Video Article

# Methods and Tips for Intravenous Administration of Adeno-associated Virus to Rats and Evaluation of Central Nervous System Transduction

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## Abstract

Adeno-associated virus (AAV) vectors are a key reagent in the neurosciences for clustered regularly interspaced short palindromic repeats (CRISPR), optogenetics, cre-lox targeting, etc. The purpose of this manuscript is to aid the investigator attempting expansive central nervous system (CNS) gene transfer in the rat via tail vein injection of AAV. Wide-scale expression is relevant for conditions with widespread pathology, and a rat model is significant due to its greater size and physiologic similarities to humans compared to mice. In this example application, a wide-scale neuronal transduction is used to mimic a neurodegenerative disease that affects the entire spinal cord, amyotrophic lateral sclerosis (ALS). The efficient wide-scale CNS transduction can also be used to deliver therapeutic protein factors in pre-clinical studies. After a post-injection expression interval of several weeks, the effects of the transduction are evaluated. For a green fluorescent protein (GFP) control vector, the amount of GFP in the cerebellum is estimated quickly and reliably by a basic imaging program. For motor disease phenotypes that are induced by the ALS related protein transactive response DNA-binding protein of 43 kDa (TDP-43), the deficits are scored by escape reflex and rotarod. Beyond disease modeling and gene therapy, there are diverse potential applications for the wide-scale gene targeting described here. The expanded use of this method will aid in expediting hypothesis testing in the neurosciences and neurogenetics.

## Video Link

The video component of this article can be found at https://www.jove.com/video/55994/

## Introduction

Recombinant adeno-associated virus (AAV) vectors are indispensable tools for CNS research because they are so efficient for transducing neurons in vivo. AAV vectors are very versatile for studying different transgenes and protein isoforms, different tissues, different host species, and different routes of administration. For instance, AAV can be administered to mice by a peripheral, relatively non-invasive, intravenous injection to transduce neurons throughout the CNS as first described in Foust *et al.* and Duque *et al.* (see JOVE paper by Gombash *et al.* (2014)).[1,2,3] This gene delivery approach is used in rats to efficiently express either green fluorescent protein (GFP) or the ALS related protein, transactive response DNA-binding protein of 43 kDa (TDP-43) in the CNS.[4,5,6,7,8,9] Working in rats is significant because the rat's physiologic and metabolic parameters are closer to humans as compared to mice and there are behavioral and toxicological assays designed specifically for rats. Furthermore, more transgenic rat lines are becoming available that can be utilized in AAV gene transfer studies.

Methods are detailed for expansive CNS gene transfer in the rat, and rapid, reliable quantification of the outcomes. Wide-scale CNS transduction is used to mimic the symptomatology of ALS in rats by expressing TDP-43 throughout the spinal cord. The method is tail vein injections of the TDP-43 vector to young adult rats as used in Jackson *et al.*[6,8,9] After several weeks, TDP-43-induced motor deficits are scored by two methods: escape reflex and rotarod as used in Dayton *et al.* and Jackson *et al.*[5,6,7,9] For the control GFP vector, in post-mortem analysis, the fluorescent area of the cerebellum is calculated as an index of transduction efficiency as used in Jackson *et al.*[6,8] The analysis of cerebellum has proven to be a rapid and reliable index of the degree of CNS transduction after peripheral gene delivery and should be applicable to a variety of approaches attempting peripheral-to-central gene transfer.

## Protocol

All procedures followed the appropriate NIH guidelines. All of the procedures followed animal protocols that were approved by the Animal Care and Use Committee at LSU Health Sciences Center in Shreveport. Female Sprague-Dawley rats at 6 weeks of age were used for this procedure.

## 1. Determine the doses/volumes of vector needed

1. Weigh the rats to be injected and determine the amount of viral vector needed for each animal based on the titer (concentration) of the vector preparation. Use vector doses that have been successful for efficient expression in the rat CNS previously (see Jackson *et al.*[6]).
   NOTE: For AAV9 TDP-43 or AAV9 GFP, a typical dose is between 3 x $10^{13}$ - 1 x $10^{14}$ vector genomes/kg. The young adult rats at 6 weeks of age weigh about 150 g.
2. Make equivalent doses per kg in all animals for systematic comparisons. Ensure the volume does not exceed standard advised injection volumes (250 - 500 µL for a 250 g rat). A typical injection volume is 200 µl to a young adult rat.

