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*Viewpoint*

# Spotlight: An Interview with Dr. Christopher A. Lowry, on the Convergence of Microbes, Nature, and Mental Health

**Alan C. Logan [1,*] and Christopher A. Lowry [2]**

[1] Nova Institute for Health, 1407 Fleet St., Baltimore, MD 21231, USA
[2] Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO 80309, USA
* Correspondence: alanxlogan@gmail.com

**Abstract:** In the ongoing series of spotlight interviews, *Challenges* Advisory Board member and Nova Institute for Health Fellow, Alan C. Logan, meets with thought leaders, scientists, scholars, healthcare professionals, artisans, and visionaries concerned about health at scales of persons, places, and the planet. Here in this interview, Dr. Christopher A. Lowry of the University of Colorado Boulder, responds to a set of questions posed by *Challenges*. For nearly twenty years, Dr. Lowry has been at the forefront of the research connecting the microbiome to mental health. Ten years ago, Dr. Lowry and his colleagues wrote a provocative article under the title 'Can we vaccinate against depression?'; Dr. Lowry updates *Challenges* on where the field has moved, with its promises and possibilities. Dr. Lowry reflects on the early influences that shaped his interest in the field and discusses the ways in which microbiome sciences are casting light on the many interconnected challenges of our time.

**Keywords:** microbiome; stress physiology; public health; personalized medicine; community health; planetary health; health inequities; non-communicable diseases; social determinants of health; serotonin



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## 1. Introduction

*Challenges* is a unique interdisciplinary journal dedicated to integrating diverse scholarly discourse related to the Grand Challenges currently facing our societies and the planet at large. To that end, the journal is continuing its series of spotlight interviews that cut across disciplines, professions, and perspectives. In collaboration with the Nova Institute for Health, the Nova Spotlight interviews seek out individuals with remarkable experience and wisdom; they are queried on their work, experiences, and ideas, and in particular, how those ideas cut across disciplines in the promotion of health and flourishing at scales of persons, places, and the planet.

Very few topics in 21st-Century science and medicine have captured the imagination like the subject of microbes, human behavior, and mental health. At the dawn of the new millennium, the idea that non-pathogenic microbes, especially commensal gut microbes, could influence cognition, behavior, and mental health was considered outlandish, if not openly mocked [1]. One area of research that helped provide legitimacy to the idea was the realm of allergic diseases—it was becoming increasingly clear that environmental factors, including shifts in human contact with non-pathogenic microbes, influence immune programming. At the same time, researchers were uncovering connections between the immune system and cognition and behavior, opening the door to the possibility that microbes might influence mood and so much more [2,3].

One researcher who was working on this convergence of ideas at a very early point was Dr. Christopher A. Lowry, now an associate professor in the Department of Integrative Physiology at the University of Colorado Boulder. Dr. Lowry is uniquely positioned to discuss the history of this work, and with hundreds of publications in the field, including many that have made media headlines, *Challenges* was honored that Dr. Lowry agreed to be the subject of the journal's ongoing Nova Spotlight interviews.

Dr. Lowry earned his Ph.D. in 1995 at Oregon State University, Corvallis. He then moved to the United Kingdom, where he was a Postdoctoral Research Fellow at the University of Bristol's University Research Centre for Neuroendocrinology. In 2007, Dr. Lowry returned to the United States and took up a post at the University of Colorado Boulder, where he continues to educate and direct multiple studies. His work is at the intersection of psychoneuroimmunology, examining environmental factors (including microbes) and the neural mechanisms underpinning emotional behavior. Included in this work is an effort to determine if select strains of bacteria and other microbes might be used for the prevention and treatment of stress-related psychiatric disorders.

## 2. The Nova Interview

**Nova: You started your academic pathway with a Bachelor of Arts degree. Can you tell us a little bit about that and whether or not it shaped your perspectives on the pursuit of science?**

**Dr. Lowry:** That's right, I received a Bachelor of Arts degree in the Department of Zoology at the University of Wyoming. My undergraduate experience had a major impact on my perspectives on the pursuit of science, in multiple ways. For context, I grew up in Wyoming, a state with an area of 97,914 square miles (approximately the size of the United Kingdom) with, in 1987, the year I started college, a population of 510,345 people. I was a first-generation college student from small towns in rural Wyoming, so the University experience was challenging but exciting. I was very fortunate to be accepted as a freshman into the Honors and Scholars program, which meant that I was able to take Honors courses with a low student-to-professor ratio and to take a series of courses that really challenged me. Two of these courses had major impacts on my perspectives on the pursuit of science. One, *Epistemology, or Ways of Knowing*, introduced me to different perspectives on how we learn about our world, including the scientific method. Another Honors course was *Modes of Understanding*, which was team-taught by Assistant Professors Dr. Kelly T. Alberts, Philosophy, and Harley A. Thronson, Jr., Physics and Astronomy, in fall, 1985. Here, we read Thomas S. Kuhn's *The Structure of Scientific Revolutions* [4], which impacted how I think about the pursuit of science and further inspired in me an interest in the philosophy of science. I later expanded on this interest by taking a graduate course, taught by National Academy of Sciences member George N. Somero, while a graduate student at Oregon State University. In this course, I learned about Karl Popper's Falsification Principle, i.e., that for a theory to be considered scientific, it must be able to be tested and conceivably proven false, and the corollary that we cannot prove something to be true using the scientific method—because it is always possible that our assumptions are incorrect. Today, I meet with every new member of the laboratory to discuss the philosophy of science, and how that impacts our day-to-day pursuits of scientific enquiry. As part of the Honors and Scholars program, I was able to engage in undergraduate research and an Honors thesis in the laboratory of Dr. William A. Gern, where we studied the control of melatonin secretion from the trout pineal gland. There, I experienced the excitement of scientific enquiry and scientific discovery and developed an understanding of the value of a good mentor.

