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Common daily activities in the virtual environment: a preliminary study in parkinsonian patients

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Abstract Patients with Parkinson's disease (PD) create behavioral motor strategies by using external cues to facilitate their movements. Virtual reality (VR) could work as an external stimulus in order to explore the motor plans by means of creation of mental images. We tested 2 women with PD aged 68 and 69 years, and 10 normal control subjects. Patients underwent a neuropsychological assessment to evaluate cognitive abilities involved in the tasks required by the VR session. VR environment reproduces common daily activities situations at home, such as eating or using the bathroom. VR describes the alterations of the motor plans in PD by a point of view different from the clinical one, by testing "pure" mental sequences of the execution of a movement, without the interference of motor disability.

Virtual reality (VR) allows medical practitioners to help their patients in a number of innovative ways, e.g., telesurgery or creating interactive systems that reduce anxiety or stress. Virtual environments are also used to train those patients with disabilities. For example, a VR program that emulates bus rides helps disabled individuals learn to use public transportation [1]. With parkinsonian patients, VR sessions could lead to an improvement in the quality of life by means of an addressed cognitive rehabilitation [2]. Indeed, PD patients create behavioral motor strategies that search for various cues to facilitate their movements [3, 4]. Thus, we supposed that VR could work as an external stimulus in order to facilitate motor plans, by means of creation of useful mental images. In this way, it would be possible to explore the motor plans of a patient in such conditions.

Advanced stages of disease include progressive motor impairment with inability in common daily activities, due to motor blocks or freezing, where cognitive deficits may also play a role. Common "paper and pencil" tests do not always describe adequately these deficits because they include short-time planning tasks, while in daily living activities patients need to perform longer plannings such as, for example, those involved in cooking or in shopping.

In this study we tested the adaptation of the parkinsonian patient in a virtual enviroment; the aim was to determine if VR could give more information in support of the clinical and neuropsychological approaches, overcoming limits inherent to the evaluations in natural environments (for example, dishomogeneity of ambients).

We tested 10 control subjects and 2 women with PD (68 and 69 years old; stage II of Hoehn & Yahr; without long-term syndrome symptoms; duration of disease, 2 and 3 years). In order to test cognitive abilities required for the tasks of the VR session, patients were evaluated by a neuropsychological assessment including: Mini-mental state examination (MMSE), bisyllable word span, Corsi's span, short tale (immediate and retarded recall), Gollin's test, and Raven's PM 38.

VR environment reproduces usual daily living situations: a furnished flat with some rooms where subjects can move around and interact with the objects (for instance, rotate a tap knob).

After briefly familiarizing themselves with the instruments, subjects were asked to perform the following tasks:

- Name objects and describe their possible use in daily life;
- Perform a trajectory by walking around the rooms. The total time of execution and the accuracy (number of failed attempts) were calculated.
- Interact with objects. Three different degrees of difficulty were considered: (1) easy, open a door; (2) medium, sit at a table and have a meal; and (3) difficult, rotate a tap knob.

The experimental procedure consisted of 6 phases:

- *Phase 1 (three minutes)*. The subject becomes familiar with the VR interface (helmet and joystick) and with the virtual environment: walking and pointing to the objects in the corridor.
- *Phase 2 (two minutes)*. Guided walk around living-room and bathroom. Subjects are asked to describe what they see on the table: pasta, spoon, fork, red wine bottle, fruit, cake, bread, omelette, water-melon. Subjects are asked to descibe the room and possible utilization of objects in the bath-room: taps, mirror, bath, shower, WC.
- *Phase 3*. Ability of pointing to targets: easy level (score, 1 point), doors and shower door; medium level (score, 2 points), food and WC; difficult level (score, 3 points) hot and cold knobs, WC button.
- *Phase 4.* Orientation and speed: walking from living-room to the shower. Orientation score (range, 0-3 points), 1 point for any correct direction. Speed score = time of execution in seconds (on-line corrections were allowed).
- *Phase 5*. Starting hesitation (speed score = time of execution in seconds): time spent to open doors and come in the living-room and bathroom.
- *Phase 6*. Incidental memory task (range of score 0-8 points; 1 point for any item recalled). Subjects were asked what kind of food was eaten, colors of bath-room tiles, number of windows in the living-room, presence and color of tablecloth.

The neuropsycological tests show absence of cognitive deficits in our patients as follows:

Patient	MMSE	Short tale	Verbal span	Gollin	Corsi	PM38
A	27	15.2	3	136	4	18/48
B	26	12.5	4	82	5	16/48

Patients and controls adapted rather easily to the virtual environment, following all items required in the time needed by the experimental procedures. They familiarized with VR interface and named objects and described rooms (phases 1 and 2).

In Fig. 1, we report the findings concerning the tasks performed. In comparison with controls, the two parkinsonian patients showed a mild difficulty in pointing tasks, incidental memory tasks and orientation. A stronger involvement was seen by the evaluation of the speed of execution: both PD patients were slower during all trials, especially when they were asked to walk through doors or narrow spaces such as in the bathroom.

Discussion

Human reactions are different according to the received information. If there is a direct visual feedback, action can be immediate. It is possible to quantify the influence of biofeedback, according to the use of an oriented paradigm of equipment and of tests, with the reliable software [5, 6].

Activities like cooking or driving a car need an involvement (in terms of quantity and duration) of superior cortical functions, especially those requiring attention, that cannot be sufficiently explored by traditional neuropsycological tests.

The two patients examinated in this study are at an early stage of disease, with a rather brief duration of symptoms and a moderate motor disability. Compared with controls, our findings show a marked difference in the speed score. The virtual narrow space exaggerates this trend, even if in real life these patients do not manifest any freezing phenomena or motor blocks. Concerning the other tasks performed, the PD patients show a mild difficulty in pointing tasks, incidental memory and orientation.

The use of VR instrumental offers new opportunities in neurorehabilitation, by:

1. Supporting the traditional clinical approach by means of the detection of predictive markers of disexecutive disorders, and
2. Establishing a rehabilitation protocol, teaching patients to approach their disabilities, in favor of autonomy, self-efficacy, social integration, and improvement of quality of life.

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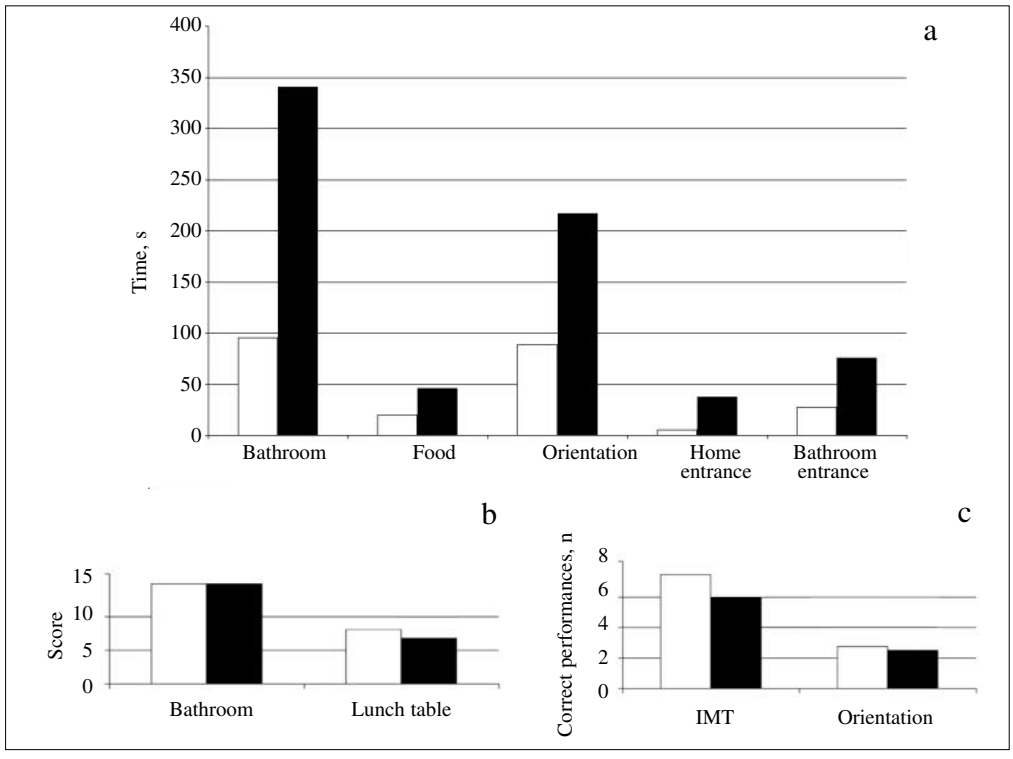

Fig. 1a-c Results obtained in the VR session for 10 normal subjects and 2 Parkinson's disease patients. **a** Speed trials. **b** Pointing tasks. **c** Number of correct performances on incidental memory task (IMT) and orientation task. ■, normal subjects; □, PD patients

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Striatal dopaminergic denervation in early and late onset Parkinson's disease assessed by PET and the tracer [^{11}C]FECIT: preliminary findings in one patient with autosomal recessive parkinsonism (Park2)

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Abstract Neuroimaging studies of striatal dopamine transporters (DAT) have shown that this measurement is a specific marker of dopaminergic degeneration in patients with Parkinson's disease. However, little data is available in subjects with early disease onset, particularly in those with autosomal recessive parkinsonism. We measured striatal DAT binding in 10 patients with early onset PD (onset <40 years) and in 10 with late onset PD (onset >50 years) using PET and the tracer [^{11}C]FECIT. One early onset subject presented a mutation in the *parkin* gene consistent with autosomal recessive parkinsonism. Data were compared with those of 15 control subjects. We found a comparable decrement of striatal DAT binding in early and late onset PD. Loss was widespread and bilateral in the patient carrying the Park2 mutation, suggesting a different pattern of denervation in these individuals.

Parkinson's disease (PD) is characterized by progressive loss of dopamine neurons in the substantia nigra. During the past decade, in vivo imaging of the nigrostriatal dopaminergic system has been developed as a research tool to monitor progressive dopaminergic loss in PD. Several reports have demonstrated that at the time of emergence of PD symptoms there is a loss of approximately 40%–60% of dopaminergic markers in the striatum. More importantly in longitudinal studies of disease progression, imaging ligands targeting dopamine metabolism with [^{11}F]fluorodopa/PET as well as FPCIT/SPECT have shown an annual rate of reduction of 6%–13% in PD patients compared to 0%–2.5% in healthy controls. Neuroimaging tracers can provide a useful tool to help the clinician in differentiating between patients with essential tremor and PD, as well as between different forms of parkinsonism [1]. Particularly, tracers that bind to the dopamine transporter (DAT) are now widely used for this purpose. Previously we have shown that [^{11}C]FECIT is a selective marker of dopamine neurons and can be used to assess dopaminergic loss in PD [3].

We studied DAT with PET and the tracer [^{11}C]FECIT in 20 PD patients with short disease duration (always <5 years; mean 2.5±3.0 years) and H&Y stages I to II. Patients were compared with 15 control subjects (age, 52±16 years). The clinical features of early onset PD patients were: age 55±13 years, disease duration 2.5±3 years. Genetic analysis revealed in one early onset patient a mutation in the *parkin* gene consistent with autosomal recessive parkinsonism. This patient developed mild generalized slowness and right leg dystonia at the age of 26 years. She was first treated with ropinirole (up to 24 mg/day) with benefit, but two years later she developed motor fluctuations and mild dyskinesias. Since then she has been receiving cabergoline (10 mg/day) with improvement of motor fluctuations. Genomic sequencing and exon dosage of *parkin* gene showed that she was a compound heterozygous carriers of *parkin* gene mutations: a point mutation on one allele, transmitted from the affected father, and a deletion in the other allele, transmitted from the healthy mother.

A dose of 5–8 mCi [^{11}C]FECIT tracer was injected in each individual. Regions of interest were placed on [^{11}C]FECIT/PET images on the caudate nucleus and the putamen of each hemisphere as well as on the cerebellum. The cerebellum was used as reference region since virtually no dopamine nerve terminals are present in this area. Values for the right and left hemispheres were averaged. Specific uptake ratios were calculated by dividing putamen and caudate nucleus activities by that for the cerebellum.

We found a significant decrement of DAT binding in the caudate nucleus and putamen of both early and late onset PD patients, compared to healthy controls. In early onset patients, values were 2.8±0.8 in the caudate nucleus and 1.2±0.4 in the putamen. Values in late onset patients were 3.2±0.7 in the caudate nucleus and 1.7±0.5 in the putamen. These values were significantly lower than those of healthy control subjects (p=0.001). Putaminal uptake values were significantly lower in early onset compared to late onset (p=0.01). In the patient with autosomal recessive parkinsonism, DAT binding loss was marked (>80% than control) in both caudate nucleus and putamen More importantly, in this patient the whole putamen was affected unlike in other early onset PD patients who revealed a reduction limited to the posterior part.

Discussion

We found that [^{11}C]FE-CIT binding to dopamine nerve terminals in the putamen completely separated PD patients

from healthy controls. Our results confirm that striatal DAT binding is a sensitive measurement of degeneration in the dopaminergic system of PD patients. Moreover, differences were found when comparing putaminal values between early and late onset PD.

The presence of greater decrements in the putamen than in the caudate nucleus is consistent with neuropathological data showing that the bulk of nigral loss affects the dopaminergic neurons projecting to the putamen. Our results are also consistent with previous studies with [^{11}F]fluorodopa/PET and [^{123}I]CIT or [^{123}I]FPCIT SPECT. Considering recent data suggesting that disease may progress at a slower pace if patients are treated early with dopamine agonists, it becomes critical to identify those individuals as soon as motor symptoms appear [4]. More importantly it may be helpful to evaluate populations at genetic or environmental risk for PD [5]. In this vein, neuroimaging markers may prove useful in supporting the clinical suspicion with a direct demonstration of dopaminergic denervation.

Finally, it is interesting that the patient with autosomal recessive parkinsonism presented with a greater denervation than other early onset counterparts. Our data suggest that dopaminergic loss is marked already a few years after symptoms onset in individuals with early onset parkinsonism, particularly if genetically determined. These findings may be relevant in the early development of motor complications (dyskinesias and motor fluctuations) in these patients.

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Body weight, levodopa pharmacokinetics and dyskinesia in Parkinson's disease

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Abstract We conducted a pharmacokinetic study in 164 patients with sporadic Parkinson's disease (PD) to address the relationship between body weight and levodopa pharmacokinetics. Patients underwent an oral acute levodopa test with 250 mg levodopa and pharmacokinetic variables were further assessed. Plasmatic levodopa area under the curve (AUC-l) and body weight were significantly and inversely correlated. Women were significantly lighter and more dyskinetic than men, and had greater AUC-l values. Our data suggest that during long-term treatment, lighter PD patients, especially women, may receive a greater cumulative dosage of levodopa per kilogram of body weight. This could explain gender differences for the development of levodopa-induced peak-dose dyskinesias observed during the course of the disease.

Levodopa is the most widely employed and the most effective treatment for Parkinson's disease (PD). However, 30%-80% of PD patients chronically treated with levodopa experience long-term complications [1], and it has been hypothesized that the emergence of levodopa-induced involuntary movements, such as peak-dose dyskinesias (PDD), may be related to the administered cumulative levodopa dose [2]. It is well known that changes in body weight may induce substantial variations in peripheral pharmacokinetics of drugs [3], although this issue has not yet been investigated for levodopa. To address whether body weight may influence the pharmacokinetic parameters of levodopa in PD, we studied the plasma levels of levodopa in a large sample of patients with PD in response to an acute oral levodopa test.

One hundred sixty-four sporadic PD patients (91 men and 73 women; mean ± SD age, 65.5±8.9 years) participated in this study. The median score on the modified Hoehn and Yahr scale was 2.5 (range, 1 to 5), and mean duration of PD was 83.7±59.8 months. All patients had received levodopa therapy prior to the study and the median duration of treatment was 51.5 months (range, 3–293 months). Clinical conditions and peripheral pharmacokinetics of levodopa were assessed following an oral acute levodopa test. Briefly, the levodopa test consisted of the oral administration of a standard dose of 250 mg levodopa plus 25 mg carbidopa (immediate release formulation). The drug was administered at 8 AM, after an overnight fast. The motor conditions were assessed by the Unified PD Rating Scale, section of Motor Examination (UPDRS-ME). The occurrence of PDD following acute levodopa was determined as previously described [4], and PDD was recorded as present or absent by the examining neurologist. Samples of venous blood for the measurement of plasmatic levodopa concentrations were drawn through an indwelling catheter immediately before and 30, 60, 120, and 240 minutes after drug intake. Plasma samples were stored immediately at -70° C and later assayed for levodopa using high performance liquid chromatography with coulometric detection [5]. Pharmacokinetic variables including peak concentration in μmol/l (Cmax), time to reach the peak concentration in hours (Tmax), elimination half-life in hours ($T_{1/2}$), and area under the curve in μmol/l h (AUC-l) were calculated using Kinetica 5.0 software (Innaphase, Champs sur Marne, France). The body weight was recorded for each patient and was measured in kilograms. Statistical analysis for differences in the examined variables was performed with Student's *t* test, Mann-Whitney *U* test, and the chi-square test, according to the distribution of data. Correlations between variables were studied by means of Pearson and Spearman tests, and a multiple linear regression model, adjusted for age and sex, was set up to investigate the relation between body weight and AUC-l.

The peripheral pharmacokinetic variables of levodopa after an acute challenge with the drug in 164 patients with PD were the following: Tmax, 0.97±0.71 hours; Cmax, 2.54±1.44 μmol/l; $T_{1/2}$, 1.51±1.21 hours; AUC-l, 5.61±3.19 μmol/l h (Table 1). The median body weight was 70.1 kg (range, 34–100 kg), and 65 patients (39.6%) exhibited PDD. Body weight and AUC-l were significantly and inversely correlated (r=-0.287, $p<0.001$). $T_{1/2}$ and body weight were also inversely and significantly correlated (r=-0.261, $p=0.001$), whereas Tmax and Cmax showed no correlation with body weight. Women were significantly lighter and more dyskinetic than men, and had greater AUC-l values.

Discussion

Our data demonstrate, in a large sample of PD patients, that peripheral pharmacokinetic parameters of levodopa, namely AUC-l and $T_{1/2}$, are inversely correlated with body weight. Thus, the present findings indicate that lighter subjects with PD are exposed to a larger amount of plasmatic levodopa after an acute challenge with the drug. Moreover, in our sample, women as compared to men were significantly lighter, had greater AUC-l values after an acute levodopa test and

Table 1 Clinical characteristics and plasmatic levodopa area under the curve (AUC-l) in 164 patients with Parkinson's disease. Values are mean (SD) unless otherwise indicated

	Men (n=91)		Women (n=73)		*p* value
Age, years	66.0	(8.9)	64.9	(9.0)	NS[b]
Body weight, kg	73.9	(9.4)	65.3	(12.2)	<0.001[b]
Duration of disease, months	78.1	(59.7)	90.7	(59.4)	NS[b]
Duration of levodopa treatment, months	59.4	(56.1)	71.3	(56.5)	NS[b]
Daily levodopa dosage, mg[a]	450	(100–1350)	500	(100–1390)	NS[c]
UPDRS-ME at baseline[a]	18	(5.0-39.5)	20	(3.5-51.0)	NS[c]
Hoehn-Yahr score[a]	2.5	(1–5)	2.5	(1–5)	NS[c]
Dyskinetic subjects, n (%)	26	(28.6)	39	(53.4)	0.001[d]
AUC-l, μmol/l h	4.94	(2.93)	6.45	(3.32)	0.002[b]

NS, not significant; *UPDRS-ME*, Unified Parkinson's Disease Rating Scale-Motor Examination
[a] Median (range); [b] Student's *t* test; [c] Mann-Whitney U test; [d] chi-square test

presented PDD more frequently. These data suggest that lighter PD patients could be at a greater risk to develop PDD during chronic therapy, since they receive a larger dose of levodopa per kilogram of body weight.

The impact of body weight on pharmacokinetics is well known [3] and, in clinical practice, drugs are often administered according to the patient's body weight. Nevertheless, patients with PD are usually treated with standard dosages of levodopa without any adjustment of the dose according to body weight. In a recent study, derived from the DATATOP trial, women were more likely to develop PDD than men (37% vs. 23%) [6], a finding that has been repeatedly reported in other observational studies [4, 7]. The authors concluded that the increased incidence of dyskinesias among women could be ascribed to the higher dose of levodopa per kilogram of body weight, administered to the women participating in the DATATOP trial, even if pharmacokinetic data of levodopa were not provided [6]. Therefore, this left substantially unresolved the question as to whether changes in the peripheral pharmacokinetics of levodopa due to the different dosage of levodopa (in mg per kilogram of body weight) between women and men could account for gender differences with respect to levodopa-induced PDD. Our findings may help to clarify this issue and suggest that variations in peripheral pharmacokinetics of levodopa due to body weight may expose lighter PD subjects to greater plasma levels during chronic levodopa therapy. On these grounds, we believe that gender differences in the frequency of PDD observed in the present study and other reports [6, 7] might be explained by differences in peripheral pharmacokinetics of levodopa between women and men.

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Serotonin syndrome: a reported case

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Abstract We describe a patient treated with SSRI and L-dopa, who developed agitation, rigidity, hyperreflexia, restlessness, autonomic instability, fever and finally death. CSF examination, MRI of the brain, laboratory investigations, except for serum CK, glycemia and WBC, were normal. His condition was thought to result from an central serotonin activity. The serotonin syndrome occurs following the use of serotomimetic agents (serotonin reuptake inhibitors, tricyclic and tetracyclic antidepressants, tryptophan alone or in combination with monoamine oxidase inhibitors).

Serotonin syndrome mostly occurs following the use of serotomimetic agents, alone or in combination with monoamine oxidase inhibitors (MAOIs). It was first described among depressed patients in 1960, by Oates and Sjoerdsma, as a consequence of the administration of high doses of tryptophan in combination with MAOIs.

Signs and symptoms vary but always tend to include agitation, confusion, disorientation, restlessness, coma, myoclonus, rigidity, hyperreflexia, low grade fever, nausea, diarrhea, headache, tachycardia, tachypnea, blood pressure changes and shivering, even if, rarely, all of them present at one same time. Electrolyte concentrations, cerebrospinal fluid analyses and brain imaging seem to be normal, with sporadic reports of increased serum CK and mild leukocytosis. In 1991 Sternbach recognized and recommended specific diagnostic criteria for serotonin syndrome. First, symptoms typically develop soon after initiation of serotomimetic agents, or even just after an increase in dosage. Second, patients must report at least three of the following signs and symptoms: confusion, agitation, incoordination, myoclonus, hyperreflexia, tremor, diarrhea or fever. Furthermore, metabolic disturbances, infections, and substance abuse or withdrawal must be excluded.

A neuroleptic agent should not be initiated, or its dosage increased before any appearance of signs and symptoms. Serotonin syndrome is due to a serotonin excess in the central nervous system (CNS) at the 5-HT_{1A} receptors. Dopamine and 5-HT_2 receptors may also be involved. The serotonin syndrome is usually mild and, if managed with drug withdrawal and supportive therapy, generally improves within hours; however, some patients have died. Causes of these deaths involved cardiopulmonary arrest and DIC with renal failure. We report a case of serotonin syndrome in a patient who had been given selective serotonin reuptake inhibitors (SSRI) in association with L-dopa.

Case report

In April 2001, a 72-year-old man came to our first aid station some days after the acute onset of limb stiffness, anxiety, trismus and dysphagia. Family history was negative for neurologic or psychiatric illness. He had been affected for years by diabetes mellitus and hepatitis C. In addition, his relatives reported frequent falls, headaches and dizziness, as well as an increasing depression, the whole occurring for one year. In fact, just because of that same depression, he had been treated for a year with SSRI and nortriptyline. During the days preceding his admission, he had been administrated L-dopa, as an initial parkinsonism had also been detected.

The first aid station testing revealed unstable blood pressure values, tachypnea, tachycardia and sweating. A general examination was conducted and reported normal. Further mental testings proved poor concentration and moderate agitation. Tone was heavily increased, this concerning legs and trunk. Clear evidence of limb hyperreflexia was reported, without the contemporary joining of Babinski's sign. Dysmetria was not present and sensation was normal. MRI, CSF and laboratory data were normal, except for glycemia, serum CK and while blood cell count. Saline, diazepam and antibiotic therapies were given during the hospitalization. The patient's neurological status worsened quite rapidly and led to death in a few days.

Discussion

The serotonin syndrome was originally described in animals pretreated with l-tryptophan and given various MAOIs or other serotonin precursors in combination with drugs that increase their bioavailability. The serotonin syndrome is thought to be induced by combined activation of 5-HT1A and the 5-HT2 receptors. Stimulation of these receptors in the dorsal and median raphe nuclei of the brainstem and spinal cord may be important in the pathophysiology of the syndrome. The dopaminergic system may be involved. It has recently been proposed that this syndrome may be mediated by presynaptic inhibition of dopamine release or synthesis, somewhat similar to neuroleptic malignant syndrome. One patient on a simultaneous bromocriptine and levodopa treatment developed a similar syndrome. Bromocriptine has been postulated to have some serotoninergic properties. Bromocriptine, in addition to being a D2-dopamine receptor agonist, activates the serotomimetic system, and l-dopa-induced myoclonus probably has a serotomimetic basis. The possible partial roles of the 5-HT2 receptor and dopamine in the serotonin syndrome are still unclear. In the present case, we remark on the extremely negative influence of SSRIs on the dopaminergic system, probably linked to their interaction with P450-cytochrome.

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Effects of the intrastriatal administration of selective dopaminergic agonists on Fos expression in the rat brain

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Abstract In this study, we mapped the cerebral expression of Fos protein following intrastriatal stimulation of D_1 or D_2 receptors, in freely moving animals. Animals treated with the D_1 agonist SKF 38393 showed massive Fos increases in the cerebral cortex, ipsilaterally to the injected striatum, which were counteracted by systemic administration of D_1 antagonist SCH 23390. Conversely, D_2 agonist quinpirole suppressed cortical expression of Fos, while systemic administration of D_2 antagonist eticlopride relieved this blockade. As for the basal ganglia, Fos was consistently expressed only in the injected striatum of rats receiving SKF 38393. These results show that striatal dopamine receptors may play a role in the modulation of cortical activity. They also provide new information on a class of drugs - the dopamine agonists - whose role in the therapeutic strategy of Parkinson's disease is continuously evolving.

According to a popular model of basal ganglia functional organization, the striatum – the main input nucleus of the circuit – communicates with the basal ganglia output nuclei, substantia nigra pars reticulata and medial globus pallidus, through two pathways: a *direct* pathway, originating from striatal neurons expressing D_1 dopamine receptors and an *indirect* pathway, originating from striatal neurons bearing D_2 receptors [1]. In the model, the two pathways remain functionally and anatomically segregated. Therefore, selective activation of either pathway would lead to opposite changes in the net output of the basal ganglia circuitry and, consequently, in the signaling to the cerebral cortex. The model is currently under partial revision: it has been suggested, for example, that the two dopamine receptor subclasses would, in fact, co-operate, thus limiting the actual segregation of the striatofugal pathways [2]. Indeed, we have recently reported that the intra-striatal administration of selective dopamine agonists reduces glucose utilization in nuclei of both the direct and indirect pathways [3].

Given the increasing role of dopamine agonists in the therapeutic approach to Parkinson's disease, we sought to further elucidate this issue, by mapping the expression of Fos protein, a nonspecific marker of neuronal activation encoded by the immediate-early gene c-*fos*, in the brain of rats receiving unilateral, intrastriatal infusions of selective (D_1 or D_2) dopamine agonists, combined with systemic administration of the corresponding antagonists.

Male Sprague-Dawley rats (270–320 g) were anesthetized with thiopental sodium (50 mg/kg body weight intraperitoneally) and placed in a stereotaxic apparatus. Through a burr hole in the skull, a 0.4-mm guide cannula (0.7 mm external diameter, Danuso, Italy) was placed in the right striatum (0.7 mm posterior and 2.6 mm lateral with respect to bregma, and -4.5 mm ventral with respect to dura) and secured to the skull with screws and dental cement (Cookson, Georgia, USA). Three days later, animals received a systemic intraperitonal injection of a D_1 (SCH 23390, 0.5 mg/kg) or a D_2 (eticlopride, 1 mg/kg) dopamine antagonist or saline, followed 30 min later by the intrastriatal infusion of a D_1 (SKF 38393, 30 mM) or a D_2 (quinpirole, 20 mM) agonist. Intrastriatal infusions were carried out using a microprocessor-controlled syringe pump (KD Scientific, USA), which delivered a total drug volume of 0.5 µl, at a rate of 0.2 µl/min. Two hours later, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially. Brains were quickly removed, post-fixed and sectioned on a microtome. Brain coronal sections (50 µm) were processed for the detection of Fos protein, using an immunocytochemical technique previously described [4].

Fos expression was quantified using a computerized image analysis system, equipped with dedicated software (NIH Image 1.62, Scion, Frederick, MA, USA). Fos-positive neurons were counted in all the cerebral areas of interest. Final values – representing the average reading from at least three consecutive sections – were expressed as number of Fos-positive cells/mm^2.

The unilateral, intrastriatal administration of SKF 38393 caused massive expression of Fos throughout the ipsilateral cerebral cortex, particularly in the motor, somatosensory, limbic, visual and auditory areas; discrete Fos expression was also detected within the injected striatum (Fig. 1). Conversely, the intrastriatal infusion of quinpirole did not induce significant Fos expression in any of the cerebral areas considered. Systemic pre-treatment with the D_1 antagonist SCH 23390 reduced the Fos response to SKF 38393, consistently, in all the cortical areas considered, as well as in the injected striatum; the average reduction was 41.3%. Conversely, systemic administration of the D_2 antagonist eticlopride induced a 5- to 50-fold increase in cortical Fos expression, ipsilaterally to the quinpirole injection. In the striatum, eticlopride pretreatment induced a 20-fold increase in Fos expression, contralaterally to the injection.

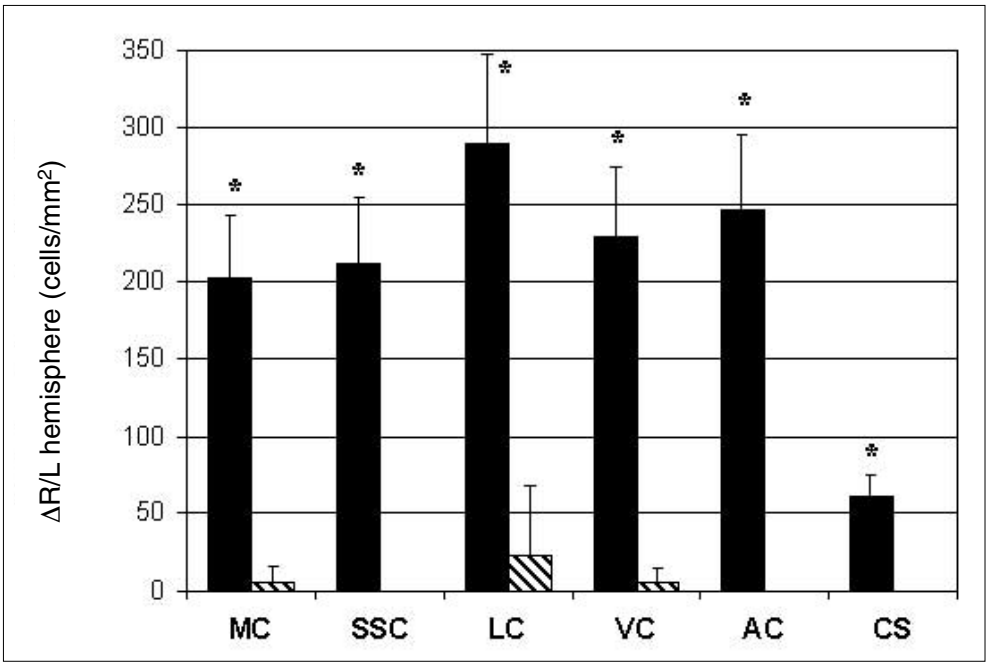

Fig. 1 Cortical and striatal Fos expression after intrastriatal administration of SKF 39393 (■, n = 5) or quinpirole (▨, n = 5), coupled with intraperitoneal saline injection. *MC*, motor cortex; *SSC*, somatosensory cortex; *LC*, limbic cortex; *VC*, visual cortex; *AC*, auditory cortex; *CS*, corpus striatum. Values are expressed as the difference (Δ) in the number of Fos-positive cells, between right (injected) and left hemispheres; *$p<0.005$ vs. quinpirole (Student's *t* test)

Discussion

In this study, we mapped the cerebral expression of Fos protein after selective stimulation of dopamine receptors (D_1 or D_2) in the striatum of rats. Stimulation of striatal D_1 receptors caused massive Fos expression throughout the entire cerebral cortex, as well as in the injected striatum. The phenomenon appeared to be receptor-mediated, since systemic administration of SCH 23390 reduced Fos expression. Conversely, striatal activation of D_2 receptors was not associated with Fos expression; discrete cortical (ipsilateral) and striatal (contralateral) Fos expression was only observed when animals were pretreated with a selective D_2 antagonist (eticlopride).

