Sex Dependent Effects of Early-Life Microbiota Depletion on Behaviour, Neuroimmune Function and Neuronal Development

Caoimhe M.K. Lynch[1,2*], Caitlin S.M. Cowan[1*], Thomaz F. S. Bastiaanssen[1], Nigel Theune[1], Eva Florensa[1], Marcel Van de Wouw[1], Timothy G. Dinan[1,2], Gerard Clarke[1,3], John F. Cryan[1,2]

[1]APC Microbiome Ireland, [2]Department of Anatomy & Neuroscience, [3]Department of Psychiatry & Neurobehavioural Sciences University College Cork, Ireland.

@CaoimheMKLynch

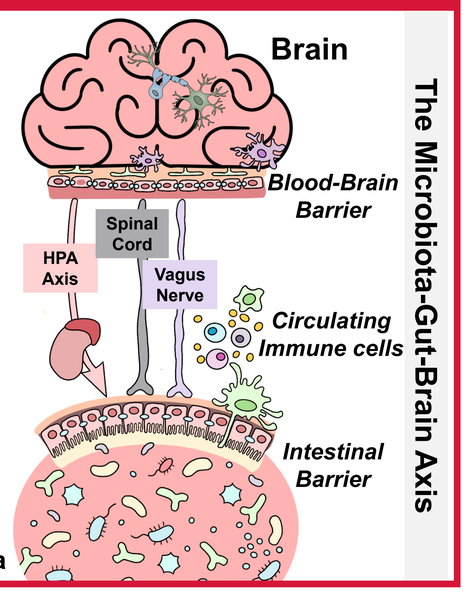
caoimhe_lynch@umail.ucc.ie

Background

There is a growing emphasis on the importance of the gut microbiota during early life and its role in modulating neurodevelopment[1,2].

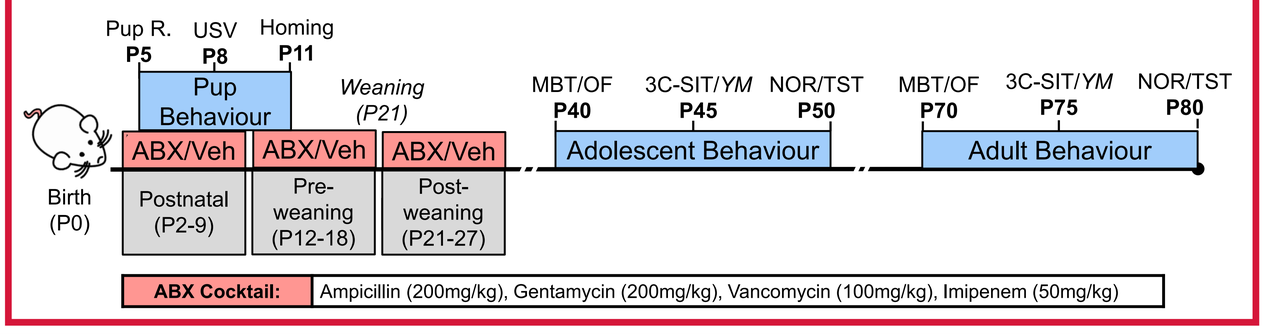
Moreover, key periods of plasticity in the gut microbiota have been found to coincide with critical windows of neurodevelopment and the timed onset of neuropsychiatric disorders[3].

Aim: To investigate the long-term effects of antibiotic-induced microbiota depletion on critical windows of brain and behavioural development.



Experimental Design

To identify critical windows in which the gut microbiota influences neurodevelopmental outcomes, an antibiotic cocktail **(ABX)** or a saline solution **(Veh)** was orally administered to NIH Swiss mice during three separate developmental windows: postnatal P2-9 **(PN)**, pre-weaning P12-18 **(PreW)** or post-weaning P21-27 **(Wean).** Behavioural readouts and neurochemical effects were assessed during adolescence.



