# EFAVIRENZ PRODUCES A DIFFERENTIAL BIOENERGETIC RESPONSE IN NEURONS AND GLIA CELLS

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## INTRODUCTION:

Neurological pathogenesis is often associated with mitochondrial dysfunction. It is known that glia cells overcome the damaging effects of mitochondrial inhibition by rapidly up-regulating AMP-kinase (AMPK)-mediated glycolysis and maintaining ATP levels, which is crucial for cell homeostasis and viability. In contrast, neurons lack this defense mechanism, and are thus more vulnerable to bioenergetic challenge. Many Efavirenz (EFV)-treated patients exhibit CNS-related effects, but the underlying mechanisms are unknown. However, recent evidence shows that this drug undermines mitochondrial function in human hepatic cells[1] and mouse brain[2].

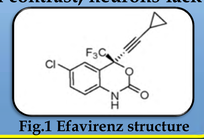


Fig.1 Efavirenz structure

## AIM:

We analyzed *in vitro* whether or not EFV interferes with the mitochondria of neurons and glia and assessed its influence on cellular bioenergetics. Since there is a substrate of CNS inflammation during HIV-related cognitive disorders, we also evaluated if EFV effects are exacerbated by nitric oxide (NO), a mediator of inflammation and inhibitor of mitochondrial respiration.

## RESULTS:

### 1. Electrochemical measurement of Oxygen ($O_2$) consumption

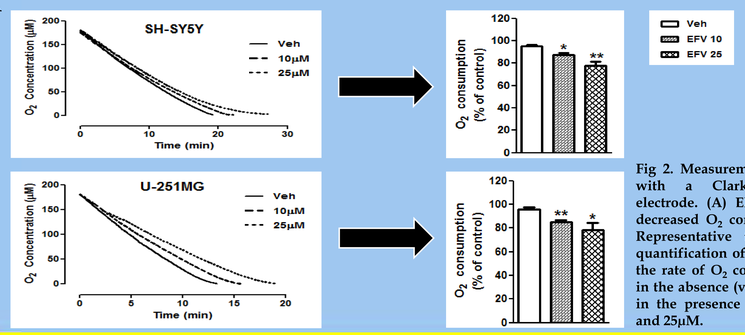


Fig 2. Measurements taken with a Clark-type $O_2$ electrode. (A) EFV acutely decreased $O_2$ consumption. Representative traces and quantification of data show the rate of $O_2$ consumption in the absence (vehicle) and in the presence of EFV 10 and 25µM.

### 2. Mitochondrial Reactive Oxygen Species (ROS) and Mitochondrial Membrane Potential ($\Delta\Psi_m$)

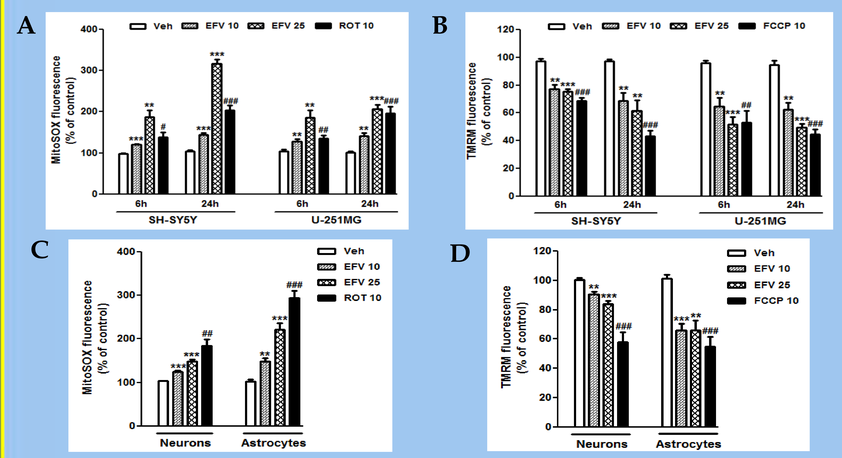


Fig 3. Effect of EFV on mitochondrial redox state studied by fluorescence microscopy. Superoxide production (A,C) and mitochondrial membrane potential (B, D) were assessed by MitoSOX and TMRM fluorescence respectively. Cells were treated with EFV for 24h (A and B: neuronal-like differentiated cells derived from SH-SY5Y and U-251MG human cells; C and D: primary cultures of neurons and astrocytes).