## 2. Prepare work station

1. Gather all required supplies: one paraffin film square (about 5 x 5 cm) per animal, one alcohol preparation pad per animal, gauze pads (2 - 3 per animal), pipet tips, and a micropipette.
2. Prepare the working surface by cleaning the surface with 70% ethanol. Place a heating pad down with the temperature set to low (~37 °C). Place a bench pad on top of the heating pad.
3. Prepare the gas anesthesia (isoflurane). Ensure adequate oxygen and gas anesthesia for the procedure. Clean the induction chamber and anesthesia mask with 70% ethanol, and place the anesthesia mask on the bench pad.

## 3. Prepare the vector

1. Thaw the vector at room temperature, and label one sterile microcentrifuge tube for each animal.
2. Pipet the volume of AAV determined in step 1.1 into the appropriate tube and bring the volume up to the desired injection volume (here, 200 µl) with lactated Ringer's solution or saline.
3. Pulse vortex the sample for 1 - 2 s and then pulse centrifuge for 5 s to bring the sample to the bottom of the tube.
4. Pipet the total injection volume for the rat out of the tube and onto the square of paraffin film.
5. Place a 30 gauge needle on a 1 mL syringe.
6. Place the needle with the bevel facing down into the vector on the paraffin film. Pull back on the syringe plunger until all the virus is in the needle and syringe. Be careful to avoid drawing air bubbles into the needle, which becomes easier with practice.

## 4. Prepare the rat

1. Turn the oxygen up to 1 L/min and set the isoflurane anesthesia to 5%. Ensure that the gas anesthesia is flowing to the induction chamber by checking all connecting hoses and stopcock orientations.
2. Place the rat in the gas anesthesia induction chamber about 3 min, until the rat is unresponsive.
3. Ensure adequate depth of anesthesia by pinching the toe. There should be no movement in the foot or leg after the toe pinch.
4. Reduce the isoflurane anesthesia to 2% for maintenance of anesthesia and adjust the stopcock so that the anesthesia is flowing to the anesthesia mask.
5. Place the rat's nose in the anesthesia mask and adjust the rat so that it is lying on its side.
6. Identify the lateral tail vein.
   NOTE: The lateral tail veins lie at approximately 10 and 2 o'clock with the dorsal top of the tail as 12 o'clock. With the rat lying on its side, the lateral tail vein should be facing up.
7. Wipe the injection area with the alcohol preparation pad. The injection site is two-thirds down the length of the tail.

## 5. Inject the vector

1. Firmly hold the tail slightly above the injection area with one finger directly over the lateral tail vein; this will dilate the vein.
2. With the bevel facing up, align the needle with the visible tail vein in the same direction.
3. Pierce the skin over the tail vein while taking great care to avoid the other hand holding the tail and pressing on the tail vein.
4. Release pressure from the tail vein to allow for normal blood flow.
5. Move the needle into the vein. To ensure that the needle has punctured the tail vein, pull back slightly on the plunger. If the needle is in the vein, blood will flow into the needle.
   1. Reposition the needle as needed. Then, to stabilize the needle, move the hand above the injection site to below the injection site and hold the end of the syringe against the tail.
6. Slowly inject the vector into the tail (approximately 20 µL/s).
   NOTE: The vein may lose color as the vector flows through it. Signs that the solution was injected outside of the vein include blebbing, blanching of the skin, or resistance when injecting the vector. With practice, extra-vascular exposure can be minimized.
7. After the vector has been administered, remove the needle from the rat and press a gauze pad against the injection site to help stem the bleeding for 30 - 60 s.

## 6. Clean-up

1. Turn off the isoflurane anesthesia and oxygen. Monitor the rat until it regains consciousness, and then return it to its cage.
2. Carefully place all needles in the sharps container. Dispose of all other disposable materials that are potentially contaminated with AAV into an appropriate biohazard container. Clean the working stations with 70% ethanol.

