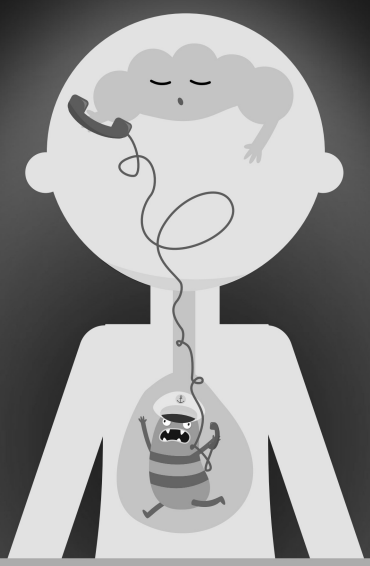
BIOMEDICAL SCIENCE JOURNAL FOR TEENS

NOVEMBER 2018

How do bacteria in the gut control the brain?



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Abstract

Bacteria are everywhere – including our bodies. This is not a bad thing as they help us in many ways. Many studies show that gut bacteria have an impact on behavior as well. During the first few months after birth the brain actively develops. At the same time, bacteria enter from the diet and environment and multiply in the infants' gut. As *Bifidobacterium* species (bifidobacteria) are the most common bacteria in the infants' gut, we wanted to see if these bacteria play a role in the development of brain functions. We examined the behavior of mice which we treated with bifidobacteria only, and compared it to the behavior of mice without any bacteria and mice that had normal mouse gut bacteria. The germ-free mice showed many important behavioral differences when compared to mice with normal bacteria, and the mice that had only bifidobacteria behaved similarly to the normal mice demonstrating how important these bacteria are. The sex of the rodents also played an important role in whether bifidobacteria could change their behavior.

Introduction

What would you say if you were told that you have trillions of life forms living inside of you? No, it's not something from a sci-fi film… Our bodies are home to trillions of bacteria. They start taking up residence from the minute we are born. Even though we think of bacteria as bad, the vast majority of them living in our bodies are actually good for us, we call these '*probiotic*' or 'commensal' bacteria. In fact, we couldn't live without them! They help us digest our food, supply us with some vitamins and nutrients, and protect us against harmful bacteria called *pathogens*.

Many studies have also demonstrated that bacteria in the intestines can affect other parts of the body, and play a role in the development of the *central nervous system* (which includes the brain and spinal cord). Germ-free mice (mice without bacteria inside them; bred in sterile conditions) for example, have shown abnormal brain activity and behavior.

Our behavior patterns and nervous system continue to develop during the first few months after we are born and it is during this same period of time that bacteria quickly begin to live and multiply in the gut. Bifidobacteria are some of the first types of bacteria that live in the gut of humans after birth (Fig. 1) and previous studies show that bifidobacteria can affect the brain function of adult mice. We think that having the right types of bacteria, like bifidobacteria, in the gut as an infant may impact the development of our brain and nervous system and may change how we behave later in life.

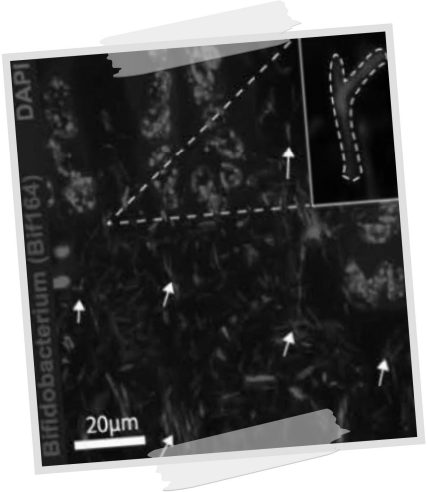

Figure 1: Bifidobacteria in the mouse gut - blue cells are mouse cells in the intestine and red cells are bifidobacteria. The rectangle on the right shows a zoomed in image of the unique shape of a *Bifidobacterium* cell.

Methods

To test our hypothesis we wanted to compare the behavior of three types of mice:

1) Germ-free mice (as a control)
2) Germ-free mice to which we gave human bifidobacteria
3) Germ-free mice to which we gave normal mouse gut bacteria

We tested both male and female mice in each group, and we looked after all of the mice in sterile isolated cages for seven weeks from the day they were born.

At seven weeks, the mice are considered adults, and at that time we examined the behavior of all the mice by conducting several experiments using special equipment. We assessed these qualities in the mice:

- Levels of anxiety
- Levels of activity
- *Motor performance* and coordination
- Memory
- Social behavior

Each piece of testing apparatus was disinfected after each mouse had used it so no microbes could spread between the mice.

To find out which microbes were in each mouse's gut we used *DNA sequencing* and *fluorescence in situ hybridization.*

Test Apparatus

But wait, how do you test mice? Well, they can't sit down and take an exam so these are the pieces of equipment we used:

Elevated plus maze (Fig.2) - a test which measures anxiety in animals. The apparatus has two open and two enclosed arms. If the mouse spends more time in the open space it is less anxious, but if it spends more time in the closed space, this indicates that the mouse is more anxious.

