Abstract: The instant disclosure describes methods for treating diseases and conditions of the central and peripheral nervous system by stimulating or increasing neurogenesis. The disclosure includes compositions and methods based on use of a SHTR agent, optionally in combination with one or more other neurogenic agents, to stimulate or activate the formation of new nerve cells.

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.

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5 HT RECEPTOR MEDIATED NEUROGENESIS

RELATED APPLICATIONS

This application is related to U.S. Provisional Applications 60/746,878, filed May 9, 2006, 60/805,436, filed June 21, 2006, and 60/882,429, filed December 28, 2006, all of which are incorporated by reference as if fully set forth.

FIELD OF THE DISCLOSURE

The instant disclosure relates to methods for treating diseases and conditions of the central and peripheral nervous system by stimulating or increasing neurogenesis via use of a 5HT receptor modulator optionally in combination with a neurogenic agent, neurogenic sensitizing agent or an anti-astrogenic agent, or another 5HT receptor modulator. The disclosure includes methods based on the application of a 5HT receptor modulator optionally in combination with a neurogenic sensitizing agent or an anti-astrogenic agent to stimulate or activate the formation of new nerve cells.

BACKGROUND OF THE DISCLOSURE


Serotonin (5-hydroxytryptamine, 5-HT or 5HT) has been proposed to assert its effects through a number of membrane-bound receptors. The 5-HT or 5HT family of receptors include multiple subtypes. The 5HT1a (or serotonin) receptor subtype is one member of the 5HT1 receptor family. Many members of the family, including 5HT1a, belong to the G protein-coupled receptor (GPCR) superfamily. According to some studies, 5HT1a receptor agonists are thought to inhibit activation of adenylate cyclase in some cells, leading to decreases in cAMP. Another study suggests that the 5HT1a receptor is coupled to N- and L-type calcium channels in some ganglion cells (see Cardenas et al. J. Neurophysiol. 77(6):3284-96, 1997).

5HT1a receptors are distributed in the CNS, and their activation has been shown to lead to neuronal hyperpolarization. The role of 5HT1a receptors has been thought to relate to modulation of anxiety, in part because knockout mice lacking 5HT1a receptors display increased anxiety. The animals also display reduced immobility in forced swimming and tail suspension tests. Agonists of 5HT1a, such as buspirone or gepirone, have been used as anxiolytics (see Tunnicliff Pharmacol. Toxicol. 69:149, 1991; and Den Boer et al. Hum Phychopharmacol. 15:315, 2000). Another 5HT1a agonist is 8-hydroxy-2-(di-n-propylamino), where the R(+)-isomer is a full agonist and the S(-)-enantiomer is a partial agonist.

Antagonists of 5HT1a may be used to accelerate the effects of selective serotonin reuptake inhibitors (SSRIs) and enhance their clinical efficacy (see Arborelius et al. Naunyn-Schmeiedebergs Arch Pharmacol 353:630-640, 1996). Examples of 5HT1a antagonists include spiperone and pindolol.

5HT4 receptor agonists have been observed as leading to increases in cAMP. The splice variants have been reported to couple positively to adenyl cyclase and have been observed to have similar pharmacological properties.

5HT4 receptors have been reported as displaying constitutive, agonist-independent, activity. The activity has been observed at relatively low receptor levels which may explain detected silent or inverse agonist activity by some putative antagonists.

Citation of the above documents is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

BRIEF SUMMARY OF THE DISCLOSURE

Disclosed herein are compositions and methods for the prophylaxis and treatment of diseases, conditions and injuries of the central and peripheral nervous systems by stimulating or increasing neurogenesis. Aspects of the methods, and activities of the compositions, include increasing or potentiating neurogenesis in cases of a disease, disorder, or condition of the nervous system. Embodiments of the disclosure include methods of treating a neurodegenerative disorder, neurological trauma including brain or central nervous system trauma and/or recovery therefrom, depression, anxiety, psychosis, learning and memory disorders, and ischemia of the central and/or peripheral nervous systems. In other embodiments, the disclosed methods are used to improve cognitive outcomes and mood disorders.

In one aspect, methods of modulating, such as by stimulating or increasing, neurogenesis are disclosed. The neurogenesis may be at the level of a cell or tissue. The cell or tissue may be present in an animal subject or a human being, or alternatively be in an in vitro or ex vivo setting. In some embodiments, neurogenesis is stimulated or increased in a neural cell or tissue, such as that of the central or peripheral nervous system of an animal or human being. In cases of an animal or human, the methods may be practiced in connection with one or more disease, disorder, or condition of the nervous system as present in the animal or human subject. Thus, embodiments disclosed herein include methods of treating a disease, disorder, or condition by administering at least one neurogenesis modulating agent having activity against a 5HT receptor, hereinafter referred to as a "5HTR agent." A 5HTR
agent may be formulated or used alone, in combination with another 5HTR agent, or in combination with one or more additional neurogenic agents.

While a 5HTR agent may be considered a "direct" agent in that it has direct activity against a 5HT receptor by interactions therewith, the disclosure includes a 5HTR agent that may be considered an "indirect" agent in that it does not directly interact with a 5HT receptor. Thus, an indirect agent acts on a 5HT receptor indirectly, or via production, generation, stability, or retention of an intermediate agent which directly interacts with a 5HT receptor.

Embodiments of the disclosure include a combination of a 5HTR agent and one or more other neurogenic agents disclosed herein or known to the skilled person. An additional neurogenic agent as described herein may be a direct 5HTR agent, an indirect 5HTR agent, or a neurogenic agent that does not act, directly or indirectly, through a 5HT receptor. Thus in some embodiments, an additional neurogenic agent is one that acts, directly or indirectly, through a mechanism other than a 5HT receptor. An additional neurogenic agent as described herein may be one which acts through a known receptor or one which is known for the treatment of a disease or condition. The disclosure further includes a composition comprising a combination of a 5HTR agent with one or more other neurogenic agents.

In a second aspect, the disclosure includes a method of lessening and/or reducing a decline or decrease of cognitive function in a subject or patient. In some cases, the method may be applied to maintain and/or stabilize cognitive function in the subject or patient. The method may comprise administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject or patient in an amount effective to lessen or reduce a decline or decrease of cognitive function.

In an additional aspect, the disclosure includes a method of treating mood disorders with use of a 5HTR agent, optionally in combination with one or more other neurogenic agents. In some embodiments, the method may be used to moderate or alleviate a mood disorder in a subject or patient. Non-limiting examples include a subject or patient having, or diagnosed with, a disease or condition as described herein. In other embodiments, the method may be used to improve, maintain, or stabilize mood in a subject or patient. Of course the method may be optionally combined with any other therapy or condition used in the treatment of a mood disorder.
In a third aspect, the disclosed methods include identifying a patient suffering from one or more diseases, disorders, or conditions, or a symptom thereof, and administering to the patient a 5HTR agent, optionally in combination with one or more other neurogenic agents, as described herein. In some embodiments, a method including identification of a subject as in need of an increase in neurogenesis, and administering to the subject a 5HTR agent, optionally in combination with one or more other neurogenic agents is disclosed herein. In other embodiments, the subject is a patient, such as a human patient.

Another aspect of the disclosure describes a method including administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject exhibiting the effects of insufficient amounts of, or inadequate levels of, neurogenesis. In some embodiments, the subject may be one that has been treated with an agent that decreases or inhibits neurogenesis. Non-limiting examples of an inhibitor of neurogenesis include opioid receptor agonists, such as a mu receptor subtype agonist like morphine. In other cases, the need for additional neurogenesis is that detectable as a reduction in cognitive function, such as that due to age-related cognitive decline, Alzheimer's Disease, epilepsy, or a condition associated with epilepsy as non-limiting examples.

In a related manner, a method may include administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject or person that will be subjected to an agent that decreases or inhibits neurogenesis. Non-limiting embodiments include those where the subject or person is about to be administered morphine or another opioid receptor agonist, like another opiate, and so about to be subject to a decrease or inhibition of neurogenesis. Non-limiting examples include administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject before, simultaneously with, or after the subject is administered morphine or other opiate in connection with a surgical procedure.

In a fifth aspect, the disclosure includes methods for preparing a population of neural stem cells suitable for transplantation, comprising culturing a population of neural stem cells (NSCs) in vitro, and contacting the cultured neural stem cells with a 5HTR agent, optionally in combination with one or more other neurogenic agents. In some embodiments, the stem cells are prepared and then transferred to a recipient host animal or human. Non-limiting examples of preparation include 1) contact with a 5HTR agent, optionally in combination with one or more other neurogenic agents, until the cells have undergone neurogenesis, such as that which is detectable by visual inspection, marking, or cell counting,
or 2) contact with a 5HTR agent, optionally in combination with one or more other neurogenic agents, until the cells have been sufficiently stimulated or induced toward or into neurogenesis. The cells prepared in such a non-limiting manner may be transplanted to a subject, optionally with simultaneous, nearly simultaneous, or subsequent administration of another neurogenic agent to the subject. While the neural stem cells may be in the form of an *in vitro* culture or cell line, in other embodiments, the cells may be part of a tissue which is subsequently transplanted into a subject.

In yet another aspect, the disclosure includes methods of modulating, such as by stimulating or increasing, neurogenesis in a subject by administering a 5HTR agent, optionally in combination with one or more other neurogenic agents. In some embodiments, the neurogenesis occurs in combination with the stimulation of angiogenesis which provides new cells with access to the circulatory system.

Another aspect of the disclosure include methods and compositions wherein two or more 5HTR agents are utilized, such as combinations of buspirone, azasetron, clozapine, cisapride, ondansetron, tandospirone, granisetron, ondansetron, mosapride, sumatriptan, agomelatine and the like. Also disclosed are 5HTR agents in combination with an opioid agent, such as naltrexone; a PDE agent, such as ibudilast; and/or a GABA agent, such as baclofen or gabapentin.

In some instances when the 5HTR agent is used alone, it cannot be a 5HTR 1a subtype, such as for stimulating or increasing neurogenesis or in treating a nervous system disorder. Preferably a 5HTR3 or 5HTR4 agent is used in stimulating or increasing neurogenesis.

Preferred combinations include azasetron with buspirone, baclofen, captopril, ibudilast, naltrexone, folic acid, methyl folate, gabapentin, methylphenidate, clozapine or carbemazepine. In addition, the combination of agents can be administered in one formulation, or concurrently or sequentially in more than one formulation. A preferred nervous system disorder is depression.

The details of additional embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the embodiments will be apparent from the drawings and detailed description, and from the claims.
BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a dose-response curve showing effect of the neurogenic agent 5-HP (5-hydroxytryptophan, a serotonin precursor) on neuronal differentiation of human neural stem cells. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. EC$_{50}$ was observed at a 5-HP concentration of 12.0 µM in test cells, compared to 4.7 µM for the positive control compound.

FIG. 2 is a dose-response curve showing the astrogenic effect of the neurogenic agent 5-HP on human neural stem cells. Astrocyte differentiation data is presented as the percentage of the astrocyte positive control, with basal media values subtracted. EC$_{50}$ was undeterminable for 5-HP (greater than concentrations tested), compared to 19.9 µM for the positive control compound.

FIG. 3 is a series of immunofluorescent microscopic images of monolayers of human neural stem cells (hNSC) after immunohistochemistry staining with the neuronal marker TUJ-I (green), the astrocyte marker GFAP (red), and a nuclear cell marker (Hoechst 33342 in blue). The upper left image is a negative control (basal media), the upper middle image is a neuronal positive control (basal media plus a known promoter of neuronal differentiation), and the upper right image is an astrocyte positive control (basal media plus a known inducer of astrocyte differentiation). The lower image shows the effect of 10.0 µM 5-HP on hNSC differentiation.

FIG. 4 is a dose-response curve showing effect of the agent buspirone, a 5-HT1A agonist, on neuronal differentiation of human neural stem cells. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. EC$_{50}$ was observed at a buspirone concentration of 22.8 µM in test cells, compared to 4.7 µM for the positive control compound.

FIG. 5 is a dose-response curve showing the astrogenic effect of the agent buspirone, a 5-HT1A agonist, on human neural stem cells. Astrocyte differentiation data is presented as the percentage of the astrocyte positive control, with basal media values subtracted. EC$_{50}$ was observed at a buspirone concentration of 13.24 µM in test cells, compared to 19.9 µM for the positive control compound.

FIG. 6 is a series of immunofluorescent microscopic images of monolayers of human neural stem cells (hNSC) after immunohistochemistry staining with the neuronal marker TUJ-I (green), the astrocyte marker GFAP (red), and a nuclear cell marker (Hoechst
33342 in blue). The upper left image is a negative control (basal media), the upper middle image is a neuronal positive control (basal media plus a known promoter of neuronal differentiation), and the upper right image is an astrocyte positive control (basal media plus a known inducer of astrocyte differentiation). The lower image shows the effect of 31.6 µM buspirone on hNSC differentiation.

FIG. 7 is a dose-response curve showing effect of the agent tandospirone, a 5-HT1A agonist, on neuronal differentiation of human neural stem cells. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. EC_{50} was observed at a tandospirone concentration of 12.05 µM in test cells, compared to 4.7 µM for the positive control compound.

FIG. 8 is a dose-response curve showing the astrogenic effect of the agent tandospirone, a 5-HT1A agonist. Astrocyte differentiation data is presented as the percentage of the astrocyte positive control, with basal media values subtracted. EC_{50} was observed at a tandospirone concentration of 8.69 µM in test cells, compared to 19.9 µM for the positive control compound.

FIG. 9 is a series of immuno fluorescent microscopic images of monolayers of human neural stem cells (hNSC) after immunohistochemistry staining with the neuronal marker TUJ-I (green), the astrocyte marker GFAP (red), and a nuclear cell marker (Hoechst 33342 in blue). The upper left image is a negative control (basal media), the upper middle image is a neuronal positive control (basal media plus a known promoter of neuronal differentiation), and the upper right image is an astrocyte positive control (basal media plus a known inducer of astrocyte differentiation). The lower image shows the effect of 31.6 µM tandospirone on hNSC differentiation.

FIG. 10 shows the effects of fluoxetine, a selective serotonin reuptake inhibitor, or SSRI, on behavior in the Novel Object Recognition (NOR) cognition assay. Data is presented as the mean percentage of the visits to the novel object in FIG. 10A and mean percentage of the number of time spent (same scale) with the novel object in FIG. 10B. The treatment populations were administered vehicle or fluoxetine at 5 and 10 mg/kg. The 10 mg/kg data in each panel are at p < 0.05. Fluoxetine increased both the number of visits to, and the amount of time spent with, the novel object, demonstrating a dose-dependent increase in cognition.

FIG. 11 shows the effects of fluoxetine, a selective serotonin reuptake inhibitors, or SSRI, on behavior in the novelty suppressed feeding (NSF) chronic depression
assay. Data is presented as the latency to eat (or feed), on a scale from 0 to 300 seconds, at intervals of 50 seconds, after treatment with vehicle or 10 mg/kg fluoxetine, with the latter at p < 0.05, upon a novel food.

FIG. 12 shows the effects of buspirone, a 5-HT1A agonist, on behavior in the Novel Object Recognition (NOR) cognition assay. Data is presented as the mean percentage of the visits to the novel object in FIG. 12A and mean percentage of the number of time spent (same scale) with the novel object in FIG. 12B. The treatment populations were administered vehicle or buspirone at 0.5 and 5.0 mg/kg. Buspirone did not demonstrate a significant effect on cognition.

FIG. 13 shows the effects of buspirone, a 5-HT1A agonist, on behavior in the novelty suppressed feeding (NSF) chronic depression assay. Data is presented as the latency to eat (feed), in seconds, upon a novel food. The treatment populations were administered vehicle or buspirone at 0.5 and 5.0 mg/kg. The 0.5 mg/kg data are at p = 0.08. Buspirone did not demonstrate a significant effect on novelty suppressed feeding.

FIG. 14 shows the effects of buspirone, a 5-HT1A agonist, on rat body weight during chronic dosing. Data is presented as the mean body weight in grams per day (vertical axis) starting with on the first day of dosing. Animals treated with buspirone showed a dose-dependent decrease in mean body weight.

FIG. 15 is a dose-response curve showing enhancement of the effects of the agent dopamine on neuronal differentiation of human neural stem cells by combination with a 5-HT1A agonist (5-HTP). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Increased efficacy was observed with a combination of dopamine and 10 µM 5-HTP or 30 µM 5-HTP than with dopamine alone.

FIG. 16 contains representations of the structures of some non-limiting neurogenic sensitizing agents of the invention.

FIG. 17 is a dose-response curve showing effect of the neurogenic agents buspirone (5HT1a receptor agonist) and melatonin (melatonin receptor agonist) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 µM to 31.6 µM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 µM buspirone and 0.01 µM melatonin). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When
used alone, EC$_{50}$ was observed at a buspirone concentration of 9.4 µM or a melatonin concentration of >31.6 µM (estimated based on extrapolation to be approximately at 49.7 µM) in test cells. When used in combination, neurogenesis is greatly enhanced: EC$_{50}$ was observed at a combination of buspirone and melatonin at concentrations of 2.2 µM each.

FIG. 18 is a dose-response curve showing effect of the agents buspirone (5HT1a receptor agonist) and melatonin (melatonin receptor agonist) in combination on astrocyte differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 µM to 31.6 µM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 µM buspirone and 0.01 µM melatonin). Data is presented as the percentage of the astrocyte positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at a buspirone concentration of 5.7 µM or a melatonin concentration of >31.6 µM (no estimation possible to lack of observed effect) in test cells. When used in combination, EC$_{50}$ was greater than all tested concentrations (>31.2 µM) and astrocyte differentiation was reduced from a maximum of 60% with buspirone alone to a maximum of 12% with the combination of buspirone and melatonin.

FIG. 19 shows the effects of buspirone alone, melatonin alone, and the combination of the two drugs on antidepressant activity in the novelty suppressed feeding assay. Male F344 rats were dosed 1x per day for 21-days with 0 (vehicle only), 0.5 mg/kg buspirone (n = 12 per dose group, Lp.), 3.0 mg/kg melatonin (n = 12 per dose group, i.p.) or the combination of the two drugs at the same doses. Behavioral testing was carried out as described in Example 14. Results shown in this figure indicate the mean latency to approach and eat a food pellet within the novel environment. Data are presented as latency to eat expressed as percent baseline. Melatonin or buspirone alone did not significantly reduce the latency to eat the food pellet. The combination of melatonin and buspirone resulted in a significant decrease in latency to eat the food pellet. The data indicate that the combination of buspirone and melatonin at doses that do not produce antidepressant activity when dosed alone, result in significant antidepressant activity when administered in combination.

FIG. 20 shows the effects of buspirone alone, melatonin alone, and the combination of the two drugs on in vivo neurogenesis. Male F344 rats were dosed 1x per day for 28-days with 0 (vehicle only), 0.5 mg/kg buspirone (n = 12 per dose group, i.p.), 3.0 mg/kg melatonin (n = 12 per dose group, ip) or the combination of the two drugs at the same
doses. BrdU was administered once daily between days 9 and 14 (100mg/kg/day, i.p., n=12 per dose group). The results show BrdU positive cell counts within the granule cell layer of the dentate gyrus. Data are presented as percent change in BrdU positive cells per cubic mm dentate gyrus. Melatonin or buspirone alone did not significantly change the number of BrdU positive cells. The combination of melatonin and buspirone resulted in a significant increase in BrdU positive cells compared to vehicle.

FIG. 21 shows the effect of chronic dosing of rats with melatonin, buspirone, or a combination of both agents on body weight (circle: vehicle; triangle: 3.0 mg/kg melatonin; diamond: 10.0 mg/kg melatonin; square: 3.0 mg/kg melatonin + 0.5 mg/kg buspirone). The combination of melatonin and buspirone resulted in decreased body weight compared to vehicle treated animals. Results are presented as the mean body weight over days.

FIG. 22 is a dose-response curve showing effect of the neurogenic agents buspirone (5HT1a receptor agonist) and baclofen (GABA receptor agonist) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 μM to 31.6 μM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 μM buspirone and 0.01 μM baclofen). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, maximal neuronal differentiation was 62% for buspirone and 74% for baclofen. When combined, the maximal neuronal differentiation was 103%.

FIG. 23 is a dose-response curve showing effect of the agents buspirone (5HT1a receptor agonist) and baclofen (GABA receptor agonist) in combination on astroocyte differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 μM to 31.6 μM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 μM buspirone and 0.01 μM baclofen). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC50 was observed at a buspirone concentration of 5.7 μM or a baclofen concentration of >31.6 μM (no estimation possible to lack of observed effect) in test cells. When used in combination, EC50 was greater than all tested concentrations (>31.2 μM) and astrocyte differentiation was reduced from a
maximum of 60% with buspirone alone to a maximum of 14% with the combination of buspirone and baclofen.

FIG. 24 is a dose-response curve showing the effect of azasetron (5HT3 receptor antagonist) (squares) on the differentiation of cultured human neural stem cells (hNSCs) along a neuronal lineage. Background media values are subtracted and data is normalized with respect to a neuronal positive control (circles). Azasetron significantly promoted neuronal differentiation, with an EC$_{50}$ value of approximately 17.8 µM compared to an EC$_{50}$ for the positive neuronal control of approximately 6.3 µM.

FIG. 25 is a dose-response curve showing the effect of azasetron (squares) on the differentiation of cultured human neural stem cells (hNSCs) along an astrocyte lineage. Background media values are subtracted and data is normalized with respect to an astrocyte positive control. Azasetron did not show a significant effect on astrocyte differentiation within the range of concentrations tested (EC50 value greater than highest concentration tested (31.6 µM)). In light of the results shown in Fig. 24, azasetron preferentially promotes differentiation of hNSCs along a neuronal lineage.

FIG. 26 is dose-response curve showing the effect of azasetron (squares) on the cell count of cultured human neural stem cells (hNSCs). Data is shown as a percent of the basal media cell count. Toxic doses typically cause a reduction of the basal cell count below 80%. Azasetron had no detectable toxicity at concentrations up to 31.6 µM.

FIG. 27 is an immunofluorescent microscopic image of a monolayer of human neural stem cells (hNSC) incubated with 30 µM azasetron and stained with the neuronal marker TUJ-I, the astrocyte marker GFAP, and a nuclear cell marker (Hoechst 33342). The hNSC comprising the monolayer are primarily of a neuronal lineage.

FIG. 28 is a full eight (8) point concentration-response curve showing effects of the 5-HT3 receptor antagonist, azasetron, on neuronal differentiation of human neural stem cells. Azasetron was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Azasetron induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation percent of positive control 57% with an EC$_{50}$ of 4.4 µM.

FIG. 29 is a dose-response curve showing effect of the 5-HT3 receptor antagonist, granisetron, on neuronal differentiation of human neural stem cells. Granisetron was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is
presented as the percentage of the neuronal positive control, with basal media values subtracted. Granisetron induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation 73% of positive control with an EC$_{50}$ of 1.2 μM.

FIG. 30 is a dose-response curve showing effect of the 5-HT3 receptor antagonist, ondansetron, on neuronal differentiation of human neural stem cells. Ondansetron was tested in a concentration response curves ranging from 0.01 μM to 31.6 μM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Ondansetron induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation 60% of positive control with an EC$_{50}$ of 23.9 μM.

FIG. 31 is a dose-response curve showing the effect of mosapride citrate (5-HT4 receptor agonist) (squares) on the differentiation of cultured human neural stem cells (hNSCs) along a neuronal lineage. Background media values are subtracted and data is normalized with respect to a neuronal positive control (circles). Mosapride citrate significantly promoted neuronal differentiation, with an EC50 value of approximately 15.8 μM, compared to an EC$_{50}$ for the positive neuronal control of approximately 6.3 μM.

FIG. 32 is a dose-response curve showing the effect of mosapride citrate (squares) on the differentiation of cultured human neural stem cells (hNSCs) along an astrocyte lineage. Background media values are subtracted and data is normalized with respect to an astrocyte positive control. Mosapride citrate did not show a significant effect on astrocyte differentiation within the range of concentrations tested (EC$_{50}$ value greater than highest concentration tested (31.6 μM)). In light of the results shown in Fig. 31, mosapride citrate preferentially promotes differentiation of hNSCs along a neuronal lineage.

FIG. 33 is dose-response curve showing the effect of mosapride citrate (squares) on the cell count of cultured human neural stem cells (hNSCs). Data is shown as a percent of the basal media cell count. Toxic doses typically cause a reduction of the basal cell count below 80%. Mosapride citrate had no detectable toxicity at concentrations up to 31.6 μM.

FIG. 34 is an immunofluorescent microscopic image of a monolayer of human neural stem cells (hNSC) incubated with 30 μM mosapride citrate and stained with the neuronal marker TUJ-I, the astrocyte marker GFAP, and a nuclear cell marker (Hoechst 33342). The hNSC comprising the monolayer are primarily of a neuronal lineage.
FIG. 35 is a full eight (8) point concentration-response curve showing effects of the 5-HT4 receptor agonist, mosapride citrate, on neuronal differentiation of human neural stem cells. Mosapride was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Mosapride induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation 92% of positive control with an EC₅₀ of 10.4 µM.

FIG. 36 is a dose-response curve showing effect of the 5-HT4 receptor agonist, cisapride, on neuronal differentiation of human neural stem cells. Cisapride was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Cisapride induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation 56% of positive control with an EC₅₀ of 7.9 µM.

FIG. 37 is a dose-response curve showing effect of the 5-HT1B/1D receptor agonist, sumatriptan, on neuronal differentiation of human neural stem cells. Sumatriptan was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Sumatriptan induced neuronal differentiation in a concentration dependent manner with a maximum neuronal differentiation 76% of positive control with an EC₅₀ of 14.9 µM.

FIG. 38 is a dose-response curve showing effect of the neurogenic agent, agomelatine (reported melatonin agonist and 5-HT2B/2C antagonist), on neuronal differentiation. Agomelatine was tested in a concentration response curves ranging from 0.01 µM to 31.6 µM. Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. Agomelatine showed a maximum neuronal differentiation 42% of positive control with an EC₅₀ of 8.7 µM.

FIG. 39 is a dose-response curve showing effect of the neurogenic agents buspirone (5HT1a receptor agonist) and modafinil in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM buspirone and 0.01 uM modafinil). Data is presented as the percentage of the neuronal
positive control, with basal media values subtracted. When used alone, EC₅₀ was observed at a buspirone concentration of 9.4 uM or a modafinil concentration of 12.5 uM in test cells. When used in combination, neurogenesis is maintained with EC₅₀ observed at a combination of buspirone and modafinil at concentrations of 4.5 uM each.

FIG. 40 is a dose-response curve showing effect of the agents buspirone (5HT₁a receptor agonist) and modafinil in combination on astrocyte differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM buspirone and 0.01 uM modafinil). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC₅₀ was observed at a buspirone concentration of 5.7 uM or a modafinil concentration of >31.6 uM in test cells. When used in combination, EC₅₀ was greater than all tested concentrations (>31.2 uM) and astrocyte differentiation was reduced from a maximum of 60% with buspirone alone to a maximum of 28% with the combination of buspirone and modafinil.

FIG. 41 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT₃ receptor antagonist) and buspirone (5HT₁a receptor agonist) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM buspirone). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC₅₀ was observed at an azasetron concentration of 5.8 uM or a buspirone concentration of 9.4 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC₅₀ was observed at a combination of azasetron and buspirone at concentrations of 0.6 uM each, resulting a synergistic combination index of 0.18.

FIG. 42 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT₃ receptor antagonist) and baclofen (GABA receptor agonist) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at
equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM baclofen). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or a baclofen concentration of 3.9 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC$_{50}$ was observed at a combination of azasetron and baclofen at concentrations of 0.19 uM each, resulting a synergistic combination index of 0.08.

FIG. 43 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and captopril (ACE inhibitor) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM captopril). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or a captopril concentration of 5.4 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC$_{50}$ was observed at a combination of azasetron and captopril at concentrations of 1.2 uM each, resulting a synergistic combination index of 0.47.

FIG. 44 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and ibudilast (PDE inhibitor) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, azasetron was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM, and ibudilast was tested in a response curve ranging from 0.003 - 10.0 uM. In combination, the compounds were combined at a 1:3.16 ratio at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.003 uM ibudilast). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or an ibudilast concentration of 0.72 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC$_{50}$ was observed at a combination of azasetron and ibudilast at concentrations of 0.1 uM each, resulting a synergistic combination index of 0.17.
FIG. 45 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and naltrexone (mixed opioid antagonist) in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM naltrexone). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or a naltrexone concentration of 0.39 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC$_{50}$ was observed at a combination of azasetron and naltrexone at concentrations of 0.16 uM each, resulting a synergistic combination index of 0.45.

FIG. 46 is a dose-response curve showing effect of the agents azasetron (5HT3 receptor antagonist) and naltrexone (mixed opioid antagonist) in combination on astrocyte differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM naltrexone). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used in combination astrocyte differentiation was reduced from a maximum of 33% with azasetron alone to a maximum of 5% with the combination of azasetron and naltrexone.

FIG. 47 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and folic acid in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM folic acid). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or a folic acid concentration of 4.5 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC$_{50}$ was observed at a
combination of azasetron and folic acid at concentrations of 0.19 uM each, resulting a
synergistic combination index of 0.08.

FIG. 48 is a dose-response curve showing effect of the agents azasetron (5HT3
receptor antagonist) and folic acid in combination on astrocyte differentiation of human
neural stem cells compared to the effect of either agent alone. When run independently, each
compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM.
In combination, the compounds were combined at equal concentrations at each point (for
example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and
0.01 uM folic acid). Data is presented as the percentage of the neuronal positive control, with
basal media values subtracted. When used in combination astrocyte differentiation was
reduced from a maximum of 33% with azasetron alone to a maximum of 8% with the
combination of azasetron and folic acid.

FIG. 49 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and gabapentin in combination on neuronal
differentiation of human neural stem cells compared to the effect of either agent alone. When
run independently, each compound was tested in a concentration response curve ranging from
0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations
at each point (for example, the first point in the combined curve consisted of a test of 0.01
uM azasetron and 0.01 uM gabapentin). Data is presented as the percentage of the neuronal
positive control, with basal media values subtracted. When used alone, EC_{50} was observed at
an azasetron concentration of 5.8 uM or a gabapentin concentration of 11.5 uM in test cells.
When used in combination, neurogenesis is greatly enhanced. EC_{50} was observed at a
combination of azasetron and gabapentin at concentrations of 0.9 uM each, resulting a
synergistic combination index of 0.24.

FIG. 50 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and methylphenidate (Ritalin®) in combination on
neuronal differentiation of human neural stem cells compared to the effect of either agent
alone. When run independently, azasetron was tested in a concentration response curve
ranging from 0.01 uM to 31.6 uM, and methylphenidate was tested in a response curve
ranging from 0.003 - 10.0 uM. In combination, the compounds were combined at a 1:3.16
ratio at each point (for example, the first point in the combined curve consisted of a test of
0.01 uM azasetron and 0.003 uM methylphenidate). Data is presented as the percentage of
the neuronal positive control, with basal media values subtracted. When used alone, EC_{50}
was observed at an azasetron concentration of 5.8 uM or a methylphenidate concentration of 2.2 uM in test cells. When used in combination, neurogenesis is greatly enhanced: EC$_{50}$ was observed at a combination of azasetron and methylphenidate at concentrations of 0.09 uM each, resulting a synergistic combination index of 0.06.

FIG. 51 is a dose-response curve showing effect of the agents azasetron (5HT3 receptor antagonist) and methylphenidate (Ritalin®) in combination on astrocyte differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, azasetron was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM, and methylphenidate was tested in a response curve ranging from 0.003 - 10.0 uM. In combination, the compounds were combined at a 1:3.16 ratio at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.003 uM methylphenidate). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used in combination astrocyte differentiation was reduced from a maximum of 33% with azasetron alone to a maximum of 4% with the combination of azasetron and methylphenidate.

FIG. 52 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and clozapine in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01 uM azasetron and 0.01 uM clozapine). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC$_{50}$ was observed at an azasetron concentration of 5.8 uM or a clozapine concentration of 3.6 uM in test cells. When used in combination, neurogenesis is greatly enhanced: EC$_{50}$ was observed at a combination of azasetron and clozapine at concentrations of 0.132 uM each, resulting a synergistic combination index of 0.06.

FIG. 53 is a dose-response curve showing effect of the neurogenic agents azasetron (5HT3 receptor antagonist) and carbemazepine in combination on neuronal differentiation of human neural stem cells compared to the effect of either agent alone. When run independently, each compound was tested in a concentration response curve ranging from 0.01 uM to 31.6 uM. In combination, the compounds were combined at equal concentrations at each point (for example, the first point in the combined curve consisted of a test of 0.01
uM azasetron and 0.01 uM carbemazepine). Data is presented as the percentage of the neuronal positive control, with basal media values subtracted. When used alone, EC_{50} was observed at an azasetron concentration of 5.8 uM or a carbemazepine concentration of 15.7 uM in test cells. When used in combination, neurogenesis is greatly enhanced. EC_{50} was observed at a combination of azasetron and carbemazepine at concentrations of 0.37 uM each, resulting a synergistic combination index of 0.09.

DEFINITIONS

"Neurogenesis" is defined herein as proliferation, differentiation, migration and/or survival of a neural cell in vivo or in vitro. In some embodiments, the neural cell is an adult, fetal, or embryonic neural stem cell or population of cells. The cells may be located in the central nervous system or elsewhere in an animal or human being. The cells may also be in a tissue, such as neural tissue, in some embodiments, the neural cell is an adult, fetal, or embryonic progenitor cell or population of cells, or a population of cells comprising a mixture of stem cells and progenitor cells. Neural cells include all brain stem cells, all brain progenitor cells, and all brain precursor cells. Neurogenesis includes neurogenesis as it occurs during normal development, as well as neural regeneration that occurs following disease, damage or therapeutic intervention, such as by the treatment described herein.

A "neurogenic agent" is defined as a chemical or biological agent or reagent that can promote, stimulate, or otherwise increase the amount or degree or nature of neurogenesis in vivo or ex vivo or in vitro relative to the amount, degree, or nature of neurogenesis in the absence of the agent or reagent. In some embodiments, treatment with a neurogenic agent increases neurogenesis if it promotes neurogenesis by at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 100%, at least about 500%, or more in comparison to the amount, degree, and/or nature of neurogenesis in the absence of the agent, under the conditions of the method used to detect or determine neurogenesis.