## 7. Evaluate hindlimb escape reflex

NOTE: Hindlimb deficits usually arise 2 - 6 weeks after TDP-43 gene transfer, depending on the vector dose.

1. On a flat surface clear of debris, with two fingers grab the rat's tail approximately 2 cm from the body. Lift the rat gently until the forelimbs are hanging while viewing the ventral underside of the rat.
    1. Perform the procedure weekly throughout the experimental time-course after gene transfer (e.g., up to 12 weeks after gene transfer).
    NOTE: Also score forelimb deficits in this manner, but hindlimb deficits are more likely to occur in this model, depending on the vector dose used.
2. Observe the hindlimbs for 10 s. Note if one or both hindlimbs clench towards the midline during the 10 s.
3. Repeat this test for a total of three times with 30 s between trials. If one or both hindlimbs are clenched for each of three trials, the rat is showing motor dysfunction. Record the data (manually in notes) as either unilateral or bilateral hindlimb deficit present if there is consistent clenching across all three trials.

## 8. Evaluate motor function on the rotarod

1. To set up the rotarod, place a bench pad beneath the rotarod and set acceleration to 4 - 40 revolutions per minute (rpm) over 2 min (the rate increases by ~0.3 rpm/s). For a 12 week time-course, run the subjects at 2, 4, 8, and 12 weeks after gene transfer.
2. Allow the rat to acclimate to the testing room for 20 min.
3. To train the rat to use the rotarod for the first time, first start the rotarod at a set speed of 4 rpm, then place the rat on the rotarod. Allow the rat to walk for one full revolution at 4 rpm, then start the acceleration and allow the rat to walk on the rotarod until it falls off. Continue training until the rat can consistently walk for at least 40 s. A healthy, young adult rat will be readily trained in this manner.
    1. In the case of a rat with a noticeable motor impairment, which may prevent the rat from achieving a baseline latency to fall of 40 s, provide the rat 3 - 5 training attempts before beginning rotarod scoring.
4. To begin rotarod testing, place the rat on the rotarod and start acceleration of the rotarod. Note the time at which the rat falls from the rotarod; this is the latency to fall. If the rat remains on the rotarod after 120 s, cap the session at this point, remove the rat from the rotarod and record a fall latency of 120 s.
5. Repeat the testing two more times for a total of three trials. To minimize the effect of fatigue, space the trials at least 5 min apart. Average the scores of the three trials. An unimpaired adult rat typically has an average latency to fall of > 40 s.
6. Place the rat back in the cage, and clean the rotarod and surrounding areas with 70% ethanol.

## 9. Quantification of GFP expression in the cerebellum

1. Anesthetize the rats and perfuse with phosphate buffered saline (PBS) and then 4% paraformaldehyde. Dissect the brain and spinal cord and soak the tissues in the fixative overnight and then move them to a 30% sucrose solution. After equilibration, cut 50 µm coronal sections on a sliding microtome with freezing stage[4,5,6].
2. Choose 3-6 sections of the cerebellum that are evenly spaced in the vermis, the central lobe of the cerebellum. Fluorescently label the GFP to maximize the signal. Use the primary antibody for GFP at 1:500 and use the Alexa-488 conjugated secondary antibody at a dilution of 1:300[4,5,6].
    NOTE: See references [4,5,6] for more detail on the sampling, sectioning, and staining procedures.
3. Photomicrograph each section in the defined region of interest with a low magnification lens using a microscope. Use a 2.5X objective (N.A. 0.12) and filter set 10, 450-490/515-565 excitation/emission). Keep the camera settings (exposure times, e.g., 2 s) the same across samples to be analyzed. Save as a .TIF file.
4. Open the photomicrograph in the widely available Scion image program; two windows will open. Close the window indicated as "Indexed Color".
5. Choose "Options", then "Density Slice"; a range of shades will be indicated in red on the Look-Up Table (LUT) window. In the window, use the cursor to fill in the specific GFP labelling only and stop when the non-specific background begins to be picked up in the image.
    NOTE: This will highlight the fluorescent area on the photomicrograph to be quantified. The settings should be optimized to specifically capture all of the visibly fluorescent cells. With practice, this method can precisely highlight the specific fluorescence.
6. Before measuring fluorescent area, first ensure that the measurements made are in the correct units (pixels). To do this, click "Analyze", then "Set Scale"; the fourth line should say "Units". From the drop-down list, choose pixels to measure in pixels, then click "OK". Toggle the analysis options to "Include Interior Holes" to ensure the inclusion of the entire cell body in the analysis.
7. To measure the fluorescent area, click "Analyze" and then click "Measure". To see this value, click "Analyze" then click "Show Results"; a results window will appear. The fluorescent area measurement will be under the heading labeled "Area".
8. To ensure standardization across the measurements, do not change the highlighted values in the LUT window.
9. Once all measurements have been recorded, average the values for each animal (from 3 - 6 sections per animal). Use an appropriate statistical test to compare the transduced areas between the groups.[6]