Novel object recognition test (Fig.3) - We placed the mouse in a chamber for five minutes to allow it to get used to the environment. → Then we placed the mouse in the chamber with two identical objects and let it get to know them. → The mouse had one hour rest in its home cage. → Meanwhile, we swapped one object for a new, different one. →We put the mouse back in the chamber for five minutes and observed whether it recognised the old object. If the mouse spends most of its time exploring the new object, we know that it recognizes the old object (and is therefore not as interested in it). If it doesn't recognize the known object it is considered to have an impaired memory.

Open field apparatus - a test for general motor activity and anxiety. It's a larger arena which the mouse can explore. Less anxious mice explore the open center area more than the anxious ones.

Rotarod performance test - the mice have to move around on a rotating rod. We evaluated their speed, balance, coordination and endurance.

Three-chambered test - a test which evaluates social behavior. We placed the mouse in the middle of three chambers. In one of them there is another mouse and in the other there is an object. If the mouse spends more time with the other mouse instead of the object, we consider it social.

Fig.2 A) **Fig.2 B)**

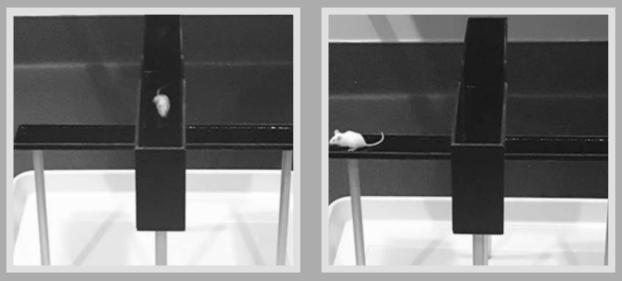

Figure 2: **A)** A mouse in the enclosed arm of an Elevated Plus Maze and **B)** a mouse in the open arm of the maze.

Fig.3

Figure 3: The chamber in which a mouse is placed for the 'novel object recognition test'. In this case it has one object which the mouse would be familiar with, and a new object. A camera is positioned above the chamber to track the mouse.

Results

Both the DNA sequencing and fluorescence analysis showed that the mice which we treated with normal gut bacteria had the richest range of microbes in the gut. As expected, bifidobacteria dominated the gut of the bifidobacteria-treated group of mice. Both this group and the germ-free adult mice gained additional species of bacteria from their environment after we transferred them out of their sterile isolated cages.

Our behavior experiments showed:

(1) Female mice with only the bifidobacteria had a normal level of anxiety.

(2) Germ-free mice were hyper-active and while colonization with bifidobacteria improved the male mice's behavior, these bacteria couldn't help the female rodents. Normal mouse bacteria helped both female and male mice though.

(3) Early bifidobacteria treatment improved the motor performance of male mice (as compared to germ-free male mice, which showed poor motor performance).

(4) Germ-free mice had trouble with memory, while the bifidobacteria-treated group of mice (and the rodents with normal gut microbes) didn't, regardless of their sex (Fig. 4).

(5) Female mice, treated with normal gut microbes, were social, while the germ-free and bifidobacteria-treated females were not very sociable.

