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(71) Applicant *(for all designated States except US)*: **KINEMED, INC.** [US/US]; 5980 Horton Street, Suite 470, Emeryville, CA 94608 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HELLERSTEIN, Marc, K.** [US/US]; 4 Anson Way, Kensington, CA 94708 (US). **SHANKARAN, Mahalakshmi** [IN/US]; 3180 Oak Road, Apt. 416, Walnut Creek, CA 94597 (US).

(74) Agents: **SILVA, Robin, M.** et al.; Morgan, Lewis & Bockius LLP, One Market, Spear Street Tower, San Francisco, CA 94105 (US).

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(54) Title: THE USE STATINS TO STIMULATE NEUROGENESIS

(57) Abstract: The present invention provides methods of treating a neurogenesis associated condition, e.g. depression or disorders of cognition, comprising administering to a patient in need thereof a therapeutically effective amount of a statin or a pharmaceutically acceptable salt, hydrate, solvate, or pro-drug thereof. These neurogenesis conditions include, but are not limited to, depression, bipolar disorder, schizophrenia, traumatic brain injury, cognitive dysfunction, memory loss, learning disorders, mild cognitive impairment, spinal cord injury, stroke, Alzheimer's disease, Parkinson's disease, Huntington Disease and multiple sclerosis.

THE USE OF STATINS TO STIMULATE NEUROGENESIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S.S.N. 60/782,374, filed March 14, 2006, of which application is hereby expressly incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Formation of new neurons in the selected regions of the adult brain occurs in a variety of species including rodents, primates and humans (Altman and Das, 1967; Eriksson et al., 1998). Adult neurogenesis occurs in the subventricular zone, giving rise to granule cells in the olfactory bulb, and in the subgranular zone generating new granule cells in the dentate gyrus of the hippocampus. Hippocampal neuronal cells in the adult are formed from pluripotent neural progenitor cells. Immature neurons migrate to the granule cell layer and mature over a period of weeks to granule cells and form functional connections (van Praag et al., 2002).

Hippocampal neurogenesis has emerged as a central therapeutic target for drug discovery, particularly for anti-depressant agents. The neurogenic theory of depression has been proposed (Jacobs et al., 2000), partly based on the finding that all known classes of clinical antidepressants (tricyclics, monoamine oxidase inhibitors, SSRIs) have been shown to increase cell proliferation in the hippocampus of normal animals (Malberg et al., 2000; Khawaja et al., 2004). Moreover, Santarelli et al. (2003) showed that hippocampal neurogenesis appears to be required to achieve the behavioral effects of antidepressants in animal models. Further support for the neurogenic model is the observation that hippocampal cell proliferation is decreased under conditions of chronic stress and that this effect is reversed by antidepressant treatment (Van der Hart et al., 2002; Malberg et al., 2003; Alonso et al., 2004). Imaging studies have shown that depressed human subjects also exhibit hippocampal volume loss and atrophy (Sheline et al., 1999, 2003). Taken together, these findings suggest that agents that are found to increase neurogenesis may potentially become novel anti-depressant drugs. In addition to mood disorders, neurogenesis has also been implicated in stroke, neuro-inflammation, learning and memory, and neurodegenerative diseases.

'Statins' are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that lower cholesterol and are widely used to treat hypercholesterolaemia. There is speculation of utility of this class of drugs in the treatment of three central nervous system (CNS) diseases including Multiple Sclerosis, Alzheimer's disease (AD), and Stroke (Menge et al., 2005, Nature Reviews Neuroscience 6:325). This speculation is driven by specific activities of statins including the inhibition of migration of T cells into the CNS (relevant to MS); the interplay of cholesterol levels and AD, and the potential that statins reduce the formation of thrombi in the left ventricle, thus potentially addressing stroke. In contrast to earlier reports that vigorous cholesterol lowering had an adverse impact on psychological well-being, recent studies have shown that long-term use of statins in patients with underlying coronary artery disease is associated with reduced risk of anxiety, depression and hostility (Young-Xu et al., 2003, J. Am. College of Cardiology 42(4):690). Moreover, in a double-blind placebo-controlled randomized clinical trial for AD, patients treated with atorvastatin for 1 year showed significant improvement on the Geriatric Depression Scale (Sparks et al., 2005, Arch. Neruol. 62:753).

In previous studies using the middle cerebral artery occlusion model (MCAO) for stroke, atorvastatin treatment has been shown to increase hippocampal neurogenesis in rats subjected to MCAO (Chen et al., 2003, Ann Neurol. 53:743). However, this effect has not been reported in normal animals treated with statins.

SUMMARY OF THE INVENTION

The present invention provides methods of treating a neurogenesis associated condition, e.g. depression, comprising administering to a patient in need thereof a therapeutically effective amount of a statin or a pharmaceutically acceptable salt, hydrate, solvate, or pro-drug thereof. These neurogenesis conditions include, but are not limited to, depression, bipolar disorder, schizophrenia, traumatic brain injury, cognitive dysfunction, memory loss, learning disorders, mild cognitive impairment, spinal cord injury, stroke, Alzheimer's disease, Parkinson's disease, Huntington Disease and multiple sclerosis.

These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the invention as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

FIG. 1 shows phenotype and proliferative characteristics of hippocampal progenitor cells. **Panel A** shows a schematic illustration of Percoll gradient isolation method. **Panel B** shows flow cytometric analysis of fixed and permeabilized progenitor cells incubated with primary antibodies for nestin and vimentin, followed by ALEXA-488 conjugated secondary antibody; propidium iodide (PI) was used to label nuclei. **Panel C** shows fractional synthesis of hippocampal progenitor cell DNA in male Swiss Webster mice labeled for either 3 days, 1, 2, 4, 16, 28 or 52 weeks with 10% ^2H_2O. **Panel D** shows progenitor cell proliferation rate in male C57/Bl6, Swiss Webster, Balb/c or 129SvEv mice labeled with 10% ^2H_2O for 1 week. $*p < 0.05$ for comparisons between C57Bl/6 and Balb/c, as well as Swiss Webster and 129SvEv, $**p < 0.01$ for comparison between C57Bl/6 and Swiss Webster, $***p < 0.001$ for comparisons of 129SvEv with C57Bl/6 or Balb/c. Data represent mean ± SEM of 6 animals per group.

FIG. 2 shows results of antidepressants increasing hippocampal progenitor cell proliferation. **Panel A** shows fractional synthesis of hippocampal progenitor cell DNA in male Swiss Webster treated with vehicle, fluoxetine (10 mg/kg/day), imipramine (20 mg/kg/day) or venlafaxine (10 mg/kg/day) in drinking water for 3 weeks and labeled with 10% ^2H_2O during the final week of treatment. $*p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 8 mice per group. **Panel B** shows fractional synthesis of hippocampal progenitor cell DNA in male Sprague-Dawley rats treated with vehicle, fluoxetine (10 mg/kg/day), imipramine (20 mg/kg/day) or venlafaxine (10 mg/kg/day) in drinking water for 3 weeks and labeled with 10% ^2H_2O during the final week of treatment. $*p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 rats per group. **Panel C** shows fractional synthesis of hippocampal progenitor cell DNA in male C57Bl/6, Swiss Webster, Balb/c or 129SvEv mice treated with fluoxetine (10 mg/kg/day) in drinking water for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. $**p < 0.01$ compared to 'Vehicle' for each of the strain, data represent mean ± SEM of 6-8 mice per group.

FIG. 3 shows flow cytometric analysis and label incorporation in neuronal DNA. Male 129SvEv mice were treated with fluoxetine (10 mg/kg/day) in drinking water for 5 weeks with 10% ^2H_2O labeling for the last 3 weeks of treatment, and then sacrificed 4 weeks after the end of treatment and label. **Panel A** on the left shows hippocampal neuronal cells isolated by Percoll gradient fractionation were fixed and incubated with tetanus toxin C fragment (TTX) and anti-TTX primary antibody, followed by ALEXA-488 conjugated secondary antibody, propidium iodide (PI) was used to identify nuclei. Panel A on the right shows fractional synthesis of neuronal cell DNA from fluoxetine-treated animals was significantly (**$p < 0.01$) different from vehicle-treated animals, data represent mean ± SEM of 8 mice per group. **Panel B** on the left shows hippocampal nuclei were isolated by ultracentrifugation, incubated with ALEXA-488 conjugated anti-NeuN primary antibody and sorted after gating on PI^{+ve} events. **Panel B** on the right shows fractional synthesis of neuronal nuclear DNA from fluoxetine-treated animals was significantly (***$p < 0.001$) different from vehicle-treated animals, data represent mean ± SEM of 8 mice per group.

FIG. 4 shows isotretinoin and prednisolone decrease hippocampal progenitor cell proliferation. **Panel A** shows sractional synthesis of hippocampal progenitor cell DNA in male 129SvEv mice treated with either vehicle or isotretinoin (1 and 3 mg/kg/day, i.p.) for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group. **Panel B** shows fractional synthesis of hippocampal progenitor cell DNA in male Swiss Webster mice treated with either vehicle or prednisolone (5 and 40 mg/kg/day) in diet, and labeled with 10% ^2H_2O for 4 weeks. **$p < 0.01$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group.

FIG. 5 shows screening of pluripotent drugs for hippocampal progenitor cell proliferation. Fractional synthesis of hippocampal progenitor cell DNA in male Swiss Webster mice treated for 3 weeks with various drugs (details are given in Table 1) and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group.

FIG. 6, **Panel A** shows dose response of topiramate for hippocampal progenitor cell proliferation. Male 129SvEv mice were treated with vehicle or topiramate (10, 30, 100, 150 mg/kg/day, p.o.) for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group. **Panel B** shows screening of anticonvulsants for

hippocampal progenitor cell proliferation. Male Swiss Webster mice were treated with different anticonvulsants (details given in 'Methods') for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group.

