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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 May 2019 (31.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/104307 A1

(51) International Patent Classification:
A61K 38/00 (2006.01) *A61K 38/17* (2006.01)
A61K 38/16 (2006.01)

(21) International Application Number:
PCT/US2018/062575

(22) International Filing Date:
27 November 2018 (27.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/590,911 27 November 2017 (27.11.2017) US
62/659,911 19 April 2018 (19.04.2018) US

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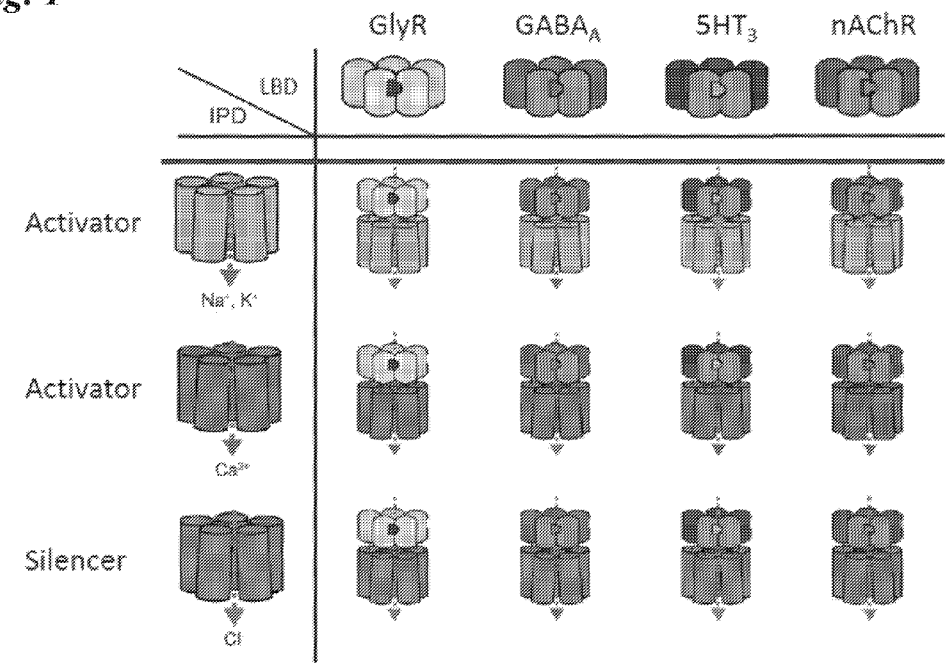
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, 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,

(54) Title: COMPOSITIONS AND METHODS FOR NEUROLOGICAL DISEASES

Fig. 1



(57) **Abstract:** Compositions and methods are provided for modulating the activity of cells using engineered receptors, polynucleotide encoded engineered receptors, and gene therapy vectors comprising polynucleotides encoding engineered receptors. These compositions and methods find particular use in modulating the activity of neurons, for example in the treatment of disease or in the study of neuronal circuits.

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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))*
— *with sequence listing part of description (Rule 5.2(a))*

COMPOSITIONS AND METHODS FOR NEUROLOGICAL DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. § 119 (e), this application claims priority to the filing date of the United States Provisional Patent Application Serial No. 62/590,911 filed November 27, 2017 and of the United States Provisional Patent Application Serial No. 62/659,911, filed April 19, 2018; the full disclosures of which are herein incorporated by reference.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The sequence listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the sequence listing is SWCH_012_04WO_ST25.txt. The text file is 178 kb, was created on November 27, 2018, and is being submitted electronically via EFS-Web.

FIELD

[0003] This disclosure pertains to engineered receptors and the use of engineered receptors and small molecule ligands to modulate the activity of cells and treat disease.

BACKGROUND

[0004] Intractable neurological disease is often associated with aberrantly acting neurons. Attempts to develop therapies to treat these conditions have been hampered by a lack of tractable target proteins associated with the disease. For example, unrelieved chronic pain is a critical health problem in the US and worldwide. A report by the Institute of Medicine estimated that 116 million Americans suffer from pain that persists for weeks to years, with resulting annual costs exceeding $560 million. There are no adequate long-term therapies for chronic pain sufferers, leading to significant cost for both society and the individual. Pain often results in disability and, even when not disabling, it has a profound effect on the quality of life. Pain treatment frequently fails even when the circumstances of care delivery are optimal, such as attentive, well-trained physicians; ready access to opioids; use of adjuvant analgesics; availability of patient-controlled analgesia; and evidence-based use of procedures like nerve blocks and IT pumps.

[0005] The most commonly used therapy for chronic pain is the application of opioid analgesics and nonsteroidal anti-inflammatory drugs, but these drugs can lead to addiction and

may cause side effects, such as drug dependence, tolerance, respiratory depression, sedation, cognitive failure, hallucinations, and other systemic side effects. Despite the wide usage of pharmaceuticals, there is a strikingly low success rate for its effectiveness in pain relief. A large randomized study with various medications found only one out of every two or three patients achieving at least 50% pain relief (Finnerup *et al.*, 2005). A follow-up study using the most developed pharmacological treatments found the same results, indicating that there was no improvement in the efficacy of medications for pain (Finnerup *et al.*, *Pain*, 150(3):573-81, 2010).

[0006] More invasive options for the treatment of pain include nerve blocks and electrical stimulation. A nerve block is a local anesthetic injection usually in the spinal cord to interrupt pain signals to the brain, the effect of which only lasts from weeks to months. Nerve blocks are not the recommended treatment option in most cases (Mailis and Taenzer, *Pain Res Manag.* 17(3):150–158, 2012). Electrical stimulation involves providing electric currents to block pain signals. Although the effect may last longer than a nerve block, complications arise with the electrical leads itself: dislocation, infection, breakage, or the battery dying. One review found that 40% of patients treated with electrical stimulation for neuropathy experienced one or more of these issues with the device (Wolter, 2014).

[0007] The most invasive, and least preferred, method for managing pain is complete surgical removal of the nerve or section thereof that is causing the pain. This option is only recommended when the patient has exhausted the former and other less invasive, treatments and found them ineffective. Radiofrequency nerve ablation uses heat to destroy problematic nerves and provides a longer pain relief than a nerve block. However, one study found no difference between the control and treatment groups in partial radiofrequency lesioning of the DRG for chronic lumbosacral radicular pain (Geurts *et al.*, 2003). Other surgical methods for surgically removing the pain nerves suffer from similar shortcomings and have serious side effects long-term, including sensory or motor deficits, or cause pain elsewhere.

[0008] Methods for treating neurological disorders should be safe, efficient and cost-effective. Gene therapy could provide non-invasive treatment options for a variety of neurological diseases, including managing pain. However, to date, gene therapy methods have not found widespread use in the treatment of neurological diseases. The present disclosure addresses these needs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The disclosure is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0010] Fig. 1 shows schematics of exemplary engineered chimeric LGIC receptors.

[0011] Fig. 2 shows a schematic of a protocol for development and characterization of engineered LGIC receptors and mutants thereof of the present disclosure.

[0012] Fig. 3 shows examples of the dose-response curves of several functional CHRNA7/GLRA1 chimeric receptors to their native ligand, acetylcholine. These receptors show an inward current in response to varying doses of the ligand acetylcholine when applied for 1 second. Voltage clamp recordings were performed in HEK293T cells transiently expressing the channel and correspond to conductance currents summed from up to 20 cells patched simultaneously using the automated patch clamp system IonFlux (Fluxion Biosciences) in Ensemble mode.

[0013] Fig. 4A- Fig. 4F show the ligand-dependent response profiles of wild type alpha-7 nAChR and various engineered chimeric LGIC receptors to acetylcholine as well as to non-native ligands TC-6987, AZD-0328, and Facinicline/RG3487. Receptors were transiently expressed in HEK293T cells and currents were measured on an automated patch clamp system (Fluxion Biosciences) following 1 second addition of drug. Fig. 4A shows results with wild type alpha-7 nAChR. Fig. 4B shows results with SEQ ID NO:16. Fig. 4C shows results with SEQ ID NO:35. Fig. 4D shows results with SEQ ID NO:33. Fig. 4E shows results with SEQ ID NO:41. Fig. 4F shows results with SEQ ID NO:37. Solid boxes: protein sequence from the human wild type α7-nAChR. Striped boxes: protein sequence from the human alpha1 glycine receptor. Doses are shown above each recording, scale bars = 1s. Currents in Fig. 4A were measured in single cell mode, where traces represent conductance from 1 cell per recording. Currents in Fig. 4B – Fig. 4F were measured in ensemble mode, where traces represent summation of conductances from up to

20 cells sampled per recording, with recordings that passed QC in grey and the composite median trace shown in black.

[0014] Fig. 5A – Fig. 5F show the ligand-dependent response profiles of wild type alpha-7 nAChR and various engineered chimeric LGIC receptors expressed in *Xenopus* oocytes to acetylcholine as well as to non-native ligands ABT-126, TC-6987, AZD-0328, and Facinicline/RG3487. Fig. 5A – Fig. 5B shows the response profile of wild type α7-nAChR to acetylcholine, nicotine, and ABT-126 (Fig. 5A) and TC-6987, AZD-0328, and Facinicline/RG3487 (Fig. 5B). Fig. 5C – Fig. 5D shows the response profile of SEQ ID NO:33 to acetylcholine, nicotine, and ABT-126 (Fig. 5C) and TC-6987, AZD-0328, and Facinicline/RG3487 (Fig. 5D). Fig. 5E – Fig. 5F shows the response profile of SEQ ID NO:41 to acetylcholine, nicotine, and ABT-126 (Fig. 5E) and TC-6987, AZD-0328, and Facinicline/RG3487 (Fig. 5F). Currents were measured on a manual patch clamp system following 5-10s applications of increasing doses of compound as listed across the top of each panel. *Left panels*, Exemplary currents in an oocyte expressing the designated receptor. EC_{50}s for each ligand as estimated from concentration-response curves shown on the right are marked with an asterisk, while shifts in EC_{50} relative to wild type α7-nAChR are marked by black arrows. *Right panels*, Concentration-response curves from the data shown on the left. Currents were normalized to unity for the maximal response. The continuous line through the data points is the best fit obtained with the Hill equation.

[0015] Fig. 6 depicts the responses of four different engineered chimeric LGIC receptors (SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:41) to increasing concentrations of acetylcholine as measure by the quench in YFP fluorescence over time. These quench plots demonstrate by a second approach that different parental chimeras have different EC_{50}s and activation kinetics to acetylcholine. The time point of acetylcholine stimulation is indicated by the arrow.

[0016] Fig. 7A – Fig. 7B provide quench plots of two engineered chimeric LGIC receptors to either acetylcholine (ACH) or AZD-0328: SEQ ID NO:33 (Fig. 7A) and SEQ ID NO:41 (Fig. 7B). The parental chimeras respond differently to similar concentrations of different ligands. Chimera #41 responds to lower doses of either ACH or AZD-0328 than Chimera #33, indicating that the inclusion of the beta1-2 loop conveys unique binding and activation properties upon the chimeric receptors.

[0017] Fig. 8A – Fig. 8I provides heat maps of the percent quench of YFP fluorescence in mutants of engineered chimera SEQ ID NO:33 that comprise single amino acid substitutions following stimulation by various doses of either acetylcholine or AZD-0328. Fig.8A – Fig. 8F show mutants that exhibit a rightward shift in responses (decreased sensitivity) to both acetylcholine and AZD-0328, demonstrating an overlap in the amino acids critical for binding and activation by both compounds. Fig. 8G shows mutants that exhibit a leftward shift in response to acetylcholine but a rightward shift in response to AZD-0328, demonstrating the ability of a single mutation to decouple responses to ligand. Fig. 8H shows mutants that exhibit a far rightward shift to acetylcholine as compared to the parental chimera but no significant change in response to AZD-0328, demonstrating the ability of a single mutation to significantly reduce activation by acetylcholine without affecting activation by other ligands. Fig. 8I shows mutants that exhibit a strong leftward shift of response (increased sensitivity) to both acetylcholine and AZD-0328. Ligand doses are written across the top of each chart (n=2 for each dose). Numbers in the boxes indicate the absolute amount of quench observed. Dark blue = 80% maximal quench of YFP reporter. Light blue = quench of 30-80%. White = 10-30%. Orange = 0-10% quench. Negative values represent non-responders that have a negative quench due to a stimulation artifact. SEQ ID NO:29 is a non-responding chimera used as a negative control.

[0018] Fig. 9A – Fig. 9B provide dose response curves of select mutants of engineered chimera SEQ ID NO:33 to acetylcholine and AZD-0328. Fig. 9A show responses of mutants comprising substitutions L131S (open square), L131T (open up triangle), and S172D (open down triangle) and demonstrate a far rightward shift (i.e., 2 log (100 fold) increase in EC_{50}) in the dose response curve for acetylcholine compared to that of unmutated SEQ ID NO:33 (open circles). In contrast, a W77M substitution (open diamond) exhibits no shift in the dose response to acetylcholine. Fig. 9B show responses of mutants comprising substitutions at L131S (open square), L131T (open up triangle), and S172D (open down triangle), and result in a slight rightward shift (i.e., 1 log increase in EC_{50}) in dose response to AZD-0328 as compared to the unmutated SEQ ID NO:33 chimera, while a W77M substitution produces a dramatic rightward shift (> 2 log increase in EC_{50}) in the dose response.

[0019] Fig. 10A – Fig. 10D provide electrophysiology data illustrating how single amino acid substitutions in an engineered chimera (SEQ ID NO:33) confer preferential responses to non-native ligands over acetylcholine. Fig. 10A shows substitution of L131S causes a significant right-

ward shift (80-fold) in the EC_{50} of acetylcholine relative to parental chimera SEQ ID NO:33 (see Fig. 4E), while the EC_{50} for AZD-0328 is only (2-fold) right-ward shifted. Fig. 10B shows substitution of S172D causes a significant rightward shift (58-fold) in the EC_{50} of acetylcholine relative to the parental chimera, while the EC_{50} for AZD-0328 on S172D is only (2-fold) right-ward shifted. Fig. 10C shows substitution of W77M causes a moderate leftward shift (2-fold) in the EC50 of acetylcholine relative to the parental chimera, while the EC_{50} for AZD-0328 on W77M is significantly rightward shifted (>2.5 log). Fig. 10D shows substitution of L131T causes a significant rightward shift (~2.5 log) in the EC50 of acetylcholine relative to the parental chimera, while the EC_{50} for AZD-0328 on this mutant shows a moderate rightward shift (~1 log). Asterisks mark estimated EC_{50} for each ligand on L131S. Arrow shows degree of shift in EC_{50} relative to SEQ ID NO:33. Triangles mark estimated EC_{50} for each ligand on the wild type α7-nAChR.

BRIEF SUMMARY

[0020] Compositions and methods are provided for modulating the activity of cells using engineered receptors, polynucleotide encoded engineered receptors, and gene therapy vectors comprising polynucleotides encoding engineered receptors. These compositions and methods find particular use in modulating the activity of neurons, for example in the treatment of disease or in the study of neuronal circuits.

[0021] In some aspects of the present disclosure, the disclosure is directed to an engineered receptor, wherein the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor and comprises a ligand binding domain derived from a first wild type Cys-loop LGIC receptor, a Cys-loop domain from a second wild-type Cys-loop LGIC receptor; and an ion pore domain derived from the second wild type Cys-loop LGIC receptor. In some embodiments, the first and the second wild type Cys-loop LGIC receptor are the same. In other embodiments, the first and the second wild type Cys-loop LGIC receptor are different. In some embodiments, the first wild type Cys-loop LGIC receptor is selected from an acetylcholine receptor (AchR), *e.g.* α7-nAChR; a Glycine receptor, (GlyR), *e.g.* GlyRα1; a λ-aminobutyric acid (GABA) receptor; and a serotonin receptor, *e.g.* 5HT3. In some embodiments, the second wild type Cys-loop LGIC receptor is selected from an acetylcholine receptor (AchR), *e.g.* α7-nAChR; a Glycine receptor, (GlyR), *e.g.* GlyRα1; a λ-aminobutyric acid (GABA) receptor; and a serotonin receptor, *e.g.* 5HT3. In certain embodiments, the first wild type Cys-loop LGIC receptor is α7-nAChR. In certain embodiments,

the second wild type Cys-loop LGIC receptor is GlyRα1. In some such embodiments, the Cys-loop domain comprises amino acids 166-180 of GlyRα1. In other such embodiments, the Cys-loop domain comprises amino acids 166-172 of GlyRα1. In some such embodiments, the engineered receptor has a sequence identity of 85% or more to SEQ ID NO:33. In some embodiments, the receptor further comprises a β1-2 loop domain from the second wild type Cys-loop LGIC receptor. In some such embodiments, the β1-2 loop domain comprises amino acids 81-84 of GlyRα1. In some such embodiments, the engineered receptor has a sequence identity of 85% or more to SEQ ID NO:41.

[0022] In some embodiments, the ligand binding domain comprises one or more amino acid substitutions at a residue corresponding to a residue of α7-nAChR selected from the group consisting of W77, Y94, R101, W108, Y115, T128, N129, V130, L131, Q139, L141, Y151, S170, W171, S172, S188, Y190, Y210, C212, C213 and Y217. In some embodiments, the one or more amino acid substitutions is a loss of function mutation that decreases the potency of acetylcholine on the receptor while substantially maintaining the potency of a non-native ligand on the receptor. In certain such embodiments, the substitution is selected from a substitution corresponding to L131S, L131T, L131D, or S172D of α7-nAChR. In certain such embodiments, the non-native ligand is selected from AZD-0328, TC6987, ABT-126 and Facinicline/RG3487. In other embodiments, the one or more amino acid substitutions is a gain of function mutation that increases the potency of acetylcholine on the receptor. In certain such embodiments, the substitution is selected from a substitution corresponding to L131N, L141W, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S172C, S188I, S188V, S188F, S188M, S188Q, S188T, S188P, or S188W of α7-nAChR.

