Advanced Review

Systems-level thinking for nanoparticle-mediated therapeutic delivery to neurological diseases

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Neurological diseases account for 13% of the global burden of disease. As a result, treating these diseases costs $750 billion a year. Nanotechnology, which consists of small (~1–100 nm) but highly tailorable platforms, can provide significant opportunities for improving therapeutic delivery to the brain. Nanoparticles can increase drug solubility, overcome the blood–brain and brain penetration barriers, and provide timed release of a drug at a site of interest. Many researchers have successfully used nanotechnology to overcome individual barriers to therapeutic delivery to the brain, yet no platform has translated into a standard of care for any neurological disease. The challenge in translating nanotechnology platforms into clinical use for patients with neurological disease necessitates a new approach to: (1) collect information from the fields associated with understanding and treating brain diseases and (2) apply that information using scalable technologies in a clinically-relevant way. This approach requires systems-level thinking to integrate an understanding of biological barriers to therapeutic intervention in the brain with the engineering of nanoparticle material properties to overcome those barriers. To demonstrate how a systems perspective can tackle the challenge of treating neurological diseases using nanotechnology, this review will first present physiological barriers to drug delivery in the brain and common neurological disease hallmarks that influence these barriers. We will then analyze the design of nanotechnology platforms in preclinical *in vivo* efficacy studies for treatment of neurological disease, and map concepts for the interaction of nanoparticle physicochemical properties and pathophysiological hallmarks in the brain.

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INTRODUCTION

Despite the significant financial investment we have made, we are still struggling to understand and adequately treat the majority of complex diseases. Neurological diseases account for 13% of the global burden of disease and cost roughly $750 billion a year to treat. Drugs that are used to treat the injured or diseased brain take 35% longer to be used in humans compared with drugs for any other type of disease.[1] Evaluating therapeutic interventions for a disease is difficult because the disease microenvironment is dynamic, heterogeneous, and variable from person to person. Delivering drugs to the diseased

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brain environment is also challenging, because the brain is protected by the strictly regulated blood–brain barrier (BBB) and blood–cerebrospinal fluid (CSF) barrier, and the brain has a complex microenvironment through which a therapeutic must move. Typically, less than 1% of a drug actually gets to the target organ, and often not just to the disease sites within the organ.[2] In addition, many drugs focus on suppressing just one aspect of the disease or have mechanisms that are complex and poorly understood, such that drug interactions within the body cannot be controlled or predicted. Administering more of a drug, or a cocktail of drugs, compensates for the inefficiency in getting a drug to a target disease site, but both of these approaches may increase side effects and harm normal healthy tissue.

Nanoparticles, which consist of small (~1–100 nm) tailorable platforms, can address some of the issues associated with ineffective drug delivery by increasing drug solubility,[3] protecting drugs from clearance or from nonspecific uptake,[4] and by providing controlled or timed release of a drug at the site of interest.[5] Nanotechnology is also a useful information-gathering tool, therapeutic intervention, or diagnostic platform for applications in the brain. An increase in the development of nanoparticle-based drug delivery to the brain has yet to translate into a therapeutic standard of care in patients for any neurological disease, despite the fact that preclinical studies have shown that nanotechnology overcomes individual barriers to drug delivery to the brain, particularly the BBB. The growing body of data from preclinical and clinical trials necessitates a new approach to integrate information generated from brain-oriented fields (i.e., neuroscience, physiology, genetics, developmental biology, psychology, neurology, and neurosurgery) with scalable technologies (i.e., via engineering, physics, chemistry, and materials science) in a clinically-relevant way.

An approach that integrates and applies information from a diverse array of fields requires systems-level thinking. The approach must account for an understanding of biological barriers to therapeutic intervention in the brain, and an understanding and leveraging of nanoparticle material properties to overcome those barriers. Given the genetic, lifestyle, and environmental complexity of the human race, a systems approach is important in engineering nanoparticles for application in neurological diseases. Systems analysis is a problem-solving method that attempts to balance holistic and reductionist thinking paradigms. The systems analysis framework is based on parts of a system being best understood in the context of relationships with each other and with other systems, rather than parts of a system being evaluated in isolation. By using nanoparticles to probe the brain, it is possible to learn and quantify how accessible the brain is to a therapy in the context of a disease, and how readily a therapy can move to the diseased cells once in the brain. Both the nanomaterial contributions and the physiological contributions play a role in therapeutic outcome, and therefore do not function in isolation. For example, it is important to understand the aspects of a brain disease that influence the ability of a nanoparticle to deliver a drug to a target cell, such as BBB impairment and cell activation. Greater BBB impairment can increase nanoparticle passage across the BBB into the brain parenchyma. However, increased phagocytic behavior of activated microglia in the injured brain can scavenge nanoparticles out of the brain parenchyma once the nanoparticle is across the BBB,[6,7] limiting uptake into other cells associated with a disease. It is equally important to evaluate how nanoparticle size and surface charge can lead to increased uptake across the impaired BBB and alter cell-specific uptake once in the brain.

To take into account multiple interacting aspects of physiology and nanomaterial properties, we can use a systems perspective to map and design nanotechnology platforms to treat neurological diseases. This review will first present physiological barriers to drug delivery in the brain and common neurological disease hallmarks that influence these barriers. We will then analyze nanotechnology platforms used in preclinical *in vivo* efficacy studies for treatment of most major neurological diseases, with an intentional focus on how nanoparticle physicochemical properties and disease hallmarks interact to overcome the collective system of barriers present in brain diseases. Synthesis of pathophysiological barriers and nanoparticle physicochemical properties into a broader systems view creates an integrated approach to future work using nanotechnology to treat neurological diseases.

PHYSIOLOGICAL BARRIERS TO NANOPARTICLE DELIVERY IN THE BRAIN

The brain is a complex organ that regulates respiration, motor control, memory, sleep, behavior, and how we relate to and interpret our environment. When the brain becomes sick or injured, the extent of damage and functional outcome are variable and heterogeneous, both within the brain itself and across species. Injury in the brain is often diffuse, in many cases affecting more than one region of the brain and more than one brain cell type. As humans age, the

brain also deteriorates naturally, making us more susceptible to certain neurological disorders.[8] For delivery to the brain, a nanoparticle must be able to (1) avoid rapid clearance by the reticuloendothelial system (RES) and (2) bypass or cross the BBB (Figure 1(a)). Once in the brain parenchyma, regardless of the administration route, a nanoparticle must (3) penetrate within the brain microenvironment to reach diffuse disease sites and (4) provide intracellular or extracellular release of the therapeutic agent at the site of disease (Figure 1(a)–(c)). Common disease hallmarks affect each of the aforementioned barriers. These disease hallmarks include extracellular matrix (ECM) changes, inflammation, oxidative stress, excitotoxicity, cell death, and impaired fluid flow, which are present in almost all neurological diseases to varying extents. In section *Physiological Barriers to Nanoparticle Delivery in the Brain*, we will discuss each barrier to therapeutic delivery in the brain. We will then provide a brief overview from a systems-level perspective of the role each hallmark plays in cancer, acute neurological injury, neurodegenerative disease, neuropsychiatric and neurodevelopmental diseases, and in infection.

