Real-Time Metabolic Profiling in Brain Tissue Biopsies Using the Seahorse XF Flex System

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Introduction

OBJECTIVE / RATIONALE

Neuronal development and function are among the most energy-demanding biological processes in the body. Disruptions in oxygen metabolism and mitochondrial function are hallmark features of aging and neurodegenerative diseases. Different brain regions have distinct functions and energy demands, yet many studies rely on cultured neurons or brain isolated mitochondria. These approaches require large tissue quantities and fail to capture the complex microenvironment and cellular interactions of intact brain tissue. To address this, we developed a novel 3D capture microplate that when used together with the Agilent Seahorse XF Flex analyzer and dedicated consumables, it enables real-time energetic metabolic analysis of small biopsy punches from discrete brain regions.

METHODS / RESULTS

Rat brains were dissected on the morning of the Seahorse XF assay and immediately placed in chilled, oxygenated artificial cerebrospinal fluid. Viable tissue slices were prepared using a Compresstome vibratome (Precisionary Instruments LLC). Biopsy punches were then collected from selected brain regions to assess the metabolic profile using the new Seahorse XF Flex 3D capture microplate-L (Agilent Technologies) in combination with the XF 3D Mito Stress Test (Agilent Technologies). The assays yielded robust basal oxygen consumption rates (OCR) and key mitochondrial function parameters. The assay demonstrated sufficient sensitivity to detect regional metabolic differences and responses to mitochondrial modulators. Representative data, workflow steps, and assay optimization strategies are presented.

Experimental

3D Brain Tissue Workflow using Seahorse XF Flex analyzer

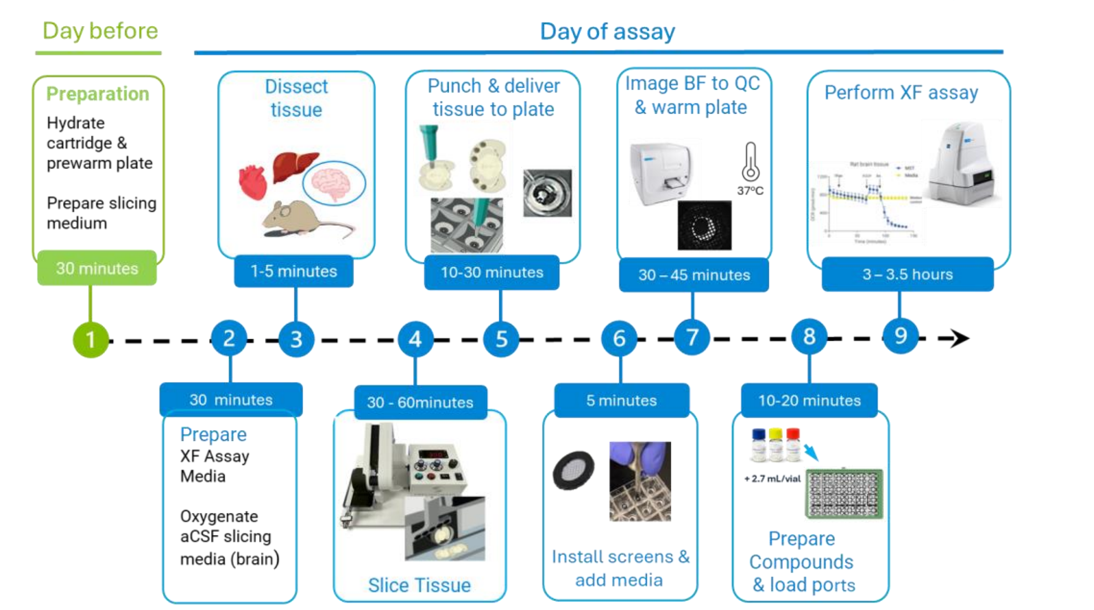

Fig 1. The Agilent Seahorse XF Flex 3D tissue workflow illustration, highlighting the key steps in the assay.

