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# **LASTING EFFECTS OF PRENATAL EXPOSURE TO CANNABIS IN THE RETINA OF THE OFFSPRING: AN EXPERIMENTAL STUDY IN MICE**

**Paulo Zantut, Mariana Veras, Sarah Benevenuto, Angélica Safatle, Ricardo Pecora,  
Victor Yariwake, Janaina Torres, Marco Martins, Aline Bolzan, Paulo Saldiva,  
Francisco Max Damico**

**Department of Ophthalmology, Universidade de Sao Paulo Medical School  
Department of Pathology, Universidade de Sao Paulo Medical School  
Department of Surgery, Veterinary Medicine College and Zootechny  
Sao Paulo SP, Brazil**

# DISCLOSURES

**Authors declare that they or their spouses/partners did not have, or will they or their spouses/partners have, a financial relationship with a commercial interest relevant to this presentation within the 12 months prior to the presentation**

# SUMMARY

## Introduction

Prenatal exposure to Cannabis is a worldwide growing problem. Although retina is part of the central nervous system, little is known about the impact of maternal cannabis use on the retinal development and its potential postnatal consequences. Considering that the prenatal period is potentially sensitive in the normal development of the retina, we hypothesized that recreational use of Cannabis during pregnancy may alter retina structure in the offspring. To test this, we developed a murine model that mimics human exposure in terms of dose and use.

## Methods

We have shown for the first time that gestational exposure to Cannabis smoke may cause structural changes in the retina of the offspring that return to normal on mice adulthood. These are experimental evidences that suggest that children and young adults whose mothers smoked Cannabis during pregnancy may require earlier and more frequently clinical care than the non-exposed population.

## Results

Pregnant BalbC mice were exposed (nose-only) daily for 5 min to either Cannabis smoke (0.2 g of Cannabis) or filtered air, from gestational day 5 to 18 (N=10/group). After weaning period, pups were separated and examined weekly. On days 60, 120, 200, and 360 after birth, 10 pups from each group were randomly selected for Spectral Domain Optical Coherence Tomography (SD-OCT) analysis of the retina. Retina layers were measured (NFL+GCL, IPL, INL, OPL, ONL, IS/OS, RPE) and the inner, outer, and total retina thickness were calculated. Other 37 mice from both groups were sacrificed on days 20, 60, and 360 for retinal stereology (total volume of the retina and volume fraction of each retinal layer) and light microscopy. Means and standard deviations were calculated and MANOVA was performed. Null hypotheses were rejected at a probability level of  $P=0.05$ .

## Conclusion

Our data show that the retina of animals which mother was exposed to Cannabis during gestation was 17% thinner on day 120 (young adult) than controls ( $P=0.003$ ) due to 21% thinning of the outer retina ( $P=0.001$ ). The analysis of each retinal layer showed that the offspring of mice from the exposed group presented thickening of the IS/OS in comparison to controls on day 200 ( $P<0.001$ ). In the volumetric analyzes by retinal stereology, the exposed mice presented transitory increase of the IS/OS total volume and volume fraction on day 60 (young adult) compared to controls ( $P=0.008$  and  $P=0.035$ , respectively). On light microscopy, exposed mice presented thickening of the IS/OS on day 360 (adult) compared to controls ( $P=0.03$ ).

# CANNABIS SMOKING AND PREGNANCY



Morning sickness



Vomiting



Back pain



Recreational use

# ENDOCANNABINOID SYSTEM AND RETINA

- Endocannabinoid receptors are present in all retinal layers and CNS
  - ✓ Neural development and modulation
- The prenatal period is potentially sensitive in the normal development of the retina
- *In utero* exposure to Cannabis may interfere with normal retina development

# PURPOSE

**To investigate structural changes in the retina of the offspring of pregnant mice exposed to Cannabis smoke during pregnancy**

- ✓ **SD-OCT retinal segmentation**
- ✓ **Histologic stereology analysis**
- ✓ **Light microscopy**

# STUDY DESIGN

Exposed to 0.2 g *Cannabis* smoke  
(CAN group)  
(5 min/day)

