BRAIN, MEET GUT

BY PETER ANDREY SMITH

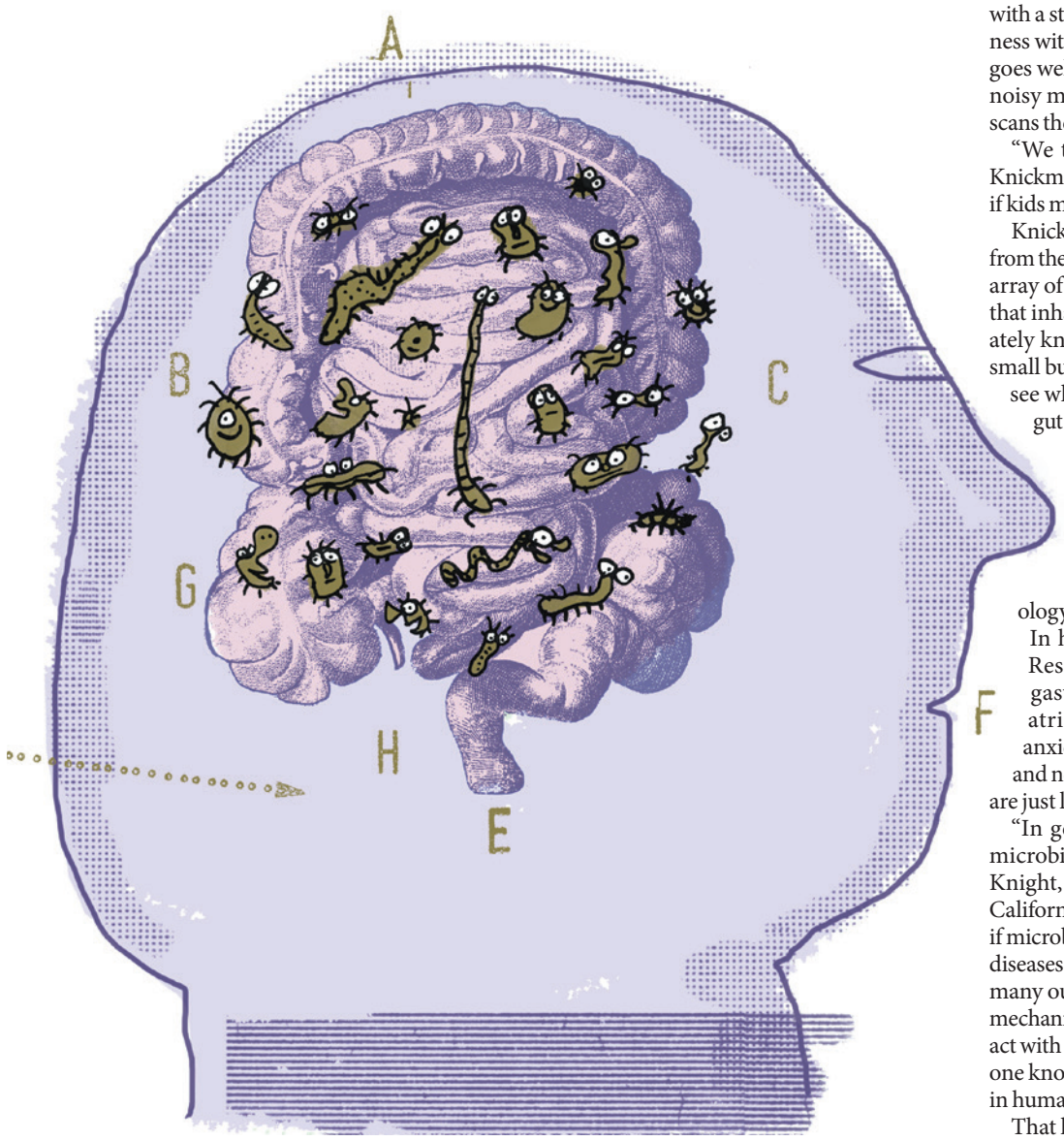


ILLUSTRATION BY SERGE BLOCH

Neuroscientists are probing the connections between intestinal microbes and brain development.

