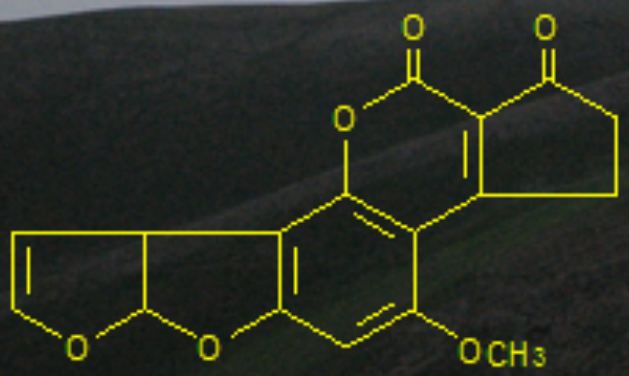
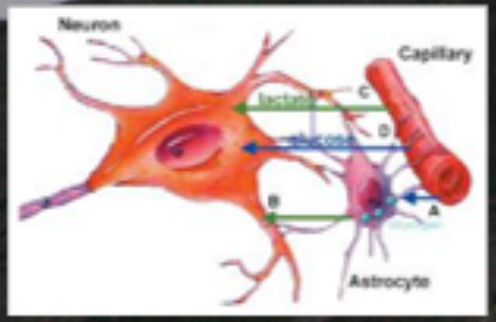
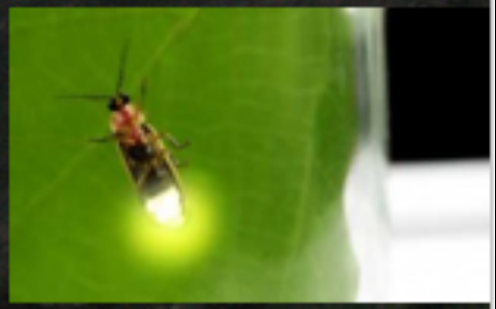
Bioluminescence-based detection of brain immune cells apoptosis and ATP depletion induced by aflatoxin B_1

By J. Mehrzad

Associate Prof. of Immunology *mehrzad@um.ac.ir*







Slide's background location:
Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3800 m above sea level

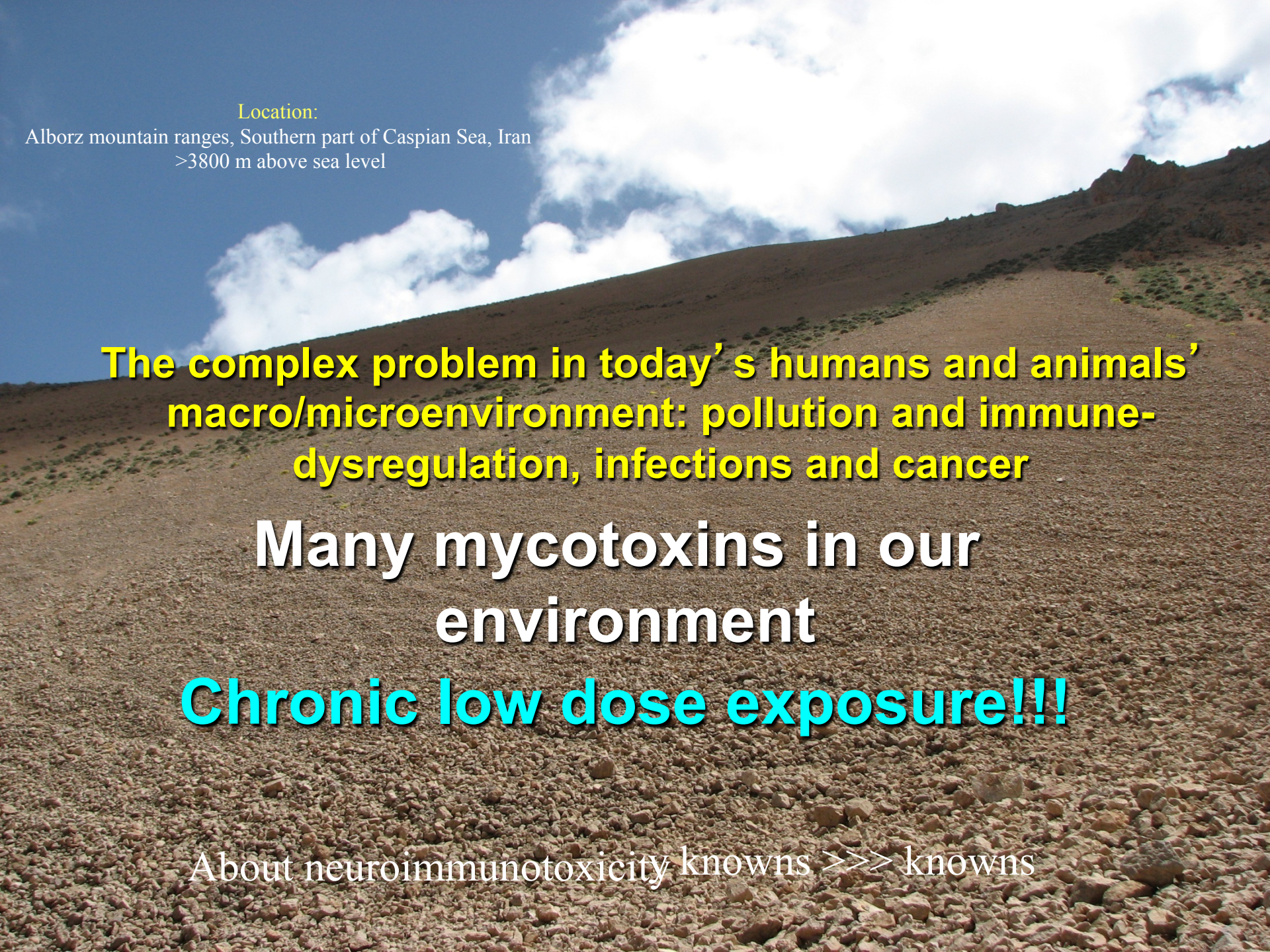
Friday , 22-07-2016, at 17:20

Contents

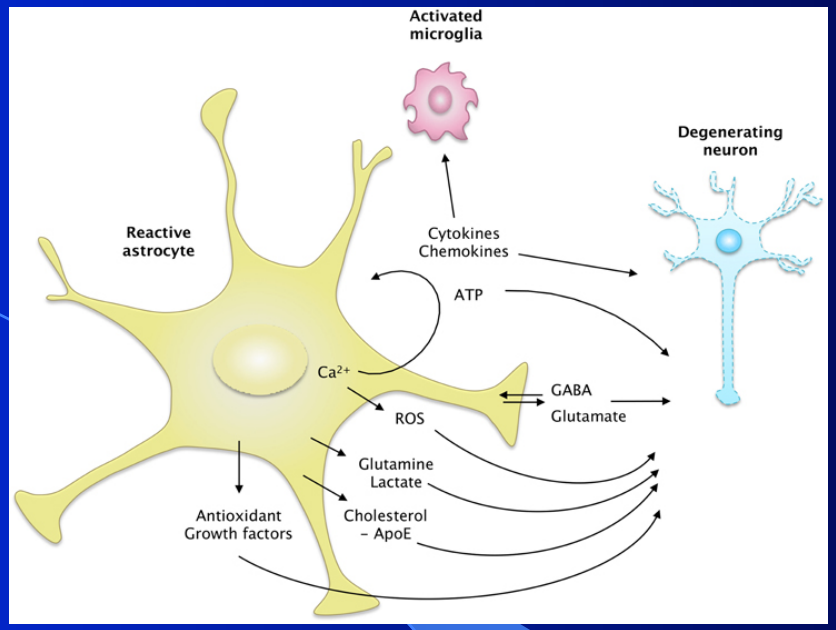
Location:
Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3800 m above sea level

