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Inventor(s)	Hartung; Thomas et al.

COMPOSITIONS AND METHODS FOR NEURALGENESIS

Abstract

The present invention relates to novel compositions and methods to produce 3D organ equivalents of the brain (i.e. “mini-brains”). The invention also relates to methods of using human induced pluripotent stem cells, a combination of growth and other soluble factors and gyratory shaking. Cells from healthy or diseased donors or animals can be used to allow testing different genetic backgrounds. The model can be further enhanced by using genetically modified cells, adding micro-glia or their precursors or indicator cells (e.g. with reporter genes or tracers) as well as adding endothelial cells to form a blood-brain-barrier.

Inventors: **Hartung; Thomas (Baltimore, MD), Pamies; David (Baltimore, MD), Hogberg; Helena T. (Baltimore, MD)**

Applicant: **The Johns Hopkins University** (Baltimore, MD)

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Background/Summary

RELATED APPLICATIONS [0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/294,112, filed Feb. 11, 2016, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING
[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Mar. 21, 2017, is named 48317-518001WO_SL.txt and is 329,024 bytes in size.
BACKGROUND OF THE INVENTION
[0004] Simple neural in vitro systems do not reflect the physiology, cellular interactions, or genetics of mammalian brain tissue. Accordingly, there is an unmet need to develop human models of brain disorders and/or diseases.
SUMMARY OF THE INVENTION
[0005] The present invention provides brain microphysiological systems (BMPS) that can be produced from induced pluripotent stem cells (iPSCs). Furthermore, the invention provides for reproducible BMPS that differentiate into mature neurons and glial cells (astrocytes and oligodendrocytes) in the central nervous system. This model is electrophysiologically active in a spontaneous manner and may be reproduced with patient cells. The derivation of 3D BMPS from iPSCs has applications in the study and treatment of neurological diseases.
[0006] In an aspect, the disclosure provides an in vitro brain microphysiological system (BMPS), comprising two or more neural cell types aggregated into a spheroid mass, wherein the spheroid mass has a diameter that is less than about 500 μm and the in vitro BMPS is electrophysiologically active in a spontaneous manner.
[0007] In an embodiment, the two or more neural cell types comprise at least a mature neuron and glial cell.
[0008] In an embodiment, the two or more neural cell types further comprise cells selected from the group consisting of astrocytes, polydendrocytes, oligodendrocytes, and combinations thereof.
[0009] In an embodiment, the in vitro BMPS has neural characteristics selected from the group consisting of synaptogenesis, neuron-neuron interactions, neuronal-glial interactions, axon myelination, and combinations thereof.
[0010] In an embodiment, two or more neural cell types of the in vitro BMPS express one or more biomarker selected from the group consisting of GRIN1, GAD1, GABA, TH, LMX1A, FOXO1, FOXA2, FOXO4, CNP, MBP, TH, TUBIII, NEUN, SLC1A6, and any combination thereof.
[0011] In an aspect, the disclosure provides a synthetic neurological organ comprising two or more neural cell types aggregated into a spheroid mass, wherein the spheroid mass has a diameter that is less than 500 μm and the in vitro BMPS is electrophysiologically active in a spontaneous manner.
[0012] In an embodiment, the two or more neural cell types comprise at least a mature neuron and glial cells.
[0013] In an embodiment, the mature neuron and glial cells further comprise cells selected from the group consisting of astrocytes, polydendrocytes, oligodendrocytes, and combinations thereof.
[0014] In an embodiment, the synthetic neurological organ further comprises neural characteristics selected from the group consisting of synaptogenesis, neuron-neuron interactions, neuronal-glial

interactions, axon myelination, and combinations thereof.
[0015] In an embodiment, the synthetic neurological organ mimics the microenvironment of the central nervous system (CNS).
[0016] In an aspect, the disclosure provides a method of reproducibly producing an in vitro brain microphysiological system (BMPS), comprising: inducing one or more pluripotent stem cell (PSC) types; differentiating the one or more PSC types to form one or more neural progenitor cell (NPC) types; exposing the one or more NPC types to gyratory shaking or stirring; and differentiating the one or more NPC types into one or more neural cell types aggregated into a spheroid mass, wherein the spheroid mass has a diameter that is less than 500 μm.
[0017] In an embodiment, the one or more pluripotent stem cells are selected from the group consisting of human or animal embryonic stem cells, iPSC, adult stem cells, fibroblasts, embryonic fibroblasts, peripheral blood mononuclear cells, neuronal precursor cells, mesenchymal stem cells, and combinations thereof.
[0018] In an embodiment, inducing further comprises: adding micro-glia or micro-glia precursor cells.
[0019] In an embodiment, the micro-glia or micro-glia precursor cells are selected from the group consisting of monocytes, human monocytes, pro-monocyte cell lines, iPSC-derived monocytes, hematopoetic stem cells, isolated microglia, immortalized microglia, and combinations thereof.
[0020] In an embodiment, gyratory shaking comprises constant or regular gyratory shaking or stirring for 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, or 8 or more weeks.
[0021] In an embodiment, the one or more growth factors are selected from the group consisting of GDNF, BDNF, GM-CSF, B27, basic FGF, basic EGF, NGF, CNTF, and any combination thereof.
[0022] In an aspect, the disclosure provides a method of cryopreserving an in vitro brain microphysiological system (BMPS), comprising: differentiating BMPS aggregates into one or more mature neurons; incubating the aggregates in a cryopreserving medium; and exposing the aggregates to freezing temperatures of −60° C. or colder.
[0023] In an embodiment, differentiating further comprises: inducing differentiation of one or more pluripotent stem cell types by incubation with one or more growth factors.
[0024] In an embodiment, the one or more pluripotent stem cells are selected from a group consisting of human or animal embryonic stem cells, iPSC, adult stem cells, fibroblasts, embryonic fibroblasts, peripheral blood mononuclear cells, neuronal precursor cells, mesenchymal stem cells, and combinations thereof.
[0025] In an embodiment, inducing further comprises: adding micro-glia precursor cells.
[0026] In an embodiment, micro-glia precursor cells are selected from the group consisting of monocytes, human monocytes, iPSC-derived monocytes, hematopoetic stem cells, pro-monocyte cell lines, isolated microglia, immortalized microglia, and combinations thereof.
[0027] In an embodiment, the one or more growth factors are selected from the group consisting of GDNF, BDNF, GM-CSF, B27, basic FGF, basic EGF, NGF, CNTF, and any combination thereof.
[0028] In an embodiment, the cryopreserving medium is a medium selected from the group consisting of regular cryopreservation medium (95% FBS and 5% DMSO), STEMdiff Neural Progenitor Freezing Medium (Stem Cells Technologies), solutions with cryoprotectants, and combinations thereof.
[0029] In an embodiment, exposing the aggregates to freezing temperatures further comprises freezing aggregates over a temperature gradient of about 1° C. per hour to below −60° C. over up to 48 hours.
[0030] In an embodiment, cryopreserving further comprises additives selected from the group consisting of DMSO, HES, glycerol, serum, and any combination or derivative thereof.
[0031] In an aspect, the disclosure provides a method of transporting a brain microphysiological system (BMPS) or mini-brain, comprising: producing the BMPS or mini-brain of claim **1**, incubating the BMPS or mini-brain at 37° C., and maintaining the temperature at 37° C. with

constant application of heat while moving the BMPS or mini-brain.
[0032] In an embodiment, maintaining the temperature comprises use of heating pads, heaters, insulation, insulated boxes, heat packs, electric blankets, chemical pads, and combinations thereof.
[0033] In an aspect, the disclosure provides a method of studying a neurological disease or disorder comprising: producing an in vitro brain microphysiological system (BMPS); exposing the in vitro BMPS to conditions that replicate or induce the neurological disease or disorder; adding an agent to treat the neurological disease or disorder; and assessing the effect of the agent on the neurological disease or disorder.
[0034] In an embodiment, the neurological disease or disorder is selected from the group consisting of neurodegenerative disorder, muscular dystrophy, Parkinson's Disease, Huntington's Disease, Autism Spectrum Disorder and other neurodevelopmental disorders, Down's Syndrome, Multiple Sclerosis, Amyotrophic lateral sclerosis, brain cancer, encephalitis, infection, trauma, stroke, and paralysis.
[0035] In an aspect, the disclosure provides a method of treating a patient having a neurological disease or disorder, comprising: extracting a stem cell from the patient with a genetic background pre-disposed for the neurological disease or disorder; producing a brain microphysiological system (BMPS) or mini-brain with the genetic background; treating the BMPS or mini-brain with an agent targeting the neurological disease or disorder; and assessing the effect of the agent on the BMPS or mini-brain.
[0036] In an embodiment, the neurological disease or disorder is selected from the group consisting of neurodegenerative disorder, muscular dystrophy, Parkinson's Disease, Huntington's Disease, Autism Spectrum Disorder and other neurodevelopmental disorders, Down's Syndrome, Multiple Sclerosis, Amyotrophic lateral sclerosis, brain cancer, encephalitis, infection, trauma, stroke, and paralysis.
[0037] In an embodiment, the BMPS includes two or more neuronal cell types that include one or more genetically modified cells. The BMPS wherein the one or more genetically modified cells include one or more reporter genes. The BMPS further comprises one or more endothelial cells capable of forming a blood-brain-barrier.
[0038] In an embodiment, the synthetic neurological organ may include two or more neural cell types that include one or more genetically modified cells. The synthetic neurological organ including one or more genetically modified cells that include one or more reporter genes. The synthetic neurological organ further comprising one or more endothelial cells capable of forming a blood-brain-barrier.
[0039] In an aspect, the disclosure provides a method of reproducibly producing an in vitro brain microphysiological system (BMPS), comprising: exposing one or more NPC types to gyratory shaking or stirring; and differentiating the one or more NPC types into one or more neural cell types aggregated into a spheroid mass, wherein the spheroid mass has a diameter that is less than 500 μm.
[0040] In an embodiment, the spheroid mass has a diameter that is less than about 450 μm, 400 μm, 350 μm, or 300 μm, or a diameter that is between about 350 μm and about 300 μm, or a diameter that is between about 330 μm and about 300 μm, or a diameter that is about 310 μm.
[0041] In an embodiment, the two or more neural cell types of the in vitro BMPS express one or more biomarker selected from the group consisting of GRIN1, GAD1, GABA, TH, LMX1A, FOXO1, FOXA2, FOXO4, CNP, MBP, TH, TUBIII, NEUN, SLC1A6, and any combination thereof.
[0042] In an embodiment, the two or more neural cell types of the in vitro BMPS express one or more biomarker selected from the group consisting of GRIN1, GAD1, GABA, TH, LMX1A, FOXO1, FOXA2, FOXO4, CNP, MBP, TH, TUBIII, NEUN, SLC1A6, and any combination thereof.
[0043] In an embodiment, the two or more neural cell types of the in vitro BMPS express one or

more biomarker selected from the group consisting of GRIN1, GAD1, GABA, TH, LMX1A, FOXO1, FOXA2, FOXO4, CNP, MBP, TH, TUBIII, NEUN, SLC1A6, and any combination thereof.

[0044] In an embodiment, inducing comprises a single PSC.

[0045] In an embodiment, the an in vitro brain microphysiological system (BMPS) may be produced according to the above described method.

[0046] It is also contemplated within the scope of the invention that the addition of other cells inside (see e.g., FIG. **6**) and outside (see e.g., FIG. **7**) the BMPS may be used to modify the structure/composition of the BMPS, such as, e.g., by forming a blood-brain-barrier. It is also contemplated that the BMPS described herein may include genetically modified pluripotent stem cells, or be combined with other organoids (see e.g., Example 11).

Definitions

[0047] By “agent” is meant any small compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

[0048] By “alteration” is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

[0049] By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

[0050] In this disclosure, “comprises,” “comprising,” “containing,” and “having” and the like may have the meaning ascribed to them in U.S. Patent law and may mean “includes,” “including,” and the like; “consisting essentially of” or “consists essentially” likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

[0051] “Detect” refers to identifying the presence, absence or amount of the analyte to be detected.

[0052] By “effective amount” is meant the amount of an agent needed to ameliorate the symptoms of a neurological disease relative to an untreated patient. The effective amount of active agent(s) used to practice the present invention for therapeutic treatment of a neurological disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

[0053] By “fragment” is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids, or more.

[0054] By “gene” is meant a locus (or region) of DNA that encodes a functional RNA or protein product, and is the molecular unit of heredity.

[0055] By “marker” is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

[0056] By “modulate” is meant alter (increase or decrease). Such alterations are detected by standard art known methods such as those described herein.

[0057] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9.

[0058] With respect to sub-ranges, “nested sub-ranges” that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.
[0059] By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.
[0060] By “reference” is meant a standard or control condition.
[0061] By “pluripotency” is meant stem cells with the potential to differentiate into any of the three germ layers: endoderm (e.g., interior stomach lining, gastrointestinal tract, the lungs), mesoderm (e.g., muscle, bone, blood, urogenital), or ectoderm (e.g., epidermal tissues and nervous system). However, one of skill in the art will understand that cell pluripotency is a continuum, ranging from the completely pluripotent cell that can form every cell of the embryo proper, e.g., embryonic stem cells and iPSCs (see below), to the incompletely or partially pluripotent cell that can form cells of all three germ layers but that may not exhibit all the characteristics of completely pluripotent cells. Induced pluripotent stem cells, commonly abbreviated as iPS cells or iPSCs are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a “forced” expression of certain genes and transcription factors. These transcription factors play a key role in determining the state of these cells and also highlight the fact that these somatic cells do preserve the same genetic information as early embryonic cells. The ability to induce cells into a pluripotent state was initially pioneered using mouse fibroblasts and four transcription factors, Oct4, Sox2, Klf4 and c-Myc; —a process called reprogramming. The successful induction of human iPSCs derived from human dermal fibroblasts has been performed using methods similar to those used for the induction of mouse cells. These induced cells exhibit similar traits to those of embryonic stem cells (ESCs) but do not require the use of embryos. Some of the similarities between ESCs and iPSCs include pluripotency, morphology, self-renewal ability, a trait that implies that they can divide and replicate indefinitely, and gene expression.
[0062] By “stem cells” is meant undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells. They are found in multicellular organisms. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells—ectoderm, endoderm and mesoderm (see induced pluripotent stem cells)—but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. There are three known accessible sources of autologous adult stem cells in humans: 1. Bone marrow, which requires extraction by harvesting, that is, drilling into bone (typically the femur or iliac crest). 2. Adipose tissue (lipid cells), which requires extraction by liposuction. 3. Blood, which requires extraction through apheresis, wherein blood is drawn from the donor (similar to a blood donation), and passed through a machine that extracts the stem cells and returns other portions of the blood to the donor. Stem cells can also be taken from umbilical cord blood just after birth. Of all stem cell types, autologous harvesting involves the least risk. By definition, autologous cells are obtained from one's own body.
[0063] By “subject” is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.
[0064] As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a neurological disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.
[0065] As used herein, the terms “prevent,” “preventing,” “prevention,” “prophylactic treatment” and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

[0066] Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a,” “an,” and “the” are understood to be singular or plural.

[0067] Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

[0068] A “therapeutically effective amount” is an amount sufficient to effect beneficial or desired results, including clinical results. An effective amount can be administered in one or more administrations.

[0069] By “GRIN1 polypeptide” (or glutamate ionotropic receptor NMDA type subunit 1) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q05586.

TABLE-US-00001 (SEQ ID NO: 1) 1 mstmrlltla llfscsvara acdpkivnig avlstrkheq mfreavnqan krhgswkiql 61 natsvthkpn aiqmalsvce dlissqvyai lvshpptpnd hftptpvsyt agfyripvlg 121 lttrmsiysd ksihlsflrt vppyshqssv wfemmrvysw nhiillvsdd hegraaqkrl 181 etlleeresk aekvlqfdpg tknvtallme akelearvii lsaseddaat vyraaamlnm 241 tgsgyvwlvg ereisgnalr yapdgilglq lingknesah isdavgvvaq avhelleken 301 itdpprgcvg ntniwktgpl fkrvlmssky adgvtgrvef nedgdrkfan ysimnlqnrk 361 lvqvgiyngt hvipndrkii wpggetekpr gyqmstrlki vtihqepfvy vkptlsdgtc 421 keeftvngdp vkkvictgpn dtspgsprht vpqccygfci dlliklartm nftyevhlva 481 dgkfgtqerv nnsnkkewng mmgellsgqa dmivapltin neraqyiefs kpfkyqglti 541 lvkkeiprst ldsfmqpfqs tlwllvglsv hvvavmlyll drfspfgrfk vnseeeeeda 601 ltlssamwfs wgvllnsgig egaprsfsar ilgmvwagfa miivasytan laaflvldrp 661 eeritgindp rlrnpsdkfi yatvkqssvd iyfrrqvels tmyrhmekhn yesaaeaiqa 721 vrdnklhafi wdsavlefea sqkcdlvttg elffrsgfgi gmrkdspwkq nvslsilksh 781 engfmedldk twvryqecds rsnapatltf enmagvfmlv aggivagifl ifieiaykrh 841 kdarrkqmql afaavnvwrk nlqdrksgra epdpkkkatf raitstlass fkrrrsskdt 901 stgggrgalq nqkdtvlprr aiereegqlq lcsrhres

[0070] By “GRIN1 nucleic acid molecule” (or glutamate ionotropic receptor NMDA type subunit 1) is meant a polynucleotide encoding an GRIN1 polypeptide. An exemplary GRIN1 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_007327.

TABLE-US-00002 (SEQ ID NO: 2) 1 gtcgccgcag cgtccggacc ggaaccagcg ccgtccgcgg agccgccgcc gccgccgccg 61 ggccctttcc aagccgggcg ctcggagctg tgcccggccc cgcttcagca ccgcggacag 121 cgccggccgc gtggggctga gccccgagcc cccgcgcacg cttcagcgcc ccttccctcg 181 gccgacgtcc cgggaccgcc gctccggggg agacgtggcg tccgcagccc gcggggccgg 241 gcgagcgcag gacggcccgg aagccccgcg ggggatgcgc cgagggcccc gcgttcgcgc 301 cgcgcagagc caggcccgcg gcccgagccc atgagcacca tgcgcctgct gacgctcgcc 361 ctgctgttct cctgctccgt cgcccgtgcc gcgtgcgacc ccaagatcgt caacattggc 421 gcggtgctga gcacgcggaa gcacgagcag atgttccgcg aggccgtgaa ccaggccaac 481 aagcggcacg gctcctggaa gattcagctc aatgccacct ccgtcacgca caagcccaac 541 gccatccaga tggctctgtc ggtgtgcgag gacctcatct ccagccaggt ctacgccatc 601 ctagttagcc atccacctac ccccaacgac cacttcactc ccacccctgt ctcctacaca 661 gccggcttct accgcatacc cgtgctgggg ctgaccaccc gcatgtccat ctactcggac 721 aagagcatcc acctgagctt cctgcgcacc gtgccgccct actcccacca gtccagcgtg 781 tggtttgaga tgatgcgtgt ctacagctgg aaccacatca tcctgctggt cagcgacgac 841 cacgagggcc gggcggctca gaaacgcctg gagacgctgc tggaggagcg tgagtccaag 901 gcagagaagg tgctgcagtt tgacccaggg accaagaacg tgacggccct gctgatggag 961 gcgaaagagc tggaggcccg ggtcatcatc ctttctgcca gcgaggacga tgctgccact 1021 gtataccgcg cagccgcgat gctgaacatg acgggctccg ggtacgtgtg gctggtcggc 1081 gagcgcgaga tctcggggaa cgccctgcgc tacgccccag acggcatcct cgggctgcag 1141 ctcatcaacg gcaagaacga gtcggcccac atcagcgacg ccgtgggcgt ggtggcccag 1201

gccgtgcacg agctcctcga gaaggagaac atcaccgacc cgccgcgggg ctgcgtgggc 1261 aacaccaaca tctggaagac cgggccgctc ttcaagagag tgctgatgtc ttccaagtat 1321 gcggatgggg tgactggtcg cgtggagttc aatgaggatg gggaccggaa gttcgccaac 1381 tacagcatca tgaacctgca gaaccgcaag ctggtgcaag tgggcatcta caatggcacc 1441 cacgtcatcc ctaatgacag gaagatcatc tggccaggcg gagagacaga gaagcctcga 1501 gggtaccaga tgtccaccag actgaagatt gtgacgatcc accaggagcc cttcgtgtac 1561 gtcaagccca cgctgagtga tgggacatgc aaggaggagt tcacagtcaa cggcgaccca 1621 gtcaagaagg tgatctgcac cgggcccaac gacacgtcgc cgggcagccc ccgccacacg 1681 gtgcctcagt gttgctacgg cttttgcatc gacctgctca tcaagctggc acggaccatg 1741 aacttcacct acgaggtgca cctggtggca gatggcaagt tcggcacaca ggagcgggtg 1801 aacaacagca acaagaagga gtggaatggg atgatgggcg agctgctcag cgggcaggca 1861 gacatgatcg tggcgccgct aaccataaac aacgagcgcg cgcagtacat cgagttttcc 1921 aagcccttca agtaccaggg cctgactatt ctggtcaaga aggagattcc ccggagcacg 1981 ctggactcgt tcatgcagcc gttccagagc acactgtggc tgctggtggg gctgtcggtg 2041 cacgtggtgg ccgtgatgct gtacctgctg gaccgcttca gccccttcgg ccggttcaag 2101 gtgaacagcg aggaggagga ggaggacgca ctgaccctgt cctcggccat gtggttctcc 2161 tggggcgtcc tgctcaactc cggcatcggg gaaggcgccc ccagaagctt ctcagcgcgc 2221 atcctgggca tggtgtgggc cggctttgcc atgatcatcg tggcctccta caccgccaac 2281 ctggcggcct tcctggtgct ggaccggccg gaggagcgca tcacgggcat caacgaccct 2341 cggctgagga acccctcgga caagtttatc tacgccacgg tgaagcagag ctccgtggat 2401 atctacttcc ggcgccaggt ggagctgagc accatgtacc ggcatatgga gaagcacaac 2461 tacgagagtg cggcggaggc catccaggcc gtgagagaca acaagctgca tgccttcatc 2521 tgggactcgg cggtgctgga gttcgaggcc tcgcagaagt gcgacctggt gacgactgga 2581 gagctgtttt tccgctcggg cttcggcata ggcatgcgca aagacagccc ctggaagcag 2641 aacgtctccc tgtccatcct caagtcccac gagaatggct tcatggaaga cctggacaag 2701 acgtgggttc ggtatcagga atgtgactcg cgcagcaacg cccctgcgac ccttactttt 2761 gagaacatgg ccggggtctt catgctggta gctgggggca tcgtggccgg gatcttcctg 2821 attttcatcg agattgccta caagcggcac aaggatgctc gccggaagca gatgcagctg 2881 gcctttgccg ccgttaacgt gtggcggaag aacctgcagg atagaaagag tggtagagca 2941 gagcctgacc ctaaaaagaa agccacattt agggctatca cctccaccct ggcttccagc 3001 ttcaagaggc gtaggtcctc caaagacacg agcaccgggg gtggacgcgg cgctttgcaa 3061 aaccaaaaag acacagtgct gccgcgacgc gctattgaga gggaggaggg ccagctgcag 3121 ctgtgttccc gtcataggga gagctgagac tccccgcccg ccctcctctg ccccctcccc 3181 cgcagacaga cagacagacg gacgggacag cggcccggcc cacgcagagc cccggagcac 3241 cacggggtcg ggggaggagc acccccagcc tcccccaggc tgcgcctgcc cgcccgccgg 3301 ttggccggct ggccggtcca ccccgtcccg gccccgcgcg tgcccccagc gtggggctaa 3361 cgggcgcctt gtctgtgtat ttctattttg cagcagtacc atcccactga tatcacgggc 3421 ccgctcaacc tctcagatcc ctcggtcagc accgtggtgt gaggcccccg gaggcgccca 3481 cctgcccagt tagcccggcc aaggacactg atgggtcctg ctgctcggga aggcctgagg 3541 gaagcccacc cgccccagag actgcccacc ctgggcctcc cgtccgtccg cccgcccacc 3601 ccgctgcctg gcgggcagcc cctgctggac caaggtgcgg accggagcgg ctgaggacgg 3661 ggcagagctg agtcggctgg gcagggccgc agggcgctcc ggcagaggca gggccctggg 3721 gtctctgagc agtggggagc gggggctaac tggccccagg cggaggggct tggagcagag 3781 acggcagccc catccttccc gcagcaccag cctgagccac agtggggccc atggccccag 3841 ctggctgggt cgcccctcct cgggcgcctg cgctcctctg cagcctgagc tccaccctcc 3901 cctcttcttg cggcaccgcc cacccacacc ccgtctgccc cttgacccca cacgccgggg 3961 ctggccctgc cctcccccac ggccgtccct gacttcccag ctggcagcgc ctcccgccgc 4021 ctcgggccgc ctcctccaga ctcgagaggg ctgagcccct cctctcctcg tccggcctgc 4081 agcccagaac gggcctcccc gggggtcccc ggacgctggc tcgggactgt cttcaaccct 4141 gccctgcacc ttgggcacgg gagagcgcca cccgcccgcc cccgccctcg ctccgggtgc 4201 gtgaccggcc cgccaccttg tacagaacca gcactcccag ggcccgagcg cgtgccttcc 4261 ccgtgcggcc cgtgcgcagc cgcgctctgc ccctccgtcc ccagggtgca ggcgcgcacc 4321 gcccaacccc cacctcccgg tgtatgcagt ggtgatgcct aaaggaatgt cacgcagttt 4381 tcaaaaaaaa aaaaaaaaaa

[0071] By “GAD1 polypeptide” (or glutamate decarboxylase 1) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q99259.

TABLE-US-00003 (SEQ ID NO: 3) 1 masstpsssa tssnagadpn ttnlrpttyd

twcgvahgct rklglkicgf lqrtnsleek 61 srlvsafker qssknllsce nsdrdarfrr tetdfsnlfa rdllpaknge eqtvqfllev 121 vdillnyvrk tfdrstkvld fhhphqlleg megfnlelsd hpesleqilv dcrdtlkygv 181 rtghprffnq lstgldiigl agewltstan tnmftyeiap vfvlmeqitl kkmreivgws 241 skdgdgifsp ggaisnmysi maarykyfpe vktkgmaavp klvlftseqs hysikkagaa 301 lgfgtdnvil ikcnergkii padfeakile akqkgyvpfy vnatagttvy gafdpiqeia 361 dicekynlwl hvdaawgggl lmsrkhrhkl ngieransvt wnphkmmgvl lqcsailvke 421 kgilqgcnqm cagylfqpdk qydvsydtgd kaiqcgrhvd ifkfwlmwka kgtvgfenqi 481 nkclelaeyl yakiknreef emvfngepeh tnvcfwyipq slrgvpdspq rreklhkvap 541 kikalmmesg ttmvgyqpqg dkanffrmvi snpaatqsdi dflieeierl gqdl

[0072] By "GAD1 nucleic acid molecule" (or glutamate decarboxylase 1) is meant a polynucleotide encoding an GAD1 polypeptide. An exemplary GAD1 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC036552.

TABLE-US-00004 (SEQ ID NO: 4) 1 agcgtgtggt agaggagaaa cgctgaaacc ggaccgaaac ctcgccctag gcttagcgat 61 ggctaaaaac cggctgggac aagagggagg caagcaacat tccgactcgc tgctttctgg 121 ctgtctggag tgcaaggtga ctgtggttct tctctggcca agtccgaggg agaacgtaaa 181 gatatgggcc tttttccccc tctcaccttg tctcaccaaa gtccctagtc cccggagcag 241 ttagcctctt tctttccagg gaattagcca gacacaacaa cgggaaccag acaccgaacc 301 agacatgccc gccccgtgcg ccctcccccc gctggcccac acgccggctg ctgagtgccc 361 aatggggctt gtagcggctc ggctggaaaa tcgctcactg agcgctcccc tgtgctccta 421 gcccagtccc ccacaccctt gcgtcttgta ctggccttgg acccccaccc cgaccccgac 481 cccgcctcgt ctcggcgctt cactccaggt cgcgccgatg caccgccaga ctcgagagcg 541 gcccagggct acgctccctg cgccccagta ccggagctag cgcgcacgtc tcctccgctg 601 cccccacccc tgcgcacccc taccaggcag gctcgctgcc tttcctccct cttgtctctc 661 cagagccgga tcttcaaggg gagcctccgt gcccccggct gctcagtccc tccggtgtgc 721 aggaccccgg aagtcctccc cgcacagctc tcgcttctct ttgcagcctg tttctgcgcc 781 ggaccagtcg aggactctgg acagtagagg ccccgggacg accgagctga tggcgtcttc 841 gaccccatct tcgtccgcaa cctcctcgaa cgcgggagcg gaccccaata ccactaacct 901 gcgccccaca acgtacgata cctggtgcgg cgtggcccat ggatgcacca gaaaactggg 961 gctcaagatc tgcggcttct tgcaaaggac caacagcctg gaagagaaga gtcgccttgt 1021 gagtgccttc aaggagaggc aatcctccaa gaacctgctt tcctgtgaaa acagcgaccg 1081 ggatgcccgc ttccggcgca cagagactga cttctctaat ctgtttgcta gagatctgct 1141 tccggctaag aacggtgagg agcaaaccgt gcaattcctc ctggaagtgg tggacatact 1201 cctcaactat gtccgcaaga catttgatcg ctccaccaag gtgctggact ttcatcaccc 1261 acaccagttg ctggaaggca tggagggctt caacttggag ctctctgacc accccgagtc 1321 cctggagcag atcctggttg actgcagaga caccttgaag tatggggttc gcacaggtca 1381 tcctcgattt ttcaaccagc tctccactgg attggatatt attggcctag ctggagaatg 1441 gctgacatca acggccaata ccaacatgtt tacatatgaa attgcaccag tgtttgtcct 1501 catggaacaa ataacactta agaagatgag agagatagtt ggatggtcaa gtaaagatgg 1561 tgatgggata ttttctcctg ggggcgccat atccaacatg tacagcatca tggctgctcg 1621 ctacaagtac ttcccggaag ttaagacaaa gggcatggcg gctgtgccta aactggtcct 1681 cttcacctca gaacagagtc actattccat aaagaaagct ggggctgcac ttggctttgg 1741 aactgacaat gtgattttga taaagtgcaa tgaaaggggg aaaataattc cagctgattt 1801 tgaggcaaaa attcttgaag ccaaacagaa gggatatgtt ccctttatg tcaatgcaac 1861 tgctggcacg actgtttatg gagcttttga tccgatacaa gagattgcag atatatgtga 1921 gaaatataac ctttggttgc atgtcgatgg atttaacttc tcacaattgg ccaataggat 1981 catctgcctt gctactgaac taatgactaa caaaggctgt gtcacgtggc atcccaacta 2041 ttcagtaaac atgcatcatg gctgcctggg gaggtgggct gctcatgtcc aggaagcacc 2101 accataaact caacggcata gaaagggcca actcagtcac ctggaaccct cacaagatga 2161 tgggcgtgct gttgcagtgc tctgccattc tcgtcaagga aaagggtata ctccaaggat 2221 gcaaccagat gtgtgcagga tacctcttcc agccagacaa gcagtatgat gtctcctacg 2281 acaccgggga caaggcaatt cagtgtggcc gccacgtgga tatcttcaag ttctggctga 2341 tgtggaaagc aaagggcaca gtgggatttg aaaaccagat caacaaatgc ctggaactgg 2401 ctgaatacct ctatgccaag attaaaaaca gagaagaatt tgagatggtt ttcaatggcg 2461 agcctgagca cacaaacgtc tgtttttggt atattccaca aagcctcagg ggtgtgccag 2521 acagccctca acgacgggaa aagctacaca aggtggctcc aaaaatcaaa gccctgatga 2581 tggagtcagg tacgaccatg

gttggctacc agccccaagg ggacaaggcc aacttcttcc 2641 ggatggtcat ctccaaccca gccgctaccc agtctgacat tgacttcctc attgaggaga 2701 tagaaagact gggccaggat ctgtaatcat ccttcgcaga acatgagttt atgggaatgc 2761 cttttccctc tggcactcca gaacaaacct ctatatgttg ctgaaacaca caggccattt 2821 cattgaggga aaacataata tcttgaagaa tattgttaaa accttactta aagcttgttt 2881 gttctagtta gcaggaaata gtgttctttt taaaaagttg cacattagga acagagtata 2941 tatgtacagt tatacatacc tctctctata tatacatgta tagtgagtgt ggcttagtaa 3001 tagatcacgg catgtttccc gctccaagag aattcacttt accttcagca gttaccgagg 3061 agctaaacat gctgccaacc agcttgtcca acaactccag gaaaactgtt tttcaaaacg 3121 ccatgtccta ggggccaagg gaaatgctgt tggtgagaat cgacctcact gtcagcgttt 3181 ctccacctga agtgatgatg gatgagaaaa aacaccacca aatgacaagt cacaccctcc 3241 ccattagtat cctgttaggg gaaaatagta gcagagtcat tgttacaggt gtactatggc 3301 tgtattttta gagattaatt tgtgtagatt gtgtaaattc ctgttgtctg accttggtgg 3361 tgggaggggg agactatgtg tcatgatttc aatgattgtt taattgtagg tcaatgaaat 3421 atttgcttat ttatattcag agatgtacca tgttaaagag gcgtcttgta ttttcttccc 3481 atttgtaatg tatcttattt atatatgaag taagttctga aaactgttta tggtattttc 3541 gtgcatttgt gagccaaaga gaaaagatta aaattagtga gatttgtatt tatattagag 3601 tgcccttaaa ataatgattt aagcatttta ctgtctgtaa gagaattcta agattgtaca 3661 taaagtcata tatatggaaa tcctgttact taaatagcat ctgctcttct cttacgctct 3721 ctgtctggct gtacgtctgg tgttctcaat gcttttctag caactgttgg ataataacta 3781 gatctcctgt aattttgtag tagttgatga ccaatctctg ttactcgctt agctgaaacc 3841 taaggcaaca tttccgaaga ccttctgaag atctcagata aagtgaccag gctcacaact 3901 gtttttgaag aagggaaatt cacactgtgc gttttagagt atgcaagaag aatataaata 3961 aataaaaata ttctccatgg agaatttgaa caaaaaaaaa aaaaaaa

[0073] By “GABA polypeptide” (or gamma-Aminobutyric acid) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P30531.

TABLE-US-00005 (SEQ ID NO: 5) 1 matngskvad gqistevsea pvandkpktl vvkvqkkaad lpdrdtwkgr fdflmscvgy 61 aiglgnvwrf pylcgknggg aflipyfltl ifagvplfll ecslgqytsi gglgvwklap 121 mfkgvglaaa vlsfwlniyy iviiswaiyy lynsftttlp wkqcdnpwnt drcfsnysmv 181 nttnmtsavv efwernmhqm tdgldkpgqi rwplaitlai awilvyfciw kgvgwtgkvv 241 yfsatypyim liilffrgvt lpgakegilf yitpnfrkls dsevwldaat qiffsyglgl 301 gslialgsyn sfhnnvyrds iivccinsct smfagfvifs ivgfmahvtk rsiadvaasg 361 pglaflaype avtqlpispl wailffsmll mlgidsqfct vegfitalvd eyprllrnrr 421 elfiaavcii syliglsnit qqgiyvfklf dyysasgmsl lflvffecvs iswfygvnrf 481 ydniqemvgs rpciwwklcw sfftpiivag vfifsavqmt pltmgnyvfp kwgqgvgwlm 541 alssmvlipg ymaymfltlk gslkqriqvm vqpsedivrp engpeqpqag sstskeayi

[0074] By “GABA nucleic acid molecule” (or gamma-Aminobutyric acid) is meant a polynucleotide encoding an GABA polypeptide. An exemplary GABA nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. U76343.

TABLE-US-00006 (SEQ ID NO: 6) 1 gtagcttcac taaggtggga tggatagcag ggtctcaggc acaaccagta atggagagac 61 aaaaccantg tatcacaaga tggagtttgt gctgtcagtg gctggggaga tcattggctt 121 aggcaacgtc tggaggtttc cctatctctg ctacaaaaat gggggaggtg ccttcttcat 181 cccctacctc gtcttcctct ttacctgtgg cattcctgtc ttccttctgg agacagcact 241 aggccagtac actagccagg gaggcgtcac agcctggagg aagatctgcc ccatctttga 301 gggcattggc tatgcctccc agatgatcgt catcctcctc aacgtctact acatcattgt 361 gttggcctgg gccctgttct acctcttcag cagcttcacc atcgacctgc cctggggcgg 421 ctgctaccat gagtggaaca cagaacactg tatggagttc cagaagacca acggctccct 481 gaatggtacc tctgagaatg ccacctctcc tgtcatcgag ttctgggagc ggcgggtctt 541 gaagatctct gatgggatcc agcacctggg ggccctgcgc tgggagctgg ctctgtgcct 601 cctgctggcc tgggtcatct gctacttctg catctggaag ggggtgaagt ccacaggcaa 661 ggtggtgtac ttcacggcca catttcctta cctcatgctg gtggtcctgt taattcgagg 721 ggtgacgttg cctggggcag cccaaggaat tcagttttac ctgtacccaa acctcacgcg 781 tctgtgggat ccccaggtgt ggatggatgc aggcacccag atattcttct ccttcgccat 841 ctgtcttggg tgcctgacag ccctgggcag ctacaacaag taccacaaca actgctacag 901 cggcaccagc tttgtggccg gctttgccat cttctccatc ctgggcttca tgtctcagga 961 gcaggggggtg cccatttctg aggtggccga gtcaggccct ggcctggctt

tcatcgctta 1021 cccgcgggct gtggtgatgc tgcccttctc tcctctctgg gcctgctgtt tcttcttcat 1081 ggtcgttctc ctgggactgg atagccagtt tgtgtgtgta gaaagcctgg tgacagcgct 1141 ggtggacatg taccctcacg tgttccgcaa gaagaaccgg agggaagtcc tcatccttgg 1201 agtatctgtc gtctccttcc ctgtggggct gatcatgctc acagagggcg gaatgtacgt 1261 gttccagctc tttgactact atgcggccag tggcatgtgc ctcctgttcg tggccatctt 1321 cgagtccctc tgtgtggctt gggtttacgg agccaagcgc ttctacgaca acatcgaaga 1381 catgattggg tacaggccat ggcctcttat caaatactgt tggctcttcc tcacaccagc 1441 tgtgtgcaca gccacctttc tcttctccct gataaagtac actccgctga cctacaacaa 1501 gaagtacacg tacccgtggt ggggcgatgc cctgggctgg ctcctggctc tgtcctcctg 1561 gtctgcattc ctgcctggag cctctacaga ctcggaaccc tcaagggccc cttcagagag 1621 agaatccgtc agctcatgtg cccagccgag gacctgcccc agcggaaccc agcaggaccc 1681 tcggctcccg ccacccccag gacctcactg ctcagactca cagagctaga gtctcactgc 1741 taggggggcag gcccttggat ggtgcctgtg tgcctggcct tggggatggc tgtggaggga 1801 acgtggcaga agcagcccca tgtgttccct gcccccgacc tggagtggat aagacaagag 1861 gggtattttg gagtccacct gctgagctgg aggcctccca ctgcaacttt tcagctcagg 1921 ggttgttgaa cagatgtgaa aaggccagtg ccaagagtgt ccctcggaga cccttgaagg 1981 c

[0075] By “TH polypeptide” (or Tyrosine Hydroxylase) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_002692.

TABLE-US-00007 (SEQ ID NO: 7) 1 mptpdattpq akgfrravse ldakqaeaim vrgqgapgps ltgspwpgta apaasytptp 61 rsprfigrrq sliedarker eaavaaaaaa vpsepgdple avafeekegk avlnllfspr 121 atkpsalsra vkvfetfeak ihhletrpaq rpraggphle yfvrlevrrg dlaallsgvr 181 qvsedvrspa gpkvpwfprk vseldkchhl vtkfdpdldl dhpgfsdqvy rqrrkliaei 241 afqyrhgdpi prveytaeei atwkevyttl kglyathacg ehleafalle rfsgyredni 301 pqledvsrfl kertgfqlrp vagllsardf laslafrvfq ctqyirhass pmhspepdcc 361 hellghvpml adrtfaqfsq diglaslgas deeieklstl ywftvefglc kqngevkayg 421 agllssygel lhclseepei rafdpeaaav qpyqdqtyqs vyfvsesfsd akdklrsyas 481 riqrpfsvkf dpytlaidvl dspqavrrsl egvqdeldtl ahalsaig

[0076] By “TH nucleic acid molecule” (or Tyrosine Hydroxylase) is meant a polynucleotide encoding an TH polypeptide. An exemplary TH nucleic acid molecule is provided at NCBI Accession No. NG_008128.

TABLE-US-00008 (SEQ ID NO: 8) 1 gcgggggggc agtgtgtgct ccagcatgtg tgtgtgtgtg tgcatgtaca cgtgtgcacc 61 tgtatcgcct gtgtgtgtgc atgtgatgtg tacacgtgtc atgcatgcac gcacatgtgt 121 agtgtgtgct cgtgtgtggt gtgtgcctgt gtcatgtatg agcacacttg tatatgttgt 181 gtgtactgtg tcatatatga gtgtgtttgc ctgtgtagtg catgcacatc cgtgtgtgca 241 tctggtgtgt ccgtgggtca ttacgagtgc atcgtatgtg tatcgtgtac atgagtacac 301 ttgtatgtgt ggtgtgtaca ggtgccatgt aagtgtgctt gtacatatat gcatgcatgt 361 gtcatatgca tctgtgtgtg catgtgtgtg gtgcacacat gtgttatgtc tgagtgtgcc 421 tgtatgtgtg ctatgtacac gtcatgtgtg agtgtgcttg catgtgcagt gtgtggatgc 481 tgcttgtacc tgtggtgtgt acctgtgtca tgggtgctca cacgtgcatg gagtgttgtg 541 tgtgtgcttg tgtgccccat gtgtgcatgt gtgtgtgcct cacacagatg cctgcatttg 601 cctaggcact tgcaagagga caccatgctg gctctcaaag atcacagggc cacctgagcc 661 ctgtgcacac cacagccagg ccatggctag accctgcaga gccacagggc gatgcctgtc 721 agccagggga cccagaacac ctcctgggct cctccccagc acatggctgg gctcctccag 781 caggcctgga tttgggaagg gcccgtggtg ggcaaggctg gtgctgggga gcaggcctgg 841 tggcctcaga gactcgccct gtgggcggag cagcctcaca gccaggtcga agtcagcact 901 ctgaccctgc cccacgcggg gagtgggcac cagtcccagg gcacagacgt gctgggtgat 961 taatctgggt gattaagcct cgggctgaga ggctgttgag agagaacacg ctccattgtg 1021 gagctggctc agcattcctt acggccatgg tggcaggggc tgtaaccaca gggacggcgg 1081 aagtggtgga gggtggtggg gtatggaggg aagcccagag ggctccgtgc aggaaggtgg 1141 agcctggtgc aatggagggg acagcaaggg ctcctcagac ctctgcgggg cccccactcc 1201 cctggtcacc tgttttgtct ctgatctggc ctgggtcggc cctcactcct ggccccacct 1261 catagccccc cctggtgggg ctccgctcca gcccttctcc ttcccagggg ccagtatgct 1321 ggccccaggg gtctcttggg gcgtgacctc ggcctccaga gaaccctgtc ccagctctgc 1381 ccttccctct ggggtctctg tagatgggac gctggtcaca gcagcctgtc

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[0077] By "LMX1A polypeptide" (or LIM homeobox transcription factor 1-alpha) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q8TE12.

TABLE-US-00009 (SEQ ID NO: 9) 1 mldglkmeen fqsaidtsas fssllgravs pksvcegcqr vildrfllrl ndsfwheqcv 61 qcasckeple ttcfyrdkkl yckydyeklf avkcggcfea iapnefvmra qksvyhlscf 121 cccvcerqlq kgdefvlkeg qllckgdyek erellslvsp aasdsgksdd eeslcksahg 181 agkgtaeegk dhkrpkrprt ilttqqrraf kasfevsskp crkvretlaa etglsvrvvq 241 vwfqnqrakm kklarrqqqq qqdqqntqrl ssaqtngggs agmegimnpy talptpqqll 301 aieqsvyssd pfrqgltppq mpgdhmhpyg aeplfhdlds ddtslsnlgd cflatseagp 361 lqsrvgnpid hlysmqnsyf ts

[0078] By "LMX1A nucleic acid molecule" (or LIM homeobox transcription factor 1-alpha) is meant a polynucleotide encoding an LMX1A polypeptide. An exemplary LMX1A nucleic acid molecule is provided at NCBI Accession No. AH011517.

