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**METHODS OF IDENTIFYING COMPOUNDS FOR TREATING DEPRESSION AND
OTHER RELATED DISEASES**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application serial number 61/824,667, filed May 17, 2013, and U.S. Provisional Patent Application serial number 61/713,085, filed October 12, 2012, each of which is hereby incorporated by reference in its entirety.

BACKGROUND

5 [0002] The N-methyl-D-aspartate (NMDA) receptor (NMDAR) has been implicated in neurodegenerative disorders including stroke-related brain cell death, convulsive disorders, and learning and memory. NMDAR also plays a central role in modulating normal synaptic transmission, synaptic plasticity, and excitotoxicity in the central nervous system. The NMDAR is further involved in Long-term potentiation (LTP).

10 [0003] The NMDAR is activated by the binding of NMDA, glutamate (Glu), and aspartate (Asp). It is competitively antagonized by D-2-amino-5-phosphonovalerate (D-AP5; D-APV), and non-competitively antagonized by phenylcyclidine (PCP), and MK-801. Most interestingly, the NMDAR is co-activated by glycine (Gly) (Kozikowski et al., 1990, *Journal of Medicinal Chemistry* 33:1561-1571). The binding of glycine occurs at an allosteric regulatory site on the
15 NMDAR complex, and this increases both the duration of channel open time, and the frequency of the opening of the NMDAR channel.

[0004] Recent human clinical studies have identified NMDAR as a novel target of high interest for treatment of depression. These studies conducted using known NMDAR antagonists CPC-101,606 and ketamine have shown significant reductions in the Hamilton Depression
20 Rating Score in patients suffering with refractory depression. Although, the efficacy was significant, but the side effects of using these NDMAR antagonists were severe.

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[0005] NMDA-modulating small molecule agonist and antagonist compounds have been developed for potential therapeutic use. However, many of these are associated with very narrow therapeutic indices and undesirable side effects including hallucinations, ataxia, irrational behavior, and significant toxicity, all of which limit their effectiveness and/or safety.

5 Further, 50% or more of patients with depression do not experience an adequate therapeutic response to known administered drugs. There currently is no single effective treatment for depression, anxiety, and other related diseases.

[0006] Thus, there remains a need for improved treatments of depression, anxiety and/or other related diseases with compounds that provide increased efficacy and reduced undesirable
10 side effects.

SUMMARY

[0007] The present disclosure relates in part to methods of identifying a candidate compound suitable for treatment of depression. In some embodiments, a candidate compound may be a NMDAR partial agonist.

[0008] In one aspect, a method for identifying a candidate compound suitable for treatment
15 of depression is provided. The method comprises exposing a cell to a potential compound in a culture medium, or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain or neural tissue of the animal, at one or more predetermined time points; analyzing the sample for increased or decreased expression levels of Wnt1 and /or identifying the candidate compound as suitable for treatment of depression based
20 on the increased expression level of Wnt1.

[0009] In another aspect, a method for identifying a candidate compound suitable for treatment of depression is provided. The method comprises exposing a cell to a potential compound in a culture medium, or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain or neural tissue of the animal, at
25 one or more predetermined time points; analyzing the sample for increased expression levels of at least one of the genes listed in Table 1 or 2 indicated with a G, or decreased expression levels or at least one of the genes listed in Table 1 or 2 indicated with a K, and identifying the compound as suitable for treatment of depression based on the increased expression level or decreased expression level.

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[0010] In some embodiments, the sample has a gene expression pattern as provided in Table 1 or 2 with the indication "G" and the identifying is based on increased expression of those genes.

5 [0011] In some embodiments, a contemplated method further comprises analyzing the candidate compound for NDMA subunit NR2B synaptic plasticity.

[0012] In another aspect, a method for identifying a compound suitable for treatment of depression is provided. The method comprises exposing a cell to a potential compound in a culture medium, or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain or neural tissue of the animal, at one or more
10 predetermined time points; analyzing the sample for NMDA receptor NR2B subunit plasticity, and identifying the compound as suitable for treatment of depression based on inducing the NR2B plasticity.

[0013] In some embodiments, a candidate compound suitable for treating depression significantly induces NR2B dependent synaptic plasticity as compared to ketamine.

15 [0014] In some embodiments, the tissue is medial prefrontal cortex.

[0015] In some embodiments, the animal is a rodent or human, and the cell is a human or rodent cell.

[0016] In some embodiments, the compound modulates the NMDA receptor.

[0017] In some embodiments, the compound suitable for treating depression has fewer side
20 effects as compared to ketamine.

[0018] In some embodiments, the compound does not have substantial addictive sensory motor grating and/or sedative effect.

[0019] In some embodiments, the cell is a eukaryotic cell.

[0020] In some embodiments, a contemplated method further comprises selecting the
25 candidate compound from a library of compounds.

[0021] In yet another aspect, a method of identifying a therapeutic compound capable of treating depression in a patient is provided. The method comprises selecting a compound that significantly induces NR2B dependent synaptic plasticity.

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BRIEF DESCRIPTION OF THE FIGURES

[0022] FIG. 1 shows the effects of GLYX-13 and Ketamine on gene expression in the medial prefrontal cortex of an adult rat at 1 hour and 24 hours post-IV injection.

[0023] FIG. 2 shows the effects of GLYX-13 on Wnt pathway specific gene expression relative to vehicle controls.

5 [0024] FIG. 3 shows a schematic of the Wnt signaling pathway.

[0025] FIG. 4 shows the anti-depressant-like effects of GLYX-13 in multiple rat models.

[0026] FIG. 5 shows the results from various tests which indicate that GLYX-13 does not show ketamine-like addictive sensory-motor gating, or sedative side effects.

10 [0027] FIG. 6 shows the results of Porsolt tests which demonstrate that GLYX-13 is antidepressant-like compared to ketamine.

[0028] FIG. 7 shows results which demonstrate the GLYX-13 induces NR2B-dependent plasticity.

[0029] FIG. 8 shows the antidepressant-like effects of GLYX-13 are synaptic-plasticity related.

15 [0030] FIG. 9 shows GLYX-13 increases *ex vivo* [³H] MK-801 binding in rat medial prefrontal cortex 1 hour after dosing.

[0031] FIG. 10 shows plots of phosphoserine 1303 NR2B (pS1303 NR2B) protein levels (left panel) and total NR2B protein levels (middle panel) as a function of post-dosing time with GLYX-13 and shows a bar graph (right panel) of the ratio of pS1303 NR2B protein levels to
20 total NR2B protein levels as a function of post-dosing time with GLYX-13.

[0032] FIG. 11 shows plots of phosphoserine 1480 NR2B (pS1480 NR2B) protein levels (left panel) and total NR2B protein levels (middle panel) as a function of post-dosing time with GLYX-13 and shows a bar graph (right panel) of the ratio of pS1480 NR2B protein levels to total NR2B protein levels as a function of post-dosing time with GLYX-13.

25 [0033] FIG. 12 shows a bar graph (left panel) of CK2 kinase activity 15 minutes post-dosing with GLYX-13 as measured by phosphorylation of a CK2 substrate and shows a plot (right panel) of a standard curve for the CK2 kinase activity assay.

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DETAILED DESCRIPTION

[0034] The present disclosure relates in part to methods of identifying a candidate compound suitable for treatment of depression. In some embodiments, a candidate compound may be a NMDAR partial agonist. In another aspect, the present disclosure relates in part to the use of identified compounds for treatment of clinically relevant depression and/or for
5 general treatment of depression and/or anxiety.

[0035] Depression is a common psychological problem and refers to a mental state of low mood and aversion to activity. Various symptoms associated with depression include persistent anxious or sad feelings, feelings of helplessness, hopelessness, pessimism, and/or
10 worthlessness, low energy, restlessness, irritability, fatigue, loss of interest in pleasurable activities or hobbies, excessive sleeping, overeating, appetite loss, insomnia, thoughts of suicide, and suicide attempts. The presence, severity, frequency, and duration of the above mentioned symptoms vary on a case to case basis. In some embodiments, a patient may have at least one, at least two, at least three, at least four, or at least five of these symptoms.

[0036] The most common depression conditions include Major Depressive Disorder and
15 Dysthymic Disorder. Other depression conditions develop under unique circumstances. Such depression conditions include but are not limited to Psychotic depression, Postpartum depression, Seasonal affective disorder (SAD), mood disorder, depressions caused by chronic medical conditions such as cancer or chronic pain, chemotherapy, chronic stress, post traumatic stress disorders, and Bipolar disorder (or manic depressive disorder). Refractory depression
20 occurs in patients suffering from depression who are resistant to standard pharmacological treatments, including tricyclic antidepressants, MAOIs, SSRIs, and double and triple uptake inhibitors and/or anxiolytic drugs, as well non-pharmacological treatments such as psychotherapy, electroconvulsive therapy, vagus nerve stimulation and/or transcranial magnetic stimulation. A treatment resistant-patient may be identified as one who fails to experience
25 alleviation of one or more symptoms of depression (e.g., persistent anxious or sad feelings, feelings of helplessness, hopelessness, pessimism) despite undergoing one or more standard pharmacological or non-pharmacological treatment. In certain embodiments, a treatment-resistant patient is one who fails to experience alleviation of one or more symptoms of depression despite undergoing treatment with two different antidepressant drugs. In other
30 embodiments, a treatment-resistant patient is one who fails to experience alleviation of one or

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more symptoms of depression despite undergoing treatment with four different antidepressant drugs. A treatment-resistant patient may also be identified as one who is unwilling or unable to tolerate the side effects of one or more standard pharmacological or non-pharmacological treatment. In certain embodiments, methods for treating refractory depression by administering
5 an effective amount of an identified compound to a treatment-resistant patient in need thereof are contemplated. In an embodiment, methods of treating depression is contemplated when a patient has suffered depression for e.g. 5, 6, 7, 8 or more weeks, or for a month or more.

[0037] In an embodiment, a method for identifying a candidate compound suitable for treatment of depression is provided comprising exposing a cell to a potential compound in a
10 culture medium, or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain or neural tissue of the animal, at one or more predetermined time points; analyzing the sample for increased expression levels of Wnt1, and /or identifying the candidate compound as suitable for treatment of depression based on the increased expression level of Wnt1.

15 [0038] In another embodiment, a method for identifying a candidate compound suitable for treatment of depression, is provided comprising: exposing a cell to a potential compound in a culture medium, and/or or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain or neural tissue of the animal, at one or more predetermined time points; analyzing the sample for increased expression levels of at
20 least one of the genes listed in Table 1 or 2 (as provided below) indicated with a G, or decreased expression levels or at least one of the genes listed in Table 1 or 2 indicated with a K, and identifying the compound as suitable for treatment of depression based on the increased expression level or decreased expression level.

[0039] A contemplated sample may have a gene expression pattern as provided in Table 1 or
25 2 with the indication "G" and the identifying is based on increased expression of those genes.

[0040] Contemplated methods may further comprising analyzing the candidate compound for NDMA subunit NR2B synaptic plasticity.

[0041] A method for identifying a compound suitable for treatment of depression or other indications is provided herein in an embodiment is provided, wherein the method may include
30 exposing a cell to a potential compound in a culture medium, or administering a potential compound to an animal; retrieving a sample from the cell and/or culture medium, or from brain

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or neural tissue of the animal, at one or more predetermined time points; analyzing the sample for NMDA receptor NR2B subunit plasticity, and identifying the compound as suitable for treatment of depression based on inducing the NR2B plasticity. A candidate compound suitable for treating depression may significantly induce NR2B dependent synaptic plasticity as compared to ketamine.

[0042] Tissues contemplated herein may be tissue of medial prefrontal cortex. Animals contemplated may be a rodent or human; cells may be a human or rodent cell. Contemplated candidate compounds may modulate a NMDA receptor, e.g. a candidate compound may be a NMDA partial agonist.

[0043] A candidate compound suitable for treating depression may have fewer side effects as compared to ketamine, for example the compound may not have substantial addictive sensory motor grating and/or sedative effect.

[0044] In an embodiment, a method of identifying a therapeutic compound capable of treating depression in a patient, is provided, comprising selecting a compound that significantly induces NR2B dependent synaptic plasticity.

[0045] Identified compounds may act predominantly at NR2B-containing NMDARs, and may not display the classic side effects of known NMDAR modulators such as CPC-101,606 and ketamine. For example, identified compounds may have markedly elevated long-term potentiation (LTP) while simultaneously reducing long-term depression (LTD) in rat hippocampal organotypic cultures. In some embodiments, identified compounds may produce an antidepressant effect essentially without dissociative side effects when administered to a subject in therapeutic amounts. In certain embodiments, an antidepressant effect with essentially no sedation may be produced by identified compounds when administered to a subject in therapeutic amounts. In still other embodiments, identified compounds may not have abuse potential (e.g., may not be habit-forming).

[0046] In some embodiments, compounds may increase AMPA GluR1 serine-845 phosphorylation or reduce expression in Wnt1 or Wnt signaling, for example as compared to ketamine.

[0047] Additionally, identified compounds may have better Blood-Brain Barrier (BBB) penetration as compared to many of the earlier glycine site ligands (Leeson & Iversen, J. Med.

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Chem. 37:4053-4067, 1994) and may cross the BBB readily. In some embodiments, identified compounds or a composition comprising same may provide better i.v. in vivo potency and/or brain level concentration, relative to plasma levels, e.g. as compared to ketamine.

[0048] A variety of depression conditions are expected to be treated with an identified compound without for example affecting behavior or motor coordination, and without inducing or promoting seizure activity. Exemplary depression conditions that are expected to be treated according to this aspect include, but are not limited to, major depressive disorder, dysthymic disorder, psychotic depression, postpartum depression, premenstrual syndrome, premenstrual dysphoric disorder, seasonal affective disorder (SAD), anxiety, mood disorder, depressions caused by chronic medical conditions such as cancer or chronic pain, chemotherapy, chronic stress, post traumatic stress disorders, risk of suicide, and bipolar disorder (or manic depressive disorder). It should be understood that depression caused by bipolar disorder may be referred to as bipolar depression. In addition, patients suffering from any form of depression often experience anxiety. Various symptoms associated with anxiety include fear, panic, heart palpitations, shortness of breath, fatigue, nausea, and headaches among others. It is expected that the methods of the present condition can be used to treat anxiety or any of the symptoms thereof.

