(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2006 (30.03.2006)

PCT

(10) International Publication Number
WO 2006/034485 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2005/034331

(22) International Filing Date:
21 September 2005 (21.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/611,749 21 September 2004 (21.09.2004) US

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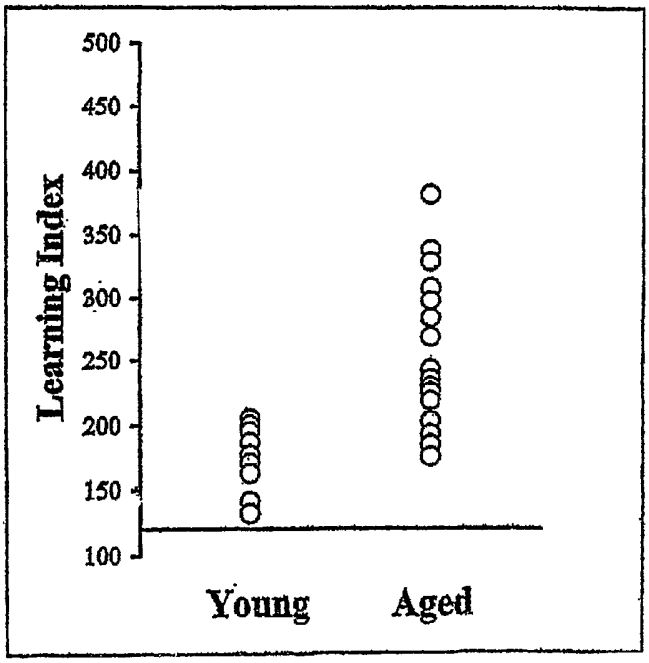
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: TREATMENT FOR AGE-RELATED COGNITIVE DECLINE AND OTHER CONDITIONS



(57) Abstract: Methods and compositions are disclosed for identifying agents useful for promoting or preserving cognitive function, or for ameliorating cognitive decline, in a mammal. Such agents are identified by screening candidate compounds for an agent that modulates the expression of a gene encoding lipoprotein lipase (LPL), or that modulates the activity of an LPL protein encoded by said gene.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Treatment for Age-Related Cognitive Decline and Other Conditions

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional application number 60/611,749 filed September 21, 2004, the disclosure of which is hereby incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates to treatment of impaired cognitive function, and has application in the fields of medicine, pharmacology and drug development.

GOVERNMENT SUPPORT

[0003] Studies described herein were conducted with government support under grant No. PO1 AG09973 awarded by the National Institutes on Aging. The government may have certain rights in the invention.

BACKGROUND

[0004] Although some decline in cognitive ability may be a normal consequence of aging, a significant population of elderly adults experiences a decline in cognitive ability that exceeds normal development yet is of insufficient magnitude to warrant a diagnosis of dementia. Some of these individuals may be diagnosed as suffering from Age-Related Cognitive Decline (ARCD) and others may be diagnosed with Mild-Cognitive Impairment (MCI).

[0005] A diagnosis of ARCD (or the equivalent construct such as age-associated Memory Impairment, AAMI) is used to define patients with a mild memory deficit that is not expected to worsen considerably over time. ARCD can also be defined as Stage 2 on the Global Deterioration Scale (GDS). The GDS is a seven-point rating system of cognitive and functional capabilities, widely used for rating cognitive performance in older adults, with scores ranging from normal aging (Stage 1) to severe dementia (Stage 7). Stage 2 is characterized by the following clinical characteristics: subjective cognitive complaints in the absence of clinically manifest deficit.

[0006] Mild Cognitive Impairment (MCI) is a condition characterized by isolated memory impairment accompanied by no other cognitive abnormality and relatively normal functional abilities. One set of criteria for a clinical characterization of MCI specifies the following characteristics: (1) memory complaint (as reported by patient, informant, or physician), (2) normal activities of daily living (ADLs), (3) normal global cognitive function, (4) abnormal memory for age (defined as scoring more than 1.5 standard deviations below the mean for a given age), and (5) absence of indicators of dementia (as defined by DSM-IV guidelines). Petersen et al., 1999, Mild cognitive impairment: clinical characterization and outcome. *Srch. Neurol.* 56: 303-308. Also see Petersen, 2003, Mild cognitive impairment: Aging to Alzheimer's Disease. New York: Oxford University Press.

[0007] MCI can also be defined as Stage 3 on the Global Deterioration Scale (GDS). Stage 3 is characterized by the following clinical characteristics: subtle, clinically manifest cognitive impairment that may be of sufficient magnitude to interfere with complex occupational or social tasks which may be accompanied by anxiety.

[0008] MCI can also be defined as a rating of 0.5 on another widely used system for rating cognitive and functional capabilities, the Clinical Dementia Rating (CDR) scale. Scores in the CDR scale range from a CDR assignment of 0 (no dementia) to 3 (severe dementia). The degree of impairment in performance is assessed within six categories of cognitive functioning: memory, orientation, judgment/problem solving, community relations, home and hobbies, and personal care.

[0009] MCI subjects also have significantly greater psychometric test deficits (Reisberg et al., 1982, The global deterioration scale for assessment of primary degenerative dementia. *Am J Psychiatry* 139:1136-39), balance and coordination deficits (Franssen et al., 1999, *J Am Geriatric Soc* 47:463-99), and deficits on motor performance tasks (Kluger et al., 1997, *J Gerontology: Psychol. Sci.* 52B: 28-39), than AAMI and normal aged subjects.

[0010] Based on these operational definitions, a diagnosis of MCI requires an objective assessment of cognitive impairment, which can be garnered through the use of well-established neuropsychological tests, including the Mini Mental State Examination (MMSE), the Cambridge Neuropsychological Test Automated Battery (CANTAB) and individual tests such as Rey Auditory Verbal Learning Test (AVLT), Logical Memory Subtest of the revised Wechsler Memory Scale (WMS-R) and the New York University (NYU) Paragraph Recall Test. See Folstein et al., 1975, The "mini-mental state": a practical method for grading the

cognitive state of patients for the clinician. *J Psychiatric Res* 12: 189-98; Robbins et al., 1994, Cambridge Neuropsychological Test Automated Battery (CANTAB): A factor analytic study of a large sample of normal elderly volunteers. *Dementia* 5: 266-81; Rey, 1964, L'examen clinique en psychologie. Paris: Presses Universitaires de France; Wechsler, 1987, Wechsler Memory-Scale-Revised. New York: Psychological Corporation; Kluger et al., 1999, Neuropsychological prediction of decline to dementia in nondemented elderly. *J Geriatr Psychiatry Neurol* 12:168-79.

[0011] Patients diagnosed with MCI based on these definitions have, in prospective studies, been reported to have a variable, but increased risk of developing Alzheimer's Disease (AD) over time, as compared with age-matched controls. It has been suggested that distinct populations of MCI patients exist, with the "progressor" population having an increased risk of developing AD and the "stable" population not having an increased risk. Genetic markers, such as the E4 allele of the glycoprotein ApoE, have been used to identify MCI patients at risk for progressing to AD. Petersen et al., 1995, Apolipoprotein E status as a predictor of the development of Alzheimer's Disease in memory-impaired individuals. *JAMA* 273: 1274-1278. A SPECT study has also demonstrated a reduction in cerebral blood flow in the left posterior cingulated cortex in MCI patients that progressed to AD and those MCI patients that were stable. See Dickerson et al., 2004, Medial temporal lobe function and structure in mild cognitive impairment. *Ann Neurol.* 56:27-35; Huang et al., 2002, Cingulate cortex hypoperfusion predicts Alzheimer's disease in mild cognitive impairment. *BMC Neurol.* 2: 9-14.

SUMMARY OF THE INVENTION

[0012] Methods are provided for the identification of agents useful for promoting or preserving cognitive function, or for ameliorating cognitive decline, in a mammal (*e.g.*, a mouse, rat or human). In one aspect, the screening methods include conducting an assay to identify an agent that modulates the expression of a gene encoding a lipoprotein lipase ("LPL") protein, or the level or activity of an LPL protein encoded by such a gene.

[0013] In one aspect, the invention provides a method for determining whether an agent is useful for treatment of cognitive impairment (CI) by determining whether the agent increases expression and/or activity of LPL in a neuronal cell, hippocampus, or a hippocampal region.

An agent that increases expression of LPL in a neuronal cell, hippocampus, or hippocampal region is identified as useful for treatment of CI.

[0014] In one aspect, the invention provides a method for determining whether an agent is useful for treatment of CI by determining whether the agent increases expression of LPL in a neuronal cell, hippocampus or a hippocampal region of an Aged Impaired (AI) mammal (e.g., a rat). An agent that increases expression of LPL in the neuronal cell, hippocampus or hippocampal region is identified as useful for treatment of CI in mammals, including humans.

[0015] In an embodiment, the agent increases the expression of LPL in the hippocampus or hippocampal region of an AI mammal to a level that is the same as the level in an Aged Unimpaired (AU) or Young (Y) mammal.

[0016] In an embodiment, the agent increases the expression of LPL in the hippocampus or hippocampal region of an AI animal, but does not significantly change the level of expression of LPL in a Y or AU animal.

[0017] In another aspect, the invention provides a method for treating CI in a mammal by administering an agent that (a) increases expression of LPL in the hippocampus or a hippocampal region of an AI mammal; (b) increases the expression of LPL in the hippocampus or hippocampal region of an AI mammal to a level that is the same as the level in an AU mammal; and/or (c) increases the expression of LPL in the hippocampus or hippocampal region of an AI mammal, but does not significantly change the level of expression of LPL in a Y or AU mammal.

[0018] In another aspect, the invention provides a method for determining an optimal or initial clinical dosage of an agent that increases LPL expression in the hippocampus or a hippocampal region of an AI mammal for treatment of CI, by (a) determining the dose of the agent that in the YUI assay (described below) increases LPL expression in the hippocampus or a hippocampal region of an AI mammal (such as a rat) to a level that is the same as the level in an AU mammal and, optionally, does not significantly change the level of expression of LPL in a Y or AU mammal, and (b) using this amount to estimate the optimal dosage and initial clinical dosage for a human subject.

[0019] In another aspect, the invention provides a method for treating CI in a human patient comprising administering a therapeutically effective amount of an agent that increases LPL expression in the hippocampus or a hippocampal region of an aged impaired mammal. In an

embodiment, the agent is administered in an amount equivalent to the amount that in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI mammal to a level that is the same as the level in an AU mammal and, optionally does not significantly change the level of expression of LPL in a Y or AU mammal.

[0020] In another aspect, the invention provides a method for treating CI in a human patient comprising administering a therapeutically effective amount of an agent identified according to the present invention, in a formulation that targets the agent to the brain.

FIGURES

[0021] Figure 1 shows the spatial learning index determined for young and aged rats in the Morris Water Maze (MWM) assessment.

[0022] Figure 2 illustrates the relationship between the MWM assessment characterization and memory performance in the Radial Arm Maze (RAM).

DETAILED DESCRIPTION

A. Definitions

[0023] The following definitions are provided to assist the reader. Unless otherwise defined, all terms of art, notations and other scientific or medical terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the biological, chemical and medical arts. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over the definition of the term as generally understood in the art.