Results

Microbiota depletion during early life dramatically alters caecal alpha and beta diversity in adolescent mice

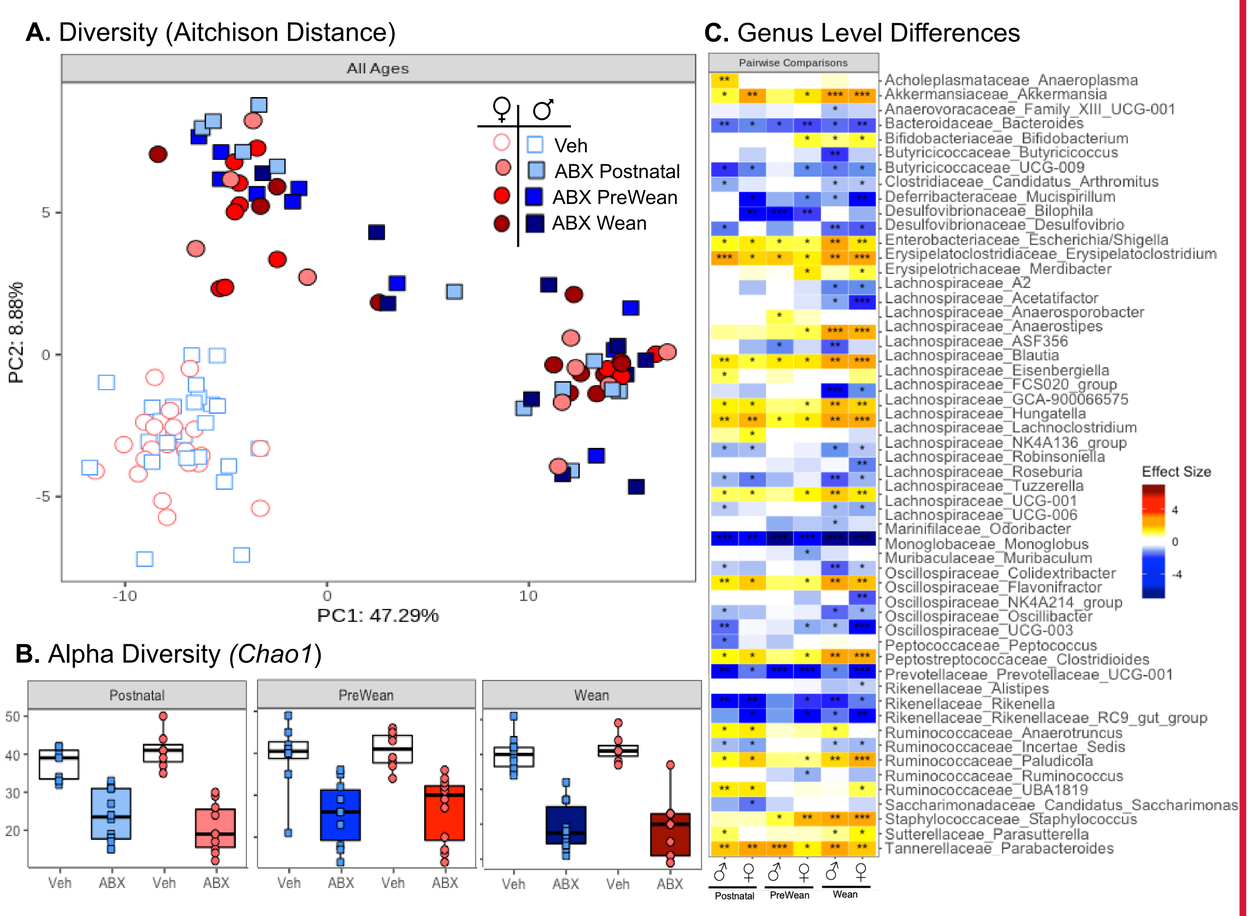


Figure 1. ***A.*** *Principle component analysis (PCA) of beta-diversity demonstrated a clear separation between microbiota depleted (ABX) and control mice (Veh) (p<0. 001).* ***B.*** *Microbial disruption during early life dramatically reduced microbial richness (Chao1: p<0.001)* ***C.*** *Enduring effects of early-life microbiota depletion on caecal microbiota composition the at genus level (Pairwise comparisons ABX vs. Veh per age and sex).*

Early-life microbiota disruption alters microglial morphology in the basolateral amygdala of adolescent mice