From a general point of view, the fact that selective stimulation of D_1 or D_2 receptors elicited opposite responses – in terms of Fos expression – is in agreement with previous observations, reporting a stimulatory and an inhibitory role for D_1 and D_2 receptors, respectively [5]. However, the exact mechanism underlying this response is unclear. The more obvious explanation, based on the current model of basal ganglia functional organization [1], is that the D_1-mediated striatal stimulation caused a polysynaptic activation of the basal ganglia circuit and, therefore, of the striatothalamocortical circuit, thus eliciting the expression of Fos in cortical neurons. However, except for the injected striatum, Fos was not detected in basal ganglia nuclei. Alternatively, cortical activation may have resulted from direct stimulation of corticostriatal neurons, via the stimulation of presynaptic dopamine receptors. It has been previously shown that systemic administration of dopamine agonists elicits Fos expression in the cerebral cortex [6]. More recently, Steiner and Kitai found that such phenomenon is blocked by intrastriatal injection of the D_1 antagonist SCH 23390 [7] and that the same effect is observed after striatal dopamine denervation [8]. They, therefore, suggested that activation of striatal dopamine receptors (particularly of the D_1 subtype) regulates cortex function. Our results support this view and add another piece of information to the understanding of basal ganglia functional organization. These findings also provide new information on the effects of a class of drugs – the dopamine agonists – whose role in the therapeutic strategy of Parkinson's disease is continuously evolving.

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Autosomal recessive early onset parkinsonism is linked to three loci: PARK2, PARK6, and PARK7

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Abstract Autosomal recessive, early onset parkinsonism (AREP) is genetically heterogeneous. Mutations in the *parkin* gene (PARK2 locus, chromosome 6q) account for up to 50% of AREP families. The parkin protein displays ubiquitin-ligase activity for different targets, which accumulate in the brain of patients with parkin defect and might cause neurodegeneration. Two new AREP loci (PARK6 and PARK7) have been recently mapped on chromosome 1p and confirmed in independent datasets, suggesting that both might be frequent. The three AREP forms display similar clinical phenotypes. Recruiting new families will help cloning the defective genes at PARK6 and PARK7 loci. This will contribute to unraveling the pathogenesis of AREP, and it is also expected to foster our understanding of molecular events underlying classic Parkinson's disease.

Clinical and molecular genetic studies have recently led to the identification of different monogenic forms of parkinsonism. Three chromosomal loci (PARK2, PARK6, and PARK7) have been so far identified in families with autosomal recessive, early onset parkinsonism (AREP). The PARK2 locus was initially mapped on the long arm of chromosome 6 in Japanese families. The defective gene was later identified and termed *parkin* [1].

A growing number of mutations in the *parkin* gene has since been identified in several families from different populations [2]. To date, more than 25 Italian families with parkin disease are known (personal observations and [2]).

In addition to point mutations, large genomic rearrangements (leading to exon deletions and multiplications) are frequently detected, in homozygous or heterozygous state, indicating the importance of gene dosage techniques for a sensitive screening of *parkin*. In the largest study published to date, combining genomic sequencing and exon dosage allowed mutations in *parkin* gene to be detected in 49% of 73 European AREP families [2].

In a few families a second mutation is not found even after gene dosage, suggesting that other mutations (likely in intronic or regulatory regions of the gene) still escape detection, or, some of the mutations might be sufficient to cause disease in heterozygous state.

A recent report of parkinsonism and heterozygous *parkin* mutation in multiple generations also suggests that parkin-related disease might sometimes be dominantly inherited [3]. However, pseudo-dominant inheritance could also explain the disease in multigeneration families. This phenomenon has already been documented in three families (one Japanese and two Italian), once again indicating that *parkin* gene mutations might be frequent in some populations [4].

The protein encoded by the *parkin* gene contains an N-terminal domain homologous to ubiquitin, and two RING finger domains separated by an IBR (in-between-RING) domain in the C-terminal part. Recent studies have shown that parkin protein is an E3-class ubiquitin-ligase. Different targets for parkin-mediated ubiquitination have recently been described: CDCrel-1, a synaptic protein; synphilin-1, an α-synuclein-interacting protein; the PAEL-receptor, a putative transmembrane polypeptide [5]; and αSp22, a new brain-specific glycosylated form of α-synuclein [6].

The ubiquitin-ligase activity of parkin is abolished by mutations found in AREP families, and the target proteins accumulate in neurons of patients with parkin-disease as a result of lack of ubiquitin-ligase activity. This, in turn, might cause neurodegeneration [5, 6].

The discovery of an interaction between parkin and the brain-specific form of α-synuclein has important implication for Parkinson's disease in general, since this reaction might underlie the deposition of ubiquitinated α-synuclein in Lewy bodies [6]. To the extent that parkin activity is essential for Lewy body formation, its defect can explain the absence of Lewy bodies in the brain of patients with *parkin* mutations.

Two new loci for AREP have recently been identified and termed PARK6 and PARK7. The two loci are close to each other on the short arm of chromosome 1, but they are separated by approximately 25 centiMorgans (PARK7 is more telomeric).

The PARK6 locus was initially identified by a genome-wide scan in a large consanguineous family originating from

Sicily (the "Marsala" kindred), with four affected individuals in three branches [7]. A maximum two-point LOD score of 4.1 was obtained for marker D1S199 ($\theta = 0$). Haplotype analysis identified a 12.5 cM homozygous region located at 1p35-p36, flanked by markers D1S483 (telomeric) and D1S247 (centromeric).

The PARK7 locus was identified in the framework of a larger research program named Genetic Research in Isolated Populations (GRIP) [8]. The subject of the GRIP project is a genetically isolated population in the southwestern area of The Netherlands. A large family with multiple consanguineous loops was identified with four individuals affected by AREP in two branches. A genome-wide scan and linkage analysis using the program MAPMAKER-HOMOZ resulted in a maximum multipoint LOD score of 4.3 for adjacent markers on 1p36. Haplotype analysis confirmed the presence in the patients of an homozygous region of about 16 cM flanked by the marker D1S243 (telomeric) and D1S244 (centromeric).

Since the initial linkage reports, evidence for both PARK6 and PARK7 have been obtained in independent datasets. Among 28 parkin-negative European families with AREP, 8 supported linkage to PARK6 [9]. In particular, a new Italian consanguineous family with two affected individuals allowed refinement of the PARK6 centromeric border, reducing the critical region to 9 cM flanked by markers D1S483 and D1S2674.

We studied four AREP families, three of which were consanguineous, and obtained support for PARK7 in three families [10]. In particular, in one Italian family with three affected individuals born from consanguineous parents, we obtained significant evidence for linkage confirmation (maximum multipoint LOD score 2.5, interval-wide $p<0.001$). In the same family, LOD scores were negative and markers were heterozygous accross PARK2 and PARK6 regions. This is therefore the second family linked to PARK7 after the original Dutch pedigree. Unfortunately, recombinants in the Italian family did not refine the PARK7 critical region.

Pathology data from PARK6 and PARK7 patients are not available.

Genotype-phenotype correlations for PARK6 and PARK7 must be considered with caution since they are based on linkage data, whereas for parkin-related disease data are based on gene testing. An early onset of parkinsonism, absence of atypical signs, slow disease progression, and good response to levodopa are unifying clinical features in all three AREP forms. However, in Europe the average age at onset is in the early thirties for the parkin-related and PARK7-linked families, whereas the onset in PARK6-linked families seems later (early forties) [2, 9, 10].

The distinction of the three AREP forms as well as other forms of young-onset parkinsonism is impossible on clinical grounds, and genetic testing is required.

The PARK6 and PARK7 critical regions contain several genes, including many of unknown function. Therefore, the identification of additional families (especially consanguineous ones) is crucial to refine the critical regions and facilitate the positional cloning of the defective genes. This in turn will reveal if the same metabolic pathway is implicated in all three AREP forms, and its relevance for the understanding of molecular pathogenesis of AREP but also of classical Parkinson's disease.

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Perfusion-weighted dynamic susceptibility (DSC) MRI: basal ganglia hemodynamic changes after apomorphine in Parkinson's disease

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Abstract Relative regional blood flow of basal ganglia was studied by means of perfusion-weighted dynamic susceptibility (DSC) MRI. Parkinson's disease (PD) patients showed a significant inter-hemispheric asymmetry due to a higher perfusion in the more affected side, while normal subjects did not. PD exhibited an abnormal "asymmetry index" in the measured nuclei. A second DSC-MRI examination performed after subcutaneous apomorphine administration did not show any significant asymmetry in PD patients. DSC-MRI of basal ganglia confirms the asymmetry observed in PET studies of PD patients, suggesting that this method is a promising and low-cost technique in neurodegenerative diseases.

A pattern of increased perfusion in the basal ganglia (BG), related to a decreased perfusion in the cortical regions, was found by positron emission tomography (PET) [1]. PET is a very expensive imaging method, while magnetic resonance imaging (MRI) is a less expensive imaging technique available almost in every neurological centre. In the last years several researchers attempted to utilise dynamic susceptibility (DSC)-MRI to measure regional cerebral blood flow (rCBF) and in epilepsy [2].

Our target was to study whether DSC-MRI perfusion method may detect an altered pattern of rCBF in patients with Parkinson's disease (PD) in comparison to normal subjects and whether this altered pattern may be normalised by apomorphine.

Fifteen subjects affected by idiopathic PD were enrolled for this study. Twelve normal subjects were included as controls. Eight of them performed a retest procedure. All included subjects gave their informed consent.

PD patients, after at least 20 days of therapy withdrawal, were submitted to perfusion DSC-MRI. Ten of them were retested after apomorphine injection (2–4.5 mg subcutaneously, motor improvement of at least 50% on UPDRS section III [6]).

MRI was performed in the dark, utilising a 1.5 T MR scanner (Philips gyroscan ACS-NT) with gradient strength of 23mT/m, rise time of 0.2 ms with sinusoidal gradient profile and echo-planar capabilities; a circularly polarized head coil with quadrature was used. T2*-weighted echo-planar sequences were used to obtain DSC-MRI images along the anteroposterior commissural (AP-CP) plane. A dose of 0.4 mmol/kg gadolinium-DTPA was injected to the subject lying with closed eyes. The bolus perfusion data were processed and converted into parameter maps for relative rCBF.

Regions of interest (ROI) of 15 pixels were manually placed on the head of caudate nucleus (CN), on the putamen (PU), on the external and internal globus pallidus (GPe/GPi) separately and on the ventrolateral nucleus of thalamus (TH). CU, PU and GPe were localised on the slice placed 3 mm above the AC-PC line, while TH and GPi were localised on the slice placed 3 mm below the AP-CP line [3]. Moreover, perfusion was evaluated in a white parieto-occipital matter (WPOM), to perform normalisation of the data.

Row flow data, calculated as the mean of each ROI in the BG nuclei and in the WPOM of each side, were logarithmically transformed, and then normalised as percentage of the value obtained from the ipsilateral WPOM. For asymmetry determination ("contrast" effect), normalised data were expressed as a ratio of the contralateral corresponding nucleus, according to the formula: [(right nucleus - left nucleus)/(right nucleus + left nucleus)*100]. In PD patients, the contrast was expressed as [(best side - worst side)/(best side + worst side)*100] according to their clinical asymmetry. An individual "contrast index" was considered abnormal when exceeding the mean ± 2 SD of normal subjects.

Data analysis was performed with STATISTICA for Windows program. Normalised data were analysed with parametric ANOVAs utilising the following main factors: "group" (between factor: PD vs. control subjects); "treatment" (within factor: before-after drug administration or test-retest); "nuclei" (within factor: CU, PU, TH GPe and GPi); "side" (within factor: right side or best side vs. left side or worst side).

The whole group of PD patients exhibited a significantly ($F(1/25)= 7.98$; $p<0.05$) different mean rCBF in the best (BS) vs. worst side (WS), in comparison to control subjects (interaction "group" x "side"). This was due to a significantly (post hoc $p<0.001$) higher mean basal rCBF in the WS (117.6) in comparison to the mean in the BS (107.3) in PD patients, not observed in control subjects (110.9 vs. 111.2). This was confirmed by "contrast" analysis showing a significant difference ($F(1/25)= 9.44$; $p<0.01$) of the mean contrast in the two groups (-4.39 vs. -0.064; PD vs. controls). The difference was similar in all the studied nuclei so that no significant interaction "group" x "nuclei" was found. Eleven out of the fifteen PD patients showed abnormal "contrast index" in the putamen and nine showed the same abnormality in the thalamus. Only three control subjects showed abnormal values in the putamen and none in the thalamus.

A significant ($F(1/16)= 5.65$; $p<0.05$) difference was found between the two groups due to a mean rCBF higher in PD patients in comparison to control subjects (116.8 vs. 112.3). More importantly, the interactions "group" x "treatment" ($F(1/16)=6.15$; $p<0.05$) and "group" x "side" ($F(1/16)= 4.53$; $p<0.05$) were also significant. The first interaction was due to a significant (post hoc $p<0.001$) difference between the mean rCBF before (111.6) and after (122.0) apomorphine in PD, while the test-retest procedure did not produce significant changes in controls. The second interaction was due to a significant (post hoc $p<0.01$) difference between the BS (114.5) and the WS (119.1) in PD which was not true in normal subjects. Finally, a significant ($F(1/16)= 4.34$; $p<0.05$) interaction among "groups" x "treatment" x "side" was found. This was related to a significant (post hoc $p<0.001$) difference of rCBF between the BS (107.4) and the WS (115.7) present in PD patients before apomorphine but not after drug administration (121.6 vs. 122.5); normal subjects never showed significant differences between the two sides (Table 1).

Contrast data (Table 1) confirmed what was observed in normalised data. There was a significant ($F(1/16)= 4.82$; $p<0.05$) effect of the factor "group" and of the factor "treatment" ($F(1/16)=4.32$; $p<0.05$). Moreover a significant ($F(1/16)= 4.57$; $p<0.05$) "group" x "treatment" interaction was found, due to a significant (post hoc $p<0.05$) decrease of asymmetry after treatment in PD patients (from -3.75 to -0.38) while normal subjects never showed a significant asymmetry (from -0.60 to -0.16).

Discussion

Our data show that rCBF in the BG of untreated PD patients is strongly asymmetric between the WS and the BS, and that this asymmetry is normalised by apomorphine. No asymmetry was present in controls in the test-retest procedure. Our study confirm previous PET findings of a relatively increased and asymmetric rCBF in the BG in unilateral or bilateral PD patients [1]. Interestingly, PET studies did not show the asymmetry between homologous BG regions but only if the rCBF of BG nuclei were compared to those of several cortical areas. On the contrary, the less expensive DSC-MRI technique seems to be able to reveal the asymmetry by examining homologous subregions of BG. This was allowed by the higher resolution of MRI in comparison to PET.

Apomorphine treatment was able to normalise the asymmetry of rCBF, probably related to a dopamine depletion-dependent mechanism. The post-apomorphine recovery was due to an increased rCBF in the BS, while the WS did not change. Dopamine receptor stimulation in the BG may selectively change the rCBF in these regions, thus accounting for this finding. However apomorphine also produces relevant vasodilatation, possibly accounting for part of the increase in rCBF observed in the BS. The larger loss of dopamine in the WS might have produced a maximal increase of rCBF already in basal conditions. Thus, apomorphine may not be able to induce further increase of rCBF in that side.

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Table 1 Basal ganglia mean rCBF and mean rCBF "contrast"

	Basal ganglia mean rCBF				Basal ganglia mean rCBF "contrast"	
	CU mean rCBF ± SD				CU mean rCBF "contrast" ± SD	
	PRE apomorphine/test		POST apomorphine/retest			
	R/B	L/W	R/B	L/W	PRE apo/test	POST apo/retest
PD n=15	111.6±7.32	121.2±1258	–	–	-3.45±5.82	–
Control n=12	115.1±6.53	116.2±6.12	–	–	-0.79±241	–
PD n=10	112.2±7.35	118.0±5.2	126.3±11.8	126.6±11.0	-2.97±3.75	-0.13±4.27
Control n=8	117.3±7.1	117.3±6.9	117.5±764	119.5±6.44	-0.34±2.554	-0.46±2.60
	PU mean rCBF ± SD				PU mean rCBF "contrast" ± SD	
	PRE apomorphine/test		POST apomorphine/retest			
	R/B	L/W	R/B	L/W	PRE apo/test	POST apo/retest
PD n=15	112.1±6.4	122.5 ± 13.4	–	–	-4.23±5.74	–
Control n=12	117.2±6.1	117.2 ± 7.62	–	–	-0.04±2.21	–
PD n=10	112.1±.25	121.2±6.2	126.6±11.1	128.2±11.4	-3.76±4.16	-0.52±3.93
Control n=8	117.3±6.08	118.7±5.1	119.1±6.11	119.7±6.26	-0.31±1.92	-0 44±1.31
	TH mean rCBF ± SD				TH mean rCBF "contrast" ± SD	
	PRE apomorphine/test		POST apomorphine/retest			
	R/B	L/W	R/B	L/W	PRE apo/test	POST apo/retest
PD n=15	111.4 ± 7.27	122.8 ± 13.0	–	–	-4.32 ± 5.51	–
Control n=12	115.2 ± 7.12	115.3 ± 7.4	–	–	0.22 ± 2.06	–
PD n=10	112.7 ± 7.73	120.0 ± 5.79	126.6 ± 12.1	128.1 ± 12.3	-3.22 ± 3.03	-0.51 ± 4.25
Control n=8	116.5 ± 6.88	116.1 ± 6.33	119.4 ± 6.23	118.1 ± 9.26	-0.023 ± 2.08	0.67 ± 4.33
	GPe mean rCBF ± SD				GPe mean rCBF "contrast" ± SD	
	PRE apomorphine/test		POST apomorphine/retest			
	R/B	L/W	R/B	L/W	PRE apo/test	POST apo/retest
PD n=15	101.2 ± 4.1	112.1 ± 12.2	–	–	-4.	–
Control n=12	106.1 ± 4.6	106.2 ± 7.6	–	–	-0.04 ± 3.14	–
PD n=10	101.6 ± 4.41	110.8 ± 5.74	114.6 ± 12.3	114.2 ± 10.4	-4.23 ± 4.44	0.066 ± 3.99
Control n=8	105.1 ± 4.04	107.2 ± 4.73	105.4 ± 3.20	106.1 ± 7.83	-0.94 ± 2.10	-0.16 ± 3.43
	GPi mean rCBF ± SD				GPi mean rCBF "contrast" ± SD	
	PRE apomorphine/test		POST apomorphine/retest			
	R/B	L/W	R/B	L/W	PRE apo/test	POST apo/retest
PD n=15	99.15 ± 12.5	109.5 ± 4.93	–	–	-4.72 ± 6.62	–
Control n=12	100.3 ± 7.4	101.1 ± 5.53	–	–	-0.14 ± 3.53	–
PD n=10	98.6 ± 5.16	107.6 ± 7.11	113.1 ± 13.32	114.5 ± 11.6	-4.53 ± 5.32	-0.78 ± 4.64
Control n=8	99.67 ± 4.71	102.1 ± 5.11	100.1 ± 2.79	102.2 ± 9.03	-1.44 ± 2.56	-0.72 ± 4.82

RCBF, regional cerebral blood flow; *CU*, caudate nucleus; *TH*, ventro lateral thalamus; *PU*, putamen; *Gpe*, external globus pallidus; *Gpi*, internal globus pallidus; *R*, right; *L*, left; *B*, best; *W*, worst. "contrast", [(right nucleus- left nucleus)/(right nucleus + left nucleus)*100] in normal subjects; "contrast", [(best side - worst side)/(best side + worst side)*100] in PD (see methods)

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Frequency, distribution and characteristics of progressive supranuclear palsy in Italy: preliminary observations

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Abstract We report the number of pathologically proven cases of progressive supranuclear palsy, described in the Italian neurological literature from 1961 until now. A discussion of the diagnostic value of downward gaze palsy is made. A comparison with the number of similar cases described in the rest of the world and with the number of Parkinsonian patients who died in the same region in the corresponding year is attempted.

Even after nearly 40 years since the discovery of progressive supranuclear palsy (PSP) [1–3], the precocious diagnosis of this disease is difficult. Recently, there is a tendency to give importance to the presence of downward supranuclear gaze palsy [4]. We have already demonstrated, from an examination of the literature, that this was not the case [5, 6]. From the frequency and the age at onset of downward gaze palsy among Italian cases, it appears that this symptom is neither frequent nor precocious (Table 1). The number of PSP cases in Italy is small, both in comparison to the number of patients described in the rest of the world and to the number of patients who died from Parkinson's disease in the same region and in the corresponding years. The small number of PSP cases is not due only to the difficulty in obtaining autopsy, because until 1980 also the number of clinical cases described in Italy was less than the corresponding number of cases reported in the rest of the world.

We also considered the number of parkinsonian patients who died in the same region, in the corresponding year (ISTAT data, available only from 1968 to 1997). The ISTAT data also include deaths due to PSP, although from this year on, deaths due to PSP will be classified separately under the code RF170. Also in this respect no correlation is useful; perhaps it will be possible in the field of the clinical cases, whose number is certainly higher. The difficulty in obtaining permission of autopsy resulted in an underestimation of the frequency of the disease.

Table 1 Pathologically proven cases of PSP reported in the literature for Italy and the rest of the world, in comparsion to direction of supranuclear ocular palsy at onset and the number of deaths due to parkinsonism for the corresponding region

Year	PSP cases in rest of world, n	PSP cases in Italy[a]			Deaths from parkinsonism Same Italian region, n[b]
		n	Region	Ocular palsy	
1961	0	1	Liguria	NR	0
1967	3	1	Liguria	Down	0
1969	7	1	Veneto + Trentino-Alto Adige	Up	81
1978	3	1	Umbria	Up + down	22
1979	5	1	Liguria	Up + down	107
1981	5	1	Piedmont	Up + down	212
	–	1	Emilia-Romagna	Up	154
	–	3	Lazio	Up	176
1983	17	1	Emilia-Romagna	Up	192
1984	18	6	Emilia-Romagna	Up + down	167
	–	3	Liguria	Up, 1; Down, 2	95
1987	58	1	Liguria	NR	158
1988	169	1	Lombardy	Up + down	389
1990	34	2	Liguria	NR	152
2000	0	3	Veneto + Trentino Alto Adige	Up + down	NA

NR, not reported; *NA*, not available; [a] Cases are reported in [7]; [b] Data from ISTAT

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Frontal intermittent rhythmic delta activity (FIRDA) in patients with dementia with Lewy bodies: a diagnostic tool?

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Abstract The accuracy of the clinical diagnosis of dementia with Lewy bodies (DLB) remains poor, especially in early phases of the disease, in spite of applying current consensus diagnostic criteria. The need for supportive diagnostic tools is therefore warranted. In this study EEG recordings showed a main pattern of bilateral frontal intermittent rhythmic delta activity (FIRDA) in 7 of 10 patients, aged 58–83 years, 8 of whom were diagnosed as affected by "probable" and 2 by "possible" DLB. Conversely, the same EEG abnormality was found only in 2 of 9 age-matched patients, 8 of whom had "probable" and 1 "possible" Alzheimer's disease, according to NINCDS-ADRDA criteria, taken as controls. The degree of cognitive impairment was comparable among the two groups of patients. If these findings will be confirmed in a larger series, FIRDA, even though an aspecific EEG pattern, could be of value in improving the diagnostic accuracy of DLB.

Renewed interest has been recently raised on the role of electroencephalography (EEG) in the diagnosis of patients with degenerative dementias. However, whereas aspecific EEG abnormalities correlating with the degree of cognitive impairment have long been recognized in patients with Alzheimer's disease (AD), in patients with dementia with Lewy bodies (DLB) a wide spectrum of EEG changes has been reported, even though to date the latter disorder remains poorly investigated with this technique. Indeed, the search for a neurophysiological dysfunction which proves useful in supporting the clinical diagnosis of DLB is strongly warranted, due to the rather low level of accuracy of the currently available diagnostic criteria [1], especially in the early phases of the disease.

We report the results of a study that investigated the patterns of EEG abnormalities in patients diagnosed with "probable" or "possible" DLB and AD.

The former group included 10 patients, 7 men and 3 women, aged 58–83 years (median 69.5 years); in 8 of these patients a diagnosis of "probable" and in the other 2, a diagnosis of "possible" DLB was made, according to the previously mentioned criteria. Duration of the disease ranged between 2 and 11 years (median 3 years), and the degree of cognitive impairment was mild in 1, moderate in 3 and severe in 6 of the patients. The second group comprised 9 patients, 4 men and 5 women, aged 58-85 years (median 73 years): 8 patients had a diagnosis of "probable" and 1 had a diagnosis of "possible" AD, according to NINCDS-ADRDA criteria [2]. In these patients the median duration of the disease was 2 years (range, 2–11 years), and the degree of cognitive impairment was moderate in 2 patients and severe in the remaining 7.

EEG recordings were performed with a 21-channel Nihon-Kohden apparatus. The electrodes were positioned according to the International 10–20 system with bipolar montages.

Slowing of the background EEG activity in the frequency band between 6 and 8 Hz, associated with poor reactivity to eyes closing, was found in all patients with DLB and in 6 of 9 patients with AD. Seven of 10 patients with DLB showed a main pattern of bilateral frontal intermittent rhythmic delta activity (FIRDA), whereas bilateral theta slow waves in the temporal regions were found in the remaining 3 patients. In contrast, FIRDA pattern occurred in only 2 of 9 patients with AD, whereas in the other 7 patients bilateral theta slow waves in the temporal regions were found.

An example of FIRDA in a patient with DLB is shown in Fig. 1.

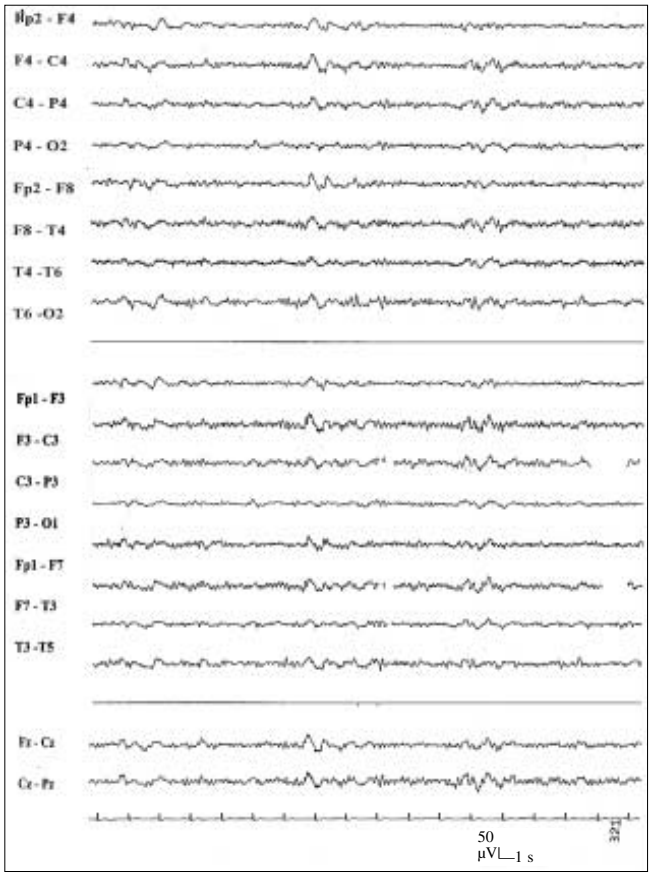

Fig. 1 EEG recording showing FIRDA pattern in a patient with DLB. The time constant was 100 msec and the signals were amplified to 50µV and filtered up to 35 Hz

Discussion

Previous anedoctical reports have shown a wide spectrum of EEG changes in DLB, including aspecific [3] and more definite abnormal patterns, i.e. FIRDA [3, 4], triphasic waves [5] and periodic synchronous discharges [6] or periodic or pseudoperiodic sharp wave complexes [7, 8] resembling those typical of Creutzfeldt-Jacob's disease. These findings likely reflect a clinicopathological heterogeneity in the reported case series.

The first formal EEG study showed in half of 14 patients with autoptically verified DLB a pattern of temporal lobe slow wave transients, which were found to be correlated with a clinical history of loss of consciousness [9]. In a more recent investigation [10], "bursts of bisynchronous, diffuse rhythmic 2- to 3-Hz waves" were reported in an unspecified proportion of 18 patients with DLB, whereas in other 6 patients "persistent diffuse delta, bisynchronous spikes or sharp waves and triphasic waves" were observed. Therefore, to date it remains unclear whether or not a distinctive abnormal EEG pattern may be recognized in DLB.

If our findings will be confirmed in a larger series, the occurrence of FIRDA in patients with degenerative dementia could be regarded as an indicator supporting the diagnosis of DLB.

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Bipolar affective disorder and Parkinson's disease: a rare, insidious and often unrecognized association

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Abstract Five patients (4 women) with Parkinson's disease (PD) and primary major psychiatric disorder (PMPD) meeting DSM-IV criteria for the diagnosis of bipolar affective disorder (BAD) were studied. Four patients had early onset PD. Four developed a severe psychiatric disorder a few years after starting dopaminergic therapy in presence of a mild motor disability and a mild cognitive impairment, with no evidence of cerebral atrophy at CT or MRI. Two patients developed a clear manic episode; the other three presented a severe depressive episode (in one case featuring a Cotard syndrome). None showed previous signs of long term L-dopa treatment syndrome (LTS), hallucinosis or other minor psychiatric disorders. The two manic episodes occurred shortly after an increase of dopaminergic therapy and in one case rapid cyclic mood fluctuations were observed. At the onset of psychiatric symptoms, all patients had an unspecific diagnosis of chronic delusional hallucinatory psychosis (CDHP).

Recent studies on the occurrence of major psychiatric disorders (MPD), e.g. delusion with or without hallucinations, major mood disorders, in subjects with Parkinson's disease (PD) provide interesting cues for discussion. The development of a psychiatric illness early in the course of PD and dopaminergic therapy, in relatively young patients without dementia or delirium, is of particular interest [1, 2]. These disorders, which usually occur in an insidious fashion with a nonspecific picture of chronic delusional hallucinatory psychosis (CDHP) in parkinsonian patients treated with dopaminergic drugs, sometimes hide a coexisting primary major psychiatric disorder (PMPD) and often represent a serious diagnostic and therapeutic problem [3]. Few studies have investigated the association between PD and bipolar affective disorders (BAD) [4, 5], although some reports evidentiated clear relationships among mood fluctuations (depression and mania), motor status and brain dopamine levels. In fact, regression of parkinsonian akinesia during a manic episode [6], occurrence of a manic episode after administration of apomorphine in parkinsonian patients with depression and, vice versa, the development of a parkinsonian syndrome in patients with BAD have been reported [7].

All patients with idiopathic PD who had been referred to our Parkinson Centre over an 8-year period and who had developed a psychotic picture with insidious onset and clinical symptoms consistent with CDHP were taken into consideration and 5 particular cases were identified. On careful evaluation of the clinical history and psychiatric manifestations of these patients, a rarely described association of PD and BAD was revealed. BAD was diagnosed retrospectively by examining all available clinical data, the typical history and DSM-IV criteria.

Each patient was examined for sex, age-at-onset and clinical variant of PD, disease duration, years of dopaminergic therapy, Hoehn/Yahr (H/Y) stage, cognitive status, Global Deterioration Scale (GDS) and Mini Mental State Examination (MMSE), brain imaging (CT or MRI), previous sign of LTS, age at psychosis onset and treatment, previous history of minor psychiatric disturbances (e.g. vivid dreams, hypersexuality, hallucinosis) and interval of onset of psychiatric symptoms.

Patients were four women and one man; three had classic variant of PD and two had an akinetic-rigid syndrome. Four had early onset PD. The mean age at disease onset was 47.2 years (range, 45-49).

BAD was diagnosed in four patients several years after diagnosis of PD; only one female patient had been previously aware of BAD. After a few years of dopaminergic therapy (range, 3–5 years), the four patients suddenly developed a clinical picture of CDHP with progressive delusional manifestations, mainly of persecutory type in depressive phase, and of megalomanic and/or erotomanic type during manic episodes. In florid periods these symptoms were associated with auditory and visual hallucinations linked to mood fluctuations. BAD was thus diagnosed in 4 of 5 patients some years after diagnosis of PD and initiation of dopaminergic treatment. In these four patients, the mean age at diagnosis of PD was 48.7 years and the mean age at diagnosis of BAD was 52.2 years. The interval between starting dopaminergic therapy and onset of the psychotic disorder consistent with BAD was 3.5 years.

The study of risk factors for development of a psychiatric disorder strictly linked to dopaminergic treatment or PD evidentiated low motor disability (H/Y stage ranged from 2 to 3), non-significant cognitive impairment (mean GDS score, 2.4; range, 2-4; mean MMSE, 26.4) and absence of brain atrophy in our patients. These findings were not consistent with an organic or pharmacotoxic psychiatric disorder. During observation, two patients experienced a manic episode, two had major depression and one had a marked mood depression featuring a Cotard syndrome. Four patients were affected by BAD with long intervals between episodes, while one patient showed a rapid-cycling BAD. Our patients manifested some interesting characteristics consistent with previous anecdotal reports: the complete remission of clinical symptoms and signs of PD in manic episodes (two patients) that led to a rapid and important reduction of dopaminergic therapy and the development of mania with

subacute onset after increase of pergolide (two patients) or levodopa (one patient).