FIG. 7 shows dose response of statins for hippocampal progenitor cell proliferation. **Panel A** shows male 129SvEv mice were treated with atorvastatin (1, 3, 10 or 30 mg/kg/day) in diet for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group. **Panel B** shows male 129SvEv mice were treated with simvastatin (1, 3, 10 or 30 mg/kg/day p.o.) for 3 weeks and labeled with 10% ^2H_2O in the final week of treatment. *$p < 0.05$ compared to 'Vehicle', data represent mean ± SEM of 6 mice per group.

Fig. 8 shows the dose-response in the forced swim test for compound 'K-001', an agent identified to have a dose-dependant stimulatory effect on hippocampal progenitor cell proliferation. Male NIH Swiss mice were treated with different doses of K-001 or imipramine 1 hour before testing. The period of immobility in the forced swim test was dose-dependently decreased by K-001, which was comparable to the effect produced by the known antidepressant drug, imipramine (the latter was used as a positive control). *p<0.05 vs 'Vehicle (ANOVA, post-hoc Dunnet's test)

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the objects outlined herein, the present invention provides methods of treating a neurogenesis associated condition, e.g. depression or a disorder of cognition, comprising administering to a patient in need thereof a therapeutically effective amount of a statin or a pharmaceutically acceptable salt, hydrate, solvate, or pro-drug thereof. These neurogenesis conditions include, but are not limited to, depression, bipolar disorder, schizophrenia, traumatic brain injury, spinal cord injury, stroke, Alzheimer's disease, cognitive dysfunction, memory loss, learning disorders, mild cognitive impairment, Parkinson's disease, Huntington Disease and multiple sclerosis.

Before the present Invention described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose

of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supercedes any disclosure of an incorporated publication to the extent there is a contradiction.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the compound" includes reference to one or more compounds and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

Overview

The present invention is directed to methods and compositions for the treatment of neurogenesis associated diseases or disorders. In particular, the present invention provides for the use of statins for the treatment of various diseases or disorders involving neurogenesis.

Applicants have discovered that (1) the hydroxymethylglutaryl-CoA (HMG-CoA) inhibitor, or statin, atorvastatin (Lipitor©) is a potent stimulator of hippocampal progenitor cell proliferation (neurogenesis, or the production of new brain cells) in the brain of healthy rodents; (2) atorvastatin is as potent in this action as clinically established anti-depressant drugs, including fluoxetine (Prozac), imipramine, and venlafaxime; (3) other HMG-CoA inhibitors of the statin class share this stimulatory action on hippocampal neurogenesis in the brain of healthy rodents; (4) this stimulatory action of statins on hippocampal neurogenesis has therapeutic utility in the treatment of depression, in that all known anti-depressants drugs stimulate hippocampal neurogensis and their anti-depressant action depends upon the stimulation of hippocampal neurogenesis; (5) this stimulatory action of statins on hippocampal neurogenesis has therapeutic utility in the treatment of conditions characterized by disorders of cognition, learning or memory, in that it is well established that the formation of new memories is dependant upon hippocampal function, including the production of new brain cells in the hippocampus and (6) this stimulatory action of statins has use in other neurologic, cognitive or psychiatric conditions as outlined herein.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B.

Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); and Mass isotopomer distribution analysis at eight years: theoretical, analytic and experimental considerations by Hellerstein and Neese (Am J Physiol 276 (Endocrinol Metab. 39) E1146-E1162, 1999), all of which are incorporated by reference for the needed techniques. Furthermore, procedures employing commercially available assay kits and reagents will typically be used according to manufacturer-defined protocols unless otherwise noted.

Definitions

By "toxic effect" is meant an adverse response by a living system to a chemical entity or known drug agent. A toxic effect can be comprised of, for example, end-organ toxicity.

By "action" is meant a specific and direct consequence of an intervention such as the administering of a drug.

"Treatment" in this context includes delay in onset or severity of symptoms and/or reduction of symptoms.

As used herein "hippocampus-A" refers to the region of the brain required for formation and consolidation of new memories and characterized by the formation of new neurons

As used herein "neurogenesis" refers to the biological process of forming new neurons from non-neurons, believed to occur rarely in adult animals except in select areas of the brain, such as the hippocampus.

As used herein "hippocampal progenitor cells" refer to cells present in the hippocampus that do not express markers of adult neurons and are capable of dividing and differentiating into adult neurons; the precursor cell for neurogenesis.

As used herein "proliferation" refers to the process of cell division, characterized by S-phase DNA replication.

As used herein "healthy brain" refers to a non-injured or non-traumatized brain, i.e. brain not exhibiting anatomically observable damage and neuronal cell death.

As used herein "depression" refers to a neuropsychiatric disorder of mood, as defined in humans by DSM IV criterea, characterized by reduced hippocampal volume radiographically in humans and, in animal models, reduced hippocampal neurogenesis.

As used herein "statins" refers to the class of HMG-CoA inhibitors widely utilized for treatment or hypercholesterolemia and other dyslipidemias, including, but not limited to, atorvastatin, simvastatin, pravastatin, lovastatin, rosuvastatin, and fluvastatin.

As used herein, "cognition" refers to mental processes involved in thought, learning, understanding, reasoning, perception, questioning and analyzing, comprehension and use of speech, visual perception and construction, calculation ability, attention (information processing), imagination, judgment, memory and other adaptations induced by experience, executive functions such as planning, problem-solving, and self-monitoring.and other aspects of mental function well known in the fields of neurology, psychology, cognitive science and psychiatry.

As used herein "anti-depressant drugs" refers to drugs approved in humans for the treatment of depression comprising several chemical classes including tricyclics (e.g. impramine), selective serotonin uptake inhibitors, mixed agents (e.g., venlafaxine), and monoamine oxidase inhibitors.

By "therapeutic action" or "therapeutic effect" is meant an effect on a biochemical or molecular process (i.e., the flow of molecules through metabolic pathways or networks) in a manner that is beneficial to the organism; e.g. any effect elicited by a compound or combination of compounds or mixtures of compounds that provides ameliorative or palliative results, or improves, even to the slightest degree, any clinical sign or symptom of a disease or condition. The effect may be responsible for, or contributing in, a causal manner to the initiation, progression, severity, pathology, aggressiveness, grade, activity, disability, mortality, morbidity, disease sub-classification or other underlying pathogenic or pathologic feature of one or more diseases wherein said effect is beneficial to health or otherwise contributes to a desirable outcome (e.g., a desirable clinical outcome).

By "therapeutically effective amount" is meant an amount effective to ameliorate the symptoms of, or ameliorate, treat or prevent neurogenesis associated condidtion including depression.

By "condition" or "medical condition" is meant the physical status of the body as a whole or of one of its parts. The term is usually used to indicate a change from a previous physical or mental status, or an abnormality not recognized by medical authorities as a disease or disorder.

As used herein "pro-drug" refers to any compound which releases an active drug in vivo when such a compound is administered to a mammalian subject. Pro-drugs can be prepared, for example, by functional group modification of a parent drug. The functional group may be cleaved in vivo to release the active parent drug compound. Pro-drugs include, for example, compounds in which a group that may be cleaved in vivo is attached to a hydroxy, amino or carboxyl group in the active drug. Examples of pro-drugs include, but are not limited to esters (e.g., acetate, methyl, ethyl, formate, and benzoate derivatives), carbamates, amides and ethers. Methods for synthesizing such pro-drugs are known to those of skill in the art.

Methods

The present invention provides a method for treating, inhibiting, or ameliorating the effects of injuries or diseases that result in neuronal degeneration or the effects of disorders that result in mental or cognitive dysfunction and a method for promoting neurogenesis. These methods involve administering to a patient in need thereof an effective amount of at least one statin to the patient.

The term "treating" in its various grammatical forms in relation to the present invention refers to preventing, curing, reversing, attenuating, alleviating, ameliorating minimizing, suppressing, or halting the deleterious effects of a neurological disorder, injury, trauma, or other abnormal condition. Symptoms of neuronal degeneration include, but are not limited to, tension, abnormal movements, abnormal behavior, tics, hyperactivity, combativeness, hostility, negativism, memory defects, sensory defects, cognitive defects, hallucinations, acute delusions, poor self-care, and sometimes withdrawal and seclusion.

Abnormal movement symptoms include a wide variety of symptoms that can range from unconscious movements that interfere very little with quality of life, to quite severe and disabling movements. Examples of symptoms which are seen

associated with neurological disorders include involuntary tongue protrusions, snake-like tongue movements, repetitive toe and finger movements, tremors of extremities or whole body sections, tics, muscular rigidity, slowness of movement, facial spasms, acute contractions of various muscles, particularly of the neck and shoulder which may eventually lead to painful, prolonged muscle contraction, restlessness, distress and an inability to remain still. Abnormal behavioral symptoms, some of which are motor in nature, include irritability, poor impulse control, distractibility, aggressiveness, and stereotypical behaviors that are commonly seen with mental impairment such as rocking, jumping, running, spinning, flaying, etc.