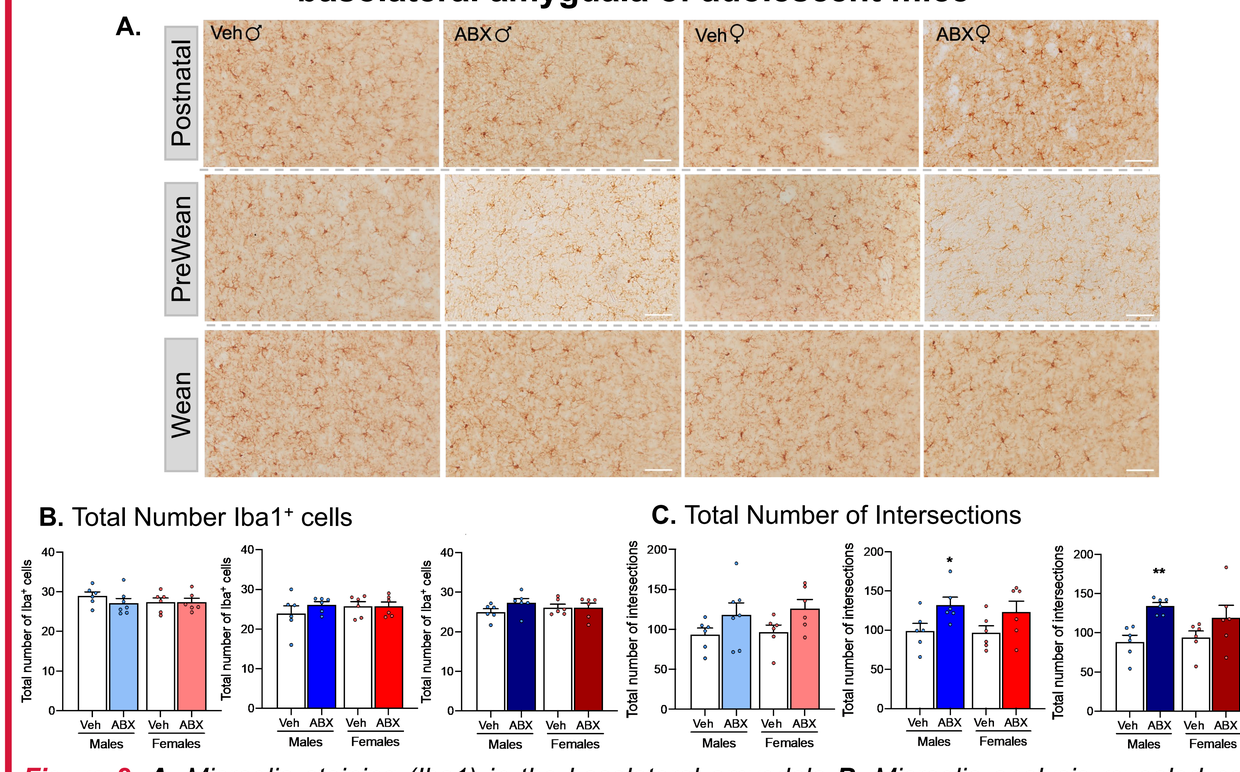


Figure 2. ***A.*** *Microglia staining (Iba1) in the basolateral amygdala* ***B.*** *Microglia analysis revealed no difference in the total number of Iba1+ cells. Scale = 50μm* ***C.*** *Microbiota depletion in early life significantly increased microglia total number of intersections. Treatment *p<0.05, **p<0.01, ***p<0.001; & Sex #p<0.05, ##p<0.01, ###p<0.001. Data presented as Mean ± SEM.*

Early-life microbiota disruption has subtle effects on anxiety-like behaviour in a sex- and time-dependent manner

