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The advent of AAV9 expands applications for brain and spinal cord gene delivery

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Abstract

Introduction—Straightforward studies compared adeno-associated virus (AAV) serotypes to determine the most appropriate one for robust expression in the CNS. AAV9 was efficient when directly injected into the brain, but more surprisingly, AAV9 produced global expression in the brain and spinal cord after a peripheral, systemic route of administration to neonatal mice.

Areas covered—Topics include AAV9 gene delivery from intraparenchymal, intravenous, intrathecal and intrauterine routes of administration, and related preclinical studies and disease models. Systemic AAV9 gene transfer yields remarkably consistent neuronal expression, though only in early development. AAV9 is versatile to study neuropathological proteins: microtubule-associated protein tau and transactive response DNA-binding protein 43 kDa (TDP-43).

Expert opinion—AAV9 will be more widely used based on current data, although other natural serotypes and recombineered vectors may also support or improve upon wide-scale expression. A peripheral-to-central gene delivery that can affect the entire CNS without having to inject the CNS is promising for basic functional experiments, and potentially for gene therapy. Systemic or intra-cerebrospinal fluid routes of AAV9 administration should be considered for spinal muscular atrophy, lysosomal storage diseases and amyotrophic lateral sclerosis, if more neuronal expression can be achieved in adults, or if glial expression can be exploited.

Keywords

adeno-associated virus; amyotrophic lateral sclerosis; frontotemporal lobar degeneration; gene therapy; gene transfer; lysosomal storage disease; microtubule-associated protein tau; spinal cord; spinal muscular atrophy; TDP-43

1. Introduction

Somatic cell gene transfer via viral vectors is an indispensable tool in the neurosciences, particularly for whole animal studies. Adeno-associated virus (AAV) vectors are efficient for gene delivery to neurons and have, therefore, been used extensively in both basic and clinical studies, due to the efficient expression, and the relatively minor immune response

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[1–3]. Natural AAV is a single-stranded parvovirus with a genome of approximately 4.7 kb [4]. It is a dependovirus because it requires helper functions from other viruses to complete its life cycle. AAV infection is not associated with any known disease [4]. Most people have been exposed to wild-type AAV and are seropositive for AAV antibodies [4]. Recombinant AAV vectors only retain 4% of the viral genome, the inverted terminal repeats, express no viral genes and are thought to persist as nonintegrated episomes [1–3]. All of these properties contribute to why gene transfer using the recombinant vector is relatively well-tolerated in various target tissues in various species. The first characterizations of AAV gene transfer in the brain of rodents were by Kaplitt *et al.* [5] and McCown *et al.* [6], which demonstrated the potential for widespread, long-term and well-tolerated transgene expression. The efficiency improved due to a number of refinements in production/purification, promoter, AAV capsid serotype (subtype) and delivery methods [1]. Gene transfer in the CNS with AAV vectors has been extensively reviewed [1–3], while here we focus on recent results with the AAV9 serotype, which has become the vector of choice for many investigators.

2. Discovery of AAV9 and intraparenchymal brain injections

Families, or clades, of natural AAV subtypes were found in humans and nonhuman primates, and characterized, and harnessed into recombinant expression vectors by Gao *et al.* [7]. Most of the AAV capsid variants are about 85 – 90% homologous to each other [7], but different serotype expression patterns are observed in the rat brain [8,9]. Most notably, the early characterized AAV2 vector has a uniquely limited spread and expression pattern in brain. Cearley and Wolfe [10] compared several serotypes in the mouse brain for levels of transgene expression (beta-glucuronidase) and concluded that AAV9 was particularly efficient, producing greater expression than AAV7 or AAV8. These conclusions were corroborated in the rat brain, both with green fluorescent protein (GFP) reporter gene expression [11] and with the neurodegenerative disease-related protein, microtubule-associated protein tau [12]. Several tau AAV vectors were injected into the substantia nigra of rats, and tau levels were analyzed by western blots at 1, 2 and 4 weeks. AAV9 expression kinetics clearly peaked earlier than AAV2 or AAV8. The work with AAV9 in the brain up until 2008 described a highly efficient vector for neurons after intraparenchymal injections to the CNS. Intrahippocampal injection of AAV9 GFP is shown in Figure 1.