Discussion

To date, the association between PD and PMPD has been poorly defined and is still scarcely considered. The need to proceed beyond anecdotal reports is supported by the important influence exerted by the correct diagnosis of these disorders, in particular on the elaboration of therapeutical strategies. The assumption that DSM-IV criteria imply both the exclusion of organic brain illness and use of psychoactive drugs in diagnosing PMPD should not discourage from investigating that association, because it is equivalent to asserting that PD could never be manifested in association with PMPD, and this is unreasonable and undemonstrated.

The association between BAD and PD has been reported only rarely [4, 5]. In our opinion, this is due both to the rarity of the association and to the fact that when a major psychiatric disorder occurs during dopaminergic treatment for PD, it may be incorrectly labelled as drug-induced CDHP; vice versa, when BAD is pre-existent to PD the latter may easily be labelled as iatrogenic parkinsonism. On the other hand, the relationship between mood fluctuation, depression or mania, and brain dopamine seems to emerge clearly in literature [8, 9]. In particular, it has been previously observed that levodopa is able to induce hypomania or mania in patients with BAD or PD [10].

In our experience PMPD in PD tends to occur early during dopaminergic therapy and is frequently characterized by an unspecified and dramatic picture of CDHP. Early onset of CDHP in early onset PD inevitably implies two alternative diagnoses: "a comorbid psychotic illness (often unrevealed by the patient initially) or an evolving parkinsonism-plus syndrome" [2].

Our findings demonstrate that the absence of atrophy in neuroimaging and or cognitive impairment in psychometric evaluation provides evidence of the lack of psychiatric organic or drug-induced disorders. Similarly, the absence of minor psychiatric disorders such as hallucinosis, hypersexuality, vivid dreams and confusional episodes (drug-induced phenomena in the advanced PD), as well as motor complications in LTS support this finding.

Meticulous investigation of family and personal history is essential for a correct nosological classification of CDHP, with respect for DSM-IV criteria, particularly when this disorder is manifested only a few years after the start of dopaminergic therapy in recently diagnosed PD patients without cerebral atrophy or cognitive impairment. Indeed, in four of our patients the interval between the start of dopaminergic therapy and onset of psychiatric disorders ascribed to BAD was less than four years. In the fifth patient BAD symptoms largely preceded the onset of PD.

The observation of a cyclic and bipolar course of a serious mood disorder in the presence of a typical family history facilitates diagnosis of undetected BAD in PD patients. The onset of PD in a patient suffering from BAD, and vice versa, seriously complicates antiparkinson therapy because of the influence that dopaminergic drugs exert over mood. The treatment of manic phases and severe depression in patients also affected by PD presents opposite problems with regard to dopaminergic therapy and common problems concerning the need to use mood-stabilizing drugs such as carbolithium, carbamazepine and valproate.

In our patients, the visual and/or auditory hallucinations in the manic phase or in Cotard syndrome were treated with atypical neuroleptics. The Cotard syndrome was successfully treated with intravenous clomipramine (50 mg/day for 20 days). In addition, the remarkable resolution of parkinsonian symptomatology during a manic phase in one patient should be mentioned. This occurrence convinced the patient that she was no longer affected by PD while a rapid cycling was induced by the administration of pergolide and levodopa.

The observations of the present study provide evidence for the underestimation of the association BAD/PD, and emphasize the need to investigate problems proposed by clinical diagnosis, physiopathological interpretation and, especially, therapeutic approach.

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Modification of respiratory function parameters in patients with severe Parkinson's disease

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Abstract Respiratory dysfunction remains one of the most common causes of death in patients with complicated Parkinson's disease (PD). The aim of this study was to investigate pulmonary function in fluctuating PD patients during "on" and "off" states of the disease. We studied 12 fluctuating, non-smoking PD patients (H&Y stages 3–5) without a history of lung or cardiovascular disease; all patients underwent Hoehn and Yahr scale (H&Y) and Unified Parkinson Disease Rating Scale (UPDRS items 18–31) to evaluate extrapyramidal impairment, as well as pulmonary function tests (PFT) and arterial blood gas analyses to assess respiratory function. All evaluations were performed during a stable on state of disease and in an off state produced by 12 hours of therapy withdrawal. A restrictive pattern of flow-volume loop was observed both in on and off states of disease. In the off state, we found a significant worsening in both FEV1 and FVC; the FEV1/FVC ratio was unmodified. These results suggest a restrictive pattern of flow-volume loop in these patients.

Pharmacological treatment and levodopa therapy provide clinical benefits and reduced mortality in Parkinson's disease, yet respiratory dysfunction remains one of the most common causes of death in these patients [1, 2]. A variety of respiratory problems have been reported in the literature: obstructive and restrictive dysfunction, respiratory dysrhythmias and abnormality of central control of ventilation [3–8]. Although there is evidence for potential pulmonary dysfunctions, most patients report no respiratory symptoms until the final stage of the disease [8]. Our aim was to investigate pulmonary function in severe, fluctuating PD patients during on and off states of the disease, using spirometry and arterial blood gas analyses.

We studied 12 consecutive, non-demented, non-smoking, fluctuating PD patients (7 women), recovered in our unit. The mean age of the patients was 67.66±5.46 years, and the duration of disease was between 8 and 25 years (mean, 14.5±0.66 years). The main inclusion criterion was the severity of disease that, according to the H&Y scale, had to be at least stage 3 (mean 4.08±0.66). All patients were on levodopa-substitution therapy (mean dosage, 704.16±145.31 mg/day), with additional administration of add-on DA-agonists for 10 patients and of ICOMT for 5 patients. Anti-parkinsonian drug intake was not changed for a period of at least 3 weeks prior to testing. None of the patients had a history of lung disease or cardiovascular disorders.

Patients were subjected to a neurological assessment of disease with Unified Parkinson Disease Rating Scale (UPDRS motor examination items 18–31), pulmonary function tests (PFT) and arterial blood gas analysis in on and off states. The off state was reached by a 12-hour withdrawal of pharmacological therapy. Pulmonary function tests were performed by a dry seal spirometer (Vmax 22 Sensor Medics, Milan, Italy) which provided measurements for forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, peak of expiratory flow (PEF), forced expiratory flow when 75%, 50% and 25% of FVC remain to be exhaled (FEF75%, FEF50%, FEF25%), and forced expiratory flow during middle half of the FVC (FEF 25%–75%), according to ATS criteria. To evaluate PaO_2, $PaCO_2$, and pH, all patients underwent an arterial blood gas analysis in the supine position at the same hour of the day.

The results were expressed as mean and standard deviation. A paired *t* test for parametric data was performed to compare the results in on and off states. A p value <0.05 was considered statistically significant. All pulmonary function parameters are expressed as percentages of normal values and have been corrected for height, sex and age.

Evaluation of the pulmonary function tests revealed a restrictive pattern in the flow-volume loop in patients with severe PD, both in on and off states. In both states of the disease, FEV1 and FVC were reduced with respect to the predicted values, whereas the FEV1/FVC ratio was normal (Table 1). The comparison of PFT and blood gas analytic values in on and in off states showed a significant reduction of values for FVC ($p<0.005$) and for FEV1 ($p<0.05$) but no significant changes in FEV1/FVC ratio ($p=0.50$). Arterial blood gas analysis showed a normal range of values in PaO_2, $PaCO_2$ and pH, both in on and off states, but a significant increase of $PaCO_2$ was observed in the off state ($p<0.05$). No significant differences were observed for the values of FEF75%, FEF50%, FEF25%, or FEF25%–75% in either the on or off state.

Discussion

We confirmed that abnormalities of pulmonary function exist in severe PD patients, as apparent by a reduction in FEV1 and FVC values, but normal FEV1/FVC ratios, in both states of disease. These results suggest a restrictive pattern of flow-volume loop in these patients. The finding of a significant worsening of spirometric parameters in the off state of disease is suggestive of a restrictive dysfunction of respiratory muscles, which is more severe in the off state than in the on state of disease.

Table 1 Pulmonary function testing and arterial blood gas analyses in on and off states in 12 fluctuating PD patients. Values are means (SD)

	On state	Off state	*p*
FVC	86.50 (26.1)	76.83 (26.74)	<0.005
FEV1	86.75 (30.63)	77.91 (28.88)	<0.05
FEV1/FVC	101.25 (15.69)	104.41 (16.36)	0.50
PEF	59.83 (20.38)	50.58 (24.73)	0.011
FEF 25%–75%	77.41 (23.36)	64.08 (25.63)	NS
FEF 25%	70.22 (13.74)	53.88 (25.01)	NS
FEF 50%	77.66 (13.38)	64.11 (25.34)	NS
FEF 75%	99.83 (52.87)	102.83 (48.18)	NS
PaO_2	80.10 (8.73)	80.83 (10.48)	NS
$PaCO_2$	39.80 (2.79)	42.31 (4.46)	<0.05
pH	7.41 (0.21)	7.40 (0.22)	NS

NS, not significant

In fact, these patients showed higher $PaCO_2$ values in the off state than in the on state, suggestive of a reduced ventilatory drive. Thus, the changes induced by dopaminergic therapy suggest that the respiratory dysfunction seen in patients with severe PD is due to abnormal activity of respiratory muscles, resulting directly from their state of rigidity and reduced range of movement [8]. Over time, PD patients tend to adapt to these changes and gradually reduce the amount of physical activity; the resultant chronic inactivity deconditions the muscles, reinforces the sedentary lifestyle and induces a progressive worsening of disability [9].

Our results suggest that spirometric studies may serve as a useful indicator of respiratory condition in PD patients. Implementation of spirometric studies could be useful in anticipating and thus preventing respiratory complications in PD, as well as for monitoring the effects of pulmonary rehabilitation programs on the respiratory dysfunction, motor performance and quality of life in PD patients.

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Visualisation of the subthalamic nucleus: a multiple sequential image fusion (MuSIF) technique for direct stereotaxic localisation and postoperative control

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Abstract A novel multiple, sequential image fusion (MuSIF) procedure merging stereotaxic CT with frameless magnetic resonance imaging (MRI) is used since June 2000 to visualise and directly localise the subthalamic nucleus (STN) on T2 images. In 13 consecutive Parkinson's cases, intraoperative recording and stimulation verified bilateral electrode implantation guided by fused T2 images. In 85% of sides, final implantation opted for visualised target track. Implanted electrode position on postoperative T2 images matched planned target. Clinical follow-up reproduces literature's best results. This MuSIF technique, effective for direct STN targeting, has practical advantages: MRI can be performed regardless of surgery time; regular MR scanning to correct real image distortion is unneeded; and the need for multiple localising tracks is reduced by enabling us to account for each patient's STN anatomy.

For functional procedures, the subthalamic nucleus (STN) is usually localised through ventriculography or stereotaxic magnetic resonance imaging (MRI). The former method is invasive and both are indirect: the STN is not visualised but its position is calculated in relation to visible anatomical landmarks. Moreover, frequent checks of the MR environment are needed to correct for real image distortion [1]. A third method uses image-fusion techniques that correct for image distortion by matching the MR image to a distortion-free image, usually computed tomography (CT) [2]. However, only thin T2 spin echo (SE) images allow for sufficient viewing of the STN [3, 4], yet a standard 1.5 T MR scanner produces a limited number of slices, too few to make T2/CT image fusion reliable for functional purposes.

The following multiple, sequential image fusion (MuSIF) technique, developed to overcome these technical problems, was used on 13 patients with Parkinson's disease for stereotaxic electrode implantation in 26 STNs from June 2000 to September 2001.

The study, performed any day before surgery in the patient's best medication condition to reduce movement artifacts, acquired frameless images on a 1.5-T MR scanner (Siemens, Concord, CA). First, a three-dimensional (3D) MPRAGE sequence was recorded in the sagittal plane (1.64 mm effective thickness, 7 min duration). Then, a thin-slice (2 mm) axial T2 SE sequence was recorded parallel to the AC-PC line (17 min duration). On surgery day, stereotaxic CT was performed using a GE Light Speed scanner with contiguous 1.25-mm slices aligned to the basal ring of the CRW frame (Radionics, Burlington, MA) and approximately parallel to the AC-PC plane. Care was taken to include as much skull as possible in the volume scanned and all landmarks were used in subsequent image-fusion procedures.

MR and CT image files were then loaded into a workstation running image-fusion and stereotaxic planning software (ImageFusion and StereoPlan 2.0, Radionics, Burlington, MA). First, the frameless MPRAGE study was fused with the stereotaxic CT data using the ImageFusion "bone matching" algorithm until visual checking and numeric results showed accurate fusion. Fused images were saved as a new set of MPRAGE images. Then, the frameless T2 study was fused onto the fused MPRAGE image set with the "intensity matching" algorithm and a fused plan of the T2 study was saved, i.e. raw data rather than a new image set. Finally, MPRAGE and T2 images that shared the stereotaxic CT study's spatial accuracy, stereotaxic volume and fiducials were available for stereotaxic localisation and planning. The initial target was selected on transverse T2 images inside the hypointensity lateral to the red nucleus, at the level of the anterior boundary of its middle portion. A thin hyperintense layer between the STN and the substantia nigra, frequently visible on reformatted coronal images, permitted refinement of target position. Entry point was set approximately 3 cm from the midline, just anterior to the coronal suture. Recording and stimulation were obtained by single coaxial microelectrodes along one or two simultaneous parallel tracks directed to the calculated target. A four-pole lead was then implanted according to recorded activity and stimulation effects. MRI was repeated after implanting the stimulator, on the same sequences as used preoperatively. A visual check of the implanted electrode's actual position with respect to the STN was easy on T2 images, while fusing the postoperative study anew with preoperative planning allowed the distance between implanted electrode and calculated target to be measured.

The STN was recognisable in all 26 sides. Frameless MRI distortion (mean scaling factors, 1.4% and 0.7% for MPRAGE and T2 images, respectively) was less than in stereotaxic MRI [1]. The microelectrode track aimed at the initially calculated target matched recorded STN activity in 21 sides and was chosen for final implantation in 22 sides according to stimulation effects. Intraoperative cerebral mass displacement due to CSF leakage may partly account for the

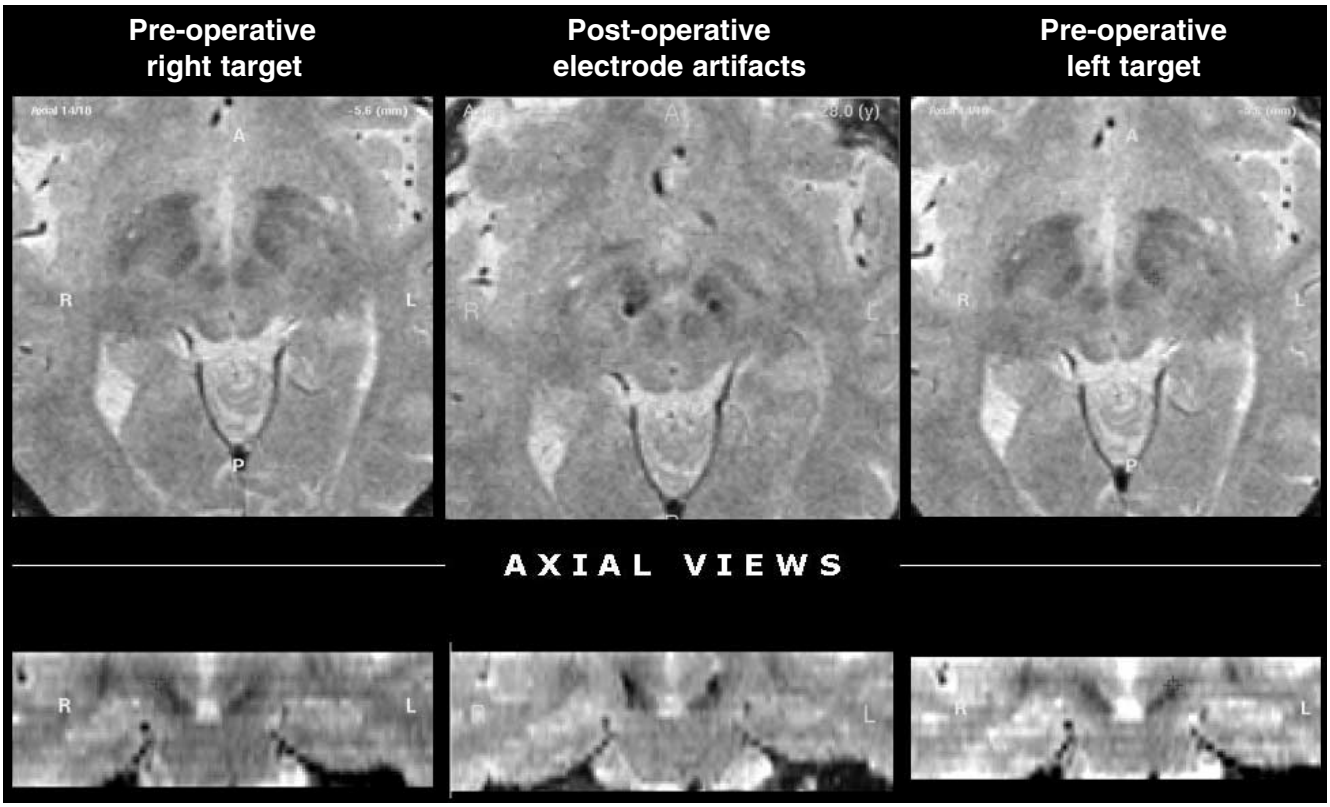

Fig. 1 T2 MR images of the pre-operative planned targets and postoperative artifacts of the implanted electrodes in the STN target

additional parallel track needed in the remaining four STNs, all of which were indeed on the second side operated. The final position of implanted electrodes in postoperative T2 images (Fig. 1) matched planned target position well: distance ranged from 0.5 to 1.5 mm, which fit very well with the application accuracy of stereotaxic frames [5]. Three-month clinical results reproduce the literature's best series.

Discussion

This MuSIF technique applied to 1.5 T frameless MR images is highly effective for STN targeting and offers some practical advantages: MRI can be performed regardless of surgery time; pre-fusion image distortion is less than in stereotaxic MR images; and regular MR scanner checks to correct for real image distortion are unneeded. With T2 images, the actual anatomy of the given patient's STN [6] can be taken into account, possibly reducing the need for multiple localising tracks. Targeting a specific portion of the STN holds promise as a potential additional instrument for studying the functional topography of the STN. Methods of validating STN targeting techniques should include postoperative T2 MRI.

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Psychophysiological approach in Parkinson's disease: L-dopa effects on preprogramming and control activity

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Abstract To investigate whether preprogramming (Bereitschaftspotential, BP) and control activity (skilled performance positivity, SPP) in a complex task are sensitive to L-dopa, movement related potentials (MRPs) were recorded in 12 non-demented Parkinson's disease (PD) patients before and after acute L-dopa administration, and in 17 control subjects. After L-dopa administration, the PD patients scored a significantly higher percentage of correct performances ($p<0.05$), linked to a decreased BP amplitude ($p<0.001$) and an increased SPP amplitude ($p<0.005$), than before therapy. Our findings suggest that preprogramming activity is impaired in untreated PD patients. Dopaminergic drug administration seems to restore their ability to use more automatic motor strategies which become more similar to that of normal subjects.

It has long been known that bradykinesia, rigidity and tremor are not the only clinical features of motor dysfunction observed in Parkinson's disease (PD) patients. Such patients exhibit other selective difficulties in performing voluntary movements, particularly if the actions are sequential, bimanual [1], time-constrained and internally guided [2]. These observations suggest that the use of highly demanding, complex movement tasks may be a better psychophysiological approach to investigate those potentials reflecting brain electric activities related to self-paced, goal-directed, time-constrained actions.

In order to investigate whether the psychophysiological pattern of a skilled motor act, evaluated by means of movement related potentials (MRPs) recording in a skilled performance task (SPT) paradigm, is sensitive to L-dopa administration, we selected 12 PD patients (8 men and 4 women; mean age, 57.5 years; SE, 2.1 years). According to the modified Hoehn and Yahr scale, the score of wash-out patients varied from 1 to 2.5 and, according to the Unified Parkinson's Disease Rating Scale (UPDRS), from 10 to 25.

All patients were treated for parkinsonian symptoms exclusively with L-dopa and dopa decarboxylase inhibitor, with a mean daily dose of 480 mg (range, 375–750 mg). The electrophysiological results obtained from these patients during the two experimental sessions were compared with those of a control group composed of 17 normal healthy volunteers (13 men and 4 women; mean age, 59.2 years; SE, 8.0 years). Patients underwent the psychophysiological examination (MRP recordings) in the morning, under fasting conditions, after administration of the minimum effective dose (determined for each patient). The following morning, a second recording was carried out in pharmacological wash-out conditions.

MRPs were recorded during the execution of the SPT [3]. The electrophysiological signals were recorded by means of Ag/AgCl electrodes placed at Fpz, Fz, Cz, Pz, P4, P3 according to the 10–20 International System, and at RPc (right precentral) and LPc (left precentral). For more details see Fattapposta et al. [7]. Independent-dependent *t* test was used for statistical analysis.

Off-therapy PD patients performed a low percentage of correct trials (12.0%±2.4%, Fig. 1). This value increased after therapy (16.9%±2.8%) and the dependent *t* test showed a statistically significant difference ($p<0.05$, t=2.94, df=11). However, PD patient scores were significantly lower than those of controls (25.2%±2.7%) in both conditions.

A well-recordable premotor negative potential (Bereitschaftspotential, BP) was observed in PD patients and controls with a maximal amplitude at the vertex (Cz). In the evaluation of the total average, the BP amplitude in off-therapy PD patients was significantly higher than in controls ($p<0.05$, t=2.55, df=27), while that of the on-therapy group did not significantly differ from controls (Fig. 1). The BP values were higher in off-therapy PD patients than in the on-therapy group ($p<0.005$, t=4.41, df = 11).

The post-motor component (skilled performance positivity, SPP) was present above all over the midline of parietal regions (Pz). In the total average analysis, a significant ($p<0.05$, t=2.07, df=27) reduction in SPP amplitude was

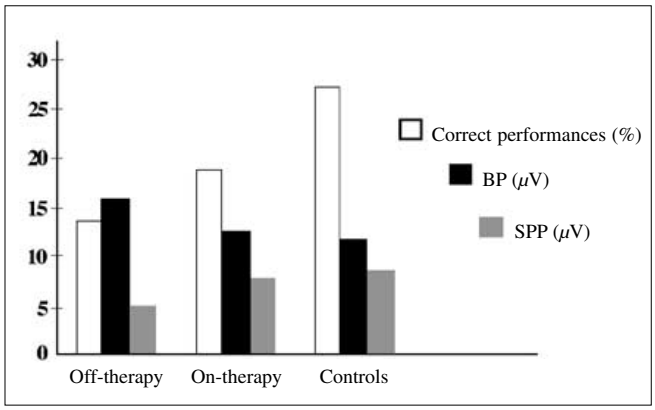

Fig. 1 Percent of correct performances, BP and SPP in Parkinson's disease patients in off-therapy and on-therapy (L-dopa) conditions, in comparison with control subjects. *BP*, Bereitschaftspotential; *SPP*, skilled performance positivity

observed in the off-therapy PD patients group when compared with controls. No differences were observed between on-therapy patients and controls. During L-dopa therapy, a significant ($p<0.005$, t=3.93, df=11) increase in SPP amplitude was observed in PD patients.

Discussion

Information about a motor task, once learned, is stored in procedural memory, and it is the basal ganglia's duty to facilitate the activity of cortical areas associated with procedural memory [4]. Thus, it is reasonable to hypothesize that the low dopamine level present in the basal ganglia of PD patients might influence their associative functions related to learning of a skilled motor task.

The analysis of performance carried out in the three experimental conditions (off-therapy, on-therapy and controls) revealed different learning profiles in each experimental session and an evident improvement of performances was seen in patients after therapy, suggesting that acute dopaminergic replacement may partially restore those cerebral processes associated with learning of a skilled motor task.

Premotor potentials (i.e. BP) reflect the activity of the supplementary motor area (SMA) [5], which receives most of the putamen output through the ventrolateral thalamus. The SMA is necessary not only for the intention and initiation of voluntary movement, but also for its "supramotor" function during the acquisition of a novel task [6], as occured in our subjects when engaged in the SPT for the first time.

In our study, off-therapy PD patients exhibited a higher BP amplitude than controls. After L-dopa administration, BP amplitude decreased and became similar to controls. One possible interpretation of these results is that the dysfunction of the SMA-basal ganglia system caused by dopaminergic deficiency requires a greater corticocortical effort to program a correct goal-directed movement sequence. This is in accordance with the suggestion that a greater "mental effort" is required in motor programming when a dopaminergic deficit is present [8].

SPP is the psychophysiological expression of the knowledge and appraisal of the results of performances and is absent when the subject is denied knowledge of the results [3]. Our results show that off-therapy PD patients had a significant lower SPP amplitude than controls, once therapy was administered, the SPP amplitude increased. On the basis of this finding, we hypothesize that untreated patients are unable to use knowledge of prior information to modify motor strategy.

The results of this study reveal that dopaminergic drug administration in PD patients modifies their electrophysiological and behavioral patterns, making them more similar to those of normal subjects by restoring the ability of such patients to use more automatic motor learning strategies.

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Noradrenergic loss enhances MDMA toxicity and induces ubiquitin-positive striatal whorls

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Abstract Movement disorders involve a number of neurodegenerative conditions, mostly affecting basal ganglia. Parkinson's disease (PD) is classically defined by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Administration of specific neurotoxins represents a common tool to reproduce this lesion. Among these, amphetamine derivatives act as powerful monoamine neurotoxins, impairing striatal dopamine (DA) axons in mice. Despite the well-investigated effects on striatal DA terminals, only sporadic studies have focused on the potential toxicity of amphetamines towards post-synaptic neurons within the striatum. In the present work we found that 3,4-methylenedioxymethamphetamine (MDMA) produces ultrastructural alterations in striatal cells, featuring as membraneous whorls, positive for ubiquitin and heat shock protein 70. These morphological alterations were enhanced in locus coeruleus-lesioned mice.

Most degenerative disorders involve the basal ganglia. Parkinson's disease (PD) is characterized by a selective loss of dopaminergic (DA) neuronal cells belonging to the nigrostriatal pathway [1].

Damage to striatal DA fibers can be produced either by microinfusing monoamine neurotoxins into the substantia nigra pars compacta (SNpc), or systemically administering specific DA neurotoxins like amphetamine derivatives [2]. These compounds are, unfortunately, largely abused by humans and produce a robust effect on the DA system, increasing at first striatal DA release [3], so leading to the damage of the nigrostriatal DA pathway, possibly due to the production of free radicals and reactive oxygen species.

Recently, a number of experimental and clinical studies documented the importance of the locus coeruleus (LC) in modulating the progression of neurodegenerative disorders; concomitantly, reduction of noradrenergic (NA) neurotransmission in various brain areas innervated by the LC is found as a constant element of the pathobiochemistry of PD. Altogether, these findings strongly suggest that NA loss could have a specific role in sustaining the disease [4]. Among amphetamine derivatives, 3,4-methylenedioxymethamphetamine (MDMA) is well known for its neurotoxic effects against monoamine axons, while the effects in striatal GABA cells are less investigated.

In this study we evaluated, at the ultrastructural level, the effects of MDMA on striatal neurons of the mouse. In addition, we investigated the role of LC in modulating the occurrence of subcellular alterations in striatal perikarya.

Male C57 black mice (C57BL/6J) 9–10 weeks old were divided into five groups of 10 mice each. To one group we administered intraperitoneally the NA neurotoxin DSP-4 (50 mg/kg). Three days later, a subgroup of DSP-4 treated mice and a group of controls received MDMA hydrochloride (three administrations of 30 mg/kg at 2-h intervals). One week after MDMA administration, one group was sacrificed and the striatum was processed in order to measure catecholamines and their metabolites by HPLC as previously described [5].

Another group of mice was anaesthetized with chloral hydrate (4 ml/kg), perfused and processed for electron microscopy and immunocytochemistry. Thin sections of 50 nm were deosmicated in a saturated solution of Na-metaperiodate for 30 min, washed in PBS and incubated in the primary antibody (Ab-I) for 24 h at 4° C. We used Ab-I anti-ubiquitin and anti-heat shock protein 70 (HSP-70), diluted 1:100; secondary antibodies were conjugated with gold particles of 10 nm diameter. Measurement of striatal whorls was carried out by counting the number of nuclear or cytosolic whorls out of the total number of neurons under observation. The same procedure was followed for each animal from each different group of treatment. Data are given as percentage of whorls and they were compared using ANOVA with Sheffè's post-hoc analysis. Null hypothesis (H_o) was rejected when $p<0.05$.

Administration of MDMA to intact mice caused a significant decrease in DA level compared with controls. In contrast, striatal DA was not affected by DSP-4 treatment, whereas in LC-lesioned mice MDMA produced significantly more severe damage.

Ultrastructural observation revealed the occurrence of morphological changes within striatal cells of MDMA-treated mice. These alterations consisted in some multilamellar bodies (Fig. 1) and they were found both in the cytosol (12.13%±1.69%) and in the nucleus of striatal neurons (29.02%±2.04%). These whorls, which were only exceptional in the striatum of control animals, constantly appeared as very electron dense inclusions, composed by multiple, concentric membranes (Fig. 1) and were found more abundantly within the nucleus of striatal cells of DSP-4- and MDMA-treated mice (41.22%±2.45%). Immunocytochemical investigation showed that whorls were positive for ubiquitin and HSP-70.

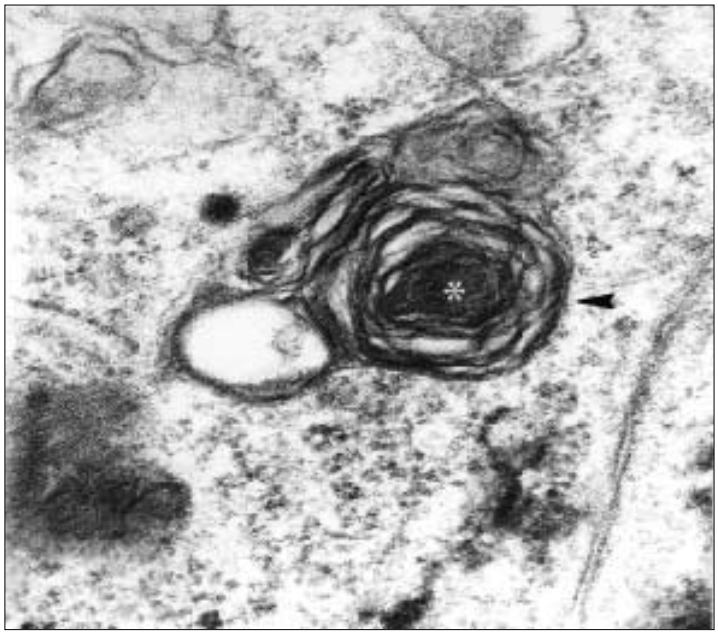

Fig. 1 Electron microscope micrograph of a cytosolic whorl within a striatal cell from MDMA-treated mouse. Multiple concentric membranes (*arrowhead*) and a more electron-dense core (∗) are evident

Discussion

This work points out the occurrence of ultrastructural alterations into striatal post-synaptic neurons following MDMA treatment, consisting of whorls of concentric membranes. Ubiquitin immunostaining suggests these formations to be functionally related to the ubiquitin-proteasome pathway, a cellular system which plays a pivotal role in proteolysis and which is involved in the regulation of cell cycle [6]. Our findings of ubiquitin-positive whorls in association with the smooth endoplasmic reticulum, as well as their positive staining for the stress-induced chaperone-like protein HSP-70, strongly support the hypothesis that they represent the morphological profile of an alteration of the ubiquitin-proteasome pathway.

Membranous whorls were described within neurons of specific brain areas in several pathological conditions; in PD typical neuronal inclusions, Lewy bodies (LB), consist of eosinophilic material found in the cytoplasm. Immunostaining shows ubiquitin as a prominent and constant component of LB. Lewy bodies are also present in other neurodegenerative disorders, such as diffuse LB disease and Alzheimer's disease.

Recently, mutant TorsinA, a novel protein identified as responsible for the early-onset torsion dystonia, has been found to stain LB [7]. TorsinA in human brain is mainly expressed in DA neurons of the SNpc; this protein, which contains an ATP-binding site, is thought to perform chaperone functions, involved in the identification of misfolded proteins and their refolding or degradation [7]. In this respect, our finding of HSP-70 immunostaining in striatal whorls might represent an example of a chaperone molecule recruited following oxidative damage induced by MDMA treatment.

In conclusion, we hypothesize that whorls represent, at the morphological level, an enhancement of a more general, physiological response of the cell to the production of altered proteins. In particular, the marked striatal DA release observed after MDMA administration, constantly associated with the production of highly reactive species, can oxidize large amounts of intracellular proteins. Moreover, a recent study [8] demonstrated that DA inhibits the proteasome pathway, making more critical the oxidative effects of DA on "proteasome-impaired" striatal cells.