### 3. Cell viability

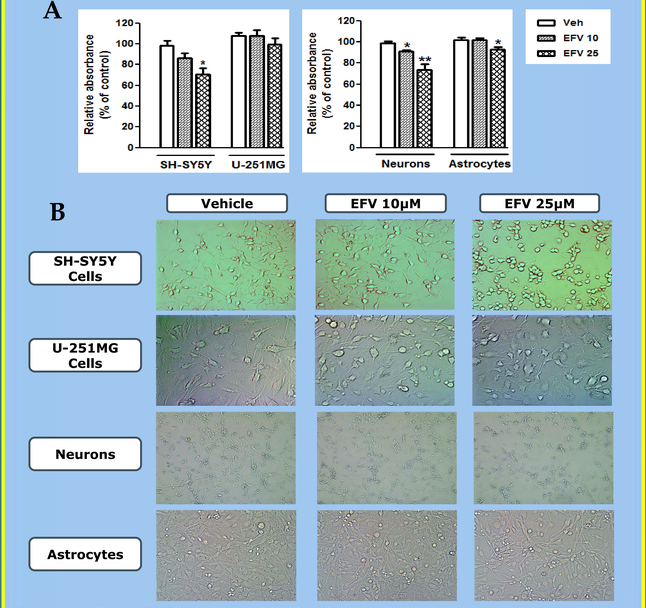


Fig 4. (A) Effect of EFV on cell viability after 24h treatment according to the MTT assay. (B) Representative inverted light microscopy images (10x) of cell culture treated with EFV for 24 h.

### 4. Differential effect on cell bioenergetics in neurons and glia cells

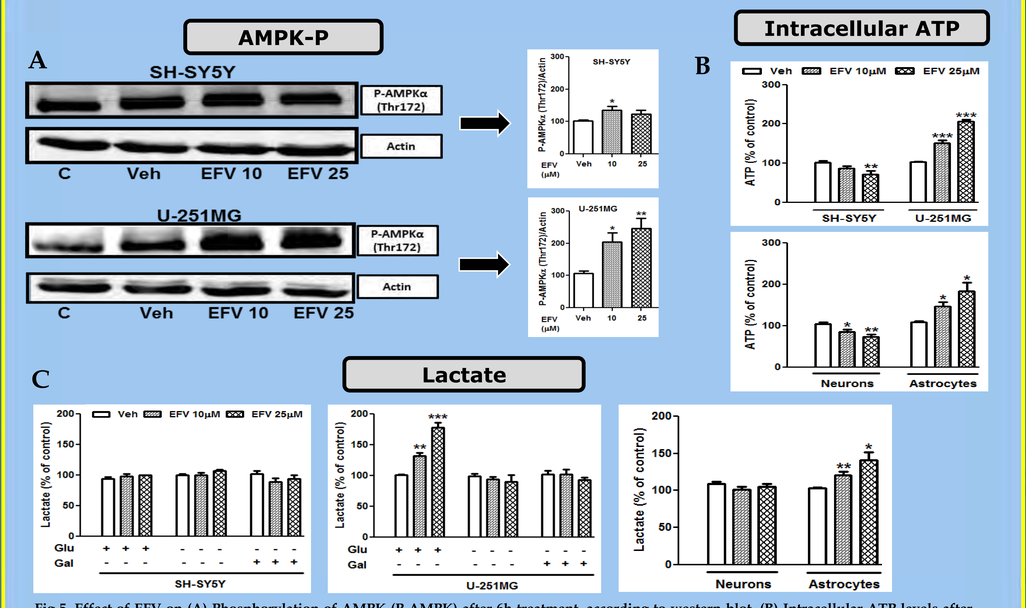


Fig 5. Effect of EFV on (A) Phosphorylation of AMPK (P-AMPK) after 6h treatment, according to western blot. (B) Intracellular ATP levels after 24h treatment, assessed by an Bioluminescence assay. (C) Lactate concentration in the extracellular medium after 24h treatment, analyzed by a spectrophotometric assay (in glucose, glucose-free and galactose-instead-of-glucose-containing media).

### 5. Effect of EFV on SH-SY5Y and U-251MG cells in the presence of exogenous Nitric Oxide (NO)

Neurons and glia cells were treated with 0.3 or 0.5mM DETA-NO respectively, concentrations capable of inhibiting cellular respiration[3].