**Nova: In your early graduate research, you focused on stress physiology. Why were you motivated to study stress and neuroendocrine regulation of behavior?**

**Dr. Lowry:** I pursued my graduate degree, beginning in the fall of 1987, with Dr. Frank L. Moore in the Department of Zoology at Oregon State University. I was attracted to Dr. Moore's research as I had read his entire compendium of work as an undergraduate while taking a course on *Animal Behavior*, taught by Dr. David J. Duvall, and was attracted to Dr. Moore's eloquent writing and methodical use of the scientific method. Dr. Moore had established his career working on an amphibian model system, the rough-skinned newt (*Taricha granulosa*), which was a useful model for investigation of neuropeptide and steroid regulation of behavior, particularly reproductive behavior. A postdoc in the laboratory, Dr. Pierre J. Deviche, provided critical training in experimental design and research methods. My fellow graduate student, Miles Orchinik, was a great day-to-day

mentor, who published in the journal *Science* the first characterization of a membrane-bound glucocorticoid receptor [5]. However, rather than focus on reproductive behavior, I quickly gravitated toward studying a neuropeptide that had recently been characterized by Dr. Wylie W. Vale and colleagues in 1981, corticotropin-releasing factor (CRF). Three years later, in 1984, Dr. Charles B. Nemeroff, working with Dr. Vale, published a paper in *Science* showing that CRF was elevated in the cerebrospinal fluid of depressed patients [6]. It soon became apparent that CRF also played a fundamental role in the neurobiology of anxiety [7]. In 1984, my supervisor, Dr. Moore, and colleagues published two papers showing that CRF altered behavioral responses in rough-skinned newts [8,9]. During a six-week period when I visited Dr. James D. Rose at the Department of Zoology at the University of Wyoming, Dr. Rose introduced me to the power of chronic single-unit recording as a window into understanding neuropeptide control of behavior, again using the rough-skinned newt as a model system [10]. These findings captured my imagination, and I focused my attention on interactions between CRF and serotonergic systems, two systems that were linked to stress-related psychiatric disorders. To this day, I've maintained a passion for understanding stress physiology and neurobiology relevant to stress-related psychiatric disorders, including anxiety disorders, mood disorders, and trauma- and stressor-related disorders, such as posttraumatic stress disorder (PTSD).

**Nova: Do you remember the first time you ever read an article or heard a discussion of microbes as a potential factor in everyday human cognition, emotions, and behavior?**

**Dr. Lowry:** As a postdoctoral research assistant at the University Research Center for Neuroendocrinology (URCN) at the University of Bristol, United Kingdom, under the mentorship of Professor Stafford L. Lightman, I was introduced to the field of psychoneuroimmunology through the work of colleagues, like Dr. Nola M. Shanks and Dr. David S. Jessop, and the work of leaders in the field, including Drs. Hymie Anisman, Robert Dantzer, Adrian J. Dunn, Steven F. Maier, Andrew H. Miller, Carmine M. Pariante, and Linda R. Watkins. A common approach in the field then was to explore how lipopolysaccharide (LPS; endotoxin) relayed signals to the central nervous system and, thus, modified behavior. I even had the opportunity, which I graciously declined, to participate in a clinical study conducted by Professor Lightman and colleagues that involved intravenous infusions of LPS. Lipopolysaccharide is a component of the outer cell membrane of Gram-negative bacteria that binds to Toll-like receptor 4 (TLR4) to induce a strong inflammatory response in immune cells and, thus, is an important molecular component of Gram-negative bacteria that can alter behavioral responses. I was influenced by the work of Drs. Ronald P.A. Gaykema, Lisa E. Goehler, and Mark Lyte, who, as early as 2004, showed that intestinal infection with the food-borne pathogen, *Campylobacter jejuni*, rapidly activated vagal afferent and central autonomic pathways [11,12]. If *C. jejuni* could rapidly alter neural pathways in the brain and potentially behavior, wouldn't it be the case that other bacteria in the gut, or elsewhere in the body, could do the same? The point is, I was interested in how bacteria or their component parts induced peripheral immune signals that communicated with the central nervous system and altered behavior, including emotional behavior relevant to stress-related psychiatric disorders. The full significance of this work may only now be becoming clear. It has recently been shown that LPS is elevated in persons with a diagnosis of PTSD [13], and this is thought to be a result of "leaky gut", where bacteria in the gut lumen (including Gram-negative bacteria) enter the body and induce an inflammatory cascade. This inflammatory cascade in turn includes microglial priming, a process that results in an "overshooting" neuroinflammatory response to LPS exposure or future stressors that we believe is central to the persistent symptoms of stress-related psychiatric disorders, including PTSD.

My first experience attending the Psychoneuroimmunology Research Society (PNIRS) meeting was in 2009 in Breckenridge, Colorado, where I met Dr. Charles L. Raison. Since then, I have had a close collaboration with Dr. Raison, who is a thought leader in understanding the role of inflammation in the etiology and pathophysiology of depression. My first exposure to the idea that components of bacteria could modulate human behavior

would have been a Viewpoint article published by Robert Dantzer and colleagues in 1992, when I was still in graduate school. The article discussed the idea that 'sickness behavior', consisting of anorexia, depressed activity, hypersomnia, inability to concentrate, hypersomnia, listlessness, malaise, weakness, and loss of interest in usual activities, including social contacts, are part of a natural homeostatic reaction that the body uses to fight infection [14].

We initiated our own studies of the mechanisms through which the soil-derived bacterium, *Mycobacterium vaccae* NCTC 11659, communicated with the central nervous system in the late 1990s, first presenting our data showing the intratracheal administration of *M. vaccae* NCTC 11659 into the lungs of mice activated afferent vagal pathways and brain serotonergic systems at the Society for Neuroscience meeting in 2000 in New Orleans, Louisiana [15,16]. Even though we were studying a whole cell heat-killed preparation of *M. vaccae* NCTC 11659, it was only much later that we began to think of *M. vaccae* NCTC 11659 as part of a larger ecosystem of bacteria, i.e., the mouse or human microbiota, associated with the potential to modulate everyday human cognition, emotions, and behavior. The recent identification of a hidden 'mycobacteriome' of the human healthy oral cavity and upper respiratory tract [17], as well as the fact that mycobacteria are the most abundant bacterial taxa in municipal water supplies [18], make this a likely possibility. Instead, we were simply interested in how peripheral immune signals were related to the central nervous system, including afferent vagal and "sympathetic afferents", i.e., afferent fibers that travel from the airways within sympathetic nerve bundles to lamina I of the spinal cord, in turn giving rise to spinoparabrachial and spinothalamic pathways that relay signals to the central nervous system, including brain regions involved in cognitive and affective function. We published this work, showing that peripheral immune stimulation with *M. vaccae* NCTC 11659 activates brain serotonergic systems and induces antidepressant-like behavioral responses in 2007 [19]. However, there is no mention of "microbiome" or "microbiota" in that paper, even though we administered *M. vaccae* NCTC 11659 to the mucosal surfaces of the airways.

