

*Analysis of volatile organic compounds in rats with
dopaminergic lesion:
possible application for early detection of Parkinson's
disease*

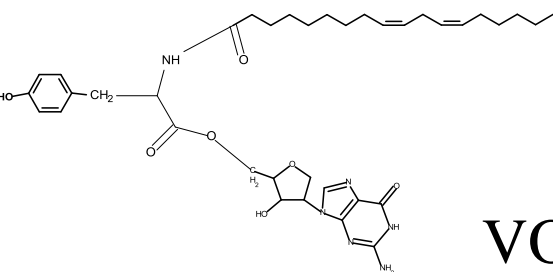
By
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Oxidative Stress and Human Diseases Research Laboratory – Galilee Research
Institute (Migal), Israel.*

Oxidative stress and human diseases laboratory activities

Markers

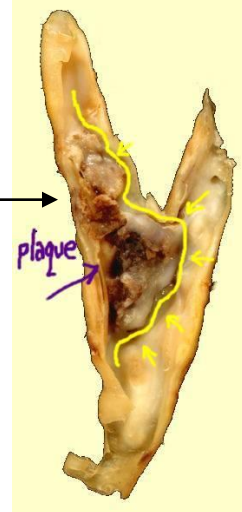
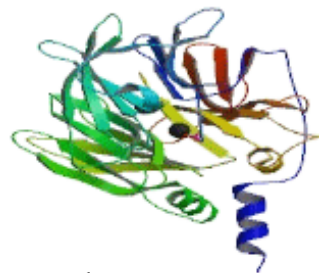
Alzheimer, Parkinson,
diabetic complications,
atherosclerosis,
depression



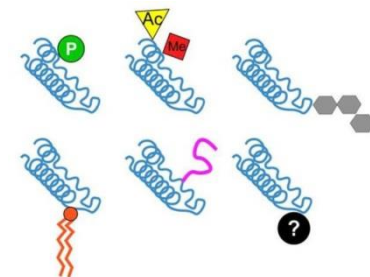
VOCs as Biomarkers

Blood

Human
carotid
plaque



Protein
oxidation and
modifications



Analytical chemistry
Isolation, Identification

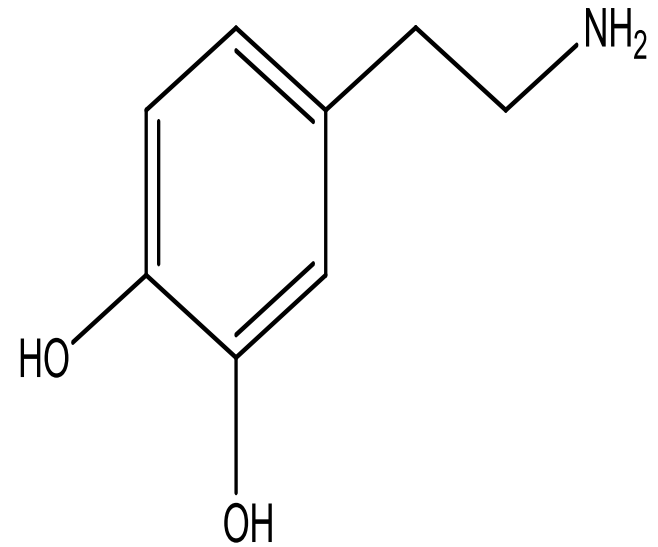
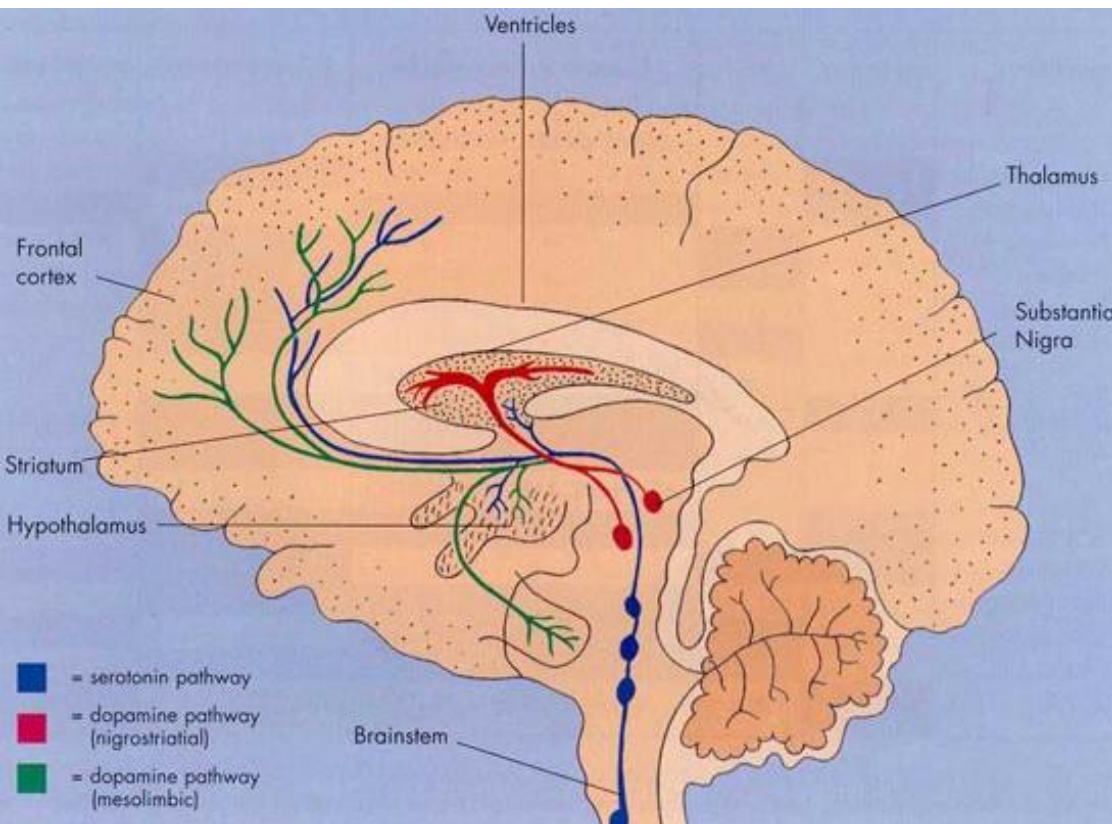
Parkinson's disease

- Parkinson's disease (PD): one of the most prevalent neurodegenerative disorders
- Affecting :
 - 0.3% of the entire population,
 - 1% of the people over 65yr,
 - 4% of the people over 80yr.



Parkinson's Disease (PD) Pathology

- Pathology is characterized with the death of **dopaminergic neurons** in the substantia nigra Para compacta and a reduction in **dopamine (DA)** levels in the striatum and the presence of "Lewy Bodies".



Symptoms of PD

- Tremor



- Bradykinesia



- Postural instability



- Rigidity





Parkinson's disease and dopamine

loss of 50-70% of **Dopaminergic** neurons



80% reduction in the striatal dopamine



Early detection of PD

- **Early detection** of PD is important as it enables slowing or stopping its progression using neuroprotective drugs.
- Although there are several biomarkers for clinical diagnosis, none of these is in routine clinical use.
- Thus, a need for an early, reliable, sensitive and specific diagnostic biomarker still exists.



