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(54) **Title**: METHODS OF TREATING OR AMELIORATING MIGRAINE

(57) **Abstract**: In certain embodiments, the invention relates to methods for treating migraine (e.g. episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache) by administering a peptide NMDAR partial agonist. In certain embodiments, the invention also relates to methods for treating or ameliorating long-term post migraine sequelae in a patient by administering a peptide NMDAR partial agonist. In certain other embodiments, the invention relates to methods for treating, suppressing, or preventing cortical spreading depression or a disease or condition caused by cortical spreading depression in a patient in need thereof, comprising administering a peptide NMDAR partial agonist. For example, provided herein are methods of treating epilepsy, traumatic brain injury, and/or stroke.

METHODS OF TREATING OR AMELIORATING MIGRAINE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit and priority to United States Provisional Application No. 62/015,727, filed June 23, 2014, and United States Provisional Application No. 62/109,386, filed January 29, 2015, each of which are hereby incorporated by reference in their entirety.

BACKGROUND

[0002] Migraine is a primary, episodic headache pain disorder associated with debilitating attacks that are recurring, and often poorly controlled by existing pharmacotherapies that focus on vascular triggers. The onset of migraine attacks is often presaged by a scintillating scotoma, or migraine aura, caused by the phenomenon of cortical spreading depression (SD; see Ayata, Headache, 50:725-30, 2010; Eikerman-Haerter et al., Curr. Neurol. Neurosci. Rep., 10:167-73, 2010; and Sánchez-del-Rio et al., Curr. Opin. Neurol., 17(3):289-93, 2004). SD is a slowly propagating suppression of electrocorticographic activity triggered by a local increase in extracellular potassium and release of glutamate that produces a self-propagating wave of slow depolarization across large regions of cortex. Migraine with aura is experienced by approximately 15-30% of migraine sufferers.

[0003] The central nervous system (CNS) of mammals employs many neuroactive peptides to effect specialized signaling within the brain and spinal cord including the neuroactive peptides somatostatin, cholecystokinin, VIP, Substance P, enkephalin, Neuropeptide Y (NPY), Neurotensin, TRH, CCK, and dynorphin. (see generally The Biochemical Basis of Neuropharmacology, Cooper, Bloom and Roth, 5th ed., Oxford University Press, New York, 1986). The careful elucidation of the complex signaling pathways, which operate in the CNS, has led to identification of specific receptors modulated by these neuroactive peptides presenting important therapeutic targets for various disorders associated with the CNS.

[0004] The N-methyl-D-aspartate (NMDA) receptor (NMDAR), is one such receptor that has been implicated in neurodegenerative disorders including stroke-related brain cell death,

2015280108 12 Nov 2019

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). LTP is the persistent strengthening of neuronal connections that underlie learning and memory (See Bliss and Collingridge, 1993, Nature 361:31-39).

[0005] Two general classes of glutamate receptors have been characterized in the central nervous system (CNS). They are the metabotropic glutamate receptors, which belong to the G-protein coupled receptor family of signaling proteins, and the ionotropic glutamate receptors (Muir and Lees, Stroke, 1995, 26, 503-513). The ionotropic class is further subdivided into the AMPA, kainate, and NMDA receptor subtypes by the selective ligands that activate them.

[0006] 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.

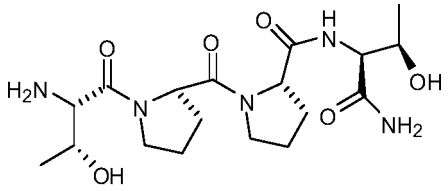
[0007] Thus, there remains a need for improved treatments of migraine and other related diseases with compounds that provide increased efficacy and reduced undesirable side effects.

SUMMARY

[0008] In certain embodiments, the disclosure relates to a method of treating migraine, comprising administering to a patient in need thereof a pharmaceutically effective amount of a GLYX peptide. In certain embodiments, the migraine may be episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, and/or cluster headache. In certain embodiments, the migraine is migraine without aura (common migraine). In certain embodiments, the migraine is migraine with aura (classical migraine). In certain embodiments, the migraine is accompanied by allodynia.

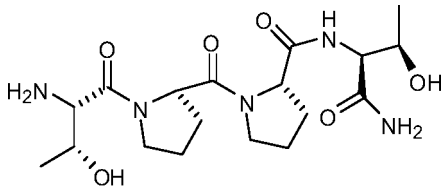
[0008a] In a first aspect, the present invention provides a method for treating migraine with aura in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:

2015280108 12 Nov 2019



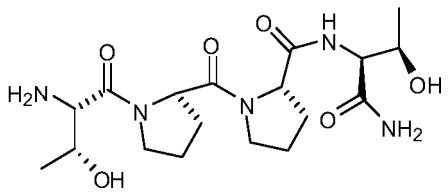
or a pharmaceutically acceptable salt thereof.

[0008b] In a second aspect, the present invention provides a method of treating, suppressing, and/or preventing cortical spreading depression in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



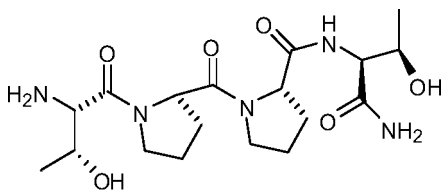
or a pharmaceutically acceptable salt thereof.

[0008c] In a third aspect, the present invention provides use of a compound represented by



or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating migraine with aura.

[0008d] In a fourth aspect, the present invention provides use of a compound represented by



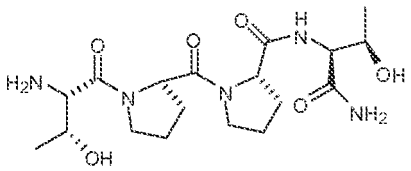
or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating, suppressing, and/or preventing cortical spreading depression.

2015280108 12 Nov 2019

[0009] For example, the disclosed compounds may functionally interact with or modulate the action of the glycine site of the NMDAR for the treatment of migraine (e.g., episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache).

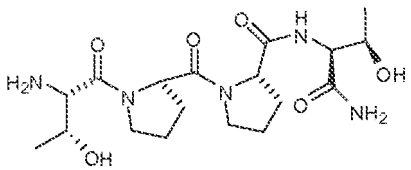
[0010] In certain embodiments, the disclosure relates to a method of treating, suppressing and/or preventing cortical spreading depression (SD), comprising administering to a patient in need thereof a pharmaceutically effective amount of a GLYX peptide. In certain embodiments, the disclosure relates to treating or ameliorating long-term post migraine sequelae in a patient in need thereof, comprising administering to the patient a pharmaceutically effective amount of a GLYX peptide.

[0011] In certain embodiments, the GLYX peptide has the following structure:



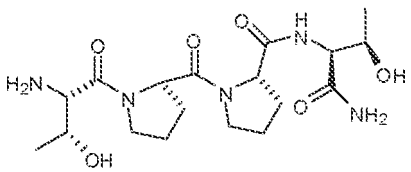
or a pharmaceutically acceptable salt thereof, or derivative thereof having NMDAR partial agonist activity.

[0012] In one aspect, the invention relates to a method for treating migraine in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



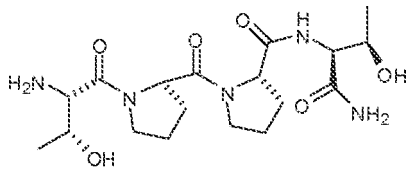
or pharmaceutically acceptable salt thereof.

[0013] In another aspect, the invention relates to a method of treating, suppressing, and/or preventing cortical spreading depression in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



or pharmaceutically acceptable salt thereof.

[0014] In another aspect, the invention relates to a method of treating or ameliorating long-term post migraine sequelae in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



[0015] or pharmaceutically acceptable salt thereof.

[0016] As described in greater detail below, drugs that are effective when administered at aura would be beneficial to a patient, e.g., by allowing intervention at an earlier stage of migraine. It is contemplated that the methods described herein are applicable to the treatment of migraine with aura comprising administration of a GLYX peptide at aura.

BRIEF DESCRIPTION OF FIGURES

[0017] Figure 1 shows focal, high [K^+]-induced spreading depression (SD) in field CA1 of hippocampal slices resulting in a change in luminance reflecting the spreading wave of mass depolarization of neurons and glia.

[0018] Figure 2 shows data for SD elicited in brain slices; there were no significant differences between the baseline areas of individual sequential episodes of SD at the initiating site (Bonferroni Multiple Comparison Test, $P > 0.20$), indicating that GLYX-13 did not alter the initiation of SD.

[0019] Figure 3 shows data demonstrating that GLYX-13 increases the refractory period for SD initiation. SD could be successfully evoked five minutes after a previous SD in a control slice (Control), but could not be elicited in a slice treated with GLYX-13 (GLYX-13 30').

[0020] Figure 4A shows that an SD "aura" of increased luminance spreads from the initiating pipette and propagates across the slice, and can be used to calculate SD conduction velocity (Figure 4B).

[0021] Figure 5 shows one-way analysis of variance (ANOVA) with repeated measures to analyze 6 subsequent SDs in order to test whether repeated episodes of SD maintained a stable speed and if GLYX-13 affected SD conduction velocity.

[0022] Figure 6A shows spine shrinkage in response to two episodes of SD in a control pyramidal neuron, while Figure 6B exemplifies the same process in the presence of 10 μM GLYX-13; Figure 6C indicates that GLYX-13 rescued recovery of spine size following SD.

[0023] Figure 7A shows spreading distance and Figure 7B shows spreading velocity of spreading depression (in estrogen-treated and non-oil treated) rats indicating that spreading speed was faster as compared to oil-treated rats.

[0024] Figure 8 shows that SD in slices from estrogen treated rats traveled longer than oil-treated rats.

[0025] Figure 9A shows that luminance changes associated with SD in a rat experiment were delayed in the presence of GLYX-13; Figure 9B indicates SD was evoked in the presence of GLYX-13.

[0026] Figure 10 shows the effect of GLYX-13 on SD propagation speed across from estrogen treated rats before and after application of GLYX-13 ($F(1,8)=3.1; p<0.05$); and the preexposure of GLYX-13 between the two groups ($F(1,8)=4.2; p<0.05$).

[0027] Figures 11A-11B show rescue of blast-induced learning deficits by rapastinel (3 mg/kg IV; 1 hour post-blast) in PEL tests 24 hours post-blast.. Figure 11A shows blast recovery time (latency to normal ambulation) data. Figure 11B shows the results of a single 3 min Positive Emotional Learning (PEL) test session conducted 24 hours post-blast using a between subjects design. N = 4-6 per group. * $P < 0.05$ (Figure 11A) ANOVA, or (Figure 11B) fisher's PLSD post hoc test, rapastinel + TBI vs. vehicle + TBI.

DETAILED DESCRIPTION

Migraine Classifications

[0028] Migraines were first comprehensively classified in 1988. The International Headache Society most recently updated their classification of headaches in 2004. According to this classification migraines are primary headaches along with tension-type headaches and cluster headaches, among others.

[0029] Migraines are divided into seven subclasses (some of which include further subdivisions):

[0030] Migraine without aura, or "common migraine", involves migraine headaches that are not accompanied by an aura.

[0031] Migraine with aura, or "classic migraine", usually involves migraine headaches accompanied by an aura. Less commonly, an aura can occur without a headache, or with a nonmigraine headache. Two other varieties are familial hemiplegic migraine and sporadic hemiplegic migraine, in which a person has migraines with aura and with accompanying motor weakness. If a close relative has had the same condition, it is called "familial", otherwise it is called "sporadic". Another variety is basilar-type migraine, where a headache and aura are accompanied by difficulty speaking, world spinning, ringing in ears, or a number of other brainstem-related symptoms, but not motor weakness. This type was initially believed to be due to spasms of the basilar artery, the artery that supplies the brainstem. Guidelines for diagnosis of migraine with aura are found, for example, in Eriksen et al., European Journal of Neurology 11 :583-591, 2004 and in the International Classification of Headache Disorders, Second Edition (ICHD-II). Subtypes of migraine with aura include those set forth in the ICHD-II such as typical aura with migraine headache (IHS 1.2.1), typical aura with non-migraine headache (IHS 1.2.2), typical aura without headache (IHS 1.2.3), familial hemiplegic migraine (IHS 1.2.4), sporadic hemiplegic migraine (IHS 1.2.5), and basilar-type migraine (IHS 1.2.6).

[0032] Childhood periodic syndromes that are commonly precursors of migraine include cyclical vomiting (occasional intense periods of vomiting), abdominal migraine (abdominal pain, usually accompanied by nausea), and benign paroxysmal vertigo of childhood (occasional attacks of vertigo).

[0033] Retinal migraine involves migraine headaches accompanied by visual disturbances or even temporary blindness in one eye.

[0034] Complications of migraine describe migraine headaches and/or auras that are unusually long or unusually frequent, or associated with a seizure or brain lesion.

[0035] Probable migraine describes conditions that have some characteristics of migraines, but where there is not enough evidence to diagnose it as a migraine with certainty (in the presence of concurrent medication overuse).