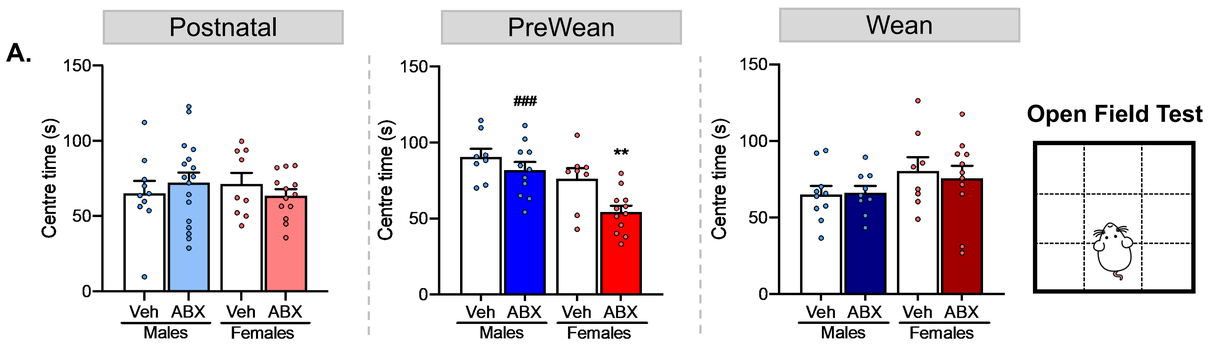


Figure 4. ***A.*** *Anxiety-like behaviour was assessed in the open field test. Microbiota depleted females (PreW) demonstrated a tendency toward increased anxiety-like behaviour (reduced time in centre). Treatment *p<0.05, **p<0.01, ***p<0.001; & Sex #p<0.05, ##p<0.01, ###p<0.001. Data presented as Mean ± SEM.*

Early-life microbiota disruption alters myelin mRNA expression in the prefrontal cortex of adolescent mice

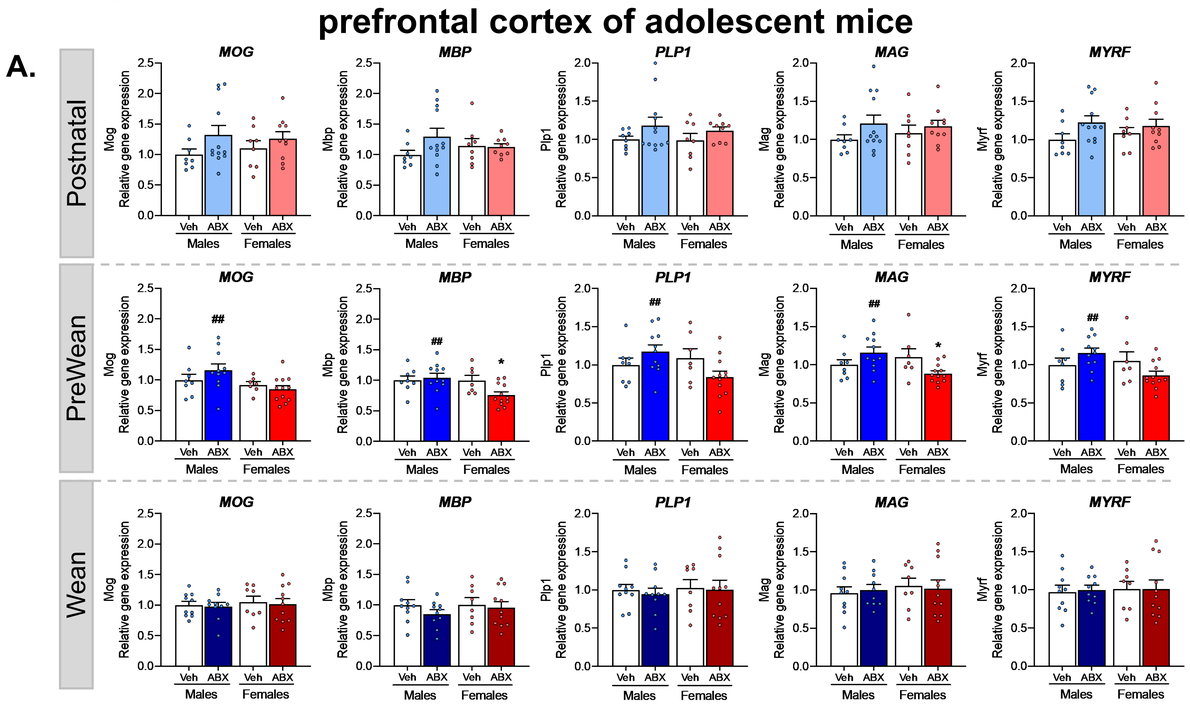
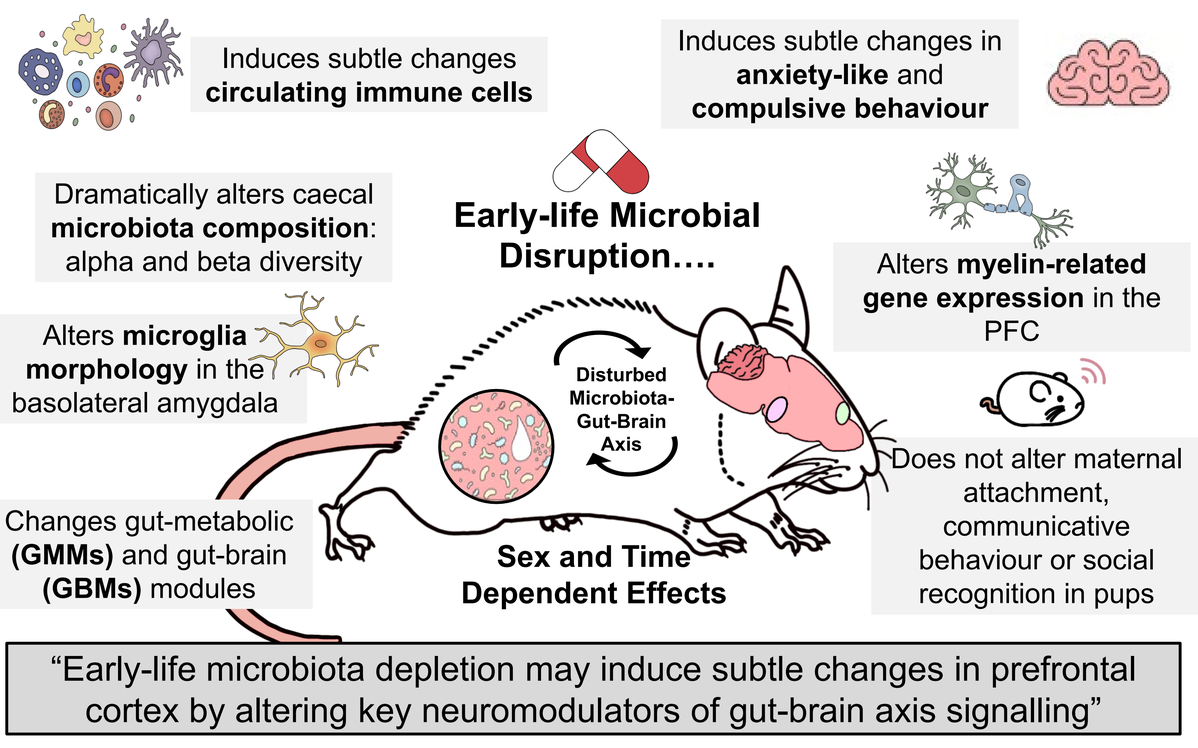