3. Expansive CNS gene transfer via intravenous administration to neonatal mice and rats

The advent of AAV9 was more fully realized, however, by Foust *et al.* [13] and Duque *et al.* [14], injecting AAV9 vectors intravenously to neonatal and adult mice in [13], and neonatal and adult mice and cats in [14]. Based on earlier work by Foust *et al.* [15] with AAV8, it was surprising that AAV9 produced such robust expression throughout the CNS after a peripheral, intravenous administration [13]. The authors demonstrated greater neuronal transduction when neonatal subjects were injected intravenously compared with that in adult subjects, which showed predominantly astroglial versus neuronal transduction, using GFP expression in mice [13]. However, Duque *et al.* [14] did report neuronal expression in the CNS after intravenous AAV9 injections to adult mice, with an efficiency of up to 28% of spinal motor neurons transduced. These studies are a breakthrough because a large fraction of the entire brain and spinal cord can be affected without having to inject the CNS; global CNS gene transfer from a peripheral delivery is now possible. It is not fully understood why neuronal transduction is not as efficient in adult subjects, although astroglial differentiation and developmental changes in the blood–brain barrier have been hypothesized [13,16]. However, aberrant glial function has been described in a number of neurodegenerative diseases, so targeting expression to glia in adults could be relevant [17].

Wang *et al.* [18] adapted the intravenous method to neonatal rats with AAV9 GFP and delineated the transduced regions in the brain and spinal cord. The use of rats has potential advantages to mice for anatomical manipulations and specific behavioral tasks (e.g., food reward paradigms). There was efficient and consistent transduction of motor neurons throughout the entire spinal cord, with an estimated 78% transduction rate. In addition to motor neurons, outstanding expression occurs in dorsal root ganglion neurons, and cerebellar Purkinje neurons (Figure 2), similar to results in mice [13,14]. In contrast to the mice studies, Wang *et al.* used a single-stranded DNA AAV9 vector, which is far less efficient for gene transfer than self-complementary double-stranded AAV vectors first developed by McCarty *et al.* [19] and used in [13,14]. Nevertheless, the spinal motor neuron transduction rate of 78% is the highest ever reported to our knowledge. Self-complementary vectors are thus not required for efficient CNS expression from intravenous injections, although their greater efficiency is important for lower effective vector doses, and easing the amounts of vector needed in production. The disadvantage of self-complementary vectors is their smaller payload, and the single-stranded vectors in Wang *et al.* [18] could thus utilize a large cytomegalovirus/chicken beta-actin hybrid promoter (1.7 kb) to express a 43 kDa functional gene product, transactive response DNA-binding protein (TDP-43) on a wide-scale basis in the spinal cord and brain.

Several studies in neonatal mice have confirmed the reproducibility of the intravenous method with either single- or double-stranded AAV9 [20–22]. Miyake *et al.* [20] demonstrated stable expression at an interval of 18 months, so the intravenous technique produces essentially permanent expression as with intraparenchymal brain injections [1]. Of note, the AAV10 serotype has also been shown to support widespread CNS gene transfer after systemic delivery to neonatal mice [20,22]. While intravenous AAV9 injections to neonatal subjects produce robust neuronal expression in the CNS, the injections also produce considerable expression in heart, liver and skeletal muscle [18,20,23,24], as expected based on earlier work administering AAV9 systemically to mice [25,26].

Hypothetically, gene therapy of a neurodegenerative disease would occur in an adult, in which widespread neuronal transduction would not be predicted from a systemic injection, though the glial transduction could have clinical relevance as mentioned [17]. Of greater currency, the neuronal transduction in neonates is a useful tool for functional studies and enhances the overall array of mammalian modeling systems.

