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Quantitative high-content screening methods reveal a regulation of cell-to-cell variability of the energy metabolism of brain cells

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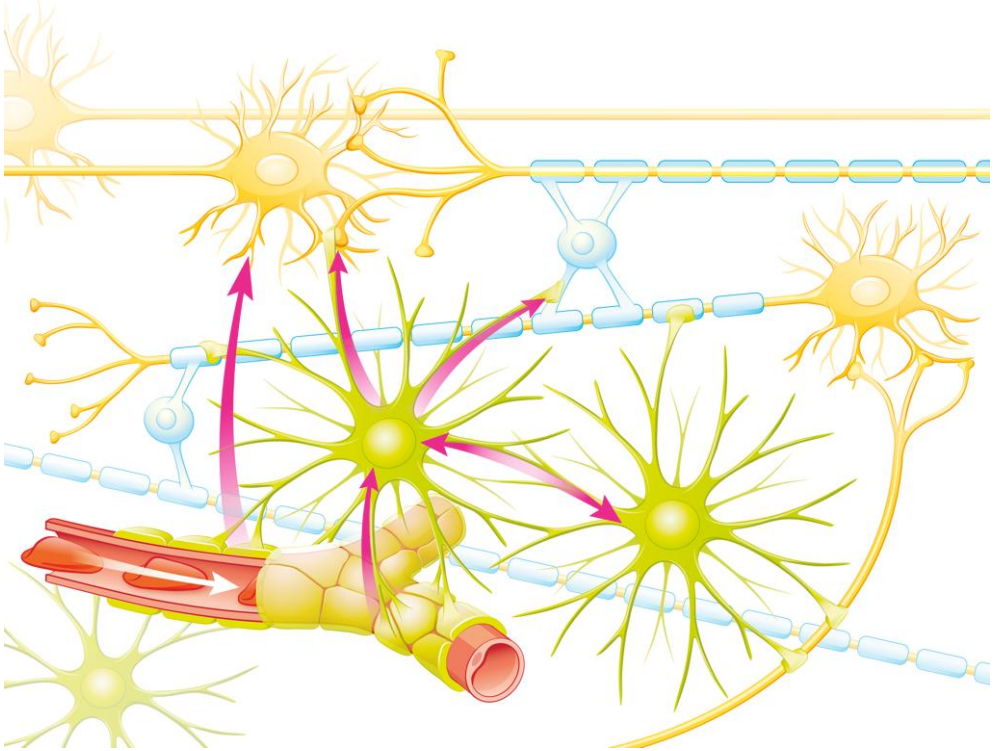
A 25-watt lightbulb in the head

Power requirement of human at rest: 116 watts (Rich, Nature 2003)

Brain represents:

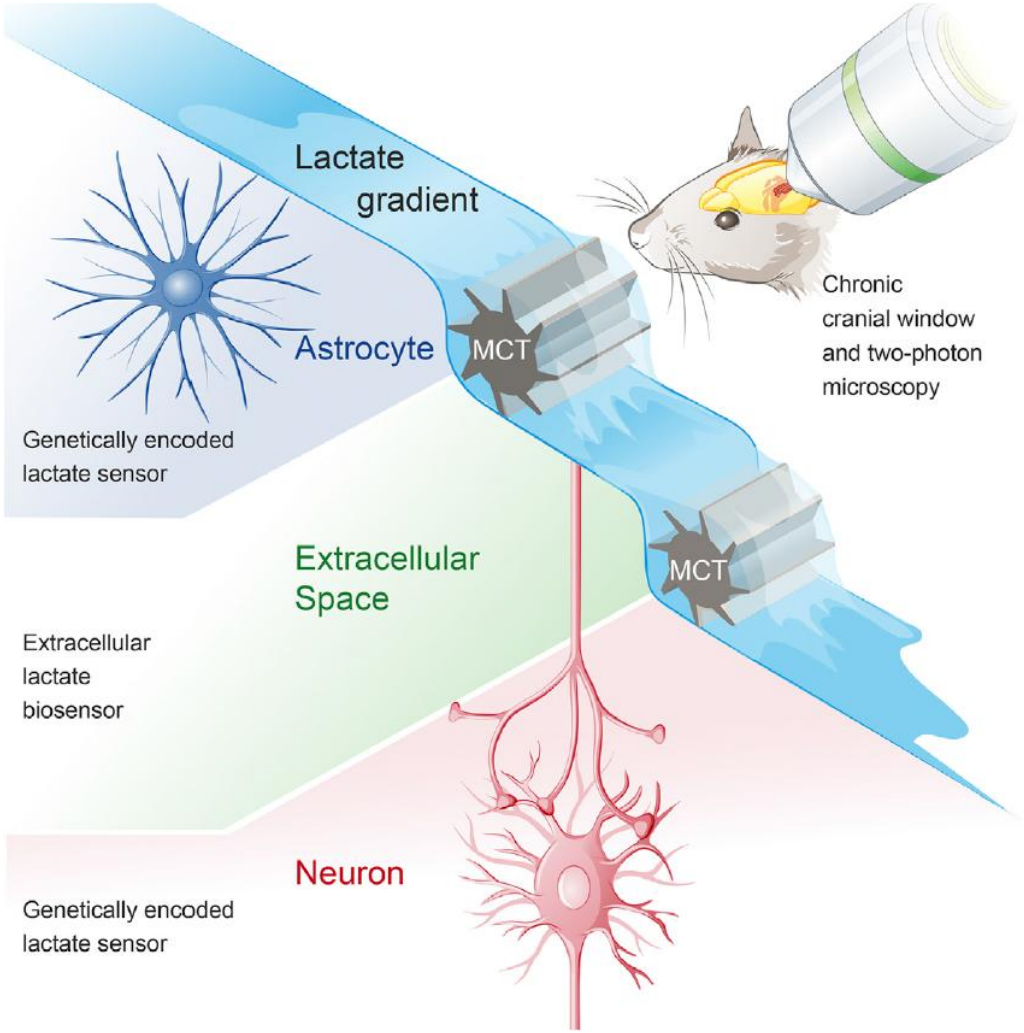
- 2% of body mass but
- 25% of total glucose consumption
- 20% of total oxygen consumption
- 20% of energy consumption

One brain requires as much power as a 25-watt lightbulb.



Neurons, astrocytes and oligodendrocytes exhibit different profiles of energy metabolism, resulting in compartmentalization of energy metabolism.