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Clinical predictors in Parkinson's disease

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Abstract Parkinson's disease is characterized by heterogeneity of clinical presentations, association of signs and symptoms, rate of progression, and response to therapy. The aim of this prospective 5-year study was to evaluate whether clinical features at onset were predictive of the subsequent progression. Two courses were identified which differed in the characteristics at onset. Slow course was characterized by earlier age at onset, lateralization of motor signs, rest tremor, and absence of gait disturbance. Rapid course presented older age, less evident lateralization of signs, predominance of bradykinesia-rigidity and gait disturbance. Our results confirmed that PD is clinically heterogeneous and specific patterns of onset seem to be associated with different rates of disease progression. Predictive models based on these clinical characteristics have a good sensitivity in indicating a slow disease progression but are not reliable in indicating a rapid evolution.

Parkinson's disease (PD) is characterised by heterogeneity of clinical presentations, association of signs and symptoms, rate of progression of the disease, and response to therapy, suggesting the existence of different subgroups, possibly related to different underlying processes. In the pre-levodopa era, the clinical data of Hoehn and Yahr [1] revealed a great heterogeneity of disease progression: 37% of the patients whose duration of disease was less than 5 years were in stage III, while 34% of patients whose duration of disease was ten years were in stage I or II. The patients in the same stage had a large variation of disease duration.

Possible tools for monitoring disease progression are evolution of clinical pattern, modality of response to therapy, neuroimaging techniques and genetic research. In the case of autosomal recessive parkinsonism, genetic tests could predict the slow progression with good response to levodopa. Sequential neuroimaging techniques seem to be a useful tool in monitoring the intra-individual progression of dopaminergic cell loss in PD but cannot predict the kind of progression when performed only at onset. Currently, disease progression rate can be evaluated with clinical indicators: rate of impairment of motor signs and their temporal association, the presence of non-motor signs, the variation in the response to therapy, the presence of motor and non-motor complications, and comorbidity.

The aim of this study was to evaluate whether clinical features at onset and their different sequence of association could be predictive of the subsequent rate of disease progression. The pattern of disease progression may define the prognosis and influence therapeutic strategies.

A prospective longitudinal study was carried out in 103 patients (69 men) with a diagnosis of PD according to the Parkinson's Disease Society Brain Research Centre's clinical diagnostic criteria. The exclusion criteria were atypical parkinsonism (at onset and follow up) and dementia at onset. At onset the following parameters were recorded: sex, age at onset, main motor sign, lateralization of motor signs, association of signs/symptoms, presence of depression, and presence of gait disturbance. At the 5-year follow-up, impairment of motor score was evaluated by Unified Parkinson's Disease Rating Scale (UPDRS) and cognitive impairment was measured by MMSE (Mini-Mental State Examination). Complications of therapy (motor fluctuations and dyskinesia), impairment of postural reflexes, functionally limiting gait disturbance and cumulative dose of levodopa were recorded to evaluate the severity of disease progression.

Cluster analysis was used to identify two groups of patients based on UPDRS motor score, presence of motor fluctuations and dyskinesia at 5 years. This distinction in two groups was used to give a prognostic meaning to the characteristics evaluated at onset. To evaluate the prognostic meaning of the variables at onset, the progress of each component of progression, the UPDRS motor score and possible relationships between each parameter of onset and presence of fluctuations and dyskinesias were analyzed. The characteristics significantly correlated with the outcome were used to obtain a predictive model using logistic regression.

For statistical analysis chi-square tests were used for all dicotomic variables and Student's *t* test was used for all continuous variables. All variables were then subjected to cluster analysis.

Two disease courses (slow and rapid) were identified by cluster analysis. Patients with a slow evolution (61%) were characterized by earlier age at onset, lateralization of parkinsonian signs, prevalence of rest tremor and absence of gait disturbance. Patients with rapid progression (39%) had an older age, absence of lateralization of parkinsonian signs, predominance of bradykinesia-rigidity and gait disturbance (Table 1).

The statistical correlation between the variables at onset and the UPDRS motor score after 5 years suggested a significant correlation of age at onset (p=0.01), main motor sign (p=0.02), lack of lateralization of clinical signs (p=0.001) and presence of gait disturbance (p=0.03). Age at onset (51.0±15.3 years vs. 61.5±9.1) was significantly correlated with presence of dyskinesias (p=0.001), while no other characteristic at onset was significantly correlated with the later presence of motor fluctuations.

Predictive models based on these clinical characteristics have a good sensitivity (87.3% of correct prediction) in indicating a slow disease progression but poor sensitivity in indi-

Table 1 Characteristics of PD patients at onset, by type of progression

	Slow progression (n=63)	Rapid progression (n=40)
UPDRS motor score[a]	11.9 (3.9)	25.1 (5.1)
Motor fluctuations, %	19.0	52.5
Dyskinesias, %	15.8	30.0
Age at onset, years[a]	56.0 (11.0)	63.5 (11.0)*
Lack of lateralization, %	17.4	45.0*
Main motor sign, %		
Tremor	58.8	42.5
Rigidity	41.2	57.5
Depression, %	9.5	12.5
Gait disturbance, %	3.2	10.0

*p=0.002

[a] Values are mean (SD)

cating a rapid evolution (37.5% prediction of correct response).

Our results confirm that PD is clinically heterogeneous and suggest that specific patterns of onset may be associated with different rates of disease progression. Several studies have addressed the question of clinical heterogeneity by proposing subgroups distinguished by age at onset, variable progression, family history, patterns of motor symptoms and associated non-motor findings such as dementia and depression. In these studies the disease progression was evaluated considering the progression of single signs, and not the sequential association of signs. Homogeneous predictive criteria were not found and their results show that early disease onset [2, 3] with tremor dominance correlates with slow progression, while older age at onset and presentation with bradykinesia are predictive of a more aggressive course [4].

Cluster analysis was used to identify subgroups [5]: in our study two different groups of patients (slow 60% and rapid 40%) were identified. Our results provide a predictive model which reliably predicts only a slow disease progression, but not a rapid evolution. We found that age at onset was significantly associated with dyskinesias, while no other characteristic at onset was significantly correlated with the later presence of motor fluctuations. This supports the hypothesis of possible different pathogenic mechanisms.

In the previous studies [6, 7], the motor complications were considered in correlation with the disease stage, the duration and the kind of exposition to levodopa more than with the progression rate of disease.

The existence of different subtypes of disease is not only revealed by the characteristics at disease onset but also by the response to therapy. In a long-term study on the efficacy of dopamine agonist vs. levodopa in the treatment of early previously untreated PD patients [8], 35% of patients in monotherapy remained clinically improved for 4 years, showing that there are patients who respond better to therapy and/or with a more slowly progressing course of disease. Our results confirm this hypothesis.

The possibility of predicting, at disease onset, the rate of progression of symptoms, the quality of therapy response and latency and severity of motor and non-motor complications may give a instrument to identify the most appropriate initial therapeutic strategy of the single subgroups. This method is useful in consideration of both efficacy in pharmacological control and presence of complications in long-term therapy.

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Screening cognitive decline in dementia: preliminary data on the Italian version of the IQCODE

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Abstract The IQCODE is a retrospective questionnaire for caregivers about changes which occurred in a patient's cognitive and functional efficiency in the previous 10 years of life. Previous studies demonstrated the validity of the IQCODE for the screening of dementia similar to that of traditional cognitive screening tests, with the additional advantage of allowing the detection of cognitive *change*, rather than just cognitive impairment. The present paper deals with the preliminary results of the validation of the Italian version of the questionnaire in a sample of 45 mild to severely demented patients and 13 patients with mild cognitive impairment (MCI), compared to 20 cognitively intact elderly subjects. The IQCODE demonstrated satisfactory discriminative power for dementia as well as for MCI and a good correlation with the MMSE.

Informant-based instruments have often proved to be as effective as brief cognitive tests for the screening of dementia [2]. In spite of some limitations (such as the need for a compliant and reliable informant and the indirect nature of the assessment), such instruments have two important properties with respect to the traditional direct neuropsychological assessment: better applicability in subjects with particularly high or low educational and pre-morbid cognitive levels and independence from patients' compliance.

The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [1] is a 26-item informant-based questionnaire which requires caregivers to compare the current patient's cognitive and functional performances with his/her 10-year earlier level of functioning. The IQCODE also has the additional advantage of allowing measurement of cognitive *decline* from previous cognitive level, which is one of the major criteria required for the clinical diagnosis of dementia.

Several studies have already verified the validity of the IQCODE as a screening test for dementia not only in its original English version, but also in some other languages [3–5]. The present paper deals with the preliminary results of a study evaluating the discriminative power of the Italian version of the IQCODE in a clinical sample of cognitively impaired patients compared to normal controls and of its concurrent validity with respect to the MMSE.

Patients selected for the study were 45 demented subjects fulfilling DSM-IV diagnostic criteria for dementia [6] and 13 subjects affected by mild cognitive impairment (MCI) according to Petersen's criteria [7]. Twenty individuals referred to our Neuropsychological Unit for subjective cognitive complaints, with normal performance at psychometric testing, were enrolled as controls. Demented patients were then divided into three subgroups according to their MMSE score: MMSE score >18, mildly demented (n=25); MMSE score = 18–12, moderately demented (n=13); MMSE score <12, severely demented (n=7).

All subjects underwent an extensive clinical, neuropsychological and neuroimaging assessment. IQCODE data were collected from caregivers by self-completion of the questionnaire during patients' testing session. Answers were always checked at the end of the session to ensure full comprehension and completion of the items. Each item could be rated from 1 (much improved) to 5 (much worse) and the final score was calculated by summing the ratings over all the items to a potential maximum score of 130 (higher scores correspond to greater cognitive decline; the cut-off point for absence of cognitive decline suggested in the literature is ≤94 [8]).

The main results of the study are shown in Table 1. First, we assessed the discriminant validity of the IQCODE in demented subjects compared to controls. The three demented subgroups and the control group did not show statistically significant differences in age, gender distribution and level of education at ANOVA. As expected, their MMSE mean scores were significantly different. As regards to the IQCODE, all demented subgroups had significantly worse ratings compared to normal controls. Moderately and severely demented patients also scored significantly worse than mildly demented, while not differing from each other.

We then considered the ability of the IQCODE to discriminate among the three more important groups from a diagnostic perspective, i.e. normal controls, MCI and patients with mild degrees of dementia. These three groups did not show statistically significant sociodemographic differences. The mean MMSE score in the MCI sample overlapped with normal subjects and was significantly better than mildly demented. By contrast, the mean IQCODE score significantly worsened from normals, to MCI, to the mildly demented. Interestingly, analysis of the individual scores within the MCI group showed a unimodal distribution for the MMSE and a bimodal distribution for the IQCODE: eight MCI subjects scored below the cut-off value, while five scored above.

Correlation analysis was finally carried out on the demented sample in order to assess the concurrent validity of the IQCODE compared to the MMSE: the IQCODE mean scores were found to be significantly negatively correlated with the MMSE scores (r=-0.71).

Table 1 Socio-demographic features and MMSE and IQCODE scores of the five study groups compared with one-way ANOVA. Values are mean (SD) unless otherwise indicated

	Controls (n=20)	MCI (n=13)	Mild dementia (n=25)	Moderate dementia (n=13)	Severe dementia (n=7)
Age, years	67.7±4.9	69.7±6.2	69.8±7.3	72.1±6.9	70.1±6.6
Males, n (%)	55	60	55	60	55
Education, years	7.2±3.4	7.0±2.6	6.6±3.7	8.0±5.2	6.7±2.9
MMSE	28.7±1.5	7.1±2.5*	22.3±3.2§	15.1±1.3§*	9.3±2.2§*
IQCODE	87±7.9	96±11§*	102.6±15§	112.1±11§*	120.9±6§*

§ $p<0.05$ vs. controls, *$p<0.05$ vs. mild dementia
MCI, mild cognitive impairment

Discussion

The present study supports the discriminant validity of the Italian version of the IQCODE in dementia, confirming the findings obtained with the original questionnaire and with previous translations into languages other than English [3–5].

The discriminant power of the questionnaire was not limited to moderate and severe stages of dementia. In fact, in the present study, the IQCODE demonstrated a satisfactory performance in mild dementia as well, maybe due to its ecological relevance. Thanks to such a good sensitivity to the early stages of cognitive deterioration, the IQCODE might indeed contribute to a more timely diagnosis of dementia. The effectiveness of the IQCODE as a screening test for dementia is further supported by its good concurrent validity with the MMSE. In addition, allowing an immediate diagnosis of cognitive decline, the IQCODE provides complementary information with respect to the MMSE. The combined use of the two instruments might thus result in improvement in diagnostic accuracy and deserves further assessment [9]. Future research should also focus on the correlations of the ICODE performance with various features of the informants (e.g. age, education and emotional state) and with the quality of their relationship with the patient, as well as on its differential diagnostic potential with respect to various kinds of dementia.

Due to the small sample size, our findings about the discriminant power of the IQCODE in MCI cannot be considered conclusive. However, they suggest that the IQCODE might have greater ability than the MMSE in discriminating between cognitively intact elderly and subjects with mild cognitive impairment. Furthermore, the bimodal distribution of the IQCODE scores within the MCI sample seems to suggest its ability in distinguishing individuals at high risk of developing dementia from nondecliners. Neuropsychological follow up will establish the actual predictive value of the questionnaire.

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Frequency of apraxia of eyelid opening in the general population and in patients with extrapyramidal disorders

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Abstract We ascertained the prevalence of apraxia of eyelid opening (AEO) in a community located in Puglia, a region of southern Italy. The crude prevalence rate was 59 per million (95% confidence interval, 24–173). AEO coexisted with adult onset blepharospasm in 75% of cases, with atypical parkinsonism in 25% of cases. Among the overall patient population seen at our movement disorders clinic from 1987 to 1997, AEO was isolated in 10 otherwise healthy individuals, associated with adult-onset dystonia in 13 cases, and associated with a parkinsonian syndrome in 9 cases. The frequency of AEO was 10.8% in the dystonia group, and 2.1% in the overall parkinsonian group (Parkinson's disease, 0.7%; progressive supranuclear palsy, 33.3%). In two patients with possible progressive supranuclear palsy, AEO worsened after increasing levodopa dosage or acute apomorphine challenge and disappeared following levodopa discontinuation. AEO developing in the setting of a parkinsonian syndrome may be either disease- or drug-related.

Apraxia of eyelid opening (AEO) is an important and often undetected cause of non-paralytic inability to maintain the eyes open and involuntary persistent eye closure [1]. The condition is thought to be due to a range of disorders involving motor activities in the orbicularis oculi (OO) and levator palpebrae superior (LPS) muscles [2, 3]. AEO may be isolated in otherwise healthy individuals [4] or associated with extrapyramidal disorders [1] including dystonia, idiopathic Parkinson's disease (IPD) and parkinsonism other than IPD. No formal epidemiologic study has focused on the frequency of AEO in the general population, and only a few studies have dealt with the frequency of AEO in extrapyramidal disorders.

We diagnosed AEO according with Lepore and Duvoisin's clinical criteria [5]. These included: (i) no sign of ongoing orbicolaris oculi (OO) contractions such as lowering of the brows beneath the superior orbital margins (Charcot's sign); (ii) marked frontalis muscle overaction during period of inability to raise eyelids; (iii) no ocular motor/ocular sympathetic nerve dysfunction or ocular myopathy. Electromyographic examinations of the OO and LPS [3] muscles were not performed since the tests are unwieldy, require expertise not readily available in our setting and, finally, seem unnecessary to the diagnosis of AEO.

The prevalence of AEO in the general population was studied in an area including 3 neighboring towns (Casamassima, Conversano and Putignano) situated in the province of Bari, Puglia, southern Italy. The overall population was 67 606 on 1 January 1998 (the prevalence day). In this area, healthcare services are provided by the Italian National Health System through about 50 general practitioners and 3 general hospitals with both neurological and ophthalmologic outpatient/inpatient departments. Patients seeking medical attention by general practitioners are usually submitted to physicians of local hospitals or the University Hospital of Bari where a tertiary referral movement disorders center and an ophthalmologic botulinum toxin unit are located. Cases were identified by reviewing records coded for appropriate diagnoses (including apraxia of eyelid opening, blepharospasm, cranial dystonia or other focal and segmental/multifocal dystonia, idiopathic Parkinson's disease, and secondary parkinsonism) of patients attending the outpatient/inpatient departments of neurology/ophthalmology of the general and university hospitals from 1987 through 1999. Diagnosis of dystonia, IPD and parkinsonism other than IPD was made according with accepted criteria [6] by experienced movement-disorder specialists based on detailed history, physical and neurologic examinations, appropriate laboratory and neuroimaging tests, and (for parkinsonian patients) the presence of convincing/unconvincing response to levodopa.

By this approach, we identified 4 cases in the study area, alive at prevalence day. Three female patients (aged 52, 67 and 70 years) suffered from AEO associated with blepharospasm (BSP). None of them reported prior neuroleptic treatment or showed neurological signs other than dystonia and abnormalities on head imaging studies. In a male patient aged 63 years, AEO developed one year after the onset of possible progressive supranuclear palsy diagnosed according with NINDS criteria. Based on this sample, a crude prevalence rate of 59 per million was calculated (95% confidence interval, 24–173).

Among the overall patient population seen at our movement disorders clinic from 1987 to 1997, AEO was observed in the following clinical settings: (i) isolated in otherwise healthy individuals (n=10); adult-onset dystonia (4 males and 9 females aged 42–69), including blepharospam (n=10), cranial dystonia (n=2) and writer's cramp (n=1); Parkinson's disease (two males and 1 female aged 53–73 years); and possible or probable (according to NINDS criteria) progressive supranuclear palsy (PSP) (3 males and 3 females aged 53–74 years). Most patients suffered from episodes of involuntary drooping of the eyelids. Although this feature was not included in the criteria used by Lepore and Duvoisin [5],

transient failure to sustain lid elevation is now considered a further manifestation of AEO [1, 2]. In the same period we obseved 120 patients with adult-onset dystonia, 399 with Parkinson's disease and 33 with possible or probable PSP. Thus, the frequency of AEO was 10.8% in the dystonia group, and 2.1% in the overall parkinsonism group (Parkinson's disease, 0.7%; PSP, 33.3%).

Clinical and demographic features of patients with isolated AEO have been described elsewhere [4]. In most patients included in the dystonia group, namely those suffering from BSP, it was not possible to assess the time elapsing between AEO and BSP onset. Among parkinsonian patients, AEO developed 2–6 years (mean, 4.1; SD, 1.3) following the onset of the parkinsonian syndrome. Two patients, one with IPD, the other with PSP, developed AEO before the introduction of levodopa or other antiparkinsonian drugs. When they were treated with benserazide/levodopa 50/200 mg thrice daily, neither PSP nor AEO improved. Seven parkinsonian patients developed AEO 1–5 years (mean, 3.2; SD, 1.6) following the introduction of levodopa. In two of such patients (both suffering from possible PSP), AEO worsened after increasing levodopa dosage and disappeared when levodopa was discontinued. Later, a dose of apomorphine (APO) widely accepted for acute tests (5 mg subcutaneously preceded by 5 days treatment with domperidone) had no significant effect on limb motor activity but induced episodes of involuntary drooping of the eyelids and prolonged (a few minutes) inability to raise eyelids following voluntary or involuntary eye closure. APO effect lasted approximately one hour. One of these patients has been described in detail elsewhere [7]. The other was a 64-year-old male subject suffering from possible PSP for 3 years who developed AEO 3 months following the introduction of benserazide/levodopa (50/200 mg daily).

Discussion

Although the number of cases was small, and confidence interval was wide, our rate suggests that AEO may be as prevalent as a number of better known neurological conditions such as Huntington's disease, myasthenia gravis, and amyotrophic lateral sclerosis. Because the study was service-based, the present rate probably underestimates the condition. The demographic and clinical features of the identified patients are of limited value because of the small number. However, the average age at onset in sixth decade, the female preponderance, and the higher frequency of AEO in the setting of dystonia are consistent with the findings of the clinical series.

Several reports have emphasized the presence of AEO in parkinsonian syndrome [1, 5], mainly PSP, but detailed description with particular reference to the relationships between antiparkinsonian medications, especially levodopa, and AEO was lacking in most cases. In the present series, AEO developed following the onset of parkinsonian syndrome and the introduction of antiparkinsonian therapy including levodopa in 7 of 9 patients. This raises the possibility of a causal relationship between levodopa and AEO. Supporting this view, we report two patients in whom AEO worsened after increasing levodopa dosage or acute APO challenge and disappeared following levodopa discontinuation.

Few data are available on the response of isolated or dystonia-associated AEO to drugs interfering with dopamine transmission. Dewey and Marangore [8] reported two patients with isolated AEO and favorable response to levodopa, but the issue of psychogenicity was not completely excluded. We recently described a patient developing isolated AEO following prolonged exposure to flunarizine, a drug known to induce extrapyramidal reactions [4]. Sustained remission when the drug was stopped was in favor of a causal relationship between isolated scAEO and flunarizine [4]. Although AEO developing in the context of a parkinsonian syndrome was known to exist before initiation of levodopa therapy [1], our observations are ground for thinking that the condition may be either disease- or drug-related. The possibility of modifying dopaminergic treatment should always be considered when dealing with AEO in patients with parkinsonism. If changes in the drug regimen do not have any benefit, pretarsal botulinum toxin treatment may be tried [4].

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Amantadine in Huntington's disease: open-label video-blinded study

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Abstract Huntington's disease (HD) is characterized by chorea, cognitive and behavioral changes. Amantadine, a non-competitive NMDA receptor antagonist, has shown an antidyskinetic effect on levodopa-induced dyskinesias, which are known to have strict pathogenetic analogies with choreic hyperkinesias. The antidyskinetic efficacy of amantadine and its effects on cognitive and behavioural symptoms were evaluated. Eight HD patients received oral amantadine (100 mg tid) unblinded for a 1-year period. A significant reduction of dyskinesias was reported ($p<0.01$). No changes were observed in neuropsychologic and psychiatric assessments after 6 and 12 months of therapy. These data may have relevance to the treatment of HD with amantadine.

Huntington's disease (HD) is a fully penetrant autosomal dominant neurodegenerative disorder characterized by psychiatric symptoms, movement disorders and progressive dementia. The glutamate neurotransmitter system may be abnormal very early in the course of illness [1] and HD hyperkinesias are thought to arise through increased glutamatergic neurotransmission in thalamocortical pathways. Amantadine is an antiglutamatergic agent that acts as a non-competitive NMDA antagonist. In double-blind clinical trials [2, 3], amantadine has demonstrated significant anti-dyskinetic efficacy in levodopa-induced dyskinesias that are remarkably similar to typical chorea. The aim of this open-label, video-blinded study was to evaluate the antidyskinetic efficacy of amantadine and its effects on cognitive and behavioral symptoms.

There were 6 men and 2 women of mean age 60.4±8.4 years, mean disease duration 5.0±1.9 years, with genetically determined HD. This study was approved by the local ethics committee. Exclusion criteria were hepatic or renal failure, other CNS disease, active medical illnesses, and alcohol or substance abuse. Before entering the study a complete blood analysis and cardiologic evaluation were performed. All the patients received oral amantadine (100 mg tid) for a 1-year period. Motor evaluations were performed at baseline and after 1 (T1), 3 (T3), 6 (T6), 12 (T12) months by means of the Unified Huntington's Disease Rating Scale (UHDRS) and the Abnormal Involuntary Movement Scale (AIMS). The motor assessments were videotaped and the videotapes were reviewed and scored by a blinded rater. Each patient was tested using a neuropsychological battery, assessing short- and long-term verbal memory (Auditory Verbal Learning Test), attention (Trial Making Test, Stroop Test), conceptual thinking (Colored Progressive Matrices) and language (Phonological Verbal Fluency Test). Standardized rating scales for depression and anxiety (Beck Depression Inventory, BDI; Hamilton Depression Rating Scale, HDRS; Hamilton Anxiety Rating Scale, HARS), and the Brief Psychiatric Rating Scale (BPRS) were also performed. Neuropsychological and neuropsychiatric assessments were repeated after 6 (T6) and 12 (T12) months.

Statistical analysis was performed with the Wilcoxon signed rank test and a 2-factor repeated-measures analysis of variance. The "live" and videotape interrater agreements for dyskinesias were evaluated with intraclass correlation coefficient [4].

One patient did not complete the study while seven patients entered the final statistical analysis. After 1 month of treatment, a moderate to marked improvement in choreic dyskinesias was observed ($p<0.05$). Amantadine maintained its antidyskinetic effect at the 1-year follow-up visit (Fig.1).

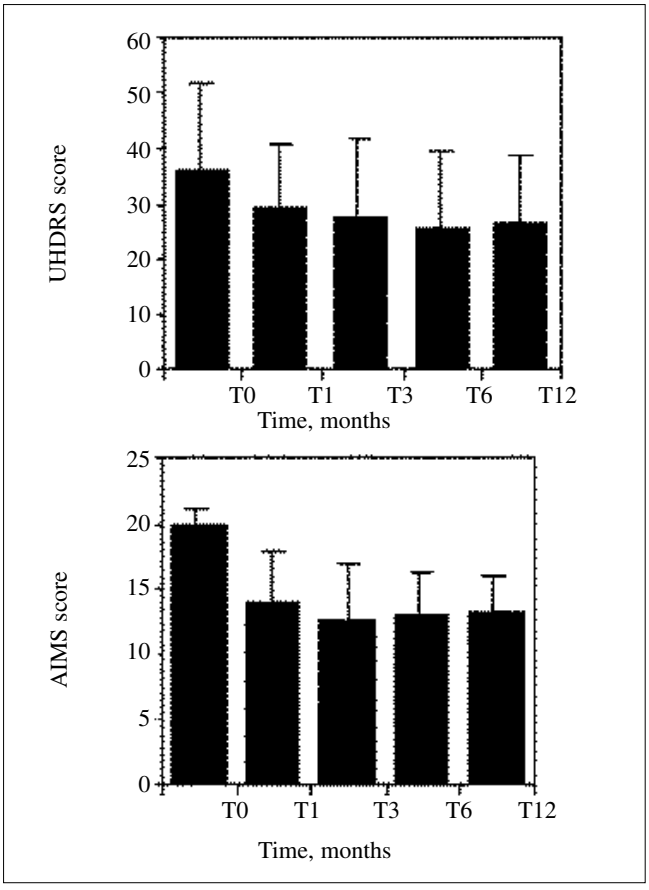

Fig. 1 Motor assessments in the study patients. Values are means and SD

Interrater agreement between "live" and "video" raters was 0.78 ("excellent"agreement beyond the range of chance) (5). No significant changes were observed in the score of both neuropsychological tests and psychiatric rating scales after 6 and 12 months of amantadine therapy.

Discussion

The results of this open-label study support the antidyskinetic effect of amantadine noted by others [5] in the past and, recently, by Verhagen et al. [6] and Bonuccelli et al. [7]. In recent years, there has been a growing interest in understanding the effect of antiglutamatergic treatments on motor disturbances of HD patients and riluzole and remacemide seem to have some benefit in choreic dyskinesias [8, 9]. The mechanism underlying the antidyskinetic action of amantadine remains elusive. As it was demonstrated that amantadine brain levels achieved with therapeutic doses are sufficient to block NMDA receptors, we can hypothesize that these antiglutamatergic properties of amantadine may well explain the effect in choreic hyperkinesias. The improvement of dyskinesias might result from a pharmacological reduction of glutamatergic transmission preferentially in the thalamocortical pathways as opposed to the other glutamatergic pathway in the subthalamic-pallidal circuits. The symptomatic effect of amantadine in HD patients corroborate the role of basal ganglia glutamatergic hyperfunction in the pathophysiology of choreic dyskinesias.

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Parkinson's disease and reproductive life events

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Abstract Onset, progression and duration of Parkinson's disease (PD) seem to be similar in men and women but gender differences have been suggested concerning clinical aspects, such as more severe disease in men and more dyskinesia in women. Taking into account the multiple influences of sex hormones, estrogens in particular, on basal ganglia function, the present work compared the characteristics of reproductive events in PD subjects and in healthy women, with regard to onset and clinical aspects of the disease with respect to the milestones of reproductive life. A total of 150 PD women and 200 healthy women matched for age were interviewed about reproductive life and disease characteristics (if patients). As a group, the women with PD had menarche later than the controls, but in the normal range. Menopause was similar to the controls for time, type (natural) and onset (slow), but with less hormonal therapies. Women with PD had fewer children, while breast feeding and gynecological diseases were comparable to controls. The characteristics of menses were similar as far as dysmenorrhea and premenstrual syndrome (PMS). The women with PD onset before menopause had a longer disease duration, with a more frequent fluctuating stage, and longer treatment with both levodopa and dopamine agonists. They had more dysmenorrhea and PMS when compared with women with PD onset after menopause and controls.

Parkinson's disease (PD) is a neurodegenerative disorder occurring in both men and women, although most studies find PD more common in men. Gender differences have been suggested concerning clinical aspects, such as more severe disease in men and more dyskinesia in women [1], and behavioral and psychiatric manifestations, such as wandering, verbal and physical abusiveness, hallucinations and delusions [2].

There is substantial evidence indicating that estrogen can modulate dopaminergic activity in the nigrostriatal system. One study suggested that estrogen has an antidopaminergic effect on motor function in postmenopausal women with PD [3]. Another study indicated that hormone replacement therapy was associated with less severe parkinsonian symptoms in postmenopausal women [4], and low-dose estrogen reduced motor disability in postmenopausal women with PD associated with motor fluctuations [5]. In a recent report, no significant dopaminergic effect of estradiol was demonstrated, but progesterone seemed to have an antidopaminergic effect [6]. In another report, no significant correlation between the objective or subjective measures of parkinsonism and estrogen and progesterone levels was demonstrated [7]. The aim of this study was to investigate the possible relationship between reproductive life events and the onset of PD, to verify the possible relationship between parkinsonism and the hormonal milieu in women. With this purpose, 150 women with PD diagnosed according to the Brain Bank criteria and attending the centres for PD of the Neurological Services of the Ospedale di Circolo of Varese and of the IRCCS C. Mondino of Pavia were recruited for the study together with 200 women of postmenopausal age attending the Menopause Unit of the Department of Obstetrics and Gynecology of the University of Pavia.

The PD women were submitted to a face to face or phone interview, designed to investigate the possible relationship between the disease and reproductive function. The interview was prepared with the collaboration of gynaecologists, and was divided into 3 sections: the first one investigated age and education level, age at onset of PD, duration and phase of disease, and pharmacological therapy; the second section investigated reproductive function, particularly the characteristics of menses, pregnancy, oral contraceptive use and menopause; finally, the last one investigated the relationship between neurological symptoms and menopause. All the data were obtained according to the current rules on confidentiality.

The data were collected into a database (Excel '97, Microsoft) and analysed with SPSS version 10.0. The qualitative variables were compared with chi-square test and quantitative variables with ANOVA.

At first the comparison between the control and patient groups was made, followed by the analysis of the patient data according to the onset before or after menopause (intending absence of menstrual bleeding >6 months). Patients with PD were between 42 and 85 years, with a mean age of 65.54 years, while the mean age of controls was 57.61 years, with a range from 44 to 80. As a group, the women with PD had menarche later than the controls, although in the normal range. Menopause in PD patients was similar to that in the controls for time, type (natural in more than 70% of the subjects), and onset (slow in more than 50% of the women), but with less hormonal therapies. Women with PD had had fewer children, but the prevalence of breast feeding and gynecological diseases was similar to that in the control women. The characteristics of menses were similar as far as dysmenorrhea and premenstrual syndrome (PMS).

In 25.3% of women, the onset of PD was before menopause. They had a longer disease duration, with a more frequent fluctuating stage, and longer treatment with both levodopa and dopamine agonists. They had more dysmenorrhea and PMS when compared with women with PD onset after menopause and with controls.

Discussion

This study compares for the first time the reproductive life events in women with PD and controls, similar for age and geographic and cultural environment. The data agree with an apparently normal step of fertile life span in women with PD onset after menopause, even though PD women had fewer children that did the controls.

In PD women with onset before menopause, the relationship with reproductive life events was more evident. In fact, they show more premenstrual symptoms and dysmenorrhea and a premenstrual worsening of motor symptoms. This last observation agrees with a previous report [8], but it seems not specific for the disease as it happenes also in various other neurological pathologies such as migraine, peripheral neuropathies, depression and spasmodic torticollis [9].

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Magnetic resonance relaxometry in Parkinson's disease

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Abstract A central role of iron in the pathogenesis of idiopathic Parkinson's disease (PD), due to its increase in substantia nigra pars compacta dopaminergic neurons and its capacity to enhance production of toxin reactive oxygen radicals, has been discussed for many years. Nuclear magnetic resonance (NMR) relaxation is considered an objective and noninvasive method of measuring regional iron concentrations. By means of this technique we investigated both controls and PD patients.

The brain shares with other organs the need for a constant and readily available supply of iron and has a similar array of proteins necessary for iron transport, storage and regulation. However, unlike other organs, the brain places demands on iron availability that are regional, cellular and age sensitive and has developed mechanisms to maintain the cellular iron homeostasis that is crucial for the viability of neurons.

