Pathogen-inspired drug delivery to the central nervous system

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Abstract

Peripherally administered molecules encounter many barriers before they reach their target. Active agents must avoid degradation and clearance while traveling through blood, cells, and extracellular matrix. These processes will direct the distribution of molecules within tissue compartments, thus determining whether an agent is capable of exerting a biological effect in its target tissue. The blood-brain barrier (BBB) remains one of the prototypical examples of a tissue barrier that restricts the action of peripherally administered substances.1 Non-fenestrated brain endothelial cells possess cell-cell junctions that inhibit passive diffusion of circulating agents into the parenchyma, while efflux transporters, such as P-glycoprotein (Pgp), actively deplete the concentration of molecules that have achieved entry into cells.

Maintenance of BBB integrity is a critical physiological process in human health, since the concentrations of ions, hormones, and metabolic products must be regulated within tight ranges to achieve proper neuronal function. It is well-appreciated that many chemicals and biomolecules that are considered innocuous in peripheral tissue are in fact neurotoxic when present in the CNS. Accordingly, the BBB is evolutionarily conserved, and known to exclude nearly 98% of small molecules and 100% of large molecules.2 The BBB thus poses an often insurmountable blockade to the preclinical evaluation and clinical translation of novel therapies to treat CNS disease.

For as long as the human BBB has evolved to exclude bloodborne agents, pathogens have adopted a multitude of strategies to bypass it.3-5 CNS entry may be achieved through various routes, which are described in Figure 1. Pathogens or pathogen-derived substances are capable of reaching the CNS by direct passage across the BBB (transcellular), through spaces created between cells (paracellular), by carriage within peripherally circulating leukocytes that engage in immune surveillance of the CNS (Trojan horse), via the axons of neurons that originate the periphery (retrograde), or through regions of the brain with distinct BBB physiology (regions of altered permeability, for example, in the choroid plexus). We propose that the mechanisms utilized by human pathogens for achieving CNS tropism could be adapted to engineer the delivery of drugs to the brain. By linking drugs or drug-loaded carriers to ligands that instruct pathogen entry into the CNS, it may be possible to improve the action of drugs in the brain while reducing systemic dose.

In this review, we will discuss the directed mechanisms that viruses, bacteria, and toxins use to enter the CNS. Our goal is to identify ligand-mediated strategies that could be used to improve the brain-specific delivery of engineered nanocarriers, including polymers, lipids, biologically sourced materials, and imaging agents. Within each main class of virus or organism, examples of a tissue barrier that restricts the action of peripherally administered substances.1

Barriers to CNS Delivery

Keywords: Drug delivery, pathogen, brain, central nervous system, toxin, peptide

Abbreviations: DGL, dendrigraft poly-l-lysine; GABA, gamma-aminobutyric acid receptor; MMP-2, matrix metalloprotease-2; nAchR, nicotinic acetylcholine receptor; NCAM, neural cell adhesion molecule; NMDA, N-methyl-D-aspartate; PEG, polyethylene glycol; PEI, polyethylenimine
drug delivery literature are discussed. In the final section, we describe in vitro and in vivo studies where payloads have been tethered to a pathogen-derived agent to achieve specific delivery to neurons or the CNS.

**Directed Mechanisms of CNS Entry by Pathogens and Toxins**

**Viruses**

Viruses are perhaps nature’s first targeted nanocarriers, depositing their bioactive payload not only within specific cell populations, but also to precisely targeted intracellular locations. Viral particle size and structure play important roles in determining viral infectivity.\(^6\)\(^-\)\(^8\) Genomic material, either DNA or RNA, is encapsulated within a 20–750 nm viral capsid, a protein coat consisting of structurally similar subunits called capsomeres, whose composition ultimately dictates capsid size and shape. Viruses are classified structurally by the symmetry of their capsid, either as icosahedral, with 20 identical equilateral triangle faces arranged with 5:3:2 rotational symmetry, or helical, for which the protein coat is tightly wound around the viral genome to form a rod. Icosahedral viruses contain a minimum of 60 capsomeres with larger viruses containing additional capsomeres, termed hexameres, that are arranged along the flat faces of the icosahedron. Thus, additional volume for containing greater quantities of genomic material may be achieved by increasing the protein content of each face. Complex virus morphologies that incorporate both icosahedral and helical symmetries are also possible.

One of the first steps in viral anchorage to target tissue is their interaction with negatively charged glycosaminoglycans (GAGs) expressed on host cells, including heparin sulfate and chondroitin sulfate.\(^9\) Many viruses are naked, or non-enveloped, in which case the capsid proteins are exposed for direct interaction with target cells.\(^10\) Naked viruses replicate within the cytoplasm and, by necessity, must destroy the host cell to release viral particles. Other viruses are enveloped, whereby a host-derived lipid bilayer is obtained by passage of the viral particle through organelle or host cell membranes, which can alter both shape and flexibility of viral particles. Because the lipid bilayer is host-derived, the viral envelope contributes to immune evasion, facilitates interaction with target cells, and enables virus propagation to occur non-destructively.\(^11\)\(^-\)\(^12\) Importantly, viral and host proteins incorporate with the envelope, either orienting centrally (matrix proteins, which engage capsid with envelope) or outwardly (glycoprotein spikes, which engage envelope with host cell). Overall viral avidity for host cells will be a balance between the receptor affinity and the number and arrangement of those receptors. Some evidence suggests that increasing glycoprotein-receptor affinity above a critical threshold does not further increase viral uptake.\(^13\)

The interaction of a virus with host membrane proteins not only brings the virus in close proximity to the host cell, but can also trigger conformational changes to exposed proteins or activate molecular machinery for viral internalization and transport. Envelope proteins are therefore important in mediating the virus release in to the cytoplasm, which makes these proteins a first lead in identifying potential ligands for receptor mediated internalization of synthetic constructs.\(^14\)\(^-\)\(^15\) However, viral tropism is not determined exclusively by surface-host interactions. This is especially evident when considering that virus receptors are frequently distributed across a much broader range of tissues than what the virus is known to actually infect. Intracellular trafficking patterns and the relative abundance of necessary transcription factors for viral replication will contribute to selectivity of infection, thus determining overall virulence.

There are many examples of viruses that are known to be infective to the nervous system (Table 1).\(^14\) Viruses achieve physical passage into the brain by a variety of mechanisms, including via transcellular, paracellular, immune cell-mediated, and retrograde routes. Some of these viruses—notably, rabies virus, human immunodeficiency virus, and West Nile virus—possess CNS tropism that is associated with their severe health burden. However, virulence typically results from the presence and replication of the virus, as opposed to acute neurotoxicity of the substances that the virus produces. Many viruses specialize in immune evasion strategies that contribute to their CNS tropism. The viral proteins that facilitate CNS invasion are therefore an interesting starting point to direct the tropism of engineered nanocarriers to treat human disease.