Aim of the study

Early detection of PD



Hypothesis

PD



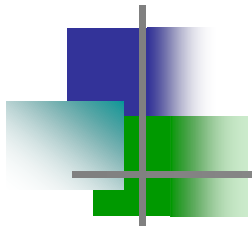
Biochemical pathways specific to the disease



Volatile organic compounds (VOCs) in
blood



Early detection of PD stage and progression

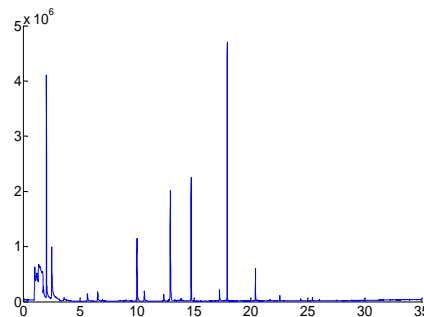
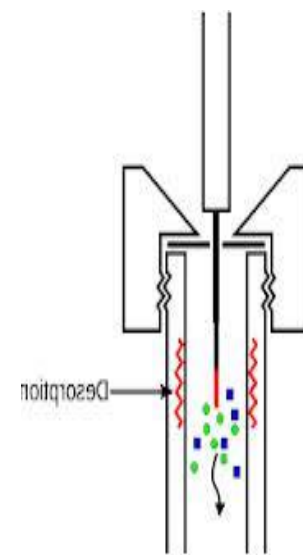
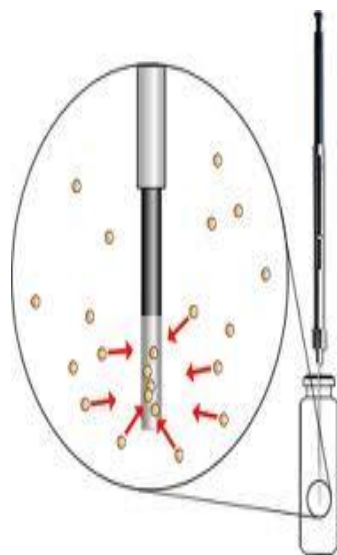


Objectives

- 1. To identify VOCs in rates blood that can be specific to Parkinson disease.
- To investigate the mechanism of such specific VOCs generation and their association with the disease.

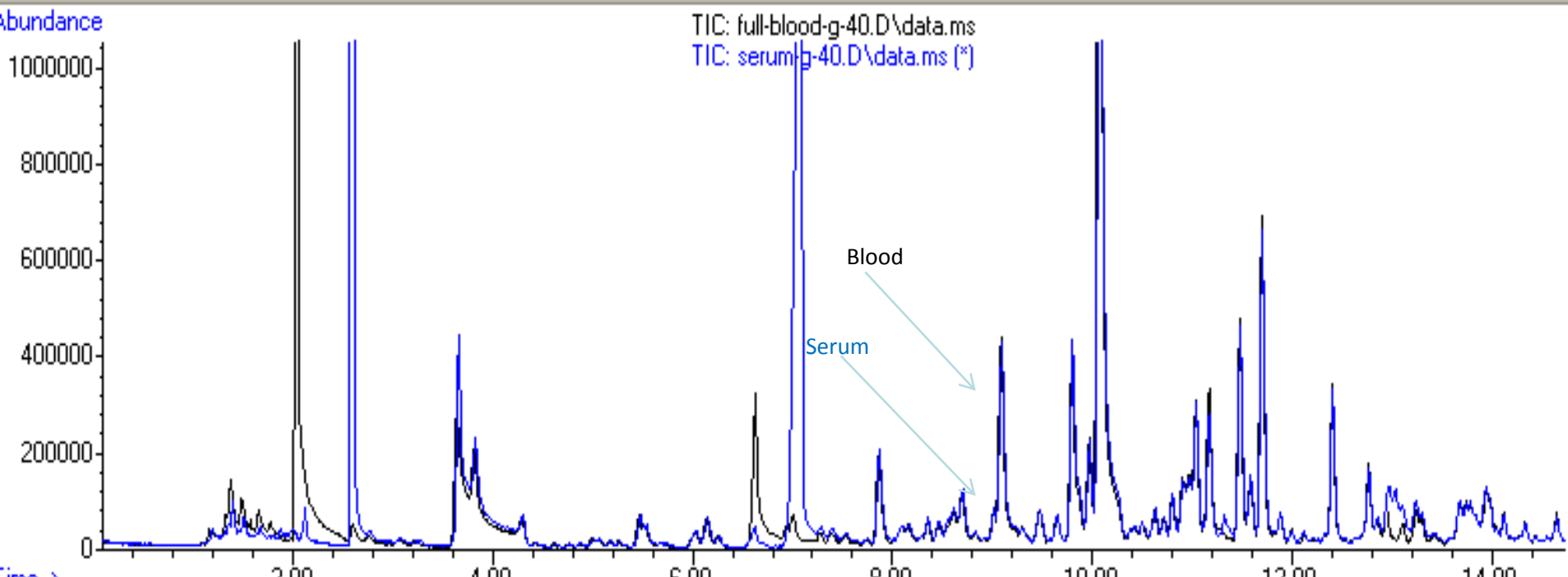
SPME Method

- VOCs can be detected by using the simple and reliable **Solid-Phase Micro Extraction (SPME)** technique, combined with **Gas-Chromatography Mass Spectrometry (GC-MS)**.



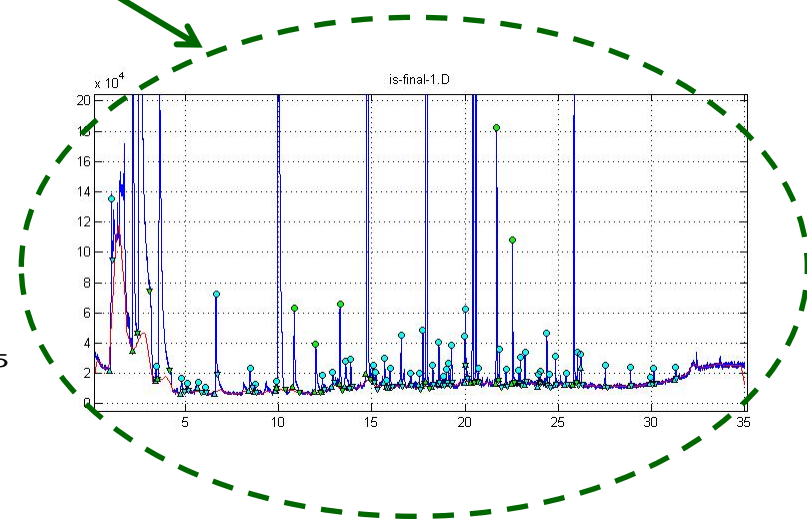
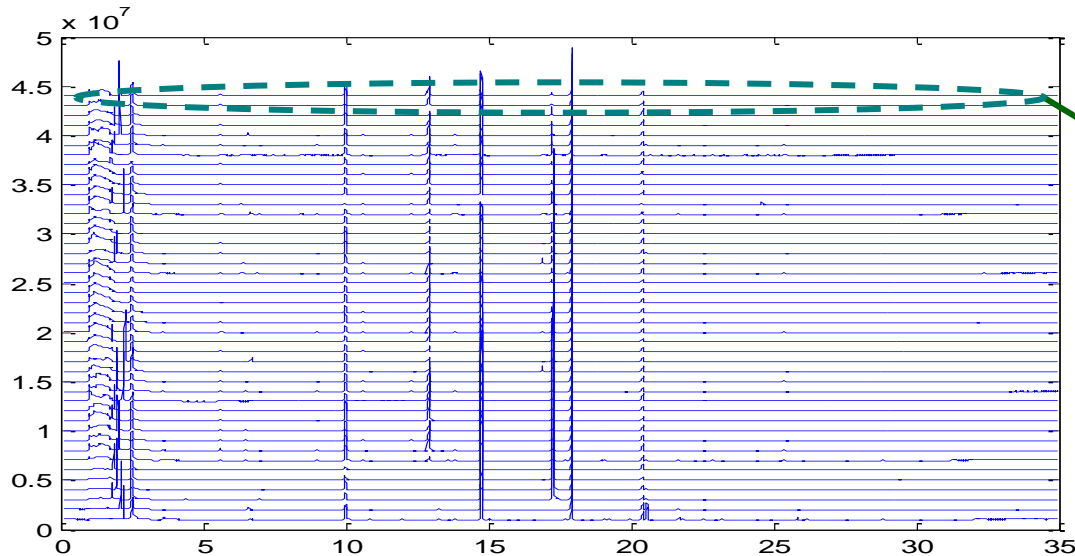
Optimization of the SPME method