[0036] Chronic migraine is a complication of migraines, and is a headache that fulfills diagnostic criteria for migraine headache and occurs for a greater time interval. Specifically, greater or equal to 15 days/month for longer than 3 months.

[0037] There are four possible phases to a migraine, although not all the phases are necessarily experienced: (1) The prodrome, which occurs hours or days before the headache; (2) The aura, which immediately precedes the headache; (3) The pain phase, also known as headache phase; and (4) The postdrome, the effects experienced following the end of a migraine attack.

[0038] Prodromal or premonitory symptoms occur in ~60% of those with migraines with an onset of two hours to two days before the start of pain or the aura. These symptoms may include a wide variety of phenomena including: altered mood, irritability, depression or euphoria, fatigue, craving for certain food, stiff muscles (especially in the neck), constipation or diarrhea, and sensitivity to smells or noise. This may occur in those with either migraine with aura or migraine without aura.

[0039] An aura is a transient focal neurological phenomenon that occurs before or during the headache. They appear gradually over a number of minutes and generally last fewer than 60 minutes. Symptoms can be visual, sensory or motor in nature and many people experience more than one. Visual effects occur most frequently; they occur in up to 99% of cases and in more than 50% of cases are not accompanied by sensory or motor effects. Vision disturbances often consist of a scintillating scotoma (an area of partial alteration in the field of vision which flickers and may interfere with a person's ability to read or drive.) These typically start near the center of vision and then spread out to the sides with zigzagging lines which have been described as looking like fortifications or walls of a castle. Usually the lines are in black and white but some people also see colored lines. Some people lose part of their field of vision (known as hemianopsia) while others experience blurring.

[0040] Sensory aurae are the second most common type; they occur in 30--40% of people with auras. Often a feeling of pins-and-needles begins on one side in the hand and arm and spreads to the nose-mouth area on the same side. Numbness usually occurs after the tingling has passed with a loss of position sense. Other symptoms of the aura phase can include: speech or language disturbances, world spinning, and less commonly motor problems. Motor

symptoms indicate that this is a hemiplegic migraine, and weakness often lasts longer than one hour unlike other auras.

[0041] An aura may also occur without a subsequent headache. Acephalgic migraines, also known as silent migraines, are relatively rare and include an aura and other symptoms but without a subsequent headache (i.e., no pain phase).

[0042] During the pain phase, the headache usually is unilateral, throbbing, and moderate to severe in intensity. It usually comes on gradually and is aggravated by physical activity. In more than 40% of cases however the pain may be bilateral, and neck pain is commonly associated. Bilateral pain is particularly common in those who have migraines without an aura. Less commonly pain may occur primarily in the back or top of the head. The pain usually lasts 4 to 72 hours in adults, however in young children frequently lasts less than 1 hour. The frequency of attacks is variable, from a few in a lifetime to several a week, with the average being about one a month.

[0043] The pain is frequently accompanied by nausea, vomiting, sensitivity to light, sensitivity to sound, sensitivity to smells, fatigue and irritability. In a basilar migraine, a migraine with neurological symptoms related to the brain stem or with neurological symptoms on both sides of the body, common effects include: a sense of the world spinning, light-headedness, and confusion. Nausea occurs in almost 90% of people, and vomiting occurs in about one-third. Other symptoms may include: blurred vision, nasal stuffiness, diarrhea, frequent urination, pallor, or sweating. Swelling or tenderness of the scalp may occur as can neck stiffness.

[0044] The effects of migraine may persist for some days after the main headache has ended; this is called the migraine postdrome. Many report a sore feeling in the area where the migraine was, and some report impaired thinking for a few days after the headache has passed. The patient may feel tired and have head pain, cognitive difficulties, gastrointestinal symptoms, mood changes, and weakness.

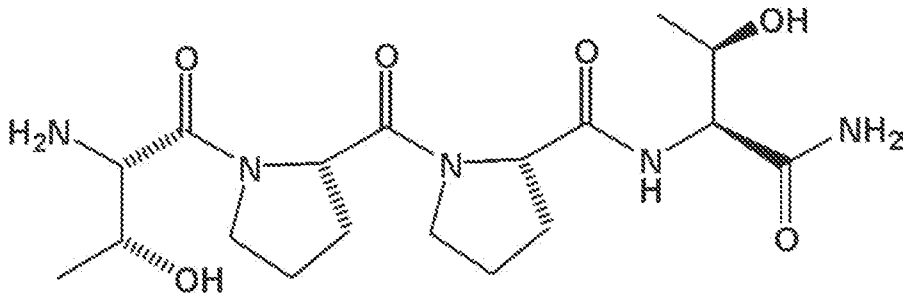
GLYX Peptides

[0045] GLYX-13 is a newly-developed rapid-acting, long-lasting antidepressant with unprecedented modulatory actions on the activation of N-methyl-D-aspartate glutamate receptors (NMDAR). This agent, which acts at the obligatory co-agonist glycine site on the

NMDA receptor required for it to be activated, normalizes activation of this critical receptor, increasing it when it is too low, and suppressing it when it is too high. Through this action, GLYX-13 can enhance the induction of long-term potentiation (LTP), while suppressing LTD, of synaptic strength, and restore normal LTP in hippocampal slices from aging animals.

[0046] 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 tetrape tide having the amino acid sequence Thr-Pro-Pro-Thr, or L-threonyl-L-prolyl-L-prolyl-L-threonine amide.

[0047] For example, GLYX-13 refers to the compound depicted as:



Formula I

[0048] 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, an 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 CH_2, OH, or NH_2 moiety. In other embodiments, GLYX-13 may be optionally substituted with one or more halogens, C_1-C_3 alkyl (optionally substituted with halogen or amino), hydroxyl, and/or amino. Glycine-site partial agonist of the NMDAR are disclosed in US 5,763,393, US 6,107,271, and Wood et al., NeuroReport, 19, 1059-1061, 2008, the entire contents of which are herein incorporated by reference.

[0049] It may be understood that the peptides disclosed here can include both natural and unnatural amino acids, e.g., all natural amino acids (or derivatives thereof), all unnatural amino acids (or derivatives thereof), or a mixture of natural and unnatural amino acids. For example,

one, two, three or more of the amino acids in GLYX-13 may each have, independently, a D- or L-configuration.

[0050] GLYX-13 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. In certain embodiments, an anti-migraine or other therapeutic effect with essentially no sedation may be produced by GLYX-13 when administered to a subject in therapeutically effective amounts. In still other embodiments, GLYX-13 may not have abuse potential (e.g., may not be habit-forming).

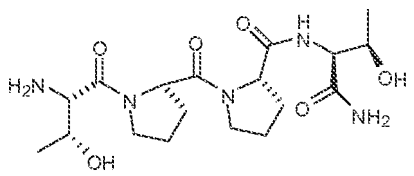
[0051] In some embodiments, GLYX-13 may increase AMPA GluR1 serine-845 phosphorylation. In certain embodiments, glycogen synthase kinase 3β (GSK-3β) may be activated by GLYX-13. In some cases, levels of β-catenin may be altered after administration of GLYX-13.

[0052] In some embodiments, GLYX-13 or a composition comprising GLYX-13 may provide better i.v. *in vivo* potency and/or brain level concentration, relative to plasma levels.

[0053] Additionally, GLYX-13 may have a wide therapeutic index compared to glycine site antagonists such as L-701,324, or other glycine site antagonists having narrow therapeutic indexes, which result in a very narrow range of dose between therapeutic effects and ataxia. For example, L-701,324 had anticonvulsant effects at doses that produced ataxia (Bristow, et al, JPET 279:492-501, 1996). Similarly, a series of Merz compounds had anticonvulsant effects at doses that produced ataxia (Parsons, et al., JPET283:1264-1275, 1997).

Methods

[0054] In one aspect, the invention relates to a method for treating migraine in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



or pharmaceutically acceptable salt thereof.

[0055] In certain embodiments, the compound is administered to the patient with a dose of about 0.01 mg/kg to about 1000 mg/kg or about 1 mg/kg to about 500 mg/kg of the compound.

[0056] In certain embodiments, the migraine is episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache.

[0057] In certain embodiments, the migraine is episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, or cluster headache.

[0058] In certain embodiments, the migraine is migraine with aura (classical migraine).

[0059] In certain embodiments, the migraine is migraine without aura (common migraine).

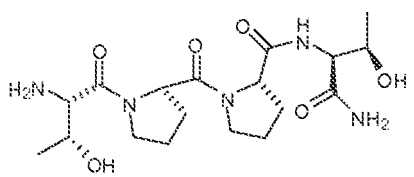
[0060] In certain embodiments, the migraine is accompanied by allodynia.

[0061] In certain embodiments, the method comprises administering about 1 to 10 mg/kg, about 10 mg/kg to about 250 mg/kg, about 20 mg/kg to about 150 mg/kg, about 30 mg/kg to about 125 mg/kg, about 40 mg/kg to about 110 mg/kg, about 50 mg/kg to about 100 mg/kg, about 60 mg/kg to about 90 mg/kg, or about 70 mg/kg to about 90 mg/kg, of the compound.

[0062] In certain embodiments, the method comprises administering about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 50 mg/kg, about 70 mg/kg, or about 100 mg/kg of the compound.

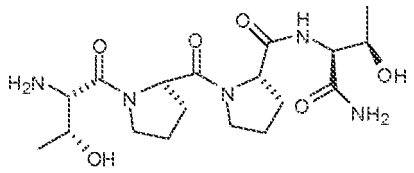
[0063] In certain embodiments, the method comprises administering the compound about twice a day, about every day, every 2 days, every 3 days, every 4 days, every 5 days, about once a week, about every two weeks, or about every four weeks.

[0064] In another aspect, the invention relates to a method of treating, suppressing, or preventing cortical spreading depression or a disease or condition caused by cortical spreading depression in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



or pharmaceutically acceptable salt thereof.

[0065] In another aspect, the invention relates to a method of treating or ameliorating long-term post migraine sequelae in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



or pharmaceutically acceptable salt thereof.

[0066] In certain embodiments, the compound is administered to the patient with a dose of about 0.01 mg/kg to about 1000 mg/kg of the compound. In certain embodiments, the compound is administered to the patient with a dose of about 1 mg/kg to about 500 mg/kg of the compound.

[0067] In certain embodiments, the method comprises administering about 1 mg/kg to 10 mg/kg, about 1 mg/kg to 20 mg/kg; about 10 mg/kg to about 250 mg/kg, about 20 mg/kg to about 150 mg/kg, about 30 mg/kg to about 125 mg/kg, about 40 mg/kg to about 110 mg/kg, about 50 mg/kg to about 100 mg/kg, about 60 mg/kg to about 90 mg/kg, or about 70 mg/kg to about 90 mg/kg, of the compound.

[0068] In certain embodiments, the method comprises administering about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 50 mg/kg, about 70 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg or about 100 mg/kg of the compound.

[0069] In certain embodiments, the method comprises administering the compound about twice a day, about every day, every 2 days, every 3 days, every 4 days, every 5 days, about once a week, or about every two weeks, or e.g., every month.

[0070] In certain embodiments, the method further comprises co-administration with an opioid, an antidepressant, an antiepileptic, a non-steroidal anti-inflammatory drug (NSAID), a serotonin 5HT1B/1D agonist, an N-methyl-D-aspartate antagonist, or an anti-inflammatory compound.

[0071] NMDAR activation may promote or even be essential for, the phenomenon of SD in many experimental situations. Therefore, compounds that prevent over-activation of NMDARs could be important new therapies for lessening, and even preventing, the onset of migraine

attacks. For example, GLYX-13, discussed below, suppresses the production and propagation of SD in hippocampal slices *in vitro*. In certain experiments, GLYX-13 may completely prevent the induction of SD by local increase in extracellular potassium concentration, and/or if not blocking it completely, may slow the rate of SD propagation. Furthermore, GLYX-13 can improve the return of dendritic spines to their original sizes following an SD. For example, provided herein are methods of treating migraine in patients prophylactiely and/or on an acute basis. Such administration may ameliorate the severity (or in some embodiments, abort) migraine attacks in patients.

[0072] In certain embodiments, the patient is a human. Contemplated patients include female patients and/or adolescent patients.

[0073] Also provided herein are methods of treating migraine (e.g., episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache) in treatment-resistant patients or treating refractory migraines, e.g., patients suffering from a migraine that does not, and/or has not, responded to adequate courses of at least one, or at least two, other compounds or therapeutics. For example, provided herein is a method of treating migraine (e.g., episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache) in a treatment resistant patient, comprising a) optionally identifying the patient as treatment resistant and b) administering an effective dose of GLYX-13 to said patient. In certain embodiments, the migraine is migraine with aura.