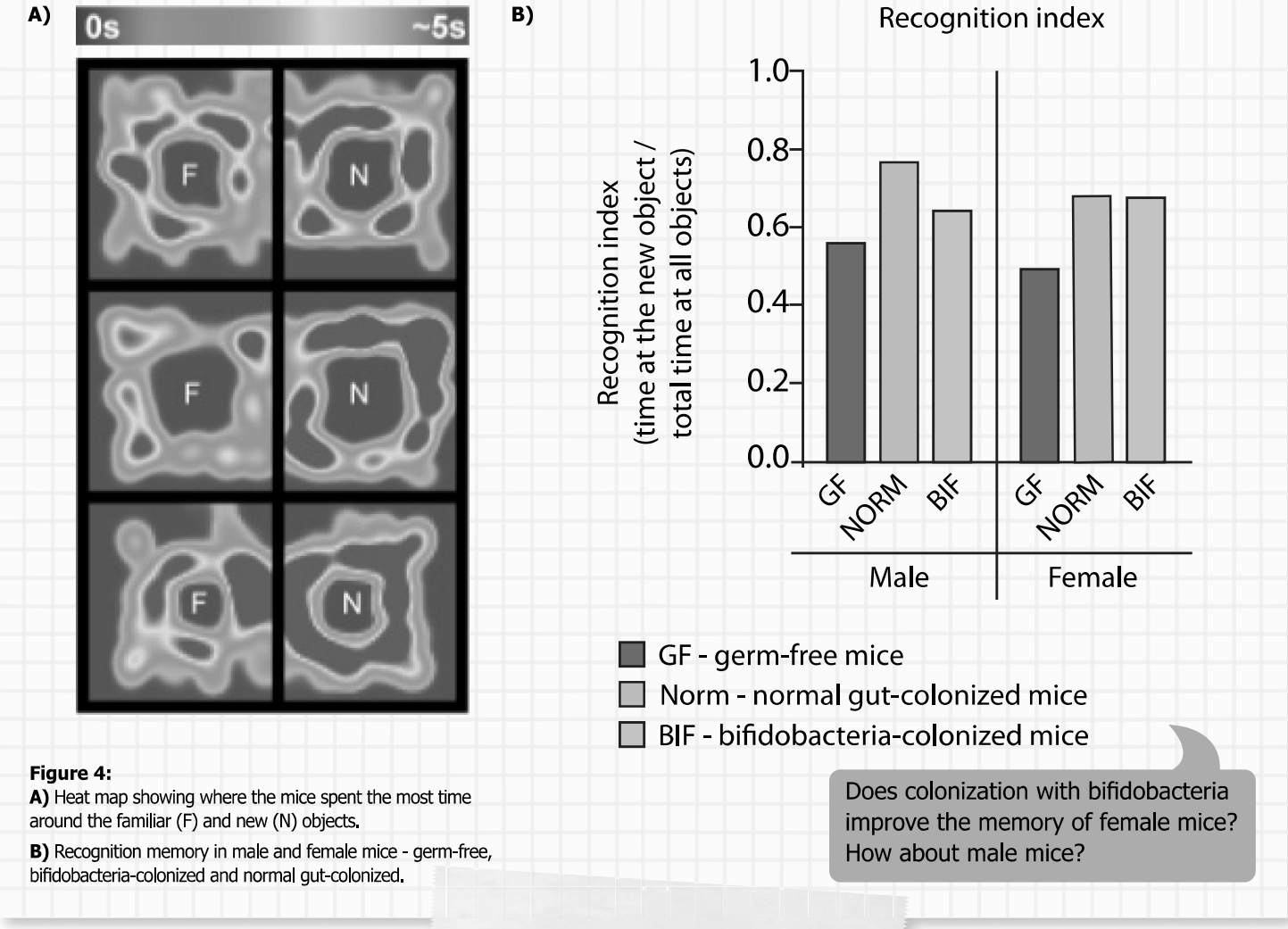

Figure 4:
A) Heat map showing where the mice spent the most time around the familiar (F) and new (N) objects.

B) Recognition memory in male and female mice - germ-free, bifidobacteria-colonized and normal gut-colonized.

Discussion

Our results show us that microbes have a great effect on the behavior of adult mice but that this behavior depends also on the sex of the mouse. Perhaps this is due to an interaction between the microbes and sex hormones. Regardless of the reason, the sex of the rodent had a great impact on their behavior. For example, all the germ-free mice had a reduced level of anxiety which could result in reckless behavior. Putting bifidobacteria in their gut raised the anxiety level to a healthy degree in female mice but not in males, and giving them normal gut microbes made both males and females appropriately anxious in order to avoid being too bold or reckless (vulnerable to predators). Similarly, the sex of the mice mattered in relation to hyper-activity: bifidobacteria improved males' behavior but not the females'. Sometimes the sex of the rodent did not make any difference, though, for example, giving normal bacteria to the previously germ-free mice improved the memory of both males and females.

The study also showed that putting bifidobacteria into the gut improved the performance on some behavioral tests but not others. Perhaps some regions of the brain are more susceptible to being changed by these bacteria than others. This could be useful in future studies to better understand the interactions between microbes and their host (whether mice, human, or another animal!).

Conclusion

We can't live without bacteria, they are vital both in our bodies and outside of them. But there are also bad bacteria which make us sick. You can avoid the majority of bacterial infections by simply washing your hands regularly. If you get sick from a bacterial infection, always use antibiotics exactly as the doctor has prescribed, making sure to take the full course of drugs even after you start feeling better – this way you could avoid bad bacteria building up resistance to the drug, and protect the trillions of good bacteria that are helping you every day.

Glossary of Key Terms

Bifidobacteria – group of bacteria which normally live in the intestines, help digest food and produce vitamins.

Central nervous system – the complex of nerve tissues that control the body's movements and activities, as well as thoughts, behavior and communication. In vertebrates (including mice and humans) the central nervous system includes the brain and the spinal cord.

DNA sequencing – the process of determining the precise order of the building blocks of DNA.

Fluorescence in situ hybridization (FISH) – a molecular technique that uses fluorescent probes to detect and localize the presence or absence of specific DNA sequences on chromosomes.

Probiotic (bacteria) – sometimes called 'friendly' bacteria, these are bacterial organisms that support the health of your gut and other parts of the body. They help maintain healthy digestion in the gut.

Microbes – microscopic organisms, which may exist in single-cell form or in a colony of cells. Bacteria are a type of microbe.

Motor performance – how well an animal or human completes movements and actions of the muscles to achieve a particular action, for example walking, climbing.

Pathogen – a virus, bacterium or other microorganism that can cause disease in another organism. For example HIV, which causes AIDS; or *Vibrio cholerae*, which causes cholera.

Check your understanding

1 What do good bacteria do and what do harmful bacteria do?

2 Did putting bifidobacteria into the gut of the germ-free mice improve their behavior?

3 Why did we use germ-free mice?

4 Why did we test the impact of bifidobacteria on mouse behavior?

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