**Rabies virus**

Rabies virus (RV) is well-adapted for CNS entry, and infection in humans without timely immunization is nearly unexceptionally fatal. Virus is introduced into muscle tissue through the saliva of an infected host, where it enters neurons for retrograde transport to the brain. Infection via aerosol exposure has also been reported.\(^15\) The virus is understood to enrich at the neuromuscular junction (NMJ) or sensory neurons, after which it...
Table 1. Examples of neurotropic viruses and their receptors

| Virus                        | Point of transmission | Known Receptor Involvement | Reference |
|------------------------------|-----------------------|---------------------------|-----------|
| Measles Virus                | Respiratory           | CD4, CXCR4, CCR5          |           |
| Cytomegalovirus              | Oronasal; Sexually transmitted | Heparin sulfate proteoglycans, EGFR, β1 integrins, TLR2 | 117, 118, 119 |
| Varicella Zoster Virus       | Oronasal              | Man6P, Insulin degrading enzyme |           |
| Human Immunodeficiency Virus | Bloodborne; Sexually transmitted | CD4, CXCR4, CCR5 | 120 |
| Rabies Virus                 | Peripheral tissue exposure; Aerosol | NCAM, NACHR, P75NTR | 16-19 |
| West Nile Virus              | Bloodborne            | TLR3, αVβ3 integrin        | 121, 122 |
| Polio Virus                  | Oronasal              | CD155                      | 123       |

HIV may be best known for its ability to infiltrate the human immune system (which is one of multiple mechanisms by which the virus achieves entry into the CNS21), the HIV-derived trans-activating regulatory protein (Tat) has gained notoriety in its own right.25 Tat (86–101 aa) is a small, positively charged protein that stabilizes transcription to support viral replication, and it is actively released from HIV-infected cells to produce a variety of biological effects. Among other functions, Tat is neuropsychotropic and immunosuppressive.23,24 Peptides derived from the full sequence are cell penetrating and potently capable of crossing the BBB. Of note, although the CNS is not the dominant target tissue for HIV virulence, Tat penetration of brain endothelial cells is well established in vitro and in vivo, with evidence presented for both active and passive (diffusion-mediated) mechanisms of entry.25,26 Three cell-surface domains have been identified for active interaction of Tat with endothelial cells, including GAGs, integrins, and the vascular endothelial growth factor receptor-2.27

Bacteria

Bacteria are ubiquitous in the human environment, displaying a broad diversity of sizes and shapes that have evolved over millions of years to facilitate their colonization and proliferation in a diverse set of environments. While bacteria are tremendously successful in achieving long-term and relatively innocuous or even beneficial residence within the human periphery, the presence of bacteria in the human CNS is almost always a severe health concern.

A typical bacterium is 500–5000 nm in length, taking on spherococcal (coci), rod-shaped (bacilli), or more complex morphologies (e.g., vibrio, spirochaetes, stella, haloarcula, or filamentous).28 Bacteria are enveloped by an exoskeletal cell wall, termed the saccus, which is composed of peptidoglycan, a sugar with alternating N-acetylglucosamine and N-acetylmuramic acid repeats.29 A variety of bacteria are capable of entering the CNS, either directly via exposed protein and receptor interactions, through immune cells, or by altering BBB permeability.4,30 It is important to note that CNS entry is not always the primary goal of bacterial pathogenesis, and in fact often results as a secondary infection (meningitis) that, if left untreated, will be rapidly fatal to the host.

Clostridium tetani and Clostridium botulinum

Perhaps the most widely appreciated neurotropic bacterial toxin is the tetanus toxin C-fragment (TTC), secreted by Clostridium tetani. Tetanus toxin enters the CNS via neuromuscular junctions to cause the spastic paralysis known as tetanus; with an LD50 of 1 ng/kg, intact toxin is quite deadly. However, when broken down into its 3 main components, one light chain (L, 50 kDa) and 2 heavy chains (H_N and H_C, 100kDa combined), independent functions for toxin pathophysiology are observed. TTC virulence originates from the L chain, which binds and cleaves the membrane protein VAMP and degrades synaptobrevin in inhibitory neurons, preventing the release of GABA and glycine and producing painful muscle spasms.31,32
The H₂ (C-terminal) and H₃ (N-terminal) chains alone are non-toxic, being responsible for different phases of neuronal transport of the toxin to target inhibitory neurons. The H₃ (TTC) fragment of tetanus toxin binds to highly enriched areas of polysialo-gangliosides on peripheral nerve terminals of motor neurons. Once endocytosed, it is transported by retrograde routes to synaptic terminals connecting to inhibitory interneurons located within the spinal cord. Once within the spinal cord, the toxin is endocytosed into the connecting neuron, where the H₃ fragment burrows into the vesicle membrane, translocating the disulfide-linked L chain into the cytosol of the inhibitory neuron.³³,³⁴

Given the neuronal-targeting action of the H fragments, TTC is an attractive candidate for facilitating drug delivery to neurons. However, one challenge to applying TTC in humans is that vaccination will promote rapid clearance of TTC linked agents by immune cells, which could prevent effective administration of TTC-linked agents. As of 2010, an estimated 64% of the United States population was reported by the CDC to have been vaccinated against tetanus.³⁵ Botulinum toxin (BoNT) is more potent than TTC, and immunization against the bacteria is infrequent in humans. Thus, the H₂ fragment of BoNT-A, which also presents strong neuronal tropism, would be expected to be a candidate for CNS delivery. However, BoNT-A is known to exert its primary effect on the peripheral nervous system on cholinergic nerve terminals. The question of whether BoNT-A can achieve CNS entry has not yet been resolved.³⁶,³⁷

**Borrelia, Escherichia coli, and others**

Members of the phylum spirochaetes include the neuroinvasive genera *Treponema* and *Borrelia*, which contain the species that cause syphilis and Lyme disease, respectively. Spirochaetes are long and slender bacteria, with a corkscrew-type, helical conformation that enables them to propel effectively through highly viscous fluids. The mechanisms that facilitate *Borrelia* infection in the CNS include immune evasion, ECM degradation via activation of host cell enzymes, and direct passage across the BBB. These activities are coordinated by a highly diverse set of surface proteins promoting pathogen interaction with host cells to achieve tissue tropism.³⁸,³⁹ *Borrelia* mode of entry into the CNS remains under discussion, with evidence presented for both paracellular (via opening of tight junctions) and transcellular (via receptor mediated mechanisms) movement across brain endothelial cells. *Borrelia* interacts with a range of extracellular or cell-surface-associated host molecules, including fibronectin, glycosaminoglycans, integrins, and receptors such as CD40.⁴⁰,⁴¹

Although it is a relatively rare complication of human infection, the bacterium *Escherichia coli* is capable of achieving physical entry by paracellular routes into the CNS to cause bacterial meningitis. Bacterial passage through the BBB is believed to involve rearrangement of the host cell actin cytoskeleton via activation of Rho GTPases,⁴²,⁴³ *E. coli* K1 invasion through human brain microvascular endothelial cells (HBMEC) was studied to identify ligands necessary for physical entry of the bacteria.⁴⁴ Outer-membrane protein A (OmpA) and type 1 fimbriae (FimH) on the surface of *E. coli* bind gp96 and CD48 on the surface of HBMEC, respectively, which will lead to cytoskeletal rearrangement to enable pathogen invasion.