TABLE-US-00010 (SEQ ID NO: 10) 1 gtataggttg gggcggagtc ggattcggga tggaaaacct ggggcaaggg atgtaggtgg 61 gggtgagggg ggcaggagaa ggagaaacgc agttgggggg cggaggccta agtacataac 121 gtgttgactt caagtgaaat cagatcagcc agagcagttc gctgtgactg atctctcctc 181 ccaccctaca ttctcttggc tggaccctat cctcctggct gattctggtc gccctggaca 241 ctccctcagt tctttcccag gagtgcggtg gctgctggcg ccgagtccca gcgggcacgg 301 acgtcagacg catcgtttct tctcctctac aggtcctccc ggcccggccc gaacatgctg 361 gacggcctaa agatggagga gaacttccaa agcgcgatcg acacctcggc ctccttctcc 421 tcgctgctgg gtgagtgttc aggccgtgcg tcctgggcgc actctctttc cgcttggcgc 481 tgagctctgg agccccgctc tctgggacct ggtccgcgat agggaagcta gcgcccctct 541 tcatacacta aattgagccc catcactatc tgtccgtcag tgcttgtggg tcgtccctac 601 ccaaataaat ccaacaagcc gccccaggcc tcacgcactg ggcaccgaat tccccaaagc 661 cgcgaggggc gggcgagctt gttcgtaggc gtctgagtgg caagtgatta aaaataccca 721 gggctggatt tttaatctcg gagctgatcg acgtctcata aatgccgccc tcttctcgcg 781 gcctagaggc aatagcatcc gagacccgag gcctggagcg cccaagttcg aggaggcttc 841 tctcccccac caactccagc cccaatttca gccatgggca aggccgagag agacttttct 901 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 961 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn gggtcccggc caggtttggc 1021 atggtctacc tgcccgggct gctcacccgc caacgtctgt tgtggctaca ggcagagcgg 1081 tgagccccaa gtctgtctgc gagggctgtc agcgggtcat cttggacagg tttctgctgc 1141 ggctcaacga cagcttctgg catgagcagt gcgtgcagtg cgcctcctgc aaagagcccc 1201 tggagaccac ctgcttctac cgggacaaga agctgtactg caagtatgac tacgagaagt 1261 aagtggccgc acccccgcag cgctccccgc gcactggcat nnnnnnnnnn nnnnnnnnnn 1321 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 1381 nnnnnnnnnn nnnnnnnnnn atcccagttc ttgaagttcc ttttgctgtt gacttcaggg 1441 gagacccagg accaagccag attttactca tggtgcatgt acttcctttc tccctgctgc 1501 caggctgttt gctgttaaat gtgggggctg cttcgaggcc atcgctccca atgagtttgt 1561 tatgcgggcc cagaagagtg tataccacct gagctgcttc tgctgctgtg tctgcgagcg 1621 acagcttcag aagggtgatg agtttgtcct gaaggagggg cagctgctct gcaaagggga 1681 ctatgagaag gagcgggagc tgctcagcct ggtgagccca gcagcctcag actcaggtga 1741 gtgccaggtg gtgggcaggg ctgcggtggg gtgggtagag tggagttggg tggctgtctg 1801 cattgtttct tccctagatg nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 1861 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 1921 catacagctc caggaactgg ctttcaggga ctcacaacat tgtcttttgc ttctttcagg 1981 taaaagtgat gatgaagaaa gtctctgcaa gtcagcccat ggggcaggga aaggaactgc 2041 tgaggaaggc aaggaccata agcgccccaa acgtccgaga accatcttga caactcaaca 2101 gaggcgagca ttcaaggcct catttgaagt atcctccaag ccctgcagga aggtatagga 2161 gggagcaggg aggaaaagga gctgggcccc acttctctgt

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[0079] By “FOXO1 polypeptide” (or Forkhead box protein O1) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q12778.

TABLE-US-00011 (SEQ ID NO: 11) 1 maeapqvvei dpdfeplprp rsctwplprp efsqsnsats spapsgsaaa npdaaaglps 61 asaaavsadf msnlsllees edfpqapgsv aaavaaaaaa

aatgglcgdf qgpeagclhp 121 appqpppgp lsqhppvppa aagplagqpr kssssrrnaw gnlsyadlit kaiessaekr 181 ltlsqiyewm vksvpyfkdk gdsnssagwk nsirhnlslh skfirvqneg tgksswwmln 241 peggksgksp rrraasmdnn skfaksrsra akkkaslqsg qegagdspgs qfskwpaspg 301 shsnddfdnw stfrprtssn astisgrlsp imteqddlge gdvhsmvypp saakmastlp 361 slseisnpen menlldnlnl lssptsltvs tqsspgtmmq qtpcysfapp ntslnspspn 421 yqkytygqss msplpqmpiq tlqdnkssyg gmsqyncapg llkelltsds pphndimtpv 481 dpgvaqpnsr vlgqnvmmgp nsvmstygsq ashnkmmnps shthpghaqq tsavngrplp 541 htvstmphts gmnrltqvkt pvqvplphpm qmsalggyss vsscngygrm gllhqeklps 601 dldgmfierl dcdmesiirn dlmdgdtldf nfdnvlpnqs fphsvkttth swvsg

[0080] By “FOXO1 nucleic acid molecule” (or Forkhead box protein 01) is meant a polynucleotide (e.g., mRNA) encoding an FOXO1 polypeptide. An exemplary FOXO1 nucleic acid molecule is provided at NCBI Accession No. NM_002015.

TABLE-US-00012 (SEQ ID NO: 12) 1 gcagccgcca cattcaacag gcagcagcgc agcgggcgcg ccgctgggga gagcaagcgg 61 cccgcggcgt ccgtccgtcc ttccgtccgc ggccctgtca gctggagcgc ggcgcaggct 121 ctgccccggc ccggcggctc tggccggccg tccagtccgt gcggcggacc ccgaggagcc 181 tcgatgtgga tggccccgcg aagttaagtt ctgggctcgc gcttccactc cgccgcgcct 241 tcctcccagt ttccgtccgc tcgccgcacc ggcttcgttc ccccaaatct cggaccgtcc 301 cttcgcgccc cctccccgtc cgcccccagt gctgcgttct ccccctcttg gctctcctgc 361 ggctggggga ggggcggggg tcaccatggc cgaggcgcct caggtggtgg agatcgaccc 421 ggacttcgag ccgctgcccc ggccgcgctc gtgcacctgg ccgctgccca ggccggagtt 481 tagccagtcc aactcggcca cctccagccc ggcgccgtcg ggcagcgcgg ctgccaaccc 541 cgacgccgcg gcgggcctgc cctcggcctc ggctgccgct gtcagcgccg acttcatgag 601 caacctgagc ttgctggagg agagcgagga cttcccgcag gcgcccggct ccgtggcggc 661 ggcggtggcg gcggcggccg ccgcggccgc caccgggggg ctgtgcgggg acttccaggg 721 cccggaggcg ggctgcctgc acccagcgcc accgcagccc ccgccgcccg ggccgctgtc 781 gcagcacccg ccggtgcccc ccgccgccgc tgggccgctc gcggggcagc cgcgcaagag 841 cagctcgtcc cgccgcaacg cgtggggcaa cctgtcctac gccgacctca tcaccaaggc 901 catcgagagc tcggcggaga agcggctcac gctgtcgcag atctacgagt ggatggtcaa 961 gagcgtgccc tacttcaagg ataagggtga cagcaacagc tcggcgggct ggaagaattc 1021 aattcgtcat aatctgtccc tacacagcaa gttcattcgt gtgcagaatg aaggaactgg 1081 aaaaagttct tggtggatgc tcaatccaga gggtggcaag agcgggaaat ctcctaggag 1141 aagagctgca tccatggaca acaacagtaa atttgctaag agccgaagcc gagctgccaa 1201 gaagaaagca tctctccagt ctggccagga gggtgctggg gacagccctg gatcacagtt 1261 ttccaaatgg cctgcaagcc ctggctctca cagcaatgat gactttgata actggagtac 1321 atttcgccct cgaactagct caaatgctag tactattagt gggagactct cacccattat 1381 gaccgaacag gatgatcttg gagaagggga tgtgcattct atggtgtacc cgccatctgc 1441 cgcaaagatg gcctctactt tacccagtct gtctgagata agcaatcccg aaaacatgga 1501 aaatcttttg gataatctca accttctctc atcaccaaca tcattaactg tttcgaccca 1561 gtcctcacct ggcaccatga tgcagcagac gccgtgctac tcgtttgcgc caccaaacac 1621 cagtttgaat tcacccagcc caaactacca aaaatataca tatggccaat ccagcatgag 1681 ccctttgccc cagatgccta tacaaacact tcaggacaat aagtcgagtt atggaggtat 1741 gagtcagtat aactgtgcgc ctggactctt gaaggagttg ctgacttctg actctcctcc 1801 ccataatgac attatgacac cagttgatcc tggggtagcc cagcccaaca gccgggttct 1861 gggccagaac gtcatgatgg gccctaattc ggtcatgtca acctatggca gccaggcatc 1921 tcataacaaa atgatgaatc ccagctccca tacccaccct ggacatgctc agcagacatc 1981 tgcagttaac gggcgtcccc tgccccacac ggtaagcacc atgccccaca cctcgggtat 2041 gaaccgcctg acccaagtga agacacctgt acaagtgcct ctgccccacc ccatgcagat 2101 gagtgccctg gggggctact cctccgtgag cagctgcaat ggctatggca gaatgggcct 2161 tctccaccag gagaagctcc caagtgactt ggatggcatg ttcattgagc gcttagactg 2221 tgacatggaa tccatcattc ggaatgacct catggatgga gatacattgg attttaactt 2281 tgacaatgtg ttgcccaacc aaagcttccc acacagtgtc aagacaacga cacatagctg 2341 ggtgtcaggc tgagggttag tgagcaggtt acacttaaaa gtacttcaga ttgtctgaca 2401 gcaggaactg agagaagcag tccaaagatg tctttcacca actccctttt agttttcttg 2461 gttaaaaaaa aaaacaaaaa aaaaaaccct ccttttttcc tttcgtcaga cttggcagca 2521 aagacatttt

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tactctgaga agtgcctgat gttgatgtac ttacagacac 5581 aagaacaatc tttgctataa ttgtataaag
ccataaatgt acataaatta tgtttaaatg 5641 gcttggtgtc tttcttttct aattatgcag aataagctct
ttattaggaa ttttttgtga 5701 agctattaaa tacttgagtt aagtcttgtc agccacaa

[0081] By “FOXA2 polypeptide” (or Forkhead box protein A2) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q9Y261.

TABLE-US-00013 (SEQ ID NO: 13) 1 mlgavkmegh epsdwssyya epegyssvsn
mnaglgmngm ntymsmsaaa mgsgsgnmsa 61 gsmnmssyvg agmspslagm spgagamagm
ggsagaagva gmgphlspsl splggqaaga 121 mgglapyanm nsmspmygqa glsrardpkt

yrrsythakp pysyislitm aiqqspnkml 181 tlseiyqwim dlfpfyrqnq qrwqnsirhs lsfndcflkv prspdkpgkg sfwtlhpdsg 241 nmfengcylr rqkrfkcekq lalkeaagaa gsgkkaaaga qasqaqlgea agpasetpag 301 tesphssasp cqehkrgglg elkgtpaaal sppepapspg qqqqaaahll gpphhpglpp 361 eahlkpehhy afnhpfsinn lmsseqqhhh shhhhqphkm dlkayeqvmh ypgygspmpg 421 slamgpvtnk tgldasplaa dtsyyqgvys rpimnss

[0082] By "FOXA2 nucleic acid molecule" (or Forkhead box protein A2) is meant a polynucleotide (e.g., mRNA) encoding an FOXA2 polypeptide. An exemplary FOXA2 nucleic acid molecule is provided at NCBI Accession No. NM_021784.

TABLE-US-00014 (SEQ ID NO: 14) 1 cccgcccact tccaactacc gcctccggcc tgcccaggga gagagaggga gtggagccca 61 gggagaggga gcgcgagaga gggagggagg aggggacggt gctttggctg actttttttt 121 aaaagagggt gggggtgggg ggtgattgct ggtcgtttgt tgtggctgtt aaatttaaa 181 ctgccatgca ctcggcttcc agtatgctgg gagcggtgaa gatggaaggg cacgagccgt 241 ccgactggag cagctactat gcagagcccg agggctactc ctccgtgagc aacatgaacg 301 ccggcctggg gatgaacggc atgaacacgt acatgagcat gtcggcggcc gccatgggca 361 gcggctcggg caacatgagc gcgggctcca tgaacatgtc gtcgtacgtg ggcgctggca 421 tgagcccgtc cctggcgggg atgtcccccg gcgcgggcgc catggcgggc atgggcggct 481 cggccggggc ggccggcgtg gcgggcatgg ggccgcactt gagtcccagc ctgagcccgc 541 tcgggggggca ggcggccggg gccatgggcg gcctggcccc ctacgccaac atgaactcca 601 tgagccccat gtacgggcag gcgggcctga gccgcgcccg cgaccccaag acctacaggc 661 gcagctacac gcacgcaaag ccgccctact cgtacatctc gctcatcacc atggccatcc 721 agcagagccc caacaagatg ctgacgctga gcgagatcta ccagtggatc atggacctct 781 tcccttcta ccggcagaac cagcagcgct ggcagaactc catccgccac tcgctctcct 841 tcaacgactg tttcctgaag gtgccccgct cgcccgacaa gcccggcaag ggctccttct 901 ggaccctgca ccctgactcg ggcaacatgt tcgagaacgg ctgctacctg cgccgccaga 961 agcgcttcaa gtgcgagaag cagctggcgc tgaaggaggc cgcaggcgcc gccggcagcg 1021 gcaagaaggc ggccgccgga gcccaggcct cacaggctca actcggggag gccgccgggc 1081 cggcctccga gactccggcg ggcaccgagt cgcctcactc gagcgcctcc ccgtgccagg 1141 agcacaagcg aggggggcctg ggagagctga aggggacgcc ggctgcggcg ctgagccccc 1201 cagagccggc gccctctccc gggcagcagc agcaggccgc ggcccacctg ctgggcccgc 1261 cccaccaccc gggcctgccg cctgaggccc acctgaagcc ggaacaccac tacgccttca 1321 accacccgtt ctccatcaac aacctcatgt cctcggagca gcagcaccac cacagccacc 1381 accaccacca accccacaaa atggacctca aggcctacga acaggtgatg cactaccccg 1441 gctacggttc ccccatgcct ggcagcttgg ccatgggccc ggtcacgaac aaaacgggcc 1501 tggacgcctc gcccctggcc gcagatacct cctactacca gggggtgtac tcccggccca 1561 ttatgaactc ctcttaagaa gacgacggct tcaggcccgg ctaactctgg caccccggat 1621 cgaggacaag tgagagagca agtgggggtc gagactttgg ggagacggtg ttgcagagac 1681 gcaagggaga agaaatccat aacaccccca ccccaacacc cccaagacag cagtcttctt 1741 cacccgctgc agccgttccg tcccaaacag agggccacac agatacccca cgttctatat 1801 aaggaggaaa acgggaaaga atataaagtt aaaaaaaagc ctccggtttc cactactgtg 1861 tagactcctg cttcttcaag cacctgcaga ttctgatttt tttgttgttg ttgttctcct 1921 ccattgctgt tgttgcaggg aagtcttact taaaaaaaaa aaaaaatttt gtgagtgact 1981 cggtgtaaaa ccatgtagtt ttaacagaac cagagggttg tactattgtt taaaaacagg 2041 aaaaaaaata atgtaagggt ctgttgtaaa tgaccaagaa aaagaaaaaa aaagcattcc 2101 caatcttgac acggtgaaat ccaggtctcg ggtccgatta atttatggtt tctgcgtgct 2161 ttatttatgg cttataaatg tgtattctgg ctgcaagggc cagagttcca caaatctata 2221 ttaaagtgtt atacccggtt ttatcccttg aatcttttct tccagatttt tcttttcttt 2281 acttggctta caaaatatac aggcttggaa attatttcaa gaaggaggga gggataccct 2341 gtctggttgc aggttgtatt ttattttggc ccagggagtg ttgctgtttt cccaacattt 2401 tattaataaa attttcagac ataaaaaa

[0083] By "FOXO4 polypeptide" (or Forkhead box protein 04) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P98177.

TABLE-US-00015 (SEQ ID NO: 15) 1 mdpgnensat eaaaiidldp dfepqsrprs ctwplprpei anqpseppev epdlgekvht 61 egrsepillp srlpepaggp qpgilgavtg prkggsrrna wgnqsyaeli sqaiesapek 121 rltlaqiyew mvrtvpyfkd kgdsnssagw knsirhnlsl hskfikvhne

atgksswwml 181 npeggksgka prrraasmds sskllrgrsk apkkkpsvlp appegatpts pvghfakwsg 241 spcsrnreea dmwttfrprs ssnassvstr lsplrpesev laeeipasvs syaggvpptl 301 neglelldgl nltsshslls rsglsgfslq hpgvtgplht yssslfspae gplsagegcf 361 sssqaleall tsdtpppad vlmtqvdpil sqaptllllg glpsssklat gvglcpkple 421 apgpsslvpt lsmiapppvm asapipkalg tpvltpptea asqdrmpqdl dldmymenle 481 cdmdniisdl mdegegldfn fepdp

[0084] By “FOXO4 nucleic acid molecule” (or Forkhead box protein 04) is meant a polynucleotide (e.g., mRNA) encoding an FOXO4 polypeptide. An exemplary FOXO4 nucleic acid molecule is provided at NCBI Accession No. NM_005938.

TABLE-US-00016 (SEQ ID NO: 16) 1 aaaaggggga gggaactgcg gctaaggaga cgttcggtga tgggagcgca atatatgagg 61 ggatacagtg cctcaggttt aaaagagcag gaagctgagt gagaggttgc agaaaaagtg 121 tcttcgctcg gcagaggtta caggtggcat ctcagaaaga gctttgaggc tacaggctgt 181 agtcgggaag gggatcggag aactgtgtga agggacagct tagggactag cgtcctggga 241 ctagggggaa gttcgcgact ttctgaagac tggcaggaat gtgcctcctg gccctcgatg 301 cttccccccct gaggggaggc atcgtgaggg actgtggcag gcttcactga acgctgagcc 361 ggggaggtcc aactccacgt atggatccgg ggaatgagaa ttcagccaca gaggctgccg 421 cgatcataga cctagatccc gacttcgaac cccagagccg tccccgctcc tgcacctggc 481 cccttccccg accagagatc gctaaccagc cgtccgagcc gcccgaggtg gagccagatc 541 tgggggaaaa ggtacacacg gaggggcgct cagagccgat cctgttgccc tctcggctcc 601 cagagccggc cgggggcccc cagcccggaa tcctgggggc tgtaacaggt cctcggaagg 661 gaggctcccg ccggaatgcc tggggaaatc agtcatatgc agaactcatc agccaggcca 721 ttgaaagcgc cccggagaag cgactgacac ttgcccagat ctacgagtgg atggtccgta 781 ctgtacccta cttcaaggac aagggtgaca gcaacagctc agcaggatgg aagaactcga 841 tccgccacaa cctgtccctg cacagcaagt tcatcaaggt tcacaacgag gccaccggca 901 aaagctcttg gtggatgctg aaccctgagg gaggcaagag cggcaaagcc ccccgccgcc 961 gggccgcctc catggatagc agcagcaagc tgctccgggg ccgcagtaaa gccccccaaga 1021 agaaaccatc tgtgctgcca gctccacccg aaggtgccac tccaacgagc cctgtcggcc 1081 actttgccaa gtggtcaggc agcccttgct ctcgaaaccg tgaagaagcc gatatgtgga 1141 ccaccttccg tccacgaagc agttcaaatg ccagcagtgt cagcacccgg ctgtcccccct 1201 tgaggccaga gtctgaggtg ctggcggagg aaataccagc ttcagtcagc agttatgcag 1261 ggggtgtccc tcccaccctc aatgaaggtc tagagctgtt agatgggctc aatctcacct 1321 cttcccattc cctgctatct cggagtggtc tctctggctt ctctttgcag catcctgggg 1381 ttaccggccc cttacacacc tacagcagct ccctttttcag cccagcagag gggcccctgt 1441 cagcaggaga agggtgcttc tccagctccc aggctctgga ggccctgctc acctctgata 1501 cgccaccacc ccctgctgac gtcctcatga cccaggtaga tcccattctg tcccaggctc 1561 cgactcttct gttgctgggg gggcttcctt cctccagtaa gctggccacg ggcgtcggcc 1621 tgtgtcccaa gcccctagag gctccaggcc ccagcagtct ggttcccacc ctttctatga 1681 tagcaccacc tccagtcatg gcaagtgccc ccatccccaa ggctctgggg actcctgtgc 1741 tcacaccccc tactgaagct gcaagccaag acagaatgcc tcaggatcta gatcttgata 1801 tgtatatgga gaacctggag tgtgacatgg ataacatcat cagtgacctc atggatgagg 1861 gcgagggact ggacttcaac tttgagccag atccctgagt catgcctgga agctttgtcc 1921 cctgcttcag atgtggagcc aggcgtgttc atatctactc tttacccttg agccctcccc 1981 aggaatttgg gaccctgctt tagagctagg gtggggtctg gtcacacaca ggtgttgaag 2041 aaattataaa gataaagctg ccccatctgg ggacgatatg gggagggaga tgggagggga 2101 aaggggagag ggtttttctc actgtgccaa ttagggggta aggccccctc tcaggagcca 2161 tcatcggctt tccccattcc tacccactta ggctttgtag caagatgagc aatgctgttg 2221 gaaatgtgaa gtcaccagtg gccttacccc tgcctttggg agcaggattt ttttgtagag 2281 agtcttatct gagctgagcc aggctagctg gagcctggga tttctatgca gtggcccctt 2341 aggccagtga tgtgcggtgg gtgggctgtt taggggatct ggaagggcca aggtctgagc 2401 actggagtgg ctcgccaggc caaatcaccc ttagaaggct gcagataaca gaaaggcttt 2461 ttataaactt ttaaagaaat ataaacacaa atatagagat tttttaacca tggcagggtg 2521 ctagtggtgg gcagaatgct ttttttctt tctgaaggct ttgtgatagt gacatgatac 2581 aaacactaca gacaataaat attaggagac acagggaagt ggggagaggt ggggagtaat 2641 agtaaacaca gggaagagct cccctacgga ccaggtatag agaaaggtct atgcagaaat 2701 aggttagagt ttccctaaca aaaaagctaa cccaggtccc ctcattcctt caacttgtgc 2761 ctgggagtgt gtggtgttag ggtgcagcca cactcttcta tgacccagca tgggttagtg 2821 ctatggtggg agagtacatt gaaggcctgg aattagcttg

gggccaggga agggactggg 2881 aggggagaga agagaaggag ggaaggattt aggatggtaa agttaggtac agagacctcc 2941 ctgttcaagg cccctgacag ctgtccctgc ccttcttccc cttccctgac tgcaggggtt 3001 atgtggaagt gtgtgtggca gcaggcagcg gggaggggag gaacagggaa gggggagctg 3061 gggagcttgg ctgagggtct gggaaatgag cagggatggg gggggatgtg gatcaggttt 3121 actagcacct gccagggagg ccatctgggg ctccttctcc accccagccc ccaaagcagc 3181 ccttccccca gtgccctttg catcgtcccc tcccccaccc ctgctgtggg ttcccatcat 3241 ttcctgtgtc agcgcctggc ctacccagat tgtatcatgt gctagattgg agtggggaag 3301 tgtgtcaaat caataaatga ataaattcaa taaatgccta taaccagcaa aaaaaaaaaa 3361 aaaaa

[0085] By “CNP polypeptide” (or 2′,3′-cyclic-nucleotide 3′-phosphodiesterase) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P09543.

TABLE-US-00017 (SEQ ID NO: 17) 1 mnrgfsrksh tflpkiffrk msssgakdkp elqfpflqde dtvatlleck tlfilrglpg 61 sgkstlarvi vdkyrdgtkm vsadaykitp gargafseey krldedlaay crrrdirilv 121 lddtnherer leqlfemadq yqyqvvlvep ktawrldcaq lkeknqwqls addlkklkpg 181 lekdflplyf gwfltkksse tlrkagqvfl eelgnhkafk kelrqfvpgd eprekmdlvt 241 yfgkrppgvl hcttkfcdyg kapgaeeyaq qdvlkksysk aftltisalf vtpkttgarv 301 elseqqlqlw psdvdklspt dnlprgsrah itlgcaadve avqtgldlle ilrqekggsr 361 geevgelsrg klyslgngrw mltlaknmev raiftgyygk gkpvptqgsr kggalqscti 421 i

[0086] By “CNP nucleic acid molecule” (or 2′,3′-cyclic-nucleotide 3′-phosphodiesterase) is meant a polynucleotide (e.g., mRNA) encoding an CNP polypeptide. An exemplary CNP nucleic acid molecule is provided at NCBI Accession No. BC011046.

TABLE-US-00018 (SEQ ID NO: 18) 1 ctccgcgcag gcgggcggcc ccggagcgct ggtgccggca gaggcggcga cggtggcgcc 61 cctcctcatc atgaggcttc tcccgaaaaa gccacacatt cctgcccaag atcttcttcc 121 gcaagatgtc atcctcaggg gccaaggaca agcctgagct gcagtttccc ttccttcagg 181 atgaggacac agtggccacg ctgctagagt gcaagacgct cttcatcttg cgcggcctgc 241 caggaagcgg caagtccacg ctggcacggg tcatcgtgga caagtaccgt gatggcacca 301 agatggtgtc ggctgacgct tacaagatca cccccggcgc tcgaggagcc ttctccgagg 361 agtacaagcg gctcgatgag gacctggctg cctactgccg ccgccgggac atcagaattc 421 ttgtgcttga tgacaccaac cacgaacggg aacggctgga gcagctcttt gaaatggccg 481 accagtacca gtaccaggtg gtgctggtgg agcccaagac ggcgtggcgg ctggactgtg 541 cccagctcaa ggagaagaac cagtggcagc tgtcggctga tgacctgaag aagctgaagc 601 ctgggctgga gaaggacttc ctgccgctct acttcggctg gttcctgacc aagaagagct 661 ctgagaccct ccgcaaagcc ggccaggtct tcctggaaga gctggggaac cacaaggcct 721 tcaagaagga gctgcgacaa ttcgtccctg gggatgagcc cagggagaag atggacttgg 781 tcacctactt tggaaagaga cccccaggcg tgctgcattg cacaaccaag ttttgtgact 841 acgggaaggc tcccggggca gaggagtacg ctcaacaaga tgtgttaaag aaatcttact 901 ccaaggcctt cacgctgacc atctctgccc tctttgtgac acccaagacg actggggccc 961 gggtggagtt aagcgagcag caactgcagt tgtggccgag tgatgtggac aagctgtcac 1021 ccactgacaa cctgccgcgg gggagccgcg cccacatcac cctcggctgt gcagctgacg 1081 tagaggccgt gcagacgggc cttgacctct tagagattct gcggcaggag aagggggggca 1141 gccgaggcga ggaggtgggc gagctaagcc ggggcaagct ctattccttg ggcaatgggc 1201 gctggatgct gaccctggcc aagaacatgg aggtcagggc catcttcacg gggtactacg 1261 ggaaaggcaa acctgtgccc acgcaaggta gccggaaggg gggcgccttg cagtcctgca 1321 ccatcatatg agtgttctca ccaccactta tgcccctaga agggaagggg agagggaaac 1381 gtgccctctg tttgatcctt gttttgtgac attttttttt ttttttttt tactcaaagt 1441 taacctacct gtaacttttt aaaaacttgt aaaataactg accctccctt cctgtccgcc 1501 ctcttccccc ctaatgctca cgctcccaac acaaggtggg cagggaggca ccattcagga 1561 acctggacca aagctgacga ggctgggcca agccagggat ggggccacag ccagaacccc 1621 gagccctact tccaggttct ggttagctca gccccagccc agcccagctg ctctgcccag 1681 agctgggtga gtggggagac acctcagagc cccgcaaaac ccactgaccg gaggcaaaag 1741 gcagtggggc tgggggtagt tttccatggt cacagagaac tagtggtggc tctgagaagg 1801 ggaggacctc tgggctttga ttccatctcc ttgtcttttt tctttgtttt tagagacagg 1861 gtcctgctat ttcccaagct ggagtgcagt ggtgcgatca tggctcactg cagcctcgaa 1921 ctcctgggct caagcaatcc

tcctgagtga tcccatttct taatcagtgt agccccaaga 1981 aggctggggc tatttaccag ggtagaaaaa ggagcttacc tcccaccttt ggtcctaagt 2041 ccctgccccc tccccttcac accataacta ggtaacagtt tgataactag ggaagaaagc 2101 agaacagtta agcagccgcc acatccccgc tggctggggg cctcactcca ggaaggggct 2161 ggactggctg tcctttccag tggcctggct ccgctgtgtg gatggggaga tcggggccag 2221 aggcagaacc ctggtgagga agctccagtc ctgctctcta cccagcccat cttgcctcca 2281 tggtgcctct ggaggcctct gggcctcctc taacaggggc tggtgggcac caagagccaa 2341 tggagtagac ccctggctgg taagggccaa gtcccaccgg ttgcttctgg gaaggggttt 2401 ctaacactag tctgtgtgct gtggttcctg gggtgccctc cactgccctc tgttcagtaa 2461 cagggccttg ctaatcgggt tgtcactcaa caaaagtgct ttggatttaa gttactatcc 2521 tggctttgcc caacctcagc aacctgtaag actgataatg aaataaatca tgttaatcct 2581 agcaaaaaaa aaaaaaaa

[0087] By “MBP polypeptide” (or myelin basic protein) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P02686.

TABLE-US-00019 (SEQ ID NO: 19) 1 mgnhagkrel naekastnse tnrgesekkr nlgelsrtts ednevfgead anqnngtssq 61 dtavtdskrt adpknawqda hpadpgsrph lirlfsrdap gredntfkdr psesdelqti 121 qedsaatses ldvmasqkrp sqrhgskyla tastmdharh gflprhrdtg ildsigrffg 181 gdrgapkrgs gkdshhpart ahygslpqks hgrtqdenpv vhffknivtp rtpppsqgkg 241 rglslsrfsw gaegqrpgfg yggrasdyks ahkgfkgvda qgtlskifkl ggrdsrsgsp 301 marr

[0088] By “MBP nucleic acid molecule” (or myelin basic protein) is meant a polynucleotide (e.g., mRNA) encoding an MBP polypeptide. An exemplary MBP nucleic acid molecule is provided at NCBI Accession No. M13577.

TABLE-US-00020 (SEQ ID NO: 20) 1 gaaaacagtg cagccacctc cgagagcctg gatgtgatgg cgtcacagaa gagaccctcc 61 cagaggcacg gatccaagta cctggccaca gcaagtacca tggaccatgc caggcatggc 121 ttcctcccaa ggcacagaga cacgggcatc cttgactcca tcgggcgctt ctttggcggt 181 gacaggggtg cgccaaagcg gggctctggc aaggactcac accacccggc aagaactgct 241 cactatggct ccctgcccca gaagtcacac ggccggaccc aagatgaaaa ccccgtagtc 301 cacttcttca agaacattgt gacgcctcgc acaccacccc cgtcgcaggg aaaggggaga 361 ggactgtccc tgagcagatt tagctggggg gccgaaggcc agagaccagg atttggctac 421 ggaggcagag cgtccgacta taaatcggct cacaagggat tcaagggagt cgatgcccag 481 ggcacgcttt ccaaaatttt taagctggga ggaagagata gtcgctctgg atcacccatg 541 gctagacgct gaaaacccac ctggttccgg aatcctgtcc tcagcttctt aatataactg 601 ccttaaaact ttaatcccac ttgcccctgt tacctaatta gagcagatga cccctcccct 661 aatgcctgcg gagttgtgca cgtagtaggg tcaggccacg gcagcctacc ggcaatttcc 721 ggccaacagt taaatgagaa catgaaaaca gaaaacggtt aaaactgtcc ctttctgtgt 781 gaagatcacg ttccttcccc cgcaatgtgc ccccagacgc acgtgggtct tcaggggccc 841 aggtgcacag acgtccctcc acgttcaccc ctccaccctt ggactttctt ttcgccgtgg 901 ctcggcaccc ttgcgctttt gctggtcact gccatggagg cacacagctg cagagacaga 961 gaggacgtgg gcggcagaga ggactgttga catccaagct tcctttgttt ttttttcctg 1021 tccttctctc acctcctaaa gtagacttca tttttcctaa caggattaga cagtcaagga 1081 gtggcttact acatgtggga gctttttggt atgtgacatg cgggctgggc agctgttaga 1141 gtccaacgtg gggcagcaca gagagggggc cacctcccca ggccgtggct gcccacacac 1201 cccaattagc tgaattcgcg tgtggcagag ggaggaaaag gaggcaaacg tgggctgggc 1261 aatggcctca cataggaaac agggtcttcc tggagatttg gtgatggaga tgtcaagcag 1321 gtggcctctg gacgtcaccg ttgccctgca tggtggcccc agagcagcct ctatgaacaa 1381 cctcgtttcc aaaccacagc ccacagccgg agagtccagg aagacttgcg cactcagagc 1441 agaagggtag gagtcctcta gacagcctcg cagccgcgcc agtcgcccat agacactggc 1501 tgtgaccggg cgtgctggca gcggcagtgc acagtggcca gcactaaccc tccctgagaa 1561 gataaccggc tcattcactt cctcccagaa gacgcgtggt agcgagtagg cacaggcgtg 1621 cacctgctcc cgaattactc accgagacac acgggctgag cagacggccc ctgtgatgga 1681 gacaaagagc tcttctgacc atatccttct taacacccgc tggcatctcc tttcgcgcct 1741 ccctccctaa cctactgacc caccttttga ttttagcgca cctgtgattg ataggccttc 1801 caaagagtcc cacgctggca tcaccctccc cgaggacgga gatgaggagt agtcagcgtg 1861 atgccaaaac gcgtcttctt aatccaattc taattctgaa tgtttcgtgt gggcttaata 1921 ccatgtctat taatatatag cctcgatgat gagagagtta caaagaacaa aactccagac 1981 acaaacctcc aaatttttca gcagaagcac tctgcgtcgc tgagctgagg

tcggctctgc 2041 gatccatacg tggccgcacc cacacagcac gtgctgtgac gatggctgaa cggaaagtgt 2101 acactgttcc tgaatattga aataaaacaa taaactttt

[0089] By “TUBIII polypeptide” (or TUBB3, tubulin beta chain 3) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_001184110.

TABLE-US-00021 (SEQ ID NO: 21) 1 mdsvrsgafg hlfrpdnfif gqsgagnnwa kghytegael vdsvldvvrk ecencdclqg 61 fqlthslggg tgsgmgtlli skvreeypdr imntfsvvps pkvsdtvvep ynatlsihql 121 ventdetyci dnealydicf rtlklatpty gdlnhlvsat msgvttslrf pgqlnadlrk 181 lavnmvpfpr lhffmpgfap ltargsqqyr altvpeltqq mfdaknmmaa cdprhgrylt 241 vatvfrgrms mkevdeqmla iqsknssyfv ewipnnvkva vcdipprglk msstfignst 301 aiqelfkris eqftamfrrk aflhwytgeg mdemefteae snmndlvsey qqyqdataee 361 egemyeddee eseaqgpk

[0090] By “TUBIII nucleic acid molecule” (or TUBB3, tubulin beta chain 3) is meant a polynucleotide (e.g., mRNA) encoding an TUBIII polypeptide. An exemplary TUBIII nucleic acid molecule is provided at NCBI Accession No. BC000748.

TABLE-US-00022 (SEQ ID NO: 22) 1 gcccggcccg cccgcgcccg tccgcagccg cccgccagac gcgcccagta tgagggagat 61 cgtgcacatc caggccggcc agtgcggcaa ccagatcggg gccaagttct gggaagtcat 121 cagtgatgag catggcatcg accccagcgg caactacgtg ggcgactcgg acttgcagct 181 ggagcggatc agcgtctact acaacgaggc ctcttctcac aagtacgtgc ctcgagccat 241 tctggtggac ctggaacccg gaaccatgga cagtgtccgc tcaggggcct ttggacatct 301 cttcaggcct gacaatttca tctttggtca gagtggggcc ggcaacaact gggccaaggg 361 tcactacacg gagggggcgg agctggtgga ttcggtcctg gatgtggtgc ggaaggagtg 421 tgaaaactgc gactgcctgc agggcttcca gctgacccac tcgctggggg gcggcacggg 481 ctccggcatg ggcacgttgc tcatcagcaa ggtgcgtgag gagtatcccg accgcatcat 541 gaacaccttc agcgtcgtgc cctcacccaa ggtgtcagac acggtggtgg agccctacaa 601 cgccacgctg tccatccacc agctggtgga gaacacggat gagacctact gcatcgacaa 661 cgaggcgctc tacgacatct gcttccgcac cctcaagctg gccacgccca cctacgggga 721 cctcaaccac ctggtatcgg ccaccatgag cggagtcacc acctccttgc gcttcccggg 781 ccagctcaac gctgacctgc gcaagctggc cgtcaacatg gtgcccttcc cgcgcctgca 841 cttcttcatg cccggcttcg ccccccctcac agcccggggc agccagcagt accgggccct 901 gaccgtgccc gagctcaccc agcagatgtt cgatgccaag aacatgatgg ccgcctgcga 961 cccgcgccac ggccgctacc tgacggtggc caccgtgttc cggggccgca tgtccatgaa 1021 ggaggtggac gagcagatgc tggccatcca gagcaagaac agcagctact tcgtggagtg 1081 gatccccaac aacgtgaagg tggccgtgtg tgacatcccg ccccgcggcc tcaagatgtc 1141 ctccaccttc atcgggaaca gcacggccat ccaggagctg ttcaagcgca tctccgagca 1201 gttcacggcc atgttccggc gcaaggcctt cctgcactgg tacacgggcg agggcatgga 1261 cgagatggag ttcaccgagg ccgagagcaa catgaacgac ctggtgtccg agtaccagca 1321 gtaccaggac gccacggccg aggaagaggg cgagatgtac gaagacgacg aggaggagtc 1381 ggaggcccag ggccccaagt gaagctgctc gcagctggag tgagaggcag gtggcggccg 1441 gggccgaagc cagcagtgtc taaacccccg gagccatctt gctgccgaca ccctgctttc 1501 ccctcgccct agggctccct tgccgccctc ctgcagtatt tatggcctcg tcctccccac 1561 ctaggccacg tgtgagctgc tcctgtctct gtcttattgc agctccaggc ctgacgtttt 1621 acggttttgt tttttactgg tttgtgttta tattttcggg gatacttaat aaatctattg 1681 ctgtcagata ccccttaaaaa aaaaaaaaaa aaaaaaaaaa

[0091] By “NEUN polypeptide” (or Feminizing Locus on X-3, Fox-3, RNA-binding protein fox-1 homolog 3, or Hexaribonucleotide Binding Protein-3) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_001076044.

TABLE-US-00023 (SEQ ID NO: 23) 1 maqpyppaqy pppqngipa eyapppphpt qdysgqtpvp tehgmtlytp aqthpeqpgs 61 eastqpiagt qtvpqtdeaa qtdsqplhps dptekqqpkr lhvsnipfrf rdpdlrqmfg 121 qfgkildvei ifnergskgf gfvtfetssd adrareklng tivegrkiev nnatarvmtn 181 kktgnpytng wklnpvvgav ygpefyavtg fpypttgtav ayrgahlrgr gravyntfra 241 apppppipty gavvyqdgfy gaeiyggyaa yryaqpaaaa aaysdsygrv yaaadpyhht 301 igpaatysig tm

[0092] By “NEUN nucleic acid molecule” (or Feminizing Locus on X-3, Fox-3, RNA-binding

protein fox-1 homolog 3, or Hexaribonucleotide Binding Protein-3) is meant a polynucleotide (e.g., mRNA) encoding an NEUN polypeptide. An exemplary NEUN nucleic acid molecule is provided at NCBI Accession No. NM_001082575.

TABLE-US-00024 (SEQ ID NO: 24)

1 gatacagcag cagctggtgc tcctggccag gctgtgcgtg ctctctctgc ctctctctct 61 cggactctct gctctctctc tctgactctc tcctctctct ctgttggcct ggtgaaatgt 121 tcttggctgt aggcacacag agccttggac tcaaggctgt tggagtcgag gacaccttga 181 cttcggtcct ggaggttgaa attctgcctc tgagaagcta acagtcttcc tgtggtcgcc 241 actcctcccc agcagccccc tccttgccaa ggacggtcca gaaggagccc cactggggcc 301 tccccgctca gcaaagcaga cctcacctcc cactaccagc ttgaagtcac agcagccaga 361 ggaaattctg ccaccatttt cccaggtctg cagcccctcc agctgggaac ctgctcctgg 421 agccatccct ctgcaaacag agagcccaga gtgcctcggg gaaaattggc tgaataaaag 481 agcgatcagg acgccacggc tccgcctgaa gcgatggccc agccctaccc ccccgcccag 541 taccccccctc cgccacagaa cggcatccct gccgagtacg ccccgccccc accgcacccc 601 acgcaggact actccggcca gaccccggtc cccacagagc atggcatgac cctgtacaca 661 ccagcacaga cccaccccga gcagccaggc tccgaggcca gcacacagcc catcgccggg 721 acccagacag tgccgcagac agacgaggcg gcacagacgg acagccagcc gctccacccc 781 tccgacccta cagagaagca gcagcccaag cggctacacg tctccaacat ccccttccgg 841 ttcagggacc ccgacttgcg gcaaatgttc gggcaattcg gaaaaatttt agacgtggag 901 atcattttta acgagcgggg ctccaagggt tttgggtttg taacttttga aactagctca 961 gatgctgacc gagcccggga gaagctgaat gggacgatcg tagagggacg gaaaattgag 1021 gtcaataatg ccacggcccg agtgatgacc aacaagaaga cggggaaccc ctacaccaac 1081 ggctggaagc taaatccagt ggtcggcgca gtctacgggc ctgaattcta tgcagtgacg 1141 gggttcccct accccaccac cggcacagcc gttgcctacc ggggcgcaca tcttcggggc 1201 cggggccggg ccgtgtataa tacatttcgg gctgcgccac ccccaccccc catcccgact 1261 tacggagcgg tcgtgtatca ggatggattt tatggtgctg agatttatgg aggctacgca 1321 gcctacagat acgctcagcc cgctgcagcg gcggcagcct acagcgacag ttacggcaga 1381 gtctacgcag ctgccgaccc gtaccatcac accatcgggc ccgcggcgac ctacagcatt 1441 ggaaccatgt gaaaccttcc accgtttcct tctcggacca tgaagggcaa aaacaaaaaa 1501 acaaaaaaaa tcacaaaaca aaaaaaacaa aaaaagatgt taagatccaa gcaacaaaaa 1561 aaaaaccaac caaaccaaga ggcatccaac caagtccaag tcccgcgtcc tggccacacg 1621 cccgcaccga gggagcacgc cggcaggggc gccgaggagc ggccccagga caggacggcc 1681 ccaccgcgtc ctggctggca gcacagtggg aacacgcccc tccgtctcag gcagtggggg 1741 agttggaggg gaaggggcct cccttgtggg acccgtgggg ggctctgttt tccatccagt 1801 cttcctttcc cagcccccaa ctcccaagac agacagtgtg gagcccagcg gcggcggagc 1861 aggcccgggc ctgagcaggc aggcgctgct agcaagactt gatctttgtg gccagctgtg 1921 ccaggggggcc ggcggggctg aggggtgcgg gcagctttca tcccaggggc tccactgggc 1981 cccgtcaccc tcctgtcgcg tcccctgcgt cccacctccc tcctgcccgg cagtcccgcc 2041 cgtgccccca gcctggcgag gaagccgtcc aacagtagcc ccggggccag ctcccaacag 2101 aaagggctga cgtggctcca ggactcaggg gcgctccatg ggaggacgaa ggaagcccag 2161 ccagccagga gccactcctc acacctccaa gtgtggccaa gtgggccctg aggccaagga 2221 cttacttgct cttcctggcc atctctccct ttctggagga ggcccggggc ctgtgtacac 2281 caaggctgac ctcgtgctgc ctgctgggac ccagccctcc ctgccgctcc cctgtgagcc 2341 cagtccaccg tgggcgccca gggccaggga cgggccagcg cccggctgca tcgcgaggtt 2401 gggagtcaca gtggctgtgg gcctggacgg gcacagccag agcaggggcc catgggaagg 2461 gcaagggatg gggaagcctg ggccggcccc ttccctgctc ccaaggcagg tgtccaggtg 2521 gcgggagcag caccaaggac agccaggctt acccggtggg aggagcagga gcagagcagg 2581 tggcagggag gaacccctgg cgaggcaggg agcactgaag tagggaagca gcaaaaaata 2641 caggctccca acgtggctcc actgtctcat gaagtgtcaa aaatttaaaa atacacctca 2701 ctttctattc agcatcagct attgaaatgg aattctcctt ttctattccc gttgtacata 2761 gccccacgcc ctgcctccgg ctttgtcctc tgtacagagc cccctgtccc ctctgctgtt 2821 ccggaccctt ttcttgcagc agctcaaccc cccgactcac tcagatcccc aggactgcag 2881 ccgagccccg ggcttccttt cttaccattc tgtatgcttc caaggtgtga ccattcaaac 2941 taacagtatt attaagatta ttaataaaga tttctttctt caaaccagga aaaaaaaaaa 3001 aaaaaa

[0093] By “SLC1A6 polypeptide” (or Excitatory amino acid transporter 4; Sodium-dependent glutamate/aspartate transporter; Solute carrier family 1 member 6) is meant a polypeptide or

fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P48664.

