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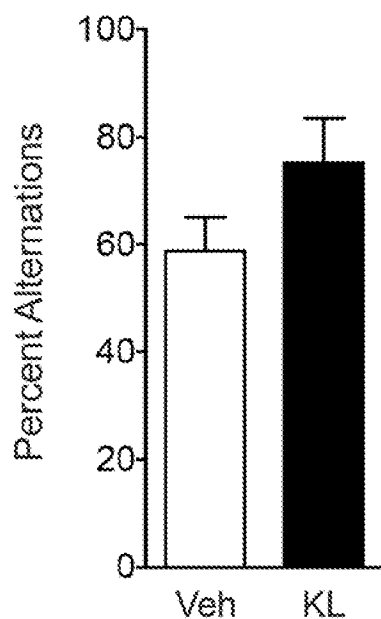
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(54) Title: METHODS AND COMPOSITIONS FOR IMPROVED COGNITION

(57) Abstract: Provided herein are klothe polypeptide compositions and methods for improving cognitive function in an individual comprising treatment of with klothe polypeptides.

FIG. 1A



— with sequence listing part of description (Rule 5.2(a))

METHODS AND COMPOSITIONS FOR IMPROVED COGNITION

5 CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] The present application claims benefit of priority to US Provisional Patent Application No. 62/113,300, filed February 6, 2015, which is incorporated by reference.

BACKGROUND OF THE INVENTION

10 [0002] Brain health is one of the biggest biomedical challenges with few if any effective medical treatments. Cognition is a highly valued and central manifestation of brain health that is impaired or becomes disrupted in normal aging, numerous neurodegenerative, neurologic, and psychiatric diseases, childhood developmental syndromes, traumatic brain injury, and stress. Cognition is also disrupted by jet lag, medication side effects, and certain medical treatments, such as those for cancer. Thus, the potential to enhance cognition or
15 counter cognitive dysfunction is of enormous relevance across the human lifespan in health and disease.

BRIEF SUMMARY OF THE INVENTION

20 [0003] Provided herein are methods and compositions for improving cognition through systemic administration of klotho or a protein comprising klotho or a functional fragment thereof. In some embodiments, the method comprises improving cognition in an individual comprising administering to the individual an effective amount of a protein comprising a Klotho polypeptide or a functional variant or fragment thereof, thereby improving cognitive function in the individual. In some embodiments, the administering is systemic, peripheral,
25 or nasal. In some embodiments, the protein is the Klotho polypeptide or a fragment thereof. In some embodiments, the administering is oral, mucosal, or carried out by injection. In some embodiments, the injection is intravenous, intraperitoneal, subcutaneous, or intramuscular. In some embodiments, the administration is by infusion, *e.g.*, continuous infusion using a reservoir or osmotic minipump.

30 [0004] In some embodiments, the individual is a human. In some embodiments, the human has at least normal cognitive function and the administering results in improved cognitive function compared to before the administering. In some embodiments, the human is 50 years

of age or older (*e.g.*, at least 60, 65, 70, 75, 80, 85, 90, 100, or older). In some embodiments, the individual is experiencing age-related cognitive decline. In some embodiments, the administering results in a reduced rate of cognitive decline, *e.g.*, so that short term memory does not decline as quickly as expected based on pre-treatment or age-cohort decline. In some embodiments, the individual is less than 50 years of age.

[0005] In some embodiments, the individual has a neurodegenerative disease, *e.g.*, Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasalar degeneration, mild cognitive impairment, vascular dementia, Lewy body dementia, multiple system atrophy, amyotrophic lateral sclerosis, prion disorder, and HIV-related dementia. In some embodiments, the individual has a mental or mood disorder, *e.g.*, depression, schizophrenia, attention deficit/ hyperactivity disorder, autism spectrum disorder, intellectual disability, a mood disorder, or a psychotic disorder. In some embodiments, the individual has a condition selected from traumatic brain injury, stroke, multiple sclerosis, neuroautoimmune disease, epilepsy, delirium, and a paraneoplastic disorder. In some embodiments, the individual has a condition selected from X-linked mental disorder, Down's syndrome, Angelman's syndrome, and Rett's syndrome. In some embodiments, the individual has a condition selected from phenylketonuria, Lesch-Nyhan, galactosemia, and adrenoleukodystrophy. In some embodiments, the individual is receiving radiation treatment or chemotherapy for cancer. In some embodiments, the individual is experiencing or is expected to experience (*e.g.*, in about 2 hours to 2 weeks, about 12-48 hours, or about 24 hours) stress, pain, sleep deprivation, or jet lag.

[0006] In some embodiments, the human has impaired motor function the administering results in improved motor function compared to before the administering.

[0007] In some embodiments, the klotho polypeptide has at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to soluble human klotho (amino acids 34-979 of SEQ ID NO:1). In some embodiments, the klotho polypeptide is a functional fragment comprising a polypeptide with at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to the KL1 domain of human klotho. In some embodiments, the klotho polypeptide is a functional fragment comprising a polypeptide with at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to the KL2 domain of human klotho. In some embodiments, the klotho

polypeptide retains at least 40, 50, 60, 70, 80, 90, or 100% of the level of at least one activity of soluble human klotho.

[0008] In some embodiments, the klotho polypeptide is administered to the individual at a dose of 0.1-50,000 µg/ kg body weight (*e.g.*, 0.5-10 µg/kg, 0.5-500 µg/kg, 1-2000 µg/kg, 1-250 µg/kg, 5-250 µg/kg, 10-100 µg/kg, 1000-20,000 µg/kg, or about 10, 25, 50, 100, or 1000 µg/kg). In some embodiments, the klotho polypeptide is administered daily, twice per week, weekly, or every two weeks. In some embodiments, the klotho polypeptide is administered prior to (*e.g.*, 2, 6, 12, 24, or 48 hours) or in response to an event requiring heightened cognition, *e.g.*, stress, jet lag, sleep deprivation, or anticipated taxing mental task.

[0009] In some embodiments, the individual is tested for cognitive ability prior to the administering. In some embodiments, the individual is tested for cognitive ability after the administering. In some embodiments, the individual is tested for cognitive ability before and after the administering, or multiple times during the course of treatment. In some embodiments, the individual is tested for semantic, episodic, procedural, priming, and/or working memory. In some embodiments, the individual is tested for language ability, executive function, visuospatial function, or dementia. In some embodiments, the dose or frequency of administration of klotho polypeptide is increased when cognitive ability does not significantly increase.

[0010] In some embodiments, a method of improving motor function is provided. In some embodiments, a method is provided for improving motor function in an individual in need thereof, the method comprising administering to the individual an effective amount of a protein comprising a Klotho polypeptide or a functional fragment thereof, wherein the administering is systemic or peripheral, thereby improving motor function in the individual compared to before the administering. In some embodiments, the klotho polypeptide has at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to soluble human klotho (amino acids 34-979 of SEQ ID NO:1). In some embodiments, the klotho polypeptide is a functional fragment comprising a polypeptide with at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to the KL1 domain of human klotho. In some embodiments, the klotho polypeptide is a functional fragment comprising a polypeptide with at least 75% identity (*e.g.*, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity) to the KL2 domain of human klotho. In some embodiments, the klotho polypeptide retains at least 40, 50, 60, 70, 80, 90,

or 100% of the level of at least one activity of soluble human klotho. In some embodiments, the klotho polypeptide is administered to the individual at a dose of 0.1-50,000 $\mu\text{g}/\text{kg}$ body weight (*e.g.*, 0.5-10 $\mu\text{g}/\text{kg}$, 0.5-500 $\mu\text{g}/\text{kg}$, 1-2000 $\mu\text{g}/\text{kg}$, 1-250 $\mu\text{g}/\text{kg}$, 5-250 $\mu\text{g}/\text{kg}$, 10-100 $\mu\text{g}/\text{kg}$, 1000-20,000 $\mu\text{g}/\text{kg}$, or about 10, 25, 50, 100, or 1000 $\mu\text{g}/\text{kg}$). In some embodiments, the klotho polypeptide is administered daily, twice per week, weekly, or every two weeks.

[0011] In some embodiments, the individual with impaired motor function has stroke to the brain or spinal cord (ischemic or hemorrhagic), neurodegenerative disease (Parkinson's disease, Lewy body dementia, multiple system atrophy, amyotrophic lateral sclerosis, prion disorder, Huntington's disease, supranuclear palsy), Parkinsonism, traumatic brain injury, neuroinfectious brain lesions, multiple sclerosis and related autoimmune and demyelinating disease, spinal cord lesions (compressive, infectious, toxic or metabolic, autoimmune, oncologic), brain tumor, epilepsy, paraneoplastic disorder, neurodevelopmental disorder (mitochondrial, autosomal genetic), muscle disease (polymyositis, dermatomyositis, inclusion body myositis, infectious, endocrine, metabolic, toxic, congenital myopathy, congenital muscular dystrophy, hereditary), neuropathies (Guillain-Barre syndrome, axonal and demyelinating, diabetic, toxic, metabolic, infectious, critical illness, entrapment), tick paralysis, myasthenia gravis, and spinal muscular atrophy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1A-J. Klotho delivery enhances cognition in mice. Mice were tested in cognitive tasks following i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (10 $\mu\text{g}/\text{kg}$) (age 4 months). Data are mean $\pm$ SEM.

[0013] FIG. 1A. Mice were tested in the Y maze 18 hrs after treatment with vehicle or klotho. Percent alternations among arms during 4 minutes of exploration of a Y maze are shown (n=4 male mice/group).

[0014] FIG. 1B-J. Mice were tested in the Morris water maze after daily treatment with vehicle or klotho (two independent cohorts shown: Cohort 1 and Cohort 2).

[0015] FIG. 1B. Spatial learning curves when the platform is hidden are shown in Cohort 1. Veh or KL was administered 4h prior to testing. Data represent the daily average of total distance traveled on Days 1-4 to reach the hidden platform. Day 0 represents distance traveled on the first trial of Day 1. On Day 5, when the platform was visible, Veh- and klotho-treated mice located it equally well. (n=4 male mice/ group)

[0016] FIG. 1C. Veh- and klotho-treated mice swam at equal speeds in Cohort 1, as measured by the average velocity from hidden training on Day 4.

[0017] FIG. 1D. Spatial learning curves when the platform is hidden are shown in an independent cohort of mice, Cohort 2. Veh or KL was administered 18h prior to testing. Data represent the daily average of distance traveled on Days 1–4 to reach the hidden platform. (n=7–10 mice/group; males and females included)

[0018] FIG. 1E-J. Results of probe trials to assess spatial memory when the platform was removed 1hr and 24hrs after completion of hidden-platform training in Veh- and klotho-treated mice in Cohort 1.

[0019] FIG. 1E. Duration at the target center in a 1hr probe shows time spent at the original platform location.

[0020] FIG. 1F. Target platform crossings in a 1hr probe shows the frequency of crossings over the original platform location.

[0021] FIG. 1G. Percent time spent in the target quadrant, compared to the average time spent in other quadrants, in a 1hr probe. *p<0.05 (t-test)

[0022] FIG. 1H. Duration at the target center in a 24hr probe.

[0023] FIG. 1I. Target platform crossings in a 24hr probe.

[0024] FIG. 1J. Percent time spent in the target quadrant in a 24hr probe.

[0025] FIG. 2A-B. Acute delivery of klotho enhances cognition in aged mice. Mice were tested for spatial and working memory in the large Y-Maze following i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (10 µg/kg) 24 hours prior to training (n=8–9 mice per experimental group, sex-balanced groups, age 18 months, from NIH colony of aging mice). 18 hours after training, mice underwent testing and duration of time spent in the novel arm and the familiar arm was measured during exploration of the maze. (FIG. 2A) Novel arm preference is shown as a ratio time spent in the novel compared to the familiar arm throughout indicated times of exploration and (FIG. 2B) at 3 minutes. *p<0.05 (t-test). Data are mean ± SEM.

[0026] FIG. 3. Acute delivery of klotho induces long-lasting cognitive enhancement. Mice were tested for spatial and working memory in the large Y-Maze at 16 days following the last i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (0.5 or 2.5 µg/kg) that was

given daily for 5 days (n=8–10 mice per experimental group, sex-balanced groups, age 5–5.5 months of age). Duration of time spent in the novel arm and the familiar arm was measured during exploration of the maze 18 hours after training. Novel arm preference is shown as a ratio of time spent in the novel compared to the familiar arm at 5 min of exploration.

5 **p=0.01 (Bonferroni-Holm test). Data are mean ± SEM.

[0027] FIG. 4A-B. Acute delivery of klotho improves cognitive deficits in transgenic hSYN mice that express the human α -synuclein protein. Mice were tested for spatial and working memory in the large Y-Maze following i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (2.5 μ g/kg) at 22 hours prior to training and then 14 hours prior to testing
10 (n=6–9 male mice per group, age 2.5–6 months of age). Duration of time and number of entries in the novel and familiar arms were measured during exploration of the maze 18 hours after training. Data are mean ± SEM.

[0028] FIG. 4A. Novel arm preference is shown as ratio of time spent in the novel compared to the familiar arm at 5 min of exploration. Two-way ANOVA: hSYN effect
15 p=0.026, KL effect p=0.07; *p<0.05, #p=0.07 (Bonferroni-Holm test)

[0029] FIG. 4B. Novel arm preference is shown as ratio of entries into the novel compared to the familiar arm at 5 min of exploration. Two-way ANOVA: hSYN effect p=0.07, KL effect p=0.03; #p=0.07 (t-test).

[0030] FIG. 5. Acute delivery of klotho improves early motor deficits in transgenic hSYN
20 mice that express the human α -synuclein protein. Mice were tested for motor function on the rotarod task following i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (2.5 μ g/kg) at approximately 17 hours prior to testing (n=6–10 male mice per experimental group, age 2.5–6 months of age). Time spent on the spinning rod without falling is depicted in seconds (s). In hSYN mice, KL-treatment improves early motor function as shown by
25 increased duration of the average time spent on the spinning rod during Trials 1 through 3. Two-way ANOVA: hSYN effect 0.0025; **p<0.01, *p<0.05 (Bonferroni-Holm test). Data are mean ± SEM.

[0031] FIG. 6. Acute delivery of klotho does not alter the overall activity of mice. Mice were tested for level of overall movements and total activity in the open field task at 16 hours
30 following i.p. delivery of vehicle (Veh) or recombinant mouse klotho (KL) (2.5 μ g/kg) (n=8

mice per experimental group; sex-balanced groups; age 5 months). Total movements over 5 minutes are depicted. Data are mean $\pm$ SEM.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

5 [0032] Klotho is a relatively large protein, approximately 130 kD in its secreted form, and is not expected to cross the blood-brain barrier. The results provided herein, however, show that Klotho exerts a positive effect on cognition within hours of systemic administration and long after its half-life elimination. These results are highly unexpected, and provide the advantage of safer, more convenient therapies compared to administration directly to the
10 brain or into the cerebrospinal fluid.

II. Definitions

[0033] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, *e.g.*, Lackie, DICTIONARY OF CELL AND MOLECULAR BIOLOGY, Elsevier (4th ed. 2007); Sambrook *et al.*,
15 MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

20 [0034] The terms “klotho” or “klotho polypeptide” refer to soluble klotho polypeptide, and functional variants and fragments thereof, unless otherwise stated. Soluble klotho is any form of klotho that circulates in fluid (*e.g.*, serum, cerebrospinal fluid, etc.), and that does not include a transmembrane or intracellular component. Klotho can be cleaved from its transmembrane form and released into fluid, or otherwise secreted or shed from a cell.
25 Klotho RNA can also be alternatively spliced and directly secreted into the surrounding fluid (*i.e.*, without forming a transmembrane protein). Both forms are encompassed in the terms soluble klotho polypeptide, klotho polypeptide, and klotho.

[0035] As used herein, the terms “systemic” or “peripheral” refer to administration by a route that does not involve direct injection (or other administration) into the cerebrospinal
30 fluid (CSF) or central nervous system (CNS). That is, systemic and peripheral administration encompasses administration to the “blood” side of the blood-brain barrier. Examples of

systemic and peripheral routes include oral and mucosal, intravenous, intraperitoneal, intramuscular, and subcutaneous injection, and intravenous drip.

[0036] The terms “cognition,” “cognitive ability,” “cognitive function,” and like terms refer to a collection of mental tasks and functions, including but not limited to: memory (e.g., semantic, episodic, procedural, priming, or working); orientation; language; problem solving; visual perception, construction, and integration; planning; organizational skills; selective attention; inhibitory control; and ability to mentally manipulate information.

[0037] The terms “improved cognition,” “increased cognitive ability,” “improved cognitive function,” and like terms refer to an improvement in cognition under a given condition (e.g., treatment with klotho) compared to cognition absent the condition (e.g., absent treatment with klotho). For an individual experiencing cognitive decline, an improvement in cognition might be a reduction in the rate of cognitive decline (*i.e.*, an improvement compared to the absence of treatment), but not an actual improvement in cognitive ability. An increase in cognitive ability can also be an increase in brain activity in a specified area, e.g., as determined by MRI, or an inhibition of brain activity that results in better overall brain function. An increase in cognitive ability can also be improvement in a cognitive performance test as described in more detail herein. An improvement or increase in cognitive ability can be in any one cognitive aspect or function, or any combination of individual cognitive functions.

[0038] An individual in need of improved cognitive function refers to individuals with age-related cognitive decline; a neurodegenerative disease; a mental or mood disorder; traumatic brain injury; developmental delay; genetic disorder resulting in reduced cognitive ability; brain injury due to stroke, brain cancer, MS, epilepsy, radiation or chemotherapy; etc. An individual in need of improved cognitive function can also include individuals that desire increased mental function to fight the effects of stress, sleep deprivation, jet lag, or pain, or to heighten ability for a particular task. A more complete and specific list of such individuals is included in the “Cognitive conditions and disorders” section herein.

[0039] The words “protein”, “peptide”, and “polypeptide” are used interchangeably to denote an amino acid polymer or a set of two or more interacting or bound amino acid polymers. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as

to naturally occurring amino acid polymers, those containing modified residues, and non-naturally occurring amino acid polymer.

[0040] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *e.g.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs may have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

[0041] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0042] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated, *e.g.*, naturally contiguous, sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. One of skill will recognize that in certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can

be modified to yield a functionally identical molecule. Accordingly, often silent variations of a nucleic acid which encodes a polypeptide is implicit in a described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0043] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. The following amino acids are typically conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g., Creighton, Proteins* (1984)).

[0044] The terms "identical" or "percent identity," in the context of two or more nucleic acids, or two or more polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides, or amino acids, that are the same (*i.e.,* about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters, or by manual alignment and visual inspection. *See e.g.,* the NCBI web site at ncbi.nlm.nih.gov/BLAST. Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a nucleotide test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the algorithms can account for gaps and the like. Typically, identity exists over a region comprising an antibody epitope, or a sequence that is at least about 25 amino acids or nucleotides in length, or over a region that is 50-100 amino acids or nucleotides in length, or over the entire length of the reference sequence.

[0045] The term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example,
5 recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0046] The term “heterologous” when used with reference to portions of a protein or nucleic acid indicates that the protein or nucleic acid comprises two or more subsequences
10 that are not found in the same relationship to each other in nature. For instance, the protein or nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source, or functional chimeric protein.

[0047] The terms “agonist,” “activator,” “inducer” and like terms refer to an agent that
15 increases activity or expression (*e.g.*, of klotho or a klotho signaling pathway) as compared to a control. Agonists are agents that, *e.g.*, stimulate, increase, activate, enhance activation, sensitize or upregulate the activity of klotho or a klotho signaling pathway. The expression or activity can be increased 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% 100% or more than that in a control. In certain instances, the activation is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-
20 fold, 10-fold, or more in comparison to a control.

[0048] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a substance that results in a detectably lower expression or activity level as compared to a control. The inhibited expression or activity can be 10%, 20%, 30%,
40%, 50%, 60%, 70%, 80%, 90% or less than that in a control. In certain instances, the
25 inhibition is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more in comparison to a control.

[0049] A “control” sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a test condition, *e.g.*, in the presence of a test compound, and compared to samples from
30 known conditions, *e.g.*, in the absence of the test compound (negative control), or in the presence of a known compound (positive control). A control can also represent an average value gathered from a number of tests or results. One of skill in the art will recognize that

controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.*, half-life) or therapeutic measures (*e.g.*, comparison of benefit and/or side effects). Controls can be designed for *in vitro* applications. One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0050] A “label” or a “detectable moiety” is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, *e.g.*, by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide. Any method known for conjugating a protein to the label may be employed, *e.g.*, using methods described in Hermanson, Bioconjugate Techniques 1996, Academic Press, Inc., San Diego.

[0051] A “labeled” molecule (*e.g.*, klotho polypeptide) is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the molecule may be detected by detecting the presence of the label bound to the molecule.

[0052] The term “diagnosis” refers to a relative probability that a disorder is present in an individual. Similarly, the term “prognosis” refers to a relative probability that a certain future outcome may occur in the individual. For example, in the context of the present disclosure, prognosis can refer to the likelihood that an individual suffer cognitive decline, or the likely severity of the disease (*e.g.*, severity of symptoms, rate of functional decline, etc.). The terms are not intended to be absolute, as will be appreciated by any one of skill in the field of medical diagnostics.

[0053] A “biological sample” can be obtained from a patient, *e.g.*, a biopsy, from an animal, such as an animal model, or from cultured cells, *e.g.*, a cell line or cells removed from a patient and grown in culture for observation. Biological samples include tissues and bodily fluids, *e.g.*, cerebrospinal fluid (CSF), blood, blood fractions, lymph, saliva, urine, feces, etc.

[0054] The terms “therapy,” “treatment,” and “amelioration” refer to any reduction in the severity of symptoms (cognitive decline), or improvement in cognitive function, or where motor function is affected, an improvement in motor function. As used herein, the terms “treat” and “prevent” are not intended to be absolute terms. Treatment and prevention can refer to any delay in cognitive decline, amelioration of symptoms (*e.g.*, confusion, delirium), etc. Treatment and prevention can be complete or partial, such that cognition is better than would be expected without treatment (*e.g.*, compared to cognition in the same individual before treatment or compared to cognition in similar non-treated individuals). The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment, or to the same patient prior to treatment or at a different time during treatment. In some aspects, cognition is improved by at least 1%, as compared, *e.g.*, to the individual before administration or to a control individual not undergoing treatment. In some embodiments, cognition is improved by at least 2, 3, 5, 7, 10, 15, 20, 25%, 50%, 75%, 80%, or 90%, or more, determined using tests of cognition, molecular proxies, or structural changes associated with brain function. In some aspects, motor function is improved by at least 1%, as compared, *e.g.*, to the individual before administration or to a control individual not undergoing treatment. In some embodiments, motor function is improved by at least 2, 3, 5, 7, 10, 15, 20, 25%, 50%, 75%, 80%, or 90%, or more, determined using tests of motor function.

[0055] The terms “effective amount,” “effective dose,” “therapeutically effective amount,” etc. refer to that amount of the therapeutic agent sufficient to ameliorate a disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of therapeutic effect at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as “-fold” increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0056] As used herein, the term “pharmaceutically acceptable” is used synonymously with physiologically acceptable and pharmacologically acceptable. A pharmaceutical composition will generally comprise agents for buffering and preservation in storage, and can include buffers and carriers for appropriate delivery, depending on the route of administration.

[0057] The terms “dose” and “dosage” are used interchangeably herein. A dose refers to the amount of active ingredient given to an individual at each administration. For the present

invention, the dose refers to the amount of Klotho polypeptide. The dose will vary depending on a number of factors, including frequency of administration; size and tolerance of the individual; type and severity of the condition; risk of side effects; and the route of administration. One of skill in the art will recognize that the dose can be modified depending on the above factors or based on therapeutic progress. The term “dosage form” refers to the particular format of the pharmaceutical, and depends on the route of administration. For example, a dosage form can be in a liquid, *e.g.*, a saline solution for injection.

[0058] “Subject,” “patient,” “individual” and like terms are used interchangeably and refer to, except where indicated, mammals such as humans and non-human primates, as well as dogs, horses, pigs, mice, rats, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision. A patient can be an individual that is seeking treatment, monitoring, adjustment or modification of an existing therapeutic regimen, etc.

