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From conifers to cognition: microbes, brain, and behavior

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Richard Lathe[1],* and David St. Clair[2],*

[1]Division of Infection Medicine, University of Edinburgh Medical School, Little France, Edinburgh, UK
[2]Institute of Medical Sciences, School of Medicine, University of Aberdeen, Aberdeen, UK

*Correspondence: richard.lathe@ed.ac.uk; d.stclair@abdn.ac.uk

Abstract

A diversity of bacteria, protozoans, and viruses ('endozoites') were recently uncovered within healthy tissues including the human brain. By contrast, it was already recognized a century ago that healthy plants tissues contain abundant endogenous microbes ('endophytes'). Taking endophytes as an informative precedent, we overview the nature, prevalence, and role of endozoites in mammalian tissues, centrally focusing on the brain, concluding that endozoites are ubiquitous in diverse tissues. These passengers often remain subclinical, but they are not silent. We address their routes of entry, mechanisms of persistence, tissue specificity, and potential to cause long-term behavioral changes and/or immunosuppression in mammals, where rabies virus is the exemplar. We extend the discussion to Herpesviridae, Coronaviridae, and *Toxoplasma*, as well as to diverse bacteria and yeasts, and debate the advantages and disadvantages that endozoite infection might afford to the host and to the ecosystem. We provide a clinical perspective in which endozoites are implicated in neurodegenerative disease, anxiety/depression, and schizophrenia. We conclude that endozoites are instrumental in the delicate balance between health and disease, including age-related brain disease, and that endozoites have played an important role in the evolution of brain function and human behavior.

MICROBES ARE UBIQUITOUS

Botanists a century ago were puzzled to discover fungal cells within healthy tissues of the Canadian spruce tree, *Picea glauca* (formerly *Picea canadensis*), and also in *Larix laricina*, the tamarack or American larch [1]. Although it was known that plant roots have a symbiotic relationship with external soil microorganisms, particularly nitrogen-fixing bacteria and mycorrhizal fungi, the surprise was to find live microbes ('endophytes'; Box 1) within normal healthy tissues of a live tree.

Emerging evidence, 100 years later, now argues that, exactly as in plants, healthy (and diseased) mammalian tissues, including the brain, harbor a multiplicity of endogenous passenger organisms ('endozoites', Box 1) that may have both detrimental and beneficial effects on their hosts. Building on findings in plants, this overview

summarizes current evidence and raises questions about the broader roles that endozoites play in human health, disease, and evolution, as well as in behavior, the central focus of this review,

THE MICROBIOME: ENDOPHYTES AND ENDOZOITES

Multicellular organisms across the tree of life – from plants to animals – are outnumbered by the constellation of microorganisms to which they give sanctuary. The microbiome – defined as the ensemble of organisms that are intimately associated with a host species – encompasses the multitude of bacteria, protozoans, and viruses that cohabit/live in close association with the host.

In plants, where the microbiota constitutes a 'hidden world' ([2] for an excellent review), the root has been a central target for studies on microbe–host symbiosis. Plant growth is crucially dependent on endogenous nutrient-assimilating and nitrogen-fixing bacteria (e.g., [3,4]). Moreover, endophytes have been reported to provide additional benefits. For example, root-colonizing *Pseudomonas* spp. produce antifungal molecules that protect the root against fungal pathogens [5]. Some plants favor colonization by insect-killing microbes (entomopathogens), including fungi and bacteria, because their presence can confer resistance to insect attack [6]. Other endophytes alter the metabolism of the host to generate defense molecules that in turn can fend off herbivore attack or reduce infection by other plant pathogens [7].

Paralleling the role of root microorganisms in plants, the microbiome in the gut of vertebrates promotes the metabolism and uptake of external nutrients from the diet [8], can have beneficial effects on the immune system [9], and can exert direct modulatory effects on brain and behavior (see below). Commonalities between root and gut, including a large surface area and a key role in nutrient assimilation, have been highlighted previously [10,11]. However, as in plants, other healthy tissues in vertebrates also contain diverse types of microbes.

Endozoites Are Widely Distributed in Healthy Tissues

In plants, endophytes are found in association with all tissues analyzed to date, and this might lead us to suspect that endozoites might also be present in multiple body tissues of vertebrates. In human, outside the intestine, attention has been paid to colonization of gastric mucosa by *Helicobacter pylori* that has also been associated with the initiation of gastric ulceration [12], but most recent work has been carried out on epithelial surfaces that are a rich repository of bacterial species (e.g., the Human Microbiome Project [13]). Bacterial species are widely reported in blood of healthy individuals (reviewed in [14]. In another study an overt pathogen, *Streptococcus pneumoniae*, was found in the nasopharyngea of 4% of adults but in 53% of children [15], blurring the distinction between endozoite and pathogen.

Diverse viruses are also present on external surfaces, including the naso-olfactory system and mouth [16], and multiple viruses are found in cervical secretions [17]. Lymphoid and neuronal cells are additional repositories for lymphotropic/neurotropic viruses. Indeed, the majority of the population is seropositive for multiple herpes viruses, including herpes simplex virus (HSV; Table 1). HSV-1 and HSV-2 seropositivity increases with age in the USA, where >60% of the elderly population is HSV-1-positive, and >20% are positive for both HSV-1 and HSV-2 [18]. Similar findings have been reported in Europe, with seropositivity rising with age to >80% in some countries [19], pointing to progressive acquisition over a lifetime. Similar high seroprevalences have been reported for multiple herpes viruses (Table 1). The widespread presence of these and other passengers, particularly of varicella zoster virus (VZV; also known as human herpes virus 3, HHV-3), Epstein–Barr virus (EBV/HHV-4),

cytomegalovirus (CMV/HHV-5), HHV-6A/B, HHV-7, and anelloviruses (Torque Teno viruses), has been substantiated by deep sequencing of blood DNA from 8000 humans [20].

By contrast, the distribution of endozoites in peripheral tissues has not been systematically addressed. However, in addition to the gut, blood, and epithelial surfaces including the lung [21], there is evidence that endozoites are present within other organs. PCR and deep sequencing of DNA from normal hamster liver revealed multiple bacterial species, confirmed by direct microbial culture from liver tissue [22]. There is evidence that the kidney also houses its own microbiota [23], and the human urinary microbiome (a proxy for the kidney) contained multiple species of Firmicutes, Actinobacteria, Bacteriodetes, and Proteobacteria [24]. Studies on other key tissues are so far lacking, for example breast, heart, ovary, pancreas, prostate, skeletal muscle, testis, thymus, and thyroid; future work will be necessary to address this issue.

Microbial Diversity

It is important to recognize that our knowledge of the spectrum of endozoites is limited. Kowarsky *et al.* performed deep sequencing of blood cell-free DNA from 188 individuals and reported that, of new bacterial and viral (including bacteriophage) sequences, the majority were absent from current databases [25]. Notably, this study almost doubled the number of human anelloviruses sequenced to date. We are only seeing the tip of the iceberg.

In addition, although we tend to refer to viruses as unique entities, this is seldom if ever the case. For example, HSV-1 and HSV-2 each comprise multiple substrains; moreover, recombinants between HSV-1 and HSV-2 have been detected worldwide [26].

GUT–BRAIN AXIS

Multiple studies report that the composition of the gut microbiota can influence brain function [27,28], partly by release of metabolites including neurotransmitters that can enter the circulation, but also via direct neuronal communication between gut and brain (notably via the vagus nerve). For an excellent review see [29]. However, our focus here is on microbes that directly enter the CNS. That endozoites are probably present in all human tissues is borne out by recent studies on the brain, discussed below.

ENDOZOITES IN THE BRAIN

We saw above that the plant root contains a high diversity of endogenous microbes, thus resembling the vertebrate gut. However, ever since Darwin's time there have been suggestions that the plant root – through demonstrable adaptive behavior – in some ways resembles the vertebrate brain (the 'root–brain hypothesis' [30]) where endophytes play a pivotal role. The presence of microbes in the brain, and whether they modulate behavior, is the central focus of this review.

Box 2 overviews current knowledge of the presence of diverse endozoites in brain, not only including bacteria, protozoans, Archaea, and viruses, but also bacteriophages, higher eukaryotes, and plant-derived agents (endogenous retroviruses/retroelements are not considered to be true endozoites; Box 3). One central conclusion emerging from this analysis is that there are parallels between the taxa found in plant tissues and those found in

both gut and brain (Table 2, discussed in Box 2), suggesting that some species may be particularly adapted to a close association with multicellular organisms including both plants and animals.

Although these combined reports confirm the widespread presence of endozoites in the brain, a complexity of all these studies is that it is not always possible to distinguish between endozoites that endogenously inhabit brain tissue versus contamination with agents borne by the circulation (including migrating lymphoid cells). Nevertheless, HSV sequences within the brain parenchyma have been confirmed by *in situ* hybridization [31,32], and *in situ* studies have directly demonstrated bacteria and protozoans within the brain (see below). However, this remains a general point of debate, and future studies on the broader brain microbiome will need to confirm the presence of endozoites by microscopy, immunohistochemistry, hybridization, and/or transcriptomics at the single-cell level.

HOW DO ENDOZOITES ENTER THE BRAIN? ROLE OF THE BLOOD–BRAIN BARRIER (BBB)

In plants, endophytes are both horizontally and vertically transmitted. In addition to sites of damage by insects and herbivores, stomata are likely to serve as a widespread transmission route, and dissemination via the xylem is thought to facilitate propagation throughout the plant (reviewed in [33]). In mammals, microbes are inhaled, ingested with the diet, or inoculated via biting insects/wounds, and can disseminate via the bloodstream. However, entry to the brain demands special mechanisms because the BBB prevents simple diffusion into brain tissue.

The BBB, generated by tight junctions between endothelial cells lining the cerebrovasculature, effectively prevents small molecules from entering the brain parenchyma. However, the BBB does not appear to constitute a barrier to many microbes. *Porphyromonas gingivalis* could be detected within the brain parenchyma following chronic oral administration to mice [34,35] (Box 1), and replication-defective HSV is found in the brain within 3 days (see below). Intranasal or intratracheal administration of the fungus *Cryptococcus neoformans* in mice led to rapid dissemination into the brain as quickly as 3 h postinfection, although the titers were low (≤1% of the inoculum) [36]. Similarly, intranasal administration of a filamentous bacteriophage to mice was followed by rapid appearance in the brain [37].

Microbes are thought to sidestep the BBB by exploiting at least four routes. First, many biologically active molecules such as polypeptide hormones are actively transported across the BBB into the brain, and many pathogens exploit host receptor-mediated transcytosis. Following receptor–ligand interactions at the endothelial cell surface, several microbes (bacteria and yeast) are internalized as vacuoles and thence pass into the brain without disrupting the integrity of the BBB (reviewed in [38,39,40]). For example, following intravenous inoculation in mice, the yeast *Candida albicans* is found within brain tissue as quickly as 4 days post-infection [41] by exploiting a specific receptor on endothelial cells [42].

Second, the BBB is not effective against migrating host cells, as exemplified by the common appearance of tumor cell metastases in brain tissue. Immune cells such as macrophages can efficiently enter the brain, and latent lymphotrophic viruses (e.g., HHV-6 and 7) borne by macrophages can thereby be delivered into the CNS. Viruses associated with mobile cells can therefore gain brain access (the 'Trojan horse mechanism'), and similar pathways may apply to intracellular bacterial pathogens (e.g., [38,39]). Passage parallels that in the gut, where intestinal dendritic, goblet, and M cells have been implicated in transporting bacteria from the gut lumen into the circulation [14].

Third, by direct neuronal pathways. Rabies virus, for example, is typically delivered to peripheral neurons following the bite of an infected animal, and then travels via axonal and trans-synaptic transport to the brain [43,44], thus bypassing the BBB. The vagus nerve has been specifically implicated as a gut–brain delivery highway for microbes in the gastrointestinal tract (e.g., [45]), and alphaherpesviruses such as HSV-1 employ a similar neuronal strategy (e.g., [46]; reviewed in [47]), principally via the olfactory system. In support, following intranasal delivery of HSV-1 to young mice, virus particles could be detected by immunohistochemistry in olfactory bulb and trigeminal nuclei in under a week, subsequently spreading to multiple brain regions including hippocampus and cortex [48]. Virus replication does not appear to be essential for dissemination. After delivery of a replication-defective marked HSV-1 either intranasally or intravenously, viral gene expression was detected in multiple brain regions as quickly as 3 days after inoculation [49].

Fourth, via the circumventricular organs. The circumventricular organs and choroid plexus lack a classical BBB, and trypanosomes (*Trypanosoma* spp.) appear to use this route for early invasion, whereas infiltration of the brain parenchyma occurs only later [50,51].

Thus, although the BBB may afford an obstacle to opportunistic pathogens, species-adapted endozoites appear to have evolved effective mechanisms to evade the BBB and gain brain entry. However, this remains to be clarified more extensively. For example, do genetically tagged endozoites introduced into the oronasal cavities, lung, or gut generally enter the brain (as demonstrated for *P. gingivalis* and HSV) or other organs?

MECHANISMS OF PERSISTENCE

At first sight, the presence of endozoites in normal healthy tissue is enigmatic. Vertebrates deploy an arsenal of defenses against pathogens, including pathogen-recognition mechanisms, antibody- and complement-mediated pathogen elimination, and cell-mediated defenses (both antigen-specific and nonspecific). How then do endozoites persist?

Entry into host cells and extrachromosomal replication (e.g., HSV) and/or genomic integration (e.g., HHV) and subsequent persistence as intracellular latent forms is the most obvious means to evade the immune system. Some normally extracellular bacteria and parasites such as *Salmonella* spp. and *T. gondii* can enter host cells where they clad themselves with host proteins inside the cell. Multiple other routes include antigenic variation (bacteria, protozoans, viruses), antigen shedding (e.g., *Leishmania*), and metabolic dormancy (e.g., *Mycobacterium tuberculosis*) (for an excellent and forward-looking review see [52]). To this list one must add biofilms, secreted layers of inert (non-immunogenic) polymers that coat the local environment of the cellular endozoites (e.g., [53]), as well as coronas of host proteins that can surround the endozoite (e.g., [54]), and that may prevent recognition by innate immune receptors, antibodies, and cell-mediated immune mechanisms.

As we will see later, several endozoites also cause local or systemic immunosuppression that further contributes to their persistence.

LATENT/DORMANT ENDOZOITES ARE NOT SILENT

A longstanding view is that endozoites, after entering tissues such as the brain, remain in a hidden 'silent' form that can persist for years. However, this view has been challenged by findings that latent HSV-1 infection is

accompanied by persistent cytokine upregulation. Following low-dose infection of mice with HSV-1, brain cytokines including IFN-γ, IL-4, IL-6, and TNF-α were chronically upregulated for up to 120 days post-infection, despite evident viral clearance [55,56].

In an important study, Halford *et al.* [57] reported that ongoing treatment of latently infected mice with a potent inhibitor of HSV-1 DNA replication, aciclovir, led to an extensive decline in brain (trigeminal ganglia) expression of IFN-γ and TNF-α by 120 days post-infection (aciclovir was started at 15 days post-infection). This demonstrates that, instead of being inert, low-level HSV-1 gene expression and DNA replication continues to take place despite viral clearance, driving chronic cytokine production [57]. Low-level neuronal expression of multiple VZV proteins was also detected during latency [58]. In support, viral sequences are detected in blood, and saliva can be source of HSV and VZV virions in otherwise healthy individuals (e.g., [59,60]).

The reported behavioral effects of *Toxoplasma gondii* infection (discussed in more detail later) also argue that the parasite is not silent, and causes changes in the host despite persisting in a subclinical state.