The 37kDa/67kDa laminin receptor (LamR) has come to the forefront within the past decade as an important receptor for multiple neurotropic pathogens, both bacterial and viral (Table 2). *E. coli*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* are bacteria known to use LamR as a receptor for entry into the BBB; their presence in the CNS will produce bacterial meningitis.⁴⁵,⁴⁶ Ligand binding domains on these organisms for LamR are conserved structurally, rather than on an amino acid level, and are typically found on surface-exposed loops of porins within the bacterial membrane.⁴⁵,⁴⁷ Competitive binding studies have revealed that most bacterial ligands target the laminin binding site (residues 161-182) or an extracellular domain (residues 263-282) on LamR.⁴⁷

**Listeria**

In both healthy and diseased brain, peripherally circulating leukocytes enter the brain parenchyma for immune surveillance of the CNS.⁴⁸ Bacteria, viruses, and protozoa are thus capable of infecting leukocytes as a means to achieve BBB passage. This process is termed the “Trojan horse” mechanism, and is a well-characterized point of entry for Listeria monocytogenes. Multiple *in vitro* and *in vivo* studies have demonstrated the ability of *L. monocytogenes* to cross the BBB via infected monocytes, macrophages, or microglia.⁴⁹,⁵¹ *In vitro*, *L. monocytogenes* is capable of directly crossing HBMECs with the aid of bacterial internalin proteins, which are thought to be the mechanism used to cross intestinal epithelium following bacterial ingestion.⁵² In the presence of human serum, however, direct passage of *L. monocytogenes* through HBMECs is grossly inhibited.⁵⁰ These data suggest that entry is primarily mediated by circulating immune cells.

**Eukaryotes: secreted toxins**

Toxins secreted in animal venoms often contain complex mixtures of organic molecules and proteins that act in a variety of ways to affect cellular function. Spider venom, for example, contains over 10 million bioactive peptides, while cone snails...
produce over 1,000 conopeptides per species.\textsuperscript{53} Given this high biological diversity, venom-derived peptides are of intense interest for therapeutic applications.\textsuperscript{54} In nature, toxins are used for both defense and as a mechanism for immobilizing food sources, and thus venom-derived peptides must often be modified to circumvent toxicity while maintaining target specificity. The efficacy of native toxin action may also be improved by peptide engineering. For example, teprotide, a nonapeptide derived from the venom of the pit viper \textit{Bothrops jaraca}, was found to have antihypertensive effects by inhibiting angiotensin converting enzyme (ACE). Its lack of oral bioavailability prompted the isolation of the peptide’s active site, and further modifications eventually brought about captopril, an FDA-approved drug now prescribed for hypertension and congestive heart failure.

Bioactive peptides can alter cellular function via interaction with cell surface receptors or channels by occlusion or by modifying the properties of ion channel gates to interfere with the kinetics of channel opening. The diversity of venom-derived peptide structures is extremely broad; however, conserved function enables grouping. For example, many spider, cone snail, and snake derived neurotoxins that bind to calcium or sodium ion channels present a similar structural organization where the peptides form compact disulfide-bonded hydrophobic cores with short loops. This conserved structure is termed the inhibitor cystine knot motif (ICK).\textsuperscript{55} It is important to recognize that tertiary peptide structure may be an important component of bioactivity and should be considered while modifying peptide chemistry. For example, many types of neurotropic peptides contain a high number of disulfide bridges for increased stability in biological fluids.\textsuperscript{56} Although stability could be an advantage for maintaining bioactivity of ligands \textit{in vitro}, disulfide-linked peptides are more difficult to produce synthetically.

### Spiders

Voltage-gated ion channels, including calcium, potassium, and sodium, are a common target for neurotropic spider venoms. Hanatoxin1 (HaTx1) is a 35 amino acid peptide with an ICK motif extracted from the venom of the tarantula \textit{Grammostola spatulata}. HaTx1 inhibits the Kv2.1 potassium channel by altering gating energetics.\textsuperscript{57} Interestingly, HaTx1 also binds to voltage-gated calcium channels, suggesting that the receptor binding motif may be conserved among different ion channel types. Another class of potassium channel toxins contains the phrixotoxins, which are derived from the venom of the Chilean copper tarantula \textit{Phrixotrichus auratus} and block Kv4 channels in a voltage-dependent manner.\textsuperscript{58} There are millions of sodium channel toxins found in taxonomically diverse species, suggesting that this channel has been evolutionarily conserved and was potentially initiated at an early stage of venom gland evolution. For example, the d-HXTX-Ar1a toxin from the Sydney funnel-web spider, \textit{Atrax robustus}, inhibits activation of the sodium ion channel, while \mu-agatoxins from the American funnel spiders, \textit{Agalenopsis aperta}, change the voltage to more negative potentials for channel activation and opening.\textsuperscript{53} The venom of the \textit{Phoneutria} genus of spiders, also known as the Brazilian wandering spiders, secrete venom containing \textit{phoneutria nigriventer} toxin-3 (PhTx3), which is a broad-spectrum calcium channel inhibitor that affects glutamate transport. Components of \textit{Phoneutria nigriventer} venom also have temporary but potent effects on the BBB, disrupting cell-cell junctions and reducing Pgp efflux pump function to increase permeability.\textsuperscript{59} Perhaps most interestingly, this disruption is spatially heterogeneous and observed primarily in the hippocampus.\textsuperscript{60} Peripherally administered dye achieved hippocampal entry via transcellular (microtubule-dependent) routes, with paracellular dye observed only in arterioles and venules, and not in capillaries. Venom neurotoxicity was not directly associated with increased BBB permeability, raising the intriguing possibility that venom components could be used for brain-region specific drug delivery.

### Snakes and snails

Both snake and snail venoms contain peptide motifs that antagonist neuronal nicotinic acetylcholine receptors (nAChR). The nAChR is a pentameric integral membrane protein complex arranged around a central pore permeable to cations. The neuronal receptors are composed of homomeric species or a mixture of \alpha- or \beta- subunits. Receptor subunit composition is specific to different tissue types, making the nAChR a promising lead for achieving CNS tropism. For example, the \alpha7-nAChR is widely expressed in the brain and spinal cord, while \alpha9- and \alpha10-nAChR have not been found in the brain, being restricted to the ear and some ganglia.\textsuperscript{61}

Snake venom members include 3-finger toxins, \alpha-neurotoxins, and muscarinic toxins, among others.\textsuperscript{62} Three-finger toxins (TFT) contain 2 major structural domains: the 3-finger domain which is responsible for binding the nAChR and forming an interaction with the target cell membrane, and a globular region which is also necessary for membrane interaction.\textsuperscript{63} NACHR-toxin complexes cross cells via endocytosis, which occurs in 2 distinct phases. In the first phase, the complex is isolated within a vesicle induced by a Rac1 GTPase-initiated actin polymerization step. This is followed by the second phase where the vesicle is delivered to the lysosome, a mechanism similar to the internalization of lipoproteins by macrophages.\textsuperscript{64}

