Neurology: Precision scRNA-seq & Metabolic Phenotyping in Parkinson's Disease

Abstract

Energy metabolism dysregulation contributes to Parkinson's disease (PD) pathogenesis. Using single-cell RNA sequencing and QSM™ metabolic phenotyping, we mapped metabolic reprogramming in PD and control samples. Key findings include altered ATP production and substrate utilization, revealing cell-type-specific vulnerabilities. This work provides insights into PD heterogeneity, identifying biomarkers and targets for precision medicine approaches.

Introduction

Energy metabolism plays a central role in brain health, with its disruption serving as a critical driver in the progression of Parkinson's disease (PD). Despite extensive investigation, the molecular and cellular mechanisms underlying PD remain poorly defined, posing significant challenges for the development of effective treatments. Mitochondrial dysfunction and oxidative stress, hallmark features of the disease, further emphasize the need to explore cellular energy pathways in PD.

Recent advances in single-cell RNA sequencing (scRNA-seq) and metabolic phenotyping have revolutionized our ability to investigate cellular heterogeneity and metabolic alterations at unprecedented resolution.

In this study, we utilize these powerful technologies to investigate disease-relevant gene expression and cell-type-specific vulnerabilities in PD, focusing on single-cell metabolic reprogramming and energy metabolism dysfunction within distinct brain cell populations.

Here, we present the first integration of scRNA-seq with single-cell metabolic phenotyping to comprehensively map cellular heterogeneity and energy metabolism in PD. Our approach aims to provide a functional understanding of energy metabolism, identify critical enzymes, and discover metabolic biomarkers associated with the disease.

By addressing the complexities of PD heterogeneity, this study seeks to pave the way for targeted therapies and precision medicine approaches for neurodegenerative diseases.

Methodology

Study Design

Human brain tissue was obtained with informed consent following elective Deep Brain Stimulation surgery in the presence of specific pathologies – PD: Parkinson's disease (9 subjects); and non-PD (5 subjects).

The patients were seen by movement disorders specialist physicians in the University Medical Center of the Johannes Gutenberg University Mainz to make / confirm / infirm the diagnosis of Parkinson's disease.

Additionally, the patients have all been tested by a clinical neuropsychologist to exclude cognitive impairment. The brain samples were obtained from the dorsolateral prefrontal cortex, directly under the skull borehole, and amounted to 50-100 mg. No compensation was provided to any subjects.

sCelLiVE® Tissue Dissociation and Single Cell Suspension Preparation

Tissues were dissociated into single-cell suspensions using the sCelLiVE® Tissue Dissociation Solution (Singleron Biotechnologies) according to manufacturer's instructions.

GEXSCOPE® scRNA-seq Library Preparation

scRNA-seq libraries were prepared using the GEXSCOPE® Single Cell RNAseq Library Kit (Singleron Biotechnologies). Briefly, single-cell partitioning was performed on the SCOPE-chip®. The microfluidic microwell-based SCOPE-chip® is particularly gentle on sensitive and fragile cell types, making it well-suited for the outlined study design. Single-cell barcoding, mRNA capture, reverse transcription, and library preparation were performed. Libraries were paired-end sequenced with 150 bp read length on NovaSeqX.

Data pre-processing and mapping was performed with CeleSCOPE® (Singleron Biotechnologies). Data was annotated using scMRMA PanglaoDB for identification of cell populations.

QSM™: A platform quantifying metabolic turnover rates at single-cell level

To date, experimental insights into the dynamics of cellular energy metabolism within the tissue context remain largely unavailable, as conventional technologies are limited to analyses of cell suspensions. Single-cell metabolic phenotyping has emerged as a powerful tool to address these previously inaccessible aspects of single-cell metabolic processes. Quantitative System Metabolism (QSM™) provides a comprehensive approach, capturing the major cellular metabolic pathways involved in energy metabolism. This includes the catabolism of key energy sources such as carbohydrates, fatty acids, branched-chain amino acids, and ketone bodies (see Fig. 1).

