

After 24 months follow up, cluster analysis showed that 47% (n=142) of PD patients remained stable, 17% (n=52) of patients were improved, 36% (n=109) were aggravated from baseline.

At baseline, aggravated PD patients were more frequently male (p=0.07), with older age (p=0.006), higher score of MMSE (p=0.06) and a less severe PD (p<0.0001). They were receiving higher dose of L-Dopa (550 mg/d) and equivalent L-Dopa (897 mg/d) as compared to improved patients (504 mg/d and 813 mg/d) and lesser dose as compared to stable patients (634 mg/d and 1028 mg/d).

**Conclusion:** We hypothesize there are three distinct subgroups that can explain PD progression.

### P3.136

#### Comorbid vascular diseases in patients with Parkinson disease and Parkinsonian syndrome – preliminary results

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**Objectives:** The cause of Parkinson disease is still unknown. Changes in cerebral blood flow are considered as one of important pathogenetic factors. Non-invasive neurosonology methods are known to be convenient tool in the assessment of vascular changes.

The aim of this study was to assess the character and frequency of comorbid vascular diseases in patients with Parkinson disease and Parkinsonian syndrome. The second aim was the comparison of changes in cerebral blood flow between these groups.

**Methods:** Eighty eight patients (36 women and 52 men, mean age 68.3±8.1 years) were included into the study. Parkinson disease was diagnosed in 66 patients and Parkinsonian syndrome in 22 of them. All patients were underwent internal and neurological examination, transcranial doppler examination.

**Results:** Arterial hypertension was observed in 14 patients (21.2%) with Parkinson disease and in 12 patients with Parkinsonian syndrome (54.5%) while coronary heart disease in 10 patients (15.2%) with Parkinson disease and in 11 patients (50%) with Parkinsonian syndrome.

Diabetes mellitus was diagnosed in 6 patients (9.1%) with Parkinson disease and in 4 patients (18.2%) with Parkinsonian syndrome.

Mean blood flow velocity and pulsatility index in patients with Parkinson disease were in normal range, while in patients with extrapyramidal syndrome mean blood flow velocity in middle cerebral artery was significantly lower and pulsatility index was significantly higher in patients with Parkinsonian syndrome.

**Conclusions:** Vascular diseases are more frequent in patients suffered from Parkinsonian syndrome then from Parkinson disease. Transcranial doppler examination is useful in differential diagnosis in patients with extrapyramidal damage.

### P3.137

#### A new high throughput PC-aided super-microisland method for primary dopaminergic neurons

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One of the most prominent features in Parkinson's disease (PD) is the degeneration of midbrain dopaminergic (DA) neurons. No treatment, which could stop the degeneration or restore the DA system, exists.

Several neurotrophic factors (NTFs) can protect and restore the dopaminergic system in *in vivo* and *in vitro* models of PD thereby giving a promising therapeutic lead. The future drugs for PD thus likely include a cocktail of NTFs to maximize the therapeutic effects. However, despite clinical relevance, methods, which can quantitatively evaluate cooperative effects of several NTFs on primary embryonic DA neurons, an essential step prior the *in vivo* studies, are currently limited due to assay reliability, arduous workflow, low throughput and low statistical power. Due to these limitations, analysis of NTF responses of DA neurons isolated from

single embryos or for example gene knock-out mice is nearly impossible.

Here we describe a new PC-aided "super-microisland" method. The method is based on minimizing the standardized culture area of DA neurons giving 8–10 data points per embryo for easy imaging and PC based quantification. The feasibility and high statistical power of the method is illustrated by confirming the survival promoting effects of GDNF and NRTN, and demonstrating the additive effects of GDNF and HBGAM cocktail. With the new method we show for the first time that DA neurons from mice lacking RET, the presumed main signaling receptor of GDNF are still, albeit to a lesser extent, GDNF responsive, suggesting presence of an alternate receptor for GDNF in the DA system.