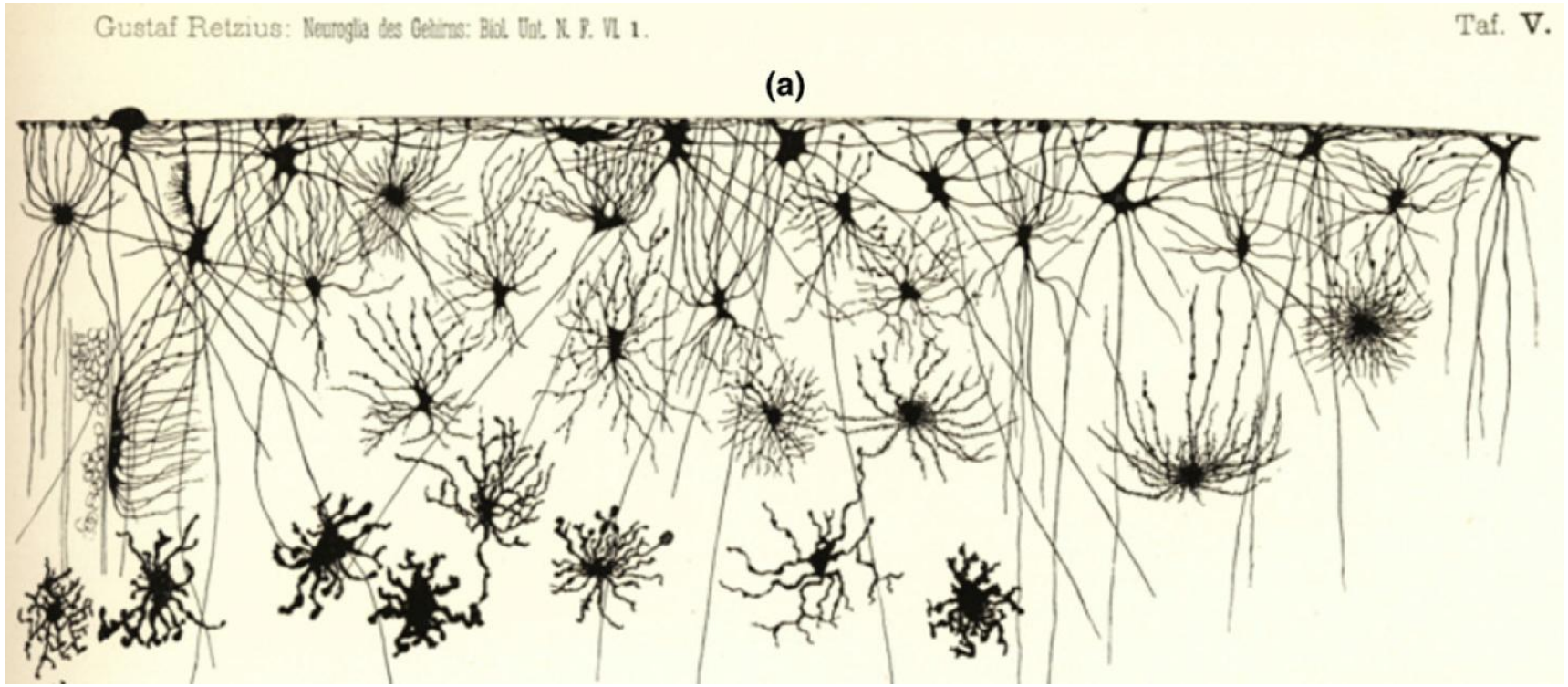
Energy metabolism in astrocytes and neurons are genetically determined

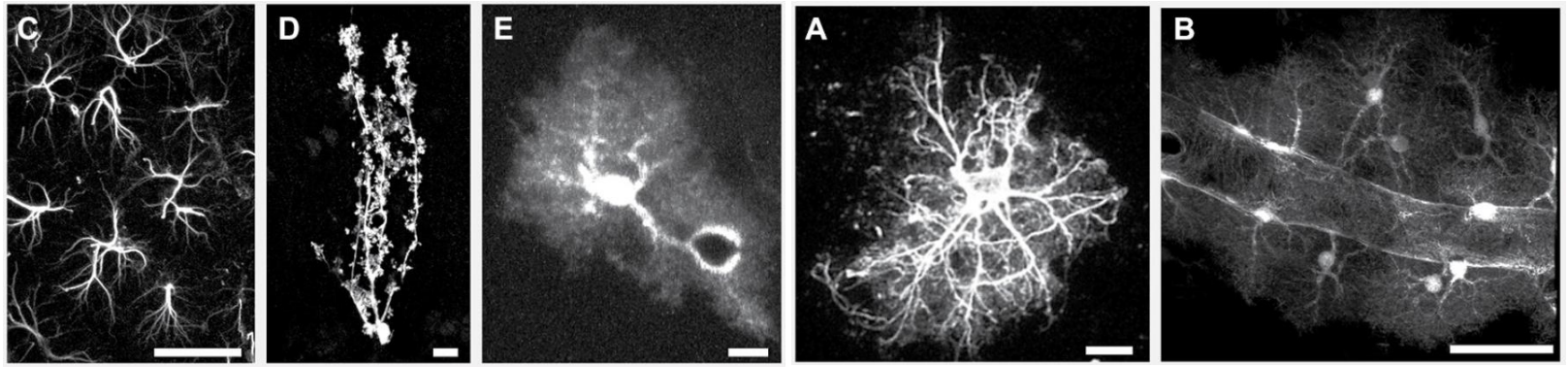


APC/C-Cdh1 stable in neurons —> **neurons** are **poorly glycolytic**.
APC/C-Cdh1 degraded in astrocytes —> **astrocytes** are **highly glycolytic**.

(Bolanos 2016)
(Mächler et al 2015)

Evidence for morphological heterogeneity of astrocytes in vivo





Hippocampus | Cerebellum | Cerebral cortex | Acutely isolated | Cerebral cortex

A role for metabolic heterogeneity in brain energy metabolism ?