[0074] Provided herein, in an embodiment, are methods of acutely treating migraine (e.g., episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache) in a patient in need thereof, comprising administering an effective amount of a GLYX peptide, for example, in a single unit dose. For example, contemplated herein in an embodiment, is a method of treating migraine in a patient in need thereof at the onset of a migraine attack comprising administering acutely (e.g., a single dose) an effective amount of GLYX-13. Such methods may relieve the patient of at least one symptom of migraine for about 2 weeks or less, 1 week or less, 1 day or less, or 1 hour or less (e.g. 15 minutes or less, half an hour or less), after said administration. In some embodiments,

such methods may relieve the patient of at least one symptom of migraine for about 1 day or more, 1 week or more, or 2 weeks or more after said administration. For example, provided herein is a method comprising administering an effective amount of a GLYX peptide to a patient suffering from migraine, wherein said patient is substantially relieved of at least one symptom of migraine substantially earlier after the first administration of a GLYX peptide, as compared to the same patient administered another medicament for treating migraine. One of skill in the art will appreciate that such methods of acute administration may be advantageous in a hospital or out-patient setting. The methods described herein can also be useful for the treatment of allodynia that occurs during migraine with aura.

[0075] The present methods may also be used in treatment of patients who have depression, suffer from traumatic brain injury, epilepsy, or are at risk of a stroke. For example, provided herein, in an embodiment, is a method for treating traumatic brain injury comprising administering an effective amount of a GLYX peptide, e.g., GLYX-13. In another embodiment, a method for treating epilepsy is provided, comprising administering an effective amount of a GLYX peptide, e.g., GLYX-13.

[0076] In addition to cardiovascular conditions, migraine sufferers with aura may be at increased risk for other neurological and/or psychological conditions and disorders. It has been shown, for example, that the co-occurrence of migraine with aura with major depression or a suicide attempt increased the risk of developing unprovoked seizure (Hesdorffer et al., Epilepsy Res. 75(2-3):220-223, 2007). Other conditions associated with migraine with aura include significantly higher markers of NO activity, increased incidence of depression, and genetic biomarker correlation for stroke substantially greater than that of the general population (Etminan et al., #MJ330(7482):63, 2005). A migraine sufferer that has, or is at risk of, any of these conditions may take medications to treat or to manage these diseases, and these medications may adversely interact with currently used medications for the treatment of migraine with aura. As shown herein, some of these conditions are contraindications for triptan therapy (e.g., stroke and sumatriptan therapy). Moreover, the FDA issued a public health advisory in 2006 regarding serotonin syndrome, a life-threatening condition that may occur when a triptan is used together with certain anti-depressants that are serotonin reuptake inhibitors (SSRIs) or selective serotonin/norepinephrine reuptake inhibitors (SNRIs).

Accordingly, the methods described herein may be useful for the treatment of patients who have depression or patients who have suffered, or are at risk of, stroke.

Dosages

[0077] The dosage of any compositions of the disclosure will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration, and the form of the subject composition. Any of the subject formulations may be administered in a single dose or in divided doses. Dosages for the compositions of the disclosure may be readily determined by techniques known to those of skill in the art or as taught herein. In general, satisfactory results can be obtained when the compound is administered to a human at a daily dosage of, for example, between 0.05 mg and 3000 mg (measured as the solid form), e.g. about 10 mg to about 500 mg, or e.g., about 1 to about 200mg/kg. Dose ranges include, for example, between 10-1000 mg (e.g., 50-800 mg). In some embodiments, 50, 100, 150, 200, 225, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 mg of the compound is administered. Alternatively, the dosage amount can be calculated using the body weight of the patient. For example, the dose of a compound, or pharmaceutical composition thereof, administered to a patient may range from 1-500 mg/kg (e.g., 5-250 mg/kg). In exemplary, non-limiting embodiments, the dose may range from 5-200 mg/kg (e.g., 1, 2, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg/kg) or from 15-100 mg/kg (e.g., 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg/kg). In exemplary, non-limiting embodiments, the dose may range from 1-15 mg/kg, 50-100 mg/kg, 60-90 mg/kg, or 70-80 mg/kg.

[0078] GLYX-13 may provide a high therapeutic index. For example, GLYX-13 may be therapeutically effective with an i.v. or subcutaneous dose range of about 1 to about 10 mg/kg, about 10 to about 200 mg/kg, e.g. about 30 mg/kg, about 75 mg/kg, or about 100 mg/kg. In some embodiments, no ataxia occurs, at for example a dose of at 500 mg/kg, i.v.

[0079] A therapeutically effective amount of GLYX peptide required for use in therapy varies with the form of the condition being treated, the length of treatment time desired, the age and the condition of the patient, and is ultimately determined by the attending physician. The desired dose may be conveniently administered in a single dose that is effective for two weeks, one week, 6, 5, 4, 3, 2, or 1 day, or as multiple doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0080] An effective dose or amount, and any possible effects on the timing of administration of the formulation, may need to be identified for any particular composition of the disclosure. This may be accomplished by routine experiment as described herein, using one or more groups of animals (preferably at least 5 animals per group), or in human trials if appropriate. The effectiveness of any subject composition and method of treatment or prevention may be assessed by administering the composition and assessing the effect of the administration by measuring one or more applicable indices, and comparing the post-treatment values of these indices to the values of the same indices prior to treatment.

[0081] The precise time of administration and amount of any particular subject composition that will yield the most effective treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a subject composition, physiological condition of the patient (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), route of administration, and the like. The guidelines presented herein may be used to optimize the treatment, e.g., determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

[0082] While the subject is being treated, the health of the patient may be monitored by measuring one or more of the relevant indices at predetermined times during the treatment period. Treatment, including composition, amounts, times of administration and formulation, may be optimized according to the results of such monitoring. The patient may be periodically reevaluated to determine the extent of improvement by measuring the same parameters. Adjustments to the amount(s) of subject composition administered and possibly to the time of administration may be made based on these reevaluations.

[0083] Treatment may be initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage may be increased by small increments until the optimum therapeutic effect is attained.

[0084] The use of the subject compositions may reduce the required dosage for any individual agent contained in the compositions because the onset and duration of effect of the different agents may be complimentary.

[0085] Toxicity and therapeutic efficacy of subject compositions may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 and the ED50.

[0086] The data obtained from the cell culture assays and animal studies may be used in formulating a range of dosage for use in humans. The dosage of any subject composition lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For compositions of the disclosure, the therapeutically effective dose may be estimated initially from cell culture assays.

[0087] In certain embodiments, the GLYX peptide is administered as a preventative measure (i.e., before the prodrome phase). In certain embodiments, the GLYX peptide is administered during prodrome. In certain embodiments, the GLYX peptide is administered at aura. By "at aura" is meant any time after the onset of aura and prior to the onset of migraine pain. In certain embodiments, the GLYX peptide is administered after the onset of migraine pain. In certain embodiments, the GLYX peptide is administered during the postdrome, e.g. to lessen the symptoms thereof.

[0088] The term "prevent," as used herein, refers to prophylactic treatment or treatment that prevents one or more symptoms or conditions of a disease, disorder, or conditions described herein (e.g., pain or migraine with aura and with or without allodynia). Preventative treatment can be initiated, for example, prior to ("preexposure prophylaxis") or following ("post-exposure prophylaxis") an event that precedes the onset of the disease, disorder, or conditions (e.g., at migraine aura). Preventive treatment that includes administration of a GLYX peptide described herein, or a pharmaceutically acceptable salt or solvate thereof, or a pharmaceutical composition thereof, can be acute, short-term, or chronic. The doses administered may be varied during the course of preventative treatment. See also: Kaniecki et al., "Treatment of Primary Headache: Preventive Treatment of Migraine." In: Standards of Care for Headache Diagnosis and Treatment. Chicago (IL): National Headache Foundation; 2004, p. 40-52.

Formulations

[0089] The GLYX peptides of the disclosure may be administered by various means, depending on their intended use, as is well known in the art. For example, if compositions of the disclosure are to be administered orally, they may be formulated as tablets, capsules,

granules, powders or syrups. Alternatively, formulations of the disclosure may be administered parenterally as injections (intravenous, intramuscular or subcutaneous), drop infusion preparations, or suppositories. For application by the ophthalmic mucous membrane route, compositions of the disclosure may be formulated as eyedrops or eye ointments. These formulations may be prepared by conventional means, and, if desired, the compositions may be mixed with any conventional additive, such as an excipient, a binder, a disintegrating agent, a lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent or a coating agent.

[0090] DNA encoding the GLYX peptides, incorporated into an expression vector, can also be administered, using any of the known administration methods, to express of the GLYX peptides *in vivo*.

[0091] In formulations of the subject invention, wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants may be present in the formulated agents.

[0092] Subject compositions may be suitable for oral, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of composition that may be combined with a carrier material to produce a single dose vary depending upon the subject being treated, and the particular mode of administration.

[0093] Methods of preparing these formulations include the step of bringing into association compositions of the disclosure with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association agents with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0094] Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia), each

containing a predetermined amount of a subject composition thereof as an active ingredient. Compositions of the disclosure may also be administered as a bolus, electuary, or paste.

[0095] In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the subject composition is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0096] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the subject composition moistened with an inert liquid diluent. Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art.

[0097] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the subject composition, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as

ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, cyclodextrins and mixtures thereof.

[0098] Suspensions, in addition to the subject composition, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0099] Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing a subject composition with one or more suitable non-irritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the body cavity and release the active agent. Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[00100] Dosage forms for transdermal administration of a subject composition includes powders, sprays, ointments, pastes, creams, lotions, gels, solutions, and patches.

[00101] For topical ocular administration compositions of this invention may take the form of solutions, gels, ointments, suspensions or solid inserts, formulated so that a unit dosage comprises a therapeutically effective amount of the active component or some multiple thereof in the case of a combination therapy.

[00102] Pharmaceutical compositions of this invention suitable for parenteral administration comprise a subject composition in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[00103] Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate and cyclodextrins. Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Combination Therapy

[00104] Any of the GLYX peptides described herein (e.g., GLYX-13) can be used alone or in combination with other agents to treat or prevent any of the diseases or conditions discussed herein. For example, in some combination treatments, the dosages of one or more of the therapeutic compounds may be reduced from standard dosages when administered alone.

[00105] The methods of the invention also comprise co-administration of a GLYX peptide with an opioid, an antidepressant, an antiepileptic, a non-steroidal anti-inflammatory drug (NSAID), a serotonin 5HT1B/1D agonist, an N-methyl-D-aspartate antagonist, or an anti-inflammatory compound.

[00106] The disclosure relates in certain embodiments to the use of a GLYX peptide or peptides alone or in combination with one or more other antidepressant treatments, such as tricyclic antidepressants, MAO-I's, SSRI's, and double and triple uptake inhibitors and/or anxiolytic drugs for manufacturing a medicament for treating migraine (e.g., episodic migraine, chronic migraine, retinal migraine, ophthalmoplegic migraine, acephalgic migraine, migrainous disorder, menstrual migraine, abdominal migraine, childhood periodic syndromes, or cluster headache). Exemplary drugs that may be used in combination with a GLYX peptide include Anafranil, Adapin, Aventyl, Elavil, Norpramin, Pamelor, Pertofrane, Sinequan, Surmontil, Tofranil, Vivactil, Parnate, Nardil, Marplan, Celexa, Lexapro, Luvox, Paxil, Prozac, Zoloft, Wellbutrin, Effexor, Remeron, Cymbalta, Desyrel (trazodone), and Ludiomill.

[00107] In certain embodiments, the opioid is selected from the group consisting of alfentanil, butorphanol, buprenorphine, dextromoramide, dezocine, dextropropoxyphene, codeine, dihydrocodeine, diphenoxylate, etorphine, fentanyl, hydrocodone, hydromorphone, ketobemidone, loperamide, levorphanol, levomethadone, meptazinol, methadone, morphine,

morphine-6-glucuronide, nalbuphine, naloxone, oxycodone, oxymorphone, pentazocine, pethidine, piritramide, propoxyphene, remifentanil, sulfentanyl, tilidine, and tramadol.

[00108] In certain embodiments, the antidepressant is selected from the group consisting of adinazolam, alaproclate, amineptine, amitriptyline/chlordiazepoxide combination, atipamezole, azamianserin, bazinaprine, befuraline, bifemelane, binodaline, bipenamol, brofaromine, caroxazone, cericlamine, cianopramine, cimoxatone, citalopram, clemeprol, clovoxamine, dazepinil, deanol, demexiptiline, dibenzepin, dothiepin, droxidopa, enefexine, estazolam, etoperidone, femoxetine, fengabine, fezolamine, fluotracen, idazoxan, indalpine, indeloxazine, iprindole, levoprotiline, lithium, litoxetine, lofepramine, medifoxamine, metapramine, metralindole, mianserin, milnacipran, minaprine, mirtazapine, montirelin, nebracetam, nefopam, nialamide, nomifensine, norfluoxetine, orotirelin, oxaflozane, pinazepam, pirlindole, pizotyline, ritanserin, rolipram, sercloremine, setiptiline, sibutramine, sulbutiamine, sulpiride, teniloxazine, thozalinone, thyroliberin, tianeptine, tiflucarbine, trazodone, tofenacin, tofisopam, toloxatone, tomoxetine, veralipride, viloxazine, viqualine, zimelidine, and zometapine.