Nearly a year has passed since Rebecca Knickmeyer first met the participants in her latest study on brain development. Knickmeyer, a neuroscientist at the University of North Carolina School of Medicine in Chapel Hill, expects to see how 30 newborns have grown into crawling, inquisitive one-year-olds, using a battery of behavioural and temperament tests. In one test, a child's mother might disappear from the testing suite and then reappear with a stranger. Another ratchets up the weirdness with some Halloween masks. Then, if all goes well, the kids should nap peacefully as a noisy magnetic resonance imaging machine scans their brains.

"We try to be prepared for everything," Knickmeyer says. "We know exactly what to do if kids make a break for the door."

Knickmeyer is excited to see something else from the children — their faecal microbiota, the array of bacteria, viruses and other microbes that inhabit their guts. Her project (affectionately known as 'the poop study') is part of a small but growing effort by neuroscientists to see whether the microbes that colonize the gut in infancy can alter brain development.

The project comes at a crucial juncture. A growing body of data, mostly from animals raised in sterile, germ-free conditions, shows that microbes in the gut influence behaviour and can alter brain physiology and neurochemistry.

In humans, the data are more limited. Researchers have drawn links between gastrointestinal pathology and psychiatric neurological conditions such as anxiety, depression, autism, schizophrenia and neurodegenerative disorders — but they are just links.

"In general, the problem of causality in microbiome studies is substantial," says Rob Knight, a microbiologist at the University of California, San Diego. "It's very difficult to tell if microbial differences you see associated with diseases are causes or consequences." There are many outstanding questions. Clues about the mechanisms by which gut bacteria might interact with the brain are starting to emerge, but no one knows how important these processes are in human development and health.

That has not prevented some companies in

the supplements industry from claiming that probiotics — bacteria that purportedly aid with digestive issues — can support emotional well-being. Pharmaceutical firms, hungry for new leads in treating neurological disorders, are beginning to invest in research related to gut microbes and the molecules that they produce.

Scientists and funders are looking for clarity. Over the past two years, the US National Institute of Mental Health (NIMH) in Bethesda, Maryland, has funded seven pilot studies with up to US$1 million each to examine what it calls the 'microbiome–gut–brain axis' (Knickmeyer's research is one of these studies). This year, the US Office of Naval Research in Arlington, Virginia, agreed to pump around US$14.5 million over the next 6–7 years into work examining the gut's role in cognitive function and stress responses. And the European Union has put €9 million (US$10.1 million) towards a five-year project called MyNewGut, two main objectives of which target brain development and disorders.

The latest efforts aim to move beyond basic observations and correlations — but preliminary results hint at complex answers. Researchers are starting to uncover a vast, varied system in which gut microbes influence the brain through hormones, immune molecules and the specialized metabolites that they produce.

"There's probably more speculation than hard data now," Knickmeyer says. "So there's a lot of open questions about the gold standard for methods you should be applying. It's very exploratory."

GUT REACTIONS

Microbes and the brain have rarely been thought to interact except in instances when pathogens penetrate the blood–brain barrier — the cellular fortress protecting the brain against infection and inflammation. When they do, they can have strong effects: the virus that causes rabies elicits aggression, agitation and even a fear of water. But for decades, the vast majority of the body's natural array of microbes was largely uncharacterized, and the idea that it could influence neurobiology was hardly considered mainstream. That is slowly changing.

Studies on community outbreaks were one key to illuminating the possible connections. In 2000, a flood in the Canadian town of Walkerton contaminated the town's drinking water with pathogens such as *Escherichia coli* and *Campylobacter jejuni*. About 2,300 people suffered from severe gastrointestinal infection, and many of them developed chronic irritable bowel syndrome (IBS) as a direct result.

