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(54) **Title**: ADENO-ASSOCIATED VIRUS VECTORS AND METHODS OF USE THEREOF

(57) **Abstract**: The present invention provides AAV vectors and methods of use thereof for delivery of transgenes or therapeutic nucleic acids to subjects.

ADENO-ASSOCIATED VIRUS VECTORS AND METHODS OF USE THEREOF

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 61/780,423, filed March 13, 2013. The foregoing application is incorporated by reference herein.

This invention was made with government support under R01NS038690 awarded by the National Institute of Neurological Disorders and Stroke (NINDS) and R01DK063973 awarded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The government has certain rights in the invention.

FIELD OF THE INVENTION

This application relates to the fields of gene therapy and molecular biology. More specifically, this invention provides adeno-associated viral vectors with improved gene transfer to the brain.

BACKGROUND OF THE INVENTION

Several publications and patent documents are cited throughout the specification in order to describe the state of the art to which this invention pertains. Each of these citations is incorporated herein by reference as though set forth in full.

Adeno-associated virus is a helper-dependent virus (Dependovirus) of the family *parvoviridae* and requires a helper virus for replication. After infection, the AAV typically enters a latent phase where the AAV genome is site specifically integrated into host chromosomes. The AAV genome is only rescued, replicated, and packaged into infectious viruses again upon an infection with a helper virus. Accordingly, natural infections take place in the context of infection with a helper virus, such as adenovirus or herpes simplex virus.

Not only are AAV vectors nonpathogenic and result in long-term expression of the encoded heterologous gene, but they are also capable of transducing nondividing cells, which is necessary for treatment of the central nervous system (CNS). Adeno-associated virus (AAV) vectors are scalable, efficient, non-cytopathic gene delivery vehicles used primarily for the treatment of genetic diseases. Indeed, a broad spectrum of animal models of human diseases has been successfully treated by AAV vectors,

including diseases of the brain, heart, lung, eye and liver (Mingozzi et al. (2011) Nat. Rev. Genet., 12:341-355). Further, numerous clinical trials with AAV vectors are currently ongoing with positive results in the treatment of a variety of diseases including, for example, Leber's Congenital Amaurosis, hemophilia, congestive heart failure, lipoprotein lipase deficiency, and Parkinson's disease (Maguire et al. (2008) New Eng. J. Med., 358:2240-2248; Bainbridge et al. (2008) New Eng. J. Med., 358:2231-2239; Hauswirth et al. (2008) Human Gene Ther., 19:979-990; Nathwani et al. (2011) New Eng. J. Med., 365:2357-2365; Jessup et al. (2011) Circulation 124:304-313; LeWitt et al. (2011) Lancet Neurol., 10:309-319). Despite the promise of AAV based gene therapy approaches for the treatment of a variety of disorders, improved AAV vectors with specific delivery to target tissues are desired.

SUMMARY OF THE INVENTION

In accordance with the present invention, compositions and methods for improved delivery of a nucleic acid molecule to the brain, particularly the neurons therein, are provided. In a particular embodiment, the method comprises administering to a subject an AAV vector comprising the nucleic acid molecule of interest, wherein the AAV vector comprises hu.32 or rh.8 capsid proteins or variants thereof. In a particular embodiment, the capsid protein comprises at least 90%, 95%, or more homology/identity with SEQ ID NO: 1 or 3 or is encoded by a nucleic acid molecule having at least 90%, 95%, or more homology/identity with SEQ ID NO: 2 or 4. The AAV may be delivered to the subject intravascularly, e.g., as part of a composition comprising at least one pharmaceutically acceptable carrier.

In accordance with another aspect of the present invention, therapeutic methods for treatment, inhibition, and/or prevention of a disease or disorder, particularly a genetic disease associated with the brain, are provided. In a particular embodiment, the disease or disorder effects more than the brain (e.g., the disease or disorder is a multi-organ disease or disorder (e.g., LSD)). In a particular embodiment, the method comprises administering to a subject an AAV vector comprising a nucleic acid molecule encoding a therapeutic protein or inhibitory nucleic acid molecule, wherein the AAV vector comprises hu.32 or rh.8 capsid proteins or variants thereof. In a particular embodiment, the capsid protein comprises at least 90%, 95%, or more homology/identity with SEQ ID NO: 1 or 3 or is encoded by a nucleic acid molecule having at least 90%, 95% or more homology/identity with SEQ ID NO: 2 or 4. The AAV may be delivered to the subject

intravascularly, e.g., as part of a composition comprising at least one pharmaceutically acceptable carrier and, optionally, at least one other therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A provides an amino acid sequence of hu.32 capsid (SEQ ID NO: 1). Figure 1B provides a nucleotide sequence of hu.32 capsid (SEQ ID NO: 2). Figure 1C provides an amino acid sequence of rh.8 capsid (SEQ ID NO: 3). Figure 1D provides a nucleotide sequence of rh.8 capsid (SEQ ID NO: 4).

Figures 2A and 2B provide images of various regions of the mouse brain depicting AAV infection as evidenced by GFP expression.

Figures 3A-3D provide images of various regions of the mouse brain depicting AAV infection as evidenced by green fluorescent protein (GFP) expression. Figure 3A is AAV2/hu32, Figure 3B is AAV2/rh8, Figure 3C is AAV2/9, and Figure 3D is AAV2/hu11.

Figure 4 provides images of various regions of the feline brain depicting AAV infection as evidenced by GFP expression.

Figure 5A provides images of brain slices from the cortex (ctx), hippocampus (hp), cerebellum (cer), and striatum (str) showing GFP expression indicating AAV infection and NeuN (Fox-3) staining indicating neurons. Figure 5B provides images of brain slices from the cortex (ctx), hippocampus (hp), and striatum (str) showing GFP expression indicating AAV infection and glial fibrillary acidic protein (GFAP) staining indicating astrocytes. Figure 5C provides images of brain slices from the cortex (ctx) and striatum (str) showing GFP expression indicating AAV infection and adenomatous polyposis coli (APC) staining indicating oligodendrocytes.

Figure 6 provides histopathology images of hippocampus, thalamus, and entorhinal cortex brain sections from normal mice, untreated MPS VII mice, and MPS VII mice transduced with AAV.hu32.hGBp.GUSB.

DETAILED DESCRIPTION OF THE INVENTION

Adeno-associated virus (AAV) vectors are among the most promising viral vectors for *in vivo* gene transfer. The prototype AAV2 vector results in relatively limited transduction of central nervous system (CNS) cells, and many humans are seropositive for AAV2, thereby limiting its use in clinical applications. However, the cross-packaging of the AAV2 genome with capsid proteins from alternative AAV serotypes

has resulted in improved gene transfer in a variety of tissues, including the brain (Davidson et al. (2000) Proc. Natl. Acad. Sci., 97:3428-3432; Passini et al. (2003) J. Virol., 77:7034-7040; Burger et al. (2004) Mol. Ther., 10:302-317; Cearley et al. (2006) Mol. Ther., 13:528-537; Taymans et al. (2007) Hum. Gene. Ther., 18:195-206; Cearley et al. (2008) Mol. Ther., 16:1710-1718). Many AAV capsid sequences have been isolated from humans and nonhuman primates by molecular rescue of sequences of endogenous AAVs. The capsid sequences have been phylogenetically characterized into six clades: A through F (Gao et al. (2002) Proc. Natl. Acad. Sci., 99:11854-11859; Gao et al. (2003) Proc. Natl. Acad. Sci., 100:6081-6086; Gao et al. (2004) J. Virol., 78:6381-6388). Certain AAV serotypes have a specific tropism for neurons and are unable to efficiently transduce other cell types within the brain such as astrocytes or oligodendrocytes while other AAV serotypes are able to undergo vector transport along neuronal projections (Davidson et al. (2000) Proc. Natl. Acad. Sci., 97:3428-3432; Burger et al. (2004) Mol. Ther., 10:302-317; Cearley et al. (2006) Mol. Ther., 13:528-537; Kaspar et al. (2003) Science 301:839–842; Passini et al. (2005) Mol. Ther., 11:754-762; Cearley et al. (2007) J. Neurosci., 27:9928-9940; Cearley et al. (2008) Mol. Ther., 16:1710-1718; Foust et al. (2009) Nat. Biotech., 27:59-65).

The instant invention demonstrates that AAV vectors comprising the hu.32 or rh.8, particularly the hu.32, capsid protein mediate AAV vector gene transfer into the brain of mice after intravascular injection. The first two letters of the nomenclature refer to the species of isolation (hu: human) followed by the number of the isolate from that species. The AAV vector specifically transduces neurons in the brain, especially the cerebral cortex, and is very widespread. The types of cells transduced by the instant AAV vectors along with the amount of distribution within the brain are unique. Further, the instant AAV vector is less efficient in transducing the liver than other AAV serotypes, thereby reducing the untoward immune response to the AAV vector *in vivo*, a clinical drawback of many AAV vectors. The distribution within the brain makes the AAV vector of the instant invention an excellent vector for the treatment of a variety of disorders including genetic disorders affecting the brain (including diseases or disorders affecting other parts of the body in addition to the brain) such as lysosomal storage diseases and neurodegenerative diseases (e.g., Alzheimer's disease).

GenBank Accession Nos. AY530597 and AAS99282 provide examples of the amino acid and nucleotide sequences of hu.32 capsid (vp1). GenBank Accession Nos. AAO88183 and AY242997 provide examples of the amino acid and nucleotide

sequences of rh.8 capsid (vp1). The AAV capsid is composed of three proteins, vp1, vp2 and vp3, which are alternative splice variants. In other words, vp2 and vp3 are fragments of vp1. Figure 1A provides SEQ ID NO: 1, which is the wild-type amino acid sequence of hu.32 vp1 capsid. Figure 1B provides SEQ ID NO: 2, which is the wild-type nucleotide sequence of hu.32 vp1 capsid. Figure 1C provides SEQ ID NO: 3, which is the wild-type amino acid sequence of rh.8 vp1 capsid. Figure 1D provides SEQ ID NO: 4, which is the wild-type nucleotide sequence of rh.8 vp1 capsid. The instant invention encompasses variants of the hu.32 and rh.8 capsids. In a particular embodiment, the capsid of the instant invention has an amino acid sequence that is at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or is 100% identical with SEQ ID NO: 1 or SEQ ID NO: 3. In a particular embodiment, the nucleic acid molecule encoding capsid of the instant invention has a nucleotide sequence that is at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or is 100% identical with SEQ ID NO: 2 or SEQ ID NO: 4.