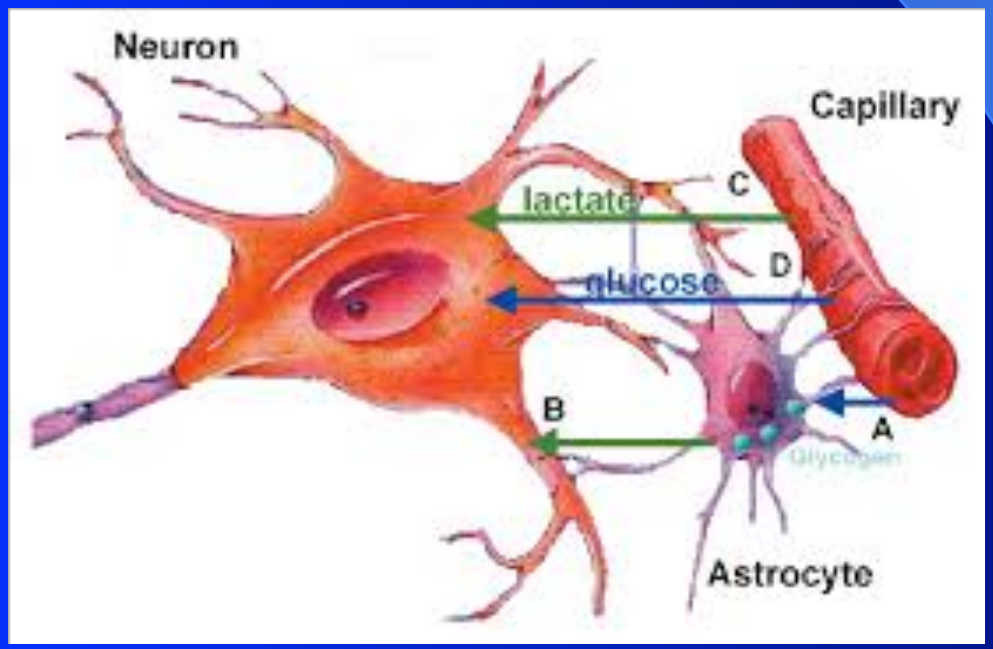
Main emphasis on neurotoxicity, astrocytes and AFB_1

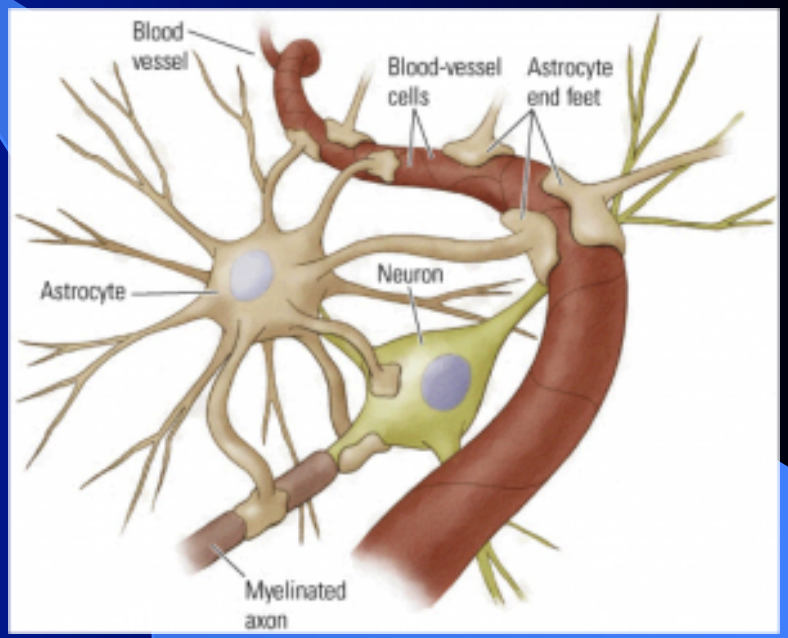

INTRODUCTION


Location:
Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3800 m above sea level
The complex problem in today's humans and animals' macro/microenvironment: pollution and immune-dysregulation, infections and cancer
Many mycotoxins in our environment
Chronic low dose exposure!!!
About neuroimmunotoxicity knowns >>> knowns

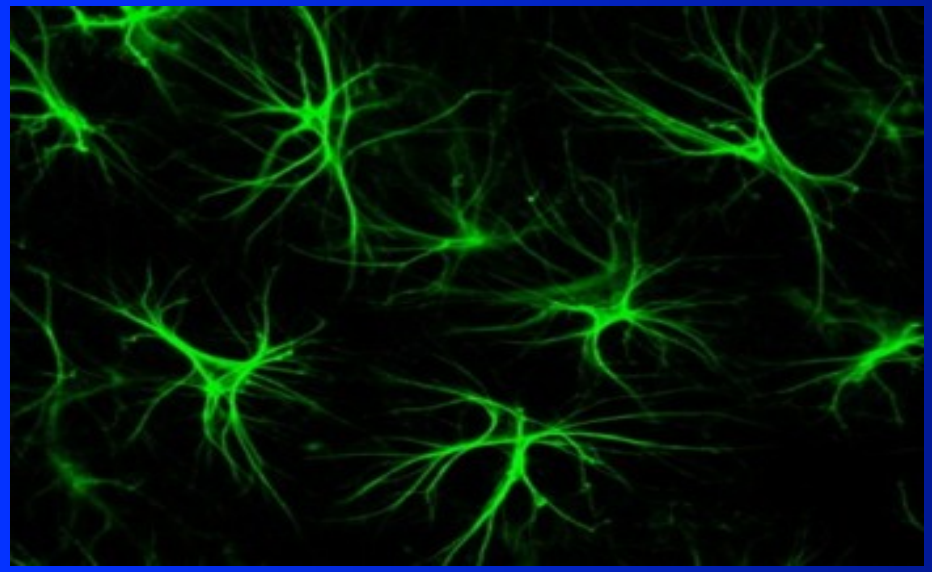
Astrocytes

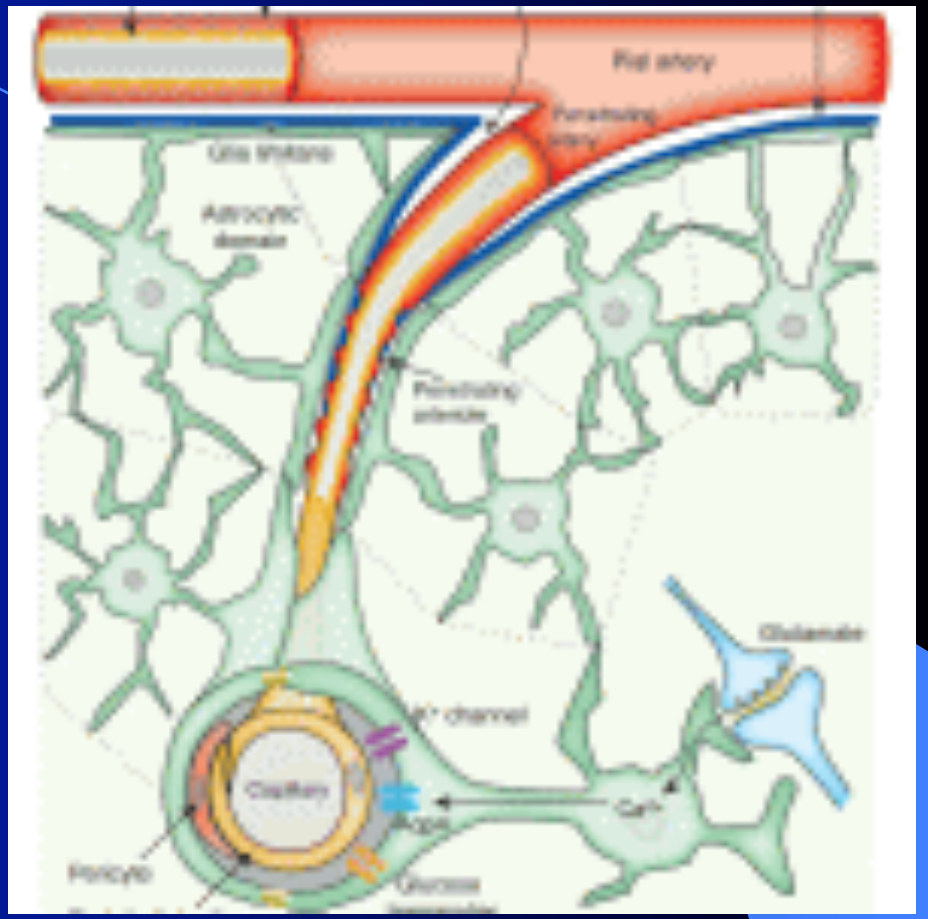

Activated microglia
Degenerating neuron
Reactive astrocyte
Cytokines Chemokines
ATP
Ca^{2+}
GABA
Glutamate
ROS
Glutamine
Lactate
Cholesterol – ApoE
Antioxidant Growth factors