## Representative Results

TDP-43 induced motor impairments should arise within 2 - 6 weeks depending on the dose of the AAV TDP-43 used (**Figure 1**). Unsuccessful tail vein injections will result in partial or no impairments; the AAV must reach the bloodstream to produce the effect. A successful injection of AAV GFP will result in GFP expression throughout the brain and spinal cord in a vector-dose dependent manner. When using the strong recombinant promoter to drive expression, the cytomegalovirus chicken beta actin hybrid promoter, also known as the CAG promoter, there is efficient expression in the CNS as well as several other tissues such as liver and heart.[4,5,10] Within the rat CNS, when using AAV9 or AAV PHP.B vectors, the pattern is predominantly neuronal transduction and only sparse, sporadic glial transduction.[4,8] Since no gender difference in transduction efficiency was observed in rats with the wide-scale method, females are typically used for intravenous gene transfer due to their lower weights.[6]

The semi-quantitative imaging method described is rapid and reliable because there is an excellent signal-to-noise ratio. A non-transduced sample is compared with a transduced sample in **Figure 2** with both samples stained with the GFP antibody. Values collected from these samples indicate 0.3% noise (from the values listed in **Figure 2B, D**) in the non-transduced sample, which can be considered negligible. Given the excellent signal-to-noise ratio, this method is valid for rapid comparisons of experimental conditions such as vector types, vector dose, gender, age, etc.

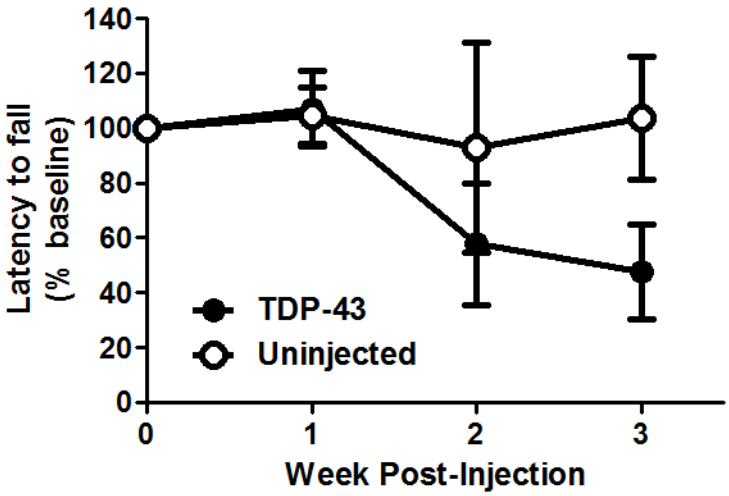


**Figure 1. Wide-scale AAV9 TDP-43 gene transfer to rats causes progressive motor impairment.** Rotarod performance at 1 - 3 weeks after gene transfer of either the control GFP or the ALS-related gene TDP-43. The subjects were tested prior to gene transfer and the subsequent measurements were expressed as a ratio to the baseline time point. A normal untreated rat will stay on the rotarod from 60 - 90 s under these conditions. This figure has been modified from[6].

