The Olfactory Bulb: An Immunosensory Effector Organ during Neurotropic Viral Infections

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Abstract

In 1935, the olfactory route was hypothesized to be a portal for virus entry into the central nervous system (CNS). This hypothesis was based on experiments in which nasophayngeal infection with poliovirus in monkeys was prevented from spreading to their CNS via transection of olfactory tracts between the olfactory neuroepithelium (ONE) of the nasal cavity and the olfactory bulb (OB). Since then, numerous neurotropic viruses have been observed to enter the CNS via retrograde transport along axons of olfactory sensory neurons whose cell bodies reside in the ONE. Importantly, this route of infection can occur even after subcutaneous inoculation of arboviruses that can cause encephalitis in humans. While the olfactory route is now accepted as an important pathway for viral entry into the CNS, it is unclear whether it provides a way for infection to spread to other brain regions. More recently, studies of antiviral innate and adaptive immune responses within the olfactory bulb suggest it provides early virologic control. Here we will review the data demonstrating that neurotropic viruses gain access to the CNS initially via the olfactory route with emphasis on findings that suggest the OB is a critical immunosensory effector organ that effectively clears virus.

Graphical Abstract

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Virus 🦠

Innate cytokines

Chemokines

Antigen presenting cell

Lymphocyte
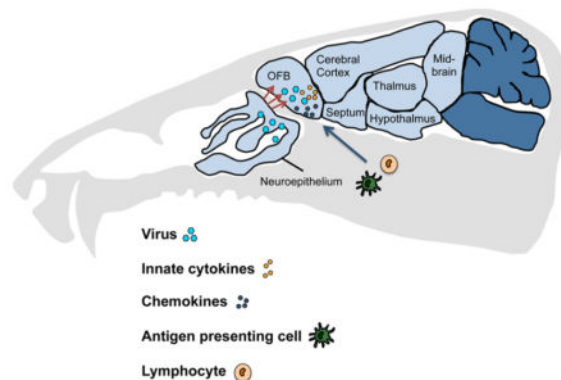
Keywords

Olfactory bulb; virus; encephalitis; olfactory sensory neurons; neuroinvasion

Viral infections of the central nervous system (CNS) are rare and often devastating, leading to death or permanent neurologic damage. Neurotropic viruses may gain access to the CNS via several routes including anterograde neuronal spread through sensory nerves,[1] across the blood-brain barrier (BBB) as free virions, or via the entry of infected immune cells.[2] However, studies examining the kinetics of neurotropic viral invasion after peripheral routes of inoculation have identified the olfactory bulb (OB) as the earliest site of CNS infection.[3] Indeed, the most direct conduit from the periphery to the brain occurs at the level of the olfactory neuroepithelium (ONE) within the nasal cavity, where cell bodies of olfactory sensory neurons (OSNs) reside and send their axons into the CNS to synapse with dendrites of mitral neurons within the olfactory bulb (OB). This route of entry was first investigated in the early 1900s in the context of poliovirus infection. Faber and Gebhardt first demonstrated that virus establishes its initial focus in the OB.[4] In 1936, Flexner reported that instillation of poliovirus into the nasal cavity, but not the stomach, leads to CNS manifestations of disease. [5] Faber and others later demonstrated that ablation of the ONE with zinc sulfate, which induces selective and rapid OSN necrosis,[6] prevents CNS infection.[5b] Evidence from a variety of animal models and human cases has since indicated that many DNA and RNA viruses, including herpesviruses,[7,7a] rhabdoviruses including vesicular stomatitis and rabies viruses (VSV, RABV),[8] neurotropic flaviviruses West Nile and Japanese encephalitis viruses (WNV, JEV),[9] paramyxoviruses parainfluenza and measles viruses (PIV, MV),[3f,10] alphaviruses Venezuelan Equine Encephalitis and chikungunya viruses (VEEV, CHIKV),[11] Bunyavirus LaCrosse virus (LACV),[12] and influenza A[13] are detected first within the OB during neuroinvasive infection. Several authors have also shown that virus within the OB is quickly cleared.[8a,14] This and the overall rarity of viral encephalitis suggests effective, neuroprotective immunity within the OB may quickly eliminate virus entering via this route, protecting the rest of the brain from infection. While the complete mechanisms of virologic control within the OB are unknown, studies demonstrate that innate immune mechanisms are specialized at this site, involving interactions between immune and neural cells and recruited leukocytes that influence viral infection and clearance at more distant brain regions. This Review will discuss the olfactory route of viral access to the CNS with

emphasis on evidence that OB innate immune response to viral infection of the CNS is an early event that controls viral entry and replication throughout the CNS.

ANATOMY AND VIRAL INFECTIONS OF THE OB

Viruses that utilize the olfactory nerve as entry into the CNS encounter many cell types progressing from the nasal cavity into the central olfactory nervous system. Cells of the ONE, which is located within the nasal cavity, include olfactory receptor neurons (ORN), supporting (sustentacular) cells, basal cells, microvillar cells, and Bowman's glands.[3j,14d,15] ORNs are unique among these cells since they establish the connective conduit between the nasal cavity and the CNS. These specialized bipolar neurons extend a single dendrite from their neuronal cell body into the ONE and an axon that crosses the basement membrane of the ONE and passes through the cribriform plate, which separates the nasal and cranial cavities. These axons terminate in the OB where they converge to form glomeruli and form synaptic contacts with neurons resident in the OB. ORN axons are supported by olfactory ensheathing cells (OECs) (i.e., Schwann cell-like glial cells) and are surrounded by mucus-secreting Bowman's glands, connective tissue, and blood and lymphatic vessels.[3j] Within the olfactory glomeruli, ORN axon terminals convey information to projected neurons such as tufted cells and mitral cells, which transmit information deeper into the CNS primarily the ipsilateral primary olfactory cortex.