Neuron
Capillary
lactate
C
D
glucose
B
A
Glycogen
Astrocyte


Blood vessel
Blood-vessel cells
Astrocyte end feet
Astrocyte
Neuron
Myelinated axon

Astrocytes protect/control neuron functions





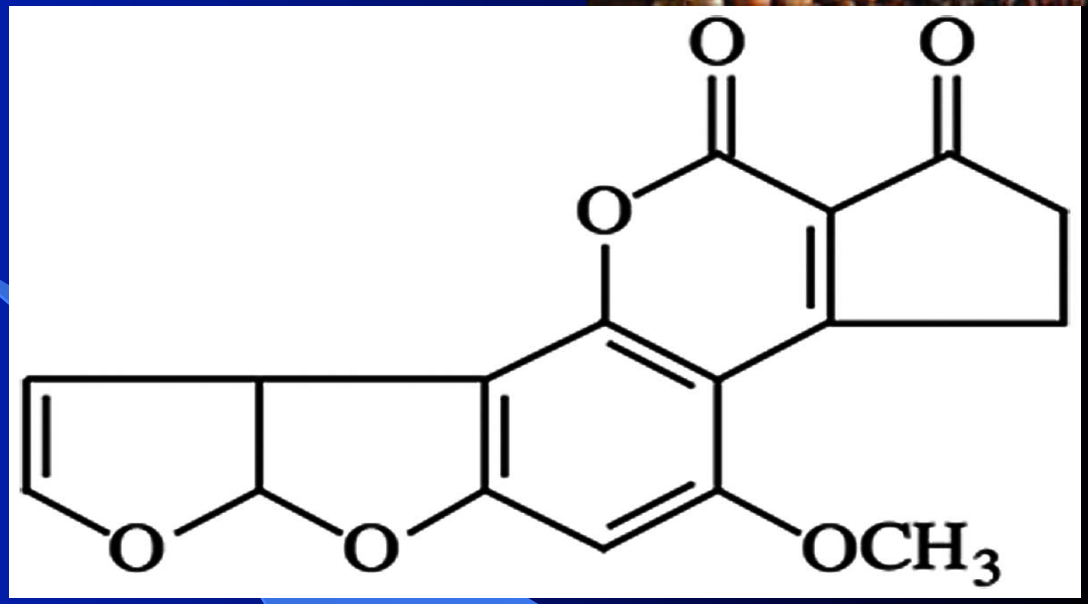
-Form BBB,

-CNS blood flow regulation

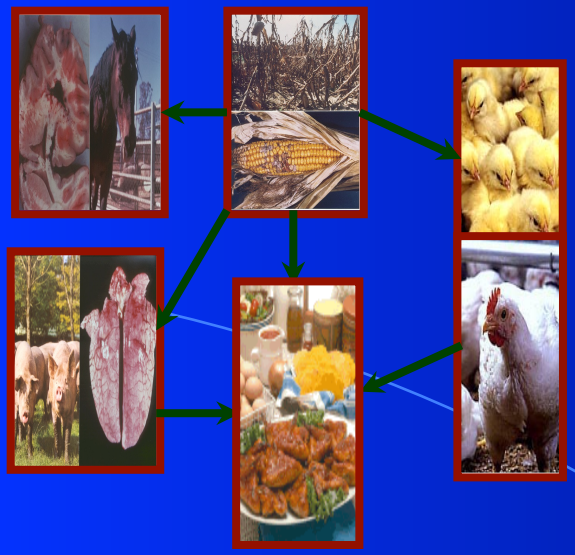
-Involve in diverse CNS and PNS physiopathology

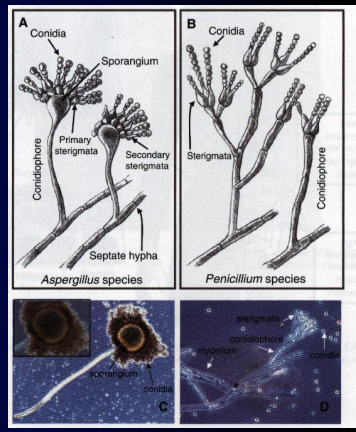
-Possess huge immunological arms again pathogens… even immunosurveillance of P/M/DAMPs ….

Aflatoxins (AFs)

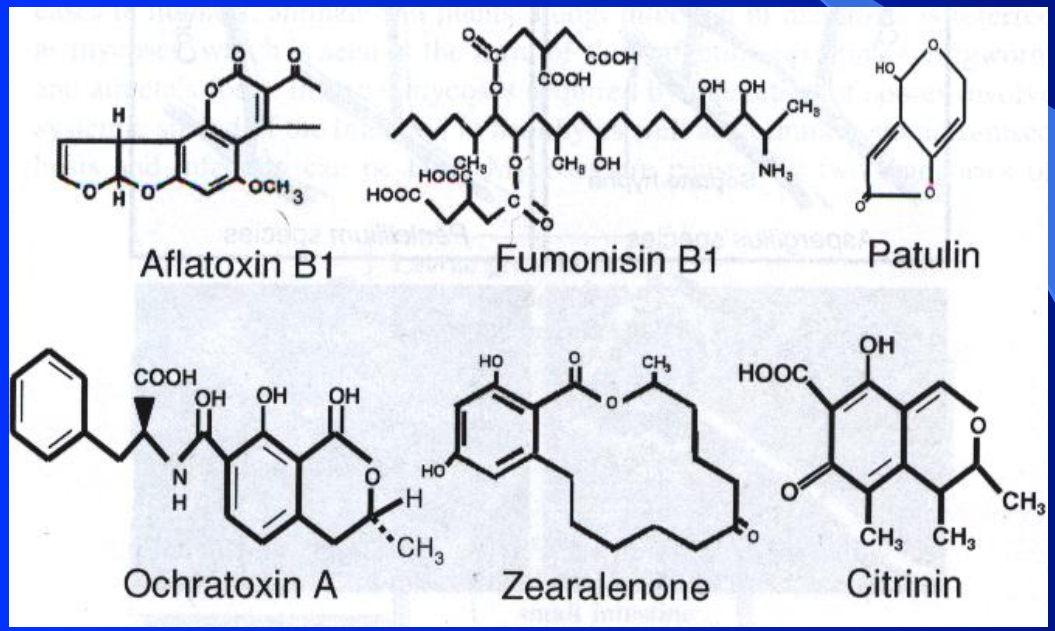


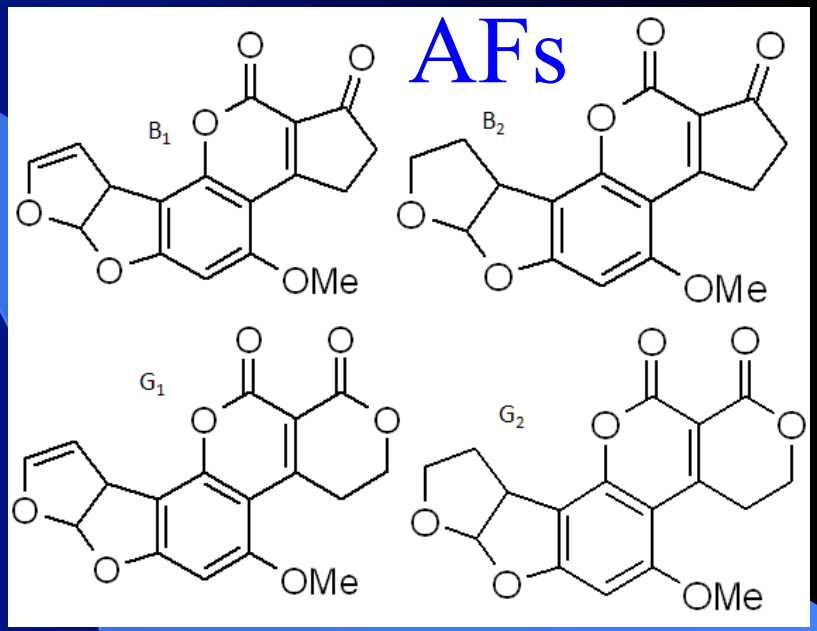
Mycotoxins, Aflatoxins (AFs)