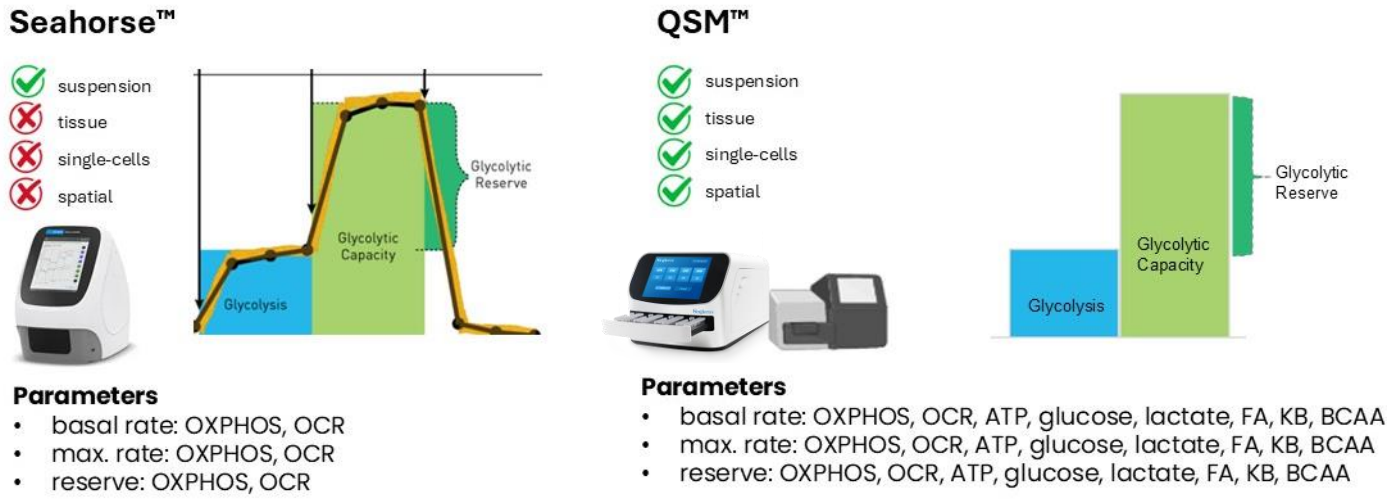

Fig. 1. Comparison of cellular energy metabolism technologies (Seahorse™ vs. QSM™)