10 pregnant mice

Exposed to filtered air  
(FA group)  
(5 min/day)

10 pregnant mice

SD-OCT

5 CAN group

5 FA group

60 days

120 days

200 days

360 days

Retinal stereology  
Light microscopy

18 CAN group

19 FA group

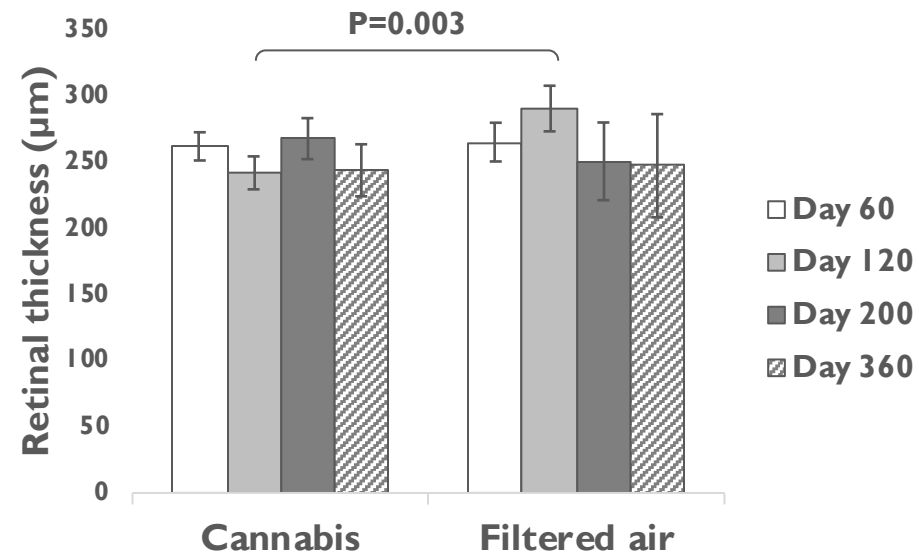
20 days

60 days

360 days

# RESULTS (SD-OCT)

On day 120, **total retina** of the offspring of the **CAN** group was **17% thinner** than in **FA**

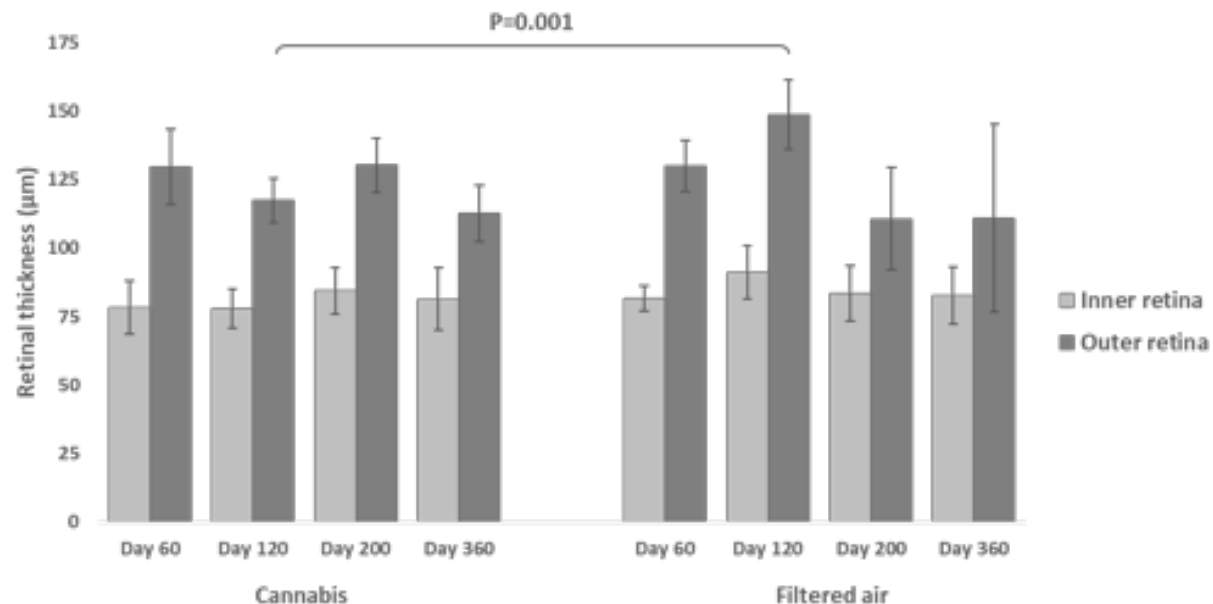


In multivariate regression analysis, **age\*exposition** was statistically significant for this outcome (95% CI: 251-263, P=0.003)



