A life without microbes: How “germ-free” research is revealing the necessity of bacteria for our brains

Jenna Herbert  
(University of Oxford)

The Science Communication Competition is now in its eighth year. As in previous years, it aims to find young talented science writers and give them the opportunity to have their work published in *The Biochemist*. In 2015, a new branch of the competition was launched to include video entries. Overall this year’s competition attracted 74 entries and these were reviewed by our external panel of expert judges. The second prize in the written category was awarded to Jenna Herbert from the University of Oxford, whose article is presented here; the second prize in the video category was Ellie Staniforth from the University of Glasgow. Ellie’s video can be viewed at [bit.ly/scicomm2018](http://bit.ly/scicomm2018).

David Vetter was a normal boy, mostly. He grew up in Texas, and he liked *Star Wars*. He was also born with a genetic defect that left his immune system completely non-functional. As a result, he was forced to live in a sterile isolator for his 12 short years of life, earning him the nickname ‘Bubble Boy’.

David, along with films like *The Boy in the Plastic Bubble* (1976) and *Bubble Boy* (2001), has inspired us to think about a life completely isolated from germs. Would we be healthier? Would our bodies, or our brains, function differently? Scientists have begun to answer these questions by studying rodents that spend their entire lives in sterile bubbles: ‘germ-free’ mice.

But you live in an incubator resembling the isolator the ‘bubble boy’ inhabited. Without a full complement of bacteria, perhaps it’s not surprising that you need additional nutrients in your diet and have a higher risk of infection because of an immature immune system. What may be more unexpected, however, is the abnormal way your brain has developed, leaving you stressed, defenseless and socially inept.

**Stressed out**

Imagine that you’re about to go through a stressful experience. When you were human, this might have been a speech or a job interview. But as a lab mouse, this more likely involves a researcher dropping you into a tepid pool of water without warning to perform the ‘forced swim test.’ You don’t like water, and when you are dipped in the tank, you panic and frantically swim to and fro to find an escape. Your brain secretes chemicals that signal the adrenal glands (located above the kidneys) to produce stress hormones called glucocorticoids, the most common of which in humans is cortisol. Glucocorticoids are then released into the blood and have a wide range of effects in the body and brain to prepare you for “fight or flight.”

Stress hormone levels that are too high or long-lasting, however, can be damaging, so evolution has developed a clever way to prevent this: glucocorticoids
inhibit their own production, creating a negative feedback loop so that under normal circumstances, hormone levels don’t get too elevated and return to baseline quickly.³

After five minutes in the tank, the researcher removes you from water and takes a blood sample. When the results come back, she finds that your stress hormone levels have sky-rocketed above those of a normal stressed mouse. This means you have a hyperactive stress response, and the heightened exposure to glucocorticoids is harmful for your body and immune system and, ultimately, your brain.⁴ We don’t understand why yet, but the presence of a microbiome seems to be critical for the correct development of your brain’s stress circuitry.

**A defenseless brain**

You’re not loving life as germ-free lab mouse. Not only are scientists putting you in tanks of water against your will, but your stress hormones are out of control. Now, the researchers want to test your brain’s immune response by administering a mild pathogen. In a normal brain, spider-like cells called microglia are constantly on patrol. Microglia wear many hats, serving as the brain’s janitors, architects, and soldiers. They work around the clock, clearing away dead or damaged cells. While the brain is growing and developing early in life, they also help to mold it, eliminating unnecessary wiring to construct an efficient machine.⁵ At the first sign of infection or damage, microglia spring into action. They retract their spidery legs (called dendrites), multiply, and call for help, releasing chemical signals to recruit other immune cells to the site of the attack.⁶

The researcher injects the pathogen. You actually have more microglia than a normal mouse, but they are sluggish to respond to the invasion, and they don’t release signals to other cells to help mount an attack.⁷ What’s more, the selectively-permeable barrier that normally isolates your brain from your blood has been compromised without the aid of the microbiome.⁸ Toxins are seeping into your brain, and you lack the

