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For *Drug Discovery Today*

Title: Targeting Delivery in Parkinson's Disease

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Keywords: Blood-brain barrier, neurodegenerative disorders, targeting ligands, nanoparticles, biomaterials

Teaser: Modifying delivery systems with targeting moieties is a relatively recent and exciting field which could enhance therapeutics for Parkinson's disease in the future.

Abstract:

Disease-modifying therapies for Parkinson's disease – with the potential to halt the neurodegenerative process and to stimulate the protection, repair and regeneration of dopaminergic neurons – remain a vital but unmet clinical need. Therapeutic targeting offers the potential for delivery of neuroprotective and disease-modifying molecules to the diseased brain region. This relatively new field of research has many opportunities – but also many challenges – for improving the quality, specificity and efficacy of the next generation of therapies for Parkinson's disease patients in the future.

Age-related neurodegenerative diseases are a group of conditions that affect patient motor and/or mental independence, and lead to a general lowering of quality of life. After Alzheimer's disease, Parkinson's disease (PD) is the second most prevalent of these neurological disorders, with an incidence of 8.6 to 19.0 per 100,000 inhabitants [1]. PD was first described by the English doctor, James Parkinson, in his 1817 essay "An Essay on the Shaking Palsy" [2], and it has since been further characterised by a progressive loss of midbrain dopaminergic neurons as well as the presence of α -synuclein protein aggregates forming intracellular Lewy bodies in affected areas. First introduced over half a century ago [3], replacement of the neurotransmitter dopamine with the precursor levodopa (L-DOPA) remains the backbone therapeutic for standard PD therapy. However, L-DOPA (as well as all other pharmacological therapies for PD) is limited in that it only treats the motor symptoms of the disease, it does not alter the relentless degeneration of the affected neurons as the disease progresses, and it is associated with significant side effects in advanced disease. Therefore, it is imperative that novel disease-modifying therapies that have the potential to halt the neurodegenerative process itself, and to stimulate the protection, repair and regeneration of dopaminergic neurons, are developed sooner rather than later.

When considering the hurdle of designing new targeted therapeutic interventions for PD, it is interesting to place the challenge into context with other neurodegenerative diseases. In comparison to other such diseases, PD offers both specific challenges as well as specific opportunities for targeted therapies. One of the challenges is that, unlike Huntington's disease (which is caused by mutations in one specific gene), the cause of sporadic PD (which accounts for the vast majority of cases) remains unknown. Having said that, in recent years, linkage analysis and genome-wide association studies have

revealed a potential genetic basis for at least some cases of “sporadic” PD [4,5]. One of the opportunities afforded by the nature of the PD pathology is that it is primarily restricted to a specific location within the brain (namely the nigrostriatal pathway) which is undoubtedly less of a challenge for targeting therapies than the widespread areas affected in many other neurodegenerative conditions, such as multiple sclerosis or Alzheimer's disease. Moreover, as PD results in the relatively specific loss of dopaminergic neurons, there is also a specific population of target neurons for neuroprotection and replacement, in contrast to most other neurodegenerative conditions with diverse patterns of cell loss, such as traumatic brain injury or stroke. The long time period over which the disease progresses also presents both challenges and opportunities. Unlike amyotrophic lateral sclerosis, which typically progresses rapidly over three years or so, the disability of PD is typically extended over 2 decades or more. This gives a window-of-opportunity suitable for therapeutic intervention and, perhaps in the future, for "personalized medicine" approaches. However, this advantage is at least in part offset by the relative plasticity of dopaminergic neurons to compensate for partial loss, such that symptoms are not observed and hence diagnosis delayed until brainstem dopaminergic cell loss exceeds 70% or more [6].

Certain growth factors, such as glial cell line-derived neurotrophic factor (GDNF), show strong promise for protecting the remaining dopaminergic neurons in PD patients. However, the long time scale of PD progression also presents a challenge for growth factor therapies, which, due to the short protein half-life will require sophisticated controlled release systems, continual infusion devices or gene therapy interventions. Moreover, since large molecules such as growth factors do not readily cross the blood-brain barrier, these requiring targeted, typically surgical, central delivery to the required

site of action. Thus, PD has certain features that give firm rationale for the development of targeted therapies, and in particular growth factor therapies. To summarise, these include the selectivity of neuron loss in a particular brain region, an array of potential therapeutic agents, and a suitable time period for neuroprotection. However, challenges include the unknown etiology, patient-to-patient variability in Parkinsonian pathology and symptoms, and the length of time over which the therapy needs to remain active to be effective.