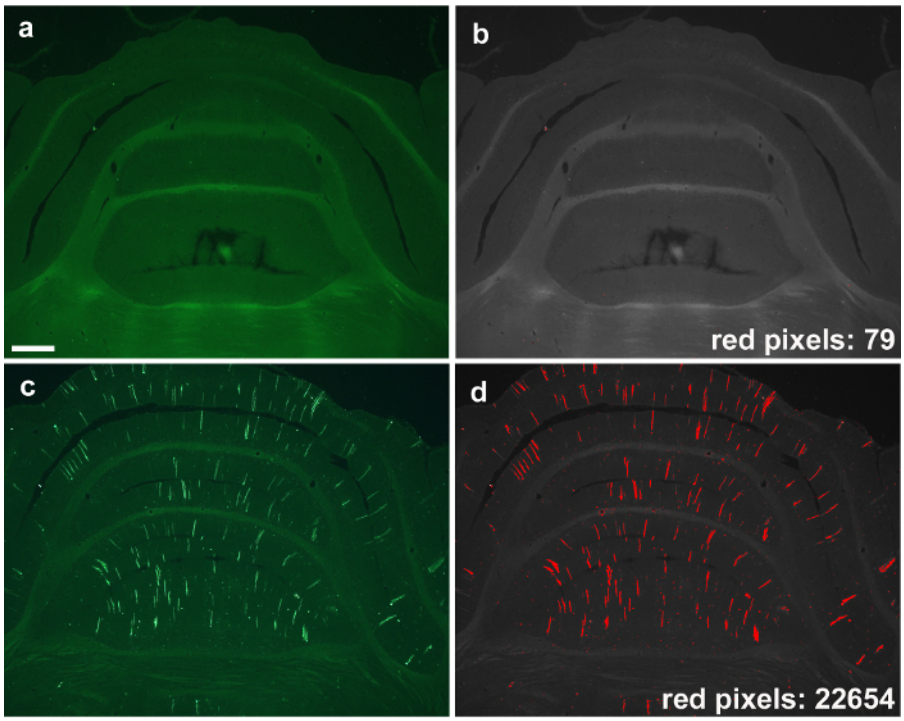


**Figure 2. Rapid quantification of the GFP-positive fluorescent area in the cerebellum after intravenous AAV administration to the rat.** A, B) Non-transduced sample that was stained with the GFP antibody. C, D) Sample from a rat receiving AAV9 GFP. B, D) The Density Slice option on Scion Image is used to selectively highlight the fluorescent area in red, and then the red pixels are counted by the program. The Density Slice option should highlight all of the visible fluorescent cells as seen in C. The red area expressed in square pixels as calculated by the program is shown in B, D. The time point was 4 weeks after a tail vein injection of AAV9 GFP to a young adult rat at a vector dose of 1 x $10^{14}$ vector genomes/kg. Scale bar in A is 536 µm; same magnification in A-D. Please click here to view a larger version of this figure.

## Discussion

A variety of delivery routes have been tested for AAV vectors: intra-parenchymal, intra-cerebroventricular, intra-thecal, intravenous, intranasal, intra-muscular, etc. Each route may have specific advantages as well as drawbacks. Tail vein administration of AAV to adult rats is a reliable method for achieving consistent transduction of the CNS. The peripheral injection method avoids the introduction of an injection cannula into the CNS tissues and is therefore minimally invasive. The efficient CNS expression that can be achieved by this method is the biggest advantage of the technique. Other practical advantages are that the injection method is very fast (about 10 min per animal from start to finish) and that the tail vein injections are not highly technical and can therefore be mastered quickly. Certainly, a major drawback is that a lot of high titer vector is needed, which will be a feasibility issue if the AAV preps are below 1 x $10^{13}$ vector genomes/ml. With further refinement of vector targeting capabilities, this approach could be advantageous for clinical gene therapy in the future, given that the gene delivery is by a peripheral route and thus relatively non-invasive. On the other hand, more direct intra-cerebrospinal fluid injections could be advantageous in preclinical and clinical approaches for two reasons: more limited spread of the vector and the use of lower vector doses.