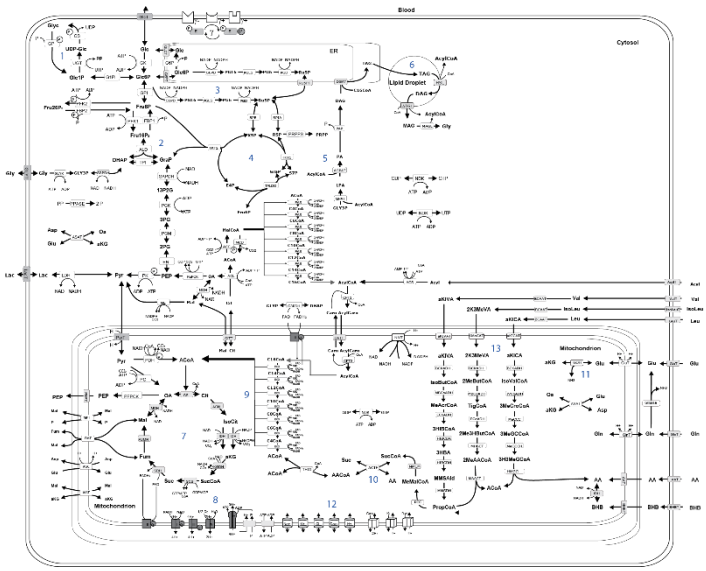

Fig. 2. QSM™ r*eaction scheme:* Arrows symbolize reactions and transport processes between compartments. (1) glycogen metabolism, (2) glycolysis, (3) oxidative pentose phosphate pathway in the endoplasmic reticulum and cytosol, (4) non-oxidative pentose phosphate pathway, (5) triglyceride synthesis, (6) synthesis and degradation of lipid droplets, (7) tricarboxylic acid cycle, (8) respiratory chain and oxidative phosphorylation, (9) β-oxidation of fatty acids, (10) ketone body utilization, (11) glutamate metabolism, (12) mitochondrial electrophysiology (membrane transport of ions, (13) Utilization of branched-chain amino acids. Small cylinders and cubes symbolize ion channels and ion transporters. Double-arrows indicate reversible reactions, which according to the value of the thermodynamic equilibrium constant and cellular concentrations of their reactants may proceed in both directions. Reactions are labeled by the short names of the catalyzing enzyme or membrane transporter given in the small boxes attached to the reactions arrow. Full names, kinetic rate laws of reaction rates, comparison of experimentally determined and calculated metabolic functions and cellular metabolite concentrations are outlined in [1].

QSM™ quantifies the key electrophysiological processes at the inner mitochondrial membrane including the membrane transport of various ions, the mitochondrial membrane potential, the generation of the proton motive force by the respiratory chain, and its utilization for mitochondrial ion homeostasis/handling and ATP production.

Individual parametrization:

Metabolic models for each individual cell were established based on single-cell transcriptomic profiles obtained with Singleron's GEXSCOPE® Single-cell RNA Library Kit to scale the maximal activities of enzymes and transporters, according to the relation:

$$v_{max}^{sc} = v_{max}^{mean\ control} \frac{E^{sc}}{E^{mean\ control}}$$

The maximal activities $v_{mx}^{mean\ control}$ for the normal control tissue were obtained from [1], by assuming that the mean single-cell activity corresponds to the metabolic activity of the mean tissue. $E^{mean\ control}$ denotes the mean transcript abundance profile in the control group, and E^{sc} denotes the abundance of transcript for enzyme E in the single-cell. If no value for E^{sc} was present, it was set to $E^{mean\ control}$.

Metabolic Phenotyping

QSM™ was employed to determine metabolic functionality under physiological conditions. Energetic capacity was measured in both resting states and under activated (energetically challenged) conditions.

Results & Discussion

Cellular Heterogeneity

In this study, we aim to investigate key drivers of PD to better understand cellular processes as well as disease development and progression. Human brain tissue samples were obtained from 5 non-PD controls and 9 patients with PD.

sCelLiVE® tissue dissociation resulted in a median cell viability of 83.7%. ScRNA-seq of these samples revealed the presence of 12 cell type clusters (Fig. 3).

The identified cell types included astrocytes, endothelial cells, macrophages, microglia, neurons, oligodendrocyte progenitor cells, oligodendrocytes, pericytes, smooth muscle cells, and T cells.

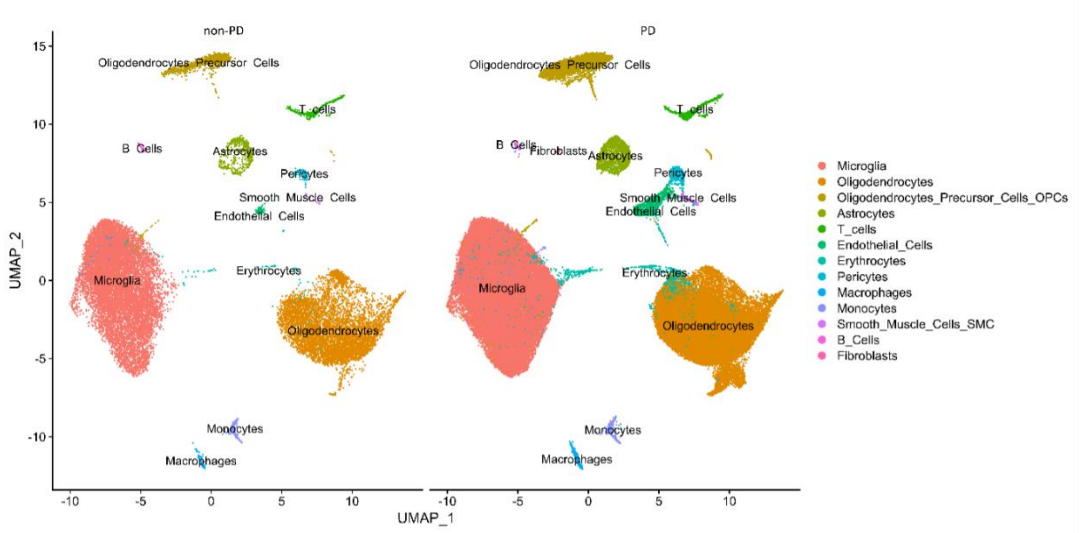

Fig. 3. Representative UMAP plots of one non-PD control sample and one PD sample with cell type identification and annotation revealed the presence of 12 clusters.