In sum, far from being silent, the albeit limited evidence suggests that endozoites in subclinical infection (paralleling endophytes in plants) display low-level gene expression and turnover that may have an ongoing influence on their host. As we will see in the following sections, endozoites can both provide benefits to the host as well as manipulating local or systemic immunity and behavior to maximize their own persistence and/or onward transmission.

THE ENEMY WITHIN: BEHAVIORAL CHANGES AND IMMUNOSUPPRESSION

Plant endophytes alter host physiology and metabolism, in some instances to promote host defense (see earlier), and in others to ensure their own propagation – for example by associating with seeds or pollen [61,33], and also by suppressing host immunity (e.g., [62]). Indeed, local immunosuppression is essential for maintenance of *Rhizobium*–legume symbiosis (e.g., [63]).

Similarly, specific endozoites cause behavioral and physiological changes that may facilitate their own proliferation. Although extensive work has been done in insects (not reviewed), the focus here is on vertebrates and the brain. Because most work has been done with acute replication, this is covered first before discussing more subtle changes taking place in subclinical infection.

Pathogens including viruses not only influence host behavior (perhaps to promote host–host transmission) but also can dampen the immune system (to prevent their elimination).

Local Immunosuppression

Multiple viruses escape immunosurveillance by downregulation MHC-mediated antigen presentation of infected cells, blockade of complement-mediated cytotoxicity, or interfering with cytokine signaling, examples being adenoviruses, poxviruses, and herpes viruses [64]. Measles virus and endozootic HHV-6A/6B interfere with key immunoregulatory circuits [65,66], and CMV is reported to disrupt signaling pathways leading to the release of immunosuppressor molecules such as arachidonic acid, prostaglandins, and cytokines (e.g., [67]).

Systemic Immunosuppression

Two routes are central. The first strategy is to directly infect immune cells including macrophages (reviewed in [68]). For lymphotropic viruses such as HIV, direct infection and inactivation of key immune cells can precipitate systemic immunosuppression.

A second route but less well studied route exploits the fact that limbic brain areas control both behavior and the immune system. Damage to temporal brain including hippocampus, amygdala, and overlying cortex can cause Klüver–Bucy syndrome that is associated in monkeys and humans with hyperorality, hypersexuality, and decreased or increased aggression [69]. Infection of limbic areas may thus modulate onward pathogen transmission.

Furthermore, the limbic brain is directly connected to the hypothalamus – and controls the hypothalamus–pituitary–adrenal (HPA) axis [70,71] and the release of glucocorticoids that have direct immunosuppressive effects (reviewed in [72]). Although the literature is mixed, hippocampal lesions are associated with upregulation of basal levels of adrenal corticosteroids, and chronic excess of cortisol is firmly linked to immunosuppression. Pivotally, a wide literature documents chronically upregulated cortisol levels in Alzheimer patients (e.g., [73]), in which early hippocampus involvement is seen, that accompany clinical progression [74]. A case may therefore be made that hippocampus targeting by endozoites can contribute to immunosuppression.

As we will see, several classes of endozoites directly enter the brain and, through effects on key brain regions such as the hippocampus, may both dampen the immune system and cause behavioral changes to promote their own proliferation. Rabies virus affords the exemplar of how a virus can influence both behavior and immunity, and we consider this case first.

Rabies Virus: The Exemplar

RV is a pathogen, and infection is predominantly lethal (unless rescued by vaccination [75]), and it is therefore not a true endozoite (related endozootic lyssaviruses of humans and animals are reviewed in Box 4), but this neurotropic negative-stranded RNA virus is the paradigm for behavioral changes.

The name of the virus is reputed to derive from the old Indian word *rabh*, meaning 'to make violent' [76]. Following peripheral infection, the virus travels in a retrograde direction via the nervous system until it reaches the brain. Target brain regions in human and animals centrally include the limbic system and hippocampus [77,78], a site that displays the highest density of RV receptors CHRNA1 (nicotinic acetylcholine receptor [79] and GRM2 (metabotropic glutamate receptor subtype 2 [80] (Figure 1A). In animals, RV infection is then accompanied by extensive virus shedding (of presumed neuronal origin) in saliva and aggressive behavior ('furious rabies') that can facilitate infection of new hosts.

RV infection is also associated with profound immunosuppression [81,82]. Kasempimolporn *et al.* reported atrophy of the spleen and thymus in RV-infected mice, but with no evidence of infection of these tissues, and the authors argued that neuronal infection by RV causes lymphoid cell apoptosis and immunosuppression by an indirect route [83].

Other Lyssaviruses, Herpes Viruses, Picornaviruses, Flaviviruses

Box 4 overviews different types of viruses and their effects on the brain and immunity. One commonality emerging from this analysis is that many agents selectively target the hippocampus (Figure 1), where they can cause behavioral changes. They also induce immunosuppression, although in many cases the underlying mechanisms remain unknown. In addition, there is some evidence that HSV-1 may target the hypothalamus (Box 4), potentially affording a further mechanism for subverting host immunity.

As in rabies, saliva may be a major route of transmission, notably for herpesviruses. Indeed, some viruses (e.g., mumps rubulavirus) target the salivary gland.

Coronaviruses: Bats as an Unusual Source of Human Infection

Human coronaviruses such as the agents of SARS (severe acute respiratory syndrome), Middle East respiratory syndrome (MERS), and more recently COVID-19, have been in the headlines as a result of recent human epidemics, but their biology is not yet fully understood. These pathogenic viruses principally cause respiratory tract disease; brain infection, although strongly suspected [84], has not yet been studied in detail. By contrast, murine coronaviruses (rat JHM virus and a derivative of murine hepatitis virus, HMV) have been reported to cause selective cell destruction in the hippocampus [85,86]. Suppression of the innate immune system by the SARS agent has been reported (e.g., [87]).

Although these specific human viruses are principally pathogens, four different types of subclinical coronavirus (229E, NL63, OC43, and HKU1) are widespread in the general population where they cause a condition that is difficult to distinguish from the common cold caused by rhinoviruses. Coronaviruses have been associated with CNS diseases such as encephalitis (e.g., [88]). Over 70% of adults worldwide are seropositive for all four virus types (e.g., [89]), demonstrating that these are true endozoites.

By contrast, the new pathogenic strains appear to derive from endozoites of bats, and the COVID-19 virus is 96% identical to a known bat virus [90]. This raises the intriguing question of why bats in particular are a rich source of human pathogens.

Viruses in Bats. There are over 1200 species of bats that comprise a quarter of mammalian species. Bats harbor more viruses per species than any other mammal [91,92,93]. How do bats coexist with so many viruses? Bats are the only mammalian species adapted to flight, and it appears that this changed their immune systems. The energy demands of flight are so great that cells in the body break down and release copious quantities of DNA and RNA into the circulation. Mammals, including bats, have sensors that respond to DNA/RNA and induce an innate immune response. To avoid damaging inflammation during flight, a key mediator of the innate immune response, STING (stimulator of interferon genes) is altered. Xie *et al.* (Wuhan) have found that the serine residue at position 358 of STING is replaced in every known bat species examined, whereas it is absolutely conserved in all other mammals [94]. They then demonstrated the S358 replacement in bat STING dampened but did not fully diminish the functionality of STING. They speculate that adaptation to flight via a weakened (but not entirely lost) functionality of the STING-mediated innate immune response may have a profound impact on the ability of bats to maintain an unusually high burden of endogenous viruses [94].

Bacteria and Archaea

Far less is known about the proclivity of bacteria for specific brain regions, but cognitive decline is commonplace in survivors of bacterial sepsis ([95] for review), and bacterial toxins including lipopolysaccharide (LPS) are known to exert negative effects on the hippocampus [96,97], where they inhibit neurogenesis and neuronal replacement (e.g., [98]). Hippocampal damage, particularly of the dentate gyrus, is a common feature of bacterial meningitis in human [99]. In addition, LPS administration to mice (a model for sepsis) can lead to immunosuppression (e.g., [100]). Further studies on the relationship between enzootic bacteria, brain physiology, and immunosuppression are warranted.

To date there have been no systematic studies on Archaea, and their potential role as brain endozoites remains unknown.

Yeast

Candida albicans is detected in the brain of Alzheimer disease patients [101], including cortex and hippocampus, and *Candida* infection has been suggested to promote functional changes in the immune system and enhance immunosuppression [102]. However, this was not confirmed in another study [103], and it remains an open question whether yeasts such as *Candida* spp. modulate local/systemic immunity and/or behavior.

Toxoplasma **and Fatal Attraction**

Infection with the protozoan *Toxoplasma gondii* is prevalent in the human population [104], and some very high rates have been reported [105], confirming that *T. gondii* is a true endozoite. In experimental rats, persistent infection remarkably blocks their innate aversion to the odor of cats ('fatal attraction'), the definitive host for *T. gondii*, thereby increasing the chance of transmission to that species [106,107]. Berenreiterova *et al.* [108] reported a significant association of *T. gondii* cysts with the limbic brain and cortex (Figure 1C), and more detailed studies highlight the amygdala, adjacent to the hippocampus, as the region responsible for the altered odor response [109]. There is also evidence for subtle behavioral changes in humans, including changes in the perception of animal pheromones [110] and in impulsivity and aggressiveness [111] (schizophrenia is discussed later). *Toxoplasma* infection can also cause immunosuppression in mice and potentially in human [112,113,114].

Trypanosomes and Sleeping Sickness

African sleeping sickness, also known as human African trypanosomiasis (HAT), is caused by infection by one of two related protozoan parasites, *Trypanosoma brucei* (*Tb*) *rhodesiensis* that is found in East Africa, and *Tb gambiensis* in West Africa. The parasite is transmitted to humans by bites from the tsetse fly (genus *Glossina*). These are painful – the fly's mouth has tiny serrations that saw into the skin and allow it suck from pooled extravasated blood. Primary infection leads to long-term persistence of the parasite. Although it has been known for over a century that trypanosomes are found in CSF of infected individuals, the skin itself is also a significant reservoir of trypanosomes [115] and the fly likely ingests the parasite from both skin and blood.

Infection appears to be a three-stage process. The first stage is a bite from the tsetse fly, leading to blood infection. In the second stage, the parasite enters the CSF and meninges. In the third stage, the protective barriers of the brain break down and a 'mass invasion' of trypanosomes crosses the BBB, attacks the brain, and is often fatal. Our appreciation of the importance of events in second stage has been highlighted by recent work of Duszenko *et al.* It

seems that the parasite keeps itself in the second stage as long as possible and actively slows disease progression, and the third stage often only occurs months, years, or even decades after infection [116].

Why do trypanosomes enter the CNS and why was this phenomenon advantageous enough to be stably passed on during evolution? Mogk *et al.* discuss this in detail and make several interesting points [116]. Escaping from the hostile blood system into a relatively immune-privileged organ may offer advantages. By occupying the pial cell layer trypanosomes are not challenged by the full immune system, but from this refuge trypanosomes can easily interfere with sleep/wake cycles [117] and at the same time easily re-enter blood vessels to ensure a permanent blood infection for onward transmission.

Sleeping sickness is an excellent example of where an endozoite deliberately manipulates host behavior. Two compounds are produced by the parasite: (i) prostaglandins including PGD2, and (ii) a tryptophan metabolite, tryptophol. PGD2 is selectively elevated in the CSF of advanced sleeping sickness patients and has somniferous properties as well as inducing immunosuppression [118]. Tryptophol is reported to induce sleep in mice [119] and also causes immune system downregulation [120]. The lethargy and somnolence that name the disease may well maximize the likelihood that an infected individual is bitten by a further tsetse fly, thus completing the insect–human–insect life cycle.

In sum, several different classes of endozoite have been shown to invade (or indirectly target) limbic regions if the brain where they can modulate both behavior and immunity. Although this suggests that brain infection and local/systemic immunosuppression are central to their life cycle, the generality of this phenomenon warrants further investigation.

We stress that behavioral changes are by no means restricted to vertebrates, and infections of mosquitoes by La Crosse virus (the agent of the most common mosquito-borne disease of US children) are reported to modify mosquito bloodfeeding behavior to enhance transmission by biting [121,122].

ENDOZOITES IN THE BRAIN: NEUROPSYCHIATRIC DISORDERS INCLUDING SCHIZOPHRENIA

Acute infections of the CNS with bacteria and viruses are associated with multiple clinical conditions including meningitis, encephalitis, and retinal necrosis (herpesvirus infections of the CNS are reviewed in [123]). Acute and post-acute infections with different types of herpes viruses have also been associated with epilepsy, including but not restricted to HSV-2, CMV, and HHV-6A/6B [124,125,126]. However, our focus here is on long-term infections by endozoites.

Neurodegeneration, Bacteria, and Herpesviruses

Host immunodeficiency inexorably leads to microbial proliferation and tissue damage in multiple organs. Thus, the persistence of endogenous microbes in healthy tissues such as the brain appears to reflect a delicate balance between microbial proliferation and elimination by the immune system. Therefore, aging, that is characterized by decline of the immune system (e.g., [127,128]), is likely to be accompanied by re-emergence of erstwhile clinically silent endozoites, as reported for HSV [129,130] and VZV [131]. Indeed, there is growing interest in the possibility that age-related reactivation of subclinical endozoites in brain might be causally linked to disorders such as Alzheimer's disease (AD) [132].

This idea has a long history. At about the same that Lewis was studying endophytes in trees, Fischer and Alzheimer discovered deposits ('Drusen') in the brain of patients with AD that they suspected ('I emphasized the peculiar similarity of the Drusen with bacterial colonies') to be associated with microbes [133]. Several reports have recently appeared that address the potential relationship between AD and different types of infection including viruses, yeasts, and bacteria [134,135,136,137,101,138,139,140], and these are not reviewed here.

Causal links are notoriously difficult to prove, but emerging population evidence argues that antiherpetic medication may reduce the incidence of AD [141], and several studies are underway to reproduce or refute this finding. Antiviral treatment is also reported to reduce the incidence of Parkinson's disease (PD) [142]. If confirmed, it would raise the prospect of eventual treatments not only in AD and PD [143,144] but also of conditions such as atherosclerosis and diabetes, among others, where an infectious trigger has long been suspected.

We raise a potential caveat regarding the brain microbiome in neurodegeneration (and other disorders) because most studies have been performed on postmortem samples from elderly patients, and it is difficult to distinguish between microbes that might play a role in brain disease such as AD versus those that invade the brain during terminal illness (e.g., the cause of death in AD is typically severe respiratory infection).

Depression/Anxiety and Infection

There have long been suggestions that chronic infections may be associated with both depression and the associated condition, anxiety. HHV infection has been associated with major depressive disorder [145,146], and we saw earlier that subclinical infection with for example HSV leads to persistently elevated levels of circulating cytokines. These predominantly target limbic brain regions, centrally including the hippocampus [147], and clinical administration of interleukins and interferons such as IL-1α, IL-2, IFN-α, IFN-β, and TNF-α has been widely reported to cause malaise and sickness behavior that resemble anxiety/depression [148,149,150,151,152,153]. Indeed, subclinical infection of several types, perhaps not only in the brain, that lead to systemic inflammation may underlie depressive and anxiety disorders [154].

Schizophrenia (SZ) and *Toxoplasma*

Intense research is presently focused on endozoite involvement in AD and PD, as well as in depression, and we therefore draw attention to a neglected condition, SZ, where infection has long been implicated.

The cause of SZ, an enigmatic condition that is typically diagnosed in late teenage years, is unknown. A possible infective contribution to the etiology and pathogenesis of SZ has been investigated intermittently for over a century. In part this is because of the textbook example of the once common form of psychosis called general paralysis of the insane (GPI). Infection of the CNS by a single agent, *Treponema pallidum*, is responsible, and the disorder can be effectively treated with penicillin.