1. Fiber type: (DVB/CAR/PDMS) (gray fiber) or (PDMS) (red fiber).
2. SPME Extraction Temperature (40C Vs 90C)
3. Blood or Serum



The requirement for the algorithm

- In each experiment – numerous GC-MS peaks
- Each chromatogram contains hundreds of peaks
- The need for an automatic and reliable algorithm for data handling, signal processing and analysis

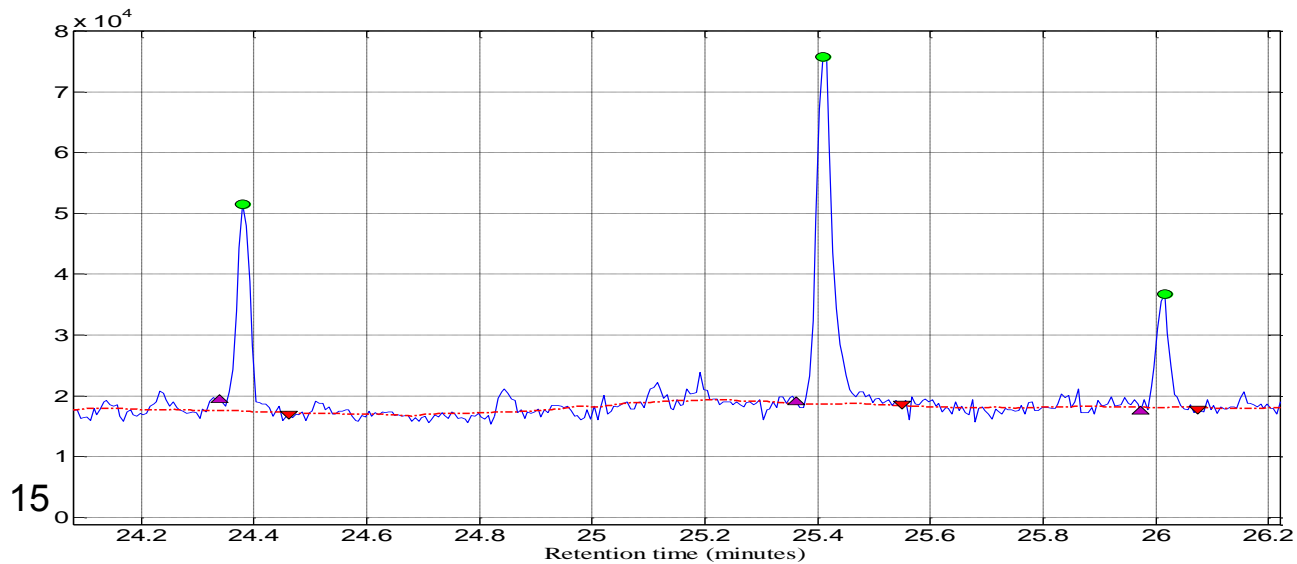
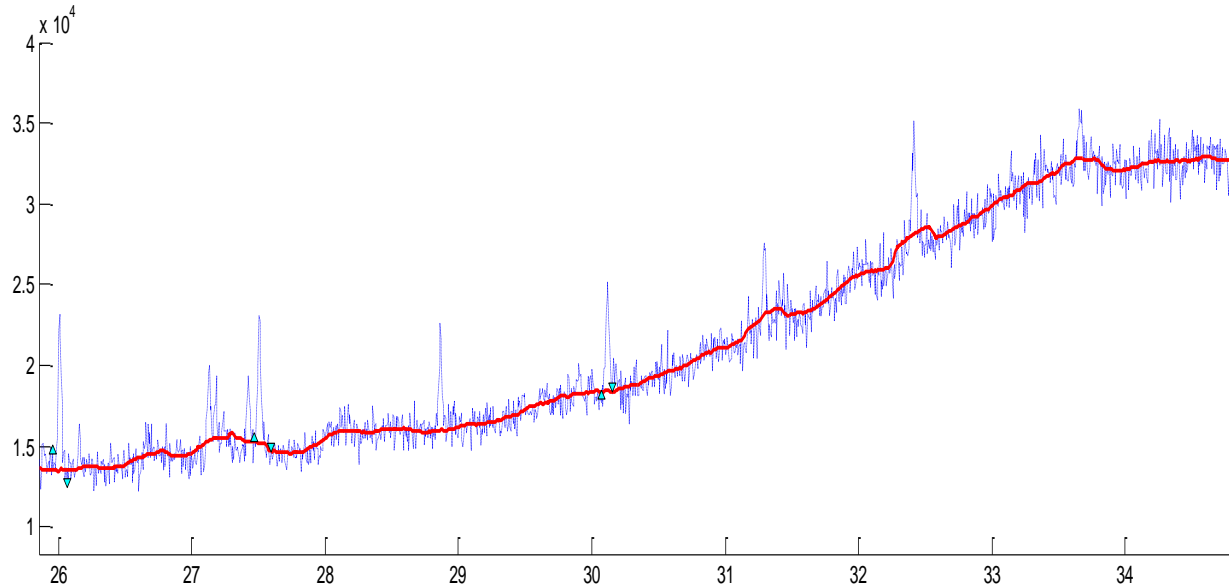




Development of an algorithm for data handling and processing.

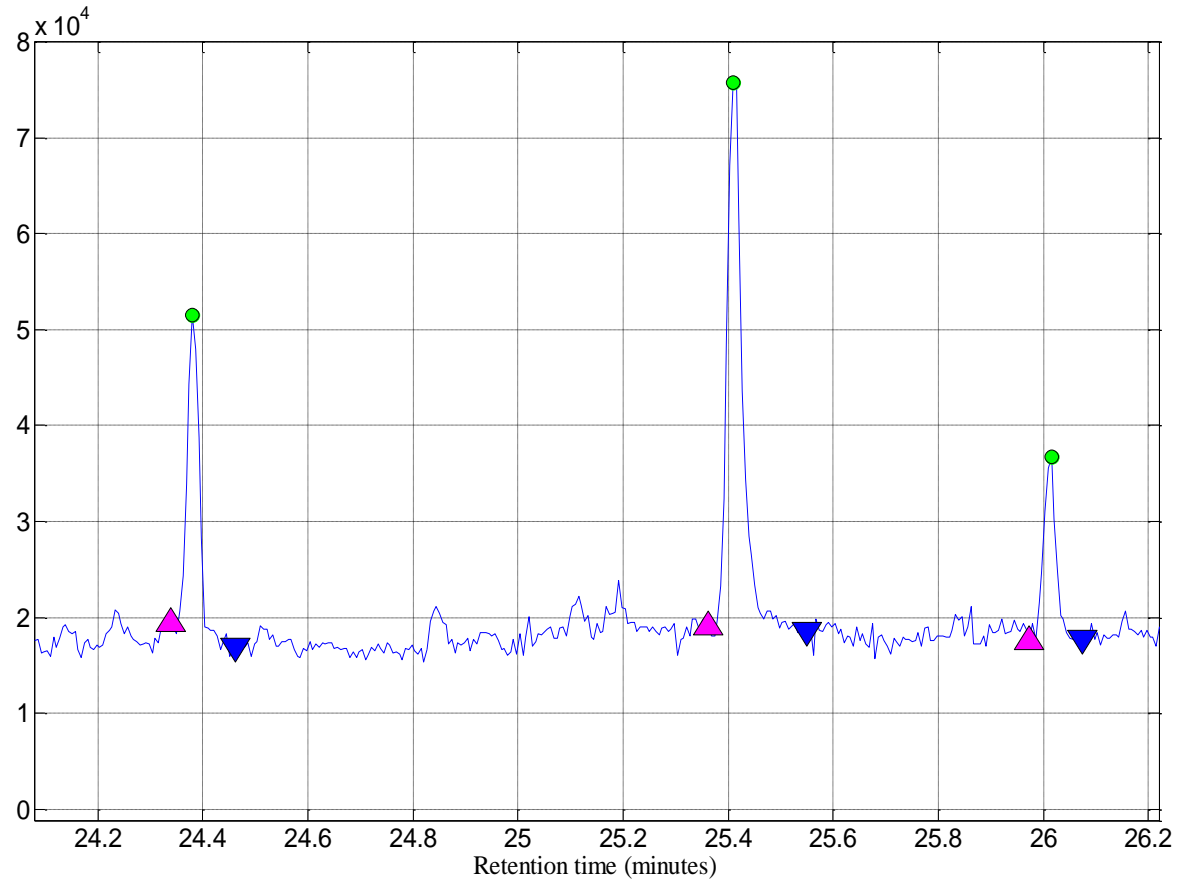
- accurately **detecting and demarcating** the peaks in the GC-MS chromatogram.
- calculating the **area under each detected peak**
- **eliminating peaks** originated from **blanks**,
- Normalization of the area under each peak using internal standards
- finding and selecting sample peaks with **areas significantly higher or lower than in control.**

