2017 International Congress of
Parkinson's Disease and Movement Disorders

Basic Science Abstracts

June 4–8, 2017

VANCOUVER

British Columbia, Canada

International Parkinson and
Movement Disorder Society

MDS-0417-480

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Circadian and Homeostatic Modulation of Multi-unit Activity in Dopaminergic and Striatal Structures

K. Fifel (Leiden, Netherlands)

Objective: The aim of this study is to investigate the circadian and homeostatic modulation of the multi-unit activity of dopamineric and striatal neuronal structures.

Background: Several neurological disorders associated with Basal Ganglia dysfunctioning, like Parkinson's and Huntington's diseases, are characterised by seriously debilitating sleep abnormalities. The involvement of Basal Ganglia in sleep modulation has been recently documented. However, the reciprocal modulation of Basal Ganglia activity by sleep-wake dependent processes is unknown.

Methods: We combined Electroencephalogram (EEG) recordings with electrical multi-unit activity (MUA) in different subdivisions of both Midbrain Dopaminergic structures [Substantia nigra lateral (SNL, n=6), Substantia nigra Medial (SNM, n=5), Ventral Tegmental area (VTA, n=6)] and striatal structures [Striatum Latero-dorsal (STR-LD, n=4), Striatum Medio-dorsal (STR-MD, n=4), Ventral striatum (STR-V, n=4)] under 12:12 light/dark (LD) and constant darkness (DD)conditions. We also investigated the effects of a 6h sleep deprivation on MUA in these areas.

Results: Both under LD and DD conditions, the MUA in the areas examined showed a vigilance state dependency with the highest firing rates during wakefulness and REM sleep compared to NREM sleep ($p<0.001$, t-test). Interestingly, striatal subdivisions displayed different sensitivities towards changes in homeostatic sleep pressure as evidenced by EEG Slow Wave Activity.

Conclusions: Our results indicate that circadian and homeostatic processes influence the activity of midbrain dopaminergic and striatal structures. These influences may contribute to behavioural changes observed in neurological disorders related to dysfunctioning in the Basal Ganglia.

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Riding the puzzle: deep brain stimulation and the non-motor symptoms in Parkinson's disease

A.C. Campos, E. Talamoni Fonoff, C. Hamani, R. Lima Pagano (São Paulo, Brazil)

Objective: To better understand the benefits of DBS in non-motor symptoms.

Background: Parkinson's disease(PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons, generating motor and non-motor symptoms, and its treatments are eminently symptomatic. The treatments begin with an effective pharmacological approach that evolves negatively with extreme complications. At this stage, the gold standard of treatment is the deep brain stimulation (DBS) of the subthalamic nucleus (STN), however the evidence of improvement of non-motor symptoms such as depression and pain has been conflicting, which corroborates to the wane of quality of life and it is been often neglected.

Methods: Male Wistar rats were submitted to an intrastriatal injection of 12 μg of 6-hydroxydopamine (6-OHDA) or saline in the left hemisphere. Then, the animals were implanted or not with stainless steel electrodes in the left STN. All animals were submitted to the paw pressure test (hyperalgesia behavior) before and after 7 days of the surgery. In the 8th day, the animals were divided in four groups: saline or hemiparkinsonian without implant; hemiparkinsonian implanted but not stimulated (control of the implant); and hemiparkinsonian rats implanted and stimulated for 5 days during 2 h (130 Hz, 60μs). After the last stimulation, animals were submitted to the apomorphine-induced rotation test, and in the other day, they were submitted to the paw pressure test, catalepsy test and forced-swimming test (depression-like behavior).

Results: The model was characterized by the increase in the contralateral rotation in hemiparkinsonian rats without stimulation, and in the immobility of these animals in the catalepsy test. The DBS animals presented less contralateral rotation to the lesion side and catalepsy behavior, showing an improvement of motor deficits. As regards to the non-motor symptoms, our DP model reproduced the finds in humans inducing a decrease in 60% of the nociceptive threshold in both posterior paws and inducing an increase of 45% in immobility in the forced swimming test. However, the DBS was able only to reverse the pain caused by the nigrostriatal lesion, without interfered with the depressive-like behavior.

Conclusions: Our preliminary results showed that STN-DBS improved the motor and painful behaviors related to PD, but more research is necessary to understand the mechanisms and ameliorate the results of the stimulation.

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Is cognitive decline similar among Parkinson's disease motor sub-types? A prospective study examining changes over time in gait, balance and cognition

T. Herman, L. Arie, S. Shema-Shiratzky, N. Giladi, J. Hausdorff (Tel Aviv, Israel)

Objective: To investigate whether changes over time in cognitive and motor functions differ across the postural instability gait difficulty (PIGD) and tremor dominant (TD) motor sub-types.

Background: Parkinson's disease (PD) is traditionally classified into PIGD and TD motor sub-types. Mild cognitive deficits are increasingly recognized as a common non-motor symptom in PD, however, cognitive differences among those two motor sub-types have not been well-described.

Methods: Non-demented patients with PD who were previously classified into PIGD (n=30) and TD (n=27) were followed for an average of 60 months. At baseline and at follow-up, participants underwent cognitive testing using the Montreal Cognitive Assessment (MoCA), and a computerized neuropsychological assessment battery. Parkinsonian motor symptoms using the motor part of the UPDRS, gait under single and dual task conditions, the Timed Up and Go (TUG) and the Berg Balance test were also evaluated.

Results: At baseline, the two sub-types were similar with respect to age ($p=0.652$), disease duration ($p=0.628$), UPDRS-III ($p=0.328$), and all cognitive functions ($p>0.29$). The Berg balance test was significantly lower in the PIGD group ($p=0.007$). At follow-up, basic characteristics, e.g., age, gender, disease duration were similar in the PIGD and TD. The global cognitive score declined by 9.5% in the PIGD group (from: 94.5±11.7 to 85.3±13.6, $p<0.001$), significantly larger ($p=0.03$) than the 4.5% decrease observed in the TD group, resulting in a significant group x time effect ($p=0.047$). Similar group differences ($p=0.006$) were observed in the decline of executive function, where a significant group x time effect was also found ($p=0.002$). Regarding motor aspects, there was a significant deterioration in the UPDRS-III, gait features and balance in both sub-types, but no interaction effect.

Conclusions: In both sub-types, development of clinically significant gait and balance problems were associated with disease progression. Conversely, the course of cognitive decline differed. These findings demonstrate that PIGD patients experience greater cognitive decline, compared to patients with TD, in specific domains of cognitive function, suggesting that perhaps certain treatments should be tailored more precisely to the motor sub-type.

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Genetic susceptibility associated to impulse control disorders and compulsive behaviors in Parkinson's disease from a Spanish population

S. Jesus, C. Tejera-Parrado, C. Cortes, M. Bonilla-Toribio, I. Bernal-Bernal, M. Labrador, L. Vargas-González, M. Bernal, A. Adarmes, F. Carrillo, M. Carballo, P. Gómez-Garre, P. Mir (Sevilla, Spain)

Objective: Our aim was to identify if there is any difference in the genetic variants linked to an increased susceptibility to suffer impulse control disorders (ICDs) or compulsive behaviors (CBs) in Parkinson's disease (PD) compared to healthy controls (HC).

Background: ICDs and CBs are non-motor symptoms that frequently appear in PD. The treatment with dopamine agonists are markedly linked to both conditions. Nevertheless, a susceptibility genetic background has been described in PD patients since some variants in dopamine metabolism genes seem to be involved in increasing the risk of develop ICDs and CBs.

Methods: We included 365 PD patients (60% males, 64.8±11.5 years) and 382 HC (50.7% males, 59.5±15.3 years). ICDs and CBs were assessed using QUIP-RS questionnaire. Demographic data were recorded in both groups. Clinical features and dopamine replacement therapy data were collected in PD group. Variants in *DRD2* (rs1800497, rs1800496), *DRD3 (*rs6280), *COMT* (rs4680), *GRIN2B* (rs1806201, rs7301328) and *HTR2A* (rs6313) were analyzed in both groups. The genotyping was carried out using the TaqMan technology. Allelic association study was done using PLINK software.

Results: ICDs and CBs were more frequent in PD compared to HC ($p<0.001$). Adjusted by age and sex this result remained significant. In HC some variants (rs6280, rs1806201, rs4680 and rs6313) had a tendency to influence in isolated ICDs or CBs. In the PD group, the variant rs6313 in *HTR2A* was associated to a higher susceptibility to suffer ICDs ($p=0.04$). Accounting by specific ICDs and CBs, this variant was significantly associated to gambling, hypersexuality, compulsive shopping and hobbysm.

Conclusions: Some variants may influence in ICDs or CBs in PD and HC. The polymorphism rs6313 in *HTR2A* (serotonin receptor) may act on the serotonin function and have an important impact on ICD and CBs in PD.

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AAV-mediated human alpha synuclein overexpression in the locus coeruleus (LC) leads to a neuronal loss in the nucleus ambiguus of mouse

B. Lee, M. Henrich, W.-H. Chiu, L. Matschke (Marburg, Germany)

Objective: To investigate the impact and temporal aspects of the neuronal loss in the LC neurons induced by alpha-synucleinopathy.

Background: In the Braak staging model of Parkinson's disease (PD), Lewy pathology progresses in a caudo-rostral pattern from the locus coeruleus (LC) to the substantia nigra (SN). The impact and temporal aspects of the neuronal loss in the LC neurons induced by alpha-synucleinopathy has neither been reported nor used as a prodromal animal model of PD.

Methods: We have performed a unilateral microinjection of recombinant adeno-associated viral vectors (rAAV) carrying human WT-aSYN, A53T-aSYN or luciferase as a control reporter in male C57Bl/6 mice. In total 1.25 µl volumes of rAAV vectors were delivered in the right hemisphere of the LC at coordinates (from dura) AP -5.4 mm, ML -0.9 mm, DV -3.7 mm. Eight animals in each group were sacrificed 3 weeks post the injection by a transcardial perfusion of PBS followed by ice-cold 4% paraformaldehyde. Immunohistochemistry for tyrosine hydroxylase (TH) for the LC and choline acetyltransferase (ChAT) for the medulla oblongata was performed to quantify neurons using stereological analysis.

Results: 3 weeks post aSYN overexpression we have found an approximate 16% neuronal loss of the LC in the injected hemisphere of rAAV WT-aSYN and A53T-aSYN compared to the non-injected side. The relative numbers of LC neurons in each hemisphere of WT-aSYN or A53T-aSYN group were significantly decreased when compared to that of rAAV-luciferase injected. In addition, we discovered that the number of ChAT positive neurons in the rostral nucleus ambiguus also significantly decreased in both rAAV WT-aSYN and A53T-aSYN injected animals, when compared to that of rAAV-luciferase injected.

Conclusions: rAAV WT-aSYN or A53T-aSYN overexpression in the LC caused a significant neuronal loss in the LC as early as three weeks post injection. Moreover, aSYN seems to play a role in the cell viability of neurons in the nucleus ambiguus. This result may imply a potential direct or indirect role of noradrenergic neurons in modulating motor innervation of the upper gastrointestinal system.

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Histological analysis of a-synuclein pathology in the circadian system in Parkinson's disease

E. De Pablo-Fernandez, J. Holton, T. Warner (London, United Kingdom)

Objective: Assessment of severity of a-synuclein pathology in the circadian system in patients with Parkinson's disease (PD).

Background: Circadian system is responsible for the 24-hour rhythm of physiological function. The central pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus which regulates the production of melatonin (the main endogenous entraining agent) by the pineal gland. Evidence suggests a disruption of the circadian system in PD with important clinical implications in motor and non-motor symptoms. Although a disruption of melatonin secretion has been shown, the neuroanatomical site of dysfunction of the circadian system in PD remains unclear.

Methods: Formalin-fixed hypothalamic and pineal tissue was obtained from patients with a histological diagnosis of PD and sex- and age-matched healthy controls from the Queen Square Brain Bank archive. Vasointestinal peptide immunohistochemistry was used for identification of the SCN. a-Synuclein immunohistochemistry severity was assessed in the SCN and pineal gland tissue using a semiquantitative score (0-4) as per neuropathological criteria.

Results: A total of 13 SCNs and 17 pineal glands (from a total of 28 PD patients) were compared with 4 SCNs and 7 pineal glands from 11 controls. a-Synuclein pathology was present in 9 (69.2%) of the SCNs of PD patients but in none of the controls ($p = 0.025$). a-Synuclein pathology was only present in the pineal gland of 2 PD cases (11.8%) but in none of the controls, and its severity did not show any significant differences ($p = 0.354$).

Conclusions: Our study shows that Lewy body pathology is significantly more severe in the SCN but not in the pineal gland in PD patients comparing with healthy controls. These findings suggest that disruption of central regulation within the SCN (rather than melatonin production by the pineal gland) may be responsible of the altered circadian melatonin output in PD.

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Long-term levodopa therapy accelerates the circadian rhythm dysfunction in 6-OHDA rat model

D. Lv, Y. Wang, F. Wang, X. Zhang, X. Gu, Y. Yang (Suzhou, People's Republic of China)

Objective: We aimed to study the effect of L-DOPA on circadian rhythms in 6-OHDA lesioned rats, and to clarify whether the disturbance of the circadian system in PD patients was associated with the disease progression itself, or the long-term L-DOPA replacement therapy.
Background: Parkinson disease (PD) patients with long-term L-DOPA treatment are suffering from circadian rhythm abnormalities, including impaired sleep-wake cycles, disrupted fluctuations of temperature, blood pressure, heart rate, hormonal levels and many other biological processes.
Methods: PD model was constructed by a bilateral stereotaxic injection of 6-OHDA into the striatum. 21days later, the rats received intraperitoneal administration of saline or 25mg/kg of L-DOPA once daily for another 21 consecutive days. Rotarod test, footprint test and open field test were carried out to evaluate the motor function. Next, we collected SCN, striatum, cortex, liver and plasma at ZT4 (Zeitgber Time), ZT10, ZT16, ZT22. Quantitative PCR was used to analyze the mRNA levels of Clock, Bmal1, Per2, Rora; ELISA detected the levels of melatonin and cortisol; HPLC analyzed the expressions of D1R, D2R in striatum and cortex.
Results: Daily injection of L-DOPA alleviated the motor deficits induced by 6-OHDA lesions. And then, we observed the expression of different clock genes in different tissues. After L-DOPA treatment, compared with 6-OHDA group. The rhythm of Clock was abolished and phase of Per2 was reversed from a nocturnal to a diurnal pattern in SCN compared with 6-OHDA group. In striatum, the expression of Bmal1, Rora was lower than that in the 6-OHDA group at ZT10, but the amplitude of Clock was elevated in cortex at four time points in L-DOPA group. In liver, L-DOPA unaltered did not affect the rhythmicity and levels expression of the four clock genes; in addition, secretion of the cortisol secretion was increased and melatonin was further inhibited after L-DOPA treatment at ZT22. Furthermore, the expression of D2R was decreased in the striatum in of 6-OHDA lesions lesioned rats but D1R remained unchanged unaltered in cortex.
Conclusions: Our research indicated that severe performance in circadian system of advanced PD patients owing to not only the progressive degeneration of the disease, but also the continuous L-DPOA treatment.

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Efficacy of tozadenant in animal models of non-motor symptoms of Parkinson's disease
C. Kostrub, C. Kenney, C. Riemer, E. Prinssen, A. Flohr, J.L. Moreau (Ardsley, NY, USA)
Objective: Investigate the potential of tozadenant (TOZ) to alleviate the non-motor symptoms of Parkinson's disease using multiple animal models.
Background: TOZ is an oral, selective adenosine A2a receptor antagonist that is being developed for the treatment of Parkinson's disease (PD). TOZ improves motor function in animal models of PD (Michel et al, 2015), has shown promise in early clinical investigations as a treatment of motor symptoms of PD (Hauser et al, 2014), and is currently in phase 3 clinical testing for the treatment of PD as an adjunct to L-dopa therapy. The biology of the A2a receptor and the mechanism of action of TOZ raises the possibility that it could also modulate the non-motor symptoms of PD. Thus, TOZ was investigated in animal models of depression and anxiety.
Methods: TOZ was tested for its effect as a monotherapy in two animal models of depression, the rat forced swim test and the rat chronic mild stress-induced anhedonia test, and one model of anxiety, the rat elevated plus-maze.
Results: In the forced swim model, single oral doses of TOZ dose-dependently reduced time spent immobile, with a low dose of 3 mg/kg showing a slight (10.6%, p=0.13) reduction, and higher doses of 10 and 30 mg/kg showing significant reductions (27.3%, p=0.02, and 31.5%, p=0.003, respectively) in immobility time versus vehicle control. In the chronic stress-induced anhedonia model, once-daily IP doses of 1 or 3 mg/kg TOZ significantly reduced the anhedonia index in stressed animals compared to vehicle controls; the anhedonia index in TOZ-treated animals returned to pre-stress baseline level, suggesting a reversal of the stress-induced depression-like state. In the elevated plus-maze test, single oral doses of TOZ showed dose dependent anxiolytic effects, with the highest dose tested of 30 mg/kg showing significant effects on all response factors measured (e.g. 152% increase in time in open arms, p=0.02).
Conclusions: The doses used in these animal models result in drug exposures similar to those achieved in clinical studies on TOZ. The antidepressant and anxiolytic-like effects demonstrated in these animal models support further investigation of TOZ for the treatment of non-motor symptoms of PD in clinical trials.

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Differential effects of ventral or dorsal predominant dopaminergic denervation of striatum on development of dopamine dysregulation syndrome and punding in a rat model of parkinsonism
E. Özkan, G. Çakmakli, B. Elibol, E. Topçuoglu (Ankara, Turkey)

Objective: To model and assess the differential effects of ventral or dorsal predominant dopaminergic denervation of striatum on development of impulsive compulsive behaviors (ICB) in rats.
Background: In Parkinson's disease (PD), dopamine replacement therapy related ICB are associated with reduced quality of life. Molecular mechanism and a detailed anatomical basis of these behavioral alterations are still not known. Few studies demonstrated that punding may stem from a dopamine-dependent sensitization to appetitive stimuli within the dorsolateral striatum and DDS from the ventral striatum.
Methods: Bilateral 6-OHDA injection to ventral tegmental area or substansia nigra were performed for development of dorsal (n=21) or vental (n=17) selective dopaminergic denervation. Controls were sham operated (n=20). Conditioned place preference (CPP) paradigm was used to model DDS. In this model, the rewarding properties of low dose apomorphine (0.1mg/kg) was explored. Chronic intermittent injection of apomorphine (1mg/kg) was performed to induce repetitive behaviors that may both model dyskinesia and punding. Behavioral studies were rated by valid scales. The extent of dopaminergic denervation is mapped with tyrosine hydroxylase immunohistochemistry staining.
Results: All the dopamine denervated rats received chronic apomorphine injection developed dyskinetic behaviors. The severity of dyskinesia increased day by day and they were very strongly and positively correlated with mean lesion volume ($r=849$, $p<0.001$). Low dose apomorphine injection induced CPP in rats with parkinsonism but conditioned place avoidance in controls. The conditioning score was strongly and positively correlated with mean ventral lesion volume ($r=0.642$, $p<0.001$). Interestingly, stereotypic behaviors were attenuated in rats with dorsal predominat dopaminergic denervation.
Conclusions: In this study we show that development of DDS in PD may be related to the severity of ventral striatal dopaminergic denervation. On the other hand, we found that stereotypies were decreased in rats with dorsal predominant dopaminergic depletion. We think that the insufficiency of classical stereotypy scales in evolution of the rich repetitive behavioral repertoire in subjects with dopamine depleted striatum may account for this finding.

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Vmat2 heterozygote mice display motor dysfunction and progressive depressive-like behaviors

K. Ma, J. Huang, L. Liu, C. Han, X. Guo, S. Guo, L. Wang, Y. Shen, Y. Xia, F. Wan, N. Xiong, T. Wang (Wuhan, People's Republic of China)

Objective: To assess motor and depressive behaviors in mice that are heterozygous for VMAT2 (VMAT2 HT).
Background: Dopamine (DA) is compartmentalized by the vesicular monoamine transporter 2 (VMAT2; SLC18A2), located in the plasma and vesicular membranes of dopaminergic neurons, which regulate levels of DA in neuronal compartments. Previous studies indicated that mice with a 50% genetic reduction in VMAT2 HT displayed depressive phenotype while performed normal locomotor activity tests at the time of 24 months.
Methods: Vmat2 HT mice were provided by professor Uhl Gr and bred from heterozygote- heterozygote crosses of KO mice. Genotypes were confirmed by PCR. Vmat2 HT and WT littermates of 16 months old and 30 months old were assessed of motor function and depressive behaviors respectively. Locomotor activity was assessed as total distance moved in the open field test and total time consumed in pole test. Tail suspension test and sucrose preference test were conducted to observe depressive behaviors.
Results: At the time of 16 months old, total distance moved in the open field test and total time consumed in pole test were similar between WT littermates and VMAT2 HT groups. VMAT2 HT mice (123.1 ± 17.57s) display significant increase in immobility times in tail suspension test ($p<0.05$) and sucrose consume ($54.73\pm3.911\%$) were significantly decreased in Vmat2 HT mice ($p<0.05$). At the time of 30 months old, both the results of open filed test and total time consumed in pole test suggested that Vmat2 HT mice displayed motor dysfunction compared with WT littermates ($p<0.05$). As for depressive-like behaviors, immobility time in tail suspension test of VMAT2 HT mice was significantly increased and sucrose consume in VMAT2 HT mice ($40.92\pm7.655\%$) groups was significantly decreased ($p<0.05$).
Conclusions: Our data demonstrated that VMAT2 HT mice didn't display motor dysfunction until 30 months old while depressive-like behaviors seemed to progress since 16 months old.

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Impact of duopa on non-motor symptoms of Parkinson's patients with deep brain stimulation

A. Wadhwa, R. Govindarajan, I. Asher (Columbia, MO, USA)

Objective: To report the role and impact of duopa on non-motor parkinsonian symptoms in patients with deep brain stimulation within a month from starting therapy.

Background: Duopa (levodopa/carbidopa gel) has shown to improve dyskinesia and off periods in patients with advanced Parkinson's disease and is an alternative to deep brain stimulation (DBS). But there is limited data on its role in patients who already have DBS especially with regards to its impact on non-motor symptoms.
Methods: This is a retrospective chart review of all adult Parkinson's patients who have DBS and have undergone duopa. Clinical, psychosocial history and demographic data pre and post therapy were reviewed. A p value of <0.05 was considered statistically significant.
Results: 4 patients all caucasian males (ages: 56 years, 57 years, 69 years, and 78 years) with STN DBS (average off time: 5 hours daily) underwent duopa therapy. Post-therapy there was significant improvement in pain with pain scales on visual analog scale improving from average 8/10 to 3/10, (0-no pain, 10-worst pain), $p<.0.5$ within a month of starting therapy. In addition, all patients reported improved interest and motivation in doing activities and tasks. All patients had an average 2 falls within a month of starting therapy (2 orthostatic, 2 mechanical).
Conclusions: The addition of duopa in patients with DBS has significant improvement in pain, and improved interest and motivation in doing activities while there is an increased risk of fall associated with it.

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Tyrosine kinase inhibition clears tau and reserves neuropathology and motor symptoms in a novel model of progressive supranuclear palsy

Y. Torres-Yaghi, F. Pagan, A. Lawler, T. Kimbason, N. Starr, B. Wilmarth, M. Arellano, M. Javidnia, M. Hebron, C. Moussa (Washington, DC, USA)
Objective: Our objective was to examine the effects of tau accumulation in transgenic mice, observing both motor function and behavior.
Background: Tau hyper-phosphorylation is a critical factor in neurodegenerative diseases. To examine the effects of tau accumulation in a novel models of tauopathy we generated a transgenic mouse via modification of an existing strain that expresses the human P301L mutation of tau.
Methods: We bred hemizygous with hemizygous transgenic mice that express P301L tau under the control of a prion promoter (TauP301L).
Results: Accumulation of hyper-phosphorylated tau (p-tau) in hemizygous mice was predominantly detected in the hippocampus, cortex, brainstem and thalamus, reminiscent of tau pathology in human progressive supranuclear palsy (PSP). TauP301L mice show a significant increase in both human and murine p-tau and display motor symptoms that mimic PSP. Homozygous mice are underweight and have severe motor symptoms and may live up to 6 months, while hemizygous mice live longer (up to 1.5 years) and look normal but begin to show anxiety-like behavior and progressive motor abnormalities resulting in paralysis from 6 months old. Daily treatment of these mice with low doses of the tyrosine kinase inhibitors Nilotinib or Bosutinib led to p-tau clearance and improvement in motor symptoms.
Conclusions: Accumulation of p-tau in the brainstem, cortex and thalamus leads to parkinsonian-like symptoms that mimic human PSP and tyrosine kinase inhibition reduces tau pathology and reverses Parkinsonism.

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Viral-mediated oligodendroglial alpha-synuclein expression models multiple system atrophy

W. Meissner, F. Bassil, P. Guerin, N. Dutheil, Q. Li, M. Klugmann, E. Bezard, P.O. Fernagut (Bordeaux, France)
Objective: We aimed to develop AAV-based models of MSA.
Background: Multiple system atrophy (MSA) is a fatal neurodegenerative disorder characterized by a combination of autonomic dysfunction, cerebellar ataxia and L-dopa unresponsive parkinsonism. The hallmark of MSA is the accumulation of a-synuclein (a-syn) forming cytoplasmic inclusions in oligodendrocytes. Adeno-associated viruses (AAV) allow efficient targeting of disease-associated genes in selected cellular ensembles and have proven efficient for the neuronal overexpression of a-syn in the substantia nigra in the context of Parkinson's disease.
Methods: Chimeric AAV1/2 vectors expressing either human wild-type a-syn or the green fluorescent protein under the control of the mouse myelin basic protein were injected in the striatum of rats and monkeys. Rats underwent a longitudinal motor assessment prior to histopathological analysis at 3 and 6 months.
Results: Injection of AAV expressing a-syn in the striatum resulted in >80% oligodendroglial selectivity in rats and >60% in monkeys. Rats developed progressive motor deficits that were L-dopa unresponsive when assessed at 6 months. Significant loss of dopaminergic neurons occurred at 3 months, further progressing at 6 months, together with a loss of striatal neurons. Prominent a-syn accumulation, including phosphorylated and proteinase-K resistant a-syn was detected in the striatum and substantia nigra.

Conclusions: AAV-mediated oligodendroglial expression of a-syn allows replicating some of the key features of MSA. This flexible strategy can be used to investigate, in several species, how a-syn accumulation in selected oligodendroglial populations contributes to the pathophysiology of MSA and offers a new framework for the pre-clinical validation of therapeutic strategies.

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a-Synuclein preformed fibrils induce disruption of myelin basic protein expression in primary oligodendrocyte culture; deciphering glial pathology in Multiple System Atrophy

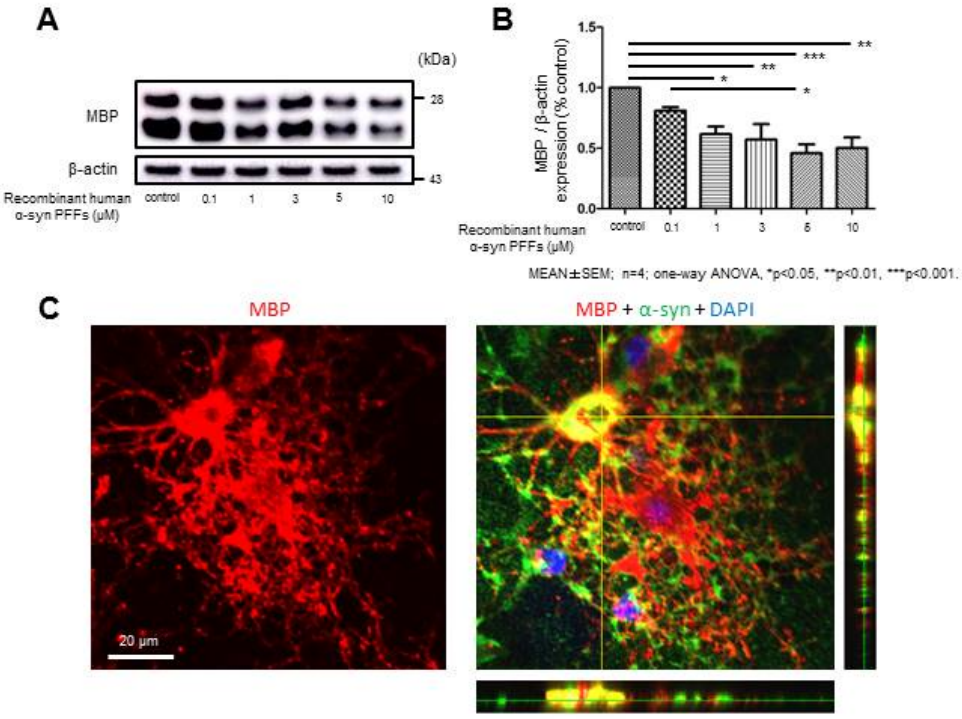
S. Kaji, T. Maki, N. Uemura, R. Takahashi (Kyoto, Japan)

Objective: This research focuses on the impact of extracellular a-synuclein preformed fibrils (a-syn PFFs) on oligodendrocytes (OLGs) as a potential contributor exacerbating the pathology of multiple system atrophy (MSA).

Background: The pathological finding in MSA is uniquely observed in OLGs, the myelin-forming cells of the central nervous system. The oligodendroglial a-syn aggregates, widely known as glial cytoplasmic inclusions (GCIs) are reportedly confirmed prior to neuronal loss in MSA brains (1). Another previous investigation detected myelin loss accompanied by preserved numbers of OLGs in MSA brains or transgenic MSA model mice brains, suggesting the existence of dysfunctional OLGs in association with a-syn accumulation (2).

Methods: Primary mixed glial cell culture was obtained from neonatal rats. OLGs culture was prepared through isolation of oligodendrocyte progenitor cells and induction of maturation. Bacterially expressed recombinant human a-syn was purified by ion exchange chromatography, followed by 3-7 days incubation with agitation at 37? for the use of a-syn PFFs. OLGs were incubated for twenty-four hours after a-syn PFFs application and subjected to immunoblot analysis as well as cell viability assays. Immunostaining was performed with confocal microscopy to evaluate the morphological change of OLGs as a result of a-syn PFFs application.

Results: Interestingly immunoblot analysis revealed unnegligible amount of endogenous a-syn in OLGs. While a-syn PFFs application did not significantly affect cell viability, the protein expression of myelin basic protein (MBP) was remarkably reduced in a concentration-dependent manner (figure 1 A, B). Immunostaining suggested that OLGs incubated with a-syn PFFs show morphological alteration characterized by fewer processes and decreased MBP expression compared with control OLGs (figure 1 C).



Conclusions: Our study indicated the possible contribution of extracellular a-syn PFFs to the production of MSA pathology in terms of OLGs dysfunction. Given that OLGs support neurons by forming myelin sheath, their dysfunction probably exacerbates neuronal activity, representing a critical pathological aspect of MSA.

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Neuroactive steroids reverse the dopaminergic neurotransmission defectiveness in chronic hepatic (HE) encephalopathy: a possible involvement in HE related parkinsonism

O. El Hiba, A. El Khiat, H. Gamrani (El Jadida, Morocco)

Objective: In the present study, we describe the changes of the dopaminergic system occurring in the cirrhotic rats and concomitantly we investigated the effect of DHEAS on this system in Sprague-Dawley rats using the expression of tyrosine hydroxylase (TH) as a neuronal marker.

Background: Hepatic encephalopathy (HE) is a neuropsychiatric disorder occurring as a consequence of both acute and chronic liver failure. Advanced HE is generally accompanied with extrapyramidal symptoms including rigidity and tremor, which may reflect alterations of the dopaminergic system. Recently we reported a beneficial effect of the neuroactive steroid dehydroepiandrosterone sulfate (DHEAS) in cirrhotic rats, however the mechanisms of such an effect by DHEAS were not addressed.

Methods: Rats were submitted to bile duct ligation (BDL) surgery and TH immunohistochemistry was assessed in the Substantia nigra pars compacta (SNc), striatum, ventral tegmental area (VTA) and the cortex.

Results: TH immunoreactivity showed a significant diminution in both SNc and VTA concomitantly with the cortical and the striatal outputs in the BDL rats vs. controls. Three daily injections of 5mg/kg of DHEAS to BDL rats significantly normalized TH expression decrease in both SNc and VTA as well as dopaminergic projections to the striatum and the cortex of BDL rats.

Conclusions: The present data support an involvement of the dopaminergic system in mild HE and a possible beneficial effect of the neurosteroid DHEAS as a potential pharmacological treatment of mild HE.

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Differential microRNA expression in a cohort of Parkinson's disease patients

A. Garvey, N. Cutfield (Dunedin, New Zealand)

Objective: To develop a blood test that will use micro RNA (miRNA) to identify patients with PD.

Background: PD is a progressive neurodegenerative disorder and by the time of clinical symptoms many midbrain structures have already been severely damaged. A need exists to identify PD before clinical symptoms arise to allow future disease modifying therapies to use early and appropriately.

Methods: 11 male patients were selected from a cohort of 51 PD patients based on gender, age and disease duration. They were age and gender matched against controls. All participants had early morning, fasting bloods collected and processed within 2 hours to separate plasma for RNA isolation. miRNA was amplified and purified using the mirVana™ miRNA isolation kit and quality assessed using the Nanodrop-1000. Pre-amplification was required before creating a cDNA library for analysis. PD samples and their control were analysed together on the same custom made array card from no replacement in drug list after reviewing the miRNA PD literature and including miRNA found from a similar Alzhiemer's study by our group.

Results: We found nine miRNAs to be differentially expressed between the PD and control groups. Five were down regulated and four up regulated. Two had an adjusted P value <0.05, the others of an unadjusted P value <0.05 with one having a 8.36 fold change. Five have been identified in relation to PD before; three in clinical cohorts, one in an autopsy series and one in both a clinical and an autopsy series.

Conclusions: We have found that miRNA expression differs between our PD group and their controls. We are currently increasing the cohort size to further validate our findings with the hope of providing a blood test that will help identify patients with PD.

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Evaluation of mitochondrial dysfunction in fibroblasts and iPSC-derived dopaminergic neurons of patients affected by Multiple System Atrophy

G. Monzio Compagnoni, E. Frattini, F. Fortunato, D. Ronchi, A. Bordoni, M. Garbellini, S. Salani, M. Guida, N. Bresolin, S. Corti, G.P. Comi, A. Di Fonzo (Milano, Italy)

Objective: The aim of the present study is to investigate mitochondrial functioning in fibroblasts and iPSC-derived dopaminergic neurons of patients affected by Multiple System Atrophy (MSA) and healthy controls.

Background: The pathogenesis of MSA is still widely unknown. Mitochondrial dysfunction is one of the pathogenetic mechanisms which have been conjectured so far.

Methods: Fibroblasts were isolated from skin biopsies of 7 MSA-P, 7 MSA-C and 5 healthy controls. Fibroblasts underwent several analyses to assess mitochondrial functioning: respiratory chain complexes' activity (spectrophotometric analyses), respiratory chain complexes amount (WB), O2 consumption (high resolution

respirometry), mitochondrial biogenesis (qPCR), mtDNA amount (qPCR). Induced pluripotent stem cells (iPSCs) were generated from 4 MSA (2 MSA-C and 2 MSA-P) and 4 controls through a viral method. iPSCs were differentiated towards dopaminergic neurons through an already described protocol. Most of the analyses described for fibroblasts (respiratory chain complexes' amount and activity, mitochondrial biogenesis, mtDNA amount) were performed also on neurons.
Results: MSA fibroblasts showed a mitochondrial deficit in comparison to controls. This observation was supported by the following findings (in some cases statistically significant): a reduction of the activity of respiratory chain (mainly in MSA-C), a reduction of O2 consumption (mainly in MSA-C) and an increase of mtDNA amount. No statistically significant differences were observed in neurons (probably also because of the limited number of cases and controls), but a trend supported many of the data observed in fibroblasts.
Conclusions: The present study strengthens the hypothesis of a mitochondrial dysfunction in MSA, outlines differences between MSA-P and MSA-C and identifies some possible mechanisms which may be responsible for the mitochondrial defect.

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Parkinsonism through astrocytic GABA induce motor symptoms
M.J. Lee, Y. Jang, J. Han, S.J. Kim, J. Kim, I. Ryu, X. Ju, M.J. Ryu, S.-Y. Choi, G.R. Kweon (Daejeon, Korea)
Objective: Although reactive gliosis is a prominent feature of PD, its role in pathogenesis has remained elusive. Here we show that aberrantly synthesized GABA from reactive astrocytes tonically inhibits neighboring dopaminergic neuronal firing in SNpc, reducing dopamine production and release, leading to parkinsonian motor symptoms.
Background: Parkinsonism is a clinical syndrome of movement abnormalities seen in Parkinson's disease (PD), which has been attributed to cell-autonomous mechanism of doparminergic neuronal death in the substantia nigra pars compacta (SNpc).
Methods: To identify the GABA from reactive astrocyte in PD, we used toxin induced model (MPP+, 6-OHDA rat model, MPTP mouse model) and overexpressed alpha synuclein mouse model. Moreover, we also assessed it in postmortem PD patients' brain.
Results: The released GABA from astrocyte tonically inhibits pacemaker action potential firing of neighboring DA neurons, resulting in reduced tyrosine hydroxylase expression and Parkinson-like motor symptoms. Impairments are fully restored by treatment with the MAO-B inhibitor, selegiline. The effects of glial GABA and selegiline were mimicked by optogenetic silencing and activation of DA firing, respectively. Brain samples of PD patients revealed a plethora of GABA-positive reactive astrocytes with a significantly increased MAO-B mRNA expression.
Conclusions: Our study proposes that glial GABA is inextricably linked to parkinsonism, which can arise even before substantial dopaminergic neuronal death.

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Rolipram, a PDE-IV inhibitor protects against experimental Parkinsonism in mice
N. Kumar, R. Khanna (Jaipur, India)
Objective: Rolipram, a specific inhibitor of the phosphodiesterase IV (PDE IV), has recently been shown to exert neuroprotective effects in an Alzheimer transgenic mouse model and in hypoxic-ischemic damage in the rat brain. It activates the cAMP-dependent protein kinase (PKA)/cAMP regulatory element-binding protein (CREB) signaling pathway and it inhibits inflammation. The cAMP mediated signaling is regulated by the activity of cyclic nucleotide phosphodiesterases (PDE) that cleave the second messenger. In the present study, we tested neuroprotective effects, if any, of rolipram drug, a specific inhibitor of the phosphodiesterase IV in 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in mice.
Background: Parkinson's disease (PD) is a neurodegenerative disease and a movement disorder characterized by loss of dopaminergic neurons in the substantia nigra causing dopamine depletion in the striatum.
Methods: Experimental animal is muscular weighing 25–30 g of 4–5-month-old. The drug was given four times at 12 h intervals by gavation (25–100 mg/kg) in animals made parkinsonian following two doses of MPTP (30 mg/kg, i.p.). Control mice were injected with the same volume of pure DMSO. MPTP-induced striatal dopamine depletion was significantly attenuated by higher dose of rolipram. MPTP-induced catalepsy and akinesia, as well as loss in swim ability, were blocked dose-dependently by rolipram. Brain was used for biochemical and histopathological study.
Results: Present study further shows that rolipram can dose-dependently attenuate both in vitro hydroxyl radical production in a Fenton-like reaction, and also ex vivo 1-methyl-4-phenylpyridinium (MPP+)-induced hydroxyl

radical generation in isolated mitochondria. These results indicate that the observed neuroprotective effects of rolipram stem from its significant antioxidant action.
Conclusions: The preliminary results suggest that rolipram is a neuroprotector, and mechanism other than lipid lowering action could be the basis of this effect. Present data show a neuroprotective effect of the PDE IV specific inhibitor rolipram against dopaminergic neuron degeneration, suggesting that PDE IV inhibitors might be a potential treatment for Parkinson's disease.

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Off-target implications of Nurr1 agonist therapy in Parkinson's disease

K. Steece-Collier, T. Collier, J. Stancati, C. Kemp, B. Daley, C. Sortwell (Grand Rapids, MI, USA)

Objective: To examine the impact of Nurr1 agonists on regulation of striatal Nurr1 and levodopa (LD)-induced dyskinesias (LIDs) in a rat model of Parkinson's disease (PD).
Background: A recent series of investigations have shown that 'Nuclear receptor related 1 protein' (Nurr1) agonist drugs or vector-mediated overexpression within nigral dopamine (DA) neurons can protect against alpha-synuclein and toxin induced death. These reports have sparked international enthusiasm that Nurr1 agonists may offer new promise for clinical neuroprotection. However, data from our labs and others demonstrate that dyskinogenic LD results in ectopic induction of striatal Nurr1, with levels correlating with LIDs severity. Whether systemic drugs that induce Nurr1 will exacerbate LIDs in parkinsonian subjects treated with LD remains unanswered.
Methods: We used the highly specific Nurr1 agonist amodiaquine (AQ) at the previously documented neuroprotective dose (20mg/kg; Kim et al., 2015). In Experiment 1 (Exp 1) intact rats received daily AQ or saline (Sal) for 28 days. In Exp 2, rats were lesioned with 6OHDA; 3 weeks after lesion, rats received 1 day of AQ or Sal pretreatment prior to receiving a single acute injection of LD (12mg/kg, sc) +/- AQ on day of sacrifice. All rats were sacrificed 60-180 mins after final AQ; brains were processed for Nurr1 RNAscope® in situ hybridization.
Results: Exp 1: Densitometry revealed widespread upregulation of Nurr1 transcript in brain including dentate gyrus ($p=0.014$), VTA ($p=0.021$), CA1 ($p=0.09$), anterior cingulate ($p=0.046$); AQ vs Sal, N=4/group. However, in the absence of DA depletion or LD, AQ had no effect on striatal Nurr1. Exp 2: When rats were rendered parkinsonian, acute pretreatment with AQ and a single dose of LD resulted in a 22-fold induction of striatal Nurr1 compared to intact striatum; LD+Sal resulted in 9-fold induction (ANOVA $p=0.0034$, Intact vs LD+Sal vs LD+AQ; LD+Sal vs LD+AQ, $p<0.05$; N=4/grp). We are currently analyzing the impact of chronic LD +/- AQ on LIDs and motor behaviors.
Conclusions: The highly specific Nurr1 agonist AQ results in robust elevation of Nurr1 in multiple brain regions including striatum where it has been linked to induction of LIDs. Aberrant Nurr1 activation in striatum and regions associated with addiction and memory following systemic agonist treatment has the potential for significant off-target side-effects in PD patients.

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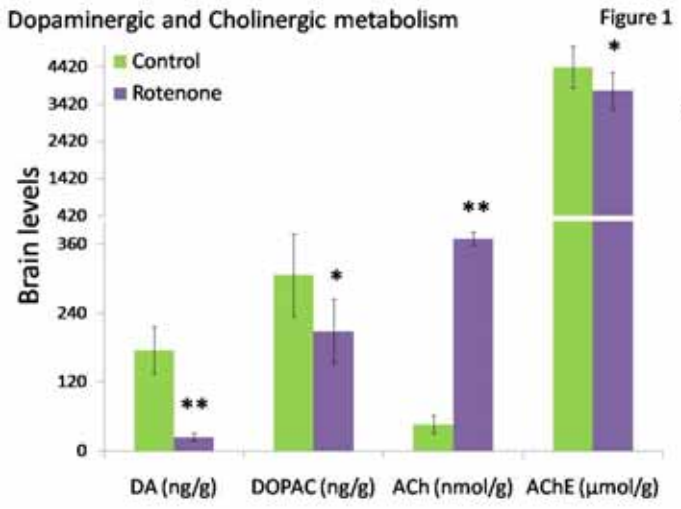
Imbalance between dopaminergic and cholinergic neurotransmission following rotenone administration suggestive of Parkinson's-like symptoms in male rats

S. Madiha, S. Haider (Karachi, Pakistan)

Objective: In the present study we analyzed rotenone-induced gait abnormalities, muscular weakness and locomotor deficits in rats. The study also evaluated the effects of rotenone on acetylcholinesterase (AChE) activity and relationship between brain dopamine (DA) and acetylcholine (ACh) levels and their association with Parkinson's-like symptoms.
Background: Rotenone (widely used as an organic pesticide and specific inhibitor of mitochondrial complex I) produces features in rats that recapitulate behaviorally, biochemically and neurochemically the symptoms of Parkinson's disease (PD). However, some aspects remain indistinct regarding the effects of rotenone as an animal model of PD.
Methods: In the study, adult male rats were administered intraperitoneally with rotenone at a dose of 1.5 mg/kg/day for eight days. Motor activity and muscular strength were monitored by the inclined plane test, footprint test, beam walking test, pole test and Kondziela's inverted screen test. Animals were decapitated after behavioral analysis and brains were dissected out for biochemical estimation such as antioxidant enzyme activities and acetylcholinesterase (AChE) activity and neurochemical estimation was also performed by HPLC-EC.
Results: Results showed that the level of DA and its metabolite were significantly decreased (figure 1) which was in turn reflected by significant impaired motor coordination in rotenone treated rats in all observed behavioral parameters (table 1). Along with these the level of reduced glutathione (GSH) and superoxide dismutase (SOD)

activity declined significantly which ultimately increased lipid peroxidation (LPO) (figure 2). Moreover, AChE activity was significantly decreased and ACh levels significantly increased in brains of rotenone administered rats (figure 1).



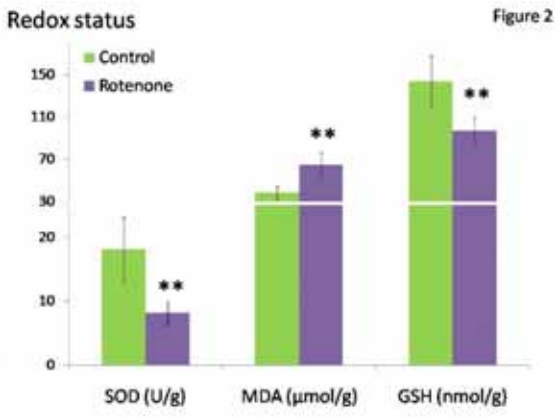
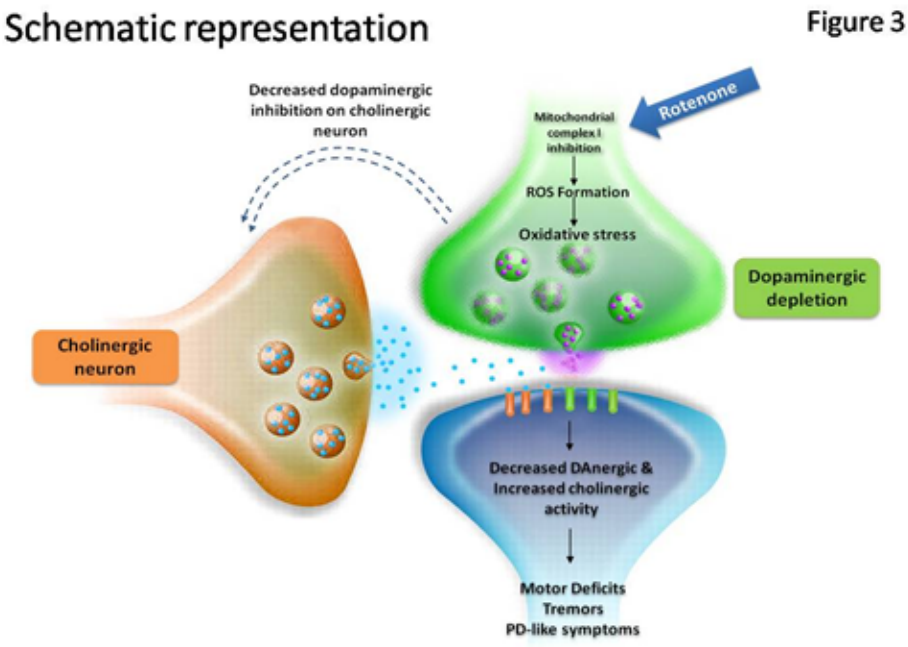


Table 1

Effects of intraperitoneal injection of rotenone on muscular strength and motor coordination.

		Control (n=6)	Test (n=8)	
Pole test Time to descend (sec)	6th day	38.33±11.2	29.20±8.4	p>0.05
	7th day	19.16±3.93	8.45±2.08	p<0.01
Konziela test Time of falling (sec)	6th day	87.85±16.5	62.59±13.2	p<0.05
	7th day	99.83±29.3	45.62±13.5	p<0.01
Inclined plane test	% Cataleptic score	2.03±0.44%	12.23±3.1%	p<0.01
	Time to scroll down (sec)	17.33±1.03%	37.44±6.02%	p<0.01
	Frozen body posture score	0±0	4.25±0.46	p<0.01
Beam walking test	Latency to cross (sec)			
	3 cm beam	9.8±2.15	36.53±8.6	p<0.01
	2 cm beam	4.1±0.98	32.07±6.2	p<0.01
	1 cm beam	7.8±2.1	46.43±9.0	p<0.01
	Number of slips			
	3 cm beam	0±0	2.75±0.7	p<0.01
	2 cm beam	0±0	1.3±0.26	p<0.01
	1 cm beam	1.8±1.1	4.6±1.30	p<0.01
Footprint	Forelimb stride length (cm)	9.91±0.5	7.62±0.5	p<0.01
	Hind limb stride length (cm)	10.06±0.6	7.72±1.2	p<0.01
	Front base width (cm)	5.47±0.31	5.48±0.13	P>0.05
	Hind base width (cm)	5.30±0.48	6.53±0.71	p<0.01
	Right overlapping (cm)	1.26±0.2	1.76±0.3	p<0.01
	Left overlapping (cm)	1.46±0.3	1.55±0.4	p>0.05



Conclusions: In conclusion, this study further provides indication that rotenone administration in rats produces PD-like symptoms which is evident by behavioral, neurochemical and biochemical changes (figure 3). This study also highlights the rotenone-induced imbalance between dopaminergic and cholinergic modulation which is exhibited in PD.

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Motor dysfunctions in rats following repeated exposure to stress on the neurotoxicity of lambda-cyhalothrin

R. Shukla, R. Gupta, A. Pant, V. Khanna (Lucknow, India)

Objective: To investigate the targets associated in regulating motor functions following subsequent exposure to stress on the neurotoxicity of lambda-cyhalothrin in rats.

Background: Stress that intensifies the toxicity of environmental chemicals has been reported in number experimental studies although the exact mechanism associated with is not clearly understood. The present study has therefore been carried out to investigate the impact of forced swim stress (FSS), a physical stressor on the neurobehavioral toxicity of lambda-cyhalothrin (LCT), a new generation type-II synthetic pyrethroid is used to control insects, pests and ectoparasites in agriculture and veterinary practices including public health programmes.

Methods: Rats were treated with FSS (placed in glass cylinder filled with water for 3 min/day) for 28 days or treated with LCT (3.0 mg/kg body weight, p.o.) for 3 days (on days 26, 27 and 28) or pre-exposed to FSS for 28 days followed by LCT treatment for 3 days. Effect on motor performance was assessed by standard protocol. Plasma corticosterone and biogenic amine levels in corpus striatum were estimated by RP-HPLC. The sensitivity of striatal dopamine receptor and related signaling was also assessed.

Results: Pre-exposure to FSS followed by LCT treatment in rats resulted to increase plasma corticosterone levels and disrupt the blood brain barrier permeability. Further, decrease expression of DA-D2 receptors, alteration in levels of DA, DOPAC and HVA in corpus striatum along with impaired in motor activity have also been observed in these rats as compared to rats exposed to either FSS or treated with LCT alone. Interesting to it, no significant effect on neurotransmitter levels of DA, DOPAC, HVA and DA-D2 receptors in corpus striatum including plasma corticosterone levels, blood brain barrier permeability associated with spontaneous locomotor activity and motor co-ordination has been observed in rats exposed to either FSS or treated with LCT alone as compared to controls.

Conclusions: The results demonstrated that stress significantly synergises the neurotoxicity of LCT through DA-D2 receptors singling which regulates the motor functions.

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D2 receptor mediated DARPP32/PP1a signaling is responsible for cadmium induced Parkinson's like behavioral alterations in rats

R. Gupta, R. Shukla, V. Khanna (Luckno, India)

Objective: To identify the specificity of dopamine D2 receptor mediated signaling in cadmium mediated motor alterations

Background: Cadmium, a heavy metal, is to known to exert the toxicity in human body because of its wild industrial and anthropogenic applications. Human exposure to cadmium may therefore occur both in occupational and non-occupational settings and poses a serious risk to health and associated problems. Epidemiological and experimental studies carried out also suggest the role of cadmium in the progression of neurodegenerative diseases like Alzheimer's and Parkinson's. The studies carried out mechanism and the targets associated with this have not clearly been understood. The present study has therefore been aimed to investigate the mechanism of cadmium induced motor dysfunction.

Methods: Male Wistar rats were exposed to cadmium at a dose of 5.0 mg/kg, bw, p.o. for 28 days. Motor assessment in rats was done by using open field activity test, Rota rod and grip strength test. The other parameters were assessed by using the specific western blotting, RTpcr and IHC studies the specific radioligand technique was used to asses the integrity of Dopamine D2 receptors. Further, computational studies were also carried out using the auto dock4.2.

Results: A decrease in the motor activity and motor coordination was significantly observed in rats exposed to cadmium. The transcriptional and translational changes in DA-D2 receptors were found to be decreased with no changes in DA-D1 type receptors in cadmium exposed rats. Further the specific radioligand technique carries out using the Spiperone also confirm the finding of involvement of DA-D2 receptor in cadmium mediated motor alterations. A change in the DARPP32/PP1a also found to target the CREB which are found to be associated with functional changes.

Conclusions: The results of the present study significantly inhibit the involvement of DA-D2 type receptors mediated signaling in regulating the Parkinson's like motor dysfunctions in rats.

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Exploiting sequence data of genes involved in synthesis of plant derived enzyme inhibitors of Acetylcholinesterase that involve in antineurodegenerative acitivity

N. No surname, M. Abdin (New Delhi, India)

Objective: Sequence analysis of genes involved in acetylcholinesterase inhibitors biosynthesis from various plant species

Background: The neurodegenerative disease like Alzheimer's disease and Parkinson's disease involve the deterioration of central nervous system caused by rapid hydrolysis of the neurotransmitter such as acetylcholine. The plant kingdom possesses a vast variety of natural compounds that have antineurodegenerative function, and have fewer side effects; extracts of a number of medicinal plants (such as Withania) have shown significant antineurodegenerative properties. The plant derived acetylcholinesterase inhibitors (AChEi), mainly alkaloids, have shown significant downregulation of acetylcholinesterase thereby increasing the acetylcholine level in brain. There is also a significant correlation between acetylcholinesterase inhibition and observed cognitive improvement.

Methods: In this study, Sequence homology and phylogenetic analysis has been performed by using NCBI database, ClustlW and MEGA 6.0 software.

Results: Sequence analysis of genes involved in acetylcholinesterase inhibitors biosynthesis from various plant species revealed their evolutionary conservation. *In silico* studies of these enzyme inhibitors also provided insight to the domain organization and various important motifs present in sequences.

Conclusions: Sequence analysis based on homology and conservation of genes for acetylcholinesterase inhibitors may lead to the effective drug designing for treatment of Alzheimer's disease and associated disorders.

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Salivary biomarkers for Huntington's disease (HD)

J. Corey-Bloom, A. Aikin, S. Park, A. Haque, A. Nathan, D. Granger, S. Granger, E. Thomas (La Jolla, CA, USA)

Objective: The objective of the current study was to assess the potential for saliva to serve as a biospecimen for accessible biomarkers for Huntington's disease (HD).

Background: Peripheral biomarkers are greatly needed in the field of neurodegenerative disorders to anticipate onset of disease symptoms, monitor disease progression, and track potential therapeutic effects. Huntington's

disease (HD) is a fatal, inherited neurodegenerative disorder caused by a CAG repeat expansion in the gene encoding the protein, huntingtin (Htt). Pathogenesis is associated with expression of the mutant Htt (mHtt) protein in the CNS; however, HD is also associated with abnormalities in peripheral tissues. The Htt protein is the most significant molecular target for disease modifying therapies, and several therapeutic approaches directed at its production, processing, and/or turnover are under development for impending clinical trials in HD. Since non-invasive methods to quantify Htt in the CNS do not exist, measuring Htt and other disease proteins in peripheral cells represents an essential step in biomarker discovery for HD.
Methods: In the current study, we measured Htt protein in saliva from manifest HD patients, gene-positive premanifest HD patients, and age- and sex-matched normal individuals (total n=178) using Western blots and ELISA methods. Additional salivary analytes, including alpha amylase, cortisol, C-reactive protein (CRP), and uric acid, were also measured using standardized ELISAs.
Results: Salivary total Htt levels were significantly increased ($p=0.0012$) in saliva from HD individuals (mean=0.775 ng/ml) compared to normal controls (mean=0.359 ng/ml). Salivary total Htt did not vary over time of day or over different days, nor were there age or gender effects. Additionally, salivary mHtt levels were higher in gene positive premanifest HD subjects compared to normal controls ($p<0.05$). CRP, a widely used biomarker of systemic inflammation, was found to be significantly ($p=0.025$) elevated in premanifest HD subjects (9,548 pg/ml) compared to normal controls (3,399 pg/ml) and may be an early marker of disease onset. Levels of other salivary proteins, alpha amylase and uric acid, were not significantly different between HD patients, premanifest subjects, or normal controls.
Conclusions: Measurement of salivary Htt and other disease proteins offers significant promise as relevant, non-invasive biomarkers of disease onset and progression in HD.