Cognitive Function

[0024] "Cognitive function" has its usual meaning in the art, and refers to higher order intellectual, brain processes involved in learning and memory, including, but not limited to, attention, acquisition, short-term memory, long-term memory and memory retrieval, and expressing an interest in one's surroundings and self-care. In animal model systems such as rat, cognitive function may be measured methods including, but not limited to, using a maze in which subjects use spatial information (e.g, Morris water maze, Barnes circular maze,

elevated radial arm maze, T maze and others), fear conditioning, active avoidance, illuminated open-field, dark activity meter, elevated plus-maze, two-compartment exploratory test or forced swimming test.

[0025] In humans, cognitive function may be measured by, without limitation, the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog); the clinical global impression of change scale (CIBIC-plus scale); the Alzheimer's Disease Cooperative Study Activities of Daily Living Scale (ADCS-ADL); the Mini Mental State Exam (MMSE); the Neuropsychiatric Inventory (NPI); the Clinical Dementia Rating Scale (CDR); the Cambridge Neuropsychological Test Automated Battery (CANTAB) or the Sandoz Clinical Assessment-Geriatric (SCAG). In addition, cognitive function may be measured using imaging techniques such as Positron Emission Tomography (PET), functional magnetic resonance imaging (fMRI), Single Photon Emission Computed Tomography (SPECT), or any other imaging technique that allows one to measure brain function.

Cognitive Impairment

[0026] "Cognitive impairment (CI)" or "impaired cognitive function" refers to cognitive function that is reduced compared to cognitive function measured in an age-matched normal subject. The reduction can be manifested in any one or more of the above-mentioned parameters by which cognitive function is measured, or in any other parameter generally understood in the art to indicate cognitive function. In certain embodiments of the present invention, a person with impaired cognitive function is a person with Age Related Cognitive Decline (ARCD). In certain aspects of the present invention, a person with impaired cognitive function is a person with Mild Cognitive Impairment (MCI).

[0027] Impaired cognitive function involving dementias may be associated with other diseases or disorders, including dementias (e.g., Lewy body dementia, vascular dementia, Alzheimer's Disease, and HIV associated dementia), Huntington's Disease, Parkinson's Disease, schizophrenia, and amyotrophic lateral sclerosis. As used herein, impaired cognitive function does not include impairments associated with hyperlipidemias (e.g., hyperchylomicronemia).

Treating

[0028] "Treating" or "treatment of" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. Beneficial or desired clinical results

for a subject with impaired cognitive function include alleviation or amelioration of one or more manifestations of cognitive impairment as well as delay of onset or slowing of progression of cognitive impairment. Beneficial results can include, but are not limited to, a change in function sufficient to result in an improved score in a test of cognitive function or increased self-sufficiency in daily life. Treating cognitive impairment includes both promoting cognitive function and preserving cognitive function.

Subject

[0029] A "subject" as used herein is any mammal, e.g., a human. Human subjects may also be referred to herein as patients. Patients are generally understood to be individuals under medical care, and are generally in need of treatment for cognitive impairment. An "animal" or mammalian subject is any mammal customarily used in experimental model systems for assessing brain or cognitive function, such as a mouse, rat, or non-human primate. For purposes of this invention, mammalian subjects should be selected from a population, such as an outbred population, in which individuals can be characterized as aged impaired (AI), aged unimpaired (AU), and young (Y). The invention also encompasses animal subjects under veterinary care, e.g., companion animals, commercially valuable animals, animals of threatened or endangered species.

Promoting cognitive function

[0030] "Promoting" cognitive function refers to improving cognitive function in a subject with impaired cognitive function so that it more closely resembles the function of an age-matched normal, unimpaired subject. Cognitive function may be promoted to any detectable degree, such as, for example, a degree sufficient to result in an increased score in a test of cognitive function.

Preserving cognitive function

[0031] "Preserving" cognitive function refers to affecting normal or impaired cognitive function such that it does not decline or does not fall below the level observed in the subject upon first presentation or diagnosis, or prior to initiation of treatment, or slowing the rate of decline of cognitive function in normal or impaired subjects (e.g., compared to untreated subjects).

A hippocampal region

[0032] "A hippocampal region" refers to a physically defined region of the hippocampus, such as the Dentate Gyrus and/orone or more Cornu Ammonis regions (e.g., the CA1, CA2, or CA3 region or a combination of any two [1-2, 1-3, 2-3] or all three CA regions.

Neuronal cell

[0033] As used herein, "neuronal cell" has its normal meaning in the art and refers to a cell in or derived from the mammalian nervous system. A neuronal cell may be a neuron (e.g., CA1 pyramidal neuron), astrocyte, oligodendrocyte, microglia cell (e.g., Bergmann glia cells) or Schwann cell. A neuronal cell may be in vivo. Alternatively, a neuronal cell may be ex vivo (e.g., in vitro) such as in primary cell culture or from a transformed cell line (for example, microglia cell line BV-2). Methods for culture of neuronal cells are well known see, e.g., Brewer et al., 1993, *J. Neuroscience Res.* 35:567 [hippocampal neurons]; Walsh et al., 1995, *Neuroscience* 69:915-29 [suprachiasmatic neurons]; Zoran et al., 1996, *Dev Biol.* 179:212-22 [motoneuronal cultures]. Preferably, a neuronal cell is a cell or line derived from the hippocampus or a hippocampal region, and preferably is from a rodent (e.g., AU/AI or Y rat) or human.

Aged

[0034] "Aged" is used herein to refer to mammals (e.g., rats) at or near the end of their average life span. Typically an aged rat would be about 24-30 months of age. An aged human would be seventy or more years of age.

Young

[0035] "Young" refers to mammals (e.g., rats) at about the age of sexual maturity and when the hippocampus has just fully matured. Typically a young rat would be 6-9 months of age.

B. Therapeutic and Screening Methods

Introduction

[0036] When aged rats of the outbred Long-Evans strain (Charles River Laboratories; Gallagher et al., 1993, *Behav. Neurosci.* 107:618-626) are tested for cognitive ability,

performance of individuals varies greatly. Notably, aged rats naturally segregate into two populations exhibiting different levels of cognitive ability relative to young rats. About half of the aged rats perform on a par with young rats in cognition assays, and approximately half fall outside the range of young performance. Gallagher et al., 1993, *Behav. Neurosci.* 107:618-26. Thus, within the aged population some animals are cognitively impaired relative to young rats and are designated Aged Impaired (AI). Other aged animals are cognitively unimpaired, and are designated Aged Unimpaired (AU). Similar segregation in aged animals may be observed in other rodents, e.g., other outbred rats (e.g., Brown-Norway x Fisher 344 rats) and mice (e.g., C57BL mice).

[0037] As described in the Example, below, genes have now been identified that are differently expressed in AI rats compared to AU and young rats (designated "Y"), thereby identifying genes for which changes in expression are implicated in age-related cognitive decline. As discussed in detail in the Example, a decrease has been detected in expression of lipoprotein lipase mRNA in AI animals compared to both Y and AU animals.

[0038] Lipoprotein lipase (EC 3.1.1.34) catalyzes the hydrolysis of the triacylglycerol component of circulating chylomicrons and very low-density lipoproteins (VLDL) resulting in nonesterified fatty acids and 2-monoacylglycerol. The nucleic acid sequence encoding the rat lipoprotein lipase is described at Genbank accession No. L03294 and in Brault et al., 1992, Sequence of rat lipoprotein lipase-encoding cDNA, Gene 121: 237-46. The nucleic acid sequence encoding the human lipoprotein lipase is described at Genbank accession No. M15856 and in Wion et al., 1987, Human lipoprotein lipase complementary DNA sequence. *Science* 235:1638-1641.

[0039] Based, in part, on these discoveries, disclosed herein are methods and reagents for treatment of cognitive impairment including age-related cognitive impairment and other conditions, and methods for identification of agents useful for treatment of cognitive impairment. *Identification of Agents Useful for Treatment of Cognitive Impairment*

1. Agents That Increase Expression of LPL

[0040] The invention provides methods for determining whether an agent is useful for treatment of cognitive impairment (CI) in a mammal. By "agent" is meant herein any molecular entity (known or novel) that has or is suspected of having biological activity in an identification method or therapeutic method of the invention. In one aspect, the invention provides a method for determining whether an agent is useful for treatment of cognitive

impairment by determining whether the agent increases expression of LPL in a neuronal cell, hippocampus, or a hippocampal region. An agent that increases expression of LPL in a neuronal cell, hippocampus, or a hippocampal region is identified as useful for treatment of CI.

[0041] A number of different assays or screening protocols can be utilized to identify agents that increase the level of expression of LPL in mammalian cells (*e.g.*, mouse cells, rat cells, non-human primate cells or human cells). In general terms, the screening methods include screening a plurality of agents ("test agents") to identify an agent that changes the expression of a gene encoding LPL protein (resulting in greater LPL mRNA abundance) or that increases the activity of an LPL protein. Thus, without limitation, useful agents may increase expression of an LPL gene, bind to an LPL polypeptide, prevent an inhibitor from binding to an LPL protein, or increase activity of an LPL protein. Methods of automating assays are known that permit screening of several thousands of compounds in a short period.

[0042] Useful test agents include compounds of a variety of general types including, but not limited to, small organic molecules (e.g., MW < 5000, e.g., < 1000, often < 500); polypeptides; carbohydrates such as oligosaccharides and polysaccharides; polynucleotides; antibodies, lipids or phospholipids; fatty acids; steroids; or amino acid analogs. Test agents can be obtained from libraries, such as natural product libraries (e.g., extracts of fungi, bacteria, yeast, algae, higher plants, insects, or any natural material comprising living organisms), and combinatorial libraries (made or modified e.g., by any synthetic means). A number of different types of libraries are commercially available and methods for preparing libraries have been described. See for example, PCT publications WO 93/06121, WO 95/12608, WO 95/35503, WO 94/08051 and WO 95/30642. Examples of chemically synthesized libraries are described in Fodor *et al.*, 1991, Science 251:767-73; Houghten *et al.*, 1991, *Nature* 354:84-86; Lam *et al.*, 1991, Nature 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-10; Gallop *et al.*, 1994, *J. Med. Chem.* 37:1233-51; Ohlmeyer *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-26; Erb *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-26; Houghten *et al.*, 1992, *Biotechniques* 13:412-21; Jayawickreme *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-18; Salmon *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-12; PCT Publication WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-83.

[0043] Screening methods can involve conducting cell-based assays in which one or more cells expressing an LPL gene are contacted with a test agent(s) then a change in LPL expression is detected by, for example, detecting an increase in levels of LPL mRNA, LPL protein abundance or LPL enzyme activity. Cells can be contacted *in vitro*, *in vivo* or *ex vivo* with a test agent. The cells may naturally express the LPL gene (i.e., the gene may be wholly endogenous to the cell or multicellular organism) or the cell may be a recombinant cell or transgenic organism comprising one or more recombinantly expressed LPL genes. Expression of a recombinant LPL gene can be accomplished using published gene and protein sequences and routine methods. See *e.g.*, Sambrook *et al.*, 2001, *Molecular Cloning: A Laboratory Manual*, 3rd ed. (Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press); Ausubel *et al.*, *Current Protocols in Molecular Biology*, 4th ed., John Wiley and Sons, New York (1999) as supplemented through June 2004. Assays can be carried out using any cell type that expresses an LPL gene including, in various embodiments, a cultured cell (*e.g.*, a cell in a primary culture or an established cell line) including a recombinant cell expressing a heterologous LPL gene (e.g., a mammalian cell such as CHO cells, HEK293 cells or COS7 cells). Particularly useful cells include neurons, glia cells, mixed neuronal cultures, primary cultures from human (e.g., fetal) or non-human hippocampus. In one embodiment, the cells are primary cells from a hippocampal region (e.g., CA1 or CA3).

