Supplementary Data

Materials and Methods

AAVrh.10hARSA-FLAG production

The vector used in this study, AAVrh.10hARSA-FLAG, is composed of an AAV2 gene transfer vector backbone (the inverted terminal repeats [ITRs] of AAV2 flanking the expression cassette) with a human cytomegalovirus (CMV) enhancer/promoter, the splice donor, and the left-hand intron sequence from chicken β-actin/right-hand intron sequence, splice acceptor from rabbit β-globin, the human ARSA cDNA (with an optimized Kozak translational initiation signal before the start codon), and a rabbit β-globin poly(A) sequence (Sondhi *et al.*, 2005, 2007; Piguet *et al.*, 2012). The AAVrh.10hARSA-FLAG vector was produced under Good Manufacturing Process (GMP) conditions by cotransfection of two plasmids, one encoding the therapeutic gene (500 μg of pAAV2-CAG-hARSA) and the other providing AAV replication and capsid functions plus the adenoviral helper functions (1 mg of pPAK-MArh.10) into a 293T cell line in the presence of PolyFect reagent (Qiagen Sciences, Germantown, MD).

AAVrh.10hARSA-FLAG vector construction

pAAV2-CAG-hARSA: The pAAV2-CAG-hARSA plasmid contains (5′ to 3′) the following: (1) the left-hand viral ITR from AAV2; (2) the human CMV enhancer; (3) the promoter, splice donor, and intron sequence from chicken β-actin; (4) the splice acceptor from rabbit β-globin; (5) the human ARSA cDNA (amino acid 507 isoform); (6) the rabbit β-globin poly(A); and (7) the right-hand ITR of AAV2 on a modified pAAV2-lacZ backbone conferring kanamycin resistance and harboring the elements for replication in *Escherichia coli* (Sondhi *et al.*, 2005). The orientation was checked by restriction mapping; the cDNA sequence was controlled by PCR; and ARSA enzymatic activity was confirmed after transfection into mammalian cells, using 4-nitrocatechol sulfate potassium salt dehydrate sulfate as the substrate. The resulting pAAV2-CAG-hARSA vector was characterized by restriction mapping and sequencing. The promoter in the AAVrh.10hARSA vector was chosen on the basis of published data on the therapeutic direct CNS gene transfer for the β-glucuronidase gene with AAV2 vectors in mucopolysaccharidosis (MPS) VII-deficient mice (Frisella *et al.*, 2001) and the efficacy and distribution data developed by our group while studying late infantile neuronal ceroid lipofuscinosis (LINCL, Batten disease) in rats, nonhuman primates, and the LINCL mouse model (Sondhi *et al.*, 2005, 2007, 2008).

pPAK-MArh.10: The helper plasmid pPAK-MArh.10, for the production of recombinant AAVrh.10 vectors, provides all of the necessary adenoviral and AAV helper functions. The *rep* gene is derived from AAV2 and therefore it is used in pseudotyping strategies where an AAV2-derived genome is packaged in the capsid of AAVrh.10. There is a fusion of the AAV2 and AAVrh.10 genomes such that the *cap* genes are expressed by the p40 promoter of AAV.

The production of the AAVrh.10hARSA-FLAG was carried out in the GMP manufacturing suite at the Belfer Gene Therapy Core Facility of the Department of Genetic Medicine at Weill Cornell Medical College (New York, NY). The AAVrh.10hARSA-FLAG vector was assembled by cotransfection of the two plasmids into a stable human embryonic kidney cell line (293T). The 293T cells were grown as anchorage-dependent cells. At 70% confluence, the two plasmids (500 μg of pAAV2-CAG-hARSA and 1 mg of pPAK-MArh.10) were added to the cells in the presence of PolyFect reagent (Qiagen Sciences) for 72 hr of incubation. The pooled cell harvest (1150 $\times$ *g*, 15 min) was subjected to multiple freeze–thaw cycles to release AAVrh.10hARSA-FLAG from the cells, producing a crude viral lysate (CVL). Digestion of any extra viral DNA was carried out with a recombinant human DNase (Benzonase, 50 U/ml; Sigma-Aldrich, St. Louis, MO) in the presence of $MgCl_2$ on thawed CVL and then centrifuged at 3300 $\times$ *g* for 20 min. The resulting AAVrh.10hARSA-FLAG vector was purified by differential density, using an iodixanol gradient. The pooled iodixanol fractions were diluted 3:1 in 1 *M* $(NH_4)_2SO_4$ in 50 m*M* NaH_2PO_4 (pH 7.0), and applied to a 5.0-ml HiTrap phenyl HP column (GE Healthcare, Piscataway, NJ). The column was washed twice in 1 *M* ammonium sulfate buffer and the vector was eluted in 50 m*M* Tris in 50 m*M* NaCl (pH 8.0). The final solution was sterile filtered through a 0.22-μm membrane filter, dispensed at prescribed volumes into cryovials, and frozen at -80°C until the day of surgery. Vector titer was determined by real-time PCR (TaqMan) with absolute quantitation. To confirm transgene function, supernatant from AAVrh.10hARSA-FLAG-infected 293-ORF6 cells were assayed for ARSA enzymatic activity (Sevin *et al.*, 2006).

Microcatheter assembly

The catheters used in the brain surgery experiments consisted of flexible fused silica capillary tubing (inner diameter [ID], 150 μm; outer diameter [OD], 363 μm; standard polyimide coating) from Polymicro Technologies (Phoenix, AZ) connected at one end to a 1-cm segment of polyethylene (PE) tubing (ID, 380 μm; OD, 1.09 mm; VWR Scientific, West Chester, PA), which acts as a coupler to join a 26-gauge needle from a Hamilton syringe by inserting the needle into the other end of the PE coupling. The entire unit was then gas sterilized. Immediately before the beginning of the brain surgery, a gas-tight Hamilton glass syringe (size, 250 μl) was attached to the 26-gauge needle end of the catheter and filled with vector (AAVrh.10hARSA-FLAG, $\sim$200 μl) under sterile conditions. The glass syringe was attached to a PHD Ultra syringe pump (Harvard Apparatus, Holliston, MA) for infusion, one catheter/syringe unit for each hemisphere.

Nonhuman primate study

Eleven adult *Chlorocebus sabaeus* (3- to 6-year-old male African green monkeys; weight, 4–7 kg) were purchased from the New Iberia Primate Center (New Iberia, LA). All monkeys were healthy and pathogen-free during the 3-month

quarantine period. No animal had previously been used for any experiments. Monkeys were maintained in individual cages after surgery to prevent injuries, fed twice daily with monkey chow (monkey diet jumbo; PMI Nutrition International, Brentwood, MO), and supplemented with fruit or vegetables daily, with access to water *ad libitum*. All experiments were approved by Institutional Animal Care and Use Committee of the Weill Cornell Medical College (New York, NY), in accordance with ethical and animal welfare issues.

AAVrh.10hARSA-FLAG at a total dose of 1.5×10^{12} genome copies (GC; $2.1–3.7\times10^{11}$ GC/kg) was administered into the CNS or cerebral vasculature of nonhuman primates via five surgical routes of delivery ($n=11$; Table 1). The dose used for the study is based on projected clinical therapeutic levels. Each group had $n=2$ male nonhuman primates, except for the intraarterial group, which had $n=1$. For the white matter administration group ($n=2$), the vector was administered to 3 sites in the white matter (centrum semiovale) in each hemisphere (see Supplementary Fig. S1) at 2 depths per burr hole, as 12 equal 15-μl deposits via a flexible fused silica catheter. For the sham surgery/vector control group ($n=2$), phosphate-buffered saline (PBS) was administered instead of AAVrh.10hARSA-FLAG via the white matter administration route. For the deep gray matter with overlying white matter administration group, the vector was administered to 3 sites in the putamen, thalamus, and caudate nucleus, plus the overlying cortex in each hemisphere, as 12 equal 15-μl deposits via a flexible fused silica catheter. For the deep gray matter convection-enhanced delivery (CED) group, vector was administered to three sites (putamen, thalamus, and caudate nucleus) in each hemisphere via a SmartFlow 16-gauge catheter (MRI Interventions, Irvine, CA) and ramped flow rate from 1 to 3 μl/min over 25 min for six equal 50-μl deposits (San Sebastian *et al.*, 2012). For the intraventricular group, a single 75 μl of vector was administered into the right frontal ventricle. For the intraarterial group ($n=1$), vector was administered as a single bolus via the right hemisphere middle cerebral artery, using fluoroscopy, after the administration of mannitol (1.5 mg/kg; APP Pharmaceuticals, Schaumburg, IL) to disrupt the blood–brain barrier. Details of all surgical and stereotactic vector administration details are given below. All nonhuman primates were assessed for biological (ARSA activity and anti-AAV antibody titers), clinical (behavior), hematological, and serum chemistry parameters at nine time points and brain immunohistochemistry was done at 13 weeks (91 days; Table 1).

Nonhuman primate magnetic resonance imaging and computed tomography scans

To determine the precise location of burr hole placement and depth of catheter tip necessary for targeting the intracranial regions with vector administration, each NHP was imaged by magnetic resonance imaging (MRI) and computer-assisted tomography scans to map their brains. Monkeys were anesthetized, intubated to place them on gas anesthesia (isoflurane) during the procedure, and provided with fluids through a catheter inserted into one of the saphenous veins. The NHP's head was then mounted in an MRI-compatible stereotaxic frame (David Kopf Instruments, Tujunga, CA) using ear bars, a pallet clamp, and ventral orbit clamps. The stereotaxic frame was the same device that was used during surgery. With the monkey mounted in the stereotaxic frame, the monkey and frame were slid together into a send/receive head MRI coil used for human imaging (MAGNETOM Trio, 3.0-T machine; Siemens Healthcare, Malvern, PA). Once a number of volumetric scans (T1 and T2 imaging) were completed the monkey was brought out of the MRI machine and transferred, attached to the head frame, over to a positron emission tomography machine for CT (Biograph mCT; Siemens Healthcare) to generate a more complete image of the NHP brain, helping to accurately guide the placement of catheters. The MRI and CT images were fused to give a three-dimensional map of the brain, from which the stereotaxic coordinates were determined with OsiriX MD software to align the delivery arms over the NHP skulls and provide the correct angle and depth needed to reach the designated targets in the white matter and deep gray matter regions of the brain. Scans were performed at the Citigroup Biomedical Imaging Center (CBIC, New York, NY); MRI images were generated by J. Borja and J. Dyke; CT images were generated by S. Morim.

