(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2017 (28.09.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/165237 A1

(51) International Patent Classification:
A61M 21/02 (2006.01) *C12Q 1/02* (2006.01)
A61N 5/06 (2006.01) *A61B 5/04* (2006.01)
A61P 25/00 (2006.01)

(21) International Application Number:
PCT/US2017/023073

(22) International Filing Date:
17 March 2017 (17.03.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/311,119 21 March 2016 (21.03.2016) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, California 94607 (US).

(72) Inventors: **DAN, Yang**; 200C Li Ka Shing Center, Berkeley, California 94720 (US). **CHUNG, Shinjae**; 200C Li Ka Shing Center, Berkeley, California 94720 (US).

(74) Agent: **BORDEN, Paula A.**; 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2017/165237 A1

(54) Title: INHIBITORY SLEEP-CIRCUIT NEURONS AND METHODS OF MODULATING A STABLE STATE BY REGULATING THE SAME

(57) Abstract: Provided herein is a neural sleep circuit that includes: a first subtype of neurons in a preoptic area (POA) of the brain, wherein neurons of the first subtype (a) are gamma aminobutyric acidproducing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and one or more second subtypes of neurons that synapse onto neurons of the first subtype, and/or onto which neurons of the first subtype synapse. Methods of promoting or suppressing a sleep circuit-regulated state of an individual by modulating the activity of a subtype of neurons of the sleep circuit, and methods of identifying agents that promote or suppress the sleep circuit-regulated state are also provided.

INHIBITORY SLEEP-CIRCUIT NEURONS AND METHODS OF MODULATING A STABLE STATE BY REGULATING THE SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/311,119, filed March 21, 2016, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] Sleep is a naturally recurring state of the body and mind characterized by altered consciousness, relatively inhibited sensory activity, inhibition of nearly all voluntary muscles, and reduced interactions with surroundings. It is distinguished from wakefulness by a decreased ability to react to stimuli, but is more easily reversed than the state of hibernation or of being comatose. Mammalian sleep occurs in repeating periods, in which the body alternates between two highly distinct modes known as non-REM and REM sleep. REM stands for "rapid eye movement" but involves many other aspects including virtual paralysis of the body.

Literature

[0003] Weber et al. (2015) *Nature* 526:435

SUMMARY

[0004] Provided herein are inhibitory neurons of a neural sleep circuit, and methods of modulating a sleep circuit-regulated state of an individual by selectively regulating activity of the neural sleep circuit. The method may include illuminating with a light stimulus a region of a brain of an individual, the brain region containing a first subtype of sleep circuit neurons, wherein a light-activated polypeptide is selectively expressed in neurons of the first subtype, and wherein the light-activated polypeptide is configured to modulate activity of neurons of the first subtype when illuminated by the light stimulus, thereby promoting or suppressing a sleep circuit-regulated state of the individual, wherein the sleep circuit includes: a second subtype of neurons in a preoptic area (POA) of the brain, wherein neurons of the second subtype (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and one or more third subtypes of neurons that synapse onto, and/or receive a synaptic connection from, neurons of the second subtype.

[0005] Also provided are methods of identifying an agent that promotes or suppresses sleep. The method may include i) administering to a non-human mammal a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in sleep-promoting and/or sleep-activated sleep circuit neurons in a POA of a brain compared to the average level of expression of the gene in neurons of the POA, wherein the sleep circuit neurons (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and ii) monitoring a sleep circuit-regulated state of the non-human mammal, wherein the sleep circuit-regulated state comprises REM sleep, non-REM sleep and wakefulness, wherein a test agent that changes the probability of REM sleep, non-REM sleep, and/or wakefulness in the non-human mammal is identified as an agent that promotes or suppresses sleep.

[0006] The present disclosure provides a method of modulating a neural sleep circuit-regulated state of an individual, the method comprising: illuminating with a light stimulus a first region of a brain of an individual, the first region comprising a first subtype of sleep circuit neurons, wherein a light-activated polypeptide is selectively expressed in neurons of the first subtype, and wherein the light-activated polypeptide modulates activity of neurons of the first subtype when illuminated by the light stimulus, thereby promoting or suppressing a sleep circuit-regulated state of the individual, wherein the sleep circuit comprises: a second subtype of neurons in a preoptic area (POA) of the brain, wherein neurons of the second subtype (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and/or one or more third subtypes of neurons that synapse onto, and/or receive a synaptic connection from, neurons of the second subtype. In some cases, the illuminating step induces i) a first change in a probability of REM sleep and/or non-REM sleep, and ii) a second change in a probability of wakefulness, wherein the first and second change are of opposite sign. In some cases, neurons of the first subtype comprise neurons in the POA that express one or more neuropeptide genes selected from the group consisting of: corticotropin releasing hormone, galanin, neurotensin, tachykinin 1, tachykinin 2, prepronociceptin, calcitonin-related polypeptide alpha, prodynorphin, and cholecystokinin. In some cases, the second subtype of neurons comprises the first subtype of neurons. In some cases, the illuminating step increases the probability of REM sleep and/or non-REM sleep, and reduces the probability of wakefulness. In some cases, the one or more third subtypes of neurons comprise the first subtype of neurons. In some cases, neurons of the third subtype comprise hypothalamic or amygdala neurons. In some cases, the amygdala neurons are GABAergic neurons of the central nucleus of the amygdala (CEA). In some cases, the illuminating step reduces the probability of REM sleep and/or non-REM sleep, and increases the probability of wakefulness. In some cases, the light-activated

polypeptide is an ion channel or an ion pump. In some cases, the method further comprises monitoring a sleep circuit-regulated state of the individual before and/or after the illuminating step, wherein the sleep circuit-regulated state comprises REM sleep, non-REM sleep and/or wakefulness. In some cases, the monitoring comprises using electroencephalography (EEG) and/or electromyography (EMG) to determine the sleep circuit-regulated state. In some cases, the individual has a sleeping disorder. In some cases, the individual is sleep deprived. In some cases, the method further comprises, before the illuminating step, genetically modifying neurons of the first subtype with a nucleic acid comprising a nucleotide sequence encoding the light-activated polypeptide, wherein the nucleic acid is configured to express the light-activated polypeptide selectively in neurons of the first subtype. In some cases, the genetically modifying step comprises administering to a second region of the brain an expression vector comprising the nucleic acid, wherein the second region comprises one or more subtypes of neurons of the sleep circuit. In some cases, the expression vector is a recombinant viral expression vector, and wherein the administering comprises administering a virion comprising the viral expression vector. In some cases, the second region is the TMN or the POA. In some cases, the first region and second region are same regions of the brain. In some cases, the first region and second region are different regions of the brain, wherein the second region comprises neurons of a third subtype that receive a synaptic connection from neurons of the second subtype, and wherein the recombinant viral vector is derived from a lentiviral vector. In some cases, the virion is pseudotyped with a rabies glycoprotein. In some cases, the individual is a transgenic, non-human mammal expressing a site-specific recombinase in GABAergic neurons or glutamatergic neurons of the brain.

[0007] The present disclosure provides a method of identifying an agent that promotes or suppresses sleep, the comprising: i) administering to a non-human mammal a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in circuit neurons in a POA of a brain compared to the average expression level of the gene in neurons of the POA, wherein the sleep circuit neurons (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and ii) monitoring a sleep circuit-regulated state of the non-human mammal, wherein the sleep circuit-regulated state comprises REM sleep, non-REM sleep and/or wakefulness, wherein a test agent that changes the probability of REM sleep, non-REM sleep, and/or wakefulness in the non-human mammal is identified as an agent that promotes or suppresses sleep. In some cases, the test agent that causes i) a first change in the probability of REM sleep and/or non-REM sleep, and ii) a second change in the probability of wakefulness, wherein the first and second change are of opposite sign, is identified as an agent that promotes or suppresses sleep. In

some cases, the test agent specifically binds to the gene product. In some cases, the test agent is a small molecule, nucleic acid, polypeptide or a combination thereof. In some cases, the gene product is a post-transcriptional gene product. In some cases, the gene product is a post-translational gene product. In some cases, the gene encodes a cell-surface receptor. In some cases, the gene encodes a G-protein coupled receptor. In some cases, the gene encodes a polypeptide gene product selected from a polypeptide gene product listed in Table 3, in Figures 18A-18AI. In some cases, the monitoring step comprises using electroencephalography (EEG) and/or electromyography (EMG) to determine the sleep circuit-regulated state. In some cases, the non-human mammal has a sleeping disorder. In some cases, the non-human mammal is sleep deprived. In some cases, the non-human mammal is a rodent (e.g., a mouse; a rat). In some cases, the non-human mammal is a non-human primate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figures 1A-1B are a collection of images, diagrams and graphs showing retrograde labeling and c-Fos staining of sleep-active preoptic area (POA) neurons, according to embodiments of the present disclosure.

[0009] Figures 2A-2C are a collection of images, diagrams and graphs showing that optogenetic activation of $GAD^{POA\rightarrow TMN}$ neurons induces sleep, according to embodiments of the present disclosure.

[0010] Figures 3A-3F are a collection of images, diagrams and graphs showing that optogenetic activation of GAD^{POA} or $VGLUT^{POA\rightarrow TMN}$ neurons promotes wakefulness.

[0011] Figures 4A-4F are a collection of images and graphs showing that optogenetically identified $GAD^{POA\rightarrow TMN}$ neurons are active during sleep, according to embodiments of the present disclosure.

[0012] Figures 5A-5F are a collection of images, diagrams and graphs showing mapping of monosynaptic inputs to $GAD^{POA\rightarrow TMN}$ neurons, according to embodiments of the present disclosure.

[0013] Figures 6A-6F are a collection of images, diagrams and graphs showing identification of molecular markers for POA sleep-promoting neurons, according to embodiments of the present disclosure.

[0014] Figures 7A-7D are a collection of images, diagrams and graphs showing identification of molecular markers for POA sleep-promoting neurons, according to embodiments of the present disclosure.

[0015] Figure 8 is a collection of images and graphs showing c-Fos staining of sleep-active POA neurons in GAD1-GFP or VGLUT2-GFP mice, according to embodiments of the present disclosure.

[0016] Figures 9A-9B are a collection of a diagram and a graph showing the effect of laser stimulation in $GAD^{POA\rightarrow TMN}$-nlsPelican control mice.

[0017] Figures 10A-10C are a collection of graphs and diagrams showing the effect of laser stimulation on transition probability between each pair of brain states in $GAD^{POA\rightarrow TMN}$-ChR2, $GAD^{POA\rightarrow TMN}$-Ctrl and GAD^{POA}-ChR2 mice, according to embodiments of the present disclosure.

[0018] Figures 11A and 11B are a collection of graphs showing optogenetic identification of $GAD^{POA\rightarrow TMN}$ neurons, according to embodiments of the present disclosure.

[0019] Figure 12 is a graph showing the firing rates of unidentified POA neurons.

[0020] Figures 13A and 13B are a collection of graphs showing the identification of differentially expressed genes in TRAP, according to embodiments of the present disclosure.

[0021] Figures 14A and 14B are a collection of images showing the overlap of identified molecular markers in POA, according to embodiments of the present disclosure.

[0022] Figure 15 is a graphs showing that optogenetic stimulation of POA GAL neurons promotes wakefulness

[0023] Figures 16 shows Table 1, showing neuropeptide-encoding genes enriched in $GAD^{POA\rightarrow TMN}$ neurons, according to embodiments of the present disclosure.

[0024] Figure 17 shows Table 2, showing G-protein coupled receptors (GPCRs) whose expression is upregulated in $GAD^{POA\rightarrow TMN}$ neurons, according to embodiments of the present disclosure.

[0025] Figures 18A-18AI shows Table 3, showing genes whose expression is upregulated in $GAD^{POA\rightarrow TMN}$ neurons, according to embodiments of the present disclosure.

[0026] Figures 19A-19B are a collection of amino acid sequences of light-activated polypeptides that find use in embodiments of the present disclosure.

DEFINITIONS

[0027] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine

bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0028] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

[0029] By "hybridizable" or "complementary" or "substantially complementary" it is meant that a nucleic acid (e.g. RNA, DNA) comprises a sequence of nucleotides that enables it to non-covalently bind, i.e. form Watson-Crick base pairs and/or G/U base pairs, "anneal", or "hybridize," to another nucleic acid in a sequence-specific, antiparallel, manner (i.e., a nucleic acid specifically binds to a complementary nucleic acid) under the appropriate in vitro and/or in vivo conditions of temperature and solution ionic strength. Standard Watson-Crick base-pairing includes: adenine (A) pairing with thymidine (T), adenine (A) pairing with uracil (U), and guanine (G) pairing with cytosine (C) [DNA, RNA]. In addition, for hybridization between two RNA molecules (e.g., dsRNA), and for hybridization of a DNA molecule with an RNA molecule: guanine (G) can also base pair with uracil (U). For example, G/U base-pairing is partially responsible for the degeneracy (i.e., redundancy) of the genetic code in the context of tRNA anti-codon base-pairing with codons in mRNA.

[0030] Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein; and Sambrook, J. and Russell, W., Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (2001). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

[0031] Hybridization requires that the two nucleic acids contain complementary sequences, although mismatches between bases are possible. The conditions appropriate for hybridization between two nucleic acids depend on the length of the nucleic acids and the degree of complementarity, variables well known in the art. The greater the degree of complementarity between two nucleotide sequences, the greater the value of the melting temperature (Tm) for hybrids of nucleic acids having those sequences. For hybridizations between nucleic acids with short stretches of complementarity (e.g. complementarity over 35 or less, 30 or less, 25 or less, 22 or less, 20 or less, or 18 or less nucleotides) the position of mismatches can become important (see Sambrook et al., supra, 11.7-11.8). Typically, the length for a hybridizable nucleic acid is 8 nucleotides or more (e.g., 10 nucleotides or more, 12 nucleotides or more, 15 nucleotides or more, 20 nucleotides or more, 22 nucleotides or more, 25 nucleotides or more, or 30 nucleotides

or more). Temperature, wash solution salt concentration, and other conditions may be adjusted as necessary according to factors such as length of the region of complementation and the degree of complementation.

[0032] It is understood that the sequence of a polynucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable or hybridizable. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a bulge, a loop structure or hairpin structure, etc.). A polynucleotide can comprise 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, 99.5% or more, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which it will hybridize. For example, an antisense nucleic acid in which 18 of 20 nucleotides of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides. Percent complementarity between particular stretches of nucleic acid sequences within nucleic acids can be determined using any convenient method. Example methods include BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656), the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), e.g., using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489), and the like.

[0033] “Gene product” as used herein, may refer to any physiologically relevant biological molecule that is produced based on transcription and/or translation of a nucleic acid (e.g., DNA) containing a nucleotide sequence. The physiologically relevant biological molecule may be an RNA (e.g., mRNA, micro RNA, other regulatory RNAs, etc.), or a polypeptide encoded by the nucleic acid.

[0034] "Binding" as used herein (e.g. with reference to an active (e.g., active compound) binding to a gene product, and the like) refers to a non-covalent interaction between molecules. Binding interactions are generally characterized by a dissociation constant (K_D) of 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, 10^{-12} M or less, 10^{-13} M or less, 10^{-14} M or less, or 10^{-15} M or less. "Affinity" refers to the strength of binding, increased binding affinity being correlated with a lower K_D. “Specific binding” as used herein may refer to a non-covalent interaction between molecules mediated by one or more features (e.g., specific sequence of nucleotides, position/presence of specific amino acid side chains,

specific structure of a molecule, etc.) from each of the molecules, where the absence of a feature (e.g., change in the nucleotide sequence, change in amino acid sequence, change in molecular structure, etc.) from one or more of the molecules reduces the binding affinity.

[0035] The term "genetic modification" refers to a permanent or transient genetic change induced in a cell following introduction into the cell of a heterologous nucleic acid (e.g., a nucleic acid exogenous to the cell). Genetic change ("modification") can be accomplished by incorporation of the heterologous nucleic acid into the genome of the host cell, or by transient or stable maintenance of the heterologous nucleic acid as an extrachromosomal element. Where the cell is a eukaryotic cell, a permanent genetic change can be achieved by introduction of the nucleic acid into the genome of the cell. Suitable methods of genetic modification include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like.

[0036] An "individual" as used herein, may be any suitable animal amenable to the methods and techniques described herein, where in some cases, the individual may be a vertebrate animal, including a mammal, bird, reptile, amphibian, etc. The individual may be any suitable mammal, e.g., human, mouse, rat, cat, dog, pig, horse, cow, monkey, non-human primate, etc.

[0037] As used herein, the terms "treat," "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a health condition, disease or symptoms thereof and/or may be therapeutic in terms of a partial or complete cure for a health condition, disease and/or adverse effect attributable to the health condition or disease. "Treatment," as used herein, covers any treatment of a health condition or disease in a mammal, particularly in a human, and includes: (a) preventing the health condition or disease from occurring in a subject which may be predisposed to the health condition or disease but has not yet been diagnosed as having it; (b) inhibiting the health condition or disease, i.e., arresting its development; and (c) relieving the health condition or disease, e.g., causing regression of the disease, e.g., to completely or partially remove symptoms of the health condition or disease.

[0038] A "therapeutically effective amount" or "effective amount" means the amount of an agent that, when administered to a cell, a tissue, a mammal or other individual for obtaining a desired change in a physiological parameter, e.g., for treating a disease, is sufficient to effect such desired change, e.g., treatment for the disease or condition. The "therapeutically effective amount" will vary depending on the agent, the disease or condition and its severity and the age, weight, etc., of the subject to be treated.

[0039] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. When the term "pharmaceutically acceptable" is used to refer to a pharmaceutical carrier or excipient, it is implied that the carrier or excipient has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0040] An "agent" as used herein, may refer to a chemical compound, or combinations of chemical compounds. The physical entity may be a chemical compound (e.g., small molecule, organic compound, nucleic acid, polypeptide, inorganic molecule, etc.), a complex of chemical compounds (e.g., antibody, nucleoprotein complex, etc.), and the like.

[0041] By "small molecule" is meant a non-peptidic, non-oligomeric organic compound that may be synthetic or natural. A small molecule may contain one or more carbon-carbon bonds, and may have a molecular weight of 1500 daltons or less than 1500 daltons.

[0042] "Synapse" as used herein, may refer to the presence of a directional, chemical communication between a first neuron and a target cell, which may be a second neuron, that causes an electrical change in the target cell.

[0043] "Subtype of neuron," or "neuronal subtype" as used herein, may refer to a classification of neurons based on a static identity of a neuron, and/or a dynamic property of a neuron. A static identity of a neuron may include marker expression (e.g., mRNA transcript or protein expression), anatomical location and/or connectivity, etc. A dynamic property of a neuron may include the pattern (e.g., frequency, duration, timing, etc.) of depolarization/action potential firing. In some cases, a region of a brain may include two or more subtypes of neurons that are intermingled with each other such that the two or more subtypes of neurons are represented in a monolithic, three-dimensional volume (e.g., a substantially spherical volume) of the brain region containing a population of neurons (e.g., a population of 10 or more, e.g., 20 or more, 50 or more, 100 or more, 1,000 or more, 10,000 or more, including 100,000 or more, neurons). "Represent" as used herein may be used to indicate that a member of an ensemble is present in the ensemble in sufficient amount so as to functionally contribute to a property of the ensemble as a whole. In some cases, the member may be present at 0.1% or more, e.g., 1% or more, 5% or more, 10% or more, 20% or more, including 50% or more, of the entire ensemble.

[0044] "Circuit" as used herein, may refer to a collection of interconnected neurons through which activity of individual neurons is propagated to produce a physiological and/or behavioral

effect on an individual. In some cases, an activity pattern (e.g., frequency, amplitude, etc.) of neurons of the circuit may represent a state, e.g., a stable but reversible state, of the circuit (e.g., sleep, rapid eye movement (REM) sleep, non-REM sleep, wakefulness, etc.) that is distinguished from another state having a different activity pattern. The neurons may be organized into different subtypes, e.g., based on their functional role in the circuit, connectivity to other neurons, and/or gene expression profile.

[0045] "Selective" as used herein, may be used to describe an action that is applied to, or a property that is present in, a larger proportion of members within a first group compared to the proportion of members of a second group to which the action is applied, or in which the property is present, where the relative proportions are such that the difference in functional consequence of the action or property between the first and second groups can be attributed to the action or the property.

[0046] A "sleeping disorder" as used herein, may refer to a condition where a temporal progression through different sleep circuit-regulated state (e.g., REM sleep, non-REM sleep and/or wakefulness) of an individual deviates from a physiologically normal progression as exhibited by average members of the species to which the individual belongs in given light-dark cycle. "Sleep deprivation" may refer to a condition where an individual is unable to or prevented from being in REM sleep and/or non-REM sleep for an amount of time due to an disruption (either physiological or external) that would have been achieved but for the disruption. A sleeping disorder or sleep deprivation may be characterized by deterioration in the ability of an individual to perform a task relative to when in the absence of the sleeping disorder of sleep deprivation.

[0047] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0048] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one

or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0049] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0050] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a neuron" includes a plurality of such neurons and reference to "the agent" includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0051] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0052] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0053] As summarized above, methods of modulating a neural sleep circuit-regulated state of an individual, and methods of identifying an agent that modulates a neural sleep circuit-regulated state in an individual are provided. In general terms, the present methods are based on the identification of a subpopulation of sleep circuit neurons in the preoptic area (POA) of the brain that can promote a sleep state (e.g., rapid-eye movement (REM) sleep or non-REM sleep) when selectively activated, e.g., activated using optogenetic tools, and/or that are selectively active when the individual is in REM or non-REM sleep state. The present subtype of sleep circuit neurons of the POA are characterized by having an inhibitory projection to the tuberomammillary nucleus (TMN). The present subtype of sleep circuit neurons of the POA are spatially intermingled among other neurons of the POA having distinct functions, and many of these other neurons promote arousal. As a result, non-specific activation of neurons in the POA suppresses sleep and increase wakefulness. Thus, a sleep-circuit regulated state (e.g. REM sleep, non-REM sleep or wakefulness) can be modulated, to specifically promote or suppress sleep, by the neuronal subtype-specific regulation of neural activity of the present subtype of sleep circuit neurons of the POA, among a plurality of subtypes of neurons that are intermingled with each other in the POA, or by the neuronal subtype-specific modulation of neural activity of other subtypes of neurons that are functionally connected to (e.g., synapse onto or receive synaptic input from) the present subtype of sleep circuit neurons of the POA.

GABAERGIC, TMN-PROJECTING NEURONS OF THE PREOPTIC AREA

[0054] Aspects of the present disclosure include a subtype of neurons in a preoptic area (POA) of a brain of an individual, which subtype is characterized by inhibitory projections to the tuberomammillary nucleus (TMN) of the brain. Thus, inhibitory, TMN-projecting neurons of the present subtype use gamma aminobutyric acid (GABA) as neurotransmitter and express genes that are used for GABA biosynthesis and processing (i.e., are GABAergic neurons). The present subtype of sleep circuit neurons are GABAergic neurons and may express any suitable gene related to the production of GABA as a neurotransmitter, such as, but not limited to, glutamate decarboxylase (GAD1/2), vesicular GABA transporter (VGAT), GABA transporter 1 (GAT1). In some cases, the present subtype of GABAergic, TMN-projecting neurons of the POA may be identified by expression of a gene operatively linked to a regulatory sequence for GAD2, and by their projection to the TMN, and may be referred to herein as $GAD^{POA \rightarrow TMN}$ neurons. In some cases, the neurons are isolated. In some cases, the neurons are genetically modified to express a

light-activated polypeptide. In some cases, the neurons are *in vitro*, and are genetically modified to express a light-activated polypeptide.

[0055] The inhibitory projection of neurons in the POA may be determined based on, e.g., anterograde or retrograde tracing methods. A suitable retrograde tracing method is described in, e.g., Cetin et al., J. Neurophysiol. *111*, 2150-2159.

[0056] GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, of the present disclosure may be characterized by a transcriptome that is distinct from the average transcriptome of neurons of the POA. "Transcriptome" as used herein, may refer to the profile of the identity and corresponding expression levels of 100 or more, e.g., 200 or more, 300 or more, 400 or more, 500 or more, 800 or more, 1,000 or more, 5,000 or more, 10,000 or more, or substantially all genes in the genome of the individual from whom the neurons originate. In some embodiments, the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, have an expression level of a gene that is higher by 1.5 fold or more, e.g., 1.8 fold or more, 2.0 fold or more, 2.2 fold or more, 2.5 fold or more, 3.0 fold or more, 4.0 fold or more, 5.0 fold or more, including 10 fold or more, and in some cases higher by 1,000 fold or less, e.g., 500 fold or less, 100 fold or less, 50 fold or less, 20 fold or less, 10 fold or less, including 5.0 fold or less than the average expression level in neurons of the POA (i.e., the average expression level across neurons of the POA regardless of the neuronal subtype, which may include a relatively small fraction of the GABAergic, TMN-projecting POA neurons compared to the fraction of neurons that are not the GABAergic, TMN-projecting POA neurons). In some embodiments, the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, have an expression level of a gene that is higher by a range of 1.5 to 1,000 fold, e.g., 1.8 fold to 500 fold, 2.0 fold to 100 fold, 2.0 fold to 50 fold, 2.0 fold to 20 fold, including 2.0 fold to 10 fold, compared to the average expression level in neurons of the POA. The fold expression of a gene may be determined, e.g., by high-throughput sequencing of gene transcripts expressed in neurons of interest, followed by comparison of the value for fragments per kilobase of exon per million fragments mapped (FPKM) for each gene.

[0057] In some cases, the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, have an upregulated expression of neuropeptide genes (i.e., genes encoding a neuropeptide gene product), such as those listed in Table 1 of Figure 16. In some cases, the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, have upregulated expression of genes encoding a polypeptide as shown in Table 3 (Figures 18A-18AI), e.g., when the individual to which the neurons belong is a murine individual, or a homologue thereof, e.g., when the individual is a non-murine individual.

[0058] A further aspect of the present disclosure includes a neural sleep circuit that includes neurons that form a neural pathway such that the activity of the neurons are reflected in the output of the circuit, which output are functionally linked (e.g., via synaptic connections, endocrine signals, etc.) to other components of the body (e.g., other neurons of the brain and body, muscle, internal organs, etc.) to promote one stable state over one or more other stable states (e.g., promote rapid eye movement (REM) and/or non-REM sleep over arousal) of the individual. The sleep circuit may include neurons that are found in any suitable anatomical region of the brain that can control a stable state (e.g. REM sleep, non-REM sleep or wakefulness) of the individual. The sleep circuit may include, without limitation, neurons of the brainstem, thalamus, hypothalamus, the basal forebrain, and the limbic system, etc. The sleep circuit may include, without limitation, neurons of the pedunculopontine and laterodorsal tegmental nucleus (PPT/LDT), locus coeruleus, dorsal and median raphe nucleus, tuberomammillary nucleus (TMN), preoptic area (POA), amygdala, ventral periaqueductal grey matter, lateral hypothalamus, etc. Neurons of the sleep circuit may be characterized by having a specific type of neurotransmitter, such as GABA, glutamate, acetylcholine, serotonin, dopamine, histamine, noradrenaline, etc., and may be characterized by having a specific peptide hormone, such as orexin/hypocretin, melatonin-concentrating hormone, etc.

[0059] In some cases, the sleep circuit includes GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, as described above. The sleep circuit may also include pre- and/or post-synaptic partners of the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. In some cases, the sleep circuit includes neurons of the POA that are characterized by having expression of one or more genes that are upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, compared to the average expression level of the respective genes in neurons of the POA overall. The upregulated genes are described, e.g., in Table 1 of Figure 16, Table 2 of Figure 17, and Table 3 of Figures 18A-18AI. In some embodiments, the upregulated gene has an expression level in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, that is 1.5 fold or more, e.g., 1.8 fold or more, 2.0 fold or more, 2.5 fold or more, including 3.0 fold or more, and in some cases, by 100 fold or less, e.g., 50 fold or less, 30 fold or less, 20 fold or less, 10 fold or less, 5.0 fold or less, including 4.0 fold or less, higher compared to its respective average expression level in the POA. In some embodiments, the upregulated gene has an expression level in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, that is in the range of 1.5 fold to 100 fold, e.g., 1.5 fold to 50 fold, 1.5 fold to 30 fold, 2.0 fold to 30 fold, 2.5 fold to 30 fold, including 3.0 fold to 30 fold, higher compared to its respective average expression level in the POA.

[0060] In some embodiments, the sleep circuit includes neurons of the POA that have upregulated expression of one or more neuropeptide genes selected from: corticotropin releasing hormone, galanin, neurotensin, tachykinin 1, tachykinin 2, prepronociceptin, calcitonin-related polypeptide alpha, prodynorphin, and cholecystokinin, compared to their respective average expression level in neurons of the POA. In some embodiments, the sleep circuit includes neurons of the POA that have upregulated expression of one or more GPCR genes selected from: 5-hydroxytryptamine (serotonin) receptor 1F; cholinergic receptor, muscarinic 4; arginine vasopressin receptor 1A, adrenergic receptor, beta 1; progestin and adipoQ receptor family member VII; G protein-coupled receptor 83; and G protein-coupled receptor 83, compared to their respective average expression level in neurons of the POA.

[0061] The sleep circuit may function as a switch between different stable behavioral, physiological and neurological states of the individual. The stable states may include rapid eye movement (REM) sleep, non-REM sleep and wakefulness, and the different states of the individual may be determined using a suitable method for measuring neuronal and/or muscle activity. In some cases, the state of the individual is monitored using electroencephalography, electromyography, or a combination thereof. Thus, in some cases, REM sleep is defined by having a high power at theta frequencies as measured by EEG; non-REM sleep is defined by a synchronized EEG with high amplitude and low frequency; and wakefulness is defined by desynchronized EEG. In some embodiments, REM sleep is defined by a low EMG activity; non-REM sleep is defined by low EMG activity; and wakefulness is defined by high EMG activity. REM sleep may be defined by a high power at theta frequencies as measured by EEG, and low EMG activity; non-REM sleep may be defined by a synchronized EEG with high amplitude and low frequency, and low EMG activity; and wakefulness may be defined by desynchronized EEG, and high EMG activity. The threshold for EEG frequency may vary depending on, e.g., the species to which the individual belongs. For example, if the individual is a mouse, the low EEG frequency may include a range of 0.5-4 Hz, and a theta frequency may include a range of 6-9 Hz. If the individual is a human, a theta frequency may include a range of 4-7 Hz.

[0062] In some cases, the state of the individual may be monitored by visual observation, e.g., visually determining whether the individual is awake or not. In some cases, the state of the individual may be monitored subjectively, e.g., based on the individual's subjective experience of sleep and/or wakefulness.

METHODS

Method of Modulating a Neural Sleep Circuit-Regulated State

[0063] As summarized above, methods modulating a neural sleep circuit-regulated state of an individual are provided. In general terms, the present method may include optogenetically stimulating a subtype of neurons of a neuronal sleep circuit of the individual to promote or suppress a sleep circuit-regulated state (e.g., REM sleep, non-REM sleep, wakefulness). The sleep circuit may include GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, and other neurons having a synaptic connection thereto, as described above. By "optogenetic stimulation" is meant any one of a number of methods of modulating the electrical activity of a neuron that expresses a light-activated polypeptide (e.g., a depolarizing light-activated polypeptide such as channelrhodopsin 2 (ChR2), C1V1, SFO, SSFO, etc., as described further below) by illuminating the neuron with a light stimulus suitable for activating the light-activated polypeptide.

[0064] A suitable optogenetic stimulation may target and selectively modulate the neural activity of a functionally-defined subtype of neurons in the sleep circuit such that the optogenetic stimulation preferentially changes the activity of the targeted subtype of neurons without directly modulating the neural activity of another functionally distinct subtype of neuron of the sleep circuit. In some cases, the optogenetically targeted subtype of neurons is intermingled within a population of different subtypes of neurons having distinct and/or opposite function as the targeted subtype, and the optogenetic stimulation can modulate the activity of the targeted subtype of neurons without substantially modulating the activity of the non-targeted subtype of neurons. Such selectivity may be achieved using any suitable method, e.g., by selectively expressing a light-activated polypeptide in the subtype of neurons of interest, as described further below.

[0065] The level of activity of neurons of the sleep circuit may control the likelihood or probability that an individual is in any given stable state. In some embodiments, the activity of a subtype of neurons in the sleep circuit promotes REM sleep, by increasing the transition probability from another state (e.g., non-REM sleep or wakefulness) to REM sleep, and/or by reducing the transition probability from REM sleep to another state, when neurons of the subtype are more active as compared to when they are less active.

[0066] In some embodiments, the activity of a subtype of neurons in the sleep circuit suppresses REM sleep, by reducing the transition probability from another state (e.g., non-REM sleep or wakefulness) to REM sleep, and/or by increasing the transition probability from REM sleep to another state, when neurons of the subtype are more active as compared to when they are less active.

[0067] In some embodiments, the activity of a subtype of neurons in the sleep circuit promotes non-REM sleep, by increasing the transition probability from another state (e.g., REM sleep or wakefulness) to non-REM sleep, and/or by reducing the transition probability from non-REM sleep to another state, when neurons of the subtype are more active as compared to when they are less active.

[0068] In some embodiments, the activity of a subtype of neurons in the sleep circuit suppresses non-REM sleep, by reducing the transition probability from another state (e.g., REM sleep or wakefulness) to non-REM sleep, and/or by increasing the transition probability from non-REM sleep to another state, when neurons of the subtype are more active as compared to when they are less active.

[0069] In some embodiments, the activity of a subtype of neurons in the sleep circuit promotes arousal, by increasing the transition probability from another state (e.g., REM sleep or non-REM sleep) to wakefulness, and/or by reducing the transition probability from wakefulness to another state, when neurons of the subtype are more active as compared to when they are less active.

[0070] In some embodiments, the activity of a subtype of neurons in the sleep circuit suppresses arousal, by reducing the transition probability from another state (e.g., REM sleep or non-REM sleep) to wakefulness, and/or by increasing the transition probability from wakefulness to another state, when neurons of the subtype are more active as compared to when they are less active.

[0071] The activity of a subtype of neurons in the sleep circuit may suppress a sleep circuit-regulated state (e.g. REM sleep, non-REM sleep, or wakefulness) at the expense of one or more of the other sleep circuit-regulated states. In some embodiments, a change in the probability that the individual is in REM sleep and/or non-REM sleep is accompanied by a change of opposite sign in the probability that the individual is in wakefulness. In some cases, an increase in the probability that the individual is in REM sleep and/or non-REM sleep is accompanied by a reduction in the probability that the individual is in wakefulness. In some cases, a reduction in the probability that the individual is in REM sleep and/or non-REM sleep is accompanied by an increase in the probability that the individual is in wakefulness. The magnitude of change in one sleep circuit-regulated state relative a change of opposite sign in another sleep circuit-regulated state may be different (e.g., 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 75% or more, including 100% or more different), similar (e.g., up to 10%, up to 5%, including up to 3% different) or may be substantially the same.

[0072] The optogenetic stimulation may include illuminating with a light stimulus, a region of the brain of the individual (e.g., a human; a non-human mammal such as a non-human primate, a

rat, a mouse, etc.), which region includes sleep circuit neurons of a particular subtype that has been genetically modified to express a light-activated polypeptide, e.g., a light-activated ion channel such as channelrhodopsin 2 (ChR2), as described further below, where activation of the light-activated polypeptide by the light stimulus induces changes in the neuronal activity (e.g., depolarization or hyperpolarization) of the neurons expressing the light-activated polypeptide. The light stimulus may vary, and may depend on, e.g., the absorption spectrum, expression level, subcellular localization, etc., of the light-activated polypeptide. For example, the light stimulus may have a wavelength that is close to the optimal wavelength for activating the light-activated polypeptide (e.g., around 480 nm or ChR2). The light stimulus may include one or more pulses of light, each pulse having a duration of 0.1 milliseconds (ms) or more, e.g., 0.5 ms or more, 1.0 ms ormore, 2.0 ms or more, including 5.0 ms or more, and in some cases a duration of 100 ms or less, e.g., 50 ms or less, 25 ms or less 20 ms or less, including 15 ms or less. In some embodiments, the light stimulus may include one or more pulses of light, each pulse having a duration in the range of 0.1 to 100 ms, e.g., 0.5 to 100 ms, 1.0 to 50 ms, including 2.0 to 25 ms.

[0073] The light stimulus may include pulses of light applied at a regular frequency. In some cases, the light stimulus may include pulses of light applied at a frequency of 1 Hz or more, e.g., 2 Hz or more, 5 Hz or more, 10 Hz or more, 15 Hz or more, including 20 Hz or more, and in some cases, at a frequency of 100 Hz or less, e.g., 80 Hz or less, 60 Hz or less, 40 Hz or less, including 30 Hz or less. In some embodiments, the light stimulus may include pulses of light applied at a frequency in the range of 1 Hz to 100 Hz, e.g., 2 Hz to 80 Hz, 5 Hz to 60 Hz, 10 Hz to 40 HZ, including 10 Hz to 30 Hz.

[0074] The light stimulus may include one or more pulses of light having any suitable power. In some embodiments, the light stimulus may include one or more pulses of light having a power of 0.1 mW or more, e.g, 0.5 mW or more, 1.0 mW or more, 2.0 mW or more, including 4.0 mW or more, and in some cases, a power of 50 mW or less, e.g., 40 mW or less, 30 mW or less, 20 mW or less, including 10 mW or less, at the tip of an optical fiber used to deliver the light stimulus to the brain region. In some embodiments, the light stimulus may include one or more pulses of light having a power in the range of 0.1 to 50 mW, e.g., 0.5 to 40 mW, 1.0 to 30 mW, 2.0 to 20 mW including 4.0 to 10 mW.

[0075] The light stimulus may be delivered to the brain region using any suitable method. In some cases, the light stimulus is delivered to the brain region using an optical fiber having an end implanted into the brain region of interest, e.g., the brain region that includes a subtype of neurons belonging to the present sleep circuit, where the subtype of neurons express a light-activated polypeptide. The optical fiber may be any suitable optical fiber for delivering the light stimulus. The optical fiber may be a multimode or single mode optical fiber.

[0076] The change in a sleep circuit-regulated state induced by optogenetic stimulation may occur within a time interval that varies depending on, e.g., the light-activated polypeptide expressed in neurons of the sleep circuit, the light stimulus properties and stimulation protocol, the nature of the neurons being activated by the optogenetic stimulation, etc. In some cases, the sleep circuit-regulated state is promoted or suppressed substantially immediately after illuminating the neurons with a light stimulus. In some cases, the sleep circuit-regulated state is promoted or suppressed within 1 second (s) or more, e.g., within 5 seconds (s) or more, within 10 s or more, within 20 s or more, within 30 s or more, within 1 minute (min) or more, within 5 minutes (min) or more, within 10 min or more, including within 30 min or more, and in some cases, within 1 day or less, e.g., within 18 hours (hr) or less, within 12 hr or less, within 6 hr or less, within 3 hr or less, within 1 hour (hr) or less, within 30 min or less, within 15 min or less, within 5 min or less, within 1 min or less, 45 s or less, including 30 s or less, upon illumination of the neurons. In some embodiments, the sleep circuit-regulated state is promoted or suppressed within a time ranging from 1 s to 30 s, 5 s to 30 s, 10 s to 30 s, 10 s to 45 s, 20 s to 45 s, 20 s to 1 min, 1min to 5 min, 5 min to 15 min, 10 min to 15 min, 10 min to 30 min, 30 min to 1 hr, 30 min to 3 hr, 30 min to 3 hr, 30 min to 6 hr, 30 min to 12 hr, 30 min to 18 hr, or 30 min to 1 day, upon illumination of the neurons.

[0077] The changes in the state of the individual, as described above, induced by activation of neurons of a subtype of neurons in the sleep circuit may be defined relative to a suitable reference condition. In some cases, the measured states (e.g., probability of states) are compared before and after performing a method step (e.g., before and after illuminating a neuron). The comparison may be made on an individual basis (i.e., comparison between before and after performing a method step for each individual), or may be made on a population basis (i.e., comparison between a population average before and a population average after performing a method step on a number of individuals). In some cases, the measured states (e.g., probability of states) are compared to a predetermined standard, e.g., a predetermined threshold value. The predetermined standard may be obtained by monitoring sleep-circuit regulated states in a control group of individuals (i.e., a group of individuals to whom a method step has not been performed).

[0078] The subtype of neurons of the sleep circuit to be targeted may vary depending on the desired outcome. In some cases, the optogenetic stimulation selectively modulates the activity of the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, as described above. An optogenetic stimulation that selectively activates GABAergic, TMN-projecting neurons of the POA, e.g., $GAD^{POA \rightarrow TMN}$ neurons, may promote REM sleep and/or non-REM sleep and suppresses arousal in the individual.

[0079] In some embodiments, the optogenetic stimulation selectively modulates the activity of GABAergic POA neurons that also express a gene that is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. Examples of such upregulated genes are shown in, e.g., Table 3, in Figures 18A-18AI. In some cases, the optogenetic stimulation selectively modulates the activity of GABAergic POA neurons that also express a gene that is upregulated by 1.5 fold or more, e.g., 1.8 fold or more, 2.0 fold or more, 2.5 fold or more, including 3.0 fold or more, and in some cases, by 100 fold or less, e.g., 50 fold or less, 30 fold or less, 20 fold or less, 10 fold or less, 5.0 fold or less, including 4.0 fold or less, compared to its respective average expression level in the POA. In some embodiments, the optogenetic stimulation selectively modulates the activity of GABAergic POA neurons that also express a gene that is upregulated by a range of 1.5 fold to 100 fold, e.g., 1.5 fold to 50 fold, 1.5 fold to 30 fold, 2.0 fold to 30 fold, 2.5 fold to 30 fold, including 3.0 fold to 30 fold, compared to its respective average expression level in the POA.

