Caesarean Section Birth, Stress Responsiveness and Cognitive Function in Young Adulthood: Implications for the Microbiota-Gut-Brain Axis and Neurodevelopmental Programming

P.J. Kennedy[1], A.B. Murphy[1,6], J. Marr[1], A.M. Cusack[1], P.W. O'Toole[1,2], P.R. Ross[1,3], C. Stanton[1,5,6], J.F. Cryan[1,4],T.G. Dinan[1,5]
[1]APC Microbiome Institute, [2]School of Microbiology, [3]College of Science, Engineering and Food Science,
[4]Department of Anatomy and Neuroscience, [5]Department of Psychiatry & Neurobehavioural Science,
University College Cork, Cork, Ireland.

EMAIL: pj.kennedy@ucc.ie

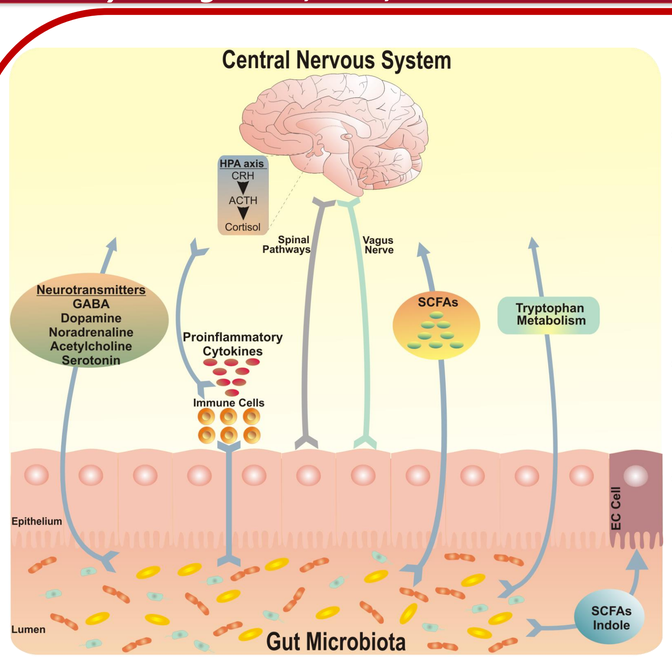


Figure 1: The microbiota-gut-brain axis

Introduction

- Over recent years it has become evident that the physiological influence of the gut microbiota extends beyond the periphery to the central nervous system (CNS) via pathways of microbiota-gut-brain axis (See **Figure 1; (1)**).
- Emerging evidence suggests that alterations in the composition of the gut microbiota across the lifespan may have a role in the pathophysiology of a number of mental health disorders (**2,3**).
- Of particular interest, is the impact of an altered gut microbial composition during critical neurodevelopmental time windows in early life and the long-term consequences for brain function and behaviour (See **Figure 2**).
- Infants born by caesarean section (C-section) have a different gut microbiota composition during the first 3 years of life which may lend vulnerability to adverse neurodevelopmental outcomes (**4**).

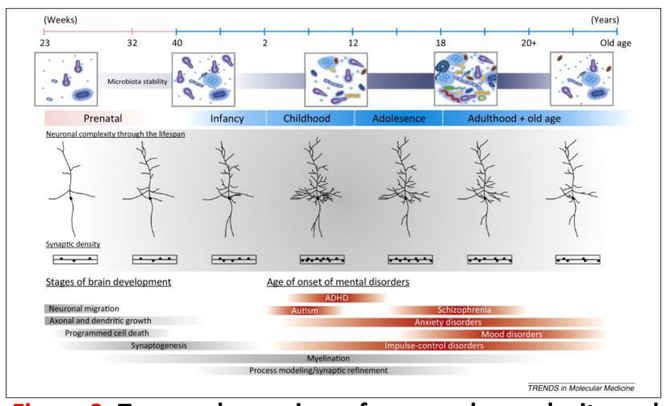


Figure 2. Temporal overview of neuronal complexity and synaptic density development in relation to gut microbiota stability/diversity and age at which a number of mental health disorders occur, across the lifespan. Figure taken from (4).