Stereotaxic administrations of AAVrh.10hARSA-FLAG into nonhuman primates

The animals were fasted for >16 hr before the procedure. Presurgical (day 0) behavior was videotaped before sedation. Anesthesia consisted of ketamine plus xylazine pretreatment (10 and 0.5 mg/kg, respectively), followed by isoflurane (1%) inhalation. Vital signs were recorded and blood was drawn for laboratory studies as indicated below. Each monkey received atropine (0.5 mg) via an intravenous line kept open with 0.9% saline, and an endotracheal tube for isoflurane (1–3%). The animals were positioned in a Kopf stereotaxic frame with bilateral ear bars. The scalp was shaved with an electric razor and the area was prepped and draped in a sterile fashion. A 4-cm midline incision was made with a no. 15 blade scalpel and the scalp was retracted with small fishhooks. Sterilized manipulators were attached to the stereotaxic frame and burr hole sites were marked with a sterile marking pen at predetermined coordinates obtained from the MRI/CT scan. Anterior/posterior (A/P) measurements were done from the ear bar set at 0. Midline (M/L) measurements were done from the central coronal suture. Burr holes were made corresponding to the predetermined tract to administration sites, using a 3-mm drill bit and Dremel drill. To guide the microcatheters into the cortex, 22-gauge $\times$ 3.5-inch Quincke spinal needles (Becton Dickinson, Franklin Lakes, NJ) were clamped to each stereotaxic arm. The microcapillary catheter was then threaded down the spinal needles to the predetermined depth coordinates. The targets were calculated by setting the visualized pia dural measurement as 0 mm. All stereotaxic arms were set at 0 degrees, unless noted. All surgical routes used bilateral vector administration (except for the intraventricular and intraarterial routes, which consisted of one injection), and the coordinates listed for one hemisphere were repeated in both hemispheres. Infusions began 2 min after the catheters were lowered to the target site, and after the injection a timed delay of 2 min was allowed to elapse before the catheter was moved for the dorsal deposit. After completion

Supplementary Table S1. Quantitative Assessment of Nonhuman Primate Behavior After Vector Administration[a]

Summary score	*Behaviors*
Anxiety score[b]	Chewing, penile erection, scratching, yawning, threaten outside
Arousal score[b]	Bipedal lookout, shift, tail flag, vertical climb, vocalization
Quiet behaviors score[b]	Cage pick, self-groom, eating, drinking
Sedation score[b]	Eyes closed, freeze (motionless), face-down
Abnormal motions score[b]	Stereotypy, dyskinesia, dystonia
Healthy sum[c]	Sum of behaviors that relate to overall good health of the nonhuman primate, which include anxiety, arousal, and quiet behaviors
Abnormal sum[d]	Sum of behaviors that relate to overall poor health of the nonhuman primate, which include sedation and abnormal motions
Food response[e]	Speed of response, delay of movement, difficulty eating
Threat response[e]	Speed of response, head tremor, limb tremor, effort of ''intention''

[a]Behavioral assessments were done presurgery (twice) and on days 0, 7, 14, 28, 56, and 91 postsurgery (±1 or 2 days), before sedation for blood draws.

[b]Specific nonhuman primate behavior (normal and abnormal) described here were visually assessed by observers blinded to treatment and route from the videotape sessions of primates in individual home cages in the absence of stimuli. The primates were given a score of ''1'' for 5 sec of each specific normal and abnormal behavior. The totals for each behavior were determined for a 3-min assessment session, and assigned into the anxiety, arousal, quiet behavior, sedation, and abnormal motion categories as described.

[c]The sum of normal typical primate behaviors (anxiety, arousal, and quiet behaviors) was calculated as the ''Healthy'' score for each session.

[d]The sum of abnormal behaviors (sedation and abnormal motions) was calculated as the ''Abnormal'' score for each session.

[e]Additional videotaped assessments were made in the presence of stimuli using specific food/threat scenarios for 1 min each to gauge their response to positive (food treats) and negative (threatening stares) reinforcements. The observers rated the monkey for delayed responses to food and threats on a scale where 0 = normal and 5 = abnormal.

of the vector administrations at each burr hole site, the catheter needle was slowly removed completely. The total dose of vector was the same in all cases, with a maximal amount of 1.5×10^{12} GC/animal, which is about the same concentration to be used in humans (10^{13} GC/ml). The volume of vector infused varied per delivery route. Galea muscles were closed with 3-0 Vicryl followed by a 4-0 resorbable subcuticular layer. The primate was then removed from the stereotaxic frame and placed in the recovery position until awake. Postoperative analgesia with carprofen (4.0 mg/kg, subcutaneous) and buprenorphine (Buprenex, 0.6 ml, subcutaneously administered into the scalp around the incision) was given when breathing and reflexes were stable and the monkey had recovered from the anesthetic. Monkeys received standard postoperative veterinary care: NHPs were assessed three times a day during recovery from surgery and daily thereafter. All monkeys recovered well from surgery and exhibited normal behavior without any apparent adverse effects up to the time of sacrifice.

White matter administration route. Vector AAVrh.10hARSA-FLAG (1.5×10^{12} GC) was delivered in a total volume of 180 μl divided equally among 12 loci (15 μl), through 6 burr holes (3 per hemisphere) at 2 sites in the frontal lobe region and 1 site in the parietal lobe region, bilaterally. White matter injections were made ventrally (−10 mm) first and then the catheter was raised to the overlying white matter (−5 mm). The catheters were then withdrawn at a rate of 0.5 mm/min and the manipulator arms were reset for the next set of burr holes. Each vector administration for this route was made at a constant rate of 1 μl/min. Stereotaxic coordinates are listed based on ear bar (A/P = 0 mm) and central coronal suture (M/L = 0): first site A/P + 23 mm, M/L −10 mm, dorsal/ventral (D/V) at −10 and −5 mm; second site A/P + 8 mm, M/L −10 mm, D/V at −10 and −5 mm; third site A/P −6 mm, M/L −10 mm, D/V at −10 and −5 mm. The same coordinates were used for both hemispheres. As sham controls, two NHPs received the same surgery with PBS (15 μl per site) replacing the AAVrh.10hARSA-FLAG vector; all criteria, assessments, and timeline were the same as for the vector-treated animals.

Deep gray matter with overlying white matter administration route. Vector AAVrh.10hARSA-FLAG (1.5×10^{12} GC) was delivered in a total of 180 μl divided equally among 12 loci (15 μl), through 6 burr holes (3 per hemisphere) directly into 6 deep brain targets (1 per thalamus, caudate nucleus, and putamen), bilaterally, with 6 overlying secondary deposits in the white matter of the centrum semiovale. Deep brain injections were made first and then the catheter was raised to the overlying white matter (−9 or −10 mm). Infusions of 15 μl of AAVrh.10hARSA-FLAG were performed at a constant rate of 1 μl/min at each location. Location of administration sites: First site (putamen) at A/P + 19 mm, M/L −12 mm, D/V at −16 and −9 mm; second site (thalamus) at A/P + 10 mm, angled at 33 degrees, M/L −16 mm, D/V at −20.5 and −9.5 mm; third site (caudate) at A/P + 24 mm, M/L −6.0 mm, D/V at −15 and −9 mm. The same coordinates were used for both hemispheres.

Deep gray matter with convection-enhanced delivery administration route. Vector AAVrh.10hARSA-FLAG (1.5×10^{12} GC) was delivered in a total of 300 μl divided equally among 6 loci (50 μl), through 6 burr holes (3 per hemisphere) directly into 6 deep brain targets (1 per thalamus, caudate nucleus, and putamen), bilaterally, with no overlying secondary deposits. For these experiments, a

Supplementary Table S2. Nonhuman Primate Serum Chemistry and CBC Statistical Analysis: Time Parameter[a]

	All groups (day 0 [presurgery] vs. postsurgery)[b]					
Testing parameter	*Day 2*	*Day 7*	*Day 14*	*Day 28*	*Day 56*	*Day 91*
Alkaline phosphatase	0.27	0.03	0.60	>0.99[c]	0.15	0.013
Alanine aminotransferase	**0.0002**	**0.0007**	0.02	0.11	0.21	0.08
Aspartate aminotransferase	**0.005**	0.12	0.49	0.52	0.35	0.68
γ-Glutamyltransferase	0.33	0.80	**0.005**	0.02	0.28	0.15
Albumin	0.42	0.31	0.06	0.013	0.03	0.03
Total protein	0.16	0.27	0.04	0.06	0.30	0.12
Globulin	0.07	0.35	0.06	0.31	0.51	0.56
Total bilirubin	0.07	0.90	0.07	0.49	>0.99	0.18
Blood urea nitrogen (BUN)	0.78	0.39	0.96	0.82	0.67	0.63
Creatinine	0.43	0.38	0.84	0.91	0.56	0.33
Triglycerides	0.09	0.20	0.20	0.03	0.06	**0.002**
Glucose	0.44	0.18	0.54	0.97	0.54	**0.007**
Lactate dehydrogenase	**0.001**	0.08	0.40	0.64	0.12	0.77
Calcium	0.02	0.15	**0.002**	**0.006**	0.012	**0.002**
Phosphorus	**0.008**	0.012	0.06	0.011	0.10	**0.004**
Bicarbonate (CO_2)	0.17	0.66	0.33	0.22	0.76	**0.009**
Creatine kinase	**0.003**	0.22	0.11	0.34	0.96	0.89
Amylase	0.012	0.02	**0.008**	**0.006**	0.03	0.08
Lipase	0.39	0.85	0.28	0.20	0.28	0.28
Sodium	0.42	0.85	0.43	0.55	0.63	0.73
Chloride	0.19	0.95	0.31	0.54	0.92	>0.99
Potassium	0.17	0.15	0.17	0.13	0.33	0.20
Sodium/potassium (Na/K) ratio	0.20	0.03	0.03	0.05	0.20	0.16
Albumin/globulin (A/G) ratio	0.02	0.52	0.21	0.69	0.19	0.75
BUN/creatinine (B/C) ratio	0.16	0.15	0.59	0.99	0.44	0.49
Anion gap	0.59	0.99	0.54	0.39	0.99	0.78
White blood cell count	0.23	0.20	0.06	0.21	0.33	0.19
Red blood cell count	0.61	0.68	0.64	0.68	0.46	0.13
Hemoglobin	0.58	0.64	0.66	0.51	0.33	0.14
Hematocrit	0.68	0.56	0.57	0.55	0.42	0.12
Mean corpuscular volume	0.29	0.53	0.49	0.43	0.88	0.97
Mean corpuscular hemoglobin	0.40	0.55	0.55	0.88	0.31	0.80
Mean corpuscular hemoglobin concentration	0.16	0.79	0.52	0.39	0.24	0.89
Neutrophil %	0.03	0.31	0.33	0.90	0.53	0.67
Lymphocyte %	0.06	0.26	0.38	>0.99	0.56	0.70
Monocyte %	0.04	0.82	0.69	0.91	0.46	0.54
Eosinophil %	0.87	0.34	0.64	0.44	0.77	0.83
Basophil %	0.22	0.22	0.59	0.59	0.78	>0.99
Neutrophil count	0.07	0.44	0.33	0.93	0.65	0.76
Lymphocyte count	0.59	0.42	0.13	0.29	0.48	0.32
Monocyte count	0.35	0.58	0.28	0.44	0.26	0.12
Eosinophil count	0.94	0.37	0.63	0.51	0.74	0.91
Basophil count	0.31	0.22	0.87	0.78	0.86	0.75
Platelet count	0.49	0.03	**0.005**	0.14	0.81	0.40
Reticulocyte %	0.02	0.77	0.12	0.45	0.81	0.89
Reticulocyte count	0.24	0.70	0.12	0.54	0.95	0.30