[0049] In addition, a variety of other neurological conditions are expected to be treated according to the methods. Exemplary conditions include, but are not limited to, a learning disorder, autistic disorder, attention-deficit hyperactivity disorder, Tourette's syndrome, phobia, post-traumatic stress disorder, dementia, AIDS dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease, spasticity, myoclonus, muscle spasm, bipolar disorder, a substance abuse disorder, urinary incontinence, and schizophrenia.

[0050] Also provided herein are methods of treating depression in treatment resistant patients or treating refractory depression, e.g., patients suffering from a depression disorder that does not, and/or has not, responded to adequate courses of at least one, or at least two, other antidepressant compounds or therapeutics. For example, provided herein is a method of treating depression in a treatment resistant patient, comprising a) optionally identifying the patient as treatment resistant and b) administering an effective dose of an identified compound to said patient.

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[0051] Symptoms of depression, and relief of same, may be ascertained by a physician or psychologist, e.g. by a mental state examination. Symptoms include thoughts of hopelessness, self-harm or suicide and/or an absence of positive thoughts or plans.

[0052] Contemplated methods include a method of treating autism and/or an autism spectrum disorder in a patient need thereof, comprising administering an effective amount of an identified to the patient. For example, upon administration, an identified compound may decrease the incidence of one or more symptoms of autism such as eye contact avoidance, failure to socialize, attention deficit, poor mood, hyperactivity, abnormal sound sensitivity, inappropriate speech, disrupted sleep, and perseveration. Such decreased incidence may be measured relative to the incidence in the untreated individual or an untreated individual(s). In some embodiments, patients suffering from autism also suffer from another medical condition, such as Fragile X syndrome, tuberous sclerosis, congenital rubella syndrome, and untreated phenylketonuria.

[0053] In another embodiment, methods of treating a disorder in a patient need thereof are contemplated, wherein the disorder is selected from group consisting of: epilepsy, AIDS dementia, multiple system atrophy, progressive supra-nuclear palsy, Friedrich's ataxia, autism, fragile X syndrome, tuberous sclerosis, attention deficit disorder, olivio-ponto-cerebellar atrophy, cerebral palsy, drug-induced optic neuritis, peripheral neuropathy, myelopathy, ischemic retinopathy, glaucoma, cardiac arrest, behavior disorders, and impulse control disorders that includes administering an identified compound.

[0054] In an embodiment, contemplated herein are methods of treating attention deficit disorder, ADHD (attention deficit hyperactivity disorder), schizophrenia, anxiety, amelioration of opiate, nicotine and/or ethanol addiction (e.g., method of treating such addiction or ameliorating the side effects of withdrawing from such addiction), spinal cord injury diabetic retinopathy, traumatic brain injury, post-traumatic stress syndrome and/or Huntington's chorea, in a patient in need thereof, that includes administering an identified compound. For example, patients suffering from schizophrenia, addiction (e.g. ethanol or opiate), autism, Huntington's chorea, traumatic brain injury, spinal cord injury, post-traumatic stress syndrome and diabetic retinopathy may all be suffering from altered NMDA receptor expression or functions.

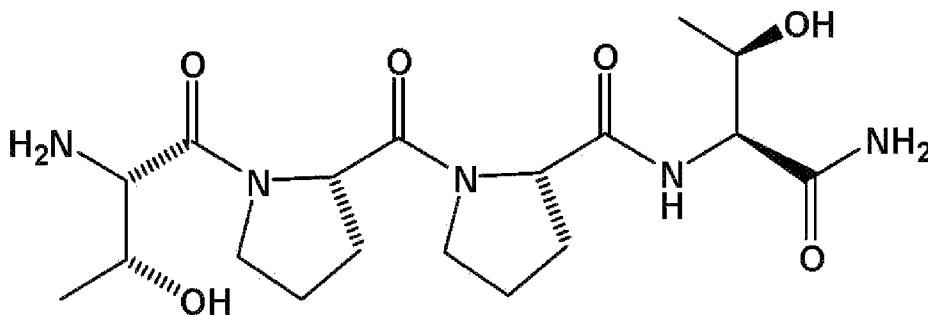
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[0055] In another embodiment, a method of treating Alzheimer's disease, or e.g., treatment of memory loss that e.g., accompanies early stage Alzheimer's disease, in a patient in need thereof is provided, comprising administering an identified compound.

[0056] Toxicity and therapeutic efficacy of subject compounds may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 and the ED50.

[0057] As used herein, the term "GLYX peptide" refers to a peptide having NMDAR glycine-site partial agonist/antagonist activity. GLYX peptides may be obtained by well-known recombinant or synthetic methods such as those described in US Patents 5,763,393 and 4,086,196 herein incorporated by reference. In some embodiments, GLYX refers to a tetrapeptide having the amino acid sequence Thr-Pro-Pro-Thr (SEQ ID NO: 13), or L-threonyl-L-prolyl-L-prolyl-L-threonine amide. In some embodiments, candidate compounds have the same microarray results as GLYX-13 and/or the below compounds.

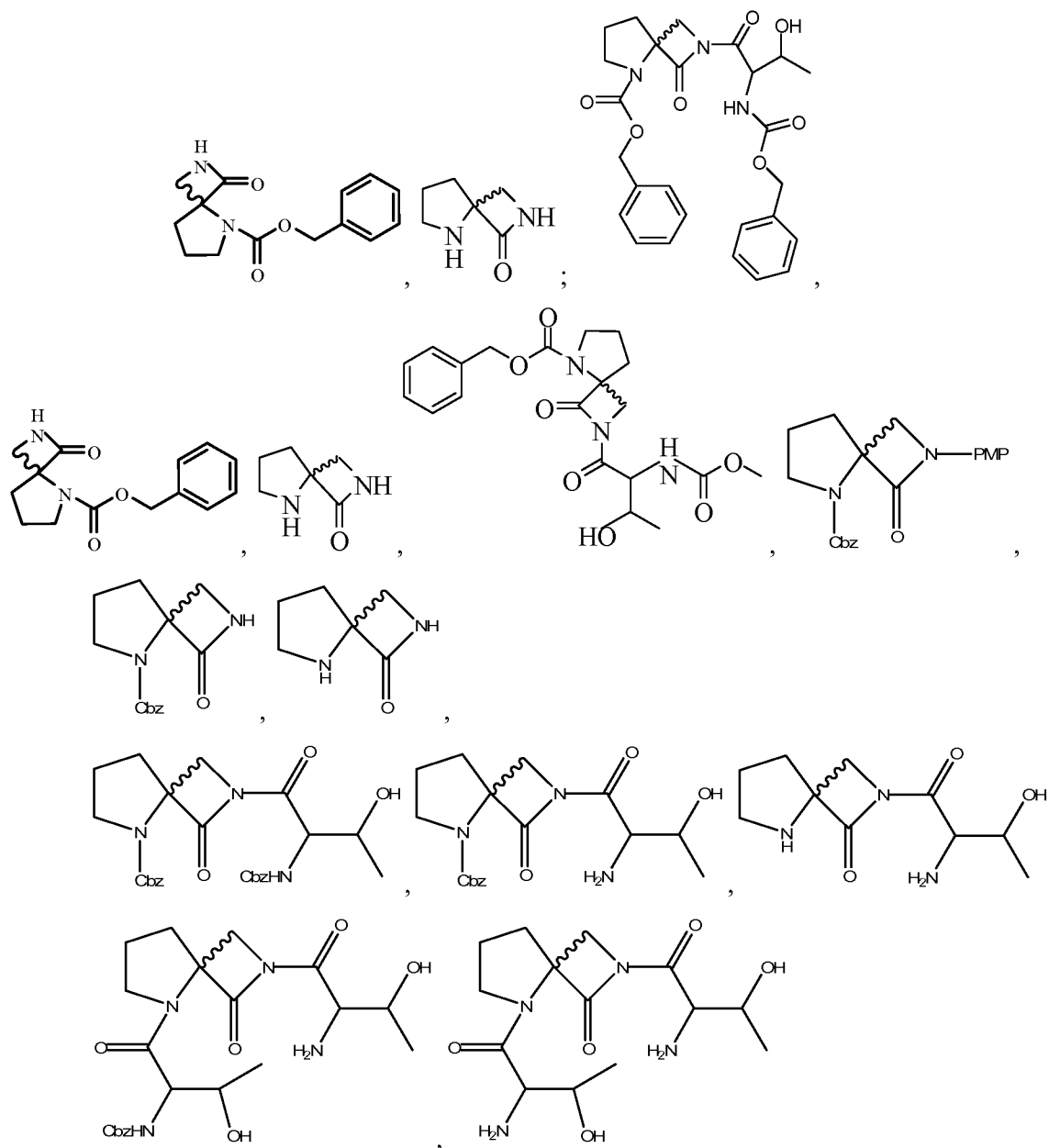
[0058] For example, GLYX-13 refers to the compound depicted as:



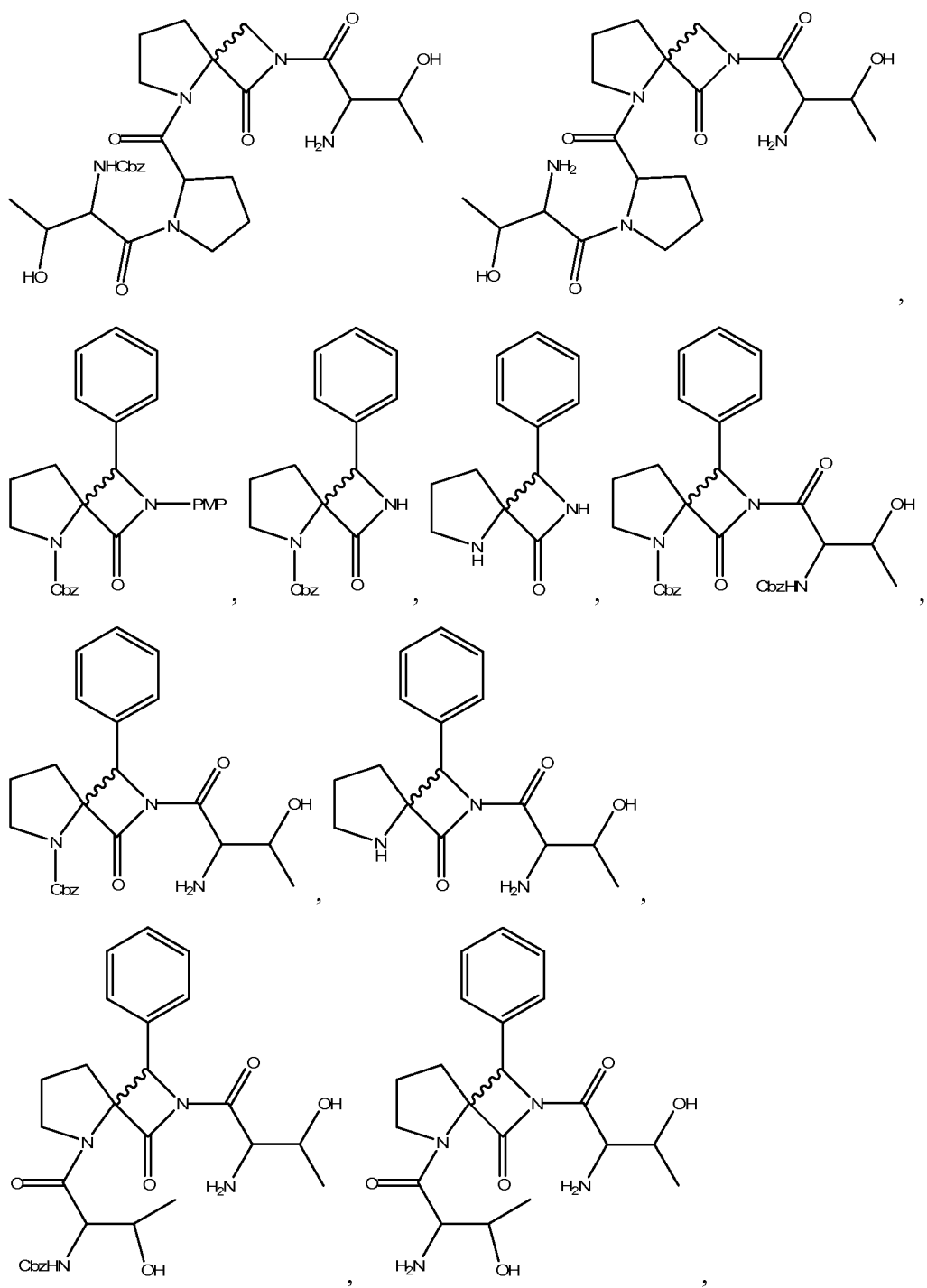
Formula I.