The term "astrogenic" is defined in relation to "astrogenesis" which refers to the activation, proliferation, differentiation, migration and/or survival of an astrocytic cell in vivo or in vitro. Non-limiting examples of astrocytic cells include astrocytes, activated microglial cells, astrocyte precursors and potentiating cells, and astrocyte progenitor and derived cells. In some embodiments, the astrocyte is an adult, fetal, or embryonic astrocyte.
or population of astrocytes. The astrocytes may be located in the central nervous system or elsewhere in an animal or human being. The astrocytes may also be in a tissue, such as neural tissue. In some embodiments, the astrocyte is an adult, fetal, or embryonic progenitor cell or population of cells, or a population of cells comprising a mixture of stem and/or progenitor cells, that is/are capable of developing into astrocytes. Astrogenesis includes the proliferation and/or differentiation of astrocytes as it occurs during normal development, as well as astrogenesis that occurs following disease, damage or therapeutic intervention.

The term "stem cell" (or neural stem cell (NSC)), as used herein, refers to an undifferentiated cell that is capable of self-renewal and differentiation into neurons, astrocytes, and/or oligodendrocytes.

The term "progenitor cell" (e.g., neural progenitor cell), as used herein, refers to a cell derived from a stem cell that is not itself a stem cell. Some progenitor cells can produce progeny that are capable of differentiating into more than one cell type.

The terms "animal" or "animal subject" refers to a non-human mammal, such as a primate, canine, or feline. In other embodiments, the terms refer to an animal that is domesticated (e.g. livestock) or otherwise subject to human care and/or maintenance (e.g. zoo animals and other animals for exhibition). In other non-limiting examples, the terms refer to ruminants or carnivores, such as dogs, cats, birds, horses, cattle, sheep, goats, marine animals and mammals, penguins, deer, elk, and foxes.

The term "5HTR agent" as used herein includes a neurogenic agent, as defined herein, that elicits an observable response upon contacting a 5HT receptor, such as one or more of the subtypes described herein. "5HTR agents" useful in the methods described herein include compounds or agents that, under certain conditions, may act as: agonists (i.e., agents able to elicit one or more biological responses of a 5HT receptor); partial agonists (i.e., agents able to elicit one or more biological responses of a 5HT receptor to a less than maximal extent, e.g., as defined by the response of the receptor to an agonist); antagonists (agents able to inhibit one or more characteristic responses of a 5HT receptor, for example, by competitively or non-competitively binding to the 5HT receptor, a ligand of the receptor, and/or a downstream signaling molecule); and/or inverse agonists (agents able to block or inhibit a constitutive activity of a 5HT receptor) of one or more subtypes of the 5HT receptor.

In some embodiments, the 5HTR agent(s) used in the methods described herein has "selective" activity under certain conditions against one or more 5HT receptor subtypes with respect to the degree and/or nature of activity against one or more other 5HT
receptor subtypes. For example, in some embodiments, the 5HTR agent has an agonist effect against one or more subtypes, and a much weaker effect or substantially no effect against other subtypes. As another example, a 5HTR agent used in the methods described herein may act as an agonist at one or more 5HT receptor subtypes and as antagonist at one or more other 5HT receptor subtypes. In some embodiments, 5HTR agents have activity against one 5HT receptor subtype, while having substantially lesser activity against one or more other 5HT receptor subtypes. In certain embodiments, selective activity of one or more 5HT receptor agonists, or antagonists, results in enhanced efficacy, fewer side effects, lower effective dosages, less frequent dosing, or other desirable attributes.

In some embodiments, the 5HTR agent(s) used in the compositions and methods described herein are substantially inactive with respect to other receptors (i.e., non-5HT receptor), such as muscarinic receptors, dopamine receptors, epinephrine receptors, histamine receptors, glutamate receptors, and the like. However, in other embodiments, 5HTR agent(s) are active against one or more additional receptor subtypes. For example, the reported 5HTR agent, agomelatine, has antagonist properties with respect to 5-HT2B/2C receptors and agonist properties with respect to MT1 and MT2 melatonin receptors under certain conditions.

In additional embodiments, a 5HTR agent as used herein includes a neurogenesis modulating agent, as defined herein, that elicits an observable neurogenic response by producing, generating, stabilizing, or increasing the retention of an intermediate agent which, when contacted with a 5HT receptor agent, results in the neurogenic response. As used herein, "increasing the retention of" or variants of that phrase or the term "retention" refer to decreasing the degradation of, or increasing the stability of, an intermediate agent.

In some cases, a 5HTR agent, optionally in combination with one or more other neurogenic agents, results in improved efficacy, fewer side effects, lower effective dosages, less frequent dosing, and/or other desirable effects relative to use of the neurogenesis modulating agents individually (such as at higher doses), due, e.g., to synergistic activities and/or the targeting of molecules and/or activities that are differentially expressed in particular tissues and/or cell-types.

The term "neurogenic combination of a 5HTR agent with one or more other neurogenic agents" refers to a combination of neurogenesis modulating agents. In some embodiments, administering a neurogenic, or neuromodulating, combination according to methods provided herein modulates neurogenesis in a target tissue and/or cell-type by at least
about 20%, about 25%, about 30%, about 40%, about 50%, at least about 75%, or at least about 90% or more in comparison to the absence of the combination. In further embodiments, neurogenesis is modulated by at least about 95% or by at least about 99% or more.

A neuromodulating combination may be used to inhibit a neural cell's proliferation, division, or progress through the cell cycle. Alternatively, a neuromodulating combination may be used to stimulate survival and/or differentiation in a neural cell. As an additional alternative, a neuromodulating combination may be used to inhibit, reduce, or prevent astrocyte activation and/or astrogenesis or astrocyte differentiation.

"IC₅₀" and "EC₅₀" values are concentrations of an agent, in a combination of a 5HTR agent with one or more other neurogenic agents, that reduce and promote, respectively, neurogenesis or another physiological activity (e.g., the activity of a receptor) to a half-maximal level. IC₅₀ and EC₅₀ values can be assayed in a variety of environments, including cell-free environments, cellular environments (e.g., cell culture assays), multicellular environments (e.g., in tissues or other multicellular structures), and/or in vivo. In some embodiments, one or more neurogenesis modulating agents in a combination or method disclosed herein individually have IC₅₀ or EC₅₀ values of less than about 10 µM, less than about 1 µM, or less than about 0.1 µM or lower. In other embodiments, an agent in a combination has an IC₅₀ or EC₅₀ of less than about 50 nM, less than about 10 nM, or less than about 1 nM, less than about 0.1 nM or lower.

In some embodiments, selectivity of one or more agents, in a combination of a 5HTR agent with one or more other neurogenic agents, is individually measured as the ratio of the IC₅₀ or EC₅₀ value for a desired effect (e.g., modulation of neurogenesis) relative to the IC₅₀/EC₅₀ value for an undesired effect. In some embodiments, a "selective" agent in a combination has a selectivity of less than about 1:2, less than about 1:10, less than about 1:50, or less than about 1:100. In some embodiments, one or more agents in a combination individually exhibits selective activity in one or more organs, tissues, and/or cell types relative to another organ, tissue, and/or cell type. For example, in some embodiments, an agent in a combination selectively modulates neurogenesis in a neurogenic region of the brain, such as the hippocampus (e.g., the dentate gyrus), the subventricular zone, and/or the olfactory bulb.

In other embodiments, modulation by a combination of agents is in a region containing neural cells affected by disease or injury, region containing neural cells associated
with disease effects or processes, or region containing neural cells affect other event injurious
to neural cells. Non-limiting examples of such events include stroke or radiation therapy of
the region. In additional embodiments, a neuromodulating combination substantially
modulates two or more physiological activities or target molecules, while being substantially
inactive against one or more other molecules and/or activities.

The term "cognitive function" refers to mental processes of an animal or
human subject relating to information gathering and/or processing; the understanding,
reasoning, and/or application of information and/or ideas; the abstraction or specification of
ideas and/or information; acts of creativity, problem-solving, and possibly intuition; and
mental processes such as learning, perception, and/or awareness of ideas and/or information.
The mental processes are distinct from those of beliefs, desires, and the like. In some
embodiments, cognitive function may be assessed, and thus optionally defined, via one or
more tests or assays for cognitive function. Non-limiting examples of a test or assay for
cognitive function include CANTAB (see for example Fray et al. "CANTAB battery:
proposed utility in neurotoxicology." Neurotoxicol Teratol. 1996; 18(4):499-504), Stroop
Test, Trail Making, Wechsler Digit Span, or the CogState computerized cognitive test (see
also Dehaene et al. "Reward-dependent learning in neuronal networks for planning and
decision making." Prog Brain Res. 2000;126:217-29; Iverson et al. "Interpreting change on
the WAIS-III/WMS-III in clinical samples." Arch Clin Neuropsychol. 2001;16(2):183-91;
and Weaver et al. "Mild memory impairment in healthy older adults is distinct from normal

DETAILED DESCRIPTION OF MODES OF PRACTICING THE DISCLOSURE

General

Methods described herein can be used to treat any disease or condition for
which it is beneficial to promote or otherwise stimulate or increase neurogenesis. One focus
of the methods described herein is to achieve a therapeutic result by stimulating or increasing
neurogenesis via modulation of 5HT receptor activity. Thus, certain methods described
herein can be used to treat any disease or condition susceptible to treatment by increasing
neurogenesis.

In some embodiments, a disclosed method is applied to modulating
neurogenesis in vivo, in vitro, or ex vivo. In in vivo embodiments, the cells may be present in
a tissue or organ of a subject animal or human being. Non-limiting examples of cells include those capable of neurogenesis, such as to result, whether by differentiation or by a combination of differentiation and proliferation, in differentiated neural cells. As described herein, neurogenesis includes the differentiation of neural cells along different potential lineages. In some embodiments, the differentiation of neural stem or progenitor cells is along a neuronal cell lineage to produce neurons. In other embodiments, the differentiation is along both neuronal and glial cell lineages. In additional embodiments, the disclosure further includes differentiation along a neuronal cell lineage to the exclusion of one or more cell types in a glial cell lineage. Non-limiting examples of glial cell types include oligodendrocytes and radial glial cells, as well as astrocytes, which have been reported as being of an "astroglial lineage". Therefore, embodiments of the disclosure include differentiation along a neuronal cell lineage to the exclusion of one or more cell types selected from oligodendrocytes, radial glial cells, and astrocytes.

In applications to an animal or human being, the disclosure includes a method of bringing cells into contact with a 5HTR agent, optionally in combination with one or more other neurogenic agents, in effective amounts to result in an increase in neurogenesis in comparison to the absence of the agent or combination. A non-limiting example is in the administration of the agent or combination to the animal or human being. Such contacting or administration may also be described as exogenously supplying the combination to a cell or tissue.

Embodiments of the disclosure include a method to treat, or lessen the level of, a decline or impairment of cognitive function. Also included is a method to treat a mood disorder. In additional embodiments, a disease or condition treated with a disclosed method is associated with pain and/or addiction, but in contrast to known methods, the disclosed treatments are substantially mediated by increasing neurogenesis. As a further non-limiting example, a method described herein may involve increasing neurogenesis ex vivo, such that a composition containing neural stem cells, neural progenitor cells, and/or differentiated neural cells can subsequently be administered to an individual to treat a disease or condition.

In further embodiments, methods described herein allow treatment of diseases characterized by pain, addiction, and/or depression by directly replenishing, replacing, and/or supplementing neurons and/or glial cells. In further embodiments, methods described herein enhance the growth and/or survival of existing neural cells, and/or slow or reverse the loss of such cells in a neurodegenerative condition.
Where a method comprises contacting a neural cell with a 5HTR agent, the result may be an increase in neurodifferentiation. The method may be used to potentiate a neural cell for proliferation, and thus neurogenesis, via the one or more other agents used with the 5HTR agent in combination. Thus the disclosure includes a method of maintaining, stabilizing, stimulating, or increasing neurodifferentiation in a cell or tissue by use of a 5HTR agent, optionally in combination with one or more other neurogenic agents that also increase neurodifferentiation. The method may comprise contacting a cell or tissue with a 5HTR agent, optionally in combination with one or more other neurogenic agents, to maintain, stabilize, stimulate, or increase neurodifferentiation in the cell or tissue.

The disclosure also includes a method comprising contacting the cell or tissue with a 5HTR agent in combination with one or more other neurogenic agents where the combination stimulates or increases proliferation or cell division in a neural cell. The increase in neuroproliferation may be due to the one or more other neurogenic agents and/or to the 5HTR agent. In some cases, a method comprising such a combination may be used to produce neurogenesis (in this case both neurodifferentiation and/or proliferation) in a population of neural cells. In some embodiments, the cell or tissue is in an animal subject or a human patient as described herein. Non-limiting examples include a human patient treated with chemotherapy and/or radiation, or other therapy or condition which is detrimental to cognitive function; or a human patient diagnosed as having epilepsy, a condition associated with epilepsy, or seizures associated with epilepsy.

Administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents, may be before, after, or concurrent with, another agent, condition, or therapy. In some embodiments, the overall combination may be of a 5HTR agent, optionally in combination with one or more other neurogenic agents.

Uses of a 5HTR agent

Embodiments include a method of modulating neurogenesis by contacting one or more neural cells with a 5HTR agent, optionally in combination with one or more other neurogenic agents. The amount of a 5HTR agent, or a combination thereof with one or more other neurogenic agents, may be selected to be effective to produce an improvement in a treated subject, or detectable neurogenesis in vitro. In some embodiments, the amount is one that also minimizes clinical side effects seen with administration of the inhibitor to a subject.
**Cognitive Function**

In other embodiments, and if compared to a reduced level of cognitive function, a method of the invention may be for enhancing or improving the reduced cognitive function in a subject or patient. The method may comprise administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject or patient to enhance, or improve a decline or decrease, of cognitive function due to a therapy and/or condition that reduces cognitive function. Other methods of the disclosure include treatment to affect or maintain the cognitive function of a subject or patient. In some embodiments, the maintenance or stabilization of cognitive function may be at a level, or thereabouts, present in a subject or patient in the absence of a therapy and/or condition that reduces cognitive function. In alternative embodiments, the maintenance or stabilization may be at a level, or thereabouts, present in a subject or patient as a result of a therapy and/or condition that reduces cognitive function.

In further embodiments, and if compared to a reduced level of cognitive function due to a therapy and/or condition that reduces cognitive function, a method of the invention may be for enhancing or improving the reduced cognitive function in a subject or patient. The method may comprise administering a 5HTR agent, or a combination thereof with one or more other neurogenic agents, to a subject or patient to enhance or improve a decline or decrease of cognitive function due to the therapy or condition. The administering may be in combination with the therapy or condition.

These methods optionally include assessing or measuring cognitive function of the subject or patient before, during, and/or after administration of the treatment to detect or determine the effect thereof on cognitive function. So in one embodiment, a methods may comprise i) treating a subject or patient that has been previously assessed for cognitive function and ii) reassessing cognitive function in the subject or patient during or after the course of treatment. The assessment may measure cognitive function for comparison to a control or standard value (or range) in subjects or patients in the absence of a 5HTR agent, or a combination thereof with one or more other neurogenic agents. This may be used to assess the efficacy of the 5HTR agent, alone or in a combination, in alleviating the reduction in cognitive function.
Mood disorders

In other embodiments, a disclosed method may be used to moderate or alleviate a mood disorder in a subject or patient as described herein. Thus the disclosure includes a method of treating a mood disorder in such a subject or patient. Non-limiting examples of the method include those comprising administering a 5HTR agent, or a combination thereof with one or more other neurogenic agents, to a subject or patient that is under treatment with a therapy and/or condition that results in a mood disorder. The administration may be with any combination and/or amount that is effective to produce an improvement in the mood disorder.

Representative and non-limiting mood disorders are described herein. Non-limiting examples of mood disorders include depression, anxiety, hypomania, panic attacks, excessive elation, seasonal mood (or affective) disorder, schizophrenia and other psychoses, lissencephaly syndrome, anxiety syndromes, anxiety disorders, phobias, stress and related syndromes, aggression, non-senile dementia, post-pain depression, and combinations thereof.

Identification of Subjects and Patients

The disclosure includes methods comprising identification of an individual suffering from one or more disease, disorders, or conditions, or a symptom thereof, and administering to the subject or patient a 5HTR agent, optionally in combination with one or more other neurogenic agents, as described herein. The identification of a subject or patient as having one or more disease, disorder or condition, or a symptom thereof, may be made by a skilled practitioner using any appropriate means known in the field.

In some embodiments, identification of a patient in need of neurogenesis modulation comprises identifying a patient who has or will be exposed to a factor or condition known to inhibit neurogenesis, including but not limited to, stress, aging, sleep deprivation, hormonal changes (e.g., those associated with puberty, pregnancy, or aging (e.g., menopause), lack of exercise, lack of environmental stimuli (e.g., social isolation), diabetes and drugs of abuse (e.g., alcohol, especially chronic use; opiates and opioids; psychostimulants). In some cases, the patient has been identified as non-responsive to treatment with primary medications for the condition(s) targeted for treatment (e.g., non-responsive to antidepressants for the treatment of depression), and a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered in a method for
enhancing the responsiveness of the patient to a co-existing or pre-existing treatment regimen.

In other embodiments, the method or treatment comprises administering a combination of a primary medication or therapy for the condition(s) targeted for treatment and a 5HTR agent, optionally in combination with one or more other neurogenic agents. For example, in the treatment of depression or related neuropsychiatric disorders, a combination may be administered in conjunction with, or in addition to, electroconvulsive shock treatment, a monoamine oxidase modulator, and/or a selective reuptake modulators of serotonin and/or norepinephrine.

In additional embodiments, the patient in need of neurogenesis modulation suffers from premenstrual syndrome, post-partum depression, or pregnancy-related fatigue and/or depression, and the treatment comprises administering a therapeutically effective amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents. Without being bound by any particular theory, and offered to improve understanding of the invention, it is believed that levels of steroid hormones, such as estrogen, are increased during the menstrual cycle during and following pregnancy, and that such hormones can exert a modulatory effect on neurogenesis.

In some embodiments, the patient is a user of a recreational drug including, but not limited to, alcohol, amphetamines, PCP, cocaine, and opiates. Without being bound by any particular theory, and offered to improve understanding of the invention, it is believed that some drugs of abuse have a modulatory effect on neurogenesis, which is associated with depression, anxiety and other mood disorders, as well as deficits in cognition, learning, and memory. Moreover, mood disorders are causative/risk factors for substance abuse, and substance abuse is a common behavioral symptom (e.g., self medicating) of mood disorders.

Thus, substance abuse and mood disorders may reinforce each other, rendering patients suffering from both conditions non-responsive to treatment. Thus, in some embodiments, a 5HTR agent, optionally in combination with one or more other neurogenic agents, to treat patients suffering from substance abuse and/or mood disorders. In additional embodiments, the 5HTR agent, optionally in combination with one or more other neurogenic agents, can used in combination with one or more additional agents selected from an antidepressant, an antipsychotic, a mood stabilizer, or any other agent known to treat one or more symptoms exhibited by the patient. In some embodiments, a 5HTR agent exerts a synergistic effect with
the one or more additional agents in the treatment of substance abuse and/or mood disorders in patients suffering from both conditions.

In further embodiments, the patient is on a co-existing and/or pre-existing treatment regimen involving administration of one or more prescription medications having a modulatory effect on neurogenesis. For example, in some embodiments, the patient suffers from chronic pain and is prescribed one or more opiate/opioid medications; and/or suffers from ADD, ADHD, or a related disorder, and is prescribed a psychostimulant, such as Ritalin®, dexedrine, adderall, or a similar medication which inhibits neurogenesis. Without being bound by any particular theory, and offered to improve understanding of the invention, it is believed that such medications can exert a modulatory effect on neurogenesis, leading to depression, anxiety and other mood disorders, as well as deficits in cognition, learning, and memory. Thus, in some preferred embodiments, a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered to a patient who is currently or has recently been prescribed a medication that exerts a modulatory effect on neurogenesis, in order to treat depression, anxiety, and/or other mood disorders, and/or to improve cognition.

In additional embodiments, the patient suffers from chronic fatigue syndrome; a sleep disorder; lack of exercise (e.g., elderly, infirm, or physically handicapped patients); and/or lack of environmental stimuli (e.g., social isolation); and the treatment comprises administering a therapeutically effective amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents.

In more embodiments, the patient is an individual having, or who is likely to develop, a disorder relating to neural degeneration, neural damage and/or neural demyelination.

In further embodiments, a subject or patient includes human beings and animals in assays for behavior linked to neurogenesis. Exemplary human and animal assays are known to the skilled person in the field.

In yet additional embodiments, identifying a patient in need of neurogenesis modulation comprises selecting a population or sub-population of patients, or an individual patient, that is more amenable to treatment and/or less susceptible to side effects than other patients having the same disease or condition, hi some embodiments, identifying a patient amenable to treatment with a 5HTR agent, optionally in combination with one or more other neurogenic agents, comprises identifying a patient who has been exposed to a factor known to enhance neurogenesis, including but not limited to, exercise, hormones or other
endogenous factors, and drugs taken as part of a pre-existing treatment regimen. In some embodiments, a sub-population of patients is identified as being more amenable to neurogenesis modulation with a 5HTR agent, optionally in combination with one or more other neurogenic agents, by taking a cell or tissue sample from prospective patients, isolating and culturing neural cells from the sample, and determining the effect of the combination on the degree or nature of neurogenesis of the cells, thereby allowing selection of patients for which the therapeutic agent has a substantial effect on neurogenesis. Advantageously, the selection of a patient or population of patients in need of or amenable to treatment with a 5HTR agent, optionally in combination with one or more other neurogenic agents, of the disclosure allows more effective treatment of the disease or condition targeted for treatment than known methods using the same or similar compounds.

In some embodiments, the patient has suffered a CNS insult, such as a CNS lesion, a seizure (e.g., electroconvulsive seizure treatment; epileptic seizures), radiation, chemotherapy and/or stroke or other ischemic injury. Without being bound by any particular theory, and offered to improve understanding of the invention, it is believed that some CNS insults/injuries leads to increased proliferation of neural stem cells, but that the resulting neural cells form aberrant connections which can lead to impaired CNS function and/or diseases, such as temporal lobe epilepsy. In other embodiments, a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered to a patient who has suffered, or is at risk of suffering, a CNS insult or injury to stimulate neurogenesis. Advantageously, stimulation of the differentiation of neural stem cells with a 5HTR agent, optionally in combination with one or more other neurogenic agents, activates signaling pathways necessary for progenitor cells to effectively migrate and incorporate into existing neural networks or to block inappropriate proliferation.

_Opialte or Opioid Based Analgesic_

Additionally, the disclosed methods provide for the application of a 5HTR agent, optionally in combination with one or more other neurogenic agents, to treat a subject or patient for a condition due to the anti-neurogenic effects of an opiate or opioid based analgesic. In some embodiments, the administration of an opiate or opioid based analgesic, such as an opiate like morphine or other opioid receptor agonist, to a subject or patient results in a decrease in, or inhibition of, neurogenesis. The administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents, with an opiate or opioid
based analgesic would reduce the anti-neurogenic effect. One non-limiting example is administration of such a combination with an opioid receptor agonist after surgery (such as for the treating post-operative pain).

Also the disclosed embodiments include a method of treating post operative pain in a subject or patient by combining administration of an opiate or opioid based analgesic with a 5HTR agent, optionally in combination with one or more other neurogenic agents. The analgesic may have been administered before, simultaneously with, or after the combination. In some cases, the analgesic or opioid receptor agonist is morphine or another opiate.

Other disclosed embodiments include a method to treat or prevent decreases in, or inhibition of, neurogenesis in other cases involving use of an opioid receptor agonist. The methods comprise the administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents, as described herein. Non-limiting examples include cases involving an opioid receptor agonist, which decreases or inhibits neurogenesis, and drug addiction, drug rehabilitation, and/or prevention of relapse into addiction. In some embodiments, the opioid receptor agonist is morphine, opium or another opiate.

In further embodiments, the disclosure includes methods to treat a cell, tissue, or subject which is exhibiting decreased neurogenesis or increased neurodegeneration. In some cases, the cell, tissue, or subject is, or has been, subjected to, or contacted with, an agent that decreases or inhibits neurogenesis. One non-limiting example is a human subject that has been administered morphine or other agent which decreases or inhibits neurogenesis. Non-limiting examples of other agents include opiates and opioid receptor agonists, such as mu receptor subtype agonists, that inhibit or decrease neurogenesis.

Thus in additional embodiments, the methods may be used to treat subjects having, or diagnosed with, depression or other withdrawal symptoms from morphine or other agents which decrease or inhibit neurogenesis. This is distinct from the treatment of subjects having, or diagnosed with, depression independent of an opiate, such as that of a psychiatric nature, as disclosed herein. In further embodiments, the methods may be used to treat a subject with one or more chemical addiction or dependency, such as with morphine or other opiates, where the addiction or dependency is ameliorated or alleviated by an increase in neurogenesis.
Transplantation

In other embodiments, methods described herein involve modulating neurogenesis in vitro or ex vivo with a 5HTR agent, optionally in combination with one or more other neurogenic agents, such that a composition containing neural stem cells, neural progenitor cells, and/or differentiated neural cells can subsequently be administered to an individual to treat a disease or condition. In some embodiments, the method of treatment comprises the steps of contacting a neural stem cell or progenitor cell with a 5HTR agent, optionally in combination with one or more other neurogenic agents, to modulate neurogenesis, and transplanting the cells into a patient in need of treatment. Methods for transplanting stem and progenitor cells are known in the art, and are described, e.g., in U.S. Patent Nos. 5,928,947; 5,817,773; and 5,800,539, and PCT Publication Nos. WO 01/176507 and WO 01/170243, all of which are incorporated herein by reference in their entirety. In some embodiments, methods described herein allow treatment of diseases or conditions by directly replenishing, replacing, and/or supplementing damaged or dysfunctional neurons. In further embodiments, methods described herein enhance the growth and/or survival of existing neural cells, and/or slow or reverse the loss of such cells in a neurodegenerative or other condition.

In alternative embodiments, the method of treatment comprises identifying, generating, and/or propagating neural cells in vitro or ex vivo in contact with a 5HTR agent, optionally in combination with one or more other neurogenic agents, and transplanting the cells into a subject. In another embodiment, the method of treatment comprises the steps of contacting a neural stem cell of progenitor cell with a 5HTR agent, optionally in combination with one or more other neurogenic agents, to stimulate neurogenesis or neurodifferentiation, and transplanting the cells into a patient in need of treatment. Also disclosed are methods for preparing a population of neural stem cells suitable for transplantation, comprising culturing a population of neural stem cells (NSCs) in vitro, and contacting the cultured neural stem cells with a 5HTR agent, optionally in combination with one or more other neurogenic agents, as described herein. The disclosure further includes methods of treating the diseases, disorders, and conditions described herein by transplanting such treated cells into a subject or patient.
Neurogenesis with Angiogenesis

In additional embodiments, the disclosure includes a method of stimulating or increasing neurogenesis in a subject or patient with stimulation of angiogenesis in the subject or patient. The co-stimulation may be used to provide the differentiating and/or proliferating cells with increased access to the circulatory system. The neurogenesis is produced by modulation of 5HT receptor activity, such as with a 5HTR agent, optionally in combination with one or more other neurogenic agents, as described herein. An increase in angiogenesis may be mediated by a means known to the skilled person, including administration of a angiogenic factor or treatment with an angiogenic therapy. Non-limiting examples of angiogenic factors or conditions include vascular endothelial growth factor (VEGF), angiopoietin-1 or -2, erythropoietin, exercise, or a combination thereof.

So in some embodiments, the disclosure includes a method comprising administering i) a 5HTR agent, optionally in combination with one or more other neurogenic agents, and ii) one or more angiogenic factors to a subject or patient. In other embodiments, the disclosure includes a method comprising administering i) a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a subject or patient with ii) treating said subject or patient with one or more angiogenic conditions. The subject or patient may be any as described herein.

The co-treatment of a subject or patient includes simultaneous treatment or sequential treatment as non-limiting examples. In cases of sequential treatment, the administration of a 5HTR agent, optionally with one or more other neurogenic agents, may be before or after the administration of an angiogenic factor or condition. Of course in the case of a combination of a 5HTR agent and one or more other neurogenic agents, the 5HTR agent may be administered separately from the one or more other agents, such that the one or more other agent is administered before or after administration of an angiogenic factor or condition.

Additional Diseases and Conditions

As described herein, the disclosed embodiments include methods of treating diseases, disorders, and conditions of the central and/or peripheral nervous systems (CNS and PNS, respectively) by administering a 5HTR agent, optionally in combination with one or more other neurogenic agents. As used herein, "treating" includes prevention, amelioration, alleviation, and/or elimination of the disease, disorder, or condition being treated or one or
more symptoms of the disease, disorder, or condition being treated, as well as improvement in the overall well being of a patient, as measured by objective and/or subjective criteria. In some embodiments, treating is used for reversing, attenuating, minimizing, suppressing, or halting undesirable or deleterious effects of, or effects from the progression of, a disease, disorder, or condition of the central and/or peripheral nervous systems. In other embodiments, the method of treating may be advantageously used in cases where additional neurogenesis would replace, replenish, or increase the numbers of cells lost due to injury or disease as non-limiting examples.

The amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents may be any that results in a measurable relief of a disease condition like those described herein. As a non-limiting example, an improvement in the Hamilton depression scale (HAM-D) score for depression may be used to determine (such as quantitatively) or detect (such as qualitatively) a measurable level of improvement in the depression of a subject.

Non-limiting examples of symptoms that may be treated with the methods described herein include abnormal behavior, abnormal movement, hyperactivity, hallucinations, acute delusions, combativeness, hostility, negativism, withdrawal, seclusion, memory defects, sensory defects, cognitive defects, and tension. Non-limiting examples of abnormal behavior include irritability, poor impulse control, distractibility, and aggressiveness. Outcomes from treatment with the disclosed methods include improvements in cognitive function or capability in comparison to the absence of treatment.

Additional examples of diseases and conditions treatable by the methods described herein include, but are not limited to, neurodegenerative disorders and neural disease, such as dementias (e.g., senile dementia, memory disturbances/memory loss, dementias caused by neurodegenerative disorders (e.g., Alzheimer's, Parkinson's disease, Parkinson's disorders, Huntington's disease (Huntington's Chorea), Lou Gehrig's disease, multiple sclerosis, Pick's disease, Parkinsonism dementia syndrome), progressive subcortical gliosis, progressive supranuclear palsy, thalamic degeneration syndrome, hereditary aphasia, amyotrophic lateral sclerosis, Shy-Drager syndrome, and Lewy body disease; vascular conditions (e.g., infarcts, hemorrhage, cardiac disorders); mixed vascular and Alzheimer's; bacterial meningitis; Creutzfeld-Jacob Disease; and Cushing's disease).

The disclosed embodiments also provide for the treatment of a nervous system disorder related to neural damage, cellular degeneration, a psychiatric condition, cellular
(neurological) trauma and/or injury (e.g., subdural hematoma or traumatic brain injury), toxic chemicals (e.g., heavy metals, alcohol, some medications), CNS hypoxia, or other neurologically related conditions. In practice, the disclosed compositions and methods may be applied to a subject or patient afflicted with, or diagnosed with, one or more central or peripheral nervous system disorders in any combination. Diagnosis may be performed by a skilled person in the applicable fields using known and routine methodologies which identify and/or distinguish these nervous system disorders from other conditions.

Non-limiting examples of nervous system disorders related to cellular degeneration include neurodegenerative disorders, neural progenitor cell disorders, degenerative diseases of the retina, and ischemic disorders. In some embodiments, an ischemic disorder comprises an insufficiency, or lack, of oxygen or angiogenesis, and non-limiting example include spinal ischemia, ischemic stroke, cerebral infarction, multi-infarct dementia. While these conditions may be present individually in a subject or patient, the disclosed methods also provide for the treatment of a subject or patient afflicted with, or diagnosed with, more than one of these conditions in any combination.

Non-limiting embodiments of nervous system disorders related to a psychiatric condition include neuropsychiatric disorders and affective disorders. As used herein, an affective disorder refers to a disorder of mood such as, but not limited to, depression, post-traumatic stress disorder (PTSD), hypomania, panic attacks, excessive elation, bipolar depression, bipolar disorder (manic-depression), and seasonal mood (or affective) disorder. Other non-limiting embodiments include schizophrenia and other psychoses, lissencephaly syndrome, anxiety syndromes, anxiety disorders, phobias, stress and related syndromes (e.g., panic disorder, phobias, adjustment disorders, migraines), cognitive function disorders, aggression, drug and alcohol abuse, drug addiction, and drug-induced neurological damage, obsessive compulsive behavior syndromes, borderline personality disorder, non-senile dementia, post-pain depression, post-partum depression, and cerebral palsy.

Examples of nervous system disorders related to cellular or tissue trauma and/or injury include, but are not limited to, neurological traumas and injuries, surgery related trauma and/or injury, retinal injury and trauma, injury related to epilepsy, cord injury, spinal cord injury, brain injury, brain surgery, trauma related brain injury, trauma related to spinal cord injury, brain injury related to cancer treatment, spinal cord injury related to cancer treatment, brain injury related to infection, brain injury related to inflammation, spinal cord
injury related to infection, spinal cord injury related to inflammation, brain injury related to environmental toxin, and spinal cord injury related to environmental toxin.

Non-limiting examples of nervous system disorders related to other neurologically related conditions include learning disorders, memory disorders, age-associated memory impairment (AAMI) or age-related memory loss, autism, learning or attention deficit disorders (ADD or attention deficit hyperactivity disorder, ADHD), narcolepsy, sleep disorders and sleep deprivation (e.g., insomnia, chronic fatigue syndrome), cognitive disorders, epilepsy, injury related to epilepsy, and temporal lobe epilepsy.

Other non-limiting examples of diseases and conditions treatable by the methods described herein include, but are not limited to, hormonal changes (e.g., depression and other mood disorders associated with puberty, pregnancy, or aging (e.g., menopause)); and lack of exercise (e.g., depression or other mental disorders in elderly, paralyze, or physically handicapped patients); infections (e.g., HIV); genetic abnormalities (down syndrome); metabolic abnormalities (e.g., vitamin B12 or fotate deficiency); hydrocephalus; memory loss separate from dementia, including mild cognitive impairment (MCI), age-related cognitive decline, and memory loss resulting from the use of general anesthetics, chemotherapy, radiation treatment, post-surgical trauma, or therapeutic intervention; and diseases of the of the peripheral nervous system (PNS), including but not limited to, PNS neuropathies (e.g., vascular neuropathies, diabetic neuropathies, amyloid neuropathies, and the like), neuralgias, neoplasms, myelin-related diseases, etc.