In support of a role of an infectious agent, genome-wide association studies of SZ have consistently reported by far the largest signal from the MHC region on chromosome six [155], and part of the risk for SZ comes from allelic variations of the complement component 4 (C4) located in the MHC region [156]. This is of particular note because the complement receptor CR2 is a receptor for EBV [157] and complement C4 directly targets viruses for inactivation [158].

We overview in Box 5 some of the best-studied potential infectious organisms that have been associated with SZ, including influenza virus, HSV-2, *Porphyromonas gingivalis*, and *Toxoplasma* spp. All are associated with CNS invasion.

Of these, the case for an involvement of *Toxoplasma* spp. in SZ is supported by genetic and pharmacological findings. First, the *DISC1* gene, that has long been recognized to be a key determinant of familial SZ [159,160], is now reported to be a pivotal modulator of immune responses to *T. gondii* [161], directly implicating *Toxoplasma* spp.

Second, SZ is widely treated with neuroleptics, but their mechanism of action is unknown. Intriguingly, these psychotropic drugs may inhibit the growth of *T. gondii*. Jones-Brando *et al.* examined the effect of a range of neuroleptic and mood stabilizing drugs on *T. gondii* cells. Valproic acid together with haloperidol showed the strongest inhibitory effect on cell proliferation, but risperidone and trimethoprim also showed some effect [162]. In rats haloperidol or valproic acid can reverse behavioral changes induced by *T. gondii* infection, such as reduced fear of cats and attraction by cat odor. However, those drugs did not prevent acute infection nor decrease the number of tissue cysts in the animal brain [163], and more recent studies have yielded less clearcut results. However, a recent study [164] confirms that antipsychotics, in particular, have antimicrobial effects. The available data suggest that some neuroleptic drugs may reduce psychosis not only through antidopaminergic action but also by direct inhibition of *T. gondii* [163,165] or other endozoites.

HOST ADVANTAGES: AN EVOLUTIONARY ROLE FOR ENDOZOITES

Healthy tissues contain a multiplicity of endozoites, from bacteria to protozoans and viruses. These are not silent. In plants, select endophytes confer protection against pathogens and herbivores. Endozoites in vertebrates can also provide advantages to the host.

Protection against Superinfection

There are many examples. Ever since the time of Jenner it was observed that infection with one pathogen (e.g., poxvirus) could confer protection against a second unrelated pathogen (e.g., herpes) [166], and the 1927 Nobel Prize in Physiology or Medicine was awarded to Julius Wagner-Jauregg for the discovery that malaria infection is protective against general paralysis of the insane (GPI; i.e., neurosyphilis): inoculation of infectious malaria into patients remitted GPI in 83% of cases [167]. Bohnhoff *et al.* in 1954 found that that, unlike control mice, mice treated with streptomycin were easily infected by *Salmonella enterica* [168], demonstrating the protective role of the normal microbiota. In a further example, experimental animals inoculated with the human symbiont *Bacteroides fragilis* were protected against colitis induced by *Helicobacter hepaticus* [169].

Similar effects have been reported for viruses. Infection with human CMV *in vitro* was reported to inhibit superinfection with HIV [170], and mice latently infected with either murine gammaherpesvirus 68 or murine CMV are more resistant to infection with the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis*, respectively [171]. Early measles virus infection in human may be associated with a twofold reduced risk of Parkinson's disease [172]. Host advantages have been well reviewed by Roossinck [173] (further discussion below).

Competition

There are likely to be other benefits, both indirect and direct, that operate at an evolutionary/ecosystem level. For example, Johnson in 1926 reported that overtly healthy potato plants harbored a latent virus that produced severe pathology in a different plant, tobacco [174]. Harboring a latent pathogen could thus favor the host in competition with other plants. The same phenomenon is evident in human: the history of human migration has reported decimation of native populations, not by conflict, but through exposure to pathogens carried by the invaders. Such effects have undoubtedly had a major impact since the dawn of the vertebrate lineage, and no doubt well before.

Ecosystem Advantages

At the ecosystem level, plant ecologists argue that many endophytes within plant tissues remain latent until natural senescence, when they proliferate to promote recycling of biomaterial, to the benefit of seedlings and saplings, and thus to the ecosystem (see earlier). This is a perhaps a strange idea in the context of vertebrates, but this cannot be formally excluded over an evolutionary timescale, particularly for fungi and bacteria.

Coevolution and Horizontal Gene Transfer

There may be more direct routes. Herpes viruses and vertebrates have coevolved for at least 200 Ma [175]. In human, where the majority of the population harbors persistent infections with herpes viruses (Table 1), the divergence of HSV-1 and HSV-2 (and recombinants) accompanied human evolution from primates, and perhaps also migration out of Africa [176,177], suggestive of functional effects.

In plants, integration of (non-retrovirus) viral sequences is commonplace [178,179], raising speculation that 'integrated viral sequences might reflect some functional advantage to the possession of the sequence' [178]. Indeed, key genes determining the evolution of land plants from precursors appear to have arisen by horizontal gene transfer from soil bacteria [180].

Intriguingly, around 1% of the human population now contains integrated HHV-6 sequences [181,182]. The fact that these insertions have expanded from a small number of ancestors is very suggestive of a host advantage, so far unknown. Of the other endozoites discussed here, BDV-related sequences are also present in both human and primate genomes [183,184], again suggesting that viral sequences might provide a selective advantage. Indeed, there is good evidence for horizontal gene transfer from endozoites, particularly parasites, to the human genome [185].

Cognitive Benefits

Given that the majority of the population harbors HSV-1 and/or HSV-2, as well as multiple other diverse endozoites, one must query whether any beneficial behavioral changes are associated with subclinical infection – or are the changes (if any) normally so subtle that we do not recognize them?

Recent meta-analysis concluded, unexpectedly, that possession of the *APOE* e4 allele (an established genetic predisposition to several types of infection, including HSV, as well as to Alzheimer disease), was associated with marked cognitive benefits in the 0–30 year age group [186], the inferred lifespan of ancestral *Homo.*

In a striking example of cognitive benefits, Trumble *et al.* studied Amazonian forager-horticulturists who harbor chronic burdens of (untyped) microbial species. Performance on a battery of cognitive tests addressing verbal memory, working memory, semantic memory, and visual scanning was significantly elevated in *APOE* e4 individuals with the highest endozooite burden (assessed by level of eosinophilia) [187]. This finding mirrors reports of cognitive deficits in germfree mice [188]. Nevertheless, how endozoites might enhance cognition remains unknown, and brain infection was not demonstrated (although this appears likely). Even so, the far-sighted study of Trumble *et al.* needs to be extended by research on other populations and microbes to determine the extent to which endozoites might promote cognitive function in the host carrier, perhaps by competing with disadvantageous microbes, thus providing an evolutionary selective pressure for their persistence.

How do we explain the cognitive benefits in Trumble's villagers? Increased 'alertness' by stimulating adrenergic/cholinergic pathways is a possibility, but how might endozoites achieve this? We are reminded of the speculative Orowan–Haldane theory that elevated levels of the caffeine-like stimulatory molecule, uric acid, in the blood of human versus other mammals may have given the lineage leading to *Homo* an edge [189], of particular note because uric acid is associated with inflammation (e.g., [190]).

HYGIENE AND MICROBIAL ECOLOGY

The findings of Trumble *et al.* contrast sharply with other reports. For example, Benros *et al.* reported that infections of diverse types are associated with compromised cognition in young Danish males [191]. However, we underline a major complicating factor – hygiene. Improved sanitation in developed countries has substantially cut infant mortality, but may have inadvertently increased other disorders. Indeed, the 'hygiene hypothesis' has been invoked to explain differential rates of autoimmune disease across the world [192,193], including AD [194], although there may be caveats.

One remarks that the situation of indigenous Amazonian villagers, who are chronically exposed over their entire lifetime to a myriad of endozoites, is a far cry from Western populations who are insulated from the vast majority of environmental microbes, and are only exposed to a restricted range of endozoites in later years. It is very possible that, despite benefits in terms of infant mortality, the lack of exposure to an 'evolutionary' spectrum of microbes may predispose the human population to 'modern' diseases including AD, PD, and SZ.

ENDOZOITES AND THE MISSING HERITABILITY

As a final note, we wonder if endozoites might explain a longstanding conundrum. Many CNS disorders, exemplified by SZ and autism, show high concordance between identical twins (a measure of 'heritability'), as well as raised concordance between siblings. However, other than for specific single-gene defects (e.g., *CFTR* mutations in cystic fibrosis), multiple genomic analyses have failed to uncover gene variants (or groups of variants) that could explain this concordance, a phenomenon dubbed 'missing heritability' [195]. Indeed, for most disorders, genes explain no more than a small fraction of the heritability.

The widespread distribution of endozoites in the human population leads us to wonder whether endozoites, clusters of endozoites, and/or specific variants thereof could explain why twins and siblings display phenotypes that are closer to each other than to the general population. We undoubtedly inherit more from our parents (and from our prenatal and postnatal environments) that merely genes. Others have suggested that the gut microbiota might play a role (e.g., [196]), but the broader spectrum of endozoites, specifically those reaching the brain, might

have greater impact on diseases such as SZ that principally affect the nervous system. Comparative studies on twin/sibling microbiomes will be necessary to address this possibility.

CONCLUSIONS

Given the precedent of plants, it comes as no surprise to discover that endozoites are widely present not only on superficial surfaces and in the GI tract, but also within healthy human tissues such as the brain. Indeed, for many host-adapted microbes it seems that there is no fundamental barrier to entering host tissues. The benefits of the close association are well documented in plants, but there is so far only limited evidence (except for the gut microbiome) that vertebrate endozoites benefit the host, and this is an area that demands further research. Instead, there is extensive evidence that endozoites manipulate host immunology and behavior to promote their own persistence and transmission.

Building on clear parallels between plants and animals, the key conclusions of this analysis are as follows.

(i) Endozoites (like endophytes) are widely present not only in the circulation but in multiple body tissues including the brain.

(ii) Endozoites have accompanied the evolution of the lineage leading to *Homo* at least since the divergence of insects and vertebrates (0.5 Ga).

(iii) These passengers are not silent, and can influence both immunity and behavior.

(iv) As in plants, some of these passengers can be beneficial, and others harmful – a delicate balance. Endozoites are directly implicated in CNS disorders including Alzheimer disease, Parkinson disease, and schizophrenia, but in other cases endozoites may give their host a cognitive advantage.

In our view, endozoite modulation of behavior is the most intriguing of all the issues we have raised here. Is this mostly an incidental correlate of immunomodulation, given that the limbic brain governs both behavior/cognition and the immune system, or do endozoites deliberately manipulate our behavior?

We also wonder if there is an optimal (beneficial) brain microbiome that – paralleling GI tract microbiome transplantation in diabetes and colitis – we could perhaps resurrect (e.g., by simple measures such as intranasal inoculation) to prevent the adverse effects of key endozoites?

In sum, both plants and animals harbor a multiplicity of endogenous microbes that inhabit multiple tissues including solid tissues such as the brain. These are not silent, and harboring particular passengers may have both advantages and disadvantages. Understanding the mechanisms, roles, and ecology of endogenous microbes in different mammalian tissues including the brain will undoubtedly be a fertile field of investigation for the future.
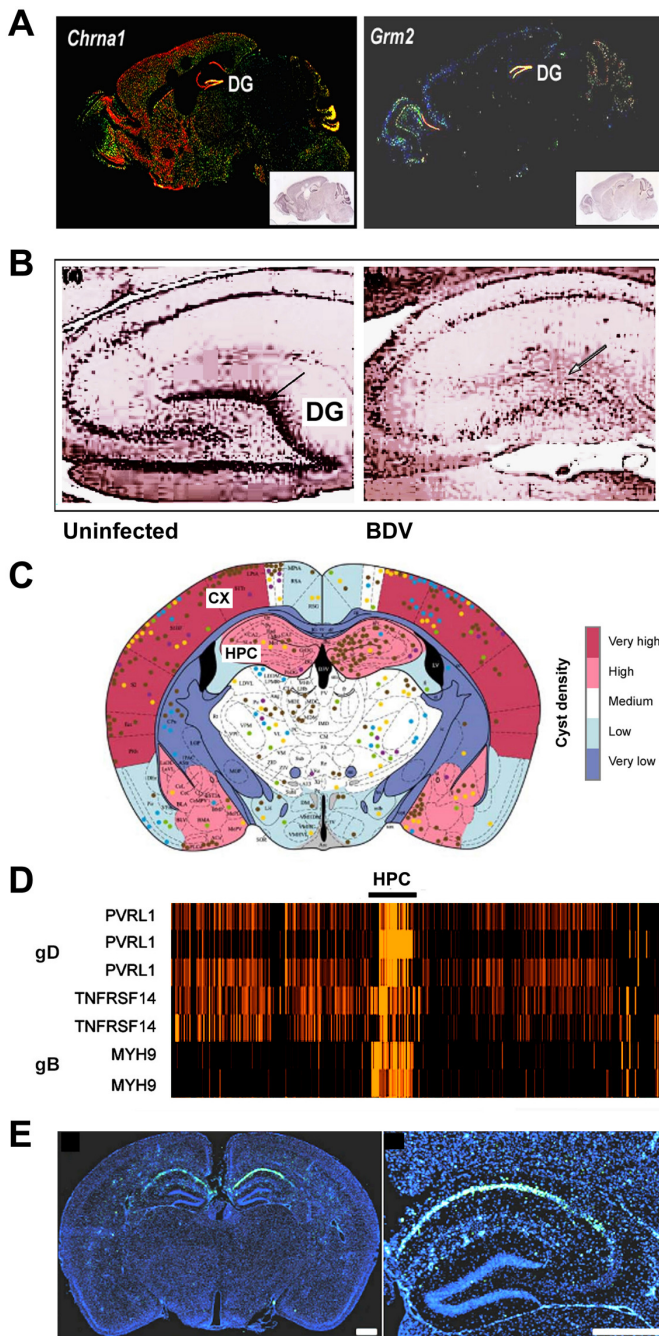
ACKNOWLEDGMENTS

Figure 1. Pathogen Localization and Neurotoxic Effects in Mammalian Brain. (A) Region-specific expression of rabies virus receptors *Chrna1* and *Grm2* in dentate gyrus (DG) of mouse hippocampus (HPC); reduced-size insets are brightfield images of the same sections (data: Allen Brain Atlas; https://mouse.brain-map.org/). (B) Selective destruction of the hippocampal DG in rats infected on postnatal day 1 with Borna disease virus (BDV); left, uninfected (age 60 days); right, infected (75 days). Figure adapted, with permission, from [197]. (C) Distribution of *Toxoplasma gondii* cysts in brain of mouse with latent *T. gondii* infection, showing enrichment in cortex (CX) and limbic brain including the HPC [108]. (D) Distribution of herpes simplex virus type 1 receptors in the human telencephalon, showing enrichment in the HPC (adapted, with permission, from [198]). (E) Picornavirus (Theiler murine encephalomyelitis virus) infection in mouse leads to selective DNA fragmentation within hippocampal CA1 neurons (white/green on blue background; 7 days post-infection) via pathways that may not involve direct virus infection (adapted, with permission, from [199]).
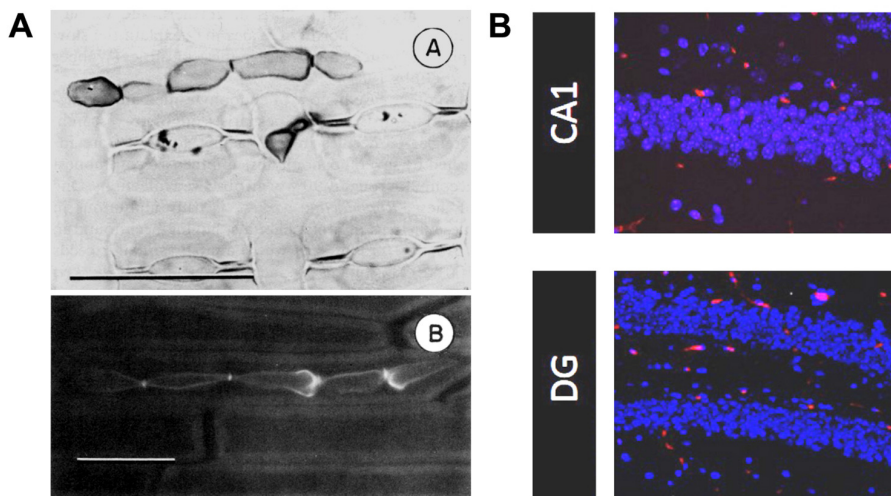
Box 1. Endophytes and Endozoites

Endophytes

Named from *endo* ('inside') and *-phyte* ('plant'), these organisms are generally described as endosymbionts/commensals that live within plant tissues without normally causing overt tissue damage (Figure IA). The formal discovery of live microbes within healthy plant tissue is generally attributed to Galippe [200], but the evidence was firmly challenged by Fernbach [201] who argued that contamination with surface material was very likely. Lewis [1] was among the first to unambiguously demonstrate the presence of fungal cells within healthy plant tissue.