[0059] Also contemplated are polymorphs, homologs, hydrates, solvates, free bases, and/or suitable salt forms of GLYX 13 such as, but not limited to, the acetate salt. The peptide may be cyclized or non-cyclized form as further described in US 5,763,393. In some embodiments, a GLYX-13 analog may include an insertion or deletion of a moiety on one or more of the Thr or Pro groups such as a deletion of CH₂, OH, or NH₂ moiety. In other embodiments, GLYX-13 may be optionally substituted with one or more halogens, C₁-C₃ alkyl (optionally substituted with halogen or amino), hydroxyl, and/or amino. Glycine-site partial agonist of the NMDAR

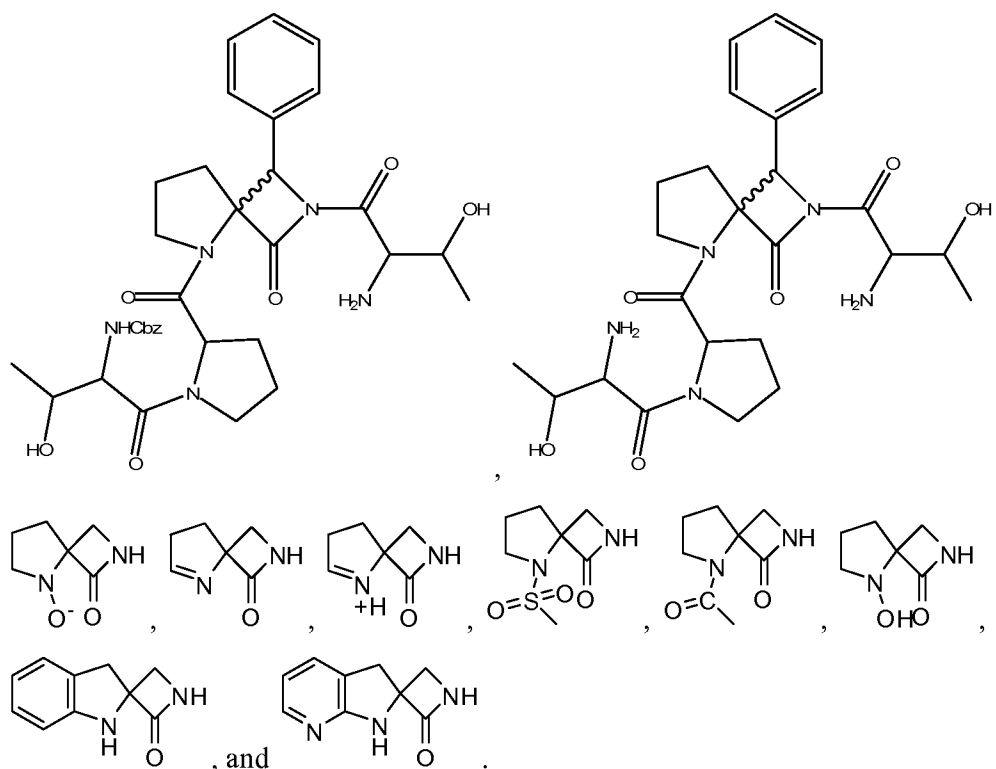
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[0061] The present disclosure has multiple aspects, illustrated by the following non-limiting examples.

EXAMPLES

Example 1: Gene Expression Patterns After Administration of GLYX-13 or Ketamine

[0062] In the present study, gene expression patterns in the medial prefrontal cortex (mPFC) were examined following either GLYX-13 (3 mg/kg, IV; the lowest dose that produces an anti-depressant effect in the Porsolt test) or ketamine (10 mg/kg, IV; a dose that produces a long-lasting anti-depressant effect in the Porsolt test) using a focused microarray platform combined with ontological analyses to identify functionally related gene sets that were differentially effected by GLYX-13 and ketamine. Among the most interesting of these was the Wnt signaling pathway. A Wnt pathway-specific qRT-PCR array was used to corroborate these findings. Using this qRT-PCR array, the results showed that at 1 hr after GLYX-13 injections, 5 genes were differentially expressed as compared to saline treated control rats. At 24hrs after GLYX-13 administration, 4 genes were upregulated. At 1 and 24 hrs following ketamine administration only 1 gene was downregulated. Taken together, these data suggest that although both GLYX-13 and ketamine produce rapid antidepressant-like effects in the

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Porsolt test, they likely effect changes in different cellular signaling pathways; one such example being the Wnt signaling pathway.

Methods:

[0063] Animals: Adult (2-3 month-old) male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed 3 to a cage and injected (IV) with one of the following - GLYX-13 (3mg/kg), ketamine (10 mg/kg), or saline vehicle (1ml/kg). At 1 and 24 hours after injection (N=5 per time point for each treatment group), rats were sacrificed, their brains were quickly dissected, frozen, and then stored at -80°C. The medial prefrontal cortex (mPFC) was dissected from frozen tissue on ice. An equal volume of the homogenized tissue was used to extract and purify RNA for microarray analysis and to make cDNA for qRT-PCR analysis. All procedures were approved by the Northwestern University IACUC committee and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

[0064] Transcriptomics: Using an in-house microarray (Kroes et al., 2006), we assayed the expression of 1,178 genes specific to the rat brain and representing more than >90% of the major gene ontological categories in the mPFC of rats at 1 or 24 hrs post-injection (IV) with GLYX-13, ketamine, or saline (N=5 adult male rats at each time-point per treatment group). Equivalent aliquots of rat reference RNA (Stratagene, La Jolla, Ca) were treated concurrently with the tissue samples. Reverse transcription of 5 ug of RNA (primed with an oligo(dT) primer bearing a T7 promoter was followed by *in vitro* transcription in the presence of amino-allyl dUTP. The aRNA was denatured, hybridized, and washed with high stringency. Fluorescence hybridization was then quantified by a high resolution confocal laser scanner utilizing QuantArray software and analyzed using GeneTraffic (Iobion Informatics, La Jolla, CA). Statistical analysis was performed using the permutation-based Significance Analysis of Microarrays (SAM) algorithm using a false discovery rate of <10%. We utilized Database for Annotation Visualization and Integrated Discovery (DAVID) gene functional classification and gene functional annotation tables to examine interrelated genes within the gene list obtained using SAM.

[0065] qRT-PCR Array: Reverse transcription of 1.0 µg of DNAsed, total RNA from 4 rats was primed with oligo(dT) and random hexamers. We utilized SuperScriptIII according to manufacturer's specifications (Invitrogen, Carlsbad, CA). A 1:10 dilution of cDNA was used as a template for quantitative real-time PCR, and the analysis was performed with Brilliant SYBR

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Green qRT-PCR Master Mix (Stratagene) on a Mx3000P Real-Time PCR System. ROX reference dye was included in all reactions. Experiments were performed in triplicate for each data point and transcript abundance was normalized to reference genes included in the rat Wnt PCR array (Qiagen, 330231).

5 Results:

[0066] As shown in FIG. 1, GLYX-13 and ketamine differentially affect gene expression patterns in the mPFC of the adult rat at 1 and 24 hrs post-injection (IV). In FIG. 1, the numbers represent the total number of genes that were shown to be significantly differentially expressed at 1 and 24 hrs following either GLYX-13 or ketamine injections (IV) as compared to vehicle control rats, using SAM analysis (FDR <10%). The sample size for each group in FIG. 1 was 5 adult male rats.

[0067] GLYX and ketamine showed a differential effect on the Wnt signaling pathway (Table 1 and FIG. 2). FIG. 2 shows Wnt signaling pathway gene expression in the mPFC following both GLYX-13 and ketamine injections at 1 hr (panel A) and 24 hrs (panel B). Using a commercially available rat Wnt qPCR array (Qiagen, 330231), a greater number of significant gene expression changes ($p < 0.05$) were observed at 1 and 24 hrs post GLYX-13 injection relative to vehicle control rats when compared to the Wnt specific gene expression changes observed following ketamine injections. Fold change values greater than 0.0 indicate an upregulation in gene expression relative to saline vehicle controls, whereas those values less than 0.0 are genes whose expression was downregulated relative to vehicle controls. The sample size for each group in FIG. 2 was 4 rats. The significant reduction in the expression of Wnt11 in the mPFC at 1 hour post GLYX-13 injections suggest that the GLYX-13 mediated effect may, at least in part, involve a reduction in non-canonical Wnt signaling.

[0068] Notably, as shown in FIG. 3, GLYX-13 produced a greater number of changes in the Wnt pathway specific gene expression 1 and 24 hours post injection relative to vehicle control rats. Significant gene expression changes observed post-GLYX-13 injection are noted in dark gray, genes present on the qPCR array that did not significantly change are noted in light gray, and genes not present on the array are noted in medium gray. Significant gene expression changes following ketamine injections were observed for Frizzled 2 (Fzd).

[0069] **Table 1. GLYX-13 and Ketamine differentially affect Wnt signaling pathway gene expression in the mPFC of the adult rat at 1 and 24 hours post injection (IV).**

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Table 1: Microarray Analysis of Wnt Signaling Pathway Gene Expression		GLYX-13 vs. Vehicle		Ketamine vs. Vehicle	
Gene Bank Accession No.	Gene Name	1 Hr	24 Hr	1 Hr	24 Hr
NM_024405	axin 1	G	-	-	-
M16112	calcium/calmodulin-dependent protein kinase II beta	-	V	-	-
NM_001042354	calcium/calmodulin-dependent protein kinase II beta	-	G	-	K
NM_012519	calcium/calmodulin-dependent protein kinase II delta	-	V	-	V
NM_053615	casein kinase 1, alpha 1	V	G	V	-
NM_053824	casein kinase 2, alpha 1 polypeptide	V	V	-	V
NM_031021	casein kinase 2, beta subunit	V	G	-	K
NM_053357	catenin, beta 1	V	G	V	V
NM_153474	frizzled homolog 3	-	-	-	V
X73653	glycogen synthase kinase 3 beta	-	V	-	-
X07286	PKC, alpha	-	V	V	-
K03486, X04139	PKC, beta	-	G	-	K
L14323, M20636	PLC, beta 1	G	G	K	K
D90035, NM_017041	Protein Phosphatase 2B	G	G	-	-
M31809	Protein Phosphatase 3	-	G	-	-
NM_001100922	protein kinase, cAMP-dependent, catalytic, alpha	-	G	-	-
XM_224987	secreted frizzled-related protein 1	V	-	-	-
AB017912	SMAD family member 2	G	V	-	V
XM_235639	Wnt 1	G	V	-	-

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Table 1: Microarray Analysis of Wnt Signaling Pathway Gene Expression		GLYX-13 vs. Vehicle		Ketamine vs. Vehicle	
Gene Bank Accession No.	Gene Name	1 Hr	24 Hr	1 Hr	24 Hr
XM_237296	Wnt 10A	-	-	V	K
NM_053402	Wnt 4	-	-	V	-
XM_226051	Wnt 8A	V	V	-	-
NM_053738	Wnt inhibitory factor 1	-	V	-	-

Significance Analysis of Microarray Data (FDR<10%). G: indicative of higher levels of gene expression in GLYX-13 treated rats; K: indicative of higher levels of gene expression in ketamine treated rats; V: indicative of a higher level of expression in saline vehicle treated rats (downregulated in GLYX-13 or ketamine treated rats as indicated). Genes present on the array that comprise the Wnt signaling pathway were defined using DAVID analysis. N = 5 rats per group.

- [0070] Data in Table 2 indicate the genes that were significantly differentially expressed in GLYX-13 and ketamine treated rats relative to either vehicle (GLYX-13 v Vehicle or Ketamine v Vehicle), or relative to each other (GLYX-13 v Ketamine) using significance analysis of microarrays (False Discovery Rate <10%). G: indicative of higher levels of gene expression in GLYX-13 treated rats; K: indicative of higher levels of gene expression in ketamine treated rats; V: indicative of a higher level of expression in saline vehicle treated rats (downregulated in GLYX-13 or ketamine treated rats as indicated). N = 5 rats per group.

- 10 [0071] Table 2. Significance Analysis of Microarray Data for GLYX-13, Ketamine, and vehicle control rats.

Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>A03913</u>	glia-derived neurite-promoting factor	V	-	-	V	-	-	-	-	-
<u>A17753</u>	D3 receptor	-	V	-	-	-	-	-	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
AB000280	solute carrier family 15, member 4	-	-	-	-	V	-	-	G	-
AB002801	cyclic nucleotide gated channel alpha 3	-	V	-	-	-	-	-	K	-
AB003478	beta 1,3- galactosyltransferase, polypeptide 4	V	-	V	V	-	V	-	-	-
AB003991 , AB003992	SNAP 25	G	G	-	-	-	K	G	G	K
AB006137	galactoside 2-alpha-L- fucosyltransferase	-	-	G	-	-	-	-	-	-
AB008682	FGF 17	-	-	-	-	V	-	-	-	-
AB010963 , AF020712	calcium-activated potassium channel beta subunit	-	G	-	-	K	-	-	-	-
AB011679	tubulin, beta 5	-	V	-	K	K	-	K	-	-
AB013130	myozenin 3; synaptopodin	-	V	-	-	V	-	K	G	-
AB015946	tubulin, gamma 1	-	V	-	V	-	V	-	-	-
AB016160	GABA B receptor 1	-	V	-	-	V	K	-	G	-
AB017656 , M16407 , M18088	mAChR 3	-	V	-	-	V	-	K	G	-
AB017912	SMAD family member 2	G	V	-	-	V	-	G	-	-
AB018049	ST3 beta-galactoside alpha- 2,3-sialyltransferase 5	-	G	-	-	K	K	-	-	-
AB018546	stress-associated endoplasmic reticulum protein 1	V	V	V	V	-	-	-	-	-
AB020978	growth arrest and DNA- damage-inducible, gamma	V	-	-	-	-	-	-	-	-
AF000423	synaptotagmin XI	-	V	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>AF000973</u>	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 1	-	V	V	-	-	V	-	-	-
<u>AF001423</u>	NMDAR 2A	V	V	-	-	V	-	K	-	-
<u>AF003598</u>	integrin, beta 7	V	-	-	-	-	-	K	-	-
<u>AF003825</u> , <u>U97143</u>	GDNF family receptor alpha 2	V	-	-	-	V	-	-	G	-
<u>AF005720</u>	chloride channel 2	-	V	-	-	V	-	-	-	-
<u>AF007583</u>	acetylcholinesterase	-	V	-	-	-	-	-	-	-
<u>AF007758</u> , <u>S73007</u>	synuclein, alpha	-	G	G	-	-	K	-	G	-
<u>AF012347</u>	SMAD family member 9	-	G	V	-	-	-	-	-	-
<u>AF013144</u>	dual specificity phosphatase 5	V	V	V	V	V	-	-	-	-
<u>AF015728</u>	cyclic nucleotide gated channel beta 1	-	-	-	-	-	-	K	-	-
<u>AF017637</u>	carboxypeptidase Z	V	-	-	-	-	-	K	-	-
<u>AF019973</u>	enolase 2, gamma, neuronal	-	G	G	-	K	-	-	-	-
<u>AF021935</u>	CDC42 binding protein kinase alpha	-	-	-	-	V	-	-	G	G
<u>AF022819</u>	potassium channel, subfamily K, member 1	-	-	-	-	V	-	-	G	-
<u>AF022935</u>	prolactin	-	V	-	-	-	-	-	-	-
<u>AF025670</u>	caspase 6	-	-	G	-	-	K	G	-	-
<u>AF025671</u> , <u>U77933</u>	caspase 2	-	G	-	-	K	-	-	G	-
<u>AF027984</u>	calcium channel, voltage-dependent, T type, alpha 1G	-	G	-	-	K	-	-	-	G