III. Klotho

[0059] Klotho is a pleiotropic protein and an aging regulator that circulates throughout the body and brain (Imura *et al.* (2004) *FEBS Letters* 565:143; Kurosu *et al.* (2005) *Science* 309:1829). Human Klotho is described in GenBank Accession No. NC_000013 and Uniprot Accession No. Q9UEF7. A number of species homologs exist, including mouse and rat Klotho which share 86% and 85% identity with the human Klotho polypeptide, which is shown as SEQ ID NO:1. It exists in a transmembrane form that can be cleaved such that the extracellular portion (amino acids 34-979) is released as a hormone (Shiraki-ika *et al.* (1998) *FEBS Letters* 424:6). Klotho also has a splice variant that results in a 549 amino acid secreted form of the protein that is also functional (Wang and Sun (2009) *Ageing Res. Rev.* 8:43). Both cleaved and secreted klotho are soluble and functional in the body, but have a sequence variation at the C-terminal end due to the splice variation. Amino acids 535-549 are DTTLSQFTDLNVYLW (SEQ ID NO:2) for cleaved, soluble human Klotho and SQLTKPISSLTKPYH (SEQ ID NO:3) for spliced, soluble human Klotho. Full length soluble Klotho includes two conserved domains (KL1 and KL2) with homology to beta glycosidase proteins. The conserved beta-glucosidase/ 6-phospho-beta-glucosidase/ beta-galactosidase motif spans 62-497 in the human protein and 64-499 in the mouse. The conserved KL1 sequence is described in Chateau *et al.* (2010) *Aging* 2:567 and Matsumura *et al.* (1998) *Biochem Biophys Res Commun*, and comprises amino acids 34-549 of the human Klotho protein, with the glycosyl hydrolase consensus region spanning amino acids 57-506 of

the human Klotho protein (59-508 of the mouse). Klotho does not have beta-glycosidase activity, but shows some beta-glucuronidase activity.

[0060] Klotho suppresses insulin and wnt signaling, regulates ion channels and their transport, and promotes function of FGF23. *See, e.g.,* Chang *et al.* (2005) *Science* 310:490; Imura *et al.* (2007) *Science* 316:1615; Kurosu (2005); Liu *et al.* (2007) *Science* 317:803; and Urakawa *et al.* (2006) *Nature* 444:770).

[0061] In mice, transgenic overexpression of klotho extends lifespan and associates with better cognitive functions in the normal and diseased brain (Kurosu (2005); Dubal *et al.* (2014) *Cell Reports* 7:1065; Dubal *et al.* (2015) *J. Neuroscience*). In humans, a single allele of the KL-VS variant of the *KLOTHO* gene, which increases secreted klotho promotes longevity (Arking *et al.*, 2002; Arking *et al.*, 2005; Invidia *et al.*, 2010) and also associates with better baseline cognitive functions in aging populations. *See, e.g.,* Arking *et al.* (2002) *PNAS* 99:856; Arking *et al.* (2005) *Circ. Res.* 96:412; Dubal *et al.* (2014) *Cell Reports* 7:1065; Yokoyama *et al.* (2015) *Ann. Clin. Translational Neurology* 2:215.

[0062] Klotho polypeptides that can be used for administration include species homologs (*e.g.*, non-human primate, mouse, rat), allelic variants, functional fragments, and functional variants of the wild type sequence that retain cognition improving activity. Examples include secreted Klotho, fragments comprising the KL1 domain, fragments comprising the KL2 domain, fragments comprising the KL1 and KL2 domains, variants comprising the KL1 domain with at least one (*e.g.*, 1-20, 5-50, 25-100) non-conserved amino acid in the KL1 domain substituted with a different amino acid or deleted, variants comprising the KL2 domain with at least one non-conserved amino acid in the KL2 domain substituted with a different amino acid.

[0063] Functional fragments of the Klotho polypeptide that can be used as described herein include the extracellular domain (*e.g.*, corresponding to or substantially identical or similar to amino acids 34-979 of human Klotho), secreted Klotho (*e.g.*, corresponding to or substantially identical or similar to 549 amino acid form), a KL1 domain (*e.g.*, corresponding to or substantially identical or similar to amino acids 34-549 of human Klotho), a glycosyl hydrolase consensus sequence (*e.g.*, corresponding to or substantially identical or similar to amino acids 57-506 of human Klotho), or a beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase consensus sequence (*e.g.*, corresponding to or substantially identical or similar to amino acids 62-497 of human Klotho). In some embodiments, the

Klotho polypeptide comprises or is substantially identical or similar to amino acids 34-549 of human Klotho. In some embodiments, the Klotho polypeptide is part of a larger fusion protein. In some embodiments, the fusion protein comprises the Klotho polypeptide as described herein and further comprises no more than 100, 75, 50, or 30 additional amino acids. In some embodiments, the Klotho polypeptide is not fused to a Fibroblast growth factor (FGF). In some embodiments, the Klotho polypeptide comprises (e.g., is fused to) an affinity tag (e.g., a histidine tag) or a conjugate to increase stability or half-life *in vivo*.

[0064] A functional variant or fragment of Klotho is a variant or fragment that retains any klotho activity, e.g., at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the level of any activity of soluble klotho. Soluble klotho activities include those described above, and include binding FGF-23, binding to FGFR1c, beta-glucuronidase activity, suppression of wnt signaling, suppression of insulin signaling, suppression of TGF-beta 1 activity, increasing GluN2B expression and/or synaptic localization, c-fos induction. Additional Klotho activities include causing changes in magnetic resonance imaging (MRI) brain scans, e.g., functional MRI, electroencephalograph (EEG), and transcranial magnetic and electrical stimulation (TMS and TES); and improved performance in neuropsychologic testing and cognitive ability.

IV. Administration of Klotho

[0065] Provided herein are methods of improving cognition and/or motor function in an individual comprising administering Klotho to the individual. In some embodiments, the method of treatment comprises administering to an individual an effective amount of a Klotho polypeptide (or functional variant or fragment thereof). In some embodiments, the treatment is prophylactic, e.g., for individual expecting stress (e.g., jet lag, military performance) or to prevent cognitive decline associated with aging. In some embodiments, the individual has been diagnosed with a cognitive disorder. In some embodiments, the individual is receiving or has received therapy for a cognitive disorder or for a condition that is related to cognitive function (e.g., cognitive decline in response to chemotherapy).

[0066] In some embodiments, the method further comprises monitoring the individual for cognitive ability, either through a molecular proxy (e.g., changes NMDA receptor or c-fos activation, or GluN2B levels in the brain), changes in MRI brain scans (e.g., functional MRI), changes in EEG, changes in TMS and TES, changes in neuropsychologic test scores, or tests of cognitive ability (e.g., for learning, short or long term memory, executive functions,

language ability, and visuospatial function). In some embodiments, the individual is monitored using more than one of the above tests in any combination. In some embodiments, the dose of the Klotho polypeptide for each administration is determined based on the therapeutic progress of the individual, *e.g.*, where a higher dose is administered if the individual is not responding sufficiently to therapy.

[0067] In some embodiments, the Klotho polypeptide is administered in a pharmaceutical composition with a physiologically (*i.e.*, pharmaceutically) acceptable carrier. The term “carrier” refers to a typically inert substance used as a diluent or vehicle for a diagnostic or therapeutic agent. The term also encompasses a typically inert substance that imparts cohesive qualities to the composition. Physiologically acceptable carriers can be liquid, *e.g.*, physiological saline, phosphate buffer, normal buffered saline (135-150 mM NaCl), water, buffered water, 0.4% saline, 0.3% glycine, glycoproteins to provide enhanced stability (*e.g.*, albumin, lipoprotein, globulin, etc.), and the like. Since physiologically acceptable carriers are determined in part by the particular composition being administered as well as by the particular method used to administer the composition, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*See, e.g.*, Remington's Pharmaceutical Sciences, 17th ed., 1989).

[0068] The presently described compositions can be sterilized by conventional, well-known sterilization techniques or may be produced under sterile conditions. Aqueous solutions can be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, and the like, *e.g.*, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, and triethanolamine oleate. Sugars can also be included for stabilizing the compositions, such as a stabilizer for lyophilized antibody compositions.

[0069] Dosage forms can be prepared for mucosal (*e.g.*, nasal, sublingual, vaginal, buccal, or rectal), parenteral (*e.g.*, subcutaneous, intravenous, intramuscular, or intraarterial injection, either bolus or infusion), oral, or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (*e.g.*,

nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (*e.g.*, aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (*e.g.*, crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

[0070] Injectable compositions can comprise a solution of the Klotho polypeptide suspended in an acceptable carrier, such as an aqueous carrier. Any of a variety of aqueous carriers can be used, *e.g.*, water, buffered water, 0.4% saline, 0.9% isotonic saline, 0.3% glycine, 5% dextrose, and the like, and may include glycoproteins for enhanced stability, such as albumin, lipoprotein, globulin, etc. In some embodiments, normal buffered saline (135-150 mM NaCl) is used. The compositions can contain pharmaceutically acceptable auxiliary substances to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, *e.g.*, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

[0071] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Injection solutions and suspensions can also be prepared from sterile powders, granules, and tablets. In some embodiments, the composition is administered by intravenous infusion, topically, intraperitoneally, intravesically, or intrathecally. The Klotho polypeptide formulation can be provided in unit-dose or multi-dose sealed containers, such as ampoules and vials.

[0072] The Klotho polypeptide composition, alone or in combination with other suitable components, can be made into aerosol formulations (“nebulized”) to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, and nitrogen.

[0073] The pharmaceutical preparation can be packaged or prepared in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component, *e.g.*, according to the dose of Klotho polypeptide. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation. The composition can, if desired, also contain other compatible therapeutic agents. In some embodiments, the Klotho polypeptide composition can be formulated in a kit for administration.

[0074] In some embodiments, a pharmaceutical composition comprising a klotho polypeptide is administered orally. In some embodiments, a pharmaceutical composition comprising a klotho polypeptide is administered mucosally, *e.g.*, nasally. In some embodiments, a pharmaceutical composition comprising a klotho polypeptide is administered by injection, *e.g.*, subcutaneous, intraperitoneal, intravenous, or intramuscular. In some embodiments, a pharmaceutical composition comprising a klotho polypeptide is administered by infusion, *e.g.*, using a reservoir or osmotic minipump.

[0075] An example of administration of a pharmaceutical composition includes storing the Klotho polypeptide at 10 mg/ml in sterile isotonic aqueous saline solution at 4°C, and diluting it in an appropriate solution for injection prior to administration to the patient. In some embodiments, the Klotho polypeptide composition can be administered by intravenous infusion over the course of 0.25-2 hours. In some embodiments, the administration procedure is via bolus injection.

[0076] In therapeutic use, the Klotho polypeptide can be administered at the initial dosage of about 0.1 µg/kg to about 1000 µg/kg daily and adjusted over time. A daily dose range of about 1 µg/kg to about 500 µg/kg, or about 10 µg/kg to about 100 µg/kg, or about 30 µg/kg to about 50 µg/kg can be used. The dosage is varied depending upon the requirements of the patient, the severity of the condition being treated, and the route of administration. For example, for injection of Klotho polypeptide, the effective dose is typically in the range of 10-100 µg/kg, while for direct delivery to the central nervous system (CNS), the effective dosage is lower, *e.g.*, 5-30 µg/kg. For oral administration, the effective dose is higher, *e.g.*, in the range of 50-10,000 µg/kg (*e.g.*, 100µg/kg-2mg/kg). The dose is chosen in order to provide effective therapy for the patient. The dose may be repeated at an appropriate frequency which may be in the range of once or twice per day, once or twice per week to once every three months, depending on the pharmacokinetics of the Klotho polypeptide

composition (*e.g.*, half-life in the circulation) and the pharmacodynamic response (*e.g.*, the duration of the therapeutic effect).

[0077] Administration can be periodic. Depending on the route of administration, the dose can be administered, *e.g.*, once every 1, 3, 5, 7, 10, 14, 21, or 28 days or longer (*e.g.*, once every 2, 3, 4, or 6 months). In some cases, administration is more frequent, *e.g.*, 2 or 3 times per day. The patient can be monitored to adjust the dosage and frequency of administration depending on therapeutic progress and any adverse side effects, as will be recognized by one of skill in the art.

[0078] Dosages can be empirically determined considering the type and severity of cognitive condition diagnosed in a particular patient. The dose administered to a patient, in the context of the present disclosure, should be sufficient to affect a beneficial therapeutic response in the patient over time. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of any particular composition in a particular patient, as will be recognized by the skilled practitioner.

[0079] In some embodiments, the Klotho polypeptide composition is administered to an (*e.g.*, human) individual having at least normal cognitive function. As described herein, it has been surprisingly discovered that not only can Klotho improve cognition in individuals with impaired cognition, Klotho can also improve cognition of individuals with at least normal cognition. Thus in some embodiments, the individual receiving the Klotho polypeptide composition begins initially with at least normal cognition and following administration of the Klotho polypeptide composition attains improved cognition compared to the initial level of cognition. The level of cognition of an individual can be determined as is known in the art. Normal cognitive functions are determined by scores from sets of cognitive tests that are compiled into global cognitive scores, as described in Dubal DB et al. (2014) *Cell Reports* 7:1065-1076. Such cognition tests include tests of executive function and working memory such as Trails A and Trails B (Dubal DB et al. (2014) *Cell Reports* 7:1065-1076). In some embodiments, administration of Klotho results in an improvement of cognition (whether initially at least normal or impaired), by at least 5%, 10%, 20% or more.

In some embodiments, administration results in improved motor function. In some embodiments, the Klotho polypeptide composition is administered to an (*e.g.*, human) individual having impaired motor function. For example, in some embodiments, the

individual has stroke to the brain or spinal cord (ischemic or hemorrhagic), neurodegenerative disease (Parkinson's disease, Lewy body dementia, multiple system atrophy, amyotrophic lateral sclerosis, prion disorder, Huntington's disease, supranuclear palsy), Parkinsonism, traumatic brain injury, neuroinfectious brain lesions, multiple sclerosis and related autoimmune and demyelinating disease, spinal cord lesions (compressive, 5 infectious, toxic or metabolic, autoimmune, oncologic), brain tumor, epilepsy, paraneoplastic disorder, neurodevelopmental disorder (mitochondrial, autosomal genetic), muscle disease (polymyositis, dermatomyositis, inclusion body myositis, infectious, endocrine, metabolic, toxic, congenital myopathy, congenital muscular dystrophy, hereditary), neuropathies 10 (Guillain-Barre syndrome, axonal and demyelinating, diabetic, toxic, metabolic, infectious, critical illness, entrapment), tick paralysis, myasthenia gravis, and spinal muscular atrophy. Changes in motor function can be assayed as known in the art. Exemplary motor function assays include but are not limited to electromyogram and nerve conduction studies, direct or device-assisted clinical testing of strength, tone, and muscle bulk, reflex examination, 15 coordination examination, and gait analysis. Assays for testing etiologies causing deficits of motor function include but are not limited to magnetic resonance imaging of the central nervous system, muscle biopsy, nerve biopsy, and laboratory studies.

[0080] Thus in some embodiments, additional administration is dependent on patient progress, *e.g.*, the patient is monitored between administrations. For example, after the first 20 administration or round of administrations, the patient can be monitored for cognitive ability or for side effects, *e.g.*, weakness, dizziness, nausea, etc.

[0081] In some embodiments, the individual has a chronic condition, so that klotho is administered over an indefinite period, *e.g.*, for the lifetime of the patient. In such cases, administration is typically periodic. Diseases that are considered long-term or chronic 25 include, but are not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, and cognitive decline associated with hypertension and heart disease.

[0082] In some embodiments, the Klotho polypeptide is linked to a stabilizing moiety such as PEG, glycosylation, or a liposome or other nanocarrier. US Patent Nos. 4,732,863 and 7892554 and Chattopadhyay *et al.* (2010) *Mol Pharm* 7:2194 describe methods for attaching 30 a polypeptide to PEG, PEG derivatives, and nanoparticles (*e.g.*, liposomes). Liposomes containing phosphatidyl-ethanolamine (PE) can be prepared by established procedures as described herein. The inclusion of PE provides an active functional site on the liposomal

surface for attachment. In some embodiments, the Klotho polypeptide is linked to an affinity tag, *e.g.*, a histidine tag (*e.g.*, 4-16 histidine residues), streptavidin, or an antibody target.

[0083] The Klotho polypeptide can also be formulated as a sustained-release preparation (*e.g.*, in a semi-permeable matrices of solid hydrophobic polymers (*e.g.*, polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides. The Klotho polypeptide can be entrapped in a nanoparticle prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly- (methylemethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions.

[0084] In some embodiments, the Klotho polypeptide is labeled, *e.g.*, for tracking in the body or *ex vivo*. The Klotho polypeptide can be labeled any diagnostic agent known in the art, as provided, for example, in the following references: Armstrong *et al.*, *Diagnostic Imaging*, 5th Ed., Blackwell Publishing (2004); Torchilin, V. P., Ed., *Targeted Delivery of Imaging Agents*, CRC Press (1995); Vallabhajosula, S., *Molecular Imaging: Radiopharmaceuticals for PET and SPECT*, Springer (2009). The diagnostic agent can be detected by a variety of ways, including as an agent providing and/or enhancing a detectable signal. Detectable signals include, but are not limited to, gamma-emitting, radioactive, echogenic, optical, fluorescent, absorptive, magnetic, or tomography signals. Techniques for imaging the diagnostic agent can include, but are not limited to, single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), optical imaging, positron emission tomography (PET), computed tomography (CT), x-ray imaging, gamma ray imaging, and the like. The terms “detectable agent,” “detectable moiety,” “label,” “imaging agent,” and like terms are used synonymously herein.

[0085] In some embodiments, the label can include optical agents such as fluorescent agents, phosphorescent agents, chemiluminescent agents, and the like. Numerous agents (*e.g.*, dyes, probes, labels, or indicators) are known in the art and can be used in the present invention. (*See, e.g.*, Invitrogen, *The Handbook—A Guide to Fluorescent Probes and Labeling Technologies*, Tenth Edition (2005)). Fluorescent agents can include a variety of organic and/or inorganic small molecules or a variety of fluorescent proteins and derivatives thereof. For example, fluorescent agents can include but are not limited to cyanines, phthalocyanines, porphyrins, indocyanines, rhodamines, phenoxazines, phenylxanthenes,

phenothiazines, phenoselenazines, fluoresceins, benzoporphyrins, squaraines, dipyrrolo pyrimidones, tetracenes, quinolines, pyrazines, corrins, croconiums, acridones, phenanthridines, rhodamines, acridines, anthraquinones, chalcogenopyrylium analogues, chlorins, naphthalocyanines, methine dyes, indolenium dyes, azo compounds, azulenes, azaazulenes, triphenyl methane dyes, indoles, benzoindoles, indocarbocyanines, benzoindocarbocyanines, and BODIPY™ derivatives. Fluorescent dyes are discussed, for example, in U.S. Pat. No. 4,452,720, U.S. Pat. No. 5,227,487, and U.S. Pat. No. 5,543,295.

[0086] The label can also be a radioisotope, *e.g.*, radionuclides that emit gamma rays, positrons, beta and alpha particles, and X-rays. Suitable radionuclides include but are not limited

to ^{225}Ac , ^{72}As , ^{211}At , ^{11}B , ^{128}Ba , ^{212}Bi , ^{75}Br , ^{77}Br , ^{14}C , ^{109}Cd , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{18}F , ^{67}Ga , ^{68}Ga , ^3H ,

^{166}Ho , ^{123}I , ^{124}I , ^{125}I , ^{130}I , ^{131}I , ^{111}In , ^{177}Lu , ^{13}N , ^{15}O , ^{32}P , ^{33}P , ^{212}Pb , ^{103}Pd , ^{186}Re , ^{188}Re , ^{47}Sc , ^{153}Sm , ^{89}Sr , $^{99\text{m}}\text{Tc}$, ^{88}Y and ^{90}Y . In some embodiments, radioactive agents can include ^{111}In -

DTPA, $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-DTPA}$, $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-ENPy2}$, $^{62/64/67}\text{Cu-TETA}$, $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-IDA}$, and $^{99\text{m}}\text{Tc}(\text{CO})_3$ triamines (cyclic or linear). In some embodiments, the agents can include DOTA and its various analogs with ^{111}In , ^{177}Lu , ^{153}Sm , $^{88/90}\text{Y}$, $^{62/64/67}\text{Cu}$, or $^{67/68}\text{Ga}$. In some embodiments, a nanoparticle can be labeled by incorporation of lipids attached to chelates, such as DTPA-lipid, as provided in the following references: Phillips *et al.*, Wiley

Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 1(1): 69-83 (2008); Torchilin, V.P. & Weissig, V., Eds. *Liposomes 2nd Ed.*: Oxford Univ. Press (2003); Elbayoumi, T.A. & Torchilin, V.P., *Eur. J. Nucl. Med. Mol. Imaging* 33:1196–1205 (2006); Mougin-Degraef, M. *et al.*, *Int'l J. Pharmaceutics* 344:110-117 (2007).

[0087] In some embodiments, the diagnostic agent can be associated with a secondary binding ligand or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and glucose oxidase. Secondary binding ligands include, *e.g.*, biotin and avidin or streptavidin compounds as known in the art.

V. Cognitive conditions and disorders

[0088] Klotho polypeptides (and functional variants and fragments thereof) can be administered to improve cognition for a number of conditions and situations. This includes

treatment of individuals with lower than normal or declining cognitive ability, or prophylactic treatment of individuals in need of improved or increased cognitive ability.

[0089] Klotho polypeptides (and functional variants and fragments thereof) can be used to prevent or reduce cognitive decline associated with aging, *e.g.* in individuals 50 years of age or older, or upon initial signs of cognitive decline.

[0090] Klotho polypeptides (and functional variants and fragments thereof) can also be used to treat individuals with age-related, non-age related, or disease related conditions including, but not limited to:

Neurodegenerative diseases and dementia: Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasalar degeneration, mild cognitive impairment, vascular dementia, Lewy body dementia, amyotrophic lateral sclerosis, prion disorder, HIV-related dementia;

Mental or mood disorders: depression, schizophrenia, attention deficit/ hyperactivity disorder, autism spectrum disorder, intellectual disability, a mood disorder, and a psychotic disorder;

Childhood neurodevelopmental syndromes and brain tumors: X-linked mental disability or retardation, astrocytoma, ependymoma, medulloblastoma, oligodendroglioma;

Genetic syndromes affecting learning: Down's syndrome, Angelman's syndrome, Rett's syndrome;

Metabolic disorders affecting cognition: phenylketonuria, Lesch-Nyhan, galactosemia, and adrenoleukodystrophy;

Cognitive decline associated with chemotherapy and/or radiation therapy; and

Additional conditions and disorders: pain-associated cognitive effects, traumatic brain injury, stroke, multiple sclerosis, neuroautoimmune disease, epilepsy, delirium, paraneoplastic disorder, developmental delay, and leukodystrophies.

[0091] Klotho polypeptides (and functional variants and fragments thereof) can be also be administered to provide increased cognition for individuals desiring improved cognition, *e.g.*, individuals exposed to stress, sleep deprivation, or jet lag, or for individuals requiring superior cognitive function, such as surgeons, air-traffic controllers, and military personnel. In such cases, the klotho polypeptide composition can be administered 2-24 hours before the

desired effect, which can last about 3-5 days for working memory and about 2 weeks for spatial memory.

[0092] Cognitive ability can be measured using any method known in the art, *e.g.*, for testing memory, language ability, executive functions, visuospatial function, dementia, or multi-parameter neuropsychological abilities. In some embodiments, Klotho administration results in at least a 1%, 2%, 5%, 7%, 10%, 15%, 20%, 30%, 50%, or greater improvement in score on a standard cognitive ability test (*e.g.*, measured 1-3 days after administration). In some embodiments, the testing is carried out more than once for an individual, *e.g.*, one or more time over the course of treatment with Klotho.

[0093] For example, standard tests for memory and learning can be applied, *e.g.*, to determine semantic, episodic, procedural, priming, and/or working (*i.e.*, short term) memory. Common tests include Cambridge prospective memory test (CAMPROMPT), memory assessment scales (MAS), Rey auditory verbal learning test, Rivermead behavioral memory test, Test of memory and learning (TOMAL), Wechsler memory scale (WMS), and Test of memory malingering (TOMM). Tests for language functions include, *e.g.*, Boston Diagnostic Aphasia Examination (BDAA), Comprehensive aphasia test (CAT), and Multilingual aphasia examination (MAE).

[0094] Executive function (*e.g.*, problem solving, planning, organization, inhibitory control) can be tested using Behavioral assessment of dysexecutive syndrome (BADS), CNS vital signs (Brief Core Battery), Controlled oral word association test (COWAT), Delis-Kaplan Executive Function System (D-KEFS), Digit vigilance test, Kaplan Baycrest neurocognitive assessment (KBNA), Hayling and Brixton tests, Tests of variables of attention (TOVA), Wisconsin card sorting test (WCST), or Test of everyday attention (TEA). Visuospatial ability (*e.g.*, visual perception, construction and integration) can be tested using the Clock Test, Hooper visual organization task (VOT), or Rey-Osterrieth complex figure tests. Dementia can be quantified using the clinical dementia rating or dementia rating scale.

[0095] Multi-parameter tests for neuropsychological function (*e.g.*, cognitive function) include but are not limited to the Barcelona neuropsychological test (BNT), Cambridge neuropsychological test automated battery (CANTAB), Cognistat, Cognitive assessment screening instrument (CASI), Cognitive function scanner (CFS), Dean-Woodcock neuropsychology assessment system (DWNAS), General practical assessment of cognition

(GPCOG) Mini mental state examination (MMSE), NEPSY, or the CDR computerized assessment system.