Endophytes include bacteria, protozoans, Archaea, and other microbes. Although the term traditionally focuses on cellular species, the endophytic compartment includes viruses that infect cellular endophytes, as well as resident viruses that persist in host tissue without causing overt damage, and this broader sense is followed here. Endophytes are acquired both vertically and horizontally from the environment. The evolutionary persistence of endophytes reflects several advantages they confer to the host (and to the ecosystem); however, the biology of endophytes is far from clear, and there is no firm distinction between endogenous (endophytes – within tissues) and exogenous (on the surface of tissues) microbes because the same species can be present in both niches.

Endozoites

The term (from *endo* and *-zoon*, 'animal') was first used the medical literature (according to PubMed) in 1975 to refer to the apicomplexan *Sarcocystis tenella* [202], and is used here to encompass the spectrum of microbes (including protozoans and viruses) that persist within healthy tissues of higher organisms including invertebrates (e.g., insects) and vertebrates, but generally without causing overt disease. The term therefore does not normally include endogenous retroviruses (see Box 3) or acute pathogens such as *Yersinia pestis*, HIV, or smallpox virus, but the frontier is blurred because known pathogen sequences have been reported (albeit at low titer) in healthy human tissue (e.g., variola virus-related sequences in human brain [140]), as well as pathogenic bacteria in Alzheimer brain (Figure I).

Box 1 Figure I. Endophytes and Endozoites. (A) Intracellular infections of the fungus *Rhabdocline parkeri* in needles of Douglas fir, *Pseudotsuga menziesii*, a member of the pine family. (Above) Hyphae (trypan blue staining). (Below) Epifluorescence micrograph (calcofluor staining). Scale bars, 25 µM. Figure adapted, with

permission, from Carroll [203]. (B) Immunostaining of Alzheimer brain (entorhinal cortex/hippocampus) with antibody against the bacterium *Chlamydophila pneumoniae* (green; the exact species stained was not established); nuclei (DAPI staining) are in blue. Figure adapted, with permission, from Pisa *et al.* [204].

Box 2. Endozoites in the Brain

Bacteria

For many years there have been reports of bacteria in brain, notably in Alzheimer's disease, such as *Borrelia* [134,205] and *Chlamydia/Chlamyophila* spp. [101,139] (Box 1 Figure I), but also in healthy tissue. Systematic surveys in both health and disease using PCR and deep sequencing have revealed that the major phyla are α-Proteobacteria and Actinobacteria, with further Firmicutes and Bacteriodetes [136,138]; similar findings were seen in macaque monkeys [136] (Table 2). Proteobacteria constitute a phylum of Gram-negative bacteria that include not only gut commensals (e.g., *Escherichia*) but also several human pathogens (e.g., *Yersinia*). The Actinobacteria also include human pathogens (e.g., *Mycobacterium*).

These are the same taxa that are seen in plants (Table 2), including the actinobacterium *Frankia* that can fix nitrogen, and several agriculturally important nitrogen-fixing α-Proteobacteria species that enter symbiotic relationships with leguminous plants (e.g., *Rhizobium* spp.). These specific taxa may be predisposed to live in association with higher eukaryotes (noting that endosymbiosis with α-proteobacteria is held to have been the primary driver for the emergence of Eukaryotes; e.g., [206]). In support, some human commensal bacteria of these phyla (e.g., Enterobacteriaceae) – gut organisms in humans – can colonize root tissues of plants such as maize, lettuce, tomato, and barley where they may persist as a reservoir for recolonization of humans [207,208], and can even promote the growth of the new plant host [207].

Protozoans

Fungi are widespread in plant tissues, but there have been few studies on human, although Pisa *et al.* [101] report intermittent detection of several fungal species, principally *Candida* spp., in brain tissue of Alzheimer patients. *Candida* spp. are also prevalent plant endophytes, and species such as *Candida metapsilosis* are found both in plants and in human infections.

The apicomplexan *Toxoplasma gondii*, an obligate intracellular eukaryote, is widely present in healthy humans and animals, and 20–50% of the population is seropositive for *Toxoplasma gondii* [209,210]. *T. gondii* can persist in a subclinical state in multiple tissues including the brain [211]. *Toxoplasma* sequences were detected in 16.5% of human brain samples [104]. A different apicomplexan, *Neospora*, is an endozoite of bovine species. Oomycetes such as *Phytophthora* spp. that are phylogenetically rooted alongside apicomplexans are important plant pathogens.

Archaea

These simple unicellular organisms are inferred to be the evolutionary precursor to all life on Earth, and are well represented in the plant root microbiome ([212]; reviewed in [213]). They are also present in human colonic, lung, nasal, pulmonary, and oral microbial flora (e.g., [214]), However, no studies to date have systematically addressed whether they enter healthy tissues such as the brain, and so far no archaeal species has been demonstrated to cause disease in human [215,216]. However, methanogenic Archaea have been directly implicated in refractory sinusitis [217], and one report described finding archaeal species in multiple brain abscesses, although only 1/27 control samples were positive [218] – possibly suggesting that archaea are not typically present in the normal CNS,

perhaps because they are predominantly anaerobic. Archaeal viruses have been intermittently reported in blood [20]. Further studies on archaea in healthy and diseased brain are certainly warranted.

Viruses

HSV in normal neuronal tissue (sensory ganglia) was reported in 1972, when virus was isolated by culture of brain tissue from 1 of 22 patients with no evidence of active HSV disease [219]. HSV in 18 of 39 normal trigeminal ganglia was demonstrated by passage and immunohistochemistry [220]. The presence of HSV sequences in normal brain samples has been confirmed by DNA-based analysis [221] and by PCR [222].

Multiple other virus sequences have also been detected. Infection with polyomaviruses is widespread in the human population, and Southern blot and PCR analysis confirmed the presence of BK and JC genomes in up to 20% of healthy brain samples [223]. Other viruses including HHV-6A/6B, EBV, CMV, varicella zoster virus (VZV), and coronavirus have been reported in human brain [221,224,136], and HHV-6A and -6B proteins were detected using specific antibodies in 22–32% of control brain samples [145]. EBV was detected by PCR in 24% of control brain samples [225]. Deep sequencing has now revealed an extraordinary diversity of viruses in normal human brain, ranging from several types of HHV and HSV to adenovirus, Duvenhage virus, hepatitis C virus, coronavirus, torque teno virus, and BK polymavirus, among others [140]. There was evidence of over-representation of HHV and HSV in Alzheimer disease brain [140].

Bacteriophages

There have reports of bacteriophages (or bacteriophage-like sequences) in multiple human tissues and that phages readily enter the brain (reviewed in [226,227,228]). For example, a sequence (dubbed Sphinx) with 70% homology to an *Acinetobacter* bacteriophage was reported in transmissible spongiform encephalopathy brain [229]. These may well be markers of bacterial coinfection rather than pathogens or endozoites in their own right because they are likely to lack the machinery for replication in higher eukaryotes. However, infection (e.g., of plastids of prokaryotic origin) may not be formally excluded. *Arabidopsis* chloroplasts contain a replication machinery similar to that of bacteriophage T7 [230] and so-called mitochondrial viruses ('mitoviruses') have been reported in several plant species [231], but not yet to our knowledge in vertebrates.

Higher Eukaryotes

Endozoites more rarely include multicellular species such as the tapeworm, *Taenia solium*. Neurocysticercosis caused by *T. solium* is the most common parasitic disease of the human CNS, and, although the parasite infects multiple body tissues, the larvae display a strong affinity for the CNS [232]. Common symptoms include headache, seizures, and meningitis, and in children include depression, social problems, and rule-breaking behavior [233].

Algae- and Plant-Derived Agents

For completeness we include agents derived from photosynthetic species. Apicomplexans such as *Toxoplasma* have a secondary plastid – the apicoplast – whose distant ancestor was probably a photosynthetic plastid that originated from a red algal cell ([234], reviewed in [235]), whereas plants emerged separately from green algae. Indeed, apicomplexans are not phylogenetically related to plants nor to any other members of the Streptophyta

(e.g., [236]). Nonetheless, the presence of plastid remnants may render particular apicomplexan species sensitive to some herbicides [237].

Regarding plant-derived agents, there has long been speculation that some plant viruses may also interact with, or reside in, humans (reviewed in [238]). An excellent illustration is provided by the work of Zhang and colleagues [239] who reported that the most abundant viral sequence in human feces is a plant virus (pepper mild mottle virus, PMMoV). Although this could be purely dietary (titers up to 10^7 particles per ml have been reported in a commercial chili product [240]), it was reported that PMMoV may predispose to gastrointestinal dysregulation in human [240], possibly indicative of host cell infection. In another example, Liu *et al.* [241] reported significant levels of antibodies against tobacco mosaic virus (TMV) in healthy volunteers (including non-users of tobacco products), potentially suggestive of persistence of TMV (or a related virus) in the population.

Other plant agents may potentially reside in mammals. Following antibiotic administration to mice, fecal DNA PCR amplicons were highly enriched in Streptophyta (a taxon comprising land plants and six main lineages of green algae) and *Zea luxurians* (a species of grass)*,* perhaps reflecting residual plant-origin DNA introduced via feed [242]. Streptophyta have been widely detected in human mucosal microbiomes (e.g. [243]) and could derive from plant pollens. However, Streptophyta have been detected in mouse seminal fluid [244], raising the possibility that green algae might potentially be true endozoites. Caution is urged, however, because the PCR apparatus might itself be contaminated with pollens.

Conversely, some human endozoites can infect plants (see earlier). Nonetheless, with the exception of apicomplexans, there have been no reports of algae- or plant-related agents in brain tissue, but future studies on the human microbiome (including the brain) should not limit themselves to known vertebrate endozoites.

Box 3. Retroviruses and Retroelements

The genomes of humans and mice are (as in plants) also vastly punctuated by integrated elements such as endogenous retroviruses (e.g., HERVs) and retroelements (e.g., long and short interspersed nuclear elements: LINES and SINES) that are inferred to have had an earlier exogenous origin. Although these may have played a major evolutionary role, they are not generally regarded as endozoites, and the focus here is on agents acquired from the environment. However, in mouse, there is evidence for active retrotransposition of LINES in brain, with suggestions that these might be of benefit (so far uncharacterized) to the host (reviewed in [245]). In human, LINE element mobilization in brain has been reported in schizophrenia [246], and upregulation of HERV-W expression and increased copy number have been reported in multiple sclerosis and neurological disease (reviewed in [247,248]). The potential benefits and adverse effects of endogenous retroviruses/elements warrant further study.

Box 4. Viral Endozoites in the Brain: Effects on Behavior and Immunity

Lyssaviruses: Duvenhage and Borna Disease Virus (BDV)

In addition to rabies virus (main text), sequences for the closely related virus, Duvenhage, have been reported in normal human brain [140]. Further studies will be necessary to address whether endogenous Duvenhage-related viruses, like rabies, modulate the immune system and alter behavior. A third Lyssavirus, BDV, is an endozoite in many vertebrate species. Infection with BDV in several non-human species is followed by long-term persistent infection, and the majority of the target animal population harbors this virus [249]. The virus exhibits tropism for the hippocampus [250]. In experimental animals BDV can cause anxiety and aggression without overt fever [251], and disintegration of the hippocampal dentate gyrus was observed in late infection [197] (Figure 1B). Immunosuppression in BDV infection has also been reported [252]. A role for BDV as an endozoite of humans (versus animals) remains uncertain [253], but the presence of integrated BDV-related sequences in primates including human [183,184] suggests a close association.

Herpes Viruses

The majority of the human population harbors several types of herpes viruses (see earlier). The peculiar proclivity of herpes viruses for the brain (specifically HSV-1 and HSV-2 – the HHV group of viruses tend to be lymphotropic) has been known for almost a century ([254]; reprinted from 1929). As noted earlier, the virus can persist lifelong in sensory ganglia, but the hippocampus displays the highest abundance of HSV-1 receptors [198] (Figure 1D) and latent virus is often found in hippocampus, amygdala, and olfactory system [255]. Regional apoptosis in hippocampus has been reported following HSV-1 infection [256]. Indeed, following reactivation or primary infection, the hippocampus is a central site for virus replication in HSV encephalitis [257]. Multiple cases of Klüver–Bucy syndrome or autism have been reported following HSV encephalitis, consistent with virus-mediated damage to the limbic brain; behavioral changes in severe infection have been discussed [258,259] that could plausibly facilitate onward transmission.

In addition to behavioral changes in acute infection, multiple other herpes viruses are known to cause immunosuppression by infecting and/or interfering with immune cell function (e.g., CMV, EBV, and Marek disease virus of birds; reviewed in [260]). In mouse, the homolog of human HHV-6/7, murine roseolovirus (MRV; also known as mouse thymic virus, MTV; that is present in ~80% of house mice), causes necrosis of the thymus and acute immunosuppression in juveniles [261].

Neurotropic herpesviruses are also reported to cause systemic immunosuppression. Following infection of mice with HSV-2, the *in vivo* response to a potent proinflammatory molecule (phytohemagglutinin) dropped dramatically shortly after inoculation, and lymphocytes remained unresponsive for several weeks [262], although the mechanisms are not understood [263].

For HSV-1 a subtle mechanism has been proposed. HSV-1 latency in the CNS is accompanied by persistent upregulation of cytokines (see earlier) and, as noted by Baker [259], chronic production of specific cytokines may have detrimental effects on endocrine function and immunity, perhaps by targeting receptors that are expressed in the brain as well as by immune cells.

There is a further potential avenue for immunomodulation. Following ocular administration of HSV-1 in rats, a selective and intense focus of viral replication was seen in the hypothalamus [264], the master regulator of body physiology including immunity. Thus, HSV-1 (and potentially other viruses) could directly target the apex of the HPA axis to cause immunosuppression.

Picornaviruses

The major cause of the common cold, rhinovirus, is among the most common viral infections in humans. However, rhinovirus infections of the brain appear to be uncommon. In mouse, a different picornavirus, Theiler's murine encephalomyelitis virus (TMEV), or murine poliovirus, is primarily an enteric endozoite that rarely causes overt disease, and reports of TMEV seropositivity in laboratory mice range from 0.1% to 48% [265]. However, acute TMEV infection of sensitive animals leads to neurological deficits and neuronal destruction in the hippocampus (Figure 1) [199]. As with other agents, infection can also lead to immunosuppression, including inhibition of innate immunity and lymphopenia [266], suggesting that persistent rhinovirus infection in humans and animals might adversely affect the immune system.