4. Intravenous AAV9 injections to nonhuman primates

Consistent with mice and rats, efficient neuronal transduction was achieved after intravenous delivery of AAV9 to a neonatal rhesus macaque [23]. Several studies have also reported gene transfer to the CNS of monkeys at various ages after intravascular AAV9 administration, with differing degrees of neuronal versus glial transduction [24,27,28]. Consistent with mice [13], the CNS transduction was mainly in glial cells after intravascular administration to adult monkeys in [24,28]. As shown in AAV immunization studies in rats [29,30], the presence of anti-AAV neutralizing antibodies in monkeys greatly reduced transgene expression [27,28]. Neutralizing antibodies may be prevalent in humans [4] and thus be a barrier to human gene therapy. On the other hand, similar to intravenous gene transfer to neonatal rodents, and more readily applicable than gene therapy, modeling strategies will be enhanced by relevant nonhuman primate models, in which intravenous gene transfer to neonates supports efficient transduction of motor neurons [23,24]. Of practical concern, overall doses of greater than 10^{14} vector genomes of AAV9 were used in macaques in [23,24]. The large vector doses per kilogram that are needed for intravenous delivery to primates underscore the importance of strategies that would lower the effective dose, such as using self-complementary AAV9 and strong promoters.

5. Intrathecal/intra-cisterna magna routes of AAV9 administration (mice, pigs, monkeys)

Though more invasive than an intravenous injection, a route of administration into the central canal of the spinal cord could better target the cord and avoid expression in peripheral organs and could require lower vector doses that those used for the intravenous delivery. Snyder *et al.* [31] demonstrated motor neuron expression in mice after intrathecal administration of AAV9, which produced more widespread spinal expression than intraparenchymal injections as expected. Intrathecal AAV9 gene delivery to pigs was described in Bevan *et al.* [24] and Federici *et al.* [32]. Both studies described efficient transduction of spinal motor neurons. Interestingly, Federici *et al.* [32] determined injection parameters and doses to achieve expression throughout the spinal cord, and in both intrathecal pig studies, expression was successfully restricted to the CNS [24,32]. In monkeys, the data demonstrated more robust expression in the CNS after intra-cisterna magna versus intravenous administration [28]. The intra-cerebrospinal fluid strategies result in more targeted CNS expression, although as with intravenous injections to monkeys [27], the presence of antibodies against AAV nearly completely blocked the expression from intra-cisterna magna injections [28].

6. Intrauterine route of administration (mice, monkeys)

Gene delivery *in utero* could potentially be considered for gene therapy of diseases with a gene defect and perinatal morbidity/mortality. Similar to intravenous injections to neonates, intravenous AAV9 delivery to fetal mice and fetal macaques has also produced extensive neuronal expression throughout the CNS [21,33], which could have potential clinical relevance. The intrauterine route of administration is even more efficient to approach complete neuronal transduction in the CNS, because a shift toward more astroglial expression was observed with postnatal injections [21]. Again, while there could be clinical relevance, advantages of intrauterine gene transfer may be of greatest importance for basic functional and developmental studies, due to the potential for even greater efficiency of neuronal transduction relative to newborns.

7. Strategies to improve expression in CNS neurons and in clinically relevant adults

Choice of promoter is critical for efficient and sustained AAV gene transfer to neurons [1–3,34,35]. Historically, most studies of intraparenchymal AAV injections to the brain have reported selective neurotropism [1–3], in contrast to the intravenous studies with AAV9 using cytomegalovirus or cytomegalovirus/chicken beta-actin promoters [13,14]. Tissue-specific promoters could help restrict expression to neurons and to specific neuronal populations. For example, a promoter region for human neurofilament heavy chain was used in transgenic rats to drive selective expression in motor neurons [36]. This sequence is too large to package into an AAV vector, but further work could potentially minimize the sequence that confers motor neuron specificity. Alternatively, incorporation of tissue-specific endogenous microRNAs could repress expression in unwanted organs [37].

The requirement of fetuses or neonates to achieve widespread neuronal expression could be considered a caveat for gene therapy, because very young subjects may not be relevant for ageing-related diseases such as Alzheimer’s disease. Some of the intravenous AAV9 studies have shown some degree of neuronal expression in adult subjects [14,24,27]. One method that has been reported to enhance neuronal expression in adults is the use of mannitol to relax the blood–brain barrier to allow vector entry into the CNS [38], although overall, results with mannitol and systemic AAV9 are inconclusive [27,39]. Laboratory-generated

vectors with empirically determined novel tropisms (directed evolution) may be necessary to improve neuronal transduction in adults, while novel recombineered vectors could also potentially evade immunosurveillance of natural AAV serotypes [17,30,40].