[00109] In certain embodiments, the antiepileptic is selected from the group consisting of carbamazepine, flupirtine, gabapentin, lamotrigine, oxcarbazepine, phenytoin, retigabine, topiramate, and valproate.

[00110] In certain embodiments, the NSAID is selected from the group consisting of acemetacin, aspirin, celecoxib, deracoxib, diclofenac, diflunisal, ethenzamide, etofenamate, etoricoxib, fenoprofen, flufenamic acid, flurbiprofen, lonazolac, lornoxicam, ibuprofen, indomethacin, isoxicam, kebuzone, ketoprofen, ketorolac, naproxen, nabumetone, niflumic acid, sulindac, tolmetin, piroxicam, meclofenamic acid, mefenamic acid, meloxicam, metamizol, mofebutazone, oxyphenbutazone, parecoxib, phenidone, phenylbutazone, piroxicam, propacetamol, propyphenazone, rofecoxib, salicylamide, suprofen, tiaprofenic acid, tenoxicam, valdecoxib, 4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide, N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide, 2-(3,4-difluorophenyl)-4-(3-hydroxy-3-methylbutoxy)-5-[4-(methylsulfonyl)phenyl]-3(2H)-pyridazinone, and 2-(3,5-difluorophenyl)-3-[4-(methylsulfonyl)phenyl]-2-cyclopenten-1-one.

[00111] In certain embodiments, the serotonin 5HT1B/1D agonist is selected from the group consisting of eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan, and zolmitriptan.

[00112] In certain embodiments, the N-methyl-D-aspartate antagonist is selected from the group consisting of amantadine, aptiganel, besonprodil, budipine, conantokin G, delucemine, dexanabinol, dextromethorphan, dextropropoxyphene, felbamate, fluorofelbamate, gacyclidine, glycine, ipenoxazone, kaitocephalin, ketamine, ketobemidone, lanicemine, licostinel, midafotel, memantine, D-methadone, D-morphine, milnacipran, neramexane, orphenadrine, remacemide, sulfazocine, FPL-12,495 (racemide metabolite), topiramate, (αR)-α-amino-5-chloro-1-(phosphonomethyl)-1H-benzimidazole-2-propanoic acid, 1-aminocyclopentane-carboxylic acid, [5-(aminomethyl)-2-[[[(5S)-9-chloro-2,3,6,7-tetrahydro-2,3-dioxo-1H-,5H-pyrido[1,2,3-de]quinoxalin-5-yl]acetyl]amino]phenoxy]-acetic acid, α-amino-2-(2-phosphonoethyl)-cyclohexanepropanoic acid, α-amino-4-(phosphonomethyl)-benzeneacetic acid, (3E)-2-amino-4-(phosphonomethyl)-3-heptenoic acid, 3-[(1E)-2-carboxy-2-phenylethenyl]-4,6-dichloro-1H-indole-2-carboxylic acid, 8-chloro-2,3-dihydropyridazino[4,5-b]quinoline-1,4-dione 5-oxide salt with 2-hydroxy-N,N,N-trimethyl-ethanaminium, N′-[2-chloro-5-(methylthio)phenyl]-N-methyl-N-[3-(methylthio)phenyl]-guanidine, N′-[2-chloro-5-(methylthio)phenyl]-N-methyl-N-[3-[(R)-methylsulfinyl]phenyl]-guanidine, 6-chloro-2,3,4,9-tetrahydro-9-methyl-2,3-dioxo-1H-indeno[1,2-b]pyrazine-9-acetic acid, 7-chlorothiokynurenic acid, (3S,4aR,6S,8aR)-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid, (−)6,7-dichloro-1,4-dihydro-5-[3-(methoxymethyl)-5-(3-pyridinyl)-4-H-1,2,4-triazol-4-yl]-2,3-quinoxalinedione, 4,6-dichloro-3-[(E)-(2-oxo-1-phenyl-3-pyrrolidinylidene)methyl]-1H-indole-2-carboxylic acid, (2R,4S)-rel-5,7-dichloro-1,2,3,4-tetrahydro-4-[[(phenylamino)carbonyl]amino]-2-quinolinecarboxylic acid, (3R,4S)-rel-3,4-dihydro-3-[4-hydroxy-4-(phenylmethyl)-1-piperidinyl]-2H-1-benzopyran-4,7-diol, 2-[(2,3-dihydro-1H-inden-2-yl)amino]-acetamide, 1,4-dihydro-6-methyl-5-[(methylamino)methyl]-7-nitro-2,3-quinoxalinedione, [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]-phosphonic acid, (2R,6S)-1,2,3,4,5,6-hexahydro-3-[(2S)-2-methoxypropyl]-6,11,11-trimethyl-2,6-methano-3-benzazocin-9-ol, 2-hydroxy-5-[[(pentafluorophenyl)methyl]amino]-benzoic acid, 1-[2-(4-hydroxyphenoxy)ethyl]-4-[(4-methylphenyl)methyl]-4-piperidinol, 1-[4-(1H-imidazol-4-yl)-3-butynyl]-4-(phenylmethyl)-piperidine, 2-methyl-6-phenylethynyl)-pyridine, 3-(phosphonomethyl)-L-phenylalanine, and 3,6,7-tetrahydro-2,3-dioxo-N-phenyl-1H,5H-pyrido[1,2,3-de]quinoxaline-5-acetamide.

[00113] In certain embodiments, the anti-inflammatory compound is selected from the group consisting of aspirin, celecoxib, cortisone, deracoxib, diflunisal, etoricoxib, fenoprofen, ibuprofen, ketoprofen, naproxen, prednisolone, sulindac, tolmetin, piroxicam, mefenamic acid,

meloxicam, phenylbutazone, rofecoxib, suprofen, valdecoxib, 4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide, N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide, 2-(3,4-difluorophenyl)-4-(3-hydroxy-3-methylbutoxy)-5-[4-(methylsulfonyl)phenyl]-3(2H)-pyridazinone, and 2-(3,5-difluorophenyl)-3-[4-(methylsulfonyl)phenyl]-2-cyclopenten-1-one.

[00114] The disclosure has multiple aspects, illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

[00115] The studies described herein utilized extracellular multi-site electrophysiological monitoring of SD rate of propagation, and two-photon laser scanning microscopy for real-time imaging of SD-evoked changes in dendritic spine size, in *in vitro* hippocampal slices. The effect of a novel NMDAR receptor functional glycine site partial agonist, GLYX-13 was examined on the threshold and rate of propagation of SD, and real-time effects of SD on dendritic spine morphology. GLYX-13 occasionally completely prevented the induction of SD by a local increase in extracellular potassium concentration, and consistently slowed its propagation rate. The passage of SD through the hippocampal CA1 region produced a rapid retraction of dendritic spines which reversed after neuronal depolarization had recovered. GLYX-13 improved the rate and extent of return of dendritic spines to their original sizes and locations following SD, indicating that the drug and others that modulate NMDA receptor activity could protect synaptic connections in the brain from possible damage from repeated migraine attacks.

General Methods

Drugs

[00116] All external and patch pipette solutions were made with deionized distilled water (resistance > 18 MΩ cm^{-2}; Milli-Q system). The chemicals for making extra- and intracellular solutions were purchased from Sigma-Aldrich (St. Louis, MO, USA). AlexaFluor 594 was purchased from Molecular Probes.

Slice Preparation and Extracellular Recordings

[00117] Experiments were performed on hippocampal slices from 14 to 21 day old Sprague-Dawley® rats (Taconic Farms, Hudson, NY). Rats were deeply anaesthetized with isofluorane, sacrificed, and the brains quickly removed and placed in oxygenated (95% O_2 – 5% CO_2), ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 11 Glucose. The brain was hemisected, the frontal lobes cut off, and individual hemispheres glued using cyanoacrylate adhesive to a stage immersed in ice-cold ACSF oxygenated continuously during slicing. 400 µm thick coronal slices containing the hippocampus were cut using a vibratome (Leica 1200s), and transferred to an interface holding chamber for incubation at room temperature for a minimum of 1 hour before transfer to a Haas-style interface chamber for recording at 32 °C. Slices were perfused with ACSF (4ml/min; ACSF mM: NaCl 126; KCl 3; NaH_2PO_4 1.25; MgCl 1.3; $CaCl_2$ 2.5; $NaHCO_3$ 26; glucose 10) saturated with 95% O_2/5% CO_2 prior to start of the experiment, and all drugs were bath-applied.

[00118] Extracellular recordings were carried out using a MultiClamp 700B (Axon Instruments) with Clampex (v. 9), filtered at 1 kHz and digitized at 3 kHz. Low resistance (1-2 MΩ after filled with ACSF) recording electrodes were made from thin-walled borosilicate glass inserted into the apical dendritic region of the Schaffer collateral termination field in *stratum radiatum* of the CA1 region, at approximately 150 mm distance intervals from the SD initiating pipette, to monitor the spread of SD. The submerged recording chamber was mounted on a Zeiss Axioskop 2FS upright microscope equipped with infrared differential interference contrast (DIC) optics. A 10x objective was used to image slice luminance change caused by SD. Luminance changes were imaged every 100 ms with a cooled CCD camera (CoolSNAP HQ) controlled by a PTI master system. Electrophysiological data were analyzed with Clampfit 9. Imaging data were digitized and reconstructed with ImageJ (NIH).

[00119] A bipolar stainless steel stimulating electrode (FHC, Bowdoin, ME) was placed in Schaffer collateral-commissural fibers in the CA3 region, and current pulses were applied with stimulus intensity adjusted to evoke approximately 50% of maximal fEPSPs once each 30 s (50 to 100 pA; 100 µs duration). Electrical stimulation from an ISO-Flex isolator was controlled by a Master eight-pulse generator (AMPI, Jerusalem, Israel) and triggered by a Multiclamp 700B (Molecular Devices, Sunnyvale, CA). Signals were digitized with a Digidata 1322 and recorded

using a Multiclamp 700B amplifier. fEPSP slope was measured by linear interpolation from 20-80% of maximum negative deflection, and slopes confirmed to be stable to within 10% for at least 15 min before commencing an experiment. Data were analyzed using Clampfit (Version 9; Axon Instrument) on an IBM-compatible personal computer. Evoked fEPSPs (50% of maximum amplitude, 2-4 mV) were recorded in the apical dendritic field in *stratum radiatum* for a stable baseline period of at least 30 min.

Induction of Hippocampal Spreading Depression

[00120] Acute coronal slices of hippocampus were transferred to a submerged recording chamber on a microscope stage and perfused with warmed ACSF (32 °C) where extracellular [K^+] was raised to 8 mM at a perfusion rate of 3 ml/min. Spreading depression was initiated by pressure-injecting puffs of 3M KCl through a glass pipette. Pressure pulses of 8-10 psi that lasted 50-100 ms were driven by a Picospritzer II (Parker Hannifin, Hollis, NH) Pipette resistances were 2 ± 0.2 MΩ when filled with 3 M KCl.

Dynamic imaging analysis of dendritic spine retraction/recovery

[00121] After loading a CA1 pyramidal neuron in a hippocampal slice with Alexa Fluor 594 (100 µM) for 15 minutes, tertiary dendrites were imaged using a using a customized two-photon laser-scanning Olympus BX61WI microscope equipped with a 60x/1.1 nA water immersion infrared objective plus 4x digital zoom, as described previously (Zhang et al., 2008). XYZ scanning mode in a range of +/- 3 µm from focused layer was used to avoid movement bias, with a z step interval of 0.5 µm, and each image took 0.45 second to finish, for a single depth profile time of 7 seconds. Great care was taken to avoid both saturation of fluorescence and any signs of phototoxicity to small dendritic spines from too much excitation light. Depth profiles were repeated at 5 min intervals to reduce possible dye photobleaching and phototoxicity. A Mai/Tai laser (Solid-State Laser, Mountain View, CA) tuned to 810 nm was used for excitation, and image acquisition controlled by Olympus FluoviewFV300 software (Olympus America, Melville, NY). Epifluorescence was detected with photomultiplier tubes of the confocal laser scan head with pinhole maximally opened and emission spectral window optimized for signal over background. In the transfluorescence pathway, a 565 nm dichroic mirror was used to separate green and red fluorescence, and passed through HQ525/50 and HQ605/50 emission filters, respectively, to eliminate transmitted or reflected excitation light (Chroma Technology, Rockingham, VT), and detected simultaneously by two photomultiplier

tubes. The figures show collapsed images from the entire 6 µm Z-profile, and these projections were used to calculate intensity as an index of spine volume.