Evidence of viral transmission along the olfactory route is based on studies in experimental animals and a few human cases. Entry into the CNS has been documented through detection of viral antigen within the olfactory mucosa and within the glomerular and mitral cell layers of the OB for many viruses including, influenza virus, HSV, poliovirus, paramyxoviruses, including canine distemper virus (CDV), Hendra virus, and Nipah virus, VSV, RABV, parainfluenza virus, adenoviruses, JEV, WNV, chikungunya virus, La Crosse virus, mouse hepatitis virus, and bunyaviruses which have been extensively reviewed previously[3j,16] (Table 1, Figure 1). Although rare, viral antigen has also been directly detected in ORNs within the olfactory mucosa following infection as is the case with influenza virus,[1i,3b,17] several herpesviruses, including HSV-1, bovine herpesvirus (BHV)-5, and equine herpesvirus (EHV)-1 and -9,[16] CDV,[18] VSV,[3h] and RABV,[19] suggesting that these viruses are transported through the axons of ORNs to access the OB.

Several studies have concluded that the initial infection of influenza A occurs at the olfactory bulb (OB).[17d,20] H5N1 is the most common form of influenza A virus detected in the olfactory bulb of patients and animal models.[20a,21] More recently, studies of HPAI H5N1 in animal models reported the entry of H5N1 virus primarily through the olfactory nerve with viral antigen detectable in the olfactory mucosa and olfactory receptor neuron.[17b,20b] Studies in H7N9-infected ferrets similarly detected viral antigen in the OB by 3 days postinfection.[22] Additional studies have demonstrated that influenza A virus infection of the OB is not strain specific.[3j,20c,21,22] Postmortem study of an immunocompromised human infant infected with H3N2 virus depicted the presence of viral load in the olfactory bulb with viral antigen detected in both neurons and glial cells.[3j] These studies strongly suggest that olfactory route is the primary route for CNS invasion in Influenza A mediated infection.

Previous studies have demonstrated that HSV, RABV, VSV, and influenza viruses are capable of transaxonal transport.[20a,23] Potentially viruses may also access the OB though direct infection of OECs or via channels in the cribriform plate. OECs are unique cells that form a continuous channel surrounding the axons of ORNs from the ONE as it passes through the cribriform plate and ends the OB. All together, numerous studies have shown that a variety of viruses are able to use the olfactory nerve as a shortcut into the CNS, however more comprehensive studies are necessary to define the mechanisms by which viruses use the olfactory nerve as a route of entry into the CNS.

INNATE IMMUNE RESPONSES OF THE OB DURING VIRAL INFECTION

Early studies examining transneuronal spread of viruses from the ONE to the OB reported that virus could no longer be detected at the latter site several days after infection. Investigators interpreted these findings as evidence that this brain region was unsatisfactory for growth, rather than postulating that it had specialized immune responses that efficiently cleared virus. In an early study, innate immune responses within the OB after application of VSV to the ONE included expression of nitric oxide and up-regulation of major histocompatibility antigens (MHC) I and II by infected astrocytes, microglia and endothelial cells.[14a] Additional studies utilizing viruses or pathogen associated molecular patterns (PAMPs) demonstrated OB expression of innate cytokines including interleukin 12,[3h] tumor necrosis factor (TNF)-α, TNFR1, interleukin (IL)-1β,[14f] and IkappaB.[24] For instance, recent studies with the flavivirus, tick-borne encephalitis virus (TBEV), and the alphavirus, Sindbis virus (SINV), confirm that pattern recognition receptor (PRR) signaling within the OB results in the upregulation of the innate cytokine interferon (IFN), which restricts viral replication in the CNS. This upregulation of IFN leads to increased expression of interferon regulatory factors (IRFs), which enhance the ability of IFN to control viral replication.[14e,25] Indeed, the expression of innate immune molecules within the OB results in rapid antiviral responses and improved survival. Similarly, intranasal inoculation of H1N1 virus, leads to upregulation of cytokines within 5–7 h post infection.[20c]

The source of some of these innate immune molecules has been traced to the OECs that envelope the olfactory nerves throughout their trajectory from the ONE to the OB. OECs, which have significant roles in OB development and repair,[26] are postulated to provide immunological protection against neutrotropic pathogens. Treatment of OECs with PAMPs or agonists of PRRs leads to production of iNOS,[27] nuclear translocation of nuclear factor kB (NK-kB) with cytokine expression.[28] Other studies implicate OB microglia in innate immune responses to PAMPs or damage associated molecular patterns (DAMPs) at this site. [29] The role of these innate immune molecules in the OB during viral infections is unclear. Studies using intranasal infection with lab adapted influenza A did not impact survival in mice deficient for iNOS, type I or II interferon (IFN) receptors, or transporter associated with antigen processing (TAP)1.[3b] However, persistent infection could be detected in 80% of surviving animals. These mice also had limited CNS recruitment of infiltrating lymphocytes suggesting that innate immunity in the OB limits viral persistence and induction of adaptive immunity within the CNS. The role of OB innate immune responses by neural and microglial cells in leukocyte trafficking and function is an active area of research.

LEUKOCYTE TRAFFICKING INTO THE OB DURING VIRAL ENCEPHALITIS

Although most viruses that invade the CNS via the olfactory nerve cause an inflammatory response characterized by an influx of neutrophils and mononuclear cells, there are few in-depth studies on their specific role. While it has been shown that type I IFN is critical for survival following intranasal infection with VSV it is also necessary for the induction of IL-12 by astrocytes and inflammatory monocytes.[8a,30] Multiple studies have demonstrated that the expression of IL-12 decreases viral titer within the OB and is strongly correlated with the rapid infiltration of both CD4+ and CD8+ T cells as well as NK cells.[8a,14a,b,30] Lymphocyte infiltration into the OB has been shown to be instrumental in limiting viral replication and spread beyond the OB as has been shown following T lymphocyte depletion during VSV[31] and MHV[32] infection. In addition, TAP-1 deficient mice were used to demonstrate that the ability to present antigens within the context of MHCI was crucial for T lymphocytes to maintain viral control within the OB following MHV infection of mice.[10] Interestingly, a recent study demonstrated that dendritic cells infiltrate into the OB during VSV infection[33] suggesting that these cells may play a role in the activation of recruited lymphocytes. In addition to T lymphocytes we recently observed that NK cell infiltration into the OB during WNV infection is crucial for viral control specifically within the hindbrain regions of the CNS (under review). Together these studies demonstrate the lymphocytic infiltration is instrumental in limiting viral replication and spread and that in their absence or inability to be full activated, viruses are able to spread from the OB into other regions of the CNS increasing damage.