During an eight-year study[1] of Walkerton residents, led by gastroenterologist Stephen Collins at McMaster University in Hamilton, Canada, researchers noticed that psychological issues such as depression and anxiety seemed to be a risk factor for persistent IBS. Premysl Bercik, another McMaster gastroenterologist, says that this interplay triggered intriguing questions. Could psychiatric symptoms be driven by lingering inflammation, or perhaps by a microbiome thrown out of whack by infection?

The McMaster group began to look for answers in mice. In a 2011 study[2], the team transplanted gut microbiota between different strains of mice and showed that behavioural traits specific to one strain transmitted along with the microbiota. Bercik says, for example, that "relatively shy" mice would exhibit more exploratory behaviour when carrying the microbiota of more-adventurous mice. "I think it is surprising. The microbiota is really driving the behavioural phenotype of host. There's a marked difference," Bercik says. Unpublished research suggests that taking faecal bacteria from humans with both IBS and anxiety and transplanting it into mice induces anxiety-like behaviour, whereas transplanting bacteria from healthy control humans does not.

"THERE'S PROBABLY MORE SPECULATION THAN HARD DATA NOW."

Such results can be met with scepticism. As the field has developed, Knight says, microbiologists have had to learn from behavioural scientists that how animals are handled and caged can affect things such as social hierarchy, stress and even the microbiome.

And these experiments and others like them start with a fairly unnatural model: germ-free — or 'gnotobiotic' — mice. These animals are delivered by Caesarean section to prevent them from picking up microbes that reside in their mothers' birth canals. They are then raised inside sterile isolators, on autoclaved food and filtered air. The animals are thus detached from many of the communal microbes that their species has evolved with for aeons.

In 2011, immunologist Sven Pettersson and neuroscientist Rochellys Diaz Heijtz, both at the Karolinska Institute in Stockholm, showed that in lab tests, germ-free mice demonstrated less-anxious behaviour than mice colonized with natural indigenous microbes[3]. (Less anxiety is not always a good thing, evolutionarily speaking, for a small mammal with many predators.)

When the Karolinska team examined the animals' brains, they found that one region in germ-free mice, the striatum, had higher turnover of key neurochemicals that are associated with anxious behaviour, including the neurotransmitter serotonin. The study also showed that introducing adult germ-free mice to conventional, non-sterile environments failed to normalize their behaviour, but the offspring of such 'conventionalized' mice showed some return to normal behaviour, suggesting that there is a critical window during which microbes have their strongest effects.

By this time, many researchers were intrigued by the mounting evidence, but results stemmed mostly from fields other than neuroscience. "The groups working on this are primarily gut folks, with a few psychology-focused people collaborating," says Melanie Gareau, a physiologist at the University of California, Davis. "So the findings tended to describe peripheral and behavioural changes rather than changes to the central nervous system."

But Pettersson and Diaz Heijtz's research galvanized the field, suggesting that researchers could get past observational phenomenology and into the mechanisms affecting the brain. Nancy Desmond, a programme officer involved in grant review at the NIMH, says that the paper sparked interest at the funding agency soon after its publication and, in 2013, the NIMH formed a study section devoted to neuroscience research that aims to unravel functional mechanisms and develop drugs or non-invasive treatments for psychological disorders.

Judith Eisen, a neuroscientist at the University of Oregon in Eugene, earned a grant to study germ-free zebrafish, whose transparent embryos allow researchers to easily visualize developing brains. "Of course, 'germ-free' is a completely unnatural situation," Eisen says. "But it provides the opportunity to learn which microbial functions are important for development of any specific organ or cell type."

CHEMICAL EXPLORATION

Meanwhile, researchers were starting to uncover ways that bacteria in the gut might be able to get signals through to the brain. Pettersson and others revealed that in adult mice, microbial metabolites influence the basic physiology of the blood–brain barrier[4]. Gut microbes break down complex carbohydrates into short-chain fatty acids with an array of effects: the fatty acid butyrate, for example, fortifies the blood–brain barrier by tightening connections between cells (see 'The gut–brain axis').