So, it's hard to recall a precise moment when I first read a particular article or heard a specific discussion of microbes as a potential factor in behavior. For me, it was layering diverse pieces of information together—a logical progression from the early views of Dantzer and colleagues on 'sickness behavior' in the early 1990s, when I was still in graduate school, to our own studies of *M. vaccae* NCTC 11659 in the late 1990s. I do recall, however, that my own thinking was clearly influenced by a Phase III clinical trial conducted by Mary O'Brien and colleagues at the Royal Marsden Hospital, assessing *M. vaccae* NCTC 11659 as a treatment in persons with a diagnosis of advanced non-small-cell lung carcinoma, published in 2004 [20]. Although treatment with *M. vaccae* NCTC 11659 didn't prolong life in this study, it unexpectedly increased overall Global Health Status, physical functioning, role limited due to emotional health, cognitive functioning, and vitality, while decreasing pain. We thought that, clearly, these effects would be dependent on the transmission of signals from the periphery (*M. vaccae* NCTC 11659 was injected intradermally in this Phase III clinical trial) to the central nervous system. Thinking of our work in psychoneuroimmunology in the context of the human microbiome was clearly influenced by Rob Knight, who was then at the University of Colorado Boulder. Indeed, our first analysis of the gut microbiome in the context of stress resilience effects of *M. vaccae* NCTC 11659 was done under the mentorship of Rob Knight and his team and was published in 2016 [21].

In 2013, I read a series of three papers on "Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances" [1,22,23], where I learned much more about the history of investigation into the microbiome and mental health. These papers further solidified my view that the gut microbiota was an important piece of the puzzle in terms of understanding the etiology and pathophysiology of stress-related psychiatric disorders.

**Nova: In 2008, you coauthored a landmark paper, 'The hygiene hypothesis and psychiatric disorders', with Dr. Graham A.W. Rook [24]. At the time it was increasingly recognized that the immune system might be involved in depression. You deepened**

**that discussion by bringing microbial exposure into the picture. Your paper was at least two years before international researchers began to take the gut microbe-brain-mental health connection seriously. What were the basic threads you and Dr. Rook were tying together?**

**Dr. Lowry:** It's impossible to overestimate the influence that Professor Graham A. W. Rook at University College London had on my thinking about the relationship between microbial exposures and mental health. By June 1999, we were collaborating with Professor Rook to evaluate the effects of *M. vaccae* NCTC 11659 on afferent signaling from the airways to the central nervous system, focusing initially on catecholaminergic systems in the nucleus of the solitary tract (the initial target of afferent vagal fibers relaying signals from the periphery to the central nervous system). Thus, we had nearly a decade in which to formulate the ideas put forward in the coauthored paper in 2008 [24]. Professor David P. Strachan first put forward the hygiene hypothesis in 1989 [25]. Five years later, Rook and colleagues put forward the 'old friends' hypothesis, reformulating the hygiene hypothesis, i.e., "This paper provides an overview of relevant work in each of these fields of medicine (though with emphasis on the allergic disorders), and concludes that the increasing failure of Treg is a consequence of diminished exposure to certain micro-organisms that are "old friends", because of their continuous presence throughout mammalian evolution." [26]. Key to the "old friends" hypothesis was the observation that certain environmental microorganisms, including *M. vaccae* NCTC 11659, have the ability to induce regulatory T cells (Treg), which produce anti-inflammatory cytokines, including interleukin (IL) 10 and transforming growth factor beta (TGFβ). The effectiveness of *M. vaccae* NCTC 11659 in this context had already been documented in a *Nature Medicine* paper by Rook and colleagues in 2002 [27]. Later, but before our 2008 paper on 'The hygiene hypothesis and psychiatric disorders', we published an article showing that the intratracheal or subcutaneous administration of *M. vaccae* NCTC 11659 activated a subset of serotonergic neurons in the dorsal raphe nucleus that we thought confers antidepressant effects (an evolutionarily conserved, hard-wired antidepressant neural system, if you will), which is located in the interfascicular part of the dorsal raphe nucleus (DRI) and projects to forebrain structures implicated in the pathophysiology of major depressive disorder, including the anterior cingulate cortex and hippocampus. The paper in *Trends in Immunology* was our first attempt (of many) to link the hygiene hypothesis to mental health.

The 2008 paper set in motion an effort to identify an animal model of stress exposure in which negative outcomes were dependent, in part, on the exaggeration of inflammation. The reasoning was, if *M. vaccae* NCTC 11659 works by limiting inappropriate inflammation (as already demonstrated in the context of allergic airway inflammation) [27], then immunizing with *M. vaccae* NCTC 11659 (even weeks before stress exposure) should set in motion an immunoregulatory response that would limit stress-induced inflammatory responses and, in turn, limit negative outcomes of stress exposure. In 2007, at the *Ninth Symposium on Catecholamines and Other Neurotransmitters in Stress*, a meeting organized by Richard Kvetnanský at Smolenice Castle, Bratislava, Slovakia, I had the good fortune of seeing a presentation by a postdoctoral researcher from the University of Regensburg, Germany, Dr. Stefan O. Reber. At this meeting, he described a model of chronic psychosocial stress in mice that resulted in glucocorticoid insensitivity, spontaneous colitis (inflammation of the colon), exaggerated chemically induced inflammation in a model of inflammatory bowel disease (IBD), exaggerated secretion of proinflammatory cytokines from freshly isolated mesenteric lymph node cells stimulated with anti-CD3 antibody ex vivo, and the development of a chronic anxiety-like state. It seemed immediately apparent that Dr. Reber's model was an ideal model to test the ideas put forward in the 2008 paper with Professor Rook, and by September 2010, we were planning the first experiments to test the efficacy of immunizations with *M. vaccae* NCTC 11659 to prevent negative outcomes of chronic psychosocial stress, work that was ultimately published in 2016 [21].

This work came full circle when Professor Reber and colleagues, Professor Rook, and I collaborated to test the "old friends" hypothesis in the context of risk factors for

stress-related psychiatric disorders. In this study, Professor Reber and colleagues recruited 20 young men who were raised for the first 15 years of life on farms with farm animals. They also recruited a second group of 20 young men who were raised for the first 15 years of life in a city of over 100,000 people, without daily exposure to pets. Both groups were then brought into the clinic and exposed to the Trier Social Stress Test (TSST), a model of acute psychosocial stress in humans. Analysis revealed that persons who grew up in cities without daily exposure to pets, and thus lacked exposure to diverse microbial environments during childhood, responded to psychosocial stress with exaggerated increases in circulating peripheral blood mononuclear cells (PBMCs), exaggerated increases in circulating interleukin 6 (IL-6), a proinflammatory cytokine, and exaggerated IL-6 secretion from isolated PBMCs stimulated ex vivo [28].

The 'Old Friends' hypothesis continues to be a useful hypothetical framework in which to understand the role of exposures to diverse microbial environments in the prevention of inflammatory conditions, including stress-related psychiatric disorders, in which inflammation is a risk factor, as outlined in a book that Professor Rook and I co-edited this year [29].