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Inhibiting sphingosine-1-phosphate lyase as a possible therapy in Huntington's disease
E. Furr Stimming, J.F. Manchon, A. Tsvetkov (Houston, TX, USA)
Objective: To advance our understanding of the process by which the mutant huntingtin (mHTT) protein contributes to neurodegeneration and translate that knowledge to potentially effective disease modifying therapies.
Background: Huntington's disease (HD) is a hereditary progressive neurodegenerative disease for which there is no approved disease modifying therapy. While significant progress has been made in understanding the pathophysiology of HD, the process by which mHtt causes cell death remains in question. Sphingosine and sphingosine-1-phosphate (S1P) are signaling lipids that differentially regulate cell fate, whereby sphingosine is associated with apoptosis while S1P generally promotes cell survival. A recent metabolomics analysis revealed that S1P is a disease-associated metabolite in HD, and that S1P levels are significantly downregulated in striatal neuronal progenitor HD cells. We recently demonstrated that sphingosine-1-phosphate lyase (S1PL), an enzyme that irreversibly metabolizes S1P, regulates neuronal autophagy, and identified S1PL as a possible therapeutic target. Novartis recently reported that S1PL inhibitors may be new possible agents against multiple sclerosis. Earlier, inhibition of S1PL in models of lung injury, myopathy, rheumatoid arthritis 8, and atherosclerosis has been suggested as a therapeutic strategy.
Methods: We use an HD model based on cultured striatal and cortical neurons, the most affected in HD, derived from embryonic rats. A novel microscopy system, an automated imaging and longitudinal analysis, enables us to track large cohorts of individual neurons over their lifetimes. We expressed S1PL in neurons, imaged them over time, and then applied statistical approaches used in clinical medicine to determine if the enzyme modulates the degradation of mHtt and affects neuronal survival.
Results: We found that S1PL decreases flux through autophagy and inhibits the degradation of mHtt. In a neuron model of HD, pharmacologically inhibiting S1PL with THI protected neurons from mHtt-induced neurotoxicity. We plan to determine if inhibiting S1PL alleviates disease phenotypes in HD mice.
Conclusions: These results identify S1PL as a novel therapeutic target in HD and provide a new target for developing therapies for neurodegenerative disorders.

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Evaluation of serum sestrin protein in Parkinson's disease: a plausible diagnostic marker
S. Dey, N. Rai, A. Singh, S. Shekhar, A. Dey (New Delhi, India)
Objective: In this study, we evaluated the concentrations of sesn1 and sesn2 in the serum of 50 PD patients and 60 elderly controls. Aging is a major risk factor for Parkinson's disease (PD).

Background: PD is characterized by loss of dopaminergic neurons of substantia nigra and presence of cytoplasmic inclusions (Lewy bodies).The muscular rigidity and atrophy with bradykinesia is common in PD. Sestrins (sesn), an antiaging protein, plays an important role in aging related diseases, owing to their antioxidative capacity, which prevents reactive oxygen species (ROS) formation and through regulation of pathways like AMPK-mTOR. Sestrins are shown to be key inducers of autophagy, which is generally considered to be beneficial for preventing age associated disease like PD.
Methods: The concentration sestrin proteins were evaluated by using surface plasmon resonance. The serum sestrin levels were further compared using western blot.
Results: The results showed significant decline ($P<0.0001$) in the levels of sesn1 protein in the sera of PD group (95% CI: 8.05-8.70 ng/µl) as compared to control group (95% CI: 12.36-14.51 ng/µl). However, an elevated level of sesn2 ($P<0.0001$) was found in the sera of PD group (95% CI: 15.52-17.11 ng/µl) but not in control group (95% CI: 13.28-14.37 ng/µl). A highly sensitive, selective cutoff values for sesn1 (9.41 ng/µl) and sesn2 (14.90 ng/µl) was determined after the construction of ROC curve to differentiate PD from controls.
Conclusions: This study opens up the important insights about the role of sestrins in the progression of PD and may help to establish sestrins as potential candidate for protein marker in detection of PD.

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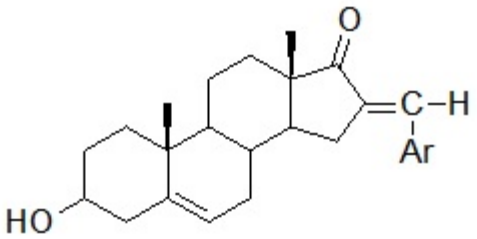
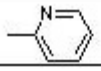
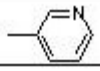
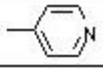
Synthesis and pharmacological evaluation of 16-aryldieno steroids as anti-parkinsonian agents in LPS induced Neuroinflammation Model of Rat

R. Bansal, R. Singh (Chandigarh, India)

Objective: The present study is aimed at design and synthesis of new therapeutically useful steroidal neuroprotective derivatives as anti-*parkinsonian* agents. The new 16-aryldieno steroidal derivatives have been synthesized by fusing pyridyl moiety at 16 position of the steroid. These D ring modified heterosteroids were then explored for their neuroprotective effects against PD.
Background: *Parkinson's disease* (*PD*) is a long-term degenerative disorder of the central nervous system mainly effecting the motor system and characterized by symptoms such as tremors, bradykinesia, rigidity, postural instability and cognitive deficits. Neuroinflammation play a key role in the pathogenesis of PD.
Methods: Aldol condensation of dehydroepiandrosterone with requisite pyridine carboxaldehyde in basic medium gave corresponding 16-arylidene steroidal derivatives **1a-c**. Rats (male wistar) were anesthetized with thiopental sodium (45 mg/kg, i.p.), stereotaxic surgery has been done and intranigral injection of LPS (10µg in 2µl) was infused into left substantia nigra using the Hamilton microsyringe.[3] Heterosteroids were evaluated against behavioral alternations using actophotometer, elevated plus maze at dose 2mg/kg after 7th, 14th and 21st day of LPS administration. Biochemical estimation of different makers for neuroinflammation, cholinergic activity and oxidative stress has also been carried out.



	a	b	c
Ar			

Results: The newly synthesized compounds were characterized by elemental and spectral analyses (IR, ^{1}H NMR). The synthesized heterosteroids **1a-c** exhibited potent neuroprotective activity against PD. The pyridin-4-yl group substituted steroid **1c** displayed activity comparable to that of standards dexamethasone and celecoxib.
Conclusions: This study suggests that 16-aryldieno steroids exhibit potent neuroprotective activity and they could be useful for the prevention of PD.

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Mutant DNAJC13 modulates accumulation and toxicity of alpha-synuclein through altered endosomal trafficking in cell and fly models of Parkinson's disease

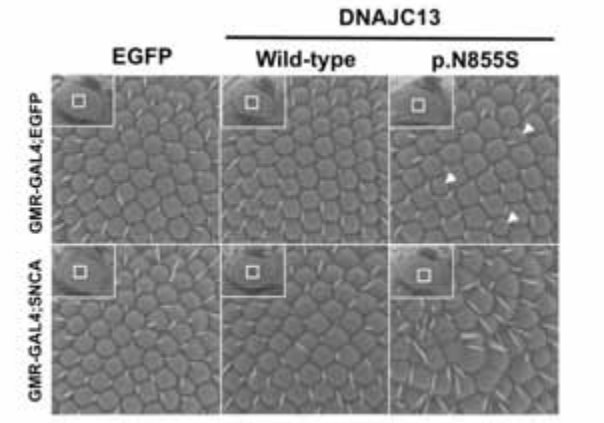
T. Hasegawa, S. Yoshida, M. Suzuki, J. Kobayashi, N. Sugeno, K. Sekiguchi, M. Edura, A. Kikuchi, A. Takeda, Y. Nagai, M. Aoki (Sendai, Japan)

Objective: The aim of this study is to investigate the effect of DNAJC13 mutation on the vesicle transport, α-synuclein (αSYN) distribution/catabolism, and neurodegeneration using a combination of cell culture and fly models.

Background: Recently, DNAJC13 have been identified as the causative gene for the PARK21-linked familial form of PD. Patients with DNAJC13 mutation represent late-onset, dopa-responsive parkinsonism with αSYN-positive Lewy bodies in the the substantia nigra and locus coeruleus. DNAJC13 is known as the mammalian homolog of receptor-mediated endocytosis 8 (RME-8), a DnaJ domain-containing protein originally identified in a screen for endocytic defect in Caenorhabditis elegans. RME-8 localizes to membrane of the early endosome and interacts with Wiscott-Aldrich and Scar Homolog (WASH) complex and sorting nexin 1 (SNX1), thereby contributes to the endosomal protein sorting. However, precise mechanism by which mutant DNAJC13 causes accumulation of αSYN and neuronal cell death leading to PD still remains unknown.

Methods: To generate human DNAJC13 transgenic (Tg) flies, pUAST plasmids encoding myc-tagged human wt or p.N855S mutant DNAJC13 were microinjected into Drosophila embryos. Eye phenotype was evaluated after crossing DNAJC13 Tg flies with control or human wt-αSYN Tg flies under eye-specific GMR-GAL4 driver. Locomotor activity was evaluated by climbing assay according to the methods described elsewhere. To investigate the effect of mutant DNAJC13 expression on intracellular trafficking, COS7 cells expressing GFP-tagged wt or p.N855S mutant DNAJC13 were co-transfected with mStrawberry-tagged Rab GTPases. The influence of mutant DNAJC13 on subcellular distribution of αSYN was determined using COS7 cells stably expressing HA-αSYN by Western blot analyses. In some experiments, cells over-expressing wt or mutant DNAJC13 were incubated with reference molecules including Alexa555-labeled transferrin or pHrodo-EGF. Time-lapse images of internalized reference molecules were acquired under confocal laser scanning microscope.

Results: The p.N855S mutant DNAJC13 Tg fly showed subtle, but significant irregularity of ommatidial array and size, locomotor impairment and loss of dopaminergic neurons, which were markedly exacerbated by the co-expression of human αSYN (Fig. 1, white arrowhead). Co-expression of mutant DNAJC13 with HA-tagged αSYN in COS7 cells resulted in the abnormal accumulation of αSYN in the endosome compartment. Intriguingly, this finding was accompanied by the impaired trafficking of reference molecules from the early to the late and/or recycling endosomes as well as disorganized assembly of intracellular actin cytoskeleton.



Conclusions: DNAJC13 mutation impairs specific endosomal trafficking and causes the aberrant endocytic retention of αSYN, which might thereby contributes to the neurodegeneration process leading to PD.

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Disease-modification in Translational Models of Parkinson's disease by the Rho Kinase Inhibitor Fasudil

L. Tönges, L. Tatenhorst, J. Koch, T. Outeiro, M. Zweckstetter, M. Bähr, P. Lingor (Bochum, Germany)

Objective: To evaluate the therapeutic potential of the Rho Kinase (ROCK) inhibitor Fasudil in translational models of Parkinson's disease (PD).

Background: There is still a strong need for a disease-modifying treatment of PD because former neuroprotective approaches have all failed. ROCK has recently been identified as a novel molecular target for PD but also for other neurodegenerative disorders such as ALS and is strongly implicated in the regulation of neuronal cell survival, axonal outgrowth and neuroinflammation.
Methods: ROCK was inhibited with Fasudil in lesioned primary midbrain dopaminergic neurons and cellular survival, neurite outgrowth as well as neuroinflammation were evaluated. In the MPTP and 6-OHDA toxin-based and in the human alpha-Synuclein (A53T) overexpressing genetic animal model of PD Fasudil was orally applied and both motor behaviour as well as neuropathological alterations were examined.
Results: In different lesioning primary neuronal cell models of PD Fasudil increased dopaminergic cell survival and stimulated neurite outgrowth which could be attributed to an increased phosphorylation of Akt or recompartmentalization of alpha-Synuclein. Chronic oral administration of Fasudil in the systemic lesion MPTP-model enhanced dopaminergic cell survival and fostered the regeneration of dopaminergic axonal terminals together with a restoration of dopamine levels in the striatum. In the local striatal lesion 6-OHDA-model Fasudil elicited an improved axonal regenerative response while under genetic overexpression of human alpha-Synuclein (A53T) its midbrain pathology was significantly reduced. All animals tolerated Fasudil very well and exhibited an improved motor behavior as evaluated in the cylinder test or Catwalk gait analysis.
Conclusions: Since Fasudil is already licensed for human use for the treatment of subarachnoid hemorrhage-induced vasospasms and has a very favorable safety profile in humans, our data strongly support the large potential of Fasudil as a pharmacological agent for disease-modification in PD.

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The novel Parkinson´s disease locus RIT2 and alpha-synuclein function in intersecting pathways
M. Volta, J. Obergasteiger, C. Überbacher, C. Ascione, C. Corti, A. Hicks, P. Pramstaller (Bolzano, Italy)
Objective: We aim to describe the molecular pathways at the crossroad of etiology and susceptibility in PD, focusing on Rin, coded by the recently associated locus RIT2, and its interaction with alpha-synuclein (aSyn).
Background: A disease-modifying therapy based on a pathogenic mechanism currently represents the main clinical need in PD. Accumulation of aSyn is the hallmark pathology of PD but its biology is still unclear. The protein Rin is a small GTPase that couples nerve growth factor (NGF) stimulation to ERK and p38 MAPK pathways. Its role in the PD context is currently unknown.
Methods: Ric (the Rin ortholog) was silenced in *D. melanogaster* expressing human aSyn and subjected to negative geotaxis. Cell lines stably expressing wild-type and A53T aSyn (SK-N-SH) or Rin (SH-SY5Y) were obtained. SH-SY5Y cells expressing wild-type and G2019S LRRK2 were kindly provided by Dr. Evy Lobbestael (University of Leuven). Protein and mRNA levels were analyzed by western blotting and real-time PCR, respectively, while immunocytochemistry was performed to assess single cell protein expression. Kinase activation was measured using the AlphaScreen SURE fire assay (Perkin Elmer).
Results: Silencing of Ric in flies ameliorated aSyn-induced climbing deficit. RIT2 mRNA levels are reduced in aSyn and LRRK2 cell lines, but NGF is able to stimulate kinase activation. Further, basal p38 activity is enhanced in Rin cells. Lastly, LRRK2-G2019S (but not wild-type) cells display pSer129-aSyn positive intracellular staining. In a preliminary experiment, transient overexpression of Rin-GFP (but not GFP alone) seems to reduce the percentage of GFP+ cells with pSer129-aSyn staining.
Conclusions: We show that Rin functionally affects aSyn biology. In addition, we confirm NGF activates Rin-ERK/p38 MAPK pathways. Future experiments will investigate the role of this pathway on synaptic transmission. Importantly, Rin appears to have a role in the phosphorylation/aggregation of aSyn in cell lines, triggered by another etiological cause of PD, (G2019S-LRRK2). Collectively, a common pathological pathway involving these proteins seems to occur in cells.

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GBA mRNA is diminished in brain and blood of Lewy body diseases
K. Beyer, J. Campdelacreu, L. Ispierto, D. Vilas, R. Reñé, R. Alvarez (Badalona, Spain)
Objective: To find out whether GBA deficiency starts at the transcriptional level and if it also involves alternative splicing. To study GBA expression changes in blood of patients with LB diseases and to check if these may be suitable as biomarkers.
Background: Parkinson's disease (PD) and dementia with Lewy Bodies (DLB) are Lewy body (LB) diseases characterized by abnormal alpha-synuclein deposits and overlapping pathological features. Several studies have shown that glucocerebrosidase (GBA) deficiency is involved in the development of LB diseases.

Methods: The expression of three *GBA* transcript variants (GBAtv1, GBAtv2 and GBAtv5) was analyzed in samples from 20 DLB, 25 PD and 17 control brains, in two areas each, and in blood of 20 DLB patients, 26 PD patients and 17 unaffected individuals. Relative mRNA expression was determined by real-time PCR with transcript-specific primers and sybr-green was used as detection dye. Beta-actin and beta-glucuronidase were used as housekeeping genes in brain, and beta-actin and porphobilinogen deaminase were the housekeeping genes in blood. Expression changes were evaluated by the deltadeltaCt method.
Results: In DLB brain, specific expression profiles were identified in the temporal cortex with the diminution of two of the three tested transcript variants. In PD brains, the caudate nucleus showed decrease of GBA mRNAs. In blood, significant GBA mRNA diminution was found in both DLB and PD patients. Of these, early PD patients showed lowest GBA levels which were normal in PD patients with advanced disease. Moreover, GBA mRNA levels were most decreased in early-onset DLB and late-onset PD corresponding in both cases to more aggressive forms of the diseases.
Conclusions: Disease group-specific GBA expression profiles were found in the most affected areas of LB diseases. In blood, GBA expression is diminished in LB diseases, especially in early onset DLB and in early PD patients. Age of disease onset exerted an opposite effect on GBA expression in DLB and PD correlating with disease progression rates.

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Impaired stress-induced mitophagy in parkinsonian LRRK2 (R1441G) knockin mutant mice

P.W.L. Ho, C.S.C. Lam, H.F. Liu, G.C.T. Leung, L. Li, M.H.-W. Kung, D. Ramsden, S.L. Ho (Hong Kong)

Objective: 1. Morphological changes and accumulation of ubiquitinated mitochondria in the striatum of aged leucine-rich-repeat kinase 2 (LRRK2) R1441G knockin mutant mice (Liu et al., 2014, 2016); 2. Mechanism of LRRK2 mutation in defective mitochondria turnover in mutant mouse embryonic fibroblasts (MEFs).
Background: Autophagy and ubiquitin-proteasome system maintain mitochondria homeostasis and turnover. Autophagic stress, a key pathological feature of Parkinson's disease (PD), perturbs mitochondrial quality and energy homeostasis in neurons. LRRK2 mutation is the most common genetic risk of PD. Studies suggested a putative role for LRRK2 in macroautophagy. We hypothesize that LRRK2 mutation disrupts mitophagy process causing defective mitochondria accumulation in aged mutant brains.
Methods: For mitochondria morphology, aged mouse striatal sections were fixed and examined under transmission electron microscopy. Total number of mitochondria were quantified and compared with wildtype (WT) controls (20 randomized photomicrographs x 3 animals). Freshly isolated striatal mitochondria were immuno-labeled by anti-ubiquitin antibody and MitoTracker™. The degree of ubiquitination in total mitochondria pool was determined by flow cytometry. MEF cultures isolated from LRRK2 mutant mice or their WT littermates were treated with rotenone (250uM) or FCCP (10uM) for 0, 30 and 120 min. Autophagic response was determined by levels of autophagic markers (LC3B-I/II and Lamp-1) and immunocytochemistry.
Results: Smaller but higher total number of mitochondria was seen in aged LRRK2 mutant mouse striatum as compared to their age-matched WT controls. The relative proportion of ubiquitinated mitochondria was higher in the mutant mice. Abnormal perinuclear clustering of enlarged lysosomes were observed in mutant but not WT MEFs under normal culture condition. Activation of autophagy was observed in both WT and mutant MEFs after exposure to rotenone or FCCP. However, mutant MEFs have significantly lower levels of total LC3B (LC3B-I/II) than the WT controls suggesting autophagosome depletion.
Conclusions: Accumulation of ubiquitinated mitochondria in aged LRRK2 mutant mice indicated impaired mitochondria turnover, possibly due to depletion of LC3B for autophagosome maturation and abnormal clustering of lysosomes.

Acknowledgement: This study was supported by the Henry G. Leong Professorship in Neurology and the Donation Fund for Neurology Research (SLH).

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Regulation of aberrant striatal oscillations by glutamate receptor blockade in parkinsonian non-human primates

A. Singh, S. Papa (Minneapolis, MN, USA)

Objective: To study the abnormal striatal oscillations and regulation of striatal oscillatory activities by micro-infusion of glutamate receptor antagonist in the non-human primate model of parkinsonism.

Background: Dopamine depletion in Parkinson's disease (PD) has been associated with abnormal oscillatory activities in the cortico-basal ganglia network (i.e. cortex, globus pallidus, and subthalamic nucleus). However, oscillatory activity in the striatum following dopamine loss and chronic replacement therapy remains poorly defined. In the current study, striatal local field potentials (LFPs) were recorded during "off", "on" and "on-with-dyskinesia" states in advanced parkinsonian primates. Additionally, glutamate receptor antagonist known to stabilize neuronal firing changes after L-dopa administration was used to regulate the abnormalities in striatal activity.
Methods: Striatal LFPs were recorded in MPTP-treated monkeys with standard techniques. The NMDA receptor antagonist LY235959 was injected into the striatum at the recording site before the systemic injection of levodopa. Striatal LFPs were analyzed during "off", "on" (after local artificial CSF or NMDA antagonist and systemic levodopa administration), and subsequently during "on"-with-dyskinesia states.
Results: A peak with the higher amplitude (relative power) in the 8-13 Hz (alpha frequency band) was observed in the "off" state. This peak significantly decreased in the "on" state (local aCSF and LY235959). However, a peak with higher amplitude in the 13-20 Hz (low-beta frequency band) was observed during "on" state. The NMDA antagonist reduced the peak amplitude in 13-20 Hz during the "on" state. No clear peak was observed in relation to dyskinesia.
Conclusions: Results indicate that the dopamine response in parkinsonian primates is associated with a reduced peak in 8-13 Hz and an increased peak in 13-20 Hz in striatal oscillations. A reduction of glutamatergic overactivity can regulate the striatal oscillations in response to levodopa in the advanced stage of PD.

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Modeling Parkinson's disease pathology by combined injection of fibrilar and monomeric a-synuclein in rat brain

P. Thakur, L. Breger, M. Lundblad, O. Wan, B. Mattsson, K. Luk, V. Lee, J. Trojanowski, A. Björklund (Frankfurt, Germany)

Objective: Development of a fast and progressive Parkinson's disease (PD) model in rat.
Background: a-Synuclein is a common link between sporadic and familial PD. Rodent PD models based on a-synuclein overexpression have been extremely helpful in elucidating the molecular pathology of disease. However, most of these models are extremely slow to develop and require unrealistically high amounts of a-synuclein to elicit disease features.
Methods: Female SD rats were injected with a low dose of AAV6-a-syn in substantia nigra (SN) to express monomeric a-synuclein. After 4 weeks, when the a-synuclein is fully expressed, sonicated pre formed fibrils of a-synuclein were injected in same spot. Some rats received either fibrils or AAV6-a-syn only. These rats were histologically evaluated at 3, 12 and 24 weeks post fibril injection.
Results: Fibrils and AAV6-a-syn alone group showed only 10-30% loss of TH positive cells in SN at 3 and 12 weeks. It took either of them 24 weeks to escalate this loss to 50%. However, the combination significantly accelerated this cell loss displaying 55% decrease in just 3 week. Further worsening of this cell loss was relatively slower and reached around 65% after 24 weeks. In contrast, TH fiber density in striatum decreased more gradually changing from 60% at 3 weeks to 40% at 24 weeks in the combination group. Similar to cell loss, fiber density loss was much slower in the fibrils or AAV6-a-syn alone groups. Further, a large number of neurons in the combination group expressed phosphorylated a-synuclein (p-syn), which is a marker for aggregated a-synuclein. These aggregates were dense, mature and spread through the nucleus, cytoplasm and dendrites at all time points. In comparison, AAV6-a-syn only group displayed diffuse cytoplasmic occurrence and small puncta in nucleus. Fibrils only group displayed far fewer aggregates occurring mostly in cytoplasm. This combination also elicited neuroinflammation, causing 2-fold increase in the number IBA-1 positive glial cells, which was not observed in other groups.
Conclusions: Ability of fibrils seeds to nucleate the aggregation of monomeric a-synuclein enhanced the pathological process both in time and intensity. Activation of neuroinflammation could be the additional mechanism acting to enhance pathology.

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Brain insulin resistance in Parkinson's disease

F. Bassil, M.H. Canron, N. Dutheil, A. Vital, E. Bezard, P.O. Fernagut, W. Meissner (Bordeaux, France)

Objective: To test the hypothesis that brain insulin resistance (i.e. decreased insulin/insulin like growth factor (IGF)-1 signaling) occurs in the substantia nigra pars compacta (SNc) and the putamen, two structures that are strongly involved in the neurodegenerative process in Parkinson's disease (PD).

Background: PD is a progressive neurodegenerative disorder characterized by the accumulation of alpha-synuclein in neurons forming Lewy bodies. Studies point to a potential involvement of abnormal insulin/IGF-1 signalling in the pathogenesis of PD as serum and cerebrospinal fluid levels of IGF-1 in PD patients are increased. Moreover, a small open-label phase 2 clinical trial assessing the effects of the glucagon-like peptide-1 (GLP-1) agonist exenatide, a well-tolerated FDA-approved antidiabetic drug that facilitates insulin/IGF-1 signalling, has reported beneficial effects on motor symptoms and cognition in PD patients. A follow up study showed persisting positive effects one year after the end of the study.
Methods: Insulin resistance was assessed by measuring protein expression levels of insulin receptor substrate-1 phosphorylated on serine 312 and serine 616 (IRS-1pS312/S616) in postmortem brain samples of PD patients and brains of AAV-SYN rat, a PD rodent model based on the overexpression of A53T mutated human alpha-synuclein (h-a-synA53T) by using adeno-associated viral vectors. The extent of dopamine loss in AAV-SYN rats was documented by stereological quantification of tyrosine hydroxylase (TH) positive neurons in the SNc.
Results: We here show increased IRS-1pS312 expression levels in PD patients and AAV-SYN rats. Specifically, neurons of the putamen and surviving TH positive neurons of the SNc in PD patients and its animal model counterpart showed increased IRS-1pS312 expression.
Conclusions: Our results suggest that insulin resistance plays a role in the pathogenesis of PD. They further provide the rationale for pursuing the clinical development of GLP-1 analogues for treating PD.

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Neuroinflammation in the substantia nigra is triggered by synucleinopathy and precedes nigral degeneration
M. Duffy, T. Collier, K. Luk, M. Tansey, K. Paumier, D. Fischer, N. Polinski, C. Kemp, C. Sortwell (Grand Rapids, MI, USA)
Objective: To determine the relationship between synucleinopathy, neuroinflammation and nigrostriatal degeneration using the rat a-syn PFF model.
Background: No therapy exists to halt or slow nigrostriatal degeneration in Parkinson's disease (PD). Neuroinflammatory markers are observed in *post mortem* PD brains, and longitudinal PET imaging reveals early microglial activation in the basal ganglia of PD patients. However, whether microglial-mediated neuroinflammation acts as a *contributor* to dopamine (DA) neuron loss or manifests as a *consequence of* nigrostriatal degeneration is debated. Our lab recently characterized a rat model in which intrastriatal injection of alpha-synuclein (a-syn) preformed fibrils (PFF) seed endogenous a-syn conversion into a pathological hyperphosphorylated form resulting in widespread, Lewy-body like pathology and ~40% nigral dopamine neuron degeneration over six months.
Methods: Male Fischer 344 rats received unilateral intrastriatal injections of mouse a-syn PFFs or vehicle. Cohorts of rats (total n= 96) were euthanized at monthly intervals up to six months. Outcome measures at each time point include quantification of nigral dopamine neurons, phosphorylated a-syn (pS129) aggregates, microglial density (Iba-1) and major histocompatibility complex-II (MHC-II) antigen-presenting microglia.
Results: Significantly higher numbers of MHC-II-immunoreactive microglia were observed in the substantia nigra (SN) of a-syn PFF-injected rats compared to control rats, indicating that the neuroinflammation observed is pSyn inclusion-specific and not related to injection damage. Nigral MHC-II immunoreactivity peaked when: 1) phosphorylated a-syn accumulations were most abundant and 2) dopaminergic neurons began to lose their phenotype, events that occur prior to overt degeneration.
Conclusions: These results suggest that pathological a-syn accumulation drives microglia-associated neuroinflammation prior to overt nigral degeneration and may serve as a contributor to nigral degeneration in PD. Future studies aimed at identifying specific inflammatory mediators will lead to greater understanding of the relationship of neuroinflammation to pathological a-syn misfolding and may identify future therapeutic targets for intervention.

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Characterizing the Bcl-2 Associated Athanogene 5 Interactome
E. Friesen, X. Wang, D. Williams, H. Chau, M. De Snoo, O. Pellerito, L. Kalia, G. Schmitt-Ulms, S. Kalia (Toronto, ON, Canada)
Objective: Characterizing the Bcl-2 Associated Athanogene 5 (BAG5) interactome will uncover the molecular pathways with which BAG5 associates and further elucidate its role in dopaminergic neurodegeneration.
Background: Monogenic PD is caused by heritable mutations to the genes encoding proteins such as a-synuclein, LRRK2, PINK1 and Parkin. BAG5 has been shown to interact with several of these proteins and to enhance protein aggregation and neurodegeneration in the substantia nigra. The pathological effect of BAG5 is thought to be due to

its inhibitory effect on the chaperone proteins Hsp70 and CHIP, which normally antagonize protein aggregation. However, BAG5 may also contribute to neurodegeneration by modulating the cellular processes responsible for maintaining mitochondrial health: a function independent of its association with Hsp70. The precise role of BAG5 in the pathobiology of PD is still unclear.
Methods: The BAG5 interactome was characterized by immunoprecipitating BAG5 from a tetracycline inducible SH-SY5Y stable cell line and identifying co-immunoprecipitated proteins via mass spectrometry. To minimize the effects of random genomic integration, the BAG5 transgene was inserted into the AAVS1 genomic safe harbor. To assess which BAG5 interactions are dependent on its association with Hsp70, the same procedure was performed with a mutated form of BAG5 [BAG5(mut)] incapable of binding Hsp70. The relative affinity of interacting proteins for either BAG5 or BAG5(mut) was gauged using isobaric tags.
Results: The interactome analysis revealed 288 high confidence BAG5 interacting proteins, including a-synuclein, LRRK2 and several other proteins commonly mutated in familial PD. Both Hsp70 and CHIP were found to preferentially associate with BAG5 relative to BAG5(mut), which was expected based on previous reports. GO term enrichment and KEGG pathway analysis revealed that BAG5 is associated with a number of diverse cellular processes including mRNA processing in the nucleus, microtubule dynamics and response to misfolded proteins.
Conclusions: This characterization of the BAG5 interactome validated previously known PD relevant BAG5-protein interactions and revealed novel interactions that may be important for further investigation into the molecular mechanisms of PD.

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Neuroprotective Potency of Tetrahydroisoquinoline a Novel Ayurveda Molecule in Experimental Parkinson's Disease

R. Banerjee, D. Nthenge-Ngumbau, R. Singh, P. Jaisankar, K. Mohanakumar, S. Biswas (Kolkata, India)
Objective: Tetrahydroisoquinoline (TIQ) an identified alkaloid from ancient Indian '*Ayurveda*' medicine for Parkinson's disease (PD) was tested for neuroprotection in a cellular system and a preclinical mouse PD model.
Background: PD treatment remains symptomatic with disabling side-effects. *Ayurveda* describes treatment for *'Kampavata'* or PD and novel herbal molecules might be neuroprotective.
Methods: Co-cultures of murine neuronal (Neuro2a), microglial (EOC20) and astrocytic (C8D30) cells were challenged with MPP+ a potent dopaminergic neurotoxin. TIQ (0.1-10 µM) incubated for 24 h post-MPP+ were subjected to MTT cell viability or Live Dead assays. Neuro2a cells, dbcAMP-differentiated to dopamine neurons were cultured in indirect contact with astrocytes and microglia through inserts. Mitochondrial superoxide radical accumulation in dopaminergic neurons was determined by MitoSOX dye flow cytometry. Adult C57/BL6 mice were acutely intoxicated with the MPTP parkinsonian neurotoxin (16 mg/kg dose, 4 times at 2 h intervals) and TIQ was gavaged (200 mg/kg body weight, bi-daily) for 7 days post MPTP intoxication. Control mice were PBS injected or fed with TIQ alone. Striatal dopamine levels on 7th day post-MPTP were measured by HPLC electrochemical detection. Striatal tyrosine hydroxylase (TH) enzyme expression was assayed by immunoblotting.
Results: *In vitro*, TIQ (10 µM) treatment significantly attenuated MPP+-induced loss of total cell viability. Live Dead Assay confirmed a significant (37%) increase in the number of live (Calcein AM-positive) differentiated neurons compared to MPP+ alone incubated for 24 h. Further, MPP+-induced mitochondrial accumulation of toxic superoxide radicals in dopamine neurons was significantly reduced (30%) by TIQ (10 µM). *In vivo,* TIQ ameliorated dopaminergic neurotoxicity in mice by causing a significant 16% increase in MPTP-induced striatal dopamine loss with 1.2-fold upregulation of the reduced expression of TH at the striatal terminals.
Conclusions: The anti-parkinsonian neuroprotective potential of TIQ is revealed in MPP+-exposed cell co-culture and in MPTP mice. TIQ protects through reduced toxic mitochondrial superoxide radicals, recovery of striatal dopamine levels and tyrosine hydroxylase expression. Molecular basis for TIQ's dopaminergic neuroprotection could translate into therapeutic benefit in PD patients.

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Alpha-synuclein enhances histone H3 lysine-9 dimethylation

N. Sugeno, S. Jäckel, A. Voigt, T. Hasegawa, P. Kahle (Tübingen, Germany)
Objective: To explore the nuclear function of alpha-synuclein (aS).
Background: aS is a protein linked to Parkinson's disease (PD) and related neurodegenerative disorders. It is mostly localized within synapses, but aS has also been suggested to play a role in the nucleus. The aim of this study is to explore epigenetic events through aS.

Methods: Histone proteins, extracted from aS transgenic Drosophila and aS inducible SH-SY5Y neuroblastoma cells, were separated by SDS-PAGE, then histone marks were analyzed by western blotting. To determine the level of histone lysine methyltransferase (HMT) and histone lysine demethylase (KDM), mRNA levels were measured by RT-PCR. Chromatin immunoprecipitation was performed by standard protocol using H3K9me2 and REST antibodies. Target genes were amplified by specific primer pairs.
Results: Overexpression of aS in male flies as well as in retinoic acid pre-treated neuroblastoma cells led to an elevation of histone H3K9 methylation, mostly mono- (H3K9me1) and di- (H3K9me2), which mean transcriptional repression. The transient increase of H3K9 methylation in SY5Y cells was slightly preceded by an induction of the euchromatic histone lysine N-methyltransferase 2 (EHMT2). Pharmacological inhibition of EHMT2 reduced the H3K9 methylations. EHMT2 and H3K9me2 can function within the REST complex. H3K9me2 chromatin immunoprecipitation (ChIP) survey of REST regulated genes showed significantly increased promoter occupancy of the synaptosomal-associated protein SNAP25 and cell adhesion molecule L1CAM genes after aS induction. Transcripts and protein levels of SNAP25 were also decreased.
Conclusions: aS overexpression enhances the histone modifications H3K9me1 and H3K9me2, likely involving EHMT2 known to catalyze these histone modifications. While, H3K9me2 strongly appears at the SNAP25 promoter, possibly affecting SNARE complex assembly and hence synaptic function regulated by aS.

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Extracellular alpha-synuclein internalizes into cells by hijacking endocytic trafficking of dopamine transporter

J. Kobayashi, T. Hasegawa, N. Sugeno, S. Yoshida, A. Kikuchi, A. Takeda, M. Aoki (Sendai, Japan)
Objective: The main purposes of this study were to: 1) investigate the possible effect of extracellular alpha-synuclein (aSYN) on the endocytic rate of dopamine transporter (DAT) and 2) determine whether the endocytic process of DAT could influence on aSYN internalization.
Background: DAT is the transmembrane protein that takes extracellular dopamine back up into dopaminergic neurons. DAT is distributed in both membrane raft and non-raft domains and the number of DATs on cell surface is controlled under the balance between endocytosis and recycling. Flotllin-1 (FLOT1), a caveolae-associated integral membrane protein, is essential for the localization of DAT within membrane microdomains.
Methods: HEK 293 cells stably expressing GFP-DAT were incubated with conditioned medium containing human monomeric aSYN for 30 minutes. Distinct endosomal structures were visualized by co-expression of mStrawberry-tagged Rab GTPases. Time-lapse imaging was performed to explore the spatiotemporal distribution of aSYN and DAT. Lipid rafts were isolated by floatation in a 5-30 % non-linear sucrose density gradient. Co-immunoprecipitation was adopted to demonstrate the molecular interaction between FLOT1 and DAT.
Results: Over-expressed GFP-DAT was localized mainly on plasma membrane, and to a much lesser degree, in the cytoplasm. Surprisingly, in the presence of extracellular aSYN, DAT was actively endocytosed and accumulated in Rab5-positine early endosome together with aSYN. Soon after reaching the early endosome, DAT was re-localized to the plasma membrane via both Rab4- and/or Rab11-mediated recycling pathway, while internalized aSYN was left behind in Rab7-mediated endo-lysosomal compartment. We further demonstrated that after addition of aSYN to the medium, DAT was dissociated from FLOT1 and shifted out of lipid raft compartment.
Conclusions: Extracellular aSYN induces re-distribution of DAT from raft to non-raft domain of cellular membrane, which possibly facilitates DAT endocytosis. Concomitantly, aSYN internalizes into cells by hijacking endocytic trafficking of DAT. These findings may suggest the possible role of DAT on cell-type specific pathological consequences in Synucleiopathies.

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New device HANABI (HANdai Amyloid Burst Inducer) is a rapid and sensitive detecting system of a synuclein fibril in CSF from Parkinson's disease patients

K. Ikenaka, K. Araki, M. So, S. Hashimoto, T. Tokuda, Y. Goto, H. Mochizuki (Suita, Japan)
Objective: Early diagnosis for PD is disturbed by the lack of sensitive and objective detection of accumulated aSyn in the patient's brain. Recently, Real-Time Quaking-Induced Conversion (RT-QuIC) analysis has been developed for a sensitive and specific detection of misfolded synuclein in the CSF of PD patients. Using these methods, we developed a new devise "HANABI (HANdai Amyloid Burst Inducer)" to induce the amplification of misfolded aSyn using ultrasonication automatically. HANABI dramatically reduced the detection time compare to the former methods.

Background: The RT-QuIC technique is based on amyloid seed–induced misfolding and aggregation of recombinant protein substrate, accelerated by alternating cycles of shaking and rest in fluorescence plate readers. Recent studies showed RT-QuIC analysis were also able to detect theaSyn amyloid in the CSF of PD patients. Although the CSF RT-QuIC has been shown to be an accurate diagnostic test for PD, with high sensitivity (95%) and specificity (100%), the protocol takes ~100 hr to complete. Using these techniques, we established a new device 'HANABI' to induce the amplification ofaSyn amyloid, which enable to shorten the assay duration for several hours.
Methods: The RT-QuiC reaction buffer (RB) was composed of 150 mmol/L NaCl, 50mM Tris HCl pH7.4, 10 lmol/L thio- flavin T (ThT), and 1.4 mg/mL human recombinant full-length (1–140 aa) aSyn. Each well of a black 96-well plate contained 90ul RB. Reactions were seeded with 10 ul of CSF. The plates were incubated in a HANABI, with which the ultrasonication-forced fibrillation of proteins can be automatically and rapidly analyzed, at 37°C for 6 h with intermittent sonication cycles: 3 min sonication, 7 min rest. ThT fluorescence measurements (450 nm excitation and 480 nm emission) were taken every 10 min.
Results: We employed 10 PD patients and 7 disease control patients. This new assay could detectaSyn aggregation in PD cerebrospinal fluid with sensitivities of 80% and with specificity of 70% when compared to control cerebrospinal fluid. From sample preparation to analysis of results, the protocol takes only ~7h to complete.
Conclusions: These results suggest that our new analysis of cerebrospinal fluid is potentially useful for the early clinical assessment of patients with a- synucleinopathy

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High-mobility group box 1 from astrocytes upregulates TH expression to maintain dopaminergic neurons via JNK pathway in human Parkinson's disease patients and MPTP induced mouse model

S.J. Kim, M.J. Ryu, J. Han, Y. Jang, J. Kim, M.J. Lee, I. Ryu, X. Ju, W. Chung, E. Oh, G.R. Kweon (Daejeon, Korea)
Objective: Supporting by glial cells could be reinforced the function of dopaminergic neurons against extracellular insults in Parkinson's disease (PD) development. However, we do not understand the molecular pathway that how they modulate the dopaminergic neurons via the actions of activated astrocytes and cytokines. We focused on the dopaminergic neurons and investigated underlying mechanism of tyrosine hydroxylase (TH) modulation in acute methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model.
Background: High-mobility group box 1 (HMGB1) can be actively secreted from inflammatory cells and is known to both promote inflammation and protect against disease propagation. Also, HMGB1 act as a mediator of neuroinflammation in subacute PD animal model.
Methods: Male C57Bl/6 mice were intraperitoneal injections of sterile saline or MPTP administered as 4 times injections of 20mg/kg at 2h intervals and were sacrificed at selected time points after the last injection (1, 3, 5 and 7 days). We measured by enzyme-linked immunosorbent assay (ELISA) that detects HMGB1 in human PD patient serum and U87MG cells (human a glioblastoma, astrocytoma cell line).
Results: The staining intensities of HMGB1 and Receptor for advanced glycation end product (RAGE) are higher in the nigral area of MPTP-treated mice, a toxin-induced PD like model, compared to saline-treated controls. HMGB1 was found to principally localize to astrocytes, and could affect the neighboring dopaminergic neurons, due to co-localization of RAGE with TH-positive cells. Treatment of a dopaminergic neuron cells with HMGB1 simultaneously induced JNK phosphorylation and TH mRNA expression. A JNK inhibitor was found to block the HMGB1-induced upregulation of TH expression.
Conclusions: Our results suggest that increased HMGB1 in astrocytes upregulates TH expression to maintain dopaminergic neuronal functions in acute MPTP mouse model. And HMGB1 could be a new research target for modulation of dopaminergic neurons.

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Interaction between PLK2 (Polo-like kinase-2) and alpha-synuclein in the non-human primate MPTP model of Parkinson's disease

R. Valenti-Azcarate, I. Martinez-Valbuena, M.-M. Carmona-Abellan, I. Marcilla-Garcia, G. Marti-Andres, M.R. Luquin-Piudo (Pamplona, Spain)
Objective: The objective is to determine the levels and interactions between PLK2 and one of its main substrates, synuclein, in the non-human primate MPTP model of Parkinson's disease (PD)
Background: The molecular mechanisms leading to the loss of specific neuronal populations of dopaminergic cells have not been delineated yet in PD, but several pathogenic pathways have been identified. One of these pathways involves mitochondrial dysfunction and dysregulation of reactive oxygen species. Matsumoto and colleagues

identified the PLK2 as a gene that is highly expressed in cells with defective respiration and increased oxidative stress. Furthermore, several studies have demonstrated that PLK2 can also phoshphorylate and promote selective autophagic clearance of synuclein (SCNA), the major component of Lewy bodies.
Methods: 15 *Macaca fascicularis* were included in the study. 10 monkeys received one weekly intravenous injection of MPTP until they developed bilateral parkinsonian features. Animals were sacrificed and perfused with saline buffer. Punches from the *Substantia Nigra* (SN) and cerebellum were collected. For quantitative analysis of PLK2 and SCNA gene expression, real time PCR was performed. Western blotting was performed using primary antibodies, against PLK2 and synuclein.
Results: In the SN of MPTP monkeys a 1.8-fold increase was observed in the PLK2 mRNA level related to untreated monkeys. Regarding SCNA expression, we found a decrease of mRNA levels nearly to 60% compared to untreated monkeys. Besides, both expression levels were negatively correlated (R=-0.76). In the cerebellum, no changes were detected and no correlation between the expression patterns was found (??Ct=0.56±0.12). To evaluate if these changes were only at RNA level, we also performed Western Blot analysis. In the SN, we found a decrease of 45% in alpha-synuclein protein levels of MPTP monkeys. A fold increase of 1,5 was found in PLK2 protein level. No changes in the cerebellum were found.
Conclusions: We demonstrate for the first time that an upregulation of PLK2 exists in the brain of MPTP-monkeys, and that this upregulation is only circumscribed to the SN. Furthermore, this upregulation correlates negatively with synuclein expression, confirming previous studies that suggested the selective autophagy clearance of synuclein by PLK2 and its role in PD pathophysiology.

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Mesencephalic astrocyte-derived neurotrophic factor alleviated 6-OHDA-induced cell damage via ROS-AMPK/mTOR mediated autophagic inhibition

J. Zhang, Q. Cai, M. Jiang, Y. Liu, H. Gu, J. Guo, H. Sun, J. Fang (Shanghai, People's Republic of China)

Objective: In this study, we investigated the role of autophagy system in MANF-mediated neuroprotection against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity.
Background: Autophagy and apoptosis are commonly involved in the dopaminergic neuron damage in the pathogenesis of Parkinson's disease. Recently, the autophagy pathway is thought to be important in the process of PD, and the regulation of autophagy may be a potential strategy for PD treatment. Mesencephalic astrocyte-derived neurotrophic factor (MANF) has been reported to have neuroprotective effects through anti-apoptosis, anti-oxidative, and anti-inflammatory mechanisms in PD. However, whether autophagic regulation is involved in MANF-mediated neuroprotection remains need to be elucidated.
Methods: The viability and apoptosis of SH-SY5Y cells were assessed by 3-(4, 5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay and Annexin V-FITC and propidium iodide (PI) double staining. Generation of intracellular reactive oxygen species (ROS) and superoxide anion were visualized and analyzed using oxidation sensitive probe DCFH-DA and DHE probes, respectively. A fluorescent dye JC-1 was applied to monitor mitochondrial membrane potential, while the intracellular ATP level was measured by ATP assay kit. The expression of autophagy related proteins were detected by western blot.
Results: MANF protected SH-SY5Y cells against 6-OHDA induced cell viability decrease and apoptosis by inhibiting autophagy. Reactive oxidative stress (ROS) accumulation triggered mitochondrion damage and energetic dysfunction were also alleviated by MANF treatment. Meanwhile, MANF downregulated phosphorylation of AMP-activated protein kinase (AMPK), a cellular energy sensor and regulator, but upregulated phosphorylation of Mammalian target of rapamycin (mTOR) under energy depletion conditions, indicating AMPK/mTOR signaling pathway was involved in the autophagic inhibition of MANF.
Conclusions: Our results suggest that autophagic inhibition is a protective mechanism of MANF in 6-OHDA induced SH-SY5Y cell death and this inhibition was associated with AMPK/mTOR pathway.

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Alpha-Synuclein knock-out mouse brain shows significant dysregulation of c-Fos expression

M. Overhoff, J. Canet-Pons, D. Meierhofer, S. Gispert, G. Auburger (Frankfurt am Main, Germany)

Objective: We aimed to understand the physiological function of alpha-synuclein (*SNCA*) for neuronal projections within the brain, particularly its effects on entire pathways and on individual molecular components.
Background: The gain-of-function of alpha-synuclein is a crucial driver of selective neurotoxicity in Parkinson's disease (PD). As an early phenotype, ultrasonic vocalizations of A53T-SNCA overexpressing mice are reduced already at the postnatal stage. Conversely, in alpha-synuclein knock-out (KO) mice at the postnatal stage, the

ultrasonic vocalizations are increased. Similarly, expansion mutations in the SNCA promoter result in Parkinsonian reduction of spontaneous motor activity, while shrinkage mutations in the SNCA promoter result in motor restlessness. These data suggest that a gain versus a loss in the physiological role of alpha-synuclein is relevant for the molecular mechanisms in PD, and that further analyses of alpha-synuclein KO mice are useful.
Methods: Alpha-synuclein KO mouse brains were dissected and subjected to oligonucleotide microarray analyses, comparing midbrain versus striatum versus cerebellum. Furthermore, global proteome analyses were conducted with label-free mass spectrometry and assessed by volcano plots. Biomathematical workup of the global transcriptome data was carried out with the STRING webserver in Heidelberg and the GSEA webserver in Cambridge/MA. Validation was performed with quantitative reverse-transcriptase polymerase chain reaction and immunoblots as well as immunohistochemistry.
Results: Several factors in the presynaptic and axonal compartments showed dysregulated expression. Overall, significant enrichment for pathway upregulation in EGF signaling, MEK signaling and DeltaFosB target pathways was observed in the aged striatum. As a representative molecule in the validation experiments, c-Fos mRNA was significantly dysregulated already at middle adult age.
Conclusions: Our striatum findings support the concept that synaptic plasticity in the dendritic spines is altered by the loss of presynaptic alpha-synuclein. Our striatum findings support the concept that synaptic plasticity in the dendritic spines is altered by the loss of presynaptic alpha-synuclein.

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The tricyclic antidepressant medication nortriptyline inhibits alpha-synuclein accumulation, aggregation and toxicity in multiple in vitro and in vivo models

T. Collier, L. Lapidus, C. Sortwell, C. Justman, P. Lansbury, K. Paumier (Grand Rapids, MI, USA)

Objective: Using preclinical models, assess the potential disease-modifying effects of nortriptyline (NOR) as a treatment in early Parkinson's disease (PD).
Background: PD and other synucleinopathies are characterized by intracellular inclusions comprised primarily of misfolded, fibrillar a-synuclein (a-syn). One therapeutic strategy to slow disease progression is to reduce the accumulation of toxic a-syn species by preventing misfolding of the native monomeric form of the protein. Previous work suggests that tricyclic antidepressants (TCAs) may alter disease progression as they are found to reduce neurodegeneration in a preclinical toxin model of parkinsonism. In a retrospective analysis of data from a cohort of patients with early PD, TCAs were specifically identified as the class of antidepressant medications associated with a significant delay in the need for dopaminergic therapy.
Methods: Interactions between the TCA compound NOR and a-syn were assessed in *in vitro* aggregation and kinetics assays and *in vivo* models including primary neuron cultures, transgenic *Drosophila* and mice, and the rat pre-formed fibril a-synucleinopathy model.
Results: Aggregation and kinetics assays demonstrate that NOR directly binds to monomeric a-syn at physiologically relevant concentrations and reduces the rate of aggregation eight-fold by increasing the rate of monomeric reconfiguration. In addition, NOR inhibits the accumulation, aggregation and neurotoxicity of a-syn in primary neuron cultures, transgenic *Drosophila* and mice, and the rat pre-formed fibril a-synucleinopathy model.
Conclusions: These findings suggest that NOR, a compound that has proven to be safe and effective in treating depression, may slow disease progression in synucleinopathies by directly binding to native a-syn, thereby inhibiting formation of toxic conformations of the protein.

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Is the GBA2 a new modifier for Gauchers's disease and GBA1-related Parkinson's disease?

E. Nakanishi, N. Uemura, H. Akiyama, M. Kinoshita, H. Yamakado, S. Takeda, Y. Hirabayashi, R. Takahashi (Kyoto, Japan)

Objective: This study was performed to determine the pathological role of GBA2 in the central nervous system(CNS) in Gaucher's disease (GD) and GBA1-related Parkinson's disease (PD).
Background: PD is one of the most prevalent neurodegenerative disorders characterized by tremor, rigidity, akinesia and postural instability. Recent genetic studies have identified that heterozygous mutations in the GBA1 gene is a strong risk factor for sporadic PD. The GBA1 mutations are responsible for GD, the common autosomal recessive lysosomal storage disease. We have reported that the GBA1 knock-out (KO) medaka can survive long enough for pathological analysis of disease progression in contrast to the perinatal death of GBA1 KO mice. These GBA1 KO medaka display abnormal swimming movement, non-selective neuronal loss, and a-synuclein accumulation in the brains. These GBA1 KO medaka are useful to investigate the mechanisms of a-synuclein

accumulation in GD and GBA1-related PD. The non-lysosomal ß-Glucosidase (GBA2), which is localized at the endoplasmic reticulum and Golgi apparatus, also cleaves glucosylceramide to glucose and ceramide. A recent study has reported that the deletion of GBA2 rescues the visceral manifestations in type1 GD mice model through reduction of sphingosine. To date, it remains unclear whether the deletion of GBA2 can modify the CNS manifestations of GD.
Methods: We generated GBA2 KO medaka by clustered regularly interspaced short palindromic repeat (CRISPR) / CRISPR-associated nuclease (Cas9) system. Then, we crossed GBA2 KO medaka with GBA1 KO medaka to examine the genetic interaction between GBA1 and GBA2 in GD and GBA1-related PD.
Results: We have successfully generated GBA2 KO medaka by CRISPR / Cas9. GBA2 KO medaka lack both GBA2 enzymatic activity and protein expression. GBA2 KO medaka did not show infertilization or apparent abnormal motor phenotypes. All GBA1 / GBA2 double KO medaka showed abnormal swimming at two months. There were no differences in life span or the loss of DA/NA cells between GBA1-/-; GBA2+/+ and GBA1-/-; GBA2-/-. Moreover, the deletion of GBA2 in GBA1 KO medaka didn't reduce the amount of sphingosine.
Conclusions: Although further analysis is needed, it seems that the deletion of GBA2 in GBA1 KO medaka does not reduce the amount of sphingosine or rescue the pathology of CNS.

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Motor effects induced by D1- or D2-like receptor agonists in experimental parkinsonism
L. Andreoli, C. Alcacer, I. Sebastianutto, A. Cenci Nilsson (Lund, Sweden)
Objective: We set out to compare movement patterns induced by systemic administration of l-dopa versus D1- or D2-class DA receptor agonists in mice with unilateral 6-OHDA lesions of the nigrostriatal pathway.
Background: Dopamine (DA) replacement therapy in Parkinson´s disease (PD) is often initiated using DA receptor agonists, which are less dyskinesiogenic than l-dopa. Mice with unilateral 6-OHDA lesions are often used to discover new symptomatic or antidyskinetic treatments for PD, but the effects of DA agonists in this animal model have not been characterized.
Methods: Mice sustained unilateral 6-OHDA injections in the medial forebrain bundle, and were divided in three groups to receive treatment with increasing doses of skf38393, quinpirole, or l-dopa. Abnormal involuntary movements (AIMs) were rated using a validated scale. Rotational, horizontal and vertical activity was evaluated using a videotracking system returning measurements of distance travelled, rearing events, rotations, and movement speed.
Results: An overall analysis of total axial, limb and orolingual (ALO) AIM scores revealed no major differences between the three treatments: ALO AIMs had increased in a dose-dependent manner. However, when different AIM subtypes were analyzed individually, we found that quinpirole had induced a dramatic increase in axial AIMs while markedly reducing horizontal and vertical activity. By contrast, skf38393 had mainly increased orofacial AIMs and induced a large increase in horizontal and vertical activity (with a similar effect magnitude to l-dopa).
Conclusions: These results indicate that the pharmacological activation of D1 and D2 receptors activation produces strikingly different movement patterns in hemiparkinsonian mice. Their relative contribution to the genesis of dyskinesias may determine the choice of antidyskinetic treatment in the future.

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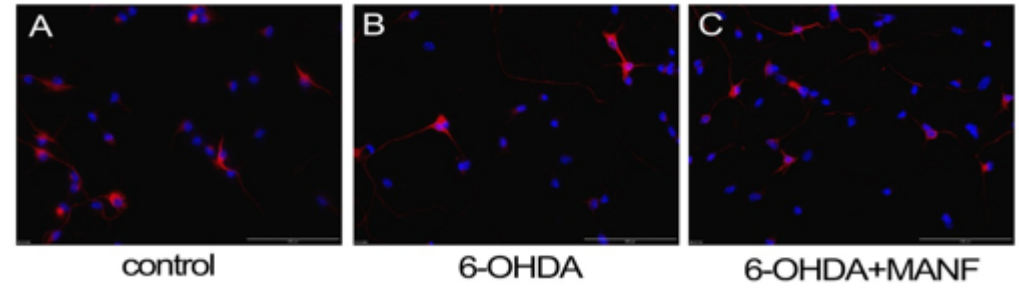
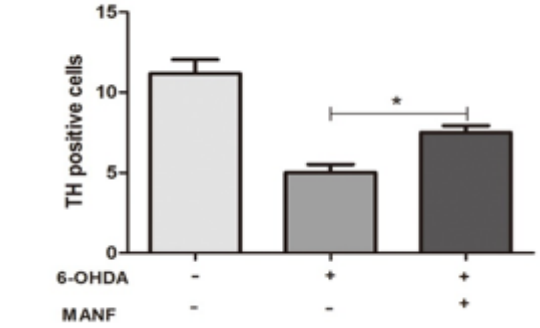
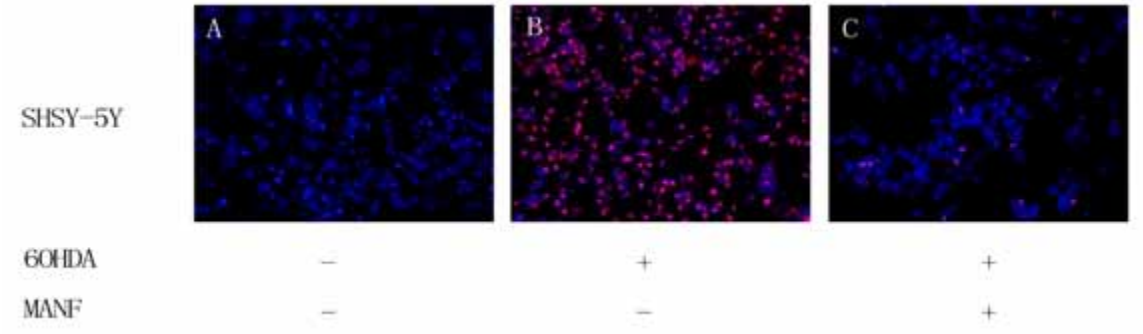
Mesencephalic astrocyte-derived neurotrophic factor reduces cell apoptosis via upregulating HSP70 in SHSY-5Y cells
H. Sun, M. Jiang, J. Zhang, L. Feng, Y. Liu, Z. Nie, J. Fang, L. Jin (Shanghai, People's Republic of China)
Objective: The newest candidate growth factor for dopaminergic neurons is Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF). Signaling mechanisms by which MANF protects dopaminergic neurons from ER stress remain unclear. HSP70s, plays a critical function in the rescue of misfolded proteins. Protein misfolding plays an important role in the development of PD . MANF is involved in the regulation of endoplasmic reticulum (ER) and unfolded protein response (UPR). Thus, we hypothesize that MANF exhibits its neuroprotection via upregulation of HSP70.
Background: Parkinson's disease (PD) is one of the most common neurodegenerative disorders of the central nervous system. It is characterized by chronic and progressive loss of midbrain dopaminergic (DA) neurons. The precise etiology and disease pathogenesis are mostly unknown. The use of neurotrophic factors represents a potential treatment strategy. They are essential to neuronal differentiation and maturation during development and adulthood.

