Examining Litter Specific Variability in Mice and its Impact on Neurodevelopmental Studies

Vanessa Valiquette[1,4], Elisa Guma[1,4], Raihaan Patel[2,4], Eric Plitman[3,4], Daniel Gallino[4], Gabriel A. Devenyi[3,4], M. Mallar Chakravarty[1,2,3,4]

[1]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada
[2]Department of Biology and Biomedical Engineering, McGill University, Montreal, QC, Canada
[3]Department of Psychiatry, McGill University, Montreal, QC, Canada
[4]Computional Brain Anatomy Laboratory, Cerebral Imaging Centre, Douglas Mental Health University Institute, Montreal, QC, Canada

Introduction

- Although mice may share genetics, the specifics of the prenatal and postnatal environment[1] impact critical periods of early development[2].
- Higher variance of measures across litters than within[3] may be attributable to maternal care, intrauterine, and postnatal environment.
- Improper handling of this litter-effect may lead to increases in the reporting of true or false positives[3,4].
- Current understanding of litter variability may limit the translation and replicability of findings.

Goal: Better understand litter variability through the observation of normal behaviour and brain anatomy development in a mouse model.

Methods

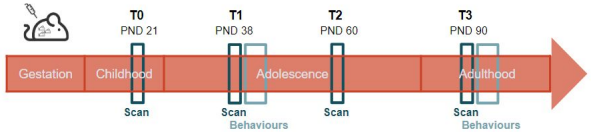

Figure 1: Experimental design. Dams injected with saline during gestation. Pups scanned at postnatal day (PND) 21, 38, 60, 90 and behaviours assessed at PND 38, 90[5].

- Data from a previous study on Maternal Immune Activation[5] of 36 C57bl/6 control mice (7 litters: 19F, 17M; litter 6-7 one sex only)

Behavioural tests
- Marble Burying test
- Open Field test
- Prepulse Inhibition test

Magnetic resonance imaging (MRI)
- T1 weighted structural MRI (100 um^3 isotropic voxels)
  - MAGeT brain[6] : extraction of brain volumes (72 brain regions volume)

Statistics

1. Levene's test : Compare measures variance across and within litters

2.i. Principal component analysis (**PCA**) : Find principal components (PC), patterns of brain regions volumes, explaining the most the variance.

2.ii. Partial least squares (**PLS**) **:** Covariant patterns between PC scores (each PC is a combination of brain regions volumes) and behaviours.

3. K-Means : Used to evaluate grouping of PLS results.

Results

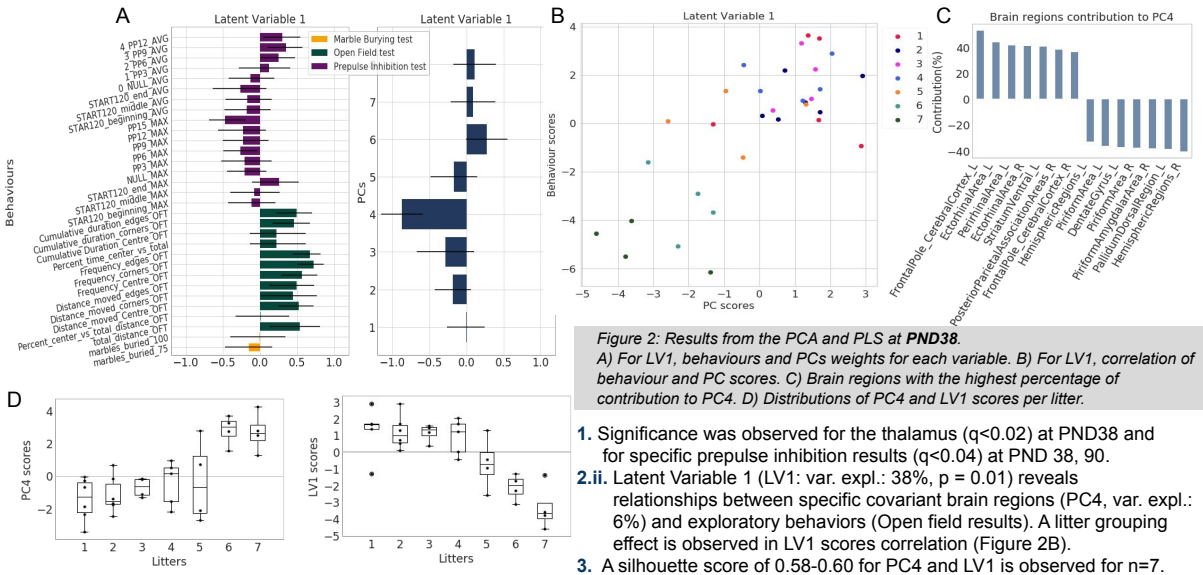

*Figure 2: Results from the PCA and PLS at **PND38**.*
A) For LV1, behaviours and PCs weights for each variable. B) For LV1, correlation of behaviour and PC scores. C) Brain regions with the highest percentage of contribution to PC4. D) Distributions of PC4 and LV1 scores per litter.

1. Significance was observed for the thalamus (q<0.02) at PND38 and for specific prepulse inhibition results (q<0.04) at PND 38, 90.

2.ii. Latent Variable 1 (LV1: var. expl.: 38%, p = 0.01) reveals relationships between specific covariant brain regions (PC4, var. expl.: 6%) and exploratory behaviors (Open field results). A litter grouping effect is observed in LV1 scores correlation (Figure 2B).

3. A silhouette score of 0.58-0.60 for PC4 and LV1 is observed for n=7.

Conclusion

- Factors specific to a litter modulate mouse development.
- Our results show greater variability between- than within- litter, mainly in the adolescence period (PND 38).
- Improved analysis decisions, such as including litter as a random effect in statistical models (eg. Linear Mixed Effect models), should be considered to better account for this litter-effect.

References

[1] Meyer U, Feldon J, Fatemi SH. (2009). Neurosci Biobehav Rev. Jul;33(7):1061-79.
[2] McCarty, R. (2017). Neurosci Biobehav. 73, 219–254.
[3] Lazic SE, Essioux L. (2013). BMC Neurosci. Mar 22;14:37.
[4] Golub MS, Sobin CA. (2020) Neurotoxicol Teratol. Jan-Feb; 77:106841.
[5] Guma E et al. (2020) bioRxiv
[6] Chakravarty MM et al., (2013). Hum Brain Mapp, 34(10):2635–54.