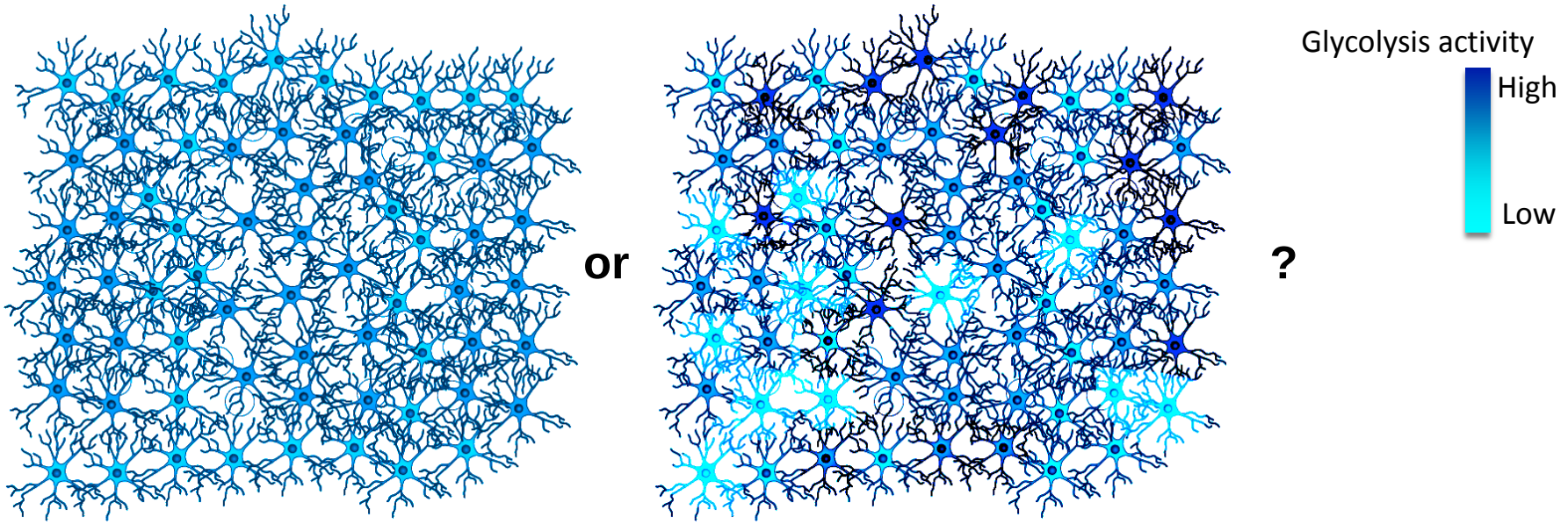
- **Hypothesis**:
  1. The **energy metabolism** is **variable** among astrocytes.
  2. The variability in energy metabolism is **regulated** by neuronal activity.

Do not treat the heterogeneity as noise but as an information coded at the population level.

Methods

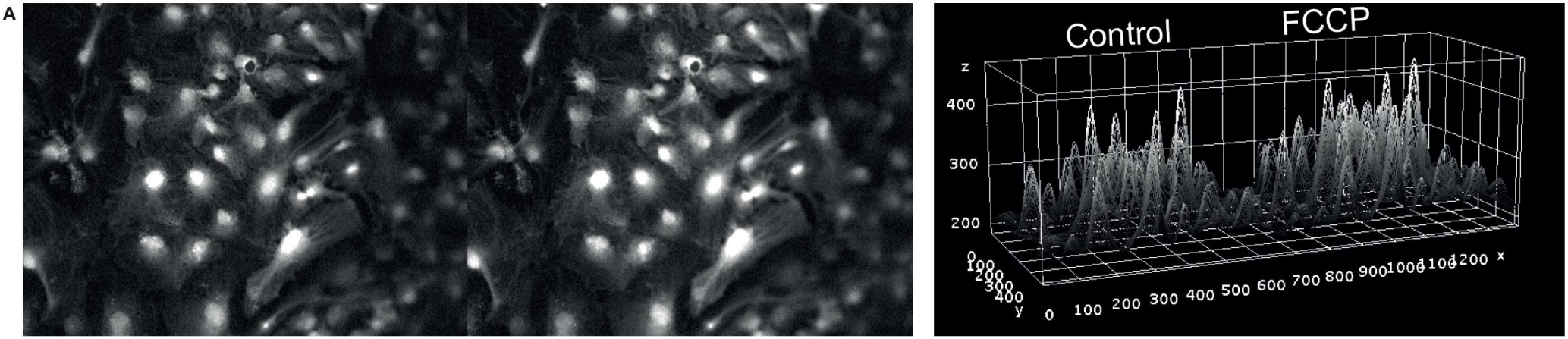
- **Intrinsic cell-to-cell variability:**
  - Normalized/calibrated metabolic parameters in primary cultures of cortical astrocytes.
- **Extrinsic cell-to-cell variability:**
  - Submit primary cultures of astrocytes to glutamate stimulation.
  - Quantitative assessment of cell-to-cell variability in vivo.

How is the metabolic profile of cortical astrocytes in unstimulated conditions ?



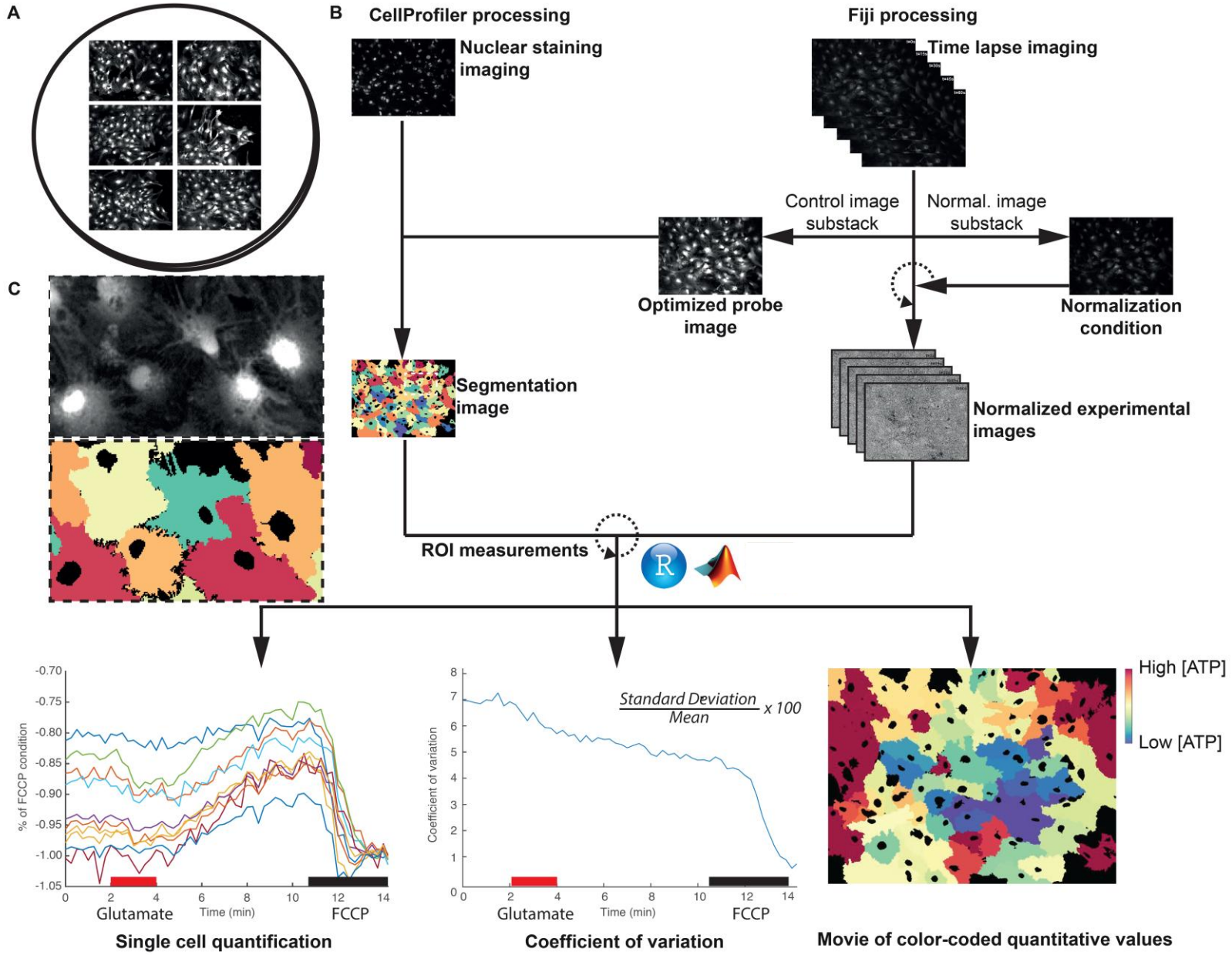
Relevance of calibration of data obtained from fluorescence microscopy