Baseline computation and removal

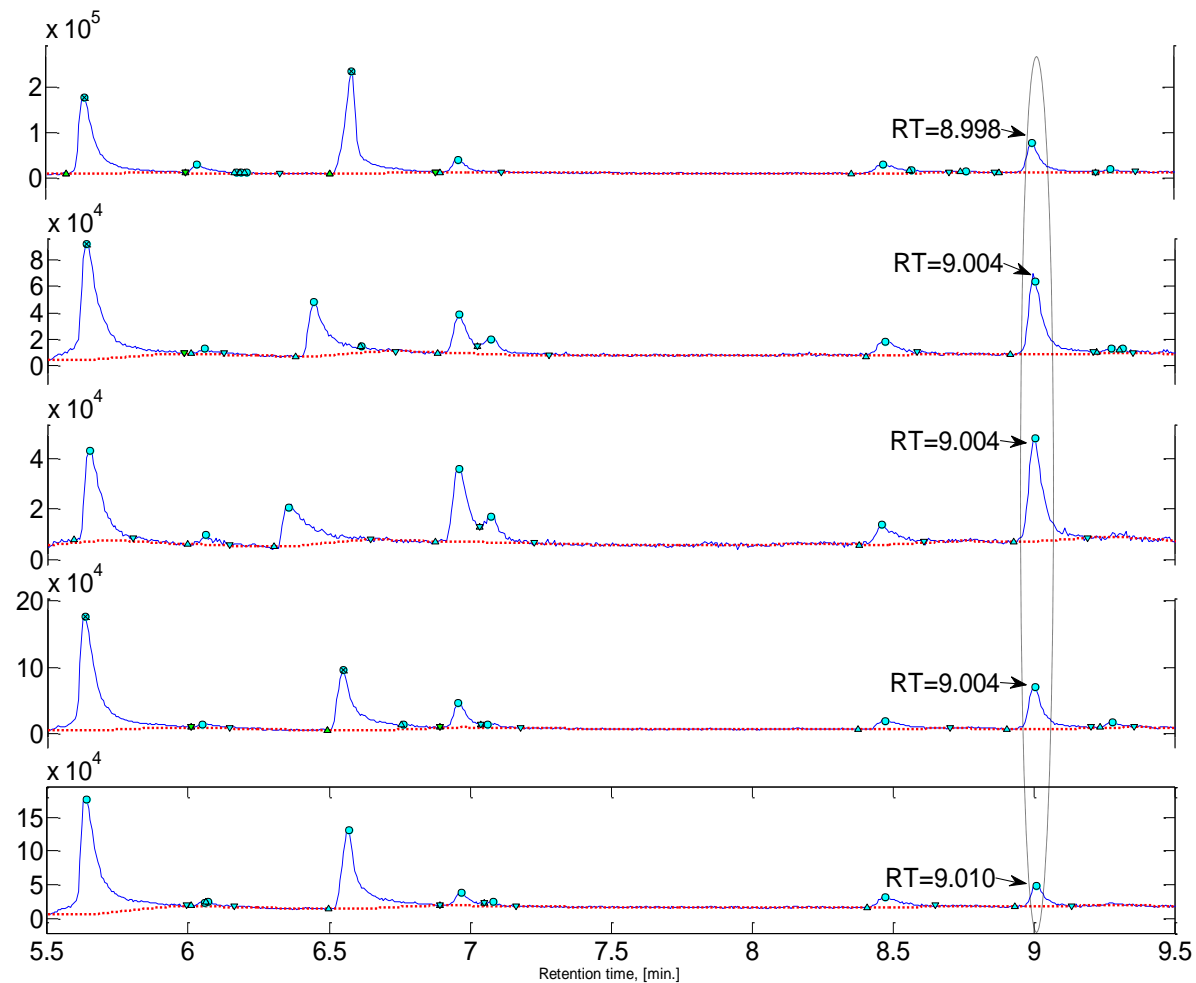


Peak detection and boundary demarcation

- “Peak picking”
- Selecting peaks: peak-to-noise ratio > 3dB
- Left and right boundaries

