

More than a gut feeling

John F. Cryan addressed the Annual Conference of the British Psychological Society's Psychobiology Section

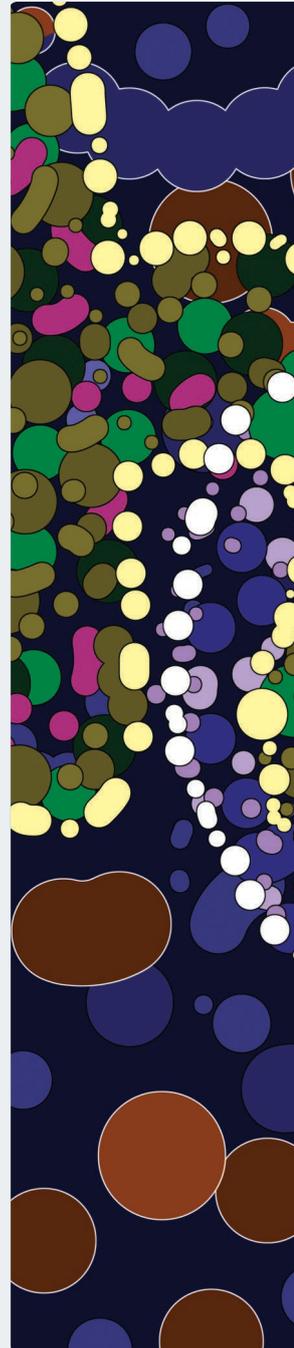
We are living in a microbial world. The microbes were there first, and in terms of genes we are more than 99 per cent microbial. For me as a neuroscientist, it's humbling to think that the weight of our gut microbes is about the same as our brain. In terms of cells we're 1.3:1 microbial (next time you go to the bathroom and get rid of some of your microbes, just think: you're becoming more human).

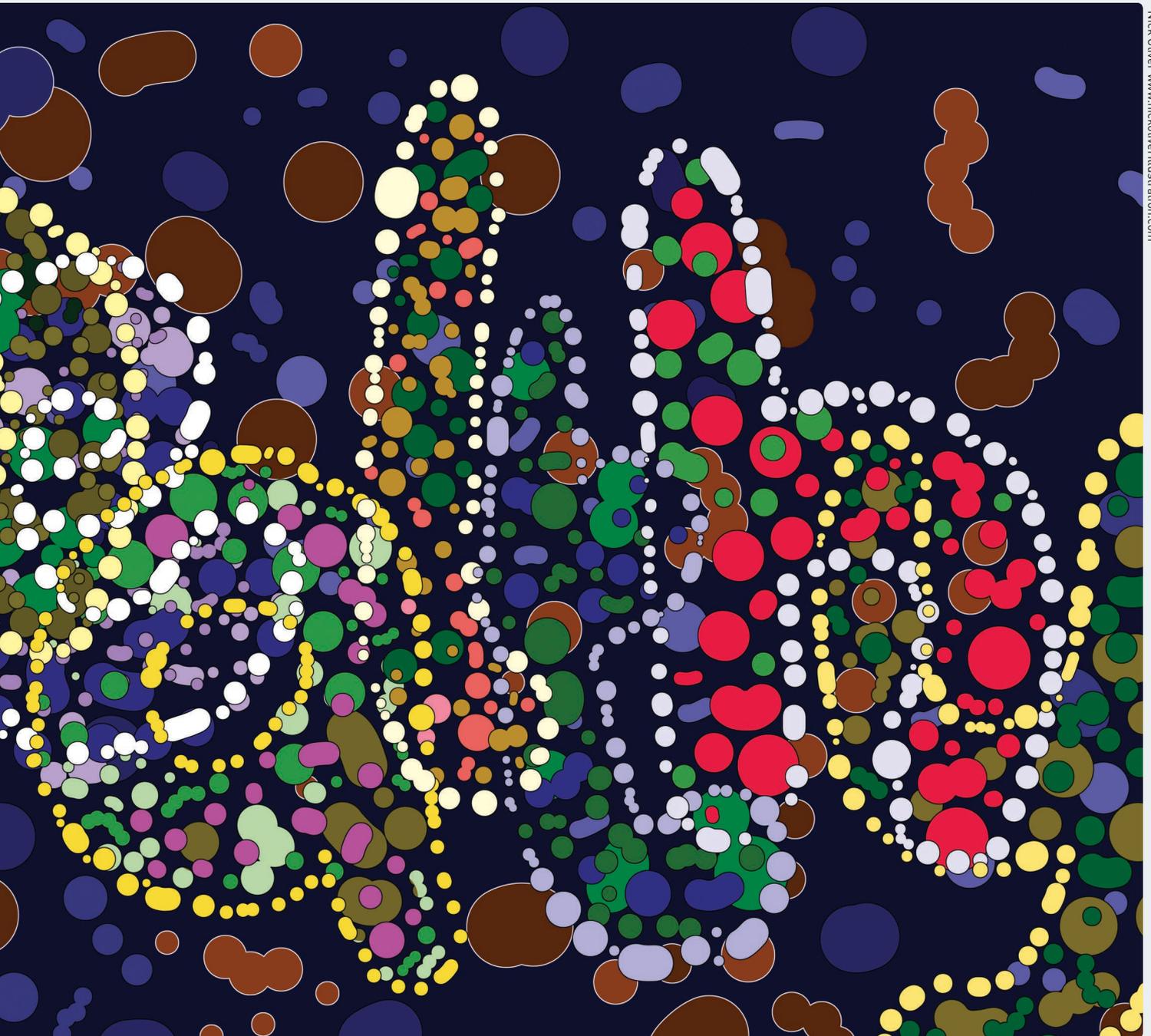
Remember the story of Pinocchio? The puppet has many adventures, but his creator Geppetto is rarely far away, guiding him along. This parallels the relationships that our brain has with our gut microbes: Who is really in charge?