[a]CBC and serum chemistry parameter within treated NHP groups. Shown are the *p* values for pairwise evaluation between each AAVrh.10hARSA-FLAG surgical cohort (1.5×10^{12}) and the PBS-mock treated controls for each time of assessment.

[b]Statistical comparisons were evaluated by two-tailed paired *t* test for all NHPs in the study at various time points to identify significant differences due to time (''aging''). Shown are the results of the comparison for day of surgery (''pre'') versus day postsurgery. Bold face *p* values are less than 0.01 and considered to be significant.

[c]*p* values >0.99 are the results from two data sets that are nearly identical.

specialized catheter (SmartFlow, 16 gauge; MRI Interventions) was used to allow increased fluid flow without retrograde reflux up the catheter. Infusions of 50 μl of AAVrh.10hARSA-FLAG were performed at rates ramped from 1 to 3 μl/min, at 0.5 μl/min every 5 min at each location, for a total administration of 1.5×10^{12} GC per brain. Location of administration sites: First site (putamen) at A/P + 20 mm, M/L − 12 mm, D/V at − 18 mm; second site (thalamus) at A/P + 13 mm, angled at 15 degrees, M/L − 12 mm, D/V at − 19.5 mm; third site (caudate) at A/P + 26 mm, M/L − 5.5 mm, D/V at − 14 mm. The same coordinates were used for both hemispheres.

SUPPLEMENTARY TABLE S3. NONHUMAN PRIMATE SERUM CHEMISTRY AND CBC STATISTICAL ANALYSIS: SURGICAL GROUP COMPARISON[a]

	PBS control vs. treated surgical groups[b]				
Testing parameter	*White matter*	*Deep gray matter with overlying white matter*	*Deep gray matter with convection-enhanced delivery*	*Intraventricular*	*Intraarterial*[c]
Alkaline phosphatase	0.09	**0.0003**	0.10	0.41	0.20
Alanine aminotransferase	>0.99[d]	0.19	0.99	0.99	0.95
Aspartate aminotransferase	0.99	**0.005**	0.96	0.59	0.99
γ-Glutamyltransferase	0.71	0.43	0.36	0.35	0.83
Albumin	0.14	0.05	0.07	0.99	0.93
Total protein	0.25	0.13	0.30	0.99	0.84
Globulin	0.82	0.60	0.56	0.99	0.81
Total bilirubin	0.56	0.88	0.61	0.57	0.87
Blood urea nitrogen (BUN)	0.17	0.63	0.13	0.34	0.58
Creatinine	0.96	0.39	0.40	0.99	0.89
Triglycerides[e]	0.99	0.39	0.91	0.32	0.22
Glucose	0.68	0.57	0.20	0.29	0.19
Lactate dehydrogenase[e]	0.90	0.66	0.37	0.67	0.98
Calcium	0.68	0.09	0.03	0.53	0.51
Phosphorus	0.20	0.48	0.11	0.81	0.66
Bicarbonate (CO2)	0.68	0.48	0.48	0.93	0.96
Creatine kinase	0.87	<**0.0001**	0.58	0.83	0.92
Amylase[e]	0.92	0.013	0.013	0.07	0.06
Lipase[e]	0.92	0.80	0.98	0.90	0.10
Sodium[e]	0.72	0.10	0.64	0.44	0.13
Chloride[e]	0.92	0.24	0.88	0.81	0.51
Potassium[e]	0.41	0.44	0.71	0.36	0.29
Sodium/potassium (Na/K) ratio[e]	0.16	0.30	0.57	0.26	0.48
Albumin/globulin (A/G) ratio	0.92	0.55	0.64	0.92	0.95
BUN/creatinine (B/C) ratio	0.88	0.46	0.88	0.62	0.99
Anion gap[e]	0.91	0.93	0.83	0.10	>0.99
White blood cell count	0.33	0.03	0.012	0.49	0.67
Red blood cell count	0.15	0.19	0.06	0.47	0.10
Hemoglobin	0.25	0.12	0.04	0.30	0.11
Hematocrit	0.24	0.20	0.07	0.49	0.04
Mean corpuscular volume	0.55	0.52	0.57	0.42	0.19
Mean corpuscular hemoglobin[e]	0.08	0.02	0.47	0.04	0.16
Mean corpuscular hemoglobin concentration	0.71	0.56	0.20	0.47	0.03
Neutrophil %	0.18	0.20	0.20	0.02	0.03
Lymphocyte %	0.25	0.41	0.14	0.013	0.07
Monocyte %	0.77	0.35	0.59	0.44	0.41
Eosinophil %	0.40	0.41	0.08	0.71	0.33
Basophil %	0.70	0.58	0.99	0.81	0.56
Neutrophil count	0.23	0.08	0.05	0.26	0.13
Lymphocyte count	0.90	0.98	0.49	0.06	0.52
Monocyte count	0.39	0.62	0.10	0.44	0.64
Eosinophil count	0.36	0.40	0.10	0.70	0.71
Basophil count	0.54	0.47	0.98	0.77	0.79
Platelet count	0.30	0.18	0.49	0.30	0.03
Reticulocyte %[e]	0.81	0.91	0.90	0.86	0.16
Reticulocyte count[e]	0.71	0.79	0.64	0.87	0.05

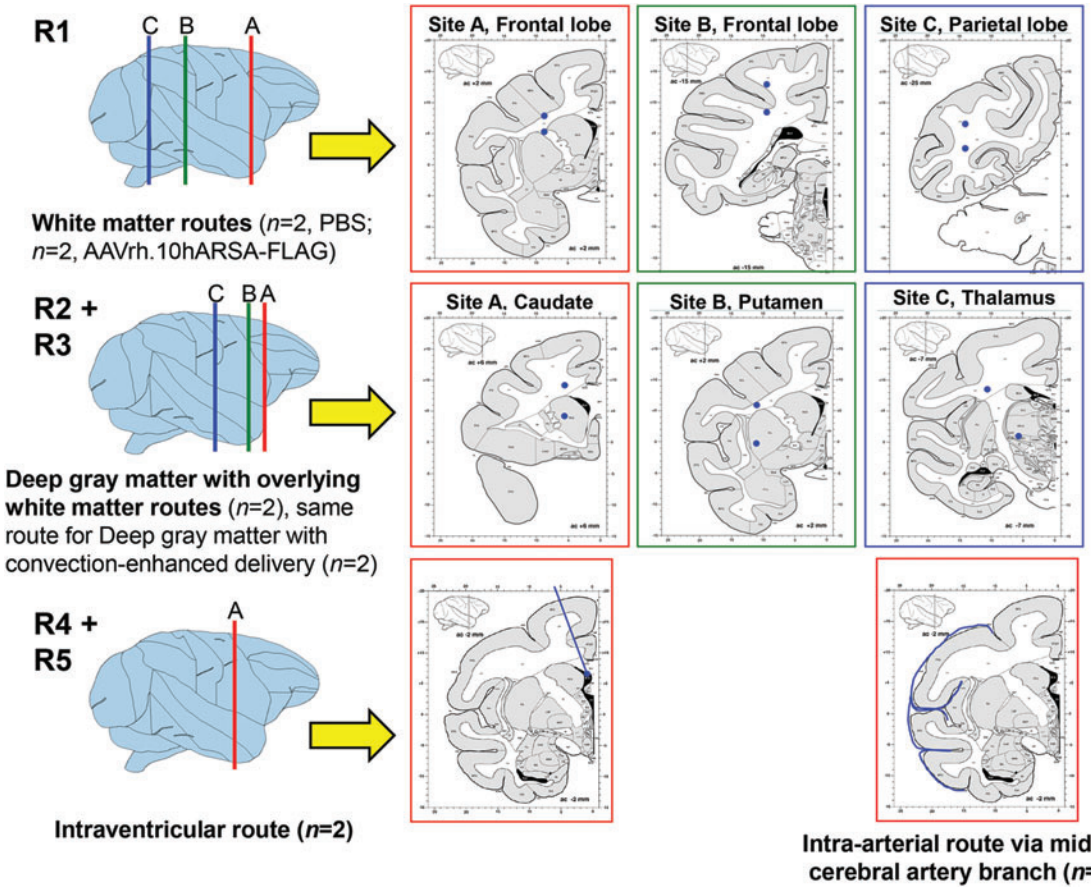
[a]CBC and serum chemistry parameter within treated NHP groups. Shown are the *p* values for pairwise evaluation between each AAVrh.10hARSA-FLAG surgical cohort (1.5×10^{12}) and the PBS-mock treated controls for each time of assessment.