In rats with darker pigmentation, the tail vein may be more difficult to identify. So, alternate routes of administration such as retro-orbital injections or intravenous injections to the saphenous vein may be used. The retro-orbital injections target a dense capillary bed and are thus similar to an intravenous injection. For mice, and perhaps for rats too, the retro-orbital method may be easier than tail vein injections, but three studies have shown great consistency of the tail vein injections in adult rats.[6,8,9] Most of this work used a strong recombinant promoter to drive transgene expression, the cytomegalovirus chicken beta actin hybrid promoter.[10] If the experimenter wishes to delimit expression to the neurons in the CNS, a cell type-specific promoter can be attempted, for example the synapsin promoter.[8,11]

With respect to the imaging method, it is a quick, semi-quantitative method for estimating the transduction efficiency. If conducted correctly, then the assay yields a reliable estimate of the transduced area. The gold standard method for counting objects in CNS tissue is called stereology. It takes about 75 min per animal to conduct a stereological probe in a region of interest in the brain whereas the described method is faster at 20 min per animal or even less when scaled up. If all conditions are kept equal and the same region is properly sampled, the consistency of the data approaches the great consistency of stereology, and large groups of samples can be quickly analyzed en masse.

There are a number of additional technical notes that warrant further discussion. For Section 1 regarding vector doses, the vector doses required for efficient transgene expression in rats were determined by trial-and-error and dose-response in Wang *et al.*, Dayton *et al.*, and Jackson *et al.*, as well as previous doses used in mice (Foust *et al.* and Duque *et al*).[1,2,4,5,6] Certainly a critical, and potentially rate-limiting step is producing high-titer AAV preps to achieve the effective doses.

For Section 3 regarding loading the vector into the needle, air bubbles in the injection solution should be avoided as best as possible. A small amount of air should not be problematic as this can be filtered out by the lungs, but excessive volumes of air injected into the vein can cause a venous air embolism potentially resulting in death.[12] If bubbles are present in the syringe, the virus should be expelled from the syringe, and a

new syringe should be loaded. To avoid the creation of bubbles, make sure to position the needle close to the drop of vector on the parafilm with the bevel facing down. When the majority of the vector has been taken up into the needle, very slowly draw up the remaining solution. A small air pocket will form at the back of the syringe. This is dead space and will not be injected. To ensure that it stays toward the back of the syringe, keep the syringe horizontal or pointed downward and do not flick the syringe.

For Section 5 regarding the injections, to ensure that the injection goes into the vein and not the tissue surrounding the vein, once the needle has been inserted, pull back slightly on the plunger to create negative pressure at the needle tip. As mentioned, if the needle is in the vein, blood should flow back into the needle and will be visible at the base of the needle. While injecting into the vein, little resistance should be felt when the needle is appropriately placed. If significant resistance is encountered, then it is likely that the injection is going into the tissue of the tail as described above. It is also possible to puncture through the vein, causing a "blown vein", when the angle that the needle is inserted is too steep, when the gauge of the needle is too large for the vein, or when the injection speed is too fast putting too much pressure on the vein. A blown vein can occur during the initial puncture of the vein or during the injection. To avoid puncturing the vein during the injection or having the needle become dislodged from the vein, the syringe should be stabilized by holding the end of the syringe against the tail as mentioned. If the needle becomes dislodged or a second injection is needed for another reason, the second injection can be made either to the lateral tail vein on the opposite side or up closer to the body on the same side.

For Section 8, the rotarod assay is run at 4-week intervals during the experiment. Normal, untreated rats have better scores when they are younger (4-6 weeks old) than at older ages. The deficits in the escape reflex are plotted as survival of limb function over time. The survival curves are analyzed by a log-rank test on Prism software.