TABLE-US-00025 (SEQ ID NO: 25) 1 msshgnslfl resgqrlgrv gwlqrlqesl qqralrtrlr lqtmtlehvl rflrrnafil 61 ltvsavvigv slafalrpyq ltyrqikyfs fpgellmrml qmlvlplivs slvtgmasld 121 nkatgrmgmr aavyymvtti iavfigilmv tiihpgkgsk eglhregrie tiptadafmd 181 lirnmfppnl veacfkqfkt qystrvvtrt mvrtengsep gasmpppfsv engtsflenv 241 tralgtlqem lsfeetvpvp gsanginalg lvvfsvafgl viggmkhkgr vlrdffdsln 301 eaimrlvgii iwyapvgilf liagkileme dmavlggqlg mytltvivgl flhagivlpl 361 iyflvthrnp fpfiggmlqa litamgtsss satlpitfrc leeglgvdrr itrfvlpvga 421 tvnmdgtaly ealaaifiaq vnnyelnlgq ittisitata asvgaagipq aglvtmvivl 481 tsvglptedi tliiavdwfl drlrtmtnvl gdsigaavie hlsqrelelq eaeltlpslg 541 kpykslmaqe kgasrgrggn esam

[0094] By "SLC1A6 nucleic acid molecule" (or Excitatory amino acid transporter 4; Sodium-dependent glutamate/aspartate transporter; Solute carrier family 1 member 6) is meant a polynucleotide (e.g., mRNA) encoding an SLC1A6 polypeptide. An exemplary SLC1A6 nucleic acid molecule is provided at NCBI Accession No. BC040604.

TABLE-US-00026 (SEQ ID NO: 26) 1 ggcatagcgc gtcccggctc cgcgccggtg cctccacggt ccggtccccg cgccggtgct 61 gcacagtccc tggcgggtcc ccgcggcccc ggccgggcgc ttcgccgggc tccggctcct 121 gcatccgggc gcagcgcgca ggccgaggcg cgggcaggcc gcccccgccg ctccggacgc 181 cgggatgtaa gaggctccga aaagcagccc acgcatctca tcagatctaa gtgtctagag 241 gtcgggagaa ccaagtggga aagacccacc ctcacccctc accttgtaga aactgggaac 301 actagaaggg acattttctg agcaggaaac ccaagagaca gggttttacg ctgtcaccca 361 agttggagtg cagtggtacg atcatagctc attgcagcct caaactcctg ggttcaagcg 421 atcctcctgc tttagcctct tgagtagcta ggactacagg cacaggccac cgtgcctggc 481 taatttttaa tttttaaaaa agagacaggg tctggctatg ttgcccaggc tggccatgaa 541 ctcctgggct caagcggttc tccagccttc acctcccaaa gtgttgggat tgcaggcatg 601 agccactgcg tctggcccac agatgctaag tgctgtctgc tcttctccag gggtcagcaa 661 atttttcag caaatggccc aagagtaaat attttgagct ttgtggcccg tacaatctct 721 gtcccaacaa ctcaactcag gcattgtagc ttgaaagcag ctgtagacaa taggtaatcc 781 atgagtgtgg ctgtgtgcca ataaaacttt atttacaaaa acaagcagta ggctgaattt 841 gactagcaga ccatagtttg tcaataccgt attatgtctt gtaaggaaga gaaaggaacc 901 agacaaaact ctagcctcgg gagttttcct gactgttcag atcttagctg aatgatctcc 961 cttggtatct acaggcaact tcctgctgtg gcttagggac tggaaacata atatcccaga 1021 gggattccct gtgtagtctg tggttcactc tttgggattt ttttttttt tttcacagca 1081 aggagaagca gcattgtggt ttcaggagat gggtccattt ggagcaggat cctaagtggg 1141 gcttggcatt gggaatttgg attagctcta gaggacgcag gatctggaaa atcagggcag 1201 atttcccatc ccttggatat ggtggggagt tgaggagggc aaggaagatc ccagaaaagc 1261 cagtggcagc aaaacacaaa ggccagggac ctacgtactg gtaaaactga gacctccaag 1321 aaacctgcag ctcgacctgg ttgaattcag atagaccatg agcagccatg gcaacagcct 1381 gttccttcgg gagagcggcc agcggctggg ccgggtgggc tggctgcagc ggctgcagga 1441 aagcctgcag cagagagcac tgcgcacgcg cctgcgcctg cagaccatga ccctcgagca 1501 cgtgctgcgc ttcctgcgcc gaaacgcctt cattctgctg acggtcagcg ccgtggtcat 1561 tggggtcagc ctggcctttg ccctgcgccc atatcagctc acctaccgcc agatcaagta 1621 cttctctttt cctggagagc ttctgatgag gatgctgcag atgctggtgt tacctctcat 1681 tgtctccagc ctggtcacag gtatggcatc cctggacaac aaggccacgg ggcggatggg 1741 gatgcgggca gctgtgtact acatggtgac caccatcatc gcggtcttca tcggcatcct 1801 catggtcacc atcatccatc ccgggaaggg ctccaaggag gggctgcacc gggagggccg 1861 gatcgagacc atccccacag ctgatgcctt catggacctg atcagaaata tgtttccacc 1921 aaaccttgtg gaggcctgct tcaaacagtt caagacgcag tacagcacga gggtggtaac 1981 caggaccatg gtgaggacag agaacgggtc tgagccgggt gcctccatgc ctcctccatt 2041 ctcagtggag aacggaacca gcttcctgga aaatgtcact cgggccttgg gtaccctgca 2101 ggagatgctg agctttgagg agactgtacc cgtgcctggc tccgccaatg gcatcaacgc 2161 cctgggcctc gtggtcttct ctgtggcctt tgggctggtc attggtggca tgaaacacaa 2221 gggcagagtc ctcagggact tcttcgacag cctcaatgag gctattatga ggctggtggg 2281 catcattatc tggtgagtcc tggtctgtgc ccacgggaag gtggagccag agctgggaag 2341 tcaggctgtg gggaagctgc cgaagggctt gctggggacc tttggtcatt catttacgta 2401 ttgggtgatt cacttaccca ctcaccaact cattcattca tgtctttctg ggatgatttc 2461 atcactagtt

cacttccttg ttcatctgtt cattcattca ttcttctatg cattggttag 2521 ttcatggaat atctcactct ttcattcatt catgtccttc tgcaatgatt cattcactgc 2581 tttgttcatc tgttcattca ctcattcttc tatgcattga tgaaatcact cattcagtga 2641 tttattcatc tatactcatg cttcaatgca ttgatttact catttcctca tgcatttatt 2701 cattcatcta tgcattggtt aaatcactgg ccaactcact aactcattca ttcattcaca 2761 cttttctgca atgatttgtt cacttgttca ctccccttgct tatctgttca ttcactcatt 2821 cttcaataca ttgaccaagc cattcactga catttattca gctacattta ttctttcatg 2881 cattggtctg gatttatttg gtcattcatt tatttatttt gcaaaattaa tgtattttta 2941 attgacaaat aaaaactgta tatattttca tgtgcaaaaa aaaaaaaaaa

[0095] By “NOGOA polypeptide” (or neurite outgrowth inhibitor A; neurite outgrowth inhibitor isoform A; human reticulon-4; human reticulon-4 isoform A) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_065393.

TABLE-US-00027 (SEQ ID NO: 27) 1 medldqsplv sssdspprpq pafkyqfvre pedeeeeeee eeedededle elevlerkpa 61 aglsaapvpt apaagaplmd fgndfvppap rgplpaappv aperqpswdp spvsstvpap 121 splsaaavsp sklpeddepp arppppppas vspqaepvwt ppapapaapp stpaapkrrg 181 ssgsvdetlf alpaasepvi rssaenmdlk eqpgntisag qedfpsvlle taaslpslsp 241 lsaasfkehe ylgnlstvlp tegtlqenvs easkevseka ktllidrdlt efseleysem 301 gssfsvspka esavivanpr eeiivknkde eeklvsnnil hnqqelptal tklvkedevv 361 ssekakdsfn ekrvaveapm reeyadfkpf ervwevkdsk edsdmlaagg kiesnleskv 421 dkkcfadsle qtnhekdses snddtsfpst pegikdrsga yitcapfnpa atesiatnif 481 pllgdptsen ktdekkieek kaqivteknt stktsnpflv aaqdsetdyv ttdnltkvte 541 evvanmpegl tpdlvqeace selnevtgtk iayetkmdlv qtsevmqesl ypaaqlcpsf 601 eeseatpspv lpdivmeapl nsavpsagas viqpsssple assvnyesik hepenpppye 661 eamsvslkkv sgikeeikep eninaalqet eapyisiacd liketklsae papdfsdyse 721 makveqpvpd hselvedssp dsepvdlfsd dsipdvpqkq detvmlvkes ltetsfesmi 781 eyenkeklsa lppeggkpyl esfklsldnt kdtllpdevs tlskkekipl qmeelstavy 841 snddlfiske aqiretetfs dsspieiide fptlisskid sfsklareyt dlevshksei 901 anapdgagsl pctelphdls lkniqpkvee kisfsddfsk ngsatskvll lppdvsalat 961 qaeiesivkp kvlvkeaekk lpsdtekedr spsaifsael sktsvvdlly wrdikktgvv 1021 fgaslfllls ltvfsivsvt ayialallsv tisfriykgv iqaiqksdeg hpfraylese 1081 vaiseelvqk ysnsalghvn ctikelrrlf lvddlvdslk favlmwvfty vgalfngltl 1141 lilalislfs vpviyerhqa qidhylglan knvkdamaki qakipglkrk ae

[0096] By “NOGOA nucleic acid molecule” (or neurite outgrowth inhibitor A; neurite outgrowth inhibitor isoform A; human reticulon-4; human reticulon-4 isoform A) is meant a polynucleotide encoding an NOGOA polypeptide. An exemplary NOGOA nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_020532.

TABLE-US-00028 (SEQ ID NO: 28) 1 agtccctgcc ctcccctggg gagggtgagt cacgccaaac tgggcggaga gtccgctggc 61 ctcactccta gctcatctgg gcggcggcgg caagtgggga cagggcgggt ggcgcatcac 121 cggcgcggag gcaggaggag cagtctcatt gttccgggag ccgtcaccac agtaggtccc 181 tcggctcagt cggcccagcc cctctcagtc ctccccaacc cccacaaccg cccgcggctc 241 tgagacgcgg ccccggcggc ggcggcagca gctgcagcat catctccacc ctccagccat 301 ggaagacctg gaccagtctc ctctggtctc gtcctcggac agcccacccc ggccgcagcc 361 cgcgttcaag taccagttcg tgagggagcc cgaggacgag gaggaagaag aggaggagga 421 agaggaggac gaggacgaag acctggagga gctggaggtg ctggagagga agcccgccgc 481 cgggctgtcc gcggccccag tgcccaccgc ccctgccgcc ggcgcgcccc tgatggactt 541 cggaaatgac ttcgtgccgc cggcgccccg gggacccctg ccggccgctc cccccgtcgc 601 cccggagcgg cagccgtctt gggacccgag cccggtgtcg tcgaccgtgc ccgcgccatc 661 cccgctgtct gctgccgcag tctcgccctc caagctccct gaggacgacg agcctccggc 721 ccggcctccc cctcctcccc cggccagcgt gagcccccag gcagagcccg tgtggacccc 781 gccagccccg gctcccgccg cgccccctc caccccggcc gcgcccaagc gcaggggctc 841 ctcgggctca gtggatgaga ccctttttgc tcttcctgct gcatctgagc ctgtgatacg 901 ctcctctgca gaaaatatgg acttgaagga gcagccaggt aacactattt cggctggtca 961 agaggatttc ccatctgtcc tgcttgaaac tgctgcttct cttccttctc tgtctcctct 1021 ctcagccgct tctttcaaag aacatgaata ccttggtaat ttgtcaacag tattacccac 1081 tgaaggaaca cttcaagaaa atgtcagtga agcttctaaa gaggtctcag agaaggcaaa 1141 aactctactc atagatagag atttaacaga gttttcagaa ttagaatact cagaaatggg 1201 atcatcgttc agtgtctctc caaaagcaga

atctgccgta atagtagcaa atcctaggga 1261 agaaataatc gtgaaaaata aagatgaaga agagaagtta
gttagtaata acatccttca 1321 taatcaacaa gagttaccta cagctcttac taaattggtt aaagaggatg
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gccttgagca aactaatcac gaaaaagata gtgagagtag 1621 taatgatgat acttctttcc ccagtacgcc
agaaggtata aaggatcgtt caggagcata 1681 tatcacatgt gctcccttta acccagcagc aactgagagc
attgcaacaa acatttttcc 1741 tttgttagga gatcctactt cagaaaataa gaccgatgaa aaaaaaatag
aagaaaagaa 1801 ggcccaaata gtaacagaga agaatactag caccaaaaca tcaaaccctt ttcttgtagc 1861
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gtacaaagat tgcttatgaa acaaaaatgg acttggttca 2041 aacatcagaa gttatgcaag agtcactcta
tcctgcagca cagctttgcc catcatttga 2101 agagtcagaa gctactcctt caccagtttt gcctgacatt
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cattagaagc 2221 ttcttcagtt aattatgaaa gcataaaaca tgagcctgaa aacccccccac catatgaaga 2281
ggccatgagt gtatcactaa aaaaagtatc aggaataaag gaagaaatta aagagcctga 2341 aaatattaat
gcagctcttc aagaaacaga agctccttat atatctattg catgtgattt 2401 aattaaagaa acaaagcttt
ctgctgaacc agctccggat ttctctgatt attcagaaat 2461 ggcaaaagtt gaacagccag tgcctgatca
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ctaaggaagc acagataaga gaaactgaaa cgttttcaga 2881 ttcatctcca attgaaatta tagatgagtt
ccctacattg atcagttcta aaactgattc 2941 attttctaaa ttagccaggg aatatactga cctagaagta
tcccacaaaa gtgaaattgc 3001 taatgccccg gatggagctg ggtcattgcc ttgcacagaa ttgccccatg
acctttcttt 3061 gaagaacata caacccaaag ttgaagagaa aatcagtttc tcagatgact tttctaaaaa 3121
tgggtctgct acatcaaagg tgctcttatt gcctccagat gtttctgctt tggccactca 3181 agcagagata
gagagcatag ttaaacccaa agttcttgtg aaagaagctg agaaaaaact 3241 tccttccgat acagaaaaag
aggacagatc accatctgct atattttcag cagagctgag 3301 taaaacttca gttgttgacc tcctgtactg
gagagacatt aagaagactg gagtggtgtt 3361 tggtgccagc ctattcctgc tgctttcatt gacagtattc
agcattgtga gcgtaacagc 3421 ctacattgcc ttggccctgc tctctgtgac catcagcttt aggatataca
agggtgtgat 3481 ccaagctatc cagaaatcag atgaaggcca cccattcagg gcatatctgg aatctgaagt 3541
tgctatatct gaggagttgg ttcagaagta cagtaattct gctcttggtc atgtgaactg 3601 cacgataaag
gaactcaggc gcctcttctt agttgatgat ttagttgatt ctctgaagtt 3661 tgcagtgttg atgtgggtat
ttacctatgt tggtgccttg tttaatggtc tgacactact 3721 gattttggct ctcatttcac tcttcagtgt tcctgttatt
tatgaacggc atcaggcaca 3781 gatagatcat tatctaggac ttgcaaataa gaatgttaaa gatgctatgg
ctaaaatcca 3841 agcaaaaatc cctggattga agcgcaaagc tgaatgaaaa cgcccaaaat aattagtagg 3901
agttcatctt taaaggggat attcatttga ttatacgggg gagggtcagg gaagaacgaa 3961 ccttgacgtt
gcagtgcagt ttcacagatc gttgttagat ctttatttttt agccatgcac 4021 tgttgtgagg aaaaattacc
tgtcttgact gccatgtgtt catcatctta agtattgtaa 4081 gctgctatgt atggatttaa accgtaatca
tatctttttc ctatctatct gaggcactgg 4141 tggaataaaa aacctgtata ttttactttg ttgcagatag
tcttgccgca tcttggcaag 4201 ttgcagagat ggtggagcta gaaaaaaaaa aaaaaaagcc cttttcagtt
tgtgcactgt 4261 gtatggtccg tgtagattga tgcagatttt ctgaaatgaa atgtttgttt agacgagatc 4321
ataccggtaa agcaggaatg acaaagcttg cttttctggt atgttctagg tgtattgtga 4381 cttttactgt
tatattaatt gccaatataa gtaaatatag attatatatg tatagtgttt 4441 cacaaagctt agacctttac
cttccagcca ccccacagtg cttgatattt cagagtcagt 4501 cattggttat acatgtgtag ttccaaagca
cataagctag aagaagaaat atttctagga 4561 gcactaccat ctgttttcaa catgaaatgc cacacacata
gaactccaac atcaatttca 4621 ttgcacagac tgactgtagt taattttgtc acagaatcta tggactgaat
ctaatgcttc 4681 caaaaatgtt gtttgtttgc aaatatcaaa cattgttatg caagaaatta ttaattacaa 4741

aatgaagatt tataccattg tggtttaagc tgtactgaac taaatctgtg gaatgcattg 4801 tgaactgtaa aagcaaagta tcaataaagc ttatagactt aaaaaaaaaa aaaaaaaaaa 4861 aaaaaaaaaa a

[0097] By "oligodendrocyte O1 polypeptide" (or oligodendrocyte marker O1; oligodendrocyte transcription factor 1: olig1) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q8TAK6.

TABLE-US-00029 (SEQ ID NO: 29) 1 myyavsqarv navpgtmlrp qrpgdlqlga slyelvgyrq ppssssssts stsstssst 61 tapllpkaar ekpeapaepp gpgpgsgahp ggsarpdake eqqqqlrrki nsrerkrmqd 121 lnlamdalre vilpysaahc qgapgrklsk iatlllarny illlgsslqe lrralgegag 181 paaprlllag lpllaaapgs vllapgavgp pdalrpakyl slaldeppcg qfalpgggag 241 gpglctcavc kfphlvpasl glaavqaqfs k

[0098] By "oligodendrocyte O1 nucleic acid molecule" (or oligodendrocyte marker O1; oligodendrocyte transcription factor 1; olig1) is meant a polynucleotide encoding an oligodendrocyte O1 polypeptide. An exemplary oligodendrocyte O1 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_138983.

TABLE-US-00030 (SEQ ID NO: 30) 1 gttctagatc gtttccccgc gcgcaggtcc gcggggaggg gcggcctgcc gaccggccca 61 ccccagggcg ttcctgaagg gcgtcctcgg ccgcccccac cgcctcccag atgtactatg 121 cggtttccca ggcgcgcgtg aacgcggtcc ccgggaccat gctgcggcca cagcggcccg 181 gagacttgca gctcggggcc tccctctacg agctggtggg ctacaggcag ccgccctcct 241 cctcctcctc ctccacctcc tccacctcct ccacttcctc ctcctccacg acggcccccc 301 tcctccccaa ggctgcgcgc gagaagccgg aggcgccggc cgagcctcca ggccccgggc 361 ccgggtcagg cgcgcacccg ggcggcagcg cccggccgga cgccaaggag gagcagcagc 421 agcagctgcg gcgcaagatc aacagccgcg agcggaagcg catgcaggac ctgaacctgg 481 ccatggacgc cctgcgcgag gtcatcctgc cctactcagc ggcgcactgc cagggcgcgc 541 ccggccgcaa gctctccaag atagccacgc tgctgctcgc ccgcaactac atcctactgc 601 tgggcagctc gctgcaggag ctgcgccgcg cgctgggcga gggcgccggg cccgccgcgc 661 cgcgcctgct gctggccggg ctgcccctgc tcgccgccgc gcccggctcc gtgctgctgg 721 cgcccggcgc cgtaggaccc cccgacgcgc tgcgccccgc caagtacctg tcgctggcgc 781 tggacgagcc gccgtgcggc cagttcgctc tccccggcgg cggcgcaggc ggccccggcc 841 tctgcacctg cgccgtgtgc aagttcccgc acctggtccc ggccagcctg ggcctggccg 901 ccgtgcaggc gcaattctcc aagtgagggc gggtctgggc ctggggcgcg acctcggccc 961 ggcctccctt cgctcagctt ctccgcgccc ctgctccctg cgtctgggag agcgaggccg 1021 agcaaggaaa gcatttcgaa ccttccagtc cagaggaagg gactgtcggg caccccttc 1081 cccgccccca cccctgggac gttaaagtga ccagagcgga tgttcgatgg cgcctcgggg 1141 cagtttgggg ttctgggtcg gttccagcgg ctttaggcag aaagtgctcg ctctcaccca 1201 gcacatctct ctccttgtcc ctggagttgc gcgcttcgcg gggccgatgt agaacttagg 1261 gcgccttgcc gtggttggcg cgccccgggt gcagcgagag gccatccccg agcgctacct 1321 ccccggagcg gagcacgcgg gctcccagta ctaggggctg cgctcgagca gtggcggggg 1381 cggaggggtg gttcttttcc ttctcctccg ccagaggcca cgggcgccct tgttcccgcc 1441 ggccaggtcc tatcaaagga ggctgccgga actcaagagg cagaaaaaga ccagttaggc 1501 ggtgcagacg gtctgggacg tggcagacgg acggaccctc ggcggacagg tggtcggcgt 1561 cggggtgcgg tgggtagggg cgaggacaac gcagggtgcg ctgggttggg acgtgggtcc 1621 acttttgtag accagctgtt tggagagctg tatttaagac tcgcgtatcc agtgttttgt 1681 cgcagagagt tttcactctt aaatcctggg ggtttcttag aaagcaactt agaactcgag 1741 attcaccttt cgtttccctt tccccaaaag tagcgtaacc aacatttaag cttgcttaaa 1801 aacgaaaacc aaccgccttg catccagtgt tcccgattta ctaaaatagg taaccaggcg 1861 tctcacagtc gccgtcctgt caagagcgct aatgaacgtt ctcattaaca cgcaggagta 1921 ccgggagccc tgaaccgccc gctgctcggc ggatcccagc tgcggtggcg acggcgggaa 1981 ggcgctttcc gctgttcctc agcgggccgg gcccttgacc agcgcggccc gcaggtcttc 2041 cttctcgccg tcttgcagtt gaagagctac atacgtagtc agtttcgatt tgttacagac 2101 gttaacaaat tcctttaccc aaggttatgc tatgaccttt ccgcagttta ctttgatttt 2161 ctatgtttaa ggttttggtt gttggtagta gccgaattta actggcactt tattttactt 2221 ctaaccttgt ttcctgacgg tgtacagaat caacaaaata aaacatttaa agtctgattt 2281 tttaaaaaaa aaaaaaaa

[0099] By "oligodendrocyte O2 polypeptide" (or oligodendrocyte marker O2; oligodendrocyte transcription factor 2; olig2) is meant a polypeptide or fragment thereof having at least about 85%

amino acid identity to NCBI Accession No. Q13516.

TABLE-US-00031 (SEQ ID NO: 31) 1 mdsdaslvss rpsspepddl flparskgss gsaftggtvs sstpsdcppe lsaelrgamg 61 sagahpgdkl ggsgfkssss stssstssaa asstkkdkkq mtepelqqlr lkinsrerkr 121 mhdlniamdg lrevmpyahg psvrklskia tlllarnyil mltnsleemk rlvseiyggh 181 hagfhpsacg glahsaplpa atahpaaaah aahhpavhhp ilppaaaaaa aaaaaaavss 241 aslpgsglps vgsirpphgl lkspsaaaaa plgggggggsg asggfqhwgg mpcpcsmcqv 301 ppphhhvsam gagslprlts dak

[0100] By "oligodendrocyte O2 nucleic acid molecule" (or oligodendrocyte marker O2; oligodendrocyte transcription factor 2; olig2) is meant a polynucleotide encoding an oligodendrocyte O2 polypeptide. An exemplary oligodendrocyte O2 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_005806.

TABLE-US-00032 (SEQ ID NO: 32) 1 gggtgcttat tatagatcga cgcgacacca gcgcccggtg ccaggttctc ccctgaggct 61 tttcggagcg agctcctcaa atcgcatcca gagtaagtgt ccccgcccca cagcagccgc 121 agcctagatc ccagggacag actctcctca actcggctgt gacccagaat gctccgatac 181 agggggtctg gatccctact ctgcgggcca tttctccaga gcgactttgc tcttctgtcc 241 tccccacact caccgctgca tctccctcac caaaagcgag aagtcggagc gacaacagct 301 ctttctgccc aagccccagt cagctggtga gctccccgtg gtctccagat gcagcacatg 361 gactctgggc cccgcgccgg ctctgggtgc atgtgcgtgt gcgtgtgttt gctgcgtggt 421 gtcgatggag ataaggtgga tccgtttgag gaaccaaatc attagttctc tatttagatc 481 tccattctcc ccaaagaaag gccctcactt cccactcgtt tattccagcc cgggggctca 541 gttttcccac acctaactga aagcccgaag cctctagaat gccacccgca ccccgagggt 601 caccaacgct ccctgaaata acctgttgca tgagagcaga ggggagatag agagagctta 661 attataggta cccgcgtgca gctaaaagga gggccagaga tagtagcgag ggggacgagg 721 agccacgggc cacctgtgcc gggaccccgc gctgtggtac tgcggtgcag gcggggagcag 781 cttttctgtc tctcactgac tcactctctc tctctctccc tctctctctc tctcattctc 841 tctcttttct cctcctctcc tggaagtttt cgggtccgag ggaaggagga ccctgcgaaa 901 gctgcgacga ctatcttccc ctggggccat ggactcggac gccagcctgg tgtccagccg 961 cccgtcgtcg ccagagcccg atgacctttt tctgccggcc cggagtaagg gcagcagcgg 1021 cagcgccttc actgggggca ccgtgtcctc gtccaccccg agtgactgcc cgccggagct 1081 gagcgccgag ctgcgcggcg ctatgggctc tgcgggcgcg catcctgtgg acaagctagg 1141 aggcagtggc ttcaagtcat cctcgtccag cacctcgtcg tctacgtcgt cggcggctgc 1201 gtcgtccacc aagaaggaca agaagcaaat gacagagccg gagctgcagc agctgcgtct 1261 caagatcaac agccgcgagc gcaagcgcat gcacgacctc aacatcgcca tggatggcct 1321 ccgcgaggtc atgccgtacg cacacggccc ttcggtgcgc aagctttcca agatcgccac 1381 gctgctgctg gcgcgcaact acatcctcat gctcaccaac tcgctggagg agatgaagcg 1441 actggtgagc gagatctacg ggggccacca cgctggcttc cacccgtcgg cctgcggcgg 1501 cctggcgcac tccgcgcccc tgcccgccgc caccgcgcac ccggcagcag cagcgcacgc 1561 cgcacatcac cccgcggtgc accaccccat cctgccgccc gccgccgcag cggctgctgc 1621 cgccgctgca gccgcggctg tgtccagcgc ctctctgccc ggatccgggc tgccgtcggt 1681 cggctccatc cgtccaccgc acggcctact caagtctccg tctgctgccg cggccgcccc 1741 gctgggggggc gggggcggcg gcagtggggc gagcgggggc ttccagcact ggggcggcat 1801 gccctgcccc tgcagcatgt gccaggtgcc gccgccgcac caccacgtgt cggctatggg 1861 cgccggcagc ctgccgcgcc tcacctccga cgccaagtga gcctactggc gccggcgcgt 1921 tctggcgaca ggggagccag gggccgcggg gaagcgagga ctggcctgcg ctgggctcgg 1981 gagctctgtc gcgaggaggg gcgcaggacc atggactggg ggtggggcat ggtggggatt 2041 tcagcatctg cgaacccaag caatgggggc gcccacagag cagtggggag tgaggggatg 2101 ttctctccgg gacctgatcg agcgctgtct ggctttaacc tgagctggtc cagtagacat 2161 cgttttatga aaaggtaccg ctgtgtgcat tcctcactag aactcatccg acccccgacc 2221 cccacctccg ggaaaagatt ctaaaaactt ctttccctga gagcgtggcc tgacttgcag 2281 actcggcttg ggcagcactt cgggggggga gggggtgtta tgggaggggg acacattggg 2341 gccttgctcg tcttcctcct ttcttggcgg gtgggagact ccgggtagcc gcactgcaga 2401 agcaacagcc cgaccgcgcc ctccagggtc gtccctggcc caaggccagg ggccacaagt 2461 tagttggaag ccggcgttcg gtatcagaag cgctgatggt catatccaat ctcaatatct 2521 gggtcaatcc acaccctctt agaactgtgg ccgttcctcc ctgtctctcg ttgatttggg 2581 agaatatggt tttctaataa atctgtggat gttccttctt caacagtatg agcaagttta 2641 tagacattca gagtagaacc acttgtggat

tggaataacc caaaactgcc gatttcaggg 2701 gcgggtgcat tgtagttatt attttaaaat agaaactacc ccaccgactc atctttcctt 2761 ctctaagcac aaagtgattt ggttattttg gtacctgaga acgtaacaga attaaaaggc 2821 agttgctgtg gaaacagttt gggttatttg ggggttctgt tggcttttta aaattttctt 2881 ttttggatgt gtaaatttat caatgatgag gtaagtgcgc aatgctaagc tgtttgctca 2941 cgtgactgcc agccccatcg gagtctaagc cggctttcct ctattttggt ttatttttgc 3001 cacgtttaac acaaatggta aactcctcca cgtgcttcct gcgttccgtg caagccgcct 3061 cggcgctgcc tgcgttgcaa actgggcttt gtagcgtctg ccgtgtaaca cccttcctct 3121 gatcgcaccg cccctcgcag agagtgtatc atctgtttta tttttgtaaa aacaaagtgc 3181 taaataatat ttattacttg tttggttgca aaaacggaat aaatgactga gtgttgagat 3241 tttaaataaa atttaaagca aaaaaaaaaa aaaaa

[0101] By “oligodendrocyte O4 polypeptide” (or oligodendrocyte marker O4; oligodendrocyte transcription factor 4; olig4) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. Q05586.

[0102] By “oligodendrocyte O4 nucleic acid molecule” (or oligodendrocyte marker O4; oligodendrocyte transcription factor 4; olig4) is meant a polynucleotide encoding an oligodendrocyte O4 polypeptide. An exemplary oligodendrocyte O4 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_007327.

[0103] By “GFAP” (or Glial fibrillary acidic protein) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P14136.

TABLE-US-00033 (SEQ ID NO: 33) 1 merritsaa rrsyvssgem mvgglapgrr lgpgtrlsla rmppplptrv dfslagalna 61 gfketraser aemmelndrf asyiekvrfl eqqnkalaae lnqlrakept kladvyqael 121 relrlrldql tansarleve rdnlaqdlat vrqklqdetn lrleaennla ayrqeadeat 181 larldlerki esleeeirfl rkiheeevre lqeqlarqqv hveldvakpd ltaalkeirt 241 qyeamassnm heaeewyrsk fadltdaaar naellrqakh eandyrrqlq sltcdleslr 301 gtneslerqm reqeerhvre aasyqealar leeegqslkd emarhlqeyq dllnvklald 361 ieiatyrkll egeenritip vqtfsnlqir etsldtksvs eghlkrnivv ktvemrdgev 421 ikeskqehkd vm

[0104] By “GFAP nucleic acid molecule” (or Glial fibrillary acidic protein) is meant a polynucleotide encoding an GFAP polypeptide. An exemplary GFAP nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_002055.

TABLE-US-00034 (SEQ ID NO: 34) 1 gcaggatgga gaggagacgc atcacctccg ctgctcgccg ctcctacgtc tcctcagggg 61 agatgatggt ggggggcctg gctcctggcc gccgtctggg tcctggcacc cgcctctccc 121 tggctcgaat gccccctcca ctcccgaccc gagtggattt ctccctggct ggggcactca 181 atgctggctt caaggagacc cgggccagtg agcgggcaga gatgatggag ctcaatgacc 241 gctttgccag ctacatcgag aaggttcgct tcctggaaca gcaaaacaag gcgctggctg 301 ctgagctgaa ccagctgcgg gccaaggagc ccaccaagct ggcagacgtc taccaggctg 361 agctgcgaga gctgcggctg cggctcgatc aactcaccgc caacagcgcc cggctggagg 421 ttgagaggga caatctggca caggacctgg ccactgtgag gcagaagctc caggatggaa 481 ccaacctgag gctggaagcc gagaacaacc tggctgccta tagacaggaa gcagatgaag 541 ccacccctggc ccgtctggat ctggagagga agattgagtc gctggaggag gagatccggt 601 tcttgaggaa gatccacgag gaggaggttc gggaactcca ggagcagctg gcccgacagc 661 aggtccatgt ggagcttgac gtggccaagc cagacctcac cgcagccctg aaagagatcc 721 gcacgcagta tgaggcaatg gcgtccagca acatgcatga agccgaagag tggtaccgct 781 ccaagtttgc agacctgaca gacgctgctg cccgcaacgc ggagctgctc cgccaggcca 841 agcacgaagc caacgactac cggcgccagt tgcagtcctt gacctgcgac ctggagtctc 901 tgcgcggcac gaacgagtcc ctggagaggc agatgcgcga gcaggaggag cggcacgtgc 961 gggaggcggc cagttatcag gaggcgctgg cgcggctgga ggaagagggg cagagcctca 1021 aggacgagat ggcccgccac ttgcaggagt accaggacct gctcaatgtc aagctggccc 1081 tggacatcga gatcgccacc tacaggaagc tgctagaggg cgaggagaac cggatcacca 1141 ttcccgtgca gaccttctcc aacctgcaga ttcgagaaac cagcctggac accaagtctg 1201 tgtcagaagg ccacctcaag aggaacatcg tggtgaagac cgtggagatg cgggatggag 1261 aggtcattaa ggagtccaag caggagcaca aggatgtgat gtgaggcagg acccacctgg 1321 tggcctctgc cccgtctcat gaggggcccg agcagaagca ggatagttgc tccgcctctg 1381 ctggcacatt tccccagacc tgagctcccc accaccccag ctgctccct ccctcctctg 1441 tccctaggtc agcttgctgc cctaggctcc gtcagtatca ggcctgcc

[0105] By “s100b” (or S-100 protein beta chain; S-100 protein subunit beta; S100 calcium-binding protein B) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P04271. [0106] 1 mselekamva lidvfhqysg regdkhklkk selkelinne Ishfleeike qevvdkvmet [0107] 61 ldndgdgecd fqefmafvam vttacheffe he (SEQ ID NO: 35)

[0108] By “s100b nucleic acid molecule” (or S-100 protein beta chain; S-100 protein subunit beta; S100 calcium-binding protein B) is meant a polynucleotide encoding an s100b polypeptide. An exemplary s100b nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_006272.

TABLE-US-00035 (SEQ ID NO: 36) 1 gggcagaggg aataagaggc tgcctctgcc caccagtcct gccgcccagg acccgcagca 61 gagacgacgc ctgcagcaag gagaccagga aggggtgaga caaggaagag gatgtctgag 121 ctggagaagg ccatggtggc cctcatcgac gttttccacc aatattctgg aagggaggga 181 gacaagcaca agctgaagaa atccgaactg aaggagctca tcaacaatga gctttcccat 241 ttcttagagg aaatcaaaga gcaggaggtt gtggacaaag tcatggaaac actggacaat 301 gatggagacg gcgaatgtga cttccaggaa ttcatggcct ttgttgccat ggttactact 361 gcctgccacg agttctttga acatgagtga gattagaaag cagccaaacc tttcctgtaa 421 cagagacggt catgcaagaa agcagacagc aagggcttgc agcctagtag gagctgagct 481 ttccagccgt gttgtagcta attaggaagc ttgatttgct ttgtgattga aaaattgaaa 541 acctctttcc aaaggctgtt ttaacggcct gcatcattct ttctgctata ttaggcctgt 601 gtgtaagctg actggcccca gggactcttg ttaacagtaa cttaggagtc aggtctcagt 661 gataaagcgt gcaccgtgca gcccgccatg gccgtgtaga ccctaacccg gagggaaccc 721 tgactacaga aattaccccg gggcaccctt aaaacttcca ctacctttaa aaaacaaagc 781 cttatccagc attatttgaa aacactgctg ttctttaaat gcgttcctca tccatgcaga 841 taacagctgg ttggccggtg tggccctgca agggcgtggt ggcttcggcc tgcttcccgg 901 gatgcgcctg atcaccaggt gaacgctcag cgctggcagc gctcctggaa aaagcaactc 961 catcagaact cgcaatccga gccagctctg ggggctccag cgtggcctcc gtgacccatg 1021 cgattcaagt cgcggctgca ggatccttgc ctccaacgtg cctccagcac atgcggcttc 1081 cgagggcact accgggggct ctgagccacc gcgagggcct gcgttcaata aaaag

[0109] By “SOX10 polypeptide” (or SRY-related HMG-box transcription factor) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_008872.1.

TABLE-US-00036 (SEQ ID NO: 37)
MAEEQDLSEVELSPVGSEEPRCLSPGSAPSLGPDGGGGGSGLRASPGPGE
LGKVKKEQQDGEADDDKFPVCIREAVSQVLSGYDWTLVPMPVRVNGASKS
KPHVKRPMNAFMVWAQAARRKLADQYPHLHNAELSKTLGKLWRLLNESDK
RPFIEEAERLRMQHKKDHPDYKYQPRRRKNGKAAQGEAECPGGEAEQGGT
AAIQAHYKSAHLDHRHPGEGSPMSDGNPEHPSGQSHGPPTPPTTPKTELQ
SGKADPKRDGRSMGEGGKPHIDFGNVDIGEISHEVMSNMETFDVAELDQY
LPPNGHPGHVSSYSAAGYGLGSALAVASGHSAWISKPPGVALPTVSPPGV
DAKAQVKTETAGPQGPPHYTDQPSTSQIAYTSLSLPHYGSAFPSISRPQF
DYSDHQPSGPYYGHSGQASGLYSAFSYMGPSQRPLYTAISDPSPSG
PQSHSPTHWEQPVYTTLSRP

[0110] By “SOX10 nucleic acid molecule” (or SRY-related HMG-box transcription factor) is meant a polynucleotide encoding an SOX10 polypeptide. An exemplary SOX10 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_006941.3.

TABLE-US-00037 (SEQ ID NO: 38) 1 gtccggccag ggtggttggt ggtaaggatt caggctccgt cctaacgagg ccgtggcctg 61 aggctcaggg ccccccgccc ctccctccca gcccaccagc gtcacctccc agccccgagc 121 tggaccgcac accttgggac acggttttcc acttcctaag gacgagcccc agactggagg 181 agaggtccga ggaggtgggc gttggactct ttgcgaggac cccggcggct ggcccggggg 241 aggcggccga ggcggcggcg gcggcggccg ggggcgacat ggcggaggag caggacctat 301 cggaggtgga gctgagcccc gtgggctcgg aggagccccg ctgcctgtcc ccggggagcg 361 cgccctcgct agggcccgac ggcggcggcg gcggatcggg cctgcgagcc agcccggggc 421 caggcgagct gggcaaggtc aagaaggagc agcaggacgg cgaggcggac gatgacaagt 481 tccccgtgtg catccgcgag gccgtcagcc

aggtgctcag cggctacgac tggacgctgg 541 tgcccatgcc cgtgcgcgtc aacggcgcca gcaaaagcaa gccgcacgtc aagcggccca 601 tgaacgcctt catggtgtgg gctcaggcag cgcgcaggaa gctcgcggac cagtacccgc 661 acctgcacaa cgctgagctc agcaagacgc tgggcaagct ctggaggctg ctgaacgaaa 721 gtgacaagcg ccccttcatc gaggaggctg agcggctccg tatgcagcac aagaaagacc 781 acccggacta caagtaccag cccaggcggc ggaagaacgg gaaggccgcc cagggcgagg 841 cggagtgccc cggtggggag gccgagcaag gtgggaccgc cgccatccag gcccactaca 901 agagcgccca cttggaccac cggcacccag gagagggctc ccccatgtca gatgggaacc 961 ccgagcaccc ctcaggccag agccatggcc cacccacccc tccaaccacc ccgaagacag 1021 agctgcagtc gggcaaggca gacccgaagc gggacgggcg ctccatgggg gagggcggga 1081 agcctcacat cgacttcggc aacgtggaca ttggtgagat cagccacgag gtaatgtcca 1141 acatggagac ctttgatgtg gctgagttgg accagtacct gccgcccaat gggcacccag 1201 gccatgtgag cagctactca gcagccggct atgggctggg cagtgccctg gccgtggcca 1261 gtggacactc cgcctggatc tccaagccac caggcgtggc tctgcccacg gtctcaccac 1321 ctggtgtgga tgccaaagcc caggtgaaga cagagaccgc ggggcccccag gggccccccac 1381 actacaccga ccagccatcc acctcacaga tcgcctacac ctccctcagc ctgccccact 1441 atggctcagc cttcccctcc atctcccgcc cccagtttga ctactctgac catcagccct 1501 caggacccta ttatggccac tcgggccagg cctctggcct ctactcggcc ttctcctata 1561 tggggccctc gcagcggccc ctctacacgg ccatctctga ccccagcccc tcagggcccc 1621 agtcccacag ccccacacac tgggagcagc cagtatatac gacactgtcc cggccctaaa 1681 gggggccctg tcgccaccac cccccgccca gcccctgccc ccagcctgtg tgccctgttc 1741 cttgcccacc tcaggcctgg tggtggcagt ggaggaggct gaggaggctg aagaggctga 1801 caggtcgggg ggctttctgt ctggctcact gccctgatga cccacccgcc ccatccaggc 1861 tccagcagca aagccccagg agaacaggct ggacagagga gaaggaggtt gactgttgca 1921 cccacactga aagatgaggg gctgcacctt cccccaggaa tgaccctcta tcccaggacc 1981 tgagaagggc ctgctcaccc tcctcgggga ggggaagcac cagggttggt ggcatcggag 2041 gccttaccac tcctatgact cctgttttct ctctcacaga tagtgagggt ctgacatgcc 2101 catgccacct atgccacagt gcctaagggc taggccaccc agagactgtg cccggagctg 2161 gccgtgtctc ccactcaggg gctgagagta gctttgagga gcctcattgg ggagtggggg 2221 gttcgaggga cttagtggag ttctcatccc ttcaatgccc cctccctttc tgaaggcagg 2281 aaggagttgg cacagaggcc ccctgatcca attctgtgcc aataacctca ttctttgtct 2341 gagaaacagc ccccagtcct cctccactac aacctccatg accttgagac gcatcccagg 2401 aggtgacgag gcaggggctc caggaaagga atcagagaca attcacagag cctccctccc 2461 tgggctcctt gccagctccc tcttccctta ctaggctcta tggcccctgc tcagtcagcc 2521 ccactccctg ggcttcccag agagtgacag ctgctcaggc cctaaccctt ggctccagga 2581 gacacagggc ccagcaccca ggttgctgtc ggcaggctga agacactaga atcctgacct 2641 gtacattctg cccttgcctc ttacccctg cctcccagtg gtatttgaat aaagtatgta 2701 gctatatctg cccctatttt cctgttctgc agccccccaa atccacatgt aactcattac 2761 tgtctcctgt tatttatctc agtagtcccc tctcctagcc actctagccc ctattaactc 2821 tgcattaagc attccacata ataaaattaa aggttccggt taaaaaaaaa aaaaaaaaaa 2881 aa

[0111] By “SYN1 protein” (or Synaptin I protein) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to GenBank: AH006533.2.