[0023] In some aspects of the present disclosure, the disclosure is directed to a polynucleotide encoding an engineered receptor as described herein. In some aspects of the disclosure, the present disclosure is directed to a vector comprising a polynucleotide encoding an engineered receptor as described herein. In some aspects of the disclosure, the present disclosure is directed to a pharmaceutical composition comprising a polynucleotide encoding an engineered receptor as described herein or a vector comprising the polynucleotide encoding an engineered receptor as described herein and a pharmaceutically acceptable vehicle.

[0024] In some aspects of the present disclosure, the disclosure is directed to an engineered receptor, wherein the engineered receptor is a ligand gated ion channel (LGIC) receptor and

comprises (a) a ligand binding domain derived from a first wild type LGIC receptor, wherein the ligand binding domain comprises at least one amino acid mutation that changes the function of the ligand binding domain relative to the wild type receptor; and (b) an ion pore domain derived from a second wild type LGIC receptor. In some embodiments, at least one amino acid mutation is a loss of function mutation. In some embodiments, the at least one amino acid mutation is a gain of function mutation. In some embodiments, the at least one amino acid mutation is both a loss of function and a gain of function mutation, i.e. the mutation that provides for the loss of function and gain of function are the same mutation. In other embodiments, the at least one loss-of-function amino acid mutation and the at least one gain-of-function amino acid mutation are mutations at different amino acid residues of the ligand binding domain. In some embodiments, the first and the second wild type LGIC receptor are the same. In some embodiments, the first and the second wild type LGIC receptor are different. In some embodiments, the first and/or second LGIC receptor is a Cys-loop receptor LGIC. In some such embodiments, the first and/or second wild type LGIC receptor is selected from the group consisting of a glycine receptor (GlyR), a λ-aminobutyric acid (GABA) receptor, a serotonin receptor, and an acetylcholine receptor (AchR). In some embodiments, the first and/or second LGIC receptor is selected from an ATP-gated cation channel receptor, an inwardly rectifying potassium channel receptor (Kir), a potassium voltage-gated channel receptor, and an ATP-binding cassette transporter receptor.

[0025] In some embodiments, the first and/or second LGIC receptor is a $GABA_A$ receptor. In some embodiments, the first and/or second LGIC receptor is a 5 hydroxytryptamine ($5\text{-}HT_3$) receptor. In some embodiments, the first and/or second LGIC receptor is a nicotinic AchR (nAchR). In some embodiments, the first and/or second LGIC receptor is a P2X receptor. In some embodiments, the first and/or second LGIC receptor is a KCNQ or K_V7 receptor. In some embodiments, the first and/or second LGIC receptor is a cystic fibrosis transmembrane conductance regulator (CFTR) receptor.

[0026] In some embodiments, the engineered receptors of the present disclosure are homomeric receptors. In some embodiments, the engineered receptors are heteromeric receptors.

[0027] In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is less than 99%, 98%, 97%, 95%, or less than 90% homologous to a native, or wild type, ligand binding domain. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain sharing a sequence identity of 85% or

more to a wild type ligand binding domain. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain sharing a sequence identity of 90% or more to a wild type, ligand binding domain. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain sharing a sequence identity of 95% or more to a wild type, ligand binding domain. In some embodiments, the engineered receptors of the present disclosure comprise one or more amino acid mutations introduced into the ligand binding domain. In some embodiments, the one or more amino acid mutations are introduced into the ligand binding domain by an error prone polymerase chain reaction (PCR). In some embodiments, the one or more amino acid mutations are introduced into the ligand binding domain by site directed mutagenesis. In some embodiments, the one or more amino acid mutations are introduced into the ligand binding domain by de novo gene synthesis.

[0028] In some embodiments, the engineered receptors of the present disclosure comprise at least one loss-of-function amino acid mutation that inhibits the binding of an endogenous ligand to the engineered receptor or decreases the binding affinity of the endogenous ligand for the engineered receptor. In some embodiments, the engineered receptors of the present disclosure comprise at least one gain-of-function amino acid mutation that increases the binding affinity of a binding agent for the engineered receptor or modulates an effect of the binding agent on the engineered receptor.

[0029] In some embodiments, the at least one gain-of function amino acid mutations increases the affinity of the engineered receptor for an agonist ligand compared to the affinity of the wild type receptor for the agonist ligand. In some embodiments, the agonist ligand is selected from the group consisting of ABT-089, ABT-126, ABT-418, ABT-894, Alfadolone, AQW-051, AZD0328, AZD7325, Basmisanil, Bilobalide, Cannabidiol, Cilansetron, CP-409092, DDP733, EVP-6124, GTS-21, Iptakalim, MK-0777/L83098, MDL-27531, MEM-3454/RG3487, MEM-63908, Moxidectin, NRX-1050, NRX-1060, P-9939, PH-399733, QH-ii-066, Rapastinel, Renzapride, Retigabine, S-18841, SL-75102, SL-651498, SR-57227, SSR-180711, and TC-5619/AT-101. In certain embodiments, the ligand is selected from the group consisting of AZD0328, ABT-126, AQW-051, Cannabidiol, Cilansetron, PH-399733, FACINICLINE/RG3487/MEM-3454, and TC-5619/AT-101.

[0030] In some embodiments, the at least one gain-of-function mutations results in an antagonist ligand functioning as an agonist ligand. In some embodiments, the antagonist ligand is

selected from the group consisting of 468816, ACEA-2085, DA-9701, Eslicarbazepine acetate, Gavestinel, GV-196771, GW-468816, HMR-2371, L-695902, L-701324, MDL-100748, MDL-102288, MDL-105519, PD-165650, Tedatioxetine, UK-315716, and ZD-9379.

[0031] In some embodiments, the at least one gain of function amino acid mutations results in a modulator ligand functioning as an agonist ligand. In some embodiments, the modulator ligand is selected from the group consisting of AQW-051, AZD7325, CTP-656, Gavestinel, and Ivermectin.

[0032] In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain derived from a GlyR. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain derived from a GABA receptor. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain is derived from an nAChR. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is derived from a $5HT_3$. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is derived from a P2X receptor. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is derived from a Kir receptor. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is derived from a KCNQ receptor. In some embodiments, the engineered receptors of the present disclosure comprise a ligand binding domain that is derived from a CFTR receptor.

[0033] In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a GlyR. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a GABA receptor. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a nAChR. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a $5HT_3$ receptor. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a P2X receptor. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a Kir receptor. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a KCNQ receptor. In some embodiments, the engineered receptors of the present disclosure comprise an ion pore domain derived from a

CFTR receptor. In some embodiments, the ion pore domain comprises a non-desensitizing mutation.

[0034] In some embodiments, the present disclosure comprises a polynucleotide encoding an engineered receptor described herein. In some embodiments, the present disclosure comprises a viral vector comprising a polynucleotide encoding an engineered receptor described herein. In some embodiments, the viral vector is an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, or a herpes simplex-1 viral vector (HSV-1). In some embodiments, the AAV vectors is selected from the group consisting of AAV1, AAV2, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAVrh10. In some embodiments, the AAV vector is selected from AAV5, AAV6, and AAV9. In some embodiments, the vector is derived from a vector selected from the group consisting of AAV1, AAV2, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAVrh10. In some embodiments, the AAV vector is derived from AAV-5, AAV-6 or AAV-9.

[0035] In some aspects of the present disclosure, a method is provided of treating a neurological disorder in a subject in need thereof comprising administering to said subject a polynucleotide encoding an engineered ligand-gated ion channel (LGIC) receptor, and administering to said subject a binding agent, wherein said binding agent acts as an agonist of the engineered LGIC receptor. In some embodiments, the polynucleotide delivered to the subject by a non-viral method. In some embodiments, the non-viral method is lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection. In some embodiments, the polynucleotide is delivered to the subject in a viral vector. In some embodiments, the viral vector is an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, or a herpes simplex-1 viral vector (HSV-1). In some embodiments, the AAV vectors is selected from the group consisting of AAV1, AAV2, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10. In some embodiments, the AAV vector is selected from AAV5, AAV6, and AAV9. In some embodiments, the vector is derived from a vector selected from the group consisting of AAV1, AAV2, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAVrh10. In some embodiments, the AAV vector is derived from AAV-5, AAV-6 or AAV-9.

[0036] In some embodiments, the method comprises expressing the engineered receptor in an excitable cell. In some embodiments, the excitable cell is a neuron or a myocyte. In some

embodiments, the neuron is a dorsal root ganglion, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

[0037] In some embodiments, the subject suffers from pain. In some embodiments, the subject suffers from a seizure disorder. In some embodiments, the subject suffers from a movement disorder. In some embodiments, the subject suffers from an eating disorder. In some embodiments, the subject suffers from a spinal cord injury. In some embodiments, the subject suffers from neurogenic bladder. In some embodiments, the subject suffers from a spasticity disorder. In some embodiments, the subject suffers from pruritus. In some embodiments, the subject suffers from Alzheimer's disease, Parkinson's disease, post-traumatic stress disorder (PTSD), gastroesophageal reflux disease (GERD), addiction, anxiety, depression, memory loss, dementia, sleep apnea, stroke, narcolepsy, urinary incontinence, essential tremor, movement disorder, or atrial fibrillation. In some embodiments, the method further comprises measuring the symptoms of the diseases after administration of the binding agent, wherein the symptoms of the diseases are reduced. In some embodiments the subject is a human.

[0038] In some embodiments, the binding agent is selected from the group consisting of 468816, ABT-089, ABT-126, ABT-418, ABT-894, ACEA-2085, Alfadolone, AQW-051, AZD0328, AZD7325, Basmisanil, Bilobalide, Cannabidiol, Cilansetron, CP-409092, CTP-656, DA-9701, DDP733, Eslicarbazepine Acetate, EVP-6124, Gavestinel, Gavestinel, GTS-21, GV-196771, GW-468816, HMR-2371, Iptakalim, L-695902, L-701324, MK-0777/L83098, MDL-100748, MDL-102288, MDL-105519, MDL-27531, MEM-3454/RG3487, MEM-63908, Moxidectin, NRX-1050, NRX-1060, P-9939, PD-165650, PH-399733, QH-ii-066, Rapastinel, Retigabine, S-18841, SL-75102, SL-651,498, SR-57227, SSR-180,711, TC-5619/AT-101, Tedatioxetine, UK-315716, and ZD-9379. In certain embodiments, the ligand is selected from the group consisting of AZD0328, ABT-126, AQW-051, Cannabidiol, Cilansetron, PH-399733, FACINICLINE/RG3487/MEM-3454, and TC-5619/AT-101. In some embodiments, the polynucleotide and/or the binding agent are administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intracranially, intraspinally, cisterna magna, or intraneurally. In some embodiments of this method, the binding agent is administered at least one week after the polynucleotide.

[0039] In some embodiments, the present disclosure comprises a composition comprising an engineered receptor described herein, a polynucleotide described herein, or a viral vector described herein.

DETAILED DESCRIPTION

A. Overview

[0040] Compositions and methods are provided for modulating the activity of cells using engineered ligand gated ion channel (LGIC) receptors, polynucleotide encoded engineered LGIC receptors, and gene therapy vectors comprising polynucleotides encoding engineered LGIC receptors. These compositions and methods find particular use in modulating the activity of neurons, for example in the treatment of disease or in the study of neuronal circuits. In addition, reagents, devices and kits thereof that find use in practicing the subject methods are provided.

[0041] In particular, the present disclosure provides engineered receptors that bind to and signal in response to known drugs, ligands, and/or binding agents. In some embodiments, the engineered receptors described herein demonstrate increased affinity for a known agonist binding agent. In some embodiments, the engineered receptors described herein demonstrate an affinity for an antagonist or modulator binding agent and respond to the antagonist and/or modulator agents as if they were agonist agents. The present disclosure further provides for methods of treating neurological diseases in subjects in need thereof. The present disclosure increases the number of clinical indications that a known drug may be used for by utilizing engineered receptors that respond to a known drug in a manner that is distinct from the wild-type endogenous receptor.

[0042] Before the present methods and compositions are described, it is to be understood that this disclosure is not limited to particular method or composition 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 disclosure will be limited only by the appended claims.

[0043] 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 limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller

ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the disclosure, 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 disclosure.

[0044] 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 disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, some potential and 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. It is understood that the present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

[0045] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0046] 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 disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

B. Definitions

[0047] As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise. Thus, for example, reference to “a cell” includes a plurality of such cells and reference to “the peptide” includes reference to one or more peptides and equivalents thereof, *e.g.* polypeptides, known to those skilled in the art, and so forth.

[0048] As used in this specification, the term "and/or" is used in this disclosure to either "and" or "or" unless indicated otherwise.

[0049] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" refers to the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers. Further, the statement of numerical ranges throughout this specification specifically includes all integers and decimal points in between.

[0050] Throughout this specification, unless the context requires otherwise, the phrase "consisting essentially of" refers to a limitation of the scope of composition, method, or kit described to the specified materials or steps that do not materially affect the basic and novel characteristic(s) of the subject disclosure. For example, a ligand binding domain "consisting essentially of" a disclosed sequence has the amino acid sequence of the disclosed sequence plus or minus about 5 amino acid residues at the boundaries of the sequence, *e.g.* about 5 residues, 4 residues, 3 residues, 2 residues or about 1 residue less than the recited bounding amino acid residue, or about 1 residue, 2 residues, 3 residues, 4 residues, or 5 residues more than the recited bounding amino acid residue.

[0051] Throughout this specification, unless the context requires otherwise, the phrase "consisting of" refers to the exclusion from the composition, method, or kit of any element, step, or ingredient not specified in the claim. For example, a ligand binding domain "consisting of" a disclosed sequence consists only of the disclosed amino acid sequence.

[0052] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0053] As used herein, the term "isolated" means material that is substantially or essentially free from components that normally accompany is as found in its native state. In some embodiments, the terms "obtained" or "derived" are used synonymously with isolated.

[0054] The terms "subject," "patient," and "individual" are used interchangeably herein to refer to any animal that exhibits pain that can be treated with the vectors, compositions, and methods contemplated herein. In some embodiments, a subject may refer to a vertebrate, preferably a mammal. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Suitable subjects (*e.g.,* patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included. In some embodiments, a "sample" may be taken from a subject.

[0055] The term "sample" refers to a volume and/or mass of biological material that is subjected to analysis. In some embodiments, a sample comprises a tissue sample, cell sample, a fluid sample, and the like. In some embodiments, a sample is taken from or provided by a subject (*e.g.*, a human subject). In some embodiments, a sample comprises a portion of tissue taken from any internal organ, a cancerous, pre-cancerous, or non-cancerous tumor, brain, skin, hair (including roots), eye, muscle, bone marrow, cartilage, white adipose tissue, and/or brown adipose tissue. In some embodiments, a fluid sample comprises buccal swabs, blood, cord blood, saliva, semen, urine, ascites fluid, pleural fluid, spinal fluid, pulmonary lavage, tears, sweat, and the like. Those of ordinary skill in the art will appreciate that, in some embodiments, a "sample" is a "primary sample" in that it is obtained directly from a source (*e.g.*, a subject). In some embodiments, a "sample" is the result of processing of a primary sample, for example to remove certain potentially contaminating components, to isolate certain components, and/or to purify certain components of interest. In some embodiments, a sample is a cell or population of cells (*e.g.*, a neuronal cell). A cell sample may be derived directly from a subject (*e.g.*, a primary sample) or may be a cell line. Cell lines may include non-mammalian cells (*e.g.*, insect cells, yeast cells, and/or bacterial cells) or mammalian cells (*e.g.*, immortalized cell lines).

[0056] "Treating" or "treatment" as used herein refers to delivering a composition (*e.g.*, an engineered receptor and/or a binding agent) to a subject and/or population of cells to affect a physiologic outcome. In particular embodiments, treatment results in an improvement (*e.g.*, reduction, amelioration, or remediation) of one or more disease symptoms. The improvement may be an observable or measurable improvement, or may be an improvement in the general feeling of well-being of the subject. Treatment of a disease can refer to a reduction in the severity of disease symptoms. In some embodiments, treatment can refer to a reduction in the severity of disease

symptoms to levels comparable to those prior to disease onset. In some embodiments, treatment may refer to a short-term (*e.g.*, temporary or acute) and/or a long-term (*e.g.*, sustained or chronic) reduction in disease symptoms. In some embodiments, treatment may refer to a remission of disease symptoms. In some embodiments, treatment may refer to the prophylactic treatment of a subject at risk of developing a particular disease in order to prevent disease development. Prevention of disease development can refer to complete prevention of the disease symptoms, a delay in disease onset, a lessening of the severity of the symptoms in a subsequently developed disease, or reducing the likelihood of disease development.

[0057] As used herein, "management" or "controlling" refers to the use of the compositions or methods contemplated herein, to improve the quality of life for an individual suffering from a particular disease. In certain embodiments, the compositions and methods described herein provide analgesia to a subject suffering from pain.

[0058] A "therapeutically effective amount" is an amount of a composition required to achieve a desired therapeutic outcome. The therapeutically effective amount may vary according to factors such as, but not limited to, disease state and age, sex, and weight of the subject. Generally, a therapeutically effective amount is also one in which any toxic or detrimental effects of a composition are outweighed by the therapeutically beneficial effects. A "therapeutically effective amount" includes an amount of a composition that is effective to treat a subject. A "prophylactically effective amount" refers to an amount of a composition required to achieve a desired prophylactic result. In some embodiments, the prophylactically effective amount may be less than the therapeutically effective amount, as a prophylactic dose is used in subjects prior to or at an earlier stage of disease.