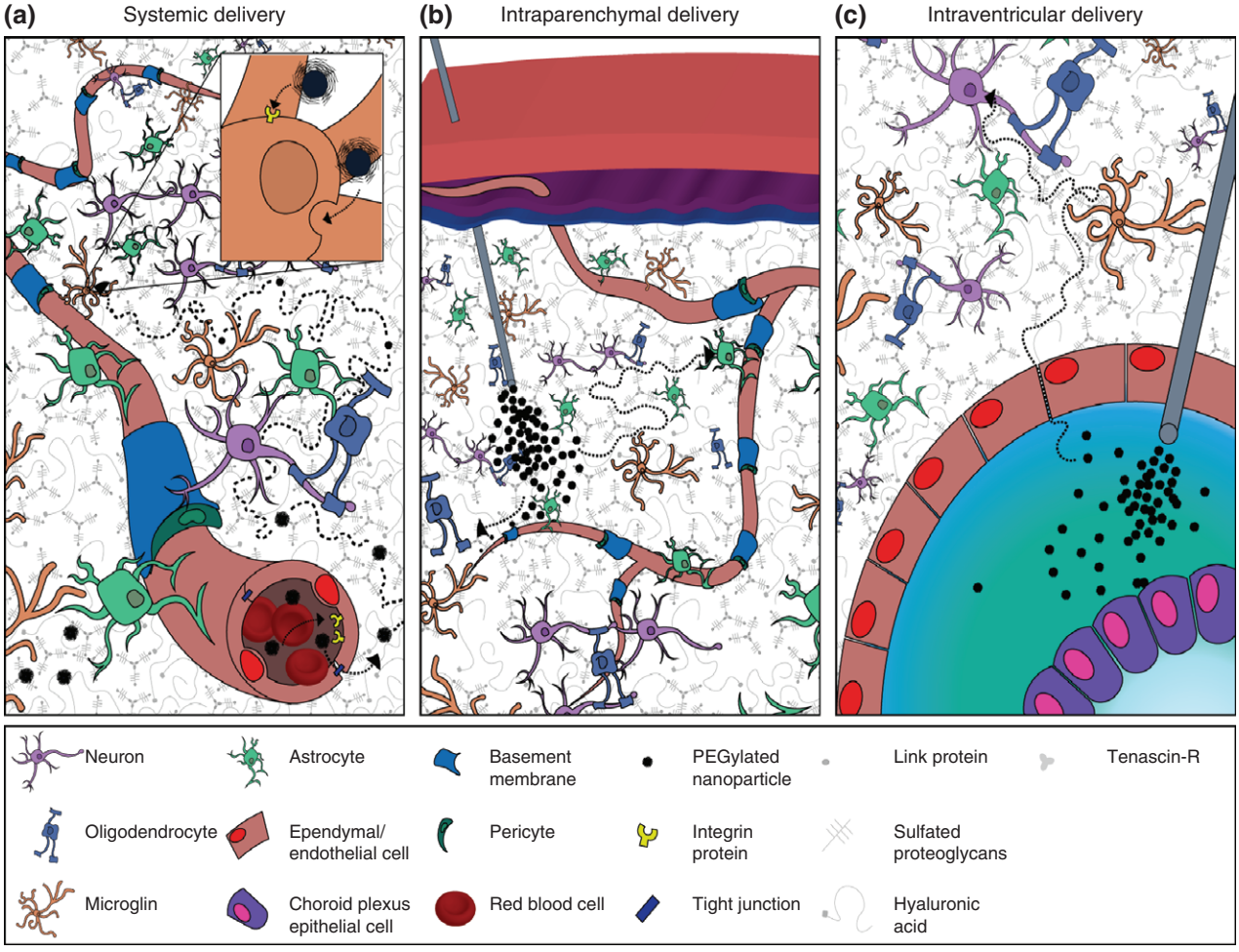

FIGURE 1 | Barriers to nanoparticle delivery to the brain. For a nanoparticle-based therapeutic delivery system to be effective in the brain, it must be able to overcome the system of barriers in the brain. (a) Following systemic administration, a nanoparticle must avoid rapid clearance by the reticuloendothelial system (RES) and cross the blood–brain barrier (BBB), for example, via receptor-mediated transcytosis or permeation across impaired tight junctions. The nanoparticle must then navigate the brain microenvironment by avoiding steric or adhesive interactions with the extracellular matrix (ECM) to reach diffuse disease sites. At the target site, the nanoparticle should provide site-specific delivery extracellularly or be internalized via nonspecific fluid-phase endocytosis, phagocytosis, or receptor-mediated transport (inset). (b) Following local delivery, a nanoparticle must still navigate the extracellular space (ECS) and ECM, avoid clearance along the perivascular space (PVS) or into the ventricles, and provide site-specific therapeutic action at a diseased cell. (c) Although the mechanism remains largely unknown, if a nanoparticle is delivered directly into the CSF, i.e., intraventricular, the nanoparticle will still need to cross the ependymal layer and navigate the ECS and ECM to reach target cells.

The Blood–Brain Barrier

The neurovascular unit (NVU) controls brain metabolism, fluid flow, and function. The NVU includes endothelial cells, pericytes, smooth muscle cells, astrocytes, oligodendrocytes, microglia, and neurons. Within the NVU, endothelial cells form the BBB, a key homeostatic site of the nervous system. The BBB is a highly regulated barrier that limits entry of neurotoxic plasma components, blood cells, and pathogens into the brain. As such, it has also been a bane for drug delivery to the brain. The endothelial cells control the transport of nutrients, energy metabolites, and other essential molecules from the blood into the brain, as well as the transport of metabolic waste products from the brain's interstitial fluid into the blood. Importantly, especially in the context of disease treatment, the development of the BBB is species-dependent and varies regionally within the brain. It is still debated whether humans or other mammals are born with a fully functioning BBB.[9] The BBB continues to mature after birth, but the exact timing of this process remains elusive, and is also species-dependent.[9,10] In most neurological disorders, BBB impairment and associated vascular dysfunction are present,[11] and are influenced by genetics, vascular risk factors, environmental factors, and lifestyle.[12–14] While the BBB is often impaired in the presence of disease, the extent of impairment is highly variable both within a single diseased brain and across patients with the same disease. Although brain capillaries are on average 10–20 μm from the nearest neuron to maximize nutrient and oxygen transport,[15,16] the BBB impairment in each capillary is not equivalent.[17] Therefore, the degree of access a therapeutic would have to reach disease sites in the brain from the blood stream would not be equal among all capillaries.

The Brain Extracellular Space and ECM

Once particles cross or bypass the BBB, they must navigate the heterogeneous extracellular space (ECS) in order to have a therapeutic effect at disease sites.[18] Diffusion is the primary driver of movement within the brain interstitium and the ECS. Transport measurements within the brain ECS agree well with diffusion theory without modifications accounting for flow.[19,20] The brain ECS is filled with extracellular interstitial fluid containing an amorphous, negatively-charged ECM.[21,22] The ECM serves as a dynamic, adaptable extracellular scaffold and regulator of ion flow and diffusion. It consists of a mesh-like network formed around a backbone of hyaluronic acid, with an assortment of proteoglycans, glycoproteins, laminin, and collagens, as well as perineuronal nets.[23] The ECM components can be either bound to cells or free-floating within the ECS. Both geometry of the ECS and the properties of the ECM affect the ability of any therapeutic to move within this space. The geometry of the ECS and ECM hinders free movement (diffusion) of molecules in general, and the ECM may increase local viscosity or cause viscous drag on molecules that undergo steric or electrostatic binding with the matrix.[18] In the presence of disease, the ECS and ECM undergo significant changes, leading to disruption of the BBB, activation of inflammation, and disruption of synaptic homeostasis.[19,24] ECM changes in individual neurological diseases are disease-specific and are covered elsewhere.[24]

The brain tissue microenvironment also includes perivascular spaces (PVSs), anatomical structures that exist on the basolateral side of arteries within the brain. The PVS, a component of the glymphatic system, plays a role in maintaining homeostasis and fluid transport within the central nervous system (CNS).[25] It allows convective fluxes of CSF to flow from the subarachnoid space along the basolateral side of parenchymal arteries and into the interstitium, clearing out metabolic waste from the brain.[26,27] Studies delivering therapeutics to the CNS have shown that large quantities of the therapeutic agent accumulate in the PVS once in the brain.[28–32] This limits the overall therapeutic distribution within brain interstitial space. Although transport in the PVS is thought to be at least 10,000-fold faster than in the ECS, transport from the PVS into the ECS remains predominantly diffusive in nature.[18] Importantly, as diffusion is the main mechanism of molecular movement within the brain parenchyma, brain size could be a potential key difference between therapeutic successes seen in preclinical studies in small animal models versus clinical trials in humans. Humans have on average 2800-fold larger brains by volume compared to a mouse, and on average four-fold larger brains compared to a non-human primate. During clinical application, the therapeutic platform will still diffuse the same distance in a given time as it did preclinically, but have a much larger area to cover.[33] In addition, while astrocytes, pericytes, and microglia sit at or adjacent to the BBB interface, and neurons are often less than 10–20 μm from their closest neighboring brain capillary,[15,16] impairment of the BBB in the presence of disease is still heterogeneous,[34] creating unequal access to the region in which a therapeutic needs to diffuse.

Brain Cell-Specific Uptake

In the presence of disease, the ECS, ECM, and PVS all experience significant disruption in normal function

due to changes in ECS volume,[19] ECM composition, architecture, and role,[24] cell death, edema formation,[35] and impaired PVS fluid flow.[36] In order to reach diseased sites, therapeutics must therefore rapidly move within the brain ECS, in the presence of disease-associated brain environment changes, many millimeters (or even further) away from the initial point of access. Diffusion alone may not be therapeutically relevant at the millimeter (or greater) scale, and facilitated diffusion mechanisms or convection-driven flow, as discussed in section *Nanoparticle Physicochemical Properties for Overcoming Physiological CNS Barriers*, can increase therapeutic distribution within the brain. Cell-specific uptake of a nanoparticle is not necessary to achieve a therapeutic effect, but is often preferred in order to achieve a site-specific effect. In some diseases, particularly those involving inflammation, astrocytes and microglia become more phagocytic,[37] and are more likely to internalize a nanoparticle platform. Neurons, which are non-phagocytic by nature, are also more susceptible to nanoparticle uptake during cell death.[38] Depending on nanoparticle size, shape, and surface functionality, nanoparticle internalization occurs via nonspecific mechanisms such as fluid-phase endocytosis or pinocytosis, or via receptor-mediated interactions (inset of Figure 1(a)). A recent review highlights different strategies for targeting nanoparticle-based platforms to specific CNS cells, including the BBB endothelium, microglia, macrophages, neurons, and astrocytes in the presence of CNS diseases.[39] We will discuss the influence of nanoparticle physicochemical properties on passage across the BBB, distribution within the brain, and cell-specific uptake in sections below.