[b]Statistical comparisons were evaluated by two-way repeated measures (RM) ANOVA, using each row as a different time point (as ''repeated measures'') with the results displayed as the ''interaction'' source of variation for *p* values. Post-test Ad hoc analysis performed with Tukey's multiple comparison test for RM-ANOVA with significance. Shown are the results of the comparison of PBS-mock controls and surgical AAV-treated groups at various time points to identify significant differences due to surgical method and/or AAV-treatment. Boldface *p* values are <0.01 and considered to be significant.

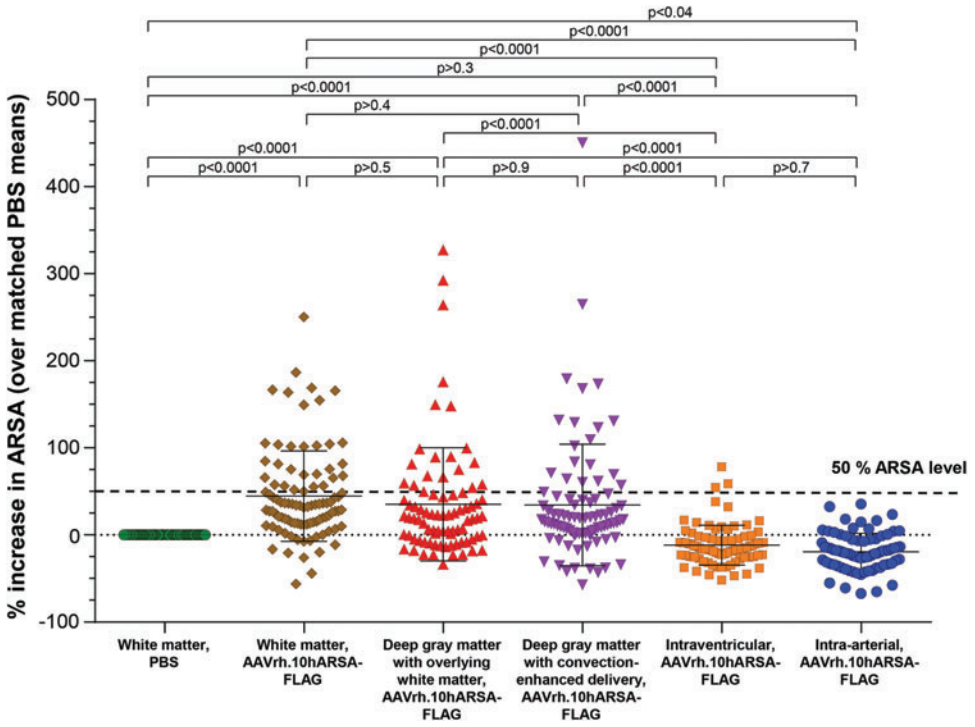
[c]Due to a clotted blood sample, it was not possible to obtain CBC for one NHP on the day of sacrifice (day 91), thus the CBC blood test parameters for PBS versus intraarterial are compared by unpaired two-tailed *t* test instead of RM-ANOVA.

[d]*p* Values >0.99 are the results from two data sets that are nearly identical.

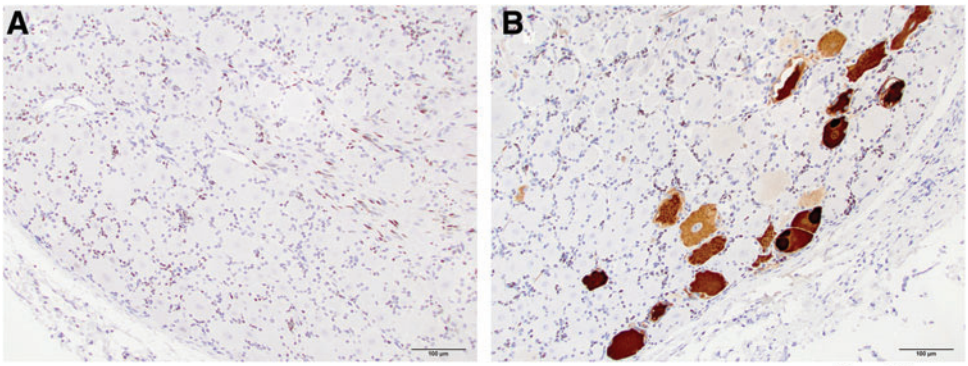
[e]Due to failed or incomplete analysis on some of blood samples, these test parameters are compared by ordinary two-way ANOVA instead of RM-ANOVA.



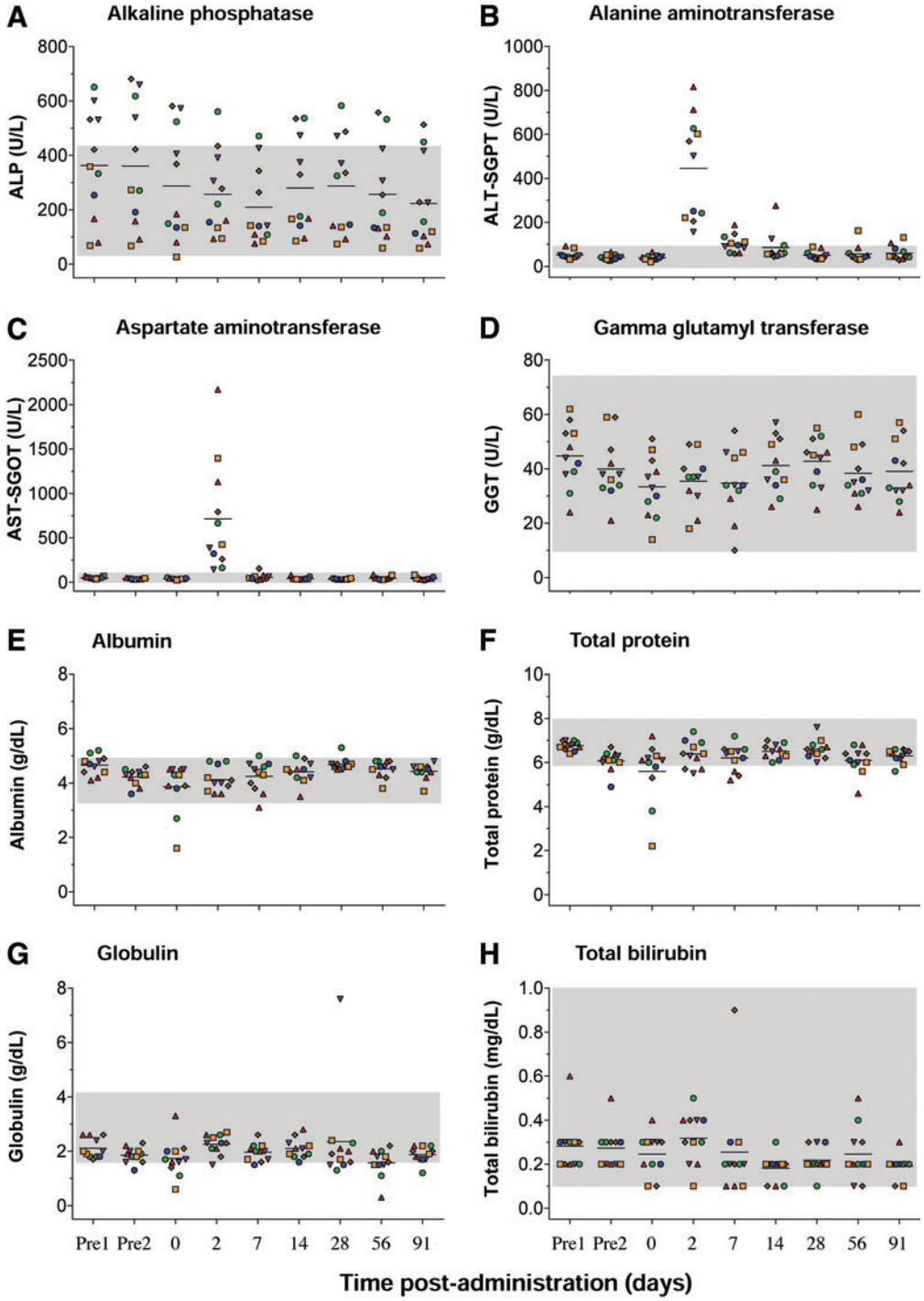
SUPPLEMENTARY FIG. S1. Sites of vector administration in nonhuman primate brains. Eleven African green monkeys were administered AAVrh.10hARSA-FLAG (1.5×10^{12} GC) or PBS to the brain via catheters, using five different surgical routes ($n=2$ per route, except for the intraarterial route, where $n=1$) to deliver vector to three sites bilaterally (six total) for white matter (R1), deep gray matter with overlying white matter (R2), and deep gray matter with convection-enhanced delivery modification (R3), or into a single location for intraventricular (R4) and intraarterial (R5). Stereotaxic locations of each site are displayed by the schematic drawings of the right hemispheres (*left drawings*) and coronal sections (*right boxes*). Blue dots indicate the approximate location of each vector deposit. In the white matter routes (R1), the vector was delivered equally to six sites and at two depths in the centrum semiovale white matter of the frontal (*A* and *B*) and parietal (*B*) lobes. In the deep gray matter with overlying white matter routes (R2), the vector was delivered equally to six sites and at two depths in the deep gray matter (caudate, putamen, and thalamus) and overlying centrum semiovale white matter of the frontal (*A–C*) lobe. In contrast, for the deep gray matter with convection-enhanced delivery routes (R3), the vector was delivered equally to six sites and at one depth in the deep gray matter (caudate, putamen, and thalamus) only in the frontal (*A–C*) lobe. For the remaining two routes, the vector was delivered in one bolus either to the lateral ventricle of the right cerebral hemisphere (intraventricular, R4) or to the proximal middle cerebral artery branch of the cerebral vasculature system (intraarterial, R5). The latter route was noninvasive and delivered via fluoroscopy-guided catheters after mannitol treatment of the middle cerebral artery.