# RESULTS (SD-OCT)

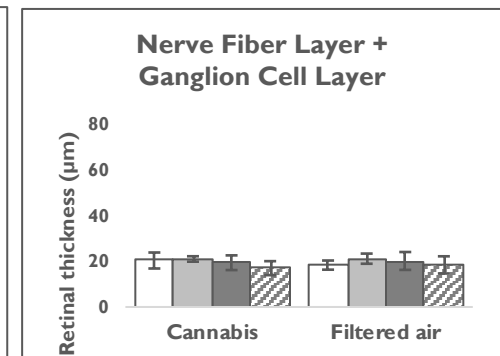
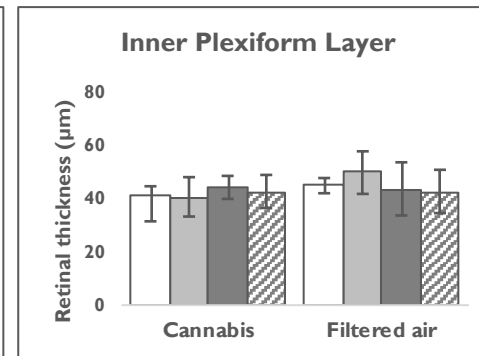
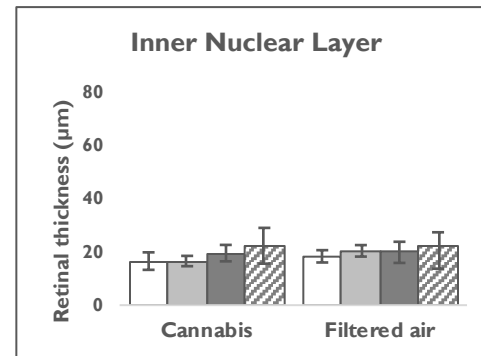
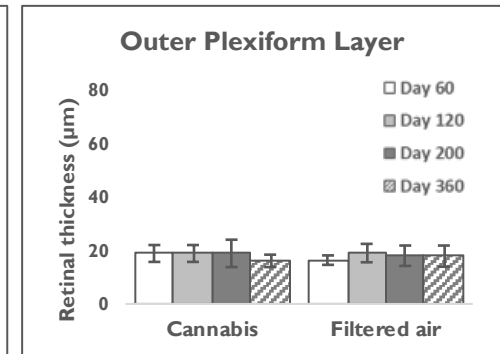
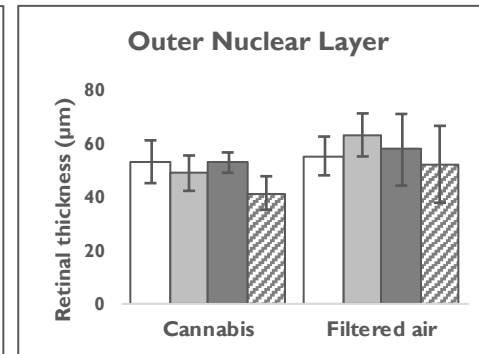
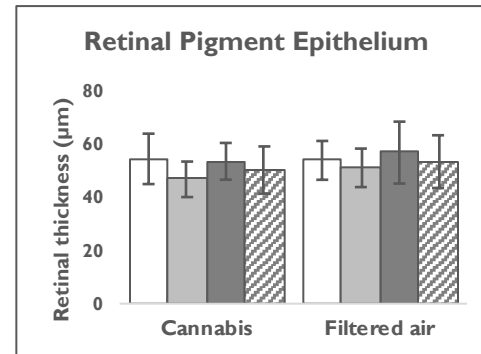
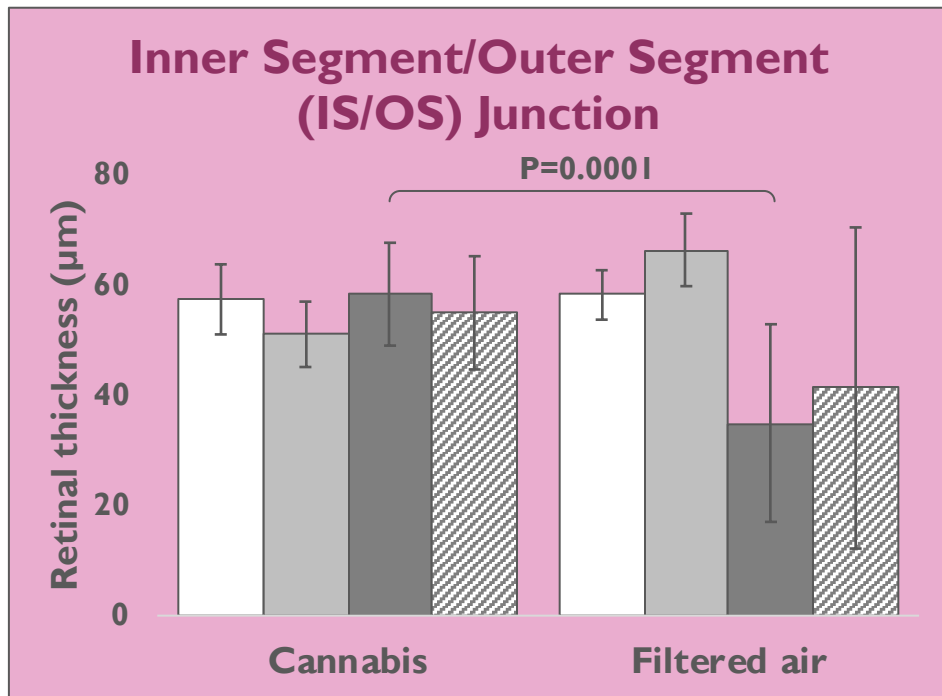
On the same time point (day 120), **outer retina** of the offspring was 21% thinner in **CAN** group than in **FA** group