[0080] In some cases, the optogenetic stimulation selectively modulates the activity of GABAergic POA neurons that also express one or more neuropeptide genes selected from corticotropin releasing hormone, galanin, neurotensin, tachykinin 1, tachykinin 2, prepronociceptin, calcitonin-related polypeptide alpha, prodynorphin, and cholecystokinin. In some cases, the gene upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, including any one or more of the neuropeptide genes listed above, may be expressed at higher level, compared to the average expression level of respective genes in the POA, in the GABAergic POA neurons selectively modulated by the optogenetic stimulation. In some embodiments, an optogenetic stimulation that selectively activates the GABAergic neurons of the POA promotes REM sleep and/or non-REM sleep and suppresses arousal in the individual.

[0081] In some embodiments, the optogenetic stimulation selectively modulates activity of neurons that synapse onto, or which receives synaptic input from, the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. Neurons that synapse onto the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, may include any neurons with such connectivity, and may include, without limitation, a subtype of neurons in other areas of the hypothalamus, or the amygdala. The functional connectivity between the neurons may be activating (e.g., glutamatergic) or inhibitory (e.g., GABAergic). In some cases, the optogenetic stimulation selectively modulates activity of GABAergic neurons of the central nucleus of the amygdala (CEA). In some embodiments, the optogenetic stimulation selectively activates GABAergic neurons of the CEA to suppress REM sleep and/or non-REM sleep, and to promote arousal. In some embodiments, the optogenetic stimulation selectively activates a

subtype of neurons of the CEA to promote REM sleep and/or non-REM sleep, and to suppress arousal.

[0082] Neurons that receive input from the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, may include any neurons with such connectivity, and may include, without limitation, a subtype of neurons in other areas of the hypothalamus (e.g., the dorsomedial hypothalamus), the TMN, or the ventrolateral periaqueductal grey (vlPAG).

Optogenetic Stimulation for Selective Modulation of the Activity of a Subtype of Sleep Circuit Neurons

[0083] An optogenetic stimulation that selectively modulate the activity of a subtype of neurons in the sleep circuit includes a light stimulus, and the subtype of neurons in the sleep circuit targeted by the light stimulus is genetically modified to express a light-activated polypeptide configured to depolarize or hyperpolarize the neuron in which the light-activated polypeptide is expressed when activated by the light stimulus. Selective modulation of the subtype of neurons in the sleep circuit may be achieved by expression of the light-activated polypeptide selectively in the subtype of neurons in the sleep circuit through, e.g., the use of appropriate regulatory elements in a nucleic acid encoding the light-activated polypeptide, and/or localized delivery of and genetic modification by the nucleic acid encoding the light-activated polypeptide, etc. Localized delivery of the light stimulus may also provide for selective modulation of the subtype of neurons in the sleep circuit.

[0084] Any suitable light-activated polypeptide may be used in the present method, as described further below. In some embodiments, the light-activated polypeptide is a light-activated ion channel (e.g., channelrhodopsin 2 (ChR2); C1V1; SFO; SSFO; etc.) where administering the light stimulus depolarizes the subtype of neurons in the sleep circuit and that express the light-activated ion channel. In some embodiments, the light-activated polypeptide is a light-activated ion pump, where administering the light stimulus hyperpolarizes the subtype of neurons in the sleep circuit and that express the light-activated ion pump.

Method of Identifying an Agent that Promotes or Suppresses Sleep

[0085] Also provided herein are methods, e.g., screening methods, for identifying an agent that promotes or suppresses sleep in an individual. The present method may include administering to an individual (e.g., a non-human mammal, such as a rat, a mouse, a non-human primate, etc.) a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, as described above; and monitoring a sleep circuit-regulated state (e.g., REM sleep, non-REM sleep and/or wakefulness) of the individual. If the test agent changes one or more

probabilities for the individual being in a sleep circuit-regulated state (e.g., REM sleep, non-REM sleep and/or wakefulness), the test agent may be determined to be an agent that modulates sleep, e.g., promotes or suppresses sleep. For example, if the test agent increases the probability of REM sleep and/or non-REM sleep, and reduces the probability of wakefulness in the individual, the test agent may be determined to be an agent that promotes sleep. Likewise, if the test agent reduces the probability of REM sleep and/or non-REM sleep, and increases the probability of wakefulness in the individual, the test agent may be determined to be an agent that suppresses sleep. Test agents identified according to the present method may find use in promoting or suppressing sleep in an individual in need, e.g., an individual with a sleeping disorder.

[0086] The gene product may be any suitable gene product of a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. Suitable genes are listed in, e.g., Table 3, in Figures 18A-18AI. In some embodiments, the test agent targets a gene product of a gene that is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, by 1.5 fold or more, e.g., 1.8 fold or more, 2.0 fold or more, 2.5 fold or more, including 3.0 fold or more, and in some cases, by 100 fold or less, e.g., 50 fold or less, 30 fold or less, 20 fold or less, 10 fold or less, 5.0 fold or less, including 4.0 fold or less, compared to its respective average expression level in the POA. In some embodiments, the test agent targets a gene product of a gene that is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, by a range of 1.5 fold to 100 fold, e.g., 1.5 fold to 50 fold, 1.5 fold to 30 fold, 2.0 fold to 30 fold, 2.5 fold to 30 fold, including 3.0 fold to 30 fold, compared to its respective average expression level in the POA.

[0087] In some embodiments, the gene product encoded by a gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, includes a cell-surface receptor, such as a G-protein coupled receptor (GPCR). The GPCR targeted by the test agent in the present method may include one or more GPCRs selected from 5-hydroxytryptamine (serotonin) receptor 1F; cholinergic receptor, muscarinic 4; arginine vasopressin receptor 1A, adrenergic receptor, beta 1; progestin and adipoQ receptor family member VII; G protein-coupled receptor 83; and G protein-coupled receptor 83 (see also, Table 2 in Figure 17).

[0088] The test agent may be any suitable agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. The test agent may be, for example, a protein or polypeptide, nucleic acid, lipid, carbohydrate, antibody, small molecule, inorganic molecule, etc., that modulates a functional activity of the gene product. In some embodiments, the test

agent selectively or specifically interacts with the gene product to modulate the function of the gene product. The specific interaction may include a specific binding interaction between the test agent and the gene product (e.g., RNA or polypeptide). The specific binding may be characterized by a dissociation constant (K_D) of 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, including 10^{-12} M or less.

[0089] The test agent of the present disclosure may specifically interact with a gene product encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, as described above, in a manner sufficient to modulate, e.g., inhibit or enhance, a functional activity of the gene product. The functional activity modulated by the test agent may vary depending on the whether the gene product with which the test agent specifically interacts is a post-transcriptional nucleic acid gene product, e.g., RNA, or post-translational gene product, e.g., polypeptide, and/or may depend on the functional role of the gene product, e.g., polypeptide, in the neuronal subtype and/or the sleep circuit. In some embodiments, where the gene product targeted by the test agent is a post-transcriptional nucleic acid gene product, e.g., RNA, the test agent may specifically interact with the gene product to reduce or prevent translation of the transcribed RNA, and/or reduce the level of the transcribed RNA in the neuron. In some embodiments, where the gene product is a post-translational gene product, e.g, polypeptide, the test agent may specifically interact with the gene product to reduce or suppress a function of the polypeptide, e.g., reduce or suppress an enzymatic function, binding function, structural function, signaling function, etc.

[0090] In some embodiments, the gene product is a nucleic acid gene product, such as an RNA transcribed from the gene, or a polypeptide gene product, such as a protein translated from an mRNA encoded by the gene. In some embodiments, the functional activity of a nucleic acid gene product, such as an RNA, may be modulated by a test agent that specifically interacts with the nucleic acid gene product. A test agent that specifically interacts with, e.g., specifically binds to, the nucleic acid gene product may be any suitable test agent, such as, but not limited to, a nucleic acid that hybridizes to the nucleic acid gene product, a nucleic acid-binding polypeptide that binds to the nucleic acid gene product in a sequence-specific manner, etc. In some cases, the test agent is an inhibitory RNA, e.g., a small-interfering RNA (siRNA) or short hairpin RNA (shRNA) that is designed to target the nucleic acid gene product, e.g., RNA, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons.

[0091] Thus, in some embodiments, the test agent can specifically bind to a post-transcriptional nucleic acid gene product, e.g., RNA, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. The specificity of

the interaction may be determined by the structural relationship between a nucleotide sequence of the post-transcriptional nucleic acid gene product, e.g., RNA, and the test agent. In some cases, the test agent is a nucleic acid containing a nucleotide sequence that can hybridize with the post-transcriptional nucleic acid gene product, e.g., RNA, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. In some cases, the test agent is an inhibitory RNA, e.g., a small-interfering RNA (siRNA) or short hairpin RNA (shRNA) that is designed to target the post-transcriptional nucleic acid gene product, e.g., RNA, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. Any suitable method may be used to design and administer a nucleic acid to target the post-transcriptional nucleic acid gene product, e.g., RNA, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. Suitable methods are described, e.g., in US App. Pub. Nos. 20100143450; 20110009468; 2011020779; 20110319475; 20120165397; 20120177723; PCT Pub. No. WO2013/138783; Baker-Herman et al., 2004 Nat Neurosci. 7:48; Pettersson et al., 2014 PLoS One 9:e100730; Jacobs et al., 1999 Neoplasia 1:402, which are incorporated herein by reference. Commercial sources of siRNA and/or shRNA include Santa Cruz Biotech and Thermo Fisher.

[0092] In some embodiments, the test agent specifically binds to a post-translational gene product, e.g., polypeptide, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. The specificity of the interaction may be determined by the structural relationship between the post-translational gene product, e.g., polypeptide, and the test agent. In some cases, the test agent is an antibody that specifically binds to an epitope on the post-translational gene product, e.g., polypeptide, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. In some cases, the test agent is a small molecule that specifically binds to one or more interaction pockets in or on the post-translational gene product, e.g., polypeptide, encoded by the gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons.

[0093] In some embodiments, the functional activity of a polypeptide gene product, such as an enzyme, scaffold protein, structural protein, signaling protein, etc., may be modulated by a test agent that specifically interacts with the polypeptide gene product. The specific interaction in some cases may be a specific binding interaction. A test agent that specifically binds to the polypeptide gene product may be any suitable test agent, such as, but not limited to, a polypeptide, an antibody or a small molecule. For example, if the polypeptide gene product binds a binding partner for functional activity, the test agent may be a polypeptide containing at

least the binding domain on the binding partner that is sufficient for the specific binding interaction. In another example, an antibody test agent may specifically bind to an epitope on the polypeptide gene product to functionally modulate the activity of the polypeptide gene product. The test agent may be an agent that functionally modulates the activity of a polypeptide gene product in any other suitable manner.

[0094] The modulation of the functional activity of the gene product by the test agent through its specific interaction with the gene product, as described above, may in turn result in selective modulation of the activity of a subtype of neurons of a sleep circuit of a brain of the individual, to promote or suppress a sleep circuit-regulated state, as described herein. In some embodiments, where the gene product normally promotes the activity of the subtype of neurons of the sleep circuit to promote a sleep circuit-regulated state, a test agent that inhibits the functional activity of the gene product may suppress the sleep circuit-regulated state. In some embodiments, where the gene product normally suppresses the activity of the subtype of neurons of the sleep circuit to promote a sleep circuit-regulated state, a test agent that inhibits the functional activity of the gene product may suppress the sleep circuit-regulated state. In some embodiments, where the gene product normally promotes the activity of the subtype of neurons of the sleep circuit to promote a sleep circuit-regulated state, a test agent that enhances the functional activity of the gene product may further promote the sleep circuit-regulated state. In some embodiments, where the gene product normally suppresses the activity of the subtype of neurons of the sleep circuit to promote a sleep circuit-regulated state, a test agent that enhances the functional activity of the gene product may suppress the sleep circuit-regulated state.

[0095] The monitoring may be done in any suitable manner to determine a sleep circuit-regulated state (e.g., REM sleep, non-REM sleep and/or wakefulness) of the individual. In some cases, the state of the individual is monitored using electroencephalography, electromyography, or a combination thereof. Thus, in some cases, REM sleep is defined by having a high power at theta frequencies as measured by EEG; non-REM sleep is defined by a synchronized EEG with high amplitude and low frequency; and wakefulness is defined by desynchronized EEG. In some embodiments, REM sleep is defined by a low EMG activity; non-REM sleep is defined by low EMG activity; and wakefulness is defined by high EMG activity. In some embodiments, REM sleep is defined by a high power at theta frequencies as measured by EEG, and low EMG activity; non-REM sleep is defined by a synchronized EEG with high amplitude and low frequency, and low EMG activity; and wakefulness is defined by desynchronized EEG, and high EMG activity. The threshold for EEG frequency may vary depending on, e.g., the species to which the individual belongs. For example, if the individual is a mouse, the low EEG frequency

may include a range of 0.5-4 Hz, and a theta frequency may include a range of 6-9 Hz. If the individual is a human, a theta frequency may include a range of 4-7 Hz.

[0096] The individual may be any suitable individual, and in some cases, the individual is an experimental animal, e.g., a mouse, rat, etc., that can be genetically manipulated to express, e.g., a heterologous gene product, such as a light-activated ion channel or ion pump, in one or more cells of interest, e.g., a subtype of neurons of a sleep circuit that includes GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. In some embodiments, the individual is a non-human individual, e.g, a non-human mammal. In some cases, the individual is a human.

[0097] The test agent may be administered to an individual in a suitable pharmaceutical composition, as desired. In some embodiments, the test agent is in a pharmaceutical composition which, when administered to the individual, specifically modulates the functional activity of a gene product encoded by a gene whose expression is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, e.g., in the brain.

Pharmaceutical compositions and methods of administration thereof

[0098] The pharmaceutical composition administered to the individual may include any other suitable components for administering, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, hydrochloride, sulfate salts, solvates (e.g., mixed ionic salts, water, organics), hydrates (e.g., water), and the like.

[0099] In some cases, a subject pharmaceutical composition will be suitable for injection into a subject, e.g., will be sterile. For example, in some embodiments, a subject pharmaceutical composition will be suitable for injection into a subject, e.g., where the composition is sterile and is free of detectable pyrogens and/or other toxins.

[00100] In some embodiments, a test agent modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, is formulated in a sustained release dosage form that is designed to release the test agent at a predetermined rate for a specific period of time. Such sustained release formulations may include, for example, formulations for use in drug delivery implants or devices, e.g., ingestible devices.

[00101] For oral preparations, a test agent modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA

neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[00102] In certain embodiments, a test agent modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00103] Unit dosage forms for oral administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, or tablet, contains a predetermined amount of the test agents of the present disclosure. Similarly, unit dosage forms for injection or intravenous administration may include one or more test agents that selectively modulate activity of a subtype of neurons of a sleep circuit in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[00104] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the test agent of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the unit dosage forms of the present disclosure depend on the particular test agent or test agents employed and the effect to be achieved, and the pharmacodynamics associated with each agent in the subject.

[00105] Any suitable pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents may be employed in the subject methods. Moreover, any suitable pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, may be used.

[00106] Any of the test agents of the present disclosure may be formulated for use in any route of administration or dosage form disclosed herein.

[00107] In practicing the methods of the present disclosure, routes of administration may be selected according to any of a variety of factors, such as properties of the test agent(s) to be delivered, the type of condition to be treated (e.g., type of sleeping disorder), and the like. For instance the pharmaceutical composition containing aa test agent of the present disclosure, e.g., a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, may be delivered systemically or locally. In some instances, pharmaceutical composition containing a test agent of the present disclosure are administered orally, such as through the digestive tract (enteral administration), buccal, sublabial, or sublingual administration. Such dosage forms may be pills, tablets, capsules, time-release formulations, osmotic controlled release formulations, solutions, softgels, hydrogels, suspensions, emulsions, syrups, orally disintegrating tablets, films, lozenges, chewing gums, mouthwashes, ointments, and the like.

[00108] Pharmaceutical compositions of the present disclosure, e.g., compositions that include a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, can be administered by direct injection into a target tissue or into the blood stream, including intradermal, subcutaneous, intravenous, intramuscular, intraosseous, or intraperitoneal injection. Pharmaceutical compositions of the present disclosure can be administered by intracavernous or intravitreal delivery to organs or tissues, or administered by intracerebral, intrathecal, or epidural delivery to tissues of the central nervous system.

[00109] For some conditions, it may be necessary to formulate test agents to cross the blood-brain barrier (BBB). One strategy for drug delivery through the blood-brain barrier (BBB) entails disruption of the BBB, either by osmotic means such as mannitol or leukotrienes, or biochemically by the use of vasoactive substances such as bradykinin. The potential for using BBB opening to target specific agents to brain regions is also an option. A BBB disrupting agent can be co-administered with test agents of the present disclosure when the test agents are administered by intravascular injection. Other strategies to go through the BBB may entail the use of endogenous transport systems, including Caveolin-1 mediated transcytosis, carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and active efflux transporters such as p-glycoprotein. Active transport moieties may also be conjugated to the test agents for use in the invention to facilitate transport across the endothelial wall of the blood vessel.

[00110] Local administration of the pharmaceutical compositions may include intrathecal administration, which may be carried out through the use of an Ommaya reservoir, in accordance

with known techniques (F. Balis et al., Am J. Pediatr. Hematol. Oncol. 11, 74, 76 (1989), *see also*, e.g. US Patent Nos. 5,222,982 and 5385582, incorporated herein by reference).

[00111] Where the pharmaceutical compositions are locally administered in the brain, one method for administration of the pharmaceutical compositions of the present disclosure is by deposition into or near the site by any suitable technique, such as by direct injection (aided by stereotaxic positioning of an injection syringe, if necessary) or by placing the tip of an Ommaya reservoir into a cavity, or cyst, for administration. Alternatively, a convection-enhanced delivery catheter may be implanted directly into the site, into a natural or surgically created cyst, or into the normal brain mass (*see e.g.* US Application No. 20070254842, incorporated here by reference). Such convection-enhanced pharmaceutical composition delivery devices greatly improve the diffusion of the composition throughout the brain mass. The implanted catheters of these delivery devices utilize high-flow microinfusion (with flow rates in the range of about 0.5 to 15.0 μl/minute), rather than diffusive flow, to deliver the test agent to the brain. Such devices are described in U.S. Patent No. 5,720,720, incorporated fully herein by reference.

[00112] In the methods of the present disclosure, the test agents may be administered to the patient using any convenient routes of administration that are capable of resulting in the desired modulation of a sleep-circuit regulated states, or treatment of a sleeping disorder. Thus, the test agents can be incorporated into a variety of formulations for therapeutic administration. More particularly, the test agents of the present disclosure can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid or liquid forms, such as tablets, capsules, powders, granules, ointments, solutions and injections.

[00113] In pharmaceutical dosage forms, the test agents of the present disclosure may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The previously-described routes of administration and dosage forms are merely exemplary and are in no way limiting.

[00114] In certain embodiments, a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, may be administered at a dosage that is sufficient to modulate the activity of the sleep circuit. Thus, the dosage of the test agent will vary, depending upon the mode of action of the test agent, the frequency of administration, the manner of administration, the clearance of the test agent from the subject, and the like.

[00115] In certain embodiments, a small molecule that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, is administered at a dose ranging from 0.1 μg/kg to 200 mg/kg body weight, e.g., 0.5 μg/kg to 175 mg/kg body weight, 1 μg/kg to 150 mg/kg body weight, 5 μg/kg to 125 mg/kg body weight, 10 μg/kg to 100 mg/kg body weight, 20 μg/kg to 50 mg/kg body weight, 50 μg/kg to 20 mg/kg body weight, 75 μg/kg to 10 mg/kg body weight, 100 μg/kg to 5 mg/kg body weight, including 100 μg/kg to 1 mg/kg body weight, to modulate the activity of the sleep circuit in an individual. In certain embodiments, a small molecule that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, may be administered at a dose of 0.1 μg/kg or more, e.g., 0.5 μg/kg or more, 1 μg/kg or more, 5 μg/kg or more, 10 μg/kg or more, 20 μg/kg or more, 50 μg/kg or more, 100 μg/kg or more, 200 μg/kg or more, 500 μg/kg or more 1 mg/kg or more, 5 mg/kg or more, 10 mg/kg or more, 20 mg/kg or more, 50 mg/kg or more, 75 mg/kg or more, 100 mg/kg or more, by body weight, and in some cases the dose may be 200 mg/kg or less, e.g., 150 mg/kg or less, 100 mg/kg or less, 75 mg/kg or less, 50 mg/kg or less, 25 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, 1 mg/kg or less, 750 μg/kg or less, 500 μg/kg or less, 250 μg/kg or less, 100 μg/kg or less, 75 μg/kg or less, 50 μg/kg or less, 20 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less or 1 μg/kg or less, by body weight, to modulate the activity of the sleep circuit in an individual. In certain embodiments, a small molecule that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, is administered at a dose or in a dosage regimen that provides for a target tissue and/or blood concentration in the range of 0.1 to 1000 nM, e.g., 1 to 500 nM, 10 to 400 nM, 20 to 300 nM, 25 to 250 nM, 30 to 200 nM, including 50 to 150 nM.

[00116] In certain embodiments, a polypeptide or antibody that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, may be administered at a dose ranging from 1 μg/kg to 150 mg/kg body weight, e.g., 5 μg/kg to 125 mg/kg body weight, 10 μg/kg to 100 mg/kg body weight, 20 μg/kg to 75 mg/kg body weight, 50 μg/kg to 50 mg/kg body weight, 75 μg/kg to 20 mg/kg body weight, 100 μg/kg to 10 mg/kg body weight, including 100 μg/kg to 1 mg/kg body weight, to modulate the activity of the sleep circuit in an individual. In certain embodiments, a polypeptide or antibody that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, may be administered at a dose of 1 μg/kg or more, e.g., 5 μg/kg or more, 10 μg/kg or more, 50 μg/kg or more, 100 μg/kg or more, 200 μg/kg or more, 500

μg/kg or more 1 mg/kg or more, 5 mg/kg or more, 10 mg/kg or more, 20 mg/kg or more, 50 mg/kg or more, 75 mg/kg or more, 100 mg/kg or more, by body weight, and in some cases the dose may be 200 mg/kg or less, e.g., 150 mg/kg or less, 100 mg/kg or less, 75 mg/kg or less, 50 mg/kg or less, 25 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, 1 mg/kg or less, 750 μg/kg or less, 500 μg/kg or less, 250 μg/kg or less, 100 μg/kg or less, 75 μg/kg or less, 50 μg/kg or less, 20 μg/kg or less, or 10 μg/kg or less, by body weight, to modulate the activity of the sleep circuit in an individual. In certain embodiments, a polypeptide that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, is administered at a dose or in a dosage regimen that provides for a target tissue and/or blood concentration in the range of 1 to 1,000 nM, e.g., 5 to 900 nM, 10 to 800 nM, 20 to 750 nM, 50 to 700 nM, 100 to 600 nM, including 200 to 600 nM. In certain embodiments, an antibody that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, is administered at a dose or in a dosage regimen that provides for a target tissue and/or blood concentration in the range of 0.01 to 1,000 μg/ml, e.g., 0.05 to 500 μg/ml, 0.1 to 250 μg/ml, 0.5 to 100 μg/ml, 1 to 50 μg/ml, 1 to 40 μg/ml, including 1 to 25 μg/ml.

[00117] A test agent modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, may be administered according to any suitable dosage regimen, including, but not limited to, daily administration, weekly administration, biweekly administration, monthly administration, semiannual administration, etc.

GENETICALLY MODIFIED ANIMALS

[00118] Also provided herein are non-human animals (e.g., non-human mammals; e.g., rats; mice; rabbits; non-human primates; etc.) that find use in performing methods of the present disclosure, and methods for generating the non-human animals. The present non-human animals have a subtype of neurons genetically modified to selectively express light-activated polypeptide, e.g. a light-activated ion channel, such as channelrhodopsin 2, where the subtype of neurons belong to a neural sleep circuit that contains GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons, as described above. In some embodiments, the light-activated polypeptide is expressed in GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. In some embodiments, the light-activated polypeptide is expressed in a subtype of neurons that are synaptically connected to the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA\rightarrow TMN}$ neurons. In some embodiments, the light-activated polypeptide is

expressed in a subtype of neurons that synapse onto the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, which subtype of neurons may include neurons of the hypothalamus or the amygdala. In some cases, the amygdala neurons are GABAergic neurons of the CEA.

[00119] Also provided herein are methods of generating the present genetically modified non-human animals, e.g., genetically modified non-human mammals. The present method of generating a genetically modified non-human mammal of the present disclosure may include administering an expression vector, e.g., a viral expression vector, containing a nucleic acid that encodes a light-activated polypeptide, to a region of the brain that includes one or more subtypes of neurons of the present sleep circuit, e.g., a sleep circuit that includes the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. To deliver the expression vector, e.g., a viral expression vector, to the target neurons, the expression vector may be packaged I any suitable viral particle (i.e., virions). In some cases, the host animal may be a transgenic animal that expresses a site-specific recombinase (e.g., Cre or Flp recombinase) under a cell type-specific promoter. In some cases, a viral vector that includes a nucleic acid encoding a site-specific recombinase may be administered to a brain region of the host animal, to provide the recombinase activity in neurons of the brain region. The viral vector may include recombination sites specific for the site-specific recombinase (e.g., Lox site, for Cre; FRT site for Flp), where the recombination sites are configured relative to a nucleic acid sequence encoding the light-activated polypeptide in such a way as to provide for expression of the light-activated polypeptide specifically in cells that express the site-specific recombinase. The viral vector may include any suitable control elements (e.g., promoters, enhancers, etc.) to control expression of the light-activated polypeptide according to neuronal subtype, timing, presence of an inducer, etc.

[00120] In some cases, the expression vector encoding the light-activated polypeptide may include a regulatory element, e.g., a promoter, that is specific for the target neurons, at least within the region of the brain in which the expression vector is administered.

[00121] Any suitable arrangement of recombination sites and other regulatory elements may be used in the viral vector, such as, but not limited to, a double inverted open reading frame (DIO) strategy. Suitable methods are described in, e.g, Fenno et al., Nat Methods. 2014 Jul;11(7):763; Gompf et al., Front Behav Neurosci. 2015 Jul 2;9:152; Cetin et al., J. Neurophysiol. *111*, 2150-2159.

[00122] Neuron-specific promoters and other control elements (e.g., enhancers) are known in the art. Suitable neuron-specific control sequences include, but are not limited to, a neuron-specific enolase (NSE) promoter (see, e.g., EMBL HSENO2, X51956; see also, e.g., U.S. Pat. No.

6,649,811, U.S. Pat. No. 5,387,742); an aromatic amino acid decarboxylase (AADC) promoter; a neurofilament promoter (see, e.g., GenBank HUMNFL, L04147); a synapsin promoter (see, e.g., GenBank HUMSYNIB, M55301); a thy-1 promoter (see, e.g., Chen et al. (1987) Cell 51:7-19; and Llewellyn et al. (2010) Nat. Med. 16:1161); a serotonin receptor promoter (see, e.g., GenBank S62283); a tyrosine hydroxylase promoter (TH) (see, e.g., Nucl. Acids. Res. 15:2363-2384 (1987) and Neuron 6:583-594 (1991)); a GnRH promoter (see, e.g., Radovick et al., Proc. Natl. Acad. Sci. USA 88:3402-3406 (1991)); an L7 promoter (see, e.g., Oberdick et al., Science 248:223-226 (1990)); a DNMT promoter (see, e.g., Bartge et al., Proc. Natl. Acad. Sci. USA 85:3648-3652 (1988)); an enkephalin promoter (see, e.g., Comb et al., EMBO J. 17:3793-3805 (1988)); a myelin basic protein (MBP) promoter; a CMV enhancer/platelet-derived growth factor-β promoter (see, e.g., Liu et al. (**2620**) Gene Therapy 11:52-60); a motor neuron-specific gene Hb9 promoter (see, e.g., U.S. Pat. No. 7,632,679; and Lee et al. (**2620**) Development 131:3295-3306); and an alpha subunit of $Ca^{(2+)}$-calmodulin-dependent protein kinase II (CaMKIIα) promoter (see, e.g., Mayford et al. (1996) Proc. Natl. Acad. Sci. USA 93:13250). Other suitable promoters include elongation factor (EF) 1α and dopamine transporter (DAT) promoters.

[00123] Any suitable viral vector may be used to deliver a nucleic acid, e.g., viral vector, encoding the light-activated polypeptide. Suitable viral vectors include, without limitation, adeno-associated viral vector and lentiviral vectors. The viral particle (i.e., virion) may be any suitable viral particle. In some cases, the envelope of the viral particle is derived from more than one type of virus. Thus, in some cases, the viral particle of one type of virus is pseudotyped with an envelope protein from another type of virus. In some embodiments, the viral particle is pseudotyped with a rabies glycoprotein. In some embodiments, the viral particle is an equine infectious anemia virus (EIAV) pseudotyped with a rabies glycoprotein.

[00124] The site-specific recombinase may be under the control of any suitable cell type-specific promoter. In some embodiments, the site-specific recombinase is expressed in, without limitation, GABAergic neurons, glutamatergic neurons, cholinergic neurons, histaminergic neurons, serotonergic neurons, or dopaminergic neurons, etc. Suitable cell type-specific promoters include, without limitation, promoter elements for GAD1, GAD1, VGAT, GAT1, VGLUT2, CCK, CRH, TAC1, GAL, etc.

[00125] The brain region to which the viral particles are administered may be any suitable brain region with functional connectivity to the sleep circuit, e.g., a sleep circuit that contains the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. In some cases, the brain region is the TMN. In some embodiments, the brain region is the POA. In some

embodiments the brain region is the CEA. The brain region may be any other region of the brain with a subtype of neurons that have synaptic connections to the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons.

[00126] The present method of generating a transgenic non-human mammal of the present disclosure can involve use of a gene-editing composition, e.g., a CRISPR/Cas system comprising a CRISPR/Cas endonuclease (e.g., Cas9) and a guide RNA. Methods of using a CRISPR/Cas system to generate make genome modifications in non-human mammals are known in the art; see, e.g., Lie et al. (2013) *Nat. Biotechnol.* 31:684; Yang et al. (2013) *Cell* 154:1370; and Niu et al. (2014) *Cell* 156:836.

LIGHT-ACTIVATED POLYPEPTIDES

[00127] A light-activated polypeptide for use in methods of the present disclosure may be any suitable light-activated polypeptide for selectively modulating activity of a subtype of neurons by illuminating the neurons with a light stimulus. In some instances, the light-activated polypeptide is a light-activated ion channel polypeptide. The light-activated ion channel polypeptides are adapted to allow one or more ions to pass through the plasma membrane of a target cell when the polypeptide is illuminated with light of an activating wavelength. Light-activated proteins may be characterized as ion pump proteins, which facilitate the passage of a small number of ions through the plasma membrane per photon of light, or as ion channel proteins, which allow a stream of ions to freely flow through the plasma membrane when the channel is open. In some embodiments, the light-activated polypeptide depolarizes the cell when activated by light of an activating wavelength. In some embodiments, the light-activated polypeptide hyperpolarizes the cell when activated by light of an activating wavelength. Suitable hyperpolarizing and depolarizing polypeptides, such as opsins, are known in the art and include, e.g., a channelrhodopsin (e.g., ChR2), variants of ChR2 (e.g., C128S, D156A, C128S + D156A, E123A, E123T), iC1C2, C1C2, GtACR2, NpHR, eNpHR3.0, C1V1, VChR1, VChR2, SwiChR, Arch, ArchT, KR2, ReaChR, ChiEF, Chronos, ChRGR, CsChrimson, and the like. In some cases, the light-activated polypeptide includes bReaCh-ES, as described herein and described further in, e.g., Rajasethupathy et al., Nature. 2015 Oct 29;526(7575):653, which is incorporated by reference. Hyperpolarizing and depolarizing opsins have been described in various publications; see, e.g., Berndt and Deisseroth (2015) *Science* 349:590; Berndt et al. (2014) *Science* 344:420; and Guru et al. (July 25, 2015) *Intl. J. Neuropsychopharmacol.* pp. 1-8 (PMID 26209858).

[00128] The present disclosure provides for the modification of light-activated polypeptides, e.g., opsins, expressed in a cell by the addition of one or more amino acid sequence motifs which

enhance transport to the plasma membranes of mammalian cells. Light-activated polypeptides having components derived from evolutionarily simpler organisms may not be expressed or tolerated by mammalian cells or may exhibit impaired subcellular localization when expressed at high levels in mammalian cells. Consequently, in some embodiments, the light-activated polypeptides expressed in a cell can be fused to one or more amino acid sequence motifs selected from the group consisting of a signal peptide, an endoplasmic reticulum (ER) export signal, a membrane trafficking signal, and/or an N-terminal golgi export signal. The one or more amino acid sequence motifs which enhance light-activated polypeptide transport to the plasma membranes of mammalian cells can be fused to the N-terminus, the C-terminus, or to both the N- and C-terminal ends of the light-activated polypeptide. Optionally, the light-activated polypeptide and the one or more amino acid sequence motifs may be separated by a linker. In some embodiments, the light-activated polypeptide can be modified by the addition of a trafficking signal (ts) which enhances transport of the protein to the cell plasma membrane. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:1).

[00129] Trafficking sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such as a trafficking sequence of human inward rectifier potassium channel Kir2.1 (e.g., KSRITSEGEYIPLDQIDINV (SEQ ID NO:1)).

[00130] A trafficking sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[00131] Signal sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such as one of the following:

[00132] 1) the signal peptide of hChR2 (e.g., MDYGGALSAVGRELLFVTNPVVVNGS (SEQ ID NO:2))

[00133] 2) the β2 subunit signal peptide of the neuronal nicotinic acetylcholine receptor (e.g., MAGHSNSMALFSFSLLWLCSGVLGTEF (SEQ ID NO:3));

[00134] 3) a nicotinic acetylcholine receptor signal sequence (e.g., MGLRALMLWLLAAAGLVRESLQG (SEQ ID NO:4)); and

[00135] 4) a nicotinic acetylcholine receptor signal sequence (e.g., MRGTPLLLVVSLFSLLQD (SEQ ID NO:5)).

[00136] A signal sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[00137] Endoplasmic reticulum (ER) export sequences that are suitable for use in a modified opsin of the present disclosure include, e.g., VXXSL (where X is any amino acid) (SEQ ID NO:6) (e.g., VKESL (SEQ ID NO:7); VLGSL (SEQ ID NO:8); etc.); NANSFCYENEVALTSK (SEQ ID NO:9); FXYENE (SEQ ID NO:10) (where X is any amino acid), e.g., FCYENEV (SEQ ID NO:11); and the like. An ER export sequence can have a length of from about 5 amino acids to about 25 amino acids, e.g., from about 5 amino acids to about 10 amino acids, from about 10 amino acids to about 15 amino acids, from about 15 amino acids to about 20 amino acids, or from about 20 amino acids to about 25 amino acids.

[00138] In some embodiments, the signal peptide sequence in the protein can be deleted or substituted with a signal peptide sequence from a different protein.

Light-activated ion channels

[00139] In some aspects, the light-activated polypeptide is an ion channel protein, e.g., a cation channel protein, that can be derived from *Chlamydomonas reinhardtii*, wherein the cation channel protein can be capable of transporting cations across a cell membrane when the cell is illuminated with light. In another embodiment, the light-activated cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:12. The light used to activate the light-activated cation channel protein derived from *Chlamydomonas reinhardtii* can have a wavelength between about 460 and about 495 nm or can have a wavelength of about 480 nm. Additionally, light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can be used to activate the light-activated polypeptide. In some embodiments, activation of the light-activated cation channel derived from *Chlamydomonas reinhardtii* with light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can cause depolarization-induced synaptic depletion of the neurons expressing the light-activated cation channel. The light-activated cation channel protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to

increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-activated cation channel protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-activated cation channel protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-activated cation channel protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to transport cations across a cell membrane. In another embodiment, the light-activated cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the C1C2 amino sequence shown in Figure 19B, and set forth in SEQ ID NO:19. A crystal structure of C1C2 is presented in Kato et al. (2012) *Nature* 482:369. In another embodiment, the light-activated cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the ReaChR amino sequence shown in Figure 19B, and set forth in SEQ ID NO:20.

[00140] In some embodiments, the light-activated cation channel comprises a T159C substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises a L132C substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises an E123T substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises an E123A substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises a T159C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises a T159C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises a T159C substitution, an L132C substitution, and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises a T159C substitution, an L132C substitution, and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises an L132C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises an L132C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:12. In some embodiments, the light-activated cation channel comprises an H143R amino acid substitution of the amino acid sequence set forth in SEQ ID NO:12.

[00141] In some embodiments, a ChR2 protein comprises at least one (such as one, two, three, or more) amino acid sequence motifs that enhance transport to the plasma membranes of target cells selected from the group consisting of a signal peptide, an ER export signal, and a membrane trafficking signal. In some embodiments, the ChR2 protein comprises an N-terminal signal peptide and a C-terminal ER export signal. In some embodiments, the ChR2 protein comprises an N-terminal signal peptide and a C-terminal trafficking signal. In some embodiments, the ChR2 protein comprises an N-terminal signal peptide, a C-terminal ER export signal, and a C-terminal trafficking signal. In some embodiments, the ChR2 protein comprises a C-terminal ER export signal and a C-terminal trafficking signal. In some embodiments, the C-terminal ER export signal and the C-terminal trafficking signal are linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER export signal is more C-terminally located than the trafficking signal. In some embodiments the trafficking signal is more C-terminally located than the ER Export signal.

Step function opsins and stabilized step function opsins

[00142] In other embodiments, the light-activated cation channel protein can be a step function opsin (SFO) protein or a stabilized step function opsin (SSFO) protein that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the protein. In some embodiments, the SFO protein can have a mutation at amino acid residue C128 of SEQ ID NO:12. In other embodiments, the SFO protein has a C128A mutation in SEQ ID NO:12. In other embodiments, the SFO protein has a C128S mutation in SEQ ID NO:12. In another embodiment, the SFO protein has a C128T mutation in SEQ ID NO:12. In some embodiments, the SFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:13.

[00143] In some embodiments, the SSFO protein can have a mutation at amino acid residue D156 of SEQ ID NO:12. In other embodiments, the SSFO protein can have a mutation at both amino acid residues C128 and D156 of SEQ ID NO:12. In one embodiment, the SSFO protein has an C128S and a D156A mutation in SEQ ID NO:12. In another embodiment, the SSFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:14. In another embodiment, the SSFO protein can comprise a C128T mutation in SEQ ID NO:12. In some embodiments, the SSFO protein comprises C128T and D156A mutations in SEQ ID NO:12.

[00144] In some embodiments the SFO or SSFO proteins provided herein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with blue light. In other embodiments, the light can have a wavelength of about 445 nm. Additionally, in some embodiments the light can be delivered as a single pulse of light or as spaced pulses of light due to the prolonged stability of SFO and SSFO photocurrents. In some embodiments, activation of the SFO or SSFO protein with single pulses or spaced pulses of light can cause depolarization-induced synaptic depletion of the neurons expressing the SFO or SSFO protein. In some embodiments, each of the disclosed step function opsin and stabilized step function opsin proteins can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

[00145] Further disclosure related to SFO or SSFO proteins can be found in International Patent Application Publication No. WO 2010/056970, the disclosure of which is hereby incorporated by reference in its entirety.

C1V1 chimeric cation channels

[00146] In other embodiments, the light-activated cation channel protein can be a C1V1 chimeric protein derived from the VChR1 protein of *Volvox carteri* and the ChR1 protein from *Chlamydomonas reinhardti,* wherein the protein comprises the amino acid sequence of VChR1 having at least the first and second transmembrane helices replaced by the first and second transmembrane helices of ChR1; is responsive to light; and is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments, the C1V1 protein can further comprise a replacement within the intracellular loop domain located between the second and third transmembrane helices of the chimeric light responsive protein, wherein at least a portion of the intracellular loop domain is replaced by the corresponding portion from ChR1. In another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1 extending to amino acid residue A145 of the ChR1. In other embodiments, the C1V1 chimeric protein can further comprise a replacement within the third transmembrane helix of the chimeric light responsive protein, wherein at least a portion of the third transmembrane helix is replaced by the corresponding sequence of ChR1. In yet another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1 extending to amino acid residue W163 of the ChR1. In other embodiments, the C1V1 chimeric protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:15.

[00147] In some embodiments, the C1V1 protein can mediate a depolarizing current in the cell when the cell is illuminated with green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 542 nm. In some embodiments, the C1V1 chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, in some embodiments, light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can be used to activate the C1V1 protein.

C1V1 chimeric mutant variants

[00148] In some aspects, the present disclosure provides polypeptides comprising substituted or mutated amino acid sequences, wherein the mutant polypeptide retains the characteristic light-activatable nature of the precursor C1V1 chimeric polypeptide but may also possess altered properties in some specific aspects. For example, the mutant light-activated C1V1 chimeric proteins described herein can exhibit an increased level of expression both within an animal cell or on the animal cell plasma membrane; an altered responsiveness when exposed to different wavelengths of light, particularly red light; and/or a combination of traits whereby the chimeric C1V1 polypeptide possess the properties of low desensitization, fast deactivation, low violet-light activation for minimal cross-activation with other light-activated cation channels, and/or strong expression in animal cells.