TFTs are often classified by toxin length. Table 3 lists BBB-specific long alpha neurotoxins, which bind to homopentameric neuronal nAChR that are typically comprised of \alpha\textsubscript{7} or \alpha\textsubscript{3} subunits.\textsuperscript{65,66} A well-known example of a TFT nAChR agonist is \alpha-bungarotoxin (\alpha-Bgtx), which is derived from the genus of venomous snakes known as \textit{Krait}.\textsuperscript{65} Studies of \alpha-Bgtx have shown the second loop interacts directly with the nAChR, directly followed by an influx of Ca\textsuperscript{2+}.\textsuperscript{67}

Conopeptides, or conotoxins, are isolated from marine cone snails from the genus \textit{Conus}. Predatory cone snails use their venom to incapacitate worms, mollusks, and small fish.\textsuperscript{69,70} Several conopeptides are in clinical trial. Ziconotide (Prialt) is a conotoxin derived drug that is clinically approved and marketed as a therapy to reduce severe pain. Conopeptides act on voltage-gated ion channels, ligand-gated ion channels, G-protein-coupled

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**Table 3** lists BBB-specific long alpha neurotoxins, which bind to homopentameric neuronal nAChR that are typically comprised of \alpha\textsubscript{7} or \alpha\textsubscript{3} sub-units.\textsuperscript{65,66} A well-known example of a TFT nAChR agonist is \alpha-bungarotoxin (\alpha-Bgtx), which is derived from the genus of venomous snakes known as \textit{Krait}.\textsuperscript{65} Studies of \alpha-Bgtx have shown the second loop interacts directly with the nAChR, directly followed by an influx of Ca\textsuperscript{2+}.\textsuperscript{67}

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**Tissue Barriers**

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**Table 3**

| Alpha Neurotoxin | BBB Permeability |
|------------------|-----------------|
| \alpha-Bgtx      | Present         |
| \alpha7-nAChR    | Limited         |
| \alpha9-nAChR    | Absent          |
| \alpha10-nAChR   | Absent          |

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**References**

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receptors and neurotransmitter receptors. The α-conotoxins are between 10–30 amino acids and typically contain disulfide bonds. Although they are not members of the TFT family, these peptides demonstrate a diverse array of bioactivity, including their ability to antagonize the nAChR by interacting with the same domain as α-Bgtx. The α-conotoxins have shown specificity to various nAChR α-subunits, with a number of α-conotoxins identified that specifically interact with α7-nAChR, which are expressed highly in brain (Table 4).

One potential challenge to conotoxin-based drug delivery strategies is that α-conotoxins containing the sequence of XCCXPACGXXXCCX are on the CDC’s select agent list, which would severely limit their laboratory investigation. This regulation was put into place due to the high affinity and acute toxicity that these peptides display. Under these regulations, no research or commercial entity may possess more than 100 mg at a time without conforming to significant US federal security protocols.

Scorpions

Scorpion venom contains hundreds of individual components, including peptides, proteins, lipids, amines, and nucleotides. The amino acid sequence of at least 250 individual neurotoxins has been determined, with the majority comprising short (23–67 aa), disulfide-bonded peptides. These peptides primarily target ion channels, and are of focused interest as biopharmaceuticals. Chlorotoxin (CTX) is one particularly interesting peptide taken from the venom of Leiurus quinquestriatus, or deathstalker scorpion, that blocks chloride-selective ion channels. CTX also appears to interact specifically with matrix metalloprotease-2 (MMP-2) that is expressed in high levels in brain tumor cells but not expressed in normal tissue. In one study, CTX demonstrated remarkable histological specificity for primary brain tumor and primitive neuroectodermal tumors.

CTX binding was undetectable in normal brain (neurological disease or healthy brain controls), skin, kidney, or lung. Evidence for the ability of CTX and CTX-bound agents to bypass the BBB is found in translational drug delivery work, discussed below. With 8 cysteines forming 4 disulfide bonds, CTX is relatively resistant to hydrolysis, but expensive to produce. It remains uncertain how disulfide binding contributes to function. Given the varied tropism of scorpion neurotoxins for specific receptors that are expressed differentially in the peripheral and central nervous system, the potential for targeted therapy is high.

Drug Delivery

Nature is a rich source of biomimicry, having evolved a multitude of strategies that could be used as inspiration for engineering CNS-targeted nanocarriers (Fig. 2). Some pathogens, notably viruses and certain bacteria, enter the CNS in whole form, achieving direct physical passage across cells to infect the brain. Other pathogens, including bacteria and multicellular eukaryotic organisms, secrete toxins that preferentially interact with neurons to exert a broad range of biological effects on peripheral and central neurons. In many instances, pathogen-type strategies would be considered dangerous. For example, whole tetanus toxin causes painful muscle spasms. However, isolation of the minimally necessary component of the protein that enables its neurotropism achieves neuronal targeting without deleterious effect.

These observations suggest that pathogen-derived strategies for achieving CNS entry could be engineered to facilitate the accumulation of synthetic or biologically derived nanocarriers in the brain. Here, we will review drug delivery literature reporting pathogenic mechanisms for achieving neuronal targeting.

Example strategies for delivering payloads to the brain via pathogenic mechanisms are summarized in Table 5. Several themes
emerge. First, pathogenic ligands may be tethered to a therapeutic (small molecule, protein, or gene), to a vehicle containing therapeutic, or to an imaging agent. Tethered agents include low molecular weight polymers, protein carriers, and viral-sized nanoparticles. Second, when the pathogenic ligand is tethered to a vehicle capable of releasing its payload before reaching its target cell, demonstrating true target specificity can be challenging. Third, the majority of characterization is performed in vitro, typically by utilizing fluorescence microscopy or competitive binding assays to determine how modification of a ligand mediates specific uptake of the payload into target cells. In vivo evaluation sometimes involves measurement of uptake or distribution in the CNS by fluorescence or radiographic methods, however, more compelling evidence is often provided by therapeutic endpoints. For example, Alvarez-Erviti et al. engineered self-derived exosomes to express the neurotropic ligand RVG on their surface.78 Exosomes were loaded with siRNA against GAPDH and delivered systemically, with knockdown observed specifically in neurons, microglia, and oligodendrocytes in the brain. In separate experiments, delivery of siRNA against BACE1 was confirmed at both the mRNA and protein level. Since siRNA must be delivered intracellularly to achieve gene silencing, these results provide compelling evidence that exosomes targeted to the CNS with RVG actually reach specific cellular targets within the brain parenchyma.