[0044] Likewise, assays can be conducted using a cell *in vivo* (e.g., by administering the agent to a multicellular organism (*e.g.*, animal, such as a non-human mammal, and including animals transgenic for a heterologous LPL gene)). For example, test agents that increase LPL gene expression, LPL RNA levels, LPL protein activity and/or LPL protein levels, can be further screened in an *in vivo* model. In an embodiment, a method is provided of screening compounds for utility in promoting cognitive function of a mammal by administering a test agent to an animal (e.g., a mammal, such as a rat) determining the level of expression of an LPL gene in neural tissue of the animal following administration of the test agent, comparing the level of expression of the LPL gene to a reference level of expression in neural tissue of an animal to which the test agent was not administered and determining whether the level of expression of the gene differs from the corresponding reference level, where a difference indicates that the test agent is a candidate therapeutic agent for preserving or promoting cognitive function.

[0045] In an exemplary embodiment, the method includes contacting a cell with a test agent and determining whether the level of expression of the gene is increased in the presence

of the test agent compared to expression in the absence of the agent, where an increase in expression is an indication that the test agent may be useful for promoting or preserving cognitive function. Typically expression is increased by at least about 10%, at least about 20%, or at least about 50% as compared to expression in the absence of the test agent. LPL gene expression and RNA levels can be assessed by art known methods including detecting changes in the abundance of LPL RNA by Northern blotting, microarray methods, or using other art known methods. *See, e.g.*, Ausubel *et al.*, *supra*; Sambrook *et al.*, *supra*.

[0046] In another exemplary embodiment, the method includes identifying agents that increase the LPL protein expression or activity. Such methods can involve conducting cell-based assays in which test agents are contacted with one or more cells expressing an LPL gene or protein and then determining whether a change in LPL expression (*e.g.*, levels of LPL polypeptide) or activity results. In another exemplary embodiment, the method includes contacting a cell with a test agent and determining whether the level of activity of the LPL protein is changed in the presence of the test agent, where a change (*e.g.*, an increase) in activity is an indication that the test agent is useful for promoting or preserving cognitive function. LPL protein activity may be assessed by art-known methods. *See, e.g.*, Pykalisto *et al.*, 1975, *Proc. Soc. Exp. Biol. Med.* 148:297; Taskinen *et al.*, 1979, *Diabetologia* 17:351; or Nilsson-Ehle and Shotz,, 1976, *J. Lipid Res.* 17:536. Typically, LPL protein activity is increased by at least about 10%, at least about 20%, or at least about 50% compared to activity in the absence of the test agent.

[0047] Usually the determination comprises comparing the expression or activity in the test cell compared to a similar cell or cells (*e.g.*, a control cell(s)) that have not been contacted with the test agent. In some embodiments the determination comprises comparing the expression or activity in an extract from the test cell compared to an extract from a control cell.

[0048] Other cell-based assays are reporter assays conducted with cells that express a reporter gene. For example, assays can be conducted with a heterologous nucleic acid construct that includes an LPL gene promoter that is operably linked to a reporter gene that encodes a detectable product. Certain LPL gene promoters are described in GenBank (http://www.ncbi.nlm.nih.gov/) (*see, e.g.*, UniGene designations HS.180878, Mm.1514, Mm.349856, or Rn.3834) and the scientific literature. A number of different reporter genes can be utilized. Exemplary reporters include green fluorescent protein, β-glucuronidase,

chloramphenicol acetyl transferase, luciferase, β -galactosidase, alkaline phosphatase, and the like. In these assays, cells harboring the reporter construct are contacted with a test agent. A test agent either activates the promoter by binding to it or triggers a cascade that produces a molecule that activates the promoter causes expression or a change in expression of the detectable reporter. A variety of different types of cells can be utilized in the reporter assays (*e.g.*, eukaryotic cells such as yeast, COS, CHO, HepG2, and HeLa cell lines). In one embodiment, the cells are neurons, glial cells, mixed neuronal cultures, primary cultures from human (e.g., fetal) or non-human hippocampus. In one embodiment, the cells are primary cells from a hippocampal region (e.g., CA1 or CA3).

[0049] Cell-based reporter assays also can be conducted to identify target agents that act synergistically with or that counteract the effect of other agents known to affect cognitive function. For example, assays can be conducted with a heterologous nucleic acid construct that includes an LPL gene promoter that is operably linked to a reporter gene that encodes a detectable product. See, *e.g.*, Tully et al., 2003, Targeting The CREB Pathway for Memory Enhancers, *Nature Reviews* 2:267-277. Exemplary reporters include green fluorescent protein, β-glucuronidase, chloramphenicol acetyl transferase, luciferase, β-galactosidase, alkaline phosphatase, and the like. In these assays, cells harboring the reporter construct are contacted with a test agent. In an embodiment, a test agent that produces little or no change in reporter gene expression by itself, but which synergizes (*i.e.*, greater than two-fold) the effect of a suboptimal dose of another agent known to affect cognitive function, is an indication that the test agent may be useful for promoting or preserving cognitive function. In another embodiment, a test agent that produces little or no change in reporter gene expression by itself, but which counteracts (i.e., reduces) the effect of dose of another agent known to negatively affect (i.e., reduce) cognitive function is an indication that the test agent may be useful for promoting or preserving cognitive function. A variety of different types of cells can be utilized in the reporter assays (*see* supra).

[0050] Identification of agents that increase activity of the LPL protein can also include screening for compounds capable of binding to an LPL protein, as at least some of the compounds so identified are likely LPL modulators. Lead compounds identified during these screens can serve as the basis for the synthesis of more active analogs. Thus, in one aspect, a method of screening an agent to determine its usefulness in reduction of cognitive impairment or decline comprises (a) contacting a polypeptide encoded by an LPL gene, or a cell expressing such a polypeptide with a test agent, and (b) determining whether the

polypeptide binds to the test agent. Such binding is an indication that the test agent is useful in reduction of cognitive impairment. The binding assays usually involve contacting an LPL polypeptide with one or more test agents and allowing sufficient time for the protein and test agent to form a binding complex. Determining the ability of the test agent to directly bind to an LPL polypeptide can be accomplished, for example, by coupling the agent to a radioisotope, enzymatic label or chemiluminescent label such that binding of the compound to the LPL polypeptide can be determined by detecting the labeled LPL polypeptide in a complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation, co-migration on non-denaturing SDS-polyacrylamide gels, and co-migration on Western blots (*see, e.g.*, Ausubel *et al.*, *supra*; Harlow and Lane, *Using Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1999); the disclosures of which are incorporated by reference herein). The LPL polypeptide utilized in such assays can be purified or recombinant. As noted above, recombinant expression and purification of LPL proteins can be accomplished using routine methods.

[0051] The LPL proteins can, *in vivo*, interact with one or more cellular and extracellular molecules (such as, without limitation, peptides, proteins, cofactors and nucleic acids) herein referred to as "binding partners." Methods are known to detect binding partners of LPLs, *e.g.*, two and three-hybrid assays. *See, e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.*, 1993, *Cell* 72:223-232; Madura *et al.*, 1993, *J. Biol. Chem.* 268:12046-54; Bartel *et al.*, 1993, *Biotechniques* 14:920-24; Iwabuchi *et al.*, 1993, *Oncogene* 8:1693-96; WO94/10300. Art-known assays can be devised to identify compounds that modulate (*e.g.*, affect either positively or negatively) interactions between an LPL protein and a binding partner. Typically, the assay for compounds that modulate the interaction between the LPL protein and a binding partner involves preparing a reaction mixture containing the LPL protein and a binding partner under conditions and for a time sufficient to allow the two products to interact and bind, thus forming a complex. In order to test an agent for modulatory activity, the reaction mixture is prepared in the presence and absence of the test agent. Also, methods can also be used for the direct detection of interactions between the LPL protein and a test agent in a homogeneous or heterogeneous assay system without further sample manipulation. For example, the technique of fluorescence energy transfer may be utilized (*see, e.g.*, U.S. Pat. No. 5,631,169; U.S. Pat. No. 4,868,103).

2. Agents That Increase Expression of LPL in AI Rats

[0052] In a related aspect, the invention provides a method for determining whether an agent is useful for treatment of CI by determining whether the agent increases expression of LPL in a neuronal cell from, or the hippocampus or a hippocampal region of an Aged Impaired (AI) rat. An agent that increases expression of LPL in the neuronal cell, hippocampus or a hippocampal region is identified as putatively useful for treatment of CI.

[0053] In a preferred embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to a level that is the same as the level in an Aged Unimpaired (AU) animal. In one embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 300% of the AI level. In one embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 200% of the AI level. In an embodiment, the expression is measured using Probe Set L03294_at (see Table 2, in the Example below). In an embodiment, the expression is measured using Probe Set L03294_g_at (see Table 2, in the Example).

[0054] In a preferred embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal, but does not significantly change the level of expression of LPL in a Young or Aged Unimpaired animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 300% of the AI level but does not significantly change the level of expression of LPL in a Young and/or Aged Unimpaired animal. In one embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 200% of the AI level but does not significantly change the level of expression of LPL in a Young and/or Aged Unimpaired animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to at least about 150% of the AI level, optionally at least about 200% of the AI level, but does not significantly change the level of expression of LPL in a Young and/or Aged Unimpaired animal.

[0055] To assess the presence and magnitude of effect of an agent on LPL gene expression in AI and AU rats, the "YUI" (Young, Aged Unimpaired, and Aged Impaired) assay can be

used. Generally, the YUI assay involves (1) administering an agent to AI animals (e.g., rats), optionally to AU and/or Y animals and (2) determining the effect of administration of the agent on expression of LPL in the hippocampus, a hippocampal region, or a cell from the hippocampus of the AI animals, and optionally the Y and AU animals. In one embodiment, the animals are outbred Long-Evans strain rats. In one embodiment, the animals are Brown-Norway x Fisher 344 rats. See Markowska and Savonenko, 2002, Retardation of cognitive aging by life-long dietary restriction: implications for genetic variance. *Neurobiol. Aging* 23:75-86. In one embodiment, the animals are C57BL mice. See Verbitsky et. al., 2004, Altered hippocampal transcript profile accompanies an age-related spatial memory deficit in mice. *Learning and Memory* 11:253-260.

[0056] Methods for identifying populations of young, AI and AU rats are known in the art, and an exemplary method is described in the Example, below. As noted above, aged rats naturally segregate into two populations, with about half of the aged rats being AI and about half being AU. To identify these populations, the cognitive ability of young and aged rats can be measured. A number of methods for assessing rat cognitive ability are know (for example, the Barnes circular maze, the radial arm maze, the Morris water maze, delayed alternation (delayed nonmatch-to-sample), novel object recognition, conditioned avoidance, fear conditioning). See Gallagher and Burwell, 1989, *Neurobiol. Aging* 10:691-708.; Ingram et al, 1981, Age and neurochemical correlates of radial maze performance in rats. *Neurobiol Aging* 2: 41-7; Morris, 1981, Spatial localization does not require the presence of local cues. *Learning and Motivation* 12: 239-260; Moore et al., 1992, Toward modeling age-related changes of attentional abilities in rats: simple and choice reaction time tasks and vigilance. Neurobiol. Aging 13: 759-772; Ennaceur and Delacour, 1988, A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav. Brain Res.* 31: 47–59; Taubenfeld et al., 2001, Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein [beta] and [delta] Co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term meomory consolidation. *J Neurosci* 21:84-91.; Quirk et al., 1995, Fear conditioning enhances short-latency auditory responses of lateral amygdale neurons: parallel recordings in the freely behaving rat. *Neuron* 15: 1029-39.