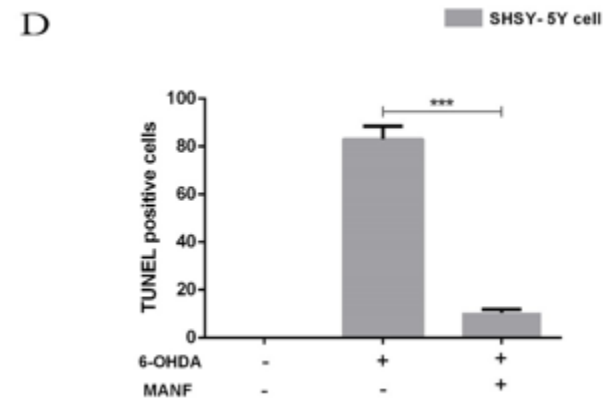
Methods: In the present study, we used a PD *in vitro* model of purified embryonic dopaminergic neurons, derived from 6-OHDA Rat model of PD and SHSY-5Y cells. Cell viability following exposure to 6-OHDA as excitotoxic stimulus was assessed by MTT assay and immunocytochemistry. We used TUNEL staining and Western blot analysis to investigate the protective effect of MANF in SHSY-5Ycells treated with 6-OHDA. Using RNA-seq analysis, realtime PCR, and RNAi, we could demonstrate that HSP70 was involved in the protection of MANF.
Results: We found the neuroprotective effects of Mesencephalic astrocyte-derived neurotrophic factor (MANF) in our *in vitro* system, which could be attributed to HSP70 upregulation. Not only due to these antiapoptosis capacities but also with respect to the increasing knowledge about the regulation of endoplasmic reticulum (ER) stress and unfolded protein response (UPR), which could be positively influenced by MANF.

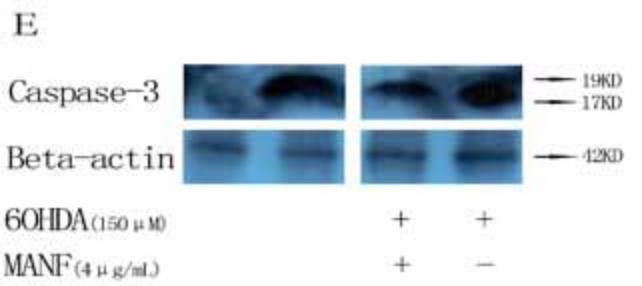


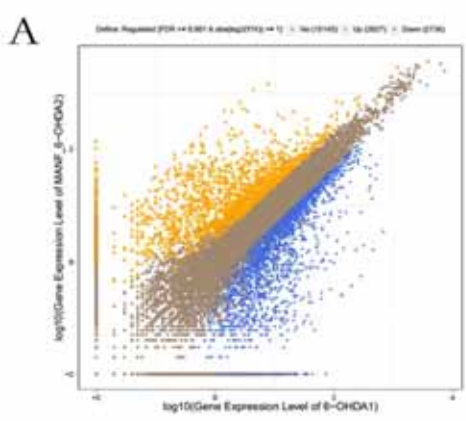
D

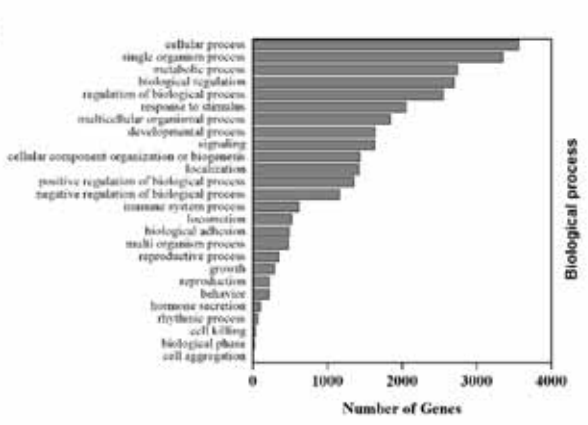


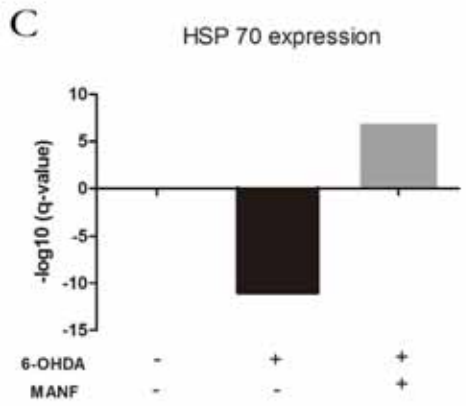


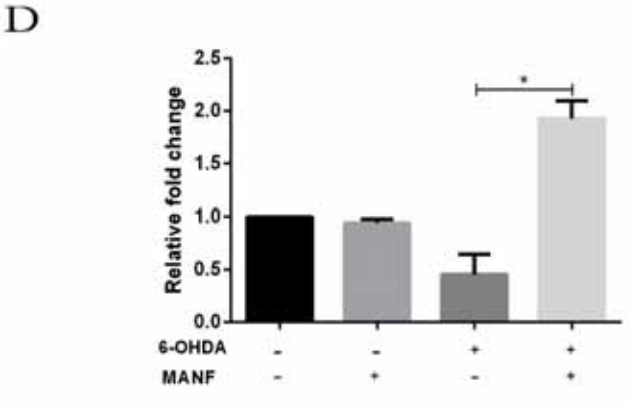

D
SHSY-5Y cell
TUNEL positive cells
100
80
60
40
20
0
***
6-OHDA - + +
MANF - - +

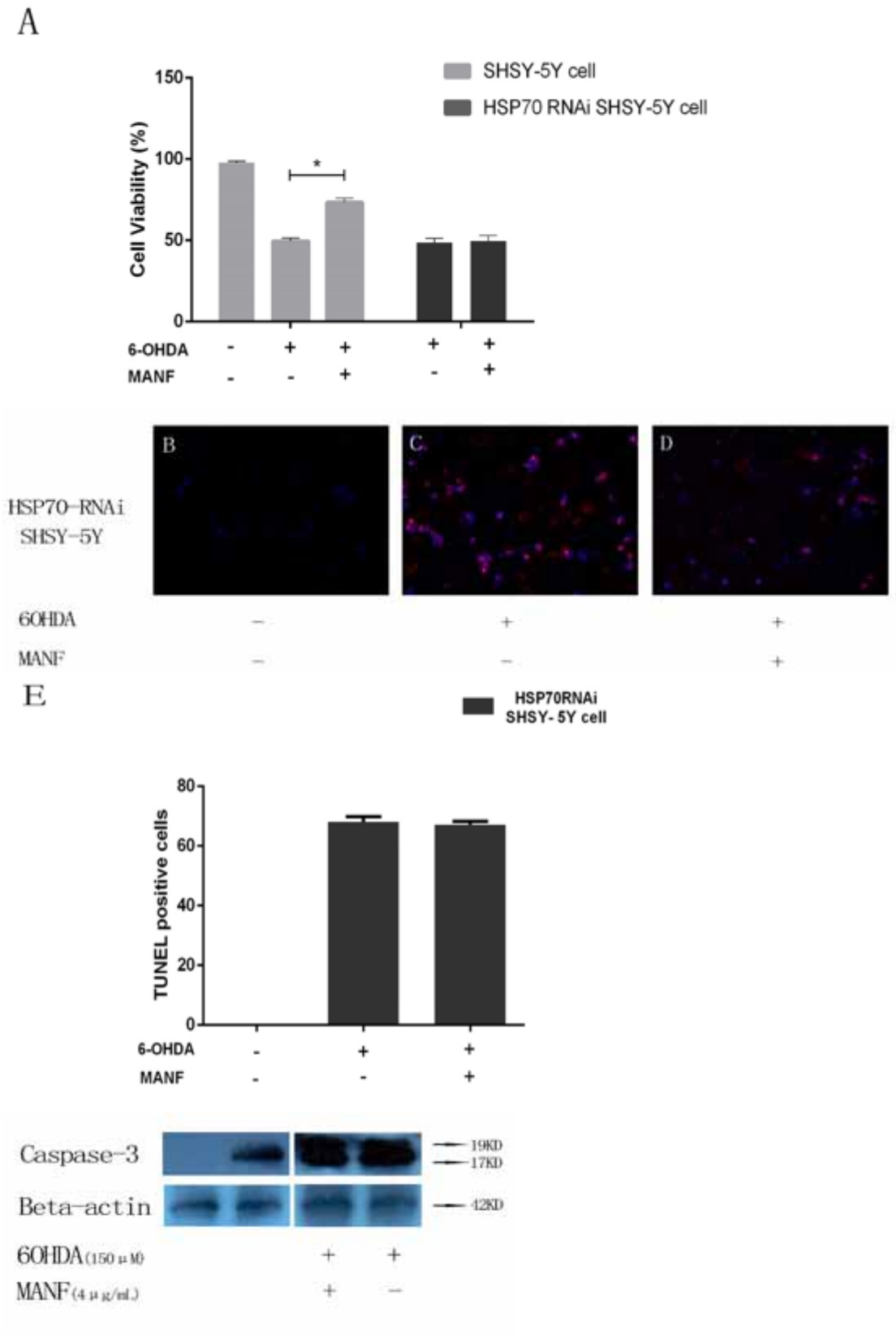

E
Caspase-3
Beta-actin
19KD
17KD
42KD
6OHDA (150 μM) + +
MANF (4 μg/mL) + −


A
log10(Gene Expression Level of MANF_6-OHDA2)
log10(Gene Expression Level of 6-OHDA1)


B
cellular process
single organism process
metabolic process
biological regulation
regulation of biological process
response to stimulus
multicellular organismal process
developmental process
signaling
cellular component organization or biogenesis
localization
positive regulation of biological process
negative regulation of biological process
immune system process
locomotion
biological adhesion
multi-organism process
reproductive process
growth
reproduction
behavior
hormone secretion
rhythmic process
cell killing
biological phase
cell aggregation
0
1000
2000
3000
4000
Number of Genes
Biological process


C
HSP 70 expression
-log10 (q-value)
10
5
0
-5
-10
-15
6-OHDA - + +
MANF - - +


D
Relative fold change
2.5
2.0
1.5
1.0
0.5
0.0
*
6-OHDA - - + +
MANF - + - +


A
SHSY-5Y cell
HSP70 RNAi SHSY-5Y cell
Cell Viability (%)
150
100
50
0
*
6-OHDA - + + + +
MANF - - + - +
HSP70-RNAi SHSY-5Y
B
C
D
6OHDA - + +
MANF - - +
E
HSP70RNAi SHSY-5Y cell
TUNEL positive cells
80
60
40
20
0
6-OHDA - + +
MANF - - +
Caspase-3
Beta-actin
19KD
17KD
42KD
6OHDA (150 μM) + +
MANF (4 μg/mL) + -

Conclusions: Our data suggest that MANF inhibits apoptosis induced by 6-OHDA in SHSY-5Y cells via upregulating HSP70 in the transcriptional pattern. MANF represents an interesting novel candidate for further *in vivo* evaluation in PD.

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Citrus extract A protects dopaminergic neuron by modulating mitochondrial respiration and reactive oxygen species

Y. Jang, J. Han, S.J. Kim, M.J. Lee, I. Ryu, X. Ju, H. Chu, M.J. Ryu, E.S. Oh, S.Y. Choi, W. Chung, J.Y. Heo, G.R. Kweon (Daejeon, Korea)

Objective: This study aims to validate the protective effect of citrus extract A (CEA) against loss of dopamine neurons in PD by dual targeting of ROS and mitochondrial respiration with citrus extract A (CEA) *in vitro* and *in vivo*. And, we address the beneficial effect of CEA administration to relieve motor symptoms in PD model.

Background: Dopamine neuronal loss in Parkinson's disease (PD) is caused by mitochondrial dysfunction and increase of reactive oxygen species (ROS). Progressive loss of dopamine neurons fails to maintain physiologic level of dopamine, which aggravates motor dysfunction. However, current therapeutics of PD targets relief of symptoms such as rigidity, tremor and bradykinesia by supplementing levodopa or dopamine agonist, instead of directly modulating mitochondrial function. Although, genetic or pharmacologic inhibition of mitochondrial respiration improve cellular function and decrease ROS production in peripheral tissues, in dopamine neuron is unclear. Therefore, we use CEA, which specifically targets mitochondrial respiration and inhibits ROS, exploring the mechanism and potency of CEA for protection of dopamine neuronal loss.

Methods: 1) Cell viability of rotenone- treated cells was measured by SRB assay with or without CEA; 2) Improvement of movement disorder was validated by open-field test and grid test after injection of CEA to MPTP induced PD

Results: Pretreatment of CEA to dopamine cell line, SN4741 significantly increased cell viability and reduced mitochondrial ROS with induction of antioxidant enzymes after rotenone administration compared to vehicle treated cells. Oxygen consumption rate and cellular ATP were decreased by CEA treatment. Blockade of mitochondrial respiration by CEA was confirmed by western blotting. As we expected, CEA injection to MPTP-induced PD mice showed mild improvement of movement. CEA injected mice showed increased number of dopamine neuron in SN region of MPTP mice.

Conclusions: These results demonstrated that CEA dual targets mitochondrial respiration and ROS to protect dopamine neuronal loss. The induction of antioxidant enzymes by CEA is indispensable for the inhibition of oxidative damage of dopamine neurons. We suggest that CEA contribute to improvement of movement and neuroprotection in PD through selective inhibition of mitochondrial respiration and ROS.

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Targeted overexpression of A53T-alpha-synuclein induces progressive neurodegeneration and electrophysiological changes of noradrenergic locus coeruleus neurons – a preclinical model of Parkinson's disease

M. Henrich, L. Matschke, A. Stoehr, W.H. Chiu, B. Lee, F. Geibl, J. Koprich, N. Decher, W. Oertel (Marburg, Germany)

Objective: In our present study, we developed a new mouse model to study the time dependent effects of cellular A53T-a-synuclein overexpression in the locus coeruleus, regarding the toxicity caused by a-synuclein accumulation, the alteration in electrophysiological properties and noradrenergic cell loss.

Background: Dysfunction of the noradrenergic locus coeruleus (LC) is an early hallmark of Parkinson's disease (PD). The extensive loss of noradrenergic LC neurons in PD is responsible for a large amount of non-motor symptoms that occur in early stages of the disease. However, the mechanisms that render LC neurons prone to a-synuclein accumulation and neurodegeneration are still unclear.

Methods: Serotype 1/2 recombinant adeno-associated viral vectors (rAAV) carrying the genome for A53T-a-synuclein or luciferase were unilaterally injected in the right LC of C57Bl/6 wildtype mice to induce continuous protein overexpression. At 1, 3, 6 and 9 weeks post injection, eight animals overexpressing either A53T or luciferase were sacrificed for immunohistochemical analysis. In addition, four animals per group and timepoint were used to study the biophysical characteristics of LC neurons by patch-clamp recordings in acute brainstem slices.

Results: We show, that targeted overexpression of A53T-a-synuclein in the LC of wildtype mice caused progressive a-synuclein accumulation and significant loss of noradrenergic LC neurons in the injected side in a time dependent manner, starting 3 weeks post-injection. Aggregated forms of a-synuclein were confirmed by Proteinase K

resistance and Ser129 phosphorylation. Furthermore, overexpression of a-synuclein led to a progressive increase of astro- and microglia density in the injected LC region. In our model, neurodegeneration of LC cells was associated with significant changes of their electrophysiological properties. Time dependently, A53T-a-synuclein overexpression induced alterations in action potential shape and an acceleration of the pacemaking frequency.
Conclusions: Our data indicate that overexpressed A53T-a-synuclein accumulates steadily in LC neurons, while simultaneously induces neuroinflammation and major changes in electrophysiological properties, which might be responsible for the observed cell death of LC neurons.

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Parkinson's disease-like pathology in the rat brain and colon following methamphetamine self-administration

B. Bradaric, A. Persons, S. Kousik, L. Kelly, S. Graves, T.C. Napier (Chicago, IL, USA)

Objective: To determine if Parkinson's disease (PD)-like pathology occurs in brain and colon following termination of methamphetamine (meth) self-administration.
Background: Meth abuse increases the risk of developing PD by three-fold. Abstinent meth abusers exhibit neuroinflammation and nigrostriatal dysfunction. We revealed that rats self-administering meth exhibit progressive, *withdrawal-time* dependent reductions in tyrosine hydroxylase (TH); where like PD, striatal dysfunction precedes nigral dysfunction[1]. The gastrointestinal system may be an early site for PD; e.g., a-synuclein (a-syn) is upregulated in the colon *prior to* clinical diagnosis. It is not known if meth abuse results in gut pathology.
Methods: Male Sprague-Dawley rats self-administered meth for 14 days; controls were saline-yoked. Forelimb akinesia was assessed every week. Brain and colon tissue was harvested one or 56 days after the last operant session and prepared for Western blotting or immunohistochemistry of a-syn and GFAP.
Results: Akinesia was not observed during, or one day after meth, but emerged after 56 days of abstinence ($p=0.0001$). One day after meth treatment, there were no changes in GFAP or a-syn in the striatum. Though previously no change in TH^+ cell number was observed[1], the substantia nigra *pars compacta* (SN_{pc}) exhibited a 50% increase in the number of a-syn^+ cells ($p=0.03$). In the colon, meth self-administering rats showed a 24% increase in GFAP ($p=0.0003$) and 69% increase in a-syn ($p=0.0001$) immunoreactivity[2]. Following 56 days of meth abstinence, when we previously observed a 22% decrease in SN_{pc} TH^+ cells[1], the number of a-syn^+ cells was still enhanced by 43% ($p=0.005$). Colonic GFAP and a-syn returned to control levels.
Conclusions: These data dovetail with our prior work to show that meth initiates subclinical nigrostriatal and colonic PD-like dysregulation, which, upon protracted abstinence, progresses to nigrostrial dysfunction that is associated with akinesia, even though the colon recovers. These studies offer insights into the enhanced vulnerability of meth abusers to develop PD.

* These authors contributed equally.

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Exploratory Clinical study of role of cerebral dopamine neurotrophic factor (CDNF) mediating endoplasmic reticulum stress (ERS) in Parkinson's disease

K. Terpstra, R. Mishra, S. Chiu (Hamilton, ON, Canada)

Objective: 1: To identify and measure endogenous CDNF levels in peripheral whole blood of healthy control subjects and to examine CNDF expression development profile across the life span of humans ; 2: To investigate differential CNDF mRNA expresion in whole blood, platelets and lymphocytes and whether the changes in CNDF expression is specific for PD subjects.
Background: There is growing evidence for the neuroprotective and neurorestorative effects of CDNF secreted by the endoplasmic reticulum selective for dopamine (DA) neurons. CDNF modulate neuroinflamamtion, protein folding and mitigates excessive ER stress and faciliates alpha-synclein aggregates clearance. CDNF infusion rescues behavioral neural repair in PD.
Methods: Part 1)For characterizing CDNF development , we recruited three groups of healthy controls: 1) children (1-18 yrs : 2) adults (18-50 yrs); 3) elderly adults (> 50 yrs). Part 2) For PD subjects, we recruited PD subjects diagnosed by research neurologists, along with normal age-matched healthy subjects and stroke patients. Venous blood was collected, lymphocytes and platelets were isolated for RNA extraction for reversed transcriptase rRT-PCR assays for both Part 1 and part 2.
Results: We found a statistically significant ($p< 0.05$, one-way ANOVA ; Tukey ;posthoc test) decrease in CDNF mRNA exprssion in whole blood in the transition from the childhood/adolescence [n=7] to the yound adulthood [n=22] , with progressive trend of significant decline in CDNF from childhood to elderly group [n=16 mean age 63

yrs] ($p< 0.05$), suggesting downregulation of CDNF mRNA expression with aging. We found that a significant increase in CDNF mRNA expression in PD patients [n=13 mean age: 72.8 yrs] compared with stroke patients [n=8 mean age: 71.5 yrs] and normal healthy control subjects [n=15, mean age: 66.8 yrs], as determined by one-way ANOVA ($F(2,33)=4.89$, p , 0.014) across the three groups. No significant difference was found in the lymphocytes. Whole blood CDNF mRNA was reduced in stroke patients compared with control but not for PD patients.
Conclusions: Our findings of specific paradoxical increase in platelet CDNF mRNA expression for PD ,and decline of CDNF mRNA with age, suggest compensatory CDNF to counteract ER stress may be the emerging potential therapeutic target in PD.

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Pharmacological modulation of mGluR5 improves dyskinesias mediated by D1 but not D2 receptor stimulation

I. Sebastianutto, N. Maslava, L. Di Menna, F. Nicoletti, M. Cenci (Lund, Sweden)

Objective: To determine whether pharmacological inhibition of metabotropic glutamate receptor type 5 (mGluR5) can improve dyskinesias mediated by D1- or D2-type dopamine (DA) receptors (D1R and D2R, respectively) in parkinsonian mice.
Background: L-DOPA-induced dyskinesia (LID) is reduced by a selective mGluR5 antagonist (Rascol et al., Parkinsonism Relat. Disord. 2014). However, the mechanisms underlying the antidyskinetic effects of this class of drugs remains unclear. In this study, the question has been explored by evaluating the effects of the selective mGluR5 antagonist MTEP on dyskinesias evoked by D1R or D2R agonists, which conceivably depend on different neuronal pathways.
Methods: Mice having a selective knockdown of mGluR5 in D1R-expressing striatal neurons ($mGluR5^{KD\text{-}D1}$; Novak et al. J. Neurosci. 2010) and Wild type (Wt) littermates sustained a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal projections. Mice were allotted to different treatment groups, receiving either L-DOPA, the D1R-like agonist SKF38393 or the D2R-like agonist Quinpirole. The potential antidyskinetic effect of MTEP was evaluated in each treatment group.
Results: In Wt mice, the selective mGluR5 antagonist MTEP ameliorated dyskinesias induced by L-DOPA ($p < 0.01$ vs. vehicle + L-DOPA) and SKF38393 ($p < 0.01$ vs. vehicle + SKF38393), but not Quinpirole. Compared to Wt controls, $mGluR5^{KD\text{-}D1}$ mice displayed lower levels of dyskinesia when treated with either L-DOPA or SKF38393 (both, $p < 0.05$ vs. Wt mice). In contrast, Quinpirole elicited comparable levels of dyskinesia in both $mGluR5^{KD\text{-}D1}$ and Wt mice. Interestingly, challenging $mGluR5^{KD\text{-}D1}$ mice with MTEP did not further reduce L-DOPA- and SKF38393-induced dyskinesia to significant levels.
Conclusions: These results are the first to substantiate that the anti-dyskinetic effect of mGluR5 antagonism is exerted on dyskinesias caused by D1R, while it is ineffective on D2R-mediated dyskinesias. This suggests the need for future treatments to take into account the patient-specific involvement of D1R or D2R in their dyskinesias.

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Beneficial and protective effects of withania Someniferais on mice brain: A therapeutic potential drug for Parkinson's disease

S. Rajput, S. Sinha (New Delhi, India)

Objective: Objective of our study was evaluate effect of Withania Someniferais leaves on Parkinson's brain of mice.
Background: Parkinson's disease (PD), an age-related disorder, is accompanied by the symptoms, tremor, bradykinesia, rigidity, stooped posture and instability. The disease progresses slowly and may ultimately produce complete akinesia. The neuropathology of the disease is based on the depigmentation and cell loss in the dopaminergic nigrostriatal tract of the brain with the corresponding decrease in the striatal dopamine concentration and the presence of eosinophilic inclusions called Lewy bodies. Withania Someniferais (WS) retard brain aging and help in regeneration of neural tissues besides producing antistress, adaptogenic and memory enhancing effect.
Methods: In present study 6-hydroxydopamine (6-OHDA) model of PD mice were used. The symptoms of PD such as tremors, akinesia, rigidity, catalepsy, and vacuous chewing movements (VCMs) were evaluated. The methanolic extract of WS was administered at doses of 200 mg and 500 mg/kg body weight followed by stress. The combination of L-dopa and carbidopa was used as a standard drug. Behavioral studies such as locomotor activity and grip strength were determined, and oxidative stress was evaluated in mice brain. ANOVA was used followed by post hoc Turkey test.

Results: Brain was used for biochemical and histopathological study. Animal exposed to stress showed significant decrease in Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and total protein. This was accompanied by simultaneous increase in Thiobarbituric acid reactive substances - TBARS evel. Treatment with Withania Somenifera had no significant but moderate effect on antioxidant enzyme (SOD and CAT). Pretreatment with WS (200 and 500 mg/kg) significantly reduced the intensity of muscular rigidity, duration of catalepsy, akinesia, the number of tremors, and increase fighting behavior. The locomotor activity and grip strength were significantly increased by WS. Treatment with WS significantly reduced LPO level and restored the defensive antioxidant enzymes SOD and CAT in mice brain.
Conclusions: Present study provides evidences that oral administration of alcoholic extract of WS leaves have shown anti-aging effect in stress.

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Altered somtatosensory cortex neuronal activity in a rat model of Parkinson`s disease and levodopa-induced dyskinesias

K. Schwabe, X. Jin, J. Krauss, M. Alam (Hannover, Germany)

Objective: In this study, we explored the neuronal firing activity of excitatory pyramidal cells and inhibitory interneurons in the forelimb region of the primary somatosensory cortex (S1FL-Ctx), along with its interaction with oscillatory activity of the primary motor cortex (MCtx) in 6-hydroxydopamine lesioned hemiparkinsonian (HP) and levodopa-primed dyskinetic (HP-LID) rats as compared to controls.
Background: Several findings support the concept that sensorimotor integration is disturbed in Parkinson`s disease (PD) and in levodopa-induced dyskinesias.
Methods: Rats were rendered hemiparkinsonian (HP) by unilateral injection of 6-OHDA in the medial forebrain bundle (MFB). Subsequently, these HP rats were divided into one group that was rendered dyskinetic by long-term injections of levodopa, in the following termed HP-LID rats, while the other HP group received no levodopa injection. Another group of rats without surgery served as naïve controls. The firing activity of excitatory pyramidal cells and inhibitory interneurons in the S1FL-Ctx, along with its interaction with oscillatory activity of the MCtx was recorded in urethane anesthetized (1.4 g/kg, i.p.) rats.
Results: While firing frequency and burst activity of S1FL-Ctx inhibitory interneurons were reduced in HP and HP-LID rats, measures of irregularity were enhanced in pyramidal cells. Further, enhanced coherence of distinct frequency bands of the theta/alpha, high-beta, and gamma frequency, together with enhanced synchronization of pyramidal cells and interneurons with MCtx oscillatory activity were observed.
Conclusions: Our study shows electrophysiological alterations in the sensorimotor cortices in a rat model of PD, which differ depending on the functional state after dopamine depletion and treatment indicating maladaptive neuroplasticity.

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Goal-directed movement in idiopathic Parkinson's disease and the effect of parkin mutations

C. Fearon, T. Munteanu, D. Birsanu, L. Newman, B. Quinlivan, i. killane, B. Magennis, J. Butler, R. Reilly, T. Lynch (Dublin,, Ireland)

Objective: To investigate goal-directed behavior in people with Parkinson's disease (with and without Parkin mutations) and healthy controls using a computer based task.
Background: In early idiopathic Parkinson's disease (PD) there is initial loss of dopaminergic innervation to the caudal putamen, which governs habitual movement. With disease progression, however, this may spread to the rostral regions of the basal ganglia involved in goal-directed behaviour. Parkin gene mutations causes more diffuse dopaminergic denervation to basal ganglia including the more rostral caudate nucleus. We investigated the effect of these topographical differences using a computer based goal-directed movement learning task.
Methods: A cohort of PD patients (with and without Parkin mutations) and age-matched healthy controls repeatedly manipulated a joystick to move an unseen cursor on a laptop screen in two tasks: Task 1 involved moving the cursor to a seen target; Task 2, to an unseen target. By examining the curves of refined movements from Task 1 to Task 2 (which requires action selection by the basal ganglia) we can quantify the rate at which goal-directed movements are learned, independently of overall speed of movement. Standard clinical and neurocognitive data were also collected, including the Wisconsin Card Sorting Test.
Results: PD patients performed Task 1 equivalently to healthy controls. However, in Task 2, early refinement of goal-directed behaviour was impaired compared with healthy controls. Final performance of Task 2 was comparable to controls implying that motor task learning, although slower, approaches that of controls over time. We found

differences in movement learning curves between patients with Parkin mutations were seen compared with non-Parkin participants.
Conclusions: This study paradigm displays the ability to quantitatively assess goal-directed movement in a manner that is independent of slowness of movement. The results above show that, in spite of bradykinesia, medicated PD patients perform goal-directed behaviour as well as controls, provided no action selection is required by the basal ganglia. If action selection is required, early refinement of goal directed behaviour becomes impaired compared to controls but approaches that of controls over time. There are subtle differences in motor learning in Parkin patients which may reflects the fact that Parkin PD is a nigrinopathy without any cortical pathology.

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CR6-interacting factor1 deficiency in dopamine neurons triggers early-onset parkinsonism in mice

I. Ryu, J. Han, Y. Jang, S.J. Kim, J. Kim, M.J. Lee, X. Ju, M.J. Ryu, S.-Y. Choi, W. Chung, E. Oh, G.R. Kweon (Daejeon, Korea)

Objective: One candidate is CR6-interacting factor1 (CRIF1), which controls translation and membrane insertion of 13 mitochondrial proteins involved in oxidative phosphorylation. Here we determined the change and impact of CRIF1 for PD progression with human postmortem brains and mice.
Background: Mitochondrial dysfunction has been implicated in Parkinson's disease (PD) progression; however, the mitochondrial factors underlying the development of PD symptoms remain unclear.
Methods: We assessed the change of CRIF1 expression using real time PCR, western blotting and immunohistochemistry in postmortem brains. And to evaluate the effect of Crif1 deficiency, we produced mice lacking the Crif1 gene in dopaminergic neurons (DAT-CRIF1-KO mice). Behavioral and biochemical changes studied with DAT-CRIF1-KO mice whether represent the human PD symptoms or not.
Results: We found that CRIF1 mRNA and protein expression were significantly reduced in postmortem brains of elderly PD patients compared to normal controls. DAT-CRIF1-KO mice began to show decreased dopamine production with progressive neuronal degeneration in the nigral area from 5 weeks of age. At ~10 weeks of age, they developed PD-like behavioral deficits, including gait abnormalities, rigidity, and resting tremor. L-DOPA, a medication used to treat PD, ameliorated these defects at an early stage, but induced stereotypic motor abnormalities in older animals.
Conclusions: These findings that down-regulation of Crif1 promotes the progression of PD could potentially form the basis of therapeutic approach through on the up-regulation of Crif1-associated functions in PD patients.

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Association of CSF biomarkers with motor and non-motor features in moderately advanced Parkinson's disease cohort: The BioFIND study

J. Goldman, A. Amara, L. Shaw, P. Taylor, H. Andrews, R. Alcalay, T. Xie, P. Tuite, C. Henchcliffe, P. Hogarth, S. Frank, M.-H. Saint Hilaire, A. Naito, M. Frasier, V. Arnedo, A. Reimer, M. Sutherland, C. Swanson-Fischer, K. Gwinn, U. Kang (Chicago, IL, USA)

Objective: Evaluate associations between CSF biomarkers of neurodegeneration and clinical phenotype in Parkinson's disease (PD) participants of the BioFIND cohort.
Background: Levels of CSF a-synuclein are lower in PD compared to healthy controls (HC) and lower levels of CSF beta-amyloid 1-42 (AB1-42) have been demonstrated to predict cognitive decline in PD. However, the relationship between CSF biomarkers and PD motor phenotype or non-motor symptoms is not well understood in moderate to advanced PD.
Methods: BioFIND is a cross-sectional, observational, case control study examining a cohort of moderate to advanced PD and matched HC participants. Clinical measures including MDS-UPDRS, Montreal Cognitive Assessment (MoCA), and REM Sleep Behavior Disorder (RBD) Questionnaire were assessed for correlations with CSF a-synuclein, AB1-42, total tau (t-tau), and phosphorylated tau (p-tau) in PD participants. We also assessed the relationship between these CSF biomarkers and PD motor phenotype (tremor dominant (TD) versus postural instability/gait disorder (PIGD).
Results: Mean CSF a-synuclein (1462.22 versus 1706.44 pg/ml, p=.012) and AB1-42 (297.62 versus 333.40, p=0.004) were lower in PD versus controls. None of the CSF biomarkers correlated with, or predicted MDS-UPDRS total or motor scores. However, CSF levels of a-synuclein were lower among PIGD participants (1210.2±485.7 pg/ml) (mean±SD) compared to those with TD phenotype (1590.6±690.4 pg/ml) (unequal variance t-test: -3.05, p=0.003). The only CSF biomarker associated with cognition was AB1-42, which was non-parametrically correlated with the remote recall subscore of the MoCA (Spearman's rho =0.215, p=0.025). There were no significant

correlations between CSF biomarkers and dichotomized RBD scores in this cohort. CSF a-synuclein was most strongly correlated with t-tau (r=0.806) and modestly with p-tau (r=0.434) and AB1-42 (r=0.402) (p's < 0.0005). **Conclusions:** Lower CSF a-synuclein is associated with PIGD motor phenotype in moderate to advanced PD participants in the BioFIND cohort, thereby extending this relationship from early to more advanced PD. CSF AB1-42, a-synuclein, t-tau, and p-tau levels do not correlate with RBD. In contrast, CSF AB1-42 remains an important potential biomarker for cognitive impairment in PD.

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Inhibition of mitochondrial complex I synthesis by chloramphenicol mitigates dopaminergic neuronal cell loss in PQ-induced parkinsonism

S.J. Kim, J. Han, Y. Jang, J. Kim, M.J. Lee, I. Ryu, X. Ju, M.J. Ryu, W. Chung, J.Y. Heo (Daejeon, Korea)

Objective: To find the potent drug for prevention of PD, we screened with above 1000 drugs in the PQ treated cells which currently used drug in clinics and hospitals. Among those drugs, chloramphenicol (CP) showed most powerful inhibitory effect.

Background: Paraquat (PQ), an herbicide regarding as an environmental factor for Parkinson's disease (PD) occurrence, induce dopaminergic neuronal loss via inhibition of mitochondrial complex I and III. Most patients of PQ-induced PD is affected by chronic exposure and need to preventional strategy for modulation of the disease progression.

Methods: We assessed the change of mitochondria complex expression using real time PCR, western blotting and immunohistochemistry in dopaminergic neuronal SN4741, mitochondrial DNA-depleted Rho cell and primary dopaminergic neuron . And to evaluate the function of mitochondria, we measured mitochondrial oxygen consumption rate using Seahorse bioscience XF24 analyzer. Also, we confirmed the ameliorate effect of CP in MPTP induced parkinson's disease mouse model.

Results: Administration of PQ after CP pretreatment has more increased cell viability in SN4741 cells and primary cultured dopaminergic neurons from rat than control group. Furthermore, reactive oxygen species (ROS) production with PQ treatment also reduced by CP pretreatment which imply the mitochondrial complex I as a target of CP. Decreased activity of mitochondrial complex I by reducing synthesis of ND1 protein with CP treatment lowered the PQ-recycling, which is mechanism of ROS production, resulting prevention of cell death and those CP effect did not observed on the Rotenone pretreatment and Rho cells. Consistent with in vitro and ex vivo results, MPTP treated mice has also ameliorate the dopaminergic neuronal cell loss and glial reactivation with CP pretreatment.

Conclusions: Our finding indicate that inhibitory action of mitochondrial complex I with CP treatment for protecting the dopaminergic neurons may provide a presentational strategy in prevention of neurotoxin induced PD.

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Alpha-synucleinopathy and mitochondrial dysfunction in a cell based model of neurodegeneration: Implications in the pathogenesis of sporadic Parkinson's disease

U. Ganguly, O. Sen, A. Ganguly, S. Chakrabarti (Kolkata, India)

Objective: To elucidate the role of a-synuclein, parkin and mitochondria in a cell-based model of neurodegeneration relevant to Parkinson's disease pathogenesis.

Background: The suggested mechanisms of PD pathogenesis have been deciphered using various experimental models,mainly the toxin-based models,which have questionable relevance in explaining the pathogenesis of sporadic PD. Thus, a model of neurodegeneration in cultured cells relevant to PD employing toxic effects of endogenous molecules like dopamine (DA) and iron would be relevant.

Methods: SHSY5Y neuroblastoma cells were treated with varying concentrations of iron (20-100 μM) and dopamine (10 - 50 μM) for a variable period of time. Cell death was assessed by trypan blue dye-exclusion test and lactate dehydrogenase release assays. The nature of cell death was analyzed by examining nuclear morphology after PI and Hoechst staining. Mitochondrial parameters were analyzed using JC-1 dye and ATP synthesis assays. a-Synuclein, parkin and Bax expressions were analyzed by Western blotting and qRT-PCR for mRNA. a-synuclein and parkin knockdown was carried out by using specific siRNA.

Results: Both DA and iron causes dose-dependent and time-dependent cell death.10 μM DA over a period of 96 h produces nearly 40% cell death along with decreases of mitochondrial membrane potential and ATP synthesis but the degree of cell death by iron was much lower. However, similar intra-cellular accumulation of a-synuclein took place in both conditions. When parkin expression levels were compared between DA-treated and iron-treated cells, a significant increase of parkin expression were noticed after iron, but not DA exposure suggesting a protective action of parkin against dysfunctional mitochondria. We are currently verifying this protective role of parkin by knock-

down experiments. The nature of cell death appears to be apoptosis and secondary necrosis.The involvement of Bax in DA or iron mediated cell death will also be explored.
Conclusions: The results strongly suggest that oxidative stress mediated by DA or iron can initiate neurodegeneration through the involvement of a-synuclein and mitochondria. Parkin may play a protective role against neurodegeneration by preventing dysfunction of mitochondria. An increased accumulation of a-synuclein and downregulation of parkin in sporadic PD brain have been documented.

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a-synuclein neurotoxicity and spreading using Lewy body extracts from Parkinson's disease brains: In vitro screening by cell-based assays

E. Bezard, F. Cavaliere, L. Cerf, B. Dehay, P. Ramos-Gonzalez, F. De Giorgi, M. Bourdenx, A. Bessede, J. Obeso, F. Ichas, C. Matute (Bordeaux, France)

Objective: To define the kinetics of neuronal and astrocytic abnormalities induced by human-derived a-syn aggregates for grounding the use of such system to identify and test putative therapeutic compounds.
Background: Synucleinopathies are a group of diseases characterized by the presence of intracellular protein aggregates containing a-synuclein (a-syn). While a-syn aggregates have been shown to induce multimodal cellular dysfunctions, uptake and transport mechanisms remain unclear.
Methods: Using high-throughput imaging on cortical neurons and astrocytes, we here define the kinetics of neuronal and astrocytic abnormalities induced by human-derived a-syn aggregates grounding the use of such system to identify and test putative therapeutic compounds. We then aimed at characterizing uptake and transport mechanisms using primary cultures of cortical neurons and astrocytes either in single well or in microfluidic chambers allowing connection between cells and cell-types.
Results: We report that astrocytes take up a-syn-aggregates far more efficiently that neurons through an endocytic event. We also highlight that active a-syn transport occurs between cells and any cell-types. Of special interest regarding the disease, we also show that uptake and spreading of a-syn from astrocytes to neurons can lead to neuronal death. We show using high-throughput screening a concentration-dependent toxicity of patients- derived a-synuclein aggregates. Then using microfluidic chambers, we report that a-syn aggregates are taken up by both neurons and astrocytes and can spread between cell-types leading to neuronal death.
Conclusions: Altogether these results lead us to propose a simple mechanism of production of a-syn toxic species and stabilization towards thermodynamically harmful species by the sole virtue of the host response to exogenous a-syn.

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Admixing augments nigral dopaminergic correlates during development to impart resistance to MPTP-toxicity at adulthood

V. D J, Y. H, R. T R, P. Anand Alladi (Bangalore, India)

Objective: Establish the developmental basis for varying nigral dopaminergic (DA) correlates in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) susceptible C57BL/6, MPTP resistant CD-1 and their resistant crossbred mice.
Background: Asian-Indians are less vulnerable to Parkinson's disease (PD) than the Caucasians. Interestingly their admixed population is at much lesser risk. Studying this phenomenon through mice strains with differential MPTP susceptibility revealed variations in nigral DA neuronal number along with other cytomorphological features in assigning resistance/susceptibility.[1,2]
Methods: Postnatal day 2 (P2), P6, P10, P14, P18, P22 and adults of C57BL/6, CD-1 and their reciprocal crossbred mice were studied. Tyrosine hydroxylase (TH) immunostained midbrain sections were evaluated for nigral volume and DA neuronal number by planimetry and stereology, respectively. TH expression and the neuronal morphological development was examined, alongside overall nigral development.
Results: Nigral volume and DA numbers were lesser in C57BL/6 compared to CD-1 and the crossbreds at birth and remained so throughout the development. A significant increase in number and nigral volume was observed in all the strains till P14. However, a drastic fall was seen thereafter only in C57BL/6 before stabilising at adulthood. Interestingly, CD-1 and the crossbreds retained their numbers from P14 to stabilize with supernumerary DA neurons at adulthood. Neuronal size significantly increased from P2 to P10 and then attained their adult morphology in CD-1 and the crossbreds, whereas it continued to increase in C57BL/6, only to stabilize at P22. TH expression and nigra attained its adult architecture at P14 in CD-1 and the crossbreds, whereas at P22 in C57BL/6.

Conclusions: We provide the first unbiased stereological estimation of nigral DA number during postnatal development in mice. CD-1 and the crossbreds by birth acquire resilient cytomorphological features; evidenced by higher number of DA neurons at P2. Absence of neuronal loss in these strains after P14 as seen in C57BL/6 indicates lesser developmental cell death. Faster maturity of DA neurons and nigral architecture provide further evidence for superior developmental characteristics, which might render MPTP resistance at adulthood. This demonstrates that variable MPTP susceptibility and heterogeneity in PD pathogenesis may arise early during development.

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Assessment of neurodegeneration and spreading of a-synuclein pathology induced by structurally defined a-synuclein assemblies in wild-type mice

E. Bezard, M. Bourdenx, L. Arcuri, L. Bousset, R. Melki, B. Dehay (Bordeaux, France)

Objective: The aim of this study was to investigate, in wild-type mice, the spreading and neurodegeneration induced by intracerebral injection of structurally well-defined a-synuclein assemblies (ribbons and fibrils), previously shown efficient in a-synuclein-overexpressing rats (Peerlaerts et al, 2015).

Background: Aggregation of a-synuclein is implicated in several neurodegenerative diseases. Emerging evidence have strongly implicated cell-to-cell transmission of misfolded a-synuclein as a pathogenetic mechanism in synucleinopathies. Several experimental paradigms have been used to study a-synuclein propagation in animals, in particular injections of exogenous *in vitro*-generated preformed a-synuclein assemblies (Bousset et al., 2013) or a-synuclein containing Lewy Bodies (LB) extracts from *postmortem* PD patient brain tissue (Recasens et al., 2014).

Methods: Wild-type mice received a stereotactic injection in the substantia nigra of synthetic a-synuclein ribbons and fibrils at various concentrations. Four months after injection, extensive analysis was performed to assess qualitatively, quantitatively and spatially in the whole brain the extent and pattern of lesion as well as the occurrence of synucleinopathy using both biochemical and histochemical procedures.

Results: Although no nigrostriatal degeneration was achieved, we observed a modest immunopositive signal for a-synuclein serine 129 phosphorylation, suggestive of an ongoing pathological process.

Conclusions: This study suggests that wild-type animals are less prone to develop a-synuclein pathology than overexpressing animals and might require a longer survival time to develop a full pathology.

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Expression of late cell cycle markers in Parkinson's disease and Lewy body dementia

I. Martinez-Valbuena, R. Valenti-Azcarate, M.M. Carmona-Abellan, I. Marcilla-Garcia, I. Gaston-Zubimendi, G. Marti-Andres, M.-T. Tuñon-Alvarez, M.R. Luquin-Piudo (Pamplona, Spain)

Objective: The objective of this study is to determine the expression of late cell cycle markers in Parkinson's disease (PD) and Lewy Body Dementia (LBD)

Background: Mechanisms that initiate and cause PD and LBD neuronal death are not fully understood. Several studies have shown that degenerating neurons, under certain stress conditions, can activate the program that normally guides cells to mitotic division. In these post-mitotic cells, the re-entry into the cell cycle will not end with neuron replication but with the activation of unknown mechanisms that will trigger neuronal death. Several studies have described abnormal expression of cell cycle markers in degenerating neurons. Furthermore, evidence for a completed S phase in PD brain, raises the question of what factors are missing in the adult neurons that lead to neuronal death just before the neuron enters mitosis.

Methods: We examined 3 brains of PD, 3 brains of LBD and 3 controls brains. Sections from *Substantia Nigra (SN), locus coeruleus (LC),* cerebellum, frontal and temporo-occipital cortex were immunostained using antibodies against PLK1 pSer 137, PLK1 pThr210, Aurora A, cyclin B and synuclein pSer 129. Double immunofluorescence techniques against the previous markers were performed. Interactions were analyzed using proximity ligation assays.

Results: We examined the expression of Aurora A and PLK1. PLK1 is crucial to regulate mitotic entry. This event depends on Aurora-A phosphorylation of PLK1 Thr 210. Besides, dephosphorylation of PLK1 Ser-137 is required for execution of cytokinesis. PLK1 pSer 137 was upregulated in all the regions both in PD and LBD brain but no in controls. Immunoreactivity against PLK1 pThr210 was found only in the *LC* in the PD brains, and in the *LC*, cerebellum, frontal and occipital cortex in the LBD brain. No expression was found in controls. All neurons immunostained against Aurora A were also immunoreactive against PLK1 Thr-210. Cyclin B1 immunoreactivity was only circumscribed to the cytoplasm, indicating that these neurons are in a late G2 phase. Cyclin B1 immunoreactive neurons, were also PLK1 pSer 137 and PLK1 pThr210 inmunoreactive.

Conclusions: This work describes for the first time the overexpression of two kinases involved in the G2/M transition, PLK1 and Aurora, whose over expression correlates with the severity of the pathology. These new PD targets could lead to the discovery of new pathways involved in neuronal death.

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Antibody binding differences in alpha-synuclein from Parkinson's disease and multiple system atrophy

T. Yamasaki, A. Welleford (Lexington, KY, USA)

Objective: Determine whether alpha-synuclein derived from Parkinson's disease (PD) and multiple system atrophy (MSA) brains has different conformations as defined by biochemical and antibody-based approaches.

Background: There is evidence in multiple neurodegenerative diseases for cell-to-cell transmission of misfolded forms of protein with the resultant spread of pathologic protein aggregates throughout the brain. In synucleinopathies such as PD and MSA there is also growing support for the idea that different conformations of alpha-synuclein underlie the clinical and pathologic differences seen in these two diseases. In prior studies we found alpha-synuclein seeding ability present in both PD and MSA brain extracts using a cell-based FRET assay. Here we use biochemical and antibody-based means to further define differences between alpha-synuclein in these two diseases.

Methods: Brain tissue from patients with PD and MSA was serially extracted to yield buffer-soluble and detergent-insoluble fractions. We used both commercially available antibodies and also novel antibodies generated to both recombinant fibrils and monomeric alpha-synuclein to test PD and MSA fractions for binding of alpha-synuclein by immunoprecipitation. The forms of alpha-synuclein bound to the antibodies were tested for ability to induce aggregation in the cell-based assay.

Results: There were distinct differences in the ability of various antibodies to bind to alpha-synuclein from PD vs MSA. Several antibodies were able to bind a form of alpha-synuclein which was capable of seeding further synuclein aggregation in the cell-based assay from MSA samples, but not from PD samples.

Conclusions: Differential antibody binding implies that different epitopes are available for binding in the aggregated state, and these findings support the idea that alpha-synuclein in PD and MSA are different in conformation. Another possibility is that post-translational modifications such as phosphorylation may interfere with antibody binding however this is less likely given the differential binding was maintained across different antibodies with different epitopes. The conformational differences seen may underlie the diverse clinical and pathologic characteristics of these two synucleinopathies.

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Development of nano-formulation containing crocetin for the protective and beneficial effect against 6-hydroxydopamine induced Parkinson's disease model via altered the genetic backgrounds

V. Kumar, A. Verma, P. Bhatt (Allahabad, India)

Objective: Parkinson's disease (PD) induced by the interaction between the number of factors viz., aging, genetics toxins, mitochondrial deformity and oxidative stress. Various evidences suggest that regular consumption of an antioxidant rich food may alter the incidence of neurodegenerative diseases. The aim of the current study was to prepare and scrutinize the nanoherbaceutical formulation containing crocetin against 6-hidroxidopamine (6-OHDA) induced PD animal model.

Background: PD is the most prevalent neurodegenerative disorder after Alzheimer's disease. The loss of dopamenergic neurons in brain results in the lower level of dopamine in the brain regions like Subtantia nigra which ultimately leads to the development of PD.

Methods: Top-down technique was used for the preparation of nanoherbaceutical suspension. Intracerebroventricular injection of 6-OHDA was used for induction the PD and mice were treated with the nano-formulation of crocetin for 28 days. The enzymatic activities viz., Catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GPx) and level of reactive oxygen species (ROS), total reactive antioxidant dopamine and its metabolites homovanillic and 3,4-dihydroxyphenylacetic acid, were scrutinized in the striatum. Behavioural parameters including locomotor, memory and depressive were estimated, respectively.

Results: The result clearly showed that nano-formulation crocetin treatment was efficient in averting the memory destruction in Morris water maze test as well as depressive like behaviour in tail suspension test. Nano-formulation crocetin attenuated 6-OHDA induced down-regulation in total reactive antioxidant, CAT and GPx, dopamine and its metabolite level in striatum of mice. We also observed the up-regulation levels of GR and reactive oxygen species in the striatum and these modulations were diminished by nano-formulation of crocetin.

Conclusions: Collectively, we can concluded that nanoherbaceutical formulation of crocetin showed a protective effect on 6-OHDA induced neurotoxicity in mice, signifying that it could be alternative therapy for the treatment of PD.

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In silico predictive analytics: accelerating identification of potential disease-modifying compounds for Parkinson's disease

N. Visanji, A. Lactoste, S. Spangler, E. Argentinis, S. Ezell, C. Marras, L. Kalia (Toronto, ON, Canada)

Objective: To use an *in silico* screen to identify compounds that have potential to reduce a-synuclein (aSyn) oligomers and are amenable to drug repurposing for Parkinson's disease (PD).

Background: Development of disease-modifying therapies for PD and translation into clinical use is expensive and slow. Repurposing of compounds, previously proven to be safe in humans and approved by regulatory agencies could reduce costs and accelerate drug development. However, methods to prioritize candidate drugs for repurposing are needed. IBM Watson for Drug Discovery (WDD) is a cognitive computing platform able to extract domain-specific text features (e.g., drugs, diseases) from the literature and identify connections between entities of interest. We used WDD to generate a predictive model to rank potential candidates for drug repurposing for PD.

Methods: We developed: 1) a training set of 15 chemical compounds known to reduce aSyn oligomers *in vitro* and/or *in vivo* based on published studies, and 2) a candidate set composed of all 620 individual active compounds in the Ontario Drug Benefit program database. WDD analyzed hundreds of thousands of Medline abstracts to learn text patterns and develop a semantic fingerprint for each compound and then, using machine learning, generated a predictive model to rank compounds from the candidate set based on their semantic similarity to the training set.

Results: Leave-one-out cross-validation demonstrated that each compound in the training set was highly ranked by the model, suggesting that highly ranked compounds from the candidate set would have properties common to the training set. Following ranking of candidate compounds, directed PubMed searches and exploration using WDD applications for the top 52 compounds revealed: Nine compounds with existing evidence for inhibition of aSyn aggregation (4 of which have not yet been studied in human clinical trials or epidemiological studies of PD), and 12 compounds not previously associated with aSyn but with biologically plausible links to aSyn aggregation.

Conclusions: Our approach using WDD to mine scientific literature to rank compounds with potential to reduce aSyn oligomers is novel and promising. Future work will perform necessary validation of prioritized compounds using both *in vitro* and *in vivo* models of aSyn aggregation and toxicity, as well as epidemiologic studies assessing incidence and outcomes in PD.

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Exposure to bifenthrin contributes to Parkinson's disease in mouse model

C. Han, S. Liu, J. Huang, L. Liu, K. Ma, X. Guo, S. Guo, L. Wang, Y. Shen, Y. Xia, F. Wan, N. Xiong, T. Wang (Wuhan, People's Republic of China)

Objective: To explore the relationship between pyrethroid bifenthrin and Parkinson's disease on mouse model.

Background: Pyrethroid is now widely used on account of its high efficiency and low toxicity compared to other pesticides. Bifenthrin, one pyrethroid used extensively, was previously demonstrated to cause developmental toxicity. Considering that some pyrethroid was demonstrated to induce dopaminergic degeneration, while the evidence for bifenthrin is still limited.

Methods: Mice were randomly divided into five groups (n=9, per group): vehicle group, 5 mg/kg bifenthrin group, 10 mg/kg bifenthrin group, 20 mg/kg bifenthrin group and 40 mg/kg bifenthrin group. Behavior assessment including pole test and rotarod test were conducted. Detection of dopamine, dopac, homovanilic acid and 5-HT by high performance liquid choromatography were performed. Besides, measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were also done. Pathologically, protein level of tyrosine hydroxylase (TH), a-synuclein and vasicular monoamine transporter 2 (VMAT2) were evaluated by western blotting and immunofluorescence or immunohistochemistry.

Results: In this study, we first demonstrated that high dose of bifenthrin was able to cause decreased body weight in mice and bradykinesia indicated by pole test and rotarod test. Pathologically, bifenthrin caused loss of TH and accumulation of a-synuclein. Furthermore, bifenthrin induced decreased expression of VMAT2. Additionally, bifenthrin caused increased oxidative index such as SOD and glutathione which might contribute to the pathological changes.

Conclusions: Our data showed that bifenthrin could induce pathological change resembling PD in mice, providing strong evidence that bifenthrin exposure could contribute to the pathogenesis of PD.

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Neuroprotective effect of a-Mangostin in restoration mitochondrial function in MPTP-induced Parkinson's disease in mice

A. Prakash, A.B. Majeed, K. Ramasamy, M. Hasan (Baltimore, MD, USA)

Objective: The aim of the present study was to explore the protective effect of alpha-mangostin against mitochondrial oxidative stress in MPTP treated mice.

Background: It has been reported that mitochondrial oxidative stress plays a pivotal role in neurodegenerative disease like Parkinson's disease (PD). Recently, medicinal plant extracts acted as traditional, complementary and potential medicine for PD. Alpha-mangostin (AM) is the main xanthone purified from mangosteen known as anti-oxidative properties.

Methods: MPTP was administered repeatedly on 1st, 7th and 14th day intranigrally for the induction of PD in mice. AM (3 and 6mg/kg) and Selegiline (10 mg/kg) were given intraperitonially, after induction of PD for 14 days. Different behavioral performances were carried on 1st, 14th, 21st, 28th days and biochemical parameters were estimated on 28th day.

Results: Central administration of MPTP showed significant impairment of motor behavior and marked increase of mitochondria oxidative damage and neuro-inflammation in mice. However, post treatment with AM (3 and 6mg/kg) significantly and dose dependently improved the motor deficits and attenuated the oxidative damage indicating decreased rise of LPO and nitrite concentration and restored the decreased activities of endogenous antioxidant enzyme (Glutathione, Catalase, SOD) and mitochondria enzymes (NADPH dehydrogenase, Succinic dehydrogenase and cytochrome oxidase) as compared to selegiline effects. In addition AM also attenuates the pro-inflammatory cytokines like TNF-a and IL-ß in striatum region of MPTP induced PD in mice.

Conclusions: These results suggested that AM exhibit Neuroprotective effect by mediating brain antioxidant defense mechanism and by up regulating of dopaminergic pathway

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Efficient control of dopamine neuron physiology for rescuing disease phenotypes

T. Rumbell, J. Kozloski (Yorktown Heights, NY, USA)

Objective: Dopamine neurons (DAs) of the substantia nigra degenerate in Parkinson's disease and are disrupted in Huntington's disease. Here we propose single cell computational modeling methods that can capture the variety of electrophysiology within this neuronal population, providing a method to understand the transition to pathology.

Background: Degeneration caused by disease may derive from altered electrophysiology that pushes DAs beyond their normal phenotype. For example, sustained engagement of calcium channels due to low-threshold L-type channel activity results in relatively high basal calcium flux and oxidative stress, constituting a risk if normal neuronal behavior is disrupted in disease.

Methods: *In vitro* data, which are variable due to differential channel regulation, can be used to understand the balance of ion channel properties required for normal DA function. Here we demonstrate a new evolutionary algorithm that generates a population of DA models with the full range of observed phenotypes. Parameters that generate these phenotypes vary widely. We tuned models of ion channels unaffected by suprathreshold channel blockers and fitted voltage to subthreshold oscillations. We performed a principal component (PC) analysis on the parameter sets in the population after this optimization, which identified combinations of parameters that covary and explain the range of acceptable models.

Results: Certain PCs were highly correlated with subthreshold oscillation amplitude and frequency, and we were able to identify separable, low-dimensional parameter combinations capable of fully controlling each oscillation feature. We term these axes in parameter space "functional regulatory units". These units provide a specific axis through parameter space which efficiently controls key features of the model. Specifically, subthreshold oscillation features in our dopamine neuron model can be fully controlled by varying several parameters related to L-type channels, calcium buffering and extrusion, and calcium-dependent potassium channels.