[00149] Accordingly, provided herein are C1V1 chimeric light-activated opsin proteins that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the VChR1 portion of the chimeric polypeptide. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E122 of SEQ ID NO:15. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E162 of SEQ ID NO:15. In other embodiments, the C1V1 protein can have a mutation at both amino acid residues E162 and E122 of SEQ ID NO:15. In other embodiments, the C1V1 protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

[00150] In some aspects, the C1V1-E122 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In other embodiments, the C1V1-E122 mutant chimeric protein can mediate a

depolarizing current in the cell when the cell is illuminated with red light. In some embodiments, the red light can have a wavelength of about 630 nm. In some embodiments, the C1V1-E122 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, in some embodiments, light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can be used to activate the C1V1-E122 mutant chimeric protein. In some embodiments, activation of the C1V1-E122 mutant chimeric protein with light pulses having a frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E122 mutant chimeric protein.

[00151] In other aspects, the C1V1-E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 535 nm to about 540 nm. In some embodiments, the light can have a wavelength of about 542 nm. In other embodiments, the light can have a wavelength of about 530 nm. In some embodiments, the C1V1-E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, in some embodiments, light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can be used to activate the C1V1-E162 mutant chimeric protein. In some embodiments, activation of the C1V1-E162 mutant chimeric protein with light pulses having a frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E162 mutant chimeric protein.

[00152] In yet other aspects, the C1V1-E122/E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated

with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein can exhibit less activation when exposed to violet light relative to C1V1 chimeric proteins lacking mutations at E122/E162 or relative to other light-activated cation channel proteins. Additionally, in some embodiments, light pulses having a temporal frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can be used to activate the C1V1-E122/E162 mutant chimeric protein. In some embodiments, activation of the C1V1- E122/E162 mutant chimeric protein with light pulses having a frequency in the range of about 5 Hz to about 100 Hz, e.g., about 10 Hz to about 80 Hz, about 10 Hz to about 60 Hz, including about 10 Hz to about 40 Hz, can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1- E122/E162 mutant chimeric protein.

UTILITY

[00153] The present methods and transgenic animals find use in many applications where it is desired to identify agents, e.g., therapeutic agents, and/or using the agents to treat an individual for a sleeping disorder, e.g., to treat insomnia or narcolepsy. The sleeping disorder may be a disorder that is associated with a neural sleep circuit that includes GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons. In some cases, the sleeping disorder is caused by aberrant activity of neurons in the sleep circuit. In some cases, selective modulation of the sleep circuit that includes GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons can compensate for a deficiency the function of another sleep circuit.

EXAMPLES

[00154] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt,

nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Materials and Methods

[00155] The following materials and methods were used in Examples 2-6.

Animals

[00156] GAD2-IRES-Cre, VGLUT2-IRES-Cre, VGAT-IRES-Cre, GAD1-eGFP, CCK-IRES-Cre, CRH-IRES-Cre and TAC1-IRES-Cre mice (Jackson stock no: 010802, 016963, 016962, 007677, 012706, 012704 and 021877 respectively) were obtained from Jackson Laboratory and VGLUT2-eGFP mice were from MMRRC (MMRRC#011835-UCD). GAL-Cre mice were obtained from GENSAT (stock no: KI87). Mice were housed in 12 hr light-dark cycle (lights on 07:00 am and off at 19:00 pm) with free access to food and water. All procedures were approved by Institutional Animal Care and Use Committees of the University of California, Berkeley, University of California, San Francisco (UCSF), Allen Institute for Brain Science and Stanford University and were done in accordance with federal regulations and guidelines on animal experimentation.

Virus Preparation

[00157] AAV_2-EF1α-DIO-ChR2-eYFP and AAV_2-EF1α-DIO-eYFP were obtained from the University of North Carolina (UNC) vector core. The final titer was estimated to be ~10^{12} gc/mL. AAV_{DJ}-EF1α-DIO-ChR2-eYFP was obtained from Stanford University virus core. Lentivirus rEIAV-DIO-TLoop-ChR2-eYFP or rEIAV-DIO-TLoop-nlsPelican was obtained from Salk virus core. cTRIO reagents: CAV-FLEx^{loxP}-Flp, AAV-FLEx^{FRT}-TVA-mCherry, AAV-FLEx^{FRT}-RG and EnvA-pseudotyped, glycoprotein (RG)-deleted, and GFP-expressing rabies viral particles (RV*dG*) were obtained from Stanford University. HSV-LoxSTOPLox-FlagHA-L10a was obtained from UCSF.

Surgery

[00158] To implant electroencephalogram (EEG) and electromyogram (EMG) recording electrodes, adult mice (6-12 weeks old) were anesthetized with 1.5-2% isoflurane and placed on a stereotaxic frame. Two stainless steel screws were inserted into the skull 1.5 mm from midline and 1.5 mm anterior to the bregma, and two others were inserted 3 mm from midline and 3.5 mm posterior to the bregma. Two EMG electrodes were inserted into the neck musculature. Insulated leads from the EEG and EMG electrodes were soldered to a 2 × 3 pin header, which was secured to the skull using dental cement.

[00159] For optogenetic activation experiments, a craniotomy was made on top of the POA in the same surgery as for EEG and EMG implant, and 0.1-0.5 µl virus was injected into the target region using Nanoject II (Drummond Scientific) via a micro pipette. Optic fibers bilaterally were then implanted into the POA. Dental cement was applied to cover the exposed skull completely and to secure the implants for EEG and EMG recordings to the screws. After surgery, mice were allowed to recover for at least 3 weeks before experiments. For retrograde tracing in Figure 1A, 0.2-0.3 µl red or green RetroBeads (Lumafluor, Inc) was injected into each target region.

[00160] For optrode recording experiments, the optrode assembly was inserted into the POA at a depth of 4.9 mm. Screws were attached to the skull for EEG recordings, and an EMG electrode was inserted into the neck musculature. The optrode assembly, screws and EEG/EMG electrodes were secured to the skull using dental cement.

[00161] For cTRIO experiments, CAV-FLEx^{loxP}-Flp (5.0×10^{12} gc/mL) was injected into either the TMN or the PFC, and AAV-FLEx^{FRT}-TVA-mCherry (2.6×10^{12} gc/mL) and AAV-FLEx^{FRT}-RG (1.3×10^{12} gc/mL) were injected into the POA. Two to three weeks later, EnvA-pseudotyped, glycoprotein (RG)-deleted, and GFP-expressing rabies viral particles (RV*dG*) (5.0×10^{8} colony forming units (cfu)/mL) were injected into the POA, and mice were sacrificed 1 week later for histology.

[00162] Stereotaxic coordinates for injections:

TMN: AP -2.45 mm, ML 1 mm, DV 5-5.2 mm from the cortical surface

POA: AP 0 mm, ML 0.7 mm, DV 5.2 mm

CEA: AP -1.5 mm, ML 2.5 mm, DV 3.8-4 mm

PFC: AP +2.0 mm, ML 0.4 mm, DV 2 mm

vlPAG: AP -4.7 mm, ML 0.7 mm, DV 2.3 mm

DMH: AP -1.8 mm, ML 0.4 mm, DV 5.2 mm

Behavior

[00163] Behavioral experiments were carried out in home cages placed in sound-attenuating boxes. Sleep recordings were carried out between 12:00 and 19:00 (light on at 7:00 and off at 19:00). EEG and EMG electrodes were connected to flexible recording cables via a mini-connector. EEG and EMG signals were recorded and amplified using AM Systems, digitally filtered (0.1-1000 Hz and 10-1000 Hz for EEG and EMG recordings respectively), and digitized at 600 Hz using LabView. Spectral analysis was carried out using fast Fourier transform (FFT), and brain states were classified into NREM, REM and wake states (wake: desynchronized EEG and high EMG activity, NREM: synchronized EEG with high-amplitude, low-frequency (0.5-4

Hz) activity and low EMG activity, REM: high power at theta frequencies (6-9 Hz) and low EMG activity).

Sleep deprivation and sleep rebound experiment

[00164] Sleep deprivation started at the beginning of the light period (7 am) and lasted till 1 pm. Mice were kept awake by a combination of cage tapping, introduction of foreign objects such as paper towels, cage rotation, and fur stroking with a paintbrush, gentle handling procedures that have been used extensively to induce sleep deprivation (Borbely et al., 1984). After 6 hr of deprivation, sleep-deprived mice were allowed rebound sleep for 4 hr before being euthanized by cervical dislocation and decapitation. c-Fos immunohistochemistry was performed as described below.

Optogenetic manipulation

[00165] Each optic fiber (200 μm diameter; ThorLabs) was attached through an FC/PC adaptor to a 473-nm blue laser diode (Shanghai laser), and light pulses were generated using a Master 8 (A.M.P.I.). All photostimulation experiments were conducted bilaterally and fiber optic cables were connected at least 2 hr before the experiments for habituation. For photostimulation experiments in ChR2- or eYFP-expressing mice, light pulses (10 ms/pulse, 10 Hz, 4-8 mW) were triggered using Master 8 that provided simultaneous input into two blue lasers.

Optrode Recording

[00166] To identify ChR2-tagged neurons in the POA, custom-made optrodes were used (Anikeeva et al., 2012). Extracellular signals recorded with a TDT RZ5 amplifier were filtered (0.3-8 kHz) and digitized at 25 kHz.

Optrode recordings

[00167] Custom-made optrodes consisted of an optic fiber (200 μm in diameter) glued together with 6 pairs of stereotrodes. Two FeNiCr wires (Stablohm 675, California Fine Wire) were twisted together and electroplated to an impedance of ~ 600 kΩ using a custom built plating device. The optrode was attached to a driver to allow vertical movement of the optrode assembly. Wires to record cortical EEG and EMG from neck musculatures were also attached for simultaneous recordings. A TDT RZ5 amplifier was used for all the recordings, signals were filtered (0.3-8 kHz) and digitized at 25 kHz. At the end of the experiment, an electrolytic lesion was made by passing a current (100 μA, 10 s) through one or two electrodes to identify the end of the recording tract.

[00168] Spikes were sorted offline based on the waveform energy and the first three principal components of the spike waveform on each stereotrode channel. For single unit isolation, all channels were separated into groups and spike waveforms were identified either manually using

Klusters (neurosuite(dot)sourceforge(dot)net/) or automatically using the software klustakwik (klustakwik(dot)sourceforge(dot)net/). The quality of each unit was assessed by the presence of a refractory period and quantified using isolation distance and L-ratio. Units with an isolation distance < 20 and L-ratio > 0.1 were discarded.

[00169] To identify ChR2-tagged neurons, laser pulse trains (10 and/or 20 Hz) were delivered intermittently every min. A unit was identified as ChR2 expressing if spikes were evoked by laser pulses with short first-spike latency (< 10 ms) and the waveforms of the laser-evoked and spontaneous spikes were highly similar (correlation coefficient > 0.9). Mean latency of all identified units was 3.05 ms. Mean correlation coefficient of all identified units was 0.99. To calculate the average firing rate of each unit in each brain state, spikes during the laser pulse trains were excluded.

Histology

[00170] Mice were deeply anesthetized and transcardially perfused using PBS followed by 4% paraformaldehyde in PBS. Brains were post-fixed in fixative, stored in 30% sucrose in PBS overnight for cryoprotection, and further processed for immunohistochemistry and fluorescence in situ hybridization (FISH).

Immunohistochemistry

[00171] Mice were deeply anesthetized and transcardially perfused using PBS followed by 4% paraformaldehyde in PBS. Brains were post-fixed in fixative and stored in 30% sucrose in PBS overnight for cryoprotection. Brains were embedded and mounted with Tissue-Tek OCT compound (Sakura finetek) and 20 μm sections were cut using a cryostat (Leica). Brain slices were washed using PBS, permeabilized using PBST (0.3% Triton X-100 in PBS) for 30 min and then incubated with blocking solution (5% normal goat serum or normal donkey serum in PBST) for 1 hr followed by primary antibody incubation overnight at 4 °C using following antibodies:

anti-GFP antibody (A-11122 or A-11120, Life technologies, 1:1000)

anti-cFos antibody (sc-52-G and sc-52, Santa cruz biotech, 1:1000)

anti-CCK-8 antibody (20078, Immunostar, 1:500)

anti-Substance P antibody (ab10353, abcam, 1:500)

anti-CRH antibody (sc-1759, Santa cruz biotech, 1:500)

anti-HA antibody (C29F4, Cell signaling tech, 1:1000)

[00172] The next day, slices were washed with PBS and incubated with appropriate secondary antibodies for 2 hr (1:500, All from Invitrogen):

A-11008, alexa fluor 488 goat anti-rabbit IgG

A-21206, alexa fluor 488 donkey anti-rabbit IgG

A-11055, alexa fluor 488 donkey anti-goat IgG

A-21202, alexa fluor 488 donkey anti-mouse IgG

A-11012, alexa fluor 594 goat anti-rabbit IgG

A-21207, alexa fluor 594 donkey anti-rabbit IgG

A-11058, alexa fluor 594 donkey anti-goat IgG

[00173] The slices were washed with PBS followed by counterstaining with DAPI or Hoechst and coverslipped. Fluorescence images were taken using a confocal microscope (LSM 710 AxioObserver Inverted 34-Channel Confocal, Zeiss) or Nanozoomer (Hamamatsu).

Fluorescence in situ hybridization (FISH)

[00174] The GAD probe was a kind gift from Dr. Olivier Civelli (University of California, Irvine). To make TAC1-FISH probes, DNA fragments containing the coding or untranslated sequences were amplified using PCR from mouse whole brain cDNA (Zyagen). A T7 RNA polymerase recognition site was added to the 3' end of the PCR product. The PCR product was purified using a PCR purification kit (QIAGEN). 1 μg of DNA was used for in vitro transcription by using digoxigenin (DIG) RNA labeling mix (Roche) and T7 RNA polymerase. After DNAse I treatment for 30 min at 37 °C, the RNA probe was purified using probeQuant G-50 Columns (GE Healthcare). 20 μm sections were pretreated with proteinase K (0.1 μg/ml), acetylated, dehydrated through ethanol (50, 70, 95, and 100%), and air dried. Pretreated sections were then incubated for 16-20 hr at 60 °C, in a hybridization buffer containing sense or antisense riboprobes. After the sections were hybridized, they were treated with RNase A (20 μg/ml) for 30 minutes at 37 °C and then washed four times in decreasing salinity (from 2× to 0.1× standard saline citrate [SSC] buffer) and a 30 min wash at 68 °C. Sections were incubated with 3% hydrogen peroxide in PBS for 1 hr and washed using PBS. After incubation in the blocking buffer for 1 hr (TNB buffer, Perkin Elmer), sections were incubated with anti-DIG-POD antibody (1:500, Roche) in TNB buffer for 2 hr. TSA-plus-Fluorescein reagent was used to visualize the signal. After washing the sections in PBS, they were incubated with blocking buffer for 2 hr followed by incubation with anti-GFP antibody overnight, and finally incubated with a secondary antibody as described above.

cTRIO data analysis

[00175] For analysis of rabies tracing data, consecutive 60 μm coronal sections were collected and stained using Hoechst. Slides were scanned using Nanozoomer (Hamamatsu). GFP+ input neurons were counted from the forebrain to the posterior brainstem except sections adjacent to the injection sites (1 mm from the injection site), and grouped into 10 regions based on Allen Mouse Brain Atlas (mouse(dot)brain-map(dot)org/static/atlas) using anatomical landmarks in the sections visualized by Hoechst staining and autofluorescence. The number of neurons were normalized in each region by the total number of input neurons in the entire brain.

Single-Cell RNA-Seq

[00176] A previously described procedure was adapted to isolate fluorescently labeled neurons from the mouse brain (Hempel et al., (2007). A manual method for the purification of fluorescently labeled neurons from the mammalian brain. Nat. Protoc. *2*, 2924-2929; Sugino et al., (2006). Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nat. Neurosci. *9*, 99-107).

[00177] *Single cell isolation*. Individual adult male mice (P56±3) were anesthetized in an isoflurane chamber, decapitated, and the brain was immediately removed and submerged in fresh ice-cold artificial cerebrospinal fluid (ACSF) containing 126 mM NaCl, 20 mM $NaHCO_3$, 20 mM dextrose, 3 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 50 μM DL-AP5 sodium salt, 20 μM DNQX, and 0.1 μM tetrodotoxin, bubbled with a carbogen gas (95% O_2 and 5% CO_2). The brain was sectioned on a vibratome (Leica VT1000S) on ice, and each slice (300-400 μm) was immediately transferred to an ACSF bath at room temperature. After the brain slicing is complete (not more than 15 minutes), individual slices of interest were transferred to a small petri dish containing bubbled room temperature ACSF. The POA was microdissected under a fluorescence dissecting microscope, and the slices before and after dissection were imaged to examine the location of the microdissected tissue and confirm its location. The dissected tissue pieces were transferred to a microcentrifuge tube and treated with 1 mg/ml pronase (Sigma, Cat#P6911-1G) in carbogen-bubbled ACSF for 70 minutes at room temperature without mixing in a closed tube. After incubation, with the tissue pieces sitting at the bottom of the tube, the pronase solution was pipetted out of the tube and exchanged with cold ACSF containing 1% fetal bovine serum. The tissue pieces were dissociated into single cells by gentle trituration through Pasteur pipettes with polished tips of 600, 300, and 150 μm diameter. Single cells were isolated by FACS into individual wells of 96-well plates or 8-well PCR strips containing 2.275 μl of Dilution Buffer (SMARTer Ultra Low RNA Kit for Illumina Sequencing, Clontech Cat#634936), 0.125 μl RNase inhibitor (SMARTer kit), and 0.1 μl of 1:1,000,000 diluted RNA spike-in RNAs (ERCC RNA Spike-In Mix 1, Life Technologies Cat#4456740). Sorting was performed on a BD FACSAriaII SORP using a 130um nozzle, a sheath pressure of 10 psi, and in the single cell sorting mode. To exclude dead cells, DAPI (DAPI*2HCl, Life Technologies Cat#D1306) was added to the single cell suspension to the final concentration of 2 ng/ml. Sorted cells were frozen immediately on dry ice and stored at -80°C.

[00178] *cDNA amplification and library construction.* The SMARTer kit above was used to reverse transcribe single cell RNA and amplify the cDNA for 19 PCR cycles. To stabilize the RNA after quickly thawing the cells on ice, an additional 0.125 μl of RNase inhibitor mixed with SMART CDS Primer II A was immediately added to each sample. All steps downstream were

carried out according to the manufacturer's instructions. cDNA concentration was quantified using Agilent Bioanalyzer High Sensitivity DNA chips. For most samples, 1 ng of amplified cDNA was used as input to make sequencing libraries with Nextera XT DNA kit (Illumina Cat#FC-131-1096). Individual libraries were quantified using Agilent Bioanalyzer DNA 7500 chips. In order to assess sample quality and adjust the concentrations of libraries for multiplexing on HiSeq, all libraries were sequenced first on Illumina MiSeq to obtain approximately 100,000 reads per library, and then on Illumina HiSeq 2000 or 2500 to generate 100 bp reads.

TRAP

[00179] A previously described procedure was adapted to perform TRAP (Knight et al., (2012). Molecular profiling of activated neurons by phosphorylated ribosome capture. Cell *151*, 1126-1137).

[00180] Mice were sacrificed and the POA was rapidly dissected on ice with a dissection buffer (1xHBSS, 2.5 mM HEPES [pH 7.4], 4 mM $NaHCO_3$, 35mM Glucose, 100 µg/ml Cycloheximide). Brains from 6 mice were then pooled, homogenized in the homogenization buffer (10 mM HEPES [pH 7.4], 150 mM KCl, 5 mM $MgCl_2$, 100 nM calyculin A, 2 mM DTT, 100 U/ml RNasin, 100 µg/ml cycloheximide and protease). Homogenates were transferred to a microcentrifuge tube and clarified at 2,000xg for 10 min at 4°C. The supernatant was transferred to a new tube, and 70 µl of 10% NP40 and 70 µl of 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (DHPC, 300mM) per 1ml of supernatant were added. This solution was mixed and then clarified at 17,000xg for 10 min at 4°C. The resulting high-speed supernatant was transferred to a new tube. This supernatant served as the input. A small amount (25 µl) was added to a new tube containing 350 µl of buffer RLT for future input RNA purification.

[00181] Immunoprecipitation was performed with an anti-FLAG antibody loaded beads. The beads were washed four times using 0.15M KCI Wash buffer (10 mM HEPES [pH 7.4], 350 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 1% NP40, 100 U/ml RNasin, and 100 µg/ml cycloheximide). After the final wash the RNA was eluted by addition of buffer RLT (350 µL) to the beads on ice, the beads removed by a magnet, and the RNA purified using the RNeasy Micro Kit (QIAGEN) and analyzed using an Agilent 2100 Bioanalyzer. cDNA libraries for RNA-seq were prepared with Ovation RNA-Seq System V2 and Ovation Ultralow Library Systems (NuGen) and analyzed on an Illumina HiSeq 2500. Gene classification shown in Table S1 was carried out using Panther (http://pantherdb.org/) (Mi et al., 2013).

Statistics

[00182] Statistical analysis was performed using Matlab, GraphPad Prism or Python. All statistical tests were two-sided. The 95% confidence intervals (CI) for brain state probabilities were calculated using a bootstrap procedure: For an experimental group of *n* mice, with mouse *i*

comprising m_i trials, the data was repeatedly resampled by randomly drawing for each mouse m_i trials (random sampling with replacement). For each of the 10,000 iterations, the mean probabilities was recalculated for each brain state across the n mice. The lower and upper confidence intervals were then extracted from the distribution of the resampled mean values. To test whether a given brain state is significantly modulated by laser stimulation, the difference between the mean probabilities during laser stimulation and the preceding period of identical duration was calculated for each bootstrap iteration.

Example 2: Optogenetic Activation of $GAD^{POA\rightarrow TMN}$ Neurons Promotes Sleep

[00183] To identify the optimal target for retrograde labeling of these neurons, fluorescent latex microspheres (RetroBeads) were injected into the TMN, dorsomedial hypothalamus (DMH), ventrolateral periaqueductal gray (vlPAG), or the prefrontal cortex (PFC). Seven to ten days later, each mouse was sleep deprived for 6 hr, allowed undisturbed rebound sleep for 4 hr, and then sacrificed for c-Fos immunohistochemistry. Elevated c-Fos expression was found in the POA (Figure 1A), consistent with previous studies. Among the POA targets tested, retrobead injection into the TMN caused the highest percentage of labeling among the c-Fos+ neurons (Figure 1A; 64.3 ± 5.1% of c-Fos+ neurons contained RetroBeads from TMN, 33.6 ± 3.5% from DMH, 9.5 ± 1.3% from vlPAG, 0% from PFC, mean ± SEM), consistent with the previous finding (Sherin et al., 1996, 1998). The TMN was thus chosen for retrograde labeling of sleep-active POA neurons.

[00184] To label the TMN-projecting neurons, a lentivirus pseudotyped with the rabies glycoprotein (RG), which can be taken up by axon terminals and retrogradely transported to the cell body, was used. Since the great majority of c-Fos+ sleep-active neurons in the POA are GABAergic (Figure 8), the pseudotyped lentivirus with Cre-dependent expression of ChR2 and eYFP (rEIAV-DIO-TLoop-ChR2-eYFP) (Cetin and Callaway, 2014) was injected into the TMN of GAD2-Cre mice. Many eYFP-labeled neurons in the POA were found, the majority of which expressed c-Fos+ following sleep rebound (73.6 ± 4.8%, Figure 1B). These neurons will be referred to as $GAD^{POA\rightarrow TMN}$ neurons.

[00185] **Figures 1A-1B. Retrograde Labeling and c-Fos Staining of Sleep-Active POA Neurons (Figure 1A)** Overlap between c-Fos expression induced by sleep rebound and retrobead (RB) labeling from TMN, DMH, vlPAG or PFC. Coronal diagram was adapted from The Mouse Brain in Stereotaxic Coordinates (Franklin and Paxinos, 2007). Percentage of c-Fos+ cells containing RB differed among target regions ($p < 0.0001$, one-way ANOVA followed by Dunnett's post-hoc test). Error bar, ± SEM. **(Figure 1B)** Expression of ChR2-eYFP in the POA

induced by lentivirus (rEIAV-DIO-TLoop-ChR2-eYFP) injection into the TMN of a GAD2-Cre mouse. Many ChR2-eYFP cells expressed c-Fos following sleep rebound (arrowheads). See also Figure 8.

[00186] **Figure 8 c-Fos Staining of Sleep-Active POA Neurons in GAD1-GFP or VGLUT2-GFP Mice.** Overlap between c-Fos expression induced by sleep rebound and GAD1-GFP (top panels) or VGLUT2-GFP (bottom panels). Many c-Fos+ cells contained GAD1-GFP (arrowheads). Graph shows percentage of c-Fos+ cells containing GAD1-GFP or VGLUT2-GFP. Error bar, ± SEM.

[00187] To test the causal role of $GAD^{POA\rightarrow TMN}$ neurons in sleep regulation, they were optogenetically activated in freely moving mice. Laser stimulation (10 Hz, 2 min/trial, through an optic fiber implanted into the POA) was applied every 15-25 min, and wakefulness, REM, and NREM states were classified based on electroencephalogram (EEG) and electromyogram (EMG) recordings (Figure 2A). Activation of the $GAD^{POA\rightarrow TMN}$ neurons caused an immediate increase in NREM sleep and a delayed increase in REM sleep at the expense of wakefulness (Figures 2B and 2C). Analysis of the probability of transitions between each pair of brain states showed that laser stimulation significantly enhanced wake→NREM and NREM→REM transitions but decreased NREM→wake transitions (Figure 10A). In control mice with $GAD^{POA\rightarrow TMN}$ neurons expressing the fluorescent protein nlsPelican without ChR2, laser stimulation had no effect (Figures 9A, 9B and 10B), and the laser-induced changes in brain states were significantly different between the ChR2 and control mice ($p < 0.001$ for wake and REM, two-way ANOVA with bonferroni post-hoc test). Thus, the observed increase in sleep was caused by the activation of $GAD^{POA\rightarrow TMN}$ neurons rather than by the laser light.

[00188] **Figures 2A-2C Optogenetic Activation of $GAD^{POA\rightarrow TMN}$ Neurons Induces Sleep.** **(Figure 2A)** Schematic of optogenetic experiment. Right panel, fluorescence image of POA (box in schematic) in a GAD2-Cre mouse with rEIAV-DIO-TLoop-ChR2-eYFP injected into the TMN. **(Figure 2B)** Two example trials in an experiment. Shown for each trial are EEG power spectrum, EMG trace, brain states (color coded), and EEG and EMG traces during selected periods (indicated by boxes) on an expanded time scale. Laser stimulation (blue shading, 10 Hz, 120 s) caused both wake→NREM (upper trial) and NREM→REM (lower trial) transitions. **(Figure 2C)** Probability of wake, NREM, or REM states before, during, and after laser stimulation (applied between 0 and 120 s, light blue shading), averaged from 9 mice ($p < 0.0001$ for change from wake to sleep states, bootstrap). Shading for each trace, 95% confidence interval (CI). See also Figures 9A, 9B, 10A-10C)

[00189] **Figures 9A-9B Effect of Laser Stimulation in $GAD^{POA\rightarrow TMN}$-nlsPelican Control Mice. (Figure 9A)** Schematic of optogenetic experiment. **(Figure 9B)** Probability of wake, NREM, or REM states before, during, and after laser stimulation (applied between 0 and 120 s, light blue shading), averaged from 4 mice ($p = 0.6104$, 0.9153 or 0.0548 for REM, NREM or wake respectively, bootstrap). Shading for each trace, 95% confidence interval (CI).

[00190] **Figures 10A-10C Effect of Laser Stimulation on Transition Probability Between Each Pair of Brain States in $GAD^{POA\rightarrow TMN}$-ChR2, $GAD^{POA\rightarrow TMN}$-Ctrl and GAD^{POA}-ChR2 mice, Related to Figures 2A-2C, 3A-3F and 9A-9B. (Figure 10A)** Transition probability within each 60 s period in $GAD^{POA\rightarrow TMN}$-ChR2 mice. Error bar, 95% CI (bootstrap). Probability of baseline transition (grey dashed line) was computed after excluding the laser stimulation period. The probability from wake to NREM and NREM to REM during laser stimulation was significantly higher than the baseline, and the probability from NREM to wake transition was significantly lower than the baseline ($p < 0.0001$, bootstrap). **(Figure 10B)** Transition probability in control mice. The probability during laser stimulation was not significantly different from baseline (left panel: $p = 0.98$, >0.99, 0.21 from top to bottom; right panel: $p > 0.99$, 0.92, 0.93, bootstrap). **(Figure 10C)** Transition probability in GAD^{POA}-ChR2 mice. The probability from NREM to wake during laser stimulation was significantly higher than the baseline, and the probability from wake to NREM was significantly lower than the baseline ($p < 0.0001$, bootstrap). Red * indicates transition probability that is significantly higher than the baseline. Green # indicates transition probability that is significantly lower than the baseline.

[00191] To test whether the $GAD^{POA\rightarrow TMN}$ neurons are functionally distinct from other nearby neurons, an AAV with Cre-dependent expression of ChR2-eYFP was injected into the POA of GAD2-Cre mice (Figure 3A), which should infect POA GABAergic neurons irrespective of their projection targets. Optogenetic activation of these neurons caused an immediate and long-lasting increase in wakefulness (Figures 3B and 3C), through an enhanced NREM→wake and decreased wake→NREM transition probability (Figure 10C). Thus, while the entire POA GABAergic population contains both sleep- and wake-promoting neurons, the effect of non-selective activation is dominated by an increased wakefulness. To test whether the sleep-promoting effect of TMN-projecting neurons is specific to GABAergic cells, the ChR2-expressing lentivirus was injected into the TMN of VGLUT2-Cre mice (Figure 3D). Optogenetic activation of the $VGLUT^{POA\rightarrow TMN}$ neurons induced an immediate increase in wakefulness (Figures 3E and 3F), indicating that the sleep-promoting effect is specific to neurons that are both GABAergic and TMN projecting.

[00192] **Figures 3A-3F Optogenetic Activation of GAD^{POA} or $VGLUT^{POA\rightarrow TMN}$ Neurons Promotes Wakefulness (Figure 3A)** Schematic for optogenetic stimulation of GAD^{POA} neurons. **(Figure 3B)** Two example trials of an experiment. Shown for each trial are EEG power spectrum, EMG trace, brain states (color coded), and EEG and EMG traces during selected periods (indicated by boxes) on an expanded time scale. Laser stimulation (blue shading, 10 Hz, 120 s) caused NREM→wake transitions in both trials. **(Figure 3C)** Probability of wake, NREM, or REM states before, during, and after laser stimulation (between 0 and 120 s), averaged from 5 mice ($p < 0.0001$ for increase in wakefulness, bootstrap). Shading for each trace, 95% CI. **(Figure 3D)** Schematic for optogenetic stimulation of $VGLUT^{POA\rightarrow TMN}$ neurons. **(Figure 3E)** Example trials of an experiment. Laser stimulation (blue shading, 10 Hz, 120 s) caused both NREM→wake (upper trial) and REM→wake (lower trial) transitions. **(Figure 3F)** Probability of wake, NREM, or REM states before, during, and after laser stimulation, averaged from 4 mice ($p < 0.0001$ for increase in wakefulness, bootstrap). See also Figures 10A-10C

Example 3: $GAD^{POA\rightarrow TMN}$ Neurons Are Sleep Active

[00193] Although the majority of $GAD^{POA\rightarrow TMN}$ neurons labeled with ChR2-eYFP expressed c-Fos following sleep rebound (Figure 1B), suggesting that they are sleep active, c-Fos expression is also modulated by non-activity-related factors. To measure directly the spiking activity of ChR2-labeled $GAD^{POA\rightarrow TMN}$ neurons across the sleep-wake cycle, optrode recordings were performed in the POA of freely moving mice. High-frequency laser pulse trains (10 or 20 Hz, 5-10 ms/pulse, 1 s/train) were applied intermittently, and single units exhibiting reliable laser-evoked spiking with short latencies and low jitter were identified as $GAD^{POA\rightarrow TMN}$ neurons (see Example 1, Figures 4A-4C and 11A-11B). For the 17 identified neurons, the mean firing rate was significantly higher during REM ($p = 8.5 \times 10^{-4}$, Wilcoxon signed rank test) and NREM ($p = 0.0014$) sleep than during wakefulness. Individually, all of the 17 neurons showed higher firing rates during REM sleep than wakefulness, and 13 of them also showed higher rates during NREM sleep ($p < 0.05$, Wilcoxon rank sum test; the remaining 4 cells showed no significant difference between NREM and wake states; Figures 4D-4F).

[00194] **Figures 4A-4F Optogenetically Identified $GAD^{POA\rightarrow TMN}$ Neurons are Active during Sleep. (Figure 4A)** Example recording of spontaneous and laser-evoked spikes from a $GAD^{POA\rightarrow TMN}$ neuron. Blue ticks, laser pulses (20 Hz). **(Figure 4B)** Comparison between laser-evoked (blue) and averaged spontaneous (grey) spike waveforms. **(Figure 4C)** Spike raster showing multiple trials of laser stimulation at 10 Hz and 20 Hz. **(Figure 4D)** Firing rates of an example $GAD^{POA\rightarrow TMN}$ neuron. **(Figure 4E)** Firing rates of 17 identified $GAD^{POA\rightarrow TMN}$ neurons

during different brain states. Each line shows firing rates of one unit; grey bar, average across units. Error bar, ± SEM. **(Figure 4F)** Summary of firing rate modulation of 17 identified (blue) and 51 unidentified (grey) units. W, wake; R, REM; NR, NREM. See also Figures 11A, 11Band 12.

[00195] **Figures 11A-11B Optogenetic Identification of $GAD^{POA\rightarrow TMN}$ Neurons. (Figure 11A)** Distribution of delays in laser-evoked spiking for all identified neurons. Delay is defined as timing of the first spike after each laser pulse. **(Figure 11B)** Distribution of correlation coefficient between laser-evoked and spontaneous spike waveforms for all identified neurons.

[00196] Compared to these identified $GAD^{POA\rightarrow TMN}$ neurons, the 51 unidentified neurons recorded in the POA showed much greater functional diversity (Figures 4F and 12), including many neurons that were maximally active during wakefulness (Figure 12). Thus, the $GAD^{POA\rightarrow TMN}$ neurons represent a subset of POA neurons that are sleep active, a property well suited for their sleep-promoting function.

[00197] **Figure 12 Firing Rates of Unidentified POA Neurons.** Firing rates of unidentified units in the three brain states. Each line represents data from one neuron. Grey bar represents average over units (n = 51). Error bar, ± SEM.

Example 4: Virus-Assisted Mapping of Inputs to $GAD^{POA\rightarrow TMN}$ Neurons

[00198] To explore the circuit mechanisms regulating the activity of $GAD^{POA\rightarrow TMN}$ neurons, monosynaptic inputs to the $GAD^{POA\rightarrow TMN}$ neurons were mapped using a newly developed cTRIO (cell type-specific tracing the relationship between input and output) method. A retrograde virus CAV-$FLEx^{loxP}$-Flp was injected into the TMN of GAD2-Cre mice to express Flp recombinase specifically in $GAD^{POA\rightarrow TMN}$ neurons, and AAV-$FLEx^{FRT}$-TVA-mCherry and AAV-$FLEx^{FRT}$-RG were injected into the POA to express TVA (the receptor for the EnvA envelope glycoprotein)-mCherry and RG in the Flp-expressing neurons. Two to three weeks later, EnvA-pseudotyped, G-deleted, and GFP-expressing rabies virus (RV*dG*) was injected into the POA (Figure 5A) to infect the TVA-expressing $GAD^{POA\rightarrow TMN}$ neurons and transsynaptically label their inputs.

[00199] GFP-labeled neurons were found in multiple brain regions (Figure 5B). Compared to the PFC-projecting POA GABAergic ($GAD^{POA\rightarrow PFC}$) neurons, which are wake promoting (ChR2-mediated activation induced a significant increase in wakefulness, p = 0.0008, bootstrap), $GAD^{POA\rightarrow TMN}$ neurons received significantly more inputs from the hypothalamus and the amygdala, but fewer inputs from the striatum, midbrain and pons (Figure 5B). In particular, the central nucleus of the amygdala (CEA) provided much more GABAergic inputs to $GAD^{POA\rightarrow TMN}$ than to $GAD^{POA\rightarrow PFC}$ neurons (Figures 5C and 5D). To test the role of this CEA GABAergic

input in modulating brain states, AAV-EF1α-DIO-ChR2-eYFP was injected into the CEA of GAD2-Cre mice and stimulated their axonal projection into the POA (Figures 5E and 5F). Laser stimulation significantly suppressed sleep and increased wakefulness, consistent with the expected effect of GABAergic inhibition of the sleep-promoting $GAD^{POA\rightarrow TMN}$ neurons (Figure 5F).

[00200] **Figures 5A-5F Mapping of Monosynaptic Inputs to $GAD^{POA\rightarrow TMN}$ Neurons Using cTRIO. (Figure 5A)** Schematic of cTRIO to map monosynaptic inputs to $GAD^{POA\rightarrow TMN}$ (left) or $GAD^{POA\rightarrow PFC}$ (right) neurons. Middle, coronal section of a mouse brain at the POA stained with Hoechst (blue). A region within the square is magnified in the inset. Arrowheads indicate starter cells (yellow) at the injection site (Scale bar in inset, 50 μm). **(Figure 5B)** Average fractional inputs in $cTRIO^{POA\rightarrow TMN}$ (purple) or $cTRIO^{POA\rightarrow PFC}$ (grey) tracing ($p = 0.0002$ for hypothalamus, $p = 0.02$ for amygdala, $p = 0.03$ for striatum, $p = 0.001$ for midbrain, $p = 0.003$ for pons, t-test). Error bar, ± SEM. **(Figure 5C)** Comparison of CEA inputs in $cTRIO^{POA\rightarrow TMN}$ and $cTRIO^{POA\rightarrow PFC}$ tracing ($p = 0.004$, t-test). **(Figure 5D)** Rabies-labeled presynaptic neurons in the CEA are largely GABAergic. Top, coronal diagram of mouse brain. Middle, coronal section at the CEA (box in coronal diagram) stained with Hoechst. Bottom, rabies-labeled presynaptic neurons and GAD1 FISH signals in the CEA. Arrowheads indicate GFP-labeled neurons expressing GAD1; 75.2 ± 4.3% of GFP+ neurons were GAD1+. **(Figure 5E)** Schematic for optogenetic stimulation of CEA GABAergic projection to the POA. **(Figure 5F)** Probability of wake, NREM, or REM states before, during, and after laser stimulation (between 0 and 120 s), averaged from 4 mice ($p < 0.0001$ for increase in wakefulness, bootstrap). Shading for each trace, 95% CI.

Example 5: Gene Expression Profiling of $GAD^{POA\rightarrow TMN}$ Neurons

[00201] Having characterized the function of $GAD^{POA\rightarrow TMN}$ neurons in sleep regulation, the molecular markers labeling these neurons were next identified. First, the translating ribosome affinity purification (TRAP) method was used in conjunction with retrograde labeling using herpes simplex virus (HSV). HSV was injected with Cre-dependent expression of the large ribosomal subunit protein Rpl10a fused with a FLAG/HA tag (HSV-LoxSTOPLox-FlagHA-L10a) into the TMN of VGAT-Cre mice. After 30-45 days of expression, the POA was dissected, and ribosome immunoprecipitation was performed using magnetic beads coated with anti-FLAG antibodies to pull down the mRNAs attached to Rpl10a for gene profiling (Figures 6A and 13A).

[00202] **Figures 6A-6F. Identification of Molecular Markers for POA Sleep-Promoting Neurons Using TRAP. (Figure 6A)** Schematic of the TRAP method. **(Figure 6B)** FPKM (fragments per kilobase of transcript per million mapped reads) IP plotted against FPKM input

on a log scale. Marker genes enriched in $GAD^{POA \rightarrow TMN}$ neurons such as *Cck*, *Crh*, *Slc32a1* and *Rpl10a* are highlighted. Red dots indicate genes that are significantly different in IP versus input ($p < 0.05$, Fisher's exact test), and blue dots indicate non-significant genes. **(Figure 6C)** Overlap between HA labeling and CCK expression. Shown is a coronal section at the POA stained with HA antibody (red) and Hoechst (blue). Region within the square is magnified in the inset. Arrowheads indicate HA-labeled neurons stained with CCK antibody (Scale bar in inset, 50 μm). **(Figure 6D)** Probability of wake, NREM, or REM states before, during, and after optogenetic stimulation of POA CCK neurons, averaged from 3 mice ($p < 0.0001$ for all states, bootstrap). Shading for each trace, 95% CI. **(Figure 6E)** HA-positive POA neurons and their overlap with CRH. Scale bar in inset, 50 μm. **(Figure 6F)** Probability of wake, NREM, or REM states before, during, and after optogenetic stimulation of POA CRH neurons, averaged from 4 mice ($p = 0.0015$ for REM, $p < 0.0001$ for NREM and wake). See also Figures 13A, 13B, 14A and 14B.

[00203] **Figures 13A-13B . Identification of Differentially Expressed Genes in TRAP. (Figure 13A)** Bioanalyzer trace of immunoprecipitated RNA. FU, fluorescence units. **(Figure 13B)** Histogram display of differentially expressed genes (IP/input).