- Without calibration, fluorescence microscopy gives semi-quantitative data informing on the dynamics of substrate variation.
- Absolute values of fluorescence intensity depends on also on biophysical properties independent from the substrate concentration (probe concentration, pH, etc.)



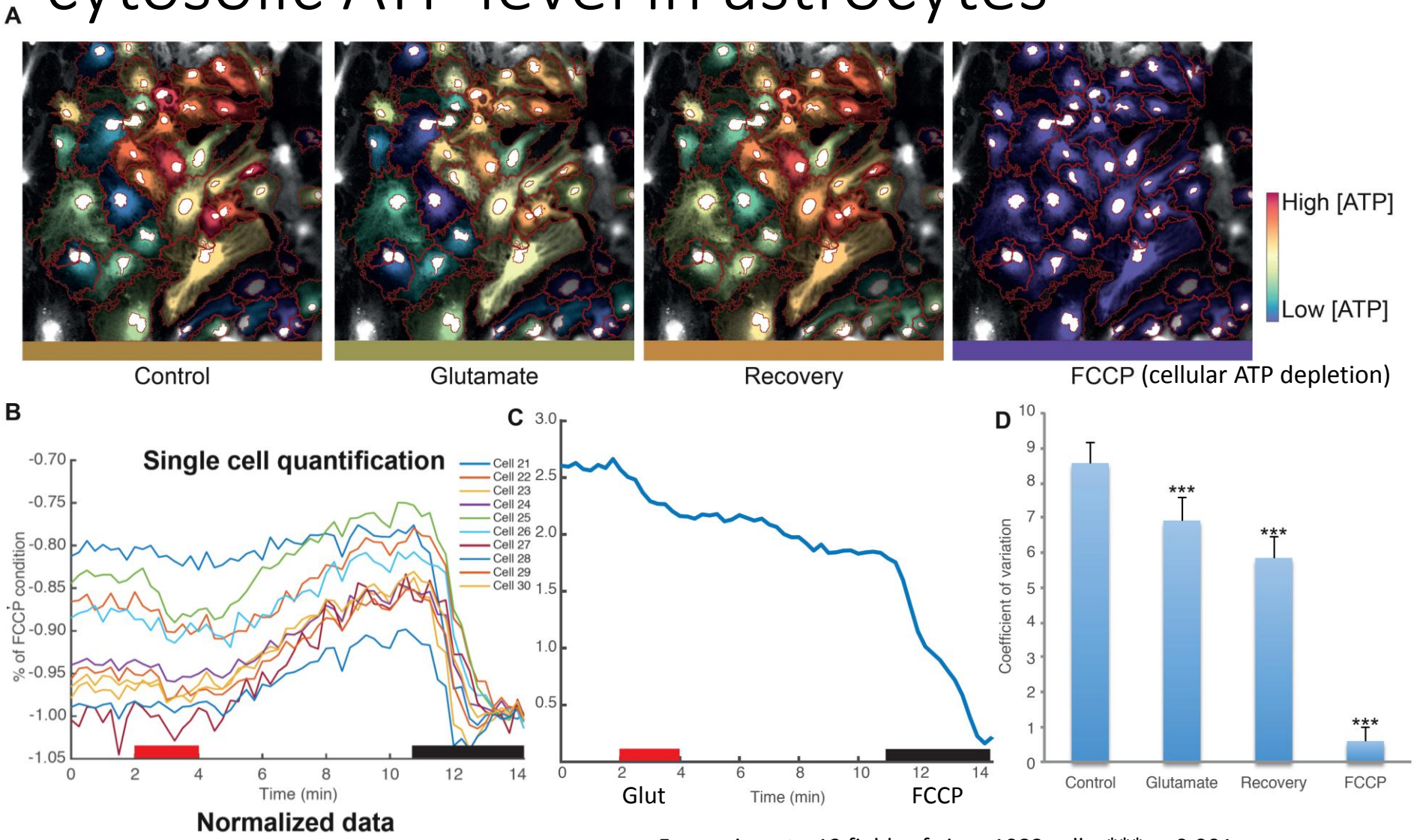
Control FCCP (Positive control)

Analysis pipeline to quantify cell-to-cell variability



25 to ~50 detected cells per field of view - up to 250 per coverslip.