Data Analyses

[00122] Recording signals were filtered through an eight-pole Bessel low-pass filter with a 1 kHz cutoff frequency and sampled by Clampex (V. 9) with an interval of 100 µs. After fEPSP slopes were calculated with Clampfit (V.9), the data were further processed with Origin 6.1 (Microcal Software, MA) and presented with CorelDraw 10 (Corel, Ottawa, Ontario, Canada). All data were analyzed by one-way analysis of variance (ANOVA), or Student's t-test using SPSS software (SPSS Inc., Chicago, IL). Significance level was preset to $P < 0.05$. Data are presented as mean ± SEM across experiments.

Results

Focal, high [K+]-induced spreading depression in field CA1 of hippocampal slices

[00123] In artificial cerebrospinal fluid (ACSF) where $[K^+]o$ was elevated to 8 mM at 32 °C, a single local puff (100 ms) of 3 M $[K^+]o$ onto a field in CA1 *stratum radiatum* (Figure 1, top left) reliably induced SDs which spread at propagation rates of 9 ± 5.4 mm/min (n = 16) over the entire CA1 region of the hippocampus. SD characterized as a negative field potential shift (Figure 1, top right) showed a maximum shift of -8 ± 1.5 mV (n=30) in the *stratum radiatum* synaptic layer of the hippocampal CA1 region which typically lasted more than 45 seconds.

[00124] SD was induced every 10 minutes to test whether the area of the negative potential shift (Figure 1, top right, hatched area), a measure of the effective magnitude and duration of extracellular space conduction of K^+, was stable with repeated SDs. While the areas of negative potential shifts recorded from the initiating electrode were larger than at the distal recording electrode 600 µm away (Figure 1, bottom), there were no significant differences in amplitude or propagation speed of the individual episodes of repeated sequential SDs elicited at 10 minute intervals at either site (Bonferroni Multiple Comparison Test, $P > 0.20$). These results indicate that reproducible SD negative potential shifts can be stably, repeatedly evoked in field CA1 of the hippocampus, at least for the first ten.

GLYX-13 suppresses the propagation and increases the refractory period of hippocampal spreading depression

[00125] To test the hypothesis that the novel NMDA receptor glycine coagonist site partial agonist GLYX-13 could raise the threshold for or prevent SD by regulating NMDA receptor activation within a physiological range and preventing excess activation, GLYX-13 was bath-applied at 1, 10, or 50 μm to hippocampal slices for 30 min prior to brief ejection of high [K+] (1 mM in patch pipette) into *stratum radiatum* of the CA1 region and attempted to elicit SD. A dose-response relation was constructed based on time of high [K+] ejection first in naive slices, and then after a 30 minute bath application of GLYX-13 at each of the three test concentrations (1,10, or 50μM), while recording DC potential to detect SD.

[00126] After at least three SDs were initially evoked in drug-free ACSF, slices were perfused with GLYX-13 and continued to elicit SDs every ten minutes. As illustrated in Figure 2, top, for 10 μM GLYX-13, there were no significant differences between the baseline areas of individual sequential episodes of SD at the initiating site (Bonferroni Multiple Comparison Test, $P > 0.20$), indicating that GLYX-13 did not alter the initiation of SD.

[00127] GLYX-13 was next tested to ascertain whether affected the relationship of SD amplitudes at near (proximal) and far (distal) recording sites with repeated SDs. The slice was perfused with 10 μM GLYX-13 and continued to elicit SDs every ten minutes (Figure 2, bottom). Bonferroni Multiple Comparison Test ($P > 0.20$) again showed no significant difference between the areas of individual sequential episodes of SD in control slices. GLYX-13 did not affect the initiation of the negative potential shift of SD at the proximal recording site.

[00128] SD amplitudes at the initiating site were correlated with areas of negative potential shifts of SD at the remote recording (Figure 2, bottom). Therefore, proximal/distal area ratio in absence and presence of GLYX-13 was compared. As illustrated in Figure 2, bottom, the area ratio between remote and initiating sites was dramatically reduced in the presence of 10 μM GLYX-13 by the application of the sixth SD (6^{th} SD). A two way ANOVA indicated three significant effects: 1) time effects repeated SDs in the same slices ($F(1, 12)=5.17$, $p < 0.05$) over all observations; 2) effects from drug treatment ($F(1,12)=5.07$, $p < 0.05$); and 3) an interaction of time with treatment ($F(1,12)=30.91$, $p < 0.01$). These results indicate that recurrent negative potential shifts of SD alter the amplitude ratio between remote and initiating sites; and that this

reduction was markedly enhanced by GLYX-13. These findings show that GLYX-13 can significantly suppress the propagation of SD through the brain, particularly after multiple SD events.

[00129] In the preceding studies, it was demonstrated that GLYX-13 can limit the propagation of SD by reducing the amplitude of the negative field potential shift and slowing the conduction speed, effects that were largest after multiple SDs. By shortening the interval between each SD initiation, it was discovered that each SD in control slices had an absolute refractory period of 3-4 minutes for the next successful SD (n = 7). 10 μM GLYX-13 prolonged this refractory period to 5-6 minutes (n = 6). As shown in Figure 3, SD could be successfully evoked five minutes after a previous SD in a control slice (Control), but could not be elicited in a slice treated with GLYX-13 (GLYX-13 30').

Measuring SD propagation using intrinsic changes in optical density produced by SD-induced extracellular space volume shifts

[00130] SD elicits profound shrinkage of the extracellular space volume as fluid rushes in to depolarized cells and they swell, and this can be seen as changes in intrinsic optical density *in vivo*. Such luminance intensity changes could also be readily detected in brain slices using transmitted light DIC microscopy. Figure 4A shows that an SD "aura" of increased luminance spreads from the initiating pipette and propagates across the slice, and can be used to calculate SD conduction velocity (Figure 4B). The time course of increased luminance correlated well with maximal negative potential shift of SD using electrophysiological recordings. Therefore, calculating the time course of luminance changes with distance provides an accurate and convenient way to measure SD conduction velocity.

[00131] To determine if repeated SDs show the same conduction velocity, the luminance changes were monitored over 5 points separated by 150 μm as diagrammed at the top of Figure 4A. A one way ANOVA with repeated measures indicated that SD maintained a steady transmitting speed from P1 to P5 ($F(5, 35)=2.42$, $p > 0.05$) within an individual slice. Therefore, the transmitting speed could be used from P1 to P5 to test SD stability over successive episodes. The average propagating speed of SD was 9.07 ± 0.55 mm/min, ranging from 7.54 to 11.14 mm/min (n=16).

[00132] To test whether repeated episodes of SD maintained a stable speed and if GLYX-13 affected SD conduction velocity, a ANOVA test was used with repeated measures to analyze 6

subsequent SDs as illustrated in Figure 5. Analysis revealed a significant difference between control and GLYX-13 treated slices ($F(1,50)=3.66$, $p<0.01$). SD sequence accounted for 38.5% of total variance, indicating that SD propagation speed significantly slowed with increasing SD number ($F(5,50)=14.01$, $p<0.01$). Although the GLYX-13 effect on SD propagation speed over all repeated SDs did not reach significance in the two-way ANOVA, there was a significant interaction between number of SD and GLYX-13 treatment ($F(5,50)=2.53$, $P<0.05$), reflecting that GLYX-13 (filled circles) significantly decreased SD propagation speed only by the seventh SD.

Spreading depression causes CA1 pyramidal neuron dendritic spines to reversibly shrink in volume

[00133] The anatomical microarchitecture of pyramidal neuron dendrites in the brain is surprisingly labile in response to a variety of stimuli. Depolarization, oxygen/glucose deprivation and N-methyl-D-aspartate have all been shown to produce retraction of dendritic spines in hippocampal CA1 pyramidal neurons *in vitro*. To examine the response of spines to SD, single CA1 pyramidal neurons were filled with the fluorescent dye AlexaFluor-594 and imaged dendritic spine shape using serial z-stack sections (0.2 µm steps spanning 5 µm) collected from 2-photon laser scanning microscopy. Thirty minutes after loading of AlexaFluror-594 into CA1 pyramidal had reached equilibrium distribution within the neuron, SDs were initiated by brief ejection of high [K+] (1M in the patch pipette) into *stratum radiatum* of the CA1 region of hippocampal slices where Schaffer collateral axons synapse on the apical dendrites of CA1 pyramidal neurons. As illustrated in Figure 6A and 6B, the depolarization produced by SD did elicit substantial spine shrinkage as measured by collapsed z-stack fluorescence amplitude, with spine volume completely recovering 20-30 minutes after the first SD. This confirms that one of the sequelae of SD-induced depolarization is alteration in dendritic spine morphology.

GLYX-13 improves dendritic spine recovery following spreading depression

[00134] Finally, the effects of GLYX-13 were examined on the dynamic morphological responses of dendritic spines to SDs. GLYX-13 was investigated whether it could either lessen the shrinkage of spines in response to SD, or improve their recovery. Figure 6A illustrates spine shrinkage in response to two episodes of SD in a control pyramidal neuron, while Figure 6B exemplifies the same process in the presence of 10 µM GLYX-13. ANOVA for repeated

measures reached significance ($F(8,64)=17.53$, $p<0.001$) for fluorescent intensity measured from dendritic spines like the one illustrated at the top of Figure 6C, and that this significance mainly came from the post-SD time courses ($F(8,64)=6.18$, $p<0.01$), revealing that, while GLYX-13 (filled circles) did not alter the shrinkage caused by SD, it did rescue the recovery of spine size following SD ($F(8,64)=2.81$, $p<0.05$).

Example 2

[00135] Hippocampal Slice Preparation: Experiments were performed using Sprague-Dawley® rats (Taconic Farms) 14 to 21 days of age. Rats were deeply anaesthetized with isofluorane, sacrificed, and the brains quickly removed and placed in oxygenated (95% O_2 – 5% CO_2), iced-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 11 Glucose. The brain was hemisected, the frontal lobes cut off, and individual hemispheres glued using cyanoacrylate adhesive to a stage immersed in ice-cold ACSF oxygenated continuously during slicing. 400μm thick coronal slices containing the hippocampus were cut using a Vibratome (Leica 1200s), and transferred to an interface holding chamber for incubation at room temperature for a minimum of 1 hour before commencing the experiment.

[00136] Induction of Hippocampal Spreading Depression: Acute coronal slices of hippocampus were transferred to a submerged recording chamber on a microscope stage and perfused with warmed ACSF (32 degree centigrade) where extracellular [K+] was raised to 8 mM at a perfusion rate of 3 ml/min. Spreading depression was initiated by pressure-injecting puffs of 3M KCl through a glass pipette. Pressure pulses of 8-10 psi that usually lasted 50 ms were driven by a picospritzer. Pipette resistances were 2 ± 0.2 MΩ when filled with 3 M KCl.

[00137] Electrophysiological Recording Methods: Extracellular recordings were carried out using a MultiClamp 700B (Axon Instruments) with Clampex (v. 9), filtered at 1 kHz and digitized at 3 kHz. Low resistance (1-2 MΩ after filled with ACSF) recording electrodes were made from thin-walled borosilicate glass inserted into the apical dendritic region of the Schaffer collateral termination field in stratum radiatum of the CA1 region, at approximately 150 mm distance intervals from the SD initiating pipette, to monitor the spread of SD. The submerged recording chamber was mounted on a Zeiss Axioskop 2FS upright microscope equipped with infrared differential interference contrast (DIC) optics. A 10x objective was used to image slice luminance change caused by SD. Luminance changes were imaged every 100 ms

with a cooled CCD camera (CoolSNAP HQ) controlled by a PTI master system. Electrophysiological data were analyzed with Clampfit 9. Imaging data were digitized and reconstructed with ImageJ (NIH). All experiments were conducted under an approved protocol in compliance with National Institutes of Health guidelines

[00138] The findings of Example 1 are investigated relating to women and adolescent patients. The experimental protocol was used to initiate spreading depolarization (SD) in the CA1 region of the hippocampus inbrain slices *in vitro*. To better observe the distance of spreading depression from the initiation site and SD propagation speed over a longer range visually, the 10x objective of Example 1was replaced with a 4x objective to enlarge the viewing domain to 1.6 mm. The recording chamber was modified to perfuse the slice from both sides, more efficient perfusion not only provided sufficient oxygen to the brain slice, but also helped to maintain stable extracellular milieu since each episode of spreading depression causes a dramatic ion composition change both extracellularly and intracellularlly.

[00139] Two groups of 2 month-old, ovariectomized Sprague-Dawley® female rats were included in the current cohort study. One group of ovariectomized rats were provided with daily injections of estrogen for 7 days since the 7th post-operation day, while the others were only injected with oil vehicle as controls for 7 days before conducting experiments.

[00140] The luminance changes were visually categorized following a puff of 3 M potassium as failure (<100 mm), local spreading (>100mm; <800 mm); or full spread (>800 mm). SD induction was attempted in 39 slices from 8 oil-treated rats and 35 slices from 7 estrogen-treated rats. Table 1 summarizes the frequency of occurrence of SD evoked by the same method in the two groups. In the oil-treated group, the failure rate was significantly higher than in the estrogen treated group (Chisquare test, P=0.014), indicating that estrogen itself enhances the propensity of brain tissue to exhibit SD upon focal depolarization.