Aims

- To determine if individuals born by C-section exhibit differences in anxiety, depression, cognitive function and stress responsiveness during young adulthood, when compared to individuals born naturally.

Methods

Study Population:

• 40 Naturally born and 35 C-section delivered male participants aged 18-24 years of age (see **Table 1** for demographics)

Stress Procedure:

•Trier Social Stress Test (TSST; See **Figure 3**)

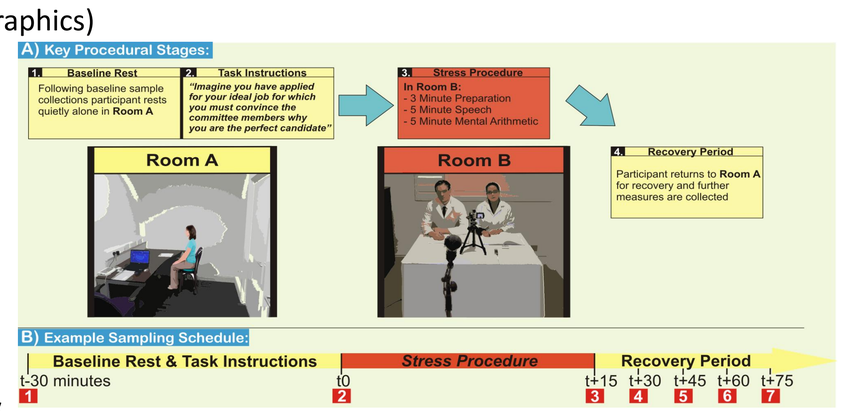


Figure 3. Procedural stages of the TSST & sampling schedule.

Measures:

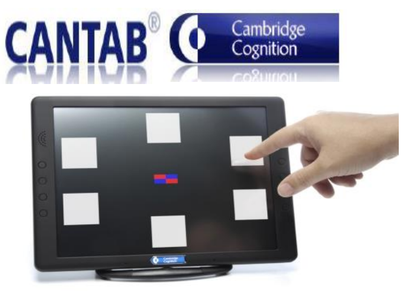
Psychological:

- State Trait Anxiety Inventory
- Pittsburgh Sleep Quality Index
- Beck Depression Inventory-II
- Cohen Perceived Stress Scale

TSST:

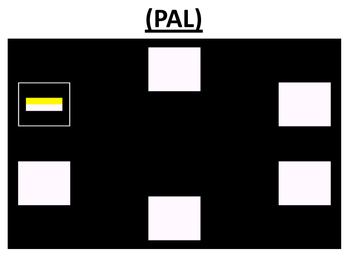
Subjective Mood & Stress:

• Positive And Negative Affect Schedule (PANAS).
• Visual analogue stress (0 no stress- 100).

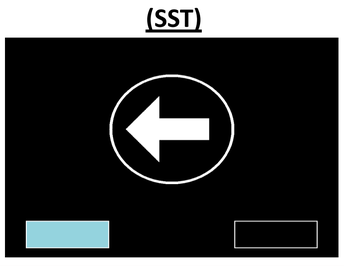


Cognitive Function:

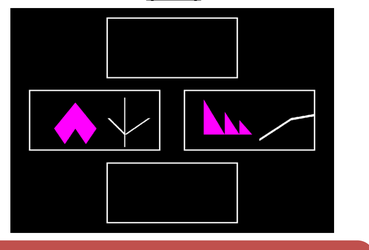
Paired Associates Learning (PAL)



Stop Signal Reaction Time (SST)



Intra/Extra Dimensional Set Shift (IED)



Results

Sample Characteristics

	Natural Born (n=40)	C-section (n=35)	p-value
Age	20.2 ± 0.24	20.3 ± 1.32	0.789
BMI	23.66 ± 0.46	24.59 ± 0.61	0.219
Years of Education	16.03 ± 0.22	15.83 ± 0.19	0.503
Units of Alcohol per week	7.43± 6.59	10.53 ± 1.62	0.103

Table 1: Comparisons between naturally born and C-section delivered participants on demographic characteristics. Study participants were matched on the basis of age ,, years of education, body mass index (BMI) and units of alcohol consumed per week. Data are expressed as mean± SEM. Independent samples t-tests using IBM SPSS V20.0 were used to determine group differences.