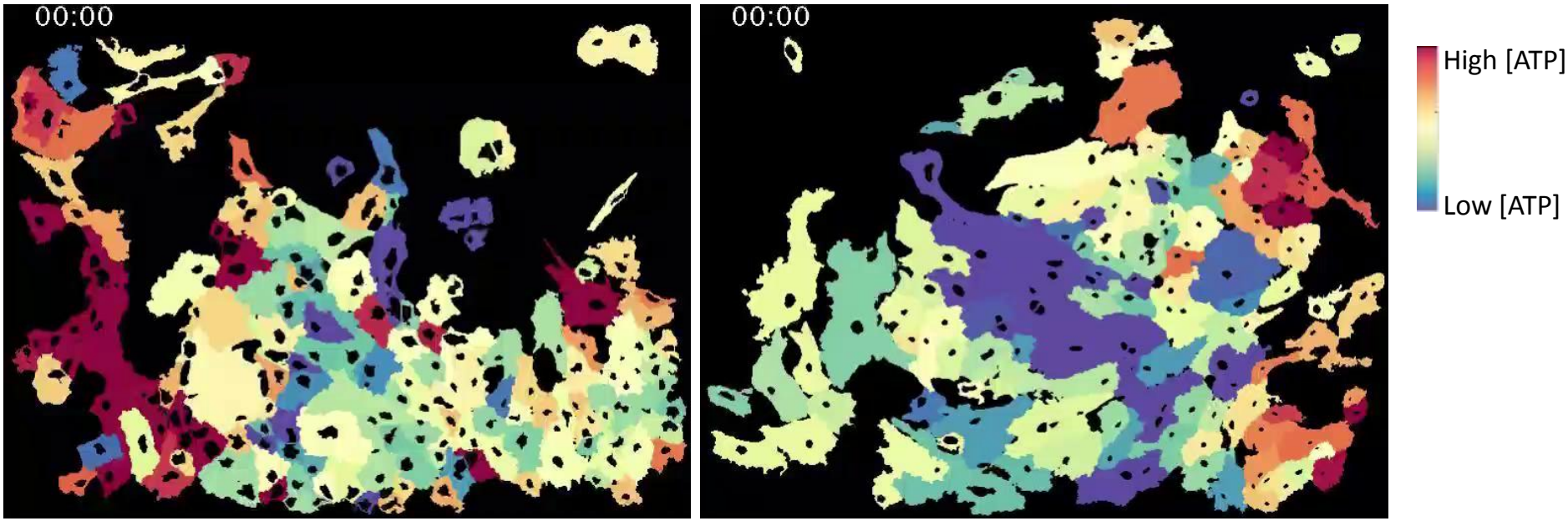
Glutamate decreases the variability in the cytosolic ATP level in astrocytes



5 experiments, 13 fields of view, 1083 cells. *** $p<0.001$

(collaboration with Jean-Yves Chatton - Department of fundamental neuroscience Lausanne)

Glutamate induces a delayed increase in the cellular ATP level

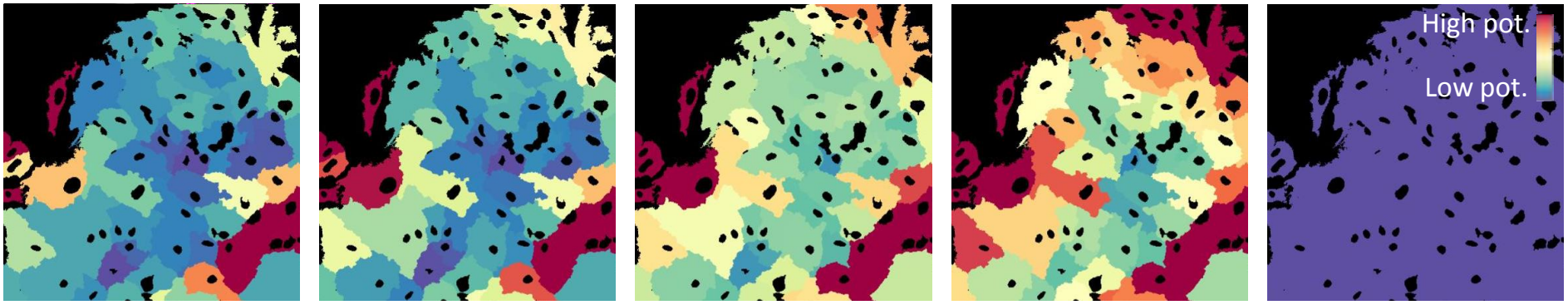


Glutamate induces two successive decreases in the variability of cellular ATP level:

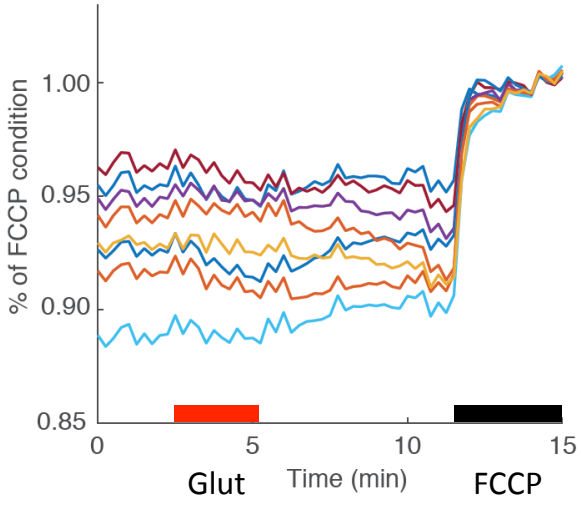
- Decrease **during glutamate stimulation** (likely mediated by the glutamate transporter)
- Decrease **after glutamate stimulation**, increasing the cellular ATP level in the cellular population.

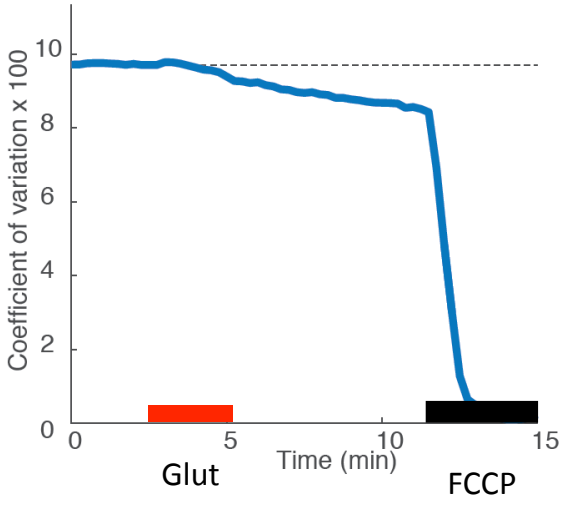
(collaboration with Jean-Yves Chatton - Department of fundamental neuroscience Lausanne)

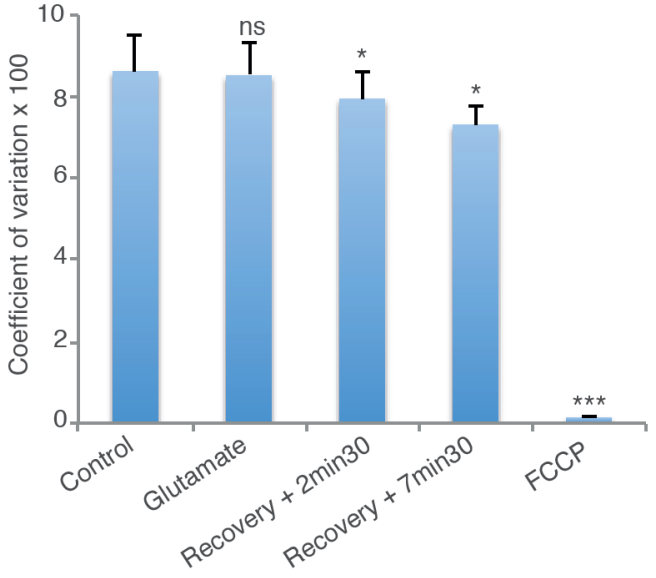
Glutamate induces a delayed decrease in the variability in the mitochondrial electrical potential