We personify our emotions in our gut. We have gut feelings, gut instincts, we make gutsy moves, we are gutted, we have butterflies in our tummies. Over the last 13 years, my lab in Cork, in close collaboration with my clinical colleague Ted Dinan, has been trying to understand the overarching biology that may link these everyday phrases.

To begin that journey, I could take you right back to Hippocrates. But I'm going to start in rural Michigan in the 1840s. Here we meet a famous army surgeon, William Beaumont – an inquisitive clinician/scientist, fascinated by how digestion worked. One day he came across a Canadian fur trader, Alexis St Martin, who had received a gunshot wound to his abdomen. Beaumont saved his life, but St Martin was left with a hole, a fistula. He was now a human guinea pig: Beaumont could actually see what was going on in the digestive tract, withdraw juices, see what affected rates of digestion. Ethics committees weren't what they are now: St Martin was in effect his slave for many years. Beaumont's became the classic text in gastroenterology. He wrote that when St Martin would, understandably, become a bit irritable or angry, it affected the rate of digestion. The emotional state was affecting the gut: we have a gut-brain axis. With the advent of brain imaging we could see the reciprocal nature of this relationship: distension of the gut will activate key brain areas involved in emotion.

Yet this is not a simple and predictable relationship. Hans Selye, the father of stress research, said: 'It's not stress that kills us, it is our reaction to it.' Why, on this rollercoaster of life, will two people exposed to the same stressors respond differently? We know that genetics is important, and the growing field of epigenetics is also important. But we are also interested in how the gut microbiome could be charging these pathways, towards susceptibility or resilience. Stress doesn't just affect a few neurons in the hippocampus. We're talking about a whole-body syndrome: it affects





our immune system, and how the immune system talks to the brain. It affects gut barrier function, driving a pro-inflammatory phenotype. We need a holistic viewpoint on what stress is doing.

Like all disorders in medicine, we need animal models to assess mechanisms underpinning all this, so I work a lot with rodent models of stress. We have to put our hands up and acknowledge the limitations of our models; we can't just put our rats and mice on the couch and ask them about their childhoods. But many of the core circuits underpinning these disorders have

been evolutionarily conserved, such as the fear circuits of the amygdala, the reward pathways, etc. We can use these rodents to tell us something about the human condition and to get to some of the mechanisms.