8. Preclinical gene therapy strategies for the brain and spinal cord using AAV9

Widespread brain and spinal gene transfer resulting from a peripheral injection is particularly important for therapeutics for two reasons: one, because a minimally invasive intervention avoids injecting the CNS; and two, because expression is achieved throughout the entire CNS, which may be necessary for a disease such as Alzheimer's disease, which affects large portions of the brain, or diseases that affect large portions of the spinal cord. There are a number of spinal cord diseases that meet the criteria for considering gene therapy, that is, life-threatening disease without cure or ameliorative, and disease forms with inherited mutations. For example, there are genetic forms of spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS), and there are no efficacious medications for these diseases involving the spinal cord [41–43]. Another reason that gene transfer is important is that a corrective protein may need to be administered on a long-term basis for a chronic disease, and a one-time gene transfer treatment can produce sustained expression. For example, repeated or chronic protein delivery to the CNS is difficult. A secretable protein factor, such as a neurotrophic factor, is particularly advantageous, as the spread of the recombinant growth factor or corrective enzyme (e.g., beta-glucuronidase for the lysosomal storage disease mucopolysaccharidosis VII [10]) could be potent over a region larger than the area of the transduced cells, thus lowering the number of gene copies needed to exert a widespread effect. Therapeutic gene delivery of a secretable factor would also bypass the need for neuronal transduction. Gene therapy paradigms in rodent models of spinal cord diseases using various AAV gene transfer modalities have been previously reviewed [44–46].

Owing to the efficient cardiac gene transfer of AAV9 [25], a number of studies have demonstrated positive effects of AAV9 vectors in models of heart disease, for example, Fechner *et al.* [47]. Likewise, the efficient expression in skeletal muscle from intravenous AAV9 has been exploited for preclinical gene therapy in a canine muscular dystrophy model [48]. Efforts with AAV9 in preclinical gene therapy paradigms for the CNS are outlined in Table 1. Intraparenchymal injections of specific AAV9 vectors have been used for protective effects in a Parkinson's disease model [49]. Intraparenchymal and combined intracerebroventricular and intravenous routes of administrations of AAV9 vectors have been successful in mouse models of lysosomal storage diseases [39,50,51], which provide the rational basis for clinical development.

SMA is attributed to mutations in the gene called survival motor neuron, and survival motor neuron knockout mouse models are used to mimic the disease [41]. Because SMA is due to a single gene defect and may be a fatal disease (depending on disease subtype) without cure, it could be a good candidate disease for experimental strategies such as gene therapy. Interestingly, survival motor neuron gene delivery with intravenous AAV9 to the mouse model of SMA restores function and either improves or fully rescues their lifespan [23,52–55], which is promising for humans. Of note, an improved therapeutic outcome resulted from an intracerebroventricular versus intravenous route of AAV9 survival motor neuron gene delivery [55], suggesting that it is more beneficial to contain expression within the CNS in SMA models. However, expression of survival motor neuron from intravenous AAV9 administration has been shown to correct cardiac abnormalities of SMA mice [52]. More clinical development of AAV9-mediated gene therapy to replace survival motor neuron function in SMA is warranted.

ALS is another spinal cord disease without cure and with a number of specific genetic etiologies [43–46]. The transgenic rodent models of ALS based on mutations in the copper zinc superoxide dismutase one (SOD1), which occur in familial ALS, are very well characterized, faithful to the spinal neurodegeneration and paralysis in human ALS and thus widely used [56]. Gene transfer was effective in improving grip strength, motor function and the lifespan when peptidergic growth factors such as insulin-like growth factor one or vascular endothelial growth factor were expressed in mSOD1 mice with AAV, by injecting the vector into brain regions that innervate the spinal cord [57,58]. Gene knockdown strategies to reduce the expression of the pathogenic mutant SOD1 in mSOD1 mice with AAV have also improved their function by preserving their grip strength, for example, Miller *et al.* [59]. Successful studies will achieve widespread spinal expression from intravenous or intrathecal AAV9 in mSOD1 models, which will underscore the utility of AAV9, and explore therapeutic opportunities for ALS that were not possible before AAV9.