**Nova: Did you ever experience any skepticism in response to that early idea that environmental microbes could influence mental health?**

**Dr. Lowry:** Indeed, our paper was ultimately published in *Neuroscience* in 2007, showing that immunization with *M. vaccae* NCTC 11659, a soil-derived bacterium with anti-inflammatory and immunoregulatory properties, activated a subset of brain serotonergic neurons and induced antidepressant-like behavioral responses, was initially submitted to Nature in 2001 and 2005, then *Proceedings of the National Academy of Sciences* (*PNAS*) in January 2006, then *Biological Psychiatry* in February 2006, then *Journal of Neuroscience* in September 2006, then *Neuroscience* in 2006, where it was finally published in 2007 [19]. Clearly, we thought that the work was impactful and important, but had difficulty convincing reviewers and editors of this. However, just after online publication on 23 March 2007, on 1 April 2007, the University of Bristol issued a press release with the whimsical title, *Getting dirty may lift your mood* [30], which captured the public's imagination. In the press release, I included a quote, "This research makes us wonder if we shouldn't spend more time playing in the dirt.", which was carefully crafted to encourage discussion, without overstating conclusions from our findings. This resulted in a cascade of live radio interviews with the BBC, newspaper articles, and magazine articles that continues, albeit with decreased frequency, to this day. The idea that injections of a bacterium isolated from soil could activate serotonergic neurons in the brain and induce antidepressant-like behavioral responses in mice seemed so unorthodox at the time that interviews frequently began with the question, "So, is this an April fool's joke?", since the press release was issued on 1 April 2007.

A common question was, "should I spend more time outdoors in nature or more time gardening". At the time it was difficult to answer this question, but, since then, innovative researchers have demonstrated that exposure to nature can increase the diversity of the human microbiome and enhance immunoregulation. Meanwhile, the link between nature exposure and well-being is well established [31]. Roslund and colleagues have shown that exposing children at daycare centers to diverse microbial environments, by introducing elements of boreal forests or sand enriched with microbially diverse soil into the outdoor play environment, increases the abundance of mycobacteria in the environment [32], while increasing Treg, and increases anti-inflammatory cytokines, such as IL-10 and TGFβ, in children [33,34]. Surprisingly, when adults engage in soil-mixing activities for ten minutes with soil that is 'spiked' with *M. vaccae* ATCC 15483, there is a rapid alteration in brain activity within the occipital cortex and alteration in the plasma metabolome, relative to soil that is not spiked with *M. vaccae* ATCC 15483 [35]; this suggests that exposures to mycobacteria not only have long-term immunoregulatory effects but also alter physiology and neurophysiology within minutes. Perhaps we all really should spend more time playing in the dirt.

**Nova: The backstory of a six-year journey to get the *M. vaccae* paper published is remarkable. It shows that you were bumping up against a dismissive scientific culture that was manifest in peer review and journal rejections. The idea just wasn't ready for prime time. Four years later, in 2012, you and Dr. Rook published an article in Drug Discovery Today with the provocative title 'Can we vaccinate against depression?' [36]. At this point, four years after your initial paper, had the research evolved enough to justify that title?**

**Dr. Lowry:** By October 2011, when the paper was first submitted, we already knew that immunization with *M. vaccae* NCTC 11659 had anti-inflammatory and stress resilience effects in the chronic subordinate colony housing model being conducted in collaboration with Dr. Stefan Reber, and in fact had already begun writing the manuscript, ultimately published in *PNAS* in 2016 [21]. Thus, we were confident in our reasoning that immunization with *M. vaccae* NCTC 11659 had the potential for stress resilience or antidepressant effects in humans. The title of the 2012 article, 'Can we vaccinate against depression?', was actually proposed by the editors of *Drug Discovery Today*, and we did not alter the title. However, the subtitle, "*Can we use microorganisms to prevent or treat depression*?" is probably a more accurate reflection of our thinking at the time. We typically do not use the term 'vaccinate' or 'vaccination' in the context of our work with *M. vaccae* NCTC 11659 as the lay understanding of 'vaccination' is an immunization that targets a pathogen or pathogens (e.g., the SARS-CoV-2 virus that causes COVID-19 is a recent example). Although it's possible that true 'vaccines' could be developed for the treatment of major depressive disorder (MDD) that is secondary to infection (for a scholarly discussion of this issue, see Garay [37]), our approach is more accurately described as an immunoregulatory approach, or, more generally, an immunomodulatory approach, as the immunizations are designed to modulate how immune cells respond to future immune stimulation (by pathogenic or non-pathogenic organisms or their components), in an effort to prevent inappropriate inflammation and the downstream sequelae, including increased risk of anxiety disorders, mood disorders, and trauma- and stressor-related disorders, such as PTSD.

Having said that, *M. vaccae* NCTC 11659 is under development as a bona fide vaccine for the treatment of tuberculosis, which is caused by the bacterium, *Mycobacterium tuberculosis* (for review, see [38]). Since recent estimates are that 24.8% of the world's population has latent tuberculosis infection (LTBI) [39], which constitutes an enormous source of potential active tuberculosis, and given evidence for the bidirectional association between tuberculosis infection and stress-related psychiatric disorders such as depression [40,41], we should not exclude the possibility that *M. vaccae* NCTC 11659 could be useful both as a bona fide vaccine (against stress-related psychiatric disorders associated with mycobacterial disease) and as an anti-inflammatory and immunoregulatory agent.

**Nova: So, here we are a decade after your suggestion that we might be able to immunize against depression. Do you think we are actually closer to immunizing against mental health disorders in general, and anxiety and depression in particular?**

**Dr. Lowry:** In 2013, Dr. Lisa A. Brenner, Ph.D., Director, VA Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Vice Chair of Research, Department of Physical Medicine and Rehabilitation, and Professor of Physical Medicine and Rehabilitation, Psychiatry, and Neurology at the University of Colorado Anschutz Medical Campus visited the University of Colorado Boulder to give a seminar in our Neuroscience seminar series. We discussed our research on *M. vaccae* NCTC 11659, the gut microbiome, and mental health. Dr. Brenner was intrigued by our research findings, and we initiated a collaboration to investigate the gut microbiome and mental health in United States Veterans with a diagnosis of PTSD. In 2016, we were awarded an Office of Research and Development (VA-ORD) Rehabilitation Research and Development (RR&D) Small Projects in Rehabilitation Research (SPiRE) (I21) (1 I21 RX002232-01) to assess the feasibility, acceptability, and safety of an eight-week probiotic intervention, as well as to begin the process of evaluating potential biological outcomes, in United States Veterans with a diagnosis of PTSD and mild traumatic brain injury. Although the study was not

powered to evaluate biological outcomes, we found a strong trend for the eight-week intervention, *Lactobacillus reuteri* DSM 17938, to reduce plasma concentrations of C-reactive protein, a biomarker of inflammation [42]. This is of interest as multiple studies have now identified baseline C-reactive protein as a biomarker of risk for the future development of PTSD [43,44], and our data support future large-scale, randomized controlled trials aimed at measuring both biological and clinical outcomes.

