News Feature: Microbes on the mind

Could the gut microbiome have a critical role in brain and behavior? The notion is starting to gain acceptance amongst both researchers and funders.

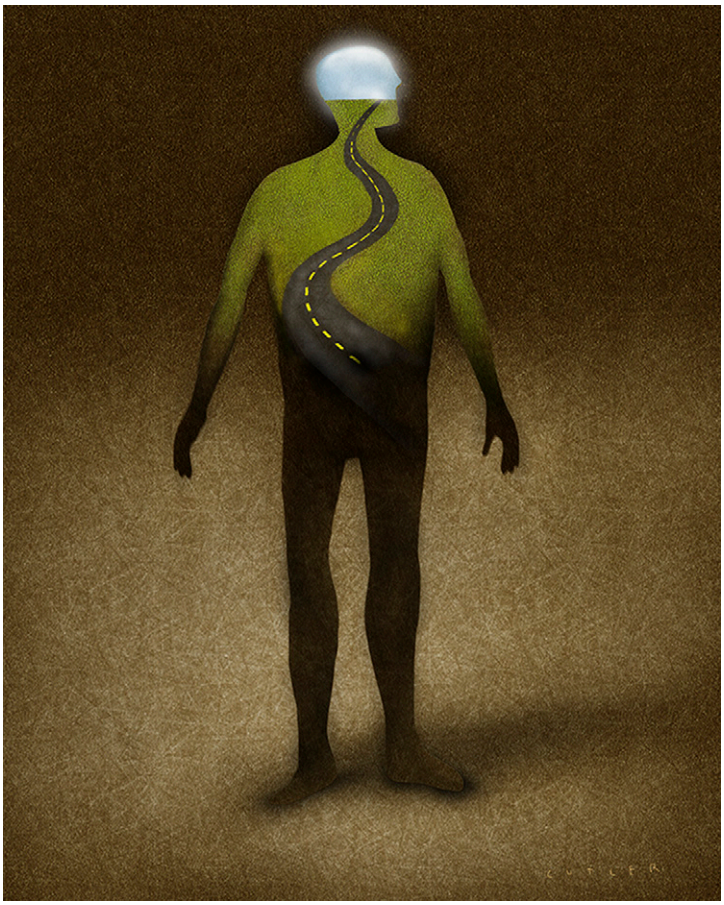
Helen H. Shen
Science Writer

Of all of the journal articles that neuroscientist Jane Foster has written in the past 10 years, one stands out. "It was the hardest paper I'd ever published in my life," says Foster, a professor at McMaster University in Ontario, Canada. Her 2011 study in *Neurogastroenterology and Motility* showed that mice produced and raised without gut bacteria display changes in anxiety-like behaviors (1). The findings supported a relatively new, and to some researchers, radical, theory: that intestinal microbes can influence the mind. Foster's manuscript met with skepticism from many reviewers, taking two years to reach publication.

But times are changing, says Foster. Most of her papers now take a more conventional four to six months to publish, bolstered by a growing number of studies in the field from Foster's laboratory and others. Mounting evidence, mostly from rodent studies, suggests that gut microbes help shape normal neural development, brain biochemistry, and behavior, especially in relation to stress, anxiety, and depression. Disruptions to gut bacteria, some researchers believe, could play a role in certain neuropsychiatric disorders and might even point to new treatments or ways of improving existing ones.

Still, scientists will have to clarify the mechanisms at work and clearly demonstrate cause and effect before the microbiome gains acceptance as a target for treating brain diseases. Numerous studies are either depleting members of the microbiota or boosting their numbers in hopes of gleaning insights into the cascade of effects on the brain.

"It could turn out that this is just the latest kid on the block and it's been way oversold, or it could turn out to be really important," says National Institute of Mental Health (NIMH) Director Thomas Insel about the brain–microbiome connection. "We have to support a lot of early-stage research and assume that we're going to be surprised."



Early work suggests the gut microbiome could have important effects on the brain and behavior. Image courtesy of Dave Cutler.