The instant invention encompasses methods of delivering a nucleic acid molecule of interest (e.g., heterologous) to cells, particularly in a subject (i.e., *in vivo*). In a particular embodiment, the method delivers the nucleic acid molecule to neurons or the brain, particularly neurons within the brain. In a particular embodiment, the method delivers the nucleic acid molecule to the olfactory bulb, striatum, cortex, hippocampus, hypothalamus, subthalamus, midbrain, brain stem, superior colliculus, inferiot colliculus, entorhinal cortex, subiculum, and/or cerebellum. The method may comprise contacting the cells with (e.g., by administering to the subject) an AAV vector comprising the hu.32 or rh.8 capsid of the instant invention, wherein the AAV vector comprises the nucleic acid molecule to be delivered. The packaged nucleic acid molecule may encode, for example, a protein of interest (e.g., a therapeutic protein) or an inhibitory nucleic acid molecule (e.g., antisense, siRNA, DsiRNA (Dicer siRNA/Dicer-substrate RNA), shRNA, miRNA (microRNA), etc.). In a particular embodiment, the nucleic acid molecule to be delivered to the subject is a gain-of-function manipulation. The delivery of a nucleic acid molecule of interest in accordance with the instant invention may be used to create a disease model (e.g., a brain disease model) in the subject (e.g., the expression of at least one protein of interest (e.g., a mutant) associated with a disease or disorder). For example, the delivery of a nucleic acid molecule of interest in accordance with the instant invention may be used to create a disease model of a neurodegenerative disease such as Alzheimer's disease (e.g., by expressing at least one gene (e.g., a mutant)

associated with Alzheimer's disease (see, e.g., Chin, J. (2011) Methods Mol. Biol., 670:169-89; Mineur et al. (2005) Neural. Plast., 12:299-310; Hall et al. (2012) Brain Res. Bulletin 88:3-12)) or Huntington's disease (e.g., by expressing a mutant huntingtin gene (also known as interesting transcript 15 (IT151) gene) associated with Huntington's disease). The instant invention also encompasses the disease models generated by the methods of the instant invention. The nucleic acid molecule of the instant invention may further comprise appropriate regulatory elements such as promoters or expression operons to express the encoded for protein or inhibitory nucleic acid molecule.

Methods of treating, inhibiting, and/or preventing a disease or disorder in a subject are also encompassed by the instant invention. In a particular embodiment, the method comprises administering to a subject in need thereof an AAV vector comprising the hu.32 or rh.8 capsid of the instant invention, wherein the AAV vector comprises a nucleic acid molecule of interest (e.g., therapeutic nucleic acid molecule) to be delivered. In a particular embodiment, the AAV vector is administered as part of a composition comprising at least one pharmaceutically acceptable carrier. The AAV vectors of the instant invention may be co-administered with any other therapeutic method for the treatment of the disease or disorder. The nucleic acid molecule of the AAV vector may encode a therapeutic protein or a therapeutic inhibitory nucleic acid molecule (e.g., siRNA). The nucleic acid molecule may further comprise appropriate regulatory elements such as promoters or expression operons to express the encoded for protein or inhibitory nucleic acid molecule.

In a particular embodiment, the disease or disorder is a genetic disease or disorder affecting the brain. Examples of the diseases or disorders that may treated include, without limitation: neurological degenerative disorders, Alzheimer's disease, Parkinson's disease, Huntington's disease (HD), stroke, trauma, infections, meningitis, encephalitis, gliomas, cancers (including brain metastasis), multiple system atrophy, progressive supranuclear palsy, Lewy body disease, neuroinflammatory disease, spinal muscular atrophy, amyotrophic lateral sclerosis, neuroAIDS, Creutzfeldt-Jakob disease, Pick's Disease, multi-infarct dementia, frontal lobe degeneration, corticobasal degeneration, HIV-1 associated dementia (HAD), HIV associated neurocognitive disorders (HAND), paralysis, amyotrophic lateral sclerosis (ALS or Lou Gerhig's disease), multiple sclerosis (MS), CNS-associated cardiovascular disease, prion disease, obesity, metabolic disorders, inflammatory disease, metabolic disorders, and lysosomal storage diseases (LSDs; such as, without limitation, Gaucher's disease, Pompe disease,

Niemann-Pick, Hunter syndrome (MPS II), mucopolysaccharidoses (MPS) (e.g., mucopolysaccharidosis I (MPS I), mucopolysaccharidosis VII (MPS VII), etc.), GM2-gangliosidoses, Sanfilippo syndrome (MPS IIIA), Tay-Sachs disease, Sandhoff's disease, Krabbe's disease, metachromatic leukodystrophy, and Fabry disease). In a particular embodiment, the disease or disorder is a lysosomal storage disease.

Gene transfer may be used to provide therapy for a variety of disease states. In general, gene transfer may be used to treat: 1) deficiency states, wherein a protein (e.g., an enzyme) is expressed at abnormally low levels or is defective (e.g., mutated) and has diminished activity, which can be treated by introducing a nucleic acid encoding for the protein (e.g., wild-type protein); and 2) over-expression states, wherein a protein is expressed to abnormally high levels or is defective (e.g., mutated) and has increased or uncontrolled activity, which can be treated by introducing an inhibitory nucleic acid molecule directed against the protein. The use of site-specific integration of nucleic acid sequences to cause mutations or to correct defects is also encompassed by the instant invention.

In a particular embodiment, a therapeutic protein is a peptide or protein that alleviates or reduces symptoms that result from an absence or defect in a protein in a cell or subject. A therapeutic protein may be a peptide or protein that may be used in the treatment of a disease or disorder. Therapeutic proteins include, but are not limited to, enzymes, antibodies, hormones, growth factors, other polypeptides, which administration to cells (e.g., neurons) can effect amelioration and/or cure of a disease, disorder, pathology, and/or the symptoms associated therewith. Neuroactive polypeptides useful in this invention include but are not limited to endocrine factors, growth factors, hypothalamic releasing factors, neurotrophic factors, paracrine factors, neurotransmitter polypeptides, antibodies and antibody fragments which bind to any of the above polypeptides (such as neurotrophic factors, growth factors, and others), antibodies and antibody fragments which bind to the receptors of these polypeptides (such as neurotrophic factor receptors), cytokines, endorphins, enzymes, polypeptide antagonists, agonists for a receptor expressed by a CNS cell, polypeptides involved in lysosomal storage diseases, and the like. In a particular embodiment, the therapeutic protein exerts its effect on the CNS, particularly the brain.

Examples of specific therapeutic proteins include, without limitation, β-glucuronidase (e.g., for the treatment of lysosomal storage disorders), catalase, telomerase, superoxide dismutase (SOD), glutathionperoxidase, glutaminase, cytokines,

endorphins (e.g., enkephalin), growth factors (e.g., epidermal growth factor (EGF)), acidic and basic fibroblast growth factor (aFGF and bFGF), insulin-like growth factor I (IGF-I; e.g., Oppenheim, RW (1996) Neuron 17:195-197; Thoenen et al. (1993) Exp. Neurol., 124:47-55; Henderson, CE (1995) Adv. Neurol., 68:235-240), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF; e.g., Li et al. (2009) Biochem. Biophys. Res. Comm., 390:947-951), neurotrophin-3 (NT-3), NT-4/5, protease nexin I (PNI; e.g., for the treatment of Alzheimer disease (Houenou et al. (1995) PNAS 92:895-899)), serine protease inhibitor protein (SPI3; e.g., Safaei, R. (1997) Brain Res Dev Brain Res., 100:5-12), platelet derived growth factor (PDGF), vascular growth factor (VGF), nerve growth factor (NGF), insulin-like growth factor-II (IGF-II), tumor necrosis factor-B (TGF-B), survival motor neuron (SMN; e.g., for the treatment of spinal muscular atrophy; Lefebvre et al. (1995) Cell 80:155-165; Roy et al. (1995) Cell 80:167-178), leukemia inhibitory factor (LIF), anti-apoptotic proteins (e.g., BCL-2, PI3 kinase), amyloid beta binders (e.g. antibodies), butyrylcholinesterase or acetylcholinesterase (e.g., Carmona et al. (1999) Drug Metab. Dispos., 28:367-371; Carmona (2005) Eur. J. Pharmacol., 517:186-190), modulators of α-, β-, and/or γ-secretases, vasoactive intestinal peptide, leptin, acid alpha-glucosidase (GAA), acid sphingomyelinase, iduronate-2-sultatase (I2S), α-L-iduronidase (IDU), β-Hexosaminidase A (HexA), β-N-acetylhexosaminidase A Acid β-glucocerebrosidase, N-acetylgalactosamine-4-sulfatase, α-galactosidase A, and neurotransmitters (e.g., Schapira, AH (2003) Neurology 61:S56-63; Ferrari et al. (1990) Adv Exp Med Biol. 265:93-99; Ferrari et al. (1991) J. Neurosci., Res. 30:493-497; Koliatsos et al. (1991) Ann. Neurol. 30:831-840; Dogrukol-Ak et al. (2003) Peptides 24:437-444; Amalfitano et al. (2001) Genet Med. 3:132-138; Simonaro et al. (2002) Am. J. Hum. Genet., 71:1413-1419; Muenzer et al. (2002) Acta Paediatr Suppl. 91:98-99; Wraith et al. (2004) J Pediatr. 144:581-588; Wicklow et al. (2004) Am J Med Genet. 127A:158-166; Grabowski (2004) J Pediatr. 144:S15-19; Auclair et al. (2003) Mol Genet Metab. 78:163-174; Przybylska et al. (2004) J Gene Med. 6:85-92). In a particular embodiment, the therapeutic protein is β-glucuronidase.

While the instant invention is generally described above for the delivery of therapeutic proteins, the AAV of the instant invention may deliver a nucleic acid molecule encoding a detectable protein (e.g., either alone or in combination with a therapeutic protein). Detectable proteins include, without limitation, fluorescent proteins (e.g., GFP), horseradish peroxidase, urease, alkaline phosphatase, glucoamylase, ferritin, dopamine receptor, and β-galactosidase.

Methods of synthesizing AAV vectors are well known in the art (see, e.g., PCT/US04/028817 and Gao et al. (2002) Proc. Natl. Acad. Sci., 99:11854-11859). In a particular embodiment, the method comprises culturing host cells comprising a nucleic acid sequence encoding hu.32 or rh.8 capsid, a nucleic acid encoding *rep*, and a nucleic acid construct comprising AAV inverted terminal repeats (ITRs) flanking at least the nucleic acid molecule of interest, such that the nucleic acid of interest is packaged in to AAV vectors. In a particular embodiment, a full length AAV genome is used. While a self-complimentary vector (scAAV; such as those typically used with AAV9) may be used in the instant invention, the full coding capacity found in rAAV is about 4.5 kb or larger, whereas scAAV typically have a capacity of about 2.3 kb. Inasmuch as certain proteins of interest (e.g., enzymes) may be encoded by a nucleic acid having a length exceeding the capacity of scAAV, the full length AAV vector would be preferred. The host cell may also provide helper functions (e.g., those supplied by a herpesvirus or adenovirus) to package the AAV vectors. The components required of the host cell to package nucleic acid molecules into AAV vectors may be provided *in trans* or by a stably transduced host cell. The *rep* gene and/or the AAV ITRs may be from any AAV serotype. For example, the *rep* gene and/or the AAV ITRs may be from, without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, etc. In a particular embodiment, the AAV ITRs are from the AAV2 serotype. The encapsulated nucleic acid molecule may encode more than one protein or polypeptide. When the nucleic acid molecule encodes more than one protein/polypeptide, the encoding regions may be separated by an internal ribozyme entry site (IRES) or nucleic acid sequence encoding a self-cleaving peptide such as a 2A peptide.

The instant invention encompasses methods of treating a disease or disorder in a subject (e.g., a neurological disease or disorder) comprising the administration of a composition comprising the AAV vectors of the instant invention and at least one pharmaceutically acceptable carrier to a subject in need thereof. The term "subject" as used herein refers to human or animal (particularly mammalian) subjects.

The AAV vectors of the invention may be conveniently formulated for administration with any pharmaceutically acceptable carrier. For example, the viral vectors may be formulated with an acceptable medium such as water, buffered saline, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), dimethyl sulfoxide (DMSO), oils, detergents, suspending agents or suitable mixtures thereof. The concentration of the AAV vectors in the chosen medium may be

varied and the medium may be chosen based on the desired route of administration of the pharmaceutical preparation. Except insofar as any conventional media or agent is incompatible with the AAV vector to be administered, its use in the pharmaceutical preparation is contemplated.