TABLE-US-00038 (SEQ ID NO: 39)

MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPPGAHSPGATPGPGTAT
AERSSGVAPAASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVG
GGSGGAGRGGAASRVLLVIDEPHTDWAKYFKGKKIHGGIDIKVEQAEFSD
LNLVAHANGGFSVDMEVLRNGVKVVRSLKPDFVLIRQHAFSMARNGDYRS
LVIGLQYAGIPSVNSLHSVYNFCDKPWVFAQMVRLHKKLGTEEFPLIDQT
FYPNHKEMLSSTTYPVVVKMGHAHSGMGKVKVDNQHDFQDIASVVALTKT
YATAEPFIDAKYDVRVQKIGQNYKAYMRTSVSGNWKTNTGSAMLEQIAMS
DRYKLWVDTCSEIFGGLDICAVEALHGKDGRDHIIEVVGSSMPLIGDHQD
EDKQLIVELVVNKMAQALPRQRQRDASPGRGSHGQTPSPGALPLGRQTSQ
QPAGPPAQQRPPPQGGPPQPGPGPQRQGPPLQQRPPPQGQQHLSGLGPPA
GSPLPQRLPSPTSAPQQPASQAAPPTQGQGRQSRPVAGGPGAPPAARPPA
SPSPQRQAGPPQATRQTSVSGPAPPKASGAPPGGQQRQGPPQKPPGPAGP

TRQASQAGPVPRTGPPTTQQPRPSGPGPAGAPKPQLAQKPSQDVPPPATA
AAGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKAETIRSLRKSFA SLFSD
[0112] By “SYN1 nucleic acid molecule” (or synapsin I gene) is meant a polynucleotide encoding an SYN1polypeptide. An exemplary SYN1nucleic acid molecule (e.g., mRNA) is provided at GenBank: AH006533.2.
TABLE-US-00039 (SEQ ID NO: 40) 1 ctcgagagag aaggagagga cattcctggc agaagttaca acacatgcaa aggtacagag 61 gttgccccct tcctacccct ctccttagag gtgggttaga gatgtatcct ttttacagat 121 gaggaaacca aatctcagaa agattaagtc actttcccaa gtgtatggtg gaggccccac 181 ttgaacccag gcactgtgtc tccagacccc acactattac tgccttgttt aaaccagcca 241 actgatttaa tgaataaagg atgaacaaat gaataagtgg atgagtcacc tgaaaattct 301 gcaggcaaag agactccata tctacttact tcttgcctat cttctgccac ctctcctagt 361 ccaccatcac tgctcactat ggtcaaggtc ctacccaatc tggcccctgc taccacaacc 421 cccttcagct tgttccagcc acattggcac tggatgtttc ctcttcctgg cacattctta 481 aaaaaatgtg ttgatcataa agtgaacatg acccctttggg aattaactgg agttcttgta 541 ttccctcatc tgtaaaatag acattatatt atccacccca ctggattgtt gtgagggtgg 601 gatgaaatga tgcatgtaaa cacgcttagc ttaagagttg ggtacaatca gtgaacaaat 661 gattatgaat tagtgctttt attgtagtca gaatcataaa gatttgacag gttcccatat 721 cccacctctg cttggactac ctcatttgct catatgcaaa gattatttgg tacctactgt 781 gtgtgcacca tgggatgggc ctgcctctgt ggaaagttct tgggtgcagg gggagacagc 841 catgggcact gatgacatca ggtagttatc gtgagttttg gcggtgtcca gagcaaaggg 901 atggtggcgt atataccaag tgtgttctgg tgtgggggtg gacacgcacc agggctaggg 961 ctgcagagaa tgtctgtgtt gcagatctag gtttctccat gatcatcggt gggaatgtgt 1021 tttgtctgca agtgtatgct catatgagtt tccctgggtc tctgtgtgtc agtgtgttac 1081 ctgtgtgtgt gggggtatgg gtgtatgcat gcatgtatgt aacatgccca tgtgtgttac 1141 tctggacttg tatgtctgta tgtataccta gattggcgtg tgttctgtct gtacatgccc 1201 tcgtatgttt cctcactttt gtgtgtgttt atatgtgtgt catttcttgt gtgccctcca 1261 ggcccccctt gccaccttgg gcaagggtgt gtacaccacc caagtgtcca cctccgcttg 1321 tctgatgctg tctgtgacgc ccccgctctc tgcctagctg agcctgtgtg gatgtgggag 1381 actaatctcc ccgcgggcac tgcgtgtgac ctcacccccc tctgtgaggg ggttatttct 1441 ctactttcgt gtctctgagt gtgcttccag tgcccccctc cccccaaaaa atgccttctg 1501 agttgaatat caacactaca aaccgagtat ctgcagactg cagagggccc tgcgtatgag 1561 tgcaagtggg ttttaggacc aggatgaggc ggggtggggg tgcctacctg acgaccgacc 1621 ccgacccact ggacaagcac ccaaccccca ttccccaaat tgcgcatccc ctatcagaga 1681 gggggagggg aaacaggatg cggcgaggcg cgtcgcgact gccagcttca gcaccgcgga 1741 cagtgccttc gcccccgcct ggcggcgcgc gccaccgccg cctcagcact gaaggcgcgc 1801 tgacgtcact cgccggtccc ccgcaaactc cccttcccgg ccaccttggt cgcgtccgcg 1861 ccgccgccgg cccagccgga ccgcaccacg cgaggcgcga gatagggggg cacgggcgcg 1921 accatctgcg ctgcggcgcc ggcgactcag cgctgcctca gtctgcggtg ggcagcggag 1981 gagtcgtgtc gtgcctgaga gcgcagctgt gctcctgggc accgcgcagt ccgccccgc 2041 ggctcctggc cagaccaccc ctaggacccc ctgccccaag tcgcagccat gaactacctg 2101 cggcgccgcc tgtcggacag caactttatg gccaatctgc caaatgggta catgacagac 2161 ctgcagcgtc cgcagccgcc cccaccgccg cccggtgccc acagccccgg agccacgccc 2221 ggtcccggga ccgccactgc cgagaggtcc tccggggtcg ccccagcggc ctctccggcc 2281 gcccctagcc ccgggtcctc ggggggcggt ggcttcttct cgtcgctgtc caacgcggtc 2341 aagcagacca cggcggcggc agctgccacc ttcagcgagc aggtgggcgg cggctctggg 2401 ggcgcaggcc gcgggggagc cgcctccagg gtgctgctgg tcatcgacga gccgcacacc 2461 gactggtaag
[0113] By “SYP protein” (or synaptophysin protein) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Reference Sequence: NM_003179.2.
TABLE-US-00040 (SEQ ID NO: 41)
MLLLADMDVVNQLVAGGQFRVVKEPLGFVKVLQWVFAIFAFATCGSYSGE
LQLSVDCANKTESDLSIEVEFEYPFRLHQVYFDAPTCRGGTTKVFLVGDY
SSSAEFFVTVAVFAFLYSMGALATYIFLQNKYRENNKGPMLDFLATAVFA
FMWLVSSSAWAKGLSDVKMATDPENIIKEMPVCRQTGNTCKELRDPVTSG
LNTSVVFGFLNLVLWVGNLWFVFKETGWAAPFLRAPPGAPEKQPAPGDAY
GDAGYGQGPGGYGPQDSYGPQGGYQPDYGQPAGSGGSGYGPQGDYGQQGY

GPQGAPTSFSNQM

[0114] By “SYP nucleic acid molecule” (or synaptophysin gene) is meant a polynucleotide encoding an SYN1polypeptide. An exemplary SYP nucleic acid molecule (e.g., mRNA) is provided at NCBI Reference Sequence: NM_003179.2.

TABLE-US-00041 (SEQ ID NO: 42) 1 gccccctgca ttgctgatgc tgctgctggc ggacatggac gtggtgaatc agctggtggc 61 tgggggtcag ttccgggtgg tcaaggagcc cctcggcttt gtgaaggtgc tgcaatgggt 121 cttcgccatc ttcgcctttg ccacatgcgg cagctacagt ggggagctcc agctgagcgt 181 ggattgtgcc aacaagaccg agagtgacct cagcatcgag gtcgagttcg agtacccctt 241 caggctgcac caagtgtact ttgatgcacc cacctgccga gggggcacca ccaaggtctt 301 cttagttggg gactactcct cgtcagccga attctttgtc accgtggccg tgtttgcctt 361 cctctactcc atgggggctc tggccaccta catcttcctg cagaacaagt accgagagaa 421 taacaaaggg cccatgctgg actttctggc cacggctgtg ttcgccttca tgtggctagt 481 tagctcatcg gcatgggcca aggggctgtc agatgtgaag atggccacag acccagagaa 541 cattatcaag gagatgcctg tctgccgcca gacagggaac acatgcaagg agctgagaga 601 ccctgtgacc tcgggactca acacctcggt ggtgttcggc ttcctgaacc tggtgctctg 661 ggtcggcaac ctgtggttcg tgtttaagga gacaggctgg gccgccccgt tcctgcgcgc 721 gcctcccggc gcccccgaga aacaaccggc acccggggac gcctacggcg atgcaggcta 781 cgggcagggc cccggcgggt acgggcccca ggattcctac gggcctcagg gcggctacca 841 gcctgactat ggtcaaccag ccggcagcgg tggcagtggc tacgggcctc agggcgacta 901 tgggcagcaa ggctacggcc cgcagggtgc acccacctcc ttctccaatc agatgtagtc 961 tggtcagtga agcccaggag gacctggggg gggcaagagc tcaggagaag gcctgccccc 1021 cttcccaccc ctatacccta ggtctccacc cctcaagcca ggagaccctg tctttgctgt 1081 ttatatatat atatattata tataaatatc tatttatctg tctgagccct gccctcactc 1141 cactcccctc atccactagg tgcccagtct tgagtgggcc ccctctctta ccccgtccct 1201 ttccctgcat cccttggccc ctctctgttt accctccctg tcccctgagg ttaaggggat 1261 ctaaaaggag gacagggagg gaacagacct cggctgtgtg gggagggtgg gcgtgacttc 1321 agactctctc ctctctctcc ctccactcct cccaactctg gccttggttc ctccagcaat 1381 gcctgcctga acaaaggccg ttagggaaat ccaactccag ggttaaagaa aggcagagat 1441 tgggggggct tggggtagag aggacagttt aggacccaag gtggtcttgg agaggaggtg 1501 tggagtggag gggtcagcag gggggttggg ttccagacag agtggatctg gagtctgaag 1561 gagaggagtg cgctagagca ttctggggtg gggcttggaa gggcgctgag ggcagggttc 1621 tagaaggggc gaggctttaa gcgaggcaga atggtgggct ccagagtagg tgggtcttgg 1681 attggtacca gagcctatgg aaagggtgtg gcttggaaca tttgggagac tgagcttgat 1741 tctaaagggg acagatcttg agcaaggcaa gaagtgggat tcaggaatgg gccaagccag 1801 ggttccagac agggtggggc ttagaatggg gcttccatgg tggtttcaga aagggcagcc 1861 cctccccatg gtgcagtgaa gaaaatgttt tacaatggct gggtttgggc agtggagagg 1921 ggacttggat aggagcttcc agatgggttt tgttaggggt gggggagaat ggctctggct 1981 acgacttggg acggaagtgg cctgagaaga gtcgagtgat atggcttgta gggtgaggcg 2041 tgggatccag agagaagcac cccaccacac acacccttcc ccactcccgt gatgaaacag 2101 ctaggttaat aggaggacag aaccaacggg tctgtgggac tggcccaccc ctcttccccc 2161 ttcccctgcg ccctccctcc ctccacacct ccacccgtcc tggggtggtt ggaggcctgg 2221 tctggagccc ctatcctgca ccctctgcta tgtctgtgat gtcagtagtg cctgtgatcg 2281 tgtgttgcca ttttgtctgg ctgtggcccc tccttctccc ctccagaccc ctacccttc 2341 ccaaaccctt cggtattgtt caaagaaccc ccctccccaa ggaagaacaa atatgattct 2401 cctctcccaa ataaactcct taaccaccta gtcaaaaaaa aaaaaaaaa

[0115] By “NOGOA polypeptide” (or neurite outgrowth inhibitor A: neurite outgrowth inhibitor isoform A: human reticulon-4; human reticulon-4 isoform A) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_065393.

TABLE-US-00042 (SEQ ID NO: 43) 1 medldqsplv sssdspprpq pafkyqfvre pedeeeeeee eeedededle elevlerkpa 61 aglsaapvpt apaagaplmd fgndfvppap rgplpaappv aperqpswdp spvsstvpap 121 splsaaavsp sklpeddepp arppppppas vspqaepvwt ppapapaapp stpaapkrrg 181 ssgsvdetlf alpaasepvi rssaenmdlk eqpgntisag qedfpsvlle taaslpslsp 241 lsaasfkehe ylgnlstvlp tegtlqenvs easkevseka ktllidrdlt efseleysem 301 gssfsvspka esavivanpr eeiivknkde eeklvsnnil hnqqelptal tklvkedevv 361 ssekakdsfn ekrvaveapm reeyadfkpf ervwevkdsk edsdmlaagg kiesnleskv 421 dkkcfadsle qtnhekdses

snddtsfpst pegikdrsga yitcapfnpa atesiatnif 481 pllgdptsen ktdekkieek kaqivteknt stktsnpflv aaqdsetdyv ttdnltkvte 541 evvanmpegl tpdlvqeace selnevtgtk iayetkmdlv qtsevmqesl ypaaqlcpsf 601 eeseatpspv lpdivmeapl nsavpsagas viqpsssple assvnyesik hepenpppye 661 eamsvslkkv sgikeeikep eninaalqet eapyisiacd liketklsae papdfsdyse 721 makveqpvpd hselvedssp dsepvdlfsd dsipdvpqkq detvmlvkes ltetsfesmi 781 eyenkeklsa lppeggkpyl esfklsldnt kdtllpdevs tlskkekipl qmeelstavy 841 snddlfiske aqiretetfs dsspieiide fptlisskd sfsklareyt dlevshksei 901 anapdgagsl pctelphdls lkniqpkvee kisfsddfsk ngsatskvll lppdvsalat 961 qaeiesivkp kvlvkeaekk lpsdtekedr spsaifsael sktsvvdlly wrdikktgvv 1021 fgaslfllls ltvfsivsvt ayialallsv tisfriykgv iqaiqksdeg hpfraylese 1081 vaiseelvqk ysnsalghvn ctikelrrlf lvddlvdslk favlmwvfty vgalfngltl 1141 lilalislfs vpviyerhqa qidhylglan knvkdamaki qakipglkrk ae

[0116] By “NOGOA nucleic acid molecule” (or neurite outgrowth inhibitor A; neurite outgrowth inhibitor isoform A; human reticulon-4; human reticulon-4 isoform A) is meant a polynucleotide encoding an NOGOA polypeptide. An exemplary NOGOA nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_020532.

TABLE-US-00043 (SEQ ID NO: 44) 1 agtccctgcc ctcccctggg gagggtgagt cacgccaaac tgggcggaga gtccgctggc 61 ctcactccta gctcatctgg gcggcggcgg caagtgggga cagggcgggt ggcgcatcac 121 cggcgcggag gcaggaggag cagtctcatt gttccgggag ccgtcaccac agtaggtccc 181 tcggctcagt cggcccagcc cctctcagtc ctccccaacc cccacaaccg cccgcggctc 241 tgagacgcgg ccccggcggc ggcggcagca gctgcagcat catctccacc ctccagccat 301 ggaagacctg gaccagtctc ctctggtctc gtcctcggac agcccacccc ggccgcagcc 361 cgcgttcaag taccagttcg tgagggagcc cgaggacgag gaggaagaag aggaggagga 421 agaggaggac gaggacgaag acctggagga gctggaggtg ctggagagga agcccgccgc 481 cgggctgtcc gcggccccag tgcccaccgc ccctgccgcc ggcgcgcccc tgatggactt 541 cggaaatgac ttcgtgccgc cggcgccccg gggacccctg ccggccgctc cccccgtcgc 601 cccggagcgg cagccgtctt gggacccgag cccggtgtcg tcgaccgtgc ccgcgccatc 661 cccgctgtct gctgccgcag tctcgccctc caagctccct gaggacgacg agcctccggc 721 ccggcctccc cctcctcccc cggccagcgt gagcccccag gcagagcccg tgtggacccc 781 gccagccccg gctcccgccg cgccccctc caccccggcc gcgcccaagc gcaggggctc 841 ctcgggctca gtggatgaga ccctttttgc tcttcctgct gcatctgagc ctgtgatacg 901 ctcctctgca gaaaatatgg acttgaagga gcagccaggt aacactattt cggctggtca 961 agaggatttc ccatctgtcc tgcttgaaac tgctgcttct cttccttctc tgtctcctct 1021 ctcagccgct tctttcaaag aacatgaata ccttggtaat ttgtcaacag tattacccac 1081 tgaaggaaca cttcaagaaa atgtcagtga agcttctaaa gaggtctcag agaaggcaaa 1141 aactctactc atagatagag atttaacaga gttttcagaa ttagaatact cagaaatggg 1201 atcatcgttc agtgtctctc caaaagcaga atctgccgta atagtagcaa atcctaggga 1261 agaaataatc gtgaaaaata aagatgaaga agagaagtta gttagtaata acatccttca 1321 taatcaacaa gagttaccta cagctcttac taaattggtt aaagaggatg aagttgtgtc 1381 ttcagaaaaa gcaaaagaca gttttaatga aaagagagtt gcagtggaag ctcctatgag 1441 ggaggaatat gcagacttca aaccatttga gcgagtatgg gaagtgaaag atagtaagga 1501 agatagtgat atgttggctg ctggaggtaa aatcgagagc aacttggaaa gtaaagtgga 1561 taaaaaatgt tttgcagata gccttgagca aactaatcac gaaaaagata gtgagagtag 1621 taatgatgat acttctttcc ccagtacgcc agaaggtata aaggatcgtt caggagcata 1681 tatcacatgt gctcccttta acccagcagc aactgagagc attgcaacaa acatttttcc 1741 tttgttagga gatcctactt cagaaaataa gaccgatgaa aaaaaaatag aagaaaagaa 1801 ggcccaaata gtaacagaga agaatactag caccaaaaca tcaaaccctt ttcttgtagc 1861 agcacaggat tctgagacag attatgtcac aacagataat ttaacaaagg tgactgagga 1921 agtcgtggca aacatgcctg aaggcctgac tccagattta gtacaggaag catgtgaaag 1981 tgaattgaat gaagttactg gtacaaagat tgcttatgaa acaaaaatgg acttggttca 2041 aacatcagaa gttatgcaag agtcactcta tcctgcagca cagctttgcc catcatttga 2101 agagtcagaa gctactcctt caccagtttt gcctgacatt gttatggaag caccattgaa 2161 ttctgcagtt cctagtgctg gtgcttccgt gatacagccc agctcatcac cattagaagc 2221 ttcttcagtt aattatgaaa gcataaaaca tgagcctgaa aacccccccac catatgaaga 2281 ggccatgagt gtatcactaa aaaaagtatc aggaataaag gaagaaatta aagagcctga 2341 aaatattaat gcagctcttc aagaaacaga agctccttat atatctattg catgtgattt 2401 aattaaagaa acaaagcttt

ctgctgaacc agctccggat ttctctgatt attcagaaat 2461 ggcaaaagtt gaacagccag tgcctgatca
ttctgagcta gttgaagatt cctcacctga 2521 ttctgaacca gttgacttat ttagtgatga ttcaatacct
gacgttccac aaaaacaaga 2581 tgaaactgtg atgcttgtga aagaaagtct cactgagact tcatttgagt
caatgataga 2641 atatgaaaat aaggaaaaac tcagtgcttt gccacctgag ggaggaaagc catatttgga 2701
atcttttaag ctcagtttag ataacacaaa agataccctg ttacctgatg aagtttcaac 2761 attgagcaaa
aaggagaaaa ttcctttgca gatggaggag ctcagtactg cagtttattc 2821 aaatgatgac ttatttattt
ctaaggaagc acagataaga gaaactgaaa cgttttcaga 2881 ttcatctcca attgaaatta tagatgagtt
ccctacattg atcagttcta aaactgattc 2941 attttctaaa ttagccaggg aatatactga cctagaagta
tcccacaaaa gtgaaattgc 3001 taatgccccg gatggagctg ggtcattgcc ttgcacagaa ttgccccatg
acctttcttt 3061 gaagaacata caacccaaag ttgaagagaa aatcagtttc tcagatgact tttctaaaaa 3121
tgggtctgct acatcaaagg tgctcttatt gcctccagat gtttctgctt tggccactca 3181 agcagagata
gagagcatag ttaaacccaa agttcttgtg aaagaagctg agaaaaaact 3241 tccttccgat acagaaaaag
aggacagatc accatctgct atattttcag cagagctgag 3301 taaaacttca gttgttgacc tcctgtactg
gagagacatt aagaagactg gagtggtgtt 3361 tggtgccagc ctattcctgc tgctttcatt gacagtattc
agcattgtga gcgtaacagc 3421 ctacattgcc ttggccctgc tctctgtgac catcagcttt aggatataca
agggtgtgat 3481 ccaagctatc cagaaatcag atgaaggcca cccattcagg gcatatctgg aatctgaagt 3541
tgctatatct gaggagttgg ttcagaagta cagtaattct gctcttggtc atgtgaactg 3601 cacgataaag
gaactcaggc gcctcttctt agttgatgat ttagttgatt ctctgaagtt 3661 tgcagtgttg atgtgggtat
ttacctatgt tggtgccttg tttaatggtc tgacactact 3721 gattttggct ctcatttcac tcttcagtgt tcctgttatt
tatgaacggc atcaggcaca 3781 gatagatcat tatctaggac ttgcaaataa gaatgttaaa gatgctatgg
ctaaaatcca 3841 agcaaaaatc cctggattga agcgcaaagc tgaatgaaaa cgcccaaaat aattagtagg 3901
agttcatctt taaaggggat attcatttga ttatacgggg gagggtcagg gaagaacgaa 3961 ccttgacgtt
gcagtgcagt ttcacagatc gttgttagat ctttatttttt agccatgcac 4021 tgttgtgagg aaaaattacc
tgtcttgact gccatgtgtt catcatctta agtattgtaa 4081 gctgctatgt atggatttaa accgtaatca
tatctttttc ctatctatct gaggcactgg 4141 tggaataaaa aacctgtata ttttactttg ttgcagatag
tcttgccgca tcttggcaag 4201 ttgcagagat ggtggagcta gaaaaaaaaa aaaaaaagcc cttttcagtt
tgtgcactgt 4261 gtatggtccg tgtagattga tgcagatttt ctgaaatgaa atgtttgttt agacgagatc 4321
ataccggtaa agcaggaatg acaaagcttg cttttctggt atgttctagg tgtattgtga 4381 cttttactgt
tatattaatt gccaatataa gtaaatatag attatatatg tatagtgttt 4441 cacaaagctt agacctttac
cttccagcca ccccacagtg cttgatattt cagagtcagt 4501 cattggttat acatgtgtag ttccaaagca
cataagctag aagaagaaat atttctagga 4561 gcactaccat ctgttttcaa catgaaatgc cacacacata
gaactccaac atcaatttca 4621 ttgcacagac tgactgtagt taattttgtc acagaatcta tggactgaat
ctaatgcttc 4681 caaaaatgtt gtttgtttgc aaatatcaaa cattgttatg caagaaatta ttaattacaa 4741
aatgaagatt tataccattg tggtttaagc tgtactgaac taaatctgtg gaatgcattg 4801 tgaactgtaa
aagcaaagta tcaataaagc ttatagactt aaaaaaaaaa aaaaaaaaaa 4861 aaaaaaaaaa a

[0117] By “GFAP” (or Glial fibrillary acidic protein) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P14136.

TABLE-US-00044 (SEQ ID NO: 45) 1 merrritsaa rrsyvssgem mvgglapgrr lgpgtrlsla
rmppplptrv dfslagalna 61 gfketraser aemmelndrf asyiekvrfl eqqnkalaae lnqlrakept
kladvyqael 121 relrlrldql tansarleve rdnlaqdlat vrqklqdetn lrleaennla ayrqeadeat 181
larldlerki esleeeirfl rkiheeevre lqeqlarqqv hveldvakpd ltaalkeirt 241 qyeamassnm
heaeewyrsk fadltdaaar naellrqakh eandyrrqlq sltcdleslr 301 gtneslerqm reqeerhvre
aasyqealar leeegqslkd emarhlqeyq dllnvklald 361 ieiatyrkll egeenritip vqtfsnlqir
etsldtksvs eghlkrnivv ktvemrdgev 421 ikeskqehkd vm

[0118] By “GFAP nucleic acid molecule” (or Glial fibrillary acidic protein) is meant a polynucleotide encoding an GFAP polypeptide. An exemplary GFAP nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_002055.

TABLE-US-00045 (SEQ ID NO: 46) 1 atcgccagtc tagcccactc cttcataaag ccctcgcatc
ccaggagcga gcagagccag 61 agcaggatgg agaggagacg catcacctcc gctgctcgcc gctcctacgt
ctcctcaggg 121 gagatgatgg tgggggggcct ggctcctggc cgccgtctgg gtcctggcac ccgcctctcc 181

ctggctcgaa tgccccctcc actcccgacc cgggtggatt tctccctggc tggggcactc 241 aatgctggct tcaaggagac ccgggccagt gagcgggcag agatgatgga gctcaatgac 301 cgctttgcca gctacatcga gaaggttcgc ttcctggaac agcaaaacaa ggcgctggct 361 gctgagctga accagctgcg ggccaaggag cccaccaagc tggcagacgt ctaccaggct 421 gagctgcgag agctgcggct gcggctcgat caactcaccg ccaacagcgc ccggctggag 481 gttgagaggg acaatctggc acaggacctg gccactgtga ggcagaagct ccaggatgaa 541 accaacctga ggctggaagc cgagaacaac ctggctgcct atagacagga agcagatgaa 601 gccaccctgg cccgtctgga tctggagagg aagattgagt cgctggagga ggagatccgg 661 ttcttgagga agatccacga ggaggaggtt cgggaactcc aggagcagct ggcccgacag 721 caggtccatg tggagcttga cgtggccaag ccagacctca ccgcagccct gaaagagatc 781 cgcacgcagt atgaggcaat ggcgtccagc aacatgcatg aagccgaaga gtggtaccgc 841 tccaagtttg cagacctgac agacgctgct gcccgcaacg cggagctgct ccgccaggcc 901 aagcacgaag ccaacgacta ccggcgccag ttgcagtcct tgacctgcga cctggagtct 961 ctgcgcggca cgaacgagtc cctggagagg cagatgcgcg agcaggagga gcggcacgtg 1021 cgggaggcgg ccagttatca ggaggcgctg gcgcggctgg aggaagaggg gcagagcctc 1081 aaggacgaga tggcccgcca cttgcaggag taccaggacc tgctcaatgt caagctggcc 1141 ctggacatcg agatcgccac ctacaggaag ctgctagagg gcgaggagaa ccggatcacc 1201 attcccgtgc agaccttctc caacctgcag attcgagaaa ccagcctgga caccaagtct 1261 gtgtcagaag gccacctcaa gaggaacatc gtggtgaaga ccgtggagat gcgggatgga 1321 gaggtcatta aggagtccaa gcaggagcac aaggatgtga tgtgaggcag gacccacctg 1381 gtggcctctg ccccgtctca tgaggggccc gagcagaagc aggatagttg ctccgcctct 1441 gctggcacat ttccccagac ctgagctccc caccacccca gctgctcccc tccctcctct 1501 gtccctaggt cagcttgctg ccctaggctc cgtcagtatc aggcctgcca gacggcaccc 1561 acccagcacc cagcaactcc aactaacaag aaactcaccc ccaaggggca gtctggaggg 1621 gcatggccag cagcttgcgt tagaatgagg aggaaggaga gaaggggagg agggcggggg 1681 gcacctacta catcgccctc cacatccctg attcctgttg ttatggaaac tgttgccaga 1741 gatggaggtt ctctcggagt atctgggaac tgtgcctttg agtttcctca ggctgctgga 1801 ggaaaactga gactcagaca ggaaagggaa ggccccacag acaaggtagc cctggccaga 1861 ggcttgtttt gtcttttggt ttttatgagg tgggatatcc ctatgctgcc taggctgacc 1921 ttgaactcct gggctcaagc agtctaccca cctcagcctc ctgtgtagct gggattatag 1981 attggagcca ccatgcccag ctcagagggt tgttctccta gactgaccct gatcagtcta 2041 agatgggtgg ggacgtcctg ccacctgggg cagtcacctg cccagatccc agaaggacct 2101 cctgagcgat gactcaagtg tctcagtcca cctgagctgc catccaggga tgccatctgt 2161 gggcacgctg tgggcaggtg ggagcttgat tctcagcact tgggggatct gttgtgtacg 2221 tggagaggga tgaggtgctg ggagggatag agggggggctg cctggccccc agctgtgggt 2281 acagagaggt caagcccagg aggactgccc cgtgcagact ggaggggacg ctggtagaga 2341 tggaggagga ggcaattggg atggcgctag gcatacaagt aggggttgtg ggtgaccagt 2401 tgcacttggc ctctggattg tgggaattaa ggaagtgact catcctcttg aagatgctga 2461 aacaggagag aaaggggatg tatccatggg ggcagggcat gactttgtcc catttctaaa 2521 ggcctcttcc ttgctgtgtc ataccaggcc gccccagcct ctgagccccct gggactgctg 2581 cttcttaacc ccagtaagcc actgccacac gtctgaccct ctccacccca tagtgaccgg 2641 ctgcttttcc ctaagccaag ggcctcttgc ggtcccttct tactcacaca caaaatgtac 2701 ccagtattct aggtagtgcc ctattttaca attgtaaaac tgaggcacga gcaaagtgaa 2761 gacactggct catattcctg cagcctggag gccgggtgct cagggctgac acgtccaccc 2821 cagtgcaccc actctgcttt gactgagcag actggtgagc agactggtgg gatctgtgcc 2881 cagagatggg actgggaggg cccacttcag ggttctcctc tcccctctaa ggccgaagaa 2941 gggtccttcc ctctccccaa gacttggtgt cctttccctc cactccttcc tgccacctgc 3001 tgctgctgct gctgctaatc ttcagggcac tgctgctgcc tttagtcgct gaggaaaaat 3061 aaagacaaat gctgcgccct tccccaaaaa aaaaaaa

[0119] By “s100b” (or S-100 protein beta chain; S-100 protein subunit beta; $100 calcium-binding protein B) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. P04271.

TABLE-US-00046 (SEQ ID NO: 47) 1 mselekamva lidvfhqysg regdkhklkk selkelinne lshfleeike qevvdkvmet 61 ldndgdgecd fqefmafvam vttacheffe he

[0120] By “s100b nucleic acid molecule” (or S-100 protein beta chain; S-100 protein subunit beta; S100 calcium-binding protein B) is meant a polynucleotide encoding an s100b polypeptide. An

exemplary s100b nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_006272.

TABLE-US-00047 (SEQ ID NO: 48) 1 gggcagaggg aataagaggc tgcctctgcc caccagtcct gccgcccagg acccgcagca 61 gagacgacgc ctgcagcaag gagaccagga aggggtgaga caaggaagag gatgtctgag 121 ctggagaagg ccatggtggc cctcatcgac gttttccacc aatattctgg aagggaggga 181 gacaagcaca agctgaagaa atccgaactg aaggagctca tcaacaatga gctttcccat 241 ttcttagagg aaatcaaaga gcaggaggtt gtggacaaag tcatggaaac actggacaat 301 gatggagacg gcgaatgtga cttccaggaa ttcatggcct ttgttgccat ggttactact 361 gcctgccacg agttctttga acatgagtga gattagaaag cagccaaacc tttcctgtaa 421 cagagacggt catgcaagaa agcagacagc aagggcttgc agcctagtag gagctgagct 481 ttccagccgt gttgtagcta attaggaagc ttgatttgct ttgtgattga aaaattgaaa 541 acctctttcc aaaggctgtt ttaacggcct gcatcattct ttctgctata ttaggcctgt 601 gtgtaagctg actggcccca gggactcttg ttaacagtaa cttaggagtc aggtctcagt 661 gataaagcgt gcaccgtgca gcccgccatg gccgtgtaga ccctaacccg gagggaaccc 721 tgactacaga aattaccccg gggcaccctt aaaacttcca ctacctttaa aaaacaaagc 781 cttatccagc attatttgaa aacactgctg ttctttaaat gcgttcctca tccatgcaga 841 taacagctgg ttggccggtg tggccctgca agggcgtggt ggcttcggcc tgcttcccgg 901 gatgcgcctg atcaccaggt gaacgctcag cgctggcagc gctcctggaa aaagcaactc 961 catcagaact cgcaatccga gccagctctg ggggctccag cgtggcctcc gtgacccatg 1021 cgattcaagt cgcggctgca ggatccttgc ctccaacgtg cctccagcac atgcggcttc 1081 cgagggcact accgggggct ctgagccacc gcgagggcct gcgttcaata aaaag

[0121] By “PAX6 polypeptide” (or paired box protein PAX6) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAK95849.1.

TABLE-US-00048 (SEQ ID NO: 49)
MQNSHSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDISRILQVSN
GCVSKILGRYYETGSIRPRAIGGSKPRVATPEVVSKIAQYKRECPSIFAW
EIRDRLLSEGVCTNDNIPSVSSINRVLRNLASEKQQMGADGMYDKLRMLN
GQTGSWGTRPGWYPGTSVPGQPTQDGCQQQEGGGENTNSISSNGEDSDEA
QMRLQLKRKLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLP
EARIQVWFSNRRAKWRREEKLRNQRRQASNTPSHIPISSSFSTSVYQPIP
QPTTPVSSFTSGSMLGRTDTALTNTYSALPPMPSFTMANNLPMQPPVPSQ
TSSYSCMLPTSPSVNGRSYDTYTPPHMQTHMNSQPMGTSGTTSTGLISPG
VSVPVQVPGSEPDMSQYWPRLQ

[0122] By “PAX6 polynucleotide” (or paired box protein PAX6) is meant a polynucleotide encoding an PAX6 polypeptide. An exemplary PAX6 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. AY047583.

TABLE-US-00049 (SEQ ID NO: 50) 1 agggggaaga ctttaactag gggcgcgcag atgtgtgagg ccttttattg tgagagtgga 61 cagacatccg agatttcaga gccccatatt cgagccccgt ggaatcccgc ggcccccagc 121 cagagccagc atgcagaaca gtcacagcgg agtgaatcag ctcggtggtg tctttgtcaa 181 cgggcggcca ctgccggact ccacccggca gaagattgta gagctagctc acagcggggc 241 ccggccgtgc gacatttccc gaattctgca ggtgtccaac ggatgtgtga gtaaaattct 301 gggcaggtat tacgagactg gctccatcag acccagggca atcggtggta gtaaaccgag 361 agtagcgact ccagaagttg taagcaaaat agcccagtat aagcgggagt gcccgtccat 421 ctttgcttgg gaaatccgag acagattact gtccgagggg gtctgtacca acgataacat 481 accaagcgtg tcatcaataa acagagttct tcgcaacctg gctagcgaaa agcaacagat 541 gggcgcagac ggcatgtatg ataaactaag gatgttgaac gggcagaccg gaagctgggg 601 cacccgccct ggttggtatc cggggacttc ggtgccaggg caacctacgc aagatggctg 661 ccagcaacag gaaggagggg gagagaatac caactccatc agttccaacg gagaagattc 721 agatgaggct caaatgcgac ttcagctgaa gcggaagctg caaagaaata gaacatcctt 781 tacccaagag caaattgagg ccctggagaa agagtttgag agaacccatt atccagatgt 841 gtttgcccga gaaagactag cagccaaaat agatctacct gaagcaagaa tacaggtatg 901 gttttctaat cgaagggcca aatggagaag agaagaaaaa ctgaggaatc agagaagaca 961 ggccagcaac acacctagtc atattcctat cagcagtagt ttcagcacca gtgtctacca 1021 accaattcca caacccacca caccggtttc ctccttcaca tctggctcca tgttgggccg 1081 aacagacaca

gccctcacaa acacctacag cgctctgccg cctatgccca gcttcaccat 1141 ggcaaataac ctgcctatgc aacccccagt ccccagccag acctcctcat actcctgcat 1201 gctgcccacc agcccttcgg tgaatgggcg gagttatgat acctacaccc ccccacatat 1261 gcagacacac atgaacagtc agccaatggg cacctcgggc accacttcaa caggactcat 1321 ttcccctggt gtgtcagttc cagttcaagt tcccggaagt gaacctgata tgtctcaata 1381 ctggccaaga ttacagtaa

[0123] By “Nestin polypeptide” is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_006608.1.

TABLE-US-00050 (SEQ ID NO: 51)

MEGCMGEESFQMWELNRRLEAYLARVKALEEQNELLSAELGGLRAQSADT
SWRAHADDELAALRALVDQRWREKHAAEVARDNLAEELEGVAGRCQQLRL
ARERTTEEVARNRRAVEAEKCARAWLSSQVAELERELEALRVAHEEERVG
LNAQAACAPRCPAPPRGPPAPAPEVEELARRLGEAWRGAVRGYQERVAHM
ETSLGQARERLGRAVQGAREGRLELQQLQAERGGLLERRAALEQRLEGRW
QERLRATEKFQLAVEALEQEKQGLQSQIAQVLEGRQQLAHLKMSLSLEVA
TYRTLLEAENSRLQTPGGGSKTSLSFQDPKLELQFPRTPEGRRLGSLLPV
LSPTSLPSPLPATLETPVPAFLKNQEFLQARTPTLASTPIPPTPQAPSPA
VDAEIRAQDAPLSLLQTQGGRKQAPEPLRAEARVAIPASVLPGPEEPGGQ
RQEASTGQSPEDHASLAPPLSPDHSSLEAKDGESGGSRVFSICRGEGEGQ
IWGLVEKETAIEGKVVSSLQQEIWEEEDLNRKEIQDSQVPLEKETLKSLG
EEIQESLKTLENQSHETLERENQECPRSLEEDLETLKSLEKENKELLKDV
EVVRPLEKEAVGQLKPTGKEDTQTLQSLQKENQELMKSLEGNLETFLFPG
TENQELVSSLQENLESLTALEKENQEPLRSPEVGDEEALRPLTKENQEPL
RSLEDENKEAFRSLEKENQEPLKTLEEEDQSIVRPLETENHKSLRSLEEQ
DQETLRTLEKETQQRRRSLGEQDQMTLRPPEKVDLEPLKSLDQEIARPLE
NENQEFLKSLKEESVEAVKSLETEILESLKSAGQENLETLKSPETQAPLW
TPEEINQGAMNPLEKEIQEPLESVEVNQETFRLLEEENQESLRSLGAWNL
ENLRSPEEVDKESQRNLEEEENLGKGEYQESLRSLEEEGQELPQSADVQR
WEDTVEKDQELAQESPPGMAGVENEDEAELNLREQDGFTGKEEVVEQGEL
NATEEVWIPGEGHPESPEPKEQRGLVEGASVKGGAEGLQDPEGQSQQVGA
PGLQAPQGLPEAIEPLVEDDVAPGGDQASPEVMLGSEPAMGESAAGAEPG
PGQGVGGLGDPGHLTREEVMEPPLEEESLEAKRVQGLEGPRKDLEEAGGL
GTEFSELPGKSRDPWEPPREGREESEAEAPRGAEEAFPAETLGHTGSDAP
SPWPLGSEEAEEDVPPVLVSPSPTYTPILEDAPGPQPQAEGSQEASWGVQ
GRAEALGKVESEQEELGSGEIPEGPQEEGEESREESEEDELGETLPDSTP
LGFYLRSPTSPRWDPTGEQRPPPQGETGKEGWDPAVLASEGLEAPPSEKE
EGEEGEEECGRDSDLSEEFEDLGTEAPFLPGVPGEVAEPLGQVPQLLLDP
AAWDRDGESDGFADEEESGEEGEEDQEEGREPGAGRWGPGSSVGSLQALS
SSQRGEFLESDSVSVSVPWDDSLRGAVAGAPKTALETESQDSAEPSGSEE
ESDPVSLEREDKVPGPLEIPSGMEDAGPGADIIGVNGQGPNLEGKSQHVN
GGVMNGLEQSEEVGQGMPLVSEGDRGSPFQEEEGSALKTSWAGAPVHLGQ
GQFLKFTQREGDRESWSSGED

[0124] By “Nestin polynucleotide” is meant a polynucleotide encoding an Nestin polypeptide. An exemplary Nestin nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_006617.