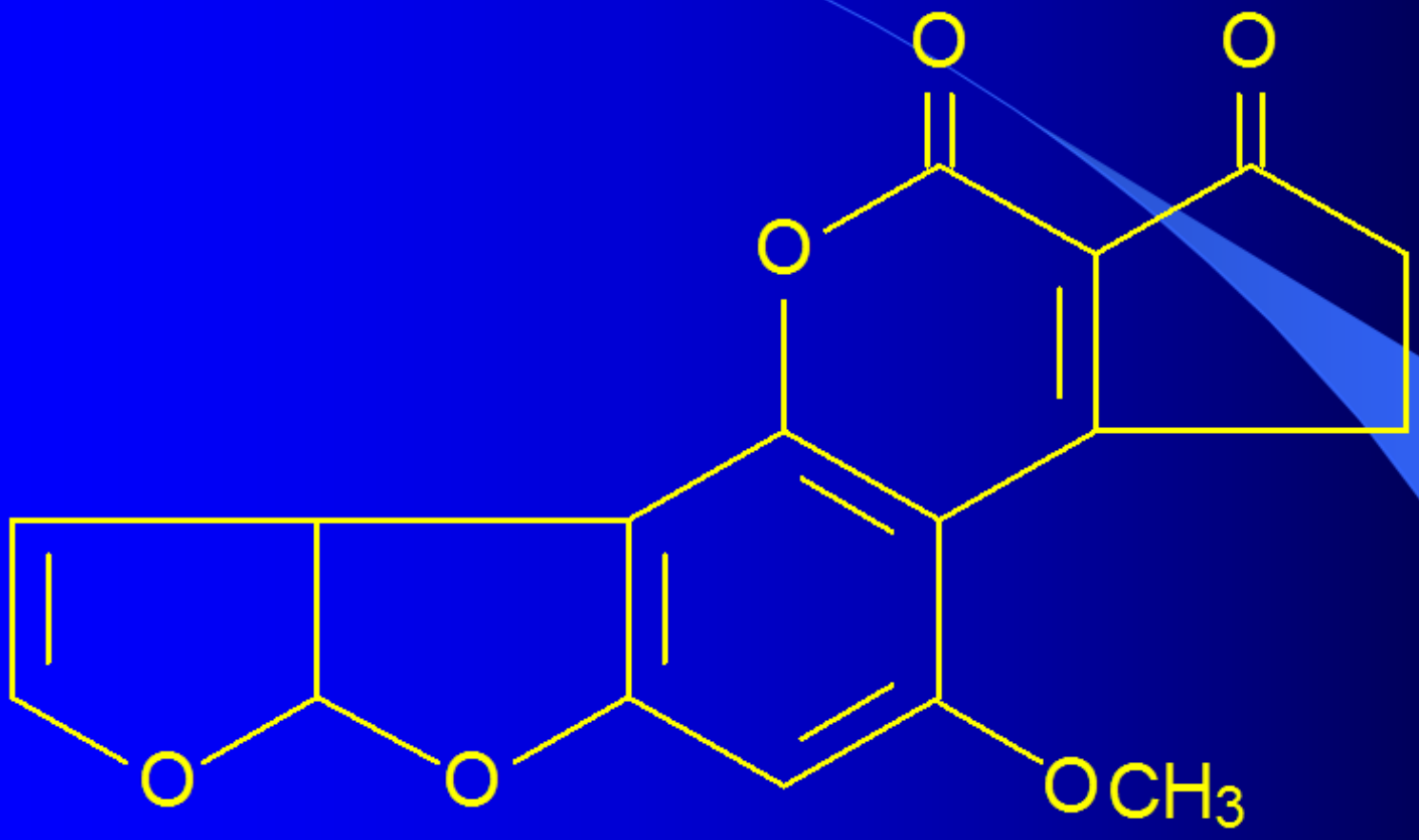
ubiquitous environmental carcinogens, and huge risk to humans and animals





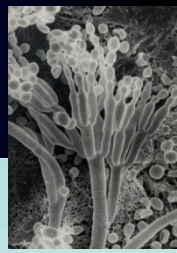
small, low MW, tasteless, colorless, highly stable and hardly destroyable…

Aflatoxin B_1



AFB_1 ($C_{17}H_{12}O_6$) is the strongest, very lipophilic and easily and fast enters even via skin….

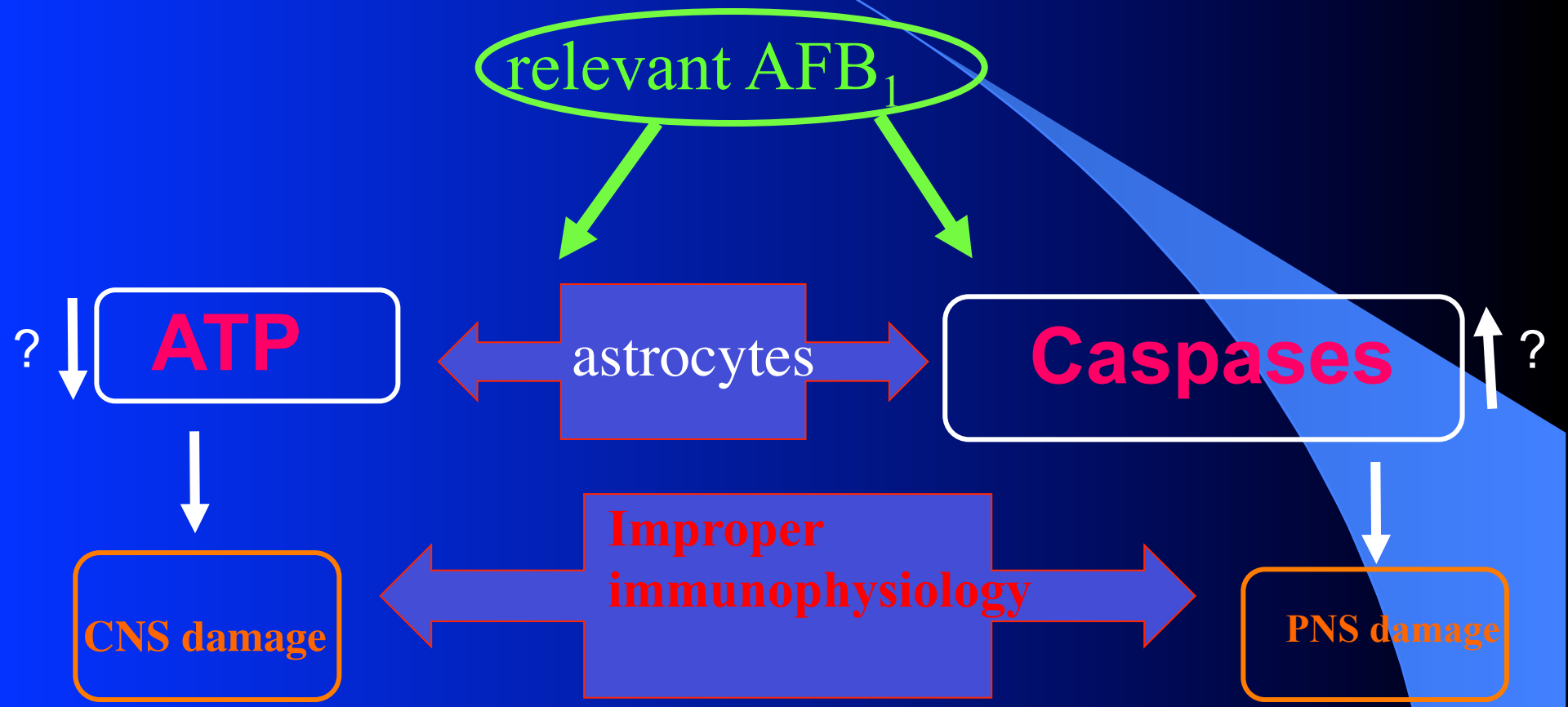
AFB_1 exposure



Very serious GLOBE issue

Chronic low dose !!

Aims/hypotheses



Cell membrane and cytosolic damage of astrocytes caused by AFB_1?

