Review

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Axonal spread of neuroinvasive viral infections

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Neuroinvasive viral infections invade the nervous system, often eliciting serious disease and death. Members of four viral families are both neuroinvasive and capable of transmitting progeny virions or virion components within the long neuronal extensions known as axons. Axons provide physical structures that enable viral infection to spread within the host while avoiding extracellular immune responses. Technological advances in the analysis of *in vivo* neural circuits, neuronal culturing, and live imaging of fluorescent fusion proteins have enabled an unprecedented view into the steps of virion assembly, transport, and egress involved in axonal spread. In this review we summarize the literature supporting anterograde (axon to cell) spread of viral infection, describe the various strategies of virion transport, and discuss the effects of spread on populations of neuroinvasive viruses.

Neuroinvasive viral infections and directional spread

The term 'neuroinvasive' can be used to describe the capacity of some viral infections to invade and spread within the host nervous system (see Glossary). Some neuroinvasive viruses infect the peripheral nervous system (PNS) with limited spread to the central nervous system (CNS) while others often spread to the CNS, eliciting significant disease [1,2]. Infection of the CNS may occur via the blood (hematogenous spread) or nerves (neurotropic spread). Many viral infections have the potential to be neuroinvasive but only a limited number do so. The rarity of neuronal spread reflects many variables, including strong intrinsic and innate defenses that protect the nervous system. However, one requirement to be neuroinvasive is that viral gene products, genomes, or virions must enter and move long distances in axons, a highly specialized neuronal compartment [3,4]. Since no viral genomes encode the molecular motors and cytoskeletal framework to move cargo in axons, they must encode adaptor or modifying gene products to repurpose neuronal components for axonal sorting and transport. In addition to directional movement in axons, for infection to spread to other neurons or cells, virions (or genomes) must leave the axon and enter cells in contact with the axons. As a result, spread of infections from axons tends to be localized, often taking apparent advantage of the tight associations of axonal membranes with their partner cells, including synaptic junctions, gap junctions, and glial/axonal interactions. The advantage of such focal axonal spread may be the avoidance of hostile extracellular immune effectors, including complement, antibodies, and phagocytes that have the potential to limit the intercellular spread of infection.

This review focuses on several neuroinvasive viruses with identified or the potential for axonal spread of infection. We review the data regarding virion component transport in axons, the sites of axonal egress, and the relationship to pathogenesis. Our goal is to identify properties shared by neuroinvasive viruses. We also emphasize how technology has improved our capacity to study the directional spread of viruses among synaptically connected neurons. Finally, we discuss recent data suggesting that spread of infection from axons represents a bottleneck that has the potential to limit the diversity of the transmitted viral population.

Four well-studied neuroinvasive viruses

Many virus families and subfamilies can be neuroinvasive but four groups are studied more intensely than others

Glossary

Axon: a specialized extension from neuronal cell bodies that facilitates the propagation of electrical signals.
Capsid/nucleocapsid (NC): an assembly of proteins that directly interacts with viral genomes. The structure often has icosahedral or helical symmetry. Enveloped viruses wrap capsids with host cell membranes either during intracellular assembly or through budding of virions from the plasma membrane.
Dynein: a family of molecular motors that facilitate minus end transport of cargo along microtubules. These motors are involved in transporting cargo, including retrograde of transporting viruses, from synapses back to the neuronal cell body.
Kinesin: a family of molecular motors that facilitate plus end-directed transport of cargo along microtubules. Certain kinesins have specialized functions within the cell; for example, kinesin-3 is associated with moving neurovesicles from the cell body to axon termini.
Neuroinvasive: viral infections with the capacity to infect neurons and spread to the CNS.
Synapse: a specialized point of contact between an axon and a dendrite or cell body. Defined by closely apposed membranes containing synaptic vesicles (in the axon) and neurotransmitter receptors (on the dendrite/cell body).
Varicosities: thickening of the axon often associated with disrupted microtubule tracks and accumulations of axonal vesicles.
Virion: the minimal infectious unit of a virus. It is a combination of proteins and sometimes host cell membrane that protects and transmits viral nucleic acids.

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with *in vivo* and *in vitro* systems to identify anterograde spread. These groups comprise: the Alpha Herpesviridae, particularly herpes simplex virus (HSV), varicella zoster virus (VZV), and pseudorabies virus (PRV); the Flaviviridae, specifically West Nile virus (WNV); the Rhabdoviridae, including vesicular stomatitis virus (VSV) and rabies virus (RV); and the Picornaviridae, specifically Theiler's murine encephalitis virus (TMEV).

