THC exposure before birth alters trajectories of brain development in young mice

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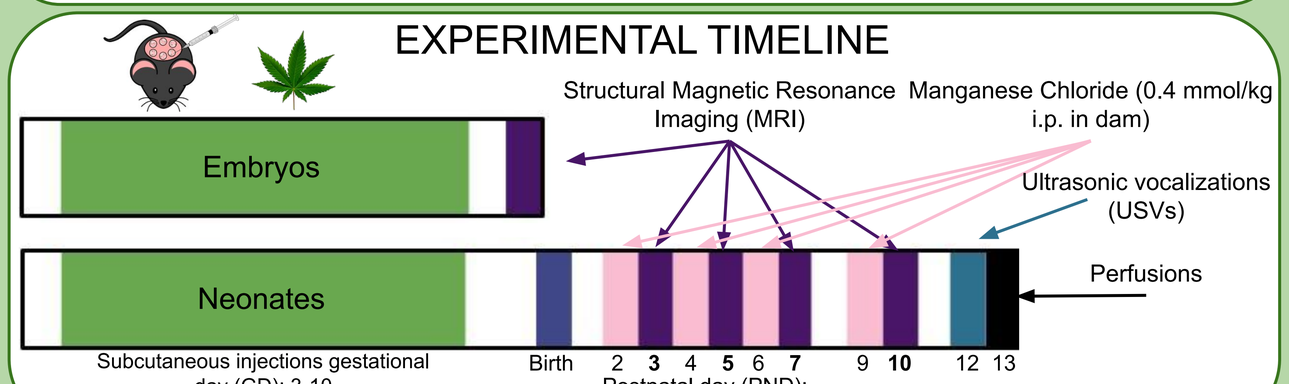
INTRODUCTION

People are **increasingly using cannabis products during pregnancy** to manage nausea and vomiting associated with morning sickness. Additionally, cannabis products contain **higher rates** of the psychoactive component, delta-9-tetrahydrocannabinol (**THC**) than in previous decades. As a result, comprehensive analyses of the impact of prenatal exposure to THC on the development of brain anatomy and behavior are increasingly urgent.

EXPERIMENTAL TIMELINE



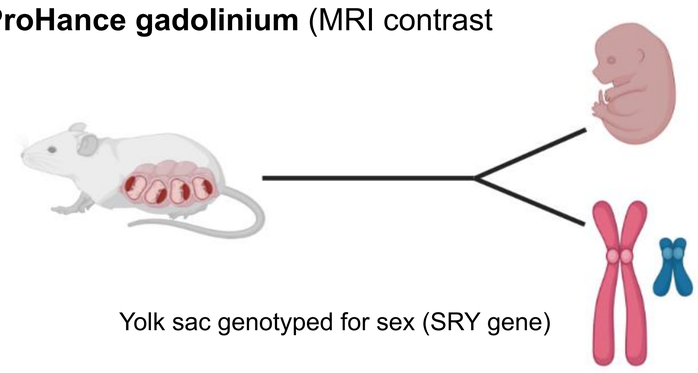
SAMPLE SIZE

	Embryos			Neonates				
	Males (MRI)	Females (MRI)	Litters	Males (MRI)	Females (MRI)	Males (USVs)	Females (USVs)	Litters
Sal	14	11	7	17	10	26	7	9
THC	9	10	7	10	14	12	15	7
Not-injected	13	14	6	NA	NA	NA	NA	NA

DATA ACQUISITION

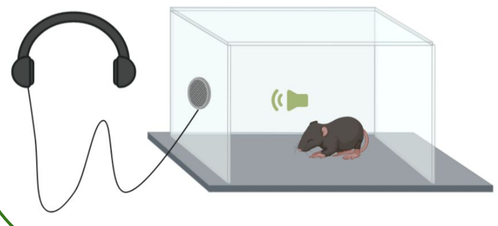
EMBRYOS

- C57BL6/J embryos extracted on **GD 17**
- Dams euthanized with cervical dislocation (no anaesthesia)
- Embryos removed from uterus and placenta
- Bled out under agitation in warm (37C) PBS
- Fixed in 4% PFA and 2% **ProHance gadolinium** (MRI contrast agent) for 1 week
- Imaged *ex-vivo* at the **Mouse Imaging Centre (Toronto)**
- T2-weighted images acquired at **40 um^3 isotropic** on Varian 7T on 16 coils overnight