More recently, we were awarded an R01 grant from the National Center for Complementary and Integrative Health (NCCIH) at the National Institutes of Health to evaluate a different probiotic, *Lactobacillus rhamnosus* GG (1R01AT010005; ClinicalTrials.gov Identifier: NCT04150380) in US Veterans with a diagnosis of PTSD in a Phase 2 double-blind, placebo-controlled, randomized controlled trial. This study is ongoing.

Obviously, we need to conduct clinical trials with *M. vaccae* NCTC 11659. At the moment, we are hampered by logistical challenges related to obtaining heat-killed preparations of *M. vaccae* NCTC 11659, prepared in compliance with Good Manufacturing Practice (GMP) that is suitable for intradermal injection (which is how *M. vaccae* NCTC 11659 has been administered in previous clinical trials). We are working toward this goal and remain optimistic that with the proper funding these clinical trials can be conducted in the near future.

**Nova: You have spent a significant portion of your career examining neurotransmitters and the ways in which serotonergic pathways might contribute, in very complex ways, to mood, fear, and mental health. What do you make of the recent headlines claiming that the idea that serotonin is involved in depression has been "debunked"? Many of these headlines have been spurred on by a well-publicized article in *Molecular Psychiatry*, a detailed review that casts doubt on the antidepressant properties of medications being the result of modifying serotonin availability and neurotransmission [45].**

**Dr. Lowry:** I view this recent systematic umbrella review by Moncrieff and colleagues [45] as part of an important dialogue in psychiatry and recognize that this article has resulted in considerable debate. I have not weighed in on this debate yet, so you are really asking me to go out on a limb here! Although I would not propose any flaws in their analysis, I do not agree with their overall conclusions. This is because I think that, at the outset, the authors' assumptions were incorrect. The systematic umbrella review identified systematic reviews, meta-analyses, and large data-set analyses in the areas of "serotonin and serotonin metabolite, 5-HIAA, concentrations in body fluids; serotonin 5-$HT_{1A}$ receptor binding; serotonin transporter (SERT) levels measured by imaging or at post-mortem; tryptophan depletion studies; SERT gene associations and SERT gene-environment interactions." I would consider each of these measures to have insufficient resolution to determine whether or not serotonin has a role in the etiology or pathophysiology of MDD, or whether or not selective-serotonin reuptake inhibitors (SSRIs) have value in the treatment of MDD, or how SSRIs actually function in the context of treatment of MDD. I would also argue that looking at the role of serotonin in the etiology, pathophysiology, and treatment of MDD through these lenses had already been determined to be problematic. One of the best examples that comes to mind is a study by Dr. Murray Esler and colleagues at the Baker Heart and Diabetes Institute in Melbourne, Australia. Dr. Esler and his colleagues inserted jugular cannulae into human volunteers and measured brain serotonin turnover [46]. The key here is that, opposed to studies reviewed by Moncrieff and colleagues, Esler and colleagues were measuring *brain* serotonin turnover, which is more relevant than simply measuring serotonin and serotonin metabolite (5-hydroxyindoleacetic acid; HIAA) concentrations in body fluids. Esler and colleagues found that depressed persons had *increased* brain serotonin turnover relative to healthy controls. Furthermore, they found that, following SSRI therapy, brain serotonin turnover was substantially reduced [46]. Thus, the study by Esler and colleagues would suggest that brain serotonin signaling is elevated in persons with MDD and that successful treatment with SSRIs restores brain serotonin signaling to normal levels. I was not surprised by this finding, and, in fact, it fit well with my understanding at the time of the role of serotonergic systems in the etiology, pathophysiology, and treatment

of MDD (more about this below). Although the paper by Esler and colleagues has been cited 236 times, it probably has not received the attention it deserves.

The reason I suggested that the assumptions in the study by Moncrieff and colleagues were incorrect is that there was an assumption that concentrations of serotonin or serotonin metabolites in plasma (or other bodily fluids) can tell us something meaningful about the role of serotonin in MDD. Furthermore, while studies of brain serotonin turnover as done by Esler and colleagues are likely more relevant, this measure still does not have sufficient resolution to reliably inform us about the role of serotonin in the etiology, pathophysiology, or treatment of MDD. This is because evidence suggests that brain serotonergic systems are not one monolithic system, but a conglomeration of anatomically and functionally distinct subsets of serotonergic neurons, each of which is likely to play a different role in the symptoms of MDD.

In the late 1990s, working with colleagues at the University of Bristol, United Kingdom, I conducted studies that laid the groundwork for the hypothesis that brainstem serotonergic systems consist of highly topographically, hodologically, and functionally distinct subpopulations of serotonergic neurons. This thesis was first published in a paper in the *Journal of Neuroscience* in 2000, which has now been cited over 250 times. In the discussion, we stated that "In marked contrast to previous hypotheses for serotonergic function, the present study suggests that topographically organized subpopulations of serotonergic neurons may be dedicated to particular functions associated with stress responses, including behavioral sensitization and behavioral adaptation to previous stress" [47]. This resulted in a phone call from Dr. Barry L. Jacobs, then a professor of psychology and neuroscience at Princeton University, who had argued that "It is important to emphasize that stressors do activate the serotonergic system, but that they do so no more than other non-stressful, activating conditions." [48]. Dr. Jacobs argued that perhaps our hypotheses related to serotonergic systems and the control of behavior were not so different, but I maintained that our hypotheses were indeed different, based in part on our unpublished work at the time suggesting that different subsets of serotonergic systems were associated with the control of anxiety-like defensive behavioral responses, panic-like physiological and behavioral responses, and antidepressant-like behavioral responses, each with direct relevance to the etiology and pathophysiology of stress-related psychiatric disorders. This was really a paradigm shift in how we viewed brainstem serotonergic systems and the control of physiology and behavior, and, today, it is common for researchers, either using single cell or subregional analysis approaches, to design studies in a way that will advance our understanding of the anatomical and functional heterogeneity of brainstem serotonergic systems, rather than a single monolithic serotonergic system.

This theoretical framework played a central role in the 2008 paper by Rook and Lowry, 'The hygiene hypothesis and psychiatric disorders', mentioned above [19]. In this paper, we noted that evidence supported the hypothesis that a subset of serotonergic neurons in the dorsal part of the caudal dorsal raphe nucleus (DRD) is involved in the facilitation of anxiety-related responses. Since MDD is highly comorbid with anxiety, perhaps this system contributed to the elevated brain serotonin turnover in MDD observed by Esler and colleagues (see, for example, Sugiyama et al. [49]). In marked contrast, we noted that *M. vaccae* NCTC 11659, the soil-derived bacterium with anti-inflammatory, immunoregulatory, and stress resilience properties, very selectively activated a subset of serotonergic neurons in the interfascicular part of the dorsal raphe nucleus (DRI), which had the potential for the prevention and treatment of MDD. The idea that DRI serotonergic neurons are responsible for antidepressant effects has not been thoroughly or rigorously tested. However, this hypothesis did lead to a novel potential antidepressant therapy, infrared whole-body heating (WBH).