For Section 9 regarding the transduction analysis, with practice, the procedure becomes relatively fast, taking about 20 min per animal or less. This assay is also highly reliable because non-transduced tissues that are processed with the GFP staining procedure a basically negligible reading (**Figure 2B**), and because there is a vast dynamic range to the readout. Most of the GFP fluorescence derives from transduced Purkinje cells, and thus their great numbers provide a large dynamic range. So far, the gene transfer efficiency achieved has not caused the readings to saturate across the entire field (i.e., 100% transduction of Purkinje cells), at least when the gene transfer is administered to adult rats. If saturation of the entire field were a problem, then the signal could be lowered by applying a lower vector dose in separate animals or by measuring only the weaker, native GFP fluorescence, forgoing the staining procedure. Certainly the transduction of other cell types in the cerebellum could also be contributing to the signal, but most of what is readily visible and counted by the program is clearly the cell bodies in the Purkinje cell layers and their dendritic trees in the molecular layers throughout the cerebellum, based on their known position and morphology. A significant challenge to the method is that air bubbles or fluorescent debris could be counted, which will thus contaminate the readings. Sections and fields with this noise are not used; clean samples free of non-specific noise are required. Since GFP is a foreign protein, there is no background noise within the tissue, so the specific fluorescence can be precisely captured and estimated. In these studies, the GFP photomicrographs were captured in color and then converted to grayscale using an imaging program other than Scion Image. However, it would be more straightforward to capture the GFP in black and white in the first place for subsequent analysis in Scion Image. Previous studies analyzed the transduction of spinal cord lower motor neurons on a percentage basis, i.e., the percentage of motoneurons expressing GFP.[4,6] However, the analysis of cerebellum is more rapid, requiring fewer sections and also appears to be more consistent and reliable too (for example, across individual observers making the assessments). The rapid cerebellar imaging method is thus a good rapid index of the success of peripheral-to-central gene transfer. Analyses of the spinal cord transduction and examples of the transduction of other sites in the rat nervous system have been reported in Wang *et al.*, Dayton *et al.*, and Jackson *et al.*[4,5,6]

In summary, the intravenous route of administration is minimally invasive; the CNS can be targeted by a peripheral administration without placing a needle into the brain or spinal cord. Along with further refinement of targeting, relevant pre-clinical and clinical gene therapies will continue to use intravenous delivery, while many to myriad future types of basic functional studies in rats can benefit by the expansive transduction in quest to expedite hypothesis testing of gene function in vivo.

## Disclosures

The authors have nothing to disclose.

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## References

1. Foust, K., Nurre, E., Montgomery, C., Hernandez, A., Chan, C., & Kaspar, B. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat. Biotechnol.* **27** (1), 59-65 (2009).
2. Duque, S., *et al.* Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. *Mol. Ther.* **17** (7), 1187-96 (2009).
3. Gombash Lampe, S., Kaspar, B., & Foust, K. Intravenous injections in neonatal mice. *J. Vis. Exp.* (93), e52037 (2014).
4. Wang, D., *et al.* Expansive gene transfer in the rat CNS rapidly produces amyotrophic lateral sclerosis relevant sequelae when TDP-43 is overexpressed. *Mol. Ther.* **18** (12), 2064-74 (2010).
5. Dayton, R., *et al.* Selective forelimb impairment in rats expressing a pathological TDP-43 25 kDa C-terminal fragment to mimic amyotrophic lateral sclerosis. *Mol. Ther.* **21** (7), 1324-34 (2013).
6. Jackson, K., Dayton, R., & Klein, R. AAV9 supports wide-scale transduction of the CNS and TDP-43 disease modeling in adult rats. *Mol. Ther. Methods Clin. Dev.* **2**, 15036 (2015).

 

7. Jackson, K., *et al.* Preservation of forelimb function by UPF1 gene therapy in a rat model of TDP-43-induced paralysis. *Gene Therapy.* **22** (1), 20-28. (2015).
8. Jackson, K., Dayton, R., Deverman, B., & Klein, R. Better targeting, better efficiency for wide-scale neuronal transduction with the synapsin promoter and AAV-PHP.B. *Front. Mol. Neurosci.* **9**, 116 (2016).
9. Jackson, K., *et al.* Severe respiratory changes at end stage in a FUS-induced disease state in adult rats. *BMC Neuroscience*. **17** (1), 69 (2016).
10. Xu, L., *et al.* CMV-beta-actin promoter directs higher expression from an adeno-associated viral vector in the liver than the cytomegalovirus or elongation factor 1 alpha promoter and results in therapeutic levels of human factor X in mice. *Hum Gene Ther.* **12** (5), 563-73 (2001).
11. Jackson, K., Dayton, R., & Klein, R. Gene vector "magic bullet": targeted expression in the central nervous system after peripheral delivery using the synapsin promoter. *Expert Opin Ther Targets.* **20** (10), 1153-4 (2016).
12. van Hulst, R., Klein, J., & Lachmann, B. Gas embolism: pathophysiology and treatment. *Clin. Physiol. Funct. Imaging.* **23** (5), 237-246 (2003).


 