The dose and dosage regimen of the compositions according to the invention that are suitable for administration to a particular patient may be determined by a physician/veterinarian/medical specialist considering the patient's age, sex, weight, general medical condition, and the specific condition for which the AAV vector is being administered and the severity thereof. The physician/veterinarian/medical specialist may also take into account the route of administration, the pharmaceutical carrier, and the AAV vector's biological activity. Exemplary doses for achieving therapeutic effects are AAV titers of at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} ,10^{16} transducing units or more, particularly about 10^8 to 10^{13} transducing units. In particular embodiments of the invention, more than one administration (e.g., two, three, four, or more administrations) may be employed to achieve desired (e.g. therapeutic) levels of gene expression.

Selection of a suitable pharmaceutical preparation will also depend upon the mode of administration chosen. The pharmaceutical preparation comprises the AAV vector preferably dispersed in a medium that is compatible with the site of injection. AAV vectors of the instant invention may be administered by any method such as injection into the blood stream, oral administration, or by subcutaneous, intracranial, intramuscular or intraperitoneal injection. The AAV vector of the invention may be administered by direct injection into an area proximal to or across the blood brain barrier. In a particular embodiment, the composition comprising the AAV vector is administered directly to or to an area proximal to a neuron(s). In a particular embodiment, the composition comprising the AAV vector is administered intravascularly or intravenously. The AAV vectors of the instant invention may be administered into any fluid space of the subject including, without limitation, blood or cerebrospinal fluid (CSF). Pharmaceutical preparations for injection are known in the art. If injection is selected as a method for administering the AAV vectors, steps must be taken to ensure that sufficient amounts of the viral vectors reach their target cells to exert a biological effect.

Pharmaceutical compositions containing an AAV vector the present invention as the active ingredient in intimate admixture with a pharmaceutically acceptable carrier

can be prepared according to conventional pharmaceutical techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravascular, direct injection, intracranial, and intramuscular.

A pharmaceutical preparation of the invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient undergoing treatment. Each dosage should contain a quantity of active ingredient calculated to produce the desired effect in association with the selected pharmaceutical carrier. Procedures for determining the appropriate dosage unit are well known to those skilled in the art.

In accordance with the present invention, the appropriate dosage unit for the administration of AAV vectors may be determined by evaluating toxicity, if any, in animal models. Various concentrations of AAV vectors in pharmaceutical preparations may be administered to mice or other animals (e.g., models of the disease to be treated), and the minimal and maximal dosages may be determined based on the beneficial results and side effects observed as a result of the treatment. Appropriate dosage unit may also be determined by assessing the efficacy of the AAV vector treatment in combination with other standard drugs. The dosage units of AAV vector may be determined individually or in combination with each treatment according to the effect detected.

The AAV vectors, reagents, and methods of the present invention can be used to direct a nucleic acid to either dividing or non-dividing cells, and to stably express the nucleic acid therein. The vectors of the present invention can thus be useful in gene therapy for disease states or for experimental modification of cell physiology.

Definitions

The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

"Gene therapy" is the insertion of nucleic acids (e.g., genes) into an individual's cells and/or tissues to treat a disease or disorder, commonly hereditary or genetic diseases (e.g., wherein a defective mutant allele is replaced or supplemented with a functional one).

The term "treat" as used herein refers to any type of treatment that imparts a benefit to a patient afflicted with a disease, including improvement in the condition of

the patient (e.g., in one or more symptoms), delay in the progression of the condition, etc.

A "therapeutically effective amount" of a compound or a pharmaceutical composition refers to an amount effective to prevent, inhibit, treat, or lessen a particular disorder or disease and/or the symptoms associated with it. The treatment of a neurological disease or disorder herein may refer to curing, relieving, inhibiting, and/or preventing the neurological disease or disorder, a symptom(s) of it, or the predisposition towards it.

An "inhibitory nucleic acid molecule" generally refers to small nucleic acid molecules which are capable of modulating expression levels of a target mRNA, (e.g., siRNA, shRNA, miRNA, DsiRNA, antisense oligonucleotides etc.). These molecules may inhibit expression of a target gene involved in mediation of a disease process, thereby preventing or alleviating the disease and/or the symptoms associated with it.

The phrase "small, interfering RNA (siRNA)" refers to a short (typically less than 30 nucleotides long, particularly 12-30 or 20-25 nucleotides in length) double stranded RNA molecule (although the siRNA may be generated by cleavage of longer dsRNA molecules). Typically, the siRNA modulates the expression of a gene to which the siRNA is targeted. siRNAs have homology (e.g., complete complementarity) with the sequence of the cognate mRNA of the targeted gene. Methods of identifying and synthesizing siRNA molecules are known in the art (see, e.g., Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Inc). Exemplary modifications to siRNA molecules are provided in U.S. Application Publication No. 2005/0032733. Expression vectors for the expression of siRNA molecules preferably employ a strong promoter which may be constitutive or regulated. Such promoters are well known in the art and include, but are not limited to, RNA polymerase II promoters, the T7 RNA polymerase promoter, and the RNA polymerase III promoters U6 and H1 (see, e.g., Myslinski et al. (2001) Nucl. Acids Res., 29:2502-09).

The term "short hairpin RNA" or "shRNA" refers to an siRNA precursor that is a single RNA molecule folded into a hairpin structure comprising an siRNA and a single stranded loop portion of at least one, typically 1-10, nucleotide. shRNA molecules are typically processed into an siRNA within the cell by endonucleases.

As used herein, the term "microRNA" or "miRNA" refers to any type of interfering RNA, including but not limited to, endogenous microRNA (naturally present in the genome) and artificial microRNA. MicroRNA typically have a length in the range

of from about 18 to about 30 nucleotides, particularly about 21 to about 25 nucleotides. MicroRNA may be single-stranded RNA molecules. The microRNA may be in the form of pre-miRNA, typically a short stem-loop structure having a length of about 50 to about 90 nucleotides, particularly about 60 to about 80 nucleotides, which are subsequently processed into functional miRNAs.

The term "RNA interference" or "RNAi" refers generally to a sequence-specific or selective process by which a target molecule (e.g., a target gene, protein or RNA) is downregulated via a double-stranded RNA. The double-stranded RNA structures that typically drive RNAi activity are siRNAs, shRNAs, microRNAs, and other double-stranded structures that can be processed to yield a small RNA species that inhibits expression of a target transcript by RNA interference.

The term "Dicer substrate RNA" or "DsiRNA" refers to oligonucleotides which comprise at least one siRNA molecule and which serve as a substrate for Dicer to release the siRNA molecule, typically 21 nucleotides in length. DsiRNA are double-stranded and comprise RNA or DNA and RNA. Typically, DsiRNA are less than about 100 nucleotides in length, less than about 50 nucleotides in length, less than about 40 nucleotides in length, less than about 35 nucleotides in length, or less than about 30 nucleotides in length. In a particular embodiment, the DsiRNA is 27 nucleotides in length. Examples of DsiRNA are provided in U.S. Patent Application Publication Nos. 2005/0244858; 2005/0277610; 2007/0265220; and 2010/0184841.

"Antisense nucleic acid molecules" or "antisense oligonucleotides" include nucleic acid molecules (e.g., single stranded molecules) which are targeted (complementary) to a chosen sequence (e.g., to translation initiation sites and/or splice sites) to inhibit the expression of a protein of interest. Such antisense molecules are typically between about 10 and about 100 nucleotides in length, particularly between about 15 and about 50 nucleotides, more particularly between about 15 and about 30 nucleotides, and often span the translational start site of mRNA molecules. Antisense constructs may also be generated which contain the entire sequence of the target nucleic acid molecule in reverse orientation. Antisense oligonucleotides targeted to any known nucleotide sequence can be prepared by oligonucleotide synthesis according to standard methods.

"Pharmaceutically acceptable" indicates approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

A "carrier" refers to, for example, a diluent, adjuvant, preservative (e.g., Thimersol, benzyl alcohol), anti-oxidant (e.g., ascorbic acid, sodium metabisulfite), solubilizer (e.g., Tween™ 80, Polysorbate 80), emulsifier, buffer (e.g., Tris HCl, acetate, phosphate), water, aqueous solutions, oils, bulking substance (e.g., lactose, mannitol), excipient, auxiliary agent or vehicle with which an active agent of the present invention is administered. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin (Mack Publishing Co., Easton, PA); Gennaro, A. R., Remington: The Science and Practice of Pharmacy, (Lippincott, Williams and Wilkins); Liberman, et al., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y.; and Kibbe, et al., Eds., Handbook of Pharmaceutical Excipients (3rd Ed.), American Pharmaceutical Association, Washington.

The term "promoter" as used herein can refer to a DNA sequence that is located adjacent to a DNA sequence that encodes a recombinant product. A promoter is preferably linked operatively to an adjacent DNA sequence. A promoter typically increases an amount of recombinant product expressed from a DNA sequence as compared to an amount of the expressed recombinant product when no promoter exists. A promoter from one organism can be utilized to enhance recombinant product expression from a DNA sequence that originates from another organism. For example, a vertebrate promoter may be used for the expression of jellyfish GFP in vertebrates. In addition, one promoter element can increase an amount of recombinant products expressed for multiple DNA sequences attached in tandem. Hence, one promoter element can enhance the expression of one or more recombinant products. Multiple promoter elements are well-known to persons of ordinary skill in the art. Inducible promoters, tissue-specific promoters, native promoters, or constitutive or high level promoters may be used. In a particular embodiment, high-level constitutive expression may be desired. Examples of such promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter/enhancer, the cytomegalovirus (CMV) immediate early promoter/enhancer, the SV40 promoter, the dihydrofolate reductase promoter, the cytoplasmic β-actin promoter and the phosphoglycerol kinase (PGK) promoter. In another embodiment, the native promoter for the transgene or nucleic acid sequence of interest is used. The native promoter may be preferred when it is desired that expression of the transgene or the nucleic acid sequence should mimic the native expression. The native promoter may be used when expression of the transgene or other nucleic acid sequence must be regulated temporally or developmentally, or in a tissue-specific

manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression. In a particular embodiment, the tissue-specific promoter is neuron specific. Examples of neuron specific protomers include, without limitation: neuron-specific enolase (NSE) promoter (Andersen et al. (1993) Cell. Mol. Neurobiol., 13:503-15); neurofilament light-chain gene (Piccioli et al. (1991) Proc. Natl. Acad. Sci., 88:5611-5); the neuron-specific vgf gene (Piccioli et al. (1995) Neuron, 15:373-84)]; and the like.

The term "enhancer" as used herein can refer to a DNA sequence that is located adjacent to the DNA sequence that encodes a recombinant product. Enhancer elements are typically located upstream of a promoter element or can be located downstream of or within a coding DNA sequence (e.g., a DNA sequence transcribed or translated into a recombinant product or products). Hence, an enhancer element can be located 100 base pairs, 200 base pairs, or 300 or more base pairs upstream or downstream of a DNA sequence that encodes recombinant product. Enhancer elements can increase an amount of recombinant product expressed from a DNA sequence above increased expression afforded by a promoter element. Multiple enhancer elements are readily available to persons of ordinary skill in the art.

"Nucleic acid" or a "nucleic acid molecule" as used herein refers to any DNA or RNA molecule, either single or double stranded and, if single stranded, the molecule of its complementary sequence in either linear or circular form. In discussing nucleic acid molecules, a sequence or structure of a particular nucleic acid molecule may be described herein according to the normal convention of providing the sequence in the 5' to 3' direction. With reference to nucleic acids of the invention, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous in the naturally occurring genome of the organism in which it originated. For example, an "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryotic or eukaryotic cell or host organism.