I recall standing in the University of Bristol library before I left Bristol in 2007, pulling an old volume of *Brain Research* from an upper shelf, dusting it off, and turning to a figure within the text. I was *very excited* to see a demonstration of heat-sensitive neurons that were highly concentrated in the DRI region of the brainstem raphe nucleus. The reasoning

was that, if our hypothesis was correct that activation of DRI serotonergic neurons was a mediator of antidepressant effects, then heat, in addition to immune stimulation using *M. vaccae* NCTC 11659, should also have antidepressant effects. Testing this hypothesis seemed possible a year later when I met Dr. Charles Raison, who had a shared interest in thermoregulation and MDD, at the PNIRS meeting in 2009 in Breckenridge, Colorado. A year later, at the 2010 PNIRS meeting at Trinity College Dublin, Ireland, we continued our open-label ions and planned preclinical validation studies and an open-label clinical trial of infrared WBH for the treatment of MDD. We published our study showing that WBH activated DRI serotonergic neurons in rats in 2011 [50] and published an open-label clinical trial showing the large effect size antidepressant effects of WBH in persons with a diagnosis of MDD in 2013 [51]. We followed this with a double-blind, sham-controlled, randomized controlled trial that showed similar large effect size antidepressant effects of WBH in 2016 [52]. The latter study demonstrated that the antidepressant effects of WBH are long-lasting, persisting for at least six weeks after a single WBH session.

In contrast to the highly publicized claim by the former director of the National Institutes of Mental Health (NIMH), Thomas Insel, that advances in neuroscience since the "Decade of the Brain" in the 1990s have yet to benefit patients [53], the studies on WBH may be an example of a novel intervention, based on basic neuroscience research, that has benefited, or at least has great potential to benefit, patients. Whether or not the activation of DRI serotonergic neurons, as observed with *M. vaccae* NCTC 11659, other peripheral immune stimuli [54], WBH, cold exposure [55], and exercise [56], continues to emerge as a biomarker of antidepressant effects remains to be thoroughly and rigorously tested.

Why would these types of stimuli result in the activation of DRI serotonergic neurons and the promotion of proactive emotional coping responses/antidepressant effects? I am an advocate of evolutionary reasoning (think, *Why We Get Sick: The New Science of Darwinian Medicine*, by George C. Williams and Randolph M. Nesse [57]) and Darwinian medicine in general. Depression is a state of increased default mode network (DMN) connectivity [58,59], which is often associated with introspective thought, including introspective negative thought in depressed persons. Shifting attention to engagement with the external environment results in a shift away from DMN activity and toward neural systems associated with goal-directed behavior [60]. There are some forms of environmental stimulation that would have demanded a shift away from introspective thought and activity of the DMN toward engagement with the environment and the activation of neural systems involved in goal-directed behavior. Failure to do so would be highly maladaptive. For example, when exposed to extremely high heat, it is adaptive to engage in behavioral thermoregulation to induce thermoregulatory cooling and to maintain body temperature in a homeostatic range. Failure to do so in extreme environments is a threat to reproduction and survival. Thus, there may be afferent neural systems that have evolved through millennia that relay salient peripheral stimuli to brain networks that promote motivated behavior, engagement with the environment, and survival.

**Nova: Critics of reductionist research, especially in mental health, often claim that the work does little to address the underlying inequities and socioeconomic predictors of non-communicable diseases. Do you think that microbiome research can actually illuminate upstream socioeconomic drivers of mental disorders and even lack of flourishing?**

**Dr. Lowry:** I would address this at two levels, which are related to each other. The first is emerging evidence that diversity of the gut microbiota (specifically a form of diversity referred to as alpha diversity) is an important feature for optimizing stress resilience. The second is the importance of pathobionts in the gut microbiome, which can wreak havoc under some conditions:

### *2.1. On the Importance of Alpha Diversity of the Human Microbiome*

First, we should define our goal, which should be "health". A classic definition of health, put forward by the World Health Organization on 7 April 1948, is "a state of

complete physical, mental and social well-being and not merely the absence of disease or infirmity." As you alluded to, noncommunicable diseases are increasing in modern urban societies. This increase was particularly alarming during the fifty-year period from 1950 to 2000. Shortly thereafter, in 2002, Jean-François Bach published an article in the *New England Journal of Medicine* reporting a striking increase in the incidence of immune disorders in the fifty-year period ending in 2000 [61]. Included were increases in autoimmune disorders, including Crohn's disease (a form of inflammatory bowel disease (IBD)), multiple sclerosis, and type 1 diabetes, as well as asthma, citing data from contemporary original research articles [62–65]. These historical trends are in alignment with a gradient of the incidence of asthma and atopy based on rural versus urban living, with, for example, the Amish (who maintain traditional farming practices, including the use of large animals for farm work) having the lowest prevalence, Swiss farmers (who have adopted modern farming practices) having intermediate levels of prevalence, and Swiss non-farmers having the highest prevalence [66].

As outlined previously by Rook and colleagues, this increase in inflammatory conditions [26,29,57–70], and stress-related psychiatric disorders, in which inflammation is a risk factor [24,71–73], is thought to be driven by a loss of exposure to diverse microbial exposures during early development but also throughout the lifespan. The 'old friends" hypothesis identifies three categories of microorganisms that can induce immunoregulation, and thus limit inappropriate inflammation (1) the commensal microbiota, including the gut microbiota, which have been altered by the Western lifestyle, including a diet that is commonly low in microbiota-accessible carbohydrates [74,75]; (2) pathogens associated with the "old infections" that were present throughout life in evolving human hunter-gatherer populations [76]; and (3) organisms from the natural environment with which humans were inevitably in daily contact with (and, consequently, had to be tolerated by the immune system) [68].