Flaviviruses

These comprise a family of principally insect-borne viruses that are endemic in tropical and subtropical regions. We focus on two flaviviruses: Dengue and its recently emerged relative, Zika. Most individuals infected with Dengue recover, but in a study on Brazilian Dengue-infected individuals, 6% had symptoms that persisted for more than 6 months, including memory loss, headache, and emotional lability [267], consistent with hippocampal involvement [268]. Zika virus (that in neonates is associated with human microcephaly), also targets the hippocampal dentate gyrus even in the absence of microcephaly [269], a finding replicated in adult mice [270].

Box 5. Endozoites and Schizophrenia (SZ)

Influenza

Influenza is one of the best-studied potential prenatal contributors to SZ. Mednick *et al.* reported an increased risk for SZ in people exposed prenatally to the 1957 influenza epidemic [271]. This was followed in rapid succession by papers from Scotland and Denmark essentially confirming the Mednick findings. National registry records were used and allowed examination of prenatal exposure to both the 1918–1919 and 1957 influenza epidemics. Unfortunately since then around 20 additional ecological studies have addressed the issue, with around half supporting the hypothesis and the other half failing to confirm. However, there are confounders that make intepretation of these studies difficult. For example, almost all studies of prenatal exposure to influenza are based solely on whether an individual was *in utero* at the time of an influenza epidemic. In these circumstances around 70% of individuals who were *in utero* during the 1957 type A2 influenza epidemic would have been misclassified as having been exposed. This increases the risk of false negative (type 2 error) associations [272,273].

In a nested case–control study [274], Brown *et al.* demonstrated a threefold elevation in risk of SZ following influenza prenatal exposure during the first half of gestation. For first trimester exposure, the risk of SZ was increased sevenfold but there was no elevated risk following exposure during the second half of gestation. These results have been difficult to interpret especially because Seltzen *et al.* [275] pointed out that serological studies may have limited validity.

HSV-2

Neonatal exposure to HSV-2 is associated with congenital anomalies and neuropsychiatric disorders [274]. Three studies have examined the relationship between prenatal exposure to HSV-2 and risk of SZ in offspring. Two were derived from selected sites of the Collaborative Perinatal Project (CPP), a multisite study of population-based birth cohorts born from 1959 to 1967. In the first study [276] raised maternal IgG antibody levels to HSV-2 were associated with a significantly elevated risk of SZ and other psychoses in offspring with odds ratios of 3.4 to 4.4. In a much larger follow-up study [277], which included 200 case subjects with psychotic disorders from three cohorts of the CPP, a 1.8-fold increased risk of SZ psychoses was observed among offspring of mothers who were seropositive for HSV-2, but only among seropositive mothers who has regular unprotected sexual intercourse. A third study based on the Child Health and Development Study (CHDS) cohort failed to replicate these positive associations [278]. Potential explanations for these discrepant findings are discussed by Brown [279], as are the limited and equivocal findings investigating measles, rubella, varicella zoster, rabies, and poliomyelitis. There have been few and mostly negative subsequent studies of HSV-2 and SZ [280], and the data so far argue that HSV-2 is not a major contributor to SZ.

Porphyromonas gingivalis

Multiple studies have demonstrated increased rates of periodontal disease in patients with SZ [281,282,283,284,285,286,287]. Indeed Fawzi *et al.* [287] demonstrated increased levels of *P. gingivalis* (the key pathogen in periodontal disease) in saliva from SZ patients compared to controls, and the severity of psychopathology was related to *P. gingivalis* levels. Although the most obvious explanation for these findings is that the periodontal changes are secondary to life style, poor oral hygiene, and medication effects associated with SZ a bidirectional

link between the two conditions cannot be ruled out, especially given observations of the presence of *P. gingivalis* in postmortem AD and its presence in brain parenchyma following chronic oral administration in mice (see earlier).

Toxoplasma

This is the topic of excellent reviews [288,289], much of which is paraphrased in the following outline. Toxoplasmosis is an infectious disease caused by the parasitic protozoan, *T. gondii*, that affects approximately one third of entire human population. *T. gondii* can be found in almost all warm-blooded animals, but cats are the only known natural hosts. *T. gondii* is highly neurotropic and, soon after the infestation, migrates within the brain tissue to localize in astrocytes, microglia, and neurons [290,291]. The dormant form or bradyzoite can persist in the host brain for many years [292,293]. The brain tissue cysts undergo continuous remodeling, but until recently were not thought to cause clinical symptoms in immunocompetent individuals [294]. However, given the high level of neurotropism and the fact that *T. gondii* is endemic in almost all cultures worldwide, it has long been postulated that there may be a link with SZ [295,296].

Increased rates of *T. gondii* infection are reported in SZ. Three meta-analyses of association between *T. gondii* exposure and SZ have been published [295,297,298]. All were conducted with necessary scientific rigor and all have demonstrated, even accounting for publication bias, an association between exposure to *T. gondii* (as measured by IgG antibodies) and SZ. The most recent analysis by Sutterland *et al.* [298] included 50 studies. Significant odds ratios (ORs) with IgG antibodies were found in SZ (OR 1.81), and to a lesser extent in bipolar disorder and obsessive compulsive disorder, but not in major depression. Increased risk of SZ was also found in the offspring of mothers with serologic signs of infection detected during pregnancy. Cohort studies of blood samples taken from mothers in the perinatal period also show a twofold increase (OR 2.61) of IgG antibodies to *T. gondii* in those whose children went on to develop SZ. None of the studies demonstrated acute infection, as detected by specific IgM antibodies: this suggests the effects are due to latent infection [278,297]. Similar results have been found assaying IgG and IgM anti-*T. gondii* antibody levels in neonatal blood spots from the Danish State Serum Institute, where increased IgG levels were present in neonates who later developed SZ (OR 1.79) [299]. Because babies only start producing IgG antibodies around 3 months after birth, IgG antibodies assayed in the neonatal blood spots must be maternal in origin and suggest that earlier maternal exposure to *T. gondii* increases the risk of SZ in offspring [299,300].

Table 1. Human Seroprevalence of Herpes Viruses

Virus	Alternative name	Prevalence[a]	Population	Ref.[b]
Herpes simplex virus 1 (HSV-1)	HHV-1	67.60%[c]	USA	[18]
		52–84%	Europe	[19]
Herpes simplex virus 2 (HSV-2)	HHV-2	21.90%[c]	USA	[18]
		4–24%	Europe	[19]
Varicella zoster virus (VZV)	HHV-3	90%	USA[d]	[301]
		>90%	Europe[d]	[302]
Epstein–Barr virus (EBV)	HHV-4	82.90%	USA	[303]
Cytomegalovirus (CMV)	HHV-5	50.40%	USA	[304]
Human herpes virus 6A/6B	HHV-6A/6B	70–100%	Worldwide	[305]
Human herpes virus 7	HHV-7	>65%	UK	[306]
Human herpes virus 8	HHV-8	3–4%	USA	[307]
		35.7–49.3%	SubSaharan Africa	[308]

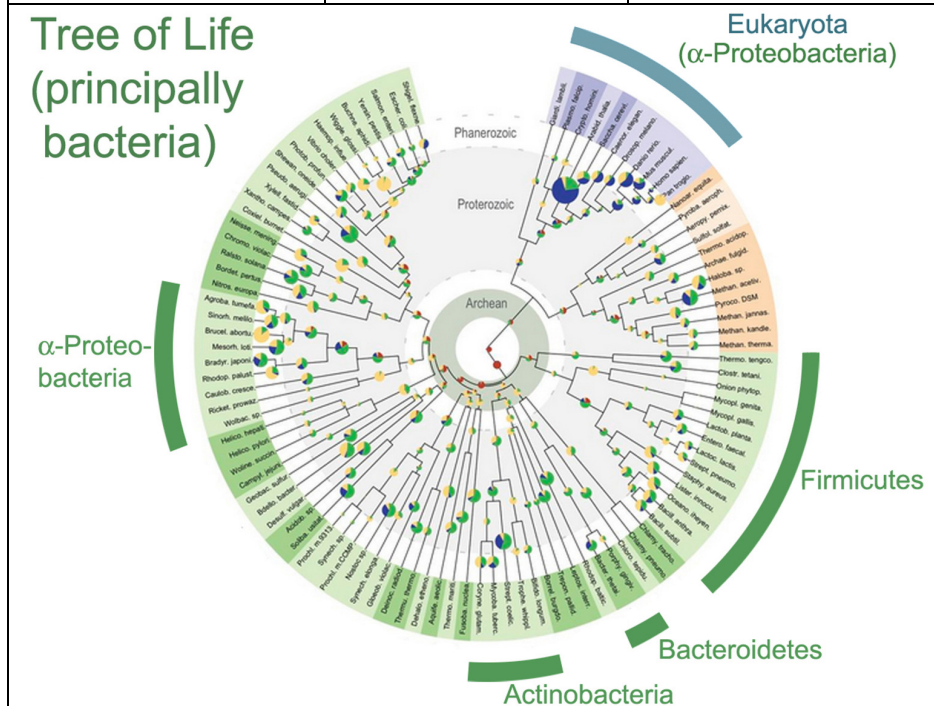[a]Seroprevalence increases with age, data are predominantly for adults.

[b]Comprehensive survey is not intended, only indicative references are given.

[c]Sequencing data suggest that ~100% of the population harbors both HSV-1 and HSV-2 [140].

[d]Before the introduction of anti-VSV vaccination campaigns.

Table 2. Predominant Bacterial Taxonomic Groups in the Microbiomes of Plants and Animals[a]

Plants[b]	Gut[c]	Brain[c]
Proteobacteria[d]	Proteobacteria[d]	Proteobacteria[d]
Actinobacteria	Actinobacteria	Actinobacteria
Bacteriodetes	Bacteriodetes	Bacteriodetes
Firmicutes	Firmicutes	Firmicutes

[a]Tree of Life, adapted from graphic https://phys.org/news/2010-12-scientists-decipher-billion-year-old-genomic-fossils.html; courtesy of Lawrence David (Duke University) [309].

[b]Rhizosphere, phyllosphere, and endosphere.

[c]Human and macaque; the same taxonomic groups are seen in blood, lung, and kidney (see text)

[d]Proteobacteria are inferred to be the precursors of the endosymbiotic organelles of eukaryotes.

REFERENCES

1. Lewis FJ. An endotropic fungus in the coniferae. *Nature* 1924;114:860.
2. Hardoim PR, van Overbeek LS, Berg G et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* 2015;79:293-320.
3. Liu H, Carvalhais LC, Crawford M et al. Inner plant values: diversity, colonization and benefits from endophytic bacteria. *Front. Microbiol.* 2017;8:2552-
4. Masson-Boivin C, Sachs JL. Symbiotic nitrogen fixation by rhizobia-the roots of a success story. *Curr. Opin. Plant. Biol.* 2018;44:7-15.
5. Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* 2002;40:309-348.
6. Vidal S, Jaber LR. Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. *Current Science.* 2015;109:46-54.
7. Gange AD, Brown VK, Aplin DM. Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecol. Lett.* 2003;6:1051-1055.
8. Krajmalnik-Brown R, Ilhan ZE, Kang DW, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. *Nutr. Clin. Pract.* 2012;27:201-214.
9. Kau AL, Ahern PP, Griffin NW et al. Human nutrition, the gut microbiome and the immune system. *Nature.* 2011;474:327-336.
10. Ramirez-Puebla ST, Servin-Garciduenas LE, Jimenez-Marin B et al. Gut and root microbiota commonalities. *Appl. Environ. Microbiol.* 2013;79:2-9.
11. Hacquard S, Garrido-Oter R, Gonzalez A et al. Microbiota and host nutrition across plant and animal kingdoms. *Cell. Host. Microbe.* 2015;17:603-616.
12. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet.* 1984;1:1311-1315.
13. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486:207-214.
14. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The healthy human blood microbiome: fact or fiction? *Front. Cell. Infect. Microbiol.* 2019;9:148-
15. Regev-Yochay G, Raz M, Dagan R et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. *Clin. Infect. Dis.* 2004;38:632-639.
16. Wylie KM, Mihindukulasuriya KA, Zhou Y et al. Metagenomic analysis of double-stranded DNA viruses in healthy adults. *BMC. Biol.* 2014;12:71-
17. Berntsson M, Dubicanac L, Tunback P et al. Frequent detection of cytomegalovirus and Epstein–Barr virus in cervical secretions from healthy young women. *Acta Obstet. Gynecol. Scand.* 2013;92:706-710.
18. Xu F, Schillinger JA, Sternberg MR et al. Seroprevalence and coinfection with herpes simplex virus type 1 and type 2 in the United States, 1988-1994. *J. Infect. Dis.* 2002;185:1019-1024.
19. Pebody RG, Andrews N, Brown D et al. The seroepidemiology of herpes simplex virus type 1 and 2 in Europe. *Sex. Transm. Infect.* 2004;80:185-191.
20. Moustafa A, Xie C, Kirkness E et al. The blood DNA virome in 8,000 humans. *PLoS Pathog.* 2017;13:e1006292-
21. Moffatt MF, Cookson WO. The lung microbiome in health and disease. *Clin. Med. (Lond.).* 2017;17:525-529.
22. Itthitaetrakool U, Pinlaor P, Pinlaor S et al. Chronic *Opisthorchis viverrini* infection changes the liver microbiome and promotes Helicobacter growth. *PLoS One.* 2016;11:e0165798-
23. Whiteside SA, Razvi H, Dave S et al. The microbiome of the urinary tract – a role beyond infection. *Nat. Rev. Urol.* 2015;12:81-90.
24. Modena BD, Milam R, Harrison F et al. Changes in urinary microbiome populations correlate in kidney transplants with interstitial fibrosis and tubular atrophy documented in early surveillance biopsies. *Am. J. Transplant.* 2017;17:712-723.
25. Kowarsky M, Camunas-Soler J, Kertesz M et al. Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA. *Proc. Natl. Acad. Sci. U. S. A.* 2017;114:9623-9628.
26. Koelle DM, Norberg P, Fitzgibbon MP et al. Worldwide circulation of HSV-2 x HSV-1 recombinant strains. *Sci. Rep.* 2017;7:44084-
27. Mayer EA, Knight R, Mazmanian SK et al. Gut microbes and the brain: paradigm shift in neuroscience. *J. Neurosci.* 2014;34:15490-15496.
28. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. *Cell. Host. Microbe.* 2015;17:565-576.
29. Smith LK, Wissel EF. Microbes and the mind: how bacteria shape affect, neurological processes, cognition, social relationships, development, and pathology. *Perspect. Psychol. Sci.* 2019;14:397-418.
30. Baluska F, Mancuso S, Volkmann D, Barlow PW. The 'root-brain' hypothesis of Charles and Francis Darwin: Revival after more than 125 years. *Plant. Signal. Behav.* 2009;4:1121-1127.
31. Sequiera LW, Jennings LC, Carrasco LH et al. Detection of herpes-simplex viral genome in brain tissue. *Lancet.* 1979;2:609-612.
32. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J. Pathol.* 2009;217:131-138.
33. Frank AC, Saldierna Guzman JP, Shay JE. Transmission of bacterial endophytes. *Microorganisms.* 2017;5:E70-
34. Ilievski V, Zuchowska PK, Green SJ et al. Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice. *PLoS One* 2018;13:e0204941-
35. Dominy SS, Lynch C, Ermini F et al. *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* 2019;5:eaau3333-

36. Coelho C, Camacho E, Salas A et al. Intranasal Inoculation of Cryptococcus neoformans in mice produces nasal infection with rapid brain dissemination. *mSphere* 2019;4:e00483-19-

37. Frenkel D, Solomon B. Filamentous phage as vector-mediated antibody delivery to the brain. *Proc. Natl. Acad. Sci. U. S. A.* 2002;99:5675-5679.