For assessment of individual metabolic functionality, we used single-cell profiles of 83,555 cells. After annotation, the cells were identified as in Tab. 1 and Fig. 4.

Cell type	Cell number
Astrocytes non-PD	454
Astrocytes PD	1636
Oligodendrocytes non-PD	5966
Oligodendrocytes PD	24232
Oligodendrocytes Precursor non-PD	937
Oligodendrocytes Precursor PD	4821
Microglia non-PD	11295
Microglia PD	34214

Tab. 1. Annotation of cell types and cell numbers.

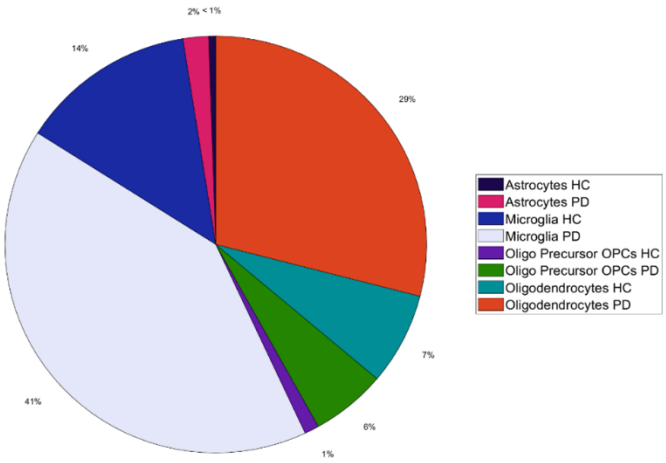

Fig. 4. Relative distribution of cell types.

Single-cell Metabolic Phenotyping

ATP production was assessed under physiological conditions. ATP production rates for maximal activation are depicted by the density plots in Fig. 5. Each cell is presented by a dot, the median value for each cell type is given by the solid line with and marked by a white dot. Interquartile distance is depicted by the colored shaded area. As can be seen PD leads to an upregulation of ATP production rates in all examined cell types, but most pronounced in Astrocytes.

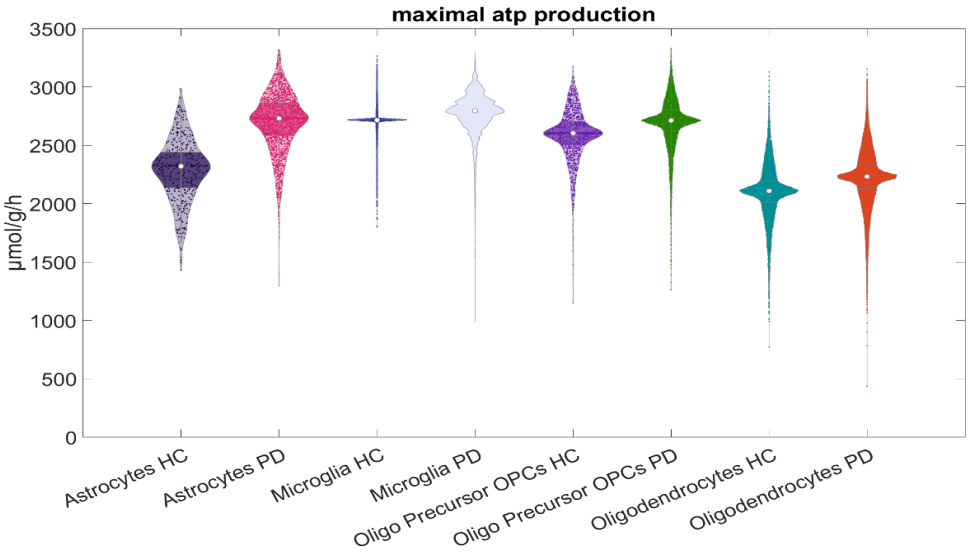

Fig. 5. ATP production capacity. Comparing non-PD conditions (HC) with Parkinson's disease (PD), ATP production capacity is significantly increased under Parkinson's disease conditions for all cell types. The difference is most prominent for astrocytes but also present in oligo precursor cells and oligodendrocytes.

