Received: 15 May 2024 | Revised: 30 July 2024 | Accepted: 5 August 2024

DOI: 10.1002/wnan.1991

SYSTEMATIC REVIEW

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY WILEY

Targeting specific brain districts for advanced nanotherapies: A review from the perspective of precision nanomedicine

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Funding information
Italian Ministry of University and Research, Grant/Award Number: PRIN 2022YJSMBX; European Union, Grant/Award Number: CUP H53D23006320006; Spanish State Research Agency, Grant/Award Number: PID2022-139267OB-I00; "Severo Ochoa" Programme for Centres of Excellence in R&D, Grant/Award Number: CEX2018-000867-S

Edited by: Olivia Merkel, Associate Editor and Christine Mayer, Co-Editor-in-Chief

Abstract

Numerous studies are focused on nanoparticle penetration into the brain functionalizing them with ligands useful to cross the blood–brain barrier. However, cell targeting is also crucial, given that cerebral pathologies frequently affect specific brain cells or areas. Functionalize nanoparticles with the most appropriate targeting elements, tailor their physical parameters, and consider the brain's complex anatomy are essential aspects for precise therapy and diagnosis. In this review, we addressed the state of the art on targeted nanoparticles for drug delivery in diseased brain regions, outlining progress, limitations, and ongoing challenges. We also provide a summary and overview of general design principles that can be applied to nanotherapies, considering the areas and cell types affected by the most common brain disorders. We then emphasize lingering uncertainties that hinder the translational possibilities of nanotherapies for clinical use. Finally, we offer suggestions for continuing preclinical investigations to enhance the overall effectiveness of precision nanomedicine in addressing neurological conditions.

Giulia Sierri and Michela Patrucco contributed equally to this study.

This article is categorized under:
Therapeutic Approaches and Drug Discovery > Nanomedicine for Neurological Disease
Therapeutic Approaches and Drug Discovery > Emerging Technologies

KEYWORDS
blood–brain barrier, brain, nanomedicine, nanoparticles, targeted drug delivery

1 | INTRODUCTION

Delivering drugs to the brain is exceptionally challenging due to the brain's protective barriers and the complex environment it presents. Meanwhile, researchers have been exploring various strategies to tackle these challenges (Pardridge, 2022), such as nanotechnology, focused ultrasound, drug modifications to enhance permeability, and the development of nanocarrier systems to ferry drugs into the brain. Each approach brings its own set of advantages and limitations, making the field dynamic and driving ongoing research and innovation. Thankfully, nanomedicine holds great promise for drug delivery to the brain, as it can overcome issues associated with delivering therapeutic agents across the blood–brain barrier (BBB) and into the central nervous system (CNS) (Rhaman et al., 2022). Indeed, in the absence of brain drug delivery technologies, nearly all large molecules (molecular weight >400 Da) and more than 98% of hydrophilic small-molecule drugs cannot penetrate the BBB (Pardridge, 2005). Drugs showing activity in the CNS are mainly used to treat affective disorders, chronic pain, insomnia, and epilepsy (Pardridge, 2007). The ability of different nanoparticles (NPs), naked or functionalized with targeting ligands boosting their interaction and crossing of the BBB, to reach the brain is extensively studied, a fact that can be confirmed by the increase in the number of publications on this topic in the last 10 years (Figure 1a).

However, it is essential to highlight that numerous studies deduce the penetration of NPs into the brain by examining the bulk brain, which encompasses blood, cerebrospinal fluid (CSF), and blood vessels within the brain. Regarding the brain comprising neurons, glial cells, and the surrounding extracellular matrix, most of the research failed to demonstrate the entry of NPs into the brain parenchyma. This topic has been recently addressed in a review by Yokel

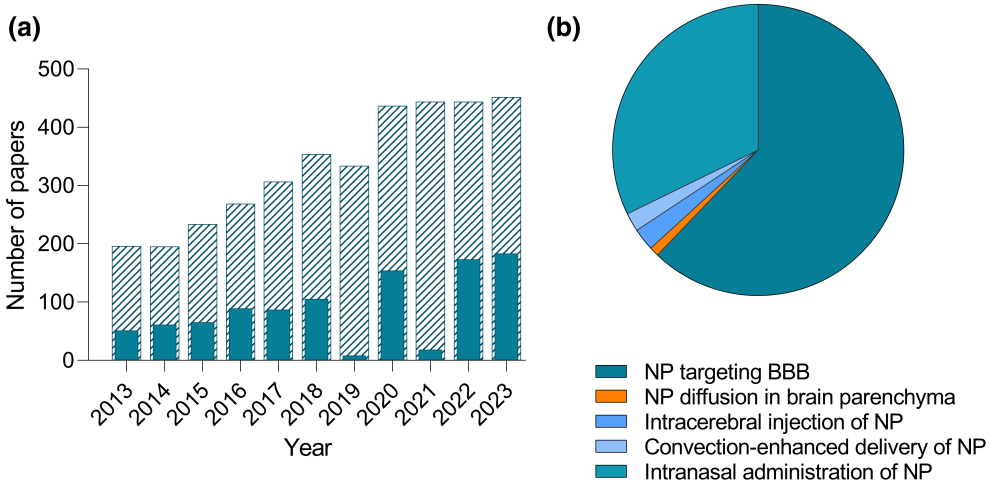


FIGURE 1 The number of publications related to the design of nanoparticles for blood–brain barrier crossing between 2013 and 2023. The bibliographic research was performed with PubMed (https://pubmed.ncbi.nlm.nih.gov/) over the publishing period of 2013–2023. (a) The search query was "nanoparticles" AND "blood–brain barrier." Bars with lines represent the total number of papers, and full bars indicate the number of reviews. (b) The aerogram represents the number of papers investigating different routes to deliver nanoparticles to the brain. Orange represents the number of papers investigating the diffusion of nanoparticles in brain parenchyma. Search queries are indicated in the figure.

(Yokel, 2020). In our opinion, a pivotal point to be discussed in the NPs' brain delivery is the targeting issue, which until now has been scarcely addressed (Figure 1b).

Since the different pathologies affecting the brain are often limited to a particular neural cell or brain area, it is crucial to functionalize NPs with appropriate targeting moieties and check their distribution in the brain parenchyma. Moreover, the profound anatomical and functional complexity of the brain and its modifications in the event of pathologies must be considered. For that, we reviewed the state-of-the-art on targeted NPs for the delivery of drugs in diseased brain regions, highlighting advantages and limitations, and raising challenges that are still open. Nevertheless, it is crucial to remember that all the information in the literature regarding the detection of NPs in the brain parenchyma is contingent upon the constraints of presently available techniques. (Box 1).