### P3.138

#### Perturbation of protein thiol homeostasis through downregulation of glutaredoxin, a protein disulfide oxidoreductase, results in loss of DJ-1 through proteolysis

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Parkinson's disease (PD) is a movement disorder with an etiology relying both on environmental and genetic causes. Most cases are sporadic although the rare familial cases have provided an insight into the pathophysiology of PD. DJ-1 is a putative gene recessively linked to early onset PD. We examined the effect of global perturbation of protein thiol homeostasis on DJ-1 function using N2a cells. While loss of cellular GSH had no apparent effect on DJ-1, downregulation of thiol disulfide oxidoreductases, such as glutaredoxin (Grx1; cytosolic thiol transferase) resulted in loss of DJ-1 protein and cell death through a Daxx translocation dependent mechanism. We exogenously expressed DJ-1 both as wild-type and redox inactive mutants along with shRNA to Grx1. Overexpressed wild-type DJ-1 protein level was also decreased but it was able to attenuate cell death and Daxx translocation while the mutant protein levels remained unaltered and did not afford protection suggesting the importance of oxidative modification of DJ-1 at the cysteine residues. Further, pretreatment with protease but not proteasomal inhibitors prevented the DJ-1 loss. In conclusion DJ-1 is susceptible to oxidative modification upon downregulation of Grx1 but not GSH and oxidatively modified DJ-1 is rapidly lost through action of protease(s). DJ-1 loss has been reported in mouse models of PD though role of protease(s) remains to be determined. Thus, loss of DJ-1 occurring through dysregulation of protein disulfide oxidoreductases may contribute to the pathogenesis of sporadic PD and links environmental challenges to the expression of this putative PD gene.

### P3.139

#### Functional and design-based stereological assessment of the MPTP-induced heart atrophy in mice: a short communication

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Functional and structural changes were evaluated in the heart of the MPTP-induced mice—a suitable model for studying Parkinson's disease. Ten 4-months-old male mice were divided into two groups: (G1) (n=5) MPTP-induced (40 mg/kg) and (G2) (n=5) saline-induced (0.9%). The following procedures were performed: open field test, haemodynamic parameters, blockade of the heart

innervation, HPLC and stereology, i.e. the left heart ventricle volume was obtained by Cavalieri's principle. Preliminary results are shown as mean (CV). The locomotion time in the central arena was: G1 = 115.3 s (0.16) and G2 = 110.9 s (0.24); locomotion time in the thigmotaxic arena: G1 = 184.7 s (0.10) and G2 = 189.1 s (0.14); total distance: G1 = 1,966.7 cm (0.28) and G2 = 1,415.2 cm (0.14); mean speed: G1 = 6.6 cm/s (0.28) and G2 = 4.7 cm/s (0.14). The heart rate was: G1 = 514.5 bpm (0.06) and G2 = 485.5 bpm (0.15); mean arterial pressure: G1 = 84.1 mmHg (0.05) and G2 = 80.3 mmHg (0.15); vagal effect: G1 = 122.9 bpm (0.36) and G2 = 153.5 bpm (0.57); sympathetic effect: G1 = 47.7 bpm (0.82) and G2 = 108.9 bpm (0.22); vagal tonus: G1 = 50.4 bpm (0.50) and G2 = 79.2 bpm (0.56); sympathetic tonus: G1 = 215.9 bpm (0.19) and G2 = 195.4 bpm (0.34); intrinsic heart rate: G1 = 451.4 bpm (0.13) and G2 = 474.1 bpm (0.12). The concentration of noradrenaline was: G1 = 0.74 pg/g (0.01) and G2 = 0.90 pg/g (0.01); adrenaline: G1 = 0.18 pg/g (0.07) and G2 = 0.20 pg/g (0.49); L-dopamine: G1 = 1.06 pg/g (1.17) and G2 = 0.59 pg/g (0.77); dopamine: G1 = 14.7 pg/g (0.10) and G2 = 91.34 pg/g (0.17). The left heart ventricle volume was: G1 = 89.7 mm<sup>3</sup> (0.05) and G2 = 99.1 mm<sup>3</sup> (0.08). The atrophy of the left heart ventricle ( $p = 0.0001$ ) may be the result of the MPTP injection, which may also be reflected in the decrease in the concentrations of the heart catecholamines.

### P3.140

#### An analysis of the relationship shared by environmental toxin exposure and movement disorders

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**Background:** Parkinson's disease (PD) is a movement disorder (MD) characterized by neurodegeneration. More than 1.5 million people in the U.S. are affected by PD, with an additional 60,000 people diagnosed each year. Both genetic and environmental factors have been analyzed as suspected causes of PD, but no definitive causal agent has been determined. Prior research has investigated the effects of exposure to environmental toxins, such as metals and pesticides, on the etiology of PD. However, there is limited research on the effect of toxin exposure on MD symptoms. Accordingly, we investigated the effects of environmental toxin exposure on the symptoms of MD.