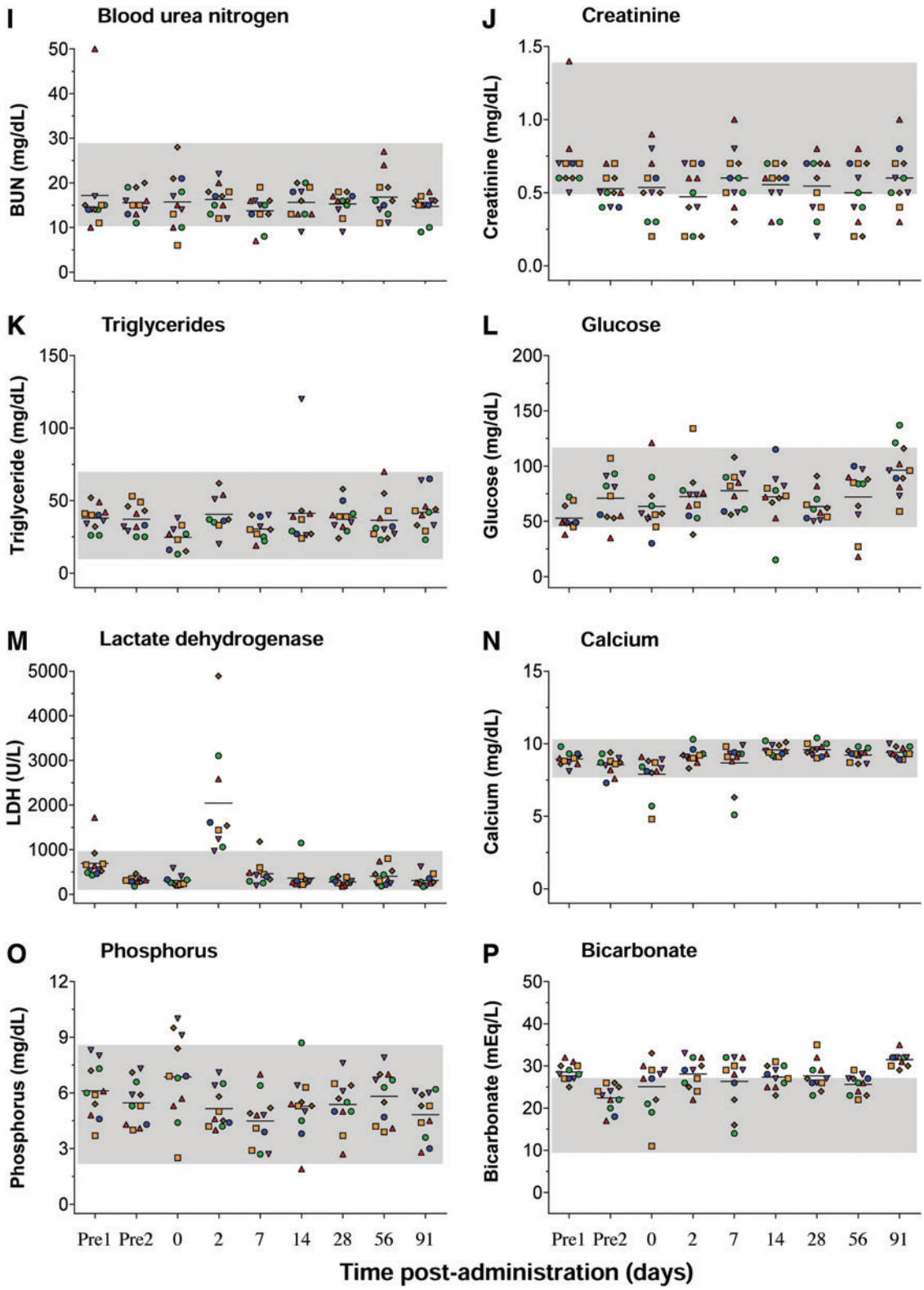
SUPPLEMENTARY FIG. S2. Comparison of the percent increase in ARSA levels in AAVrh.10hARSA-FLAG-treated nonhuman primate CNS. The raw ARSA value for each brain cube was corrected and the percent increase was determined with a corresponding matched brain cube set from PBS-treated nonhuman primate controls. All of the cube ARSA activity values have been grouped into one set of values per treatment and compared with each other as the percent increase in ARSA over matched PBS cube means. Bars indicate the mean ARSA values ± SD of each treatment group. The dashed line indicates a 50% increase in ARSA levels over endogenous background. Values of *p* were generated by one-way ANOVA, with Dunnett's multiple comparisons test, with ''adjusted'' *p* values reported.



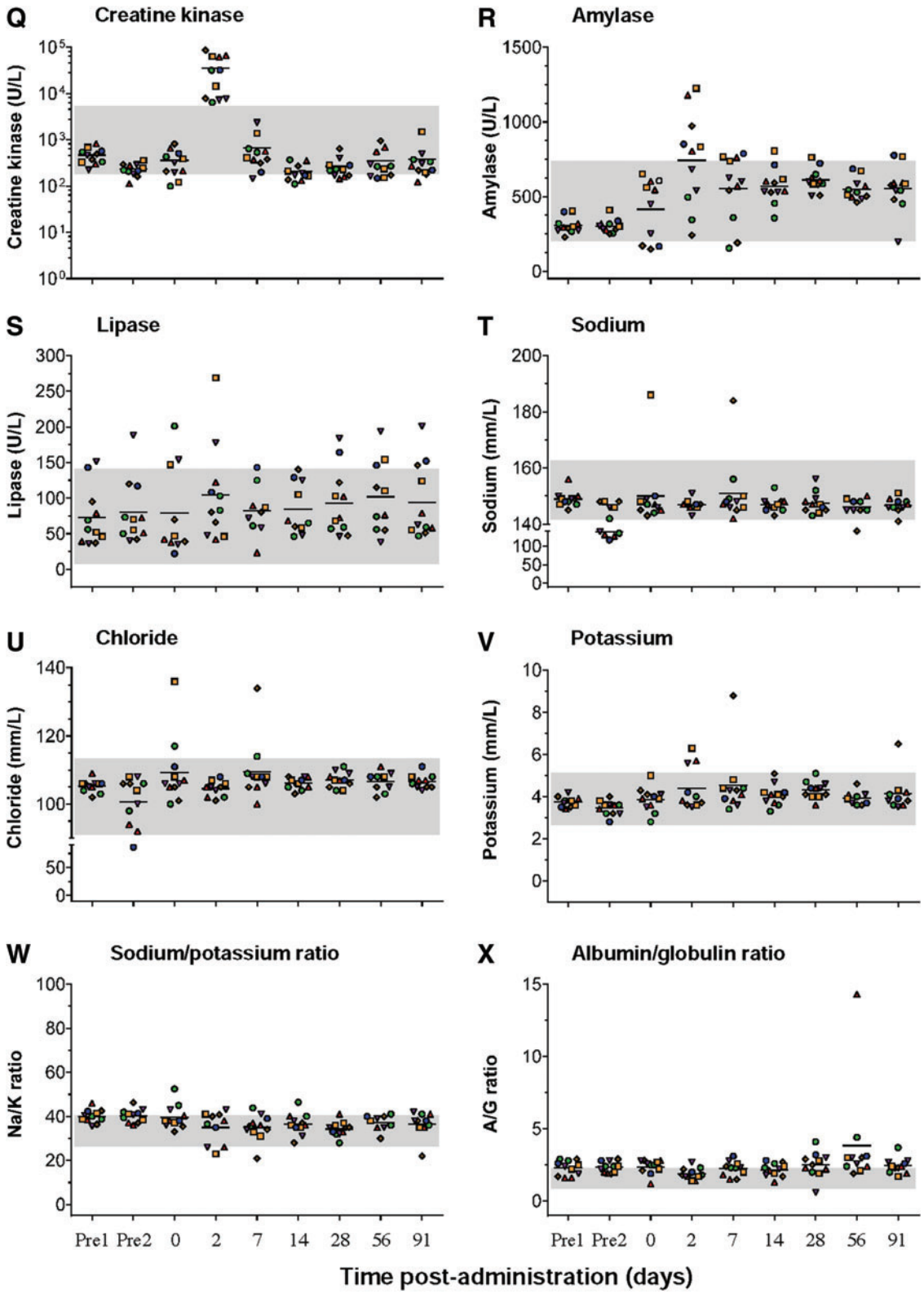
SUPPLEMENTARY FIG. S3. ARSA-FLAG detection in treated nonhuman primate dorsal root ganglion (DRG). To assess the tangential spread of the AAV vector and/or ARSA enzyme to the distal nervous system, spinal cord segments (C3, T4, L4) were isolated from each monkey during necropsy and were examined by anti-FLAG antibody immunohistochemistry for ARSA-FLAG transgene expression. **(A** and **B)** Representative images from the FLAG staining of treated nonhuman primate lumbar spinal dorsal root ganglion samples. **(A)** Enlarged section from the lumbar (L4) DRG of a nonhuman primate that received AAVrh.10hARSA-FLAG via white matter administration. **(B)** Enlarged section from the lumbar (L4) dorsal root ganglion of a nonhuman primate that received AAVrh.10hARSA-FLAG via intraventricular administration. Intense staining of neurons (cell bodies) was observed in the lumbar dorsal root ganglia with the following routes of delivery: deep gray matter with overlying white matter, AAVrh.10ARSA-FLAG; deep gray matter with convection-enhanced delivery, AAVrh.10ARSA-FLAG; and intraventricular, AAVrh.10hARSA-FLAG. Scale bar, 100 μm.