Bacterial Beginnings

Already, scientists probing the complex human microbiome have uncovered associations with conditions such as obesity, diabetes, and malnutrition. Some see the brain and behavior as the next, most promising area for microbiome research. The Society for Neuroscience held its first-ever annual meeting symposium on the brain–microbiome connection last year. The NIMH recently funded its first related research program, supporting about $1 million in grants, and the Office of Naval Research is ramping up to a projected $3.3 million in funding by September 2016.

Various studies suggest that bacteria and their byproducts could have major effects on the brain by activating nerve fibers, or possibly by modulating immune, hormonal, and neurochemical systems in the body. "The idea that microbes can affect our metabolism is intuitively acceptable to lots of people," says Rob Knight, cofounder of the American Gut Project, a crowd-sourced, crowd-funded effort to study the gut microbiome of the United States population. "That microbes can affect our personality and cognition, or that we could improve cognitive performance by

Box 1. Communication Break-Down

The potential pathways connecting intestinal bacteria to brain physiology are many and complex; researchers are a long way from pinning down the detailed, step-by-step processes that may link specific microbes all of the way to the brain. But the players are starting to come into view (11).

One pathway appears fairly well traveled: at least some bacteria seem to exert their influence via the vagus nerve, a conduit between the brain and the gut's own dense network of nerves. Cyran and colleagues have found that cutting this nerve in mice blocked the anxiety-relieving effects of the bacteria *Lactobacillus rhamnosus* and prevented treatment-related changes in brain chemistry (12).

But the actual messengers may be bacterial byproducts, which include neuroactive molecules. Researchers have found that bacteria themselves are capable of generating neurotransmitters and other neuromodulators, although it's unclear precisely how these signals act locally on the gut or how they might be relayed to or interpreted by the brain.

And all of these players may be intertwined with the immune system via, for example, circulating levels of signaling molecules called cytokines, which gut microbiota can help regulate. Among their many functions, cytokines in the blood help the body respond to infections and injury; in the brain, cytokines support neuron growth and help remodel connections between neurons. Elevated blood levels of inflammatory cytokines have been associated with neuropsychiatric disorders, such as depression (13).

One theory suggests that cytokines and some bacterial cell wall components stimulate certain cells in the brain such as immune cells called microglia. This interaction, in turn, generates even more cytokines, along with molecules such as nitric oxide, which is toxic to neurons. The result: neurotransmitter signaling problems that affect mood and possibly other aspects of brain function (13).

But it could also be that the bacteria themselves, or parts of their cell walls, are escaping from the gut and making trouble. In a condition called "leaky gut," chronic stress and other conditions can compromise the gut lining, which normally tightly regulates passage of nutrients and microbes into the blood (14). Rogue bacteria that slip out of the gut can trigger harmful inflammatory responses. Interestingly, some probiotics appear to block this unwanted effect by restoring the gut's integrity.

"There really are a lot of mechanisms being uncovered right now," says Knight. If different bacteria are affecting different circuits and behaviors via different mechanisms, attempts at translational research will prove exceedingly complex. "What matters is what's relevant for what condition and for what species of bacteria," he adds. "We're getting into a situation where there's almost too many mechanisms where the gut can affect the brain."

altering our microbes or diet—that's still very much a new frontier."

Much of today's interest in the gut microbiome-brain link traces back to a study in 2004 by Nobuyuki Sudo et al. at Kyushu University in Japan (2). The researchers showed that mice produced and raised without gut bacteria appear unusually sensitive to stress. When researchers temporarily placed these "germ-free" mice and normal mice in restraints, both groups boosted production of the stress hormones adrenocorticotrophin and corticosterone. Compared with normal mice, the animals that lacked gut microbes pumped out roughly twice the amount of those hormones.

The findings prompted several groups to investigate the peculiar behavior and neurobiology of germ-free mice. Tests by Sudo and others suggest that the mice have brain biochemistry changes affecting molecules suspected to have roles in some aspects of anxiety and depression in humans. For example, a few groups have reported lower-than-normal levels of the nerve growth factor BDNF in the germ-free mouse cortex and hippocampus (2, 3).