Conclusions: By identifying how to combine changes to multiple parameters simultaneously to produce a desired functional change, we propose a method to rescue pathological phenotypes. We propose that this method can provide great insight into neuronal function, dysfunction, and therapeutic design by identifying functional regulatory units for control and rescue of behavioral features of neuron models.

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Optimization of synucleinopathy and nigrostriatal degeneration induced by injection of alpha-synuclein preformed fibrils into rat striatum

C. Sortwell, T. Collier, M. Duffy, K. Luk, C. Kemp, N. Kanaan, K. Paumier, J. Patterson (Grand Rapids, MI, USA)

Objective: Optimization of the surgical parameters (intrastriatal placement and concentration of alpha-synuclein preformed fibrils; a-syn PFFs) to increase the magnitude of nigrostriatal a-syn pathology and resultant degeneration.

Background: Existing models of Parkinson's disease (PD) have been unable to predict clinical translation. Specifically, no animal model of PD has simultaneously incorporated widespread a - a-syn pathology, protracted and significant nigrostriatal degeneration and consistent motor impairments. Previously, we demonstrated that injection of mouse a-syn PFFs unilaterally into rats results in significant accumulation of a-syn pathology and bilateral nigrostriatal degeneration (˜ 40% loss, Paumier et al., 2015). However, the nigrostriatal degeneration was not of sufficient magnitude to produce consistent motor deficits. In the present study we analyzed whether more precisely targeting the nigrostriatal terminal field within the dorsal striatum and increasing the amount of mouse a-syn PFFs could augment synucleinopathy, loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) and produce motor impairments.

Methods: Male Fischer 344 rats (n=88) were injected with 8 µg a-syn PFFs, 16 µg a-syn PFFs, 16 µg a-syn monomer or an equal volume of vehicle in two sites of the dorsal striatum. Post-mortem pathology was evaluated at 2, 4, and 6 months after surgery. Unbiased stereological assessment was used to quantify tyrosine hydroxylase immunoreactive, total neurons and phosphorylated a-syn (pSer129) in the SNpc. Motor performance of the 6 month cohort was assessed to examine forelimb use in the cylinder and adjusting steps task as well as locomotor activity in the open field and modified cylinder.

Results: Targeting of a-syn PFF placement to the dorsal striatum significantly concentrated a-syn pathology to the SNpc and decreased syn pathology in the ventral tegmental area (VTA). Stereological and behavioral analyses are ongoing and will be presented at the meeting.

Conclusions: Optimization of a-syn PFF-induced synucleinopathy in rats will leverage the distinct advantages of the rat model system compared to mice, i.e. more complex motor behaviors, greater synaptic complexity and sampling capability. Once successfully optimized, the a-syn rat PFF PD model will facilitate preclinical assessment of novel disease-modifying therapies for PD.

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Genetic knock-down of HDAC4 attenuates rotenone-induced abnormal expression of a-synuclein by affecting autophagic flux in SH-SY5Y Cells

L. Wang, J. Huang, L. Liu, C. Han, K. Ma, X. Guo, S. Guo, Y. Shen, Y. Xia, F. Wan, N. Xiong, T. Wang (Wuhan, People's Republic of China)

Objective: To assess whether histone deacetylase 4 (HDAC4) is related to the abnormal expression of a-synuclein (a-syn) via the autophagy pathway.

Background: The overexpression and aggregation of a-syn play central roles in the pathogenesis of Parkinson's disease (PD). Therefore, the clearance of a-syn is a feasible and promising therapeutics alternative for PD. Previous studies indicated that aberrant histone acetylation could inhibit the autophagy process, which influence the abnormal overexpression and aggregation of a-syn.

Methods: The shRNA of HDAC4 and vehicle were transfected into SH-SY5Y cells respectively, then the cells were treated with 1µmol rotenone for 48 hours. The protein levels of HDAC4, a-syn, LC3-?, p62 and beclin-1 were evaluated by Western blot.

Results: After exposure in rotenone, the SH-SY5Y cells showed an abnormal increase in the expression of a-syn. However, after knock-down of HDAC4, the abnormal expression of a-syn was reversed. Moreover, the protein levels of LC3-? and belcin-1 were increased, while the level of p62 was decreased.

Conclusions: Reduced expression of HDAC4 reverses the abnormal expression of a-syn via activation of autophagy. HDAC4 is related to rotenone-induced elevated level of a-syn.

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Alpha-synuclein protein homeostasis and oligomerization in iron-overloaded cells expressing mutant HFE

Y. Kim, J. Connor, M. Stahl (Hershey, PA, USA)

Objective: Study the direct and indirect effects of iron overload on alpha synuclein homeostasis in a genetic model of hemochromatosis (*HFE*)

Background: Parkinson's disease (PD) is characterized by the presence of alpha synuclein-containing Lewy bodies with selective vulnerability of particular neuronal populations, especially the dopaminergic neurons in the substantia nigra (SN). Increased levels of iron and ferritin in the SN also appear to be consistent features of the disease, but it remains unclear whether this accumulation is pathologic and if it relates to synuclein aggregation. Population genotype studies have returned conflicting results in regards to correlation between polymorphisms in iron metabolism genes (transferrin, transferrin receptor 1, HFE, frataxin, and lactoferrin) and PD. Nevertheless, a direct effect on synuclein can be postulated due to the known effect of metal ions on protein aggregation in vitro, the presence of a putative iron responsive element (IRE) in the 5'- untranslated region of the alpha synuclein messenger RNA, and the effects of iron on synuclein disposal via autophagy and proteasome activity.
Methods: Human neuroblastoma SH-SY5Y cell lines with stable transfection of two common *HFE* mutations (C282Y and H63D) were used to study alpha synuclein in iron-overloaded states. Total synuclein protein was assessed by Western analysis, while oligomers were studied using blue native PAGE. Autophagic flux was assessed via Western blot for LC3 and proteasome activity via effects on a artificial fluorescent/luminescent substrates. Transcripts were measured using RT-PCR.
Results: Western blot analysis showed that H63D and C282Y *HFE*-expressing cells had higher levels of total alpha synuclein compared to wild type. Addition of the iron chelator deferoxamine had varying effects on the intracellular labile iron pool and alpha synuclein levels. The impact of the *HFE* mutants on transcription of alpha synuclein, autophagic flux and proteasome activity and ultimately the levels of higher molecular weight alpha synuclein oligomers was also assessed.
Conclusions: These findings support the concept that iron may play a synuclein-mediated role in PD neurodegeneration.

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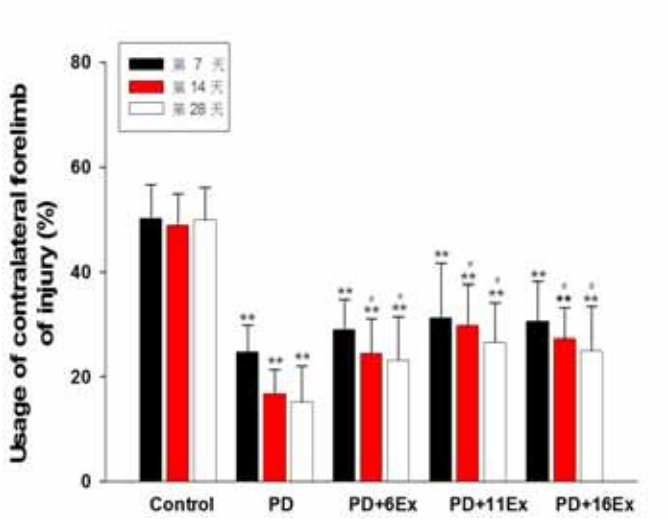
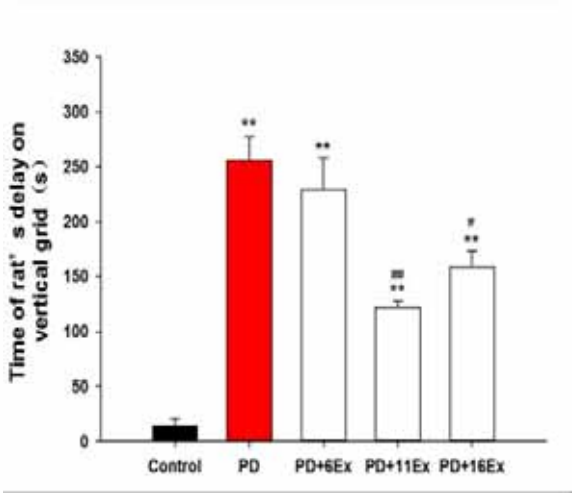
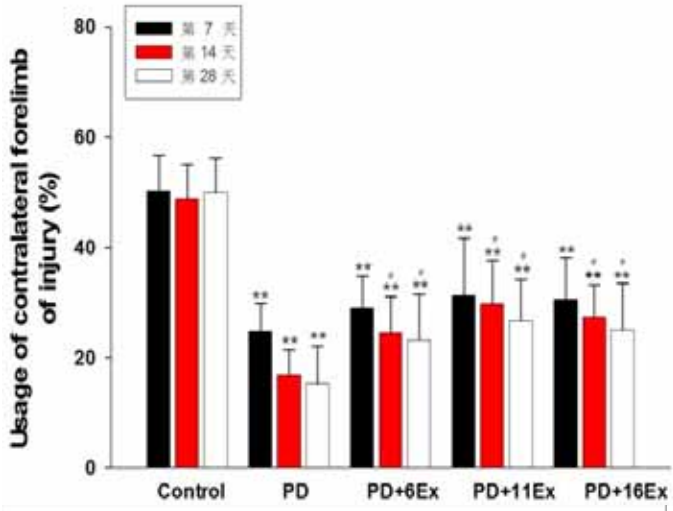
Effects of different intensity exercises on motor function of PD rats as well as the regulation of DA

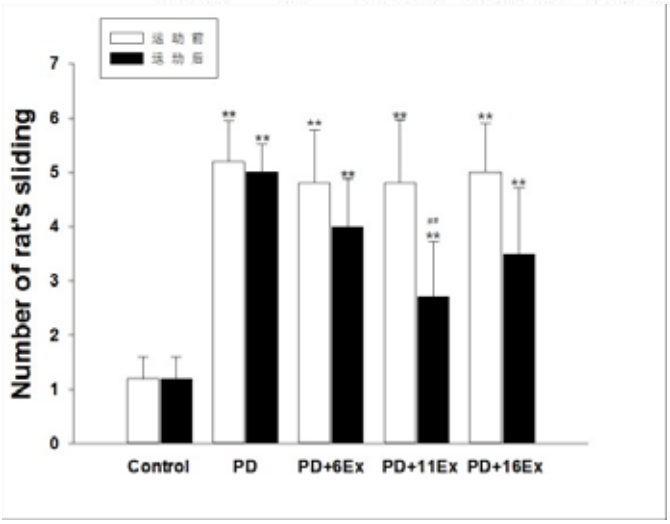
X. Liu, P. Chen, D. Qiao, M. Wang (Beijing, People's Republic of China)

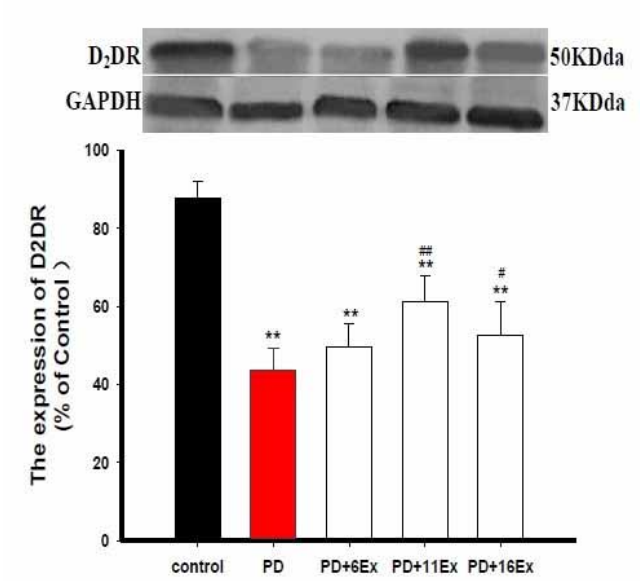
Objective: This paper research the intervention effect of the different intensity treadmill exercise on motor function of Parkinson's 's diseases (PD) model rats, and explore the most suitable intensity treadmill exercise intervention for prevention and treatment of PD.
Background: Parkinson's disease (PD) is the second most common progressive, irreversible neurodegenerative disorder in the World. Leads to difficulty with activities of daily living (ADLs) and decline in quality of life (QOL). Treatment options for PD are limited to pharmacologic management and surgery. Less invasive alternatives, such as exercise, have captured the attention of scientists and clinicians as possible adjunctive therapy.
Methods: Clean level male SD rats were randomly divided into Control group, PD group,PD+6Ex group,PD+11Ex group and PD+16Ex group. PD model rats were established by injection of 6-OHDA in right medial forebrain bundle single point. Control groups rats received same dose of saline. The exercise groups were intervened by 4 weeks of treadmill exercise at 24 hours after the surgery, the corresponding running speed of each group is 6m/min,11m/min and 16m/min,30 min/day,5day/week.
Results: Cylinder test showed that, the effect of the intervention became more and more obvious with the time prolonged. Grid test results showed, after 4 weeks exercise intervention, compared with PD group of rats, the slip times of PD+11Ex group significantly reduced. Immunohistochemical results showed that, compared with PD group, the expression of TH and D2DR in striatum of PD+11Ex group and PD+16Ex group rats increased significantly .The variation trend of the results of Western Blot was in accordance with the results of immunohistochemistry. The expression levels of TH and D2DR in striatum in low intensity exercise group rats have low correlations with the changes of motor ability ;but in medium intensity exercise group rats have high correlations with the changes of motor ability.

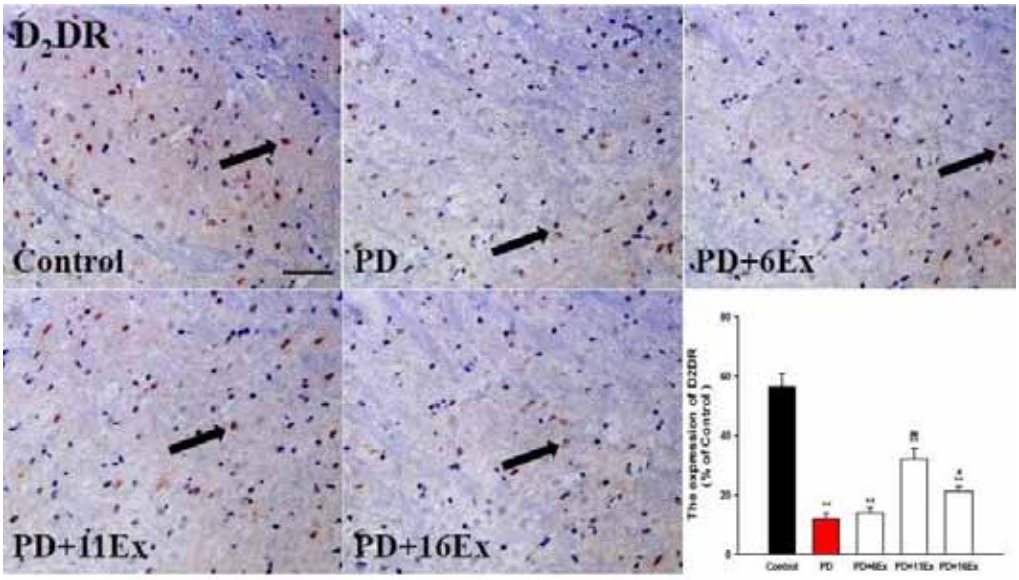

Usage of contralateral forelimb of injury (%)
第 7 天
第 14 天
第 28 天
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex

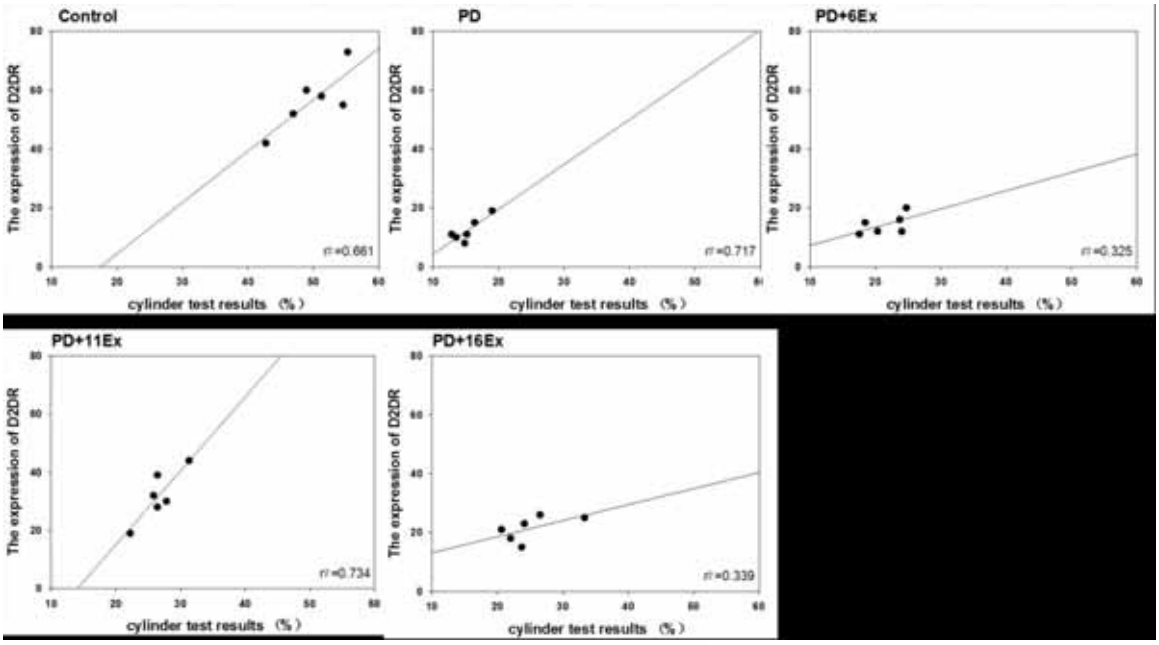

Time of rat' s delay on vertical grid (s)
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex

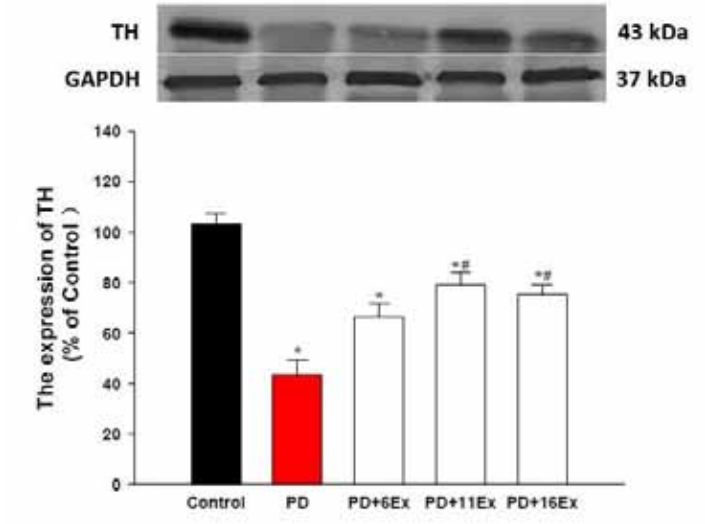

Usage of contralateral forelimb of injury (%)
第 7 天
第 14 天
第 28 天
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex

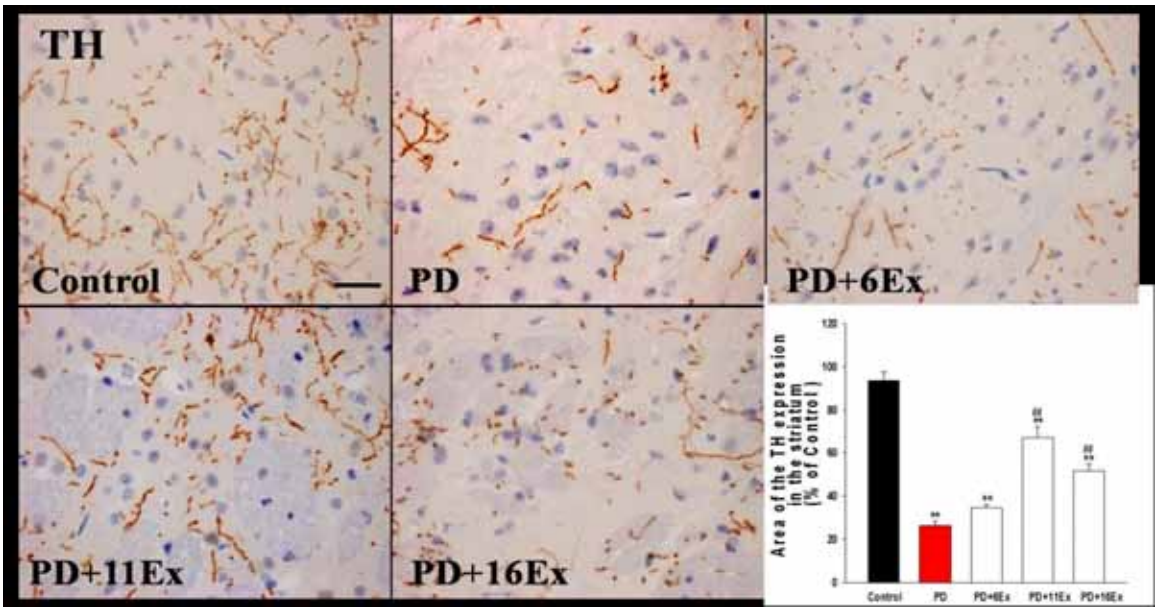

Number of rat's sliding
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex

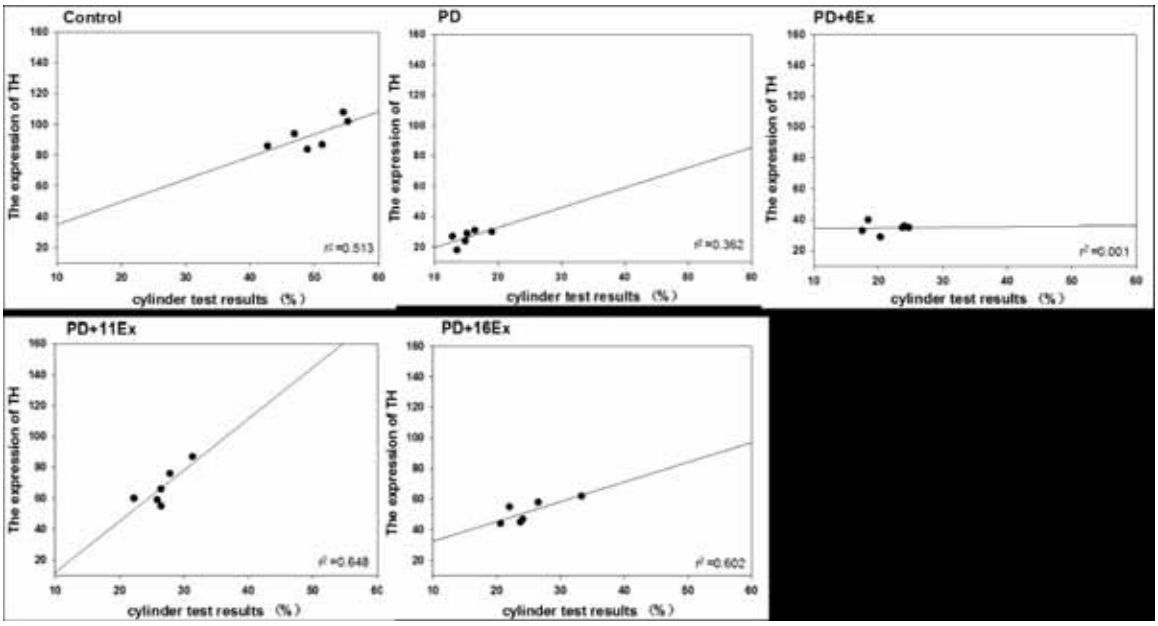

D2DR
50KDda
GAPDH
37KDda
The expression of D2DR
(% of Control)
100
80
60
40
20
0
control
PD
PD+6Ex
PD+11Ex
PD+16Ex

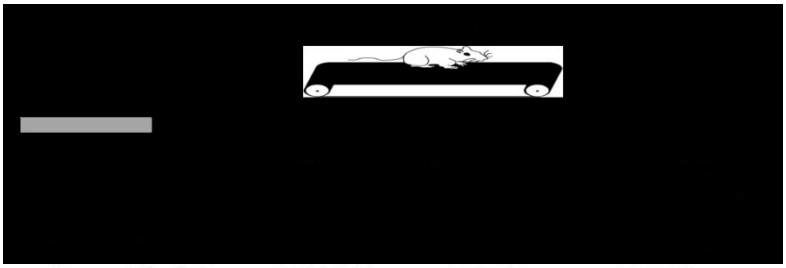

D2DR
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex

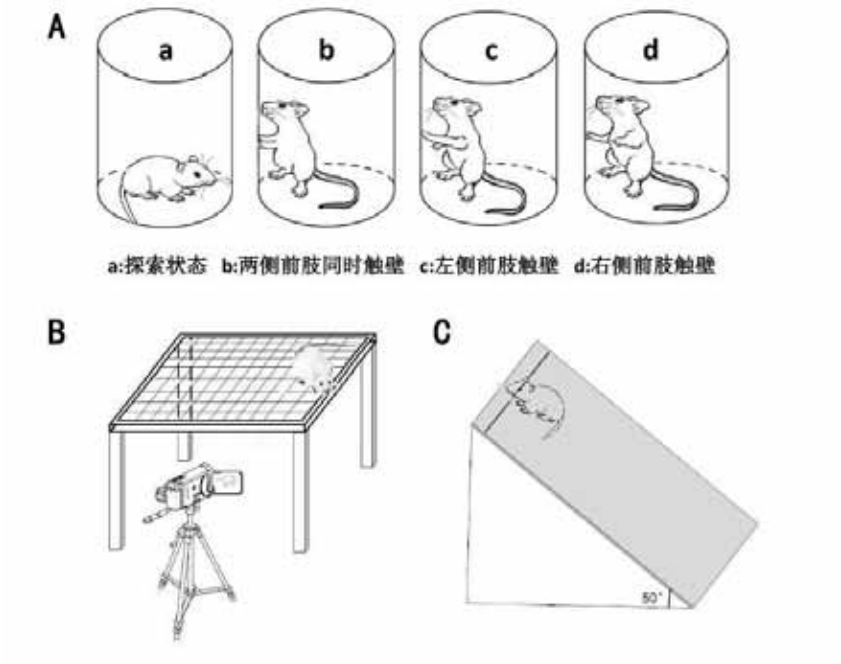

Control
PD
PD+6Ex
PD+11Ex
PD+16Ex
The expression of D2DR
cylinder test results （%）
r=0.661
r=0.717
r=0.325
r=0.734
r=0.339

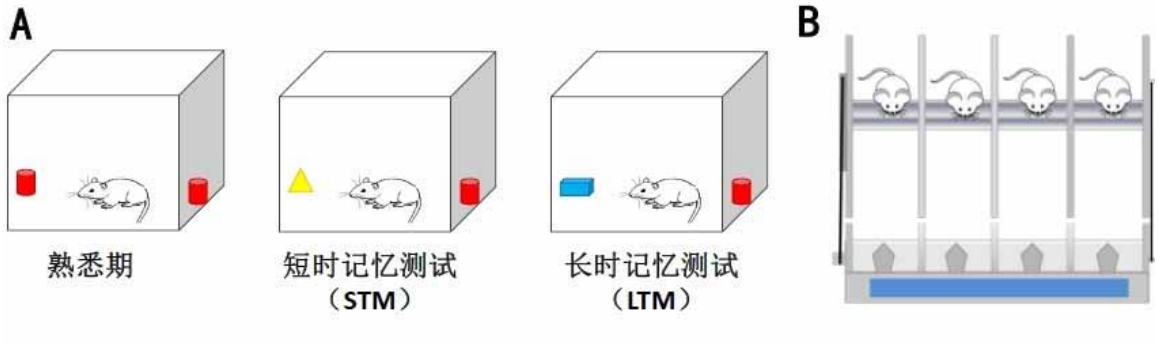

TH
GAPDH
43 kDa
37 kDa
The expression of TH
(% of Control)
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex


TH
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex
Area of the TH expression in the striatum (% of Control)
Control
PD
PD+6Ex
PD+11Ex
PD+16Ex


Control
PD
PD+6Ex
PD+11Ex
PD+16Ex
The expression of TH
cylinder test results (%)
r² =0.513
r² =0.362
r² =0.001
r² =0.648
r² =0.602







Conclusions: Four weeks treadmill exercise intervention can reduce movement dysfunction of PD model rats, and significantly increased TH and D2DR expression level in striatum. This suggests that DA systems involved in the regulation of motor function in rats; and the neurobiological mechanisms of exercise intervention reduced the motor dysfunction in PD model rats may related to the regulation effect of TH and D2DR.

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Inhibition of long term memory and induction of biochemical deficits in rats following protofibrillar Aß 1-42 injection

B. Nehru, S. Sharma (Chandigarh, India)

Objective: The present study was undertaken to study the effects of single intracerebroventricular (i.c.v.) injection of protofibrillar Aß 1-42 on the long term memory

Background: Amyloid-beta (Aß) peptide deposition into insoluble plaques is a pathological hallmark of Alzheimer's disease (AD) but the soluble oligomeric Aß has been hypothesized to directly impair the learning and memory in AD. Evidences from some clinical studies indicated that Aß protofibril formation is induced by the Arctic mutation (E22G) and is the major cause for early AD onset. However, the biochemical mechanism involved in the protofibril-induced toxicity is not very well addressed.
Methods: Rats were divided into two groups (n = 5 per group): (1) sham control group and (2) Aß 1-42 injected group. A single dose of protofibrillar Aß 1-42 (5 ul) through i.c.v. injection was bilaterally administered to 2nd group animals while sham control animals were administered with 5 ul of vehicle
Results: The results demonstrated that protofibrillar Aß significantly induced reactive oxygen species (ROS) production, acetylcholinesterase activity, nitrite levels and lipid peroxidation in hippocampus, cortex and striatum regions after six weeks. Also, the behavioral studies have shown an increase in the anxiety levels and inhibition of long term memory after protofibrillar Aß injection. The activity of antioxidant enzymes was also significantly reduced after protofibrillar Aß injection in hippocampus, cortex and striatum regions. Thioflavin-T staining confirmed the presence of amyloid deposits and Nissl's staining have shown the neuronal loss after six weeks of protofibrillar Aß injection
Conclusions: The present study indicated that protofibrillar Aß 1-42 injection inhibits the long term memory and also leads to various biochemical alterations.

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Perrault syndrome: CLPP-Knock-Out mouse brain shows accumulation of mitoribosomes

J. Key, J. Heidler, S. Torres-Odio, G. Auburger, I. Wittig, S. Gispert (Frankfurt am Main, Germany)

Objective: We aimed to document the alterations of pathways and molecules that underlie brain pathology in the Perrault syndrome.
Background: Perrault syndrome is an autosomal recessively inherited rare disorder with early sensorineural deafness, accompanied by complete infertility and progressive cerebellar ataxia. Loss-of-function mutations in the CLPP (Caseinolytic Mitochondrial Matrix Peptidase Proteolytic Subunit) gene are responsible. Although this peptidase is highly conserved from bacteria until the mitochondrial matrix of human species, the protein substrates that are cleaved by CLPP rather than by the other two mitochondrial matrix proteases (LONP1 and m-AAA, the latter being responsible for Spinocerebellar Ataxia type 28) are still unclear.
Methods: Clpp-knock-out (KO) mouse brains were dissected and subjected to global proteome profiling via label-free mass spectrometry and volcano plot analysis. Biomathematical workup was carried out with the STRING webserver in Heidelberg. Validation experiments included quantitative reverse-transcriptase polymerase chain reactions and immunoblots.
Results: A systematic accumulation of practically all mitoribosomal subunits was observed, in the absence of transcriptional induction, suggesting decreased turnover of the mitoribosomal proteins in the absence of CLPP peptidase. The molecular chaperone ERAL1 in the mitochondrial matrix is involved in mitoribosomal assembly and showed protein accumulation in the absence of transcriptional induction, as well. These data confirm analogous observations of an independent team in Clpp-KO heart tissue, which were recently published (Szczepanowska K et al 2016 EMBO J) and also showed mitoribosome and ERAL1 accumulation.
Conclusions: The mitoribosomal dysregulation in Clpp-deficient neural tissue is likely to explain the progressive hearing impairment in Perrault syndrome. Previous observations of mitochondrial rRNA and tRNA variants as the cause of sensorineural deafness point in the same direction. Mitoribosomes are also the target of aminoglycoside antibiotics, which cause deafness upon chronic administration.

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Development of a prototype alpha-synuclein vaccine to induce T regulatory cells for the treatment of synucleonopathy

E. Rockenstein, G. Ostroff, F. Dikengil, F. Rus, M. Mante, J. Florio, A. Adame, I. Trinh, E. Masliah, R. Rissman (La Jolla, CA, USA)

Objective: Test the hypothesis that combining humoral and immunosuppressive cellular asyn immunization will enhance asyn clearance, and reduce inflammation and neuropathological symptoms in a human asyn tg animal model system.
Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive accumulation of alpha-synuclein (asyn; synucleinopathy) and currently no disease modifying treatments are

available. Previous studies in transgenic (tg) mice have shown that active and passive immunization to asyn reduces asyn accumulation and disease symptoms. It has been recently reported that the co-delivery of an antigen + Rapamycin (Rap) in nanoparticles induced antigen-specific T regulatory (Treg) cells. We adapted this immunization strategy to asyn using the antigen-presenting cell targeting glucan particle (GP) vaccine delivery system.
Methods: Glucan particles were loaded with recombinant full-length human-asyn (h-asyn), Rap or h-asyn + Rap. A macrophage cell line expressing LC3-GFP was used to demonstrate Rap delivery and induction of autophagy. PDGF-asyn tg and control non-tg mice were immunized with: Groups 1) GP alone, 2) GP + human h-asyn, 3) GP + Rap and 4) GP + Rap + h-asyn vaccines and analyzed by immunological, biochemical and neuropathological markers.
Results: Group 2 - GP + h-asyn or Group 4 - GP + Rap + h-asyn immunized mice showed strong total IgG, IgG1 and IgG2 anti-asyn titers, levels were slightly higher in the combined asyn + Rap group. Asyn levels in the frontal cortex and hippocampus in Groups 2 and 4 asyn immunized mice were reduced between 30-50% when compared to control Groups 1 and 3 non-Tg immunized mice. Asyn reduction in the combined asyn + Rap group was greater. Combined immunotherapy in Group 4 - GP + Rap + h-asyn mice resulted in increased levels of CD25, FoxP3 and CD4 positive Treg cells and TGFb1, and reduced neuro-inflammation (Iba-1, GFAP, IL6, TNFalpha) by microglial and astroglial cells.
Conclusions: *In vivo* vaccination studies targeting alpha-synuclein support the hypothesis that immunization to generate Treg cells might enhance the effects of active immunotherapy for the treatment of synucleionopathies. Future work will focus on the asyn antigen specificity of induced Tregs and application to other neurodegenerative proteinopathy-mediated diseases. *Supported by grants from NIH grant AG18440*

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Design and Discovery of 1,2,4 Triazole Derivatives as Adenosine A2A Receptor Antagonist Against Parkinson's Disease

A. Verma, V. Kumar, U. Singh (Allahabad, India)

Objective: In the present study, we intended to develop novel 1,2,4 triazole as potent A(2A)AR antagonists against Parkinson's disease.
Background: Parkinson's disease (PD) is a general, age-associated, progressive, neurodegenerative, neurological disease and its effective treatment is seriously jeopardized with loss of drug action, motor problem and failure to circumvent disease progression. Thus, novel approaches to therapy are urgently required. In this regard, agents targeting adenosine A(2A) receptors (A(2A)ARs) provides a viable option. The expected introduction of ST1535 and ST4206, a 1,2,4 triazole based antagonist in clinical trial provide a rational for the development of new A(2A)AR antagonists.
Methods: The skeleton of 1,2,4 triazole was developed using cyclo-condensation reaction with primary amines. These molecules were subsequently evaluated for in vitro anti-Parkinson's activity by free radical scavenging assay. The crystal structure of adenosine A2A receptor was undertaken with the aim to selectively antagonize its effect.
Results: The synthesized molecules were found in accordance with Drug Likeliness recommendations for new chemical entity (NCE) exhibiting good bioavailability. Anti-Parkinson's screening suggested, compound 6b, 6d and 6a having electron withdrawing group as potent inhibitors with 80%, 92% and 96 % free radical scavenging activity, respectively. In selective inhibition study of compounds with the adenosine A2A receptor, it has been suggested that, above molecules selectively and efficiently create interaction with Ala59, Ile66, Phe168, Ile80, Tyr271 and Ile274 of A2A receptor protein domain with Ki (Inhibition constant) raging from 427.37nM to 4.89µM disclosing 6a as a most potent analogue.
Conclusions: The results of study enumerated 6a as a potent A(2A)ARs antagonist providing benefit against Parkinson's disease via excellent free radical scavenging activity with excellent bioavailability.

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Neuroprotection and Alleviation of Parkinsonian Phenotypes by Ayurvedic Herbs

S. Singh, J. Prakash, S. Yadav (Varanasi, India)

Objective: Our laboratory has focused on the herbal treatment of Parkinson's disease especially by the above two common herbs *Withania somnifera (Ws)* and *Mucuna pruriens (Mp)*. Both the extracts are capable of inhibiting the oxidative stress occurring in nigrostriatal tissues and simultaneously increase the counts of TH positive cells in SN region of the MB–PQ induced PD mouse brain.
Background: PD is one of the most common neurodegenerative disorders after Alzheimer's found in the elderly. L-dopa therapy is a major medical treatment for the symptoms of PD, though it has several side effects. The two herbs,

Ashwagandha (*Withania somnifera*) and Kapikachhu (*Mucuna pruriens*), have been used to treat PD for several hundred years in the traditional system of Indian medicine..
Methods: We have chemically induced PD in mice, using either MPTP or Paraquat. and tested our hypothesis by biochemical estimations, Western blotting, immunohistochemical methods (e.g TH, GFAP) and behavior tests (e.g rotarad, narrow beam walking & hanging).
Results: In the maneb-paraquat (MB+PQ) model of PD in mice, *Ws co*-treatment improved PD-induced behavioural deficits, rescued dopaminergic neuron degeneration and had a potent antioxidant effect in the nigrostriatal region. Similar results were observed for *Mp* co-treatment with a PQ-induced model of PD. Further, the synergistic effect of both *Ws* and *Mp* resulted in drastic improvements in all the symptoms of PD. Finally, using an acute model of toxin-induced PD (MPTP),we obtained that one of the components, Ursolic Acid (UA), of Mp seed extract has a neuroprotective effect against PD.This study suggest that the UA has a strong antioxidant property. The treatment of the MPTP-intoxicated mice with UA, improved motor behaviour impairment by reduction of oxidative stress in SN, improved the expression of TH in SN region of the brain and protected the dopaminergic neurons.
Conclusions: It is evident from this study that UA has strong antioxidant potential and it is known to have partial MAO-B inhibitor activity. Altogether, this study demonstrates that UA could be used as a potential drug for the prevention/treatment of PD by both reversing the symptoms and correcting the underlying cause.It is evident that traditional ayurvedic herbs have the ability to treat, prevent and reverse the symptoms of the most debilitating neurodegenerative diseases, without side effects.

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Antisense Oligonucleotides Containing Amido-Bridged Nucleic Acid Downregulate SNCA Expression and Improve Motor Function in Parkinson's disease Mouse Models

T. Uehara, C.J. Choong, H. Hayakawa, Y. Kasahara, T. Nagata, T. Yokota, K. Baba, M. Nakamori, S. Obika, H. Mochizuki (Osaka, Japan)

Objective: In this study, we used gapmer-type antisense oligonucleotides (ASOs) containing amido-bridged nucleic acid (AmNA). ASO, short single-stranded stretches of DNA or RNA with complementary sequence of their target mRNA, is one of the most commonly used strategy for silencing gene expression. AmNA is a novel locked nucleic acid (LNA) analogue based on a cyclic amide structure, which shows high nuclease resistance and binding affinities towards complementary strands. The aim of this study is to investigate the effectiveness in downregulation of SNCA, administering ASO to SNCA transgenic mouse models.
Background: a-synuclein (aSyn) plays important roles in the pathogenesis of Parkinson's disease (PD). Both missense mutations and increased copy number of the SNCA gene encoding aSyn cause early onset autosomal dominant PD. Though previous studies have shown that nucleic acid therapies inhibit SNCA expression in some animal models, there still remain issues in both effectiveness and sustainablity.
Methods: We transfected ASOs which have 50 different sequences encoding SNCA's mRNA to human embryonic kidney cells 293. Total RNA from the cells was extracted 1 day after transfection, reverse transcribed, and evaluated SNCA expression by quantitative PCR. We determined "the best" sequence of the 50 ASOs. Then, we injected the ASO intracerebroventricularly to human SNCA transgenic mice. Seven days after injection, we extracted RNA from striatum and evaluated SNCA expression by quantitative PCR, similarly. Thirty days after injection, protein was also extracted, and the level of aSyn was determined by ELISA. As the behavioral test, we gave several 1cm-pieces of spaghetti noodles to human SNCA transgenic mice which had motor deficit (Line 61), and evaluated the number of bites per gnawing episode and the frequency (pasta gnawing test).
Results: The mice which were injected the AmNA-ASO showed significantly reduction of SNCA expression and the level of aSyn in the striatum. The result of pasta gnawing test indicated that the ASO-treated group significantly improve motor function better than non-treated group.
Conclusions: We revealed that gapmer AmNA-ASO downregulates SNCA expression, and improve motor function of the PD animal model. We think this nucleic acid medicine has the potential to treat PD.

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CB2R Agonist AM1241 Reverses the Development of MPTP-Induced PD and Activates the Regeneration of DA Neurons

R. Zhu (Shanghai, People's Republic of China)

Objective: A promising and potential direction to treat PD is to develop new non-dopaminergic drugs without producing dyskinesia, as well as arresting and even reversing the degeneration of dopaminergic neurons. In this

study, we investigated the role and mechanism of CB2R agonist AM1241 on MPTP-induced neurotoxicity and regeneration of DA neurons.
Background: PD is a kind of neurodegenerative movement disorder, which is characterized primarily by a massive loss and degeneration of DA neurons in the substantianigra compact and a significant reduction of striatal dopamine. Current pharmacotherapies relieve PD symptoms but fail to prevent or slow down the disease progression.
Methods: The research was studied on MPTP-induced PD mice. Behavioral tests were assessed the treatment function of AM1241, and using HPLC to detect the striatal DA and 5-HT levels. To explore the mechanism, the expressions of CB1, CB2, Parkin, PINK1, pPI3K and pAkt were determined by both western blot and immunofluorescence. And RT-PCR detected the mRNA levels of CB1, CB2, Parkin and PINK1. The neurogenesis of AM1241 on DA neurons through immunofluorescence with DA neurons, astrocytes and microglia markers.
Results: Upon treatment with AM1241, the behavior score markedly elevated and accompanied with the increase of DA and 5-HT. After administration of MPTP, the experssion of CB2R in substantianigra was significantly down-regulated, meanwhile, treatment with AM1241 reversed the down-regulation. Besides, the western blot and immuno staining results both suggested that AM1241 significantly activated PI3K/Akt/MEK phosphorylation and increase the expressions of Parkin and PINK1 both in substantianigra and hippo. The results of mRNA expressions further demonstrated that AM1241 activated CB2 receptor and Parkin/PINK1 signaling pathways. Furthermore, the increase of TH-positive cells and the co-localization of CB2R and DA neurons suggested that treatment of AM1241 induced the regeneration of DA neurons combined with the activation of CB2 receptor, which confirmed that AM1241 could be a non-dopaminergic potential candidate for PD treatment.
Conclusions: The selective CB2 agonists AM1241 has significant therapeutic effect on PD and can regenerate DA neurons which could alleviate the neurotoxicity of MPTP, and the one possible mechanism underlying the neurogenesis effect of AM1241 on DA neurons might be via modulating PI3K/AKT signaling pathways.

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Comparison of Neurotoxic Potency of a Newly Produced Botulinum Neurotoxin Type A with OnabotulinumtoxinA, IncobotulinumtoxinA and BotulinumtoxinA

Y. Feng, W. Liu, L. Pan, Z. Nie (Shanghai, People's Republic of China)

Objective: This study aims to compare the neurotoxic potency of four different kinds of toxins, accompanied with expression level changes of key factors after BoNT/A treatment.
Background: Botulinum toxins are the most toxic exotoxins widely used for clinical diseases including neuromuscular diseases. Four botulinumtoxins type A (BoNT/A) formulations, onabotulinumtoxinA (A/Ona, Botox), incobotulinumtoxinA (A/Inco, Xeomin), BotulinumtoxinA (BTA) and Botulinum Neurotoxin type A (BNT-A, 150KD) are applied in the present study among which BNT-A is newly produced. All four preparations are manufactured via different methods and demonstrate unique features, making them not interchangeable.
Methods: 189 male Sprague-Dawley rats were randomized to injection with onabotulinumtoxinA, incobotulinumtoxinA, BotulinumtoxinA, or Botulinum Neurotoxin type A in the gastrocnemius. Muscle strength were measured at 1w, 4w, and 12w after injection. Fresh muscles were harvested simultaneously at each specific time point for the detection of key factors associated with neuromuscular junction formation. In addition, fluorescent staining of histological frozen sections were used to investigate the effect of four kinds of toxins on AChR clustering after injection.
Results: Muscle strength of all four toxins reduced significantly 7 days after BoNT/A administration among which BNT-A achieved the most decrease. This reduction was gradually recovered due to wore-off of toxins and increase of body weight, and no obvious difference was observed 12 weeks after toxins injection. Accordingly, genes including nicotinic acetylcholine receptor (nAChR), Myogenic regulatory factors (MRFs) and muscle-specific receptor tyrosine kinase (MuSK) were up-regulated after BoNT/A injection. BNT-A induced the most significant up-regulation of genes expression. We also found local inflammation response following BoNT/A administration. Due to the lack of complexing proteins, both A/Inco and BNT-A stimulated a relatively less inflammation response comparing to A/Ona and BTA formulations.
Conclusions: We demonstrated that BoNT/A injection had a significant role in inducing muscle paralysis, accompanied with induction of key factors. Due to the different characterization of the four botulinum toxin A formulations, they presented specific toxic potency in inducing muscle paralysis and key factors expression.

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The Therapeutic Effects of Cortical Electrical Stimulation in an Animal Model of Parkinson's Disease

T.H. Hsieh, W.S. Chang Chien, C.W. Peng, Y.Z. Hunag, J.J. Chen (Taoyuan, Taiwan)

Objective: In this study, we first designed the long-term implantable cortical electrical stimulation (CES) module in a rat model of Parkinson's disease (PD). With the help of this model, the therapeutic effects of long-term CES intervention were examined from motor behaviors and electrophysiological findings in advanced PD rats.

Background: PD is the second most common age-related neurodegenerative disorder. Although pharmaceutical agents have been quite successful in the management of PD, the symptom relief is often incomplete, and chronic drug therapy is often limited by side-effects. Thus, new therapeutic and alternative strategies are clearly needed for PD. CES has been developed for modulating cortical excitability through plasticity-like mechanisms which are considered having therapeutic potentials for PD. However, the therapeutic values of such approach for PD are still uncertain. Accordingly, an animal model of PD may be useful to clarify the existence of treatment effect and explore an effective therapeutic strategy using CES protocols.

Methods: A hemiparkinsonian rat model, generated by unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle, was applied to evaluate the long-term treatment of CES-intermittent theta burst stimulation (CES-iTBS) in the motor behaviors and motor cortical plasticity. The detailed functional behavior investigations including gait pattern and apomorphine-induced rotational analysis were assessed up to four weeks after daily administration of CES-iTBS over the motor cortex in chronic PD rats.

Results: In comparison with sham-CES treated PD rats, long-term CES-iTBS treatment progressively improved locomotor function but failed to reduce the rotational behavior over a 4-week observation. Furthermore, the amount of motor cortical plasticity induced by CES-iTBS were impaired in sham treated PD rats, but relatively normal in CES treated PD rats.

Conclusions: We showed that the locomotor dysfunction and impaired motor plasticity can be improved after long-term CES-iTBS treatment. The developed CES animal treatment model may serve as a translational platform bridging human and animal research for developing therapeutic strategies of CES for PD or other neurological disorders.

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Targeting alpha-Synuclein Aggregation for the Treatment of Parkinson's Disease

E. Tsika, E. Gabellieri, A. Davranche, C. Boudou, M. Ravache, L. Grasso, S. Nampally, H. Haddouk, J. Wettstein, A. Pfeifer, A. Muhs (Lausanne, Switzerland)

Objective: To develop small molecular weight compounds (SMEs) as disease-modifying agents, which are designed to arrest or even reverse the aggregation process of alpha-synuclein (aSyn) protein.

Background: The aberrant accumulation of alpha-synuclein (aSyn) aggregates is a common pathological feature of Parkinson's disease (PD) and other synucleinopathies. An increasing amount of evidence from cellular and animal models indicates that the active processes of aSyn aggregation and fibril growth are actually contributing to neuronal toxicity. Agents and small molecules that can interfere with these processes and either disaggregate or inhibit the formation of ß-sheet-rich aggregates are therefore expected to have therapeutic effects.

Methods: SMEs were synthesized based on the rationale of the Morphomer™ Technology platform developed by AC Immune in which compounds are designed to target and modify ß-sheet structures of aggregation-prone proteins rendering them harmless. SMEs are evaluated *in vitro systems* for their ability to reduce or inhibit aSyn aggregation by traditional methods for monitoring ß-sheet content such as Thioflavin T-based assays as well as the effects on the morphology of recombinant fibrils visualized by electron microscopy. SMEs were further characterized by their ability to rescue neuroblastoma cells and primary neurons from aSyn-mediated toxicity. Finally, the efficacy of selected compounds was evaluated in transgenic (Tg) mice overexpressing aSyn.

Results: New SMEs have been identified with the potential to inhibit aSyn aggregation and alter the conformation of aSyn aggregates. Importantly, these SMEs can rescue neurons from aSyn-induced toxicity and reduce the burden of intracellular aSyn aggregates in brains of treated Tg mice. Biomarkers for neuroinflammation and synaptic connectivity as well as the pathways involved in clearance of misfolded proteins and protein quality control have been evaluated to shed light into the molecular pathways involved into the reversal of aSyn-induced toxicity by the SMEs.

Conclusions: The data from this battery of *in vitro* and *in vivo* assays show that the SMEs have a dual function, arresting the fibrillation process of aSyn into non-toxic species and reducing ß-sheet content of preformed aSyn

aggregates. Such small molecule compounds hold promise in providing therapeutic benefit for PD and related synucleinopathies.

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Fibroblasts Implanted in Internal Pallidum of Parkinsonian Macaques Exert L-Dopa-Sparing Activity

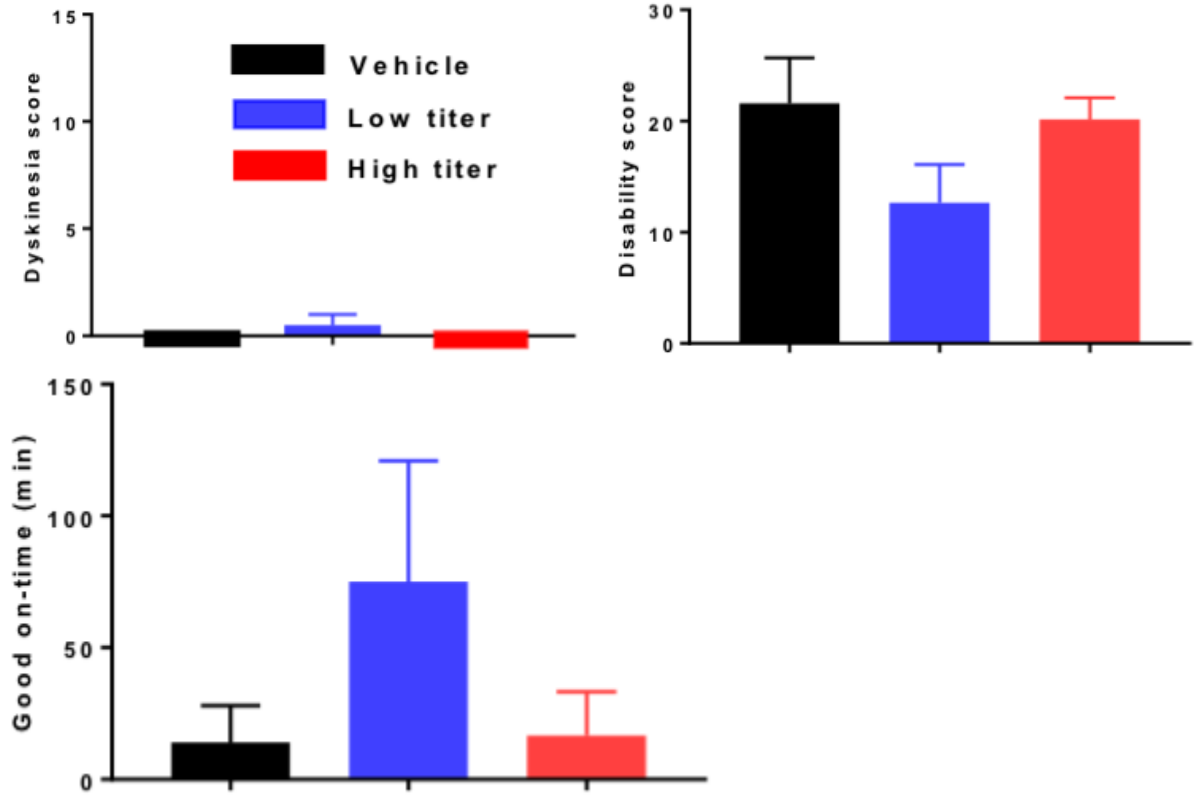
E. Bezard, J. Finberg, W.K. Ko, Q. Li, B. Dehay, E. Pioli, Y. Yair (Bordeaux, France)

Objective: To assess the effects of autologous implantation of fibroblasts in the internal globus pallidus of dyskinetic MPTP-lesioned monkeys upon motor behaviour and L-DOPA response.

Background: Autologous Implantation of fibroblasts (AIF) in the entopeduncular nucleus of 6-hydroxydopamine-treated rats inhibits L-dopa-induced dyskinesia (LID), and unilateral AIF to internal globus pallidus (GPi) of MPTP-induced parkinsonian macaques reduces LID on the contralateral side. We now present the effect of bilateral AIF to GPi on LID and parkinsonian behavior of MPTP-induced parkinsonian macaques.

Methods: Seventeen macaques were divided into 3 groups; control (n=5), therapeutic dose of fibroblasts as determined in previous experiment (n=6) and double this dose of fibroblasts (n=6). Parkinsonism was induced by daily MPTP administration until the animals attained a score of 7 on an accepted parkinsonism scale for MPTP-treated non-human primates. Daily treatment with Madopar (50% of the optimal dose for suppression of parkinsonism) was commenced until dyskinesia developed and became stable. Autologous fibroblasts or medium (control) were administered bilaterally to GPi.

Results: In months 1-7, both doses of fibroblasts increased the locomotor response to Madopar but enhanced dyskinesia. In months 8-9, a 25% dose of Madopar reduced parkinsonian symptoms without causing dyskinesia, and increased good on-time (GOT). In month 11, in addition to Madopar, pramipexole was administered at 100% and 33% optimal dose, which also improved parkinsonian symptomatology without causing dyskinesia and with increased GOT (Fig. 1).



Conclusions: Fibroblast implantation potentiates good effects of L-dopa and enables use of a lower, non-dyskinetic, dose.

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Intracerebroventricular Administration of Dopamine: a New Therapeutic Concept for Parkinson's Disease

C. Laloux, F. Gouel, C. Lachaud, K. Timmerman, A. Jonneaux, C. Moreau, R. Bordet, J.C. Devedjian, D. Devos (Lille, France)

Objective: In this study, we aim to demonstrate that continuous i.c.v. infusion of dopamine close to the striatum is a feasible and highly efficient treatment as compared to classical peripheral L-dopa therapy.

Background: In Parkinson's disease (PD) depletion of dopamine in the nigro-striatal pathway, as the main pathological hallmark, requires continual circadian and focal restoration. Current predominant treatment with intermittent oral administration of its precursor, Levodopa (L-dopa), fails to restore this dopaminergic striatal neurotransmission due to pharmacological drawbacks that trigger dyskinesia. Equally, continuous intracerebroventricular (i.c.v.) administration of dopamine previously failed through unresolved accelerated dopamine oxidation and tachyphylaxia. We aim to overcome prior challenges.

Methods: We prepare dopamine in anaerobia (A-dopamine) as compared with aerobic-prepared dopamine (O-dopamine) with and without the conservator (sodium metabisulfite, SMBS). We assessed the dose response curves of continuous i.c.v infusion of A- and O- dopamine in the acute MPTP mice model (24 hours a day) and in the 6-OHDA-lesioned rat (circadian administration).

Results: Continuous i.c.v infusion of A-dopamine during 7 days restored MPTP mice motor function with a larger therapeutic index compared to intra-peritoneal L-dopa treatment. A-dopamine induced a dose dependent neuroprotection of the nigro-striatal dopaminergic neurons, which was not observed with O-dopamine and L-dopa. A-dopamine in the 6-OHDA-lesioned rat improved motor activity during 30 days without occurrence of tachyphylaxia. Importantly, A-dopamine did not induce dyskinesia or behavioral sensitization, conversely to peripheral L-dopa treatment. Nigro-striatal pathways and peripheral organs analysis demonstrated histological safety of A-dopamine.