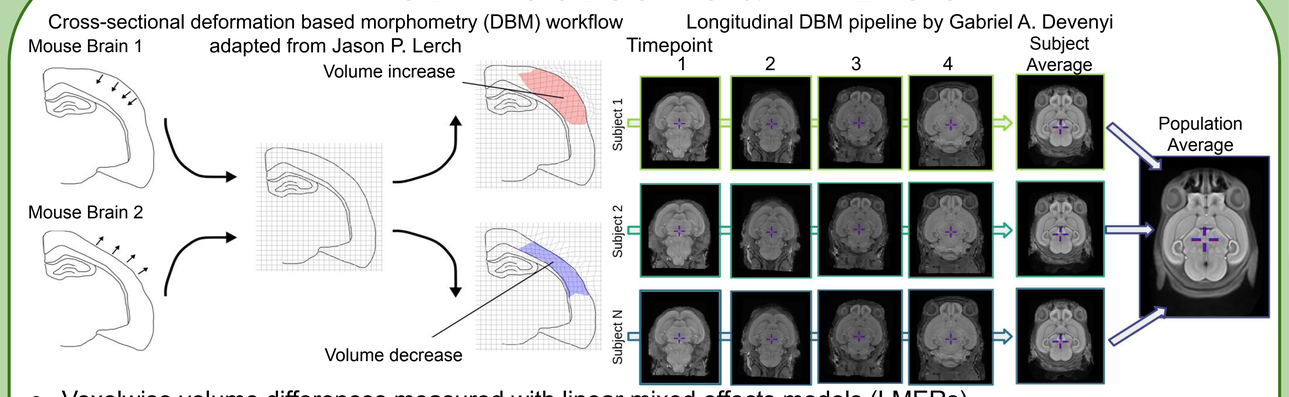
NEONATES

- On PND 2, litters culled to 6
- 2 males and 2 females included for scanning per litter
- 24 hours prior to each scan dams injected with 0.04 mmol/kg **$MnCl_2$** (MRI contrast agent) for pups to absorb through nursing
- Imaged at the **Cerebral Imaging Center (Montreal)** at **70 um^3 isotropic** on Bruker 7T with a **cryogenically-cooled** surface coil
- Perfused on GD 13 for **immunohistochemistry** and **electron microscopy** (future directions)



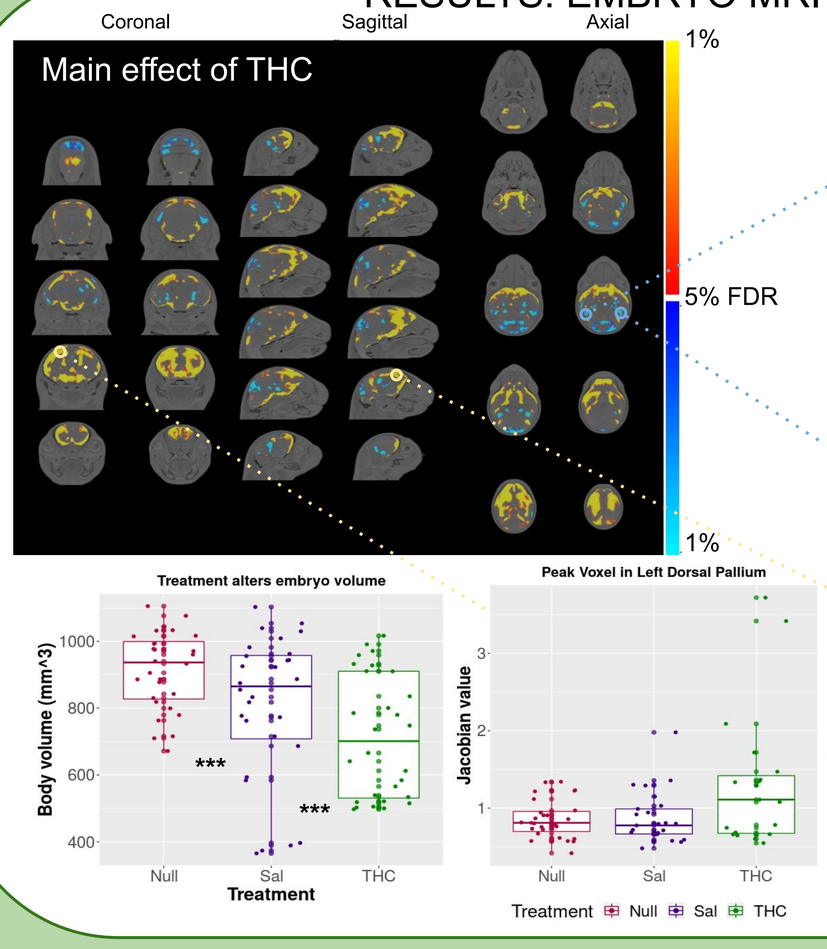
- On PND 12, **USVs were acquired**
- Pups separated from dams & littermates
- Calls recorded for **5 minutes**
- **UltraVox** software used for data collection (Noldus Information Technology, Leesburg, VA)
- Differences between distributions by condition statistically examined with **shift function**[1], performing a percentile-based bootstrap comparison.