Results

Baseline Mood and Self-Reported Stress Levels

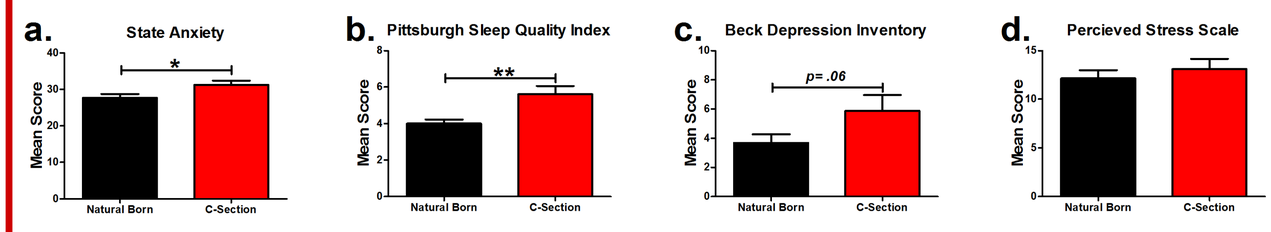


Figure 4. a. Participants born by C-section scored significantly higher on state anxiety (t(71)= 2.25, *p*= 0.027); and **b.** reported significantly poorer sleep quality during the prior month (t(68)= 3.17, *p*= 0.003) when compared to participants born naturally. **c.** The difference in self-reported levels of depression between C-section and natural born participants approached significance (t(67)= 1.86, *p*= 0.068)**; d.** there was no difference in perceived stress levels between C-section when compared to healthy controls (t(70)= 0.71, *p*= 0.48)**;** Data are expressed as mean± SEM.

Neuropsychological Performance

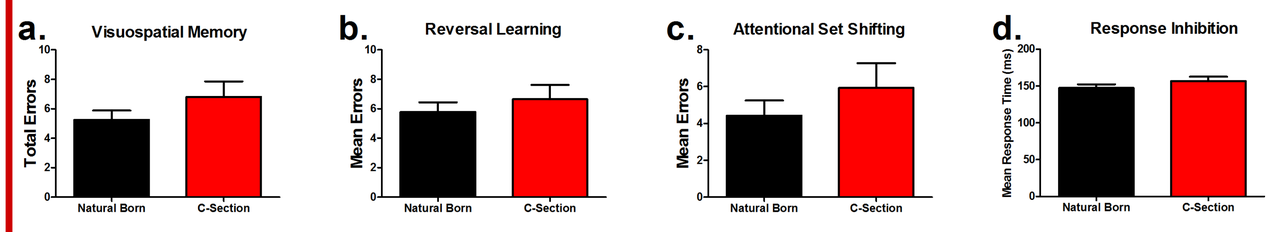


Figure 5. There was no significant difference **a**. visuospatial memory performance on the Paired Associates Learning (**PAL**) test (t(71)= 1.33, *p*= 0.19) **b.** reversal learning on the Intra/Extradimensional Set Shift Test (**IED**) (t(71)= 0.75, *p*= 0.46); **c.** attentional set shifting on the Intra/Extradimensional Set Shift Test (**IED**) (t(71)= 1.01, *p*= 0.32); **d.** or response inhibition on the Stop Signal Reaction Time (**SSRT**) test (t(71)= 1.25, *p*= 0.22). Data are expressed as mean± SEM.