Preparation of viable and consistent tissue samples

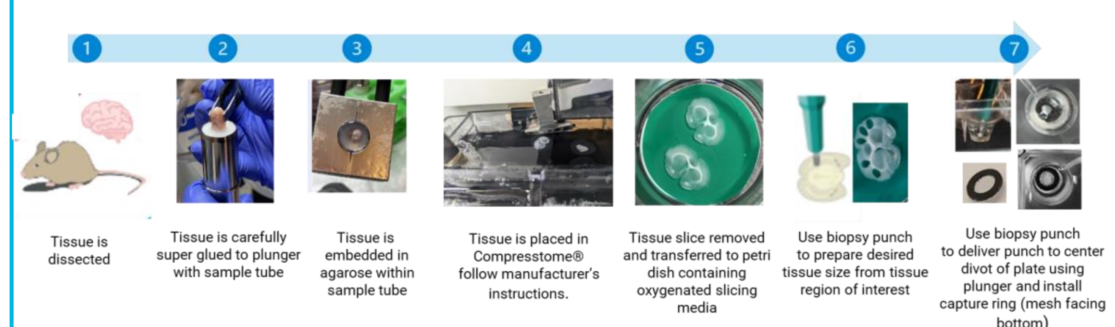

Fig 2. Illustration of the key steps involved in the preparation of rat brain tissue slices, using the Compresstome® tissue slicer from Precisionary Instruments LLC.

Results and Discussion

Robust correlation is observed between basal OCR and rat/mouse brain tissue sample size

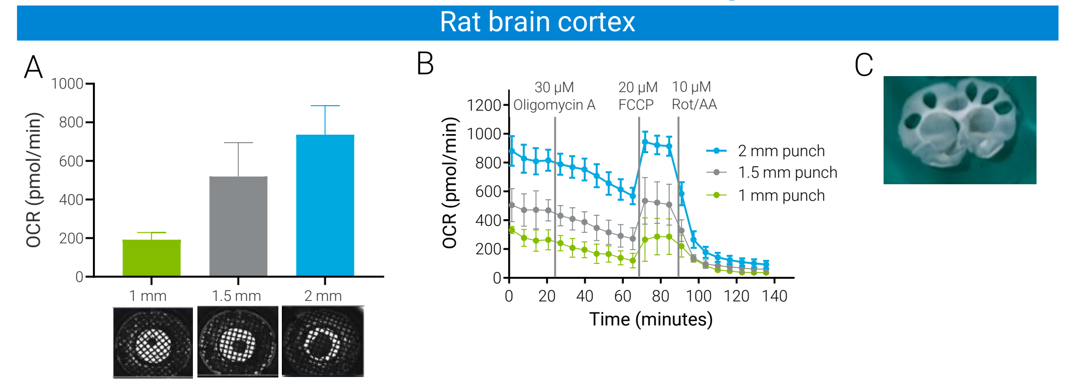

Fig 3. Evaluation of correlation between OCR and rat brain punch sizes. (A) Basal OCR obtained using different tissue punch sizes. The images below the chart were taken using an Agilent BioTek Cytation 1 cell imaging multimode reader, showing the tissue discs under the capture ring in the well. (B) OCR kinetic graph of the XF 3D Mito Stress Test. (C) Coronal brain tissue slice with 2 mm punches of cortex. Representative data average ± STD of n= 3 to 12.