The methods of the invention may be used to alleviate a symptom of a neurological disease or disorder such as Parkinson's disease (shaking palsy), including primary Parkinson's disease, secondary parkinsonism, and postencephalitic parkinsonism; drug-induced movement disorders, including parkinsonism, acute dystonia, tardive dyskinesia, and neuroleptic malignant syndrome; Huntington's disease (Huntington's chorea; chronic progressive chorea; hereditary chorea); delirium (acute confusional state); dementia; Alzheimer's disease; non-Alzheimer's dementias, including Lewy body dementia, vascular dementia, Binswanger's dementia (subcortical arteriosclerotic encephalopathy), dementia pugilistica, normal-pressure hydrocephalus, general paresis, frontotemporal dementia, multi-infarct dementia, and AIDS dementia; age-associated memory impairment (AAMI); amnesias, such as retrograde, anterograde, global, modality specific, transient, stable, and progressive amnesias, and posttraumatic amnesias, and Korsakoff's disease

In one embodiment, the invention provides methods of treating a neurogenesis-associated disease comprising administering to a patient in need of treatment a pharmaceutical composition comprising a statin, or pharmaceutically acceptable salt, hydrate, solvate, or pro-drug thereof. The compositions of the invention comprising one or more statins are administered to patients in need of treatment. In one embodiment, the patient has a level of cholesterol that does not warrant therapeutic intervention with the statin to lower the cholesterol levels. In one embodiment, the patient suffers from depression.

In one embodiment, the patient suffers from depression and is not in need of cholesterol-lowering medication. In one embodiment, the patient suffers from bipolar disorder. In one embodiment, the patient suffers from bipolar disorder and is not in

need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from bipolar disorder. In one embodiment, the patient suffers from schizophrenia. In one embodiment, the patient suffers from schizophrenia and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from schizophrenia. In one embodiment, the patient suffers from traumatic brain injury. In one embodiment, the patient suffers from traumatic brain injury and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from traumatic brain injury. In one embodiment, the patient suffers from spinal cord injury. In one embodiment, the patient suffers from spinal cord injury and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from spinal cord injury. In one embodiment, the patient suffers from stroke. In one embodiment, the patient suffers from stroke and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from stroke. In one embodiment, the patient suffers from Alzeheimer's disease (AD) and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from AD. In one embodiment, the patient suffers from Parkinson's disease (PD) and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from PD. In one embodiment, the patient suffers from Huntington's disease (HD) and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from HD. In one embodiment, the patient suffers from multiple sclerosis (MS) and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from MS. In one embodiment, the patient suffers from or is at risk for cognitive dysfunction or other disturbance of higher mental activity and is not in need of cholesterol-lowering medication. In one embodiment, the patient does not suffer from cognitive dysfunction or other disturbance of higher mental activity.

Formulations

In therapeutic use for the treatment of neurogenesis-associated conditions, diseases, or disorders, the compound(s) utilized in the pharmaceutical method of the invention are administered to patients diagnosed with one or more neurogenesis associated conditions, diseases, or disorders at dosage levels suitable to achieve therapeutic benefit. By "therapeutic benefit" is meant that the administration of compound(s) leads to a beneficial effect in the patient over time.

One statin may be administered alone or in combination with another statin. In addition, statins can be administered in combinations with other drugs/agents that are used to treat neurogenesis associated conditions. For example, one appropriate combination utilizes both a statin and an anti-depressant.

For combinations, the compositions can be administered together in a single dosage form (e.g., oral formulations that combine the two drugs) or singly, in any of the dosage forms outlined below, simultaneously or sequentially. For example, one drug can be administered orally and the other intraperitoneally, either together or sequentially. In addition, when dosed separately, the dosages may be at different times or frequencies. Alternatively, the two drugs may be administered separately but in the same dosage form.

Initial dosages suitable for administration to humans may be determined from in vitro assays or animal models. For example, an initial dosage may be formulated to achieve a serum concentration that includes the IC_{50} of the particular metabolically active agent of the compound(s) being administered, as measured in an in vitro assay. Alternatively, an initial dosage for humans may be based upon dosages found to be effective in animal models. As one example, the initial dosage for each component of the pharmaceutical compositions outlined herein may be in the range of about 0.01 mg/kg/day to about 200 mg/kg/day, or about 0.1 mg/kg/day to about 100 mg/kg/day, or about 1 mg/kg/day to about 50 mg/kg/day, or about 10 mg/kg/day to about 50 mg/kg/day, can also be used. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound(s) being employed. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound(s) in a particular patient. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound(s). Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

The concentration of active compound in the drug composition will depend on absorption, distribution, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also

vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound(s) suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; (d) suitable emulsions and (e)aerosol formulations. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The active compound or pharmaceutically acceptable salt thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. Syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

As many of the retinoids described herein are acidic, they may be included in any of the above-described formulations as the free acid, a pharmaceutically

acceptable salt, a pro-drug, solvate or hydrate. Pharmaceutically acceptable salts substantially retain the activity of the free acid and may be prepared by reaction with bases. Pharmaceutically acceptable salts include any known suitable salts of retinoids known in the art for administration to mammals. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than is the corresponding free acid form. Similarly, the retinoids may be included in any of the above-described formulations as a solvate, hydrate or pro-drug. Preferred pro-drugs include hydrolyzable ester derivatives such as aromatic esters, benzyl esters and lower alkyl esters such as ethyl, cyclopentyl etc. Other pro-drugs are known to those of skill in the pharmaceutical arts.

The active compound or pharmaceutically acceptable salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action.

As used herein, the term "pharmaceutically acceptable salt(s)" refers to salts that retain the desired biological activity of the above-identified compounds and exhibit minimal or no undesired toxicological effects. Examples of such salts include, but are not limited to acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, naphthalenedisulfonic acid, and polygalacturonic acid. The compounds can also be administered as pharmaceutically acceptable quaternary salts known by those skilled in the art, which specifically include the quaternary ammonium salt of the formula --NR+Z-, wherein R is hydrogen, alkyl, or benzyl, and Z is a counter-ion, including chloride, bromide, iodide, --O-alkyl, toluenesulfonate, methylsulfonate, sulfonate, phosphate, or carboxylate (such as benzoate, succinate, acetate, glycolate, maleate, malate, citrate, tartrate, ascorbate, benzoate, cinnamoate, mandeloate, benzyloate, and diphenylacetate).

The compound(s) of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. In one embodiment, the pharmaceutically acceptable carrier is suitable for a

electrohydrodynamic aerosol device, a nebulizer device or a aerosol device. In one preferred embodiment, the pharmaceutically acceptable carrier is a liquid such as water, alcohol, polyethylene glycol or perfluorocarbon.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the packaged nucleic acid with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the compound(s) of choice with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intra-articular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration, oral administration, subcutaneous administration and intravenous administration are the preferred methods of administration. A specific example of a suitable solution formulation may comprise from about 0.1-100 mg/ml compound(s) and about 1000 mg/ml propylene glycol in water. Another specific example of a suitable solution formulation may comprise from about 0.1 or about 0.2 to about100 mg/ml compound(s) and from about 800-1000 mg/ml polyethylene glycol 400 (PEG 400) in water.

A specific example of a suitable suspension formulation may include from about 0.2-30 mg/ml compound(s) and one or more excipients selected from the group consisting of: about 200 mg/ml ethanol, about 1000 mg/ml vegetable oil (e.g., corn oil), about 600-1000 mg/ml fruit juice (e.g., grape juice), about 400-800 mg/ml milk, about 0.1 mg/ml carboxymethylcellulose (or microcrystalline cellulose), about 0.5 mg/ml benzyl alcohol (or a combination of benzyl alcohol and benzalkonium chloride) and about 40-50 mM buffer, pH 7 (e.g., phosphate buffer, acetate buffer or citrate buffer or, alternatively 5% dextrose may be used in place of the buffer) in water.

A specific example of a suitable liposome suspension formulation may comprise from about 0.5-30 mg/ml compound(s), about 100-200 mg/ml lecithin (or other phospholipid or mixture of phospholipids) and optionally about 5 mg/ml cholesterol in water. For subcutaneous administration of a compound(s), a liposome suspension formulation including 5 mg/ml compound(s) in water with 100 mg/ml lecithin and 5 mg/ml compound(s) in water with 100 mg/ml lecithin and 5 mg/ml cholesterol provides good results.

The formulations of compound(s) can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the compound(s). The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents, discussed in more detail, below.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation (CA) and Gilford Pharmaceuticals (Baltimore, Md.). Liposomal suspensions may also be pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidylcholine, arachadoyl phosphatidylcholine, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. Aqueous

solutions of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Materials and Methods

The following methods and materials were used in the Examples below.

Animals

All animal studies were carried out within the NIH guidelines for the care and use of laboratory animals and received approval from the institutional committee. 10-12 week old male C57Bl/6, Swiss Webster, or Balb/c mice were obtained from Charles River Laboratories (Wilmington, MA) and 10-12 week old male 129SvEv mice were obtained from Taconic (Oxnard, CA). The outbred Swiss Webster strain of mice was used for the initial drug-screening experiments. Subsequent experiments were performed in the inbred 129SvEv strain, as these showed the least inter-animal variability. Male Sprague-Dawley rats (250 g) were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in a climate-controlled environment and fed standard rodent chow and water *ad libitum*.

^2H_2O Labeling

For ^2H_2O labeling, animals received a priming intraperitoneal bolus of 49 ml/kg >99% ^2H_2O (Spectra Stable Isotopes, Columbia, MD) containing 0.9% NaCl and were maintained on 10% ^2H_2O in drinking water for the duration of the labeling period. In previous studies (reviewed in Jones and Leatherdale, *Clin Sci (Lond)* 80:277-280 (1991)), intake of up to 20% ^2H_2O has no apparent phenotypic or behavioral effects. For protocols involving label incorporation in hippocampal progenitor cells, mice were labeled continuously for up to 1 year. In a separate study, total hippocampal tissue was isolated after 3, 7, or 14 days of labeling. Animals that received drug treatment were labeled during the final 7-10 days of treatment. For studies that assessed proliferation of mature neurons, animals were labeled with 10% ^2H_2O for 3 weeks, after which time the label was discontinued and animals were sacrificed 4 weeks later.