Both RVG and CTX have been recent but highly popular candidates for improving brain-specific delivery of systemically administered carriers, the former achieving impressive preclinical success in carrying therapeutics to the CNS in rodent models, and the latter being a component of a targeted radiotherapy that reached phase II clinical trial (NCT00114309). One reason that RVG has risen as a surface-modifying ligand may be the fact that the portion of glycoprotein understood to be responsible for its highly specific CNS tropism is known and of reasonably short length (29-aa). The peptide is commercially available and can be conjugated to the surface of a nanocarrier with well-defined chemistry that would be expected to preserve orientation of the ligand for interaction with its cell surface receptor. The short sequence of RVG should be taken in direct contrast to what is – or isn’t, as the case may be – known about other viral envelope proteins, whose full sequences can be hundreds of amino acids long and may possess multiple binding sites for attachment to the surface of a nanocarrier.

![Figure 2. Bio-inspired nanocarriers can be engineered to improve drug delivery to the CNS. In this example, a solid polymer nanoparticle encapsulates a therapeutic (small molecule, nucleic acid, or protein) or imaging agent. The surface of the viral-sized nanoparticle is modified to display a pathogen-derived peptide (e.g., rabies virus glycoprotein) that will facilitate passage of nanoparticle with cargo across the BBB. Therapeutic compounds that have been encapsulated in biodegradable nanoparticles will be released slowly over time for targeted treatment. A solid polymer nanoparticle is shown as one example of a targeted drug carrier; many other types of carriers or conjugates can be similarly modified to improve CNS delivery (for example, liposomes, micelles, drug-antibody conjugates, and others).](image-url)

| Table 5. Examples of CNS-directed delivery achieved by pathogenic strategies |
|---------------------------------|---------------------------------|
| **Ligand**                      | **Pathogen**                   | **Entry**                | **Payload**                        | **Vehicle**                          |
| Rabies Virus Glycoprotein (RVG) | Rabies Virus                   | nAChR, P75, NCAM, GABA;  | Nucleic Acids79, 138-140            | Exosomes139                          |
|                                 |                                | presumed direct passage   | Proteins141,142                    | particles143                         |
|                                 |                                | across the BBB; retrograde| Small Molecules141                 | dendrimers134,144                    |
|                                 |                                | transport is possible      |                                  | other78,142                          |
| Chlorotoxin (CTX)               | Scorpion Venom (Leiurus       | Cl- channels and MMP-2;  | Nucleic Acids83-88                  | Particles95,87, 89, 90, 95, 96, 101,103|
|                                 | quinquestriatus)               | presumed direct passage   | Proteins89                         | Lipids83,91,92,94                    |
|                                 |                                | across the BBB             | Small Molecules88, 90-103           | DGL83                              |
|                                 |                                |                            | Quantum Dots104                     | Dendrimers84                         |
|                                 |                                |                            | Other105                            | PEG48                               |
|                                 |                                |                            |                                     | PEI88                               |
| Dendrotoxin                     | Mamba Snake                   | K+ channel                 | Quantum Dots104                     |                                     |
| Conantokin-G                    | Snail Venom                   | NMDA receptors             |                                     |                                     |
|                                 |                                |                            |                                     |                                     |
| Tetanus toxin                   | C. tetani                    | Peripheral nerve polysialogangliosides | Nucleic Acids145-148 | Particles155                         |
|                                 |                                |                            | Protein104, 110,140-154             | Lipids145                           |
|                                 |                                |                            |                                     | PEI98                               |
| Hannah toxin                    | King Cobra Snake              | nAChR                     | Small Molecules131                  | Micelles131                         |

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The first report describing delivery of a therapeutic payload linked to RVG was published in 2007 by Kumar and colleagues and serves as an excellent example of the direct translation of viral strategies for CNS entry to improve the brain-specific delivery of synthetic nanocarriers.\(^7\) RVG-pseudotyped lentivirus was observed to be infective to neuronal (Neuro2A) but not peripheral (HeLa) cells, whereas vesicular stomatitis virus (VSV) pseudotyped vectors were infective to both cell types. This result is perhaps not unsurprising, given the broad peripheral tropism of VSV. Correspondingly, RVG-pseudotyped virus encoding antiviral siRNA protected against Japanese encephalitis virus challenge, whereas VSV pseudotyped vectors did not provide protection. RVG was then linked to a 9-arginine repeat to carry antiviral siRNA from peripheral circulation into the brain; 80% of mice treated with RVG-linked antiviral siRNA survived JEV challenge, which was fatal in mice treated with antiviral siRNA linked to a control rabies virus matrix protein (RVMAT). Since this initial report, RVG-linked polymers, liposomes, and biologically derived nanocarriers have been used for successful delivery of proteins, small molecules, and nucleic acids to the CNS, with functionally improved outcomes in several distinct disease models. This research presents compelling evidence that pathogenic strategies for achieving CNS entry can be adapted to improve delivery of engineered drug carriers.

The first experiments demonstrating affinity of CTX for brain tumor cells were published in 1998 by Soroceanu et al.\(^8\) I-125-labeled CTX was shown to bind to at least two sites (high and low affinity) on glioma cells, with impressive specificity of delivery to orthotopic glioma xenografts (39% of ID/g in tumor bearing versus 12% ID/g in healthy brain). Subsequent studies have suggested very high specificity of CTX binding to malignant brain tumor cells in patient biopsy samples compared to non-target tissue, with CTX staining more than 90% of brain tumor cells, compared to almost no detectable staining in uninvolved brain tissue.\(^8\) When CTX-linked Cy5.5 was delivered systemically to mice bearing orthotopic medulloblastoma, fluorescence was detected widely in tumor and not in healthy brain, in spite of an apparently intact BBB, which was demonstrated by the lack of Evan’s Blue dye extravasation from the vascular compartment and albumin immunostaining.\(^8\) These data suggest that CTX agents may be capable of bypassing intact BBB, although specific mechanisms of entry have yet to be characterized. I-131-labeled chlorotoxin (TM-601) reached phase II clinical trial for targeted radiotherapy by direct application to the tumor resection cavity. Phase I results demonstrated that intracavitary administration of TM-601 was safe, with no dose-limiting toxicity observed and some evidence of therapeutic benefit.\(^8\) In the time since the phase I results were reported, additional preclinical work has not only confirmed specificity of CTX for a range of malignant cell types, but also demonstrated the ability of CTX to ferry systemically administered agents into the brain.\(^8\) \(^3\)\(^-\)\(^5\)

KC2S, a synthetic peptide derived from toxin b of the king cobra (Ophiophagus hannah), demonstrates similar affinity for neuronal nAChR as RVG, as judged by competitive inhibition assay against α-Bgtx (IC50s of 33nM and 28nM for KC2S and RVG, respectively). KC2S-linked micelles were taken up in brain capillary endothelial cells but not in HeLa cells.\(^6\) When delivered systemically, dye-loaded KC2S-micelles were observed to accumulate slowly in the brain, and drug-loaded KC2S-micelles provided a modest survival benefit in an orthotopic glioma model. Interestingly, although blood pharmacokinetic parameters were nearly identical for KC2S-targeted and non-targeted micelles, \(C_{\text{max}}\) and AUC in the brain was significantly higher for the targeted formulation, suggesting active transport of toxin linked micelles from blood to brain. Toxins from snails have also been used to deliver tethered payload to neuronal cells, although work remains to test delivery effectiveness in vivo.\(^9\)\(^4\)\(^7\)