Exp time	6-OHDA rat Groups	Dyskinesia types			Dyskinesias score	Locomotive AIM
		Limb	Axial	Orolingual		
D7	Vehicle	0	0	0	0	0
	L-dopa	11.1 ± 1.99 *	8.4 ± 1.67 *	9.9 ± 1.66 *	29.40 ± 5.25 *	4.8 ± 1.39 *
	A-DA 1 mg/d	0	0	0	0	1 ± 1 #
	A-DA 2 mg/d	0	0	0	0	2.14 ± 0.91
	A-DA 3 mg/d	0	0	0	0	5.4 ± 1.12 *
D15	Vehicle	0	0	0	0	0
	L-dopa	12.2 ± 1.67 *	7.9 ± 1.38 *	10.9 ± 1.75 *	31.0 ± 4.63 *	6.1 ± 1.0 *
	A-DA 1 mg/d	0	0	0	0	0.7 ± 0.7 #
	A-DA 2 mg/d	0	0	0	0	1.56 ± 0.67 #
	A-DA 3 mg/d	0	0	0	0	2.71 ± 1.02 #
D30	Vehicle	0	0	0	0	0
	L-dopa	13.6 ± 2.03 *	8.6 ± 1.85 *	13.5 ± 2.15 *	35.7 ± 5.89 *	7.2 ± 1.33 *
	A-DA 1 mg/d	0	0	0	0	1 ± 1 #
	A-DA 2 mg/d	0	0	0	0	2.25 ± 1.31 #
	A-DA 3 mg/d	0	0	0	0	0.6 ± 0.4 #

Table : Lack of dyskinesia during cerebral infusion of A-dopamine in 6-OHDA rats.
The table differentiates dyskinesias score (Limb, axial and orolingual) and locomotive abnormal involuntary movement (AIM) observations each 20 minutes over 2 hours (beginning 30 min after daily i.p. L-dopa treatment or during A-dopamine i.c.v) at 7, 15 and 30 days of treatments. Data are expressed in means ± SEM (n=10 animal per group). Significant differences * *vs.* untreated 6-OHDA rats, # *vs.* L-dopa treated 6-OHDA rats, $p<0.05$ (non-parametric Kruskal-Wallis variance analysis and Mann-Whitney comparison).

Figure :

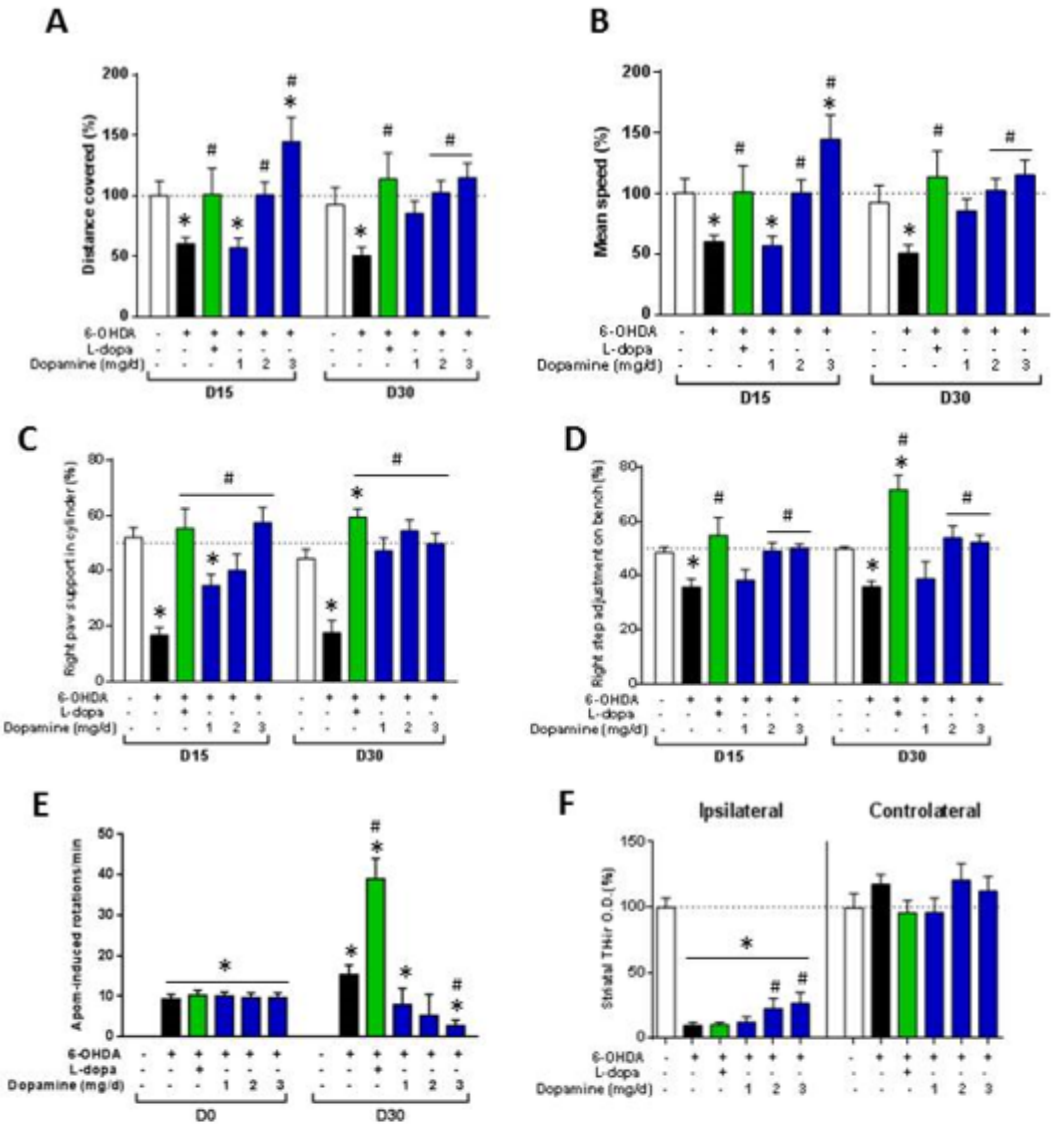

Figure : Functional recovery without tachyphylaxia or dopaminergic sensitization following chronic continuous and circadian A-dopamine stimulation in 6-OHDA rats. In 6-OHDA rats after 15 or 30 days of i.p. L-dopa (12 mg/kg/day) or i.c.v. anaerobically prepared dopamine (A-dopamine; 1-3 mg/day), distance covered (**A**) and mean speed (**B**) in the actimetry arena as well as right paw support in the cylinder test (**C**) and right step adjustment in the stepping test (**D**) were evaluated. All data was compared to vehicle control set at 100% within the actimetry arena (**A&B**) or 50% representing equal forelimb preference (**C&D**). Number of Apomorphine induced rotations were measured immediately after 6-OHDA insult but before treatment (D0) as well as 30 days after treatments (D30) using the same treatment parameters (**E**). TH-ir+ optical density in dorsal striatum (ipsilateral and contralateral) (**F**) was also measured. All data are expressed in percentage from Vehicle rats, means ± SEM (n=10/group). Significant differences * *vs.* vehicle rats, # *vs.* untreated 6-OHDA rats, $p<0.05$ (one-way ANOVA and LSD Fisher post-hoc tests or Mann-Whitney comparison test).

Conclusions: Continuous i.c.v infusion of A-dopamine is highly efficient on the motor handicap without induction of dyskinesia and tachyphylaxia and is safe with a large therapeutic index. This could represent a new therapeutic strategy aiming at restoring the dopamine neurotransmission.

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AAV-mediated Over-expression of VEGF-B in PINK1 Gene Knockout Rats Increases Striatal Dopamine Content

T. Falk, M. Bartlett, D. Muller, B. Silashki, D. Farrell, K. Parent, M. Heien, S. Sherman (Tucson, AZ, USA)

Objective: To evaluate overexpression of the neuroprotective vascular endothelial growth factor B (VEGF-B) in a genetic Parkinson's disease (PD) model.

Background: We have shown in prior work that VEGF-B is neuroprotective in toxin-induced (rotenone; 6-OHDA) rat models *in vitro* and *in vivo* (Yue et al., 2014). Here, we further assess the neuroprotective effects of VEGF-B using a PTEN-induced putative kinase 1 (PINK1) knockout (KO) rat model, a novel genetic PD model. Mutations in the *PINK1* gene have been shown to be a cause of human familial PD cases. PINK1 KO rats gradually develop motor impairment, evident at 7-8 month of age, and 50% dopaminergic cell loss in the substantia nigra (SN) at 8 months, reflecting a slow and progressive degeneration.

Methods: In a preliminary study (n=5-6), at 5 months of age PINK1 KO rats were injected unilaterally with an adeno-associated virus expressing human VEGF-B (AAV2/1-CAG-hVEGF-B) into two sites in the striatum (AP +1.0, ML +3.0, DV -5.0 and AP -0.6, ML +3.5, DV -5.0) and one in the SN (AP -5.0, ML -2.0, DV -7.2). Behavioral analyses were compared to PINK1 KO rats and Wild-Type Long-Evans controls. Changes in motor function were tracked monthly using an array of behavioral tests, including forelimb adjusting steps to evaluate forepaw function, as well as the tapered balance beam to evaluate hindlimb function. At 13 months the animals were sacrificed, striatal protein was isolated and striatal dopamine (DA) levels were measured with HPLC-EC.

Results: Our data show behavioral deficits in PINK1 KO rats reach maximum level at 8-months. Further aging does not increase deficits, indicating its utility as an early PD model. An increase in bilateral hindlimb slips is evident in PINK1 KO rats at 7-10 months. PINK1 KO rats show strong trends of improved motor behavior after unilateral VEGF-B over-expression in SN and striatum, not reaching significance in our small pilot study. We quantified striatal DA content and are currently testing for the integrity of the dopaminergic system, by analyzing tyrosine hydroxylase content in the SN and striatum. Striatal DA levels were reduced by 20-30% in PINK1 KO rats compared to WT, but in the AAV2/1-CAG-hVEGF-B injected hemisphere DA was restored close to WT level (n=3-5).

Conclusions: Our pilot data indicate that VEGF-B overexpression might have a neuroprotective effect in PINK1 KO rats.

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Induced Pluripotent Stem Cells Based in-vitro Modelling of Spinocerebellar Ataxia Type -12 (SCA-12)

D. Kumar, M. Faruq, A. Srivastava, M. Mukerji, O. Mukherjee (Delhi, India)

Objective: To derive neuronal lineages from patient's peripheral blood mononuclear cells (PBMCs) and exploration of disease biology.

Background: Spinocerebellar ataxia type-12 (SCA-12) is a progressive cerebellar and purely neurodegenerative genetic disorder caused by (CAG) expansion in 5'UTR of PPP2R2B gene. SCA12 is the second common genetic ataxia type and has unique prevalence in North Indian population. Transgenic or any other appropriate neuronal-cell/animal models are lacking to understand the underlying cellular perturbations in SCA12. Human-induced-pluripotent-stem cell based (HiPSC) based disease modeling overcomes the limitation of non-availability of specific cell type for studying such disorders.

Methods: Lymphoblastoid-cell-lines (LCLs) derived from patient's PBMCs were used to generate non-integrated iPSCs using episomal plasmids (Yamanaka-factors). These iPSCs were then de-differentiated into NSCs/Neuronal lineage. Appropriate cellular characterization was conducted for respective cell lines. A comprehensive candidate gene transcriptomic-profiling was conducted in cell lines and compared with appropriate controls

Results: PPP2R2B transcripts levels were found down-regulated in blood samples of patients compared to controls (N=10), however, in HiPSCs lines, we observed a significant variation of all the isoforms except brain-specific isoform-4. In neural progenitor cells (NSCs) and differentiated neurons, we observed the expression of brain-specific isoform-4. This suggest that these patient iPSC derived neuronal model are good for mechanistic analysis. We observed that CAG-containing non-coding PPP2R2B transcripts (isoform-12) significantly expressed (fc>30) lines compared to control in both NSCs and neurons.

Conclusions: We have generated patient derived HiPSCs and their differentiated derivatives to study SCA12 pathogenesis. Our preliminary data shows that HiPSC based model system can serve as model to study such adult onset complex disorders. We were able to identify expression pattern of candidate gene PPP2R2B, mimicking brain-specific transcripts. Our preliminary data shows some trend towards disease relevant transcriptional signatures.

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Ataxia-Teleangiectasia-Mutated-Knock-Out Mouse Cerebellum Shows Significant Reduction of Calcium Homeostasis Factors

J. Canet-Pons, R. Schubert, U. Warnken, M. Schnoelzer, S. Zielen, G. Auburger (Frankfurt am Main, Germany)

Objective: We aim to identify sensitive and specific molecular markers of the cerebellar Purkinje neuron loss in Ataxia Teleangiectasia.

Background: Ataxia teleangiectasia is an autosomal recessively inherited disorder with loss of cerebellar Purkinje neurons and a deficient immune defense. The causative loss-of-function mutations in the Ataxia-Teleangiectasia-Mutated (*ATM*) gene hinder the repair of DNA-damage events. The corresponding mouse model with *Atm*-knock-out dies around the age of 4-6 months due to thymic lymphomas, without showing histological or behavioral signs of cerebellar damage.

Methods: We assessed a number of molecular markers of cerebellar Purkinje neuron loss, which were previously defined in the autosomal dominantly inherited Spinocerebellar Ataxia type 2 (SCA2) cerebellar tissue from patient autopsies and mouse models. As a sensitive detection method with near-linear correlation, quantitative reverse-transcriptase polymerase chain reaction was employed to measure the expression of candidate factor expression. Tata-binding-protein (*Tbp*) mRNA was used as loading control.

Results: Two known markers of cerebellar Purkinje neurons, Calbindin-1 (*Calb1*) and Inositol-Tris-Phosphate-Receptor-1 (*Itpr1*) showed significant reductions to 80% expression of the respective transcripts already at age 3 months (n = 8 KO vs 8 WT). Additional analyses of Reelin pathway components and DNA-damage markers are ongoing. Different ages and a comparison of homozygotes with heterozygotes are under study, to define the usefulness of these molecules as progression markers.

Conclusions: Our identification of two calcium homeostasis factors as pre-symptomatic molecular markers of cerebellar Purkinje neuron loss will be useful to assess the neuroprotective potential of novel therapeutic approaches, such as bone marrow transplantation and anti-oxidative drugs.

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Investigating the Lipidomic Signature of Spinocerebellar Ataxia (SCA1) Using A Liquid-Microjunction Surface Sampling Probe

V. Vedam-Mai, E. Gill, M. Marks, R. Yost, T. Garrett (Gainesville, FL, USA)

Objective: Lipidomic analysis of mice with spinocerebellar ataxia by mass spectrometry.

Background: Ataxia is a movement disorder affecting balance and coordination of limbs, gait, eyes and speech. Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominantly inherited neurodegenerative disorder for which there is no available disease modifying treatment or cure. Lipid profiling (lipidomics) in neurodegenerative disorders such as Parkinson's disease and Alzheimer's recently suggest that there are lipid anomalies associated with a wide spectrum of neurodegenerative diseases. Liquid-microjunction surface sampling probe (LMJ-SSP) is an emerging ambient ionization mass spectrometry technique based on the continuous flow of solvent using an *in situ* micro-extraction device. It provides a unique opportunity to sample thick tissue sections with no prior sample preparation on an ambient platform, whist allowing direct analysis of the metabolic profile related to the disease state.

Methods: Transgenic SCA1 mice (B05) were sacrificed and whole brains were harvested immediately from the skull fresh, without perfusion. The fresh whole mouse brain was sectioned using a high-grade aluminum alloy 1 mm coronal rodent matrix (ASI Instruments, Warren, MI, USA) immediately post sacrifice. Sections were placed directly onto glass slides and frozen at -80 ºC prior to sampling. The cerebellum of wild type and diseased mice were sampled for 1 minute on a Prosolia FlowprobeTM (Indianapolis, IN, USA). A Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer was used for all experiments. All data were analyzed using MetaboAnalyst.

Results: LMJ-SSP showed good promise for sampling mouse brains. Using MetaboAnalyst, the data was subject to Principal Component Analysis (PCA), an unsupervised multivariate statistical analysis technique. There were distinct differences observed between the wild type and transgenic mice in glycerophospholipids (e.g. phosphatidylcholine) and phospholipids (e.g. sphingomylein) between the two groups.

Conclusions: To the best of our knowledge, this is the first lipidomic study of the ataxic brain, utilizing FlowProbeTM technology. We detected several significant differences in lipid features in the ataxic brain, suggesting that lipid metabolism is altered in several progressive ataxic conditions.

791

Targeting the Intracellular Localization of Ataxin-3 as Novel Treatment Strategy for Spinocerebellar Ataxia Type 3

T. Schmidt, Z. Wang, A. Sowa, J. Schmidt, M. Anders, M.I. Martins, A. de Castro, D. Weishäupl, J. Weber, H. Tricoire, P. Maciel, O. Riess (Tübingen, Germany)

Objective: Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited neurodegenerative disorder caused by a CAG expansion in the *ATXN3* gene leading to a polyglutamine expansion in the encoded ataxin-3 protein. There is still no treatment available for this disease. We therefore aim to develop a novel treatment strategy for SCA3/MJD.

Background: Characteristic for SCA3 are the so called neuronal intranuclear inclusion bodies (NII). As ataxin-3 is predominantly located in the cytoplasm, the formation of protein aggregates in the nucleus require a nucleocytoplasmic shuttling of ataxin-3. We've already demonstrated *in vivo* that the toxicity of expanded ataxin-3 is linked to its intracellular localization: Only nuclear ataxin-3 gave rise to a phenotype while purely cytoplasmic ataxin-3, however, even with a highly expanded polyglutamine repeat, remained harmless. We then dissected the nucleocytoplasmic transport mechanisms of ataxin-3 and identified a transport protein with critical importance for the nuclear import of ataxin-3.

Methods: As pathologically, ataxin-3 remains harmless as long as it is kept in the cytoplasm. We further anticipated the intracellular localization of ataxin-3 as a target for a possible therapeutical intervention. For this reason, we generated an essay allowing us to easily monitor the intracellular localization of normal or expanded ataxin-3 and used our essay to screen a library of FDA-approved compounds.

Results: We identified compounds impacting the nuclear translocation of ataxin-3 and validated them *in vitro* and *in vivo*. As the compounds we identified are already FDA-approved and on the market, they could be transferred to the clinics comparatively fast. We believe that our results will improve the understanding of pathological mechanisms influencing the progression of the disease and are an important contribution towards a treatment of SCA3.

Conclusions: Our results demonstrate that nuclear transport processes are critical for the pathophysiology of SCA3/MJD and that it is possible to impact and thereby alleviate symptoms induced by expanded ataxin-3 using specific compounds. We believe that our results will improve the understanding of mechanisms influencing the pathogenesis of SCA3/MJD and are an important contribution towards a treatment of this disease.

799

Effectiveness of Deep Brain Stimulation (DBS) in Mice with Spinocerebellar Ataxia (SCA1)

V. Vedam-Mai, K. McFarland, R. Nathu, S. Kurtovic, Q. Zhang, T. Ashizawa, M. Okun (Gainesville, FL, USA)

Objective: To evaluate the effectiveness of Deep Brain Stimulation (DBS) in a mouse model of Spinocerebellar Ataxia type 1 (SCA1).

Background: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominantly inherited neurodegenerative disorder resulting in Purkinje cell loss and atrophy of the brainstem and cerebellum. There is no cure for SCA1 and typically, symptoms worsen over time and death results from complications related to brainstem dysfunction. An absence of the inhibitory signals to the deep cerebellar nuclei (DCN) is postulated to result in hyperexcitability and disinhibition of DCN neurons thereby affecting the cerebellar-thalamic-cortical pathways. Regulating firing frequency of DCN neurons could potentially ameliorate symptoms of the disease. We hypothesize that DBS can be used to alleviate ataxia in SCA1 mice.

Methods: Symptomatic B05/SCA1 mice were stereotactically implanted in the DCN with stainless steel, single channel, DBS electrodes (Plastics One). Mice in the experimental "STIM" group underwent acute High Frequency Stimulation (HFS) for one week post recovery and mice in the "SHAM" group did not undergo any HFS. Mice were tested for improvements in gait by comparing gait signal before DBS implantation, after DBS implantation and again after HFS. At the end of the experimental paradigm, mice were sacrificed, brains dissected and flash frozen for transcriptomic analyses to assess gene expression changes occurring from HFS.

Results: We were able to implant DBS electrodes in DCN of B05 mice and perform acute HFS. Gait assessment of movement control and coordination demonstrated improvements in the experimental group of mice following HFS DBS. Hind paw area and power generation of the hind paw during propulsion (MAX dA/dT) was returned to values similar to wild-type siblings. This implies that our treatment at least corrects B05 mice for their lack of control and deceleration. Transcriptomic analysis is ongoing.

Conclusions: In this study, we were able to target the deep cerebellar nucleus in ataxic mice with DBS electrodes and perform acute HFS. We demonstrate improvement in the gait of stimulated animals. Further work including

chronic stimulation paradigms will be necessary to confirm our initial observations in order to translate this therapy to humans.

845

Adding cues to a rat gambling task potentiates the increase in premature responding in response to chronic D2/3 agonist ropinirole without mitigating preference for risk

M. Tremblay, M. Barrus, P. Cocker, S. Kaur, C. Winstanley (Vancouver, BC, Canada)

Objective: Investigating the effect of chronic administration of the dopamine $D_{2/3}$ agonist ropinirole on a cued model of gambling behaviour in rats.

Background: Dopamine $D_{2/3}$ agonists are successfully used to treat the motor symptoms of Parkinson's Disease (PD), either as adjunct to L-DOPA, or as stand alone treatments. However, these dopamine agonists may lead to impulse control disorders (ICD) including pathological gambling in some patients. We have previously observed that chronic ropinirole increased choice of uncertain options on the rat Betting Task (rBT), a paradigm that captures aspects of risk aversion. In contrast, ropinirole transiently increased premature responses, a measure of motor impulsivity observed in models of addiction, without influencing choice on the rat Gambling Task (rGT). The rGT is a rodent analogue of the Iowa Gambling Task used clinically to assess decision making under risk in which rats choose between four options, each associated with differing probabilities of reward and punishment. Repeated exposure to cues that predict reward with maximal uncertainty may sensitize the dopamine system and predispose subjects to addiction disorders such as gambling. Although win-associated stimuli are salient in casinos, they are not featured on the original version of the rGT, which may explain the lack of effect of ropinirole on choice in the uncued task. We therefore tested if chronic ropinirole would increase choice of risky options on a cued version of the rGT. Win-associated cues were previously shown to increase rats' preference for the riskier options on this task.

Methods: Subjects were 40 male rats performing the cued rGT and implanted with an osmotic pump delivering either ropinirole (5mg/kg/day) or saline for 28 days.

Results: Ropinirole led to a larger and more long-lasting increase in premature responses on the cued rGT with no effect on choice, consistent with results on the original rGT.

Conclusions: Together with other data, our results suggest that the rGT and the rBT may model decision-making deficits linked to different disorders. Chronic ropinirole increases preference for uncertainty on the rBT, potentially akin to problem gambling behaviour, whereas this drug increases impulsivity on the rGT, which may represent an endophenotype for impulse control disorders or drug addiction.

897

Identification of a trehalose treatment regimen with potential to be translated into a therapeutic for the treatment of Parkinson's disease

J. Koprich, P. Howson, T. Johnston, M. Hill, P. Ravenscroft, J. Brotchie (Toronto, ON, Canada)

Objective: To evaluate the preclinical efficacy of trehalose and to identify a dosing regimen that can be used to clinically evaluate trehalose as a disease-modifying therapy for Parkinson's disease (PD).

Background: Trehalose, a disaccharide approved for human use and an autophagy-enhancer, showed potential for disease-modification in several models of PD. In these studies, trehalose was administered in drinking water and therefore the profile and total amount of trehalose consumed are unknown, impeding translation of the preclinical results into an appropriate clinical study. Using an alpha-synuclein (aSYN) rat model of PD, we evaluated three regimens of trehalose administration.

Methods: Thirty female, Sprague-Dawley rats (280-325 g) split into 5 groups received unilateral injections of AAV1/2 delivering A53T aSYN or empty vector (EV) into the substantia nigra (SN). Commencing on day of surgery and continuing for 6 weeks, rats received vehicle (sterile drinking water) or trehalose (2.67 g/kg/day) administered in the drinking water (2% w/v), as three separate administrations 8 h apart (0.89 g/kg/t.i.d., p.o.) or as a single administration (2.67 g/kg/day, p.o). Behaviour was assessed pre-surgery, 3 and 6 weeks post-surgery using the cylinder test of forelimb asymmetry. After 6 weeks the rats were killed and striatal tissue analysed for dopamine (HPLC) and aSYN (Western blotting) levels. The number of TH^{+ve} cells in the SN was assessed stereologically.

Results: Rats receiving A53T aSYN and vehicle exhibited increased forelimb asymmetry at Weeks 3 and 6 compared to rats receiving EV. Furthermore, striatal dopamine levels were reduced and A53T aSYN load per TH^{+ve} neuron were increased cf. rats receiving EV. Administration of trehalose as a single oral administration (2.67 g/kg/day, p.o) reduced asymmetry to a level similar to rats receiving EV, increased striatal dopamine levels (by 54%) and reduced the A53T aSYN load per TH^{+ve} neuron (by 41%) cf. rats receiving A53T aSYN alone.

Administration of the same amount of trehalose as either 3 doses, 8 h apart or *ad libitum* in the drinking water did not alter behaviour, striatal dopamine levels or the amount of A53T aSYN per TH^{+ve} neuron.
Conclusions: In AAV aSYN rats, a single daily oral administration of trehalose was efficacious and thus is the most appropriate regimen to use in the clinical evaluation of trehalose in PD.

900

Using cognitive computing to identify compounds with antidyskinetic potential for Parkinson's disease
N. Visanji, A. Lacoste, S. Ezell, S. Spangler, E. Argentinis, J. Brotchie, A. Lang, T. Johnston (Toronto, ON, Canada)
Objective: IBM Watson for Drug Discovery (WDD) is a platform that analyzes vast quantities of scientific data to help researchers identify hidden connections and formulate evidence-based hypotheses. We applied WDD to discover compounds with potential for repurposing to L-DOPA-induced dyskinesia (LID).
Background: Current treatment options for LID are limited. Repurposing compounds with regulatory approval is an attractive approach to accelerate availability of novel therapeutic options. WDD uses cognitive capabilities to extract domain specific text features from published literature and identify new connections between entities of interest, including drugs. We used WDD to analyze published abstracts of a set of training compounds known to prevent LID, and applied machine learning to rank a candidate set of drugs according to similarity of linguistic context.
Methods: 16 training compounds were identified, via literature search, with demonstrated ability to reduce LID in animal models or human clinical trials (Table 1). Candidate drugs were filtered from 905 FDA-approved drugs with binding data available (https://www.bindingdb.org/bind/ByFDAdrugs.jsp) cross referenced with the human brain proteome (http://www.proteinatlas.org/humanproteome/brain). Dopamine agonists, antipsychotics and drugs with <5 published abstracts were also removed, leaving 385 final candidates. WDD analyzed ~450,000 Medline abstracts to generate a semantic fingerprint for each compound and then, using machine learning, create a predictive model to rank candidate compounds based on semantic similarity to the training set.

Table 1. Training compounds with demonstrated ability to reduce LID in animal models or human clinical trials.

Class of compound	Compound name
NMDA receptor antagonists	Amantadine
Tyrosine kinase inhibitor	Nilotinib
mGlu5 NAMs	MTEP
	Dipraglurant
	Mavoglurant
AMPA antagonists	Topiramate
alpha2 adrenergic antagonists	Fipamezole
5-HT1A agonists	Eltoprazine
	Sarizotan
alpha 7 containing nicotinic agonists	TC-8831
	ABT-126
	ABT-894
	ABT-107
	AQW051
	AZD1446
mu-opioid receptor antagonist	ADL5510

Results: Leave-one-out cross-validation demonstrated a strong predictive power of training entities over each other compared to candidates, confirming that highly ranked candidates share many properties with the training set. The top 50 ranked candidates comprised 20 compounds with preliminary evidence of antidyskinetic action with several not fully explored, 2 untested compounds with plausible antidyskinetic mechanisms of action and 6 compounds with as yet unexplored mechanisms of predicted antidyskinetic action.
Conclusions: We have employed a powerful text mining approach using WDD to identify drugs predicted to have the potential to prevent LID. Critical validation of the most promising novel compounds will be performed in rodent and non-human primate models of LID.

902

Mechanisms of sub-anesthetic ketamine infusions to reduce L-DOPA-induced dyskinesia: effects on striatal mTOR signaling and beta band oscillations in striatum and motor cortex

M. Bartlett, A. Flores, T. Ye, H. Dollish, K. Doyle, S. Cowen, S. Sherman, T. Falk (Tucson, AZ, USA)

Objective: To evaluate mechanism of long-term activity of sub-anesthetic ketamine infusion to reduce L-DOPA-induced dyskinesia (LID).

Background: We have published preclinical evidence and patient case reports showing a long-term reduction of LID after sub-anesthetic ketamine infusion (Bartlett et al. 2016; Sherman et al., 2016). Although the mechanisms are unknown, data from recent literature suggest that high-frequency oscillations (HFO; >100 Hz) and beta-band oscillations (15-30 Hz) in the striatum and cortex could be involved in ketamine's effects, as well as changes in dendritic spines.

Methods: Preclinical LID model: escalating L-DOPA doses (2 weeks: 6 mg/kg + 2 weeks: 12 mg/kg) to prime unilaterally 6-OHDA-lesioned rats. To mimic a 10-hr patient infusion ketamine (20 mg/kg) was injected 5 x *i.p.* two hrs apart, L-DOPA was co-injected at the 5th injection. In a separate pilot study we conducted *in vivo* electrophysiology (1-hr baseline period followed by the 10-hr ketamine protocol) in awake freely behaving 6-OHDA-lesioned rats implanted with electrode arrays targeting dorsolateral striatum (DLS) and motor cortex (M1).

Results: Ketamine infusion once a week reduced the development of LID and increased phosphorylation of striatal mTOR (n=9 per group, *p<0.05, ANOVA). BDNF levels and dendritic spine density in the striatum are currently investigated. In a separate pilot study TrkB receptors were blocked with ANA-12 during ketamine-exposure. Co-injection of ANA-12 did not reduce the acute, but the sustained anti-dyskinetic effect seen in ketamine-only injected LID rats after 4 days, indicating an involvement of BDNF in the sustained anti-dyskinetic effects of ketamine (n=9-10). In the PD rats ketamine induced sustained gamma (30-60 Hz) and HFO (130-160 Hz) in the DLS and M1, and reduced beta power (n=5, one way ANOVA). Ketamine triggered strong HFO coherence and a progressive reduction in coherence at bands <30 Hz in M1/DLS, illustrated by an inverse relationship between HFO and beta coherence.

Conclusions: Our pilot data indicate that the anti-dyskinetic activity of sub-anesthetic ketamine infusion is accompanied by activation of striatal mTOR signaling, and reduction of beta band activity and coherence in DLS and M1, supporting the hypothesis of ketamine acting as a "chemical DBS".

904

Basal Ganglia and Limbic Striatal Regions are Differentially Affected by Pramipexole: D3 receptor – Mediated Changes in Markers of Synaptic Strength

M. Bailey, A. Persons, T.C. Napier (Chicago, IL, USA)

Objective: To determine whether pramipexole (PPX) differentially upregulates AMPA receptor trafficking in limbic vs. motor striatal regions of rats.

Background: Impulse control disorders (ICD) are a side effect of PPX, a dopamine agonist, used in Parkinson's disease and restless leg syndrome. Mechanisms that underlie these disorders are poorly understood. PPX has a high affinity for D2 and D3 receptors (D2/D3R). These receptors are expressed throughout the forebrain, but D3R are higher in limbic regions implicated in ICD, whereas D2R are higher in basal ganglia regions involved in motor control. Increases in limbic striatal AMPA receptor (AMPAR)-mediated synaptic strength is associated with addictions, which often involve impulse control. D2R/D3R signaling can involve Akt/GSK3ß, and GSK3ß promotes insertion of AMPAR into cytosolic membranes to strengthen glutamatergic synapses. We hypothesized PPX-activation of D3R will alter Akt/GSK3ß and AMPAR trafficking in limbic brain regions involved in addiction (nucleus accumbens), but not in motor-related basal ganglia regions (dorsal striatum).

Methods: Rats were administered saline or PG01037 (D3R antagonist); 30min later, saline or PPX was administered. One hour later, they were killed and striatal regions were extracted. Western blot protocols determined tissue levels of Akt/GSK3ß, and surface and intracellular levels of AMPAR subunits (GluA1, GluA2). Data were analyzed using a one-way ANOVA followed by a *post hoc* Newman-Keuls test.

Results: PPX significantly reduced the ratio of pAkt (active)/Akt (total) and pGSK3ß (inactive)/GSK3ß (total) and increased the surface/intracellular (S/I) ratio for GluA1 and GluA2 in the nucleus accumbens. These effects were reversed by PG01037. In the dorsal striatum, no change occurred in pAkt/Akt, pGSK3ß/GSK3ß or GluA2 S/I. Unexpectedly, PG01037 decreased GluA1 S/I, (likely reflecting blockade of endogenous dopamine activation of D3R).

Conclusions: PPX upregulates AMPA receptor trafficking to the cytosolic membrane in nucleus accumbens (i.e., limbic striatum) via D3R-mediated Akt/GSK3ß signaling, but this does not occur in basal ganglia striatum. This may be a mechanism that underlies dopamine agonist-induced ICD.

906

All-trans-retinoic Acid pretreatment exhibits neuroprotective effect against 6-hydroxydopamine-induced hemi-parkinsonian rats

A. Morad Ganjeh (Karaj, Iran)

Objective: This study examined the effect of pretreatment with All-trans-retinoic Acid (ATRA) on the 6-hydroxydopamine-induced Parkinsonism in Wistar rats in order to find out whether ATRA could attenuate oxidative stress, behavioural and structural abnormalities in an experimental model of PD in rat.

Background: Parkinson's disease refers to a progressive neurodegenerative disorder involving degeneration of dopaminergic neurons particularly in substantia nigra. The loss of dopaminergic cells results in complex motor syndrome. One of the hypothesis in pathogenesis of PD is oxidative stress via NADPH-dependent oxidases. ATRA have been reported to increase oxidative burden.

Methods: 48 male wistar rats were divided into four groups: (1) sham, normal saline was injected in the left SNC (substantia nigra pars compacta) , (2) 6-OHDA, 6-hydroxydopamine was injected into left SNC, (3) 6-OHDA+ATRA (10 mg/kg, p.o), (4) 6-OHDA+ATRA (20 mg/kg, p.o). ATRA were given rats daily from eight dayes before the surgery to a day after. At the end of the experiment, oxidative stress markers, apomorphine-induced rotational asymmetry were measured and the number of Nissl-stained and tyrosine-hydroxylase (TH)-positive neurons on the left side of the substantia nigra after 2 weeks were counted.

Results: ATRA administration could attenuate the rotational behavior and also restore malondialdehyde and nitrite content and catalase activity in lesioned rats pretreated with ATRA and protect the neurons of SNC against 6-OHDA toxicity in comparison with the group which received only neurotoxin.

Conclusions: In conclusion, the results of the present study suggest that ATRA administration has a protective effect against 6-OHDA toxicity and may be useful for as an adjuvant therapy for management of PD at its early stages.

907

Piperine potentiate the neuroprotective effect of quercetin against MPTP induced neurotoxicity in rats

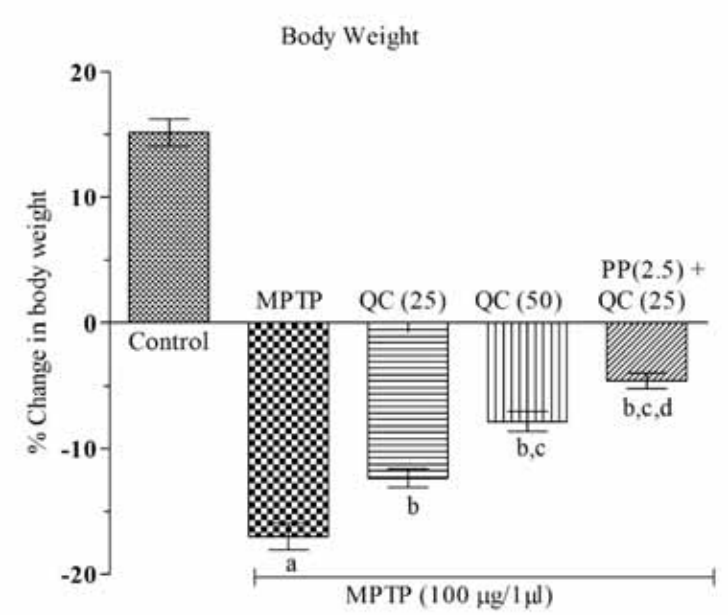
S. Singh, P. Kumar (Moga, India)

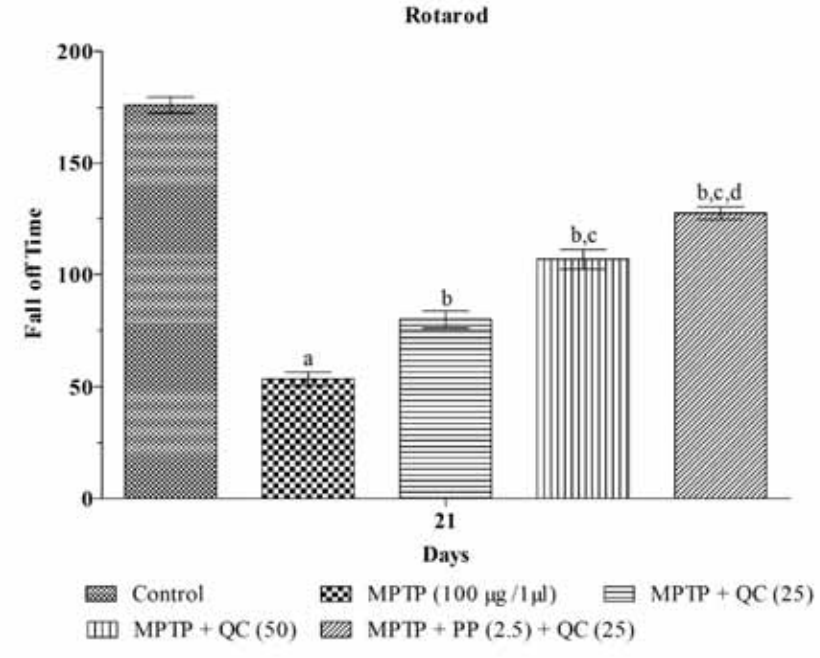
Objective: (1) Quercetin is well tolerated bioflavonoid used as supplement for various disorders but problem is its low oral bioavailability and (2) Piperine is combined to enhance bioavailability of quercetin used as neuromodulatory, neuroprotective in movement disorders like Parkinson's disease.

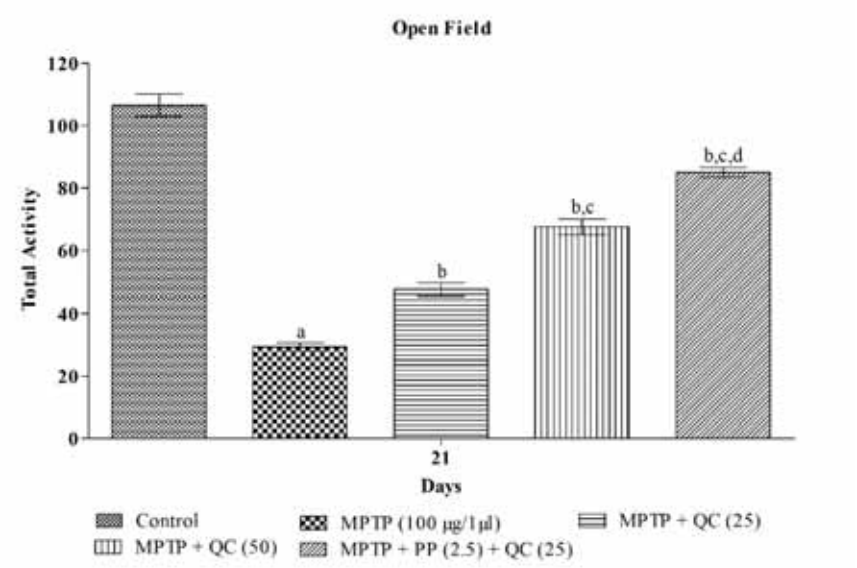
Background: MPTP is a neurotoxin which cause destruction of dopaminergic neurons, produces Parkinson's like manifestations both in human and animals. Quercetin possesses good antioxidant and neuroprotective activity but major complication is its poor oral bioavailability. So to overcome this hindrance the present study was designed to investigate the effect of quercetin along with bioenhancer piperine against MPTP induced neurotoxicity in rats.

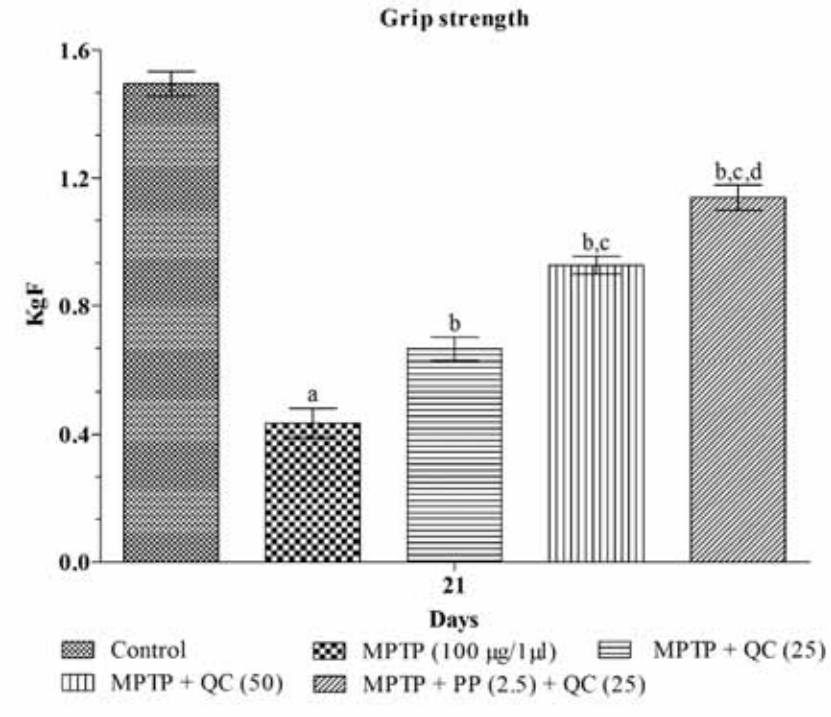
Methods: Rats were administered MPTP (100 µg/1 µL bilaterally) for 3 days (i.e. 1st, 4th and 7th). Quercetin (25 and 50 mg/kg) and combination of quercetin (25 mg/kg) with piperine (2.5 mg/kg) was administered daily for 21 days starting from the 7th day of 1st MPTP injection. Body weight and behavioral observations (locomotor, Rotarod, Grip Strength and Narrow beam walk performance) were recorded at weekly intervals after MPTP treatment. On the 22nd day, the animals were sacrificed and the rat striatum was isolated for the estimation of biochemical parameters (lipid peroxidation, glutathione and nitrite), determination of pro-inflammatory cytokine levels (TNF-a, IL-6 and IL-1b) and neurochemical analysis (Norepinephrine, 5-HT, GABA, glutamate, dopamine).

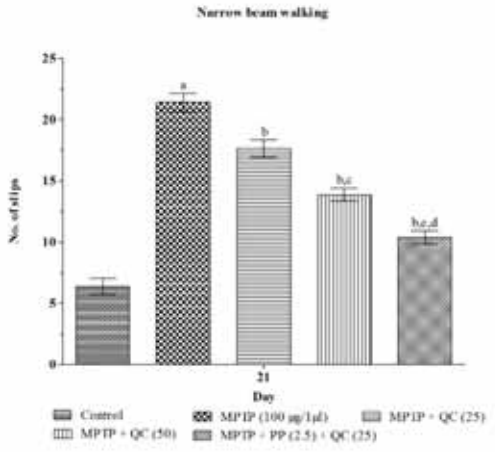
Results: The present finding had showed that chronic quercetin treatment for the 14 days significantly ameliorated the MPTP induced motor deficit, biochemical and neurochemical alterations in rats. Moreover combination of piperine (2.5 mg/kg) with quercetin (25 mg/kg) significantly potentiates the protective effect as compared to curcumin alone treated group.

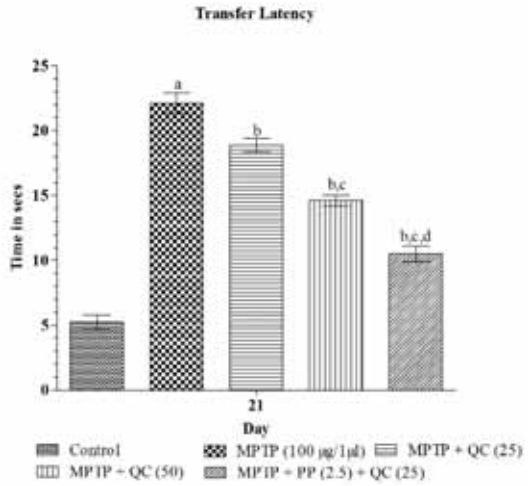

Body Weight
% Change in body weight
20
10
0
-10
-20
Control
MPTP
QC (25)
QC (50)
PP(2.5) + QC (25)
a
b
b,c
b,c,d
MPTP (100 µg/1µl)

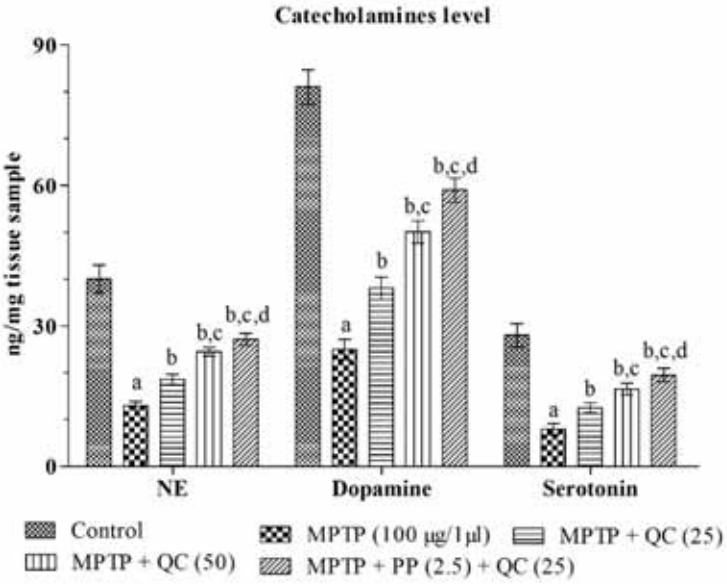

Rotarod
Fall off Time
200
150
100
50
0
a
b
b,c
b,c,d
21
Days
Control
MPTP (100 µg /1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)

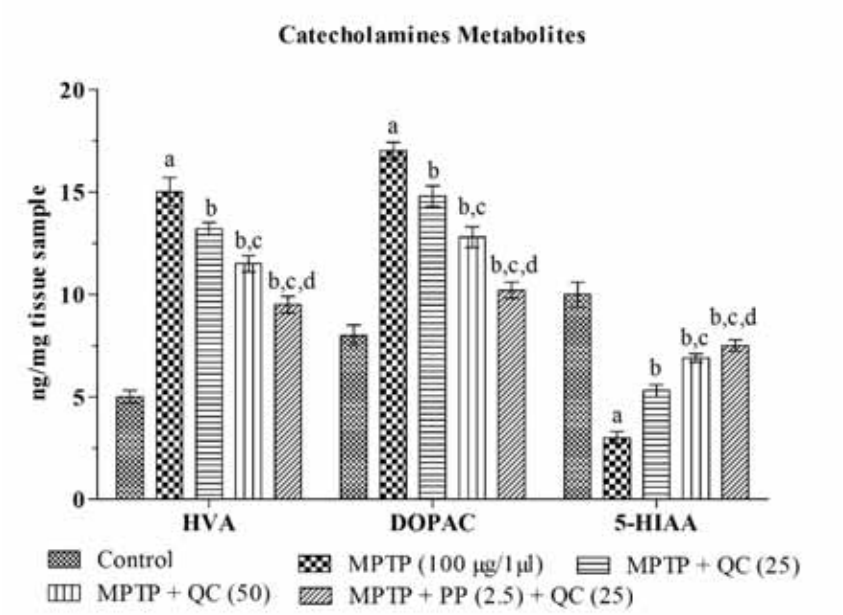

Open Field
Total Activity
120
100
80
60
40
20
0
a
b
b,c
b,c,d
21
Days
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)

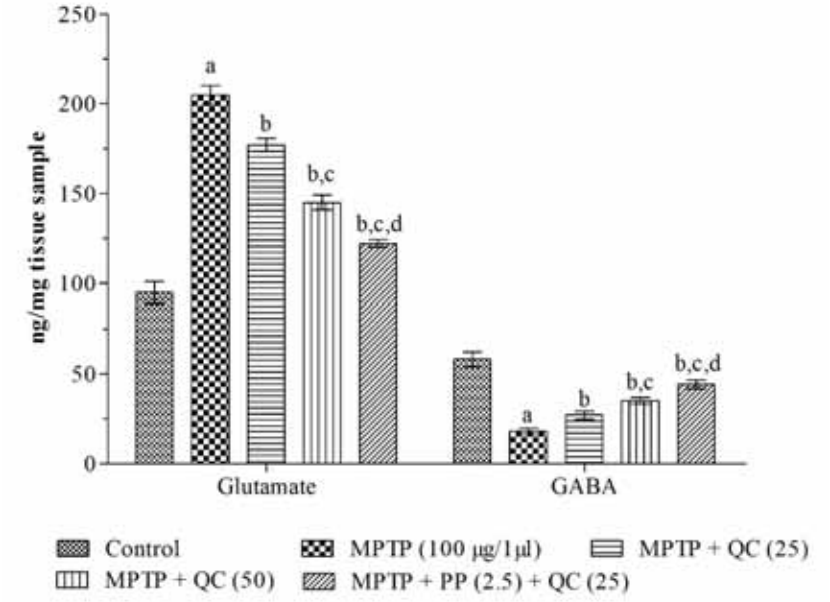

Grip strength
KgF
1.6
1.2
0.8
0.4
0.0
a
b
b,c
b,c,d
21
Days
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)


Narrow beam walking
No. of slips
25
20
15
10
5
0
a
b
b,c
b,c,d
21
Day
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)


Transfer Latency
Time in secs
25
20
15
10
5
0
a
b
b,c
b,c,d
21
Day
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)


Catecholamines level
ng/mg tissue sample
90
60
30
0
a
b
b,c
b,c,d
NE
Dopamine
Serotonin
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)


Catecholamines Metabolites
ng/mg tissue sample
20
15
10
5
0
a
b
b,c
b,c,d
HVA
DOPAC
5-HIAA
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)


ng/mg tissue sample
250
200
150
100
50
0
a
b
b,c
b,c,d
Glutamate
GABA
Control
MPTP (100 µg/1µl)
MPTP + QC (25)
MPTP + QC (50)
MPTP + PP (2.5) + QC (25)

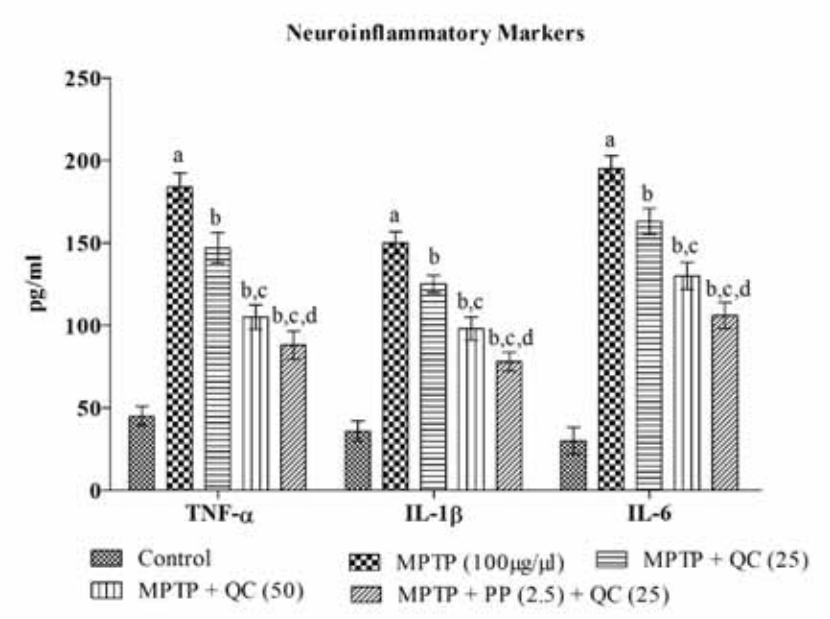


Table.1

Treatment (mg/kg)	MDA (nmol/mg protein) % of control	Nitrite level (μg/ml protein) % of control	GSH (nmol/μg protein) % of control
Control	100 ± 7.81	100 ± 6.23	100 ± 5.23
MPTP	227 ± 6.45 [a]	217 ± 6.54 [a]	26.4 ± 4.17 [a]
MPTP + QC (25)	172 ± 8.27 [b]	178 ± 5.9 [b]	43 ± 6.41 [b]
MPTP + QC (50)	145 ± 5.76 [b,c]	148 ± 6.7 [b,c]	62.5 ± 5.17 [b,c]
MPTP + PP (2.5) + QC (25)	126 ± 4.38 [b,c,d]	124 ± 5.4 [b,c,d]	81.3 ± 4.38 [b,c,d]

Protective effect quercetin in combination with piperine on MDA, nitrite and GSH in MPTP-treated rats: Values are given as mean ± SEM (n = 3). Values are statistically significant at $p < 0.05$ according to two-way ANOVA followed by Tukey's post hoc test. [a]P <0.001 vs C, [b]P < 0.05 vs MPTP, [c]P < 0.05 vs QC 25, [d]P < 0.05 vs QC 50 respectively.

Conclusions: In conclusion the administration of combination of quercetin and piperine had significantly prevented the MPTP induced behavioral, biochemical and neurological alteration by enhancing antioxidant and anti-inflammatory properties in rats.

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Protective effect of Myristicin against neurodegeneration in a 6-hydroxydopamine induced model of Parkinson's disease in rats

A. Morad Ganjeh (Karaj, Iran)

Objective: This study examined the effect of pretreatment with Myristicin on the severity of 6-hydroxydopamine-induced Parkinsonism in Wistar rats in order to find out whether Myristicin could attenuate oxidative stress markers, behavioral and structural abnormalities in an experimental model of PD in rat.

Background: Parkinson's disease refers to a progressive neurodegenerative disorder involving degeneration of dopaminergic neurons particularly in substantia nigra. The loss of dopaminergic cells results in complex motor syndrome. One of the hypothesis in pathogenesis of PD is oxidative stress via NADPH-dependent oxidases. Myristicin have been reported to increase oxidative burden.
Methods: 72 male wistar rats were divided into six groups: (1) sham, normal saline was injected in the left SNC (substantia nigra pars compacta) , (2) 6-OHDA, 6-hydroxydopamine was injected into left SNC, (3) 6-OHDA+Captopril (5 mg/kg, i.p), (4) 6-OHDA+Myristicin (5 mg/kg, i.p), (5) 6-OHDA+Myristicin (20 mg/kg, i.p), (6) 6-OHDA+Myristicin (100 mg/kg, i.p). Myristicin and captopril were injected rats daily from six days before the surgery to a day after. At the end of the experiment brain's oxidative stress markers; Lipid peroxidation and protein oxidation, apomorphine-induced rotational asymmetry were measured and the number of Nissl-stained neurons in SNC after 2 weeks were counted.
Results: Myristicin administration could attenuate the rotational behavior (p?0,05). and Myristicin also decrease the amounts of oxidative stress markers in lesioned rats and protect the neurons of SNC against 6-OHDA toxicity in comparison with the group which received only neurotoxin (p?0,01).
Conclusions: In conclusion, the results of the present study suggest that Myristicin administration has a protective effect against 6-OHDA toxicity and may be useful for treatment of Parkinson's disease.