9. Expressing a neuropathological protein (AAV9 Tau and AAV9 TDP-43)

Viral vector expression of neuropathological proteins in the CNS is an alternative modeling system of specific neurodegenerative diseases compared with more standard methods of neurochemical lesioning or germ-line transgenic mice [1,60]. Vector-based methods are particularly useful to target expression of the most relevant brain regions and to avoid embryonic lethality. Modeling neurodegenerative diseases in rats with AAV9 is outlined in Table 2. As mentioned, Klein *et al.* [12] utilized the high gene transfer efficiency of AAV9 to express the microtubule-associated protein tau in the substantia nigra, which is relevant to diseases with tau neurofibrillary tangles in the brain, such as frontotemporal lobar degeneration (FTLD) with tau pathology [61–63]. AAV9 Tau led to more pronounced and near-complete striatal dopamine loss compared with AAV2 or AAV8 Tau vectors, thus graded, early- and late-stage disease models are possible via different gene transfer efficiencies [12]. Of note, an advantage of the somatic cell gene transfer approach is the ability to induce expression at any age to produce age-related models relevant to age-related neurodegenerative diseases [64]. In terms of tau neurofibrillary tangle pathology, earlier work confirmed the presence of straight tau filaments by electron microscopy, using an AAV2 Tau vector in the substantia nigra with the P301L disease-related tau mutation [1].

To mimic subtypes of the neurodegenerative disease FTLD-TDP with pathology in the nigrostriatal system [62,65], Tatom *et al.* [65] expressed TDP-43 in the substantia nigra of rats with AAV9 to develop an assay of relevant TDP-43 neuropathology and neurodegeneration. The human wild-type TDP-43 vector produced consistent, dose-dependent gliosis, dopaminergic neuron loss and motor deficits. There were only few examples of cells with disease-relevant cytoplasmic TDP-43 deposition using the wild-type TDP-43, which expressed mainly in the nucleus as expected [65]. TDP-43 is a major pathological protein in both FTLD-TDP and in ALS, with pathology found in the spinal cord and brain [62,63]. Both lower motor neurons in the spinal cord and upper motor neurons in the motor cortex degenerate during ALS progression. For these reasons, the AAV9 transduction patterns in Foust *et al.* [13] were intriguing because it appeared that the neuronal populations affected in ALS were efficiently transduced.

One concern about attempting to model a spinal cord disease state that would be consistent with germ-line transgenic rodent models was variability of the transduction produced by an intravenous gene delivery. However, extensive experience with the technique has confirmed consistent intravenous delivery, circulation to the brain and spinal cord, and gene transfer in the CNS. Relevant aspects of ALS rapidly manifested when wild-type TDP-43 was expressed, with severe muscle atrophy and limb paralysis, and moderate loss of spinal motor neurons. The intravenous AAV9 method was thus successful for modeling a spinal cord

disease state comparable with germ-line TDP-43 transgenic mice and rats [44]. There was great reproducibility of the TDP-43 phenotype, which is due in part to the potent neurotoxicity of wild-type TDP-43 hyperexpression, but also to the highly consistent intravenous delivery technique. The relevant and consistent models are relatively cost-effective compared with developing and maintaining transgenic lines, which is important in light of new discoveries of genes associated to ALS [43], which could be assayed individually and in combination more rapidly by a somatic cell gene transfer approach than with transgenics. The approach will also be useful for gene structure–function studies in rodents, because designed gene variants can be assayed rapidly relative to making transgenic lines for each hypothesis.