TTC bound agents are popular tools for studying neuronal transport and have been used to achieve targeting in vivo. The specificity of TTC for gangliosides predominantly concentrated in neuronal membranes allows TTC-tethered agents to selectively bind neurons. For example, a recombinant fusion of TTC and wild type SOD-1, a protein whose mutant form is implicated in the disease pathology of amyotrophic lateral sclerosis (ALS), was found to have a significantly higher spread and retention compared to free SOD-1 when infused into the brain of mice.\(^8\) In another study, Ciriza et al. demonstrated that weekly limb injection of a TTC-GDNF fusion, a neurotrophic factor known to increase the survival and proliferation of neurons, was able to increase survival and quality of life in SOD-1 mutant mice, while the injection of an insect recombinant TTC-IGF-1 fusion protein in SOD-1 mutant mice was able to sufficiently reach the spinal cord but not affect the overall survival in treatment groups.\(^9\)\(^3\)\(^4\)\(^7\) Perhaps most interestingly, evidence for retrograde transport of SOD-1-TTC to the brain was found after intramuscular injection in mice, raising the question of whether a larger carrier (for example, a drug loaded nanoparticle), could achieve CNS entry by retrograde route.\(^1\)\(^1\) For a more comprehensive review of therapeutic recombinant fusion TTC proteins, the authors refer the reader to Toivonen et al.\(^3\)

We focus here primarily on ligand-receptor strategies for receptor-mediated delivery of therapeutic payloads. However, it is important to note that for certain pathogenic sequences, including Tat, peptide-modified nanoparticle entry may occur via non-receptor mediated mechanisms.\(^1\)\(^2\) In several intriguing and recent reports, non-receptor mediated biomimicry has been used to engineer the interaction of nanocarriers with cell targets. For example, Ng and colleagues mimicked bacterial surface composition to target intracellular actin networks, with the ultimate goal of overcoming diffusional barriers to actively propel polymer or lipid nanoparticles through the cytoplasm.\(^1\)\(^3\) In other work, Niu et al. mimicked viral surface topography to dramatically enhance silica nanoparticle internalization by cells.\(^1\)\(^4\) Viral and bacterial derived carriers have also been used as vehicles for targeted drug delivery, although the emerging field of biologically sourced nanocarriers remains beyond the scope of this review. These and other examples highlight the potential broad range of pathogen-derived strategies that could be used as inspiration for engineering targeted drug delivery vehicles.
Conclusion

Drug delivery remains a critical challenge to achieving better therapy of disease in the CNS. Most systemically delivered drugs do not cross the BBB, and those that do are often poorly bioavailable. However, many viruses, bacteria, and neurotoxins are well-adapted for achieving CNS entry. A variety of pathogenic mechanisms have already been used to improve the brain or neuronal tropism of drugs, drug-loaded vehicles, or imaging agents. These strategies have the potential to improve delivery of peripherally administered agents to the CNS, which could facilitate the translation of novel therapeutics to the clinic to treat human disease.

References

1. Friden PM, Walus LR, Musso GF, Taylor MA, Maloff B, Starzyk RM. Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. Proc Natl Acad Sci 1991; 88:4771-5.
2. Partridge WM. The blood-brain barrier: bottleneck in brain drug development. NeuroRx 2005; 2:5-13; PMID:15717053; http://dx.doi.org/10.1016/j.nrrx.2004.02.005
3. Bencuurova E, Mlynarcik P, Blidh M. An insight into the ligand-receptor interactions involved in the translocation of pathogens across blood-brain barrier. FEMS Immunol Med Microbiol 2011; 63:97-118; PMID:22092557; http://dx.doi.org/10.1111/j.1574-695X.2011.01086.x
4. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. Nat Rev Microbiol 2008; 6:625-34; PMID:18604221
5. Salinas S, Schiavo G, Kremer EJ. A hitchhiker’s guide to the nervous system: the complex journey of the mammalian p75 neurotrophin receptor. J Biol Chem 1999; 274:401-33; PMID:1038/formin296.
6. Moody MF. Geometry of phage head construction. J Mol Biol 1999; 293:401-33; PMID:10529353
7. Morton VL, Stockley PG, Stonehouse NJ, Ashcroft JB. The neural cell adhesion molecule is a receptor for rabies viruses. Arch Biochem Biophys 2013; 531:65-76; PMID:2341584; http://dx.doi.org/10.1016/j.abb.2013.04.029
8. Antonucci F, Rossi C, Gianfranceschi L, Rossetto O, Caleo M. Long-distance retrograde effects of botulinum toxin. J Physiol 2013; 591:1031-43; PMID:23109108; http://dx.doi.org/10.1113/jphysiol.2012.242131
9. Lentz TL. Rabies virus binding to an acetylcholine receptor alpha-subunit peptide. J Mol Recognit 1990; 3:82-8
10. Superti F, Derer M, Tsang H. Mechanism of rabies virus entry into CER cells. J Gen Virol 1984; 65(Pt 4):6781-9; PMID:6423770; http://dx.doi.org/10.1099/0022-1317-65-4-74.1
11. Ivey NS, Maclean AG, Lackner AA. Acquired immunodeficiency syndrome and the blood-brain barrier. J Neurol 2002; 277:3657-62; PMID:12163480; http://dx.doi.org/10.1007/s00702-002-0620-9
12. Thomasen M, Lafage M, Schachner M, Hartmann U, Cremer H, Lafora M. The neural cell adhesion molecule is a receptor for viruses. J Virol 1998; 72:7181-90; PMID:9096812
13. McGavern DB, Kang SS. Illuminating viral infections of the CNS. Nat Rev Immunol 2012; 12:123-36; PMID:22262421
14. Johnson N, Phillpotts R, Fooks AR. Airborne transmission of Riftia pachyptila. Trends Parasitol 2008; 24:226-30; PMID:18234786; http://dx.doi.org/10.1016/j.pt.2008.04.003
15. Johnson N, Phillpotts R, Fooks AR. Airborne transmission of Riftia pachyptila. Trends Parasitol 2008; 24:226-30; PMID:18234786; http://dx.doi.org/10.1016/j.pt.2008.04.003
16. Antonucci F, Rossi C, Gianfranceschi L, Rossetto O. Tetanus toxin fragment forms channels in lipid vesicles at low pH. Proc Natl Acad Sci U S A 1982; 79:7614-8; PMID:6296842; http://dx.doi.org/10.1073/pnas.79.24.7614
17. Centers for Disease Control and Prevention. Adult vaccination coverage—United States, 2010. MMWR Morbidity and mortality weekly report 2012; 61:66-72.
18. Leong JM, Wang H, Magoun L, Field JA, Morrissey PE, Robbins D, Coburn J, Parveen N, Catoe M. Long-distance retrograde effects of botulinum neurotoxin A. J Neurosci 2008; 28:3689-96; PMID:18385327; http://dx.doi.org/10.1523/JNEUROSCI.0264-08.2008
19. Different classes of proteoglycans contribute to the attachment of Borrelia burgdorferi to cultured endothelial and brain cells. Infect Immun 1998; 66:994-9; PMID:9488847
20. Mortarj TJ, Shi M, Lin YP, Ebyad R, Zhou H, Odiulo T, Hardy PO, Saliman-Dilgimen A, Wu J; Acknowledgments