Control | Glutamate | Recovery +2min30 | Recovery +7min30 | FCCP (~~elec. pot.~~)

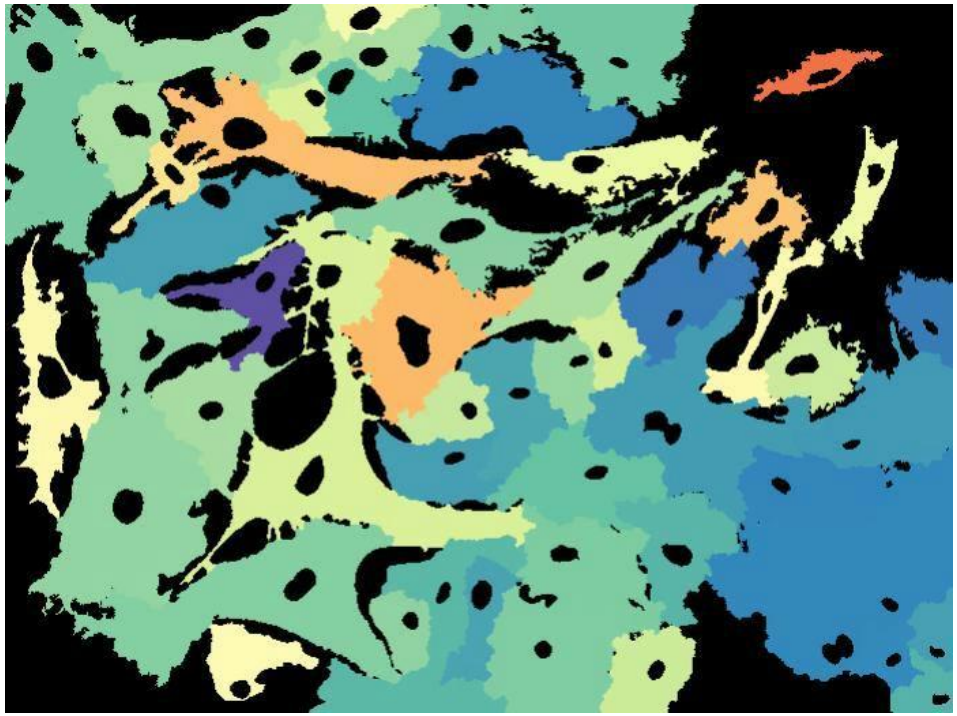


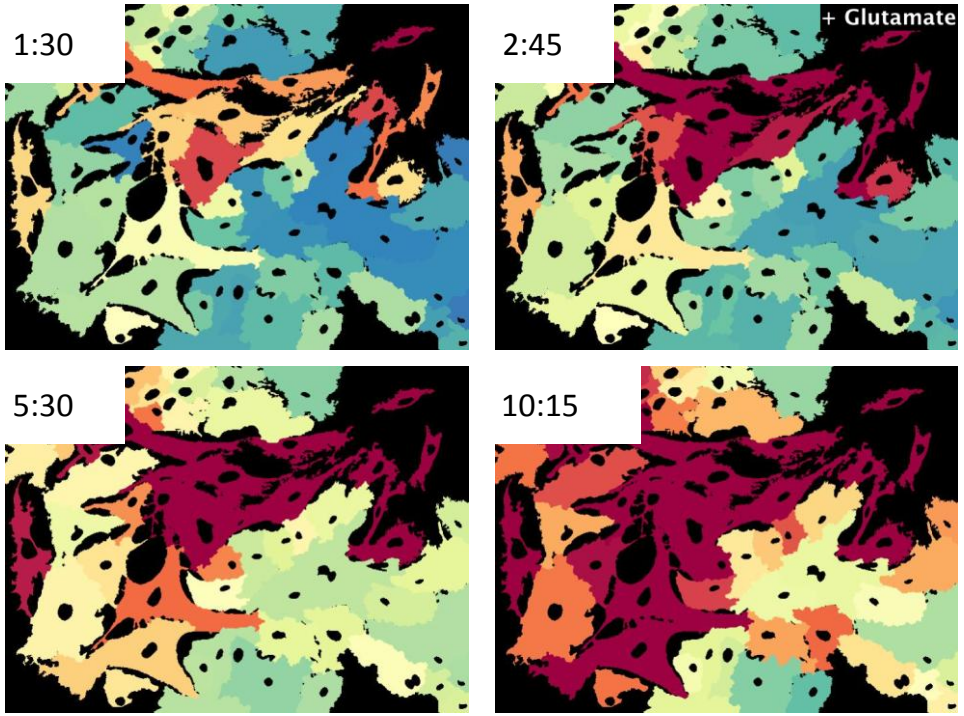




4 experiments, 24 fields of view, 1852 cells. * p<0.05 *** p<0.001 using paired t-test.

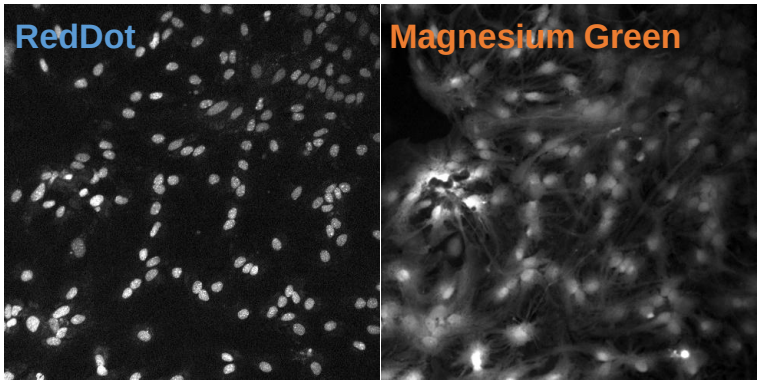
Slow propagation of glutamate-induced mitochondrial hyperpolarization