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The clinically-available anti-depressant mirtazapine alleviates both psychosis and dyskinesia in the MPTP-lesioned marmoset

I. Frouni, S. Nuara, N. Veyres, C. Kwan, M.-J. Harraka, V. Nafade, L. Sid-Otmane, J. Gourdon, H. Adjia, P. Huot (Montreal, QC, Canada)
Objective: To determine the effect of mirtazapine, a clinically-available anti-depressant, on psychosis and dyskinesia in Parkinson's disease (PD).
Background: Psychosis and dyskinesia cause significant morbidity to as many as 50-95% of patients with advanced PD. There is increasing evidence indicating that antagonising serotonin 2A receptors (5-HT_{2A}R) may alleviate PD psychosis and dyskinesia. Mirtazapine is a clinically-available anti-depressant that acts through complex interactions with a breadth of pharmacological targets, including 5-HT_{2A}R. Here, we hypothesised that mirtazapine is potentially efficacious to reduce PD psychosis and dyskinesia.
Methods: Five common marmosets were rendered parkinsonian by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Stable and reproducible psychosis-like behaviours (PLBs) and dyskinesia were induced by chronic administration of L-3,4-dihydroxyphenylalanine (L-DOPA). Mirtazapine (vehicle, 0.1, 1 and 10 mg/kg) was then administered to the animals, in combination with L-DOPA, after which its effects on L-DOPA-induced dyskinesia, PLBs and parkinsonism were determined.
Results: We found that, in combination with L-DOPA, mirtazapine (10 mg/kg) significantly reduced PLBs severity, by 50% ($P < 0.05$), when compared to L-DOPA/vehicle treatment. Moreover, mirtazapine (10 mg/kg) significantly reduced duration of on-time with disabling PLBs, when compared to L-DOPA/vehicle (by 64%, $P < 0.01$). Mirtazapine (10 mg/kg) also reduced dyskinesia severity, by 29%, when compared to vehicle ($P < 0.01$). Accordingly, mirtazapine (10 mg/kg) significantly reduced duration of on-time with disabling dyskinesia, by 71%, when compared to vehicle. Importantly, mirtazapine did not alter the anti-parkinsonian effect of L-DOPA, as measured by parkinsonian disability scores and on-time duration.
Conclusions: These results suggest that mirtazapine is a potential drug candidate to effectively reduce the severity of PD psychosis and dyskinesia. Because it is already available in the clinic, our results could rapidly lead to proof-of-concept clinical trials where the anti-psychotic and anti-dyskinetic actions of mirtazapine would be assessed.

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The highly-selective mGluR2 positive allosteric modulator LY-487,379 alleviates L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease

C. Kwan, I. Frouni, V. Nafade, D. Gagnon, M.-J. Wallman, L. Sid-Otmane, M. Parent, A. Parent, C. Rouillard, H. Adjia, P. Huot (Montreal, QC, Canada)
Objective: To determine the effectiveness of highly-selective metabotropic glutamate 2 receptor ($mGluR_2$) activation at alleviating and preventing the development of L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia.
Background: Chronic administration of L-DOPA, the current mainstay of Parkinson's disease (PD) treatment, is marred by motor complications such as dyskinesia. Modulation of glutamatergic transmission with amantadine is an

effective strategy to alleviate dyskinesia. Here, we hypothesised that $mGluR_2$ activation is a new and efficacious way to reduce the severity of established, and prevent the development of, dyskinesia.
Methods: Female Sprague-Dawley rats were rendered hemi-parkinsonian by administration of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. Two sets of experiments were then conducted. In the first set, rats were primed with L-DOPA to elicit stable and reproducible axial, limbs and oro-lingual (ALO) abnormal involuntary movements (AIMs), after which they were administered L-DOPA in combination with the highly-selective $mGluR_2$ positive allosteric modulator LY-487,379 (vehicle, 0.1, 1 and 10 mg/kg) and ALO AIMs severity was determined. In the second series of experiments, rats were divided in 2 groups, L-DOPA/vehicle and L-DOPA/LY-487,379 0.1 mg/kg, after which they were primed for 3 weeks. After a 2-day washout period, they were all administered an acute challenge of L-DOPA and ALO AIMs severity was assessed. The effect of LY-487,379 on L-DOPA anti-parkinsonian action was determined by the cylinder test.
Results: In combination with L-DOPA, LY-487,379 0.1 mg/kg significantly diminished the severity of ALO AIMs, by 40% ($P < 0.05$), compared to L-DOPA alone. We also demonstrate that LY-487,379 0.1 mg/kg, started concurrently with L-DOPA, attenuates the priming process leading to the development of dyskinesia, when compared to L-DOPA alone, by 82% ($P < 0.05$). Importantly, administration of LY-487,379 did not hinder L-DOPA anti-parkinsonian action.
Conclusions: These results suggest that selective $mGluR_2$ activation is a new and effective therapeutic strategy to reduce the severity, and attenuate the development, of L-DOPA-induced dyskinesia.

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Piper betle L. Attenuates 6-OHDA Induced Apoptosis in SH-SY5Y Cells and Neuronal Injury in Caenorhabditis elegans Parkinson's Model

G. Shanmugam, A. Mohankumar, P. Sundararaj (Coimbatore, India)

Objective: To explore the anti-apoptotic effects of *Piper bitle* leaf extract (PLE) on 6-OHDA induced cellular injury in human neuroblastoma cell SH-SY5Y. To analyse the neuroprotective effects of PLE in Parkinson's disease *C. elegans* model.
Background: The molecular pathogenesis leading to neurodegeneration of dopaminergic neurons in PD is largely unknown. There is a need of an effective therapy to control the progression of proteinopathies. PLE attenuates 6-OHDA induced apoptotic events by regulating various signalling cascades in SH-SY5Y cells, and PLE ameliorates the a-synuclein accumulation, DAergic neurodegeneration, restore the lipid content and increase the lifespan of *C. elegans* treated with 6-OHDA.
Methods: SH-SY5Y: Cell viability, LDH release, nuclear condensation, detection of apoptosis, mitochondrial membrane potential, cell cycle and western blot analysis. *C.elegans :* a-synuclein aggregation, lipid deposition, DAergic neurodegeneration and lifespan analysis.
Results: The results revealed that pre-treatment with PLE in SH-SY5Y cells prior to 6-OHDA exposure improves the cell viability, decrease the LDH release, reverse mitochondrial transmembrane abnormalities, restore the Bax/Bcl2 ratio, and cytochrome c release. Meanwhile, PLE inhibits the phosphorylation of MAPKs (p38, JNK and ERK) pathway and p53 activation. Moreover, PLE could increase the expression of PI3K/Akt. In addition, PLE could significantly decrease the a-synuclein aggregation; attenuates the DAergic neurodegeneration; ameliorate the lipid content in NL5901 worms; increased the lifespan and decreased the lipofuscin in 6-OHDA treated N2 wild type worms.
Conclusions: Findings demonstrated that PLE exerts its neuroprotective effect by anti-oxidative and anti-apoptotic ability. Moreover, PLE has a potent anti-parkinsonism effects and this effect was driven by the inhibition of neurotoxicity, apoptotic cascade events, and ROS-mediated activation of downstream signalling pathways, MAPKs and PI3K/Akt. Studies were strongly proves that PLE exhibits strong ameliorative futures against a-synuclein accumulation, DAergic neurodegeneration and physiological impairments induced by 6-OHDA. Taken together, we believe that PLE was a potent candidate for the prevention of neurodegeneration in the patients with PD.

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The highly-selective metabotropic glutamate receptor 2 positive allosteric modulator LY-487,379 alleviates psychosis-like behaviours and dyskinesia in the MPTP-lesioned marmoset

L. Sid-Otmane, S. Nuara, A. Hamadjida, N. Veyres, C. Rouillard, M. Panisset, J. Gourdon, P. Huot (Montreal, QC, Canada)

Objective: To investigate the effect of metabotropic glutamate receptor 2 (mGluR2) activation on L-3,4-dihydroxyphenylalanine (L-DOPA)-induced psychosis-like behaviours (PLBs) and dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset model of Parkinson's disease (PD).
Background: Psychosis and dyskinesia undermine the quality of life of as many as 50-95% of patients with advanced PD. Available therapies are few, their efficacy is partial and they may elicit important side effects. Serotonergic 2A receptor (5-HT2AR) blockade is a validated approach to alleviate both psychosis and dyskinesia, but the effectiveness of this approach is also limited. 5-HT2AR forms a functional hetero-complex with mGluR2 involved in psychotic symptoms of schizophrenia and hallucinogenic effects of psychotomimetic drugs. We hypothesised that mGluR2 activation is a new and effective approach to reduce both L-DOPA-induced dyskinesia and psychosis in PD.
Methods: Six common marmosets were rendered parkinsonian by MPTP administration. Dyskinesia and PLBs were induced by chronic administration of L-DOPA. The potent and highly-selective mGluR2 positive allosteric modulator (PAM) LY-487,379 (vehicle, 0.1, 1 and 10 mg/kg) was then administered in combination with L-DOPA and its effects on each of dyskinesia, PLBs and parkinsonism were assessed.
Results: LY-487,379 1 and 10 mg/kg significantly alleviated PLBs (by 35% and 42%, respectively, both $P < 0.05$), when compared to vehicle. LY-487,379 1 and 10 mg/kg also significantly reduced the severity of dyskinesia (by 46% and 53%, respectively, both $P < 0.05$). LY-487,379 also significantly improved the quality of on-time, by reducing the duration of on-time with disabling PLBs/dyskinesia. Importantly, LY-487,379 did not hinder with L-DOPA anti-parkinsonian action.
Conclusions: Selective mGluR2 activation stands as a new and promising approach to alleviate both PD psychosis and dyskinesia without negative impact on parkinsonian symptoms.

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Role of Apocyanin in modulating glial cell functions and associated inflammatory response in Lipopolysaccharide induced Parkinson's disease model.

N. Sharma, B. Nehru (Chandigarh, India)

Objective: To explore the effect of apocyanin on glia mediated inflammatory response and a-synuclein aggregation in single intranigral LPS induced PD model.
Background: Converging lines of evidence suggest that glia associated neuroinflammatory processes may account for the progressive death of dopaminergic neurons in Parkinson's disease (PD). Also, Apocyanin, an established microglial NADPH oxidase inhibitor has been proved to have beneficial effects in modulating anti-inflammatory as well as anti-oxidative effects in case of lipopolysaccharide induced PD model.
Methods: LPS (5µg/kg b.wt) was injected intranigral stereotaxically and apocyanin (10mg/kg/ day) was given i.p. for a period of 21 days.
Results: Following LPS injection significant augmentation in the gene as well as protein expression of transcription factor NF?B as well as proinflammatory cytokines (TNF-a, IL-1a, IL-1ß), NADPH oxidase subunits gp90PHOX and gp21PHOX were observed in the glial fraction thus suggesting the prevalence of inflammatory responses and activation of NADPH oxidase complex. IHC for microglial activation and a-synuclein revealed that apocyanin significantly inhibited microglial activation as well as alpha synuclein aggregation in dopaminergic cells. With this significantly compromised glutathione system as well as other antioxidant enzymes (SOD,Catalase) were also observed. However, with apocyanin treatment marked improvement in NF?B activation and related parameters was observed. This was further reflected in histopathological studies showing no evidence of inflammation in case of apocyanin treated animals.
Conclusions: This can be concluded that apocyanin play an important role in modulating glial cell functions thus revealing its potential anti-inflammatory role along with its NADPH oxidase inhibiting property. Therefore, its neuroprotective role could be further evaluated in other toxicological conditions.

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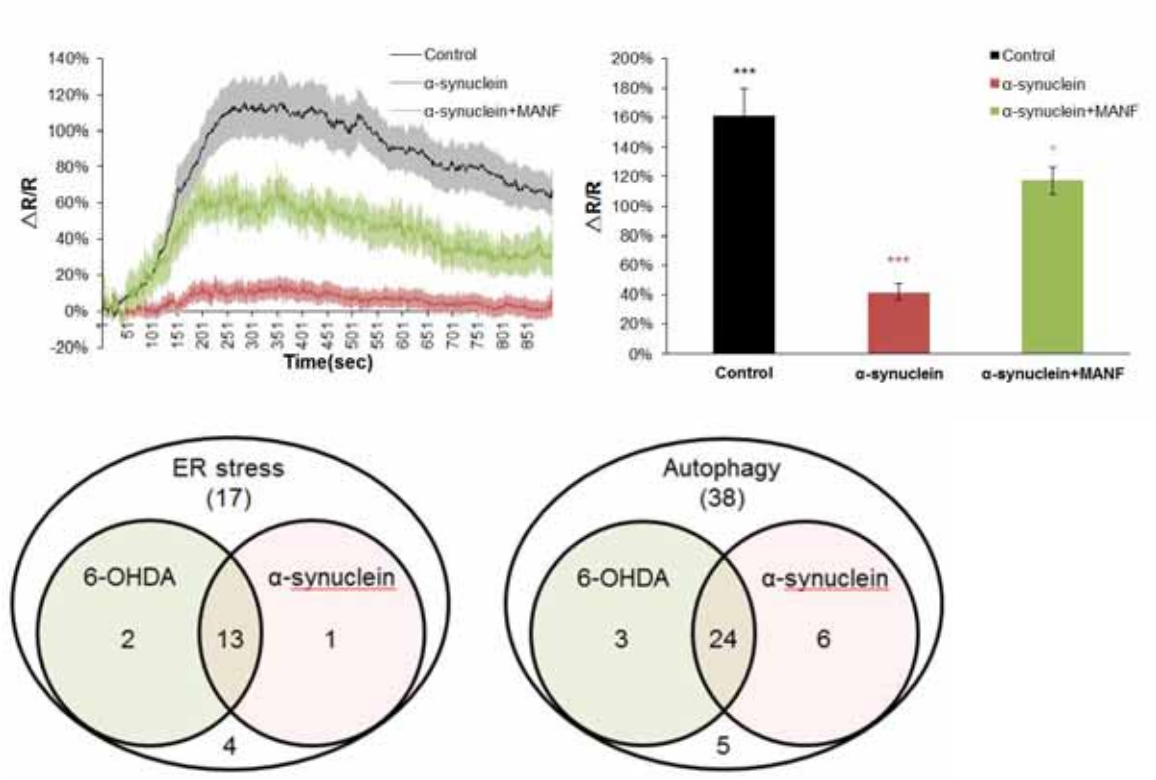
MANF protects dopamine neurons and locomotion defect from Neurotoxin/Human alpha-synuclein-induced progressive Parkinson's disease models in C.elegans.

Z. Zhang (Shanghai, People's Republic of China)

Objective: To study whether Mesencephalic astrocyte-derived neurotrophic factor (MANF) has beneficial effects in Parkinson's disease and its intracellular mechanisms.
Background: The distinguishing feature of Parkinson's disease is due to the degeneration of dopaminergic(DA) neurons. Caenorhabditis elegans (*C.elegans*) has a defined neural architecture and it has been demonstrated to be a

good in vivo model in the study of neurodegenerative diseases. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a novel neuron factor which exhibits a survival-promoting effect to dopaminergic neurons in vitro. However, to date, it remains unclear whether MANF can rescue DA neurons in a-synuclein (a-Syn) model of PD and the neuroprotective mechanisms of MANF on dopaminergic neurons remain unclear.
Methods: 1. We construct neurotoxin/a-synuclein induced dopamine neurodegeneration model and observe the neuronal morphology includes cell body fluorescence, cilia, dendrite, axon, nuclear morphology and the locomotory index includes bending angle, length of worms, omega bending, speed, grid counting, track length. 2. We feeded MANF/a-Syn treated worms on plates seeded with HT115 E.coli bacteria expressing dsRNA for knocking down autophagy and ER stress related genes. Then we observed the dopamine neuron survival rate of 6-OHDA induced worms treated with MANF and locomotory capacity of a-Syn induced worms treated with MANF.
Results: 1. Dopaminergic neurons show time-related degeneration in 6-OHDA and a-synuclein induced model. 2. The abnormal behavior of C. elegans occurs in both two models. Also, the neuronal morphology damaged at first, then the motor symptoms emerged. 3. MANF has protective effects in two models. At the same time, MANF rescues the function of dopamine neurons by calcium imaging (shown - [Fig1]). 4. We screening all of autophagy and ER stress related genes so far identified in C.elegans, and find that almost all genes are involved in the molecular mechanism of MANF (shown - [Fig2]).



Conclusions: MANF has protective effects in neurotoxin/Human a-Syn-induced PD models by regulating autophagy and ER stress pathway.

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Rotenone induces astrocyte-mediated non-cell autonomous dopaminergic neurotoxicity

I. Miyazaki, S. Murakami, R. Kikuoka, N. Isooka, Y. Kitamura, M. Asanuma (Okayama, Japan)

Objective: In this study, we examined involvement of astrocytes in rotenone-induced dopaminergic neurotoxicity using primary cultured cells.

Background: Several studies reported that pesticide exposure, such as rotenone or paraquat, increased the incidence of Parkinson's disease (PD). Therefore, exposure to pesticides is thought as a potential environmental factor to play important roles in the pathogenesis of PD. Rotenone is often used to induce PD-like pathology in the central nervous system and enteric nervous system. We previously reported that subcutaneous infusion of rotenone in mice resulted in neurodegeneration and glial activation not only in the nigrostriatal pathway but also in the olfactory bulb and ascending colon, and that these effects were region-specific but not serial changes.
Methods: Primary cultured neurons and astrocytes were prepared from the mesencephalon of Sprague-Dawley rat embryos at 15 days of gestation. Enriched neuronal cultures or neuron-astrocyte co-cultures were treated with rotenone for 48 h. The treated cells were fixed and reacted with the mouse anti-tyrosine hydroxylase antibody. We also examined the effects of rotenone-treated astrocytes on survival of dopaminergic neurons using conditioned media from rotenone-treated astrocytes. Furthermore, we examined differentially displayed molecules and antioxidative property in astrocytes after rotenone treatment.
Results: Rotenone exposure significantly decreased dopaminergic neurons in neuron-astrocyte co-cultures, but not in enriched neuronal cultures. In addition, dopaminergic neurotoxicity was induced by treatment with conditioned media from rotenone-treated astrocytes. Rotenone treatment induced moderate up-regulation of inflammatory cytokines in conditioned media of astrocytes. Furthermore, the pesticide significantly decreased the secretion of metallothionein, which is an antioxidative molecule, from astrocytes.
Conclusions: These results showed that rotenone induced astrocyte-mediated non-cell autonomous dopaminergic neurodegeneration, and suggested that astrocyte dysfunction plays an important role in rotenone neurotoxicity. (The part of this paper was presented at 90th Annual Meeting of the Japanese Pharmacological Society on March 16, 2017.)

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Neuroprotective effects of antidepressant mirtazapine against dopaminergic neurodegeneration in cultured cells and in parkinsonian mice possibly by targeting astrocytes

M. Asanuma, I. Miyazaki, R. Kikuoka, S. Murakami, N. Isooka, Y. Kitamura (Okayama, Japan)

Objective: In this study, we examined neuroprotective effects of mirtazapine and involvement of astrocytes in the effects using primary cultured cells and parkinsonian mice.
Background: We previously reported that 8-OH-DPAT, a full serotonin 1A (5-HT1A) agonist, upregulated an antioxidative molecule metallothionein (MT) in the striatal astrocytes and protected dopaminergic neurons in parkinsonian mice. Mirtazapine, a noradrenergic and specific serotonergic antidepressant (NaSSA), shows multiple pharmacological actions such as inhibiting presynaptic a2 noradrenergic receptors and activating indirectly 5-HT1A receptors.
Methods: Primary cultured neurons and astrocytes were prepared from the mesencephalon and striata of Sprague-Dawley rat embryos at 15 days of gestation. Mesencephalic neurons or neuron-astrocyte co-cultures were treated with mirtazapine (10 μM) for 24 h, followed by exposed to 6-hydroxydopamine (6-OHDA). Changes in the number of dopaminergic neurons and MT expression in astrocytes were evaluated. The hemi-parkinsonian mice unilaterally lesioned by intrastriatal injections of 6-OHDA were treated with mirtazapine (5 or 16 mg/kg, i.p.) for 8 days, and immunohistochemical analyses were performed using brain slices.
Results: Pretreatment with mirtazapine significantly prevented 6-OHDA-induced dopaminergic neurotoxicity in neuron-astrocyte cocultures, but not in enriched neuronal cultures. Mirtazapine did not affect MT expression in cultured astrocytes directly, but conditioned medium from mirtazapine-treated mesencephalic neurons upregulated MT expression in astrocytes. The neuroprotective effects of mirtazapine in neuron-astrocyte cocultures were annulled by 5-HT1A antagonist. The treatment with mirtazapine (16 mg/kg) reduced neurodegeneration of nigrostriatal dopaminergic neurons in parkinsonian mice, with increasing striatal MT expression.
Conclusions: These results suggest that mirtazapine upregulates MT expression in astrocytes via secreted molecules from mesencephalic neurons and the drug exerts neuroprotective effects against dopaminergic neurodegeneration possibly by targeting astrocytes. (The part of this paper was presented at 46th Annual Meeting of the Japanese Society of Neuropsychopharmacology on July 2-3, 2016.)

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Curcumin I prevents the developmental defective sensorimotor patterns resulted from Lead poisoning in rat

H. Benammi, O. El Hiba, H. Gamrani (Marrakech, Morocco)

Objective: In the present investigation, we evaluated the effect of prenatal exposure of rats to lead from neonatal to adult age on the sensorimotor performances during development. Also, we evaluated the neuroprotective effect of curcumin I against lead neurotoxicity.

Background: Lead (Pb) is a heavy metal with no apparent biological function [1]. Pb is known to cause several damages in the central and peripheral nervous system [2], which could be manifested by several neurophysiological and behavioral outcomes [3-5].

Methods: Using grasping and cliff avoidance tests and Catwalk task, we evaluated the impairment of the sensorimotor functions in neonatal and young rats exposed prenatally to Pb.

Results: Our results showed that rats exhibit an impaired locomotor gait in Pb-treated from puppy and until young age. These abnormalities were strongly attenuated by curcumin I co-administration.

Conclusions: The present finding have brought evidence of a potent neuroprotective and restorative action of curcumin I against neuronal damages occurring during developmental exposition to heavy metals especially Pb, therefore, consumption of Curcumin I can be recommended as a possible pharmacological strategy for preventing Pb neurotoxicity.

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Application of a Qualitative Model to Elucidate the Role of the Alpha-Synuclein System in Parkinson's Disease

C. Friedrich, W. Zago, S. Gardai, G. Tonn, M. Reed (Cupertino, CA, USA)

Objective: In order to improve the understanding of the alpha-synuclein system and its role in Parkinson's disease, Elan and Rosa collaborated in the development of a Synuclein PhysioMap®, a graphical model to support hypothesis generation and testing. The objectives of the project were to provide insight into alpha-synuclein function in vesicle trafficking by creating a PhysioMap, memorialize and communicate the current state of knowledge within Elan in a Synuclein Model Qualification Method Document (MQM), and to recommend experiments to test hypotheses, resolve uncertainties, and identify and prioritize potential targets.

Background: Elan was developing inhibitors of alpha-synuclein formation for the treatment of Parkinson's disease and wanted to improve the design and interpretation of *in vitro* assays to evaluate candidate compounds.

Methods: The PhysioMap was developed using both publically available literature and proprietary Elan data. The Synuclein PhysioMap was designed and curated by a multidisciplinary team to represent synuclein synthesis and distribution within a neuron, SNARE complex formation, phagocytosis, cytokine release, neurotrophic factor release, and mitochondrial function.

Results: During the development of the PhysioMap, the team identified key biological uncertainties and hypotheses and recommended focused laboratory experiments to gain a better understanding of synuclein function in Parkinson's disease.

Conclusions: Development of the PhysioMap helped provide insight into the alpha-synuclein system and its function in Parkinson's disease, and helped identify a more efficient experimental path for evaluating therapeutic compounds.

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Curcumin I protect against copper induced neurobehavioral features of Parkinson's disease in rat

A. Abbaoui, O. EL Hiba, H. Gamrani (Marrakech, Morocco)

Objective: evaluate the impact of acute Cu intoxication (10 mg/kg B.W. i.p) for 3 days on the dopaminergic system and locomotor performance, together with the possible restorative effect of oral administration of curcumin (30 mg/kg B.W.).

Background: Parkinson's disease is a progressive disorder of the nervous system that affects movement. The classic motor symptoms of Parkinson's disease result from progressive dopaminergic neurons death within substantia nigra. Some finding support the involvement of heavy metals, as an exogenous risk factor such as copper (Cu), excessive levels of this element are responsible for profound physiological alterations including the central nervous system.

Whereas, different pharmacological trials have shown a beneficial role against Cu neurotoxicity, curcumin (*Curcuma longa*) is one of the powerful medicinal plants with an array of therapeutic effects.
Methods: Behavioral study (open field) and Immunohistochemistry.
Results: We noted, in the Cu intoxicated rats, a significant loss of TH (tyrosine hydroxylase) expression within substantia nigra compacta (SNc), ventral tegmental area (VTA) and the subsequent striatal outputs, those alterations were correlated to behavioral abnormalities such as a severe drop of locomotor performance. While curcumin administration to Cu intoxicated rats showed a noticeable beneficial effect; this potential was featured by a complete recovery of the TH expression and locomotor behavior deficiencies in the intoxicated rats.
Conclusions: The present investigation have brought, on the one hand, an experimental evidence of an altered dopaminergic innervations following Cu intoxication and on the other hand, a new pharmacological property of curcumin that may be used as a neuroprotective plant for neurodegenerative disorders touching the dopaminergic system trigged by heavy metals.

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Cortical excitability during a movement compared with postural control task in healthy subjects
F. Chang, P. Menon, M. VanDen Bos, M. Kiernan, S. Vucic, V. Fung (Sydney, Australia)
Objective: To investigate cortical excitability and surround cortical inhibition during movement compared with postural control task in healthy subjects.
Background: Motor control has traditionally been divided into the pyramidal and the extrapyramidal systems. The pyramidal system is conceptualized as the corticospinal pathways, thought to be responsible for fine motor and goal-directed movements, whereas the extrapyramidal system is responsible for the speed, selection, amplitude of movement and posture1. More recently separate systems within M1 for postural and movement control were discovered in healthy primate2.
Methods: Short interval cortical inhibition (SICI), resting motor threshold (RMT) and cortical silent period (CSP) are measures derived from motor evoked potentials measured over the resting abductor pollicis brevis muscle (APB) of the pronated hand. We used threshold tracking technique and transcranial magnetic stimulation(TMS) over M1, recording over the contralateral resting abductor pollicis brevis muscle (APB) of the pronated hand: while at rest, performance of a postural control task (constant extension of ipsilateral extensor indicis (EI)) and movement control task(activation of EI at 1Hz) in healthy subjects. A separate channel monitored APB muscle activity ensured its resting state during data acquisition. Studies were undertaken on 17 healthy controls were recruited (13 women; 4 men; age 35±1.5). All results are expressed as mean±SEM.
Results: SICI, between ISI 1 to 7ms, was significantly higher during the postural task (1.6±3.4%) when compared to the movement control task (-3.5±5.6%, p=0.03). In addition, cortical silent period during movement (151.0±13.7ms) was lower than the postural task (172.8±9.7ms, p=0.04). Of further relevance, resting motor threshold was significantly lower during movement control (39.3±7.2%) than the postural task (43.7±7.8%, p=0.01). MEP amplitude was higher at rest (1.9±0.3mV,p=0.03) than during postural task (1.3±0.8mV) or movement task (1.8±1.3mV,p=0.07). There was no difference between postural and movement control task MEP amplitude.
Conclusions: Voluntary limb movement is associated with motor cortical disinhibition, contrasting with limb posture. Taken together, the present finding supports the notion that distinct motor control systems mediate movement and postural tasks.

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Network for parallel gamma synchronizations during upper limb movement
G. Tamas, A. Anwar, G. Deuschl, J. Raethjen, S. Groppa, M. Muthuraman (Budapest, Hungary)
Objective: Our aim was to identify the sources of parallel gamma synchronizations (GS) and analyze the direction of information flow in their network, at the beginning of simple and combined upper limb movements.
Background: GS at the onset of movements may promote the processing between functionally related cortico-subcortical neural populations.
Methods: We measured 64-channel EEG in 11 healthy volunteers; surface EMG detected the movements of the dominant hand. In Task1 subjects kept a constant medium-strength contraction of the first dorsal interosseus muscle and superimposed on this they performed a repetitive voluntary self-paced brisk squeeze of an object. They executed brisk contraction in Task2 and constant contraction in Task3. Time-frequency analysis of the EEG signal was performed with multitaper method. GS sources were identified in five frequency bands (30-49Hz, 51-75Hz, 76-100Hz, 101-125Hz and 126-150Hz) with the beamformer inverse solution dynamic imaging of coherent sources by taking the EMG as the reference signal. The direction of information flow between the sources was estimated by

renormalized partial directed coherence for each frequency band. To identify significant connections, the data driven surrogate test and the time reversal technique was performed.
Results: The first three sources in consecutive order in each movement task, in every frequency band, were as follows: contralateral primary sensorimotor cortex (S1M1), dorsolateral prefrontal cortex (dPFC) and supplementary motor cortex (SMA). Gamma activity was detected in narrower low- and high-frequency bands in the contralateral thalamus (TH) and ipsilateral cerebellum (C), in all three tasks. In the combined Task1 additional low gamma activity appeared in the contralateral posterior parietal cortex (PPC). In every task, S1M1 had efferent information flow to the SMA and the dPFC; the latter had no afferent relation to the network. S1M1 and SMA had a bidirectional connection with the TH, and the C. Afferent information flow was detected from the PPC to the SMA and bidirectional flow between PPC and the TH, in the combined Task1.
Conclusions: The same network could be identified for the parallel gamma synchronizations in the tasks; it was complemented by the PPC in the combined Task1. S1M1 drove the other cortical sources and had afferent activity from the TH and the C, which activated in variable frequency bands in the tasks.

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Patient-derived GBA1-PARK2 double-mutant cellular models to study the effect of GBA1 as a modifier of familial Parkinson's disease

Z. Hanss, I. Boussaad, P. Barbuti, S. Goldwurm, R. Krüger (Belvaux, Luxembourg)
Objective: In this study, we propose to decipher the role of *GBA1* as a modifier of familial Parkinson's disease (PD) using double-mutant patient-derived cellular models.
Background: Mutations in more than 20 genes have been identified as being causative for PD. Nevertheless, heterogeneity in penetrance, phenotype and age of onset of patients carrying the same mutations lead to the search of modifying factors that could influence disease onset and progression. Mutations in the *GBA1* gene, encoding the glucocerebrosidase (GCase), are an important and common risk factor for familial and sporadic PD. Indeed, 5-10% of all PD patients are carrying a *GBA1* mutation [1]. These patients are more likely to progress to dementia, develop earlier axial motor symptoms and have a slightly earlier age of onset compared to non-carrier PD patients. Consequently, mutation in *GBA1* could influence PD causal genes and modify the pathophysiology.
Methods: Fibroblasts from a PD patient harbouring homozygous mutation in the *PARK2* gene and a point mutation in the *GBA1* gene have been donated. Patient's fibroblasts have been reprogrammed into induced pluripotent stem cells (iPSC) using synthetic RNA encoding for reprogramming factors (Oct4, Nanog, Klf4, Glis1). These iPSC have been differentiated into small neuronal precursor cells to finally generate midbrain-specific dopaminergic neurons [2]. At iPSC stage, CRISPR Cas9 technology will be used to correct the *GBA1* mutation to obtain an isogenic control free from any effect caused by this mutation and only harbouring the deletion in *PARK2*.
Results: Three cellular models derived from patient's fibroblasts have been successfully generated. Enriched neuronal culture were obtained, containing more than 15% of dopaminergic neurons. The characterisation of the cellular models confirmed the quality and suitability of our cell lines to study PD relevant phenotypes. Further characterisation of the cells revealed an impairment in GCase activity and a loss of Parkin. We now aim to investigate *GBA1* mutation specific effect in the frame of the double mutation focusing on mitochondrial features, autophagy-lysosomal function and a-synuclein interplay.
Conclusions: We have established the first double-mutant *GBA1-PARK2* patient-derived cellular models. Phenotypic difference were identified and specific effect of GBA will be further studied particularly with the help of isogenic control.

1020

Parkinson's disease in untreated Gaucher patients is associated with reduced glucosylsphingosine (Lyso-Gb1) serum levels

D. Arkadir, T. Dinur, A. Rolfs, A. Zimran (Jerusalem, Israel)
Objective: To assess the role of glycosphingolipids in increased risk of Parkinson's disease (PD) in patients with Gaucher disease (GD).
Background: Patients with GD are at increased risk of PD. Controversy exists regarding the biochemical pathways linking the two diseases. It remains unclear whether neuronal death is secondary to increased levels of glycosphingolipids or to accumulation of the misfolded glucocerebrosidase. Glucosylsphingosine (Lyso-Gb1), a sensitive and specific biomarker for GD, can help resolve this issue.

Methods: We tested Lyso-Gb1 serum levels (Centogene, Rostock, Germany) in GD patients with PD and compared the result of each patient with a mutation-matched control group. Participants were not on enzyme-replacement therapy. To normalize the distribution of Lyso-Gb1 values they were log-transformed and then z-scored.
Results: Lyso-Gb1 levels were tested in four GD-PD patients with a N370S mutation in the *GBA* gene and an additional in-trans mutation (N370S, L444P, V394L or RecTL). Lyso-Gb1 values were lower in all GD-PD individuals when compared with their relevant control group (z-scores: -0.55, -1.53, -3.47, -3.75, respectively to in-trans mutation).
Conclusions: The hypothesis that high levels of glycosphingolipids are a major cause of increased risk of PD in GD patients is not supported by our results.

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Association Of Single Nucleotide Polymorphism In Maob And Risk Of Levodopa-induced Dyskinesias in Parkinson's Disease

B. Santos-Lobato, M. Capelari, N. Novaretti, Â. Vieira, V. Borges, H. Ferraz, I. Mata, C. Zabetian, V. Tumas (Ribeirão Preto, Brazil)

Objective: To identify genetic risk factors for developing levodopa-induced dyskinesias (LID) in patients with Parkinson's disease (PD).
Background: LID are common complications in PD, but there are conflicting data about genetic risk factors associated with its onset.
Methods: A cross-sectional study was conducted with epidemiological and clinical data from Brazilian PD patients enrolled from two centers as part of the Latin American Research consortium on the Genetics of PD (LARGE-PD). All PD patients were submitted to neurological examinations and semi-structured interviews performed by a neurologist with experience on Movement Disorders. Presence of LID was confirmed if UPDRS Part IV had a score = 1 on item 32. Based on previous studies, we chose eight Single Nucleotide Polymorphisms (SNP) in the following genes: *COMT, MAOB, ANKK1, DRD3, DAT1, BDNF, ADORA2A* and *NOS1*. Genotyping was performed using TaqMan SNP genotyping assays. Association between SNPs and LID was tested using multivariate logistic regression under an additive model adjusting for sex, age at onset of PD and levodopa therapy duration.
Results: 186 Brazilian PD patients were enrolled (males - 58%; mean age 60 years). Of these patients, 91 (48.9%) presented LID. Only *MAOB* SNP rs1799836 was associated with LID, with the A allele increasing the risk of developing LIDs (OR 1.51, CI95% 1.00-2.28; p = 0.05). However, when analyzed independently in male and females (MAOB gene is located in chromosome X), these differences were not significant.
Conclusions: *MAOB* SNP rs1799836 is a probable genetic risk factor for LID although further studies in larger samples are required to explore the influence of *MAOB* polymorphisms on LID.

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SNCA multiplication consortium: Clinicogenetic analysis of SNCA multiplication probands and families.

A. Book, M. Farrer, T. Candido, I. Guella, D. Evans, E. Gustavsson (Vancouver, BC, Canada)

Objective: Clinicogenetic analysis of 57 affected probands with *SNCA* multiplication, 3 unaffected carriers, and their 60 families. To use informative pedigrees to identify penetrance modifiers within the *SNCA* locus, neighboring regions, and known variability associated with PD and cognition.
Background: *SNCA* dosage has been correlated with alpha-synuclein expression levels, age of motor onset and disease severity in PD. Patients with *SNCA* duplications present a wide range of phenotypes, while those with triplications have an earlier presentation, accompanied by psychiatric problems and cognitive decline.
Methods: DNA samples and clinical measures of disease progression and cognitive decline were collected from 57 probands and 3 unaffected individuals carrying *SNCA* multiplication mutations. Further clinical evaluation, with analysis of intra- and inter-familial variance, and biospecimen collection is planned. To date, DNA has been genotyped with a 1.8 million SNP Multi-Ethnic Genotyping Array (MEGA). 5', 3' and intron 4 *SNCA* STR markers are assessed along with APOE, MAPT and GBA risk factors using TaqMan genotyping and Sanger sequencing.
Results: Of the 60 individuals (30 male/30 female), 51 had three copies of *SNCA* (3C), and 9 had four copies (4C). Average age of motor symptom onset for those affected was 45.3±11.1 years (3C: 47.2±10.6, 4C: 34.5±7.9). 25 of those affected have a secondary diagnosis of dementia, with average dementia onset at 51.1±12 years (3C: 56.5±9.6, 4C: 39.6±5.5) where 50% of dementia diagnoses came within 5 years of PD diagnosis (3C: 14, 4C: 1). The average length of multiplication was 2.18±2.54Mb (range: 0.16-13.83Mb) and is not correlated with age of motor onset (r=0.21).

Conclusions: With coordinating sites in Asia, Europe, and North America (lead by Nobutaka Hattori, Alexis Brice and Matt Farrer, respectively) the SNCA Multiplication Consortium uniquely serves to: 1) screen and identify new *SNCA* multiplication carriers; 2) harmonize clinical phenotyping; 3) expand pedigrees and; 4) develop biomarkers for alpha-synuclein-targeted therapies (see http://www.geopd.org/projects/6).

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A neuronal model of PARK20 (SYNJ1 mutation) using patient derived iPSCs

R. Masius, M. Minneboo, M. Grochowska, M. Quadri, M. Picillo, P. Barone, V. Bonifati, W. Mandemakers (Rotterdam, Netherlands)

Objective: The SYNJ1 homozygous mutation (p.Arg258Gln) leads to juvenile Parkinsonism (PARK20). SYNJ1 plays an important role in synaptic vesicle cycling, and regulating autophagic flux. We generated an *in vitro* model of PARK20 using small molecule induced dopaminergic neurons (iDA) from SYNJ1 p.Arg258Gln patient derived and control induced pluripotent stem cells (IPSCs), to investigate the disease molecular mechanisms.

Background: Our lab and others have identified a SYNJ1 homozygous mutation (p.Arg258Gln) segregating with disease in consanguineous families with Parkinsonism, dystonia, and cognitive deterioration. The mutation impairs the Sac1 domain phosphatase activity of SYNJ1 protein *in vitro*. Another homozygous mutation within the Sac1 domain (p.Arg459Pro), as well as compound heterozygous mutations have been recently described as a cause of autosomal recessive juvenile Parkinsonism Strikingly, mutations in the SYNJ1 gene that result in complete loss of SYNJ1 expression lead to more severe phenotypes, including early onset refractory seizures and juvenile lethality, suggesting a phenotype-genotype correlation. Although mechanistic insight is lacking, these studies point towards a strong link between SYNJ1 mutations and neurodegeneration.

Methods: Patient derived SYNJ1 p.Arg258Gln and control induced pluripotent stem cells (IPSCs) were exposed to small molecules and differentiated for 4 weeks to induced dopaminergic (iDA) neurons. After 4 weeks, iDA neurons were exposed to starvation and control conditions and probed for markers of autophagy (i.e. LC3B, WIPI2) and synaptic vesicle recycling pathways (i.e. clathrin, endophilin) by immunocytochemistry and Western blotting techniques. Expression levels were quantified using FIJI software.

Results: Tyrosine Hydroxylase expressing neurons could be detected after 4 weeks of small molecule treatment both in control and patient cell lines. Immunohistochemistry analysis showed increased expression levels of the autophagy marker WIPI2 in patient derived iDA neurons as compared to control neurons before and after starvation.

Conclusions: Our preliminary data indicate that the SYNJ1 p.Arg258Gln mutation affects the autophagy pathway as indicated by increased levels of WIPI2 expression in patient derived iDA neurons. Further research is needed to clarify the role of SYNJ1 in the autophagy and synaptic vesicle cycling pathway.

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eEF1A2 promotes cell survival and protects against MPP+-induced apoptotic neuronal death through the PI3K/Akt/mTOR pathway

K. Khwanraj, P. Dharmasaroja, B. Morris (Ratchathewi, Thailand)

Objective: To assess the correlation of eEF1A2 and PI3K/Akt/mTOR expression and investigate the pro-survival role of eEF1A2 in NG108-15 rodent neuroglioma cells.

Background: Eukaryotic protein elongation factor 1 alpha 2 (eEF1A2) is one of the members of the eEF1A family, which plays a canonical role in protein synthesis during the elongation phase of protein translation. A non-canonical role in pro-survival has been shown in many cancers and neurodegenerative diseases. An increase of eEF1A2 expression causes cancer progression whereas a decrease of eEF1A2 has been linked to neuronal death in many neurodegenerative diseases. eEF1A2 is proposed to contribute protection against apoptotic cell death, likely through activation of the PI3K/Akt pathway. Akt is activated by various toxins and involved in cell survival and proliferation as well as exerting an anti-apoptotic effect. Previous evidence has also shown correlated expression of eEF1A2 with the PI3K/Akt/mTOR pathway in a cellular model of Parkinson's disease.

Methods: We studied the expression of eEF1A2 in both undifferentiated and differentiated NG108-15 after treatment with the neuronal toxin MPP+. To study the pro-survival role of eEF1A2 we also checked the expression of PI3K/Akt/mTOR both before and after RNAi knockdown of eEF1A2 by using quantitative real time PCR and western blot analysis.

Results: There was a trend towards an increase of the expression of eEF1A2 after treatment with MPP+ in both undifferentiated and differentiated cells, when compared with control. Meanwhile, the expression of PI3K/Akt/mTOR proteins were increased in both differentiated and undifferentiated NG108-15 cells treated with MPP+, especially for the phosphorylated Akt protein. After eFE1A2 knockdown, the expression of

PI3K/Akt/mTOR was significantly increased, but the ratio of p-Akt/total Akt was lesser than that of non eEF1A2 knockdown.
Conclusions: The present findings suggested that eEF1A2 may promote survival of NG108-15 cells through the PI3K/Akt/mTOR pathway by the enhanced expression of phosphorylated Akt in order to protect MPP+ neurotoxin induced cell death. Further investigation is required to confirm our findings, such as the expression of apoptosis and oxidative stress markers.

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Neuroprotection of indole-derivative compound NC001-8 in Parkinson's disease cell model by regulatory of NRF2 pathway

P.C. Wei (Tao-Yuan, Taiwan)
Objective: The goal of this study is to investigate the effects of indole-derivative compound NC001-8 on neuroprotection in PD disease cell model.
Background: Parkinson's disease (PD) is a common neurodegenerative disorder with selected loss of midbrain dopaminergic (DAergic) neurons. The lack of treatment to prevent the disease from progressing urges the development of new agents that may halt the rate of PD progression. Similar to other neurodegenerative diseases, there is increasing evidence that chaperones and oxidative stress participating to the pathogenesis of PD, while chaperone inducers and antioxidants may be relevant therapeutic strategies for PD. In previous study, we have found that indole derivative NC001-8, a Chinese herb, displayed prominent anti-oxidative and mitochondria biogenesis activities by up-regulatory of chaperones and/or autophagy in ataxia neurodegenerative cell models with ATXN3/Q75 or TBP/Q79 expression[1,2].
Methods: To identify the neuroprotection of NC001-8, we attempted to evaluate the chaperone and oxidative stress marker expressions, mitochondrial functions, cell survival, and apoptosis on DAergic cells derived from SH-SY5Y.
Results: NC001-8 showed lower cytotoxicity and no impact on the neural induction by 12-O-tetradecanoyl-phorbol-13-acetate (TPA). However, treatment of NC001-8 not only gave rise to a remarkable reduce of ROS level but also was shown to a better survival rate in PD cell model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Moreover, the activation of cleavaged Caspase 3, the downstream of apoptotic pathway, was diminished through the cause of NC001-8. Subsequently, we used in-house Q-PCR array to find that NC001-8 elevated both of NRF2 and NQO1 on RNA and protein levels. Furthermore, an addition of NC001-8 for knockdown of NRF2 in PD cell model significantly eliminated oxidative stress and raised up the cell viability.
Conclusions: Indole-derivative compound NC001-8 was capable of reducing ROS level and apoptotic activity in the PD cell model. The neuroprotective effect of NC001-8 was useful for maintaining cell survival. Therefore, these results might imply that the NC001-8 is able to develop a new therapeutics in treating PD as well.

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Physical and cognitive stimulation through environmental enrichment prevents early molecular pathology in a Parkinson´s disease model

Z. Wassouf, T. Hentrich, S. Samer, O. Riess, N. Casadei, J. Schulze-Hentrich (Tübingen, Germany)
Objective: Understand the molecular principles and identify genetic drivers underlying and mitigating the preventative effects of environmental enrichment on the unfolding of Parkinson´s disease (PD) pathology.
Background: While genomic mutations and multiplications have been linked to rare familial forms of PD, the preponderance of PD cases cannot be explained by genetics alone and seems to occur sporadically. Moreover, age as well as environmental factors correlate with onset and progression of the disease. This multi-factorial interplay suggests a highly complex pathomechanism that has remained largely enigmatic.
Methods: A transgenic mouse model over-expressing the human wildtype *SNCA* gene was generated for this study and showed early non-motor symptoms of PD. Both wildtype (WT) and transgenic (TG) animals were exposed to either a standard (SE) or enriched environment (EE), the latter modeled by enhancing social and cognitive complexity and stimulating physical activity over a period of 12 months. RNA-Seq was used to profile gene expression in hippocampal tissue of all four experimental groups in order to (i) identify genes and cellular processes disturbed through SNCA-overexpression and to (ii) reveal effects on gene activity induced through the EE.
Results: Differentially expressed genes in transgenic animals housed in SE pointed to disturbances in synaptic processes and several other cellular pathways linked to SNCA before. Intriguingly, transgenic animals housed in EE were largely protected from these disturbances in gene activity. Bioinformatic analyses revealed specific transcription factors, kinases, and phosphatases that likely drove the observed protection by activating an array of downstream targets that counterbalanced the influence of the SNCA transgene. Together with first epigenetic data,

we integrated these data to a system's view of environmental protection and its mechanisms along the gene-environment axis in PD.
Conclusions: Our study identifies candidate genes and suggests potential cascades of activation underlying the protective effect of environmental enrichment in PD. We consider these efforts to be highly valuable in assessing whether changes in life style can delay or ameliorate PD symptoms in human, and whether these candidate genes offer opportunities to mimic the environmental influence towards much-needed novel therapies of PD.

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The therapeutic effect of MANF in the MPTP/MPP+-induced model of Parkinson's disease

Y. Liu (Shanghai City, People's Republic of China)

Objective: To observe therapeutic effect of MANF in the attenuation of neurotoxin MPTP/MPP+-induced model of Parkinson's disease
Background: Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a novel neurotrophic factors and had proved that MANF has neuroprotective and neurorestorative effect.
Methods: 1. Male C57BL/6 mice were treated with MPTP intraperitoneal injection, MPTP-induced mice were randomly divided. mice were injected bilaterally into striatum with MANF (10 μg/side) or phosphate buffered saline 2 μl/side. 2. Rotarod test was used to record the latency on the rotor. Immunohistochemistry was used to detect the tyrosine hydroxylase (TH) positive cells in SNpc. Dopamine (DA) and its metabolites 3,4-dihydroxyphenylaceticacid (DOPAC) and homovanillic acid (HVA) were determined by high performance liquid chromatography (HPLC) analysis. Superoxide dismutase (SOD), Glutathione (GSH), and Malondialdehyde (MDA) in substantial nigra were evaluated using assay kits. 3. A cell model of PD was structured by incubating SH-SY5Y cells with MPP+, cells were pretreated with different concentrations of MANF before incubation in medium containing MPP+, then the cell viability was measured by MTT assay. JC-1 was used to detect mitochondrial transmembrane potential changes in SH-SY5Y cells under a flow cytometry.
Results: 1. The latency reduction caused by MPTP injection were partially rescued in the MANF-treatment group relative to PBS-treatment group after two weeks. 2. MANF significantly reduced the number of TH-positive dopaminergic neurons loss in MANF-treatment group relative to PBS-treatment group in the SNpc. 3.Mice that received MANF injection had signi?cantly higher striatal DA, DOPAC and HVA levels than mice that received PBS injection. 4.Compared with PBS-treatment group, MANF-treatment group increased the activity of SOD and the yield of GSH, and decreased the yield of MDA. 5. MANF for 2 h followed by 2mM MPP+ for 24 h significantly decreased MPP+-induced loss of cell viability . MANF alone did not cause a significant affect in cell viability in SH-SY5Y cells after 24 h treatment. 6. Pretreatment with MANF reversed MPP+-induced mitochondrial membrane potential loss.
Conclusions: MANF can attenuate the neuronal lesion in MPTP/MPP+-induced model of Parkinson's disease; MANF may play a role in improving mitochondrial function and inhibiting oxidative stress.

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Alteration of early endosomal trafficking causes neurodegeneration in PARK20

C. Criscuolo, D. Fasano, V. Coppola, G. Amodio, M. Picillo, A. De Rosa, V. Bonifati, P. Barone, M. Pellecchia, G. De Michele, P. Remondelli, S. Paladino (Naples, Italy)

Objective: To elucidate the molecular basis of the Park20 by evaluating the role of synaptojanin 1 in intracellular trafficking.
Background: Recently a new form of autosomic recessive parkinsonism (PARK20) has been reported due to mutation in *SYNJ1*. This gene encodes for synaptojanin 1, a phosphoinositide phosphatase, involved in recycling of synaptic vesicles in neurons.
Methods: We assessed the effects of *SYNJ1* knockdown on the endocytic pathway evaluating the morphology and function of endosomal compartments through quantitative confocal analysis and internalization assays. We carried out the study in human neural (SH-SY5Y) and non-neural (HeLa) cells and in fibroblasts (from skin biopsies) of patients with homozygous *SYNJ1* missense mutation1,2.
Results: We observed that the loss of Synj1 causes a drastic alteration of the early endosomes labeled with EEA1 antibody, which result expanded and more numerous; while it does not affect the morphology of late endosomes labelled with Rab7 antibody both in HeLa and SH-SY5Y cells. In contrast, the morphology of exocytic organells is comparable to wild-type cells. We observed a slight effect on lysosomal compartments stained with a fluorescent dye or with Lamp1 antibody. Through endocytosis assays we analyzed the trafficking of two receptors (transferrin and EGF), which, once internalized, follow different routes: TfR recycles back to the cell surface, while EGFR

move to late endosomes and undergo lysosomal degradation. Strikingly, in Synj1-depleted cells the TFR trafficking is inhibited with progressive accumulation of transferrin inside the cells. In contrast, we observed no difference in the kinetic of internalization of EGFR in scrambled and silenced cells. Moreover, we found the same alterations in patient fibroblasts: early endosomal compartments result enlarged and the recycling of transferrin is impaired.
Conclusions: Our data indicate that the synaptojanin 1 plays a crucial role in regulating the homeostasis and functions of early endosomal compartments in neural and non-neural cells alterating in particular the recycling pathways from early endosomes. Therefore, we suggest that endosomal pathways dysfunction might underlie neurodegeneration in PARK20.

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The identification of molecular-genetic background of familial atypical parkinsonism in "Hornacko", a specific region of the south-eastern Moravia, Czech Republic.

K. Mensikova, R. Vodicka, K. Kolarikova, T. Bartonikova, P. Kanovsky (Olomouc, Czech Republic)

Objective: To assess the genetic background in the pedigrees with identified autosomal-dominant inheritance of parkinsonism.
Background: Increased prevalence of parkinsonism with cognitive deterioration was detected in a small region of south-eastern Czech Republic. Three large pedigrees with probable autosomal-dominant inheritance patterns of parkinsonism were identified.
Methods: Molecular genetic examinations were performed in 12 clinically positive probands. Coding sequences, exon/intron regions and 5´/3´UTR sequences of *ADH1C, ATP13A3, EIF4G1, FBXO7, GBA+GBAP1, GIGYF2, HTRA2, LRRK2, MAPT, PARK2, PARK7, PINK1, PLA2G6, SNCA, UCHL1*, and *VPS35*genes were tested with a massive parallel sequencing method using Ion Torrent technology and confirmed by Sanger sequencing. In total, 93 % of gene sequences were covered. Variants were filtered using parameter MAF?0.01. SIFT; PolyPhen-2 prediction software was used for missense variants evaluation. PhyloP algorithm was used to assess the phylogenetical conservation.
Results: 31 rare heterozygous variants have been identified. The most interesting variants (PhyloP score =2 and/or missense variants) included: one variant located in coding sequence - c.143C>T in *ADH1C* gene, one variant in coding sequence - c.689A>G in *MAPT* gene, one variant in UTR-3 region sequence - c.*77G>T in *SNCA* gene, one variant in exon - c.1180C>T in *PARK2* gene, one variant in coding sequence - c.344A>T in *PINK1* gene, three exon variants - c.2167A>G, c.6241A>G, c.4541G>A in *LRRK2* gene, one variant in noncoding sequence - c.*1593G>A and one in coding sequence - c.3662C>T in *GIGYF2* gene, one exon variant - c.1027C>T in *PLA2G6* gene and one variant in noncoding - c.-109G>A and one in coding sequence - c.3706C>G in *EIF4G1* gene.
Conclusions: It seems that several molecular-genetic factors play role in the genetic background of this newly detected atypical familial neurodegenerative parkinsonism.

Supported by AZV- Ministry of Health of the Czech Republic grant Nr. 15-32715A and the MH CZ – DRO (FNOL 00098892) – 2016.

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Genetic, epigenetic and expression profiles in alpha-synucleinopathies

E. Scott, I. Guella, A. Rajput, A. Rajput, L. Parkkinen, M. Kobor, M. Farrer (Vancouver, BC, Canada)

Objective: To determine whether each of the alpha-synucleinopathies has distinct methylation and/or expression profiles that distinguishes them from other disorders in this class.
Background: The alpha-synucleinopathies, encompassing Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), are adult-onset neurodegenerative disorders characterized by *post-mortem* alpha-synuclein (*SNCA*) aggregate pathology. The clinical features and progression of these disorders, and the brain regions affected, are distinct. *SNCA* genetic variability has been insightful in monogenic and idiopathic PD but our understanding of gene/protein expression in disease pathogenesis remains limited. How aggregated alpha-synuclein pathology is propagated and why specific cell types are more vulnerable remains enigmatic. Nevertheless, endogenous alpha-synuclein expression modulates the induction of Lewy-like pathology. Genetic variability influences epigenetic marks and allele-specific expression in human induced pluripotent stem cells and differentiated neurons[1]. Concomitantly, we have shown that an expanded *SNCA* ($TTTC_n$) repeat is associated with dementia in PD[2]. Hence, we hypothesize genetic, epigenetic and expression changes in *SNCA* influence the distribution and burden of neuropathology in distinct alpha-synucleinopathies.

Methods: Using striatal (affected in PD and MSA-P), cerebellar (affected in MSA-C), entorhinal cortex (affected in DLB) and occipital cortex (unaffected) tissue from brains of clinically- and pathologically-confirmed PD, DLB and MSA along with unaffected control subjects (n=32, 8/group), we pilot genome-wide SNP, methylation and expression analyses. Brain regions are powdered for correlative DNA, RNA and protein extraction. Ampliseq Transcriptome Ion Proton sequencing enables differential gene expression analyses between tissues, patients and control subjects, adjusting for SNPs while considering the burden of inclusion pathology.
Results: We will present a comparison of gene expression profiles of the three alpha-synucleinopathies. Future studies will also assess genetic variability and methylation to determine whether there are distinct profiles for each alpha-synucleinopathy.
Conclusions: The study is to provide insight into the propagation of alpha-synucleinopathies and inform the development of novel therapeutics.

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Association analyses of three susceptibility loci for Alzheimer's disease in Parkinson's disease, amyotrophic lateral sclerosis, and multiple system atrophy

Y. Chen, R. Ou, X. Chen, B. Cao, Q. Wei, B. Zhao, Y. Wu, H. Shang (Chengdu, People's Republic of China)
Objective: Considering the overlapping of clinical manifestation and pathologic characteristics of Alzheimer's disease (AD) and Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and multiple system atrophy (MSA), we conducted a large-sample study to investigate the associations between these variants and other three common neurodegenerative diseases (PD, ALS, MSA) in a Chinese population.
Background: A number of genetic variants were identified to be associated with the risk for AD, including rs10838725 in the *CELF1*, rs28834970 in the *PTK2B*, rs17125944 in the *FERMT2* and rs1041544 in *SIRT2* based on the genome-wide association studies (GWAS) data.
Methods: A total of 2449 patients, including 1219 PD, 870 SALS, and 360 MSA patients, and 821 healthy controls (HCs) were examined in the current study. All cases were genotyped for SNPs using Sequenom iPLEX Assay technology.
Results: The genotype distributions of rs28834970 in the *PTK2B* were significant different between ALS patients and HCs, the minor allele "C" carriers have an increased risk of ALS (OR=1.26); Interestingly, the minor allele "C" of this variant increased the risk for abnormal cognitive function in PD patients(OR=1.84). In addition, the minor allele frequency of rs10838725 in *CELF1* was significant high in MSA than that in HCs, the homozygous "CC" carriers have increased the risk for MSA than the homozygous "TT" carriers(OR=1.70). However, no significant differences in the genotype distributions and minor allele frequency(MAF) of rs17125944 in the *FERMT2* and rs1041544 in *SIRT2* were found between PD, ALS, or MSA patients and HCs.
Conclusions: This study provided new clue that these four neurodegenerative diseases shared some of common pathogenesis.

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Expression of OX40 and OX40 serum level in patients with Parkinson's disease

F. Alsahebfosoul, A. SeyedJoodaki, N. Eskandari, V. Shaygannejad, M. Salehi, M. Kazemi, M.T. Kardi, O. Mirmossayeb (Isfahan, Iran)
Objective: Study the expression of OX40 gene and its serum levels could be measured to predict and confirm diagnosis of Parkinson's disease and help develop new treatment and prevention approaches through suppression of this interaction.
Background: Parkinson's disease is a common neurodegenerative disease that mainly affects central nervous system (CNS) and consequently motor system. Inflammation of immune system and CNS has been known as an important predisposing factor for Parkinson's disease. OX40 protein (CD134) is from family of tumor necrosis receptors that acts on T cells surface. Increased expression of this protein has been known as a factor for increase in inflammation and initiation of NF-kappa B signaling pathway in different diseases. This study investigates OX40 gene expression in mRNA and serum levels.
Methods: Twenty people with Parkinson's disease and 20 healthy people, as controls, were enrolled in the study. Measurement of OX40 gene expression was conducted by real-time PCR and serum protein level measured by enzyme-linked immunosorbent assay.
Results: The mean expression rate of OX40 gene in the patients increased compared to the controls yet *in*significantly ($p>0.05$). The mean serum concentration of OX40 protein increased in the patients yet *in*significantly compared to the controls ($p>0.05$).