Methodology

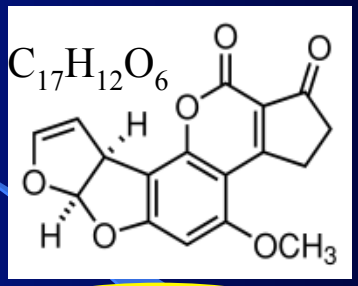
Location: Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3500 m above sea level

Functional analyses on astrocytes

With some apparatus for analytical assays

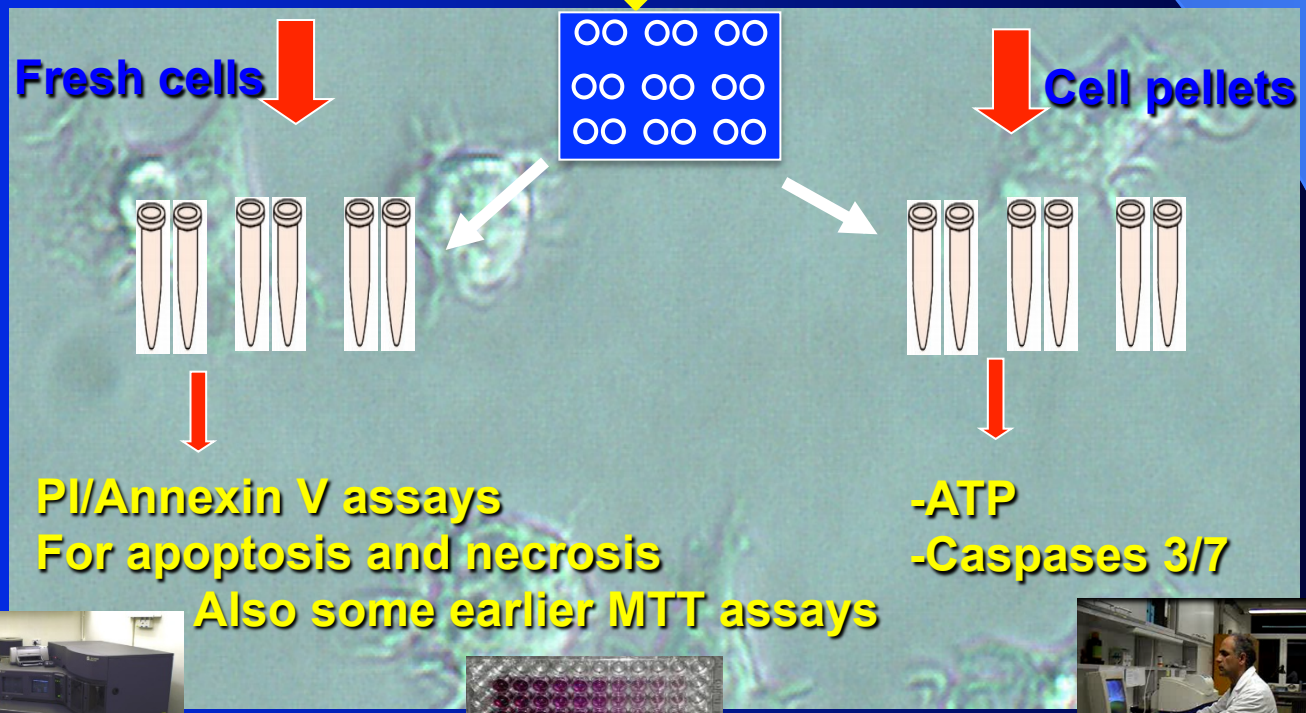
Murine astrocytes

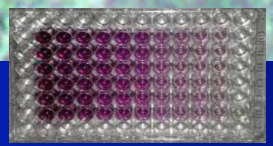
+/-



FACS

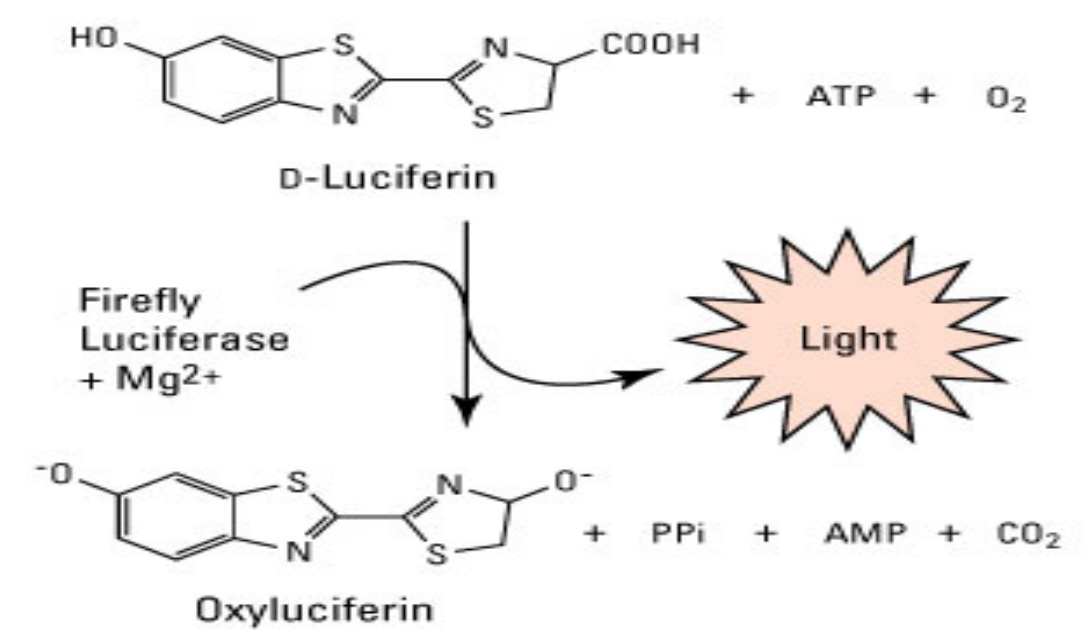
Bioluminescence





With various doses and exposure time

Functional analyses on astrocytes



BL probes

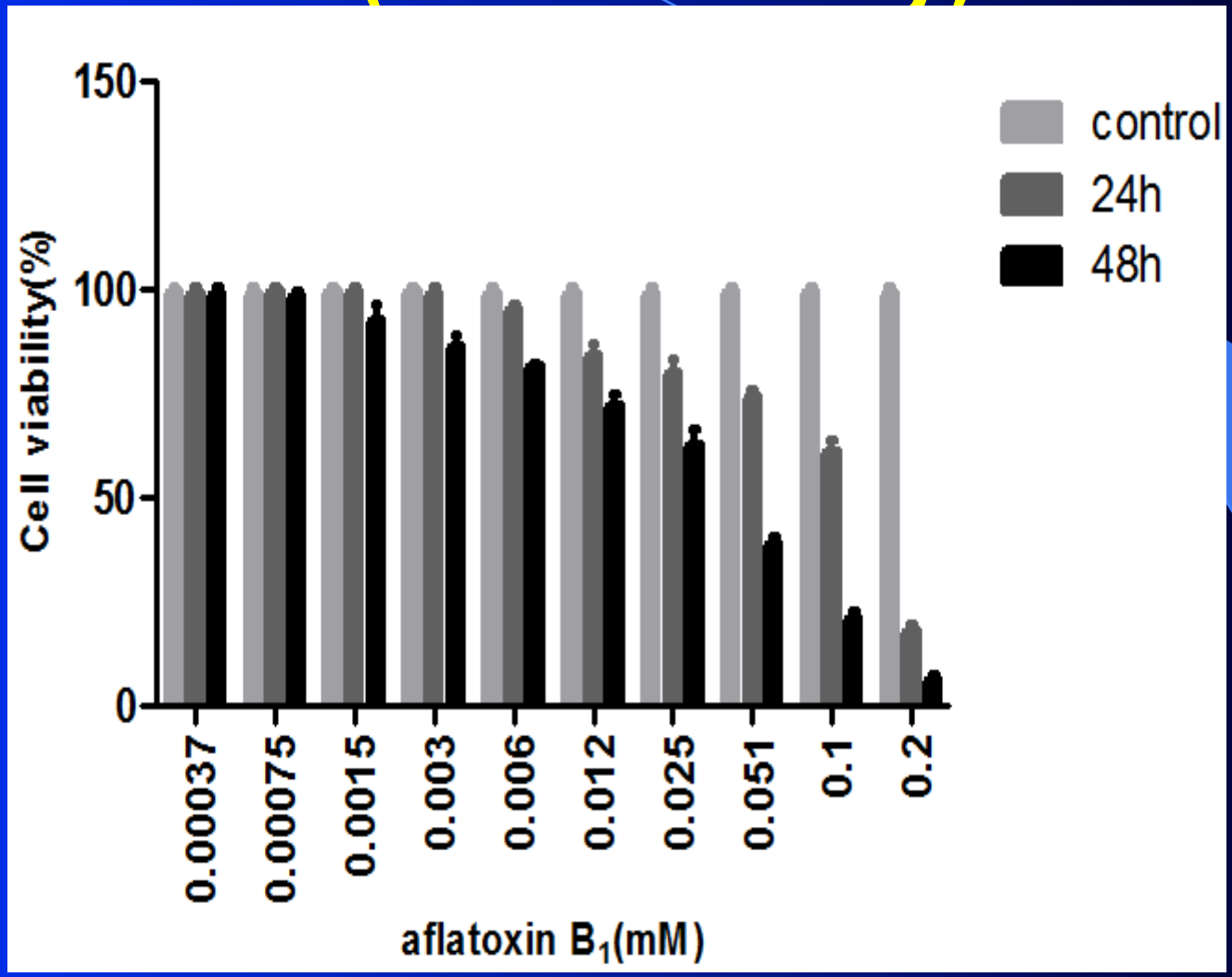
Luciferin, luciferase, and ATP ➡ Key.....

