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Effects of transplacental *Fusobacterium nucleatum* infection in the postnatal neurological health of offspring

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INTRODUCTION

Fusobacterium nucleatum:

- A Gram-negative anaerobic bacterium commonly found within the oral cavity and closely associated with periodontal disease
- Capable of spreading systemically to extra-oral sites and thus implicated in a wide spectrum of human diseases including GI disorders and adverse pregnancy outcomes such as preterm birth and neonatal sepsis.

Although *F. nucleatum* is one of the few organisms that can cross both placental and blood-brain barriers, no studies have been conducted to assess the postnatal neurological health of mice exposed to transplacental infection.

OBJECTIVE

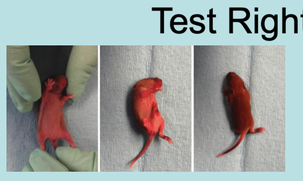
We aimed to investigate the effects of transplacental *F. nucleatum* infection on the developing mouse brain through neurobehavioral tests of the offsprings.

METHODS

Infect pregnant mice with 100ul of $1x10^7$-$5x10^7$ cfu of *F. nucleatum* via tail-vein injection.

At 1-week and 2-weeks of age:

Test Righting Reflex

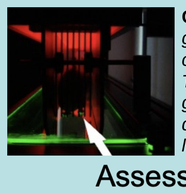


Mouse is placed on its back on a flat surface. The time taken to right itself (up to a maximum of 30 sec) onto its supine position is measured.

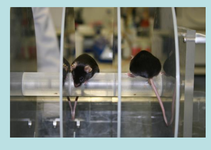
Assess motor strength and coordination at early developmental ages

At 15-weeks and 26-weeks of age:

Test Catwalk and Rotarod



Catwalk: *Mouse is placed in a glass platform with a video camera that records from below. The Catwalk is an automated gait analysis system for quantitate assessment of locomotion.*



Rotarod: *Mouse is placed on a rotating horizontal rod and the time it takes the mouse to fall of the rod is recorded. Three separate trials are run on two consecutive days.*

Assess neuromuscular function and learning ability

RESULTS

Figure 1:

A) **Righting Reflex: Average of Two Trials**

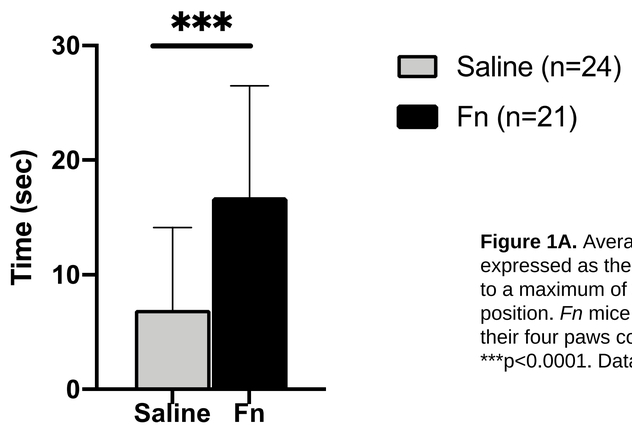


Figure 1A. Average of two trials of righting reflex. Results expressed as the time taken by the mouse to right itself (up to a maximum of 30 sec) after being placed in a supine position. *Fn* mice (n=24) significantly took longer to return to their four paws compared to saline mice (n=21). ***p<0.0001. Data are given as mean+SD.

B) **Righting Reflex: Proportion of Mice with Trial >30 sec**

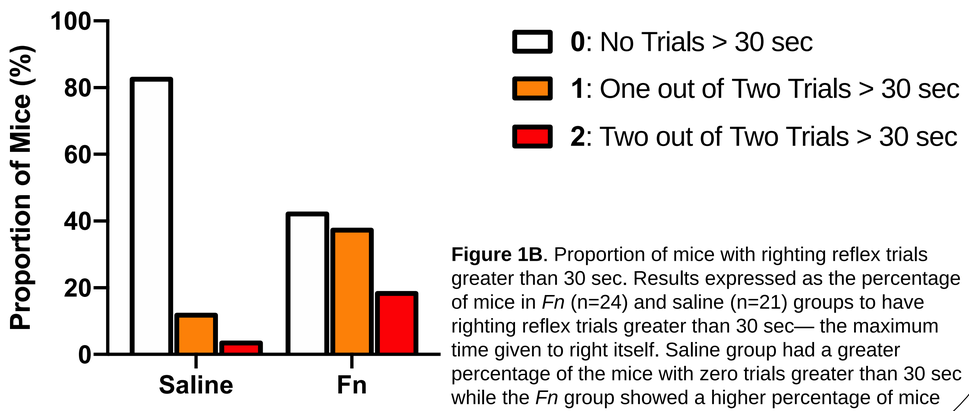


Figure 1B. Proportion of mice with righting reflex trials greater than 30 sec. Results expressed as the percentage of mice in *Fn* (n=24) and saline (n=21) groups to have righting reflex trials greater than 30 sec— the maximum time given to right itself. Saline group had a greater percentage of the mice with zero trials greater than 30 sec while the *Fn* group showed a higher percentage of mice with one or two trials greater than 30 sec.