Route to the Target Area

When designing a targeted therapy for any disease, it is essential to consider by which means it is expected to reach the target site. The majority of current PD drugs are administered orally which results in system-wide distribution via the bloodstream despite an effect being desired in the nigrostriatal pathway only. The nature of absorption through the gastrointestinal track inevitably results in periods of high drug concentration in the blood, or a low concentration, depending on when the last administration took place. This escalation and reduction in dopamine concentration can later result in an “on-off” effect, whereby patient mobility is severely compromised before the next dose is taken. In addition, this rise and fall of drug-derived dopamine levels may cause a worsening of motor complications such as involuntary movements (dyskinesias) [7].

Figure 1 and Table 1 about here

Stereotactic surgery offers an alternative to the blood stream as a route to the nigrostriatal dopaminergic pathway. Intraparenchymal administration bypasses the blood-brain barrier (selective permeability barrier separating blood flow from the brain

extracellular fluid (see Figure 1)) to target sites such as the striatum or substantia nigra directly. Alternatively, stereotactic intraventricular injection also bypasses the blood-brain barrier, but due to the large volume of the ventricles within the brain, the drug can still act upon a large region of the brain (i.e. areas in proximity to the ventricles). As with most aspects of drug design, there are advantages and disadvantages to either delivery route (systemic vs. stereotactic) which are considered in Table 1.

The above consideration of the administration route shows that the design of targeted therapies for PD alters considerably depending on whether systemic or stereotactic administration is envisaged. Therefore, recent developments towards novel therapeutics for PD are considered separately below.

Targeting Delivery with Stereotactic Surgery

When considering targeted therapies for PD, one strategy might be the development of nanoparticles designed to carry payloads across the blood-brain barrier. This is indeed a large focus of novel therapeutic research, however stereotactic injection of controlled release devices directly into the brain provide another means to directly target the ascending dopamine pathways and their striatal terminals. In addition, intracerebral injection can allow for the delivery of larger biomaterial devices such as microspheres, which otherwise would not be able to cross the blood brain barrier, directly into host neuropil. This section will review recent work involving growth factor and gene delivery to the brain, highlighting the materials used and therapeutic molecules delivered.

Growth factors such as GDNF have been extensively shown to protect dopaminergic neurons from toxic insult [8,9], so represent a major promise for future disease-

modifying PD therapies (i.e. to reduce the death rate of dopaminergic neurons). However, as GDNF requires a long time frame of action in order to slow the disease progression, some form of continual delivery (e.g. pump) or controlled release (e.g. biomaterial or other vectors) system must be considered to overcome the short half-life of the protein. A recent review of the different biomaterials used for therapeutic delivery to the CNS highlights poly(lactic-co-glycolic acid) (PLGA) as a material which has been studied extensively for the delivery of therapeutics [10]. With regulatory approval for use in humans and a biodegradation profile that can be tailored easily (via monomer composition), PLGA is a good candidate for the preparation of GDNF-loaded microspheres that can be used to deliver sustained neurotrophic factor release in the brain. PLGA microspheres can be prepared in emulsion and loaded with GDNF. Using the 6-hydroxydopamine (6-OHDA) model of PD, the neuroprotective property of GDNF-loaded microspheres injected into the striatum can yield an impressive functional motor benefit (reversal of amphetamine-induced rotational behaviour) in rats [11,12], lasting up to at least 30 weeks [13]. However, care should be taken when extrapolating these results to the human condition. The 6-OHDA model is an acute toxic model which selectively destroys the midbrain dopaminergic neurons, but does not simulate the slow progressive nature of PD. Whilst GDNF release can be observed over a period of days or weeks [11], the design of materials for constant release over months and years remains a formidable task.