Table 1

Group	Non-SD	Localized SD	Full SD	Total
Oil Treated	13	21	5	39
Estrogen Treated	6	23	6	35

[00141] Luminance changes were used to estimate the transmitting velocity of spreading depression as shown in Figure 7 below. Propagation velocities were calculated from both localized and full SD induced by focal 3M potassium puffs, by dividing the distance of SD between observation sites by time that SD took to travel between these observation sites. As illustrated in Figure 7, mean spreading speed in slices from estrogen-treated rats was 0.121± 0.013 mm/s, significantly faster than the speed of propagation in slices from of oil-treated control rats (0.083 ± 0.005 mm/s, $P<0.05$, Student's t-test). These data indicate that estrogen plays a significant role in enhancing both the propensity and speed of SD in hippocampus, and probably in neocortex as well.

[00142] Testing of how estrogen affects the severity of SD by measuring the maximum distance of propagation of SD away from the initiation site was conducted. As shown in Fig 8, the spreading end point along the Schaffer collateral pathway estimated by averaging the distance of the observation site that showed minimum detectable luminance changes (>5% change from baseline) from the initiation site with the distance of the close neighboring observation site that exhibited undetectable luminance changes, and measuring this distance from the initiation site. As shown in Figure 8, the SD in slices from estrogen-treated rats traveled a significantly longer distance (0.594 ± 0.071 mm; n=18) than SD in slices from oil-treated rats (0.394 ± 0.051mm; n=16, $P<0.05$, Student's t-test). Hence, SD in estrogen-treated rats was not only more readily elicited, but propagated longer distances.

[00143] 10 μM GLYX-13 was then added to the perfusate after 2 Sds were induced 10 minutes apart, and continued to induce SD each 10 minutes to determine whether the velocity and scale of SD were affected. Figure 9 shows the typical differences of evoked SD in a slice from an estrogen-treated rat before application of GLYX-13 and after a 50 minute exposure to 10 μM bath-applied GLYX-13. The luminance changes at 10 observation sites were measured to assess the effects of GLYX-13 on SD. Although SD was still readily evoked, as shown in Figure 9B, Figure 9A clearly shows that luminance changes associated with SD were delayed in the presence of GLYX-13. A paired t-test showed that GLYX-13 significantly reduced mean SD transmission velocity from 6.56± 0.57 mm/min to 4.96 ± 0.28 mm/min (n=5, $P<0.01$, paired ttest). The same experiments in hippocampal slices from oil-treated rats were conducted. While GLYX-13 decreased mean SD transmission velocity from 5.09 ± 0.61 mm/min to 4.50 ± 0.39 mm/min (n=5), this decrease did not reach statistical significance.

[00144] A two-way repeated ANOVA was also carried out to explore the effect of GLYX-13 on SD propagation speed across the two groups. As Figure 10 illustrates, the first significance comes from estrogen treated rats before and after application of GLYX-13 ($F(1,8)=3.1;p<0.05$); and the second significance arises from preexposure of GLYX-13 between the two groups ($F(1,8)=4.2$; $p<0.05$). These results indicate that GLYX-13 has a stronger effect in estrogen treated rats, significantly slowing transmission velocity of SD to the level of oil-treated rats.

Example 3

Methods

Animals

[00145] Adult male (2-3 month old) Sprague-Dawley® (SD) rats were purchased from Harlan (USA). Rats were housed in Lucite cages with aspen wood chip bedding, maintained on a 12 hour:12 hour light:dark cycle (lights on at 5 AM), and given ad libitum access to Purina® rat chow (USA) and tap water throughout the study.

Traumatic brain injury induction

[00146] Single blast-induced traumatic brain injury was induced, modified for use in rats according to the protocol of Goldstein et al. (Goldstein, L.E., et al. (2012) "Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model," *Science Translational Medicine* 4:134ra160.) Adult male Sprague-Dawley® rats were anesthetized by isoflurane, ear plugs were inserted into both of the rat's ears, and the rat received a single ~ 42 PSI blast of helium generated by puncturing 0.014 inches of polyester film. Sham controls were placed outside of the blast radius. As follows Animals were dosed with rapastinel (3 mg/kg IV) or 0.9% sterile saline vehicle (1 ml/kg IV) 1 hour post-blast Rats were first anesthetized using 3.5-4% isoflurane, then their ears were protected with 1.5 x 1.5 mm foam plugs (Pura-Fit ear plugs, Moldex-Metric INC, Culver City, California). The rats were placed into head access rodent thoracic restrainers (Stoelting, USA) to protect their bodies while allowing the heads to move freely, with the heads 10 cm from the end of the aluminum shock tubes (183 x 61 cm; L-3 Applied Technologies, USA). Rats received a single ~42 PSI blast of helium generated by puncturing 0.014 inches of polyester film. Sham controls were placed outside of the blast radius. Animals were dosed with rapastinel (3 mg/kg IV) or 0.9%

sterile saline vehicle (1 ml/kg IV) 1 hour post-blast. Latency to recover from anesthesia was recorded (Figure 11A). Recovery was defined as displaying eyeblink and righting reflexes, and normal ambulation (normal rhythmic gate, non-circular gate, fully supporting body weight, evidence of some sniffing and exploratory behaviors). Animals were dosed with GLYX-13 (3 mg/kg IV) or 0.9% sterile saline vehicle (1 ml/kg IV) 1 hour post-blast. N = 4-6 per group; * $P < 0.05$ (Figure 11A) ANOVA.

[00147] Positive emotional learning (PEL) Figure 11B shows the results of a single 3 min Positive Emotional Learning (PEL) test session conducted 24 hours post-blast using a between subjects design. N = 4-6 per group. Fisher's PLSD post hoc test was used to evaluate results (rapastinel + TBI vs. vehicle + TBI).

[00148] Heterospecific rough-and-tumble play was conducted as previously described (Burgdorf, J., et al. (2011) "Positive emotional learning is regulated in the medial prefrontal cortex by GluN2B-containing NMDA receptors," *Neuroscience* 192:515-523), and testing occurred 3 hours and 2 weeks post-dosing. Heterospecific rough-and-tumble play stimulation was administered by the experimenter's right hand. Animals received 3 min of heterospecific rough-and-tumble play that consisted of alternating 15 sec blocks of heterospecific play and 15 sec of non-stimulation. High frequency ultrasonic vocalizations (USVs) were recorded and analyzed by sonogram with Avasoft SASlab Pro (Germany) as previously described (Id.). Frequency modulated 50-kHz USVs that occurred during each of the non-stimulation periods were quantified to measure PEL. Animals were not habituated to play stimulation before testing.

Results

[00149] As shown in Figure 11A, animals that received TBI showed longer recovery times from anesthesia in comparison to sham control animals [$F(1, 13) = 41.7$, $P < 0.05$]. As shown in Figure 11B, rapastinel (3 mg/kg IV) rescued TBI-induced suppression of positive emotional learning [$F(2, 12) = 10.0$, $P < .05$; Fisher's PLSD post hoc test rapastinel + TBI vs. vehicle + TBI, sham vs. vehicle + TBI vs., $P < 0.05$]. In addition, anesthesia recovery time was not significantly correlated with rates of PEL [$r(15) = -0.14$, $P > .05$]. In a positive emotional learning test, a single dose of rapastinel (3 mg/kg IV) completely rescued the deficits in learning and emotion induced by TBI. Thus, in this preclinical model, rapastinel was effective

2015280108 12 Nov 2019

in treating the core cognitive and affective symptoms of TBI and rapastinel appears the have therapeutic potential for the treatment of PTSD.

EQUIVALENTS

[00150] 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.

[00151] 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." 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 disclosure.

[00152] 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.

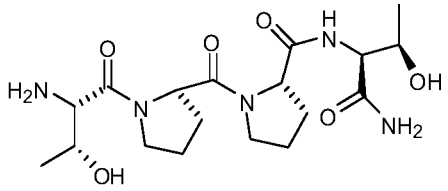
[00153] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[00154] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

2015280108 12 Nov 2019

The claims defining the invention are as follows:

1. A method for treating migraine with aura in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:



or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the compound or a pharmaceutically acceptable salt thereof is administered to the patient with a dose of about 0.01 mg/kg to about 1000 mg/kg or about 1 mg/kg to about 500 mg/kg of the compound.

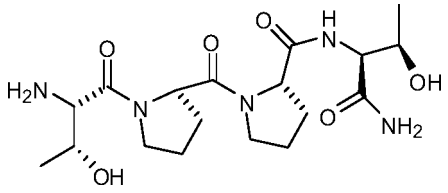
3. The method of claim 1 or 2, comprising administering about 1 mg/kg to about 10 mg/kg, about 10 mg/kg to about 250 mg/kg, about 20 mg/kg to about 150 mg/kg, about 30 mg/kg to about 125 mg/kg, about 40 mg/kg to about 110 mg/kg, about 50 mg/kg to about 100 mg/kg, about 60 mg/kg to about 90 mg/kg, or about 70 mg/kg to about 90 mg/kg, of the compound or a pharmaceutically acceptable salt thereof.

4. The method of any one of claims 1 to 3, comprising administering about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 50 mg/kg, about 75 mg/kg, or about 100 mg/kg of the compound or a pharmaceutically acceptable salt thereof.

5. The method of any one of claims 1 to 4, comprising administering the compound or a pharmaceutically acceptable salt thereof twice a day, about every day, every 2 days, every 3 days, every 4 days, every 5 days, about once a week, about every two weeks, or about once a month.

6. A method of treating, suppressing, and/or preventing cortical spreading depression in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound represented by:

2015280108 12 Nov 2019



or a pharmaceutically acceptable salt thereof.

7. The method of claim 6, wherein the compound or a pharmaceutically acceptable salt thereof is administered to the patient with a dose of about 0.01 mg/kg to about 1000 mg/kg or about 1 mg/kg to about 500 mg/kg of the compound.

8. The method of any one of claims 6 or 7, comprising administering about 1 to about 20 mg/kg, about 2 to about 15 mg/kg; about 5 to about 10 mg/kg, about 10 mg/kg to about 250 mg/kg, about 20 mg/kg to about 150 mg/kg, about 30 mg/kg to about 125 mg/kg, about 40 mg/kg to about 110 mg/kg, about 50 mg/kg to about 100 mg/kg, about 60 mg/kg to about 90 mg/kg, or about 70 mg/kg to about 90 mg/kg, of the compound or a pharmaceutically acceptable salt thereof.

9. The method of any one of claims 6 to 8, comprising administering about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, about 10 mg/kg, about 21 mg/kg, about 25 mg/kg, about 30 mg/kg, about 50 mg/kg, about 70 mg/kg, or about 10 mg/kg of the compound or a pharmaceutically acceptable salt thereof.

10. The method of any one of claims 6 to 9, comprising administering the compound or a pharmaceutically acceptable salt thereof twice a day, about every day, every 2 days, every 3 days, every 4 days, every 5 days, about once a week, about every two weeks, or about once a month.

11. The method of any one of claims 1 to 10, further comprising co-administration with an opioid, an antidepressant, an antiepileptic, a non-steroidal anti-inflammatory drug (NSAID), a serotonin 5HT1B/1D agonist, an N-methyl-D-aspartate antagonist, or an anti-inflammatory compound.

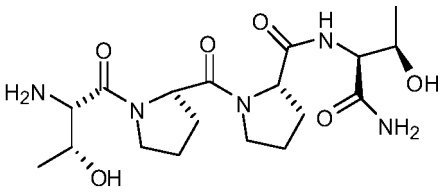
12. The method of any one of claims 1 to 11, wherein the patient is a human.

2015280108 12 Nov 2019

13. The method of any one of claims 1 to 12, wherein the patient is female.

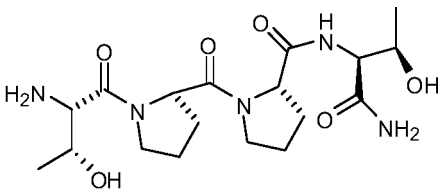
14. The method of any one of claims 1 to 13, wherein the patient is a pediatric or adolescent patient.

15. Use of a compound represented by



or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating migraine with aura.

16. Use of a compound represented by



or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating, suppressing, and/or preventing cortical spreading depression.