38. Kim KS. Microbial translocation of the blood-brain barrier. *Int. J. Parasitol.* 2006;36:607-614.

39. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. *Nat. Rev. Microbiol.* 2008;6:625-634.

40. Pulzova L, Bhide MR, Andrej K. Pathogen translocation across the blood-brain barrier. *FEMS Immunol. Med. Microbiol.* 2009;57:203-213.

41. Wu Y, Du S, Johnson JL et al. Microglia and amyloid precursor protein coordinate control of transient *Candida cerebritis* with memory deficits. *Nat. Commun.* 2019;10:58-

42. Liu Y, Mittal R, Solis NV et al. Mechanisms of *Candida albicans* trafficking to the brain. *PLoS Pathog.* 2011;7:e1002305-

43. Tsiang H, Ceccaldi PE, Lycke E. Rabies virus infection and transport in human sensory dorsal root ganglia neurons. *J. Gen. Virol.* 1991;72:1191-1194.

44. MacGibeny MA, Koyuncu OO, Wirblich C et al. Retrograde axonal transport of rabies virus is unaffected by interferon treatment but blocked by emetine locally in axons. *PLoS Pathog.* 2018;14:e1007188-

45. Breen DP, Halliday GM, Lang AE. Gut–brain axis and the spread of alpha-synuclein pathology: vagal highway or dead end? *Mov. Disord.* 2019;34:307-316.

46. Bearer EL, Breakefield XO, Schuback D et al. Retrograde axonal transport of herpes simplex virus: evidence for a single mechanism and a role for tegument. *Proc. Natl. Acad. Sci. U. S. A.* 2000;97:8146-8150.

47. Miranda-Saksena M, Denes CE, Diefenbach RJ, Cunningham AL. Infection and transport of herpes simplex virus type 1 in neurons: role of the cytoskeleton. *Viruses* 2018;10:E92-

48. Tomlinson AH, Esiri MM. Herpes simplex encephalitis. Immunohistological demonstration of spread of virus via olfactory pathways in mice. *J. Neurol. Sci.* 1983;60:473-484.

49. Jiang Y, Wei N, Zhu J et al. A new approach with less damage: intranasal delivery of tetracycline-inducible replication-defective herpes simplex virus type-1 vector to brain. *Neuroscience.* 2012;201:96-104.

50. Mogk S, Meiwes A, Bosselmann CM et al. The lane to the brain: how African trypanosomes invade the CNS. *Trends Parasitol.* 2014;30:470-477.

51. Bentivoglio M, Kristensson K, Rottenberg ME. Circumventricular organs andparasite neurotropism: neglected gates to the brain? *Front. Immunol.* 2018;9:2877-

52. Mackowiak PA. Microbial latency. *Rev. Infect. Dis.* 1984;6:649-668.

53. Parsek MR, Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* 2003;57:677-701.

54. Ezzat K, Pernemalm M, Palsson S et al. The viral protein corona directs viral pathogenesis and amyloid aggregation. *Nat. Commun.* 2019;10:2331-

55. Halford WP, Gebhardt BM, Carr DJ. Persistent cytokine expression in trigeminal ganglion latently infected with herpes simplex virus type 1. *J. Immunol.* 1996;157:3542-3549.

56. Shimeld C, Whiteland JL, Williams NA et al. Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1. *J. Gen. Virol.* 1997;78:3317-3325.

57. Halford WP, Gebhardt BM, Carr DJ. Acyclovir blocks cytokine gene expression in trigeminal ganglia latently infected with herpes simplex virus type 1. *Virology.* 1997;238:53-63.

58. Grinfeld E, Kennedy PG. Translation of varicella-zoster virus genes during human ganglionic latency. *Virus. Genes.* 2004;29:317-319.

59. Slots J, Slots H. Bacterial and viral pathogens in saliva: disease relationship and infectious risk. *Periodontol. 2000.* 2011;55:48-69.

60. Levin MJ. Varicella-zoster virus and virus DNA in the blood and oropharynx of people with latent or active varicella-zoster virus infections. *J. Clin. Virol.* 2014;61:487-495.

61. Shahzad R, Khan AL, Bilal S et al. What is rhere in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. *Front. Plant. Sci.* 2018;9:24-

62. Khan M, Seto D, Subramaniam R, Desveaux D. Oh, the places they'll go! A survey of phytopathogen effectors and their host targets. *Plant. J.* 2018;93:651-663.

63. Luo L, Lu D. Immunosuppression during Rhizobium-legume symbiosis. *Plant. Signal. Behav.* 2014;9:e28197-

64. Gooding LR. Virus proteins that counteract host immune defenses. *Cell.* 1992;71:5-7.

65. Gerlier D, Valentin H. Measles virus interaction with host cells and impact on innate immunity. *Curr. Top. Microbiol. Immunol.* 2009;329:163-191.

66. Dagna L, Pritchett JC, Lusso P. Immunomodulation and immunosuppression by human herpesvirus 6A and 6B. *Future. Virol.* 2013;8:273-287.

67. Nokta MA, Hassan MI, Loesch K, Pollard RB. Human cytomegalovirus-induced immunosuppression. Relationship to tumor necrosis factor-dependent release of arachidonic acid and prostaglandin E2 in human monocytes. *J. Clin. Invest.* 1996;97:2635-2641.

68. Rouse BT, Horohov DW. Immunosuppression in viral infections. *Rev. Infect. Dis.* 1986;8:850-873.

69. Lanska DJ. The Kluver–Bucy Syndrome. *Front. Neurol. Neurosci.* 2018;41:77-89.

70. Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic–pituitary–adrenocortical axis. *Endocr. Rev.* 1991;12:118-134.

71. Haas HS, Schauenstein K. Neuroimmunomodulation via limbic structures – the neuroanatomy of psychoimmunology. *Prog. Neurobiol.* 1997;51:195-222.

72. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol. Cell. Endocrinol.* 2011;335:2-13.

73. Balldin J, Gottfries CG, Karlsson I et al. Dexamethasone suppression test and serum prolactin in dementia disorders. *Br. J. Psychiatry.* 1983;143:277-281.

74. Weiner MF, Vobach S, Olsson K et al. Cortisol secretion and Alzheimer's disease progression. *Biol. Psychiatry.* 1997;42:1030-1038.

75. Kieny MP, Desmettre P, Soulebot JP, Lathe R. Rabies vaccine: traditional and novel approaches. *Prog. Vet. Microbiol. Immunol.* 1987;3:73-111.

76. Awasthi M, Parmar H, Patankar T, Castillo M. Imaging findings in rabies encephalitis. *Am. J. Neuroradiol.* 2001;22:677-680.

77. Mrak RE, Young L. Rabies encephalitis in humans: pathology, pathogenesis and pathophysiology. *J. Neuropathol. Exp. Neurol.* 1994;53:1-10.

78. Stein LT, Rech RR, Harrison L, Brown CC. Immunohistochemical study of rabies virus within the central nervous system of domestic and wildlife species. *Vet. Pathol.* 2010;47:630-633.

79. Lafon M. Rabies virus receptors. *J. Neurovirol.* 2005;11:82-87.

80. Wang J, Wang Z, Liu R et al. Metabotropic glutamate receptor subtype 2 is a cellular receptor for rabies virus. *PLoS Pathog.* 2018;14:e1007189-

81. Wiktor TJ, Doherty PC, Koprowski H. Suppression of cell-mediated immunity by street rabies virus. *J. Exp. Med.* 1977;145:1617-1622.

82. Hirai K, Kawano H, Mifune K et al. Suppression of cell-mediated immunity by street rabies virus infection. *Microbiol. Immunol.* 1992;36:1277-1290.

83. Kasempimolporn S, Tirawatnapong T, Saengseesom W et al. Immunosuppression in rabies virus infection mediated by lymphocyte apoptosis. *Jpn. J. Infect. Dis.* 2001;54:144-147.

84. Desforges M, Le CA, Dubeau P et al. Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? *Viruses.* 2019;12:E14-

85. Sorensen O, Dales S. In vivo and in vitro models of demyelinating disease: JHM virus in the rat central nervous system localized by in situ cDNA hybridization and immunofluorescent microscopy. *J. Virol.* 1985;56:434-438.

86. Kakizaki M, Kashiwazaki H, Watanabe R. Mutant murine hepatitis virus-induced apoptosis in the hippocampus. *Jpn. J. Infect. Dis.* 2014;67:9-16.

87. Siu KL, Chan CP, Kok KH et al. Suppression of innate antiviral response by severe acute respiratory syndrome coronavirus M protein is mediated through the first transmembrane domain. *Cell. Mol. Immunol.* 2014;11:141-149.

88. Li Y, Li H, Fan R et al. Coronavirus infections in the central nervous system and respiratory tract show distinct features in hospitalized children. *Intervirology.* 2016;59:163-169.

89. Zhou W, Wang W, Wang H et al. First infection by all four non-severe acute respiratory syndrome human coronaviruses takes place during childhood. *BMC. Infect. Dis.* 2013;13:433-

90. Zhou P, Yang X-L, Wang X-G et al. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *BioRxiv.* 2020;Published online January 23 2020:http://dx.doi.org/10.1101/2020.01.22.914952-

91. Brook CE, Dobson AP. Bats as 'special' reservoirs for emerging zoonotic pathogens. *Trends Microbiol.* 2015;23:172-180.

92. Luis AD, Hayman DT, O'Shea TJ et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proc. Biol. Sci.* 2013;280:20122753-

93. Olival KJ, Hosseini PR, Zambrana-Torrelio C et al. Host and viral traits predict zoonotic spillover from mammals. *Nature.* 2017;546:646-650.

94. Xie J, Li Y, Shen X et al. Dampened STING-dependent interferon activation in bats. *Cell Host Microbe.* 2018;23:297-301.

95. Sankowski R, Mader S, Valdes-Ferrer SI. Systemic inflammation and the brain: novel roles of genetic, molecular, and environmental cues as drivers of neurodegeneration. *Front. Cell. Neurosci.* 2015;9:28-

96. Johansson S, Bohman S, Radesater AC et al. *Salmonella* lipopolysaccharide (LPS) mediated neurodegeneration in hippocampal slice cultures. *Neurotox. Res.* 2005;8:207-220.

97. Zhang J, Li A, Song Z. Systemic LPS resulted in a transient hippocampus malfunction but a prolonged corpus callosum injury. *BMC. Anesthesiol.* 2017;17:105-

98. Ekdahl CT, Claasen JH, Bonde S et al. Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. U. S. A.* 2003;100:13632-13637.

99. Meli DN, Christen S, Leib SL, Tauber MG. Current concepts in the pathogenesis of meningitis caused by *Streptococcus pneumoniae.* *Curr. Opin. Infect. Dis.* 2002;15:253-257.

100. Uchiyama T, Kamagata Y, Yoshioka M. Mechanism of lipopolysaccharide-induced immunosuppression: immunological activity of B cell subsets responding to T-dependent or T-independent antigens in lipopolysaccharide-preinjected mice. *Infect. Immun.* 1984;45:367-371.

101. Pisa D, Alonso R, Rabano A et al. Different brain regions are infected with fungi in Alzheimer's disease. *Sci Rep.* 2015;5:15015-

102. Angulo I, Jimenez-Diaz MB, Garcia-Bustos JF et al. *Candida albicans* infection enhances immunosuppression induced by cyclophosphamide by selective priming of suppressive myeloid progenitors for NO production. *Cell. Immunol.* 2002;218:46-58.

103. Kirchner FR, Littringer K, Altmeier S et al. Persistence of *Candida albicans* in the Oral Mucosa Induces a Curbed Inflammatory Host Response That Is Independent of Immunosuppression. *Front. Immunol.* 2019;10:330-

104. Samojlowicz D, Twarowska-Malczynska J, Borowska-Solonynko A et al. Presence of *Toxoplasma gondii* infection in brain as a potential cause of risky behavior: a report of 102 autopsy cases. *Eur. J. Clin. Microbiol. Infect. Dis.* 2019;38:305-317.

105. Desmonts G, Couvreur J. Congenital toxoplasmosis. A prospective study of 378 pregnancies. *N. Engl. J. Med.* 1974;290:1110-1116.

106. Berdoy M, Webster JP, Macdonald DW. Fatal attraction in rats infected with *Toxoplasma gondii. Proc. Biol. Sci.* 2000;267:1591-1594.

107. Flegr J, Markos A. Masterpiece of epigenetic engineering– how *Toxoplasma gondii* reprogrammes host brains to change fear to sexual attraction. *Mol. Ecol.* 2014;23:5934-5936.

108. Berenreiterova M, Flegr J, Kubena AA, Nemec P. The distribution of *Toxoplasma gondii* cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PLoS One.* 2011;6:e28925-

109. Hari Dass SA, Vyas A. *Toxoplasma gondii* infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. *Mol. Ecol.* 2014;23:6114-6122.

110. Flegr J, Lenochova P, Hodny Z, Vondrova M. Fatal attraction phenomenon in humans: cat odour attractiveness increased for *Toxoplasma*-infected men while decreased for infected women. *PLoS Negl. Trop. Dis.* 2011;5:e1389-

111. Martinez VO, de Mendonca Lima FW, de Carvalho CF, Menezes-Filho JA. *Toxoplasma gondii* infection and behavioral outcomes in humans: a systematic review. *Parasitol. Res.* 2018;117:3059-3065.

112. Kobayashi A, Suzuki Y. Suppression of antibody responses by *Toxoplasma* infection in mice. *Zentralbl. Bakteriol. Mikrobiol. Hyg. A.* 1987;264:312-318.

113. Khan IA, Matsuura T, Kasper LH. IL-10 mediates immunosuppression following primary infection with *Toxoplasma gondii* in mice. *Parasite. Immunol.* 1995;17:185-195.

114. Kankova S, Holan V, Zajicova A et al. Modulation of immunity in mice with latent toxoplasmosis – the experimental support for the immunosuppression hypothesis of *Toxoplasma*-induced changes in reproduction of mice and humans. *Parasitol. Res.* 2010;107:1421-1427.

115. Capewell P, Cren-Travaille C, Marchesi F et al. The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *Elife* 2016;5:e17716-

116. Mogk S, Bosselmann CM, Mudogo CN et al. African trypanosomes and brain infection - the unsolved question. *Biol. Rev. Camb. Philos. Soc.* 2017;92:1675-1687.

117. Wolburg H, Mogk S, Acker S et al. Late stage infection in sleeping sickness. *PLoS One.* 2012;7:e34304-

118. Pentreath VW, Rees K, Owolabi OA et al. The somnogenic T lymphocyte suppressor prostaglandin D2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. *Trans. R. Soc. Trop. Med. Hyg.* 1990;84:795-799.

119. Feldstein A, Chang FH, Kucharski JM. Tryptophol, 5-hydroxytryptophol and 5-methoxytryptophol induced sleep in mice. *Life Sci.* 1970;9:323-329.

120. Ackerman SB, Seed JR. The effects of tryptophol on immune responses and it implications toward trypanosome-induced immunosuppression. *Experientia.* 1976;32:645-647.

121. Grimstad PR, Ross QE, Craig GB, Jr. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. II. Modification of mosquito feeding behavior by virus infection. *J. Med. Entomol.* 1980;17:1-7.

122. Jackson BT, Brewster CC, Paulson SL. La Crosse virus infection alters blood feeding behavior in *Aedes triseriatus* and Aedes albopictus (Diptera: Culicidae). *J. Med. Entomol.* 2012;49:1424-1429.