Multivariate regression analysis showed the influence of **age\*exposition** over these outcomes (95% CI: 117-126, P=0.001)

# RESULTS (SD-OCT)

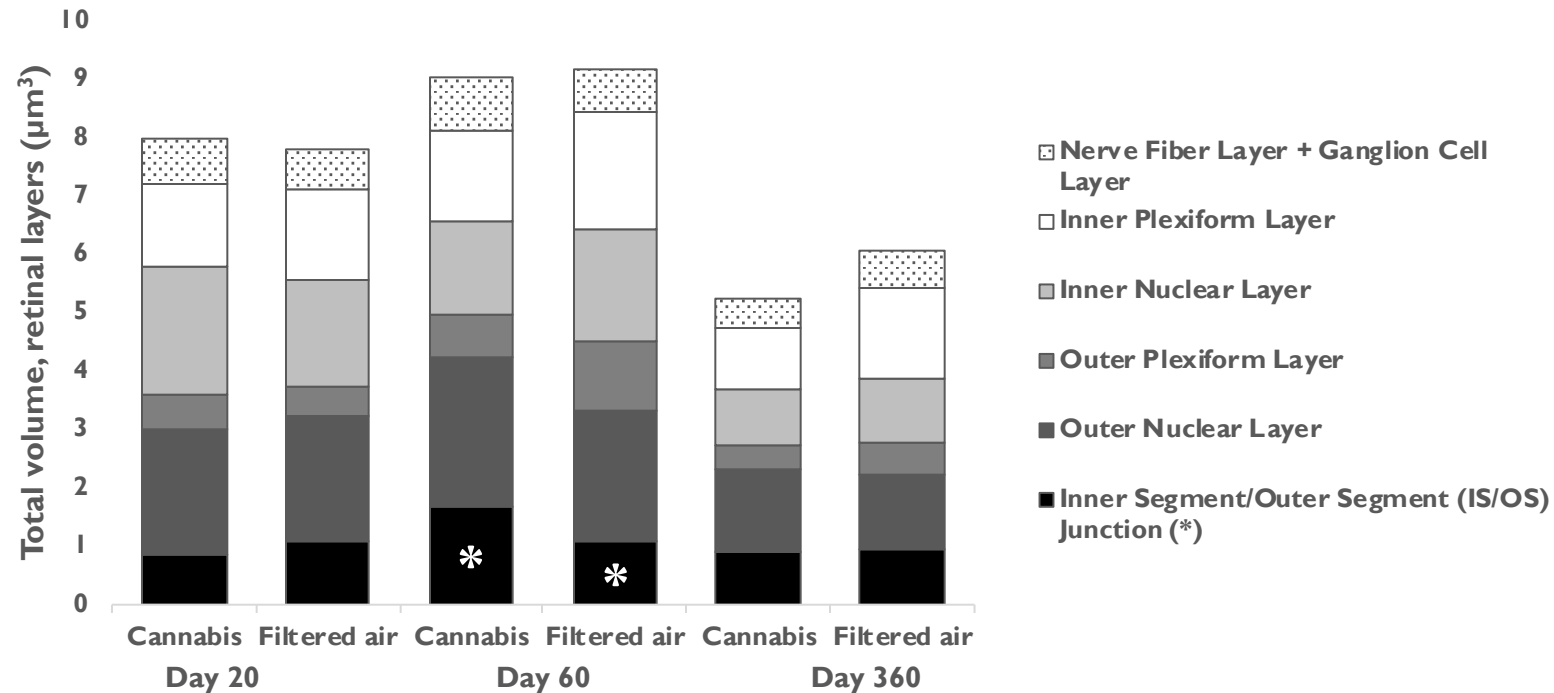
On day 200 the **IS/OS** from the **CAN** group was **68%** thicker than in the **FA** group