In a number of common neurodegenerative disorders, there appears to be a loss of these homeostatic mechanisms with following excess accumulation of iron in the brain. Presently the mechanisms involved in the disturbances of iron metabolism in PD remain obscure, but numerous studies using a variety of analytic techniques demonstrated that iron levels are increased in PD, primarily within the substantia nigra pars compacta. Laser microprobe (LAMMA) studies capable of providing subcellular localization indicate that iron normally accumulates within neuromelanin granules of dopaminergic neurons in the substatia nigra and that iron levels within neuromelanin granules are significantly increased in PD [1]. Information pertaining to the availability of ferritin in the brain of PD patients would be useful, as iron bound to ferritin is relatively unreactive and unlikely to induce tissue damage. There are conflicting reports in the literature, with indications that ferritin is both increased [2] and decreased [3] in PD. These differences probably reflect differences in the isoform of ferritin that was studied. More recently, no increase in neuronal isoferritin was detected when specific brain isoforms of ferritin were evaluated [4].

Since the introduction in clinical practice of NMR at 1.5 tesla, a basal ganglia hypointensity has been described in spin echo (SE) T2-weighted images. Drayer et al. [5] and Rutledge et al. [6] observed that in T2-weighted images obtained at high field intensity MRI, the distribution of low signal intensity in the basal ganglia and other areas of the brain corresponded roughly with the ferric iron (Fe^{+++}) distribution demonstrated by Perls' stain in brain sections [6]. Iron deposits would create local inhomogeneities in the magnetic field which, in turn, would result in loss of the T2 signal. So, if T2 hypointensity of extrapyramidal nuclei is due to iron presence in these regions, we expect a further reduction in signal intensity when there is iron accumulation.

NMR studies have reported several patterns of decreased signal intensity in the basal ganglia and in substantia nigra of patients with PD, using a visual analysis of T2-weighted images [6, 7]. The estimation of T2 relaxation time might be more reliable than visual analysis in differentiating healthy volunteer subjects from PD patients. The aim of this study was to evaluate the T2 relaxation time of the extrapyramidal nuclei in PD patients and in healthy subjects.

For this study, 25 PD patients (age range, 39–76 years; mean age, 63 years) were selected. PD diagnosis was based on clinical criteria including the presence of bradykinesia with tremor or rigidity or postural instability, asymmetric symptoms onset, good response to L-dopa treatment, and absence of signs or symptoms of atypical or secondary parkinsonism. They had a mean disease duration of 8.4±5.5 years and were evaluated according to subscores I and III of Unified Parkinson's Disease Rating Scale (UPDRS) showing a cognitive-performances score of 4.0±2.6 and a motor examination score of 28.92±13.55. All patients were taking anti-PD medications: L-dopa or an oral dopaminergic agonist or both.

Twenty-seven age-mached healthy control participants (age range, 46–90 years; mean age, 66 years) were also studied. Selection criteria were no history of psychiatric or neurologic disease, no abnormal signs at general medical and neurologic examinations, and a previous normal neuroimaging (CT or NMR).

Informed consent was obtained from each person.

Subjects were studied with an MR Gyroscan (Philips) scanner operating at 1.5 T. After conventional sagittal T1-weighted scout images were acquired, a trial set of axial spin-echo sequences with a short TR, 600/20 (TR/TE) and a long TR, 2500/20, 80 (TR/first-echo TE, second-echo TE) were were taken parallel to the orbitomeatal plane, as determined on the sagittal section.

The relaxometric study was performed by using two 5-mm single sections: one including the head of caudate nucleus, putamen and globus pallidus, the other including the midbrain at the level of substantia nigra and nucleus ruber. A 5-mm section thickness was chosen as the best compromise to maintain good signal-to-noise ratios and reasonable imaging times (which cannot be prolonged too much in patients with PD).

At each of these levels, a multiecho sequence (eight echoes; echo times 30–240 ms) was applied, and mean T2

values for the following regions of interest (ROIs) were calculated on each hemisphere: frontal cortex, temporal cortex, frontal white matter, head of caudate, globus pallidus, anterior and posterior putamen, nucleus ruber, substantia nigra pars compacta and pars reticulata. ROIs were irregular and were drawn by hand on the screen around the outline of the corresponding structures. Frontal and temporal cortex and frontal white matter ROIs were placed on the plane at the basal ganglia level. In each subject, T2 values derived from the corresponding ROIs on the right and left brain hemispheres were pooled. T2 relaxation time measurements were performed by work station "Easy Vision".

Using an unpaired the two-tailed *t* test, no significant difference between groups was found in T2 values on temporal cortex, frontal white matter, head of caudate, globus pallidus, anterior and posterior putamen, red nucleus and substantia nigra (neither pars compacta nor pars reticulata). The only significant difference was found in T2 values of the frontal cortex that were significantly shorter in the PD patient group on both right (p=0.0089) and left (p=0.0367) sides. Moreover, both groups showed a significant decline of T2 signal with age in frontal cortex (p<0.02, Spearman's rank), while no significant age decline was found in other regions. However, in frontal cortex as in all other regions, no correlation was found between T2 signal and subscores and duration of disease.

In conclusion, we did not find any significant difference in T2 relaxation time both in basal ganglia and in substantia nigra of PD patients compared with healthy controls. We tempted various explanations. First of all, up to now we cannot say what relative contribution the various forms of iron (i.e. free versus bound, Fe^{++} versus Fe^{+++}) make to the NMR signal, so it is possible that the form of iron which is increased in PD is not a very paramagnetic form so it doesn't modify T2 relaxation time. In second place, it is unclear if T2 hypointensity is due to iron itself or to iron-binding proteins [G] as ferritin (there are data about neuromelanin) which is not increased in PD and so doesn't modify signal intensity. Finally, in PD it could be no iron absolute increment but its redistribution within the cell (according to recent theories) and so no NMR differences may be seen.

We only found a significant shortening of T2 relaxation time in frontal cortex of PD group that didn't present any correlation with score and duration of disease and that presented a large overlap of its values between healthy subjects and PD patients. We don't know the meaning of this result, even if it may be interesting since frontal cortex is involved in part of PD symptoms. In any case, T2 shortening of frontal cortex and signal intensity of extrapyramidal nuclei shouldn't be used to support clinical PD diagnosis.

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Quetiapine versus clozapine: a preliminary report of comparative effects on dopaminergic psychosis in patients with Parkinson's disease

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Abstract This study investigated the efficacy and safety of quetiapine versus clozapine in parkinsonian patients with dopaminergic psychosis. All patients fulfilling the inclusion criteria were randomly assigned to receive either quetiapine or clozapine. The duration of the trial was 12 weeks. The severity of psychosis was assessed using the BPRS and the Clinical Global Impression Scale-Severity subscale (CGI-S). The UPDRS III was used to monitor the progression of PD during the study period. Twenty patients, 10 on clozapine, and 10 on quetiapine, completed the study. The psychopathological state, as assessed by the BPRS and by the CGI-S, improved significantly ($p<0.001$) from baseline in both treatment groups. No differences were found between clozapine and quetiapine at each assessment time. The UPDRS score decreased significantly ($p<0.05$) in the clozapine group, while was almost unchanged in the quetiapine group.

Several studies have established that psychotic symptoms occur in 16%–37% of parkinsonian patients chronically treated with dopaminergic drugs [1, 2]. They are more frequent in patients with cognitive impairment [3], depression [1] and a longer disease duration [4]. So far, clozapine is the most effective drug for treating psychosis in Parkinson's disease (PD) and it does not cause a significant exacerbation of parkinsonism. Nevertheless its use is limited by the potential risk of developing agranulocytosis which requires weekly white blood cell monitoring. Quetiapine fumarate is a new dibenzothiazepine atypical antipsychotic similar to clozapine, so it may be useful in the treatment of dopaminergic psychosis in PD. This study aimed to investigate the efficacy and safety of quetiapine versus clozapine in parkinsonian patients with dopaminergic psychosis.

Patients attending the Parkinson Unit at the Institute of Neurology, University of Messina, were eligible for the study if they had idiopathic Parkinson's disease and psychosis defined by the DSM-IV induced by antiparkinsonian drugs. To be included in the study, each patient had to have a documented history of psychosis of at least four weeks before study entry and required a baseline score of 3 or greater on the items hallucinations and/or delusions of the Brief Psychiatric Rating Scale (BPRS). All patients fulfilling the inclusion criteria were included in the study which was a randomized, open-label, blinded-rater, parallel group trial. Each patients was assigned to receive either quetiapine or clozapine. Both drugs were given at a low initial dose (6.25 mg/day for clozapine and 25 mg/day for quetiapine), administered orally. The antipsychotic dose was then titrated according to the individual clinical response and tolerability up to a maximum of 50 mg/day for clozapine and 200 mg/day for quetiapine. The duration of the trial was 12 weeks. During the study, the dosage of antiparkinsonian drugs was kept constant. All patients were assessed at baseline and after 2, 4, 8 and 12 weeks by the same investigators, a neurologist and a psychiatrist. The severity of psychosis was assessed using the BPRS and the Clinical Global Impression Scale-Severity (CGI-S) subscale. The Unified Parkinson's Disease Rating Scale (UPDRS III) was used to monitor the progression of PD during the study period. Dyskinesias were assessed using the Abnormal Involuntary Movement Scale (AIMS). Adverse effects were evaluated at each study visit. Electrocardiography (ECG) and laboratory tests, including hematology, clinical chemistry and urine analysis, were performed on admission and at the end of treatment. Blood pressure and pulse rate were measured at each study visit. In the clozapine group, a complete blood count was performed weekly. The within- and between-group differences in efficacy and safety variables were analyzed using an analysis of covariance (ANCOVA).

Twenty-three patients fulfilled the inclusion criteria and participated in the study: 12 received clozapine and 11 quetiapine. Two patients in the clozapine group did not complete the trial: one dropped out for severe hypotension at week 2 and the other for oversedation at week 3. One patient in the quetiapine group dropped out for confusional state after 2 weeks. Twenty patients, 10 (5 males and 5 females) on clozapine and 10 (5 males and 5 females) on quetiapine completed the study and were included in the clinical analysis (Table 1). In the clozapine group, the final dose of clozapine ranged from 12.5 to 50 mg/day, while in the quetiapine group, final dose ranged from 25 to 200 mg/day. There were no significant differences between the clozapine and quetiapine groups in any of the efficacy parameters at baseline. The psychopathological state, as assessed by the BPRS, and by the CGI-S, improved significantly ($p<0.001$) from baseline to week 12 in both treatment groups. No differences were found between clozapine and quetiapine at each assessment time. The UPDRS III score decreased significantly ($p<0.05$) in the clozapine group, while was almost unchanged in the quetiapine group. However, in the 3 patients receiving a quetiapine dose higher than 100 mg/day, a slight worsening of parkinsonism was observed. The AIMS score also decreased significantly ($p<0.05$) in both treat-

Table 1 Demographic and clinical features of the 20 patients completing the study. Values are means (SD). Differences between treatment groups were not significant

	Quetiapine (n = 10)	Clozapine (n = 10)
Dosage, mg/day	90.0 (51.7)	27.5 (14.2)
Age, years	69 (12)	68 (11)
Duration of illness, months	112 (59)	121 (60)
Hoehn & Yahr stage	3.1 (0.5)	3.2 (0.6)
L-Dopa dosage, mg/day	675 (188)	705 (123)
BPRS total		
Baseline	37.7 (6.9)	38.2 (6.2)
Endpoint	28.4 (5.2)**	27.5 (4.7)**
CGI-S		
Baseline	3.6 (0.7)	3.8 (0.8)
Endpoint	2.1 (0.6)**	1.9 (0.6)**
UPDRSIII		
Baseline	53.3 (9.7)	57.8 (11.2)
Endpoint	54.9 (11.5)	55.7 (14.9)*
AIMS		
Baseline	7.9 (2.2)	7.7 (2.3)
Endpoint	6.3 (1.3)*	5.9 (1.6)*

**Statistically significant difference between endpoint and baseline $p<0.001$; *Statistically significant difference between endpoint and baseline $p<0.05$; *BPRS*, Brief Psychiatric Rating Scale; *CGI-S*, Clinical Global Impression Scale, severity subscale; *UPDRS III*, Unified Parkinson's Disease Rating Scale; *AIMS*, Abnormal Involontary Movement Scale

ment groups during the study period. Side effects were mild and transient in both groups.

Discussion

Some uncontrolled, open-label studies have shown that quetiapine is a useful drug in the management of PD psychosis [5]. This is the first controlled open-label, blinded-rater, trial of quetiapine versus another atypical antipsychotic in PD patients with dopaminergic psychosis. Clozapine is considered the most effective agent for treating this condition. Preliminary observations with the new antipsychotics risperidone and olanzapine were positive and they were proposed as an alternative for clozapine. However, recent double-blind randomized studies [6, 7] have shown that olanzapine and risperidone may worsen extrapyramidal symptoms and, therefore, should not be used in PD psychosis. Our results confirm that quetiapine may be an effective and well tolerated agent in treating drug-induced psychosis in PD patients. Nevertheless, the three patients treated with a quetiapine dose higher than 100 mg/day showed a slow and gradual increase in UPDRS III score. Based on our findings and on literature data, we believe that reduction of the antiparkinsonian medication dosage and discontinuation of those agents with the highest risk/benefit ratio is the first therapeutic strategy in this condition. If psychosis persists and/or parkinsonism worsens, we suggest the use of the atypical antipsychotics quetiapine and clozapine at low doses.

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Incidence of RBD and hallucination in patients affected by Parkinson's disease: 8-year follow-up

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Abstract We describe the 8-years follow-up of 80 patients affected by idiopathic, L-dopa-responsive Parkinson's disease. All patients were evaluated at baseline and during the follow-up with visual evoked potential, P300 event related potentials and polysomnography. The patients and their relatives compiled sleep and hallucination questionnaires. Statistical analysis was performed to evaluate if visual abnormalities, abnormal P300 recordings or sleep disturbances were linked to the development and hallucinations. Our results show that abnormal vision and abnormal P300 did not correlate with the incidence of hallucinations. However, the presence of REM sleep behavioral disorder (RBD) was significantly related to the development of hallucinations, independently of age, gender or duration of disease but dependent on the amount of dopaminoagonist treatment.

Many studies have reported visual abnormalities with electroretinogram and visual evoked potential (VEP) alterations in Parkinson's disease (PD) [1–3], showing that visual abnormalities are due to dopamine deficiency and related to the perception of the spatial frequency of stimuli [4]. Oddly enough, with the exception of one study based on color and contrast perception [5], visual abnormalities were not studied in relation to the occurrence of the well known "visual" complication of PD, i.e. visual hallucinations. Visual hallucinations are attributed to abnormalities of the ascending cholinergic and serotoninergic brainstem (and thalamic) pathways involved in the control of sleep-waking state, or to the defective visual processing accompanied by abnormal cortical release phenomenon [6].

The renovated approach to sleep studies in PD and parkinsonism has evidenced that a peculiar disturbance of rapid eye movement (REM) sleep phase, consisting of the loss of normal muscle inhibition during REM sleep and enacting of dreams, i.e. REM sleep behavior disorder (RBD), is present in idiopathic PD [7], in some parkinsonism states and in Lewy body disease (LBD). The presence of RBD has been associated with the occurrence of daytime hallucinations [8]. In order to understand whether hallucinations in PD are related to visual or sleep disturbances, we performed an 8-year follow-up of 80 patients affected by idiopathic, L-dopa responsive PD. All patients were evaluated for VEP alterations and contrast sensitivity, underwent visual P3 event related potential (ERP) recordings and polysomnography, and responded to sleep and hallucinations questionnaires, in order to assess the possible correlation with the onset or severity of hallucinations.

Consecutive patients with probable PD and their caregivers who were followed in the movement disorders outpatients offices were invited to participate in the study. Initial selections included 166 patients. At the end of the study, eight years after admission, 80 patients were considered to be affected by idiopathic PD because, despite occurrence of wearing-off or similar phenomenon, they were (1) still experiencing a benefit from dopaminomimetic therapies that had been increased by 1.5- to 3-times the amount administered at admission, (2) cognitive and behavioral functions were not altered during dopaminomimetic treatment, and (3) tremor was a consistent, although variable, feature in off periods.

Electroretinography (ERG) and polysomnography were performed, and VEPs, contrast sensitivity and visual P300 ERPs were recorded according to methods described in detail elsewhere [4, 9–11]. All electrophysiological recordings were performed at admission, before any treatment regimen was initiated in 52 patients, and 24 hours after the last L-dopa, dopaminoagonist or anticholinergic treatment in the other patients. Recordings were repeated with the same method at the end of the study. Criteria for VEP abnormality were set by the mean latency + 2 standard deviations (SD), for contrast sensitivity abnormality on 2 dB loss of 2–5 cycles per degree (cpd) spatial frequency, for ERG abnormality on the ratio below 50% of 1 vs 3 cpd evoked ERG, for visual P3 ERP abnormality on the mean latency + 1.5 SD. At admission, patients and family members were interviewed on their sleep behavior and sleep history using a simple sleep questionnaire, respecting the minimal ICSD (1997) criteria for RBD in agreement with the spouse's report [8], developed in our center: the questionnaire is proposed to patients and family members and investigates items such as quality of sleep, medication, vocalization, movements during sleep, nightmares, dream enactment, hallucinations when awaking from nightmares and frequency of movements during sleep. Patients and family members were asked with a questionnaire, part of the present state examination [12], about hallucinations and, if present, about the kind of hallucinations.

Questionnaires were administered once every year until the end of the study. Polysomnography in night sleep was performed whenever the sleep questionnaire suggested RBD and at the end of the study. All patients were evaluated at the beginning of the study with the Hoehn/Yahr scale [13], UPDRS [14] and MMSE [15].

Total daily intakes of L-dopa and dopaminoagonist were

compared with the other parameters; for dopaminoagonists an equivalent bromocriptine dose was calculated as 10 mg bromocriptine = 1 mg pergolide, 1 mg pramipexolo, 5 mg ropinirole or 1.5 mg cabergoline.

Systematic differences between hallucinating and non-hallucinating patients were evaluated using χ^2 test, Mantel-Haenzel tests for linear associations and Fisher's exact test. Factors independently associated with hallucinatory status were identified using logistic regression models (CATMOD procedure).

We forced in the logistic model two variables (visual abnormalities and P300), also if the univariated analysis reached a *p* value greater than 0.10. Our intention was to demonstrate the role that both visual abnormalities and P300 play on hallucinatory status development. The saturated logistic model also contained the variables age and sex. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated from the estimated coefficients in the model. To evaluate the hierarchically best model, the conditional maximum likelihood ratio (CMLR) was performed, with or without interactions between main predictors. The Hosmer-Lemeshow test χ^2 (8 df), goodness of fit, was also performed. All analyses were performed using SAS package [16].

The RBD questionnaire was validated in comparison with polysomnography recordings: all patients classified as having RBD by questionnaire had polysomnographic evidence of RBD, only 2 patients classified as non-RBD by the questionnaire had 1 or 2 episodes of RBD evidenced by polysomnography (sensitivity, 100%; specificity, 96.3%).

According to the Hoehn/Yahr scale at baseline, 44 patients were at stage 1, 14 patients at stage 1.5, 10 patients at stage 2, and 12 patients at stage 2.5. After 8 years, the staging of the same study population was as follows: 20 patients at stage 2, 14 patients at stage 2.5, 39 patients at stage 3, and 7 patients at stage 4.

At baseline, visual abnormalities were found in 28 PD patients (Table 1). The chronic dopaminergic treatment in "on" state reduced visual abnormalities to normal limits in the majority of patients and at the end of the study visual abnormalities were observed in "off" and "on" state in only 27 patients. Linear regressions of VEP latencies and amplitudes vs. duration and stage of Parkinson's disease did not reach statistical significance. The prevalence of hallucinations was below 40% both in patients with and without visual abnormalities (not significant at χ^2). A delay of P300 was recorded in 2 patients at baseline and in 15 patients during "on" state and 27 patients during "off" state at the 8-year fol-

Table 1 Prevalence of visual and P300 abnormalities, RBD and hallucinations in 80 patients with idiopathic L-dopa-responsive Parkinson's disease. Values are number of patients

	Baseline	3 years	6 years		8 years	
			On	Off	On	Off
Vision						
Normal	52	66	67	59	58	53
Abnormal	28	14	13	21	22	27
P300						
Normal	78	71	69	59	65	53
Abnormal	2	9	11	21	15	27
RBD	5	9	23	–	27	–
Hallucinations	5	8	19	–	31	–

RBD, REM sleep behavioral disorder

Table 2 Prevalence of visual and P300 abnormalities and RBD in patients with hallucinations, identified from 80 patients with idiopathic Parkinson's disease and followed for 8 years. Value are number of patients

	Baseline (n=5)	3 years (n=8)	6 years (n=19)	8 years (n=31)
Vision				
Normal	3	6	11	19
Abnormal	2	2	7	12
P300				
Normal	5	7	13	24
Abnormal	0	1	5	7
RBD	3	8	19	27

RBD, REM sleep behavior disorder

low-up. P300 delay was distributed equally in patients with and without hallucinations, (NS at χ^2). CMLR excluded P300 as a main predictor.

Throughout the follow-up observation, 27 patients were classified to be clinically affected by RBD (questionnaire plus PSG).

At the last visit, only 4 patients describing hallucinations were classified among the normal sleepers (n=53), while 27 PD patients had RBD and experienced hallucinations independently of age or gender. There was a higher incidence of hallucinations in stages 3 and 4 PD patients, not reaching statistical significance ($p<0.6$). CMLR showed that the presence of RBD was significantly related and predictive of the development of hallucinations ($p<0.001$), independently of MMSE score, Hoehn/Yahr stage or UPDRS evaluation. RBD was not correlated with visual abnormalities ($p<0.7$), just as hallucinations were not correlated with visual abnormalities ($p<0.2$). RBD and hallucinations were related to the amount of dopaminoagonist drugs administered: patients who experienced both RBD and hallucinations assumed 32±6 mg/day bromocriptine equivalent, while those with neither RBD nor hallucinations assumed 11±6 mg/day bromocriptine equivalent ($p<0.001$). Table 2 shows the prevalence of visual P300 abnormalities and RBD in patients with hallucinations throughout the 8-year follow-up.

Discussion

Visual abnormalities or abnormalities of cognitive ERPs do not correlate with the presence of visual hallucinations, and do not correlate with the presence of RBD. RBD is significantly correlated with hallucinations independently of duration, gender, age, stage of disease and is correlated with the administered amount of dopaminoagonist therapy. This finding is not surprising as visual abnormalities are corrected by L-dopa or dopaminoagonist therapy, while hallucinations and RBD are precipitated by dopaminoagonist therapy and L-dopa [8].

Many authors have suggested that sleep disruptions, vivid dreams and hallucinations may have a common anatomical locus [17, 18], but only a few studies reported visual hallucinations that occur early in the course of PD. In the nineteenth century, some authors reported hallucinosis in untreated parkinsonian patients [19, 20]. McKee et al. [21] suggested that the hallucinations should be attributed to midbrain alterations, at one time, disturbing sleep or giving rise to visual hallucinations, as the connections of the substantia nigra pars reticulata to brain stem nuclei regulating REM sleep and to the limbic structures may provide an anatomical explanation for both clinical manifestations.

The relationship between hallucinations and sleep disturbances has recently been undergoing a controversial debate. Some authors have suggested that hallucinations, together with sleep disturbances and psychosis, are part of a kindling phenomenon [22], while others stressed the early occurrence of hallucinations, independently of major mental disturbances [23]. The debate has also recently been focused on the hypothesis that hallucinations might be dependent on an alteration of cholinergic pathways [24]. Our study does not address the origin of the disturbances, but undoubtedly shows that the visual abnormalities of PD are not linked to hallucinations. This lack of correlation suggests that the visual abnormalities are not linked to the receptorial neurotrasmitter abnormalities that generate hallucinations and RBD.

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Cerebrospinal fluid levels of biomarkers and activity of acetylcholinesterase (AChE) and butyrylcholinesterase in AD patients before and after treatment with different AChE inhibitors

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Abstract In order to evaluate the biochemical effects of long-term treatment with inhibitors of acetylcholinesterase (AChE) in patients with Alzheimer's disease (AD), we measured the activities of AChE and butyrylcholinesterase (BuChe) and the concentrations of β-amyloid (1–42), τ and phosphorylated τ proteins in the cerebrospinal fluid (CSF). A total of 91 patients suffering from probable AD of mild to moderate degree were treated for 6 months with donepezil (n=59), galantamine (n=15), rivastigmine (n=10), or placebo (n=7). AChE activity in CSF was significantly increased after treatment with donepezil and galantamine; the opposite was observed in the rivastigmine-treated group. Untreated patients did not show any AChE activity variation. BuChE did not show any change in any of the groups studied. Mean values of β-amyloid(1–42), total τ and phosphorylated τ also did not vary significantly. We conclude that AChE inhibitors induce different effects on CSF AChE activity, while other CSF biomarkers are not significantly affected by treatment.

Acetylcholinesterase (AChE) colocalizes with β-amyloid in neuritic plaques and accelerates the assembly of amyloid β-peptides into fibrils deposited in the brain of patients with Alzheimer's disease (AD). Conversely, the β-amyloid protein regulates AChE expression, assembly and glycosylation in cell cultures, transgenic mice and Alzheimer brain, thus creating a vicious circle leading to an increased accumulation of β-amyloid. AChE inhibitors have been suggested to enhance the release of nonamyloidogenic soluble derivatives of amyloid precursor proteins (APPs) in vitro and in vivo and possibly to slow down formation of amyloidogenic compounds in brain [1–3]. Therefore, inhibition of AChE activity might influence APP processing and β-amyloid deposition.

With respect to the potential influence of AChE inhibitors on τ protein, there is a recent in vitro study showing that these drugs are able to modulate phosphorylation and levels of τ protein in SH-SY5Y cells through an interaction with nicotinic receptors [4]. According to these premises, the biochemical effects of AChE inhibitors can be monitored by means of biological markers in the cerebrospinal fluid (CSF), e.g. AChE and butyrylcholinesterase (BuChE) activities and the concentrations of β-amyloid and total and phosphorylated τ proteins. Therefore, we carried out an explorative study by measuring activities of AChE and BuChE and concentrations of β-amyloid(1–42), τ and phospho-τ proteins in CSF of AD patients before and after long-term treatment with different AChE inhibitors.

We studied 91 patients suffering from probable AD of mild to moderate degree, recruited in Malmö and Piteå, Sweden and in Perugia, Italy. A total of 84 subjects were treated with one of three AChE inhibitors for 6 months: 59 patients received donepezil, 15 were treated with galantamine, and 10 received rivastigmine. As control group, 7 AD patients enrolled in a previous double-blind placebo controlled clinical trial who underwent lumbar puncture before and 6 months after treatment with placebo were included. AChE and BuChE activities in the CSF were measured spectrophotometrically. β-Amyloid(1–42), τ and phospo-τ [5] were determined using a specifically constructed sandwich ELISA (Innogenetics, Ghent, Belgium).

All the patients (or their nearest relatives) gave informed consent to participating in the study. At the time of enrollment in the study, none of the patients were being treated with any drug interfering with cognitive functions. For statistical analysis, normal distributions were tested using the Shapiro-Wilk test; if normality was rejected, non-parametric tests were employed. Subgroup comparisons were done using the *t* test, after Bonferroni correction for multiple comparisons.

Treatment with donepezil caused a significant and dose-related increase of CSF AChE activity as opposite to completely unmodified BuChE activity. None of the other CSF markers (β-amyloid(1–42), total τ, phospho-τ) showed a significant change after treatment.

Similarly to that observed for donepezil, treatment with galantamine caused a significant increase of AChE activity in CSF, while BuChE activity remained unchanged. No variations were observed in the concentrations of the other biochemical markers.

Although only 10 patients were treated with rivastigmine, a significant reduction of AChE activity was documented in this group. BuChE activity and levels of the other biomarkers did not show major variations.

No significant variations were observed in any of the biochemical parameters studied in the 7 control subjects.

With respect to the effect on AChE activity, the drugs tested behaved differently: donepezil and galantamine caused a marked (donepezil>galantamine) increase; rivastigmine induced a significant decrease. These findings are in agreement with two recent reports [6, 7]. The different mechanisms of action of these drugs might explain this result: donepezil and galantamine are reversible inhibitors, while rivastigmine is a pseudo-irreversible inhibitor, implying that in the process of inactivating AChE a cleavage of the parent molecule takes place. Donepezil caused a strong and dose-dependent up-regulation of the enzyme activity, probably due to its non-competitive (i.e. non-compensatory) action; galantamine has a competitive action, which does not depend on the absolute concentration of the drug but more on the relationship with the substrate concentration. None of the drugs tested influenced BuChE activity. This was expected for donepezil and, to a less extent, also for rivastigmine [8], while, to our knowledge, no data are available for galantamine relative to human CSF studies. The other CSF biomarkers for AD - β-amyloid(1–42), τ and phospho-τ - did not show any significant change after treatment.

In conclusion, this study showed that: (i) AChE inhibitors induced different effects on AChE activity in the CSF and, at least for donepezil, the effect was dose-dependent; (ii) the biochemical effects of these drugs were detected in CSF and different treatments were distinguished; (iii) other CSF biomarkers of AD were not significantly affected by treatment with AChE inhibitors. The possibility to detect in CSF the biochemical processes taking place in the central nervous system of AD patients treated with anti-dementia drugs confirms the importance of this approach for a better knowledge of the pathophysiology of the disease and will allow us to demonstrate the actual impact of the new therapeutic strategies (e.g. anti-β-secretase drugs, anti-amyloid vaccine) aimed at interfering with the pathogenetic events of the disease.

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Cytogenetic alterations in lymphocytes of Alzheimer's disease and Parkinson's disease patients

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Abstract We investigated the presence of cytogenetic alterations in peripheral blood lymphocytes of Alzheimer's disease (AD) and Parkinson's disease (PD) patients. Detection of spontaneous structural and/or numerical chromosome damage has been assessed by micronucleus (MN) assay coupled with fluorescence in situ hybridization (FISH). The cytogenetic investigation was performed on 22 AD patients, 18 PD patients, and 20 controls. The spontaneous frequencies of micronuclei (MN) in human lymphocytes of both AD and PD patients were significantly higher than in controls. The majority of MN was composed of whole chromosomes in AD patients, while a prevalence of MN arising from chromosome breakage was observed in PD patients. Different molecular mechanisms underlie cytogenetic alterations observed in peripheral lymphocytes of AD and PD patients.

There is increasing interest in evaluating, at peripheral level, the presence of chromosome damage in somatic cells of patients with neurodegenerative diseases. Cytogenetic studies in cultured cells of Alzheimer's disease (AD) patients showed an increase in aneuploid metaphases [1, 2], but not always confirmed [3]. Elevated levels of micronuclei (MN) in peripheral lymphocytes [4] and fibroblasts [5] were also reported in AD patients. No cytogenetic study has been performed until now in Parkinson's disease (PD) patients. The aim of this study was to confirm the presence of chromosome malsegregation in lymphocytes of AD patients, and to assess, in the same cell system, the spontaneous level of chromosome alteration in PD patients.

The cytogenetic investigation was performed on two groups of patients: 22 untreated AD patients (4 men and 18 women of mean age 68.8 years; SD, 7.0) diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria; 18 untreated PD patients (3 men and 15 women of mean age 65.5 years; SD, 10.5), diagnosed according to UK Parkinson's Disease Society Brain Bank criteria. We also enrolled 20 healthy subjects including 4 men and 16 women of mean age 67.1 years (SD, 6.6), matched for age, sex and smoking habits to the patient groups. No previous exposure to toxic metals or recent X-ray examination was reported, and all patients were drug-free for at least two months before the examination.

We used the MN assay for detecting the presence of chromosome damage in cytochalasin β-blocked binucleated lymphocytes [6]. MN are chromatin structures readily visible in the cytoplasm of interphase binucleated cells. The application of fluorescence in situ hibridization (FISH) technique with an alphoid DNA probe specific for the centromere of all human chromosomes allowed us to discriminate between MN arising from chromosomal fragments (C-MN, not incorpored into the daughter nuclei at the anaphase because lacking of the centromere) and MN that derive from lagging chromosomes (C+MN) during the anaphase stage (chromosome loss).

The average spontaneous MN frequency in human lymphocytes of both AD and PD patients was higher compared with that of controls ($p<0.001$). FISH analysis showed 75.4% and 34.8% C+MN in AD and in PD patients, respectively. C-MN was 24.6% in AD and 65.2% in PD (Table 1).

Table 1 Results of cytogenetic analyses in lymphocytes of AD and PD patients. Values are means (SD)

Parameter	AD (n=24)	PD (n=20)	Controls (n=22)
MN (%)	1.82 (0.48)**	1.75 (0.37)**	0.85 (0.42)
C+MN (%)	75.4 (5.3)*	34.8 (4.4)	49.3 (3.7)
C-MN (%)	24.6 (5.3)	65.2 (4.4)*	50.7 (3.7)

MN, micronuclei, *C+MN*, micronuclei derived from whole chromosome(s); *C-MN*, micronuclei derived from chromosomal fragments; **$p<0.001$ vs. controls; *$p<0.05$ vs. controls

Discussion

High levels of spontaneous MN were observed in lymphocytes of AD and PD patients. Our results indicate the presence of cytogenetic alterations, at peripheral level, in both these neurodegenerative diseases. However, the application of FISH analysis revealed that the majority of MN in AD patients was composed preferentially of whole chromosomes. The presence of higher percentage of C+MN in AD patients indicates a more frequent involvement of aneuploidy in the origin of spontaneous MN, supporting the hypothesis that microtubule impairment might be associated with the disease. Altered microtubule stability may regulate critical neuronal functions, perhaps related to length, synaptic complexity and/or neuronal plasticity.

