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(54) Title: TREATMENT FOR PSYCHIATRIC DISORDERS

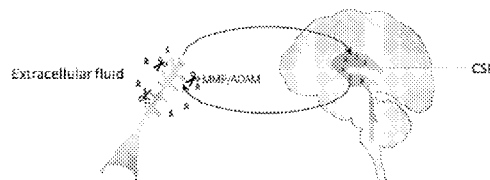
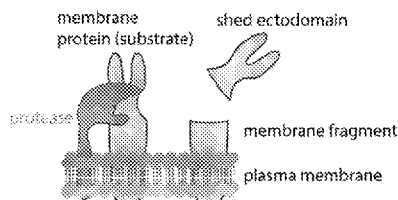


FIG. 1

(57) Abstract: Disclosed herein are a novel peptide and treatment for neurodevelopmental and psychiatric disorders. More specifically, disclosed herein is a composition and treatment based on administering to a subject an ectodomain that showed reduced levels in subjects with the disorder to treat negative and cognitive symptoms of said disorder.



TREATMENT FOR PSYCHIATRIC DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application i claims priority to U.S. Provisional Application No. 63/597,461, filed November 9, 2023, and U.S. Provisional Application No. 63/607,719, filed December 8, 2023, the contents of which are incorporated herein by reference in their entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant Nos. MH097216 and NS100785, both of which were awarded by the National Institutes of Health (NIH). The United States government has certain rights in the invention.

TECHNICAL FIELD

[0003] This disclosure relates to a composition to treat psychiatric or neurodevelopmental disorders and a method of treatment using said composition. More specifically, the disclosure relates to the use of a recombinant protein or a portion thereof to treat psychiatric disorders.

BACKGROUND

[0004] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

[0005] Abnormalities in synapses on excitatory and inhibitory neurons play key roles in the pathophysiology of psychiatric disorders, including schizophrenia (SCZ). This is supported by neuroanatomical studies showing morphological abnormalities in both excitatory and inhibitory neurons. In addition, large-scale human genomic studies have shown that genes encoding synaptic proteins play a key role in the etiology of psychiatric disorders. Rodent models with manipulations of these risk genes similarly present synaptic and behavioral phenotypes. Indeed, a large fraction of psychiatric disorder risk genes encode synaptic proteins. Many of these synaptic risk gene-encoded proteins are transmembrane proteins.

[0006] Despite the efficacy of antipsychotics for treating positive symptoms of psychiatric disorders, such as schizophrenia, negative and cognitive symptoms remain difficult to address.

Thus, treatment options that target the neural pathways and synaptic proteins may improve cognition and other symptoms exhibited by individuals with such disorders.

SUMMARY

[0007] The present application discloses a novel peptide and a pharmaceutical composition comprising a soluble Cav α 2 δ -1 protein or variant or portion thereof and a method of treatment for neurodevelopmental or psychiatric disorders using said protein, variant, or portion thereof. As shown herein, the soluble Cav α 2 δ -1 protein can modulate the cognitive effects of neurodevelopmental or psychiatric disorders by interacting with the synaptic domains of various neurons. It is to be understood that the disclosed embodiments are merely exemplary, and accordingly, the invention may be embodied in various and alternative forms. The specific structural and functional details disclosed herein are not to be interpreted as limiting but merely as a basis for the claims and as a representative basis for teaching one skilled in the art to variously employ the embodiments described herein.

[0008] In a first aspect, the present disclosure provides a polypeptide, comprising or consisting of the amino acid sequence TYEDSFYKRSLDN.

[0009] In a second aspect, the present disclosure provides a pharmaceutical composition, comprising (a) a soluble Cav α 2 δ -1 protein or a variant or a portion thereof, or a nucleic acid encoding the soluble Cav α 2 δ -1 protein or a variant or a portion thereof, and (b) a pharmaceutically acceptable carrier. In some embodiments of this second aspect, the soluble Cav α 2 δ -1 protein comprises a fusion of the C-terminal region of an α 2 subunit of a Cav α 2 δ -1 protein to the N-terminal region of the δ subunit of the same Cav α 2 δ -1 protein. In some embodiments of this second aspect, the soluble Cav α 2 δ -1 protein or a variant or a portion thereof comprises, consists of, or consists essentially of the amino acid sequence TYEDSFYKRSLDN. In some embodiments of this second aspect, the soluble Cav α 2 δ -1 protein is recombinant. In some embodiments of this second aspect, the structure of the active binding region of the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is stabilized. In some further embodiments of this aspect, the stabilized soluble Cav α 2 δ -1 protein or a variant or a portion thereof is a stapled protein or peptide. In some other further embodiments of this aspect, the stabilized soluble Cav α 2 δ -1 protein or a variant or a portion thereof is a circularized protein or peptide. In some

embodiments of this second aspect, the nucleic acid encoding the soluble Cava α 2 δ -1 protein or a variant or a portion thereof is selected from an mRNA, a plasmid, and a vector. In some further embodiments of this aspect, the vector is selected from an adenoviral vector, an adeno-associated viral (AAV) vector, a retroviral vector, a lentiviral vector, and a herpes simplex viral vector. In some embodiments of this second aspect, the composition is formulated as an injectable.

[0010] In a third aspect, the present disclosure provides a method of treating a subject with a neurodevelopmental or psychiatric disorder, comprising administering to the subject a soluble Cava α 2 δ -1 protein or a variant or portion thereof, or a nucleic acid encoding the soluble Cava α 2 δ -1 protein or a variant or a portion thereof. In some embodiments of this third aspect, the soluble Cava α 2 δ -1 protein or a variant or a portion thereof comprises a fusion of the C-terminal region of an α 2 subunit of a Cava α 2 δ -1 protein to the N-terminal region of the δ subunit of the same Cava α 2 δ -1 protein. In some embodiments of this third aspect, the soluble Cava α 2 δ -1 protein or a variant or a portion thereof comprises, consists of, or consists essentially of the amino acid sequence of TYEDSFYKRSLDN. In some embodiments of this third aspect, the structure of the active binding region of the soluble Cava α 2 δ -1 protein or a variant or a portion thereof is stabilized. In some further embodiments of this aspect, the soluble Cava α 2 δ -1 protein or a variant or a portion thereof is a stapled protein or peptide or a circularized protein or peptide. In some embodiments of the third aspect, the nucleic acid encoding the soluble Cava α 2 δ -1 protein or a variant or a portion thereof is selected from an mRNA, a plasmid, and a vector. In some further embodiments of this aspect, the vector is selected from an adenoviral vector, an adeno-associated viral (AAV) vector, a retroviral vector, a lentiviral vector, and a herpes simplex viral vector. In some embodiments of this third aspect, the neurodevelopmental or psychiatric disorder is schizophrenia (e.g., a form of schizophrenia that is genetically linked or idiopathic schizophrenia). In some embodiments of this third aspect, the neurodevelopmental or psychiatric disorder is an autism spectrum disorder. In some embodiments of this third aspect, the composition is administered to the subject via injection. In some further embodiments of this aspect, the injection is an intrathecal injection, an intravenous injection, an intracerebroventricular injection, or an intracerebral injection. In some embodiments of this third aspect, a level of soluble Cava α 2 δ -1 protein is detected in the subject prior to administration of the soluble Cava α 2 δ -1 protein or a variant or portion thereof. In some embodiments of this third aspect, the level of soluble Cava α 2 δ -1 protein is detected in a cerebrospinal fluid (CSF) sample

from the subject. In some embodiments of this third aspect, the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is administered to the subject if the level of soluble Cav α 2 δ -1 protein detected in the subject is lower as compared to a similar sample from a subject without the neurodevelopmental or psychiatric disorder.

[0011] In a fourth aspect, the present disclosure provides a method of detecting a soluble Cav α 2 δ -1 protein, comprising obtaining a cerebrospinal fluid (CSF) sample from a subject having or suspected of having a neurodevelopmental or psychiatric disorder, and measuring a level of soluble Cav α 2 δ -1 protein in the CSF sample. In some embodiments of this fourth aspect, the neurodevelopmental or psychiatric disorder is schizophrenia. In some embodiments of this fourth aspect, the neurodevelopmental or psychiatric disorder is an autism spectrum disorder.

[0012] In a fifth aspect, the present disclosure provides a method of diagnosing a neurodevelopmental or psychiatric disorder, comprising collecting a biological sample from a subject; measuring a level of soluble Cav α 2 δ -1 protein in the biological sample; comparing the level of soluble Cav α 2 δ -1 protein in the sample from the subject to a reference level; and diagnosing the subject with the neurodevelopmental or psychiatric disorder when the level of soluble Cav α 2 δ -1 protein in the sample from the subject is less than the reference level. In some embodiments of this fifth aspect, the reference level corresponds to a level of soluble Cav α 2 δ -1 protein in a sample from an individual known to not have the disorder. In some embodiments of this fifth aspect, the neurodevelopmental or psychiatric disorder is schizophrenia. In some embodiments of this fifth aspect, the neurodevelopmental or psychiatric disorder is an autism spectrum disorder. In some embodiments of this fifth aspect, the biological sample is a cerebrospinal fluid (CSF) sample. In some embodiments of this fifth aspect, the levels of soluble Cav α 2 δ -1 protein are measured using liquid chromatography mass spectrometry (LC-MS), high-performance liquid chromatography, ELISA, protein immunoprecipitation, immunoelectrophoresis, western blot, or protein immunostaining. In some embodiments of this fifth aspect, the method further comprises administering to the subject a soluble Cav α 2 δ -1 protein or a variant or portion thereof when the level of soluble Cav α 2 δ -1 protein in the sample from the subject is less than the reference level. In some embodiments, the soluble Cav α 2 δ -1 protein or variant or portion thereof administered to the subject comprises, consists of, or consists essentially of the amino acid sequence of TYEDSFYKRSLDN.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1** depicts the shedding of ectodomains into the cerebrospinal fluid (CSF). **FIG. 1** shows a drawing of a membrane bound substrate that can be cleaved by a protease to generate a membrane-bound fragment and an ectodomain that is released into the extracellular fluid surrounding the neuron. **FIG. 1** further shows how cleavage of proteins bound to neurons can then enter the CSF.

[0014] **FIGS. 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, and 2L** illustrate the CSF quantitative proteomics experiments and results. **FIG. 2A** is an experimental schematic for multiplex tandem mass tag proteomics. For these experiments, 5 unaffected control CSF samples and 5 CSF samples from subjects with schizophrenia (SCZ) were digested with trypsin, labeled with tandem mass tag (TMT), and analyzed on liquid chromatography tandem mass spectrometry (LC-MS/MS). **FIG. 2B** is a volcano plot presenting the proteins significantly dysregulated in the SCZ CSF samples compared to samples from unaffected controls. **FIG. 2C** depicts the brain region specific expression analysis diagram, showing enrichment of differentially expressed proteins (DEPs) in SCZ CSF samples in Layer 5b and 6 cortical genes. Gray coloring indicates no statistically significant expression. **FIG. 2D** is the plot of pLi organized per DEPs. pLi scores are a depiction of the probability of being loss-of-function intolerant, where genes under strong selection have higher scores. In red are the pLi>0.9 genes that present transmembrane domains. **FIG. 2E** shows the 58 genes that overlap between the DEPs from the SCZ CSF samples and high confidence CSF brain-expressed proteins. **FIG. 2F** depicts the 58 proteins that were analyzed on SynGo, revealing that 3 proteins are present in both pre- and post-synaptic compartments. **FIG. 2G** depicts the overlap of genes that were enriched in the cortex, in pre- and post-synaptic neurons, and that had a pLi greater than 0.9. **FIG. 2H** illustrates the peptide mapping of $\alpha 2\delta$ -1, encoded by the CACNA2D1 gene, showing that only extracellular peptides were found in CSF. **FIG. 2I** shows the validation of $\alpha 2\delta$ -1 reduction in an independent group of individuals with early SCZ as compared to unaffected controls using western blot analysis (n = 26 healthy individuals, n = 26 SCZ individuals, unpaired t-test p = 0.0022). **FIG. 2J** show secondary validation of $\alpha 2\delta$ -1 reduction in SCZ CSF in independent post-mortem CSF samples between 7 unaffected controls and 11 SCZ samples using the ELISA method (*p<0.05). **FIG. 2K** shows a schematic representation of hippocampal soluble fraction

preparation and Western blot analysis of $\alpha 2\delta$ -1 in the soluble fraction of the hippocampus of mice in their home cage versus mice exposed to an enriched environment. The β -actin blot was used as a loading control. **FIG. 2L** depicts the quantification of the optical density of $\alpha 2\delta$ -1 normalized per the optical density of β -actin, showing an increase of soluble $\alpha 2\delta$ -1 in the hippocampus of mice exposed to enriched environments compared to the home cage ($n = 5$ mice per condition, t-test, $*p < 0.05$).

[0015] FIG. 3 depicts the brain region specific expression analysis for total proteins identified in the CSF samples from SCZ patients. Gray coloring indicates no statistically significant expression.

[0016] FIGS. 4A, 4B, 4C, 4D, and 4E show the experimental setup to test whether neural stress induces $\alpha 2\delta$ -1 shedding and the results of said experiments. **FIG. 4A** depicts the schematic representation of acute live cortical brain slice stimulation in a bath of high KCl (30 mM) or the GABA-A receptor antagonist Bicuculline (30 μ M). **FIG. 4B** shows the levels of soluble $\alpha 2\delta$ -1 in control brain slices and slices treated with KCl. **FIG. 4C** shows the levels of soluble $\alpha 2\delta$ -1 in control brain slices and those treated with Bicuculline. **FIG. 4D** depicts the level of soluble extracellular $\alpha 2\delta$ -1 as assessed by western blot when cells were treated with either the cAMP pathway activator Forskolin (FSK) or the GLPD1 inhibitor 1, 10 PNT. **FIG. 4E** shows the quantification of the optical density for the soluble $\alpha 2\delta$ -1 for samples treated with FSK and/or with 1, 10 PNT.

[0017] FIGS. 5A, 5B, 5C, 5D, 5E, 5F, 5G, 5H, 5I, and 5J depict the synthetic soluble $\alpha 2\delta$ -1 fusion protein ($\alpha 2\delta$ -1-Fc-His6) and experiments testing the localization of said fusion protein and the effects on protein expression. **FIG. 5A** shows a cartoon schematic of the synthetic $\alpha 2\delta$ -1-Fc-His6 design, tagged with the Fc fragment and 6xHis. **FIG. 5B** depicts a representative 3D reconstruction from confocal images of GFP cell fill, in gray, in excitatory neurons and PV+ neurons, along with the binding of $\alpha 2\delta$ -1-Fc-His6, in purple. **FIG. 5C** shows quantitation of $\alpha 2\delta$ -1-Fc-His6 puncta density per μm^3 for PV+ and excitatory neurons, which reveals preferential binding on Parvalbumin positive (PV+) dendrites ($n = 10$ neurons per condition, t-test, $**p < 0.01$). **FIG. 5D** shows the median expression of genes present in CSF in excitatory neurons vs. Parvalbumin interneurons, indicating an enrichment of DEPs in candidates for

excitatory to Parvalbumin neuron signaling. **FIG. 5E** depicts a cartoon illustrating the ChABC enzyme injection to digest the PNN unilaterally in the cortex of adult mice. **FIG. 5F** shows the representative 3D reconstruction of $\alpha 2\delta$ -1 presence in the PNN. **FIG. 5G** depicts the representative confocal images of immunostaining $\alpha 2\delta$ -1-Fc-His6 puncta binding to PV+ neurons, shown in gray, in the presence or absence of PNN following ChABC digestion. **FIG. 5H** shows the measurement of the average $\alpha 2\delta$ -1-Fc-His6 intensity around PV+ neurons ($n = 16$ -22 neurons, t-test, $P < 0.0001$). **FIG. 5I** depicts the immunostaining for Parvalbumin in brains fixed 6 hours after injection with 150 ng of $\alpha 2\delta$ -1-Fc-His6. **FIG. 5J** shows the number of cells expressing high levels of PV is increased on the $\alpha 2\delta$ -1-Fc-His6 -injected side compared to the control Fc-injected hippocampus ($n = 5$ mice, paired t-test, $**p < 0.01$).