Common Disease Hallmarks in Neurological Disease

Pathophysiology influences the BBB, the brain microenvironment, and cell-specific behavior, which also vary from disease to disease and across individuals with the same clinically defined disease. A systems-level perspective of how disease hallmarks can influence each barrier to nanoparticle delivery in the brain (Figure 2) can help understand how nanoparticle-based therapeutics can overcome these barriers to decrease disease burden. Inflammation, oxidative stress, cell death, and excitotoxicity change the brain microenvironment by increasing cell debris within the ECS, disrupting BBB function and integrity, and altering the composition and structural geometry of the ECM.[11,40,41] These disease hallmarks are present in cancer, acute neurological injury, neurodegenerative disease, neuropsychiatric and neurodevelopmental diseases, and in CNS infection. In primary brain cancers, such as malignant gliomas (MGs), medulloblastomas, and oligodendrocytomas, changes in BBB permeability and an overproduction of ECM components are seen, as well as increased inflammation, macrophage infiltration, and cell death.[42] These disease processes increase the tortuosity and decrease the volume fraction of the ECS. Increased interstitial pressure within the tumor microenvironment can also inhibit movement and distribution of a therapeutic within that environment.[43] In acute neurological injury, such as traumatic brain or spinal cord injury (TBI or SCI) and stroke [hypoxic-ischemic (HI) or hemorrhagic], destruction of blood vessel integrity, cell death around the area of injury, and associated edema formation, lead to inflammation and oxidative stress, and in some cases increased intracranial pressure.[44,45] These changes reduce therapeutic distribution through increased tortuosity and decreased volume fraction. In neurodegenerative diseases, including Alzheimer's (AD), Parkinson's (PD), Huntington's diseases (HD), amytrophic lateral sclerosis (ALS), and multiple sclerosis (MS), inflammation involving activation of microglia and astrocytes leads to decreased immune surveillance and homeostatic function. The inflammatory process results in impaired fluid flow and an increase in oxidative stress, which leads to cell death and ongoing chronic injury.[46] Similarly, it is thought that inflammation, oxidative stress, and excitotoxicity play a critical role in mediating neuropsychiatric and neurodevelopmental diseases, including depression, migraines, obsessive compulsive disorder (OCD), bipolar, schizophrenia/hallucinations, eating disorders, autism spectrum disorder (ASD), intellectual disability (ID), fetal alcohol syndrome, perinatal asphyxia, cerebral palsy (CP), and epilepsy.[47,48] Lastly, microglia, astrocytes, and peripheral macrophages appear to be the cells most susceptible to CNS infections, including human immunodeficiency virus (HIV), tuberculosis, and meningitis, resulting in chronic oxidative stress, neuroinflammation, and BBB impairment.[49]

NANOPARTICLE PHYSICOCHEMICAL PROPERTIES FOR OVERCOMING PHYSIOLOGICAL CNS BARRIERS

A variety of materials have been utilized for nanoparticle delivery to the brain, including dendrimers,[50] polymers,[51,52] micelles,[53] hydrogels,[54] liposomes and solid lipids,[55,56] gold,[57] silica,[58] silver-based inorganics,[59]

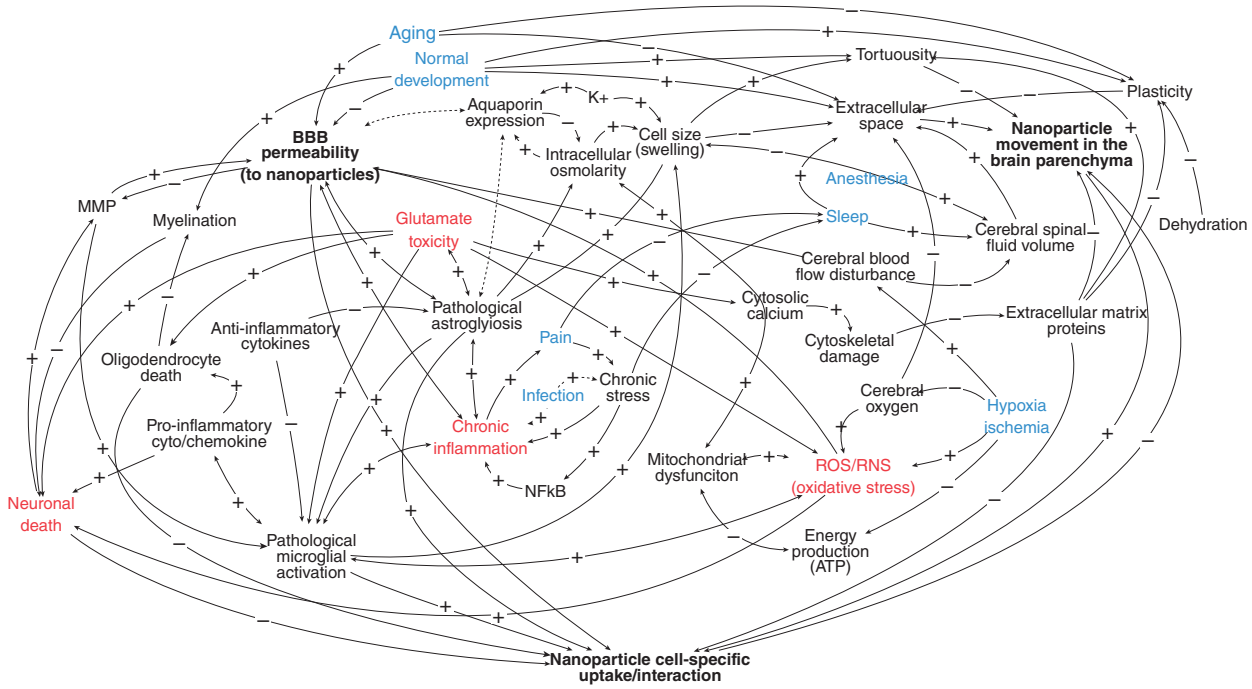

FIGURE 2 | Physiology and biology-based factors influencing nanoparticle delivery in the brain. The system of factors that change in the presence of an input (examples in blue) can positively or negatively impact common disease hallmarks (examples in red) and changes in blood–brain barrier (BBB) permeability, brain microenvironment, and cell behavior in the brain. These changes influence the ability of a nanoparticle to penetrate across the BBB, move within the brain parenchyma, and uptake into specific cells; however, as the map shows, none of the factors influencing nanoparticle delivery of a therapeutic can be viewed in isolation. Double-headed arrows indicate a two-way effect of similar directionality (i.e., pathological astrogliosis increases chronic inflammation, and chronic inflammation increases pathological astrogliosis). ROS/RNS, reactive oxygen/nitrogen species; MMP, matrix metalloproteases; NMDA, *N*-methyl-D-aspartate.

iron or iron oxides,[60,61] quantum dots,[62] and carbon-based particles.[63,64] For each of these nanoparticle systems, important physicochemical characteristics that affect drug delivery and efficacy include size, surface charge, composition, molecular weight (MW), material structure, elasticity, shape, and material porosity (Table 1). The combination of nanoparticle physicochemical properties influences absorption, distribution, metabolism, and excretion (ADME profiles) of these particles from the body.[81] In addition, the physicochemical nanoparticle properties will play a role in whether the nanoparticle can bypass the BBB, move through the brain microenvironment, and uptake into disease-specific cells, which directly influences therapeutic.

To bypass the BBB and move within the brain parenchyma, nanoparticles must be small and possess negative or neutral surface functionality.[18,51,82,83] Depending on the disease or delivery route and the nanoparticle surface coating, successful brain uptake across the BBB has been accomplished with nanoparticles ranging in hydrodynamic diameter from 2 to 250 nm. The size of the nanoparticle will dictate circulation and clearance from target tissues, with sub-20 nm nanoparticles rapidly cleared by the spleen and kidneys, and >200 nm particles rapidly cleared by Küpffer cwnan1422-fig-0002ells in the liver. In the case of local or intranasal delivery, which bypasses the BBB, it is possible that larger nanoparticles or microparticles can reach disease sites within the brain. However, if movement in the brain microenvironment is necessary to achieve efficacy, particles that are sub-200 nm will have optimal diffusive capabilities.[82] Shape also plays a role in whole-body distribution and tissue-specific uptake,[65] and various shapes of nanoparticles can be manufactured from top-down and bottom-up approaches.[84] Although most nanoparticles are spherical or rod-like, nonspherical nanoparticles show deviating hydrodynamic behavior in the presence of shear flow (i.e., in blood), altered degradation rates, and have different interactions with cells based on their aspect ratio.[85,86] There are limited studies on the effect of nanoparticle shape on delivery to the brain, but a recent publication highlights the effect of nanoparticle shape on uptake into neurons and microglia. In this study, spherical and urchin-like gold nanoparticles demonstrate

TABLE 1 | Examples of Nanoparticle Platforms and the Most Common Physicochemical Properties Exhibited by Nanoparticle Platforms Used for Therapeutic Delivery

Nanoparticle	Core Material Examples	Size Range (nm)	Common Core Material Polarity	Common Shapes	Reported Young's Modulus (MPa)	Common Surface Functionalities or Coatings
Polymer	PLGA, PBCA, PLA	50–450	Hydrophobic	Spherical; wide range from PRINT or lithography techniques[65,66]	3–110[67]	Hydroxyl, carboxylate, amine, acid, surfactants, PEG, zwitterions
Dendrimer	Polyamidoamine, polylysine, phosphorus	1.5–14	Hydrophilic	Spherical/globular	150–700[68]	Hydroxyl, carboxylate, amine, acid (i.e., phosphoric, succinamic), PEG
Silica	Mesoporous silica	5–450	Hydrophilic	Spherical	<0.001[69]	Carboxylate, amine, phosphonate, PEG, octadecyl[70]
Quantum dots	CdSe, ZnO, CdS, ZnS	2–10	Hydrophobic	Spherical, wires, rods	ZnO: 3.3×10^6, CdSe[71]: 1.3×10^5	Thiols, carboxyl, PEG, methoxy, amine, mercaptopropionic acid
Lipid [i.e., liposomes, solid-lipid (SLNs)]	Phosphatidylcholine, phospholipids, cholesterol, fatty acids, waxes	50–400	Liposomes: amphipathic, SLNs: hydrophobic core	Spherical	Liposome[72]: 2–10	Lipid end groups: triglycerides, cationic/anionic (depending on core composition), PEG, gangliosides
Metallic	Silver, gold, iron and iron oxides	Gold: 5–400, silver: 1–100, iron oxide: 4–100	Hydrophobic	Spherical, diamond	Gold[73,74]: $2.5–1 \times 10^5$	General: thiol, PEG; gold[75]: mercapto(acetic, propionic, succinic) acid; iron[76]: galactose, mannose, folic acid
Carbon	Carbon nanotubes (CNTs), fullerenes	<1–4 nm single dimension	Hydrophobic	CNTs: rod, fullerenes: spherical cages	CNT[77]: $0.40–4.2 \times 10^6$	PEG, biotin, poly(vinyl pyrrolidone), esters, SiO_2[78]
Hydrogels	Chitosan, alginate, polyethylene oxides, PEG	10–1000	Hydrophilic	Spherical	15–42[79]	Cationic/anionic depending on composition (i.e., cationic: chitosan, anionic: alginate), tripolyphosphate, polysaccharides[80]

PLGA, poly(lactic-*co*-glycolic acid); PBCA, poly(butyl cyanoacrylate); PLA, poly(lactic acid); CdSe, cadmium selenide; ZnO, zinc oxide; CdS, ZnS, cadmium or zinc sulfide; PEG, poly(ethylene glycol); PRINT, particle replication in nonwetting templates; SiO_2, silicon oxide.