Results were influenced by **age\*exposition** (95% CI: 47-54, P=0.0001)

# RESULTS (RETINAL STEREOLOGY)

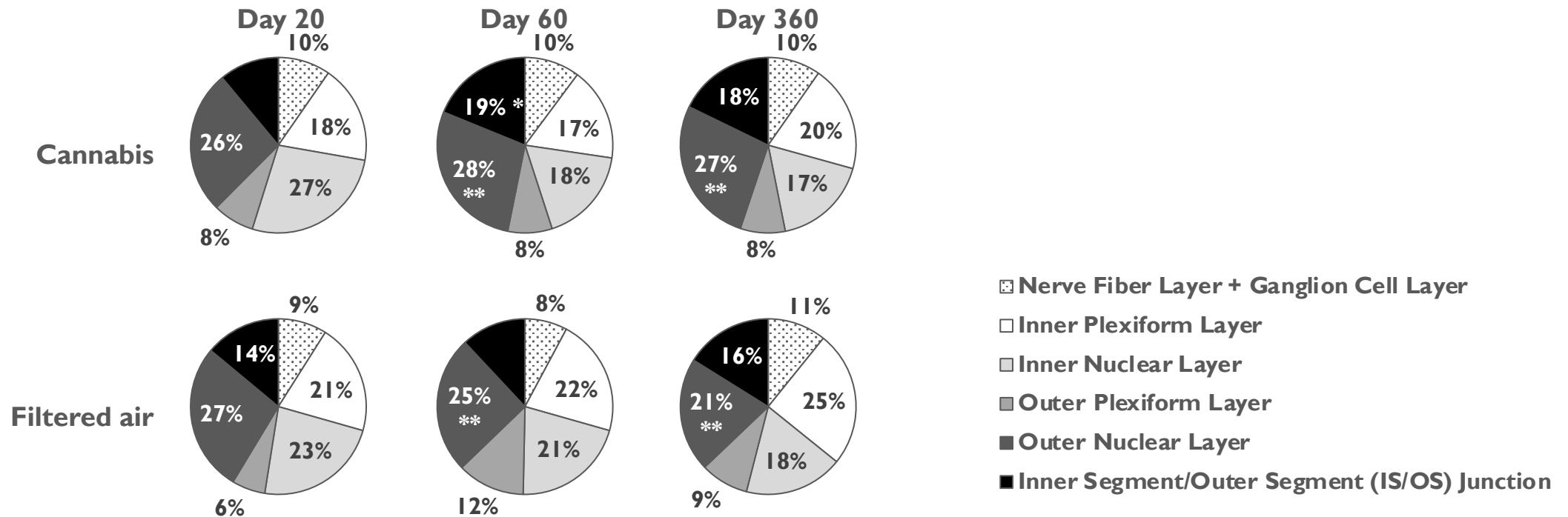
Total volume of the **IS/OS** increased during young adulthood (day 60) in the **CAN** group and returned to normal on adulthood (day 360)