Another important discriminant among cells and cell types lies in the substrates utilized for ATP production. Fig. 6 shows the mean ATP production rates and substrate utilization rates under resting and activated conditions. Importantly, differences in glucose and fatty acid utilization account for the majority of substrate utilization differences. The relative contribution of the different substrates is also depicted in Fig. 7. As can be seen, maximal activation leads to a severe shift towards carbohydrate utilization.

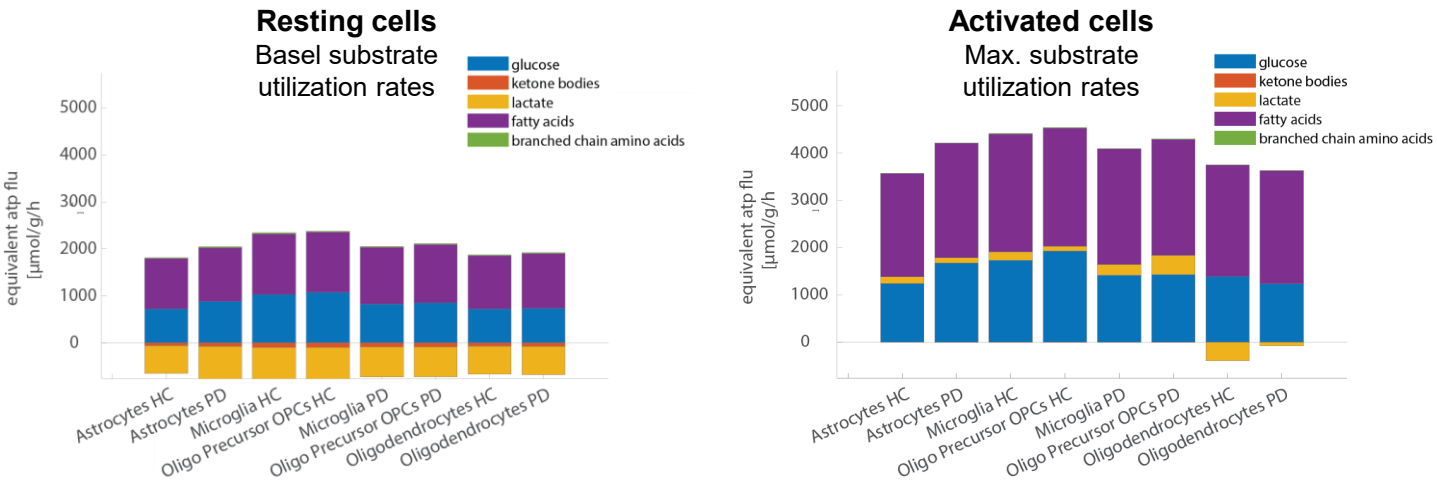

Fig. 6. Basal and maximal substrate utilization rates.