Neuronal infections by the alpha herpesviruses HSV-1, and PRV have been studied using a combination of model systems including *in vivo* infection of defined neuronal circuits and *in vitro* infection of neuronal cultures, capable of distinguishing the directionality of viral spread within neurons [5–7]. Alpha herpesvirus infections can spread from infected neurons in both anterograde and retrograde directions (Box 1), with viral gene products controlling directional spread. Early characterization of an attenuated vaccine strain of PRV, known as PRV Bartha, revealed that three gene products dictate the direction of PRV infection and spread. In the absence of the glycoproteins gE and gI and the membrane-associated US9 protein, intracellular progeny virions do not enter axons and, as a result, anterograde spread to synaptically connected postsynaptic neurons or to epithelial cell targets is completely abrogated. Retrograde spread is only modestly affected, indicating that progeny virions can spread from neuronal cell bodies and dendrites to synaptically connected axons [8–10]. These same genes are important for the spread of HSV and VZV among connected neurons and cells, but the mechanism of spread may differ [11–13] In either case, anterograde spread of infection is important for diseases associated with both HSV and VZV infection [14,15].

Among rhabdoviruses, RV is the most extensively studied with regard to neuronal spread of infection. It is highly neuroinvasive and has often been said to be restricted to retrograde spread of infection [16,17]. There is evidence that RV is also capable of anterograde spread [18–20]. Using an *in vitro* system, researchers cultured dorsal root ganglion (DRG) neurons in a three-compartment device and observed RV transmission via axons to a distal compartment following infection of cell bodies [19]. Supporting *in vivo* evidence for RV anterograde spread comes from extensive antigenic RV reactivity in ipsilateral and contralateral DRG neurons following footpad injection [20]. This work suggests axonal transmission of either virions moving in the retrograde direction or progeny virions spreading from the neuronal soma. Similarly, the rhabdovirus VSV is competent for bidirectional transport in axons and spread between neurons, although its exquisite sensitivity to interferon and other innate antiviral immune responses typically restricts viral replication to sites of primary replication. Using an intranasal *in vivo* model of VSV infection, researchers showed extensive neuroinvasive spread of VSV into olfactory bulb neurons via axons [21].

There is no consensus on how the *Flavivirus* WNV infects and spreads in the nervous system [22,23]. However, an *in vitro* system of compartmentalized neuronal cultures may help to clarify this situation. In these compartmentalized cultures, embryonic superior cervical ganglion (SCG) neurons were grown such that cell bodies extended axons that penetrate underneath two physical barriers resulting in axonal termini in a separate hydrostatically isolated compartment. First pioneered in the 1970s to understand the role of nerve growth factor in neuronal development [24,25], the system was later adapted to study alpha herpesvirus transport and spread [26,27]. With this system, investigators showed that WNV infection can spread from axon terminals by long-distance axonal transport [28].

Persistent infection by the picornavirus TMEV is a remarkable example of specialized spread of infection from axons. TMEV infection results in a progressive disease of myelinating oligodendrocytes. This unusual mode of axonal spread of infection was identified using intravitreal eye infections, taking advantage of the restricted spread of the primary inoculum and direct infection of retinal ganglion cells that project axons into the optic nerve. In this model, infection spreads from infected axons to oligodendrocytes that myelinate and support the optic nerve [29]. The capacity of TMEV to infect and kill myelinating oligodendrocytes provides a disease model for the progressive demyelination of sensory and motor neurons [30,31]. In addition, the physical separation of the primary inoculum in the eye from secondary spread events in distant axons is strong support for the model of axon-to-oligodendrocyte cell spread.

Box 1. Distinguishing directional spread and virion transport in neurons

The directional transport of progeny virions within a neuron underlies the directional spread of infection within the PNS and CNS. Directional spread of infection in circuits of neurons is defined as follows.

Retrograde spread of infection. Proceeds from a postsynaptic neuron to uninfected presynaptic neurons. Virions attach to and enter axons, where virus particles undergo retrograde transport on microtubules toward the cell body using dynein motor complexes. Retrograde spread continues by transport of progeny virions into neuronal dendrites or cell bodies, with subsequent virion transmission to the presynaptic cell.

Anterograde spread of infection. Proceeds from a presynaptic neuron to an uninfected postsynaptic neuron. Progeny virions or virion components are sorted into axons and undergo anterograde transport away from the cell body on microtubules using kinesin motor complexes. Infection spreads to postsynaptic cells from axons. This review focuses on viral infections with a demonstrated capacity for anterograde transport and spread.

Moving virions and proteins into and out of axons

Although axons contain more than 95% of the neuron's cytoplasm, they are highly specialized compartments [32,33]. They not only transmit nerve impulses, but also move a remarkable number of specialized cellular structures along microtubules, including cytoskeletal elements, mitochondria, endosomes, lysosomes, and ribonucleoprotein complexes [34,35]. Axonal transport of virion components over long distances requires specificity in cargo loading and motor engagement. The subsequent egress of infectious particles and spread of viral infection then requires another level of specificity and targeting. For some viruses, particularly the Rhabdoviridae, it may be that viral structural components are sorted into axons and