Common nanoparticle core materials lead to nanoparticles with a range of sizes and shapes. Core material polarity is an indicator of the aqueous solubility of the nanoparticle core, without any surface functionalization or surface coating. Where available, Young's moduli are provided to demonstrate the range of nanoparticle elasticity. Common surface functionalities and surface coatings are listed as examples of modifications or surface presentations added to the particle after core material formation. Examples of surface-targeting ligands are presented in Table 3.

enhanced microglial uptake *in vivo*, while rod-shaped gold nanoparticles were the only shape to internalize into neurons.[6] In addition, rod-shaped nanoparticles enhance the targeting capability of transferrin (Tf) to BBB endothelium compared with Tf-conjugated spherical nanoparticles.[87] Shape and size, as well as core material and particle composition, can impact particle elasticity (a property of the material) and stiffness (a property of the structure). While the role of particle stiffness is well studied in the tissue engineering field,[88] it has only recently begun to be systematically investigated in nanoparticle-based drug delivery.[89] The role of particle stiffness is complex and dependent on other particle properties, such as elasticity as evidenced by the range of Young's moduli in Table 1, but general trends do appear. For example, less stiff particles persist longer in circulation, increasing the chance of crossing an impaired BBB, and stiffer particles are more likely to get 'stuck' in the spleen and lungs.[89] There are currently no studies evaluating the effect of particle stiffness and elasticity on passage across the BBB and movement within the brain. Therefore, future analysis should evaluate how nanoparticle stiffness and elasticity play a role, in addition to size, surface charge, and shape, in overcoming each barrier to drug delivery to the brain.

Functional end groups, surfactants, or targeting ligands can alter or control nanoparticle surface functionality. There are an extensive number of potential targeting ligands, and with the ongoing debate about their necessity,[90] the effect of targeting ligands on nanoparticle brain uptake will be discussed in subsequent sections from an efficacy-oriented point-of-view only. Functional end groups and surfactants can change a particle's surface charge, composition, and MW, all of which can influence other material and physical properties, such as nanoparticle size and porosity. Along with size and shape, surface charge determines biodistribution, penetration within the brain microenvironment, and cellular uptake. Positively charged (cationic, >+10 mV) surface functionalities, independent of the core material, generally lead to more rapid clearance, greater macrophage uptake, and increased nonspecific cell internalization.[91,92] Cationic nanoparticles lyse cell membranes and induce autophagy through lysosomal impairment and oxidative stress,[93,94] an effect that is exacerbated with increasing nanoparticle size and concentration. Negatively-charged (anionic, <−10 mV) and neutral (nonionic, −10 to +10 mV) surface functionalities have shown successful in nanoparticle brain uptake and penetration within the brain microenvironment, as long as the material is amphipathic or hydrophilic,[95] or hydrophobic domains on a particle platform with a hydrophobic core are well shielded.[82] For sub-10 nm nanoparticles, negative surface functionality directs toward neuronal uptake,[96] whereas neutral surface functionality leads primarily to glial uptake.[97]

Surface coatings, including poly(ethylene glycol) (PEG), zwitterions, and surfactants, can provide neutral or protective surface functionalities to reduce protein adsorption and opsonization, and shield charge–charge, hydrophobic, or van der Waals interactions with a tissue, cell, or protein. PEG is a hydrophilic polymer long utilized to achieve stealth behavior in the body.[98,99] PEG MWs between 2 and 5 kDa are optimal for reducing protein adsorption, increasing circulation, and improving penetration within the brain microenvironment.[82,98] However, PEG can also lead to decreased cell-specific uptake for some nanoparticles, and to less effective platforms in the case of repeat administrations, due to development of anti-PEG immunity.[100] Zwitterions are a promising replacement for PEG. The surface engineering of zwitterionic-coated nanoparticles is a rapidly growing area for drug delivery,[101,102] but has limited investigations in the brain thus far.[96] Coatings such as chitosan,[103] polysorbate 80 (P80),[104] polyvinyl alcohol (PVA),[83] and polaxamer 188 (P188)[105] can provide a protective shell and near-neutral surface charge. In particular, P80 leads to adsorption of apolipoprotein-A to the nanoparticle surface, which can act as a brain-targeting agent through receptor-mediated transcytosis across the BBB.[106,107] The use of surfactants has also allowed polymeric nanoparticles up to 250 nm in size to cross the BBB.[108] While these studies show promise for increasing brain uptake via passage across the BBB,[109] it is still unclear what effect different surface coatings have on a particle's ability to escape the endothelium or lumen, and move within the brain microenvironment. There are extensive mechanistic studies on how surface coating/functionality influences passage across the BBB or cell-specific uptake and intracellular trafficking; yet, there remains a critical need for understanding the middle step: getting from the blood vessel to the cell of interest by navigating the brain microenvironment.

NANOPARTICLE ADMINISTRATION ROUTES TO OVERCOME BARRIERS FOR *IN VIVO* EFFICACY IN BRAIN DISEASES

The desired or available administration method for therapeutic intervention in the brain can determine

the nanoparticle physicochemical properties needed to deliver a therapeutic. In the following section, organized by route of administration, we focus on therapeutic strategies used to treat common disease hallmarks in preclinical brain disease models. The use of nanoparticles (abbreviated as NPs in this section) in brain cancer treatment has been discussed in several recent publications,[110–112] and therefore will not be addressed in this review. In addition, the use of NPs for diagnostic applications in the brain is outside the scope of this review, and we refer the reader to other recent publications.[113–115] However, the design-mapping concept presented in the next section is relevant to diagnostic NPs, because these particles require site-specific delivery to provide accurate diagnostic information. Therefore, this section focuses on non-cancer applications of NPs that show promising therapeutic outcomes (improvement in phenotype, survival, or neurological deficits) in preclinical efficacy studies. We first present studies that utilized intravenous (i.v.) delivery to achieve efficacy, including passive delivery and active targeting (i.e., ligand-mediated), as well as oral delivery and intraperitoneal (i.p.) delivery. We next discuss preclinical efficacy studies using local delivery strategies, including intraparenchymal and direct CSF administration, and lastly, we conclude with intranasal delivery methods.

Intravenous Targeting

Systemic administration of NPs consists of i.v., oral, intramuscular, and i.p. routes of delivery. With all systemic delivery methods, NPs will reach the blood stream, and must be able to avoid clearance from the circulation and achieve passage across the BBB to selectively uptake into the brain. Direct i.v. administration is one of the most commonly studied administration routes for NP delivery to the brain because it is noninvasive, yet still avoids the barriers presented by the skin and gastrointestinal tract. In addition, i.v. dosages provide rapid absorption and delivery of fluids or therapeutics too irritating for injection in another manner. NP delivery via i.v. administration is one of the preferred administration routes in the clinic, and many preclinical studies have demonstrated efficacy by administering drug- and gene-loaded NPs in this manner. Given the number of studies involving NPs administered i.v. for *in vivo* efficacy, we discuss a few studies that highlight common disease hallmarks, and provide additional *in vivo* efficacy studies in Table 2 for reference.

Under ischemic conditions in the brain (i.e., a stroke), there is an imbalance in reactive oxygen species (ROS) generation and scavenging, leading to oxidative stress. In order to increase the half-life and BBB permeability of ROS scavengers such as superoxide dismutase (SOD), Reddy and Labhasetwar delivered SOD encapsulated in poly(lactide-*co*-glycolide) (PLGA) (291 nm, –24.5 mV) to rats after experimental stroke,[131] and showed a decrease in infarct volume and reduced neurological deficit scores. In a thoracic contusion SCI model, Kwon et al. demonstrated that magnesium salts, which often have to be given at high doses to achieve a therapeutic effect, could be encapsulated with PEG to reduce the required dose, while still achieving the same therapeutic effect.[132] PEG extends circulation time of the therapeutic and limits off-site toxicity; however, PEG can also reduce cell-specific uptake, which could limit further improvement in efficacy. NP applications for ROS scavenging require passage across the BBB and movement within the brain parenchyma, as well as sustained release of the scavenger to achieve an optimal balance of ROS generation in the brain. While PEG-coated particles might decrease cell-specific uptake, retention and localization of PEG-coated particles in the brain microenvironment should be analyzed to reduce the risk of overscavenging ROS, which are also critical to normal brain function.