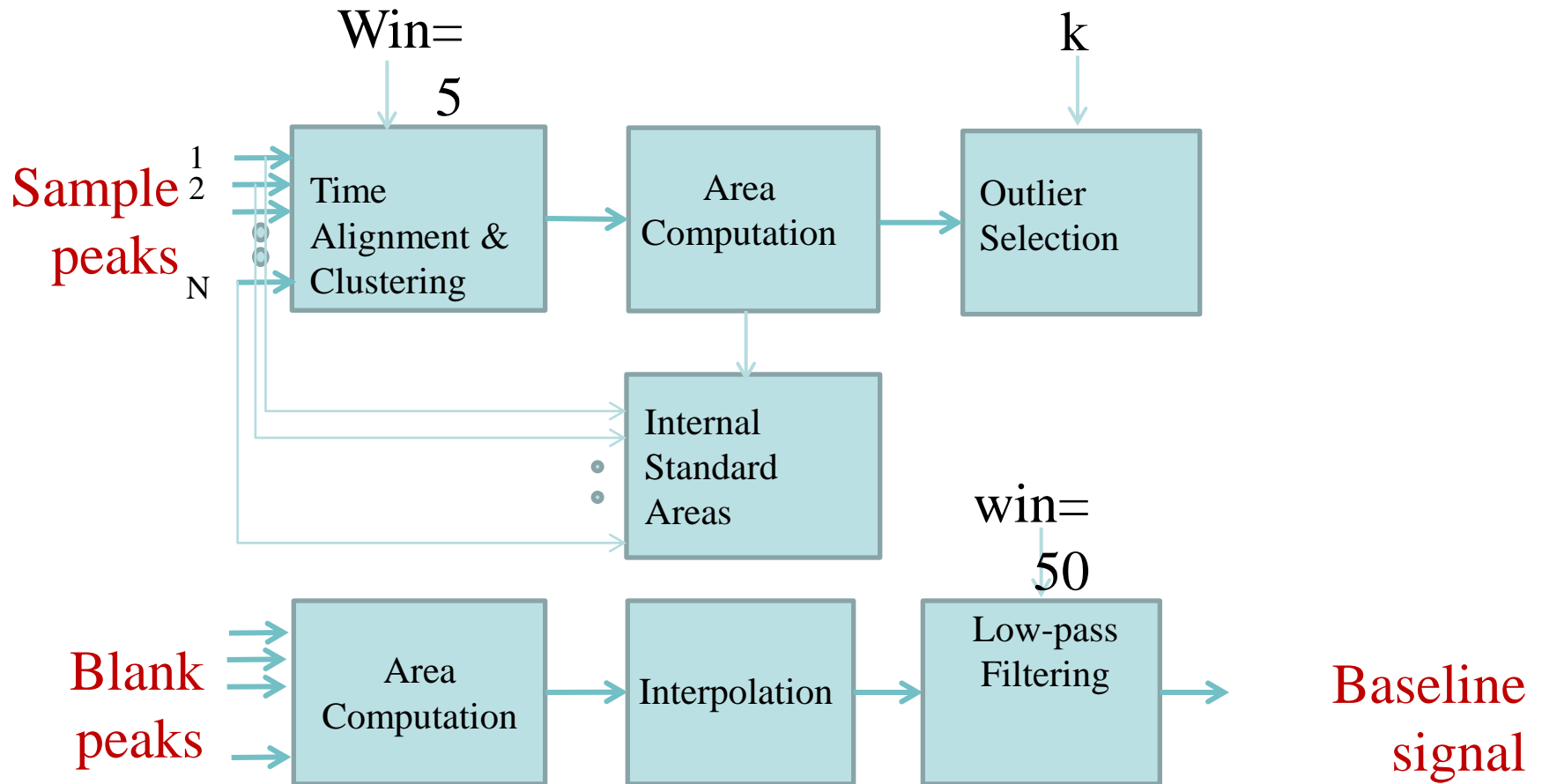


Peaks alignment and grouping

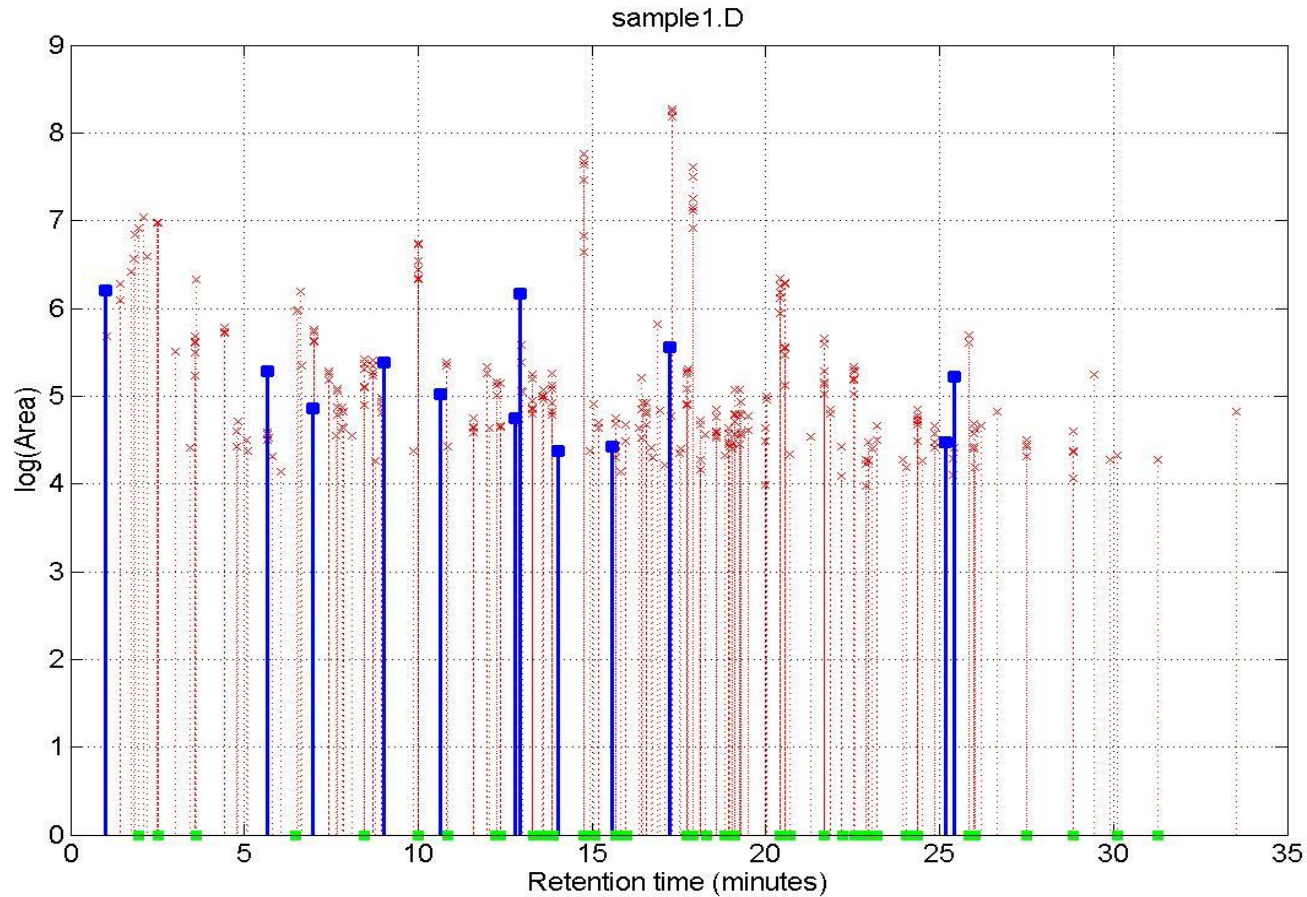


Aim: targeting peaks partial GC/MS signals from 5 different samples, each contains several local maxima, aligned on a common time-scale. The retention times of peaks which stem from the same compound are shown for all samples, demonstrating the small shifts between different samples.

Area computation and Internal Standard Correction



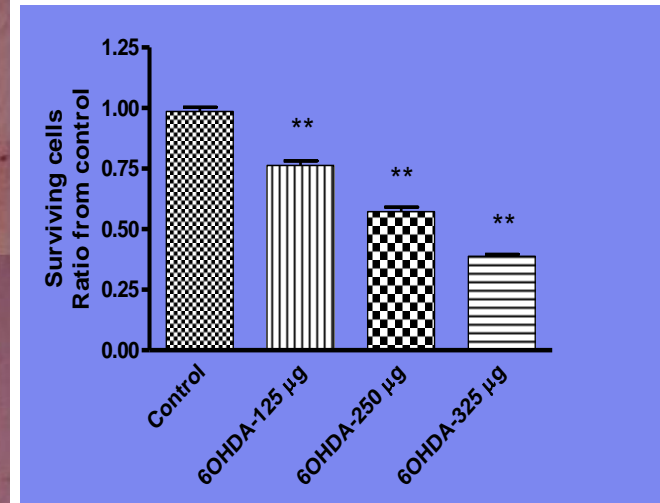
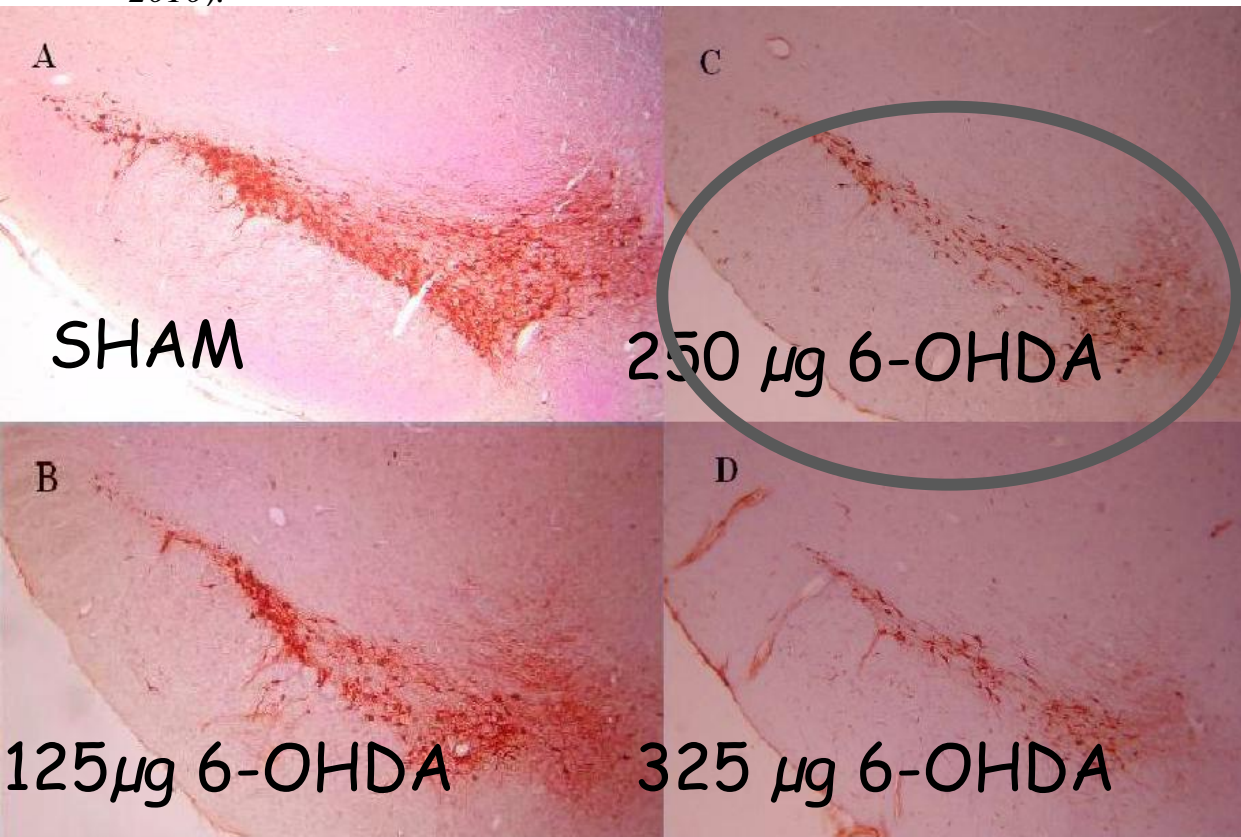
Peaks vs. Blanks