---

**Further reading**

- Haberman, C. (2015) ‘The Boy in the Bubble’ Moved a World He Couldn’t Touch. New York Times
- Luczynski, P., Neufeld, K.A.M.V., Oric, C., Clarke, G., Dinan, T.G. and Cryan, J.F. (2016) Growing up in a bubble: using germ-free animals to assess the influence of the gut microbiota on brain and behavior. Int. J. Neuropsychopharmacol. 19, 1–17
- Spencer, R.L. and Deak, T. (2017) A user’s guide to HPA axis research. Physiology and Behavior 178, 43–65
- Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N.X. et al. (2004) Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. 558, 263–275
- Nayak, D., Roth, T.L. and McGavern, D.B. (2014) Microglia development and function. Annu. Rev. Immunol. 32, 367–402
- Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E. et al. (2015) Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18, 965–977
- Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M. et al. (2014) The gut microbiota influences blood-brain barrier permeability in mice. Sci. Transl. Med. 6, 263ra158
- Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G., and Cryan, J.F. (2014) Microbiota is essential for social development in the mouse. Mol. Psychiatry 19, 146–148
- Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A. et al. (2011) Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. 108, 3047–3052
- Tazume, S., Umehara, K., Matsuura, H., Aikawa, H., Hashimoto, K. and Sasaki, S. (1991) Effects of germfree status and food restriction on longevity and growth of mice. Jikken Dobutsu 40, 517–522
- Rabot, S., Membrez, M., Bruneau, A., Gerard, P., Harach, T., Moser, M. et al. (2010) Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J. 24, 4948–4959
- Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., McCoy, K. D., Frisoni, G. et al. (2017) Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci. Rep. 7, 41802
- Sampson, T. R., Debelius, J. W., Thron, T., Wittung-stafshede, P., Knight, R., Mazmanian, S. K. et al. (2016) Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson’s disease. Cell 167, 1469–1480
- Arentsen, T., Raith, H., Qian, Y., Forsberg, H. and Heijtz, R. D. (2015) Host microbiota modulates development of social preference in mice. Microbial Ecology in Health & Disease 26, 1–8
- The Microbiome in Health and Disease (2017) Edited by Julian Marchesi, Emerging Topics in Life Sciences 1, (4)
proper protection of the microglia. This was just one exposure to a pathogen, but in the real world, you would be exposed to bacteria and viruses constantly without defenses, leaving your body and brain vulnerable.

**Not a social butterfly**

For one final test in the lab, you are placed in between two chambers. One is empty, and one contains another mouse. Mice are social animals, and they typically prefer a new four-legged friend over the empty compartment. You, however, are more timid than the usual mouse. You would much rather escape to the vacant chamber than sniff and explore another animal.

In a second test, you are put in the same chamber, but now you have the choice between the mouse you met earlier and a new mouse. In this task, there's no private section to escape to. The best option, you decide, is to choose what is familiar and spend more time with the old acquaintance. Perhaps you want to avoid the awkwardness of an entirely new social interaction. You don't realize that normal mice usually choose to meet new rodents with new smells. They are curious creatures that explore new things, but you favor familiarity.

Perhaps the most surprising trait under the influence of the microbiome is social behaviour. It may seem like a stretch (and perhaps a bit like science fiction) that our behaviour could be affected by bacteria. But our microbes can alter the levels of a number of chemicals in the brain, including some you may have heard of, such as serotonin and dopamine. Ultimately, it's these chemicals that determine how we think and act.

**Our microbes: small but mighty**

A life without microbes is still something we can only imagine. But based on studies in germ-free mice, it doesn't sound ideal. It's clear that the microbiome is extremely important to our brain health. We don't know how exactly the microbiome communicates with the brain, but the middlemen are likely the vagus nerve (which allows the gut and the brain to communicate with each other), by-products of bacterial digestion of fibre that can alter brain function, and interactions with immune cells.

It's important to note that although many characteristics of the germ-free brain are abnormal, being abnormal isn't always clearly a bad thing. Mice without microbes are not as anxious as normal rodents. They live longer and are more resistant to weight gain. Also, mice that have been genetically modified to have Alzheimer's- or Parkinson's-like syndromes do not show symptoms if they grow up without a microbiome. And finally, as is often the case in science, studies sometimes contradict each other. One study, for example, found that germ-free mice are actually more sociable than normal animals, the exact opposite of the findings discussed in this article.

So there isn't a nice story here about gut bacteria as the perfect symbiotic pals. What the brain and behaviour of germ-free mice can show us, however, is which aspects of our neurobiology may be influenced by our microbes. The next step will be understanding how to target our microbiomes, using dietary modifications or even faecal microbiota transplants (the transfer of good bacteria from a healthy donor to a patient), to optimize our brain health and combat disease.

---

**Shaping Your Career in Molecular Biosciences: Taking a Wider View**

**16 November 2018**

Technology and Innovation Centre, Glasgow, UK

Register at Bit.ly/shapingyourcareer2018