CONCLUDING REMARKS

Many viruses are able to invade the CNS via the olfactory route. In general, if a viral infection is not contained locally (due to inefficient intrinsic and innate immune responses), it can spread to vital organs, causing severe pathologies. Viral spread within the CNS can be severe as well as deadly not only due to the fact that infected neurons may die, but also because of the immune-mediated pathology in the brain. The OB, although commonly recognized as a sensory organ for olfaction, also serves as an immunoeffector organ within the CNS. The CNS encounters an unknown number of pathogens primarily through the nasal cavity. Since this sensory organ is intimately exposed and particularly vulnerable it is likely there was high evolutionary pressure for neuroprotective mechanisms within the olfactory system. Use of genetic approaches to deplete OSNs[34] via temporally controlled diphtheria toxin A expression or conditional deletion of innate immune signaling in response to type I or II IFNs[35] will elegantly address the role of these neurons and innate immune responses in virologic control within the OB. In addition, the role of supporting cells, such as the OECs, during CNS viral infection is an area not well explored. It is unclear whether OECs are susceptible to certain viral infections or whether they have a definitive role in immunoprotection and spread of viruses from the OB to the rest of the CNS. As further studies are accomplished focusing on this vital yet vulnerable organ, it will become more clear that the OB is a complex sentinel immune organ that is instrumental in preventing passage of pathogens to other vital regions of the CNS preventing injury of neural cells and/or immunopathology.

Acknowledgments

References

1. (a) Antinone SE, Smith GA. Two modes of herpesvirus trafficking in neurons: membrane acquisition directs motion. J Virol. 2006; 80(22):11235–11240. [PubMed: 16971439] (b) Collins JJ, Lin CE, Berthoud HR, Papka RE. Vagal afferents from the uterus and cervix provide direct connections to the brainstem. Cell Tissue Res. 1999; 295(1):43–54. [PubMed: 9931352] (c) Cunningham AL, Diefenbach RJ, Miranda-Saksena M, Bosnjak L, Kim M, Jones C, Douglas MW. The cycle of human herpes simplex virus infection: virus transport and immune control. J Infect Dis. 2006; 194:S11–S18. [PubMed: 16921466] (d) Dups J, Middleton D, Yamada M, Monaghan P, Long F, Robinson R, Marsh GA, Wang LF. A new model for Hendra virus encephalitis in the mouse. PLoS One. 2012; 7(7):e40308. [PubMed: 22808132] (e) Hafezi W, Eing BR, Lorentzen EU, Thanos S, Kuhn JE. Reciprocal transmission of herpes simplex virus type 1 (HSV-1) between corneal epithelium and trigeminal neurites in an embryonic chick organ culture. FASEB J. 2002; 16(8):878–880. [PubMed: 11967226] (f) Penfold ME, Armati P, Cunningham AL. Axonal transport of herpes simplex virions to epidermal cells: evidence for a specialized mode of virus transport and assembly. Proc Natl Acad Sci U S A. 1994; 91(14):6529–6533. [PubMed: 7517552] (g) Samuel MA, Wang H, Siddharthan V, Morrey JD, Diamond MS. Axonal transport mediates West Nile virus entry into the central nervous system and induces acute flaccid paralysis. Proc Natl Acad Sci U S A. 2007; 104(43):17140–17145. [PubMed: 17939996] (h) Tsiang H, Lycke E, Ceccaldi PE, Ermine A, Hirardot X. The anterograde transport of rabies virus in rat sensory dorsal root ganglia neurons. J Gen Virol. 1989; 70(8):2075–2085. [PubMed: 2475581] (i) Yamada M, Bingham J, Payne J, Rookes J, Lowther S, Haining J, Robinson R, Johnson D, Middleton D. Multiple routes of invasion of wild-type Clade 1 highly pathogenic avian influenza H5N1 virus into the central nervous system (CNS) after intranasal exposure in ferrets. Acta Neuropathol. 2012; 124(4):505–516. [PubMed: 22763823]

2. (a) Chaves AJ, Vergara-Alert J, Busquets N, Valle R, Rivas R, Ramis A, Darji A, Majo N. Neuroinvasion of the highly pathogenic influenza virus H7N1 is caused by disruption of the blood brain barrier in an avian model. PLoS One. 2014; 9(12):e115138. [PubMed: 25506836] (b) Cosby SL, Brankin B. Measles virus infection of cerebral endothelial cells and effect on their adhesive properties. Vet Microbiol. 1995; 44(2–4):135–139. [PubMed: 8588307] (c) Coyne CB, Kim KS, Bergelson JM. Poliovirus entry into human brain microvascular cells requires receptor-induced activation of SHP-2. EMBO J. 2007; 26(17):4016–4028. [PubMed: 17717529] (d) Dai J, Wang P, Bai F, Town T, Fikrig E. Icam-1 participates in the entry of west nile virus into the central nervous system. J Virol. 2008; 82(8):4164–4168. [PubMed: 18256150] (e) Daniels BP, Holman DW, Cruz-Orengo L, Jujjavarapu H, Durrant DM, Klein RS. Viral pathogen-associated molecular patterns regulate blood-brain barrier integrity via competing innate cytokine signals. mBio. 2014; 5(5):e01476. [PubMed: 25161189] (f) Drevets DA, Leenen PJ. Leukocyte-facilitated entry of intracellular pathogens into the central nervous system. Microbes Infect. 2000; 2(13):1609–1618. [PubMed: 11113380] (g) Fletcher NF, Bexiga MG, Brayden DJ, Brankin B, Willett BJ, Hosie MJ, Jacque JM, Callanan JJ. Lymphocyte migration through the blood-brain barrier (BBB) in feline immunodeficiency virus infection is significantly influenced by the pre-existence of virus and tumour necrosis factor (TNF)-alpha within the central nervous system (CNS): studies using an in vitro feline BBB model. Neuropathol Appl Neurobiol. 2009; 35(6):592–602. [PubMed: 19486302] (h) Hurwitz AA, Berman JW, Lyman WD. The role of the blood-brain barrier in HIV infection of the central nervous system. Adv Neuroimmunol. 1994; 4(3):249–256. [PubMed: 7533041] (i) Krakowka S, Cork LC, Winkelstein JA, Axthelm MK. Establishment of central nervous system infection by canine distemper virus: breach of the blood-brain barrier and facilitation by antiviral antibody. Vet Immunol Immunopathol. 1987; 17(1–4):471–482. [PubMed: 2963430] (j) Liu TH, Liang LC, Wang CC, Liu HC, Chen WJ. The blood-brain barrier in the cerebrum is the initial site for the Japanese encephalitis virus entering the central nervous system. J NeuroVirol. 2008; 14(6):514–521. [PubMed: 19023687] (k) Maslin CL, Kedzierska K, Webster NL, Muller WA, Crowe SM.