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invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. Infect Immun 1991; 66:5260-7; PMID:9784531
53. Klint JK, Senff S, Ruipainghe DB, Er SY, Herzig V, Nicholson GH. Discovery of venom peptides that target voltage-gated sodium channels: pharmacological tools and potential therapeutic leads. Toxicon: Off J Int Soc Toxicol 2012; 60:478-91; PMID:22554387; http://dx.doi.org/10.1016/j.
toxicon.2012.01.037
54. King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. Expert Opin Biol Ther 2011; 11:1469-84; PMID:21939428; http://dx.doi.org/10.1517/14712598.2011.621940
55. Norsøe KS, Pallagy PK. The cysteine knot structure of ion channel toxins and related polypeptides. Toxicon: Off J Int Soc Toxicol 1998; 36:1573-83; PMID:9797213; http://dx.doi.org/10.1006/jbpe.1998.0104
56. Mouhout S, Jonquère M, Abba D, Waer M, Sabatier JM. Diversity of fields in animal toxins acting on ion channels. Biochem J 2004; 378:717-26; PMID:14674883; http://dx.doi.org/10.1042.
57. Takahashi H, Kim JI, Min HJ, Sato K, Szwarc KW, Shimada I. Solution structure of hanatoxin1, a gating modifier of voltage-dependent K+ channels: common surface features of gating modifiers toxins. J Mol Biol 2008; 378:717-26; PMID:18068802; http://dx.doi.org/10.1016/j.
58. Norsøe KS, Pallagy PK. The cysteine knot structure of ion channel toxins and related polypeptides. Toxicon: Off J Int Soc Toxicol 1998; 36:1573-83; PMID:9797213; http://dx.doi.org/10.1006/jbpe.1998.0104
59. Chagot B, Escoubas P, Villages E, Bernard C, Ferrat G, Corzo G, Lauzuncis M, Darbon H. Solution structure of phorotoxin 1, a specific peptidic inhibitor of Kv potassium channels from the venom of the thalassoid spider Phoridoxus australis. Protein Sci 2004; 13:1197-208; PMID:15906662; http://dx.doi.org/10.1110.
60. Rapoport K, Oddorosi PA, Oliveira AL, Ayama H, Ferreira CV, Verinaud I, Fontana K, Ruela-de-Sousa RR, da Cruz-Holm MA. Effect of Phoneu-
61. Van Rooijen N, Perl A, Mohrs M, van der Heijden F, Reutelingsperger C. Efficient transvascular delivery of peptides, siRNA, and other biological macromolecules. Nat Immunol 2003; 4:569-75; PMID:12787465; http://dx.doi.
62. Norsøe KS, Pallagy PK. The cysteine knot structure of ion channel toxins and related polypeptides. Toxicon: Off J Int Soc Toxicol 1998; 36:1573-83; PMID:9797213; http://dx.doi.org/10.1006/jbpe.1998.0104
63. Kini RM, Doley R. Structure, function and evolution of the human cytotoxin GABA receptor binding site. Toxicon: Off J Int Soc Toxinol 2010; 56:1709-18; PMID:20783231; http://dx.doi.org/10.1016/j.
64. Hone AJ, Ruiz M, Scadden M, Christensen S, Gajewiak J, Atam L, McIntosh JM. Positional scanning mutagenesis of alpha-conotoxin PeIA identifies critical residues that confer potent binding and selectivity for α7 neuropeptide receptors. Mol Pharmacol 2003; 63:562-70; PMID:12876559; http://dx.doi.org/10.1124/j.
65. Klint JK, Senff S, Ruipainghe DB, Er SY, Herzig V, Nicholson GH. Discovery of venom peptides that target voltage-gated sodium channels: pharmacological tools and potential therapeutic leads. Toxicon: Off J Int Soc Toxicol 2012; 60:478-91; PMID:22554387; http://dx.doi.org/10.1016/j.
toxicon.2012.01.037
66. Alama A, Bruzzo C, Cavaleri Z, Forlani A, Urkin Y, Casciano I, Romani M. Inhibition of the nicotinic acetylcholine receptors by ophidian venom alpha-neurotoxins: is there a perspective in lung cancer treatment. J Photochem Photobiol B: Biol 2011; 6;20695; PMID:21695184
67. Lopes do M, Barbozar EV, Lyuynanov EN, Kosinsky YA, Shulepko MA, Dolgikh DA, Kirpich-
nikov MP, Efferm RF, Arseniev AS. Specific microvascular binding of neurotoxin II can facilitate its delivery to acetylcholine receptor. Biophys J 2009; 97:2089-97; PMID:19804741; http://dx.doi.org/10.1016.
bj.2009.07.037
68. Colpaudson D, Unwin N, Shelley C, Barton C, Sivoloni L. Burger’s medicinal chemistry and drug discovery. New York: Wiley-Interscience; 2003.
69. Redwan el RM. Animal-derived pharmaceutical proteins. J Immunol Assay Immunochrom 2009; 30:262-6; PMID:18738913; http://dx.doi.org/10.1080/15321810903808498
70. Essak M, Bajc VB, Archer JA. Conotoxins that confer therapeutic possibilities. Marine Drugs 2012; 10:124-45; PMID:22822570; http://dx.doi.org/10.3390/md1001.
71. Lewis RJ, Dutertre MA, Kerthie I, Munday MA, Atam L, McIntosh JM. Conus venom peptide pharmacology. Marine Rev 2012; 64:259-98; PMID:22407615; http://dx.doi.org/10.1111/j.1539.
72. Honjo K, Ruiz M, Scotten A, Christensen S, Gajewiak J, Atam L, McIntosh JM. Positional scanning mutagenesis of alpha-conotoxin PeIA identifies critical residues that confer potent binding and selectivity for α7 neuropeptide receptors. Mol Pharmacol 2003; 63:562-70; PMID:12876559; http://dx.doi.org/10.1124/j.
73. National Select Agent Registry. Centers for Disease Control, 2013; http://www.selectagents.gov/SelectAgentList.html
74. Ding J, Chua P, Bay BH, Gopalakrishnakone P. Scorpion venoms as a potential source of novel cancer therapeutic compounds. Exp Biol Med (Maywood) 2014; 339:54-66; PMID:25691905; http://dx.doi.org/10.1177/1535370214551575
75. Lyons SA, O’Neill J, Sontheimer H. Cholorotoxin, a scorpion-derived, peptide specifically binds to glia-
76. Hone AJ, Ruiz M, Scadden M, Christensen S, Gajewiak J, Atam L, McIntosh JM. Positional scanning mutagenesis of alpha-conotoxin PeIA identifies critical residues that confer potent binding and selectivity for α7 neuropeptide receptors. Mol Pharmacol 2003; 63:562-70; PMID:12876559; http://dx.doi.org/10.1124/j.
77. Chijiana C, Feng L, Yingliang W, Xin M, Wexnin L. Genetic mechanisms of scorpion venom pep-
78. Alvarez-Enri1 L, Seow Y, Yin H, Betts C, Lakhal S, Davidson BL, Lee SK, Shankar P, Manjunath N. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011; 29:341-5; PMID:21423189; http://dx.doi.org/10.
79. Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, Lee SK, Shankar P, Manjunath N. Transvascular delivery of small interfering RNA to the central nervous system. Nature 2007; 448:39-43; PMID:17577266; http://dx.doi.org/10.1038.
80. Sorocoeau L, Gillespie K, Khazaell MB, Sontheimer H. Use of chlorotoxin for targeting of primary brain tumors. Cancer Res 1998; 58:4738-55; PMID:9731848; http://dx.doi.org/10.1158/0008-5472.
81. Veiseh M, Gabrakian P, Bahrami SB, Veiseh O, Zhang M, Hackman RC, Ravanyac PC, Stroud MB, Kusuma Y, Hansen SJ, et al. Tumor painting with a chlorotoxin: C5.5 bioconjugate for intraperitoneal
120. Dunfe R, Thomas ER, Gorry PR, Wang J, Ancuta P, Gabazda D. Mechanisms of HIV-1 tropism. Curr HIV Res 2006; 4:267-78; PMID:16842080; http://dx.doi.org/10.2174/157016206777795095.