We're particularly interested in stress at key times across the lifespan, such as in the perinatal period. We have worked on a well-known animal model of early stress, the maternal separated rat model. This is based around clear human data showing that adverse life events in childhood are a predisposing factor for many psychiatric disorders, but also for disorders



Germ-free mice

In science, one of the easiest ways to study something is to take it out and see what happens. The concept of being germ-free has captured the imagination for a long time: Louis Pasteur wrote about it, and I have a sci-fi magazine from the 1920s featuring a germ-free man. So we have a germ-free facility in our lab: the mice are never exposed to any bacteria, they grow up with each other and in their normal cages, but in effect in a 'bubble'.

A group in Japan showed that these animals have an exaggerated stress response, and that was more or less ignored at the time. But when we found that stress was affecting the microbiome, we thought that maybe in our germ-free animals the brain areas underlying the stress response would be out of kilter. Other groups were working on it too, and we all found the same thing: specific neurodevelopmental changes in these germ-free mice. Their brains didn't wire properly, for example in terms of neurogenesis in the hippocampus and in the morphology of the amygdala. There were also behavioural changes relevant to anxiety in particular. Curiously, the effects were much more prominent in males.

We then found something we never would have predicted: that a lot of the genes that were operating in germ-free mice were involved in myelination. That's the insulation that nerve cells require for appropriate conductance. We hear about it myelination mostly in the context of demyelinating disorders such as multiple sclerosis, but here what we found in the electron microscope was that there was hyper-myelination. That's really intriguing, because it doesn't occur that often in nature. Again, the sex-dependent effect came through: it was only evident in males. This led us once more to the world of autism, around the same time we were doing our epidemiology studies. Autism is very much comorbid with gastrointestinal symptoms. So, do our mice have behavioural problems? They are by nature very social – but not if they're germ-free. They will usually prefer a new playmate over an existing one – again, not if they're germ-free. These mice also had increased repetitive behaviour. That was telling us that for normal, appropriate behaviour in a mouse you need to have appropriate microbes in your gut.

such as irritable bowel syndrome, one of the most common gastrointestinal disorders. So rat pups are separated from their mothers, and when they grow up they have a whole-body syndrome: changes in visceral pain, neurochemistry, the stress response, gut barrier function, depression-like behavioural and cognitive changes, immune changes.

My colleague Ted Dinan and I had a PhD student, Siobhain O'Mahony, and she (thankfully) did something we tell every PhD student not to do. We advise them to focus, and she did a crazy experiment with someone down the hallway, who happened to be a microbiologist. She looked at the microbiome in animals who had been stressed in early life, and found a reduction in diversity... a signature of this early life trauma that persisted in the microbes of these animals. That could be completely epiphenomenological, but it got us thinking and set us on this path we've been on ever since. We subsequently validated some other models and it has been shown in human cohorts as well: mums with high perceived stress during pregnancy have offspring with a different microbiome. People are teasing out the mechanisms.

Beginnings

So *how* does the microbiome shape and influence behaviour across the lifespan?

For the most part we're thought to be sterile *in utero*, and we get our microbes as we emerge from the birth canal – 'frontier microbes' from our mother. It's like an evolutionary relay race, and we are handed the baton at birth. During pregnancy, a mum's microbiome changes so that it is optimal for this handover at the right time. These microbes inform the developing immune system and are important for gut health.

What happens if you bypass this handover, due to C-section delivery? There are now 14 studies to show that your microbiome will be different. We were intrigued by that, because it's well established from epidemiological studies that infants born by C-section have an increased relative risk of allergies, asthma, type 1 diabetes. But we know less about the relationship between mode of delivery and psychological outcomes.

The first step was to do a systematic review, and we found an increased relative risk of autism of 23 per cent in children born by C-section. That got a lot of headlines, mainly for the wrong reasons. There are health warnings to be associated with this type of analysis: many of these studies were old, and 'relative risk' gets lost in translation. We're going from 10 in 1000 to 12 in 1000, so it's not causing an epidemic in autism. But it got us thinking, and we found in our animal model that these animals grow up and have increased anxiety, and an elevated stress response.

So we took a cohort of healthy volunteers, stratified them by mode of delivery, and stressed them in the lab. We saw a significant increase in stress response to an acute stressor amongst those born by C-section, and then in a later study we saw an increase in the response

to examination stress. These people are mid-20s, C-section happened a long time ago and many other things could have happened in between, but it was very interesting to us. Back in epidemiology, we looked at everyone born in Stockholm from 1970 onwards: 2.7 million people. Again using autism as a read out, we again found a 20 per cent increase in relative risk. This looked like a huge public health concern, particularly with C-section rates going up. In places like Brazil you have 70 per cent C-section in certain provinces. In China it can be 60 per cent. In Ireland rates have doubled in 30 years. If people knew, for elective C-section in particular, that there might be long-term effects...