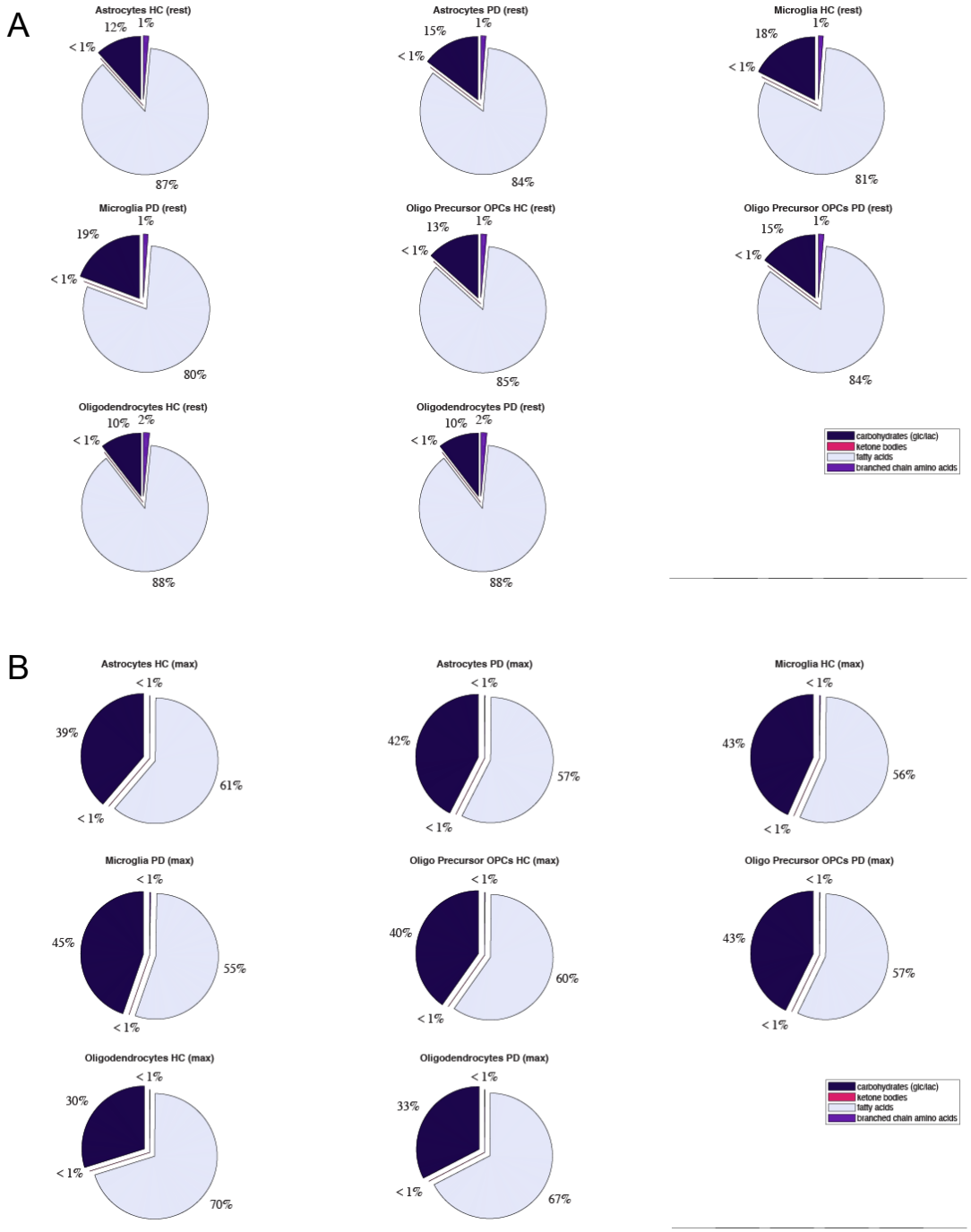

Fig. 7. Relative substrate utilization for ATP production: Basal rate (A) and maximum rate (B).

In addition to revealing cell type-specific, disease-specific, and activation-dependent differences in metabolic functionality, single-cell metabolic profiling enables the discrimination and identification of subpopulations within a specific cell type based on their metabolic functions. Fig. 8 illustrates the dependence of ATP production rate at maximal activation on lactate exchange in individual cells, visualized as density plots separated by cell type. Notably, distinct subpopulations emerge within certain cell types based on variations in ATP production and lactate exchange rates. Furthermore, lactate exchange rate demonstrates a strong correlation with ATP production rate, suggesting its potential as a marker for metabolic fitness.