BOX 1 Techniques to detect nanoparticles in brain parenchyma

The choice of the imaging technique employed to follow the NPs distribution in the brain parenchyma is a pivotal aspect of any therapeutic research study. Magnetic resonance imaging, positron emission tomography, mass spectrometry imaging and in vivo imaging systems, are employed to track the NPs within the brain parenchyma. Nevertheless, most of the detected NPs were found to be still embedded in the brain vessels after transcardial perfusion (Yokel, 2020). Indeed, the spatial resolution is insufficient to distinguish NPs in the brain parenchyma from the ones in the bulk brain, comprising the brain microvessels. More suitable techniques are confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM). CLSM individuated gold/iron NPs on vessel walls, in the perivascular tissue and in deeper regions of brain parenchyma. Through TEM, NPs were tracked at subcellular levels inside trans/endocytotic vesicles of cells lining the BBB or, after their extravasation, in the basal lamina and in the synaptic region of astrocytic endfeet (Sanavio et al., 2018). Raman confocal spectroscopy is a label-free technique which produces a map distribution of chemical features within the cells, recognizing chemical groups of the nanoformulation against the cellular background signal (Vanden-Hehir et al., 2019) with promising results in the ex-vivo NPs localization (Fisusi et al., 2016). X-ray phase-contrast tomography is an emerging non-invasive technique to achieve a tridimensional and multiscale understanding of the NPs' fate after administration (Endrizzi, 2018) which identified gadolinium-based NPs in mice brain metastasis both extravasated and as clusters in the circulatory system (Longo et al., 2021). Lastly, intravital imaging (Lin et al., 2020) allows spatiotemporal information about the NPs–brain cell interaction at a single-cell level in live animals (Zinger et al., 2021).

2 | FACTORS AFFECTING THE NPs DISTRIBUTION IN BRAIN PARENCHYMA

Several factors, including systemic and local biological barriers, the physico-chemical NPs properties, and the physiological characteristics of the brain tissue can influence the distribution of NPs in brain parenchyma. Understanding these elements is crucial for effective targeted drug delivery and therapeutic outcomes.

2.1 | The BBB and its alterations in brain pathologies

The BBB is a highly selective semipermeable membrane barrier that separates circulating blood from the brain's extracellular fluid in the CNS. It is a crucial physiological feature that acts as a customhouse, regulating the passage of substances between the bloodstream and the brain parenchyma. The BBB is formed by tightly packed non-fenestrated endothelial cells that line the capillaries in the brain, which are linked together by tight junctions' proteins. In addition to endothelial cells, the BBB comprises supporting cells such as pericytes and astrocytes, which further contribute to its structural integrity and functional properties (Baghirov et al., 2018). The presence of the BBB strongly limits drugs and therapeutics access to the brain, including NPs. Therefore, the BBB affects the net amount of NPs reaching the brain parenchyma. In this context, NPs can be designed by optimizing the interplay between shape, size, charge, and surface functionalization to target and cross the BBB, thus delivering drugs to the brain parenchyma. Generally, NPs smaller

than 100 nm, with a neutral or slightly negative surface charge have been found to exhibit better penetration across the BBB compared to larger particles and positively charged ones (Blanco et al., 2015; Ding et al., 2020). Moreover, NPs surfaces can be modified with ligands (peptides, antibodies, aptamers, small molecules) that can specifically bind to receptors or transporters present on the endothelial cells of the BBB, such as lactoferrin, leptin, insulin growth factor, transferrin, and low-density lipoprotein receptor, facilitating the transport of NPs across the barrier (Pérez-López et al., 2023), thus improving the BBB crossing. All these aspects were already extensively reviewed (Hersh et al., 2022; Lombardo et al., 2020).

Additionally, the specificity of endothelial targeting can be further enhanced by engineering the shape of NPs. Kohlar et al., taking into consideration the shear rates, flow geometries and vascular features, assessed that polystyrene-targeted nanorods (both at low and high flow rates) are more suitable for brain endothelium adhesion applications than nanospheres with the same targeting ligand (Kolhar et al., 2013). Similar results were obtained comparing different subclasses of human plasma high-density lipoproteins (HDLs) in BBB crossing, where discoidal HDLs displayed a superior capability to cross the BBB versus spherical ones (Dal Magro et al., 2019).

A summary of the ideal NPs features for the BBB crossing is represented in Figure 2.

Even if the BBB represents the first step that limits the NP distribution in the brain parenchyma, the efficiency of BBB crossing is strongly affected by the uptake/internalization and release mechanisms used by endothelial cells. In fact, not only the uptake, but also the release of NPs from the BBB toward the brain are essential factors in determining the effective amount of NPs reaching the brain parenchyma. Also in this case the physico-chemical parameters of NPs can influence their release from the BBB (Sakhtianchi et al., 2013). For instance, NPs functionalized with large amounts of transferrin remain strongly attached to brain endothelial cells. In contrast, those with less transferrin can interact with the transferrin receptor on the luminal side of the BBB and detach from it on the brain side of the BBB, allowing the passage of NPs through endothelial layers (Wiley et al., 2013).

NPs with a size between 30 and 50 nm, rod-shaped and non-targeted are more efficiently secreted by endothelial cells toward the brain in comparison to bigger, spherical, targeted ones. The intracellular localization of NPs after internalization also affects the release rate, thus determining their diffusion into the brain parenchyma. Free NPs in the cytosol are released from cells more efficiently than those located in endo-lysosomal compartments (Muscetti et al., 2023). Accordingly, recent studies are focused on strategies aimed at promoting endosomal/lysosomal escape, such as the incorporation of cationic polymers or membrane-destabilizing peptides into the NPs (Martens et al., 2014).

In the design of NPs for brain drug delivery, it is crucial to consider that in several brain pathologies, such as tumors, stroke, brain infections, and neurodegenerative diseases, the BBB is altered (Meyer et al., 2008; Sweeney et al., 2019), and its modifications could change the NPs performance in reaching the brain (Wu et al., 2023). Most scientific papers addressing this issue defined the diseased BBB as leaky and more permeable, but this is not directly associated with an increased drug permeability. For example, in glioblastoma, the most common brain malignancy