But what I learned from working with epidemiologists is that they love to show non-causality. They interrogated the data, and with a sibling design analysis the whole association fell apart. It tells us the association is due to confounding factors; I'm trying to work out what those factors could be, but it does seem that whatever is driving C-section is perhaps also what's driving the increased risk of autism.

That doesn't mean that C-section is off the hook, and we started looking at it in other disorders like ADHD, psychosis. We still think that by shifting and disturbing the gut microbes we could be leaving enduring effects. But C-section is a life-saving procedure, so what can we do? Is there anything we can offer to prevent or reverse this misfortune? Can we put in pre- or probiotics, or use other strategies, to prevent these effects? People are even doing vaginal swabs, and anointing infants in that way, to try to reverse it.

A brain under construction

What microbes are there is one thing, but it's what they are doing that's really important. They're little factories, producing all kinds of weird and wonderful chemicals that our bodies wouldn't produce without them. One of the most intriguing examples of this is in human breast milk. It has a higher complexity of sugars, by about 20-fold, than any other mammalian system. These sugars cannot be broken down by the infant, but they are totally broken down by the microbes. This is probably the best example of co-evolution... if you don't have the microbes you can't extract the good nourishment from the sugars. The chemicals that these sugars make include sciatic acid, which is crucial for



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brain development. We know the cognitive effects of breastfeeding on IQ and various other aspects, and it's largely thought to be due to the sugars.

What about adolescence, another vulnerable time? It's a brain under construction. Basic neuroscience is excited about neuronal and glial interactions, pruning and long-term changes. We know that a lot of psychological disorders begin to emerge in this period. Any time you have change, you have the capacity for things to go wrong. A lot of focus is on trying

to understand the impact of a range of insults: alcohol, stress, poor nutrition, lack of sleep, drugs. We need to also understand what these are doing to the adolescent gut and gut microbes, and how they can then talk to the brain. That could also play a role in the trajectory towards these disorders.

It's early days, and there's little work going on with

human adolescents. We've been showing that if we deplete microbes in animals during the adolescent period we see changes in anxiety, changes in memory, changes in social memory. In the context of alcohol we've been working with colleagues in the NIH, looking at the impact of vaporised alcohol on the microbiome – an increase in bacteria that are seen in inflammatory conditions. With colleagues in Lausanne we've been

looking at dopamine receptors in the striatum, linking that to microbial competition in an animal model of alcohol seeking.

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Ageing

Élie Metchnikoff won the Nobel Prize in 1908. All scientists, later in their careers, start coming up with crazy ideas, and Metchnikoff was full of them. One was around why people in some parts of rural Bulgaria lived longer. He noted that they ate a lot of fermented foods, containing lactic acid bacteria. Metchnikoff has been more or less forgotten about for 70–80 years (although in Korea, you can get a yoghurt drink with Metchnikoff's face on it: with that Nobel seal of approval, it must be good for you!).

During the ageing process, the brain goes into an inflammatory state, and stress will really add fuel to that fire. Our studies showed recently that in a group of around 180 elderly people, health outcomes – frailty in particular – correlated with the diversity of their microbiome. We went one step further to show that it was diversity of diet that was driving it. These findings are reinforcing Metchnikoff's writings: that the secret

to healthy ageing may lie in the gut. We've revisited these ideas in our animal studies, and we're now trying to delve into it to see if we could reverse the effects of ageing by changing the diversity of the microbiome by nutritional provision or even transplants.

Changing the microbiome

Could we modulate the microbiome to attenuate the effects of stress? It turns out that most strains of bacteria will do diddly-squat to behaviour. It's important to work out what the ones that do affect anxiety and other aspects of behaviour have that others don't.

We've also been working on prebiotics, showing that if we pre-treat stressed mice with saccharides we're able to restore certain bacteria, and functional behaviour. The anxiety and depressive behaviour is attenuated. So again through a dietary intervention in an animal we are able to see that the effects of chronic stress are reduced.