[0059] An "increase" refers to an increase in a value (*e.g.*, increased binding affinity, increased physiologic response, increased therapeutic effect, etc.) of at least 5% as compared to a reference or control level. For example, an increase may include a 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000% or more increase. Increase also means an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) higher than a reference or control level.

[0060] A "decrease" refers to a decrease in a value (*e.g.*, decreased binding affinity, decreased physiologic response, decreased therapeutic effect, etc.) of at least 5% as compared to a reference or control level. For example, a decrease may include a 5, 6, 7, 8, 9, 10, 15, 20, 25, 30,

35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000% or more decrease. Decrease also means a decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) lower than a reference or control level.

[0061] By "maintain," or "preserve," or "maintenance," or "no change," or "no substantial change," or "no substantial decrease" refers generally to a physiologic and/or therapeutic effect that is comparable to an effect caused by either vehicle, or a control molecule/composition. A comparable response is one that is not significantly different or measurable different from the reference response

[0062] The terms "reference" or "control" level are used interchangeably herein and refer the value of a particular physiologic and/or therapeutic effect in a subject or sample that has not been treated with a composition described herein, or a subject or sample that has been treated with a vehicle control. In some embodiments, a reference level refers to a value of a particular physiologic and/or therapeutic effect that is measure in a subject or sample prior to the administration of a composition described herein (*e.g.*, a baseline level).

[0063] "Receptor-ligand binding" and "ligand binding" are used interchangeably herein and refer to the physical interaction between a receptor (*e.g.*, a LGIC) and a ligand. The term "ligand" as used herein may refer to an endogenous or naturally occurring ligand. For example, in some embodiments, a ligand refers to a neurotransmitter (*e.g.*, λ-aminobutyric acid (GABA), acetylcholine, serotonin, and others) and signaling intermediate (*e.g.*, phosphatidylinositol 4,5-bisphosphate (PIP_2)), amino acids (*e.g.*, glycine), or nucleotides (*e.g.*, ATP). In some embodiments, a ligand may refer to a non-native, i.e. synthetic or non-naturally occurring, ligand (*e.g.*, a binding agent). For example, in some embodiments, a ligand refers to a small molecule. Ligand binding can be measured by a variety of methods known in the art (*e.g.*, detection of association with a radioactively labeled ligand).

[0064] "Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a receptor and a ligand. Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, receptor and ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein.

[0065] The terms "specific binding affinity" or "specific binding" are used interchangeably throughout the specification and claims and refer to binding which occurs between a paired species of molecules, *e.g.,* receptor and ligand. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is typically electrostatic, hydrogen-bonding, or the result of lipophilic interactions. In various embodiments, the specific binding between one or more species is direct. In one embodiment, the affinity of specific binding is about 2 times greater than background binding (non-specific binding), about 5 times greater than background binding, about 10 times greater than background binding, about 20 times greater than background binding, about 50 times greater than background binding, about 100 times greater than background binding, or about 1000 times greater than background binding or more.

[0066] "Signaling" refers to the generation of a biochemical or physiological response as a result of ligand binding to a receptor (*e.g.*, as a result of a binding agent binding to an engineered receptor described herein).

[0067] The terms "native" and "wildtype" are used interchangeably throughout the specification and claims to refer a composition found in nature.

[0068] The terms "non-native", "variant", and "mutant" are used interchangeably throughout the specification and the claims to refer to a mutant of a native, or wild type, composition, for example a variant polypeptide having less than 100% sequence identity with the native, or wild type, sequence.

[0069] The term "parental" or "starter" are used interchangeably throughout the specification and claims to refer to an initial composition that is modified, or derivatized, to create an engineered composition having novel properties.

[0070] The term "engineered" is used throughout the specification and claims to refer to a non-naturally occurring composition having properties that are distinct from the parental composition from which it was derivatized.

[0071] In general, "sequence identity" or "sequence homology" refers to the nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining

their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin And Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (generally nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the proteins being compared. Default parameters are provided to optimize searches with short query sequences in, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, Computers and Chemistry 17:149-163 (1993). Ranges of desired degrees of sequence identity are approximately 80% to 100% and intervening integer values. Typically, the percent identities between a disclosed sequence and a claimed sequence are at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%.

[0072] As used herein, "substantially identical" refers to having a sequence identity that is 85% or more, for example 90% or more, *e.g.* 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100%, wherein the activity of the composition is unaltered by the modifications in the sequence that result in the difference in sequence identity.

C. Engineered Receptors

[0073] The present disclosure is directed to engineered receptors, engineered receptor mutants, and methods for their use. The term "receptor" as used herein refers to any protein that is situated on the surface of a cell and that can mediate signaling to and/or from the cell. The term "engineered receptor" is used herein to refer to a receptor that has been experimentally altered such that it is physically and/or functionally distinct from a corresponding parental receptor. In some embodiments, the parental receptor is a wild-type receptor. The term "wild-type receptor" is used herein to refer to a receptor having a polypeptide sequence that is identical to the polypeptide

sequence of a protein found in nature. Wild-type receptors include receptors that naturally occur in humans as well as orthologs that naturally occur in other eukaryotes, *e.g.* protist, fungi, plants or animals, for example yeast, insects, nematodes, sponge, mammals, non-mammalian vertebrates. In other embodiments, the parental receptor is a non-native receptor; that is, it is a receptor that does not occur in nature, for example, a receptor that is engineered from a wild type receptor. For example, a parental receptor may be an engineered receptor comprising one or more subunits from one wild-type receptor with one or more subunits from a second wild-type receptor. The resulting proteins are therefore comprised of subunits from two or more wild-type receptors. Engineered receptors of the present disclosure include, for example, parental receptors, parental receptor mutants, and switch receptors.

[0074] In some aspects, an engineered receptor of the present disclosure comprises at least one amino acid mutation relative to the corresponding parental receptor, *e.g.* one or more mutations in one or more domains of a wild-type receptor. By an "amino acid mutation" it is meant any difference in an amino acid sequence relative to a corresponding parental sequence, *e.g.* an amino acid substitution, deletion, and/or insertion. In some embodiments, the engineered receptor shares a sequence identity of 99%, 98%, 95%, 90%, 85%, 80%, 70%, 60%, 50%, or less with the corresponding parental receptor. In some embodiments, the parental receptor mutant has a sequence identity of 85% or more with the corresponding parental receptor, *e.g.* 90% or more or 95% or more, for example, 96%, 97%, 98% or 99% identity with the corresponding parental receptor. In some embodiments, an engineered receptor (*e.g.*, a parental receptor mutant) is generated by error prone PCR.

[0075] In some embodiments, the amino acid mutation is a loss-of-function amino acid mutation relative to a corresponding parental receptor. "Loss-of-function" amino acid mutations refer to one or more mutations that reduce, substantially decrease, or abolish the function of the engineered receptor relative to the parental receptor, for example by reducing the binding of an endogenous ligand to an engineered receptor relative to the binding of endogenous ligand to the parental receptor, or by reducing the activity of signaling pathway(s) downstream of the engineered receptor that are typically activated in response to the binding of a binding agent to the corresponding parental receptor.

[0076] In some embodiments, the amino acid mutation is a gain-of-function amino acid mutation relative to a corresponding parental receptor. "Gain-of-function" amino acid mutations

refer to one or more mutations that modify the function of the engineered receptor relative to the parental receptor, for example by altering or enhancing the affinity of an engineered receptor for a binding agent relative to the binding of endogenous ligand to the parental receptor, or by altering or enhancing the activity of the signaling pathways that are activated in response to the binding of a binding agent to an engineered receptor relative to the binding of the endogenous ligand to the corresponding parental receptor. In some embodiments, a gain-of-function mutation results in an increased affinity of the engineered receptor for a binding agent. In particular embodiments, a gain-of-function mutation results in an increased affinity of the engineered receptor for an agonist binding agent. In some embodiments, a gain-of-function mutation results in an antagonist binding agent acting as an agonist binding agent upon binding to the engineered receptor (*e.g.*, results in the activation of agonist signaling pathways instead of antagonist signaling pathways). In some embodiments, a gain-of-function mutation results in a modulator binding agent acting as an agonist binding agent upon binding to the engineered receptor. In some embodiments, the subject engineered receptor of the present disclosure comprises one or more loss-of-function amino acid mutations and one or more gain-of-function amino acid mutations relative to a corresponding parental receptor.

[0077] In some embodiments, the loss of function mutation and the gain of function mutation are at the same residue, i.e. they are the same mutation. In other embodiments, the loss of function mutation and the gain of function mutation are mutations at different amino acid residues. In some embodiments, the subject engineered receptor comprising the loss of function mutation and/or gain of function mutation shares a sequence identity of 99%, 98%, 95%, 90%, 85%, 80%, 70%, 60%, 50%, or less with the corresponding parental receptor, *e.g.* wild type receptor or non-native receptor. In some embodiments, the subject engineered receptor shares a sequence identity of 85% or more with the corresponding parental receptor, for example 85%, 90%, or 95% or more sequence identity, in some instances 96%, 97%, 98% or more sequence identity, *e.g.* 99% or 99.5% sequence identity.

[0078] In some aspects, engineered receptors of the present disclosure include receptors produced by the combination of one or more amino acid sequences, *e.g.* subunits, from one wild-type receptor with one or more amino acid sequences, *e.g.* subunits, from a second wild-type receptor. In other words, the engineered receptor comprises amino acid sequences that are heterologous to one another, where by "heterologous", it is meant not occurring together in nature.

Such receptors are referred to herein as "chimeric receptors". In some embodiments, chimeric receptors serve as parental receptors from which an engineered receptor of the present disclosure is generated.

[0079] In some embodiments, a parental receptor mutant demonstrates increased affinity for an agonist binding agent. In some embodiments, a ligand or a binding agent that functions as an antagonist or modulator when binding to a wild type receptor functions as an agonist when binding to a parental receptor mutant.

[0080] In some embodiments, the engineered receptor is a "ligand-gated ion channel" or LGIC. An LGIC refers to a large group of transmembrane proteins that allow passage of ions upon activation by a specific ligand (*e.g.*, chemical or binding agent). LGIC are composed of at least two domains: a ligand binding domain and a transmembrane ion pore domain. Ligand binding to an LGIC results in activation of the LGIC and opening of the ion pore. Ligand binding causes a drastic change in the permeability of the channel to a specific ion or ions; effectively no ions can pass through the channel when it is inactive or closed but up to 10^7 ions/second can pass through upon ligand binding. In some embodiments, LGICs respond to extracellular ligands (*e.g.*, neurotransmitters) and facilitate an influx of ions into the cytosol. In some embodiments, LGICs respond to intracellular ligands (*e.g.*, nucleotides such at ATP and signaling intermediates such as PIP_2) and facilitate an efflux of ions from the cytosol into the extracellular environment. Importantly, activation of LGIC results in the transport of ions across the cellular membrane (*e.g.*, Ca^{2+}, Na^+, K^+, Cl^-, etc.) and does not result in the transport of the ligand itself.

[0081] LGIC receptors are comprised of multiple subunits and can be either homomeric receptors or heteromeric receptors. A homomeric receptor is comprised of subunits that are all the same type. A heteromeric receptor is comprised of subunits wherein at least one subunit is different from at least one other subunit comprised within the receptor. For example, the glycine receptor is comprised of 5 subunits of which there are two types: α-subunits, of which there are four isoforms ($\alpha_1 - \alpha_4$) and β-subunits, of which there is a single known isoform. An exemplary homomeric GlyR is a GlyR comprised of 5 α_1-GlyR subunits. Similarly, a homomeric $GABA_A$ receptor may be comprised of β_3-$GABA_A$ subunits, and an nAchR receptor may be comprised of α_7-nAchR subunits. An exemplary heteromeric GlyR may be comprised of one or more α-subunits and one or more of β-subunits (*e.g.*, an $\alpha_1\beta$-GlyR). Subunits of example LGIC receptors are shown in Table 1.

Table 1: LGIC Receptors and Subunits

Receptor	Subunits	Subunit Isoforms
GlyR	GLRA1	
	GLRA2	
	GLRB	
$5HT_3$	5-HT3A	
	5-HT3B	
	5-HT3C	
	5-HT3D	
	5-HT3E	
nAChR	α	α1
		α2
		α3
		α4
		α5
		α6
		α7
		α8
		α9
		α10
	β	β1
		β2
		β3
		β4
	γ	γ
	δ	δ
	ε	ε
$GABA_A$	α	α1
		α2
		α3
		α4
		α5
$GABA_A$	α	α6
	β	β1
		β2
		β3
	γ	γ1
		γ2
		γ3
	δ	δ
	ε	ε
	π	π
	ρ	ρ1
		ρ2

Receptor	Subunits	Subunit Isoforms
		ρ3
P2X	P2X1	
	P2X2	
	P2X3	
	P2X4	
	P2X5	
	P2X6	
	P2X7	
KCNQ	α	K_vα1
		K_vα2
		K_vα3
		K_vα4
		K_vα5
		K_vα6
		K_vα7
		K_vα8
		K_vα9
		K_vα10
		K_vα11
		K_vα12
	β	K_vβ1
		K_vβ2
		K_vβ3
		minK
		MiRP1
		MiRP2
		MiRP3
		KCNE1-like
		KCNIP1
		KCNIP2
		KCNIP3
		KCNIP4

[0082] Illustrative examples of families of LGICs suitable for use in particular embodiments include, but are not limited to Cys-loop receptors such as Glycine receptors (GlyR), serotonin receptors (*e.g.*, 5-HT3 receptors), λ-Aminobutyric Acid A (GABA-A) receptors, and Nicotinic acetylcholine receptors (nAchR); as well as Acid-sensing (proton-gated) ion channels (ASICs), Epithelial sodium channels (ENaC), Ionotropic glutamate receptors, IP3 receptor, P2X receptors, the Ryanodine receptor, and Zinc activated channels (ZAC).

[0083] Specific non-limiting examples of LGICs that are suitable for use with the methods described herein include: HTR3A; HTR3B; HTR3C; HTR3D; HTR3E; ASIC1; ASIC2; ASIC3; SCNN1A; SCNN1B; SCNN1D; SCNN1G; GABRA1; GABRA2; GABRA3; GABRA4; GABRA5; GABRA6; GABRB1; GABRB2; GABRB3; GABRG1; GABRG2; GABRG3; GABRD; GABRE; GABRQ; GABRP; GABRR1; GABRR2; GABRR3; GLRA1; GLRA2; GLRA3; GLRA4; GLRB; GRIA1; GRIA2; GRIA3; GRIA4; GRID1; GRID2; GRIK1; GRIK2; GRIK3; GRIK4; GRIK5; GRIN1; GRIN2A; GRIN2B; GRIN2C; GRIN2D; GRIN3A; GRIN3B; ITPR1; ITPR2; ITPR3; CHRNA1; CHRNA2; CHRNA3; CHRNA4; CHRNA5; CHRNA6; CHRNA7; CHRNA9; CHRNA10; CHRNB1; CHRNB2; CHRNB3; CHRNB4; CHRNG; CHRND; CHRNE; P2RX1; P2RX2; P2RX3; P2RX4; P2RX5; P2RX6; P2RX7; RYR1; RYR2; RYR3; and ZACN.

[0084] TRPV1, TRPM8 and $P2X_2$ are members of large LGIC families that share structural features as well as gating principles. For example TRPV4, similar to TRPV1, is also triggered by heat, but not by capsaicin; and $P2X_3$, is triggered by ATP, but desensitizes more rapidly than $P2X_2$. TRPV1, TRPM8 and $P2X_2$ are, therefore, non-limiting examples of LGIC suitable for use in particular embodiments.

[0085] In one embodiment, the engineered receptor is a TRPV1 or TRPM8 receptor or a mutein thereof. TRPV1 and TRPM8, are vanilloid and menthol receptors expressed by nociceptive neurons of the peripheral nervous system. Both channels are thought to function as non-selective, sodium- and calcium-permeable homotetramers. In addition, both channels and their principal agonists--capsaicin and cooling compounds, such as menthol, respectively--are virtually absent from the central nervous system. Capsaicin and some cooling compounds, including menthol and icilin, contain potential acceptor sites for photolabile blocking groups. Association of a photolabile blocking group with such an acceptor would result in a ligand-gated ion channel in which light acts as an indirect trigger by releasing the active ligand.

[0086] In one embodiment, the engineered receptor is a $P2X_2$ receptor or a mutein thereof. $P2X_2$ is an ATP-gated non-selective cation channel distinguished by its slow rate of desensitization. $P2X_2$ may be used as a selectively addressable source of depolarizing current and present a platform for the generation of engineered channel-ligand combinations that lack natural agonists altogether.

[0087] Non-limiting examples of sequences of wild-type LGIC receptor that find use in the generation of engineered receptors of the present disclosure include the following. In the sequences, the signal peptide is italicized, the ligand binding domain is bolded, and the ion pore domain is underlined:

[0088] In some embodiments, the wild-type LGIC receptor is a human alpha 1 glycine receptor (GlyRα1) (GenBank Accession No. NP_001139512.1, SEQ ID NO:2), encoded by the *GLRA1* gene (GenBank Accession No. NM_001146040.1 (SEQ ID NO:1):

(a) *MYSFNTLRLYLWETIVFFSLAASKEAEA***ARSAPKPMSPSDFLDKLMGRTSG YDARIRPNFKGPPVNVSCNIFINSFGSIAETTMDYRVNIFLRQQWNDPRLAYNEYPD DSLDLDPSMLDSIWKPDLFFANEKGAHFHEITTDNKLLRISRNGNVLYSIRITLTLAC PMDLKNFPMDVQTCIMQLESFGYTMNDLIFEWQEQGAVQVADGLTLPQFILKEEK DLRYCTKHYNTGKFTCIEARFHLERQ**MGYYLIQMYIPSLLIVILSWISFWINMDAAPA RVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQ HKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNS NTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:2).