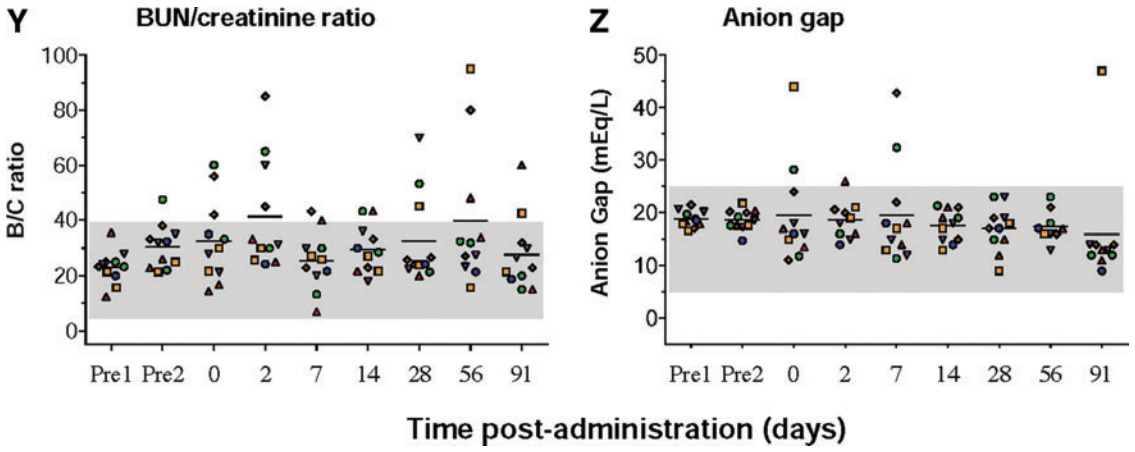
SUPPLEMENTARY FIG. S4. Assessment of AAVrh.10hARSA-FLAG administration and surgical treatment on nonhuman primate serum chemistries. The effect of CNS administration of AAVrh.10hARSA-FLAG on serum chemistries was analyzed at multiple time points, pre- and postsurgery, in treated nonhuman primates. Shown are data from individual nonhuman primates ($n = 11$ treated) from blood draws at 9 time points (presurgery [Pre1 and Pre2], day of surgery [day 0], and days 2, 7, 14, 28, 56, and 91 postsurgery). Health surveillance was monitored for all monkeys pre- and postsurgery by monitoring their blood to evaluate whether the brain surgeries had any long-term effect on the health of the monkeys. Each surgery cohort is represented by different color schemes, as follows: (1) white matter (brown diamonds); (2) deep gray matter with overlying white matter (red triangles); (3) deep gray matter using convection-enhanced delivery (CED; inverted purple triangles); (4) intraventricular (gold squares); (5) intraarterial (blue circles). Monkeys in a subset were designated controls and received PBS in place of AAVrh.10hARSA-FLAG via white matter administration (green circles). The gray shaded area indicates the adult African green monkey normal range of test parameters (Liddie *et al*., 2010). **(A–Z)** Serum chemistry tests. **(A)** Alkaline phosphatase (ALP); **(B)** alanine aminotransferase (ALT); **(C)** aspartate aminotransferase (AST); **(D)** γ-glutamyltransferase (GGT); **(E)** albumin; **(F)** total protein; **(G)** globulin; **(H)** total bilirubin; **(I)** blood urea nitrogen (BUN); **(J)** creatinine; **(K)** triglycerides; **(L)** glucose; **(M)** lactate dehydrogenase (LDH); **(N)** calcium; **(O)** phosphorus; **(P)** bicarbonate; **(Q)** creatine kinase (CK); **(R)** amylase; **(S)** lipase; **(T)** sodium; **(U)** chloride; **(V)** potassium; **(W)** sodium/potassium (Na/K) ratio; **(X)** albumin/globulin (A/G) ratio; **(Y)** BUN/creatinine (B/C) ratio; **(Z)** anion gap.



SUPPLEMENTARY FIG. S4. (Continued)



SUPPLEMENTARY FIG. S4. (Continued)



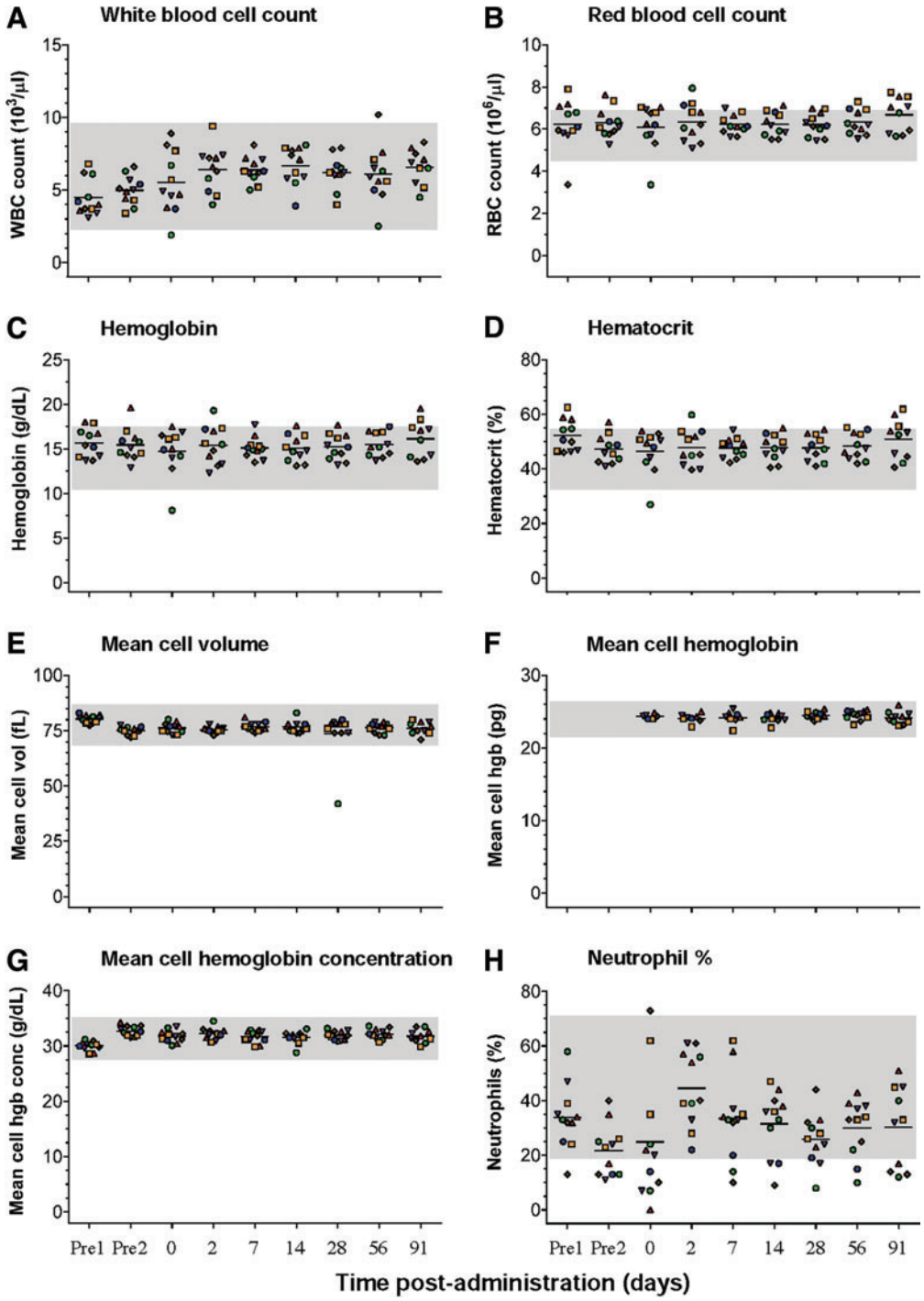
SUPPLEMENTARY FIG. S4. (Continued)

Intraventricular administration route. For the intraventricular administration route, vector was administered directly into the cerebral–ventricular system via one burr hole into right frontal ventricle, unilaterally. A 22-gauge (1-inch) intravenous catheter and needle was inserted into the right frontal burr hole to approximately 18 mm, and the stylet was removed from the catheter. Approximately 200 μl of cerebrospinal fluid (CSF) was removed and then the microcatheter was threaded through the intravenous catheter and placed for injection of viral vector. Seventy-five microliters of virus was injected into the right ventricle at a constant rate of 15 μl/min, for a total administration of 1.5×10^{12} GC per brain. Location of administration site: A/P + 21 mm, angled at 13 degrees, M/L −5.5 mm, D/V at −18.5 mm. This route used a single infusion of vector into the intracerebral ventricular system.