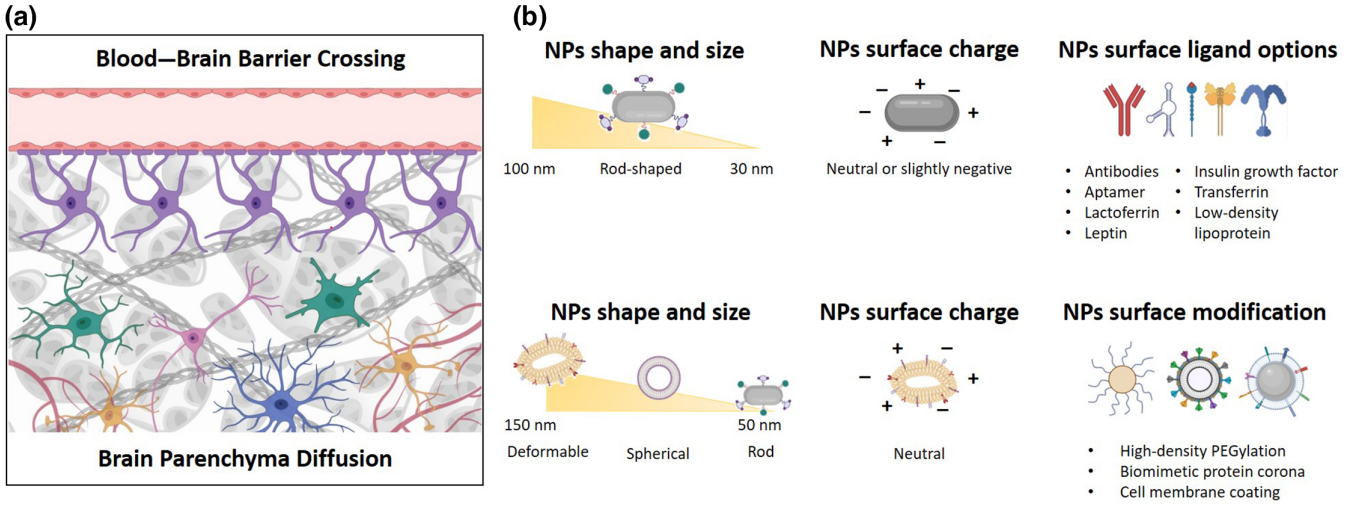


FIGURE 2 Graphical representation of ideal features to consider in the design of NPs for brain targeting, in terms of size, shape, surface charge, and surface chemistry (PEGylation, ligand conjugation). (a) Graphical representation of ideal NPs features for BBB crossing. (b) Graphical representation of ideal NPs features to improve their diffusion in brain parenchyma.

(Lucifero et al., 2020), the BBB and the new vessels originating from tumor-induced neo-angiogenesis remain impermeable to almost all large and small drug molecules, limiting the available therapies (Hendricks et al., 2015). We have recently shown that the molecular alterations in the brain vasculature of transgenic mouse models of Alzheimer's disease (AD) dramatically reduced the number of NPs reaching the brain, in comparison to healthy animals (Magro et al., 2018). Therefore, identifying the BBB changes in aging and disease conditions is essential to deepening the knowledge of disease pathophysiology and helping NP designers refine drug delivery systems.

One possible alternative, is represented by the intranasal administration route that has the potential to circumvent the BBB, allowing the direct entry of NPs to the CNS mainly through the sensory neuronal pathway (Battaglia et al., 2018; Biddlestone-Thorpe et al., 2012; Dighe et al., 2023). In this case, the factors affecting NPs diffusion in brain parenchyma are those described in the following paragraphs.

Once they arrive in the brain, the NPs should ideally distribute in the brain parenchyma, reaching the target precisely. For example, neurons are typically located within 10–15 μm from ECs (Zlokovic, 2005), and the brain's extracellular matrix poses an obstacle that restricts the extensive spread of NPs and their capacity to reach target cells. Despite the pressure-induced flow, facilitated by convection-enhanced delivery (CED), a technique that generates a pressure gradient at the tip of an infusion catheter to deliver therapeutics directly through the interstitial spaces of the CNS, treatments are still limited to the site of administration or movement within the perivascular regions (Papa et al., 2013; Peviani et al., 2019; Salegio et al., 2014), lacking efficient penetration into the brain tissue to access target cells (Mehta et al., 2017). Another potential approach is the design of implantable biomaterials for the in situ release of NPs (Di Mascolo et al., 2023; Viale et al., 2024). This is a very compelling approach, even if applicable only to brain pathologies requiring surgical intervention, such as brain tumors.

2.2 | Physical parameters of NPs (size, charge, shape, and flexibility)

In general, the size, surface charge, shape, and composition of NPs are key factors that can be manipulated to control their pharmacokinetics, biodistribution, and cellular uptake. For example, smaller, spherical, and neutrally charged NPs typically have longer circulation times and more predictable distribution patterns, while larger, non-spherical, and charged NPs interact differently with biological systems. Few data are available on this topic in the context of NPs brain distribution. The distribution of NPs in brain parenchyma is dominated by diffusion, and the effective diffusion coefficient estimates the NPs spread in the brain (Holter et al., 2017). Interstitial fluid (IF), present in the spaces between cells and extracellular matrix (Chary & Jain, 1989; Stine & Munson, 2019), provides a pathway for NPs to move within the brain tissue (Soltani & Chen, 2012). Its movement is influenced by various factors such as hydrostatic pressure, osmotic pressure, and the presence of obstacles like cells and extracellular matrix components (Chary & Jain, 1989; Stine & Munson, 2019). Size, surface charge, shape, and flexibility are key factors that can affect the NPs diffusion coefficient. The human brain extracellular space (ECS) has ∼85% of pores ≤125 nm, and ∼15% in the range of 126–225 nm (Nance et al., 2012). Accordingly, polystyrene NPs with a diameter in the range of 40–114 nm easily diffused within the human and rat brain, compared to bigger ones (Nance et al., 2012). Similar results were obtained in another work, where lipid-based NPs with a diameter <200 nm, demonstrated the capacity to disseminate within mouse brain tissue (Di et al., 2022). NPs with a near-neutral net surface charge (less negative than −4 mV) display a greater percent diffusive fraction in rat brain tissue ex vivo, in comparison to those with a ζ-potential in the −20 and −40 mV range (Nance et al., 2012). In another study, it was found that negatively charged NPs interact with the neuronal membranes, particularly at the synaptic cleft, while positively and neutrally charged NPs consistently avoid localization on neurons. This study establishes the role of negatively charged NPs in modulating neuronal excitability, underscoring their potential utility for manipulating and controlling neuron activity (Dante et al., 2017). At the same time, these results open the possibility to exploit NPs charges for cell targeting. Recently Yuan et al. (Yuan et al., 2022) have developed a mathematical model to investigate the relationship between NPs size/surface charge and their brain diffusion coefficients. Results showed that increasing the absolute value of ζ-potential could significantly increase the NPs diffusivity. When the absolute value of ζ-potential is increased by 5 mV, the order of magnitude of diffusivity is 10^{-9}–10^{-8} m^2/s. Moreover, unlike the NPs diffusion in white matter, which exhibits a particle size threshold, NPs diffusing within the pure IF do not demonstrate such a limit (Yuan et al., 2022).