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	subunit									
AF028784	glial fibrillary acidic protein	-	-	-	-	-	-	G	-	-
AF030086	neurotransmitter-induced early gene 1 (ania-1)	-	V	G	-	V	K	-	K	-
AF030087	activity and neurotransmitter-induced early gene 2 (ania-2) mRNA	-	-	-	-	K	-	-	K	-
AF030088	homer homolog 1	-	-	-	K	-	-	-	-	-
AF030089	doublecortin-like kinase 1	-	-	-	-	K	-	-	-	-
AF030091	cyclin L1	V	V	-	-	-	-	-	K	-
AF030253	GABA vesicular transporter	-	-	G	-	-	K	-	-	-
AF030358	chemokine (C-X3-C motif) ligand 1	-	G	-	-	-	K	-	-	-
AF031384	potassium channel, subfamily K, member 3	-	-	G	-	-	-	-	-	G
AF031522	galanin receptor 3	-	-	G	-	-	-	-	-	-
AF032872	protein inhibitor of activated STAT, 3	-	-	-	-	V	-	-	G	-
AF035632	syntaxin 12	-	-	-	-	V	-	-	G	-
AF037067	TNF superfamily, member 4	-	V	-	-	V	-	-	-	-
AF037071, X59949	NOS 1, neuronal	G	-	-	K	V	K	G	G	K
AF038571	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	-	V	-	-	-	-	-	K	-
AF041244	hypocretin (orexin) receptor 1	-	-	-	-	K	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
AF041373	phosphatidylinositol binding clathrin assembly protein	-	-	-	-	-	-	K	-	-
AF042499	SMAD family member 7	-	-	-	-	V	-	-	-	-
AF042713	neurexophilin 3	G	-	-	-	V	-	-	-	-
AF042714	neurexophilin 4	V	-	-	-	-	-	-	-	-
AF044581	syntaxin 12	-	G	-	-	K	-	-	-	-
AF044910	survival motor neuron 1	-	V	-	-	V	-	-	-	-
AF048828	voltage-dependent anion channel 1	V	-	-	V	-	-	-	-	-
AF049239	sodium channel, voltage gated, type VIII, alpha subunit	-	V	G	-	V	-	-	-	G
AF049344	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5 (GalNAc-T5)	-	V	-	-	V	-	K	-	-
AF049882	Cd82 molecule	-	V	-	V	V	-	-	-	-
AF050659	activity and neurotransmitter-induced early gene 7 (ania-7) mRNA	-	V	-	-	-	-	K	-	-
AF050660	activity and neurotransmitter-induced early gene 8 (ania-8) mRNA	-	V	-	-	-	-	-	K	-
AF050661	activity and neurotransmitter-induced early gene 9 (ania-9) mRNA	-	-	-	-	V	-	-	-	-
AF050663	activity and neurotransmitter-induced early gene 11 (ania-11)	G	-	G	-	-	-	-	-	G
AF052596	SNAP 23	-	V	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
AF054586	ring finger protein 112	V	V	-	-	-	-	K	K	-
AF057308	hypoxia-inducible factor 1, alpha subunit	-	G	V	-	K	-	-	-	-
AF058795	GABA B receptor 2	-	G	-	-	K	K	-	-	K
AF060879	neurocan	-	V	-	-	-	-	-	-	-
AF064541, D45400, U27322	arginine vasopressin receptor 1B	-	V	-	-	V	-	-	-	-
AF076167	UDP-N-acetyl-alpha-D- galactosamine:polypeptide N- acetylgalactosaminyltransfer ase 7 (GalNAc-T7)	-	-	-	-	-	V	-	-	G
AF078779	sodium leak channel, non- selective	G	G	-	K	K	-	-	G	-
AF081366	potassium inwardly-rectifying channel, subfamily J, member 1	-	-	-	-	-	-	-	G	-
AF081557	glial cells missing homolog 1 (Drosophila)	-	-	G	-	-	-	-	-	G
AF087431	glucosidase 1	-	-	-	-	K	-	-	-	-
AF087453	potassium voltage-gated channel, subfamily Q, member 2	-	V	-	-	V	-	-	-	-
AF087454	potassium voltage-gated channel, subfamily Q, member 3	-	V	-	-	-	-	-	K	-
AF087839	ATP-binding cassette, sub- family C (CFTR/MRP), member 9	-	V	-	-	-	-	-	-	-
AF089730	potassium channel, subfamily T, member 1	-	G	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
AF090113	glutamate receptor interacting protein 2	-	-	-	-	V	-	-	-	-
AF096291	Bcl2-like 2; poly(A) binding protein, nuclear 1	-	-	-	-	-	-	G	-	-
AF159803	Non-specific lipid-transfer protein 6	-	-	-	-	K	-	-	-	-
AF191028	Xylem cysteine proteinase 2	-	-	-	-	V	-	-	G	-
AF205717	transmembrane 4 L six family member 4	-	V	-	-	-	-	-	-	-
AF247559	Triosephosphate isomerase, chloroplastic; Triosephosphate isomerase	V	V	-	-	-	-	-	-	-
AF264018	transferase	-	V	-	-	-	-	-	-	-
AF459021	tubulin, beta 3	G	G	G	V	K	K	G	-	-
AJ000515	cyclic nucleotide gated channel beta 1	G	V	-	-	-	-	G	-	-
AJ000556	Janus kinase 1	V	-	-	-	V	-	K	-	-
AJ001029	SRY (sex determining region Y)-box 10	V	-	-	V	-	-	-	-	-
AJ002942	retinoic acid receptor, beta	-	-	-	-	V	-	-	G	-
AJ003065	potassium inwardly-rectifying channel, subfamily J, member 14	-	-	-	-	V	-	-	-	-
AJ006519	amiloride-sensitive cation channel 2, neuronal	-	-	-	-	V	-	-	-	-
AJ006710	phosphoinositide-3-kinase, class 3	-	-	-	-	V	-	-	G	-
AJ006855	synaptojanin 1	G	-	G	-	-	K	-	-	-
AJ007627 , AJ007628	potassium voltage-gated channel, subfamily H (eag- related), member 4	-	V	V	-	-	-	-	K	K

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>AJ007632</u>	potassium voltage-gated channel, subfamily H (eag-related), member 8	-	V	-	-	V	-	-	-	-
<u>AJ012603</u>	ADAM metallopeptidase domain 17	-	-	V	-	-	V	-	-	-
<u>AJ222813</u> , <u>U77776</u>	interleukin 18	-	-	-	-	K	-	-	K	-
<u>AJ277828</u>	endothelial PAS domain protein 1	-	V	-	-	V	-	-	-	-
<u>AJ295749</u>	xylosyltransferase II	-	-	-	-	V	-	-	-	-
<u>D00634</u>	adrenergic, beta-1-, receptor	-	G	-	-	-	-	-	-	G
<u>D00698</u> , <u>M15480</u> , <u>M15481</u> , <u>X06107</u>	IGF 1	V	V	-	V	-	V	K	K	G
<u>D00833</u> , <u>NM_013133</u>	glycine receptor, alpha 1	-	V	-	-	V	-	-	-	-
<u>D10106</u>	platelet-derived growth factor alpha polypeptide	-	G	V	-	K	V	-	-	-
<u>D12519</u>	syntaxin 1A	-	G	-	-	-	K	-	G	K
<u>D12524</u>	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	-	V	-	-	V	-	-	-	-
<u>D12573</u>	hippocalcin	G	-	-	-	K	-	-	-	-
<u>D13212</u> , <u>NM_012575</u> , <u>U08259</u>	NMDAR 2C	-	G	-	-	K	K	G	-	-
<u>D13417</u>	hairy and enhancer of split 1	-	-	-	-	-	V	-	K	G
<u>D13418</u>	hairy and enhancer of split 3	G	-	-	-	-	-	G	-	-
<u>D13871</u>	solute carrier family 2 (facilitated glucose/fructose	-	V	-	-	V	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	transporter), member 5									
<u>D13985</u>	chloride channel, nucleotide-sensitive, 1A	V	-	-	V	-	-	-	-	-
<u>D14048</u>	heterogeneous nuclear ribonucleoprotein U	-	G	V	V	K	-	G	-	-
<u>D14480</u>	calpain 8	-	-	-	-	V	-	K	-	-
<u>D14869</u> , <u>D16443</u>	prostaglandin E receptor 3 (subtype EP3)	V	V	-	V	V	-	-	-	-
<u>D17469</u>	thyrotropin releasing hormone receptor	-	-	-	-	V	-	-	-	-
<u>D17521</u>	chloride channel 3	-	V	-	-	V	-	G	-	-
<u>D17764</u>	synuclein, beta	-	G	G	-	-	K	-	G	-
<u>D25224</u>	similar to 40S ribosomal protein SA	V	V	G	-	-	K	K	-	-
<u>D25233</u>	retinoblastoma 1	-	G	G	-	K	-	G	-	-
<u>D25290</u>	cadherin 6	V	V	-	-	V	-	-	-	-
<u>D26111</u>	chloride channel Kb	-	V	-	-	-	-	-	-	-
<u>D26154</u>	RB109	-	-	-	V	-	-	G	-	-
<u>D28560</u>	ectonucleotide pyrophosphatase/phosphodiesterase 2	-	-	G	-	-	K	-	-	-
<u>D28562</u>	solute carrier family 2 (facilitated glucose/fructose transporter), member 5	-	V	-	-	V	-	-	-	-
<u>D30666</u>	acyl-CoA synthetase long-chain family member 3	-	G	-	-	-	K	-	-	-
<u>D32045</u> , <u>M60655</u>	adrenergic, alpha-1B-, receptor	G	-	-	-	V	-	-	-	-
<u>D32249</u>	praja 2, RING-H2 motif containing	-	G	G	-	K	-	G	-	G

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>D38380</u>	signal recognition particle receptor, B subunit; transferrin	-	G	V	V	-	-	G	G	-
<u>D44495</u>	APEX nuclease (multifunctional DNA repair enzyme) 1	-	-	V	V	V	V	G	G	-
<u>D45187</u>	cathepsin E	-	V	-	-	-	-	-	-	-
<u>D50093</u>	prion protein	-	G	-	-	-	-	-	G	-
<u>D50497</u>	chloride channel 5	-	-	-	-	V	-	-	G	-
<u>D63772</u>	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	-	-	V	-	-	-	-	-	K
<u>D84450</u>	ATPase, Na ⁺ /K ⁺ transporting, beta 3 polypeptide	-	-	G	-	-	K	-	-	-
<u>D86039</u>	potassium inwardly rectifying channel, subfamily J, member 11	-	V	-	-	V	-	-	-	-
<u>D87839</u>	4-aminobutyrate aminotransferase	-	V	-	-	V	-	G	-	-
<u>D88672</u>	phospholipase D2	-	-	-	K	-	-	-	-	-
<u>D89655</u>	scavenger receptor class B, member 1	-	-	G	-	-	-	-	-	G
<u>D90035</u> , <u>NM_017041</u>	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform	G	G	G	-	-	K	G	G	G
<u>D90048</u> , <u>J04629</u>	ATPase, Na ⁺ /K ⁺ transporting, beta 2 polypeptide	V	V	G	-	V	-	K	G	-
<u>D90258</u>	proteasome subunit, alpha type 3	-	G	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>E00988</u>	IGF II	-	V	-	-	V	-	-	-	-
<u>E01789</u>	rat C-kinase type-II (beta-2)	-	G	-	-	-	-	-	G	-
<u>E01884</u>	rat IL-1-beta(interleukin-1 beta).	-	-	-	-	-	-	-	G	-
<u>E05646</u>	rat hippocampal cholinergic neurostimulating	V	-	-	V	V	-	-	-	-
<u>E12625</u>	novel protein which is expressed with nerve injury	-	-	-	V	-	V	-	-	-
<u>E12746</u>	Rat cholecystokinin-A receptor gene	-	V	-	-	-	-	-	-	-
<u>E13644</u>	Neurodap-1	G	G	-	-	-	-	G	-	G
<u>E13732</u>	CC chemokine receptor protein	-	V	-	-	V	-	-	-	-
<u>J02722</u>	heme oxygenase (decycling) 1	V	-	-	-	-	-	-	-	-
<u>J03624</u>	galanin prepropeptide	-	G	-	K	-	-	K	-	-
<u>J03754</u>	ATPase, Ca++ transporting, plasma membrane 2	V	-	-	V	-	-	-	-	K
<u>J03933</u>	thyroid hormone receptor beta	-	-	-	V	-	-	-	-	-
<u>J04024</u>	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	-	G	-	-	K	-	-	-	-
<u>J04532</u>	PKC, zeta	-	G	-	-	-	-	-	G	-
<u>J04563, M25350</u>	phosphodiesterase 4B, cAMP specific	-	V	-	-	V	-	-	-	-
<u>J04625</u>	carboxypeptidase E	-	G	-	-	K	-	G	-	-
<u>J04636</u>	nACh receptor, beta 3	-	G	-	-	-	-	-	G	-
<u>J04731</u>	potassium voltage-gated channel, shaker-related	G	-	G	-	-	-	G	-	G