Gene therapy could be an alternative means of maintaining an elevated level of striatal GDNF over a sustained period. A general outline for successful gene therapy for PD involves using a vector capable of delivering a gene encoding the neurotrophic factor to cells in the nigrostriatal pathway in order to allow the transfected/transduced cells to

secrete the neurotrophic factor. To date, the most effective vectors remain viral vectors, despite much research into developing non-viral alternatives. For example, lentiviral-mediated GDNF overexpression has impressively been shown to ameliorate the effects of a 6-OHDA lesion, including behaviour benefits in a wide range of behavioural tasks [14]. Indeed, adeno-associated virus (AAV)-mediated gene delivery has reached clinical trials for the delivery of the growth factor GDNF-analogue, neurturin, to patients with relatively advanced PD [15].

Non-viral vector counterparts lag behind, both in terms of transfection efficiency in the brain [16] and clinical development, and this has been reviewed extensively elsewhere [17]. However, polymer-based non-viral vectors offer the potential for systemic administration (see below), repeat administrations, addition of targeting ligands, and feasible up-scale/ease of handling. The addition of targeting ligands (sometimes termed 'moieties') to non-viral vectors is not limited to those proposed for systemic administration. Specific targeting of gene therapy to neurons has been attempted using the Tet1 peptide attached to a polymeric vector. Intraventricular delivery resulted in the specific transfection of neural progenitor cells in the sub-ventricular zone [18]. A targeting peptide has also been used to specifically target the neurotensin receptor of dopaminergic neurons [19]. The neurotensin peptide sequence has been added to a poly-L-lysine gene vector in order to deliver the GDNF encoding gene to the 6-OHDA rat model of PD [20]. Intranigral administration of the neurotensin modified gene vector resulted in robust protection of the dying neurons, both in the substantia nigra and in the striatum. Such studies mark how non-viral gene transfection has progressed from standard cationic polymers [21] to peptide modified vectors capable of bringing about functional recovery in a rodent model of PD. However, if the design of non-viral gene

vectors is restricted to the stereotactic route of administration, they will either have to become as efficient as viral vectors, be proven safer, and/or become cheaper to produce/handle than their viral counterparts. The development of non-viral vectors which can target the brain, by using bound ligands to mediate their transfer across the blood brain barrier, opens up the possibility of systemic administration for future nanomedicine therapies for PD. This line of research could provide significant advantages over viral gene delivery, and drug/gene carriers and their respective targeting ligands are discussed below.

Targeting Delivery with Systemic Administration

The development of biomaterials to improve drug delivery for PD has focused heavily on sustained delivery of L-dopa in a non site-specific manner (reviewed elsewhere [10]). However, drug delivery systems designed to translocate small molecule drugs across the blood brain barrier have also been developed. In 1984, a key study showed that if monoclonal antibodies (mAb) against transferrin (Tf) receptors were systemically administered to the rat, they labelled the capillaries in the brain, but not other tissues [22]. Researchers have now extensively investigated the use of targeting ligands such as mAb against Tf receptors, the Tf glycoprotein itself, or glycoproteins from the transferrin family (e.g. Lactoferrin) for translocation of nanomedicines across the blood brain barrier. Whilst many of the recent studies have been focused on non-viral gene vectors, lactoferrin has also been conjugated to PLGA nanoparticles containing urocortin [23]. Urocortin is a corticotrophin releasing hormone which was shown to be neuroprotective in the rat 6-OHDA and lipopolysaccharide (LPS) models of PD [24] after intranigral injection. Since urocortin cannot transverse the blood brain barrier, Hu and

co-workers used PLGA nanoparticles, modified with lactoferrin via a polyethylene glycol (PEG) spacer, to successfully deliver urocortin to the brain following systemic administration in the Parkinsonian mouse [23]. Lactoferrin has also been used as a brain-targeting ligand for cationic polymers designed for gene delivery. Poly(amidoamide) (PAMAM) dendrimers, functionalized with lactoferrin or transferrin, again by a PEG spacer, allowed the delivery of the GDNF encoding gene to the brain of rodent PD models [25,26]. Lactoferrin modified PAMAM showed greater GDNF expression levels in the brain, than transferrin modified PAMAM, so was used for further study into a multiple dosing regime in the rotenone rat model of PD [25]. Five injections resulted in higher GDNF expression levels than three injections (Figure 2), but behavioural improvement was similar between these groups, as was the density of tyrosine hydroxylase (TH) immunopositive staining (dopaminergic terminals) in the striatum. Studies such as these prove the concept that non-viral gene delivery can be achieved in the rodent brain, and that multiple dosing can be used. However, we must also not overlook the short duration of transgene expression, as can be observed in the progressive reduction of expression in the 2, 6 and 10 day analyses (Figure 2).