Shape, especially aspect ratio, is also a key parameter in determining the NPs diffusion in the brain parenchyma because it can change the hydrodynamic diameter and influence charge distribution on the NPs surface. Cognet and co-workers have shown that single-wall carbon nanotubes (more than 100 nm in length and 1 nm in diameter) can

readily diffuse in brain slices. Interestingly, the trajectories of single-walled carbon nanotubes have been recently used to explore the ECS in the live brain showing that it is highly heterogeneous. In the brain extracellular matrix, local rheological properties can change drastically within a few nanometers and ECS width may vary between brain tissue models (Paviolo et al., 2020). Considering this information and the littleness of data about the effect of NPs shape on brain diffusion, we can hypothesize that deformable NPs with high flexibility may be the most suitable for navigating a tortuous environment such as the cerebral one. Deformable NPs are flexible vesicles, mostly similar to liposomes in their structural aspects but unique in their functional aspect (Sapkota et al., 2023). They are widely used because their morphology allows them to go through pores much smaller than their size and they can spontaneously reconfigure during assembly in response to environmental cues. The stiffness of deformable NPs affects their diffusion properties in polymeric gels through decoupling their deformation and transportation during diffusion (Yu et al., 2022). Moreover, it has been demonstrated that cell internalization and transport of elastic NPs with low stiffness occur faster than those of rigid NPs (Chen et al., 2018). Additionally, the flexibility of deformable NPs can also be exploited to make NPs interchangeable through a distinctive form of cell-to-cell interaction, namely tunneling nanotubes. This possibility could enhance the spread of NPs between neuronal cells that are distant from each other (Box 2).

BOX 2 Intercellular tunneling nanotubes to improve nanoparticles distribution in the brain parenchyma

Tunneling nanotubes (TNTs) were first described in the literature in 2004 by Rustom et al. (Rustom et al., 2004) and represent a means of cell-to-cell communication (Khattar et al., 2022). TNTs are open membranous channels with diameters of several hundred nanometers, which contain F-actin and hovering over the substrate in the extracellular space they directly connect close and far-away cells. They have been detected in various cell types in vitro, where they show a variability in their morphology in terms of length and thickness, and in different biological conditions, such as normal physiology, tissue injury, and cancer (Melwani & Pandey, 2023). They are able to transfer calcium waves, macromolecules, organelles such as mitochondria and lysosomes and virus. Many researchers focused their attention on inhibiting TNTs formation to reduce the spreading of diseases. However, most of the compounds used to stop TNTs formation mostly block the mobility of the whole cytoskeleton (Dash et al., 2021). A promising alternative could be the exploitation of TNTs intercellular communication to enhance the intercellular distribution of therapeutic nanocarriers. In 2014, Tosi et al. (Tosi et al., 2014) demonstrated the versatility of TNT-mediated transfer of PLGA NPs between different cell types, particularly glial and neuronal cells. Moreover, as reported by Formicola et al. (Formicola et al., 2019) the type of TNTs formed, whether "thick" or "thin," influences cargo transport efficiency, with implications for drug delivery efficacy. These studies emphasize the interaction between NPs and TNTs, offering opportunities to optimize nanomedicine delivery and diffusion in brain parenchyma (Han & Wang, 2021).

2.3 | Surface modifications of NPs

Surface functionalization of NPs for brain delivery often involves modifying the outer layer of NPs to enhance their ability to cross the BBB (see Section 2.1) and target specific brain regions (see Section 4). This process typically entails attaching ligands or coatings on the NPs surface, and currently the most utilised brain targeting ligands are peptides, proteins, aptamers, small molecules and antibodies. The type of ligands and the surface density of these moieties are key features useful to improve the drug delivery efficacy for neurological disorders while minimizing off-target effects. A detailed description of advantages and disadvantages of brain targeting ligands, and their clinical development phase is presented in a recent review by Moreira (Moreira et al., 2024). Furthermore, another essential point is that the initial interaction between NPs and biological fluids triggers the rapid assembly of a diverse array of resident biomolecules on the NPs surface, encompassing lipids, proteins, and carbohydrates, collectively forming a (bio)corona (Mishra et al., 2021) that confers to the NPs a novel biological identity (Westmeier et al., 2016). Zhang et al. developed a corona-mediated brain targeting system, designing lipid NPs functionalized with the peptide $A\beta_{25-35}$, which can mediate the absorption of circulating apolipoproteins on the surface of the NPs, exposing Apo targeting domains. This strategy

results in a significant enhancement of brain distribution of $A\beta_{25-35}$ functionalized NPs in the hippocampus and cortex than non-functionalized ones (Rip et al., 2009; Z. Zhang et al., 2019).

In vivo results showed that 40- and 100-nm, but not 200-nm, NPs spread rapidly within brain tissue only if densely coated with polyethylene glycol (PEG), a non-ionic polyether compound commonly used to improve the pharmacokinetic and biocompatibility of a variety of nanomedicines (Hyldbakk et al., 2024). Similar results were observed in rat brain tissue with paclitaxel-loaded biodegradable NPs of similar size (85 nm) and surface properties. All uncoated NPs, even those with 40 nm particle size, were essentially immobile in human, rat, and mouse brains. The dense PEG coatings on NPs confer them minimally adhesive properties, enhancing their brain diffusion and movement in the brain extracellular matrix (Nance et al., 2012).

A recent study showed that the surface modification of 160 nm-sized NPs with poly(amidoamine), imparting a positive charge to the NPs, substantially enhances their ability to accumulate in the brains of healthy mice (Wiwatchaitawee et al., 2022).

A summary of the key physico-chemical features of NP to improve their brain distribution is represented in Figure 2.

2.4 | Nanoparticle composition and formulation procedures

The chemical composition of NPs, such as gold, silver, lipids, proteins, polymers, or silica, plays a crucial role in their biocompatibility, biodegradability, and stability thus affecting their ability to reach the brain parenchyma. For example, it has been shown that, given the same size, transferrin-based NPs have a 9-fold higher uptake than serum albumin particles in vitro on BBB model (Brown et al., 2020). Moreover, the same Authors have shown that transferrin can induce high transport even for larger particles (in terms of diameter), suggesting that particle composition dominates the outcome over particle dimension.

Moreover, the potential interaction with cells (particularly with those composing the BBB), and the potential toxicity, greatly depend on the actual composition of the NPs. The interaction with cells for some biological components like phospholipids will be quite different compared to the non-biological components such as metals (De Jong & Borm, 2008).

NPs composition and formulation procedures are critical parameters affecting their tendency to aggregate. Aggregated NPs may have reduced ability to penetrate brain tissues and could accumulate in certain areas, potentially leading to toxicity or reduced therapeutic efficacy (Abbasi et al., 2023).

Improving the technological analysis of material composition and formulation procedures are key factors that can affect the performance of different Active Pharmaceutical Ingredients (APIs) delivered.

However, as far as we know, there are not in vivo data about the study of the effect of NPs composition and formulation procedure on their distribution in brain parenchyma.