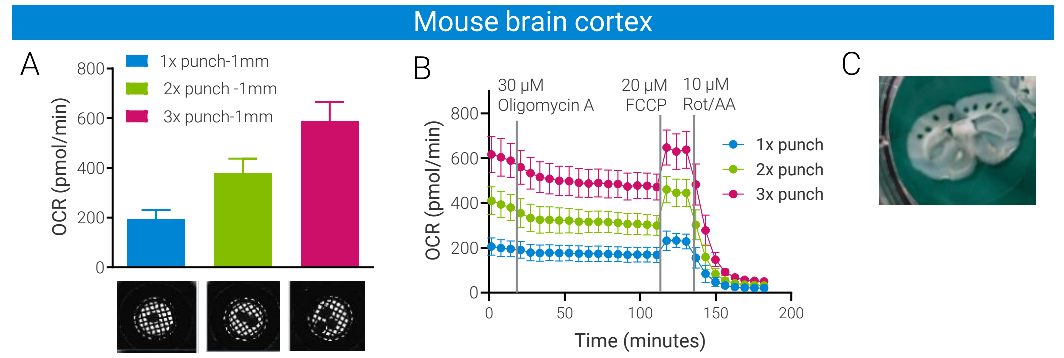

Fig 4. Evaluation of correlation between OCR and number of brain punches per well. (A) Basal OCR obtained using 1, 2 or 3 punches of one-mm diameter per well. The images below the chart were taken using an Agilent BioTek Cytation 1 cell imaging multimode reader, showing the tissue discs under the capture ring in the well. (B) OCR kinetic graph the XF 3D Mito Stress Test. (C) Coronal brain tissue slice with 1 mm punches of cortex. Representative data average ± STD of n= 4 to 6.

Optimization of modulator concentration and cycles in tissue specific conditions for reliable XF assay results