DEVD (caspase 7/3 substrate)-conjugated luciferin

Both in ATP and caspases assays, with high quantum yields BL is generated and measured

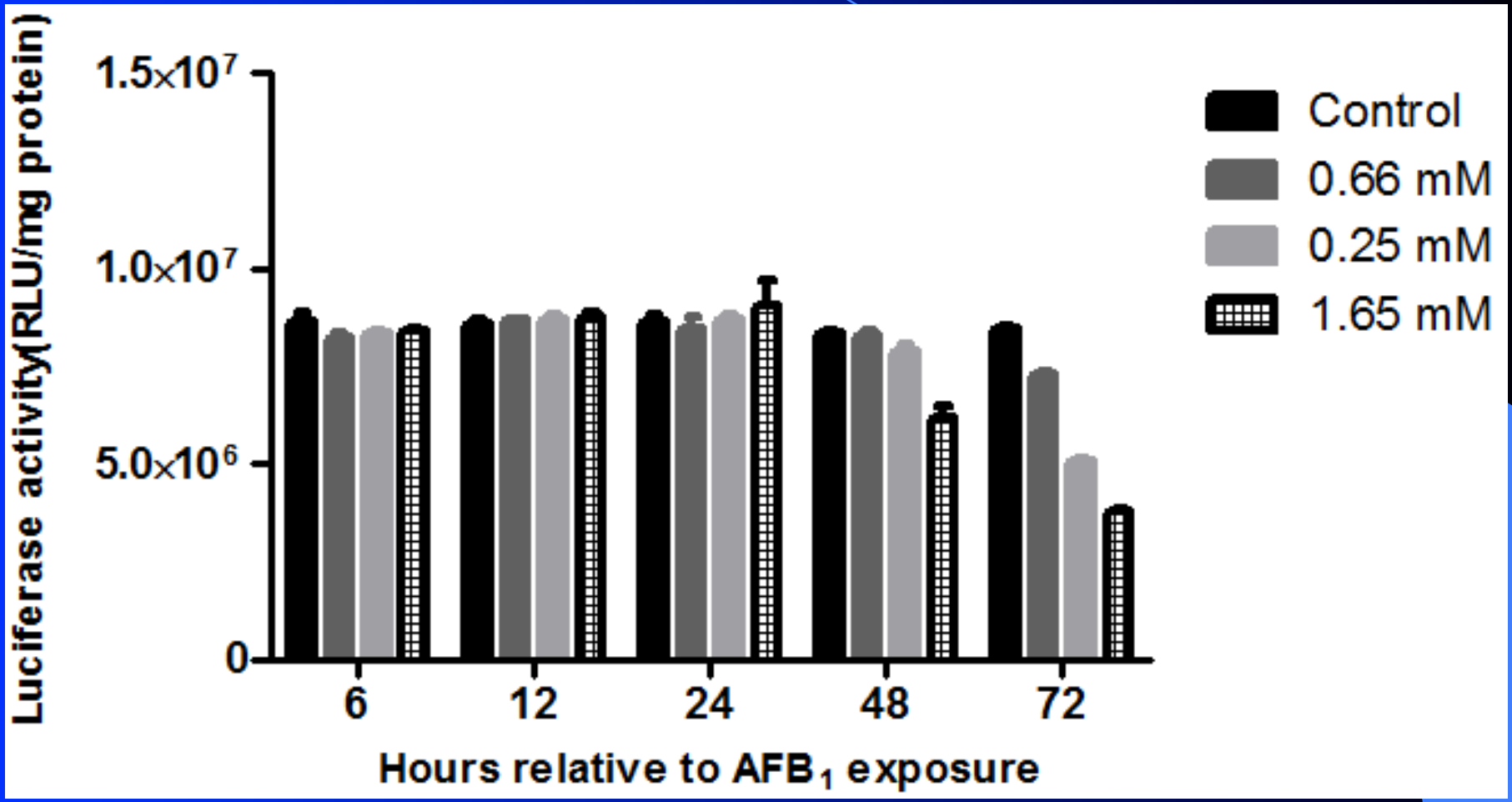

Some results and
discussions

AFB_1 decreases viability of astrocytes (in MTT assay)



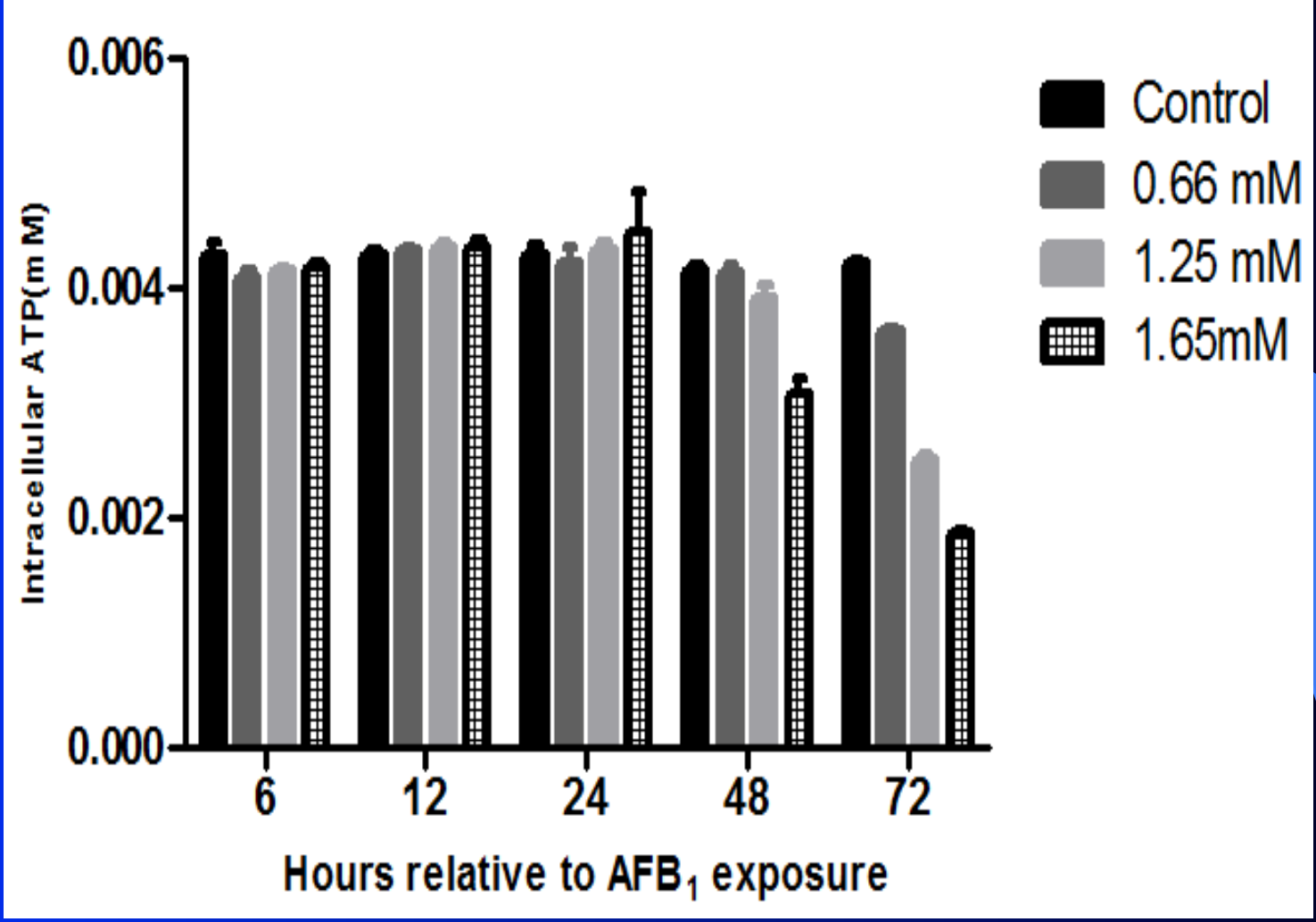
With LC50 of 0.11 and 0.03 mM for 24 and 48h, respectively