Conclusions: The expression of this protein could be measured to predict and confirm diagnosis of Parkinson's disease and help develop new treatment and prevention approaches through suppression of this interaction. However, additional clinical, cellular, and interventional studies should be conducted to confirm the treatment approaches.

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Genetic and pharmacological rescue of DJ-1 loss-of-function caused by a c.192G>C mutation in PARK7

I. Boussaad, C. Obermaier, Z. Hanss, N. Weisschuh, B. Schmid, S. Hoffmann, L. Burbulla, C. Klein, S. Duga, D. Krainc, T.G. Gasser, B. Wissinger, R. Krüger (Esch-sur-Alzette, Luxembourg)

Objective: In this study we investigate the cellular mechanism underlying the Parkinson's disease (PD)-associated mutation c.192G>C in PARK7 and present a compound treatment that rescues the cellular phenotype in a patient-based cell model.

Background: Homozygous loss-of-function mutations in the DJ-1 gene PARK7 cause a rare form of inherited early-onset Parkinson's disease (PD). DJ-1 covers a wide range of biological functions. It acts as sensor of oxidative stress, as chaperone, glyoxylase and as transcriptional regulator. Patient-derived cellular models harboring the homozygous c.192G>C mutation display specific cellular phenotypes due to loss of function of DJ-1. This mutation was predicted to cause an E64D amino acid change, however, using patient-based material we show a different mechanism leading to loss of DJ-1.

Methods: Patient's fibroblasts were obtained from skin biopsy and reprogrammed into induced pluripotent stem cells (iPSCs). Fibroblasts and iPSC-derived neurons, were used to study the effect of the mutation and to identify compounds that rescue cellular phenotypes.

Results: We identified the c.192G>C mutation to cause mis-splicing of DJ-1 pre-mRNA instead of an amino acid change. We identified impaired U1 mediated recognition of the splice-donor site at exon 3 of PARK7 leading to skipping of exon 3 (?Ex3). Although the levels of ?Ex3 mRNA in patient cells are comparable to wild-type DJ-1 mRNA in control cells, DJ-1 protein levels in patient cells are dramatically reduced. Genetic intervention restored DJ-1 protein levels only when cells were transduced with full-length DJ-1 vectors. Translation of full-length mutant DJ-1 could be rescued when patient cells were transduced with genetically engineered U1 snRNA in patient cells. Moreover, we identified a combination of two compounds that rescues mis-splicing of DJ-1 mRNA and cellular function in patient-derived cell models.

Conclusions: In contrast to current notion we have discovered that the c.192G>C mutation in PARK7 does not cause an E>D missense mutation but a loss of protein due to exon skipping and provide strategies for genetic and pharmacological rescue. Treatment with a combination of two compounds rescues correct splicing of mutant DJ-1 mRNA as well as cellular function in patient cells.

1054

Full sequencing and GWAS markers analysis of SNCA in RBD and progression to synucleinopathies.

Z. Gan-Or, J. Ruskey, I. Arnulf, Y. Dauvilliers, B. Hogl, A. Stefani, C. Charley Monaca, P. Dion, A. Desautels, J.-F. Gagnon, N. Dupre, R. Postuma, J. Montplaisir, G. Rouleau (Montreal, QC, Canada)

Objective: To study the role of *SNCA* variants in REM sleep Behavior Disorder (RBD) and progression to synucleinopathies.

Background: Individuals with properly diagnosed idiopathic RBD will progress to a synucleinopathy within 10-12 years in average. Coding variants in *SNCA* may cause familial PD or DLB, and non-coding variants are associated with risk for PD and DLB.

Methods: A total of 351 individuals with RBD, 525 individuals with PD and 691 controls were studied. Data on *SNCA* variants was produced using the NeuroX SNP chip (Illumina). The entire coding sequence of *SNCA* was captured and sequenced using molecular inversion probes (MIPs).

Results: The strongest *SNCA* SNPs associated with RBD were at the 5' of the gene (strongest marker rs2737014, p=2.9xE-3). In PD they were around the 3' (rs356182, p=3.9E-4). In PD, no point mutations were identified, but two controls carried the p.N122T variant (predicted to be benign). In the 3' UTR region of *SNCA*, rs3857053 and rs1045722 were in full Linkage Disequilibrium. and were associated with an increased risk for PD (allele frequency in PD and controls 0.081 and 0.059, respectively, OR 1.39, 95% CI 1.02-1.91, p=0.03), consistent with its effect in PDgene (OR 1.34, p=4.64E-25). This association did not exist in RBD. There was a trend towards a faster progression from RBD to synucleinopathies in carriers of the rs2245804 SNP in the 5' UTR of *SNCA*. This SNP was nominally associated with both AAO of RBD (p=0.048) and progression rate (p=0.015). The occurrence of probable RBD in a subset of our PD cohort with available data (n=260) was associated with both rs1045722 and rs2301135.

Carriage of either one or two alleles (dominant model) of the rs1045722 SNP was associated with an OR of 2.78 for probable RBD among PD patients (95%CI 1.35 – 5.75, p=0.005). Homozygous carriage of the rs2301135 SNP (recessive model) was associated with an OR of 1.95 for probable RBD among PD patients (95% CI 1.004 – 3.78, p=0.047).
Conclusions: *SNCA* variants are associated with both RBD and PD, but in PD they are concentrated around the 3' of the gene, and in RBD they are at the 5'. This may suggest that RBD is genetically more similar to DLB, in which the associated markers are also at the 5' of *SNCA*. The potential associations with rate of progression from RBD to PD, with AAO of RBD and with probable RBD in the PD cohort, need to be replicated in additional cohorts.

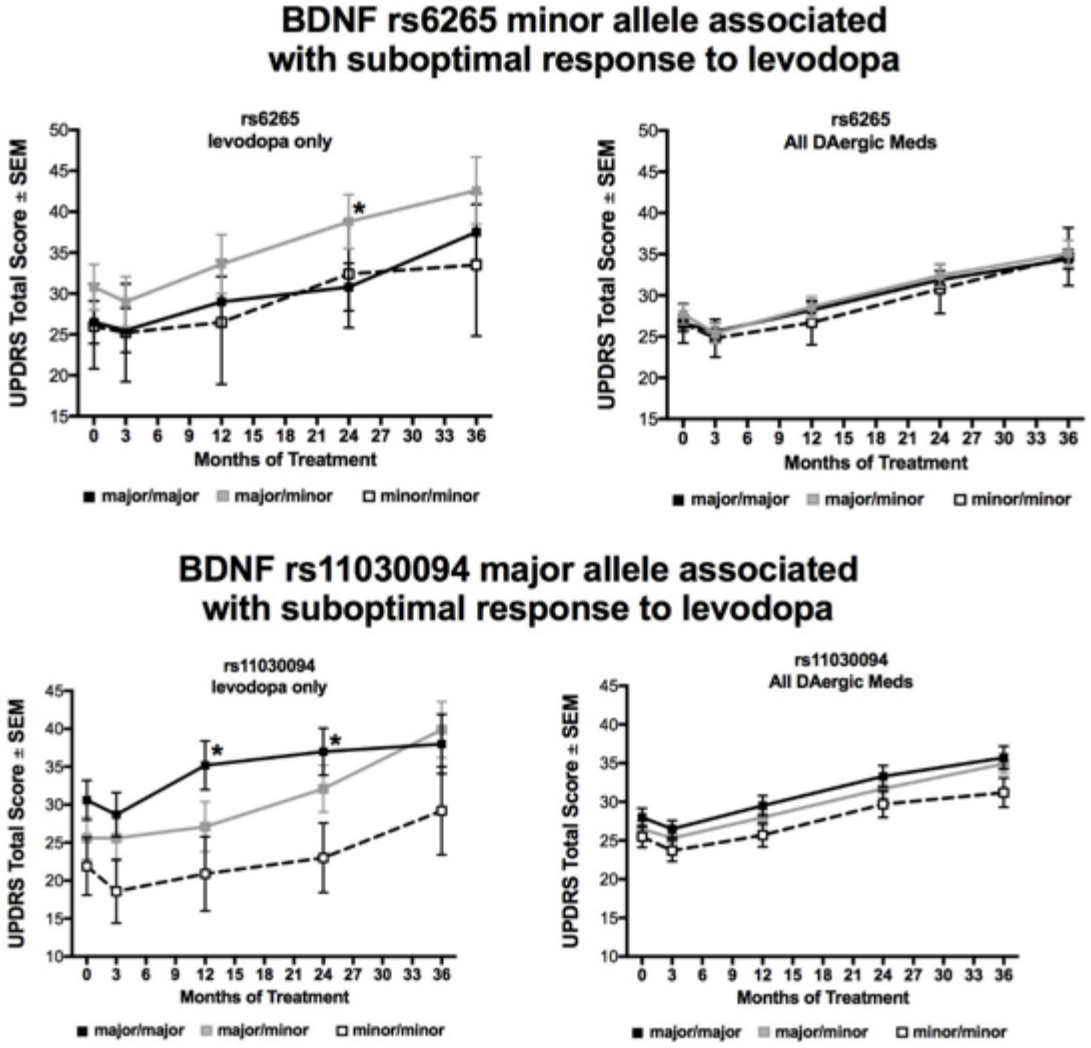
1059

Genome-wide DNA methylation analysis reveals epigenetic perturbations in Parkinson's disease
J. Young, S. Sivasankaran, L. Wang, A. Ali, A. Mehta, D. Davis, K. Belle, D. Dykxhoorn, C. Petito, G. Beecham, E. Martin, D. Mash, W. Scott, J. Vance (Miami, FL, USA)
Objective: Several environmental exposures are known to affect the risk for PD. Because DNA methylation is affected by environmental variables, we investigated whether brain region-specific DNA methylation patterns differ between PD cases and controls.
Background: Genetic variations have been associated with Parkinson's disease (PD), but only explain part of the disease risk. Studies have shown that there are many environmental exposures that modify the risk of PD. As DNA methylation is one the known mechanisms through which environment can influence biological process, we investigated whether methylation differences exist between PD and controls. Because DNA methylation profiles vary between brain regions, we investigated areas of the brain relevant for PD. These included the dorsal motor nucleus of the vagus (DMV), the substantia nigra (SN) and the cingulate gyrus (CG).
Methods: We investigated the association of DNA methylation patterns with PD by performing a genome-wide screen of DNA methylation on the DMV, SN and CG in 12 autopsy-confirmed PD and 11 autopsy-confirmed controls. We used Illumina 450K beadchips to interrogate gene promoters and CpG islands.
Results: We found 9,621 CpG sites that were differentially methylated between PD and controls at false discovery rate (FDR) of <0.05 and absolute differential methylation (|?M|) of = 1.5 fold in the DMV, with none and 1 CpG site differentially methylated in the SN and CG, respectively. The methylation status of neighbor CpG sites has been shown to be highly correlated, leading to the identification of differentially methylated regions (DMRs), which are less subject to identification artifacts than single CpG site methylation. We identified 1,775 DMRs in the DMV (FDR<0.05), including some in genes previously reported as associated to PD, like *MAPT*. A combined analysis of both methylation and RNA-seq data revealed 90 genes with significant DMRs that were also significantly differentially expressed. Pathway analysis of these genes showed significant enrichment for synaptic or neurotransmitter function (P value < 0.001).
Conclusions: Our data suggest that an epigenetic-load mechanism affecting genes involved in neurotransmission and synaptic pathways is a likely factor in neurodegenerative processes of PD.

1060

Specific Bdnf variants are associated with suboptimal response to levodopa but not to other dopaminergic medications or deep brain stimulation in Parkinson's disease
C. Sortwell, P. Auinger, J. Goudreau, B. Pickut, B. Berryhill, M. Hacker, D. Charlesdavid.charles@Vanderbilt.Edu, J. Lipton, A. Cole-Strauss, J. Elm, D. Fischer (Grand Rapids, MI, USA)
Objective: We examined the impact of rs6265 and other brain-derived neurotrophic factor (*Bdnf*) variants in two subject cohorts: 1) early-stage Parkinson's disease (PD) subjects from the NIH NINDS Exploratory Trials in PD Long-term Study-1 (NET-PD LS-1) treated with either levodopa alone (l-dopa, n=56) or any combination of dopaminergic medications (DA meds, n=540), and 2) 15 mid/late-stage PD subjects treated with subthalamic nucleus deep brain stimulation (STN DBS).
Background: The ability to use patient genotype to design optimal antiparkinsonian treatment strategies would be a powerful approach. BDNF is a critical modulator of neurotransmission and plasticity in the basal ganglia. Preclinical work has implicated BDNF signaling in the antiparkinsonian efficacy of both levodopa and deep brain stimulation (DBS). Several single nucleotide variants exist in the *Bdnf* gene, one in particular (rs6265) results in reduced activity-dependent BDNF release. Previously we showed that early-stage PD subjects carrying the rs6265 minor allele exhibit a less robust response to l-dopa therapy, whereas rs6265 variant status did not alter patient response to STN DBS.

Methods: Subjects were genotyped for six *Bdnf* variants, including rs6265. Response to therapy was assessed by UPDRS scores at various visits over 36 months. Mean UPDRS scores in NET-PD LS-1 were stratified by variant allele status and adjusted for site, age, race, PD duration, therapy dose, and treatment group.
Results: In l-dopa treated subjects, rs6265 minor allele carriers and rs11030094 major allele carriers exhibited higher mean total UPDRS scores compared to subjects without the risk allele at 24 months (rs6265: 37.6 vs. 30.7, p=0.04; re11030094: 35.0 vs. 23.8, p=0.01 compared to minor/minor). Neither allele was associated with an altered response to DA meds or STN DBS. Possession of multiple BDNF variant risk alleles was associated with a significantly worse therapeutic response to l-dopa (mean total UPDRS at 24 months for 0-1, 2-3, 3-6 risk alleles=21.0 , 31.7, 38.8, respectively, p=0.001), but not DA meds.



Conclusions: Two of the six risk alleles for *Bdnf* variants were associated with a worsened therapeutic response to levodopa. Genotyping for specific *Bdnf* variants may be a useful precision medicine approach to identify optimal antiparkinsonian treatment strategies.

1061

Parkinson's disease GWAS risk loci and symptom progression

K. Paul, J. Schulz, J. Bronstein, C. Lill, B. Ritz (Los Angeles, CA, USA)

Objective: To assess whether GWAS identified Parkinson's disease (PD) risk loci also influence symptom progression among patients.
Background: Genetic factors have a considerable influence on PD susceptibility. The largest genome-wide association study identified 26 independent single-nucleotide polymorphisms (SNPs) associated with PD risk.

Among patients, the course and severity of symptom progression is variable, and little is known about the potential impact of these genetic factors on phenotypic variance.
Methods: We genotyped 23 SNPs in our longitudinal cohort of 246 incident PD patients, followed on average over 5 years and 7.5 years into disease. Movement disorder specialists repeatedly assessed PD symptom progression. The combined impact of PD risk loci on fast motor symptom progression and cognitive decline was assessed by logistic regression analyses using a weighted genetic risk score.
Results: The weighted genetic risk score was significantly associated with fast motor symptom decline, defined as those in the top quartile of UPDRS-III change over follow-up (OR=1.47, 95% CI=1.02, 2.12). Furthermore, the risk score was associated with fast cognitive decline, those in the top quartile of change in MMSE score (OR=1.43, 95% CI=1.01, 2.03).
Conclusions: Overall, our study indicates that a cumulative genetic risk, based on PD susceptibility SNPs, is associated with faster motor and cognitive symptom decline.

1063

Alterations in lipid metabolism modify GBA1-mediated neurodegeneration in a Drosophila model of Parkinson's disease

M. Davis, R. Thomas, A. Germanos, S. Yu, B. Whitley, L. Pallanck (Seattle, WA, USA)
Objective: To understand how glucocerebrosidase (*GBA1*) mutations increase susceptibility to Parkinson's disease (PD).
Background: Our understanding of the pathogenesis PD remains limited, and currently no disease-modifying therapies exist. Mutations in *GBA1* are the strongest genetic risk factor for PD, and *GBA1* encodes glucocerebrosidase, an important enzyme in lipid metabolism. However, most *GBA1* carriers do not develop PD, suggesting the presence of modifiers. To investigate how *GBA1* influences PD pathogenesis, we created a *Drosophila* model of *GBA1* deficiency ($GBA1^{del}$) that has neurodegeneration and impaired lysosomal protein degradation (Davis, et al.).
Methods: A pilot screen using our $GBA1^{del}$ model was conducted to identify genetic modifiers. Chromosomal deletions were screened for suppression or enhancement of the climbing deficit present in $GBA1^{del}$ homozygotes compared to controls. The modifier locus was identified by narrowing candidate regions using publicly available smaller overlapping deletions and mutated alleles. Modifiers were further characterized by enhancement/suppression of other $GBA1^{del}$ phenotypes, including impairment in autophagy. Targeted lipidomic analysis was used to evaluate alterations in lipid metabolism due to genetic perturbations of modifiers in $GBA1^{del}$ mutants.
Results: Eleven chromosomal deletions were identified as modifiers. Glucosylceramide transferase (*GlcT-1*), which encodes an enzyme with the reverse enzymatic activity to GBA1, was identified as a modifying locus. A publicly available mutation of *GlcT-1* suppressed accelerated insoluble protein aggregation in $GBA1^{del}$ homozygotes. Ectopic expression of *GlcT-1* enhanced the climbing deficit, increased insoluble ubiquitinated protein aggregation, and shortened lifespan of $GBA1^{del}$ homozygotes.
Conclusions: *GlcT-1* is a modifier of *GBA1*-mediated pathogenesis in *Drosophila*. Loss of function of *GlcT-1* suppresses *GBA1* mutant phenotypes, and is predicted to increase ceramide and decrease glucosylceramide levels, suggesting that decreased levels of ceramide and/or increased levels of glucosylceramide may lead to neurodegeneration in $GBA1^{del}$ homozygotes. Further studies characterizing modifiers, including targeted lipidomics, will elucidate whether alterations in lipid metabolite levels are responsible for the pathogenic mechanisms causing PD and may reveal new targets for disease-modifying therapies in PD.

1068

Pure ATXN10 repeat expansion causes Parkinson's disease

F. Jimenez Gil, K. McFarland, K. Lee, Y.-C. Tsai, C. Byrne, R. Gopi, N. Huang, J. Langston, T. Clark, T. Ashizawa, B. Schuele (Guadalajara, Mexico)
Objective: Clinical and genetic characterization of a multigenerational family with spinocerebellar ataxia type 10 (SCA10) and parkinsonism.
Background: Pentanucleotide repeat expansions of ATTCT in intron 9 of the *ATXN10* gene typically cause a distinct clinical phenotype of progressive spinocerebellar ataxia with or without seizures and present neuropathologically with Purkinje cell loss resulting in symmetrical cerebellar atrophy.
Methods: We clinically characterized several affected and unaffected family members of a large 4-generation Mexican kindred with 28 affected family members with *ATXN10* expansions using standardized clinical assessment tools. Furthermore, to fully understand the genetic architecture of the *ATXN10* repeat expansion, we used a novel

technology combining single molecule real time (SMRT) sequencing and CRISPR/Cas9-based capture method, and sequenced the entire span of ~5.1kb-6.5kb repeat expansions as one continuous fragment.
Results: Four of the affected family members examined showed clinical features of progressive ataxia and seizures with cognitive and psychological changes including dementia. However, one affected individual presented with early-onset L-Dopa responsive parkinsonism and no signs of ataxia, and one sibling was clinically unaffected indicating reduced penetrance or delayed onset for this allele. Interestingly, no repeat interruptions were detected in the patient presenting with Parkinson's disease and his sister with reduced or delayed penetrance. However, in the siblings with typical ataxia, we found *ATXN10* repeat interruptions which have not been associated with seizures previously.
Conclusions: This is the first reported case with clinically typical L-dopa responsive parkinsonism and an *ATXN10* repeat expansion. We propose that the absence of repeat interruptions is responsible for the clinical presentation of Parkinson's disease. It will be important to understand the underlying genetic and molecular differences that lead to the changes in the neurodegenerative process in this family with different clinical and presumably neuropathological phenotypes.

1156

Genotype-Phenotype correlations and expansion of the molecular spectrum of AP4M1-related Hereditary Spastic Paraplegia

S. Efthymiou, C. Bettencourt, V. Salpietro Damiano, H. Houlden (London, United Kingdom)

Objective: To identify possible novel variants in a HSP family from Greece.
Background: Autosomal recessive hereditary spastic paraplegia (HSP) due to *AP4M1* mutations is a very rare neurodevelopmental disorder with a few reported patients presenting the combination of infantile hypotonia, severe intellectual disability, progressive hypertonia and spasticity, coupled with variable cerebellar involvement and white matter loss on brain magnetic resonance imaging (MRI).
Methods: We investigated a Greek family with three siblings affected with a phenotype characterized by the combination of: (a) febrile seizures with onset in the first year of life (followed by epileptic non-febrile seizures); (b) distinctive facial appearance (e.g., coarse features, bulbous nose and hypomimia); (c) developmental delay and intellectual disability; (d) early-onset spastic weakness of the lower limbs; (e) cerebellar hypoplasia/atrophy on brain MRI.
Results: Genetic analysis of the family using whole exome sequencing (WES) identified a novel compound heterozygous mutation of the *AP4M1*A gene segregating with the phenotype in all the three probands (c.521dupT, p.V174fs and c.T955C, p.C319R). The mutation was confirmed by sanger sequencing and the unaffected parents were found to be carriers of the variants.
Conclusions: We reported on a Greek family with a phenotype of early-onset epilepsy, developmental delay and progressive spastic paraplegia, due to a previously unreported *AP4M1* mutation. The *AP4M1* gene encodes a subunit of the heterotetrameric adaptor protein (AP) complex that mediates vesicle trafficking of glutamate receptors between the trans-Golgi network (TGN) to the synaptic membrane, and thereby contribute to regulate brain development and neurotransmission.

1162

Stimulation in the nucleus entopeduncularis affects neuronal activity in the nucleus accumbens and medial prefrontal cortex after apomorphine-induced deficient prepulse inhibition in rats

K. Schwabe, J. Krauss, M. Alam (Hannover, Germany)

Objective: The aim of our study was to investigate the effects of stimulation in the rat entopeduncular nucleus (EPN) on single neuronal activity of the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC) and coherence of oscillatory activity with sensorimotor cortex.
Background: Deficient sensorimotor gating induced by dopamine receptor agonists is used as an endophenotype for certain neuropsychiatric disorders, such as Tourette´s syndrome. Deep brain stimulation (DBS) of the globus pallidus internus (GPi) is experimentally used to alleviate tics in Tourette`s syndrome. One operational measure of sensorimotor gating is prepulse inhibition (PPI) of the acoustic startle response (ASR). We recently showed that DBS of the rat EPN (the equivalent to the human GPi) alleviates an apomorphine induced PPI deficit.
Methods: Neuronal recordings were carried out in urethane anesthetized (1.4 g/kg, i.p.) male Sprague-Dawley rats. A concentric bipolar electrode for stimulation was stereotaxically implanted in the EPN. Single neuronal recordings were acquired from the mPFC and NAC before and after apomorphine injection (1mg/kg BW). Thereafter, 60 sec

EPN stimulation (130 Hz, 100 μA current, with 120 μs biphasic square wave pulses) was applied and the neuronal activity recorded.
Results: Neuronal firing rate was not affected by apomorphine injection in both regions, but enhanced after stimulation in the NAC. Measures of irregularity were enhanced after apomorphine injection in both regions. Stimulation normalized this measure in the NAC, but had no effect in the mPFC. Coherence of oscillatory theta (4-8 Hz) and alpha (8-12 Hz) band activity between the mPFC and NAC local field potentials and sensory motor cortical field potentials was enhanced after apomorphine injection. EPN stimulation reduced theta and alpha coherence in the NAC, while in the PFC only alpha activity was reduced.
Conclusions: These investigations shed new light on the effect of DBS on disturbed neuronal network activity in an animal model with sensorimotor gating deficit, which may be used to understand and improve this experimental therapy in neuropsychiatric disorders.

(Data have been presented at the ESSFN 2016 in Madrid)

1169

Basal ganglia circuits for motor and behavioral, emotional performances
F. Fujiyama (Kyotanabe, Japan)
Objective: The present study aimed to identify which neurons in the basal ganglia (BG) target dopaminergic or GABAergic neurons in SN pars compacta (SNc) or reticulata (SNr), respectively.
Background: Electrophysiological studies in monkeys have shown that dopaminergic neurons respond to the reward prediction error. In addition, striatal neurons alter their responsiveness to cortical or thalamic inputs in response to the dopamine signal, via the mechanism of dopamine-regulated synaptic plasticity. These findings have led to the hypothesis that the striatum exhibits synaptic plasticity under the influence of the reward prediction error and conduct reinforcement learning throughout the basal ganglia circuits.
Methods: In the present study, we generated a novel parvalbumin (PV)-Cre rat model and conducted a detailed morphological and electrophysiological investigation of axons from PV-globus pallidus (GP). For the striatofugal pathways, we performed the single neuron tracing using the recombinant virus.
Results: We initially found that 1) 57% of PV neurons co-expressed Lim-homeobox 6, 2) the PV-GP terminals were preferentially distributed in the ventral part of dorsal tier of SNc, 3) PV-GP neurons formed basket-like appositions with the somata of tyrosine hydroxylase, PV, calretinin and cholecystokinin immunoreactive neurons in the SN, and 4) in vitro whole cell recording during optogenetic photo-stimulation of PV-GP terminals in SNc demonstrated that PV-GP neurons strongly inhibited dopamine neurons via GABAA receptors. For the striatofugal pathways, we found that the neurons in the striosome compartment, but not matrix compartment, projected to SNc.
Conclusions: These results suggest that dopamine neurons receive direct focal inputs from PV-GP prototypic neurons. On the other hand, striatum-SNc inhibition originates from direct pathway MSNs that express the D1 receptor, whereas the D2, D3, D4, and D5 receptors are expressed in the perikarya and dendrites of GP neurons . Therefore, these pathways might exert different modes of dopaminergic modulation; and their timing, plasticity, and functional significance will be investigated further in future.

1171

MitoQ and reduced glutathione protects against dopamine induced brain mitochondrial electron transport chain inhibition during extended in vitro incubation: Involvement of free radicals and quinone products
A. Maiti (Bengaluru, India)
Objective: To study the impact of MitoQ and GSH against DA induced rat brain mitochondrial electron transport chain inhibition during extended *in vitro* incubation.
Background: Studies on mitochondrial functions following extended exposure to dopamine (DA) *in vitro* have produced variable results. Our previous study suggests the predominant involvement of quinone products in long-term DA-mediated inactivation of complex I and IV(1). Reduced glutathione (GSH), a quinone scavenger, almost completely abolished the DA effect on mitochondrial complex I and IV activities, but was not prevented by catalase or metal-chelator diethylenetriaminepentaacetic acid (DTPA) or the hydroxyl radical scavengers like mannitol and dimethyl sulphoxide (DMSO) indicating the non-involvement of •OH radicals and Fenton's chemistry in this process. In our current *in vitro* study, MitoQ, a mitochondria specific antioxidant (known for peroxynitrite and superoxide scavenging) has been included for the first time as there exists no previous data on such study.
Methods: Isolated rat brain mitochondria suspended in 50mM phosphate buffer, pH 7.4 (200μl containing 200μg protein) were incubated with varying concentrations of DA (100–400μM) for up to 2 hours with or without

additions such as reduced glutathione (5mM), MitoQ (50nM), catalase (50µg/ml), mannitol (20mM), DMSO (20mM) at 37ºC in 50 mM phosphate buffer, pH 7.4 in a total volume of 400 µl. At the end of the incubation, the mitochondrial membranes were washed with an excess of ice-cold 50mM phosphate buffer, pH 7.4, collected by centrifugation at 4ºC and resuspended in the same buffer. Aliquots of mitochondrial suspension were used for measurement of complex I (Hatefi 1978) and complex IV (Wharton and Tzagoloff, 1967) activities.

Results: The inhibition of complex I and IV activities were prevented by reduced glutathione (5mM) and MitoQ (50nM), however, catalase, DTPA, mannitol and DMSO failed to provide protection (Figure1). These results indicate the contribution of DA-derived quinone products along with peroxynitrite (ONOO-) and superoxide (O2º-) in the inhibition of complex I and IV activities.

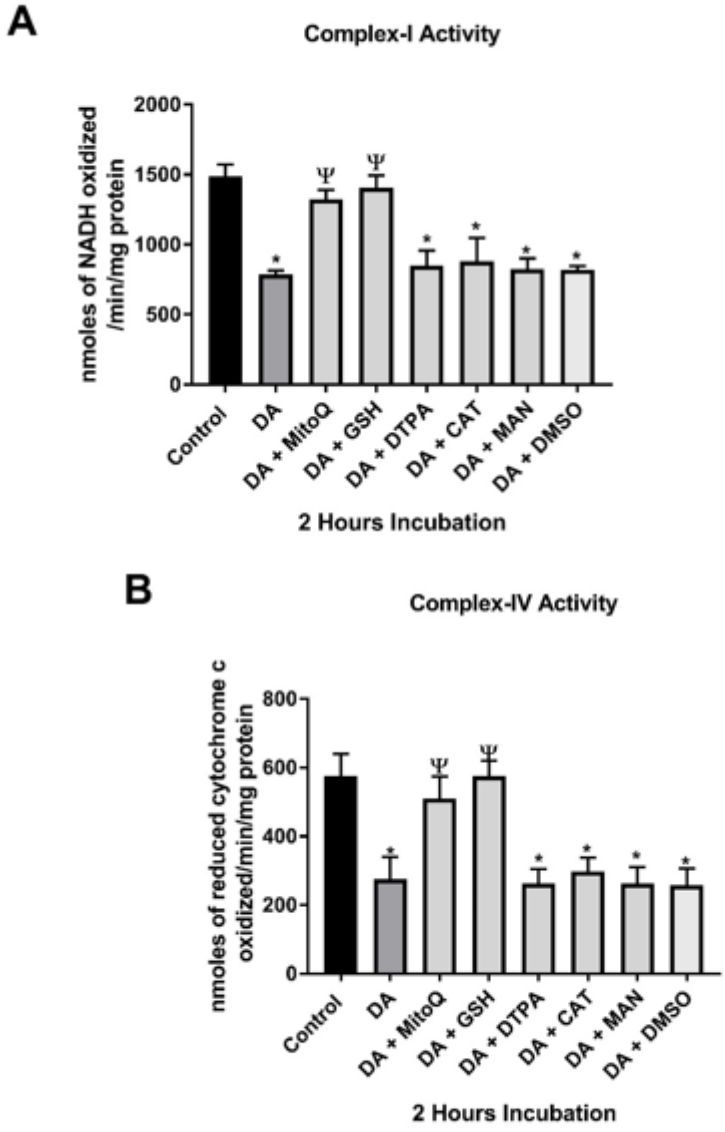


Figure.1 Effects of antioxidants, radical and quinone scavengers on DA-mediated inhibition of mitochondrial complex I and complex IV activities.The values are means ± SEM of 6 observations for complex I (A) and 6 observations for complex IV (B). Statistical significance was calculated by Student's t test paired. *P <0.05 vs. control, $^{\Psi}$P < 0.05 vs. DA

Conclusions: Quinone products and ONOO-/O2º- are involved in the long-term DA-mediated inactivation of complex I and complex IV

1204

Motor stress elicits dystonia-like movements in a pharmacological mouse model for Rapid-Onset Dystonia-Parkinsonism (DYT12)

L. Rauschenberger, J. Volkmann, C.W. Ip (Wüerzburg, Germany)

Objective: To study if a mild stressful trigger by motor activity leads to development of dystonia-like movements in a pharmacological mouse model for DYT12.

Background: DYT12 dystonia, linked to loss-of-function of the ATP1a3 gene, is characterized by generalized dystonic postures and parkinsonian symptoms that abruptly develop after a stressful event. Previous publications reported the development of a pharmacological DYT12 model by perfusion of ouabain, a selective ATP1a3-blocker, into basal ganglia and cerebellum of wt mice via osmotic pumps that lead to dystonia-like postures and parkinsonian symptoms if mice were subjected to severe stress by electric foot shocks.

Methods: Ouabain concentrations between 9 - 36 ng/h in cerebellum and basal ganglia were tested with a final concentration of 11.2 ng/h being used. As motor stressors, ouabain-perfused mice were repeatedly subjected to the Rotarod Performance test and Pole test. The exhibited phenotype was longitudinally assessed through Open Field analysis, video recordings of spontaneous movements and tail suspension test over 72 h. Motor symptoms were evaluated by an adapted Motor Behavioral Scale with 0-2 points per category to assess general activity, postural instability, hind limb dystonia and truncal dystonia. A new 8-point scoring system to assess dystonia-like movements was applied for the tail suspension test: crossing of hyperextended forelimbs as well as tonic flexion, scored from 0-4 points depending on severity and duration; hyperextension of hindlimbs and clasping behavior was scored from 0-3 points; 1 point was given for truncal distortion.

Results: Abnormal motor behavior observed in ouabain-perfused mice were hyperextensions of limbs, kyphosis, reduced locomotion and postural instability. The Motor Behavioral Scale in stressed mice was significantly higher than in unstressed mice (5.06 ± 0.22 vs 3.19 ± 0.36, respectively; $P<0.0001$). Open Field analysis showed reduced locomotion in stressed mice with 16.7 % less distance moved than unstressed animals. Scoring of the mice in tail suspension placed the stressed group at 5.50 ± 0.32 compared to 3.77 ± 0.53 in the unstressed group ($P<0,005$).

Conclusions: This modified pharmacologic mouse model for DYT12 with reduced ouabain-dosage exhibits dystonia-like movements and a parkinsonian phenotype with significant increase of symptoms when subjected to motor stress.

1215

Multiple Neural Networks dysfunction in Primary Blepharospasm: An Independent Components Analysis Study

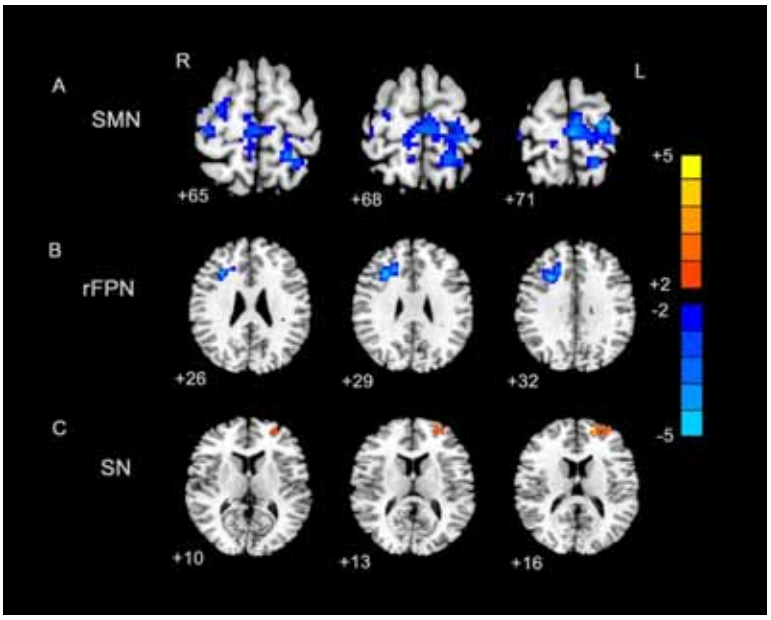
X. Huang, Z.H. Liang, M.R. Zhu (Dalian, People's Republic of China)

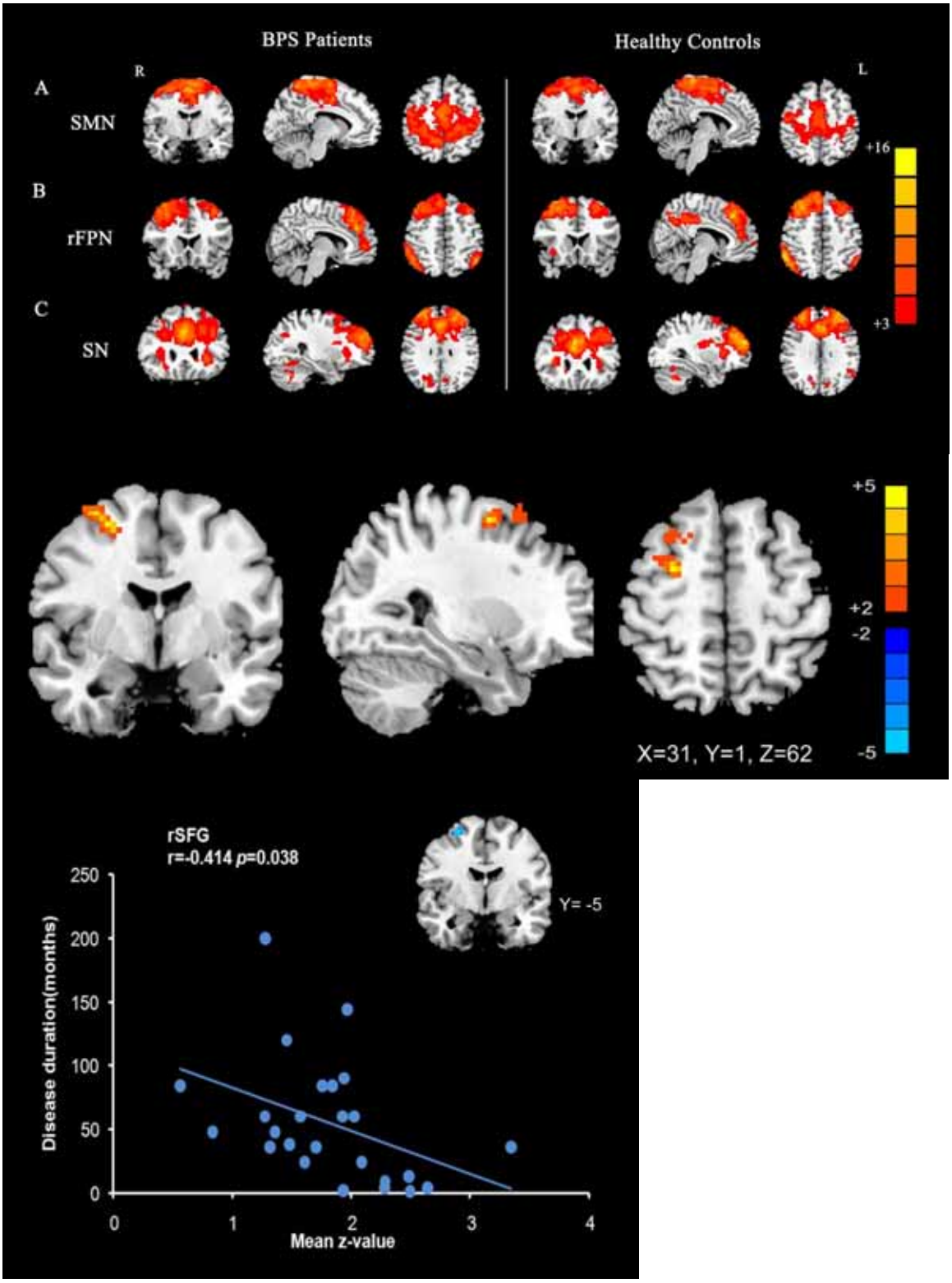
Objective: This study aimed to explore altered functional brain connectivity and the possible correlations of these networks with clinical variables in BPS.

Background: Primary blepharospasm (BPS) is a focal dystonia characterized by involuntary blinking and eyelid spasms. The pathophysiology of BPS remains unclear.

Methods: Twenty-five patients with BPS and 25 age-and gender-matched healthy controls received resting-state fMRI scan. Group ICA was performed with the GIFT toolbox.

Results: (1) There were no significant differences in the demographic variables between two groups besides the HAMA scores [table 1]. (2) Comparing with healthy control group, BPS patients exhibited different resting-state connections within sensory-motor network (SMN), right frontoparietal network (rFPN) and salience network (SN) [figure 1, figure 2]. BPS patients exhibited decreased connectivity within SMN that involved regions of the bilateral primary sensorimotor cortex, supplementary motor area, right superior frontal gyrus (BA 6), bilateral precuneus and left superior parietal cortex [table 2]. Within rFPN, decreased connections were observed in the middle frontal gyrus, DLPFC and inferior frontal gyrus [table 3]. Regarding salience network, increased connectivity was observed in the left superior frontal gyrus and middle frontal gyrus (including the DLPFC) [table 4]. We found positive correlation between the left superior frontal gyrus and HAMA scores, but this result was not significant. (3) Among the BPS patients, 12 performed sensory tricks positive (ST+), 9 perform sensory tricks negative (ST-). ST+ as compared to ST- patients exhibited significant higher connectivity in right premotor cortex within SMN [figure 3, table 5]. The results also showed a significant negative correlation between the right superior frontal gyrus and disease duration (Pearson's correlation $r = -0.414$, $p = 0.038$)[figure 4]. (4) We compared the SMN connectivity maps from pre- and after- treatment of 6 patients, but found no significant result.


A
SMN
R
L
+65
+68
+71
+5
+2
-2
-5
B
rFPN
+26
+29
+32
C
SN
+10
+13
+16



Conclusions: Multiple neural networks dysfunction may play roles in BPS.

1217

Dystonia-like phenotype in a DYT1 rat model after peripheral trauma

S. Knorr, K. Grundmann-Hauser, J. Volkmann, C.W. Ip (Würzburg, Germany)

Objective: Establishment and characterization of a DYT1 rat model with dystonia-like movements after sciatic nerve crush injury.

Background: Penetrance of DYT1 dystonia is markedly reduced with 30-40%. To verify if environmental factors can trigger dystonia in genetically predisposed DYT1 gene carrier (second hit hypothesis), we aimed to induce dystonia in a transgenic DYT1 rat model (?ETorA), that harbors the full human mutant *Tor1A* gene and is asymptomatic per se, by a peripheral nerve injury.

Methods: Dystonia-like movements of the hindlimbs during tail suspension were scored before and after unilateral sciatic nerve crush injury with a new scoring system at week 2, 5, 9 and 12. Nerve conduction velocity (NCV) and compound muscle action potentials (CMAP) of wildtype (wt) and ?ETorA rat sciatic nerves were evaluated by in vivo electroneurographic recordings (ENG) 12 weeks after nerve injury.

Results: Both wt and ?ETorA rats developed dystonia-like movements after nerve injury with a maximum score at week 2. In wt rats, the score then continuously decreased to a minimum at week 9 (0.4 ± 0.3) and stayed at this low level, that was comparable to naïve rats, until week 12 (0.6 ± 0.3). However, compared to this group a significantly higher dystonia-like movement score was observed in nerve injured ?ETorA rats at week 9 (1.9 ± 0.4) ($p< 0.01$) and at week 12 (1.9 ± 0.4) ($p< 0.05$). Moreover, after nerve injury a higher penetrance of dystonia-like movements was found in ?ETorA rats (70%) compared to wt rats (30%). Additionally, a spreading of dystonia-like movements to the contralateral hindlimb was seen in 35% of nerve injured ?ETorA rats but not in nerve injured wt rats. ENG recordings didn`t show any significant differences in NCV or CMAP comparing wt rats with ?ETorA rats, 12 weeks after nerve injury, although both genotypes demonstrated slower NCV and reduced CMAP amplitudes after nerve crush compared to naïve controls of both genotypes.

Conclusions: Our data indicate that a peripheral nerve trauma can trigger dystonia-like movements in genetically predisposed ?ETorA rats, which supports the “second hit” hypothesis. Functional assessment by ENG excluded impaired nerve regeneration after injury as a reason for a higher dystonia-like movement score in ?ETorA rats.

1222

RAB12 variants and their role in dystonia

K. Lohmann, E. Hebert, F. Borngräber, A. Schmidt, A. Rakovic, A. Weissbach, J. Hampf, E.-J. Vollstedt, S. Schaake, H. Manzoor, H.C. Jabusch, M. Kasten, V. Kostic, T. Gasser, K. Zeuner, P. Bauer, E. Altenmüller, C. Klein (Lüebeck, Germany)

Objective: To evaluate the role of mutations in RAB12 in different forms of dystonia.

Background: By next generation sequencing, we recently identified an extremely rare missense variant (Ile196Val) in RAB12 in two of three small families with musician’s dystonia (MD) and writer’s dystonia (WD). While MD and WD are task-specific movement disorders, other dystonias persistently affect postures as in the case of cervical dystonia. RAB12 encodes a small GTPase which regulates the degradation of transmembrane proteins including the transferrin receptor 1 (TFRC).

Methods: We performed Sanger sequencing of RAB12 in 814 additional dystonia patients, 333 Parkinson´s disease patients, and 461 healthy controls. Functional characterization of two RAB12 variants (Ile196Val, Gly13Asp) was performed using patient-derived fibroblasts and two RAB12-overexpressing cell models.

Results: We identified 4 additional carriers of rare, missense changes among dystonia patients (0.5%) but only one carrier among the non-dystonia individuals (0.1%). The detected variants comprised Gly13Asp, Ala148Thr, Arg181Gln, and Ile196Val and were found in patients with MD, WD, and cervical dystonia. Of note, the Ile196Val mutation has previously also been found in two families with MD/WD. RAB12 is a gene with only about 50 reported missense variants in the about 60,000 exomes of the Exome Aggreggation Consortium (ExAC). Functional characterization revealed increased GTPase activity in mutants compared to wildtype. Further, subcellular distribution of RAB12 and lysosomes in mutants were altered in fibroblasts. Soluble Transferrin receptor 1 levels were reduced in blood of all three tested Ile196Val carriers.

Conclusions: Our data suggest a role of RAB12 variants in the etiology of MD and other dystonias.

1224

Head turning onset and velocity in response to a peripheral visual stimulus in cervical dystonia patients, their unaffected relatives with and without abnormal temporal discrimination and healthy controls

I. Beiser, B. Quinlivan, E. McGovern, L. Williams, S. Narasimham, O. Killian, R. Beck, S. O'Riordan, J. Butler, R. Reilly, M. Hutchinson (Dublin, Ireland)

Objective: We postulate that cephalomotor responses (onset of head turning and speed of head turning) to the appearance of a novel peripheral visual stimulus will be impaired in patients with cervical dystonia and their unaffected first-degree relatives with abnormal temporal discrimination (TD) (reflecting abnormal sensory processing in the superior colliculus) compared to unaffected relatives with normal TD and healthy controls.

Background: Abnormal TD reflects disordered sensory processing within the superior colliculus and, as a mediational endophenotype, indicates a pathomechanism for the generation of cervical dystonia via disinhibited motor output from the superior colliculus to brain stem and cervical cord motor pathways. By examining head turning, in response to the appearance of a novel visual stimulus we aimed to demonstrate that unaffected first-degree relatives with abnormal TD have abnormalities in the initiation of, and speed of head turning compared to unaffected relatives with normal TD and healthy control participants. This will confirm the relevance of abnormal sensory processing in the superior colliculus in this disorder.

Methods: A lightweight head mounted display (Oculus Rift DK2) was employed for simultaneous data collection and task presentation. All participants completed a modified Posner task in which head movements towards validly- and invalidly-cued eccentric targets were employed as the response. Patients, relatives and control participants were balanced by gender and age.

Results: A delay in onset of head movement was observed during invalid trials (compared to valid trials) in patients and healthy controls. Head turns were earlier during valid trials. This novel representation of the behavioural impact of orientation of attention (Posner effect) was clearly visible in both cohorts but more than doubled in the patient group. We will demonstrate that unaffected relatives with abnormal TD will show abnormalities in head turning initiation and velocity.

Conclusions: We hypothesise that abnormal TD represents delayed sensory processing within the superficial layers of the superior colliculus, manifested in a subclinical disordered motor output from the deeper layers of the superior colliculus.

1225

Brain volume changes in relation to intracortical inhibition and clinical benefit of pallidal stimulation in dystonia: a combined VBM and TMS study

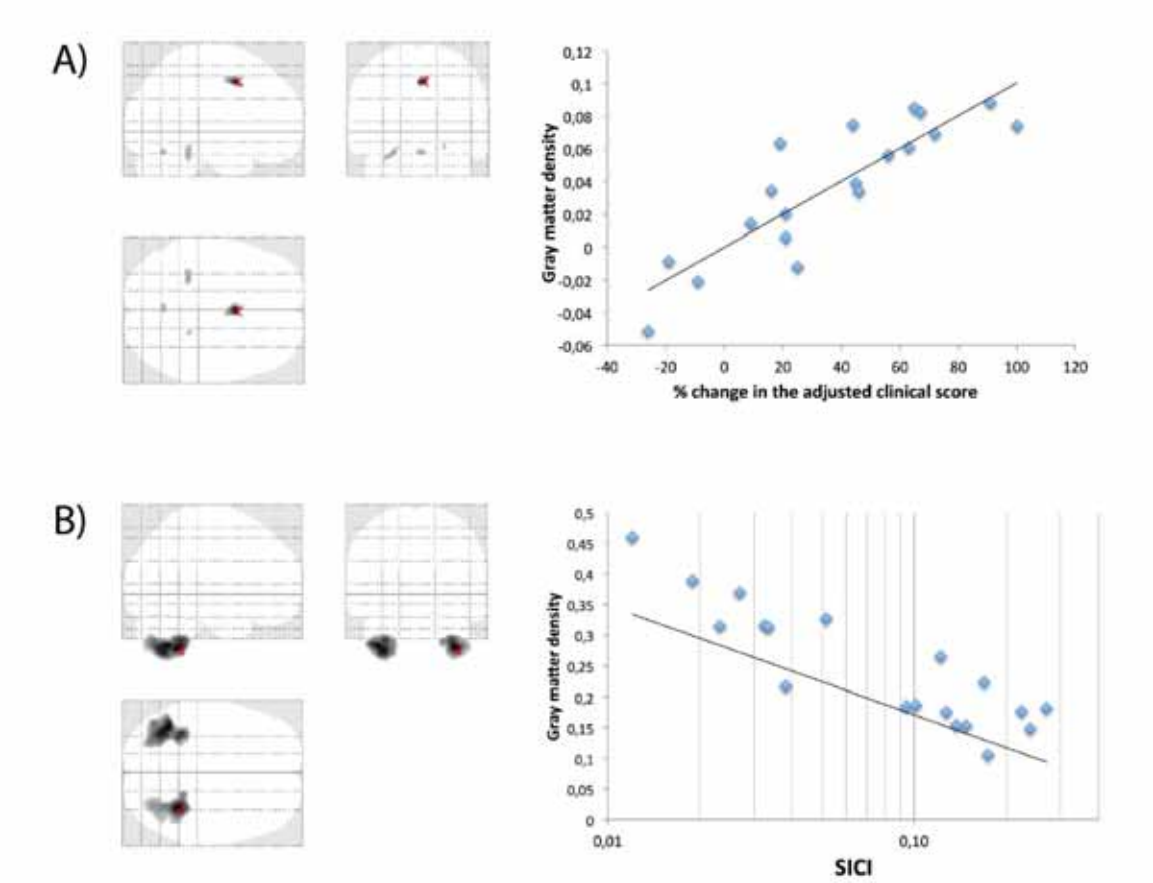
R. Jech, A. Fecíková, V. Cejka, V. Capek, D. Šťastná, F. Ružicka, D. Urgošík, E. Ružicka (Prague, Czech Republic)

Objective: To explore the relationship between brain morphology, clinical effects of bilateral pallidal stimulation (GPi DBS) and intracortical inhibition of the motor cortex in patients with dystonia.

Background: Dystonia patients have trouble suppressing unwanted movement that is potentially related to less effective intracortical inhibition, which can be restored with GPi DBS. However, the treatment efficacy varies and the benefit increases relatively slowly, suggesting induction of slow plastic processes in regions involved with motor control.

Methods: We examined 19 patients (mean age 48±(SD)18 years) with cervical (N=7) or generalized dystonia (N=12) of various origin by chronic GPi DBS for 57±28 months. Voxel-based morphometry of postoperative T1-weighted images (MPRAGE, 1x1x1 mm) was calculated for gray matter (GM) density in each voxel using CAT12/SPM12 software in every patient and compared with 20 matched controls. Paired TMS with subthreshold conditioning stimulus followed by a supratreshold testing stimulus were applied to the motor cortex to elicit short-latency intracortical inhibition (SICI) of the motor evoked potential. The clinical effect of GPi DBS was expressed as a change in the dystonic score (BFMDS or TWSTRS) between actual GPi DBS ON condition and the preoperative state.

Results: Dystonia patients showed increased GM density in the supplementary motor area (SMA) and middle cingulate in comparison with healthy controls ($p<0.05$ corrected). The GM density in this region positively correlated with the clinical effect of GPi DBS ($p<0.001$)(Fig. 1A). The SICI was lower in patients than in controls regardless of the ON and OFF conditions ($p<0.001$) and its mean amplitude correlated with GM density in both cerebellar hemispheres ($p<0.05$ corrected)(Fig. 1B).[figure1]



Conclusions: Brain changes of chronically GPi DBS treated patients possibly reflect "hardwire" rebuilding of motor regions associated with functional improvement. Cortically, they showed that the SMA hypertrophy was quantitatively related to the clinical benefit suggesting a compensatory mechanism. Subcortically, they showed hypertrophy of the cerebellar hemispheres growing with the effectiveness of intracortical inhibition of the primary motor cortex which possibly allows better motor control. *Supported by the grants GACR 16-13323S and PRVOUK P26/LF1/4.*

1237

Impaired pain processing in functional dystonia

F. Morgante, A. Matinella, E. Andrenelli, C. Allegra, C. Terranova, P. Girlanda, M. Tinazzi (Messina, Italy)

Objective: Aim of the present study was to assess the sensory-discriminative and cognitive-emotional aspects of pain in patients with functional and idiopathic dystonia respectively assessing pain threshold and pain tolerance in affected and unaffected limbs.

Background: Pain is often experienced by patients with functional dystonia and might occur also in body segments not affected by involuntary movements. We hypothesized an alteration of cognitive-emotional component of pain in patients with functional dystonia.

Methods: We enrolled 10 patients with idiopathic cervical dystonia (CD), 10 patients with functional dystonia (F-dys) and 15 age and gender matched healthy controls (HC). All patients with F-Dys had symptoms restricted to one body side, except for 2 patients with predominant right side involvment. Exclusion criteria were presence of cognitive impairment according to MMSE=24, presence of lesions in peripheral and central sensory pathways , diagnosis of diabetes. Each patient underwent clinical evaluation by means of the Burke-Fahn-Marsden Rating Scale (BFMRS), the pain score of the TWTRS, Hamilton's depression and anxiety rating scales (HDRS, HARS). We assessed tactile threshold, pain threshold (P-Th) (intensity at which sensation changed from unpainful to faintly painful) and pain tolerance (P-Tol) (intensity at which painful sensation was intolerable) by delivering electrical pulses of increasing intensity to the index finger of each hand.

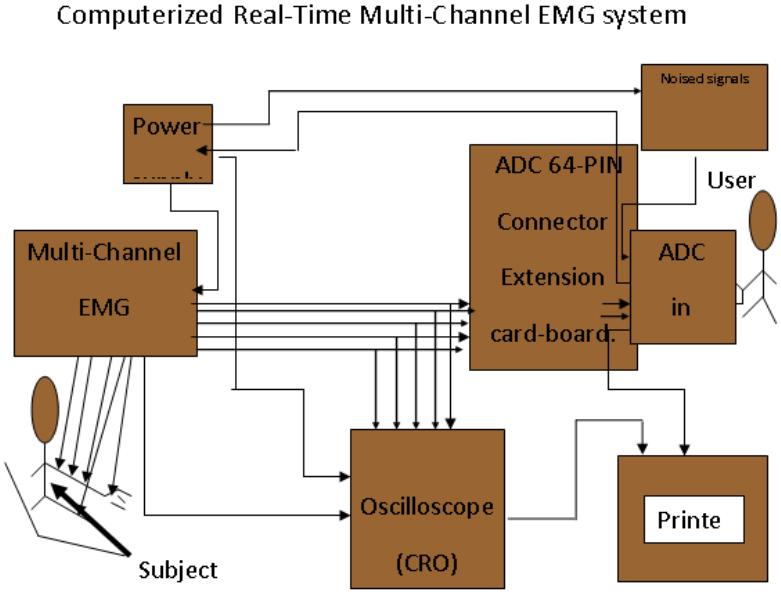
Results: No difference was found between groups for tactile threshold for both hands. P-Th was significantly increased only in the affected hand of patients with F-Dys compared to CD (p=0.04). P-Tol was significantly increased in both the affected (p = 0.03) and unaffected (p=0.04) hand of F-Dys compared to CD. No difference was found between idiopathic CD and HC in pain thresholds, regardless of the presence of pain in CD. Spearman Rank Correlation did not demonstrate any correlation between P-Th or P-Tol with pain score, HDRS, HARS, BFMRS (movement and disability scores), disease duration, age at onset.
Conclusions: Patients with functional dystonia have an impairment of the sensory-discriminative component of pain in the affected hand and of the cognitive-emotional component of pain in both hands, regardless of the presence of abnormal movements. We hypothesize that the abnormal connectivity between the motor and the limbic system might account for abnormal pain processing in functional dystonia.