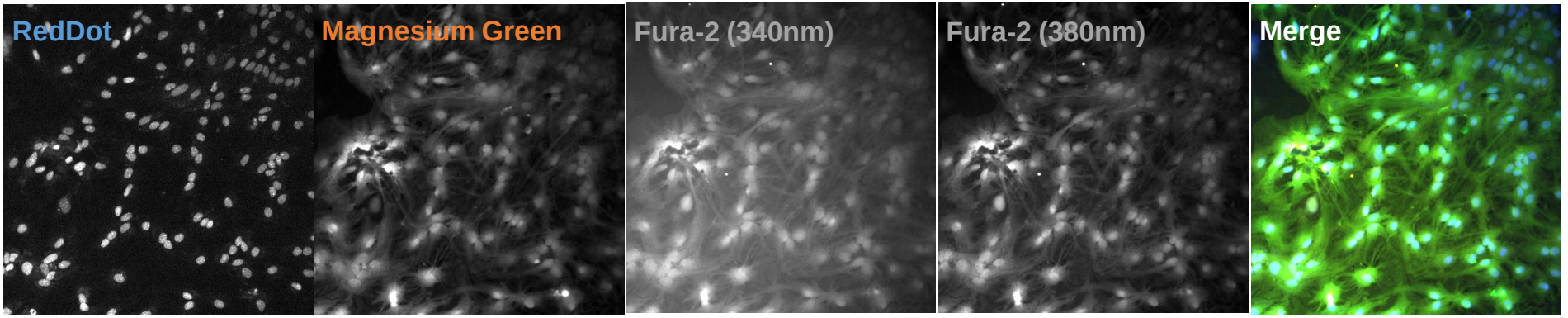
- Glutamate induces a mitochondrial hyperpolarization in **some cells**, not all.
- Cells in the **neighborhood** of cells having hyperpolarized mitochondria will **also exhibit** a mitochondrial hyperpolarization.
- The glutamate-induced mitochondria **persists after** glutamate stimulation.

No match between the spatio-temporal signaling pattern of cytosolic calcium and cellular ATP level



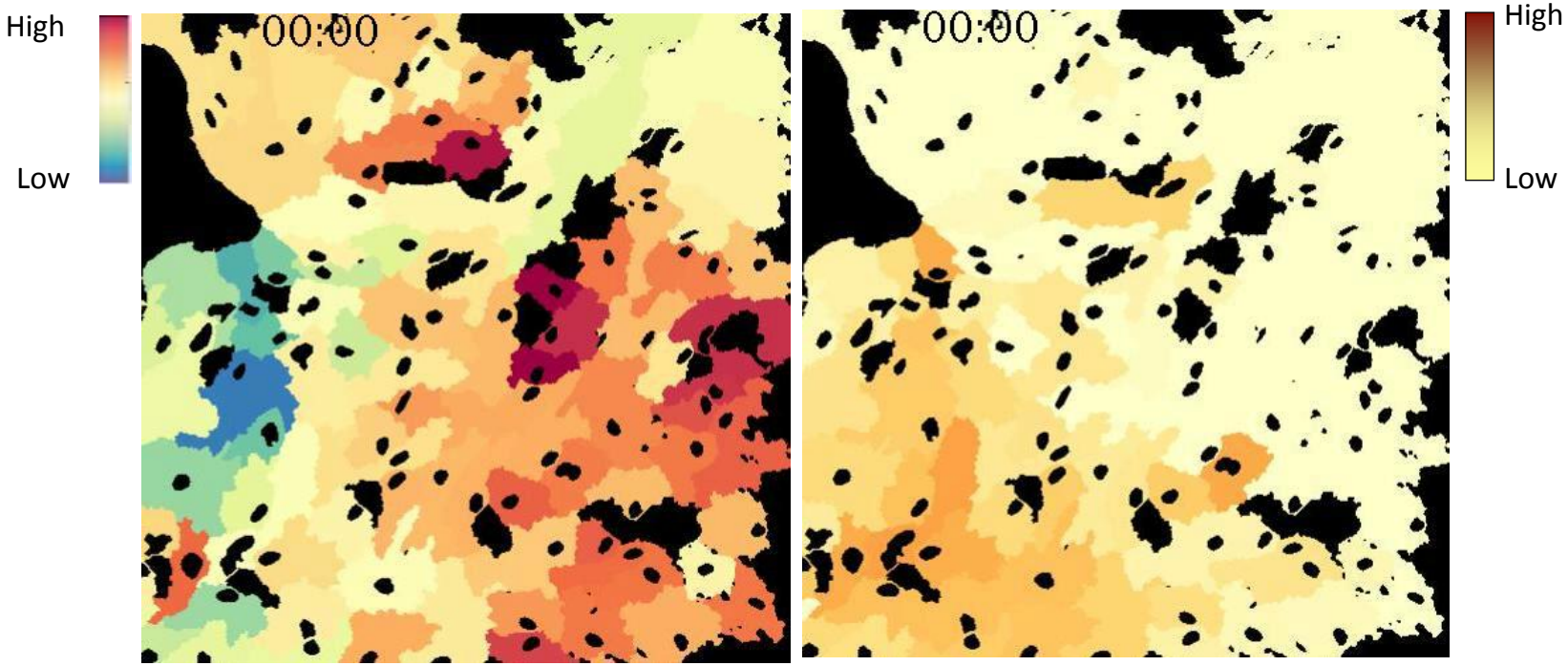
5 experiments, 23 fields of view

No match between the spatio-temporal signaling pattern of cytosolic calcium and cellular ATP level



Cellular ATP level (MagG)

Cytosolic Ca^{2+} concentration (Fura-2 F_{340nm}/F_{380nm})