Figure 2 about here

Aside from lactoferrin, other targeting moieties have been used for nanoparticle delivery to the brain (reviewed elsewhere [27]). Some examples include angiopep, a ligand which specifically binds to the low-density lipoprotein receptor-related protein present on the blood brain barrier [28], and the rabies virus glycoprotein peptide, a 29 amino-acid peptide which binds to the nicotinic acetylcholine receptor which is widely expressed in

the brain and blood brain barrier [29]. There are few direct comparisons between the many different brain targeting moieties, however, van Rooy and colleagues [30] used liposomes as the vector and varied the targeting functionality. This study showed that RI7217, a monoclonal antibody against the mouse transferrin receptor was preferable to the antibody OX-26, transferrin itself, angiopep, and other moieties designed to cross the blood brain barrier [30]. This study can also serve to highlight the current limitation of systemic delivery to the brain, namely the problem of off-target accumulation. Incremental improvements in brain uptake are achieved via correct selection of targeting ligands (Figure 3B), however, the degree of off-target accumulation is considerably larger, particularly in the liver and spleen (Figure 3A) [30]. This is a common observation in studies where delivery from the blood stream to the brain is desired, and a hurdle which must be overcome for further progress in this field.

Figure 3 about here

These studies have focused primarily on targeting the brain in general; however, PD affects a specific brain region and a specific type of cell. When designing therapies specifically targeted at the regions affected by PD, gene therapies hold an advantage in that they can contain two means of targeting the transgene effect. The first is the targeting ligand present on the polymer/nanoparticle/liposome etc., as mentioned above. The second is that a region specific promoter might be used, so that transgene expression would only be driven in the desired region or specific population of cells, for example in TH-positive catecholaminergic neurons. TH-specific expression of GDNF has been successfully achieved in rodent PD models using PEGylated liposomes known as

“Trojan Horse Liposomes” [31,32]. These liposomes are functionalized with the mouse transferrin receptor monoclonal antibody OX-26 to assist crossing the blood brain barrier, and the GDNF encoding gene contains a TH-specific promoter instead of a more widely expressed promoter. Together, such systems allow an acceptable level of brain uptake, yet may provide a means of circumventing the problem of off-target accumulation of the therapeutic.

Could Cell Therapies be Better Targeted?

As highlighted in the previous sections, designing targeting strategies for better delivery of drugs, growth factors and genes to the brain is a relatively recent research area but is of growing interest. An alternative possibility is the use of cell transplantation to deliver targeted cells or therapeutic molecules on a sustainable basis into the Parkinsonian brain. Cell therapies are being considered as a potential means to replace the neurons lost in PD [33,34] or as a means of providing neurotrophic support [35]. Supportive biomaterials are being considered to improve transplant survival [10,17], but they can also provide a means of better stabilisation and positioning of the graft within the target area (e.g. striatum; see figure 4). Striatal placement of cell transplants do not always result in all of the cells remaining in the striatum after the needle is withdrawn. In the authors' experience, grafted cells can frequently be observed in the corpus callosum and/or in the overlying cortex, whether through inadvertent deposition at the time of injection, pressure extrusion as the needle is withdrawn, or by graft expansion and growth in the weeks subsequent to transplantation surgery (for examples see figure 4 and reference [36]). Hydrogels which form *in situ*, i.e. in the brain post-injection, such as those formed from collagen used previously for delivery of stem cells to the brain [37],

have the potential to form a means of retaining the grafted cells in a single transplant site. Alternatively, biomaterial spheres or scaffolds have been used to deliver GDNF to promote engraftment by improving graft integration in the brain [38,39]. Moreover, when fetal ventral mesencephalon cells were pre-adhered to PLGA spheres, the survival was improved [40], showing that perhaps materials designed to be cell adherent could serve a dual purpose of holding the graft in a specific position and improving survival. One problem of such strategies is that such materials result in a “dead space” in the brain, during the material degradation time, and the scaffold size is limited to the small injection cannula diameter. To overcome these problems, we are currently developing highly macroporous microscale cryogel particles to which neurons can adhere, but, in a manner similar to a sponge, they can collapse to fit through a needle, and re-expand to the original shape if volume allows [41].