Figure 3. ***A.*** *Microbiota depletion resulted in significant changes in myelin-related gene expression in a sex-and time-dependent manner. Microbiota depleted females (PreW) demonstrated a tendency toward decreased myelin-related gene expression relative to vehicle treated and microbiota depleted males. Treatment *p<0.05, **p<0.01, ***p<0.001; & Sex #p<0.05, ##p<0.01, ###p<0.001. Data presented as Mean Δ Δ Ct ± SEM.*

Conclusions



"Early-life microbiota depletion may induce subtle changes in prefrontal cortex by altering key neuromodulators of gut-brain axis signalling"

Acknowledgements

This work was supported, in part, by research grants from Science Foundation Ireland to APC Microbiome Ireland (grant no. RC/2012/2273_P2). C.S.M.C. was supported by a European Union H2020 Marie Skłodowska-Curie Individual Fellowship (grant no. 797592).

References

[1]Cryan, J.F., & Dinan, T.G. (2015) More than a gut feeling: The microbiota regulates neurodevelopment and behavior. *Neuropsychopharm, 40,* 241-242.
[2]Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., *et al.* (2019) The microbiota-gut-brain axis. *Phys Reviews, 99,* 1877-2013.
[3]Cowan, C.S.M., Dinan, T.G., & Cryan, J.F. (2020) Critical windows: The microbiota-gut-brain axis in neurocognitive development. *J. Child Psychology and Psychiatry.*