishnan A, Banquet JP, Burnod Y, Contreras-vidal JL (2011) Parkinson's disease differentially affects adaptation to gradual as compared to sudden visuomotor distortions. Hum Mov Sci 30:760–769.

Weiner MJ, Hallett M, Funkenstein HH (1983) Adaptation to lateral displacement of vision in patients with lesions of the central nervous system. Neurology 33:766–772.