[0018] FIG. 6 shows an illustration of the cortical microcircuit, with the PV+ GABAergic interneuron's axons targeting the pyramidal (Pyr) neurons and the soluble Cava $\alpha 2\delta$ -1 ectodomains localized near the dendrites of the PV+ interneurons.

[0019] FIGS. 7A, 7B, 7C, 7D, and 7E show the 3D structure of the $\alpha 2\delta$ -1 protein and localization of $\alpha 2\delta$ -1-Fc-His6 in mice brains. **FIG. 7A** is a rendering of the structure of the $\alpha 2\delta$ -1-Fc-His6 protein with conserved positively-charged motifs predicted to have high affinity for extracellular matrix protein surround inhibitory neurons called perineuronal net (PNN) depicted in red. **FIG. 7B** shows a schematic of the treatment of the mice brain live slice with $\alpha 2\delta$ -1-Fc-His6 or Fc control. **FIG. 7C** is a comparison of the localization of the control Fc (left panel) and $\alpha 2\delta$ -1-Fc-His6 (right panel) in red around neurons with the PNN in green. **FIG. 7D** shows a representative 3D reconstruction of $\alpha 2\delta$ -1-Fc-His6 localization in red with the PNN shown in green. **FIG. 7E** shows brain slices with the PNN shown in green and the PV+ neurons shown in red, with the brain slices treated with an enzyme degrading the PNN, Chondroitinase ABC (ChABC) in the left panel and with control BSA treatment in the right panel.

[0020] FIGS. 8A, 8B, 8C, 8D, 8E, 8F, 8G, 8H, 8I, 8J, 8K, 8L, and 8M illustrate the $\alpha 2\delta$ -1-Fc-His6 interaction experiments and results along with the effects of $\alpha 2\delta$ -1 on GABAergic interneurons. **FIG. 8A** shows schematics of the pulldown of cortical membrane proteins using agarose AG-beads coated with $\alpha 2\delta$ -1-Fc-His6. **FIG. 8B** is an illustration of the spectral count plot of ranked $\alpha 2\delta$ -1 membrane interactors with synaptic proteins highlighted. **FIG. 8C**

illustrates the SynGo top 10 significant biological synaptic compartments from the list of $\alpha 2\delta$ -1 interactors. **FIG. 8D** is representative super-resolution imaging of recombinant $\alpha 2\delta$ -1-Fc-His6 on PV+ interneuron dendrites in rat primary cortical cultures in vitro, with $\alpha 2\delta$ -1-Fc-His6 in magenta, VGLUT1 in cyan, and the dendrites in gray. **FIG. 8E** shows the schematics of line-scan intensity profiles for triplets of GFP- $\alpha 2\delta$ -1-VGLUT1. **FIG. 8F** depicts examples of normalized intensity profiles in line scans. Distances were measured from the maximum intensity locations for $\alpha 2\delta$ -1-Fc-His6 and VGLUT1 (value = 1), and the border of GFP selected by using the value 0.8. **FIG. 8G** shows the average distance between VGLUT1-GFP and $\alpha 2\delta$ -1-Fc-His6 ($n = 12$ neurons per condition, t-test, $p < 0.0001$). **FIG. 8H** is the representative 3D reconstructed image from a confocal stack of Parvalbumin immunostaining in vitro and surface GluA2 in cyan, showing increased sGluA2 density in PV+ dendrites incubated 24 hours with $\alpha 2\delta$ -1-Fc-His6 ($n = 27$ -31 neurons per condition, $**p < 0.01$). **FIG. 8I** is a representative image of Dlx-Gcamp6f neuron acquired on a widefield microscope. **FIG. 8J** shows representative spontaneous dendritic Ca^{2+} traces, with control Fc treatment in dark and $\alpha 2\delta$ -1-Fc-His6 treatment in blue. **FIG. 8K** illustrates the measurement of dendritic Ca^{2+} amplitude, revealing a potentiation of spontaneous dendritic activity after $\alpha 2\delta$ -1-Fc-His6 incubation ($n = 81/89$ neurons, t-test, $**p < 0.01$). **FIG. 8L** is the measurement of Ca^{2+} amplitude, showing no difference in somatic Ca^{2+} events amplitude ($n = 81/89$ neurons, t-test, n.s. [not significant]). **FIG. 8M** is the quantification of the number of VGLUT1 contacts with PV+ neurons for neurons treated with $\alpha 2\delta$ -1-Fc-His6 and Fc control, which indicates an increase in the number of contacts after treatment of $\alpha 2\delta$ -1.

[0021] FIGS. 9A, 9B, 9C, and 9D depict the effect of $\alpha 2\delta$ -1-Fc-His6 on somatic and dendritic calcium events in GABAergic interneurons. **FIG. 9A** shows the quantification of the number of dendritic Ca^{2+} events for Fc control samples and $\alpha 2\delta$ -1-Fc treated samples. **FIG. 9B** shows the quantification of the duration of dendritic Ca^{2+} events for Fc control samples and $\alpha 2\delta$ -1-Fc-His6 treated samples. **FIG. 9C** shows the quantification of the number of somatic Ca^{2+} events for Fc control samples and $\alpha 2\delta$ -1-Fc-His6 treated samples. **FIG. 9D** shows the quantification of the duration of somatic Ca^{2+} events for Fc control samples and $\alpha 2\delta$ -1-Fc-His6-treated samples.

[0022] FIGS. 10A, 10B, 10C, 10D, 10E, 10F, 10G, 10H, 10I, and 10J show experiments testing soluble $\alpha 2\delta$ -1 modulation of neuronal network dynamics. **FIG. 10A** shows representative Raster

plots of action potentials detected in the microelectrode array (MEA) from cortical neurons in culture exposed for 1 hour with $\alpha 2\delta$ -1-Fc-His6. **FIG. 10B** depicts MEA measurements of firing rate and neuronal network activity in culture, showing a reduction in the number of action potentials per network event after treatment with $\alpha 2\delta$ -1-Fc-His6 as compared to control (n = 23 wells per condition, t-test, **p<0.001, *p<0.05). **FIG. 10C** is representative images of AAV-Syn-GCAMP6 in green and AAV-Dlx-NLS-tdTomato-P2-hM4di, with red nuclear staining indicated with an arrow. **FIG. 10D** is examples traces of H4MDi silencing effects on Dlx-positive and -negative neurons, showing a reduction of interneuronal activity and increase of Dlx-negative neuron activity. **FIG. 10E** shows the percentage of co-active neurons per network event, which is reduced by $\alpha 2\delta$ -1-Fc-His6. This effect is rescued by silencing the activity of the interneurons (n = 8 coverslips per condition, Two-way ANOVA, posthoc Tukey, *p<0.05, ****p<0.0001, **p<0.01). **FIG. 10F** shows Ca²⁺ events amplitude reduction by $\alpha 2\delta$ -1-Fc-His6 and rescue by silencing the activity of the interneurons. **FIG. 10G** is a representative image of CA1 hippocampal neurons expressing GCAMP6f calcium indicator under the Thy1 promoter. **FIG. 10H** depicts representative traces of Ca²⁺ activity in control (Fc) and $\alpha 2\delta$ -1-Fc-His6 conditions. **FIG. 10I** shows soluble $\alpha 2\delta$ -1-induced reduction in spontaneous events rate in hippocampal acute slices (n = 158-186 neurons, Two-way ANOVA, posthoc Tukey, p<0.01), normalized by adding the GABA-A receptor antagonist Bicuculline (30 μ M) to the ACSF (n = 37-45 neurons, t-test, n.s.). **FIG. 10J** shows soluble $\alpha 2\delta$ -1-induced reduction in the amplitude of calcium events in hippocampal acute slices, which was normalized by adding Bicuculline to the ACSF.

[0023] FIGS. 11A, 11B, and 11C show the quantitation of the effects of $\alpha 2\delta$ -1-Fc-His6 on astrocytic calcium events. **FIG. 11A** is the quantitation of the astrocyte peak duration for Fc control samples and $\alpha 2\delta$ -1-Fc-His6-treated samples (labeled cav2d1 on the figure). **FIG. 11B** is the quantitation of the number of calcium evens in two minutes for Fc control samples and $\alpha 2\delta$ -1-Fc-His6-treated samples (labeled cav2d1 on the figure). **FIG. 11C** is the quantitation of the peak amplitude for Fc control samples and $\alpha 2\delta$ -1-Fc-His6-treated samples (labeled cav2d1 on the figure). No significant effects were observed in astrocytes in any of their calcium activity metrics.

[0024] **FIG. 12** shows a western blot comparing levels of soluble $\alpha 2\delta$ -1 protein in CSF collected from wild-type and 16p11.2 duplicate mice.

[0025] **FIGS. 13A, 13B, 13C, 13D, 13E, and 13F** depict experiments testing the effects of soluble $\alpha 2\delta$ -1 injection in a mouse model of 16p11.2 duplication syndrome. **FIG. 13A** shows representative images of Parvalbumin staining in magenta and Hoechst in cyan for nuclei on the ACC region of wild-type (+/+) and 16p11.2 (dup/+) brains treated with Fc or $\alpha 2\delta$ -1-Fc-His6. **FIG. 13B** is quantitation of high expressing PV+ neuron density, revealing a reduction in the density on the 16p11.2 duplicate mice and rescue to wild-type levels by treatment with $\alpha 2\delta$ -1-Fc-His6 (n = 5 mice per condition, each point represents the average data from 3 slices, ANOVA, posthoc Tukey, *p<0.05, **p<0.001). **FIG. 13C** shows schematics of the $\alpha 2\delta$ -1-Fc-His6 *in vivo* injection on the ACC of 16p11.2 duplicate mice and following behavioral and immunohistochemical assays. **FIG. 13D** is a heatmap of the time spent by the mice in the different zones of the three chambers. **FIG. 13E** shows the preference index in male (left panel) and female (right panel) 16p11.2 duplicate mice and the effect of $\alpha 2\delta$ -1-Fc-His6 treatment (ANOVA, posthoc Tukey, ****p<0.0001). **FIG. 13F** shows the novel object preference index rescue in 16p11.2 duplicate mice by the $\alpha 2\delta$ -1-Fc-His6 treatment (ANOVA, posthoc Tukey, *p<0.05, ***p<0.001).

[0026] **FIGS. 14A, 14B, 14C, 14D, and 14E** depicts the effects of $\alpha 2\delta$ -1-Fc-His6 treatment on basal locomotion. **FIG. 14A** shows the traces of mouse locomotion for wild-type mice injected with the vehicle (left panel), for 16p11.2 duplicate mice injected with the vehicle (middle panel), and for 16p11.2 duplicate mice injected with $\alpha 2\delta$ -1-Fc (right panel). **FIG. 14B** shows quantitation of the percentage of time the mice spent in the center of the cage for the three conditions. **FIG. 14C** is the quantitation of the distance traveled by the mice for the three conditions. **FIG. 14D** illustrates the y-maze experimental set up. **FIG. 14E** shows the quantitation of the percent alternations for the mice to determine the willingness of the mice to explore new environments for the three conditions.

[0027] **FIGS. 15A, 15B, 15C, 15D, 15E, 15F, and 15G** depict the experiments to generate and validate the peptide fragment of soluble Cava $\alpha 2\delta$ -1. **FIG. 15A** shows the comparison of the normalized firing rate measured from rat cortical neurons on a Multi Electrode Array treated

with the various peptide fragments of soluble Cav α 2 δ -1 generated. **FIG. 15B** is the dose response curve of peptide 2. **FIG. 15C** shows the data of the normalized firing rate of neurons treated with the peptide 2, with neurons treated with peptide 2 illustrated as green dots, red squares and blue triangles, each puncta representing a culture well from 3 independent experiments. **FIG. 15D** shows somatic calcium signaling in neurons treated with peptide 2, with calcium visualized with Syn-GCamp6f in green. **FIG. 15E** shows the amplitude for the calcium imaging for neurons treated with a control and with peptide 2. **FIG. 15F** is the quantification of the data in **FIG. 15E**. **FIG. 15G** shows the pairwise correlation for neurons treated with a control and with peptide 2.

DETAILED DESCRIPTION

[0028] The present disclosure provides a novel peptide and approach to treating neurodevelopmental or psychiatric diseases. As shown herein, treatment with soluble Cav α 2 δ -1 protein or a novel peptide derived from the binding domain of the soluble Cav α 2 δ -1 protein is sufficient to bind to interneurons and their synapses to modulate neuronal network dynamics and alter negative and cognitive symptoms of neurodevelopmental or psychiatric diseases.

[0029] Some neuronal and synaptic proteins are known to undergo ectodomain shedding, which is a general biological process by which many membrane proteins are cleaved by proteases called sheddases (**FIG. 1**). This cleavage results in the release of soluble extracellular fragments of the membrane-associated protein, or ectodomains, into the interstitial fluid within the brain and into the cerebrospinal fluid (CSF) (**FIG. 1**). Global unbiased proteomic analyses of the human CSF (hCSF) have defined the synaptic sheddome, which is the totality of the shed ectodomains originating from synaptic proteins detectable in the CSF. While the shedding of most ectodomains results in termination of activity, a few soluble shed ectodomains are known to diffuse away from the site of origin and may act as paracrine signals.

[0030] By comparing the ectodomains present in CSF samples from subjects known to have schizophrenia with samples from subjects without the disorder, soluble Cav α 2 δ -1 found to be significantly reduced in the CSF from afflicted subjects. Further studies uncovered a novel role for soluble Cav α 2 δ -1 as a treatment for neuron dysfunction and cognitive impairments associated with neurodevelopmental or psychiatric disorders.

[0031] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology. It is to be understood that the present disclosure is not limited to particular uses, methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein for the purpose of describing particular embodiments only and is not intended to be limiting.

I. Definitions

[0032] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, reference to a “a cell” includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[0033] As used herein, “about” when used with a numerical value means the numerical value stated as well as plus or minus 10% of the numerical value (except where such number would be less than 0% or exceed 100% of a possible value). For example, “about 10” should be understood as both “10” and “9-11.”

[0034] As used herein, the “administration” of or “administering” an agent to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically. Administration includes self-administration and the administration by another.

[0035] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount which results in the prevention of,

or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein.

[0036] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) relieving a disease or disorder, i.e., causing regression of the disorder; (ii) slowing progression of the disorder; and/or (iii) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, e.g., alleviated, reduced, cured, or placed in a state of remission.

[0037] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[0038] As used herein, the term “peptide” refers to a polymer of amino acid residues joined by amide linkages, which may optionally be chemically modified to achieve desired characteristics. The term “amino acid residue,” includes but is not limited to amino acid residues contained in the group consisting of alanine (Ala or A), cysteine (Cys or C), aspartic acid (Asp or D), glutamic acid (Glu or E), phenylalanine (Phe or F), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), lysine (Lys or K), leucine (Leu or L), methionine (Met or M), asparagine (Asn or N), proline (Pro or P), glutamine (Gln or Q), arginine (Arg or R), serine (Ser or S), threonine (Thr or T), valine (Val or V), tryptophan (Trp or W), and tyrosine (Tyr or Y) residues.

The term “amino acid residue” also may include unnatural amino acids or residues contained in the group consisting of homocysteine, 2-Aminoadipic acid, N-Ethylasparagine, 3-Aminoadipic acid, Hydroxylysine, β -alanine, β -Amino-propionic acid, allo-Hydroxylysine acid, 2-Aminobutyric acid, 3-Hydroxyproline, 4-Aminobutyric acid, 4-Hydroxyproline, piperidinic acid, 6-Aminocaproic acid, Isodesmosine, 2-Aminoheptanoic acid, allo-Isoleucine, 2-Aminoisobutyric acid, N-Methylglycine, sarcosine, 3-Aminoisobutyric acid, N-Methylisoleucine, 2-Aminopimelic acid, 6-N-Methyllysine, 2,4-Diaminobutyric acid, N-Methylvaline, Desmosine, Norvaline, 2,2'-Diaminopimelic acid, Norleucine, 2,3-Diaminopropionic acid, Ornithine, and N-Ethylglycine. Typically, the amide linkages of the peptides are formed from an amino group of the backbone of one amino acid and a carboxyl group of the backbone of another amino acid.