Transendothelial migration of monocytes: the underlying molecular mechanisms and consequences of HIV-1 infection. Curr HIV Res. 2005; 3(4):303–317. [PubMed: 16250878] (l) Miner JJ, Daniels BP, Shrestha B, Proenca-Modena JL, Lew ED, Lazear HM, Gorman MJ, Lemke G, Klein RS, Diamond MS. The TAM receptor Mertk protects against neuroinvasive viral infection by maintaining blood-brain barrier integrity. Nat Med. 2015; 21(12):1464–1472. [PubMed: 26523970] (m) Miner JJ, Diamond MS. Mechanisms of restriction of viral neuroinvasion at the blood-brain barrier. Curr Opin Immunol. 2016; 38:18–23. [PubMed: 26590675] (n) Ohka S, Nomoto A. The molecular basis of poliovirus neuro-virulence. Dev Biol (Basel). 2001; 105:51–58. [PubMed: 11763337] (o) Roe K, Orillo B, Verma S. West Nile virus-induced cell adhesion molecules on human brain microvascular endothelial cells regulate leukocyte adhesion and modulate permeability of the in vitro blood-brain barrier model. PLoS One. 2014; 9(7):e102598. [PubMed: 25036379] (p) Wang S, Welte T, McGargill M, Town T, Thompson J, Anderson JF, Flavell RA, Fikrig E, Hedrick SM, Wang T. Drak2 contributes to West Nile virus entry into the brain and lethal encephalitis. J Immunol. 2008; 181(3):2084–2091. [PubMed: 18641347] (q) Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med. 2004; 10(12):1366–1373. [PubMed: 15558055]

3. (a) Allan GM, McNeilly F, Walker I, Linne T, Moreno-Lopez J, Hernandez P, Kennedy S, Carroll BP, Herron B, Foster JC, Adair B. A sequential study of experimental porcine paramyxovirus (LPMV) infection of pigs: immunostaining of cryostat sections and virus isolation. J Vet Diagn Invest. 1996; 8(4):405–413. [PubMed: 8953523] (b) Aronsson F, Robertson B, Ljunggren HG, Kristensson K. Invasion and persistence of the neuroadapted influenza virus A/ WSN/33 in the mouse olfactory system. Viral Immunol. 2003; 16(3):415–423. [PubMed: 14583155] (c) Charles PC, Walters E, Margolis F, Johnston RE. Mechanism of neuroinvasion of Venezuelan equine encephalitis virus in the mouse. Virology. 1995; 208(2):662–671. [PubMed: 7747437] (d) Dinn JJ. Transolfactory spread of virus in herpes simplex encephalitis. Br Med J. 1980; 281(6252):1392.(e) Durrant DM, Daniels BP, Pasieka T, Dorsey D, Klein RS. CCR5 limits cortical viral loads during West Nile virus infection of the central nervous system. J Neuroinflammation. 2015; 12:233. [PubMed: 26667390] (f) Mori I, Komatsu T, Takeuchi K, Nakakuki K, Sudo M, Kimura Y. Parainfluenza virus type 1 infects olfactory neurons and establishes long-term persistence in the nerve tissue. J Gen Virol. 1995; 76(5):1251–1254. [PubMed: 7730810] (g) Munster VJ, Prescott JB, Bushmaker T, Long D, Rosenke R, Thomas T, Scott D, Fischer ER, Feldmann H, de Wit E. Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. Sci Rep. 2012; 2:736. [PubMed: 23071900] (h) Reiss CS, Plakhov IV, Komatsu T. Viral replication in olfactory receptor neurons and entry into the olfactory bulb and brain. Ann N Y Acad Sci. 1998; 855:751–761. [PubMed: 9929681] (i) Ryzhikov AB, Ryabchikova EI, Sergeev AN, Tkacheva NV. Spread of Venezuelan equine encephalitis virus in mice olfactory tract. Arch Virol. 1995; 140(12):2243–2254. [PubMed: 8572944] (j) van Riel D, Leijten LM, Verdijk RM, GeurtsvanKessel C, van der Vries E, van Rossum AM, Osterhaus AD, Kuiken T. Evidence for influenza virus CNS invasion along the olfactory route in an immunocompromised infant. J Infect Dis. 2014; 210(3):419–423. [PubMed: 24550441]

4. Faber HK, Gebhardt LP. Localizations of the Virus of Poliomyelitis in the Central Nervous System during the Preparalytic Period, after Intranasal Instillation. J Exp Med. 1933; 57(6):933–954. [PubMed: 19870171]

5. (a) Flexner S. Respiratory Versus Gastro-Intestinal Infection in Poliomyelitis. J Exp Med. 1936; 63(2):209–26. [PubMed: 19870468] (b) Faber HK, Silverberg RJ, Dong L. Poliomyelitis in the Cynomolgus Monkey: Iii. Infection by Inhalation of Droplet Nuclei and the Nasopharyngeal Portal of Entry, with a Note on This Mode of Infection in Rhesus. J Exp Med. 1944; 80(1):39–57. [PubMed: 19871397]