Drug Treatments and ^2H_2O Labeling

Male rats and mice were treated with antidepressants of different classes such as an SSRI - fluoxetine, a tricyclic – imipramine, or a serotonin-norepinephrine reuptake inhibitor (SNRI) -venlafaxine, administered in drinking water. The drug solutions were dissolved at a concentration of 100, 200 and 100 mg/L, for fluoxetine, imipramine and venlafaxine, respectively. These concentrations were calculated to achieve a dose of 10 mg/kg/day for fluoxetine and venlafaxine and 20 mg/kg/day for imipramine, based on the average cage consumption of water (3 ml / 30g mouse or 25 ml / 250g rat). Treatment with anti-depressant drugs was continued for 3-5 weeks.

Various approved drugs were screened for neurogenic activity (Table 1). These agents were selected based on long-standing use in humans and on the recognition of having several therapeutic actions (i.e., pleiotropic effects). This effort was intended to be a proof-of-concept study to demonstrate 'indications discovery', i.e. ability to find unexpected actions of agents with potential use in a new indication. The doses selected were based on use in published preclinical studies with these agents, and the concentrations in food or water were calculated based on the average cage consumption of food (3 g / 30g mouse) and water (3 ml / 30g mouse). As a follow-up study after initial screening, a potential 'class' effect for one of the agents (topiramate) tested in the initial screen was investigated by treating mice

orally for 3 weeks with one of 11 other anticonvulsants: valproate (1 g/kg), clonazepam (3 mg/kg), gabapentin (100 mg/kg), carbamazepine (30 mg/kg), ethosuximide (300 mg/kg), levetiracetam (30 mg/kg), oxcarbazepine (100 mg/kg), phenytoin (100 mg/kg), primidone (100 mg/kg), tiagabine (30 mg/kg) or zonisamide (50 mg/kg).

Dose response studies were also done for topiramate (10, 30, 100, 150 mg/kg, p.o.), atorvastatin (1, 3, 10 and 30 mg/kg in diet), simvastatin (1, 3, 10 and 30 mg/kg, p.o.). In addition, mice received chronic treatment with potential inhibitors of neurogenesis such as isotretinoin (1 and 3 mg/kg, i.p.) or prednisolone (5 and 40 mg/kg, in diet).

Isolation and characterization of hippocampal progenitor cells

Animals were euthanized by CO_2 asphyxiation, brains were immediately removed and hippocampus dissected out and progenitor cells were isolated by a modification of the method described previously (Palmer et al., *J Neurosci* 19:8487-8497 (1999)). Briefly, tissues were finely minced and digested in a solution of papain (4 U/ml; Worthington Biochemical Corporation, Lakewood, NJ) and DNase (250 U/ml; Roche Applied Science, Indianapolis, IN) dissolved in Hibernate-A (BrainBits LLC, Springfield, IL). The digested tissue was then mechanically triturated and thoroughly mixed with an equal volume of Percoll solution, made by mixing nine parts of Percoll (Amersham Biosciences, Piscataway, NJ) with one part 10X PBS. The cell suspension was fractionated by centrifugation for 30 min, 18°C at 20,000 X g. Density beads were run in parallel and cells fractionating between 1.064 – 1.075 g/ml were collected and washed free of Percoll and stored frozen at -20°C until DNA isolation.

Characterization of progenitor cells and neurons by flow cytometry

The gradient-purified progenitor cells were immunofluorescently stained for intracellular markers, nestin and vimentin, and analyzed by flow cytometry. The cells were fixed and permeabilized with IntraCyte™ buffers (Orion Biosolutions, Vista, CA) and incubated overnight at 4°C with mouse anti-nestin (1:50, Rat401, BD Pharmingen, San Diego, CA) or mouse anti-vimentin (1:50, BD Pharmingen, San Diego, CA) primary antibodies. Gradient-purified hippocampal neurons were stained

for tetanus toxin C fragment (TTX), a cell surface marker for neurons. The cells were fixed with 4% paraformaldehyde and incubated with tetanus toxin C fragment followed by anti-TTX mouse monoclonal antibody (Roche applied Science, Indianapolis, IN). After washing, cells were incubated with ALEXA488-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes, Eugene, OR). Propidium iodide (PI) was used to stain DNA, and ALEXA488 staining of PI-positive single cells (gated on plots of forward scatter peak area vs. peak height) was analyzed on a Coulter EpicsXL cytometer (Beckman Coulter).

Isolation of nuclei from mature neurons and sorting NeuN positive nuclei by flow cytometry

Neuronal nuclei were isolated from frozen hippocampal tissue by a modification of a recently-described method (Spalding et al., *Cell* 122:133-143 (2005)). Briefly, tissue was homogenized in 1 ml lysis buffer (0.32 M sucrose, 5 mM $CaCl_2$, 3 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris-HCl [pH 8.0], 0.1% Triton X and 1mM DTT). Homogenized samples were gently suspended in 1.8 ml of sucrose solution (1.8 M sucrose, 3 mM magnesium acetate, 1 mM DTT, 10 mM Tris-HCl [pH 8.0]), layered onto a cushion of 1 ml sucrose solution, and centrifuged at 30,000 g for 2.5 hr at 4° C. The isolated nuclei were resuspended in 1 ml PBS and stored overnight at 4° C.

To identify neuronal nuclei, anti-NeuN antibodies were directly conjugated with Zenon mouse IgG labeling reagent (Alexa 488, Molecular Probes) by mixing 10 µl of ALEXA 488 conjugate in 100 µl blocking buffer (PBS / 0.5% BSA / 10% normal goat serum) with 300 µl NeuN antibody (1 mg/ml; diluted 1:250 in PBS) and incubating at room temperature for 5 minutes. The suspension of nuclei (1 ml) was added and incubated at 4°C for 1 hour. The nuclei were then washed twice with 3 ml PBS by centrifuging at 1000 x g for 10 min. Propidium iodide was used to stain DNA, and neuronal nuclei were sorted as a homogeneous population of $NeuN^{bright}$ cells, using a Coulter Epics Elite sorter (Beckman Coulter) with gates set for PI-positive single nuclei.

Analysis of cell proliferation

Gas chromatographic / mass spectrometric (GC/MS) analysis was performed as described previously (Neese et al., *Proc. Natl. Acad. Sci. U S A* 99:15345-15350 (2002); Busch et al., *J. Immunol. Methods* 286:97-109 (2004)) to measure ^2H incorporation from ^2H_2O into purine deoxyribonucleotides in genomic DNA. Briefly, genomic DNA was isolated from hippocampal tissue, isolated progenitor cells or sorted neurons, using a DNEasy tissue kit (Qiagen, Valencia, CA) and hydrolyzed enzymatically. The deoxyribose was converted to the pentane tetraacetate derivative and analyzed by GC/MS. The fraction of newly labeled cells was calculated as the ratio of excess ^2H enrichment in hippocampal cells to the corresponding enrichment in bone marrow DNA (a fully turned-over tissue).

Behavioral Assay Using the Forced Swim Test

Male NIH Swiss mice (n=10) were placed in clear glass or plastic cylinders (diameter approximately 10 cm; height approximately 25 cm) filled with 10 cm of water (22-25°C) for 6 minutes. The duration of floating ("immobility") was scored by an observer during the last 4 minutes of the 6 minute test period. The period of immobility is considered to be when the mouse is floating motionless or making only those movements necessary to keep its head above water. This test is widely used in psychopharmacology research for prediction of antidepressant drug activity.

Statistical Analysis

For comparison between multiple groups, one way ANOVA was used, with a post-hoc Dunnet's test for comparisons with vehicle-treated animals (SigmaStat). Data were considered significant at $p < 0.05$

Example 1

Flow Cytometric Analysis Of Gradient-Enriched Hippocampal Progenitor Cells

Measurement of ^2H incorporation into the DNA of hippocampal progenitor cells requires isolation of this cell population from intact tissue. To this end, progenitor cells were isolated from the hippocampus of Swiss Webster mice by Percoll gradient fractionation (FIG. 1, Panel A); the isolated cells were fixed and permeabilized, stained for specific intracellular markers of progenitor cells, nestin

and vimentin, and analyzed by flow cytometry after gating on nucleated (propidium iodide-stained) cells and excluding doublets. A majority of cells stained positively for either nestin (65% positive in FIG. 1, Panel B) or vimentin (72% positive), as compared to isotype controls (2.1% positive). A broad distribution of fluorescence intensity was observed, and the cells remaining in the negative region of the dot plot appeared to be weakly stained.

Example 2

Label Incorporation Kinetics in Progenitor Cells

In order to characterize the proliferation kinetics of gradient-enriched hippocampal progenitor cells, Swiss Webster mice were labeled continuously with 10% ^2H_2O in drinking water, starting at 10 weeks of age; hippocampal progenitor cells were isolated after various labeling times, and ^2H incorporation into DNA, analyzed by GC/MS, was used to determine the fraction of cells that had incorporated the label through cell division (FIG. 1, Panel C). Approximately 16% of cells incorporated label at plateau, with a half-life within this dividing population of about 2.7 weeks; the majority of cells continued to be unlabeled over the course of a year. This is consistent with a "kinetic subpopulation" pattern wherein a preponderance of non-dividing precursors is present with a subset of actively dividing cells that enters and exits the pool (by differentiation or death). The baseline rate of progenitor proliferation was strain-dependent (FIG. 1, Panel D). The initial rate of labeling, measured after 1 week of ^2H_2O intake, varied about threefold among four mouse strains tested and were significantly ($p < 0.001$) different from each other. The highest proliferation rate was observed in C57Bl/6 mice, whereas the 129SvEv mice had the lowest rate of proliferation.

The fraction of new cells in whole hippocampal tissue of C57Bl/6 mice after 3, 7 or 14 days of labeling was 0.7 ± 0.01%, 1.3 ± 0.3% and 2.9 ± 0.6% respectively. Thus, gradient isolation enriched for proliferating cells, and the consistency of the labeling results indicated that the cell isolation method was highly consistent in this regard.