[0057] In a preferred embodiment, cognitive ability is determined using the Morris water maze assay (MWM). In the Morris water maze assay, rats learn and remember the location of an escape platform guided by a configuration of spatial cues surrounding the maze. The cognitive basis of performance is tested in probe trials using measures of the animal's spatial

bias in searching the location of the escape platform. Aged rats in the study population have no difficulty swimming to a visible platform, but an age-dependent cognitive impairment can be detected when the platform is camouflaged, requiring the use of spatial information.

[0058] When reassessed using the MWM in a new spatial environment several weeks after the original characterization, the AI animals are consistently impaired, whereas the AU animals again perform proficiently. Colombo et al., 1997, *Proc. Natl. Acad. Sci.* 94:14195-99. The difference in cognitive ability in the MWM assessment for AI and AU rats is reliable even over an interval of 3 months. Gallagher and Burwell, 1989, *Neurobiol.* Aging 10:691-708. Further, AI and AU characterization in the MWM differentiates the performance of the same aged subjects in other behavioral tasks that require the same cognitive function, such as the Barnes circular maze (Gallagher and Burwell, 1989, *Neurobiol.* Aging 10:691-708) and the radial arm maze (RAM). This naturally occurring impairment in an aged population of rodents indicates that cognitive aging is not inevitable or strictly linked to chronological age, and, importantly, it affords the opportunity to compare the trajectory of changes in the brain that lead to decline or preserved memory.

[0059] After rats are characterized for cognitive status in the MWM, the effect of a test agent on LPL expression in AI and/or AU and/or Y rats can be tested. Usually, control animals within each group are administered vehicle alone rather than the agent. The amount of agent administered, and mode of administration, will vary depending on the characteristics of the agent, and can be determined by the practitioner. Typically between 0.1 and 1000 mg/kg is administered daily, for one or more days.

[0060] The hippocampus is obtained from the AI and/or AU rats, and optionally from Y rats. The hippocampus may be frozen or immediately processed to isolate RNA, or may be dissected and RNA isolated from a particular hippocampal region (e.g., the CA1 and/or CA3 region). Labeled cRNA probes are prepared and hybridized to immobilized LPL sequences. The relative abundance of LPL RNA in AI, AU and Y rats is then calculated based on the quantity of hybridization LPL probe.

[0061] Conveniently, RNA abundance can be determined using any quantitative method including, but not limited to, microarrays. See, e.g., Schena et al., 1995, Quantitative monitoring of gene expression patterns with a complementary DNA microarray, *Science* 270:467-470; DeRisi et al., 1996, Use of a cDNA microarray to analyze gene expression patterns in human cancer, *Nature Genetics* 14:457-460; Shalon et al., 1996, A DNA

microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization, *Genome Res.* 6:639-645; Schena et al., 1995, Parallel human genome analysis; microarray-based expression of 1000 genes, *Proc. Natl. Acad. Sci. USA* 93:10539-11286; Fodor et al., 1991, Light-directed spatially addressable parallel chemical synthesis, *Science* 251:767-773; Pease et al., 1994, Light-directed oligonucleotide arrays for rapid DNA sequence analysis, *Proc. Natl. Acad. Sci. USA* 91:5022-5026; Lockhart et al., 1996, Expression monitoring by hybridization to high-density oligonucleotide arrays, *Nature Biotech* 14:1675; U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270; Blanchard et al., 1996, High-density oligonucleotide arrays, *Biosensors & Bioelectronics* 11: 687-90). In a preferred embodiment, oligonucleotide probes are used, such as those listed in Table 2 below. In one embodiment, the Affymetrix Genechip® U34A (or an equivalent) is used.

[0062] Hybridization conditions will depend on the cRNA label selected and on the nature of the probe used but can readily be determined by one of ordinary skill. The conditions of the assay are as generally described in the Example below.

[0063] As used in this context of the YUI assay, an agent "does not significantly change" the level of expression of LPL (e.g., in a Young and/or Aged Unimpaired rat) when administered at a specified dose if the change in expression in the Y and/or AU rat is less than 15%, or in some embodiments, less than 10%.

[0064] As used in this context of the YUI assay, the level of expression in an AU rat and an AI rat is "the same" when the values are within 15%, or in some embodiments, within 10% of each other.

[0065] It will be appreciated that the assays will be conducted using sufficient animals and trials to produce statistically significant values. Generally, similarities and differences will be supported by data with a p value less than or equal to 0.05; optionally less than or equal to 0.01.

3. Other targets

[0066] The methods described above are also applicable to identification of therapeutic agents that increase expression of other genes for which changes in expression are implicated in age-related cognitive decline. For example, given a gene for which changes in expression are implicated in age-related cognitive decline, agents useful for treatment of CI can be identified by identifying agents that modulate (increase or decrease) the expression of the gene in the hippocampus or hippocampal region of an AI animal, but does not significantly

change the level of expression in a Y or AU animal. Useful agents increase expression of genes that are underexpressed in the AI animals compared to the AU animals, or decrease expression of genes that are overexpressed in the AI animals compared to the AU animals.

4. Further Assays

[0067] Agents identified by assay(s) described above can be administered to experimental animals to measure their cognition promoting and preserving activities. Suitable assays for cognition include, for example, escape latency tests or passive avoidance tests (*see, e.g.,* U.S. Pat. No. 6,300,373). Other assays for cognitive impairment include, for example, spatial memory impairments (*see, e.g.*, Brightwell *et al.*, 2004, *Neurobiol. Learn Mem.* 81:19-26).

[0068] In a preferred embodiment a YUI-based assay is used for further testing of an LPL activating agent. By way of illustration, the cognitive status of rats is characterized in the MWM, and AI rats are assigned to one of two treatment conditions (control vehicle or agent) that were equated with respect to their MWM learning index scores. Initially rats in both treatment conditions are trained on the RAM task (habituation, no-delay version, and then delay of 60 seconds). Then the agent or vehicle alone is administered (e.g., by injection). Rats receive daily testing on the radial-arm maze with the delay extended to 3 hr. Critical tests at the 3 hour delay occur on days 8-10 (after 7 days of injection). The effect of the agent is demonstrated when memory errors for aged rats receiving vehicle alone are significantly elevated relative to a young group tested concurrently.

Determination of Dosage of Agents Useful for Treatment of Cognitive Impairment

[0069] In one aspect, the invention provides a method for determining an optimal dosage or an initial clinical dose of an agent for treatment of CI. The optimal dosage for treatment of CI is the dose in the YUI rat model that increases LPL expression in the hippocampus or a hippocampal region of an AI animal. In one embodiment, the method involves determining the dose of the agent that in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI animal to a level that is the same as the level in an AU animal and, optionally, does not significantly change the level of expression of LPL in a Young or AU animal. In one embodiment, the method involves determining the dose of the agent that in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI animal to from about 150% to about 300% of the untreated AI level and, optionally, does not significantly change the level of expression of LPL in a Y or AU animal. In one

embodiment, the method involves determining the dose of the agent that in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI animal to from about 150% to about 200% of the untreated AI level and, optionally, does not significantly change the level of expression of LPL in a Y or AU animal. In one embodiment, the method involves determining the dose of the agent that increases the expression of LPL in the hippocampus or a hippocampal region of an AI animal to at least about 150% of the untreated AI level, optionally at least about 200% of the untreated AI level, but does not significantly change the level of expression of LPL in an untreated Y and/or AU animal.

[0070] Having determined an optimal dosage or dosage range in the YUI rat model, art known methods for determining the initial clinical dose of the agent for administration to humans are used. See, e.g., Guidance for Industry and Reviewers, Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, U.S. Dept of Health and Human Services, Dec. 2002, the teachings of which are incorporated herein by reference. Briefly, a dose defined as the maximum recommended starting dose (MRSD) for human clinical trials is derived using the no observed adverse effect levels (NOAELs) in relevant animal models. Once toxicity testing is completed in one or more experimental animal model systems (e.g., rat, non-human primate), the NOAEL is determined by inspection of the data for each animal species. The NOAEL is the highest dose level (usually in mg/kg) that does not produce a significant increase in adverse effects. Each NOAEL is converted into a human equivalent dose (HED) using an appropriate scaling factor, usually related to body surface area (mg/m^2). In the case of rats, the scaling factor usually applied is 6 for systemically administered drugs. Different scaling factors are required where drug is administered locally or into a defined body compartment. Once all HEDs are calculated, the most appropriate experimental animal species is selected for predicting the experience anticipated in human clinical trials. Generally, the most appropriate species is the one yielding the lowest HED, although other factors can be taken into account such as comparative metabolism, similarities in biochemistry, toxicology, and physiology, and prior experience with the class of compounds to which a particular drug belongs. For purposes of this invention, the YUI model system in outbred rats is considered to be the most appropriate species. Then, a safety factor is applied, typically reducing the HED in the most appropriate species by a factor of 10. The MRSD so derived is then compared to the pharmacologically active dose (PAD) derived from preclinical pharmacodynamic models. If the pharmacologic

HED is lower than the MRSD, the initial dose for clinical trials is lowered accordingly. It is expected that the actual dose for patients in need of treatment for cognitive impairment will be within the range 0.10 - 5-fold the initial clinical dose. A "therapeutically effective" dose or amount of the agent is accordingly understood to be any dose that produces a detectable benefit in any parameter of cognitive function, including surrogate markers of cognitive function (including novel markers as discovered and defined according to this invention).

Therapeutic Administration of Agents That Increase LPL Expression/Activity

[0071] In another aspect, the invention provides a method for treating CI in a patient comprising administering a therapeutically effective amount of an agent that increases LPL expression in the hippocampus or a hippocampal region of an AI animal. Preferably the agent when administered in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI animal to a level that is the same as the level in an AU animal and, optionally does not significantly change the level of expression of LPL in a Y or AU animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an AI animal to from about 150% to about 300% of the untreated AI level but does not significantly change the level of expression of LPL in a Y and/or AU animal. In one embodiment, the agent increases the expression of LPL in the hippocampus or hippocampal region of an AI animal to from about 150% to about 200% of the untreated AI level but does not significantly change the level of expression of LPL in a Y and/or AU animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to at least about 150% of the untreated AI level, optionally at least about 200% of the untreated AI level, but does not significantly change the level of expression of LPL in a Y and/or AU animal.

[0072] In an embodiment, the agent is administered in an amount that is a human equivalent to the amount that in the YUI assay increases LPL expression in the hippocampus or a hippocampal region of an aged impaired animal to a level that is the same as the level in an AU animal and, optionally does not significantly change the level of expression of LPL in a Y or AU animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 300% of the untreated AI level but does not significantly change the level of expression of LPL in a Y and/or AU animal. In one embodiment, the agent increases the

expression of LPL in the hippocampus or a hippocampal region of an Aged Impaired animal to from about 150% to about 200% of the untreated AI level but does not significantly change the level of expression of LPL in a Y and/or AU animal. For example, in an embodiment, the agent increases the expression of LPL in the hippocampus or a hippocampal region of an AI animal to at least about 150% of the untreated AI level, optionally at least about 200% of the untreated AI level, but does not significantly change the level of expression of LPL in a Y and/or AU animal.