[0096] Alternatively, cognition can be determined using structural or molecular proxies for cognitive activity, *e.g.*, compared over time to detect changes. Cognitive changes can be detected, *e.g.*, by observing changes to brain structure, connectivity, activation, inhibition, or synaptic plasticity, *e.g.*, by MRI, fMRI, EEG, TMS and TES, and/or any combination of these. In some embodiments, brain activity is observed. In some embodiments, Klotho administration results in a 1.5-fold, 2-fold, 5-fold, 7-fold, 10-fold, or greater increase in brain activity (*e.g.*, measured 1-3 days after administration). Molecular proxies for improved cognition include, but are not limited to: increased levels of GluN2B, increased GluN2B synaptic localization, increased NMDA receptor activation, and/or increased c-fos activation in the brain. These measures are particularly relevant to cognition. Such method can include, *e.g.*, obtaining a sample of neuronal tissue or CSF from an individual and using standard assays to determine gene expression or activation.

[0097] Similarly, in mice and other non-human animals, cognitive ability can be tested with measures of executive function (working memory, attention, processing speed, set shifting), visiospatial learning and memory, object memory, pattern recognition, fear memory, passive avoidance memory, habituation, and novel object recognition, for example. Common tests include but are not limited to the Morris water maze, Barnes maze, radial arm water maze, y-maze, T-maze, and open field habituation. Brain imaging techniques are similarly applicable.

VI. Examples

Example 1:

A. Materials and Methods

[0098] *Mice.* All mice were on a C57BL/6J background and were kept on a 12-h light/dark cycle with *ad libitum* access to food (Picolab Rodent Diet 20, Labdiet) and water. The standard housing group was five mice per cage except for single housing during water maze studies. All cognitive and behavioral studies were carried out during the light cycle. All studies were conducted in a blinded manner.

[0099] *Treatments.* Vehicle or klotho (recombinant mouse klotho, amino acids 35-982, with a C-terminal His tag, R&D Systems) was injected intraperitoneally (i.p.) before behavioral testing of mice.

[0100] *Morris water maze cognitive behavior test.* Water maze studies were carried out as described in Dubal *et al.* (2014) *Cell Reports* 7:1065; Dubal *et al.* (2015) *Journal of Neuroscience* 35:2358. Mice were treated with either vehicle or klotho (10µg/kg) i.p. 4h prior to testing daily for 5 days. Briefly, mice were tested in a pool (diameter, 122 cm) with white, opaque water (21°± 1°C). A square, 14-cm² platform was 2 cm below the surface. Before hidden platform training, mice underwent two pre-training trials by swimming through a channel to mount a hidden platform. Over the course of hidden platform training, the platform location stayed consistent while the drop location was varied between trials. Mice underwent two training sessions, consisting of two trials of 60 s each, daily for four days. For the probe trial testing, the platform was removed and the mice were allowed to swim for 60 s. After 1h and 24h probe trials, mice were tested for their ability to find a visible platform marked with a cue (15-cm pole on the platform) over two sessions. As part of the studies, swim velocities were also recorded.

[0101] *Y-maze cognitive behavior test.* Y-maze studies were carried out as described in Dubal *et al.* (2014) *Cell Reports* 7:1065. Mice were treated with either vehicle or klotho (10µg/kg) i.p. 18h prior to testing. Briefly, mice were acclimated to the room 30 min prior to testing. Then, mice were placed in one arm of the Y-maze (three identical arms, 120° apart) and explored for 4 min. Arm entries were recorded and an alternation was noted any time the mouse entered each of the three arms in successive arm entries; chance alternation was 22%. The apparatus was cleaned with 70% alcohol between testing sessions. Percent alternation was calculated from recorded data.

B. Systemic klotho delivery enhances working memory in young mice

[0102] To investigate whether therapeutic delivery of klotho can enhance cognition, we injected mice with recombinant klotho (i.p.) and 18h later, tested working memory in the Y-maze. Compared to vehicle-treated mice, klotho-treated mice showed more alternations, indicating superior working memory (FIG. 1A). Thus, systemic klotho delivery enhanced working memory, a process that involves frontal cortical brain regions.

C. Systemic klotho delivery enhances spatial learning in young mice

[0103] We next tested whether klotho treatment enhances spatial learning in the Morris water maze. We injected mice with recombinant klotho (i.p.) 4h prior to testing daily for five days in a group of mice (Cohort 1). Compared to vehicle-treated mice, klotho-treated mice performed better in spatial learning of the hidden platform location (FIG. 1B). Both groups swam at equal speeds (FIG. 1C). In an independent group of mice (Cohort 2), we injected recombinant klotho (i.p.) 18h prior to testing daily. Again, compared to vehicle-treated mice, klotho-treated mice performed better in spatial learning (FIG. 1D). Thus, systemic klotho delivery enhanced spatial learning, a process that involves frontal cortical and hippocampal brain regions.

D. Systemic klotho delivery enhances spatial memory in young mice

[0104] To determine whether klotho treatment can enhance spatial memory, we removed the platform and performed probe testing following hidden training in the Morris water maze. In probe trials of Cohort 1, spatial memory retention was assessed by measuring the affinity of mice for the target area. In probe testing at 1h, klotho-treated mice spent more time at the target center (FIG. 1E), showed increased frequency of crossing the target (FIG. 1F), and increased time in the target quadrant (FIG. 1G), compared to vehicle-treated controls. In probe testing at 24h, klotho-treated mice continued to show more center duration (FIG. 1H), crossing frequency (FIG. 1I), and percent time in the target quadrant (FIG. 1J), compared to vehicle-treated mice. Thus, systemic klotho delivery enhanced spatial memory, a process that engages hippocampal brain regions.

[0105] The data show that systemic delivery of klotho enhances normal cognition in mice. Klotho treatment improved learning and memory in multiple tests and measures including working memory, spatial learning, and spatial memory. These findings provide a direct therapeutic application for boosting cognitive functions, a therapy that is relevant, but not limited to, cognitive enhancement of the normal brain and cognitive dysfunction due to normal aging, numerous neurodegenerative, neurologic, and psychiatric diseases, childhood developmental syndromes, traumatic brain injury, and stress.

Example 2

[0106] Methods.

[0107] Mice. All mice were on a C57BL/6J background and were kept on a 12-h light/dark cycle with ad libitum access to food (Picolab Rodent Diet 20, Labdiet) and water. The standard housing group was five mice per cage except for single housing during water maze studies. All motor, cognitive and behavioral studies were carried out during the light cycle.

5 All studies were conducted in a blinded manner.

[0108] Aged mice (18 months) were obtained from the NIH aging colony and were used in experiments. Transgenic mice that model human neurodegenerative diseases related to α -synuclein toxicity were utilized in experiments; these mice express full length human α -synuclein from the mouse Thy-1 promoter (Rockenstein E, *et al.* (2002) *J Neurosci Res* 68:568–578) and exhibit motor, cognitive and behavioral deficits. Increased expression of the human α -synuclein protein contributes to several neurodegenerative diseases in the human condition including, but not limited to, Parkinson's disease (PD), Alzheimer's disease (AD), Lewy body dementia (LBD), and multiple system atrophy (MSA).

10 [0109] Treatments. Vehicle or α -klotho (recombinant mouse α -klotho, amino acids 35-982, with a C-terminal His tag, R&D Systems) was injected intraperitoneally (i.p.) before behavioral testing of mice as indicated. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco and conducted in compliance with NIH guidelines.

[0110] Cognitive and Motor Behavior

20 [0090] Morris water maze. Water maze studies were carried out as described (Zarei M, *et al.* (2013) *J Neurol Neurosurg Psychiatry* 84:875-881; Dubal DB et al. (2014) *Cell Reports* 7:1065-1076). Mice were treated with either vehicle or klotho (10 μ g/kg) i.p. 4h prior to testing daily for 5 days. Briefly, mice were tested in a pool (diameter, 122 cm) with white, opaque water (21 $\pm$ 1 $^{\circ}$ C). A square, 14-cm² platform was 2 cm below the surface. Before
25 hidden platform training, mice underwent two pre-training trials by swimming through a channel to mount a hidden platform. Over the course of hidden platform training, the platform location stayed consistent while the drop location was varied between trials. Mice underwent two training sessions, consisting of two trials of 60 s each, daily for four days. For the probe trial testing, the platform was removed and the mice were allowed to swim for 60 s.
30 After 1h and 24h probe trials, mice were tested for their ability to find a visible platform marked with a cue (15-cm pole on the platform) over two sessions. As part of the studies, swim velocities were also recorded.

[0090] Y-maze. Y-maze studies were carried out as described (Dubal DB et al. (2014) *Cell Reports* 7:1065-1076). Mice were treated with either vehicle or klotho (10µg/kg) i.p. 18h prior to testing. Briefly, mice were acclimated to the room 30 min prior to testing. Then, mice were placed in one arm of the Y-maze (three identical arms, 120° apart) and explored for 4 min. Arm entries were recorded and an alternation was noted any time the mouse entered each of the three arms in successive arm entries; chance alternation was 22%. The apparatus was cleaned with 70% alcohol between testing sessions. Percent alternations was calculated from recorded data.

[0111] Large Y-maze. Large Y-maze studies were performed as described (Dellu F, et al. (1992) *Brain Res* 588:132-139) with minor modifications. The large Y-maze apparatus consists of three identical arms, 120° apart, with a distinct and different visual cue at the back end of two arms; the third arm without a visual cue is the start arm. Prior to training or testing, mice were acclimated to the dimly lit room for 60 minutes. During training, one arm with a visual cue was blocked with a solid divider and mice were placed at the end of the start arm. Mice were allowed to explore the open arm for five minutes and then returned to their cages. The open arm used in training (the familiar arm) was counterbalanced throughout the cohort testing. 16 hours after training, mice underwent testing. During testing, the divider was removed. Mice were placed in the start arm and allowed to explore the arms with novel and familiar visual cues (novel arm and familiar arm) for 5 minutes. Number of entries and duration of time spent in the novel and familiar arms was recorded and measured. The ratio of novel to familiar arm entries or duration was calculated to assess spatial and working memory. The apparatus was cleaned with 70% alcohol between sessions.

[0112] Rota Rod. Mice were acclimated to the room 60 minutes prior to each session. Five mice were simultaneously put on the rotating rod (Rota Rod, Med Associates Inc, VT) at a constant speed of 16 rpm for a maximum of 300 seconds. Latency to fall was recorded in each trial. Three trials were performed consecutively with a 10 minute rest between trials. The apparatus was cleaned with 70% alcohol between testing sessions.

[0113] Open field. Total activity in the open field was measured as described (Dubal DB, et al. (2015) *J Neurosci* 35:2358-2371) with an automated Flex-Field/Open Field Photobeam Activity System (San Diego Instruments, San Diego, CA). Mice were acclimated to the testing room for 30 minute and then tested in a clear plastic chamber (41 x 30 cm) for 5 min,

with two photobeam arrays measuring movements. The apparatus was cleaned with 70% alcohol between testing sessions.

[0114] Results.

[0115] Systemic klotho delivery enhances cognition in aged mice. To determine whether therapeutic delivery of klotho can enhance cognition in aged mice, we injected mice with recombinant klotho (i.p., 10µg/kg) once and then trained them for a task to measure working and spatial memory in the large Y-maze 24h later. Then, 18h following training (42h after klotho delivery), mice underwent testing. At baseline, and without klotho treatment, the aged mice did not show novel arm preference, indicating a cognitive deficit. Compared to vehicle-treated mice, klotho-treated mice showed a persistent preference for the novel arm largely throughout exploration (FIG. 2A,B), indicating better cognition that involves frontal and hippocampal brain regions. These data show that klotho enhances cognition in mice with age-induced cognitive deficits. These data suggest that therapeutic delivery of klotho enhances cognition in the aging brain.

[0116] Systemic klotho delivery enhances cognition in a long-lasting manner. To determine whether therapeutic delivery of klotho can induce long-lasting cognitive enhancement, in a manner that extends beyond its half-life of 7 hours (Hu MC, *et al.* (2015) *J Am Soc Nephrol.*), we tested young mice approximately two weeks after 5 days of daily klotho treatment and cognitive testing (i.p., 0.5 or 2.5 µg/kg). As expected, vehicle-treated mice did not show preference for the novel arm, indicating a loss of memory for the visual cue after two weeks. In contrast, klotho-treated mice showed a clear preference for the novel arm of the maze – even two weeks after the last treatment and in a dose-dependent manner (FIG. 3). These data indicate that klotho-mediated cognitive enhancement in normal, young mice was long-lasting and extended at least two weeks beyond its half-life. These data suggest that therapeutic delivery of klotho induces organizational changes in the brain that enhance neural function.

[0117] Systemic klotho delivery improves cognitive dysfunction in mice that model neurodegenerative disease. We next tested whether acute, therapeutic delivery of klotho can improve existing cognitive deficits in a mouse model of neurodegenerative disease. In this well-characterized model of disease, mice express human α -synuclein (hSYN), a pathogenic protein that causes cognitive and motor deficits and contributes to Parkinson's disease, Alzheimer's disease, Lewy body dementia, and multiple system atrophy. In these mice,

cognitive function was assessed in the large-Y maze. This task is optimal since it utilizes the mouse's natural tendency to explore, avoids testing-induced stressors, and is not affected by motor difficulties that limit other tests (such as inability to swim appropriately in a water maze). Compared to nontransgenic (NTG) mice, hSYN mice showed decreased preference for the novel arm, as shown by decreased duration (FIG. 4A). Remarkably, klotho treatment of hSYN mice (i.p., 2.5 µg/kg,) increased novel arm preference (FIG. 4A,B), indicating acute and therapeutic cognitive improvement. There was an overall effect of klotho treatment in enhancing memory as indicated by increasing novel arm preference (Two-way ANOVA KL effect $p < 0.05$) (FIG. 4B). These data suggest that therapeutic delivery of klotho can reverse or improve cognitive deficits caused by α -synuclein toxicity in neurodegenerative diseases. Of note, klotho-mediated cognitive enhancement could contribute to improved motor learning.

[0118] Systemic klotho delivery improves early motor dysfunction in mice that model neurodegenerative disease. Since α -synuclein toxicity induces deficits in motor function, a major clinical problem in neurodegenerative diseases, we tested whether therapeutic delivery of klotho can improve this key measure in hSYN mice. Motor function was assessed on a spinning rod, the Rota rod. Compared to NTG mice, hSYN mice showed decreased motor function (FIG. 5). Remarkably, klotho treatment (i.p., 2.5 µg/kg) of hSYN mice acutely increased motor function, as shown by longer latency on the spinning rod (FIG. 5). These data indicate that klotho treatment acutely improves early motor function in hSYN mice. These data suggest that therapeutic delivery of klotho can improve deficits related to motor problems caused by α -synuclein toxicity in neurodegenerative diseases.

[0119] Systemic klotho delivery does not alter total movements or activity of mice. To examine whether klotho delivery alters total movements and activity of mice, we examined mouse exploration of an open field. Total movements did not differ between Veh- or KL-treated (i.p., 2.5 µg/kg) mice (FIG. 6).. These data suggest that the therapeutic effects of klotho are specific to cognitive and motor functions and are not influenced by non-specific actions such as hyperactivity.

[0120] Discussion

[0121] Our data show that systemic delivery of klotho, which does not cross the blood brain barrier, enhances normal cognition in young mice in a manner that is long-lasting, improves cognitive deficits in normal young mice and in aging mice; it also improves

cognitive and motor deficits in transgenic mice that model major neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Lewy body dementia, and multiple system atrophy.

[0122] These findings provide a direct therapeutic application for boosting cognitive functions in the normal brain and improving brain function in aging and neurodegenerative disease. This therapeutic application is also relevant, but not limited to, cognitive dysfunction due to numerous neurologic, and psychiatric diseases, childhood developmental syndromes, traumatic brain injury, and stress.

[0123] Our data show that:

1. Systemic administration of mouse recombinant klotho forms enhance normal cognition across the lifespan from young (2–7 mos) to aged (18mos) male and female mice.
2. Systemic klotho therapy is effective in enhancing cognition when given from 4 hours to 16 days prior to testing. The cognitive-enhancing effects last for at least two weeks following extensive cognitive training.
3. Systemic doses of mouse klotho between 0.5 µg/kg and 10 µg/kg enhance cognition in conditions tested.
4. Systemic therapy with recombinant klotho enhances cognition in a mouse model of neurodegenerative disease relevant to, but not limited to, Alzheimer's disease, Parkinson's disease, Lewy body dementia, and multiple system atrophy.
5. In addition to improving cognitive deficits in a mouse model of neurodegenerative disease, klotho therapy also improved early motor function.
6. The therapeutic effects of klotho on enhancing cognition extend far beyond its half-life of approximately 7 hours.
7. The therapeutic effects of klotho appear specific to cognitive and motor functions and are not influenced by non-specific actions such as hyperactivity.

[0124] The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, databases, internet sources, patents, patent applications, and accession numbers cited herein are hereby incorporated by reference in their entireties for all purposes.

VII. Informal sequence listing

SEQ ID NO:1: Human Klotho Protein

	10	20	30	40	50
5	MPASAPPRRP	RPPPPSLSLL	LVLLGLGGRR	LRAEPGDGAQ	TWARFSRPPA
	60	70	80	90	100
	PEAAGLFQGT	FPDGFLWAVG	SAAYQTEGGW	QQHGKGASIW	DTFTHHPLAP
	110	120	130	140	150
	PGDSRNASLP	LGAPSPLQPA	TGDVASDSYN	NVFRDTEALR	ELGVTHYRFS
10	160	170	180	190	200
	ISWARVLPNG	SAGVPNREGL	RYYRRLLERL	RELGVQPVVT	LYHWDLPQRL
	210	220	230	240	250
	QDAYGGWANR	ALADHFRDYA	ELCFRHFGGQ	VKYWITIDNP	YVVAWHGYAT
	260	270	280	290	300
15	GRLAPGIRGS	PRLGYLVAHN	LLLAHAKVWH	LYNTSFRPTQ	GGQVSIALSS
	310	320	330	340	350
	HWINPRRMTD	HSIKECQKSL	DFVLGWFAKP	VFIDGDYPES	MKNNLSSILP
	360	370	380	390	400
	DFTESEKKFI	KGTADFFALC	FGPTLSFQLL	DPHMKFRQLE	SPNLRQLLSW
20	410	420	430	440	450
	IDLEFNHPQI	FIVENGWFVS	GTTKRDDAKY	MYYLKKFIME	TLKAIKLDGV
	460	470	480	490	500
	DVIGYTAWSL	MDGFEWHRGY	SIRRGIFYVD	FLSQDKMLLP	KSSALFYQKL
	510	520	530	540	550
25	IEKNGFPPLP	ENQPLEGTFP	CDFAWGVVDN	YIQVDTTLSQ	FTDLNVYLWD
	560	570	580	590	600
	VHHSKRLIKV	DGVVTKKRKS	YCVDFAAIQP	QIALAQEMHV	THFRFSLDWA
	610	620	630	640	650
	LILPLGNQSQ	VNHTILQYYR	CMASELVRVN	ITPVVALWQP	MAPNQGLPRL
30	660	670	680	690	700
	LARQGAWENP	YTALAFAEYA	RLCFQELGHH	VKLWITMNEP	YTRNMTYSAG
	710	720	730	740	750
	HNLLKAHALA	WHVYNEKFRH	AQNGKISIAL	QADWIEPACP	FSQKDKEVAE
	760	770	780	790	800
35	RVLEFDIGWL	AEPIFGSGDY	PWVMRDWLNQ	RNNFLLPYFT	EDEKKLIQGT
	810	820	830	840	850
	FDFLALSHYT	TILVDSEKED	PIKYNDYLEV	QEMTDITWLN	SPSQVAVVPW
	860	870	880	890	900
	GLRKVLNWLK	FKYGDLPYI	ISNGIDDGLH	AEDDQLRVYY	MQNYINEALK

910 920 930 940 950
AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
960 970 980 990 1000
NGFPGPETLE RFCPEEFTVC TECSEFFHTRK SLLAFIAFLF FASIISLSLI
5 1010
FYYSKKGRRS YK

1 WHAT IS CLAIMED IS:

2 1. A method for improving cognitive function in an individual in need
3 thereof comprising
4 administering to the individual an effective amount of a protein comprising a
5 Klotho polypeptide or a functional fragment thereof, wherein the administering is systemic or
6 peripheral,
7 thereby improving cognitive function in the individual.

8
9 2. The method of claim 1, wherein the protein is a Klotho polypeptide or
10 a functional fragment thereof.

11 3. The method of claim 1, wherein the administering is oral, mucosal, or
12 carried out by injection.

13 4. The method of claim 3, wherein the injection is intravenous,
14 intraperitoneal, subcutaneous, or intramuscular.

15 5. The method of any one of the proceeding claims, wherein the
16 individual is a human.

17 6. The method of claim 5, wherein the human has at least normal
18 cognitive function and the administering results in improved cognitive function compared to
19 before the administering.

20 7. The method of claim 5, wherein the human is 50 years of age or older.

21 8. The method of claim 7, wherein the human has age related cognitive
22 decline.

23 9. The method of claim 5, wherein the human is less than 50 years of age.

24 10. The method of any one of the proceeding claims, wherein the
25 individual is a human having a neurodegenerative disease.

26 11. The method of claim 10, wherein the neurodegenerative disease is
27 selected from the group consisting of: Alzheimer's disease, Parkinson's disease, Huntington's
28 disease, frontotemporal dementia, progressive supranuclear palsy, corticobasalar

29 degeneration, mild cognitive impairment, vascular dementia, Lewy body dementia, multiple
30 system atrophy, amyotrophic lateral sclerosis, prion disorder, and HIV-related dementia.

31 12. The method of any one of the foregoing claims, wherein the individual
32 is a human having a condition selected from the group consisting of: depression,
33 schizophrenia, attention deficit/ hyperactivity disorder, autism spectrum disorder, intellectual
34 disability, a mood disorder, and a psychotic disorder.

35 13. The method of any one of the foregoing claims, wherein the individual
36 is a human having a condition selected from the group consisting of traumatic brain injury,
37 stroke, multiple sclerosis, neuroautoimmune disease, epilepsy, delirium, and a paraneoplastic
38 disorder.

39 14. The method of any one of the foregoing claims, wherein the individual
40 is a human having a condition selected from the group consisting of: an X-linked mental
41 disorder, Down's syndrome, Angelman's syndrome, Rett's syndrome, phenylketonuria,
42 Lesch-Nyhan, galactosemia, and adrenoleukodystrophy.

43 15. The method of any one of the foregoing claims, wherein the individual
44 is a human having a condition selected from astrocytoma, ependymoma, medulloblastoma,
45 and oligodendroglioma.

46 16. The method of any one of the foregoing claims, wherein the individual
47 is a human receiving radiation treatment or chemotherapy for cancer.

48 17. The method of any one of the foregoing claims, wherein the individual
49 is a human that is experiencing, or will experience within 24 hours, sleep deprivation or jet
50 lag.

51 18. The method of any one of the foregoing claims, wherein the Klotho
52 polypeptide has at least 85% identity to amino acids 34-534 of SEQ ID NO:1.

53 19. The method of any one of the foregoing claims, wherein the Klotho
54 protein is a functional fragment comprising a KL1 domain.

55 20. The method of any one of the foregoing claims, wherein the Klotho
56 protein is a functional fragment comprising KL1 and KL2 domains.

1 21. The method of any one of the foregoing claims, wherein the Klotho
2 protein has at least 85% identity to amino acids 34-979 of SEQ ID NO:1.

3 22. The method of any one of the foregoing claims, wherein the effective
4 amount is 1 ug to 1000 ug per kg body weight of the individual.

5 23. The method of any one of the foregoing claims, wherein the Klotho
6 polypeptide or a functional fragment thereof is administered more than once as part of a
7 course of treatment.

8 24. The method of claim 23, wherein the Klotho polypeptide or a
9 functional fragment thereof is administered once every 1-7 days.

10 25. The method of any one of the foregoing claims, further comprising
11 testing the cognitive function of the individual after administering.

12 26. The method of claim 25, further comprising testing the cognitive
13 function of the individual prior to administering, and comparing the cognitive function of the
14 individual prior to and after administering.

15 27. The method of claim 25 or 26, wherein cognitive function is
16 determined by testing the individual for semantic, episodic, procedural, priming, and/or
17 working memory.

18 28. The method of claim 25 or 26, wherein cognitive function is
19 determined by testing the individual for language ability, executive function, visuospatial
20 function, or dementia.

21 29. A method for improving motor function in an individual in need
22 thereof comprising
23 administering to the individual an effective amount of a protein comprising a
24 Klotho polypeptide or a functional fragment thereof, wherein the administering is systemic or
25 peripheral,

26 thereby improving motor function in the individual compared to before the
27 administering.