Example 3

Effects of Anti-Depressant Drugs on Progenitor Cell Proliferation In Rodent Hippocampus

Male Swiss-Webster mice (FIG. 2, Panel A) or Sprague-Dawley rats (FIG. 2, Panel B) were treated for 3 weeks with anti-depressant drugs of different classes: an SSRI (fluoxetine, 10 mg/kg/day), a tricyclic, (imipramine, 20 mg/kg/day) or an SNRI (venlafaxine, 10 mg/kg/day). After labeling with ^2H_2O during the last week of treatment, antidepressant-treated animals from all groups showed a significant increase in the progenitor cell proliferation rate in the hippocampus (FIG. 2, Panels A and B). Both baseline proliferation and the magnitude of the drug effects were somewhat different between mice and rats, however. Fluoxetine treatment produced a significant ($p < 0.01$) increase in the hippocampal progenitor cell proliferation in C57Bl/6, 129SvEv, Swiss Webster, and Balb/c mice (FIG. 2, Panel C). The percent stimulation of progenitor cell proliferation by fluoxetine was similar across strains.

Example 4

Effects of Anti-Depressant Treatment on Survival and Differentiation of Newly Divided Cells

A "pulse-chase" protocol was used to assess the survival and differentiation of progenitor cells into neurons in vehicle- and fluoxetine-treated animals. Male 129SvEv mice were treated with fluoxetine (10 mg/kg/day) or vehicle for 5 weeks and labeled with 10% ^2H_2O for the last 3 weeks of treatment. A longer labeling time was used in this protocol, to allow greater numbers of newly divided progenitor cells to be labeled, allowing for some label loss due to death of labeled cells and differentiation into non-neuronal progeny. The antidepressant treatment and ^2H_2O intake were then discontinued, and the animals were followed for another 4 weeks, after which time intact neurons or neuronal nuclei were isolated for analysis of DNA labeling. FIG. 3, Panel A shows that > 80% of gradient-enriched hippocampal neurons stained positively for tetanus toxoid (TTX), a surface marker for neuronal cells. Further purification of neurons by flow cytometric sorting proved difficult, however, because of the large size range of neuronal cell bodies and large amount of axonal debris present in these gradient fractions (data not shown). Successful resolution of $NeuN^{bright}$ and $NeuN^{dim}$ nuclei obtained from frozen mouse brain was possible (FIG. 3, Panel B), allowing sorting of brightly stained nuclei of mature

neurons to > 98% purity upon reanalysis (not shown). GC/MS analysis of extracted DNA from both preparations showed that fluoxetine treatment produced a significant increase in the fraction of ^{2}H-labeled neurons, 4 weeks after the end of a 3-week labeling period (FIG. 3, Panels A and B). ^{2}H incorporation into DNA of gradient-enriched neurons exceeded that of $NeuN^{bright}$ neuronal nuclei; although Percoll gradients efficiently resolve proliferating neural progenitors from neuronal cells, the gradient-enriched population may include a greater proportion of immature neurons derived from dividing precursors that do not yet express high levels of NeuN. Taken together, these results confirm that differentiation of recently divided precursors into mature neurons is enhanced by fluoxetine and show that this antidepressant drug effect is detectable by $^{2}H_2O$ labeling.

Example 5

Effect of Retinoid or Glucocorticoid Administration on Progenitor Cell Proliferation

We also evaluated the activity of a retinoid and a glucocorticoid on hippocampal progenitor cell proliferation. Male 129SvEv mice were treated with isotretinoin (1 or 3 mg/kg, i.p.) for 3 weeks and labeled with 10% $^{2}H_2O$ in the final 10 days of treatment. There was a dose-dependent decrease in hippocampal progenitor cell proliferation following retinoid treatment (FIG. 4, Panel A). Treatment with 3 mg/kg dose of isotretinoin produced a 38% reduction over baseline which was statistically significant ($p < 0.05$) compared to vehicle-treated controls.

Male Swiss Webster mice were treated with prednisolone (5 or 40 mg/kg in diet) and labeled with 8% $^{2}H_2O$ for 4 weeks. These mice received a slightly different treatment and labeling protocol since they were part of a study investigating the effects of glucocorticoid treatment on several metabolic pathways concurrently. Prednisolone treatment produced a dose-dependent significant ($p < 0.01$) decrease in hippocampal progenitor cell proliferation (FIG. 4, Panel B). Progenitor cell proliferation was significantly (52%) reduced at the 40 mg/kg dose, compared to untreated controls, and showed a trend toward reduction at the 5 mg/kg dose.

Example 6

Use of The Hippocampal Progenitor Cell Proliferation Assay to Screen Drugs

Next, the feasibility of using hippocampal progenitor cell proliferation as a screen for candidate neurogenic agents was explored. To this end, a panel of approved drugs with known or suspected pleiotropic actions (Table 1), but not previously known to have neurogenic activity in normal murine hippocampus, was tested for their effects on proliferation of hippocampal progenitor cells in Swiss Webster mice (FIG. 5). Two of these agents were found to increase the rate of progenitor cell proliferation. The hydroxymethyl-glutaryl CoA (HMGCoA) reductase inhibitor (statin), atorvastatin (10 mg/kg p.o.), and the anticonvulsant, topiramate (100 mg/kg in diet), each given daily for 3 weeks, significantly increased progenitor cell proliferation, measured by ^2H_2O labeling during the third week. The increases were of a similar magnitude as observed with the positive controls, fluoxetine and imipramine (FIG. 5). No stimulatory effects were seen for 9 other drugs given by various routes (diet, drinking water, oral gavage, or i.p.) at daily doses reported in the literature to be pharmacologically active for other actions (Table 1).

Table 1

Drug	Class	Dose	Route
Aspirin	NSAID	60 mg/kg/day	Diet
Atorvastatin	HMG CoA Reductase Inhibitor	10 mg/kg/day	Oral gavage
Calcitriol	Vitamin D analog	2.5 μg/kg/day	Diet
Clofibrate	PPAR-α agonist	50 mg/kg/day	Diet
Enalapril Maleate	ACE Inhibitor	2.5 mg/kg/day	Drinking water
Etiocholanedione	Steroid Analog	1.5 g/kg/day	Diet
Flurbiprofen	NSAID	50 mg/kg/day	Intraperitoneal
Ketoconazole	Anti-fungal	100 mg/kg/day	Diet
Methotrexate	Folate Inhibitor	20 mg/kg/2days	Intraperitoneal
Rosiglitazone	PPAR-γ agonist	6 mg/kg/day	Diet
Topiramate	Anti-convulsant	100 mg/kg/day	Diet

Abbreviations: HMG CoA, hydroxyl-methylglutaryl coenzyme A; NSAID, non-steroidal anti-inflammatory drug; PPAR, peroxisome proliferator-activated receptor; ACE, angiotensin converting enzyme.

In follow-up studies, we assessed dose-response relationships of the compounds with stimulatory activity and tested other agents in their classes (statins and anticonvulsants). Topiramate produced a dose-dependent increase in the proliferation of hippocampal progenitor cells of 129SvEv mice (FIG. 6, Panel A). Oral treatment with 30 mg/kg/day produced an approximately 70% increase over baseline proliferation, approaching a plateau at higher doses (100 and 150 mg/kg/day). We also screened 11 other anticonvulsant drugs at single doses known to have anticonvulsant activity in animals (FIG. 6, Panel B). Treatment of Swiss Webster mice for 3 weeks with either valproate (1 g/kg in diet) or oxcarbazepine (100 mg/kg, p.o.) significantly increased progenitor cell proliferation. Treatment with valproate doubled the number of proliferating cells as compared to vehicle, while oxcarbazepine treatment produced a 67% increase over baseline proliferation. The other anticonvulsants either had no effect on progenitor cell proliferation or were slightly inhibitory at the single doses tested. We conclude that a subset of anticonvulsants is neurogenic, as judged by DNA labeling of hippocampal progenitors.

The stimulatory action of statins on hippocampal progenitor cell proliferation was also confirmed and extended in dose-response studies, using atorvastatin or simvastatin. Treatment of 129SvEv mice with increasing doses of atorvastatin (1, 3, 10, 30 mg/kg in diet) produced a dose-dependent increase in hippocampal progenitor cell proliferation, with the maximal (68% increase over baseline) effect seen at the 3 mg/kg dose (FIG. 7, Panel A). Chronic oral treatment with another HMGCoA reductase inhibitor, simvastatin, also produced a dose-dependent increase in the proliferation of precursor cells (FIG. 7, Panel B) in the hippocampus of 129SvEv mice. This more lipophilic statin showed significant stimulatory activity at the 10 and 30 mg/kg doses.

Example 7

Use of the Hippocampal Progenitor Cell Proliferation Assay to Identify Drugs That Have Antidepressant Activity in Animal Models

Next, the use of the hippocampal progenitor cell proliferation assay to identify drugs with functional effects on behavior, particularly in the areas of antidepressant activity, was explored. The forced-swim test (FST) is well established in

psychopharmacology research as a test that has utility for predicting antidepressant activity of drugs. A compound that was screened by the hippocampal progenitor cell proliferation assay (compound K-001) and was shown to have dose-dependant stimulatory actions on hippocampal progenitor cell proliferation was then used in the FST in rats. The compound K-001 altered the FST in a dose-dependant manner (FIG. 8). The known anti-depressant drug imipramine gave similar effects on the FST in this study (FIG. 8) and served as a positive control.

WHAT IS CLAIMED IS:

1. A method of treating depression in a patient comprising administering an effective amount of a hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitor to a subject with depression.

2. A method according to claim 1, wherein said inhibitor is a statin.

3. A method according to claim 2, wherein said statin is atorvastatin.

4. A method of treating depression in a patient who is not in need of treatment for elevated cholesterol comprising administering an effective amount of a hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitor to a subject with depression.