Recent studies also demonstrate that gut microbes directly alter neurotransmitter levels, which may enable them to communicate with neurons. For example, Elaine Hsiao, a biologist now at the University of California, Los Angeles, published research[5] this year examining how certain metabolites from gut microbes promote serotonin production in the cells lining the colon — an intriguing finding given that some antidepressant drugs work by promoting serotonin at the junctions between neurons. These cells account for 60% of peripheral serotonin in mice and more than 90% in humans.

Like the Karolinska group, Hsiao found that germ-free mice have significantly less serotonin floating around in their blood, and she also showed that levels could be restored by introducing to their guts spore-forming bacteria (dominated by *Clostridium*, which break down

short-chain fatty acids). Conversely, mice with natural microbiota, when given antibiotics, had reduced serotonin production. "At least with those manipulations, it's quite clear there's a cause–effect relationship," Hsiao says.

But it remains unclear whether these altered serotonin levels in the gut trigger a cascade of molecular events, which in turn affect brain activity — and whether similar events take place in humans, too. "It will be important to replicate previous findings, and translate these findings into human conditions to really make it to the textbooks," Hsiao says.

For John Cryan, a neuroscientist at University College Cork in Ireland, there is little question that they will. His lab has demonstrated[6] that germ-free mice grow more neurons in a specific brain region as adults than do conventional mice. He has been promoting the gut–brain axis to neuroscientists, psychiatric-drug researchers and the public. "If you look at the hard neuroscience that has emerged in the last year alone, all the fundamental processes that neuroscientists spend their lives working on are now all shown to be regulated by microbes," he says, pointing to research on the regulation of the blood–brain barrier, neurogenesis in mice and the activation of microglia, the immune-like cells that reside in the brain and spinal cord.

At the 2015 Society for Neuroscience meeting in Chicago, Illinois, this month, Cryan and his colleagues plan to present research showing that myelination — the formation of fatty sheathing that insulates nerve fibres — can also be influenced by gut microbes, at least in a specific part of the brain. Unrelated work[7] has shown that germ-free mice are protected from an experimentally induced condition similar to multiple sclerosis, which is characterized by demyelination of nerve fibres. At least one company, Symbiotix Biotherapies in Boston, Massachusetts, is already investigating whether a metabolite produced by certain types of gut bacterium might one day be used to stem the damage in humans with multiple sclerosis.

A MOVE TO THERAPY

Tracy Bale, a neuroscientist at the University of Pennsylvania in Philadelphia, suspects that simple human interventions may already be warranted. Bale heard about Cryan's work on the radio programme *Radiolab* three years ago. At the time, she was researching the placenta, but wondered how microbes might fit into a model of how maternal stress affects offspring.

In research published this year[8], Bale subjected pregnant mice to stressful stimuli. She found that it noticeably reduced the levels of *Lactobacilli* present in the mice's vaginas, which are the main source of the microbes that colonize the guts of offspring. These microbial shifts carried over to pups born vaginally, and Bale detected signs that microbiota might affect neurodevelopment, especially in males.

In work that her group plans to present at the Society for Neuroscience meeting, Bale has shown that by feeding vaginal microbiota from stressed mice to Caesarean-born infant mice, they can recapitulate the neurodevelopmental effects of having a stressed mother. Bale and her colleagues are now wrapping up research investigating whether they can treat mice from stressed mums with the vaginal microbiota of non-stressed mice.