FIG. 1A

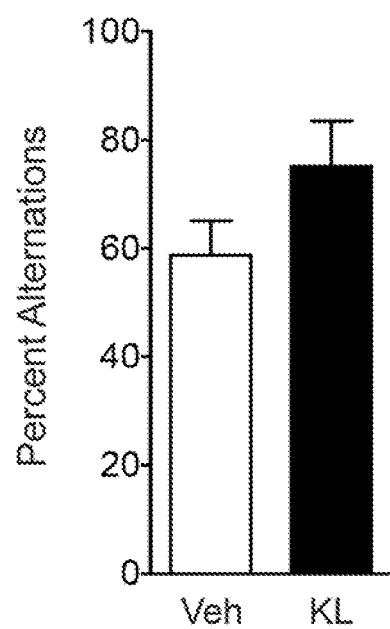


FIG. 1B

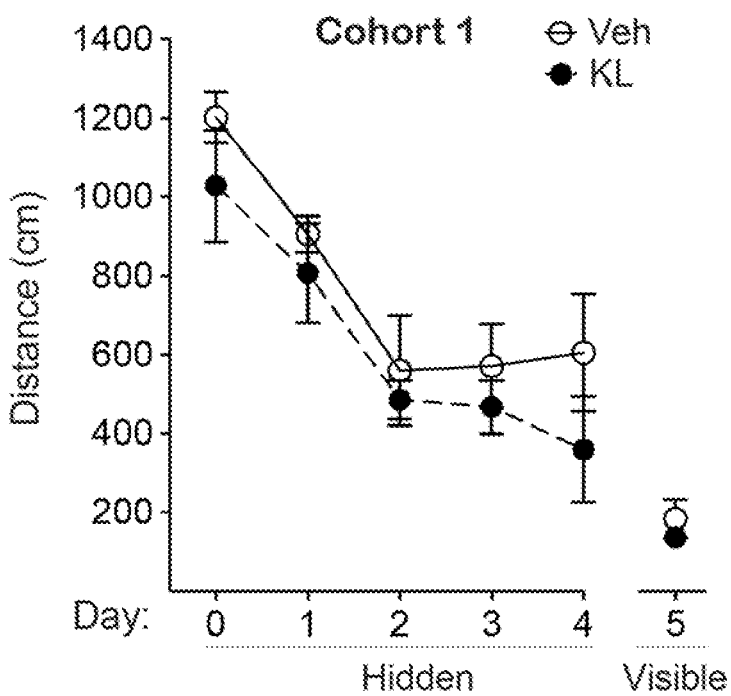


FIG. 1C

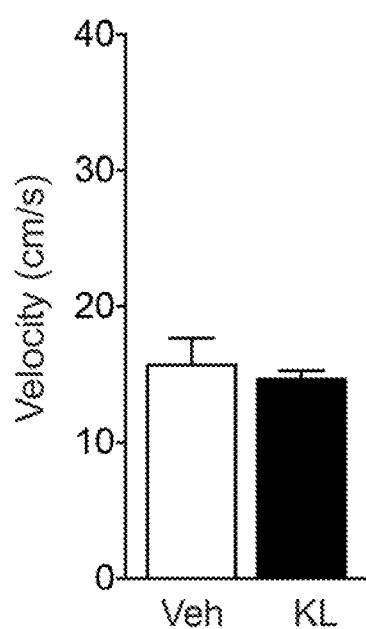


FIG. 1D

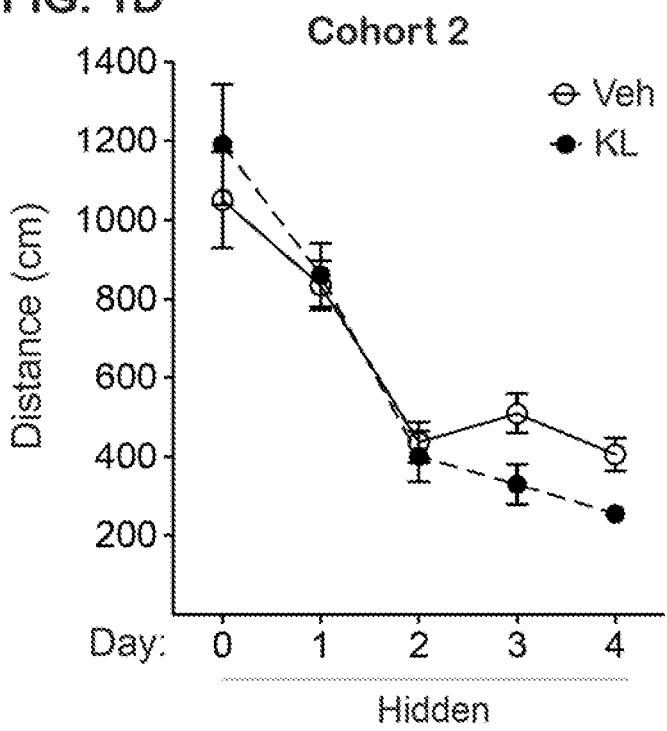


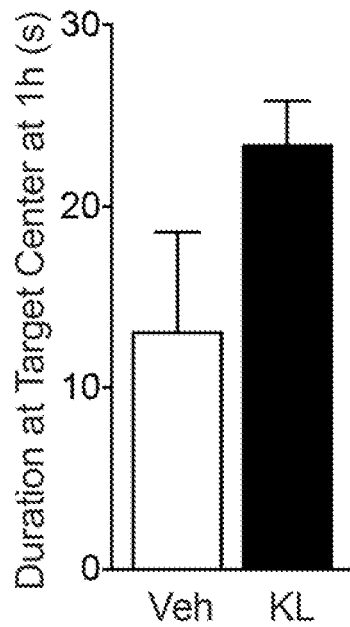
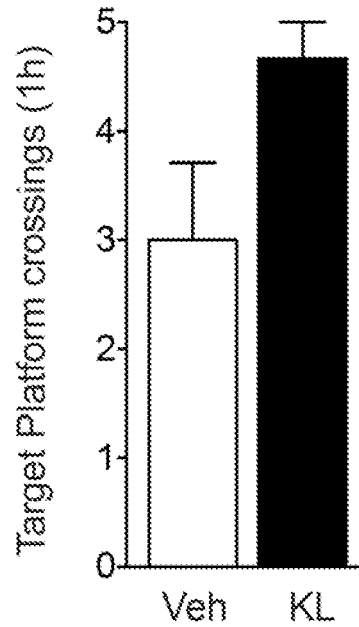
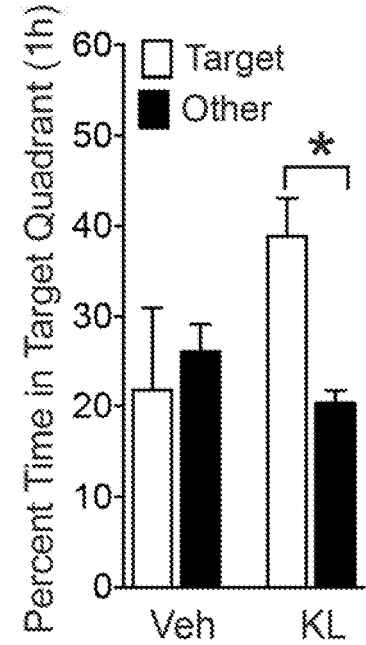
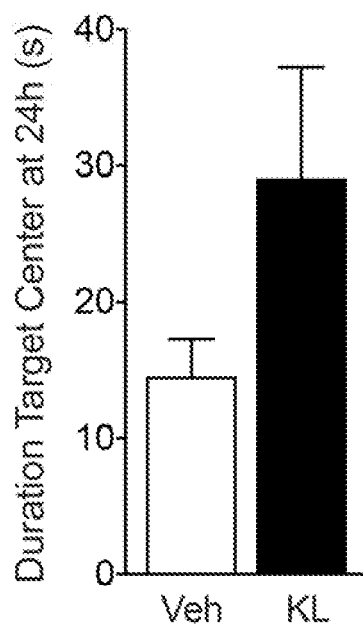
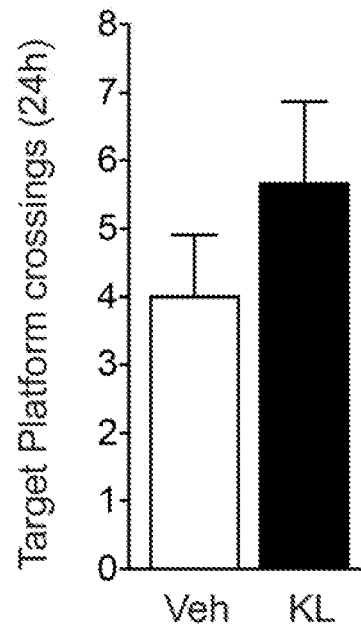
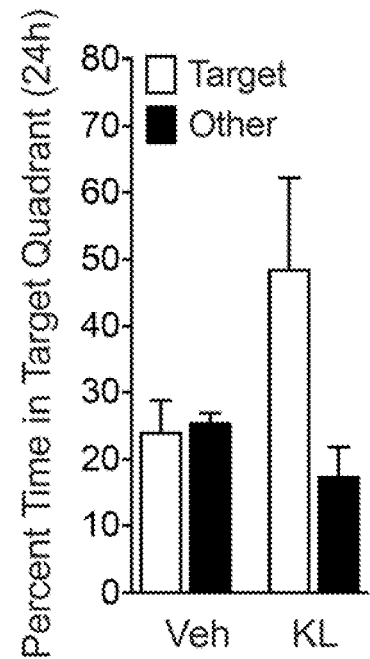
FIG. 1E**FIG. 1F****FIG. 1G****FIG. 1H****FIG. 1I****FIG. 1J**

FIG. 2A

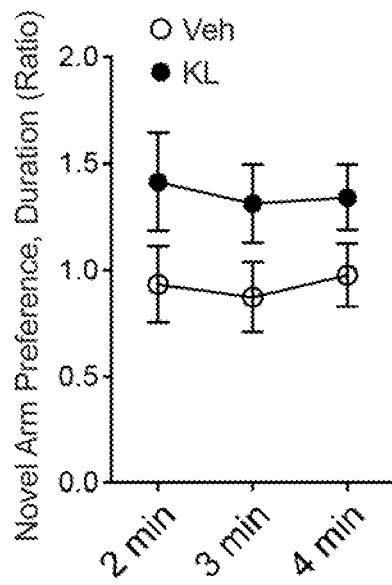


FIG. 2B

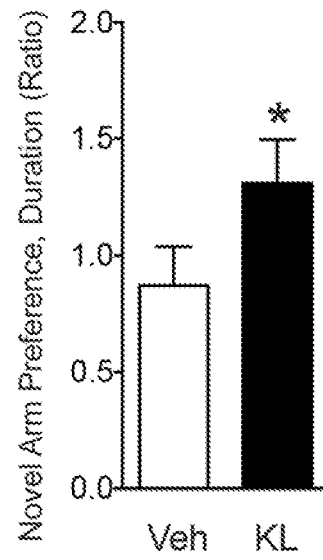


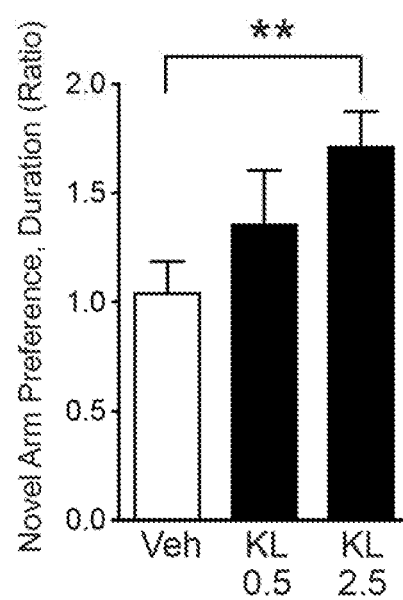
FIG. 3 Long Lasting Effect

FIG. 4A

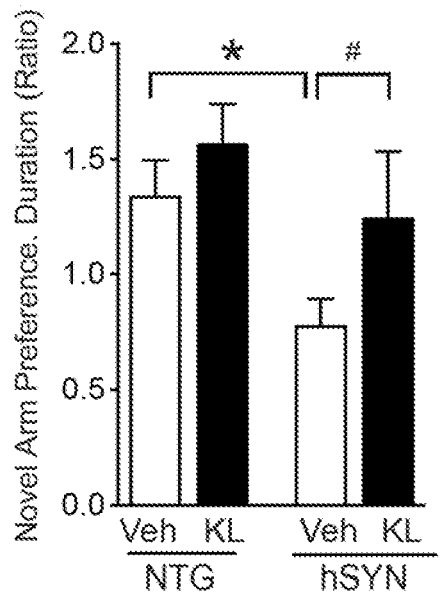


FIG. 4B

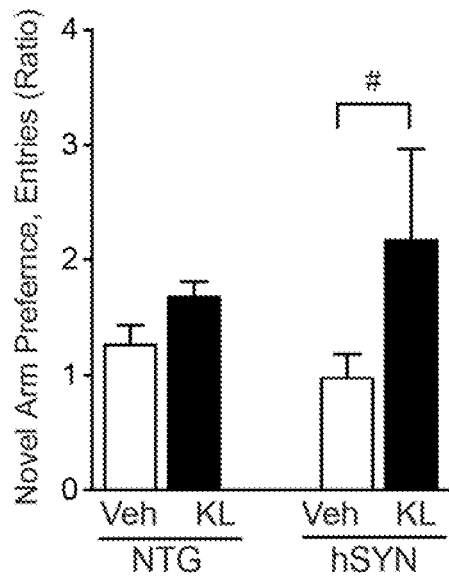


FIG. 5

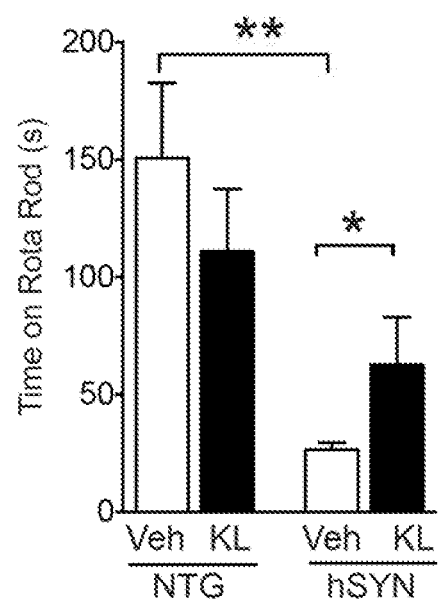
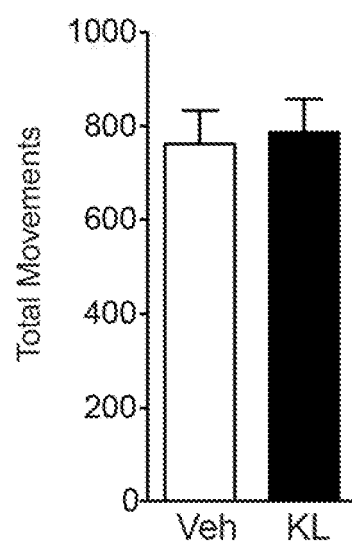


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/16842

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/17, 38/22, 38/47; C07K 14/50 (2016.01)

CPC - A61K 38/17, 38/22, 38/47; C07K 14/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (8) - A61K 38/00, 38/17, 38/22, 38/47; C07K 14/50 (2016.01); CPC -A61K 38/00; 38/02, 38/17, 38/22, 38/47; C07K 14/50, 14/72, 14/435; USPC - 424/94.61; 514/9.1, 21.2; 530/399

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH), EBSCO, Google/Google Scholar, PubMed; Klotho, Klotho polypeptide, cognitive function, cognitive enhancer, parenteral, oral, mucosal, injection, intravenous, human, patient, age related cognitive, cognitive, dementia, memory

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 8481031 B2 (GLASS, D et al.) 9 July 2013; column 4, lines 52-62; column 5, lines 1-2; column 12, lines 5-15 and 66-67; column 13, lines 1-6; column 17, lines 9-13; column 22, lines 15-18, 28-33, and 51-65	29 --- 1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 8/7/5/1-4, 9/5/1-4
Y	↖ Dubal DB et al. "Life Extension Factor Klotho Enhances Cognition" Cell Reports 22 May 2014, vol. 7, no. 4 pages 1065-1076, DOI: 10.1016/j.celrep.2014.03.076 (open access), pages 1-12; page 2, second column, second and third paragraph; page 8, second column, second paragraph	1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 8/7/5/1-4, 9/5/1-4
Y	↖ FOSTER, TC "Biological Markers of Age-Related Memory Deficits" CNS Drugs 2006, vol. 20, no. 2, pages 153-166; page 154, first column, second paragraph and second column, first paragraph	9/5/1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 March 2016 (30.03.2016)

Date of mailing of the international search report

03 MAY 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/16842

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 10-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.



(12)发明专利申请

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W02016/127097 EN 2016.08.11

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代理人 陈晓娜

(51)Int.Cl.

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A61K 38/22(2006.01)

A61K 38/47(2006.01)

C07K 14/50(2006.01)

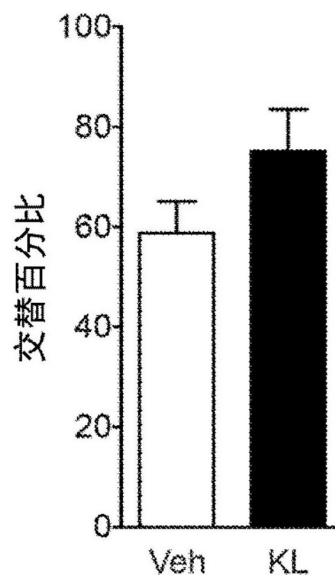
权利要求书2页 说明书22页
序列表5页 附图9页

(54)发明名称

用于改善认知的方法和组合物

(57)摘要

本文提供了用于改善个体的认知功能的klotho多肽组合物和方法,所述方法包括利用klotho多肽的治疗。



1. 一种用于改善有需要的个体的认知功能的方法,所述方法包括:
向所述个体施用有效量的包含Klotho多肽或其功能片段的蛋白,其中所述施用是全身性的或外周性的,由此改善所述个体的认知功能。
2. 权利要求1所述的方法,其中所述蛋白是Klotho多肽或其功能片段。
3. 权利要求1所述的方法,其中所述施用是口服的、粘膜的或通过注射执行。
4. 权利要求3所述的方法,其中所述注射是静脉内注射、腹膜内注射、皮下注射或肌肉注射。
5. 前述任一权利要求所述的方法,其中所述个体是人。
6. 权利要求5所述的方法,其中所述人至少具有正常的认知功能,并且与所述施用之前相比,所述施用导致改善的认知功能。
7. 权利要求5所述的方法,其中所述人的年纪为50岁以上。
8. 权利要求7所述的方法,其中所述人具有年龄相关的认知减退。
9. 权利要求5所述的方法,其中所述人的年纪小于50岁。
10. 前述任一权利要求所述的方法,其中所述个体是患有神经变性疾病的人。
11. 权利要求10所述的方法,其中所述神经变性疾病选自由下列各项组成的组:阿尔茨海默病、帕金森病、亨廷顿舞蹈病、额颞叶痴呆、进行性核上性麻痹、皮质基底节变性、轻度认知功能损害、血管性痴呆、雷维小体痴呆、多系统萎缩、肌萎缩性侧索硬化症、朊病毒病症和HIV相关痴呆。
12. 前述任一权利要求所述的方法,其中所述个体是具有选自由下列各项组成的组的病况的人:抑郁症、精神分裂症、注意力缺陷/多动症、自闭症谱系障碍、智力障碍、心境障碍和精神病性障碍。
13. 前述任一权利要求所述的方法,其中所述个体是具有选自由下列各项组成的组的病况的人:创伤性脑损伤、卒中、多发性硬化症、神经自身免疫性疾病、癫痫、谵妄和副肿瘤病症。
14. 前述任一权利要求所述的方法,其中所述个体是具有选自由下列各项组成的组的病况的人:X-连锁精神障碍、唐氏综合征、安吉尔曼综合征、雷特综合征、苯丙酮尿症、莱施-奈恩病、半乳糖血症和肾上腺脑白质营养不良。
15. 前述任一权利要求所述的方法,其中所述个体是具有选自由下列各项组成的组的病况的人:星形细胞瘤、室管膜瘤、髓母细胞瘤和少突神经胶质瘤。
16. 前述任一权利要求所述的方法,其中所述个体是接受放射治疗或化学疗法以治疗癌症的人。
17. 前述任一权利要求所述的方法,其中所述个体是正在经历或将在24小时内经历睡眠剥夺或飞行时差反应的人。
18. 前述任一权利要求所述的方法,其中所述Klotho多肽与SEQ ID NO:1的氨基酸34-534具有至少85%的同一性。
19. 前述任一权利要求所述的方法,其中所述Klotho蛋白是包含KL1结构域的功能片段。
20. 前述任一权利要求所述的方法,其中所述Klotho蛋白是包含KL1和KL2结构域的功能片段。

21. 前述任一权利要求所述的方法, 其中所述Klotho蛋白与SEQ ID NO:1的氨基酸34-979具有至少85%的同一性。

22. 前述任一权利要求所述的方法, 其中所述有效量是所述个体每kg体重1ug-1000ug。

23. 前述任一权利要求所述的方法, 其中所述Klotho多肽或其功能片段作为疗程的一部分施用超过一次。

24. 权利要求23所述的方法, 其中所述Klotho多肽或其功能片段每1-7天施用一次。

25. 前述任一权利要求所述的方法, 其进一步包括在施用后测试所述个体的认知功能。

26. 权利要求25所述的方法, 其进一步包括在施用前测试所述个体的认知功能, 并且比较所述个体在施用前后的认知功能。

27. 权利要求25或26所述的方法, 其中认知功能通过测试所述个体的语义记忆、情节记忆、程序记忆、启动记忆和/或工作记忆来确定。

28. 权利要求25或26所述的方法, 其中认知功能通过测试所述个体的语言能力、执行功能、视觉空间功能或痴呆来确定。

29. 一种改善有需要的个体的运动功能的方法, 所述方法包括:

向所述个体施用有效量的包含Klotho多肽或其功能片段的蛋白, 其中所述施用是全身性的或外周性的, 由此与所述施用之前相比改善所述个体的运动功能。

用于改善认知的方法和组合物

[0001] 相关专利申请的交叉引用

[0002] 本申请要求于2015年2月6日提交的美国临时专利申请号62/113,300的优先权权益,该申请通过引用并入。

[0003] 发明背景

[0004] 脑健康是最大的生物医学挑战之一,如果有的话,有效医疗手段也很少。认知是脑健康的很重要的和主要的表现,在正常衰老、众多的神经变性疾病、神经疾病和精神疾病、儿童发育综合征、创伤性脑损伤和应激中其受损或被扰乱。认知还被飞行时差反应(jet lag)、药物副作用和某些医疗手段,诸如用于癌症的那些所扰乱。因此,增强认知或对抗认知紊乱的潜力在整个人类生命中在健康和疾病方面具有巨大的关联性。

[0005] 发明简述

[0006] 本文提供了通过全身施用klotho或包含klotho或其功能片段的蛋白来改善认知的方法和组合物。在一些实施方案中,该方法包括改善个体的认知,包括向所述个体施用有效量的包含Klotho多肽或其功能变体或片段的蛋白,由此改善所述个体的认知功能。在一些实施方案中,所述施用是全身性的、外周性的或经鼻的。在一些实施方案中,所述蛋白是Klotho多肽或其片段。在一些实施方案中,所述施用是口服的、粘膜的或通过注射执行。在一些实施方案中,所述注射是静脉内注射、腹膜内注射、皮下注射或肌肉注射。在一些实施方案中,所述施用借助于输注,例如,使用储蓄库或微型渗透泵连续输注。

[0007] 在一些实施方案中,所述个体是人。在一些实施方案中,所述人至少具有正常的认知功能并且与所述给药之前相比所述施用导致认知功能改善。在一些实施方案中,所述人为50岁或更大的年纪(例如,至少60岁、65岁、70岁、75岁、80岁、85岁、90岁、100岁或更大的年纪)。在一些实施方案中,所述个体正在经受年龄相关的认知减退。在一些实施方案中,所述施用导致认知减退的速率减小,例如,使得短期记忆不如基于治疗前或年龄分组下降所期望的那样快速地下降。在一些实施方案中,所述个体小于50岁。

[0008] 在一些实施方案中,所述个体具患有神经变性疾病,例如,阿尔茨海默病(Alzheimer's disease)、帕金森病(Parkinson's disease)、亨廷顿舞蹈病(Huntington's disease)、额颞叶痴呆(frontotemporal dementia)、进行性核上性麻痹(progressive supranuclear palsy)、皮质基底节变性(corticobasalar degeneration)、轻度认知功能损害(mild cognitive impairment)、血管性痴呆(vascular dementia)、雷维小体痴呆(Lewy body dementia)、多系统萎缩(multiple system atrophy)、肌萎缩性侧索硬化症(amyotrophic lateral sclerosis)、朊病毒病症(prion disorder)和HIV相关痴呆(HIV-related dementia)。在一些实施方案中,所述个体患有精神或心境障碍,例如,抑郁症(depression)、精神分裂症(schizophrenia)、注意力缺陷/多动症(attention deficit/hyperactivity disorder)、自闭症谱系障碍(autism spectrum disorder)、智力障碍(intellectual disability)、心境障碍(mood disorder)或精神病性障碍(psychotic disorder)。在一些实施方案中,所述个体具有选自下列的病况:创伤性脑损伤(traumatic brain injury)、卒中(stroke)、多发性硬化症(multiple sclerosis)、神经自身免疫性疾