[0089] In some embodiments, the wild-type LGIC receptor is a human nicotinic cholinergic receptor alpha 7 subunit (α7-nAchR) (GenBank Accession No. NP_000737.1, SEQ ID NO:4), encoded by the *CHRNA7* gene (GenBank Accession No. NM_000746.5 (SEQ ID NO:3):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRR**TLYYGLNLLIPCVLISALALLVFLLPADSGEKISLGITVLLSLTVFML LVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLQYHHHDPDGGKMPKWTRVILLN WCAWFLRMKRPGEDKVRPACQHKQRRCSLASVEMSAVAPPPASNGNLLYIGFRGLDG VHCVPTPDSGVVCGRMACSPTHDEHLLHGGQPPEGDPDLAKILEEVRYIANRFRCQDES EAVCSEWKFAACVVDRLCLMAFSVFTIICTIGILMSAPNFVEAVSKDFA (SEQ ID NO:4).

[0090] In some embodiments, the wild-type LGIC receptor is a human 5-hydroxytryptamine receptor 3A (5HT3A, GenBank Accession No. NP_998786.2, SEQ ID NO:6), encoded by the *HTR3A* gene (GenBank Accession No. NM_213621.3, SEQ ID NO:5):

(a) *MLGKLAMLLWVQQALLALLLPTL***LAQGEARRSRNTTRPALLRLSDYLLT NYRKGVRPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWN PEDFDNITKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTA CSLDIYNFPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMNQGEWELLG VLPYFREFSMESSNYYAEMKFYVVIRRR**PLFYVVSLLLPSIFLMVMDIVGFYLPPNSGE RVSFKITLLLGYSVFLIIVSDTLPATAIGTPLIGKAPPGSRAQSGEKPAPSHLLHVSLASAL GCTGVYFVVCMALLVISLAETIFIVRLVHKQDLQQPVPAWLRHLVLERIAWLLCLREQS TSQRPPATSQATKTDDCSAMGNHCSHMGGPQDFEKSPRDRCSPPPPPREASLAVCGLLQ ELSSIRQFLEKRDEIREVARDWLRVGSVLDKLLFHIYLLAVLAYSITLVMLWSIWQYA (SEQ ID NO:6).

[0091] In some embodiments, the wild-type LGIC receptor is a human 5-hydroxytryptamine receptor 3B (5HT3B GenBank Accession No. NP_006019.1, SEQ ID NO:57), encoded by the *HTR3B* gene (GenBank Accession No. NM_006028.4, SEQ ID NO:56):

(a) *MLSSVMAPLWACILVAAGILA***TDTHHPQDSALYHLSKQLLQKYHKEVRP VYNWTKATTVYLDLFVHAILDVDAENQILKTSVWYQEVWNDEFLSWNSSMFDEIR EISLPLSAIWAPDIIINEFVDIERYPDLPYVYVNSSGTIENYKPIQVVSACSLETYAFPF DVQNCSLTFKSILHTVEDVDLAFLRSPEDIQHDKKAFLNDSEWELLSVSSTYSILQSS AGGFAQIQFNVVMRRHP**LVYVVSLLIPSIFLMLVDLGSFYLPPNCRARIVFKTSVLVGY TVFRVNMSNQVPRSVGSTPLIGHFFTICMAFLVLSLAKSIVLVKFLHDEQRGGQEQPFLC LRGDTDADRPRVEPRAQRAVVTESSLYGEHLAQPGTLKEVWSQLQSISNYLQTQDQTD QQEAEWLVLLSRFDRLLFQSYLFMLGIYTITLCSLWALWGGV (SEQ ID NO:57).

[0092] In some embodiments, the wild-type LGIC receptor is a human Gamma-aminobutyric acid receptor A (GABA-A), subunit beta-3 (GABA-A β3) (GenBank Accession No. NP_000805.1, SEQ ID NO:8), encoded by the *GABRB3* gene (GenBank Accession No. NM_000814.5, SEQ ID NO:7):

(a) *MWGLAGGRLFGIFSAPVLVAVVCCA***QSVNDPGNMSFVKETVDKLLKGYD IRLRPDFGGPPVCVGMNIDIASIDMVSEVNMDYTLTMYFQQYWRDKRLAYSGIPLN**

LTLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGLRITTTAA CMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGGDKAVTGVERIELPQFSIVEH RLVSRNVVFATGAYPRLSLSFRLKRNIGYFILQTYMPSILITILSWVSFWINYDASAARV ALGITTVLTMTTINTHLRETLPKIPYVKAIDMYLMGCFVFVFLALLEYAFVNYIFFGRGP QRQKKLAEKTAKAKNDRSKSESNRVDAHGNILLTSLEVHNEMNEVSGGIGDTRNSAISF DNSGIQYRKQSMPREGHGRFLGDRSLPHKKTHLRRRSSQLKIKIPDLTDVNAIDRWSRIV FPFTFSLFNLVYWLYYVN (SEQ ID NO:8).

[0093] In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho1 (ρ1) (GABA-A ρ1) (GenBank Accession No. NP_002033.2, SEQ ID NO:10), encoded by the *GABRR1* gene (GenBank Accession No. NM_002042.4, SEQ ID NO:9):

(a) *MLAVPNMRFGIFLLWWGWVLA***TESRMHWPGREVHEMSKKGRPQRQRR EVHEDAHKQVSPILRRSPDITKSPLTKSEQLLRIDDHDFSMRPGFGGPAIPVGVDVQ VESLDSISEVDMDFTMTLYLRHYWKDERLSFPSTNNLSMTFDGRLVKKIWVPDMF FVHSKRSFIHDTTTDNVMLRVQPDGKVLYSLRVTVTAMCNMDFSRFPLDTQTCSLE IESYAYTEDDLMLYWKKGNDSLKTDERISLSQFLIQEFHTTTKLAFYSSTGWYNRL YINFTLRRHIFF**FLLQTYFPATLMVMLSWVSFWIDRRAVPARVPLGITTVLTMSTIITGV NASMPRVSYIKAVDIYLWVSFVFVFLSVLEYAAVNYLTTVQERKEQKLREKLPCTSGLP PPRTAMLDGNYSDGEVNDLDNYMPENGEKPDRMMVQLTLASERSSPQRKSQRSSYVS MRIDTHAIDKYSRIIFPAAYILFNLIYWSIFS (SEQ ID NO:10).

[0094] In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho2 (ρ2) (GABA-A ρ2) (GenBank Accession No. NP_002034.3, SEQ ID NO:12), encoded by the GABRR2 gene (GenBank Accession No. NM_002043.4, SEQ ID NO:11):

(a) *MPYFTRLILFLFCLMVLVES***RKPKRKRWTGQVEMPKPSHLYKKNLDVT KIRKGKPQQLLRVDEHDFSMRPAFGGPAIPVGVDVQVESLDSISEVDMDFTMTLYL RHYWKDERLAFSSASNKSMTFDGRLVKKIWVPDVFFVHSKRSFTHDTTTDNIMLR VFPDGHVLYSMRITVTAMCNMDFSHFPLDSQTCSLELESYAYTDEDLMLYWKNGD ESLKTDEKISLSQFLIQKFHTTSRLAFYSSTGWYNRLYINFTLRRHIFF**FLLQTYFPAT LMVMLSWVSFWIDRRAVPARVSLGITTVLTMTTIITGVNASMPRVSYVKAVDIYLWVSF VFVFLSVLEYAAVNYLTTVQERKERKLREKFPCMCGMLHSKTMMLDGSYSESEANSLA

GYPRSHILTEEERQDKIVVHLGLSGEANAARKKGLLKGQTGFRIFQNTHAIDKYSRLIFP ASYIFFNLIYWSVFS (SEQ ID NO:12).

[0095] In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho3 (ρ3) (GABA-A ρ3) (GenBank Accession No. NP_001099050.1, SEQ ID NO: 14), encoded by the GABRR3 gene (GenBank Accession No. NM_001105580.2, SEQ ID NO:13):

(a) *MVLAFQLVSFTYIWIILKPNVCA***ASNIKMTHQRCSSSMKQTCKQETRMK KDDSTKARPQKYEQLLHIEDNDFAMRPGFGGSPVPVGIDVHVESIDSISETNMDFT MTFYLRHYWKDERLSFPSTANKSMTFDHRLTRKIWVPDIFFVHSKRSFIHDTTMEN IMLRVHPDGNVLLSLRITVSAMCFMDFSRFPLDTQNCSLELESYAYNEDDLMLYW KHGNKSLNTEEHMSLSQFFIEDFSASSGLAFYSSTGWYNRLFINFVLRRHVFF**FVLQ TYFPAILMVMLSWVSFWIDRRAVPARVSLGITTVLTMSTIITAVSASMPQVSYLKAVDV YLWVSSLFVFLSVIEYAAVNYLTTVEERKQFKKTGKISRMYNIDAVQAMAFDGCYHDS EIDMDQTSLSLNSEDFMRRKSICSPSTDSSRIKRRKSLGGHVGRIILENNHVIDTYSRILFPI VYILFNLFYWGVYV (SEQ ID NO:14).

[0096] In some aspects, the subject engineered receptor is a chimeric receptor. In some embodiments, the chimeric receptor comprises a ligand binding domain sequence from at least at first LGIC and an ion pore conduction domain sequence, or more simply, "ion pore domain sequence" from a least a second LGIC. In some embodiments, the first and second LGIC are Cys-loop receptors. Ligand binding domain sequences and ion pore domain sequences of the Cys-loop receptors are well known in the art and can be readily identified from the literature by use of publicly available software, *e.g.* PubMed, Genbank, Uniprot, and the like. In the sequences described above, the ligand binding domain is bolded, and the ion pore domain is underlined.

[0097] In some embodiments, the ligand binding domain of the chimeric receptor comprises the ligand binding domain sequence of a human glycine receptor. In some embodiments, the human glycine receptor is human GlyRα1 (SEQ ID NO:2). In some such embodiments, the ligand binding domain comprises about amino acids 29-235 of GlyRα1, *e.g.* amino acids 29-235, amino acids 29-240, amino acids 29-246, amino acids 29-248, amino acids 29-250, or amino acids 29-252 of SEQ ID NO:2. In certain such embodiments, the ligand binding domain consists essentially of amino acids 29-235 of SEQ ID NO:2, consists essentially of amino acids 29-240 of SEQ ID NO:2, consists essentially of amino acids 29-246 of SEQ ID NO:2, consists essentially of

amino acids 29-248 of SEQ ID NO:2, consists essentially of amino acids 29-250 of SEQ ID NO:2, consists essentially of amino acids 29-252 of SEQ ID NO:2. In some embodiments, the ion pore domain sequence is from a Cys-loop receptor other than the human GlyRα1.

[0098] In some embodiments, the ligand binding domain of the chimeric receptor comprises the ligand binding domain sequence of a human nicotinic cholinergic receptor. In some embodiments, the human nicotinic cholinergic receptor is human α7-nAChR. In some such embodiments, the ligand binding domain comprises about amino acids 23-220 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 23-220, amino acids 23-226, amino acids 23-229, amino acids 23-230, in some instances amino acids 23-231 of SEQ ID NO:4. In certain such embodiments, the ligand binding domain consists essentially of amino acids 23-220 of SEQ ID NO:4, consists essentially of amino acids 23-226 of SEQ ID NO:4, consists essentially of amino acids 23-229 of SEQ ID NO:4, consists essentially of amino acids 23-230 of SEQ ID NO:4, or consists essentially of amino acids 23-231 of SEQ ID NO:4. In some embodiments, the ion pore domain sequence is from a Cys-loop receptor other than the human α7-nAChR.

[0099] In some embodiments, the ligand binding domain of the chimeric receptor comprises the ligand binding domain sequence of a human serotonin receptor. In some embodiments, the human serotonin receptor is human 5HT3A or 5HT3B. In some such embodiments, the ligand binding domain comprises about amino acids 23-247 of 5HT3A (SEQ ID NO:6), *e.g.* amino acids 23-240, amino acids 30-245, amino acids 23-247, amino acids 23-250, in some instances amino acids 30-255 of SEQ ID NO:6. In certain embodiments, the ligand binding domain consists essentially of amino acids 23-240 of SEQ ID NO:6, consists essentially of amino acids 23-245 of SEQ ID NO:6, consists essentially of amino acids 30-247 of SEQ ID NO:6, consists essentially of amino acids 23-250 of SEQ ID NO:6, consists essentially of amino acids 23-255 of SEQ ID NO:6. In some such embodiments, the ligand binding domain comprises about amino acids 21-239 of 5HT3B (SEQ ID NO:57), *e.g.* amino acids 21-232, amino acids 21-235, amino acids 21-240, amino acids 21-245, in some instances amino acids 21-247 of SEQ ID NO:57. In certain embodiments, the ligand binding domain consists essentially of amino acids 21-239 of SEQ ID NO:57, consists essentially of amino acids 21-232 of SEQ ID NO:57, consists essentially of amino acids 21-235 of SEQ ID NO:57, consists essentially of amino acids 21-240 of SEQ ID NO:57, consists essentially of amino acids 21-245 of SEQ ID NO:57. In some embodiments, the

ion pore domain sequence is from a Cys-loop receptor other than the human 5-hydroxytryptamine receptor 3.

[0100] In some embodiments, the ligand binding domain of the chimeric receptor comprises the ligand binding domain sequence of a human GABA receptor. In some embodiments, the human GABA receptor is human GABA-A β3. In some such embodiments, the ligand binding domain comprises about amino acids 26-245 of GABA-A β3 (SEQ ID NO:8), *e.g.* amino acids 26-240, amino acids 26-245, amino acids 26-248, amino acids 26-250, in some instances amino acids 26-255 of SEQ ID NO:8. In certain such embodiments, the ligand binding domain consists essentially of amino acids 26-240 of SEQ ID NO:8, consists essentially of amino acids 26-245 of SEQ ID NO:8, consists essentially of amino acids 26-248 of SEQ ID NO:8, consists essentially of amino acids 26-250 of SEQ ID NO:8, or consists essentially of amino acids 26-255 of SEQ ID NO:8. In some embodiments, the ion pore domain sequence is from a Cys-loop receptor other than the human GABA-A receptor.

[0101] In some embodiments, the ion pore domain to which the ligand binding domain is fused conducts anions, *e.g.* it comprises an ion pore domain sequence of a human glycine receptor or a human serotonin receptor. In other embodiments, the ion conduction pore domain to which the ligand binding domain is fused conducts cations, *e.g.* it comprises an ion pore domain sequence of a human acetylcholine receptor or a human gamma-aminobutyric acid receptor A.

[0102] In some embodiments, the ion pore domain comprises the ion pore domain sequence of a human glycine receptor. In some embodiments, the human glycine receptor is human GlyRα1. In some such embodiments, the ion pore domain comprises about amino acids 245-457 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 240-457, amino acids 245-457, amino acids 248-457, amino acids 249-457, amino acids 250-457, amino acids 255-457, or amino acids 260-457 of SEQ ID NO:2. In certain such embodiments, the ion pore domain consists essentially of amino acids 245-457 of SEQ ID NO:2, consists essentially of amino acids 248-457 of SEQ ID NO:2, consists essentially of amino acids 249-457 of SEQ ID NO:2, or consists essentially of amino acids 250-457 of SEQ ID NO:2.

[0103] In some embodiments, the ion pore domain comprises the ion pore domain sequence of a human nicotinic cholinergic receptor. In some embodiments, the human nicotinic cholinergic receptor is human α7-nAChR. In some such embodiments, the ion pore domain comprises about amino acids 230-502 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 227-502,

amino acids 230-502, amino acids 231-502, amino acids 232-502, or amino acids 235-502. In certain such embodiments, the ion pore domain consists essentially of amino acids 227-502 of SEQ ID NO:4, consists essentially of amino acids 230-502 of SEQ ID NO:4, consists essentially of amino acids 231-502 of SEQ ID NO:4, consists essentially of amino acids 232-502 of SEQ ID NO:4, or consists essentially of amino acids 235-502 of SEQ ID NO:4.

[0104] In some embodiments, the ion pore domain comprises the ion pore domain sequence of a human serotonin receptor. In some embodiments, the human serotonin receptor is human 5HT3A or 5HT3B. In some such embodiments, the ion pore domain comprises about amino acids 248-516 of 5HT3A (SEQ ID NO:6), *e.g.* amino acids 240-516, amino acids 245-516, amino acids 248-516, amino acids 250-516, or amino acids 255-516 of SEQ ID NO:6. In certain such embodiments, the ion pore domain consists essentially of amino acids 240-516 of SEQ ID NO:6, consists essentially of amino acids 245-516 of SEQ ID NO:6, consists essentially of amino acids 248-516 of SEQ ID NO:6, consists essentially of amino acids 250-516 of SEQ ID NO:6, or consists essentially of amino acids 253-516. In some such embodiments, the ion pore domain comprises about amino acids 240-441 of 5HT3B (SEQ ID NO:57), *e.g.* amino acids 230-441, amino acids 235-441, amino acids 240-441, amino acids 245-441, or amino acids 250-441 of SEQ ID NO:57. In certain such embodiments, the ion pore domain consists essentially of amino acids 230-441 of SEQ ID NO:57, consists essentially of amino acids 235-441 of SEQ ID NO:57, consists essentially of amino acids 240-441 of SEQ ID NO:57, consists essentially of amino acids 245-441 of SEQ ID NO:57, or consists essentially of amino acids 250-441.