6. (a) Cancalon P. Degeneration and regeneration of olfactory cells induced by ZnSO4 and other chemicals. Tissue Cell. 1982; 14(4):717–733. [PubMed: 6189263] (b) Tronitskaia VT, Gladysheva OS. Morphofunctional research on the olfactory organ of the mouse after zinc sulfate exposure. Neirofiziologiia. 1987; 19(6):796–802. [PubMed: 3448494] (c) Matulionis DH. Light and electron microscopic study of the degeneration and early regeneration of olfactory epithelium in the mouse. Am J Anat. 1976; 145(1):79–99. [PubMed: 1246968]

7. (a) Esiri MM. Herpes simplex encephalitis. An immunohistological study of the distribution of viral antigen within the brain. J Neurol Sci. 1982; 54(2):209–226. [PubMed: 6284882] (b) Harberts E,

Yao K, Wohler JE, Maric D, Ohayon J, Henkin R, Jacobson S. Human herpesvirus-6 entry into the central nervous system through the olfactory pathway. Proc Natl Acad Sci U S A. 2011; 108(33): 13734–13739. [PubMed: 21825120]

8. (a) Christian AY, Barna M, Bi Z, Reiss CS. Host immune response to vesicular stomatitis virus infection of the central nervous system in C57BL/6 mice. Viral Immunol. 1996; 9(3):195–205. [PubMed: 8890478] (b) Conomy JP, Leibovitz A, McCombs W, Stinson J. Airborne rabies encephalitis: demonstration of rabies virus in the human central nervous system. Neurology. 1977; 27(1):67–69. [PubMed: 556820]

9. (a) Goverdhan MK, Kulkarni AB, Gupta AK, Tupe CD, Rodrigues JJ. Two-way cross-protection between West Nile and Japanese encephalitis viruses in bonnet macaques. Acta Virol. 1992; 36(3): 277–283. [PubMed: 1360756] (b) Suen WW, Prow NA, Hall RA, Bielefeldt-Ohmann H. Mechanism of West Nile virus neuroinvasion: a critical appraisal. Viruses. 2014; 6(7):2796–2825. [PubMed: 25046180]

10. Urbanska EM, Chambers BJ, Ljunggren HG, Norrby E, Kristensson K. Spread of measles virus through axonal pathways into limbic structures in the brain of TAP1 –/– mice. J Med Virol. 1997; 52(4):362–369. [PubMed: 9260681]

11. (a) Danes L, Kufner J, Hruskova J, Rychterova V. The role of the olfactory route on infection of the respiratory tract with Venezuelan equine encephalomyelitis virus in normal and operated Macaca rhesus monkeys. I. Results of virological examination. Acta Virol. 1973; 17(1):50–56. [PubMed: 4405396] (b) Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol. 2007; 88(9):2363–2377. [PubMed: 17698645]

12. Bennett RS, Cress CM, Ward JM, Firestone CY, Murphy BR, Whitehead SS. La Crosse virus infectivity, pathogenesis, and immunogenicity in mice and monkeys. Virol J. 2008; 5:25. [PubMed: 18267012]

13. (a) Park CH, Ishinaka M, Takada A, Kida H, Kimura T, Ochiai K, Umemura T. The invasion routes of neurovirulent A/Hong Kong/483/97 (H5N1) influenza virus into the central nervous system after respiratory infection in mice. Arch Virol. 2002; 147(7):1425–1436. [PubMed: 12111416] (b) Reinacher M, Bonin J, Narayan O, Scholtissek C. Pathogenesis of neurovirulent influenza A virus infection in mice. Route of entry of virus into brain determines infection of different populations of cells. Lab Invest. 1983; 49(6):686–692. [PubMed: 6656200]

14. (a) Bi Z, Barna M, Komatsu T, Reiss CS. Vesicular stomatitis virus infection of the central nervous system activates both innate and acquired immunity. J Virol. 1995; 69(10):6466–6642. [PubMed: 7545248] (b) Bi Z, Quandt P, Komatsu T, Barna M, Reiss CS. IL-12 promotes enhanced recovery from vesicular stomatitis virus infection of the central nervous system. J Immunol. 1995; 155(12): 5684–5689. [PubMed: 7499854] (c) Chesler DA, Dodard C, Lee GY, Levy DE, Reiss CS. Interferon-gamma-induced inhibition of neuronal vesicular stomatitis virus infection is STAT1 dependent. J NeuroVirol. 2004; 10(1):57–63. [PubMed: 14982729] (d) Kalinke U, Bechmann I, Detje CN. Host strategies against virus entry via the olfactory system. Virulence. 2011; 2(4):367–370. [PubMed: 21758005] (e) Kurhade C, Zegenhagen L, Weber E, Nair S, Michaelsen-Preusse K, Spanier J, Gekara NO, Kroger A, Overby AK. Type I Interferon response in olfactory bulb, the site of tick-borne flavivirus accumulation, is primarily regulated by IPS-1. J Neuroinflammation. 2016; 13(1):22. [PubMed: 26819220] (f) Leyva-Grado VH, Churchill L, Wu M, Williams TJ, Taishi P, Majde JA, Krueger JM. Influenza virus- and cytokine-immunoreactive cells in the murine olfactory and central autonomic nervous systems before and after illness onset. J Neuroimmunol. 2009; 211(1–2):73–83. [PubMed: 19410300]

15. Morrison EE, Costanzo RM. Morphology of the human olfactory epithelium. J Comp Neurol. 1990; 297(1):1–13. [PubMed: 2376627]

16. Mori I, Nishiyama Y, Yokochi T, Kimura Y. Olfactory transmission of neurotropic viruses. J NeuroVirol. 2005; 11(2):129–137. [PubMed: 16036791]