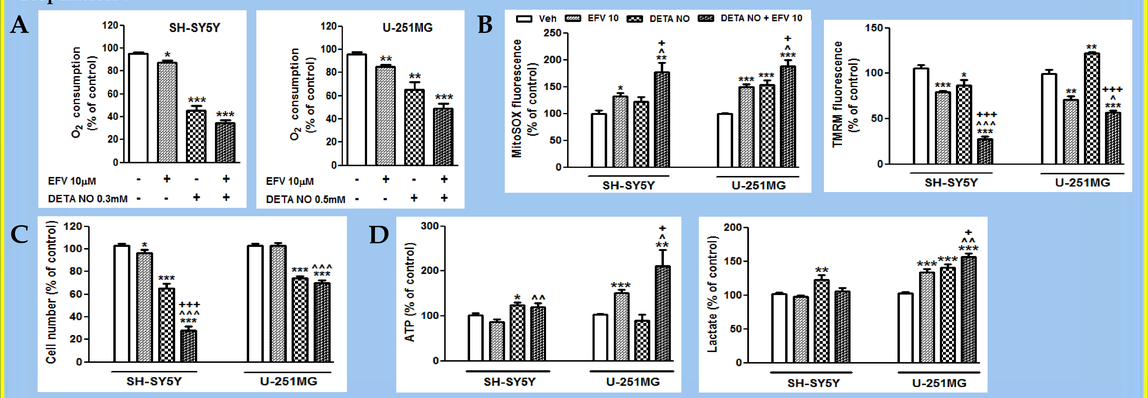


Fig 6. Histograms represent data obtained in the presence of EFV 10µM, DETA-NO or with co-treatment of DETA-NO and EFV 10µM. (A) Rate of $O_2$ consumption (B) Superoxide production and $\Delta\Psi_m$ (C) Cell number (cell count determined by static cytometry) (D) Intracellular ATP level and lactate production.

### 6. Effect of EFV on primary cultures of neurons and astrocytes in the presence of exogenous NO

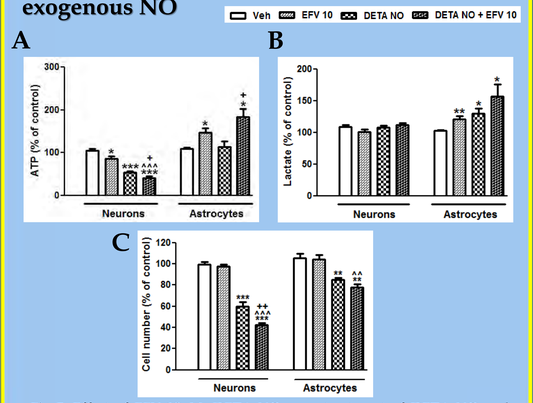


Fig 7. Effect of EFV 10µM, DETA-NO or co-treatment of DETA-NO and EFV 10µM on: (A) Intracellular ATP levels (B) Lactate production (C) Cell number.

## Statistical analysis:

Data are represented as % of control (considered 100%) and were analyzed using GraphPad Prism v.5 software with "Student's t-test". All values are mean±S.E.M and statistical significance was: *p<0.05, **p<0.01 and ***p<0.001 vs Vehicle. ^p<0.05, ^^p<0.01, ^^^p<0.001 EFV 10µM vs co-treatment of DETA-NO with EFV 10µM. $^{+}$p<0.05, $^{++}$p<0.01 $^{+++}$p<0.001 DETA-NO vs co-treatment of DETA-NO with EFV 10µM.

## References:

[1]Blas-García A, *et al.*, Hepatology 2010; 52(1):115-125. [2]Streck EL, *et al.*, Neurochem Res 2011; 36(6):962-966. [3]Almeida A, *et al.*, Proc Natl Acad Sci USA 2001; 98(26):15294-15299. [4]Saha RN, *et al.*, Free Radic Biol Med 2007; 42(12):1866-1878.

## CONCLUSION:

EFV exerts a direct and specific effect on the energetic balance and viability of neurons and glia through a mechanism involving acute mitochondrial inhibition, an action that could be exacerbated in neuroinflammatory conditions like those often present in HIV-patients. This is relevant given that neurocognitive disorders, including HIV-related dementia, have been associated with a similar scenario of glia cell survival and degeneration of neurons[4].