TABLE-US-00051 (SEQ ID NO: 52) 1 gctactccca ccccgccccg ccccgtcatt gtccccgtcg gtctcttttc tcttccgtcc 61 taaaagctct gcgagccgct cccttctccc ggtgccccgc gtctgtccat cctcagtggg 121 tcagacgagc aggatggagg gctgcatggg ggaggagtcg tttcagatgt gggagctcaa 181 tcggcgcctg gaggcctacc tggcccgggt caaggcgctg gaggagcaga atgagctgct 241 cagcgcggag ctcgggggggc tccgggcaca atccgcggac acctcctggc gggcgcatgc 301 cgacgacgag ctggcggccc tgcgggccct cgttgaccaa cgctggcggg agaagcacgc 361 ggccgaggtg gcgcgcgaca acctggctga

agagctggag ggcgtggcag gccgatgcca 421 gcagctgcgg ctggcccggg agcggacgac
ggaggaggta gcccgcaacc ggcgcgccgt 481 cgaggcagag aaatgcgccc gggcctggct gagtagccag
gtggcagagc tggagcgcga 541 gctagaggct ctacgcgtgg cgcacgagga ggagcgcgtc ggcctgaacg
cgcaggctgc 601 ctgtgccccc cgctgccccg cgccgccccg cgggcctccc gcgccggccc cggaggtaga
661 ggagctggca aggcgactgg gcgaggcgtg gcgcggggca gtgcgcggct accaggagcg 721
cgtggcacac atggagacgt cgctgggcca ggcccgcgag cggctgggcc gggcggtgca 781 gggtgcccgc
gagggccgcc tggagctgca gcagctccag gctgagcgcg gaggcctcct 841 ggagcgcagg gcagcgttgg
aacagaggtt ggagggccgc tggcaggagc ggctgcgggc 901 tactgaaaag ttccagctgg ctgtggaggc
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cacctcaaga tgtccctcag 1021 cctggaggtg gccacgtaca ggaccctcct ggaggctgag aactcccggc
tgcaaacacc 1081 tggcggtggc tccaagactt ccctcagctt tcaggacccc aagctggagc tgcaattccc 1141
taggacccca gagggccggc gtcttggatc tttgctccca gtcctgagcc caacttccct 1201 cccctcaccc
ttgcctgcta cccttgagac acctgtgcca gcctttctta agaaccaaga 1261 attcctccag gcccgtaccc
ctaccttggc cagcaccccc atccccccca cacctcaggc 1321 accctctcct gctgtagatg cagagatcag
agcccaggat gctcctctct ctctgctcca 1381 gacacagggt gggaggaaac aggctccaga gcccctgcgg
gctgaagcca gggtggccat 1441 tcctgccagc gtcctgcctg gaccagagga gcctgggggc cagcggcaag
aggccagtac 1501 aggccagtcc ccagaggacc atgcctcctt ggcaccaccc ctcagccctg accactccag
1561 tttagaggct aaggatggag aatccggtgg gtctagagtg ttcagcatat gccgagggga 1621
aggtgaaggg caaatctggg ggttggtaga gaaagaaaca gccatagagg gcaaagtggt 1681 aagcagcttg
cagcaggaaa tatgggaaga agaggatcta aacaggaagg aaatccagga 1741 ctcccaggtt cctttggaaa
aagaaaccct gaagtctctg ggagaggaga ttcaagagtc 1801 actgaagact ctggaaaacc agagccatga
gacactagaa agggagaatc aagaatgtcc 1861 gaggtcttta gaagaagact tagaaacact aaaaagtcta
gaaaaggaaa ataaagagct 1921 attaaaggat gtggaggtag tgagacctct agaaaaagag gctgtaggcc
aacttaagcc 1981 tacaggaaaa gaggacacac agacattgca atccctgcaa aaggagaatc aagaactaat 2041
gaaatctctt gaaggtaatc tagagacatt tttatttcca ggaacggaaa atcaagaatt 2101 agtaagttct
ctgcaagaga acttagagtc attgacagct ctggaaaagg agaatcaaga 2161 gccactgaga tctccagaag
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cagagtattg tgagacctct 2341 agaaacagag aatcacaaat cactgaggtc tttagaagaa caggaccaag
agacattgag 2401 aactcttgaa aaagagactc aacagcgacg gaggtctcta ggggaacagg atcagatgac
2461 attaagaccc ccagaaaaag tggatctaga accactgaag tctcttgacc aggagatagc 2521
tagacctctt gaaaatgaga atcaagagtt cttaaagtca ctcaaagaag agagcgtaga 2581 ggcagtaaaa
tctttagaaa cagagatcct agaatcactg aagtctgcgg gacaagagaa 2641 cctggaaaca ctgaaatctc
cagaaactca agcaccactg tggactccag aagaaataaa 2701 tcagggggca atgaatcctc tagaaaagga
aattcaagaa ccactggagt ctgtggaagt 2761 gaaccaagag acattcagac tcctggaaga ggagaatcag
gaatcattga gatctctggg 2821 agcatggaac ctggagaatt tgagatctcc agaggaggta gacaaggaaa
gtcaaaggaa 2881 tctggaagag gaagagaacc tgggaaaggg agagtaccaa gagtcactga ggtctctgga
2941 ggaggaggga caggagctgc cgcagtctgc agatgtgcag aggtgggaag atacggtgga 3001
gaaggaccaa gaactggctc aggaaagccc tcctgggatg gctggagtgg aaaatgagga 3061 tgaggcagag
ctgaatctga gggagcagga tggcttcact gggaaggagg aggtggtaga 3121 gcagggagag ctgaatgcca
cagaggaggt ctggatccca ggcgaggggc acccagagag 3181 ccctgagccc aaagagcaga
gaggcctggt tgagggagcc agtgtgaagg gaggggctga 3241 gggcctccag gaccctgaag ggcaatcaca
acaggtgggg gccccaggcc tccaggctcc 3301 ccaggggctg ccagaggcga tagagcccct ggtggaagat
gatgtggccc caggggggtga 3361 ccaagcctcc ccagaggtca tgttggggtc agagcctgcc atgggtgagt
ctgctgcggg 3421 agctgagcca ggcccggggc agggggtggg agggctgggg gacccaggcc atctgaccag
3481 ggaagaggtg atggaaccac ccctggaaga ggagagtttg gaggcaaaga gggttcaggg 3541
cttggaaggg cctagaaagg acctagagga ggcaggtggt ctggggacag agttctccga 3601 gctgcctggg
aagagcagag acccttggga gcctcccagg gagggtaggg aggagtcaga 3661 ggctgaggcc
cccaggggag cagaggaggc gttccctgct gagaccctgg gccacactgg 3721 aagtgatgcc ccttcacctt
ggcctctggg gtcagaggaa gctgaggagg atgtaccacc 3781 agtgctggtc tcccccagcc caacgtacac

cccgatcctg gaagatgccc ctgggcctca 3841 gcctcaggct gaagggagtc aggaggctag ctgggggtg caggggaggg ctgaagccct 3901 ggggaaagta gagagcgagc aggaggagtt gggttctggg gagatccccg agggccccca 3961 ggaggaaggg gaggagagca gagaagagag cgaggaggat gagctcgggg agacccttcc 4021 agactccact cccctgggct tctacctcag gtcccccacc tcccccaggt gggaccccac 4081 tggagagcag aggccacccc ctcaagggga gactggaaag gagggctggg atcctgctgt 4141 cctggcttcc gagggccttg aggccccacc ctcagaaaag gaggaggggg aggagggaga 4201 agaggagtgt ggccgtgact ctgacctgtc agaagaattt gaggacctgg ggactgaggc 4261 acctttttctt cctggggtcc ctggggaggt ggcagaacct ctgggccagg tgccccagct 4321 gctactggat cctgcagcct gggatcgaga tggggagtcc gatgggtttg cagatgagga 4381 agaaagtggg gaggagggag aggaggatca ggaggagggg agggagccag gggctgggcg 4441 gtgggggcca gggtcttctg ttggcagcct ccaggccctg agtagctccc agagagggga 4501 attcctggag tctgattctg tgagtgtcag tgtcccctgg gatgacagct tgaggggtgc 4561 agtggctggt gcccccaaga ctgccctgga aacggagtcc caggacagtg ctgagccttc 4621 tggctcagag gaagagtctg accctgtttc cttggagagg gaggacaaag tccctggccc 4681 tctagagatc cccagtggga tggaggatgc aggcccaggg gcagacatca ttggtgttaa 4741 tggccagggt cccaacttgg aggggaagtc acagcatgtg aatgggggag tgatgaacgg 4801 gctggagcag tctgaggaag tggggcaagg aatgccgcta gtctctgagg gagaccgagg 4861 gagccccttt caggaggagg aggggagtgc tctgaagacc tcttgggcag gggctcctgt 4921 tcacctgggc cagggtcagt tcctgaagtt cactcagagg gaaggagata gagagtcctg 4981 gtcctcaggg gaggactagg aaaagaccat ctgcccggca ctggggactt aggggtgcgg 5041 ggaggggaag gacgcctcca agcccgctcc ctgctcagga gcagcactct taacttacga 5101 tctcttgaca tatggtttct ggctgagagg cctggcccgc taaggtgaaa aggggtgtgg 5161 caaaggagcc tactccaaga atggaggctg taggaatata acctcccacc ctgcaaaggg 5221 aatctcttgc ctgctccatc tcataggcta agtcagctga atcccgatag tactaggtcc 5281 ccttccctcc gcatcccgtc agctggaaaa ggcctgtggc ccagaggctt ctccaaaggg 5341 agggtgacat gctggctttt gtgcccaagc tcaccagccc tgcgccacct cactgcagta 5401 gtgcaccatc tcactgcagt agcacgccct cctgggccgt ctggcctgtg gctaatggag 5461 gtgacggcac tcccatgtgc tgactccccc catccctgcc acgctgtggc cctgcctggc 5521 tagtccctgc ctgaataaag taatgcctcc gcttcaaaaa aaaaaaaaaa aaaaaaaaaa 5581 aaaaaaaaaa a

[0125] By “LHX6 polypeptide” (or LIM homeobox 6) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAI03937.1.

TABLE-US-00052 (SEQ ID NO: 53)

MAQPGSGCKATTRCLEGTAPPAMAQSDAEALAGALDKDEGQASPCTPSTP
SVCSPPSAASSVPSAGKNICSSCGLEILDRYLLKVNNLIWHVRCLECSVC
RTSLRQQNSCYIKNKEIFCKMDYFSRFGTKCARCGRQIYASDWVRRARGN
AYHLACFACFSCKRQLSTGEEFGLVEEKVLCRIHYDTMIENLKRAAENGN
GLTLEGAVPSEQDSQPKPAKRARTSFTAEQLQVMQAQFAQDNNPDAQTLQ
KLADMTGLSRRVIQVWFQNCRARHKKHTPQHPVPPSGAPPSRLPSALSDD
IHYTPFSSPERARMVTLHGYIESHPFSVLTLPALPHLPVGAPQLPLSR

[0126] By “LHX6 polynucleotide” (or LIM homeobox 6) is meant a polynucleotide encoding an LHX6 polypeptide. An exemplary LHX6 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC103936.

TABLE-US-00053 (SEQ ID NO: 54) 1 cccgccaccg accaggtgat ggcccagcca gggtccggct gcaaagcgac cacccgctgt 61 cttgaaggga ccgcgccgcc cgccatggct cagtctgacg ccgaggccct ggcaggagct 121 ctggacaagg acgagggtca ggcctcccca tgtacgccca gcacgccatc tgtctgctca 181 ccgccctctg ccgcctcctc cgtgccgtct gcaggcaaga acatctgctc cagctgcggc 241 ctcgagatcc tggaccgata tctgctcaag gtcaacaacc tcatctggca cgtgcggtgc 301 ctcgagtgct ccgtgtgtcg cacgtcgctg aggcagcaga acagctgcta catcaagaac 361 aaggagatct tctgcaagat ggactacttc agccgattcg ggaccaagtg tgcccggtgc 421 ggccgacaga tctacgccag cgactgggtg cggagagctc gcggcaacgc ctaccacctg 481 gcctgcttcg cctgcttctc gtgcaagcgc cagctgtcca ctggtgagga gttcggcctg 541 gtcgaggaga aggtgctctg ccgcatccac tacgacacca tgattgagaa cctcaagagg 601 gccgccgaga acgggaacgg cctcacgttg gagggggcag tgccctcgga acaggacagt

661 caacccaagc cggccaagcg cgcgcggacg tccttcaccg cggaacagct gcaggttatg 721
caggcgcagt tcgcgcagga caacaacccc gacgctcaga cgctgcagaa gctggcggac 781 atgacgggcc
tcagccggag agtcatccag gtgtggtttc aaaactgccg ggcgcgtcat 841 aaaaagcaca cgccgcaaca
cccagtgccg ccctcggggg cgcccccgtc ccgccttccc 901 tccgccctgt ccgacgacat ccactacacc
ccgttcagca gccccgagcg ggcgcgcatg 961 gtcaccctgc acggctacat tgagagtcat ccttttcag
tactaacgct gccggcactt 1021 ccgcatctgc ccgtgggcgc cccacagctg cccctcagcc gctgagatcc
agtgtccaag 1081 ctgcggccag gagtccaccc acctccgcat ccacccccgt ccgccatcct gcccaccacc
1141 aggtcggttc ccgaggcctg gcctttccct ctcctgctga gaaccagaac ccaccaggag 1201
caccacagag tcctcctctt ggaaggcaga actccctgaa atctggaatc agggtggaaa 1261 cagcctgttt
ttcccattta aacaggagtc ctcttcaact tcagctgatt acaataacaa 1321 aaggcggaat tgaattgtgc
gatgccaacg gccttctcat ttacaggttt ttttccccca 1381 cattggcctt tatttactac ttccttggaa
ccatctctga attctgaata gctgacaacc 1441 cccaatgtta tccactctgt tgcttttgtc tggaaaactc
tacagtgttt gtgggatgtc 1501 cccaaaggta agctatgttc taattttatc atttccatct gtctggttat gtcaagttaa
1561 ttcagaaaga gaagagacag tgaccaaccc tgagaggcct aatagggcag agatggaggc 1621
ctgcccagac taggaggcag cggggataga cagggaatgg ggagaagaaa gacccccatt 1681 ggtttggaaa
tcaaggagag ggcggtgaca tattggacca gaagaggcac tagccatttt 1741 aaggagagga aagagaaaac
tctggggtca gggagagacc ctacccccac ctaattatcc 1801 agcatatatg taagaaacat agcagcgatg
gtattcgatc tgtgccatga ctcttctgaa 1861 tgtttggaca ggttagagtt ggggacccct gttggccact
tgttgacctc tcatagtggt 1921 gcttgggcca ggtcttctca atggaagggg aatcccttat aggggagagg
gaacagagcc 1981 cagtgaaatg gcagtcagaa tgttaaccct ggatccatct ctaagtagag agagggtgcc 2041
cattgcctag gtgagtgtgc caagctcagg attccaactg gtgcctctga gcttcccaat 2101 caatacttcc
tggagccagc cccacccacc cctgagaaca gaggtcagac acagctgcgt 2161 aacatccatc ctgctacaac
tcttccaccc caaacaaaag ggctcaggct acacacgacc 2221 atgatttatg ttttcagggg atgcccattt
gtcccaagct tatcctgtaa ttctagaatt 2281 acctggtgtc ctgatgcatt ttccactaga ggttgctaat
cagcatgttt tagcccaagt 2341 ccaccttcct gctgtggtta acctgttatg ttgcttttgg aaggagactc
taagacaggg 2401 aaagcaagtt catggtacat acgcagccat tgtctctgtt tttacccatg gcagacattg 2461
ctaatcaatg gcagctctat ttcactgagt ctggataagg tttcagagtt caaatgcttg 2521 acgttggcac
ttaacatgaa agcctatagg tcattcttgc tctgggatct acaggcaggg 2581 taggcacagg tgcagcctaa
gaagggaacc tgcttcctct cccttccaaa gacagtgaca 2641 gctgactgag ggcaaagagc aggcaccact
cagaacgtgg tgagtacagc tcagctcagc 2701 actcagtcag tggtaacttg tgcccagccc tgtgctaggc
gctgacatta acaggagcaa 2761 ccagggccca attcctggcc ttggagctca aatctttcct ttgatttttg
ctcctgatca 2821 tcaaggcccc agtgg

[0127] By “LHX8 polypeptide” (or LIM homeobox 8) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAH40321.1.

TABLE-US-00054 (SEQ ID NO: 55)

MQILSRCQGLMSEECGRTTALAAGRTRKGAGEEGLVSPEGAGDEDSCSSS
APLSPSSSPRSMASGSGCPPGKCVCNSCGLEIVDKYLLKVNDLCWHVRCL
SCSVCRTSLGRHTSCYIKDKDIFCKLDYFRRYGTRCSRCGRHIHSTDWVR
RAKGNVYHLACFACFSCKRQLSTGEEFALVEEKVLCRVHYDCMLDNLKRE
VENGNGISVEGALLTEQDVNHPKPAKRARTSFTADQLQVMQAQFAQDNNP
DAQTLQKLAERTGLSRRVIQVWFQNCRARHKKHVSPNHSSSTPVTAAPPS
RLSPPMLEEMAYSAYVPQDGTMLTALHSYMDAHSPTTLGLQPLLPHSMTQ LPISHT

[0128] By “LHX8 polynucleotide” (or LIM homeobox 8) is meant a polynucleotide encoding an LHX8 polypeptide. An exemplary LHX8 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC040321.

TABLE-US-00055 (SEQ ID NO: 56) 1 agcggcaaga ggctagcggc tggaccactt gtgctggagt
ggtaaagaac tatcatgaat 61 ccatttactg aaagtgtcca tttctgaact caccctaaag aggacaaaca
ccgcaaagta 121 gttaaaagtc aggcattcgc gtcggacgtc tgggtttgaa ttctgccctg gcttgactgg 181
aaacgcttcc cctatttctt ccgtagcgga ccgggagagc ttactggcgc tctgcgaacc 241 ggctggaaag
aaacaccgag tcactcgtac agactcttgg tcgcagaact tggctttccg 301 ctattggtcc tccagaaccg

cttgaaacaa ctggccccag ctggcgcatc agaccgcagt 361 gaggaatgcc gcggggcggg tggcgaaggc
agggtctgcc cgccagtgga ttcccgggtg 421 tcccgcgtgg agcaggcttg cccagctggg aagcccatca
aacctcagtc ttggcccaca 481 gtgggagaga gaccagtggg tcccagacgg aggccatcgc ccgcttttgg
cgacctccac 541 tggcgtgaat aaaagcaccc ctctcttacc ctcagaaact gtgggtagca aggtataaaa 601
cggagtctgg gaccggtaag tcccaaggtg agcccgtata cagctctgcc atctctgagg 661 ggttatgcag
attctgagca ggtgtcaggg gctcatgtca gaggagtgcg ggcggactac 721 agccctggcg gccgggagga
ctcgcaaagg cgccggggaa gagggactgg tgagccccga 781 gggagcgggg gacgaggact cgtgctcctc
ctcggccccg ctgtccccgt cgtcctcgcc 841 ccggtccatg gcctcgggct ccggctgccc tcctggcaag
tgtgtgtgca acagttgcgg 901 cctggagatc gtggacaaat accttctcaa ggtgaatgac ctatgctggc
atgtccggtg 961 tctctcctgc agtgtttgca gaacctccct aggaaggcac accagctgtt atattaaaga 1021
caaagacatt ttctgcaaac ttgattattt cagaaggtat ggaactcgct gctctcgatg 1081 tgggagacac
atccattcta ctgactgggt ccggagagcc aaggggaatg tctatcactt 1141 ggcatgcttt gcctgctttt
cctgcaaaag gcaactttcc acaggagagg agtttgcttt 1201 ggtggaagag aaagtcctct gcagagtaca
ttatgactgc atgctggata atttaaaaag 1261 agaagtagaa aatgggaatg ggattagtgt ggaaggtgcc
ctcctcacag agcaagatgt 1321 taaccatcca aaaccagcaa aaagagctcg gaccagcttt acagcagatc
agcttcaggt 1381 tatgcaagca caatttgctc aggacaacaa cccagatgca cagacactcc agaaattggc 1441
agaaaggaca ggcttgagca gacgtgtgat acaggtgtgg tttcagaatt gtagagcacg 1501 ccacaagaaa
cacgtcagtc ctaatcactc atcctccacc ccagtcacag cagccccacc 1561 ctccaggctg tctccaccca
tgttagaaga aatggcttat tctgcctacg tgccccaaga 1621 tggaacgatg ttaactgcgc tgcatagtta
tatggatgct cattcaccaa caactcttgg 1681 actccagccc ttgttacccc attcaatgac acaactgcca
ataagtcata cctaattctt 1741 ttttcaggga tagacttgat taaggatata aatttgtcat ttattatgta taaaatacca
1801 ttgaaaagat attactgtta atttttttatt taacacctaa agcatttcca acatcacttt 1861 gctgcccagg
tatgtatcta tagttggcct gcaagacact tttattaatt cttcattttt 1921 tgtaaaactt atgtttacaa
gaagaaaaca aatcaaaaca ttttttgtat tgtctggaaa 1981 tagttcactc tagtgtgtat ctgttaattt
atttgtcatc aaaagagcac tttgcctaaa 2041 agaaaggact gacaagtgtg caaaatgttt acaatctttt
gtgaaattgt agtttatcat 2101 tagtttgtat ctgtaagtta ttgtaataaa tattacctgt atttttttgtt atatacaact
2161 ttatactttg aagcttgtat ctgtgaattt gcaactgaaa tttattttgc caatgttttc 2221 tgaatgaact
gaataaagct tctgttgtag catgccatgc aaacacatta ttgtgtttgt 2281 ggttgatgaa ttatggctgt
aaataacact atagtttaat aagcccacca ttctgagttt 2341 attaaacatt ttccattctt gtgaaaattt
caaaaaaaaa aaaaaaaaaa aaagaaaaaa 2401 aaaaaaaaaa a

[0129] By “TBR1 polypeptide” (or T-box, brain 1 (TBR1)) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_006584.1.

TABLE-US-00056 (SEQ ID NO: 57)

MQLEHCLSPSIMLSKKFLNVSSSYPHSGGSELVLHDHPIISTTDNLERSS
PLKKITRGMTNQSDTDNFPDSKDSPGDVQRSKLSPVLDGVSELRHSFDGS
AADRYLLSQSSQPQSAATAPSAMFPYPGQHGPAHPAFSIGSPSRYMAHHP
VITNGAYNSLLSNSSPQGYPTAGYPYPQQYGHSYQGAPFYQFSSTQPGLV
PGKAQVYLCNRPLWLKFHRHQTEMIITKQGRRMFPFLSFNISGLDPTAHY
NIFVDVILADPNHWRFQGGKWVPCGKADTNVQGNRVYMHPDSPNTGAHWM
RQEISFGKLKLTNNKGASNNNGQMVVLQSLHKYQPRLHVVEVNEDGTEDT
SQPGRVQTFTFPETQFIAVTAYQNTDITQLKIDHNPFAKGFRDNYDTIYT
GCDMDRLTPSPNDSPRSQIVPGARYAMAGSFLQDQFVSNYAKARFHPGAG
AGPGPGTDRSVPHTNGLLSPQQAEDPGAPSPQRWFVTPANNRLDFAASAY
DTATDFAGNAATLLSYAAAGVKALPLQAAGCTGRPLGYYADPSGWGARSP
PQYCGTKSGSVLPCWPNSAAAAARMAGANPYLGEEAEGLAAERSPLPPGA
AEDAKPKDLSDSSWIETPSSIKSIDSSDSGIYEQAKRRRISPADTPVSES
SSPLKSEVLAQRDCEKNCAKDISGYYGFYSHS

[0130] By “TBR1 polynucleotide” (or T-box, brain 1 (TBR1)) is meant a polynucleotide encoding an TBR1 polypeptide. An exemplary TBR1 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_006593.

TABLE-US-00057 (SEQ ID NO: 58) 1 gtcgctacca ggagccaggt gattatccta attaatgtct atctaattaa attactgtca 61 gcagctaacc aatggcagga gccgtttcat cggctgcaca agcagcaaga tcaaaagtga 121 gccttttctg attgctgcat agtgtcaatt ggccaatctc ttctcccagg gaaaaaaaaa 181 agtaaatcaa acctttgaga agcatttgct ggttgaagtg ctttctgtct agtgaggggg 241 tctgtggatt tctagtttat gataaatagg actttaaaaa ccagggacgg gagggcgagt 301 gttcaggttc tagagctatg cagctggagc actgcctttc tccttctatc atgctctcca 361 agaaatttct caatgtgagc agcagctacc cacattcagg cggatccgag cttgtcttgc 421 acgatcatcc cattatctcg accactgaca acctggagag aagttcacct ttgaaaaaaa 481 ttaccagggg gatgacgaat cagtcagata cagacaattt tcctgactcc aaggactcac 541 caggggacgt ccagagaagt aaactctctc ctgtcttgga cggggtctct gagcttcgtc 601 acagtttcga tggctctgct gcagatcgct acctcctctc tcagtccagc cagccacagt 661 ctgcggccac tgctcccagt gccatgttcc cgtaccccgg ccagcacgga ccggcgcacc 721 ccgccttctc catcggcagc cctagccgct acatggccca ccacccggtc atcaccaacg 781 gagcctacaa cagcctcctg tccaactcct cgccgcaggg ataccccacg gccggctacc 841 cctacccaca gcagtacggc cactcctacc aaggagctcc gttctaccag ttctcctcca 901 cccagccggg gctggtgccc ggcaaagcac aggtgtacct gtgcaacagg ccccctttggc 961 tgaaatttca ccggcaccaa acggagatga tcatcaccaa acagggaagg cgcatgtttc 1021 ctttttaag ttttaacatt tctggtctcg atcccacggc tcattacaat atttttgtgg 1081 atgtgatttt ggcggatccc aatcactgga ggtttcaagg aggcaaatgg gttccttgcg 1141 gcaaagcgga caccaatgtg caaggaaatc gggtctatat gcatccggat tcccccaaca 1201 ctggggctca ctggatgcgc caagaaatct cttttggaaa attaaaactt acgaacaaca 1261 aaggagcttc aaataacaat gggcagatgg tggttttaca gtccttgcac aagtaccagc 1321 cccgcctgca tgtggtggaa gtgaacgagg acggcacgga ggacactagc cagcccggcc 1381 gcgtgcagac gttcactttc cctgagactc agttcatcgc cgtcaccgcc taccagaaca 1441 cggatattac acaactgaaa atagatcaca acccttttgc aaaaggattt cgggataatt 1501 atgacacgat ctacaccggc tgtgacatgg accgcctgac cccctcgccc aacgactcgc 1561 cgcgctcgca gatcgtgccc ggggcccgct acgccatggc cggctctttc ctgcaggacc 1621 agttcgtgag caactacgcc aaggcccgct tccacccggg cgcgggcgcg ggccccgggc 1681 cgggtacgga ccgcagcgtg ccgcacacca acgggctgct gtcgccgcag caggccgagg 1741 acccgggcgc gccctcgccg caacgctggt ttgtgacgcc ggccaacaac cggctggact 1801 tcgcggcctc ggcctatgac acggccacgg acttcgcggg caacgcggcc acgctgctct 1861 cttacgcggc ggcgggcgtg aaggcgctgc cgctgcaggc tgcaggctgc actggccgcc 1921 cgctcggcta ctacgccgac ccgtcgggct ggggcgcccg cagtcccccg cagtactgcg 1981 gcaccaagtc gggctcggtg ctgccctgct ggcccaacag cgccgcggcc gccgcgcgca 2041 tggccggcgc caatccctac ctgggcgagg aggccgaggg cctggccgcc gagcgctcgc 2101 cgctgccgcc cggcgccgcc gaggacgcca agcccaagga cctgtccgat tccagctgga 2161 tcgagacgcc ctcctcgatc aagtccatcg actccagcga ctcggggatt tacgagcagg 2221 ccaagcggag gcggatctcg ccggccgaca cgcccgtgtc cgagagttcg tccccgctca 2281 agagcgaggt gctggcccag cgggactgcg agaagaactg cgccaaggac attagcggct 2341 actatggctt ctactcgcac agctaggccg cccctgcccg cccggccccg ccgcggcccg 2401 gacccccagc cagcccctca cagctcttcc ccagctccgc ctccccacac tcctccttgc 2461 gcacccactc attttatttg accctcgatg gccgtctgca gcgaataagt gcaggtctcc 2521 gagcgtgatt ttaacctttt ttgcacagca gtctctgcaa ttagctcacc gaccttcaac 2581 tttgctgtaa acctttttggt tttcctactt actcttcttc tgtggagtta tcctcctaca 2641 attcccctcc ccctcgtctt tctcttacct cctacttctc tttcttgtaa tgaaactctt 2701 caccttttagg agacctgggc agtcctgtca ggcagcagcg attccgaccc gccaagtctc 2761 ggcctccaca ttaaccatag gatgttgact ctagaacctg gacccaccca gcgcgtcctt 2821 tcttatcccc gagtggatgg atggatggat ggatggtagg gatgttaata attttagtgg 2881 aacaaagcct gtgaaatgat tgtacatagt gttaatttat tgtaacgaat ggctagtttt 2941 tattctcgtc aaggcacaaa accagttcat gcttaacctt tttttccttt cctttctttg 3001 cttttctttc tctcctctca tactttctct tctctctctt ttaattttct tgtgagataa 3061 tattctaaga ggctctagaa acatgaaata ctcagtagtg atgggtttcc cacttctcct 3121 caatccgttg catgaaataa ttactatgtg ccctaatgca cacaaatagc taaggagaat 3181 ccacccaaac acctttaaag gataggtgtc tgttcatagg caagtcgatt aagtggcatg 3241 atgcctgcaa agcaaagtca actggagttg tatgttcccc ccaccttcta aatagaatag 3301 ctcgacatca gcaatattat tttgccttat ttgtttttcc ccaaagtgcc aaatccatta 3361 ctggtctgtg caggtgccaa atatgctgac aaactgtttc tgaatatctt tcagtacccc 3421 ttcaccttta tatgctgtaa atctttgtaa tgaatactct

attaatgata tagatgactg 3481 aattgttggt aactatagtg tagtctagtg aagatgaatt gtgtgagttg tatattttac 3541 tgcattttag ttttgaaaat gacttcccca ccacctagaa acagctgaaa tttgacttcc 3601 ttgggagaac actagcatta atgcaagtaa gactgatttt cccctaagtc ttgttatatt 3661 tgataaggag cattaatccc cctggaaata gattagtagg atttctaatg ttgtgtagca 3721 aacctatact tttttgtatt taaaaattaa tgtgaaatat gcatcataca caatattcaa 3781 tctagattcc agtccatggg gggatttttc ctaataggaa ttcagggtct aaacgtgtgt 3841 atattttggc tcttctgtaa atctaatgtt gtgattttta tatttgtttc gttttgtctg 3901 tgaactgaat aatttataca agaacacact ccattgagaa acgttttgtt ttttgctcgt 3961 ttgtatcgtc tgtgtataac aagtaaaata aacctggtaa aaacgc

[0131] By "SLC1A3 polypeptide" (or solute carrier family 1; glial high affinity glutamate transporter member 3 (SLC1A3)) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. BAG35230.1.

TABLE-US-00058 (SEQ ID NO: 59)

MTKSNGEEPKMGGRMERFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAF
VLLTVTAVIVGTILGFTLRPYRMSYREVKYFSFPGELLMRMLQMLVLPLI
ISSLVTGMAALDSKASGKMGMRAVVYYMTTTIIAVVIGIIIVIIIHPGKG
TKENMHREGKIVRVTAADAFLDLIRNMFPPNLVEACFKQFKTNYEKRSFK
VPIQANETLVGAVINNVSEAMETLTRITEELVPVPGSVNGVNALGLVVFS
MCFGFVIGNMKEQGQALREFFDSLNEAIMRLVAVIMWYAPVGILFLIAGK
IVEMEDMGVIGGQLAMYTVTVIVGLLIHAVIVLPLLYFLVTRKNPWVFIG
GLLQALITALGTSSSSATLPITFKCLEENNGVDKRVTRFVLPVGATINMD
GTALYEALAAIFIAQVNNFELNFGQIITISITATAASIGAAGIPQAGLVT
MVIVLTSVGLPTDDITLIIAVDWFLDRLRTTTNVLGDSLGAGIVEHLSRH
ELKNRDVEMGNSVIEENEMKKPYQLIAQDNETEKPIDSETKM

[0132] By "SLC1A3 polynucleotide" (or solute carrier family 1; glial high affinity glutamate transporter member 3 (SLC1A3)) is meant a polynucleotide encoding an SLC1A3 polypeptide. An exemplary SLC1A3 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. AK312304.

TABLE-US-00059 (SEQ ID NO: 60) 1 gatagtaact tgcagtttca gagcacatgc acactgtcag ggctagcctg cctgcttacg 61 cgcgctgcgg attgttgctc cgttgtacct gctggggaat tcacctcgtt actgcttgat 121 atcttccacc ccttacaaaa tcagaaaagt tgtgttttct aataccaaag aggaggtttg 181 gctttctgtg ggtgattccc agacactgaa gtgcaaagaa gagaccctcc tagaaaagta 241 aaatatgact aaaagcaatg gagaagagcc caagatgggg ggcaggatgg agagattcca 301 gcagggagtc cgtaaacgca cacttttggc caagaagaaa gtgcagaaca ttacaaagga 361 ggatgttaaa agttacctgt ttcggaatgc ttttgtgctg ctcacagtca ccgctgtcat 421 tgtgggtaca atccttggat ttaccctccg accatacaga atgagctacc gggaagtcaa 481 gtacttctcc tttcctgggg aacttctgat gaggatgtta cagatgctgg tcttaccact 541 tatcatctcc agtcttgtca caggaatggc ggcgctagat agtaaggcat cagggaagat 601 gggaatgcga gctgtagtct attatatgac taccaccatc attgctgtgg tgattggcat 661 aatcattgtc atcatcatcc atcctgggaa gggcacaaag gaaaacatgc acagagaagg 721 caaaattgta cgagtgacag ctgcagatgc cttcctggac ttgatcagga acatgttccc 781 tccaaatctg gtagaagcct gctttaaaca gtttaaaacc aactatgaga agagaagctt 841 taaagtgccc atccaggcca acgaaacgct tgtgggtgct gtgataaaca atgtgtctga 901 ggccatggag actcttaccc gaatcacaga ggagctggtc ccagttccag gatctgtgaa 961 tggagtcaat gccctgggtc tagttgtctt ctccatgtgc ttcggttttg tgattggaaa 1021 catgaaggaa caggggcagg ccctgagaga gttctttgat tctcttaacg aagccatcat 1081 gagactggta gcagtaataa tgtggtatgc ccccgtgggt attctcttcc tgattgctgg 1141 gaagattgtg gagatggaag acatgggtgt gattgggggg cagcttgcca tgtacaccgt 1201 gactgtcatt gttggcttac tcattcacgc agtcatcgtc ttgccactcc tctacttctt 1261 ggtaacacgg aaaaaccctt gggtttttat tggagggttg ctgcaagcac tcatcaccgc 1321 tctggggacc tcttcaagtt ctgccaccct acccatcacc ttcaagtgcc tggaagagaa 1381 caatggcgtg gacaagcgcg tcaccagatt cgtgctcccc gtaggagcca ccattaacat 1441 ggatgggact gccctctatg aggctttggc tgccattttc attgctcaag ttaacaactt 1501 tgaactgaac ttcggacaaa ttattacaat cagcatcaca gccacagctg ccagtattgg 1561 ggcagctgga attcctcagg

cgggcctggt cactatggtc attgtgctga catctgtcgg 1621 cctgcccact gacgacatca cgctcatcat cgcggtggac tggttcctgg atcgcctccg 1681 gaccaccacc aacgtactgg gagactccct gggagctggg attgtggagc acttgtcacg 1741 acatgaactg aagaacagag atgttgaaat gggtaactca gtgattgaag agaatgaaat 1801 gaagaaacca tatcaactga ttgcacagga caatgaaact gagaaaccca tcgacagtga 1861 aaccaagatg tag

[0133] By “TH polypeptide” (or tyrosine hydroxylase) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAI43612.1.

TABLE-US-00060 (SEQ ID NO: 61)

MPTPDATTPQAKGFRRAVSELDAKQAEAIMSPRFIGRRQSLIEDARKERE
AAVAAAAAAVPSEPGDPLEAVAFEEKEGKAVLNLLFSPRATKPSALSRAV
KVFETFEAKIHHLETRPAQRPRAGGPHLEYFVRLEVRRGDLAALLSGVRQ
VSEDVRSPAGPKVPWFPRKVSELDKCHHLVTKFDPDLDLDHPGFSDQVYR
QRRKLIAEIAFQYRHGDPIPRVEYTAEEIATWKEVYTTLKGLYATHACGE
HLEAFALLERFSGYREDNIPQLEDVSRFLKERTGFQLRPVAGLLSARDFL
ASLAFRVFQCTQYIRHASSPMHSPEPDCCHELLGHVPMLADRTFAQFSQD
IGLASLGASDEEIEKLSTLYWFTVEFGLCKQNGEVKAYGAGLLSSYGELL
HCLSEEPEIRAFDPEAAAVQPYQDQTYQSVYFVSESFSDAKDKLRSYASR
IQRPFSVKFDPYTLAIDVLDSPQAVRRSLEGVQDELDTLAHALSAIG

[0134] By “TH polynucleotide” (or tyrosine hydroxylase) is meant a polynucleotide encoding an TH polypeptide. An exemplary TH nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC143611.

TABLE-US-00061 (SEQ ID NO: 62) 1 acccagaggg ggctttgacg tcagctcagc ttataagagg ctgctgggcc agggctgtgg 61 agacggagcc cggacctcca cactgagcca tgcccacccc cgacgccacc acgccacagg 121 ccaagggctt ccgcagggcc gtgtctgagc tggacgccaa gcaggcagag gccatcatgt 181 ccccgcggtt cattgggcgc aggcagagcc tcatcgagga cgcccgcaag gagcgggagg 241 cggcggtggc agcagcggcc gctgcagtcc cctcggagcc cggggacccc ctggaggctg 301 tggcctttga ggagaaggag gggaaggccg tgctaaacct gctcttctcc ccgagggcca 361 ccaagccctc ggcgctgtcc cgagctgtga aggtgtttga gacgtttgaa gccaaaatcc 421 accatctaga gacccggccc gcccagaggc cgcgagctgg gggccccccac ctggagtact 481 tcgtgcgcct cgaggtgcgc cgaggggacc tggccgccct gctcagtggt gtgcgccagg 541 tgtcagagga cgtgcgcagc cccgcggggc ccaaggtccc ctggttccca agaaaagtgt 601 cagagctgga caagtgtcat cacctggtca ccaagttcga ccctgacctg gacttggacc 661 acccgggctt ctcggaccag gtgtaccgcc agcgcaggaa gctgattgct gagatcgcct 721 tccagtacag gcacggcgac ccgattcccc gtgtggagta caccgccgag gagattgcca 781 cctggaagga ggtctacacc acgctgaagg gcctctacgc cacgcacgcc tgcggggagc 841 acctggaggc ctttgctttg ctggagcgct tcagcggcta ccgggaagac aatatccccc 901 agctggagga cgtctcccgc ttcctgaagg agcgcacggg cttccagctg cggcctgtgg 961 ccggcctgct gtccgcccgg gacttcctgg ccagcctggc cttccgcgtg ttccagtgca 1021 cccagtatat ccgccacgcg tcctcgccca tgcactcccc tgagccggac tgctgccacg 1081 agctgctggg gcacgtgccc atgctggccg accgcacctt cgcgcagttc tcgcaggaca 1141 ttggcctggc gtccctgggg gcctcggatg aggaaattga gaagctgtcc acgctgtact 1201 ggttcacggt ggagttcggg ctgtgtaagc agaacgggga ggtgaaggcc tatggtgccg 1261 ggctgctgtc ctcctacggg gagctcctgc actgcctgtc tgaggagcct gagattcggg 1321 ccttcgaccc tgaggctgcg gccgtgcagc cctaccaaga ccagacgtac cagtcagtct 1381 acttcgtgtc tgagagcttc agtgacgcca aggacaagct caggagctat gcctcacgca 1441 tccagcgccc cttctccgtg aagttcgacc cgtacacgct ggccatcgac gtgctggaca 1501 gccccccaggc cgtgcggcgc tccctggagg gtgtccagga tgagctggac acccttgccc 1561 atgcgctgag tgccattggc taggtgcacg gcgtccctga gggcccttcc caacctcccc 1621 tggtcctgc

[0135] By “Neurofilament 200 polypeptide” (or neurofilament heavy (NEFH)) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_066554.2.

TABLE-US-00062 (SEQ ID NO: 63)

MMSFGGADALLGAPFAPLHGGGSLHYALARKGGAGGTRSAAGSSSGFHSW

TRTSVSSVSASPSRFRGAGAASSTDSLDTLSNGPEGCMVAVATSRSEKEQ
LQALNDRFAGYIDKVRQLEAHNRSLEGEAAALRQQQAGRSAMGELYEREV
REMRGAVLRLGAARGQLRLEQEHLLEDIAHVRQRLDDEARQREEAEAAAR
ALARFAQEAEAARVDLQKKAQALQEECGYLRRHHQEEVGELLGQIQGSGA
AQAQMQAETRDALKCDVTSALREIRAQLEGHAVQSTLQSEEWFRVRLDRL
SEAAKVNTDAMRSAQEEITEYRRQLQARTTELEALKSTKDSLERQRSELE
DRHQADIASYQEAIQQLDAELRNTKWEMAAQLREYQDLLNVKMALDIEIA
AYRKLLEGEECRIGFGPIPFSLPEGLPKIPSVSTHIKVKSEEKIKVVEKS
EKETVIVEEQTEETQVTEEVTEEEEKEAKEEEGKEEEGGEEEEAEGGEEE
TKSPPAEEAASPEKEAKSPVKEEAKSPAEAKSPEKEEAKSPAEVKSPEKA
KSPAKEEAKSPPEAKSPEKEEAKSPAEVKSPEKAKSPAKEEAKSPAEAKS
PEKAKSPVKEEAKSPAEAKSPVKEEAKSPAEVKSPEKAKSPTKEEAKSPE
KAKSPEKEEAKSPEKAKSPVKAEAKSPEKAKSPVKAEAKSPEKAKSPVKE
EAKSPEKAKSPVKEEAKSPEKAKSPVKEEAKTPEKAKSPVKEEAKSPEKA
KSPEKAKTLDVKSPEAKTPAKEEARSPADKFPEKAKSPVKEEVKSPEKAK
SPLKEDAKAPEKEIPKKEEVKSPVKEEEKPQEVKVKEPPKKAEEEKAPAT
PKTEEKKDSKKEEAPKKEAPKPKVEEKKEPAVEKPKESKVEAKKEEAEDK
KKVPTPEKEAPAKVEVKEDAKPKEKTEVAKKEPDDAKAKEPSKPAEKKEA
APEKKDTKEEKAKKPEEKPKTEAKAKEDDKTLSKEPSKPKAEKAEKSSST
DQKDSKPPEKATEDKAAKGK

[0136] By “Neurofilament 200 polynucleotide” (or neurofilament heavy (NEFH)) is meant a polynucleotide encoding an Neurofilament 200 polypeptide. An exemplary Neurofilament 200 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_021076.