Other neurochemical systems involved in mood appear to be affected in germ-free mice, although the few studies done so far have not offered a clear consensus on what's going on. Some researchers have found elevated levels of the mood-modulating neurotransmitter serotonin in the hippocampus (4), whereas others have reported fewer hippocampal serotonin receptors (1). And various subunits of the NMDA glutamate receptor—linked to some mood disorders—appear to be produced in lower-than-normal numbers in some parts of the brain. The absence of microbes throughout development, and the resulting constellation of physiological, immunological, and neurological defects, appear to alter mood, but diagramming the cause and effect has proved elusive.

Behavioral tests have yielded some perplexing findings as well. Despite their elevated hormonal responses to stress, for example, germ-free mice move about in a less-anxious or less-cautious manner. They spend more time in open, exposed spaces that normal mice tend to avoid (1).

"It's difficult to say what this means," says neuroscientist John Cryan, a professor at the University College Cork in Ireland. "Germ-free mice are a good model to ask the very simple question: is the microbiota involved in this process or not? That's a yes or no answer." But the total lack of gut microbes throughout life, which is unlike any real human condition, probably affects many biological processes, thus accounting for some of the conflicting results. Even so, says Cryan, germ-free mouse studies do suggest that gut bacteria are required to form normal anxiety and stress responses.

To better understand these dynamics, some researchers are manipulating the microbiotal composition with bacteria transplants. In one striking demonstration, scientists at McMaster University used a gut bacteria makeover to turn timid mice into intrepid explorers. The researchers transferred intestinal microbes from the bold NIH Swiss strain into germ-free mice of the more anxious BALB/c strain. BALB/c mice normally balk when placed on an elevated platform, taking several minutes to step over the precipice. And yet, three weeks after receiving NIH Swiss gut bacteria, the BALB/c mice were quicker to take the plunge. When the bold NIH Swiss mice received BALB/c gut bacteria, they hesitated about three times longer than normal before stepping down (5).

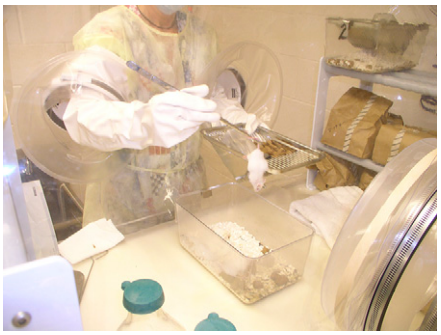
Cryan and colleagues have tried another approach: feeding mice different types of bacteria. The researchers found that two strains, *Bifidobacterium longum* 1714 and *Bifidobacterium breve* 1205, both reduced anxiety-related responses (6), although each exerted slightly different effects. *B. breve* 1205 emboldened the animals to explore open, exposed parts of a maze, and *B. longum* 1714 suppressed spikes in body temperature that normally occur in response to stress.

Deepening the brain–microbiome mystery, all of these effects—whether triggered with probiotics, fecal transplants, or the lack of any actual microbiome—appear to be happening via a panoply of mechanisms (See Box 1).

A Matter of Time

Why are scientists so intent on untangling this intricate web linking bacteria, behavior, and biochemistry? One big motivation: targeting the microbiome could prove to be a powerful tool for treating mental health problems, in part because of when the microbiome develops.

Increasing evidence suggests that there are limited, crucial windows for intervening in many mental health disorders: autism, for example, arises from problems in early brain development, and schizophrenia may arise, at least in part, due to defects in brain maturation during adolescence and early adulthood. Meanwhile, there appear to be critical periods when microbes influence neural circuits related to stress and anxiety. This could turn out to be more than a coincidence; if the microbiome proves to play a key role during



Germ-free mice, which are born without a microbiome and raised in sterile isolators, could prove a crucial tool in understanding the role of bacteria in cognitive function. Image courtesy of Caroline Westwater (Medical University of South Carolina, Gnotobiotic Animal Core, Charleston, SC).

sensitive stages of brain development, its potential as a therapeutic target would be greatly magnified.

