The Neurological Development of Offspring Exposed to Transplacental Infection of *Fusobacterium Nucleatum*

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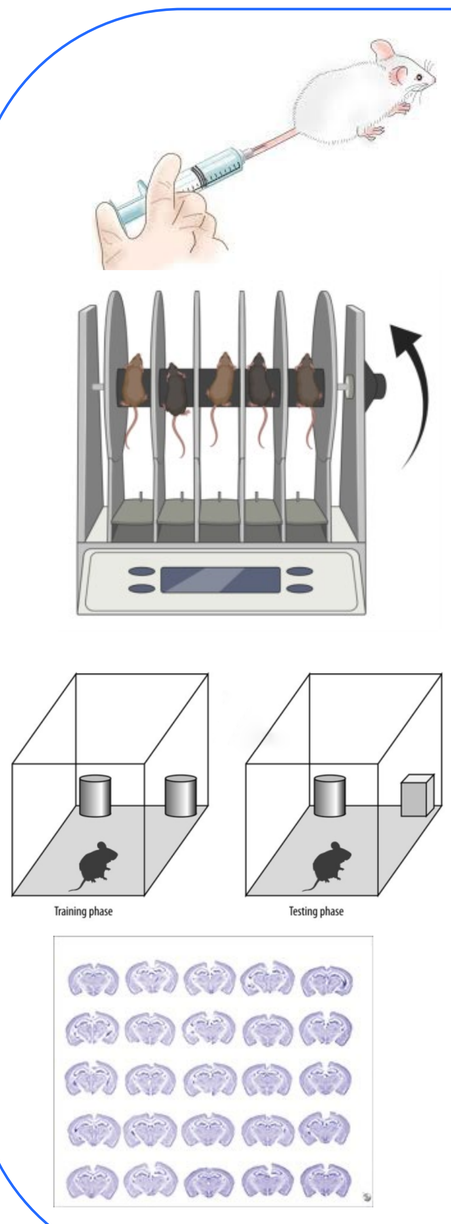
BACKGROUND

Fusobacterium nucleatum: gram-negative anaerobic bacterium
•commensal in the oral cavity
•closely associated with periodontal disease and infection to extraoral sites leading to several human diseases including colorectal cancer and adverse pregnancy outcomes
No studies have assessed *Fn*'s role in neurological disease in mice.
Preliminary studies in Dr. Han's lab have found mouse pups born following *F. nucleatum* infection exhibited abnormal neurobehavior in cognition, learning, and memory.

OBJECTIVES

We aimed to examine the brain pathologies of these mice and determine if and where *F. nucleatum* is located in the brain using immunohistochemical staining.

MATERIALS AND METHODS



16th day of gestation
•Pregnant mice were infected with 100 µl of 8 x 10^5 cfu of *F. nucleatum* via intravenous tail injection.
Week 1-2: Eye Opening
•Mouse is monitored from day of birth until both eyes open to assess cortical circuit assembly and maturation
Week 1-2: Righting Reflex
•Mouse is placed in supine position on a flat surface. The time taken right itself (maximum 30 seconds) to the prone position is measured to assess motor strength and coordination.
Week 15: Rotarod
•Mouse is placed on a rotating rod. The time mouse can stay on the rod is measured to test neuromuscular function and learning ability. Data is collected over 3 trials per day for two consecutive days.
Week 20: Novel Object Recognition
•Mouse is placed in an arena with two identical objects on day 1 and then on day 2 with the same familiar object and a novel object. The time spent exploring the objects is recorded to evaluate recognition memory.
Neuroanalysis: Immunohistochemical Staining
•Paraffin-embedded sections (5 µm) of 28-week-old mice brains were prepared from surgically resected tissues fixed in 4% paraformaldehyde.

RESULTS

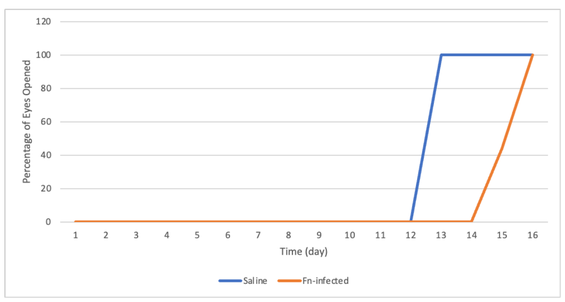


Figure 1: Eye Opening
Results expressed as the percentage of eyes opened in each litter opened on each day of the 16-day observation period. *Fn* mice on average (n=9) took longer to open both eyes compared to saline mice (n=7) with p<0.05 (t-test). Data given as mean.

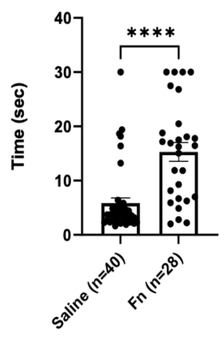


Figure 2: Righting Reflex
Average of trials of righting reflex conducted one week after birth. Results indicate time taken by the mouse to right itself (max. 30 sec) after being placed in a supine position. *Fn* mice (n=24) took significantly longer to return to their four paws compared to saline mice (n=21); p<0.0001 (t-test). Data given as mean+SD.