[00204] RNA-seq revealed multiple genes that were enriched in immunoprecipitated (IP) RNA from the $GAD^{POA \rightarrow TMN}$ neurons relative to the input RNA (the remaining lysates after IP, Figures 6B and 13B). Genes encoding neuropeptides were studied further, because they play important roles in interneuronal communication and behavioral regulation along with classical neurotransmitters and have proved to be very useful markers for specific cell types. Among the highly enriched neuropeptides (Table 1 in Figure 16), corticotropin-releasing hormone (CRH) and cholecystokinin (CCK), both of which have been implicated in brain state regulation, were closely examined. Immunohistochemistry showed that CCK and CRH are expressed in 49.1 ± 7.5 and 6.6 ± 0.9%, respectively, of HA-labeled neurons in the POA (Figures 6C and 6E), indicating that each marker indeed labels a subset of $GAD^{POA \rightarrow TMN}$ neurons. The two markers also showed partial overlap in their POA expression (Figure 14A). To test whether the CCK- or CRH-expressing POA neurons are sleep promoting, AAV-EF1α-DIO-ChR2-eYFP was injected into the POA of CCK- or CRH-Cre mice. Laser stimulation significantly increased NREM and REM sleep while suppressing wakefulness in both CCK-Cre and CRH-Cre mice (Figures 6D and 6F), indicating that both markers label sleep-promoting POA neurons.

[00205] **Figures 14A-14B Overlap of Identified Molecular Markers in POA. (Figure 14A)** Overlap between CCK and CRH: 55.1 ± 9.4% of CRH+ neurons contained CCK, and 19.8 ± 7.6% of CCK+ neurons contained CRH. **(Figure 14B)** Overlap between CCK and substance P:

55.4 ± 10.5% of substance P+ neurons contained CCK, and 12.6 ± 1.1% of CCK+ neurons contained substance P.

[00206] Notably, a significant enrichment of galanin (GAL, Table 1 in Figure 16), a neuropeptide highly expressed in VLPO sleep-active neurons, was also found. However, in GAL-Cre mice (obtained from GENSAT) with AAV-EF1α-DIO-ChR2-eYFP injected into the POA, optogenetic activation increased wakefulness and decreased sleep (Figure 15), suggesting that GAL also labels some wake-promoting neurons.

[00207] **Figure 15 Optogenetic Stimulation of POA GAL Neurons Promotes Wakefulness.** Probability of wake, NREM, or REM states before, during, and after laser stimulation (applied between 0 and 60 s, light blue shading), averaged from 4 mice ($p < 0.0001$ for increase in wakefulness, bootstrap). Shading for each trace, 95% confidence interval (CI).

[00208] As a complement to the TRAP method, transcriptome analysis of $GAD^{POA \rightarrow TMN}$ neurons at the single-cell level was also performed. Four weeks after injecting the retrograde lentivirus rEIAV-DIO-TLoop-nlsPelican into the TMN of GAD2-Cre mice, nlsPelican-labeled POA neurons were dissociated for single-cell RNA-seq (Figure 7A). high-level expression of *Tac1* (Figure 7B), a gene encoding several members of the tachykinin neuropeptide family, some of which have been implicated in sleep regulation, was found. Fluorescent in situ hybridization showed that *Tac1* was expressed in 48.4 ± 8.4% of nlsPelican-labeled neurons (Figures 7C), but only in ~10% of all GAD1+ neurons in the POA (connectivity(dot)brain-map(dot)org/transgenic/experiment/180304064), thus confirming its preferential labeling of the $GAD^{POA \rightarrow TMN}$ subpopulation. In TAC1-Cre mice injected with AAV-EF1α-DIO-ChR2-eYFP in the POA, optogenetic stimulation significantly increased NREM sleep and decreased wakefulness (Figure 7D), indicating that TAC1 also labels sleep-promoting POA neurons. Notably, TAC1 was not identified by the TRAP method, indicating that using both gene-profiling methods in parallel can significantly enhance the likelihood of identifying useful markers for sleep-promoting neurons.

Example 6: Pdyn-expressing neurons promote non-REM sleep when activated

[00209] Pdyn (encoding prodynorphin) was identified as a gene whose expression is elevated in the $GAD^{POA \rightarrow TMN}$ subpopulation. Similar to TAC1, in Pdyn-Cre mice injected with AAV-EF1α-DIO-ChR2-eYFP in the POA, optogenetic stimulation significantly increased NREM sleep and decreased wakefulness.

Example 7: Method of identifying an agent that promotes or suppresses sleep

[00210] A pharmaceutical composition that includes a test agent, e.g., a small molecule, nucleic acid, polypeptide, etc., that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in circuit neurons in a POA of a brain compared to the average expression level of the gene in neurons of the POA, is administered to a non-human mammal, e.g., a mouse. The test agent is administered at a dose sufficient to modulate the functional activity of the gene product in the brain, e.g., in the POA, of the non-human mammal.

[00211] The test agent specifically modulates the functional activity of a gene product encoded by a gene that is upregulated in the GABAergic, TMN-projecting POA neurons, e.g., $GAD^{POA \rightarrow TMN}$ neurons, where the gene encodes a polypeptide gene product selected from Table 3, in Figures 18A-18AI.

[00212] After administering the pharmaceutical composition, the sleep circuit-regulated state of the non-human mammal is monitored, e.g., by monitoring a sleep circuit-regulated state (e.g., REM sleep, non-REM sleep, wakefulness) using, e.g., EEG, EMG or a combination thereof.

[00213] If administration of the pharmaceutical composition that contains the test agent increases the probability of REM sleep, and/or non-REM sleep, and reduces the probability of wakefulness in the non-human mammal, the test agent is identified as an agent that promotes sleep. If administration of the pharmaceutical composition that contains the test agent reduces the probability of REM sleep, and/or non-REM sleep, and increases the probability of wakefulness in the non-human mammal, the test agent is identified as an agent that suppresses sleep.

[00214] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method of modulating a neural sleep circuit-regulated state of an individual, comprising:

illuminating with a light stimulus a first region of a brain of an individual, the first region comprising a first subtype of sleep circuit neurons, wherein a light-activated polypeptide is selectively expressed in neurons of the first subtype, and wherein the light-activated polypeptide modulates activity of neurons of the first subtype when illuminated by the light stimulus, thereby promoting or suppressing a sleep circuit-regulated state of the individual,

wherein the sleep circuit comprises:

a second subtype of neurons in a preoptic area (POA) of the brain, wherein neurons of the second subtype (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and/or

one or more third subtypes of neurons that synapse onto, and/or receive a synaptic connection from, neurons of the second subtype.

2. The method of claim 1, wherein the illuminating induces i) a first change in a probability of REM sleep and/or non-REM sleep, and ii) a second change in a probability of wakefulness, wherein the first and second change are of opposite sign.

3. The method of claim 1 or 2, wherein neurons of the first subtype comprise neurons in the POA that express one or more neuropeptide genes selected from the group consisting of: corticotropin releasing hormone, galanin, neurotensin, tachykinin 1, tachykinin 2, prepronociceptin, calcitonin-related polypeptide alpha, prodynorphin, and cholecystokinin.

4. The method of any one of claims 1 to 3, wherein the second subtype of neurons comprises the first subtype of neurons.

5. The method of claim 4, wherein the illuminating increases the probability of REM sleep and/or non-REM sleep, and reduces the probability of wakefulness.

6. The method of any one of claims 1 to 3, wherein the one or more third subtypes of neurons comprise the first subtype of neurons.

7. The method of claim 6, wherein neurons of the third subtype comprise hypothalamic or amygdala neurons.

8. The method of claim 7, wherein the amygdala neurons are GABAergic neurons of the central nucleus of the amygdala (CEA).

9. The method of any one of claims 6 to 8, wherein the illuminating reduces the probability of REM sleep and/or non-REM sleep, and increases the probability of wakefulness.

10. The method of any one of claims 1 to 9, wherein the light-activated polypeptide is an ion channel or an ion pump.

11. The method of any one of claims 1 to 10, further comprising monitoring a sleep circuit-regulated state of the individual before and/or after the illuminating, wherein the sleep circuit-regulated state comprises REM sleep, non-REM sleep and/or wakefulness.

12. The method of claim 11, wherein the monitoring comprises using electroencephalography (EEG) and/or electromyography (EMG) to determine the sleep circuit-regulated state.

13. The method of any one of claims 1 to 12, wherein the individual has a sleeping disorder.

14. The method of any one of claims 1 to 12, wherein the individual is sleep deprived.

15. The method of any one of claims 1 to 14, further comprising, before the illuminating, genetically modifying neurons of the first subtype with a nucleic acid comprising a nucleotide sequence encoding the light-activated polypeptide, wherein the nucleic acid is configured to express the light-activated polypeptide selectively in neurons of the first subtype.

16. The method of claim 15, wherein the genetically modifying comprises administering to a second region of the brain an expression vector comprising the nucleic acid, wherein the second region comprises one or more subtypes of neurons of the sleep circuit.

17. The method of claim 16, wherein the expression vector is a recombinant viral expression vector, and wherein the administering comprises administering a virion comprising the viral expression vector.

18. The method of claim 16 or 17, wherein the second region is the TMN or the POA.

19. The method of any one of claims 16 to 18, wherein the first region and second region are same regions of the brain.

20. The method of claim 17 or 18, wherein the first region and second region are different regions of the brain, wherein the second region comprises neurons of a third subtype that receive a synaptic connection from neurons of the second subtype, and wherein the recombinant viral vector is derived from a lentiviral vector.

21. The method of claim 20, wherein the virion is pseudotyped with a rabies glycoprotein.

22. The method of any one of claims 15 to 21, wherein the individual is a transgenic, non-human mammal expressing a site-specific recombinase in GABAergic neurons or glutamatergic neurons of the brain.

23. A method of identifying an agent that promotes or suppresses sleep, comprising:

i) administering to a non-human mammal a test agent that modulates a functional activity of a gene product encoded by a gene whose expression is upregulated in circuit neurons in a POA of a brain compared to the average expression level of the gene in neurons of the POA, wherein the sleep circuit neurons (a) are gamma aminobutyric acid-producing (GABAergic), and (b) project to a tuberomammillary nucleus (TMN) of the brain; and

ii) monitoring a sleep circuit-regulated state of the non-human mammal, wherein the sleep circuit-regulated state comprises REM sleep, non-REM sleep and/or wakefulness,

wherein a test agent that changes the probability of REM sleep, non-REM sleep, and/or wakefulness in the non-human mammal is identified as an agent that promotes or suppresses sleep.

24. The method of claim 15, wherein the test agent that causes i) a first change in the probability of REM sleep and/or non-REM sleep, and ii) a second change in the probability of wakefulness, wherein the first and second change are of opposite sign, is identified as an agent that promotes or suppresses sleep.

25. The method of claim 15 or 24, wherein the test agent specifically binds to the gene product.

26. The method of any one of claims 15 to 25, wherein the test agent is a small molecule, nucleic acid, polypeptide or a combination thereof.

27. The method of any one of claims 15 to 26, wherein the gene product is a post-transcriptional gene product.

28. The method of claim 27, wherein the gene product is a post-translational gene product.

29. The method of any one of claims 15 to 28, wherein the gene encodes a cell-surface receptor.

30. The method of claim 29, wherein the gene encodes a G-protein coupled receptor.

31. The method of any one of claims 15 to 30, wherein the gene encodes a polypeptide gene product selected from a polypeptide gene product listed in Table 3, in Figures 18A-18AI.

32. The method of any one of claims 15 to 31, wherein the monitoring comprises using electroencephalography (EEG) and/or electromyography (EMG) to determine the sleep circuit-regulated state.

33. The method of any one of claims 15 to 32, wherein the non-human mammal has a sleeping disorder.

34. The method of any one of claims 15 to 32, wherein the non-human mammal is sleep deprived.

FIG. 1A

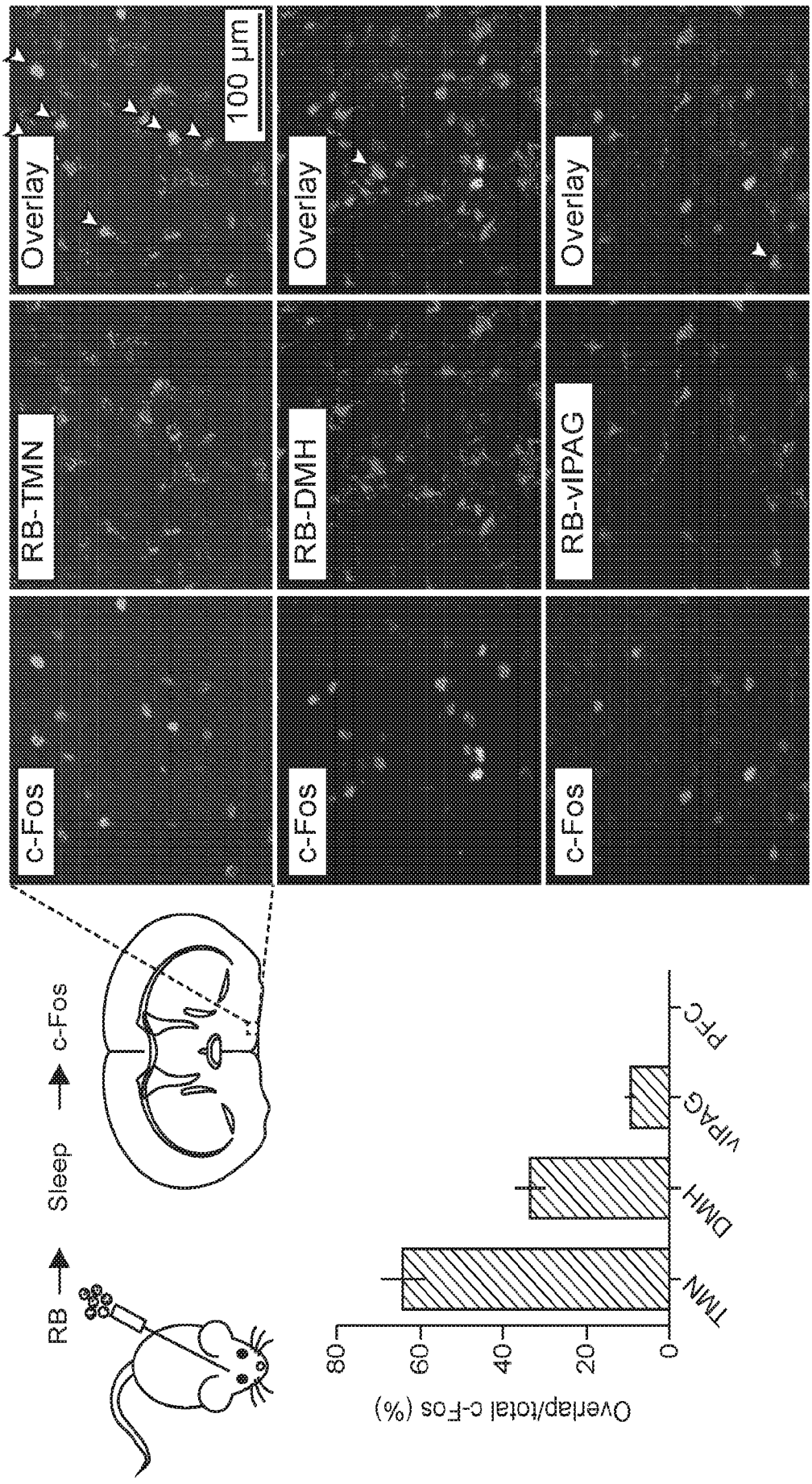

c-Fos
RB-TMN
Overlay
100 µm
c-Fos
RB-DMH
Overlay
c-Fos
RB-vlPAG
Overlay
RB
Sleep
c-Fos
Overlap/total c-Fos (%)
0
20
40
60
80
TMN
DMH
VlPAG
PFC

FIG. 1B

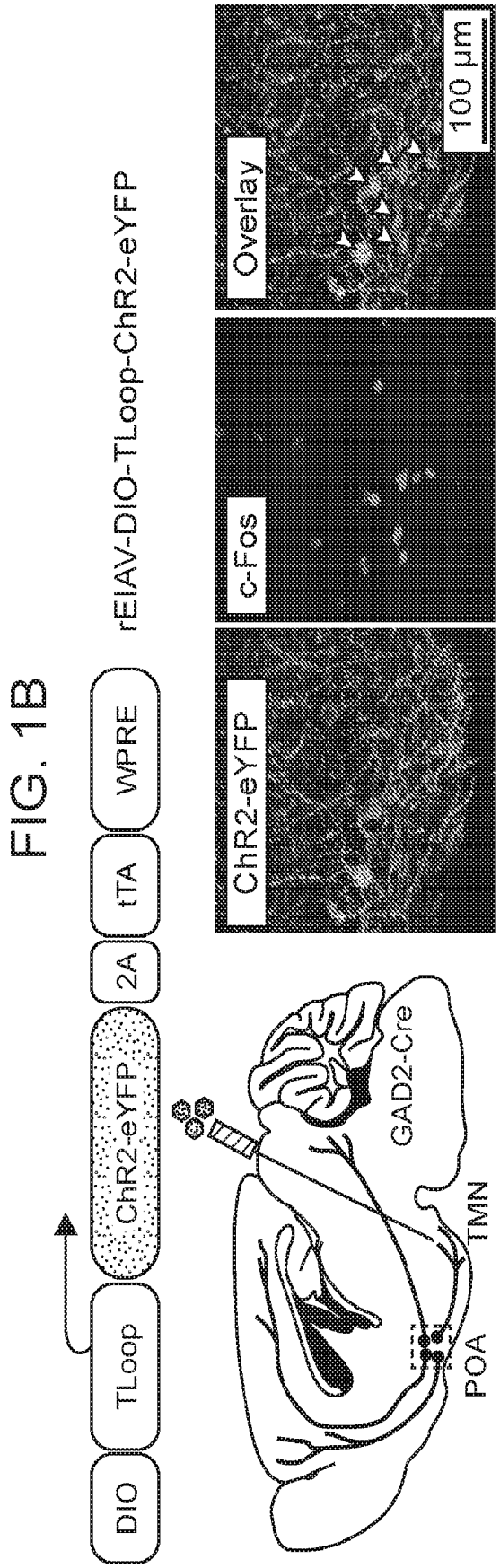

DIO
TLoop
ChR2-eYFP
2A
tTA
WPRE
rEIAV-DIO-TLoop-ChR2-eYFP
ChR2-eYFP
c-Fos
Overlay
100 µm
GAD2-Cre
TMN
POA

FIG. 2A

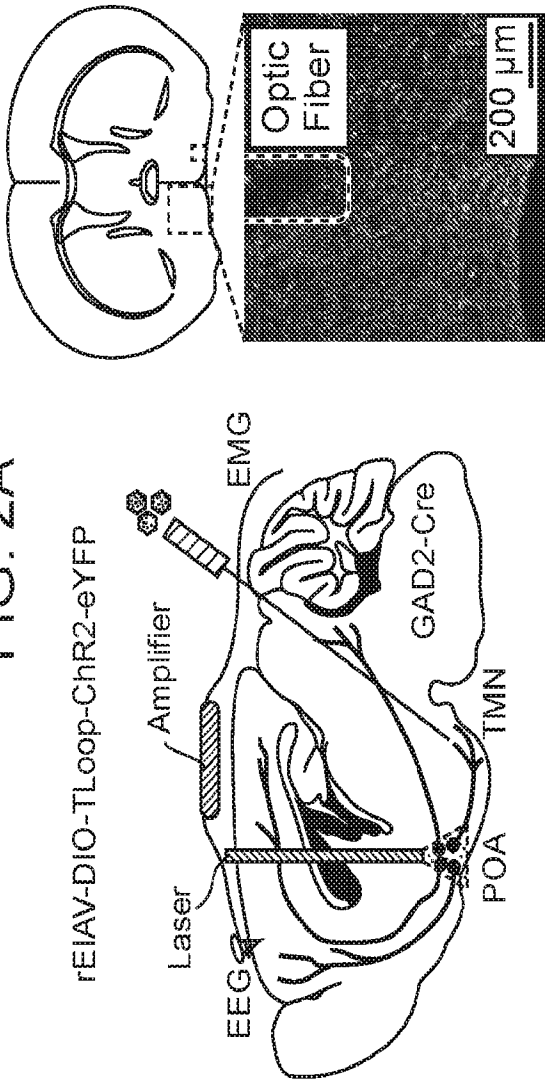

rEIAV-DIO-TLoop-ChR2-eYFP
Amplifier
EMG
Laser
EEG
GAD2-Cre
TMN
POA
Optic Fiber
200 µm

FIG. 2B

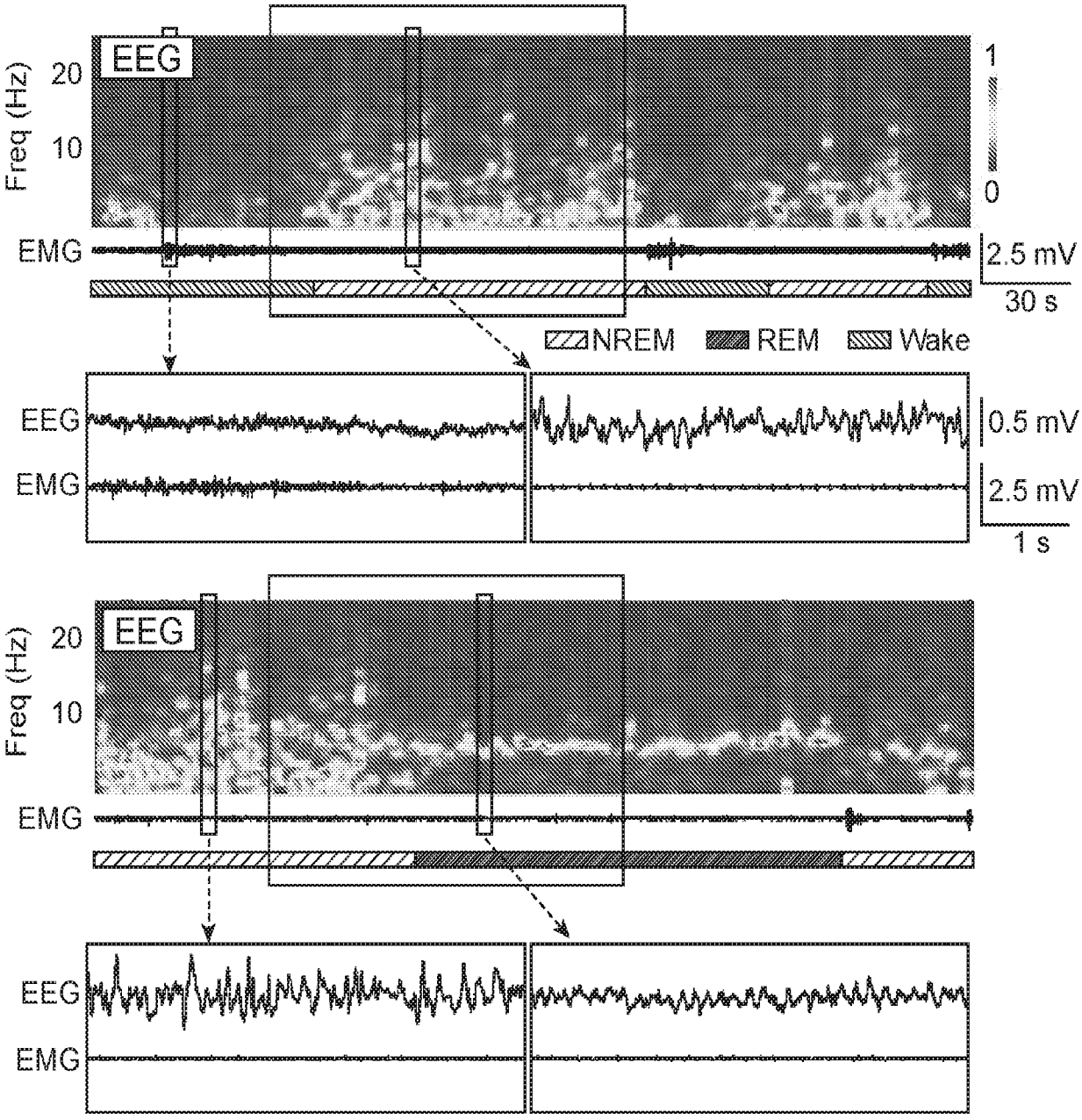

EEG
Freq (Hz)
20
10
EMG
1
0
2.5 mV
30 s
NREM
REM
Wake
EEG
EMG
0.5 mV
2.5 mV
1 s
EEG
Freq (Hz)
20
10
EMG
EEG
EMG

FIG. 2C

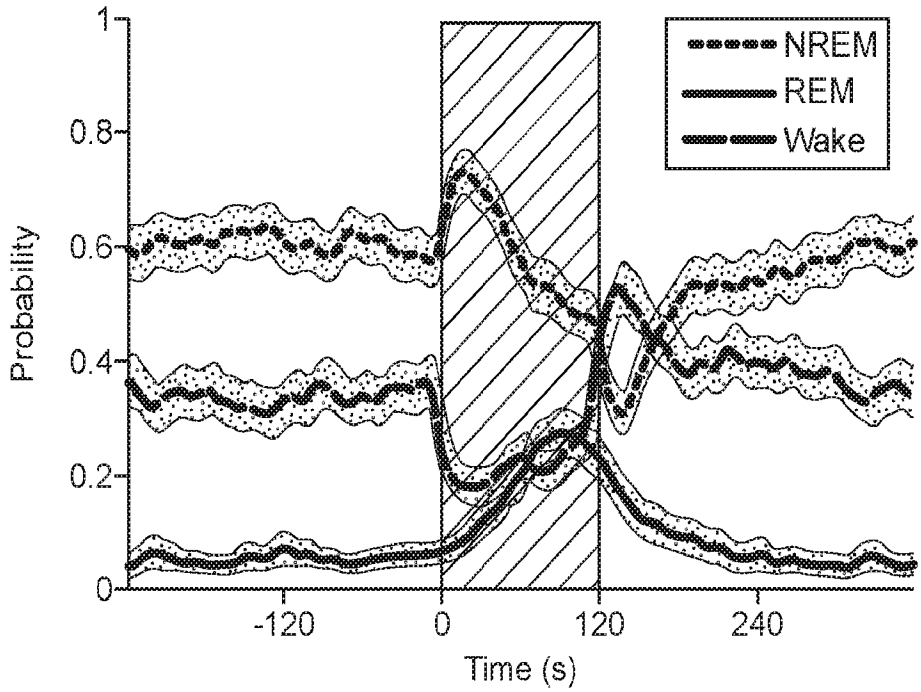

NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 3A

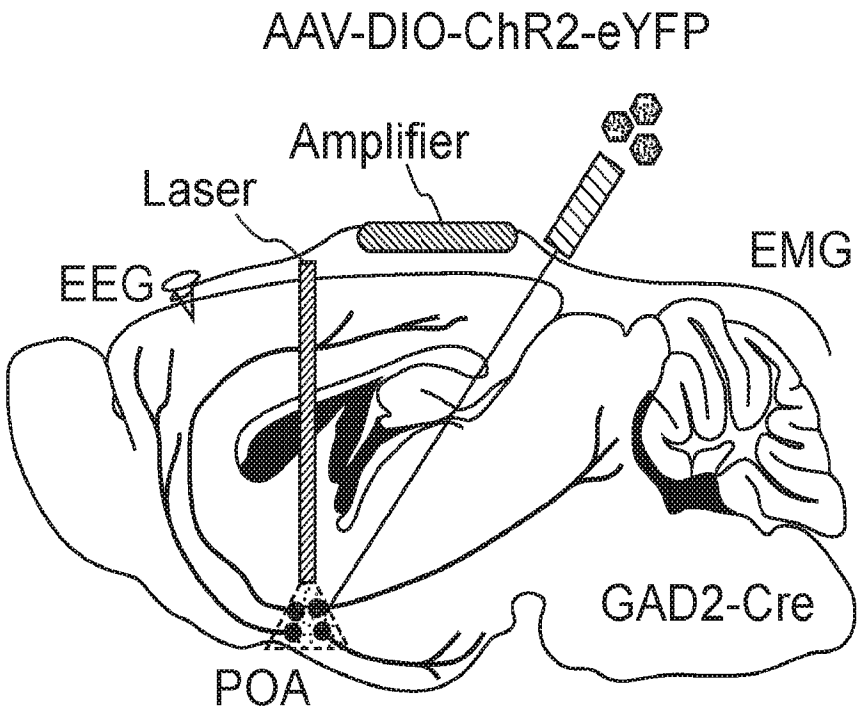

AAV-DIO-ChR2-eYFP
Amplifier
Laser
EMG
EEG
GAD2-Cre
POA

FIG. 3B

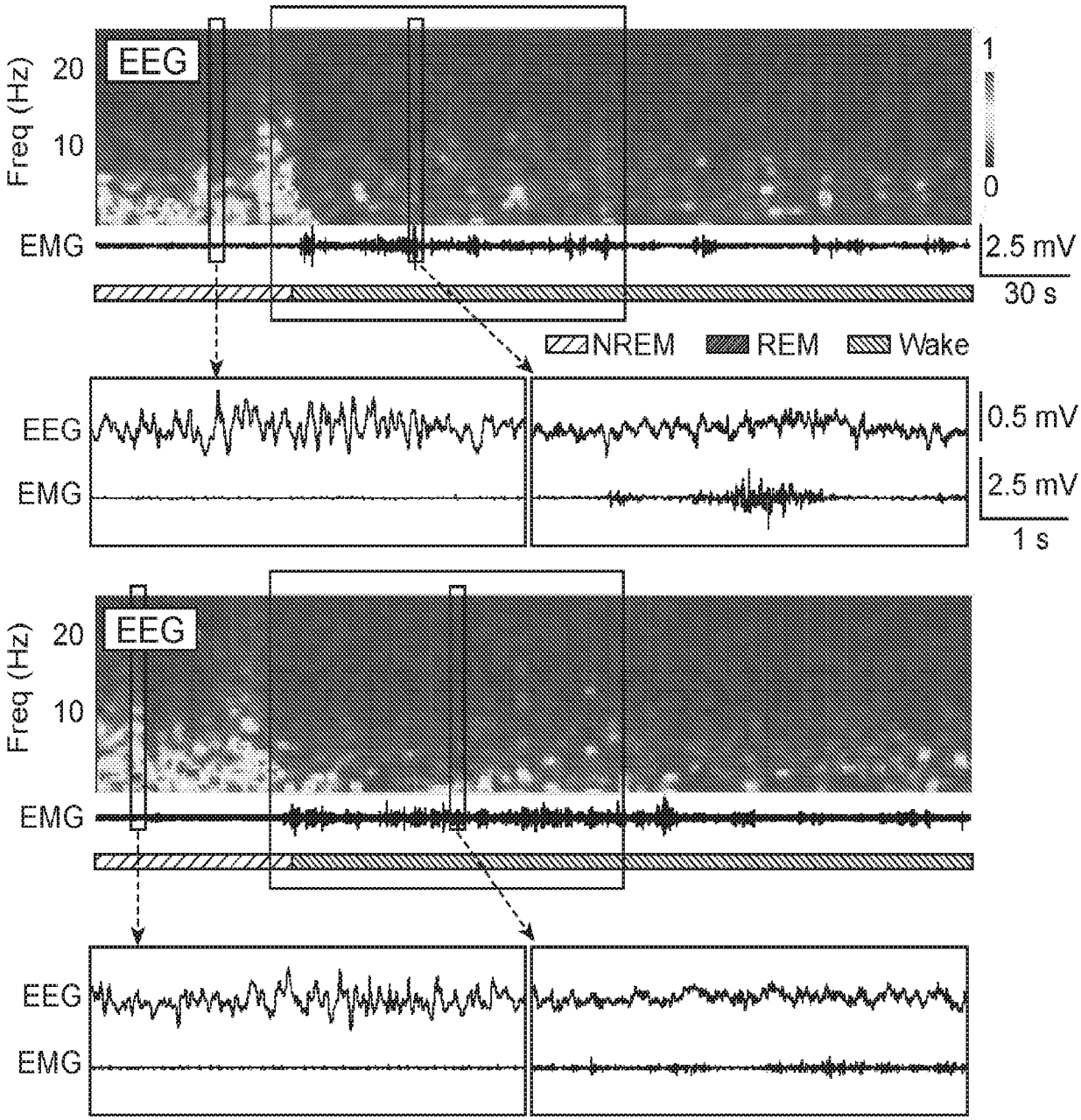

EEG
Freq (Hz)
20
10
EMG
1
0
2.5 mV
30 s
NREM
REM
Wake
EEG
EMG
0.5 mV
2.5 mV
1 s
EEG
Freq (Hz)
20
10
EMG
EEG
EMG

FIG. 3C

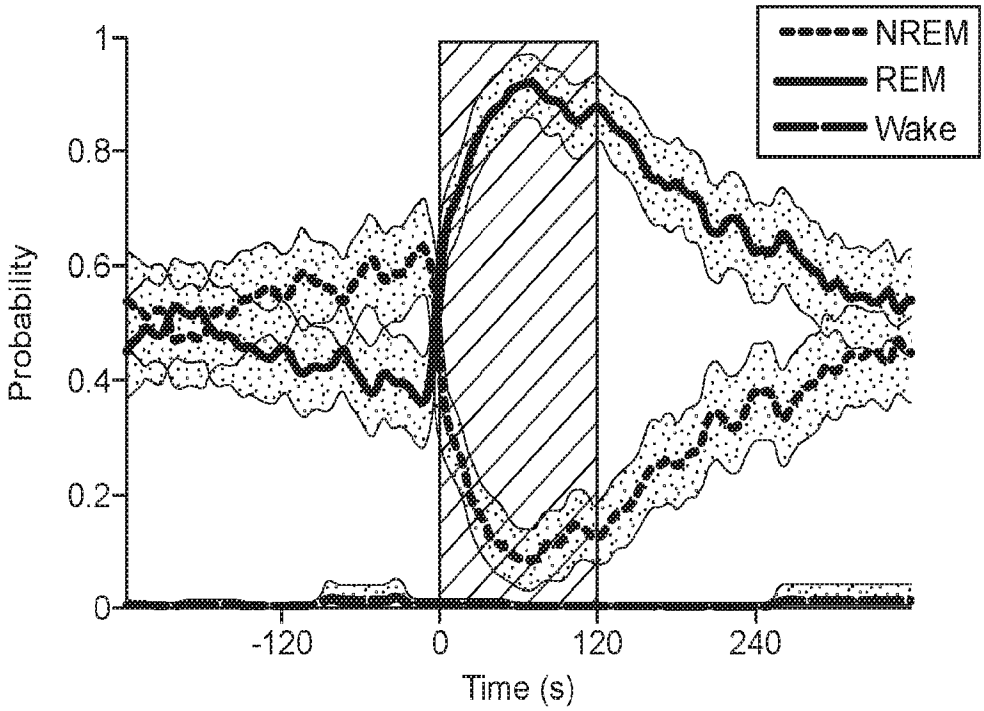

NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 3D

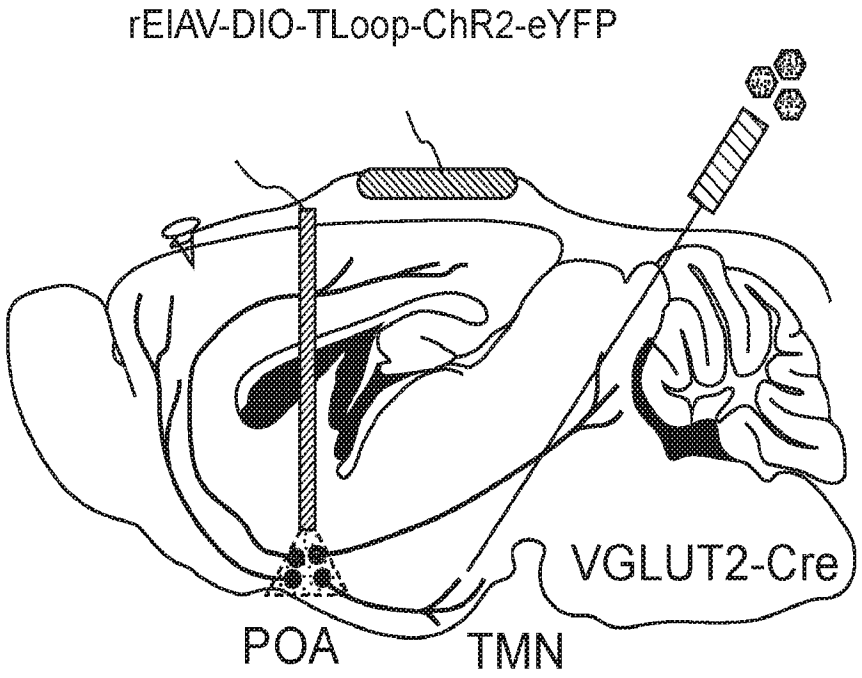

rEIAV-DIO-TLoop-ChR2-eYFP
VGLUT2-Cre
POA
TMN

FIG. 3E

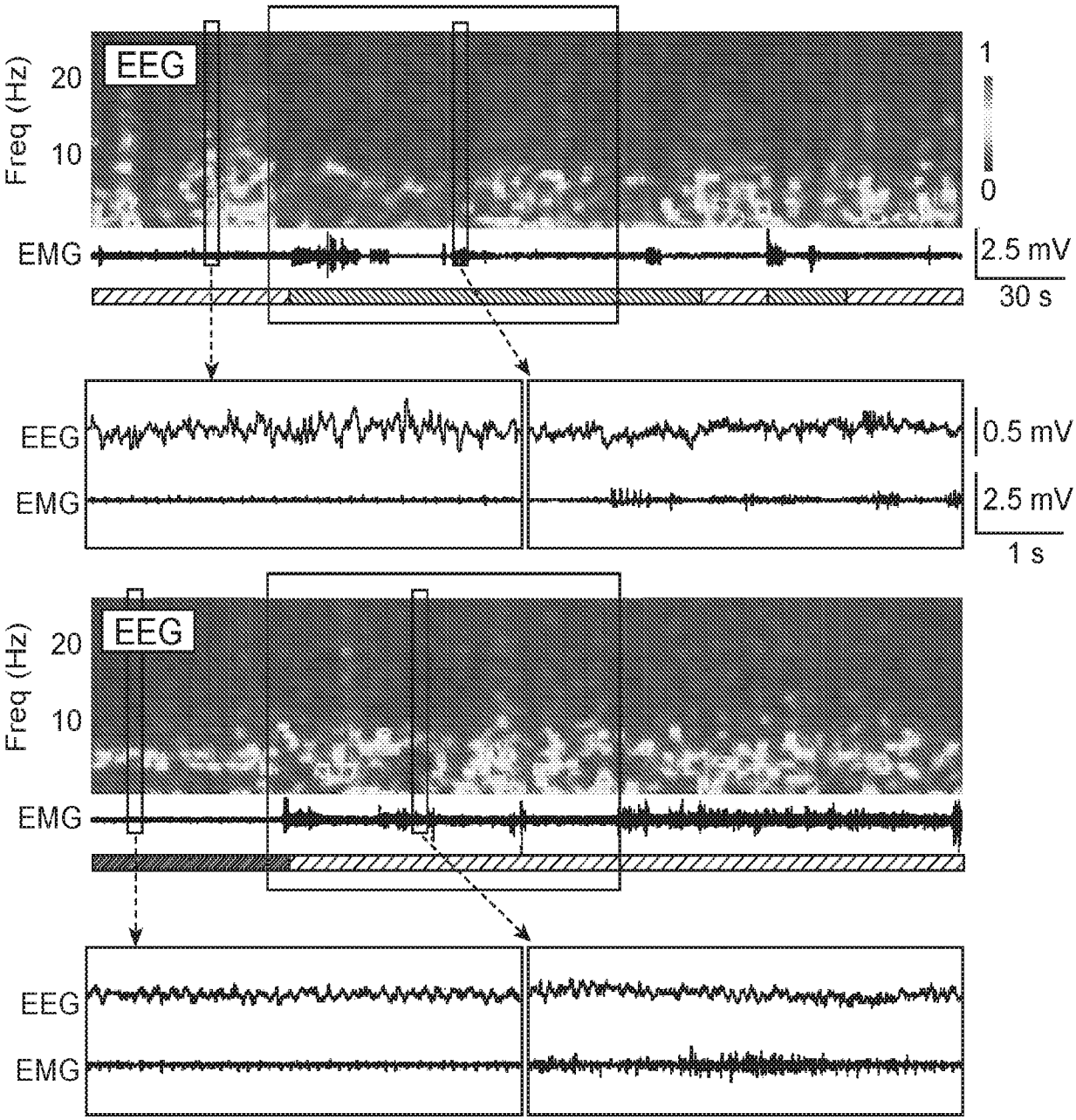

EEG
Freq (Hz)
20
10
EMG
1
0
2.5 mV
30 s
EEG
EMG
0.5 mV
2.5 mV
1 s
EEG
Freq (Hz)
20
10
EMG
EEG
EMG