[0073] In certain embodiments, the agent is administered in a form or formulation targeted to the brain generally, or to the hippocampus particularly.

[0074] Exemplary agents that activate LPL expression and are expected to be useful in treatment of CI when administered in accord with the invention include, but are not limited to, those described below.

[0075] In one embodiment, ibrolipim is administered to a subject in need thereof to treat CI. Ibrolipim (also called NO-1886; CAS Registry No. 133208-93-2; diethyl [4-[(4-bromo-2- cyanophenyl)carbamoyl] benzyl]phosphonate) has been developed for protection against atherosclerosis. Also useful are analogs, derivatives and metabolites of ibrolipim that increase lipoprotein lipase activity. For a description of NO-1886, including a description of useful analogs, derivatives and metabolites, see: Tsutsumi et al., 1993, The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. *J. Clin. Invest.* 92: 411-417; Yin and Tsutsumi, 2003, Lipoprotein lipase activator NO-1886. *Cardiovascular Drug Reviews* 21: 133-142.; Goto et al., 1996, Synthesis and biological activity of the metabolites of diethyl 4-[(4-bromo-2-cyanophenyl) carbamoyl] benzylphosphonate (NO-1886). *Chem Pharm Bull (Tokyo)* 44: 547-551; Kurogi et al., 1996, Synthesis and hypolipidemic activities of novel 2-[4-[diethoxyphosphoryl) methyl]phenyl] quinazolines and 4(3H)-quinazolinones. *J Med Chem* 39: 1433-1447; Morioka et al., 1996, Metabolism of diethyl 4-[(4-bromo-2-cyanophenyl)-carbamoyl]benzylphosphonate in the rat. *Xenobiotica* 26: 853-861. Effective amounts (doses) for increasing lipoprotein lipase activity in human tissues are described in these and other publications, and can be determined as described hereinabove.

[0076] In one embodiment, an agonist of a PPAR (peroxisome proliferator-activated receptor) is administered to a subject in need thereof to treat CI. The agonist may be specific

for PPARalpha, PPARgamma, or may be a dual activator of PPARalpha and PPARgamma. Activated peroxisome proliferator activated receptors (PPARs) alter the transcription of the LPL and ApoC-III genes. Both the LPL gene and the ApoC-III gene contain a peroxisome proliferator response element (PPRE) that PPARs specifically bind to as a transcription factor. PPAR agonists increase transcription of the LPL gene and increase expression and activity of the LPL protein. PPAR-alpha agonists also decrease transcription of the ApoC-III gene and thereby decrease the expression and activity of ApoC-III, which is an inhibitor of LPL activation. See, e.g., Staels et al, 1997, The effects of fibrates and thiazolidinediones on plasma triglyceride metabolism are mediated by distinct peroxisome proliferator activated receptors (PPARs). *Biochimie* 79: 95-99. Also see Shearer and Hoekstra, 2002, "Peroxisome Proliferator-Activated Receptors (PPARs): Choreographers of Metabolic Gene Transcription" in *Celltransmissions* 18:3 (Sigma-Aldrich) published at http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Signaling/Celltransmissions/Celltransmissions__Vol__18__No__3.html; Shearer and Hoekstra, 2003, "Recent advances in peroxisome proliferator-activated receptor science." *Curr Med Chem.* 10:267-80

[0077] In one embodiment, the agent is a fibrate drug such as fenofibrate. Fenofibrate (CAS Registry Number: 49562-28-9) is a selective ligand for peroxisome proliferator-activated receptors alpha (PPARα). Also useful are analogs, derivatives and metabolites of fibrates that increase lipoprotein lipase activity. Exemplary analogs include clofibrate (CAS Reg. No.: 637-07-0), gemfibrozil (CAS RN: 25812-30-0), bezafibrate (CAS Reg. No.: 41859-67-0), ciprofibrate (CAS Reg. No.: 52214-84-3) and others known in the art (see, e.g., Grundy and Vega, 1987, Fibric acids: effects on lipids and lipoprotein metabolism. *Am J Med* 83(5B): 9-20.) For a description of fenofibrate, including a description of useful analogs, derivatives and metabolites, see: Kloer, 1987, Structure and biochemical effects of fenofibrate. *Am J Med.* 83(5B): 3-8; Guo et al., 2001, Regulation of lipid metabolism and gene expression by fenofibrate in hamsters. *Biochim Biophys Acta* 1533: 220-232; Staels and Auwerx, 1992, Perturbation of developmental gene expression in rat liver by fibric acid derivatives: lipoprotein lipase and K-fetoprotein as models. *Development* 115: 1035-1043; Caldwell, 1989, The biochemical pharmacology of fenofibrate. *Cardiology* 76 (suppl. 1): 33-44; Inoue et al., 2003, Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferators-activated receptor alpha in mice. *Neurosci Letters* 352: 203-206; U.S. Pat. US4859703 (Lipid regulating compositions); U.S. Patent application

US20040005339A1 (Formulations of fenofibrate and/or fenofibrate derivatives with improved oral bioavailability).

[0078] In one embodiment, GW9578 [(2-[4-{2-[3-(2,4-difluorophenyl)-1-heptylureido]ethyl}phenylsulfanyl]-2-methylpropanoic acid] is administered to a subject in need thereof to treat CI. GW9578 is a subtype-selective PPARalpha agonist (*see* Brown et al., 1999, A ureido-thioisobutyric acid (GW9578) is a subtype-selective PPARalpha agonist with potent lipid-lowering activity. *J Med Chem* 42: 3785-3788).

[0079] In one embodiment, GW7647 [2-(4-(2-(1-Cyclohexanebutyl-3-cyclohexylureido) ethyl)phenylthio) -2-methylpropionic acid; CAS Registry Number: 265129-71-3] is administered to a subject in need thereof to treat CI. GW7647 is a subtype-selective PPARalpha agonist (see Brown et al., 2001, Identification of a subtype selective human PPARalpha agonist through parallel-array synthesis. *Bioorg Med Chem Lett* 11: 1225-1227.).

[0080] In one embodiment, WY-14643 [pirinixic acid; 4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid; CAS Registry Number: 50892-23-4] is administered to a subject in need thereof to treat CI. WY-14643 is a subtype-selective PPARalpha agonist (see Santilli et al., 1974, A potent antihypercholesterolemic agent: (4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid (Wy-14643). *Experientia* 30: 1110-1111.

[0081] In one embodiment, BM-17.0744 is administered to a subject in need thereof to treat CI. BM-17.0744 is a PPARalpha agonist (see Meyer et al., 1999, Species differences in induction of hepatic enzymes by BM 17.0744, an activator of peroxisome proliferator-activated receptor a (PPARa). *Arch Toxicol* **73**: 440–450;.

[0082] In one embodiment, GW409544 is administered to a subject in need thereof to treat CI. GW409544 is a PPARalpha agonist (see WO03075911A1: Use of PPAR Alpha Agonists for the Treatment of Vascular and Renal Diseases).

[0083] Other agents useful for treatment of CI include the PPARgamma agonists GI262570 (Farglitazar; CAS Reg. No.: 196808-45-4); GW1929; GW7845; SB-219994; JTT-501; and KRP-297. See Shearer and Hoekstra WJ. Peroxisome proliferator-activated receptors (PPARs): Choreographers of metabolic gene transcription. *Celltransmissions (by Sigma-Aldrich)* 18(3): 3-10.In one embodiment, the agent is an agent that is a dual activator (agonist) of both PPAR-alpha and PPAR-gamma agonist. In one embodiment, the agent is DRF-2519, shown in ex vixo studies to increase LPL protein expression and activity in

adipose tissue. See Chakrabarti et al., 2004, Antidiabetic and hypolipidemic potential of DRF 2519-a dual activator of PPAR-alpha and PPAR-gamma. *Eur J Pharmacol.* 491:195-206. In one embodiment, the agent is DRF-2525 (Ragaglitzar), a co-ligand of PPAR-alpha and PPAR-gamma that significantly increases both liver and fat LPL activity as well as reducing plasma ApoCIII levels. See Chakrabarti et al., 2003, Ragaglitazar: a novel PPAR alpha PPAR gamma agonist with potent lipid-lowering and insulin-sensitizing efficacy in animal models. *Br J Pharmacol.*140:527-37.

[0084] In one embodiment, a thiazolidinedione is administered to a subject in need thereof to treat CI. In one embodiment, the thiazolidinedione is rosiglitazone (BRL49653; CAS Registry No.: 122320-73-4; 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-2,4-thiazolidinedione). In one embodiment the thiazolidinedione is pioglitazone (Actos; CAS RN: 111025-46-8). In one embodiment the thiazolidinedione is troglitazone (Rezulin; CAS RN: 97322-87-7). See Hauner, 2002, The mode of action of thiazolidinediones. *Diabetes Metab Res Rev* 18 (Suppl. 2): S10-5; Schoonjans et al., 1996, PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 15: 5336-5348; Lefebvre et al., 1997, Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler Thromb Vasc Biol* 17: 1756-1764. Analysis of the influence of BRL49653 (rosiglitazone) on the expression of LPL and apo C-III, two key players in triglyceride catabolism, showed a dose-dependent increase in mRNA levels and activity of LPL in epididymal adipose tissue, whereas liver apo C-III mRNA levels remained constant. Furthermore, addition of BRL49653 to primary cultures of differentiated adipocytes increased LPL mRNA levels, indicating a direct action of the drug on the adipocyte.

[0085] In one embodiment, Resveratrol (CAS Registry Number: 501-36-0) is administered to a subject in need thereof to treat CI. See Inoue et al., 2003, Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferators-activated receptor alpha in mice. *Neurosci Letters* 352: 203-206.

Administration of Therapeutic Agents

[0086] Therapeutic agents for treatment of MCI and ARCD may be administered to a patient in need of therapeutic or prophylactic treatment by a variety of routes, including orally, intravenously, arterially, transdermally, suppository, ocular, inhalation, subcutaneously, intramuscularly, and sublingually.

[0087] In one embodiment, the agent is administered in a form or formulation targeted to the brain generally, and optionally to the hippocampus particularly. It is believed that targeted drug delivery is advantageous as it permits the administration of higher concentrations of therapeutic agents to the brain (e.g., hippocampus) and reduces the effect on LPL expression in non-brain tissues.

[0088] A variety of carriers may be used to facilitate targeted drug delivery across the blood brain barrier to brain tissues, including but not limited to the use of liposomes, nanoparticles, microparticles, microspheres, encapsulated microbubbles or similar structures which envelope biologically or pharmaceutically active agents, carrier molecules including polymers, and protein including hydrophile proteins.