2.5 | Brain clearance mechanisms

Clearance mechanisms, including active and passive processes, also influence the NPs distribution in the brain (Gu et al., 2019). Unfortunately, limited research and few preclinical data have been conducted on studying the NPs brain clearance and metabolism. NPs can be degraded by neurons or glia, including phagocytic microglia and astrocytes. From non-degradable nanocarriers, such as quantum dots and polystyrene NPs (Maysinger et al., 2007), to biodegradable NPs, such as liposomes and polymerosomes (Gu et al., 2020), were detected in astrocytes in association with intracellular organelles involved in degradative processes, showing that NPs can be eliminated from the brain through a clearance mechanism mediated by astrocytes thus limiting their efficacy. Moreover, enzyme-mediated degradation can also occur (K. Y. Choi et al., 2011). Passive clearance mechanisms involve the drainage of IF and solutes through the glymphatic pathway and the BBB (Hablitz & Nedergaard, 2021). The glymphatic system relies on the movement of CSF through perivascular spaces surrounding arteries, facilitated by glial cells. (Louveau et al., 2015; Thomas, 2019). Studies performed by Gu et al. (Gu et al., 2020) showed that organic NPs reconstituted high-density lipoprotein and poly(ethylene glycol)-b-poly(lactic acid) NPs displayed an half-life <5 h in the brain, and that the 80% of NPs brain clearance was due to the perivascular glymphatic pathway. Little is known about the mechanisms responsible for the NPs removal from the brain, but Gu et al. suggested that microglial extracellular vesicles and astrocytic aquaporin-4

water channels can be involved in the NPs elimination from the brain (Gao et al., 2024; Gu et al., 2020). To the best of our knowledge, no data are available on the brain-to-blood efflux of NPs, a clearance mechanism well known for endogenous and exogenous macromolecules (Ueno et al., 2010).

3 | THE MOST COMMON BRAIN DISORDERS AND THE CORRESPONDING AFFECTED BRAIN CELLS OR AREAS

The intricate and dynamic distribution of NPs within the brain is poised to become a focal point of research in nanomedicine. Researchers are continuously working to develop NPs and new delivery strategies that optimize the passage of NPs through the BBB, losing sight of the targeting of the brain districts affected by the disease under investigation.

There are several common brain disorders, each with specific manifestations and, in some cases, identifiable brain areas affected. It is worth noting that these disorders can often involve multiple areas and networks in the brain, making NPs-based treatments more complex. Some, like AD or Parkinson's disease (PD), have relatively more localized impacts, while others, such as epilepsy or schizophrenia, can have more diffuse or varied effects. Understanding these disorders could help in the rational design of NPs.

In the following paragraphs, we summarized the most common brain disorders highlighting the brain areas and cell populations affected.

3.1 | Alzheimer's disease

AD is a complex neurological condition characterized by progressive cognitive decline, memory loss, and behavioral changes. It represents the most common cause of dementia, affecting 3%–4% of older adults, and its prevalence increases with age, with most cases occurring in individuals over 65 years old. (Fiest et al., 2016; Reitz et al., 2011). The main pathological hallmarks of AD are the formation of extracellular amyloid plaques and neuritic plaques between neurons and the intracellular aggregates of hyperphosphorylated tau protein called neurofibrillary tangles. The disease is also characterized by progressive neuronal loss, particularly in brain regions involved in memory and cognitive function, such as the hippocampus and cortex. This neuronal loss leads to brain atrophy, granulovacuolar degeneration, astrocytic gliosis, and microglial activation (Serrano-Pozo et al., 2011).

3.2 | Parkinson's disease

PD is a neurodegenerative disorder characterized by motor symptoms such as tremors, rigidity, bradykinesia (slowness of movement), and postural instability. It affects 1% of the population above 60 years, and its prevalence increases with age, affecting young people too, albeit less frequently (Armstrong & Okun, 2020). The main pathological hallmarks are the formation of abnormal protein aggregates of alpha-synuclein (called Lewy Bodies) within neurons and the deposition of other inclusion bodies consisting of aggregated proteins (van Vliet et al., 2023). PD is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta region and then it spreads to neocortical and cortical regions. In advanced stages of the disease, secondary changes such as gliosis, axonal degeneration, and neuronal loss may extend beyond the substantia nigra to the striatum and cortex, contributing to a wider range of symptoms and complications (Ramesh & Arachchige, 2023).

3.3 | Epilepsy

Epilepsy is a neurological disorder affecting over 70 million people that involves a predisposition to frequent spontaneous epileptic seizures, with related cognitive, psychological, and social impairments, and it is the third most common neurologic disease (Beghi, 2020; Fisher et al., 2017). This disorder is caused by an imbalance between excitatory and inhibitory activity in a neuronal network, thus resulting in disruption of normal neuron processing, axonal sprouting, synaptic reorganization, and glial alterations. It can affect various areas depending on the type of seizure (Thijs

et al., 2019). In generalized epilepsy, seizures involve thalamocortical neurons bilaterally; in focal epilepsy, neurons of one hemisphere (usually limbic or neocortical) are affected. Brain damage should result in hippocampal cell loss, axonal sprouting and synapsis reorganization (Thijs et al., 2019).

3.4 | Schizophrenia

Schizophrenia is a common psychiatric disorder that has a lifetime prevalence of approximately 1%, and it is associated with reduced life expectancy (15 years shorter than the general population) and a high risk of suicide (McCutcheon et al., 2020). The main factors responsible for the disease are two neurotransmitters with essential physiological functions, dopamine and glutamate. The brain areas involved in the pathology are the frontal lobe, the temporal lobe, and the hippocampus. Some studies also showed a reduction in brain volume, but it mainly concerns the gray matter. Schizophrenia presents not only a reduction in the activity of the prefrontal cortex, but also an increased frontal activation during the performance of cognitive tasks (Jauhar et al., 2022).

3.5 | Stroke

Stroke is a complex cerebrovascular disease affecting approximately 16.9 million people and is considered one of the first causes of disabilities and cognitive deficits, accounting for 5.2% of all deaths worldwide (Béjot et al., 2016). Two different types of stroke can be identified: hemorrhagic stroke, which occurs when a blood vessel in the brain leaks or ruptures, and ischemic stroke, which is the most common and happens when there is a transient or permanent occlusion of cerebral vessels, that is, the blood supply to the brain is interrupted. Since each area of the brain is supplied by specific arteries, the localization of the disease depends on the exact position of the obstruction or bleeding in the brain (Zhao et al., 2022). Astrocytes could be promising therapeutic targets for improving functional outcomes after stroke, as they limit the extent of injury by exerting anti-excitotoxic effects and releasing neurotrophins (Zhao et al., 2022).