**Methods:** We collected data from 47 participants with PD and essential tremor (ET) in the areas of general demographics, symptoms, social and medical history, changes in cognition, sleep habits, and other areas before and after deep brain stimulation surgery.

**Results:** Analysis of 47 participants with PD and ET (13 females, mean age 67.38; 34 males, mean age 64.03) revealed differences in MD between those exposed to toxins and those not exposed. There were significant differences between the groups in the symptoms of akinesia, salivation, action tremor, and years of instability. There was also a significant difference in diagnosis type. Of those with PD, 40.7% reported exposure to toxins compared with 0.07% of ET participants.

**Discussion:** Our data suggest a relationship between toxin exposure and diagnosis type. However, further research is needed to elucidate the causal effects of toxin exposure on MD symptoms to better understand their progression.

### P3.141

#### Swift purification of tyrosine hydroxylase from leaves of *Mucuna prurens* and its implication to gene therapy in Parkinson's disease

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**Background:** Deficiency in tyrosine hydroxylase (TH, EC 1.14.16.2) gene expression leads Parkinson's disease and an autosomal

recessive form of dopa-responsive dystonia (DRD). We have purified a highly efficient and catalytically active TH from the leaves of *Mucuna prurens* for their implication in Parkinson patient's gene therapy.

**Methods:** The enzyme was purified by ammonium sulphate precipitation and gel filtration chromatography (Heparin-Sepharose). Enzyme purity was analyzed by western blot augmented with IgG mouse monoclonal antibody (Santa Cruz Biotechnology; USA) followed by 2D gel electrophoresis.

**Results:** The purified enzyme catalyzed the formation of L-dopa from tyrosine required the tetrahydrobiopterin as co-enzyme. The specific activity of purified enzyme was 1023.518 nKat mg<sup>-1</sup>. The molecular mass of TH was estimated to be 55 kDa by SDS-PAGE. The enzyme displayed a pH optimum of 6.0–7.5 in 100 mM tris buffer. Catalytic activity in vitro displayed a linear time course (60 min) and reached its half maximum value at 25 μM L-tyrosine. The enzyme was activated by Fe(II) while higher concentration of Mg(II) reduced the activity.

**Conclusion:** TH from *Mucuna* showed very high catalytic activity as compared to the reported literature. The molecular mass of the enzyme showed similarity with molecular mass of mouse as well as human. Purified enzyme reacts efficiently with antibody raised from mouse. The antisense DNA synthesized on the basis of sequence analysis of purified enzyme might be an effective tool for the gene therapy in patients suffering from Parkinson's disease.

### P3.142

#### Synaptic plasticity of corticostriatal and thalamostriatal systems in Parkinson's disease

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The striatum is the entrance of information to the basal ganglia circuitry. It receives glutamatergic innervation from the cerebral cortex and thalamus. Both cortical and thalamic terminals contact dendritic spines of striatal projection neurons, except for thalamic inputs from the caudal intralaminar nuclei, that terminate predominantly on dendritic shafts. Despite evidence for functional plasticity of glutamatergic transmission in the striatum, very little is known about the morphological and neurochemical substrate underlying these effects. In this presentation, I will review evidence from our laboratory and others showing that both cortical and thalamic glutamatergic synapses in the striatum are endowed with a significant degree of structural plasticity, which likely underlies functional changes in these two neural systems in Parkinson's disease. Striatal dopamine denervation results in as much as 50% loss of dendritic spines on both direct and indirect striatofugal neurons in the sensorimotor striatum of MPTP-treated monkeys. This spine loss is an early feature of parkinsonism correlated with the degree of dopamine denervation, but not parkinsonian motor symptoms. The striatum expresses a higher level of vesicular glutamate transporter 1 (vGluT1), a specific marker of corticostriatal terminals, in parkinsonian condition. The remaining spines contacted by cortical or thalamic inputs in the striatum of parkinsonian monkeys undergo ultrastructural remodeling characterized by an increased spine head volume and postsynaptic density area accompanied with an increased volume of pre-synaptic glutamatergic terminals. Thus, striatal projection neurons undergo major structural changes in Parkinson's disease, which likely underlie functional alterations in corticostriatal and thalamostriatal glutamatergic transmission.