[0089] Liposomes include unilamellar and multilamellar lipid vesicles. Unilamellar lipid vesicles, include molecules made by techniques described by Batzri and Korn, *Biochem. Biophys. Acta.* 298: 1015-1019, 1973; Deamer and Bangham, *Biochem. Biophys. Acta.* 443: 629-634, 1976; Weder and Zumbuehl, in "Liposome Technology" ed. G. Gregoriadis, CRC Press Inc. Boca Raton, Fla., Vol. I, Ch. 7, pg 79-107, 1984, and in U.S. Pat. No. 4,016,100. Multilammelar lipid vesicles, first described by Bangham, et al., *J. Mol. Biol.* 13: 238:252, 1965, may be made by techniques not limited to the techniques described U.S. Pat. No. 4,485,054, U.S. Pat. No. 4,234,871, and Barenholz *et al.*, *FEBS Lett.* 99: 210-214, 1979.

[0090] Liposomes may comprise targeting molecules on the liposome surface to as described in US 4,920,016, US 6,562,318, and US Patent Application Publication 20020054902. Immunoliposomes use antibodies as the targeting agents. This activity can be used to target specific tissues in the brain.

[0091] Antibodies that bind to the transferrin receptor have been shown to selectively target brain-blood barrier endothelium due to the high levels of TfR expressed by these cells (Friden *et al.*, *Neurosurgery* 35: 294-298 (1994), Bickel et al., *Proc Natl Acad Sci USA* 90: 2618-2622 (1993), and Moos and Morgan, *J Neurochem* 79: 119-129 (2001). As described by Pardridge *et al.*, a liposome may comprise two antibodies on the liposome surface, an antibody to the transferrin receptor to allow passage through the blood brain barrier, and a second antibody to target a specific tissue within the brain. *Clinical Cancer Research*, 10(11):3667-77 (2004).

[0092] Nanoparticles, as described in US Application 08/203,326, US Patent Publication 20040131692, US Patent Publication 20040131692, WO 95/22963 are advantageous in that

they can transport any hydrophilic or hydrophobic biologically or pharmaceutically active or diagnostic agent without modification of the agent.

[0093] Microparticles may be used for targeted delivery to the brain. Microparticles exist in a variety of formations, as disclosed in US 4,675,189, US 6,117,454, and US 6,419,949. US 6,565,888 discloses methods using microparticles for sustained release composition for the targeted delivery of biologically active agents to specific tissues and cells. US 6,455,733 US 6,410,517 discloses microparticles comprising a linking molecule or a targeting ligand, including hormones, antibodies, cell-adhesion molecules, saccharides, drugs, and neurotransmitters, attached to the microparticle surface.

[0094] Protein, polysaccharide, and synthetic polymer-based microspheres are also effective carriers in targeted drug delivery systems as discussed by Cummings, *et al.*, *Biochem. Pharm.*, 41:1849-54 (1991); Verrijik, *et al.*, *Cancer Chemother. and Pharm.*, 29:117-21 (1991); Tabata, *et al.*, *Jpn. J. Cancer Res.*, 79:636-646(1988), Rongved, *et al.*, *Carbohydrate Res.*, 145:83-92 (1991); Eldridge, *et al.*, *Molec. Immunology*, 28:287-94 (1991); Pappo, *et al.*, *Immunology*, 73:277-80 (1991), and US 6,410,517.

[0095] U.S. Pat. No. 5,849,727, and US Patent Application Publication 20040126400, and 20040141922, discuss the conjugation of therapeutic agents to gas filled, protein-encapsulated microbubbles, conventionally employed as contrast agents in ultrasonic imaging. US 20020044959 describes biocarrier molecules, including microbubbles, bearing molecules that bind to a cellular adhesion molecule expressed on endothelial cell; and a pharmaceutical, as well as methods of treating a pathophysiological state in an individual comprising irradiating a target tissue or organ in said individual; and administering the biomolecular carrier.

[0096] Carrier molecules may also be tethered to a biologically or pharmaceutically active molecule to traverse the blood brain barrier and target brain tissue. WO 95/22963 and US 20030152636 describe the use the proteins, including hydrophile proteins or polymeric materials for such applications. US 6,759,387 describes delivery of a drug across an endothelial tissue, including the blood brain barrier using a conjugate of defined chemical structure in combination with a delivery-enhancing transporter such as a polyarginine peptide. US 4,479,932, US 4,727,079, US 4,824,850, US 4,880,816, US 5,008,257, US 5,017,566, US 5,187,158,and US 5,525,727 describe various brain-specific drugs tethered to

a reduced blood brain barrier penetrating lipoidal form [D-DHC] of a dihydropyridine revreaction pyridinium salt type redux carrier, e.g. 1,4-dihydrotrigorielline.

[0097] Antibodies may be used as carrier molecules as well, as discussed by Friden et al. and Pardridge, in US 5,182,107 ,US 5,154,924, US 5,004,697, and WO 89/01343. Specifically, antibodies directed against the transferring receptor may be conjugated to a drug and used to transport the drug across the blood brain barrier.

[0098] Other methods of targeted drug delivery to the brain rely upon disrupting the blood brain barrier by chemical means (such as increasing permeability of the blood brain barrier by treatment with osmotic agents or drugs that act upon the CNS including cholinommimetic arecolines) or mechanical means (implantation or injection of the pharmaceutically active agent).

C. Experimental Example

[0099] Behavioral tests were performed on 9 young (4-6 mo) and 18 aged (25-27 months) pathogen-free male Long-Evans rats with the MWM protocol and used the same animals for microarray analysis. An additional 10 aged rats were tested in the MWM, followed by training and testing in the RAM to assess test-retest reliability for individual differences in cognitive function across the two tasks.

Morris Water Maze Apparatus

[0100] The MWM apparatus consists of a large, circular pool (diameter 1.83 m; height, 0.58 m) filled with water (27°C) that has been made opaque through the addition of non-toxic pigment or some other substance. In the typical "hidden platform" version of the task, rats are trained to find a camouflaged white escape platform (height, 34.5 cm) that is positioned in the center of one quadrant of the maze just 1.0 cm below the water surface. This platform could be retracted to the bottom of the tank or raised to its normal position from outside the maze during behavioral testing. The location of this platform remained constant from trial to trial. Because there were no local cues that marked the position of the platform, the rat's ability to locate it efficiently from any starting position at the perimeter of the pool depended on using information surrounding the maze. The maze was surrounded by black curtains with white patterns affixed to provide a configuration of spatial cues. A second platform (height 37.5 cm) with its surface painted black was elevated 2 cm above the water surface during cue training, the version of the task used to control for factors unrelated to cognition.

The behavior of a rat in the pool was recorded by a camera suspended 2.5 m above the center of the pool, connected to a video tracking system (HVS Image Advanced Tracker VP200) and a PC computer running HVS software developed by Richard Baker of HVS Image, Hampton, UK.

Morris Water Maze Procedure

[0101] The MWM protocol was optimized for sensitivity to the effects of aging on cognition and for measures of reliable individual differences within the aged population of out-bred Long-Evans rats (Gallagher et al., 1993, *Behav. Neurosci.* 107:618-626).

[0102] Rats received three trials per day for 8 consecutive days, using a 60 sec intertrial interval. On each training trial, the rat was released in the maze from one of four equally spaced starting positions around the perimeter of the pool. The starting position varied from trial to trial, thus preventing the use of a response strategy (e.g. always turning left from the start location to locate the escape platform). If a rat did not locate the escape platform within 90 sec on any trial, the experimenter guided the rat to the platform, where it remained for 30 sec. Every sixth trial consisted of a probe trial to assess the development of spatial bias in the maze. During these trials, the rat swam with the platform retracted to the bottom of the pool for 30 sec, at which time the platform was raised to its normal position for completion of an escape trial. At the completion of the protocol using the hidden platform, rats were assessed for cue learning using the visible platform. The location of this platform varied from trial to trial in a single session of 6 training trials.

[0103] The proximity of the animal's position with respect to the goal was used for analysis of training trial and probe trial performance. The proximity measure was obtained by sampling the position of the animal in the maze (10X/sec) to provide a record of distance from the escape platform in 1 sec averages. For both probe trials and training trials, a correction procedure was implemented so that trial performance was relatively unbiased by differences in distance to the goal from the various start locations at the perimeter of the pool. In making this correction the average swimming speed was calculated for each trial (pathlength/latency). Then the amount of time required to swim to the goal at that speed from the start location used on the trial was removed from the record prior to computing trial performance, i.e. cumulative distance on training trials and average distance from the goal on probe trials. Thus, scores obtained using the proximity measure are designed to reflect search

error, representing deviations from an optimal search, i.e. direct path to the goal and search in the immediate vicinity of that location during probe trials.

Morris Water Maze Analysis

[0104] Computer records of video-tracking were compiled to provide data on each rat's performance in the maze. Measures on training trials and probe trials were analyzed by Analysis of Variance.

Morris Water Maze Data Results

[0105] The performance during training with the hidden, camouflaged platform differed between the groups of young and aged rats [$F(1,23)= 12.69$, $p<.002$]. No difference between the groups occurred for the cue training trials with a visible platform. Latencies to escape during cue training averaged 9.36 seconds for young and 10.60 seconds for the aged rats.

[0106] The average proximity measure on interpolated probe trials was used to calculate a spatial learning index for each individual subject as described in detail in Gallagher et al., 1993, Behav. Neurosci. 107:618-626. When a rat rapidly learned to search for the platform close to its position, it's spatial learning index is low. Overall, aged rats differed from young [$F(1,23) = 15.18$, $p<.001$]. Aged rats were classified as either unimpaired or impaired relative to the learning index profile of the young study population. Aged rats that fall within the normative range of young rats (index scores <241) were designated aged unimpaired (Figure 1). The remaining aged subjects that have index scores outside the range of young performance were designated aged impaired.

Radial Arm Maze Apparatus

[0107] Each arm (7 x 75 cm) of the elevated eight arm radial maze projected from each facet of an octagonal center platform (30 cm diameter, 51.5 cm height). Clear side walls on the arms were 10 cm high and were angled at 65° to form a trough. A food well (4 cm diameter, 2 cm deep) was located at the distal end of each arm. Blocks constructed of Plexiglas (30 cm H x 12 cm W) could be positioned to block entry to any arm. Numerous extra maze cues were provided in the room surrounding the apparatus and lighting was provided by overhead fixtures.

Radial Arm Maze Procedures

[0108] Rats were first habituated to the maze for an 8 min session on four consecutive days. In each of these sessions food rewards were scattered on the RAM, initially on the center platform and arms and then progressively confined to the arms. After this habituation phase, a standard training protocol was used in which a food pellet was located at the end of each arm. Rats received one trial each day for 18 days; each daily trial terminated when all eight food pellets had been obtained or when either 16 choices were made or 15 min had elapsed. An error consisted of returning to an arm (all four paws on the arm) from which food had already been obtained. After completion of this phase, the memory demand of the task was increased by imposing a delay during the trial. At the beginning of each trial three arms were blocked. The identity and configuration of the blocked arms was varied across trials. Rats were allowed to obtain food on the five arms to which access was permitted at the beginning of the trial. The rat was then removed from the maze for 60 s, during which time the barriers on the maze were removed, thus allowing access to all eight arms. Rats were then placed back onto the center platform and allowed to obtain the remaining food rewards.

Radial Arm Maze Analysis

[0109] A memory error occurred during test trials using a 60 second delay when a rat returned to one of the five arms that was already visited prior to the delay. Each rat's performance was averaged across four consecutive test trials. Parametric statistics (unpaired t-tests) were used to compare performance between young and aged groups. Correlational analysis (Pearson's r) was used to examine the relationship between performance of aged rats (N=10) in the Morris Water maze (learning index scores) and radial-arm maze (memory errors).