TABLE-US-00063 (SEQ ID NO: 64) 1 aaaagggccg gcgccctggt gctgccgcag tgcctcccgc cccgtcccgg cctcgcgcac 61 ctgctcaggc catgatgagc ttcggcggcg cggacgcgct gctgggcgcc ccgttcgcgc 121 cgctgcatgg cggcggcagc ctccactacg cgctagcccg aaagggtggc gcaggcggga 181 cgcgctccgc cgctggctcc tccagcggct tccactcgtg gacacggacg tccgtgagct 241 ccgtgtccgc ctcgcccagc cgcttccgtg gcgcaggcgc cgcctcaagc accgactcgc 301 tggacacgct gagcaacggg ccggagggct gcatggtggc ggtggccacc tcacgcagtg 361 agaaggagca gctgcaggcg ctgaacgacc gcttcgccgg gtacatcgac aaggtgcggc 421 agctggaggc gcacaaccgc agcctggagg gcgaggctgc ggcgctgcgg cagcagcagg 481 cgggccgctc cgctatgggc gagctgtacg agcgcgaggt ccgcgagatg cgcggcgcgg 541 tgctgcgcct gggcgcggcg cgcggtcagc tacgcctgga gcaggagcac ctgctcgagg 601 acatcgcgca cgtgcgccag cgcctagacg acgaggcccg gcagcgagag gaggccgagg 661 cggcggcccg cgcgctggcg cgcttcgcgc aggaggccga ggcggcgcgc gtggacctgc 721 agaagaaggc gcaggcgctg caggaggagt gcggctacct gcggcgccac caccaggaag 781 aggtgggcga gctgctcggc cagatccagg gctccggcgc cgcgcaggcg cagatgcagg 841 ccgagacgcg cgacgccctg aagtgcgacg tgacgtcggc gctgcgcgag attcgcgcgc 901 agcttgaagg ccacgcggtg cagagcacgc tgcagtccga ggagtggttc cgagtgaggc 961 tggaccgact gtcggaggca gccaaggtga acacagacgc tatgcgctca gcgcaggagg 1021 agataactga gtaccggcgt cagctgcagg ccaggaccac agagctggag gcactgaaaa 1081 gcaccaagga ctcactggag aggcagcgct ctgagctgga ggaccgtcat caggccgaca 1141 ttgcctccta ccaggaagcc attcagcagc tggacgctga gctgaggaac accaagtggg 1201 agatggccgc ccagctgcga gaataccagg acctgctcaa tgtcaagatg gctctggata 1261 tagagatagc cgcttacaga aaactcctgg aaggtgaaga gtgtcggatt ggctttggcc 1321 caattccttt ctcgcttcca gaaggactcc ccaaaattcc ctctgtgtcc actcacataa 1381 aggtgaaaag cgaagagaag atcaaagtgg tggagaagtc tgagaaagaa actgtgattg 1441 tggaggaaca gacagaggag acccaagtga ctgaagaagt gactgaagaa gaggagaaag 1501 aggccaaaga ggaggagggc aaggaggaag aaggggtga agaagaggag gcagaagggg 1561 gagaagaaga aacaaagtct cccccagcag aagaggctgc atccccagag aaggaagcca 1621 agtcaccagt aaaggaagag gcaaagtcac cggctgaggc caagtcccca gagaaggagg 1681 aagcaaaatc cccagccgaa gtcaagtccc ctgagaaggc caagtctcca gcaaaggaag 1741 aggcaaagtc accgcctgag gccaagtccc cagagaagga ggaagcaaaa tctccagctg 1801

aggtcaagtc ccccgagaag gccaagtccc cagcaaagga agaggcaaag tcaccggctg 1861 aggccaagtc tccagagaag gccaagtccc cagtgaagga agaagcaaag tcaccggctg 1921 aggccaagtc cccagtgaag gaagaagcaa aatctccagc tgaggtcaag tccccggaaa 1981 aggccaagtc tccaacgaag gaggaagcaa agtcccctga gaaggccaag tccccagaga 2041 aggaagaggc caagtcccct gagaaggcca agtccccagt gaaggcagaa gcaaagtccc 2101 ctgagaaggc caagtcccca gtgaaggcag aagcaaagtc ccctgagaag gccaagtccc 2161 cagtgaagga agaagcaaag tcccctgaga aggccaagtc cccagtgaag gaagaagcaa 2221 agtcccctga gaaggccaag tccccagtga aggaagaagc aaagaccccc gagaaggcca 2281 agtccccagt gaaggaagaa gctaagtccc cagagaaggc caagtcccca gagaaggcca 2341 agactcttga tgtgaagtct ccagaagcca agactccagc gaaggaggaa gcaaggtccc 2401 ctgcagacaa attccctgaa aaggccaaaa gccctgtcaa ggaggaggtc aagtccccag 2461 agaaggcgaa atctcccctg aaggaggatg ccaaggcccc tgagaaggag atcccaaaaa 2521 aggaagaggt gaagtcccca gtgaaggagg aggagaagcc ccaggaggtg aaagtcaaag 2581 agcccccaaa gaaggcagag gaagagaaag cccctgccac accaaaaaca gaggagaaga 2641 aggacagcaa gaaagaggag gcacccaaga aggaggctcc aaagcccaag gtggaggaga 2701 agaaggaacc tgctgtcgaa aagcccaaag aatccaaagt tgaagccaag aaggaagagg 2761 ctgaagataa gaaaaaagtc cccaccccag agaaggaggc tcctgccaag gtggaggtga 2821 aggaagacgc taaacccaaa gaaaagacag aggtagccaa gaaggaacca gatgatgcca 2881 aggccaagga acccagcaaa ccagcagaga agaaggaggc agcaccggag aaaaaagaca 2941 ccaaggagga gaaggccaag aagcctgagg agaaacccaa gacagaggcc aaagccaagg 3001 aagatgacaa gaccctctca aaagagccta gcaagcctaa ggcagaaaag gctgaaaaat 3061 cctccagcac agaccaaaaa gacagcaagc ctccagagaa ggccacagaa gacaaggccg 3121 ccaaggggaa gtaaggcagg gagaaaggaa catccggaac agccaaagaa actcagaaga 3181 gtcccggagc tcaaggatca gagtaacaca attttcactt tttctgtctt tatgtaagaa 3241 gaaactgctt agatgacggg gcctccttct tcaaacagga atttctgtta gcaatatgtt 3301 agcaagagag ggcactccca ggcccctgcc cccaggccct ccccaggcga tggacaatta 3361 tgatagctta tgtagctgaa tgtgatacat gccgaatgcc acacgtaaac acttgactat 3421 aaaaactgcc cccctccttt ccaaataagt gcatttattg cctctatgtg caactgacag 3481 atgaccgcaa taatgaatga gcagttagaa atacattatg cttgagatgt cttaacctat 3541 tcccaaatgc cttctgtttt ccaaaggagt ggtcaagccc ttgcccagag ctctctattc 3601 tggaagagcg gtccaggtgg ggccggggac tggccactga attatgccag ggcgcacttt 3661 ccactggagt tcactttcaa ttgcttctgt gcaataaaac caagtgctta taaaatgaaa 3721 a

[0137] By “Map2” (or microtubule-associated protein 2) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAH38857.1.

TABLE-US-00064 (SEQ ID NO: 65)

MADERKDEAKAPHWTSAPLTEASAHSHPPEIKDQGGAGEGLVRSANGFPY
REDEEGAFGEHGSQGTYSNTKENGINGELTSADRETAEEVSARIVQVVTA
EAVAVLKGEQEKEAQHKDQTAALPLAAEETANLPPSPPPSPASEQTVTVE
EAAGGESALAPSVFKQAKDKVSNSTLSKIPALQGSTKSPRYSSACPSTTK
RATFSDSLLIQPTSAGSTDRLPYSKSGNKDGVTKSPEKRSSLPRPSSILP
PRRGVSGDRDENSFSLNSSISSSARRTTRSEPIRRAGKSGTSTPTTPGST
AITPGTPPSYSSRTPGTPGTPSYPRTPHTPGTPKSAILVPSEKKVAIIRT
PPKSPATPKQLRLINQPLPDLKNVKSKIGSTDNIKYQPKGGQVRILNKKI
DFSKVQSRCGSKDNIKHSAGGGNVQIVTKKIDLSHVTSKCGSLKNIRHRP
GGGRVKIESVKLDFKEKAQAKVGSLDNAHHVPGGGNVKIDSQKLNFREHA
KARVDHGAEIITQSPGRSSVASPRRLSNVSSSGSINLLESPQLATLAEDV TAALAKQGL

[0138] By “Map2 polynucleotide” (or microtubule-associated protein 2) is meant a polynucleotide encoding an Map2 polypeptide. An exemplary Map2 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC038857.

TABLE-US-00065 (SEQ ID NO: 66) 1 ggcgctcggg ctgcgcgggc tctgggcagc agcagcagca gcagcagcat cctctcttcc 61 tttacttccc ttccgcttct ttctcttcct tctccttctt tttccccccc ctcccccttct 121 tcccctaacc cttctacccc tctccttttt ctccggaggg cgctaagtcc gtgagcggtg 181 gcagtcgcga ccgcgggtgc atccagtttc tgcgcccaga ttttattgat ctaatccaaa 241 gtatcttata

acttctggct ggaattaaga ttcttcagct tgtctctaac cgaggaagca 301 ttgattggga gctactcatt cagaaaatta aaagaaagaa gccagaaaat attatcaacc 361 ctttgagaac acgacacaac gaactttata ttttaccact tccttgaata gttgcaggag 421 aaataacaag gcattgaaga atggcagatg aacggaaaga cgaagcaaag gcacctcact 481 ggacctcagc accgctaaca gaggcatctg cacactcaca tccacctgag attaaggatc 541 aaggcggagc aggggaagga cttgtccgaa gcgccaatgg attcccatac agggaggatg 601 aagagggtgc ctttggagag catgggtcac agggcaccta ttcaaatacc aaagagaatg 661 ggatcaacgg agagctgacc tcagctgaca gagaaacagc agaggaggtg tctgcaagga 721 tagttcaagt agtcactgct gaggctgtag cagtcctgaa aggtgaacaa gagaaagaag 781 ctcaacataa agaccagact gcagctctgc ctttagcagc tgaagaaaca gctaatctgc 841 ctccttctcc acccccatca cctgcctcag aacagactgt cacagtggag gaagcagcag 901 gtggggaatc agctctggct cccagtgtat ttaaacaggc aaaggacaaa gtctctaatt 961 ctaccttgtc aaagattcct gctttacagg gtagcacaaa gtccccaaga tacagctcag 1021 cctgccctag cacgactaaa agggctacat tttctgacag tttattaata cagcccacct 1081 cagcaggctc cacagaccgt ttgccatact caaaatcagg gaacaaggac ggagtaacca 1141 agagcccaga aaagcgctct tctctcccaa gaccttcctc cattctccct cctcggcgag 1201 gtgtgtcagg agacagagat gagaattcct tctctctcaa cagttctatc tcttcttcag 1261 cacggcggac caccaggtca gagccaattc gcagagcagg gaagagtggt acctcaacac 1321 ccactacccc tgggtctact gccatcactc ctggcacccc accaagttat tcttcacgca 1381 caccaggcac tcctggaacc cctagctatc ccaggacccc tcacacacca ggaacccccа 1441 agtctgccat cttggtgccg agtgagaaga aggtcgccat catacgtact cctccaaaat 1501 ctcctgcgac tcccaagcag cttcggctta ttaaccaacc actgccagac ctgaagaatg 1561 tcaaatccaa aatcggatca acagacaaca tcaaatacca gcctaaaggg gggcaggtta 1621 ggattttaaa caagaagatc gattttagca aagttcagtc cagatgtggt tccaaggata 1681 acatcaaaca ttcggctggg ggcggaaatg tacaaattgt taccaagaaa atagacctaa 1741 gccatgtgac atccaaatgt ggctctctga agaacatccg ccacaggcca ggtggcggac 1801 gtgtgaaaat tgagagtgta aaactagatt tcaaagaaaa ggcccaagct aaagttggtt 1861 ctcttgataa tgctcatcat gtacctggag gtggtaatgt caagattgac agccaaaagt 1921 tgaacttcag agagcatgct aaagcccgtg tggaccatgg ggctgagatc attacacagt 1981 ccccaggcag atccagcgtg gcatcacccc gacgactcag caatgtctcc tcgtctggaa 2041 gcatcaacct gctcgaatct cctcagcttg ccactttggc tgaggatgtc actgctgcac 2101 tcgctaagca gggcttgtga atatttctca tttagcattg aaataataat atttaggcat 2161 gagctcttgg caggagtggg ctctgagcag ttgttatatt cattctttat aaaccataaa 2221 ataaataatc tcatccccaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2281 aaaaaa

[0139] By “DCX” (or doublecortin) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_835366.1.

TABLE-US-00066 (SEQ ID NO: 67)

MELDFGHFDERDKTSRNMRGSRMNGLPSPTHSAHCSFYRTRTLQALSNEK
KAKKVRFYRNGDRYFKGIVYAVSSDRFRSFDALLADLTRSLSDNINLPQG
VRYIYTIDGSRKIGSMDELEEGESYVCSSDNFFKKVEYTKNVNPNWSVNV
KTSANMKAPQSLASSNSAQARENKDFVRPKLVTIIRSGVKPRKAVRVLLN
KKTAHSFEQVLTDITEAIKLETGVVKKLYTLDGKQVTCLHDFFGDDDVFI
ACGPEKFRYAQDDFSLDENECRVMKGNPSATAGPKASPTPQKTSAKSPGP
MRRSKSPADSANGTSSSQLSTPKSKQSPISTPTSPGSLRKHKDLYLPLSL DDSDSLGDSM

[0140] By “DCX polynucleotide” (or doublecortin) is meant a polynucleotide encoding an DCX polypeptide. An exemplary DCX nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. NM_178153.

TABLE-US-00067 (SEQ ID NO: 68) 1 ctggcaggaa tttcttgctt ggagctcaga caacaaaggc atagagagat tggttttctt 61 tctctcagca tctccaccca accagcagaa aaccggtctc tgaggttcca ccaaaatatg 121 gaacttgatt ttggacactt tgacgaaaga gataagacat ccaggaacat gcgaggctcc 181 cggatgaatg ggttgcctag ccccactcac agcgcccact gtagcttcta ccgaaccaga 241 accttgcagg cactgagtaa tgagaagaaa gccaagaagg tacgtttcta ccgcaatggg 301 gaccgctact tcaaggggat tgtgtacgct gtgtcctctg accgttttcg cagctttgac 361 gccttgctgg ctgacctgac gcgatctctg tctgacaaca tcaacctgcc tcagggagtg 421 cgttacattt acaccattga tggatccagg aagatcggaa

gcatggatga actggaggaa 481 ggggaaagct atgtctgttc ctcagacaac ttctttaaaa aggtggagta caccaagaat 541 gtcaatccca actggtctgt caacgtaaaa acatctgcca atatgaaagc cccccagtcc 601 ttggctagca gcaacagtgc acaggccagg gagaacaagg actttgtgcg ccccaagctg 661 gttaccatca tccgcagtgg ggtgaagcct cggaaggctg tgcgtgtgct tctgaacaag 721 aagacagccc actcttttga gcaagtcctc actgatatca cagaagccat caaactggag 781 accggggttg tcaaaaaact ctacactctg gatggaaaac aggtaacttg tctccatgat 841 ttctttggtg atgatgatgt gtttattgcc tgtggtcctg aaaaatttcg ctatgctcag 901 gatgattttt ctctggatga aaatgaatgc cgagtcatga agggaaaccc atcagccaca 961 gctggcccaa aggcatcccc aacacctcag aagacttcag ccaagagccc tggtcctatg 1021 cgccgaagca agtctccagc tgactcagca aacggaacct ccagcagcca gctctctacc 1081 cccaagtcta agcagtctcc catctctacg cccaccagtc ctggcagcct ccggaagcac 1141 aaggacctgt acctgcctct gtccttggat gactcggact cgcttggtga ttccatgtaa 1201 aggaggggag agtgctcaga gtccagagta caaatccaag cctatcattg tagtagggta 1261 cttctgctca agtgtccaac agggctattg gtgctttcaa gtttttattt tgttgttgtt 1321 gttattttga aaaacacatt gtaatatgtt gggtttattt tcctgtgatt tctcctctgg 1381 gccactgatc cacagttacc aattatgaga gatagattga taaccatcct ttggggcagc 1441 attccaggga tgcaaaatgt gctagtccat gacctttcaa tggaaagctt aggtgcctgc 1501 gttatatttg ccctgtctaa ttttgcccat acagtcttcc ttctgtagag ggctgtttac 1561 atatacagca cttaaaatgt ttgtgtggga aaaaaaaaac tcattggcag atccaagaat 1621 gacaaacaca agtgcccctt ttctctggat ctcaagaatg gtggaggacc ctggaaggac 1681 agcaaggcag ctccccagcc tcactcttca ctcctgattg aggcccgggt ttgttgtcca 1741 gcaccaattc tggctgtcaa tggggagaaa taaaccaaca acttataatt gtgacaccag 1801 atgcttagga tcctggtgct gggttagcta agagaataga cagaattgga aaatactgca 1861 gacatttccg aagagtttat aaagcacagt gaattcctgg tcaatctctc cactgaggca 1921 atttggaatc aataagcaat tgataatagt ttggagtaag ggacttcata tacctgattc 1981 ctctagaagg ctgtctaaca taccacatga ttacatgaac tgtatggtat ccatctatct 2041 ctgttctatt gaatgccttg ttaacagcca acactgaaaa cactgtgaga atttgttttc 2101 aggtctgaca cctttcagtc tctttttata gcaagaaatc aatatccttt ttataaaaat 2161 tcatgtctgt atttcaggag caaactcttc aggctccttt tttataaact ggtgattttt 2221 cttttgtcta aaaaacacat gaagaaaatt taccaaaaaa aaaaaaaaaa gcagaagaat 2281 aatgtagttt agaaattatg ctgtcactgc caaacagtaa cctccaggag aaaacaagat 2341 gaatagcaga ggccaattca atagaatcag ttttttgata gctttttaac agttatgctt 2401 gcattaataa tttcaatgtg gaccagacat tctaattata ttttaaatga aatgttacag 2461 catattttaa gcaactcttt ttatctataa tcctaatatt tcatactgaa gacacagaaa 2521 tctttcactt gtctttaaca ttagaaagga tttctcttta ctaaggactg atcatttgaa 2581 atagttttca gtcttttgag atacaggttt ataacactgc ttttttttt ctgtaatcat 2641 agcccataat ggcaaagaca actaaattta agtgaaggtc atgcatgcca attctgtgtt 2701 tgcttttagc agatatgaag atttccttat ttctttgtaa ttgtgcagat attttgaaag 2761 gcacagcatt cgaagccaag ctgctgtttg gctactgaat ggcttgcagt tgttcctcca 2821 ctctaaatgg aatgagcttg ctgtgtgtgt gtgtggtggt ggtgggaggg ggtggtgcat 2881 gtgtgtgtgt gtgtgtgcat ctgcagctgc ttcaaaatta ggaaatacta caggacaccc 2941 ctgtaatgga ttggtggcaa ctgggtggca ctgctgatgt gcactgtgta ggggggaacc 3001 cagtggtggt ggggtagctc agatgcccct agacaagctt cagatgtctg tagctaccag 3061 aaacattttc ggttcaggaa aagtgagatg atggtagtac tggtttctgg tgaaattgaa 3121 gaaccccaaa tgatgaggat ctctttttgc cccctctcct ttttttgtag acccattcaa 3181 aaccattaat aagcccattt tactaagccc ctatttcttt ctagaagctc agggttttct 3241 tagtgcctcc cagaacattt tgtagttaat tgggaaaaag tgatacttgg attagggggt 3301 gtgggcataa agaatggtgg gaggcctgat tttaaacttc aggccagaac ccccaatgac 3361 tccacccata gtctcacttt aggtctcatt tagtccatca cctttatttt aagttgagga 3421 agtggaggct ggtaaagagc aggaccagag gaagaatcca gatttcctta tgcttgggcc 3481 tcacactagc tctctgagta tttccttgat tgcggtatat gtactactag aaaataccaa 3541 atggatatat tttctttagg ataacctttg aaccaacaat cttcaataac aatagtacat 3601 cttccatctt acttttaatc gagtataagg aaatgtttct ttatggccat tttggaggga 3661 gcaggggatg aggcttggca tagtccaaaa tttaagtctc caataattaa ttgcatttta 3721 aattggccca ctttcaaggc aatttttttt gtgtgtctgt aactgagctc ctccacccct 3781 gtcattcact tccaatttta cccaatccaa ttttagcact caagttccat tgtgttaatt 3841 tctgcacggt caacaaacat caagtcagca agcatttgcc accactccct atacttctcc 3901 ctccttctta cacacacaca cacacacaca cacacaatcc atctcttgct tgttcctacc 3961 tcctgatttt tcttccctac agaaatagaa atagggacaa agaaggggaa

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[0141] By “GABRA1” (or gamma-aminobutyric acid (GABA) A receptor) is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. AAH30696.1.

TABLE-US-00068 (SEQ ID NO: 69)

MRKSPGLSDCLWAWILLLSTLTGRSYGQPSLQDELKDNTTVFTRILDRLL
DGYDNRLRPGLGERVTEVKTDIFVTSFGPVSDHDMEYTIDVFFRQSWKDE
RLKFKGPMTVLRLNNLMASKIWTPDTFFHNGKKSVAHNMTMPNKLLRITE
DGTLLYTMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEVVYEWTR
EPARSVVVAEDGSRLNQYDLLGQTVDSGIVQSSTGEYVVMTTHFHLKRKI
GYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFGVTTVLTMTTLSISA
RNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDGKSVV
PEKPKKVKDPLIKKNNTYAPTATSYTPNLARGDPGLATIAKSATIEPKEV
KPETKPPEPKKTFNSVSKIDRLSRIAFPLLFGIFNLVYWATYLNREPQLK APTPHQ

[0142] By “GABRA1 polynucleotide” (or gamma-aminobutyric acid (GABA) A receptor) is meant a polynucleotide encoding an GABRA1 polypeptide. An exemplary GABRA1 nucleic acid molecule (e.g., mRNA) is provided at NCBI Accession No. BC030696.

TABLE-US-00069 (SEQ ID NO: 70) 1 agcggagcgg gcgagcaagg gagcgagcag gacaggagcc tgatcccaca gctgctgctc 61 cagcccgcga tgaggaaaag tccaggtctg tctgactgtc tttgggcctg gatcctcctt 121 ctgagcacac tgactggaag aagctatgga cagccgtcat tacaagatga acttaaagac 181 aataccactg tcttcaccag gattttggac agactcctag atggttatga caatcgcctg 241 agaccaggat tgggagagcg tgtaaccgaa gtgaagactg atatcttcgt caccagtttc 301 ggacccgttt cagaccatga tatggaatat acaatagatg tatttttccg tcaaagctgg 361 aaggatgaaa ggttaaaatt taaaggacct atgacagtcc tccggttaaa taacctaatg 421 gcaagtaaaa tctggactcc ggacacattt ttccacaatg gaaagaagtc agtggcccac 481 aacatgacca tgcccaacaa actcctgcgg atcacagagg atggcacctt gctgtacacc 541 atgaggctga cagtgagagc tgaatgtccg atgcatttgg aggacttccc tatggatgcc 601 catgcttgcc cactaaaatt tggaagttat gcttatacaa gagcagaagt tgtttatgaa 661 tggaccagag agccagcacg ctcagtggtt gtagcagaag atggatcacg tctaaaccag 721 tatgaccttc

ttggacaaac agtagactct ggaattgtcc agtcaagtac aggagaatat 781 gttgttatga ccactcattt ccacttgaag agaaagattg gctactttgt tattcaaaca 841 tacctgccat gcataatgac agtgattctc tcacaagtct ccttctggct caacagagag 901 tctgtaccag caagaactgt ctttggagta acaactgtgc tcaccatgac aacattgagc 961 atcagtgcca gaaactccct ccctaaggtg gcttatgcaa cagctatgga ttggtttatt 1021 gccgtgtgct atgcctttgt gttctcagct ctgattgagt ttgccacagt aaactatttc 1081 actaagagag gttatgcatg ggatggcaaa agtgtggttc cagaaaagcc aaagaaagta 1141 aaggatcctc ttattaagaa aaacaacact tacgctccaa cagcaaccag ctacacccct 1201 aatttggcca ggggcgaccc gggcttagcc accattgcta aaagtgcaac catagaacct 1261 aaagaggtca agcccgaaac aaaaccacca gaacccaaga aaacctttaa cagtgtcagc 1321 aaaattgacc gactgtcaag aatagccttc ccgctgctat ttggaatctt taacttagtc 1381 tactgggcta cgtatttaaa cagagagcct cagctaaaag cccccacacc acatcaatag 1441 atcttttact cacattctgt tgttcagtcc tctgcactgg gaatttattt atgttctcaa 1501 cgcagtaatt cccatctgct ttattgcctc tgtcttaaag aatttgaaag tttccttatt 1561 ttcataattc atttaagaac aagagacccc tgtctggcag tctggagcaa agcagactat 1621 gcagcttgga gacaggattc tgacagagca agcgaaagag caaagtcatg tcagaaggag 1681 acagaatgag agagaaaaga gggggaagat ggttcaaaga tacaagaaaa agtagaaaaa 1741 aaaataacac ttaactaaaa cccctaggtc atttgtagat atatatttcc aaatattcta 1801 aaaaagatac tgtatatgtc aaaaatattt ttatgtgaag gtgtttcaaa gggtaaatta 1861 taaatgtttc atgaagaaaa aattttaaaa atctacgtct ttattacaca aactatggtg 1921 tgcttatgtt tttgttttgc tttttaaact gatgtatagc tttaacattt tgtttccaaa 1981 gctgaagatc cccattcttt ctctttgaaa aaaaaaaagg cctaatgcat tattttgtca 2041 taaaatgcta ttttaaaatt catggaactt tcatacgtaa aggtgcagtt gctcattgta 2101 gagcacattt agtccaatga agataaatgc tttaaatagt ttacttcact ttcatctgag 2161 cttttaccac tagactcaag gaagaataat tttaacagac atgtatactc catagaaact 2221 aaattaaaat agtttaaaaa tattcccttt ttcaccctat tttcagatag cacatgagcc 2281 caacactcac ttaattctca ttatgaagat gtttttagag gggcaaaaat attttgcaag 2341 ctctggaatt gttgaatgta ttcttttata taactacatt aaaagcttta gattgaaatt 2401 tatgactagc aaacaaaaat agaatatata aacgatatat gtaaatatac agcatgagat 2461 tgtacatttt ttacttttt aaaattgtgt tcttaaaata ttgtgtaaga atcactgcac 2521 ttagctgttg gaatgttgtt aaatgctatg gaaatacatt tagaacctgc atttaagaac 2581 agaacagcaa gtatgaacca catggaactt aaaacatatg ggtgtgaagt ccacttatgt 2641 agacaaaact tataatttcc aaactgttgt ctagtataca gtgatcagtt gctctctgtt 2701 caagtcattc cacacatttc cctattttag gctattataa tatagaaaga aaatgggaag 2761 cattagttgg agctagaaaa tgaactgtat attattgcta tatttgctaa taccaactat 2821 ttcaataagt gttgtaccat atgtagcatt aaatataaaa tacataaaag aatgtacaga 2881 aaatagcttt tattgagtaa tattacattt catttatact gtagcaatat atttgtaggt 2941 atactatgta agggctttaa ataaaagagg tccattaata cttccttata aaaattctag 3001 tctgtttcat tactgcccag atgttttaga gataaatatt tatgcagaag gtatttttga 3061 agtctccttt tgtctgatag agtttaacag atatttaaat ttagtgctca gaatccacaa 3121 gtcacggtct aaacacactt agaatactac agcataaatc tgttagcatt attgccaaat 3181 aagacagttg ggatccaaac ccaagtcttg agcaatgttt ttctcaaaaa gctgctatcc 3241 aatgatatag gaaaatacat tgtgttttcc taaacacact tttcttttta aatgtgcttc 3301 attgtttgat ttggtcctgc ctaaatttca caagctaggc caatgaaggc tgaatcaaag 3361 acatttcatc caccaatatc atgtgtagat attatgtata gaaaataaaa taaattatgg 3421 ctccaaaaaa aaaaaaaaaa

[0143] Other features and advantages of the invention will be apparent to those skilled in the art from the following detailed description and claims.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0144] FIGS. **1**A-**1**D depict characterization of BMPS during differentiation. FIG. **1**A depicts a diagram of a differentiation protocol. FIG. **1**B depicts size of aggregates measured during the 3D neuronal differentiation. Negative days on the x-axis represent 3D cells cultured in NPC medium while positive days represent 3D cells cultured in differentiation medium. FIG. **1**C**1**-C**5** depicts

BMPS mRNA and miRNA expression of different markers during differentiation. FIG. **1**D depicts flow cytometry population analysis of BMPS at different stages of differentiation.

[0145] FIGS. **2**A-**2**C depict morphological characterization of BMPS. FIG. **2**A depicts co-immunostaining of neurons with markers. MAP2+ neurons were co-immunostained with the maturation marker Nestin at 2, 4, and 8 weeks of differentiation, which showed progressive increase of MAP2+ neurons and decrease of Nestin+ cells over time (panels a, b, c), demonstrating neuronal maturation. Co-immunostaining of neurons (NF-H) with the myelin marker MBP at 2, 4, and 8 weeks of differentiation (d,e,f, respectively) showed progressive increase of MBP+ cells in association with axonal processes. An increasing number of MBP+ cells (oligodendrocytes) was observed in association with axons (panels d, e, f). FIG. **2**B depicts neuronal and glial cell diversity was evaluated at 8 weeks. Neurons (MAP2, NF, SYP and SMI32) were visualized interacting with glia (GFAP and NOGOA). Neurons disclosed characteristic perykaria, dendrites (MAP2, panels a, b) and axons (NF, SMI32, panels c-f) associated with glia. Neurons exhibited diverse neurotransmitter identities shown by identification of glutamatergic VGLUT1+ (panels g, h), GABAergic CALB+ (panels i, j) and dopaminergic TH (panels k, 1) neurons. FIG. **2**C depicts that GFAP+ astroglia and CNPase+, O1+ and MBP+ oligodendroglia were identified. Oligodendroglia appeared mixed among astrocytes (panels a, b). 01+(panels c, d) and MBP+(panels e, f) oligodendrocytes were associated with axonal processes. Astrocytes established relationships with oligodendrocytes and exhibited characteristic multipolar processes (panels g, h). MBP+ oligodendrocytes issued processes in association with axons (panel i) 3D-reconstruction demonstrated myelinating processes resembling human myelination (panels j, k). Electron microscopy analysis of BMPS at 4 and 8 weeks of differentiation identified morphology of axonal structures and cells (e.g., oligodendrocytes) (panel 1). Myelinating-like processes, which closely resembled cross-sections of myelinated axons of the CNS were identified at 8 weeks of differentiation (panel m). FIG. **2**D depicts MBP+ oligodendrocytes issued processes in close association with axons and seemed to enwrap them at 8 weeks (a,b,c). Myelination calculated as the mean percentage MBP positive oligodendrocyte processes coverage of NF-H-positive axons (a,b,c) at 2, 4 and 8 weeks in at least 2 independent experiments showed significant increase of myelination observed with time of differentiation ($p<0.001$) (d). FIG. **2**E depicts 3D-reconstruction based on confocal z-stacks at 8 weeks demonstrating a “wrapping” myelinating process, which resembled the myelination of axons in human CNS. FIG. **2**F depicts a comparison of expression of neuronal and glial markers at 2 and 8 weeks. At 2 weeks, oligodendrocytes (O1, CNPase, NOGOA) were identified without a preferential localization (a,b,e,f,i,j), later they resemble human oligodendrocytes and localize in close proximity with axons (c,d, g,h, k,l). At 2 weeks there are few MAP2-positive cells without identifiable neuronal shape (I,j) whereas at 8 weeks, the MAP2+ cells acquire a well-defined dendritic network (k,l). The amount of astrocytes and density of the astroglial network increases with time of differentiation (GFAP, g,h). FIG. **2**G depicts variation in the nuclear morphology. Co-immunostaining of neurons (MAP2) with cell-division marker KI67 showed that some cells are dividing (a,b), there was also a small degree of apoptosis demonstrated by positive staining with CASP3 (c). CASP 3-positive nuclei did not co-localize with mature neurons (d). FIG. **2**H depicts ultrastructure analysis by electron microscopy of 4 week BMPS showed evidence of cell to cell junctions demonstrating functional interactions between the cells (arrows, a,b). Nuclear variation was confirmed by the presence of a few apoptotic nuclei (c) and normal healthy nuclei (d). NF: Neurofilament-heavy-chain, MAP2: Microtubule-associated-protein 2, MBP: myelin-basic-protein, VGLUT1: Vesicular-glutamate-transporter 1, GFAP: Glial-fibrillary-acidic-protein, CALB: Calbindin, NOGOA: Neurite-outgrowth-inhibitor, SYP: Synaptophysin, SMI32: Nonphosphorylated-neurofilament, TH: Tyrosine-hydroxylase, O1: Olig1, CNPase: 2′,3′-Cyclic-nucleotide-3′-phosphodiesterase. Scale Bar: 10 μm.

[0146] FIGS. **3**A-**3**F depict electrical activity of BMPS. Cells were cultured in 3D for 8 weeks and then cultured in 12-well and 48-well MEA plates for 4 more weeks. FIG. **3**A depicts heat map

recordings from a 48-well plate. FIG. **3**B depicts illustration of an active well showing spike morphology and FIG. **3**C depicts spike activity. FIGS. **3**D and **3**E depicts phase-contrast imaging of the mini-brains on MEAs, electrode diameter is 40-50 μm and inter-electrode space is 350 μm. FIG. **3**F depicts activity pattern recordings over 0.05 spikes/sec of the electrode over 10 min.

[0147] FIGS. **4**A-**4**G depict Parkinson's disease (PD) application of BMPS. BMPS were differentiated for 4 weeks and exposed to rotenone and MPP+ for 12 and 24 hours. FIG. **4**A depicts viability (resazurin assay) of BMPS after 24 hours rotenone exposure. FIG. **4**B depicts ROS (OxiSelect™ In Vitro ROS/RNS Assay Kit) production of BMPS after 12 and 24 hours rotenone exposure. FIG. **4**C depicts viability (resazurin assay) of BMPS after 24 hours MPP+ exposure. FIG. **4**D depicts ROS (OxiSelect™ In Vitro ROS/RNS Assay Kit) production of BMPS after 12 and 24 hours MPP+ exposure. FIGS. **4**E and **4**F depict confocal images of BMPS exposed to different concentrations of rotenone and MPP+ for NF200 (Red), TH (Green) and Hoechst nucleus staining (Blue). FIG. **4**G depicts expression of genes associated with oxidative stress and PD by real time RT-PCR. Graphs represent the relative expression of different markers compared to control (cells not treated) after 24 hours exposure to 5 μM rotenone and 1 mM MPP+. Genes of interest: mitochondrial complex 5 (ATP50, ATP5C1), mitochondrial complex 1 (NDUFB1), oxidative stress (KEAP1) and genes related to PD (TH, SNCA, TBR1, CASP1). Data are presented as mean±SD, of 3 independent experiments performed in 3 replicates. *$P<0.05$ comparing to control (untreated).

[0148] FIGS. **5**A-**5**D depict Down's Syndrome application of BMPS. BMPS were produced with iPSCs derived from a patient with Down's Syndrome. FIG. **5**A depicts morphological characterization with immunostaining of neurons (MAP2, Syn1, TH, SYP), neural precursor cells (nestin) and glial cells (GFAP) at 8 weeks of differentiation. FIG. **5**B depicts expression of genes in healthy BMPS vs. Down's Syndrome BMPS before and after treatment with 5 μM rotenone, after 24 hours exposure. Genes of interest include CNS markers (TH, OLIG2, NEFH), mitochondrial markers (ATP5C1, ATPSJ, ATP50) and ROS markers (NFE2L2, SOD1) which were measured by comparing control with exposed cells to rotenone on both healthy and Down syndrome derived mini-brains. FIGS. **5**C and **5**D depict karyotyping of iPSCs derived from the patient with Down's Syndrome. aCGH+SNP results for Down syndrome iPSC line are shown.

[0149] FIG. **6** depicts viability of pre-frozen NT2 human teratocarcinoma cell line and iPSC derived mini-brains. Fmedium corresponds to 95% FBS and 5% DMSO. NPC fmedium corresponds to STEMdiff™ Neural Progenitor Freezing Medium. Viability was measured by resazurin cell viability assay. Non-frozen cells at the same stage of differentiation were used as control aggregates.

[0150] FIG. **7** depicts an example of a BMPS covered with other cell types. LUHMES fluorescent cells (red) were incorporated to a BMP using gravity systems to cover the surface of the aggregate.

[0151] FIGS. **8**A-**8**E depict morphologic characterization of mature human BMPS. FIG. **8**A shows at 8 weeks, neuronal populations exhibited a diversity of neurotransmitter identities as shown by identification of dopaminergic TH+ (a,b), glutamatergic VGLUT1+ (c,d) and gabaergic calbindin+ (e,f) neurons. Neurons disclosed characteristic axons (NF) and synaptic proteins (SYN) (g,h). FIG. **8**B depicts two distinctive glial populations were identified in close interaction with neuronal populations, GFAP+ astroglia and CNPase+, O1+, NOGOA+ oligodendroglia. O1+ oligodendrocytes were closely associated with axonal processes (NF) (a,b), CNPase+ oligodendroglia appeared mixed among GFAP+ astroglia (c,d) and exhibited the characteristic multipolar glial processes, which extended from the perykaria (e,f). NOGOA+ cells were associated with MAP+ neurons (g,h). FIG. **8**C depicts example of custom algorithm created using the Cellomics Target Activation image-analysis software package to study astrocytes and oligodendrocytes (a,b,c,d). Quantification of cell populations as a percentage of the total nuclei count showed 3% NOGOA+ positive cells, 9% CNPase+ cells and 19% GFAP+ cells at 8 weeks (e). FIG. **8**D shows Co-expression of mature oligodendroglia markers (MBP and 02). FIG. **8**E shows expression of neuronal markers (VGLUT, TUJ1, SYN). Scale Bar: 10 μm.

DETAILED DESCRIPTION OF THE INVENTION

[0152] The present invention is based, at least in part, upon the discovery that brain microphysiological systems (BMPS) can be produced from induced pluripotent stem cells (iPSCs). Furthermore, the invention provides for reproducible BMPS that differentiate into mature neurons and glial cells (astrocytes and oligodendrocytes) in the central nervous system. This model is spontaneously electrophysiological active and may be reproduced with patient or genetically modified cells. The derivation of 3D BMPS from iPSCs has applications in the study and treatment of neurological and neurodevelopmental diseases. In some embodiments, the present disclosure provides for compositions and methods to study and/or treat neurodevelopmental and neurodegenerative disorders. In some cases, the neurodevelopmental and neurodegenerative disorders treated and/or studied by the present disclosure include, but are not limited to, autism, encephalitis, trauma, brain cancer, stroke, Amyotrophic lateral sclerosis, Huntington's Disease, muscular dystrophy, neurodegenerative disorder, neurodevelopmental disorder, Multiple Sclerosis, infection, Parkinson's Disease and Alzheimer's Disease.

[0153] As described herein, the present disclosure provides for the derivation of a multitude of identical brain microphysiological systems (BMPS) from stem cells, preferably of human origin, but including stem cells from animal origin. The preferred starting material are human induced pluripotent stem cells or embryonic stem cells, although other pluripotent stem cells such as, for example, neuronal precursor cells and mesenchymal stem cells may also be employed. Human in-vitro models of brain neurophysiology are needed to investigate molecular and cellular mechanisms associated with neurological disorders and neurotoxicity. The techniques herein provide a reproducible iPSC-derived human 3D BMPS that includes differentiated mature neurons and glial cells (astrocytes and oligodendrocytes) that reproduce neuronal-glial interactions and connectivity. BMPS mature over about eight weeks and show the critical elements of neuronal function including, but not limited to, synaptogenesis and neuron-to-neuron (e.g. spontaneous electric field potentials) and neuronal-glial interactions (e.g. myelination). Advantageously, the BMPS described herein include mature neurons (e.g., glutamatergic, dopaminergic and GABAergic neurons) and glial cells (e.g., astrocytes and oligodendrocytes). Quantification of the different cell types exhibited high reproducibility between experiments. Moreover, the BMPS disclosed herein present neuron and glial functions such as spontaneous electrical activity and axon myelination. The BMPS described herein are able to mimic the microenvironment of the central nervous system, which is a significant advance in the field of neurobiology as this ability has not been achieved at this level of functionality, reproducibility, and consistency in prior art in vitro systems.

[0154] In particular, the high amount of myelination of axons (up to 40%) in the disclosed BMPS represents a significant improvement over the prior art. Myelin pathology is a rather frequent condition in demyelinating and inflammatory disorders such as multiple sclerosis and post-infection diseases as well as other neurological diseases such as acute and post-traumatic brain injury, stroke and neurodegenerative disorders (see e.g., Fumagalli et al., 2016; Tse and Herrup, 2016). Moreover, the myelination process can be perturbed by exposure to chemicals and drugs (see e.g., Garcia et al., 2005; Brubaker et al., 2009; Creeley et al., 2013) during brain development and adulthood. For example, the BMPS disclosed herein show 40% overall myelination after 8 weeks of differentiation. Myelin was observed by immunohistochemistry and confirmed by confocal microscopy 3D reconstruction and electron microscopy. These findings are of particular relevance since myelin is crucial for proper neuronal function and development. The ability to assess oligodendroglia function and mechanisms associated with myelination in this BMPS model provide an excellent tool for future studies of neurological disorders such as multiple sclerosis and other demyelinating diseases. Thus, the BMPS provides a suitable and reliable model to investigate neuron-neuroglia function in neurotoxicology or other pathogenic mechanisms that has heretofore not been available in the prior art.

[0155] The method disclosed combines gyratory shaking or regular stirring and the addition of

growth factors to obtain the basic model. Suitable conditions as to how to achieve reproducible brain composition are disclosed herein. In contrast to earlier models, identical units of BMPS are produced, which allow comparative testing for the purpose of product development or safety assessments.

[0156] According to the techniques herein, a number of additional measures complement the basic BMPS to increase their completeness in modeling the human brain and improve its usefulness for such testing, for example:

[0157] 1. The addition of microglia: All stem-cell-derived brain models described so far lack micro-glia. The techniques herein provide that the addition of micro-glia precursor cells and suitable growth factors may allow microglia to be added to the model. Suitable cells may be monocytes (e.g., human monocytes), hematopoetic stem cells, respective (pro-)monocyte cell lines, and isolated microglia.

[0158] 2. The addition of a blood-brain-barrier: The human brain is protected by a tight blood-brain-barrier that excludes many substances from the brain. For the first time, the techniques herein provide a method to form a blood-brain-barrier to the BMPS via cells such as, for example, human endothelial cells.

[0159] 3. Addition of reporter and reporter cells: During the generation of the BMPS, cells carrying reporter for testing purposes may be used or added. These include, but are not limited to, fluorescent or luminescent markers to indicate a certain cell lineage or cell response. Genetic transient or permanent transfections are the primary, but not only, method of choice.

[0160] 4. The BMPS may also be produced, entirely or in its components, from cells from a specific genetic background, e.g. from patients with a specific disease or after selective genetic manipulation of the cells.

[0161] 5. The versatility of the BMPS may be improved by combining it with electrodes including, but not limited to, micro-electrode arrays (MEA).

[0162] 6. The versatility of the BMPS may be improved by combining it with other MPS (organ models) platforms such as, for example, microfluidic human-on-chip systems, perfusion chambers and others.

[0163] 7. Transportability of BMPS: Methods to cryopreserve BMPS were developed, which allow transport to other laboratories and testing or integration into multi-MPS platforms.

[0164] Simplified neural in vitro systems do not reflect physiology, interactions between different cell types, or human genetics. Induced pluripotent stem cells (iPSC)-derived human-relevant microphysiological systems (MPS) better mimic the organ level, but are too complex for chemical and drug screening. As described herein, a reproducible 3D brain MPS (BMPS) that differentiates into mature neurons and glial cells (astrocytes and oligodendrocytes), which reproduces the topology of neuronal-glial interactions and connectivity in the central nervous system was developed. BMPS from healthy donors or patients evolve from a period of differentiation to maturity over about 8 weeks, including synaptogenesis, neuron-neuron interactions (e.g. spontaneous electric field potentials) and neuronal-glial interactions (e.g. myelination of axons), which mimic the microenvironment of the central nervous system. Effects of substances on neurodevelopment may be studied during this phase of BMPS development. In an exemplary embodiment, the techniques herein were used to study Parkinson's disease (PD) by evaluating neurotoxicants with a link to PD pathogenesis. Exposure to 5 μM rotenone or 100 μM 1-methyl-4-phenylpyridinium (MPP+) (or 1 mM 1-methyl-4-phenylpyridinium (MPP+) for gene expression studies) disrupted dopaminergic neurons, as observed by immunohistochemistry and altered expression of PD-related genes (TH, TBR1, SNCA, KEAP1, NDUFB1, ATP5C1, ATP50 and CASP1), thus recapitulating hallmarks of PD pathogenesis linked to toxicant compounds in the respective animal models. The BMPS, as described herein, provide a suitable and reliable model to investigate neuron-neuroglia function in neurotoxicity or other pathogenic mechanisms.

[0165] There is growing concern about the continuing increase in neurodevelopmental and -

degenerative disorders such as autism [1, 2], Parkinson's [3] and Alzheimer disease [4]. Although genetic factors play an important role, environmental factors such as pesticides, air pollution, cigarette smoke, and dietary toxicants appear to contribute [5, 6, 7]. Due to a lack of mechanistic understanding, it is difficult to study their contributions and interactions with respect to neurotoxicity and neurological disorders. The complexity of the CNS makes it challenging to find appropriate in vitro human-relevant models, ideally from different genetic backgrounds, that are able to recapitulate the relevant pathophysiology. The poor predictive ability of animal-based models for human health, which may fail to mimic human pathology as outlined in the costly and time-consuming current developmental neurotoxicity (DNT) guidelines, contributes to the lack of reliable information on DNT mechanisms [8]. At the same time, more than 90% of all drugs fail clinical trials after extensive animal testing [9] due, in part, to the fact that animal studies often do not reflect human physiology and inter-individual differences. Simple in vitro systems do not represent physiology and organ function [10], which creates a critical demand for better models in drug development, study of disease mechanisms and progression, bioengineering and toxicological testing.

[0166] Attempts to generate more complex organotypic cultures or microphysiological systems (MPS) [11, 12, 13, 14] have resulted in more physiological multicellular 3D co-culture models able to simulate a functional part of the brain [15, 16]. 3D MPS have shown increased cell survival, differentiation, cell-cell interactions and can reproduce the complexity of the organ more closely [18]. Recent US research programs by NIH, FDA, DARPA, and DTRA have initiated the systematic development of MPS, including the model presented here, and their combinations to human-on-a-chip technologies to assess the safety and efficacy of countermeasures to biological and chemical terrorism and warfare [19].

[0167] The discovery of induced pluripotent stem cells (iPSC) and new protocols to differentiate them into various cell types have boosted the development of human in vitro models [20, 21]. iPSC from healthy or patient donors with a specific disease [22, 23, 24, 12] used in MPS promise more human-representative models, e.g. the brain organoids by Lancaster et al. and Kadoshima et al., have been able to recapitulate features of human cortical development [15, 16]. These complex systems present novel tools to study biological mechanisms in the CNS, however, they have certain limitations: 1) an elaborate and complex protocol, 2) size differences between organoids, 3) necrosis in the center of the organoid, 4) low reproducibility in cell differentiation. The human BMPS described herein overcomes these limitations. The reproducible in vitro iPSC-derived human 3D brain microphysiological system (BMPS) is comprised of differentiated and mature neurons and glial cells (astrocytes and oligodendrocytes).