RESULTS (cont.)

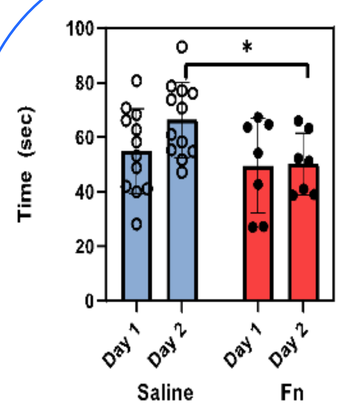


Figure 3: Rotarod
The results represent the average of three trials conducted on two consecutive days. Day 1 and day 2 data were compared to assess the learning ability in both groups. No significant difference was found in the *Fn* mice (n=7), while significant difference was found in the saline mice (n=12) with p<0.05 (ANOVA). Data given as mean+SD.

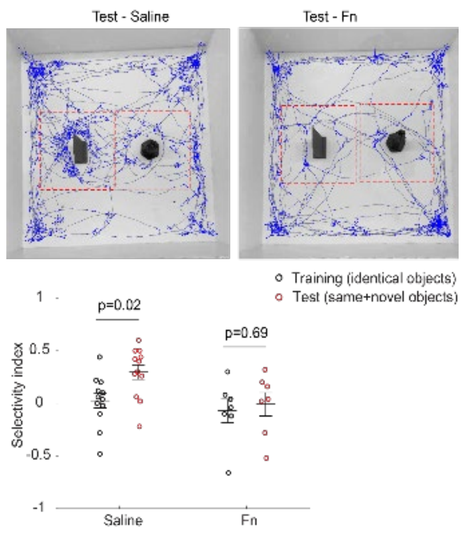


Figure 4: Novel object recognition
The selectivity index of calculated (time spent in area 1 - time spent in area 2)/(time spent in both areas). No significant difference in the positivity index was found between day 1 and day 2 in *Fn* mice, but significant difference was found in the saline mice, indicating preference towards the novel object with p<0.05 (t-test). Data given as mean+SD.

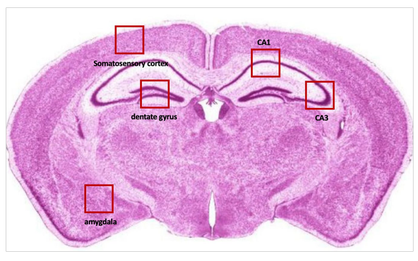
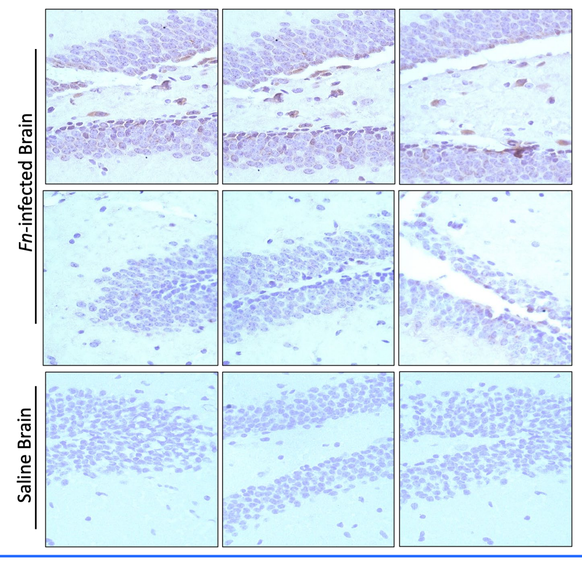


Figure 5: Immunohistochemistry
DAB peroxidase substrate was used to perform IHC on 28-week-old mice brains (saline and *Fn*-infected) with *F. nucleatum* polyclonal rabbit serum primary antibody (1:2000 dilution in 2.5% horse serum) and biotinylated goat anti-rabbit IgG secondary antibody (1:200 dilution in 2.5% horse serum), showing detection of *Fn* in the dentate gyrus region. Images taken at 40X.



DISCUSSION

•The offspring born to mothers infected with *F. nucleatum* exhibited significant defects in neuromotor function, learning ability, and memory
•The span of the tests reveal the neurological effect in the offspring takes place throughout the developmental and maturation process
•IHC detected *F. nucleatum* in the dentate gyrus region of the hippocampus, which is consistent with the neurological abnormalities

CONCLUSIONS

The next steps of the project include Western blot to confirm FadA, and consequently *F. nucleatum*, in the hippocampus region and transcriptomic analysis of isolated hippocampuses to elucidate the molecular mechanisms of *Fn* infection in the mouse brain.

ACKNOWLEDGEMENTS

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