In multivariate regression analysis, results were influenced by **age\*exposition** (95% CI: 0.8-1.5, P=0.008)

# RESULTS (RETINAL STEREOLOGY)

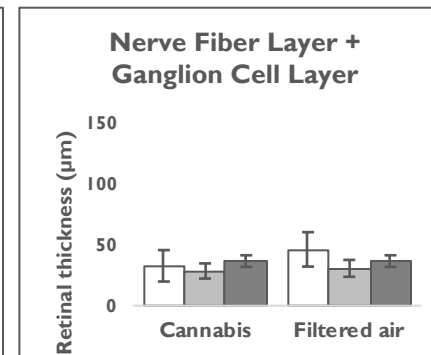
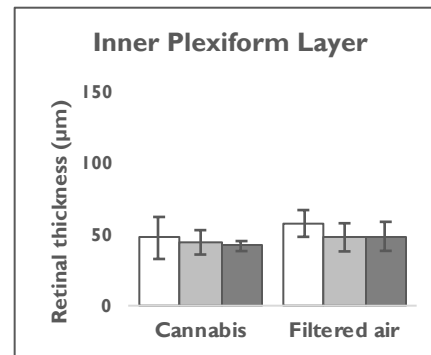
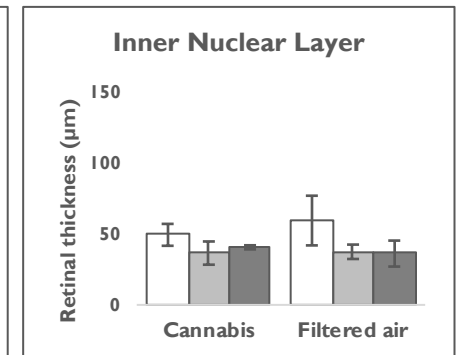
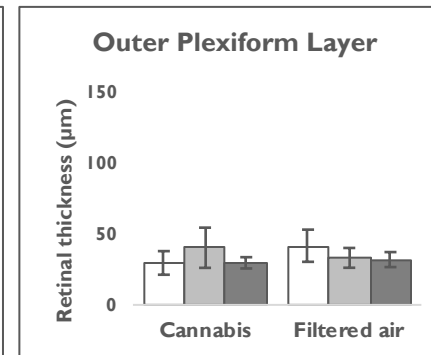
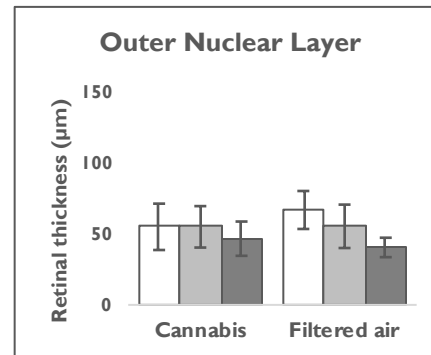
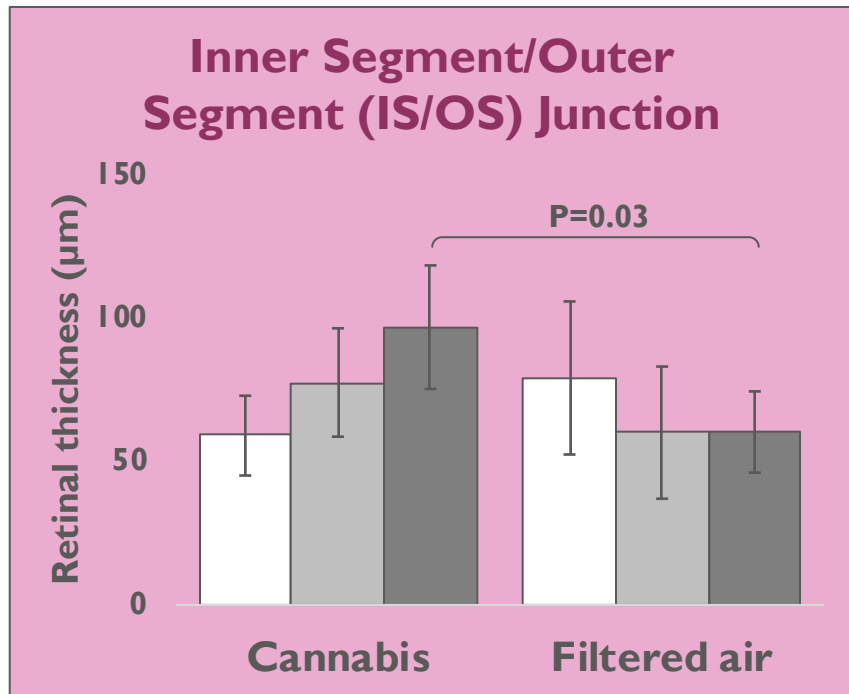
Volume fraction of the **IS/OS** increased during young adulthood (day 60) in the **CAN** group and returned to normal on adulthood (day 360)