Other conditions that can be beneficially treated by increasing neurogenesis are known in the art (see e.g., U.S. Publication Nos. 2002/0106731, 2005/0009742 and 2005/0009847, 20050032702, 2005/0031538, 2005/0004046, 2004/0254152, 2004/0229291, and 2004/0185429, herein incorporated by reference in their entirety).

5HTR agents

A 5HTR agent can be is a ligand which modulates activity at one or more 5HT receptor subtypes. In some cases, the ligand may bind or interact with one or more subtypes. In other cases, the ligand may modulate activity indirectly as described herein. In some embodiments, the agent is an agonist of one or more subtypes. In other embodiments, the agent is an antagonist of one or more subtypes. In additional embodiments, the agent is an agonist of at least one subtype as well as an antagonist of at least one other subtype.
A 5HT receptor agent for use in embodiments of the invention includes a reported 5HT1a receptor agonist (or partial agonist) such as buspirone hydrochloride (BuSpar®). In some embodiments, a reported 5HT1a receptor agonist is an azapirone, such as buzipine. Non-limiting examples of additional reported 5HT1a receptor agonists include flesinoxan (CAS RN 98206-10-1), MDL 72832 hydrochloride, U-92016A, (+)-UH 301, F 13714, F 13640, 6-hydroxy-buspirone (see US 2005/0137206), S-6-hydroxy-buspirone (see US 2003/0022899), R-6-hydroxy-buspirone (see US 2003/0009851), adatanserin, buspirone-saccharide (see WO 00/12067) or 8-hydroxy-2-dipropylaminotetralin (8-OHDPAT).

Additional non-limiting examples of reported 5HT1a receptor agonists include OPC-14523 (1-[(3-[4-[(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2[H]-quinolinone monomethanesulfonate); BMS-181 100 or BMY 14802 (CAS RN 105565-56-8); flibanserin (CAS RN 167933-07-5); repinotan (CAS RN 144980-29-0); lesopitron (CAS RN 132449-46-8); piclozotan (CAS RN 182415-09-4); aripiprazole, Org-13011 (1-[(4-trifluoromethyl)-2-pyridinyl]4-[2-o xo-1-pyrrolidinyl]butyl)piperazine (E)-2-butenedioate); SDZ-MAR-327 (see Christian et al. "Positron emission tomographic analysis of central dopamine D1 receptor binding in normal subjects treated with the atypical neuroleptic, SDZ MAR 327." Int J Mol Med. 1998 l(l):243-7; MKC-242 ((S)-5-[(3-[1,4-benzodioxan-2-ylmethyl]amino]propoxy]-l,3-benzodioxole HCl); vilazodone; sarizotan (CAS RN 177975-08-5); roxindole (CAS RN 112192-04-8) or roxindole methanesulfonate (CAS RN 119742-13-1); alnespiron (CAS RN 138298-79-0); bromerguride (CAS RN 83455-48-5); xaliproden (CAS RN 135354-02-8); mazapertine succinate (CAS RN 134208-18-7) or mazapertine (CAS RN 134208-17-6); PRX-00023; F-13640 ((3-chloro-4-fluorophenyl)-[4-fluoro-4-[[5-methyl-pyridin-2-ylmethyl]-amino]methyl]piperidin-1-yl)methane, fumaric acid salt); eptapiron (CAS RN 179756-85-5); ziprasidone (CAS RN 146939-27-7); sunepitron (see Becker et al. "G protein-coupled receptors: In silico drug discovery in 3D" PNAS 2004 101(31):l 1304-1 1309); umesperone (CAS RN 107736-98-1); SLV-308; bifeprunox; and zalospirone (CAS RN 114298-18-9).

Yet further non-limiting examples include AP-521 (partial agonist from AsahiKasei) and Du-123015 (from Solvay).

Alternatively, a 5HTR agent may be a reported 5HT4 receptor agonist (or partial agonist). In some embodiments, a reported 5HT4 receptor agonist or partial agonist is a substituted benzamide, such as cispamide; individual, or a combination of, cispamide.
enantiomers ((+) cisapride and (-) cisapride); mosapride; and renzapride as non-limiting examples. In other embodiments, the chemical entity is a benzofuran derivative, such as prucalopride. Additional embodiments include indoles, such as tegaserod, or benzimidazolones. Other non-limiting chemical entities reported as a 5HT4 receptor agonist or partial agonist include zacopride (CAS RN 90182-92-6), SC-531 16 (CAS RN 141 196-99-8) and its racemate SC-49518 (CAS RN 146388-57-0), BIMUI (CAS RN 127595-43-1), TS-951 (CAS RN 174486-39-6), or MLI 0302 CAS RN 148868-55-7). Additional non-limiting chemical entities include metoclopramide, 5-methoxytryptamine, RS67506, 2-[l-(4-piperonyl)piperazinyl]benzothiazole, RS66331, BIMU8, SB 205149 (the n-butyl quaternary analog of renzapride), or an indole carbazimidamide as described by Buchheit et al. ("The serotonin 5-HT4 receptor. 2. Structure-activity studies of the indole carbazimidamide class of agonists." J Med Chem. (1995) 38(13):2331-8). Yet additional non-limiting examples include norcisapride (CAS RN 102671-04-5) which is the metabolite of cisapride; mosapride citrate; the maleate form of tegaserod (CAS RN 189188-57-6); zacopride hydrochloride (CAS RN 99617-34-2); mezacopride (CAS RN 89613-77-4); SK-951 ((+-)-4-amino-N-(2-(1-azabicyclo(3.3.0)octan-5-yl)ethy1)-5-chloro-2,3-dihydro-2-methylbenzo(b)furan-7-carboxamide hemifumarate); ATI-7505, a cisapride analog from ARYx Therapeutics; SDZ-216-454, a selective 5HT4 receptor agonist that stimulates cAMP formation in a concentration dependent manner (see Markstein et al. "Pharmacological characterisation of 5-HT receptors positively coupled to adenyly cyclase in the rat hippocampus." Naunyn Schmiedebergs Arch Pharmacol. (1999) 359(6):454-9); SC-54750, or aminomethylazadamantane; Y-36912, or 4-amino-N-[l-[3-(benzylsulfonyl)propyl]piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide as disclosed by Sonda et al. ("Synthesis and pharmacological properties of benzamide derivatives as selective serotonin 4 receptor agonists." Bioorg Med Chem. (2004) 12(10):2737-47); TKS 159, or 4-amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl] benzamide, as reported by Haga et al. ("Effect of TKS 159, a novel 5-hydroxytryptamine4 agonist, on gastric contractile activity in conscious dogs." RS67333, or l-(4-amino-5-chloro-2-methoxyphenyl)-3-(l-n-butyl-4-piperidinyl)-l-propanone; KDR-5169, or 4-amino-5-chloro-N-[l-(3-fluoro-4-methoxybenzyl)piperidin-4-yl]-2-(2-hydroxyethoxy)benzamide hydrochloride dihydrate as reported by Tazawa, et al. (2002) "KDR-5169, a new gastrointestinal prokinetic agent, enhances gastric contractile and emptying activities in dogs and rats." Eur J Pharmacol 434(3): 169-76); SL65.0155, or 5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[l-
(2-phenyl ethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3 \( H \))-onemonohydrochloride; and Y-34959, or 4-amino-5-chloro-2-methoxy-N-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide.

Other non-limiting reported 5HT4 receptor agonists and partial agonists for use in combination with a 5HTR agent include metoclopramide (CAS RN 364-62-5), 5-methoxytryptamine (CAS RN 608-07-1), RS67506 (CAS RN 168986-61-6), 2-[(4-piperonyl)piperazinyl]benzothiazole (CAS RN 155106-73-3), RS66331 (see Buccafusco et al. "Multiple Central Nervous System Targets for Eliciting Beneficial Effects on Memory and Cognition." (2000) Pharmacology 295(2):43 8-446), BIMU8 (endo-N-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dehydro-2-oxo-3-(prop-2-yl)-lH-benzimidazole-carboxamide), or SB 205149 (the n-butyl quaternary analog of renzapride). Compounds related to metoclopramide, such as metoclopramide dihydrochloride (CAS RN 2576-84-3) or metoclopramide dihydrochloride (CAS RN 5581-45-3) or metoclopramide hydrochloride (CAS RN 7232-21-5 or 54143-57-6) may also be used in a combination or method as described herein.

Additionally, a 5HTR agent may be a reported 5HT3 receptor antagonist such as azasetron (CAS RN 123039-99-6); ondansetron (CAS RN 99614-02-5) or ondansetron hydrochloride (CAS RN 99614-01-4); cilansetron (CAS RN 120635-74-7); aloxi or palonosetron hydrochloride (CAS RN 135729-62-3); palonosetron (CAS RN 135729-61-2 or 135729-56-5); cisplatin (CAS RN 15663-27-1); lotronex or alosetron hydrochloride (CAS RN 122852-69-1); azemetric or dolasetron mesylate (CAS RN 115956-13-3); zacopride or R-zacopride; E-3620 ([3(S)-endo]-4-amino-5-chloro-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2[(1-methyl-2-butylnyl)oxy]benzamide) or E-3620 HCl (3(S)-endo-4-amino-5-chloro-N-(8-methyl-8-azabicyclo [3.2.1] oct-3-yl)-2-(1-methyl-2-butylxyloxy)-benzamide-HCl); YM 060 or ramosetron hydrochloride (CAS RN 132907-72-3); a thieno[2,3-d]pyrimidine derivative antagonist described in U.S. Patent 6,846,823, such as DDP 225 or MCI 225 (CAS RN 135991-48-9); marinol or dronabinol (CAS RN 1972-08-3); or lac hydrin or ammonium lactate (CAS RN 515-98-0); kytril or granisetron hydrochloride (CAS RN 107007-99-8); bemesetron (CAS RN 40796-97-2); tropisetron (CAS RN 89565-68-4); zatosetron (CAS RN 123482-22-4); mirisetron (CAS RN 135905-89-4) or mirisetron maleate (CAS RN 14861-75-0); or renzapride (CAS RN 112727-80-7).

Additionally, a 5HT2A/2C receptor antagonist such as ketanserin (CAS RN 74050-98-9) or ketanserin tartrate; risperidone; olanzapine;
adatanserin (CAS RN 127266-56-2); ritanserin (CAS RN 87051-43-2); etoperidone;
nefazodone; deramciclane (CAS RN 120444-71-5); geoden or ziprasidone hydrochloride
(CAS RN 138982-67-9); zeldox or ziprasidone or ziprasidone hydrochloride; EMD 281014
(7-[4-[2-(4-fluoro-phenyl)-ethyl]-piperazine-1-carbonyl]-IH-indole-3-carbonitrile HCl);
MDL 100907 or M100907 (CAS RN 139290-65-6); effexor XR (venlafaxine formulation);
zomaril or iloperidone; quetiapine (CAS RN 111974-69-7) or quetiapine fumarate (CAS RN
111974-72-2) or seroquel; SB 228357 or SB 243213 (see Bromidge et al.
"Biarylcarbamoyleindolines are novel and selective 5-HT(2C) receptor inverse agonists:
identification of 5-methyl-1-[2-[(2-methyl-3-pyridyl)oxy]- 5-pyriddy]carbamoyl]-6-
trifluoromethylindoline (SB-243213) as a potential antidepressant/anxiolytic agent." J Med
Chem. 2000 43(6): 1123-34); SB 220453 or tonabersat (CAS RN 175013-84-0); sertindole
(CAS RN 106516-24-9); eplivanserin (CAS RN 130579-75-8) or eplivanserin fumarate (CAS
RN 130580-02-8); lubazodone hydrochloride (CAS RN 161178-10-5); cyproheptadine (CAS
RN 129-03-3); pizotyline or pizotifen (CAS RN 15574-96-6); mesulergine (CAS RN 64795-
35-3); irindalone (CAS RN 96478-43-2); MDL 11939 (CAS RN 107703-78-6); or
pruvanserin (CAS RN 443144-26-1).

Additional non-limiting examples of modulators include reported 5-HT2C
agonists or partial agonists, such as m-chlorophenylpiperazine; or 5-HT2A receptor inverse
agonists, such as ACP 103 (CAS RN: 868855-07-6), APD 125 (from Arena Pharmaceuticals),
AVE 8488 (from Sanofi-Aventis) or TGWOOAD/AA(from Fabre Kramer Pharmaceuticals).

Additionally, a 5HTR agent may be a reported 5HT6 receptor antagonist such
as SB-357134 (N-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-
ylbenzenesulfonamide); SB-27 1046 (5-chloro-N-(4-methoxy-3-(piperazin- 1-yl)phenyl)-3-
methylbenzo[b]thiophene-2-sulfonamide); Ro 04-06790 (N-(2,6-bis(methylamino)pyrimidin-
4-yl)-4-aminobenzenesulfonamide); Ro 63-0563 (4-amino-N-(2,6 bis-methylamino-pyridin-
4-yl)-benzene sulfonamide); clozapine or its metabolite N-desmethyl clozapine; olanzapine
(CAS RN 132539-06-1); fluperlapine (CAS RN 67121-76-0); seroquel (quetiapine or
quetiapine fumarate); clomipramine (CAS RN 303-49-1); amitriptyline (CAS RN50-48-6);
doxepin (CAS RN 1668-19-5); nortryptiline (CAS RN 72-69-5); 5-methoxytryptamine (CAS
RN 608-07-1); bromocryptine (CAS RN 25614-03-3); octoclothein (CAS RN 13448-22-1);
chlorpromazine (CAS RN 50-53-3); loxapine (CAS RN 1977-10-2); fluphenazine (CAS RN
69-23-8); or GSK 742457 (presented by David Witty, "Early Optimisation of in vivo
Activity: the discovery of 5-HT6 Receptor Antagonist 742457" GlaxoSmithKline at

As an additional non-limiting example, the reported 5HT6 modulator may be SB-258585 (4-Iodo-N-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulphonamide); PRX 07034 (from Predix Pharmaceuticals) or a partial agonist, such as E-6801 (6-chloro-N-(3-((2-(dimethylamino)ethyl)-1H-indol-5-yl)imidazo[2,1-b]thiazole-5-sulphonamide) or E-6837 (5-chloro-N-(3-(2-(dimethylamino)ethyl)-1H-indol-5-yl)naphthalene-2-sulfonamide).

A 5HTR agent as described herein includes pharmaceutically acceptable salts, derivatives, prodrugs, and metabolites of the agent. Methods for preparing and administering salts, derivatives, prodrugs, and metabolites of various agents are well known in the art.

Compounds described herein that contain a chiral center include all possible stereoisomers of the compound, including compositions comprising the racemic mixture of the two enantiomers, as well as compositions comprising each enantiomer individually, substantially free of the other enantiomer. Thus, for example, contemplated herein is a composition comprising the S enantiomer of a compound substantially free of the R enantiomer, or the R enantiomer substantially free of the S enantiomer. If the named compound comprises more than one chiral center, the scope of the present disclosure also includes compositions comprising mixtures of varying proportions between the diastereomers, as well as compositions comprising one or more diastereomers substantially free of one or more of the other diastereomers. By "substantially free" it is meant that the composition comprises less than 25%, 15%, 10%, 8%, 5%, 3%, or less than 1% of the minor enantiomer or diastereomer(s). Methods for synthesizing, isolating, preparing, and administering various stereoisomers are known in the art.

In some embodiments, a 5HTR agent used in the methods described herein is substantially inactive with respect to other receptors, such as muscarinic receptors, nicotinic receptors, dopamine receptors, and opioid receptors as non-limiting examples.

As described herein, a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered to an animal or human subject to result in neurogenesis. A combination may thus be used to treat a specified disease, disorder, or condition.

Methods for assessing the nature and/or degree of neurogenesis in vivo and in vitro, for detecting changes in the nature and/or degree of neurogenesis, for identifying
neurogenesis modulating agents, for isolating and culturing neural stem cells, and for preparing neural stem cells for transplantation or other purposes are disclosed, for example, in U.S. Provisional Application No. 60/697,905, and U.S. Publication Nos. 2005/0009742 and 2005/0009847, 2005/0031538, 2005/004046, 2004/0254152, 2004/022929, and 2004/0185429, all of which are herein incorporated by reference in their entirety.

Formulations and Doses

In some embodiments of the disclosure, a 5HTR agent, optionally in combination with one or more other neurogenic agents, is in the form of a composition that includes at least one pharmaceutically acceptable excipient. As used herein, the term "pharmaceutically acceptable excipient" includes any excipient known in the field as suitable for pharmaceutical application. Suitable pharmaceutical excipients and formulations are known in the art and are described, for example, in Remington's Pharmaceutical Sciences (19th ed.) (Genarro, ed. (1995) Mack Publishing Co., Easton, Pa.). Preferably, pharmaceutical carriers are chosen based upon the intended mode of administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents. The pharmaceutically acceptable carrier may include, for example, disintegrants, binders, lubricants, glidants, emollients, humectants, thickeners, silicones, flavoring agents, and water.

A 5HTR agent, optionally in combination with one or more other neurogenic agents, or with another 5HTR agent, may be incorporated with excipients and administered in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, or any other form known in the pharmaceutical arts. The pharmaceutical compositions may also be formulated in a sustained release form. Sustained release compositions, enteric coatings, and the like are known in the art. Alternatively, the compositions may be a quick release formulation.

The amount of a combination of a 5HTR agent, or a combination thereof with one or more other neurogenic agents, may be an amount that also potentiates or sensitizes, such as by activating or inducing cells to differentiate, a population of neural cells for neurogenesis. The degree of potentiation or sensitization for neurogenesis may be determined with use of the combination in any appropriate neurogenesis assay, including, but not limited to, a neuronal differentiation assay described herein. In some embodiments, the amount of a combination of a 5HTR agent, optionally in combination with one or more other
neurogenic agents, is based on the highest amount of one agent in a combination, which amount produces no detectable neuroproliferation \textit{in vitro} but yet produces neurogenesis, or a measurable shift in efficacy in promoting neurogenesis \textit{in vitro}, when used in the combination.

As disclosed herein, an effective amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents, in the described methods is an amount sufficient, when used as described herein, to stimulate or increase neurogenesis in the subject targeted for treatment when compared to the absence of the combination. An effective amount of a 5HTR agent alone or in combination may vary based on a variety of factors, including but not limited to, the activity of the active compounds, the physiological characteristics of the subject, the nature of the condition to be treated, and the route and/or method of administration. General dosage ranges of certain compounds are provided herein and in the cited references based on animal models of CNS diseases and conditions. Various conversion factors, formulas, and methods for determining human dose equivalents of animal dosages are known in the art, and are described, e.g., in Freireich et al., Cancer Chemother Repts 50(4): 219 (1966), Monro et al., Toxicology Pathology, 23: 187-98 (1995), Boxenbaum and Dilea, J.Clin.Pharmacol. 35: 957-966 (1995), and Voisin et al., Reg. Toxicol. Pharmacol, 12(2): 107-116 (1990), which are herein incorporated by reference.

The disclosed methods typically involve the administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents, in a dosage range of from about 0.001 ng/kg/day to about 200 mg/kg/day. Other non-limiting dosages include from about 0.001 to about 0.01 ng/kg/day, about 0.01 to about 0.1 ng/kg/day, about 0.1 to about 1 ng/kg/day, about 1 to about 10 ng/kg/day, about 10 to about 100 ng/kg/day, about 100 ng/kg/day to about 1 µg/kg/day, about 1 to about 2 µg/kg/day, about 2 µg/kg/day to about 0.02 mg/kg/day, about 0.02 to about 0.2 mg/kg/day, about 0.2 to about 2 mg/kg/day, about 2 to about 20 mg/kg/day, or about 20 to about 200 mg/kg/day. However, as understood by those skilled in the art, the exact dosage of a 5HTR agent, optionally in combination with one or more other neurogenic agents, used to treat a particular condition will vary in practice due to a wide variety of factors. Accordingly, dosage guidelines provided herein are not limiting as the range of actual dosages, but rather provide guidance to skilled practitioners in selecting dosages useful in the empirical determination of dosages for individual patients. Advantageously, methods described herein allow treatment of one or more conditions with reductions in side effects, dosage levels, dosage frequency, treatment duration, safety,
tolerability, and/or other factors. So where suitable dosages for a 5HTR agent to modulate a 5HT receptor activity are known to a skilled person, the disclosure includes the use of about 75%, about 50%, about 33%, about 25%, about 20%, about 15%, about 10%, about 5%, about 2.5%, about 1%, about 0.5%, about 0.25%, about 0.1%, about 0.05%, about 0.025%, about 0.02%, about 0.01%, or less than the known dosage.

In other embodiments, the amount of a 5HTR agent used in vivo may be about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 18%, about 16%, about 14%, about 12%, about 10%, about 8%, about 6%, about 4%, about 2%, or about 1% or less than the maximum tolerated dose for a subject, including where one or more other neurogenic agents is used in combination with the 5HTR agent. This is readily determined for each muscarinic agent that has been in clinical use or testing, such as in humans.

Alternatively, the amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents, may be an amount selected to be effective to produce an improvement in a treated subject based on detectable neurogenesis in vitro as described above. In some embodiments, such as in the case of a known 5HTR agent, the amount is one that minimizes clinical side effects seen with administration of the agent to a subject. The amount of an agent used in vivo may be about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 18%, about 16%, about 14%, about 12%, about 10%, about 8%, about 6%, about 4%, about 2%, or about 1% or less of the maximum tolerated dose in terms of acceptable side effects for a subject. This is readily determined for each 5HTR agent or other agent(s) of a combination disclosed herein as well as those that have been in clinical use or testing, such as in humans.

In other embodiments, the amount of an additional neurogenic sensitizing agent in a combination with a 5HTR agent of the disclosure is the highest amount which produces no detectable neurogenesis when the sensitizing agent is used, alone in vitro, or in vivo, but yet produces neurogenesis, or a measurable shift in efficacy in promoting neurogenesis, when used in combination with a 5HTR agent. Embodiments include amounts which produce about 1%, about 2%, about 4%, about 6%, about 8%, about 10%, about 12%, about 14%, about 16%, about 18%, about 20%, about 25%, about 30%, about 35%, or about 40% or more of the neurogenesis seen with the amount that produces the highest level of neurogenesis in an in vitro assay.
In some embodiments, the amount may be the lowest needed to produce a desired, or minimum, level of detectable neurogenesis or beneficial effect. Of course the administered 5HTR agent, alone or in a combination disclosed herein, may be in the form of a pharmaceutical composition.

As described herein, the amount of a 5HTR agent, optionally in combination with one or more other neurogenic agents, may be any that is effective to produce neurogenesis, optionally with reduced or minimized amounts of astrogenesis. As a non-limiting example described herein, the levels of astrogenesis observed with the use of certain 5HTR agents alone may be reduced or suppressed when the 5HTR agent is used in combination with a second agent such as baclofen (or other GABA modulator with the same anti-astrogenesis activity) or melatonin. This beneficial effect is observed along with the ability of each combination of agents to stimulate neurogenesis. So while certain 5HTR agents have been observed to produce astrogenesis, their use with a second compound, such as baclofen and melatonin, advantageously provides a means to suppress the overall level of astrogenesis.

Therefore, the methods of the disclosure further include a method of decreasing the level of astrogenesis in a cell or cell population by contacting the cell or population with a 5HTR agent and a second agent that reduces or suppresses the amount or level of astrogenesis caused by said 5HTR agent. The reduction or suppression of astrogenesis may be readily determined relative to the amount or level of astrogenesis in the absence of the second agent. In some embodiments, the second agent is baclofen or melatonin.

In some embodiments, an effective, neurogenesis modulating amount of a combination of a 5HTR agent, optionally in combination with one or more other neurogenic agents, is an amount of a 5HTR agent (or of each agent in a combination) that achieves a concentration within the target tissue, using the particular mode of administration, at or above the IC\textsubscript{50} or EC\textsubscript{50} for activity of target molecule or physiological process. In some cases, a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered in a manner and dosage that gives a peak concentration of about 1, about 1.5, about 2, about 2.5, about 5, about 10, about 20 or more times the IC\textsubscript{50} or EC\textsubscript{50} concentration of the 5HTR agent (or each agent in the combination). IC\textsubscript{50} and EC\textsubscript{50} values and bioavailability data for a 5HTR agent and other agent(s) described herein are known in the art, and are described, e.g., in the references cited herein or can be readily determined using
established methods. In addition, methods for determining the concentration of a free compound in plasma and extracellular fluids in the CNS, as well pharmacokinetic properties, are known in the art, and are described, e.g., in de Lange et al., AAPS Journal, 7(3): 532-543 (2005). In some embodiments, a 5HTR agent, optionally in combination with one or more other neurogenic agents, described herein is administered, as a combination or separate agents used together, at a frequency of at least about once daily, or about twice daily, or about three or more times daily, and for a duration of at least about 3 days, about 5 days, about 7 days, about 10 days, about 14 days, or about 21 days, or about 4 weeks, or about 2 months, or about 4 months, or about 6 months, or about 8 months, or about 10 months, or about 1 year, or about 2 years, or about 4 years, or about 6 years or longer.

In other embodiments, an effective, neurogenesis modulating amount is a dose that produces a concentration of a 5HTR agent (or each agent in a combination) in an organ, tissue, cell, and/or other region of interest that includes the ED_{50} (the pharmacologically effective dose in 50% of subjects) with little or no toxicity. IC_{50} and EC_{50} values for the modulation of neurogenesis can be determined using methods described in PCT Application US06/026677, filed July 7, 2006, incorporated by reference, or by other methods known in the art. In some embodiments, the IC_{50} or EC_{50} concentration for the modulation of neurogenesis is substantially lower than the IC_{50} or EC_{50} concentration for activity of a 5HTR agent and/or other agent(s) at non-targeted molecules and/or physiological processes.

In some methods described herein, the application of a 5HTR agent in combination with one or more other neurogenic agents may allow effective treatment with substantially fewer and/or less severe side effects compared to existing treatments. In some embodiments, combination therapy with a 5HTR agent and one or more additional neurogenic agents allows the combination to be administered at dosages that would be sub-therapeutic when administered individually or when compared to other treatments. In other embodiments, each agent in a combination of agents may be present in an amount that results in fewer and/or less severe side effects than that which occurs with a larger amount. Thus the combined effect of the neurogenic agents will provide a desired neurogenic activity while exhibiting fewer and/or less severe side effects overall. In further embodiments, methods described herein allow treatment of certain conditions for which treatment with the same or similar compounds is ineffective using known methods due, for example, to dose-limiting side effects, toxicity, and/or other factors.
Routes of Administration

As described, the methods of the disclosure comprise contacting a cell with a 5HTR agent, optionally in combination with one or more other neurogenic agents, or administering such an agent or combination to a subject, to result in neurogenesis. Some embodiments comprise the use of one 5HTR agent, such as buspirone, tandospirone, azasetron, granisetron, ondansetron, mosapride, cisapride, or sumatriptan, in combination with one or more other neurogenic agents. In other embodiments, a combination of two or more agents, such as two or more of buspirone, tandospirone, azasetron, granisetron, ondansetron, mosapride, cisapride, and sumatriptan, is used in combination with one or more other neurogenic agents.

In some embodiments, methods of treatment disclosed herein comprise the step of administering to a mammal a 5HTR agent, optionally in combination with one or more other neurogenic agents, for a time and at a concentration sufficient to treat the condition targeted for treatment. The disclosed methods can be applied to individuals having, or who are likely to develop, disorders relating to neural degeneration, neural damage and/or neural demyelination.

Depending on the desired clinical result, the disclosed agents or pharmaceutical compositions are administered by any means suitable for achieving a desired effect. Various delivery methods are known in the art and can be used to deliver an agent to a subject or to NSCs or progenitor cells within a tissue of interest. The delivery method will depend on factors such as the tissue of interest, the nature of the compound (e.g., its stability and ability to cross the blood-brain barrier), and the duration of the experiment or treatment, among other factors. For example, an osmotic minipump can be implanted into a neurogenic region, such as the lateral ventricle. Alternatively, compounds can be administered by direct injection into the cerebrospinal fluid of the brain or spinal column, or into the eye. Compounds can also be administered into the periphery (such as by intravenous or subcutaneous injection, or oral delivery), and subsequently cross the blood-brain barrier.

In some embodiments, the disclosed agents or pharmaceutical compositions are administered in a manner that allows them to contact the subventricular zone (SVZ) of the lateral ventricles and/or the dentate gyrus of the hippocampus. The delivery or targeting of a 5HTR agent, optionally in combination with one or more other neurogenic agents, to a neurogenic region, such as the dentate gyrus or the subventricular zone, may enhances efficacy and reduces side effects compared to known methods involving administration with
the same or similar compounds. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Intranasal administration generally includes, but is not limited to, inhalation of aerosol suspensions for delivery of compositions to the nasal mucosa, trachea and bronchioli.

In other embodiments, a combination of a 5HTR agent, optionally in combination with one or more other neurogenic agents, is administered so as to either pass through or by-pass the blood-brain barrier. Methods for allowing factors to pass through the blood-brain barrier are known in the art, and include minimizing the size of the factor, providing hydrophobic factors which facilitate passage, and conjugation to a carrier molecule that has substantial permeability across the blood brain barrier. In some instances, an agent or combination of agents can be administered by a surgical procedure implanting a catheter coupled to a pump device. The pump device can also be implanted or be extracorporally positioned. Administration of a 5HTR agent, optionally in combination with one or more other neurogenic agents, can be in intermittent pulses or as a continuous infusion. Devices for injection to discrete areas of the brain are known in the art. In certain embodiments, the combination is administered locally to the ventricle of the brain, substantia nigra, striatum, locus ceruleous, nucleus basalis of Meynert, pedunculopontine nucleus, cerebral cortex, and/or spinal cord by, e.g., injection. Methods, compositions, and devices for delivering therapeutics, including therapeutics for the treatment of diseases and conditions of the CNS and PNS, are known in the art.

In some embodiments, a 5HTR agent and/or other agent(s) in a combination is modified to facilitate crossing of the gut epithelium. For example, in some embodiments, a 5HTR agent or other agent(s) is a prodrug that is actively transported across the intestinal epithelium and metabolized into the active agent in systemic circulation and/or in the CNS.

In other embodiments, a 5HTR agent and/or other agent(s) of a combination is conjugated to a targeting domain to form a chimeric therapeutic, where the targeting domain facilitates passage of the blood-brain barrier (as described above) and/or binds one or more molecular targets in the CNS. In some embodiments, the targeting domain binds a target that is differentially expressed or displayed on, or in close proximity to, tissues, organs, and/or cells of interest. In some cases, the target is preferentially distributed in a neurogenic region of the brain, such as the dentate gyrus and/or the SVZ. For example, in some embodiments, a 5HTR agent and/or other agent(s) of a combination is conjugated or complexed with the fatty
acid docosahexaenoic acid (DHA), which is readily transported across the blood brain barrier and imported into cells of the CNS.

Representative Conditions and Agents

The disclosure includes methods for treating depression and other neurological diseases and conditions. In some embodiments, a method may comprise use of a combination of a 5HTR agent and one or more agents reported as anti-depressant agents. Thus a method may comprise treatment with a 5HTR agent and one or more reported anti-depressant agents as known to the skilled person. Non-limiting examples of such agents include an SSRI (selective serotonin reuptake inhibitor), such as fluoxetine (Prozac®; described, e.g., in U.S. Pat. 4,314,081 and 4,194,009), citalopram (Celexa®; described, e.g., in U.S. Pat. 4,136,193), escitalopram (Lexapro®; described, e.g., in U.S. Pat. 4,136,193), fluvoxamine (described, e.g., in U.S. Pat. 4,085,225) or fluvoxamine maleate (CAS RN: 61718-82-9) and Luvox®, paroxetine (Paxil®; described, e.g., in U.S. Pat. 3,912,743 and 4,007,196), or sertraline (Zoloft®; described, e.g., in U.S. Pat. 4,536,518), or alaproclate; the compound nefazodone (Serozone®; described, e.g., in U.S. Pat. 4,338,317); a selective norepinephrine reuptake inhibitor (SNRI) such as reboxetine (Edronax®), atomoxetine (Strattera®), milnacipran (described, e.g., in U.S. Pat. 4,478,836), sibutramine or its primary amine metabolite (BTS 54 505), amoxapine, or maprotiline; a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) such as venlafaxine (Effexor®; described, e.g., in U.S. Pat. 4,761,501), and its reported metabolite desvenlafaxine, or duloxetine (Cymbalta®; described, e.g., in U.S. Pat. 4,956,388); a serotonin, noradrenaline, and dopamine "triple uptake inhibitor", such as DOV 102,677 (see Popik et al. "Pharmacological Profile of the "Triple"

Monoamine Neurotransmitter Uptake Inhibitor, DOV 102,677." Cell Mol Neurobiol. 2006 Apr 25; Epub ahead of print),

DOV 216,303 (see Beer et al. "DOV 216,303, a "triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile." J Clin Pharmacol. 2004 44(12): 1360-7),


NS-2330 or tesofensine (CAS RN 402856-42-2), or NS 2359 (CAS RN 843660-54-8);
and agents like dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS), CP-122,721 (CAS RN 145742-28-5).

Additional non-limiting examples of such agents include a tricyclic compound such as clomipramine, dosulepin or dothiepin, lofepramine (described, e.g., in U.S. Pat. 4,172,074), trimipramine, protriptyline, amitriptyline, desipramine (described, e.g., in U.S. Pat. 3,454,554), doxepin, imipramine, or nortriptyline; a psychostimulant such as dextroamphetamine and methylphenidate; an MAO inhibitor such as selegiline (Emsam®); an ampakine such as CX516 (or Ampalex®, CAS RN: 154235-83-3), CX546 (or 1-(1,4-benzodioxan-6-ylcarbonyl)pi peridine), and CX614 (CAS RN 191744-13-5) from Cortex Pharmaceuticals; a Vlb antagonist such as SSR149415 ((2S,4R)-l-[5-chloro-l-[((2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-IR-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide),

\[
[1-(\text{beta-mercapto-beta,beta-cyclopentamethylenepropionic acid}), 2-O-ethyltyrosine, 4-valine] \text{arginine vasopressin (d(CH}_2\text{)}_5[Tyr(Et}_2\text{)]VAVP (WK 1-1),}
\]

9-desglycine [1-(beta-mercapto-beta,beta-cyclopentamethylenepropionic acid), 2-O-ethyltyrosine, 4-valine] arginine vasopressin desGly9d(CH}_2\text{)}_5 [Tyr(Et}_2\text{)]-VAVP (WK 3-6), or

9-desglycine [1-(beta-mercapto-beta,beta-cyclopentamethylenepropionic acid),2-D-(0-ethyl)tyrosine, 4-valine] arginine vasopressin desGly9d(CH}_2\text{)}_5 [D-Tyr(Et}_2\text{)]VAVP (AO 3-21); a corticotropin-releasing factor receptor (CRF) R antagonist such as CP-154,526 (structure disclosed in Schulz et al. "CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors." Proc Natl Acad Sci U S A. 1996 93(19):10477-82), NBI 30775 (also known as R121919 or 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5-a]pyrimidine), a stressin (CAS RN 170809-5 1-5), or a photoactivatable analog thereof as described in Bonk et al. "Novel high-affinity photoactivatable antagonists of corticotropin-releasing factor (CRF)" Eur. J. Biochem. 267:3017-3024 (2000), or AAG561 (from Novartis); a melanin concentrating hormone (MCH) antagonist such as 3,5-dimethoxy-N-(l-(naphthalen-2-ylmethyl)piperidin-4-yl)benzamide or (R)-3,5-dimethoxy-N-(l-(naphthalen-2-ylmethyl)-pyrrolidin-3-yl)benzamide (see Kim et al. "Identification of substituted 4-aminopiperidines and 3-aminopyrrolidines as potent MCH-R1 antagonists for the treatment of obesity." Bioorg Med Chem Lett. 2006 Jul 29; [Epub ahead of print] for both), or any MCH antagonist disclosed in U.S. Patent 7,045,636 or published U.S. Patent Application US2005/0171098.
Further non-limiting examples of such agents include a tetracyclic compound such as mirtazapine (described, e.g., in U.S. Pat. 4,062,848; see CAS RN 61337-67-5; also known as Remeron®, or CAS RN 85650-52-8), mianserin (described, e.g., in U.S. Pat. 3,534,041), or setiptiline.