AFB_1 decreases intracellular ATP of astrocytes (in bioluminescence assay)



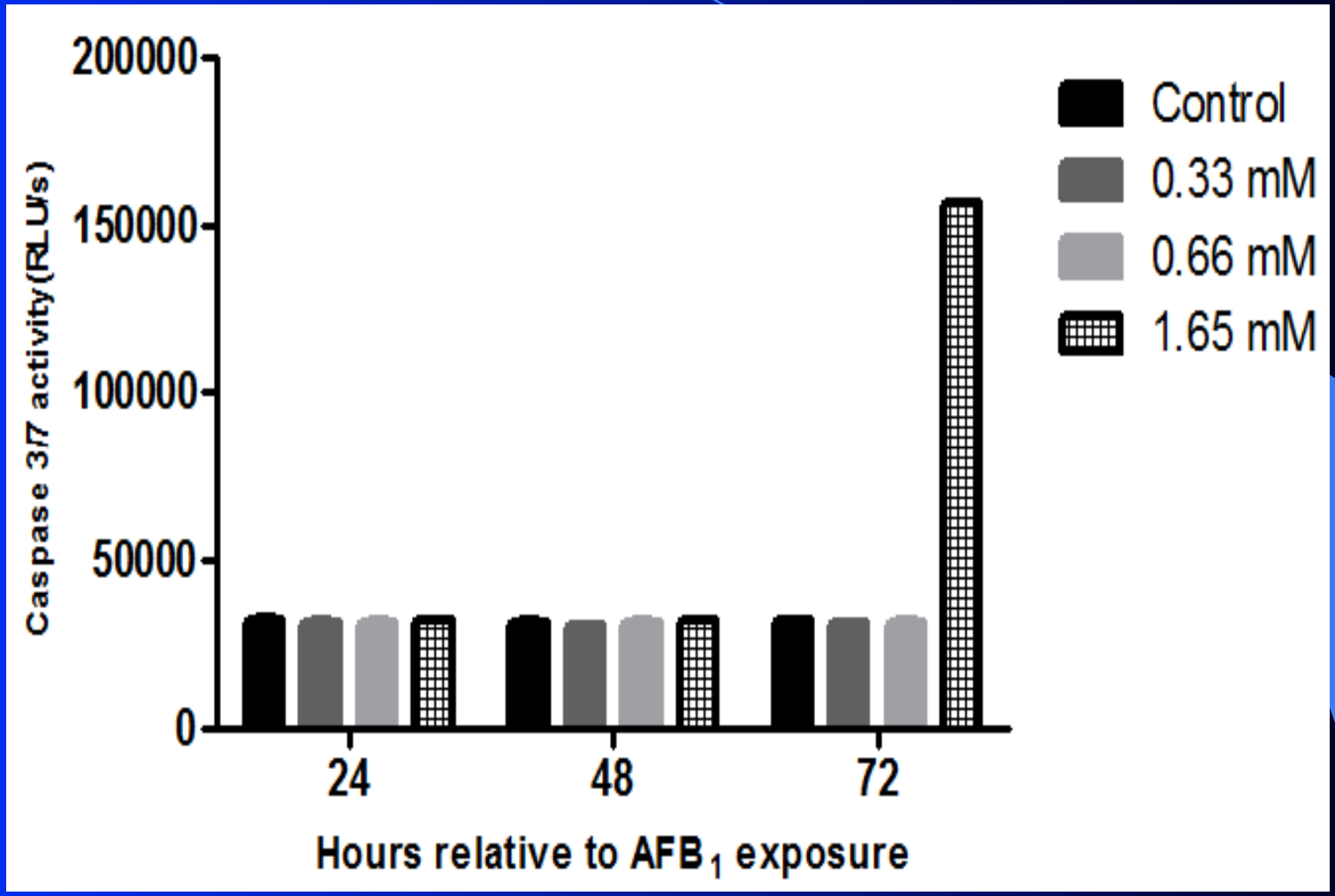
Dose-and-time dependent manner

AFB_1 decreases intracellular ATP of astrocytes (in bioluminescence assay)



Dose-and-time dependent manner

AFB_1 increases caspases activity of astrocytes (in bioluminescence assay)



Dose-and-time dependent manner

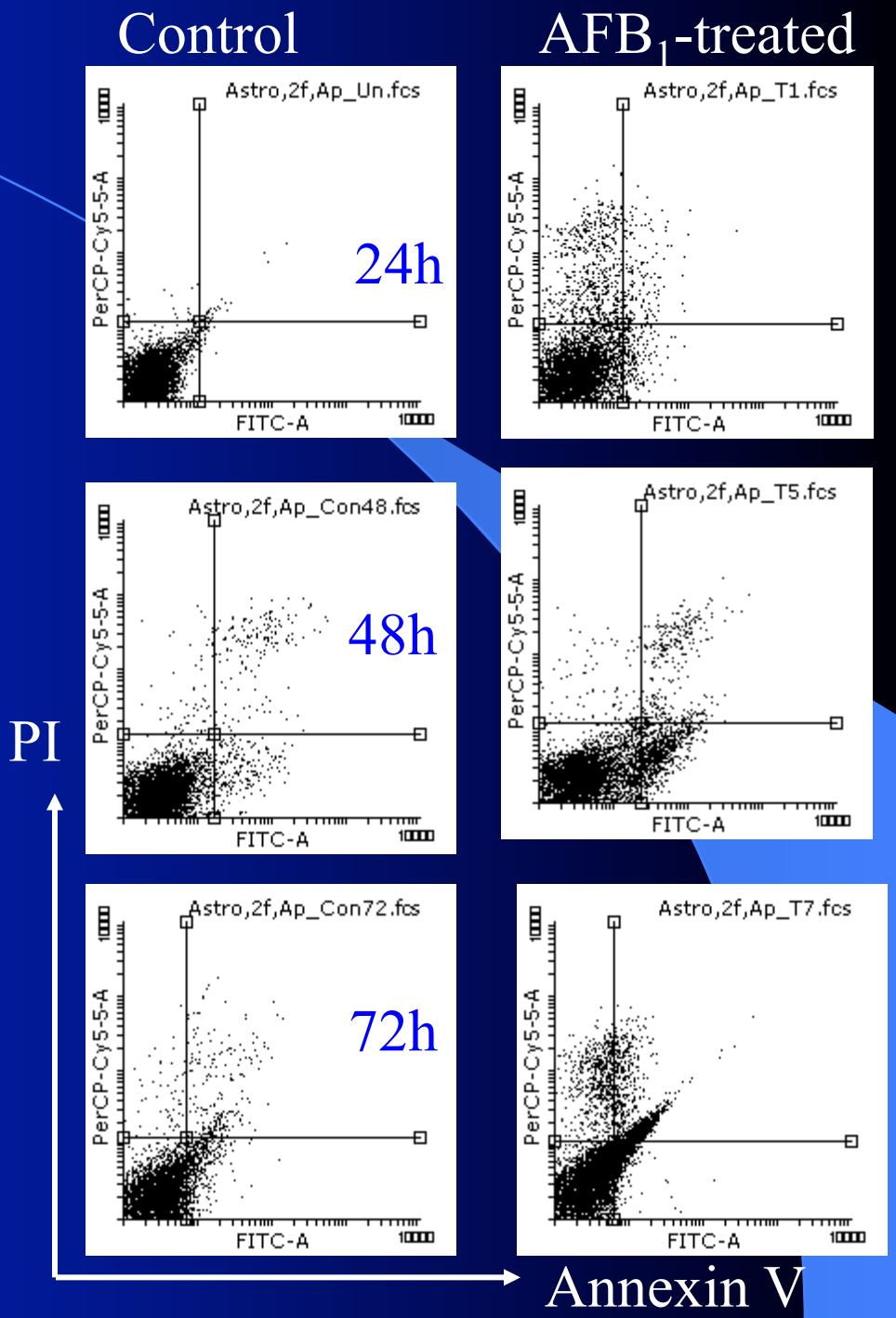
AFB_1 and apoptosis /necrosis in astrocytes (in FACS assay)