Area versus retention-time of all peaks in all blood samples. Dotted red lines represent peaks originated from blanks, and solid blue lines represent peaks where no blanks were present.

PD Rates model

Rat model: Stable- 50% depletion of dopaminergic neurons by injection of 6-OHDA i.c.v into the left lateral ventricle (Aluf et al. *FRR*, 2010).



(** $P < 0.01$, one way ANOVA, $n = 7$)



Method

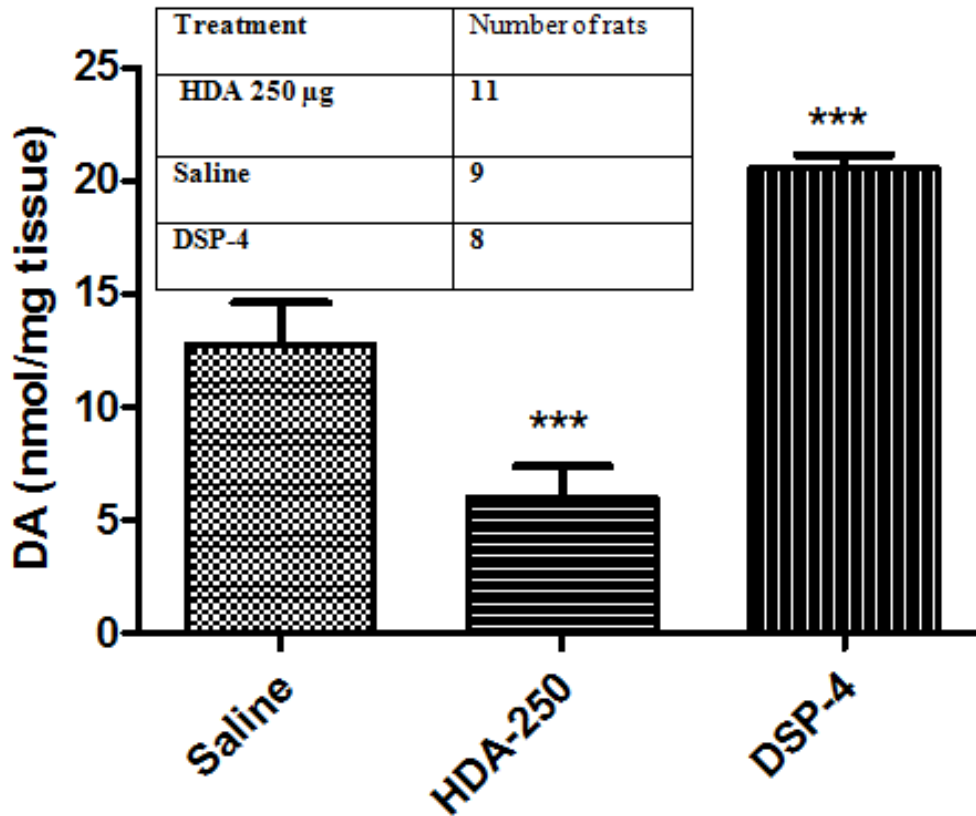
- 1- Rats were injected by i.c.v into the left lateral ventricle with 250 μg 6-OHDA, or i.p injection of (DSP-4), 50 mg/kg or seline.
- 2- after 5 weeks blood was collected.
- 3- rats were decapitated, the brain removed and the striatum dissected , weighted and homogenized into PBS.
- 4- dopamine content in striatum determined using HPLC connected to ECD.
- 5- VOCs from Blood and striatum homogenate were determined using SPME-GC-MS method PBS was used as blank.
- 6- data processed and analyzed using the developed algorithm.

Dopamine levels in the striatum homogenate of rats

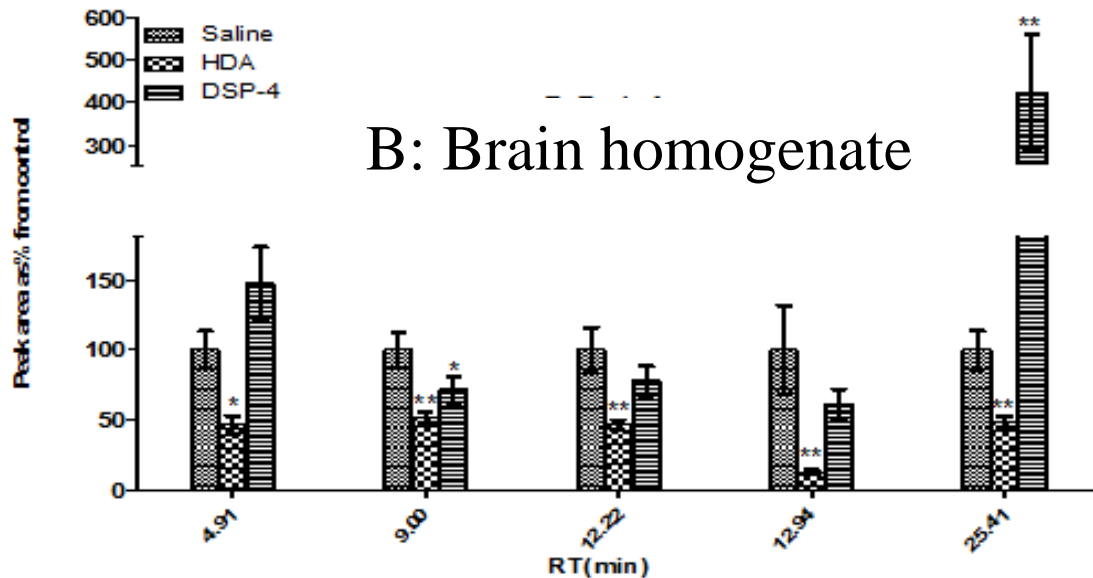
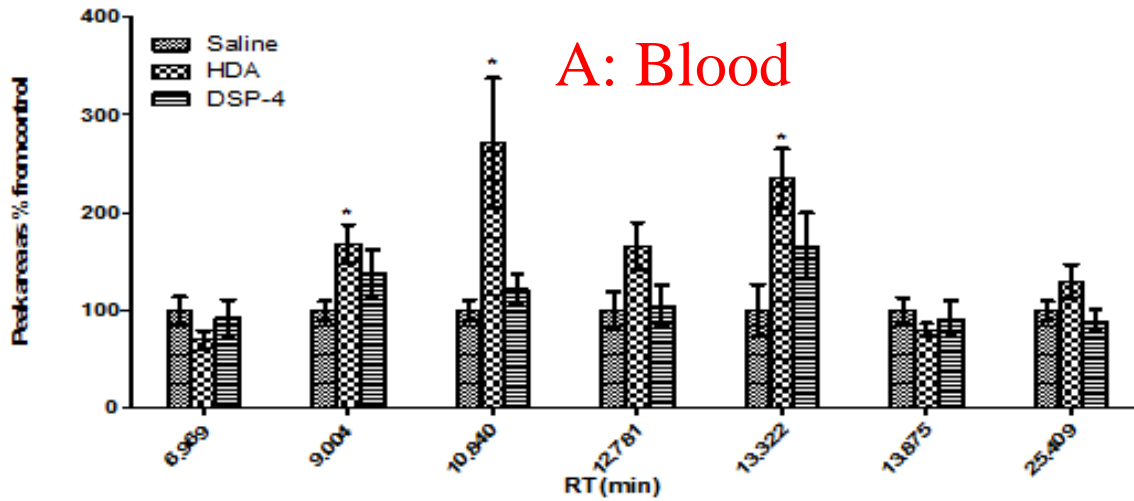
DA= Dopamine