121. Wang T, Town T, Alexopoulos 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; 10:1366-73; PMID:15588055; http://dx.doi.org/10.1038/nm1104.

122. Chu J, Ng ML. Interaction of West Nile virus with alpha v beta 3 integrin mediates virus entry into cells. J Biol Chem 2004; 279:54533-41; PMID:15477534; http://dx.doi.org/10.1074/jbc.M401020200.

123. Racinielle VR. One hundred years of poliovirus pathogenesis. Virusology 2006; 349:9-16; PMID: AMBIGUOUS.

124. Chung JW, Hong SJ, Kim KG, Gori D, Simns MF, Shin S, Dawson VL, Dawson TM, Kim KS. 37-kDa laminin receptor precursor modulates cytotoxic necrotizing factor 1-mediated RhoA activation and bacterial uptake. J Biol Chem 2003; 278:16857-62; PMID:14015923; http://dx.doi.org/10.1074/jbc.M304839200.

125. Sheppart C, Smith DR. Serotype-specific entry of dengue virus into liver cells: identification of the 37-kilodalton/67-kilodalton high-affinity laminin receptor as a dengue virus serotype 1 receptor. J Virol 2004; 78:12647-56; PMID:15507651; http://dx.doi.org/10.1128/JVI.78.22.12647-12656.2004.

126. Bogachev MV, Protopopova EV, Loktev VB, Zaitsev BN, Favrè M, Sekarzki SK, Dieteler G. Immunochemical and single molecule force spectroscopy studies of specific interaction between the laminin binding protein and the West Nile virus surface glycoprotein E domain II. J Mol Recog 2008; 21:75-82; PMID:18061925; http://dx.doi.org/10.1002/jmr.106.

127. Protopopova EV, Sorokin AV, Konovalova SN, Kachko AV, Netroev SV, Loktev VB. Human laminin binding protein as a cell receptor for the tick-borne encephalitis virus. Zbl Bakter 1999; 289:632-8; PMID:10465533.

128. Hurtado S, Acín JC, Boivin C, Levin B, Yee H, Protopopova EV, Sorokin AV, Konovalova SN, Racaniello VR. One hundred years of poliovirus receptor molecules on microglial cells. J Med Chem 2002; 45:3053-62; PMID:12021532; http://dx.doi.org/10.1021/jm010426m.

129. Inserca MC, Kompella SN, Vetter I, Brust A, Daly NL, Cuny H, Craik DJ, Alewof PD, Adams DJ, Lewis RJ. Isolation and characterization of α-conotoxin LiAα with potent activity at nicotinic acetylcholine receptors. Biochimica Biophysica Acta 2013; 1831:869-76; PMID:23942607; http://dx.doi.org/10.1016/j.bpc.2013.07.016.

130. Yu R, Craik DJ, Kaas Q. Blockade of neuronal α7-nAChR by n-conotoxin ImI explained by computational binding energy calculations. PLoS Comput Biol 2011; 7:e1001201; PMID:AMBIGUOUS.

131. Liu Y, Guo Y, An S, Kuang Y, He X, Ma H, Li J, Ly J, Zhang J, Jiang N, Cang T. Targeting caspase-3 as dual therapeutic benefits by RNAs facilitating brain-targeted gene delivery into peripheral sensorial neurons mediated by cell-surface binding domain and Bcl-x for protection of peripheral nerve neurons. Neurosurgery 2008; 63:1130-42; PMID:17854886; http://dx.doi.org/10.1227/01.NEU.0000334145.45033.EA.

132. Orfani MA, Hui SC, Fishman PS. CuZn superoxide dismutase (SOD-1): tetanus toxin fragment C hybrid protein for target delivery of SOD-1 to neuronal cells. J Biol Chem 1995; 270:15434-42; PMID:7797532; http://dx.doi.org/10.1074/jbc.270.25.15434.

133. Grunhich PA, Remington MP, Amin J, Berenbecher MJ, Fishman PS. Tast-tetanus toxin fragment C: a novel protein delivery vector and its use with photochemical internalization. J Drug Target 2013; 21:662-74; PMID:23697582; http://dx.doi.org/10.1080/10613030.2013.796954.

134. Larsen KE, Benn SC, Ay I, Chian R, Celia SA, Remington MP, Bejarano M, Liu M, Ross J, Carmillo P, et al. A glial cell line-derived neurotrophic factor (GDNF): tetanus toxin fragment C protein conjugate improves delivery of GDNF to spinal cord motor neurons in mice. Brain Res 2006; 1120:1-12; PMID:17020749; http://dx.doi.org/10.1016/j.brainres.2006.08.079.

135. Li J, Chian RY, Ay I, Kasbi BB, Celia SA, Tamrizzani E, Pepinsky RB, Fishman PS, Brown RH Jr, Jr, Francis JW. Insect GD1F:TTG fusion protein improves delivery of GDNF to mouse CNS. Biochim Biophys Acta 2009; 1798:547-51; PMID:19582934; http://dx.doi.org/10.1016/j.bbr.2009.10.083.

136. Townsend SA, Evrony GD, Gu FX, Schulte MP, Brown RH Jr, Jr, Langer R. Tetanus toxin fragment C conjugated nanoparticles for targeted drug delivery to neurons. Biomaterials 2007; 28:5176-84; PMID:17854886; http://dx.doi.org/10.1016/j.biomaterials.2007.08.011.