5. A method according to claim 4, wherein said inhibitor is a statin.

6. A method according to claim 5, wherein said statin is atorvastatin.

7. A method of treating a disorder of cognition in a patient who is not in need of treatment for elevated cholesterol comprising administering an effective amount of a hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitor to a subject with a cognitive disorder.

8. A method according to claim 7, wherein said inhibitor is a statin.

9. A method according to claim 8, wherein said statin is atorvastatin.

10. A method of treating a patient at risk for a cognitive disorder who is not in need of treatment for elevated cholesterol comprising administering an effective amount of a hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitor to a subject at risk for a cognitive disorder.

11. A method according to claim 10, wherein said inhibitor is a statin.

12. A method according to claim 11, wherein said statin is atorvastatin.

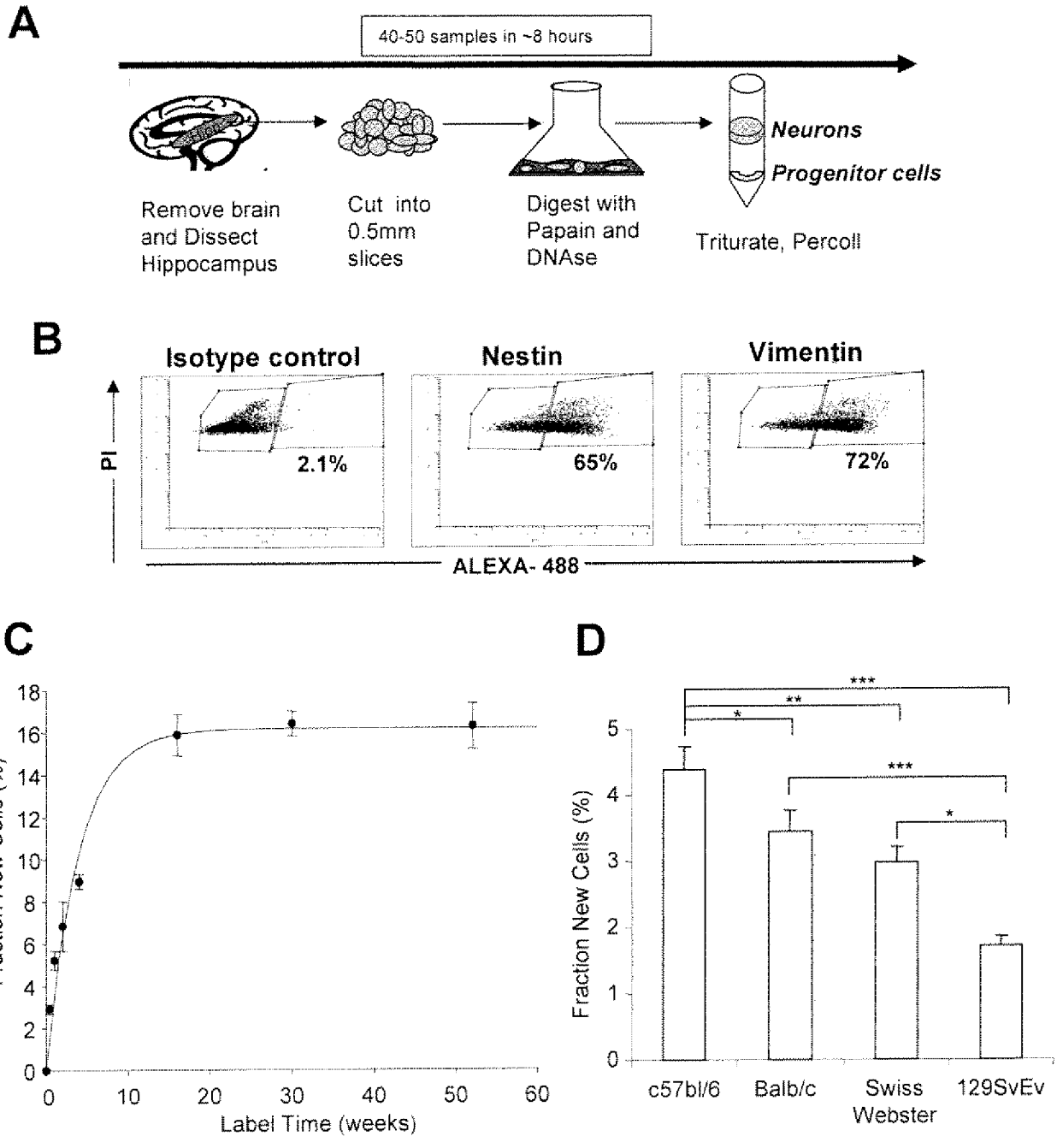

A
40-50 samples in ~8 hours
Neurons
Progenitor cells
Remove brain and Dissect Hippocampus
Cut into 0.5mm slices
Digest with Papain and DNAse
Triturate, Percoll
B
Isotype control
Nestin
Vimentin
PI
2.1%
65%
72%
ALEXA- 488
C
Fraction New Cells (%)
Label Time (weeks)
D
Fraction New Cells (%)
c57bl/6
Balb/c
Swiss Webster
129SvEv

FIG. 1

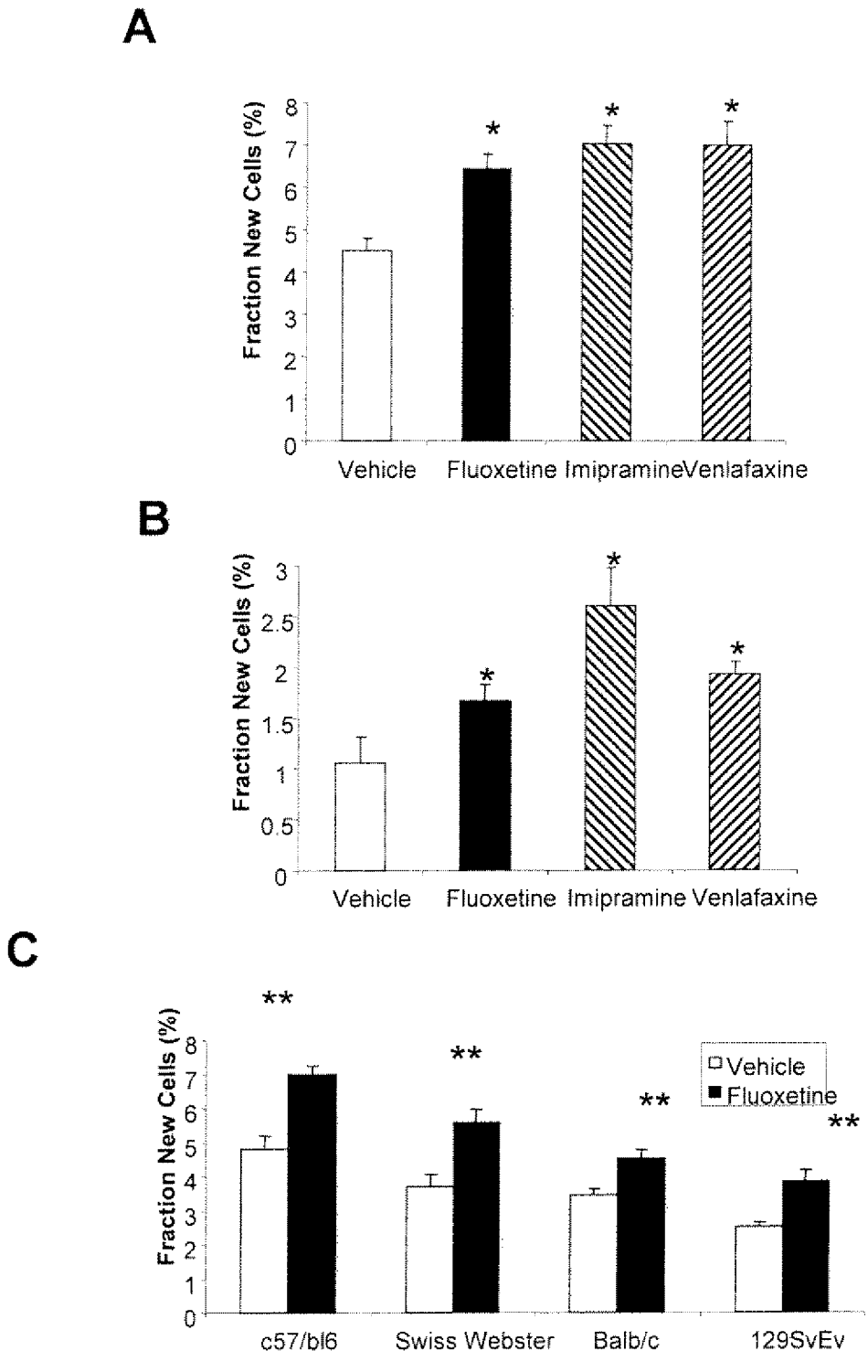

A
Fraction New Cells (%)
0
1
2
3
4
5
6
7
8
*
*
*
Vehicle
Fluoxetine
Imipramine
Venlafaxine
B
Fraction New Cells (%)
0
0.5
1
1.5
2
2.5
3
*
*
*
Vehicle
Fluoxetine
Imipramine
Venlafaxine
C
Fraction New Cells (%)
0
1
2
3
4
5
6
7
8
**
**
**
**
Vehicle
Fluoxetine
c57/bl6
Swiss Webster
Balb/c
129SvEv