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	subfamily, member 2									
<u>J04811</u>	growth hormone receptor	-	V	-	-	V	-	-	-	-
<u>J04963</u>	carcinoembryonic antigen- related cell adhesion molecule 1 (biliary glycoprotein)	V	V	-	-	-	-	-	-	-
<u>J05107</u>	hydroxysteroid 11-beta dehydrogenase 1	V	-	V	-	-	-	-	-	K
<u>J05122</u>	translocator protein	V	V	-	-	-	-	K	K	-
<u>J05510</u>	IP3 receptor, type 1	-	G	G	-	-	-	-	-	G
<u>K00512</u>	myelin basic protein	G	-	-	-	-	-	G	-	-
<u>K01701</u>	oxytocin, prepropeptide	G	-	-	K	-	-	-	-	-
<u>K03486</u> , <u>X04139</u>	PKC, beta	-	G	G	-	K	K	-	-	-
<u>L02926</u>	interleukin 10	-	V	-	-	V	-	-	-	-
<u>L04535</u>	somatostatin receptor 5	-	-	-	V	-	V	-	-	-
<u>L04684</u>	calcium channel, voltage- dependent, L type, alpha 1S subunit	-	-	-	-	V	-	-	-	-
<u>L04739</u>	ATPase, Ca++ transporting, plasma membrane 1	G	G	-	-	-	K	G	-	K
<u>L04796</u>	glucagon receptor	-	V	-	-	V	-	G	K	-
<u>L05435</u>	synaptic vesicle glycoprotein 2a	-	G	-	-	-	K	-	G	-
<u>L05596</u>	serotonin receptor 1F	G	-	-	-	-	-	G	-	-
<u>L06894</u>	platelet-derived growth factor alpha polypeptide	-	V	-	-	V	-	-	-	-
<u>L08228</u> , <u>X63255</u>	NMDAR 1	G	G	V	K	-	-	G	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
L08492	GABA A receptor, alpha 3	V	-	-	V	-	-	-	G	-
L08493	GABA A receptor, alpha 4	-	-	-	-	-	-	-	G	-
L08497	GABA A receptor, gamma 2	-	G	-	-	-	K	-	G	-
L09119	neurogranin	-	G	-	-	-	K	-	-	K
L09120	calpain 2	-	V	-	-	-	-	-	-	-
L10072	serotonin receptor 5A	-	V	-	-	V	K	-	-	-
L10326	GNAS complex locus	-	-	-	-	-	K	G	-	-
L14323, M20636	PLC, beta 1	G	G	G	K	K	-	-	-	-
L14851	neurexin 3	V	-	-	V	V	-	-	G	-
L16532	2',3'-cyclic nucleotide 3' phosphodiesterase	-	-	-	V	-	-	G	-	-
L16764	heat shock 70kD protein 1B	-	-	-	K	-	-	K	-	-
L18889	calnexin	-	-	-	-	V	-	-	-	-
L20821	syntaxin 4	-	V	-	-	-	-	-	-	-
L20822	syntaxin 5	V	V	-	V	V	-	-	G	-
L21192, NM_017195	GAP 43	G	G	G	-	K	K	G	G	K
L22339	sulfotransferase family, cytosolic, 1C, member 3	-	-	-	-	-	V	-	-	-
L23088	selectin, platelet	-	-	-	V	-	-	-	-	-
L24907, NM_134468	calcium/calmodulin- dependent protein kinase I	-	G	-	-	V	K	-	G	K
L25925	prostaglandin-endoperoxide synthase 2	-	V	-	-	-	-	-	K	-
L27059	phosphodiesterase 4D, cAMP-specific	-	-	-	-	V	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>L27487</u>	calcitonin receptor-like	V	V	-	-	V	-	K	-	-
<u>L31546</u>	serotonin receptor 2A	G	G	-	-	K	K	-	-	-
<u>L31620</u>	nAChR, alpha 4	-	-	-	K	-	-	-	-	-
<u>L31622</u>	nAChR, beta 2	-	-	-	-	K	-	-	-	-
<u>L31771</u> , <u>M60654</u>	adrenergic, alpha-1D-, receptor	-	V	-	-	-	-	-	G	-
<u>L36884</u>	protein tyrosine phosphatase, receptor type, V	-	V	-	-	V	-	-	-	-
<u>L41623</u>	platelet-derived growth factor beta polypeptide	-	V	-	-	-	-	-	-	-
<u>M10088</u>	prodynorphin	-	-	-	K	V	-	K	-	-
<u>M10244</u>	tyrosine hydroxylase	-	-	-	-	K	-	-	-	-
<u>M11794</u>	metallothionein 2A	V	V	-	V	-	-	-	-	-
<u>M12492</u>	protein kinase, cAMP dependent regulatory, type II beta	-	G	-	-	K	-	-	-	K
<u>M15191</u>	tachykinin 1	-	G	-	-	-	-	-	-	-
<u>M15880</u>	neuropeptide Y	-	G	-	-	K	-	-	-	-
<u>M16112</u>	calcium/calmodulin- dependent protein kinase II beta	-	V	-	-	-	-	-	-	-
<u>M16406</u>	mAChR 1	-	G	-	-	-	-	G	-	-
<u>M16409</u>	mAChR 4	-	-	-	-	-	K	-	-	-
<u>M17069</u> , <u>X13817</u> , <u>X14265</u>	calmodulin	-	G	-	-	K	V	-	G	-
<u>M17526</u>	G protein alpha activating activity polypeptide O	G	-	-	-	-	K	G	-	K

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>M18416</u>	early growth response 1	G	-	-	K	K	-	-	K	-
<u>M19257</u>	retinol binding protein 1, cellular	G	-	-	-	-	-	-	-	-
<u>M19533</u>	peptidylprolyl isomerase A	V	G	-	-	K	-	-	-	-
<u>M20133</u>	androgen receptor	G	-	G	-	-	-	K	-	G
<u>M20636</u>	PLC, beta 1	V	-	-	-	-	-	-	-	-
<u>M21060</u> , <u>M25157</u> , <u>Y00404</u>	SOD 1	V	G	V	-	K	V	-	-	-
<u>M22253</u>	sodium channel, voltage- gated, type I, alpha	-	G	-	K	-	-	-	G	-
<u>M22254</u>	sodium channel, voltage- gated, type II, alpha 1	-	G	G	-	-	K	-	G	-
<u>M22357</u>	myelin-associated glycoprotein	G	G	-	-	-	K	-	-	-
<u>M22412</u>	potassium voltage-gated channel, Isk-related subfamily, member 1	-	V	-	-	-	-	-	K	-
<u>M23601</u>	monoamine oxidase B	-	-	V	-	-	-	-	-	K
<u>M23697</u>	plasminogen activator, tissue	-	-	-	-	-	-	-	-	K
<u>M24104</u>	vesicle-associated membrane protein 1	-	V	-	-	-	K	-	-	-
<u>M24852</u>	Purkinje cell protein 4	-	G	-	-	K	-	G	-	-
<u>M25638</u>	neurofilament, light polypeptide	-	G	-	-	-	-	-	G	-
<u>M25646</u>	arginine vasopressin	-	-	-	-	V	V	-	-	-
<u>M25888</u>	proteolipid protein 1	G	G	-	-	-	K	G	G	K
<u>M25890</u>	somatostatin	-	G	-	-	K	-	-	-	K

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>M26161</u>	potassium voltage-gated channel, shaker-related subfamily, member 1	G	G	-	-	-	-	G	-	-
<u>M26715</u>	phosphodiesterase 4A, cAMP-specific	-	G	-	-	-	-	-	G	-
<u>M26744</u> , <u>M26745</u>	interleukin 6	-	V	-	V	V	-	-	-	-
<u>M27293</u>	IGF 1 receptor	-	-	-	-	V	-	-	-	-
<u>M27925</u>	synapsin II	-	G	-	-	-	-	-	G	-
<u>M30312</u>	potassium voltage-gated channel, shaker-related subfamily, member 3	-	V	G	-	V	K	-	-	-
<u>M31174</u> , <u>X12744</u>	thyroid hormone receptor alpha	-	G	-	-	K	-	G	-	-
<u>M31176</u>	gastrin releasing peptide	-	-	-	-	V	-	-	G	-
<u>M31178</u>	calbindin 1	-	G	-	K	K	-	-	-	-
<u>M31433</u>		-	-	-	-	V	-	-	G	-
<u>M31809</u>	protein phosphatase 3, catalytic subunit, beta isoform	-	G	G	-	-	K	-	G	-
<u>M32061</u>	adrenergic, alpha-2B-, receptor	-	V	-	-	V	-	-	-	-
<u>M34253</u>	interferon regulatory factor 1	-	V	-	-	V	-	-	-	-
<u>M34445</u>	glutamate decarboxylase 1	-	G	-	K	K	K	-	-	-
<u>M35162</u> , <u>NM_017289</u>	GABA A receptor, delta	-	-	G	-	-	-	K	-	G
<u>M36074</u>	nuclear receptor subfamily 3, group C, member 2	-	-	-	-	V	-	-	G	-
<u>M36831</u> , <u>X56065</u> , <u>NM_012547</u>	D2 receptor	V	-	V	V	-	-	G	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>M37394</u>	epidermal growth factor receptor	-	V	-	-	V	-	-	-	-
<u>M38061</u>	AMPA 2 receptor	G	G	G	-	K	K	G	G	-
<u>M55291</u>	neurotrophic tyrosine kinase, receptor, type 2	G	-	G	-	V	K	G	G	-
<u>M57664</u>	creatine kinase, brain	-	G	-	-	K	-	-	-	-
<u>M58040</u>	transferrin receptor	-	G	-	-	K	-	G	-	-
<u>M58316</u> , <u>X57659</u>	adrenergic, alpha-2C-, receptor	G	-	-	-	-	-	-	G	-
<u>M58634</u>	IGF binding protein 1	-	V	-	-	-	-	K	-	-
<u>M59313</u> , <u>X62839</u>	potassium voltage gated channel, Shaw-related subfamily, member 2	-	G	V	-	V	K	-	-	K
<u>M59980</u>	potassium voltage gated channel, Shal-related family, member 2	-	V	-	-	-	-	-	-	-
<u>M60525</u> , <u>M74223</u>	VGF nerve growth factor inducible	G	G	-	K	K	-	-	-	K
<u>M60753</u>	catechol-O- methyltransferase	V	-	-	V	-	-	-	-	-
<u>M61099</u>	mGluR 1	-	G	-	-	-	-	-	-	-
<u>M61177</u> , <u>NM_017347</u>	MAPK 3	V	-	-	-	K	-	-	-	-
<u>M62781</u>	IGF binding protein 5	-	V	-	-	V	-	-	-	-
<u>M63101</u>	IL 1 receptor antagonist	-	-	-	-	V	-	-	G	-
<u>M63122</u>	tumor necrosis factor receptor superfamily, member 1a	-	V	-	-	-	-	-	K	-
<u>M64300</u>	MAPK 1	-	-	-	-	V	-	-	G	-
<u>M68971</u>	hexokinase 2	-	-	-	-	-	K	-	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>M69055</u>	IGF binding protein 6	V	V	-	-	-	-	-	K	-
<u>M77482</u>	potassium voltage gated channel, Shab-related subfamily, member 2	-	V	-	-	-	-	-	-	-
<u>M77809</u>	transforming growth factor, beta receptor III	-	-	-	-	V	-	G	G	-
<u>M80545</u>	calcium channel, voltage- dependent, beta 2 subunit	V	-	-	-	-	-	-	-	-
<u>M81783</u>	potassium voltage-gated channel, subfamily F, member 1	-	-	-	-	V	-	-	G	-
<u>M82824</u>	NF1	-	-	-	K	V	-	-	-	-
<u>M84009</u> , <u>NM_012944</u>	D4 receptor	G	V	G	K	V	-	-	-	G
<u>M84725</u>	transgelin 3	-	G	-	-	-	K	-	G	-
<u>M85035</u>	AMPA 2	V	V	-	V	V	-	-	-	-
<u>M85183</u>	NLR family, pyrin domain containing 6	-	-	-	-	-	-	G	-	-
<u>M86389</u> , <u>S45392</u> , <u>X54793</u>	heat shock protein 1	V	V	V	-	K	-	K	K	-
<u>M86621</u>	calcium channel, voltage- dependent, alpha2/delta subunit 1	G	G	-	-	-	K	G	G	K
<u>M86742</u>	neurotrophin 4	-	V	-	-	-	-	-	-	-
M86835	vasoactive intestinal peptide receptor 1	-	-	-	V	-	K	G	-	K
M88096	cholecystokinin A receptor	-	V	-	-	-	-	-	-	-
<u>M88751</u>	calcium channel, voltage- dependent, beta 3 subunit	-	-	-	-	V	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
M89924	calcium channel, voltage-dependent, L type, alpha 1C subunit	-	-	G	-	-	K	-	-	-
<u>M89953</u>	serotonin receptor 1D	-	V	-	-	V	-	-	-	-
<u>M89954</u> , <u>X62944</u>	serotonin receptor 1B	-	-	-	-	K	K	-	-	-
<u>M91466</u>	adenosine A2B receptor	-	V	-	-	-	-	-	-	-
<u>M91599</u>	FGFR 4	-	V	-	-	-	-	-	-	-
<u>M91652</u>	glutamate-ammonia ligase (glutamine synthetase)	-	G	-	-	K	K	-	-	K
<u>M91808</u>	sodium channel, voltage-gated, type I, beta	-	-	-	-	V	-	-	G	-
<u>M92075</u>	mGluR 2	G	G	-	-	K	-	-	-	-
<u>M92076</u>	mGluR 3	-	-	-	-	V	-	-	-	-
<u>M92905</u>	calcium channel, voltage-dependent, N type, alpha 1B subunit	G	G	-	-	-	-	G	-	-
<u>M95735</u>	syntaxin 1B	-	-	-	K	-	-	K	-	-
<u>M95762</u>	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	-	-	-	K	-	-	-	-	-
<u>M96375</u>	neurexin 1	-	-	-	K	K	K	-	-	-
<u>M96376</u>	neurexin 2	-	G	-	-	K	K	-	-	-
<u>M96853</u>	discs, large homolog 4 (Drosophila)	-	-	-	-	-	K	-	-	-
<u>M98820</u>	interleukin 1 beta	-	V	-	-	V	-	-	-	-
<u>NM_001003</u> <u>964</u>	DNA (cytosine-5-)-methyltransferase 3-like	V	-	V	V	-	V	-	-	-
<u>NM_001007</u>	catenin (cadherin associated	V	-	V	-	K	-	K	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>145</u>	protein), alpha 1									
<u>NM_001008335</u>	eukaryotic translation initiation factor 4A2	-	G	G	-	K	K	-	-	-
<u>NM_001017374</u>	eukaryotic translation initiation factor 4, gamma 2, pseudogene 1	-	G	-	-	K	-	-	G	-
<u>NM_001024870</u>	catenin, beta like 1	-	-	-	-	K	-	-	K	-
<u>NM_001024964</u>	exocyst complex component 3	-	G	V	-	-	-	-	-	K
<u>NM_001033069</u>	eukaryotic translation initiation factor 4E binding protein 2	-	V	-	-	V	V	-	-	-
<u>NM_001033870</u>	casein kinase 1, gamma 2	-	-	V	-	-	-	-	-	-
<u>NM_001042354</u>	calcium/calmodulin-dependent protein kinase II beta	-	G	-	-	K	-	-	-	-
<u>NM_001100158</u>	eukaryotic translation initiation factor 4A, isoform 3	-	-	-	-	-	V	-	G	G
<u>NM_001100922</u>	protein kinase, cAMP-dependent, catalytic, alpha	-	G	-	-	-	-	-	-	-
<u>NM_001104528</u>	Eph receptor B1	G	G	-	-	-	K	-	G	K
<u>NM_001106259</u>	AKT1 substrate 1 (proline-rich)	G	-	-	-	-	-	G	-	-
<u>NM_001106598</u>	catenin (cadherin associated protein), alpha 2	-	G	-	-	K	K	-	-	-
<u>NM_001106693</u>	eukaryotic translation initiation factor 4 gamma, 3	-	G	-	-	K	K	-	-	-
<u>NM_001106797</u>	chromobox homolog 5 (HP1 alpha homolog, Drosophila)	G	-	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>NM_001107511</u>	adaptor-related protein complex 2, alpha 1 subunit	-	G	-	-	K	K	-	-	-
<u>NM_001108065</u>	SHC (Src homology 2 domain containing) transforming protein 2	-	G	-	-	K	-	-	-	-
<u>NM_001108577</u>	protein phosphatase 2A activator, regulatory subunit 4	-	G	V	-	K	-	-	-	K
<u>NM_001108808</u>	eukaryotic translation initiation factor 4E member 2	-	-	-	-	-	-	-	K	-
<u>NM_001109302</u>	c-fos serum response element-binding transcription factor	-	G	-	-	-	-	-	-	-
<u>NM_001130441</u>	Transforming protein p21	-	G	-	-	K	-	-	G	-
<u>NM_001134499</u>	regulatory associated protein of MTOR, complex 1	-	-	-	-	K	K	-	-	K
<u>NM_001135561</u>	son of sevenless homolog 2	-	G	V	-	K	V	-	-	-
<u>NM_012512</u>	beta-2 microglobulin	V	-	V	-	K	V	K	-	-
<u>NM_012519</u>	calcium/calmodulin-dependent protein kinase II delta	-	V	-	-	V	-	-	-	-
<u>NM_012528</u>	nAChR, beta 1 (muscle)	-	V	-	-	-	-	-	-	-
<u>NM_012734</u>	hexokinase 1	-	G	-	-	K	K	G	-	-
<u>NM_012752</u>	CD24 molecule	-	-	-	-	K	-	-	-	K
<u>NM_012813</u>	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	-	-	-	-	V	-	-	G	-
<u>NM_012821</u>	adenylate cyclase 6	-	-	-	-	-	K	-	-	-
<u>NM_012957</u>	GABA A receptor, beta 2	G	G	-	-	-	-	G	-	-