[0168] The techniques herein provide a reproducible BMPS that contains several different cell types of the human brain, such as glutamatergic, dopaminergic and GABAergic neurons, astrocytes and oligodendrocytes. Moreover, the system has shown neural functionality as observed by spontaneous electrical activity and myelination of axons. Furthermore, the BMPS is reproducible from batch to batch and displays differences between healthy and patient donors. In addition, the obtained results demonstrate the application of such BMPS to the study of neurological disorders such as, for example, Parkinson's Disease (PD).

[0169] The brain MPS described herein is a versatile tool for more complex testing platforms and strategies as well as research into neurotoxicity (e.g., developmental), CNS physiology and pathology. Some stem cell-derived brain microphysiological systems have been developed in the latest years showing the capability to recapitulate some of the in vivo biological process [36, 37, 38]. These models have an enormous advantage over the classical in vitro models to study various differentiation mechanisms, developmental processes and diseases [15]. However, they are mostly based on human embryonic stem cells raising ethical concerns and not allowing the use of patient cells. Moreover, they require complicated protocols that may reduce the reproducibility of the system and make it difficult to use in other fields such as chemical and drug screening. Some of

these complex organoids have a large diameter, which can lead to extensive cell death, visible in the core of these tissues [15]. This may be due to insufficient diffusion of nutrients and oxygen in these non-vascularized systems, which may generate artifacts in toxicological and disease measurements and make it difficult to study different endpoints in a medium- to high-throughput manner. In addition, it will be challenging to adapt endpoints, established for relative simple 2D cultures, to such complex models. In the study described herein, the ability to generate a high number of viable (about 800 per batch), BMPS that are homogeneous in size (e.g., about 300 μm) and shape using iPSC by applying a constant or regular gyratory shaking or stirring technique as described earlier for rat re-aggregating brain cell cultures [40] is shown. Control of the size using specific shaker speed allowed the aggregates to be maintained below 350 μM in diameter (FIG. **1**B) and avoid disparate morphology and/or necrosis in the middle of the organoids. Moreover, a spherical homogeneous shape facilitates fluorescent quantification and further imaging-based endpoints as well as reproducibility between aggregates. The BMPS had reproducible cell composition by immunomorphological quantification, assessment of imaging-based endpoints and neurophysiological testing.

[0170] The 3D differentiation protocol described herein covered stages from neuronal precursors to different cell types of the mature CNS. After 2 weeks, BMPS consisted of an immature population of cells, showing minimal neuronal networks, low percentage of mature astrocytes and oligodendrocytes, with no myelin basic protein expression (FIG. **1**C). Cell populations in the BMPS were further differentiated and matured over time (FIG. **2**A). Evidence of iPSC differentiation into mature BMPS was supported by decreased Nestin expression over time. Nestin is normally expressed in embryonic tissue and its expression decreases with age in humans, therefore its decrement is a sign of maturation towards the adult phenotype [41, 42]. Also, the increasing presence of mature neuronal and glial markers such as MAP2, GFAP, Olig1 and MBP corroborate differentiation of the system. Different markers of pluripotency and proliferation decreased during the differentiation process, indicating maturing of the in vitro system (FIGS. **1**C and **1**D). Neuronal precursor markers such as Nestin, SOX1, SOX2 and the proliferation marker Ki67 decreased at the gene expression level and in flow cytometry measurements during the differentiation process (FIGS. **1**C and **1**D). Gene expression studies, flow cytometry, image analysis, immunostaining and miRNA studies have demonstrated an increase of cell maturation markers, which follows the BMPS differentiation (FIGS. **1**A-**1**D, **2**A-**2**H and **9**A-**9**C). Obtained data demonstrate that this simple protocol is sufficient to generate representative CNS cell phenotypes that can reproduce various stages of differentiation. The presence of GABAergic neurons, dopaminergic neurons and glutamatergic neurons was observed by immunohistochemistry and real-time-PCR data (FIG. **1**C and FIG. **2**B). In addition, miRNAs such as mir-124, mir-132, mir-128, mir-137 and mir133b with a role in nervous system differentiation and neuronal degeneration [43, 44] increased during differentiation in patterns consistent with the in vivo situation. Moreover, the BMPS described herein produced spontaneous electrical activity (FIG. **3**) confirming neuronal functionality of the system. However, further optimizations of the electrophysiological measurements using MEAs in 3D systems are needed.

[0171] Most of the brain MPS published so far are entirely focused on neurons and not glia populations [45, 46]; the brain MPS described herein is the first 3D model with fully characterized mature human oligodendrocytes, astrocytes and neurons, derived from iPSC. Astrocytes and oligodendrocytes play an important role during neuronal development, plasticity and neuronal injury. Astrocytes have a role in protecting neurons, increasing neuronal viability and mitochondrial biogenesis from both exogenous (e.g. chemicals) or endogenous (such as glutamate-induced excitotoxicity or the Alzheimer related Aβ1-42) toxicity [47, 48, 49, 50]. Astrocytes have an especially important role in neuroprotection from oxidative stress. Oxidative stress is known to be involved in a number of neuropathological conditions (such as neurodegenerative diseases) [51, 52, 53]. Thus, the presence of astrocytes in a biological system to study disease is crucial due to their

role in detoxification and neuronal protection. Immunochemistry results from the iPSC-derived BMPS showed low numbers of astrocytes (GFAP-positive cells) at 2 weeks of differentiation, which increased continuously throughout differentiation (FIG. **2**F-**2**H, and FIG. **2**A). Real-time RT-PCR data supports these findings, as a continuous increase in both s100b and GFAP mRNA levels could be observed from 2 weeks up to 8 weeks old BMPS. Immunohistochemistry and RT-PCR data results showed increasing numbers of astrocytes (GFAP-positive cells) in the BMPS model, reaching 19% astrocytes of the total cell population at 8 weeks. After 4 weeks of differentiation, astrocytes demonstrated increased positive staining for GFAP and the presence of glial network was observed (FIG. **2**C, panels g, h). At the same time, the presence of oligodendrocytes and myelination of axons could be observed in the system described herein. This process is highly important, since it is known to be involved in many degenerative diseases such as multiple sclerosis [54], congenital hypomyelination [55], progressive multifocal leukoencephalopathy caused by JC virus infection [56], periventricular leukomalacia (PVL) [57] and Alzheimer's disease [58]. Moreover, several chemicals such as ethanol [59], tellurium [60] and lead [(61, 62, 63, 64, 65] have shown to have an effect on the myelination process.

[0172] The presence of astroglia and oligodendroglia in the model described herein brings the system closer to the in vivo brain physiology, which is a crucial component to study neurodegeneration and neurotoxicity. In addition, the system has shown functionality as seen by imaging of cell-cell junctions, myelination, a rich astroglial network and electrical activity (FIG. **3**). These characteristics make the BMPS described herein a promising tool to study interactions between human neuronal cells in neurological diseases. The use of iPSCs makes it possible to study genetic factors and gene/environment interactions.

[0173] An assessment of the myelination process by quantification of MBP immunostaining along axons showed an increase over time reaching 42% of myelinated axons at 8 weeks (FIG. **2**D). 3D reconstruction of confocal z-stacks images (FIGS. **2**C and **2**E) and electron microscopy confirmed the wrapping of axonal structures after 8 weeks of differentiation (FIG. **2**C). These findings are of particular relevance since myelin is a critical element for proper neuronal function and development, the ensheathment of axons by myelin allows faster action potential transmission, reduces axonal energy consumption and protects the axons from degeneration[79]. Furthermore, recent evidence suggests that oligodendrocytes and myelin have a role in the metabolic support of axons independent of their role in action potential conduction, highlighting their importance in neuronal survival[80]. The ability of assessing oligodendroglia function and mechanisms associated with myelination in the BMPS model provide an excellent tool for future studies of neurological disorders such as multiple sclerosis and other demyelinating disorders.

[0174] In one embodiment, the model described herein is useful for studying Parkinson's disease (PD). Traditionally, PD has been described as a pre-synaptic degenerative process that affects dopaminergic neurons and induces a fundamental motor disorder [66], however, non-motor symptoms can also be present [67]. Research in Parkinson's disease is experiencing an upswing at the moment, owing to a lack of curative drugs for the large number of patients. Drug testing is nearly exclusively performed in vivo in the so-called MPTP (the parent compound to the metabolite MPP+ used here), rotenone, methamphetamine and 6-hydroxydopamine models requiring tens of thousands of animals [68, 69, 70]. These model toxins are mainly used in mice and primates (and less in cell cultures) to model a disease state resembling PD. Human neurons, which would be most relevant, are not usually available and existing cell lines are only very poor substitutes. The model described herein shows that treatment with MPP+ or rotenone induced specific degeneration of dopaminergic neurons in agreement with Parkinson patients and current animal models of the disease (FIGS. **4**E and **4**F). The BMPS PD model has shown to recapitulate some of the molecular mechanisms of the human disease, e.g. increase in ROS production (FIGS. **4**B and **3**D) and changes in genes related to PD (FIG. **4**G). BMPS treated with rotenone or MPP+ had decreased TH gene expression compared to controls, supporting the results presented in FIGS.

4E and **4**F where the dopaminergic neuronal phenotype is altered after treatment with the two chemicals. TBR1 encodes a transcription factor involved in the regulation of developmental processes. It also plays a role in major neurological diseases such as Alzheimer Disease and PD [71]. This gene was down-regulated after treatment with non-cytotoxic concentrations of MPP+ and rotenone. At the same time, mRNA levels of SNAC were altered. α-Synucleinopathy (common in Parkinson) is a neurodegenerative disease, which consists of the abnormal accumulation of aggregates of alpha-synuclein protein in neurons, nerve fibers or glial cells [72]. Alpha-synuclein plays regulatory roles such as synaptic maintenance, mitochondrial homeostasis, proteasome function, dopamine metabolism [73]. Reduction of SNCA (the alpha-synuclein encoding gene) after treatment with 5 μM rotenone and to a lesser extent after 1 mM MPP+ exposure could be explained by the alteration of alpha-synuclein protein metabolism. However, it may be that longer exposure times are required to produce an increase in gene expression. Caspase-1 (CASP1) expression increased significantly after 24 h exposure to 1 μM MPP+. Recently, some studies have identified human enzyme caspase-1 as the protease that cleaves α-synuclein in vivo [74]. This cleavage generates α-synuclein fragments that are prone to toxic aggregate formation. Finally, effects upon genes related with mitochondrial function (such as NDUFB1, ATP5C1 and ATP50) were down-regulated, more strongly in BMPS treated with MPP+ than rotenone. Changes in NDUFB1, indicate an alteration in mitochondrial function, agreeing with the phenomena already described in Parkinson's disease. This downregulation is linked to the increase in KEAP1 expression (oxidative stress marker) after 24 h exposure to 1 mM MPP+. The high variability in some of the genes may be explained by the selective effects of these chemicals (especially MPP+) to dopaminergic neurons, which represent only a subpopulation within the BMPS. While rotenone and MPP+ alter gene expression of this cell population, the other populations presented in BMPS appear not to be affected. Further studies using cell sorting could identify cell-specific effects.

[0175] This disclosure provides for a description of a brain microphysiological system aiming to study various aspects of brain development, pathophysiology and disturbance by genetic and environmental factors. The possibilities to study developmental and neurodegenerative disorders, infections, toxicity and trauma are emerging with such a system. Furthermore, the potential to use iPSC from different donors adds a personalized component to these studies. The high reproducibility and relatively easy protocol, enables future higher throughput testing of chemicals, and drugs and their potential to induce or treat diseases.

Autism

[0176] Autism is a highly variable neurodevelopmental disorder that first appears during infancy or childhood, and generally follows a steady course without remission. Patients with autism may be severely impaired in some respects but normal, or even superior, in others. Overt symptoms gradually begin after the age of six months, become established by age two or three years, and tend to continue through adulthood, although often in more muted form. It is distinguished not by a single symptom, but by a characteristic triad of symptoms: impairments in social interaction; impairments in communication; and restricted interests and repetitive behavior. Other aspects, such as atypical eating, are also common but are not essential for diagnosis. Autism's individual symptoms occur in the general population and appear not to associate highly, without a sharp line separating pathologically severe from common traits.

[0177] While autism is highly heritable, researchers suspect both environmental and genetic factors as causes. In rare cases, autism is strongly associated with agents that cause birth defects. Controversies surround other proposed environmental causes; for example, the vaccine hypotheses have been disproven. Autism affects information processing in the brain by altering how nerve cells and their synapses connect and organize; how this occurs is not well understood. It is one of three recognized disorders in the autism spectrum (ASDs), the other two being Asperger syndrome, which lacks delays in cognitive development and language, and pervasive developmental disorder, not otherwise specified (commonly abbreviated as PDD-NOS), which is diagnosed when the full

set of criteria for autism or Asperger syndrome are not met.
[0178] Globally, autism is estimated to affect 21.7 million people as of 2013. As of 2010, the number of people affected is estimated at about 1-2 per 1,000 worldwide. It occurs four to five times more often in boys than girls. About 1.5% of children in the United States (one in 68) are diagnosed with ASD as of 2014, a 30% increase from one in 88 in 2012. The rate of autism among adults aged 18 years and over in the United Kingdom is 1.1%. The number of people diagnosed has been increasing dramatically since the 1980s, partly due to changes in diagnostic practice and government-subsidized financial incentives for named diagnoses; the question of whether actual rates have increased is unresolved.
[0179] Autism has a strong genetic basis, although the genetics of autism are complex and it is unclear whether ASD is explained more by rare mutations with major effects, or by rare multigene interactions of common genetic variants. Complexity arises due to interactions among multiple genes, the environment, and epigenetic factors which do not change DNA but are heritable and influence gene expression. Studies of twins suggest that heritability is 0.7 for autism and as high as 0.9 for ASD, and siblings of those with autism are about 25 times more likely to be autistic than the general population. However, most of the mutations that increase autism risk have not been identified. Typically, autism cannot be traced to a Mendelian (single-gene) mutation or to a single chromosome abnormality, and none of the genetic syndromes associated with ASDs have been shown to selectively cause ASD. Numerous candidate genes have been located, with only small effects attributable to any particular gene. The large number of autistic individuals with unaffected family members may result from copy number variations-spontaneous deletions or duplications in genetic material during meiosis. Hence, a substantial fraction of autism cases may be traceable to genetic causes that are highly heritable but not inherited: that is, the mutation that causes the autism is not present in the parental genome.
[0180] Several lines of evidence point to synaptic dysfunction as a cause of autism. Some rare mutations may lead to autism by disrupting some synaptic pathways, such as those involved with cell adhesion. Gene replacement studies in mice suggest that autistic symptoms are closely related to later developmental steps that depend on activity in synapses and on activity-dependent changes. All known teratogens (agents that cause birth defects) related to the risk of autism appear to act during the first eight weeks from conception, and though this does not exclude the possibility that autism can be initiated or affected later, there is strong evidence that autism arises very early in development.
[0181] Exposure to air pollution during pregnancy, especially heavy metals and particulates, may increase the risk of autism. Environmental factors that have been claimed to contribute to or exacerbate autism, or may be important in future research, include certain foods, infectious diseases, solvents, diesel exhaust, PCBs, phthalates and phenols used in plastic products, pesticides, brominated flame retardants, alcohol, smoking, illicit drugs, vaccines, and prenatal stress, although no links have been found, and some have been completely disproven.
[0182] Autism does not have a clear unifying mechanism at either the molecular, cellular, or systems level; it is not known whether autism is a few disorders caused by mutations converging on a few common molecular pathways, or is (like intellectual disability) a large set of disorders with diverse mechanisms. Autism appears to result from developmental factors that affect many or all functional brain systems, and to disturb the timing of brain development more than the final product. Neuroanatomical studies and the associations with teratogens strongly suggest that autism's mechanism includes alteration of brain development soon after conception. This anomaly appears to start a cascade of pathological events in the brain that are significantly influenced by environmental factors. Just after birth, the brains of children with autism tend to grow faster than usual, followed by normal or relatively slower growth in childhood. It is not known whether early overgrowth occurs in all children with autism. It seems to be most prominent in brain areas underlying the development of higher cognitive specialization. Hypotheses for the cellular and

molecular bases of pathological early overgrowth include the following: an excess of neurons that causes local over connectivity in key brain regions, disturbed neuronal migration during early gestation, unbalanced excitatory-inhibitory networks, and abnormal formation of synapses and dendritic spines, for example, by modulation of the neurexin-neuroligin cell-adhesion system, or by poorly regulated synthesis of synaptic proteins.

[0183] The immune system is thought to play an important role in autism. Children with autism have been found by researchers to have inflammation of both the peripheral and central immune systems as indicated by increased levels of pro-inflammatory cytokines and significant activation of microglia. Biomarkers of abnormal immune function have also been associated with increased impairments in behaviors that are characteristic of the core features of autism such as deficits in social interactions and communication. Interactions between the immune system and the nervous system begin early during the embryonic stage of life, and successful neurodevelopment depends on a balanced immune response. It is thought that activation of a pregnant mother's immune system such as from environmental toxicants or infection can contribute to causing autism through causing a disruption of brain development. This is supported by recent studies that have found that infection during pregnancy is associated with an increased risk of autism.

[0184] The relationship of neurochemicals to autism is not well understood; several have been investigated, with the most evidence for the role of serotonin and of genetic differences in its transport. The role of group I metabotropic glutamate receptors (mGluR) in the pathogenesis of fragile X syndrome, the most common identified genetic cause of autism, has led to interest in the possible implications for future autism research into this pathway. Some data suggests neuronal overgrowth potentially related to an increase in several growth hormones or to impaired regulation of growth factor receptors. Also, some inborn errors of metabolism are associated with autism, but probably account for less than 5% of cases.

[0185] The mirror neuron system (MNS) theory of autism hypothesizes that distortion in the development of the MNS interferes with imitation and leads to autism's core features of social impairment and communication difficulties. The MNS operates when an animal performs an action or observes another animal perform the same action. The MNS may contribute to an individual's understanding of other people by enabling the modeling of their behavior via embodied simulation of their actions, intentions, and emotions. Several studies have tested this hypothesis by demonstrating structural abnormalities in MNS regions of individuals with ASD, delay in the activation in the core circuit for imitation in individuals with Asperger syndrome, and a correlation between reduced MNS activity and severity of the syndrome in children with ASD. However, individuals with autism also have abnormal brain activation in many circuits outside the MNS and the MNS theory does not explain the normal performance of children with autism on imitation tasks that involve a goal or object.

[0186] The under connectivity theory of autism hypothesizes that autism is marked by under functioning high-level neural connections and synchronization, along with an excess of low-level processes. Evidence for this theory has been found in functional neuroimaging studies on autistic individuals and by a brainwave study that suggested that adults with ASD have local over connectivity in the cortex and weak functional connections between the frontal lobe and the rest of the cortex. Other evidence suggests the under connectivity is mainly within each hemisphere of the cortex and that autism is a disorder of the association cortex.

[0187] From studies based on event-related potentials, transient changes to the brain's electrical activity in response to stimuli, there is considerable evidence for differences in autistic individuals with respect to attention, orientation to auditory and visual stimuli, novelty detection, language and face processing, and information storage; several studies have found a preference for nonsocial stimuli. For example, magnetoencephalography studies have found evidence in children with autism of delayed responses in the brain's processing of auditory signals.

[0188] Relations have been found between autism and schizophrenia based on duplications and

deletions of chromosomes; research showed that schizophrenia and autism are significantly more common in combination with 1q21.1 deletion syndrome. Research on autism/schizophrenia relations for chromosome 15 (15q13.3), chromosome 16 (16p13.1) and chromosome 17 (17p12) are inconclusive.

[0189] Diagnosis is based on behavior, not cause or mechanism. Under the DSM-5, autism is characterized by persistent deficits in social communication and interaction across multiple contexts, as well as restricted, repetitive patterns of behavior, interests, or activities. These deficits are present in early childhood, typically before age three, and lead to clinically significant functional impairment. Sample symptoms include lack of social or emotional reciprocity, stereotyped and repetitive use of language or idiosyncratic language, and persistent preoccupation with unusual objects. The disturbance must not be better accounted for by Rett syndrome, intellectual disability or global developmental delay. ICD-10 uses essentially the same definition. A pediatrician commonly performs a preliminary investigation by taking developmental history and physically examining the child. If warranted, diagnosis and evaluations are conducted with help from ASD specialists, observing and assessing cognitive, communication, family, and other factors using standardized tools, and taking into account any associated medical conditions. A pediatric neuropsychologist is often asked to assess behavior and cognitive skills, both to aid diagnosis and to help recommend educational interventions.

[0190] Clinical genetics evaluations are often done once ASD is diagnosed, particularly when other symptoms already suggest a genetic cause. Although genetic technology allows clinical geneticists to link an estimated 40% of cases to genetic causes, consensus guidelines in the US and UK are limited to high-resolution chromosome and fragile X testing. Metabolic and neuroimaging tests are sometimes helpful, but are not routine.

[0191] Many medications are used to treat ASD symptoms that interfere with integrating a child into home or school when behavioral treatment fails. More than half of US children diagnosed with ASD are prescribed psychoactive drugs or anticonvulsants, with the most common drug classes being antidepressants, stimulants, and antipsychotics. Antipsychotics, such as risperidone and aripiprazole, have been found to be useful for treating some conditions associated with autism, including irritability, repetitive behavior, and sleeplessness. A person with ASD may respond atypically to medications, the medications can have adverse effects, and no known medication relieves autism's core symptoms of social and communication impairments. Experiments in mice have reversed or reduced some symptoms related to autism by replacing or modulating gene function, suggesting the possibility of targeting therapies to specific rare mutations known to cause autism. Although many alternative therapies and interventions are available, few are supported by scientific studies. Some alternative treatments may place the child at risk. A 2008 study found that compared to their peers, autistic boys have significantly thinner bones if on casein-free diets; in 2005, botched chelation therapy killed a five-year-old child with autism. There has been early research looking at hyperbaric treatments in children with autism.

Parkinson's Disease

[0192] Parkinson's disease (PD, also known as idiopathic or primary parkinsonism, hypokinetic rigid syndrome (HRS), or paralysis agitans) is a degenerative disorder of the central nervous system mainly affecting the motor system. The motor symptoms of Parkinson's disease result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain. The causes of this cell death are poorly understood. Early in the course of the disease, the most obvious symptoms are movement-related; these include shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, thinking and behavioral problems may arise, with dementia commonly occurring in the advanced stages of the disease, and depression is the most common psychiatric symptom. Other symptoms include sensory, sleep and emotional problems. Parkinson's disease is more common in older people, with most cases occurring after the age of 50; when it is seen in young adults, it is called young onset PD (YOPD).

[0193] The main motor symptoms are collectively called “parkinsonism,” or a “parkinsonian syndrome.” The disease can be either primary or secondary. Primary Parkinson's disease is referred to as idiopathic (having no known cause), although some atypical cases have a genetic origin, while secondary parkinsonism is due to known causes like toxins. The pathology of the disease is characterized by the accumulation of a protein into Lewy bodies in neurons, and insufficient formation and activity of dopamine in certain parts of the midbrain. Where the Lewy bodies are located is often related to the expression and degree of the symptoms of an individual. Diagnosis of typical cases is mainly based on symptoms, with tests such as neuroimaging being used for confirmation.

[0194] Diagnosis of Parkinson's disease involves a physician taking a medical history and performing a neurological examination. There is no lab test that will clearly identify the disease, but brain scans are sometimes used to rule out disorders that could give rise to similar symptoms. People may be given levodopa and resulting relief of motor impairment tends to confirm diagnosis. The finding of Lewy bodies in the midbrain on autopsy is usually considered proof that the person had Parkinson's disease. The progress of the illness over time may reveal it is not Parkinson's disease, and some authorities recommend that the diagnosis be periodically reviewed. Other causes that can secondarily produce a parkinsonian syndrome are Alzheimer's disease, multiple cerebral infarction and drug-induced parkinsonism. Parkinson plus syndromes such as progressive supranuclear palsy and multiple system atrophy must be ruled out. Anti-Parkinson's medications are typically less effective at controlling symptoms in Parkinson plus syndromes. Faster progression rates, early cognitive dysfunction or postural instability, minimal tremor or symmetry at onset may indicate a Parkinson plus disease rather than PD itself. Genetic forms are usually classified as PD, although the terms familial Parkinson's disease and familial parkinsonism are used for disease entities with an autosomal dominant or recessive pattern of inheritance.

[0195] The PD Society Brain Bank criteria require slowness of movement (bradykinesia) plus either rigidity, resting tremor, or postural instability. Other possible causes for these symptoms need to be ruled out prior to diagnosis with PD. Finally, three or more of the following features are required during onset or evolution: unilateral onset, tremor at rest, progression in time, asymmetry of motor symptoms, response to levodopa for at least five years, clinical course of at least ten years and appearance of dyskinesias induced by the intake of excessive levodopa. Accuracy of diagnostic criteria evaluated at autopsy is 75-90%, with specialists such as neurologists having the highest rates. Computed tomography (CT) and conventional magnetic resonance imaging (MRI) brain scans of people with PD usually appear normal. These techniques are nevertheless useful to rule out other diseases that can be secondary causes of parkinsonism, such as basal ganglia tumors, vascular pathology and hydrocephalus. A specific technique of MRI, diffusion MRI, has been reported to be useful at discriminating between typical and atypical parkinsonism, although its exact diagnostic value is still under investigation. Dopaminergic function in the basal ganglia can be measured with different PET and SPECT radiotracers. Examples are ioflupane (123I) (trade name DaTSCAN) and iometopane (Dopascan) for SPECT or fluorodeoxyglucose (18F) and DTBZ for PET. A pattern of reduced dopaminergic activity in the basal ganglia can aid in diagnosing PD.

[0196] Treatments, typically the medications L-DOPA and dopamine agonists, improve the early symptoms of the disease. As the disease progresses and dopaminergic neurons continue to be lost, these drugs eventually become ineffective at treating the symptoms and at the same time produce a complication marked by involuntary writhing movements. Surgery and deep brain stimulation have been used to reduce motor symptoms as a last resort in severe cases where drugs are ineffective. Although dopamine replacement alleviates the symptomatic motor dysfunction, its effectiveness is reduced as the disease progresses, leading to unacceptable side effects such as severe motor fluctuations and dyskinesias. Furthermore, there is no therapy that will halt the progress of the disease. Moreover, this palliative therapeutic approach does not address the underlying mechanisms of the disease.

[0197] The term parkinsonism is used for a motor syndrome whose main symptoms are tremor at rest, stiffness, slowing of movement and postural instability. Parkinsonian syndromes can be divided into four subtypes according to their origin: primary or idiopathic, secondary or acquired, hereditary parkinsonism, and Parkinson plus syndromes or multiple system degeneration. Usually classified as a movement disorder, PD also gives rise to several non-motor types of symptoms such as sensory deficits, cognitive difficulties or sleep problems. Parkinson plus diseases are primary parkinsonisms which present additional features. They include multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration and dementia with Lewy bodies.
[0198] In terms of pathophysiology, PD is considered a synucleiopathy due to an abnormal accumulation of alpha-synuclein protein in the brain in the form of Lewy bodies, as opposed to other diseases such as Alzheimer's disease where the brain accumulates tau protein in the form of neurofibrillary tangles. Nevertheless, there is clinical and pathological overlap between tauopathies and synucleinopathies. The most typical symptom of Alzheimer's disease, dementia, occurs in advanced stages of PD, while it is common to find neurofibrillary tangles in brains affected by PD. Dementia with Lewy bodies (DLB) is another synucleinopathy that has similarities with PD, and especially with the subset of PD cases with dementia. However, the relationship between PD and DLB is complex and still has to be clarified. They may represent parts of a continuum or they may be separate diseases.
[0199] Mutations in specific genes have been conclusively shown to cause PD. These genes encode alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2. In most cases, people with these mutations will develop PD. With the exception of LRRK2, however, they account for only a small minority of cases of PD. The most extensively studied PD-related genes are SNCA and LRRK2. Mutations in genes including SNCA, LRRK2 and glucocerebrosidase (GBA) have been found to be risk factors for sporadic PD. Mutations in GBA are known to cause Gaucher's disease. Genome-wide association studies, which search for mutated alleles with low penetrance in sporadic cases, have now yielded many positive results.
[0200] The role of the SNCA gene is important in PD because the alpha-synuclein protein is the main component of Lewy bodies. The histopathology (microscopic anatomy) of the substantia nigra and several other brain regions shows neuronal loss and Lewy bodies in many of the remaining nerve cells. Neuronal loss is accompanied by death of astrocytes (star-shaped glial cells) and activation of the microglia (another type of glial cell). Lewy bodies are a key pathological feature of PD.

Alzheimer's Disease

[0201] Alzheimer's disease (AD) accounts for 60% to 70% of cases of dementia. It is a chronic neurodegenerative disease that often starts slowly, but progressively worsens over time. The most common early symptom is short-term memory loss. As the disease advances, symptoms include problems with language, mood swings, loss of motivation, disorientation, behavioral issues, and poorly managed self-care. Gradually, bodily functions are lost, ultimately leading to death. Although the speed of progression can vary, the average life expectancy following diagnosis is three to nine years. The cause of Alzheimer's disease is poorly understood. About 70% of the risk is believed to be genetic with many genes involved. Other risk factors include a history of head injuries, hypertension, or depression. The disease process is associated with plaques and tangles in the brain.
[0202] Alzheimer's disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Alzheimer's disease has been hypothesized to be a protein misfolding disease (proteopathy), caused by accumulation of abnormally folded A-beta and tau proteins in the brain. Plaques are made up of small peptides, 39-43 amino acids in length, called beta-amyloid (also written as A-beta

or Aβ). Beta-amyloid is a fragment from a larger protein called amyloid precursor protein (APP), a transmembrane protein that penetrates through the neuron's membrane. APP is critical to neuron growth, survival and post-injury repair. In Alzheimer's disease, an unknown process causes APP to be divided into smaller fragments by enzymes through proteolysis. One of these fragments gives rise to fibrils of beta-amyloid, which form clumps that deposit outside neurons in dense formations known as senile plaques.

[0203] A probable diagnosis is based on the history of the illness and cognitive testing with medical imaging and blood tests to rule out other possible causes. Initial symptoms are often mistaken for normal ageing. Examination of brain tissue is needed for a definite diagnosis. Alzheimer's disease is diagnosed through a complete medical assessment. There is no one clinical test that can determine whether a person has Alzheimer's. Usually several tests are performed to rule out any other cause of dementia. The only definitive method of diagnosis is examination of brain tissue obtained from a biopsy or autopsy. Tests (such as blood tests and brain imaging) are used to rule out other causes of dementia-like symptoms. Laboratory tests and screening include: complete blood cell count; electrolyte panel; screening metabolic panel; thyroid gland function tests; vitamin B-12 folate levels; tests for syphilis and, depending on history, for human immunodeficiency antibodies; urinalysis; electrocardiogram (ECG); chest X-ray; computerized tomography (CT) head scan; and an electroencephalogram (EEG). A lumbar puncture may also be informative in the overall diagnosis.

[0204] There are no known medications or supplements that decrease risk of Alzheimer's. Additionally, no known treatments stop or reverse Alzheimer's progression, although some may temporarily improve symptoms.

[0205] This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the figures, are incorporated herein by reference.

EXAMPLES

Example 1: Characterization of BMPS by Expression of Neural Specific Genes During Differentiation

[0206] According to the techniques herein, the BMPS model established herein follows a stepwise differentiation protocol (FIG. **1**A). In the final step, cells were differentiated into various neuronal and glial cell types during constant gyratory shaking. Briefly, the BMPS were established as follows: cells were differentiated, by addition of B27, GDNF and BDNF and withdrawal of stempro, basic FGF and EGF, into different neuronal and glial cell types with CNS functions during constant gyratory shaking. Advantageously, the techniques herein provide that the BMPS that were produced were of a spherical shape and a consistent size. For example, the BMPS showed spherical shapes and controlled sizes that were below 350 μm after 17 days in culture, a size that avoids necrosis in the center of the aggregate (FIG. **1**B) that occurs in larger spheroids (e.g., >350 μm) due to nutrient and oxygen deprivation. Nutrient and oxygen deprivation-induced necrosis could produce artifacts in the different endpoints measured, especially in disease and toxicity studies. Five days after initiation of aggregation in NPC medium, spheres were on average 130±5 μm in diameter; the size increased to 300±40 μm during the first 17 days in differentiation medium. From day 17 onwards size remained constant around 310 μm. Advantageously, this technique significantly increases throughput of BMPS production by allowing simultaneous production of several batches with different conditions. Without the shaking condition, aggregates tend to stick together, grow in different shapes, attach to the bottom and in some point get necrotic in the middle of the sphere. Thus, constant gyratory shaking technology is a suitable method to control the shape and size of BMPS.

[0207] In order to characterize different stages of the differentiation and maturation process, BMPS were collected every week up to 8 weeks of differentiation (FIGS. **1**C**1**-C**5**). Analysis of different neuronal and glial cell-specific genes by real-time reverse transcription polymerase chain reaction

(RT-PCR) was performed to characterize the presence of neurons, astrocytes, oligodendrocytes and neural precursor cells (NPC). NPC are self-renewing and proliferating multi-potent cells able to generate different cell types of the central nervous system. The differentiation of NPC in 3D was initiated by changing the medium to differentiation medium. Gene expression of the cell proliferation marker Ki67 decreased 95% after 2 weeks of differentiation (FIG. **1**C**1**, proliferation and stem cell markers). The remaining Ki67 expression appears to be due to the presence of a small population of NPC and other proliferating cell types such as oligodendrocytes and astrocytes (FIG. **1**C**2**, astroglia and oligodendroglia). Astrocyte-specific genes (S100B and GFAP) showed a constant increase after two weeks, while, differentiation of oligodendrocytes was induced later, after six weeks of differentiation as shown by OLIG2 gene expression (FIG. **1**C**2**).

[0208] Gene expression of specific neurotransmitters or their receptors was used to characterize the identity of different neuronal populations and the differentiation patterns of the human iPSC derived BMPS (FIG. **1**C**4**, neuronal markers; right y-axis relative quantification of GRIN1 and GABRA1; MBP, FOXA2, and SLC1A3). GRIN1 encodes the essential Glutamate [NMDA] receptor subunit zeta-1 [25] was increased at very early stages of differentiation (one week after induction of differentiation) and continued to increase up to 5 weeks when it reached a plateau (FIG. **1**C**4**). Similarly, GAD1, a GABAergic neuronal gene marker which encodes the Glutamate decarboxylase 1, and catalyzes decarboxylation of glutamate to GABA, showed an increase in expression during the first 4 weeks of differentiation, reaching a plateau thereafter (FIG. **1**C**4**). The expression of tyrosine hydroxylase (TH) a gene, which identifies dopaminergic neurons, was observed first after three weeks, showing delayed differentiation compared to glutamatergic neurons. The expression of TH increased constantly thereafter reaching an 86-fold increase at seven weeks compared to NPC (week 0; FIG. **1**C**4**). GABRA1, which encodes the gamma-aminobutyric acid (GABA) receptor, showed a steady increase of expression after 2 weeks and reached its maximum increase of a 150-fold change at 8 weeks compared to week 0 (FIG. **1**C**4**). Moreover other markers for specific part of the brain, such as ventral midbrain neuron marker LMX1A, FOXO1 and FOXA2 (Hedlund et al., 2016; Stott et al., 2013), cerebral cortex marker FOXO4, or markers for myelination CNP and MBP (Li and Richardson, 2008; Agrawal et al., 1994) and L-glutamate transport SLC1A6 (Sery et al., 2015) has been studied (FIG. **1**D d). Based on the patterns of expression of neuronal genes, the iPSC-derived BMPS model closely represents the different neuronal populations of different cortical and subcortical areas of the human CNS, suggesting that some of the mechanisms implicated in the early stages of nervous system development are reflected.

[0209] To prove that BMPS can be generated from different IPCs, another healthy line (IPS IMR90) and Down syndrome line (DYP0730) were used (FIG. **1**C**5**). Both lines were able to generate BMPS and differentiated to neurons (MAP2 marker), astrocytes (GFAP marker) and oligodendrocytes (OLIG1 marker).

Example 2: Characterization of BMPS by Flow Cytometry Analysis Shows Neuronal Maturation of the Human Induced Pluripotent Stem Cells Over Time

[0210] In order to quantify cell populations in the iPSC-derived BMPS and verify the reproducibility between experiments and batches of the cell line (C1, CRL-2097), flow cytometry was performed using CNS-specific antibodies for identification of neural markers (Table 1). Flow cytometry allowed quantifying 60% of cells with proliferation marker (Ki67) at the NPCs stage (week 0), which was reduced during differentiation down to 9% at 2 weeks, 7% at 4 weeks and 1% at 8 weeks (FIG. **1**D), indicating a fast reduction of proliferating cells after induction of differentiation. This confirms the gene expression data and indicates a fast reduction of proliferating cells after induction of differentiation. This result was confirmed by further analysis of NPC markers such as SOX1, SOX2 and Nestin. SOX1 and SOX2 are known to be involved in the maintenance of neural progenitor cell identity. The number of SOX1-, SOX2- and NES-positive (NPC marker) cells in the NPC population (week 0) was 46%, 68% and 60%, respectively. SOX1,

SOX2 and NES expression was reduced dramatically with differentiation, showing very low positive populations at eight weeks (2%, 3% and 2%, respectively). This loss in the NPC population during differentiation was corroborated by Doublecortin (DCX), a microtubule-associated protein expressed in neuroblasts and immature neurons: the number of DCX-positive cells in NPC (week o) was around 30%, which reduced to 22% at two, 17% at four and 4% at eight weeks, respectively. On the other hand, the marker for mature neurons, Tuj1 (Neuron-specific class III beta-tubulin) presented the opposite pattern. Analysis showed low levels of Tuj1-positive cells at the NPC stage (week 0). The expression of this marker in the cell population increased to 70% after 2 weeks of differentiation and remained constant up to 8 weeks. These flow cytometry experiments indicate differentiation and maturation of the BMPS over time.

[0211] Quantification of the cell population in at least three independent experiments showed low variability between cultures, demonstrating the reproducibility of the system. The variation (standard deviation, SD) between experiments decreased with the cell differentiation process and was very small at the latest maturation stage (eight weeks); DCX SD 0.9%, Ki67 SD 0.2%, SOX1 SD 0.7%, SOX2 SD 1.2%, NES SD 0.7% and Tuj1 SD 9.8% (FIG. **1**E). These results indicate that after eight weeks of differentiation the cellular composition is similar and shows high reproducibility between different BMPS experiments.

TABLE-US-00070 TABLE 1 Gene and miRNAs Taqman Assays. List of the primers used for the experiments. Assay ID Assay Type Availability Catalog Number Assay Name Gene Expression Taqman Primers Hs01060665 TaqMan ® Gene Expression Assay Inventoried 4331182 BACT Hs99999901 TaqMan ® Gene Expression Assay Inventoried 4331182 18S Hs04187831 TaqMan ® Gene Expression Assay Inventoried 4331182 NES Hs01032443 TaqMan ® Gene Expression Assay Inventoried 4331182 Ki67 Hs01088112 TaqMan ® Gene Expression Assay Inventoried 4331182 PAX6 Hs00909233 TaqMan ® Gene Expression Assay Inventoried 4331182 GFAP Hs00300164 TaqMan ® Gene Expression Assay Inventoried 4331182 OLIG2 Hs00902901 TaqMan ® Gene Expression Assay Inventoried 4331182 S100B Hs00609557 TaqMan ® Gene Expression Assay Inventoried 4331182 GRIN1 Hs00165941 TaqMan ® Gene Expression Assay Inventoried 4331182 TH Hs00971228 TaqMan ® Gene Expression Assay Inventoried 4331182 GABRA1 Hs01065893 TaqMan ® Gene Expression Assay Inventoried 4331182 GAD1 Hs00199577 TaqMan ® Gene Expression Assay Inventoried 4331182 SYN1 Hs00232429 TaqMan ® Gene Expression Assay Inventoried 4331182 TBR1 Hs01003383 TaqMan ® Gene Expression Assay Inventoried 4331182 SNCA Hs01003430 TaqMan ® Gene Expression Assay Inventoried 4331182 KEAP1 Hs00929425 TaqMan ® Gene Expression Assay Inventoried 4331182 NDUFB1 Hs01101219 TaqMan ® Gene Expression Assay Inventoried 4331182 ATP5C1 Hs00919163 TaqMan ® Gene Expression Assay Inventoried 4331182 ATP50 Hs00354836 TaqMan ® Gene Expression Assay Inventoried 4331182 CASP1 Hs00263981 TaqMan ® Gene Expression Assay Inventoried 4331182 CNP Hs01054576 TaqMan ® Gene Expression Assay Inventoried 4331182 FOXO1 Hs00188193 TaqMan ® Gene Expression Assay Inventoried 4331182 SLC1A3 Hs00936217 TaqMan ® Gene Expression Assay Inventoried 4331182 FOXO4 Hs00892663 TaqMan ® Gene Expression Assay Inventoried 4331182 LMX1A Hs00232764 TaqMan ® Gene Expression Assay Inventoried 4331182 FOXA2 miRNA Taqman Assays 1182 TaqMan ® microRNA Assay Inventoried 4427975 mmu-miR-124a 2216 TaqMan ® microRNA Assay Inventoried 4427975 hsa-miR-128a 457 TaqMan ® microRNA Assay Inventoried 4427975 hsa-miR-132 2247 TaqMan ® microRNA Assay Inventoried 4427975 hsa-miR-133b 1129 TaqMan ® microRNA Assay Inventoried 4427975 mmu-miR-137 1094 Control miRNA Assay Inventoried 4427975 RNU44

Example 3: MicroRNAs as Neuronal Differentiation Markers in Human iPSC-Derived BMPS

[0212] MicroRNAs (miRNA), known as posttranscriptional regulators of developmental timing, have recently been established as markers to study the differentiation process [26]. Expression of neural-specific miRNAs showed strong induction of miRNAs involved in neurogenesis (FIG. **1**C**3**, miRNA). mir-124, the most abundant brain miRNA, was strongly induced in the earlier stages of

differentiation, then slightly down-regulated at eight weeks of differentiation. This finding correlates with previous studies, where mir-124 was shown to promote neuronal lineage commitment at earlier stages of neural stem cells specification by targeting anti-neuronal factors [26]. mir-128, a modulator of late neural differentiation, was strongly up-regulated after 5 weeks of differentiation. mir-137, the most induced miRNA over time in the system described herein, is known as a regulator of neural differentiation of embryonic stem cells (ESCs) [27]. mir-132 and mir-133b which are involved in regulation of dopaminergic neuron maturation and function, were induced in week three of differentiation, a finding which correlates with the expression pattern of TH. Moreover, mir-132 is involved in dendritic spine formation [28]. These results support the view of a coordinated mechanism of neuronal differentiation as reflected by the patterns of neuronal gene and miRNA expression and neuronal and neurotransmitter identity.

Example 4: Characterization of Human BMPS by Immunohistochemistry and Electron Microscopy Shows Evidence of Differentiation into Mature Brain Cell Types

[0213] In order to assess the cellular composition and the process of maturation of the cells within the human BMPS, the expression of markers for different CNS cell populations including neurons and glial cells at 2, 4 and 8 weeks of differentiation were evaluated using immunohistochemistry and electron microscopy techniques. A reproducible pattern of expression consistent with maturation of the BMPS towards mature neural phenotypes was found. After 4 weeks of differentiation, the BMPS showed positive staining for mature neuronal markers such as microtubule-associated protein 2 (MAP2), neurofilament-heavy chain (NF, SMI32) and synaptophysin (FIG. **2**A, **2**B). Furthermore, different neuronal subtypes in the BMPS including dopaminergic (TH-positive neurons), glutamatergic (VGLUT1-positive neurons) and GABAergic interneurons (calbindin-positive neurons) (FIG. **2**B, FIG. **8**A) were observed. Moreover, the BMPS matured over time of differentiation as seen by decreased NES-positive cells (FIG. **2**A) and increased cell-cell interactions (neuron-neuron and neuron glia) as subsets of neurons showed several processes, which resembled dendritic and axonal projections (FIG. **8**A).