FIGURES

FIGURE 1

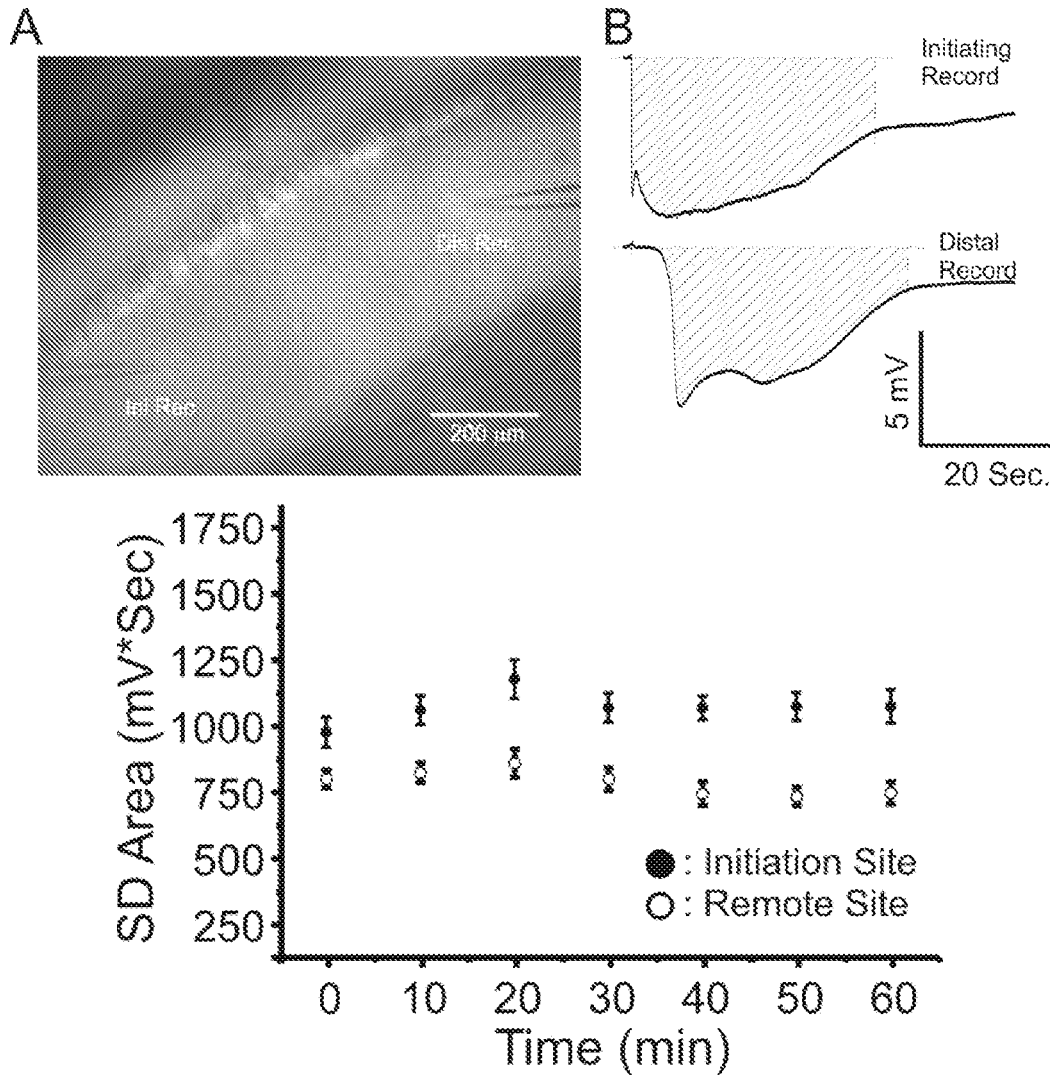


FIGURE 2

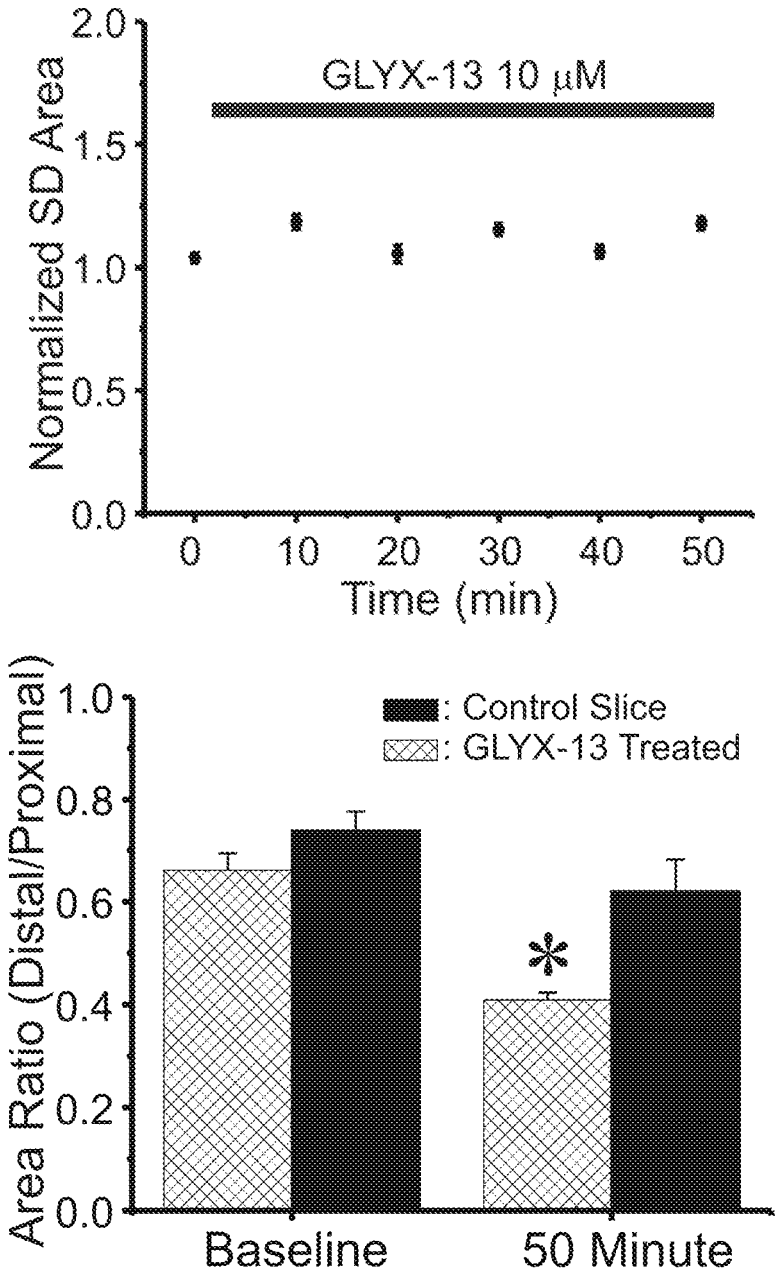

GLYX-13 10 μM
Normalized SD Area
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
Time (min)
Area Ratio (Distal/Proximal)
1.0
0.8
0.6
0.4
0.2
0.0
: Control Slice
: GLYX-13 Treated
*
Baseline
50 Minute

FIGURE 3

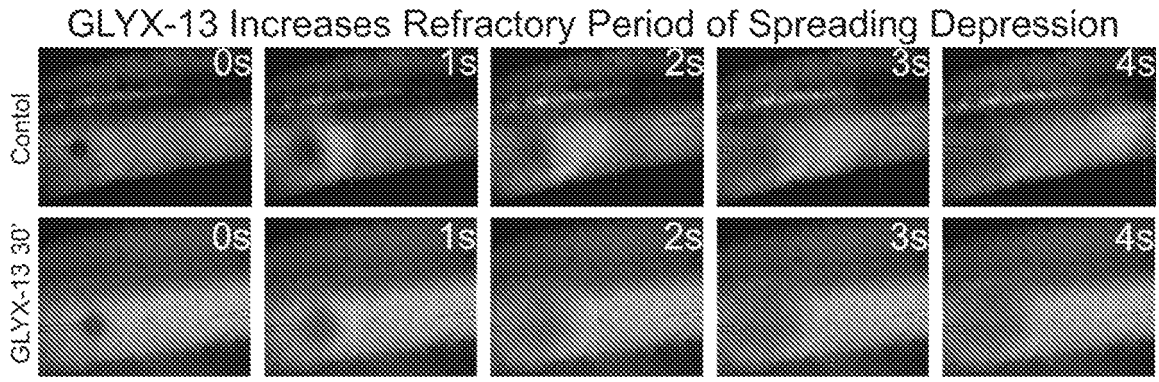

GLYX-13 Increases Refractory Period of Spreading Depression
Contol
GLYX-13 30'
0s
1s
2s
3s
4s
0s
1s
2s
3s
4s

FIGURE 4

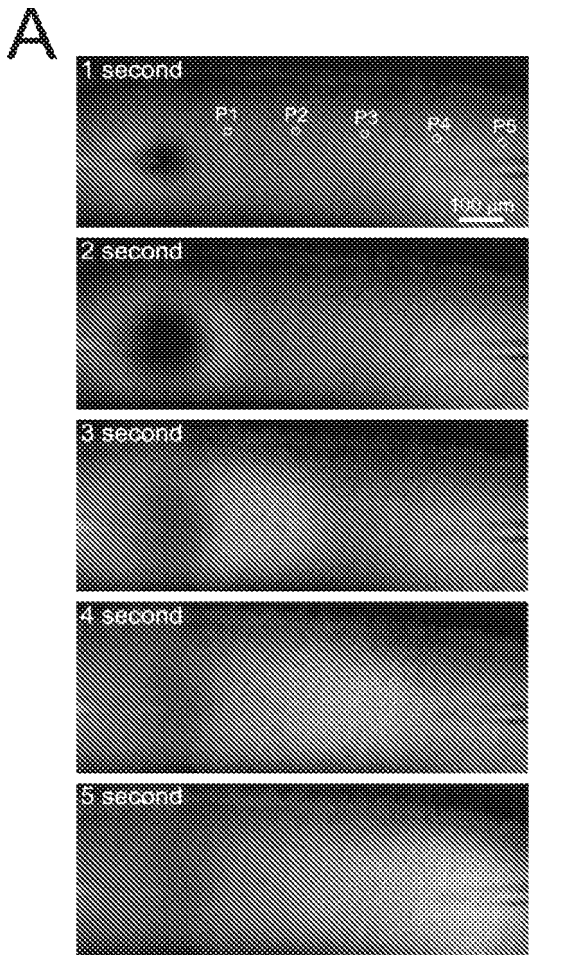

A
1 second
P1
P2
P3
P4
P5
2 second
3 second
4 second
5 second
B
Relative Luminance Intensity
1.3
1.2
1.1
1.0
0.15mm/s
0.16mm/s
0.17mm/s
0.14mm/s
0.16mm/s
P1
P2
P3
P4
P5
2
3
4
5
6
7
Time (Second)

FIGURE 5

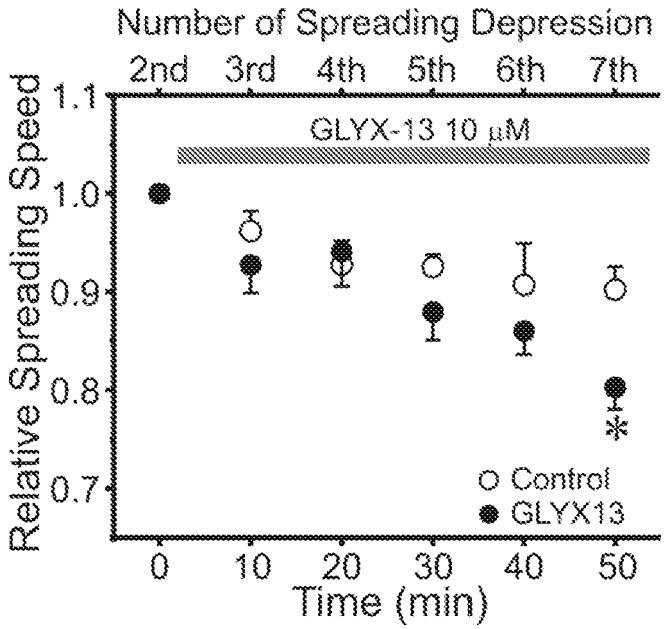

Number of Spreading Depression
2nd
3rd
4th
5th
6th
7th
GLYX-13 10 μM
Relative Spreading Speed
1.1
1.0
0.9
0.8
0.7
Control
GLYX13
*
0
10
20
30
40
50
Time (min)