3.6 | Migraine

Migraine is a chronic and complex neurological disorder (Edvinsson et al., 2018) involving a constellation of symptoms characterized by headache (Aguilar-Shea & Diaz-de-Teran, 2022). The pathogenesis of the pain is still debated due to the episodic and unpredictable nature of migraine (Ferrari et al., 2022). However, several studies highlight the involvement of cranial blood vessels (DosSantos et al., 2014): a transient increase in the permeability of the BBB to small molecules was observed in the cortex but not in the brainstem, caused by changes in cerebral blood flow and dysregulated neurovascular coupling (Palomino et al., 2022). Animal models have shown that the neurogenic inflammation of migraine originates in the meninges and is mediated by a variety of factors (Christensen et al., 2022). Such factors include the trigeminal sensory nerves and calcitonin gene-related peptide, a vasoactive neuropeptide (Edvinsson et al., 2018).

3.7 | Multiple sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disorder characterized by inflammation, demyelination, and neurodegeneration within the CNS. It is estimated that around 2.8 million people worldwide are diagnosed with MS (Walton et al., 2020). The specific brain areas or cells affected by MS can vary depending on the subtype of the disease and the individual's unique pathology. However, commonly affected regions and cell types include white and gray matter, oligodendrocytes, astrocytes, and microglia (Faissner et al., 2019).

3.8 | Brain tumors

Brain and CNS tumors contribute to significant mortality and morbidity across all age groups because of their histological complexity (Fan et al., 2022). Based on the international classification of oncology diseases written by the World

Health Organization, over 100 types of these tumors are recognized (Barnholtz-Sloan et al., 2018). Among them, gliomas represent the largest category of adult brain tumors, accounting for 78% of malignant cases. They grow from the brain's supporting cells known as glia, composed of astrocytes, ependymal, and oligodendroglial cells. They are most commonly found at the level of the cerebral hemispheres and less frequently in the trunk and cerebellum. Non-malignant meningioma represents the predominant nonmalignant tumors, accounting for 54% of nonmalignant cases, and it most commonly occurs in the cerebral meninges (Miller et al., 2021).

4 | CURRENT LANDSCAPE OF NPs TARGETING SPECIFIC BRAIN AREAS OR CELLS

Currently, only a few nanomedicines are already in clinical use or in the clinical pipeline to treat brain disorders (clinicaltrials.gov), and the efforts to target specific brain areas or neuronal cells constitute a significant hurdle. In some of these studies, there is no evidence of selective uptake of NPs by a specific cell population, despite the therapeutic cargo providing pharmacological effects.

The nano-approaches currently utilized for brain targeting are:

1. Naked NPs or NPs functionalized with ligands to cross the BBB. These approaches assume that the cargo is accumulated in the brain and released near the target cells, evidencing pharmacological improvement.
2. NPs functionalized with ligands able to target specific brain cell populations. This approach improves drug efficiency, enhancing the targeting of disease-associated brain cells or districts.
3. Biomimetic NPs, engineered to mimic biological structures or processes found in the body, particularly those relevant to cross the BBB and targeting specific cells or regions within the brain. Recently dual and multi-targeting strategies have been investigated not only to improve the brain site-specific drug delivery but also to achieve the desidered drug release. In most cases, NPs are engineered with stimulus-sensitive bonds or molecules in order to make them responsive to variations of environmental factors (Luo et al., 2020).

4.1 | NPs naked or functionalized with BBB ligands

Different antipsychotic drugs useful for the treatment of schizophrenia were loaded in naked lipid- or polymer-based NPs. Details about the development of these nanoformulations from 2011 to 2016 are presented in the review of Sun et al. (Sun et al., 2016). Three of them have been approved to treat schizophrenia and schizoaffective disorder (Chang et al., 2021; Chue & Chue, 2012; Preda & Shapiro, 2020). In these cases, as well as for epilepsy (Bonilla et al., 2022), the loading of compounds in NPs improved their therapeutic efficacy increasing the amount of drugs reaching the brain, overcoming the drug resistance mediated by P-glycoprotein and improving drug bioavailability and safety profile. In another example, FK506(Tacrolimus)-liposomes injected intravenously in t-MCAO (Transient Middle cerebral artery occlusion) rats were shown to cross the disrupted BBB and to diffuse into the brain parenchyma only in the ischemic hemisphere, ameliorating the motor function deficits caused by ischemia (Ishii et al., 2013). In this case, the localization of NPs in the damaged brain district is due to the vascular alterations occurring in ischemia.

A potential parameter that can be exploited to target specific brain areas could be the NPs' size. In a paper published in 2010 by Sousa et al. positively charged gold NPs have been coated with a polyelectrolyte multilayer and human serum albumin, intravenously injected in mice and their precise distribution in the brain was studied ex-vivo by different advanced imaging techniques. Results showed a non-uniform particle distribution in the brain, even in the absence of targeting ligands, with the highest accumulation in the hippocampus, thalamus and hypothalamus (Sousa et al., 2010). Similarly, the encapsulation of Thymoquinone, a phytochemical compound with analgesic, anticancer, antioxidant, anti-inflammatory, and antipyretic activity, in negatively charged mesoporous silica NPs enhanced its delivery to cortex, thalamus, hypothalamus, and midbrain, reducing its delivery to the cerebellum (Fahmy et al., 2019). In both examples, the NPs size was in the range of 100–115 nm. The specific localization of these NPs in the cortex and hypothalamus is particularly intriguing, given their proximity to the regions associated with the onset of AD (cerebral cortex), PD (substantia nigra), and prion diseases. In another investigation, spherical acidic poly-lactic-co-glycolic acid (PLGA)-based NPs with a mean diameter of 150 nm were administered via intracerebral injections into a mouse model of PD. Results showed that these NPs were able to target lysosomes of nigral dopaminergic cells specifically (Arotcarena et al., 2022).

4.2 | Targeted

NPsThe majority of brain-targeting ligands used to functionalize NPs exhibit the ability to target a variety of brain cells, ECs of the BBB included, rather than being restricted to a single specific cell type. Below, we summarise the ligands with the highest specificity for the brain that have been tested in vivo. Examining how targeted delivery systems interact with biological tissues and achieve site-specific drug release, it is possible to state that the main mechanisms of targeting specific brain areas or cells are: i) receptor-mediated endocytosis, where NPs surface is functionalized with ligands targeting specific receptors specifically distributed in diseased areas/cells; ii) disease markers, where NPs surface is functionalized with molecules able to co-localized with molecular signatures, such as β-amyloid peptide in AD, that are overexpressed in pathological sites.