FIG. 2

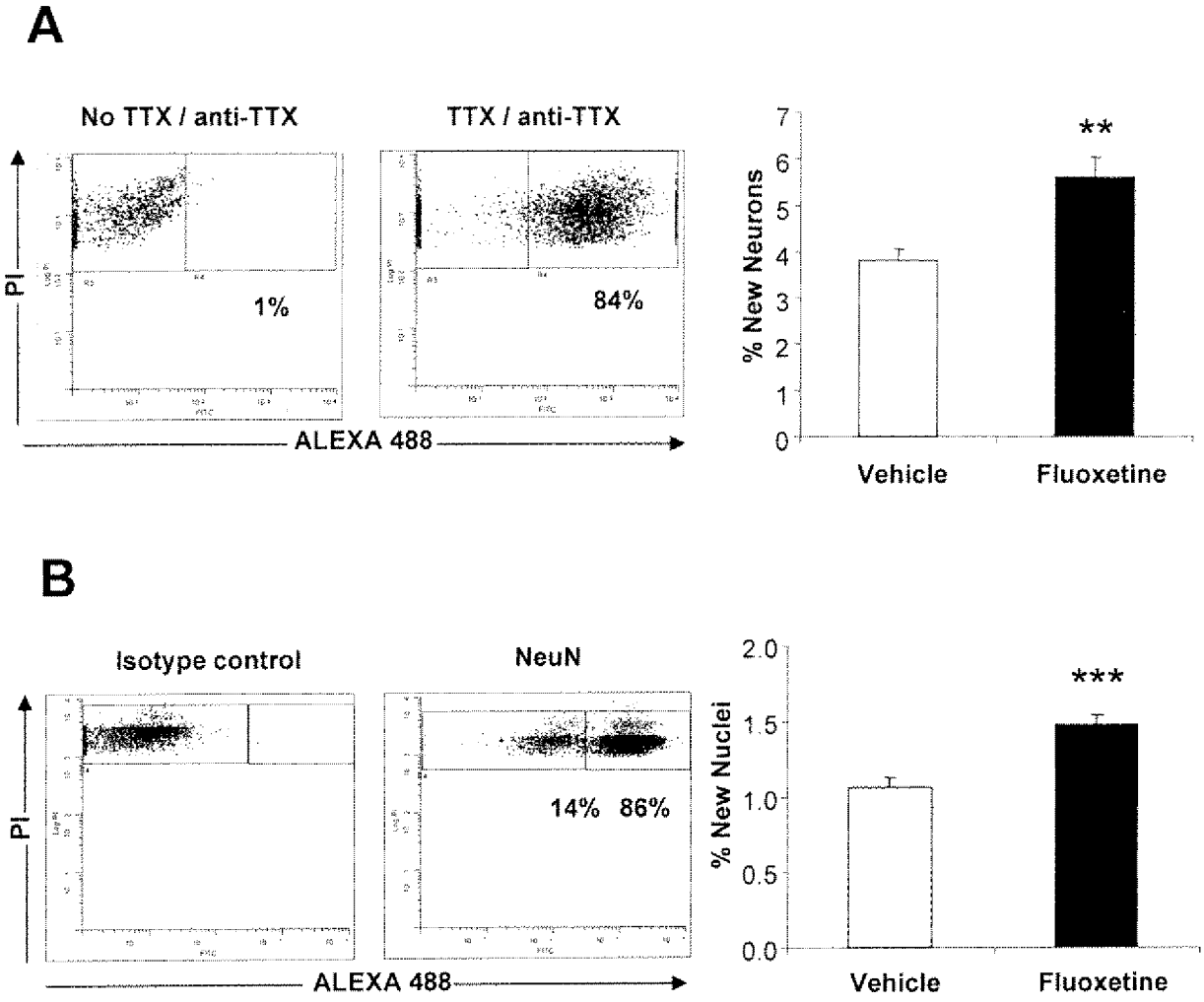
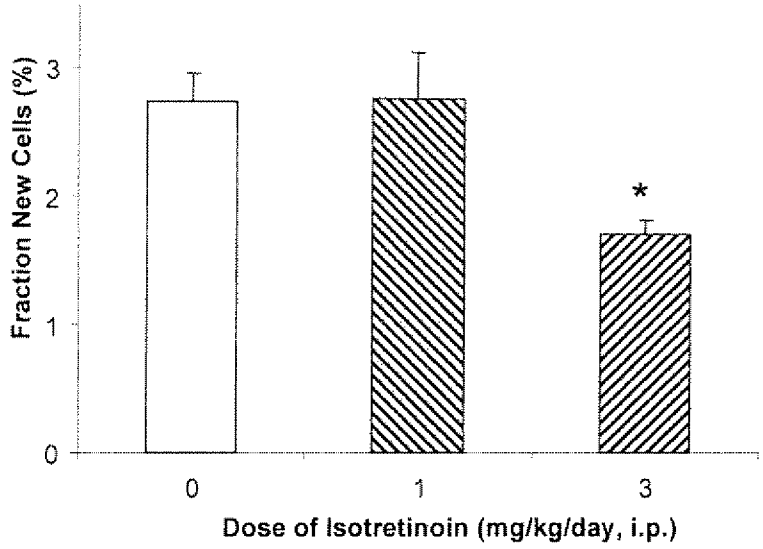

A
No TTX / anti-TTX
TTX / anti-TTX
PI
1%
84%
ALEXA 488
% New Neurons
**
Vehicle
Fluoxetine
B
Isotype control
NeuN
PI
14%
86%
ALEXA 488
% New Nuclei
***
Vehicle
Fluoxetine

FIG. 3

A


Fraction New Cells (%)
0
1
2
3
*
0
1
3
Dose of Isotretinoin (mg/kg/day, i.p.)

B

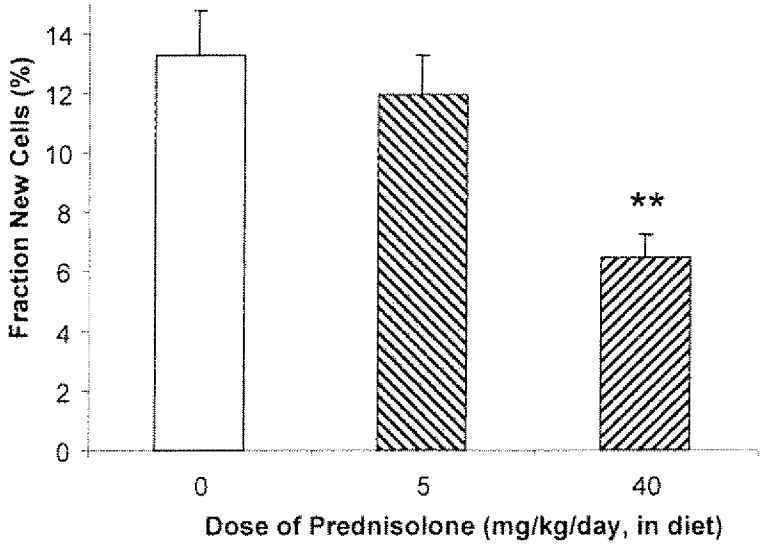

Fraction New Cells (%)
0
2
4
6
8
10
12
14
**
0
5
40
Dose of Prednisolone (mg/kg/day, in diet)

FIG. 4

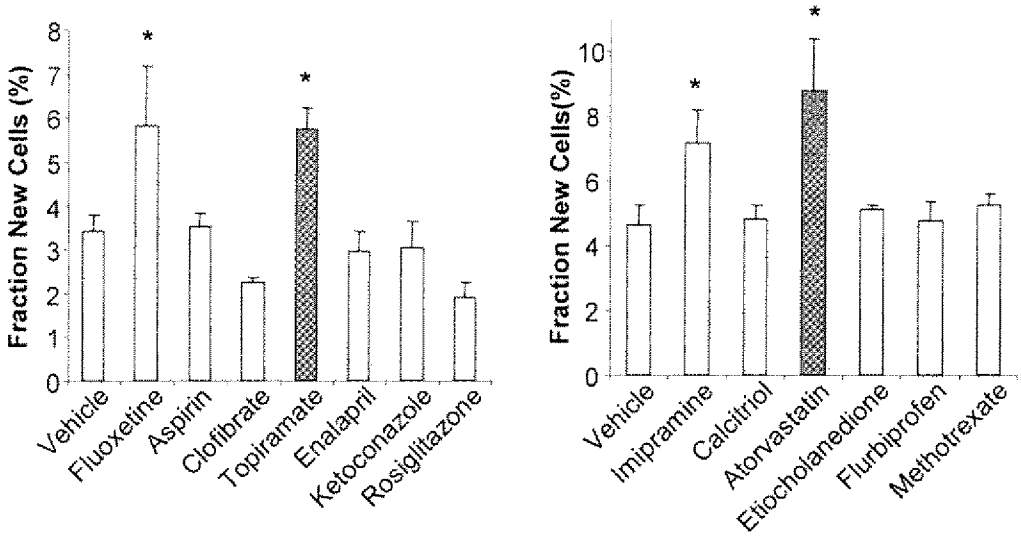
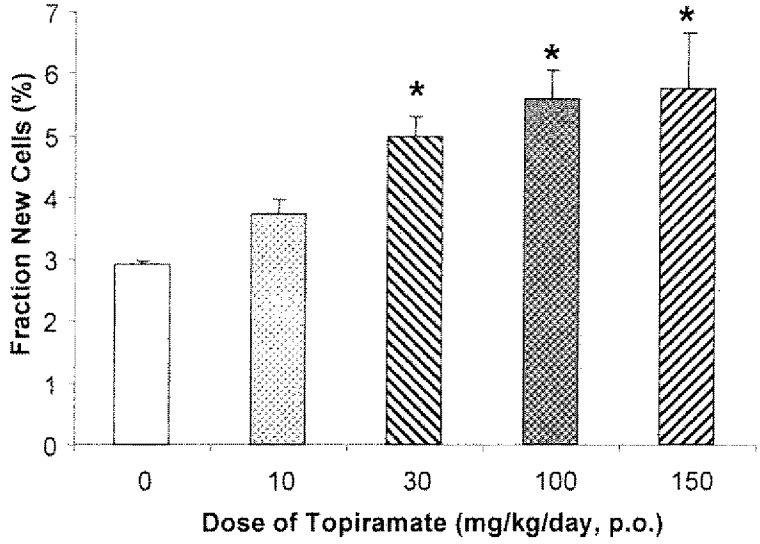

Fraction New Cells (%)
0
1
2
3
4
5
6
7
8
*
*
Vehicle
Fluoxetine
Aspirin
Clofibrate
Topiramate
Enalapril
Ketoconazole
Rosiglitazone
Fraction New Cells(%)
0
2
4
6
8
10
*
*
Vehicle
Imipramine
Calcitriol
Atorvastatin
Etiocholanedione
Flurbiprofen
Methotrexate

FIG. 5

A


Fraction New Cells (%)
0
1
2
3
4
5
6
7
*
*
*
0
10
30
100
150
Dose of Topiramate (mg/kg/day, p.o.)