Cell membrane and cytosolic damage

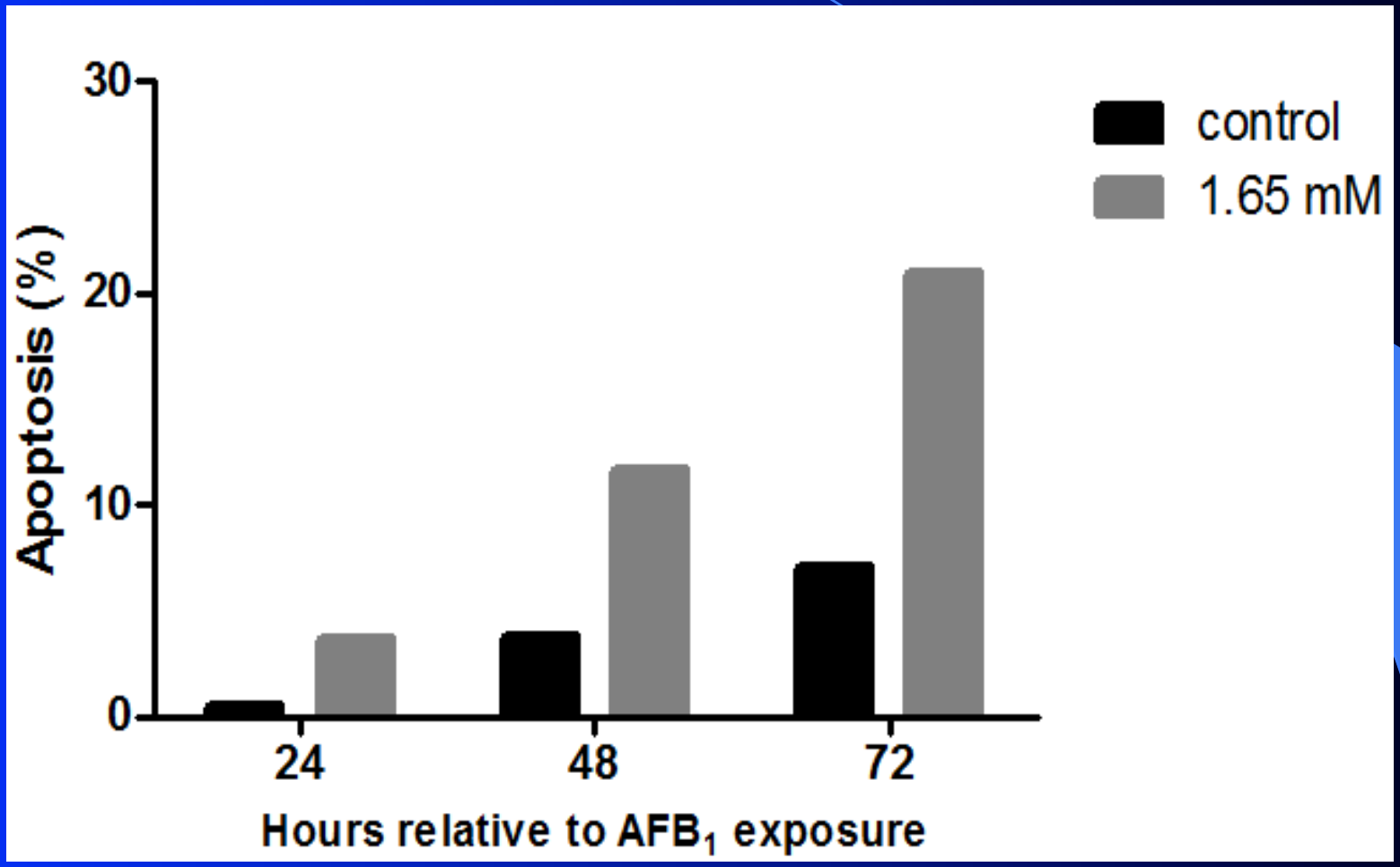
Two dimentional plots

Four distinct cell populations

Representative

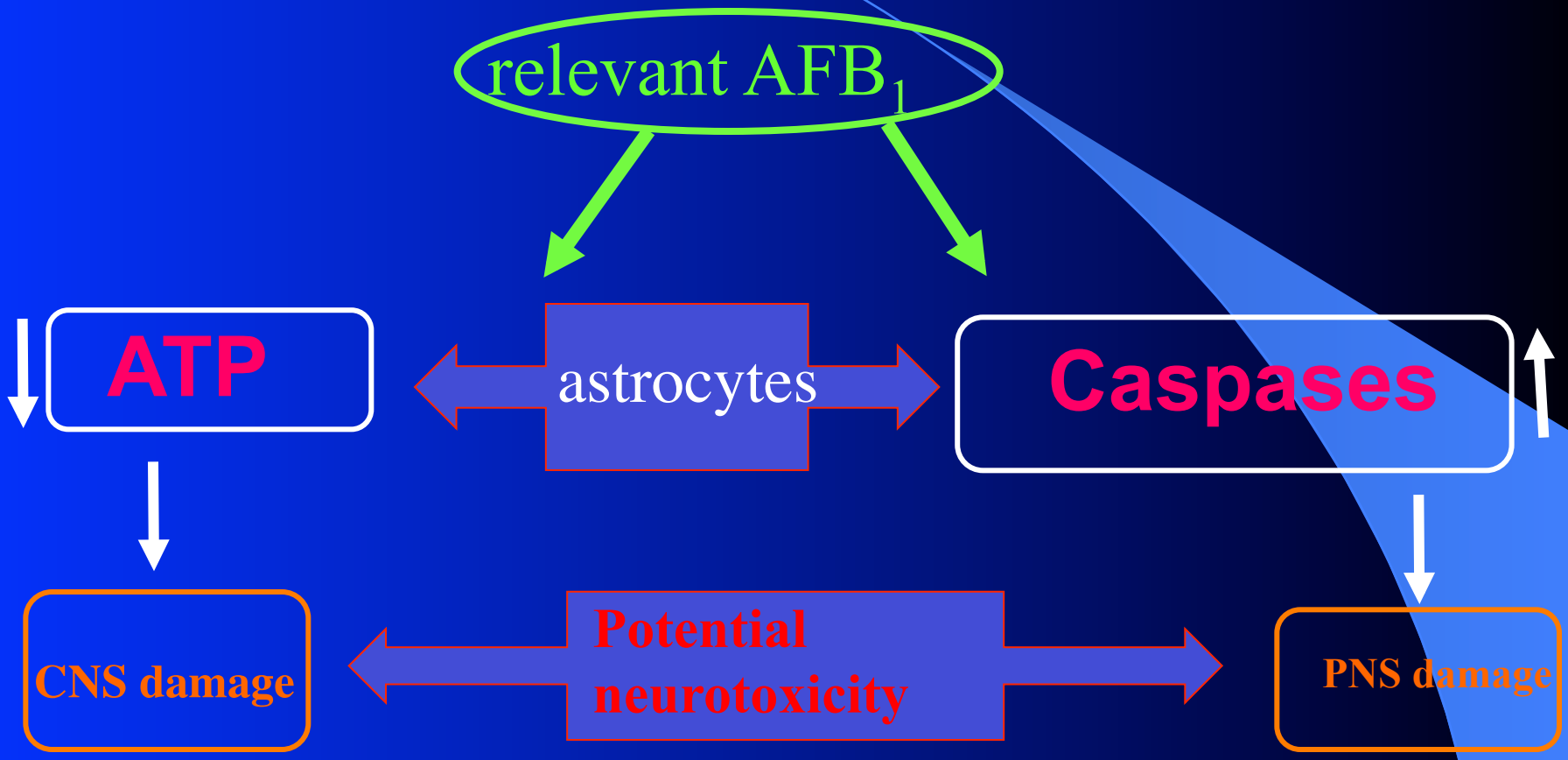


AFB_1 and apoptosis/necrosis in astrocytes (in FACS assay)




Conclusions

Potential neurotoxicity of AFB_1 on CNS/ PNS via astrocytes



this *in vitro* could potentially translate to *in vivo… more work*

MSc student, Parisa Vahidi Ferdowsi
Prof. Dr Saman Hosseinkhani

From Tarbiat Modares University, Tehran.

Thanks for your attention

Location: Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3500 m above sea level