[0039] The terms “protein,” “peptide,” “polypeptide,” and “amino acid sequence” are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymers may be linear or branched. The polymers may comprise modified amino acids or amino acid analogs and may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

[0040] The term “recombinant” with reference to a nucleic acid or polypeptide refers to one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. A recombinant polypeptide may also refer to a polypeptide that has been made using recombinant nucleic acids, including recombinant nucleic acids transferred to a host organism that is not the natural source of the polypeptide. The term “recombinant” when used with reference to a cell, virus, or vector indicates that the cell, virus, or vector has been modified by or is the result of laboratory methods. A recombinant cell, virus, or vector can include a cell, virus, or vector that has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein. Thus, for example, recombinant cells include cells that

express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed, or not expressed at all.

[0041] As used herein, the terms “subject,” “patient,” or “individual” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient or individual is a human.

[0042] A “fragment” is a portion of an amino acid sequence or a polynucleotide which is identical in sequence to but shorter in length than a reference sequence. A fragment may comprise up to the entire length of the reference sequence, minus at least one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or contiguous amino acid residues of a reference polynucleotide or reference polypeptide, respectively. In some embodiments, a fragment may comprise at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous amino acid residues of a reference peptide, respectively. Fragments may be preferentially selected from certain regions of a molecule. The term encompasses the full-length polynucleotide or full-length polypeptide.

[0043] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same nucleobase or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A polypeptide or polypeptide region has a certain percentage (for example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In some embodiments, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, program is BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by =HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS

translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the National Center for Biotechnology Information. Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same or similar biological activity. Two sequences are deemed “unrelated” or “non-homologous” if they share less than 40% identity, or less than 25% identity, with each other.

II. Recombinant Cava α 2 δ -1 Protein and s α 2 δ -1-derived Peptide

[0044] The Cava α 2 δ -1 or α 2 δ -1 protein is a calcium channel subunit that is encoded by the CACNA2D1 gene. There are four known α 2 δ proteins, all of which are attached to the intracellular β subunit of the calcium channel via the transmembrane α subunit. The four different α 2 δ isoforms are expressed selectively in different tissues, with the α 2 δ -1 protein known to be present in neurons, axonal terminals, and dendrites.

[0045] Cava α 2 δ -1 was identified in the hCSF samples as an ectodomain that is shed from the synapses. Levels of soluble Cava α 2 δ -1 (s α 2 δ -1), or Cava α 2 δ -1 ectodomains that have been shed and are present in the CSF samples, increase following increased neuronal activity or environmental enrichment. hCSF samples from subjects with schizophrenia contained reduced levels of s α 2 δ -1.

[0046] Disclosed herein is a recombinant Cava α 2 δ -1 protein derived from the s α 2 δ -1 protein. The recombinant Cava α 2 δ -1 protein may be a fusion protein. The fusion protein may include a fusion between the C-terminal region of an α 2 subunit of a Cava α 2 δ -1 protein and the N-terminal region of the δ subunit of the same Cava α 2 δ -1 protein. This novel fusion occurs at the region where the subunits are naturally joined by disulfide bonds. The recombinant Cava α 2 δ -1 protein may be a fragment of the whole Cava α 2 δ -1 protein. The recombinant Cava α 2 δ -1 protein may also include additional tags. The additional tags may include an Fc tag. The additional tags may include a polyhistidine (His) tag. The recombinant Cava α 2 δ -1 protein may include the amino acid sequence TYEDSFYKRSLDN. The recombinant Cava α 2 δ -1 protein may exhibit similar localization and binding abilities as s α 2 δ -1 protein.

[0047] Disclosed herein is a peptide derived from the s α 2 δ -1 protein. The s α 2 δ -1-derived peptide may include the amino acid sequence TYEDSFYKRSLDN. The s α 2 δ -1-derived peptide

may exhibit similar localization and binding abilities as the recombinant Cava2 δ -1 protein or the sa2 δ -1 protein.

[0048] The recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may exhibit preferential binding to the PV+ neurons. The recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may bind to the perineuronal nets surrounding the PV+ neurons. The recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may induce the surface density of GluA2 on PV+ neurons and increase the intensity of dendritic Ca²⁺ peaks within PV+ neurons. The recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may reduce the number of co-active neurons within the neural circuit of the PV+ neuron targeted by the recombinant Cava2 δ -1 protein or sa2 δ -1-derived peptide. The recombinant Cava2 δ -1 protein and the sa2 δ -1-derived peptide may reduce the spontaneous event rates in brain slices or whole brains. The recombinant Cava2 δ -1 or the sa2 δ -1-derived peptide may be capable of mitigating negative or cognitive consequences of a neurodevelopmental or psychiatric disorder.

[0049] The recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may include features to enhance stability. The stabilized recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may be a stapled peptide. The stapled peptide may include one or more staple or synthetic brace. The staples or synthetic braces may stabilize or force the active binding region of the recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide into an alpha helical structure. The staples or synthetic braces may be attached at or near the active binding region of the recombinant Cava2 δ -1 protein or sa2 δ -1-derived peptide. The staples or synthetic braces may not interfere with the activity of the recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide. The staples or synthetic braces may be prepared via ring closing metathesis, copper catalyzed azide alkyne cycloaddition, lactamization reaction, cysteine-xylene stapling, cysteine-perfluorobenzene stapling, thiol-yne/-ene click chemistry, selenocysteine stapling, tryptophan condensation, C-H activation, triazole-stapling, or any alternative method of peptide stapling. The stabilized recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide may be a circularized protein. The circularized protein may include a connector. The connector may be an engineered polypeptide segment that connects the C-terminus of the recombinant Cava2 δ -1 protein or the sa2 δ -1-derived peptide with its N-terminus. The connector may be optimized for conformational stability of the active binding region of the Cava2 δ -1 protein or the sa2 δ -1-

derived peptide. The circularized protein may be prepared utilizing intein-mediated protein cyclization, transpeptidase-mediated protein cyclization, side chain-mediated cyclization, or any alternative method of peptide circularization. The circularized protein may not exhibit interference with the active binding region of the recombinant Cav α 2 δ -1 or s α 2 δ -1-derived peptide. The circularized protein may exhibit increased blood-brain barrier permeability when administered to a subject.

III. Composition to Treat Neurodevelopmental or Psychiatric Disorder

[0050] Disclosed herein is a pharmaceutical composition to treat neurodevelopmental or psychiatric disorders. The pharmaceutical composition may include a s α 2 δ -1 protein or a variant or portion thereof and a pharmaceutically acceptable carrier. The s α 2 δ -1 protein may be a recombinant protein. The recombinant protein may be a fusion protein. The fusion protein may include a fusion of the C-terminal region of an α 2 subunit of a Cav α 2 δ -1 protein and the N-terminal region of the δ subunit of the same Cav α 2 δ -1 protein. The portion of the Cav α 2 δ -1 protein may be a peptide with the amino acid sequence of TYEDSFYKRSLDN. The active binding region of the s α 2 δ -1 protein or variant or portion thereof may be stabilized. The stabilized s α 2 δ -1 protein may be a stapled protein or peptide. The stapled peptide may include one or more staple or synthetic brace. The staples or synthetic braces may stabilize or force the active binding region of the recombinant Cav α 2 δ -1 protein or the s α 2 δ -1-derived peptide into an alpha helical structure. The staples or synthetic braces may be attached at or near the active binding region of the recombinant Cav α 2 δ -1 protein or s α 2 δ -1-derived peptide. The staples or synthetic braces may not interfere with the activity of the recombinant Cav α 2 δ -1 protein or the s α 2 δ -1-derived peptide. The staples or synthetic braces may be prepared via ring closing metathesis, copper catalyzed azide alkyne cycloaddition, lactamization reaction, cysteine-xylylene stapling, cysteine-perfluorobenzene stapling, thiol-yne/-ene click chemistry, selenocysteine stapling, tryptophan condensation, C-H activation, triazole-stapling, or any alternative method of peptide stapling. The stabilized s α 2 δ -1 protein may be a circularized protein or peptide. The circularized protein may include a connector. The connector may be an engineered polypeptide segment that connects the C-terminus of the recombinant Cav α 2 δ -1 protein or the s α 2 δ -1-derived peptide with its N-terminus. The connector may be optimized for conformational stability of the active binding region of the Cav α 2 δ -1 protein or the s α 2 δ -1-derived peptide. The circularized

protein may be prepared utilizing intein-mediated protein cyclization, transpeptidase-mediated protein cyclization, side chain-mediated cyclization, or any alternative method of peptide circularization. The circularized protein may not exhibit interference with the active binding region of the recombinant Cav α 2 δ -1 or s α 2 δ -1-derived peptide. The pharmaceutical composition may be formulated as an injectable.

[0051] Formulations of the present disclosure include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal 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 active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the disclosed Cav α 2 δ -1 proteins/polypeptides and fragments thereof which produces a therapeutic effect.

[0052] Formulations of the disclosed Cav α 2 δ -1 proteins/polypeptides and fragments thereof may include, but are not limited to, one or more solvents, such as an organic phosphate-based solvent, bulking agents, coloring agents, pharmaceutically acceptable excipients, a preservative, pH adjuster, buffer, chelating agent, etc. The additional compounds can be admixed into a previously formulated composition, or the additional compounds can be added to the original mixture to be further formulated.

[0053] Suitable preservatives in the disclosed composition include, but are not limited to, cetylpyridinium chloride, benzalkonium chloride, benzyl alcohol, chlorhexidine, imidazolidinyl urea, phenol, potassium sorbate, benzoic acid, bronopol, chlorocresol, paraben esters, phenoxyethanol, sorbic acid, alpha-tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, sodium ascorbate, sodium metabisulphite, citric acid, edetic acid, semi-synthetic derivatives thereof, and combinations thereof. Other suitable preservatives include, but are not limited to, benzyl alcohol, chlorhexidine (bis (p-chlorophenyldiguanido) hexane), chlorphenesin (3-(-4-chlorophenoxy)-propane-1,2-diol), Kathon CG (methyl and methylchloroisothiazolinone), parabens (methyl, ethyl, propyl, butyl

hydrobenzoates), phenoxyethanol (2-phenoxyethanol), sorbic acid (potassium sorbate, sorbic acid), Phenonip (phenoxyethanol, methyl, ethyl, butyl, propyl parabens), Phenoroc (phenoxyethanol 0.73%, methyl paraben 0.2%, propyl paraben 0.07%), Liquipar Oil (isopropyl, isobutyl, butylparabens), Liquipar PE (70% phenoxyethanol, 30% liquipar oil), Nipaguard MPA (benzyl alcohol (70%), methyl & propyl parabens), Nipaguard MPS (propylene glycol, methyl & propyl parabens), Nipasept (methyl, ethyl and propyl parabens), Nipastat (methyl, butyl, ethyl and propyl parabens), Elestab 388 (phenoxyethanol in propylene glycol plus chlorphenesin and methylparaben), and Killitol (7.5% chlorphenesin and 7.5% methyl parabens).

[0054] The disclosed composition may further comprise at least one pH adjuster. Suitable pH adjusters in the disclosed composition include, but are not limited to, diethanolamine, lactic acid, monoethanolamine, triethanolamine, sodium hydroxide, sodium phosphate, semi-synthetic derivatives thereof, and combinations thereof.

[0055] In addition, the disclosed composition can comprise a chelating agent. In one embodiment of the disclosed, the chelating agent is present in an amount of about 0.0005% to about 1%. Examples of chelating agents include, but are not limited to, ethylenediamine, ethylenediaminetetraacetic acid (EDTA), phytic acid, polyphosphoric acid, citric acid, gluconic acid, acetic acid, lactic acid, and dimercaprol, and a preferred chelating agent is ethylenediaminetetraacetic acid.

[0056] The neurodevelopmental or psychiatric disorder treated by any composition disclosed herein may be schizophrenia. The form of schizophrenia may be genetic and associated with a known mutation or genotype that increases the risk of disorder manifestation. The form of schizophrenia may be idiopathic and not associated with a known mutation or genotype that increases the risk of disorder manifestation. The neurodevelopmental or psychiatric disorder treated by any of the methods disclosed herein may be an autism spectrum disorder.

IV. Method of Treating a Subject with a Neurodevelopmental or Psychiatric Disorder

[0057] Disclosed herein is a method of treating a subject with a neurodevelopmental or psychiatric disorder. The method may include administering to the subject a $\alpha 2\delta$ -1 protein or a variant or portion thereof. The $\alpha 2\delta$ -1 protein or variant or portion thereof administered to the

patient may be a recombinant protein. The recombinant protein may be a fusion protein. The fusion protein may include a fusion of the C-terminal region of an $\alpha 2$ subunit of a Cav $\alpha 2\delta$ -1 protein and the N-terminal region of the δ subunit of the same Cav $\alpha 2\delta$ -1 protein. The portion of the $\alpha 2\delta$ -1 protein administered to the subject may be a peptide with the amino acid sequence of TYEDSFYKRSLDN.

[0058] The active binding region of the $\alpha 2\delta$ -1 protein or variant or portion thereof may be stabilized. The stabilized $\alpha 2\delta$ -1 protein may be a stapled protein or peptide. The stapled peptide may include one or more staple or synthetic brace. The staples or synthetic braces may stabilize or force the active binding region of the recombinant Cav $\alpha 2\delta$ -1 protein or the $\alpha 2\delta$ -1-derived peptide into an alpha helical structure. The staples or synthetic braces may be attached at or near the active binding region of the recombinant Cav $\alpha 2\delta$ -1 protein or $\alpha 2\delta$ -1-derived peptide. The staples or synthetic braces may not interfere with the activity of the recombinant Cav $\alpha 2\delta$ -1 protein or the $\alpha 2\delta$ -1-derived peptide. The staples or synthetic braces may be prepared via ring closing metathesis, copper catalyzed azide alkyne cycloaddition, lactamization reaction, cysteine-xylene stapling, cysteine-perfluorobenzene stapling, thiol-yne/-ene click chemistry, selenocysteine stapling, tryptophan condensation, C-H activation, triazole-stapling, or any alternative method of peptide stapling. The stabilized $\alpha 2\delta$ -1 protein may be a circularized protein or peptide. The circularized protein may include a connector. The connector may be an engineered polypeptide segment that connects the C-terminus of the recombinant Cav $\alpha 2\delta$ -1 protein or the $\alpha 2\delta$ -1-derived peptide with its N-terminus. The connector may be optimized for conformational stability of the active binding region of the Cav $\alpha 2\delta$ -1 protein or the $\alpha 2\delta$ -1-derived peptide. The circularized protein may be prepared utilizing intein-mediated protein cyclization, transpeptidase-mediated protein cyclization, side chain-mediated cyclization, or any alternative method of peptide circularization. The circularized protein may not exhibit interference with the active binding region of the recombinant Cav $\alpha 2\delta$ -1 or $\alpha 2\delta$ -1-derived peptide.

[0059] The neurodevelopmental or psychiatric disorder treated by any of the methods disclosed herein may be schizophrenia. The form of schizophrenia may be genetic and associated with a known mutation or genotype that increases the risk of disorder manifestation. The form of schizophrenia may be idiopathic and not associated with a known mutation or genotype that

increases the risk of disorder manifestation. The neurodevelopmental or psychiatric disorder treated by any of the methods disclosed herein may be an autism spectrum disorder.

[0060] Any of the methods disclosed herein may involve administering the $\alpha 2\delta$ -1 protein or variant or portion thereof to the subject via injection. The method of administration may be via intrathecal injection, intravenous injection, intracerebroventricular injection, or intracerebral injection.

[0061] Any of the methods disclosed herein may involve administering a nucleic acid encoding the $\alpha 2\delta$ -1 protein or variant or portion thereof to the subject. The nucleic acid is selected from an mRNA, a plasmid, and a vector. When the nucleic acid is in the form of a vector, the vector may be selected from an adenoviral vector, an adeno-associated viral (AAV) vector, a retroviral vector, a lentiviral vector, and a herpes simplex viral vector. When the vector is an AAV vector, the AAV vector may be an AAV type 2.1 vector.

