

**Title**

Perinatal microbial colonization of the mouse colon and corresponding hypothalamic activation

**Authors**

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**Background/Synopsis**

At birth, the mammalian fetus exits a sterile womb and enters a world teeming with microbes.

These microorganisms, primarily bacteria, colonize the GI tract, as well as other body sites, and influence immune function, behavior, and disease states. We previously reported significant differences between the brains of germ-free (GF; those never having been exposed to microbes) and conventionally colonized mice (CC) on the day of birth, but not one day earlier.

Interestingly, CC mice show brain activation within a few hours after birth, in regions that receive neural input from the gut. This raised the questions of exactly when the mouse colon, which harbors the largest microbial community, is colonized by microbes, and if brain activation is caused by their arrival.

**Objectives/Purpose**

Our current work aims to answer – when exactly do microbes arrive in the colon, which species of bacteria are present during colonization, and what impact does this event have on the brain?

**Design/Methods**

Timed pregnancies were established in Swiss Webster mice, and samples were collected at time points of interest in the perinatal period. We specifically looked at brains and colons of male and female CC offspring either one day before birth, or after birth at 3h, 6h, 12h, 24h, postnatal day (P)3, and P7. Additionally, we examined GF offspring at key time points (3h, 24h, and P3) to understand if our findings are bacteria-dependent. Colons were sectioned and underwent quadruple-tagged fluorescent *in situ* hybridization and immunohistochemistry, and subsequent confocal imaging, to visualize at which time points bacteria began to arrive in the colon. Colonic

contents underwent DNA extraction, qPCR, and 16S rRNA gene sequencing to uncover both quantity and type of bacteria present. Finally, we used c-Fos immunohistochemistry, a marker of neural activation, and subsequent imaging to examine brain regions that receive input from the vagus nerve, a primary route of gut-brain communication; including the paraventricular nucleus of the hypothalamus.

## **Results**

Our imaging revealed that in CC mice, bacteria begin to arrive in the colon as early as 3h after birth, with a stark increase in bacterial quantity between 24h and P3, which was confirmed by qPCR. The absence of bacteria were confirmed in our GF mice at all time points, and again in qPCR, confirming the CC data were not a result of contamination. Via 16S rRNA gene sequencing, we find in CC mice that the microbiota becomes less diverse days after birth, as *Lactobacillus spp.* begin to dominate. c-Fos immunohistochemistry did not show any significant differences in brain activation between GF and CC mice, suggesting that the activation previously observed shortly after birth in CC mice does not require the arrival of microbes.

## **Conclusion**

This pre-clinical model of microbiota arrival in the neonatal colon allows for a more tightly controlled study than is possible in humans. Through our imaging and microbiota analyses, we showed microbes arriving to the mouse colon as soon as 3h after birth. This adds to a growing body of work debating the sterility of the womb. Our work is ongoing to further uncover the complete microbiota community present in the neonatal period, including other taxa outside of bacteria, and to understand further the potential impact that this community could have on the brain.