[0105] In some embodiments, the ion pore domain comprises the ion pore domain sequence of a human GABA receptor. In some embodiments, the human GABA receptor is human GABA-A β3. In some such embodiments, the ion pore domain comprises about amino acids 246-473 of GABA-A β3 (SEQ ID NO:8), *e.g.* amino acids 240-473, amino acids 245-473, amino acids 247-473, amino acids 250-473, or amino acids 253-473 of SEQ ID NO:8. In certain such embodiments, the ion pore domain consists essentially of amino acids 240-473 of SEQ ID NO:8, amino acids 245-473 of SEQ ID NO:8, amino acids 247-473 of SEQ ID NO:8, amino acids 250-473 of SEQ ID NO:8, or amino acids 253-473 of SEQ ID NO:8.

[0106] In some embodiments, the ion pore domain of the subject chimeric ligand-gated ion channel comprises an M2-M3 linker domain that is heterologous to the M2-M3 linker domain of the ion pore domain. By an "M2-M3 linker domain", or "M2-M3 linker", it is meant the sequence

within an ion pore domain of a LGIC that is flanked at its amino (N) terminus by the C-terminal end of transmembrane domain 2 (M2) of the receptor and at its carboxy (C) terminus by the N-terminal end of transmembrane domain 3 (M3) of the receptor. The M2-M3 linker of a LGIC may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. Typically, when the ion pore domain of a chimeric receptor comprises a heterologous M2-M3 linker, the M2-M3 linker is derived from the same receptor as the ligand binding domain of the chimeric receptor. For example, when the subject ligand-gated ion channel comprises a ligand binding domain from an AChR and an ion pore domain from a GlyR, the subject ligand-gated ion channel may comprise an ion pore domain sequence from a GlyR except for the M2-M3 linker, which would instead be derived from a AChR. In some embodiments, the ion pore domain is from GlyRα1 and the M2-M3 linker is from α7-nAChR. In some such embodiments, the M2-M3 linker sequence that is removed from the GlyRα1 is about amino acids 293-311 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 304-310, 293-306, 298-310, 305-311, etc. In some such embodiments, the M2-M3 linker that is inserted is about amino acids 281-295 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 290-295, 281-290, 281-295, 287-292, etc. or a sequence having about 95% identity or more to amino acids 281-295 of α7-nAChR.

[0107] In some embodiments, the ligand binding domain of the subject chimeric ligand-gated ion channel comprises a Cys-loop domain sequence that is heterologous to the Cys-loop sequence of the ligand binding domain. By a "Cys-loop domain sequence", or "Cys-loop sequence", it is meant the domain within a ligand binding domain of a Cys-loop LGIC that forms a loop structure flanked by a cysteine at the N-terminus and the C-terminus. Without wishing to be bound by theory, it is believed that upon binding of the ligand to the ligand binding domain, the Cys-loop structurally moves to be in close proximity to the M2-M3 loop, this movement mediating the biophysical translation of ligand binding in the extracellular domain to signal transduction in the ion pore domain (as reviewed in Miller and Smart, Trends in Pharmacological Sci 2009:31(4)). The substitution of an endogenous Cys-loop sequence with a heterologous Cys-loop sequence may increase the conductivity of the LGIC by 1.5-fold or more, *e.g.* at least 2-fold, 3-fold or 4-fold, in some instances at least 5-fold or 6-fold, and at certain doses, at least 7-fold, 8-fold, 9-fold or 10-fold. The Cys-loop domain of a Cys-loop receptor may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. Typically, when the ligand binding domain of a chimeric receptor comprises a heterologous Cys-loop sequence,

the Cys-loop sequence is derived from the same receptor as the ion pore domain of the chimeric receptor. For example, when the subject chimeric ligand-gated ion channel comprises a ligand binding domain from an AChR and an ion pore domain from a GlyR, the subject ligand-gated ion channel may comprise ligand binding domain sequence from an AChR except for the sequence of the Cys-loop domain, which is instead derived from a GlyR. In some embodiments, the ligand binding domain is from α7-nAChR and the Cys-loop sequence is from GlyRα1. In some such embodiments, the Cys-loop sequence that is removed from the α7-nAChR is about amino acids 150-164 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 150-157 of α7-nAChR. In some such embodiments, the Cys loop sequence that is inserted is about amino acids 166-180 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 166-172 of GlyRα1, or a sequence having about 95% identity or more to amino acids 166-180 of GlyRα1.

[0108] In some embodiments, the ligand binding domain of the subject chimeric ligand-gated ion channel comprises a β1-2 loop domain sequence that is heterologous to the β1-2 loop domain sequence of the ligand binding domain. By a "β1-2 loop domain sequence", or "β1-2 loop, or β1- β2 loop", it is meant the domain within a ligand binding domain of a Cys-loop LGIC that is flanked at its N-terminus by the C-terminus of the β1 sheet and, at its C-terminus, by the N-terminus of the β2 sheet. Without wishing to be bound by theory, it is believed that the β1-2 loop helps to mediate biophysical translation of ligand binding in the extracellular domain to the ion pore domain and subsequent signal transduction (i.e. chloride influx in case of GlyR). It is believed that upon binding of ligand, the β1-2 loop, together with the Cys-loop, come in close proximity to the M2-M3 loop to mediate the biophysical translation of ligand binding in the extracellular domain to signal transduction in the ion pore domain where the M2-M3 loop resides (as reviewed in Miller and Smart, *supra*). The substitution of an endogenous β1-2 loop sequence with a heterologous β1-2 loop sequence may increase the conductivity of the LGIC by 1.5-fold or more, *e.g.* at least 2-fold, 3-fold or 4-fold, in some instances at least 5-fold or 6-fold, and at certain doses, at least 7-fold, 8-fold, 9-fold or 10-fold. The β1-2 loop of a Cys-loop receptor may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. Typically, when the ligand binding domain of a chimeric receptor comprises a heterologous β1-2 loop sequence, the β1-2 loop sequence is derived from the same receptor as the ion pore domain of the chimeric receptor. For example, when the subject chimeric ligand-gated ion channel comprises a ligand binding domain from an AChR and an ion pore domain from a

GlyR, the subject ligand-gated ion channel may comprise ligand binding domain sequence from an AChR except for the sequence of the β1-2 loop domain, which is instead derived from a GlyR. In some embodiments, the ligand binding domain is from α7-nAChR and the β1-2 loop sequence is from GlyRα1. In some embodiments, the β1-2 loop sequence that is removed from the α7-nAChR is about amino acids 67-70 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 67-70, 66-71 or 64-72 of α7-nAChR. In some embodiments, the β1-2 loop sequence that is inserted is about amino acids 79-85 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 81-84, 79-85, or 81-84 of GlyRα1, or a sequence having about 95% identity or more to amino acids 79-85 of GlyRα1.

[0109] Non-limiting examples of sequences of chimeric LGIC receptors of the present disclosure include the sequences disclosed herein as SEQ ID NO:15 – SEQ ID NO:52. In some embodiments, the chimeric LGIC receptor or the polynucleotide that encodes it has a sequence identity of 85% or more to a sequence provided in SEQ ID NO:15 – SEQ ID NO:52 herein, e.g. a sequence identity of 90% or more, 93% or more, or 95% or more, i.e. 96%, 97%, 98%, 99% or 100% to a sequence provided in SEQ ID NO:15 - SEQ ID NO:52. In the sequences, the signal peptide is italicized, the ligand binding domain is bolded, and the ion pore domain is underlined.

[0110] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera (R229 junction), comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined) (*e.g.* SEQ ID NO:16 of Fig. 3):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPLTV YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKR SERFYECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAA PARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAV NFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAK DGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFY WIIYKIVRREDVHNQ (SEQ ID NO:16, encoded by SEQ ID NO:15).

[0111] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (R228 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPLTV YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKR SERFYECCKEPYPDVTFTVTMRR**QMGYYLIQMYIPSLLIVILSWISFWINMDAA PARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAV NFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAK DGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFY WIIYKIVRREDVHNQ (SEQ ID NO:17).

[0112] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (V224 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPLTV YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKR SERFYECCKEPYPDVTFTV**HLERQMGYYLIQMYIPSLLIVILSWISFWINMDAAP ARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAV NFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAK DGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFY WIIYKIVRREDVHNQ (SEQ ID NO:18).

[0113] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (Y233 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPLTV YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR**

WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKR SERFYECCKEPYPDVTFTVTMRRRTLYYLIQMYIPSLLIVILSWISFWINMDAAP ARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAV NFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAK DGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFY WIIYKIVRREDVHNQ (SEQ ID NO:19).

[0114] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera (R229 junction), comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined) comprising an α7-nAChR M2-M3 linker (lowercase):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSeimpatsdsvSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHH KSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEE MRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:21, encoded by SEQ ID NO:20);

(b) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:23, encoded by SEQ ID NO:22);

(c) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD**

GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRRMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSRASLPKVsdsvplIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:25, encoded by SEQ ID NO:24);

(d) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:27, encoded by SEQ ID NO:26);

(e) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRRT**GYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:29, encoded by SEQ ID NO:28); or

(f) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV QHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP YPDVTFTVTMRRRTL**YYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQ

SSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKS PMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMR KLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:31, encoded by SEQ ID NO:30).

[0115] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) (see, *e.g.* SEQ ID NO:34 of Fig. 3):

[0116] *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtc KLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPD VTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSS GSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSP MLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMR KLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:33, encoded by SEQ ID NO:32).

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknFPFDVQ HCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPY PDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQ SSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:35, encoded by SEQ ID NO:34).

[0117] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDG QIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQ HCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPY PDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQ SSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHK SPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEM RKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:37, encoded by SEQ ID NO:36).

[0118] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase) and Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) (*e.g.* SEQ ID NO:41 of Fig. 3):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDiaettmdLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQI WKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtcKLK FGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVTFT VTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSSGSRAS LPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLF QEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQR AKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:39, encoded by SEQ ID NO:38).

(b) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDG QIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtcKL KFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVT FTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSSGSR ASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPML NLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLF

IQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:41, encoded by SEQ ID NO:40).

(c) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDG QIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtcKL KFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVT FTVTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSSGSR ASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPML NLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLF IQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:43, encoded by SEQ ID NO:42).

(d) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDiaettmdLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQI WKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtcKLK FGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVTFT VTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSSGSRAS LPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLF QEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQR AKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:45, encoded by SEQ ID NO:44).

[0119] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) comprising human α7-nAChR M2-M3 linker (lowercase):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDG QIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQ HCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPY PDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQ SSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKS

PMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMR KLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:47, encoded by SEQ ID NO:46).

[0120] In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising a GlyRα1 Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) comprising a human α7-nAChR M2-M3 linker (lowercase):

(a) *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQ PLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmdvqtc KLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYPD VTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSS GSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPM LNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKL FIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:49, encoded by SEQ ID NO:48).

[0121] In some embodiments, the chimeric LGIC receptor is a HTR3A/GLRA1 chimera (R241 junction), comprising the human 5HT3A serotonin receptor signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

(a) *MLLWVQQALLALLLPTLLAQGEA***RRSRNTTRPALLRLSDYLLTNYRKGV RPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPEDFDNI TKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTACSLDIYN FPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMNQGEWELLGVLPYFR EFSMESSNYYAEMKFYVVIRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLG ITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELL RFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNP PPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:50).

[0122] In some embodiments, the chimeric LGIC receptor is a HTR3A/GLRA1 chimera (V236 junction) comprising the human 5HT3A serotonin receptor signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

(a) *MLLWVQQALLALLLPTLLAQGEA***RRSRNTTRPALLRLSDYLLTNYRKGV RPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPEDFDNI TKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTACSLDIYN FPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMNQGEWELLGVLPYFR EFSMESSNYYAEMKFYV**HLERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLG ITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELL RFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNP PPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:51).

[0123] In some embodiments, the chimeric LGIC receptor is a GABRB3/GLRA1 chimera (Y245 junction), comprising the human GABA-A β3 signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

(a) *MWGLAGGRLFGIFSAPVLVAVVCCA***QSVNDPGNMSFVKETVDKLLKGYD IRLRPDFGGPPVCVGMNIDIASIDMVSEVNMDYTLTMYFQQYWRDKRLAYSGIPLN LTLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGLRITTTAA CMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGGDKAVTGVERIELPQFSIVEH RLVSRNVVFATGAYPRLSLSFRLKRNIGY**MGYYLIQMYIPSLLIVILSWISFWINMDAA PARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVS RQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGAN NSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHN Q (SEQ ID NO:52).

[0124] As discussed above, in some aspects, the subject engineered receptor comprises at least one amino acid mutation that alters the potency of a ligand on the engineered receptor relative to its potency on the unmutated parental receptor. Put another way, the one or more amino acid mutations, *e.g.* a loss-of-function mutations or a gain-of-function mutations, shift the responsiveness of the engineered receptor to the ligand relative to the responsiveness of the unmutated parental receptor. In some such embodiments the one or more mutations is in the ligand

binding domain of the engineered receptor. In some embodiments, as when the ligand binding domain of the engineered receptor is a Cys-loop receptor protein, the one or more amino acid mutations is a substitution at a residue corresponding to a residue of α7-nAChR (SEQ ID NO:4) selected from the group consisting of W77, Y94, R101, W108, Y115, T128, N129, V130, L131, Q139, L141, Y151, S170, W171, S172, S188, Y190, Y210, C212, C213 and Y217. In some embodiments, one residue is substituted. In some embodiments, 2, 3, 4, or 5 or more residues are substituted, *e.g.* 6, 7, 8, 9 or 10 residues are substituted. In certain embodiments, the residue corresponds to a residue of α7-nAChR (SEQ ID NO:4) that is selected from the group consisting of W77, R101, Y115, N129, L131, S170, S172, and S188. In certain embodiments, the one or more substitutions is selected from a substitution listed in Figure 8A-I. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence.

[0125] In some embodiments, the one or more substitutions decreases, *e.g.* 2-fold or more, 3-fold or more, 4-fold or more. 5-fold or more, 10-fold or more, 20-fold or more, 30-fold or more, 50-fold or more, or 100-fold, the responsiveness of an engineered receptor to acetylcholine and a non-native ligand. In some such embodiments, the one or more substitutions corresponds to an amino acid substitution in an α7-nAChR sequence described in Figure 8A-F and 8H. In certain embodiments, the one or more substitutions is a substitution corresponding to R101I, R101S, R101D, Y115L, Y115M, Y115D, Y115T, T128M, T128R, T128I, N129I, N129V, N129P, N129W, N129T, N129D, N129E, L131P, L131T, L131D, L131S, L141S, L141R, W171F, W171H, S172F, S172Y, S172R, S172D, C212A, C212L, or C213P of α7-nAChR. In other instances, the one or more substitutions decreases the potency of acetylcholine on the engineered receptor selectively. In other words, the one or more substitutions decreases the responsiveness of the engineered receptor to acetylcholine while essentially maintaining responsiveness to non-native ligand or otherwise decreasing the responsiveness of the engineered receptor to acetylcholine 2-fold or more, *e.g.* 3-fold, 4-fold, 5-fold or more, in some instances 10-fold, 20-fold, 50-fold, or 100-fold or more, than it decreases the responsiveness of the engineered receptor to non-native ligand. Exemplary substitutions include those provided in Figure 8H, namely, a substitution corresponding to L131S, L131T, L131D, or S172D of α7-nAChR. In yet other embodiments, the one or more substitutions decreases the potency of a non-native ligand on the engineered receptor selectively. In other words, the one or more substitutions decreases the responsiveness of the engineered receptor to non-native ligand while essentially maintaining

responsiveness to acetylcholine or otherwise decreasing the responsiveness of the engineered receptor to non-native ligand 2-fold or more, *e.g.* 3-fold, 5-fold or more, in some instances 10-fold, 20-fold or 50-fold or more, than it decreases the responsiveness of the engineered receptor to acetylcholine. Exemplary substitutions include those in Figure 8G, namely, a substitution corresponding to W77M, Y115W, S172T, or S172C of α7-nAChR. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence. In certain embodiments, the non-native ligand is selected from AZD-0328, TC6987, ABT-126 and Facinicline/RG3487.

[0126] In other embodiments, the one or more substitutions increases, *e.g.* 2-fold or more, 3-fold or more, 4-fold or more. 5-fold or more, 10-fold or more, 20-fold or more, 30-fold or more, 50-fold or more, or 100-fold, the responsiveness of the engineered receptor to acetylcholine and/or non-native ligand. Exemplary substitutions include those described in Figure 8G or 8I, i.e. a substitution corresponding to L131N, L141W, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S172C, S188I, S188V, S188F, S188M, S188Q, S188T, S188P or S188W. In some instance, the one or more substitutions increases potency of both acetylcholine and non-native ligand, *e.g.* substitutions corresponding to L131N, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S188I, S188V, S188F, S188M, S188Q and S188T of α7-nAChR. In other instances, the one or more substitutions increases the potency of acetylcholine on the engineered receptor selectively. In other words, the one or more substitutions increases the responsiveness of the engineered receptor to acetylcholine 2-fold or more, *e.g.* 3-fold, 4-fold, or 5-fold or more, in some instances 10-fold, 20-fold, 50-fold, or 100-fold, than it increases the responsiveness of the engineered receptor to non-native ligand, *e.g.* substitutions corresponding to L141W, S172T, S172C, S188P or S188W, of α7-nAChR. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence. In certain embodiments, the non-native ligand is selected from AZD-0328, TC6987, ABT-126 and Facinicline/RG3487. In yet other instances, the one or more substitutions increases the potency of the non-native ligand on the engineered receptor selectively. In other words, the one or more substitutions increases the responsiveness of the engineered receptor to non-native ligand 2-fold or more, *e.g.* 3-fold, 5-fold or more, in some instances 10-fold, 20-fold or 50-fold or more, than it increases the responsiveness of the engineered receptor to acetylcholine.