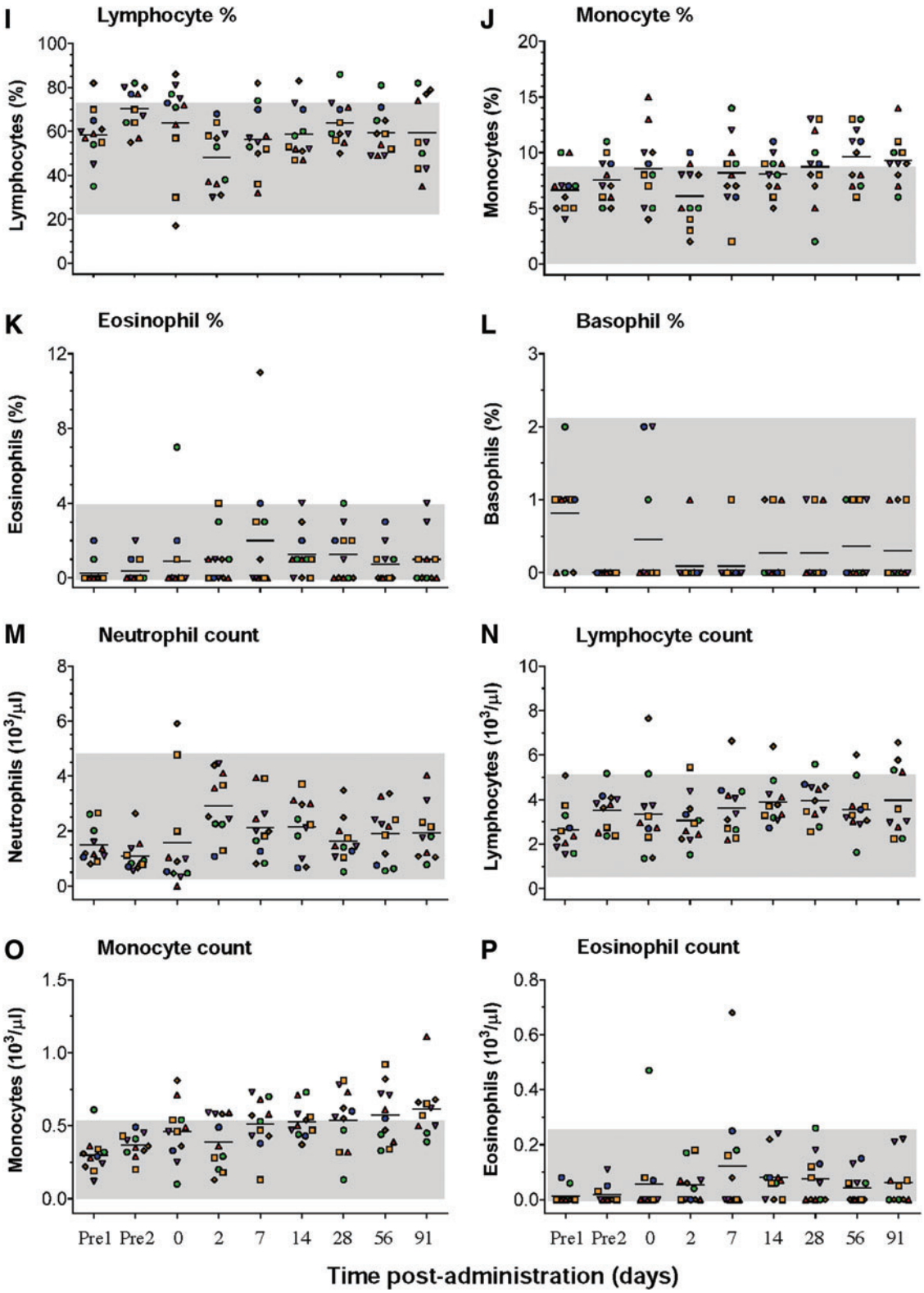
Intraarterial administration route. For the intraarterial administration route, vector was delivered to the major blood vessels of the brain via a neonatal French catheter (1.2F) during an angiogram procedure. Instead of brain surgery, the catheter was threaded up the femoral artery until it reached the carotid arteries. A French Foley catheter was inserted into the right common femoral artery of the NHP, using a 19-gauge single-wall needle, which was exchanged for a 4F sheath for the procedure. The sheath was connected to a continuous heparin saline flush at a low flow rate to keep the sheath patent (3 ml/hr). A heparin bolus to obtain an automated activated coagulation time of approximately three times baseline (~50 U/kg) was administered. Each monkey received atropine (0.05 mg/kg, subcutaneous) via an intravenous line (kept open with isotonic fluid [lactated Ringer's solution] at a rate of approximately 5 ml/kg/hr) and an endotracheal tube. Under fluoroscopic guidance, a Magic® 1.2F intravascular infusion microcatheter (BALT extrusion, Montmorency, France) was advanced over a 0.008-inch guidewire (Mirage, Dublin, Ireland), and the diagnostic angiography catheter was then passed through the introducer sheath up the femoral artery, the iliac artery, and into the descending aorta with the use of the guidewire. The catheter and wire were then advanced together from the descending aorta to the thoracic aorta and into the aortic arch, and then used to cannulate the innominate artery, right common carotid artery, right subclavian artery, and right vertebral artery. The guidewire was then advanced into the right common carotid artery and the diagnostic catheter was advanced over it to obtain angiographic images of the carotid bifurcation. All images were obtained via injection of iohexol (Omnipaque; GE Healthcare) contrast agent at a flow rate determined by visualization of contrast agent in the target vessels. After cannulation of the major brain arterial supply (right middle cerebral artery branch), we delivered an injection of 25% filtered mannitol (12 ml; APP Pharmaceuticals) via the endovascular catheters. The total dose of mannitol (1.5 mg/kg) was delivered to induce blood–brain barrier disruption of the cerebral artery. One minute after the injection of mannitol, a dose of 1.5×10^{12} GC of AAV (180 μl) was administered to the intracranial vessel over a 2-min period. On completion of the infusion the microcatheter was slowly removed and an angiogram was performed from the guiding catheter to verify cerebral circulation before the catheter and introducer sheath were removed. The guiding catheter was removed and homeostasis was achieved at the femoral artery by manual compression for 10–20 min. This route used a single infusion of vector to the right side of the nonhuman primate cerebral vascular system.

Necropsy and sample collection

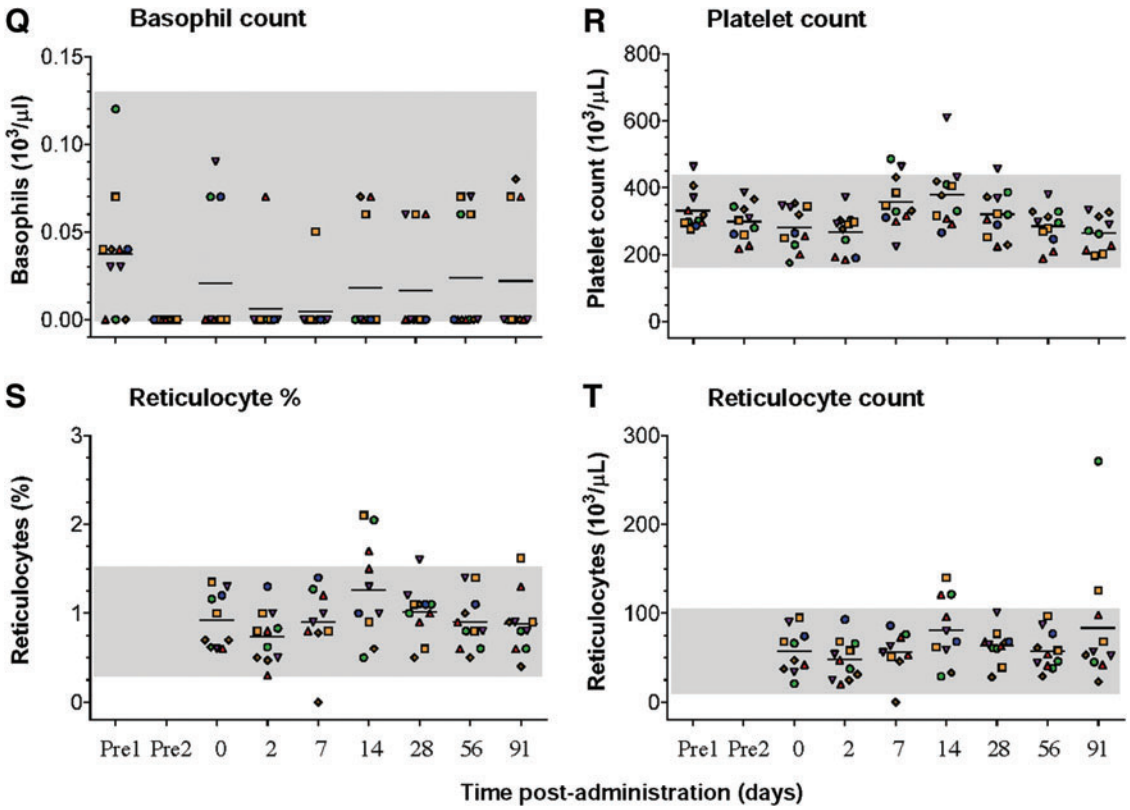
At sacrifice (day 91 ± 2 days), euthanasia was carried out with intravenous pentobarbital until loss of corneal reflexes was achieved. No perfusion was performed. Sections of organs were collected at necropsy and fixed with 10% neutral buffered formalin for immunohistochemistry; additional sections were flash-frozen in liquid nitrogen and stored frozen (unfixed) for ARSA enzyme assays. The brain was bisected into two hemispheres. The right hemisphere was processed by sectioning into 1-cm coronal slabs (seven to nine slices) and then subdividing the slabs into 1-cm^3 cubes for the ARSA enzymatic assay. The left hemisphere was used for assessment of the distribution of ARSA protein by immunohistochemistry for the FLAG peptide tag.



SUPPLEMENTARY FIG. S5. Assessment of AAVrh.10hARSA-FLAG administration and surgical treatment on nonhuman primate blood cell counts. The effect of CNS administration of AAVrh.10hARSA-FLAG on the complete blood count (CBC) was analyzed at multiple time points, pre- and postsurgery, in treated nonhuman primates. Shown are data from individual nonhuman primates ($n = 11$ treated) from blood draws at 9 time points (presurgery [Pre1 and Pre2], day of surgery [day 0], and days 2, 7, 14, 28, 56, and 91 postsurgery). Health surveillance was monitored for all monkeys pre- and postsurgery by monitoring their blood to evaluate whether the brain surgeries had any long-term effect on the health of the monkeys. Each surgery cohort is represented by a unique color scheme, as follows: (1) white matter (brown diamonds); (2) deep gray matter with overlying white matter (red triangles); (3) deep gray matter using convection-enhanced delivery (CED; inverted purple triangles); (4) intraventricular (gold squares); and (5) intraarterial (blue circles). Monkeys in a subset were designated controls and received PBS in place of AAVrh.10hARSA-FLAG via white matter administration (green circles). The gray shaded area indicates the adult African green monkey normal range of test parameters (Liddie *et al*., 2010). **(A–T)** Complete blood tests. **(A)** White blood cell count (WBC); **(B)** red blood cell count (RBC); **(C)** hemoglobin; **(D)** hematocrit; **(E)** mean corpuscular volume (MCV); **(F)** mean corpuscular hemoglobin (MCH); **(G)** mean corpuscular hemoglobin concentration (MCHC); **(H)** segmented neutrophils (% of total cells); **(I)** lymphocytes (%); **(J)** monocytes (%); **(K)** eosinophils (%); **(L)** basophils (%); **(M)** segmented neutrophil count (absolute); **(N)** lymphocyte count (absolute); **(O)** monocyte count (absolute); **(P)** eosinophil count (absolute); **(Q)** basophil count (absolute); **(R)** platelet count; **(S)** reticulocytes (%); **(T)** reticulocyte count (absolute).



SUPPLEMENTARY FIG. S5. (Continued)



SUPPLEMENTARY FIG. S5. (Continued)

ARSA enzymatic activity assays

To determine the spread of the transgene product in the brain, brain cubes were assessed by an *in vitro* functional ARSA assay that catalyzes the hydrolysis of a synthetic chromogenic substrate, *p*-nitrocatechol sulfate (pNCS). Brain cubes from the right hemispheres were thawed and homogenized in 4 ml of lysis buffer (100 m*M* Tris–150 m*M* NaCl–0.5% Triton X-100, pH 7.0). Aliquots of clarified supernatants were assayed in parallel for protein content and for ARSA activity. Brain lysates were tested in triplicate, using 40 μl of brain lysate in 80 μl pNCS solution (9 mM pNCS –0.84 mM sodium pyrophosphate –1.7 M sodium chloride –0.5 M sodium acetate, pH 5.0). The conversion of pNCS to 4-nitrocatechol (4-NC) was measured at 1 and 3 hr, optical density at 515 nm, with the difference in optical density recorded. The activity was calculated using a 4-NC standard curve (0–100 nmol). Total protein concentration assayed by bicinchoninic acid was used to normalize the enzyme activity (Micro BCA protein assay kit; Thermo Scientific Pierce, Rockford, IL). Activity of ARSA is reported as nanomoles per hour per milligram of protein. The baseline endogenous ARSA activity was specified by the average of the matching brain cubes of the two PBS-administered monkeys. Significant increases in ARSA activity for each location across the entire CNS in the AAVrh.10hARSA-FLAG-treated NHPs was determined by the difference in average ARSA activity levels in the corresponding matching brain cubes for each method of treatment ($n = 2$) and the corresponding PBS-treated nonhuman primate cube mean and expressed as the percent increase over the corresponding PBS cube mean. Where a corresponding cube was lacking in the PBS control set, the global PBS mean was used.