Figure 4 about here

The majority of transplantation studies in the parkinsonian animal brain use intra-striatal injection because nigral grafts fail to project as far as the striatum, to make relevant reconnections. However, supportive tracts from the substantia nigra to the striatum have been formed either with a stimulatory amino acid (kainic acid), or Schwann cells to promote ventral mesencephalic grafts to project from the substantia nigra to the striatum [42,43]. Such nigrostriatal “bridges” could be supported by biomaterial scaffolds, such as PEG hydrogel rods [44]. To reduce the dead space occupied by such materials, one could look to the recent developments in cryogel technology to develop highly porous

and shape memory materials, which can be injected and allow neuron growth through the scaffold matrix [45,46].

Conclusion

The lack of disease modifying strategies for PD provides strong rationale for the delivery of neuroprotective growth factors, gene therapies or replacement cells. Targeting the delivery begins with the question of systemic or stereotactic administration, and depending on which is most desirable for this therapy, a variety of targeting ligands can be considered. The problem of off-target accumulation may be easier to circumvent with gene therapies by the use of site-specific promoters, whereas growth factor delivery may benefit from controlled release devices delivered directly to the target area. Lastly, we speculate as to whether biomaterials could be used for better positioning of cells transplanted into the nigrostriatal pathway. This relatively new field of research has many challenges, but also many opportunities, for improving the quality of therapy of PD patients in the future.

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Tables

Table 1. The advantages and disadvantages of systemic vs. stereotactic drug delivery.

Systemic Drug Administration	
Disadvantages:	Advantages:
<ol style="list-style-type: none">1. Significant barriers to circumvent (see Figure. 1) such as opsonisation, removal by the reticulo-endothelial system (RES), and the blood-brain barrier.2. Off-target effects.	<ol style="list-style-type: none">1. Low cost.2. Ease of repeat administration.3. High patient acceptance.
Stereotactic Drug Administration	
Disadvantages:	Advantages:
<ol style="list-style-type: none">1. High cost.2. Requires skilled surgeon.3. Risk of infection.4. Increased risks with repeat administrations.5. Possibility of low patient acceptance.	<ol style="list-style-type: none">1. Bypasses the blood-brain barrier.2. Suitable for large cargo such as protein/cells, or therapeutics unsuitable for brain-barrier permeation such as viral vectors.3. Can target the affected region directly, so targeting moieties only need to be tailored to specific cell types.4. No or reduced off-target effects.

Figure Legends

Figure 1. Schematic depiction of the barriers to targeting systemically administered therapies to the brain as opposed to direct stereotactic injection. Many of the barriers to effective delivery via systemic administration (such as phagocytosis, removal by the reticulo-endothelial system (RES), degradation and crossing the blood brain barrier) can be overcome by stereotactic injection. However, the advantages and disadvantages of both routes are discussed further below.

Figure 2. GDNF expression levels in the rat brain after systemic gene therapy. Top panel (A) shows the effect of various modifications made to the PAMAM vector (NP) on transgene efficiency, with poly(ethylene glycol)(PEG), Transferrin (Tf) and Lactoferrin (Lf) all improving the GDNF expression in the brain as detected by ELISA. Lower panel (B) shows GDNF expression: (1) two days post single saline injection; (2) two days post single Lf-modified NP injection; (3) six days post single Lf-modified NP injection; (4) ten days post single Lf-modified NP injection; (5) two days post triple injections of Lf-modified NPs, one injection every other day; (6) two days post five injections of Lf-modified NPs, one injection every other day. This study shows the short transgene expression window, but also the additive effect of repeat administrations (5 and 6). Image reproduced with permission from [25].

Figure 3. Accumulation of liposomes with different targeting proteins/peptides, reproduced here with permission from [30], to highlight the high level of off-target accumulation in mice (in particular the liver) as opposed to the cerebellum/cerebrum.

Figure 4. A schematic diagram modified from [10], to show a side view of the substantia nigra, striatum, and surrounding ventricles (in grey). A typical needle tract is shown and potential regions for off-target therapeutic delivery are shown in red. The insert shows an image reproduced with permission from [36], showing a graft residing in the rat striatum as desired (left) and a graft with a large percentage in the cortex (right), representing the possibilities for improving graft positioning within the brain.