[0127] Typically, the amino acid residue that is mutated in the subject engineered receptor is not an amino acid corresponding to R27, E41, Q79, Q139, 141, G175, Y210, P216, Y217, or

D219 of wild type a7 nAChR (SEQ ID NO:4). More typically still, the substitution is not a substitution corresponding to W77F, W77Y, W77M, Q79A, Q79Q, Q79S, Q79G, Y115F, L131A, L131G, L131M, L131N, L131Q, L131V, L131F, Q139G, Q139L, G175K, G175A, G175F, G175H, G175M, G175R, G175S, G175V, Y210F, P216I, Y217F, or D219A in wild type α7 nAChR. Alternatively, if such a substitution does exist within the engineered receptor, it exists in combination with one or more of the amino acid mutations described herein.

[0128] For example, it has been discovered that residues Y94, Y115, Y151, and Y190 of α7-nAChR (SEQ ID NO:4) mediate binding of the native ligand acetylcholine. Mutations at these residues will reduce binding of acetylcholine and hence are loss of function mutations. In contrast, residues W77, Y115, N129, V130, L131, Q139, L141, S170, Y210, C212, C213 and Y217 of the α7-nAChR mediate the binding of non-native ligand AZD0328 to this receptor, and mutation of these residues may increase the affinity of AZD0328 and/or other ligands for this receptor and hence be gain-of-function mutations. In some embodiments, the subject engineered receptor comprises a mutation in one or more amino acid residues of the ligand binding domain region of α7-nAChR (SEQ ID NO:4) or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR, where the one or more amino acid residues is selected from the group consisting of W77, Y94, Y115, N129, V130, L131, Q139, L141, Y151, S170, Y190, Y210, C212, C213 and Y217. In certain embodiments, the mutation in the one or more amino acid residues of the ligand binding domain region of α7-nAChR (SEQ ID NO:4) or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR is a substitution at one or more amino acid residues selected from the group consisting of W77, Y94, Y115, N129, V130, L131, Q139, L141, Y151, S170, Y190, Y210, C212, C213 and Y217.

[0129] As another example, it has been discovered that residues Y115, L131, L141, S170, W171, S172, C212, and Y217 of α7-nAChR (SEQ ID NO:4) mediate binding of acetylcholine and/or nicotine, and mutations at one or more of these residues will reduce binding of acetylcholine and/or nicotine. R101, Y115, L131, L141, W171, S172, S188, Y210, and Y217 of α7-nAChR mediate binding of the non-native ligand ABT126, and mutation of one or more of these residues is expected to increase the affinity of ABT126 and/or other ligands for α7-nAChR. R101, Y115, T128, N129, L131, L141, W171, S172, Y210, C212, C213 and Y217 of α7-nAChR mediate binding of the non-native ligand TC6987, and mutation of one or more of these residues is expected

to increase the affinity of TC6987 and/or other ligands for α7-nAChR. R101, N120, L131, L141, S170, W171, S172, Y210, and Y217 of α7-nAChR mediate binding of the non-native ligand Facinicline/RG3487, and mutation of one or more of these residues is expected to increase the affinity of Facinicline/RG3487and/or other ligands for α7-nAChR. In some embodiments, the subject engineered receptor comprises a mutation in one or more amino acid residues of the ligand binding domain region of α7-nAChR or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR, where the one or more amino acid residues is selected from the group consisting of R101, Y115, T128, N120, N129, L131, L141, S170, W171, S172, S188, Y210, C212, C213 and Y217. In some embodiments, the one or more amino acid residues alters the binding of acetylcholine and/or nicotine to α7-nAChR, wherein the amino acid is selected from the group consisting of Y115, L131, L141, S170, W171, S172, C212 and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from C212 and S170. In some embodiments, the mutation in the one or more amino acid residues alters the binding of ABT126 to α7-nAChR, wherein one or more amino acid residues is selected from the group consisting of R101, Y115, L131, L141, W171, S172, S188, Y210, and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from R101, S188, and Y210. In some embodiments, the mutation in the one or more amino acid residues alters the binding of TC6987 to α7-nAChR, wherein one or more amino acid residues is selected from the group consisting of R101, Y115, T128, N129, L131, L141, W171, S172, Y210, C212, C213 and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from R101, T128, N129, Y210 and C213. In some embodiments, the mutation in the one or more amino acid residues alters the binding of Facinicline/RG3487 to α7-nAChR, wherein one or more amino acid residues is selected from the group consisting R101, N120, L131, L141, S170, W171, S172, Y210, and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from Y210, R101, and N129.

[0130] As another example, it has been discovered that residues W85, R87, Y136, Y138, G146, N147, Y148, K149, S177, S178, L179, Y228, and Y229 of 5HT3 (SEQ ID NO:6) mediate binding of serotonin, and mutations at one or more of these residues will reduce binding of serotonin to 5HT3. D64, I66, W85, R87, Y89, N123, G146, Y148, T176, S177, S178, W190, R191, F221, E224, Y228, Y229 and E231 of 5HT3 mediate binding of the non-native ligand Cilansetron, and mutation of one or more of these residues is expected to increase the affinity of Cilansetron and/or other ligands for 5HT3. In some embodiments, the subject engineered receptor

comprises a mutation in one or more amino acid residues of the ligand binding domain region 5HT3A or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of 5HT3, where the one or more amino acid residues is selected from the group consisting of D64, I66, W85, R87, Y89, N123, Y136, Y138, G146, N147, Y148, K149, T176, S177, S178, L179, W190, R191, F221, E224, Y228, Y229, and E231. In some embodiments, the mutation in the one or more amino acid residues alters the binding of serotonin to 5HT3, wherein the amino acid is selected from the group consisting of W85, R87, Y136, Y138, G146, N147, Y148, K149, S177, S178, L179, Y228, and Y229 of 5HT3A. In certain such embodiments, the amino acid is selected from Y136, Y138, N147, K149, and L179. In some embodiments, the mutation in the one or more amino acid residues alters the binding of Cilansetron to 5HT3 wherein one or more amino acid residues is selected from the group consisting of D64, I66, W85, R87, Y89, N123, G146, Y148, T176, S177, S178, W190, R191, F221, E224, Y228, Y229 and E231 of 5HT3A. In certain such embodiments, the amino acid is selected from D64, I66, Y89, N123, T176, W190, R191, F221, E224, and E231.

[0131] In some embodiment, the one or more mutations that affects the ability of a ligand to modulate the activity of the LGIC is located in the ion pore domain of the LGIC. For example, residue T279 of the serotonin receptor 5HT3A mediates the way in which the ligand modulates the activity of the channel, such that mutation of this residue to, *e.g.* serine (T279S), converts the effect from being antagonistic (i.e., reducing the activity of the LGIC) to agonistic (i.e. promoting the activity of the channel). In some embodiments, the subject ligand gated ion channel comprises a mutation in one or more amino acid residues of the ion pore domain of the human 5HT3A (SEQ ID NO:6) or the ion pore domain of a chimeric LGIC receptor that comprises the ion pore domain of 5HT3A, where the substitution is in an amino acid corresponding to 279 of SEQ ID NO:6. In certain embodiments, the substitution is a T279S substitution relative to SEQ ID NO:6.

[0132] In some aspects, the subject ligand-gated ion channel comprises one or more non-desensitizing mutations. When used in the context of a ligand-gated ion channel, "desensitization" refers to the progressive reduction in ionic flux in the prolonged presence of agonist. This results in a progressive loss of responsiveness of the neuron to the ligand. By a non-desensitizing mutation, it is meant an amino acid mutation that prevents the LGIC from becoming desensitized to ligand, thereby preventing the neuron from becoming less responsive or nonresponsive to ligand. Non-desensitizing mutations can be readily identified by introducing the LGIC carrying

the mutation into a neuron and analyzing the current flux over time during prolonged exposure to ligand. If the LGIC does not comprise a non-desensitizing mutation, the current will restore from peak to steady state during prolonged exposure, whereas if the LGIC comprises a non-desensitizing mutation, the current will remain at peak flux for the duration of exposure to ligand. Exemplary amino acid mutations that result in desensitization include a V322L mutation in the human GlyRα1 (V294L post-processing of the pro-protein to remove the signal peptide) and a L321V mutation in human GABA-A receptor GABRB3 (L296V post-processing of the pro-protein to remove the signal peptide). In some embodiments, the desensitizing mutation is the replacement of amino acid residues at or near the C-terminus of the LGIC with a desensitizing sequence, for example, a sequence having 90% identity or more to IDRLSRIAFPLLFGIFNLVYWATYLNREPQL (SEQ ID NO:53) derived from the C terminus of the protein encoded by GABAR1, *e.g.* the replacement of residues 455-479 in GABRR1 with IDRLSRIAFPLLFGIFNLVYWATYLNREPQL (SEQ ID NO:53). LGIC desensitization, methods for measuring desensitization of LGICs, and mutations that are non-desensitizing are well known in the art; see, *e.g.* Gielen et al. Nat Commun 2015 Apr 20, 6:6829, and Keramidas et al. Cell Mol Life Sci. 2013 Apr;70(7):1241-53, the full disclosures of which are incorporated herein by reference.

[0133] In some aspects, the subject ligand-gated ion channel comprises one or more conversion mutations. By a conversion mutation, it is meant a mutation that changes the permeability of the ion pore domain of the LGIC such that it becomes permissive to the conductance of a non-native ion, i.e. an ion that does not naturally allow to pass through. In some cases, the mutation converts the permeability from cation to anion, for example the replacement of amino acid residues 260-281 in human α7-nAChR (CHRNA7) (EKISLGITVLLSLTVFMLLVAE, SEQ ID NO:54) or the corresponding amino acids in another cation-permeable LGIC with the peptide sequence PAKIGLGITVLLSLTTFMSGVAN (SEQ ID NO:55). In some cases, the mutation converts the permeability from anion to cation, for example, the substitution of amino acid residue 279 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC to glutamic acid (E), (which, as an A293E substitution in GLRA1 converts the LGIC from being anion-permissive to calcium-permissive), or the deletion of amino acid residue 278 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC, the substitution of amino acid residue 279 of GLRA1 or the corresponding amino acid in another

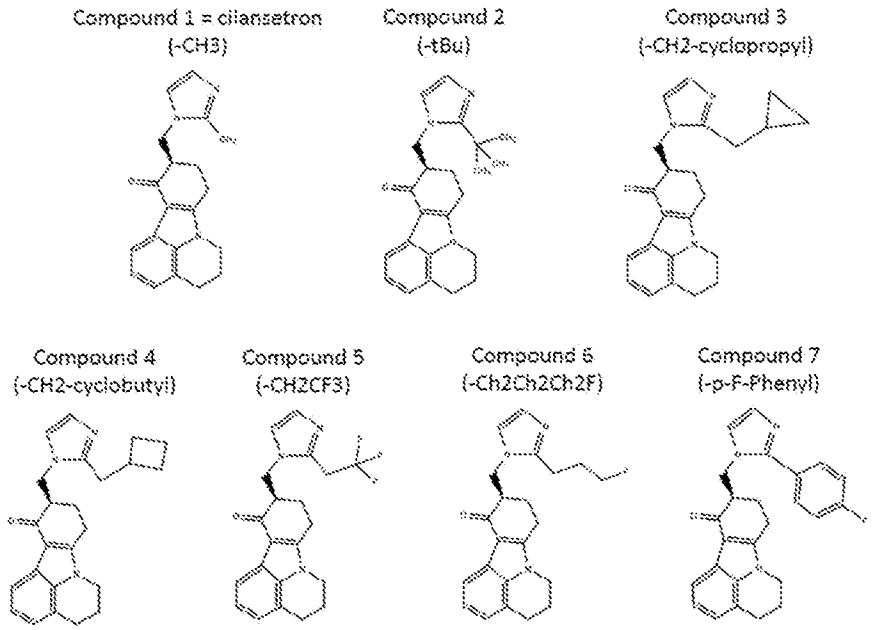
anion-permeable LGIC to glutamic acid (E), and the substitution of amino acid residue 293 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC to valine (V) (which, as a P278Δ, A279E, T293V in GLRA1 converts the LGIC from being anion-permissive to cation-permissive).

[0134] Additional engineered receptors beyond those described herein can be readily identified by *in vitro* screening and validation methods, for example as described in Fig. 2 and the working examples herein. In some embodiments, a library of parental receptor mutants is generated from a limited number of parental receptors, such as those shown in Fig. 1 and described elsewhere herein. The parental receptors can be mutated using methods known in the art, including error prone PCR. In some embodiments, the library of parental receptor mutants is then transfected into yeast or mammalian cells and screened in high throughput to identify functional receptors (*e.g.*, to identify parental receptor mutants that are capable of signaling in response to a binding agent or ligand). In some embodiments, the functional parental receptor mutants identified in this primary screen is then expressed in mammalian cells and screened for responsiveness to binding agents or ligands, *e.g.* by the plate reader and/or electrophysiology assays described herein. The parental receptor mutants that demonstrate either increased binding affinity for agonist binding agents, or that enable the use of antagonist or modulator binding agents as agonists in the secondary screen can then be selected and carried though further *in vitro* and/or *in vivo* validation and characterization assays. Such screening assays are known in the art, for example Armbruster, B.N. *et al.* (2007) PNAS, 104, 5163–5168; Nichols, C.D. and Roth, B.L. (2009) Front. Mol. Neurosci. 2, 16; Dong, S. *et al.* (2010) Nat. Protoc. 5, 561–573; Alexander, G.M. *et al.* (2009) Neuron 63, 27–39; Guettier, J.M. *et al.* (2009) PNAS 106, 19197–19202; Ellefson J.W. *et al.* (2014) Nat Biotechnol. 32(1):97-101; Maranhao AC and Ellington AD. (2017) ACS Synth Biol. 20;6(1):108-119; Talwar S *et al.* (2013) PLoS One;8(3):e58479; Gilbert D.F. *et al.* (2009) Front Mol Neurosci. 30;2:17; Lynagh and Lynch, (2010), Biol Chem. 14:285(20), 14890–14897; Islam R. *et al.* (2016) ACS Chem Neurosci. 21;7(12):1647-1657; and Myers *et al.* (2008) Neuron. 8:58(3): 362–373.

D. Binding Agents

[0135] The terms "binding agent" or "agent" are used interchangeably herein and refer to exogenous drugs or compounds with a known mechanism of action on a mammalian cell (*e.g.*, are known to act as an agonist, antagonist, or modulator of a receptor). Binding agents can include

proteins, lipids, nucleic acids, and/or small molecules. In some embodiments, binding agents include drugs or compounds that have been approved by the US Food and Drug Administration (FDA) for clinical use in the treatment of a particular disease (*e.g.*, a neurological disease). In some embodiments, binding agents include drugs or compounds that have not been approved by the FDA for clinical use, but have been tested in one or more clinical trials, are currently being tested in one or more clinical trials, and/or are anticipated to be tested in one or more clinical trials. In some embodiments, binding agents include drugs or compounds that have not been approved by the FDA for clinical use, but are routinely used in laboratory research. In some embodiments, the binding agent is an analog of one of the aforementioned agents. In particular embodiments, a binding agent is selected from any one of the agents in Tables 2 – 9. In some such embodiments, the binding agent is selected from the group consisting of AZD0328, ABT-126, AQW-051, Cannabidiol, Cilansetron, PH-399733, FACINICLINE/RG3487/MEM-3454, and TC-5619/AT-101. In particular embodiments, the binding agent is an analog of Cilansetron, *e.g.* as described by one of the compound formulas 2-7 below in either its R or S enantiomer:



[0136] In some embodiments, the binding agent acts as an agonist. The term "agonist" as used herein refers to a ligand or binding agent that induces a signaling response. In some embodiments, the binding agent acts as an antagonist. The term antagonist is used herein to refer to an agent that inhibits a signaling response.

[0137] In some embodiments, the binding agent is an anxiolytic, anticonvulsant, antidepressant, antipsychotic, antiemetic, nootropic, antibiotic, antifungal, antiviral, or an antiparasitic.