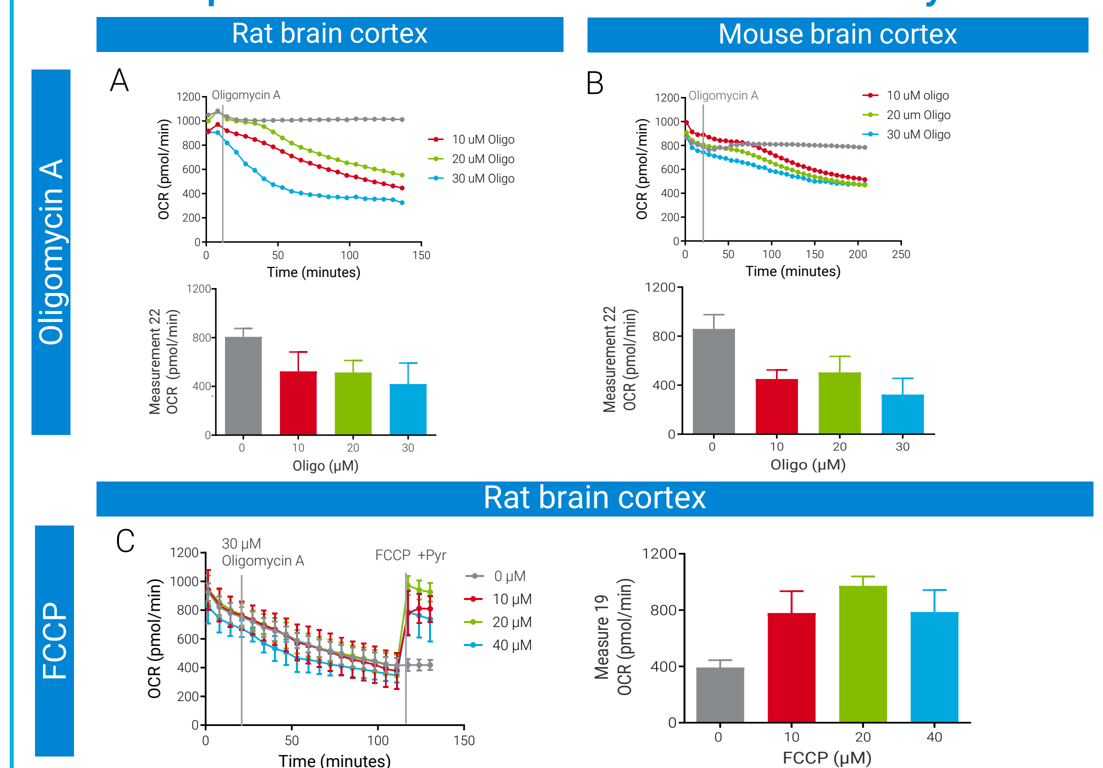

Fig 5. Example of XF Mito Stress Test compound concentration optimization. The experiment was performed using the Seahorse XF 3D Mito Stress Test kit in rat or mouse cortex tissue discs of 200 µm thickness and 2 mm diameter. Oligomycin A titration identified 30 µM as optimal inhibitor concentration for rat (A) and mouse (B) brain tissue. Representative data average n= 4 to 6. (C) FCCP titration determine 30 µM as optimal concentration for rat brain. Representative data average n = 4 to 6.

Results and Discussion

Real-time metabolic interrogation of intact brain tissue

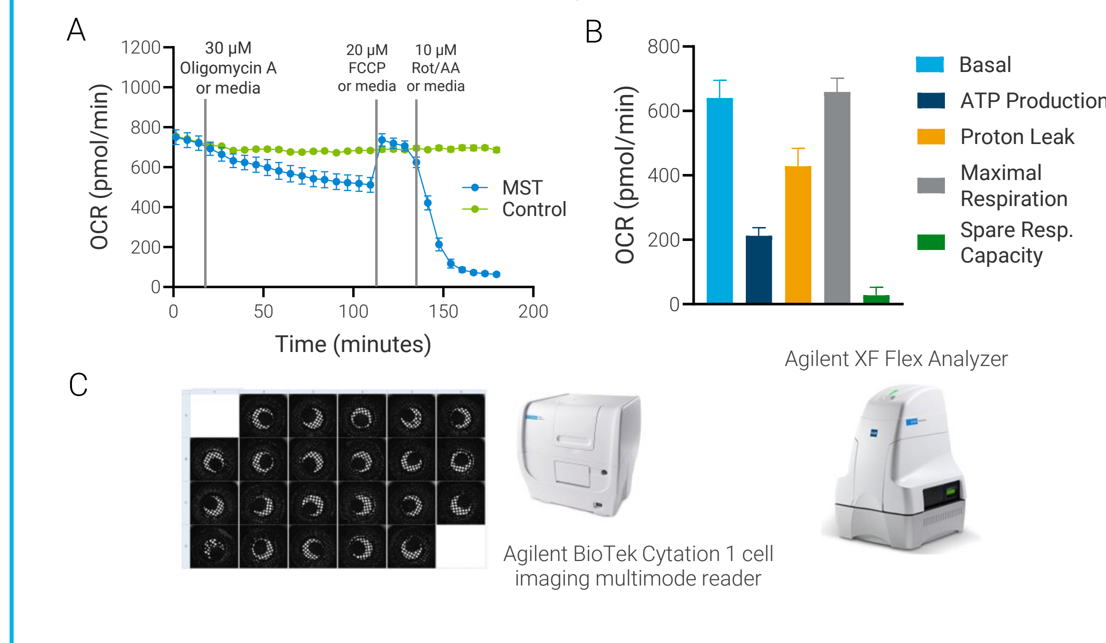

Fig 6. Agilent Seahorse XF 3D Mito Stress Test in rat brain cortex. 200 µm × 2 mm tissue punch were used in each well of the 3D capture microplate-L on XF Flex analyzer. (A) OCR Kinetic graph including tissue control with stable OCR throughout run indicating health of tissue. (B) Key parameters of mitochondrial function, Representative data average ± STD of control n = 2 and MST n =10. (C) Brightfield Images of 3D capture microplate (4x BFHC montage) after 3D capture ring installation showing placement of tissue punches in each well.