Figure 1.

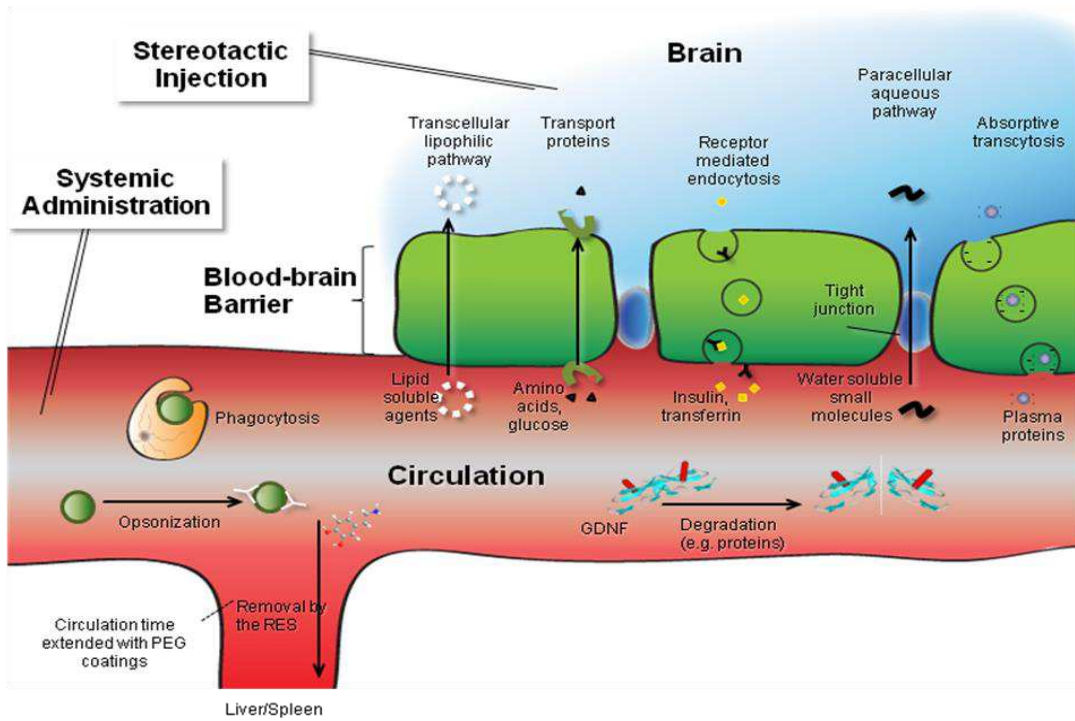


Figure 2.

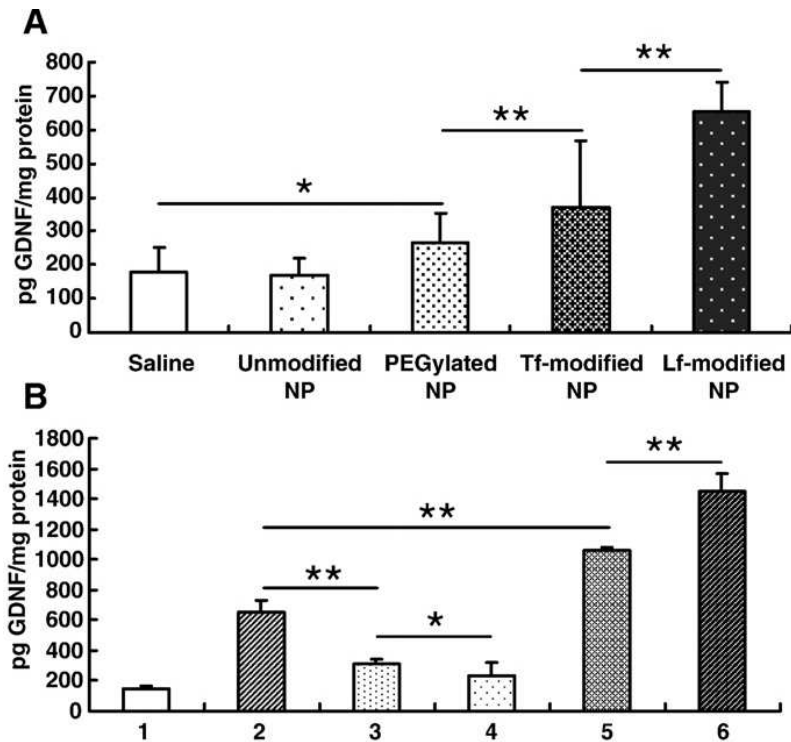


Figure 3.