Radial Arm Maze Results

[0110] The performance of young adult rats in the delay version of the RAM varies as a function of the delay interval, ranging from 60 seconds to eight hours (Chappell et al. Neuropharmacology 37: 481-488, 1998). Aged rats previously characterized in the MWM, committed more memory errors after a 60 second delay relative to young rats ($p < .025$). On average young rats committed 0.17 errors, whereas aged rats committed an average of 1.52 errors. The ten aged rats, however, exhibited a wide range of performance on the RAM. A significant relationship was found between the initial MWM characterization and memory performance in the RAM (r value = .82, data shown in Figure 2).

Gene expression analysis of the young, aged-impaired (AI) and aged-unimpaired (AU) animals

Preparation of RNA from behaviorally characterized animals

[0111] Twenty-seven behaviorally characterized rats (data shown in Figure 1) were killed by rapid decapitation. The hippocampus was dissected bilaterally and frozen (-80°C). One hippocampus from each animal was weighed and homogenized in the appropriate volume of phenol-guanadine isothiocyanate (Trizol reagent; 1ml per 100mg of tissue with a minimum volume of 1 ml). Each sample was extracted with chloroform (200µl per ml of Trizol) and precipitated with isopropanol. RNA pellets were air dried and resuspended in DEPC treated water. All samples were stored at -80°C. A portion of the RNA was further purified using Qiagen's RNeasy mini RNA extraction kit according to manufacturer's instructions and subsequently stored at -80°C. Samples were quantified by absorbance at 260 nm and purity determined by ratio of absorbance at 260nm and 280nm. Sample integrity and concentration was confirmed by agarose gel electrophoresis. Photographs of agarose gels were scanned, the pixels were inverted and quantified using NIH-image. Concentrations were then adjusted if needed.

[0112] For analysis on gene microarrays, samples from three rats of the same phenotype, either Y, AI or AU, were pooled to yield independent microarray analysis for a sample size of three GeneChip® arrays/phenotype. With respect to behavioral characterization by spatial learning index in the MWM, tissue was pooled as set forth in Table 1

Table 1

Young	Aged Unimpaired	Aged Impaired
Y1: 143.1, 171.4, 194.6	AU1: 181.5, 202.9, 229.5	AI1: 268.3, 283.2, 381
Y2:139.5, 169.4, 186.6	AU2: 183.9, 218.4, 229.3	AI2: 285.5, 303.4, 337.7
Y3: 169, 173.9, 196.5	AU3: 193.3, 228.2, 230.6	AI3: 282.2, 307.2, 329.3

Reverse transcription and hybridization to microarray

[0113] Labeled cRNA probes for hybridization were prepared using the Affymetrix Enzo Bioarray high yield RNA transcript labeling system. RNAs were reverse transcribed into

cDNA and converted to biotin labeled cRNA. Internal standards provided with each labeling system were added to test RNA prior to reverse transcription. cRNAs were then tested on control chips to ensure that reverse transcription and labeling were optimal before performing hybridization onto experimental GeneChips®. cRNAs were applied to U34A Affymetrix GeneChip® arrays. These arrays included specific sequences for 7000 expressed rat genes and 1000 EST clusters, and included all genes represented on a recently developed, smaller, neuroscience gene microarray. A GeneChip Fluidics Station automated introduction of the labeled cRNAs on to the gene arrays and hybridization as conducted in a GeneChip hybridization oven. Hybridization, washing, staining and scanning were performed according to the Affymetrix GeneChip Expression Analysis Manual (P/N 70022 rev.3) chapters 5 and 6. A GeneArray scanner was used to detect and quantify hybridization signals for each oligomer set based on confocal laser scanning.

Data analysis of microarray

[0114] The power of the present model lies, in part, in the ability to compare across the three groups Y, AU and AI to identify those genes which either change between young and aged hippocampus and thus generally relate to the aging process and those which discriminate AU and AI rats. The genes identified through this process relate specifically to aging-cognitive impairment or preservation of cognitive function.

[0115] In generating the data disclosed herein, a series of analytic steps were conducted to identify 3 sets of genes informative for the model of age and cognitive impairment. Set 1 comprised genes of interest that differ from young as a function of age alone. Set 2 comprised genes of interest that differ in the impaired aged rats relative to both young and aged unimpaired. Set 3 (referred to as Aged Unimpaired genes) consisted of genes that differ in the aged unimpaired relative to both young and aged impaired and may, therefore, related to age-induced preservation of cognitive function.

[0116] Herein are described the steps for generating the set of genes altered specifically in aged impaired rats, in which lipoprotein lipase was included. A Genechip Analysis Suite and the Affymetrix MAS 5.0 algorithms was used in our analysis of data (Technical Note ' New Statistical Algorithms for Monitoring Gene Expression on GeneChip Probe Arrays' http://www.wi.mit.edu/CMT/protocols/statisticalalgorithms.pdf). Each set was generated from the full microarray dataset following the MAS 5.0 analysis. In the first step the values for all probe sets on the chips representing gene expression in the young rats (N=3) and the

aged unimpaired (N=3) were examined for a detection criterion based on an absolute call in the MAS 5.0 analysis for present or marginal expression. All probe pair sets that did not meet the detection criterion using the absolute call from the MAS 5.0 analysis were eliminated from further consideration. A simple effects analysis was then conducted to determine the values on the chips for the two groups (young and aged unimpaired) that did not differ by an effect size of greater than 1.0. All probe sets that met both detection criterion and the criterion for pooling in the comparison group (young and aged unimpaired not differing by an effect size of 1.0 or greater) were then treated as a single group for comparison with the aged impaired. A simple effects analysis was then conducted on the pooled probe sets for young and aged unimpaired along with the corresponding probe sets from the aged impaired. Power analysis for inferential statistical tests of significance indicated that sample sizes of the microarray experiment (3 aged impaired chips and 6 chips in the pooled comparison group) would detect a difference at $p<.05$ with 80% power for genes with an effect size of 2.5 or greater. In follow-up analysis with sample sizes of 8 aged impaired and 8 subjects in each of the comparison groups (young and aged unimpaired) that are now underway, statistical power at 80% is expected for effect sizes of 1.25 and greater. Three independent probe sets for lipoprotein lipase yielded a similar pattern of results. As shown in Table 2 values did not differ for young and aged unimpaired but values were consistently lower in aged impaired.

Results from the microarray

[0117] As summarized below in Table 2, a decrease in LPL expression was reliably seen in AI rats compared to AU rats in three separate probes sets for that gene. LPL RNA levels in AU rats was comparable to that of Y rats. The three probe sets used in this study were composed as follows (all sequences read 5' to 3'):

Probe Set ID: L03294_at

GTTCTATCTCAGAGGCTGTTGCTGG (SEQ ID NO: 1)

TGAACACCTACACACAAGCAAAGCC (SEQ ID NO: 2)

CCCACAAGAGTCTTTGTCATTCAAT (SEQ ID NO: 3)

AGAGTCTTTGTCATTCAATGTCATT (SEQ ID NO: 4)

TGGTTGTGCCTATGTAATATAGGAC (SEQ ID NO: 5)

GTTTCATTAGGCTCAGTGTCATTCT (SEQ ID NO: 6)

TTAGGCTCAGTGTCATTCTAACAAT (SEQ ID NO: 7)

TCATTCTAACAATAAAATGATGTAC (SEQ ID NO: 8)

GATGTACCATATGTCACCAGCATCC (SEQ ID NO: 9)

AGCATCCCCATTATTGCTAATTCAT (SEQ ID NO: 10)

CCCATTATTGCTAATTCATGGCAGT (SEQ ID NO: 11)

ATTGCTAATTCATGGCAGTATATAC (SEQ ID NO: 12)

TATATACGTACATATCTCAATGATG (SEQ ID NO: 13)

TATCTCAATGATGCTTTGACTTTAA (SEQ ID NO: 14)

ACTGGAAACGCTGTTGTGGCTATCT (SEQ ID NO: 15)

TTGTGGTGCTAACTCTGTGTCCACC (SEQ ID NO: 16)

Probe Set ID: L03294_g_at

CACCTCCATCAGTGATTGTCTCACT (SEQ ID NO: 17)

CTCCATCAGTGATTGTCTCACTGAG (SEQ ID NO: 18)

CATCAGTGATTGTCTCACTGAGCCA (SEQ ID NO: 19)

CAGTGATTGTCTCACTGAGCCAACT (SEQ ID NO: 20)

TGATTGTCTCACTGAGCCAACTCAC (SEQ ID NO: 21)

TCTCACTGAGCCAACTCACTCTGAT (SEQ ID NO: 22)

CACTGAGCCAACTCACTCTGATGAA (SEQ ID NO: 23)

TGAGCCAACTCACTCTGATGAACAG (SEQ ID NO: 24)

GCCAACTCACTCTGATGAACAGCAC (SEQ ID NO: 25)

TCACTCTGATGAACAGCACAATGGA (SEQ ID NO: 26)

CTCTGATGAACAGCACAATGGAATA (SEQ ID NO: 27)

TGATGAACAGCACAATGGAATAGCT (SEQ ID NO: 28)

AACTCACCTGTGTGAAGAAATGGGA (SEQ ID NO: 29)

TCACCTGTGTGAAGAAATGGGATCT (SEQ ID NO: 30)

TGGGATCTGCTTTCAATAAAATCGA (SEQ ID NO: 31)

GATCTGCTTTCAATAAAATCGAGAA (SEQ ID NO: 32)

Probe Set ID: rc_AI23773

TTCAGGCTTACCTTGAACTCTCAAC (SEQ ID NO: 33)

TCTTAGTCATTTTCACCAATAGAAC (SEQ ID NO: 34)

TTCACCAATAGAACACATTCAATGC (SEQ ID NO: 35)

TGCCCAATCGTTAGCATTTCGTTTG (SEQ ID NO: 36)

TAGCATTTCGTTTGAGACTCATCTT (SEQ ID NO: 37)

TTGAGACTCATCTTGACCGTACCTC (SEQ ID NO: 38)

TTGACCGTACCTCTGTCACACGTCT (SEQ ID NO: 39)

TCTGTCACACGTCTAACACATCACA (SEQ ID NO: 40)

TCTAACACATCACATTAATTTCTAG (SEQ ID NO: 41)

CTGCACTGCGCAAAGTACAAGTTTT (SEQ ID NO: 42)

GTATATCGATGCTTGTACACTGTTG (SEQ ID NO: 43)

AAAGTGAGGAGCCTTCTATTGTGAT (SEQ ID NO: 44)

CCTTCTATTGTGATAGCCATAGACA (SEQ ID NO: 45)

TAGCCATAGACAGTACCAGGCTCGT (SEQ ID NO: 46)

AGTACCAGGCTCGTTGCCGCTCTTT (SEQ ID NO: 47)

GATCTCATATGTTCAGATTGCTTTT (SEQ ID NO: 48)

TABLE 2

Lipoprotein Lipase

Probe Set ID	Y*	AU*	AI*
L03294_at	633.8 (34.7)	603.1 (42.0)	339.8 (96.2)
L03294_g_at	433.1 (25.9)	482.6 (77.4)	345.2 (44.4)
rc_AI23773	546.2 (40.8)	489.1 (49.6)	414.0 (41.8)

Average (standard error)

[0118] Although the present invention has been described in detail with reference to specific embodiments, those of skill in the art will recognize that modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. All publications and patent documents (patents, published patent applications, and unpublished patent applications) cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the invention.