Next, I'll comment on the gut microbiota, and address your question, "Do you think that microbiome research can actually illuminate upstream socioeconomic drivers of mental disorders, and even lack of flourishing?" Independent of the arguments put forward by Rook and colleagues (above), there is increasing consensus that diversity of the gut microbiota is a key feature of a 'healthy microbiome'. For example, at a meeting of the North American branch of the ILSI North America's conference, "Can a Healthy Gut Microbiome Be Defined through Quantifiable Characteristics?" held on 17 December 2018, in Washington, DC, the authors of the consensus statement concluded that " . . . diversity is likely more important than the presence of specific taxa", and that " . . . Ecosystem functions are probably more important than specific individual members (which may even show functional redundancy)." [77]. If we assume for a moment that this is true, then a main objective to promote health, regardless of socioeconomic status, is to promote a diverse gut microbial ecosystem in all persons. How best do we achieve that? One approach that now has a lot of empirical research supporting it is a diet that includes the intake of a diversity of plants. The relationship between the number of different plants that a person eats in a given week and the alpha diversity of the gut microbiome was first described by Rob Knight and colleagues using data from the American Gut Project. The authors reported that "The self-reported dietary data suggested, unexpectedly, that the number of unique plant species that a subject consumes is associated with microbial diversity, rather than self-reported categories such as "vegan" or "omnivore" (Figure 2C,D)" [78].

This has subsequently been replicated in a number of studies showing that diversity of plant intake is one of the most important features contributing to higher alpha diversity of the gut microbiota [79,80]. This makes sense, as each healthy plant consumed has its own microbiome [81–83]. Even a three to four-leaf spinach plant has over 800 different bacterial species in its microbiome [84]. These are called endophytes and exist inside the plant (i.e., you can't wash them off). Thus, eating thirty plants a week may introduce upwards of 30,000 different bacterial species/strains to the gut microbiome of a healthy person.

While it's not possible to attribute mental health benefits to the diversity of plant intake and associated increases in alpha diversity of the gut microbiota, meta-analysis has revealed that whole dietary interventions have been shown to significantly reduce depressive symptoms [85]. These findings have led to the development of a new field in psychiatry, i.e., Nutritional Psychiatry. While different studies have used different whole dietary interventions, common features of dietary interventions that increase mental health outcomes " . . . reduce the intake of "junk" foods (e.g., high-fat, high-sugar discretionary foods and takeaways), while replacing these with high-fiber, nutrient-dense alternatives, such as vegetables" [85].

Although the question is framed in the context of the gut microbiota, we should not forget other microbiota, including the skin microbiota, oral microbiota, and microbiota of the upper airways. Exposure to diverse microbial environments has been shown to increase skin microbial diversity, in concert with increased immunoregulation [32–34].

### *2.2. On the Importance of Pathobionts*

One consequence of low alpha diversity is that it provides an opportunity for pathobionts to proliferate and, under some conditions, drive negative health outcomes for the host. The term 'pathobiont' has been proposed to describe resident microorganisms with pathogenic potential [86,87]. As an example of how pathobionts may influence health outcomes, including mental health outcomes, let's consider our own studies using the chronic subordinate colony housing model in mice. In this model, chronic psychosocial stress induces a three order of magnitude increase in the relative abundance of *Helicobacter* species [88]. Stress-induced increases in *Helicobacter* are thought to be due to the stress-induced secretion of glucocorticoid hormones, acting on glucocorticoid receptors [21]. In our model, stress-induced increases in *Helicobacter* spp. were associated with stress-induced decreases in alpha diversity [88]. However, the host's capacity for immunoregulation (i.e., to respond with a balanced expression of Treg and effector T cells that drive inflammatory responses), seems to determine the health outcomes. For example, mice that lack the anti-inflammatory cytokine IL-10 respond to inoculation with *Helicobacter* spp. with spontaneous colitis [89,90]. When mice are exposed to the chronic subordinate colony housing model in a specified pathogen-free environment, in the absence of *Helicobacter* spp., typical negative consequences of chronic psychosocial stress are absent [88,91,92]. Importantly, however, even in the presence of *Helicobacter* spp., prior immunization with *M. vaccae* NCTC 11659 prevents the stress-induced exaggeration of secretion of proinflammatory cytokines from freshly isolated mesenteric lymph node cells stimulated with anti-CD3 antibody ex vivo and dramatically increases the secretion of the anti-inflammatory cytokine IL-10. Immunization with *M. vaccae* NCTC 11659, while it does not prevent stress-induced increases in the relative abundance of *Helicobacter* spp., prevents the stress-induced exaggeration of colitis in a model of inflammatory bowel disease and prevents stress-induced increases in anxiety-like defensive behavioral responses [88].

Together, these data suggest that the presence of *Helicobacter* spp. in the context of a healthy gut microbiota, i.e., in the context of high diversity and adequate immunoregulation, does not result in negative outcomes. However, in marked contrast, the presence of *Helicobacter* spp. in the context of an unhealthy gut microbiota, i.e., in the context of low diversity and inadequate immunoregulation, does result in negative outcomes.

Back to your question, this suggests that individuals with low socioeconomic backgrounds, often associated with poor access to healthy, fresh foods, including a diet with diverse fresh fruits and vegetables, may be at higher risk for stress-induced increases in the relative abundance of pathobionts, low gut microbiome diversity, and consequently, negative health outcomes, including negative mental health outcomes (i.e., low stress resilience).

Until recently, I believed that *Helicobacter* spp. was not a major concern in modern urban societies, due to the well-documented eradication of *Helicobacter* in high-income countries [93,94]. However, there is an important detail that requires more attention, i.e., in a cross-sectional analysis in Canada, "Ethnic minorities accounted for 42% of the *H. pylori*-

positive patients." Low socioeconomic status is also a factor [95,96]. Eradicating *H. pylori* in communities with low socioeconomic status is proving to be challenging [97,98].

Thus, multiple factors seem to tip the balance toward negative health outcomes in communities with low socioeconomic status: (1) poor access to healthy, fresh foods, including a diet with diverse fresh fruits and vegetables, nuts, seeds, and fish; (2) low gut microbiome diversity; and (3) higher risk for stress-induced increases in the relative abundance of pathobionts, including *Helicobacter* spp. Thus, in line with, but perhaps expanding, our previous conclusions [72,99], this convergence of factors has the potential to increase the risk of negative health outcomes, including mental health outcomes in persons in low socioeconomic positions.

**Nova: Psychology has come a long way since the Rorschach test. Can you see a day where we will have better ways to screen children and adults for various mental health vulnerabilities using biological markers and perhaps oral and gut microbiome markers?**

**Dr. Lowry:** We have recently engaged in efforts to develop better ways to screen children and adults for various mental health vulnerabilities using biological markers and perhaps oral and gut microbiome markers. This effort was led by Peter Templeton at The William Templeton Foundation for Young People's Mental Health, in collaboration with the University of Cambridge's knowledge transfer company Institute for Manufacturing (IfM) Engage. The main aims of the project were to: (1) identify the systems that cause depression; and (2) use this understanding as the basis for identifying opportunities to intervene early to interrupt the biological dysfunctions that can cause depression. The findings from this effort are available online in the report, *Changing Hearts, Changing Minds, Evidence-based approaches to the prevention, diagnosis and treatment of depression in young people*. [100]. The next phase of this project, which is designed to innovate preventative and early interventions for depression in young people: *A game-changing approach to depression in young people*, is ongoing.