[0062] Any of the methods disclosed herein may involve administering an effective amount of the $\alpha 2\delta$ -1 protein or variant or portion thereof to the subject. The $\alpha 2\delta$ -1 protein or variant or portion thereof may be administered to the patient in a single dose. The $\alpha 2\delta$ -1 protein or variant or portion thereof may be administered to the patient in two, three, four, or more doses. The $\alpha 2\delta$ -1 protein or variant or portion thereof may be administered to the patient at specific time intervals.

[0063] Any of the methods disclosed herein may involve detecting a level of endogenous $\alpha 2\delta$ -1 protein in the subject prior to the administration of the $\alpha 2\delta$ -1 protein or variant or portion thereof. The level of the endogenous $\alpha 2\delta$ -1 protein in the subject may be detected in a CSF sample from the subject. Any of the methods disclosed herein may involve administering the $\alpha 2\delta$ -1 protein or variant or portion thereof to the subject if the level of endogenous $\alpha 2\delta$ -1 protein detected in the subject is lower as compared to a similar sample from a subject without the neurodevelopmental or psychiatric disorder. For all the above-described methods, optimal doses and routes of administration may vary.

V. Method of Detecting a Soluble Cava2δ-1 Protein

[0064] Disclosed herein are the methods for detecting a $\alpha 2\delta$ -1 protein. The present disclosure provides a method of detecting a $\alpha 2\delta$ -1 protein, including obtaining a CSF sample from a subject having or suspected of having a neurodevelopmental or psychiatric disorder and measuring a level of $\alpha 2\delta$ -1 protein in the CSF sample.

[0065] The neurodevelopmental or psychiatric disorder may be schizophrenia. The form of schizophrenia may be genetic and associated with a known mutation or genotype that increases the risk of disorder manifestation. The form of schizophrenia may be idiopathic and not associated with a known mutation or genotype that increases the risk of disorder manifestation. The neurodevelopmental or psychiatric disorder may be an autism spectrum disorder.

[0066] The levels of $\alpha 2\delta$ -1 protein in the samples can be measured via liquid chromatography mass spectrometry (LC-MS/MS), high-performance liquid chromatography, ELISA, protein immunoprecipitation, immunoelectrophoresis, western blot, single-molecule augmented capture (SMAC), protein immunostaining, or any alternative protein detection method.

[0067] Previously, Cava2δ-1 was not known to undergo shedding. However, as disclosed herein, soluble forms of Cava2δ-1 were detected in CSF samples from subjects with and without neurodevelopmental or psychiatric disorders. The soluble forms of Cava2δ-1 detected in CSF samples lacked the transmembrane and intracellular peptides found in the canonical $\alpha 2\delta$ -1 protein.

[0068] Any of the methods disclosed herein may be utilized to monitor synapse health by analyzing the synaptic ectodomains in the CSF. The ectodomain may be $\alpha 2\delta$ -1 protein.

[0069] Any of the methods disclosed herein may be utilized using biological samples other than CSF, including, but not limited to, blood samples or nasal biopsy samples.

VI. Method of Diagnosing Neurodevelopmental or Psychiatric Disorder in a Subject

[0070] Disclosed herein are the methods for diagnosing a subject with a neurodevelopmental or psychiatric disorder. The present disclosure provides a method of diagnosis a subject with a neurodevelopmental or psychiatric disorder, including collecting a biological sample from a

subject; measuring a level of $\alpha 2\delta$ -1 protein in the biological sample; comparing the level of $\alpha 2\delta$ -1 protein in the sample from the subject to a reference level; and diagnosing the subject with the neurodevelopmental or psychiatric disorder when the level of $\alpha 2\delta$ -1 protein in the sample from the subject is less than the reference level. The disclosed methods are in vitro methods that can be performed on a biological sample taken from or obtained from a subject (i.e., an individual suspected of having a neurodevelopmental or psychiatric disorder).

[0071] Based on the disclosure herein, levels of $\alpha 2\delta$ -1 may act as a biomarker for neurodevelopmental or psychiatric disorders, including schizophrenia. There are few reliable biomarkers for schizophrenia and other disorders.

[0072] The reference level may correspond to a level of $\alpha 2\delta$ -1 protein in a biological sample from an individual known to not have the neurodevelopmental or psychiatric disorder. The neurodevelopmental or psychiatric disorder may be schizophrenia. The form of schizophrenia may be genetic and associated with a known mutation or genotype that increases the risk of disorder manifestation. The form of schizophrenia may be idiopathic and not associated with a known mutation or genotype that increases the risk of disorder manifestation. The neurodevelopmental or psychiatric disorder treated by any of the methods disclosed herein may be an autism spectrum disorder.

[0073] The biological sample may be a cerebrospinal fluid (CSF) sample. CSF is a biological fluid that surrounds the brain and spinal cord and is in open communication with the interstitial fluid that surrounds neurons and synapses. The CSF can be accessed in live humans through a spinal tap, which offers a more convenient, safer, and less invasive readout of normal and pathophysiological processes in live human brains and, therefore, possible avenues for diagnosing subjects with neurodevelopmental or psychiatric disorders. The biological sample may also be a blood sample and a nasal biopsy sample.

[0074] The levels of $\alpha 2\delta$ -1 protein in the samples can be measured via liquid chromatography mass spectrometry (LC-MS/MS), high-performance liquid chromatography, ELISA, protein immunoprecipitation, immunoelectrophoresis, western blot, protein immunostaining, or any alternative protein detection method.

[0075] Any of the methods disclosed herein may further comprise administering to the subject a $\alpha 2\delta$ -1 protein or a variant or portion thereof when the level of $\alpha 2\delta$ -1 protein in the sample from the subject is less than the reference level. Any of the methods disclosed herein may include use of the $\alpha 2\delta$ -1 protein that includes the amino acid sequence TYEDSFYKRSLDN.

[0076] The methods disclosed herein may also be utilized to determine whether a candidate subject would be expected to respond well to the treatment with $\alpha 2\delta$ -1, as described above.

EXAMPLES

[0077] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way.

[0078] Example 1: A CSF-Derived Mechanism and Therapeutic Strategy in Psychiatric Disorders

[0079] *Introduction*

[0080] The cerebrospinal fluid (CSF) is a biological fluid that surrounds the brain and the spinal cord. It is in open communication with the interstitial fluid that surrounds neurons and synapse, and it contains small molecules and proteins derived from the brain and glial cells. Most importantly, the CSF can be accessed in live humans through a spinal tap, offering the potential for a readout of normal and pathophysiological processes in the brain of living humans.

[0081] Some neuronal and synaptic proteins undergo ectodomain shedding, which is a general biological process in which many membrane proteins are cleaved by proteases called sheddases, resulting in the release of a soluble extracellular fragment, called the ectodomain. Global unbiased proteomic analysis of the human CSF has defined the “synaptic sheddome,” or the totality of shed ectodomains originating from synaptic proteins detectable in the CSF. In most cases, shedding results in the termination of activity of the ectodomains. However, there are a few examples where soluble, shed ectodomains diffuse away from the site of origin and may act as paracrine signals.

[0082] Because the CSF communicates with the brain milieu and some synaptic ectodomains can be detected in the human CSF, the Applicants asked whether synaptic sheddomes are altered

in psychiatric disorders, and if so, whether such altered synaptic ectodomains can provide insight into basic and pathological brain processes.

[0083] To answer these questions, the Applicants compared the synaptic sheddomes of schizophrenia (SCZ) patients with unaffected controls. Using quantitative proteomics, the Applicants found that ectodomains of several synaptic proteins were reduced in human CSF (hCSF) of SCZ patients. Among these, the $\alpha 2\delta$ subunits of voltage-gated Ca^{2+} channels, previously unknown to undergo shedding, were prominent. Neuronal activity and environmental enrichment increased Cava $\alpha 2\delta$ -1 shedding. A recombinant form of soluble Cava $\alpha 2\delta$ -1 (sa $\alpha 2\delta$ -1) binds preferentially to parvalbumin positive (PV+) interneurons and their synapses and interacts with pre- and post-synaptic proteins. Treatment with recombinant sa $\alpha 2\delta$ -1 increases PV+ neuron activity, GluA2 synaptic content, and PV protein levels. Through this action, sa $\alpha 2\delta$ -1 modulates neuronal network dynamics, revealing a novel function of $\alpha 2\delta$ 1 as an activity-regulated paracrine factor that regulates synapses and circuits. Finally, the Applicants provide evidence that introducing altered sa $\alpha 2\delta$ -1 can normalize anatomical and behavioral endpoints in a mouse model of 16p11.2 duplication disorder, a neurodevelopmental condition associated with a 14-fold increase in SCZ risk. Taken together, it appears that synaptic ectodomains altered in patients' CSF can uncover novel mechanisms and therapies.

[0084] *Results*

[0085] To explore the CSF proteome in SCZ patients, a multiplexed tandem mass tag (TMT) quantitative proteomics method was used to measure the relative abundance of approximately 1,400 proteins in CSF samples from individuals with SCZ and unaffected controls (**FIG. 2A**). The analysis revealed a reduction in the levels of several synaptic proteins and an increase in IgG levels in the SCZ-CSF samples compared to controls (**FIG. 2B**). As CSF proteins can originate in various regions of the brain, the putative origin of the CSF differentially expressed proteins (DEPs) was retraced in SCZ samples using the Cell-Type Specific Expression Analysis (CSEA) tool. This analysis showed that, whereas total CSF proteins have widespread brain origins, DEPs in SCZ samples are enriched in deep layers (L5 and L6) cortical neuron genes (**FIGS. 2C and 3**).

[0086] To unbiasedly identify DEPs that may be important for brain function, proteins expressed by genes under high constraints ($p\text{Li} > 0.9$) were examined using the Genome Aggregation

Database Consortium (GnomAD) database (**FIG. 2D**). pLi scores are a depiction of the probability of being loss-of-function intolerant, where genes under strong selection have higher scores. Interestingly, total CSF proteins are already significantly enriched in the high pLi category compared to the whole genome (hypergeometric test, enrichment 1.25, $p=0.0370$). However, DEPs in SCZ CSF showed an even greater enrichment for $pLi>0.9$ (enrichment 1.4, $p<0.0001$) (**FIGS. 2D-2E**). Finally, the DEPs originating from plasma membranes, called the “SCZ-sheddome,” represented the highest enrichment in $pLi>0.9$ genes among the three categories (enrichment 2.2, $p = 0.0051$) (**FIG. 2D**). The DEPs identified in the SCZ CSF samples were compared to genes that had a high confidence of being expressed in the human brain to identify genes that may be relevant for brain function, identifying 58 genes that were in both categories (**FIG. 2E**). SynGo analysis, using a public knowledge base for synapse research based on gene ontologies and gene or protein annotations, further showed enrichment for post-synaptic genes in the SCZ-sheddome (**FIG. 2F**). These results suggested that CSF proteins shed and released from the plasma membrane may be the ones with the highest functional impacts on brain and synapse physiology.

[0087] By combining the proteomic analysis with public available single-cell RNA sequencing data obtained from post-mortem human cortexes, DEPs in SCZ CSF were found to be present in genes expressed by pyramidal neurons (Pyr) and absent in PV+ cells, suggesting that these proteins may play a role in mediating Pyr neuron to PV+ neuron signaling, as observed with other proteins such as neuronal pentraxins.

[0088] Remarkably, only one gene, CACNA2D1 encoding the Cav α 2 δ -1 protein, was found to be differentially expressed and common to all three gene categories: $pLi>0.9$, Pyr to PV, and genes with enriched expression in the neocortex (**FIG. 2G**). The membrane bound Cav α 2 δ -1 protein is classically observed in excitatory synapses and acts as an auxiliary subunit of voltage-gated calcium channels (VGCCs), one of the gene families where mutations are most consistently associated with neurodevelopmental disorders and SCZ. Peptide mapping from the MS sequencing shows that the CSF Cav α 2 δ -1 is, however, devoid of transmembrane and intracellular peptides in the CSF as compared to the canonical α 2 δ -1 protein (**FIG. 2H**). A reduction of ~40% α 2 δ -1 protein in SCZ samples as compared to unaffected control samples was authenticated using an independent group of CSF samples from healthy and individuals with

SCZ (**FIG. 2I**). This reduction in $\alpha 2\delta$ -1 was confirmed independently by measuring the levels of $\alpha 2\delta$ -1 via ELISA in post-mortem CSF samples from unaffected controls and individuals with SCZ (**FIG. 2J**).

[0089] The release of $\text{Cav}\alpha 2\delta$ -1 in the extracellular milieu was investigated to determine whether it was regulated process, influenced by neuronal activity and/or proteolytic enzymes. Acute live cortical brain slices were stimulated with bath application of high KCl (30 mM) or the GABA-A receptor antagonist Bicuculline (Bic, 30 μ M) for three hours, and the levels of $\alpha 2\delta$ -1 released into the media was measured via western blot (**FIG. 4A**). Both stimulation methods resulted in increased extracellular $\alpha 2\delta$ -1 levels (**FIGS. 4B-4C**). These findings were further confirmed in vivo by exposing mice to an enriched environment for 30 minutes as compared to mice in their home cage and then measuring $\alpha 2\delta$ -1 levels from the hippocampal soluble fraction using western blots (**FIGS. 2K-2L**).

[0090] Structurally, $\text{Cav}\alpha 2\delta$ -1 presents a GPI-anchor site, which can be cleaved by endogenous enzymes, such as Glycosylphosphatidylinositol Specific Phospholipase D1 (GPLD1), which itself is implicated in aging and cognition. HEK293 cells were used to determine if GPLD1 was involved in cleavage of $\text{Cav}\alpha 2\delta$ -1. Levels of soluble extracellular $\alpha 2\delta$ -1 were found to be increased in the cells by a cAMP pathway activator, Forskolin, and reduced by the GPLD1 inhibitor, 1, 10 PNT (**FIGS. 4D-4E**).

[0091] Based on the tryptic sequences of human CSF $\alpha 2\delta$ -1 observed in the LC-MS/MS screen, a synthetic recombinant form of $\alpha 2\delta$ -1 protein, termed $\alpha 2\delta$ -1-Fc-His6, was designed (**FIG. 5A**). The recombinant form of the soluble $\alpha 2\delta$ -1 generated is a fusion protein with the C-terminal region of the $\alpha 2$ subunit of the $\text{Cav}\alpha 2\delta$ -1 protein fused to the N-terminal region of the δ subunit of the same $\text{Cav}\alpha 2\delta$ -1 protein, replacing a disulfide bond linking the two subunits. Further, the recombinant protein includes additional Fc and polyhistidine (His6) tags to facilitate detection of the protein.

[0092] To examine the recombinant protein's binding to neurons, live cortical neurons were treated in culture with $\alpha 2\delta$ -1-Fc-His6 for 30 minutes, washed, fixed, and then analyzed via surface immunostaining (**FIG. 5B**). The presence of $\alpha 2\delta$ -1-Fc-His6 fluorescent puncta colocalized with the surface of the dendrites of the two main types of cortical neurons were

analyzed. The types of cortical neurons are Pyr, which are the main excitatory neurons, and PV+, which are the GABAergic neurons (**FIG. 6**). Puncta were visualized via high magnification, deconvolved confocal microscopy. Both Pyr and PV+ cells showed $\alpha 2\delta$ -1-Fc-His6 puncta on their dendrites' surfaces, but there was a significant increase (~40%) in puncta density around PV+ neurons (**FIG. 5C**). Super-resolution microscopy was then used to determine the subcellular binding location of $\alpha 2\delta$ -1-Fc-His6 on PV+ neurons. After incubating the neurons with $\alpha 2\delta$ -1-Fc-His6 and immunostaining for the Vesicular Glutamate Transporter 1 (VGLUT1), the distance between the center of the $\alpha 2\delta$ -1-Fc-His6 or VGLUT1 puncta and the post-synaptic membrane of the neurons was measured using structured illumination microscopy (SIM) (**FIGS. 8D-8F**). This revealed that $\alpha 2\delta$ -1-Fc-His6 was located at an even distance between the pre-synaptic boutons and the dendritic plasma membrane (**FIG. 8G**).