Normalization strategies for brain tissue depends on experimental workflow and study objective

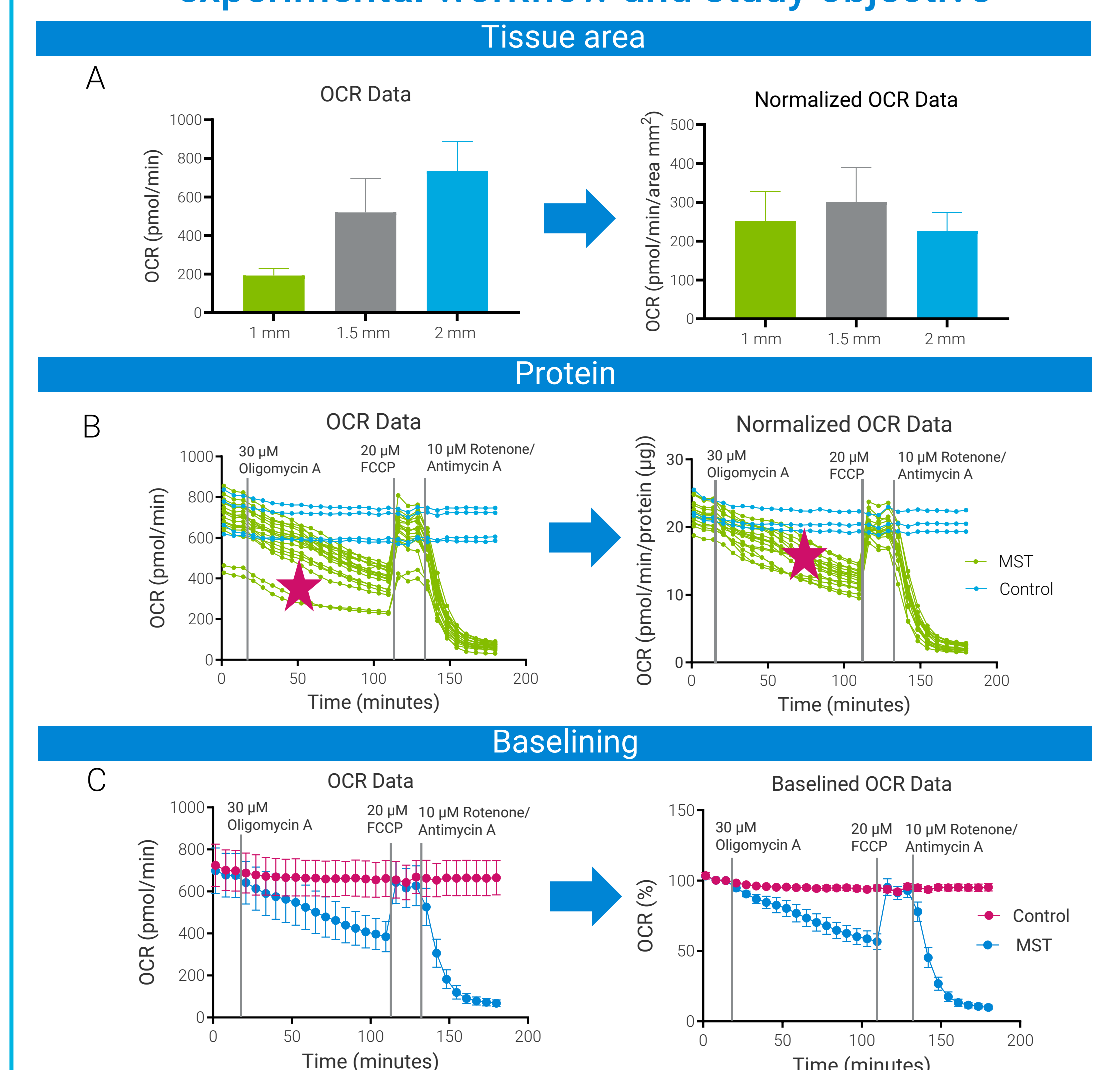

Fig 7. Several option can be used for normalization depending on study objective. (A) various size punches with OCR ranging from 190 to 700 normalized to area brings normalized OCR data within 30% variability. Representative data average ± STD of n = 3 to 12. (B) Normalization using protein concentration brings two outlier samples in line with other samples. Representative data of n = 4 to18. (C) Data normalized to basal signal expressed as % OCR of baselined rate is appropriate when comparing responses to acute injection helping to reduce well to well variability. Representative data of data average ± STD of control n = 4, MST n =18.

