DIFFERENTIAL PROTEIN AND NEUROPEPTIDE EXPRESSION IN EXPERIMENTAL PARKINSON'S DISEASE.

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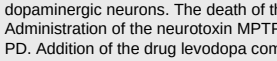
INTRODUCTION
Parkinson's disease (PD) is a common neurodegenerative disorder characterized by a selective degeneration of the nigrostriatal dopaminergic neurons. The death of these neurons causes symptoms as slowness of movement, muscular rigidity and resting tremor. Administration of the neurotoxin MPTP causes similar degeneration, and this procedure has been widely used to generate animal models for PD. Addition of the drug levodopa compensates for the lack of dopamine and temporarily reduces the motor symptoms of PD.

In the present study two-dimensional fluorescence difference gel electrophoresis (DIGE) was used to pinpoint significant differences in protein abundance during dopamine denervation after MPTP treatment in the striatum. Secondly, we have developed a peptidomic approach to study a large number of neuropeptides and employed it to an investigation of the endogenous neuropeptide content of PD. Finally, direct molecular profiling of the biological samples using MALDI-MS was shown to be a powerful tool for investigating the spatial distribution of peptides and proteins directly on tissue samples.

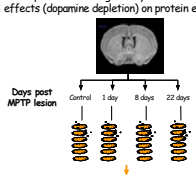
PROTEOMICS

Application of DIGE (Differential Imaging Gel Electrophoresis) - MS system to Parkinson's disease model



- **Mouse model of Parkinson's disease**
  - Mice treated with neurotoxin to create brain lesions that mimic Parkinson's disease (degeneration of dopamine neurons)
  - Dissect striatum region of brain
- **Experimental design with internal standard**
  - 6 biological replicates for each sample measures inter-animal biological variation
  - Control (untreated) vs. MPTP treated samples after 1 / 8 / and 22 days

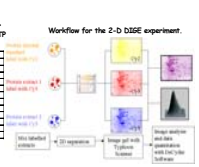
DIGE experimental design: analysis of MPTP treatment effects (dopamine depletion) on protein expression



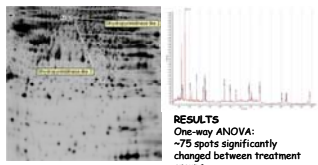
4 GROUPS
a) saline treated controls, b) MPTP 1 day, c) MPTP 8 days, d) MPTP 22 days

DIGE experimental design for analysis of the effect of MPTP treatment.

Gel	Cy3	Cy5	Cy2
1	Ref	Cy5	1D
2	Ref	Cy5	8D
3	Ref	Cy5	22D
4	Ref	Cy5	1D
5	Ref	Cy5	8D
6	Ref	Cy5	22D
7	Ref	Cy5	1D
8	Ref	Cy5	8D
9	Ref	Cy5	22D
10	Ref	Cy5	1D
11	Ref	Cy5	8D
12	Ref	Cy5	22D



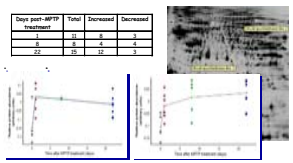
- DIGE gel image showing the location of spots picked for identification by mass spectrometry (left)
- MALDI-ToF Pro PMF trace of dihydropyrimidinase-like protein 2 (right)



RESULTS
One-way ANOVA:
~75 spots significantly changed between treatment groups

DIGE analysis Exp. Parkinson's disease

Days post-MPTP treatment	Total	Increased	Decreased
1	21	8	3
8	8	4	4
22	25	12	3



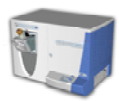
DeCyder graph showing the variation of dihydropyrimidinase-like protein 2 and dihydropyrimidinase-like protein 3 following MPTP treatment (identified after PMF database searching).

Peptide Identification

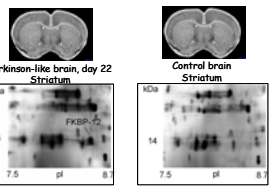
Software for automated Peptide Mass Fingerprinting (PMF) analysis

MALDI-TOF MS

Electrospray MS/MS analysis



INCREASED EXPRESSION OF FKBP-12 IN PARKINSON



CONCLUSIONS
- This study demonstrates significant changes ($p<0.05$) in 50 gel spots following dopamine depletion using an animal model of Parkinson's disease. 16 proteins have been identified.
- 34 differentially expressed protein spots were identified as significantly changed in comparison to the saline treated controls.
- Using the 2-D DIGE integrated system, a number of proteins potentially important in the development of Parkinson's disease have been identified by using MALDI MS and nano ESI MSMS. Work is ongoing to discern their biological importance in Parkinson's disease.