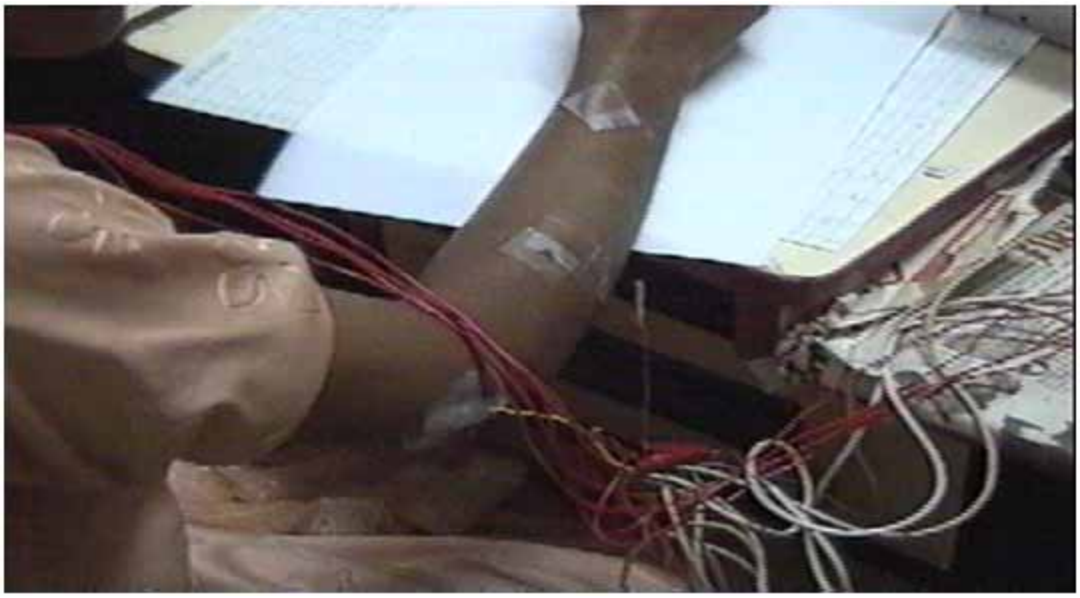
1243

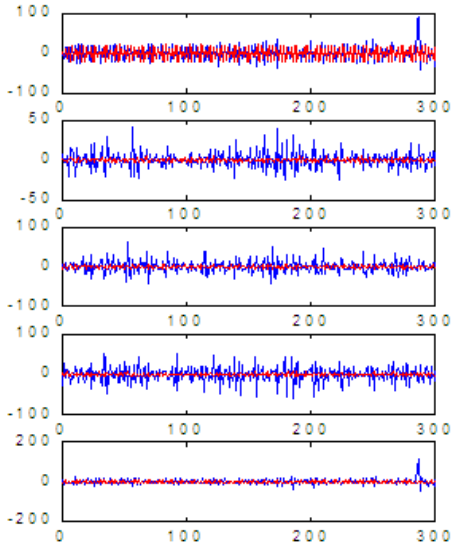
Mirror dysonia in writer`s aramp: a study with advanced multi-channel micro-electrode recording EMG system

V. Rama Raju, R. Borgohain, R. Kandadai (Hderabad, India)

Objective: To differentiate between those with concordant(C) and discordant (D) MMs in WC, in order to establish that there is a quantifiable difference between these two groups and to design/fabricate a sophisticated multichannel microelectrode-recording EMG system.
Background: The main hypothesis is that when a Writer's cramp(WC) patient inscribes with an abnormal posture, it is difficult to determine if that posture is because of the primary dystonic-force or if a compensatory-force applied by the WC-patient has overcome the primary dystonic-force and has resulted in that posture. One way to differentiate those two would be is to look at the mirror-movements (MMs).
Methods: Multivariate statistical analysis for 12 patients in their means, differences in means, standard deviations, variances, t, F and p-values between RHWS and LHWS were compared using student *t*, χ^2 (*Chi-square*) and Fisher's tests. RootMeanSquare(RMS) measured the amplitudes of WC signals and its complexity. decomposition,latent variate factorial analysis principal component analysis, clustering, Canonical correlation/multidimensional scaling,entropy.
Results: 12 patients with writer's cramp (8 with concordant and 4 with discordant MMs) were assessed. On comparison of the measures of dispersion; D group had statistically significant difference between LHWS and RHWS (variance, standard deviation and F ratio) with a larger variance in RHWS, as compared to C group where variances and SD were equal or smaller in the RHWS compared to LHWS. Mean amplitudes for RHWS and LHWS for the same muscles though differ significantly in statistical terms, showed a consistent pattern only in the fifth muscle with a larger mean-amplitude on left-side in all patients and were not of value in differentiating between concordant (C) and discordant (D) groups of patients.



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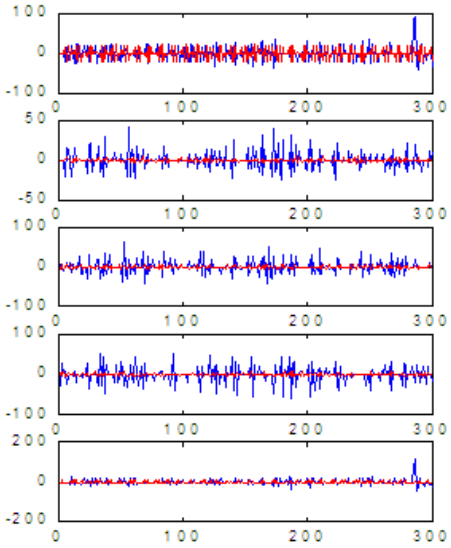
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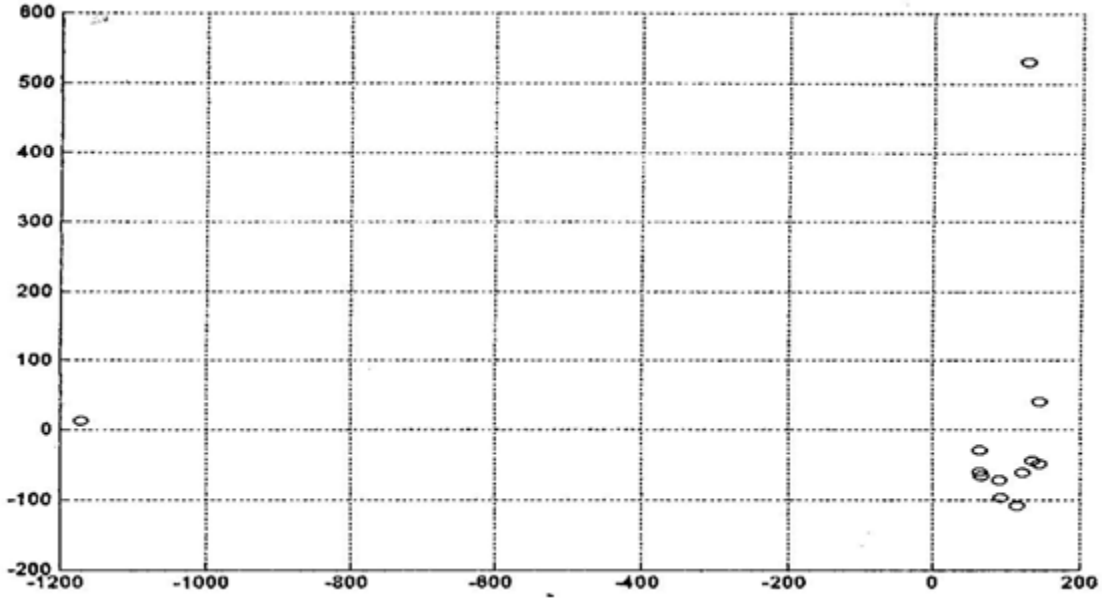
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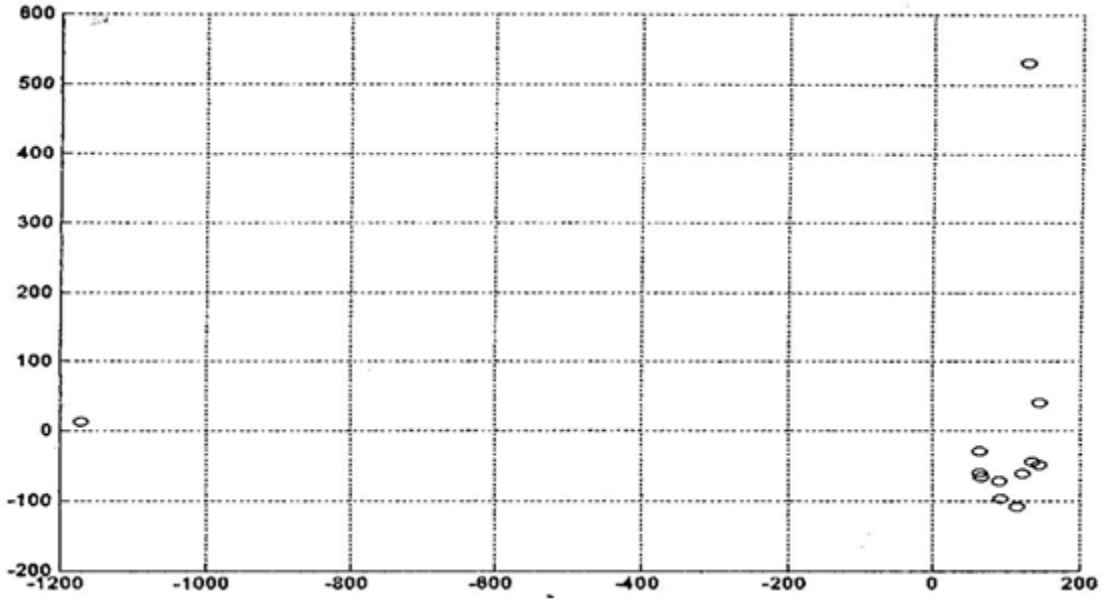
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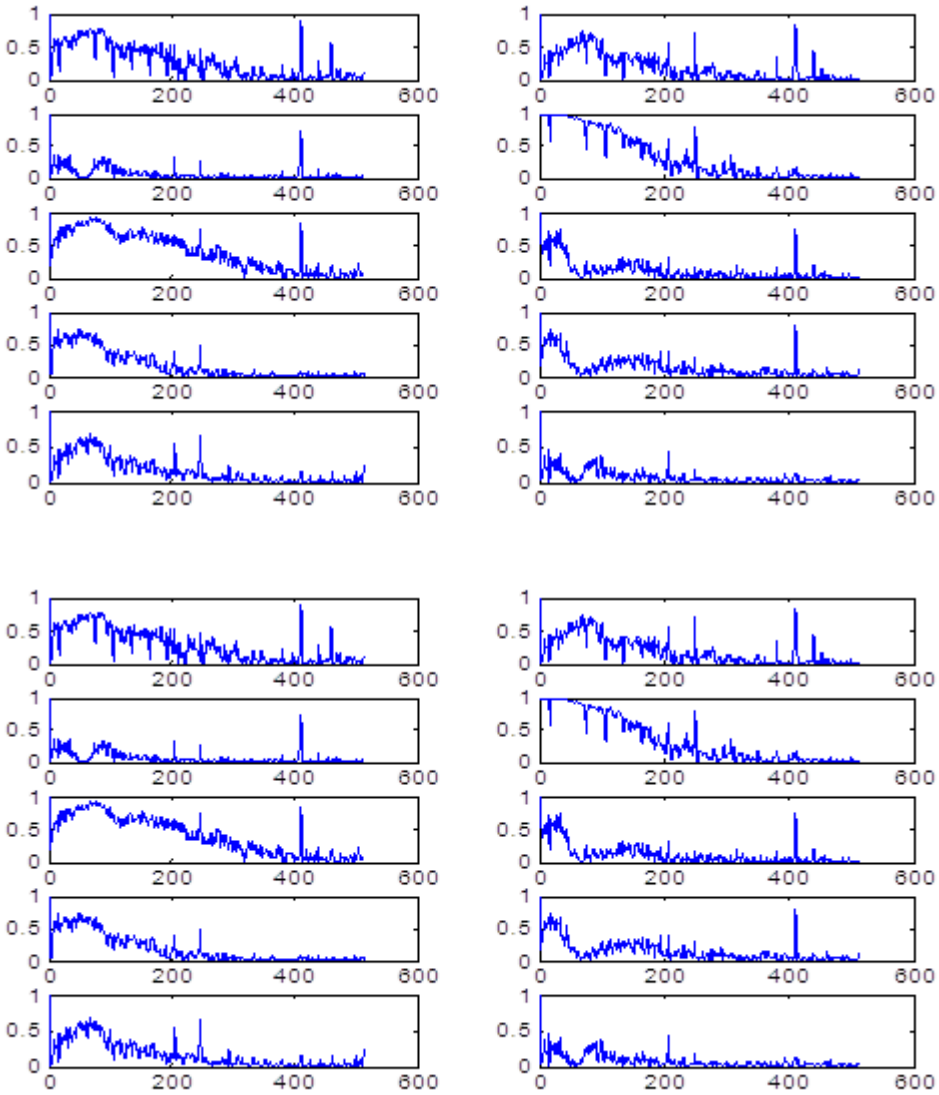
Table Squared canonical correlations

Patient	λ_1	λ_2	λ_3	λ_4	λ_5	
A1	**0.6527**	**0.3015**	0.0006	0.0003	0.0000	D
A2	0.1566	0.0023	0.0004	0.0001	0.0000	
A3	0.0021	0.0015	0.0002	0.0002	0.0000	
A4	**0.1734**	0.0018	0.0004	0.0000	0.0000	
A5	0.0383	0.0018	0.0015	0.0001	0.0000	
A6	0.0509	0.0033	0.0011	0.0004	0.0001	
A7	0.0022	0.0014	0.0009	0.0008	0.0000	D
A8	**0.0799**	0.0018	0.0004	0.0002	0.0000	
A9	**0.2615**	0.0707	0.0030	0.0002	0.0000	
A10	0.0502	0.0069	0.0009	0.0004	0.0001	
A11	0.0034	0.0021	0.0006	0.0002	0.0000	D
A12	0.0013	0.0006	0.0002	0.0002	0.0000	D

Patient	(1,3)	(1,4)	(1,5)	(2,3)	(2,4)	(2,5)	(3,4)	(3,5)	(4,5)
A1	* *	* *	+ *	+ +	* *	- -	* -	- *	* *
A2	* *	* *	+ *	+ +	* -	* *	* -	* *	* *
A3	* *	* *	+ +	* *	* *	* *	- -	* *	* *
A4	←			N	I	L			→
A5	←			N	I	L			→
A6	* *	* *	+ +	* *	* *	* *	- -	* *	* *
A7	←			N	I	L			→
A8	+ *	--	* +	+ *	* *	* *	- -	* *	* -
A9	* *	* *	+ +	* *	* *	* *	* *	* *	* *
A10	* *	* *	+ *	* *	* -	* *	* *	* -	* *
A11	* *	* *	+ +	* *	- *	* *	* *	* *	* *
A12	* *	* *	+ +	* +	-	* *	- *	* -	* *

Patient	Nearest two Patients	Farthest two Patients
1	5, 2	3,11
2	1,10	3,11
3	7, 4	2,10
4	6, 7	2,11
5	1,10	11,3
6	4, 9	2,11
7	6,4	2,10
8	4,12	11,10
9	6,4	2,8
10	1,2	3,4
11	12,7	2, 8
12	11,7	2,10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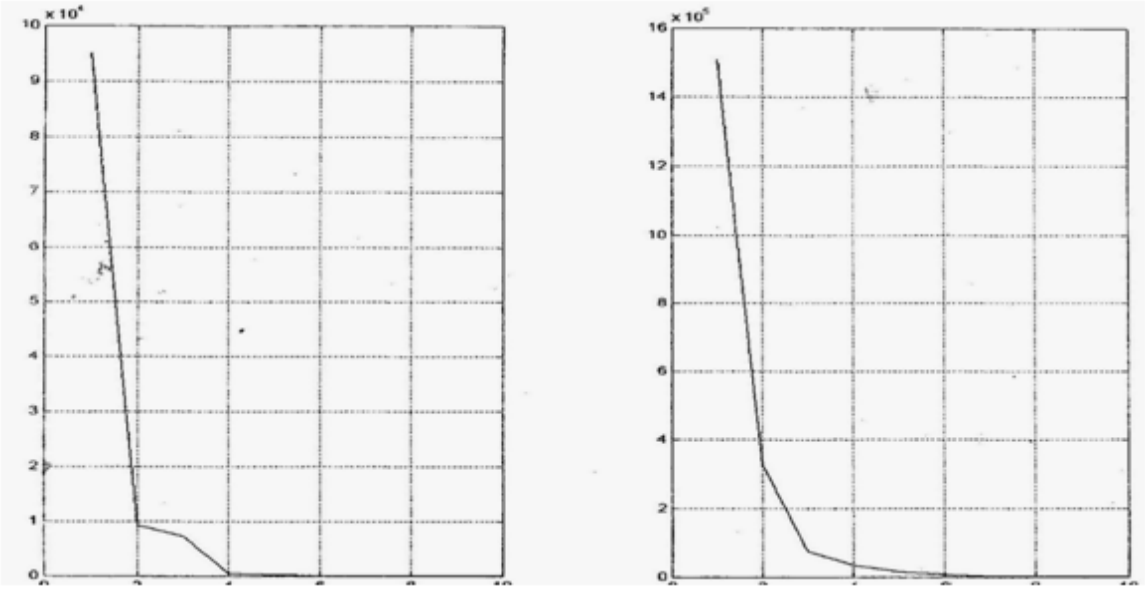


Group	Y	N	
RH	4	0	4
LH	1	3	4
	5	3	8

Conclusions: This study showed significant quantifiable EMG differences in the signals seen while inscribing with the right and left hands between those writer's cramp patients with concordant mirror movements (C group) versus those with discordant mirror movements (D group). This was mainly seen in the measures of dispersion of the signal i.e., standard dispersion, variances and their ratio (F-ratio). These were statistically significantly different between the two groups, C and D, and the pattern of differences were consistent with the hypothesis that the discordant group had a compensatory force which overcame the dystonic force resulting in the final abnormal posture. These analyses could possibly be applied to longitudinal follow-ups and correlations with a normal control population in future to better comprehend the WC phenomenon.

1268

Cinnamomum verum on Learning and Memory in Wistar Albino Rats

N. Ahmed, D. Agrawal, A. Chughtai (Aligarh, India)

Objective: Several studies have been performed on Cinnamomum verum,but not much is known about the activity of Cinnamomum verum on Memory Enhancing.Therefore Cinnamomum verum is selected for this study to understand its efficacy on Learning and Memory.

Background: Learning and Memory are the basic need through which we try to interact with surrounding, but with stress, depression in our socio-economical and sedentary lifestyle which affects our mind and behaviour and in turn they also alter our thinking process and dampens our learning and memory. In Unani medicine,many drugs are described to be effective in enhancing the memory but only very few of them have been scientifically evaluated. So, one of the single drug is, Cinnamomum verum. In the present study it was evaluated for its learning & memory enhancing effect in experimentally induced stress in Male wistar albino rats.

Methods: Animals were divided into 4 groups with 6 animals in each group, Group I Control, Group II Acute Noise Stress alone, Group III treated with C. verum and Group IV Stress and Drug Treated. Group I and II received equal amount of saline, whereas Group III and IV was administered with C. verum powder in a dose of 100 mg/Kg/body weight dissolved in distilled water (1ml) and administrated orally for 22 consecutive days. Reference Memory Error, Working Memory Error, Triad Error and Total Time Taken were noted using 8-Arm and Y-Maze were evaluated

and statistically compared with the similar values obtained in all the groups for same duration respectively. Phytochemical studies were also done to know the active compound & constituent of C. verum.

Results: Oral administration of C. verum in the treated group exhibit high percentage of correct responses when compared to the control rats, C. verum treated group also exhibit decreased Reference Memory Error, Working Memory Error, Total Time Taken and Latency period compared to control and stress rats. Both 8 Arm and Y Maze showed the memory enhancing property of C. verum. After withdrawal from the drug for 10 days, the test rats showed an improved performance compared to the control rats, which can be interpreted as good retention performance.

Kingdom:	**Plantae**
Division:	**Magnoliophyta**
Class:	**Magnoliopsida**
Order:	**Laurales**
Family:	**Lauraceae**
Genus:	**Cinnamomum**
Species:	**Cinnamomum verum**
Common names:	**Darchini, Laungpattai, Cinnomon**

Figure: 1. Cinnamomum verum, as illustrated by Franz Eugen Köhler, in his Medicinal- Plants in Natural Garden Abbildungen and kurzerläuterndem Text, published in 1887.

Cinnamomum verum is a very common traditional Indian Spice known as **Darchini** in Hindi/Urdu and **Lavangapatta, Karuvapattai** in Tamil & Telugu. The word cinnamon comes from the Greek word "**kinnamomon**".

Fig: 2. CINNAMOMUM VERUM PLANT

Fig: 3. CINNAMOMUM BARK

Fig: 4. EIGHT ARM RADIAL MAZE

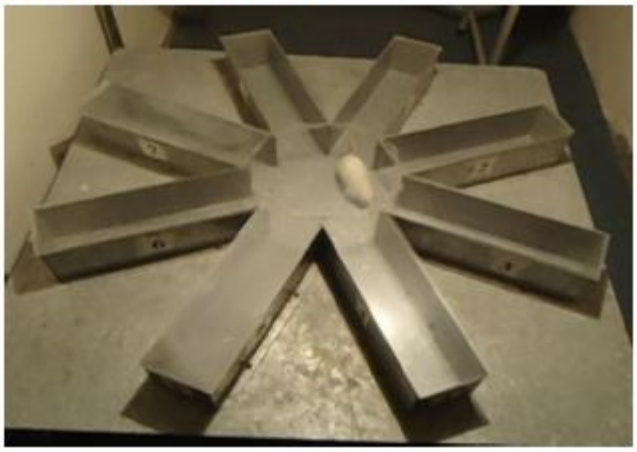
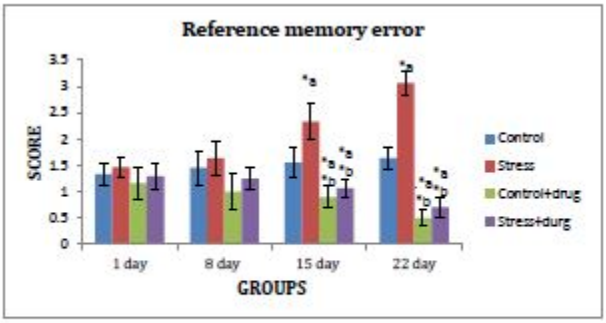


Fig: 5. Y-MAZE

Table 1: **8-ARM RADIAL MAZE- REFERENCE MEMORY ERROR**

S. No	GROUPS	REFERENCE MEMORRY ERROR			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	1.33 ± 0.21	1.33 ± 0.21	2.17 ± 0.31	2.33 ± 0.21
2	STRESS	2.66 ± 0.21	2.83 ± 0.17	2.83 ± 0.17	2.67 ± 0.21
3	CONTROL + DRUG TREATED	1.17 ± 0.17	1.50 ± 0.22	2.50 ± 0.22	0.50 ± 0.22
4	STRESS + DRUG TREATED	2.50 ± 0.22	2.67 ± 0.21	2.67 ± 0.21	1.67 ± 0.17

Figure 8: **REFERENCE MEMORY ERROR-8 ARM RADIAL MAZE**

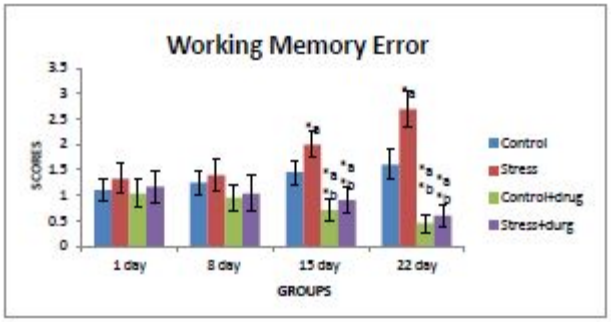


The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Table 2:**8-ARM RADIAL MAZE- WORKING MEMORY ERROR**

S. No	GROUPS	WORKING MEMORY ERROR			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	0.50 ± 0.22	0.50 ± 0.22	1.33 ± 0.21	2.83 ± 0.17
2	STRESS	1.33 ± 0.21	1.50 ± 0.22	2 ± 0.26	3 ± 0.26
3	CONTROL + DRUG TREATED	0.33 ± 0.21	0.50 ± 0.22	1.17 ± 0.17	1.33 ± 0.21
4	STRESS + DRUG TREATED	1.17 ± 0.17	1.33 ± 0.21	1.50 ± 0.22	0.50 ± 0.22

Figure 9: **WORKING MEMORY ERROR-8 ARM RADIAL MAZE**

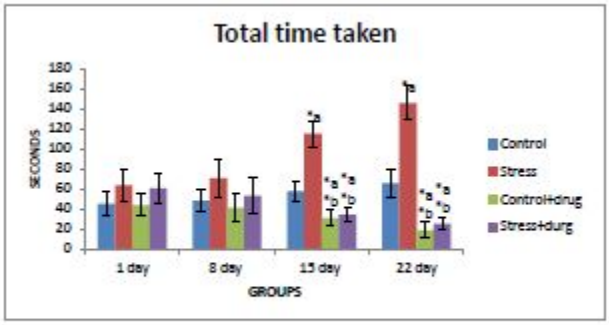


The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Table 3: **8-ARM RADIAL MAZE - TOTAL TIME TAKEN**

S. No	GROUPS	TOTAL TIME TAKEN			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	45.17 ± 5.19	48 ± 5.13	67.50 ± 3.82	90.83 ± 5.83
2	STRESS	93.33 ± 8.82	106.67 ± 7.49	115 ± 7.64	131.67 ± 9.46
3	CONTROL+ DRUG TREATED	46.67 ± 4.41	45.83 ± 5.69	66.67 ± 4.41	40.83 ± 5.83
4	STRESS+ DRUG TREATED	92.50 ± 6.42	73.33 ± 8.82	115 ± 6.19	75 ± 7.64

Figure 10: **TOTAL TIME TAKEN-8 ARM RADIAL MAZE**

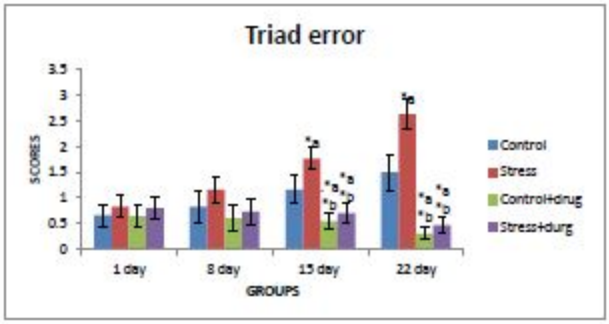


The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Table 4: **Y MAZE- TRIAD ERROR**

S. No	GROUPS	TRIAD ERROR			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	0.66 ± 0.21	0.83 ± 0.17	1.17 ± 0.17	1.50 ± 0.22
2	STRESS	0.83 ± 0.17	1.17 ± 1.67	1.67 ± 0.21	1.83 ± 0.17
3	CONTROL + DRUG TREATED	0.67 ± 0.21	0.67 ± 0.21	0.50 ± 0.22	0.33 ± 0.21
4	STRESS + DRUG TREATED	0.83 ± 0.17	0.83 ± 0.17	1.17 ± 0.17	0.33 ± 0.21

Figure 11: **TRIAD ERROR-Y MAZE**

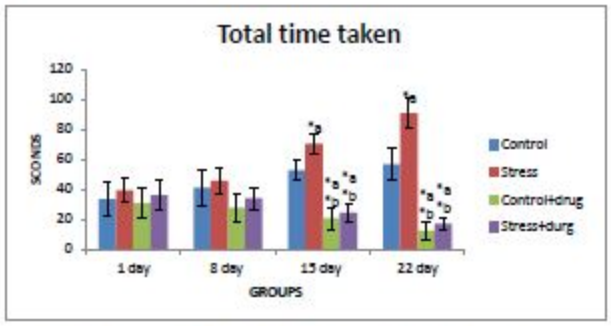


The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Table 5: **Y MAZE- TOTAL TIME TAKEN**

S. No	GROUPS	TOTAL TIME TAKEN			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	33.33 ± 4.41	45.83 ± 4.72	52.50 ± 3.82	56.67 ± 4.41
2	STRESS	39.17 ± 5.39	52.50 ± 3.82	53.33 ± 4.41	66.67 ± 4.41
3	CONTROL + DRUG TREATED	37.50 ± 4.23	42.50 ± 3.82	37.50 ± 3.81	33.33 ± 4.41
4	STRESS + DRUG TREATED	40.83 ± 5.07	50.83 ± 3.00	43.33 ± 4.41	30.82 ± 5.83

Figure 12: **TOTAL TIME TAKEN-Y MAZE**

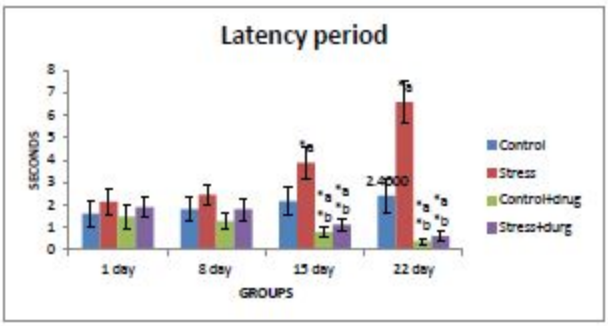


The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Table 6: **Y MAZE- LATENCY PERIOD**

S. No	GROUPS	LATENCY PERIOD			
		DAY 1	DAY 8	DAY 15	DAY 22
1	CONTROL	1.62 ± 0.23	2.03 ± 0.22	2.17 ± 0.25	2.40 ± 0.30
2	STRESS	2.35 ± 0.23	2.55 ± 0.23	3.48 ± 0.28	5.28 ± 0.39
3	CONTROL + DRUG TREATED	1.67 ± 0.22	1.77 ± 0.14	1.08 ± 0.18	0.78 ± 0.15
4	STRESS + DRUG TREATED	1.60 ± 0.18	1.22 ± 0.19	0.88 ± 0.14	2.10 ± 0.30

Figure 13: **LATENCY PERIOD -Y MAZE**



The data are expressed as mean ± SEM. *a compared to control, *b compared to acute noise stress group, *c compared to drug treated group and *d has compared to the stress with drug treated group.

Conclusions: Present study shows that Cinnamomum verum improves learning and memory it may be due to the presence of Cinnamon being an exceptional source of anti-oxidant; it also contains phyto-chemicals that assist the brain in metabolizing glucose, an essential form of energy for mental function.

1273

Neural correlates of movement sequence kinematics in substantia nigra dopaminergic cells

M. Mendonça, J. Alves Silva, L. Hernandez, J. Obeso, R. Costa (Lisboa, Portugal)

Objective: To develop a new behavioral task for assessment of mice forelimb movements, and evaluate the substantia nigra *pars compacta* dopaminergic cells' (SNpc) correlates of movement kinematics.

Background: Models of basal ganglia function frequently focus on initiation deficits. This contrasts with what is observed in Parkinson's Disease (PD) where chronic dopamine depletion is associated not only with "slowness of initiation" but also with "progressive reduction in speed and amplitude of repetitive actions" (Bradykinesia). The role of basal ganglia on movement speed and amplitude remains unclarified, but it seems reasonable to hypothesize that dopamine has a pivotal role.

Methods: Repetitive finger tapping is commonly used to assess movement speed and amplitude in PD. Using this as an inspiration we developed a novel self-paced operant task, in which mice learn to perform a particular sequence of actions, using only one forelimb. The task was designed to collect data regarding the spatial position, speed and acceleration of the forelimb of the mouse. A miniature epifluorescence microscope (~1.9g) was used to image GCaMP6f fluorescence (a calcium indicator) in dopaminergic SNpc cells while TH-cre mice performed the task. After animals learned the task we induced partial dopamine depletion by unilateral intrastriatal 6-Hydroxydopamine injection.

Results: Preliminary results showed that after depletion there is a redistribution of movement speed with an increase in slower movements and with longer within-sequence inter-press intervals. We also found changes in the sequence

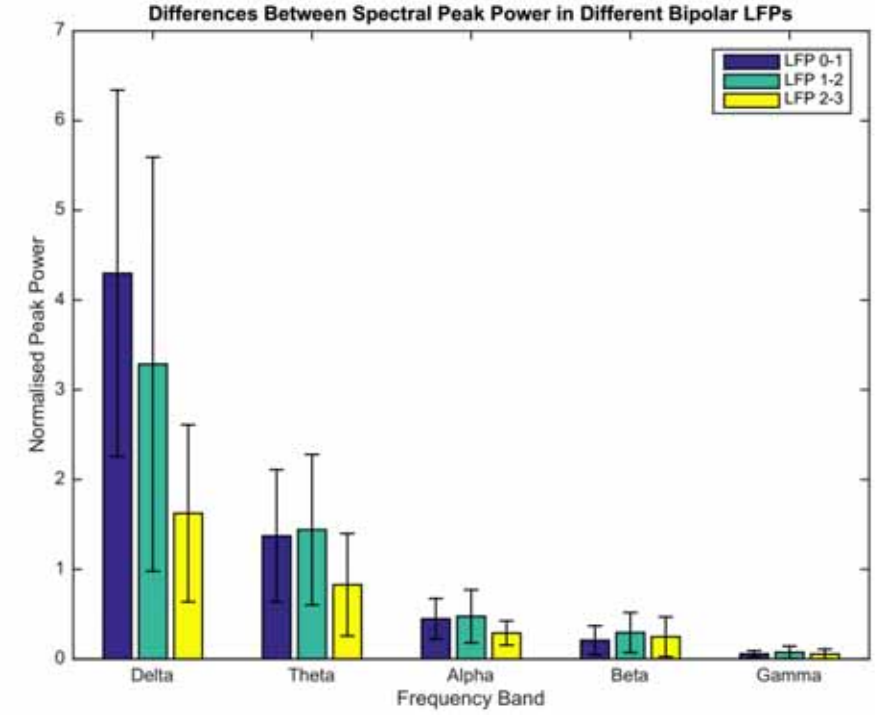
microstructure, including the number of lever presses/sequence. Using in vivo calcium imaging we identified phasic activity of SNpc dopaminergic accompanying the start of a learned lever-press sequence both in healthy and partially dopamine depleted animals.
Conclusions: We developed a clinically-relevant task for movement sequence kinematics assessment in mice, and identified SNpc correlates of movement. Ongoing analysis using the combination of these 2 tools will allow us to clarify the role of SNpc dopaminergic neurons in different type of movements (slow vs. fast movements), in healthy and chronic dopamine depleted mice. This will have impact in our comprehension on the role of basal ganglia dysfunction in PD symptoms.

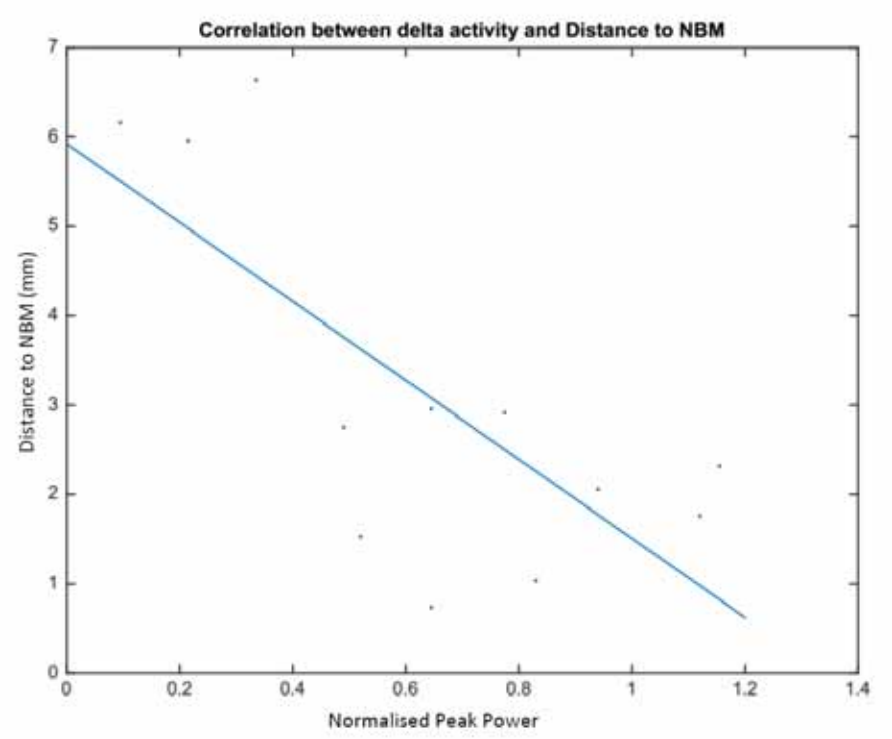
1279

Oscillatory Activity in the Nucleus Basalis of Meynert

M. Nazmuddin, D. Oterdoom, J. van Zijl, A. Kampman, J. van Dijk, G. Drost, T. van Laar, M. Beudel (Groningen, Netherlands)

Objective: Characterize oscillatory activity in and around the nucleus basalis of Meynert (NBM).
Background: The NBM is the major source of cholinergic innervation to the neocortex. It is a new potential target of deep brain stimulation (DBS) for cognitive sequelae in Parkinson's disease (PD) dementia and Lewy Body dementia (1, 2). In order to effectively stimulate the NBM, more knowledge about its anatomical delineation and local neurophysiological activity is needed. PD patients with implanted globus pallidus interna (GPi) electrodes often have their most distal contact points in the vicinity of the NBM. This enables the recording of neural activity in this area.
Methods: Bipolar LFPs from adjacent DBS contact pairs (bottom to top: 0-1, 1-2, 2-3) were recorded in two patients during the replacement surgery of an implanted pulse generator. For each bipolar derivation (n=12), frequency spectra were calculated over 15-sec epochs of resting state. Stereotactic coordinates of each contact and their distance to the NBM were calculated based on CT-MRI fusion images and correlated with spectral power in different frequency bands.
Results: Normalized peak power in delta band (1-4 Hz) was significantly higher in the 0-1 contact compared to the 2-3 contact pair (p=0.02, Fig. 1). The distance of the middle of the bipolar contact pairs to the NBM correlated negatively with delta peak power (cc=-0.72, p=0.007, Fig. 2) and positively with gamma (30-100 Hz) peak power (cc=0.6, p=0.03).





Conclusions: The increased delta and decreased gamma activity might help to functionally delineate the NBM and might be applied for directing stimulation of the NBM and the development of biomarkers for adaptive algorithms.

1280

New method to evaluate new motor skill learning

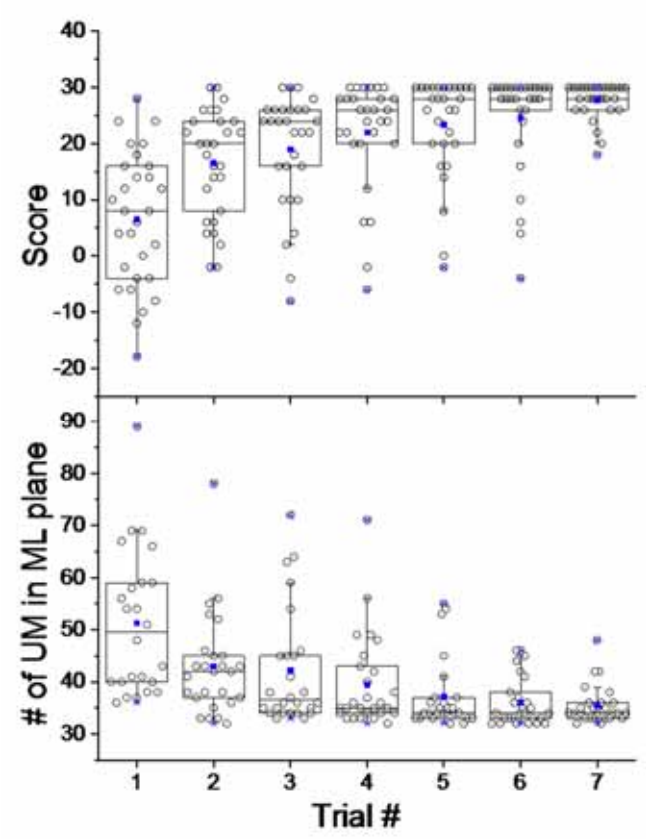
A.P. Quixadá, V. Sotero, J.F. Daneault, G. Diaz, Â. Torres, J.P. Vieira, M. Fonseca, P. Bonato, N. Peña, J.G. Miranda (Salvador, Brazil)

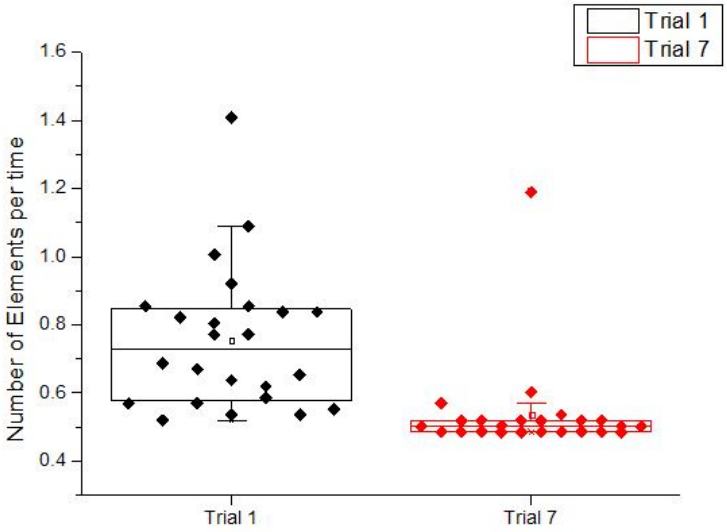
Objective: To present a novel approach to assessing improvements in new motor skill learning.

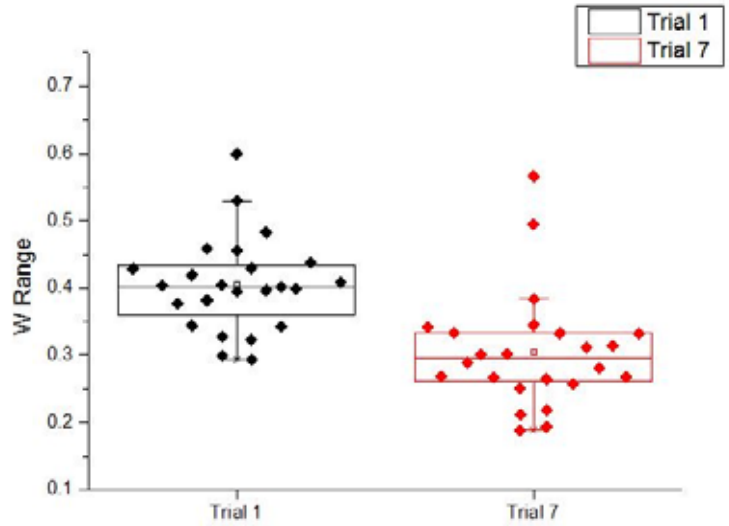
Background: Several methods have been employed over the years to try to understand how the central nervous system plans and controls voluntary movements. Despite the work that has been done over the last decades, studies have provided conflicting results and have continued to focus on simple reaching tasks. In the current study, we propose a novel method (movement elements decomposition - MED) that enables the analysis of simple and complex motor tasks to further understand how the brain manages the computational load associated with motor planning and control. We present an experiment that suggest that we can measure movement complexity and smoothness from the elements decomposition point of view.

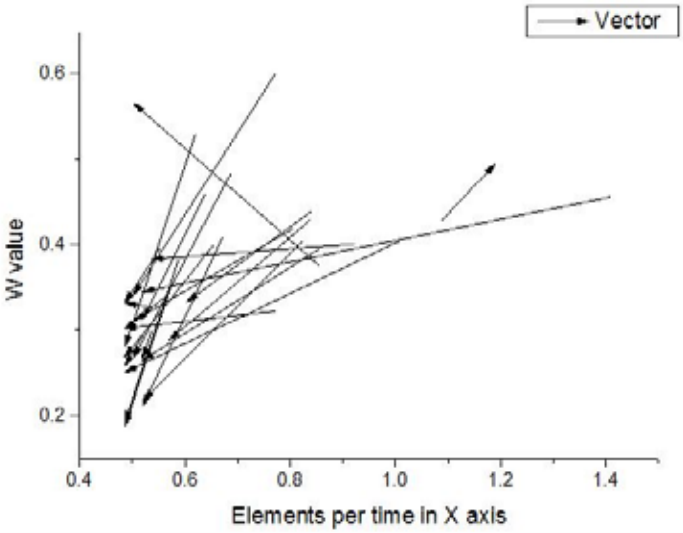
Methods: 29 Participants positioned themselves on the Wii Balance board® in order to catch the arrows in a game specifically developed for the purpose of this experiment. Participants were asked to control an on-screen target through lateral weight transfer on the Wii Balance Board® (Figure 1). Each participant performed seven trials; Participants had never used a Wii Balance board® prior to this study and no information about the game was previously provided to them. A GoPro camera was positioned in front of the participants in order to track the anatomical landmarks using the CvMob Software. In order to follow the optimization rule, all human movements have to begin and end with zero velocity. This is the basis of the hypothesis suggesting that complex movements need to be segmented by the CNS during motor planning and performance. To segment movements into ME we opted to define ME as a trajectory between two points with zero velocity. The number of movement elements were considered the complexity of movement and the natural response to motor learning is decrease complexity. To evaluate the smoothness we proposed a model as a neutral model of the intended movement and measured the irregularity *W*.

Results: Our results shows that the number of elements segmented in the horizontal axis and the W were significantly different between the first and last trial of the game ($p<0.01$), demonstrating a tendency to simplify and smooth the movement with practice (Figures 2, 3, 4 and 5).


Score
# of UM in ML plane
Trial #


Trial 1
Trial 7
Number of Elements per time
Trial 1
Trial 7





Conclusions: The number of elements and the irregularity W are good measures to identify improvement on new motor skills acquisition.

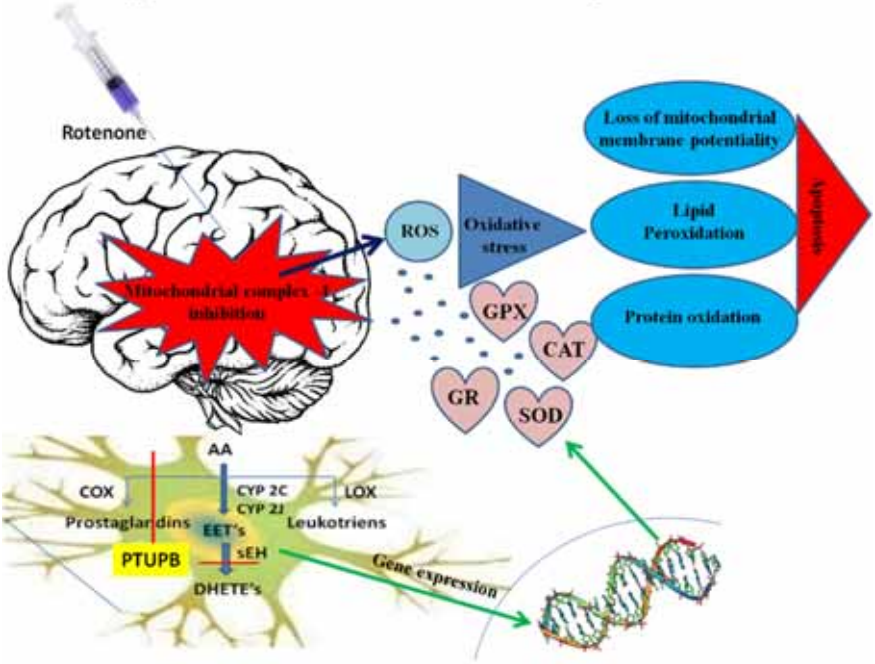
1286

Neuroprotective propensity of PTUPB, a dual inhibitor of sEH and COX-2 against rotenone induced neurotoxicity in cell line and Drosophila model of Parkinson's disease

N. Lakkappa, P. Thaggikuppe Krishnamurthy, P.D. Mirazkar, N. Lakkappa, P. Krishnamurthy, P.M.D., M.M. Srinivas Bharath, B. Hammock, S.H. Hwang (Udhagamandalam, India)

Objective: To evaluate the potential anti-parkinson activity of PTUPB (4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide) a dual inhibitor of soluble Epoxide hydrolase (sEH) and cyclooxygenase-2 (COX-2) against rotenone induced mitochondrial dysfunction, oxidative stress and neuroinflammation in N27 dopaminergic cell lines and *Drosophila melanogaster*.

Background: The metabolites of arachidonic acid cascade such as epoxyeicosatrienoic acids (EETs), is reported to play a crucial role in cytoprotection, due to their ability to attenuate oxidative stress, in ammation, and apoptosis. However EETs are subjected to rapid *in vivo* metabolism by sEH. The progressive neuroinflammation induces the release of COX-2 which is rapidly expressed in several cell types in response to cytokines, and pro-inflammatory mediators. The potential downstream toxic effects of COX-2 are further increase in progression of inflammation and oxidative stress via production of oxidizing reactive species during the peroxidase activity. Therefore, one of the novel strategy is inhibition of both sEH and COX-2 (Fig 1).



Methods: Cytoprotective role of PTUPB was confirmed in N27 cells against rotenone (400nM) treatment by MTT and LDH release assays. Further, total intracellular ROS, protein oxidation, lipid peroxidation, mitochondrial membrane potential, antioxidant, anti-inflammatory, and anti-apoptotic status was evaluated as per the standard protocols. Further, *in vivo* neuroprotective ability of PTUPB was confirmed against rotenone induced toxicity in *Drosophila*. Survival rate, negative geotaxis, antioxidant status, dopamine level was evaluated as per the standard protocols.

Results: Our results indicated that PTUPB pre-treatment significantly improved cell viability as confirmed by cytotoxicity assays. Further, neuroprotective role of PTUPB was associated with amelioration of ROS production, proteins oxidation and lipids peroxidation against rotenone induced toxicity. PTUPB also significantly protect the mitochondrial damage and improved the enzymatic antioxidant, anti-inflammatory, anti-apoptotic status which was declined with rotenone treatment in both N27 cell line and *Drosophila model of PD*.

Conclusions: These results substantiate the neuroprotective effect of PTUPB indicating its potential therapeutic benefits in the treatment of PD.

1323

Antiparkinson and antioxidant effect of n-methanesulfonylpyrazolinyl substituted heterosteroids in lps induced neuroinflammation model of rats

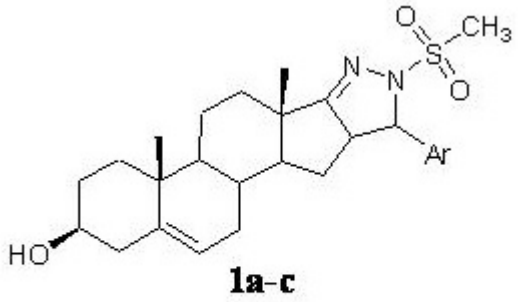
R. Singh (Chandigarh, India)

Objective: This present study is mainly focused at design and synthesis of new therapeutically useful steroidal neuroprotective agents against neuroinflammation which involved in the progression of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. The new androstane derivative has been synthesized by fusing *N*-methylsulfonyl substituted pyridylpyrazoline moieties at [17,16-*c*] position of the steroid. These D ring modified heterosteroids were then explored for their antiparkinson and antioxidant effect.

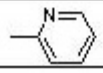
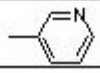
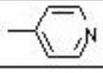
Background: Parkinson's disease (PD) is a progressive, disabling neurodegenerative disorder characterized by an insidious onset with variable expression of motor, vegetative, sensory and psychopathological symptoms. Recent literature reports indicated that neuroinflammation cause dopaminergic neuron death that involved in progression of PD.

Methods: Aldol condensation of dehydroepiandrosterone with requisite pyridine carboxaldehyde in basic medium gave corresponding 16-arylidene steroidal derivatives, treatment of which with hydrazine hydrate in refluxing methanol afforded pyrazoline substituted steroids. Pyrazoline intermediates were further treated with mesyl chloride at 0° C to afford target *N*-methyl sulfonyl substituted pyrazoline steroids **1a-c**. Rats (male wistar) were anesthetized with thiopental sodium (45 mg/kg, i.p.), stereotaxic surgery has been done and intranigral injection of LPS (10µg in 2µl) was infused into left substantia nigra using the Hamilton microsyringe.[3] Heterosteroids were evaluated against behavioral alternations using actophotometer, elevated plus maze at dose 2mg/kg after 7th, 14th and 21st day of LPS administration. Biochemical estimation of different makers for neuroinflammation, cholinergic activity and oxidative stress has also been carried out.

Results: The synthetic heterosteroids were characterized using IR, ^{1}H NMR. All heterosteroids **1a-c** exhibited potent activity against PD. The pyridin-4-yl group substituted steroid **1c** displayed activity comparable to that of standards dexamethasone and celecoxib.



1a-c

	a	b	c
Ar			

Conclusions: The findings suggests that *N*-methanesulfonylpyrazolinyl substituted exhibit potent anti-neuroinflammatory activity and could be useful for the prevention of PD and oxidative stress.

1326

Noladin ether attenuates MPTP -induced motor deficit by abrogating pro-inflammatory cytokines and striatal neurochemical alterations in rats

R. Deshmukh, S. Singh (Moga, India)

Objective: The present study was designed to investigate the therapeutic potential of noladin ether- a putative endocannabinoid against MPTP-induced behavioral, biochemical and neurochemical alterations in rats.

Background: Cannabinoid CB1 receptors are densely expressed in striatal neurons and suggested to influence dopaminergic signaling in basal ganglionic circuitry. Noladin ether is known to be a putative endocannabiod and is reported to modulate cannabinoid receptors. However, least is known about is neuroprotective potential.

Methods: MPTP (100µg/µl; intranigral) was infused in to substantia nigra pars compacta [SNpc, repeatedly on 1st, 4th and 7th day] in rats. Noladin ether, WIN55, 212-2 as cannabinoid receptor agonists and AM251 a CB1 receptor

antagonist was used. Following 1st MPTP infusion, rats were treated with noladin ether (0.01 and 0.1mg/kg, ip), WIN55, 212-2 (0.1mg/kg, ip), alone or in combination with AM251 (0.025mg/kg, ip) for 21 days to confirm the involvement of the CB_1 receptors. Motor abnormalities were assessed by grip strength, narrow beam walk, open field and rotarodtests on a weekly basis.On 22nd day rats were sacrificed, and the striatal brain region was used for determining the levels of inflammatory cytokines, dopamine (DA) and its metabolites, GABA and glutamate.
Results: MPTP produced significant motor deficit, which was accompanied by increase in striatal cytokines and glutamate and a significant deficiency in DA and GABA levels. Both,noladin ether and WIN55, 212-2 attenuated the MPTP-infused motor deficit and restored striatal neurochemical alterations in rats. 30min prior administration of AM251 significantly abrogated the beneficial effects of noladin ether, indicating CB_1 receptor dependency.
Conclusions: The current findings clearly indicate the neuroprotective potential of noladin ether and demonstrate important role of CB1 receptor in neuroinflammation and the striatal neurochemistry. Together, our results suggest that the therapeutic potential of CB1 receptor ligands in the treatment of Parkinson's disease.

1394

Focused ultrasound subthalamotomy for Parkinson's disease: A pilot study

R. Martinez-Fernandez, R. Rodriguez-Rojas, M. del Álamo, F. Hernandez, J.A. Pineda-Pardo, M. di Leone, F. Alonso, I. Obeso, C. Gasca-Salas, E. de Luis, L. Vela, J.A. Obeso (Mostoles, Spain)

Objective: To evaluate feasibility, safety and effectiveness of unilateral subthalamotomy performed with MRIgHIFU to treat the cardinal motor features of PD.
Background: Ablative functional neurosurgery for the treatment of Movement Disorders and, particularly, of Parkinson`s disease (PD) has been applied for decades. However, after the resurgence of deep brain stimulation, lesional procedures were practically abandoned. In the case of subthalamotomy, the main reason was the concern of inducing hemichorea-hemiballism. This is now revisited due to the development of MRI guided high intensity focused ultrasound (MRIgHIFU).
Methods: This is an open-label, single center study. Ten PD patients with markedly asymmetric parkinsonism who were not optimally controlled with medication were entered into the study. Motor features were assessed by the MDS-UPDRS III at baseline, 1 month, 3 months and 6 months after treatment. Motor complications were evaluated through MDS-UPDRS IV and the Dyskinesia rating scale (DRS). PDQ39 provided an estimation of patient's quality of life. Levodopa daily equivalents were measured. Adverse events were recorded.
Results: Ten patients (6 males) were treated. Mean age was 59.5 and disease evolution 6.3±2.5 years (range, 3 to 10). MDS-UPDRS III score for the treated hemibody presented a reduction of 55 and 51% between baseline and 6-month visit in the off and on-drug states respectively (from 16.6±2.9 to 7.5 ± 3.9 and from 11.9±3.1 to 5.8±3.5, $p<0.001$). Total motor MDS-UPDRS improved up to 35 and 32% (from 32.7±5.4 to 21.2±8.2 $p<0.005$, and from 21.5±6.3 to 14.5±5.3 $P=0.02$). In both off and on conditions rigidity was the most improved motor feature (72% and 91% $p<0.001$, respectively) whereas akinesia was the least (40% $p=0.01$, and 28% $p=0.07$, respectively). Subthalamotomy resulted in no change in dyskinesia scores whereas off-dystonia improved significantly in both the MDS-UPDRS IV and DRS assessments. No significant changes were found in the PDQ39SI. Levodopa equivalents were reduced by 23% ($p=0.003$). There was no hemichorea-ballism immediately after treatment. One patient developed upper limb chorea 5 days after treatment that progressively reduce and was minimal at 6 months
Conclusions: This pilot study suggests that MRIgHIFU subthalamotomy is feasible, safe and effective for the treatment of PD motor features. Randomized control trial with a larger sample is mandatory to confirm this promising evidence.