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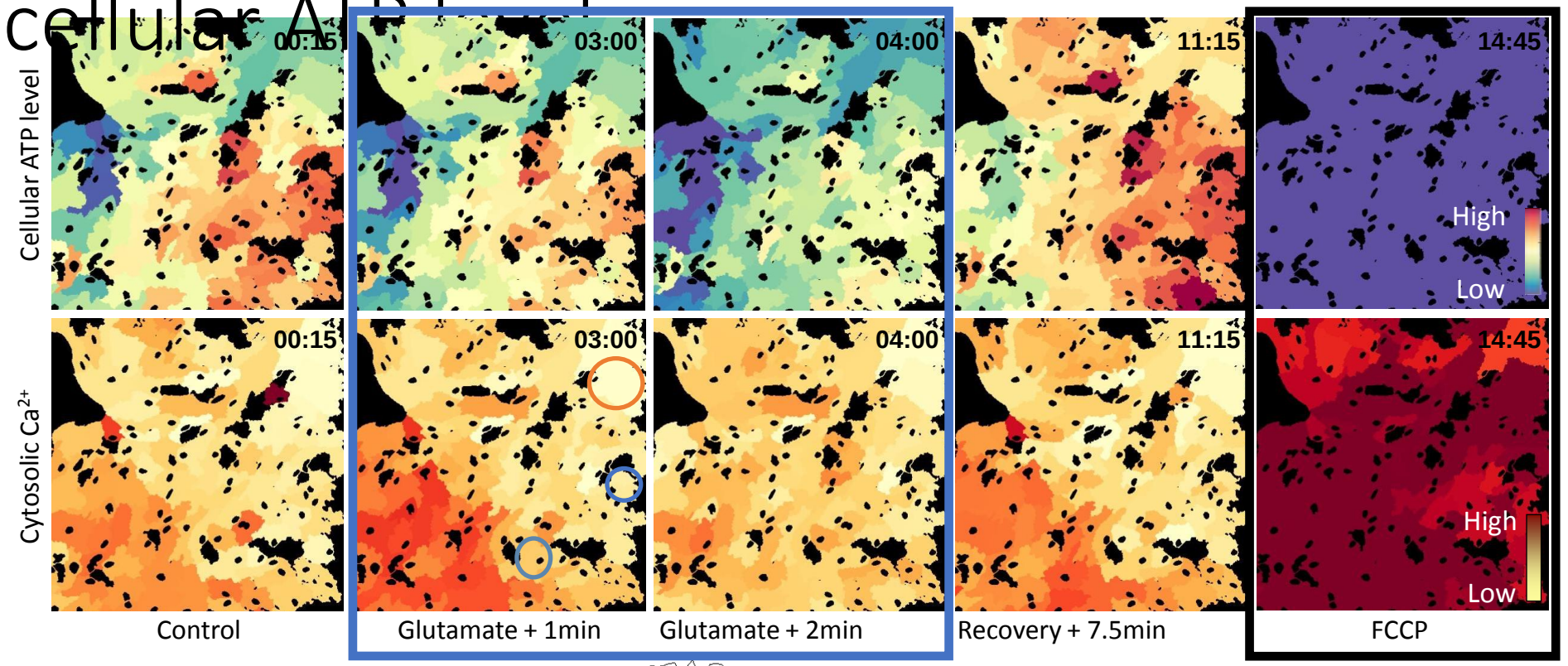
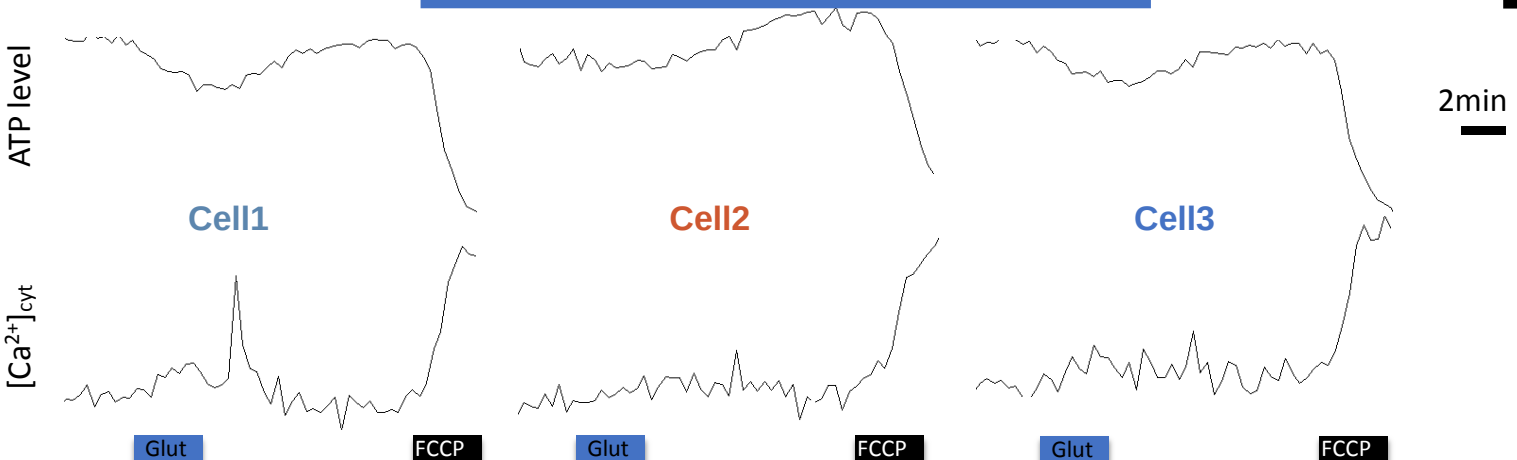


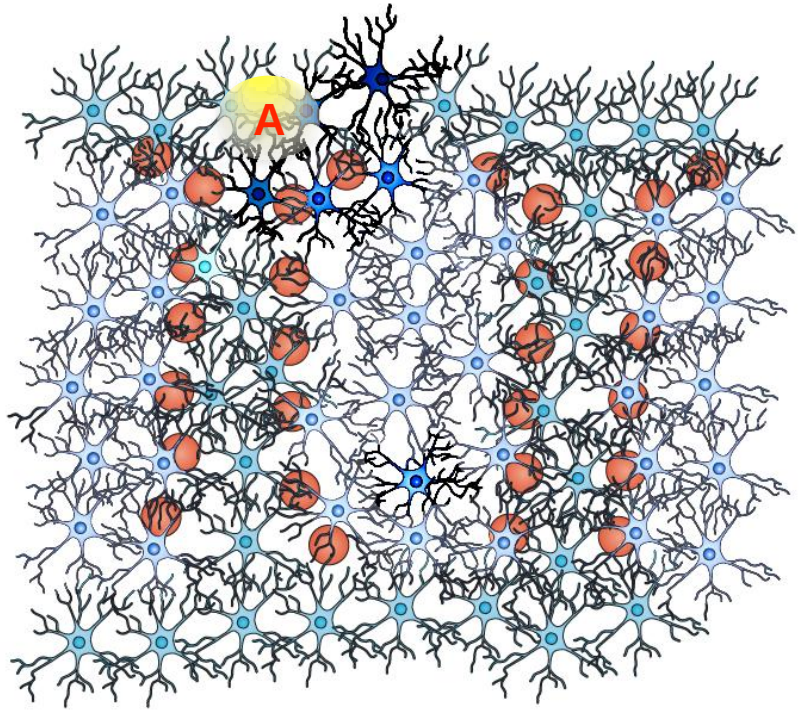


5 experiments, 23 fields of view

Outlook

- Normalization of fluorescence imaging data reveals a **significant cell-to-cell heterogeneity** in cell populations (e.g. astrocyte).
- The cell-to-cell variability can be **regulated** by extrinsic factors.

- In the case of the brain:
  - Astrocytes are **heterogeneous** with respect to cellular ATP level, mitochondrial electrical potential and mitochondrial ROS concentration.
  - Glutamate induces a **change** in the cellular energy status of astrocytes and in the **variability in energy metabolism**.



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