Already there's evidence that bacteria may influence early brain wiring. Researchers have found that gut bacteria can reverse some behavioral abnormalities in germ-free mice, but only when they administered treatment before 10 weeks of age (7). Sudo et al. showed that the elevated stress hormone response could be reversed by colonizing the guts of germ-free mice at 6 weeks of age, but not during adulthood at 14 weeks (2). The results suggest that the presence or absence of bacteria shapes how neural circuits wire up to control stress and anxiety. But those circuits, once established incorrectly in germ-free mice, could be difficult to modify with bacterial interventions later in life.

Looking to probe the earliest stages of microbiome formation, neuroscientist Tracy Bale at the University of Pennsylvania in Philadelphia has been investigating stress-related changes in the maternal vaginal microbiome, which seeds the baby's initial microbiome. In unpublished experiments, Bale has found that mice that are stressed early in pregnancy show reduced *Lactobacillus* in their vaginas, and their pups exhibit the same changes in their guts. In their brains, the offspring have decreased availability of several amino acids related to neural signaling. Although maternal stress can affect the fetus and newborn through many channels, Bale is starting to look for mechanisms that could link the bacterial and the neural changes. She plans to attempt to mitigate the neural defects by treating pups from stressed mothers with vaginal bacteria from nonstressed mothers.

Ultimately, if Bale and other researchers can establish causal links between a mother's vaginal microbiome and her baby's brain, they could find new ways to address developmental problems early on in humans, when the brain circuits involved are most malleable. "If you have a mom who experienced stress during pregnancy, you could look at the vaginal microbiome, see that it looks to be troublesome, and know that it is going to be an issue for the developing brain," says Bale.

Missing Links

Bridging the rather vast gap between animal studies and human treatments will be the next big challenge. Already, researchers are attempting to uncover clues. And already, they have some tantalizing leads.

In one influential autism study, California Institute of Technology microbiologist Sarkis Mazmanian and his colleagues modeled the disorder in mice by injecting pregnant animals with a chemical that mimicked a viral infection. The resulting offspring showed autism-like symptoms, such as increased anxiety-like behaviors, decreased social tendencies, and abnormal, repetitive movements. The mice also had leaky guts, which have been reported in some studies of humans with autism (see Box 1). Feeding the mice a particular strain of the human gut microbe, *Bacteroides fragilis*, repaired their guts and ameliorated the behavioral symptoms (8, 9).

Of course, such animal evidence has its limits. "We don't kid ourselves thinking these mouse models truly have autism," says Mazmanian. To move toward results that could apply more closely to humans, Mazmanian's group is preparing to test whether fecal transplants from people with autism into otherwise healthy, germ-free mice can reproduce autism-related symptoms. If so, he hopes to use these mice to test hypotheses about the mechanisms by which bacteria contribute to the development of autism, as well as test potential interventions.

Although no one yet knows whether gut bacteria can actually be turned into psychiatric therapies, it does appear as though certain strains of bacteria or combinations of strains could influence human brains and behavior. One experiment had 55 healthy volunteers drink a probiotic mix of *Lactobacillus helveticus* R0052 and *B. longum* R0175 for one month. In psychological tests, participants showed significantly greater reductions in depression, anger, and hostility, compared with placebo-treated volunteers (10).

Such results are intriguing. But with the work still in its early stages, many scientists caution against overhyping the promise of probiotics. "There's very little evidence that currently available probiotics have any real health benefits," says Mazmanian.

To begin with, commercially available probiotics often contain a mix of bacterial species. And those species can include an unspecified and unregulated mix of strains, each of which can affect the body differently. Some strains of *Escherichia coli* can cause serious illness, for example, whereas many strains are harmless or even beneficial.

"Most bacteria will not have any positive effect on behavior. It doesn't mean they'll be negative, but they won't do anything," says Cryan. "Understanding what's specific about the bacteria that do positive things, what they produce, what's on their cell walls—we need to figure that all out."

These are some of the many unknowns when it comes to understanding how the microbiome might affect the brain. But if researchers are able to resolve even a portion of them, the brain–microbiome connection could shift from the "latest kid on the block," in the words of NIMH's Insel, to a powerful formula for helping to treat mental illness.

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