A "vector" is a replicon, such as a plasmid, cosmid, bacmid, phage or virus, to which another genetic sequence or element (either DNA or RNA) may be attached so as to bring about the expression and/or replication of the attached sequence or element.

The term "gene" refers to a nucleic acid comprising an open reading frame encoding a polypeptide, including exon and (optionally) intron sequences. The nucleic acid may also optionally include non-coding sequences such as promoter or enhancer sequences. The term "intron" refers to a DNA sequence present in a given gene that is not translated into protein and is generally found between exons.

An "expression operon" refers to a nucleic acid segment that may possess transcriptional and translational control sequences, such as promoters, enhancers, translational start signals (e.g., ATG or AUG codons), polyadenylation signals, terminators, and the like, and which facilitate the expression of a polypeptide coding sequence in a host cell or organism.

The term "operably linked" means that the regulatory sequences necessary for expression of the coding sequence are placed in the DNA molecule in the appropriate positions relative to the coding sequence so as to effect expression of the coding sequence. This same definition is sometimes applied to the arrangement of transcription units and other transcription control elements (e.g. enhancers) in an expression vector.

The term "oligonucleotide" as used herein refers to sequences, primers and probes of the present invention, and is defined as a nucleic acid molecule comprised of two or more ribo- or deoxyribonucleotides, preferably more than three. The exact size of the oligonucleotide will depend on various factors and on the particular application and use of the oligonucleotide.

The term "isolated" may refer to protein, nucleic acid, compound, or cell that has been sufficiently separated from the environment with which it would naturally be associated, e.g., so as to exist in "substantially pure" form. "Isolated" does not necessarily mean the exclusion of artificial or synthetic mixtures with other compounds or materials, or the presence of impurities that do not interfere with the fundamental activity, and that may be present, for example, due to incomplete purification.

The term "percent identity" refers to the percentage of sequence identity found in a comparison of two or more nucleic acid sequences. Percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Search Tool (BLAST) described by Altshul et al. (J. Mol. Biol. (1990) 215:403-10) as well as GAP, BESTFIT, FASTA, and TFASTA (available as part of the GCG® Wisconsin Package® (Accelrys Inc., Burlington, MA)).

"Polypeptide" and "protein" are sometimes used interchangeably herein and indicate a molecular chain of amino acids. The term polypeptide encompasses peptides,

oligopeptides, and proteins. The terms also include post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

The following examples are provided to illustrate certain embodiments of the invention. They are not intended to limit the invention in any way.

EXAMPLE I

AAV hu.32 capsid was cloned into an AAV2-based packaging plasmid to obtain a hybrid construct with AAV2 *rep* and the alternative *cap* in frame as described (Gao et al. (2002) Proc. Natl. Acad. Sci., 99:11854-11859). All vectors comprised the cytomegalovirus promoter and enhanced GFP transgene and were cross-packaged into an AAV2 recombinant genome with heterologous cap sequence from the tested AAV variant using a triple-transfection procedure as described (Gao et al. (2002) Proc. Natl. Acad. Sci., 99:11854-11859). The packaging, purification, and determination of vector titers were performed by the University of Pennsylvania Vector Core. All recombinant vectors were purified using the CsCl sedimentation method and genome copy titers were determined as described (Gao et al. (2000) Hum. Gene Ther., 11:2079-2091).

Adult mice were injected intravenously with the hu.32 AAV vector comprising the GFP transgene. After injection, mice were anesthetized with a mixture of ketamine and xylazine (~0.15 ml per mouse) and perfused transcardially with a solution of phosphate-buffered saline followed by 4% paraformaldehyde. Brains from animals were then removed and put in 4% paraformaldehyde overnight, following which they were transferred to 30% sucrose for cryoprotection. Once the brains sank in the sucrose, they were mounted in optimum cutting temperature solution (Sakura, Torrance, CA) and frozen at -20°C until sectioning. Sectioning was done at a thickness of 20 μm using a cryostat (Leica Microsystems, Wetzlar, Germany) and the sections were mounted on three sets of slides which were then kept at -20°C until imaging by confocal microscopy.

As seen in Figure 2, GFP was expressed intensely throughout the brain after intravenous injection. More specifically, GFP expression was detected in neurons in the olfactory bulb, cortex, striatum, hippocampus, midbrain, superior colliculus, entorhinal cortex, and cerebellum. These results demonstrate substantially greater levels of transduction than observed with AAV9 (Foust et al. (2009) Nat. Biotechnol., 27:59-65).

Further, the widespread expression of GFP has been observed in Balb/c, C3H, and C57Bl/6 mice.

Figure 3 shows a comparison of gene transfer for AAV2/9, AAV2/hu11, AAV2/rh8, and AAV2/hu32. Mice were injected intravenously with the same quantity of virus. However, as evidenced by Figure 3, hu32 dramatically increased the delivery to the brain over the other strains. Indeed, hu11 showed minimal targeting to the brain, AAV9 showed weak targeting, rh8 showed improved targeting, and hu32 showed unexpectedly robust targeting.

EXAMPLE II

The targeting of the AAV vectors of the instant invention was also tested in cats. Six week old cats (n=3) were injected in the carotid artery with 2.88 x 10^{13} vector genomes (vg)/kg of AAV.hu32.hGBp.GFP, where hGBp is the human β-glucuronidase (hGUSB) promoter (378 bp fragment) and GFP is green fluorescent protein. GFP expression was monitored 8 weeks post-infection. As seen in Figure 4, GFP was expressed intensely throughout the brain after intravascular (carotid) injection. More specifically, GFP expression was detected in neurons in the prefrontal cortex, caudate nucleus, putamen, cortex, hippocampus, midbrain, cerebellum, and brain stem.

EXAMPLE III

To demonstrate that the hu32 AAV vectors of the instant invention are infecting neurons, cells of infected brain regions were studied for GFP expression (indicating infection by the AAV vector) and cell-type specific markers. Specifically, expression of NeuN (Fox-3) was used to identify neurons, expression of glial fibrillary acidic protein (GFAP) was used to identify astrocytes, and expression of adenomatous polyposis coli (APC) was used to identify oligodendrocytes. Figure 5A shows the double staining of neurons (GFP+, NeuN+) in the cortex, hippocampus, cerebellum, and striatum, indicating that the neurons were infected with GFP encoding hu32 AAV vector. In contrast, Figures 5B and 5C show that there is no double staining of astrocytes or oligodendrocytes, respectively, thereby indicating that the hu32 AAV vector did not transduce these cell types. Accordingly, these results demonstrate that the AAV vector of the instant invention is able to selectively infect neurons to the exclusion of astrocytes and oligodendrocytes.

EXAMPLE IV

Adeno-associated virus serotype 9 (AAV9) can cross the blood-brain barrier and infect neurons and astrocytes and other tissues (Foust et al. (2009) Nat Biotechnol., 27:59-65; Cearley et al. (2008) Mol. Ther., 16:1710-1718). However, it has recently been determined that AAV9 was unable to transduce CNS neurons in a mouse model of the lysosomal storage disease (LSD) mucopolysaccharidosis (MPS) VII (Chen et al. (2012) Mol. Ther., 20:1393-1399).

In stark contrast, the hu32 AAV vectors of the instant invention were capable of transducing neurons upon systemic administration. Table 1 shows β-glucuronidase (GUSB) activity of lysates of cryostat cut brain sections from 4 MPS VII mice treated with AAV.hu32.hGBp.GUSB. Briefly, GUSB enzyme activity was determined by the cleavage of a substrate to 4-methylumbelliferone (4-MU) by GUSB, where 4-MU can be detected fluorometrically. As seen in Table 1, the intravascular delivery of the hu32 AAV vector leads to transduction of brain neurons and very high - well above therapeutic levels - expression of GUSB.

	nMoles/mg/hr	% normal
24617	0.41	13.69
24734	0.31	10.45
24736	0.40	13.46
24740	0.44	14.82

Table 1: β-glucuronidase activity as percent of normal is provided from 4 cryostat cut brain samples obtained from 4 MPS VII mice transduced with AAV.hu32.hGBp.GUSB.

Figure 6 provides histopathology images of normal mice, untreated MPS VII mice, and MPS VII mice transduced with AAV.hu32.hGUSB.GFP. Sections of the hippocampus, thalamus, and entorhinal cortex were examined. The untreated MPS VII mice brain slices show the characteristic lesions observed with MPS VII. In stark contrast, the MPS VII mice treated with AAV.hu32.hGBp.GUSB show a histopathology similar to normal mice without the hallmark lesions of MPS.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A method for delivering a nucleic acid molecule to the brain of a subject, said method comprising administering an adeno-associated virus (AAV) vector to said subject, wherein said AAV vector comprises said nucleic acid molecule and comprises a capsid protein comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 1 or 3.

2. The method of claim 1, wherein said capsid protein comprises an amino acid sequence having at least 95% identity with SEQ ID NO: 1.

3. The method of claim 1, wherein said capsid protein comprises SEQ ID NO: 1.

4. The method of claim 1, wherein said nucleic acid molecule encodes a therapeutic protein or inhibitory nucleic acid molecule.

5. The method of claim 1, wherein said nucleic acid molecules are delivered to the neurons within the brain.

6. A method for treating a disease or disorder affecting the brain of a subject, said method comprising administering an adeno-associated virus (AAV) vector to said subject, wherein said AAV vector comprises a nucleic acid molecule encoding a therapeutic protein or inhibitory nucleic acid molecule and comprises a capsid protein comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 1 or 3.

7. The method of claim 6, wherein said capsid protein comprises an amino acid sequence having at least 95% identity with SEQ ID NO: 1.

8. The method of claim 6, wherein said capsid protein comprises SEQ ID NO: 1.

9. The method of claim 6, wherein said nucleic acid molecule encodes a therapeutic protein.

10. The method of claim 6, wherein said disease or disorder is a lysosomal storage disease.

11. The method of claim 6, wherein said disease or disorder is a neurodegenerative disease.

12. The method of claim 6, wherein said nucleic acid molecule encodes a β-glucuronidase.

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MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY  50
KYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF 100
QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSP 150
QEPDSSAGIGKSGSQPAKKKLNFGQTGDTESVPDPQPIGEPPAAPSGVGS 200
LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALP 250
TYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR 300
LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDY 350
QLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYF 400
PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKT 450
INGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSE 500
FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGR 550
DNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG 600
ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIK 650
NTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQ 700
YTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL               736
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Figure 1A