IMAGE PROCESSING & ANALYSIS

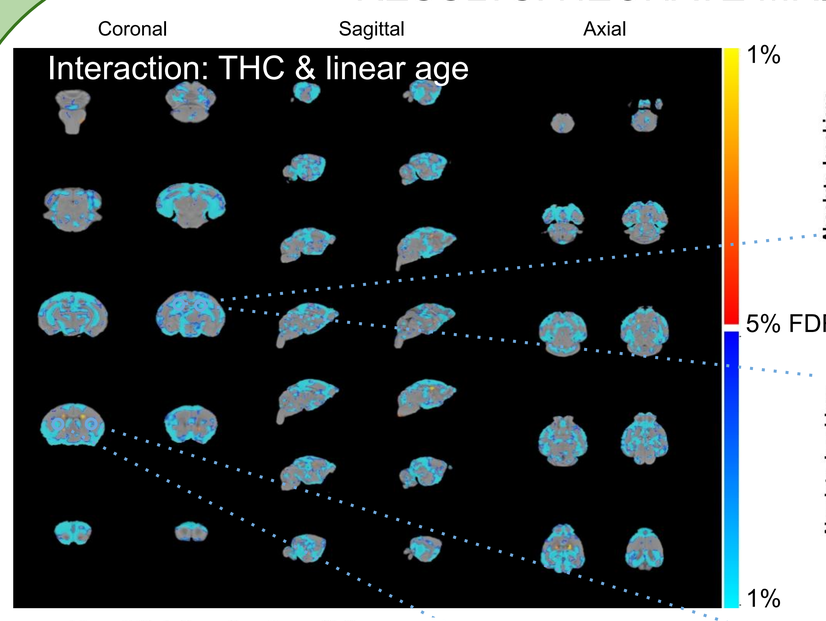


- Voxelwise volume differences measured with linear mixed effects models (LMERs)
- **Cross-sectional (embryos):** main effects-**treatment** & **sex**; fixed effects-**litter size** & **coil**
- **Longitudinal (neonates):** Interaction-**treatment***quadratic(**age**); main effect-**sex**; fixed effects-**litter** & **ID**

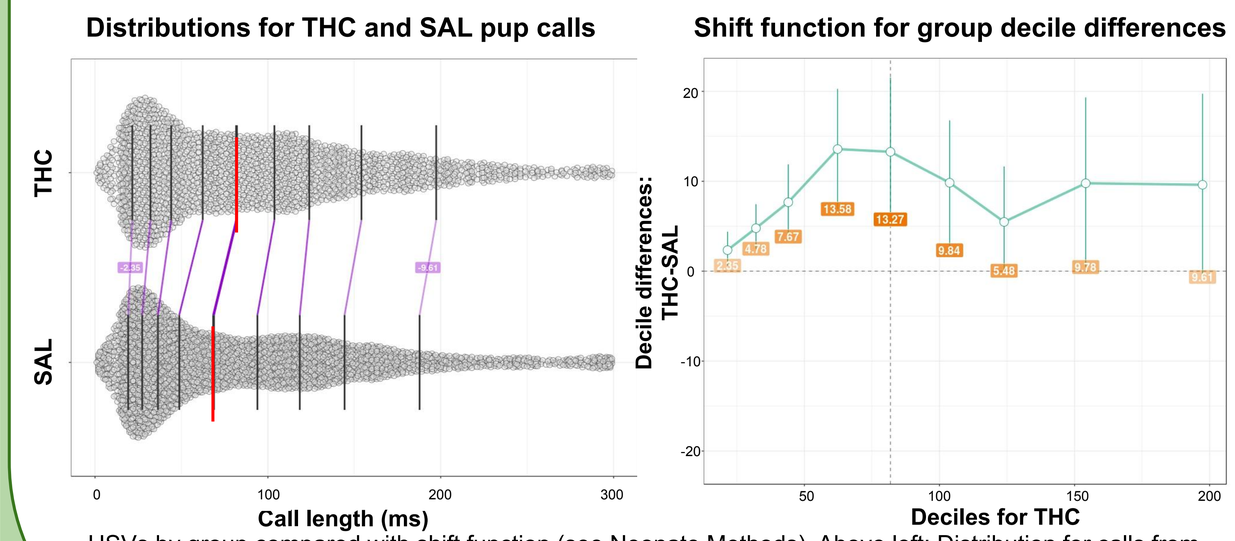
RESULTS: EMBRYO MRI



RESULTS: NEONATE MRI



RESULTS: USVs



USVs by group compared with shift function (see Neonate Methods). Above left: Distribution for calls from THC and SAL offspring. Median = red bar. Deciles = black bar. Violet bars link deciles across group showing relatively lower call length in SAL pups. Above Right: Decile differences between THC and SAL (y-axis) with THC deciles (x-axis). Confidence intervals generated from bootstrap resampling.

CONCLUSIONS

Prenatal exposure to THC alters brain volume late in gestation and changes the trajectories of development over the first two weeks of life. Additionally, THC pups exhibit anxiety-like phenotypes through increased medium-length ultrasonic vocalizations. Future directions include post-mortem analyses including immunofluorescence to stain for synapses (synaptophysin and PSD-95) and CB1 and CB2 receptors in embryos and pups and extension of analyses into adolescence and adulthood.

REFERENCES & ACKNOWLEDGEMENTS

1. Rousselet et al., *Eur J Neurosci.,* 2017. DBM Github: https://github.com/CobraLab/twolevel_ants_dbm

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