Yet additional non-limiting examples of such agents include CX717 from Cortex Pharmaceuticals, TGBAOIAD (a serotonin reuptake inhibitor, 5-HT2 agonist, 5-HTIA agonist, and 5-HT1D agonist) from Fabre-Kramer Pharmaceuticals, Inc., ORG 4420 (an NaSSA (noradrenergic/specific serotonergic antidepressant) from Organon, CP-316,311 (a CRF1 antagonist) from Pfizer, BMS-562086 (a CRF1 antagonist) from Bristol-Myers
Squibb, GW876008 (a CRFl antagonist) from Neurocrine/GlaxoSmithKline, ONO-2333Ms (a CRFl antagonist) from Ono Pharmaceutical Co., Ltd., JNJ-19567470 or TS-041 (a CRFl antagonist) from Janssen (Johnson & Johnson) and Taisho, SSR 125543 or SSR 126374 (a CRFl antagonist) from Sanofi-Aventis, Lu AA21004 and Lu AA24530 (both from H. Lundbeck A/S), SEP-225289 from Sepracor Inc., ND7001 (a PDE2 inhibitor) from Neuro3d, SSR 411298 or SSR 101010 (a fatty acid amide hydrolase, or FAAH, inhibitor) from Sanofi-Aventis, 163090 (a mixed serotonin receptor inhibitor) from GlaxoSmithKline, SSR 241586 (an NK2 and NK3 receptor antagonist) from Sanofi-Aventis, SAR 102279 (an NK2 receptor antagonist) from Sanofi-Aventis, YKP581 from SK Pharmaceuticals (Johnson & Johnson), R1576 (a GPCR modulator) from Roche, or ND1251 (a PDE4 inhibitor) from Neuro3d.

In other embodiments, a method may comprise use of a combination of a 5HTR agent and one or more agents reported as anti-psychotic agents. Non-limiting examples of a reported anti-psychotic agent as a member of a combination include olanzapine, quetiapine (Seroquel®), clozapine (CAS RN 5786-21-0) or its metabolite ACP-104 (N-desmethylclozapine or norclozapine, CAS RN 6104-71-8), reserpine, aripiprazole, risperidone, ziprasidone, sertindole, trazodone, paliperidone (CAS RN 144598-75-4), mifepristone (CAS RN 84371-65-3), bifeprunox or DU-127090 (CAS RN 350992-10-8), asenapine or ORG 5222 (CAS RN 65576-45-6), iloperidone (CAS RN 133454-47-4), ocaperidone (CAS RN 129029-23-8), SLV 308 (CAS RN 269718-83-4), licarbazepine or GP 47779 (CAS RN 29331-92-8), Org 34517 (CAS RN 189035-07-2), ORG 34850 (CAS RN 162607-84-3), Org 24448 (CAS RN 211735-76-1), lurasidone (CAS RN 367514-87-2), blonanserin or lonasen (CAS RN 132810-10-7), talnetan or SB-223412 (CAS RN 174636-32-9), secretin (CAS RN 1393-25-5) or human secretin (CAS RN 108153-74-8) which are endogenous pancreatic hormones, ABT 089 (CAS RN 161417-03-4), SSR 504734 (see compound 13 in Hashimoto "Glycine Transporter Inhibitors as Therapeutic Agents for Schizophrenia." Recent Patents on CNS Drug Discovery, 2006 1:43-53), MEM 3454 (see Mazurov et al. "Selective alpha7 nicotinic acetylcholine receptor ligands." Curr Med Chem. 2006 13(13):1567-84), a phosphodiesterase 10A (PDE10A) inhibitor such as papaverine (CAS RN 58-74-2) or papaverine hydrochloride (CAS RN 61-25-6), paliperidone (CAS RN 144598-75-4), trifluoperazine (CAS RN 117-89-5), or trifluoperazine hydrochloride (CAS RN 440-17-5).

Additional non-limiting examples of such agents include trifluoperazine, fluphenazine, chlorpromazine, perphenazine, thioridazine, haloperidol, loxapine,
mesoridazine, molindone, pimoxide, or thiothixene, SSR 146977 (see Emonds-Alt et al. "Biochemical and pharmacological activities of SSR 146977, a new potent nonpeptide tachykinin NK3 receptor antagonist." Can J Physiol Pharmacol. 2002 80(5):482-8), SSR181507 (5-(3-exo)-8-benzoyl-N-[[2 s]-7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl]methyl]-8-azabiclo[3.2.1]octane-3-methanamine monohydrochloride), or SLV313 (1-(2,3-dihydrobenz[1,4]dioxin-5-yl)-4-[5-(4-fluorophenyl)-pyridin-3-ylmethyl]-piperazine).

Further non-limiting examples of such agents include Lu-35-138 (a D4/5-HT antagonist) from Lundbeck, AVE 1625 (a CBl antagonist) from Sanofi-Aventis, SLV310,313 (a 5-HT2A antagonist) from Solvay, SSR 181507 (a D2/5-HT2 antagonist) from Sanofi-Aventis, GW07034 (a 5-HT6 antagonist) or GW773812 (a D2, 5-HT antagonist) from GlaxoSmithKline, YKP 1538 from SK Pharmaceuticals, SSR 125047 (a sigma receptor antagonist) from Sanofi-Aventis, MEMI 003 (a L-type calcium channel modulator) from Memory Pharmaceuticals, JNJ-17305600 (a GLYT1 inhibitor) from Johnson & Johnson, XY2401 (a glycine site specific NMDA modulator) from Xytis, PNU 170413 from Pfizer, RGH-188 (a D2, D3 antagonist) from Forrest, SSR 180711 (an alpha7 nicotinic acetylcholine receptor partial agonist) or SSR 103800 (a GLYT1 (Type 1 glycine transporter) inhibitor) or SSR 241586 (aNK3 antagonist) from Sanofi-Aventis.

In other disclosed embodiments, a reported anti-psychotic agent may be one used in treating schizophrenia. Non-limiting examples of a reported anti-schizophrenia agent as a member of a combination with a 5HTR agent include molindone hydrochloride (MOBAN®) and TC-1 827 (see Bohme et al. "In vitro and in vivo characterization of TC-1827, a novel brain α4β2 nicotinic receptor agonist with pro-cognitive activity." Drug Development Research 2004 62(l):26-40).

In some embodiments, a method may comprise use of a combination of a 5HTR agent and one or more agents reported for treating weight gain, metabolic syndrome, or obesity, and/or to induce weight loss or prevent weight gain. Non-limiting examples of the reported agent include various diet pills that are commercially or clinically available. In some embodiments, the reported agent is orlistat (CAS RN 96829-58-2), sibutramine (CAS RN 106650-56-0) or sibutramine hydrochloride (CAS RN 84485-00-7), phetermine (CAS RN 122-09-8) or phetermine hydrochloride (CAS RN 1197-21-3), diethylpropion or amfepramone (CAS RN 90-84-6) or diethylpropion hydrochloride, benzphetamine (CAS RN 156-08-1) or benzphetamine hydrochloride, phendimetrazine (CAS RN 634-03-7 or 21784-30-5) or phendimetrazine hydrochloride (CAS RN 17140-98-6) or phendimetrazine tartrate,
rimonabant (CAS RN 168273-06-1), bupropion hydrochloride (CAS RN: 31677-93-7),
topiramate (CAS RN 97240-79-4), zonisamide (CAS RN 68291-97-4), or APD-356 (CAS
RN 846589-98-8).

In other non-limiting embodiments, the agent may be fenfluramine or
Pondimin® (CAS RN 458-24-2), dexfenfluramine or Redux® (CAS RN 3239-44-9), or
levofenfluramine (CAS RN 37577-24-5); or a combination thereof or a combination with
phentermine. Non-limiting examples include a combination of fenfluramine and phentermine
(or "fen-phen") and of dexfenfluramine and phentermine (or "dexfen-phen").

The combination therapy may be of one of the above with a 5HTR agent as
described herein to improve the condition of the subject or patient. Non-limiting examples of
combination therapy include the use of lower dosages of the above additional agents, or
combinations thereof, which reduce side effects of the agent or combination when used alone.
For example, an anti-depressant agent like fluoxetine or paroxetine or sertraline may be
administered at a reduced or limited dose, optionally also reduced in frequency of
administration, in combination with a 5HTR agent.

Similarly, a combination of fenfluramine and phentermine, or phentermine
and dexfenfluramine, may be administered at a reduced or limited dose, optionally also
reduced in frequency of administration, in combination with a 5HTR agent. The reduced
dose or frequency may be that which reduces or eliminates the side effects of the
combination.

In light of the positive recitation (above and below) of combinations with
alternative agents to treat conditions disclosed herein, the disclosure includes embodiments
with the explicit exclusion of one or more of the alternative agents or one or more types of
alternative agents. As would be recognized by the skilled person, a description of the whole
of a plurality of alternative agents (or classes of agents) necessarily includes and describes
subsets of the possible alternatives, such as the part remaining with the exclusion of one or
more of the alternatives or exclusion of one or more classes.

Representative Combinations

As indicated herein, the disclosure includes combination therapy, where a
5HTR agent in combination with one or more other neurogenic agents is used to produce
neurogenesis. When administered as a combination, the therapeutic compounds can be
formulated as separate compositions that are administered at the same time or sequentially at
different times, or the therapeutic compounds can be given as a single composition. The methods of the disclosure are not limited in the sequence of administration.

Instead, the disclosure includes methods wherein treatment with a 5HTR agent and another neurogenic agent occurs over a period of more than about 48 hours, more than about 72 hours, more than about 96 hours, more than about 120 hours, more than about 144 hours, more than about 7 days, more than about 9 days, more than about 11 days, more than about 14 days, more than about 21 days, more than about 28 days, more than about 35 days, more than about 42 days, more than about 49 days, more than about 56 days, more than about 63 days, more than about 70 days, more than about 77 days, more than about 12 weeks, more than about 16 weeks, more than about 20 weeks, or more than about 24 weeks or more. In some embodiments, treatment by administering a 5HTR agent, occurs at least about 12 hours, such as at least about 24, or at least about 36 hours, before administration of another neurogenic agent. Following administration of a 5HTR agent, further administrations may be of only the other neurogenic agent in some embodiments of the disclosure. In other embodiments, further administrations may be of only the 5HTR agent.

In some cases, combination therapy with a 5HTR agent and one or more additional agents results in an enhanced efficacy, safety, therapeutic index, and/or tolerability, and/or reduced side effects (frequency, severity, or other aspects), dosage levels, dosage frequency, and/or treatment duration. Examples of compounds useful in combinations described herein are provided above and below. Structures, synthetic processes, safety profiles, biological activity data, methods for determining biological activity, pharmaceutical preparations, and methods of administration relating to the compounds are known in the art and/or provided in the cited references, all of which are herein incorporated by reference in their entirety. Dosages of compounds administered in combination with a 5HTR agent can be, e.g., a dosage within the range of pharmacological dosages established in humans, or a dosage that is a fraction of the established human dosage, e.g., 70%, 50%, 30%, 10%, or less than the established human dosage.

In some embodiments, the neurogenic agent combined with a 5HTR agent may be a reported opioid or non-opioid (acts independently of an opioid receptor) agent. In some embodiments, the neurogenic agent is one reported as antagonizing one or more opioid receptors or as an inverse agonist of at least one opioid receptor. A opioid receptor antagonist or inverse agonist may be specific or selective (or alternatively non-specific or non-selective) for opioid receptor subtypes. So an antagonist may be non-specific or non-
selective such that it antagonizes more than one of the three known opioid receptor subtypes, identified as \( \text{OP}_1 \), \( \text{OP}_2 \), and \( \text{OP}_3 \) (also known as delta, or \( \delta \), kappa, or \( \kappa \), and mu, or \( \mu \), respectively). Thus an opioid that antagonizes any two, or all three, of these subtypes, or an inverse agonist that is specific or selective for any two or all three of these subtypes, may be used as the neurogenic agent in the practice. Alternatively, an antagonist or inverse agonist may be specific or selective for one of the three subtypes, such as the kappa subtype as a non-limiting example.

Non-limiting examples of reported opioid antagonists include naltrindol, naloxone, naloxene, naltrexone, JDTic (Registry Number 785835-79-2; also known as 3-isoquinolinonecarboxamide, 1,2,3,4-tetrahydro-7-hydroxy-N-[(1S)-l-][(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-l-piperidinyl]methyl]-2-methylpropyl]-dihydrochloride, (3R)-(9CI), nor-binaltorphimine, and buprenorphine. In some embodiments, a reported selective kappa opioid receptor antagonist compound, as described in US 20020132828, U.S. Patent 6,559,159, and/or WO 2002/053533, may be used. All three of these documents are herein incorporated by reference in their entireties as if fully set forth. Further non-limiting examples of such reported antagonists is a compound disclosed in U.S. Patent 6,900,228 (herein incorporated by reference in its entirety), arodyn (Ac[Phe(l,2,3),Arg(4),d-Ala(8)]Dyn A-(I-1 1)NH(2), as described in Bennett, et al. (2002) J Med. Chem. 45:5617-5619), and an active analog of arodyn as described in Bennett et al. (2005) J Pept Res. 65(3):322-32, alvimopan.

In some embodiments, the neurogenic agent used in the methods described herein has "selective" activity (such as in the case of an antagonist or inverse agonist) under certain conditions against one or more opioid receptor subtypes with respect to the degree and/or nature of activity against one or more other opioid receptor subtypes. For example, in some embodiments, the neurogenic agent has an antagonist effect against one or more subtypes, and a much weaker effect or substantially no effect against other subtypes. As another example, an additional neurogenic agent used in the methods described herein may act as an agonist at one or more opioid receptor subtypes and as antagonist at one or more other opioid receptor subtypes. In some embodiments, a neurogenic agent has activity against kappa opioid receptors, while having substantially lesser activity against one or both of the delta and mu receptor subtypes. In other embodiments, a neurogenic agent has activity against two opioid receptor subtypes, such as the kappa and delta subtypes. As non-limiting examples, the agents naloxone and naltrexone have nonselective antagonist activities against
more than one opioid receptor subtypes. In certain embodiments, selective activity of one or more opioid antagonists results in enhanced efficacy, fewer side effects, lower effective dosages, less frequent dosing, or other desirable attributes.

An opioid receptor antagonist is an agent able to inhibit one or more characteristic responses of an opioid receptor or receptor subtype. As a non-limiting example, an antagonist may competitively or non-competitively bind to an opioid receptor, an agonist or partial agonist (or other ligand) of a receptor, and/or a downstream signaling molecule to inhibit a receptor's function.

An inverse agonist able to block or inhibit a constitutive activity of an opioid receptor may also be used. An inverse agonist may competitively or non-competitively bind to an opioid receptor and/or a downstream signaling molecule to inhibit a receptor's function. Non-limiting examples of inverse agonists for use in the disclosed methods include ICI-174864 (AyV-diallyl-Tyr-Aib-Aib-Phe-Leu), RTI-5989-1, RTI-5989-23, and RTI-5989-25 (see Zaki et al. J. Pharmacol. Exp. Therap. 298(3): 1015-1020, 2001).

Additional embodiments of the disclosure include a combination of a 5HTR agent with an additional agent such as acetylcholine or a reported modulator of an androgen receptor. Non-limiting examples include the androgen receptor agonists ehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS).

Alternatively, the neurogenic agent in combination with a 5HTR agent may be an enzymatic inhibitor, such as a reported inhibitor of HMG CoA reductase. Non-limiting examples of such inhibitors include atorvastatin (CAS RN 134523-00-5), cerivastatin (CAS RN 145599-86-6), crilvastatin (CAS RN 120551-59-9), fluvastatin (CAS RN 93957-54-1) and fluvastatin sodium (CAS RN 93957-55-2), simvastatin (CAS RN 79902-63-9), lovastatin (CAS RN 75330-75-5), pravastatin (CAS RN 81093-37-0) or pravastatin sodium, rosuvastatin (CAS RN 287714-41-4), and simvastatin (CAS RN 79902-63-9). Formulations containing one or more of such inhibitors may also be used in a combination. Non-limiting examples include formulations comprising lovastatin such as Advicor® (an extended-release, niacin containing formulation) or Altocor® (an extended release formulation); and formulations comprising simvastatin such as Vytorin® (combination of simvastatin and ezetimibe).

In other non-limiting embodiments, the neurogenic agent in combination with a 5HTR agent may be a reported Rho kinase inhibitor. Non-limiting examples of such an inhibitor include fasudil (CAS RN 103745-39-7); fasudil hydrochloride (CAS RN 105628-
07-7); the metabolite of fasudil, which is hydroxyfasudil (see Shimokawa et al. "Rho-kinase-mediated pathway induces enhanced myosin light chain phosphorylations in a swine model of coronary artery spasm." Cardiovasc Res. 1999 43:1029-1039, Y 27632 (CAS RN 138381-45-0); a fasudil analog thereof such as (S)-Hexahydro-l-(4-ethylenisoquinoline-5-sulfonyl)-2-methyl-IH-1,4-diazepine, (S)-hexahydro-4-glycyl-2-methyl-1-(4-methylisoquinoline-5-sulfonyl)-IH-1,4-diazepine, or (S)-(++)-2-methyl-l-[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine (also known as H-1 152P; see Sasaki et al. "The novel and specific Rho-kinase inhibitor (S)-(++)-2-methyl-l-[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine as a probing molecule for Rho-kinase-involved pathway." Pharmacol Ther. 2002 93(2-3):225-32); or a substituted isoquinolinesulfonamide compound as disclosed in U.S. Patent 6,906,061.

Furthermore, the neurogenic agent in combination with a 5HTR agent may be a reported GSK-3 inhibitor or modulator. In some non-limiting embodiments, the reported GSK3-beta modulator is a paullone, such as alsterpaullone, kenpaullone (9-bromo-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one), kgwennpaullone (see Knockaert et al. "Intracellular Targets of Paullones. Identification following affinity purification on immobilized inhibitor." J Biol Chem. 2002 277(28):25493-501), azakenpaullone (see Kunick et al. "1-Azakenpaullone is a selective inhibitor of glycogen synthase kinase-3 beta." Bioorg Med Chem Lett. 2004 14(2):413-6), or the compounds described in U.S. Publication No. 20030181439; International Publication No. WO 01/60374; Leost et al., Eur. J. Biochem. 267:5983-5994 (2000); Kunick et al., J Med Chem.; 47(1): 22-36 (2004); or Shultz et al., J. Med. Chem. 42:2909-2919 (1999); an anticonvulsant, such as lithium or a derivative thereof (e.g., a compound described in U.S. Patent Nos. 1,873,732; 3,814,812; and 4,301,176); carbemazepine, valproic acid or a derivative thereof (e.g., valproate, or a compound described in Werstuck et al., Bioorg Med Chem Lett., 14(22): 5465-7 (2004)); lamotrigine; SL 76002 (Progabide), gabapentin; tiagabine; or vigabatrin; a maleimide or a related compound, such as Ro 31-8220, SB-216763, SB-41011 l t, SB-495052, or SB-415286, or a compound described, e.g., in U.S. Pat. No. 6,719,520; U.S. Publication No. 20040010031; International Publication Nos. WO-2004072062; WO-03082859; WO-03103663; WO-03095452; WO-200500836; WO 0021927; WO-03076398; WO-00021927; WO-00038675; or WO-03076442; or Coghlan et al., Chemistry & Biology 7: 793 (2000); a pyridine or pyrimidine derivative, or a related compound (such as 5-iodotubercidin, GI 179186X, GW 784752X and GW 784775X, and compounds described, e.g., in U.S. Pat. Nos.
WO 01/09106; WO 00/21927; WO01/41768; WO 00/17184; WO 04/037791; WO-
04065370; WO 01/37819; WO 01/42224; WO 01/85685; WO 04/072063; WO-2004085439;
Chem. Lett. 12, 1525 (2002); CP-79049, GI 179186X, GW 784752X, GW 784775X, AZD-
1080, AR-014418, SN-8914, SN-3728, OTDZT, Aloisine A, TWSI 19, CHIR98023,
CHIR99021, CHIR98014, CHIR98023, 5-iodotubercidin, Ro 31-8220, SB-216763, SB-
4101 11, SB-495052, SB-415286, alstef aullone, kenpaullone, gwennpaullone, LY294002,
wortmannin, sildenafil, CT98014, CT-99025, flavoperidol, or L803-mts.

In yet further embodiments, the neurogenic agent used in combination with a
5HTR agent may be a reported glutamate modulator or metabotropic glutamate (mGlu)
receptor modulator. In some embodiments, the reported mGlu receptor modulator is a Group
II modulator, having activity against one or more Group II receptors (mGlu₂ and/or mGlu₃).
Embodiments include those where the Group II modulator is a Group II agonist. Non-
limiting examples of Group II agonists include: (i) (1S,3R)-1-aminocyclopentane-1,3-
dicarboxylic acid (ACPD), a broad spectrum mGlu agonist having substantial activity at
Group I and II receptors; (ii) (−)-2-thia-4-aminobicyclo-hexane-4,6-dicarboxylate
(LY389795), which is described in Monn et al., J. Med. Chem., 42(6):1027-40 (1999); (iii)
compounds described in US App. No. 20040102521 and Pellicciari et al., J. Med. Chem., 39,
2259-2269 (1996); and (iv) the Group II-specific modulators described below.

Non-limiting examples of reported Group II antagonists include: (i)
phenylglycine analogues, such as (RS)-alpha-methyl-4-sulphonophenylglycine (MSPG),
(RS)-alpha-methyl-4-phosphonophenylglycine (MPPG), and (RS)-alpha-methyl-4-
tetrazolylphenylglycine (MTPG), described in Jane et al., Neuropharmacology 34: 851-856
(1995); (ii) LY366457, which is described in O’Neill et al., Neuropharmacol., 45(5): 565-74
(2003); (iii) compounds described in US App Nos. 20050049243, 200501 19345 and
20030157647; and (iv) the Group II-specific modulators described below.

In some non-limiting embodiments, the reported Group II modulator is a
Group II-selective modulator, capable of modulating mGlu₂ and/or mGlu₃ under conditions
where it is substantially inactive at other mGlu subtypes (of Groups I and III). Examples of
Group II-selective modulators include compounds described in Monn, et al., J. Med. Chem.,
40, 528-537 (1997); Schoepp, et al., Neuropharmacol., 36, 1-1 1 (1997) (e.g., 1S,2S,5R,6S-2-
aminobicyclohexane-2,6-dicarboxylate); and Schoepp, Neurochem. Int., 24, 439 (1994).
Non-limiting examples of reported Group II-selective agonists include (i) (+)-2-aminobicyclohexane-2,6-dicarboxylic acid (LY354740), which is described in Johnson et al., Drug Metab. Disposition, 30(1): 27-33 (2002) and Bond et al., NeuroReport 8: 1463-1466 (1997), and is systemically active after oral administration (e.g., Grillon et al., Psychopharmacol. (Berl), 168: 446-454 (2003)); (ii) (-)-2-oxa-4-aminobicyclohexane-4,6-dicarboxylic acid (LY379268), which is described in Monn et al., J. Med. Chem. 42: 1027-1040 (1999) and US Pat. No. 5,688,826. LY379268 is readily permeable across the blood-brain barrier, and has EC\textsubscript{50} values in the low nanomolar range (e.g., below about 10 nM, or below about 5 nM) against human mGlu2 and mGlu3 receptors in vitro; (iii) (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylic acid ((2R,4R)-APDC), which is described in Monn et al., J. Med. Chem. 39: 2990 (1996) and Schoepp et al., Neuropharmacology, 38: 1431 (1999); (iv) (1S,3S)-l-aminocyclopentane-1,3-dicarboxylic acid ((1S,3S)-ACPD), described in Schoepp, Neurochem. Int., 24: 439 (1994); (v) (2i?,4i?)4-aminopyrrolidine-2,4-dicarboxylic acid ((2R,4R)-APDC), described in Howson and Jane, British Journal of Pharmacology, 139, 147-155 (2003); (vi) (2S,rS,2'S)-2-(carboxycyclopropyl)-glycine (L-CCG-I), described in Brabet et al., Neuropharmacology 37: 1043-1051 (1998); (vii) (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV), described in Hayashi et al., Nature, 366, 687-690 (1993); (viii) IS,2S,5R,6S-2-aminobicyclohexane-2,6-dicarboxylate, described in Monn, et al., J. Med. Chem., 40, 528 (1997) and Schoepp, et al., Neuropharmacol., 36, 1 (1997); and (ix) compounds described in US App. No. 20040002478; US Pat. Nos. 6,204,292, 6,333,428, 5,750,566 and 6,498,180; and Bond et al., Neuroreport 8: 1463-1466 (1997).

Non-limiting examples of reported Group II-selective antagonists useful in methods provided herein include the competitive antagonist (2S)-2-amino-2-((1S,2S)-carboxycyclopropyl)-l-yl)-3-(xanth-9-yl)-propanoic acid (LY341495), which is described, e.g., in Kingston et al., Neuropharmacology 37: 1-12 (1998) and Monn et al., J Med Chem 42: 1027-1040 (1999). LY341495 is readily permeably across the blood-brain barrier, and has IC\textsubscript{50} values in the low nanomolar range (e.g., below about 10 nM, or below about 5 nM) against cloned human mGlu2 and mGlu3 receptors. LY341495 has a high degree of selectivity for Group II receptors relative to Group I and Group III receptors at low concentrations (e.g., nanomolar range), whereas at higher concentrations (e.g., above 1µM), LY341495 also has antagonist activity against InGlu7 and mGlu4, in addition to mGlu2/3. LY341495 is substantially inactive against KA, AMPA, and NMDA iGlu receptors.
Additional non-limiting examples of reported Group II-selective antagonists include the following compounds, indicated by chemical name and/or described in the cited references: (i) \( \alpha \)-methyl-L-(carboxycyclopropyl) glycine (CCG); (ii) \((2S,3S,4S)-2\)-methyl-2-(carboxycyclopropyl) glycine (MCCG); (iii) \((1R,2R,3R,5R,6R)-2\)-amino-3-(3,4-dichlorobenzoxyl)-6 fluorobicyclohexane-2,6-dicarboxylic acid (GLA) (Group II antagonist/Group III partial agonist/antagonist); (iv) \((2S,2'R,3'R)-2\)-(2',3'-heptyl, n-octyl, 5-methylbutyl, or 6-methylpentyl ester prodrug of MGS0039; (v) MGS0210 (3-(3,4-dichlorobenzoxyl)-2-amino-6-fluorobicyclohexane-2,6-dicarboxylic acid n-heptyl ester); (vi) \((2S,1'S,2'S,3'R)-2\)-(2'-carboxy-3'-phenylcyclopropyl)glycine (PCCG-IV); and (ix) compounds described in US Pat No. 6,107,342 and US App No. 200400061 14. APICA has an IC_{50} value of approximately 30 \( \mu \)M against mGluR \(_2\) and mGluR \(_3\), with no appreciable activity against Group I or Group III receptors at sub-mM concentrations.

In some non-limiting embodiments, a reported Group II-selective modulator is a subtype-selective modulator, capable of modulating the activity of mGlu \(_2\) under conditions in which it is substantially inactive at mGlu \(_3\) (mGlu \(_2\)-selective), or vice versa (mGlu \(_3\)-selective). Non-limiting examples of subtype-selective modulators include compounds described in US Pat Nos. 6,376,532 (mGlu \(_2\)-selective agonists) and US App No. 2004002478 (mGlu \(_3\)-selective agonists). Additional non-limiting examples of subtype-selective modulators include allosteric mGlu receptor modulators (mGlu \(_2\) and mGlu \(_3\)) and NAAG-related compounds (mGlu \(_3\)), such as those described below.

In other non-limiting embodiments, a reported Group II modulator is a compound with activity at Group I and/or Group III receptors, in addition to Group II receptors, while having selectivity with respect to one or more mGlu receptor subtypes. Non-limiting examples of such compounds include: (i) \((2S,3S,4S)-2\)-(carboxycyclopropyl)glycine (L-CCG-I) (Group I/Group II agonist), which is described in Nicoletti et al., Trends Neurosci. 19: 267-271 (1996), Nakagawa, et al., Eur. J. Pharmacol, 184, 205 (1990), Hayashi, et al., Br. J. Pharmacol, 107, 539 (1992), and Schoepf et al, J. Neurochem., 63., page 769-772 (1994); (ii) \((S)-4\)-(carboxy-3-hydroxyphenyl)glycine \((4C_3HPG)\) (Group II agonist/Group I competitive antagonist); (iii) gamma-carboxy-L-glutamic acid (GLA) (Group II antagonist/Group III partial agonist/antagonist); (iv) \((2S,2'R,3'R)-2\)-(2,3-
dicarboxycyclopropyl)glycine (DCG-IV) (Group II agonist/Group III antagonist), which is described in Ohfune et al., Bioorg. Med. Chem. Lett., 3: 15 (1993); (v) (RS)-a-methyl-4-carboxyphenylglycine (MCPG) (Group I/Group II competitive antagonist), which is described in Eaton et al., Eur. J. Pharmacol., 244: 195 (1993), Collingridge and Watkins, TiPS, 15: 333 (1994), and Joly et al., J. Neurosci., 15: 3970 (1995); and (vi) the Group II/III modulators described in US Pat Nos. 5,916,920, 5,688,826, 5,945,417, 5,958,960, 6,143,783, 6,268,507, 6,284,785.

In some non-limiting embodiments, the reported mGlu receptor modulator comprises (S)-MCPG (the active isomer of the Group I/Group II competitive antagonist (RS)-MCPG) substantially free from (R)-MCPG. (S)-MCPG is described, e.g., in Sekiyama et al., Br. J. Pharmacol., 117: 1493 (1996) and Collingridge and Watkins, TiPS, 15: 333 (1994).

Additional non-limiting examples of reported mGlu modulators useful in methods disclosed herein include compounds described in US Pat Nos. 6,956,049, 6,825,211, 5,473,077, 5,912,248, 6,054,448, and 5,500,420; US App Nos. 20040077599, 20040147482, 20040102521, 20030199533 and 20050234048; and Intl Pub/App Nos. WO 97/19049, WO 98/00391, and EP0870760.

In some non-limiting embodiments, the reported mGlu receptor modulator is a prodrug, metabolite, or other derivative of N-acetylaspartylglutamate (NAAG), a peptide neurotransmitter in the mammalian CNS that is a highly selective agonist for mGluR₃ receptors, as described in Wroblewska et al., J. Neurochem., 69(1): 174-181 (1997). In other embodiments, the mGlu modulator is a compound that modulates the levels of endogenous NAAG, such as an inhibitor of the enzyme N-acetylated-alpha-linked-acidic dipeptidase (NAALADase), which catalyzes the hydrolysis of NAAG to N-acetyl-aspartate and glutamate. Examples of NAALADase inhibitors include 2-PMPA (2-(phosphonomethyl)pentanedioic acid), which is described in Slusher et al., Nat. Med., 5(12): 1396-402 (1999); and compounds described in J. Med. Chem. 39: 619 (1996), US Pub. No. 20040002478, and US Pat Nos. 6,313,159, 6,479,470, and 6,528,499. In some embodiments, the mGlu modulator is the mGlu₃-selective antagonist, beta-NAAG.

Additional non-limiting examples of reported glutamate modulators include memantine (CAS RN 19982-08-2), memantine hydrochloride (CAS RN 41100-52-1), and riluzole (CAS RN 1744-22-5).

In some non-limiting embodiments, a reported Group II modulator is administered in combination with one or more additional compounds reported as active.
against a Group I and/or a Group III mGlu receptor. For example, in some cases, methods comprise modulating the activity of at least one Group I receptor and at least one Group II mGlu receptor (e.g., with a compound described herein). Examples of compounds useful in modulating the activity of Group I receptors include Group I-selective agonists, such as (i) trans-azetidine-2,4,6-tricarboxylic acid (tADA), which is described in Kozikowski et al., J. Med. Chem., 36: 2706 (1993) and Manahan-Vaughan et al., Neuroscience, 72: 999 (1996); (ii) (RS)-3,5-dihydroxyphenylglycine (DHPG), which is described in Ito et al., NeuroReport 3: 1013 (1992); or a composition comprising (S)-DHPG substantially free of (R)-DHPG, as described, e.g., in Baker et al., Bioorg.Med.Chem.Lett. 5: 223 (1995); (iii) (RS)-2-amino-2-methyl-4-phosphonobutanoic acid; (iv) the Group III-selective antagonists (RS)-α-methyl-2-(phenylazo)-3-pyridinol which is described in Birse et al., Neuroscience 52: 481 (1993); or a composition comprising (S)-3-hydroxyphenylglycine substantially free of (R)-3-hydroxyphenylglycine, as described, e.g., in Hayashi et al., J.Neurosci., 14: 3370 (1994); (iv) and (S)-homoquisqualate, which is described in Porter et al., Br. J. Pharmacol., 106: 509 (1992).