病(neuroautoimmune disease)、癫痫(epilepsy)、谵妄(delirium)和副肿瘤病症(paraneoplastic disorder)。在一些实施方案中,所述个体具有选自下列的病况:X-连锁精神障碍(X-linked mental disorder)、唐氏综合征(Down's syndrome)、安吉尔曼综合征(Angelman's syndrome)和雷特综合征(Rett's syndrome)。在一些实施方案中,所述个体具有选自下列的病况:苯丙酮尿症(phenylketonuria)、莱施-奈恩病(Lesch-Nyhan)、半乳糖血症(galactosemia)和肾上腺脑白质营养不良(adrenoleukodystrophy)。在一些实施方案中,所述个体正在接受放射治疗或化学疗法以治疗癌症。在一些实施方案中,所述个体正在经历或预期经历(例如,在大约2小时至2周,约12-48小时,或约24小时)应激、疼痛、睡眠剥夺或飞行时差反应。

[0009] 在一些实施方案中,所述人具有受损的运动功能,与施用前相比,所述施用导致改善的运动功能。

[0010] 在一些实施方案中,所述klotho多肽与可溶的人klotho(SEQ ID NO:1的氨基酸34-979)具有至少75%的同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%的同一性)。在一些实施方案中,所述klotho多肽是包含与人klotho的KL1结构域具有至少75%同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%同一性)的多肽的功能片段。在一些实施方案中,所述klotho多肽是包含与人klotho的KL2结构域具有至少75%同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%同一性)的多肽的功能片段。在一些实施方案中,所述klotho多肽保留了可溶性人klotho的至少一种活性水平的至少40、50、60、70、80、90或100%。

[0011] 在一些实施方案中,所述klotho多肽以0.1-50,000μg/kg体重(例如,0.5-10μg/kg,0.5-500μg/kg,1-2000μg/kg,1-250μg/kg,5-250μg/kg,10-100μg/kg,1000-20,000μg/kg,或约10,25,50,100,或1000μg/kg)的剂量施用于所述个体。在一些实施方案中,所述klotho多肽每天一次、每周两次、每周一次、或每两周一次施用。在一些实施方案中,所述klotho多肽在需要改善认知的事件之前(例如,2、6、12、24或48小时)或响应于需要改善认知的事件而施用,所述需要改善认知的事件例如,应激、飞行时差反应、睡眠剥夺或预期繁重的脑力劳动。

[0012] 在一些实施方案中,在施用前测试所述个体的认知能力。在一些实施方案中,在施用后测试所述个体的认知能力。在一些实施方案中,在施用前后,或在疗程期间多次测试所述个体的认知能力。在一些实施方案中,测试所述个体的语义记忆、情节记忆、程序记忆、启动记忆、和/或工作记忆。在一些实施方案中,测试所述个体的语言能力、执行功能、视空间功能或痴呆。在一些实施方案中,当认知能力不再显著增加时,增加klotho多肽的施用剂量或频率。

[0013] 在一些实施方案中,提供了改善运动功能的方法。在一些实施方案中,提供了改善有需要的个体的运动功能的方法,该方法包括向所述个体施用有效量的包含Klotho多肽或其功能片段的蛋白,其中所述施用是全身性的或外周性的,由此与所述施用之前相比改善所述个体的认知功能。在一些实施方案中,所述klotho多肽与可溶的人klotho(SEQ ID NO:1的氨基酸34-979)具有至少75%的同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%的同一性)。在一些实施方案中,所述klotho多肽是包含与人klotho的KL1结构域具有至少75%同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%同

一性)的多肽的功能片段。在一些实施方案中,所述klotho多肽是包含与人klotho的KL2结构域具有至少75%同一性(例如,至少80、85、90、91、92、93、94、95、96、97、98、99或100%同一性)的多肽的功能片段。在一些实施方案中,所述klotho多肽保留了可溶性人klotho的至少一种活性的水平的至少40、50、60、70、80、90或100%。在一些实施方案中,所述klotho多肽以0.1-50,000 μ g/kg体重(例如,0.5-10 μ g/kg,0.5-500 μ g/kg,1-2000 μ g/kg,1-250 μ g/kg,5-250 μ g/kg,10-100 μ g/kg,1000-20,000 μ g/kg,或约10、25、50、100、或1000 μ g/kg)的剂量施用于所述个体。在一些实施方案中,所述klotho多肽每天一次、每周两次、每周一次、或每两周一次施用。

[0014] 在一些实施方案中,运动功能受损的个体患有脑或脊髓的卒中(缺血性或出血性)、神经变性疾病(帕金森病、雷维小体痴呆、多系统萎缩、肌萎缩性侧索硬化症、朊病毒病、亨廷顿舞蹈病、核上性麻痹)、震颤麻痹(Parkinsonism)、创伤性脑损伤、神经传染性脑损伤、多发性硬化症以及相关的自身免疫和脱髓鞘疾病、脊髓损害(压缩性、传染性、毒性或代谢性、自身免疫、肿瘤性)、脑肿瘤、癫痫、副肿瘤病症、神经发育病症(线粒体、常染色体遗传)、肌肉疾病(多肌炎(polymyositis)、皮肌炎(dermatomyositis)、包涵体肌炎(inclusion body myositis)、传染性、内分泌、代谢性、毒性、先天性肌病、先天性肌营养不良(congenital muscular dystrophy)、遗传性)、神经病(吉兰-巴雷综合征(Guillain-Barre syndrome)、轴突和脱髓鞘性、糖尿病性、毒性、代谢性、传染性、危重病性、卡压性(entrapment))、蜱性麻痹(tick paralysis)、重症肌无力(myasthenia gravis)和脊髓性肌萎缩症(spinal muscular atrophy)。

[0015] 附图简述

[0016] 图1A-J.在小鼠中Klotho递送增强认知。在腹腔内(i.p.)递送赋形剂(Veh)或重组小鼠klotho(KL)(10 μ g/kg)(4月龄)后以认知任务测试小鼠。数据为平均数 $\pm$ SEM。

[0017] 图1A.在用赋形剂或klotho治疗后18小时在Y迷宫中测试小鼠。显示了4分钟探索Y迷宫期间各臂间的交替百分比(percent alternation)(n=4只雄性小鼠/组)。

[0018] 图1B-J.在每天用赋形剂或klotho治疗后在Morris水迷宫中测试小鼠(显示两个独立群组:群组1和群组2)。

[0019] 图1B.显示在群组1中当平台被隐藏时的空间学习曲线。在测试前4小时施用Veh或KL。数据代表在第1-4天到达隐藏平台所行走的总距离的每天平均值。第0天代表第1天的第一次试验所行走的距离。在第5天,当可见到平台时,Veh-和klotho-治疗的小鼠同样好地查找到该位置。(n=4只雄性小鼠/组)

[0020] 图1C.在群组1中Veh-和klotho-治疗的小鼠以相同的速度游泳,如通过在第4天来自隐藏训练的平均速率来测量。

[0021] 图1D.显示在群组2的独立小鼠群组中当平台被隐藏时的空间学习曲线。在测试前18小时施用Veh或KL。数据代表在第1-4天到达隐藏平台所行走的距离的每天平均值。(n=7-10只小鼠/组;包括雄性和雌性)

[0022] 图1E-J.在群组1中在Veh-和klotho-治疗的小鼠中在隐藏平台训练结束后1小时和24小时用来评估当移除平台时的空间记忆的探索实验的结果。

[0023] 图1E.在1小时探索中在目标中心处的逗留时间显示了在原始平台位置处所花费的时间。

[0024] 图1F在1小时探索中的目标平台跨越显示了跨越原始平台位置的频率。

[0025] 图1G.在1小时探索中,与在其他象限中所花费的平均时间相比,在目标象限中所花费的时间百分比。 $*p < 0.05$ (t-检验)

[0026] 图1H.在24小时探索中在目标中心处的逗留时间。

[0027] 图1I.在24小时探索中的目标平台跨越。

[0028] 图1J在24小时探索中在目标象限中所花费的时间百分比。

[0029] 图2A-B.短期递送klotho增强老年小鼠的认知。在训练前24小时腹膜内递送赋形剂(Veh)或重组小鼠klotho(KL) (10 μ g/kg)后测试小鼠在大型Y-迷宫中的空间和工作记忆(每个实验组n=8-9只小鼠,性别平衡组,18月龄,获自NIH colony of aging mice (NIH衰老小鼠种群))。训练后18小时,小鼠经历测试并且在探索迷宫期间测量在新异臂和熟悉的臂中所经历的逗留时间。(图2A)新异臂偏好性显示为在整个指示的探索时间内以及在(图2B)3分钟时与熟悉的臂相比在新异臂中所花费的时间比率。 $*p < 0.05$ (t-检验)。数据为平均数 $\pm$ SEM。

[0030] 图3.短期递送klotho诱导长期认知增强。在最后一次腹膜内递送赋形剂(Veh)或重组小鼠klotho(KL) (0.5或2.5 μ g/kg)后的16天时测试小鼠在大型Y-迷宫中的空间和工作记忆,其中赋形剂(Veh)或重组小鼠klotho(KL)每天给药一次持续5天(每个实验组n=8-10只小鼠,性别平衡组,5-5.5月龄)。训练后18小时,在探索迷宫期间测量在新异臂和熟悉的臂中所经历的逗留时间。新异臂偏好性显示为在5分钟探索时与熟悉的臂相比在新异臂中所花费的时间比率。 $**p = 0.01$ (Bonferroni-Holm检验)。数据为平均数 $\pm$ SEM。

[0031] 图4A-B.短期递送klotho改善表达人 α -突触核蛋白的转基因hSYN小鼠中的认知缺陷。在训练前22小时以及之后的测试前14小时腹膜内递送赋形剂(Veh)或重组小鼠klotho(KL) (2.5 μ g/kg)后,测试小鼠在大型Y-迷宫中的空间和工作记忆(每组n=6-9只雄性小鼠,2.5-6月龄)。训练后18小时,在探索迷宫期间测量在新异臂和熟悉的臂中的逗留时间和进臂次数。数据为平均数 $\pm$ SEM。

[0032] 图4A.新异臂偏好性显示为在5分钟探索时与熟悉的臂相比在新异臂中所花费的时间比率。双向ANOVA:hSYN效应 $p = 0.026$,KL效应 $p = 0.07$; $*p < 0.05$, $\#p = 0.07$ (Bonferroni-Holm检验)

[0033] 图4B.新异臂偏好性显示为在5分钟探索时与熟悉的臂相比进入新异臂的次数比率。双向ANOVA:hSYN效应 $p = 0.07$,KL效应 $p = 0.03$; $\#p = 0.07$ (t-检验)。

[0034] 图5.短期递送klotho改善表达人 α -突触核蛋白的转基因hSYN小鼠中的早期运动缺陷。在测试前大约17小时腹膜内递送赋形剂(Veh)或重组小鼠klotho(KL) (2.5 μ g/kg)后测试小鼠在旋转任务(rotarod task)中的运动功能(每个实验组n=6-10只雄性小鼠,2.5-6月龄)。在旋转棒上没有掉落所经过的时间以秒(s)表示。在hSYN小鼠中,KL-治疗改善了早期运动功能,如在试验1-3期间在旋转棒上所经过的平均逗留时间增加所示。双向ANOVA:hSYN效应0.0025; $**p < 0.01$, $*p < 0.05$ (Bonferroni-Holm检验)。数据为平均数 $\pm$ SEM。

[0035] 图6.短期递送klotho不改变小鼠的总活动。在腹膜内递送赋形剂(Veh)或重组小鼠klotho(KL) (2.5 μ g/kg)后的16小时,测试小鼠在旷场任务中的总运动和总活动水平(每个实验组n=8只小鼠;性别平衡组;5月龄)。绘制了5分钟内的总运动。数据为平均数 $\pm$

SEM。

[0036] 发明详述

[0037] I. 介绍

[0038] Klotho是相对大的蛋白,其分泌形式大约130kD,且预期不会穿越血脑屏障。然而,本文提供的结果显示Klotho在几小时的全身给药内以及其半衰期清除后长时间地对认知发挥阳性作用。这些结果是非常出人意料地,并且与直接向大脑给药或直接进入脑脊液相比提供了更安全、更便利的疗法的优势。

[0039] II. 定义

[0040] 除非另外规定,否则本文所用的技术和科学术语具有与本领域普通技术人员所普遍理解相同含义。参见,例如,Lackie,DICTIONARY OF CELL AND MOLECULAR BIOLOGY(细胞生物学词典),Elsevier(第4版,2007);Sambrook等人,MOLECULAR CLONING,A LABORATORY MANUAL(分子克隆,实验室手册),Cold Springs Harbor Press(Cold Springs Harbor,NY 1989)。与本文所述的那些相似或等效的任何方法、装置和材料可以用于本发明的实施中。提供下列定义以促进对本文中频繁使用的某些术语的理解并且不意在限制本公开的范围。

[0041] 除非另外指明,术语“klotho”或“klotho多肽”是指可溶性klotho多肽,以及其功能变体和片段。可溶性klotho是在流体(例如,血清、脑脊液等)中循环的任何形式的klotho,其不包括跨膜或细胞内成分。Klotho可以由其跨膜形式裂解并释放到流体中,或以另外的方式由细胞分泌或脱落。Klotho RNA也可以备选地被剪接并且直接分泌至周围流体中(即,不形成跨膜蛋白)。两种形式包括在术语可溶性klotho多肽、klotho多肽和klotho中。

[0042] 如本文所用,术语“全身性”或“外周性”是指通过不涉及直接注射(或其他给药)至脑脊液(CSF)或中枢神经系统(CNS)的途径的给药。即,全身性和外周性给药涵盖给药至血脑屏障的“血液”侧。全身和外周途径的实例包括口服和粘膜、静脉内、腹膜内、肌肉内和皮下注射,以及静脉内滴注。

[0043] 术语“认知”、“认知能力”、“认知功能”和类似术语是指思维任务和功能的集合体,包括但不限于:记忆(例如,语义记忆、情节记忆、程序记忆、启动记忆或工作记忆);定向;语言;解决问题;视觉感知,构造和整合;计划;组织技能;选择性注意;抑制性控制;和心理处理信息的能力。

[0044] 术语“改善的认知”、“增加的认知能力”、“改善的认知功能”和类似术语是指在给定的条件(例如,用klotho治疗)下与缺少该条件(例如,缺少利用klotho的治疗)下的认知相比,认知有改善。对于经历认知减退的个体来说,认知的改善可以是认知减退的速率减慢(即,与缺少治疗相比的改善),而不是认知能力的实际改善。认知能力的增加也可以是在特定区域中脑活动的增加,例如,如通过MRI测定,或抑制脑活动导致更好的全脑功能。认知能力的增加也可以是如本文更详细描述的认知表现的改善。认知能力的改善或增加可以是在任一个认知方面或功能中,或单个认知功能的任意组合中。

[0045] 需要改善认知功能的个体是指具有下述的个体:年龄相关的认知减退;神经变性疾病;精神或心境障碍;创伤性脑损伤;发育迟缓;导致认知能力下降的遗传性疾病;由于卒中、脑癌、MS、癫痫、放疗或化疗引起的脑损伤等。需要改善认知功能的个体还可以包括希望

增加心理机能以对抗应激、睡眠剥夺、飞行时差反应、或疼痛的作用,或为了加强对特定工作的能力的个体。这类个体的更完全和特定的列表包括在本文的“认知病况和障碍”章节中。

[0046] 词语“蛋白”、“肽”和“多肽”可互换地用来说明氨基酸聚合物或一组两种以上相互作用的或结合的氨基酸聚合物。这些术语适用于其中一个或多个氨基酸残基是相应的天然存在的氨基酸的人工化学模拟物的氨基酸聚合物,以及天然存在的氨基酸聚合物,含有修饰残基的那些,以及非天然存在的氨基酸聚合物。

[0047] 术语“氨基酸”是指天然存在的和合成的氨基酸,以及功能上与天然存在的氨基酸相似的氨基酸类似物和氨基酸模拟物。天然存在的氨基酸是由遗传密码编码的那些,以及以后被修饰的那些氨基酸,例如,羟脯氨酸, γ -羧基谷氨酸盐和O-磷酸丝氨酸。氨基酸类似物是指与天然存在的氨基酸具有相同的基本化学结构的化合物,例如,与氢、羧基、氨基、和R基团结合的 α 碳,例如,高丝氨酸、正亮氨酸、蛋氨酸亚砷、蛋氨酸甲基砷。这些类似物可以具有修饰的R基团(例如,正亮氨酸)或修饰的肽主链,但保留了与天然存在的氨基酸相同的基本化学结构。氨基酸模拟物是指具有与氨基酸的一般化学结构不同的结构但功能与天然存在的氨基酸相似的化学化合物。

[0048] 在本文中氨基酸可以由它们普遍已知的三字母符号或由IUPAC-IUB生物化学命名委员会(IUPAC-IUB Biochemical Nomenclature Commission)所推荐的单字母符号来表示。同样地,核苷酸可以由它们普遍接受的单字母代码来表示。

[0049] “保守修饰的变体”适用于氨基酸和核酸序列两者。关于特定的核酸序列,保守修饰的变体是指编码相同的或基本上相同的氨基酸序列的那些核酸,或其中所述核酸不编码氨基酸序列的情况下,是指基本上相同的或相关联的(例如,天然相邻的)序列。由于遗传代码的简并性,大量功能上相同的核酸编码大多数蛋白质。例如,密码子GCA,GCC,GCG和GCU均编码氨基酸丙氨酸。因此,在密码子指定丙氨酸的每一个位置处,该密码子可以被改变成所述的对应密码子中的另一个,而不会改变所编码的多肽。这样的核酸变异是“沉默变异”,其是保守修改变异中的一类。本文中的编码多肽的每个核酸序列还描述了核酸的沉默变异。技术人员将认识到在某些背景下,核酸中的每个密码子(除了AUG和TGG以外,AUG通常是仅用于蛋氨酸的密码子,TGG通常是仅用于色氨酸的密码子)可以被修饰以产生功能上相同的分子。因此,编码多肽的核酸的沉默变异在所述的序列中关于表达产物经常是不明确的,但关于实际的探针序列则是明确的。

[0050] 对于氨基酸序列,技术人员将认识到对于核酸、肽、多肽或蛋白序列的单个置换、缺失或添加(其在所编码的序列中改变、添加或删除单一氨基酸或小百分比的氨基酸)是“保守修饰的变体”,其中所述改变导致氨基酸被化学上相似的氨基酸置换。提供功能上相似的氨基酸的保守置换表在本领域中是众所周知的。这样的保守修饰变体是另外的,并且不排除本发明的多形态变体、种间同系物和等位基因。下列的氨基酸是典型的相互的保守置换:1) 丙氨酸(A),甘氨酸(G);2) 天冬氨酸(D),谷氨酸(E);3) 天冬酰胺(N),谷氨酰胺(Q);4) 精氨酸(R),赖氨酸(K);5) 异亮氨酸(I),亮氨酸(L),蛋氨酸(M),缬氨酸(V);6) 苯丙氨酸(F),酪氨酸(Y),色氨酸(W);7) 丝氨酸(S),苏氨酸(T);和8) 半胱氨酸(C),蛋氨酸(M)(参见,例如,Creighton,Proteins(蛋白)(1984))。

[0051] 在两个以上核酸,或两个以上多肽的上下文中,术语“相同的”或“同一性百分比”

是指这样的两条以上序列或子序列：它们是相同的或具有指定百分比的相同的核苷酸或氨基酸（即，当在比较窗上或标注区域上比较和比对最大对应性时，在特定区域上约60%的同一性，优选65%，70%，75%，80%，85%，90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或更高的同一性），如使用具有缺省参数的BLAST或BLAST 2.0序列比较算法测量，或通过人工比对和目测测量。参见，例如，NCBI网址ncbi.nlm.nih.gov/BLAST。这样的序列然后称为“基本上相同”。此定义也称为，或可以适用于核苷酸测试序列的编辑。该定义还包括具有缺失和/或添加的序列，以及具有置换的那些序列。如下所述，算法可以说明空位等。典型地，同一性在包括抗体表位的区域上存在，或在长度为至少约25个氨基酸或核苷酸的序列上存在，或在长度为50-100个氨基酸或核苷酸的区域上存在，或在参比序列的整个长度上存在。

[0052] 术语“重组”当关于例如，细胞、或核酸、蛋白或载体使用时，指示所述细胞、核酸、蛋白或载体已经通过引入异源核酸或蛋白或改变天然核酸或蛋白而被修饰，或所述细胞来源于如此被修饰的细胞。因此，例如，重组细胞表达所述细胞的天然（未重组）形式内不存在的基因或表达以另外的方式被异常表达、表达下调或根本不表达的天然基因。

[0053] 术语“异源”当关于蛋白或核酸的部分使用时，指示所述蛋白或核酸包含两条以上在自然界中没有发现彼此处于相同关系的子序列。例如，所述蛋白或核酸典型地被重组产生，具有两条以上来自不相关基因的序列，所述序列排列以组成新的功能核酸，例如，来自一个来源的启动子，和来自另一个来源的编码区，或功能性嵌合蛋白。

[0054] 术语“激动剂”、“激活剂”、“诱导剂”和类似术语是指与对照相比增加（例如，klotho或klotho信号传导途径）活性或表达的试剂。激动剂是例如，刺激、增加、激活、增强激活、敏化或上调klotho或klotho信号传导途径的活性的试剂。所述表达或活性可以比对照中增加10%、20%、30%、40%、50%、60%、70%、80%、90%、100%或更多。在某些情况下，与对照相比，所述激活是1.5-倍、2-倍、3-倍、4-倍、5-倍、10-倍或更高。

[0055] 术语“抑制剂”、“阻遏物”或“拮抗剂”或“下调节剂”可互换地指与对照相比导致可检测到地较低表达或活性水平的物质。被抑制的表达或活性可以是对照的10%，20%，30%，40%，50%，60%，70%，80%，90%或更少。在某些情况下，与对照相比，所述抑制是1.5-倍、2-倍、3-倍、4-倍、5-倍、10-倍或更高。

[0056] “对照”样品或值是指充当参比的样品，通常是已知的参比，用于与测试样品比较。例如，测试样品可以取自测试条件，例如，在测试化合物的存在下，并且与来自已知条件的样品进行比较，例如，在缺少测试化合物的条件下（阴性对照），或在已知化合物的存在下（阳性对照）。对照还可以代表由多个测试或结果收集的平均值。本领域技术人员将认识到对照可以设计用于评估任何数量的参数。例如，对照可以被设计成基于药理学数据（例如，半衰期）或治疗措施比较治疗益处（例如，比较益处和/或副作用）。对照可以被设计用于体外应用。本领域技术人员将理解哪种对照在给定的情况下是有价值的并且能够基于与对照值的比较分析数据。对照还有价值的用于确定数据的显著性。例如，如果用于给定参数的值在对照中大幅变化，则测试样品中的变化将不被认为是显著的。

[0057] “标记”或“可检测部分”是可通过光谱学、光化学、生物化学、免疫化学、化学或其他物理手段检测到的组成成分。例如，可用的标记包括³²P、荧光染料、电子致密试剂、酶（例如，如ELISA中所普遍使用的）、生物素、洋地黄毒苷、或半抗原和蛋白或可以制成为可检测

到的其他实体,例如,通过将放射性标记掺入到可与靶肽特异性反应的肽或抗体中。可以采用用于将蛋白缀合到标记上的任何已知方法,例如,使用Hermanson,Bioconjugate Techniques (生物缀合技术) 1996, Academic Press, Inc., San Diego中所述的方法。

[0058] “标记的”分子(例如,klotho多肽)是通过接头或化学键共价结合,或通过离子键、范德华力、静电或氢键非共价结合标记,使得分子的存在可以通过检测与所述分子结合的标记的存在来检测到。

[0059] 术语“诊断”是指在个体中存在病症的相对可能性。相似地,术语“预后”是指某种未来结果可能在所述个体中发生的相对可能性。例如,在本公开的背景下,预后可以指个体患有认知减退的可能性,或疾病的可能严重性(例如,症状的严重性,功能减退的速率等)。所述术语不意在是绝对的,如医学诊断领域中任一技术人员所理解的那样。

[0060] “生物样品”可以获自患者,例如,活检,获自动物,诸如动物模型,或获自培养的细胞,例如,从患者移除并且在培养物中生长的用于观察的细胞系或细胞。生物样品包括组织和体液,例如,脑脊液(CSF)、血液、血液级分、淋巴、唾液、尿液、粪便等。