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   1 atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga
  61 cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac
 121 gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccggcaa cggactcgac
 181 aaggggggagc cggtcaacgc agcagacgcg gcggccctcg agcacgacaa ggcctacgac
 241 cagcagctca aggccggaga caacccgtac ctcaagtaca accacgccga cgccgagttc
 301 caggagcggc tcaaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag
 361 gccaaaaaga ggcttcttga acctcttggt ctggttgagg aagcggctaa gacggctcct
 421 ggaaagaaga ggcctgtaga gcagtctcct caggaaccgg actcctccgc gggtattggc
 481 aaatcgggtt cacagcccgc taaaaagaaa ctcaatttcg gtcagactgg cgacacagag
 541 tcagtccccg accctcaacc aatcggagaa cctcccgcag ccccctcagg tgtgggatct
 601 cttacaatgg cttcaggtgg tggcgcacca gtggcagaca ataacgaagg tgccgatgga
 661 gtgggtagtt cctcgggaaa ttggcattgc gattcccaat ggctgggga cagagtcatc
 721 accaccagca cccgaacctg ggccctgccc acctacaaca atcacctcta caagcaaatc
 781 tccaacagca catctggagg atcttcaaat gacaacgcct acttcggcta cagcaccccc
 841 tgggggtatt ttgacttcaa cagattccac tgccacttct caccacgtga ctggcagcga
 901 ctcatcaaca acaactgggg attccggcct aagcgactca acttcaagct cttcaacatt
 961 caggtcaaag aggttacgga caacaatgga gtcaagacca tcgccaataa ccttaccagc
1021 acggtccagg tcttcacgga ctcagactat cagctcccgt acgtgctcgg gtcggctcac
1081 gagggctgcc tcccgccgtt cccagcggac gttttcatga ttcctcagta cgggtatctg
1141 acgcttaatg atgggagcca ggccgtgggt cgttcgtcct tttactgcct ggaatatttc
1201 ccgtcgcaaa tgctaagaac gggtaacaac ttccagttca gctacgagtt tgagaacgta
1261 cctttccata gcagctacgc tcacagccaa agcctggacc gactaatgaa tccactcatc
1321 gaccaatact tgtactatct ctcaaagact attaacggtt ctggacagaa tcaacaaacg
1381 ctaaaattca gcgtggccgg acccagcaac atggctgtcc agggaagaaa ctacatacct
1441 ggacccagct accgacaaca acgtgtctca accactgtga ctcaaaacaa caacagcgaa
1501 tttgcttggc ctggagcttc ttcttgggct ctcaatggac gtaatagctt gatgaatcct
1561 ggacctgcta tggccagcca caaagaagga gaggaccgtt tctttccttt gtctggatct
1621 ttaatttttg gcaaacaagg aactggaaga gacaacgtgg atgcggacaa agtcatgata
1681 accaacgaag aagaaattaa aactactaac ccggtagcaa cggagtccta tggacaagtg
1741 gccacaaacc accagagtgc ccaagcacag gcgcagaccg gctgggttca aaaccaagga
1801 atacttccgg gtatggtttg gcaggacaga gatgtgtacc tgcaaggacc catttgggcc
1861 aaaattcctc acacggacgg caactttcac ccttctccgc taatgggagg gtttggaatg
1921 aagcacccgc ctcctcagat cctcatcaaa aacacacctg tacctgcgga tcctccaacg
1981 gctttcaata aggacaagct gaactctttc atcacccagt attctactgg ccaagtcagc
2041 gtggagattg agtgggagct gcagaaggaa aacagcaagc gctggaaccc ggagatccag
2101 tacacttcca actattacaa gtctaataat gttgaatttg ctgttaatac tgaaggtgta
2161 tatagtgaac cccgccccat tggcaccaga tacctgactc gtaatctgta a
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Figure 1B