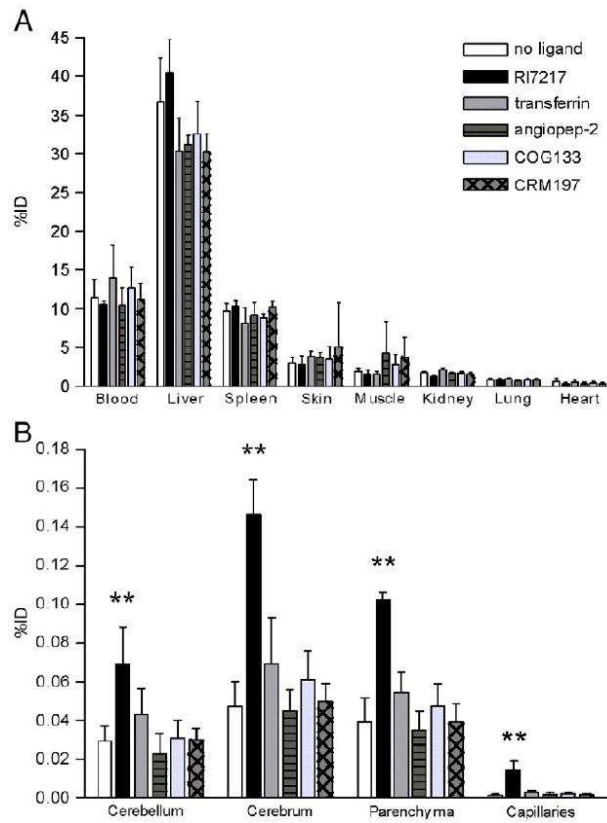
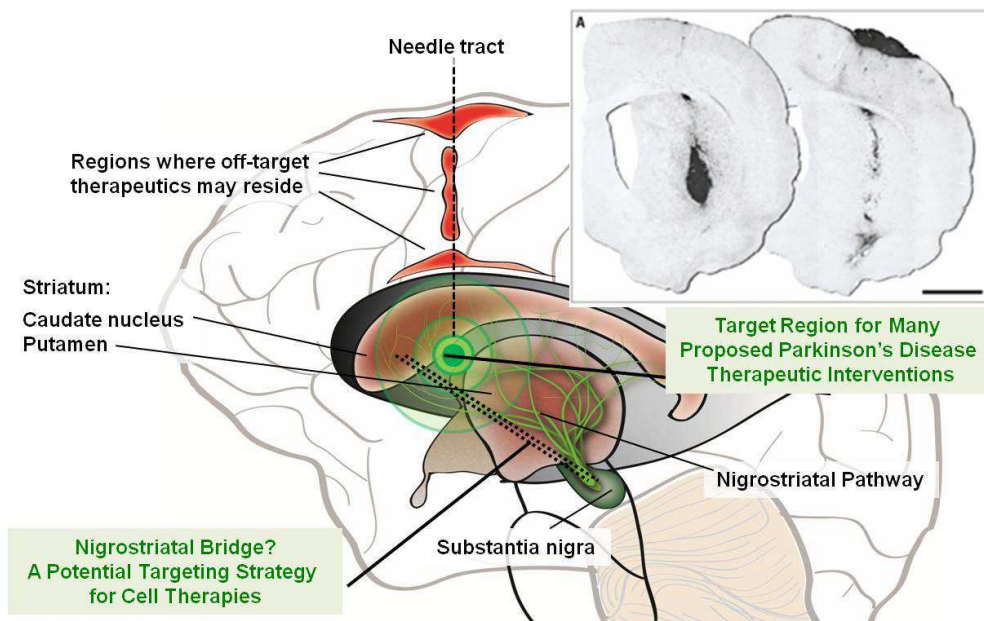


Figure 4.



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