[0061] 术语“疗法”、“治疗”和“改善”是指症状(认知减退)的严重性的任何减少,或认知功能的改善,或在运动功能受影响的情况下,指运动功能的改善。如本文所用,术语“治疗”和“预防”不意在是绝对的术语。治疗和预防可以指认知减退的任何延缓,症状(例如,思维混乱、谵妄)的改善等。治疗和预防可以是完全的或部分的,使得认知好于不治疗情况下所预期的认知(例如,与治疗前同一个体中的认知相比或与相似的未治疗个体中的认知相比)。治疗的作用可以与不接受治疗的个体或大量个体相比较,或与治疗前或处于治疗期间不同时间的同一患者相比较。在一些方面,与例如,给药前的个体或与不经历治疗的对照个体相比,认知提高至少1%。在一些实施方案中,使用认知测试、分子代用品(molecular proxies)、或与脑功能相关联的结构变化确定,认知提高至少2,3,5,7,10,15,20,25%,50%,75%,80%,或90%或更高。在一些方面,与例如,给药前的个体或与不经历治疗的对照个体相比,运动功能提高至少1%。在一些实施方案中,使用运动功能的测试确定,运动功能提高至少2,3,5,7,10,15,20,25%,50%,75%,80%,或90%,或更高。

[0062] 术语“有效量”、“有效剂量”、“治疗有效量”等是指足以改善如上所述的病症的治疗剂的量。例如,对于给定的参数,治疗有效量将显示疗效增加或减少至少1%,2%,5%,10%,15%,20%,25%,40%,50%,60%,75%,80%,90%,或至少100%。治疗效力也可以表示为“-倍”增加或降低。例如,治疗有效量可以产生对照的至少1.2-倍、1.5-倍、2-倍、5-倍或更多的作用。

[0063] 如本文所用,术语“药学上可接受的”与生理学上可接受的和药理学上可接受的作为同义词使用。药学组合物通常将包含用于缓冲和储存保存的试剂,并且可以包含缓冲液和用于适宜地递送的载体,这取决于给药途径。

[0064] 术语“剂量”和“药量”在本文中可互换使用。剂量是指在每次给药时给予个体的活性成分的量。对于本发明,剂量是指Klotho多肽的量。剂量将根据许多因素而变化,包括给药频次;个体的尺寸和耐受性;病况的类型和严重性;副作用的风险;以及给药途径。本领域技术人员将认识到可以根据上述因素或基于治疗进展修改剂量。术语“剂型”是指药物的特定形式,并且取决于给药途径。例如,剂型可以是液体,例如注射用盐水溶液。

[0065] “受试者”、“患者”、“个体”等术语可互换使用,并且除了指示的情况以外,是指哺

乳动物诸如人和非人灵长类动物,以及犬、马、猪、小鼠、大鼠和其他哺乳动物物种。所述术语不必要指示所述受试者已经被诊断为特定的疾病,但典型地是指处于医疗监督下的个体。患者可以是正在寻求治疗、监测、调整或修改现有治疗方案等的个体。

[0066] III.Klotho

[0067] Klotho是一种多效蛋白和衰老调节剂,其在整个身体和大脑内循环(Imura等人(2004)FEBS Letters 565:143;Kurosu等人(2005)Science 309:1829)。人Klotho记述于GenBank登录号NC_000013和Uniprot登录号Q9UEF7。存在多种物种同源物,包括小鼠和大鼠Klotho,它们与人Klotho多肽(显示为SEQ ID NO:1)具有86%和85%的同一性。其以跨膜形式存在,所述跨膜形式可以被裂解使得细胞外部分(氨基酸34-979)作为激素释放(Shiraki-ika等人(1998)FEBS Letters 424:6)。Klotho还具有剪接变体,所述剪接变体产生了蛋白的549个氨基酸分泌形式,该分泌形式也是有功能的(Wang和Sun(2009)Ageing Res.Rev.8:43)。裂解的和分泌的klotho两者都是可溶的并且在身体中都是有功能的,但由于剪接变化在C-末端处具有序列变化。氨基酸535-549是用于裂解的可溶性人Klotho的DTTLSQFTDLNVYLW(SEQ ID NO:2),以及用于剪接的可溶性人Klotho的SQLTKPISSLTKPYH(SEQ ID NO:3)。全长可溶性Klotho包含两个与β糖苷酶蛋白具有同源性的保守结构域(KL1和KL2)。保守的β-葡萄糖苷酶/6-磷酸-β-葡萄糖苷酶/β-半乳糖苷酶基序在人蛋白中跨越62-497,在小鼠中跨越64-499。保守的KL1序列记述于Chateau等人(2010)Aging 2:567和Matsumura等人(1998)Biochem Biophys Res Commun中,并且包含人Klotho蛋白的氨基酸34-549,其中糖苷水解酶共有区跨越人Klotho蛋白的氨基酸57-506(小鼠的59-508)。Klotho不具有β-糖苷酶活性,但显示一些β-葡萄糖醛酸糖苷酶活性。

[0068] Klotho抑制胰岛素和wnt信号传导,调节离子通道和它们的运输,并且促进FGF23的功能。参见,例如,Chang等人(2005)Science 310:490;Imura等人(2007)Science 316:1615;Kurosu(2005);Liu等人(2007)Science 317:803;和Urakawa等人(2006)Nature 444:770)。

[0069] 在小鼠中,klotho的转基因过表达延长寿命并且与正常和患病大脑中更好的认知功能相关(Kurosu(2005);Dubal等人(2014)Cell Reports 7:1065;Dubal等人(2015)J.Neuroscience)。在人类中,KLOTHO基因的KL-VS变体的单一等位基因增加分泌的klotho,促进长寿(Arking等人,2002;Arking等人,2005;Invidia等人,2010)并且也与衰老人群中较好的基线认知功能相关。参见,例如,Arking等人(2002)PNAS 99:856;Arking等人(2005)Circ.Res.96:412;Dubal等人(2014)Cell Reports 7:1065;Yokoyama等人(2015)Ann.Clin.Translational Neurology 2:215。

[0070] 可以用于给药的Klotho多肽包括物种同源物(例如,非人灵长类动物,小鼠,大鼠),等位基因变体,功能片段,以及保留了认知改善活性的野生型序列的功能变体。实例包括分泌的Klotho,包含KL1结构域的片段,包含KL2结构域的片段,包含KL1和KL2结构域的片段,包含KL1结构域的变体(其中KL1结构域中的至少一个(例如,1-20个,5-50个,25-100个)非保守氨基酸被不同的氨基酸置换或缺失),包含KL2结构域的变体(其中KL2结构域中的至少一个非保守氨基酸被不同的氨基酸置换)。

[0071] 如本文所述可以使用的Klotho多肽的功能片段包括细胞外结构域(例如,与人Klotho的氨基酸34-979对应或基本上相同或相似),分泌的Klotho(例如,与549个氨基酸的

形式对应或基本上相同或相似), KL1结构域(例如, 与人Klotho的氨基酸34-549对应或基本上相同或相似), 糖基水解酶共有序列(例如, 与人Klotho的氨基酸57-506对应或基本上相同或相似), 或 β -葡萄糖苷酶/6-磷酸- β -葡萄糖苷酶/ β -半乳糖苷酶共有序列(例如, 与人Klotho的氨基酸62-497对应或基本上相同或相似)。在一些实施方案中, Klotho多肽包含人Klotho的氨基酸34-549或与人Klotho的氨基酸34-549基本上相同或相似。在一些实施方案中, Klotho多肽是较大融合蛋白的一部分。在一些实施方案中, 所述融合蛋白包含如本文所述的Klotho多肽并且进一步包含不超过100、75、50或30个额外的氨基酸。在一些实施方案中, Klotho多肽不与成纤维细胞生长因子(FGF)融合。在一些实施方案中, Klotho多肽包含(例如, 融合至)亲和标签(例如, 组氨酸标签)或缀合物以增加体内的稳定性或半衰期。

[0072] Klotho的功能变体或片段是保留klotho任意活性的变体或片段, 例如, 可溶性klotho的任意活性的水平的至少20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 或100%。可溶性klotho活性包括上述的那些, 并且包括结合FGF-23, 结合至FGFR1c, β -葡萄糖醛酸糖苷酶活性, 抑制wnt信号传导, 抑制胰岛素信号传导, 抑制TGF- β 1活性, 增加GluN2B表达和/或突触定位, c-fos诱导。其他的Klotho活性包括导致核磁共振成像(MRI)脑扫描的改变, 例如, 功能性MRI、脑电图(EEG)、和经颅磁电刺激(TMS和TES); 以及在神经心理测试和认知能力方面的改善的表现。

[0073] IV. Klotho的给药

[0074] 本文提供了改善个体的认知和/或运动功能的方法, 包括向所述个体施用Klotho。在一些实施方案中, 治疗方法包括向个体施用有效量的Klotho多肽(或其功能变体或片段)。在一些实施方案中, 所述治疗是预防性的, 例如, 用于预期应激(例如, 飞行时差反应, 军事表演(military performance))的个体或用来预防与衰老相关的认知减退。在一些实施方案中, 所述个体已经被诊断患有认知病症。在一些实施方案中, 所述个体正在接受或已经接受了用于认知病症的疗法或用于与认知功能相关的病况(例如, 响应于化疗的认知减退)的疗法。

[0075] 在一些实施方案中, 所述方法进一步包括通过分子代用品(例如, 改变脑中的NMDA受体或c-fos激活, 或GluN2B水平), MRI脑扫描的变化(例如, 功能性MRI), EEG的改变, TMS和TES的改变, 神经心理测试评分的改变, 或认知能力测试(例如, 用于学习、短期或长期记忆、执行功能、语言能力和视空间功能)来监测所述个体的认知能力。在一些实施方案中, 使用上述测试中的一种以上以任意组合监测所述个体。在一些实施方案中, 基于所述个体的治疗进展来确定每次给药Klotho多肽的剂量, 例如, 如果所述个体对疗法反应不足则施用较高的剂量。

[0076] 在一些实施方案中, Klotho多肽以含有生理学上(即, 药学上)可接受的载体的药学组合物施用。术语“载体”是指用作诊断或治疗药剂的稀释剂或赋形剂的典型惰性物质。该术语还涵盖对所述组合物施加粘聚性的典型惰性物质。生理学上可接受的载体可以是液体, 例如, 生理盐水, 磷酸盐缓冲液, 生理缓冲盐水(normal buffered saline, 135-150mM NaCl), 水, 缓冲水, 0.4%盐水, 0.3%甘氨酸, 糖蛋白以提供增强的稳定性(例如, 白蛋白, 脂蛋白, 球蛋白等)等。由于生理学可接受的载体部分地由被施用的特定组合物以及用来施用该组合物的特定方法来决定, 因此本发明的药物组合物存在大量的合适制剂(参见, 例如, Remington's Pharmaceutical Sciences, 第17版, 1989)。

[0077] 本发明所述的组合物可以通过常规的、众所周知的灭菌技术来灭菌,或可以在无菌条件下制备。水溶液可以被包装用于使用或在无菌条件下过滤并冻干,在给药前所述冻干的制剂与无菌水溶液混合。所述组合物可以根据需要含有药学上可接受的辅料物质以接近生理条件,诸如pH调节剂和缓冲剂、渗透压调节剂、润湿剂,等等,例如,乙酸钠、乳酸钠、氯化钠、氯化钾、氯化钙、失水山梨醇单月桂酸酯和油酸三乙醇胺。也可以包含糖类用于稳定所述组合物,诸如用于冻干的抗体组合物的稳定剂。

[0078] 剂型可以制备用于粘膜(例如,鼻的、舌下、阴道、含服、或直肠)、胃肠外(例如,皮下、静脉内、肌肉内或动脉内注射,推注或输注)、口服或透皮给药给患者。剂型的实例包括,但不限于:分散体;栓剂;油膏;泥敷剂(膏状药);糊剂;粉针剂;敷料;乳膏剂;贴膏剂;溶液剂;贴剂;气雾剂(例如,喷鼻剂或吸入剂);凝胶剂;适合于口服或粘膜施用给患者的液体剂型,包括混悬剂(例如,水性或非水性液体混悬液、水包油乳液,或油包水液体乳液)、溶液剂和酏剂;适合于胃肠外施用至患者的液体剂型;和可以被重构以提供适合用于胃肠外施用至患者的液体剂型的无菌固体(例如,结晶固体或非晶态固体)。

[0079] 可注射组合物可以包括悬浮在可接受的载体诸如水性载体中的Klotho多肽的溶液。可以使用多种水性载体中的任一种,例如,水,缓冲水,0.4%盐水,0.9%等渗盐水,0.3%甘氨酸,5%葡萄糖等,并且可以包含糖蛋白用于增加稳定性,诸如白蛋白,脂蛋白,球蛋白等。在一些实施方案中,使用生理缓冲盐水(135-150mM NaCl)。所述组合物可以包含药学上可接受的辅料物质以接近生理条件,诸如pH调节剂和缓冲剂、张力调节剂、润湿剂,例如,乙酸钠、乳酸钠、氯化钠、氯化钾、氯化钙、失水山梨醇单月桂酸酯、油酸三乙醇胺等。

[0080] 适合于胃肠外给药(诸如,例如,通过关节内(在关节内)、静脉内、肌肉内、皮内、腹膜内和皮下途径给药)的剂型,包括水性和非水性、等渗无菌注射液,其可以包含抗氧化剂、缓冲液、抑菌剂和使得所述制剂与预期接受者的血液等渗的溶质,以及水性和非水性无菌混悬液,其可以包含混悬剂、增溶剂、增稠剂、稳定剂和防腐剂。注射液和混悬液也可以由无菌粉针剂、颗粒剂和片剂制备。在一些实施方案中,所述组合物通过静脉内输注、局部地、腹膜内、膀胱内或鞘内给药。Klotho多肽制剂可以以单位剂量或多剂量密封容器,诸如安瓿和小瓶来提供。

[0081] Klotho多肽组合物,单独地或与其他合适的组分组合,可以制成为气雾剂制剂(“被雾化”)从而经吸入给药。气雾剂制剂可以被置于可接受的加压推进剂中,诸如二氯二氟甲烷、丙烷和氮中。

[0082] 药物制剂可以以单位剂型的方式包装或制备。以这种形式,制剂被细分成含有适当量的活性成分(例如,根据Klotho多肽的剂量)的单位剂量。单位剂型可以是包装的制剂,所述包装含有不同量的制剂。如果需要,所述组合物也可以含有其他配伍的治疗剂。在一些实施方案中,Klotho多肽组合物可以配制在用于给药的试剂盒中。

[0083] 在一些实施方案中,包含klotho多肽的药物组合物口服给药。在一些实施方案中,包含klotho多肽的药物组合物粘膜给药,例如,经鼻给药。在一些实施方案中,包含klotho多肽的药物组合物通过注射给药,例如,皮下、腹膜内、静脉内或肌肉内注射。在一些实施方案中,包含klotho多肽的药物组合物通过输注给药,例如,使用储蓄库或微型渗透泵进行。

[0084] 药物组合物的给药实例包括将Klotho多肽以10mg/ml在4℃保存在无菌等渗盐水中,并在向患者给药前将其稀释在适宜的注射用溶液中。在一些实施方案中,Klotho多

肽组合物可以通过静脉内输注在0.25-2小时的时程内给药。在一些实施方案中,所述给药程序经由推注进行。

[0085] 在治疗应用中,Klotho多肽可以以约0.1 μ g/kg至约1000 μ g/kg每天一次的初始剂量给药并随时间推移而进行调整。可以使用约1 μ g/kg至约500 μ g/kg,或约10 μ g/kg至约100 μ g/kg,或约30 μ g/kg至约50 μ g/kg的日剂量范围。剂量根据患者的需求、被治疗的病况的严重性和给药途径而变化。例如,对于Klotho多肽的注射,有效剂量典型地在10-100 μ g/kg的范围内,而对于向中枢神经系统(CNS)的直接递送,有效剂量较低,例如,5-30 μ g/kg。对于口服给药,有效剂量较高,例如,在50-10,000 μ g/kg(例如,100 μ g/kg-2mg/kg)的范围内。选择剂量以便为患者提供有效的治疗。剂量可以以适宜的频率重复,所述频率可以在每天一次或两次,每周一次或两次至每三个月一次的范围内,取决于Klotho多肽组合物的药代动力学(例如,循环中的半衰期)和药物动力学响应(例如,治疗效应的持续时间)。

[0086] 给药可以是周期性的。取决于给药途径,所述剂量可以例如,每1、3、5、7、10、14、21或28天或更长时间施用一次(例如,每2、3、4或6个月一次)。在一些情况下,给药更为频繁,例如,每天2或3次。可以监测患者以根据治疗进展和任何不良的副作用调整给药的剂量和频次,如本领域技术人员所认识的那样。

[0087] 可以考虑特定患者所诊断的认知病况的类型和严重性来经验性地确定剂量。在本公开的背景下,给药至患者的剂量应当足以随时间推移影响患者中的有益治疗反应。剂量的大小也将通过伴随任何特定组合物在特定患者中的给药的任何不良副作用的存在、性质和程度来确定,如熟练的执业医师所认识的那样

[0088] 在一些实施方案中,将Klotho多肽组合物给药于至少具有正常认知功能的个体(例如,人)。如本文所述,已经出人意料地发现Klotho不仅可以改善认知受损的个体的认知,而且Klotho还可以改善至少具有正常认知的个体的认知。因此在一些实施方案中,接受Klotho多肽组合物的个体初始地以至少具有正常认知开始,并且在施用Klotho多肽组合物后,与初始认知水平相比获得提高的认知。个体的认知水平可以如本领域中已知的那样确定。正常认知功能通过来自多组认知测试的评分确定,所述评分被编译成总认知评分,如Dubal DB等人(2014) Cell Reports 7:1065-1076所述。这样的认知测试包括执行功能和工作记忆的测试,诸如实验A和实验B(Dubal DB等人(2014) Cell Reports 7:1065-1076)。在一些实施方案中,Klotho的给药导致认知(无论初始是至少正常的还是受损的)提高至少5%、10%、20%或更多。

[0089] 在一些实施方案中,给药导致改善的运动功能。在一些实施方案中,Klotho多肽组合物给药于运动功能受损的个体(例如,人)。例如,在一些实施方案中,所述个体具有脑或脊髓的卒中(缺血性或出血性)、神经变性疾病(帕金森病、雷维小体痴呆、多系统萎缩、肌萎缩性侧索硬化症、朊病毒病症、亨廷顿舞蹈病、核上性麻痹)、震颤麻痹、创伤性脑损伤、神经传染性脑损伤、多发性硬化症和相关的自身免疫和脱髓鞘疾病,脊髓病变(压缩性、传染性、毒性或代谢性、自身免疫、肿瘤性)、脑肿瘤、癫痫、副肿瘤病症、神经发育病症(线粒体、常染色体遗传)、肌肉疾病(多肌炎、皮肌炎、包涵体肌炎、传染性、内分泌、代谢性、毒性、先天性肌病、先天性肌营养不良、遗传性)、神经病(吉兰-巴雷综合征、轴突和脱髓鞘性、糖尿病性、毒性、代谢性、传染性、危重病性、卡压性)、痹性麻痹、重症肌无力和脊髓性肌萎缩症。运动功能改变可以如本领域中已知的那样评估。示例性的运动功能测定包括但不限于肌电图和

神经传导研究,对强度、张力和肌肉体积的直接或装置辅助的临床测试,反射检查、协调检查和步态分析。用于测试引起运动功能缺陷的病因学的测定包括但不限于对中枢神经系统的磁共振成像、肌肉活检、神经活检和实验室研究。

[0090] 因此,在一些实施方案中,额外的给药取决于患者的进展,例如,在给药之间监测患者。例如,在第一次给药或第一轮给药后,监测患者的认知能力或副作用,例如,虚弱、眩晕、恶心等。

[0091] 在一些实施方案中,所述个体具有慢性病症,使得klotho无限期地给药,例如,持续患者的一生。在这种情况下,给药典型地是周期性的。被认为是长期的或慢性的疾病包括,但不限于阿尔茨海默病、帕金森病、亨廷顿舞蹈病,和与高血压和心脏病相关的认知减退。

[0092] 在一些实施方案中,Klotho多肽与稳定部分相连,所述稳定部分诸如PEG、糖基化,或脂质体或其他纳米载体。美国专利号4,732,863和7892554以及Chattopadhyay等人(2010)Mol Pharm 7:2194描述了将多肽连接至PEG、PEG衍生物和纳米粒子(例如,脂质体)的方法。含有磷脂酰-乙醇胺(PE)的脂质体可以通过如本文所述的已建立的程序制备。PE的纳入在脂质体表面上提供了活性功能位点用于连接。在一些实施方案中,Klotho多肽连接到亲和标签,例如,组氨酸标签(例如,4-16组氨酸残基)、链霉抗生物素或抗体靶标。

[0093] Klotho多肽也可以被配制成缓释制剂(例如,在固体疏水聚合物(例如,聚酯、水凝胶(例如,聚(2-羟乙基-甲基丙烯酸酯),或聚(乙烯醇),聚乳酸)的半渗透基质中)。Klotho多肽可以包埋在例如通过凝聚技术或通过界面聚合制备的纳米粒子中,例如,分别处于胶体药物递送体系(例如,脂质体、白蛋白微球、微乳液、纳米粒子和纳米胶囊)或处于粗乳液中的羟甲基纤维素或明胶微胶囊和聚-(甲基丙烯酸甲酯)微胶囊。

[0094] 在一些实施方案中,Klotho多肽被标记,例如,用于在身体内或离体追踪。Klotho多肽可以用本领域中已知的任何诊断剂标记,如例如下面文献中所提供的:Armstrong等人,Diagnostic Imaging,第5版,Blackwell Publishing(2004);Torchilin,V.P.,编,Targeted Delivery of Imaging Agents,CRC Press(1995);Vallabhajosula,S.,Molecular Imaging:Radiopharmaceuticals for PET and SPECT,Springer(2009)。诊断剂可以被多种方式检测到,包括作为提供和/或增强可检测信号的试剂。可检测信号包括,但不限于, γ -发射,放射性,回声的,光学的,荧光的,吸收性的,磁性的,或断层扫描信号。用于对诊断剂成像的技术可以包括,但不限于,单光子发射计算机断层成像术(SPECT),磁共振成像(MRI),光学成像,正电子发射计算机断层扫描术(PET),计算机断层扫描术(CT),x-线成像, γ 射线成像等。术语“可检测试剂”,“可检测部分”,“标记”,“成像剂”等术语在本文中同义地使用。

[0095] 在一些实施方案中,标记可以包括光学试剂诸如荧光剂,磷光剂,化学发光剂等。大量的试剂(例如,染料、探针、标记或指示剂)在本领域中是已知的并且可以用在本发明中。(参见,例如,Invitrogen,The Handbook—A Guide to Fluorescent Probes and Labeling Technologies(手册——荧光探针和标记技术指南),第十版(2005))。荧光剂可以包括多种多样的有机和/或无机小分子或多种多样的荧光蛋白及其衍生物。例如,荧光剂可以包括但不限于青色素,酞菁,卟啉,靛青(indocyanines),若丹明,吩噻嗪,苯基咕吨(phenylxanthenes),吩噻嗪,吩硒嗪(phenoselenazines),荧光素,苯并卟啉,方酸

(squaraines), 二吡咯并嘧啶酮, 并四苯, 喹啉, 吡嗪, 可啉 (corrins), 克酮酸 (croconiums), 吡啶酮, 菲啶, 若丹明, 吡啶类、葱醌类, chalcogenopyrylium 类似物, 二氢卟吩, 萘酞菁 (naphthalocyanines), 次甲基染料, 吲哚染料 (indolenium dyes), 偶氮化合物, 甘菊蓝, 氮杂甘菊蓝 (azaazulenes), 三苯甲烷染料, 吲哚, 苯并吲哚, 吲哚羰花青 (indocarbocyanines), 苯并吲哚羰花青, 和 BODIPYTM 衍生物。荧光染料在例如美国专利号 4,452,720、美国专利号 5,227,487 和美国专利号 5,543,295 中讨论。

[0096] 所述标记还可以是放射线同位素, 例如, 释放 γ 射线、正电子、 β 和 α 粒子和 X-射线的放射性核素。合适的放射性核素包括但不限于 ^{225}Ac , ^{72}As , ^{211}At , ^{11}B , ^{128}Ba , ^{212}Bi , ^{75}Br , ^{77}Br , ^{14}C , ^{109}Cd , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{18}F , ^{67}Ga , ^{68}Ga , ^3H , ^{166}Ho , ^{123}I , ^{124}I , ^{125}I , ^{130}I , ^{131}I , ^{111}In , ^{177}Lu , ^{13}N , ^{15}O , ^{32}P , ^{33}P , ^{212}Pb , ^{103}Pd , ^{186}Re , ^{188}Re , ^{47}Sc , ^{153}Sm , ^{89}Sr , $^{99\text{m}}\text{Tc}$, ^{88}Y 和 ^{90}Y 。在一些实施方案中, 放射性试剂可以包括 ^{111}In -DTPA, $^{99\text{m}}\text{Tc}(\text{CO})_3$ -DTPA, $^{99\text{m}}\text{Tc}(\text{CO})_3$ -ENPy2, $^{62/64/67}\text{Cu}$ -TETA, $^{99\text{m}}\text{Tc}(\text{CO})_3$ -IDA, 和 $^{99\text{m}}\text{Tc}(\text{CO})_3$ 三胺 (环状的或线性的)。在一些实施方案中, 所述试剂可以包括 DOTA 及其具有 ^{111}In , ^{177}Lu , ^{153}Sm , $^{88/90}\text{Y}$, $^{62/64/67}\text{Cu}$, 或 $^{67/68}\text{Ga}$ 的各种类似物。在一些实施方案中, 纳米粒子可以通过掺入与螯合物连接的脂质 (如 DTPA-脂质) 来标记, 如下面文献中所提供的那样: Phillips 等人, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 1(1):69-83 (2008); Torchilin, V.P. & Weissig, V., 编辑. Liposomes, 第 2 版: Oxford Univ. Press (2003); Elbayoumi, T.A. & Torchilin, V.P., Eur. J. Nucl. Med. Mol. Imaging 33:1196-1205 (2006); Mougin-Degraef, M. 等人, Int'l J. Pharmaceutics 344:110-117 (2007)。