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  1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD
 61 KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFQ
121 AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE
181 SVPDPQPLGE PPAAPSGLGP NTMASGGGAP MADNNEGADG VGNSSGNWHC DSTWLGDRVI
241 TTSTRTWALP TYNNHLYKQI SNGTSGGSTN DNTYFGYSTP WGYFDFNRFH CHFSPRDWQR
301 LINNNWGFRP KRLNFKLFNI QVKEVTTNEG TKTIANNLTS TVQVFTDSEY QLPYVLGSAH
361 QGCLPPFPAD VFMVPQYGYL TLNNGSQALG RSSFYCLEYF PSQMLRTGNN FQFSYTFEDV
421 PFHSSYAHSQ SLDRLMNPLI DQYLYYLVRT QTTGTGGTQT LAFSQAGPSS MANQARNWVP
481 GPCYRQQRVS TTTNQNNNSN FAWTGAAKFK LNGRDSLMNP GVAMASHKDD DDRFFPSSGV
541 LIFGKQGAGN DGVDYSQVLI TDEEEIKATN PVATEEYGAV AINNQAANTQ AQTGLVHNQG
601 VIPGMVWQNR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGL KHPPPQILIK NTPVPADPPL
661 TFNQAKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ YTSNYYKSTN VDFAVNTEGV
721 YSEPRPIGTR YLTRNL
```

Figure 1C

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   1 atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc
  61 gagtggtggg acttgaaacc tggagccccg aaacccaaag ccaaccagca aaagcaggac
 121 gacggccggg gtctggtgct tcctggctac aagtacctcg gacccttcaa cggactcgac
 181 aaggggggagc ccgtcaacgc ggcggacgca gcggccctcg agcacgacaa agcctacgac
 241 cagcagctca aagcgggtga caatccgtac ctgcggtata atcacgccga cgccgagttt
 301 caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag
 361 gccaagaagc gggttctcga acctctcggt ctggttgagg aaggcgctaa gacggctcct
 421 ggaaagaaga gaccggtaga gcagtcgcca caagagccag actcctcctc gggcatcggc
 481 aagacaggcc agcagcccgc taaaaagaga ctcaattttg gtcagactgg cgactcagag
 541 tcagtccccg acccacaacc tctcggagaa cctccagcag ccccctcagg tctgggacct
 601 aatacaatgg cttcaggcgg tggcgctcca atggcagaca ataacgaagg cgccgacgga
 661 gtgggtaatt cctcgggaaa ttggcattgc gattccacat ggctgggga cagagtcatc
 721 accaccagca cccgaacctg ggccctgccc acctacaaca accacctcta caagcaaatc
 781 tccaacggca cctcgggagg aagcaccaac gacaacacct attttggcta cagcaccccc
 841 tgggggtatt ttgacttcaa cagattccac tgtcactttt caccacgtga ctggcaacga
 901 ctcatcaaca acaattgggg attccggccc aaaagactca acttcaagct gttcaacatc
 961 caggtcaagg aagtcacgac gaacgaaggc accaagacca tcgccaataa tctcaccagc
1021 accgtgcagg tctttacgga ctcggagtac cagttaccgt acgtgctagg atccgctcac
