Manganese chloride as a contrast agent and its interaction with prenatal exposure to THC in mice

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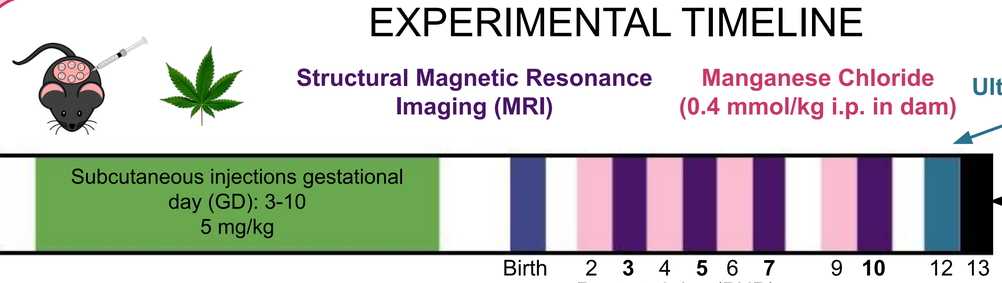
INTRODUCTION

- Lack of axon myelination, thus contrast, in neonatal mouse MRI
- Manganese chloride ($MnCl_2$) is used as a contrast agent, uptaken through voltage-gated Ca_{2+} channels
- $MnCl_2$ may impact pup physical and brain development
- Potential interaction between experimental methods and $MnCl_2$ exposure
- Using data from a previous study[3], we investigate the influence of $MnCl_2$ and prenatal cannabinoid (THC) exposure on mouse pup development.

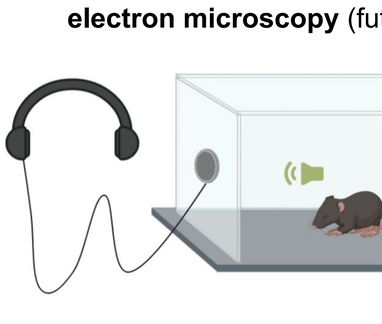
EXPERIMENTAL TIMELINE



SAMPLE SIZE

	Manganese-enhanced			Not Manganese-enhanced		
	Males (MRI)	Females (MRI)	Litters	Males (MRI)	Females (MRI)	Litters
Sal	15	11	9	4	4	2
THC	9	14	7	6	2	2

HYPOTHESES



- $MnCl_2$-exposed TBV will be about 10% smaller than controls[2]
- $MnCl_2$-exposed pup weight will be up to 20% less than controls[2]
- We expect to see lower brain volume and weight in the $MnCl_2$-exposed pups than the saline controls

Recreated from Szulc et al., *NeuroImage*, 2015.

DATA ACQUISITION

ORIGINAL EXPERIMENT

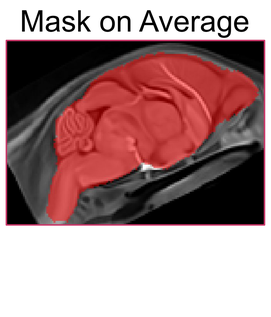
- On PND 2, litters culled to 6
- 2 males and 2 females included for scanning per litter
- 24 hours prior to each scan dams injected with 0.04 mmol/kg **$MnCl_2$** (MRI contrast agent) for pups to absorb through nursing
- Imaged at the **Cerebral Imaging Center (Montreal)** at **70 um^3 isotropic** on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

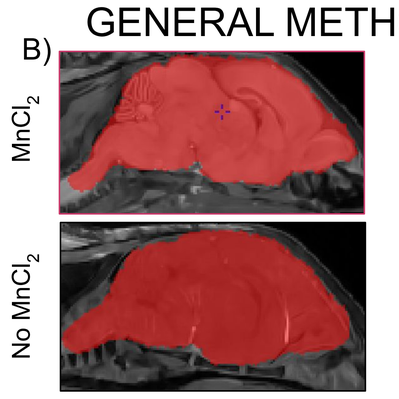
- On PND 12, **USVs were acquired**
- Pups separated from dams & littermates
- Calls recorded for **5 minutes**
- **UltraVox** software used for data collection (Noldus Information Technology, Leesburg, VA)
- Differences between distributions by condition statistically examined with **shift function**[1],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

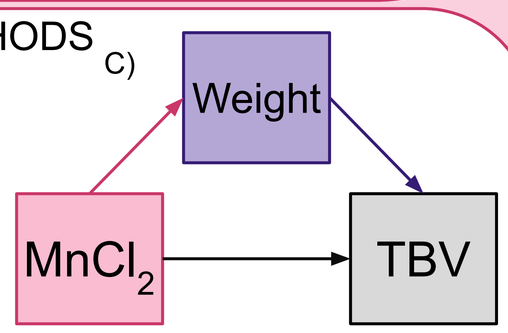
MANGANESE FOLLOW UP

- Small sample follow-up
- Instead of $MnCl_2$, saline (SAL) injected 24 hours prior to each scan