Spinal and peripheral nervous tissue. To determine the spread of vector from the injection site in the brain to the peripheral organs and spinal cord, we compared the transgene expression of ARSA at three sites in the spinal cord (cervical, thoracic, and lumbar) plus adjacent dorsal root ganglia (DRG), sciatic nerve (proximal and distal), and common fibular nerve (proximal and distal) for all vector administration methods. Spinal cord and nerve samples were harvested by a pathologist and flash frozen. Before enzymatic analysis the samples were thawed and homogenized in 400 μl of lysis buffer (Tris–NaCl–Triton). Clarified supernatants were assayed for protein content and for ARSA activity (20 μl of lysates, in triplicate). Similar to brain lysates, the conversion of pNCS to 4-NC was measured at 1 and 3 hr, optical density at 515 nm, with the difference in optical density recorded. ARSA activity is reported as nanomoles per hour per milligram (nmol/hr·mg) of protein.

Immunohistochemistry of nonhuman primate CNS

Nonhuman primates were killed 13 weeks postsurgery (day 91 ± 2 days), and the brain and spinal cord segments (cervical, C3; thoracic, T4; lumbar, L4) with adjacent DRG were excised to examine the extent of vector and transgene

spread across the CNS and PNS by immunohistochemical detection of ARSA enzyme, using the transgene 3′-terminal FLAG peptide tag. The formalin-fixed brains (left hemispheres) were divided into 3-mm coronal slices, processed in alcohol and xylene, embedded in paraffin blocks, and sectioned at 4 μm with a sliding microtome into serial free-floating sections. The areas surrounding and adjacent to the site of vector administration were examined by immunohistochemistry, using an anti-FLAG antibody and counterstain. Tissue sections were blocked for endogenous activity with 3% H_2O_2 (Thermo Fisher Scientific, Hampton, NH) and prepared for staining, using heat-induced epitope retrieval (10 m*M* citrate, 15 min in microwave; Dako, Carpinteria, CA). Nonspecific binding was blocked with mouse IgG blocking reagent (Vector Laboratories, Burlingame, CA) for 1 hr, 23°C. Primary antibody (anti-FLAG IgG1 mouse monoclonal, clone M2; Sigma-Aldrich) was diluted at 1:1500 in 4.5 ml of Mouse-on-Mouse (M.O.M.) diluent (Vector Laboratories) and incubated on tissues overnight at 4°C. Negative control antibody used on matching brain slices was a mouse IgG1, diluted at 1:1500 (Dako). Sections were washed and incubated for 30 min, 23°C with the secondary antibody, biotinylated anti-mouse IgG, diluted 1:250 in M.O.M. diluent (Vector Laboratories), followed by avidin–biotin–peroxidase detection for 30 min, 23°C (VECTASTAIN Elite ABC kit; Vector Laboratories). The ARSA-FLAG/tissue-bound peroxidase was visualized by a 3,3′-diaminobenzidine tetrahydrochloride (DAB) reaction (DAB in 0.3% H_2O_2–PBS, 4 min; Sigma-Aldrich). Nuclei were counterstained (blue) with Harris modified hematoxylin (2 min, 23°C; Thermo Fisher Scientific). Additional staining was performed on all spinal cord–DRG sections and nerve samples to assess vector spread to the PNS. Replacement of the primary antibody by another mouse immunoglobulin, if the same isotype, was used as negative reagent control for each set of tissue staining. Immunohistochemical slides were scanned in digitally, using a ScanScope AT Turbo scanner (Aperio/Leica Microsystems, Vista, CA) at $\times 20$ magnification (0.50 μm/pixel), and analyzed with ImageScope software (Aperio/Leica Microsystems).

All slides were evaluated by a board-certified pathologist and observations were recorded. In the brain and spinal cord, discrete areas of FLAG expression (positive staining) were identified and the following observations were recorded: size, anatomic location, intensity of staining in the white and gray matters, intensity of staining in cellular components of the gray and white matter (neurons, glial cells, gray matter neuropil, white matter axons), and cellular compartment of staining (cytoplasmic or nuclear). In the dorsal root ganglia, intensity and cellular component of staining were recorded. For brain areas denoted by the pathologist as containing white matter lesions, the extent of the lesion size was recorded (in mm^2) with Ariol software (Applied Spectral Imaging, Vista, CA), and the white spaces representing spongiosis in the lesions were then calculated with ImageJ software (Rasband, 1997–2014), by converting the white spaces to black, measuring the bare outlines, and analyzing the circular shapes between 2 and 50 pixels in size. The percentage of lesions containing spaces was calculated by multiplying the lesion size by the percentage of area with degeneration. The total lesion volume versus brain mass was subsequently measured by sum of all lesion volumes per individual nonhuman primate brain mass, as determined at necropsy.

Pre- and postsurgical safety monitoring of nonhuman primates

Health assessments. The nonhuman primates were observed and monitored daily by the experienced husbandry technicians and research specialists for general appearance, signs of toxicity, distress, and changes in behavior. The animals were housed singly after surgery and paired for the rest of the time, with behavioral assessments recorded with monkeys in separate cages to minimize interference. At 1–4 weeks before surgery (twice), on the day of administration, and on days 2, 7, 14, 28, 56, and 91 (± 1 or 2 days) postsurgery, animals were sedated for general health assessment and for blood draw for complete blood count (CBC), serum chemistries, and anti-AAV neutralizing antibody titers. Serum chemistry tests included the following: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), albumin, total protein, globulin, total bilirubin, blood urea nitrogen (BUN), creatine kinase (CK), triglycerides, glucose, lactate dehydrogenase (LDH), calcium, phosphorus, bicarbonate, amylase, lipase, sodium, chloride, potassium, sodium/potassium (Na/K) ratio, albumin/globulin (A/G) ratio, BUN/creatinine (B/C) ratio, and anion gap. Complete blood tests included the following: white blood cell count (WBC), red blood cell count (RBC), reticulocytes (percentage and absolute), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), segmented neutrophils (percentage and absolute), lymphocytes (percentage and absolute), monocytes (percentage and absolute), eosinophils (percentage and absolute), basophils (percentage and absolute), and platelet count. The analyses were performed by the Laboratory of Comparative Pathology (Memorial Sloan-Kettering Cancer Center, New York, NY) in a blinded fashion. The values from PBS control monkeys ($n = 2$) were used as normal controls, and the upper and lower limits of normal African green monkey values were used to compare pre- and postsurgery test results (Liddie *et al.*, 2010).

Anti-AAV neutralizing titer analysis. AAVrh.10 neutralizing antibody titers were assessed at each blood draw. Neutralizing antibody titer was determined by an *in vitro* assay with 293-ORF6 cells in 96-well plates. AAVrh.10Luc (10^9 genome copies) was incubated with serial dilutions of serum from AAVrh.10hARSA-FLAG-administered or PBS-administered control nonhuman primates at 37°C for 45 min and then used to infect cells at a multiplicity of infection of 10,000. At 48 hr postinfection, luciferase activity was assessed with the luciferase assay system (Promega, Madison, WI). The neutralizing antibody titer was expressed as the reciprocal of that serum dilution at which 50% inhibition of AAVrh.10Luc was observed (De *et al.*, 2006).

Behavioral assessments of nonhuman primates.

At eight time points (-28, -7, 0, 7, 14, 28, 56, and 91 days postsurgery, ± 1 or 2 days) before sedation for blood draws, all nonhuman primates were visually assessed for behavioral changes by videotaping the monkeys separated in individual home cages in the absence of stimuli and then with specific food/threat scenarios. Each NHP was videotaped for 3 min in the absence of outside stimuli to observe nonstimulated ''normal'' behavioral activity, and then for another 2 min to gauge their response to positive (food treats) and

negative (threatening stares) reinforcements. Behaviors were scored by observers ($n=2$) blinded to treatment and route from the video tape sessions for number of times the NHPs performed specific normal ($n=14$) and abnormal ($n=6$) behaviors, scoring ''1'' for 5 sec of each behavior. The sum of normal behaviors (anxiety, arousal, and quiet behaviors) was calculated as the ''Healthy'' score (defined in Supplementary Table S1) and the sum of abnormal behaviors (sedation and abnormal motions) was calculated as the ''Abnormal'' score for each session. Additional assessments were made to rate the monkeys' response to food and threats for delayed responses over a 1-min period (0, normal; 5, abnormal). The normal behaviors were assessed on the basis of a previous data set (Hackett *et al.*, 2005).

Statistical analysis

All statistical analyses were performed with GraphPad Prism version 6.02 (GraphPad Software, San Diego, CA). Statistical analyses comparing the various surgical cohorts with PBS-treated controls for ARSA levels in the CNS were generated by one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test; and for primate behavior by two-way repeated measures of ANOVA (RM-ANOVA) with Dunnett's multiple comparisons test. $p<0.05$ was considered significant. Evaluations of blood counts and serum chemistries were performed by a two-tailed paired t test at various time points to identify significant differences due to time (''aging''), and by two-way RM-ANOVA, using each row as a different time point (as ''repeated measures'') with the results displayed as the ''interaction'' source of variation p values. Post-test ad hoc analysis was performed with Tukey's multiple comparison test for RM-ANOVA results displaying significance. Because of daily variations in cell numbers and serum chemistries, comparisons that were at least $p<0.01$ were considered significant. Because of the small scale of the study, only the white matter route of vector administration included sham controls for vector- or transgene-induced changes.

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