1081 cagggatgtc tgcctccgtt cccggcggac gtcttcatgg ttcctcagta cggctattta
1141 actttaaaca atggaagcca agccctggga cgttcctcct tctactgtct ggagtatttc
1201 ccatcgcaga tgctgagaac cggcaacaac tttcagttca gctacacctt cgaggacgtg
1261 cctttccaca gcagctacgc gcacagccag agcctggaca ggctgatgaa tcccctcatc
1321 gaccagtacc tgtactacct ggtcagaacg caaacgactg gaactggagg gacgcagact
1381 ctggcattca gccaagcggg tcctagctca atggccaacc aggctagaaa ttgggtgccc
1441 ggaccttgct accggcagca gcgcgtctcc acgacaacca accagaacaa caacagcaac
1501 tttgcctgga cgggagctgc caagtttaag ctgaacggcc gagactctct aatgaatccg
1561 ggcgtggcaa tggcttccca caaggatgac gacgaccgct tcttcccttc gagcggggtc
1621 ctgatttttg gcaagcaagg agccgggaac gatggagtgg attacagcca agtgctgatt
1681 acagatgagg aagaaatcaa ggctaccaac cccgtggcca cagaagaata tggagcagtg
1741 gccatcaaca accaggccgc caatacgcag gcgcagaccg gactcgtgca caaccagggg
1801 gtgattcccg gcatggtgtg gcagaataga gacgtgtacc tgcagggtcc catctgggcc
1861 aaaattcctc acacggacgg caactttcac ccgtctcccc tgatgggcgg ctttggactg
1921 aagcacccgc ctcctcaaat tctcatcaag aacacaccgg ttccagcgga cccgccgctt
1981 accttcaacc aggccaagct gaactctttc atcacgcagt acagcaccgg acaggtcagc
2041 gtggaaatcg agtgggagct gcagaaagaa aacagcaaac gctggaatcc agagattcaa
2101 tacacttcca actactacaa atctacaaat gtggactttg ctgtcaacac ggaggggggtt
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Figure 1D

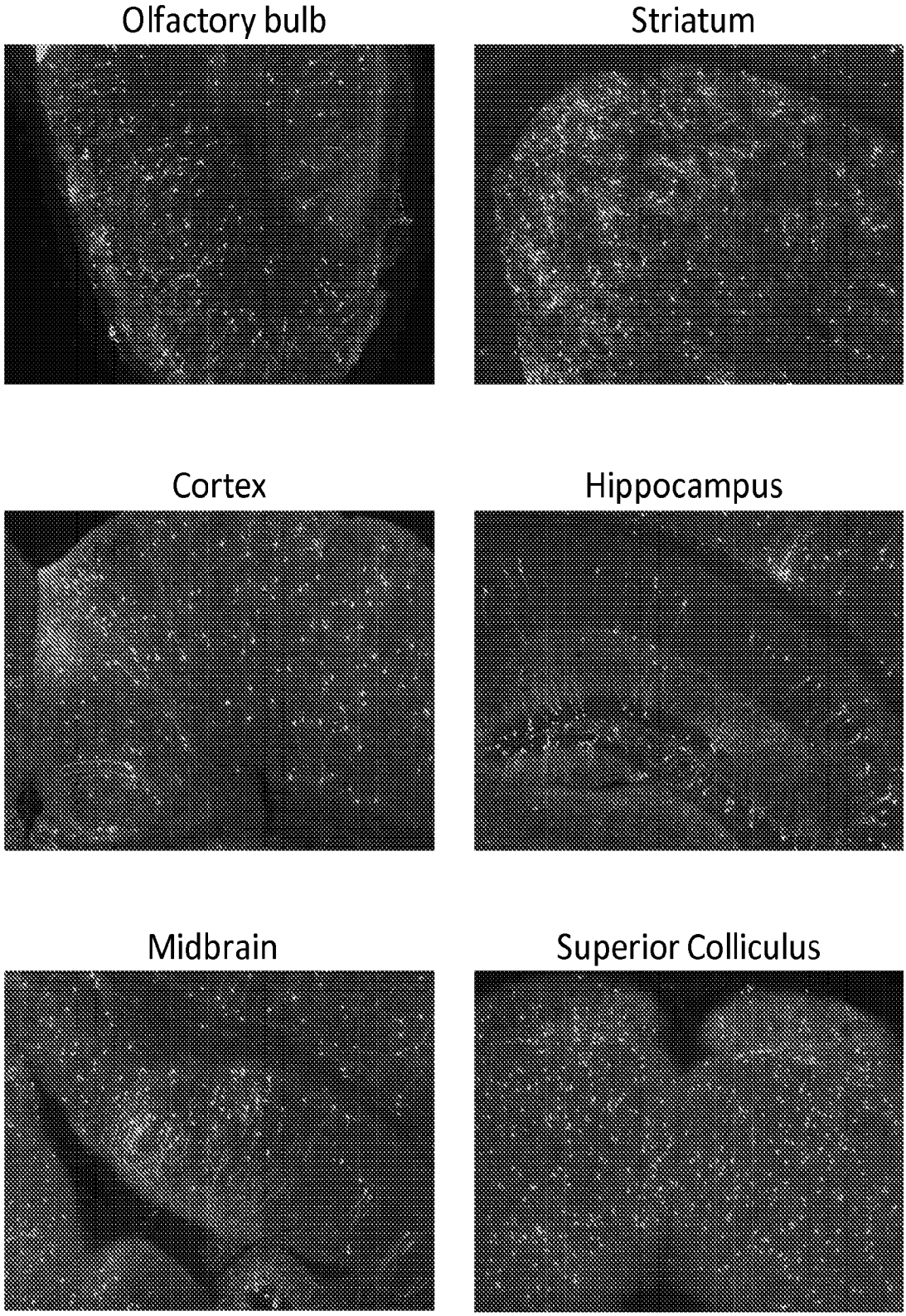
Olfactory bulb
Striatum
Cortex
Hippocampus
Midbrain
Superior Colliculus

Figure 2A

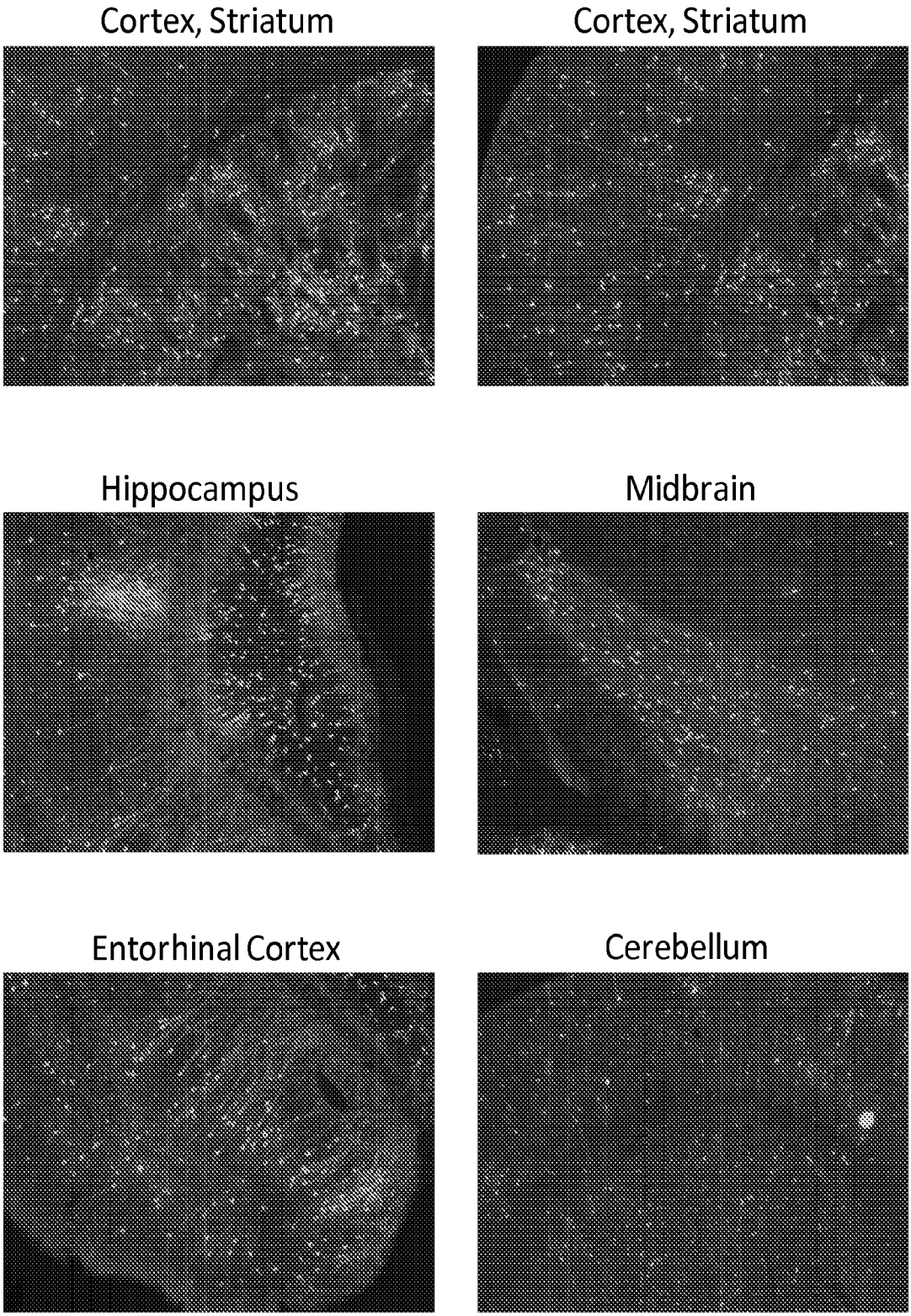
Cortex, Striatum
Cortex, Striatum
Hippocampus
Midbrain
Entorhinal Cortex
Cerebellum

Figure 2B

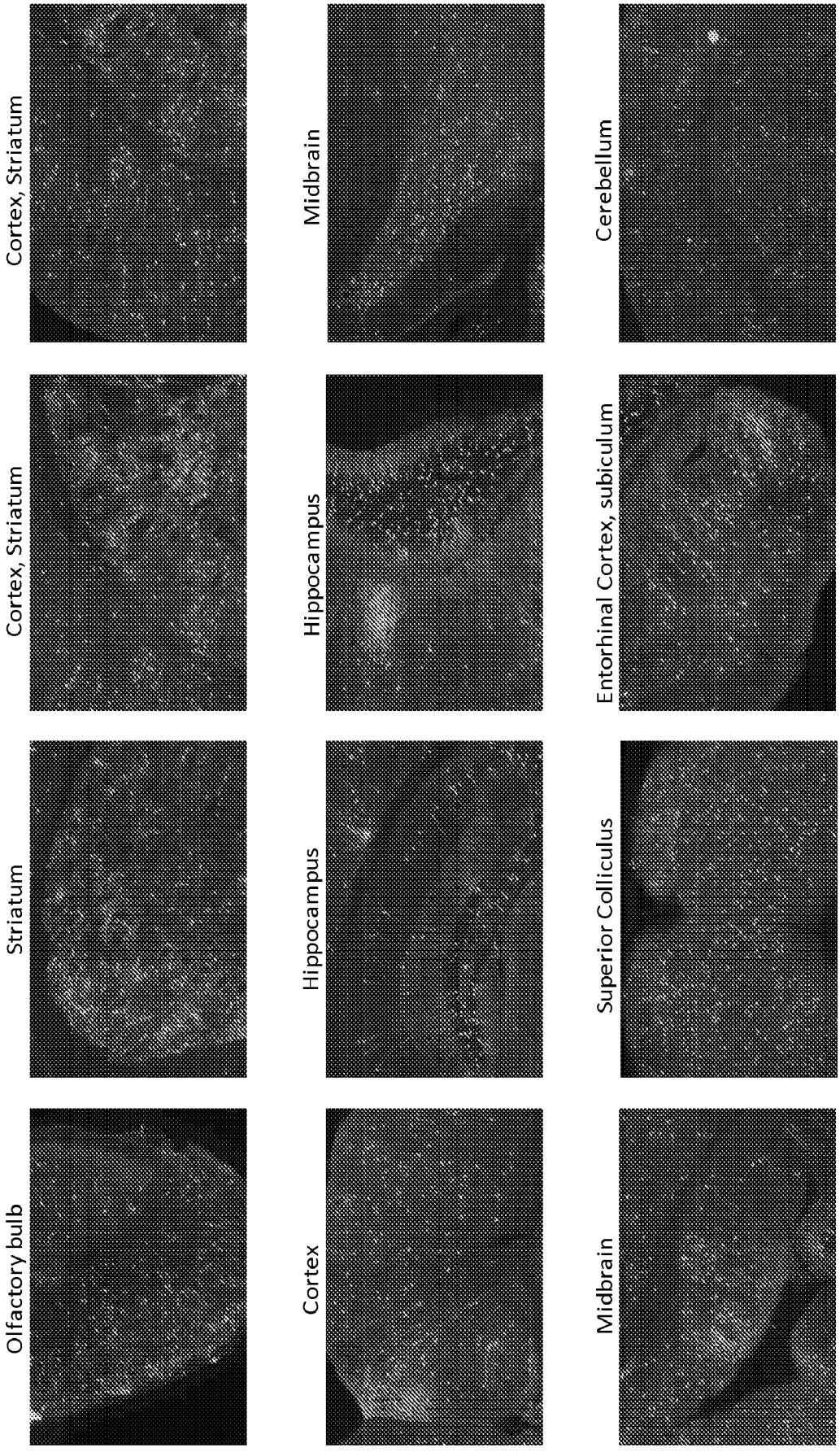
Cortex, Striatum
Midbrain
Cerebellum
Cortex, Striatum
Hippocampus
Entorhinal Cortex, subiculum
Striatum
Hippocampus
Superior Colliculus
Olfactory bulb
Cortex
Midbrain

Figure 3A

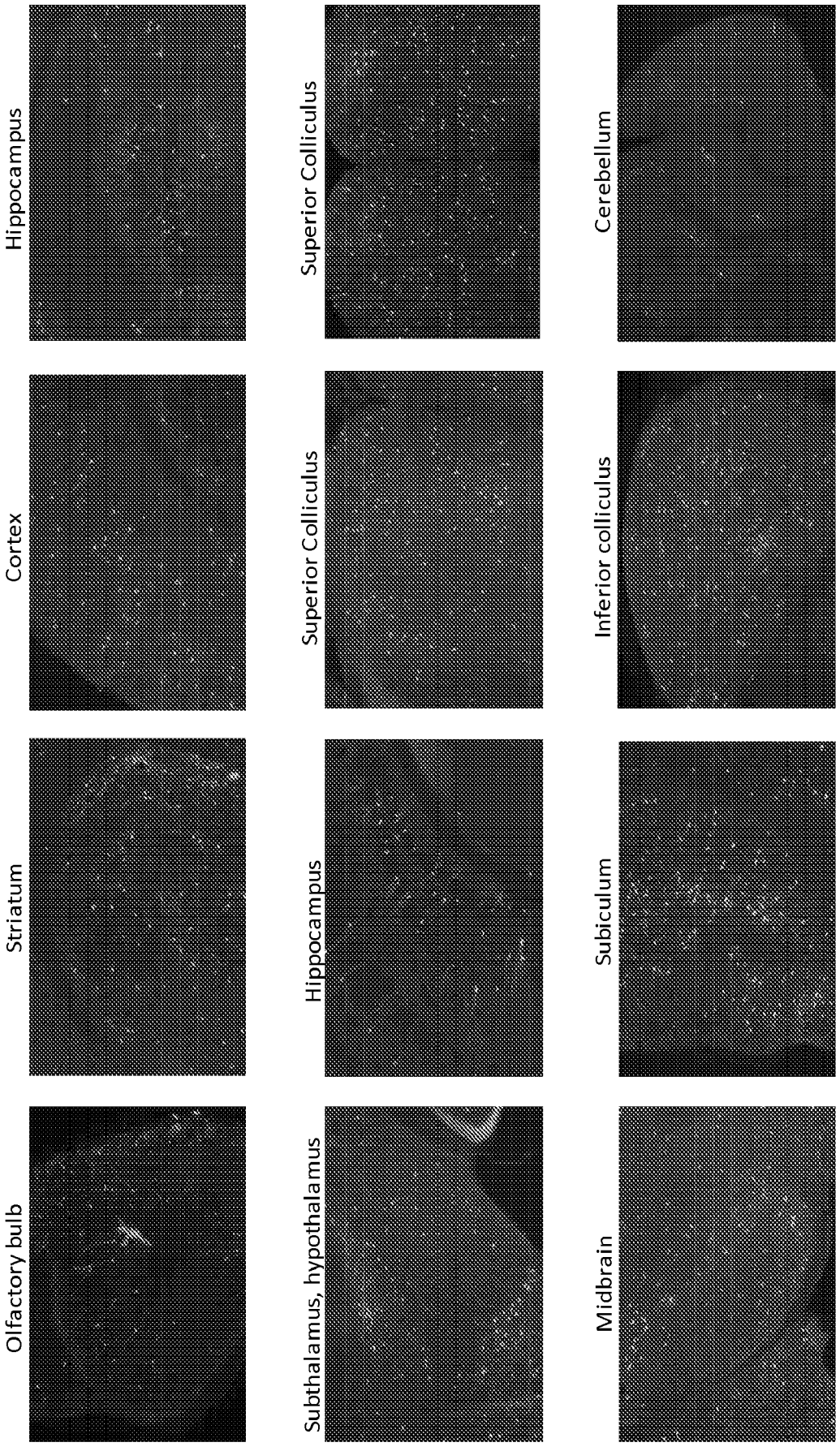
Hippocampus
Superior Colliculus
Cerebellum
Cortex
Superior Colliculus
Inferior colliculus
Striatum
Hippocampus
Subiculum
Olfactory bulb
Subthalamus, hypothalamus
Midbrain

Figure 3B

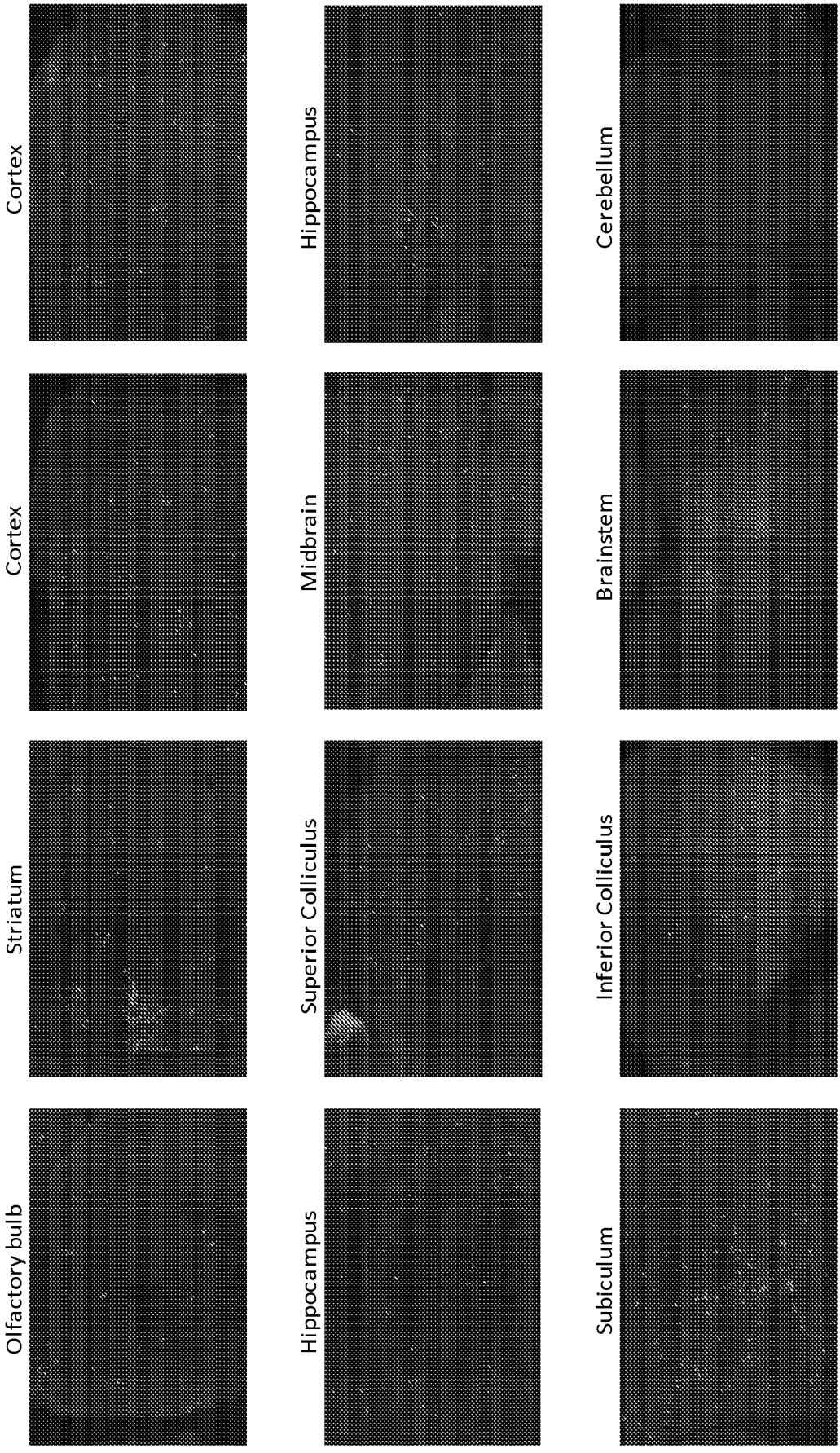
Cortex
Hippocampus
Cerebellum
Cortex
Midbrain
Brainstem
Striatum
Superior Colliculus
Inferior Colliculus
Olfactory bulb
Hippocampus
Subiculum

Figure 3C

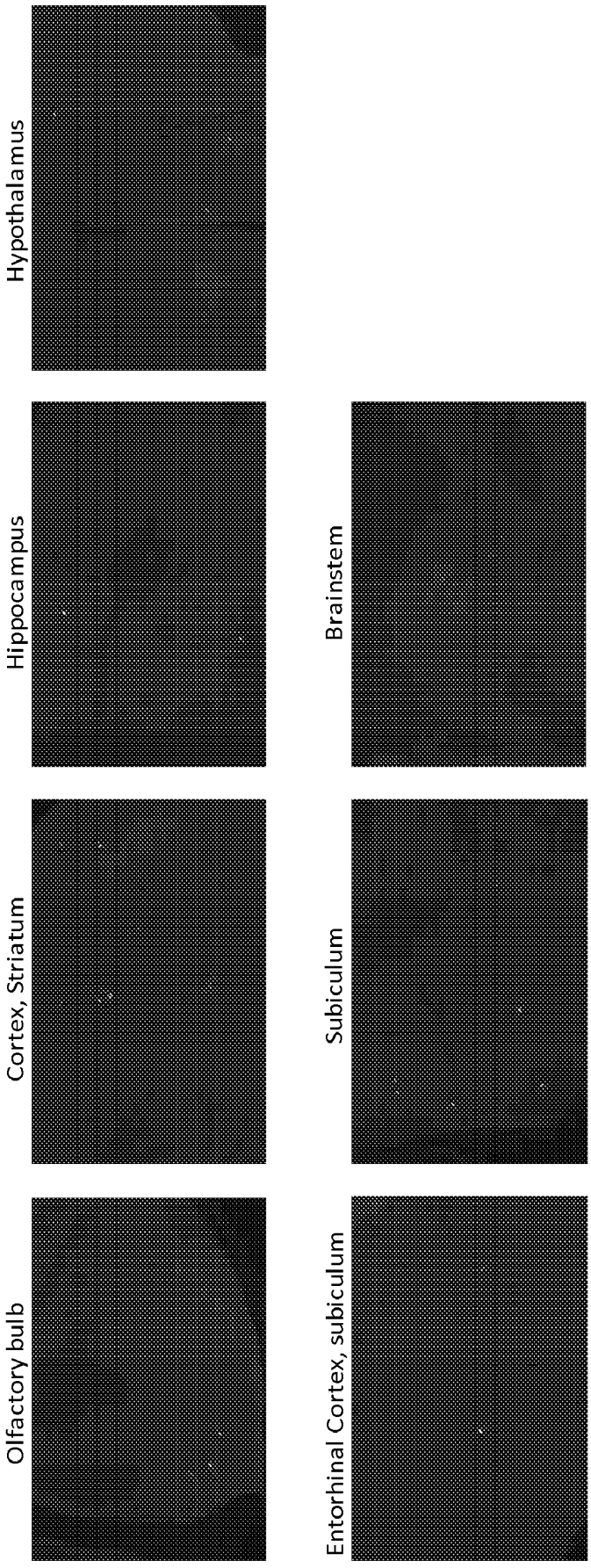
Hypothalamus
Hippocampus
Brainstem