[0093] The canonical membrane-bound Cav $\alpha 2\delta$ -1 is known to impact synapse physiology, but its membrane interactome remains incomplete. To identify protein targets of $\alpha 2\delta$ -1, an affinity pull-down assay was performed using mouse cortical membrane preparations followed by LC-MS/MS using established methods (**FIGS. 8A-8B**). Remarkably, it was found that around 70% of the proteins identified to interact with $\alpha 2\delta$ -1 were present on a publicly available human PSD proteome list versus the ~34% that was expected (hypergeometric test, enrichment 1.9, $p < 0.001$). A SynGo analysis was performed to corroborate this synaptic enrichment, which revealed several Gene Ontology terms related to synapses and trans-synaptic signaling (**FIG. 8C**, **Table 1**). The interactome assay revealed novel $\alpha 2\delta$ -1 synaptic interactors, including neuronal adhesion molecules, such as CNTN1, NCAM2, Cadherin-13, L1CAM, and IGSF8, and transporters, such as the potassium (K⁺)/chloride (Cl⁻) symporter KCC2 and calcium extruder pump ATP2B2 (**FIG. 8B**).

Table 1:

<u>Synapse GO Category (synGO)</u>	<u>q value</u>	<u>-log q-value</u>	<u>n-proteins</u>	<u>Significant</u>
synapse	2.33E-05	4.632448	113	Yes
postsynaptic ribosome	4.05E-05	4.392209	28	Yes
presynapse	9.95E-05	4.00206	72	Yes

anchored component of synaptic vesicle membrane	0.002683	2.571306	11	Yes
presynaptic ribosome	0.002896	2.538271	18	Yes
translation at presynapse	0.00618	2.209001	18	Yes
translation at postsynapse	0.00618	2.209001	19	Yes
process in the synapse	0.006663	2.176314	79	Yes
postsynapse	0.010678	1.971502	55	Yes
synaptic vesicle membrane	0.011611	1.935119	23	Yes
postsynaptic density	0.025048	1.601221	22	Yes
presynaptic active zone membrane	0.027121	1.566701	11	Yes
postsynaptic specialization	0.027121	1.566701	25	Yes
integral component of presynaptic active zone membrane	0.05159	1.287431	8	Yes
presynaptic active zone	0.076196	1.118071	12	No
synaptic membrane	0.106678	0.971926	4	No
presynaptic membrane	0.119738	0.921768	14	No
process in the presynapse	0.122371	0.912322	32	No
synaptic vesicle cycle	0.122371	0.912322	27	No

[0094] Despite being mainly expressed by Pyr neurons, it was observed that recombinant $\alpha 2\delta$ -1-Fc-His6 preferentially bound the surface of PV+ interneurons, suggesting a paracrine Pyr to PV role for $\alpha 2\delta$ -1 that could modulate synaptic activity of PV+ interneurons. Thus, the mechanism underlying the preferential binding was investigated. PV+ interneurons are surrounded by a higher density of perineuronal nets (PNNs) than other cortical neurons (FIG. 7E, right panel). PNNs are dense extracellular networks known to bind to extracellular proteins. The analysis of human, mouse, and rabbit Cav $\alpha 2\delta$ -1's amino acid motifs revealed at least two conserved, positively-charged motifs of the glycan-binding domain that have been previously published, suggesting that $\alpha 2\delta$ -1 may strongly interact with PNNs around PV+ interneurons (FIG. 7A). Fc control and $\alpha 2\delta$ -1-Fc-His6 were injected into brains to determine if $\alpha 2\delta$ -1 was

localized in the PNN. The samples were visualized via immunohistochemistry, showing Fc and $\alpha 2\delta$ -1-Fc-His6 localization in red and the PNN in green (**FIG. 7C**). This hypothesis was tested by co-injecting $\alpha 2\delta$ -1-Fc-His6 and the enzyme Chondroitinase ABC (ChABC) into the somatosensory cortex (S1) of one side of the brain to digest the PNNs, while $\alpha 2\delta$ -1-Fc-His6 and bovine serum albumin (BSA) were co-injected on the contralateral side as a control, and the brains were then immunostained for Fc (**FIGS. 5E-5H, 7B, 7E**). Digestion of the matrix by ChABC significantly reduced the binding of $\alpha 2\delta$ -1-Fc-His6 to PV+ interneurons, demonstrating the role of the PNN for $\alpha 2\delta$ -1 selective binding.

[0095] The next aspect investigated was whether the high affinity of $\alpha 2\delta$ -1 for PV+ interneurons could increase the number of excitatory synapses on them. It was found that incubation with the recombinant $\alpha 2\delta$ -1-Fc-His6 significantly increased the density of surface GluA2, one of the synaptic $\alpha 2\delta$ -1 interactors, on the PV+ interneuron dendrites in cultured neurons (**FIG. 8H**). Presynaptic bouton density (VGLUT1) on Dlx5/6-GFP-expressing PV+ interneurons was also increased (**FIG. 8M**).

[0096] Consistent with this, imaging of single-cell spontaneous Ca^{2+} activity in Dlx5/6-GCamp6f-expressing GABAergic interneurons revealed a significant increase in Ca^{2+} activity, specifically in the dendrites of PV+ interneurons upon $\alpha 2\delta$ -1-Fc-His6 treatment as compared to untreated controls, which exhibited no effect in soma (**FIGS. 8J-8L**), indicating GABAergic neuron potentiation. There was no significant difference in the number of dendritic Ca^{2+} events or event rates for control samples and samples treated with $\alpha 2\delta$ -1-Fc-His6 (**FIGS. 9A-9B**). While there was no significant difference in the somatic Ca^{2+} event rates between treatment conditions, there was an increase in the somatic Ca^{2+} event duration following treatment. (**FIGS. 9C-9D**).

[0097] Parvalbumin (PV) protein expression is dynamically modulated by PV+ interneuron activity in the hippocampus. Hence, the impact of in vivo $\alpha 2\delta$ -1-Fc-His6 unilateral injection was measured via PV immunostaining in the adult wild-type mouse hippocampus. A significantly higher number of cells with high levels of PV were observed on the $\alpha 2\delta$ -1-Fc-His6-injected CA1 side as compared to the control side injected with Fc (**FIGS. 5I-5J**). All

together, these findings demonstrate that soluble $\alpha 2\delta$ -1 enhances intraneuronal dendritic activity and excitatory synapses.

[0098] GABAergic interneurons are key regulators of neuronal microcircuit dynamics, and modulation of their activity by $\alpha 2\delta$ -1-Fc-His6 may affect neuronal network dynamics. The effect of $\alpha 2\delta$ -1-Fc-His6 on network activity was assessed in neuronal cultures plated on multi-electrode arrays (MEAs). A significant reduction in the number of action potentials per network burst was found for neurons treated for 1 hour with $\alpha 2\delta$ -1-Fc-His6 as compared to Fc-treated controls (**FIGS. 10A-10B**). To assess dependence on GABAergic interneurons, a chemogenetic strategy was employed, using the hM4Di-designed receptor expressed under Dlx5/6 promoter to reduce GABAergic neuron firing upon Clozapine-N-Oxide (CNO) application (**FIG. 10C**), followed by imaging Ca^{2+} activity reported by co-expressed GCaMP6f. A reduction in the percentage of co-active cells and in the amplitude of the Ca^{2+} -transients was observed during network events after $\alpha 2\delta$ -1-Fc-His6 incubation. Remarkably, silencing interneurons using CNO eliminated the difference between Fc and $\alpha 2\delta$ -1-Fc-His6 treatments, indicating that $\alpha 2\delta$ -1's effect on interneurons was causing the changes in network activity (**FIGS. 10D-10F**). It appeared that this effect was specific to the GABAergic interneurons as $\alpha 2\delta$ -1-Fc-His6 treatment did not affect the calcium dynamics of astrocytes (**FIGS. 11A-11C**).

[0099] To validate these findings in a more complex brain microcircuit, acute hippocampal slices from 1-month-old Thy1-GCaMP6f mice were incubated with $\alpha 2\delta$ -1-Fc-His6 for three hours before neuronal activity was imaged under a multiphoton microscope (**FIG. 10G**). A significant reduction in spontaneous Ca^{2+} events was observed after $\alpha 2\delta$ -1-Fc-His6 treatment, which was completely reversed by bath application of the GABA-A receptor antagonist, Bicuculline (30 μM) (**FIGS. 10H-10J**). This corroborated the in vitro observations of the essential role of GABAergic neurons for $\alpha 2\delta$ -1-Fc-His6's effect on network activity.

[0100] The data presented support a role for $\alpha 2\delta$ -1 in modulating microcircuits by increasing inhibitory neuron activity. Excitatory/inhibitory (E/I) imbalance is a hallmark of several neurodevelopmental disorders and is thought to contribute to cognitive impairments in sociability and cognitive function. It was hypothesized that boosting inhibitory neuron activity using $\alpha 2\delta$ -1-Fc-His6 may restore E/I balance and improve functional and behavioral endpoints

in genetic mouse models of neurodevelopmental disorders. To test this, a single $\alpha 2\delta$ -1-Fc-His6 injection was evaluated to determine if it could restore behavioral and anatomical endpoints in 16p11.2dup/+ mice, a genetic model of 16p11.2 duplication syndrome. The 16p11.2 microduplication is a human copy number variant (CNV) that significantly increases the susceptibility to develop schizophrenia and autism spectrum disorders. Mice carrying this mutation show brain connectivity alterations characterized by both hyperglutamatergic and hypogabaergic cortical circuits and reduced Parvalbumin protein in the prefrontal cortex. To determine if 16p11.2dup/+ mice had altered levels of $\alpha 2\delta$ -1, CSF samples from wild-type and 16p11.2dup/+ mice were evaluated via western blot (**FIG. 12**). Similar to samples from SCZ patients, the 16p11.2dup/+ samples had lower levels of $\alpha 2\delta$ -1 as compared to wild-type samples.

[0101] 16p11.2dup/+ mice were injected bilaterally with $\alpha 2\delta$ -1-Fc-His6 or Fc control into the anterior cingulate cortex (ACC) (**FIG. 13A**), a subregion of the prefrontal cortex important for sociability, cognitive function, and implicated in SCZ. A significant reduction in sociability was observed in a three-chamber test in 16p11.2dup/+ male mice compared to wild-type, which was completely rescued by $\alpha 2\delta$ -1-Fc-His6 injection (**FIGS. 13D-13E**). No effect of the genotype on sociability was observed in females, revealing a potential predictive effect of the female sex, as observed in other mouse models of neurodevelopmental disorder. Novel object recognition was also reduced in 16p11.2dup/+ mice as compared to wild-type, and remarkably, was restored to wild-type levels by $\alpha 2\delta$ -1-Fc-His6 injection (**FIG. 13F**). Basal locomotion and working memory in y-maze assays were not altered in 16p11.2dup/+ mice, and $\alpha 2\delta$ -1-Fc-His6 did not alter these endpoints (**FIGS. 14A-14E**). Moreover, the number of high-expressing PV+ interneurons were reduced in the ACC of 16p11.2dup/+ mice, and $\alpha 2\delta$ -1-Fc-His6 injection restored high-expressing PV+ interneuron density to wild-type levels (**FIGS. 13B-13C**).

[0102] *Discussion*

[0103] In all, it was found that multiple ectodomains originating from synaptic proteins are reduced in the CSF of SCZ patients. The CSF is one of the few biofluids that is accessible in live humans and could thus allow the monitoring of synapse health in humans by analyzing synaptic ectodomains in the CSF. Further, large-scale unbiased proteomic analyses can identify global

changes in synaptic ectodomain shedding in various physiological and clinical conditions, revealing novel biological and pathological mechanisms and guiding novel treatment options.

[0104] Synaptic ectodomains can also serve as biomarkers in psychiatric disorders. The findings of reduced synaptic ectodomains and increased IgG is consistent with previous findings of reduced synapse numbers and increased neuroinflammation in individuals with SCZ. While other proteomic studies found reduced synaptic membrane proteins, they did not realize their importance for mechanistic insight and treatment of such disorders.

[0105] Identified herein is a novel non-canonical, soluble form of $\alpha 2\delta$ -1 protein, which opens up a new aspect of the protein's biology. Previously, only membrane-bound roles of $\alpha 2\delta$ proteins were known. In addition to further understanding the role of $\alpha 2\delta$ proteins, a recombinant form of this protein, $\alpha 2\delta$ -1-Fc-His6, was designed and tested, which showed potently enhanced PV+ interneuron function and modulation of E/I balance and microcircuit properties. Using affinity proteomics, several novel synaptic interFOGactors of $\alpha 2\delta$ -1 were identified, supporting novel VGCC-independent roles in synaptic regulation. This is consistent with previous reports of membrane-attached $\alpha 2\delta$ interactions with AMPAR subunits, LRP1, and neuroligins, which affect presynaptic function. Interaction of $\alpha 2\delta$ -1 with both pre- and post-synaptic proteins, along with equidistant localization between pre- and post-synaptic sites, suggests a role for $\alpha 2\delta$ -1 as a trans-synaptic bridge at excitatory synapses on PV+ interneurons.

[0106] The paracrine network modulating properties of $\alpha 2\delta$ -1 were utilized to normalize phenotypes in a mouse model of the 16p11.2 duplication syndrome, a prominent risk factor that increases the risk of developing neurodevelopmental disorders and schizophrenia in humans. The 16p11.2 microduplication region spans 27 genes, making gene therapy or finding drug targets difficult. Thus, targeting a circuit level alteration may present a better strategy for CNV disorders.

[0107] While the 16p11.2 duplication may increase the risk of developing neurodevelopmental disorders and schizophrenia, schizophrenia may arise idiopathically in subjects that do not have such genetic predispositions to the disorder. Subjects with idiopathic schizophrenia also exhibit reduced levels of $\alpha 2\delta$ -1 in their CSF, indicating that $\alpha 2\delta$ -1 treatment may benefit this population as well as populations that carry the 16p11.2 duplication mutation.

[0108] Soluble $\alpha 2\delta$ -1 has the advantage of mimicking the endogenous activity of the protein without the adverse effects of a synthetic drug or gene therapy. This could have profound implications for the treatment of psychiatric disorders. Despite the efficacy of antipsychotics for treating positive symptoms in SCZ, negative and cognitive symptoms remain difficult to address. Synapse enhancers have shown promise as novel treatments for depression, but developing approaches to restore synapse physiology suitable for SCZ remains a challenge. Recent studies have identified proteins in blood plasma and CSF with pro-cognitive properties in aged mice. Thus, $\alpha 2\delta$ -1 treatment may complement existing antipsychotics targeting the dopamine system for the improvement of cognition and other negative symptoms, especially in older individuals with SCZ. Because inhibitory neuron dysfunction, altered E/I balance, and reduced sociability are shared among several neurodevelopmental disorders, $\alpha 2\delta$ -1 treatment may be a potential therapeutic option for a broader range of disease that present these features.

[0109] Example 2: Generation and Validation of an Effective Peptide Derived from Soluble Cav $\alpha 2\delta$ -1 protein

[0110] To identify peptide fragments of the soluble Cav $\alpha 2\delta$ -1 that may also be capable of modulating neuronal function, peptides derived from the $\alpha 2\delta$ -1 protein were screened via a multi-electrode array using cortical cultures. Candidate charged peptides were selected by screening for sequences that were conserved in proteins for all species and that were not present near the voltage-gated calcium channel regulation region of the protein. Four candidate peptides were tested along with a vehicle-only control to test the normalized firing rate 1 hour after treatment as compared to before said treatment (**FIG. 15A**). Only peptide 2 exhibited a significant decrease in firing rate. Peptide 2 has the amino acid sequence of TYEDSFYKRSLDN.

[0111] To further understand the dynamics of peptide 2, a dose response curve was generated using concentrations of 1 nM, 10 nM, 100 nM, 1 μ M, and 10 μ M (**FIG. 15B**). The IC₅₀, or concentration of inhibitor where the response is reduced by half, was determined to be at 50nM. The normalized firing rate of neurons from three independent experiments treated with TYEDSFYKRSLDN were compared before peptide incubation and one hour after peptide 2

incubation (**FIG. 15C**). Neurons exhibited 30-40% inhibition of firing when peptide 2 was administered at a concentration of 1 μ M for 1 hour.