Additional non-limiting examples of reported Group I modulators include (i) Group I agonists, such as (RS)-3,5-dihydroxyphenylglycine, described in Brabet et al., Neuropharmacology, 34, 895-903, 1995; and compounds described in US Pat Nos. 6,399,641 and 6,589,978, and US Pub No. 20030212066; (ii) Group I antagonists, such as (S)-4-carboxy-3-hydroxyphenylglycine; 7-(hydroxylimino)cyclopropa-β-chromen-1 α-carboxylate ethyl ester; (RS)-1-aminooindan-1,5-dicarboxylic acid (AIDA); 2-methyl-6(phenylethynyl)pyridine (MPEP); 2-methyl-6-(2-phenylethenyl)pyridine (SIB-1 893); 6-methyl-2-(phenylazo)-3-pyridinol (SIB-1 757); (Sα-amino-4-carboxy-2-methylbenzeneacetic acid; and compounds described in US Pat Nos. 6,586,422, 6,583,575, 5,843,988, 5,536,721, 6,429,207, 5,696,148, and 6,218,385, and US Pub Nos. 20030109504, 20030013715, 20050154027, 20050004130, 20050209273, 20050197361, and 20040082592; (iii) mGlu5-selective agonists, such as (RS)-2-chloro-5-hydroxyphenylglycine (CHPG); and (iv) mGlus-selective antagonists, such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP); and compounds described in US Pat No. 6,660,753; and US Pub Nos. 20030195139, 20040229917, 20050153986, 20050085514, 20050065340, 20050026963, 20050020585, and 20040259917.

Non-limiting examples of compounds reported to modulate Group III receptors include (i) the Group III-selective agonists (L)-2-amino-4-phosphonobutyric acid (L-AP4), described in Knopfel et al., J. Med Chem., 38, 1417-1426 (1995); and (S)-2-amino-2-methyl-4-phosphonobutanoic acid; (ii) the Group III-selective antagonists (RS)-α-
cyclopropyl-4-phosphonophenylglycine; (RS)-α-methylserine-O-phosphate (MSOP); and compounds described in US App. No. 20030109504; and (iii) (IS,3R,4S)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-I).

In additional embodiments, the neurogenic agent used in combination with a 5HTR agent may be a reported alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) modulator. Non-limiting examples include CX-516 or ampalex (CAS RN 154235-83-3), Org-24448 (CAS RN 211735-76-1), LY451395 (2-propanesulfonamide, N-[(2R)-2-[4'-(2-[methylsulfonyl]amino)ethyl][l,l'-biphenyl]-4-yl]propyl]). LY-450108 (see Jhee et al. "Multiple-dose plasma pharmacokinetic and safety study of LY450108 and LY451395 (AMPA receptor potentiaters) and their concentration in cerebrospinal fluid in healthy human subjects." J Clin Pharmacol. 2006 46(4):424-32), and CX717. Additional examples of reported antagonists include irampanel (CAS RN 206260-33-5) and E-2007.

Further non-limiting examples of reported AMPA receptor antagonists for use in combinations include YM90K (CAS RN 154164-30-4), YM872 or zonampanel (CAS RN 210245-80-0), NBQX (or 2,3-dioxo-6-nitro-7-sulfamoylbenzo(f)quinoxaline; CAS RN 118876-58-7), PNQX (1,4,7,8,9,10-hexahydro-9-methyl-6-nitropyrido[3,4-f]quinoxaline-2,3-dione), and ZK200775 ([l,2,3,4-tetrahydro-7-morpholinyl-2,3-dioxo-6-(fluoromethyl) quinoxalin-1-yl] methylphosphonate).

In additional embodiments, a neurogenic agent used in combination with a 5HTR agent may be a reported muscarinic agent. Non-limiting examples of a reported muscarinic agonist include a muscarinic agonist such as milameline (CI-979), or a structurally or functionally related compound disclosed in U.S. Patent Nos. 4,786,648, 5,362,860, 5,424,301, 5,650,174, 4,710,508, 5,314,901, 5,356,914, or 5,356,912; or xanomeline, or a structurally or functionally related compound disclosed in U.S. Patent Nos. 5,041,455, 5,043,345, or 5,260,314.

Other non-limiting examples include a muscarinic agent such as alvameline (LU 25-109), or a functionally or structurally compound disclosed in U.S. Pat. Nos. 6,297,262, 4,866,077, RE36,374, 4,925,858, PCT Publication No. WO 97/17074, or in Moltzen et al., J Med Chem. 1994 Nov 25;37(24):4085-99; 2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (YM-796) or YM-954, or a functionally or structurally related compound disclosed in U.S. Patent Nos. 4,940,795, RE34,653, 4,996,210, 5,041,549, 5,403,931, or 5,412,096, or in Wanibuchi et al., Eur. J. Pharmacol., 187, 479-486 (1990); cevimeline (AF102B), or a functionally or structurally compound disclosed in U.S. Pat. Nos.
4,855,290, 5,340,821, 5,580,880 (American Home Products), or 4,981,858 (optical isomers of AF102B); sabcomeline (SB 202026), or a functionally or structurally related compound described in U.S. Patent Nos. 5,278,170, RE35,593, 6,468,560, 5,773,619, 5,808,075, 5,545,740, 5,534,522, or 6,596,869, U.S. Patent Publication Nos. 2002/0127271, 2003/0129246, 2002/0150618, 2001/0018074, 2003/0157169, or 2001/0003588, Bromidge et al, J Med Chem. 19:40(26):4265-80 (1997), or Harries et al, British J Pharm., 124, 409-415 (1998); talsaclidine (WAL 2014 FU), or a functionally or structurally compound disclosed in U.S. Patent Nos. 5,451,587, 5,286,864, 5,508,405, 5,451,587, 5,286,864, 5,508,405, or 5,137,895, or in Pharmacol. Toxicol., 78, 59-68 (1996); or a 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazole derivative, such as tetra(ethyleneglycol)(4-methoxy-1,2,5-thiadiazol-3-yl)[3-(1-methyl-1,2,5,6-tetrahydropyrid-3-yl)-1,2,5-thiadiazol-4-yl]ether, or a compound that is functionally or structurally related to a 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazole derivative as provided by Cao et al. ("Synthesis and biological characterization of 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazole derivatives as muscarinic agonists for the treatment of neurological disorders." J. Med. Chem. 46(20):4273-4286, 2003).

Yet additional non-limiting examples include besipiridine, SR-46559, L-689,660, S-9977-2, AF-102, thiopilocarpine, or an analog of clozapine, such as a pharmaceutically acceptable salt, ester, amide, or prodrug form thereof, or a diaryl[a,d]cycloheptene, such as an amino substituted form thereof, or N-desmethylclozapine, which has been reported to be a metabolite of clozapine, or an analog or related compound disclosed in US 2005/0192268 or WO 05/63254.

In other embodiments, the muscarinic agent is an m1 receptor agonist selected from 55-LH-3B, 55-LH-25A, 55-LH-30B, 55-LH-4-1A, 40-LH-67, 55-LH-15A, 55-LH-16B, 55-LH-11C, 55-LH-31A, 55-LH-46, 55-LH-47, 55-LH-4-3A, or a compound that is functionally or structurally related to one or more of these agonists disclosed in US 2005/0130961 or WO 04/087158.

In additional embodiments, the muscarinic agent is a benzimidazolidinone derivative, or a functionally or structurally compound disclosed in U.S. Patent 6,951,849, US 2003/0100545, WO 04/089942, or WO 03/028650; a spiroazacyclic compound, or a functionally or structurally related related compound like 1-oxa-3,8-diaza-spiro[4,5]decan-2-one or a compound disclosed in U.S. Patent 6,911,452 or WO 03/057698; or a
tetrahydroquinoline analog, or a functionally or structurally compound disclosed in US 2003/0176418, US 2005/0209226, or WO 03/057672.

In yet additional embodiments, the neurogenic agent in combination with a 5HTR agent is a reported HDAC inhibitor. The term "HDAC" refers to any one of a family of enzymes that remove acetyl groups from the epsilon-amino groups of lysine residues at the N-terminus of a histone. An HDAC inhibitor refers to compounds capable of inhibiting, reducing, or otherwise modulating the deacetylation of histones mediated by a histone deacetylase. Non-limiting examples of a reported HDAC inhibitor include a short-chain fatty acid, such as butyric acid, phenylbutyrate (PB), 4-phenylbutyrate (4-PBA), pivaloyloxymethyl butyrate (Pivanex, AN-9), isovalerate, valerate, valproate, valproic acid, propionate, butyramide, isobutyramide, phenylacetate, 3-bromopropionate, or tributyrin; a compound bearing a hydroxamic acid group, such as suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), trichostatin C (TSC), salicylhydroxamic acid, oxamflatin, suberic bishydroxamic acid (SBHA), m-carboxy-cinnamic acid bishydroxamic acid (CBHA), pyroxamide (CAS RN 382 180-17-8), diethyl bis-(pentamethylene-N,N-dimethylcarboxamide) malonate (EMBA), azelaic bishydroxamic acid (ABHA), azelaic-1-hydroxamate-9-anilide (AAHA), 6-(3-chlorophenylureido) carpoic hydroxamic acid, or A-161906; a cyclic tetrapeptide, such as depsipeptide (FK228), FR225497, trapoxin A, apicidin, chlamydocin, or HC-toxin; a benzamide, such as MS-275; depudecin, a sulfonamide anilide (e.g., diallyl sulfide), BL1521, curcumin (diferuloylmethane), CI-994 (N-acetyldinaline), spiruchostatin A, scriptaid, carbamazepine (CBZ), or a related compound; a compound comprising a cyclic tetrapeptide group and a hydroxamic acid group (examples of such compounds are described in U.S. Patent Nos. 6,833,384 and 6,552,065); a compound comprising a benzamide group and a hydroxamic acid group (examples of such compounds are described in Ryu et al., Cancer Lett. 2005 Jul 9 (epub), Plumb et al, Mol Cancer Ther., 2(8):721-8 (2003), Ragno et al., J Med Chem, 47(6):1351-9 (2004), Mai et al., J Med Chem., 47(5):1098-109 (2004), Mai et al, J Med Chem., 46(4):512-24 (2003), Mai et al., J Med Chem., 45(9):1778-84 (2002), Massa et al., J Med Chem., 44(13):2069-72 (2001), Mai et al, J Med Chem., 48(9):3344-53 (2005), and Mai et al, J Med Chem, 46(23):4826-9 (2003)); a compound described in U.S. Patent Nos. 6,897,220, 6,888,027, 5,369,108, 6,541,661, 6,720,445, 6,562,995, 6,777,217, or 6,387,673, or U.S. Patent Publication Nos. 20050171347, 20050165016, 20050159470, 20050143385, 20050137234, 20050137232, 200501477225, 20050113732, 20050107445, 20050107384, 20050096468, 20050085515,
20050032831, 20050014839, 20040266769, 20040254220, 20040229889, 20040198830, 20040142953, 20040106599, 20040092598, ... in methods described herein include triazolophthalazine derivatives, such as those disclosed in WO 99/25353, and modes reported include limiting translational receptor tacedinaline Derivatives American Journal of Metabolites, prodrugs, and stereoisomers thereof; and a molecule that inhibits the transcription and/or translation of one or more HDACs.

Additional non-limiting examples include a reported HDac inhibitor selected from ONO-2506 or arundic acid (CAS RN 185517-21-9); MGCD0103 (see Gelmon et al. 2005: 3147 and Kalita et al. "Pharmacodynamic effect of MGCD0103, an oral isotype-selective histone deacetylase (HDAC) inhibitor, on HDAC enzyme inhibition and histone acetylation induction in Phase I clinical trials in patients (pts) with advanced solid tumors or non-Hodgkin's lymphoma (NHL)" Journal of Clinical Oncology, 2005 ASCO Annual Meeting Proceedings. 23(16S, June 1 Supplement), 2005: 9631), a reported thiophenyl derivative of benzamide HDac inhibitor as presented at the 97th American Association for Cancer Research (AACR) Annual Meeting in Washington, DC. in a poster titled "Enhanced Isotype-Selectivity and Antiproliferative Activity of Thiophenyl Derivatives of BenzamideHDAC Inhibitors In Human Cancer Cells," (abstract #4725), and a reported HDac inhibitor as described in U.S. Patent 6,541,661; SAHA or vorinostat (CAS RN 149647-78-9); PXDIO1 or PXD 101 or PX 105684 (CAS RN 414864-00-9), CI-994 or tacedinaline (CAS RN 112522-64-2), MS-275 (CAS RN 209783-80-2), or an inhibitor reported in WO2005/108367.

In other embodiments, the neurogenic agent in combination with a 5HTR agent is a reported GABA modulator which modulates GABA receptor activity at the receptor level (e.g., by binding directly to GABA receptors), at the transcriptional and/or translational level (e.g., by preventing GABA receptor gene expression), and/or by other modes (e.g., by binding to a ligand or effector of a GABA receptor, or by modulating the activity of an agent that directly or indirectly modulates GABA receptor activity). Non-limiting examples of GABA-A receptor modulators useful in methods described herein include triazolophthalazine derivatives, such as those disclosed in WO 99/25353, and
WO/98/04560; tricyclic pyrazolo-pyridazinone analogues, such as those disclosed in WO 99/00391; fenamates, such as those disclosed in 5,637,617; triazolo-pyridazine derivatives, such as those disclosed in WO 99/37649, WO 99/37648, and WO 99/37644; pyrazolo-pyridine derivatives, such as those disclosed in WO 99/48892; nicotinic derivatives, such as those disclosed in WO 99/43661 and 5,723,462; muscimol, thiomuscimol, and compounds disclosed in 3,242,190; baclofen and compounds disclosed in 3,471,548; phaclofen; quisqualamine; ZAPA; zaleplon; THIP; imidazole-4-acetic acid (IMA); (+)-bicuculline; gabalinoleamide; isoguvicaine; 3-aminopropane sulphonic acid; piperidine-4-sulphonic acid; 4,5,6,7-tetrahydro-[5,4-c]-pyridin-3-ol; SR 95531; RU5315; CGP 55845; CGP 35348; FG 8094; SCH 5091 1; NG2-73; NGD-96-3; pricrotoxin and other bicyclophosphates disclosed in Bowery et al., Br. J. Pharmacol., 57; 435 (1976).

Additional non-limiting examples of GABA-A modulators include compounds described in 6,503,925; 6,218,547; 6,399,604; 6,515,140; 6,451,809; 6,448,259; 6,448,246; 6,423,711; 6,414,147; 6,399,604; 6,380,209; 6,353,109; 6,297,252; 6,268,496; 6,21,1365; 6,166,203; 6,177,569; 6,194,427; 6,156,898; 6,143,760; 6,127,395; 6,103,903; 6,103,731; 6,723,735; 6,479,506; 6,476,030; 6,337,331; 6,730,676; 6,730,681; 6,828,322; 6,872,720; 6,699,859; 6,696,444; 6,617,326; 6,608,062; 6,579,875; 6,541,484; 6,500,828; 6,355,798; 6,333,336; 6,319,924; 6,303,605; 6,303,597; 6,291,460; 6,255,305; 6,233,255; 6,872,731; 6,900,215; 6,642,229; 6,593,325; 6,914,060; 6,914,063; 6,914,065; 6,936,608; 6,534,505; 6,426,343; 6,313,125; 6,310,203; 6,200,975; 6,071,909; 5,922,724; 6,096,887; 6,080,873; 6,013,799; 5,936,095; 5,925,770; 5,910,590; 5,908,932; 5,849,927; 5,840,888; 5,817,813; 5,804,686; 5,792,766; 5,750,702; 5,744,603; 5,744,602; 5,723,462; 5,696,260; 5,693,801; 5,668,283; 5,637,725; 5,625,063; 5,610,299; 5,608,079; 5,606,059; 5,604,235; 5,585,490; 5,510,480; 5,484,944; 5,473,073; 5,463,054; 5,451,585; 5,426,186; 5,367,077; 5,328,912 5,326,868; 5,312,822; 5,306,819; 5,286,860; 5,266,698; 5,243,049; 5,216,159; 5,212,310; 5,185,446; 5,185,446; 5,182,290; 5,130,430; 5,095,015; 20050014939; 20040171633; 20050165048; 20050165023; 20040259818; and 20040192692.

In some embodiments, the GABA-A modulator is a subunit-selective modulator. Non-limiting examples of GABA-A modulator having specificity for the alpha1 subunit include alpidem and Zolpidem. Non-limiting examples of GABA-A modulator having specificity for the alpha2 and/or alpha3 subunits include compounds described in 6,730,681; 6,828,322; 6,872,720; 6,699,859; 6,696,444; 6,617,326; 6,608,062; 6,579,875;
6,541,484; 6,500,828; 6,355,798; 6,333,336; 6,319,924; 6,303,605; 6,303,597; 6,291,460; 6,255,305; 6,133,255; 6,900,215; ... in Yasumatsu et al., Br J Pharmacol 111: 1170-1178 (1994); and compounds described in U.S. Patent 4,513,135.

bromazepam, benzodiazepine related examples described in (1980).


In some embodiments, the GABA-A receptor modulator is a reported allosteric modulator. In various embodiments, allosteric modulators modulate one or more aspects of the activity of GABA at the target GABA receptor, such as potency, maximal effect, affinity, and/or responsiveness to other GABA modulators. In some embodiments, allosteric modulators potentiate the effect of GABA (e.g., positive allosteric modulators), and/or reduce the effect of GABA (e.g., inverse agonists). Non-limiting examples of benzodiazepine GABA-A modulators include alprazolam, bentazepam, bretazenil, bromazepam, brotizolam, cannazepam, chlordiazepoxide, clobazam, clonazepam, cinolazepam, clotiazepam, cloxazolam, clozasin, delorazepam, diazepam, dibenzepin, dipotassium chlorazepate, divaplon, etazolam, ethyl-loflazepat, etizolam, fludiazepam, flumazenil, flunitrazepam, flurazepam HCl, flutoprazepam, halazepam, haloxazolam, imidazenil, ketazolam, lorazepam, loprazolam, lormetazepam, medazepam, metaclazepam, mexoxolam, midazolam-HCl, nabanezil, nimetazepam, nitrazepam, nordazepam, oxazepam-tazepam, oxazolam, pinazepam, prazezam, quazepam, sarmazenil, suriclone, temazepam, tetrazepam, tofisopam, triazolam, zaleplon, zalezepam, Zolpidem, zopiclone, and zopielon.

Non-limiting examples of barbiturate or barbituric acid derivative GABA-A modulators include phenobarbital, pentobarbital, pentobarbitone, primidone, barbexaclon, dipropyl barbituric acid, eunarcon, hexobarbital, mephobarbital, methohexital, Na-methohexital, 2,4,6(1H,3H,5)-pyrimidintrion, secbutabarbital and/or thiopental.


Non-limiting examples of beta-carboline GABA-A modulators include abecarnil, 3,4-dihydro-beta-carboline, gedocarnil, 1-methyl-1-vinyl-2,3,4-trihydro-beta-carboline-3-carboxylic acid, 6-methoxy-1,2,3,4-tetrahydro-beta-carboline, N-BOC-L-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid, tryptoline, pinoline, methoxyharmalan, tetrahydro-beta-carboline (THBC), 1-methyl-THBC, 6-methoxy-THBC, 6-hydroxy-THBC, 6-methoxyharmalan, norharman, 3,4-dihydro-beta-carboline, and compounds described in Nielsen et al., Nature, 286: 606 (1980).

In some embodiments, the GABA modulator modulates GABA-B receptor activity. Non-limiting examples of reported GABA-B receptor modulators useful in methods described herein include CGP36742; CGP-64213; CGP 56999A; CGP 54433A; CGP 36742; SCH 50911; CGP 7930; CGP 13501; baclofen and compounds disclosed in 3,471,548; saclofen; phaclofen; 2-hydroxysaclofen; SKF 97541; CGP 35348 and related compounds described in Olpe et al, Eur. J. Pharmacol., 187, 27 (1990); phosphinic acid derivatives described in Hills, et al, Br. J. Pharmacol., 102, pp. 5-6 (1991); and compounds described in 4,656,298, 5,929,236, EP0463969, EP 0356128, Kaupmann et al., Nature 368: 239 (1997).

In some embodiments, the GABA modulator modulates GABA-C receptor activity. Non-limiting examples of reported GABA-C receptor modulators useful in methods described herein include cis-aminocrotonic acid (CACA); 1,2,5,6-tetrahydropyridine-4-yl methyl phosphinic acid (TPMPA) and related compounds such as P4MPA, PPA and SEPI; 2-methyl-TACA; (+/-)-TAMP; muscimol and compounds disclosed in 3,242,190; ZAPA; THIP and related analogues, such as aza-THIP; picrotoxin; imidazole-4-acetic acid (IMA); and CGP36742.

In some embodiments, the GABA modulator modulates the activity of glutamic acid decarboxylase (GAD).

In some embodiments, the GABA modulator modulates GABA transaminase (GTA). Non-limiting examples of GTA modulators include the GABA analog vigabatrin, and compounds disclosed in 3,960,927.

In some embodiments, the GABA modulator modulates the reuptake and/or transport of GABA from extracellular regions. In other embodiments, the GABA modulator modulates the activity of the GABA transporters, GAT-I, GAT-2, GAT-3 and/or BGT-I. Non-limiting examples of GABA reuptake and/or transport modulators include nipecotic acid and related derivatives, such as CI 966; SKF 89976A; TACA; stiripentol; tiagabine and GAT-I inhibitors disclosed in 5,010,090; (R)-1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid and related compounds disclosed in 4,383,999; (R)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid and related compounds disclosed in Anderson et al., J. Med. Chem. 36, (1993) 1716-1725; guvacine and related compounds disclosed in Krogsgaard-Larsen, Molecular & Cellular Biochemistry 31, 105-121 (1980); GAT-4 inhibitors disclosed in 6,071,932; and compounds disclosed in 6,906,177 and Ali, F. E., et al. J. Med. Chem. 1985, 28, 653-660. Methods for detecting GABA reuptake inhibitors are known in the art, and are described, e.g., in 6,906,177; 6,225,1 15; 4,383,999; Ali, F. E., et al. J. Med. Chem. 1985, 28, 653-660.

In some embodiments, the GABA modulator is the benzodiazepine clonazepam, which is described, e.g., in 3,121,076 and 3,116,203; the benzodiazepine diazepam, which is described, e.g., in 3,371,085; 3,109,843; and 3,136,815; the short-acting diazepam derivative midazolam, which is a described, e.g., in 4,280,957; the imidazodiazepine flumazenil, which is described, e.g., in 4,316,839; the benzodiazepine

Additionally, the neurogenic agent in combination with a 5HTR agent may be a neurogenic sensitizing agent that is a reported anti-epileptic agent. Non-limiting examples of such agents include carbamazepine or tegretol (CAS RN 298-46-4), clonazepam (CAS RN 1622-61-3), BPA or 3-(p-boronophenyl)alanine (CAS RN 90580-64-6), gabapentin or neuronin (CAS RN 60142-96-3), phenytoin (CAS RN 57-41-0), topiramate, lamotrigine or lamictal (CAS RN 84057-84-1), phenobarbital (CAS RN 50-06-6), oxcarbazepine (CAS RN 28721-07-5), primidone (CAS RN 125-33-7), ethosuximide (CAS RN 77-67-8), levetiracetam (CAS RN 102767-28-2), zonisamide, tiagabine (CAS RN 115103-54-3),

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depakote or divalproex sodium (CAS RN 76584-70-8), felbamate (Na-channel and NMDA receptor antagonist), or pregabalin (CAS RN 148553-50-8).

In further embodiments, the neurogenic sensitizing agent may be a reported direct or indirect modulator of dopamine receptors. Non-limiting examples of such agents include the indirect dopamine agonists methylphenidate (CAS RN 113-45-1) or methylphenidate hydrochloride (also known as Ritalin® CAS RN 298-59-9), amphetamine (CAS RN 300-62-9) and methamphetamine (CAS RN 537-46-2), and the direct dopamine agonists sumanirole (CAS RN 179386-43-7), roprinirole (CAS RN 91374-21-9), and rotigotine (CAS RN 99755-59-6). Additional non-limiting examples include 7-OH-DPAT, quinpirole, haloperidole, or clozapine.

Additional non-limiting examples include bromocriptine (CAS RN 25614-03-3), adrogolide (CAS RN 171752-56-0), pramipexole (CAS RN 104632-26-0), roprinirole (CAS RN 91374-21-9), apomorphine (CAS RN 58-00-4) or apomorphine hydrochloride (CAS RN 314-19-2), lisuride (CAS RN 18016-80-3), sibenadet hydrochloride or viozan (CAS RN 154189-24-9), L-DOPA or levodopa (CAS RN 59-92-7), melevodopa (CAS RN 7101-51-1), etilevodopa (CAS RN 37178-37-3), talipexole hydrochloride (CAS RN 36085-73-1) or talipexole (CAS RN 101626-70-4), nolomirole (CAS RN 90060-42-7), quinelorane (CAS RN 97466-90-5), pergolide (CAS RN 66104-22-1), fenoldopam (CAS RN 67227-56-9), carmoxirole (CAS RN 98323-83-2), terguride (CAS RN 37686-84-3), cabergoline (CAS RN 81409-90-7), quinagolide (CAS RN 87056-78-8) or quinagolide hydrochloride (CAS RN 94424-50-7), sumanirole, docarpamine (CAS RN 74639-40-0), SLV-308 or 2(3H)-benzoxazolone, 7-(4-methyl-l-piperazinyl)-monohydrochloride (CAS RN 269718-83-4), aripiprazole (CAS RN 129722-12-9), bifeprunox, lisdexamfetamine dimesylate (CAS RN 608137-33-3), safmamide (CAS RN 133865-89-1), or adderall or amfetamine (CAS RN 300-62-9).

In further embodiments, the neurogenic agent used in combination with a 5HTR agent may be a reported dual sodium and calcium channel modulator. Non-limiting examples of such agents include safmamide and zonisamide. Additional non-limiting examples include enecadin (CAS RN 259525-01-4), levosemotiadil (CAS RN 116476-16-5), bisaramil (CAS RN 89194-77-4), SL-34.0829 (see U.S. Patent 6,897,305), lifarizine (CAS RN 119514-66-8), JTV-519 (4-[3-(4-benzylpiperidin-l-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride), and delapril.
In further embodiments, the neurogenic agent in used in combination with a 5HTR agent may be a reported calcium channel antagonist such as amlodipine (CAS RN 88150-42-9) or amlodipine maleate (CAS RN 88150-47-4), nifedipine (CAS RN 21829-25-4), MEM-1003 (CAS RN see Rose et al. "Efficacy of MEM 1003, a novel calcium channel blocker, in delay and trace eyeblink conditioning in older rabbits." Neurobiol Aging. 2006 Apr 16; [Epub ahead of print]), isradipine (CAS RN 75695-93-1), felodipine (CAS RN 72509-76-3; 3,5-Pyridinedicarboxylic acid, 1,4-dihydro-4-(2,3-dichlorophenyl)-2,6-dimethyl-, ethyl methyl ester) or felodipine (CAS RN 86189-69-7; 3,5-Pyridinedicarboxylic acid, 4-((2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ethyl methyl ester, (+--)), lemlidipine (CAS RN 125729-29-5 or 94739-29-4), clevidipine (CAS RN 166432-28-6 or 167221-71-8), verapamil (CAS RN 52-53-9), ziconotide, cinnarizine (CAS RN 298-57-7), monatepil maleate (CAS RN 132046-06-1), manidipine (CAS RN 89226-50-6), furnidipine (CAS RN 138661-03-7), nitrendipine (CAS RN 39562-70-4), loperamide (CAS RN 53179-1-1-6), amiodarone (CAS RN 1951-25-3), bepridil (CAS RN 64706-54-3), diltiazem (CAS RN 42399-41-7), nimodipine (CAS RN 66085-59-4), lamotrigine, cinnarizine (CAS RN 298-57-7), lacipidine (CAS RN 103890-78-4), nilvadipine (CAS RN 75530-68-6), dotarizine (CAS RN 84625-59-2), cilnidipine (CAS RN 132203-70-4), oxodipine (CAS RN 90729-41-2), aranidipine (CAS RN 86780-90-7), anipamil (CAS RN 83200-10-6), ipenoxazone (CAS RN 104454-71-9), efondipine hydrochloride or NZ 105 (CAS RN 11101-1-53-1) or efondipine (CAS RN 11101-1-63-3), temiverine (CAS RN 173324-94-2), pranidipine (CAS RN 99522-79-9), dopropidil (CAS RN 79700-61-1), lercanidipine (CAS RN 100427-26-7), terodiline (CAS RN 15793-40-5), fantofarone (CAS RN 114432-13-2), azelnidipine (CAS RN 123524-52-7), mibebradil (CAS RN 116644-53-2) or mibebradil dihydrochloride (CAS RN 116666-63-8), SB-237376 (see Xu et al. "Electrophysiologic effects of SB-237376: a new antiarrhythmic compound with dual potassium and calcium channel blocking action." J Cardiovasc Pharmacol. 2003 41(3):414-21), BRL-32872 (CAS RN 113241-47-7), S-2150 (see Ishibashi et al. "Pharmacodynamics of S-2150, a simultaneous calcium-blocking and alpha 1-inhibiting antihypertensive drug, in rats." J Pharm Pharmacol. 2000 52(3):273-80), nisoldipine (CAS RN 63675-72-9), semotiadil (CAS RN 116476-13-2), palonidipine (CAS RN 96515-73-0) or palonidipine hydrochloride (CAS RN 96515-74-1), SL-87.0495 (see U.S. Patent 6,897,305), YM430 (4(((S)-2-hydroxy-3-phenoxypropyl)amino)butyl methyl 2,6-dimethyl-((S)-4-(m-nitrophenyl))-1,4-dihydropyridine-3,5-dicarboxylate), barnidipine (CAS RN 104713-75-9), and AM336 or CVID (see Adams et al. "Omega-Conotoxin CVID Inhibits a

In other embodiments, the neurogenic agent used in combination with a 5HTR agent may be a reported modulator of a melatonin receptor. Non-limiting examples of such modulators include the melatonin receptor agonists melatonin, LY-156735 (CAS RN 118702-11-7), agomelatine (CAS RN 1381 12-76-2), 6-chloromelatonin (CAS RN 63762-74-3), ramelteon (CAS RN 196597-26-9), 2-Methyl-6,7-dichloromelatonin (CAS RN 104513-29-3), and ML 23 (CAS RN 108929-03-9).

In yet further embodiments, the neurogenic agent in combination with a 5HTR agent may be a reported modulator of a melanocortin receptor. Non-limiting examples of such agents include a melanocortin receptor agonists selected from melanotan II (CAS RN 121062-08-6), PT-141 or bremelanotide (CAS RN 189691-06-3), HP-228 (see Getting et al. "The melanocortin peptide HP228 displays protective effects in acute models of inflammation and organ damage." Eur J Pharmacol. 2006 Jan 24), or AP214 from Action Pharma A/S.

Additional embodiments include a combination of a 5HTR agent and a reported modulator of angiotensin II function, such as at an angiotensin II receptor. In some embodiments, the neurogenic sensitizing agent used with a 5HTR agent may be a reported inhibitor of an angiotensin converting enzyme (ACE), or a rennin inhibitor, such as aliskerin. Non-limiting examples of such reported inhibitors include a sulphydryl-containing (or mercapto-containing) agent, such as alacepril, captopril (Capoten®), fentiapril, pirepropril, pivalopril, or zofenopril; a dicarboxylate-containing agent, such as enalapril (Vasotec® or Renitec®) or enalaprilat, ramipril (Altace® or Trifluraz® or Ramace®), quinapril (Accupril®) or quinapril hydrochloride, perindopril (Coversyl®) or perindopril erbumine (Aceon®), lisinopril (Lisodur® or Prinivil® or Zestril®); a phosphate-containing (or phosphate-containing) agent, such as fosinopril (Monopril®), fosinoprilat, fosinopril sodium (CAS RN 88889-14-9), benazepril (Lotensin®) or benazepril hydrochloride, imidapril or imidapril hydrochloride, moexipril (Univasc®), or trandolapril (Mavik®). In other embodiments, a modulator is administered in the form of an ester that increases bioavailability upon oral administration with subsequent conversion into metabolites with greater activity.

Further embodiments include reported angiotensin II modulating entities that are naturally occurring, such as casokinins and lactokinins (breakdown products of casein and
whey) which may be administered as such to obviate the need for their formation during digestion. Additional non-limiting embodiments of reported angiotensin receptor antagonists include candesartan (Atacand® or Ratacand®, 139481-59-7) or candesartan cilexetil; eprosartan (Teveten®) or eprosartan mesylate; irbesartan (Aprove® or Karvea® or Avapro®); losartan (Cozaar® or Hyzaar®); olmesartan (Benicar®, CAS RN 144689-24-7) or olmesartan medoxomil (CAS RN 144689-63-4); telmisartan (Micardis® or Pritor®); or valsartan (Diovan®).

Additional non-limiting examples of a reported angiotensin modulator that may be used in a combination include nateglinide or starlix (CAS RN 105816-04-4); tasosartan or its metabolite enoltasosartan; omapatrilat (CAS RN 167305-00-2); or a combination of nateglinide and valsartan, amoldipine and benazepril (Lotrel 10-40 or Lotrel 5-40), or delapril and manidipine (CHF 1521).

Additionally, the agent used in combination with a 5HTR agent may be a reported compound (or "monoamine modulator") that modulates neurotransmission mediated by one or more monoamine neurotransmitters (referred to herein as "monoamines") or other biogenic amines, such as trace amines (TAs) as a non-limiting example. TAs are endogenous, CNS-active amines that are structurally related to classical biogenic amines (e.g., norepinephrine, dopamine (4-(2-aminoethyl)benzene-1,2-diol), and/or serotonin (5-hydroxytryptamine (5-HT), or a metabolite, precursor, prodrug, or analog thereof. The methods of the disclosure thus include administration of one or more reported TAs in a combination with a 5HTR agent. Additional CNS-active monoamine receptor modulators are well known in the art, and are described, e.g., in the Merck Index, 12th Ed. (1996).

Certain food products, e.g., chocolates, cheeses, and wines, can also provide a significant dietary source of TAs and/or TA-related compounds. Non-limiting examples of mammalian TAs useful as constitutive factors include, but are not limited to, tryptamine, p-tyramine, m-tyramine, octopamine, synephrine or β-phenylethylamine (β-PEA). Additional useful TA-related compounds include, but are not limited to, 5-hydroxytryptamine, amphetamine, bufotenin, 5-methoxytryptamine, dihydromethoxytryptamine, phenylephrine, or a metabolite, precursor, prodrug, or analogue thereof.

In some embodiments, the constitutive factor is a biogenic amine or a ligand of a trace amine-associated receptor (TAAR), and/or an agent that mediates one or more biological effects of a TA. TAs have been shown to bind to and activate a number of unique receptors, termed TAARs, which comprise a family of G-protein coupled receptors (TAAR1-
TAAR9) with homology to classical biogenic amine receptors. For example, TAAR1 is activated by both tyramine and β-PEA.