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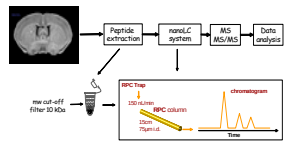
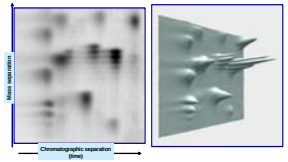
PEPTIDOMICS

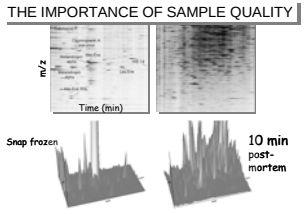
Schematic flowchart of the peptidomics experimental setup

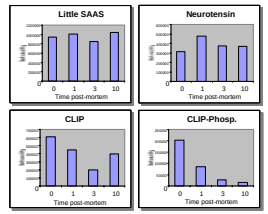


"Signal Intensity Map" representation

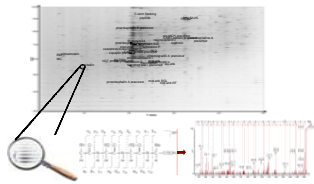


THE IMPORTANCE OF SAMPLE QUALITY

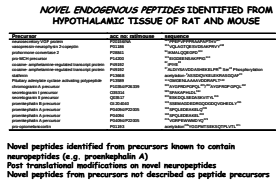




Discovery of Novel Neuropeptides

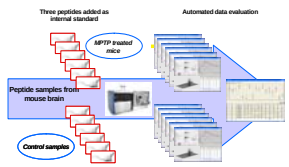


NOVEL ENDOGENOUS PEPTIDES IDENTIFIED FROM HYPOTHALAMIC TISSUE OF RAT AND MOUSE

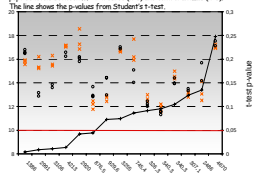


- Novel peptides identified from precursors known to contain neuropeptides (e.g. proenkephalin A)
- Post translational modifications on novel neuropeptides
- Novel peptides from precursors not described as peptide precursors

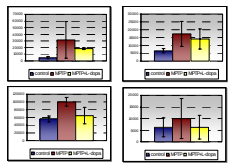
Neuropeptide application - Parkinson



Preliminary results from a fully automated analysis of the differences in peptide levels between MPTP treated ($n=3$) and control animals ($n=3$). The line shows the p-values from Student's t-test.

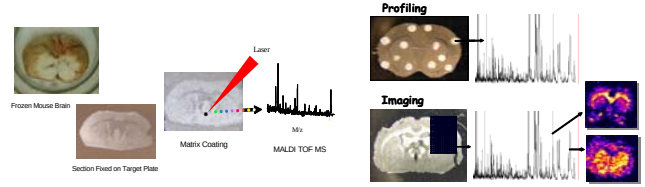


Novel Peptides derived from proenkephalin A

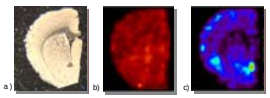


CONCLUSIONS
- Novel peptides identified from precursors known to contain neuropeptides (e.g. proenkephalin A)
- Novel peptides identified from precursors not described as peptide precursors
- Post-translational modifications of known and novel neuropeptides identified
- The rel. concentration for several novel and known neuropeptides differs between 6-OHDA treated (Parkinson-like) and untreated (control) side of the brain (striatum)

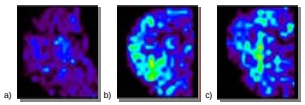
MALDI IMAGING/PROFILING



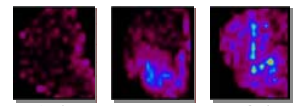
The present results show that the MALDI imaging technique provides unique information about the expression and distribution of proteins and peptides in the brain of an experimental model of Parkinson's disease.



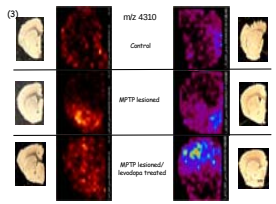
The figures above show the orientation of one of the hemispheres from a mouse brain. a) The digital photo of the mounted tissue, b) The total ion intensity from all the pixels, c) The distribution of the peptide at m/z 4280 in one of the samples.



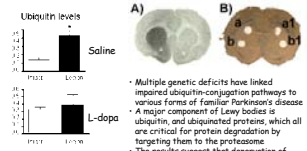
The ion density map of the peptide at m/z 6310. a) Saline treated. b) MPTP-lesioned. c) MPTP-lesioned/levodopa treated.



The figure above displays the distribution of the peptide m/z 2770 in a control (saline treated), MPTP-lesioned animal and an animal that were treated with levodopa after MPTP-lesioning.

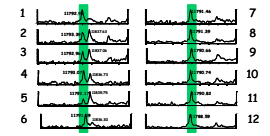


Increased levels of ubiquitin in the 6-OHDA-lesioned striatum

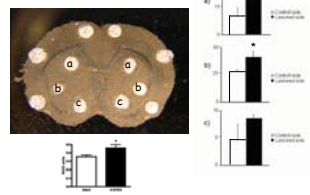


- Multiple genetic deficits have linked impaired ubiquitin-conjugation pathways to various forms of familiar Parkinson's disease
- A major component of Lewy bodies is ubiquitin, and ubiquinated proteins, which all are critical for protein degradation by targeting them to the proteasome
- The results suggest that denervation of dopamine neurons *per se* is implicated in the regulation of ubiquitin pathways

Protein FKBP-12 in brain tissue sections.
MW 11791, 107 AA



MALDI Profiling of FKBP-12 Striatum



FKBP12 mRNA levels in the intact and the denervated hemisphere

CONCLUSIONS
Using the combination of 2-D DIGE (proteomics), nanoLC MS (peptidomics), and MALDI Imaging, a number of peptides and proteins potentially important in the development and treatment of Parkinson's disease have been identified.