FIG. 3F

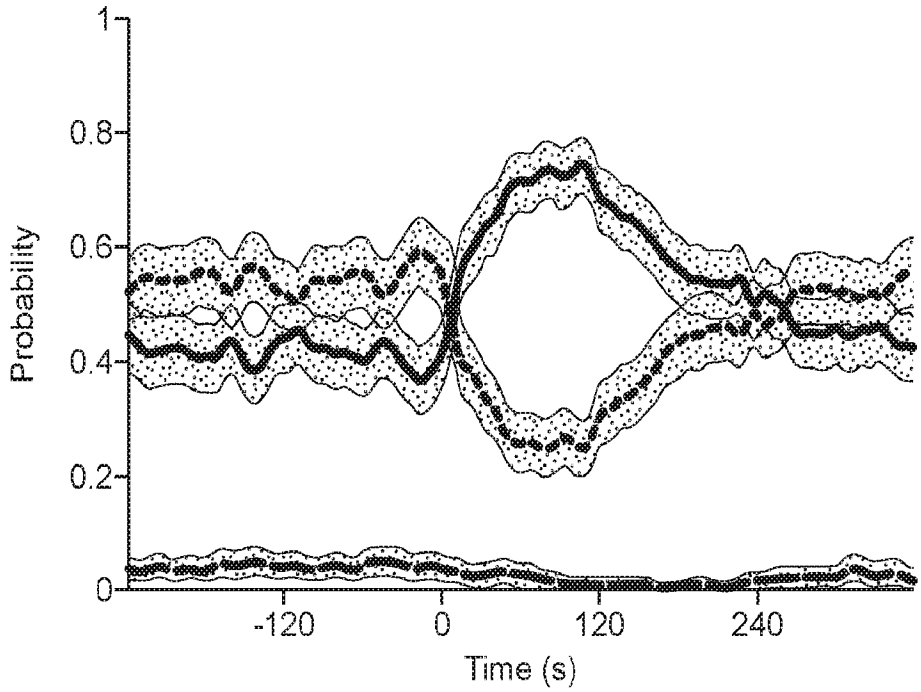


FIG. 4A

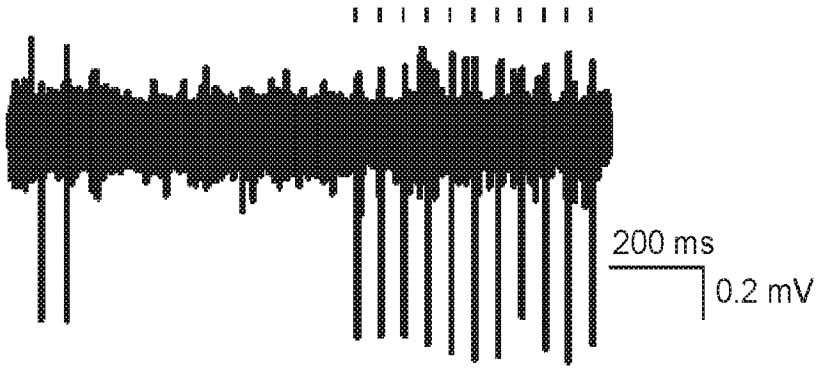
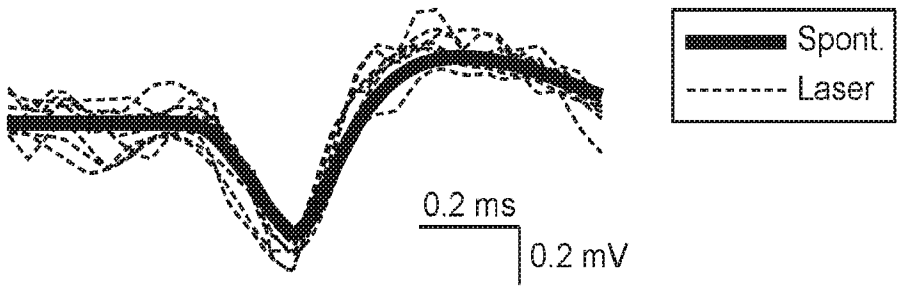


FIG. 4B



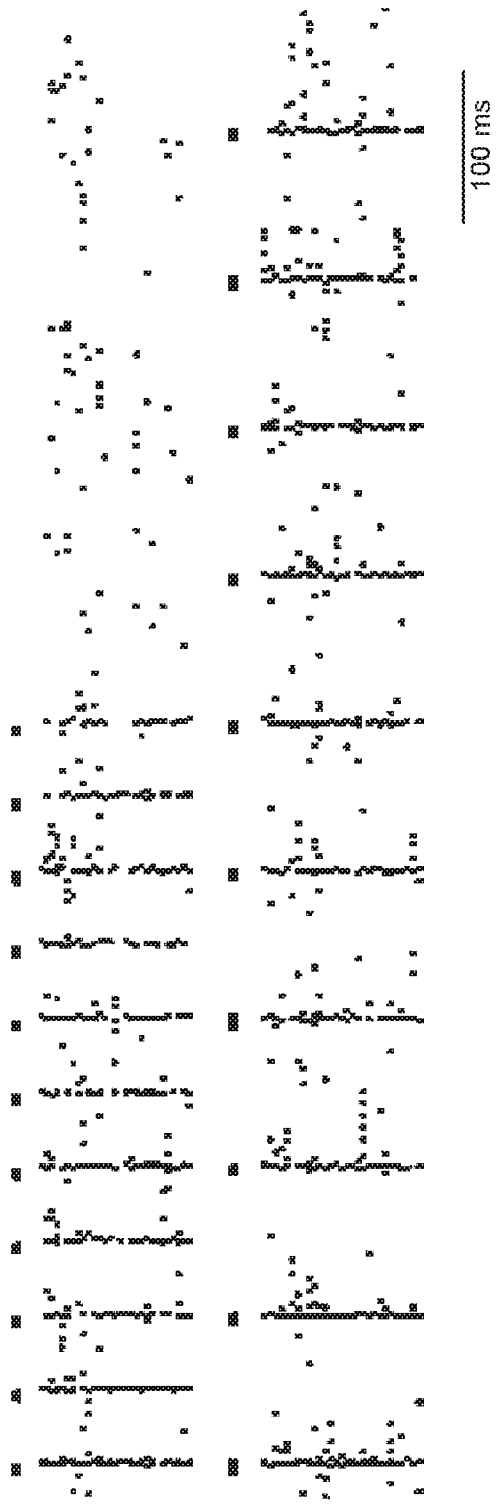

100 ms

FIG. 4C

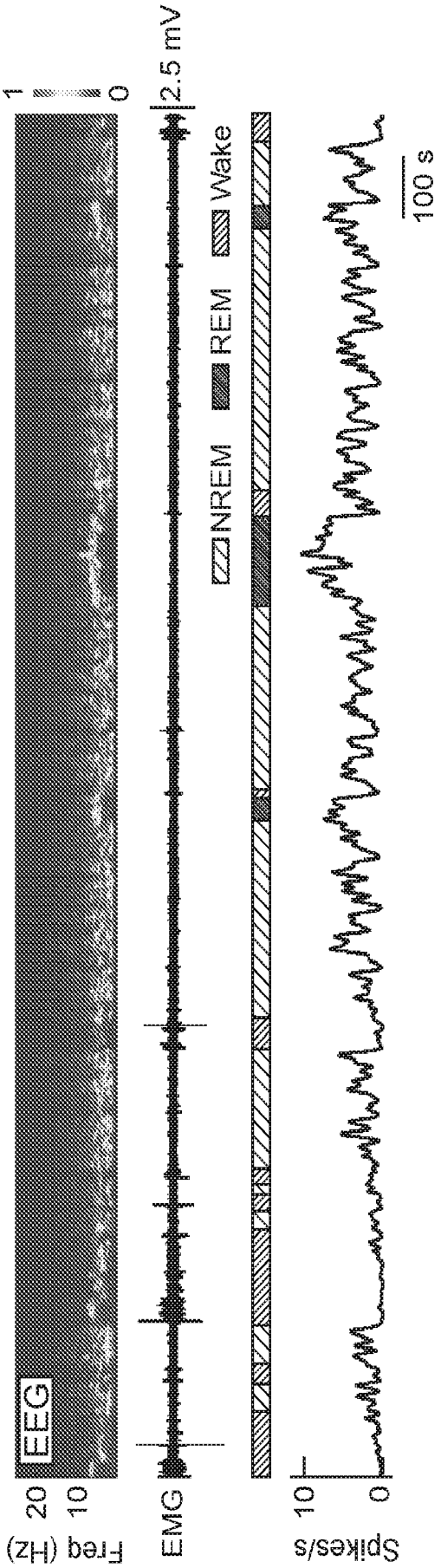

1
0
2.5 mV
NREM
REM
Wake
EEG
20
10
Freq (Hz)
EMG
10
0
Spikes/s
100 s

FIG. 4D

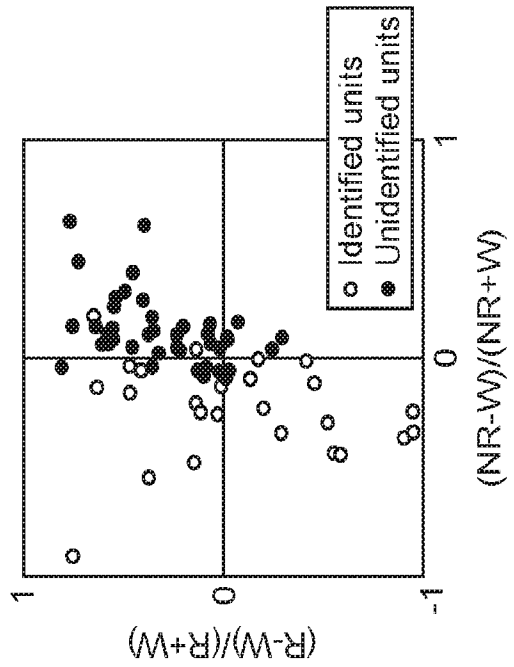


FIG. 4F

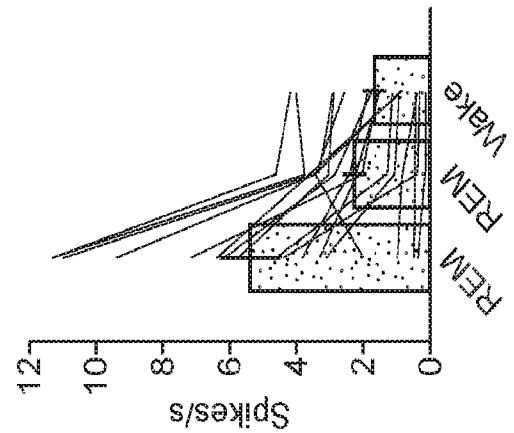


FIG. 4E

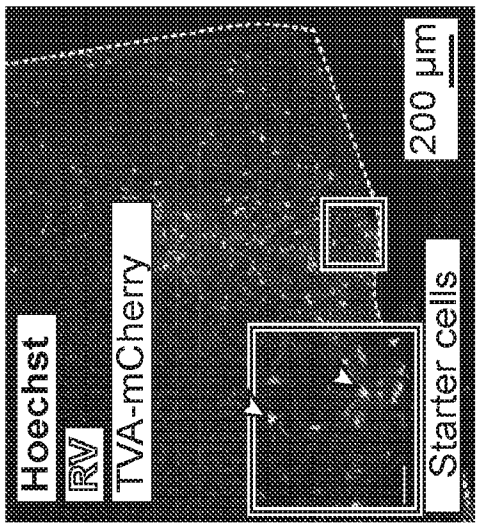
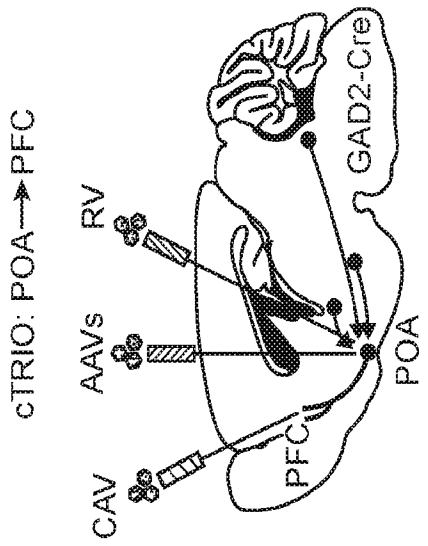


FIG. 5A



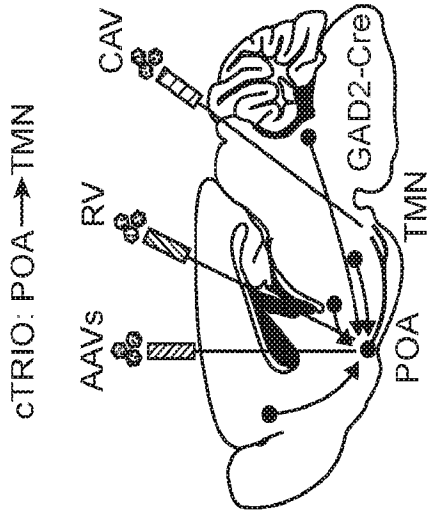


FIG. 5B

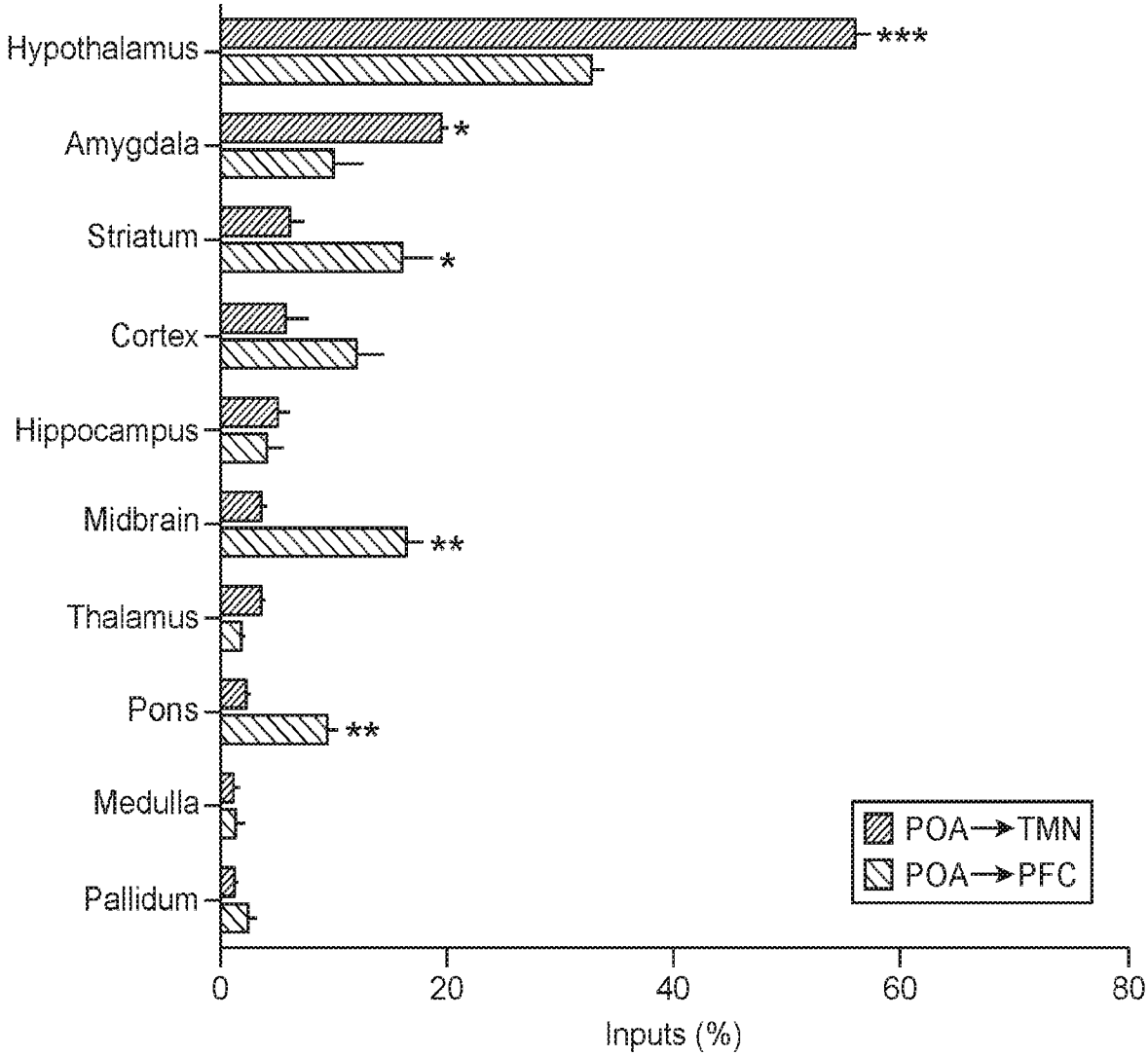

Hypothalamus
Amygdala
Striatum
Cortex
Hippocampus
Midbrain
Thalamus
Pons
Medulla
Pallidum
***
*
*
**
**
POA→TMN
POA→PFC
0
20
40
60
80
Inputs (%)

FIG. 5C

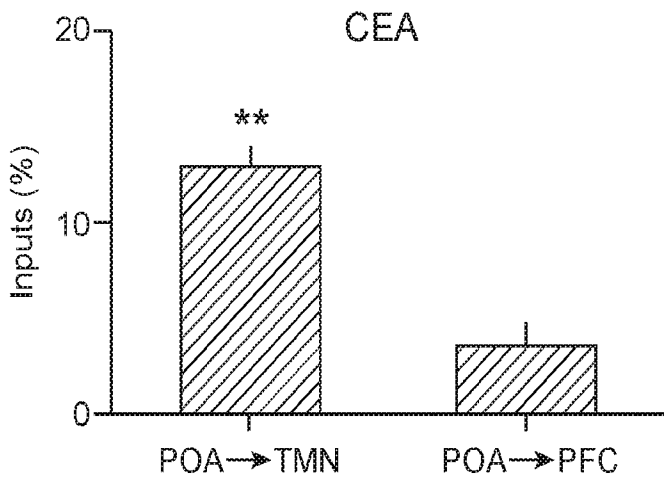

CEA
20
10
0
Inputs (%)
**
POA→TMN
POA→PFC

FIG. 5E

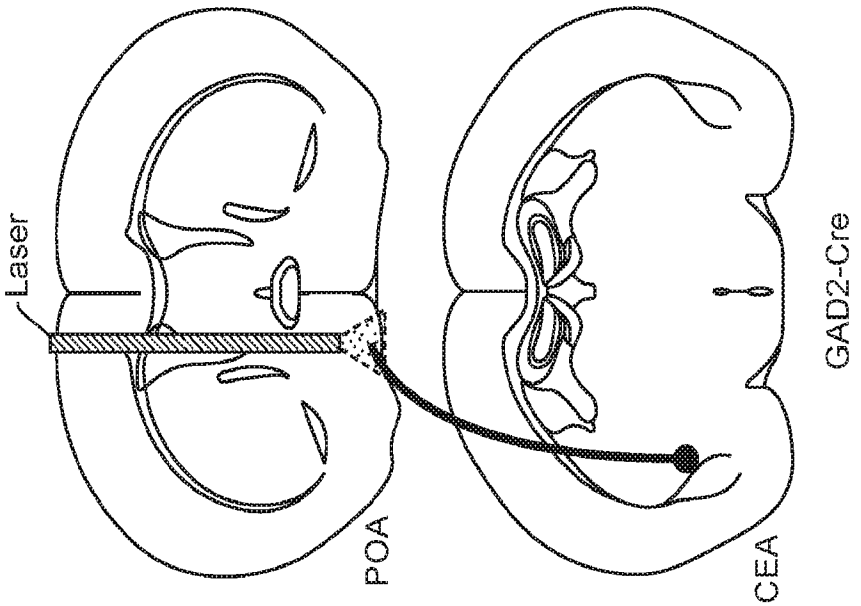
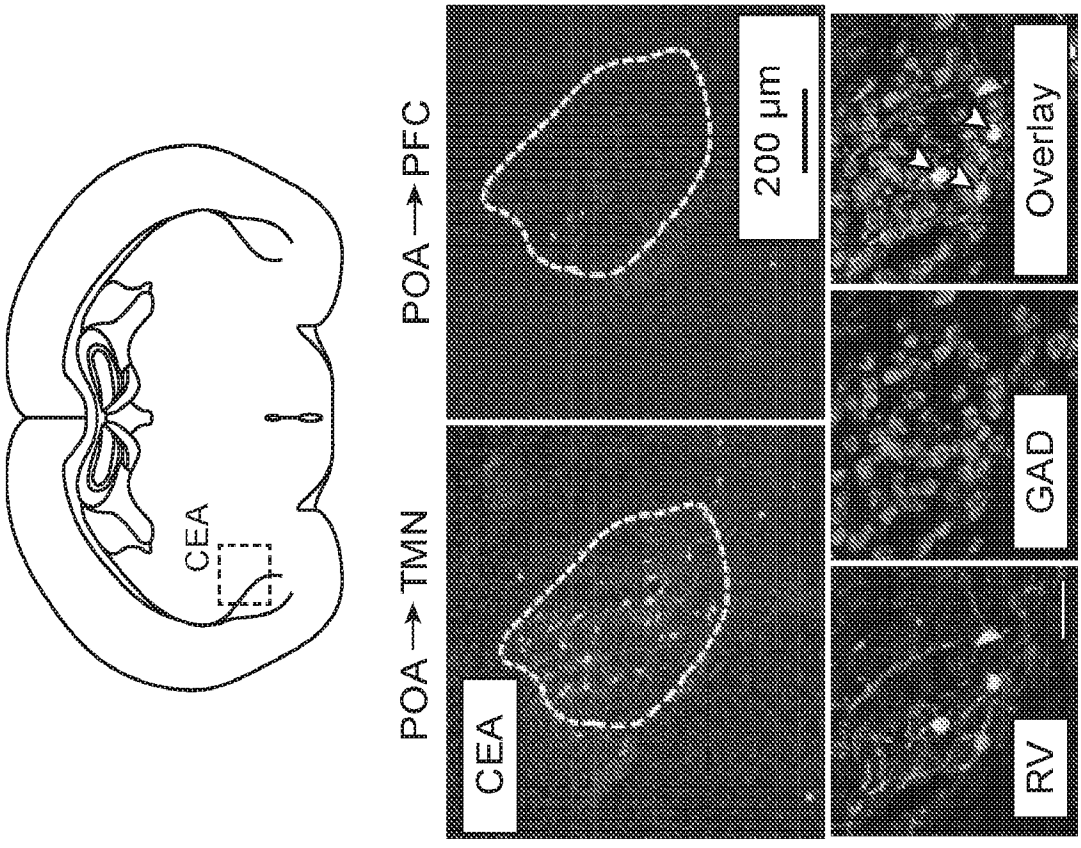

Laser
POA
CEA
GAD2-Cre

FIG. 5D


CEA
POA → TMN
POA → PFC
200 µm
RV
GAD
Overlay

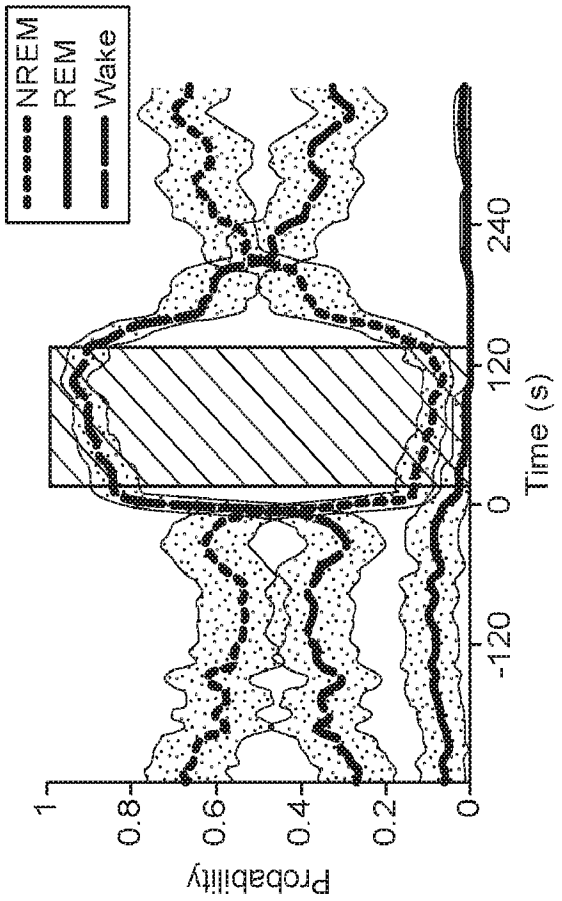
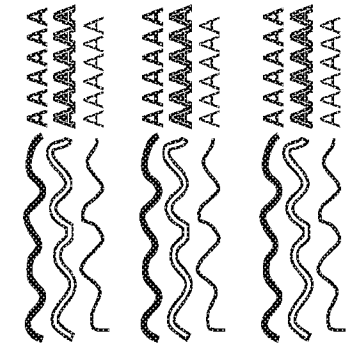
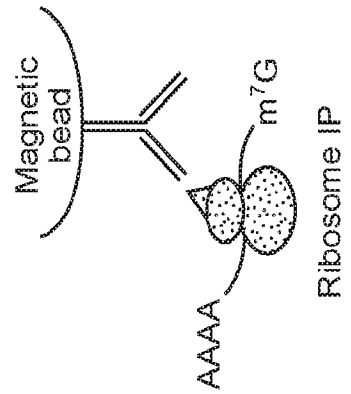

NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 5F


AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA


Magnetic bead
m7G
AAAA
Ribosome IP

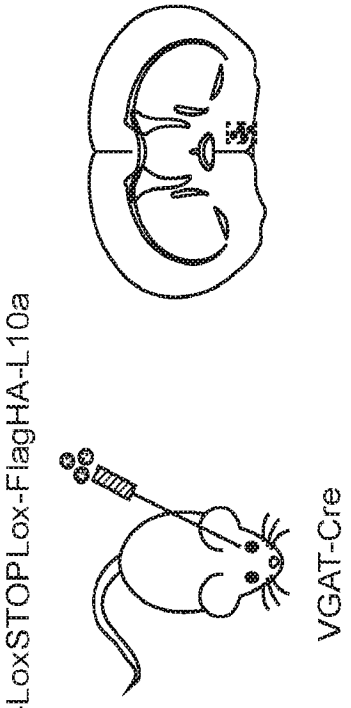

Injection
Dissection
Immunoprecipitation
RNA-seq
HSV-LoxSTOPLox-FlagHA-L10a
VGAT-Cre

FIG. 6A

FIG. 6B

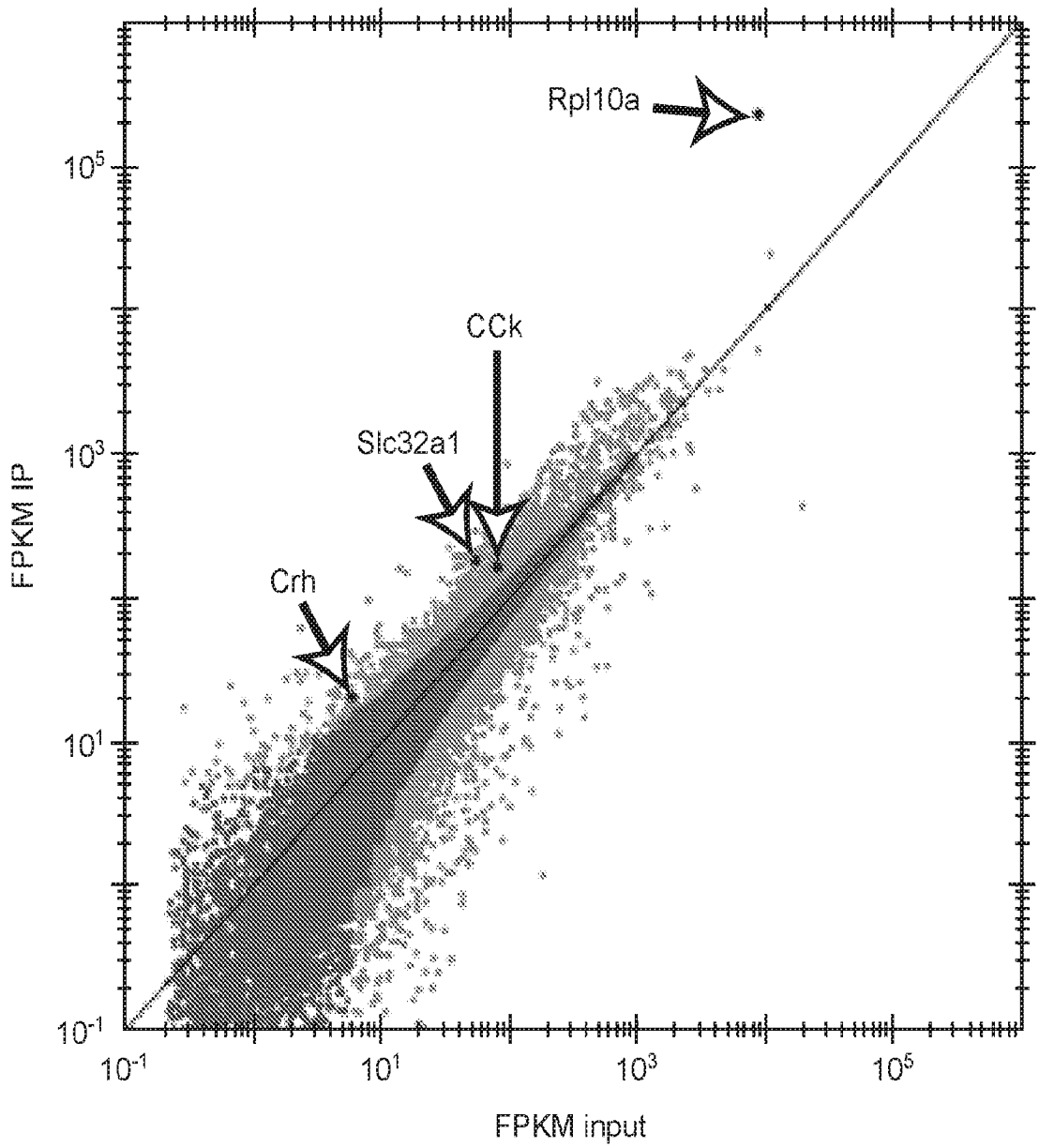

Rpl10a
CCk
Slc32a1
Crh
FPKM IP
FPKM input
10^{5}
10^{3}
10^{1}
10^{-1}
10^{-1}
10^{1}
10^{3}
10^{5}

FIG. 6C

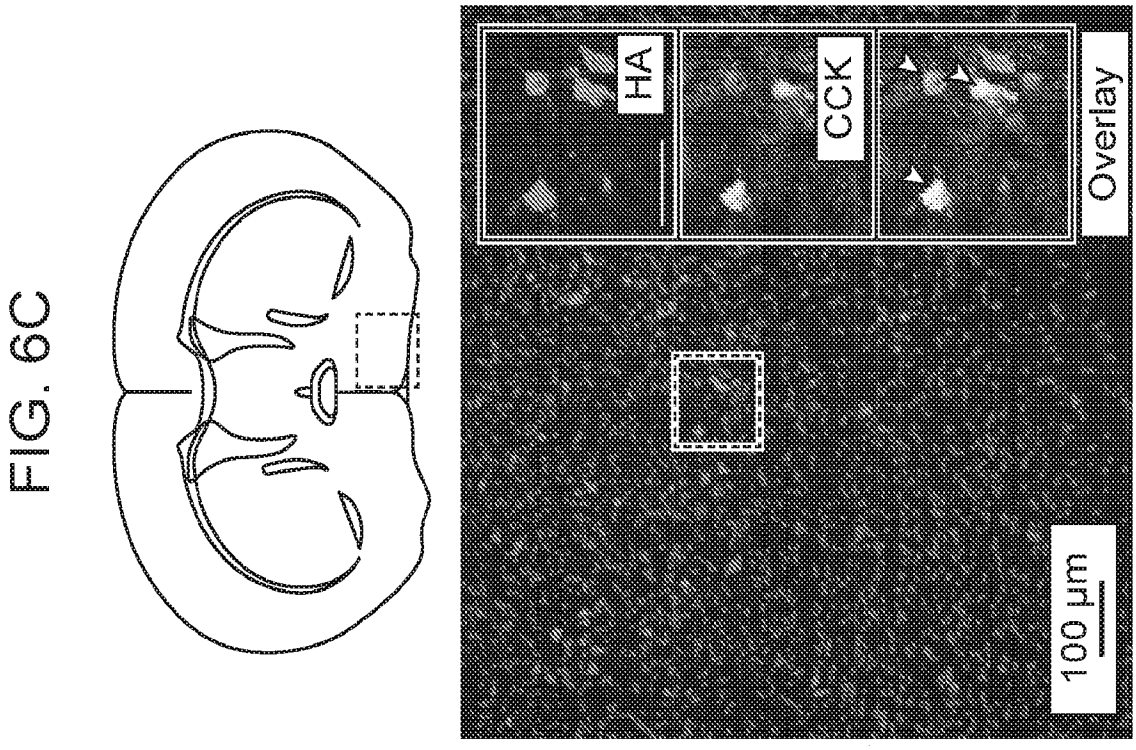

HA
CCK
Overlay
100 µm

FIG. 6D

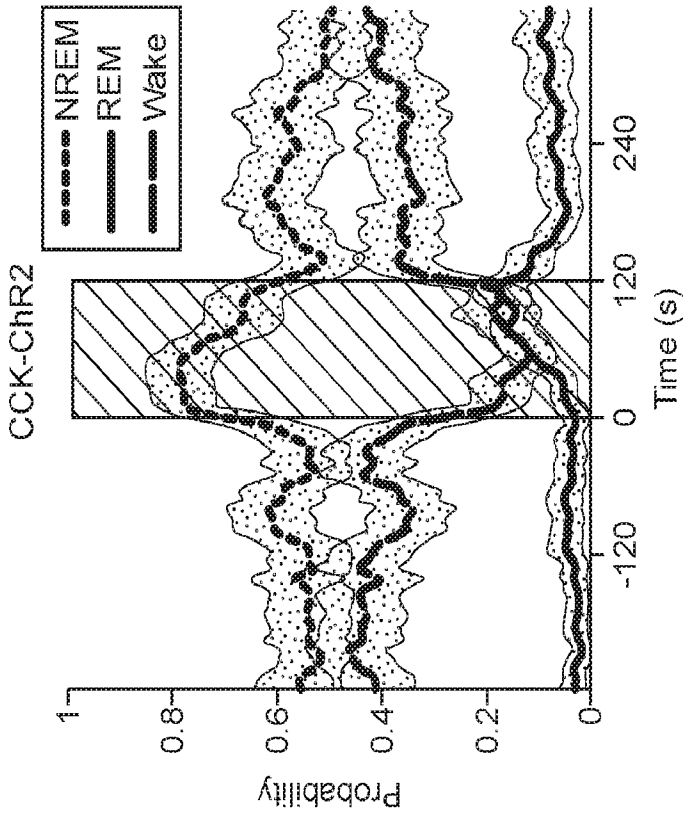

CCK-ChR2
NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 6E

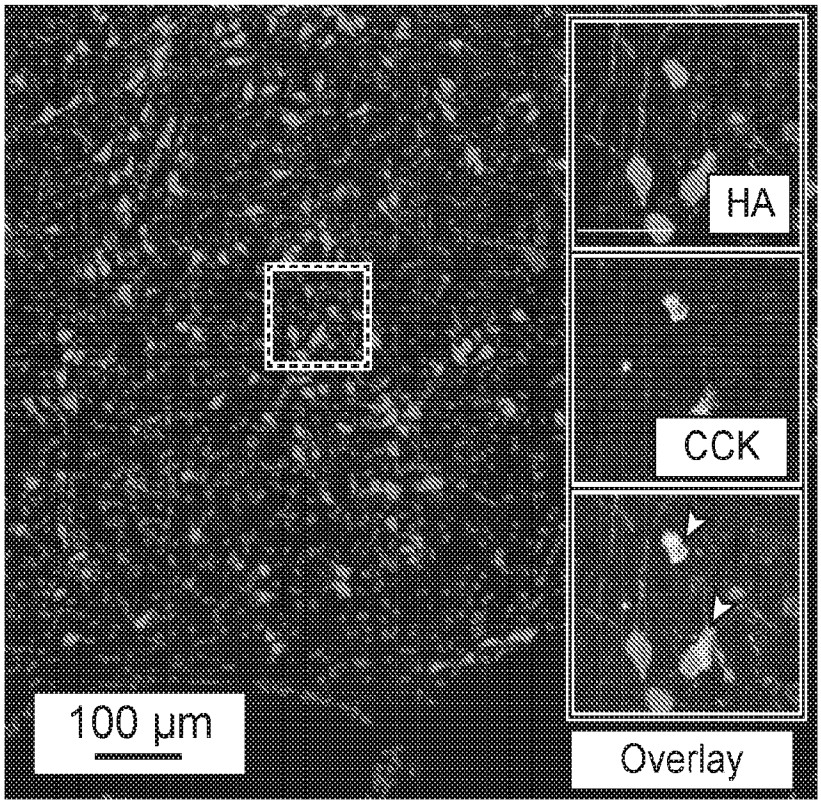

HA
CCK
100 µm
Overlay

FIG. 6F

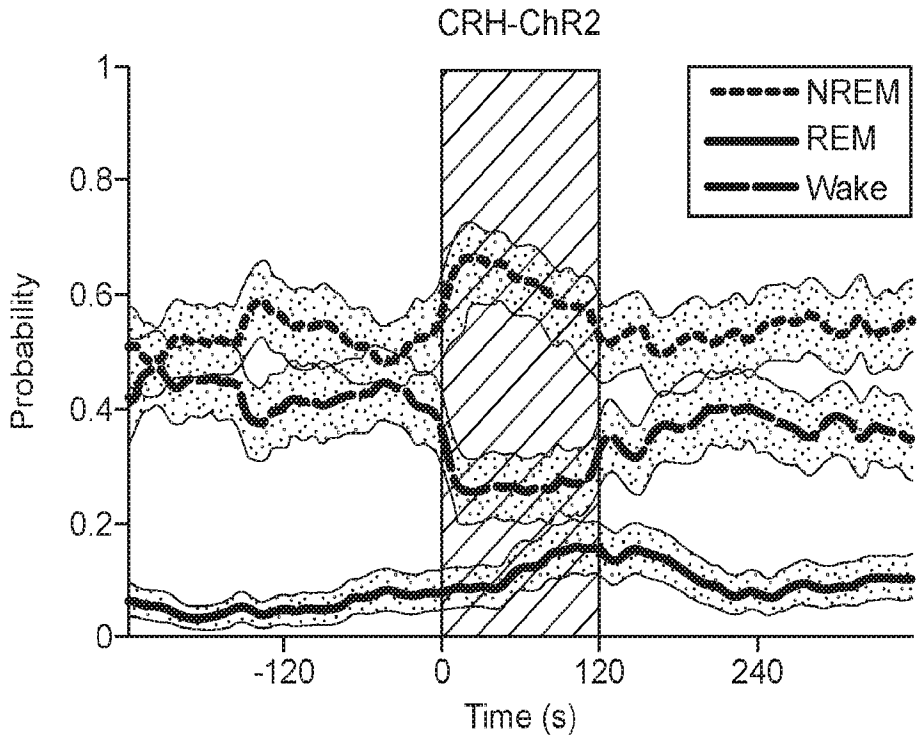

CRH-ChR2
NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 7A

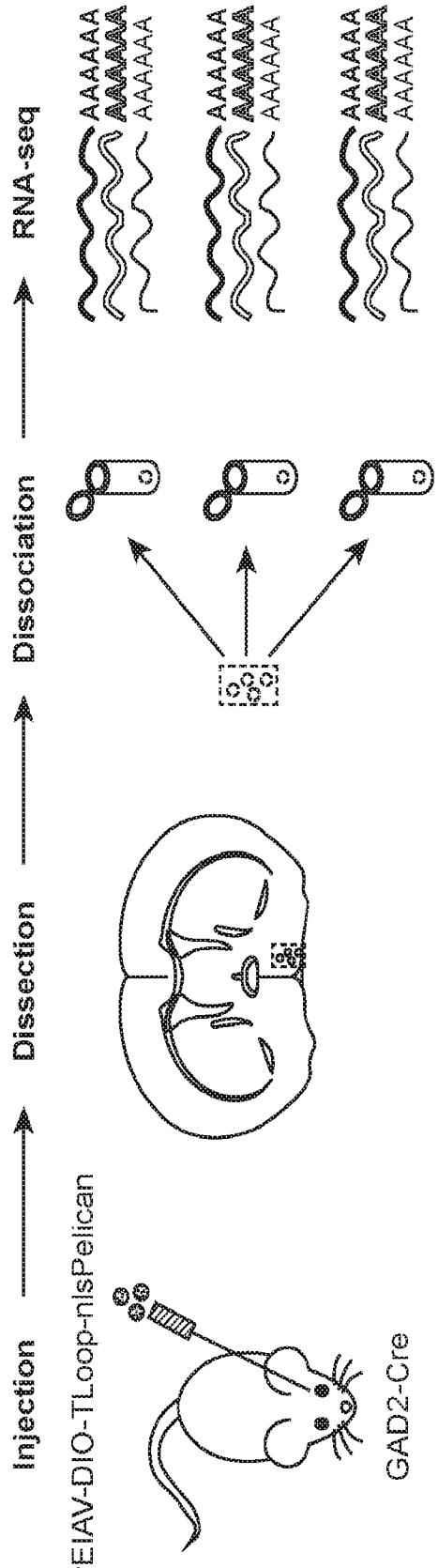

Injection
Dissection
Dissociation
RNA-seq
rEIAV-DIO-TLoop-nlsPelican
GAD2-Cre
AAAAAA
AAAAAA
AAAAAA