Thus non-limiting embodiments include methods and combination compositions wherein the constitutive factor is β-PEA, which has been indicated as having a significant neuromodulator role in the mammalian CNS and is found at relatively high levels in the hippocampus (e.g., Taga et al., Biomed Chromatogr., 3(3): 118-20 (1989)); a metabolite, prodrug, precursor, or other analogue of β-PEA, such as the β-PEA precursor L-phenylalanine, the β-PEA metabolite β-phenylacetic acid (β-PAA), or the β-PEA analogues methylphenidate, amphetamine, and related compounds.

Most TAs and monoamines have a short half-life (e.g., less than about 30 s) due, e.g., to their rapid extracellular metabolism. Thus embodiments of the disclosure include use of a monoamine "metabolic modulator," which increases the extracellular concentration of one or more monoamines by inhibiting monoamine metabolism, h some embodiments, the metabolic modulator is an inhibitor of the enzyme monoamine oxidase (MAO), which catalyzes the extracellular breakdown of monoamines into inactive species. Isoforms MAO-A and/or MAO-B provide the major pathway for TA metabolism. Thus, in some embodiments, TA levels are regulated by modulating the activity of MAO-A and/or MAO-B. For example, in some embodiments, endogenous TA levels are increased (and TA signaling is enhanced) by administering an inhibitor of MAO-A and/or MAO-B, in combination with a 5HTR agent as described herein.

Non-limiting examples of inhibitors of monoamine oxidase (MAO) include reported inhibitors of the MAO-A isoform, which preferentially deaminates 5-hydroxytryptamine (serotonin) (5-HT) and norepinephrine (NE), and/or the MAO-B isoform, which preferentially deaminates phenylethylamine (PEA) and benzylamine (both MAO-A and MAO-B metabolize Dopamine (DA)). In various embodiments, MAO inhibitors may be irreversible or reversible (e.g., reversible inhibitors of MAO-A (RIMA)), and may have varying potencies against MAO-A and/or MAO-B (e.g., non-selective dual inhibitors or isoform-selective inhibitors). Non-limiting examples of MAO inhibitors useful in methods described herein include clorgyline, L-deprenyl, isocarboxazid (Marplan®), ayahuasca, nialamide, iproniazide, iproclozide, moclobemide (Aurorix®), phenelzine (Nardil®), tranylcypromine (Parnate®) (the congeneric of phenelzine), toclofamine, levo-deprenyl (Selegiline®), harmala, RIMAs (e.g., moclobemide, described in Da Prada et al., J Pharmacol Exp Ther 248: 400-414 (1989); brofaromine; and befloxatone, described in Curet et al., J

In additional embodiments, the monoamine modulator is an "uptake inhibitor," which increases extracellular monoamine levels by inhibiting the transport of monoamines away from the synaptic cleft and/or other extracellular regions. In some embodiments, the monoamine modulator is a monoamine uptake inhibitor, which may selectively/preferentially inhibit uptake of one or more monoamines relative to one or more other monoamines. The term "uptake inhibitors" includes compounds that inhibit the transport of monoamines (e.g., uptake inhibitors) and/or the binding of monoamine substrates (e.g., uptake blockers) by transporter proteins (e.g., the dopamine transporter (DAT), the NE transporter (NET), the 5-HT transporter (SERT), and/or the extraneuronal monoamine transporter (EMT)) and/or other molecules that mediate the removal of extracellular monoamines. Monoamine uptake inhibitors are generally classified according to their potencies with respect to particular monoamines, as described, e.g., in Koe, J. Pharmacol. Exp. Ther. 199: 649-661 (1976). However, references to compounds as being active against one or more monoamines are not intended to be exhaustive or inclusive of the monoamines modulated in vivo, but rather as general guidance for the skilled practitioner in selecting compounds for use in therapeutic methods provided herein.

In embodiments relating to a biogenic amine modulator used in a combination or method with a 5HTR agent as disclosed herein, the modulator may be (i) a norepinephrine and dopamine reuptake inhibitor, such as bupropion (described, e.g., in U.S. Pat. 3,819,706 and 3,885,046), or (S,S)-hydroxybupropion (described, e.g., in U.S. Pat. 6,342,496); (ii) selective dopamine reuptake inhibitors, such as medifoxamine, amineptine (described, e.g., in U.S. Pat. 3,758,528 and 3,821,249), GBR12909, GBR12783 and GBR13069, described in Andersen, Eur J Pharmacol, 166:493-504 (1989); or (iii) a monoamine "releaser" which stimulates the release of monoamines, such as biogenic amines from presynaptic sites, e.g., by modulating presynaptic receptors (e.g., autoreceptors, heteroreceptors), modulating the packaging (e.g., vesicular formation) and/or release (e.g., vesicular fusion and release) of monoamines, and/or otherwise modulating monoamine release. Advantageously, monoamine releasers provide a method for increasing levels of one or more monoamines within the synaptic cleft or other extracellular region independently of the activity of the presynaptic neuron.
Monoamine releasers useful in combinations provided herein include fenfluramine or p-chloroamphetamine (PCA) or the dopamine, norepinephrine, and serotonin releasing compound amineptine (described, e.g., in U.S. Pat. 3,758,528 and 3,821,249).

The agent used with a 5HTR agent may be a reported phosphodiesterase (PDE) inhibitor. In some embodiments, a reported inhibitor of PDE activity include an inhibitor of a cAMP-specific PDE. Non-limiting examples of cAMP specific PDE inhibitors useful in the methods described herein include a pyrrolidinone, such as a compound disclosed in U.S. Pat. 5,665,754, US20040152754 or US20040023945; a quinazolineone, such as a compound disclosed in U.S. Pat. 6,747,035 or 6,828,315, WO 97/49702 or WO 97/42174; a xanthine derivative; a phenylpyridine, such as a compound disclosed in U.S. Pat. 6,410,547 or 6,090,817, or WO 97/22585; a diazepine derivative, such as a compound disclosed in WO 97/36905; an oxime derivative, such as a compound disclosed in U.S. Pat. 5,693,659 or WO 96/00215; a naphthyridine, such as a compound described in U.S. Patents, 5,817,670, 6,740,662, 6,136,821, 6,331,548, 6,297,248, 6,541,480, 6,642,250, or 6,900,205, or Trifilieff et al., Pharmacology, 301(1): 241-248 (2002), or Hersperger et al., J Med Chem., 43(4):675-82 (2000); a benzofuran, such as a compound disclosed in U.S. Patents 5,902,824, 6,211,203, 6,514,996, 6,716,987, 6,376,535, 6,080,782, or 6,054,475, or EP 819688, EP685479, or Perrier et al., Bioorg. Med. Chem. Lett. 9:323-326 (1999); a phenanthridine, such as that disclosed in U.S. Patents 6,191,138, 6,121,279, or 6,127,378; a benzoxazole, such as that disclosed in U.S. Pat. 6,166,041 or 6,376,485; a purine derivative, such as a compound disclosed in U.S. Pat. 6,228,859; a benzamide, such as a compound described in U.S. Pat. 5,981,527 or 5,712,298, or WO95/01338, WO 97/48697 or Ashton et al., J. Med Chem 37: 1696-1703 (1994); a substituted phenyl compound, such as a compound disclosed in U.S. Patents. 6,297,264, 5,866,593,65 5,859,034, 6,245,774, 6,197,792, 6,080,790, 6,077,854, 25 5,962,483, 5,674,880, 5,786,354, 5,739,144, 5,776,958, 5,798,373, 5,891,896, 5,849,770, 5,550,137, 5,340,827, 5,780,478, 5,780,477, or 5,633,257, or WO 95/35283; a substituted biphenyl compound, such as that disclosed in U.S. Pat. 5,877,190; or a quinilinone, such as a compound described in U.S. Pat. 6,800,625 or WO 98/14432.

Additional non-limiting examples of reported cAMP-specific PDE inhibitors useful in methods disclosed herein include a compound disclosed in U.S. Patents 6,818,651, 6,737,436, 6,613,778, 6,617,357, 6,146,876, 6,838,559, 6,884,800, 6,716,987, 6,514,996, 6,376,535, 6,740,655, 6,559,168, 6,069,151, 6,365,585, 6,313,116, 6,245,774, 6,011,037, 6,127,363, 6,303,789, 6,316,472, 6,348,602, 6,331,543, 6,333,354, 5,491,147, 5,608,070,
In some embodiments, the reported cAMP-specific PDE inhibitor is cilomilast (SB-207499); filaminast; tibenelast (LY-186655); ibudilast; piclamilast (RP 73401);
doxofylline; cipamfylline (HEP-688); atizoram (CP-80633); theophylline;
isobutylmethylxanthine; mesopram (ZK-1 17137); zardaverine; vinpocetine; rolipram (ZK-
doxofylline (LAS-31025); roflumilast (BY-217); pumafentrin (BY-343); denbufylline;
EHNA; milrinone; siguazodon; zaprinast; tolafentrine; Isbufylline; IBMX; IC-485;
dyphylline; verolyllyline; bampyffilline; pentoxyfilline; enprofylline; lirilimilast (BAY 19-8004);
filaminast (WAY- PDA-641); benafentrine; trequinsin; nitroquazone; cistostamide;
vesnarinine; piroximone; enoximone; amrinone; olprinone; imazodan or 5-methyl-imazodan;
indolidan; anagrelide; carbazeran; ampizone; emoradan; motapizone; phthalazinol; lixazinone
(RE 82856); quazinone; bemorand (RWJ 22867); adibendan (BM 14,478); pimobendan
(MCI-154); saterinone (BDF 8634); tetovalast (OPC-6535); benzafentrine; sulmazole (ARL
115); revizinone; 349-U-85; AH-21-132; ATZ-1993; AWD-12-343; AWD-12-281; AWD-
12-232; BRL 50481; CC-7085; CDC-801; CDC-998; CDP-840; CH-422; CH-673; CH-928;
CH-3697; CH-3442; CH-2874; CH-4139; Chiroscience 245412; CI-930; CI-1018; CI-1044;
CI-11 18; CP-353164; CP-77059; CP-146523; CP-293321; CP-220629; CT-2450; CT-2820;
CT-3883; CT-5210; D-4418; D-22888; E-4021; EMD 54622; EMD-53998; EMD-57033;
GF-248; GW-3600; IC-485; ICI 63197; ICI 153,1 10; IPL-4088; KF-19514; KW-4490; L-
PDE. Non-limiting examples of a cGMP specific PDE inhibitor for use in the combinations and methods described herein include a pyrimidine or pyrimidinone derivative, such as a compound described in U.S. Pat. 6,677,335, 6,458,951, 6,251,904, 6,787,548, 5,294,612, 5,250,534, or 6,469,012, WO 94/28902, WO96/16657, EP0702555, and Eddahibi, Br. J. Pharmacol., 125(4): 681-688 (1988); a griseolic acid derivative, such as a compound disclosed in U.S. Pat. 4,460,765; a 1-arilnaphthalene lignan, such as that described in Ukita, J. Med. Chem. 42(7): 1293-1305 (1999); a quinazoline derivative, such as 4-[[3',4'- (methylenedioxy)benzyl] amino]-6-methoxyquinazoline] or a compound described in U.S. Pats. 3,932,407 or 4,146,718, or RE31,617; a pyrroloquinolone or pyrrolopyridinone, such as that described in U.S. Pat. 6,686,349, 6,635,638, 6,818,646, US20050113402; a carboline derivative, such a compound described in U.S. Pats. 6,492,358, 6,462,047, 6,821,975, 6,306,870, 6,178,881, 6,043,252, or 3,819,631, US20030166641, WO 97/43287, Daugan et al, J Med Chem., 46(21):4533-42 (2003), or Daugan et al, J Med Chem., 9;46(21):4525-32 (2003); an imidazoo derivative, such as a compound disclosed in U.S. Pats. 6,130,333, 6,566,360, 6,362,178, or 6,582,351, US20050070541, or US20040067945; or a compound described in U.S. Pats. 6,825,197, 5,719,283, 6,943,166, 5,981,527, 6,576,644, 5,859,009, 6,943,253, 6,864,253, 5,869,516, 5,488,055, 6,140,329, 5,859,006, or 6,143,777, WO 96/16644, WO 01/19802, WO 96/26940, Dunn, Org. Proc. Res. Dev., 9: 88-97 (2005), or Bi et al, Bioorg Med Chem Lett, 11(18):2461-4 (2001).
In some embodiments, the PDE inhibitor used in a combination or method disclosed herein is caffeine. In some embodiments, the caffeine is administered in a formulation comprising a 5HTR agent. In other embodiments, the caffeine is administered simultaneously with a 5HTR agent. In alternative embodiments, the caffeine is administered in a formulation, dosage, or concentration lower or higher than that of a caffeineinated beverage such as coffee, tea, or soft drinks. In further embodiments, the caffeine is administered by a non-oral means, including, but not limited to, parenteral (e.g., intravenous, intradermal, subcutaneous, inhalation), transdermal (topical), transmucosal, rectal, or intranasal (including, but not limited to, inhalation of aerosol suspensions for delivery of compositions to the nasal mucosa, trachea and bronchioli) administration. The disclosure includes embodiments with the explicit exclusion of caffeine or another one or more of the described agents for use in combination with a 5HTR agent.

In further alternative embodiments, the caffeine is in an isolated form, such as that which is separated from one or more molecules or macromolecules normally found with caffeine before use in a combination or method as disclosed herein. In other embodiments, the caffeine is completely or partially purified from one or more molecules or macromolecules normally found with the caffeine. Exemplary cases of molecules or macromolecules found with caffeine include a plant or plant part, an animal or animal part, and a food or beverage product.

Non-limiting examples of a reported PDE1 inhibitor include IBMX; vinpocetine; MMPX; KS-505a; SCH-51866; W-7; PLX650; PLX371; PLX788; a phenothiazines; or a compound described in U.S. Pat. 4,861,891.

Non-limiting examples of a PDE2 inhibitor include EHNA; PLX650; PLX369; PLX788; PLX 939; Bay 60-7550 or a related compound described in Boess et al., Neuropharmacology, 47(7):1081-92 (2004); or a compound described in US20020132754.

Non-limiting examples of reported PDE3 inhibitors include a dihydroquinolinolone compound such as cilostamide, cilostazol, vesnarinone, or OPC 3911; an imidazolone such as piroximone or enoximone; a bipyridine such as milrinone, amrinone or olprinone; an imidazoline such as imazodan or 5-methyl-imazodan; a pyridazinone such as indolidan; LY1 81512 (see Komas et al. "Differential sensitivity to cardiotonic drugs of cyclic AMP phosphodiesterases isolated from canine ventricular and sinoatrial-enriched tissues." J Cardiovase Pharmacol. 1989 14(2):213-20); ibudilast; isomazole; motapizone; phthalazinol; trequinsin; lixazinone (RS 82856); Y-590; SKF 94120; quazinone; ICI 153,1 10; bemorandan.
(RWJ 22867); siguazodan (SK&F 94836); adibendan (BM 14,478); pimobendan (UD-CG 115, MCI-154); saterinone (BDF 8634); NSP-153; zardaverine; a quinazoline; benzafentrine; sulmazole (ARL 115); ORG 9935; CI-930; SKF-95654; SDZ-MKS-492; 349-U-85; EMD-53998; EMD-57033; NSP-306; NSP-307; Revizitone; NM-702; WIN-62582; ATZ-1993; WIN-63291; ZK-6271; PLX650; PLX369; PLX788; PLX939; anagrelide; carbazeran; amipzone; emoradan; or a compound disclosed in 6,156,753.

Non-limiting examples of reported PDE4 inhibitors include a pyrrolidinone, such as a compound disclosed in U.S. Pat. 5,665,754, US20040152754 or US20040023945; a quinazolineone, such as a compound disclosed in U.S. Pats. 6,747,035 or 6,828,315, WO 97/49702 or WO 97/42174; a xanthine derivative; a phenylpyridine, such as a compound disclosed in U.S. Pat. 6,410,547 or 6,090,817 or WO 97/22585; a diazepine derivative, such as a compound disclosed in WO 97/36905; an oxime derivative, such as a compound disclosed in U.S. Pat. 5,693,659 or WO 96/00215; a naphthyridine, such as a compound described in U.S. Pats. 5,817,670, 6,740,662, 6,136,821, 6,331,548, 6,297,248, 6,541,480, 6,642,250, or 6,900,205, Trifilieff et al., Pharmacology, 301(1): 241-248 (2002) or Hersperger et al., J Med Chem., 43(4):675-82 (2000); abenozofuran, such as a compound disclosed in U.S. Pats. 5,902,824, 6,211,203, 6,514,996, 6,716,987, 6,376,535, 6,080,782, or 6,054,475, EP 819688, EP685479, or Perrier et al., Bioorg. Med. Chem. Lett. 9:323-326 (1999); a phenanthridine, such as that disclosed in U.S. Pats. 6,191,138, 6,121,279, or 6,127,378; a benzoxazole, such as that disclosed in U.S. Pats. 6,166,041 or 6,376,485; a purine derivative, such as a compound disclosed in U.S. Pat. 6,228,859; abenzamid, such as a compound described in U.S. Pats. 5,981,527 or 5,712,298, WO95/01338, WO 97/48697, or Ashton et al., J. Med Chem 37: 1696-1703 (1994); a substituted phenyl compound, such as a compound disclosed in U.S. Pats. 6,297,264, 5,866,593,65, 5,859,034, 6,245,774, 6,197,792, 6,080,790, 6,077,854, 5,962,483, 5,674,880, 5,786,354, 5,739,144, 5,776,958, 5,798,373, 5,891,896, 5,849,770, 5,550,137, 5,340,827, 5,780,478, 5,780,477, or 5,633,257, or WO 95/35283; a substituted biphenyl compound, such as that disclosed in U.S. Pat. 5,877,190; or a quininilone, such as a compound disclosed in U.S. Pat. 6,800,625 or WO 98/14432.

Additional examples of reported PDE4 inhibitors useful in methods provided herein include a compound disclosed in U.S. Pats. 6,716,987, 6,514,996, 6,376,535, 6,740,655, 6,559,168, 6,069,151, 6,365,585, 6,313,116, 6,245,774, 6,011,037, 6,127,363, 6,303,789, 6,316,472, 6,348,602, 6,331,543, 6,333,354, 5,491,147, 5,608,070, 5,622,977, 5,580,888, 6,680,336, 6,569,890, 6,569,885, 6,500,856, 6,486,186, 6,458,787, 6,455,562,
Non-limiting examples of a reported PDE5 inhibitor useful in a combination or method described herein include a pyrimidine or pyrimidinone derivative, such as a compound described in U.S. Pat. 6,677,335, 6,458,951, 6,251,904, 6,787,548, 5,294,612, 5,250,534, or 6,469,012, WO 94/28902, WO96/16657, EP0702555, or Eddahibi, Br. J. Pharmacol., 125(4): 681-688 (1988); a griseolic acid derivative, such as a compound disclosed in U.S. Pat. 4,460,765; a 1-arylnaphthalene lignan, such as that described in Ukita, J. Med. Chem. 42(7): 1293-1305 (1999); a quinazoline derivative, such as 4-[[3',4'-methyleneoxy]benzyl] aminol-6-methoxyquinazoline or a compound described in U.S. Pats. 3,932,407 or 4,146,718, or RE31,617; a pyrroloquinolones or pyrrolopyridinone, such as that described in U.S. Pats. 6,686,349, 6,635,638, or 6,818,646, US200501 13402; a carboline derivative, such a compound described in U.S. Pats. 6,492,358, 6,462,047, 6,821,975, 6,306,870, 6,117,881, 6,043,252, or 3,819,631, US200301 66641, WO 97/43287, Daugan et al., J Med Chem., 46(21):4533-42 (2003), and Daugan et al., J Med Chem., 9;46(21):4525-32 (2003); an imidazo derivative, such as a compound disclosed in U.S. Pats. 6,130,333, 6,566,360, 6,362,178, or 6,582,351, US20050070541, or US20040067945; or a compound described in U.S. Pats. 6,825,197, 6,943,166, 5,981,527, 6,576,644, 5,859,009, 6,943,253, 6,864,253, 5,869,516, 5,488,055, 6,140,329, 5,859,006, or 6,143,777, WO 96/16644, WO 01/19802, WO 96/26940, Dunn, Org. Proc. Res. Dev., 9: 88-97 (2005), or Bi et al., Bioorg Med Chem Lett., 11(18):2461-4 (2001).

In some embodiments, a reported PDE5 inhibitor is zaprinast; MY-5445; dipyridamole; vinpocetine; FR229934; l-methyl-3-isobutyl-8-(methylamino)xanthine; furazlocillin; Sch-51866; E4021; GF-196960; IC-351; T-1032; sildenafil; tadalafil;
vardenafil; DMPPO; RX-RA-69; KT-734; SKF-96231; ER-21355; BF/GP-385; NM-702; PLX650; PLX134; PLX369; PLX788; or vesnarinone.

In some embodiments, the reported PDE5 inhibitor is sildenafil or a related compound disclosed in U.S. Pats. 5,346,901, 5,250,534, or 6,469,012; tadalafil or a related compound disclosed in U.S. Pat. 5,859,006, 6,140,329, 6,821,975, or 6,943,166; or vardenafil or a related compound disclosed in U.S. Pat. 6,362,178.

Non-limiting examples of a reported PDE6 inhibitor useful in a combination or method described herein include dipyridamole or zaprinast.

Non-limiting examples of a reported PDE7 inhibitor for use in the combinations and methods described herein include BRL 50481; PLX369; PLX788; or a compound described in U.S. Pats. 6,818,651; 6,737,436, 6,613,778, 6,617,357; 6,146,876, 6,838,559, or 6,884,800; US20050059686; US20040138279; US2005022138; US200402 14843; US20040106631; US 20030045557; US 20020198198; US20030162802, US20030092908, US 20030104974; US20030100571; 20030092721; or US20050148604.

A non-limiting examples of a reported inhibitor of PDE8 activity is dipyridamole.

Non-limiting examples of a reported PDE9 inhibitor useful in a combination or method described herein include SCH-51866; IBMX; or BAY 73-6691.

Non-limiting examples of a PDE10 inhibitor include sildenafil; SCH-51866; papaverine; zaprinast; dipyridamole; E4021; vinpocetine; EHNA; milrinone; rolipram; PLX107; or a compound described in U.S. Pat. 6,930,114, US20040138249, or US20040249148.

Non-limiting examples of a PDE11 inhibitor includes IC-351 or a related compound described in WO 9519978; E4021 or a related compound described in WO 9307124; UK-235,187 or a related compound described in EP 579496; PLX788; zaprinast; dipyridamole; or a compound described in US20040106631 or Maw et al., Bioorg Med Chem Lett. 2003 Apr 17;13(8):1425-8.

In some embodiments, the reported PDE inhibitor inhibits dual-specificity PDE. Non-limiting examples of a dual-specificity PDE inhibitor useful in a combination or method described herein include a cAMP-specific or cGMP-specific PDE inhibitor described herein; MMPX; KS-505a; W-7; a phenothiazine; Bay 60-7550 or a related compound described in Boess et al., Neuropharmacology, 47(7):1081-92 (2004); UK-235,187 or a related compound described in EP 579496; or a compound described in U.S. Pats. 6,930,114 or 4,861,891, US20020132754, US20040138249, US20040249148, US2004016631, WO 951997, or Maw et al., Bioorg Med Chem Lett. 2003 Apr 17;13(8):1425-8.

In some embodiments, a reported PDE inhibitor exhibits dual-selectivity, being substantially more active against two PDE isozymes relative to other PDE isozymes. For example, in some embodiments, a reported PDE inhibitor is a dual PDE4/PDE7 inhibitor, such as a compound described in US20030104974; a dual PDE3/PDE4 inhibitor, such as zardaverine, tolafentrine, benafentrine, trequinsine, Org-30029, L-686398, SDZ-ISQ-844, Org-20241, EMD-54622, or a compound described in U.S. Pats. 5,521,187, or 6,306,869; or a dual PDE1/PDE4 inhibitor, such as KF19514 (5-phenyl-3-(3-pyridyl)methyl-3H-imidazo[4,5-c][1,8]naphthyridin-4 (5H)-one).

Furthermore, the neurogenic agent in combination with a 5HTR agent may be a reported neurosteroid. Non-limiting examples of such a neurosteroid include pregnenolone and allopregnenolone.

Alternatively, the neurogenic sensitizing agent may be a reported non-steroidal anti-inflammatory drug (NSAID) or an anti-inflammatory mechanism targeting agent in general. Non-limiting examples of a reported NSAID include a cyclooxygenase inhibitor, such as indomethacin, ibuprofen, celecoxib, nimesulide, naproxen, or aspirin. Additional non-limiting examples for use in combination with a 5HTR agent include rofecoxib, meloxicam, piroxicam, valdecoxib, parecoxib, etoricoxib, etodolac, nimesulide, acemetacin, bufexamac, diflunisal, ethenzamide, etofenamate, flobufen, isoxicam, kebuzone, lonazolac, meclofenamic acid, metamizol, mofebutazone, niflumic acid, oxyphenbutazone, paracetamol, phenidine, propacetamol, propyphenazon, salicylamide, tenoxicam, tiaprofenic acid, oxaprozin, lornoxicam, nabumetone, minocycline, benorylate, alopixrin, salsalate, flurbiprofen, ketoprofen, fenoprofen, fenbufen, benoxaprofen, suprofen, piroxicam, meloxicam, diclofenac, ketorolac, fenclofenac, sulindac, tolmetin, xiphyphenbutazone, phenylbutazone, feprazone, azapropazone, flufenamic acid or mefenamic acid.
In additional embodiments, the neurogenic agent in combination with a 5HTR agent may be a reported agent for treating migraines. Non-limiting examples of such an agent include a triptan, such as almotriptan or almotriptan malate; naratriptan or naratriptan hydrochloride; rizatriptan or rizatriptan benzoate; sumatriptan or sumatriptan succinate; zolmitriptan or zolmitriptan hydrobromide. Embodiments of the disclosure may exclude combinations of triptans and an SSRI or SNRI that result in life threatening serotonin syndrome.

Other non-limiting examples include an ergot derivative, such as dihydroergotamine or dihydroergotamine mesylate, ergotamine or ergotamine tartrate; diclofenac or diclofenac potassium or diclofenac sodium; flurbiprofen; amitriptyline; nortriptyline; divalproex or divalprox sodium; propranolol or propranolol hydrochloride; verapamil; methysergide (CAS RN 361-37-5); metoclopramide; prochlorperazine (CAS RN 58-38-8); acetaminophen; topiramate; GW274150 ([2-[(1-iminoethyl) amino]ethyl]-L-homocysteine); or ganaxalone (CAS RN 38398-32-2).

Additional non-limiting examples include a COX-2 inhibitor, such as celecoxib.

In other embodiments, the neurogenic agent in combination with a 5HTR agent may be a reported modulator of a nuclear hormone receptor. Nuclear hormone receptors are activated via ligand interactions to regulate gene expression, in some cases as part of cell signaling pathways. Non-limiting examples of a reported modulator include a dihydrotestosterone agonist such as dihydrotestosterone; a 2-quinolone like LG121071 (4-ethyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-g] quinoline); a non-steroidal agonist or partial agonist compound described in U.S. Pat. No.6,01 7,924; LGD2226 (see WO 01/16108, WO 01/16133, WO 01/16139, and Rosen et al. "Novel, non-steroidal, selective androgen receptor modulators (SARMs) with anabolic activity in bone and muscle and improved safety profile." J Musculoskelet Neuronal Interact. 2002 2(3):222-4); or LGD2941 (from collaboration between Ligand Pharmaceuticals Inc. and TAP Pharmaceutical Products Inc.).

Additional non-limiting examples of a reported modulator include a selective androgen receptor modulator (SARM) such as andarine, ostarine, prostarin, or andromustine (all from GTx, Inc.); bicalutamide or a bicalutamide derivative such as GTx-007 (U.S. Pat. 6,492,554); or a SARM as described in U.S. Pat. 6,492,554.
Further non-limiting examples of a reported modulator include an androgen receptor antagonist such as cyproterone, bicalutamide, flutamide, or nilutamide; a 2-quinolone such as LGI 20907, represented by the following structure:

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      CF3
   O      N
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or a derivative compound represented by the following structure:

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    CF3
  O      N
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(see Allan et al. "Therapeutic androgen receptor ligands" Nucl Recept Signal 2003; 1:e009); a phthalamide, such as a modulator as described by Miyachi et al. ("Potent novel nonsteroidal androgen antagonists with phthalimide skeleton." Bioorg. Med. Chem. Lett. 1997 7:1483-1488); osaterone or osaterone acetate; hydroxyflutamide; or a non-steroidal antagonist described in U.S. Pat. No.6,017,924.

Other non-limiting examples of a reported modulator include a retinoic acid receptor agonist such as all-trans retinoic acid (Tretinoin®); isotretinoin (13-cis-retinoic acid); 9-cis retinoic acid; bexarotene; TAC-101 (4-1,3,5-bis (trimethylsilyl) benzamide] benzoic acid); AC-261066 (see Lund et al. "Discovery of a potent, orally available, and isoform-selective retinoic acid beta2 receptor agonist." J Med Chem. 2005 48(24):7517-9); LGDl 550 ((2E,4E,6E)-3-methyl-7-(3,5-di-ter-butyl phen-yl)octatrienoicacid); E6060 (E6060 [4- {5-[7-fluoro-4-(trifluoromethyl)benzo[b]furan-2-yl]-IH-2-pyrrrolyl]benzoic acid]; agonist 1 or 2 as described by Schapira et al. ("In silico discovery of novel Retinoic Acid Receptor agonist structures." BMC Struct Biol. 2001; 1:1 (published online 2001 June 4) where "Agonist 1 was purchased from Bionet Research (catalog number 1G-433S). Agonist 2 was purchased from Sigma-Aldrich (Sigma Aldrich library of rare chemicals. Catalog number S08503-1")); a synthetic acetylenic retinoic acid, such as AGN 190121 (CAS RN: 132032-67-8), AGN 190168 (or tazarotene or CAS RN 118292-40-3), or its metabolite AGN 190299 (CAS RN 118292-41-4); etretinate; acitretin; an acetylenic retinoate, such as AGN 190073 (CAS 132032-68-9), or AGN 190089 (or 3-pyridinecarboxylic acid, 6-(4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-1-ynyl)- ethyl ester or CAS RN 116627-73-7).
In further embodiments, the additional agent for use in combination with a 5HTR agent may be a reported modulator selected from thyroxin, tri-iodothyronine, or levothyroxine.


Furthermore, the additional agent may be a reported Cortisol receptor modulator, such as methylprednisolone or its prodrug methylprednisolone sulpeptanate; PI-1020 (NCX-1020 orbudesonide-21-nitrooxymethylbenzoate); fluticasone furoate; GW-215864; betamethasone valerate; beclomethasone; prednisolone; or BVT-3498 (AMG-311).

Alternatively, the additional agent may be a reported aldosterone (or mineralocorticoid) receptor modulator, such as spironolactone or eplerenone.

In other embodiments, the additional agent may be a reported progesterone receptor modulator such as asoprisnil (CAS RN 199396-76-4); mesoprogestin or J1042; J956; medroxyprogesterone acetate (MPA); R5020; tanaproget; trimegestone; progesterone; norgestomet; melengestrol acetate; mifepristone; onapristone; ZKI 373 16; ZK23021 1 (see Fuhrmann et al. "Synthesis and biological activity of a novel, highly potent progesterone receptor antagonist."  J Med Chem. 2000 43(26):5010-6); or a compound described in Spitz "Progesterone antagonists and progesterone receptor modulators: an overview."  Steroids 2003 68(10-13):981-93.

In further embodiments, the additional agent may be a reported i) peroxisome proliferator-activated receptor (PPAR) agonist such as muraglitazar; tesaglitazar; reglitazar; GW-409544 (see Xu et al. "Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors."  Proc Natl Acad Sci U S A. 2001 98(24):13919-24); or DRL 11605 (Dr. Reddy's Laboratories); ii) a peroxisome proliferator-activated
receptor alpha agonist like clofibrate; ciprofibrate; fenofibrate; gemfibrozil; DRF- 10945 (Dr. Reddy's Laboratories); iii) a peroxisome proliferator-activated receptor delta agonist such as GW501516 (CAS RN 317318-70-0); or iv) a peroxisome proliferator-activated gamma receptor agonist like a hydroxyoctadecadienoic acid (HODE); (v) a prostaglandin derivative, such as 15-deoxy-Delta2,14-prostaglandin J2; a thiazolidinedione (glitazone), such as pioglitazone, troglitazone; rosiglitazone or rosiglitazone maleate; ciglitazone; balaglitazone or DRF-2593; AMG 131 (from Amgen); or G1262570 (from GlaxoWellcome). In additional embodiments, a PPAR ligand is a PPARγ antagonist such as T0070907 (CAS RN 313516-66-4) or GW9662 (CAS RN 22978-25-2).

In additional embodiments, the additional agent may be a reported modulator of an "orphan" nuclear hormone receptor. Embodiments include a reported modulator of a liver X receptor, such as a compound described in U.S. Pat. 6,924,311; a farnesoid X receptor, such as GW4064 as described by Maloney et al. ("Identification of a chemical tool for the orphan nuclear receptor FXR." J Med Chem. 2000 43(16):2971-4); a RXR receptor; a CAR receptor, such as 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP); or a PXR receptor, such as SR-12813 (tetra-ethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethenyl-1, 1-bisphosphonate).

In additional embodiments, the agent in combination with a 5HTR agent is ethyl eicosapentaenoate or ethyl-EPA (also known as 5,8,11,14,17-eicosapentaenoic acid ethyl ester or miraxion, CAS RN 86227-47-6), docosahexaenoic acid (DHA), or a retinoid acid drug. As an additional non-limiting example, the agent may be omacor, a combination of DHA and EPA, or idebenone (CAS RN 58186-27-9).

In further embodiments, a reported nootropic compound may be used as an agent in combination with a 5HTR agent. Non-limiting examples of such a compound include piracetam (Nootropil®), aniracetam, xixiracetam, pramiracetam, pyritinol (Enerbol®), ergoloid mesylates (Hydergine®), galantamine or galantamine hydrobromide, selegiline, centrophenoxine (Lucidril®), desmopressin (DDAVP), nicergoline, vinpocetine, picamilone, vasopressin, milacemide, FK-960, FK-962, levetiracetam, nefiracetam, or hyperzine A (CAS RN: 102518-79-6).