The high percentage of MN containing acentric fragments in lymphocytes of PD patients suggests that MN are mainly due to chromosome (chromatid) breakage. Active oxygen radicals are known to induce chromosomal aberrations, and evidence of oxidative damage are reported at peripheral level in PD patients [7, 8]. With this in mind, we can hypothesize that the chromosomal breakage observed in somatic cells of PD patients might be related to an abnormally high oxidative stress.

Finally, our findings indicate that, although both neurological diseases share some common clinical and etiological features, different molecular mechanisms underlie the observed chromosome damage in AD and PD patients.

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Controlled-release transdermal apomorphine treatment for motor fluctuations in Parkinson's disease

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Abstract This study evaluated the efficacy in Parkinson's disease (PD) of a new pharmacologic preparation of apomorphine included in microemulsions and administered by transdermal route, which provides a constant release of the drug for several hours (Apo-TD). Twenty-one PD patients with motor fluctuations were treated with L-dopa alone, with L-dopa plus oral dopamine-agonists, or with L-dopa plus Apo-TD. Apo-TD improved UPDRS-III and tapping test scores in "off" conditions, and reduced duration of "off" periods; no improvement in "on" conditions occurred. We conclude that Apo-TD shows its efficacy particularly by reducing "off" period duration and disability rather than improving motor performances in "on" conditions and therefore it seems a promising treatment for uncontrolled "off" phases in PD patients.

Several strategies have been proposed to control "off" periods in advanced Parkinson's disease, but at present these remain a major problem. Apomorphine is a well-known potent, short-acting agonist on D1 and D2 dopamine receptors, used by subcutaneous or sublingual route for the treatment of "off" periods. However, its clinical use is limited by its short half-life of approximately 30 minutes and local side effects. In order to exploit the favourable pharmacodynamic characteristics of apomorphine and overcome the limits, we studied a peculiar pharmacologic preparation of apomorphine, dissolved in microemulsions, which may be administered by an epicutaneous-transdermal route (Apo-TD) and may provide a constant release of the drug for several hours, depending on few variables (concentration of the drug and application area over the skin), as demonstrated in vitro with hairless mouse skin [1].

We selected 21 consecutive patients with idiopathic Parkinson's disease, according to the following inclusion criteria: age between 55 and 75 years, stages III-IV Hoehn-Yahr, presence of long-term L-dopa syndrome characterized by "wearing-off" or predictable "off" periods; and positive response to subcutaneous apomorphine test without severe side effects. Mean age was 59.5 years and mean duration of illness was 7.9 years with a mean Hoehn-Yahr stage of 3.4; mean daily L-dopa dosage was 645 mg. Patients were evaluated on T0 (L-dopa therapy + oral dopamine agonists), on T1 (L-dopa alone) and on T2 (L-dopa + Apo-TD). UPDRS–part III and tapping test at regular intervals from 8 a.m. until 10 p.m. were performed and mean score values in "off" and "on" conditions were calculated. A diary recording duration of "off" and "on" periods was also obtained. Apo-TD (30 mg) was applied to a 100 cm^2 area over the anterior part of the chest using a bandage, and left for 12 hours from 8 a.m. until 8 p.m.

Differences between motor performances during APO-TD treatment (T2 evaluation) and motor performances obtained on T1 and T0 evaluations are summarized in Table 1.

Mean UPDRS-III and tapping test scores obtained in "on" conditions in T2 and T0 were not significantly different. In "off" conditions, mean UPDRS-III score on T2 was lower and tapping test score was higher compared to those on T0, but these differences did not reach statistical significance. Conversely mean duration of "off" periods on T2 (3.1±1.2 h) was shorter compared to that on T0 (4.6±2.6 h) and this difference was statistically significant (p=0.02).

Mean UPDRS-III and mean tapping test scores in "on" conditions in T2 were not statistically different from the same scores in T1. On the contrary mean UPDRS-III score in "off" condition was lower in T2 compared to T1 (respectively 30.1±10.7 vs. 36.6±9.8; p<0.05). Tapping test score in "off" condition was higher on T2 compared to T1 (8.7±3.5 vs. 6.3±3.1) and also this difference was statistically significant (p<0.05). Mean duration of "off" periods in T2 was shorter than that in T1 (6.6±3.4 h, p=0.0001).

In our study, the overall tolerability of Apo-TD was good: 6 patients referred sleepiness during treatment, 1 patient presented mild orthostatic hypotension, and 3 patients had transient nausea controlled with domperidone. Only in one case was this side effect not tolerated and Apo-TD had to be discontinued. No hallucinations occurred. Regarding local side effects, 71.4% of patients had transient, mild erythema in the site of Apo-TD application, with complete regression within 48 hours; in 2 patients this erythema lasted more than three days and required local therapy.

Discussion

Our study showed an improvement in mean UPDRS scores in "off" conditions and a reduction of "off" period durations on T2 compared to T1. These findings are somehow expected, as on T1 patients were treated with L-dopa alone, while on T2 Apo-TD was added. Nevertheless, these results are important as Apo-TD demonstrated its efficacy by shortening the duration and disability of "off" periods.

Table 1 Clinical evaluation during L-dopa + Apo-TD treatment (T2) compared to L-dopa therapy (T1) and L-dopa + dopamine agonists therapy (TO). Values are mean (SD)

	T0		T1		T2	
	"On"	"Off"	"On"	"Off"	"On"	"Off"
UPDRS-III score	12.6 (8.9)	32.3 (8.4)	13.3 (5.1)	36.6 (9.8)	11.8 (7.6)	30.1 (10.7)*
Tapping test score	18.3 (12.6)	7.7 (3.8)	17.4 (13.5)	6.3 (3.1)	20.1 (12.9)	8.7 (3.5)*

*$p<0.05$ vs. T1 in "off" conditions

Analysing T2 versus T0 clinical evaluation, Apo-TD demonstrated important advantages compared to standard oral dopamine-agonist treatments. Similarly to what was seen on T1, mean UPDRS-III scores and tapping test scores in "on" conditions obtained on T2 did not show significant differences from those obtained on T0. On the contrary, in "off" conditions motor performance evaluated by UPDRS-III scores and tapping test score improved on T2 compared to T0, even if a significant difference was not achieved. Remarkable is the fact that mean duration of "off" periods was shorter on T2 compared to T0; this difference was statistically significant. This means that Apo-TD, compared to other add-on oral dopamine-agonists, has a longer action and is able to further prolong "on" periods when added to L-dopa therapy. In conclusion, our study shows that Apo-TD may be effective, particularly in reducing "off" period duration and disability rather than improving motor performances in "on" conditions. This fact makes Apo-TD a promising treatment for uncontrolled wearing-off and prolonged "off" phases in parkinsonian patients. Nevertheless, further studies are needed to verify the efficacy and tolerability of Apo-TD in prolonged treatments.

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Movement-related modulation of neural activity in human basal ganglia and its L-DOPA dependency: recordings from deep brain stimulation electrodes in patients with Parkinson's disease

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Abstract Through electrodes implanted for deep brain stimulation in three patients (5 sides) with Parkinson's disease, we recorded the electrical activity from the human basal ganglia before, during and after voluntary contralateral finger movements, before and after L-DOPA. We analysed the movement-related spectral changes in the electroencephalographic signal from the subthalamic nucleus (STN) and from the internal globus pallidus (GPi). Before, during and after voluntary movements, signals arising from the human basal ganglia contained two main frequencies: a high β (around 26 Hz), and a low β (around 18 Hz). The high β (around 26 Hz) power decreased in the STN and GPi, whereas the low β (around 18 Hz) power decrease was consistently found only in the GPi. Both frequencies changed their power with a specific temporal modulation related to the different movement phases. L-DOPA specifically and selectively influenced the spectral power changes in these two signal bands.

This is a preliminary report of a study aimed to test the role of the subthalamic nucleus (STN) and the globus pallidus internus (GPi) in human motor control by exploring their movement-related spectral changes on electroencephalography (EEG) recorded through deep brain stimulation (DBS) electrodes and their dopaminergic dependence in free-moving patients with Parkinson's disease (PD).

After local ethical committee approval, the study was conducted in 3 patients (5 sides) with idiopathic PD undergoing stereotactic procedures for bilateral implantation of electrodes (3389 quadripolar lead, Medtronic, Minneapolis, USA) for chronic DBS in the STN. One patient had also bilateral implantation in the GPi. The precise electrode placement was verified postoperatively by magnetic resonance imaging (MRI). The methods have been reported elsewhere [1].

The task involved the repetitive execution of brisk voluntary second finger extensions. The inter-trial interval was >8 s. The mean number of non-corrupted trials per side was 115±30 (mean±SD). For each side we recorded the surface electromyographyc signal using Ag/AgCl 9-mm diameter electrodes over the extensor indicis muscle as a trigger signal; EEG signals were recorded from the contralateral STN electrodes. In patient 1, the EEG signal was also recorded from the contralateral GPi nuclei.

The variables extrapolated were the spectral power and central frequency values during the trial (in the time window between -4 s and +4 s with respect to the EMG onset) in two EEG rhythms of interest: low β (mean central frequency 18 Hz) and high β (mean central frequency 26 Hz). The spectral power was expressed as a percent (%) increase or decrease in instant power with respect to the mean power measured at rest (4 s to 2.4 s before EMG onset). The same procedure for recording and estimating variables was repeated 30–40 minutes after patients received a 100-mg dose of a fast-acting L-DOPA preparation (Madopar Dispersibile, Roche) (number of non-corrupted trials per side, 99±7).

Before and after L-dopa administration the three movement phases, preparation, execution and recovery (after the movement ended) elicited specific neural activity patterns in the ST and GPi (Table 1).

The changes in the basal ganglia neural activity started during *movement preparation* (about 0.5 s before EMG onset), and reached their minimum or maximum values during *movement execution*. Power in the high β rhythm decreased (STN, 5 nuclei, min value, -66.4%±22.4%; GPi, 2 nuclei, min values, -83.5%, -91.6%). At the same time, whereas power in the low β rhythm decreased also in the GPi (min values, -66.6%, -69,9%), it varied widely in the STN. In patient 1, it decreased but only slightly (2 nuclei, min values -31.0%, -20.7%), in patient 2 (the most bradykinetic) it increased markedly (1 nucleus, max value +288.1%), and in patient 3 it decreased markedly (2 nuclei, min values, -82.2%, -64.8%).

During *movement recovery*, the power spectrum of the neural activity returned to values at rest. In addition, the high β rhythm in the STN and the low β rhythm in the GPi both showed a post-movement power increase (5 STN nuclei, max value, +100.9%±70.4%; 2 GPi nuclei, max values, +88.1%, +172.9%) about 1–2 s after movement onset.

L-DOPA, though inducing some dyskinesias, clinically improved all the patients and also led to changes in the movement related neural activity of the basal ganglia.

During movement preparation and execution, L-DOPA slightly, though not significantly, reduced the high β power decrease in the STN (5 nuclei, min value before L-DOPA, -66.4%±22.4%; after L-DOPA, -57.5%±14.0%) and in the GPi (1 nucleus, min value before L-DOPA, -91.6%; after L-DOPA, -88.6%). Conversely, it had variable effects on the low β rhythm. In patient 1 it reduced the GPi power decrease (1 nucleus, min value before L-DOPA, -69.9%; after L-DOPA, -51.8%) but intensified the power decrease in the STN (1 nucleus, min value before L-DOPA, -20.7%; after L-DOPA, -43.0%). In patient 2, it removed the large power increase in the STN observed before L-DOPA, and substituted it with a more "normal" pattern of power decrease (1 nucleus, before L-dopa, +288.1%; after L-DOPA, -66.9%). In patient 3, it reduced the STN power decrease (2 nuclei, before L-DOPA, -82.2%, -64.8%; after L-DOPA, -21.6%, -39.7%).

A more consistent effect observed was that L-dopa significantly reduced the power rebounds during movement recovery in the high β rhythm in the STN (5 nuclei, max value before L-DOPA, +100.9%±70.4%; after L-DOPA, +49.5%±40.0%; paired t test, $p<0.05$) and in the low β rhythm in the GPi (1 nucleus, max value before L-dopa, 172.9%; after L-dopa, +44.4%).

Table 1 Movement-related modulation of electrical activity of the human basal ganglia before and after the administration of L-dopa. Changes in high and low b power were recorded from electrodes implanted for deep brain stimulation in patients with Parkinson's disease

	STN		GPi	
	Baseline (5 nuclei)	After L-DOPA (5 nuclei)	Baseline (2 nuclei)	After L-DOPA (1 nucleus)
Movement preparation and execution				
High β	Decreased	No change	Decreased	No change
Low β	Increased or decreased	Increased or decreased	Decreased	Increased
Recovery				
High β	Increased	Decreased	Rest power level	No change
Low β	Rest power level	No change	Increased	Decreased

STN, subthalamic nucleus; *GPi*, internal globus pallidus. Note that changes after L-DOPA are relative to baseline

Discussion

Our preliminary observations show that electrodes for DBS can open a unique window on the basal ganglia scenario in humans. The main finding is the high β (around 26 Hz) power decrease in the STN and in the GPi, whereas the low b (around 18 Hz) decreases consistently only in the GPi, but not in the STN. EEG power decreases are interpreted as neural activation [2].

In the classic basal ganglia model [3, 4], the STN excites the GPi. A coherent power decrease in the same frequency range in both the STN and GPi suggests increased output activity in the STN, exciting the GPi and, consequently, inhibiting the thalamocortical circuit. Hence, the high β (26 Hz) power decrease observed in this study might reflect movement-related activity in the indirect pathway with a final inhibitory effect on the thalamocortical circuit.

The second distinctive finding in this study is the relatively specific low β (around 18 Hz) GPi power decrease. This 18-Hz GPi power decrease was not correlated with STN power changes in the same frequency and may have primarily reflected striatal input, which has an inhibitory effect on the GPi. The net result would be to reduce the output inhibitory activity of the GPi, thereby reducing inhibition on the thalamocortical circuit. From this point of view, the 18-Hz GPi power decrease may represent activity on the direct pathway. In the Gpi, L-DOPA reduced the decrease in the low β power, which means a reduction in the activity of a structure with inhibitory action and, hence, a net disinhibition of the final target of the system (i.e. the thalamus). Thus L-DOPA faclitated the action of the direct pathway.

If the STN belongs to the indirect pathway and if the 18 Hz activity reflects the direct pathway, one could wonder why some of this - speculatively - direct pathway-related activity spreads also to the STN. A possibility is that the two pathways are not completely segregated, particularly in Parkinson's disease. There are several possible shunts, but one likely possibility is the pedunculopontine nucleus which receives input from the GPi and sends an output to the STN [4].

Whether these large power increases related to movement are pathological is unclear. STN increases in the low β rhythm during movement preparation and execution may be important in bradykinesia, as the clinical and electrical characteristics in Patient 2 suggest. An increase in both low and high β rhythms during movement recovery suggests impaired recovery of basal ganglia function after movement. Our observation that L-DOPA reduced the post-movement power rebound could explain the drug's clinical benefit on sequential movements that are impaired in Parkinson's disease [5]. Without L-DOPA, the abnormally large increase in post-movement power may engage the circuit thus preventing the system from accomplishing the rapid power decrease required for the next movement. In contrast, after L-DOPA the absence of a large post-movement rebound might render the system more quickly and easily excitable, thereby increasing the rate of sequential movements.

In conclusion, we speculate that the human basal ganglia motor circuit probably comprises various subcircuits, tuned onto different frequencies. These subcircuits extend to the anatomical structures classically thought to belong to the direct or indirect pathways: a subsystem operating in the high β range (around 26 Hz) predominantly distributed in structures of the indirect pathway, and a different subsystem operating in the low β range (around 18 Hz) predominantly distributed in the GPi, a structure of the direct pathway. Large power increases during movement preparation and execution or during recovery seem to reflect a pathological instability of the basal ganglia circuit, especially related to a bradykinetic clinical condition. L-DOPA removes the abnormal power increases, probably by stabilizing the basal ganglia circuit and reducing the abnormal movement-related synchronization.

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High-frequency electrical stimulation of the subthalamic nucleus in Parkinson's disease: kinetic and kinematic gait analysis

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Abstract In the advanced phase of Parkinson's disease (PD), gait disturbances represent one of the main causes of disability. Several studies demonstrated that high-frequency electrical stimulation (HFS) of the subthalamic nucleus (STN) significantly improves the motor symptoms of PD. This study was finalised to quantitatively analyze the effect of STN HFS on gait of PD patients, through a three-dimensional gait analysis system. Ten PD patients were studied, with and without STN HFS. The results demonstrated that STN HFS significantly improves all the main gait parameters in PD patients.

Gait disorders play an important role in the determination of the disability in patients with Parkinson's disease (PD). The main characteristics of PD gait are slowness and shuffling, with a shortening of the stride length and a reduction of velocity despite the maintenance of a normal cadence. The range of motion at the joint level is tipically reduced, with a loss of the synergistic arm movements [1, 2]. Moreover, a severe incapability to start walking and sudden freezing are characteristics of the advanced phase of PD. These symptoms are due to an alteration of basal ganglia function relative to the loss of dopaminergic neurons in the substantia nigra pars compacta. In the advanced phase of PD, it is often impossible to improve motor symptoms despite the best pharmacological treatment. Therefore, the surgical approach in these advanced phases has received increasing interest. Bilateral high-frequency stimulation (HFS) of the globus pallidus internus or subthalmic nucleus (STN) significantly improves PD symptoms through a functional, reversible inhibition of the hyperactive targets [3–5]. Nevertheless, a few studies have focused on the effects of HFS on PD patients' gait. In this study, we analysed the effects of bilateral HFS of the STN on PD gait, using three-dimensional (3D) gait analysis techniques.

We studied 9 patients (5 woman, 4 men) with idiopathic PD and 9 age- and sex-matched controls. The mean age of the PD patients was 59.3 years, the mean disease duration was 16.6 years (SD 5.7) and the mean Hoehn and Yahr off-stage score was 3.6 (SD 0.7). All patients were bilaterally implanted for HFS of the STN at least three months before the study, as previously described [5]. All patients and controls gave written informed consent to the study and the protocol was approved by the ethics committee. All the patients were studied in two conditions, without stimulation and without medication (Stim-off/Med-off) and with bilateral stimulation and without medication (Stim-on/Med-off), in order to evaluate the effects of the HFS alone on gait. All antiparkinsonian drugs were stopped at least 12 hours before the study. The stimulation parameters used during the test were the same used for chronic stimulation (monopolar cathodic stimulation; mean amplitude 3.1 volts (SD, 0.4); mean rate 144.4 Hz (SD 18.0); pulse width 60 or 90 μs).

Three-dimensional kinematic and kinetic gait analyses were performed as previously described [6, 7] using an optoelectronic system (ELITE System, BTS, Corsico, Italy) [8] to measure the 3D coordinates of retroreflective markers and a dynamometric force platform (Kistler, Winterthur, Switzerland) for ground reaction force (GRF) detection.

Four video cameras, with a sampling rate of 50 Hz, were placed posterolaterally of the subject. The working volume (2x3x1 m^3) was calibrated by means of a precision grid. The acquired markers' coordinates were digitally low-pass filtered to estimate the optimal cut-off frequency and minimize the residual noise (resultant cut-off frequency within the range of 3–7 Hz). Nineteen markers (10 mm diameter) were glued on the subjects' bony landmarks: the fifth metatarsal heads, the lateral malleoli, the lateral femoral condyles, the posterior superior iliac spines, the acromions, the lateral humeral condyles, the ulnar prominences, the base of the occipital bone, the sacrum, the seventh thoracic vertebra and the seventh cervical vertebra. The force platform (60x40 cm^2) was embedded in the floor within the calibrated volume and in the middle of a 10-m pathway. Ground reaction forces were sampled at 50 Hz.

Marker coordinates and ground reactions were processed using specific analysis programs [7] to compute spatio-temporal gait parameters, joint and body segment kinematics, joint moment and powers, and body baricenter kinematics.

For the analysis of stride length, step length and mean gait velocity were expressed as the percentage of body height (%h and %h/s respectively) and the percentage of height per second (% /s), respectively. Ground reaction components, joint moments and joint powers were normalised by dividing by body weight (N/kg, Nm/kg and W/kg, respectively). For the analysis we considered the following parameters: the spatiotemporal gait parameters (stride length, cadence, mean gait velocity, percentage of the stance phase on the stride duration); the range of amplitude during the gait cycle of the hip, knee and ankle joint angles, of the pelvis orientation in the frontal plane, of trunk lateral flexion and trunk torsion; the maximal values within the gait cycle of the hip and ankle

joint moments and powers; the mean forward inclination of the trunk in the sagittal plane. All variables were averaged among the two sides. Two-tailed Student's *t*-test for paired measures was applied to examine differences between the two conditions (Stim-off/Med-off and Stim-on/Med-off). Unpaired measures tests were used for the comparisons between patients and controls. The minimum 0.05 level of significance was adopted.

The mean gait velocity of patients in Stim-off/Med-off was significantly reduced with respect to that of controls (34.5±14.6 %h/s vs. 69.1±10.3 %h/s), mainly for the reduction of the stride length (41.5%±11.9% h vs. 77.0%±6.9% h) with little reduction of cadence (96.4±18.3 steps/min vs. 107.3±7.7 steps/min) and little increase in stance phase (65.4%±4.8% vs. 59.4%±1.8%). In the Stim-on/Med-off condition, there was a significant increase of both the mean gait velocity and the mean stride length (52.5±16.0% h/s and 59.9±10.6 %h/s, respectively), while the cadence increase (103.1±16.1 steps/min) and the stance phase reduction (61.7%± 3.8%) were not significant.

All subjects walked in the Stim-on/Med-off condition with a larger range of motion (ROM) of all lower limb joints with respect to the Stim-off/Med-off condition (hip angle ROM, 39.1°±7.6° vs. 29.3°±8.2°; knee angle ROM, 47.0°±7.7° vs. 37.0°±10.3°; ankle angle ROM, 18.8°±3.9° vs. 13.7°±3.3). In the Stim-on/Med-off condition, the range of pelvic obliquity increased with respect to the Stim-off/Med-off condition (7.6°±3.6° vs. 5.9°±2.3°). Also the trunk lateral flexion and torsion increased (8.0°±3.2° vs. 5.9°±1.5° and 8.5°±4.5° vs. 5.0°±2.3°, respectively), while the forward inclination of the trunk significantly decreased towards controls values (11.2°±5.1° vs. 15.1°±8.1°).

For the kinetic variables, the peak values of moments and powers at hip and ankle joints increased in all patients when the Stim-on/Med-off was on compared to the Stim-off/Med-off (hip moment peak: 0.955 Nm/kg ± 0.338 vs. 0.846 Nm/kg ± 0.269; ankle moment peak: 1.135 Nm/kg ± 0.262 vs. 1.082 Nm/kg ± 0.220; hip power peak: 1.570 W/kg ± 0.843 vs. 0.893 W/kg ± 0.487; ankle power peak: 1.241 W/kg ± 0.567 vs. 0.710 W/kg ± 0.408).

Discussion

In this study, we analysed the effects of HFS of the STN on PD patients' gait using a 3D system. The data collected in the Stim-off/Med-off condition confirmed, as observed in other studies, that PD gait is slow and with short steps, while the cadence and the duration of the stride sub-phases are quite similar to controls; these findings validate the adopted protocol of analysis.

The main effect of the STN HFS is the large increase in stride length, which is mostly responsible for the increase in gait velocity, while the increase in cadence and the reduction of stance phase duration were not significant. In the Stim-on/Med-off condition, the angular excursion at lower limb joints, reduced in PD patients, was increased, with a larger variation at hip level; also the abnormal forward inclination of the trunk and the reduced range of amplitude of trunk torsion and lateral flexion, typical of PD patients were improved.

These changes observed in the Stim-on/Med-off condition suggest that STN HFS is capable of significantly improving PD gait, leading toward a more physiological mechanism of walking speed modulation. More studies, with a comparison of the effects of stimulation and levodopa, are needed to better investigate the role of basal ganglia in gait mechanisms.

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Cognitive and psychiatric characterization of patients with Huntington's disease and their at-risk relatives

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Abstract We examined cognitive and psychiatric disturbances in patients with Huntington's disease (HD) in comparison to at risk asymptomatic subjects. Cognitive and psychiatric scales and an HD motor scale were administered to 40 HD patients, 17 pre-symptomatic HD gene carriers (AR^+) and 28 non gene carriers (AR^-). HD patients did worse than AR^+ and AR^- in all motor, cognitive and psychiatric measures, while AR^+ and AR^- subjects did not differ between each other. HD patients had high scores for negative psychiatric symptoms, but there was no correlation between illness duration and psychiatric or cognitive performance. In HD, disease course and symptomatology are heterogeneous and negative psychiatric symptoms are common.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterised by motor, cognitive and psychiatric disturbances. Motor and cognitive impairment have been fairly extensively investigated, while behavioural and psychiatric aspects have been neglected. However, the latter have a major impact on the lives of patients and their relatives, and render disease management problematic. Psychiatric disturbances are particularly frequent (40% to 70%) at all disease stages [1]. Some studies indicate that psychiatric disturbances are often a prelude to the onset of chorea and cognitive decline [2]. However, this finding was not confirmed on pre-symptomatic gene mutation carriers, who as a group, did not have psychiatric or cognitive impairment [3].

The aim of this study was to examine cognitive and psychiatric disturbances in HD patients in comparison to a group of asymptomatic offspring of HD patients who were therefore at risk (AR) for the disease. We also sought correlations between motor, cognitive and behavioural alterations in patients with HD.

Patients and methods

We studied 85 adults (39 men) from 35 HD families: 40 HD patients (24 men) diagnosed from family history, chorea, or characteristic impairment of voluntary movement on the Quantified Neurological Scale (QNE) for HD, and confirmed by the genetic test for HD; 17 asymptomatic at risk subjects (7 men) who tested positive for the pathological HD gene (AR^+); and 28 asymptomatic at risk subjects (8 men) who tested negative (AR^-).

The neuropsychological tests administered were the Mini Mental State Examination (MMSE) to assess global cognitive function; the Raven Progressive Matrices 1947 (PM47) to investigate deductive reasoning and visuo-perceptive ability; the Short Tale Test to assess long term verbal memory; the Visual Search Test which examines focused attention; the Benton Visual Orientation Line Test to assess orientation and spatial perception; the Verbal Fluency Test (phonemic) to assess strategic word searching and the Nelson simplified form of the Wisconsin Card Sorting Test to assess categorization and set shifting abilities. All except the Nelson Test – for which a correction is not available – were corrected for age and education.

The psychiatric scales were: the Brief Psychiatric Rating Scale (BPRS) a screening test for psychiatric disturbances; the Hamilton Anxiety Scale (HAM-A) which evaluates anxiety, the Hamilton Psychiatric Rating Scale for Depression (HAM-D) whose score is a combination of subjective depressive feelings and the examiner's judgement, the Scale for the Assessment of Positive Symptoms (SAPS) which quantifies positive schizophrenic symptoms (hallucinations, delusions, bizarre behaviour and disturbances of thought), the Scale for the Assessment of Negative Symptoms (SANS) which quantifies negative schizophrenic symptoms (emotional flatness, abulia, apathy, retiring social behaviour, impaired attention, etc).

The genetic test, performed in all subjects, determines the number of CAG repeats (pathological value >36) on the IT 15 gene of chromosome 4. The statistical tests used were one-way ANOVA with post-hoc Scheffè comparison as appropriate, and Pearson's correlation test.

Results

Patients with chorea were significantly older than the AR^+ and AR^- subjects; mean duration of education was higher in the AR^+ group. CAG expansion length did not differ between patients and AR^+ subjects (Table 1).

As expected, patients did significantly worse than AR^+ and AR^- in all cognitive tests ($p<0.0001$). There was no significant difference in cognitive test scores between AR^+ and AR^- subjects, although AR^+ subjects' scores were consistently lower than those of AR^- subjects. Thirteen HD patients had a MMSE score below 24, indicating dementia, while none of the AR^+ or AR^- subjects was demented.

Similarly, psychiatric scores were significantly worse in HD patients than AR^+ and AR^- subjects. We considered a score of ≥17 as indicating definite depressive disturbance on the Hamilton depression scale; by this criterion nine HD patients and one AR^+ subject had depression while none of the AR^- subjects was depressed. However, AR^+ subjects had higher (but not significantly so) depression and anxiety scores than AR^- (Table 1).

Correlation analysis of severity of illness parameters (illness duration, QNE), psychiatric scales and cognitive tests in

Table 1 Demographic data and psychiatric scores in HD patients, at-risk carriers (AR^+) and at-risk non carriers (AR^-)

	HD (n=40)	AR^+ (n=17)	AR^- (n=28)	*p*
Men, n (%)	24 (60)	7 (14)	8 (29)	–
Age, years	43.0 (11.7)	30.2 (7.2)	31.6 (12.3)	<0.0001*†
Education, years	9.3 (3.7)	12.7 (2.8)	10.7 (3.2)	<0.005‡
Illness duration, months	43.8 (24.2)	NA NA	–	
QNE score	31.7 (13.7)	0.9 (2.0)	0.5 (1.1)	<0.0001*†
CAG repeats, n	47.8 (5.0)	45.9 (4.6)	20.4 (4.3)	<0.0001*†
Psychiatric scale scores				
BPRS	31.0 (11.0)	24.1 (4.4)	20.6 (5.0)	<0.0001*†
HAM-D	11.4 (8.5)	5.8 (6.0)	1.7 (3.4)	<0.0001
HAM-D	9.6 (1.0)	6.2 (5.9)	3.3 (4.7)	0.0019
SANS	26.4 (25.5)	2.1 (5.6)	0.1 (0.4)	<0.0001
SAPS	3.1 (10.0)	0.3 (1.0)	0 (0)	>0.05

*HD vs. AR^+; †HD vs. AR^-; ‡ AR+ vs. AR^-
NA, not applicable
Numbers in brackets are standard deviations unless otherwise indicated

the HD group showed that QNE correlated with illness duration (0.58, p<0.001), that psychiatric scale scores correlated among each other, and that the cognitive tests MMSE, Benton Test, Raven Test and Short Tale Test also inter-correlated. Severity of illness did not correlate with psychiatric or cognitive status. Length of CAG expansion did not correlate with cognitive performance, psychiatric scales or illness severity, but correlated inversely with age at disease onset (-0.75, p<0.001). In general the psychiatric scales did not correlate with cognitive tests except the Visual Search score that correlated significantly with depression score (-0.61, p<0.001) and negative schizophrenia symptoms score (-0.49, p<0.01). There were no correlations between negative and positive schizophrenia symptoms.

Discussion

In AR^+ subjects we found no cognitive or psychiatric alterations suggesting that disease onset might be occurring before the development of motor impairment. The most interesting findings concern HD patients. We found that psychiatric symptoms are prominent in HD, and combine with the usual motor and cognitive compromise to contribute to overall disability. The most frequent psychiatric problems were depression, anxiety and apathy, while positive symptoms such as delusions, hallucinations and mania were less frequent. The lack of correlation between illness severity, as evaluated by illness duration and motor compromise, and cognitive and psychiatric impairments indicates that disease course is not uniform in HD. In addition, and as reported previously [4-5], we found that the severity of the cognitive and behavioural disturbances were not related to GAG length.

It is noteworthy that apathy was frequent in our HD patients, and that negative psychiatric symptoms (including apathy) correlated with depression and lack of attention. These findings suggest that apathy may arise from a combination of cognitive, affective and motivational factors [6]. In HD the circuits joining the striatal structures to the fronto-orbital, dorso-lateral and cingulate cortices – concerned with cognitive, affective and motivational aspects – are impaired [7]. The apathy may be the expression of an underlying affective disorder, or of an initial cognitive impairment starting as an attentional defect. Alternatively the attention deficit may be a consequence of the apathy. It is also possible that the nature of the apathetic disorder may vary with disease stage. Since our HD patients were all at an intermediate disease stage, it is necessary to investigate cognitive, psychiatric and behavioural modifications in HD patients with early and advanced illness in order to provide indications as to source of apathy in HD.

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CAG mutation effect on rate of progression in Huntington's disease

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Abstract Huntington's disease (HD) is progressively invalidating and caused by a CAG expanded mutation. We tested the effect of the mutation length on the rate of progression in a cohort of 80 patients clinically followed-up and genetically characterized. Two patients presenting an infantile and aggressive HD form starting under 10 years had over 90 repeats; the other patients did not show any influence of the CAG expanded number on the rate of progression. In conclusion, the CAG expanded repeat affects the disease progression only at a very upper pathological range and in rare cases initiating very early in the life, while it does not seem to affect in any way the severity of the phenotype in most HD patients. Other factors affecting the motor symptom progression, other than the expanded repeats, therefore have to be investigated.