FIG. 7B

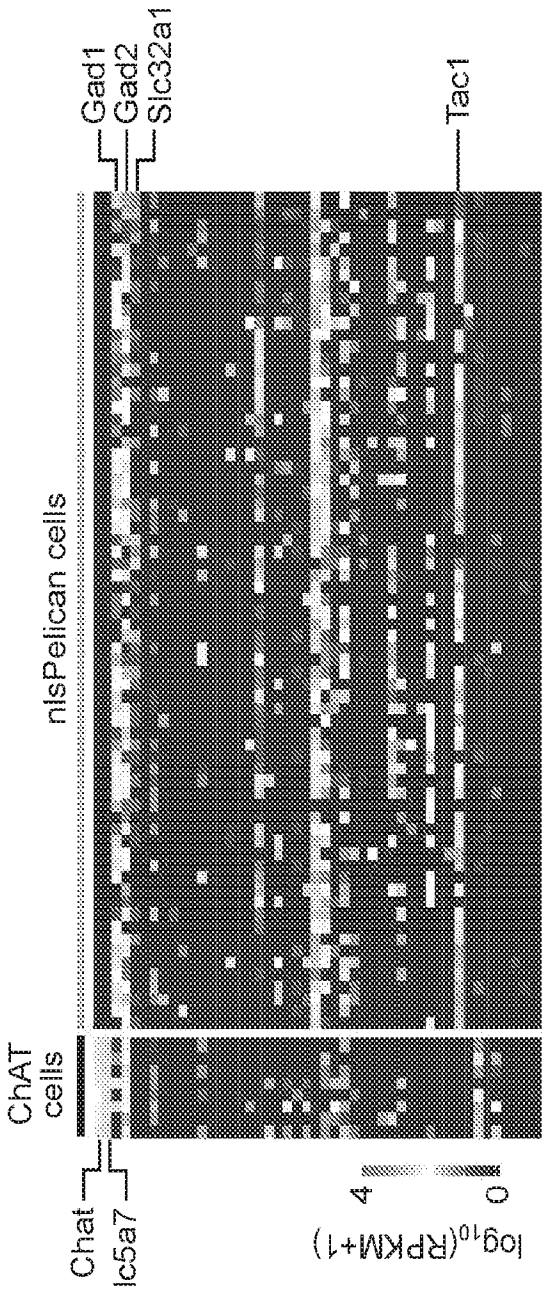

ChAT cells
nlsPelican cells
Gad1
Gad2
Slc32a1
Tac1
Chat
Slc5a7
4
0
log10(RPKM+1)

FIG. 7C

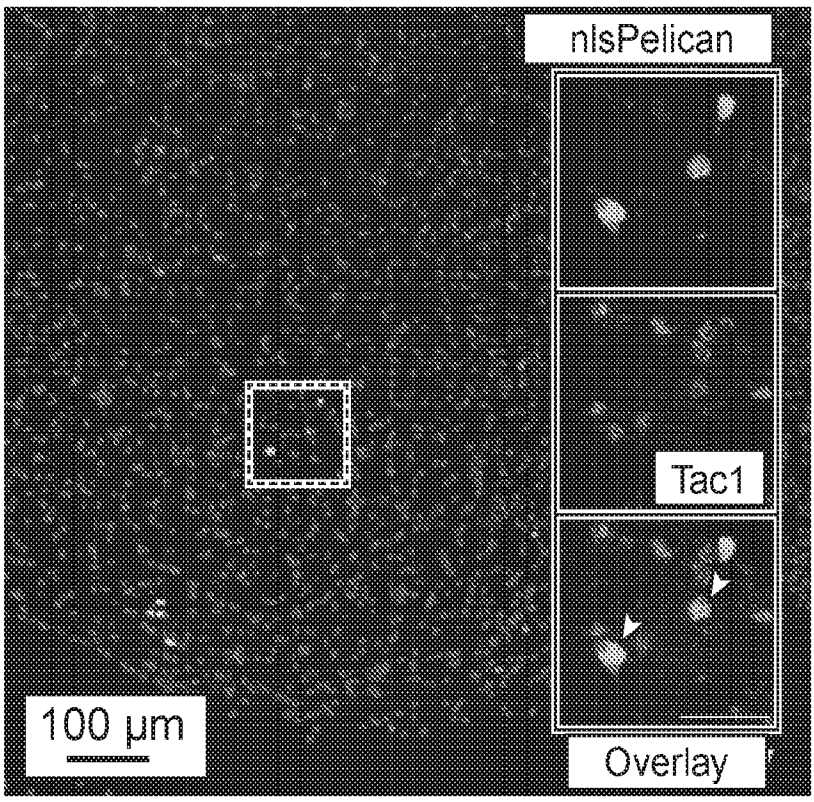

nlsPelican
Tac1
Overlay
100 µm

FIG. 7D

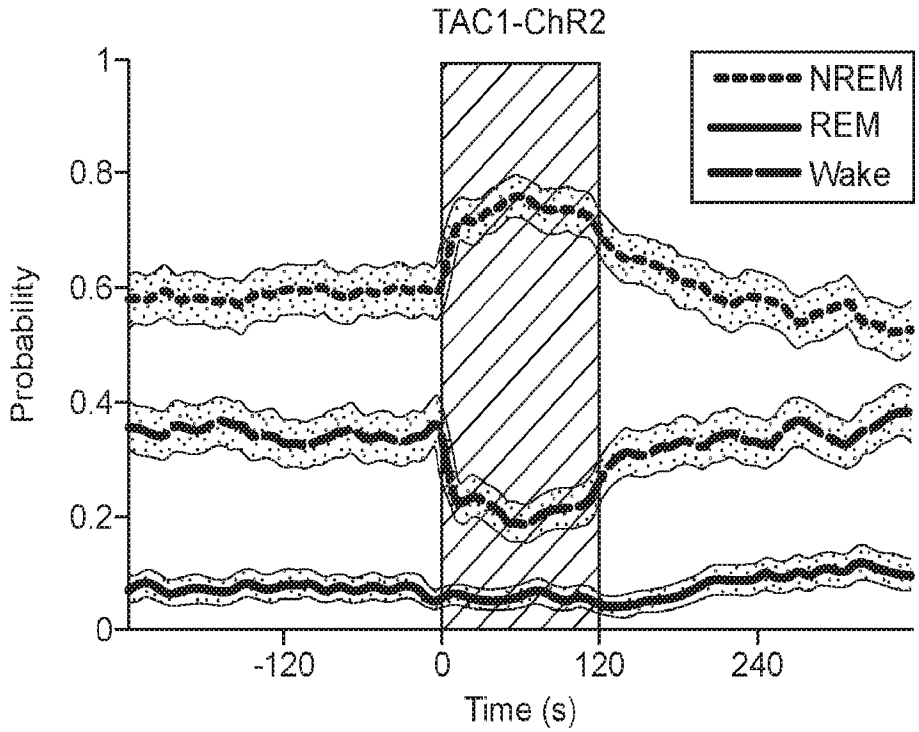

TAC1-ChR2
NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 8

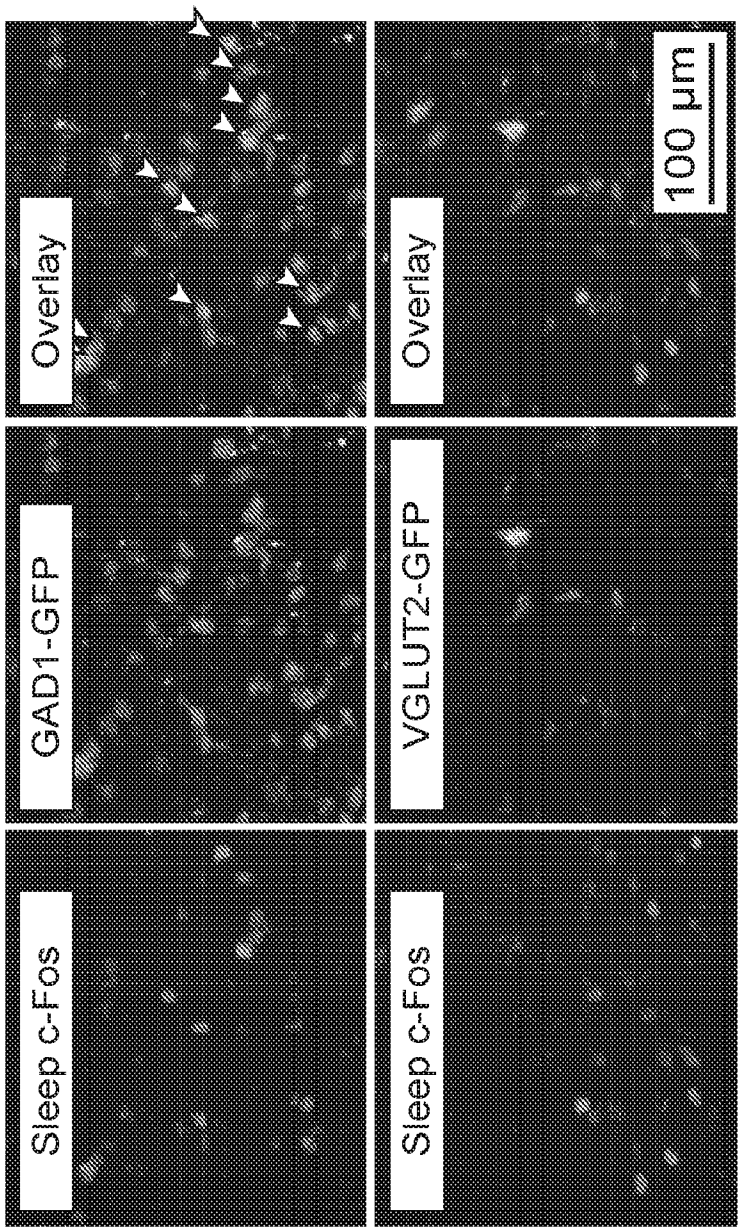

Sleep c-Fos
GAD1-GFP
Overlay
Sleep c-Fos
VGLUT2-GFP
Overlay
100 µm
Overlap/total c-Fos (%)
80
60
40
20
0
GAD1
VGLUT2

FIG. 9A

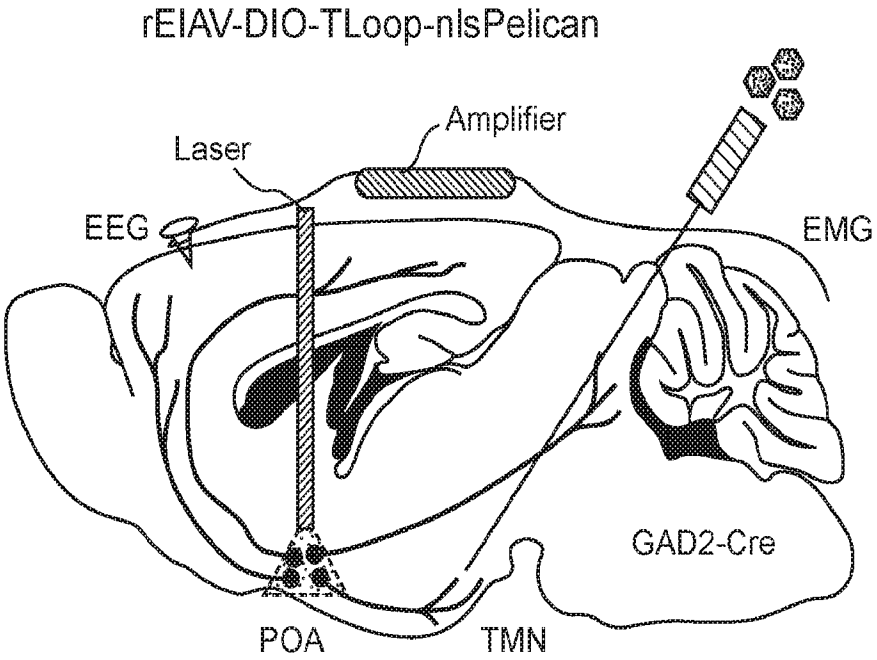

rEIAV-DIO-TLoop-nlsPelican
Amplifier
Laser
EEG
EMG
GAD2-Cre
POA
TMN

FIG. 9B

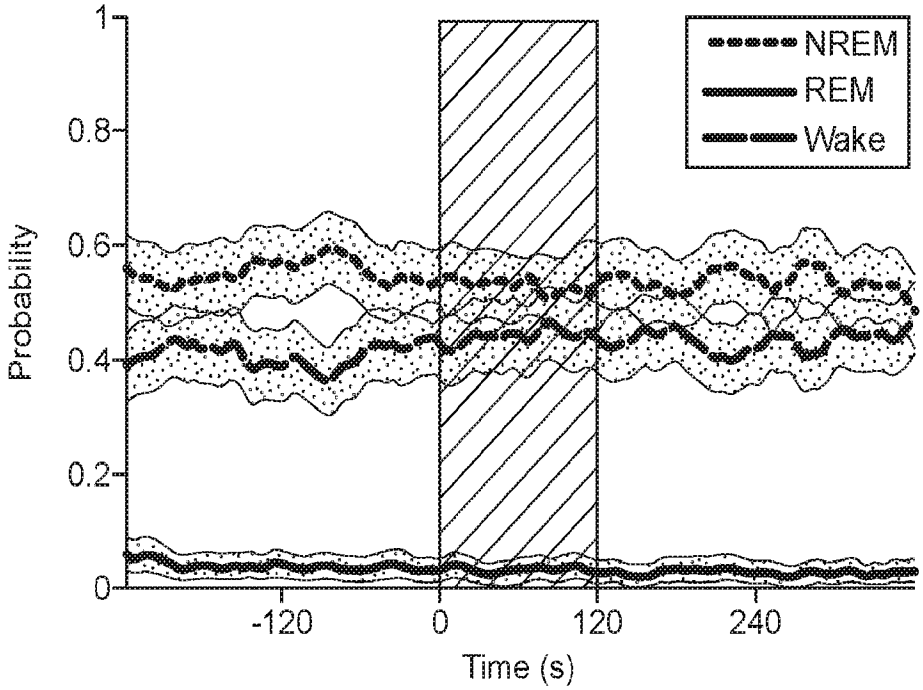

NREM
REM
Wake
Probability
1
0.8
0.6
0.4
0.2
0
-120
0
120
240
Time (s)

FIG. 10A

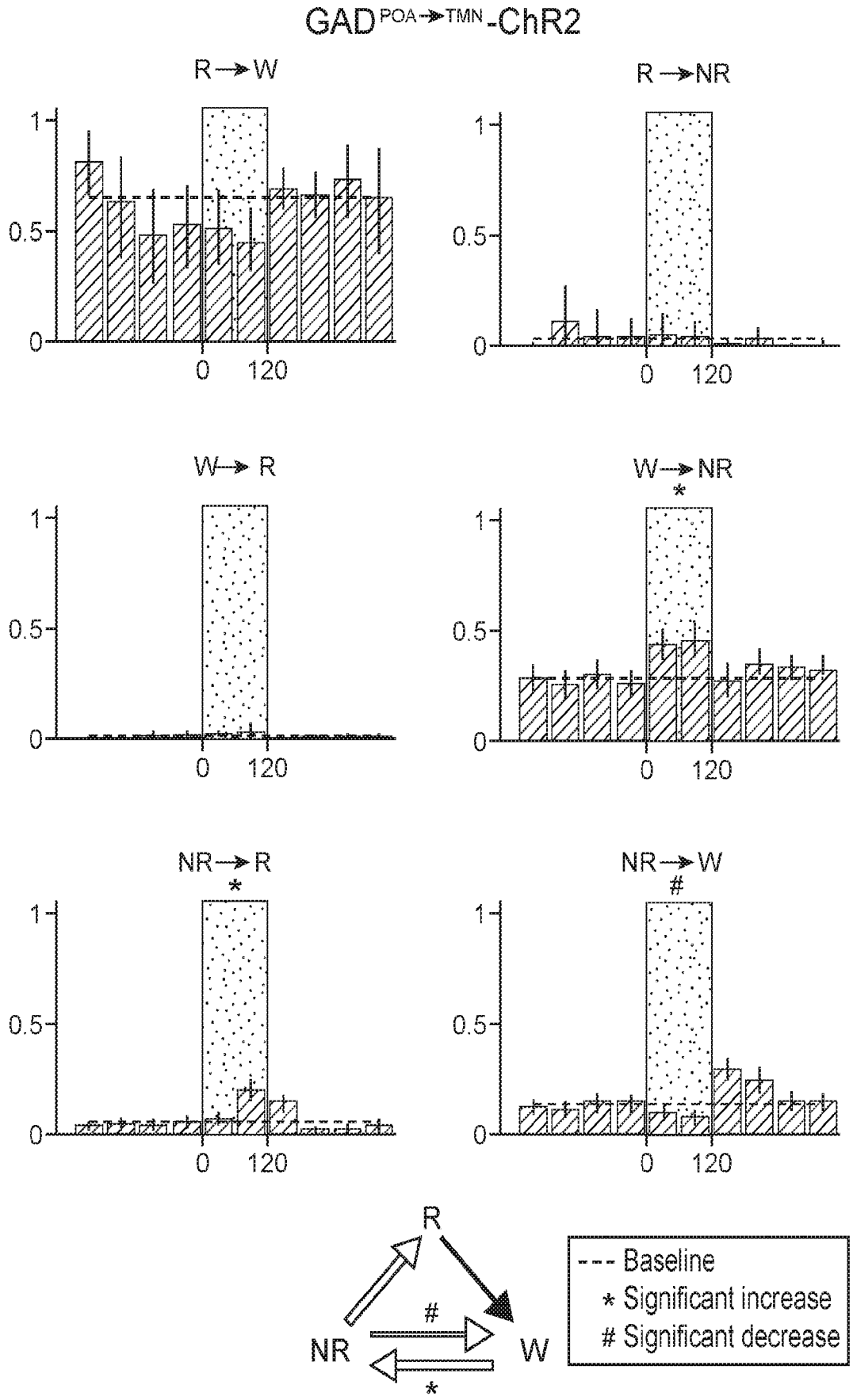

GAD POA→TMN -ChR2
R→W
R→NR
W→R
W→NR
*
NR→R
*
NR→W
#
1
0.5
0
0
120
R
NR
W
#
*
--- Baseline
* Significant increase
# Significant decrease

FIG. 10B

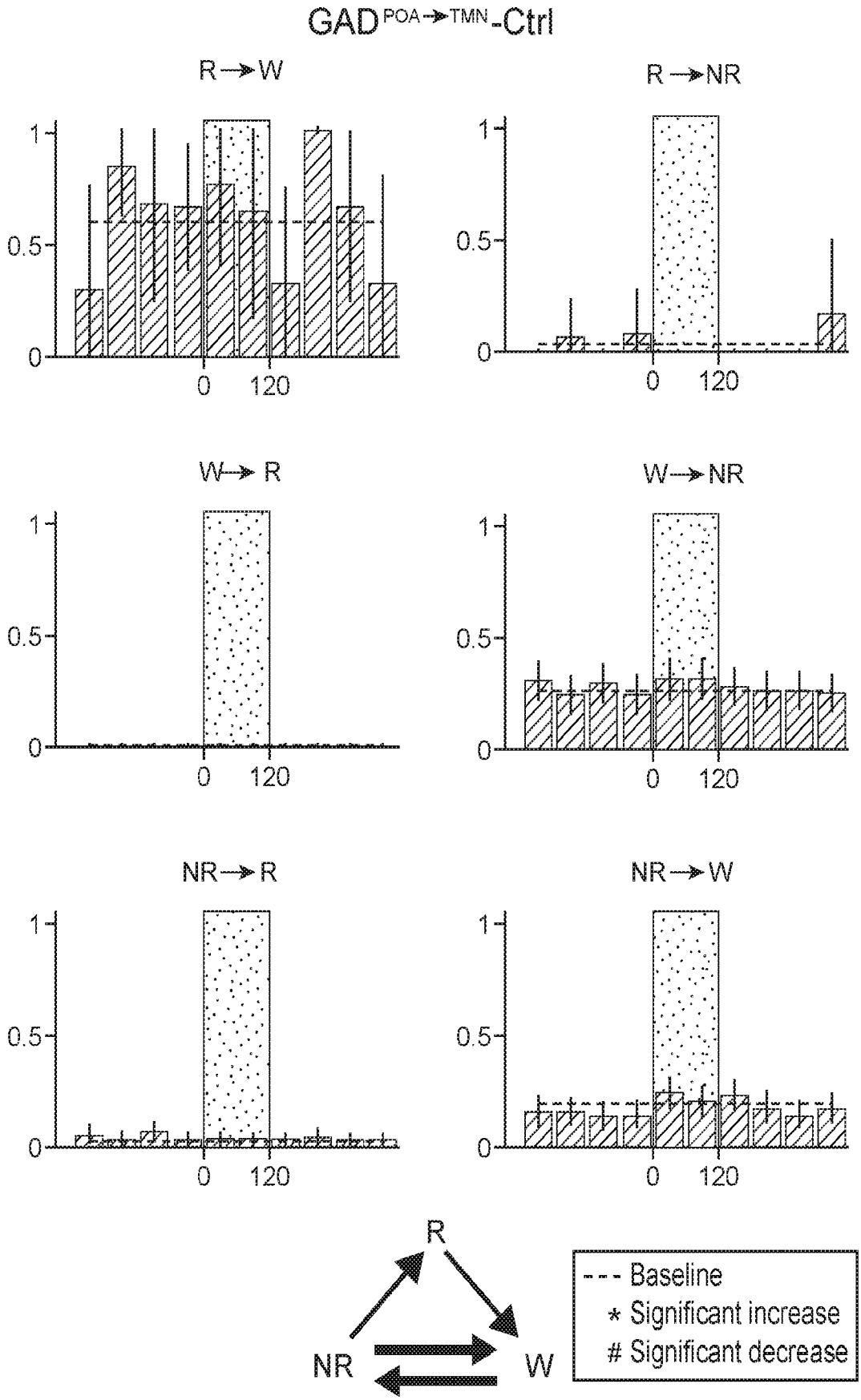

GAD POA→TMN-Ctrl
R→W
R→NR
W→R
W→NR
NR→R
NR→W
1
0.5
0
0
120
R
NR
W
--- Baseline
* Significant increase
# Significant decrease

FIG. 10C

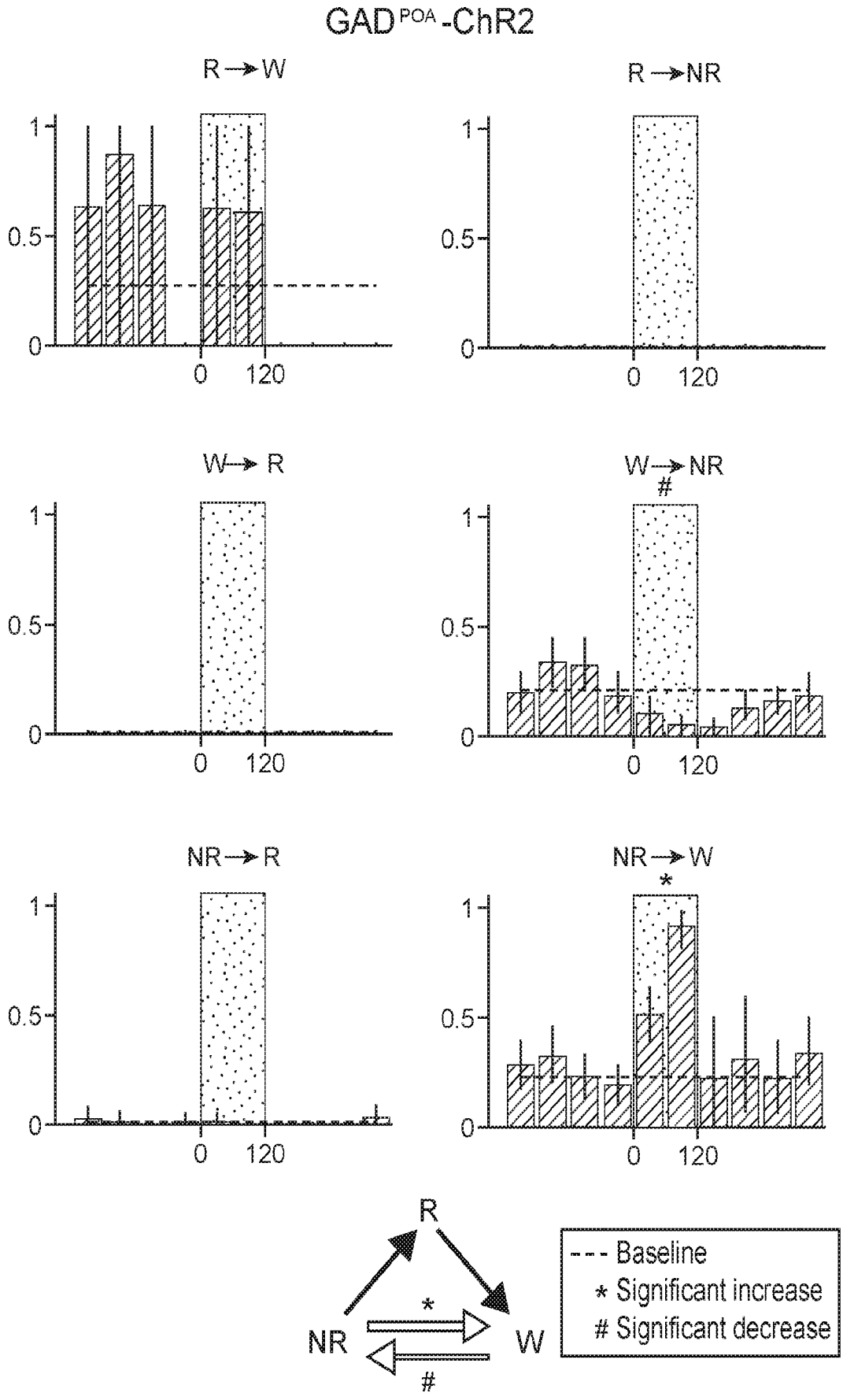

GAD POA -ChR2
R→W
R→NR
W→R
W→NR
#
NR→R
NR→W
*
1
0.5
0
0
120
R
NR
W
*
#
Baseline
* Significant increase
# Significant decrease

FIG. 11A

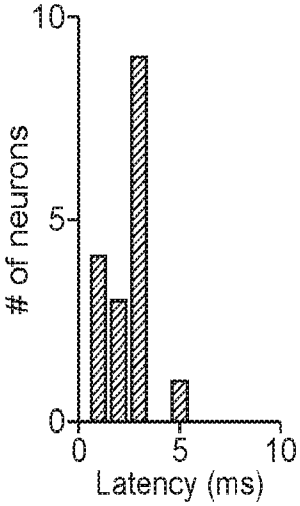


FIG. 11B

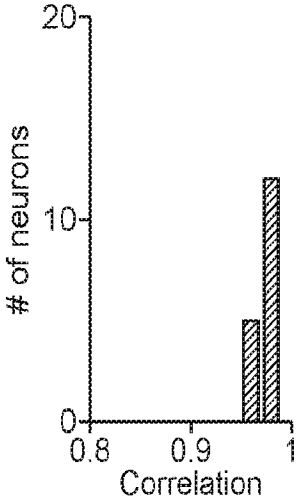


FIG. 12

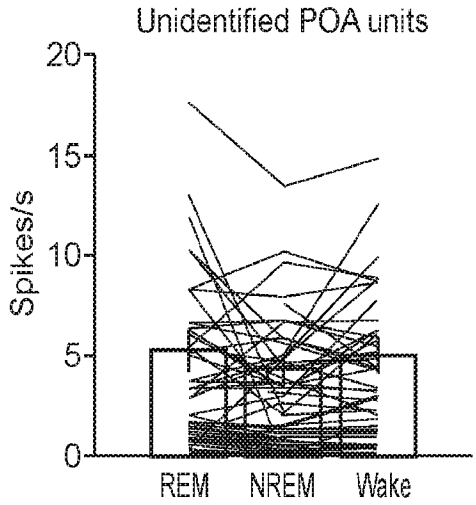


FIG. 13A

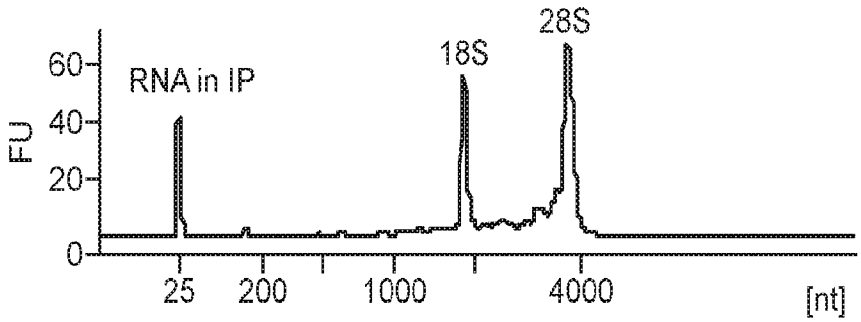


FIG. 13B

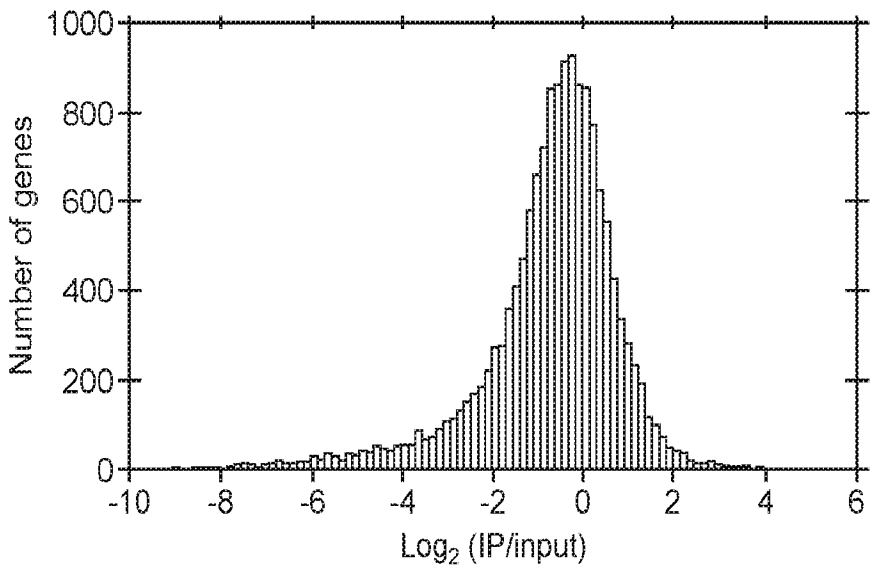


FIG. 14A

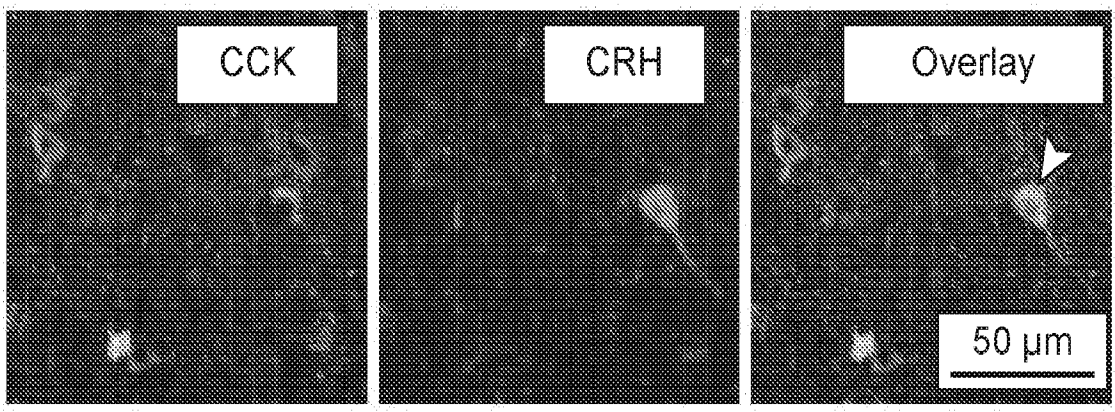


FIG. 14B

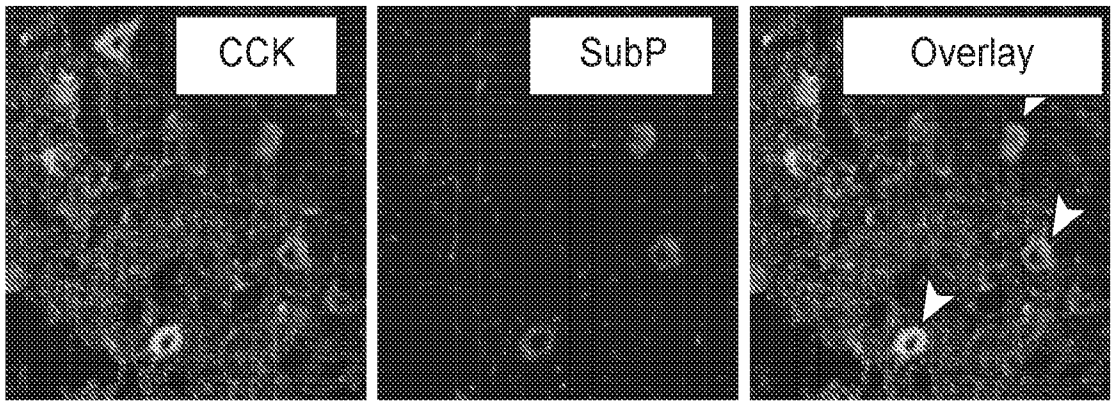


FIG. 15

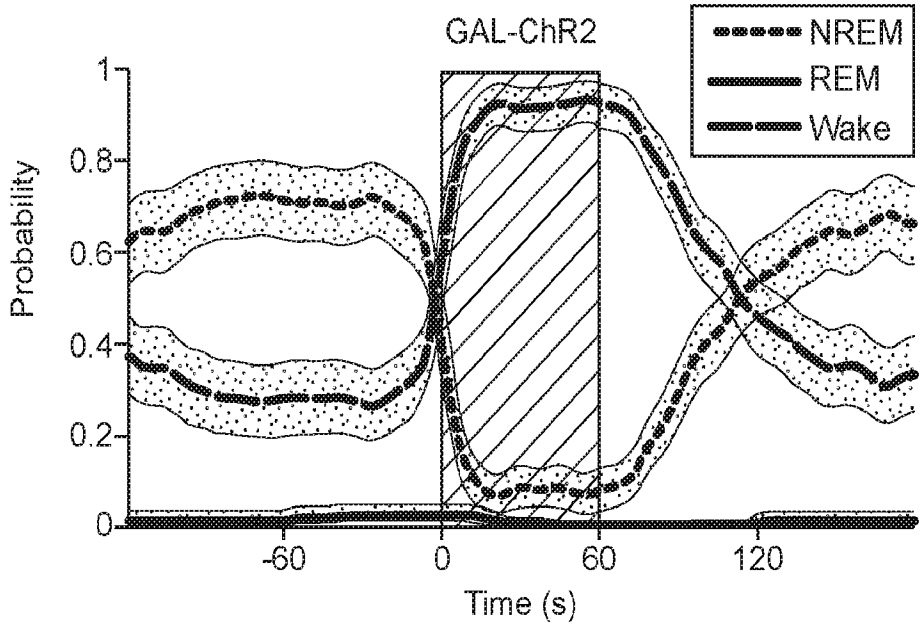


FIG. 16

Table 1. Neuropeptide-encoding genes enriched in $GAD^{POA \rightarrow TMN}$ neurons identified by TRAP

Gene	Fold-change	Neuropeptide
Crh	3.46261603	corticotropin releasing hormone
Gal	3.09623078	galanin
Nts	2.822915	neurotensin
Tac2	2.584122	tachykinin 2
Pnoc	2.350308	prepronociceptin
Calca	2.26167755	calcitonin-related polypeptide alpha
Cck	2.039715	cholecystokinin

FIG. 17

Table 2 Up-regulated GPCRs in $GABA^{POA \rightarrow TMN}$ neurons identified by TRAP

Gene	Fold-change	GPCRs
Htr1f	6.74	5-hydroxytryptamine (serotonin) receptor 1F
Chrm4	5.26	cholinergic receptor, muscarinic 4
Avpr1a	4.63	arginine vasopressin receptor 1A
Adrb1	3.86	adrenergic receptor, beta 1
Paqr7	2.36	progestin and adipoQ receptor family member VII
Gpr83	2.21	G protein-coupled receptor 83
Gabbr1	1.39	G protein-coupled receptor 83

*Genes whose level is FPKM<1 in either IP and Input are not shown

FIG. 18A

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Hist1h4m	30.9465	1.1705	26.43870141
Rpl10a	114679.29	4403.7775	26.04111765
Tmem121	15.01	1.0755	13.9562994
Hist1h2bb	48.3165	3.9265	12.30523367
1700021C14Rik	14.8625	1.266	11.73973144
Hist2h4	79.9145	6.9545	11.49104896
3110009E18Rik	13.2115	1.1545	11.44348203
Tmod3	74.5665	7.754	9.616520506
Tarbp2	14.8365	1.606	9.238169365
Gm13072	426.7765	47.819	8.924831134
Hist1h2bj	19.082	2.295	8.31459695
Thg1l	20.995	2.842	7.387403237
Gm13981	9.4585	1.328	7.122364458
Fam131c	32.4305	4.8395	6.701208803
Hist3h2ba	112.701	17.0155	6.623431577
Atp5l	1601.9725	243.986	6.565837794
2310039H08Rik	19.4495	2.9715	6.545347468
Cox6b2	29.0345	4.5345	6.403021281
LOC100046034	7.8605	1.23	6.390650407
Hist2h2ac	26.474	4.6935	5.640566741
Hist1h1b	10.8375	1.981	5.470721858
Sec61g	145.782	27.032	5.392941699
Ripply2	14.431	2.6995	5.345804779
Hist1h2bm	17.6915	3.331	5.311167817
Kazald1	7.23	1.3615	5.310319501
Gm1673	196.871	37.7115	5.220449995
Lsm4	96.964	18.702	5.18468613
Tpm2	11.5445	2.2395	5.1549453
Tceb2	1118.8645	217.27	5.149650205
LOC100503724	18.896	3.676	5.140369967
Dok5	19.914	3.888	5.12191358
Zfand2b	11.3	2.2185	5.093531666
Gm10221	959.7975	189.045	5.077084821
Hist1h4c	23.187	4.613	5.026446998
Tomm7	99.485	20.2315	4.917331883
LOC100503172	4.8475	1	4.8475
Atp5j2	794.921	164.7975	4.823622931
Ndufa5	519.466	109.497	4.744111711
Refbp2	10.402	2.195	4.738952164
Usmg5	905.3675	193.094	4.688739681
Fkbpl	10.465	2.2605	4.629506746
Gm6900	14.4895	3.138	4.617431485
Uqcr11	562.0235	121.9935	4.606995455
Ndufc1	1029.66	223.8245	4.600300682

FIG. 18B

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
C1qtnf4	23.7265	5.17	4.58926499
Scyl2	67.2215	14.7345	4.56218399
Atp5e	884.0335	194.6755	4.541061921
Nop10	552.92	121.8365	4.538213097
Gm561	207.151	45.702	4.532646274
Med11	15.6685	3.4975	4.479914224
Gm3244	142.6045	31.9815	4.458968466
Dpm3	97.691	22.02	4.436466848
Rpa3	23.536	5.3055	4.436151164
1810022K09Rik	213.1715	48.6205	4.384395471
Sprr1a	8.503	1.942	4.378475798
Efcab10	23.106	5.2935	4.364975914
Rtn4r	5.283	1.2115	4.360709864
Rundc2a	5.6745	1.302	4.358294931
Pfdn4	98.098	22.597	4.341195734
Ndufa2	442.895	102.053	4.339852822
Uqcrq	895.4335	206.533	4.335546862
LOC100503459	186.453	43.153	4.320742474
Ndufa7	397.406	92.807	4.28206924
Gm10029	8.967	2.0965	4.277128548
Cox7a2	701.558	164.2645	4.270904547
Hist1h4d	383.8715	89.964	4.266945667
Romo1	558.065	130.8035	4.266437825
Rps27l	81.3645	19.193	4.239279946
Chchd1	103.97	24.789	4.19419904
Hist1h4i	12.2045	2.9135	4.188948001
Psmg4	31.823	7.6255	4.173234542
Thumpd2	5.453	1.31	4.16259542
Gm9803	84.6665	20.4375	4.142703364
Znrd1as	17.02	4.151	4.100216815
Ndufb4	669.6945	164.4095	4.073332137
Ndufb2	345.829	85.383	4.050326177
Pafah1b3	133.1685	32.939	4.042882298
Bnip1	30.48	7.5765	4.022965749
Gm6788	23.385	5.8225	4.016316015
Hist1h3a	4.8405	1.208	4.007036424
Dynll1	351.472	87.826	4.001912873
Nudt17	14	3.5085	3.990309249
Ubl5	246.934	62.4455	3.95439223
Mif	1387.941	351.8165	3.945070797
Mrpl33	157.8685	40.1515	3.931820729
Ndufa4	1127.489	286.923	3.929587381
Dnajc15	185.236	47.4605	3.902950875
Cox6b1	1172.932	301.2515	3.893530821
Dctpp1	34.417	8.869	3.880595332
Dnajc19	84.2545	21.7635	3.871367197
2900010M23Rik	162.7415	42.0995	3.865639734