17. (a) Plourde JR, Pyles JA, Layton RC, Vaughan SE, Tipper JL, Harrod KS. Neurovirulence of H5N1 infection in ferrets is mediated by multifocal replication in distinct permissive neuronal cell regions. PLoS One. 2012; 7(10):e46605. [PubMed: 23056366] (b) Schrauwen EJ, Herfst S, Leijten LM, van Run P, Bestebroer TM, Linster M, Bodewes R, Kreijtz JH, Rimmelzwaan GF, Osterhaus AD, Fouchier RA, Kuiken T, van Riel D. The multibasic cleavage site in H5N1 virus is critical for systemic spread along the olfactory and hematogenous routes in ferrets. J Virol. 2012; 86(7):3975–3984. [PubMed: 22278228] (c) van den Brand JM, Stittelaar KJ, van Amerongen G, Reperant L,

de Waal L, Osterhaus AD, Kuiken T. Comparison of temporal and spatial dynamics of seasonal H3N2, pandemic H1N1 and highly pathogenic avian influenza H5N1 virus infections in ferrets. PLoS One. 2012; 7(8):e42343. [PubMed: 22905124] (d) Iwasaki T, Itamura S, Nishimura H, Sato Y, Tashiro M, Hashikawa T, Kurata T. Productive infection in the murine central nervous system with avian influenza virus A (H5N1) after intranasal inoculation. Acta Neuropathol. 2004; 108(6): 485–492. [PubMed: 15480712] (e) Yoshida T, Goshima F, Imai Y, Kohsaka S, Sugiyama T, Yoshida T, Yokochi T, Nishiyama Y, Kimura Y, Mori I. Olfactory receptor neurons prevent dissemination of neurovirulent influenza A virus into the brain by undergoing virus-induced apoptosis. J Gen Virol. 2002; 83(9):2109–2116. [PubMed: 12185263]

18. Rudd PA, Cattaneo R, von Messling V. Canine distemper virus uses both the anterograde and the hematogenous pathway for neuroinvasion. J Virol. 2006; 80(19):9361–9370. [PubMed: 16973542]

19. Lafay F, Coulon P, Astic L, Saucier D, Riche D, Holley A, Flamand A. Spread of the CVS strain of rabies virus and of the avirulent mutant AvO1 along the olfactory pathways of the mouse after intranasal inoculation. Virology. 1991; 183(1):320–330. [PubMed: 2053286]

20. (a) Jang H, Boltz D, Sturm-Ramirez K, Shepherd KR, Jiao Y, Webster R, Smeyne RJ. Highly pathogenic H5N1 influenza virus can enter the central nervous system and induce neuroinflammation and neurodegeneration. Proc Natl Acad Sci U S A. 2009; 106(33):14063–14068. [PubMed: 19667183] (b) Shinya K, Makino A, Hatta M, Watanabe S, Kim JH, Hatta Y, Gao P, Ozawa M, Le QM, Kawaoka Y. Subclinical brain injury caused by H5N1 influenza virus infection. J Virol. 2011; 85(10):5202–5207. [PubMed: 21389133] (c) Majde JA, Bohnet SG, Ellis GA, Churchill L, Leyva-Grado V, Wu M, Szentirmai E, Rehman A, Krueger JM. Detection of mouse-adapted human influenza virus in the olfactory bulbs of mice within hours after intranasal infection. J NeuroVirol. 2007; 13(5):399–409. [PubMed: 17994424]

21. Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine. 2008; 26(Suppl 4):D59–D66. [PubMed: 19230162]

22. Xu L, Bao L, Deng W, Dong L, Zhu H, Chen T, Lv Q, Li F, Yuan J, Xiang Z, Gao K, Xu Y, Huang L, Li Y, Liu J, Yao Y, Yu P, Li X, Huang W, Zhao X, Lan Y, Guo J, Yong W, Wei Q, Chen H, Zhang L, Qin C. Novel avian-origin human influenza A(H7N9) can be transmitted between ferrets via respiratory droplets. J Infect Dis. 2014; 209(4):551–556. [PubMed: 23990570]

23. (a) Koyuncu OO, Hogue IB, Enquist LW. Virus infections in the nervous system. Cell Host Microbe. 2013; 13(4):379–393. [PubMed: 23601101] (b) Smith G. Herpesvirus transport to the nervous system and back again. Annu Rev Microbiol. 2012; 66:153–176. [PubMed: 22726218] (c) Diefenbach RJ, Miranda-Saksena M, Douglas MW, Cunningham AL. Transport and egress of herpes simplex virus in neurons. Rev Med Virol. 2008; 18(1):35–51. [PubMed: 17992661] (d) Pernet O, Wang YE, Lee B. Henipavirus receptor usage and tropism. Curr Top Microbiol Immunol. 2012; 359:59–78. [PubMed: 22695915]

24. Mori K, Kaneko YS, Nakashima A, Nagatsu I, Takahashi H, Ota A. Peripheral lipopolysaccharide induces apoptosis in the murine olfactory bulb. Brain Res. 2005; 1039(1–2):116–129. [PubMed: 15781053]

25. Schultz KL, Vernon PS, Griffin DE. Differentiation of neurons restricts Arbovirus replication and increases expression of the alpha isoform of IRF-7. J Virol. 2015; 89(1):48–60. [PubMed: 25320290]

26. (a) Roet KC, Verhaagen J. Understanding the neural repair-promoting properties of olfactory ensheathing cells. Exp Neurol. 2014; 261:594–609. [PubMed: 24842489] (b) Ekberg JA, Amaya D, Mackay-Sim A, StJohn JA. The migration of olfactory ensheathing cells during development and regeneration. Neurosignals. 2012; 20(3):147–158. [PubMed: 22456085]

27. Harris JA, West AK, Chuah MI. Olfactory ensheathing cells: nitric oxide production and innate immunity. Glia. 2009; 57(16):1848–1857. [PubMed: 19455713]

28. Vincent AJ, Choi-Lundberg DL, Harris JA, West AK, Chuah MI. Bacteria and PAMPs activate nuclear factor kappaB and Gro production in a subset of olfactory ensheathing cells and astrocytes but not in Schwann cells. Glia. 2007; 55(9):905–916. [PubMed: 17427933]

29. (a) Lalancette-Hebert M, Phaneuf D, Soucy G, Weng YC, Kriz J. Live imaging of Toll-like receptor 2 response in cerebral ischaemia reveals a role of olfactory bulb microglia as modulators of inflammation. Brain. 2009; 132(4):940–954. [PubMed: 19153151] (b) Loseva E, Yuan TF,

Karnup S. Neurogliogenesis in the mature olfactory system: a possible protective role against infection and toxic dust. Brain Res Rev. 2009; 59(2):374–387. [PubMed: 19027790]