Additional non-limiting examples of such a compound include anapsos (CAS RN 75919-65-2), nebracetam (CAS RN 97205-34-0 or 116041-13-5), metrifonate, ensaculin (or CAS RN 155773-59-4 or KA-672) or ensaculin HCl, rokan (CAS RN 122933-57-7 or EGb 761), AC-3933 (5-(3-methoxyphenyl)-3-(5-methyl-1,2,4-oxadiazol-3-yl)-2-oxo-1,2-
dihydro-1,6-naphthyridine) or its hydroxylated metabolite SX-5745 (3-(5-hydroxymethyl-1,2,4-oxadiazol-3-yl)-5-(3-methoxyphenyl)-2-oxo-1,2-dihydro-1,6-naphthyridine), JTP-2942 (CAS RN 148152-77-6), sabeluzole (CAS RN 104383-17-7), ladostigil (CAS RN 209394-27-4), choline alphoscerate (CAS RN 28319-77-9 or Gliatilin®), dimebon (CAS RN 3613-73-8), tramiprosate (CAS RN 3687-18-1), omigapil (CAS RN 181296-84-4), cebaracetam (CAS RN 113957-09-8), fasoracetam (CAS RN 110958-19-5), PD-151832 (see Jaen et al. "In vitro and in vivo evaluation of the subtype-selective muscarinic agonist PD 151832." Life Sci. 1995 56(11-12):845-52), vinconate (CAS RN 70704-03-9), PYM-50028 (Cogane) or PYM-50018 (Myogane) as described by Harvey ("Natural Products in Drug Discovery and Development. 27-28 June 2005, London, UK." IDrugs. 2005 8(9):719-21), SR-46559A (3-[N-(2 diethyl-amino-2-methylpropyl)-6-phenyl-5-propyl) dihydroergocristine (CAS RN 17479-19-5), dabelotine (CAS RN 118976-38-8), zanapezil (CAS RN 142852-50-4).

Further non-limiting examples include NBI-113 (from Neurocrine Biosciences, Inc.), NDD-094 (from Novartis), P-58 or P58 (from Pfizer), or SR-57667 (from Sanofi-Synthelabo).

Moreover, an agent in combination with a 5HTR agent may be a reported modulator of the nicotinic receptor. Non-limiting examples of such a modulator include nicotine, acetylcholine, carbamylcholine, epibatidine, ABT-418 (structurally similar to nicotine, with an ixoxazole moiety replacing the pyridyl group of nicotine), epiboxidine (a structural analogue with elements of both epibatidine and ABT-418), ABT-594 (azetidine analogue of epibatidine), lobeline, SSR-591813, represented by the following formula:

![Chemical Structure](image)

In additional embodiments, an agent used in combination with a 5HTR agent is a reported aromatase inhibitor. Reported aromatase inhibitors include, but are not limited to, nonsteroidal or steroidal agents. Non-limiting examples of the former, which inhibit aromatase via the heme prosthetic group, include anastrozole (Arimidex®), letrozole (Femara®), or vorozole (Rivisor®). Non-limiting examples of steroidal aromatase inhibitors AIs, which inactivate aromatase, include, but are not limited to, exemestane (Aromasin®), androstenedione, or formestane (Lentaron®).
Additional non-limiting examples of a reported aromatase for use in a combination or method as disclosed herein include aminoglutethimide, 17α-estradiol (or "6-OXO"), or zoledronic acid or Zometa® (CAS RN 118072-93-8).

Further embodiments include a combination of a 5HTR agent and a reported selective estrogen receptor modulator (SERM) may be used as described herein. Non-limiting examples include tamoxifen, raloxifene, toremifene, clomifene, bazedoxifene, arzoxifene, or lasofoxifene. Additional non-limiting examples include a steroid antagonist or partial agonist, such as centchroman, clomiphene, or droloxfene.

In other embodiments, a combination of a 5HTR agent and a reported cannabinoid receptor modulator may be used as described herein. Non-limiting examples include synthetic cannabinoids, endogenous cannabinoids, or natural cannabinoids. In some embodiments, the reported cannabinoid receptor modulator is rimonabant (SR141716 or Acomplia), nabilone, levonantradol, marinol, or sativex (an extract containing both THC and CBD). Non-limiting examples of endogenous cannabinoids include arachidonyl ethanolamine (anandamide); analogs of anandamide, such as docosatetraenylethanolamide or homo-γ-linoenylethanolamide; N-acyl ethanolamine signalling lipids, such as the noncannabimimetic palmitoyl ethanolamine or oleoyl ethanolamine; or 2-arachidonyl glycerol. Non-limiting examples of natural cannabinoids include tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabivarol (CBV), tetrahydrocannabivar (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), or cannabigerol monoethyl ether (CBGM).

In yet further embodiments, an agent used in combination with a 5HTR agent is a reported FAAH (fatty acid amide hydrolase) inhibitor. Non-limiting examples of reported inhibitor agents include URB597 (3′-carbamoyl-biphenyl-3-y1-cyclohexylcarbamate); CAY10401 (1-oxazolo[4,5-b]pyridin-2-yl-9-octadecyn-l-one); OL-135 (1-oxo-l[5-(2-pyridyl)-2-yl]-7-phenylheptane); anandamide (CAS RN 94421-68-8); AA-5-HT (see Bisogno et al. "Arachidonoylserotonin and other novel inhibitors of fatty acid amide hydrolase." Biochem Biophys Res Commun. 1998 248(3):515-22); 1-Octanesulfonyl fluoride; or O-2142 or another arvanil derivative FAAH inhibitor as described by Di Marzo et al. ("A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid." J Pharmacol Exp Ther. 2002 300(3):984-91).
Further non-limiting examples include SSR 411298 (from Sanofi-Aventis), JNJ28614118 (from Johnson & Johnson), or SSR 101010 (from Sanofi-Aventis).

In additional embodiments, an agent in combination with a 5HTR agent may be a reported modulator of nitric oxide function. One non-limiting example is sildenafil (Viagra®).

In additional embodiments, an agent in combination with a 5HTR agent may be a reported modulator of prolactin or a prolactin modulator.

In additional embodiments, an agent in combination with a 5HTR agent is a reported anti-viral agent, with ribavirin and amantadine as non-limiting examples.

In additional embodiments, an agent in combination with a 5HTR agent may be a component of a natural product or a derivative of such a component. In some embodiments, the component or derivative thereof is in an isolated form, such as that which is separated from one or more molecules or macromolecules normally found with the component or derivative before use in a combination or method as disclosed herein. In other embodiments, the component or derivative is completely or partially purified from one or more molecules or macromolecules normally found with the component or derivative.

Exemplary cases of molecules or macromolecules found with a component or derivative as described herein include a plant or plant part, an animal or animal part, and a food or beverage product.

Non-limiting examples such a component include folic acid; a flavinoid, such as a citrus flavonoid; a flavonol, such as quercetin, kaempferol, myricetin, orisorhamnetin; a flavone, such as luteolin or apigenin; a flavanone, such as hesperetin, naringenin, or eriodictyol; a flavan-3-ol (including a monomelic, dimeric, or polymeric flavanol), such as (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin 3-gallate, (-)-epigallocatechin 3-gallate, theaflavin, theaflavin 3-gallate, theaflavin 3’-gallate, theaflavin 3,3’ digallate, thearubigin, or proanthocyanidin; an anthocyanidin, such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, or petunidin; an isoflavone, such as daidzein, genistein, or glycitein; flavopiridol; a prenylated chalcone, such as xanthohumol; a prenylated flavanone, such as isoxanthohumol; a non-prenylated chalcone, such as chalconaringenin; a non-prenyalted flavanone, such as naringenin; resveratrol; or an anti-oxidant neutraceutical (such as any present in chocolate, like dark chocolate or unprocessed or unrefined chocolate).

Additional non-limiting examples include a component of *Gingko biloba*, such as a flavo glycoside or a terpene. In some embodiments, the component is a flavanoid, such
as a flavonol or flavone glycoside, or a quercetin or kaempferol glycoside, or rutin; or a
terpenoid, such as ginkgolides A, B, C, or M, or bilobalide.

Further non-limiting examples include a component that is a flavanol, or a
related oligomer, or a polyphenol as described in US2005/245601AA, US2002/018807AA,
procyanidin or derivative thereof or polyphenol as described in US2005/171029AA; a
procyanidin, optionally in combination with L-arginine as described in US2003/104075AA; a
low fat cocoa extract as described in US2005/031762AA; lipophilic bioactive compound
containing composition as described in US2002/107292AA; a cocoa extract, such as those
containing one or more polyphenols or procyanidins as described in US2002/004523AA; an
extract of oxidized tea leaves as described in US Pat. 5,139,802 or 5,130,154; a food
supplement as described in WO 2002/024002.

Of course a composition comprising any of the above components, alone or in
combination with a 5HTR agent as described herein is included within the disclosure.

In additional embodiments, an agent in combination with a 5HTR agent may
be a reported calcitonin receptor agonist such as calcitonin or the Orphan peptide' PHM-27
(see Ma et al. "Discovery of novel peptide/receptor interactions: identification of PHM-27 as

In an alternative embodiment, the agent may be a reported modulator of
parathyroid hormone activity, such as parathyroid hormone, or a modulator of the parathyroid
hormone receptor.

In additional embodiments, an agent in combination with a 5HTR agent may a
reported antioxidant, such as N-acetylcysteine or acetylcysteine; disufenton sodium (or CAS
RN 168021-79-2 or Cerovive); activin (CAS RN 104625-48-1); selenium; L-methionine; an
alpha, gamma, beta, or delta, or mixed, tocopherol; alpha lipoic acid; Coenzyme Q;
benzimidazole; benzoic acid; dipyriramole; glucosamine; IRFI-016 (2(2,3-dihydro-5-
acetoxy-4,6,7-trimethylbenzofuranyl) acetic acid); L-carnosine; L-Histidine; glycine;
flavocoxid (or LIMBREL®); baicalin, optionally with catechin (3,3',4', 5,7-
pentahydroxyflavan (2R,3S form)), and/or its stereo-isomer; masoprocol (CAS RN 27686-
84-6); mesna (CAS RN 19767-45-4); probucol (CAS RN 23288-49-5); silibinin (CAS RN
22888-70-6); sorbinil (CAS RN 68367-52-2); spermine; tangeretin (CAS RN 481-53-8);
butylated hydroxyanisole (BHA); butylated hydroxytoluene (BHT); propyl gallate (PG);
tertiary-butyl-hydroquinone (TBHQ); nordihydroguaiaretic acid (CAS RN 500-38-9); astaxanthin (CAS RN 472-61-7); or an antioxidant flavonoid.

Additional non-limiting examples include a vitamin, such as vitamin A (Retinol) or C (Ascorbic acid) or E (including tocotrienol and/or tocopherol); a vitamin cofactors or mineral, such as coenzyme Q10 (CoQ10), manganese, or melatonin; a carotenoid terpenoid, such as lycopene, lutein, alpha-carotene, beta-carotene, zeaxanthin, astaxanthin, or canthaxanthin; a non-carotenoid terpenoid, such as eugenol; a flavonoid polyphenols (or bioflavonoid); a flavonol, such as resveratrol, pterostilbene (methoxylated analogue of resveratrol), kaempferol, myricetin, isorhamnetin, a proanthocyanidin, or a tannin; a flavone, such as quercetin, rutin, luteolin, apigenin, or tangeritin; a flavanone, such as hesperetin or its metabolite hesperidin, naringenin or its precursor naringin, or eriodictyol; a flavan-3-ols (anthocyanidins), such as catechin, gallocatechin, epicatechin or a gallate form thereof, epigallocatechin or a gallate form thereof, theaflavin or a gallate form thereof, or a thearubigin; an iso flavone phytoestrogens, such as genistein, daidzein, or glycitein; an anthocyanins, such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, or petunidin; a phenolic acid or ester thereof, such as ellagic acid, gallic acid, salicylic acid, rosmarinic acid, cinnamic acid or a derivative thereof like ferulic acid, chlorogenic acid, chicoric acid, a gallotannin, or an ellagitannin; a nonflavonoid phenolic, such as curcumin; an anthoxanthin, betacyanin, citric acid, uric acid, R-α-lipoic acid, or silymarin.

Further non-limiting examples include 1-(carboxymethylthio)tetradecane; 2,2,5,7,8-pentamethyl-1-hydroxychroman; 2,2,6,6-tetramethyl-4-piperidinol-N-oxyl; 2,5-dimethyl-2,6-dihydroxyquinone; 2-tert-butylhydroquinone; 3,4-dihydroxyphenylethanol; 3-hydroxypyridine; 3-hydroxytamoxifen; 4-coumaric acid; 4-hydroxyanisole; 4-hydroxyphenylethanol; 4-methylecatechol; 5,6,7,8-tetrahydrobiopterin; 6,6′-methylenebis(2,2-dimethyl-4-methanesulfonic acid-1,2-dihydroquinoline); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; 6-methyl-2-ethyl-3-hydroxypyridine; 6-O-palmitoylascorbic acid; acetovanillone; acteoside; actovegin; allicin; allyl sulfide; alphapentyl-3-(2-quinolinylmethoxy)benzenemethanol; alpha-tocopherol acetate; apolipoprotein A-IV; betemethyl; boldine; bucillamine; calcium citrate; canthaxanthin; crocetin; diallyl trisulfide; dicarbine; dihydrolipoic acid; dimephosphon; ebselen; efamol; enkephalin-Leu, Ala(2)-Arg(6)-; ergothioneine; esculetin; essential 303 forte; ethonium; etofyllinclofibrate; fenozan; glaucine; H290-5 I; histidyl-proline diketopiperazine; hydroquinone; hypotaurine; idebenone; indole-3-carbinol; isoascorbic acid; kojic acid, lacidipine, lodoxamide
tromethamine; mexidol; morin; N,N'-diphenyl-4-phenylenediamine; N-isopropyl-N-phenyl-4-phenylenediamine; N-monoacetylcysteine; nicaraven, nicotinoyl-GABA; nitecapone; nitroxyll; nobiletin; oxymethacil; p-tert-butyl catechol; phenidone; pramipexol; proanthocyanidin; procyanidin; prolinedithiocarbamate; propyl gallate; purpurogallin; pyrrolidine dithiocarbamic acid; rebamipide; retinol palmitate; salvin; selenious acid; sesamin; sesamol; sodium selenate; sodium thiosulfate; theaflavin; thiazolidine-4-carboxylic acid; tirilazad; tocopherolquinone; tocotrienol, alpha; a tocotrienol; tricyclodecane-9-yl-xanthogenate; turmeric extract; U 74389F; U 74500A; U 78517F; ubiquinone 9; vanillin; vinpocetine; xylometazoline; zeta carotene; zilascorb; zinc thionein; or zonisamide.

5 In additional embodiments, an agent in combination with a 5HTR agent may be a reported modulator of a norepinephrine receptor. Non-limiting examples include atomoxetine (Strattera®); a norepinephrine reuptake inhibitor, such as talsupram, tomoxetine, nortriptyline, nisoxetine, reboxetine (described, e.g., in U.S. Pat. 4,229,449), or tomoxetine (described, e.g., in U.S. Pat. 4,314,081); or a direct agonist, such as a beta adrenergic agonist.

10 Additional non-limiting examples include an alpha adrenergic agonist such as etilefrine or a reported agonist of the \( \alpha_2 \)-adrenergic receptor (or \( \alpha_2 \) adrenoceptor) like clonidine (CAS RN 4205-90-7), yohimbine, mirtazepine, atipamezole, carvedilol; dexmedetomidine or dexmedetomidine hydrochloride; ephedrine, epinephrine; etilefrine; lidamidine; tetramethylpyrazine; tizanidine or tizanidine hydrochloride; apraclonidine; bitolterol mesylate; brimonidine or brimonidine tartrate; dipivefrin (which is converted to epinephrine \textit{in vivo}); guanabenz; guanfacine; methylodopa; alphamethylnoradrenaline; mivazerol; natural ephedrine or D(-)-ephedrine; any one or any mixture of two, three, or four of the optically active forms of ephedrine; CHF1035 or nolomirole hydrochloride (CAS RN 138531-51-8); or lofexidine (CAS RN 31036-80-3).

15 Alternative non-limiting examples include an adrenergic antagonist such as a reported antagonist of the \( \alpha_2 \)-adrenergic receptor like yohimbine (CAS RN 146-48-5) or yohimbine hydrochloride, idazoxan, fluparoxan, mirtazepine, atipamezole, or RX781094 (see Elliott et al. "Peripheral pre and postjunctional alpha 2-adrenoceptors in man: studies with RX781094, a selective alpha 2 antagonist." J Hypertens Suppl. 1983 l(2):109-1 1).

20 Other non-limiting embodiments include a reported modulator of an \( \alpha_1 \)-adrenergic receptor such as cirazoline; modafinil; ergotamine; metaraminol; methoxamine; midodrine (a prodrug which is metabolized to the major metabolite
desglymidodrine formed by deglycination of midodrine; oxymetazoline; phenylephrine; phenylpropanolamine; or pseudoephedrine.

Further non-limiting embodiments include a reported modulator of a beta adrenergic receptor such as arbutamine, befunolol, cimaterol, higenamine, isoxsuprine, methoxyphenamine, oxyfedrine, ractopamine, tretoquinol, or TQ-1016 (from TheraQuest Biosciences, LLC), or a reported β1-adrenergic receptor modulator such as prenalterol, Ro 363, or xamoterol or a reported β1-adrenergic receptor agonist like dobutamine.

Alternatively, the reported modulator may be of a β2-adrenergic receptor such as levosalbutamol (CAS RN 34391-04-3), metapreterenol, MN-221 or KUR-1246 ((-)bis(2-
{(2S)-2-((2R)-2-hydroxy-2-[4-hydroxy-3-(2-hydroxyethyl) phenyl] ethyl]amino)-1,2,3,4-tetrahydro-1-naphthalen-7-yl]oxy}-N,N-dimethylacetamide)monosulfate orbis(2-[(2S)-2-
((2R)-2-hydroxy-2-[4-hydroxy-3-(2-hydroxyethyl)phenyl]ethyl]amino)-l, 2,3,4-
tetrahydro-1-naphthalen-7-yl]oxy}-N,N-dimethylacetamide) sulfate or CAS RN 194785-31-4), nylidrin, orciprenaline, pirbuterol, procarterol, reproterol, ritodrine, salmeterol, salmeterol xinafoate, terbutaline, tulobuterol, zinterol or bromoacetylnaphrolololthane, or a reported β2-adrenergic receptor agonist like albuterol, albuterol sulfate, salbutamol (CAS RN 35763-26-9), clenbuterol, broxaterol, dopexamine, formoterol, formoterol fumarate, isoetharine, levalbuterol tartrate hydrofluoroalkane, or mabuterol.

Additional non-limiting embodiments include a reported modulator of a β3-adrenergic receptor such as AJ-9677 or TAK677 [(3-[(2R)-[(2R)-(3-chlorophenyl)-2-

Further alternative embodiments include a reported nonselective alpha and beta adrenergic receptor agonist such as epinephrine or ephedrine; a reported nonselective alpha and beta adrenergic receptor antagonist such as carvedilol; a β1 and β2 adrenergic receptor agonist such as isopreoterenol; or a β1 and β2 adrenergic receptor antagonist such as CGP 12177, fenoterol, or hexoprenaline.

Non-limiting examples of reported adrenergic agonists include albuterol, albuterol sulfate, salbutamol (CAS RN 35763-26-9), clenbuterol, adrafinil, and SR5861 IA (described in Simiand et al., Eur J Pharmacol, 219:193-201 (1992)), clonidine (CAS RN 4205-90-7), yohimbine (CAS RN 146-48-5) or yohimbine hydrochloride, arbutamine;
befunolol; BRL 26830A; BRL 35135; BRL 37344; bromoacetylalprenololmenthane;
broxaterol; carvedilol; CGP 12177; cimaterol; cirazoline; CL 316243; clenbuterol;
denopamine; dexmedetomidine or dexmedetomidine hydrochloride; dobutamine,
dopexamine, ephedrine, epinephrine, etilefrine; fenoterol; formoterol; formoterol fumarate;
hexoprenaline; higenamine; ICI D71 14; isoproterenol; isoxsuprine; levabalbuterol
hdrofluoroalkane; lidamidine; mabuterol; methoxyphenamine; modafnil; nylidrin;
orciprenaline; oxyfedrine; pirbuterol; prenalterol; procaterol; ractopamine; reproterol;
ritodrine; ro 363; salmeterol; salmeterol xinafoate; terbutaline; tetramethylpyrazine;
tizanidine or tizanidine hydrochloride; tretoquinol; tulobuterol; xamoterol; or zinterol.

Additional non-limiting examples include apraclonidine, bitolterol mesylate, brimonidine or
brimonidine tartrate, dipivefrin (which is converted to epinephrine in vivo), epinephrine,
ergotamine, guanabenz, guanfacine, metaproterenol, metaraminol, methoxamine,
methyldopa, midodrine (a prodrug which is metabolized to the major metabolite
desglymidodrine formed by deglycination of midodrine), oxymetazoline, phenylephrine,
phenylpropanolamine, pseudoephedrine, alphamethylnoradrenaline, mivazerol, natural
ephedrine or D(-)ephedrine, any one or any mixture of two, three, or four of the optically
active forms of ephedrine, CHF1035 or nolomirole hydrochloride (CAS RN 138531-51-8),
AJ-9677 or TAK677 (3-[2R]-[(3-chorophenyl)-2-hydroxyethyl]amino)propyl]-IH-
indol-7-yloxy]acetic acid), MN-221 or KUR-1246 (2-[(2S)-2-((2R)-2-hydroxy-2-[4-
hydroxy-3-(2-hydroxyethyl) phenyl]ethyl]amino)-1,2,3,4-tetrahydronaphthalen-7-yl]oxy]-
N,N-dimethy lacetamide)monosulfate or bis-[2-[(2S)-2-((2R)-2-hydroxy-2-[4-hydroxy-3-(2-
hydroxyethyl)-phenyl]ethyl]amino)-1,2,3,4-tetrahydronaphthalen-7-yl]oxy]-N,N-
dimethy lacetamide) sulfate or CAS RN 194785-31-4), levosalbutamol (CAS RN 34391-04-
3), lofexidine (CAS RN 31036-80-3) or TQ-1016 (from TheraQuest Biosciences, LLC).

In further embodiments, a reported adrenergic antagonist, such as idazoxan or
fluparoxan, may be used as an agent in combination with a nootropic agent as described
herein.

In further embodiments, an agent in combination with a 5HTR agent may be a
reported modulator of carbonic anhydrase. Non-limiting examples of such an agent include
acetazolamide, benzenesulfonamide, benzolamide, brinzolamide, dichlorphenamidine,
dorzolamide or dorzolamide HCl, ethoxzolamide, flurbiprofen, mafenide, methazolamide,
sezolamide, zonisamide, bendroflumethiazide, benzthiazide, chlorothiazide, cyclothiazide,
dansylamide, diazoxide, ethinamate, furosemide, hydrochlorothiazide, hydroflumethiazide,
mercuribenzoic acid, methyclothiazide, trichloromethazide, amlodipine, cyanamide, or a benzenesulfonamide. Additional non-limitinge examples of such an agent include (4S-Trans)-4-(Ethylamino)-5,6-dihydro-6-methyl-4H-thieno(2,3-B)thiopyran-2-sulfonamide-7,7-dioxide; (4S-trans)-4-(methylamino)-5,6-dihydro-6-methyl-4H-thieno(2,3-B)thiopyran-2-sulfonamide-7,7-dioxide; (R)-N-(3-indol-1-Yl-2-methyl-propyl)-4-sulfamoyl-benzamide; (S)-N-(3-indol-1-Yl-2-methyl-propyl)-4-sulfamoyl-benzamide; 1,2,4-triazole; 1-methyl-3-oxo-1,3-dihydro-benzo[C]isothiazole-5-sulfonic acid amide; 2,6-difluorobenzensulfonamide; 3,5-difluorobenzensulfonamide; 3-mercuri-4-aminobenzensulfonamide; 3-nitro-4-(2-oxo-pyridolin-1-Yl)-benzenesulfonamide; (aminosulfonyl)-N-[(2,3,4-trifluorophenyl)methyl]-benzamide; 4-(aminosulfonyl)-N-[(2,4,6-trifluorophenyl)methyl]-benzamide; 4-(aminosulfonyl)-N-[(2,4-difluorophenyl)methyl]-benzamide; A-(aminosulfonyl)-N-[(3,4,5-trifluorophenyl)methyl]-benzamide; 4-(aminosulfonyl)-N-[(4-fluorophenyl)methyl]-benzamide; 4-(hydroxymercury)benzoic acid; A-flourobenzenesulfonamide; 4-methylimidazole; 4-sulfonamide-[1-(4-aminobutane)]benzamide; 4-sulfonamide-[4-(thiomethylaminobutane)]benzamide; 5-acetamido-1,3,4-thiadiazole-2-sulfonamide; 6-oxo-8,9,10,1-tetrahydro-7H-cyclohepta[c][I]benzopyran-3-O-sulfamate; (4-sulfamoyl-phenyl)-thiocarbamic acid O-(2-thiophen-3-yl-ethyl) ester; (R)-4-ethylamino-3,4-dihydro-2-(2-methoxethyl)-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; 3,4-dihydro-4-hydroxy-2-(2-thienylmethyl)-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; 3,4-dihydro-4-hydroxy-2-(4-methoxyphenyl)-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; N-[4-methoxyphenyl)methyl]2,5-thiophenedesulfonamide; 2-(3-methoxyphenyl)-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; (R)-3,4-didhydro-2-(3-methoxyphenyl)-4-methylamino-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; (S)-3,4-dihydro-2-(3-methoxyphenyl)-4-methylamino-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; 3,4-dihydro-2-(3-methoxyphenyl)-2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide-1,1-dioxide; [2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide,2-(3-hydroxyphenyl)-3-(4-morpholinyl)]-, 1,1-dioxide; [2H-thieno[3,2-E]-1,2-thiazine-6-sulfonamide,2-(3-methoxyphenyl)-3-(4-morpholinyl)-], 1,1-dioxide; aminodi(ethyloxy)ethylaminocarbonylbenzenesulfonamide; N-(2,3,4,5,6-pentafluorobenzyl)-4-sulfamoyl-benzamide; N-(2,6-difluoro-benzyl)-4-sulfamoyl-benzamide; N-(2-fluoro-benzyl)-4-sulfamoyl-benzamide; N-(2-thienylmethyl)-2,5-thiophenedisulfonamide; N-
[2-([H-indol-5-yl]-butyl)-4-sulfamoyl-benzamide; N-benzyl-4-sulfamoyl-benzamide; or sulfamic acid 2,3-O-(1-methylethylidene)-4,5-O-sulfonyl-beta-fractopyranose ester.]

In yet additional embodiments, an agent in combination with a 5HTR agent may be a reported modulator of a catechol-O-methyltransferase (COMT), such as floproprine, or a COMT inhibitor, such as tolcapone (CAS RN 134308-13-7), nitecapone (CAS RN 116313-94-1), or entacapone (CAS RN 116314-67-1 or 130929-57-6).

In yet further embodiments, an agent in combination with a 5HTR agent may be a reported modulator of hedgehog pathway or signaling activity such as cyclopamine, jervine, ezetimibe, regadenoson (CAS RN 313348-27-5, or CVT-3146), a compound described in U.S. Pat. 6,683,192 or identified as described in U.S. Pat. 7,060,450, or CUR-61414 or another compound described in U.S. Pat. 6,552,016.

In other embodiments, an agent in combination with a 5HTR agent may be a reported modulator of IMPDH, such as mycophenolic acid or mycophenolate mofetil (CAS RN 128794-94-5).

In yet additional embodiments, an agent in combination with a 5HTR agent may be a reported modulator of a sigma receptor, including sigma-1 and sigma-2. Non-limiting examples of such a modulator include an agonist of sigma-1 and/or sigma-2 receptor, such as (+)-pentazocine, SKF 10,047 (N-allylnormetazocine), or 1,3-di-O-tolylguanidine (DTG). Additional non-limiting examples include SPD-473 (from Shire Pharmaceuticals); a molecule with sigma modulatory activity as known in the field (see e.g., Bowen et al., Pharmaceutica Acta Helvetiae 74: 211-218 (2000)); a guanidine derivative such as those described in U.S. Pat. Nos. 5,489,709; 6,147,063; 5,298,657; 6,087,346; 5,574,070; 5,502,255; 4,709,094; 5,478,863; 5,385,946; 5,312,840; or 5,093,525; WO9014067; an antipsychotic with activity at one or more sigma receptors, such as haloperidol, rimcazole, perphenazine, fluphenazine, (-)-butaclamol, acetophenazine, trifluoperazine, molindone, pimozide, thioridazine, chlorpromazine and trifluromazine, BMY 14802, BMY 13980, remoxipride, tiospirene, cinuperone (HR 375), or WY47384.

Additional non-limiting examples include igmesine; BD1008 and related compounds disclosed in U.S. Publication No. 20030171347; cis-isomers of US0488 and related compounds described in de Costa et al, J. Med. Chem., 32(8): 1996-2002 (1989); U101 958; SKF10,047; apomorphine; OPC-14523 and related compounds described in Oshiro et al., J Med Chem.; 43(2): 177-89 (2000); arylcyclohexamines such as PCP; (+)-morphinanis such as dextrallorphan; phenylpiperidines such as (+)-3-PPP and OHBQs; neurosteroids such
as progesterone and desoxycorticosterone; butyrophenones; BD614; or PRX-00023. Yet additional non-limiting examples include a compound described in U.S. Pat. Nos. 6,908,914; 6,872,716; 5,169,855; 5,561,135; 5,395,841; 4,929,734; 5,061,728; 5,731,307; 5,086,054; 5,158,947; 5; 16,995; 5; 149,817; 5; 109,002; 5; 162,341; 4; 4956,368; 4; 4831,031; or 4; 4957,916; U.S. Publication Nos. 20050132429; 20050107432; 2005003801 i. 20030105079; 20030171355; 20030212094; or 20040019060; European Patent Nos. EP 503 411; EP 362 001-A1; or EP 461 986; International Publication Nos. WO 92/14464; WO 93/09094; WO 92/22554; WO 95/15948; WO 92/18127;91/06297;WO01/02380;WO91/18868; or WO 93/00313; or in Russell et al, J Med Chem.; 35(11): 2025-33 (1992) or Chambers et al, J. Med Chem.; 35(11): 2033-9 (1992).

Further non-limiting examples include a sigma-1 agonist, such as IPAG (l-(4-iodophenyl)-3-(2-adamantyl)guanidine); pre-084; carbetapentane; 4-IBP; L-687,384 and related compounds described in Middlemiss et al., Br. J. Pharm., 102: 153 (1991); BD 737 and related compounds described in Bowen et al., J Pharmocol Exp Ther., 262(1): 32-40 (1992); OPC-14523 or a related compound described in Oshiro et al., J Med Chem.; 43(2): 177-89 (2000); a sigma-1 selective agonist, such as igmesine; (+)-benzomorphans, such as (+)-pentazocine and (+)-ethylketocyclazocine; SA-4503 or a related compound described in U.S. Pat. No. 5,736,546 or by Matsuno et al., Eur J Pharmacol., 306(1-3): 271-9 (1996); SK&F 10047; or ifenprodil; a sigma-2 agonist, such as haloperidol, (+)-5,8-disubstituted morphan-7-ones, including CB 64D, CB 184, or a related compound described in Bowen et al., Eur. J. Pharmacol. 278:257-260 (1995) or Bertha et al., J Med. Chem. 38:4776-4785 (1995); or a sigma-2 selective agonist, such as l-(4-fluorophenyl)-3-[4-[3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-en-8-yl]-l-butyl]-lH-indole, Lu 28-179, Lu 29-253 or a related compound disclosed in U.S. Pat. Nos. 5,665,725 or 6,844,352, U.S. Publication No.


Alternative non-limiting examples include a sigma-1 antagonist such as BD-1047 (N-)[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamin-o)ethylamine, BD-1063 (l-)[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, rimcazole, haloperidol, BD-1047, BD-1063, BMY 14802, DuP 734, NE-100, AC915, or R-(+)-3-PPP. Particular non-limiting examples include fluoxetine, fluvoxamine, citalopram, sertaline, clorgyline, imipramine,
igesine, opipramol, siramesine, SL 82.0715, imcazole, DuP 734, BMY 14802, SA 4503, OPC 14523, panamasine, or PRX-00023.

Other non-limiting examples of an agent in combination with a 5HTR agent include acamprosate (CAS RN 77337-76-9); a growth factor, like LIF, EGF, FGF, bFGF or VEGF as non-limiting examples; octreotide (CAS RN 83150-76-9); an NMDA modulator like ketamine, DTG, (+)-pentazocine, DHEA, Lu 28-179 ([4-[l-(4-fluorophenyl)-lH-indol-3-yl]-l-butyl]-spiro[isobenzofuran-l(3H), 4'-piperidine]), BD 1008 (CAS RN 138356-08-8), ACEA1021 (Licostinel or CAS RN 153504-81-5), GV150526A (Gavestinel or CAS RN 153436-22-7), sertraline, clorgyline, or memantine as non-limiting examples; or metformin.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the disclosed invention, unless specified.

EXCEPTIONS

Example 1 - Effect on neuronal differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of 5-HTP (test compound), and stained with TUJ-I antibody, as described in PCT Application No. US06/026677 (hereby incorporated by reference as if fully set forth). Mitogen-free test media with a positive control was used for neuronal differentiation, and basal media without growth factors served as a negative control.

Results are shown in Figure 1, which shows dose response curves of neuronal differentiation after background media values are subtracted. The dose response curve of the neuronal positive control is included as a reference. The data is presented as a percent of neuronal positive control. The data indicate that 5-HTP weakly promoted neuronal differentiation.

Example 2 - Effect on astrocyte differentiation of hNSCs

Experiments were carried out as described in Example 1, except the positive control for astrocyte differentiation contained mitogen-free test media with a positive control, and cells were stained with GFAP antibody. Results are shown in Figure 2, which shows
dose response curves of astrocyte differentiation after background media values are subtracted. 5-HTP showed no significant increase in astrocyte differentiation above basal media values.

**Example 3 - Immunohistochemistry with neuronal and astrocyte markers**

Immunohistochemistry was carried out as described above using TUJ-I as a neuronal cell marker and GFAP as an astrocyte marker. The results are shown in Figure 3, with control images included at the top for reference, and cells treated with 10.0 µM 5-HTP shown at the bottom.

**Example 4 - Effect of 5-HT1a agonists on neuronal differentiation of human neural stem cells**

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of test compounds, and stained with TUJ-1 antibody, as described in Example 1. Mitogen-free test media with a positive control for neuronal differentiation was used, and basal media without growth factors served as a negative control.