In modeling neurodegenerative diseases, there are two potential philosophies: one, to express the neuropathological protein only in the neuronal populations affected in disease; and two, to ubiquitously express the protein and study selective vulnerability. The intravenous TDP-43 vector model from Wang *et al.* [18] expresses in peripheral tissues as well as in many brain regions, so changes in vector design or delivery would be needed to approach selective expression in motor neurons. However, unpublished studies by Klein *et al.* have shown that expression of TDP-43 in the spinal cord is necessary to induce ALS-relevant paralysis because when a different promoter was used to express TDP-43 (tet-off promoter, Haberman *et al.*, [66]), which expresses in liver and heart but not the spinal cord, there was no observable disease state induced. Results with AAV9-expressing neuropathological proteins either with intraparenchymal [12,64,65,67] or intravenous [18] vector delivery have been impressively consistent, which support the use of AAV9 for versatile assays of neurodegenerative diseases. The intrauterine route of administration of AAV9 [21,33] could have utility for modeling, by further increasing neuronal transduction efficiency. AAV TDP-43 vectors can be used to generate nonhuman primate models of neurodegenerative diseases. For example, focal injections of an AAV1 TDP-43 vector to the cervical spinal cord of macaques has recently been reported, which produced ALS-relevant TDP-43 neuropathology in the cervical spinal cord and forelimb paresis [68]. It will be interesting to study intrathecal or intravenous delivery of AAV1 or AAV9 TDP-43 vectors to see whether ALS-like neuropathology and paralysis will be more widespread throughout the spinal cord and brain, which should be highly relevant to human and which we are attempting.

10. Expert opinion

There is a consensus that AAV9 is an efficient serotype for gene transfer in the brain, so more studies using AAV9 are expected. The widespread gene transfer derived from intravenous or intrathecal AAV9 has advanced potential applications of global CNS gene therapy. Further refinements will be necessary to achieve neuronal-specific expression in clinically relevant, symptomatic adult paradigms. It will be interesting to determine whether AAV9 has the unique attribute for wide-scale transduction after systemic or intra-cerebrospinal fluid routes of administration. When we injected AAV1 or AAV10 directly into the hippocampus or substantia nigra, they appeared to work as well as AAV9 [9,11,12], which is consistent with other studies [69–71]. In addition to AAV9, AAV10 yields efficient CNS gene transfer from intravenous injections to neonates [20,22].

Due to the widespread spinal and brain expression that is now possible, more preclinical work with AAV9 in animal models of ALS is expected, while clinical development of AAV9 gene therapy for fatal forms of SMA and lysosomal storage diseases should be pursued. Functional translation of preclinical gene therapy in rodents and dogs to primates and humans has been historically difficult [72], but we are hopeful that the widespread AAV9 expression can be harnessed for clinical utility of specific conditions. Recent

successes in human gene therapy for hemophilia B and a form of blindness called Leber's congenital amaurosis with AAV suggest there will be a growing number of specific indications that work in humans [73].

Nonetheless in consideration of potential caveats, if therapy was pursued in a young individual with a wide-scale approach, the efficient expression in the developing CNS could be dangerous in ways that we do fully not understand. The concept of gene doping is unnerving by any route of administration, and even more with a wide-scale approach involving the CNS during development. More practically speaking, developmental side effects in basic studies involving young subjects must be considered.

While human AAV9 gene therapy might be worth considering someday, AAV9 is an important vector for modeling neurodegenerative diseases in animals today. Disease models are facilitated with AAV9 due to its efficiency and versatility for either focal [12,64,65,67] or widespread [18] expression, as well as expression in different species such as nonhuman primates. The rapid and cost-effective models are consistent and should expedite gene variant structure–function comparisons and gene combinations that would take longer and cost more in transgenicmice, which is an important point considering the growing list of genes mutated in neurodegenerative diseases, and the specific pathological isoforms and comorbidities that are found. The ultimate goal in this regard is an improved model with a mechanism that is more relevant to human disease because a more faithful mechanism could enable discovery and development of an efficacious small molecule that would otherwise be missed in models with irrelevant mechanisms.

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Declaration of interest

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Article highlights

- AAV9 is an efficient natural adeno-associated virus (AAV) serotype for transgene expression in neurons.
- Surprisingly, the AAV9 serotype produced wide-scale neuronal transduction in the CNS from a peripheral, systemic injection in neonatal subjects.
- AAV9 has been used in a number of gene therapy and neurodegenerative disease modeling paradigms, by several routes of administration.
- There are remaining caveats to overcome in approaching human gene therapy with AAV9. On the other hand, the remarkably consistent and efficient gene transfer with AAV9 has enhanced basic research on neurodegenerative diseases.