30. Komatsu T, Reiss CS. IFN-gamma is not required in the IL-12 response to vesicular stomatitis virus infection of the olfactory bulb. J Immunol. 1997; 159(7):3444–3452. [PubMed: 9317143]

31. Huneycutt BS, Bi Z, Aoki CJ, Reiss CS. Central neuropathogenesis of vesicular stomatitis virus infection of immunodeficient mice. J Virol. 1993; 67(11):6698–6706. [PubMed: 8105106]

32. Pearce BD, Hobbs MV, McGraw TS, Buchmeier MJ. Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo. J Virol. 1994; 68(9):5483–5495. [PubMed: 8057431]

33. D'Agostino PM, Kwak C, Vecchiarelli HA, Toth JG, Miller JM, Masheeb Z, McEwen BS, Bulloch K. Viral-induced encephalitis initiates distinct and functional CD103+ CD11b+ brain dendritic cell populations within the olfactory bulb. Proc Natl Acad Sci U S A. 2012; 109(16):6175–6180. [PubMed: 22474352]

34. Goldstein BJ, Goss GM, Hatzistergos KE, Rangel EB, Seidler B, Saur D, Hare JM. Adult c-Kit(+) progenitor cells are necessary for maintenance and regeneration of olfactory neurons. J Comp Neurol. 2015; 523(1):15–31. [PubMed: 25044230]

35. (a) Detje CN, Meyer T, Schmidt H, Kreuz D, Rose JK, Bechmann I, Prinz M, Kalinke U. Local type I IFN receptor signaling protects against virus spread within the central nervous system. J Immunol. 2009; 182(4):2297–2304. [PubMed: 19201884] (b) Lee SH, Carrero JA, Uppaluri R, White JM, Archambault JM, Lai KS, Chan SR, Sheehan KC, Unanue ER, Schreiber RD. Identifying the initiating events of anti-Listeria responses using mice with conditional loss of IFN-gamma receptor subunit 1 (IFNGR1). J Immunol. 2013; 191(8):4223–4234. [PubMed: 24048899]

36. (a) Gao P, Watanabe S, Ito T, Goto H, Wells K, McGregor M, Cooley AJ, Kawaoka Y. Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from humans in Hong Kong. J Virol. 1999; 73(4):3184–3189. [PubMed: 10074171] (b) Gubareva LV, McCullers JA, Bethell RC, Webster RG. Characterization of influenza A/HongKong/156/97 (H5N1) virus in a mouse model and protective effect of zanamivir on H5N1 infection in mice. J Infect Dis. 1998; 178(6):1592–1596. [PubMed: 9815209] (c) Lu X, Tumpey TM, Morken T, Zaki SR, Cox NJ, Katz JM. A mouse model for the evaluation of pathogenesis and immunity to influenza A (H5N1) viruses isolated from humans. J Virol. 1999; 73(7):5903–5911. [PubMed: 10364342] (d) Shinya K, Shimada A, Ito T, Otsuki K, Morita T, Tanaka H, Takada A, Kida H, Umemura T. Avian influenza virus intranasally inoculated infects the central nervous system of mice through the general visceral afferent nerve. Arch Virol. 2000; 145(1):187–195. [PubMed: 10664417]

37. (a) Al-Mubarak A, Zhou Y, Chowdhury SI. A glycine-rich bovine herpesvirus 5 (BHV-5) gE-specific epitope within the ectodomain is important for BHV-5 neurovirulence. J Virol. 2004; 78(9):4806–4816. [PubMed: 15078962] (b) Boggian I, Buzzacaro E, Calistri A, Calvi P, Cavaggioni A, Mucignat-Caretta C, Palu G. Asymptomatic herpes simplex type 1 virus infection of the mouse brain. J NeuroVirol. 2000; 6(4):303–313. [PubMed: 10951554] (c) Chowdhury SI, Onderci M, Bhattacharjee PS, Al-Mubarak A, Weiss ML, Zhou Y. Bovine herpesvirus 5 (BHV-5) Us9 is essential for BHV-5 neuropathogenesis. J Virol. 2002; 76(8):3839–3851. [PubMed: 11907224] (d) El-Habashi N, El-Nahass E, Fukushi H, Hibi D, Sakai H, Sasseville V, Yanai T. Experimental intranasal infection of equine herpesvirus 9 (EHV-9) in suckling hamsters: kinetics of viral transmission and inflammation in the nasal cavity and brain. J NeuroVirol. 2010; 16(3):242–248. [PubMed: 20500017] (e) Lee BJ, Weiss ML, Mosier D, Chowdhury SI. Spread of bovine herpesvirus type 5 (BHV-5) in the rabbit brain after intranasal inoculation. J NeuroVirol. 1999; 5(5):474–484. [PubMed: 10568884] (f) Milho R, Frederico B, Efstathiou S, Stevenson PG. A heparan-dependent herpesvirus targets the olfactory neuroepithelium for host entry. PLoS Pathog. 2012; 8(11):e1002986. [PubMed: 23133384] (g) Narita M, Uchimura A, Kawanabe M, Fukushi H, Hirai K. Invasion and spread of equine herpesvirus 9 in the olfactory pathway of pigs after intranasal inoculation. J Comp Pathol. 2001; 124(4):265–272. [PubMed: 11437502] (h) Shivkumar M, Milho R, May JS, Nicoll MP, Efstathiou S, Stevenson PG. Herpes simplex virus 1 targets the murine olfactory neuro-epithelium for host entry. J Virol. 2013; 87(19):10477–10488. [PubMed: 23903843]

38. (a) Kristensson K, Leestma J, Lundh B, Norrby E. Sendai virus infection in the mouse brain: virus spread and long-term effects. Acta Neuropathol. 1984; 63(2):89–95. [PubMed: 6328837] (b)

Lundh B, Kristensson K, Norrby E. Selective infections of olfactory and respiratory epithelium by vesicular stomatitis and Sendai viruses. Neuropathol Appl Neurobiol. 1987; 13(2):111–122. [PubMed: 3039392]

39. Weingartl H, Czub S, Copps J, Berhane Y, Middleton D, Marszal P, Gren J, Smith G, Ganske S, Manning L, Czub M. Invasion of the central nervous system in a porcine host by nipah virus. J Virol. 2005; 79(12):7528–7234. [PubMed: 15919907]