The question remains how, and we're only beginning to tease that apart. We know that the immune system plays a role, but I'll draw your attention to the short-chain fatty acids. These are

really important products that we wouldn't have in our bodies without microbes. They support gut health, immune health, and we wanted to see if they support aspects of brain health and stress response. In an animal study where we bypassed the microbes and gave metabolites, we found that chronic stress-induced changes in anxiety and the animal version of cortisol were significantly attenuated when we fed these animals short-chain fatty acids.

The vagus nerve is also an important pathway for gut-brain signalling. In an animal study some years ago we collaborated with John Bienenstock's group in McMaster University in Canada to show that all of the effects of a specific lactobacillus were absent when the vagus was severed. So this means that 'what happens in vagus doesn't stay in vagus' but can affect our emotions.

What happens if you transplant a microbiome? We found that in people with resistant major depression, there is a reduction in the diversity of the microbiome. Then we took that microbiome and transplanted it to a rat, and much to our surprise we were able to emulate many of the core symptoms of depression in the rodent. They developed anxiety, anhedonia, increased inflammation, changes in triptan levels... things you wouldn't expect compared to controls. Again this helps us move away from correlation and towards causation.

Of course, no introduction to the microbiome is complete without mentioning faecal transplants. They are now used in every Western gastrointestinal medicine centre, as a last resort in treating *C. diff.* – it has a 90 per cent efficacy rate in what can be a deadly condition. The idea goes right back to ancient China, where Ge Hong called it 'yellow soup'. It's challenging our view of what medicine is. People are innovating, finding new ways to deliver it – including the 'capsule'.

Towards a 'psychobiotic revolution'

The field now needs to move more and more towards humans, with targeted interventions of the microbiome to support brain health. My clinical colleague Ted Dinan has coined the word *psychobiotic* for such interventions. It's early days, but we're starting to get the data. In work with Andrew Allen, we took healthy volunteers and gave them probiotics over a month, along with cognitive testing and EEG. Remarkably, we found that when we stressed them, those that had taken the psychobiotic had an attenuated behavioural response as well as a distinct EEG signature. And in another study, a fermented milk drink containing four or five different bacterial strains was able to dampen down an emotional network in the brain. It's very gratifying to see work from animal models translate.

Key questions...

...for a psychology of the brain–gut–microbiome axis (Allen, Dinan, Clarke and Cryan, 2017, in *Social and Personality Psychology Compass*)

Cognitive psychology

How does the composition and function of the microbiota impact upon cognitive performance?

Can the neurotransmitters produced by the gut microbiota impact upon stress and cognitive performance, and through what mechanism, if they cannot cross the blood–brain barrier?

How do visceral factors associated with the gastrointestinal tract impact upon cognitive function?

Social and cultural psychology

How does the composition and function of the microbiota impact upon social behaviour?

Does social interaction impact upon the microbiota?

How does culture interact with the presentation and treatment of disorders of the brain–gut–microbiota axis?

Clinical psychology

How is the composition and function of the microbiota altered under conditions of psychological disorder?

Can interventions designed to target psychological wellbeing alter the microbiota?

Can interventions that ameliorate dysregulation of the microbiota improve psychological wellbeing?

How do functional gastrointestinal disorders interact with cognition, emotion and stress?

Yet when we took our best bacteria, those that had the best results and that we know were working through the important vagus pathway, and used a similar design, you could not have seen more negative data. This highlights some of the challenges that we have in translating animal work to humans; this is common to all aspects of biopsychology.

We will continue to follow the data. It's a journey that has taken me to weird and wonderful places that I never expected to go to. In the field in rural Tanzania, investigators have looked at the microbiome of hunter gatherers, and found that they have a very diverse microbiome. We can chart what agriculture as a process has done to the microbiome by looking at parts of rural Venezuela and Malawi, and see that we start to lose part of our microbiome. Then we look at our diets, our stress, our antibiotic exposure... we have extinguished microbes that our ancestors had. That's a critical part of trying to understand where we are. We need to feed our microbes in order to feed our brain.