123. Gilden DH, Mahalingam R, Cohrs RJ, Tyler KL. Herpesvirus infections of the nervous system. *Nat. Clin. Pract. Neurol.* 2007;3:82-94.

124. Cornford ME, McCormick GF. Adult-onset temporal lobe epilepsy associated with smoldering herpes simplex 2 infection. *Neurology.* 1997;48:425-430.

125. Dunin-Wasowicz D, Kasprzyk-Obara J, Jurkiewicz E et al. Infantile spasms and cytomegalovirus infection: antiviral and antiepileptic treatment. *Dev. Med. Child. Neurol.* 2007;49:684-692.

126. Wipfler P, Dunn N, Beiki O et al. The viral hypothesis of mesial temporal lobe epilepsy – is human herpes virus-6 the missing link? A systematic review and meta-analysis. *Seizure.* 2018;54:33-40.

127. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. *J. Clin. Invest.* 2013;123:958-965.

128. Valiathan R, Ashman M, Asthana D. Effects of Ageing on the Immune System: Infants to Elderly. *Scand. J. Immunol.* 2016;83:255-266.

129. Stowe RP, Kozlova EV, Yetman DL et al. Chronic herpesvirus reactivation occurs in aging. *Exp. Gerontol.* 2007;42:563-570.

130. Stowe RP, Peek MK, Cutchin MP, Goodwin JS. Reactivation of herpes simplex virus type 1 is associated with cytomegalovirus and age. *J. Med. Virol.* 2012;84:1797-1802.

131. Thomas SL, Hall AJ. What does epidemiology tell us about risk factors for herpes zoster? *Lancet. Infect. Dis.* 2004;4:26-33.

132. Itzhaki RF, Lathe R, Balin BJ et al. Microbes and Alzheimer's disease. *J. Alzheimers. Dis.* 2016;51:979-984.

133. Fischer 0. Die presbyophrene Demenz, deren anatomische Grundlage und klinische Abgrenzung. *Zeitschrift Gesamte Neurologie Psychiatrie.* 1910;3:371-471.

134. MacDonald AB. Borrelia in the brains of patients dying with dementia. *JAMA.* 1986;256:2195-2196.

135. Miklossy J. Alzheimer's disease – a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. *J. Neuroinflammation.* 2011;8:90-

136. Branton WG, Ellestad KK, Maingat F et al. Brain microbial populations in HIV/AIDS: alpha-proteobacteria predominate independent of host immune status. *PLoS One.* 2013;8:e54673-

137. Itzhaki RF. Herpes simplex virus type 1 and Alzheimer's disease: increasing evidence for a major role of the virus. *Front Aging Neurosci.* 2014;6:202-

138. Emery DC, Shoemark DK, Batstone TE et al. 16S rRNA next generation sequencing analysis shows bacteria in Alzheimer's post-mortem brain. *Front. Aging Neurosci.* 2017;9:195-

139. Balin BJ, Hammond CJ, Little CS et al. *Chlamydia pneumoniae*: an etiologic agent for late-onset dementia. *Front. Aging. Neurosci.* 2018;10:302-

140. Readhead B, Haure-Mirande JV, Funk CC et al. Multiscale analysis of independent Alzheimer's cohorts finds disruption of molecular, genetic, and clinical networks by human herpesvirus. *Neuron* 2018;99:64-82.

141. Tzeng N-S, Chung CH, Lin FH et al. Antiherpetic medications and reduced risk of dementia in patients with herpes simplex virus infections - a nationwide population-based cohort study in Taiwan. *Neurotherapeutics* 2018;15:417-429.

142. Lin WY, Lin MS, Weng YH et al. Association of antiviral therapy with risk of Parkinson disease in patients with chronic hepatitis C virus infection. *JAMA Neurol.* 2019;76:1019-1027.

143. Park SC, Moon JC, Shin SY et al. Functional characterization of alpha-synuclein protein with antimicrobial activity. *Biochem. Biophys. Res. Commun.* 2016;478:924-928.

144. Olsen LK, Dowd E, McKernan DP. A role for viral infections in Parkinson's etiology. *Neuronal Signaling.* 2018;2:NS20170166-

145. Prusty BK, Gulve N, Govind S et al. Active HHV-6 infection of cerebellar purkinje cells in mood disorders. *Front. Microbiol.* 2018;9:1955-

146. Toro CT, Eliassen E, Prusty BK. Does infection of cerebellar Purkinje neurons with human herpes virus 6A or 6B (HHV-6) increase the risk of developing mood disorders? *Future. Microbiol.* 2019;14:85-88.

147. Lathe R, Singadia S, Jordan C, Riedel G. The interoceptive hippocampus: mouse brain endocrine receptor expression highlights a dentate gyrus (DG)-cornu ammonis (CA) challenge-sufficiency axis. *PLoS One.* 2020;15:e0227575-

148. Smith JW, Urba WJ, Curti BD et al. The toxic and hematologic effects of interleukin-1 alpha administered in a phase I trial to patients with advanced malignancies. *J. Clin. Oncol.* 1992;10:1141-1152.

149. Capuron L, Ravaud A, Gualde N et al. Association between immune activation and early depressive symptoms in cancer patients treated with interleukin-2-based therapy. *Psychoneuroendocrinology.* 2001;26:797-808.

150. Valentine AD, Meyers CA, Kling MA et al. Mood and cognitive side effects of interferon-alpha therapy. *Semin. Oncol.* 1998;25:39-47.

151. Raison CL, Demetrashvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. *CNS. Drugs.* 2005;19:105-123.

152. Exton MS, Baase J, Pithan V et al. Neuropsychological performance and mood states following acute interferon-beta-1b administration in healthy males. *Neuropsychobiology.* 2002;45:199-204.

153. Creaven PJ, Plager JE, Dupere S et al. Phase I clinical trial of recombinant human tumor necrosis factor. *Cancer. Chemother. Pharmacol.* 1987;20:137-144.

154. Bullmore E. *The Inflamed Mind.* London: Short Brooks; 2018.

155. Schizophrenia Working Group. Biological insights from 108 schizophrenia-associated genetic loci. *Nature.* 2014;511:421-427.

156. Sekar A, Bialas AR, de RH et al. Schizophrenia risk from complex variation of complement component 4. *Nature.* 2016;530:177-183.

157. Nemerow GR, Wolfert R, McNaughton ME, Cooper NR. Identification and characterization of the Epstein–Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). *J. Virol.* 1985;55:347-351.

158. Bottermann M, Foss S, Caddy SL et al. Complement C4 prevents viral infection through capsid inactivation. *Cell. Host. Microbe.* 2019;25:617-629.

159. St CD, Blackwood D, Muir W et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet.* 1990;336:13-16.

160. Millar JK, Wilson-Annan JC, Anderson S et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* 2000;9:1415-1423.

161. Kano SI, Hodgkinson CA, Jones-Brando L et al. Host-parasite interaction associated with major mental illness. *Mol. Psychiatry.* 2019;25:194-205.

162. Jones-Brando L, Torrey EF, Yolken R. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii. Schizophr. Res.* 2003;62:237-244.

163. Webster JP, Lamberton PH, Donnelly CA, Torrey EF. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour. *Proc. Biol. Sci.* 2006;273:1023-1030.

164. Maier L, Pruteanu M, Kuhn M et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature.* 2018;555:623-628.

165. Fond G, Macgregor A, Tamouza R et al. Comparative analysis of anti-toxoplasmic activity of antipsychotic drugs and valproate. *Eur. Arch. Psychiatry. Clin. Neurosci.* 2014;264:179-183.

166. Kern AB, Schiff BL. Smallpox vaccinations in the management of recurrent herpes simplex: a controlled evaluation. *J. Invest. Dermatol.* 1959;33:99-102.

167. Raju TN. The Nobel chronicles. 1927: Julius Wagner-Jauregg (1857–1940). *Lancet.* 1998;352:1714-

168. Bohnhoff M, DRAKE BL, Miller CP. Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. *Proc. Soc. Exp. Biol. Med.* 1954;86:132-137.

169. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature.* 2008;453:620-625.

170. King CA, Baillie J, Sinclair JH. Human cytomegalovirus modulation of CCR5 expression on myeloid cells affects susceptibility to human immunodeficiency virus type 1 infection. *J. Gen. Virol.* 2006;87:2171-2180.

171. Barton ES, White DW, Cathelyn JS et al. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature.* 2007;447:326-329.

172. Sasco AJ, Paffenbarger RS, Jr. Measles infection and Parkinson's disease. *Am. J. Epidemiol.* 1985;122:1017-1031.

173. Roossinck MJ. The good viruses: viral mutualistic symbioses. *Nat. Rev. Microbiol.* 2011;9:99-108.

174. Johnson J. Transmission of viruses from apparently healthy potatoes. *Research Bulletin 63 of the Agricultural Experiment Station of the University of Wisconsin.* 1926;

175. Davison AJ. Evolution of the herpesviruses. *Vet. Microbiol.* 2002;86:69-88.

176. Wertheim JO, Smith MD, Smith DM et al. Evolutionary origins of human herpes simplex viruses 1 and 2. *Mol. Biol. Evol.* 2014;31:2356-2364.

177. Burrel S, Boutolleau D, Ryu D et al. Ancient recombination events between human herpes simplex viruses. *Mol. Biol. Evol.* 2017;34:1713-1721.

178. Hull R, Harper G, Lockhart B. Viral sequences integrated into plant genomes. *Trends Plant Sci.* 2000;5:362-365.

179. Harper G, Hull R, Lockhart B, Olszewski N. Viral sequences integrated into plant genomes. *Annu. Rev. Phytopathol.* 2002;40:119-136.

180. Cheng S, Xian W, Fu Y et al. Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell.* 2019;179:1057-1067.

181. Hall CB, Caserta MT, Schnabel K et al. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. *Pediatrics.* 2008;122:513-520.

182. Tweedy J, Spyrou MA, Hubacek P et al. Analyses of germline, chromosomally integrated human herpesvirus 6A and B genomes indicate emergent infection and new inflammatory mediators. *J. Gen. Virol.* 2015;96:370-389.

183. Horie M, Kobayashi Y, Suzuki Y, Tomonaga K. Comprehensive analysis of endogenous bornavirus-like elements in eukaryote genomes. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2013;368:20120499-

184. Belyi VA, Levine AJ, Skalka AM. Unexpected inheritance: multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes. *PLoS Pathog.* 2010;6:e1001030-

185. Huang W, Tsai L, Li Y et al. Widespread of horizontal gene transfer in the human genome. *BMC Genomics.* 2017;18:274-
186. O'Donoghue MC, Murphy SE, Zamboni G et al. APOE genotype and cognition in healthy individuals at risk of Alzheimer's disease: A review. *Cortex.* 2018;104:103-123.
187. Trumble BC, Stieglitz J, Blackwell AD et al. Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden. *FASEB. J.* 2017;31:1508-1515.
188. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. *J. Psychiatr. Res.* 2015;63:1-9.
189. Haldane JB. Origin of man. *Nature.* 1955;176:169-170.
190. Shi Y. Caught red-handed: uric acid is an agent of inflammation. *J. Clin. Invest.* 2010;120:1809-1811.
191. Benros ME, Sorensen HJ, Nielsen PR et al. The Association between infections and general cognitive ability in young men - a nationwide study. *PLoS One.* 2015;10:e0124005-
192. Strachan DP. Hay fever, hygiene, and household size. *BMJ.* 1989;299:1259-1260.
193. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 2002;347:911-920.
194. Fox M, Knapp LA, Andrews PW, Fincher CL. Hygiene and the world distribution of Alzheimer's disease: epidemiological evidence for a relationship between microbial environment and age-adjusted disease burden. *Evol. Med. Public. Health.* 2013;2013:173-186.
195. Maher B. Personal genomes: the case of the missing heritability. *Nature* 2008;456:18-21.
196. Sandoval-Motta S, Aldana M, Martinez-Romero E, Frank A. The human microbiome and the missing heritability problem. *Front. Genet.* 2017;8:80-
197. Carbone KM. Borna disease virus and human disease. *Clin. Microbiol. Rev.* 2001;14:513-527.
198. Lathe R, Haas JG. Distribution of cellular HSV-1 receptor expression in human brain. *J. Neurovirol.* 2017;23:376-384.
199. Buenz EJ, Sauer BM, Lafrance-Corey RG et al. Apoptosis of hippocampal pyramidal neurons is virus independent in a mouse model of acute neurovirulent picornavirus infection. *Am. J. Pathol.* 2009;175:668-684.
200. Galippe V. Bactériologie. Note sur la présence des micro-organismes dans les tissus végétaux. *Journal des Connaissances Médicales.* 1887;1887:203-205 and 252-254-
201. Fernbach MA. De l'absence des microbes dans les tissus végétaux. *Annales de l'Institut Pasteur.* 1888;II:567-570.
202. Porchet-Hennere E. Ultrastructure of *Sarcocystis tenella*. I. Endozoite (after negative staining). *J. Protozool.* 1975;22:214-220.
203. Carroll G. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology.* 1988;68:2-9.
204. Pisa D, Alonso R, Fernandez-Fernandez AM et al. Polymicrobial infections in brain tissue from Alzheimer's disease patients. *Sci. Rep.* 2017;7:5559-
205. Miklossy J. Alzheimer's disease – a spirochetosis? *Neuroreport.* 1993;4:841-848.
206. Lang BF, Gray MW, Burger G. Mitochondrial genome evolution and the origin of eukaryotes. *Annu. Rev. Genet.* 1999;33:351-397.
207. Tyler HL, Triplett EW. Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annu. Rev. Phytopathol.* 2008;46:53-73.
208. Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 2013;37:634-663.
209. Jones JL, Kruszon-Moran D, Elder S et al. *Toxoplasma gondii* infection in the United States, 2011–2014. *Am. J. Trop. Med. Hyg.* 2018;98:551-557.
210. Flegr J, Prandota J, Sovickova M, Israili ZH. Toxoplasmosis – a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PLoS One.* 2014;9:e90203-
211. Hill DE, Chirukandoth S, Dubey JP. Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Anim. Health. Res. Rev.* 2005;6:41-61.
212. Chelius MK, Triplett EW. The diversity of Archaea and Bacteria in association with the roots of *Zea mays* L. *Microb. Ecol.* 2001;41:252-263.
213. Moissl-Eichinger C, Pausan M, Taffner J et al. Archaea are interactive components of complex microbiomes. *Trends Microbiol.* 2018;26:70-85.
214. Koskinen K, Pausan MR, Perras AK et al. First insights into the diverse human archaeome: specific detection of Archaea in the gastrointestinal tract, lung, and nose and on skin. *MBio* 2017;8:e00824-17-
215. Eckburg PB, Lepp PW, Relman DA. Archaea and their potential role in human disease. *Infect. Immun.* 2003;71:591-596.
216. Aminov RI. Role of archaea in human disease. *Front. Cell. Infect. Microbiol.* 2013;3:42-
217. Sogodogo E, Fellag M, Loukil A et al. Nine cases of methanogenic archaea in refractory sinusitis, an emerging clinical entity. *Front. Public. Health.* 2019;7:38-
218. Drancourt M, Nkamga VD, Lakhe NA et al. Evidence of archaeal methanogens in brain abscess. *Clin. Infect. Dis.* 2017;65:1-5.
219. Bastian FO, Rabson AS, Yee CL, Tralka TS. Herpesvirus hominis: isolation from human trigeminal ganglion. *Science.* 1972;178:306-307.
220. Warren KG, Devlin M, Gilden DH et al. Isolation of herpes simplex virus from human trigeminal ganglia, including ganglia from one patient with multiple sclerosis. *Lancet.* 1977;2:637-639.
221. Fraser NW, Lawrence WC, Wroblewska Z et al. Herpes simplex type 1 DNA in human brain tissue. *Proc. Natl. Acad. Sci. U. S. A.* 1981;78:6461-6465.
222. Jamieson GA, Maitland NJ, Wilcock GK et al. Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains. *J. Med. Virol.* 1991;33:224-227.
223. Elsner C, Dorries K. Evidence of human polyomavirus BK and JC infection in normal brain tissue. *Virology.* 1992;191:72-80.
224. Lin WR, Wozniak MA, Wilcock GK, Itzhaki RF. Cytomegalovirus is present in a very high proportion of brains from vascular dementia patients. *Neurobiol. Dis.* 2002;9:82-87.
225. Hassani A, Corboy JR, Al-Salam S, Khan G. Epstein-Barr virus is present in the brain of most cases of multiple sclerosis and may engage more than just B cells. *PLoS One.* 2018;13:e0192109-
226. Navarro F, Muniesa M. Phages in the human body. *Front. Microbiol.* 2017;8:566-
227. Tetz G, Tetz V. Bacteriophages as new human viral pathogens. *Microorganisms.* 2018;6:E54-

228. Huh H, Wong S, St Jean J, Slavcev R. Bacteriophage interactions with mammalian tissue: therapeutic applications. *Adv. Drug. Deliv. Rev.* 2019;145:4-17.