Cortex, Striatum
Subiculum
Olfactory bulb
Entorhinal Cortex, subiculum

Figure 3D

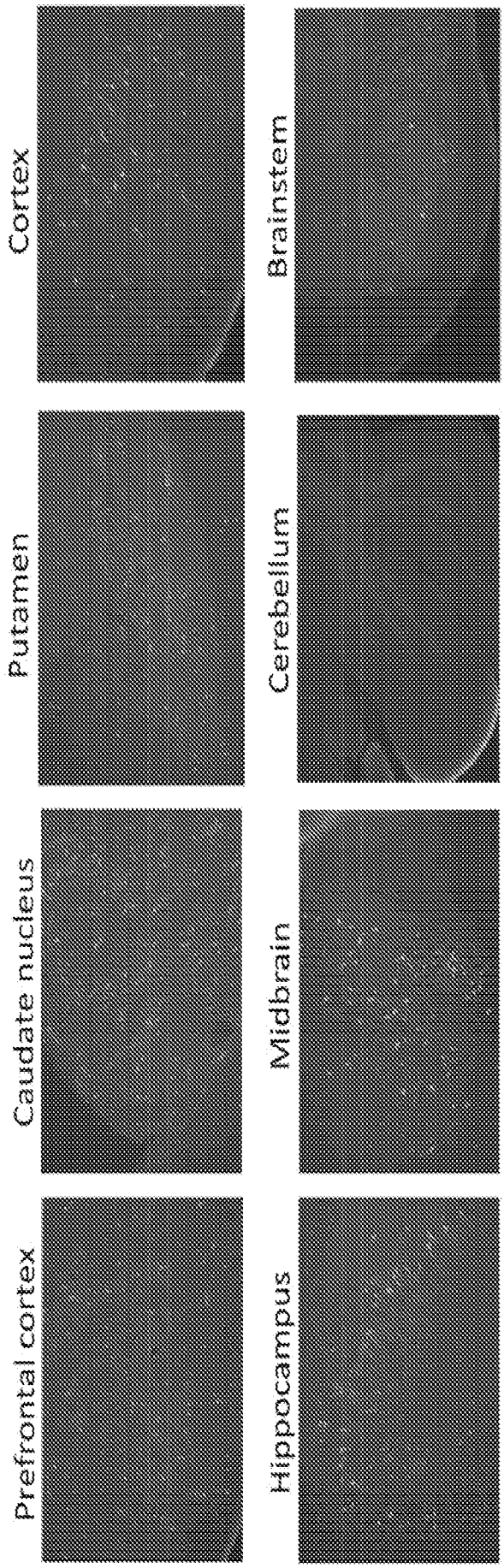
Cortex
Brainstem
Putamen
Cerebellum
Caudate nucleus
Midbrain
Prefrontal cortex
Hippocampus

Figure 4

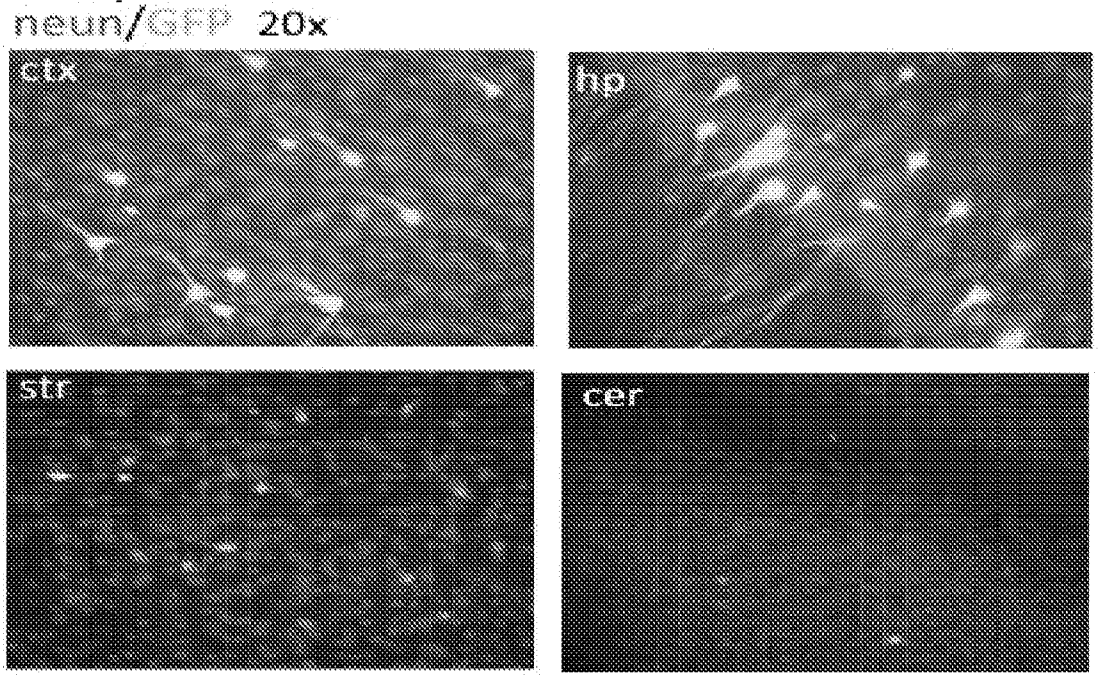

Figure 5A

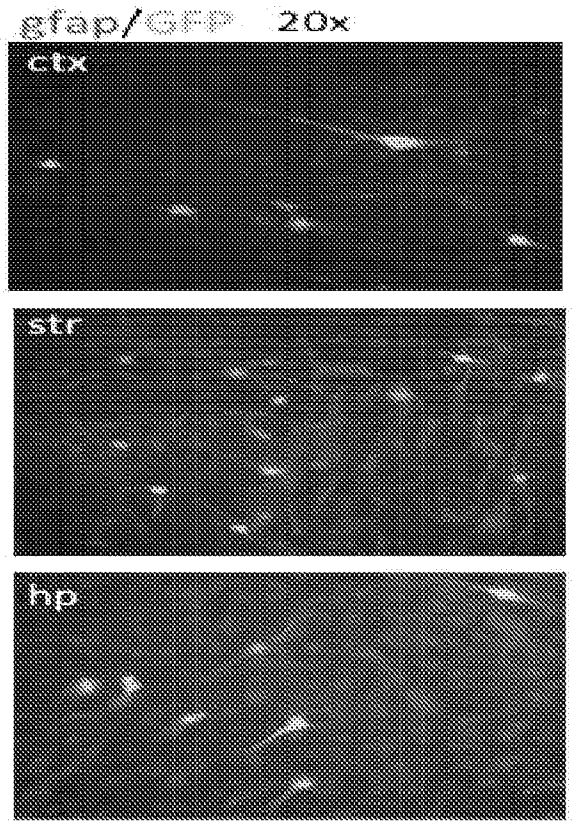

Figure 5B

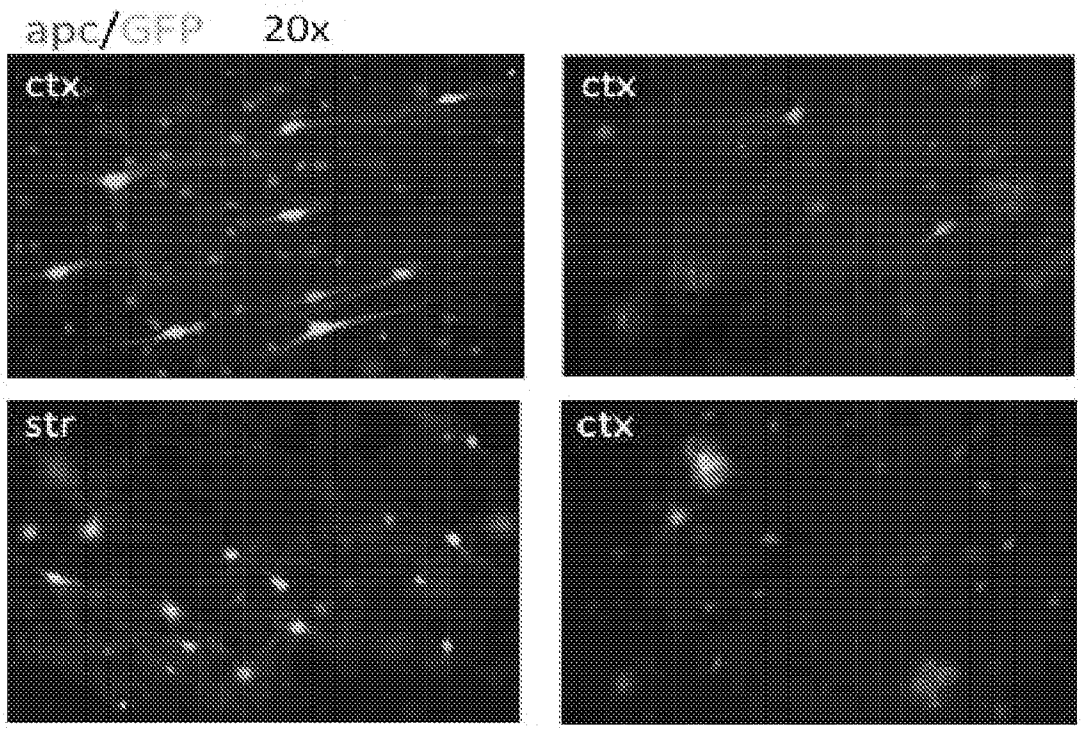

Figure 5C

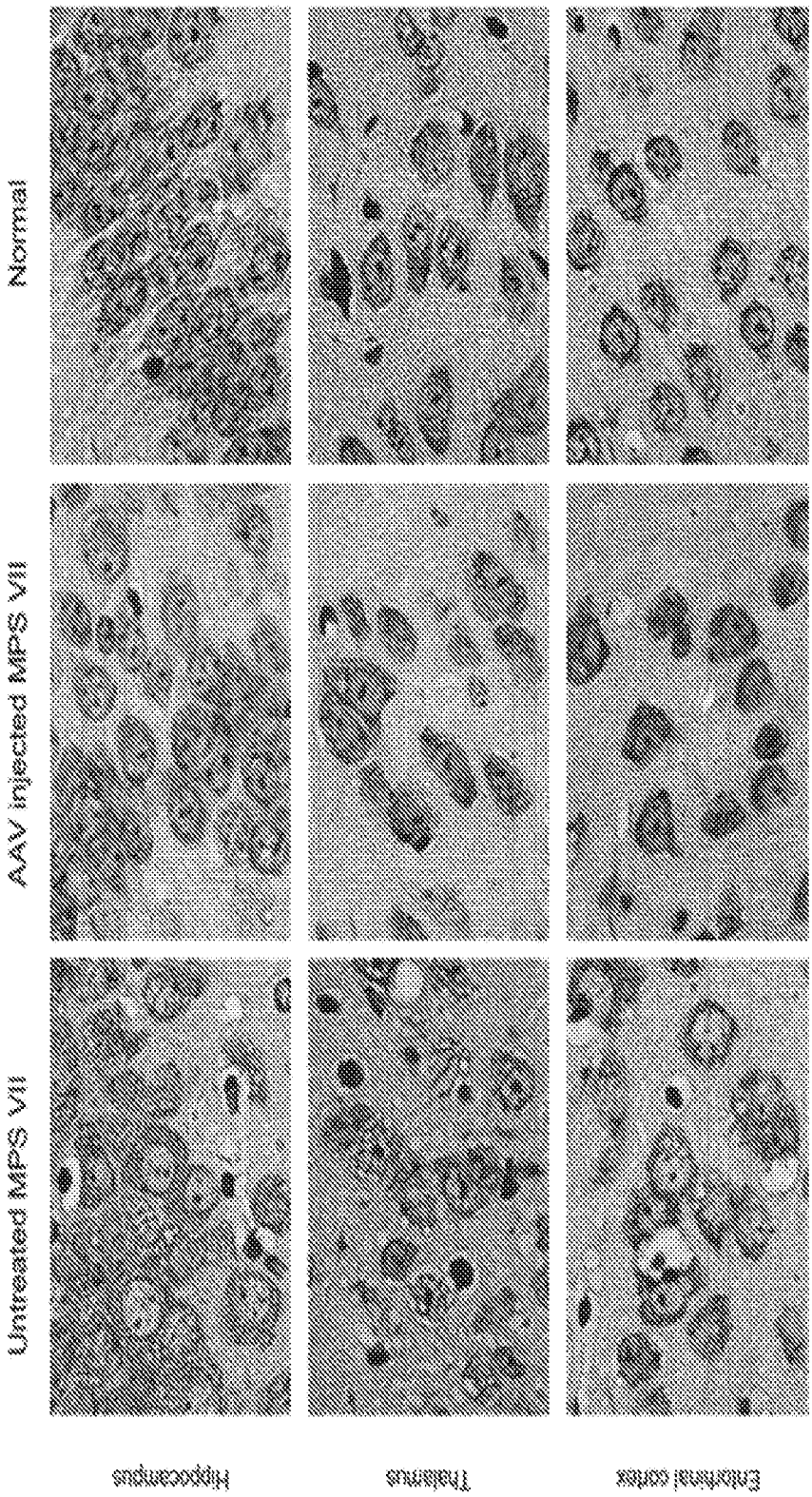
Normal
AAV injected MPS VII
Untreated MPS VII
hippocampus
thalamus
entorhinal cortex

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/25794

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00, C12N 15/86, A61K 39/23, A61K 39/235 (2014.01)
USPC - 435/320.1, 435/456, 424/233.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 435/320.1, 435/456, 424/233.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 435/455, 514/44R, 424/93.2; 424/199.1; 435/325

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: Adeno-associated virus, nucleic acid delivery, brain, capsid protein, brain neuron/nerve cell, AAV, VP1, AAV-9, AAV 9 Clade, b-glucoronidase, dopaminergic neurons, clone hu.32, Q6JC22, Clade F. GenCore 6.4.1: SEQ ID NO:1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/057363 A1 (Muramatsu) 03 MAY 2012 (03.05.2012) para [0067], [0082], [0084], SEQ ID NO:6 and 8	1, 2, 4, 5
X – Y	Cearley, et al. Expanded repertoire of AAV vector serotypes mediate unique patterns of transduction in mouse brain. Mol Ther. 2008, 16(10):1710-8; Abstract, pg 6 [of the posted document], col 2, last para; pg 7, GenBank AY243003	1-3 ----- 6-12
A	GenBank Submission AY243003. Non-human primate Adeno-associated virus isolate AAVrh.32 capsid protein (VP1) gene, complete cds. 14 May 2003. [Retrieved from the Internet 14 June 2014: <http://www.ncbi.nlm.nih.gov/nuccore/AY243003>]; in entirety	1-12
Y	WO 2011/143557 A2 (Wolfe, et al.) 17 November 2011 (17.11.2011) pg 1, ln 23-30; pg 2, ln 25-35; pg 5, ln 20-35; pg 16, ln 8 to pg 17, ln 10	6-12
A	Ross, et al. Development of small alginate microcapsules for recombinant gene product delivery to the rodent brain. J Biomater Sci Polym Ed. 2002;13(8):953-62; pg 954, 2nd para	12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2014 (13.06.2014)	10 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)