Results are shown for the 5-HT1a agonist buspirone in Figure 4 and for the 5-HT1a agonist tandospirone in Figure 7, which show dose response curves of neuronal differentiation after background media values are subtracted. The dose response curve of the neuronal positive control is included as a reference. The data is presented as a percent of neuronal positive control. The data indicate that 5-HT1a agonists promoted neuronal differentiation.

**Example 5 - Effect of 5-HT1a agonists on astrocyte differentiation of hNSCs**

Experiments were carried out as described in Example 2, except the positive control for astrocyte differentiation contained mitogen-free test media with a different positive control. Results are shown for the 5-HT1a agonist buspirone in Figure 5 and for the 5-HT1a agonist tandospirone in Figure 8, which show dose response curves of astrocyte differentiation after background media values are subtracted. The data indicate that 5-HT1a agonists promoted astrocytic differentiation, compared to 5-HTP as described in Example 2 and Figure 2.
Example 6 - Immunohistochemistry with neuronal and astrocyte markers on hNSCs treated with 5-HT1a agonists

Immunohistochemistry was carried out as described in Example 3. The results are shown for the 5-HT1a agonist buspirone in Figure 6 and for the 5-HT1a agonist tandospirone in Figure 9, with control images included at the top of each figure for reference, and cells treated with 31.6 μM test compound shown at the bottom of each figure.

Example 7 - Effect of the SSRI Fluoxetine in the in vivo novel object recognition cognition assay

Male F344 rats were dosed 1x per day for 28-days with 0 (vehicle only) or 5.0 and 10.0 mg/kg fluoxetine (n = 12 per dose group, p.o.). The apparatus consisted of an open field (45 x 45 x 50 cm high) made of polycarbonate. Triplicate copies were used of the objects to be discriminated. Care was taken to ensure that the pair of objects tested were made from the same material so that they could not be distinguished readily by olfactory cues although they had very different appearances.

Each test session consisted of two phases. In the initial familiarization phase, two identical objects (A1 and A2) were placed in the far corners of the box arena. A rat was then placed in the middle of the arena and allowed 15 minutes to explore both objects. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. After a delay of 48-hours the rat was reintroduced to the arena ("test phase"). The box now contained a third identical copy of the familiar object (A3) and a new object (B). These were placed in the same locations as the sample stimuli, whereby the position (left or right) of the novel object in the test phase was balanced between rats. For half the rats, object A was the sample and object B was the novel alternative. The test phase was 15 minutes in duration, with the first 30 seconds of object interaction used to determine preference scores. Any animal with less than 15 seconds of object exploration were excluded from analysis. Figure 10A shows the mean preference based on visits to the novel object. Figure 10B shows the mean preference based on time spent exploring the novel object. Fluoxetine produced a dose dependent increase in preference for the novel object suggesting cognitive enhancement with chronic fluoxetine.

Example 8 - Effect of the SSRI Fluoxetine in the in vivo novelty suppressed feeding depression assay

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Male F344 rats were dosed 1x per day for 21-days with 0 (vehicle only) or 10.0 mg/kg fluoxetine (n = 12 per dose group, p.o.). Twenty-four hours prior to behavioral testing, all food is removed from the home cage. At the time of testing a single pellet is placed in the center of a novel arena. Animals are placed in the corner of the arena and latency to eat the pellet is recorded. Compounds are generally administered 30 minutes prior to testing. Animals receive compound daily for 21 days and testing is performed on day 21. A decreased latency to eat the food pellet is indicative of both neurogenesis and antidepressant activity. Figure 11 shows the mean latency to approach and eat a food pellet within the novel environment. Fluoxetine treatment reduced the latency to eat the food pellet indicating anti-depressant activity of chronic fluoxetine.

**Example 9 - Effect of the 5-HT1a agonist Buspirone in the in vivo novel object recognition cognition assay**

Male F344 rats were dosed 1x per day for 28-days with 0 (vehicle only) or 0.5 and 5.0 mg/kg buspirone (n = 12 per dose group, i.p.). Behavioral testing was carried out as described in Example 7. The results are shown for the 5-HT1a agonist buspirone in Figure 12. Figure 12A shows the mean preference based on visits to the novel object. Figure 12B shows the mean preference based on time spent exploring the novel object. Buspirone did not increase preference for the novel object indicating an absence of cognitive enhancement with chronic buspirone.

**Example 10 - Effect of the 5-HT1a agonist Buspirone in the in vivo novelty suppressed feeding depression assay**

Male F344 rats were dosed 1x per day for 21-days with 0 (vehicle only) or 0.5 and 5.0 mg/kg buspirone (n = 12 per dose group, i.p.). Behavioral testing was carried out as described in Example 8. Figure 13 shows the mean latency to approach and eat a food pellet within the novel environment. Buspirone treatment did not significantly reduce the latency to eat the food pellet indicating an absence of anti-depressant activity with chronic buspirone.

**Example 11 - Effect of chronic dosing of the 5-HT1a agonist Buspirone on rat body weight**

Male F344 rats were dosed 1x per day for 28-days with 0 (vehicle only) or 0.5 and 5.0 mg/kg buspirone (n = 12 per dose group, i.p.). Body weights were collected daily.
Figure 14 shows the mean body weight over the 28-days of dosing. Buspirone treatment led to a gradual decrease in body weight relative to vehicle treated controls starting after 7 days of treatment and lasting throughout the length of the experiment.

Example 12 - Effect of 5-HTP in combination with an additional agent upon differentiation of human neural stem cells

Experiments with various concentrations of 5-HTP with dopamine were carried out generally as described in Example 1 for neuronal differentiation. The results are shown in Figure 15, which shows dose response curves for neuronal differentiation after background media values are subtracted. Dopamine alone did not significantly enhance neuronal differentiation. The combination of 10 µM or 30 µM 5-HTP with the non-neurogenic, but sensitizing, agent dopamine enhanced the stimulation of neuronal differentiation by dopamine in a dose-dependent manner. These data demonstrate that the combination of a 5HT1a receptor agonist and a neurogenic sensitizing agent can significantly enhance the combined neurogenic effects of the agents.

Example 13 - Effects of the 5-HT1a agonist Buspirone in combination with the melatonin agonist melatonin on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of buspirone in the presence or absence of the melatonin agonist melatonin, and stained with TUJ-1 antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 17 and 18, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of buspirone with melatonin are shown with the concentration response curves of buspirone or melatonin alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of buspirone with melatonin resulted in enhanced neuronal differentiation and concomitant reduced astrocyte differentiation relative to either agent alone.
Example 14 - Effects of the 5-HT1a agonist Buspirone in combination with the melatonin agonist melatonin on in vivo rat behavior and neurogenesis

Male F344 rats were dosed 1x per day for 21-days with 0 (vehicle only), 0.5 mg/kg buspirone (n = 12 per dose group, Lp.), 3.0 mg/kg melatonin (n = 12 per dose group, Lp.), or the combination of the two drugs at the same doses. Twenty-four hours prior to behavioral testing, all food is removed from the home cage. At the time of testing a single pellet is placed in the center of a novel arena. Animals are placed in the corner of the arena and the latency (in time) to eat the pellet is recorded. Compounds are generally administered 30 minutes prior to testing. Animals receive compound daily for 21 days and testing is performed on day 21. A decreased latency to eat the food pellet is indicative of both neurogenesis and antidepressant activity.

The results are in Figure 19 and show the mean latency to approach and eat a food pellet within the novel environment. Data are presented as latency to eat expressed as percent baseline. Melatonin or buspirone alone did not significantly reduce the latency to eat the food pellet. The combination of melatonin and buspirone resulted in a significant decrease in latency to eat the food pellet. The data indicate that the combination of buspirone and melatonin at doses that do not produce antidepressant activity (when each compound is dosed alone), results in significant antidepressant activity when administered in combination.

For the in vivo neurogenesis assays, male F344 rats were dosed 1x per day for 28-days with 0 (vehicle only), 0.5 mg/kg buspirone (n = 12 per dose group, Lp.), 3.0 mg/kg melatonin (n = 12 per dose group, ip) or the combination of the two drugs at the same doses. BrdU was administered once daily between days 9 and 14 (100mg/kg/day, Lp., n=12 per dose group). Figure 20 shows BrdU positive cell counts within the granule cell layer of the dentate gyrus. Data are presented as percent change in BrdU positive cells per cubic mm dentate gyrus. Melatonin or buspirone alone did not significantly change the number of BrdU positive cells. The combination of melatonin and buspirone resulted in a significant increase in BrdU positive cells compared to vehicle.

Example 15 - Effect of combined dosing the 5-HT1a agonist buspirone and melatonin on body weight in in vivo rodent studies

Male Fischer F344 rats are chronically injected with test compound(s) + vehicle or vehicle only (negative control) once daily. Rats are weighed daily, immediately
prior to injections. The results, shown in Figure 21, indicate that the combination of melatonin and buspirone resulted in decreased body weight over time.

Example 16 - Effects of the 5-HT1a agonist Buspirone in combination with the GABA agonist baclofen on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of buspirone in the presence or absence of the GABA agonist baclofen, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 22 and 23, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of buspirone with baclofen are shown with the concentration response curves of buspirone or baclofen alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of buspirone with baclofen resulted in selective reduction of astrocyte differentiation while retaining neuronal differentiation, indicating a lineage specific promotion of neuronal differentiation.

Example 17 - Effect of 5-HT3 antagonists on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of a 5-HT3 antagonist and stained with TUJ-I antibody, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 24, 25, 26, 27, 28, 29 and 30, which show concentration response curves of neuronal or astrocyte differentiation after background media values are subtracted for the 5-HT3 antagonists azasetron, granisetron, and ondansetron. The effect of azasetron on toxicity/survival of neural stem cells is shown in Figure 26. The data are presented as percents of neuronal positive control. The data indicate that azasetron,
granisetron, and ondansetron each promote differentiation of neural stem cells into neurons, and azasetron showed no detectable toxicity at concentrations up to 31.6 µM.

Example 18 - Effect of 5-HT4 agonists on neuronal differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of a 5-HT4 agonist and stained with TUJ-I antibody, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control. Results are shown in Figures 31, 32, 33, 34, 35 and 36, which show concentration response curves of neuronal or astrocyte differentiation after background media values are subtracted for the 5-HT4 agonists mosapride and cisapride. The effect of mosapride on toxicity/survival of neural stem cells is shown in Figure 33. The data are presented as percents of neuronal positive control. The data indicate that mosapride and cisapride each promote differentiation of neural stem cells into neurons, and mosapride showed no detectable toxicity at concentrations up to 31.6 µM.

Example 19 - Effect of sumatriptan on neuronal differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of the 5-HT1B/1D agonist sumatriptan and stained with TUJ-I antibody, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control. Results are shown in Figure 37, which shows concentration response curves of neuronal differentiation after background media values are subtracted for sumatriptan. The data are presented as percents of neuronal positive control. The data indicate that sumatriptan promotes differentiation of neural stem cells into neurons.

Example 20 - Effect of agomelatine on neuronal differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of the melatonin agonist agomelatine and...
stained with TUJ-I antibody, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 38, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The data is presented as a percent of neuronal positive control. The data indicate that agomelatine promotes differentiation of neural stem cells into neurons.

Example 21 - Effects of the 5-HT1a agonist Buspirone in combination with modafinil on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of buspirone in the presence or absence of modafinil, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 39 and 40, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of buspirone with modafinil are shown with the concentration response curves of buspirone or modafinil alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of buspirone with modafinil resulted in consistent neuronal differentiation with a simultaneous decrease in astrocyte differentiation relative to that produced by buspirone alone.

Example 22 - Effects of the 5-HT3 antagonist azasetron in combination with the 5-HT1a agonist buspirone on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of buspirone, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.
Results are shown in Figure 41, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with buspirone are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with buspirone resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone.

Example 23 - Effects of the 5-HT3 antagonist azasetron in combination with the GABA receptor agonist baclofen on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of buspirone, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 42, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with baclofen are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with baclofen resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone.

Example 24 - Effects of the 5-HT3 antagonist azasetron in combination with the ACE inhibitor captopril on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of captopril, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.
Results are shown in Figure 43, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with captopril are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with captopril resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone.

Example 25 - Effects of the 5-HT3 antagonist azasetron in combination with the PDE inhibitor ibudilast on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of ibudilast, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 44, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with ibudilast are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with ibudilast resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone.

Example 26 - Effects of the 5-HT3 antagonist azasetron in combination with the mixed opioid antagonist naltrexone on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of naltrexone, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.
Results are shown in Figures 45 and 46, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of buspirone with modafinil are shown with the concentration response curves of azasetron or naltrexone alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of azasetron with naltrexone resulted resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone concomitant with a simultaneous decrease in astrocyte differentiation relative to that produced by azasetron alone.

Example 27 - Effects of the 5-HT3 antagonist azasetron in combination with folic acid on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of folic acid, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 47 and 48, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of buspirone with modafinil are shown with the concentration response curves of azasetron or folic acid alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of azasetron with folic acid resulted resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone concomitant with a simultaneous decrease in astrocyte differentiation relative to that produced by azasetron alone.

Example 28 - Effects of the 5-HT3 antagonist azasetron in combination with gabapentin on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of
gabapentin, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 49, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with gabapentin are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with gabapentin resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone.

**Example 29 - Effects of the 5-HT3 antagonist azasetron in combination with methylphenidate on differentiation of human neural stem cells**

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of methylphenidate, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figures 50 and 51, which show concentration response curves of neuronal and astrocyte differentiation respectively, after background media values are subtracted. The concentration response curves of the combination of azasetron with methylphenidate are shown with the concentration response curves of azasetron or methylphenidate alone. The data is presented as a percent of neuronal or astrocyte positive control. The data indicate that the combination of azasetron with methylphenidate resulted in synergistically enhanced neuronal differentiation relative to that that produced by either agent alone concomitant with a simultaneous decrease in astrocyte differentiation relative to that produced by azasetron alone.
Example 30 — Effects of the 5-HT3 antagonist azasetron in combination with the anti-psychotic drug clozapine on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of clozapine, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described above. Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 52, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with clozapine are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with clozapine resulted in synergistically enhanced neuronal differentiation relative to that produced by either agent alone.

Example 31 — Effects of the 5-HT3 antagonist azasetron in combination with the GABA modulator carbamazepine on differentiation of human neural stem cells

Human neural stem cells (hNSCs) were isolated and grown in monolayer culture, plated, treated with varying concentrations of azasetron in the presence or absence of carbamazepine, and stained with TUJ-I antibody for the detection of neuronal differentiation or GFAP antibody for the detection of astrocyte differentiation, as described in U.S. Provisional Application No. 60/697,905 (incorporated by reference). Mitogen-free test media with a positive control for neuronal differentiation was used along with basal media without growth factors as a negative control.

Results are shown in Figure 53, which shows concentration response curves of neuronal differentiation after background media values are subtracted. The concentration response curves of the combination of azasetron with carbamazepine are shown with the concentration response curves either agent alone. The data is presented as a percent of neuronal positive control. The data indicate that the combination of azasetron with carbamazepine resulted in synergistically enhanced neuronal differentiation relative to that produced by either agent alone.
Example 32 - Determination of Synergy

The presence of synergy was determined by use of a combination index (CI). The CI based on the EC$_{50}$ was used to determine whether a pair of compounds had an additive, synergistic (greater than additive), or antagonistic effect when run in combination.

The CI is a quantitative measure of the nature of drug interactions, comparing the EC$_{50}$s of two compounds, when each is assayed alone, to the EC$_{50}$ of each compound when assayed in combination. The combination index (CI) is equal to the following formula:

$$CI = \frac{C1}{IC1} + \frac{C2}{IC2} + \frac{(CI \times C2)}{(IC1 \times IC2)}$$

where C1 and C2 are the concentrations of a first and a second compound, respectively, resulting in 50% activity in neuronal differentiation when assayed in combination; and IC1 and IC2 are the concentrations of each compound resulting in 50% activity when assayed independently. A CI of less than 1 indicates the presence of synergy; a CI equal to 1 indicates an additive effect; and a CI greater than 1 indicates antagonism between the two compounds.

Non-limiting examples of combinations of a 5HT agent and an additional agent as described herein were observed to result in synergistic activity. The exemplary results are shown in the following table:

<table>
<thead>
<tr>
<th>Combination</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azasetron + Buspirone</td>
<td>0.18</td>
</tr>
<tr>
<td>Azasetron + Baclofen</td>
<td>0.08</td>
</tr>
<tr>
<td>Azasetron + Captopril</td>
<td>0.47</td>
</tr>
<tr>
<td>Azasetron + Ibudilast</td>
<td>0.17</td>
</tr>
<tr>
<td>Azasetron + Naltrexone</td>
<td>0.45</td>
</tr>
<tr>
<td>Azasetron + Folic Acid</td>
<td>0.08</td>
</tr>
<tr>
<td>Azasetron + Gabapentin</td>
<td>0.24</td>
</tr>
<tr>
<td>Azasetron + Methylphenidate</td>
<td>0.06</td>
</tr>
<tr>
<td>Azasetron + Clozapine</td>
<td>0.06</td>
</tr>
<tr>
<td>Azasetron + Carbemazepine</td>
<td>0.09</td>
</tr>
</tbody>
</table>

As the CI is less than 1 for each of these combinations, the two compounds have a synergistic effect in neuronal differentiation.

The above is based on the selection of EC$_{50}$ as the point of comparison for the two compounds. The comparison is not limited by the point used, but rather the same
comparison may be made at another point, such as EC_{20}, EC_{30}, EC_{40}, EC_{60}, EC_{70}, EC_{80}, or any other EC value above, below, or between any of those points.

All references cited herein, including patents, patent applications, and publications, are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not.

Having now fully provided the instant disclosure, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the disclosure and without undue experimentation.

While the disclosure has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the disclosure following, in general, the disclosed principles and including such departures from the disclosure as come within known or customary practice within the art to which the disclosure pertains and as may be applied to the essential features hereinbefore set forth.
WHAT I S CLAIMED IS:

1. A method of stimulating or increasing neurogenesis in a cell or tissue, said method comprising contacting said cell or tissue with a 5HTR agent, optionally in combination with one or more other neurogenic agents, wherein the 5HTR agent or combination is effective to produce neurogenesis in said cell or tissue, and wherein the 5HTR agent when used alone is not a 5HTR 1a subtype agent.

2. The method of Claim 1, wherein said cell or tissue is in an animal subject or a human patient.

3. The method of Claim 2, wherein said patient is in need of neurogenesis or has been diagnosed with a disease, condition, or injury of the central or peripheral nervous system.

4. The method of any one of Claims 1-3, wherein said one or more other neurogenic agent is an opioid, or non-opioid, neurogenic agent.

5. The method of Claim 4, wherein said non-opioid neurogenic agent is dopamine.

6. The method of Claim 1, wherein said neurogenesis comprises differentiation of neural stem cells (NSCs) along a neuronal lineage.

7. The method of Claim 1, wherein said neurogenesis comprises differentiation of neural stem cells (NSCs) along a glial lineage.

8. The method of Claim 4, wherein said opioid is a kappa opioid receptor antagonist.

9. The method of Claim 8, wherein said opioid is a kappa opioid receptor selective antagonist.

10. The method of Claim 9, wherein said opioid is JDTic, nor-binaltorphimine, or buprenorphine.
11. The method of Claim 2 or 3, wherein said initial cell or tissue exhibits decreased neurogenesis or is subjected to an agent which decreases or inhibits neurogenesis.

12. The method of Claim 11, wherein said agent which decreases or inhibits neurogenesis is an opioid receptor agonist.

13. The method of Claim 12, wherein said opioid receptor agonist is morphine or another opiate.

14. The method of Claim 2 or 3, wherein said subject or patient has one or more chemical addictions or dependencies.

15. A method of treating a nervous system disorder related to cellular degeneration, a psychiatric condition, cellular trauma and/or injury, or another neurologically related condition in a subject or patient, said method comprising administering a 5HTR agent, optionally in combination with one or more other neurogenic agents, to produce an improvement in said disorder in said subject or patient, and wherein the 5HTR agent when used alone is not a 5HTR 1a subtype agent.

16. The method of Claim 15, wherein said nervous system disorder related to cellular degeneration is a neurodegenerative disorder, a neural stem cell disorder, a neural progenitor cell disorder, a degenerative disease of the retina, an ischemic disorder, or a combination thereof.

17. The method of Claim 15, wherein said nervous system disorder related to a psychiatric condition is a neuropsychiatric disorder, an affective disorder, depression, hypomania, panic attack, anxiety, excessive elation, bipolar depression, bipolar disorder (manic-depression), seasonal mood (or affective) disorder, schizophrenia or other psychosis, lissencephaly syndrome, anxiety syndrome, anxiety disorder, phobia, stress and related syndrome, cognitive function disorder, aggression, drug and alcohol abuse, obsessive compulsive behavior syndrome, borderline personality disorder, non-senile dementia, post-pain depression, post-partum depression, cerebral palsy, or a combination thereof.

18. The method of Claim 15, wherein said nervous system disorder related to cellular trauma and/or injury is a neurological trauma and injury, surgery related trauma and/or injury, retinal injury and trauma, injury related to epilepsy, spinal cord injury, brain...
injury, brain surgery, trauma related brain injury, trauma related to spinal cord injury, brain injury related to cancer treatment, spinal cord injury related to cancer treatment, brain injury related to infection, brain injury related to inflammation, spinal cord injury related to infection, spinal cord injury related to inflammation, brain injury related to environmental toxin, spinal cord injury related to environmental toxin, or a combination thereof.

19. The method of Claim 15, wherein said neurologically related condition is a learning disorder, memory disorder, autism, attention deficit disorder, narcolepsy, sleep disorder, cognitive disorder, epilepsy, temporal lobe epilepsy, or a combination thereof.

20. The method of Claim 17, wherein said psychiatric condition comprises depression.

21. The method of Claim 20, wherein said one or more other neurogenic agents comprises an anti-depressant agent.

22. The method of Claim 20, wherein said depression is due to morphine, alcohol, or drug use by the subject or patient.

23. The method of any one of Claims 15-22, wherein said 5HTR agent is buspirone, tandospirone, azasetron, granisetron, ondansetron, mosapride, cisapride, or sumatriptan.

24. The method of Claim 15, wherein said 5HTR agent is in a combination with one or more other neurogenic agents.

25. The method of Claim 15, wherein said 5HTR agent, optionally in combination with one or more other neurogenic agents, is in a pharmaceutically acceptable formulation.

26. A method of preparing a cell or tissue for transplantation to a subject or patient, said method comprising

   contacting said cell or tissue with a 5HTR agent, optionally in combination with one or more other neurogenic agents, to stimulate or increase neurogenesis in said cell or tissue.
27. A method of decreasing the level of astrogenesis in a cell or cell population, said method comprising contacting the cell or population with a 5HTR agent and a second agent that reduces or suppresses the amount or level of astrogenesis caused by said 5HTR agent.

28. The method of Claim 27, wherein said second agent is baclofen or melatonin.

29. The method of Claim 1, 15, 26 or 27, wherein there are two or more 5HTR agents.

30. The method of Claim 29, wherein the 5HTR agents are buspirone, azasetron, clozapine, cisapride, ondansetron, and/or tandospirone.

31. The method of Claim 1, 15, 26, 27, or 28 wherein the 5HTR agent is buspirone, tandospirone, azasetron, granisetron, ondansetron, mosapride, cisapride, sumatriptan, clozapine, agomelatine, or a combination thereof.

32. The method of Claim 1, 15 or 26, wherein the neurogenic agent is an opioid, or a PDE or GABA agent.

33. A composition comprising a 5HTR agent and a PDE agent.

34. The composition of Claim 33 wherein the 5HTR is 5HT3.

35. The composition of Claim 34 wherein the 5HTR agent is azasetron.

36. The composition of Claim 33 wherein the PDE agent is ibudilast.

37. A composition comprising a 5HTR agent and a GABA agent.

38. The composition of Claim 37 wherein the 5HTR agent is azasetron or buspirone.

39. The composition of Claim 38 wherein the GABA agent is baclofen or gabapentin.

40. A composition comprising a 5HTR agent and an opioid agent.
41. The composition of Claim 40 wherein the 5HTR agent is azasetron or buspirone and the opioid agent is naltrexone.

42. The composition of Claim 33, 37 or 40 wherein the agents are administered in a single formulation.

43. A composition comprising azasetron in combination with buspirone, baclofen, captopril, ibudilast, naltrexone, folic acid, methyl folate, gabapentin, methylphenidate, clozapine, or carbemazepine.

44. The method of Claim 1 or 15 wherein the 5HTR agent is a 5HTR3 antagonist or a 5HTR4 agonist.

45. The method of Claim 44 wherein the nervous system disorder is depression.

46. A method of stimulating or increasing neurogenesis in a cell or tissue, said method comprising contacting said cell or tissue with a 5HTR3 or 5HTR4 agent wherein the agent is effective to produce neurogenesis in said cell or tissue.

47. The method of Claim 1, 15, or 26 wherein the 5HTR agent is azasetron and the other agent is buspirone, baclofen, captopril, ibudilast, naltrexone, folic acid, methyl folate, gabapentin, methylphenidate, clozapine, or carbemazepine.
Human Neurogenesis Assay: 5-HTP
Neuronal Differentiation (TUJ1)

- 5-HTP
- Neuronal Positive Control

FIG. 1
Human Astrocyte Differentiation Assay: 5-HTP

Astrocyte Differentiation (GFAP)

- □ 5-HTP
- ▲ Astrocyte Positive Control

![Graph showing the percent of positive control against log conc (M) for 5-HTP and astrocyte positive control.](image-url)

**FIG. 2**
Human Neurogenesis Assay: Buspirone

Neuronal Differentiation (TUJ1)

- Buspirone
- Neuronal Positive Control

Percent of Positive Control vs. Log Conc(M)

FIG. 4
Human Astrocyte Differentiation Assay: Buspirone

Astrocyte Differentiation (GFAP)

- Buspirone
- Astrocyte Positive Control

FIG. 5
Human Neurogenesis Assay: Tandospirone

Neuronal Differentiation (TUJ1)

- Tandospirone
- Neuronal Positive Control

FIG. 7
Human Astrocyte Differentiation Assay: Tandospirone

Astrocyte Differentiation (GFAP)

- Tandospirone
- Astrocyte Positive Control

FIG. 8
Rat Cognition Assay: Fluoxetine Novel Object Recognition

FIG. 10
Rat Depression Assay: Fluoxetine
Novelty Suppressed Feeding

Novelty Suppressed Feeding: Latency to Eat

Fluoxetine 10

Vehicle

Latency to Eat (seconds)

* p < 0.05

FIG. 11
Rat Cognition Assay: Buspirone Novel Object Recognition

**Fig. 12**

**A**

Novel Object Recognition (Visits)

- 90%
- 80%
- 70%
- 60%
- 50%
- 40%
- 30%
- 20%
- 10%
- 0%

Vehicle

0.05 mg/kg

5.0 mg/kg

**B**

Novel Object Recognition (Time)

- 90%
- 80%
- 70%
- 60%
- 50%
- 40%
- 30%
- 20%
- 10%
- 0%

Vehicle

0.05 mg/kg

5.0 mg/kg

Mean Preference (%)
Human Neuronal Differentiation Assay

- ■ Dopamine
- ● Dopamine with 10uM 5-HTP
- ▲ Dopamine with 30uM 5-HTP

Percent of Positive Control

Log Conc (M)

FIG. 15
Human Astrocyte Differentiation Assay: Buspirone + Melatonin

Astrocyte Differentiation (GFAP)

- Buspirone + Melatonin
- Buspirone
- Melatonin

Percent of Positive Control

Conc (M)

FIG. 18
NSF: Buspirone + Melatonin

Novelty Suppressed Feeding (at 21 days)

![Bar graph showing latency to eat (% baseline) for different groups.
Vehicle, Melatonin 3.0 mg/kg, Buspirone 0.5 mg/kg, Melatonin + Buspirone.

* p = 0.003

FIG. 19
In Vivo Neurogenesis
Buspirone + Melatonin

BrdU Positive Cells in Dorsal Granule Cell Layer

* p < 0.01 (11% increase)

FIG. 20
In Vivo Weight Gain Studies
Buspirone + Melatonin

Body Weight (grams)

Days of Treatment

- ○ Vehicle
- ▲ 3 mg/kg Melatonin
- ◇ 10 mg/kg Melatonin
- ▼ 3 mg/kg Melatonin + 0.5 mg/kg Buspirone

FIG. 21
Human Neurogenesis Assay: Buspirone + Baclofen

Neuronal Differentiation (TUJ1)

- Buspirone + Baclofen
- Buspirone
- Baclofen

Percent of Positive Control

Conc (M)

FIG. 22
Human Astrocyte Differentiation Assay: Buspirone + Baclofen

**Astrocyte Differentiation (GFAP)**

- **Buspirone + Baclofen**
- **Buspirone**
- **Baclofen**

**FIG. 23**

**Percent of Positive Control**

**Conc (M)**

10^{-8.5} 10^{-8.0} 10^{-7.5} 10^{-7.0} 10^{-6.5} 10^{-6.0} 10^{-5.5} 10^{-5.0} 10^{-4.5} 10^{-4.0}
FIG. 24
Azasetron - Astrocyte Differentiation

Percent of Positive Control

Log Conc (M)

FIG. 25
FIG. 26

- Azasetron

Percent of Control Cell Count

Log Conc (M)

-6.25 -6.00 -5.75 -5.50 -5.25 -5.00 -4.75 -4.50 -4.25

Control Cell Count

120
110
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
FIG. 27
Human Neurogenesis Assay: Azasetron

Human Neurogenesis Assay

- Azasetron

Percent of Positive Control

Conc (M)

FIG. 28
Human Neurogenesis Assay: Granisetron

Human Neurogenesis Assay

- Granisetron

Percent of Positive Control

Conc (M)

FIG. 29
Human Neurogenesis Assay: Ondansetron

Human Neurogenesis Assay

- Ondansetron

![Graph showing the effect of Ondansetron on neurogenesis across different concentrations.](image)

**FIG. 30**
FIG. 31
Mosapride Citrate - Astrocyte Differentiation

FIG. 32
FIG. 33
Human Neurogenesis Assay: Mosapride

Neuronal Differentiation (TUJ1)

- Mosapride

Percent of Positive Control

Log Conc (M)

FIG. 35
Human Neurogenesis Assay: Cisapride

Neuronal Differentiation (TUJ1)

Cisapride

![Graph showing the effect of Cisapride concentration on neuronal differentiation](image-url)

FIG. 36
Human Neurogenesis Assay: Sumatriptan

Neuronal Differentiation (TUJ1)

- Sumatriptan

![Graph showing the effect of Sumatriptan concentration on neuronal differentiation](image)

FIG. 37
Human Neurogenesis Assay: 4-P-PDOT

Agomelatine
Neuronal Differentiation (TUJ1)

![Graph showing the effect of Agomelatine on neuronal differentiation.](image-url)

FIG. 38
Human Neurogenesis Assay: Buspirone + Modafinil

Neuronal Differentiation (TUJ1)

Percent of Positive Control

Conc (M)

FIG. 39
Human Astrocyte Differentiation Assay: Buspirone + Modafinil

Astrocyte Differentiation (GFAP)

- • Buspirone
- ■ Modafinil
- ◆ Combination

Percent of Positive Control

Conc (M)

10^{-8.5} 10^{-7.5} 10^{-6.5} 10^{-5.5} 10^{-4.5}

FIG. 40
Human Neurogenesis Assay: Azasetron + Buspirone

Neuronal Differentiation (TUJ1)

- Azasetron
- Buspirone
- Combination

Percent of Positive Control vs. Conc (M)

FIG. 41
Human Neurogenesis Assay: Azasetron + Baclofen

Neuronal Differentiation (TUJ1)

- • Azasetron
- ■ Baclofen
- ♦ Combination

Percent of Positive Control

Conc (M)

FIG. 42
Human Neurogenesis Assay: Azasetron + Captopril

Neuronal Differentiation (TUJ1)

- Azasetron
- Captopril
- Combination

FIG. 43
Human Neurogenesis Assay: Azasetron + Ibudilast

Neuronal Differentiation (TUJ1)

- • Azasetron
- ■ Ibudilast
- ▲ Combination

Percent of Positive Control

Azasetron Concentration

Ibudilast Concentration

Conc (M)

FIG. 44
Human Neurogenesis Assay: Azasetron + Naltrexone

Neuronal Differentiation (TUJ1)

- • Azasetron
- ■ Naltrexone
- ◆ Combination

Percent of Positive Control

Conc (M)

FIG. 45
Human Astrocyte Differentiation Assay: Azasetron + Naltrexone

Astrocyte Differentiation (GFAP)

- - - Azasetron
- - - Naltrexone
- - - Combination

Percent of Positive Control

Conc (M)

FIG. 46
Human Neurogenesis Assay: Azasetron + Folic Acid

Neuronal Differentiation (TUJ1)

- • - Azasetron
- ■ - Folic Acid
- ◆ - Combination

![Graph showing neuronal differentiation](image)

**FIG. 47**
Human Astrocyte Differentiation Assay: Azasetron + Folic Acid

Astrocyte Differentiation (GFAP)

- Azasetron
- Folic Acid
- Combination

Percent of Positive Control

 Conc (M)  $10^{-9}$  $10^{-8}$  $10^{-7}$  $10^{-6}$  $10^{-5}$  $10^{-4}$

FIG. 48
Human Neurogenesis Assay:
Azasetron + Gabapentin

Neuronal Differentiation (TUJ1)

- - - - Azasetron
- - - - Gabapentin
- - - - Combination

Percent of Positive Control

Conc (M)

FIG. 49
Human Neurogenesis Assay: Azasetron + Methylphenidate

Neuronal Differentiation (TUJ1)

- Azasetron
- Methylphenidate
- Combination

Percent of Positive Control

Azasetron Concentration

Methylphenidate Concentration

Conc (M)

FIG. 50
Human Astrocyte Differentiation Assay: Azasetron + Methylphenidate

Astrocyte Differentiation (GFAP)

- Azasetron
- Methylphenidate
- Combination

Percent of Positive Control

Azasetron Concentration

Conc (M)

Methylphenidate Concentration

FIG. 51
Human Neurogenesis Assay: Azasetron + Clozapine

Neuronal Differentiation (TUJ1)

- Azasetron
- Clozapine
- Combination

Percent of Positive Control

Conc (M)

FIG. 52
Human Neurogenesis Assay: Azasetron + Carbemazepine

Neuronal Differentiation (TUJ1)

- Azasetron
- Carbemazepine
- Combination

Percent of Positive Control

Conc (M)

FIG. 53