Kannan et al. demonstrated the efficacy of i.v. administered dendrimer–drug conjugates in neuroinflammation-mediated models of brain injury. Hydroxyl-terminated polyamidoamine (PAMAM) dendrimers (4 nm, +4.5 mV) conjugated with *N*-acetyl cysteine (NAC, anti-inflammatory) or valproic acid (anticytotoxic) showed reduction in neurological injury in a rabbit model of CP[95] and a hypothermic circulatory arrest (HCA)-induced canine model of brain injury.[38] There was significant improvement in inflammation, neuronal damage, and motor function in CP kits treated with dendrimer–NAC compared to kits treated with free NAC, with nearly equivalent behavior to age-matched healthy controls. As seen in the CP model, development of dendrimer-based therapy for postnatal treatment of a prenatal insult can have significant future implications for fetal and perinatal brain diseases. In the HCA model, canines treated with dendrimer–drug conjugates had lower neurobehavioral scores, no adverse side effects, and required a tenth of the drug dose to achieve the therapeutic effect compared with a free drug. The PAMAM dendrimers used in the CP and HCA models were 4 nm with a near-neutral surface charge, and were capable of bypassing an impaired BBB, moving within the brain microenvironment, and selectively localizing into disease-associated cells.[97]

TABLE 2 | Therapeutic Nanoparticle Platforms Delivered i.v. for *In Vivo* Efficacy in Noncancer Neurological Diseases

Platform	Therapeutic	Disease Model	Outcome
P188-coated PLGA NPs (200 nm)	Brain-derived neurorophic factor (BDNF)	TBI mice[116]	• Increased BDNF half-life and BDNF permeability across the BBB, and improved neurological outcome and cognitive deficits
PLGA NPs (250–330nm, –13 mV)	Cerebrolysin	TBI rat[117]	• Slowed edema formation and BBB breakdown. Behavioral changes were not evaluated
P80-coated PLA–PEG NPs (100 nm), P80-coated PBCA NPs (69 nm)	Amphotericin B (AmB)	Meningitis[118,119]	• PLA–PEG NPs enhanced drug concentration in brain, reduced toxicity of AmB to liver, kidney, and blood system, and increased survival of mice by twofold. • PBCA NPs led to 80% survival compared to 60% with free AmB and 0% survival for untreated animals
P80-coated PBCA NPs (250 nm)	Nerve growth factor (NGF)	Scopolamine-induced amnesia mice and PD mice[120]	• Reversed amnesia, improved recognition and memory, reduced rigidity and tremor
Squaline-lipid NPs (120 nm, –25 mV)	Adenosine	SCI rat[121]	• Dose-dependent decrease in infarct volume and improved neurological function
PEG–liposome (230 nm)	Hemoglobin	Ischemic brain injury[122]	• Reduced injury in cortex, striatum, hippocampus, and pyriform lobe, likely where regions of BBB impairment were present
Liposome	Phenytoin	Epileptic mice[123]	• Reduced epileptic symptoms via accumulation in the amygdala, possibly due to presence of BBB impairment and cell death
PEG–liposomes (80 nm)	Tempamine	MS mice[124]	• Decreased disease severity, as indicated by improved clinical scores and decreased duration of symptoms
PEG-functionalized carbon clusters (HCC) (2–3 nm wide, 30–40 nm long)	Carbon	TBI accompanied by hemorrhagic shock[125,126]	• Restored superoxide levels to normal in the brain and in vasculature, and normalized nitric oxide (NO) levels
Platinum NPs (2–3 nm)	Platinum	MCAO mice[127]	• Reduced injury-induced increases in matrix metalloproteinase and ROS
Cerium oxide (CeO) NPs (<5 nm, –23.5 mV)	Cerium	MS mice,[128] ischemic stroke[129]	• Reduced disease severity and ROS levels, and improved motor function. • Studies suggest that 5-nm particles were more toxic than 30-nm CeO NPs. To note, 30-nm CeO NPs were impeded by the BBB[130]

BBB, blood–brain barrier; NP, nanoparticle; MCAO, middle cerebral artery occlusion; MS, multiple sclerosis; PBCA, poly(butyl cyanoacrylate); PD, Parkinson's disease; PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic); ROS, reactive oxygen species; SCI, spinal cord injury; TBI, traumatic brain injury.
Several nanoparticle platforms have been used as therapeutic carriers or therapeutics themselves (i.e., carbon, platinum, and cerium oxide NPs), and demonstrated significant efficacy in ischemia, injury, infection, and inflammation-mediated neurological disease models.

Large animal model efficacy and efficacy of the same NP platform across multiple, yet similar, models of neuroinflammation-mediated brain injury represents a critical and promising step toward clinical translation.

Targeting ligands, osmotic agents, and focused ultrasound (FUS) techniques can enhance NP uptake across the BBB. We will not cover these topics in this review, but reviews on NP targeting to the brain using osmotic agents[133] and ultrasound techniques like FUS[134,135] to disrupt the BBB have been published recently. Owing to the heterogeneity of BBB impairment in many brain diseases,[17] active targeting using ligands or coatings on the NP surface has become commonplace, and can reduce toxicity by minimizing off-target uptake, while increasing drug delivery to the target site. Many active targeting strategies, summarized in Table 3, take advantage of receptor-mediated transcytosis, which involves targeting native receptor proteins on the surface of cerebral endothelial cells at the BBB. Some common receptor targets include Tf receptors, insulin receptors, and endothelial growth factor receptors (EGFRs). Surfactants such as chitosan, P80, and P188 can also open tight junctions, act as brain-targeting agents, and initiate a receptor-mediated transcytosis across the BBB endothelium.[105] However, the effectiveness of actively targeting NPs to the brain, or any tissue, is still a controversial topic.[90] Regardless of any targeting ligands, the combination of NP physicochemical properties (i.e., size, surface charge, and shape), bulk fluid flow, and nonspecific uptake in the body, as governed by the physiology, influences the tortuous route a NP navigates *in vivo*. In addition, with the exception of local delivery approaches, the path a NP must take from the point of administration to its target site is often several orders of magnitude greater in distance compared with the distance in which ligands recognize their receptors (several microns).

Oral Delivery

Oral delivery is another preferred method for delivery of therapeutics due to high patient compliance, ease of administration, and lower cost. Therapeutic NPs should reach the bloodstream after oral delivery (p.o.), and avoid being denatured before absorption or cleared out otherwise.[147,148] NP design for oral applications needs to overcome denaturation in the digestive tract due to acid and bile salts, and accommodate for highly-regulated absorption followed by first-pass metabolism by the liver. Synthetic and natural polymeric NP platforms are biodegradable, controlled-release platforms that can provide pH-responsive or bile- and acid-resistant stabilization of drugs in the gastrointestinal (GI) tract.[147,149] Das and Lin evaluated P80-coated PEG20k–poly(butyl cyanoacrylate) (PBCA) NPs for p.o. delivery of dalargin, an antinociceptive hexapeptide that does not penetrate the BBB by itself.[150] Orally-administrated PBCA NPs doubly coated with P80 and PEG20k showed increased dalargin-induced analgesia compared to either coating alone in mice exposed to a heat stimulus. The ability to cross the BBB was thought to be due to particle size (100 nm), near-neutral zeta potential (ZP) (–2.4 mV), and the use of both P80 and PEG. The double coating provided synergistic benefits, where PEG provided protection from gastric and intestinal degradation, extended circulation, and reduced clearance, while P80 increased cell-specific uptake. Orally-administered pH-sensitive, 'redox polymers' like PEG-*b*-poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl) aminomethylstyrene] (40 nm) scavenge ROS in the brain through protonation of the amino groups of the hydrophobic segment of the polymers.[151] Redox NPs reduced levels of oxidative stress in the brain of senescence-accelerated mice, even though only 0.5–1.0% injected dose (ID) internalized in the brain. Redox NPs led to amelioration of cognitive impairment, an increased number of surviving neurons, and no detectable toxicity.[151] Similar to the studies by Kannan et al.,[38,95,152] large amounts of total brain uptake are not necessary if the NPs act in a cell-specific manner. Sordet et al. studied the activity of atovaqueone-loaded poly(lactic acid) (PLA) NPs (206 nm) in the treatment of acute and chronic murine toxoplasmosis.[153] Seventy-five percent of mice treated with atovaquone-loaded PLA NPs survived with no detectable parasites in the blood compared to almost no survival in the untreated group. In chronic toxoplasmosis, there was a marked reduction of cyst counts in comparison to the controls. Using oral delivery of NPs in infection models offers promise for expansion into infectious disease in the brain, including treatment of HIV, which currently uses orally administered therapies.

Lipid-based nanoparticles have the ability to enhance absorption and bioavailability for poorly water-soluble therapeutics. Solid-lipid NPs (SLN) are thought to provide protection of drug payload from chemical degradation in the GI tract.[154,155] Leyva-Gomez et al. tested the efficacy of oral delivery of clonazepam (CLZ)-loaded SLN (CLZ-SLN, 300 nm) for seizures.[156] CLZ-SLN p.o. showed significant inhibition of convulsive behaviors in rats and mice compared to i.p. administration of CLZ-SLN. Hansraj

TABLE 3 | Nanoparticle Platforms With Active Targeting Ligands That Have Demonstrated *In Vivo* Efficacy in Preclinical Animal Models of Noncancer Neurological Diseases