B

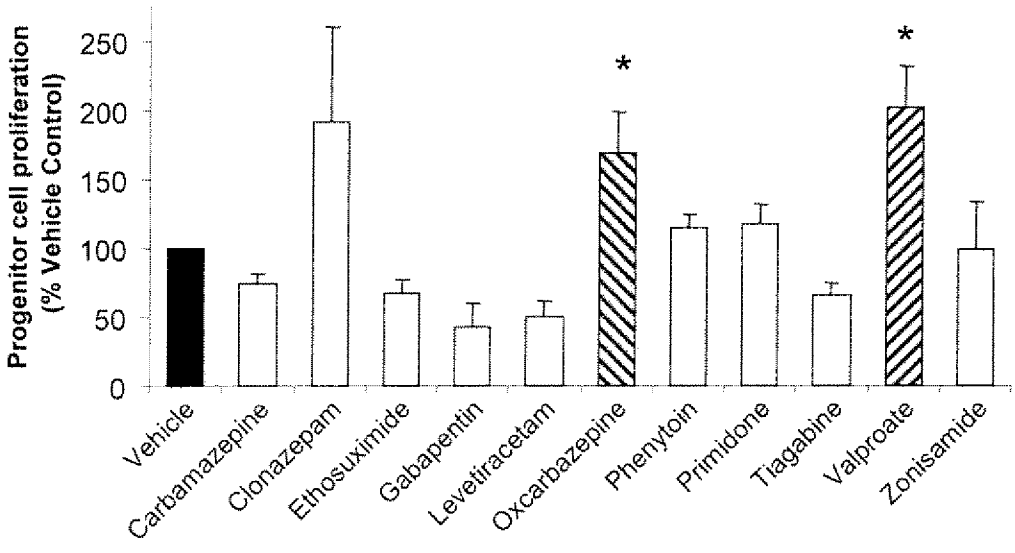
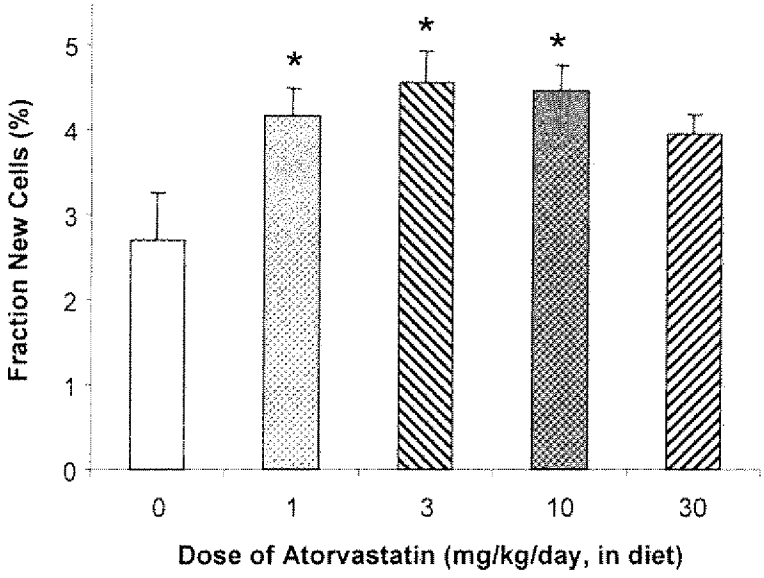

Progenitor cell proliferation
(% Vehicle Control)
0
50
100
150
200
250
*
*
Vehicle
Carbamazepine
Clonazepam
Ethosuximide
Gabapentin
Levetiracetam
Oxcarbazepine
Phenytoin
Primidone
Tiagabine
Valproate
Zonisamide

FIG. 6

A


Fraction New Cells (%)
0
1
2
3
4
5
*
*
*
0
1
3
10
30
Dose of Atorvastatin (mg/kg/day, in diet)

B

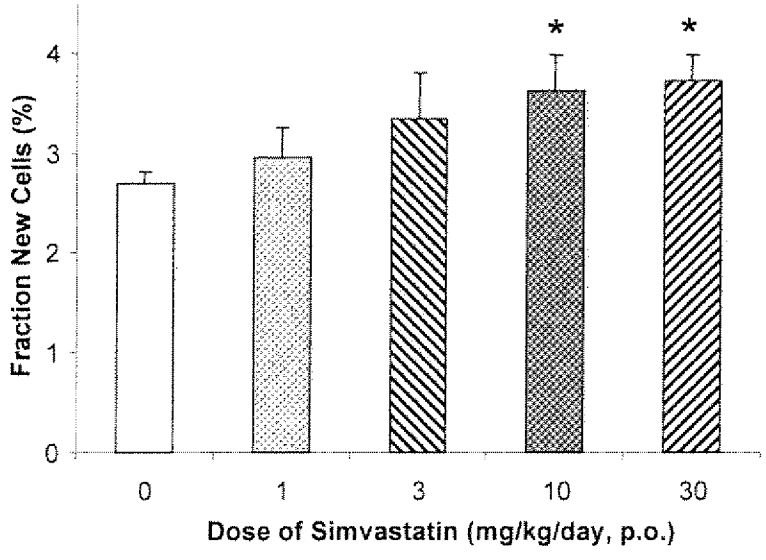

Fraction New Cells (%)
0
1
2
3
4
*
*
0
1
3
10
30
Dose of Simvastatin (mg/kg/day, p.o.)

FIG. 7

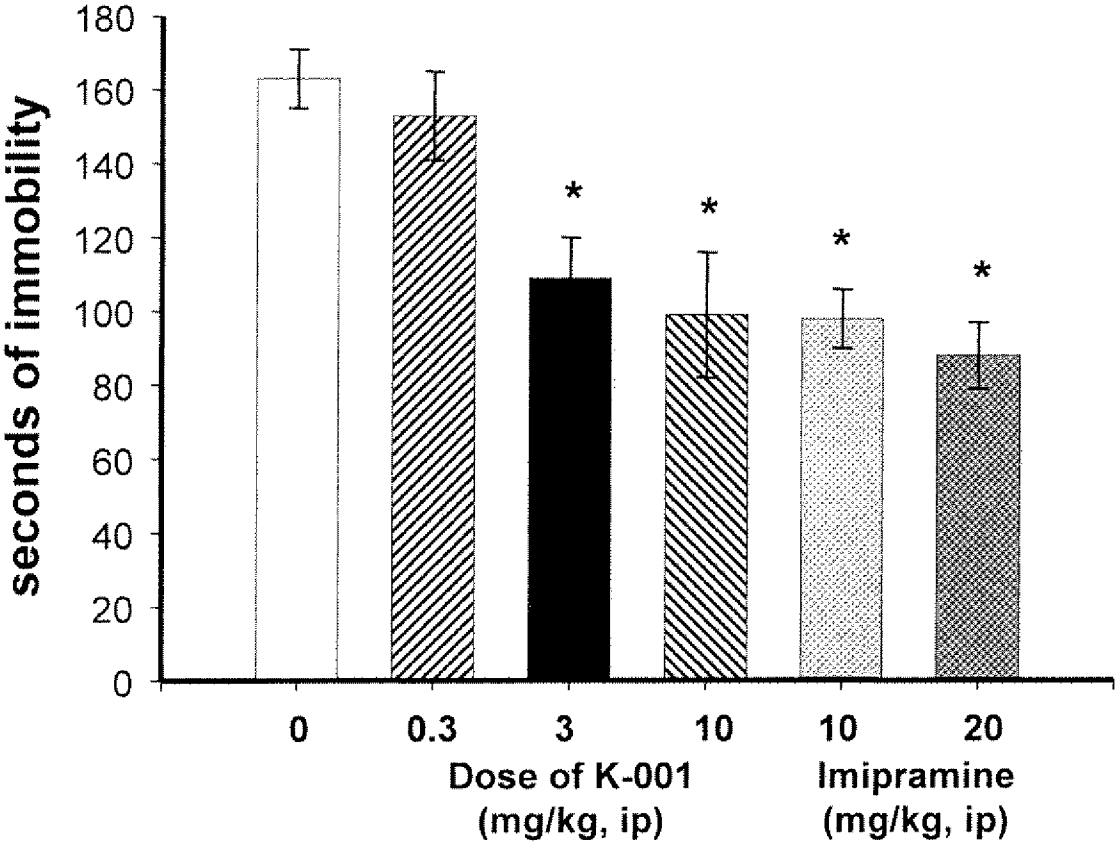

seconds of immobility
180
160
140
120
100
80
60
40
20
0
*
*
*
*
0
0.3
3
10
10
20
Dose of K-001
(mg/kg, ip)
Imipramine
(mg/kg, ip)

FIG. 8