Self-Reported Mood & Stress Response to TSST

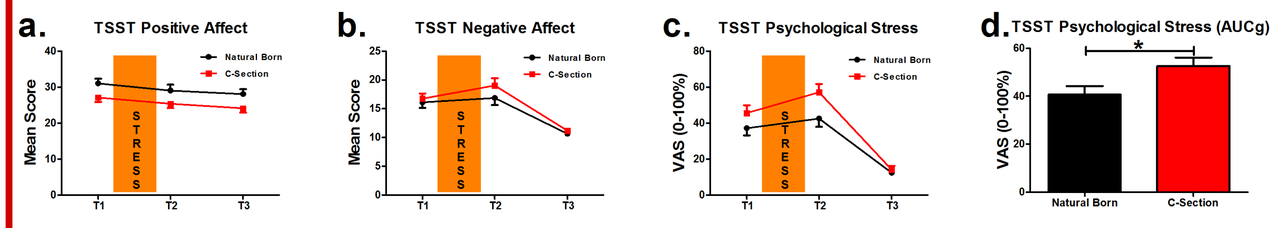


Figure 6. a. The TSST significantly reduced positive affect (F(2,126)= 7.08, *p*= 0.001, h_p^2 = 0.101) and across all time points, C-section participants reported lower positive affect in comparison to natural born participants (F(1,63)= 5.38, *p*< 0.001, h_p^2 = 0.08); **b.** negative affect increased in response to the TSST (F(1.79,112.51)= 60.21, *p*< 0.001, h_p^2 = 0.49) with no difference between groups; **c.** self-reported psychological stress increased in response to the TSST (F(2,128)= 69.17, *p*< 0.001, h_p^2 = 0.52), and across all time points, C-section participants reported significantly higher levels of psychological stress (F(1,64)= 4.8, *p*= 0.03, h_p^2 = 0.07); **d**. area under the curve with respect to ground (AUCg) analysis showed that psychological stress levels were higher in C-section participants in comparison to controls throughout the experimental session (t(64)=2.3, *p*= 0.025). Data are expressed as mean± SEM.

Conclusions

- Our results suggest that individuals born by C-section exhibit a differential psychological response to stress, in addition to reporting higher state anxiety and poorer sleep quality.
- These results are a preliminary analysis of a larger study. Future analysis will aim to determine if there is a difference between individuals born by C-section when compared to those born naturally, in the gut microbiota composition, and hypothalamic-pituitary-adrenal (HPA) axis, proinflammatory cytokine, lipopolysaccharide binding protein and zonulin response to the TSST.
- Given the significant rise in C-section births worldwide, the outcomes of this study may have significant public health and clinical implications. These data will also provide the first attempt to translate preclinical findings, indicating the importance of the gut microbiota in programming brain development during early life, with adverse long-term neurodevelopmental consequences.

Acknowledgements & Disclosure

The authors and their work were supported by supported by the European Commission under the Food, Agriculture and Fisheries, and Biotechnology Theme of the 7th Framework Programme for Research and Technological Development (MyNewGut, FFP7- KBBE/2013–2018, grant agreement no 613979); and Dept Agriculture, Food & the Marine, Ireland funded SMARTFOOD**:** Science Based 'Intelligent'/Functional and Medical Foods for Optimum Brain Health, Targeting Depression and Cognition ((2013-2017); and are supported in part by a research grant from Science Foundation Ireland (SFI) under Grant Number SFI/12/RC/2273.

References

1. Cryan, J.F. and Dinan, T.G., (2012). *Nature Reviews Neuroscience*, 13 (10) , 701-712.
2. Sampson, T.R. and Mazmanian, S.K. *Cell host & microbe*, 17, 565-576)
3. Mayer et al., *The Journal of Neuroscience*, 34 (46),, 15490-15496).
4. Borre et al., (2014). *Trends in Molecular Medicine*, 20, 509-518.