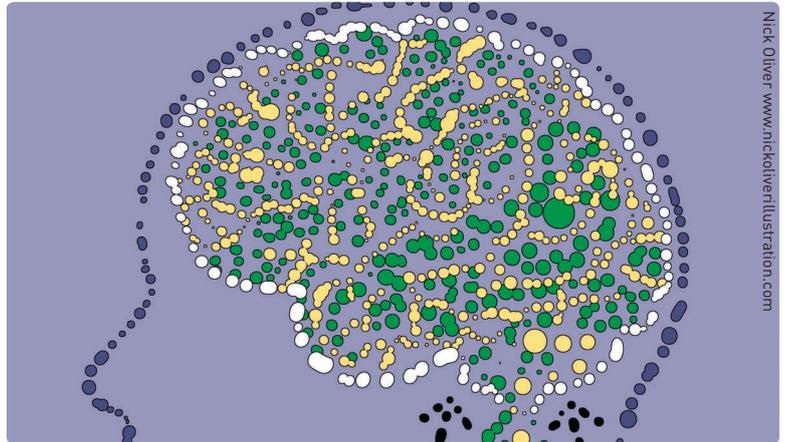
A role for psychologists

Remember Pinocchio? It's a story of who is in control. Often the person who is really in control is the partner of the person who thinks they are in control. We've been so focused on the brain, but perhaps it is the microbiome pulling the strings.

I'm hugely enthusiastic about this area, but also wary of the 'hype cycle' (see box). A lot of what we do is reinventing the wheel: if you had the *British Journal of Psychiatry* from 1910, you would have read about the treatment of melancholia with lactic acid bacteria. We still have too many small, underpowered studies, many without good dietary information, or good psychological or psychiatric phenotyping. That's why this is interdisciplinary work, and there's an important role for psychologists moving forward (see box, 'Key questions'). There's a whole interesting area around mental health and the microbiome of the built environment; how where we live and work influences our psychological wellbeing.

It's also worth noting that when we talk about the microbiome we're often just talking about the bacteriome. We haven't even scratched the surface in understanding the relationship between archaea, viruses, bacteriophage, the fungi... all of these make up our microbiome. And it was the microbial evolutionary biologist Seth Bordenstein who reminded me that these microbes were there first. We've never existed without them. Our brains have never evolved without any microbial signals. It's a co-evolution, which gives us a different perspective.

The 20th century was all about killing germs, saving lives. I still talk to older doctors and they can't get the germ idea out of their heads. But we're starting to appreciate the role that a healthy gut plays in a healthy brain: perhaps we're now living in the psychobiotic century.



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Hope or hype?

It's not an overstatement to say that the microbiome-to-brain concept has been a complete paradigm shift in neuroscience and biological psychiatry. I'm hugely enthusiastic about it, but I'm equally wary of where we are on the 'hype cycle'. It's still early days, and caution is needed in over-interpreting studies. Any field where there are more review articles than primary papers requires careful consideration. There are many open questions...

To what extent can animal studies be translated to complex human behaviour, if at all? More interventional studies are needed with probiotic strains, prebiotics and even potentially faecal microbiota transplants. These will be important for the field to move away from correlative analysis towards causative and potentially therapeutic approaches. Most of the studies to date have been in relatively healthy cohorts. Can any of these studies translate to clinical populations?

What is a normal, healthy microbiome? There's not an accepted definition, and inter-individual differences in microbiome composition can be vast. This makes a 'one size fits all' approach to targeting the microbiome challenging. It also, though, offers opportunities – the microbiome may be the conduit for effective personalised medicine approaches in the future.

Is there anything that the microbiome isn't involved in? I've often been asked this by sceptical colleagues. The answer is probably no, since there has never been at time in evolutionary history where the brain existed without signals from the microbiome.

Do we have the right computational/biostatistical tools? Microbiome science is the epitome of big data. Most measures reflect relative abundance and can be made at different levels of granularity from phylum down to strain level, thus there are many ways to report alterations. Moreover, there are constraints on all of the currently used tools used to analyse such data; however, new bioinformatic pipelines and algorithms are being generated at a great pace.

Is it what's there or what it's doing? We must get a better understanding of what the microbiome is doing in terms of metabolites generated and interactions with the host. What are the mechanisms of communication? Despite much research this is still a very open question. The field must take advantage of recent technical advances in neuroscience to map circuits that mediate the effects of microbes from the periphery to the brainstem and from the brainstem to corticolimbic structures that underpin complex human behaviours.