4.2.1 | Targeting specific brain areas

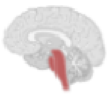
The functionalization of NPs with ligands for opioid receptors can be exploited to selectively target different brain regions. Indeed, μ opioid receptors are localized predominantly in the hypothalamus and thalamus, δ opioid receptors are localized predominantly in the striatum, limbic system, and cerebral cortex, and κ opioid receptors are widely distributed in the neocortex, striatum, amygdala, and thalamus (Leung, 2004). Lewicky et al. (Lewicky et al., 2021) showed that mannosylated glycoliposomes targeting κ opioid receptors were able to improve brain levels of the dynorphin peptide by approximately 3–3.5-fold in the striatum of BALB/C mice. In a recent study, negatively charged albumin-PLGA NPs with a size of about 400 nm and loaded with dopamine were administered intraperitoneally (i.p.) in a PD mouse model. These nanosystems are able to cross the BBB and distribute throughout the brain, particularly in the hippocampus and striatum (Monge-Fuentes et al., 2021), regions highly affected in PD. In the context of AD, liposomes functionalized with a peptide derived from apolipoprotein E were localized in the brain parenchyma, in particular in the hippocampus, with amyloid plaques (Balducci et al., 2014; Conti et al., 2017) due to the high affinity of the apoE-derived peptide for β-amyloid (Gobbi et al., 2010). A design strategy that has been suggested to improve the brain targeting is the dual or multi-targeted nanomedicines, where two or more targeting moieties are utilized to funtionalize NPs. A review of Barnabas (Barnabas, 2019) presents an overview of this approach. Although significant progress has been made in the design of multi-targeted NPs, their practical implementation remains mainly in the preliminary stage, primarily as proof of concept.

4.2.2 | Targeting specific brain cells

The surface decoration with a fusion dual-ligand peptide that combined a BBB-penetrating peptide with the Tet1 peptide (HLNILSTLWKYR, able to bind to the GT1b receptor, which exists abundantly in neuronal membranes) has allowed the accumulation of polymeric NPs predominantly adjacent to the neurites, with the neuron targeting specificity of >90% in mice (Guo et al., 2020). In addition, the functionalization of polymeric micelles with the C3 peptide (the neural cell adhesion molecule mimicking peptide), allowed their co-localization with neurons and rarely with microglia and astrocytes in the hippocampus or cortex of APP/PS1 transgenic mice (Yang et al., 2020). The conjugation of RVG29 peptide (a peptide derived from Rabies Virus Glycoprotein that binds to the α-7 subunit of nicotinic acetylcholine receptors of neuronal cells) has favored the localization of gold NPs near or inside the neurons, rather than in blood microvessels or endothelial cells, demonstrating the RVG29-mediated active neuron targeting in C57BL/6 mice (You et al., 2018). Despite that, some concerns have been raised about the stability of the RVG29 peptide and its neuron-targeting ability. Alvarez-Erviti et al. showed that the intravenous injection in C57BL/6 mice of RVG-exosomes loaded with GADPH siRNA resulted in significant ($p < 0.05$) knockdown of GAPDH mRNA in neurons, microglia and oligodendrocytes, suggesting the weak targeting capability of RVG peptide (Alvarez-Erviti et al., 2011). A promising ligand to target neurons is the neuro-specific peptide, neurotensin. Indeed, the functionalization of graphene oxide NPs with this peptide enhanced the ability to target neurons and achieve high gene transfection, thus representing an interesting nanoplatform for brain gene therapy (Hsieh et al., 2016). Although significant progress has been made, directing drugs to neurons remains a formidable task, due to NPs design complexities and also because neurons make up just 10% of the brain's total cell population and are non-phagocytic cells. Recently, Bai et al. have functionalized liposomes with phosphatidylserine to target and bind to the overexpressed phosphatidylserine-specific receptors on the surface of

astrocytes and microglia. This targeting strategy allowed liposomes to selectively target injured astrocytes and microglia (Bai et al., 2023). In 2005, Chekhonin et al. (2005) showed that liposomes functionalized with monoclonal antibodies against human gliofibrillary acidic protein (GFAP) were able to target astrocytes in vitro specifically, but they were not able to penentrate the BBB. Thus, they suggested using these NPs as drug delivery vehicle in the brain area with damaged BBB. PLGA-based NPs of ~120 nm diameter loaded with the leukemia inhibitory factor and functionalized with antibodies against NG-2 chondroitin sulfate proteoglycan, expressed on oligodendrocyte precursor cells (OPCs), were able to selectively target OPC stimulating myelin repair and reducing the inflammation (Rittchen et al., 2015). In 2024, Huang et al. showed that biomimetic NPs engineered with DSPE-PEG2000-levodopa can cross the BBB and specifically bind to dopamine neurons, thereby potentially improving the accumulation of therapeutic agents for PD treatment (Huang et al., 2024).

TABLE 1 Representation of the data found in the literature relating to the use of NPs for targeting specific brain districts, presenting in vivo results.

Brain region	Disease	Targeted cells	Nanoparticle	References
 Subventricular zone	Glioma	Glial cells	Glioma cell membrane coated ICG loaded solid lipid NPs	Zhang et al., 2023
 Cerebellum	Medulloblastoma	Neurons and glial cells	DNA plasmid encoding suicide gene HSVtk loaded NPs	Choi et al., 2020
 Brain hemispheres	Stroke	Astrocytes and microglia cells	Phosphatidylserine functionalised liposomes	Bai et al., 2023
	Stroke	Neurons	Macrophage membrane coated FTY-loaded MNO_2 nanospheres	Li et al., 2021
 Frontal lobe	Schizofrenia	Neurons	Drug nanocrystals of paliperidone palmitate	Panda et al., 2016
	Alzheimer's disease	Neurons	PEG–PLA NPs	Guo et al., 2020
 Hyppocampus	Alzheimer's disease	Neurons (motor, dopaminergic, and cholinergic) and microglia cells	Metallic NPs and organic NPs	Yang et al., 2020
 Striatum	Parkinson's disease	Neurons	Albumin/PLGA nanosystems loaded with dopamine	Monge-Fuentes et al., 2021
 Substancia Nigra	Parkinson's disease	Neurons	Retinoic acid-loaded polymeric NPs	Esteves et al., 2015
 Temporal lobe	Epilepsy	Neurons	Oxcarbazepine loaded polymeric NPs	Musumeci et al., 2018
 Brain stem	Multiple sclerosis	Glial cells	PLGA NPs	Rittchen et al., 2015