Huntington disease's (HD), dominantly transmitted and caused by a CAG expanded mutation beyond 36 repeats [1], is highly and progressively invalidating. The severity of the symptom progression is variable among patients, with juvenile (onset <20 years) [2] and homozygous subjects (F. Squitieri, unpublished results) showing the most accelerated course towards high disability. However, although the age at onset is affected by the CAG mutation length [3] (the low expanded number is associated with a variable penetrance of the mutation [4]), no genetic factors have so far been described as possible modifiers of the disease severity [5]. In this study we analyzed the possible contribution of the expanded CAG repeat length on symptom progression in a cohort of 80 patients of identical ethnic origin, whose genetic and clinical data of age at onset and follow-up were available from the Italian HD Databank [5].

All 80 patients (37 males and 43 females) were seen at the Neurogenetics Unit of the IRCCS Neuromed of Pozzilli (IS), Italy and clinically analyzed on the Unified Huntington Disease Rating Scale (UHDRS), which includes motor, behavioral, cognitive and independence assessments. The rate of disability was assessed with the Total Functional Capacity (TFC) [6] and by the disability scale (DS) [7] and calculated as loss of units per year (TFC, mean 0.6±0.5, range 0.2–3.2; DS, mean 3.4±2.8, range 0.8–13.7). The onset of disease was defined as the time when clinical motor manifestations (i.e. choreic movements) first became noticeable (mean 41.3±14.0 years, range 3–73). To investigate the size of the CAG repeat expanded mutation, a molecular genetic test on patients' DNA was performed, after informed consent, at Neuromed by published techniques [8] (mean 46.3±10.5 CAG repeats, range 39-100). For statistical analysis, a nonparametric test (Mann-Whitney U) and a linear regression approach were used (significance at $p<0.05$). Among these 80 patients, two had an infantile form (starting at an age less than 10 years) with age at onset at three and seven years, 100 and 95 repeats, respectively, and a particularly devastating progression towards a high loss of independence (mean loss of units per year was 2.7±0.7 (range 2.2-3.2) at TFC and 12.7±1.4 (range 11.7–13.7) at DS). The mean loss-of-unit values per year obtained from these two little patients was significantly different from the cohort of juvenile (onset under 20 years, n=4, mean 0.7±0.7 units per year at TFC, $p=0.016$ and 4.7±3.9 at the DS; $p=0.017$) and adult subjects (0.6±0.3 units per year at TFC, $p=0.008$ and 3.0±2.3 at DS, $p=0.009$). In order to examine a possible influence of the mutation length on the HD symptom progression, we plotted the expanded CAG repeat number with the mean loss of units per year at TFC and DS and found a significant correlation (CAG/TFC, $p=0.02$; CAG/DS, $p=0.01$) if the two patients were included in the cohort of all 80 subjects (Fig. 1, panels A and B). Conversely, there was no significant correlation after excluding the two patients with the infantile form (Fig. 1, $p>0.05$, panels C and D).

Discussion

We found an influence of the mutation length on HD severity only when the mutation was much expanded, the CAG repeat number at the upper edge of the pathological range so far described [1]. In these rare cases, the increased toxic effect of the mutation likely contributes to the devastating severity of the phenotype. This was not the case of the adult affected population whose accurate clinical follow-up consented to obtain data of symptom progression from our databank. The juvenile patients (onset between 11 and 20 years), whose assessment of HD progression was available, were only four and did not differ substantially from the adults. After the discovery of the gene mutation, only few reports studied the influence of the very expanded CAG repeat number on HD severity [10]. This was likely due to the difficulty of studying large affected HD cohorts of patients both accurately followed-up and genetically characterized. Our study confirms the effect of large CAG expansions on the rate of HD progression [10]. Differently from Illarioshkin *et al*. [10], we did not find any significant correlation after excluding from statistical analysis patients carrying the most expanded mutations (Fig. 1). It likely depended on either the different genetic background of the patient cohorts analyzed and on the diverse clinical methodology used to calculate the rate of progression. We have recently reported the occurrence of a worse HD prognosis in case of at onset atypical

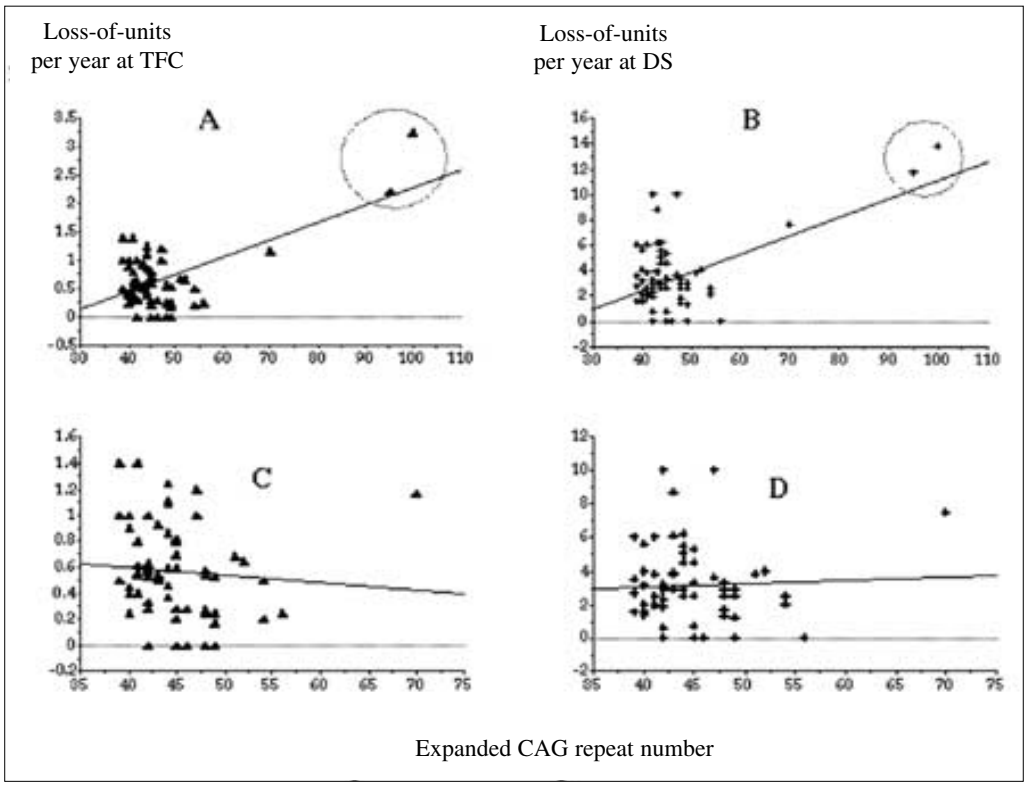

Fig. 1a-d. Linear regression analysis between expanded CAG repeat number (x-axis) and loss of units per year (y-axis) at the TFC and DS. **a,b** Analysis including the two patients with infantile form. **c,d** Excluding the 2 patients with infantile Huntington's disease. The infantile patients are highlighted by a circle in **a** and **b**

movement disorder [9]. In that study, we found mean expanded repeats longer in the juvenile rigid patients than in the juveniles initiating with chorea, predicting therefore the occurrence of an influence of large expansions on HD phenotype. We confirm such hypothesis as both patients affected with infantile form here described presented at onset movement disorder other than chorea, characterized by rigidity in one and dystonia and limb ataxia in the other. In these cases, as in the rigid juvenile patients, the particularly expanded and toxic mutation leads to a highly invalidating phenotype. Conversely, in most patients the CAG repeat contributes to the age at onset for about 50%–60%, the remaining percentage is influenced by other genetic factors probably of familial origin [3], but not to the rate of disease progression. Other factors affecting the severity of the phenotype have to be studied on cohorts of patients well characterized clinically and genetically. The discovery of factors influencing the symptom progression and, possibly predicting the HD prognosis, will offer new opportunities in the therapeutic strategies for this devastating disease.

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Anatomo-clinical correlation of intraoperative stimulation-induced side-effects during HF-DBS of the subthalamic nucleus

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Abstract The efficacy of deep brain stimulation of the subthalamic nucleus (STN) is dependent on the accuracy of targeting. In order to reduce the number of passes and, consequently, the duration of surgery and risk of bleeding, we have set up a new method based on direct magnetic resonance imaging (MRI) localisation of the STN. This procedure allows a short duration of the neurophysiological session (one or two initial tracks). Whenever a supplementary track is needed, the stimulation-induced side effects are analysed to choose from one of the remaining holes in Ben's gun. A good knowledge of anatomical structures surrounding the STN is mandatory to relate side effects to the actual position of the track. In our series of 11 patients (22 sides, 37 tracks), the most common and reproducible side effects were those characterised by motor, sensorial, oculomotor and vegetative signs and symptoms. Moreover, the therapeutic window (distance between the current intensity needed to obtain the best clinical effect and the intensity capable to induce side effects) predicted clinical efficacy in the long-term, and contributed to the choice of which among the examined tracks had to be implanted with the chronic macroelectrode.

The efficacy of high frequency deep brain stimulation (HF-DBS) of the subthalamic nucleus (STN) for the treatment of advanced Parkinson's disease (PD) is strictly dependent on the precision of reaching the target. This concept was well clear to the first authors when they proposed a multitrack approach to determine target location in DBS [1]. Afterwards, several authors have replicated their good clinical results. Various different strategies have been developed to reach the target, generally with a multiple simultaneous or independent trajectories approach to cover with both recording and stimulation a wider area. Recently a review on STN-implanted patients suggested that the higher the number of microelectrode passes, the higher the risk of intracranial bleeding during DBS maneuvers [2].

In order to improve the risk-benefit ratio, we proposed a novel technique guided by magnetic resonance imaging (MRI) and based on multiple sequential image fusion (MuSIF), which allows a direct localisation of the subthalamic nucleus [3]. In our experience, the high targeting precision reached by this technique made it possible to reduce the number of intraoperative tracks. We present our DBS method and discuss how the analysis of stimulation-induced side effects evoked during the initial neurophysiological session (one or two tracks) can help address a supplementary track, if needed, and limit the total number of passes.

Twelve consecutive PD patients were selected for bilateral STN implantation of electrodes. The operative session was started after a navigation session in which the stereotactic coordinates were obtained by means of the fusion of stereotactic computed tomography (CT) and frameless MRI data. Through a burr hole located 2 cm anteriorly to the coronal suture, one microelectrode inserted into the central hole of the Ben's gun was advanced with a micrometric drive towards the target (patients 1–7). For patients 8–12, two parallel microelectrodes were simultaneously inserted into the brain. The Ben's gun has 5 holes 2.0 mm apart from each other and arranged in a cross-fashion. Recording and then semi-microstimulation were performed at several levels with a coaxial electrode starting 6 mm before and ending 6 mm after the theoretical target. The results were collected and then discussed during a brain storm session. If the required criteria were satisfied, i.e. recording and stimulation data met our standard, the chronic macroelectrode was implanted; otherwise a new neurophysiological session was started with a supplementary track.

Position of chronic electrodes was regularly checked a few days after stimulators implantation, by means of a postoperative MR image, which was eventually compared with the preoperative MR image. The electrode was visualised as a 1.5-mm diameter artefact which is an acceptable size to reliably indicate the electrode's actual position. We have arbitrarily subdivided the STN into nine regions: 1 central and 8 peripheral (Fig. 1). All the electrodes were classified as central (C), anteromedial (AM), anterior (A), anterolateral (AL), lateral (L), posterolateral (PL), posterior (P), posteromedial (PM), or medial (M), according to the position of the artefact.

We analysed the side effects evoked by intraoperative semi-microstimulation at 5 levels (-2, -1, 0, +1, +2; level 0 was the target), thereby covering a 4-mm area along the trajectory of each track.

The therapeutic window for each side effect was considered, i.e. the ratio between the current intensity needed for eliciting the side effect and the current intensity required at the same level to obtain the maximum clinical effect.

At the 3-month follow-up, all patients benefited from chronic stimulation as shown by improvement of the UPDRS III score in med-off conditions and reduction in off-time and dyskinesia scores and levodopa equivalent daily dose (LEDD). The first patient did not undergo postoperative MRI and was not enrolled in this study. In the remaining consecutive 11 patients (22 sides), we used 37 passes with a mean of 1.7 per side. No intracranial bleeding occurred during the procedures.

In the 12 patients (24 sides), the central track was accepted for definitive implantation in 20 sides (83.3%). In 6 sides,

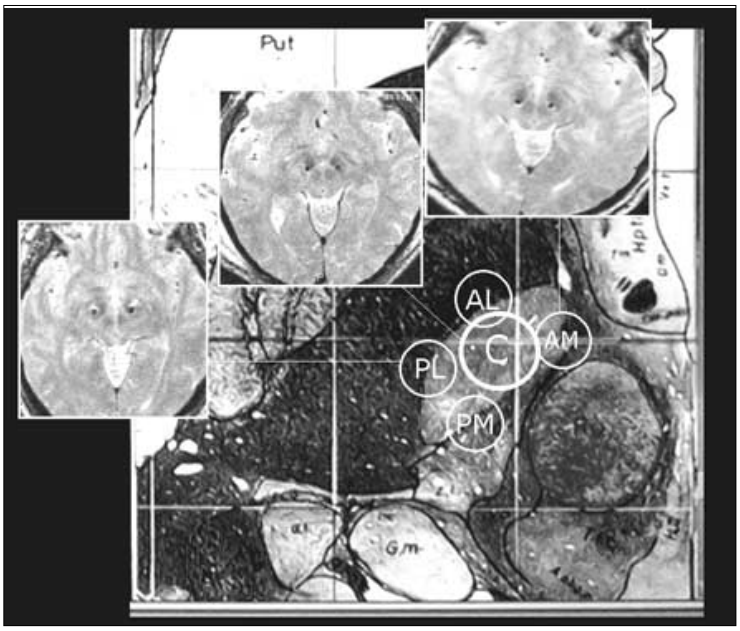

Fig. 1 The position of the definitive electrocatheter as visualised on MRI is then reported onto the Schaltenbrandt Atlas axial view of the subthalamic area. Each circle corresponds to a discrete part of this area

in which clinical and neurophysiological results were mildly consistent with our criteria, a supplementary trajectory was explored and eventually chosen for definitive implantation in 2 sides. The side effects evoked during stimulation addressed the choice of which of the available holes in the gun had to be used and consequently the orientation of the additional track. We never needed a fourth pass.

The most common side effects elicited during the 37 tracks stimulation were pyramidal, sensorial, oculomotor and vegetative. Unspecific side effects, like chest constriction, malaise and dizziness, were hardly related to specific structures, so they were not useful to locate the position of the track.

Motor contractions in the contralateral hemibody were due to stimulation of the corticobulbar and corticospinal tracts. They were frequent (30 tracks, 81%), particularly in C and AL regions, but not in M and PM regions. They were time-locked to stimulation and of paramount importance for the definition of the therapeutic window. We presumed that horizontal gaze deviation can be considered to be a motor side effect deriving from stimulation of the corticofugal pathways which traverse the anterior limb of the internal capsule. Moreover we considered both reduced ipsilateral gaze and contralateral gaze deviations to be part of the same phenomenon, since they were evoked at the same level and since the latter comes out as the current intensity is increased.

Sensorial side effects were perceived by the patient as paraesthesic sensations and were due to stimulation of the medial lemniscus fibres. They were fairly common (12 tracks, 32.5%; persistent in 6 of 12) and more frequent in M and PM regions and at lower levels.

Oculomotor side effects were quite uncommon (9 tracks, 24%), exclusive of C, M and AM regions. They were due to stimulation of the third nerve (adduction or reduced abduction, elevation of superior eyelid in ipsilateral eye) or of the rostral interstitial nucleus (oblique, i.e. lateral and upward, gaze deviation).

Vegetative side effects (nausea, heat sensation, sweating, bradycardia, observed in 15 tracks, 40.5%) were frequent in the anteromedial area and less common in C region. They were not reproducible in the long-term stimulation.

Discussion

In order to reduce the risk of bleeding, we used only one or two trajectories during the initial neurophysiological session of STN surgery. Stimulation-induced side effects are a reliable and reproducible tool for determining the actual localisation of the trajectories. Therefore, if a supplementary trajectory is needed, the analysis of side effects can help in deciding which one among the available holes in the Ben's gun has to be used. Moreover, the ratio of side effects to clinical effects (therapeutic window) is important in deciding where to implant the definitive electrode. It is a marker of the distance between the tip of the electrode and the structure which is responsible for that side effect. The lower is the value of the therapeutic window, the nearer is the tip of the electrode to structures other than STN. The amplitude of the therapeutic window predicts the safety of increasing current intensity in the long term.

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Deep brain stimulation of the subthalamic nucleus in Parkinson's disease: long-term follow-up

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Abstract Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been shown to be an effective therapy for the treatment of advanced Parkinson's disease (PD). Forty-seven patients were bilaterally implanted for STN DBS and clinically evaluated according to the Core Assessment Program for Intracerebral Transplantations before surgery and 3, 12 and 24 months after surgery. Electrical stimulation led to a significant improvement in motor symptoms and in the quality of life, allowing a significant reduction of dopaminergic drugs with a consequent improvement of drug-induced dyskinesias. Statistical differences were observed between UPDRS parts II, III and IV values and daily levodopa dosage in the pre- and postoperative periods, while no differences were evident between the 3 postoperative conditions.

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is effective for the control of the main motor symptoms and of drug-related dyskinesias in advanced Parkinson's disease (PD) [1–4]. The aim of this study was to evaluate the effectiveness of high frequency electrical stimulation in 47 patients (18 women and 29 men) affected by idiopathic PD, who underwent surgery for the implantation of a bilateral stimulating system of STN.

The mean age of the patients was 62.8 years, the mean duration of the disease was 15.6 years, the average duration of levodopa therapy was 14.5 years, and the mean score on the Hoehn and Yahr scale in off phase was 4. A clinical evaluation of the stimulation effectiveness was performed in 39 patients at the 3-month follow-up, in 21 patients at 1 year and in 7 patients at 2 years after surgery according to the Core Assessment Program for Intracerebral Transplantations (CAPIT) [5, 6]. The patients were evaluated in the preoperative period in the off phase (med off) after a 12-hour withdrawal of all antiparkinsonian drugs and after the administration of a supramaximal dose of levodopa (150–400 mg) (med on). After surgery the clinical assessment was performed in four different conditions: stim on-med off, stim off-med off, stim off-med on (with the same dose of levodopa) and stim on-med on. The statistical analysis was performed using the ANOVA test, with a level of significance of p=0.05.

The analysis of the stim on-med off condition showed that STN DBS was responsible for a 58% improvement in the UPDRS part II score (activities of daily life) at 3-month follow-up, 57% at 1 year and 55% at 2 years with respect to the preoperative med off condition. The differences between the preoperative UPDRS part II scores and the corresponding postoperative values were significant (p<0.05); no differences were observed between the 3 postoperative stim on-med off conditions.

As far as the UPDRS part III (motor score) was concerned, a 56.4% improvement at 3 months, 58.2% at 1 year and 63.4% at 2 years were observed. Also in this case a statistical difference was evident between med off condition and stim on-med off postoperative conditions, in absence of statistical differences between the 3 postoperative controls (3 months, 1 year, 2 years).

The improvement of motor fluctuations and drug-induced dyskinesias (UPDRS IV, complications of the therapy) was 80% at 3 months and 1 year and 90% at 2 years.

The clinical effectiveness of STN DBS was responsible for a reduction in the mean daily levodopa dosage from 850 mg/day to 172 mg/day (80%) at 3 months of follow-up, to 232 mg/day (73%) at 1 year and to 160.7 mg/day (81%) at 2 years after surgery. These values showed a significant difference between preoperative and postoperative levodopa dosages, while no differences emerged between the 3 postoperative values.

At the 3-month follow-up, 12 patients (31%) no longer took levodopa and 6 of them took no dopaminergic therapy; 9 patients were taking only levodopa and 18 patients assumed levodopa and small doses of dopaminoagonist drugs. At the 1-year follow-up, 6 patients (29%) no longer took levodopa and 3 of them took only dopaminoagonists; 4 patients took only levodopa and 11 patients were took levodopa and dopaminoagonists.

As far as the stimulation parameters is concerned, the voltage is progressively increased from a mean value of 2.56 V immediately after surgery to 2.96 V at 3 months, 3.21 V at 1 year and 3.34 V at 2 years.

After surgery, 7 of the 47 patients evaluated showed transitory mental confusion; 2 patients showed hypophonia and 1 patient had transitory eye opening apraxia. In addition, there was 1 case of thrombophlebitis and 1 case of subcutaneous infection.

Another aspect of our study was the evaluation of the influence of the patient's age on DBS effectiveness. In fact, a not advanced age at the moment of the operation and a short duration of the complicated phase of the disease are related to a better clinical outcome. For this purpose the patients were divided in two groups, younger and older than 60 years.

No significant differences were observed between the two groups when UPDRS II, III and IV scores were considered. The two groups showed, in fact, the same degree of clinical improvement.

The mean daily levodopa dosage taken by patients older than 60 years was 30% greater at the 3-month follow-up and 40% greater at 1 year. However, these differences were not significant. In the same way no differences in the voltage values utilized in the two groups were observed in the immediate postoperative period or during the follow-up.

Another analysis concerned the modification of the UPDRS III score in off phase after surgery (21 patients in the preoperative period and 3 and 12 months after the surgical treatment). There was a slight increment in the mean value of the motor score from 59/108 in preoperative med off condition to 62.2/108 at 3 months and 64.1/108 at 1 year of follow-up, without any significant differences.

Discussion

Our findings confirm that STN DBS represents an effective therapy for advanced PD. The clinical improvement of the parkinsonian symptoms was relevant, with a remarkable reduction of the daily levodopa dosage and a progressive disappearance of drug-induced dyskinesias and motor fluctuations. In addition, no differences between the two groups of PD patients younger and older than 60 years were observed, suggesting that an age of more than 60 years cannot be, probably, an exclusion criterion for the selection of patients as candidates for STN surgery.

The analysis of the modifications of the off motor scores after surgery seems to suggest a substantial stability of the clinical picture of the patients. However, the period analyzed was undoubtedly very short but the preliminary data at 2 and 3 years of follow-up seem to confirm this observation.

In our pool of patients the surgical complications were not relevant, confirming that in selected PD patients the operatory risk of STN DBS can be acceptable in consideration of the significant motor improvement.

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The role of somatosensory feedback in dystonia: a psycophysical evaluation

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Abstract Ten patients with idiopathic dystonia and twelve healthy controls were tested with pairs of non-noxious electrical stimuli separated by different time intervals. Stimuli were delivered (i) to the pad of the index finger (same-point condition), (ii) to the pad and to the base of the index finger (same-finger condition) and (iii) to the pad of the index and ring fingers (different-fingers condition). Subjects were asked to report if they perceived single or double stimuli in the first condition and synchronous or asynchronous stimuli in the second and third conditions. STDTs were significantly higher in dystonic than control subjects in all three conditions. Results extend current knowledge on deficits of somesthetic temporal discrimination in dystonia by showing that temporal deficits are not influenced by spatial variables.

Idiopathic dystonia is a poorly understood neurological syndrome in which the most dramatic symptoms appear to be motor in nature. It is largely believed that a dysfunction of cortical-striato-thalamo-cortical motor circuits plays a major role in the pathophysiology of primary dystonia [1]. Several lines of evidence, however, hint at the involvement of the somatosensory system in dystonia [2–6]. Using a psychophysical approach, we recently found that the capability to perceive as temporally separate two sequential somesthetic stimuli was highly impaired in dystonic patients [2, 3]. This result has been confirmed in subsequent studies on patients with focal hand dystonia, who performed like normal controls in a single-touch, gross localization task that purportedly tapped spatial discrimination abilities [4, 5]. It is not known, however, if there is any interaction between spatial and temporal deficits observed in dystonia.

We tested 10 patients with idiopathic dystonia (5 women and 5 men; mean age, 39.2 years) and 12 healthy subjects matched for age and sex (6 women and 6 men; mean age, 38 years). In all patients, dystonia involved at least one upper limb (8 patients had generalized dystonia, 1 patient had a right-sided writer's cramp and 1 patient had segmental dystonia involving the right arm and the trunk). Motor impairment of the arm was graded by evaluating the arm subscore of the Burke-Fahn-Marsden scale.

Each stimulus consisted in a pair of square wave electrical pulses, of 0.2 ms duration and intensity three-times the sensory threshold (non-painful). Surface skin electrodes were used.

For each hand, three experimental conditions were studied. In the first, a single electrode was positioned on the volar surface of the pad of the index finger (same-point condition). In the second (same-finger condition) two electrodes, one on the base and one on the pad of the index finger, were used. In the third condition (different-fingers) two electrodes, positioned on the pad's volar surface of the index and of the ring finger, were used.

In same-point condition, the shortest temporal interval at which subjects reported two separate stimuli (instead of a single stimulus) was taken as threshold value. In the same-finger and different-fingers conditions, the first interval at which subjects judged the two stimuli to be asynchronous was taken as thresholds value. For each experimental condition, ascending and descending temporal discrimination threshold (ATDT, DTDT) were determined for both left and right hands.

For each subject, mean threshold values were entered in a 2 (hand: left and right) x 3 (experimental condition: same-point, same-finger, different fingers) ANOVA for mixed design, where the group (controls or dystonic patients) was the between-subjects factor. A correlational analysis between STDT values (average of ATDT and DTDT) and motor impairment of the dystonic hand was carried out by linear regression analysis.

As shown in Figure 1 the factor group was significant, threshold values being much lower in controls (35.7 ms) than in dystonic patients (107.3 ms). The factor experimental condition was also significant. Post-hoc comparisons, carried out by means of the Student-Newman-Kuels test, showed that STDT in the different-finger conditions (82.6 ms) was significantly higher than in the same-finger (66.1 ms) and same-point (65.9 ms) conditions, which in turn were not different from each other.

There was no significant correlation between the severity of motor impairment of the dystonic hand and the increase of temporal discrimination thresholds.

Discussion

Idiopathic dystonia is currently thought of as a model of dysfunction of neostriatal parts of the basal ganglia and of their relationships with thalamic and cortical motor structures [1]. Functional connectivity of basal ganglia, however, does not involve only cortical motor structures, but also somatosensory areas. For example, heavy connections between the puta-

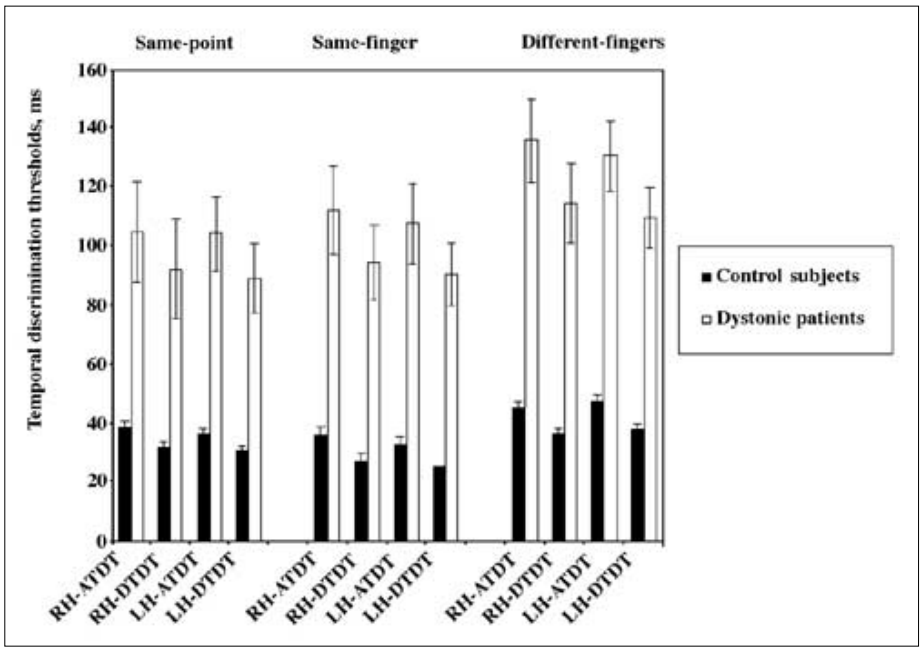

Fig. 1. Mean values (over five blocks) of ascending and descending temporal discrimination thresholds in dystonic and control groups in the three experimental conditions. Error bars indicate standard errors. *LH*, left hand; *RH*, right hand; *ATDT*, ascending temporal discrimination threshold; *DTDT*, descending temporal discrimination threshold

minal portions of the neostriatum and S1 and posterior parietal cortices have been traced [1]. A possible role for sensory areas in dystonia is also supported by PET studies documenting that, in addition to a hypermetabolism in the striatum, dystonic patients show a reduced activation of the sensorimotor cortices contralateral to the hand where vibrotactile stimulation was delivered [6]. Psychophysical studies in brain-damaged patients have shown that focal lesions not only of S1 but also of basal ganglia induced significant impairments in the experimental task of judging as simultaneous or sequential pairs of tactile stimuli [1]. Deficits of both spatial and temporal aspects of somatosensory processing have been reported recently in focal-hand dystonic patients. This study not only confirms previous studies reporting temporal discrimination deficits in idiopathic dystonia and in focal hand-dystonia patients [2-5], but also adds significantly to previous knowledge by showing that temporal discrimination in dystonic patients is largely independent from spatial variables. Indeed, although performance was significantly worse in the different-fingers conditions than in the same-point and same-finger conditions, this figure was comparable in dystonic patients and control subjects. Previous studies in normal subjects reported that reaction times for identifying pairs of spatial patterns presented to two fingers were longer than for patterns presented to one finger. This effect has been attributed to subject's limitations of shifting attention effectively across two spatial locations. Since mechanisms responsible for the advantage of having temporal stimuli delivered close in space appear similarly spared in patients and controls, the discrimination deficit observed in dystonic patients does not appear to interact with the spatial distance between the loci on the hand, where tactile stimuli were delivered. The increase of temporal discrimination thresholds observed in dystonic patients was not significantly related to the severity of motor impairment and it was also observed at the side of the clinically normal hand. This means that dystonic symptoms are not a direct consequence of these somatosensory abnormalities, but the deficit reported here may contribute at least partially to the motor impairment present in dystonia.

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Combination of two different dopamine agonists in the management of Parkinson's disease

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Abstract An alternative approach to the symptomatic treatment of parkinsonian patients with and without motor fluctuations is to use dual dopamine agonists. The aim of this study was to investigate the symptomatic effect of administrating a second dopamine agonist to parkinsonian patients already assuming pramipexole or ropinirole. As the second dopamine agonist we chose cabergoline, a drug with a long half life, whose pharmacological profile differs from that of the newer non-ergot-derived dopamine-receptor agonists. In this pilot study we enrolled 27 patients: 21 patients had motor fluctuations and were receiving levodopa plus a dopamine agonist, and 6 patients without motor fluctuations were receiving a dopamine agonist without levodopa. This open study shows that dual dopamine-agonist therapy (cabergoline plus pramipexole or ropinirole) may be used in the symptomatic treatment of patients with Parkinson's disease receiving therapy with or without levodopa.

Different studies have demonstrated that dopamine agonists induce less motor complications than levodopa in patients with Parkinson's disease (PD) [1–3]. Moreover, motor complications appear or worsen when levodopa is added to the agonists. Other studies showed that dyskinesia is more severe in patients taking higher doses of levodopa. Unfortunately, dopamine agonist monotherapy has a limited span of efficacy that varies from 3 to 6 years, and then levodopa must be added. In patients taking levodopa plus a dopamine agonist, levodopa dosage must be increased during the course of the disease to maintain good control of symptoms. This strategy is often necessary despite the agonist being used at fairly high dose. Since dopamine agonists have different receptor binding proprieties and different pharmacokinetic characteristics, it may be possible that two dopamine agonists induce a better clinical effect than one with the advantage of a prolonged dopamine agonist monotherapy or an increased levodopa-sparing effect. Epilepsy therapy is an example of how the combination of two drugs with similar modes of action may be more effective than one for the patient. We report the preliminary results of a pilot study to determine the usefulness of the combination of two agonists. Cabergoline was chosen as the agonist to add to ropinirole or pramipexole because of its unique half life and different dopamine receptor binding properties [4–6].

The study was conducted as a three-month prospective, open label pilot trial in patients with PD. After signing an Institutional Review Board (IRB)-approved informed consent from, 27 patients (16 men) with idiopathic PD were included in the study. All the patients reported unsatisfactory control of their symptoms. The 27 patients were subgrouped according to their previous therapy. The first group comprised 21 patients (12 men) of mean age 60.8±6.8 years, mean disease duration 9.1±4.1; they were treated with a dopamine agonist (11 pramipexole, 10 ropinirole) plus levodopa. The mean daily dosage of levodopa was 471.4 mg/day (SD, 239.0), and the mean daily dosages of dopamine agonists were 3.7 mg/day (SD, 0.9) for pramipexole and 17.3 mg/day (SD, 6.1) for ropinirole. These 21 patients suffered from motor fluctuations (wearing OFF), and 10 of 21 showed also mild dyskinesia.