[0112] Calcium signaling was detected in neurons treated with peptide 2, as visualized through Syn-GCamp6f (**FIG. 15D**). Amplitudes and frequencies of the action potentials for the neurons treated with either the control or peptide 2 were measured (**FIG. 15E**). The neurons treated with peptide 2 exhibited fewer action potentials for the testing period, and the action potentials were lower as well. The quantitation of the maximum amplitudes for the action potentials are shown in **FIG. 15F**. The neurons treated with peptide 2 exhibited a significantly lower maximum amplitude as compared to the control neurons. To further understand the effects of peptide 2 on neuronal activity, the pairwise correlation for neurons for both treatment conditions were compared (**FIG. 15G**). Neurons treated with 1 μ M of peptide 2 exhibited a significantly lower pairwise correlation or lower underlying network connectivity, which is expected as peptide 2 treatment reduced firing rates. Taken as a whole, peptide 2 is a novel peptide derived from the soluble Cav α 2 δ -1 protein that exhibits similar effects on neurons as the full protein. Thus, peptide 2 is a candidate for use in the treatment of neurodevelopmental or psychiatric disease, along with full-length soluble Cav α 2 δ -1 protein. Peptide 2 presents further benefits as it may be able to traverse the blood brain barrier (BBB) more easily.

EQUIVALENTS

[0113] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0114] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent that are not inconsistent with the explicit teachings of this specification.

CLAIMS

What is claimed is:

1. A polypeptide, comprising or consisting of the amino acid sequence
TYEDSFYKRSLDN.
2. A pharmaceutical composition, comprising:
 - (a) a soluble Cav α 2 δ -1 protein or a variant or a portion thereof, or a nucleic acid encoding the soluble Cav α 2 δ -1 protein or a variant or a portion thereof; and
 - (b) a pharmaceutically acceptable carrier.
3. The pharmaceutical composition of claim 2, wherein the soluble Cav α 2 δ -1 protein or a variant or a portion thereof comprises a fusion of the C-terminal region of an α 2 subunit of a Cav α 2 δ -1 protein to the N-terminal region of the δ subunit of the same Cav α 2 δ -1 protein.
4. The pharmaceutical composition of claims 2 or 3, wherein the soluble Cav α 2 δ -1 protein or a variant or a portion thereof comprises or consists of the amino acid sequence
TYEDSFYKRSLDN.
5. The pharmaceutical composition of any one of claims 2-4, wherein the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is recombinant.
6. The pharmaceutical composition of any one of claims 2-5, wherein the structure of active binding region of the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is stabilized.
7. The pharmaceutical composition of claim 6, wherein the stabilized soluble Cav α 2 δ -1 protein or a variant or a portion thereof is a stapled protein or peptide.
8. The pharmaceutical composition of claim 6, wherein the stabilized soluble Cav α 2 δ -1 protein or a variant or a portion thereof is a circularized protein or peptide.

9. The pharmaceutical composition of any one of claims 2-5, wherein the nucleic acid encoding the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is selected from an mRNA, a plasmid, and a vector.
10. The pharmaceutical composition of claim 9, wherein the vector is selected from an adenoviral vector, an adeno-associated viral (AAV) vector, a retroviral vector, a lentiviral vector, and a herpes simplex viral vector.
11. The pharmaceutical composition of any one of claims 2-10, wherein the composition is formulated as an injectable.
12. A method of treating a subject with a neurodevelopmental or psychiatric disorder, comprising administering to the subject a soluble Cav α 2 δ -1 protein or a variant or portion thereof, or a nucleic acid encoding the soluble Cav α 2 δ -1 protein or a variant or a portion thereof.
13. The method of claim 12, wherein the soluble Cav α 2 δ -1 protein or a variant or portion thereof comprises a fusion of the C-terminal region of an α 2 subunit of a Cav α 2 δ -1 protein to the N-terminal region of the δ subunit of the same Cav α 2 δ -1 protein.
14. The method of claim 12 or 13, wherein the soluble Cav α 2 δ -1 protein or a variant or portion thereof comprises, consists of, or consists essentially of the amino acid sequence TYEDSFYKRSLDN.
15. The method of any of claims 12-14, wherein the structure of active binding region of the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is stabilized.
16. The method of claim 15, wherein the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is a stapled protein or peptide or a circularized protein or peptide.
17. The method of claims 12-14, wherein the nucleic acid encoding the soluble Cav α 2 δ -1 protein or a variant or a portion thereof is selected from an mRNA, a plasmid, and a vector.

18. The method of claims 17, wherein the vector is selected from an adenoviral vector, an adeno-associated viral (AAV) vector, a retroviral vector, a lentiviral vector, and a herpes simplex viral vector.
19. The method of any one of claims 12-18, wherein the neurodevelopmental or psychiatric disorder is schizophrenia.
20. The method of any one of claims 12-19, wherein the neurodevelopmental or psychiatric disorder is an autism spectrum disorder.
21. The method of any one of claims 12-20, wherein the composition is administered to the subject via injection.
22. The method of claim 21, wherein the injection is intrathecal injection, intravenous injection, intracerebroventricular injection, or intracerebral injection.
23. The method of any one of claims 12-22, wherein a level of soluble Cava α 2 δ -1 protein is detected in the subject prior to administration of the recombinant form of the soluble Cava α 2 δ -1 protein or a variant or portion thereof.
24. The method of claim 23, wherein the level of soluble Cava α 2 δ -1 protein is detected in a cerebrospinal fluid (CSF) sample from the subject.
25. The method of claim 23 or 24, wherein the soluble Cava α 2 δ -1 protein is administered to the subject if the level of soluble Cava α 2 δ -1 protein detected in the subject is lowered as compared to a similar sample from a subject without the neurodevelopmental or psychiatric disorder.
26. A method of detecting a soluble Cava α 2 δ -1 protein, comprising:
 - (a) obtaining a cerebrospinal fluid (CSF) sample from a subject having or suspected of having a neurodevelopmental or psychiatric disorder; and
 - (b) measuring a level of soluble Cava α 2 δ -1 protein in the CSF sample.
27. The method of claim 26, wherein the neurodevelopmental or psychiatric disorder is schizophrenia.

28. The method of claim 26, wherein the neurodevelopmental or psychiatric disorder is an autism spectrum disorder.
29. A method of diagnosing neurodevelopmental or psychiatric disorders, the method comprising:
- (a) collecting a biological sample from a subject;
 - (b) measuring a level of soluble Cava α 2 δ -1 protein in the biological sample;
 - (c) comparing the level of soluble Cava α 2 δ -1 protein in the sample from the subject to a reference level; and
 - (d) diagnosing the subject with the neurodevelopmental or psychiatric disorder when the level of soluble Cava α 2 δ -1 protein in the sample from the subject is less than the reference level.
30. The method of claim 29, wherein the reference level corresponds to a level of soluble Cava α 2 δ -1 protein in a sample from an individual known to not have the neurodevelopmental or psychiatric disorder.
31. The method of claim 29 or 30, wherein the neurodevelopmental or psychiatric disorder is schizophrenia.
32. The method of claim 29 or 30, wherein the neurodevelopmental or psychiatric disorder is an autism spectrum disorder.
33. The method of any one of claims 29-32, wherein the biological sample is a cerebrospinal fluid (CSF) sample.
34. The method of any one of claims 29-33, wherein the levels of soluble Cava α 2 δ -1 protein are measured using liquid chromatography mass spectrometry (LC-MS), high-performance liquid chromatography, ELISA, protein immunoprecipitation, immunoelectrophoresis, western blot, or protein immunostaining.
35. The method of any one of claims 29-34, further comprising administering to the subject a soluble Cava α 2 δ -1 protein or a variant or portion thereof or a nucleic acid encoding the

soluble Cav α 2 δ -1 protein or a variant or a portion thereof, when the level of soluble Cav α 2 δ -1 protein in the sample from the subject is less than the reference level.

36. The method of claim 35, wherein the soluble Cav α 2 δ -1 protein or a variant or portion thereof comprises, consists of, or consists essentially of the amino acid sequence TYEDSFYKRSLDN.

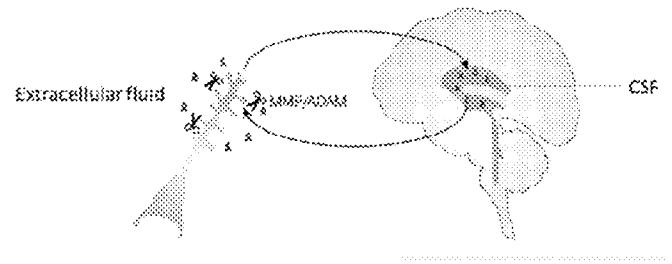
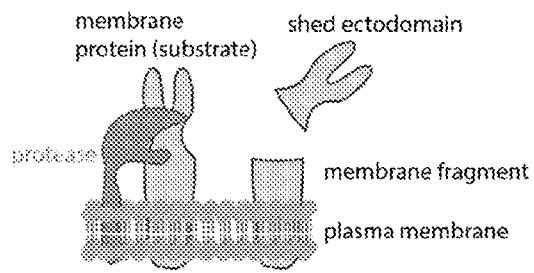