FIG. 18C

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Adrb1	7.9425	2.056	3.863083658
Lypla11	10.1655	2.6335	3.860072147
2010107E04Rik	829.43	215.4745	3.849318597
Nubp1	5.169	1.346	3.840267459
Gm10053	106.7795	27.9305	3.82304291
Ndufa1	260.1105	68.1745	3.815363516
Atpif1	943.722	247.695	3.810016351
Ndufa8	397.685	105.1755	3.781156258
2310030G06Rik	4.262	1.1275	3.780044346
Chchd10	919.0805	243.336	3.777001759
Mrpl12	91.7185	24.3105	3.772793649
Gm10177	136.083	36.1795	3.761328929
Cacng8	23.781	6.3285	3.757762503
A230065H16Rik	21.8015	5.838	3.73441247
Als2cr4	13.823	3.7175	3.718359112
Pvalb	29.033	7.853	3.697058449
Sac3d1	10.68	2.8915	3.693584645
Csrp2	13.949	3.78	3.69021164
Rrp36	28.8155	7.8225	3.683668904
Sncb	306.3235	83.6175	3.663389841
Med31	62.377	17.0655	3.655152208
1110032A04Rik	6.6325	1.8195	3.645232207
Mrps34	46.6085	12.792	3.643566291
Mrpl53	111.517	30.649	3.638520017
Mrpl34	245.71	68.006	3.613063553
Ndufa3	429.3675	119.0985	3.605146161
Tstd1	4.6395	1.2915	3.592334495
Slc25a10	5.735	1.6025	3.578783151
2310016M24Rik	185.8115	51.962	3.575911243
Cox5a	538.115	151.293	3.556773942
Ndufa11	116.921	32.8785	3.556153717
Gm10269	5.034	1.4255	3.531392494
Ndufv3	283.1175	80.255	3.527724129
Uqcr10	936.83	266.121	3.520315947
Hist1h3e	17.704	5.0405	3.512349965
1810035L17Rik	270.3785	77.1005	3.506831992
Pfdn1	96.259	27.7075	3.474113507
Dhrs7	13.473	3.89	3.463496144
Crh	10.258	2.9625	3.462616034
Pgam2	5.257	1.5215	3.455142951
Atp6v1f	309.981	90.0715	3.441499253
Robld3	215.405	62.835	3.428105355
Zfp771	9.2435	2.704	3.418454142
Chmp6	18.375	5.3775	3.417015342
Yjefn3	14.928	4.3885	3.401617865
Grhl3	3.6075	1.0645	3.388914984
Gm14166	38.471	11.3665	3.384595082

FIG. 18D

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gtpbp3	9.2395	2.736	3.377010234
Ap3b2	66.1715	19.6025	3.375666369
Atox1	151.137	45.0185	3.35721981
Coq4	19.075	5.691	3.351783518
Higd2a	537.3105	161.155	3.334122429
Slc32a1	89.945	26.9835	3.333333333
Tmub1	13.4975	4.0515	3.331482167
Thap3	29.049	8.735	3.32558672
Uck1	18.016	5.4465	3.307812357
Ndufs7	437.7275	132.355	3.307222999
Spa17	23.504	7.1075	3.3069293
Gm7536	102.3315	30.986	3.302507584
Ndufs6	370.673	112.2485	3.302253482
Rnasek	1259.338	382.285	3.294238592
Gm2000	132.873	40.4095	3.288162437
1110001J03Rik	204.405	62.3255	3.279636746
Slc29a1	6.839	2.09	3.272248804
Setx	24.6525	7.5895	3.248237697
Zfp787	8.7665	2.706	3.239652624
Cda	10.26	3.173	3.233532934
Rpusd4	4.9825	1.5415	3.232241323
Cox5b	955.902	296.0255	3.229120464
Sssca1	121.4445	37.619	3.228275605
Erh	212.669	66.26	3.209613643
Hcfc1r1	90.5105	28.2195	3.207374333
Uqcrh	1024.428	319.768	3.203660154
Tmem203	20.6155	6.4395	3.201413153
Neil2	3.21	1.004	3.197211155
Polr2j	113.0315	35.4185	3.19131245
Amdhd2	8.4855	2.6655	3.183455262
Cox6c	1169.184	367.3745	3.182539888
Tmem115	7.5355	2.369	3.180878008
Ndufs5	556.87	175.1975	3.178527091
Akr7a5	8.509	2.684	3.170268256
3110001D03Rik	71.866	22.804	3.151464655
Gm3934	41.1595	13.0795	3.146871058
Mrpl23	120.5825	38.3315	3.145780885
Aacs	8.351	2.6555	3.144793824
Fxyd2	17.076	5.4605	3.127186155
Fabp3	201.4065	64.438	3.125585834
Gpn2	6.3545	2.0355	3.121837386
Smcr7	8.1415	2.6085	3.121142419
Gng13	34.468	11.07	3.11364047
1700023F06Rik	12.3255	3.962	3.110928824
Timm8b	582.267	187.1765	3.110791152
Cox6a1	1259.033	405.38	3.105809364
0910001L09Rik	61.816	19.904	3.105707396

FIG. 18E

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Mrpl20	123.253	39.7875	3.097781967
Gal	165.235	53.3665	3.096230781
Ext2	45.0985	14.62	3.084712722
1810006K21Rik	77.5565	25.195	3.078249653
Ankrd37	6.8325	2.2215	3.075624578
Rpp21	89.0795	28.966	3.075312435
Fam183b	29.623	9.639	3.073244112
Mrpl52	231.916	75.4635	3.073220829
Plvap	13.111	4.268	3.071930647
6330527O06Rik	26.8755	8.7975	3.054901961
Gabarapl2	93.432	30.6985	3.043536329
Tomm6	183.46	60.291	3.04290856
Ndufb5	253.883	83.477	3.041352708
Vps37d	14.171	4.6595	3.041313446
Scand1	26.3855	8.684	3.038403961
Lsm7	66.373	21.8525	3.037318385
Sec61b	98.2725	32.541	3.019959436
Mrpl36	152.6785	50.693	3.01182609
Grcc10	372.4755	123.888	3.006550271
Serf1	26.694	8.8835	3.004896719
Yif1b	19.432	6.469	3.003864585
Atp5k	717.4725	239.2265	2.999134711
Srp14	328.308	109.564	2.996495199
Chchd2	873.471	291.535	2.996110244
Sirt4	7.408	2.474	2.994341148
Hist1h2bg	4.9485	1.6535	2.992742667
Fxc1	25.846	8.6365	2.992647485
Tm2d1	13.792	4.61	2.99175705
Eepd1	4.6815	1.568	2.98565051
Rpusd1	24.703	8.279	2.98381447
Dem1	9.462	3.175	2.98015748
Ccdc124	69.304	23.2565	2.97998409
B9d2	16.1645	5.4245	2.979905982
Gm6181	14.1095	4.741	2.976059903
Krtcap2	97.895	32.9065	2.97494416
Ogg1	4.4665	1.503	2.97172322
Gm4184	23.592	7.958	2.964563961
Atp5o	696.3875	235.365	2.95875555
Fbxo36	11.5315	3.899	2.957553219
Clpp	73.673	24.9465	2.953239933
Med29	14.0985	4.778	2.950711595
Cpsf4	9.0705	3.0765	2.948317894
Exoc3l	7.0715	2.3985	2.948301021
Snrpd2	313.486	106.388	2.946629319
Gga1	15.5245	5.275	2.943033175
Hspe1	497.7365	169.167	2.942278931
Nup37	5.3885	1.832	2.941320961

FIG. 18F

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Mrps28	51.903	17.651	2.940513285
Gm5453	4.136	1.409	2.935415188
Cox7c	1456.53	496.616	2.932909934
Ftsj1	19.625	6.7015	2.928448855
Abhd14a	17.4085	5.9675	2.917218266
Tmsb15b2	9.4325	3.2335	2.917117674
Tmem147	52.0075	17.88	2.908696868
Emg1	68.5575	23.5775	2.907751034
Tmtc4	17.9835	6.191	2.904781134
Ap2s1	190.141	65.7045	2.893880937
Ndufb11	198.029	68.503	2.890807702
Pam16	132.821	46.069	2.883088411
Zfp526	8.2695	2.87	2.881358885
Mpdu1	22.532	7.8395	2.874162893
Magohb	15.055	5.2505	2.867345967
Ccne1	8.7895	3.07	2.863029316
Cox4i1	1328.1925	464.274	2.860794488
Inpp1	18.2865	6.4035	2.855703912
Cstb	186.609	65.3505	2.855509904
Chchd6	62.848	22.0685	2.847860072
Mrpl42	200.2255	70.3335	2.846801311
1700021F05Rik	52.319	18.46	2.834182015
Commd3	128.821	45.4835	2.832257852
Lsm5	29.329	10.3715	2.827845538
Pcdhgc5	14.1865	5.022	2.824870569
H2afj	22.4	7.932	2.824004034
Nts	150.985	53.4855	2.822914622
Arl2	224.412	79.578	2.820025635
LOC100503508	16.9975	6.0295	2.819056306
Sip1	7.621	2.709	2.813215209
Gm13202	3.758	1.336	2.812874252
Fbxl6	4.8475	1.7235	2.812590659
Mansc1	5.409	1.9245	2.810600156
Lsmd1	207.3635	74.0745	2.799391154
Snx17	63.6795	22.752	2.798852848
Lrrc24	13.3515	4.7755	2.795832897
Atp5g1	1218.382	435.859	2.795358132
Hist1h2bf	12.347	4.431	2.786504175
Ranbp1	204.155	73.3515	2.783242333
Fads6	12.434	4.48	2.775446429
Rplp2	1203.59	435.3495	2.764652308
Asphd1	36.7955	13.31	2.764500376
Cox8a	1076.501	390.349	2.757791105
Nudt8	7.8945	2.8645	2.755978356
B3galt6	5.5375	2.0095	2.755660612
Lor	8.2815	3.0195	2.742672628
Rnf126	41.1985	15.031	2.740902136

FIG. 18G

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Abcb9	5.6705	2.07	2.739371981
Poc1a	6.5305	2.386	2.737007544
Ndufs3	221.8755	81.1255	2.734966194
2410015M20Rik	123.2695	45.261	2.723525773
Dusp6	12.8255	4.71	2.723036093
Gm5617	51.565	18.95	2.721108179
Ndufa12	477.701	175.92	2.71544452
Pcx	4.6715	1.723	2.711259431
Spns1	4.6405	1.712	2.71057243
Dhps	41.826	15.448	2.707534956
2310003F16Rik	218.517	80.764	2.705623793
Rwdd1	42.5915	15.746	2.704909183
Fam125a	19.6555	7.2685	2.704203068
Ndufb3	324.9015	120.2415	2.702074575
Cmc1	28.1175	10.4105	2.70087892
1700040I03Rik	8.4925	3.1445	2.700747337
Wdr3	6.4465	2.387	2.700670297
Taf10	36.5955	13.553	2.700177083
Gm10175	301.928	111.8685	2.698954576
Klhdc3	76.3715	28.336	2.695211039
Hagh	57.2915	21.2725	2.693218945
Hist2h2bb	6.768	2.514	2.692124105
Cetn2	62.3415	23.165	2.691193611
Pcp4	1592.2515	592.1915	2.688744266
2410002I01Rik	4.053	1.5105	2.683217478
1810049H13Rik	8.466	3.1585	2.680386259
Sdf2l1	24.347	9.0895	2.678585181
2310028O11Rik	114.253	42.762	2.671834807
H1fx	6.1285	2.294	2.671534438
Immp2l	4.347	1.6285	2.669327602
Mrpl14	50.355	18.8755	2.667743901
Dpy30	117.9415	44.3065	2.661945764
Pop5	59.863	22.4945	2.6612283
Snrpd3	43.4085	16.3205	2.659753071
Cgref1	30.081	11.3175	2.657919152
Psenen	410.1285	154.8025	2.649366128
Mrps16	52.6855	19.904	2.646980506
Dapk3	26.782	10.123	2.645658402
Gng3	1687.7025	639.2355	2.640188944
Mrps21	198.2895	75.1915	2.637126537
Gm3837	17.4095	6.604	2.63620533
Tmem218	9.9925	3.7915	2.635500462
1110008F13Rik	29.6895	11.285	2.630881701
Bola2	153.476	58.337	2.630851775
Uba52	1437.8195	546.9185	2.628946543
Ndufa6	154.226	58.7555	2.624877671
Zfp637	30.885	11.77	2.62404418

FIG. 18H

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gm10154	96.5545	36.8205	2.622302793
Necrp1	5.5275	2.1115	2.617807246
Hist1h2bl	2.7865	1.065	2.616431925
Ndufb6	204.2125	78.2415	2.610027926
Dync1h1	81.697	31.304	2.609794275
Lin7b	99.8805	38.4075	2.600546768
Uqcrb	128.8765	49.6065	2.597976072
Clptm1l	21.4905	8.288	2.592965734
Ndufab1	96.0515	37.052	2.592343193
Psmd3	49.3505	19.0635	2.588742886
Tnfrsf8	2.902	1.1225	2.585300668
Ndufb7	216.105	83.628	2.584122543
Tac2	424.6965	164.3485	2.584121547
Rpl36	1209.866	469.321	2.577907232
1110058L19Rik	53.296	20.7195	2.572262844
Tmem86a	7.9325	3.085	2.571312804
Kctd21	15.5865	6.0665	2.569273881
1700029F09Rik	3.319	1.293	2.566898685
Ddt	149.2415	58.1835	2.565014136
Tmsb10	1099.414	428.6385	2.564897927
Ppp4c	21.8285	8.518	2.562632073
Ccdc56	175.002	68.355	2.56019311
Pde11a	4.5205	1.767	2.558290889
2410022L05Rik	161.1775	63.017	2.557682847
Hyal2	5.3595	2.102	2.549714558
Psmb3	191.649	75.2245	2.547693903
Rpp30	30.274	11.885	2.547244426
Ccdc52	3.547	1.393	2.546302943
Lig4	8.675	3.4075	2.545854732
Prdx5	580.7205	228.138	2.545479052
6330512M04Rik	7.2885	2.869	2.540432206
Cdipt	29.175	11.488	2.539606546
Pdlim7	36.7385	14.483	2.536663675
Jak1	63.437	25.056	2.531808748
Slc10a3	6.0785	2.4015	2.531126379
Rpl34	182.276	72.095	2.528275192
2610528E23Rik	27.1505	10.754	2.524688488
Slc2a6	5.5655	2.2065	2.522320417
Gtf2h3	10.608	4.2065	2.521811482
Rcc1	4.993	1.98	2.521717172
Gm5526	92.5135	36.7385	2.518162146
5730403M16Rik	5.602	2.226	2.516621743
3110040N11Rik	42.525	16.8995	2.516346637
Rpl39	607.093	241.3915	2.514972565
Cdc42ep3	11.8005	4.6945	2.513686229
Abtb1	7.28	2.901	2.50947949
Gm10196	7.3495	2.938	2.501531654

FIG. 18I

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Dbn1	25.44	10.1785	2.499385961
Polr2i	44.8585	17.95	2.49908078
Znrd1	55.4675	22.1975	2.498817434
Cltb	154.256	61.79	2.496455737
B4galt3	11.1075	4.453	2.494385807
Ube2s	97.654	39.1855	2.49209529
Mrpl51	77.8825	31.257	2.491681863
Npdc1	66.47	26.7125	2.488348152
Brp44	177.95	71.5395	2.48743701
Cdk4	54.9745	22.101	2.487421384
Hint2	78.615	31.626	2.485771201
Ube2d1	137.102	55.1595	2.485555525
Gm10047	8.051	3.241	2.484109843
Rpl38	1187.776	478.229	2.483697141
D4Bwg0951e	4.4195	1.7805	2.48216793
Pgp	43.94	17.7245	2.479054416
Atp5d	673.6085	272.0185	2.476333411
Srrm2	267.34	108.001	2.47534745
5430437P03Rik	64.4715	26.0715	2.472872677
Utp18	7.927	3.211	2.468701339
Raly	14.342	5.811	2.468077784
Mrps18a	111.0805	45.01	2.467907132
Qsox2	14.0355	5.691	2.46626252
Ccdc12	30.7025	12.467	2.462701532
Slc7a4	9.3885	3.8155	2.460621151
Abhd11	32.8335	13.344	2.460544065
Ndufv2	149.3165	60.7085	2.45956497
1810014F10Rik	57.7955	23.594	2.44958464
Lipt1	6.976	2.853	2.445145461
H2afx	100.475	41.154	2.441439471
Spr	16.9085	6.9375	2.437261261
Slc35b2	5.1445	2.112	2.435842803
Dnajc17	24.3815	10.0125	2.435106117
Lrrc48	4.5845	1.8835	2.434032387
Pex16	11.2925	4.6415	2.432941937
Ssu72	136.468	56.1055	2.432346205
Ly6h	32.5455	13.3925	2.430128803
Glrx5	100.3455	41.3595	2.426177783
Gbx2	5.175	2.133	2.426160338
2900010J23Rik	193.64	79.9105	2.423210967
Fabp5	114.8795	47.4205	2.422570407
Sepx1	15.7665	6.511	2.421517432
Moxd1	2.8435	1.1745	2.421030226
Bloc1s2	55.889	23.086	2.420904444
Mrpl32	58.2575	24.111	2.416220812
Timm13	45.307	18.7975	2.410267323
Clasp2	163.716	67.941	2.409678986

FIG. 18J

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Nit2	16.0925	6.6785	2.409597964
Med30	25.6065	10.635	2.407757405
Atf6b	6.675	2.7725	2.407574391
Thyn1	80.562	33.471	2.406919423
Mtpap	18.63	7.742	2.406354947
Crhbp	8.326	3.462	2.404968226
1700037H04Rik	197.9145	82.317	2.404296804
Gm6136	55.644	23.1615	2.402435075
Nenf	71.8125	29.899	2.401836182
Fam53a	8.8805	3.698	2.401433207
Bud31	82.307	34.287	2.400530813
Dctn3	259.444	108.1235	2.399515369
Apex1	34.4755	14.3785	2.397711861
Pdcd5	191.3465	79.817	2.397315108
Rpl22l1	312.9475	130.652	2.395275235
Ccdc107	11.5365	4.819	2.393961403
Tmem132a	5.7475	2.401	2.393794252
Nudt1	8.1525	3.406	2.39357017
Tbca	520.2655	217.7865	2.388878558
Ephx1	7.9945	3.3485	2.387486934
Rxrg	10.388	4.352	2.386948529
BC048546	7.584	3.1775	2.386782061
Pusl1	15.131	6.346	2.38433659
1110001A16Rik	33.9535	14.258	2.381364848
Slc22a5	4.762	2	2.381
Coro1a	54.127	22.7725	2.376858052
Osbpl10	6.8565	2.8855	2.376191301
Krt25	2.564	1.0795	2.375173692
Fggy	9.516	4.0075	2.374547723
Txn1	241.7655	101.8745	2.37316993
Micall2	3.3345	1.4055	2.372465315
Grtp1	13.7785	5.8085	2.372127055
Mpp3	15.8845	6.706	2.368699672
N6amt2	28.6045	12.091	2.365767927
Mrpl21	72.4075	30.617	2.364944312
Paqr7	12.195	5.159	2.3638302
Mrpl43	87.838	37.162	2.363651041
G0s2	5.5335	2.343	2.361715749
Tpra1	11.6395	4.9285	2.361671908
Ramp2	4.0985	1.7375	2.358848921
Iscu	289.4815	122.8275	2.356813417
Zfp385b	81.039	34.391	2.356401384
Khk	15.2935	6.4905	2.356289962
Tmem222	88.6795	37.677	2.35367731
Ssr4	54.53	23.172	2.353271189
Tuba8	8.321	3.5375	2.352226148
Praf2	190.779	81.132	2.35146428

FIG. 18K

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Pnoc	29.9535	12.7445	2.350307976
Grwd1	8.139	3.465	2.348917749
Pofut2	19.935	8.4895	2.348194829
Hebp1	14.255	6.0765	2.345922817
Spcs1	75.2695	32.0875	2.345757694
Rps28	613.903	261.7175	2.345670427
Sra1	51.3925	21.9165	2.344922775
D430042O09Rik	3.7845	1.62	2.336111111
Pde6d	101.826	43.6175	2.334521694
Uchl3	46.371	19.8725	2.333425588
Smn1	21.9285	9.399	2.333067348
Hspb11	26.7	11.4465	2.332590748
Alk	3.703	1.592	2.326005025
C330021F23Rik	2.5285	1.088	2.323988971
Atp5j	464.389	199.8405	2.323798229
Siva1	25.0135	10.7655	2.323487065
Pgls	13.7485	5.921	2.321989529
Fbll1	40.9755	17.6715	2.318733554
Rnaseh2c	64.015	27.6155	2.318082236
Cycs	156.661	67.599	2.317504697
Hint1	397.7925	171.7615	2.315958466
Ndufb8	314.5345	135.9525	2.313561722
Cox17	337.403	146.046	2.310251565
1110008P14Rik	108.2935	46.9595	2.306104196
Kcnj11	7.965	3.456	2.3046875
Cxx1b	106.9445	46.4215	2.303770882
Yrdc	8.686	3.7705	2.303673253
Ube2m	40.2	17.452	2.303460921
Acn9	29.723	12.9075	2.302769708
Ndufs4	94.7155	41.136	2.302496597
Zfp628	6.9795	3.036	2.298913043
Hist1h1e	46.1265	20.07	2.298281016
Shh	5.7565	2.5055	2.2975454
Pcdhga9	4.3935	1.914	2.295454545
Ndufc2	447.346	195.1075	2.292818062
Prmt10	4.5225	1.9755	2.28929385
Fancf	4.3255	1.8895	2.289229955
Pin4	91.829	40.14	2.287717987
Dcun1d5	25.518	11.1575	2.287071477
Dis3l2	9.683	4.234	2.286962683
Cacna2d3	8.716	3.8135	2.285564442
Egfl7	3.8705	1.6965	2.281461833
0610037L13Rik	51.99	22.799	2.280363174
Lgmn	31.9265	14.004	2.279812911
Vsig10l	3.395	1.4895	2.279288352
Mid1	28.7535	12.652	2.272644641
P4htm	19.256	8.4795	2.270888614

FIG. 18L

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Suclg1	92.775	40.8705	2.269974676
Ypel3	236.823	104.33	2.269941532
Eif1	347.8985	153.268	2.269870423
Trappc6a	6.218	2.74	2.269343066
Lsm1	14.044	6.19	2.268820679
6330409D20Rik	9.656	4.257	2.268264036
Tm2d3	77.623	34.243	2.266828257
Gm4604	153.767	67.8405	2.266595912
Coq7	61.1855	27.0155	2.26482945
Nme2	220.0635	97.1715	2.264691808
Tyw3	4.9125	2.17	2.263824885
Btf3	237.828	105.0975	2.262927282
Tspan4	6.8745	3.038	2.262837393
Lrrc42	10.6845	4.723	2.262227398
Gm5908	5.088	2.2505	2.260830926
Ccdc85c	5.393	2.3885	2.257902449
Anapc11	11.848	5.248	2.257621951
Map1lc3a	129.101	57.221	2.256182171
Tpd52l1	45.7385	20.2885	2.254405205
Xylt2	4.0635	1.803	2.25374376
Ctxn2	54.564	24.212	2.25359326
0610011F06Rik	104.527	46.3845	2.253489851
Atp5g3	747.394	331.6825	2.253341675
Myl6b	185.817	82.5035	2.252231724
Mgst3	98.63	43.801	2.251775074
Pcbd1	133.656	59.36	2.251617251
Slc46a1	4.715	2.0945	2.251133922
Ostc	31.529	14.015	2.249661077
Pqbp1	117.5945	52.3535	2.246163103
Nme1	232.9525	103.7895	2.24447078
Evl	71.2415	31.7735	2.242167215
Mrpl13	80.225	35.7825	2.242017746
LOC100504116	35.8755	16.0095	2.240888223
Lars2	12300.984	5490.047	2.240597121
Dexi	24.1895	10.803	2.239146533
Ydjc	15.3445	6.8535	2.238929014
Rai12	60.5165	27.05	2.237208872
Cuta	347.189	155.194	2.237129013
Fkbp1b	56.61	25.3945	2.229222863
Sh3bgrl3	460.0025	206.409	2.228597106
Gm4459	230.0995	103.4165	2.224978606
Rpl27	487.231	219.118	2.223600982
Fam36a	39.0665	17.5715	2.22328771
Dact2	2.4445	1.1	2.222272727
Myeov2	221.9385	99.9425	2.220661881
Arhgdig	45.014	20.294	2.218094018
Comtd1	5.7205	2.583	2.214672861

FIG. 18M

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Car4	3.6885	1.6655	2.214650255
Lage3	42.4925	19.1915	2.214131256
Gpr83	12.02	5.4315	2.213016662
Ube2v1	332.4115	150.299	2.211668075
Slc2a8	5.102	2.3085	2.210093134
Zfp579	8.392	3.7975	2.209874918
4930538K18Rik	2.2485	1.0185	2.207658321
Alg13	2.998	1.3585	2.206845786
Krt10	2.912	1.32	2.206060606
Ctbp1	53.0495	24.078	2.203235319
4930427A07Rik	2.736	1.2445	2.198473282
Gale	3.5185	1.6005	2.198375508
Cisd3	64.6655	29.439	2.196592955
Commd4	75.1155	34.2215	2.194979764
Mt3	4.494	2.0485	2.193800342
Ict1	77.7785	35.462	2.193291411
Prelid1	236.986	108.108	2.192122692
Tmem11	47.113	21.547	2.186522486
Dusp14	12.026	5.5075	2.183567862
Polr2f	86.8295	39.806	2.181316887
Tmem141	10.325	4.7365	2.179879658
Pls3	29.0345	13.3205	2.179685447
Mrpl41	54.7555	25.129	2.178976481
Chrna3	2.4555	1.1285	2.175897209
Apitd1	12.617	5.8025	2.174407583
Ssna1	47.6515	21.931	2.172791938
Gm10051	90.1155	41.5335	2.169706382
Med26	8.0585	3.716	2.168595264
C1rl	3.752	1.731	2.167533218
Nhp2	23.2785	10.746	2.166247906
Cisd1	138.856	64.102	2.166172662
Ankrd9	2.828	1.306	2.165390505
Gpr135	3.115	1.4395	2.163945815
Pfdn5	501.0695	231.5625	2.163862888
Nedd8	291.22	134.6295	2.163121753
Polg2	2.92	1.35	2.162962963
Gm9843	526.221	243.433	2.16166666
Rrp9	5.8785	2.7205	2.160816026
Mrpl54	37.396	17.319	2.15924707
Gm11847	98.417	45.5855	2.158954053
Vgf	37.141	17.2355	2.154912825
Atp5g2	89.5585	41.5865	2.153547425
Sh2d7	2.422	1.125	2.152888889
Tceb1	59.2575	27.531	2.152391849
Park7	197.391	91.7605	2.151154364
Prpf8	66.1315	30.7425	2.151142555
Banf1	91.952	42.754	2.150722739

FIG. 18N

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Fgf18	20.547	9.557	2.149942451
Etnk2	3.381	1.575	2.146666667
Ppa2	50.7465	23.6645	2.14441463
Rab4b	54.1035	25.249	2.142797735
CK137956	5.042	2.3555	2.140522182
2310007A19Rik	10.3555	4.84	2.139566116
Pdap1	74.803	34.967	2.139245574
Dlx2	12.4645	5.827	2.139093873
Foxo6	8.7805	4.107	2.137935233
Cbx8	4.28	2.0025	2.13732834
Tmem91	93.7105	43.846	2.137264517
Zdhhc1	12.5745	5.888	2.13561481
Mrpl37	45.364	21.2645	2.133320793
Dynlrb1	1513.6345	709.7735	2.132559894
Srm	67.8175	31.808	2.132089411
Snrpg	119.7715	56.182	2.131848279
Polr3c	14.01	6.579	2.129502964
Acpl2	6.847	3.2155	2.129373348
Ngfrap1	511.5735	240.424	2.127797142
Mrps18c	50.963	23.9585	2.127136507
Dad1	250.5625	117.963	2.124077041
3110007F17Rik	3.352	1.5785	2.123535002
Fam195a	7.184	3.3845	2.122617817
Gmppb	4.263	2.009	2.12195122
Polr2k	43.287	20.401	2.121807755
Jmjd7	3.747	1.7665	2.121143504
Rps14	242.904	114.572	2.120099152
Rinl	2.7605	1.3025	2.119385797
Phpt1	119.4205	56.3775	2.11822979
Rpl29	596.507	281.7985	2.116785576
Timm9	91.3935	43.1785	2.1166437
Snrpe	52.283	24.7075	2.116078114
2010005H15Rik	16.4305	7.7685	2.115015769
Drd1a	2.7635	1.307	2.114384086
Ccdc106	16.702	7.902	2.113642116
Gpx7	3.145	1.488	2.113575269
Sema6b	21.1335	9.999	2.113561356
Rasgef1c	4.7935	2.272	2.109815141
Mrpl55	44.8745	21.289	2.10787261
Cox18	17.5445	8.326	2.107194331
2810408M09Rik	10.8805	5.169	2.104952602
Esr1	12.739	6.059	2.10249216
Maf1	29.431	14.0035	2.101688864
Dlx5	77.982	37.11	2.101374293
Fam173a	66.261	31.5505	2.100156891
Pttg1	45.6205	21.7275	2.099666321
Arpc4	165.191	78.678	2.099583111

FIG. 18O

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Sdhb	131.9685	62.875	2.098902584
Hist2h2ab	18.9565	9.0385	2.097305969
0610009B22Rik	101.3635	48.372	2.095499463
Satb2	12.8755	6.147	2.094598991
Lyrm2	30.3035	14.4715	2.094012369
Nhp2l1	198.6	94.913	2.092442553
Blm	2.191	1.0475	2.091646778
BC030500	50.567	24.1765	2.091576531
Gm5107	4.7045	2.2505	2.09042435
Rps21	637.2735	305.1755	2.088219729
Mrpl11	31.351	15.024	2.086727902
Wbscr22	34.1525	16.381	2.084884928
Asna1	242.8505	116.503	2.08449997
Mogs	5.5245	2.652	2.083144796
Fhl2	4.8015	2.3055	2.082628497
Pcsk1n	156.198	75.006	2.082473402
Fkbp1a	1107.568	532.213	2.08106153
Xylt1	6.8575	3.296	2.080552184
Vkorc1	48.4175	23.2765	2.080102249
Mrpl22	51.3635	24.697	2.079746528
Kpna2	28.2195	13.582	2.07771315
Slc19a2	5.987	2.886	2.074497575
Rrad	5.0135	2.418	2.073407775
Macrod1	3.838	1.852	2.072354212
Rars2	11.1375	5.3745	2.072285794
Galntl4	5.071	2.449	2.070641078
Spint2	19.0845	9.218	2.070351486
Pmf1	9.3545	4.5195	2.069808607
1810063B05Rik	52.3965	25.3305	2.068514242
Fam165b	47.0115	22.7425	2.067121029
Ubl4	33.7825	16.3465	2.066650353
Gm13841	5.843	2.8285	2.065759236
Hspbp1	34.2145	16.5785	2.063787436
Hmx2	3.99	1.9335	2.063615206
Nrgn	91.075	44.134	2.063601758
1810030N24Rik	10.91	5.288	2.063161876
1110049F12Rik	75.6775	36.69	2.062619242
Dmrtc1a	6.5695	3.186	2.061989956
Gfm1	28.564	13.861	2.060745978
Gba	23.8105	11.557	2.060266505
Tmem14c	33.4615	16.2465	2.05961284
Arx	17.223	8.363	2.059428435
Hsbp1	1087.814	528.3625	2.058840285
Dctd	6.0835	2.956	2.058017591
Ints1	9.1305	4.4395	2.056650524
Fank1	4.033	1.961	2.056603774
Gm5561	6.6905	3.254	2.056084819

FIG. 18P

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Cox7b	451.4055	219.7035	2.054612239
Pomp	919.5195	447.615	2.054264267
Bola1	35.052	17.1025	2.049524923
LOC100503055	3.8485	1.878	2.049254526
Hmgn1	105.1575	51.3455	2.048037316
BC056474	277.3625	135.4435	2.047809603
Sema3f	2.29	1.1195	2.045556052
U2af2	48.8	23.8635	2.044964066
Polr2l	25.731	12.583	2.044901852
Rpl35	462.4805	226.203	2.044537429
Necab2	24.4505	11.9635	2.043758098
Rpp25	52.495	25.6915	2.043282798
Ndufa13	232.699	113.886	2.043262561
Polr2c	38.3865	18.817	2.039990434
Shfm1	78.513	38.489	2.039881525
Cck	81.6865	40.048	2.039714842
Dydc2	2.2725	1.115	2.038116592
Parp4	3.383	1.66	2.037951807
Anapc13	193.612	95.051	2.036927544
Mtrf1	9.257	4.547	2.035847812
Eif1b	168.2135	82.6335	2.035657451
Commd2	20.4845	10.063	2.035625559
Lrrc14	8.647	4.25	2.034588235
Myl12b	1868.7535	918.503	2.034564394
Slc16a2	6.5535	3.2215	2.034300792
Rps26	828.79	407.4645	2.034017687
Psmb6	190.4435	93.7005	2.032470478
Fkbp3	591.866	291.3145	2.031707999
Mtch1	290.2245	142.882	2.031218068
Arf5	39.099	19.254	2.030694921
Mrps33	165.3375	81.4425	2.03011327
C1qbp	87.7145	43.2165	2.029653026
Apoa1bp	89.3665	44.0395	2.029235118
Nxt1	4.1875	2.0655	2.027354152
Atp5h	765.9775	378.185	2.025404233
A230050P20Rik	17.1245	8.4565	2.025010347
Cited4	4.1595	2.057	2.022119592
Mrps12	82.997	41.0465	2.022023802
Ndfip1	236.3535	116.9815	2.020434855
0610007C21Rik	69.461	34.393	2.019626087
Yif1a	6.743	3.339	2.019466906
A430005L14Rik	12.933	6.4085	2.01810096
Wbp11	65.9765	32.745	2.01485723
Polr2h	56.8755	28.2455	2.013612788
Gstp1	371.4985	184.5075	2.013460157
Rpl35a	477.663	237.444	2.011686966
Mtx2	38.987	19.3805	2.011661206

FIG. 18Q

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Pard6g	3.4085	1.6945	2.011507819
Polr2d	28.505	14.1795	2.010296555
Rnf208	132.1865	65.7725	2.009753316
Ppdpf	125.2735	62.335	2.009681559
Ttc27	14.8645	7.398	2.009259259
Dlx6	24.415	12.1725	2.005750667
Nudt14	8.68	4.3325	2.003462204
Mrpl39	86.4685	43.22	2.000659417
Atp5f1	159.253	79.614	2.000314015
Hebp2	6.018	3.0135	1.99701344
Psmd8	91.8475	45.995	1.996901837
Itpa	40.999	20.5335	1.996688339
2310003C23Rik	20.6845	10.367	1.995225234
Ift27	16.235	8.137	1.995207079
Clec11a	4.2085	2.1095	1.995022517
Pigf	5.509	2.762	1.994569153
Plod2	2.4255	1.2165	1.993834772
Mrps36	118.0735	59.2365	1.993255847
Fam96b	58.009	29.122	1.991930499
Gpr162	5.849	2.938	1.990810075
Zmynd19	9.354	4.699	1.990636306
2010107G23Rik	25.3605	12.74	1.990620094
Mrpl30	235.1995	118.1815	1.990154973
Pxmp3	7.6095	3.824	1.989932008
Nell1	24.925	12.535	1.988432389
2510049J12Rik	3.871	1.947	1.988186954
Mitd1	5.3785	2.706	1.987620103
Slc35b1	27.5345	13.853	1.98762001
1810009A15Rik	44.002	22.151	1.986456593
Selm	236.4475	119.119	1.984968813
Smg5	24.2475	12.221	1.984084772
Zc3h3	5.6015	2.8235	1.983885249
Unc119	20.043	10.104	1.983669834
Mlf2	268.0745	135.2615	1.981898027
Tsen34	50.886	25.684	1.981233453
Sf3b5	65.5875	33.1045	1.981226117
Mrpl10	54.639	27.6025	1.979494611
Pard6a	10.591	5.352	1.978886398
Hmx3	3.014	1.5235	1.97833935
Gabarap	173.3735	87.654	1.977930271
Brp44l	102.2925	51.7215	1.977755866
Triap1	21.4015	10.824	1.977226534
Iffo1	12.5875	6.3685	1.976525084
Commd1	44.569	22.556	1.975926583
Nat14	6.924	3.5045	1.97574547
Acot9	6.4525	3.268	1.974449204
Mlx	15.483	7.8505	1.972231068

FIG. 18R

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Il34	10.8705	5.512	1.972151669
Fmnl1	13.133	6.662	1.971329931
2410003K15Rik	37.0435	18.798	1.970608575
Hmg20b	4.582	2.3255	1.970328962
Cno	5.331	2.707	1.969338751
Frs3	14.067	7.1445	1.968927147
Lmo2	9.326	4.7375	1.968548813
Rabl2	22.001	11.19	1.966130474
Timm17a	274.133	139.449	1.965829802
Med25	16.8895	8.592	1.965723929
Gtf2h4	5.9595	3.032	1.965534301
Rpl36al	834.713	424.9745	1.964148437
2010305A19Rik	3.2385	1.649	1.963917526
Tmem48	3.958	2.016	1.963293651
Cox16	17.2365	8.7865	1.961702612
Ap1s1	88.506	45.1225	1.961460469
Sharpin	30.919	15.7685	1.96080794
Arpc5l	73.6695	37.572	1.96075535
Tceal6	89.8965	45.8775	1.959489946
Znhit1	113.4965	57.934	1.959065488
Snx3	117.867	60.2175	1.95735459
Chchd7	24.2685	12.4	1.957137097
Zfpl1	12.3825	6.332	1.955543272
Vstm2l	8.9015	4.5545	1.954440663
Fam159b	2.176	1.115	1.951569507
Gm14173	6.129	3.141	1.951289398
Snrpf	42.4855	21.7975	1.949099667
Gm4987	3.056	1.568	1.948979592
Chst10	13.7785	7.0705	1.948730641
Tomm22	133.7695	68.7075	1.946941746
Bcam	4.2375	2.177	1.94648599
Spin2	17.421	8.954	1.9456109
Manf	15.193	7.812	1.944828469
Snip1	7.209	3.712	1.942079741
Dctn1	49.8125	25.661	1.941175324
Endog	8.0405	4.1425	1.94097767
Aamp	67.483	34.79	1.939724059
Rheb	126.6215	65.2965	1.939177444
Slc9a5	4.835	2.496	1.937099359
1700001L19Rik	19.691	10.166	1.936946685
Ndufaf3	91.2515	47.121	1.936535727
Zscan29	2.7905	1.4415	1.935830732
D8Ertd738e	93.7315	48.4235	1.935661404
Ndufaf2	95.732	49.493	1.934253329
Trappc2l	66.0195	34.143	1.933617433
Bax	21.1515	10.942	1.933056114
Anapc7	9.711	5.0245	1.932729625

FIG. 18S

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Psmb4	370.8915	191.906	1.932672767
Bak1	21.897	11.3325	1.932230311
Cox19	30.8	15.945	1.931640013
Pla2g6	8.3095	4.3025	1.931319001
Tesc	8.8325	4.5755	1.930390121
Ppil1	19.3475	10.024	1.930117717
Ap2m1	343.4635	178.025	1.929299256
Gm3724	161.6305	83.7815	1.929190812
Pex6	19.014	9.8665	1.927127147
Spna2	90.044	46.726	1.927064161
Lhx8	65.368	33.9345	1.926299194
Harbi1	3.5955	1.8675	1.925301205
Gemin4	5.2275	2.7155	1.925059842
1700019E19Rik	15.0865	7.8395	1.9244212
Pcdhgb6	4.348	2.2595	1.92431954
Rabac1	100.9765	52.484	1.923948251
B230118H07Rik	17.174	8.927	1.923826593
Cpa2	12.6495	6.583	1.921540331
Rps6kl1	12.6505	6.5895	1.919796646
Sephs2	19.008	9.9075	1.918546556
Dennd2a	4.873	2.5405	1.918126353
Ppia	2342.097	1222.1005	1.916452043
Lpcat4	68.445	35.728	1.915724362
Gm7589	13.798	7.2025	1.915723707
2410002F23Rik	56.323	29.4115	1.914999235
Arl6ip5	17.515	9.1465	1.914940141
Tm4sf5	8.077	4.219	1.9144347
Gpr161	2.314	1.2095	1.913187267
Ppa1	105.1395	54.958	1.913088176
Tk2	10.213	5.344	1.911115269
Cyth2	15.124	7.919	1.909837101
Izumo4	21.5285	11.2765	1.909147342
Nudt7	3.82	2.001	1.909045477
Fau	96.879	50.7875	1.907536303
Dut	20.37	10.6835	1.906678523
Penk	165.007	86.625	1.904842713
Pcbd2	79.447	41.7375	1.903492063
Sod1	2463.7695	1294.6865	1.902985395
Mapksp1	73.085	38.421	1.902214935
Armc6	5.6065	2.9475	1.902120441
Gm10288	212.3485	111.65	1.901912226
Ndufs8	128.873	67.78	1.901342579
Radil	3.4725	1.83	1.897540984
Tmem192	21.053	11.1035	1.896068807
Xrcc6bp1	8.186	4.322	1.894030541
Bcl7b	36.6825	19.37	1.89377904
Kcnab3	15.6955	8.288	1.893762066