4.3 | Biomimetic NPs

Biomimetic NPs often emulate natural structures, processes, or functions found in biological entities to improve their biocompatibility, targeting efficiency, and therapeutic efficacy. In recent studies, it has been investigated the neuroprotective efficacy of biomimetic NPs for multitargeted combined treatment of ischemic stroke in rat models via iv injection. The macrophage-membrane-coated Fingolimod(FTY)-loaded MnO_2 nanospheres have been synthesized to consume the excess of hydrogen peroxide and convert it into oxygen to reduce the oxidative stress and also reverse the proinflammatory microenvironment to reinforce the survival of damaged neuron. The results confirmed the ischemic region targeting capability of these NPs, coupled with an extended circulation lifespan attributed to the biomimetic coating on their surface (Li et al., 2021). Ma et al. investigated the targeted delivery of glyburide through PLGA-NPs coated with neural stem cells (NSC) membrane engineered to overexpress CXCR4 and administered intravenously in mice. The over-expression of CXCR4 on the NPs surface allows a strong interaction with the stromal cell-derived factor 1, which is highly expressed in the injured brain. Compared to the free drug, NSC membrane-coated PLGA-NPs demonstrated significantly greater effects in reducing infarct volume, as shown by brain sections imaged through IVIS (Ma et al., 2019). A recent study explores a novel approach using glioma cell membranes to encapsulate NPs loaded with indocyanine green. Cell membrane modification enhanced the cellular uptake of NPs by glioma cells when administered intravenously, reinforcing the bio-distribution of the formulation within the tumor area (Zhang et al., 2023).

A summary of the data herein described is presented in Table 1.

4.4 | Administration Routes

The route of administration for NPs targeting the brain is crucial in determining their effectiveness in crossing the BBB and reaching the brain parenchyma. The primary routes of administration used for brain-targeted nanoparticle delivery are intravenous injection (i.v.); intraperitoneal injection, which can be translated to humans as a slow i.v. infusion (De Smet et al., 2013); and intranasal administration, where NPs are delivered through the nasal cavity, exploiting the olfactory and trigeminal nerve pathways to bypass the BBB (Kashyap & Shukla, 2019). In preclinical experiments, the direct injection of NPs into the brain ventricles, brain parenchyma or into the CSF are also utilised, but is it important to remember that these are invasive approaches with a high risk of infections. An innovative strategy that has recently been investigated is the possibility of using implantable biomaterials for controlled NPs release (Viale et al., 2024), (Di Mascolo et al., 2023). However, the main limitation of this approach is that it is more suitable for brain disorders requiring surgery; otherwise, it would be too invasive. Overall, it is important to highlight that the choice of delivery route for NPs targeting the brain primarily affects the quantity of NPs that reach the brain, rather than their distribution within the brain parenchyma.

5 | CONCLUSIONS

Targeted delivery of therapeutics to disease brain sites is one of the biggest challenges in medicine, as it determines the treatment efficacy of virtually all chemical and biological drugs. In the last years, research focused the attention on engineering NPs to improve their brain penetration, losing sight of the NPs distribution in the brain parenchyma. This is supported by the few studies on this topic, summarized in this review. However, examining the current status of targeted NPs for drug delivery to specific brain areas or cells, we have identified the fundamental design principles for customized NPs. In particular, we underline that not only the surface targeting ligand is essential, but also the physical parameters of NPs (i.e., size, shape, and surface charge) play a key role in determining the nanomedicines distribution in different brain districts.

The little information available on this topic and the limitation of the imaging techniques available to accurately identify NPs in the brain parenchyma hinder the translational prospects of nanotherapies for clinical development.

Currently, there are not yet clinically approved nanomedicines for brain indications, and this failure can be due to the challenges of scale-up, regulatory issues, and the inability to sample brain tissue to quantify drug uptake. There are few successful examples where NPs have been demonstrated to cross the BBB and reach the brain in humans. Clinical trials have investigated the use of liposomal formulations for treating brain tumours (i.e. GBM) and neurodegenerative diseases (NCT03020017; NCT01869837; NCT03389273), showing that these NPs can cross the BBB and deliver cargoes

to the brain tumour site. Advanced imaging techniques have been used to track NPs in the human brain, confirming their ability to cross the BBB (NCT02788981).

Although some BBB-crossing capabilities have been demonstrated in research studies, translating these nanomaterials and methods into the clinical setting has been enormously challenging. Therefore, new strategies and ideas are sought after, and advances in nanorobotics, machine learning tools and artificial intelligence can be useful to predict and design the next-generation NPs and implantable biomaterials to address the treatment of neurological disorders.

The investigation of NPs brain distribution is still in the initial phase of progress and the discovery of new arrangements in NPs design, the combination of bio-nano materials and the applications of bioinspired approaches represent a novel alternative direction for developing brain-tailored nanomedicines.

AUTHOR CONTRIBUTIONS

Giulia Sierri: Data curation (equal); methodology (equal); writing – original draft (equal). **Michela Patrucco:** Data curation (equal); methodology (equal); writing – original draft (equal). **Davide Ferrario:** Data curation (lead); writing – original draft (equal). **Antonio Renda:** Data curation (equal); writing – original draft (equal). **Susanna Comi:** Data curation (equal); writing – original draft (equal). **Matilde Ciprandi:** Data curation (equal); writing – original draft (equal). **Veronica Fontanini:** Data curation (equal); writing – original draft (equal). **Francesco Saverio Sica:** Data curation (lead); writing – original draft (equal). **Silvia Sesana:** Project administration (equal). **Marta Costa Verdugo:** Data curation (equal); writing – original draft (equal). **Marcelo Kravicz:** Supervision (equal); writing – review and editing (equal). **Luca Salassa:** Supervision (lead); writing – review and editing (equal). **Marta Busnelli:** Supervision (lead); writing – review and editing (equal). **Francesca Re:** Conceptualization (lead); funding acquisition (lead); methodology (lead); writing – review and editing (lead).

ACKNOWLEDGMENTS

L.S. and M.C.V. thank the Spanish State Research Agency (Grants PID2022-139267OB-I00). The Donostia International Physics Center is supported by the "Severo Ochoa" Programme for Centres of Excellence in R&D (MINECO, CEX2018-000867-S). Figure 2 was created with BioRender.com. Open access publishing facilitated by Universita degli Studi di Milano-Bicocca, as part of the Wiley - CRUI-CARE agreement.

FUNDING INFORMATION

The work was supported by National Recovery and Resilience Plan (NRRP), Mission 4-Component 2-Investment 1.1-Call for tender No. 104 published on 2.2.2022 by the Italian Ministry of University and Research (MUR), funded by the European Union – Next Generation EU– PRIN2022 to F.R. and M.B. (NOXY4B) – CUP H53D23006320006.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Sierri, G., Patrucco, M., Ferrario, D., Renda, A., Comi, S., Ciprandi, M., Fontanini, V., Sica, F. S., Sesana, S., Costa Verdugo, M., Kravicz, M., Salassa, L., Busnelli, M., & Re, F. (2024). Targeting specific brain districts for advanced nanotherapies: A review from the perspective of precision nanomedicine. *WIREs Nanomedicine and Nanobiotechnology*, *16*(5), e1991. https://doi.org/10.1002/wnan.1991