FIG. 1

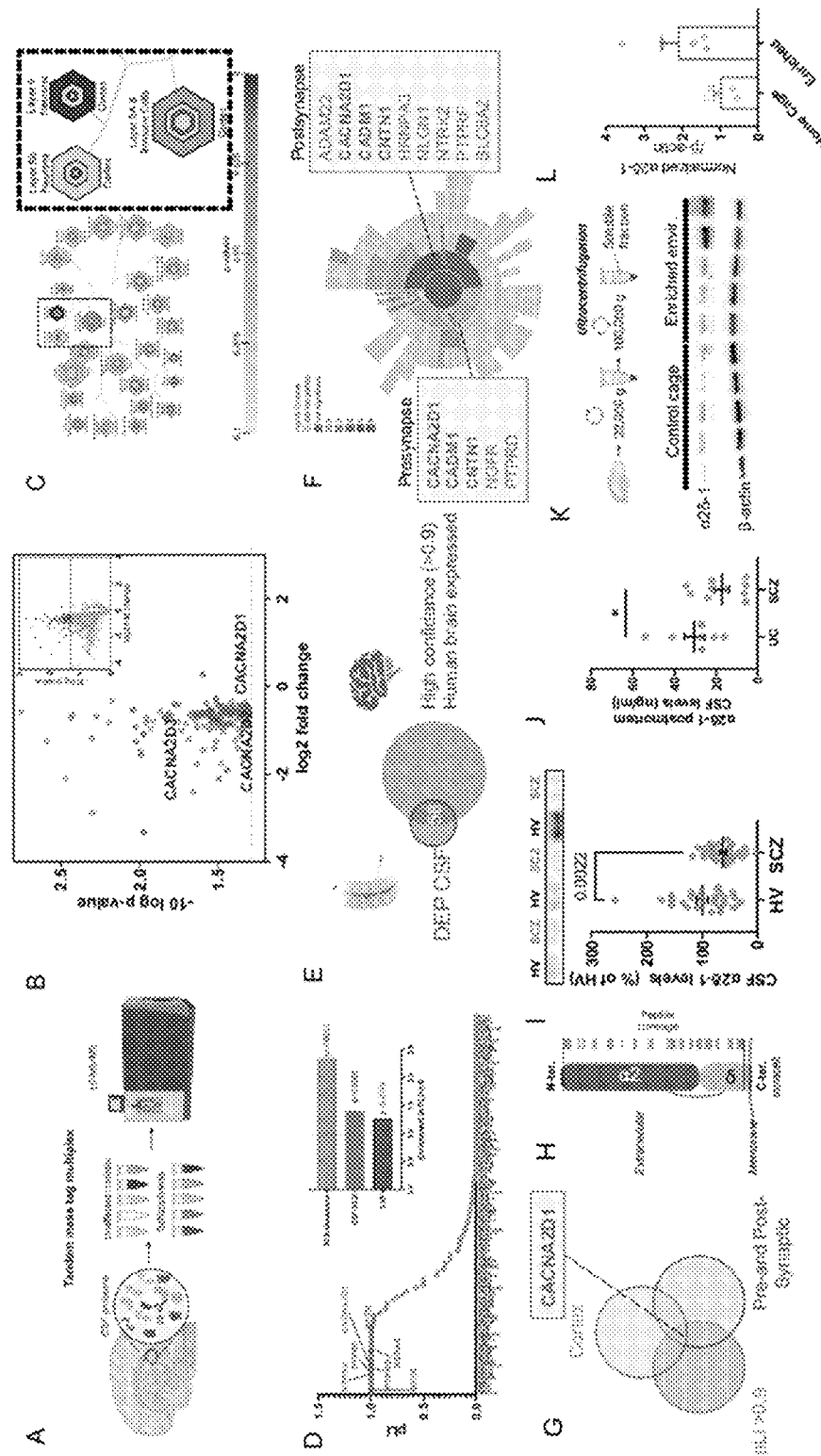


FIG. 2

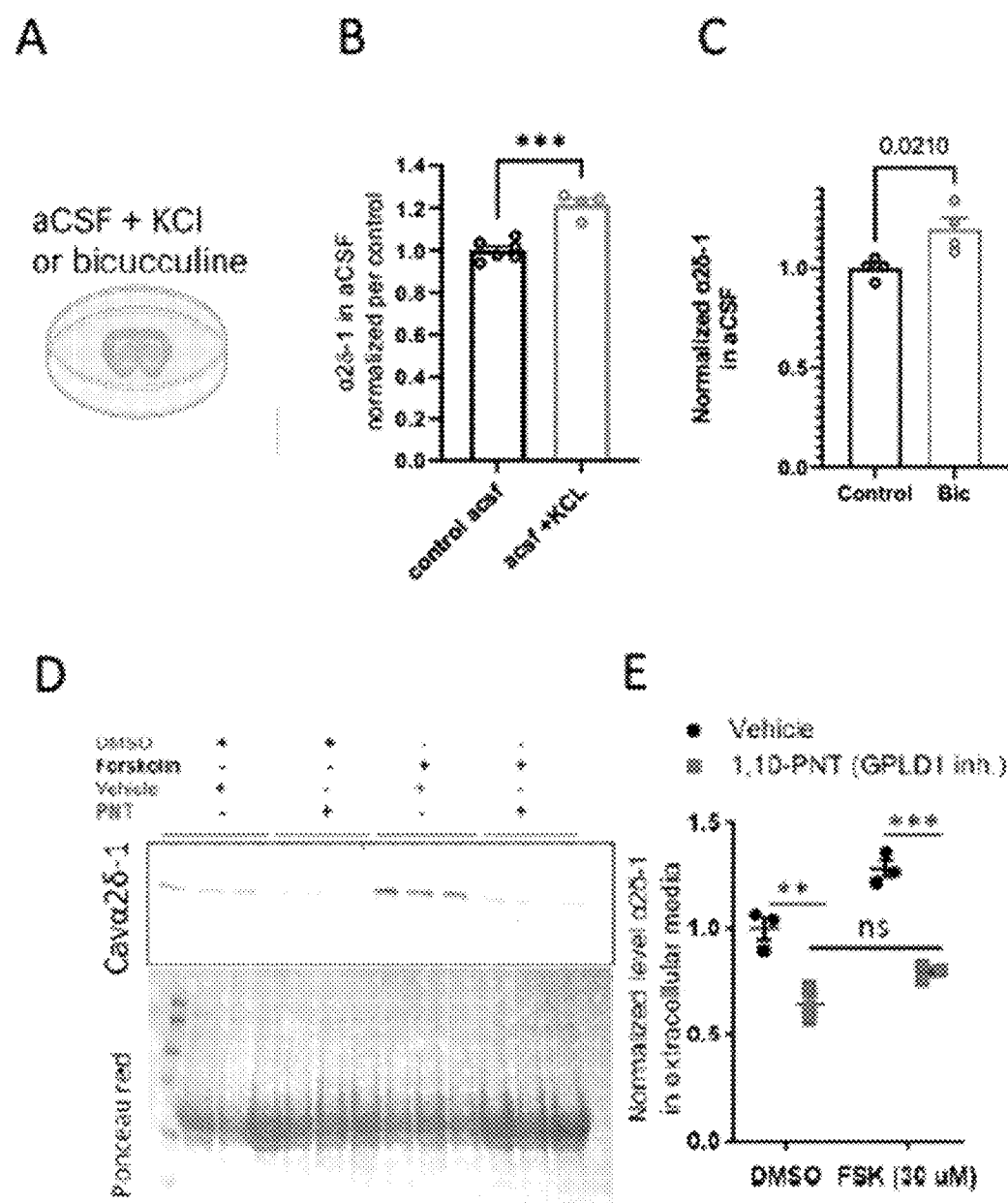


FIG. 4

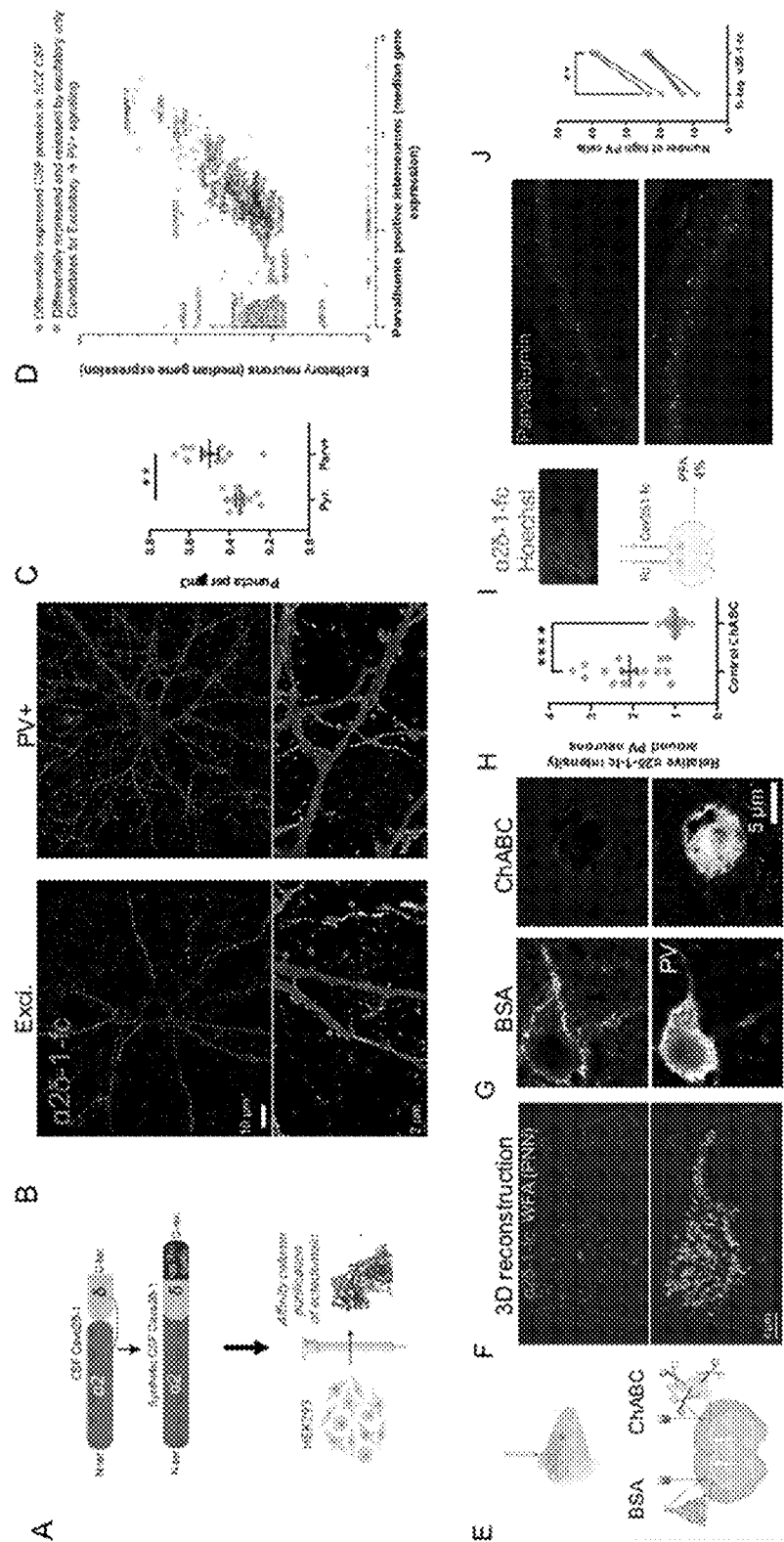


FIG. 5

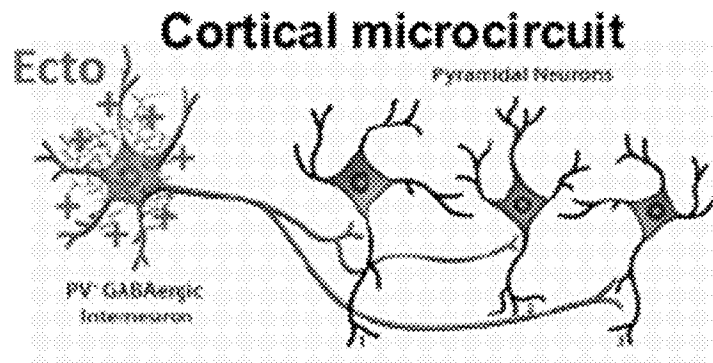


FIG. 6

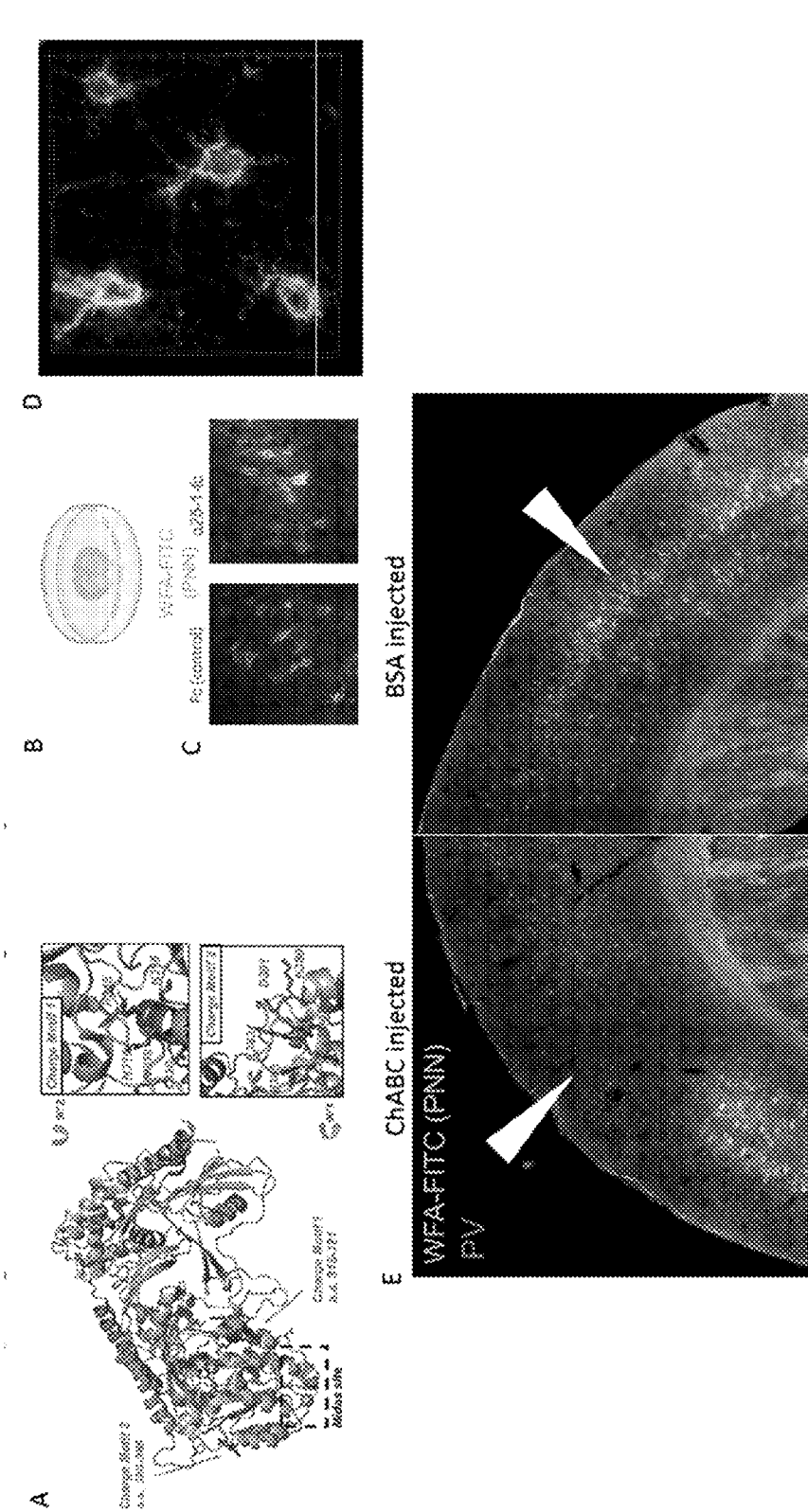


FIG. 7

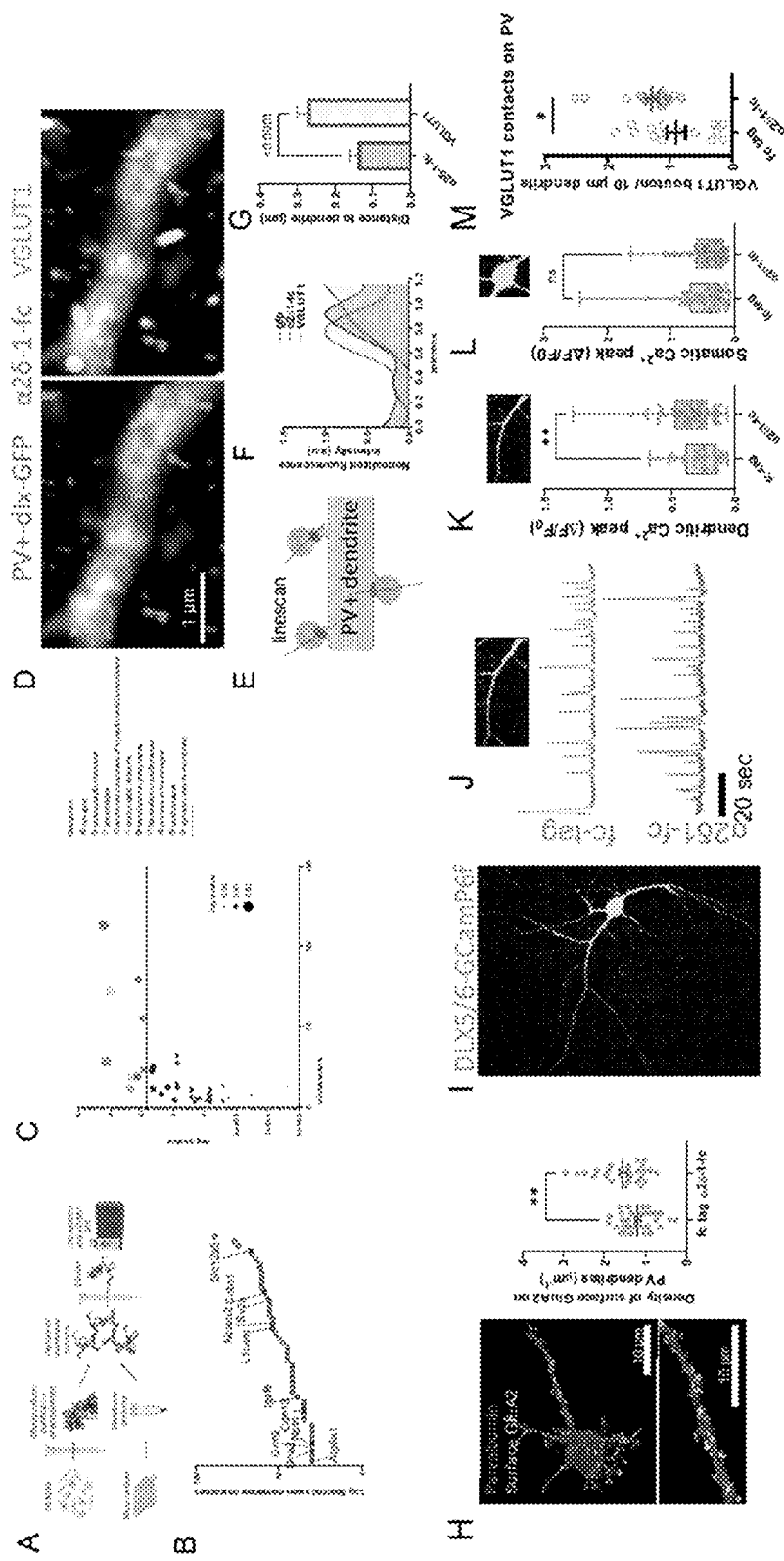


FIG. 8

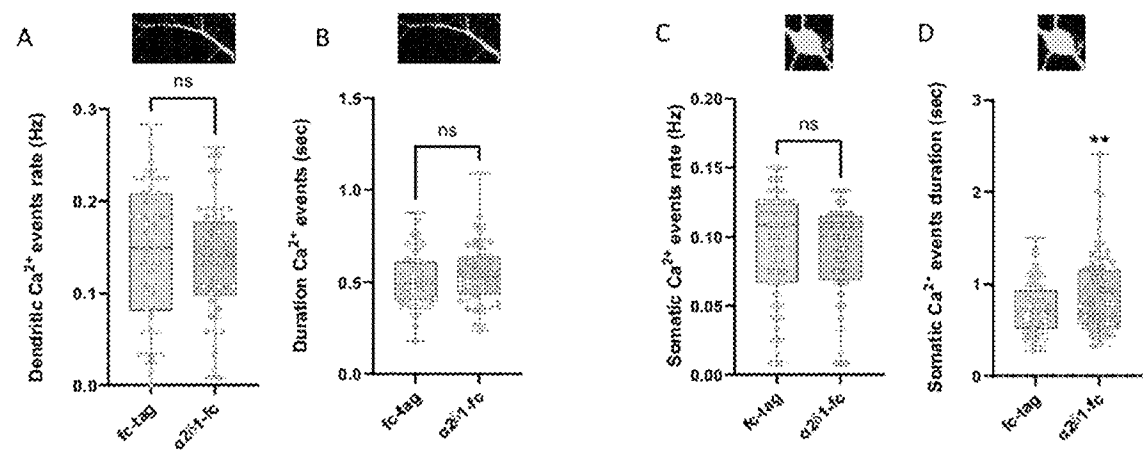