FIG. 18T

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Dhrs7b	7.5755	4.005	1.891510612
Scn1b	45.4695	24.043	1.891174146
Psmg1	27.567	14.582	1.890481415
Rgs10	165.034	87.2985	1.890456308
Imp3	36.8975	19.5215	1.890095536
LOC100502641	10.0445	5.32	1.88806391
Taf12	23.133	12.255	1.887637699
9430023L20Rik	16.1025	8.5315	1.887417219
Lrfn1	5.3035	2.8105	1.887030777
1810027O10Rik	42.2575	22.398	1.886663988
Fundc2	29.8105	15.8075	1.885845327
Aldh1l2	2.163	1.1475	1.88496732
Entpd3	4.3465	2.3065	1.884456969
Nfatc2	13.078	6.9445	1.883216934
Ogdhl	9.904	5.2605	1.882710769
Brd9	33.303	17.6935	1.882216633
Arl3	182.8295	97.1495	1.881939691
Csnk2b	140.3015	74.5755	1.881335023
Ptdss2	7.3025	3.8845	1.879907324
Ube2f	19.332	10.308	1.875436554
Tmed9	63.2585	33.732	1.8753261
Rab1b	74.971	39.98	1.875212606
Ier3	14.8895	7.941	1.875015741
Begain	33.009	17.6105	1.874393118
Ndufb10	181.1685	96.6715	1.874063193
Tusc3	47.5725	25.385	1.874039787
Rexo2	90.262	48.168	1.873899684
Eri3	82.097	43.82	1.873505249
Mc4r	3.042	1.624	1.873152709
Nutf2	68.8755	36.7805	1.872609127
Atp6v0a2	31.9075	17.0455	1.871901675
Sdr39u1	123.333	65.904	1.87140386
Naca	380.809	203.6015	1.870364413
Timm23	99.8645	53.4175	1.869509056
Dnajc30	124.939	66.836	1.869336884
Naa10	38.3155	20.508	1.86831968
Wdr61	33.7835	18.0885	1.867678359
Rhobtb2	16.612	8.9015	1.866202325
Commd9	25.256	13.536	1.865839244
Grhpr	33.672	18.0565	1.864813225
Cbr1	122.06	65.461	1.864621683
Cxx1c	788.8375	423.227	1.863863837
Mib2	9.582	5.151	1.860221316
Psme2	78.6375	42.3105	1.858581203
Chrac1	13.629	7.3345	1.858204377
1810046J19Rik	122.686	66.082	1.856572138
Rab3a	566.623	305.2275	1.856395639

FIG. 18U

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Bmyc	401.539	216.3435	1.856025256
Gm13889	16.244	8.753	1.855820861
Sdhd	118.6755	63.9585	1.855507868
Ica1	18.819	10.1425	1.855459699
Zfp35	6.954	3.748	1.855389541
Psma2	144.592	77.9735	1.854373601
Mrps9	24.3225	13.1375	1.851379638
Jtb	11.307	6.1075	1.851330332
Mmp11	2.127	1.149	1.851174935
BC024814	21.784	11.772	1.850492695
Rwdd2a	89.211	48.219	1.850121321
Ddost	27.8035	15.03	1.849866933
Pramef8	5.5995	3.028	1.849240423
Pip5k1c	35.789	19.3605	1.84855763
Zbtb45	8.993	4.865	1.848509764
1500032L24Rik	174.6585	94.493	1.848375012
Dohh	29.0755	15.7375	1.847529786
Asb6	13.579	7.352	1.846980413
Dnajc8	89.7575	48.621	1.846064458
Itga3	9.918	5.373	1.845896147
LOC100046290	37.793	20.4755	1.845766892
4930452B06Rik	9.4925	5.146	1.844636611
Snf8	63.5875	34.488	1.843757249
Fam100a	91.0395	49.3855	1.843445951
Rph3al	2.79	1.514	1.842800528
Stra13	32.8325	17.8225	1.842193856
Bre	35.231	19.139	1.84079628
Ppp1r1a	20.968	11.395	1.840105309
Ap4s1	41.2775	22.4355	1.839829734
Psma6	134.1485	72.917	1.839742447
Uchl1	575.5775	312.925	1.839346489
2310036O22Rik	80.873	43.982	1.838774953
Nudt2	23.2765	12.6605	1.838513487
Gprasp1	309.9	168.562	1.838492661
Ensa	269.9945	147.001	1.836684784
Coq9	19.428	10.591	1.834387688
Nudt16l1	32.2675	17.591	1.834318686
Fam171a2	4.9645	2.707	1.833949021
Narg2	6.4145	3.4985	1.833500071
Gm8186	35.9265	19.6035	1.832657434
Nr2c2ap	8.079	4.4085	1.832596121
Ppp1r11	77.399	42.257	1.83162553
Polm	7.8825	4.3095	1.829098503
Pddc1	23.2225	12.7045	1.827895628
Atp9a	35.235	19.2805	1.8274941
Psma7	332.1595	181.847	1.826587736
A2ld1	27.227	14.906	1.826579901

FIG. 18V

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Dhdpsl	4.622	2.531	1.82615567
Mcts2	34.72	19.019	1.825542878
St8sia5	6.3585	3.4835	1.825319363
Fam136a	17.2395	9.4455	1.825154836
Sepn1	5.796	3.181	1.822068532
Gm6816	36.0605	19.7975	1.821467357
2610524H06Rik	27.5465	15.124	1.82137662
Cyp4x1	3.5425	1.945	1.821336761
Rdm1	2.1115	1.1595	1.821043553
Mea1	158.536	87.0955	1.820254778
Fkbp2	242.888	133.5	1.819385768
Nmbr	4.036	2.219	1.818837314
Slc35c2	13.344	7.3405	1.817859819
AI316807	19.8485	10.921	1.817461771
Ndufs1	35.8505	19.742	1.815950765
Chmp4b	117.4105	64.682	1.815195881
Myst1	17.408	9.591	1.815034929
D230037D09Rik	11.001	6.0645	1.813999505
Kctd8	7.557	4.166	1.813970235
Wdr36	7.781	4.2905	1.813541545
Rps19bp1	42.427	23.395	1.81350716
1110065P20Rik	18.713	10.3435	1.809155508
Rpl22	61.3445	33.917	1.808665271
Mycbp	20.8395	11.5245	1.808278016
Gadd45gip1	25.668	14.195	1.808242339
LOC100503996	2.0775	1.149	1.808093995
Scyl1	22.5205	12.457	1.807859035
Rfc5	2.666	1.475	1.807457627
Pcdhgb7	2.432	1.346	1.806835067
Panx1	6.5255	3.6125	1.806366782
Bcl11a	32.883	18.2115	1.80561733
Ankrd13d	15.2635	8.4585	1.804516167
Nup85	12.147	6.732	1.804367201
Rnase6	1.997	1.107	1.803974706
Fam58b	24.711	13.699	1.803854296
0610010O12Rik	25.352	14.0545	1.803835071
Cartpt	52.5785	29.1615	1.803010819
Nell2	40.896	22.6945	1.802022516
Pak6	15.9905	8.888	1.799111161
Timm10	54.0325	30.0595	1.797518255
Tmem55b	40.256	22.398	1.797303331
Pdxp	158.0165	87.946	1.796744593
Top2b	12.712	7.0755	1.796622147
Prdx3	57.0845	31.786	1.795900711
Cpsf3l	7.181	4.0025	1.79412867
Spire2	13.1855	7.35	1.793945578
Vps29	151.8415	84.6515	1.79372486

FIG. 18W

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Ccdc28a	21.7485	12.129	1.793099184
Rpl32	932.051	520.175	1.791802759
Wwp2	14.6385	8.17	1.791738066
Acta1	4.2915	2.3955	1.791484033
Acot7	323.206	180.457	1.791041633
2010107G12Rik	2.4355	1.3605	1.79015068
Srp19	70.9215	39.6215	1.78997514
U2af1l4	29.539	16.5035	1.789862756
Cdh22	3.1315	1.75	1.789428571
Pias3	9.731	5.4385	1.789280132
Mad2l1	6.0185	3.3655	1.788292973
Ddb1	49.904	27.9445	1.785825476
Snrpd1	83.0325	46.5275	1.784589759
Gm12715	79.177	44.389	1.783707675
Mgat4c	4.7835	2.682	1.783557047
Avpi1	9.9915	5.6045	1.78276385
Slc35e4	1.8635	1.0475	1.778997613
Tigd2	5.4155	3.047	1.777321956
Pin1	16.6885	9.393	1.776695411
C1d	22.593	12.719	1.776318893
Tsc22d1	158.659	89.3265	1.776169446
Atp6v0c	683.4585	384.936	1.775512033
Frat1	5.428	3.059	1.77443609
Mrpl38	29.5055	16.632	1.774019962
Rpl28	625.7955	352.834	1.773625841
Cnpy2	61.6985	34.787	1.773607957
Pdcd2l	11.2545	6.3495	1.772501772
Stmn3	545.117	307.773	1.771165762
Bcas2	89.072	50.296	1.770955941
Serpinb6a	14.7095	8.3075	1.77062895
Lyar	19.193	10.846	1.769592476
Nt5dc2	4.634	2.619	1.769377625
Pfdn2	356.3345	201.444	1.768901035
Drap1	72.365	40.9135	1.768731592
Marcksl1	61.1865	34.5955	1.768625977
Gas6	22.9755	12.9915	1.768502482
Tmem160	7.1745	4.057	1.768424945
Gm6344	11.1165	6.2865	1.768313052
Ckmt1	240.565	136.127	1.767210032
Senp3	15.9245	9.014	1.766640781
Psmb1	147.1705	83.312	1.766498224
Ccdc28b	31.0815	17.5955	1.766445966
Lrpprc	43.012	24.3585	1.765790176
Dnpep	15.6975	8.892	1.765350877
Gm6251	2.041	1.1565	1.764807609
2610029G23Rik	8.513	4.824	1.764718076
Agpat1	91.1655	51.6955	1.763509396

FIG. 18X

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Shf	3.6405	2.065	1.762953995
Ntan1	74.334	42.187	1.762011994
Psmb2	192.524	109.2985	1.761451438
Dus1l	9.4215	5.3505	1.760863471
Gm8430	108.4995	61.627	1.760583835
Pcdhb15	3.0295	1.7225	1.758780842
Cstf3	41.9135	23.834	1.758559201
Cul9	5.0065	2.848	1.757900281
Skp1a	256.533	145.985	1.757255882
St3gal5	49.9815	28.4465	1.757035136
Cacna1a	14.085	8.018	1.756672487
Ttc9b	17.3675	9.89	1.756066734
Acyp2	83.544	47.586	1.755642416
Gm5623	21.706	12.367	1.755154848
Mrps23	35.9705	20.502	1.754487367
Gsto1	32.5115	18.5335	1.754201851
LOC100503890	32.1955	18.355	1.754045219
Siah3	12.205	6.964	1.752584721
Fam175b	11.2635	6.427	1.752528396
Txndc17	75.063	42.8415	1.75210952
Pcdhga4	2.635	1.504	1.751994681
Ccdc72	321.734	183.7095	1.751319338
2310022A10Rik	16.679	9.528	1.750524769
Rps27a	112.387	64.2155	1.750153779
Oxtr	4.353	2.488	1.749598071
Abhd10	10.6865	6.1095	1.749161142
Abcc8	1.762	1.008	1.748015873
Nme3	59.6325	34.1205	1.747702994
Masp1	2.127	1.2175	1.747022587
Bmp3	3.0055	1.7205	1.746875908
Fam135b	11.075	6.343	1.746019234
1700106N22Rik	36.3585	20.842	1.744482295
Pcdh8	5.1435	2.9495	1.743854891
Zfp324	4.6755	2.6825	1.742963653
Cops6	192.4275	110.4025	1.742963248
Wdr6	58.086	33.3295	1.74278042
Tomm40	32.9495	18.9365	1.739999472
Emd	56.887	32.695	1.739929653
Abhd8	81.5575	46.8875	1.739429485
Gpaa1	4.1255	2.372	1.739249578
Abhd12	69.615	40.0355	1.738831787
Atp5c1	300.521	172.8685	1.73843702
Thap4	26.284	15.1275	1.737497934
9230110C19Rik	12.915	7.4335	1.737404991
Ccl17	2.198	1.2655	1.7368629
Fxn	13.3665	7.6975	1.736472881
Fam134a	37.992	21.883	1.736142211

FIG. 18Y

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Resp18	434.7045	250.4155	1.73593288
Tubb3	242.6445	139.901	1.73440147
Rps17	860.5925	496.2165	1.734308513
Mrpl28	20.3145	11.7185	1.733540982
2810428I15Rik	83.8945	48.404	1.733214197
Pacrg	22.7135	13.111	1.732400275
Pfn1	389.6485	225.0855	1.731113288
Tmsb15b1	6.0915	3.5195	1.730785623
Dusp15	39.3715	22.752	1.730463256
Gnl3	12.0645	6.974	1.729925437
Bahd1	5.977	3.4565	1.729205844
Hdac5	57.1005	33.0255	1.72898215
2900006K08Rik	2.3125	1.3375	1.728971963
Bex4	64.257	37.166	1.728918904
Chchd4	85.191	49.285	1.728538095
Tagln3	240.0475	138.921	1.7279425
Usp39	11.297	6.5385	1.727766307
Rac3	24.206	14.01	1.727765882
AI462493	12.4925	7.2315	1.727511581
Fam122a	36.7995	21.3115	1.726743777
Fam192a	41.5855	24.0855	1.726578232
Taf11	56.2305	32.5765	1.726106242
Rab4a	69.174	40.0805	1.725876673
Vps18	8.0845	4.6895	1.723957778
Wdr18	23.7725	13.7895	1.723956634
Hras1	43.131	25.029	1.72324104
Gm3552	3.5935	2.0855	1.723087988
Fam195b	37.9715	22.037	1.723079367
Gm8526	5.257	3.051	1.723041626
Gnb2	34.805	20.2015	1.722891864
Cdkn2aipnl	35.147	20.403	1.722638828
Tbc1d7	32.491	18.8715	1.721696738
Dync1i1	28.7315	16.6905	1.721428357
Fkrp	31.117	18.089	1.720216706
Lysmd2	24.746	14.3855	1.720204372
Fam98b	36.334	21.136	1.719057532
Incenp	5.8265	3.392	1.71771816
Cacybp	119.6575	69.6795	1.717255434
Tcerg1l	20.3985	11.8855	1.716250894
Rragc	55.941	32.5985	1.716060555
Glrx	93.1945	54.3245	1.715515099
0610010K14Rik	40.8985	23.844	1.715253313
C230052I12Rik	12.6195	7.3575	1.715188583
B4galnt1	51.105	29.802	1.714817797
Opn3	2.6225	1.53	1.714052288
Rpl27a	191.9195	111.9945	1.713651117
Tmsb4x	1033.615	603.526	1.712627128

FIG. 18Z

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gm10001	5.835	3.4105	1.710892831
Fam98c	12.5685	7.3485	1.710349051
Mapk8ip2	27.108	15.853	1.70996026
Aldh1b1	2.2085	1.292	1.709365325
Rundc3a	43.0715	25.2	1.709186508
Creld2	9.3345	5.4615	1.709145839
Pmm1	71.2715	41.701	1.709107695
Ufsp1	24.066	14.094	1.707535121
Ddx54	8.129	4.7645	1.706160143
Crem	5.4135	3.1735	1.705845281
Snrpb2	36.657	21.491	1.705690754
Pop7	24.375	14.2945	1.705201301
Fxyd7	41.57	24.401	1.703618704
Armc9	9.6635	5.679	1.701620004
Smarcd3	33.401	19.648	1.699969463
Txn2	65.9775	38.8585	1.697891066
Gprasp2	143.114	84.3225	1.697221975
Smyd5	14.807	8.7255	1.696980116
Pinx1	16.5275	9.746	1.695823928
Tmem151a	19.806	11.686	1.694848537
Kcnh6	2.7465	1.6205	1.694847269
Gipc1	14.166	8.3615	1.694193626
Rps16	378.7555	223.618	1.693761236
Sf3b4	63.4455	37.473	1.693099031
Gm3386	5.1805	3.0645	1.690487845
Rpl37a	740.0525	437.778	1.690474396
H2afz	1008.4405	596.783	1.68979428
Calm2	1814.7365	1074.833	1.688389266
Apeh	6.3285	3.749	1.688050147
Tmem97	4.6325	2.7445	1.687921297
Mrpl2	24.2225	14.3505	1.687920282
Mrps7	55.1705	32.689	1.687738995
Acp6	8.5245	5.051	1.687685607
Dpcd	68.3935	40.533	1.687353514
Ap2a1	34.4755	20.433	1.687246121
Thap11	14.002	8.3045	1.686073815
Gde1	46.198	27.425	1.684521422
Pdzrn4	9.666	5.744	1.682799443
Gm88	14.071	8.3635	1.682429605
Elmod1	106.207	63.15	1.681821061
BC037034	27.178	16.161	1.681702865
Rasgrp2	12.1855	7.2465	1.681570413
Osgepl1	11.779	7.0105	1.680193995
Slc29a2	4.8755	2.902	1.680048243
1110038F14Rik	12.354	7.3535	1.680016319
Tnfrsf12a	2.815	1.676	1.679594272
Susd1	2.385	1.4215	1.677805135

FIG. 18AA

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Cab39l	11.388	6.789	1.677419355
Fnta	64.5485	38.491	1.676976436
Mpv17l2	9.766	5.824	1.676854396
Tctn2	6.295	3.757	1.675538994
Stk19	13.9755	8.3415	1.67541809
Clns1a	94.248	56.272	1.674864942
2700062C07Rik	40.6585	24.281	1.674498579
Mrps15	111.757	66.7535	1.674174388
Grm7	9.6045	5.74	1.67325784
Tmem53	6.0535	3.618	1.673161968
Pik3r2	8.2625	4.9395	1.672740156
Rcl1	12.529	7.491	1.672540382
Hmga1	152.9905	91.481	1.672374591
Haghl	35.3635	21.155	1.671637911
Mrps35	16.0915	9.635	1.670108978
Ppih	20.7925	12.4585	1.668940884
Mbip	9.412	5.64	1.668794326
Sumo2	137.1645	82.2035	1.668596836
Tmem17	4.106	2.461	1.668427469
Mtmr14	6.3875	3.832	1.666884134
Cetn3	118.5795	71.208	1.665255308
Carkd	15.4625	9.287	1.664961775
Ifnar2	15.063	9.048	1.664787798
Tmem5	13.493	8.106	1.664569455
Dbndd1	12.4555	7.484	1.664283805
Get4	29.5135	17.737	1.663951063
Cedc71	22.0115	13.229	1.66388238
Rps2	280.5415	168.76	1.662369637
Acot13	198.19	119.2775	1.661587475
Bend5	9.484	5.7095	1.661091164
Nans	6.147	3.701	1.660902459
Stambpl1	24.9065	15.0015	1.660267307
Nudc	113.072	68.106	1.660235515
Ppp1ca	125.7295	75.7405	1.660003565
Clasrp	5.096	3.07	1.659934853
2310061I04Rik	78.929	47.5495	1.659933333
Clstn3	43.0265	25.9275	1.659492817
Zkscan14	5.7415	3.462	1.658434431
Rab40c	8.2045	4.949	1.657809659
Myl6	274.0135	165.2895	1.65777923
Cars2	5.834	3.5195	1.657621821
Sap18	74.075	44.7065	1.656917898
Spag7	63.2515	38.1795	1.656687489
Bscl2	44.211	26.692	1.656338978
Cntnap5a	9.2985	5.615	1.656010686
Grin1	125.161	75.58	1.65600688
2310030N02Rik	10.213	6.168	1.65580415

FIG. 18AB

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gm5633	2.167	1.3095	1.654830088
Atp6v0b	117.699	71.152	1.654191028
Prr13	64.153	38.7875	1.653960683
Akr1a4	340.227	205.8285	1.652963511
Ccnl1	13.5935	8.2245	1.652805642
1190007I07Rik	17.065	10.3285	1.652224428
Tubg1	33.196	20.0945	1.651994327
E2f4	14.8445	8.992	1.650856317
Bcas3	10.1425	6.145	1.650528885
Atg9a	24.9275	15.106	1.650172117
Upf1	14.222	8.6185	1.650171143
Prdx2	197.703	119.816	1.650055084
Samd14	16.3175	9.8905	1.64981548
Pmvk	51.3805	31.144	1.649772027
Dnajc4	22.9645	13.926	1.649037771
Pitpnm1	13.888	8.4315	1.647156496
Gtf2a2	45.215	27.4505	1.647146682
Snurf	13.0695	7.9385	1.646343768
Bri3	26.3185	15.9925	1.645677661
Pofut1	4.4395	2.7005	1.643954823
Rag1ap1	14.097	8.5805	1.642911252
Cnot8	16.6075	10.109	1.642843011
Ebp	3.2145	1.9575	1.642145594
Tmem90b	8.3705	5.1	1.64127451
Fzr1	17.042	10.388	1.640546785
Rps25	743.597	453.373	1.640143987
1500011H22Rik	107.848	65.767	1.639849773
Armc10	15.1255	9.2365	1.63757917
Tmem198	4.282	2.615	1.637476099
Cxx1a	44.9125	27.4375	1.63690205
Med10	20.709	12.663	1.635394456
2700060E02Rik	55.3365	33.8375	1.635360177
Pigx	8.987	5.4975	1.634743065
Tssc4	14.6275	8.9515	1.634083673
Top3b	8.9215	5.461	1.633675151
Gm8894	16.1305	9.8745	1.633551066
Rfc3	9.211	5.64	1.633156028
Fastk	21.9055	13.4185	1.632485002
Trpm4	1.8585	1.1385	1.632411067
2810422O20Rik	4.121	2.527	1.630787495
Samd1	7.6675	4.7025	1.630515683
Gltpd1	11.6	7.115	1.630358398
Phf1	30.847	18.927	1.629788133
Mdh1	577.9295	354.6965	1.629363414
Wdyhv1	18.6445	11.4495	1.628411721
Zfp523	14.886	9.142	1.628308904
Ormdl1	3.9655	2.4355	1.62820776

FIG. 18AC

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Lyrm5	13.4285	8.2535	1.627006724
Cdc16	24.823	15.2625	1.626404586
Tbcb	137.4745	84.5645	1.625676259
Fam131a	19.367	11.92	1.624748322
Edf1	584.609	359.8475	1.624602088
Mcee	31.768	19.555	1.624546152
2410004B18Rik	18.6285	11.47	1.624106364
Hbxip	73.0505	44.993	1.623597004
Leng1	4.4375	2.7335	1.623376623
Pomc	3.992	2.4595	1.623094125
Gtf2f2	7.062	4.351	1.623075155
Sumo3	63.6	39.192	1.622780159
Rab24	36.963	22.792	1.621753247
2810004N23Rik	36.984	22.81	1.621394125
Mrpl27	80.435	49.6445	1.620219763
Jmjd8	21.9355	13.539	1.620171357
Eif3b	18.9725	11.7105	1.620127236
Tsg101	30.7695	18.995	1.619873651
Pck2	5.5435	3.423	1.619485831
Imp4	30.162	18.6245	1.619479718
Stub1	49.294	30.4475	1.618983496
Fam5b	11.1485	6.888	1.618539489
Nfu1	70.786	43.7435	1.618206133
Dda1	43.13	26.659	1.617840129
Lyrm7	14.0925	8.7115	1.617689261
Bok	9.368	5.7945	1.616705497
Ptprz1	16.7855	10.386	1.616165993
Hsd17b12	10.8265	6.6995	1.616016121
Mtvr2	10.8835	6.7365	1.615601574
rp9	25.317	15.677	1.614913568
Alg5	14.1715	8.7795	1.614157982
Hspa14	12.242	7.5875	1.613443163
Fam162a	46.884	29.0585	1.613434967
Kri1	14.9155	9.2505	1.61239933
Cdk2ap2	11.452	7.103	1.612276503
Rgl2	1.8185	1.128	1.61214539
Chst12	7.434	4.6115	1.612056814
2310079N02Rik	11.9215	7.3975	1.611557959
Urm1	10.2365	6.3535	1.611159204
Utp20	3.3795	2.098	1.610819828
Usp10	37.2285	23.113	1.610716913
Sirt5	9.934	6.1715	1.609657296
Meig1	2.8265	1.756	1.609624146
D10Jhu81e	13.5445	8.417	1.609183795
Tmem59	76.8445	47.7895	1.60797874
Rap1gap	33.4245	20.7925	1.607526752
Armcx6	5.8735	3.655	1.606976744

FIG. 18AD

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Cyb5r4	7.6225	4.7435	1.606935807
Wdr89	13.5545	8.4365	1.606649677
D1Bwg0212e	12.0605	7.508	1.606353223
Cul2	14.8175	9.2255	1.606146008
Lhpp	7.1255	4.4385	1.605384702
Supt4h1	138.8505	86.5575	1.604141755
Gm3362	6.217	3.876	1.603973168
Timm8a1	13.4135	8.3665	1.603239108
Phax	47.182	29.43	1.60319402
Ppm1m	24.14	15.06	1.602921647
Psma1	60.424	37.698	1.602843652
Fuca1	19.7495	12.322	1.602783639
Jakmip1	20.1385	12.5655	1.602681947
Rcan1	51.336	32.034	1.602547294
Deb1	100.7815	62.8895	1.602517113
1810043G02Rik	16.433	10.2555	1.602359709
Tle1	7.7695	4.8495	1.60212393
Pigyl	37.9675	23.7	1.602004219
Wdr74	41.277	25.7665	1.60196379
Prim2	9.5775	5.9815	1.601186993
Med21	64.2655	40.147	1.600754726
Tspan7	209.477	130.9495	1.599677738
Pdrg1	69.3845	43.3925	1.598997523
Tm2d2	35.0675	21.934	1.598773594
Caly	115.774	72.418	1.598690933
Fam20c	8.4715	5.3015	1.597943978
Mettl8	7.9465	4.973	1.597928816
Rims4	3.179	1.9895	1.597888917
Ufsp2	26.0955	16.335	1.597520661
Mettl1	5.0825	3.1825	1.597014925
Nme6	8.4555	5.295	1.596883853
Klc2	58.1185	36.3975	1.596771756
Slc39a7	19.345	12.131	1.5946748
Tbc1d25	10.4805	6.576	1.59375
Gm6988	2.854	1.7915	1.593078426
Phf5a	34.3045	21.545	1.592225574
Higd1a	74.9685	47.086	1.592161152
Zfp213	1.896	1.191	1.591939547
Tfpt	25.493	16.0145	1.591869868
Gap43	1380.721	867.6385	1.591355155
Nr2f1	7.8075	4.9065	1.591256496
Plk1s1	16.003	10.0575	1.591150882
Mthfsd	4.754	2.989	1.590498494
Dcps	12.6015	7.9235	1.590395658
Pycrl	12.325	7.75	1.590322581
Zfp622	16.302	10.251	1.590283875
Psma5	132.9445	83.602	1.590207172

FIG. 18AE

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Prpf19	125.458	78.907	1.58994766
Ttc30a1	3.9055	2.4565	1.589863627
C1galt1c1	11.44	7.2025	1.588337383
Pldn	16.8505	10.611	1.588021864
Tmem59l	114.613	72.2035	1.587360724
Tmem159	8.709	5.487	1.587206124
Sf3b3	19.7775	12.4665	1.586451691
Ftsjd1	2.328	1.468	1.585831063
Ndufb9	173.7215	109.552	1.585744669
Tmem62	4.56	2.876	1.585535466
Rnf112	24.815	15.6535	1.58526847
Nubpl	4.7005	2.966	1.584794336
Hap1	138.108	87.1515	1.584688732
Lcmt1	62.1475	39.227	1.584304178
Rgs9	12.667	7.996	1.584167084
Atp6v1g1	126.7275	80.013	1.583836377
Exosc6	6.9115	4.364	1.583753437
Hn1	50.404	31.847	1.582692247
Zfp688	4.428	2.7995	1.58171102
Tmem126b	33.7735	21.3615	1.581045339
Thap1	8.0925	5.1195	1.580720774
Hax1	73.5605	46.541	1.580552631
Gm10086	7.733	4.8945	1.579936664
Camk2n2	144.4645	91.5215	1.578476096
Mettl6	17.288	10.9545	1.578164225
Zfp414	105.7955	67.0545	1.577753917
Rassf5	18.256	11.5765	1.576987863
Gstm4	6.5455	4.152	1.576469171
Parl	25.403	16.1145	1.576406342
LOC100503185	2.0635	1.309	1.576394194
Ctps	15.9935	10.146	1.576335502
Pom121	44.834	28.442	1.576330778
Trappc2	23.267	14.764	1.575927933
Unc45a	5.02	3.1865	1.575396203
Nppc	5.111	3.2475	1.57382602
Gm5528	5.9675	3.792	1.573707806
Etfb	72.0165	45.7635	1.573666787
Hbs1l	27.77	17.6485	1.57350483
Krt1	26.9435	17.128	1.573067492
Vps4a	48.853	31.0575	1.572985591
Aes	124.989	79.4885	1.572416136
Mapkapk5	9.389	5.9745	1.57151226
Ikbkap	11.996	7.6335	1.571494072
Polr2e	44.49	28.3155	1.571224241
Glt1d1	4.428	2.8185	1.57104843
Fam100b	14.526	9.2465	1.570972801
Zfp13	5.096	3.246	1.569932224

FIG. 18AF

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gm7866	9.905	6.3155	1.56836355
Fasn	29.085	18.554	1.567586504
Tceal3	126.045	80.4095	1.567538661
Thoc3	26.94	17.19	1.567190227
Lsm2	54.9125	35.076	1.565529137
Rplp1	711.495	454.4855	1.565495489
Trim28	49.8375	31.837	1.565395609
Kdelr1	23.71	15.155	1.564500165
Paqr4	14.1775	9.0685	1.563378729
Dtnb	19.9465	12.761	1.563082831
Vwa5b2	9.249	5.9175	1.562991128
2210016L21Rik	185.798	118.896	1.562693446
Tmem14a	21.032	13.4665	1.561801507
Dyrk3	3.6835	2.3585	1.561797753
Polr1d	158.5635	101.547	1.561478921
1700020C11Rik	55.583	35.5995	1.561342154
LOC100503815	25.5285	16.3565	1.560755663
Myg1	29.539	18.9265	1.560721739
Ahsa1	97.435	62.4475	1.560270627
5730494M16Rik	31.082	19.9235	1.560067257
Snapc5	23.763	15.2345	1.559814894
Cfdp1	160.3625	102.8245	1.55957481
2310016C08Rik	4.6365	2.973	1.559535822
Dcaf12l1	17.561	11.2615	1.559383741
Dnttip1	15.4705	9.9225	1.559133283
Gm14176	8.0725	5.1785	1.558849088
Flii	30.6615	19.6915	1.557093162
Esrra	4.4145	2.836	1.556593794
Clcn6	32.0925	20.619	1.556452786
6330403K07Rik	1242.2925	799.239	1.554344195
LOC100503409	12.274	7.897	1.554261112
Rnps1	170.7985	109.9195	1.553850773
Tnfrsf19	2.068	1.331	1.553719008
Fam69b	31.0745	20.0215	1.552056539
Adcy2	13.152	8.4745	1.551949968
Pcyox1l	4.5665	2.943	1.551647978
Cyc1	194.663	125.4775	1.551377737
Tekt2	4.2075	2.7135	1.550580431
Tmbim4	30.934	19.9545	1.550226766
AW551984	39.4185	25.4465	1.549073546
Hs3st2	4.665	3.013	1.54829074
Txnrd2	4.076	2.634	1.54745634
Tceal5	136.5885	88.267	1.547446951
Nup107	6.029	3.897	1.547087503
Ssr2	35.4965	22.952	1.546553677
Ntn4	1.8505	1.197	1.545948204
Ethe1	24.178	15.6435	1.545562055

FIG. 18AG

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Gls2	5.3205	3.4425	1.545533769
Hbq1	1.662	1.0755	1.545327755
Bod1	102.7725	66.5505	1.544278405
Adam15	13.5755	8.7955	1.543459724
Pcdhgb4	1.645	1.066	1.54315197
Slc25a14	33.2345	21.5465	1.542454691
Chmp2b	26.793	17.372	1.542309464
Drg2	27.6365	17.9195	1.542258434
Eif3d	58.181	37.7255	1.542219454
Pcyt2	11.986	7.7745	1.541706862
Gm16379	2.432	1.578	1.541191382
Chrna7	7.729	5.0165	1.540715638
Spsb2	2.719	1.765	1.540509915
Magee1	72.225	46.8935	1.540192138
Csmd1	4.3055	2.796	1.539878398
Rbx1	130.8335	84.9695	1.539770153
Zbtb8os	16.3725	10.635	1.539492243
Dhx9	22.638	14.707	1.539267016
Ints7	4.5825	2.9785	1.538526104
Rpl24	725.855	471.835	1.538366166
Rnf19b	26.511	17.2345	1.538251762
Pgbd1	1.882	1.224	1.537581699
Zswim1	20.76	13.513	1.536298379
Gm15450	259.3375	168.8605	1.535809144
Sec11c	33.8935	22.0735	1.535483725
Tro	40.1175	26.1365	1.534922426
Nup62-il4i1	3.139	2.0455	1.53458812
Fam40b	2.99	1.9485	1.534513729
Pqlc1	5.1055	3.3275	1.534335086
Iqsec2	10.9075	7.109	1.53432269
Gnb5	41.459	27.023	1.534211598
Lta4h	16.667	10.864	1.534149485
Evpl	2.8025	1.827	1.533935413
Gsbs	3.327	2.1695	1.533533072
Atp5sl	22.0335	14.3705	1.53324519
Fbxo16	13.103	8.5475	1.532962855
Gm7429	2.4035	1.5685	1.532355754
Hdac6	6.993	4.569	1.530531845
6430527G18Rik	13.1795	8.6135	1.530098102
Aph1a	3.251	2.125	1.529882353
Psmb10	48.685	31.829	1.529579943
Spryd3	54.0435	35.3365	1.52939595
Zfp422-rs1	5.079	3.323	1.528438158
Usp21	20.8325	13.637	1.527645377
Tctex1d2	43.7405	28.637	1.527412089
Maged1	595.0535	389.667	1.527082098
Rpl8	329.6675	215.939	1.526669569

FIG. 18AH

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Mllt1	9.553	6.258	1.526526047
Tmem9	59.492	38.977	1.526336044
Fkbp8	105.5075	69.1305	1.52620768
Prkrip1	30.594	20.0485	1.525999451
Nkg7	4.6145	3.024	1.525958995
Vta1	37.7275	24.7315	1.525483695
Wbp5	101.8665	66.782	1.52535863
Tsen15	16.2985	10.6865	1.525148552
Slc1a6	4.422	2.9	1.524827586
1500009C09Rik	87.58	57.4425	1.52465509
Zfp358	11.7135	7.686	1.524004684
Ccdc101	19.4145	12.7415	1.523721697
Meis3	26.921	17.669	1.523628955
Rpl36a	331.877	217.8225	1.523612115
Mtch2	27.605	18.119	1.523538827
Slc45a1	17.22	11.303	1.523489339
Bad	22.773	14.9505	1.523226648
Hdac3	24.345	15.9935	1.522180886
3110002H16Rik	15.065	9.9035	1.521179381
Hspa9	60.535	39.7985	1.521037225
0610007P14Rik	56.566	37.2085	1.52024403
6720456B07Rik	127.5525	83.9625	1.519160339
Rpl18	369.0465	242.9735	1.518875515
Gm8210	14.8655	9.792	1.518127042
Slc8a2	9.983	6.576	1.518096107
AI118078	1.578	1.0395	1.518037518
Cd83	22.4685	14.8065	1.517475433
Gpr63	1.96	1.292	1.517027864
3110082D06Rik	1.9235	1.268	1.516955836
Btg1	8.688	5.73	1.516230366
Hspa11	3.582	2.3635	1.515548974
Fry	28.7485	18.972	1.515312039
Magee2	65.537	43.264	1.514816013
Chmp5	88.874	58.729	1.513289857
Paip2	170.7485	112.837	1.513231475
Mrps11	20.468	13.5275	1.513065977
Aen	18.5935	12.2895	1.512958216
Alg1	2.025	1.3385	1.512887561
Brms1	10.8445	7.1685	1.512799051
Ubb	1700.11	1123.872	1.512725648
Snrnp200	27.9005	18.4485	1.512345177
Vdac3	166.4475	110.072	1.512169307
2310003L22Rik	23.936	15.832	1.511874684
Srprb	10.9925	7.272	1.511619912
Hn1l	8.1415	5.386	1.511604159
Med9	27.2125	18.0145	1.510588692
Zdhhc8	12.861	8.5145	1.510482119

FIG. 18AI

Table 3. Summary of TRAP Data

Gene	IP	Input	IP/Input
Cdc42	108.286	71.7095	1.510064915
Mrps26	39.0695	25.8845	1.509378199
Pdss2	8.778	5.8185	1.508636246
Rsl1d1	105.1445	69.7155	1.508194017
Gm10073	96.02	63.692	1.507567669
Kcnq5	7.1485	4.742	1.507486293
Rps13	474.281	314.6465	1.507345545
Pigp	33.0295	21.9145	1.50719843
Eif4g1	39.627	26.305	1.506443642
Eid2	24.0065	15.9365	1.506384714
Aurkaip1	149.279	99.125	1.505967213
Pla2g12a	13.457	8.9455	1.504331787
Nmd3	13.2355	8.7985	1.504290504
Ppp1r8	37.091	24.658	1.504217698
Mrps14	48.5115	32.2555	1.503976066
Chmp2a	108.6455	72.2805	1.503109414
Klhl22	36.174	24.0665	1.503085201
Polh	2.094	1.3935	1.502691066
Lmna	5.9105	3.9335	1.502605822
Rasgrf1	297.604	198.1395	1.501992283
Alg14	10.2155	6.8025	1.501727306
Galk2	9.628	6.413	1.501325433
Lpcat3	5.7385	3.823	1.501046299
Nsmce1	14.0085	9.3335	1.500883913
Chic2	8.377	5.584	1.500179083

FIG. 19A

Amino acid sequence of ChR2:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAAGFSILLLMFY
AYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLRYAEWLLTCPVILIHLSNLTG
LSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIFFCLGLCYGANTFFHAAKAYIEGYHTVPKGRCRQ
VVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGD
IRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:12).

amino acid sequence of a SFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAAGF
SILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLRYAEWLL
TSPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIFFCLGLCYGANTFFH
AAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLM
SKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:13).

amino acid sequence of an SSFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAAGF
SILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLRYAEWLL
TSPVILIHLSNLTGLSNDYSRRTMGLLVSAIGTIVWGATSAMATGYVKVIFFCLGLCYGANTFFH
AAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLM
SKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID
NO:14).

amino acid sequence of C1V1:

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENN
GSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIY
VATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVLLIHLSNLTGLKDDYSKRT
MGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGICRELV
RVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHI
LLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ ID NO:15).

amino acid sequence of C1V1 (E122T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENNGS
VICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWETIYVAT
IEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVLLIHLSNLTGLKDDYSKRTMGLLV
SDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGICRELVRVMAWT
FFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHILLYGDIRK
KQKITIAGQEMEVETLVAEEED (SEQ ID NO:16).

FIG. 19B

amino acid sequence of C1V1 (E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENN
GSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIY
VATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHLSNLTGLKDDYSKRT
MGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGICRELV
RVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHI
LLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ ID NO:17).

amino acid sequence of C1V1 (E122T/E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENN
GSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWETIY
VATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHLSNLTGLKDDYSKRT
MGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGICRELV
RVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHI
LLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ ID NO:18).

Amino acid sequence of C1C2

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENNGSV
ICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIYVATI
EMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVILIHLSNLTGLANDYNKRTMGLL
VSDIGTIVWGTTAALSKGYVRVIFFLMGLCYGIYTFFNAAKVYIEAYHTVPKGRCRQVVTGMAW
LFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDI
RKTTKLNIGGTEIEVETLVEDEAEAGAV (SEQ ID NO:19)

Amino acid sequence of ReaChR

MVSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENNGSVICIPN
NGQCFCLAWLKSNGTNAEKLAANILQWVTFALSVACLGWYAYQAWRATCGWEEVYVALIEMMKSIIEAFH
EFDSPATLWLSSGNGVVWMRYGEWLLTCPVILIHLSNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCT
GWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGLCRQLVRAMAWLFFVSWGMFPVLFLLGPEGFGHI
SPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVETLVAEEEDKYESS
(SEQ ID NO:20)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/23073

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61M 21/02, A61N 5/06, A61P 25/00, C12Q 1/02, A61B 5/04 (2017.01)
CPC - A61M 21/02, A61N 5/0618, A61N 2005/0662, C12Q 1/02, A61B 5/4806
A61M 21/00, A61M 2021/0055, A61M 2021/0083, A61M 2230/18, A61B 5/4809, A61B 5/4812

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0037533 A1 (CIVELLI et al.) 28 March 2002 (28.03.2002) Abstract; para [0140]; para [0153]; Claim 1	23
Y	US 2014/0235826 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 21 August 2014 (21.08.2014) Especially Abstract; para [0011]; para [0014]; para [0044]; para [0121]	1-3
Y	SHERIN et al., Innervation of Histaminergic Tuberomammillary Neurons by GABAergic and Galaninergic Neurons in the Ventrolateral Preoptic Nucleus of the Rat. The Journal of Neuroscience. 15 June 1998, Vol 18, No 12, pp 4705-4721. Especially Abstract; p4705, Col 1, last para - Col 2, first para	1-3
Y	WU et al., Galanin neurons in the medial preoptic area govern parental behavior. Nature. 15 May 2014, Vol 509, No 7500, pp 325-330. Especially Abstract	3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 June 2017	06 JUL 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/23073

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-22, 24-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.