This box summarizes key points contained in the article.

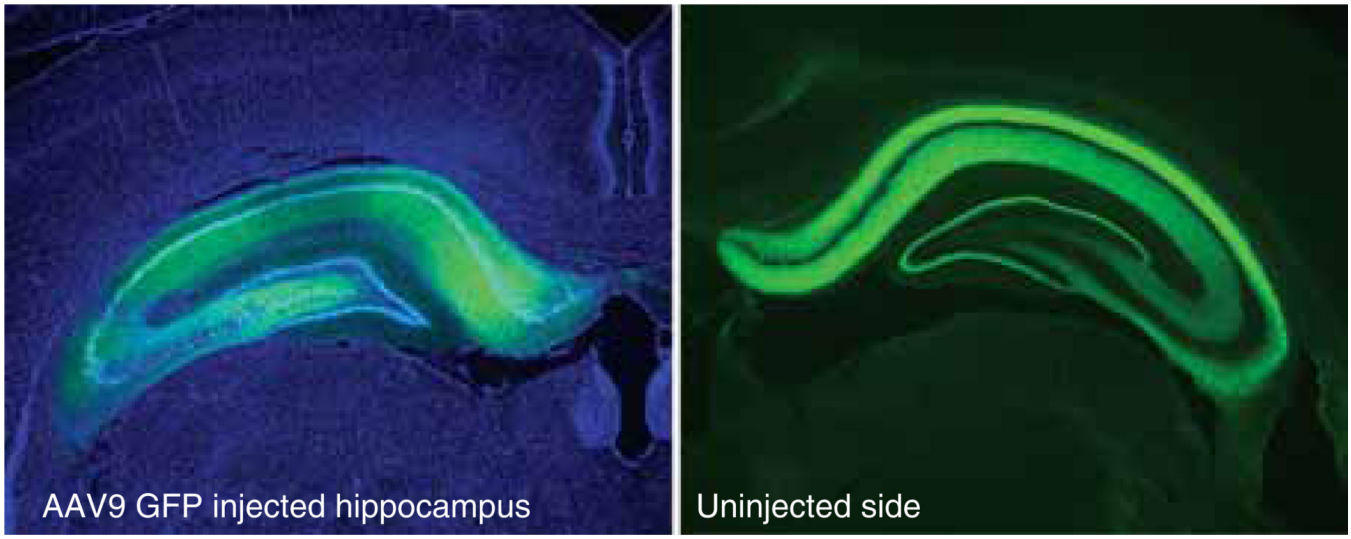

Figure 1. Intraparenchymal (intrahippocampal) injection of an adeno-associated virus 9 (AAV9) vector for green fluorescent protein (GFP) in the rat

Left) Injected side with nuclear counterstain in blue. Right) Contralateral, uninjected side showing anterograde projections from the injected side. There are three points worth noting about the panel on the left: i) efficient gene transfer throughout the dorsal–ventral and medial–lateral extents of the hippocampus; ii) targeting, lack of GFP expression in the cortex above or thalamus below; and iii) lack of needle track damage in the overlying (injected) cortex. Investigators must consider all of these points in evaluating their injections. For example, the memory-related behavioral assays ran in Dayton *et al.* [67] after hippocampal injections of AAV9 would be difficult to interpret if there were expression in, or damage to, the cortex. Three-week expression interval. Images from Klein *et al.* [11].

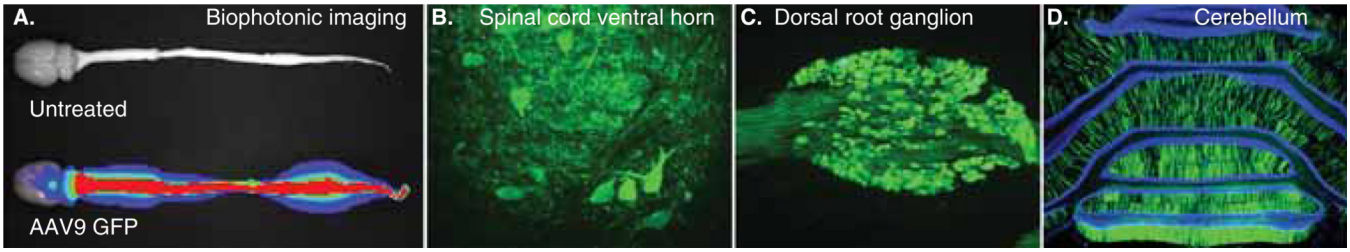

Figure 2. Intravenous injection of an adeno-associated virus 9 (AAV9) vector for green fluorescent protein (GFP) to a neonatal rat