[illegible]

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	(Drosophila)									
<u>NM_022855</u>	casein kinase 1, gamma 3	-	G	-	-	K	K	-	-	-
<u>NM_022952</u>	adaptor-related protein complex 2, sigma 1 subunit	-	-	V	-	-	-	-	-	-
<u>NM_024405</u>	axin 1	G	-	V	-	-	-	-	-	-
<u>NM_031007</u>	adenylate cyclase 2	G	-	-	K	-	-	-	G	-
<u>NM_031008</u>	adaptor-related protein complex 2, alpha 2 subunit	G	-	V	-	-	-	G	-	-
<u>NM_031021</u>	casein kinase 2, beta subunit	V	G	-	-	K	-	-	-	-
<u>NM_031338</u>	calcium/calmodulin-dependent protein kinase kinase 2, beta	-	-	-	-	-	K	-	-	-
<u>NM_031515</u> <u>, U09793</u>	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	V	V	-	-	V	-	-	-	-
<u>NM_031527</u>	protein phosphatase 1, catalytic subunit, alpha isoform	-	G	V	-	K	-	-	-	-
<u>NM_031575</u>	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	V	V	-	-	V	-	K	-	-
<u>NM_031590</u>	WNT1 inducible signaling pathway protein 2	-	V	-	-	-	-	-	K	-
<u>NM_031622</u>	MAPK 6	V	G	-	-	-	-	-	-	-
<u>NM_031639</u>	discs, large homolog 3 (Drosophila)	-	-	V	-	K	-	-	K	K
<u>NM_031662</u>	calcium/calmodulin-dependent protein kinase kinase 1, alpha	-	G	-	-	-	K	-	-	-
<u>NM_031985</u>	ribosomal protein S6 kinase,	-	-	-	-	K	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	70kDa, polypeptide 1									
<u>NM_032058</u>	eukaryotic translation initiation factor 2B, subunit 2 beta	V	G	V	-	-	V	-	G	-
<u>NM_033230</u>	v-akt murine thymoma viral oncogene homolog 1	-	-	V	-	K	-	-	K	-
<u>NM_053346</u> <u>, U88958</u>	neuritin 1	G	G	-	-	K	K	G	-	K
<u>NM_053351</u>	calcium channel, voltage-dependent, gamma subunit 2	G	G	-	K	-	K	-	G	K
<u>NM_053354</u>	DNA (cytosine-5-)-methyltransferase 1	G	-	-	-	K	K	-	K	-
<u>NM_053357</u>	catenin (cadherin associated protein), beta 1	V	G	V	V	V	-	K	G	-
<u>NM_053400</u>	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	V	-	-	-	-	-	K	-	-
<u>NM_053402</u>	wingless-type MMTV integration site family, member 4	-	-	-	V	-	-	G	-	-
<u>NM_053615</u>	casein kinase 1, alpha 1	V	G	-	V	-	-	-	G	-
<u>NM_053649</u>	kringle containing transmembrane protein 1	-	-	V	-	V	V	-	G	-
<u>NM_053698</u>	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	-	-	-	K	K	V	K	K	-
<u>NM_053738</u>	Wnt inhibitory factor 1	-	V	-	-	-	-	-	-	-
<u>NM_053824</u>	casein kinase 2, alpha 1 polypeptide	V	V	-	-	V	-	-	-	-
<u>NM_053837</u>	adaptor-related protein	-	G	V	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	complex 2, mu 1 subunit									
<u>NM_053857</u>	eukaryotic translation initiation factor 4E binding protein 1	V	-	V	-	-	V	-	K	G
<u>NM_053861</u>	tenascin C	-	-	-	-	-	V	-	-	-
<u>NM_080394</u>	reelin	-	G	-	-	K	-	-	-	-
<u>NM_080583</u>	adaptor-related protein complex 2, beta 1 subunit	-	G	-	-	K	-	-	-	-
<u>NM_130779</u>	adenylate cyclase 3	G	-	-	-	K	-	-	-	-
<u>NM_133605</u>	calcium/calmodulin- dependent protein kinase II gamma	-	-	-	-	-	K	-	-	K
<u>NM_133609</u>	eukaryotic translation initiation factor 2B, subunit 3 gamma	G	-	-	-	-	-	-	-	-
<u>NM_138905</u>	phosphatidic acid phosphatase type 2B	G	G	-	-	K	-	G	-	-
<u>NM_139060</u>	casein kinase 1, delta	-	G	G	-	K	K	-	-	-
<u>NM_153474</u>	frizzled homolog 3	-	-	V	-	V	-	-	-	K
<u>NM_172029</u>	eukaryotic translation initiation factor 2B, subunit 1 alpha	-	V	-	-	V	-	G	G	-
<u>NM_173331</u>	MAPK 15	-	V	-	-	-	-	-	-	-
<u>NM_173337</u>	calcium/calmodulin- dependent protein kinase II inhibitor 1	-	G	G	-	-	K	G	G	-
<u>NM_182842</u>	calcium/calmodulin- dependent protein kinase IG	-	G	-	-	-	-	-	G	-
<u>NM_199372</u>	eukaryotic translation initiation factor 4A1	-	-	-	-	K	-	-	-	-
<u>S42358</u>	neurotransmitter transporter,	-	G	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	GABA, member 11									
<u>S44606</u>	beta-integrin	V	-	V	-	-	-	K	-	-
<u>S48813</u>	adrenergic, beta, receptor kinase 1	-	G	-	-	K	-	-	-	-
<u>S49491</u>	proenkephalin 1	G	G	-	-	-	V	-	-	-
<u>S53527</u>	S100 calcium binding protein B	G	G	-	-	K	-	G	G	-
<u>S54008</u>	FGFR 1	-	-	-	-	-	K	-	-	-
<u>S59158</u> , <u>X63744</u>	glial high affinity glutamate transporter, member 3	G	G	-	-	K	K	G	-	-
<u>S62043</u>	serotonin receptor 6	-	V	-	-	-	-	-	-	-
<u>S67770</u>	transforming growth factor, beta receptor II	-	V	-	-	V	-	-	-	-
<u>S68944</u>	solute carrier family 6 (neurotransmitter transporter), member 17	-	-	-	-	-	-	-	-	G
<u>S70690</u> , <u>X01032</u>	cholecystokinin	-	G	-	-	K	K	-	-	K
<u>S71570</u>	calcium/calmodulin- dependent protein kinase II gamma	-	-	-	-	-	K	-	-	K
<u>S72505</u> , <u>X78848</u>	glutathione S-transferase A3	-	-	-	-	V	-	G	-	-
<u>S75952</u>	glucagon-like peptide 1 receptor	-	-	-	-	V	-	-	-	-
<u>S76145</u>	neurotransmitter transporter, dopamine, member 3	-	-	G	-	-	K	-	-	-
<u>S76779</u>	apolipoprotein E	G	G	-	-	-	K	G	-	-
<u>S77528</u> , <u>X60769</u>	CCAAT/enhancer binding protein (C/EBP), beta	V	V	V	K	-	-	K	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>S79263</u>	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	-	-	-	-	V	-	K	G	-
<u>S82649</u> , <u>XM 221901</u>	neuronal pentraxin 2	-	V	-	-	V	K	-	-	-
<u>S83194</u>	calcium/calmodulin- dependent protein kinase kinase 1, alpha	-	G	-	-	-	K	-	G	K
<u>U03390</u>	G protein, beta polypeptide 2 like 1	V	V	V	-	-	V	K	-	-
<u>U04738</u>	somatostatin receptor 4	-	-	-	-	V	-	-	G	-
<u>U05784</u>	microtubule-associated protein 1 light chain 3 beta	-	G	-	-	K	-	-	-	-
<u>U08141</u>	Ferritin light chain 2	-	-	-	-	V	-	-	G	-
<u>U08255</u>	glutamate receptor, ionotropic, delta 1	V	G	V	-	-	-	-	-	-
<u>U08256</u>	glutamate receptor, ionotropic, delta 2	V	V	-	-	-	-	-	K	-
<u>U08260</u>	NMDAR 2D	-	G	-	-	-	-	-	-	-
<u>U08290</u>	neuronatin	-	G	-	-	K	K	-	-	-
<u>U10071</u>	CART prepropeptide	-	-	-	V	-	-	-	-	-
<u>U11031</u>	contactin 3	-	V	-	-	V	-	-	K	-
<u>U12336</u>	nAChR, alpha 9	-	-	-	-	-	K	-	-	K
<u>U13368</u>	adrenergic, alpha-1A-, receptor	-	V	-	-	-	K	-	-	-
<u>U14533</u>	nuclear receptor subfamily 1, group H, member 2	V	-	-	-	K	-	-	-	-
<u>U15211</u>	retinoic acid receptor, alpha	-	V	-	-	-	-	-	-	-
<u>U17254</u>	nuclear receptor subfamily 4, group A, member 1	-	-	-	K	K	-	K	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>U17607</u>	nuclear transcription factor-Y gamma	-	-	-	-	-	V	-	-	-
<u>U18650</u>	huntingtin	-	V	-	-	V	-	-	-	G
<u>U18982</u>	FBJ osteosarcoma oncogene B	-	-	-	-	-	K	-	-	-
<u>U20105</u>	synaptotagmin VI	-	V	-	-	-	-	-	K	-
<u>U20283</u>	syntaxin binding protein 2	V	-	-	-	-	-	-	-	-
<u>U21662</u>	mannosyl (alpha-1,6-)- glycoprotein beta-1,2-N- acetylglucosaminyltransferas e	V	G	-	-	K	-	-	-	-
<u>U22414</u>	chemokine (C-C motif) ligand 3	V	-	-	-	-	-	-	-	-
<u>U26402</u>	synaptotagmin V	-	V	-	-	V	-	-	-	-
<u>U26541</u>	PDGFA associated protein 1	-	G	-	V	-	-	-	-	-
<u>U29701</u>	4-aminobutyrate aminotransferase	-	G	-	-	-	K	-	G	-
<u>U30290</u>	galanin receptor 1	V	V	-	-	V	-	K	-	-
<u>U30938</u> , <u>X53455</u>	microtubule-associated protein 2	-	G	-	-	K	K	-	-	K
<u>U31203</u>	noggin	-	-	-	-	-	-	-	G	-
<u>U31554</u>	limbic system-associated membrane protein	-	G	-	-	V	-	-	G	-
<u>U33472</u>	serine/threonine kinase 10	-	V	-	V	-	-	-	K	-
<u>U35365</u>	FYN oncogene related to SRC, FGR, YES	-	-	-	-	V	-	-	-	-
<u>U37058</u>	neuromedin B receptor	-	-	-	-	V	-	-	-	-
<u>U37142</u>	brevican	V	V	-	-	-	-	K	-	-
<u>U38306</u>	arylalkylamine N-	-	V	-	-	-	-	-	K	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	acetyltransferase									
<u>U38653</u>	IP3 receptor, type 1	-	V	-	-	V	-	-	-	-
<u>U48829</u> , <u>S71597</u> , <u>U03699</u> , <u>U16359</u>	NOS 2	-	V	V	-	V	-	K	-	-
<u>U49729</u>	Bcl2-associated X protein	V	-	-	-	-	-	-	-	-
<u>U49953</u>	p21 protein (Cdc42/Rac)- activated kinase 1	-	-	G	-	-	K	-	-	-
<u>U50147</u>	discs, large homolog 3 (Drosophila)	-	-	-	-	V	-	-	G	-
<u>U50194</u>	tripeptidyl peptidase II	-	G	-	-	K	-	-	-	-
<u>U52948</u>	complement component 9	-	-	-	V	-	-	-	-	-
<u>U53927</u>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	V	-	-	-	-	K	K	-	-
<u>U57715</u>	FGF receptor activating protein 1	V	V	-	V	V	-	-	-	-
<u>U59809</u>	IGF 2 receptor	G	-	V	-	-	V	G	-	-
<u>U61696</u>	arginyl aminopeptidase (aminopeptidase B)	-	-	-	V	V	-	G	G	-
<u>U63740</u>	fasciculation and elongation protein zeta 1 (zygin I)	V	G	-	-	-	K	-	G	-
<u>U67140</u>	discs, large homolog- associated protein 4	-	G	V	-	-	-	-	G	-
<u>U72350</u>	similar to Bcl2-like 1 isoform 3; Bcl2-like 1	-	-	-	V	-	-	-	-	-
<u>U72353</u>	lamin B1	-	V	-	-	-	-	-	-	-
<u>U73142</u>	MAPK 14	G	V	-	-	-	-	G	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>U73859</u>	hexokinase 3	-	V	-	-	-	-	G	-	G
<u>U75899</u>	heat shock protein 2	-	V	-	-	V	-	-	-	-
<u>U81492</u>	interleukin 3	V	V	-	V	V	-	-	-	-
<u>U83112</u>	forkhead box M1	V	G	V	-	-	-	-	G	-