In multivariate regression analysis, results were influenced by **age\*exposition** (95% CI: 10.6-17.4, P=0.035)

# RESULTS (LIGHT MICROSCOPY)

**IS/OS** became thicker on day 360 (adult) in the **CAN** group compared to the **FA** group



□ Day 60  
■ Day 120  
■ Day 360

In multivariate regression analysis, results were influenced by **age\*exposition** (95% CI: 251-263, P=0.03)

# DISCUSSION

- **Mother exposure to Cannabis smoke during pregnancy causes morphological retinal changes in the offspring that are transitory and return to normal by adulthood**
  - ✓ **Retinal thinning in young adulthood due to outer retina thinning**
  - ✓ **Returns to normal values in adult life**

# DISCUSSION

- **IS/OS thickening on day 200 is transitory**
  - ✓ **Secondary to the effects of Cannabis on remodeling, apoptosis, and neuroprotection?**
  - ✓ **An attempt to decrease cellular losses by apoptosis?**
    - **Endocannabinoid system plays a role on neuroplasticity and neuroprotective action in traumatic and ischemic injuries, inflammation, and neurological damage to the CNS**
- **Increase on ONL volume fraction in the young adult mice**
  - ✓ **The same process that occurs in IS/OS may also occur in the cell bodies of the photoreceptors**

# DISCUSSION

- Prenatal inhalation of Cannabis smoke by pregnant mice interferes with retinal development of the offspring
  - ✓ Critical stages of cell elimination (apoptosis) to remove cells that have not established communications or compromised cells?

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Fernández-Ruiz JJ et al. *Life Sci.* 1999;65:725-36.

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# LIMITATIONS

- **Absence of macula in the murine model**
- **Drug susceptibility differences among species**
- **Low concentration of THC in the Cannabis used in this study**
  - ✓ **Values our findings and reinforces the potential toxic effects of Cannabis in the retina of the offspring of pregnant mice exposed to Cannabis smoke**

# CONCLUSIONS

- **Gestational exposure to Cannabis smoke may cause structural changes in the retina of the offspring that return to normal on mice adulthood**
- **These experimental evidences suggest that children and young adults whose mothers smoked Cannabis during pregnancy may require earlier and more frequently clinical care than the non-exposed population**