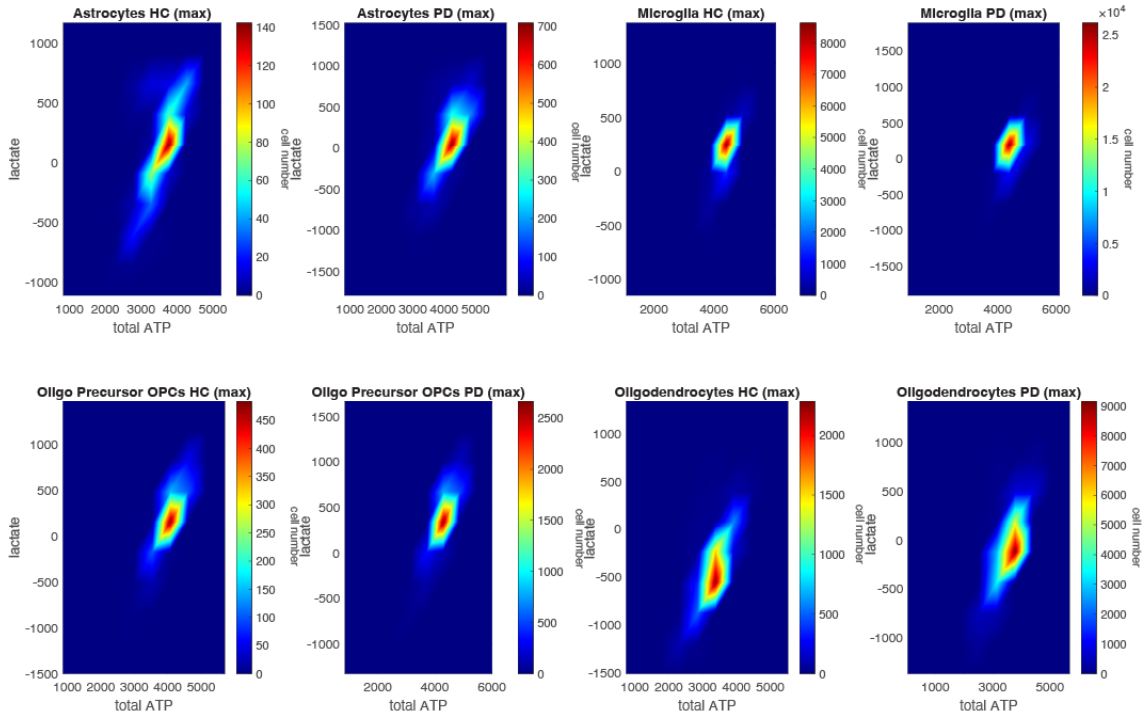

Fig. 8. Relative contribution of substrates to ATP production at rest and maximal activation.

Similarly, Fig. 9 illustrates the dependence of carbohydrate and fatty acid utilization for ATP production at maximal activation, presented as density plots for individual cells separated by cell type. While some heterogeneity is observed among individual cells within a given cell type, no distinct subpopulations can be identified. However, notable differences between disease conditions are evident, reinforcing the condition-specific metabolic variations previously observed in Fig. 5-7.