Figure 2:

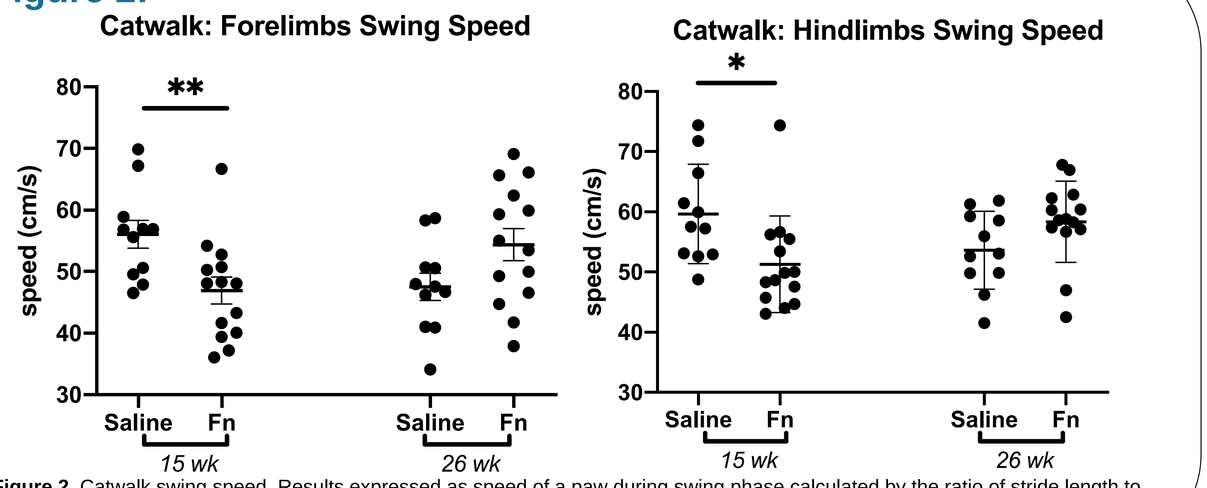


Figure 2. Catwalk swing speed. Results expressed as speed of a paw during swing phase calculated by the ratio of stride length to swing duration. At 15-weeks of age, *Fn* mice (n=14) had significantly slower swing speeds in both forelimb and hindlimb paws compared to saline mice (n=11). **p<0.01, *p<0.05. No significant differences were found at 26-weeks of age.

Figure 3:

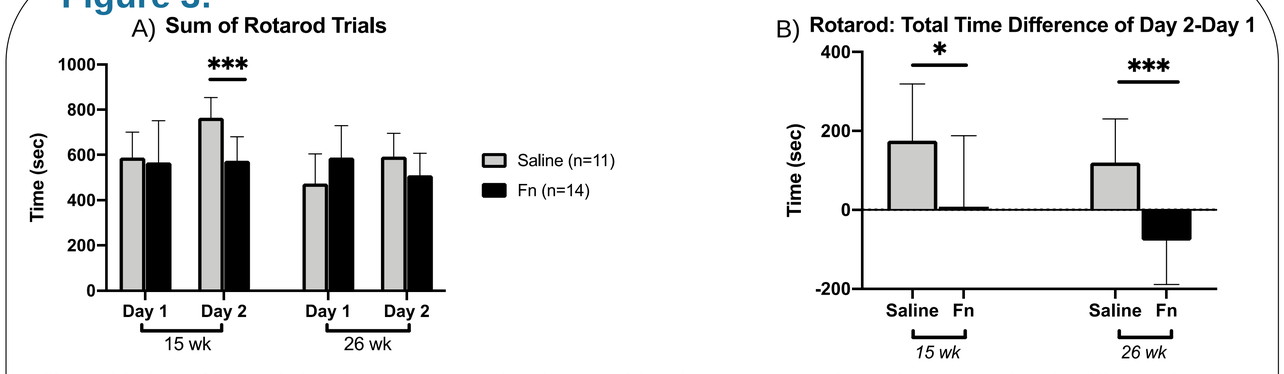


Figure 3A. Sum of Rotarod trials. Results expressed as the sum of 3 trials conducted on two consecutive days. There was significant difference between *Fn* (n=14) and saline (n=11) mice on Day 2 at 15-weeks of age. ***p<0.0001. No difference was found on Day 1, indicating no neuromuscular differences between the two groups. Thus, in relation to Figure 3B, the difference between the two days is reflective of learning ability, only. Data are given as mean+SD.

Figure 3B. Rotarod. Results expressed as the difference between day 2 and day 1 of the total time (sum of 3 trials) a mouse is able to maintain itself on a rotating rod. A positive difference indicates an increased ability of the mouse to remain on the rod on Day 2. Significant differences were found between *Fn* (n=14) and saline (n=11) mice at both 15-weeks and 26-weeks of age. *p<0.05, ***p<0.0001. Data are given as mean+SD.

CONCLUSION

- Mice exposed to transplacental infection of *F. nucleatum* exhibit significant behavioral changes reflecting neuromotor function and learning ability.
- Righting reflex tests gross neuromuscular defects, which was present at 1-week of age but absent at 2-weeks of age. The Catwalk tests more refined and specific neuromuscular defects which was present at 15-weeks but not at 26-weeks of age. However, learning disability and memory impairment, tested by Rotarod, exists at 15-weeks and persists through 26-weeks of age.
- We thus discover for the first time a neurological effect of transplacental *F. nucleatum* infection on the offspring. These findings are consistent with previous epidemiological studies that intrauterine infection may impact neurodevelopment and increase the risk for CNS disorders such as cerebral palsy and autism.
- Further research will be conducted by analyzing brain tissue harvested from the offspring mice to examine the possible presence of *F. nucleatum* and *Fusobacterium* adhesin FadA.

ACKNOWLEDGEMENTS

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