HDA= 6-hydroxydopamine (HDA)

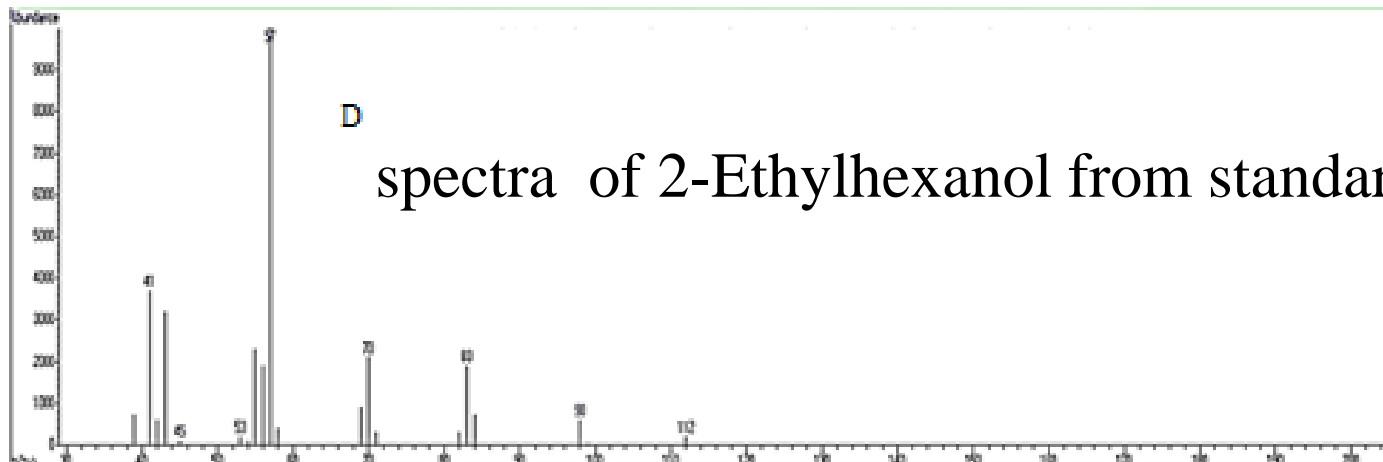
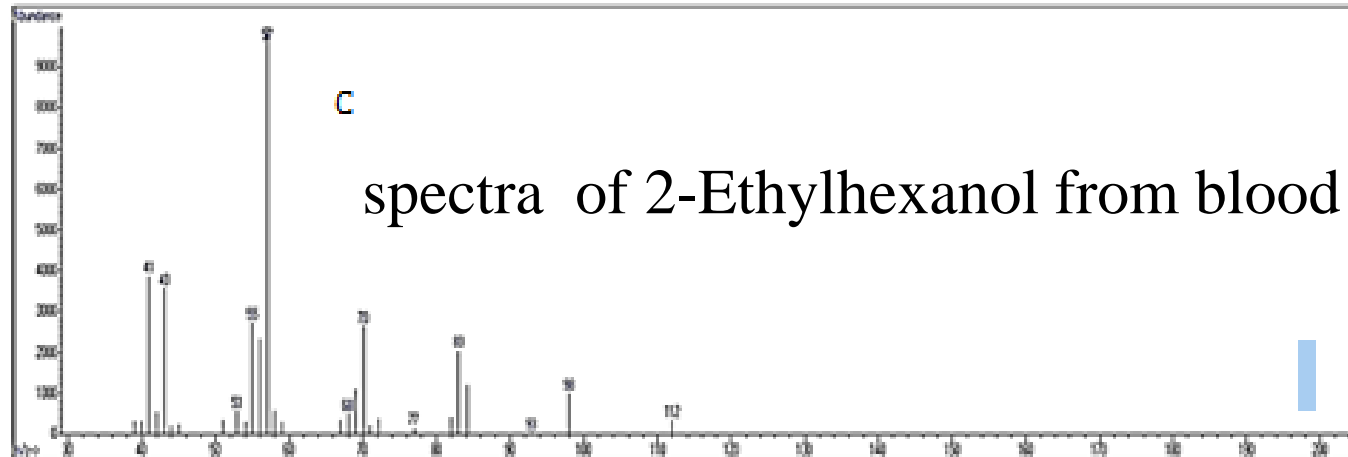
DSP-4= N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine



The level of VOCs in treated versus control rats as analyzed by SPME/GC-MS.



The MS fragmentations of peaks





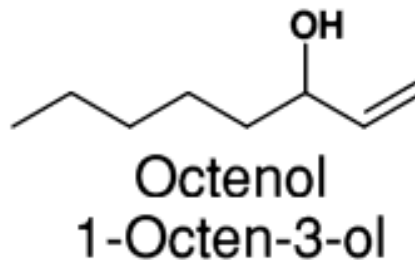
VOCs detected in blood or Brain Homogenate

Table 1: VOCs detected in either the blood or brain which were higher/lower or specific in HDA Rats as compared to control rats. RT= retention time of the material on the GC-MS chromatogram.

compound		RT(min)	% of change between rats and controls	P value
Hexanoic acid, methyl ester	Blood	6.97	-30%	0.08
1-OCTEN-3-OL	Blood	9	+70%	0.04
2-Ethylhexanol	Blood	10.84	+171%	0.04
n-Hexanol	Brain	4.9	-53%	0.01
1-OCTEN-3-OL	Brain	9	-50%	0.002
2-Octen-1-ol	Brain	12.22	-54%	0.003
Methyl benzoate	Brain	12.94	-87%	0.004
Methyl palmitate	Brain	25.4	-45%	0.002



VOCs detected in blood or Brain Homogenate



1-Octen-3-ol is neurotoxic in the *Drosophila melanogaster* model and to human embryonic stem cells. ([Inamdar et al Neurotox Res 2014](#))

it reduces dopamine levels, causes dopamine neuron degeneration. ([Inamdar et al. PNAS, 2013;](#))

1-Octen-3-ol has been detected in cow milk ([van Aardt et al., 2005](#)), human sweat ([Cork and Park, 1996](#)), and feces ([Garner et al., 2007](#)).



Summary

- Three groups of rats were studied: DA-lesioned rats injected with 6-hydroxydopamine (n=11); control rats injected with saline (n=9); and control rats injected with DSP-4 (n=8), a specific noradrenergic neuron toxin.
- The SPME method was optimized for testing Blood VOCs
- An algorithm for data processing was developed
- In the blood, 1-octen-3-ol and 2-ethylhexanol were found at significantly higher concentrations in HDA-treated versus sham rats.