Table 2: Binding agents for Glycine Receptor (GlyR)

Agonists		Modulators/Binders	Antagonists	
Bilobalide	L-Serine	Gavestinel	468816	Lindane
Cannabidiol	MDL-27531	Halothane	ACEA-2085	MDL-100748
D-Alanine	Methoxyflurane		Bicuculline	MDL-102288
D-Serine	Milacemide		Brucine	MDL-105519
Desflurane	Moxidectin		Caffeine	PD-165650
Doramectin	NRX-1050		Gavestinel	Picrotoxin
Emamectin	NRX-1060		Ginkgo biloba	Strychnine
Eprinomectin	P-9939		GV-196771	Thiocolchicoside
Ethanol	Quisqualamine		GW 468816	Tutin
Glycine	Rapastinel		HMR-2371	UK-315716
Hypotaurine	S-18841		L-695902	ZD-9379
Isoflurane	Sarcosine		L-701324	
Ivermectin	Sevoflurane			
L-Alanine	Taurine			
L-Proline	β-Alanine			

Table 3: Binding Agents for λ-Aminobutyric Acid A Receptor (GABA-A)

Agonists	Modulators/Binders			Antagonists
3-acyl-4-quinolone	(−)Epigallo-catechin-3-Gallate	Etifoxine	Pentobarbital	(±)-cis-(3-Aminocyclopentyl) butylphosphinic acid
Acamprosate	10-Methoxy-yangonin	Etizolam	Petrichloral	(S)-(4-Aminocyclopent-1-enyl)butylphosphinic acid
Alfadolone	11-Hydroxy-yangonin	Etomidate	PF-4480682	Amoxapine
Bamaluzole	11-Methoxy-12-Hydroxy-dehydrokavain	Evt-201	Phenazepam	Bicuculline
Basmisanil	11-Methoxy-yangonin	Fasiplon	Phenobarbital	CGP-36742 (3-aminopropyl-n-butyl-phosphinic acid)
Bretazenil	123i-Iomazenil	Fg-8205	Pinazepam	Flumazenil
CACA	2-Oxoquazepam	Fletazepam	Pipequaline	Gabazine
CAMP	3-Hydroxy-phenazepam	Flubromazepam	Pivoxazepam	Ginkgo biloba

Agonists	Modulators/Binders			Antagonists
CP-409092	5-Hydroxykavain	Flubromazolam	Potassium Bromide	Lindane
Doramectin	5,6-Dehydro-methysticin	Fludiazepam	Prazepam	Methohexital
Emamectin	5,6-Dihydro-yangonin	Flumazenil	Premazepam	Picrotoxin
Eprinomectin	5,6,7,8-Tetrahydro-yangonin	Flunitrazepam	Primidone	SKF-97541 (3-Aminopropyl (methyl)phosphinic acid)
Eszopiclone	7,8-Dihydrokavain	Flurazepam	Proflazepam	TPMPA
Ethanol	7,8-Dihydro-methysticin	Flutazolam	Propanidid	ZAPA ((Z)-3-[(Aminoiminomethyl)thio]prop-2-enoic acid)
Etomidate	7,8-Dihydro-yangonin	Flutemazepam	Propofol	
Flunitrazepam	Abecarnil	Flutoprazepam	PWZ-007A	
GABA	Adinazolam	Fosazepam	PWZ-009A1	
Gabamide	Allobarbital	Fospropofol	Pwz-029	
GABOB	Allo-pregnanolone	Ganaxolone	Pyrazolam	
Gaboxadol	Alphaxolone	Gbld-345	PZ-II-028	
Gamma Hydroxybutyric Acid	Alphenal	Gedocarnil	PZ-II-029	
Glutethimide	Alpidem	Gidazepam	Qh-Ii-66	
Ibotenic acid	Alprazolam	Girisopam	Quazepam	
Imidazenil	Amentoflavone	Glutethimide	Quinidine Barbiturate	
Isoflurane	Amobarbital	Gyki-52466	Reclazepam	
Isoguvacine	Apigenin	Gyki-52895	Remimazolam	
Isonipecotic acid	Aprobarbital	Halazepam	Rilmazafone	
Ivermectin	Arfendazam	Haloxazolam	Ripazepam	
L-830982	Avizafone	Heptabarbital	Ro15-4513	
Meprobamate	AZD7325	Hexobarbital	Ro48-6791	
Methoxyflurane	Baicalein	Iclazepam	Ro48-8684	
MK-0777/L83098	Baicalin	Imidazenil	Ro4938581	
Methyprylon	Barbital	Indiplon	Rwj-51204	
Moxidectin	Barbituric Acid Derivative	Irazepine	Saripidem	
Muscimol	Bentazepam	Kavain	Sarmazenil	
N4-Chloroacetyl-	Brallobarbital	Kenazepine	Sb-205,384	

Agonists	Modulators/Binders			Antagonists
cytosine arabinoside				
Pagoclone	Bretazenil	Ketazolam	Scutellarein	
Phenibut	Bromazepam	L-655708	Secobarbital	
Picamilon	Brotizolam	L-838,417	Sevoflurane	
Piperazine	Butalbital	Lanthanum	Sh-053-R-Ch3-2′F	
Piperidine-4-sulfonic acid	Butethal	LAU 156	Skull-capflavone II	
Progabide	Butobarbital	LAU 157	Sl-651,498	
QH-ii-066	Camazepam	LAU 159	Sodium Amytal	
Quisqualamine	Carburazepam	LAU 161	Sodium Pentothal	
Sevoflurane	Carisoprodol	LAU 162	Stiripentol	
SL 75102	CGS 20625	LAU 163	Sulazepam	
SL-651,498	CGS 20625	LAU 176	Sulfonmethane	
Thiamylal	CGS 8216	LAU 177	Suproclone	
Thiomuscimol	CGS 9895	LAU 206	Suriclone	
Tolgabide	CGS 9896	Lofendazam	Sx-3228	
Topiramate	Chloral Hydrate	Lopirazepam	Talampanel	
Zolpidem	Chloralose	Loprazolam	Talbutal	
α5IA	Chlordiazepoxide	Lorazepam	Taniplon	
	Chlormezanone	Lorbamate	Temazepam	
	Chloroform	Loreclezole	Tetrazepam	
	Ciclotizolam	Lorediplon	Tetronal	
	Cinazepam	Lormetazepam	Thdoc	
	Cinolazepam	Meclonazepam	Theanine	
	Cl-218,872	Medazepam	Thiamylal	
	Clazolam	Menitrazepam	Thieno-diazepine	
	Climazolam	Mephobarbital	Thiopental	
	Clobazam	Meprobamate	Tofisopam	
	Clomethiazole	Metaclazepam	Tolufazepam	
	Clonazepam	Methaqualone	Tp-003	
	Clonazolam	Metharbital	Tp-13	
	Clorazepate	Methohexital	Tpa-023	
	Clotiazepam	Methyl-phenobarbital	Triazolam	
	Cloxazolam	Methyprylon	Triflubazam	
	Cp-1414s	Methysticin	Triflunor-dazepam	
	CTP-354	Metizolam	Trional	

Agonists	Modulators/Binders			Antagonists
	Cyclobarbital	Mexazolam	Tuclazepam	
	Cyprazepam	Midazolam	Uldazepam	
	Delorazepam	Motrazepam	Valerenic Acid	
	Demoxepam	N-Desalkyl-flurazepam	Valeric Acid	
	Deschloro-etizolam	Necopidem	Wogonin	
	Desmethoxy-yangonin	Nerisopam	XHe-II-006	
	Desmethyl-flunitrazepam	Niacin	XHe-II-019	
	Diazepam	Niacinamide	XHe-II-087c	
	Diclazepam	Nifoxipam	XHe-II-094	
	Diethyl Ether	Nimetazepam	XHe-II-098b	
	Dihydro-ergotoxine	Nitrazepam	XHe-II-17	
	Dihydroquinidine Barbiturate	Nitrazepate	XHe-III-006c	
	Diproqualone	Nitrazolam	XHe-III-063	
	Divaplon	Nordazepam	XHe-III-24	
	Doxefazepam	Nortetrazepam	XHe/ON-I	
	Elb-139	Ns-2664	Y-23684	
	Elfazepam	Ns-2710	Yangonin	
	Estazolam	Ocinaplon	Zaleplon	
	Eszopiclone	Oroxylin A	Zapizolam	
	Etaqualone	Oxazepam	Zinc	
	Etazepine	Oxazolam	Zk-93423	
	Etazolate	Pagoclone	Zolazepam	
	Ethyl Carfluzepate	Panadiplon	Zolpidem	
	Ethyl Dirazepate	Pazinaclone	Zomebazam	
	Ethyl Loflazepate	PB-XHe	Zopiclone	

Table 4: Binding Agents for 5-Hydroxytryptamine Receptor (5-HT3)

Agonists	Modulators/ Binders	Antagonists	
2-methyl-5-HT	5-chloroindole	3-Tropanyl indole-3-carboxylate	Mianserin
Alpha-Methyltryptamine	Trimipramine	Adr-851	Mirisetron Maleate
Bufotenin		Adr-882	Mirtazapine
Chlorophenyl-biguanide		Alosetron	Ml-1035
Cisapride		Amoxapine	Mm-218

Agonists	Modulators/ Binders	Antagonists	
DDP733		Anpirtoline	N-3256
Ethanol		Aripiprazole	Netupitant
Ethanol		AS-8112	Olanzapine
Ibogaine		Azasetron	Ondansetron
Metoclopramide		Batanopride	Org-4419
Phenylbiguanide		Bimu-1	Org-4419-2
Quipazine		Chloroprocaine	Palonosetron
RS-56812		Cilansetron	Pancopride
SR-57227		Clozapine	Procaine
Tapentadol		Cp-81386	Quetiapine
Varenicline		Cp-93318	R-093777
YM-31636		Cr-3124	R-Zacopride
		DA-9701	Ramosetron
		Daizac	Renzapride
		Dat-582	Rg-12915
		Dau-6285	Ricasetron
		Ddp-225	Rocuronium
		Dolasetron	Rs-16566
		E-3620	Rs-33800
		Fabesetron	Rs-56532
		Facinicline/RG3487 Hydrochloride	Rs-56812
		Galdansetron	S-21007
		Gastroprokinetics	Sc-50410
		Gk-128	Sc-52150
		Gr-65630	Sc-52246
		Granisetron	Sc-52491
		Gyki-46903	Sc-54750
		Itasetron	Sdz-Icm-567
		Kb-6806	Sep-226332
		Kf-18259	Srss-021
		Kf-20170	Tedatioxetine
		Kga-0941	Thujone
		L-683877	Topiramate
		Lamotrigine	Tropisetron
		Lerisetron	Tubocurarine
		Lintopride	Tzb-30878
		Litoxetine	Va-21B7

Agonists	Modulators/ Binders	Antagonists	
		Loxapine	Vi-0134
		Lurosetron	Vortioxetine
		Ly-278584	Vortioxetine Hydrobromide
		M1, the major active metabolite of mosapride	Way-100289
		Mci-225	Ym-114
		Mci-225	Ym-26103-2
		Memantine	Ym-26308-2
		Menthol	Zacopride
		Methadone	Zatosetron
		Metoclopramide	Ziprasidone

Table 5: Binding Agents for Nicotinic Acetylcholine Receptor (nAchR)

Agonists	Modulators/Binders	Antagonists
(+)-N-(1-azabicyclo[2.2.2]oct-3-yl)benzo[b]furan- 2-carboxamide	A-867744	(−)-7-methyl-2-exo-[3'-(6-[18F]fluoropyridin-2-yl)-5'-pyridinyl]-7-azabicyclo[2.2.1]heptane
3-Bromocytisine	AQW-051	2-fluoro-3-(4-nitro-phenyl)deschloroepibatidine
A-366,833	AVL-3288	Acv-1
A-582941	Desformylflustrabromine	ACVx
A-82695	Ethanol	Amobarbital
A-84,543	Galantamine	Anandamide
ABT-089	Ivermectin	Anq-9040
ABT-126	Nefiracetam	Aprobarbital
Abt-202	NS-1738	ATG-001
ABT-418	NS-9283	ATG003
Abt-418	PNU-120,596	Atracurium besylate
Abt-560	Tetraethylammonium	Barbital
ABT-894		Barbituric acid derivative
Acetylcholine		Biperiden
Altinicline		Bupropion
Altinicline		Butabarbital
Aminoethoxypyridine		Butalbital
Anabasine		Butethal
AR-R17779		Chloroprocaine
Asm-024		Cisatracurium Besilate
AZD-0328		Cisatracurium besylate

Agonists	Modulators/Binders	Antagonists
Bupropion Hydrochloride		Coclaurine
Carbachol		Dehydronorketamine
Choline		Deuterated Bupropion
Cm-2433		Dextromethorphan
CP-601927		Doxacurium chloride
CP-601932		Ethanol
Cp-810123		Gallamine Triethiodide
Cytisine		Heptabarbital
DBO-83		Hexobarbital
Decamethonium		Hydroxybupropion
Dianicline		Hydroxynorketamine
Dianicline		Inaperisone
Encenicline		Iptakalim
Epibatidine		Isoflurane
EVP-6124		Ketamine
Evp-6124		Kynurenic acid
Galantamine		Levomethadyl Acetate
GTS-21		Lobeline
Gts-21		Mecamylamine
ICH-3		Mecamylamine
Ispronicline		Memantine
Levamisole		Methadone
Lobeline		Metharbital
Lobeline Sulphate		Methyllycaconitine
Mem-3454		Methylphenobarbital
MEM-63908		Metocurine
Mem-63908		Metocurine Iodide
N-(3-pyridinyl)-bridged bicyclic diamine		Mivacurium
Nicotine		Neramexane
PH-399733		Nic-002
Ph-399733		Nitrous oxide
PHA-543,613		Norketamine
Pha-543613		Org-9991
PHA-709829		Pancuronium
PNU-282,987		Pentobarbital
Pnu-282987		Pentolinium
Pozanicline		Phenobarbital

Agonists	Modulators/Binders	Antagonists
Pozanicline		Pipecuronium
Rivanicline		Pipecuronium
Rivanicline		PNU-120,596
Rjr-1401		Primidone
Sar-130479		Procaine
Sazetidine A		Quinolizidine (–)-1-epi-207I
Sib-1663		RJR-2531
Sib-1765f		Rocuronium
Sib-3182		RPI-78M
Sofinicline		Secobarbital
SSR-180,711		Talbutal
Succinylcholine		Thiopental
Suvn-F90101		Trimethaphan
Suvn-F91201		Tubocurarine
Tacrine-huperzine A		U-2902
TC-1698		Vecuronium
TC-1827		α-Bungarotoxin
Tc-2216		α-Conotoxin
Tc-2403		
Tc-2429		
Tc-2559		
Tc-2696		
TC-5619		
Tebanicline		
Tropisetron		
Ub-165		
Varenicline		
WAY-317,538		

Table 6: Binding Agents for ATP-Gated P2X Receptor Cation Channel (P2X)

Agonists	Modulators /Binders	Antagonists	
ATP	Ivermectin	4-benzoyl-1-substituted-piperazin-2-ones	MK-801
BzATP		5-methyl-6,7-dihydro-5Hcyclopentapyrazine	MRS2159
α,β-meATP		5-oxo-3-pyrrolidinecarboxamides	NF023
		5,6,7,8-tetrahydropyrido[4,3,d]-pyrimidines	NF279

Agonists	Modulators /Binders	Antagonists	
		A-317491	NF449
		Amitriptyline	Nortriptyline
		Azaindole-3-carboxamides	Oxoisoquinoline carboxamides
		AZD-9056	Paroxetine
		Benzamide 6	Piperazines
		Benzofuro-1,4-diazepin-2-ones	Polycyclic guanines
		Benzoimidazoles	PPADS
		Biaryl benzamides	PPNDS
		Carboxamides	Pyrazolo-[1,5,a]-pyridine carboxamides
		CE-224535	Pyridazinones
		Desipramine	Pyridoxal-5-phosphate
		Diaminopyridines	Pyrrolinones
		Doxepin	Pyrrolo-[2,3,b]-pyridine carboxamides
		Eslicarbazepine acetate	Pyrrolopyrimidin-7-ones
		EVT-401	Quinoline carboxamides
		Fluoxetine	RO-3
		Hyaluronic acid derivatives	RO-4
		Imipramine	RO-51
		Indole carboxamides	Spinorphins
		Indole-3-carboxamides	Suramin
		Ip5I	Tetrahydro-2H-1,2-thiazine 1,1-dioxides
		Isoquinoline carboxamides	TNP-ATP
		Isothiazolidine 1,1-dioxides	

Table 7: Binding Agents for Inwardly Rectifying Potassium Channel (Kir)

Agonists	Modulators / Binders	Antagonists
Diazoxide		Acetohexamide
Iptakalim		Carbutamide
Minoxidil		Chlorpropamide
Nicorandil		Glibenclamide
Phosphatidylinositol 4,5-Bisphosphate		Glibenclamide
Pinacidil		Glibornuride
		Gliclazide
		Glimepiride
		Glipizide
		Gliquidone

Agonists	Modulators / Binders	Antagonists
		Glisoxepide
		Glyburide
		Glyclopyramide
		Glycyclamide
		Metahexamide
		Tolazamide
		Tolbutamide
		Tolhexamide

Table 8: Binding Agents for Voltage Dependent Potassium Channel (KCNQ/Kv7)

Agonists	Modulators / Binders	Antagonists
Diazoxide		Azimilide
Flupirtine		Amiodarone
Minoxidil		Bretylium
Nicorandil		Clofilium
Pinacidil		Dalfampridine
Retigabine		Dofetilide
		E-4031
		Ibutilide
		Nifekalant
		Sematilide
		Sotalol
		Sulfonylureas
		Tedisamil

Table 9: Binding Agents for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Agonists	Modulators / Binders	Antagonists
	8-cyclopentyl-1,3-dipropylxanthine	Bumetanide
	8-methoxypsoralen	Crofelemer
	Apigenin	Glyburide
	CTP-656	Ibuprofen
	Genistein	
	IBMX	
	Ivacaftor	
	Lumacaftor	

E. Polynucleotides

[0138] In various illustrative embodiments, the present disclosure contemplates, in part, polynucleotides, polynucleotides encoding engineered receptor polypeptides including LGICs, and subunits and muteins thereof, and fusion polypeptides, viral vector polynucleotides, and compositions comprising the same..

[0139] As used herein, the terms "polynucleotide," "nucleotide," "nucleotide sequence" or "nucleic acid" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Polynucleotides may be deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or DNA/RNA hybrids. Polynucleotides may be single-stranded or double-stranded. Polynucleotides include, but are not limited to: pre-messenger RNA (pre-mRNA), messenger RNA (mRNA), RNA, short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), ribozymes, synthetic RNA, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)), synthetic RNA, genomic DNA (gDNA), PCR amplified DNA, complementary DNA (cDNA), synthetic DNA, or recombinant DNA. Polynucleotides refer to a polymeric form of nucleotides of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 1000, at least 5000, at least 10000, or at least 15000 or more nucleotides in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, as well as all intermediate lengths. It will be readily understood that "intermediate lengths" in this context, means any length between the quoted values, such as 6, 7,

8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.* In particular embodiments, polynucleotides or variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence described herein or known in the art, typically where the variant maintains at least one biological activity of the reference sequence unless otherwise stated.