<u>U88036</u>	solute carrier organic anion transporter family, member 1a4	-	G	-	-	-	-	-	G	-
<u>U88324</u> , <u>AF022083</u>	G protein, beta polypeptide 1	V	G	V	V	K	K	-	-	K
<u>U92469</u>	gonadotropin releasing hormone receptor	V	-	-	-	-	-	K	-	-
<u>U92655</u>	potassium voltage-gated channel, subfamily Q, member 1	G	G	-	K	K	-	-	-	-
<u>V01217</u>	actin, beta	V	G	-	-	K	-	K	-	-
<u>V01227</u>	tubulin, alpha	V	G	V	V	K	-	G	G	-
<u>X00336</u>	interferon alpha family	-	G	-	-	-	K	-	-	K
<u>X00911</u> , <u>X16703</u> , <u>X17012</u>	IGF 2	G	G	-	-	K	K	G	-	K
<u>X01454</u>	thyroid stimulating hormone, beta	-	-	-	-	V	K	-	-	-
<u>X02231</u>	GAPDH	V	V	G	V	V	-	K	G	G
<u>X03347</u>	FBR-murine osteosarcoma provirus genome	V	-	-	-	-	-	-	-	-
<u>X03475</u>	ribosomal protein L35a	V	-	-	-	-	-	K	-	-
<u>X04979</u>	apolipoprotein E	-	-	V	K	-	-	K	-	-
<u>X06554</u>	myelin-associated glycoprotein	-	V	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>X06769</u>	FBJ osteosarcoma oncogene	-	-	V	-	K	V	K	K	-
<u>X06827</u>	hydroxymethylbilane synthase	V	V	V	-	-	-	-	K	-
<u>X06942</u>	v-raf murine sarcoma 3611 viral oncogene homolog	-	-	V	-	-	-	-	-	-
<u>X07286</u>	PKC, alpha	-	V	-	V	-	-	-	-	-
<u>X07467</u>	glucose-6-phosphate dehydrogenase	-	-	-	-	K	-	-	-	-
<u>X07729</u>	enolase 2, gamma, neuronal	-	V	-	-	-	-	-	-	-
<u>X13016</u>	Cd48 molecule	V	G	V	-	K	V	K	K	-
<u>X13804</u>	neurofilament, heavy polypeptide	-	G	-	-	-	-	-	-	-
<u>X15013</u>	ribosomal protein L7a	V	-	-	-	-	V	-	-	G
<u>X17184</u>	AMPA 1	-	V	-	-	V	K	-	-	K
<u>X17621</u>	potassium voltage gated channel, shaker related subfamily, member 6	-	V	-	-	V	-	-	-	-
<u>X51992</u>	GABA A receptor, alpha 5	-	-	-	K	-	-	K	G	-
<u>X55812</u>	CB1 receptor	G	G	-	-	K	K	-	-	K
<u>X56917</u>	IP3 3-kinase A	-	G	-	-	-	K	-	G	K
<u>X57514</u>	GABA A receptor, gamma 1	V	-	-	-	-	-	K	-	-
<u>X58149</u>	Phosphoribulokinase, chloroplastic	-	-	-	-	-	-	-	G	-
<u>X61159</u>	glycine receptor, alpha 2	-	G	-	-	K	-	K	-	-
<u>X62085</u>	hypoxanthine phosphoribosyltransferase 1	-	G	-	V	K	-	-	G	-
<u>X62295</u>	angiotensin II receptor, type 1a	-	V	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>X62314</u>	somatostatin receptor 1	-	-	-	K	-	-	-	G	-
<u>X62840</u>	potassium voltage gated channel, Shaw-related subfamily, member 1	V	-	G	-	-	-	K	-	-
<u>X62841</u>	potassium voltage gated channel, Shaw-related subfamily, member 4	-	-	G	-	-	-	-	-	G
<u>X62952</u>	vimentin	V	V	G	-	-	-	K	K	G
<u>X63143</u>	syndecan 3	-	-	-	-	-	V	-	-	-
<u>X63995</u>	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	-	-	-	-	V	-	-	-	K
<u>X66842</u>	serotonin receptor 2B	-	V	-	-	-	-	-	-	-
<u>X66870</u>	lamin A	V	V	-	V	V	V	G	G	-
<u>X69903</u>	interleukin 4 receptor, alpha	-	-	-	-	V	-	-	-	-
<u>X70521</u>	sodium channel, nonvoltage- gated, type I, alpha	-	-	-	-	-	K	-	-	-
<u>X70662</u>	potassium voltage-gated channel, shaker-related subfamily, beta member 1	-	-	-	-	-	-	-	G	-
<u>X73653</u>	glycogen synthase kinase 3 beta	-	V	-	-	-	-	-	-	-
<u>X73683</u>	similar to H3 histone, family 3B	V	V	V	-	-	-	K	K	-
<u>X74833</u>	cholinergic receptor, nicotinic, beta 1 (muscle)	-	V	-	-	-	-	-	-	-
<u>X76489</u>	CD9 molecule	V	-	-	V	-	-	K	-	-
<u>X78606</u>	RAB28, member RAS oncogene family	-	G	-	-	K	-	-	-	-
<u>X79321</u>	microtubule-associated	-	G	-	-	K	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	protein tau									
<u>X83094</u>	heat shock transcription factor 1	G	-	-	-	-	K	-	-	-
X83580, X87635	potassium inwardly-rectifying channel, subfamily J, member 4	G	G	-	-	K	K	G	G	K
<u>X83585</u>	potassium inwardly-rectifying channel, subfamily J, member 10	-	-	-	-	-	K	-	-	-
<u>X86789</u>	synuclein, gamma (breast cancer-specific protein 1)	-	-	-	-	-	V	-	-	-
<u>X91810</u>	signal transducer and activator of transcription 3	-	V	-	V	V	-	-	-	-
X92070	purinergic receptor P2X, ligand-gated ion channel, 6	-	-	-	-	V	-	-	G	-
X95096	Macrophage stimulating 1 (hepatocyte growth factor-like)	-	-	-	-	-	V	-	-	G
<u>X95466</u>	spectrin repeat containing, nuclear envelope 1	G	G	-	-	K	K	G	-	K
X95579	GABA receptor, rho 1	-	V	-	-	V	K	-	-	-
<u>X96488</u>	mitogen-activated protein kinase 12	-	-	-	-	V	-	-	G	-
<u>X97121</u>	neurotensin receptor 2	-	V	-	-	-	-	-	K	-
<u>X97374</u>	prepronociceptin	-	-	-	-	-	-	G	-	-
<u>X98564</u>	potassium channel, subfamily V, member 1	-	-	-	-	-	K	-	-	-
<u>XM_001060756</u>	eukaryotic translation initiation factor 4 gamma, 1	-	-	-	-	-	K	-	-	K
<u>XM_001066554</u>	eukaryotic translation initiation factor 4E family	V	-	-	-	-	-	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
	member 1B									
<u>XM_217346</u>	PTK7 protein tyrosine kinase 7	V	G	-	-	-	-	K	G	-
<u>XM_217785</u>	Rattus norvegicus TATA box binding protein (Tbp),	-	V	-	-	-	-	-	-	-
<u>XM_224987</u>	secreted frizzled-related protein 1	V	-	V	-	-	-	-	-	-
<u>XM_226051</u>	wingless-type MMTV integration site family, member 8A	V	V	-	-	-	-	-	-	-
<u>XM_234422</u>	c-fos	V	V	V	V	-	V	-	-	-
<u>XM_235454</u>	forkhead box H1	-	V	-	-	V	-	-	K	-
<u>XM_235639</u>	wingless-type MMTV integration site family, member 1	G	V	-	-	-	-	G	-	-
<u>XM_237295</u>	wingless-type MMTV integration site family, member 6	-	-	-	-	-	-	-	-	K
<u>XM_237296</u>	wingless-type MMTV integration site family, member 10A	-	-	-	V	K	-	-	K	-
<u>XM_575489</u>	neurogenic differentiation 6	-	G	-	-	-	-	-	G	-
<u>Y11433</u>	pyrimidinergic receptor P2Y, G-protein coupled, 4	-	V	-	-	-	-	-	-	-
<u>Y14635</u>	amiloride-sensitive cation channel 1, neuronal	-	-	-	-	-	-	-	-	K
<u>Y16563</u>	bassoon	-	-	-	-	V	-	-	G	-
<u>Z11558</u>	glia maturation factor, beta	G	V	-	-	-	-	G	-	-
<u>Z12152</u>	neurofilament, medium polypeptide	-	V	-	-	V	K	-	-	-

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Gene Bank Accession No.	Gene Name	Glyx v Vehicle			Ketamine v Vehicle			Glyx v Ketamine		
		1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks	1 Hr	24 Hr	2 wks
<u>Z24721</u>	SOD 3, extracellular	-	-	V	-	V	V	-	-	-

Example 2: GLYX-13 induces rapid antidepressant-like effects without dissociative side effects

[0072] The present study examined GLYX-13 for its potential as a clinically relevant antidepressant using multiple rat models of depression, and tested for ketamine-like side effects in rats. The study also examined whether the antidepressant-like effects of GLYX-13 required AMPA glutamate receptor activation, and whether GLYX-13 could facilitate metaplasticity.

Methods:

[0073] Behavioral Pharmacology: Male Sprague-Dawley (SD) rats (2-3 Months old) were given injections of GLYX-13 (1-56 mg/kg IV; 1-100 mg/kg SC; 0.1-10 µg MPFC), ketamine (10 mg/kg IV; 0.1-10 µg MPFC), fluoxetine positive control (three doses at 10 mg/kg SC) or sterile 0.9% saline vehicle, either 20-60 min or 24 hrs before Porsolt testing. Pretreatment with NBQX (10 mg/kg IP) was used to test the role of AMPAR in the antidepressant-like effect of GLYX-13 (3 mg/kg IV) in the Porsolt test. Antidepressant-like drug effects were measured by decrease in floating time in the Porsolt test, decreased feeding latency in a novel but not familiar environment for the novelty-induced hypophagia (NIH) test, and decreased number of escape failures in the learned helplessness (LH) test. Ketamine like abuse potential and reward was measured by ketamine-like responding in drug discrimination testing and time spent in the drug paired side in the conditioned place preference assay. Ketamine-like disruptions in sensory-motor gating were measured by decreased pre-pulse inhibition. Ketamine-like sedation was measured by decreases in open field locomotor activity and operant response rate in a drug discrimination study. Molecular Pharmacology: Adult male SD rats were dosed with GLYX-13 (3 mg/kg IV), ketamine (10 mg/kg IV) or saline vehicle and sacrificed 24 hrs post dosing. MPFC and hippocampal slices were prepared, and cell surface expressing proteins were cross-linked by biotinylation. Cell surface expression of GluR1 and NR2B were measured by Western blot. Electrophysiology: Hippocampal slices were prepared from adult male SD rats 24 hours

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after a single injection of GLYX-13 (3 mg/kg IV), ketamine (10 mg/kg IV) or vehicle. LTP at Schaffer collateral-CA1 synapses was measured in response to three submaximal bouts of high-frequency Schaffer collateral stimulation (2x100Hz/800ms). The percent contribution of NR2B and NR2A-containing NMDARs to pharmacologically isolated total NMDAR conductance were measured in Schaffer collateral-evoked EPSCs of CA1 pyramidal neurons by using the NR2B-selective NMDAR antagonist ifenprodil (10 μ M), and the NR2A-NMDAR selective antagonist NVP-AM077 (100nM).