GENERAL METHODS







A) Generated a full brain mask on a model of neonatal scans in-house B) Transformed the mask to individual scans and manually corrected each C) Weight and total brain volume (TBV) differences measured longitudinally with linear mixed effects models (LMERs)**: Weight** or **TBV:** Interaction-**treatment***quadratic(**age**)***sex**; fixed effects-**litter** & **ID. Weight** examined as potential mediator of **$MnCl_2$** on **TBV.**

REFERENCES & ACKNOWLEDGEMENTS

1. Rousselet, et al., *Eur J Neurosci*, 2017.
2. Szulc, et al., *NeuroImage*, 2015.
3. Cupo, et al., *OHBM,* 2022.

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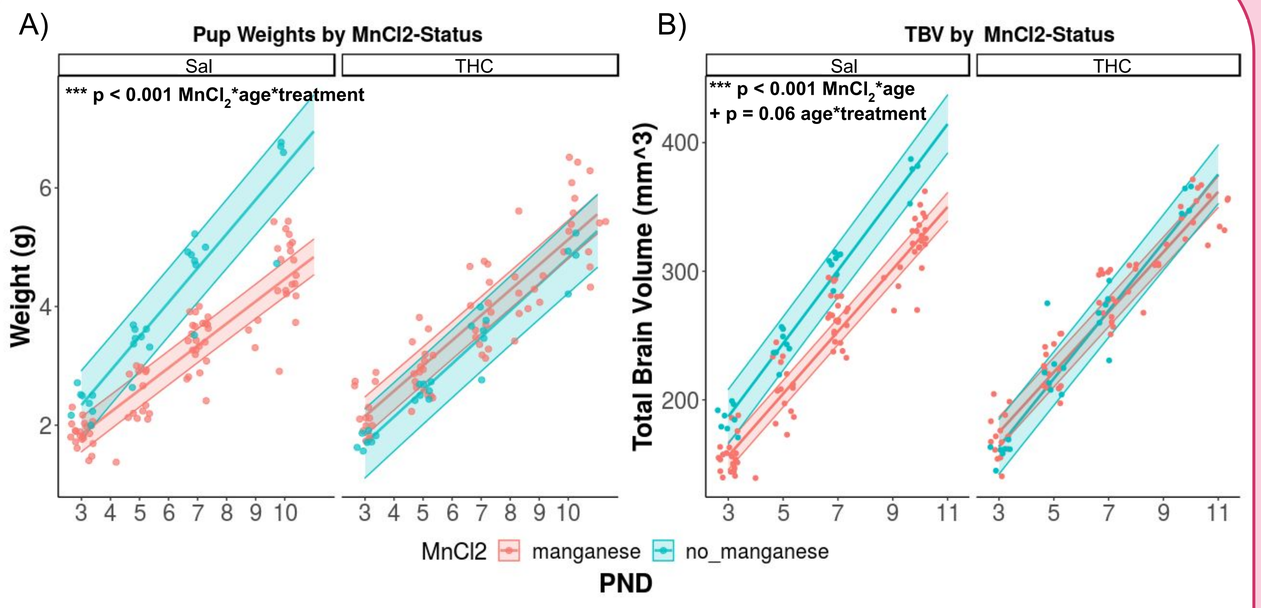
RESULTS: WEIGHT AND TBV

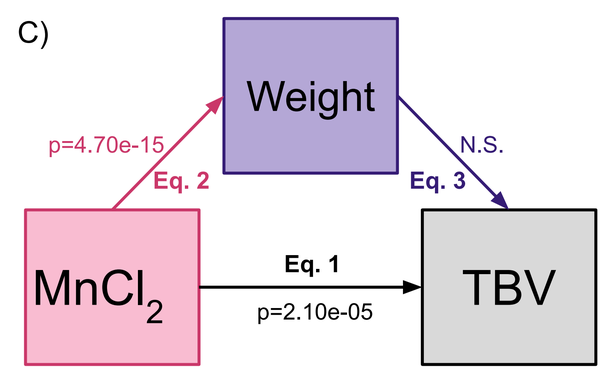


Weight as a mediator of $MnCl_2$ and Age Interaction

Eq. 1: lmer(TBV ~ $MnCl_2$*age*condition + (1|ID) + (1|mom_id))

Eq. 2: lmer(weight ~ $MnCl_2$*age*condition + (1|ID) + (1|mom_id))