What is claimed is:

1. A method for determining whether an agent is useful for treatment of cognitive impairment (CI), comprising the step of determining whether the agent increases expression of lipoprotein lipase (LPL) in a neuronal cell.

2. A method of determining whether an agent is useful for treatment of CI, comprising the step of determining whether the agent increases activity of LPL in a neuronal cell.

3. The method of claim 1 or 2 wherein the neuronal cell is disposed in a hippocampus.

4. The method of claim 3 wherein the neuronal cell is disposed in a hippocampal region.

5. A method for determining whether an agent is useful for treatment of CI, comprising the step of determining whether the agent increases expression of LPL in a neuronal cell of an Aged Impaired (AI) mammal.

6. The method of claim 5 wherein the agent increases expression of LPL in said mammal to a level that is the same as the level in an Aged Unimpaired (AU) mammal.

7. The method of claim 5 wherein the agent increases expression of LPL in said mammal to a level that is the same as the level in a Young (Y) mammal.

8. The method of claim 5 wherein the agent increases expression of LPL in the AI mammal but does not significantly change the level of expression of LPL in an AU or Y mammal.

9. The method of claim 5 wherein the mammal is a rat.

10. A method for treating CI in a mammal, comprising the step of administering to said mammal an agent that (a) increases expression of LPL in the hippocampus or a hippocampal region of an AI mammal; (b) increases the expression of LPL in the hippocampus or a hippocampal region of an AI mammal to a level that is the same as the level in an AU mammal; or (c) increases the expression of LPL in the hippocampus or a hippocampal region of an AI mammal but does not significantly change the level of expression of LPL in a Y or AU mammal.

11. A method for determining an initial clinical dosage of an agent that increases LPL expression in the hippocampus or a hippocampal region of an AI mammal for treatment of CI, comprising the steps of (a) determining the dose of the agent that in a YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI mammal to a level that is the same as the level in an AU or Y mammal, and (b) estimating from said dose the initial clinical dosage for a human subject.

12. The method of claim 11 wherein the dose determined in step (a) does not significantly change the level of expression of LPL in a Y or AU mammal.

13. A method for treating CI in a patient, comprising the step of administering to said patient a therapeutically effective amount of an agent that increases LPL expression in the hippocampus or a hippocampal region of an AI mammal.

14. The method of claim 13 wherein the agent is administered in an amount that is the human equivalent to the amount that in a YUI assay increases LPL expression in the hippocampus or a hippocampal region of an AI mammal to a level that is the same as the level of expression in a Y or AU mammal.

15. The method of claim 14 wherein said amount is the human equivalent to the amount that in a YUI assay does not significantly change the level of expression of LPL in a Y or AU mammal.

16. The method of claim 13 wherein said agent is administered in a formulation that targets the agent to the brain.

17. A method for treating CI in a patient, comprising the step of administering to said patient a therapeutically effective amount of an agent that (a) increases LPL gene expression, (b) increases LPL protein, or (c) increases LPL protein activity, in the hippocampus or a hippocampal region of an AI mammal.

18. The method of claim 17 wherein said agent is administered in an amount that does not significantly (a) increase LPL gene expression, (b) increase LPL protein, or (c) increase LPL protein activity, in the hippocampus or a hippocampal region of an AU or Y mammal.

19. The method of claim 17 wherein the patient is afflicted with age related cognitive decline (ARCD), age associated memory impairment (AAMI) or mild cognitive impairment (MCI).

20. The method of claim 17 wherein the patient is afflicted with dementia, Huntington's Disease, Parkinson's Disease, schizophrenia, or amyotrophic lateral sclerosis.

21. The method of claim 17 wherein the agent is administered systemically.

22. The method of claim 21 wherein systemic administration is accomplished by oral, intravenous, arterial, transdermal, suppository, ocular, inhalation, subcutaneous, intramuscular, or sublingual delivery.

23. The method of claim 17 wherein the agent is administered in a form or formulation targeted to the brain.

24. The method of claim 23 wherein the agent is administered in a form or formulation targeted to the hippocampus.

25. The method of claim 23 wherein the agent is formulated with a carrier that facilitates targeted drug delivery across the blood brain barrier.

26. The method of claim 17 wherein the agent is ibrolipim or an analog, derivative, or metabolite thereof.

27. The method of claim 17 wherein the agent is a peroxisome proliferator-activated receptor (PPAR) agonist.

28. The method of claim 27 wherein the agent is a PPAR alpha agonist.

29. The method of claim 27 wherein the agent is a PPAR gamma agonist.

30. The method of claim 17 wherein the agent is resveratrol.

31. A composition comprising a therapeutically effective amount of an agent that (a) increases LPL gene expression, (b) increases LPL protein, or (c) increases LPL protein activity, in the hippocampus or a hippocampal region of an AI mammal.

32. The composition of claim 31 wherein the amount is effective for treating cognitive impairment in a patient.

33. The composition of claim 31 wherein the amount is effective for promoting or preserving cognitive function in a patient afflicted with ARCD, AAMI or MCI.

34. The composition of claim 31 wherein the amount is effective for promoting or preserving cognitive function in a patient afflicted with dementia, Huntington's Disease, Parkinson's Disease, schizophrenia, or amyotrophic lateral sclerosis.

35. The composition of claim 31 wherein the agent is dispersed in a vehicle suitable for systemic administration to a mammal.

36. The composition of claim 31 wherein the agent is dispersed in a formulation targeted to the brain.

37. The composition of claim 31 further comprising a carrier that facilitates targeted delivery of the agent across the blood brain barrier.

38. A composition comprising an amount of ibrolipim or an analog, derivative, or metabolite thereof, that is therapeutically effective for treatment of cognitive impairment.

39. A composition comprising an amount of a PPAR agonist that is therapeutically effective for treatment of cognitive impairment.

40. A composition comprising an amount of resveratrol that is therapeutically effective for treatment of cognitive impairment.

41. The composition of claim 38, 39, or 40 further comprising a vehicle suitable for systemic administration to a mammal.

42. The composition of claim 38, 39, or 40 further comprising a vehicle suitable for targeted delivery to the brain.

43. The composition of claim 38, 39, or 40 further comprising a carrier that facilitates targeted drug delivery across the blood brain barrier.

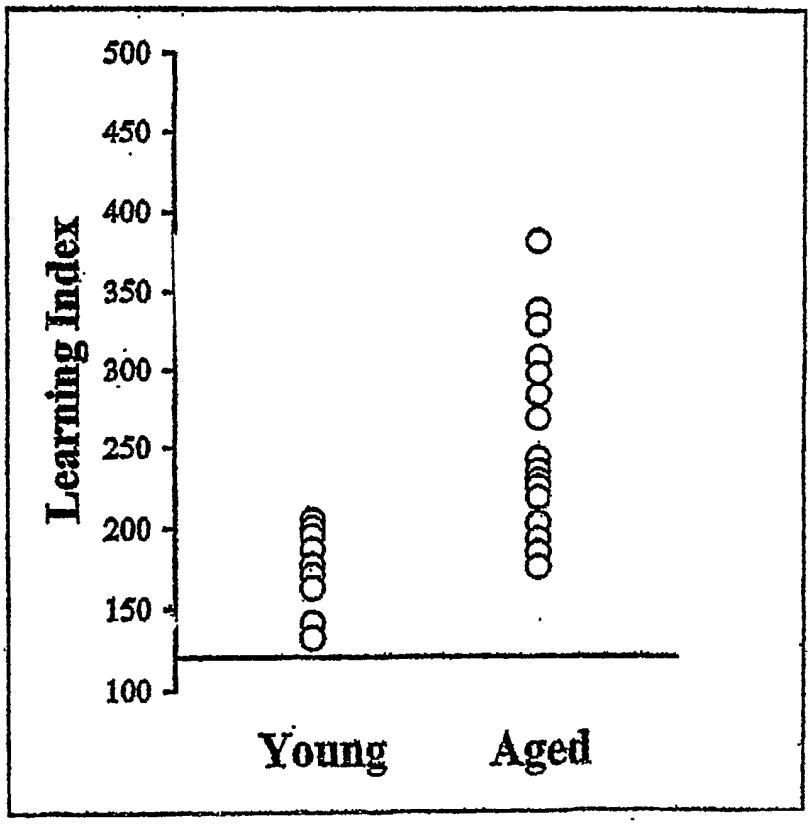

FIG 1

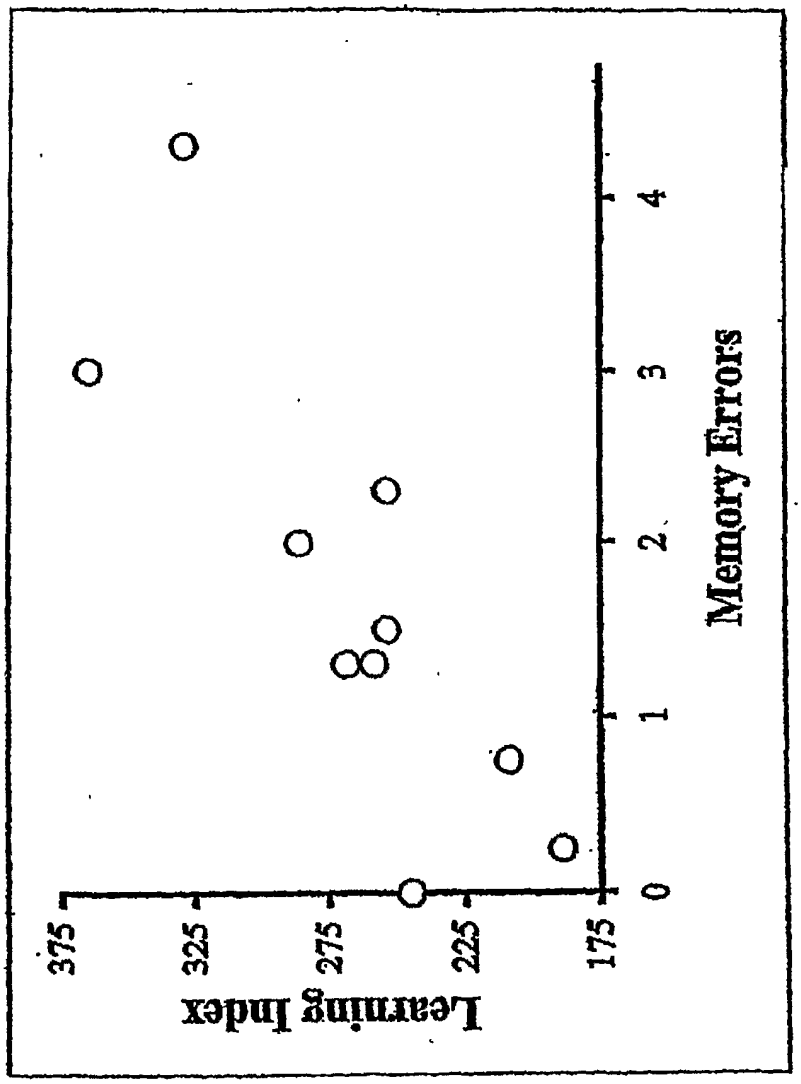

FIG 2