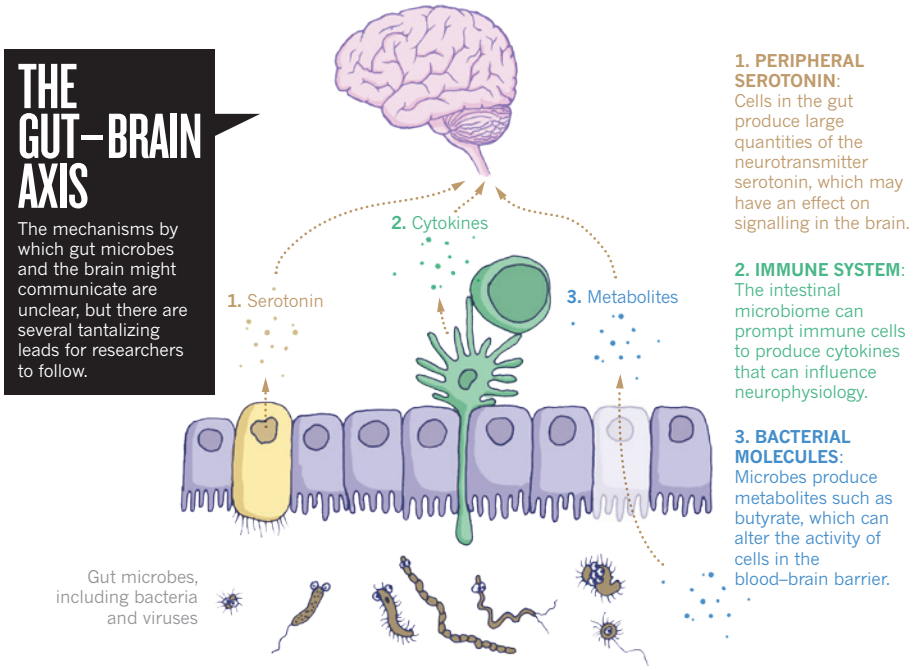
The work, Bale says, has "immediate translational effects". She points to a project headed by Maria Dominguez-Bello, a microbiologist at the New York University School of Medicine, in which babies born by means of Caesarean section are swabbed on the mouth and skin with gauze taken from their mothers' vaginas. Her team wants to see whether these offspring end up with microbiota similar to babies born vaginally. "It's not standard of care," Bale says, "but I will bet you, one day, it will be."

Many are still sceptical about the link between microbes and behaviour and whether it will prove important in human health — but scientists seem more inclined to entertain the idea now than they have in past. In 2007, for example, Francis Collins, now director of the US National Institutes of Health, suggested that the Human Microbiome Project, a large-scale study of the microbes that colonize humans, might help to unravel mental-health disorders. "It did surprise a few people who assumed we were talking about things that are more intestinal than cerebral," Collins says. "It was a little bit of leap, but it's been tentatively backed up."

Funding agencies are supporting the emerging field, which spans immunology, microbiology and neuroscience, among other disciplines. The NIMH has offered seed funding for work on model systems and in humans to probe whether the area is worth more-substantial investment, a move that has already brought more researchers into the fold. The MyNewGut project in Europe has an even more optimistic view of the value of such research, specifically seeking concrete dietary recommendations that might alleviate brain-related disorders.

Today, Knickmeyer's project on infants represents what she calls "a messy take-all-comers kind of sample". Among the brain regions that Knickmeyer is scanning, the amygdala and prefrontal cortex hold her highest interest; both have been affected by microbiota manipulations in rodent models. But putting these data together with the dozens of other infant measures that she is taking will be a challenge. "The big question is how you deal with all the confounding factors." The children's diets, home lives and other environmental exposures can all affect their microbiota and their neurological development, and must be teased apart.

Knickmeyer speculates that tinkering with microbes in the human gut to treat mental-health disorders could fail for other reasons. Take, for instance, how microbes might interact with the human genome. Even if scientists were to find the therapeutic version of a "gold Cadillac of microbiota", she points out, "maybe your body rejects that and goes back to baseline because your own genes promote certain types of bacteria." There is much more to unravel, she says. "I'm always surprised. It's very open. It's a little like a Wild West out there." ■



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