FIGURE 6

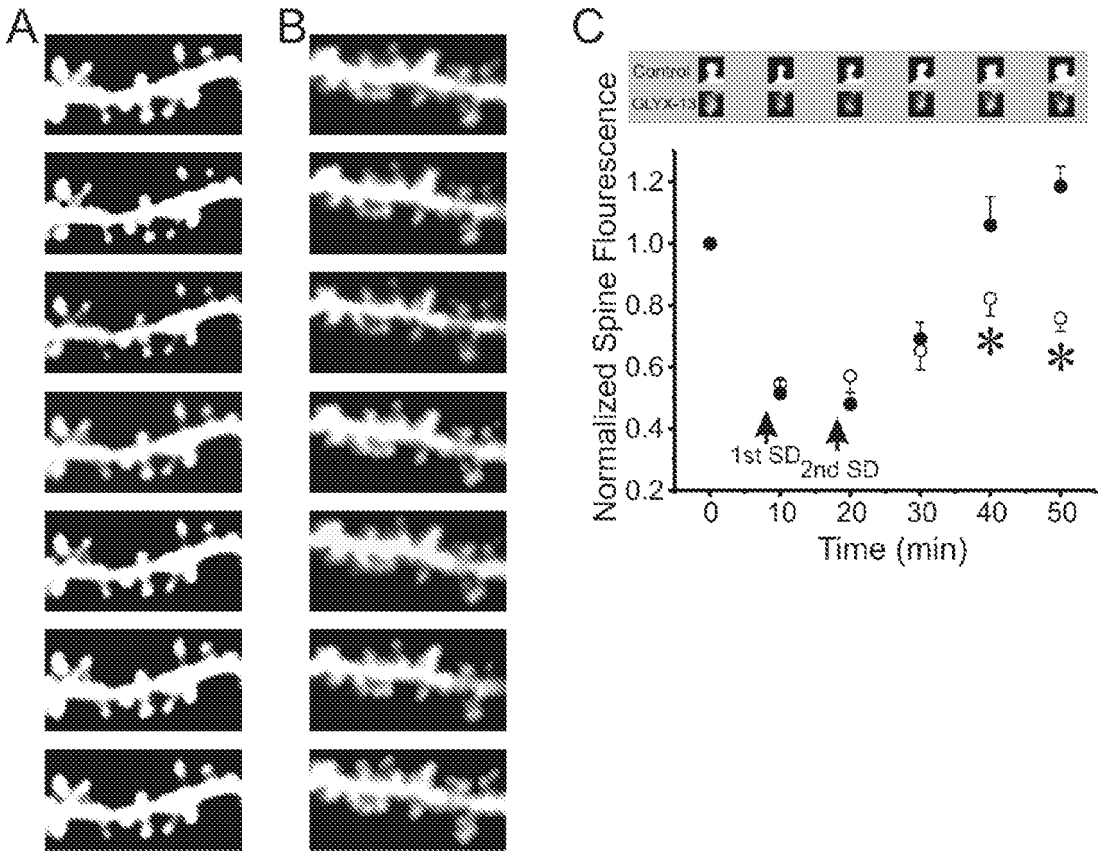

A
B
C
Control
GLYX-13
Normalized Spine Flourescence
1.2
1.0
0.8
0.6
0.4
0.2
1st SD
2nd SD
0
10
20
30
40
50
Time (min)

FIGURE 7

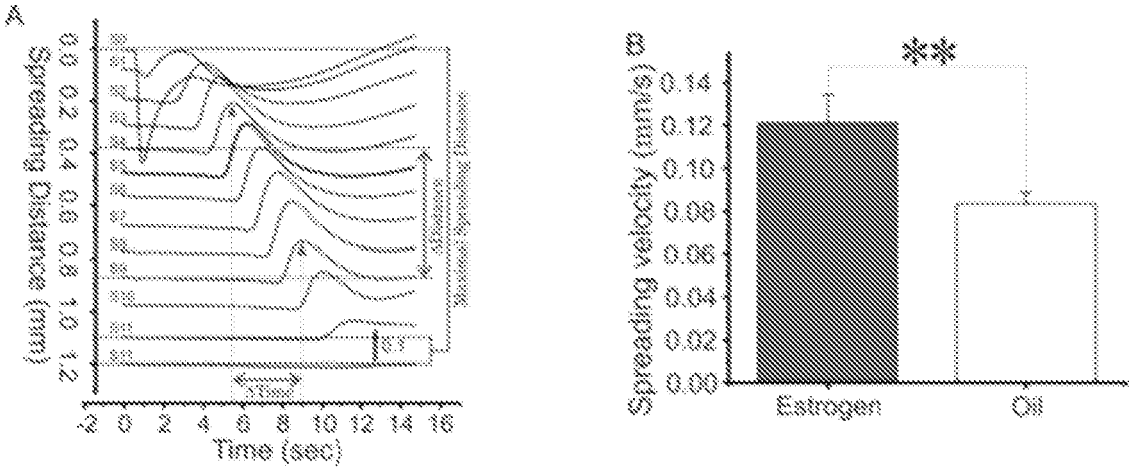

A
Spreading Distance (mm)
0.0 0.2 0.4 0.6 0.8 1.0 1.2
-2 0 2 4 6 8 10 12 14 16
Time (sec)
B
Spreading velocity (mm/s)
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14
**
Estrogen
Oil

FIGURE 8

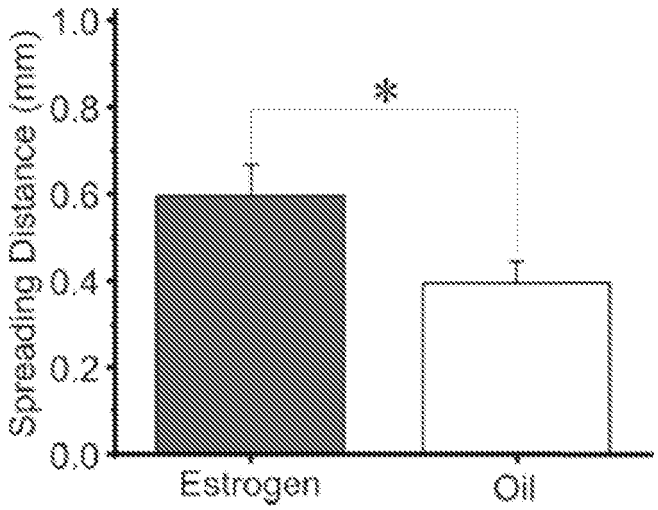
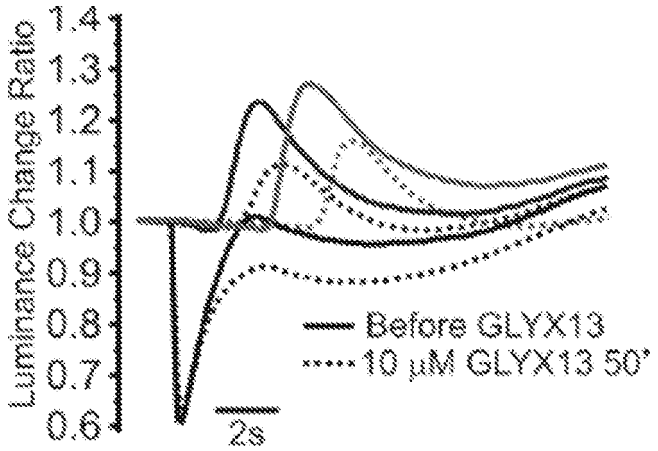
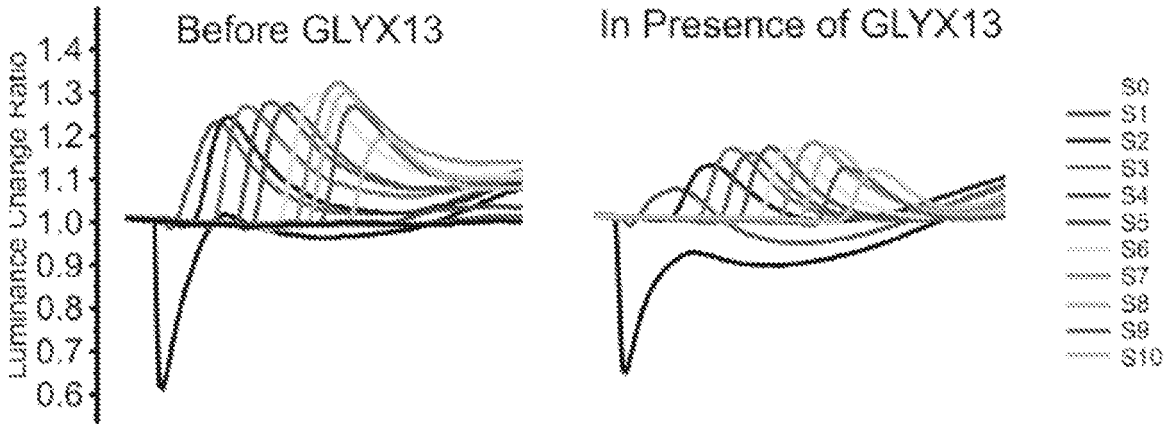


FIGURE 9


Luminance Change Ratio
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
Before GLYX13
10 µM GLYX13 50'
2s

A


Luminance Change Ratio
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
Before GLYX13
In Presence of GLYX13
S0
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10

B

FIGURE 10

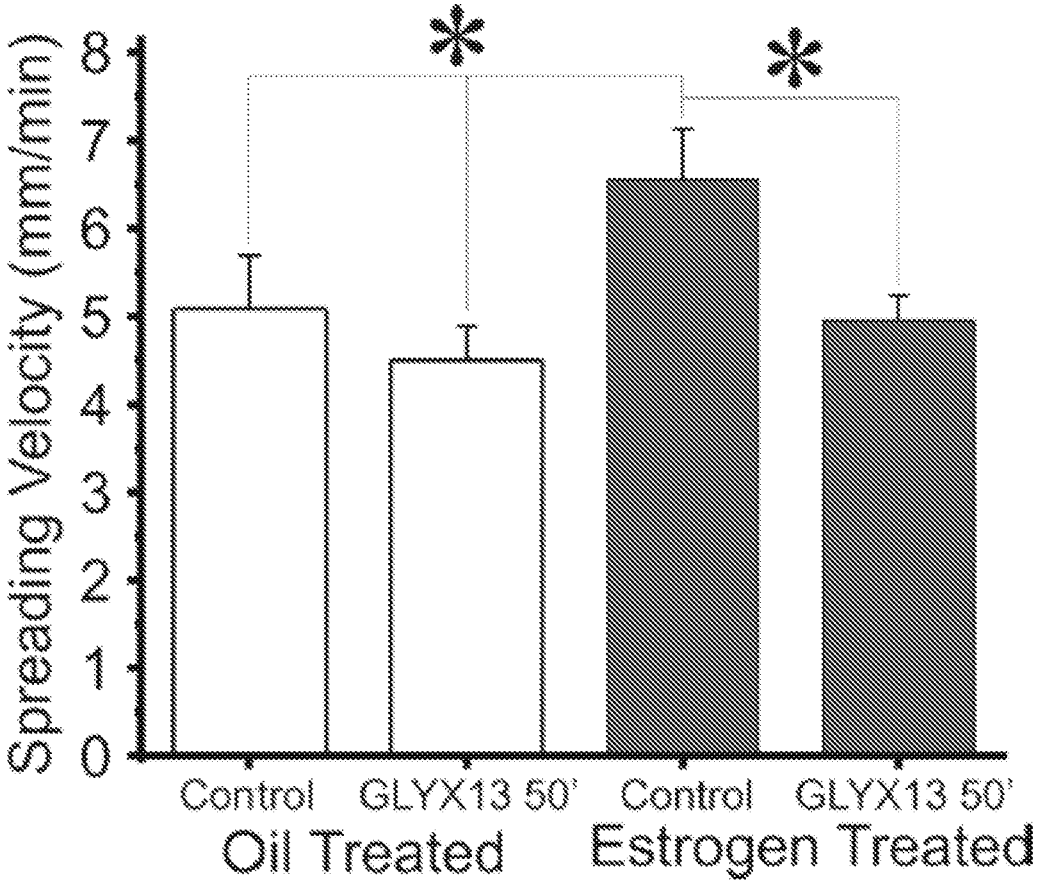
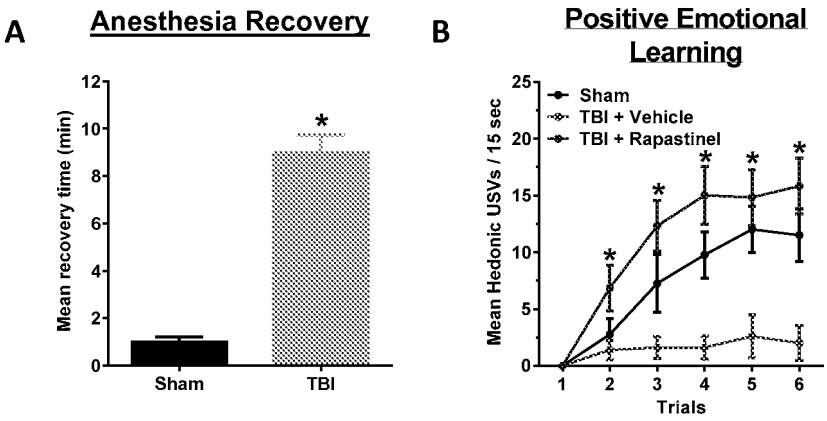

Spreading Velocity (mm/min)
0
1
2
3
4
5
6
7
8
*
*
Control
GLYX13 50'
Control
GLYX13 50'
Oil Treated
Estrogen Treated

FIGURE 11


A
Anesthesia Recovery
Mean recovery time (min)
12
10
8
6
4
2
0
*
Sham
TBI
B
Positive Emotional Learning
Mean Hedonic USVs / 15 sec
25
20
15
10
5
0
Sham
TBI + Vehicle
TBI + Rapastinel
*
1
2
3
4
5
6
Trials