40. Phillips AT, Stauft CB, Aboellail TA, Toth AM, Jarvis DL, Powers AM, Olson KE. Bioluminescent imaging and histopathologic characterization of WEEV neuroinvasion in outbred CD-1 mice. PLoS One. 2013; 8(1):e53462. [PubMed: 23301074]

41. Roy CJ, Reed DS, Wilhelmsen CL, Hartings J, Norris S, Steele KE. Pathogenesis of aerosolized Eastern Equine Encephalitis virus infection in guinea pigs. Virol J. 2009; 6:170. [PubMed: 19852817]

42. Plakhov IV, Arlund EE, Aoki C, Reiss CS. The earliest events in vesicular stomatitis virus infection of the murine olfactory neuroepithelium and entry of the central nervous system. Virology. 1995; 209(1):257–262. [PubMed: 7747478]

43. Sabin AB, Olitsky PK. Fate of Nasally Instilled Poliomyelitis Virus in Normal and Convalescent Monkeys with Special Reference to the Problem of Host to Host Transmission. J Exp Med. 1938; 68(1):39–62. [PubMed: 19870774]

44. Yamada M, Nakamura K, Yoshii M, Kaku Y, Narita M. Brain lesions induced by experimental intranasal infection of Japanese encephalitis virus in piglets. J Comp Pathol. 2009; 141(2–3):156–162. [PubMed: 19523649]

45. Monath TP, Cropp CB, Harrison AK. Mode of entry of a neurotropic arbovirus into the central nervous system. Reinvestigation of an old controversy. Lab Invest. 1983; 48(4):399–410. [PubMed: 6300550]

46. (a) Brown AN, Kent KA, Bennett CJ, Bernard KA. Tissue tropism and neuroinvasion of West Nile virus do not differ for two mouse strains with different survival rates. Virology. 2007; 368(2):422–430. [PubMed: 17675128] (b) Klein RS. Regulation of neuroinflammation: the role of CXCL10 in lymphocyte infiltration during autoimmune encephalomyelitis. J Cell Biochem. 2004; 92(2):213–222. [PubMed: 15108349] (c) Nir Y, Beemer A, Goldwasser RA. West Nile Virus infection in mice following exposure to a viral aerosol. Br J Exp Pathol. 1965; 46(4):443–449. [PubMed: 5317964]

47. McMinn PC, Dalgarno L, Weir RC. A comparison of the spread of Murray Valley encephalitis viruses of high or low neuroinvasiveness in the tissues of Swiss mice after peripheral inoculation. Virology. 1996; 220(2):414–423. [PubMed: 8661392]
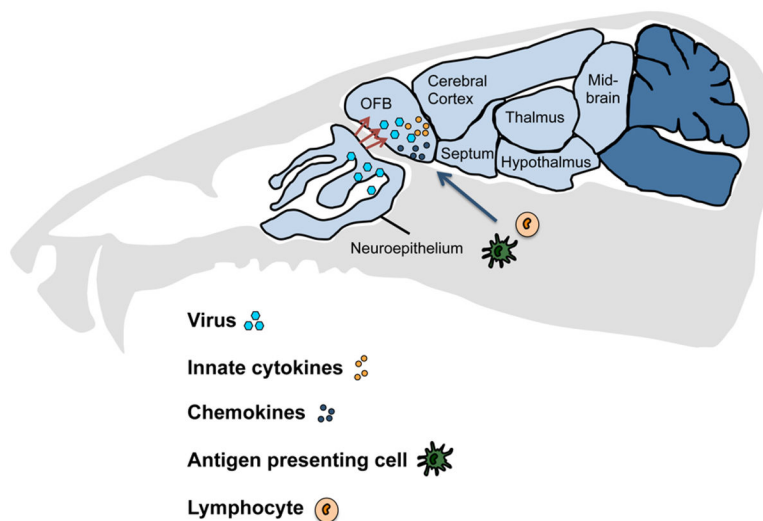
Figure 1.
Viral entry via the olfactory neuroepithelium induces antiviral responses in the olfactory bulb. Depicted is a cartoon of a mouse brain in which viral particles enter the CNS via axons of olfactory receptor neurons within the neuroepithelium of the nasal cavity. Infection of neurons within the olfactory bulb (OB) leads to expression of innate cytokines and chemokines, which recruit lymphocytes and antigen presenting cells.

Table 1

Neurotropic Virus Detection in the Olfactory Neuroepithelium (ONE), Olfactory Sensory Nerve (OSN), Olfactory Bulb (OB), and Other Regions of the CNS Following Infection in Various Animal Models via Isolation or by Immunohistochemistry (IHC)[a]

virus (strain)	route of infection	virus detection				ref
		ONE	OSN	OB	other CNS regions	
Influenza A (PR8)	i.n.		+	+	+	13b, 20c
Influenza A (R404BP)	i.n.		+	+		17e
Influenza A (H5N1)	i.n.		+	+	+	1i, 13a, 17a, d, 36
Influenza A (WSN/33)	i.n.	+		+	+	3b
Herpesvirus	i.n.	+	+	+	+	7b, 37
Parainfluenza (Sendai)	i.n.		+	+	n.d.	3f, 38
Nipah virus	i.n.	+	+	+	+	3g, 39
Hendra virus	i.n.		+	+	+	1d
Western Equine encephalitis virus	i.n.	+		+	+	40
Venezuelan Equine encephalitis virus	f.p.	+	+	+	+	3c
Eastern Equine encephalitis virus	i.n.	+	+	+	+	41
Rabies virus (CVS)	i.n.	+		+		19
Vesicular stomatitis virus	i.n.	+	+	+		42
Poliovirus	i.n.	+	+	+	+	4, 5b, 43
Japanese encephalitis virus	i.n.		+	+	n.d.	44
St. Louis encephalitis virus	i.p.	+		+	+	45
West Nile virus	i.p., f.p., i.n.	+	+	+	+	46
Murray Valley encephalitis virus	f.p.			+	+	47

[a]
n.d.: none detected.