229. Szigeti-Buck K, Manuelidis L. Prokaryotic SPHINX replication sequences are conserved in mammalian brain and participate in neurodegeneration. *J. Cell. Biochem.* 2019;120:17687-17698.

230. Morley SA, Ahmad N, Nielsen BL. Plant organelle genome replication. *Plants.* 2019;8:E358-

231. Nibert ML, Vong M, Fugate KK, Debat HJ. Evidence for contemporary plant mitoviruses. *Virology.* 2018;518:14-24.

232. Gripper LB, Welburn SC. Neurocysticercosis infection and disease-A review. *Acta Trop.* 2017;166:218-224.

233. Prasad R, Shambhavi, Mishra OP et al. Cognitive and behaviour dysfunction of children with neurocysticercosis: a cross-sectional study. *J. Trop. Pediatr.* 2014;60:358-362.

234. Wilson RJ, Williamson DH, Preiser P. Malaria and other apicomplexans: the 'plant' connection. *Infect. Agents. Dis.* 1994;3:29-37.

235. Nair SC, Striepen B. What do human parasites do with a chloroplast anyway? *PLoS Biol.* 2011;9:e1001137-

236. Burki F, Roger AJ, Brown MW, Simpson AGB. The new tree of eukaryotes. *Trebds Ecol. Evol.* 2020;35:43-55.

237. Zuther E, Johnson JJ, Haselkorn R et al. Growth of *Toxoplasma gondii* is inhibited by aryloxyphenoxypropionate herbicides targeting acetyl-CoA carboxylase. *Proc. Natl. Acad. Sci. U. S. A.* 1999;96:13387-13392.

238. Balique F, Lecoq H, Raoult D, Colson P. Can plant viruses cross the kingdom border and be pathogenic to humans? *Viruses.* 2015;7:2074-2098.

239. Zhang T, Breitbart M, Lee WH et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biol.* 2006;4:e3-

240. Colson P, Richet H, Desnues C et al. Pepper mild mottle virus, a plant virus associated with specific immune responses, Fever, abdominal pains, and pruritus in humans. *PLoS One.* 2010;5:e10041-

241. Liu R, Vaishnav RA, Roberts AM, Friedland RP. Humans have antibodies against a plant virus: evidence from tobacco mosaic virus. *PLoS One.* 2013;8:e60621-

242. Ericsson AC, Franklin CL. Manipulating the gut microbiota: methods and challenges. *ILAR J.* 2015;56:205-217.

243. Knights D, Costello EK, Knight R. Supervised classification of human microbiota. *FEMS Microbiol. Rev.* 2011;35:343-359.

244. Javurek AB, Spollen WG, Ali AM et al. Discovery of a novel seminal fluid microbiome and influence of estrogen receptor alpha genetic status. *Sci. Rep.* 2016;6:23027-

245. Erwin JA, Marchetto MC, Gage FH. Mobile DNA elements in the generation of diversity and complexity in the brain. *Nat. Rev. Neurosci.* 2014;15:497-506.

246. Doyle GA, Crist RC, Karatas ET et al. Analysis of LINE-1 elements in DNA from postmortem brains of individuals with schizophrenia. *Neuropsychopharmacology.* 2017;42:2602-2611.

247. Morandi E, Tanasescu R, Tarlinton RE et al. The association between human endogenous retroviruses and multiple sclerosis: a systematic review and meta-analysis. *PLoS One.* 2017;12:e0172415-

248. Christensen T. Human endogenous retroviruses in neurologic disease. *APMIS* 2016;124:116-126.

249. Richt JA, Pfeuffer I, Christ M et al. Borna disease virus infection in animals and humans. *Emerg. Infect. Dis.* 1997;3:343-352.

250. Gosztonyi G, Ludwig H. Borna disease--neuropathology and pathogenesis. *Curr. Top. Microbiol. Immunol.* 1995;190:39-73.

251. Carbone KM, Rubin SA, Nishino Y, Pletnikov MV. Borna disease: virus-induced neurobehavioral disease pathogenesis. *Curr. Opin. Microbiol.* 2001;4:467-475.

252. Carbone KM, Park SW, Rubin SA et al. Borna disease: association with a maturation defect in the cellular immune response. *J. Virol.* 1991;65:6154-6164.

253. Ludlow M, Kortekaas J, Herden C et al. Neurotropic virus infections as the cause of immediate and delayed neuropathology. *Acta Neuropathol.* 2016;131:159-184.

254. Goodpasture EW. Herpetic infection, with especial reference to involvement of the nervous system. 1929. *Medicine. (Baltimore.).* 1993;72:125-132.

255. Baringer JR, Pisani P. Herpes simplex virus genomes in human nervous system tissue analyzed by polymerase chain reaction. *Ann. Neurol.* 1994;36:823-829.

256. Kopp SJ, Banisadr G, Glajch K et al. Infection of neurons and encephalitis after intracranial inoculation of herpes simplex virus requires the entry receptor nectin-1. *Proc. Natl. Acad. Sci U. S. A.* 2009;106:17916-17920.

257. Damasio AR, Van Hoesen GW. The limbic system and the localisation of herpes simplex encephalitis. *J. Neurol. Neurosurg. Psychiatry.* 1985;48:297-301.

258. Becker Y. HSV-1 brain infection by the olfactory nerve route and virus latency and reactivation may cause learning and behavioral deficiencies and violence in children and adults: a point of view. *Virus. Genes.* 1995;10:217-226.

259. Baker M, Noisakran S, Gebhardt BM et al. The relationship between interleukin-6 and herpes simplex virus type 1: implications for behavior and immunopathology. *Brain. Behav. Immun.* 1999;13:201-211.

260. Banks TA, Rouse BT. Herpesviruses – immune escape artists? *Clin. Infect. Dis.* 1992;14:933-941.

261. Morse SS. Mouse thymic necrosis virus: a novel murine lymphotropic agent. *Lab. Anim. Sci.* 1987;37:717-725.

262. Cappel R, Henry C, Thiry L. Experimental immunosuppression induced by herpes simplex virus. *Arch. Virol.* 1975;49:67-72.

263. Rinaldo CR, Jr., Torpey DJ, III. Cell-mediated immunity and immunosuppression in herpes simplex virus infection. *Immunodeficiency.* 1993;5:33-90.

264. Cleator GM, Klapper PE, Lewis AG et al. Specific neuro-radiological diagnosis of herpes encephalitis in an animal model. *Arch. Virol.* 1988;101:1-12.

265. Nicklas W, Bleich A, Maehler B. *Viral Infections of Laboratory Mice.* 2012;2nd edn:427-480.

266. Cusick MF, Libbey JE, Fujinami RS. Picornavirus infection leading to immunosuppression. *Future Virol.* 2014;9:475-482.

267. Teixeira LAS, Nogueira FPDS, Nascentes GAN. Prospective study of patients with persistent symptoms of dengue in Brazil. *Rev. Inst. Med. Trop. Sao. Paulo.* 2017;59:e65-

268. Yeo PS, Pinheiro L, Tong P et al. Hippocampal involvement in dengue fever. *Singapore. Med. J.* 2005;46:647-650.

269. Adams Waldorf KM, Nelson BR, Stencel-Baerenwald JE et al. Congenital Zika virus infection as a silent pathology with loss of neurogenic output in the fetal brain. *Nat. Med.* 2018;24:368-374.

270. Li H, Saucedo-Cuevas L, Regla-Nava JA et al. Zika virus infects neural progenitors in the adult mouse brain and alters proliferation. *Cell. Stem. Cell.* 2016;19:593-598.

271.   Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult schizophrenia following prenatal exposure to an influenza epidemic. *Arch. Gen. Psychiatry.* 1988;45:189-192.
272.   Kilbourne ED. *Influenza.* New York: Plenum; 1987.
273.   Flegr J, Horacek J. *Toxoplasma*-infected subjects report an obsessive-compulsive disorder diagnosis more often and score higher in obsessive-compulsive inventory. *Eur. Psychiatry.* 2017;40:82-87.
274.   Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am. J. Psychiatry.* 2010;167:261-280.
275.   Selten JP, Termorshuizen F. The serological evidence for maternal influenza as risk factor for psychosis in offspring is insufficient: critical review and meta-analysis. *Schizophr. Res.* 2017;183:2-9.
276.   Buka SL, Tsuang MT, Torrey EF et al. Maternal infections and subsequent psychosis among offspring. *Arch. Gen. Psychiatry.* 2001;58:1032-1037.
277.   Buka SL, Cannon TD, Torrey EF, Yolken RH. Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring. *Biol. Psychiatry.* 2008;63:809-815.
278.   Brown AS, Schaefer CA, Quesenberry CP, Jr. et al. No evidence of relation between maternal exposure to herpes simplex virus type 2 and risk of schizophrenia? *Am. J. Psychiatry.* 2006;163:2178-2180.
279.   Brown AS. The environment and susceptibility to schizophrenia. *Prog. Neurobiol.* 2011;93:23-58.
280.   Tanaka T, Matsuda T, Hayes LN et al. Infection and inflammation in schizophrenia and bipolar disorder. *Neurosci. Res.* 2017;115:59-63.
281.   Gurbuz O, Alatas G, Kurt E et al. Periodontal health and treatment needs among hospitalized chronic psychiatric patients in Istanbul, Turkey. *Community. Dent. Health.* 2011;28:69-74.
282.   Arnaiz A, Zumarraga M, Diez-Altuna I et al. Oral health and the symptoms of schizophrenia. *Psychiatry. Res.* 2011;188:24-28.
283.   Kebede B, Kemal T, Abera S. Oral health status of patients with mental disorders in southwest Ethiopia. *PLoS One.* 2012;7:e39142-
284.   Eltas A, Kartalci S, Eltas SD et al. An assessment of periodontal health in patients with schizophrenia and taking antipsychotic medication. *Int. J. Dent. Hyg.* 2013;11:78-83.
285.   Shetty S, Bose A. Schizophrenia and periodontal disease: an oro-neural connection? A cross-sectional epidemiological study. *J. Indian. Soc. Periodontol.* 2014;18:69-73.
286.   Morales-Chavez MC, Rueda-Delgado YM, Pena-Orozco DA. Prevalence of bucco-dental pathologies in patients with psychiatric disorders. *J. Clin. Exp. Dent.* 2014;6:e7-e11.
287.   Fawzi MM, El-Amin HM, Elafandy MH. Detection and quantification of *Porphyromonas gingivalis* from saliva of schizophrenia patients by culture and Taqman real-time PCR: a pilot study. *Life Sci. J.* 2011;8:65-74.
288.   Fuglewicz AJ, Piotrowski P, Stodolak A. Relationship between toxoplasmosis and schizophrenia: a review. *Adv. Clin. Exp. Med.* 2017;26:1031-1036.
289.   Tedford E, McConkey G. Neurophysiological changes induced by chronic *Toxoplasma gondii* infection. *Pathogens.* 2017;6:E19-
290.   Henriquez SA, Brett R, Alexander J et al. Neuropsychiatric disease and *Toxoplasma gondii* infection. *Neuroimmunomodulation.* 2009;16:122-133.
291.   Kamerkar S, Davis PH. *Toxoplasma* on the brain: understanding host-pathogen interactions in chronic CNS infection. *J. Parasitol. Res.* 2012;2012:589295-
292.   Vyas A, Sapolsky R. Manipulation of host behaviour by *Toxoplasma gondii*: what is the minimum a proposed proximate mechanism should explain? *Folia. Parasitol. (Praha).* 2010;57:88-94.
293.   Hurley RA, Taber KH. Latent Toxoplasmosis gondii: emerging evidence for influences on neuropsychiatric disorders. *J. Neuropsychiatry. Clin. Neurosci.* 2012;24:376-383.
294.   Lyons RE, McLeod R, Roberts CW. *Toxoplasma gondii* tachyzoite–bradyzoite interconversion. *Trends Parasitol.* 2002;18:198-201.
295.   Torrey EF, Bartko JJ, Lun ZR, Yolken RH. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr. Bull.* 2007;33:729-736.
296.   Carruthers VB, Suzuki Y. Effects of *Toxoplasma gondii* infection on the brain. *Schizophr. Bull.* 2007;33:745-751.
297.   Torrey EF, Bartko JJ, Yolken RH. *Toxoplasma gondii* and other risk factors for schizophrenia: an update. *Schizophr. Bull.* 2012;38:642-647.
298.   Sutterland AL, Fond G, Kuin A et al. Beyond the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta Psychiatr. Scand.* 2015;
299.   Mortensen PB, Norgaard-Pedersen B, Waltoft BL et al. *Toxoplasma gondii* as a risk factor for early-onset schizophrenia: analysis of filter paper blood samples obtained at birth. *Biol. Psychiatry.* 2007;61:688-693.
300.   Brown AS, Patterson PH. Maternal infection and schizophrenia: implications for prevention. *Schizophr. Bull.* 2011;37:284-290.
301.   Reynolds MA, Kruszon-Moran D, Jumaan A et al. Varicella seroprevalence in the U.S.: data from the National Health and Nutrition Examination Survey, 1999-2004. *Public. Health. Rep.* 2010;125:860-869.
302.   Bollaerts K, Riera-Montes M, Heininger U et al. A systematic review of varicella seroprevalence in European countries before universal childhood immunization: deriving incidence from seroprevalence data. *Epidemiol. Infect.* 2017;145:2666-2677.
303.   Dowd JB, Palermo T, Brite J et al. Seroprevalence of Epstein–Barr virus infection in U.S. children ages 6–19, 2003–2010. *PLoS One.* 2013;8:e64921-
304.   Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004. *Clin. Infect. Dis.* 2010;50:1439-1447.
305.   De Bolle L, Naesens L, De CE. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin. Microbiol. Rev.* 2005;18:217-245.
306.   Ward KN. The natural history and laboratory diagnosis of human herpesviruses-6 and -7 infections in the immunocompetent. *J. Clin. Virol.* 2005;32:183-193.
307.   Martro E, Bulterys M, Stewart JA et al. Comparison of human herpesvirus 8 and Epstein-Barr virus seropositivity among children in areas endemic and non-endemic for Kaposi's sarcoma. *J. Med. Virol.* 2004;72:126-131.

308. Butler LM, Were WA, Balinandi S et al. Human herpesvirus 8 infection in children and adults in a population-based study in rural Uganda. *J. Infect. Dis.* 2011;203:625-634.
309. David LA, Alm EJ. Rapid evolutionary innovation during an Archaean genetic expansion. *Nature.* 2011;469:93-96.