Disease	Targeting Ligand	Platform	Outcome
PD	• Lactoferrin (Lf)	• Urocortin-loaded PEG–PLGA NPs (120 nm)[136] • Human glial cell line-derived neurotrophic factor gene (hGDNF) PAMAM–PEG NPs (196 nm, +29.4 mV)[137,138]	• Increased brain delivery of urocortin by threefold,[136] improved locomotor activity and reduced dopaminergic neuronal loss,[137] and enhanced monoamine neurotransmitter levels in PD rats[138]
Stroke	• Transferrin receptor (TfR) • Chlorotoxin (CTX)/ lexiscan (LEX)	• Caspase-3 PLGA NPs (640 nm)[139] • VEGF-liposomes[140] • Nogo-66 receptor antagonist (NEP1-40)-loaded PLGA NPs (152 nm, −22.5 to −25.1 mV)[141]	• TfR PLGA NPs decreased infarct volume, neurological deficit scores, and caspase-3 activity at the highest dose[139]; yet, the particles were likely to be too large to move readily within the brain microenvironment based on current steric limitations.[82] Liposomes resulted in higher levels of VEGF mRNA and protein, higher vascular density, and reduced infarct volume.[140] However, these particles rely on the successful conjugation of multiple agents, resulting in large particles with lower success rates and higher cost. • CTX/LEX PLGA NPs reduced infarct volumes, improved neurological function, and enhanced survival of stroke mice.[141] CTX and LEX more efficiently crossed the BBB in the ischemic brain. The negatively charged PLGA–CTX/LEX NPs may bind to MMP in the ECM, instead of localizing in damaged neurons
AD	• Triphenylphosphonium (TPP)	• TPP-ceria NPs (3 nm, +45 mV)[142]	• Ceria NPs localized to mitochondria, ameliorated neuronal damage and Aβ-induced mitochondrial ROS accumulation in astrocytes in AD mice, and restored mitochondrial morphology after oxidative damage
Seizures	• Angiopep-2 (ANG)	• Phenytoin sodium (PHT)-loaded hydrogel NPs[143]	• Hydrogel NPs led to higher distribution in the CNS, lowered the effective therapeutic doses of PHT, and improved antiseizure effects
Meningitis	• Trans-activator of transcription (TAT) • TfR	• Self-assembling amphiphilic cationic antimicrobial peptides[144] • Amphotericin B (AmB) PLA–PEG NPs (121.8 nm, −18.1 mV)[145]	• TAT NPs led to significant reductions in fungal counts and leukocyte concentrations in the CSF, as well as reduced histopathologic scores, but results were not statistically significant when compared to free fluconazole, which readily penetrates the blood CSF and BBB.[146] To provide increased clinical benefit, a NP must improve cell-specific uptake and provide sustained action to increase efficacy compared to a free drug. • HPLC measurements indicated significantly higher concentrations of AmB in the brain using AMB NPs than free AmB[145]

AD, Alzheimer's disease; BBB, blood–brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; ECM, extracellular matrix; HPLC, high-performance liquid chromatography; MMP, matrix metalloprotease; NP, nanoparticle; PAMAM, polyamidoamine; PD, Parkinson's disease; PEG, poly(ethylene glycol); PLGA, poly(lactic-*co*-glycolic); VEGF, vascular endothelial growth factor; ZP, zeta potential.
Active targeting ligands, including transferrin, growth factors, and small peptides, are commonly used to increased cell-specific uptake in the brain. This table provides several examples of the use of active targeting ligands. The outcomes of NP therapeutic delivery to the brain and limitations associated with each study are discussed. Physicochemical properties including size (nm) and ZP (mV) are provided when available from the reference.

et al. showed that sumatriptan-loaded chitosan SLN improved antimigraine potential and improved the bioavailability of sumatriptan via p.o. delivery compared with i.p. delivery, leading to a 4.5-fold increase in uptake in the brain, and reduced acetic acid-induced writhing and light aversive behavior.[157] Elnaggar et al. first utilized brain-targeted glyceryl monooleate cubosomes with bioactive excipients for oral piperine (PIP),[158] demonstrating significant improvement in cognitive function in a rat model of sporadic AD. In addition, several novel nonpolymeric- and nonlipid-based NP platforms are in early-stage preclinical development. Yang et al. evaluated the organelle targets of carbon nanotubes (CNTs) in rats after gavage administration, showing that CNTs deliver acetylcholine to mitochondria and recover learning ability in AD rats to normal levels.[159] CNTs have many interesting properties that could be explored in drug delivery platforms, but their toxicological effects are still not fully understood, which limits their current clinical application.[160] In general, given that oral dosing is preferred for many medications for psychiatric diseases, continued investigation into oral nanomedicine approaches for treatment of neurological diseases is needed.[155]

Intraperitoneal

Intraperitoneal delivery is used for chemotherapeutic delivery to peritoneal cancers or delivery of antibiotics for peritonitis episodes in PD patients. Preclinically, i.p. delivery is a time-effective way to administer particles to the large numbers of animals required to get statistically significant and clinically relevant outcomes in brain disease models.[161] Kurakhmaeva et al. examined the antiparkinsonian effect of nerve growth factor (NGF) adsorbed to the surface of P80-coated PBCA NPs in a model of PD in mice.[162] A single i.p. injection of P80-coated NGF-NP significantly reduced rigidity and freezing episodes. Valenza et al. studied the i.p. delivery of PLGA NPs modified with a glycopeptide (g7) and loaded with cholesterol in a mouse HD model.[163] Cholesterol normalized GABAergic activity, partially normalized glutamatergic synaptic activity, and protected mice from cognitive decline, but did not rescue motor deficits. The low drug loading (1%) of g7-NPs in this study could be a potential limitation. Even though behavior and neuronal activity improved, the very low dose of exogenous cholesterol delivered to the brain of HD mice (21 μg) cannot easily be discriminated from endogenously produced cholesterol.

Delivery via i.p. administration is also beneficial for newborn or young animals, particularly rodents, where a vein is not readily accessible. Therapeutic studies in fetal, perinatal, or neonatal animal models are limited, yet current studies using nanotechnology are promising. Nance et al. demonstrated the effectiveness of i.p. administered PAMAM dendrimer–NAC conjugates to newborn mice with cerebral ischemia,[152] showing suppression of proinflammatory cytokine production, reduced glial activation, and improved myelination. In this study, dendrimer-mediated cellular uptake in the brain was dependent on timing of administration after injury, tracking changes in cellular function as the injury progressed. Erythropoietin (EPO), a hematopoietic cytokine, provides broad-acting neuroprotection in the newborn injured brain. A single i.p. injection of EPO-loaded oligochitosan NPs (EPO-NPs) was investigated by Wang et al. as a treatment for periventricular leukomalacia (PVL),[164] a pathological precursor for CP and ASD. NPs displayed a long circulation half-life and reduced the therapeutic dose of EPO to 1% of equivalent free EPO. These studies targeting disease hallmarks in perinatal models provide a foundation for NP-mediated delivery in perinatal and pediatric brain diseases, an often-underserved area in technology development.

NP technologies that can allow for rapid absorption into systemic circulation from the peritoneum, and sustained circulation following absorption, are beneficial. PIP SLNs (~300 nm) were injected i.p. in animals with AD and showed decreased plaques and acetylcholinesterase (AChE) activity. Owing to increased brain uptake, treated AD animals performed as well as age-matched healthy control animals at the forced swim test, and outperformed those treated with Donepezil, a clinically available AChE inhibitor.[165] Frozza et al. demonstrated the neuroprotective effects of resveratrol (RSV) against exogenous amyloid beta (Aβ)-induced cognitive dysfunction by encapsulation in lipid-core nanocapsules (LNC).[166] Free RSV given i.p. failed to improve spontaneous alternation behavior in Aβ-infused rats. However, treatment with RSV-LNC at the same drug dosage attenuated impairment, increased ability to distinguish between familiar and new objects, and reduced microglial activation. Bernardi et al. loaded the nonsteroidal anti-inflammatory drug indomethacin (IndOH) into LNCs (IndOH-LNCs; 240 nm), which increased its delivery across the BBB when delivered i.p. in a rat model of AD.[167] IndOH-LNCs increased IndOH delivery to the brain more than fourfold and reduced glial activation, while improving memory function and behavior. In a double-transgenic AD mouse model, Muhs et al. tested the difference between two Aβ liposome-based treatments delivered i.p., one that was palmitoylated (PALM-Aβ1–15) and

one that was PEGylated (PEG-Aβ1–16).[168] They demonstrated that the PALM-Aβ1–15 formulation increased immunoglobulin G (IgG) response compared to that of the PEG-Aβ1–16 treatment, and restored memory.

Xie et al. demonstrated that zinc oxide (ZnO) NPs had neuroprotective effects in an LPS-induced model of depression in mice.[169] However, these results were not strictly consistent with prior reports. In fact, a similar study by Han et al. reported that ZnO NPs damage spatial cognition,[170] though the enhancement of long-term potentiation (LTP) and escape latency in the Morris water maze (MWM) test by ZnO NPs was the same in these two studies. This may indicate a bidirectional effect of ZnO NPs, but a remaining concern regarding ZnO NPs is toxicity through increased ROS generation.[171] The conflicting results could be dose- and exposure-dependent. Xie et al. gave 5.6 mg/kg ZnO NPs every other day for 8 days and Han et al. administered 4 mg/kg biweekly over 8 weeks. Although ZnO NPs cross the BBB, given that the ZnO NPs ranged from 20 to 80 nm, and possess a +6 mV surface charge, dose-dependent and exposure time studies could elucidate mechanisms of ZnO toxicity in brain disease models.

Local Delivery

Local delivery of NPs, including parenchymal, ventricular, subarachnoid, or cisternal, is often a common strategy in cases where surgical intervention or access to the CSF (i.e., catheter or shunt implantation) is an option. Local delivery directly bypasses the BBB, delivering higher concentrations of therapeutic to the brain, as well as allowing administration of therapeutics that would be intolerable in concentrations necessary for systemic delivery.[172] This approach would require NP properties that reduce clearance from the interstitial space, allow for movement within the brain parenchyma, and increase cell-specific activity.