[0097] 在一些实施方案中, 诊断剂可以与二级结合配体缔合或与酶 (酶标签) 缔合, 所述第二结合配体或酶在与显色底物接触时生成有色产物。合适的酶的实例包括尿素酶, 碱性磷酸酶, (辣根) 过氧化氢酶和葡萄糖氧化酶。二级结合配体包括, 例如, 如本领域中已知的生物素和抗生物素蛋白或链霉抗生物素化合物。

[0098] V. 认知病况和病症

[0099] Klotho 多肽 (及其功能变体和片段) 可以被施用以提高许多病况和状况的认知。这包括治疗具有低于正常或减退的认知能力的个体, 或预防性治疗需要改善或提高的认知能力的个体。

[0100] Klotho 多肽 (及其功能变体和片段) 可以用来防止或减少与衰老相关的认知减退, 例如, 在 50 岁以上的个体中, 或在有认知减退的初步征象时。

[0101] Klotho 多肽 (及其功能变体和片段) 还可以用来治疗具有年龄相关的、非年龄相关的或疾病相关的病况的个体, 所述病况包括, 但不限于:

[0102] 神经变性疾病和痴呆: 阿尔茨海默病, 帕金森病, 亨廷顿舞蹈病, 额颞叶痴呆, 进行性核上性麻痹, 皮质基底节变性, 轻度认知功能损害, 血管性痴呆, 雷维小体痴呆, 肌萎缩性侧索硬化症, 朊病毒病症, HIV 相关痴呆;

[0103] 精神或心境障碍: 抑郁症, 精神分裂症, 注意力缺陷/多动症, 自闭症谱系障碍, 智力障碍, 心境障碍和精神病性障碍;

[0104] 儿童神经发育性综合征和脑肿瘤: X-连锁的智力障碍或迟钝, 星形细胞瘤 (astrocytoma), 室管膜瘤 (ependymoma), 髓母细胞瘤 (medulloblastoma), 少突神经胶质瘤 (oligodendroglioma);

[0105] 影响学习的遗传综合征:唐氏综合征,安吉尔曼综合征,雷特综合征;

[0106] 影响认知的代谢紊乱:苯丙酮尿症,莱施-奈恩病,半乳糖血症和肾上腺脑白质营养不良;

[0107] 与化疗和/或放疗相关的认知减退;和

[0108] 其他的病况和病症:疼痛相关的认知效应,创伤性脑损伤,卒中,多发性硬化症,神经自身免疫性疾病,癫痫,谵妄,副肿瘤病症,发育迟缓,和脑白质营养不良。

[0109] Klotho多肽(及其功能变体和片段)还可以被施用从而为需要改善认知的个体提供提高的认知,例如,所述需要改善认知的个体为暴露于应激、睡眠剥夺或飞行时差反应的个体,或对需要较佳认知功能的个体,诸如外科医生、空中交通管制员和军事人员提供提高的认知。在这样的情况下,klotho多肽组合物可以在所需的效应之前2-24小时施用,所述效应对应于工作记忆可以持续约3-5天,对空间记忆可以持续约2周。

[0110] 可以使用本领域中已知的任何方法测量认知能力,例如,测试记忆,语言能力,执行功能,视觉空间功能,痴呆,或多参数神经心理能力。在一些实施方案中,Klotho施用为标准认知能力测试(例如,施用后1-3天测量)评分方面导致至少1%,2%,5%,7%,10%,15%,20%,30%,50%或更高的改善。在一些实施方案中,所述测试对于个体执行多于一次,例如在用Kloth治疗的过程中执行一次或多次。

[0111] 例如,可以实施用于记忆和学习标准测试,例如,以确定语义记忆、情节记忆、程序记忆、启动记忆和/或工作(即,短期)记忆。常见的测试包括剑桥前瞻性记忆测试(Cambridge prospective memory test,CAMPROMPT),记忆评估量表(memory assessment scales,MAS),雷氏听觉语言学习测试(Rey auditory verbal learning test),Rivermead行为记忆测试,记忆学习测试(Test of memory and learning,TOMAL),Wechsler记忆量表(WMS),和记忆伪装测试(Test of memory malingering,TOMM)。用于语言功能的测试包括,例如,波士顿诊断性失语症检查(Boston Diagnostic Aphasia Examination,BDAE),综合失语症测试(Comprehensive aphasia test,CAT),和多语种失语症检查(Multilingual aphasia examination,MAE)。

[0112] 执行功能(例如,问题解决、计划、组织、抑制性控制)可以使用执行功能缺陷综合征的行为学评价(Behavioral assessment of dysexecutive syndrome,BADS),CNS生命体征(Brief Core Battery),控制的口语词汇联想测试(Controlled oral word association test,COWAT),Delis-Kaplan执行功能系统(Delis-Kaplan Executive Function System,D-KEFS),数字警戒测试,Kaplan Baycrest神经认知评价(Kaplan Baycrest neurocognitive assessment,KBNA),Hayling和Brixton测试,注意力变量测试(Tests of variables of attention,TOVA),威斯康星卡片分类测试(Wisconsin card sorting test,WCST),或日常注意力测试(Test of everyday attention,TEA)。视觉空间能力(例如,视觉感知、构造和整合)可以使用时钟测试、Hooper视觉组织任务(VOT)或Rey-Osterrieth复杂图形测试来检测。痴呆可以使用临床痴呆评级或痴呆评级量表来量化。

[0113] 用于神经心理功能(例如,认知功能)的多参数测试包括但不限于巴塞罗那神经心理测试(Barcelona neuropsychological test,BNT),剑桥自动化成套神经心理测试(Cambridge neuropsychological test automated battery,CANTAB)、认知量表(Cognistat)、认知评价筛查测试(Cognitive assessment screening instrument,CASI)、

认知功能扫描仪 (Cognitive function scanner, CFS)、Dean-Woodcock神经心理评价系统 (Dean-Woodcock neuropsychology assessment system, DWNAS)、全科医生认知评价 (General practical assessment of cognition, GPCOG)、简短精神状态检查 (Minimal state examination, MMSE)、NEPSY或CDR计算机化评价系统。

[0114] 备选地, 可以使用用于认知活动的结构性或分子代用品测定认知, 例如, 随时间推移比较来检测变化。认知改变可以例如通过观察脑结构、连通性、激活、抑制或突触可塑性的变化来检测, 例如, 通过MRI, fMRI, EEG, TMS和TES, 和/或这些的任意组合来检测。在一些实施方案中, 观察脑活动。在一些实施方案中, Klotho给药导致脑活动的1.5-倍、2-倍、5-倍、7-倍、10-倍、或更大的增加 (例如, 给药后1-3天测量)。用于认知提高的分子代用品包括, 但不限于: 大脑中的GluN2B的水平增加, 增加的GluN2B突触定位, 增加的NMDA受体激活, 和/或增加的c-fos激活。这些测量与认知特别相关。这样的方法可以包括, 例如, 从个体获取神经组织或CSF的样品并且使用标准测定来测定基因表达或激活。

[0115] 类似地, 在小鼠和其他非人动物中, 认知能力可以利用, 例如, 执行功能 (工作记忆, 注意力, 处理速率, 定势转移), 视觉空间学习和记忆, 客体记忆, 模式识别, 恐惧记忆, 被动回避记忆, 习惯化和新物体识别的量度来检测。常见的测试包括但不限于Morris水迷宫, Barnes迷宫, 放射状水迷宫, y-迷宫, T-迷宫和旷场习惯化。脑成像技术类似地适用。

VI. 实施例

[0116] 实施例1:

[0117] A. 材料和方法

[0118] 小鼠。所有小鼠均为C57BL/6J背景并且保持于12-小时光/暗周期, 随意进食 (Picolab Rodent Diet 20, Labdiet) 和饮水。除了水迷宫研究期间的单只圈养以外, 标准圈养组为每笼5只小鼠。在光周期期间执行所有认知和行为研究。所有研究均以设盲的方式进行。

[0119] 治疗。在小鼠的行为测试之前经腹膜 (i.p.) 注射赋形剂或klotho (重组小鼠klotho, 氨基酸35-982, 具有C-末端His标签, R&D Systems)。

[0120] Morris水迷宫认知行为测试。如Dubal等人 (2014) Cell Reports 7:1065; Dubal等人 (2015) Journal of Neuroscience 35:2358所述进行水迷宫研究。小鼠在测试前4小时用赋形剂或klotho (10 μ g/kg) 腹膜内注射进行治疗, 每天一次持续5天。简言之, 小鼠在具有白色不透明水 (21 $^{\circ}$ $\pm$ 1 $^{\circ}$ C) 的池 (直径, 122cm) 中测试。正方形的14-cm²平台在表面下2cm处。在隐藏平台训练之前, 小鼠通过游动穿过一个通道从而爬上隐藏平台而经历两个训练前实验。在隐藏平台训练的过程中, 平台位置保持恒定, 而下降位置在各实验间是变化的。小鼠经历两个训练期, 所述训练期由每个60秒的两个实验组成, 每天一次持续4天。对于探索实验测试, 去除平台并且使小鼠游泳60秒。在1小时和24小时探索实验后, 测试小鼠在经过两个训练期发现标记以线索 (平台上的15-cm杆) 的可见平台的能力。作为研究的一部分, 还记录了游泳速度。

[0121] Y-迷宫认知行为测试。如Dubal等人 (2014) Cell Reports 7:1065中所述执行Y-迷宫研究。在测试前18小时对小鼠用赋形剂或klotho (10 μ g/kg) 腹膜内注射进行治疗。简言之, 在测试前30分钟使小鼠适应房间。然后, 将小鼠置于Y-迷宫 (三个相同的臂, 120 $^{\circ}$ 分隔)

的一个臂中并探索4分钟。记录进入臂次数,并且在小鼠以连续的臂进入进入三个臂中的任何一个的任何时候记录交替(alternation);交替机会为22%。在测试期之间,将装置用70%酒精清洗。由记录的数据计算交替百分比。

[0122] B.全身klotho递送增强幼鼠中的工作记忆

[0123] 为了调查klotho的治疗递送是否能够增强认知,我们用重组klotho(腹膜内)注射小鼠,并且18小时后,测试在Y-迷宫中的工作记忆。与赋形剂治疗的小鼠相比,klotho治疗的小鼠显示更多的交替,指示更佳的工作记忆(图1A)。因此,全身klotho递送增强工作记忆,工作记忆是一个涉及额叶皮质脑区的过程。

[0124] C.全身klotho递送增强幼鼠中的空间学习

[0125] 我们下一步测试了klotho治疗是否增强在Morris水迷宫中的空间学习。我们在小鼠(群组1)中在测试前4小时向小鼠注射重组klotho(腹膜内),每天一次持续5天。与赋形剂治疗的小鼠相比,klotho治疗的小鼠在隐藏平台位置的空间学习中表现更好(图1B)。两组以相等的速度游泳(图1C)。在独立的一组小鼠(群组2)中,我们在测试前18小时每天注射一次重组klotho(腹膜内)。同样,与赋形剂治疗的小鼠相比,klotho-治疗的小鼠在空间学习方面表现更好(图1D)。因此,全身klotho递送增强空间学习,空间学习是一个涉及额叶皮质和海马脑区的过程。

[0126] D.全身klotho递送增强幼鼠中的空间记忆

[0127] 为了确定klotho治疗是否可以增强空间记忆,我们去除了平台,并且在Morris水迷宫中的隐藏训练后进行探索测试。在群组1的探索实验中,通过测量小鼠对靶区域的亲近性(affinity)评估空间记忆保留。在1小时时的探索测试中,klotho-治疗的小鼠在靶标中心处花费更多的时间(图1E),与赋形剂治疗的对照相比,显示了穿越靶标的频率增加(图1F),在靶标象限中的时间增加(图1G)。在24小时时的探索测试中,与赋形剂治疗的小鼠相比,klotho-治疗的小鼠继续显示更多的中心逗留时间(图1H),穿越频率(图1I),和在靶标象限中的时间百分比(图1J)。因此,全身klotho递送增强空间记忆,空间记忆是一个与海马脑区有密切关系的过程。

[0128] 数据显示全身递送klotho增强了小鼠中的正常认知。Klotho治疗在多个测试和测量中改善了学习和记忆,包括工作记忆、空间学习和空间记忆。这些发现提供了用于增强认知功能的直接治疗应用,相关的但不限于下列的疗法:对正常大脑和由于正常衰老引起的认知障碍、大量的神经变性疾病、神经疾病和精神疾病、儿童发育综合征、创伤性脑损伤和应激的认知增强。

[0129] 实施例2

[0130] 方法。

[0131] 小鼠。所有小鼠均为C57BL/6J背景并且保持于12-小时光/暗周期,随意进食(Picolab Rodent Diet 20,Labdiet)和饮水。除了水迷宫研究期间的单只圈养以外,标准圈养组为每笼5只小鼠。在光周期期间执行所有运动、认知和行为研究。所有研究均以设盲的方式进行。

[0132] 年老的小鼠(18月龄)获自NIH衰老种群并且在实验中使用。在实验中利用对与 α -突触核蛋白毒性相关的人神经变性疾病建模的转基因小鼠;这些小鼠由小鼠Thy-1启动子表达全长人 α -突触核蛋白(Rockenstein E,等人(2002) J Neurosci Res 68:568-578)并且

表现出运动、认知和行为缺陷。在人的病况中,人 α -突触核蛋白的表达增加促成几种神经变性疾病,包括但不限于,帕金森病(PD)、阿尔茨海默病(AD)、雷维小体痴呆(LBD)和多系统萎缩(MSA)。

[0133] 治疗。如指示的那样,在小鼠的行为测试之前腹膜内(i.p.)注射赋形剂或 α -klotho(重组小鼠 α -klotho,氨基酸35-982,具有C-末端His标签,R&D Systems)。所有动物研究得到旧金山加利福尼亚大学的机构动物管理和使用委员会(Institutional Animal Care and Use Committee of the University of California, San Francisco)批准并且按照NIH指南进行。

[0134] 认知和运动行为

[0135] Morris水迷宫。水迷宫研究如所述的那样进行(Zarei M,等人(2013) J Neurol Neurosurg Psychiatry 84:875-881;Dubal DB等人(2014) Cell Reports 7:1065-1076)。小鼠在测试前4小时用赋形剂或klotho(10 μ g/kg)腹膜内注射进行治疗,每天一次持续5天。简言之,小鼠在具有白色不透明水(21 $^{\circ}$ ±1 $^{\circ}$ C)的池(直径,122cm)中测试。正方形的14-cm²平台在表面下2cm处。在隐藏平台训练之前,小鼠通过游动穿过一个通道从而爬上隐藏平台而经历两个训练前实验。在隐藏平台训练的过程中,平台位置保持恒定,而下降位置在各实验间是变化的。小鼠经历两个训练期,所述训练期由每个60秒的两个实验组成,每天一次持续4天。对于探索实验测试,去除平台并且使小鼠游泳60秒。在1小时和24小时探索实验后,测试小鼠经过两个训练期发现标记以线索(平台上的15-cm杆)的可见平台的能力。作为研究的一部分,还记录了游泳速度。

[0136] Y-迷宫。如所述的那样(Dubal DB等人(2014) Cell Reports 7:1065-1076)执行Y-迷宫研究。在测试前18小时对小鼠用赋形剂或klotho(10 μ g/kg)腹膜内注射进行治疗。简言之,在测试前30分钟使小鼠适应房间。然后,将小鼠置于Y-迷宫(三个相同的臂,120 $^{\circ}$ 分隔)的一个臂中并探索4分钟。记录进入臂次数,并且在小鼠以连续的臂进入进入三个臂中的每一个的任何时候记录交替;交替机会为22%。在测试期之间,将装置用70%酒精清洗。由记录的数据计算交替百分比。

[0137] 大型Y-迷宫。如所述的那样(Dellu F,等人(1992) Brain Res 588:132-139)执行大型Y-迷宫研究(稍加改动)。大型Y-迷宫设备由三个相同的臂组成,分隔120 $^{\circ}$,在两个臂的后端具有明显的和不同的视觉线索;第三个没有视觉线索的臂是起始臂。在训练或测试之前,使小鼠对灯光灰暗的房间适应60分钟。在训练期间,用实心分隔物挡住带有视觉线索的一个臂,并将小鼠置于起始臂的末端。使小鼠探索开放臂5分钟,然后使其返回到它们的笼中。在训练中使用的开放臂(熟悉臂)在整个群组测试中是平衡的。训练后16小时,小鼠经历测试。在测试期间,去除分隔物。将小鼠置于起始臂中并使其探索带有新的和熟悉的视觉线索的臂(新异臂和熟悉臂)5分钟。记录并测量在新异臂和熟悉臂中进入的次数和经历的逗留时间。计算新异臂与熟悉臂进入次数或逗留时间的比率以评估空间和工作记忆。在测试期之间将装置用70%酒精清洗。

[0138] 转棒。在每期之前使小鼠适应房间60分钟。将5只小鼠同时放在以16rpm的恒定速率旋转的旋转棒(转棒(Rota Rod), Med Associates Inc, VT)上持续最大300秒。在每个实验中记录到掉落的潜伏期。连续进行三次实验,实验间休息10分钟。在测试期之间将装置用70%酒精清洗。

[0139] 旷场。如所述 (Dubal DB, 等人 (2015) J Neurosci 35:2358-2371) 的那样利用自动 Flex-Field/Open Field光子束活动系统 (San Diego Instruments, San Diego, CA) 测量旷场中的总活动。使小鼠对测试房间适应30分钟, 然后在明亮的塑料室 (41x30cm) 中用两个测量运动的光子束阵列测试5min。在测试期之间将装置用70%酒精清洗。

[0140] 结果。

[0141] 全身klotho递送增强年老小鼠的认知。为了确定治疗递送klotho是否可以增强年老小鼠的认知, 我们用重组klotho (i.p., 10 μ g/kg) 注射小鼠一次, 然后训练它们进行任务从而在24小时后测量在大型Y-迷宫中的工作和空间记忆。然后, 训练后18小时 (klotho递送后42小时), 小鼠经历测试。在基线处, 并且在不利用klotho治疗的条件下, 年老小鼠没有显示新异臂偏好性, 这指示认知缺陷。与赋形剂治疗的小鼠比较, klotho-治疗的小鼠在整个探索期间显示主要对新异臂的持久偏好性 (图2A, B), 这指示更好的认知, 这涉及额叶和海马脑区。这些数据显示klotho增强具有年龄引起的认知缺陷的小鼠的认知。这些数据表明klotho的治疗递送增强了衰老大脑的认知。

[0142] 全身性klotho递送以持久的方式增强认知。为了确定klotho的治疗递送是否可以以延长超出其7小时的半衰期的方式引起持久的认知增强 (Hu MC, 等人 (2015) J Am Soc Nephrol.), 在5天的每天一次klotho治疗 (i.p., 0.5或2.5 μ g/kg) 和认知测试后大约两周, 我们测试了幼鼠。如期望的那样, 赋形剂治疗的小鼠没有显示对新异臂的偏好性, 这指示在两周后对视觉线索的记忆丧失。相反, klotho-治疗的小鼠显示对迷宫的新异臂具有明显的偏好性——甚至在最后一次治疗后两周并且以剂量依赖的方式 (图3)。这些数据指示在正常的幼鼠中的klotho-介导的认知增强是持久的并且在其半衰期以外延长至少两周。这些数据表明klotho的治疗递送引起大脑中的组织结构变化, 这些变化增强了神经功能。

[0143] 全身klotho递送在神经变性疾病的建模小鼠中改善了认知障碍。我们下一步测试klotho的短期治疗递送是否可以在神经变性疾病的小鼠模型中改善现有的认知缺陷。在这个充分表征的疾病模型中, 小鼠表达人 α -突触核蛋白 (hSYN), 其是一种导致认知和运动缺陷并且促进帕金森病、阿尔茨海默病、雷维小体痴呆和多系统萎缩的致病蛋白。在这些小鼠中, 在大-Y迷宫中评估认知功能。这个任务是最适的, 因为其利用了小鼠探索的天然倾向, 避免了测试诱导的应激源, 并且不受限制其他测试的运动困难 (诸如不能在水迷宫中适当地游泳) 的影响。与非转基因 (NTG) 小鼠相比, hSYN小鼠显示对新异臂的偏好性减少, 如减少的逗留时间所示 (图4A)。显著地, 对hSYN小鼠的klotho治疗 (i.p., 2.5 μ g/kg) 增加新异臂偏好性 (图4A, B), 这指示极大的和治疗性的认知改善。klotho治疗的总体效应是增强记忆, 如增加新异臂偏好性所指示 (双向ANOVA KL效应 $p < 0.05$) (图4B)。这些数据提示klotho的治疗递送可以逆转或改善神经变性疾病中由 α -突触核蛋白毒性所引起的认知缺陷。要注意的是, klotho-介导的认知增强可以促进改善的运动学习。

[0144] 全身klotho递送在建模神经变性疾病的小鼠中改善早期运动障碍。由于 α -突触核蛋白毒性诱导运动功能缺陷 (这是神经变性疾病中的主要临床问题), 因此我们测试了klotho的治疗递送是否可以改善hSYN小鼠中的此关键量度。在旋转棒 (即Rota rod) 上评估运动功能。与NTG小鼠相比, hSYN小鼠显示出减少的运动功能 (图5)。显著地, 对hSYN小鼠的klotho治疗 (i.p., 2.5 μ g/kg) 极大地增加了运动功能, 如旋转棒上的较长潜伏期所示 (图5)。这些数据指示klotho治疗极大地改善了hSYN小鼠中的早期运动功能。这些数据提示klotho的治

疗递送可以改善神经变性疾病中由 α -突触核蛋白毒性所引起的与运动问题相关的缺陷。

[0145] 全身klotho递送不改变小鼠的总运动或活动。为了检验klotho递送是否改变小鼠的总运动和活动,我们检查了小鼠对旷场的探索。在Veh-或KL-治疗的(i.p., 2.5 μ g/kg)小鼠之间总运动没有差异(图6)。这些数据提示klotho的治疗效应特异性针对认知和运动功能,并且不受非特异的动作诸如多动的影响。

[0146] 讨论

[0147] 我们的数据显示全身递送klotho(其不穿越血脑屏障)以持久的方式增强幼鼠中的正常认知,改善正常幼鼠和年老小鼠中的认知缺陷;其还改善对主要的神经变性疾病建模的转基因小鼠中的认知和运动缺陷,所述神经变性疾病诸如阿尔茨海默病、帕金森病、雷维小体痴呆和多系统萎缩。

[0148] 这些发现提供了用于增强正常脑的认知功能和改善年老和神经变性疾病中的脑功能的直接治疗应用。此治疗应用还与下列相关但不限于下列:由于大量的神经疾病和精神疾病、儿童发育综合征、创伤性脑损伤和应激引起的认知障碍。

[0149] 我们的数据显示:

[0150] 1. 在从幼鼠(2-7月龄)至年老(18月龄)雄性和雌性小鼠一生中,全身施用小鼠重组klotho形式增强正常认知。

[0151] 2. 当在测试前4小时至16天给药时,全身klotho治疗有效地增强认知。在大量的认知训练后,所述认知增强效应持续至少两周。

[0152] 3. 小鼠klotho的在0.5 μ g/kg至10 μ g/kg之间的全身剂量在所测试的条件下增强认知。

[0153] 4. 利用重组klotho的全身治疗增强神经变性疾病的小鼠模型中的认知,所述神经变性疾病与阿尔茨海默病、帕金森病、雷维小体痴呆和多系统萎缩相关但不限于这些。

[0154] 5. 除了改善神经变性疾病的小鼠模型中的认知缺陷以外,klotho治疗还改善早期运动功能。

[0155] 6. klotho在增强认知方面的治疗效应延长远远超出其大约7小时的半衰期。

[0156] 7. klotho的治疗效应似乎特异性针对认知和运动功能,并且不受非特异性的动作(诸如多动)影响。

[0157] 提供上面的实施例以说明本发明但不限制其范围。本发明的其他变化形式对于本领域普通技术人员是显而易见的,并且由附带的权利要求涵盖。本文所引用的所有出版物、数据库、互联网资源、专利、专利申请和登录号整体地通过引用并入本文用于所有目的。