The second group comprised 6 patients (3 men) treated with dopamine agonist monotherapy (3 pramipexole, 3 ropinirole); their mean age was 51.7±9.9 years and the mean disease duration was 4.5±1.6. The mean daily dosages of dopamine agonists were 3.5 mg/day (SD, 0.7) for pramipexole and 22.6 mg/day (SD, 6.8) for ropinirole. These patients had no motor fluctuations or dyskinesia, and their clinical conditions were stable though not optimal.

In both subgroups, cabergoline was started at 0.5 mg in a single daily dose and then titrated up to the optimal dose. The optimal dose was defined as that able to induce the best improvement in the clinical state without inducing adverse effects. Levodopa dosage was kept unchanged during the study period. Patients were assessed every two weeks during the titration phase and every four weeks once the optimal dose was reached. During the week before entering the study, patients in the subgroup treated with a dopamine agonist plus levodopa were asked to maintain a home-diary, recording in a flow-chart the number of hours spent OFF. The motor and functional assessment included the Unified Parkinson's disease rating scale (UPDRS motor score), assessed during the ON and OFF states, and the abnormal involuntary movements (AIMS) rating scale. Patients in the subgroup treated with a dopamine agonist without levodopa were assessed for the best motor state using the UPDRS scale. Clinical assessments were done by an independent blind observer at baseline, every two weeks thereafter, and four weeks after the

optimal treatment was reached. During the last assessment, patients and observers were asked to rate changes in each subject's condition after cabergoline had been added to therapy; they used the global clinical impression (GCI) scale to select among: worse, no change, slightly improved, or markedly improved.

All data are expressed as means and standard deviations. Student's paired two-tailed test was used to assess the significance of changes in continuous or normalized data.

In the subgroup of 21 patients receiving a dopamine agonist plus levodopa, when cabergoline was added to therapy (4.04±1.62 mg, administered in 12 patients once a day, and in the other 9 patients twice a day), the UPDRS motor score measured in the OFF period decreased from 38.9±12.6 to 35.4±10.22 ($p<0.0001$) while that in the ON period decreased from 18.07±6.63 to 16.61±7.22. The time spent OFF decreased from 2.9±1.1 hours to 1.2±1.1 hours ($p<0.0001$). Involuntary movements were present in 10 patients with a severity of 1.6±0.8 (AIMS scale 0-4) and they did not change significantly after cabergoline treatment (1.8±1.0). At the GCI, only 1 patient considered the clinical state worsened, 1 reported no change, 6 reported that it had slightly improved, 9 much improved, and 4 very much improved. No differences were found between the clinical results in patients treated with pramipexole and ropinirole. In the subgroup of 6 patients receiving a dopamine agonist without levodopa, when cabergoline (3.8±0.7 mg once a day) was added to therapy, the UPDRS motor score for the best motor state decreased from 21.5±5.3 at baseline to 13.5±3.8 ($p=0.0001$). At the GCI, 4 patients considered their clinical state much improved, and 2 very much improved. All 27 patients tolerated adjunctive cabergoline well. Although 6 of the 27 patients reported adverse effects (2 patients experienced nausea, 2 had swollen legs, and 1 had orthostatic hypertension), no patient stopped the treatment. The symptoms were transient and improved with domperidone (10 or 20 mg three times daily).

Discussion

In all the patients with Parkinson's disease treated with a dopamine agonist in monotherapy or with supplementary levodopa, adjunctive cabergoline induced a significant clinical improvement. All patients tolerated the addition well and none of them experienced major adverse effects. In conclusion, an alternative approach to the symptomatic treatment of parkinsonian patients with and without motor fluctuations is to use dual dopamine agonists. Two agonists combined probably give better results than a single agonist because pharmacological profiles converge. The encouraging preliminary results obtained in this open trial now need to be confirmed in a double-blind study.

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PARK6 is a common cause of familial parkinsonism

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Abstract The Parkin gene is responsible for about 50% of autosomal recessive juvenile parkinsonism (ARJP) and less than 20% of sporadic early onset cases. We recently mapped a novel ARJP locus (PARK6) on chromosome 1p. Linkage to PARK6 was confirmed in 8 families from 4 different European countries. These families share some clinical features with the European Parkin-positive cases, with a wide range of ages at onset and slow progression. However, features typical of ARJP, such as dystonia and sleep benefit, were not observed, making the clinical presentation of late-onset cases indistinguishable from that of idiopathic PD. The determination of the smallest region of homozygosity in one consanguineous family allowed reducing the candidate interval to 9 cM. PARK6 appears to be an important locus for ARJP in Europe.

Autosomal recessive juvenile parkinsonism (ARJP) shares many clinical features with Parkinson's disease (PD), but is characterized by early onset of symptoms, slow progression, good response to levodopa and early levodopa-induced dyskinesias. Hyperreflexia, dystonia at onset and sleep benefit may be present. Pathological studies have shown a selective degeneration of dopaminergic neurons in the substantia nigra and, usually, the absence of Lewy bodies [1, 2]. A gene for ARJP (Parkin) has been identified on human chromosome 6q [3]. The Parkin gene codes for a protein with ubiquitin-ligase activity [4]. In a recent study on the prevalence of Parkin in Europe, mutations were detected in about 50% of ARJP families and only 18% of sporadic cases with early onset parkinsonism. Some patients with Parkin mutations had a later age of onset (up to 65 years) and were clinically indistinguishable from patients with idiopathic PD [5].

We have recently mapped a novel ARJP locus (PARK6) to a 12.5 cM interval on chromosome 1p35-p36 [6]. The clinical presentation was similar to that of idiopathic PD, the only distinctive features being an earlier age at onset (range, 32–48 years) and a slower progression of the disease. A third ARJP locus (PARK7) has also been mapped on chromosome 1p36, 25 cM telomeric to PARK6 [7]. To assess the role of PARK6 in Europe, we studied 28 ARJP Parkin-negative families [8]. Of 28 families, 8 were British, 6 were Dutch, 6 were Italian, 5 were German and 3 were French. All affected individuals from the 28 families were genotyped for 11 microsatellite markers spanning the PARK6 region. The 11 loci are ordered as follows: tel-D1S483-0 cM-D1S199-3.2 cM-D1S2732-0 cM-D1S2828-0 cM-D1S478-0.6 cM-D1S2702-3.6 cM-D1S2734-0 cM-D1S2698-1.6 cM-D1S2674-0.8 cM-D1S2885-2.7 cM-D1S247-cen. Microsatellite markers were PCR-amplified from genomic DNA and electrophoresed on a capillary using a 3100 DNA Sequencer (ABI Prism). Pairwise LOD scores were obtained using the MLINK program, assuming equal male-female recombination rate, autosomal recessive inheritance, reduced penetrance (0.90) and a gene frequency of 0.001. Cumulative multipoint LOD scores were generated by use of the SIMWALK2 program. Marker order and genetic distances were based on the Marshfield chromosome 1 genetic map.

Eight families generated positive LOD scores at all recombination fractions (thetas) and the affected members of each family shared identical haplotypes. The other families generated negative LOD scores at all thetas and affected individuals had different haplotypes across the entire region; these were therefore excluded from further analysis.

In the 8 families showing evidence of linkage, the 11 markers were genotyped in all available unaffected family members and haplotypes were phased based on the minimum number of recombinants (Fig. 1). Linkage analysis including unaffected family members gave a cumulative maximum pairwise LOD score of 5.39 for marker D1S478. The maximum cumulative multipoint LOD score obtained on the 8 families using 6 markers (D1S483, D1S478, D1S2734, D1S2674, D1S2885 and D1S247) was 6.29 at locus D1S478.

No recombination events were detected at the telomeric end of the region. The determination of the smallest region of homozygosity in consanguineous family IT-GR identified a recombination event between markers D1S2698 and D1S2674, allowing the refinement of the candidate interval to 9 cM between flanking markers D1S483 and D1S2674. Each family showed a different haplotype.

Of the 8 PARK6-linked families, 3 were Dutch, 2 were German, 2 were Italian and 1 was British. Mean age at onset was 42.1±9.4 years, and at least 1 of the 2 affected siblings had onset before the age of 45 years. However, 4 affected individuals had an age at onset above 45 years, with the latest onset at 68 years. Clinical features were indistinguishable from Parkin-positive European ARJP cases, being characterized by a mild to moderate parkinsonian syndrome with good response to levodopa and slow progression (mean disease duration, 12.0±6.9 years). Levodopa-induced dyskinesias occurred in 8 (50%) of 16 patients. Dystonia at onset and sleep benefit were not detected. Hyperreflexia was observed only in 2 patients [8].

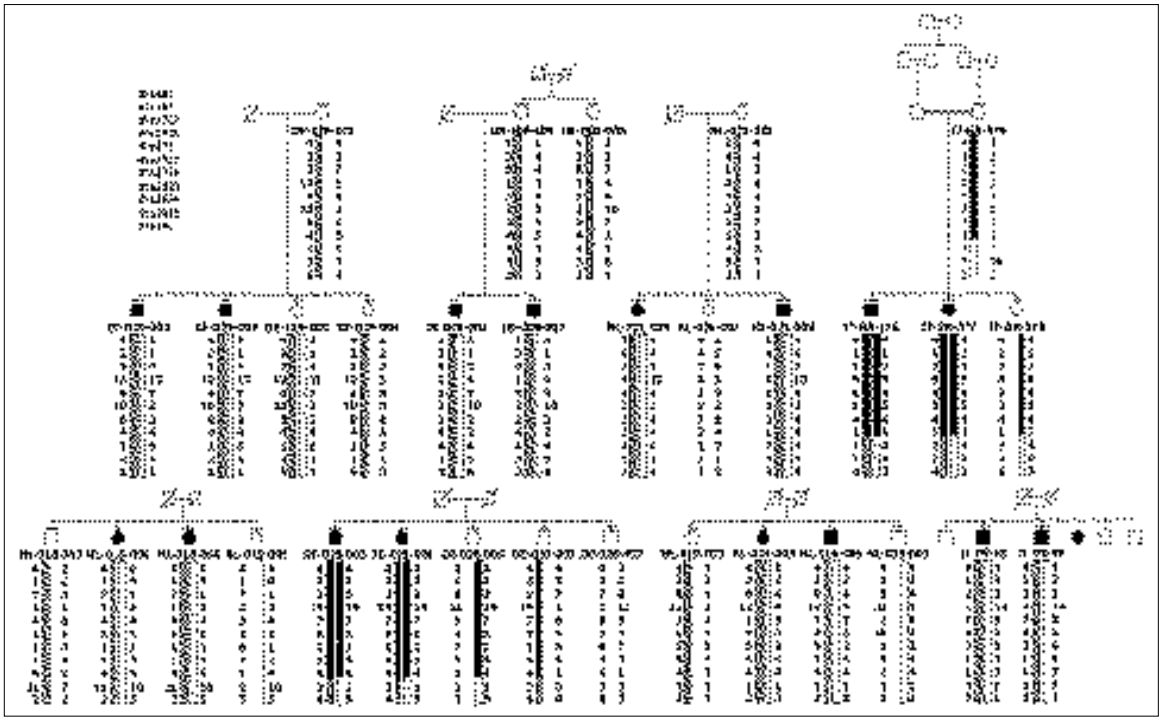

Fig. 1 Pedigrees of the 8 linked families with haplotypes spanning the PARK6 region

Discussion

Three loci for ARJP have been identified so far, indicating genetic heterogeneity [8]. The Parkin gene is responsible for about 50% of ARJP European cases [5]. We have analyzed 28 Parkin-negative ARJP families and detected linkage to PARK6 in 8 families from 4 different European countries. No common haplotype could be detected, suggesting independent mutational events. These results indicate that PARK6 is not restricted to Italy, thus confirming the role of this locus in determining ARJP in European Parkin-negative cases. The PARK6-associated phenotype does not resemble the typical ARJP presentation, as is often seen in Japanese patients [1, 2]. The phenotype is more similar to that described in Parkin-positive European ARJP patients, with a broader clinical spectrum and range of ages at onset [5]. In the late-onset cases, clinical features are indistinguishable from idiopathic PD. However, no pathological data on PARK6-linked patients are available at present. It is still unclear whether PARK6 shares the same neuropathological features as Parkin or idiopathic PD, or represents a distinct disorder within familial parkinsonisms.

Parental consanguinity was detected only in 1 (IT-GR) of 8 families. The 2 affected siblings were homozygous across the entire linked region except for the 3 centromeric markers. This finding, which strongly suggests a recombination between markers D1S2698 and D1S2674, allows the refinement of the candidate interval from 12.5 to 9 cM.

A large number of genes map within the candidate interval but none of them is a striking candidate for parkinsonism. The linked region is still too large to allow searching for candidate genes. Additional work is needed to test a larger group of ARJP families for linkage to PARK6, in order to further refine the linked interval.

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Evaluation of risk of Parkinson's disease in a cohort of licensed pesticide users

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Abstract In the last two years, the environmental theory on the aetiology of Parkinson disease has acquired new data. From an experimental point of view, a new model of parkinsonism induced by rotenone, a diffuse insecticide, has been proposed, and in vitro studies have provided proof that several pesticides stimulate the formation of α-synuclein fibrils (one of the principal constituents of Lewy bodies). Moreover, a meta-analysis of all case-control studies so far performed showed a positive, statistically significant association between pesticide exposure and PD. In this context, we are performing a cohort study on 5575 licensed pesticide users in the province of Viterbo. After 27 years of follow-up, 4788 subjects are still alive. The aim of this study is to measure the prevalence of Parkinson's disease in a large group of workers with theoretically increased risk.

In the early 1980s, the environmental theory on the aetiology of Parkinson's disease (PD) was lent support by the discovery of MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine), a potent neurotoxin for the substantia nigra, which caused an extrapyramidal syndrome in a group of young Californian drug-addicts shortly after exposure. This molecule is chemically similar to paraquat, a diffuse herbicide, and its toxic metabolite, $MPP^{+,}$ has been sold as a herbicide under the name of cyperquat.

More recently, an experimental model of parkinsonism induced by rotenone, a diffuse insecticide, has been proposed [1]. Rotenone inhibits complex I of the mitochondrial respiratory chain. In rats, it induces a nigral degeneration with fibrillar cytoplasmic inclusions similar to the Lewy bodies observed in the substantia nigra of PD patients [1].

Some pesticides (e.g. diethyldithiocarbamate, rotenone, dieldrin and paraquat) stimulate the formation of α-synuclein fibrils and induce a conformational change in α-synuclein [2]. α-Synuclein is a protein that accumulates in Lewy bodies; a mutation in its gene (Ala53Thr) is responsible for a monogenic form of PD.

In the last twenty years, numerous case-control studies have been published on the association between occupational pesticide exposure and PD (Fig. 1) [3]. A meta-analysis of these studies estimated the risk for PD to be approximately twice as high for subjects with previous pesticide exposure (OR=1.85; 95% CI, 1.31–2.60) compared with the general population [4]. However, an overall evaluation of other environmental factors associated with PD onset (e.g. rural living, well-water drinking, farming) indicated that it is likely that all people living in rural areas have an increased risk of PD [4]. Moreover, in a study of a cohort of orchardists, a high prevalence ratio (PR) for parkinsonism was found in those exposed to pesticides for more than 50 years (PR=2; 95% CI, 1.0–4.2) [5].

The fact that epidemiological evidence for association between pesticide exposure and PD is supported by previous experimental data suggests that this theory is biologically plausibile. This is a crucial criterium in the evaluation of a possible association between exposure and disease in way of a cause-effect relationship. Nevertheless, no specific pesticide has yet been associated with disease onset in parkinsonian patients, even if it is possible that several pesticides induce the typical dopaminergic degeneration of PD through a different mechanism of action.

The methodological aspects of case-control studies performed on PD patients must, however, be evaluated carefully. The results of a case-control study may in fact be influenced by the clinical definition of cases, the ascertainment of cases, the recall bias in elderly subjects, the definition of exposure and the selection of appropriate controls. These potential sources of variability should, therefore, be considered. In this context, we propose another epidemiological approach which is similar to the cohort study but permits a better clarification of the relationship between exposure and disease. In epidemiological research on PD, this study design has recently been recommended by the National Institute of Environmental Health Sciences (NIEHS) [6].

We identified a cohort of licensed pesticide users between 1971 and 1973 in the province of Viterbo, a town near Rome. This cohort included male farmers (n=2977) and their wives (n=2598), for a total of 5575 subjects. On 31 December 2000, after 27 years of follow-up (1974–2000), 4788 subjects were still alive, 757 were deceased (including 7 for whom the specific cause on death certificate was missing), and 28 were lost to follow-up. In accordance with the International Classification of Diseases (ICD, IX Revision), the specific causes of death (ICD code) were: 326 malignant neoplasms (140–208), 16 diseases of the nervous system (320–359), 215 diseases of the circulatory system (390–459), 23 disease of the respiratory system (460–519), 48 diseases of the digestive system (520–579), 11 diseases of the genitourinary system (580–629), 14 cases of symptoms, signs and ill-defined conditions (780–799), 56 cases of injury and poisoning (800–999), and 42 infectious diseases (209–319, 629–780).

The 16 specific diseases of the nervous system comprised: 1 bacterial meningitis (320.9), 2 encephalitis-myelitis (323.9),

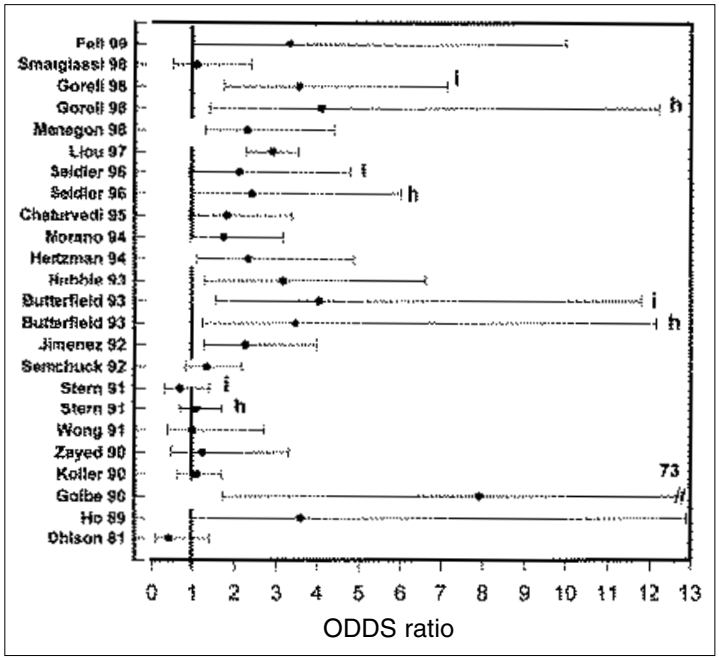

Fig. 1 Association between pesticide exposure and Parkinson's disease. Case-control studies. *i*, exposure to insecticides; *h*, exposure to herbicides

5 Alzheimer's disease (331.0), 2 unspecified cerebral degeneration (331.9), 2 Parkinson's disease (332.0), 1 motor neuron disease (335.2), 1 epilepsy (345.9), 1 unspecified encephalopathy (348.3), and 1 progressive muscular dystrophy (359.1).

To evaluate the risk of PD in this cohort, we are carrying out a study with a two-phase design: in the first, a questionnaire for the screening of parkinsonisms, previously validated on the Italian population [7], is mailed to all subjects in the cohort; in the second phase, all subjects with at least one positive item in the questionnaire are visited for a clinical diagnosis of PD according to the United Kingdom PD Brain Bank criteria. The ratio between the numbers of observed and expected PD cases in this cohort is the standardized prevalence ratio (SPR) and may be interpreted as a new index of the association between pesticide exposure and PD.

In conclusion, future epidemiological research on PD will probably be conducted in the following three ways:

1. Cohort studies on workers exposed to neurotoxins (e.g. solvents, metals, pesticides) that must consider as outcome not the death certificate, which does not accurately estimate the frequency of neurodegenerative diseases, but rather the SPR or a similar index;
2. Studies on the relationship between genetic and environmental factors that consider the data on differences in the genotype profile of detoxificant enzymes between PD patients and the general population (NAT2, GSTT1, CYP2D6) [8, 9];
3. Follow-up studies on subjects with previous, acute pesticide intoxication to evaluate the possible presence of neurological sequelae [10].

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Clinical assessment of dysphagia in early phases of Parkinson's disease

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Abstract Dysphagia is a frequent symptom in parkinsonism, but it is less commonly reported by patients with idiopathic Parkinson's disease (IPD), especially in the early phases. Sixty-five patients with IPD were questioned about symptoms of dysphagia and an objective swallowing test was administered. Reduced swallowing speed for food and complaints of food sticking in the throat, wet voice and cough after liquid intake and nocturnal sialorrhea were reported, respectively, by 35%, 20% and 15% of patients. On objective examination, oral-phase (facial, tongue and palatal musculature) abnormalities were found in 70% of patients. Lingual transfer movements, mainly propulsion, and palatal elevation were severely hypokinetic. Wet voice after liquid intake and cough reflex after solid/liquid intake were detected in 40% of patients. On the other hand, severe dysphagia with frequent food aspiration and chest infections requiring antibiotics in the last 12 months was not found; cough reflex was retained in all patients. On the basis of these results, a regular assessment on swallowing abilities in patients with IPD is warranted in the clinical setting because with simple dietary advice and a short rehabilitative training, the quality of life in these patients can be improved.

Dysphagia is a symptom reported by 20%–40% of patients with idiopathic Parkinson's disease (IPD) when questioned [1], even though it is seldom self-reported by the patients themselves. How disabling symptom it appears after 10–14 years from the beginning of the disease [2].

Using videofluoroscopy, swallowing problems have been demonstrated up to 100% of IPD patients [3]. Dysphagia, especially when unrecognized, plays a role in weight loss in IPD [4] and in the recurrence of airway infections. The mean survival from the beginning of an overt dysphagia is about 2–3 years [2]. The aim of this study was to determine the usefulness of a clinically objective test in detecting dysphagia in IPD patients, even in asymptomatic cases.

Sixty-five patients with IPD (36 women, 29 men) were studied. Mean age was 66.3±9.1 years (range, 33–81), mean illness duration was 6.6±4.1 years (range, 1–13), mean UPDRS score was 28.2±6.9 (range, 17–38); and Hoehn-Yahr scale ranged from 1.5 to 3.0.

Patients were interviewed about symptoms of dysphagia by means of a short questionnaire (food, liquid and tablet swallowing difficulties; food sticking in the throat; cough and wet voice after solid/liquid intake; nocturnal sialorrhea; recent history of weight loss or chest infections requiring antibiotics) [5].

An objective swallowing test, based on Kennedy et al.'s [6] dysphagia rating scale, was administered. In the 12-item modified dysphagic rating scale (mDRS, Table 1) each item is scored from 1 to 5, in which 1 represents the worst performance and 5 the normal condition.

From the analysis of the questionnaire we obtained the following results: 35% of patients, when questioned, complained of occasional liquid and solid dysphagia; 30% of patients reported the sensation of food sticking in the throat; 20% complained of wet voice and cough after liquid intake; 15% had nocturnal sialorrhea; 1% experienced weight loss; but no chest infections requiring antibiotics in the last 12 months were reported.

The results from the objective swallowing test were: mean score (SD) of mDRS was 55.3±3.1 (normal score for the mDRS is 60). No correlation was found between mDRS score and age of patients as well as between mDRS and illness duration. mDRS score showed a trend towards a correlation with UPDRS score (R=-0.7) and the correlation was significant (p=0.004). Mouth opening and palatal elevation were impaired in 60%, lingual protrusion in 70% of patients. Wet voice after liquid intake was observed in 40% of cases. Cough reflex after liquid intake was found in 40%, while cough after solid intake was hardly ever observed (5%). Cough reflex was retained in all patients.

Table 1 Modified dysphagia rating scale (mDRS), based on the work of Kennedy et al. [6]

1.	Maintaining lip closure throughout the oral phase
2.	Mouth opening
3.	Lateral movements of the tongue during mastication
4.	Protrusion of the tongue
5.	Palatal elevation
6.	Triggering of the swallow for liquid/solid intake
7.	Triggering of cough before, during or after liquid intake
8.	Triggering of cough before, during or after solid intake
9.	Solid bolus swallowing test (6 times)
10.	Liquid bolus swallowing test (6 times)
11.	Voice quality after liquid intake
12.	Reflux of material through cricopharyngeal sphincter leading to oral or nasal emission

Discussion

Only 35% of the IPD patients reported subjective dysphagia. However, a higher prevalence of objective disorder was found. The main findings were the abnormalities of the oral phase, either for prevalence (around 70%) or severity. These changes, being correlated to the UPDRS motor scores, are likely to express bradykinesia of oropharyngeal muscles. Lingual protrusion and palatal elevation were severely hypokinetic, explaining difficulties in bolus preparation, bolus directioning, posterior propulsion of the bolus by the tongue and correct opening of the pharynx.

Videofluoroscopic data support the evidence that derangements of the oral phase are greatly responsible for dysphagia in IPD patients. Indeed, oral transit duration is usually longer than normal, while the duration of the pharyngeal phase is only slightly impaired [7].

mDRS can supply only indirect information about pharyngeal and esophageal phases. Wet voice and cough after liquid intake, observed in 40% of patients, can be considered the clinical expression of deficient epiglottic positioning and range of motion and laryngeal dismobility. Complaints of reduced swallowing speed for food and food sticking in the throat can be referred to the abnormalities of pharyngo-esophageal motility. Prolonged transit time for solid food and retained cough reflex may explain lack of true aspiration and chest infection in our patients. Therefore, dysphagia can be seen as a frequent but not severe dysfunction in IDP patients, at least in the early stage of the disease. However it may interfere with an adequate nutrition [4].

In conclusion, monitoring swallowing abilities by a regular clinical assessment is useful in detecting manifest as well as silent swallowing impairments. mDRS is an easy and reliable tool to evaluate mainly the oral and upper pharyngeal phases of swallowing, which are actually the most impaired phases in IPD, also in asymptomatic patients. Since the disorders of the oral phase seem to be the most responsive to dopaminergic therapy [8] and amenable to short rehabilitative training as well as to simple dietary advice, detecting them by means of an objective swallowing test could provide a further opportunity to monitor the general health and to improve the quality of life of these patients.

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ALS-plus: 5 cases of concomitant amyotrophic lateral sclerosis and parkinsonism

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Abstract According to El Escorial criteria, amyotrophic lateral sclerosis (ALS), combined with other neurologic disorders, such as dementia and parkinsonism, is defined as ALS-plus. These overlaping syndromes are extremely rare. Here we report 5 cases (3 men, 2 women) of ALS-plus; mean age at the onset of symptoms was 67 years (range, 65-72). In 3 patients, motoneuronal signs preceded the onset of parkinsonian syndrome. In 4 cases, the clinical picture was characterized by the prevalence of motoneuronal signs. Parkinsonism was poorly responsive to L-dopa treatment in all patients. The clinical course did not differ from that expected in patients with only ALS. Our clinical observations and neuropathological reports of nigral neuronal loss in ALS patients suggest a common pathogenic mechanism underlying these disorders.

According to the El-Escorial classification [1], amyotrophic lateral sclerosis (ALS) combined with other neurologic disorders, such as parkinsonism and dementia, is defined as ALS-plus. These overlaping syndromes are rare outside of the isles of Guam and Kii peninsula. The most common finding is ALS associated with dementia. Clinical and neuropathological studies of patients presenting ALS and parkinsonism supported the hypothesis of a common pathogenesis [2–6] Here we describe 5 patients, observed in Puglia (southern Italy), who developed both ALS and parkinsonism.

Case 1. A 67-year-old woman presented with progressive dysarthria and dysphagia. After 1 year, the patient became anarthric and developed weakness and wasting of upper limb muscles; at that time ALS was diagnosed. In the following months, dysphagia became extremely severe requiring the application of percutaneous endoscopic gastrostomy (PEG). Two years after the onset of neurological symptoms, she began to complain of rest tremor of her right hand, which in a few months involved the remaining limbs. On neurological examination, she was anarthric, dysphagic and dyspnoic, with hyperactive jaw jerk. Upper limb examination revealed distal hypotrophy and brisk tendon reflexes, associated with rest tremor and rigidity. On lower limb examination, there were rest tremor and rigidity mainly on the right side, with sparing of strength and trophism. Coordination and all sensory modalities were normal. Electromyography (EMG) revealed a diffuse pattern of chronic denervation with fasciculations. Magnetic resonance imaging (MRI) of the brain showed mild cortical atrophy. Treatment with L-dopa, benserazide, and riluzole was started. In the following months, tremor and rigidity continued to increase despite L-dopa therapy. After five years, the patient is anarthric and severely tetraparethic and needs subcontinuous positive pressure ventilation.

Case 2. A 66-year-old man presented with progressive weakness of the lower limbs. After 2 years, he began to complain of rest tremor of his left upper limb, rapidly involving the leg. Neurological examination showed rest tremor and rigidity of left upper and lower limbs; lower limbs were markedly paraparethic with increased deep tendon reflexes and bilateral Babinski's sign. Coordination and all sensory modalities were preserved. EMG showed diffuse denervation with fasciculations in the four limbs; MRI of the brain revealed some hyperintense areas of the deep white matter on T2-weighted images. Levodopa treatment did not improve the parkinsonian symptoms. The neurological condition progressively worsened in the following months; the patient died 4 years after the onset of motoneuron disease.

Case 3. A 65-year-old woman presented muscle weakness and hypotrophy of her left upper limb, progressively involving the remaining limbs. Two years later, rest tremor of the left hand became evident, associated with mild rigidity of the left limbs. In the following few months, tremor involved the right side of the body. In addition, neurological examination showed muscle weakness, hypotrophy and fasciculations in the four limbs, associated with hyperactive deep tendon reflexes. Bilateral Hoffmann's and Babinski's signs were present. Coordination and all sensory modalities were preserved. EMG revealed denervation with fibrillations in the four limbs; MRI of the brain showed mild cortical atrophy. Levodopa treatment was ineffective on tremor and rigidity. The clinical course was rapidly progressive; the patient died 3 years after the onset of symptoms.

Case 4. A 65-year-old man presented with start hesitation and freezing on turning on the left side. Neurological examination revealed mild rigidity and bradykinesia of left upper and lower limbs. L-Dopa treatment was poorly effective on parkinsonism. In the following 6 months, he became dysarthric and dysphagic and developed progressive weak-

ness of both upper and lower limbs. On examination, dysarthria and dysphagia, with hypotrophy and fasciculations of the tongue, were present. He presented marked muscle weakness and hypotrophy in the four limbs, associated with fasciculations and brisk deep tendon reflexes. Babinski's sign was present bilaterally. EMG revealed active denervation with diffuse fasciculations in all four limbs; MRI of the brain and spinal cord were normal. After one year, the patient became wheelchair-bound. The clinical course was rapidly progressive; the patient died 4 years after the onset of symptoms, because of respiratory failure.

Case 5. A 73-year-old man presented with a 2-year history of diffuse bradykinesia. Neurological examination revealed bradykinesia and rigidity in the four limbs, associated with brisk deep tendon reflexes. In addition, fasciculations and hypotrophy of the left upper limb were evident. EMG revealed diffuse denervation and fasciculations. MRI of the brain showed mild cortical atrophy. L-Dopa and riluzole therapies were initiated. The clinical course of the patient was slowly progressive; after one year he developed impairment of short-term memory. Nearly two years after the diagnosis of motor neuron disease and parkinsonism, neurological examination reveals mild weakness of upper limbs, associated with marked and diffuse rigidity and bradykinesia.

Discussion

In the 5 cases here reported, the mean age at the onset of symptoms was slighly higher (67 years; range, 65–72) than that commonly reported for ALS patients. In 3 of these cases, motoneuronal signs preceded the onset of parkinsonism. In 3 cases, the parkinsonian picture was characterized mainly by rest tremor and rigidity, while the other 2 cases presented with bradykinesia. Parkinsonism was poorly responsive to L-dopa treatment in all patients. In 4 cases the clinical picture was characterized by the prevalence of motoneuronal signs; in these patients the clinical course did not differ from that usually expected in ALS.

The association between ALS and parkinsonism, although rare, occurred more frequently than expected by chance [2, 3]. Epidemiological studies showed a higher prevalence of parkinsonism in family members of ALS patients. [5]. Some authors reported that ALS is associated with parkinsonism in 5% of cases, during the course of the disease [2]. The mean age at the onset of this overlaping syndrome is almost 10 years greater than in ALS without parkinsonism [2, 4].

The most common extrapyramidal signs are bradykinesia and rigidity; usually these signs respond poorly to levodopa treatment. The clinical course does not differ from that expected in ALS without parkinsonism [4].

Neuropathological evidence of neuronal loss, with neurofilamentous and Lewy body inclusions in the basal ganglia, has been demonstrated in some ALS patients [3, 6]. In addition, in recent years, positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies revealed a progressive midbrain dopaminergic deficit in ALS patients, even in the absence of extrapyramidal signs [6, 7].

These findings suggest that the disorders share a common pathogenic mechanism. Mitochondrial dysfunction, oxidative stress and neurotoxic effects of free radicals have been supposed to be involved in neuronal death in Parkinson's disease and ALS [7]. In addition, other pathogenic mechanisms have been proposed in these disorders, including glutamatergic neuroexcitotoxicity, as well as apoptotic neuronal death [8]. However, the real pathogenesis still remains to be proved and the occurrence of parkinsonism in elderly ALS patients needs to be determined.

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