A) Biophotonic imaging of GFP throughout the entire spinal cord in a GFP rat, while an untreated rat is blank. Red indicates higher levels of GFP epifluorescence relative to blue. **B**) GFP expression in large neurons in the ventral horn of the lumbar spinal cord. **C**) Dorsal root ganglia. **D**) Cerebellum (with nuclear counterstain in blue). The most evident GFP-expressing cells in the cerebellum are Purkinje neurons. In contrast to focal, intraparenchymal brain injections, which transduce a circumscribed population of neurons, the intravenous AAV9 injections transduce neurons throughout the entire brain and spinal cord. 4 (**B–D**) or 12 (**A**) week expression intervals. Epifluorescence in **A**, **C** and immunofluorescence in **B**, **D**. **A**, **B** from Wang *et al.* [18].

Table 1

Preclinical gene transfer strategies in the central nervous system using the adeno-associated virus serotype 9 (AAV9) vector.

Disease model	Subjects	Route	AAV9 vector	Therapeutic outcomes	Ref.
Parkinson's disease	Adult	Intraparenchymal Striatum	EPO	Protection of dopamine neurons and motor function	[49]
Mucopolysaccharidosis VII	Adult	Intraparenchymal VTA	GUSB	Reduced lysosomal storage pathology	[50]
Mucopolysaccharidosis IIIB	Juvenile	IV	NAGLU	Reduced lysosomal storage pathology and neurodegeneration	[39]
Multiple sulfatase deficiency	Neonatal	IV and ICV	SUMF1	Reduced inflammation and lysosomal storage pathology, behavioral correction, improved lifespan	[51]
Spinal muscular atrophy	Neonatal	IV	SMN	Improved motor and neuromuscular function, motor neuron survival, improved body weight and lifespan	[23,52–55]
Spinal muscular atrophy	Neonatal	ICV	SMN	Improved motor function and muscle size, improved body weight and lifespan	[55]
Visceral	Adult	IV	GLT-1	Increased glutamate uptake, anti-nociception	[74]

All studies were conducted in mice except [49], which used rats.

EPO: Erythropoietin; GLT-1: Glutamate transporter; GUSB: Beta-glucuronidase; ICV: Intracerebroventricular; IV: Intravenous; NAGLU: alpha-*N*-acetylglucosaminidase; SMN: Survival motor neuron; SUMF1: Sulfatase-modifying factor 1; VTA: Ventral tegmental area.

Table 2

Neurodegenerative disease modeling using the adeno-associated virus serotype 9 (AAV9) vector.

Disease model	Transgene	Subjects	Injection method	Relevant outcomes	Ref.
FTLD-Tau	Tau	Adult	Intraparenchymal substantia nigra	Neuronal loss, motor deficit	[12]
FTLD-Tau	Tau	Aged adult	Intraparenchymal substantia nigra	Neuronal loss, motor deficit Neuronal loss, motor deficit, microgliosis, age-related effects	[64]
FTLD-TDP	TDP-43	Adult	Intraparenchymal substantia nigra	Neuronal loss, apoptosis, astrogliosis, microgliosis, motor deficit	[65]
FTLD-TDP	TDP-43	Adult	Intraparenchymal hippocampus	Neuronal loss, microgliosis, memory-related deficit	[67]
ALS	TDP-43	Adult	IV	Lower motor neuron loss, paralysis, muscle wasting, microgliosis	[18]

Human wild-type forms of tau or TDP-43 were expressed in rats, except [12], which used the P301L form of tau related to a subtype of FTLD-Tau.

ALS: Amyotrophic lateral sclerosis; FTLD: Frontotemporal lobar degeneration; IV: Intravenous; TDP: Transactive response DNA-binding protein.