FIG. 9

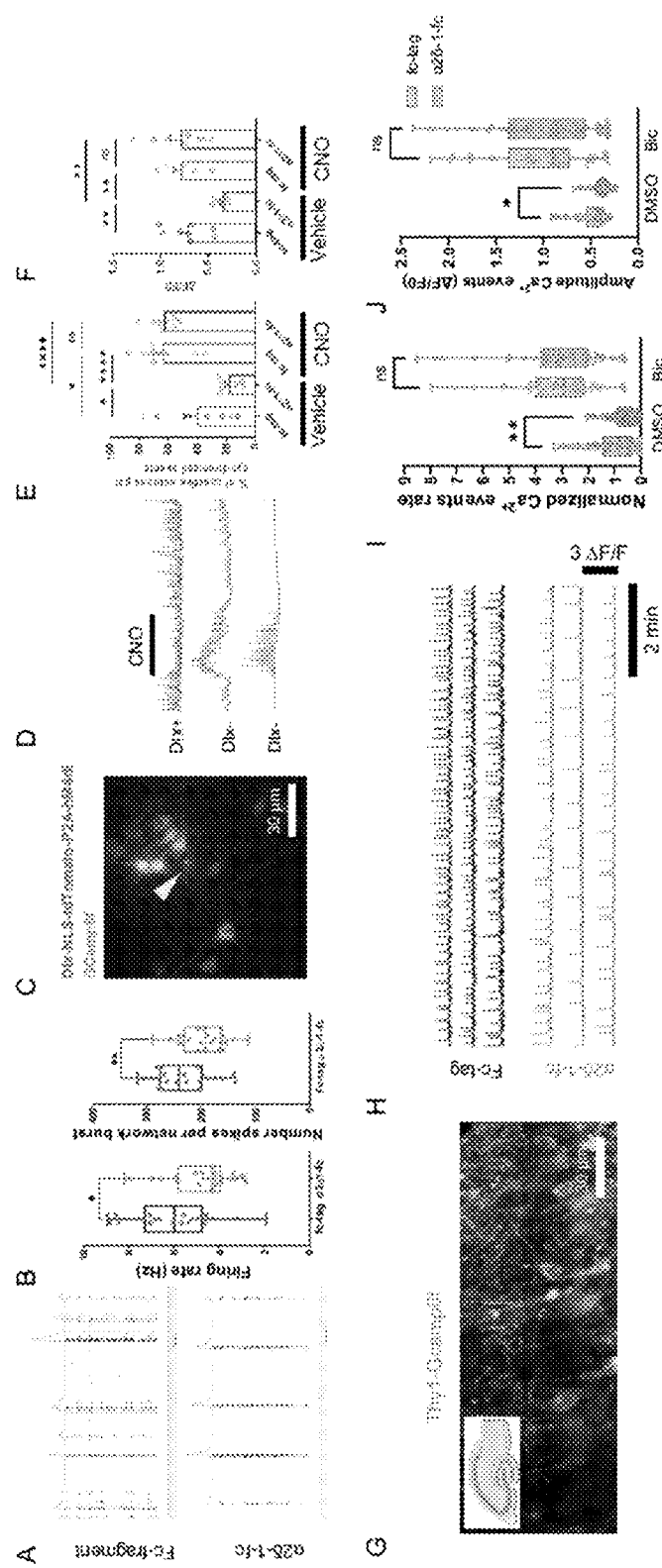


FIG. 10

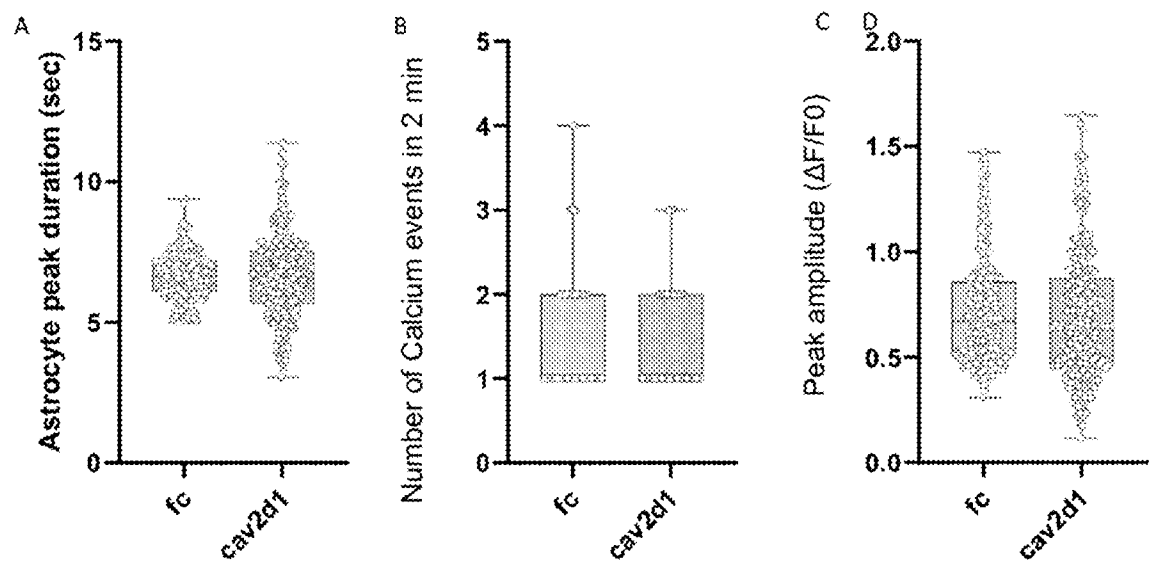


FIG. 11

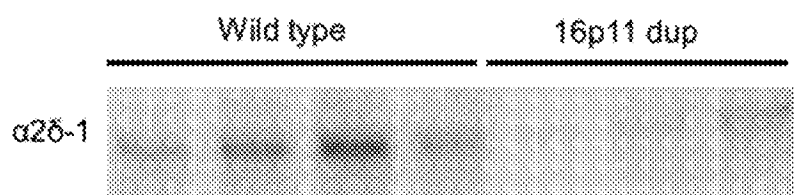


FIG. 12

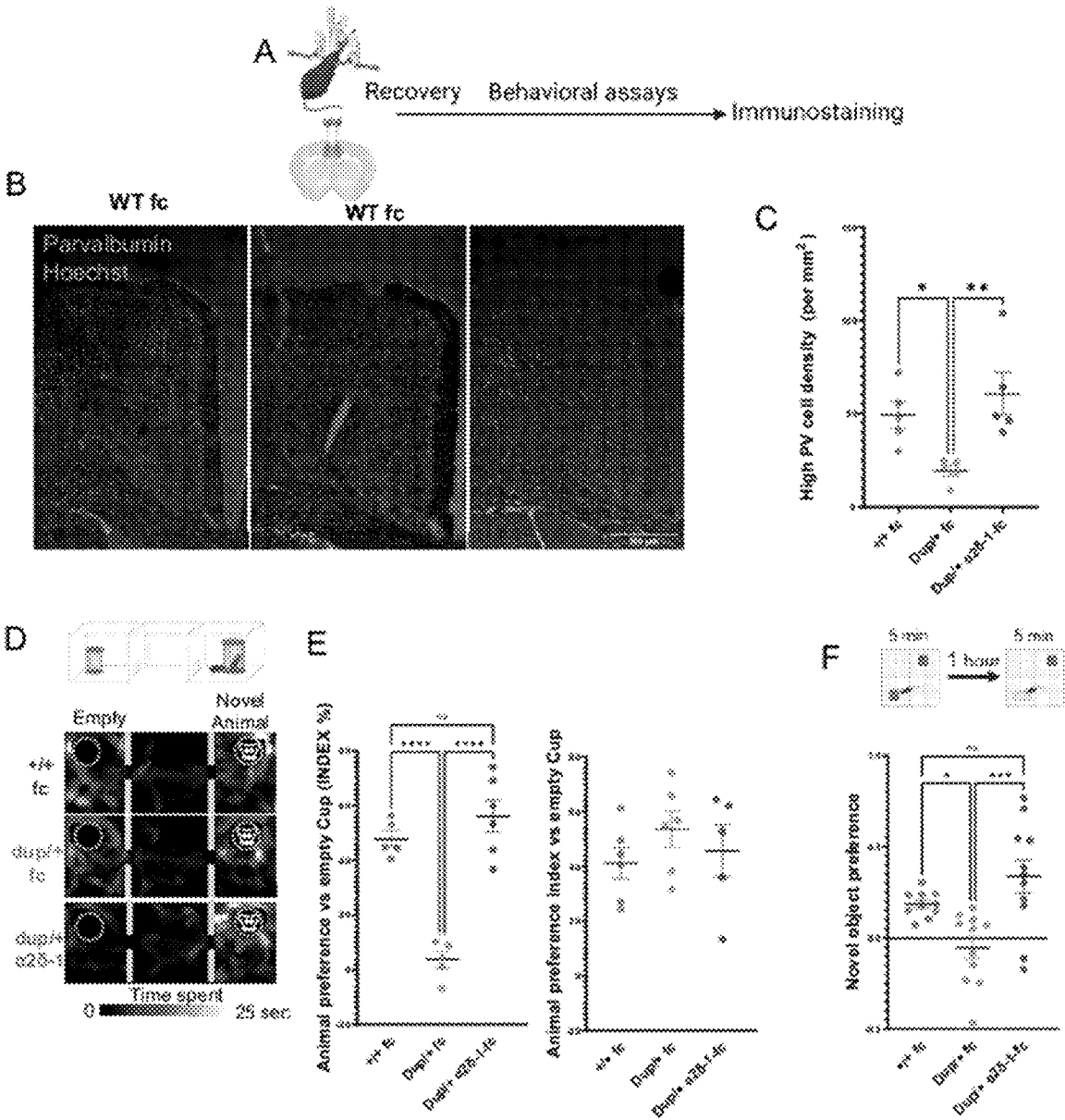


FIG. 13

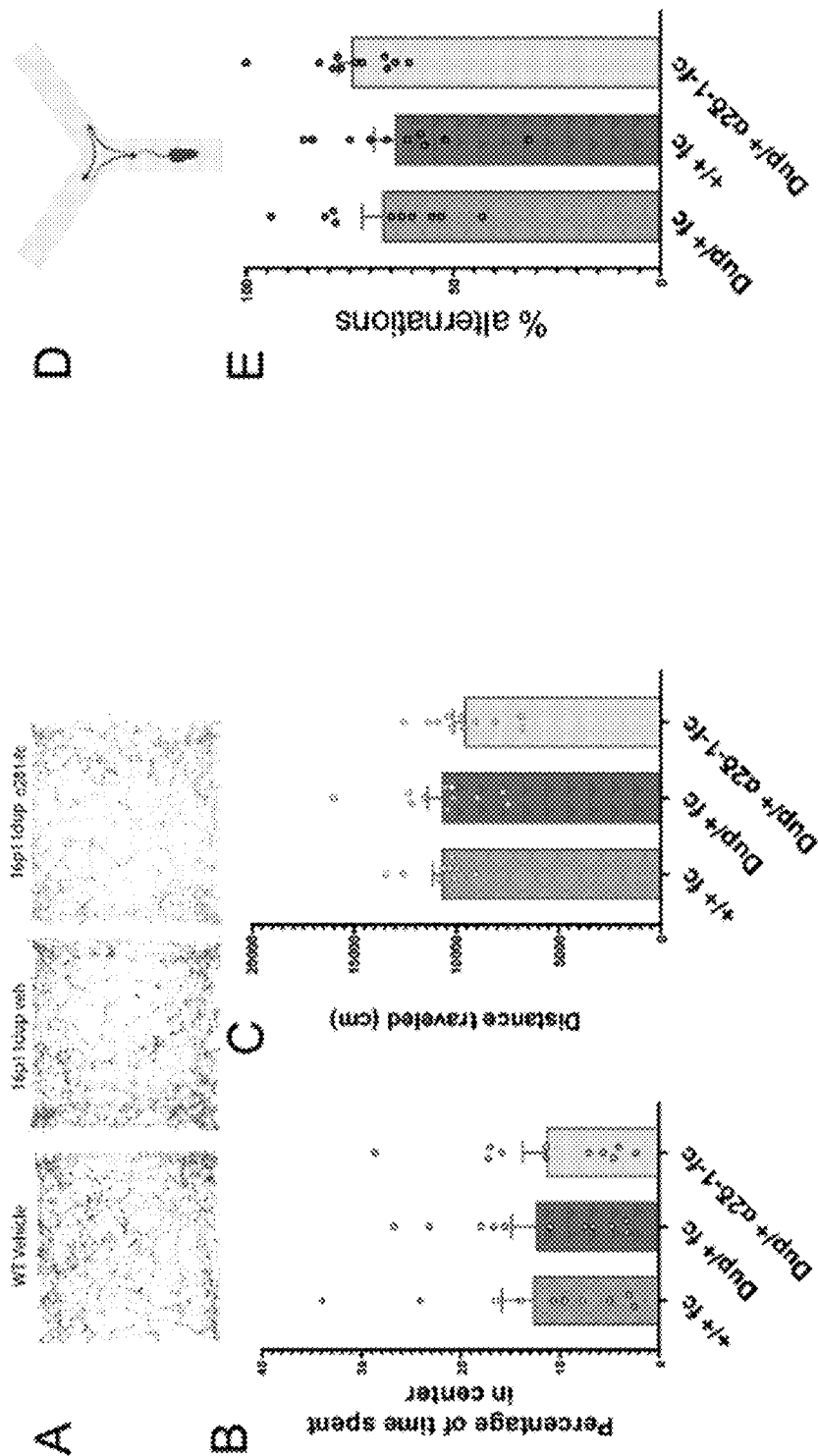


FIG. 14

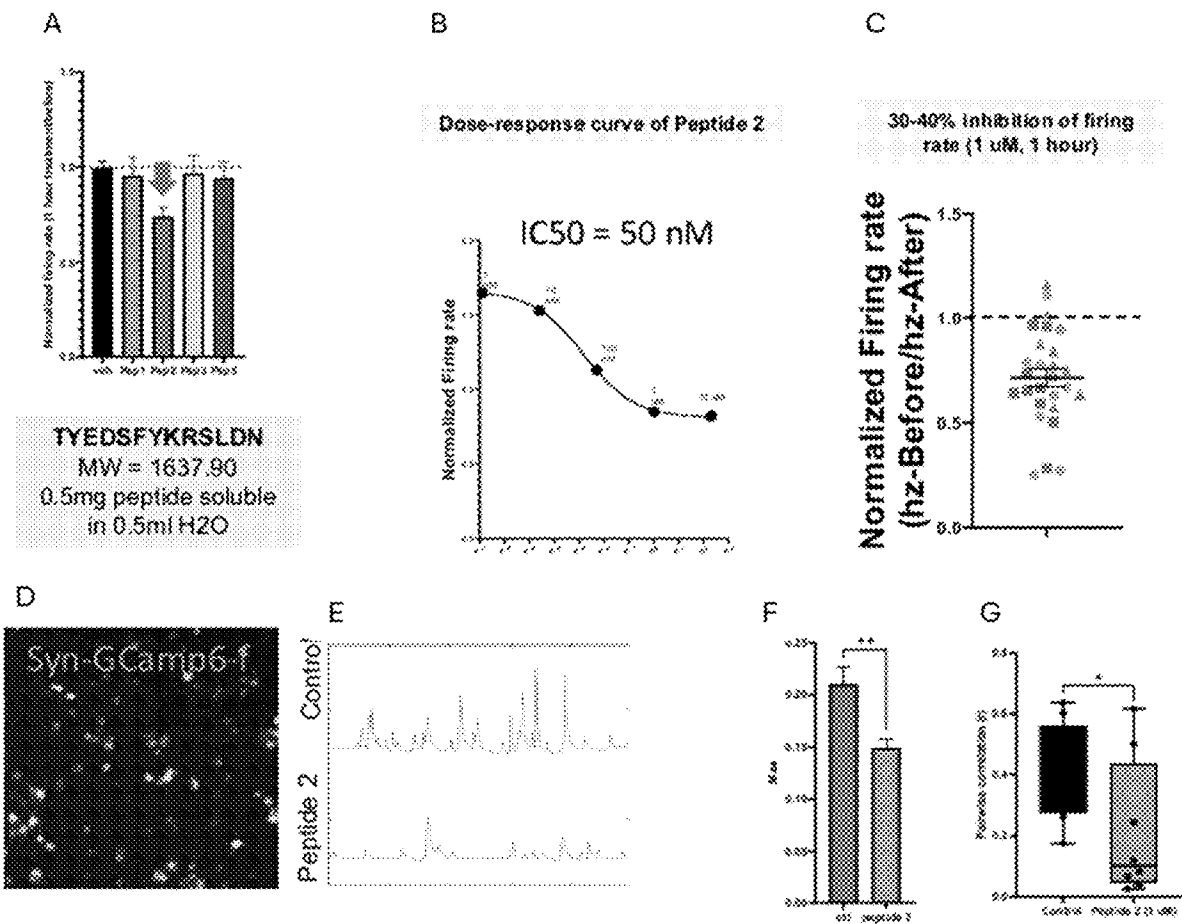


FIG. 15