Summary

- In the striatal homogenate 1-octen-3-ol and other four compounds were found at significantly lower concentrations in HDA rate

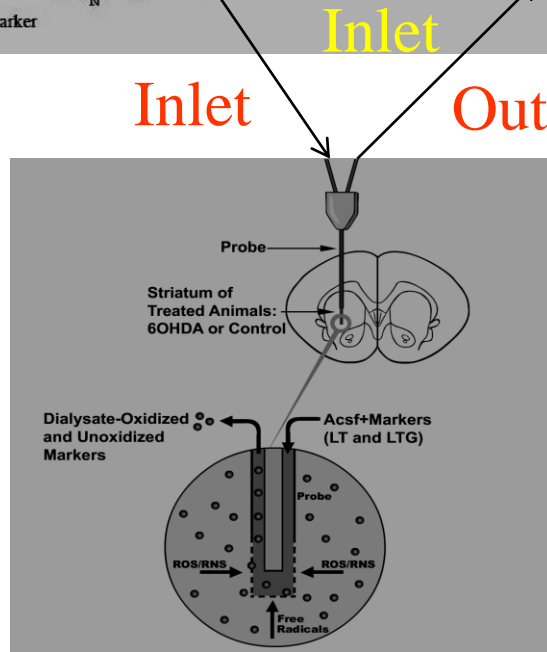
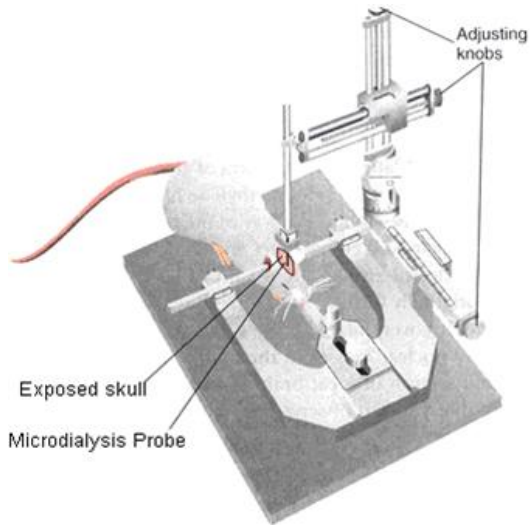
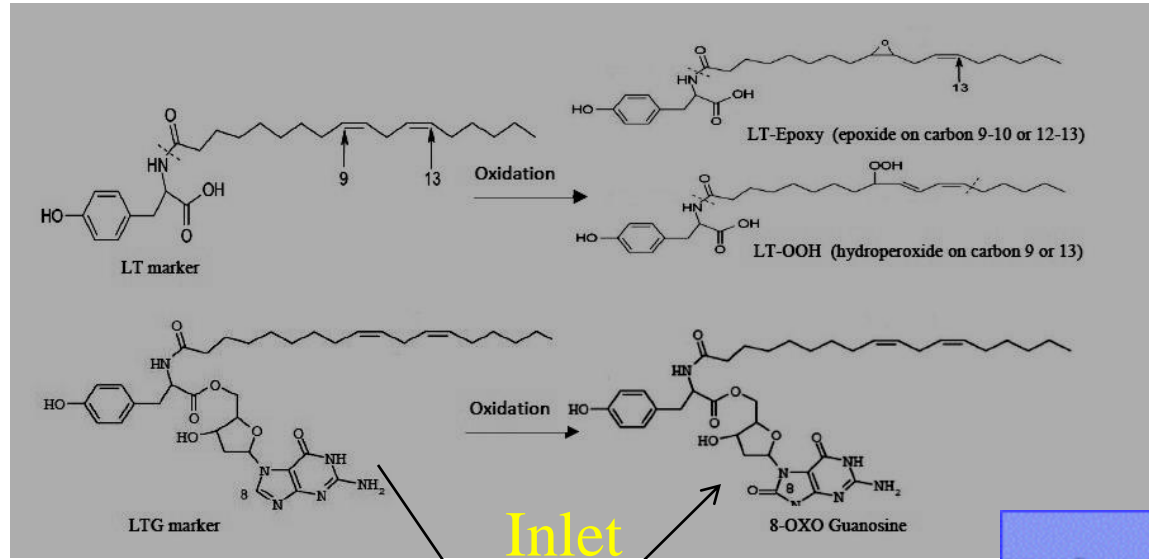


Future plans

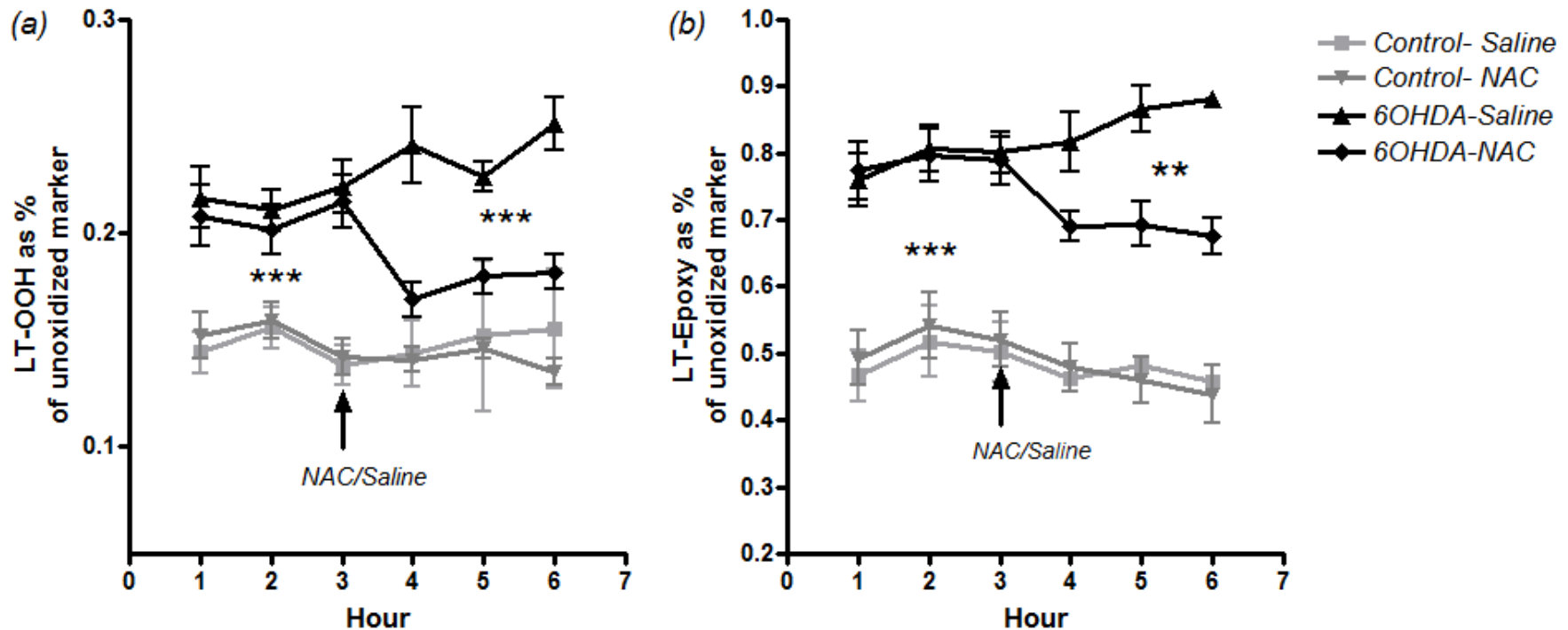
- To examine the presence of volatile materials in Parkinson human blood.
- To investigate the mechanism of the obtained VOCs generation and their association with the disease.

III. Novel markers for extra cellular OS

2. Extra cellular OS: Microdialysis of a non dialyzable marker (ROS/RNS trap).



Oxidative stress- extra cellular marker oxidation



- Increased oxidative stress is observed in our rat model (44% and 28% increase in the level of oxidized products of the marker),
- NAC decreased the increment almost back to normal



Thanks

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Dr. Yuval Aluf.



- Dr. Yizhar Lavner.