Intraparenchymal

While direct injection into the parenchyma is intrusive, this method is clinically relevant when used during a surgical procedure where the brain is already exposed. The Gliadel wafer, an 8-mm diameter, biodegradable polymer wafer impregnated with carmustine, is an excellent example of a 'leave behind' approach that was successful in becoming part of the standard of care for glioblastoma (GBM) treatment.[173,174] However, the limited ability of free drug to penetrate beyond the wafer-tissue interface into the heterogeneous GBM microenvironment has led to the need to develop novel approaches that can aid a therapy's access to the diffuse disease cells associated with most brain diseases.[173] Microspheres have high drug loading and sustained drug release, and are intraparenchymally injected for noncancer brain diseases. Studies have included the use of bethanechol-encapsulated copolymer of poly(bis(*p*-carboxy-phenoxy)propane) anhydride and sebacic acid (PCPP-SA)[175] or NGF-encapsulated microspheres in AD,[176,177] and carbidopa (CD)- and levodopa (LD)-loaded PLGA[178] and dopamine (DA)- or norepinephrine (NE)-encapsulated PLGA for PD.[179]

While these studies are promising, microspheres are not capable of penetrating into the brain microenvironment. Further research to scale down microsphere formulations to the nanoscale could lead to new avenues and treatments, and most initial studies thus far have focused on gene delivery vehicles. Yurek et al. used 10 kDa PEG-lysine 30-mers (8–11 nm) to intrastriatally deliver a glial cell line-derived neutrophic factor (GDNF) plasmid to rats with unilateral 6-hydroxydopamine (6-OHDA) lesions in a PD model, leading to an increase in tyrosine hydroxylase-positive dopaminergic cells.[180] Lysine-based DNA NP complexes are stable, nonimmunogenic, and noninflammatory, and the small size is an important factor in transfection of the brain, due to the ability to penetrate the brain microenvironment, uptake into cells, and traffic across the 25-nm nuclear membrane pore. PLGA NPs (50–100 nm) can be detected in neurons after intracerebral injection and attenuated PD-related neurodegeneration by reacidification of impaired lysosomes.[181] Hyun et al. delivered reducible poly(oligo-D-arginine) NPs (150–430 nm, >+25 mV) containing a heme oxygenase-1 gene plasmid through intraparenchymal injection to a middle cerebral artery occlusion (MCAO) stroke rat model to reduce infarct volume and inflammation.[182] Kim et al. delivered siRNA in biodegradable arginine ester PAMAM dendrimers (100–200 nm, >+30 mV) to silence high mobility group box-1 (HMGB-1) in normal and MCAO rats, silencing HMGB-1 in 40% of neurons and astrocytes in a normal brain, and leading to reduced infarct volume in the ischemic brain.[183] These NPs also achieved efficacy upon intranasal administration.[184] Overall, there are limited studies in intraparenchymal delivery for noncancer diseases, and efforts exploring local administration for diseases like epilepsy, PD, TBI, and stroke could provide mechanistic insights into NP behavior once in the brain, as well as additional treatment platforms.

Convection-Enhanced Delivery

Pressure-driven infusion, or convection-enhanced delivery (CED), can provide clinically relevant volumes of distribution of therapeutic agents, including nanoparticles, via local delivery in the brain.[185] The manner in which CED parameters and nanoparticle characteristics can affect delivery in the brain have been reviewed elsewhere[30,186,187] and are not covered here. Thus far, CED has been used almost exclusively in MG models with multiple NP platforms[83,188,189] and most extensively with gene therapy in clinical trials. Gene therapy applications using CED in treating MG have been significantly hindered in clinical trials due to lethal hepatotoxicity.[190] In noncancer applications, investigations involving CED delivery of NPs are limited. Ksendzovsky et al. tested the feasibility and safety of CED delivery of M13 bacteriophages (900 nm) for AD and PD treatment in a primate model.[191] M13 bacteriophages bind to Aβ and α-synuclein proteins to trigger plaque disaggregation, with histological studies indicating no evidence of toxicity. Given the success of preclinical studies in MG models, exploration of the use of CED in PD, epilepsy, TBI, and stroke models is a potential area of further research, in conjunction with continued development focused on standardizing surgical implantation and infection risk-reduction methods.

Intraventricular, Intracisternal, or Subarachnoid

Direct administration into the CSF is a current therapeutic approach for management of pain or muscle spasms in CP, MS, chronic pain, and spinal injuries. Several preclinical studies have utilized CSF administration for increased therapeutic uptake into the brain.[192] Shyam et al. found that infusion of rod-shaped polyethyleneimine (PEI)–PEG copolymer-based NPs (<100 nm) improved levels of *BACE1* (an enzyme involved in cleaving Aβ precursors) in a mouse model of AD compared to worm-like and spherical PEI NPs.[193] Dengler et al. were the first to demonstrate that positively-charged amorphous mesoporous silica NPs containing plasmid DNA encoding the IL-10 gene (240 nm, +24 mV) showed therapeutic efficacy after intrathecal delivery in a model of pain hypersensitivity (allodynia).[194] Pain sensitivity returned to baseline for almost 2 weeks, with minimal toxicity. Tian et al. showed that intracisternal administration of Tat peptide-decorated gelatin–siloxan NPs (170 nm, +36 mV) carrying the calcitonin gene-related peptide (a potent vasodilator) attenuated cerebral vasospasm and improved neurological outcomes in a rat model of subarachnoid hemorrhage.[195] However, there is a physical difference in subarachnoid hemorrhage between rats and humans, which necessitates studies using larger mammal or nonhuman primate models of vasospasm. Amine-modified single-walled carbon nanotubes (SWCNTs, 4–10 nm diameter) also exhibited therapeutic and prophylactic abilities in a rat stroke model.[196] Treated rats showed less tissue damage than controls and displayed better motor function after injury. When administered into the CSF, nanoparticles must cross the ependymal layer to move into brain tissue and uptake into cells. Appropriate size and surface functionalization of particles may help overcome this barrier. For instance, Dai et al. confirmed that neutral dendrimers target neuroinflammatory cells after subarachnoid administration, despite an absence of targeting ligands.[197] The PAMAM dendrimers crossed the ependymal layer and localized in activated microglia and astrocytes in the presence of neuroinflammation. Oligodendrocytes and neurons, cells not thought to be directly involved in the inflammatory process, did not take up dendrimers to an appreciable extent.

Intranasal Delivery

Intranasal administration is another method to bypass the BBB and deliver drugs to the brain. Intranasal delivery benefits from a more direct pathway to the brain compared with systemic administration as well as reduced toxicity, and can have lower dosage requirements.[198] It is minimally-invasive and requires little training for administration, making it a viable route for large-scale treatment. Klementieva et al. used poly(propylene imine) (PPI) glycodendrimers coated with cationic maltose or maltotriose shells (7–8 nm) in an AD mouse model.[199] Fourth-generation cationic PPI maltose glycodendrimers can glue together Aβ fibrils, changing the surface charge of these fibrils and reducing interaction of Aβ with cell membranes.[200] After administration to male AD mice and wild-type littermates, Aβ levels were reduced in the brain, but memory issues were not reversed. The cationic maltose coating was also harmful when administered chronically.[199] Zhang et al. encapsulated basic fibroblast growth factor (bFGF) in lectin-modified PLGA–PEG NPs (STL–bFGF–NP) (104 nm, −31 mV) and rats received injections of Aβ and ibotenic acid to induce AD-like symptoms. Treatment with STL–bFGF–NP improved spatial learning and memory.[201]

A large number of preclinical intranasal studies have also been done for PD. Ropinirole hydrochloride (RH) is offered as an oral tablet on the market,

but has been loaded into chitosan-coated NPs (150–400 nm, +32 mV),[202] polymer-lipid hybrid NPs (PLNs, 98–287 nm, −20 to +45 mV),[203] and SLNs (66–271 nm, −16 to +47 mV)[204] for use in animal models. All formulations caused extended release of RH, and both PLN and SLN RH NP systems resulted in a reduction in tremors, rigidity, and immobility versus free oral drug.[203,204] Haney et al. used a catalase-encapsulating exosome (100–200 nm), which reduced ROS and inflammation, and extended catalase release in PD mice, compared with mice given PBS control.[205] Lu et al. treated male PD rats with substance P-loaded gelatin NPs (172 nm, −30 mV), leading to higher neuronal cell viability and lower levels of proapoptotic caspase-3.[206] Wen et al. conjugated odorranalectin-coated PEG–PLGA NPs (110 nm) with urocortin and administered them intranasally to 6-OHDA-induced PD rats.[207] Rats treated with urocortin NPs exhibited improved motor function, but dopamine levels were still significantly lower than control. However, dopamine levels in urocortin NP-treated rats were ninefold higher than in animals treated with blank NPs, with significant reduction in dopaminergic neuronal loss.

Both Kubek et al. and Veronesi et al. used anticonvulsant thyrotropin-releasing hormone polylactide NPs (108 nm) to treat electrode-induced seizures/epilepsy in rats, demonstrating that NPs crossed the BBB and increased the seizure threshold.[208,209] Natural polymeric NPs, like chitosan, gelatin, or alginate, can provide improved delivery and sustained drug release, are readily available on a large scale, and are low cost.[210] Haque et al. used alginate NPs (167 nm, +23 mV) loaded with the antidepressant venlafaxine in a rat depression model,[211] which improved neurobehavioral function compared to free-drug treated rats. Singh et al. conducted a similar study using selegiline hydrochloride, another antidepressant, encapsulated in thiolated chitosan NPs (215 nm, +17 mV), which improved forced swimming and decreased immobility time after tail suspension compared to control animals.[212] Although limited in the number of infection-focused studies, Mahajan et al. used saquinavir mesylate, a protease inhibitor with activity against HIV, in a P80-PEG (400 Da) nanoemulsion (176 nm, −10 mV) to increase brain concentrations of saquinavir, which in free form is effluxed by P-glycoprotein expressed in BBB endothelial cells.[213] While intranasal delivery is a promising delivery route for NPs to reach the brain, preclinical study outcomes should be interpreted with caution. There are physiological differences in the nose between humans and animal models, including nonhuman primates. In order to translate NP technologies administered intranasally, the role of the mucosal barrier, the surface area of the nose, the vascularity, the physical nasal cavity structure, and the length scale of transport from the nasal surface to the brain in humans need to be evaluated.