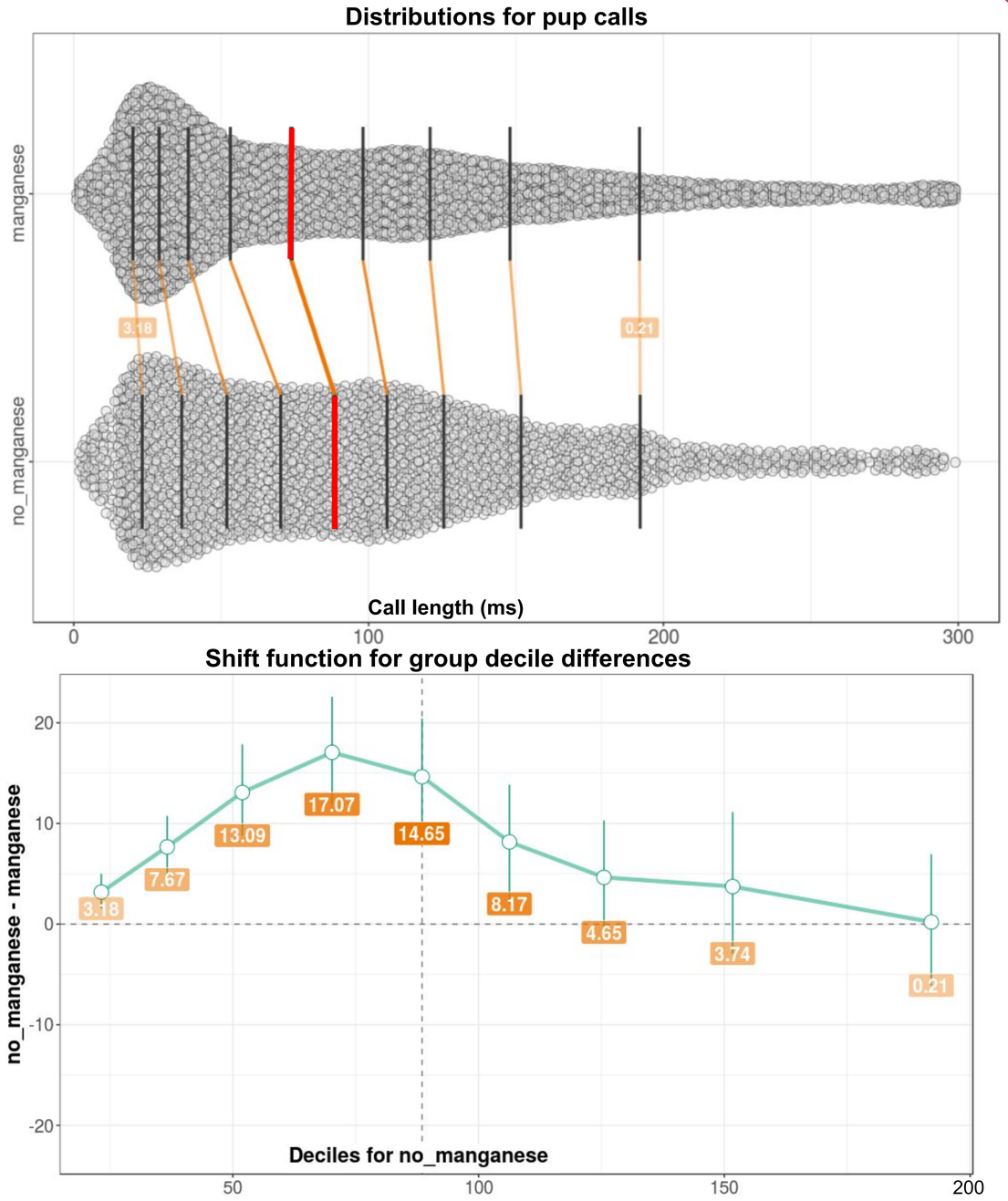
Eq. 3: lmer(TBV~$MnCl_2$*age*condition+weight + (1|ID) + (1|mom_id))



a) A significant (p<0.001) 3-way interaction between $MnCl_2$ status, treatment, and age on weight, with no difference between $MnCl_2$ and Sal in the THC group. b) A significant (p<0.001) 2-way interaction between $MnCl_2$ status and age on TBV. c) Weight is a full mediator of the relationship between the $MnCl_2$*age interaction and TBV.

RESULTS: USVs

Distributions for pup calls



USVs by group compared with shift function (see Original Experiment). Top: Distribution for calls from $MnCl_2$ and no $MnCl_2$ offspring. Median = red bar. Deciles = black bar. Orange lines link deciles across group showing relatively lower call length in $MnCl_2$ pups. Bottom: Decile differences between $MnCl_2$ and no $MnCl_2$ (y-axis) with no $MnCl_2$ deciles (x-axis). Confidence intervals generated from bootstrap resampling.

CONCLUSIONS

Surprisingly, repeated exposure to $MnCl_2$ early in post-natal life impacted pup weight only in the Sal group. While $MnCl_2$*age impacts weight and TBV, weight is a full mediator of the relationship between the interaction and TBV. $MnCl_2$ exposure is associated with fewer medium-length calls compared to no-$MnCl_2$ pups, potentially indicating increased anxiety-like behavior in those *not* exposed to $MnCl_2$. The interaction of $MnCl_2$ and prenatal treatment may be an important factor in other studies examining neonatal brain development.