**Nova: In 1988, OMNI magazine asked well-known personalities, some in science and medicine, about their own utopian thinking, or the world they would like to live in. Contemporary research on utopian thinking indicates that it can be a healthy process, increasing both personal and social hope, yielding an abstract mindset that bridges the psychological distance between the status quo ("here and now") and a better possible future: What type of world would you like to live in?**

**Dr. Lowry:** This is a great question. Given the focus of this article, I'd start with the universal recognition that each of us, human beings, is an ecosystem, and that our health (i.e., "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity") depends on maintaining a healthy ecosystem in and on our bodies. This would extend to the universal recognition that maintaining a healthy ecosystem in and on our bodies, in turn, depends on nurturing diverse and healthy natural ecosystems that allow us to live and thrive. Our socioeconomic systems would be designed to optimize, particularly in children, access to lifestyle and diet factors that have been shown to correlate most strongly with diverse microbiomes, and thus resilience, including access to nature (Table 1)—excluding caffeinated beverages and alcohol, for children, of course. Our policy decisions would be based on the universal recognition that we need to maintain healthy air, water, soil, and natural ecosystems.

In a paper published in 1986, Fujimuri demonstrated that within one hour of inoculating the ileum of rabbits with the mycobacterium, *M. bovis* bacillus Calmette-Guérin (BCG), the bacteria are found adhering specifically to specialized cells called microfold cells (M cells) associated with Peyer's patches, inside these cells, in the intercellular space between the M cells and adjacent columnar epithelial cells, withing macrophages enfolded by M cells, and in macrophages further away [101]. Fujimuri explained his perception that "Electron microscopically in 1 h post inoculated specimens, the bacteria were found adhering specifically to M cells, and the microfolds of the M cells were seen to stretch like tentacles toward the bacteria to catch them." Since seeing this article and Fujimuri's amazing photomicrographs, I've often wondered, who benefits here? Is this simply a

bacterium that has evolved to enter mammalian systems, using the M cell as a portal into the fantastical world of the mammalian body, or are the mammalian systems "sampling" the environment as a way of optimizing host physiology to environmental conditions at that moment in time? Is this a true symbiosis?

**Table 1.** Lifestyle and diet factors associated with gut microbiome diversity.

| Lifestyle or diet factor [68,69] |
| --- |
| Raw vegetables (frequency per week) [69]; vegetables [68] |
| Vigorous activity (duration) [69] |
| Vigorous activity (frequency per week) [69] |
| Oil-rich fish (frequency per week) [69]; fish [68] |
| Caffeinated beverages (frequency per week) [69]; |
| tea/coffee [68] |
| Cruciferous vegetables (frequency per week) [69] |
| Walk or bike [69] |
| Vegetables (frequency per week) [69] |
| Alcohol (frequency per week); |
| Fruits (frequency per week) [69]; fruit [68] |

Over time, I've come to view bacteria on a spectrum of "safety signals" to "danger signals". Bacteria acting as "safety signals" include those that induce anti-inflammatory and immunoregulatory effects, decrease anxiety and fear, and promote prosocial behavior, reproduction, and growth. Think, for example, Susan Erdman's discovery that *Lactobacillus reuteri* MM4-1A (ATCC PTA 6475) sustains youthful serum testosterone concentrations and testicular size in aging mice [102], increases oxytocin while decreasing plasma concentrations of corticosterone, a stress hormone [103], and induces thick lustrous skin and hair, while enhancing reproductive fitness [104]. Further studies highlight a potential role in the prevention of age-related bone loss [105]. Yet, further studies demonstrate that the same strain has protective effects in multiple mouse models of endophenotypes relevant to autism spectrum disorder, including the promotion of social behavior [106,107]. Mammalian encounters with such bacteria with anti-inflammatory and immunoregulatory properties might be most frequent in environments where resources are abundant, as reflected in the abundant availability of a diversity of fruits and vegetables, nuts, seeds, and perhaps, occasionally, fish. Foraging in a boreal forest, short grass prairie, or long grass prairie, savanna, or rain forest, drinking from pristine streams and lakes, i.e., exposure to healthy, diverse, natural ecosystems, would increase exposures to these types of bacteria, promote a sense of "safety", and provide a physiological signal that this is a good time to invest in social behavior, reproduction, and growth.

In contrast, bacteria acting as "danger signals" include those that induce inflammation and pose a risk of infection, increase anxiety, fear, and social isolation, and divert resources away from reproduction and growth and toward immune function to fight off potential pathogens. Mammalian encounters with such bacteria with proinflammatory and pathogenic potential might be most frequent in environments where resources are scarce, as reflected in the low availability of a diversity of fruits and vegetables, nuts, seeds, and fish. One might be limited to the consumption of poor-quality food and water, including rotting vegetables or meat. Exposure to resource-poor, unhealthy ecosystems, would increase exposures to these types of bacteria, promote a sense of "danger", and provide a physiological signal that this is not a good time to invest in social behavior, reproduction, and growth. These conditions might also lead to competition for limited resources.

Where are we in modern urban environments? What is clear is that there is an increasing prevalence of non-communicable, inflammatory conditions in modern urban environments. Individuals raised in urban environments in the absence of daily contact with pets respond to psychosocial stress with an exaggerated and prolonged inflammatory response. A number of hypotheses have been put forward to explain the increasing incidence and prevalence of non-communicable diseases in modern urban societies. These

include the hygiene hypothesis [25,108], the "Farm Effect" [109], the biodiversity hypothesis [75,110], the disappearing microbiota hypothesis [111,112], and the "Old Friends" hypothesis [113]. What all of these hypotheses share, however, is that they propose, in one manner or another, that reduced exposures to diverse microbial ecosystems are responsible for increases in noncommunicable diseases in modern urban environments. Are reduced exposures to diverse microbial ecosystems also impacting human social behavior?

Every summer, Officer Marc Hodges, Denton Police Department, and the Denton Police Youth Summer Program and School Resource Officers lead a summer program for "at-risk" youth aimed at positively impacting the participants' lives and teaching them civic responsibility, leadership, and teamwork. Denton is a city in Texas within the Dallas-Fort Worth metro area. This summer, while I was in Rocky Mountain National Park, I ran into this team, who had brought a group of "at-risk" youths to the park for a week-long immersive experience in nature. Three of the youths had never been out of the inner city. In my Utopian world, everyone would have a shared appreciation of the importance of preserving diverse natural ecosystems, and everyone would have the opportunity to benefit from that diversity.

> "In wildness is the preservation of the world,"
>
> Henry David Thoreau, "Walking"

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