[0158] VII. 非正式的序列表

[0159] SEQ ID NO:1:人Klotho蛋白

	10	20	30	40	50
	MPASAPRRRP	RPPPPSLSLL	LVLLGLGGRR	LRAEPGDGAQ	TWARFSRPPA
	60	70	80	90	100
	PEAAGLFQGT	FPDGFLWAVG	SAAYQTEGGW	QQHGKGASIW	DTFTHHPLAP
	110	120	130	140	150
	PGDSRNASLP	LGAPSPLQPA	TGDVASDSYN	NVFRDTEALR	ELGVTHYRFS
	160	170	180	190	200
	ISWARVLPNG	SAGVPNREGL	RYYRLLERL	RELGVQPVVT	LYHWDLPQRL
	210	220	230	240	250
	QDAYGGWANR	ALADHFRDYA	ELCFRHFGGQ	VKYWITIDNP	YVVAWHGYAT
	260	270	280	290	300
	GRLAPGIRGS	PRLGYLVAHN	LLLAHAKVWH	LYNTSFRPTQ	GGQVSIALSS
	310	320	330	340	350
	HWINPRRMTD	HSIKECQKSL	DFVLGWFAKP	VFIDGDYPES	MKNNLSSILP
	360	370	380	390	400
	DFTSEKKFI	KGTADFFALC	FGPTLSFQLL	DPHMKFRQLE	SPNLRQLLSW
	410	420	430	440	450
[0160]	IDLEFNHPQI	FIVENGWFS	GTTKRDDAKY	MYYLKKFIME	TLKAIKLDGV
	460	470	480	490	500
	DVIGYTAWSL	MDGFEWHRGY	SIRRGIFYVD	FLSQDKMLLP	KSSALFYQKL
	510	520	530	540	550
	IEKNGFPPLP	ENQPLEGTFP	CDFAWGVVDN	YIQVDTTLSQ	FTDLNVYLWD
	560	570	580	590	600
	VHHSKRLIKV	DGVVTKKRKS	YCVDFAAIQP	QIALLOEMHV	THFRFSLDWA
	610	620	630	640	650
	LILPLGNQSQ	VNHTILQYYR	CMASELVRVN	ITPVVALWQP	MAPNQGLPRL
	660	670	680	690	700
	LARQGAWENP	YTALAFAEYA	RLCFQELGHH	VKLWITMNEP	YTRNMTYSAG
	710	720	730	740	750
	HNLLKAHALA	WHVYNEKFRH	AQNGKISIAL	QADWIEPACP	FSQKDKEVAE
	760	770	780	790	800
	RVLEFDIGWL	AEPIFGSGDY	PWVMRDWLNQ	RNNFLLPYFT	EDEKKLIQGT
	810	820	830	840	850
	FDFLALSHYT	TILVDSEKED	PIKYNDYLEV	QEMTDITWLN	SPSQVAVVPW
	860	870	880	890	900
	GLRKVLNWLK	FKYGDLPYI	ISNGIDDGLH	AEDDQLRVYY	MQNYINEALK

	910	920	930	940	950
	AHILDGINLC	GYFAYSFNDR	TAPRFGLYRY	AADQFEPKAS	MKHYRKIIDS
	960	970	980	990	1000
[0161]	NGFPGPETLE	RFCPEEFTVC	TECSFFHTRK	SLLAFIAFLF	FASIISLSLI
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	FYYSKKGRRS	YK			

序列表

<110> 加利福尼亚大学董事会

德娜·杜波

<120> 用于提高认知的方法和组合物

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<151> 2015-02-06

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<400> 1

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          20          25          30
Ala Glu Pro Gly Asp Gly Ala Gln Thr Trp Ala Arg Phe Ser Arg Pro
          35          40          45
Pro Ala Pro Glu Ala Ala Gly Leu Phe Gln Gly Thr Phe Pro Asp Gly
          50          55          60
Phe Leu Trp Ala Val Gly Ser Ala Ala Tyr Gln Thr Glu Gly Gly Trp
65          70          75          80
Gln Gln His Gly Lys Gly Ala Ser Ile Trp Asp Thr Phe Thr His His
          85          90          95
Pro Leu Ala Pro Pro Gly Asp Ser Arg Asn Ala Ser Leu Pro Leu Gly
          100          105          110
Ala Pro Ser Pro Leu Gln Pro Ala Thr Gly Asp Val Ala Ser Asp Ser
          115          120          125
Tyr Asn Asn Val Phe Arg Asp Thr Glu Ala Leu Arg Glu Leu Gly Val
          130          135          140
Thr His Tyr Arg Phe Ser Ile Ser Trp Ala Arg Val Leu Pro Asn Gly
          145          150          155          160
Ser Ala Gly Val Pro Asn Arg Glu Gly Leu Arg Tyr Tyr Arg Arg Leu
          165          170          175
Leu Glu Arg Leu Arg Glu Leu Gly Val Gln Pro Val Val Thr Leu Tyr
          180          185          190

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His Trp Asp Leu Pro Gln Arg Leu Gln Asp Ala Tyr Gly Gly Trp Ala		
195	200	205
Asn Arg Ala Leu Ala Asp His Phe Arg Asp Tyr Ala Glu Leu Cys Phe		
210	215	220
Arg His Phe Gly Gly Gln Val Lys Tyr Trp Ile Thr Ile Asp Asn Pro		
225	230	235
Tyr Val Val Ala Trp His Gly Tyr Ala Thr Gly Arg Leu Ala Pro Gly		
	245	250
		255
Ile Arg Gly Ser Pro Arg Leu Gly Tyr Leu Val Ala His Asn Leu Leu		
	260	265
		270
Leu Ala His Ala Lys Val Trp His Leu Tyr Asn Thr Ser Phe Arg Pro		
	275	280
		285
Thr Gln Gly Gly Gln Val Ser Ile Ala Leu Ser Ser His Trp Ile Asn		
290	295	300
Pro Arg Arg Met Thr Asp His Ser Ile Lys Glu Cys Gln Lys Ser Leu		
305	310	315
Asp Phe Val Leu Gly Trp Phe Ala Lys Pro Val Phe Ile Asp Gly Asp		
	325	330
		335
Tyr Pro Glu Ser Met Lys Asn Asn Leu Ser Ser Ile Leu Pro Asp Phe		
	340	345
		350
Thr Glu Ser Glu Lys Lys Phe Ile Lys Gly Thr Ala Asp Phe Phe Ala		
	355	360
		365
Leu Cys Phe Gly Pro Thr Leu Ser Phe Gln Leu Leu Asp Pro His Met		
370	375	380
Lys Phe Arg Gln Leu Glu Ser Pro Asn Leu Arg Gln Leu Leu Ser Trp		
385	390	395
Ile Asp Leu Glu Phe Asn His Pro Gln Ile Phe Ile Val Glu Asn Gly		
	405	410
		415
Trp Phe Val Ser Gly Thr Thr Lys Arg Asp Asp Ala Lys Tyr Met Tyr		
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		430
Tyr Leu Lys Lys Phe Ile Met Glu Thr Leu Lys Ala Ile Lys Leu Asp		
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		445
Gly Val Asp Val Ile Gly Tyr Thr Ala Trp Ser Leu Met Asp Gly Phe		
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		460
Glu Trp His Arg Gly Tyr Ser Ile Arg Arg Gly Leu Phe Tyr Val Asp		
465	470	475
		480
Phe Leu Ser Gln Asp Lys Met Leu Leu Pro Lys Ser Ser Ala Leu Phe		
	485	490
		495
Tyr Gln Lys Leu Ile Glu Lys Asn Gly Phe Pro Pro Leu Pro Glu Asn		

500	505	510
Gln Pro Leu Glu Gly Thr Phe	Pro Cys Asp Phe Ala Trp	Gly Val Val
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Asp Asn Tyr Ile Gln Val Asp Thr Thr	Leu Ser Gln Phe Thr Asp	Leu
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560	565	570
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Ser Gln Val Asn His Thr Ile Leu Gln	Tyr Tyr Arg Cys Met Ala Ser	
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Glu Leu Val Arg Val Asn Ile Thr Pro	Val Val Ala Leu Trp Gln Pro	
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Met Ala Pro Asn Gln Gly Leu Pro Arg	Leu Leu Ala Arg Gln Gly Ala	
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Trp Glu Asn Pro Tyr Thr Ala Leu Ala	Phe Ala Glu Tyr Ala Arg Leu	
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Cys Phe Gln Glu Leu Gly His His Val	Lys Leu Trp Ile Thr Met Asn	
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Glu Pro Tyr Thr Arg Asn Met Thr Tyr	Ser Ala Gly His Asn Leu Leu	
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Lys Ala His Ala Leu Ala Trp His Val	Tyr Asn Glu Lys Phe Arg His	
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Ala Gln Asn Gly Lys Ile Ser Ile Ala	Leu Gln Ala Asp Trp Ile Glu	
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Pro Ala Cys Pro Phe Ser Gln Lys Asp	Lys Glu Val Ala Glu Arg Val	
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Leu Glu Phe Asp Ile Gly Trp Leu Ala	Glu Pro Ile Phe Gly Ser Gly	
755	760	765
Asp Tyr Pro Trp Val Met Arg Asp Trp	Leu Asn Gln Arg Asn Asn Phe	
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Leu Leu Pro Tyr Phe Thr Glu Asp Glu	Lys Lys Leu Ile Gln Gly Thr	
785	790	795
800	805	810
Phe Asp Phe Leu Ala Leu Ser His Tyr	Thr Thr Ile Leu Val Asp Ser	
815		

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 Met Thr Asp Ile Thr Trp Leu Asn Ser Pro Ser Gln Val Ala Val Val
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 Pro Trp Gly Leu Arg Lys Val Leu Asn Trp Leu Lys Phe Lys Tyr Gly
 850 855 860
 Asp Leu Pro Met Tyr Ile Ile Ser Asn Gly Ile Asp Asp Gly Leu His
 865 870 875 880
 Ala Glu Asp Asp Gln Leu Arg Val Tyr Tyr Met Gln Asn Tyr Ile Asn
 885 890 895
 Glu Ala Leu Lys Ala His Ile Leu Asp Gly Ile Asn Leu Cys Gly Tyr
 900 905 910
 Phe Ala Tyr Ser Phe Asn Asp Arg Thr Ala Pro Arg Phe Gly Leu Tyr
 915 920 925
 Arg Tyr Ala Ala Asp Gln Phe Glu Pro Lys Ala Ser Met Lys His Tyr
 930 935 940
 Arg Lys Ile Ile Asp Ser Asn Gly Phe Pro Gly Pro Glu Thr Leu Glu
 945 950 955 960
 Arg Phe Cys Pro Glu Glu Phe Thr Val Cys Thr Glu Cys Ser Phe Phe
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 His Thr Arg Lys Ser Leu Leu Ala Phe Ile Ala Phe Leu Phe Phe Ala
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1	5	10	15
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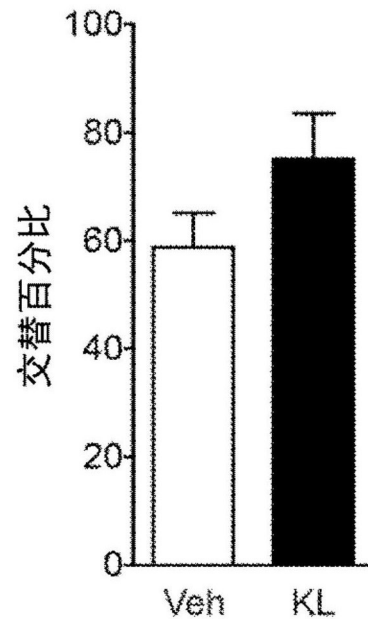


图1A

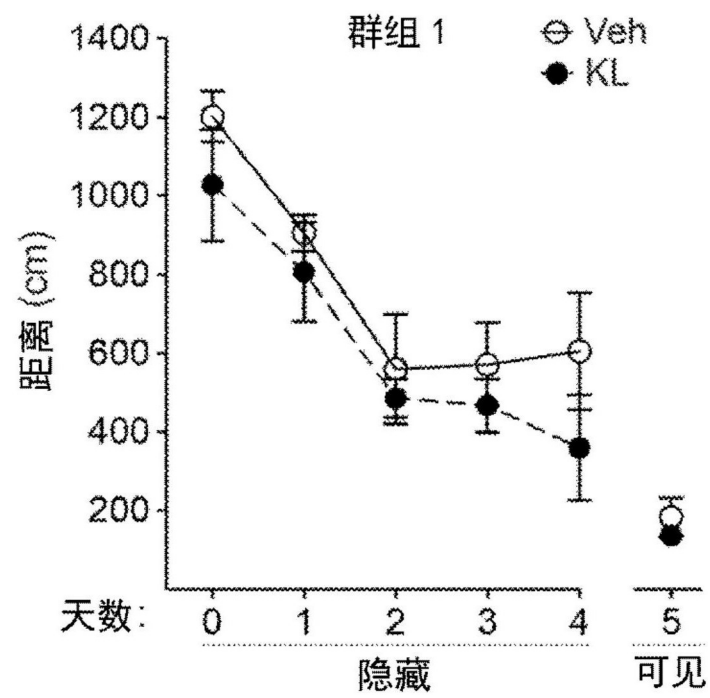


图1B

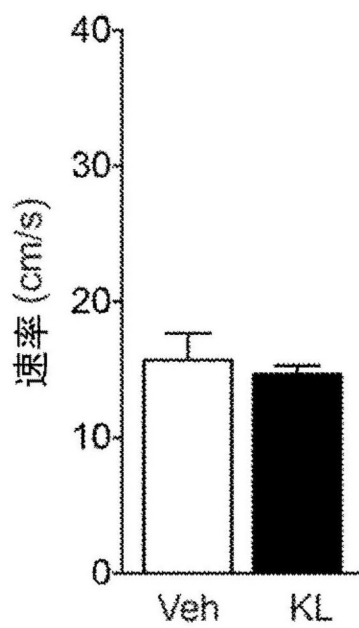


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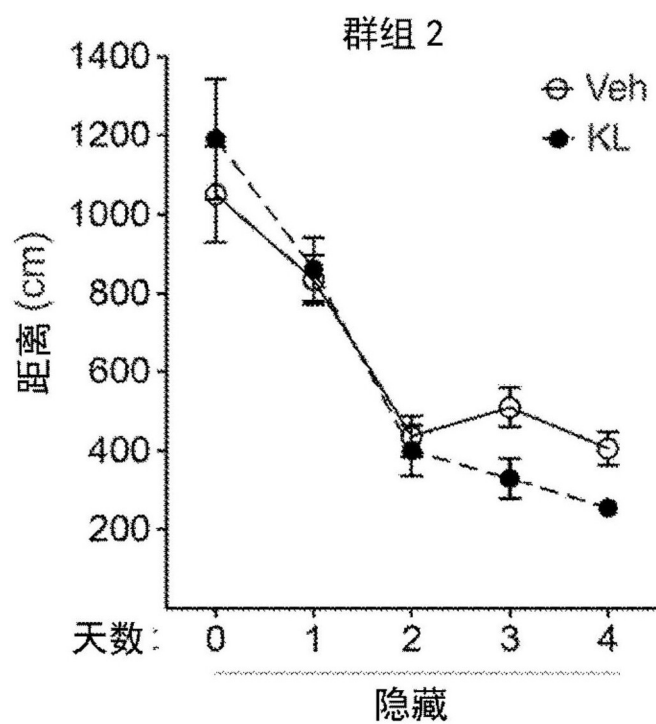


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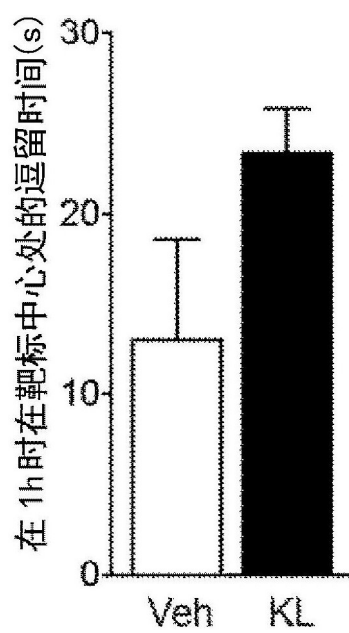


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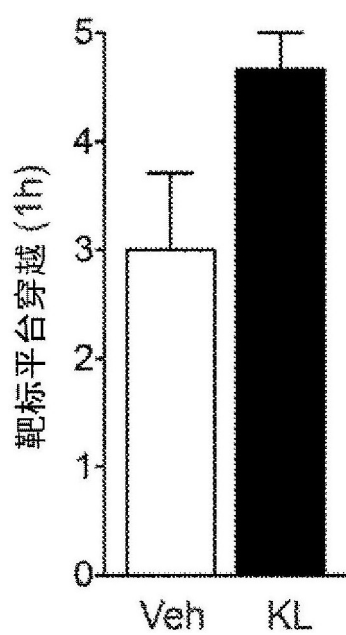


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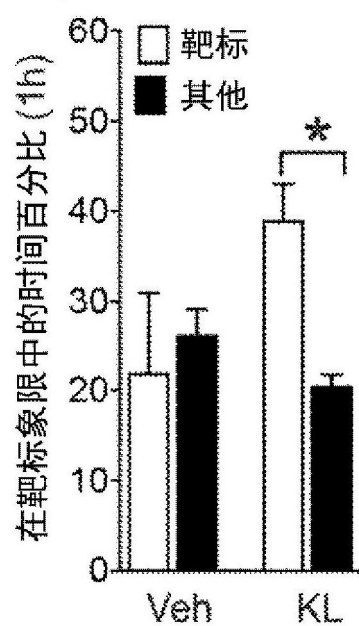


图1G

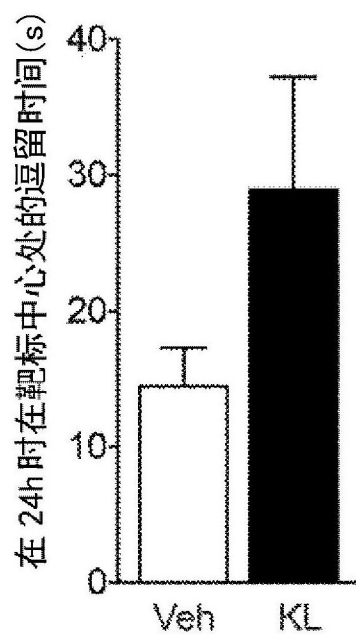


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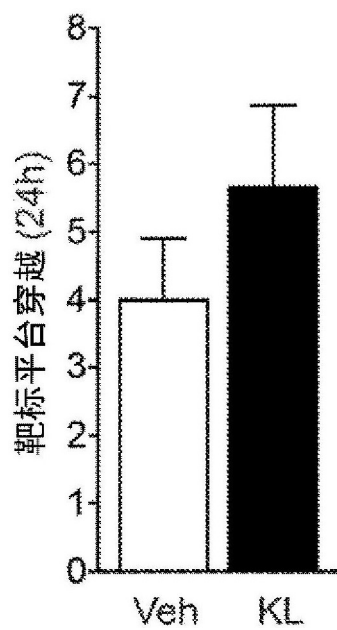


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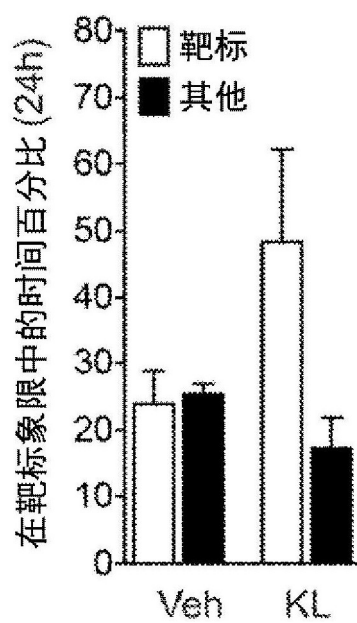


图1J

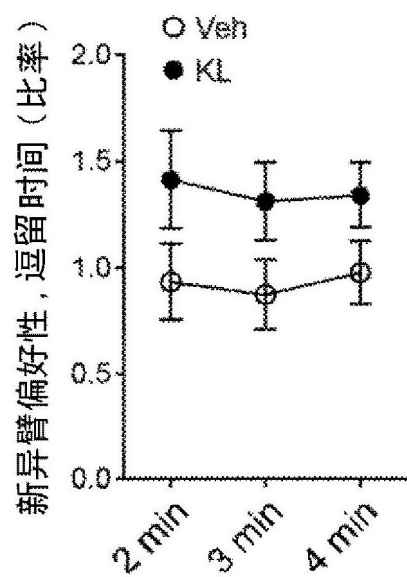


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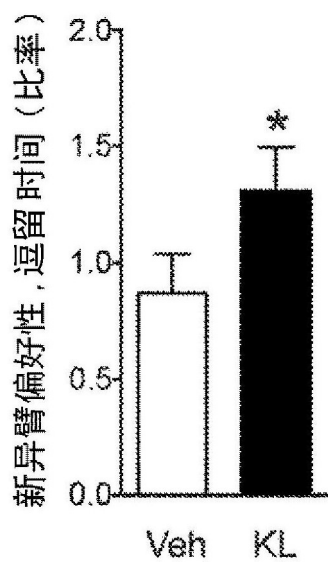


图2B

长久持续效应

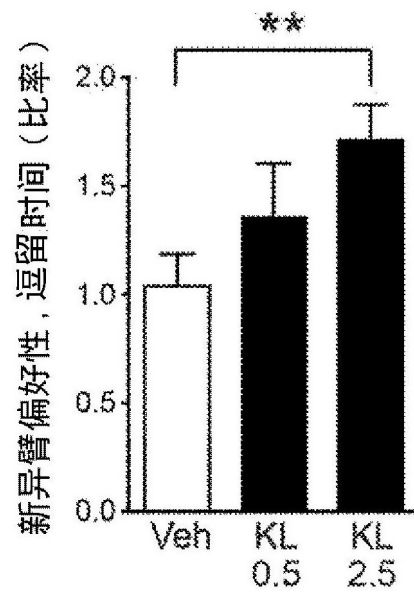


图3

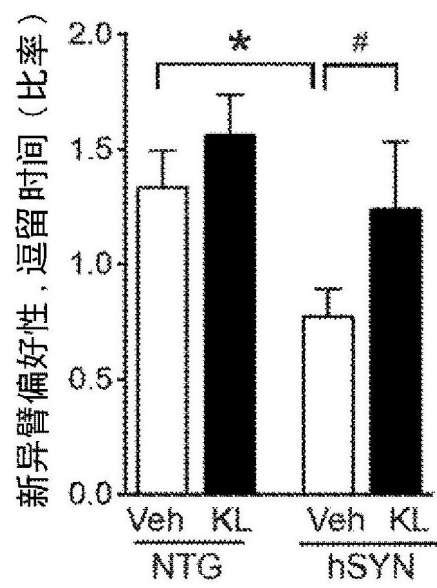


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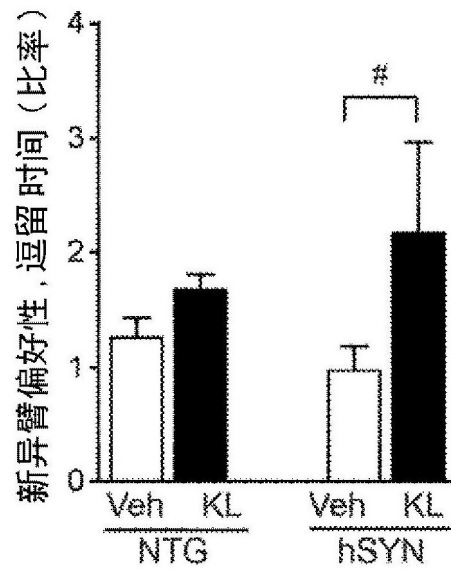


图4B

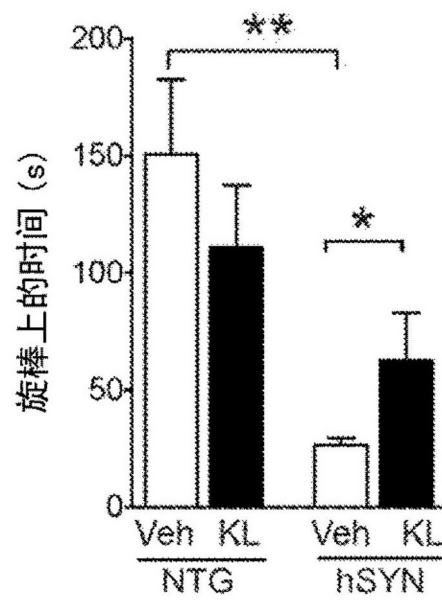


图5

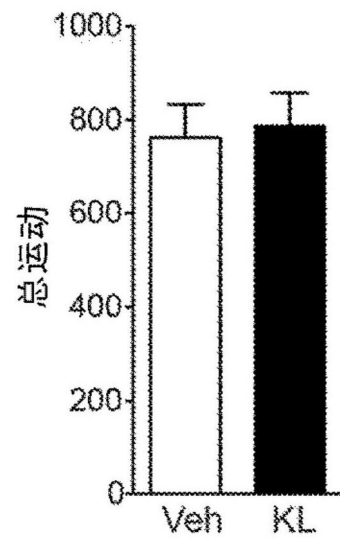


图6