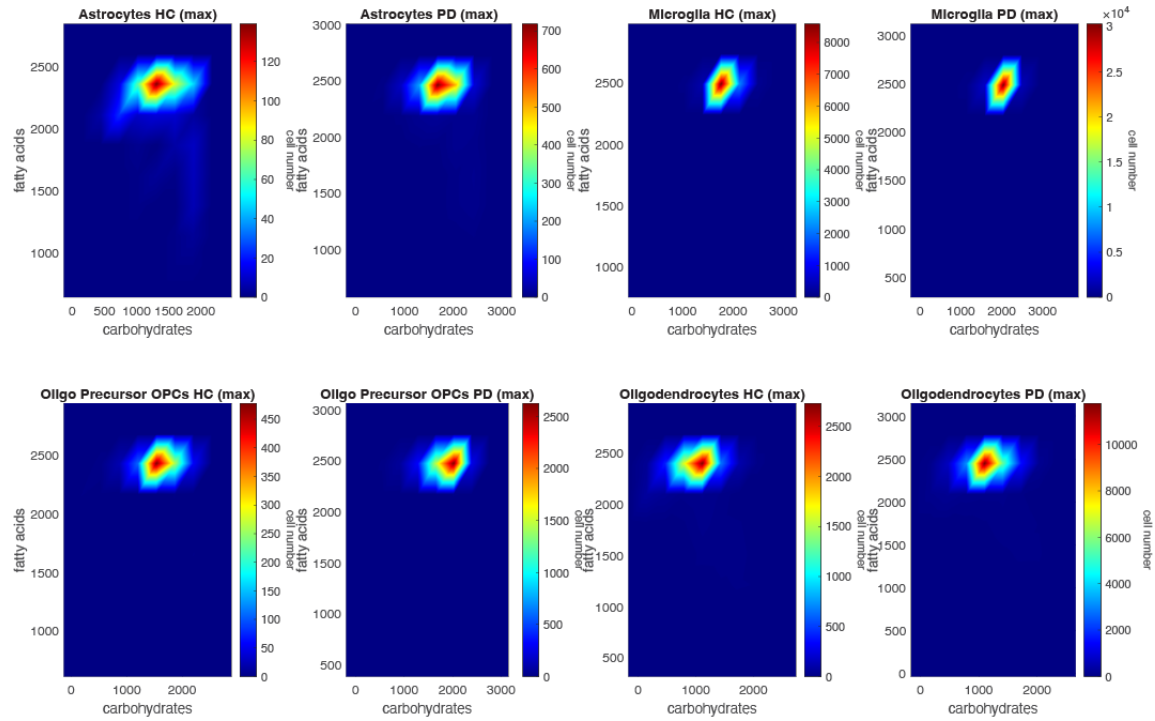

Fig. 9. Interdependence of carbohydrate and fatty acid utilization for ATP production at maximal activation.

Discussion

Parkinson's disease impacts the human brain, which is composed of diverse cell types such as neurons, astrocytes, microglia, and oligodendrocytes, each characterized by distinct transcriptional profiles and critical roles in PD pathology. To study the functions and contributions of these delicate cell types at the single-cell level, gentle tissue dissociation and single-cell processing are paramount. The sCelLiVE® Tissue Dissociation Solution was specifically developed to enable gentle yet effective dissociation, yielding highly viable single-cell suspensions from challenging tissues like the brain. This approach provides a robust foundation for precise cell identification and metabolic phenotyping.

To complement this, the SCOPE-chip®, a microwell-based technology, facilitates single-cell barcoding with minimal cellular stress. Unlike pressurized droplet-based systems, the SCOPE-chip employs gravity to partition cells, minimizing selective loss of fragile cell types and preserving accurate cell representation. These features support reliable downstream analyses of individual cell phenotypes and functions.

While Seahorse™ technology (Agilent, USA) revolutionized real-time metabolic analysis of cell suspensions, QSM™ represents a significant advancement by enabling single-cell and spatial metabolic profiling. This novel platform provides unprecedented insights into Parkinson's disease (PD)-related metabolic reprogramming in brain cells.

Specifically, QSM™ reveals that the often-described "glycolytic switch" in PD may instead represent a "shift in gear," where glucose is utilized as an additional substrate rather than entirely replacing fatty acids. This nuanced metabolic adjustment underscores the complexity of energy dynamics in PD and highlights the potential for targeted therapeutic interventions.

Conclusion

This case study highlights the transformative potential of integrating single-cell RNA sequencing (scRNA-seq) with QSM™ metabolic phenotyping to unravel the complexities of Parkinson's disease (PD). By providing an unprecedented resolution in mapping cellular heterogeneity and single-cell energy metabolism, our findings shed light on the metabolic reprogramming and energy dysfunction that underpin neurodegeneration.

Although the diagnostic application of these insights is yet to be fully realized, single-cell metabolic phenotyping holds significant promise for advancing clinical research and enabling the development of personalized therapeutic strategies.

By targeting specific metabolic pathways or biomarkers unique to individual patients, this precision medicine approach could redefine PD management offering a paradigm shift to implementing interventions that directly address the disease's underlying biology.

Reference

[1] Mirzac, D., Bange, M., Kunz, S. *et al.* Targeting pathological brain activity-related to neuroinflammation through scRNA-seq for new personalized therapies in Parkinson's disease. *Sig Transduct Target Ther* **10**, 10 (2025). https://doi.org/10.1038/s41392-024-02086-7