Results and Discussion

Real-time metabolic interrogation in distinct regions of intact brain tissue

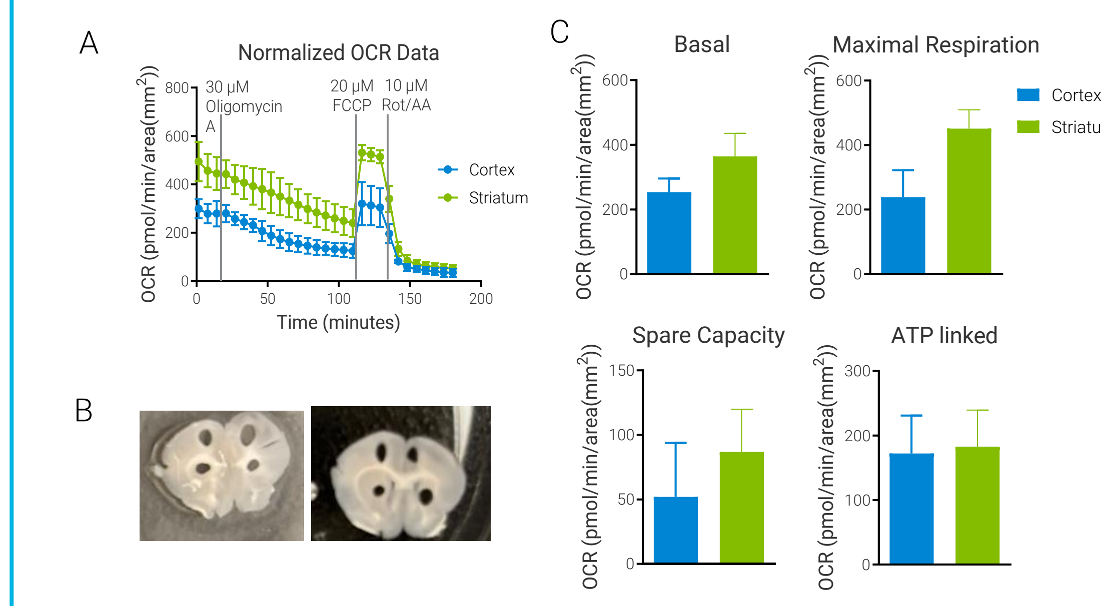

Fig 8. Real-time metabolic comparison of intact tissue isolated from striatum and cortex regions of day 10 C57BL/6 female mouse brain. (A) OCR kinetic graph of 200 µm x 1.5 mm diameter tissue punches normalized to tissue area. (B) Image of coronal tissue slice with regions of punch samples. (C) Key parameters of mitochondrial function. Data analyzed using Seahorse Analytics (SHA). Representative data average ± STD, n = 3.

Conclusions

- The new Seahorse XF Flex analyzer, in combination with the XF 3D capture microplate-L, enables physiologically relevant metabolic analysis in three-dimensional brain tissue models.
- This system provides the sensitivity needed to detect robust tissue respiration signals and dynamic responses to mitochondrial perturbations.
- With optimized and streamlined workflows, this approach facilitates real-time metabolic interrogation of intact brain tissue, offering a valuable tool for studying regional bioenergetics in health and disease models.

References

1. A Superior System for Real-Time Metabolic Analysis with Brain Tissue and Other 3D Models. https://www.agilent.com/cs/library/applications/an-superior-system-for-real-time-metabolic-analysis-5994-8309EN-agilent.pdf