[0214] A subset of neuronal cells exhibited immunoreactivity for markers such as NOGOA, O1, O2, and CNPase (FIG. **8**B, panels a-j; FIG. **1**C**5**), which identifies the presence of mature oligodendrocytes in the BMPS [31, 33]. Automatic image quantification showed that oligodendrocytes (CNPase, NOGOA, and Olig1) comprised 3, 9, and 11% of the total cell population, respectively, at 8 weeks of differentiation (FIG. **8**C; FIG. **1**C**5**). Similar to the in vivo physiology, these cells were immunoreactive for myelin basic protein (MBP) (FIG. **2**), which characterizes myelinating oligodendrocytes [32]. Moreover, they had morphological features of normal human oligodendrocytes in vivo and appeared in close contact with neuronal processes (FIG. **8***a-b*, FIG. **2**C, **2**D)

[0215] Similarly, populations of neuroglia such as astrocytes and oligodendrocytes were identified using specific antibody markers. A subset of neuroglial cells exhibit immunoreactivity for markers such as NOGOA, Olig1 and CNPase (FIG. **2**C, panels a-f and **2**C, panel i), which identify the presence of mature oligodendrocytes in the BMPS [29, 30, 31, 32]. This pattern of immunostaining suggests that oligodendrocytes within the BMPS are functional and myelinate axons. Similar to the in vivo physiology, these cells were also immunoreactive for myelin basic protein (MBP) (FIG. **2**C panel i and **2**C panel j), which characterizes myelinating oligodendrocytes [33, 30]. These cells had morphological features of normal human oligodendrocytes and appeared in close contact with neuron processes, which resemble axonal structures (FIG. **2**C, panels j-m). In addition, a high number of mature astrocytes (FIG. **2**Ca, **2**Cb, **2**Cg, **2**Ch and **2**F) at 4 and 8 weeks of differentiation were observed. Morphometric studies of neuronal processes identified by immunostaining with NF antibodies and MBP markers were used to estimate the percentage of myelinated axons within the BMPS with an average of 4% at 2 weeks, 25% at 4 weeks and 42% at 8 weeks of differentiation ($p<0.001$) (FIG. **2**D). All analyzed BMPS showed similar extent of myelination at the same differentiation window. Percentages were calculated as the mean of at least 18 microscopy fields

from at least 3 individual BMPS in 2 different experiments. Ultrastructural analysis by electron microscopy demonstrated cell projections, which enwrapped cell processes resembling axons after 8 weeks of differentiation (FIG. **2**C).

[0216] GFAP-positive cells formed numerous cell processes organized in a network typical for human astrocyte glial processes in vivo, which established contacts with other glial cells and neurons (FIG. **2**Cg, **2**Ch, **2**F, and FIG. **8**B). Image quantification revealed 19% of astrocytes in the total population (FIG. **8**C). Altogether, the patterns of cell morphology, immunostaining and cell-cell interactions shown by neuronal and glial cell populations demonstrates that the BMPS recapitulates the cellular types and pattern of interactions seen in the human CNS and is, therefore, considered organotypic.

[0217] The morphology of cell nuclei observed by immunocytochemistry and electron microscopy showed some variation in nuclear morphology attributed to (i) cell proliferation as seen by positive staining for Ki67 and Nestin markers, and (ii) nuclear fragmentation likely associated with apoptosis as indicated by caspase 3 staining (FIG. **2**G, **2**H) was observed. These observations were also confirmed by electron microscopy studies at 4 and 8 weeks of differentiation (FIG. **2**H). The variation of nuclei morphology likely reflects the active stages of cell differentiation that BMPS exhibited during all stages of development. The presence of apoptotic nuclei likely resemble stages of cell death seen in normal neurodevelopment [34, 35]. Importantly, Caspase 3-positive nuclei did not concentrate in the center of the spheres and BMPS did not present necrosis in the center of the 3D structures (FIG. **2**G). Thus, Caspase3-positive nuclei do not appear linked to deprivation of oxygen or nutrients. Caspase has been quantified at eight weeks in BMPS (FIG. **8**C). Additionally, FIGS. **8**D and **8**E depict co-expression of mature oligodendroglia markers (MBP and O2) and expression of neuronal markers (VGLUT, TUJ1, SYN), respectively.

[0218] Further analysis of neuronal cell populations and morphology presented a pattern of evolution that suggests BMPS maturation as seen by Nestin-positive cells decreasing over time of differentiation while MBP expressing cells increased (FIG. **2**A). There was also evidence of cell-cell interactions as subsets of neurons showed several processes, which resemble dendritic and axonal projections that interact with other neurons as well as glial cells (FIG. **2**B, FIG. **2**H). Furthermore, cells immunostained with myelin binding protein (MBP) antibodies issued projections, which appear to enwrap neuronal processes, which resemble axons (FIG. **2**C, panels i-k, **2**C, panel m). The pattern of immunostaining with MBP and its association with neuronal processes suggests that oligodendrocytes within the BMPS exhibit myelinating properties such as in the human CNS in vivo. Ultrastructural analysis by electron microscopy demonstrated cell projections, which enwrapped cell processes resembling axons (FIG. **2**C, panel m).

Example 5: Microelectrode Array Recording of Spontaneous Electrical Activity of BMPS

[0219] To test the neurophysiological properties of the cells within the BMPS model, spontaneous electrical activity in BMPS was analyzed by micro-electrode array (MEA) (see FIG. **3** generally). BMPS were plated in 12-well or 48-well MEA plates at 8 weeks of differentiation. The aggregates were attached to the MEAs using Matrigel coating. Spontaneous electrical activity was measured starting one week after plating up to two weeks. The activity was measured for 20 minutes on 7 different days. Electrodes were considered active when the recorded activity was above 0.05 spikes/sec. FIG. **3**A shows a representative heatmap of a 48-well MEA plate measurement from one 20 minute recording. The heatmap represents the spike amplitude (V) with a minimum of 0 μV and maximum of 40 μV (FIG. **3**A). The spikes showed a common waveform between different electrodes and measurements (FIG. **3**B) and neurons were repeatedly firing. 25 electrodes, distributed over 19 wells, were included after the first step of data analysis. 20 to 40% of these 25 electrodes reached the threshold of 0.05 spikes/sec during each recording. FIG. **3**F shows the spike events of active electrodes from one representative 20 minutes recording. These data show potential for the use of MEA to measure electrical activity of the 3D BMPS. Further optimization of the protocol may increase the measurement of the neuronal activity on the electrodes.

Example 6: A Human 3D Model to Study Parkinson's Disease

[0220] Due to the presence of TH-positive dopaminergic neurons in the iPSC-derived BMPS (FIG. **2**B, panels k, l, and FIG. **8**), the possibility of using this model to study Parkinson's Disease (PD), a neurodegenerative disorder known to specifically affect dopaminergic neurons, was further explored. Two well-known neurotoxicants, which induce pathogenic processes resembling the mechanism associated with neurodegeneration in PD: the illicit drug MPTP's toxic metabolite MPP+ and the broadly used pesticide rotenone, were selected. Both MPP+ and rotenone interfere with oxidative phosphorylation in mitochondria by inhibiting complex I [36]. Initially, cytotoxicity experiments were performed to estimate sub-cytotoxic concentrations of these two compounds affecting only dopaminergic neurons (FIGS. **4**A and **4**C). Selective disruption of dopaminergic neurons but not of any other cell types in the systems described herein were observed with immunohistochemistry after exposure to 1 μM rotenone and 100 μM MPP+ for 24 h (FIGS. **4**E and **4**F). This effect was likely selective even at cytotoxic concentrations of 10 μM rotenone and 1000 μM MPP+as these concentrations did not show any alterations in other neurofilament 200-positive neurons. Lower concentrations of these compounds may induce effects in dopaminergic neurons, however, the effect was not as obvious by immunocytochemistry. Higher concentrations of rotenone and MPP+(up to 50 μM and 5000 μM, respectively) led to general cytotoxicity and affected also other neuronal types stained positive for neurofilament 200 (FIGS. **4**E and F). 5 μM of rotenone and 1000 μM of MPP+ were selected for further studies as these concentrations induced clear and selective dopaminergic effects. Reactive oxygen species (ROS) were measured in the cellular medium using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Cellbiolabs, San Diego, CA) as an indication of oxidative stress. Exposure to rotenone at 5 μM and MPP+ at 1000 μM showed an increase in ROS production after 24 hours exposure, while 12 hours showed no statistically significant changes. Real time RT-PCR was performed in order to determine effects of both chemicals on genes related to PD, mitochondrial dysfunction and oxidative stress. Tyrosine hydroxylase (TH, Dopaminergic neuronal marker) mRNA expression decreased by 84%±11 after exposure to 5 μM rotenone and 70%±9 after exposure to 1000 μM MPP+ for 24 hours. Additional genes related to PD also showed changes at sub-cytotoxic concentrations of MPP+ and rotenone. The expression of genes that encode T-box brain 1 (TBR1) and Alpha-synuclein (SNCA) protein decreased after 24 hours exposure. The reduction of TBR1 was 70±13% (rotenone) and 76±22% (MPP+) and the reduction of SNCA was 72±6% (rotenone) and 41±40% (MPP, however, BMPS exposed to 1 mM MPP+ led to no statistically significant changes in SNCA expression). Expression of genes related to mitochondrial function complex I (NDUFB1) or complex 0 (ATP5C1 or ATP50) tended to decrease in expression but these changes were not statistically significant. Caspase-1 gene expression, which has been related to SNCA, increased after 24 hours exposure to MPP+. These results demonstrate the potential of BMPS for studies elucidating molecular mechanisms of PD, lending itself to PD drug and neurotoxicity screening.

Example 7: Addition of Microglia

[0221] Peripheral blood mononuclear cells (PBMCs) are isolated from fresh or commercially available cryo-preserved whole blood of pooled healthy donors by Ficoll or Percoll gradient centrifugation. Monocyte populations are obtained by negative magnet-antibody selection after Ficoll or Percoll gradient and then re-suspend in RPMI 1640. Monocytes are cultured in macrophage serum-free medium, stimulated with a cocktail of cytokines, GM-CSF and IL-34. Monocytes may also be obtained by differentiation of iPSCs, hematopoetic or other stem cells. The microglia-like cells are combined with neuronal precursor cells in shaker cultures to preferably arrive at a final concentration of 5-8% microglia.

[0222] Primary monocytes or iPSC-derived monocytes may be incorporated into the system, both at early and later stages of BMPS differentiation. For the early stages, a number of 2×10.sup.6 NPCs mixed with 2×10.sup.4 monocytes are plated per 1 well (6 well-plate). Gyratory shaking is used at 88 rpms to generate spheres. After 2 days media are replaced with ½ CNS differentiation

medial (Neurobasal® electro Medium (Gibco) supplemented with 5% B-27® Electrophysiology (Gibco), 1% glutamax (Gibco), 10 μg human recombinant GDNF (Gemini), 10 μg human recombinant BDNF (Gemini)) and ½ macrophage differentiation media (Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% FCS, 0.055 mM β-mercaptoethanol, M-CSF (50 ng/ml), and IL-3 (25 ng/ml) (R&D Systems). The medium is replaced every 3 days.

[0223] Monocytes can also be incorporated after BMPS differentiation. For that, BMPS are differentiated up to 8 weeks. BMPS spheres are separated in 500 μl Eppendorf tubes. 2×10.sup.4 monocytes are added to the Eppendorf with the BMPS. Tubes are shaking manually every hour, up to 8 hours. After that, BMPS-monocytes are collected and plated in 6 well plates. Cells are kept on constant shaking until use.

[0224] The characterization of the immune-competent human organoids can be carried out by immunocytochemically assessing the presence of markers such as HLA-DR, and the ionized calcium-binding adapter molecule 1 (Iba1), specific microglial markers. Measures of cytokines and chemokines release and expression of receptors associated with microglia function (e.g., CCL2 and CX3CL) demonstrates successful engrafting of the microglia cells. This modified model is more suitable to investigate the neuroimmunological component associated with many substance exposures and diseases.

Example 8: Addition of a Blood Brain Barrier

[0225] The blood brain barrier (BBB) has a crucial role in neurotoxicity, being the last barrier for substances before reaching the brain. Moreover, the BBB is the bottleneck in brain drug development and is the single most important factor limiting the future growth of neurotherapeutics [81]. Most of the in vitro models do not incorporate BBB.

[0226] Human brain microvascular endothelial cells (hBMECs) from human iPSCs are incorporated into the BMPS by two techniques. In the first approach, mature BBB endothelial cells and neuronal precursors cells (NPCs) are combined in a single cells suspension in a ratio of 1:5, gyratory shaking or stirring are used to generated spheroids and aggregates are cultured up to 8 weeks. In the second technique, mature BMPS (8 weeks of differentiation) are covered by BBB endothelial cells using gravity systems (aggrewell, gravity well or hanging drops). Cells may be covered as well with other cell types, such as fluorescent LUHMES cells (FIG. **7**).

Example 9: Addition of Reporters

[0227] The BMPS gives the opportunity to develop cell-based assays allowing for high-content imaging (HCI) that can be adapted to high-throughput platforms, to evaluate the effects of toxicants on key cellular processes of neural development and physiology in the culture system.

[0228] Example of establishing fluorescent iPSC cell line: Creation of reporter cells lines greatly assists imaging efforts by allowing us to avoid complications associated with staining 3D cultures, to image subsets of cells, and to perform functional assays. Differentiated 3D aggregates from iPSC cultures spiked with 1-2% of iPSCs ubiquitously expressing fluorescent protein allow visualizing individual cells within the aggregates aiding quantification of phenotypic parameters, including neurite outgrowth and migration. Lines expressing markers allow measurement of synapse formation (PSD95, Synapsin 1), proliferation (Ki67), glial maturation (GFAP), and calcium signaling (GCaMP). Clustered Regularly Interspaced Short Palindromic Repeats/Cas (CRISPR) were used to create the various lines. Similar in function to the well-established zinc-finger (ZFNs) and TALEN nucleases, the Cas9-CRISPR system is a new entrant into the rapidly emerging field of genome engineering and has been quickly adopted and validated across a wide array of human stem cells. Gene-editing in hiPSCs has traditionally been a technically difficult task but with these advances it is now possible to generate reporter and mutant cell lines with genetically matched controls [83, 84, 85, 86]; essential tools not only for this project but also for the future success of using human iPSC-derived cells in quantitative live-cell phenotypic assays of toxicant testing.

[0229] Using the CRISPR-Cas9 system, fluorescent protein (FxP) reporter cell lines were generated by generating gRNAs targeting the gene of interested. In this system as described herein,

an RNA guided Cas9 endonuclease is used in conjunction with customizable small guide RNAs (gRNAs) to target and cleave any DNA template with a GN21GG sequence; the first G is for the U6 polymerase promoter while the N21GG is for the protospacer adjacent motif (PAM) sequence requirement of Cas9 [86, 87, 89].

[0230] For reporter cell generation, homology-directed repair (HDR) guides the insertion of the appropriate DNA donor fragment into a target site at regions of homology between the donor fragment and the genomic DNA target. An ES line that ubiquitously expresses GFP was created by introducing CAG promoter-driven GFP into the AAVS1 safe harbor locus, and can use these constructs to transfect iPSC cells. For other reporters, constructs may be created that will direct the integration of a self-cleaving P2A peptide sequence [90] targeted fluorescent protein cassette in frame at the stop codon of the gene of interest. The P2A sequence engineered between the C-terminus of the endogenous protein and the fluorescent protein may minimize possible fusion protein functional defects. Plasmids encoding the Cas9 nuclease, the targeting gRNA, and appropriate donor DNA will be introduced by electroporation, recombinant hiPSC clones will be manually selected and screened for the desired insertion by PCR, and the genotype may be verified by sequencing. Reporter hiPSCs will be subjected to a differentiation protocol and expression of the reporter validated by examining expression patterns and through immunohistochemistry experiments where it may be determined whether the FxP expressing cells co-label with known markers.

Example 10: Using Cells with Specific Genetic Backgrounds

[0231] The use of iPSCs, as described herein, has created new opportunities to study human diseases and gene/environment interaction [20, 21]. Fibroblasts or other somatic cells from healthy and diseased individuals can be reprogrammed into iPSCs, and subsequently be differentiated into all neural cell types. Similarly, iPSC can be genetically modified before creating the BMPS. As a proof-of-principle, iPSCs were obtained from patients with Down's syndrome (FIGS. **1**C**5** and **5**A-D), Rett Syndrome and from individuals with mutations in disrupted in schizophrenia 1 (DISC1). DISC1 may have some functional overlap with TSC-iPSCs as both are involved in the mTOR cell signaling pathway.

[0232] The Down's syndrome model is further characterized (see FIGS. **5**A-**5**D). Down's syndrome iPSCs have been successfully differentiated into neural precursor cells (NPCs). Currently the cells are differentiated in 3D and characterization by gene expression and immunohistochemistry is being performed. The Down's syndrome model has been exposed to compounds that induce oxidative stress (rotenone and paraquat). The response was compared to the model from healthy donors, which were more sensitive to these compounds than the healthy model.

Example 11: Combining the BMPS with Other Organoids

[0233] In some embodiments, BMPS may be combined with other organs and/or organ model systems. Several groups have been developing organ-on-a-chip platforms for different organs by using microfluidic techniques. Those platforms are designed to mimic in-vivo fluidic flows in the organs by separating cell culture chambers and perfusion channels, and successfully demonstrate recapitulation of iPSC-based organ functions. Together with other organ models on these platforms, the BMPS can be integrated, which allow us to untwine the complex toxicology from organ interactions. Such platforms allow (1) in-situ and high-throughput production of mini-brains on chip, (2) in-vivo like fluidic flow around mini-brains with enough supply of nutrient and small molecule through diffusion, (3) a large number of parallel test of toxic materials, and (4) a real-time monitoring of electrophysiological activities from BMPS with integrated electrodes. Companies such as TissUse GmbH have designed microfluidics platform that allow culture of floating spheres like the BMPS as described herein.

Example 12: Cryopreservation and Other Modes of Transportability

[0234] In order to e.g. incorporate the BMPS into platforms or enable any use in other laboratories, transportability of the system was optimized. Preliminary studies have shown possible recovery of

the neuronal 3D aggregates after cryopreservation (FIG. **6**). A human embryonal carcinoma stem cell line, (hNT2), and iPSC derived-aggregates were differentiated into mature neurons (8 weeks of differentiation for each cell line) and then cryopreserved with regular cryopreservation medium (95% FBS and 5% DMSO) or STEMdiff™ Neural Progenitor Freezing Medium (Stem cells technologies). After 2 days in liquid nitrogen, cells were thawed. Freezing media was removed and fresh media was added. One day later, viability was measured using the resazurin cell viability assay. hNT2 aggregates presented a 70% decrease in viability in both freezing medias while iPSC derived mini-brains showed a 20%-35% reduction in viability (FIG. **6**). However, viability recovery of the 3D aggregates is currently optimized using other viability and functional assays. Optimization of this protocol will vary additives (DMSO, HES, glycerol, serum etc.), the cooling temperature gradient as well as thawing protocol.

[0235] Human iPSC derived mini-brains are kept in culture at 37° C. In order to transport the live mini-brains, temperature must be controlled. Different methods can be used to control temperature during transport. Heating pads combined with an insulated box have been used to transport live biological material. Disposable chemical pads employ a one-time exothermic chemical reaction such as catalyzed rusting of iron, or dissolving calcium chloride. The most common reusable heat pads are based on a chemical reaction that transforms a liquid into a solid thus releasing energy. Some new heating pads (such as Deltaphase Isothermal Pad 3SET, from Braintree Scientific, Inc.) have been able to maintain 37° C. for more than 6 hours. 3D mini-brains cultured up to 8 weeks are sent in an insulated material box with heating pads. After transport, viability may be measured.

Example 13: Overview

[0236] The techniques herein provide a human BMPS model that is a versatile tool for more complex testing platforms, as well as for research into CNS physiology, mechanisms associated with (developmental) neurotoxicity, and pathogenesis of neurological disorders. Prior art stem cell-derived brain model systems developed in the past few years have shown the capability to recapitulate some of the in vivo biological processes (Juraver-Geslin and Durand, 2015; Nakano et al., 2012; Krug et al., 2014) and have an advantage over other classical in vitro models as they facilitate the study of various differentiation mechanisms, developmental processes and diseases (Lancaster et al., 2013). Unfortunately, these prior art systems require complicated protocols that reduce the reproducibility of the system and make it difficult to use in other fields such as chemical toxicity and drug screening. Additionally, these prior art models are also limited by large diameters, which lead to extensive cell death in the interior regions due to insufficient diffusion of oxygen and nutrients (Lancaster et al., 2013) and other artifacts.

[0237] The techniques herein overcome the limitations of the prior art by developing a human in vitro model by the gyratory shaking technique that enables reliably generation of a high number (about 500 per six-well plate) of viable BMPS that are homogeneous in size and shape. Control of size makes it possible to keep cell aggregates below 350 μM in diameter (FIG. **1**C) and thereby avoid disparate morphology and/or necrosis in the center of the spheres. Moreover, the BMPS showed reproducible cell composition by immunomorphological quantification, assessment of imaging-based endpoints and flow cytometry analysis.

[0238] As described herein, the 3D differentiation protocol for the BMPS covers stages from neuronal precursors to different cell types of the mature CNS. As discussed in detail above, at two weeks, BMPS consisted of an immature population of cells, showing minimal neuronal networks, a low percentage of mature astrocytes and oligodendrocytes, and minimal but early stages of myelin basic protein (MBP) expression. iPSC differentiation into mature BMPS was indicated by decreasing NES expression over time and a progressive expression of mature neuronal and glial markers such as MAP2, GFAP, O1 and MBP. Gene expression studies, flow cytometry, image analysis, immunostaining and miRNA studies have shown increase of cell maturation markers, which follow the BMPS differentiation. The presence of GABAergic neurons, dopaminergic neurons and glutamatergic neurons was documented by immunohistochemistry and real-time PCR

data. Moreover, the BMPS showed spontaneous electrical activity, indicating neuronal functionality of the system.

[0239] Since astrocytes and oligodendrocytes play important roles during neuronal development, plasticity and injury, the presence of glial cell populations in the presently disclosed BMPS model provides an excellent opportunity for the evaluation of neuronal-glial interactions and the role of glia in pathogenesis and toxicity processes. Astrocytes have an important role in protecting neurons, increasing neuronal viability and mitochondrial biogenesis from both exogenous (e.g. chemicals) and endogenous toxicity (Shinozaki et al., 2014; Aguirre-Rueda et al., 2015), especially against oxidative stress (Shao et al., 1997; Schwab and McGeer, 2008). Thus, their presence in a biological system to study disease and neurotoxicity is crucial. Immunohistochemistry and RT-PCR results showed increasing numbers of astrocytes (GFAP-positive cells) in the BMPS model reaching 19% astrocytes of the total cell population at eight weeks, which is earlier than in previously described cortical spheroids, where similar proportions of GFAP-positive cells were observed first at day 181, at day 86 the number of GFAP+ cells was below 10% (Pasca et al., 2015).

[0240] The most novel element of this BMPS is the presence of mature human oligodendrocytes with myelination properties, which has not been achieved in the prior art. Immunocytochemical and ultrastructural studies confirmed the morphological identity of these cells (FIG. **2**D) as multiple markers for mature oligodendrocytes were expressed by rounded cells with branching processes and membrane sheaths that are similar to the ones found in humans in vivo. The structure and morphology was further confirmed by electron microscopy. Quantitative assessment of the myelination process of MBP immunostaining along axons showed an increase over time of differentiation reaching 42% of myelinated axons at eight weeks (FIG. **2**D). 3D reconstruction of confocal z-stacks images (FIG. **2**A) and electron microscopy confirmed the wrapping of axonal structures after eight weeks of differentiation (FIG. **2**C). These findings are of particular relevance since myelin is a critical element for proper neuronal function and development, and the covering of axons by myelin allows faster action potential transmission, reduces axonal energy consumption and protects the axons from degeneration (Nave, 2010). Furthermore, recent evidence suggests that oligodendrocytes and myelin have a role in the metabolic support of axons independent of their role in action potential conduction, highlighting their importance in neuronal survival (Saab et al., 2013). This is the first time that a 3D human microphysiological system, consisting of different types of neurons and glial cells, has achieved such a high percentage of myelination. The ability to assess oligodendroglia function and mechanisms associated with myelination in this BMPS model provides an excellent tool for future studies of neurological disorders such as multiple sclerosis and other demyelinating disorders. As an illustration it was recently discovered that astroglia cells could promote oligodendrogenesis via secreted molecules (Jiang et al., 2016). A human BMPS that consist of neurons, astrocytes and oligodendrocytes is essential to evaluate this mechanism further and to develop a potential therapy for demyelinating disorders.

[0241] In conclusion, the techniques herein provide a BMPS that replicates crucial aspects of brain physiology and functionality. The potential for studying developmental and neurodegenerative disorders, brain infections, toxicity and trauma with such a system is growing. Furthermore, the potential to use iPSCs from different donors adds a personalized component to these studies. The high reproducibility and relatively simple protocol, enables future medium-throughput (96-well format) testing of chemicals, drugs and their potential to induce or treat diseases.

Methods and Materials

Chemicals

[0242] Rotenone and MPP+ were supplied from Sigma-Aldrich (St. Louis, MO). A 10 mM rotenone stock was prepared in DMSO Hybri-Max (Sigma) while MPP+ was diluted in water to a concentration of 30 mM.

iPSC Generation

[0243] CCD1079Sk (ATCC® CRL2097™), IPS IMR90 (WiCELL) and ATCCDYP0730 Human (IPS) Cells (ATCC® ACS1003™) fibroblasts were originally purchased from ATCC. All studies followed institutional IRB protocols approved by the Johns Hopkins University School of Medicine. Human fibroblasts and mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium (DMEM, Mediatech Inc.) supplemented with 10% fetal bovine serum (FBS, HyClone) and 2 mM L-glutamine (Invitrogen). MEFs were derived from E13.5 CF-1 mouse embryos. Human iPCS cells were generated with the EBV-based vectors as previously described [75]. iPSC from other sources were used as well. Colonies of iPSCs were manually picked after 3-6 weeks for further expansion and characterization. iPSCs (passage ≤20) were cultured on irradiated MEFs in human embryonic stem cell (hESC) medium comprising D-MEM/F12 (Invitrogen), 20% Knockout Serum Replacement (KSR, Invitrogen), 2 mM L-glutamine (Invitrogen), 100 μM MEM NEAA (Invitrogen), 100 μM β-mercaptoethanol (Invitrogen), and 10 ng/mL human basic FGF (bFGF, PeproTech). Media were changed daily and iPSC lines were passaged using collagenase (Invitrogen, 1 mg/ml in D-MEM/F12 for 1 hr at 37° C.). These iPSC lines have been previously fully characterized [75].

Neuronal Progenitor Cells (NPC) Production

[0244] NPC generated followed the previous published protocol [75]. Briefly, iPSCs colonies were detached from the feeder layer with collagenase (1 mg/ml) treatment for 1 hr and suspended in EB medium, comprising of FGF-2-free hESC medium supplemented with Dorsomorphin (2 μM) and A-83 (2 μM), in non-treated polystyrene plates for 4 days with a daily medium change. After 4 days, EB medium was replaced by neural induction medium (hNPC medium) comprising of DMEM/F12, N2 supplement, NEAA, heparin (2 μg/ml) for 15 more days. The floating neurospheres were then dissociated to single cells in Accutase and plated in 175 mm flasks and were allowed to expand for 7 days. NPCs were expanded in poly-1-ornithine and laminin-coated 175 mm flask on StemPro® NSC SFM (Life Technologies). Half of the media was changed every day. Cultures were maintained at 37° C. in an atmosphere of 5% CO.sub.2. After NPC generation, iPSCs colonies were detached and NPCs were expanded in poly-1-ornithine and laminin-coated 175 mm flask in StemPro® NSC SFM (Life Technologies). Half of the media was changed every day. Cultures were maintained at 37° C. in an atmosphere of 5% CO2.

BMPS Differentiation

[0245] At 100% confluence NPCs were detached mechanically and counted. 2×10.sup.6 cells per well were plated in 2 ml of medium in non-treated 6 well-plates. Cells were grown in NPC media for two days under constant gyratory shaking. Subsequently, medium was changed to differentiation medium (Neurobasal® electro Medium (Gibco) supplemented with 5% B-27® Electrophysiology (Gibco), 1% glutamax (Gibco), 0.02 μg/ml human recombinant GDNF (Gemini), 0.02 μg/ml human recombinant BDNF (Gemini)). Cultures were maintained at 37° C., 5% CO.sub.2 under constant gyratory shaking for up to 8 weeks. Differentiation medium was routinely changed every 2 days.

Size Measurement

[0246] Aggregates (n=20) from 3 independent experiments were randomly selected per time point for obtaining pictures and measuring size using SPOT software 5.0. Results were expressed as mean±SD. Cells were kept two days in NPC medium, indicated as NPC med. 2d in FIG. **1**B.

RNA and miRNA Extraction

[0247] Total RNA was extracted from aggregates every week up to 8 weeks of differentiation using Tripure (Roche, Switzerland) according to Chomczynski and Sacchi (1987) [76]. The same RNA extraction method was used to isolate RNA after toxicant treatment. RNA quantity and purity was determined using NanoDrop 2000c (Thermo Scientific). One microgram of RNA was reverse-transcribed using the M-MI V Promega Reverse Transcriptase (Promega) according to the manufacturer's recommendations. For miRNA reverse-transcription 60 ng of RNA were reverse transcribed using TaqMan microRNA Reverse transcription kit in combination with miRNA

specific stem-loop primers, which are a part of TaqMAn microRNA expression assay. Upto eight stem-loop primers were multiplexed in one reaction.

Quantitative RT-PCR

[0248] The expression of genes was evaluated using specific Taqman® gene expression assays (Life Technologies). miRNA expression was analyzed using TaqMAn microRNA expression assay in combination with TaqMan miRNA Reverse Transcription kit using protocol described in [77]. Table 1 shows a summary of the genes assayed. Real time RT-PCRs were performed using a 7500 Fast Real Time system machine (Applied Biosystems). Fold changes were calculated using the $2(-\Delta\Delta Ct)$ method [78]. β-actin and 18 s were used as a housekeeping genes for mRNA and RNU44 for miRNA. There were no statistically significant differences in expression for β-actin, 18 s, and RNU44. Data were presented as mean±SD, normalized to housekeeping genes and week 0.

Immunocytochemistry of the BMPS

[0249] BMPS aggregates were collected at 2, 4 and 8 weeks. BMPS were fixed in 4% paraformaldehyde for 1 hour, washed 3 times in PBS, then incubated for 1 hour in blocking solution consisting of 5% normal goat serum (NGS) in PBS with 0.4% TritonX (Sigma). BMPS were then incubated at 4° C. for 48 hours with a combination of primary antibodies (Table 2) diluted in PBS containing 3% NGS and 0.1% TritonX. BMPS were washed in PBS 3 times after which they were incubated with the appropriate fluorophore-tagged secondary antibody for 1 hour in PBS with 3% NGS at room temperature. Double immunostaining was visualized using the proper combination of secondary antibodies (e.g., goat anti-rabbit secondary antibody conjugated with Alexa 594 and goat ant-mouse secondary antibody conjugated with Alexa 488 (Molecular Probes). Nuclei were counterstained with DRAQ5 dye (Cell Signaling; 1:5000 in 1×PBS) or NucRed Live (Molecular Probes) for 15 minutes before mounted on slides with coverslips and Prolong Gold antifade reagent (Molecular Probes); BMPS used as negative controls for immunostaining were processed omitting the primary antibody. Images were taken using a Zeiss UV-LSM 510 confocal microscope. The experiments were performed in duplicates; at least three different fields of view were analyzed for each combination of antibodies. 3D reconstruction was done using Imaris 7.6.4 software for scientific imaging.

TABLE-US-00071 TABLE 2 Primary Antibodies. Antibody Host Type Source Dilution NF-H Rabbit Polyclonal Enzo 1:1000 GFAP Rabbit Polyclonal Dako 1:500 Olig 1 Mouse Monoclonal Millipore 1:500 CNPase Mouse Monoclonal Millipore 1:500 Calbindin Mouse Monoclonal SIGMA 1:500 NOGO-A Rabbit Polyclonal Santa Cruz 1:500 Map2 Mouse Monoclonal Chemicon 1:1000 MBP/SMI99 Mouse Monoclonal COVANCE 1:1000 SMI-32 Mouse Monoclonal Stenberger 1:2000 Monoclonals Synaptophysin Mouse Monoclonal SIGMA 1:500 VGLUT1 Rabbit Polyclonal Alpha Diagnostic 1:500 TH Mouse Monoclonal Millipore 1:250 Nestin Rabbit Polyclonal Millipore 1:200 Ki67 Rabbit Polyclonal abcam 1:100 Caspase3 Rabbit Polyclonal R&D 0.2 μg/ml OLIG1 Mouse Monoclonal Millipore 1:200 TUJ1 Mouse Monoclonal Stemcell 1:200 technologies S100B Rabbit Polyclonal Santa Cruz 1:200

Automated Quantitation of Cell Types

[0250] BMPS was differentiated for 8 weeks. Randomly selected pictures from three experiments were acquired by confocal imaging and then analyzed with a custom algorithm created with the Cellomics TargetActivation (Thermo Fisher Scientific, Pittsburgh, PA) image-analysis software package. With this algorithm, cells were identified based on DRAQ5 stained nucleus and quantified oligodendrocytes and astrocytes based on staining of CNPase, NOGO1 and GFAP.

Myelination Assessment and Quantification

[0251] To calculate the percentage of axonal myelination, a semi-automated computer platform was used, termed computer-assisted evaluation of myelin formation (CEM) [82], which uses NIH Image J built-in tools as well as a Math lab processing functions. The results were generated as pixel counts and percent values. The percent of myelinated axons was calculated by dividing the pixel count for myelin by the pixel count for axons after cell body removal and multiplying by 100.

For each time point at least 18 fields from at least two independent experiments were analyzed.

Electron Microscopy

[0252] BMPS aggregates were collected at 2, 4 and 8 weeks and were fixed in 2% glutaraldehyde and 4% formaldehyde in 0.1M Sodium Cacodylate buffer (EMS, electron microscopy sciences) pH 7.4 with 3% sucrose and 3 mM CaCl.sub.2. Post-fixation was done with 2% osmium for 2 hours. The BMPS aggregates were then stained en bloc with 2% uranyl acetate in distilled water for 30 min and subsequently dehydrated in graded ethanol. Embed 812 (EMS) was used as the embedding media. Thin sections (70-80 nm) were cut on a Reichert Jung Ultracut E microtome and placed on formvar coated 100 mesh copper grids. The grids were stained with uranyl acetate and followed by lead citrate. All imaging was performed on a Zeiss Libra 120 electron microscope with a Veleta (Olympus) camera.

Treatment and Cytotoxicity Assay

[0253] BMPS was exposed to different concentrations of rotenone and MPP+ for 24 and 48 hours after 4 weeks of differentiation. Rotenone working solutions were prepared in differentiation medium from 10 nM or 100 μM stocks to reach final concentrations of 0.1, 1, 10, 25 and 50 μM. DMSO was used as vehicle control. MPP+ working solutions were prepared in differentiation medium from 30 mM stocks to reach final concentrations of 10, 50, 100, 500, 1,000, 5,000 and 10,000 μM. Four independent experiments in 3 replicates were performed for each experimental condition (control and toxicant exposure for the different time points). Resazurin reduction assay was performed in order to determine cell viability after rotenone and MPP+ treatment. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye that is reduced into red fluorescent resorufin by redox reactions in viable cells. 100 μl Resazurin (2 mg/ml stock) were added directly to the 6 well plates (2 ml/well). Plates were incubated for 3 h at 37° C., 5% CO.sub.2. Subsequently, 50 μl of medium were transferred from each well in triplicates to a 96-well plate and fluorescence was measured at 530 nm/590 nm (excitation/emission) using a multi-well fluorometric reader CytoFluor series 4000 (PerSeptive Biosystems, Inc). Data were presented as mean±SD. Statistical analysis was performed using Dunnett's test.

Reactive Oxygen Species Measurement

[0254] Reactive oxygen species (ROS) were measured in cell media collected 24 hours after treatment with 5 μM rotenone or 1,000 μM MPP+ using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Cell Biolabs, San Diego, CA). This is a fluorescence-based assay measuring the presence of total free radicals within a sample and was used according to the manufacturer's protocol. The quenched fluorogenic dye dichlorodihydrofluorescin-DiOxyQ (DCFH-DiOxyQ) which is similar to the popular 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) is first primed with a quench removal reagent. The resulted highly reactive non-fluorescent DCFH can react with present ROS species in the cell supernatant and is then oxidized to the highly fluorescent DCF (2′,7′-dichlorodihydroxyfluorescein). At every time point, 50 μl of the cell supernatant was added to a 96-well plate in triplicates and was mixed and incubated with the DCFH-DiOxyQ for 45 minutes. The fluorescence intensity was measured with a fluorescence microplate reader at 480 nm/530 nm (excitation/emission) and was proportional to the total ROS/RNS levels within the sample.

Flow Cytometry

[0255] In order to quantify percentage of NPCs, and neurons within the aggregates, flow cytometry with NPC and neuronal markers was performed. Flow cytometry was performed according to previously published protocol [77] with some optimization steps for 3D cultures. Aggregates were washed once with PBS/1 mM EDTA and trypsinized directly in the well using TrypLE Express containing 4 units/ml DNAse for 30 min at 37° C. on the shaker. Pipetting the aggregates up and down with a 1 ml syringe and a 26G3/8 needle ensured generation of single cell suspension. Cells were counted, washed once with PBS/1 mM EDTA, fixed with 2% PFA for 20 min at 4° C., washed twice with PBS/1% BSA (wash solution I, WS I) and blocked for 30 min in blocking solution (PBS/1% BSA/0.15% saponin/10% NGS). 1×10.sup.6 cells were stained for one hour at 4°

C. with fluorochrome-conjugated antibodies dissolved in blocking solution (Table 3). Unstained cells as well as cells incubated with isotype controls were used as negative controls to set the gates for measurements. Cells were washed twice with PBS/1% BSA/0.15% saponin, once with PBS/1% BSA. Flow cytometry was performed using a Becton Dickinson FACSCalibur system by measuring 10.sup.4 gating events per measurement. Data was analyzed using FlowJo v10 software.
TABLE-US-00072 TABLE 3 Antibodies for flow cytometry analysis Antibodies Host type Source Dilution Alexa Fluor ® 647 Nestin Mouse Monoclonal, clone 25 BD Pharmingen 1:05 Alexa Fluor ® 488 β-III-Tubulin Mouse Monoclonal, clone TUJ1 BD Pharmingen 1:05 PerCP-Cy ™ 5.5 Sox2 Mouse Monoclonal, clone 030-678 BD Pharmingen 1:20 PerCP-Cy ™ 5.5 Sox1 Mouse Monoclonal, clone N23-844 BD Pharmingen 1:20 PE Doublecortin Mouse Monoclonal, clone 30 BD Pharmingen 1:20 Alexa Fluor ® 647 Ki67 Mouse Monoclonal, clone B56 BD Pharmingen 1:20
Microelectrode Array (MEA) Recordings
[0256] After 8 weeks of differentiation, BMPS were plated on 48-well MEA plates previously coated with Matrigel. During two weeks spontaneous electrical activity was recorded using the 'Maestro' MEA platform and Axion's Integraded Studio (AXIS) software [Axion Biosystems inc.; Atlanta, US]. Each well of the 48-well MEA plate contains 16 individual microelectrodes (˜40-50 μm diameter, center-to-center spacing 350 μm) with integrated ground electrodes, resulting in a total of 768 electrodes/plate. The 'Maestro' MEA platform has an integrated heating system, which can be controlled by AXIS software. All recordings were performed at a constant temperature of 37° C. Prior to a twenty minutes recording, the MEA plates were placed in the Maestro MEA platform and equilibrated for five min. AXIS software was used to control heating system and monitor the recordings, which includes simultaneously sampling of the channels at 12.5 kHz/channel with a gain of 1200× and a band pass filter of 200-5000 Hz. The recordings were converted into RAW files. After a recording the RAW-files were re-recorded with AXIS to convert the data into a spike file, which includes spike timing and profile information. A variable threshold spike detector was used for the spike-file, it was set at 6 times standard deviations of the rms-noise on each channel. The spike file was later used for data analysis with NeuroExplorer® [Nex Technologies, Madison (AL), US] to convert data into Microsoft Excel files. Using the function rate histogram, a summary of the spikes of all electrodes of one plate was put into one Excel sheet. Only electrodes that recorded activity higher than 0.05 spikes/sec at least once over the time measured were included for data analysis.
Statistical Analysis
[0257] Statistical analysis was performed using GraphPad InStat 3. The Dunnett's test was applied to all the experiments shown here that compare to a control group. Statistically significant values ($p<0.01$) are marked with an asterisk (*). For myelination quantification at the different time points, a Kruskal-Wallis test was employed, statistical significance was considered for p values <0.05.
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EQUIVALENTS

[0348] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

1-**47**. (canceled)

48. An in vitro brain microphysiological system (BMPS), comprising: at least two neural cell types aggregated into a spheroid mass and endothelial cells capable of forming a blood brain barrier, wherein the spheroid mass has a diameter that is less than about 500 μm and the in vitro BMPS is electrophysiologically active in a spontaneous manner.

49. The BMPS of claim 48, further comprising one or more microglia-like cells.

50. The BMPS of claim 49, wherein the micro-glia like cells comprise microglia, microglia precursor cells, or a combination thereof.

51. The BMPS of claim 48, wherein the in vitro BMPS has neural characteristics selected from the group consisting of synaptogenesis, neuron-neuron interactions, neuronal-glial interactions, axon myelination, and combinations thereof.

52. The BMPS of claim 48, wherein at least one neural cell type comprises a mature neuron, a glial cell, or a combination thereof.
53. The BMPS of claim 48, wherein at least one neural cell type comprises astrocytes, polydendrocytes, oligodendrocytes, or combinations thereof.
54. The BMPS of claim **481**, wherein the BMPS mimics the microenvironment of the central nervous system (CNS).
55. A synthetic neurological organ comprising a mature neuron, at least one glial cell aggregated into a spheroid mass, and a population of microglia-like cells, wherein the spheroid mass has a diameter that is less than 500 μm and the synthetic neurological organ is electrophysiologically active in a spontaneous manner.
56. The synthetic neurological organ of claim 55, further comprising one or more endothelial cells capable of forming a blood-brain-barrier.
57. The synthetic neurological organ of claim 55, wherein the micro-glia like cells comprise microglia, microglia precursor cells, or a combination thereof.
58. The synthetic neurological organ of claim 55, wherein the mature neuron and glial cells further comprise cells selected from the group consisting of astrocytes, polydendrocytes, oligodendrocytes, and combinations thereof.
59. The synthetic neurological organ of claim 55, wherein synthetic neurological organ further comprises neural characteristics selected from the group consisting of synaptogenesis, neuron-neuron interactions, neuronal-glial interactions, axon myelination, and combinations thereof.
60. The synthetic neurological organ of claim 55, wherein the synthetic neurological organ mimics the microenvironment of the central nervous system (CNS).
61. A method of reproducibly producing an in vitro brain microphysiological system (BMPS) that is electrophysiologically active in a spontaneous manner, comprising: exposing one or more NPC types to gyratory shaking or stirring; and differentiating the one or more NPC types into one or more neural cell types aggregated into a spheroid mass.
62. The method of claim 61, wherein the spheroid mass has a diameter that is less than about 450 μm, less than about 400 μm, less than about 350 μm, or less than about 300 μm.
63. The method of claim 61, wherein gyratory shaking comprises constant or regular gyratory shaking or stirring for 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, or 8 or more weeks.
64. The method of claim 61, further comprising adding one or more microglia-like cells.
65. The method of claim 64, wherein the micro-glia like cells comprise microglia, microglia precursor cells, or a combination thereof.
66. The method of claim 61, wherein at least one neural cell type comprises a mature neuron, at least one neuronal cell type comprises a glial cell, or a combination thereof.
67. The method of claim 61, further comprising adding one or one or more endothelial cells capable of forming a blood-brain-barrier.