SYNTHESIZING NANOMATERIAL PROPERTIES AND PATHOPHYSIOLOGY HALLMARKS USING SYSTEMS-LEVEL THINKING

Nanoparticle physicochemical properties and administration routes can be fine-tuned to leverage disease hallmarks and overcome barriers in the brain. A variety of factors can determine or influence the therapeutic effect and impact on disease burden. Some factors are specific to the disease (i.e., etiology, age of onset/injury, and progression of the disease), and some factors are specific to susceptibility or risk of disease (i.e., genetics, environment, diet, lifestyle, age). Regardless, common disease hallmarks, including ECM changes, inflammation, oxidative stress, excitotoxicity, cell death, and impaired fluid flow, are present in almost all neurological diseases, albeit to varying extents. Rather than try to increase brain uptake in a purely iterative manner, which may result in poorer outcomes and slower progress, systems-level thinking maps the disease and the nanoparticle as part of the same dynamic environment. Tailoring nanoparticle properties to specific disease commonalties (i.e., inflammation) in the brain could lead to more rapid and effective translation into the clinic. In brain diseases, particle properties must be fine-tuned to achieve uptake in the brain, distribution within the brain parenchyma, and cell-specific interaction (Figure 3). Material properties can be readily controlled and tailored to overcome certain physical barriers, specifically the BBB and cell uptake, and must account for the influence of common disease hallmarks on these barriers, which shapes the heterogeneity across patients and fluctuations in the brain environment as the disease progresses. Changes in barriers in the brain and common disease hallmarks can dictate a nanoparticle's ability to reach the brain and move within the brain to specific cells, more so than any one aspect of the nanoparticle design. Therefore, it is critical to assess nanoparticle behavior in the brain in the context of these interconnected physiological factors, and to design nanoparticles with disease characteristics as the driving principles, as we have mapped in Figure 3.

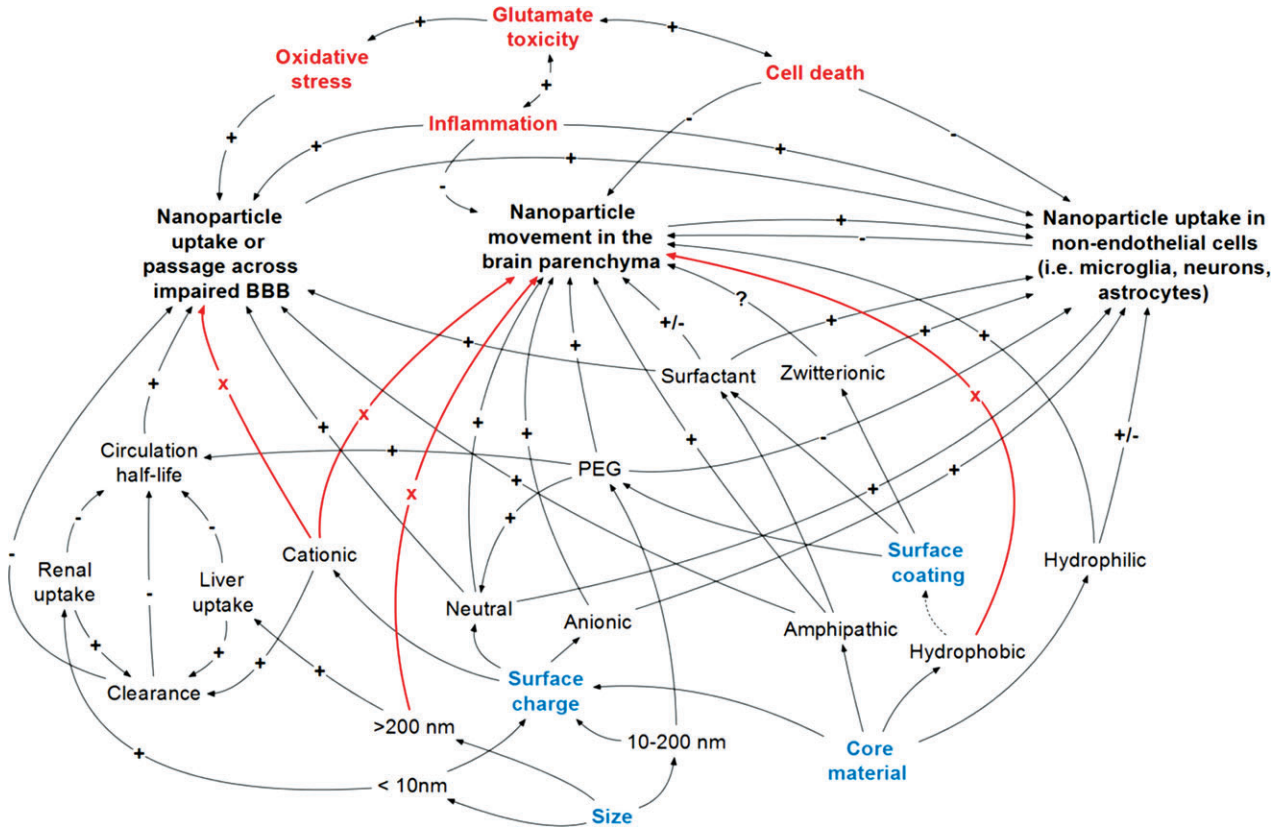

FIGURE 3 | Predictive design mapping of nanoparticle behavior as a function of nanoparticle physicochemical properties to overcome barriers to therapeutic delivery in the brain. Nanoparticle physicochemical properties (blue), including size, core material, surface charge, and surface coating, can influence the behavior of the nanoparticle in the brain, specifically the ability of a nanoparticle to overcome barriers to therapeutic delivery. However, pathophysiological hallmarks (red) influence these barriers, potentially altering the nanoparticle behavior. By looking at both nanoparticle properties and disease hallmarks, nanoparticles can be designed to avoid dead ends (red arrows) that will prevent the particle from achieving a therapeutic effect in the brain. Additional factors, such as shape and particle stiffness, will also affect therapeutic delivery, but these are less thoroughly studied in the brain.

Systems thinking can be applied to design nanoparticles for specific neurological diseases. For example, in PD the therapeutic goal is often to save dying dopaminergic neurons in the presence of BBB impairment, oxidative stress, and microglial cell activation.[214] Nanoparticles can more easily cross an impaired BBB to access the brain parenchyma. However, nanoparticles are likely to be scavenged out of the brain parenchyma by phagocytic activated microglia.[37] This could limit uptake into dopaminergic neurons, particularly in the substantia nigra. A nanoparticle could be designed to avoid microglial uptake by utilizing a negative surface charge to specifically target neurons[96] and improve site-specific drug delivery. To achieve specific delivery only to disease sites in the brain, i.e., dopaminergic neurons in PD, and not to normal healthy tissue, the focus for nanoparticle design should not be only on increasing uptake across the BBB. Instead, the design should also consider movement away from the BBB into the parenchyma. For example, a sub-200 nm particle with a hydrophobic core might require PEG or a zwitterionic coating to diffuse in the brain ECS, while a sub-10 nm amphiphatic or hydrophilic particle with neutral or anionic surface charge is more likely to penetrate to reach target cells.

In the case of more poorly-defined neurological diseases, like neurodevelopmental disorders (i.e., ASD), characterization of nanoparticle behavior from a systems point-of-view helps better understand common disease hallmarks that are accessible to therapeutic intervention. For example, neutral, hydroxyl-modified dendrimer nanoparticles (<10 nm) rapidly uptake into activated microglia and astrocytes in inflammation-mediated neonatal brain injury models,[95,152] compared to cationic or anionic dendrimers, linear dextrans, or polystyrene beads.[97] Interestingly, microglia are more phagocytic, but also have decreased scavenging ability in the presence of neuroinflammation.[215] Therefore, nanoparticles must have physicochemical properties

that allow penetration from impaired capillaries to activated glial cells. The combination of small particle size and neutral surface functionality with BBB impairment and glial activation allows the dendrimer to achieve site-specific delivery,[97] and significant efficacy, with no off-site toxicity.[95]

Several considerations are necessary when applying a systems approach to engineer the design of a nanoparticle to treat a complex disease. First, it is important that a nanoparticle formulation is reproducible, scalable, and well characterized. Taking advantage of changes in barriers in the disease, such as increased BBB permeability and cell uptake, can help lower the complexity of the particle design, without simplifying the disease to a point of clinical irrelevance. In addition, the interdisciplinary nature of nanoparticle-associated fields offers the advantage to test nanoparticle platforms across multiple species and multiple models of the same disease. This can help standardize ways of quantifying and reporting distribution, pharmacokinetic, and efficacy studies, which will aid in comparison to current standard of care treatments and translation to clinical application. More importantly, for pediatric or chronic neurological diseases, study design and funding efforts should focus on the long-term impact of nanoparticle platforms, including longitudinal studies that look at sustained efficacy and safety, and the effect on symptom-free survival.

This review is the first to take an intentional focus on nanoparticle design from the disease perspective, and starts to integrate how nanoparticle physicochemical properties and disease physiology hallmarks interact to overcome the system of barriers present in brain diseases. Systems-level thinking can allow for evaluation of the disease and the nanomaterial in tandem, integrating information across multiple disciplines and applying a combination of engineering, neuro, molecular, and cellular biology, and clinical tools. However, the fields involved with understanding and treating brain disease evolve rapidly, providing new information and insights into the mechanisms of disease and potential therapeutic interventions. In addition, the nano and biomaterial fields continue to leverage advances in fabrication and synthesis to generate data and provide nanoscale control of material properties. Therefore, a systems-level approach must also be dynamic and adaptable, much like the system the approach is modeling. It is important to move toward a paradigm of testing a nanoparticle platform across multiple species and multiple models of the same disease, in order to maintain relevance to the human patient population. Thoughtful and intentional collaborations with medicine, basic science, and engineering, examples of which are rapidly appearing around the world, can increase the applicability of systems-level thinking for complex disease. With a systems-level approach, there is great potential to leverage resources across the multiple fields working to understand and treat neurological diseases.

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