Results:

[0074] As shown in FIG. 4, GLYX-13 produces antidepressant-like effects in multiple rat models. The data were collected as described in the Methods and below.

[0075] Porsolt test: 2-3 month old Sprague Dawley (SD) rats treated with a single dose of GLYX-13 (TPPT-NH₃; 1-56 mg/kg, IV), scrambled GLYX-13 (PTTP-NH₃; 3 mg/kg, IV), ketamine (10 mg/kg, IP), 3 doses of fluoxetine (20 mg/kg SC; 24, 5, and 1 hr before testing;(Detke et al., 1995)), or sterile saline vehicle (1 ml/kg, IV) 30-60 min before testing, or a single dose of GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV) or 3 doses of fluoxetine (20 mg/kg SC; last dose 24 hrs before testing) or saline vehicle treated rats tested 24 hrs post dosing. NIH test: latency to eat in the novelty induced hypophagia (NIH) test in SD rats dosed with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV) or saline and tested 1 hr post dosing. LH test: escape failures in the footshock induced learned helplessness (LH) test in SD rats dosed with single dose of GLYX-13 (3 mg/kg IV; 24 hrs before testing), 3 doses Fluoxetine (20 mg/kg SC; last dose 1 hr before testing), or sterile saline vehicle (1 ml/kg IV; tail vein) 24 hrs before testing. Naïve control animals did not receive pre-shock or injection before LH testing. USVs test: Hedonic and Aversive USVs in adult SD rats receiving 2 min of heterospecific play (alternating blocks of 15 sec stimulation followed by 15 sec no stimulation). Data expressed as Mean ($\pm$ SEM). N = 7 - 21 per group. P < .05 Fishers PLSD post hoc test vs. vehicle.

[0076] Results of the various tests presented in FIG. 5 demonstrate that GLYX-13 does not show ketamine-like addictive sensory motor gating, or sedative side effects. The data were collected as described in the Methods and below.

[0077] Drug discrimination: Percentage ketamine-lever responding and for different doses of ketamine (IP and SC) and GLYX-13 (SC) in SD rats trained to discriminate 10 mg/kg ketamine (Ket), IP, from saline (Sal). Values above Sal and Ket are the results of control tests

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conducted before testing each dose response curve. Place Preference: Ketamine (10 mg/kg IV) but not GLYX-13 (10 mg/kg IV) induced conditioned place preference as measured by % time in drug paired chamber. Prepulse Inhibition: Ketamine (10 mg/kg IP) but not GLYX-13 (10 mg/kg IV) decreased sensory-motor gating as measured by prepulse inhibition. Open field: A
5 sedating dose of ketamine (10 mg/kg SC) but not GLYX-13 (10 mg/kg IV) reduced locomotor activity in the open field as measured by line crosses. N = 8-11 per group. Data are expressed as Mean ($\pm$ SEM). * P < .05 Fishers PLSD post hoc test vs. vehicle.

[0078] As indicated in FIG. 6, injection of GLYX-13 into the prefrontal cortex shows antidepressant-like effects in the Porsolt test. The data were collected as described below.

10 [0079] Mean ($\pm$ SEM) time (sec) spent immobile in the Porsolt test in 2-3 month old male rats implanted with (a) medial prefrontal or motor cortex (dorsal control) cannulae and injected with GLYX-13 (0.1, 1, 10 μ g / side) or sterile saline vehicle (0.5 μ L/ 1 min) and tested 1 hr post dosing or rats given MPFC injections of ketamine (0.1, 1, 10 μ g), GLYX-13 (1 μ g), or saline and tested 20 min and 24 hrs post dosing. Animals received a 15 min training swim
15 session one day before dosing. Mean ($\pm$ SEM) line crosses in the open field 20 min following MPFC infusion of GLYX-13 (1 μ g), ketamine (0.1 μ g) or sterile saline vehicle. Given that 0.1 μ g dose of ketamine increased locomotor activity, the Porsolt data for that dose were not included in the analysis given that increasing locomotor activity produces a false positive antidepressant-like response. A representative H&E stained section depicting MPFC cannulae
20 placements, arrow indicates injection site. N = 5-10 per group. * P < .05, Fisher PLSD vs. vehicle

[0080] The data in FIG. 7 demonstrate that GLYX-13 induces NR2B-dependent synaptic plasticity. The data were collected as described in the Methods and below.

ex vivo cell surface protein levels: Biotinylated cell surface GluR1 protein levels in the medial
25 prefrontal cortex (MPFC) or hippocampus as measured by western blot in SD rats treated with GLYX-13 (3 mg/kg IV) ketamine (10 mg/kg IV) or sterile saline vehicle 24 hours prior to sacrifice. ex vivo NMDAR current: NMDA receptor-dependent single shock-evoked EPSCs in the presence of the NR2B-selective NMDA receptor antagonist ifenprodil (10 μ M), in CA1 pharmacological isolated NMDA current in rats that were dosed with GLYX-13 (3 mg/kg IV)
30 ketamine (10 mg/kg IV) or sterile saline vehicle (IV) 24 hrs before ex-vivo NMDA current measurement. ex vivo LTP: GLYX-13 (3 mg/kg IV) or ketamine (10 mg/kg IV) 24 hrs post

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dosing enhances the magnitude of ex vivo long-term potentiation (LTP) of synaptic transmission at Schaffer collateral-CA1 synapses. Data are expressed as Mean ($\pm$ SEM). N = 5-11 per group. *P < .05, **P < .01 Fishers PLSD post hoc test vs. vehicle.

[0081] In FIG. 8, the data show that the antidepressant-like effects of GLYX-13 are synaptic-plasticity related. The data were collected as described in the Methods and below.

[0082] Ex vivo cell surface protein levels: Biotinylated cell surface GluR1 protein levels in the medial prefrontal cortex (MPFC) or hippocampus as measured by western blot in SD rats treated with GLYX-13 (3 mg/kg IV) or sterile saline vehicle 24 hours prior to sacrifice.

AMPA antagonism: Mean ($\pm$ SEM) Floating time in the Porsolt test in animals pretreated with the AMPA receptor antagonist NBQX (10 mg/kg IP) before GLYX-13 (3 mg/kg IV) dosing and tested 1 hr post dosing.

Summary:

[0083] In total, the data show that (i) GLYX-13 produces a robust antidepressant-like effect without dissociative side effects; and (ii) GLYX-13 produce an antidepressant-like effect by facilitating synaptic plasticity in the MPFC.

Example 3: GLYX-13 increases ex vivo [³H] MK-801 binding, a non-competitive antagonist of the NMDA receptor, in the rat medial prefrontal cortex 1 hour post dosing

[0084] FIG. 9 shows that ex vivo [³H] MK-801 binding in the rat medial prefrontal cortex increases one hour after dosing with GLYX-13. The data were collected as described below.

[0085] Mean $\pm$ SEM specific [³H] MK-801 binding (5 nM; 22.5 Ci / mmol) to well washed rat MPFC membranes (200 μ g) in 2-3 month old Male SD rats treated with GLYX-13 (3 mg/kg IV) or sterile saline vehicle (1 ml/kg tail vein) and decapitated without anesthesia 1 hr post dosing, and brain rapidly removed (60 sec), frozen on dry ice, and stored at -80 °C until assay. [³H]MK-801 binding for was measured under equilibrium conditions (2 hrs) in the presence 1 mM glycine. Non-specific binding was were determined in the absence of any glycine ligand and in the presence of 30 μ M 5,7 DCKA. Maximal stimulation was measured in the presence of 1mM glycine. 50 μ M glutamate was present in all reactions. n = 5-6 per group. * P < .05 vs. respective vehicle.

Example 4: Rapid antidepressant effects of GLYX-13 may be mediated by an E-LTP-like mechanism

[0086] To examine the rapid-acting effects of GLYX-13, the biochemical processes that underlie the induction of early stage long term potentiation (E-LTP) were studied.

5 [0087] Without wishing to be bound by any theory, E-LTP is dependent upon the persistent activation of protein kinases, including Ca^{2+} /calmodulin-dependent protein kinase (CAMKII), protein kinase C (PKC), and casein kinase II (CK2). GLYX-13 (3 mg/kg, IV), or vehicle, were administered to adult (2-3 months old) male Sprague-Dawley rats, and medial prefrontal cortex samples were collected at 15, 30, 60, and 120 min post-dosing (n= 7-9 per group). Total
10 cellular proteins were subjected to 7.5% SDS-PAGE and probed with antibodies directed against GluN2B (4207S, Cell Signaling, MA), pS-1303 GluN2B (Millipore, MA), or pS-1480 GluN2B (ab73014, Abcam, MA). Enhanced chemiluminescence was used to quantitate individual bands. CK2 activity was measured by phosphorylation of a CK2 substrate peptide using the transfer of the gamma-phosphate of [γ - ^{32}P]-ATP (Millipore, MA). Total
15 protein (7.5 micrograms) was incubated with CK2 substrate peptide for 10 min in the presence of 0.1 microliters of stock [γ - ^{32}P]-ATP (100 nCi/reaction).

[0088] GLYX-13 led to a significant increase in total GluN2B protein within 15 min (1.53 fold vs. vehicle, $P < .05$) of administration that peaked at 30 min (1.71 fold, $P < .05$) and returned to control levels by 60 min (60 min, 1.13 fold, $P > .05$; 120 min, 1.16 fold, $P > .05$)
20 (FIGs. 10 and 11). CAMKII / PKC-mediated serine-1303 phosphorylation of GluN2B levels were increased at the 30 min (1.93 fold, $P < .05$), 60 min (2.23 fold, $P < .05$), and 120 min (2.67 fold, $P < .05$) timepoints but did not change at the 15 min timepoint (1.02 fold, $P > .05$) (FIG. 10). CK2-mediated serine-1480 phosphorylation of GluN2B levels peaked within 15 min (2.01 fold, $P < .05$) and remained significantly elevated up to 120 min (30 min, 1.80 fold, $P < .05$; 60 min, 1.48 fold, $P < .05$; 120 min 1.50 fold, $P < .05$) after administration of GLYX-13
25 (FIG. 11). A significant increase in CK-2 specific activity was also observed at 15 min (3.08 fold, $P < .05$) (FIG. 12). CK2 and CAMKII activity has been shown to be rapidly increased at the onset of LTP (Charriaut-Marlangue et al., 1991, *PNAS*, 88, 10232; Fukunaga et al., 1993, *JBC*, 268, 7863). This observation, along with the results reported here, suggest that the rapid
30 onset of the antidepressant effect of GLYX-13 is mediated, at least in part, by the same mechanisms that regulate E-LTP.

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EQUIVALENTS

[0089] While specific embodiments of the subject disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of this specification. The full scope of the disclosure should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

[0090] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, parameters, descriptive features and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.”

10 Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention.

INCORPORATION BY REFERENCE

[0091] All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

[0092] What is claimed is:

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1 1. A method for identifying a candidate compound suitable for treatment of depression,
2 comprising:

3 exposing a cell to a potential compound in a culture medium, or administering a
4 potential compound to an animal;

5 retrieving a sample from the cell and/or culture medium, or from brain or neural tissue
6 of the animal, at one or more predetermined time points;

7 analyzing the sample for increased expression levels of Wnt1, and

8 identifying the candidate compound as suitable for treatment of depression based on the
9 increased expression level of Wnt1.

1 2. A method for identifying a candidate compound suitable for treatment of depression,
2 comprising:

3 exposing a cell to a potential compound in a culture medium, or administering a
4 potential compound to an animal;

5 retrieving a sample from the cell and/or culture medium, or from brain or neural tissue
6 of the animal, at one or more predetermined time points;

7 analyzing the sample for increased expression levels of at least one of the genes listed in
8 Table 1 or 2 indicated with a G, or decreased expression levels or at least one of the genes
9 listed in Table 1 or 2 indicated with a K, and

10 identifying the compound as suitable for treatment of depression based on the increased
11 expression level or decreased expression level.

1 3. The method of claim 2, wherein the sample has a gene expression pattern as provided in
2 Table 1 or 2 with the indication "G" and the identifying is based on increased expression of
3 those genes.

1 4. The method of any one of claims 1-3, further comprising analyzing the candidate
2 compound for NDMA subunit NR2B synaptic plasticity.

1 5. A method for identifying a compound suitable for treatment of depression, comprising:

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- 2 exposing a cell to a potential compound in a culture medium, or administering a
3 potential compound to an animal;
- 4 retrieving a sample from the cell and/or culture medium, or from brain or neural tissue
5 of the animal, at one or more predetermined time points;
- 6 analyzing the sample for NMDA receptor NR2B subunit plasticity, and
7 identifying the compound as suitable for treatment of depression based on inducing the
8 NR2B plasticity.
- 1 6. The method of claim 5, wherein a candidate compound suitable for treating depression
2 significantly induces NR2B dependent synaptic plasticity as compared to ketamine.
- 1 7. The method of any one of claims 1-6, wherein the tissue is medial prefrontal cortex.
- 1 8. The method of any one of claims 1-7, wherein the animal is a rodent or human, and the
2 cell is a human or rodent cell.
- 1 9. The method of any one of claims 1-7, wherein the compound modulates the NMDA
2 receptor.
- 1 10. The method of any one of claims 1-8, wherein the compound suitable for treating
2 depression has fewer side effects as compared to ketamine.
- 1 11. The method of claim 10, wherein the compound does not have substantial addictive
2 sensory motor grating and/or sedative effect.
- 1 12. The method of any one of claims 1-11, wherein the cell is a eukaryotic cell.
- 1 13. The method of any one of claims 1-12, further comprising selecting the candidate
2 compound from a library of compounds.
- 1 14. A method of identifying a therapeutic compound capable of treating depression in a
2 patient, comprising selecting a compound that significantly induces NR2B dependent synaptic
3 plasticity.