[0140] As used herein, the term "gene" may refer to a polynucleotide sequence comprising enhancers, promoters, introns, exons, and the like. In particular embodiments, the term "gene" refers to a polynucleotide sequence encoding a polypeptide, regardless of whether the polynucleotide sequence is identical to the genomic sequence encoding the polypeptide.

[0141] As used herein, a "cis-acting sequence, "cis-acting regulatory sequence", or "cis-acting nucleotide sequence" or equivalents refers to a polynucleotide sequence that is associated with the expression, e.g. transcription and/or translation, of a gene. In one embodiment, the cis-acting sequence regulates transcription because it is a binding site for a polypeptide that represses or decreases transcription or a polynucleotide sequence associated with a transcription factor binding site that contributes to transcriptional repression. Examples of cis-acting sequences that regulate the expression of polynucleotide sequences and that may be operably linked to the polynucleotides of the present disclosure to regulate the expression of the subject engineered receptors are well known in the art and include such elements as promoter sequences (e.g CAG, CMV, SYN, CamKII, TRPV1), Kozak sequences, enhancers, posttranscriptional regulatory elements, miRNA binding elements, and polyadenylation sequences.

[0142] As one non-limiting example, a promoter sequence is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Various promoters may be used to drive the various vectors of the present invention. For example, the promoter may be a constitutively active promoter, i.e. a promoter that is active in the absence

externally applied agents, e.g. the CMV IE1 promoter, the SV40 promoter, GAPDH promoter, Actin promoter. The promoter may be an inducible promoter, i.e. a promoter whose activity is regulated upon the application of an agent to the cell, e.g. doxycycline, the tet-on or tet-off promoter, the estrogen receptor promoter, etc. The promoter may be a tissue-specific promoter, i.e. a promoter that is active on certain types of cells.

[0143] In some embodiments, the promoter is active in an excitable cell. By an "excitable cell", it is meant a cell that is activated by a change in membrane potential, e.g. a neuron or myocyte, e.g. a dorsal root ganglion neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron. Promoters that are active in an excitable cell that would find use in the present polynucleotide compositions would include neuronal promoters, for example, the synapsin (SYN), TRPV1, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, CamKII, NSE, and Advillin promoters; myocyte promoters, e.g. the desmin (Des), alpha-myosin heavy chain (α-MHC), myosin light chain 2 (MLC-2) and cardiac troponin C (cTnC) promoters; and ubiquitous acting promoters, e.g. CAG, CBA, E1Fa, Ubc, CMV, and SV40 promoters.

[0144] As used herein, a "regulatory element for inducible expression" refers to a polynucleotide sequence that is a promoter, enhancer, or functional fragment thereof that is operably linked to a polynucleotide to be expressed and that responds to the presence or absence of a molecule that binds the element to increase (turn-on) or decrease (turn-off) the expression of the polynucleotide operably linked thereto. Illustrative regulatory elements for inducible expression include, but are not limited to, a tetracycline responsive promoter, an ecdysone responsive promoter, a cumate responsive promoter, a glucocorticoid responsive promoter, an estrogen responsive promoter, an RU-486 responsive promoter, a PPAR-γ promoter, and a peroxide inducible promoter.

[0145] A "regulatory element for transient expression" refers to a polynucleotide sequence that can be used to briefly or temporarily express a polynucleotide nucleotide sequence. In particular embodiments, one or more regulatory elements for transient expression can be used to limit the duration of a polynucleotide. In certain embodiments, the preferred duration of polynucleotide expression is on the order of minutes, hours, or days. Illustrative regulatory elements for transient expression include, but are not limited to, nuclease target sites, recombinase recognition sites, and inhibitory RNA target sites. In addition, to some extent, in particular

embodiments, a regulatory element for inducible expression may also contribute to controlling the duration of polynucleotide expression.

[0146] As used herein, the terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion, substitution, or modification of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or modified, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. In particular embodiments, polynucleotides or variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence described herein or known in the art, typically where the variant maintains at least one biological activity of the reference sequence unless otherwise stated.

[0147] In one embodiment, a polynucleotide comprises a nucleotide sequence that hybridizes to a target nucleic acid sequence under stringent conditions. To hybridize under "stringent conditions" describes hybridization protocols in which nucleotide sequences at least 60% identical to each other remain hybridized. Generally, stringent conditions are selected to be about 5ºC lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium.

[0148] The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned

sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.,* 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0149] An "isolated polynucleotide," as used herein, refers to a polynucleotide that has been purified from the sequences which flank it in a naturally-occurring state, *e.g.*, a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment. In particular embodiments, an "isolated polynucleotide" refers to a complementary DNA (cDNA), a recombinant DNA, or other polynucleotide that does not exist in nature and that has been made by the hand of man.

[0150] Terms that describe the orientation of polynucleotides include: 5' (normally the end of the polynucleotide having a free phosphate group) and 3' (normally the end of the polynucleotide having a free hydroxyl (OH) group). Polynucleotide sequences can be annotated in the 5' to 3' orientation or the 3' to 5' orientation. For DNA and mRNA, the 5' to 3' strand is designated the "sense," "plus," or "coding" strand because its sequence is identical to the sequence of the pre-messenger (premRNA) [except for uracil (U) in RNA, instead of thymine (T) in DNA]. For DNA and mRNA, the complementary 3' to 5' strand which is the strand transcribed by the RNA polymerase is designated as "template," "antisense," "minus," or "non-coding" strand. As used herein, the term "reverse orientation" refers to a 5' to 3' sequence written in the 3' to 5' orientation or a 3' to 5' sequence written in the 5' to 3' orientation.

[0151] The term "flanked" refers to a polynucleotide sequence that is in between an upstream polynucleotide sequence and/or a downstream poylnucleotide sequence, *i.e.*, 5' and/or 3', relative to the sequence. For example, a sequence that is "flanked" by two other elements (*e.g.*, ITRs), indicates that one element is located 5' to the sequence and the other is located 3' to the sequence; however, there may be intervening sequences therebetween.

[0152] The terms "complementary" and "complementarity" refer to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the complementary strand of the DNA sequence 5' A G T C A T G 3' is 3' T C A G T A C 5'. The latter sequence is often written as the reverse complement with the 5' end on the left and the 3' end on the right, 5' C A T G A C T 3'. A sequence that is equal to its reverse complement is said to be a palindromic sequence. Complementarity can be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there can be "complete" or "total" complementarity between the nucleic acids.

[0153] The terms "nucleic acid cassette" or "expression cassette" as used herein refers to polynucleotide sequences within a larger polynucleotide, such as a vector, which are sufficient to

express one or more RNAs from a polynucleotide. The expressed RNAs may be translated into proteins, may function as guide RNAs or inhibitory RNAs to target other polynucleotide sequences for cleavage and/or degradation. In one embodiment, the nucleic acid cassette contains one or more polynucleotide(s)-of-interest. In another embodiment, the nucleic acid cassette contains one or more expression control sequences operably linked to one or more polynucleotide(s)-of-interest. Polynucleotides include polynucleotide(s)-of-interest. As used herein, the term "polynucleotide-of-interest" refers to a polynucleotide encoding a polypeptide or fusion polypeptide or a polynucleotide that serves as a template for the transcription of an inhibitory polynucleotide, *e.g.*, LGICs, and subunits and muteins thereof, as contemplated herein. In a particular embodiment, a polynucleotide-of-interest encodes a polypeptide or fusion polypeptide having one or more enzymatic activities, such as a nuclease activity and/or chromatin remodeling or epigenetic modification activities.

[0154] Vectors may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more nucleic acid cassettes. In a preferred embodiment of the disclosure, a nucleic acid cassette comprises one or more expression control sequences (*e.g.*, a promoter or enhancer operable in a neuronal cell) operably linked to a polynucleotide encoding a engineered receptor, *e.g.*, an LGIC, or subunit or muteins thereof. The cassette can be removed from or inserted into other polynucleotide sequences, *e.g.*, a plasmid or viral vector, as a single unit.

[0155] In one embodiment, a polynucleotide contemplated herein comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or more nucleic acid cassettes any number or combination of which may be in the same or opposite orientations.

[0156] Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that may encode a polypeptide, or fragment of variant thereof, as contemplated herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure, for example polynucleotides that are optimized for human and/or primate codon selection. In one embodiment, polynucleotides comprising particular allelic sequences are provided. Alleles are endogenous polynucleotide sequences that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides.

F. Vectors

[0157] In some aspects of the disclosure, a nucleic acid molecule, i.e., a polynucleotide encoding an engineered receptor is delivered to a subject. In some cases, the nucleic acid molecule encoding the engineered receptor is delivered to a subject by a vector. In various embodiments, a vector comprises a one or more polynucleotide sequences contemplated herein. The term "vector" is used herein to refer to a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule. The transferred polynucleotide is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. A vector can deliver a target polynucleotide to an organism, a cell or a cellular component. In some cases, the vector is an expression vector. An "expression vector" as used herein refers to a vector, for example, a plasmid, that is capable of promoting expression, as well as replication of a polynucleotide incorporated therein. Typically, the nucleic acid sequence to be expressed is operably linked to cis-acting regulatory sequence, e.g. a promoter and/or enhancer sequence, and is subject to transcription regulatory control by the promoter and/or enhancer. In particular cases, a vector is used to deliver a nucleic acid molecule encoding an engineered receptor of the disclosure to a subject.

[0158] In particular embodiments, any vector suitable for introducing an expression cassette or polynucleotide encoding an engineered receptor into a neuronal cell can be employed. Illustrative examples of suitable vectors include plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. In some cases, the vector is a circular nucleic acid, for *e.g.*, a plasmid, a BAC, a PAC, a YAC, a cosmid, a fosmid, and the like. In some cases, circular nucleic acid molecules can be utilized to deliver a nucleic acid molecule encoding an engineered receptor to a subject. For example, a plasmid DNA molecule encoding an engineered receptor can be introduced into a cell of a subject whereby the DNA sequence encoding the engineered receptor is transcribed into mRNA and the mRNA "message" is translated into a protein product. The circular nucleic acid vector will generally include regulatory elements that regulate the expression of the target protein. For example, the circular nucleic acid vector may include any number of promoters, enhancers, terminators, splice signals, origins of replication, initiation signals, and the like.

[0159] In some cases, the vector can include a replicon. A replicon may be any nucleic acid molecule capable of self-replication. In some cases, the replicon is an RNA replicon derived from a virus. A variety of suitable viruses (*e.g.* RNA viruses) are available, including, but not limited to, alphavirus, picornavirus, flavivirus, coronavirus, pestivirus, rubivirus, calcivirus, and hepacivirus.

[0160] In some embodiments, the vector is a non-viral vector. By a "non-viral vector", it is meant any delivery vehicle that does not comprise a viral capsid or envelope, *e.g.* lipid nanoparticles (anionic (negatively charged), neutral, or cationic (positively charged)), heavy metal nanoparticles, polymer-based particles, plasmid DNA, minicircle DNA, minivector DNA, ccDNA, synthetic RNA, exosomes, and the like. Non-viral vectors may be delivered by any suitable method as would be well understood in the art, including, *e.g.*, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection. See, *e.g.* Chen et al. Mol. Therapy, Methods and Clinical Development. 2016 Jan; Vol 3, issue 1; and Hardy, CE et al. Genes (Basel). 2017 Feb; 8(2): 65

[0161] In other embodiments, the vector is a viral vector. By a "viral vector" it is meant a delivery vehicle that comprises a viral capsid or envelop surrounding a polynucleotide encoding an RNA or polypeptide of interest. In some cases, the viral vector is derived from a replication-deficient virus. Non-limiting examples of viral vectors suitable for delivering a nucleic acid molecule of the disclosure to a subject include those derived from adenovirus, retrovirus (*e.g.*, lentivirus), adeno-associated virus (AAV), and herpes simplex-1 (HSV-1). Illustrative examples of suitable viral vectors include, but are not limited to, retroviral vectors (*e.g.,* lentiviral vectors), herpes virus based vectors and parvovirus based vectors (*e.g.*, adeno-associated virus (AAV) based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors).

[0162] The term "parvovirus" as used herein encompasses all parvoviruses, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus, Erythrovirus, Densovirus, Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, mouse minute virus, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.*, Fields *et al.*, 1996 *Virology*, volume 2, chapter 69 (3d ed., Lippincott-Raven Publishers).

[0163] The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type rh10, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV.

[0164] In a preferred embodiment, the vector is an AAV vector. In particular cases, the viral vector is an AAV-6 or AAV-9 vector.

[0165] The genomic organization of all known AAV serotypes is similar. The genome of AAV is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides (nt) in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VPl, -2 and -3) form the capsid and contribute to the tropism of the virus. The terminal 145 nt ITRs are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild-type (wt) AAV infection in mammalian cells the Rep genes are expressed and function in the replication of the viral genome.

[0166] In some cases, the outer protein "capsid" of the viral vector occurs in nature, *e.g.* AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In particular cases, the capsid is synthetically engineered (*e.g.* through directed evolution or rational design) to possess certain unique characteristics not present in nature such as altered tropism, increased transduction efficiency, or immune evasion. An example of a rationally designed capsid is the mutation of one or more surface-exposed tyrosine (Y), serine (S), threonine (T), and lysine (K) residues on the VP3 viral capsid protein. Non-limiting examples of viral vectors whose VP3 capsid proteins have been synthetically engineered and are amenable for use with the compositions and methods provided herein include: AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV5(Y436+693+719F), AAV6(Y705+731F+T492V), AAV8(Y733F), AAV9(Y731F), and AAV10(Y733F). Non-limiting examples of viral vectors that have been engineered through directed evolution and are amenable for use with the compositions and methods provided herein include AAV-7m8 and AAV-ShH10.

[0167] A "recombinant parvoviral or AAV vector" (or "rAAV vector") herein refers to a vector comprising one or more polynucleotides contemplated herein that are flanked by one or more AAV ITRs. Such polynucleotides are said to be "heterologous" to the ITRs, as such combinations do not ordinarily occur in nature. Such rAAV vectors can be replicated and packaged into infectious viral particles when present in an insect host cell that is expressing AAV rep and cap gene products (*i.e.*, AAV Rep and Cap proteins). When an rAAV vector is incorporated into a larger nucleic acid construct (*e.g.*, in a chromosome or in another vector such as a plasmid or baculovirus used for cloning or transfection), then the rAAV vector is typically referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and necessary helper functions.

[0168] In particular embodiments, any AAV ITR may be used in the AAV vectors, including ITRs from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, and AAV16. In one preferred embodiment, an AAV vector contemplated herein comprises one or more AAV2 ITRs.

[0169] rAAV vectors comprising two ITRs have a payload capacity of about 4.4 kB. Self-complementary rAAV vectors contain a third ITR and package two strands of the recombinant portion of the vector leaving only about 2.1 kB for the polynucleotides contemplated herein. In one embodiment, the AAV vector is an scAAV vector.

[0170] Extended packaging capacities that are roughly double the packaging capacity of an rAAV (about 9kB) have been achieved using dual rAAV vector strategies. Dual vector strategies useful in producing rAAV contemplated herein include, but are not limited to splicing (trans-splicing), homologous recombination (overlapping), or a combination of the two (hybrid). In the dual AAV trans-splicing strategy, a splice donor (SD) signal is placed at the 3′ end of the 5′-half vector and a splice acceptor (SA) signal is placed at the 5′ end of the 3′-half vector. Upon co-infection of the same cell by the dual AAV vectors and inverted terminal repeat (ITR)-mediated head-to-tail concatemerization of the two halves, trans-splicing results in the production of a mature mRNA and full-size protein (Yan *et al.*, 2000). Trans-splicing has been successfully used to express large genes in muscle and retina (Reich *et al.*, 2003; Lai *et al.*, 2005). Alternatively, the two halves of a large transgene expression cassette contained in dual AAV vectors may contain homologous overlapping sequences (at the 3′ end of the 5′-half vector and at the 5′ end of the 3′-half vector, dual AAV overlapping), which will mediate reconstitution of a single large genome

by homologous recombination (Duan *et al.*, 2001). This strategy depends on the recombinogenic properties of the transgene overlapping sequences (Ghosh *et al.*, 2006). A third dual AAV strategy (hybrid) is based on adding a highly recombinogenic region from an exogenous gene (*i.e.*, alkaline phosphatase; Ghosh *et al.*, 2008, Ghosh *et al.*, 2011)) to the trans-splicing vectors. The added region is placed downstream of the SD signal in the 5′-half vector and upstream of the SA signal in the 3′-half vector in order to increase recombination between the dual AAVs.

[0171] A "hybrid AAV" or "hybrid rAAV" refers to an rAAV genome packaged with a capsid of a different AAV serotype (and preferably, of a different serotype from the one or more AAV ITRs), and may otherwise be referred to as a pseudotyped rAAV. For example, an rAAV type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 genome may be encapsidated within an AAV type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 capsid or variants thereof, provided that the AAV capsid and genome (and preferably, the one or more AAV ITRs) are of different serotypes. In certain embodiments, a pseudotyped rAAV particle may be referred to as being of the type "x/y ", where "x" indicates the source of ITRs and "y" indicates the serotype of capsid, for example a 2/5 rAAV particle has ITRs from AAV2 and a capsid from AAV6.

[0172] A "host cell" includes cells transfected, infected, or transduced *in vivo, ex vivo,* or *in vitro* with a recombinant vector or a polynucleotide of the disclosure. Host cells may include virus producing cells and cells infected with viral vectors. In particular embodiments, host cells *in vivo* are infected with viral vector contemplated herein. In certain embodiments, the term "target cell" is used interchangeably with host cell and refers to infected cells of a desired cell type.

[0173] High titer AAV preparations can be produced using techniques known in the art, *e.g.*, as described in U.S. Pat. Nos. 5,658,776; 6,566,118; 6,989,264; and 6,995,006; U.S. 2006/0188484; WO98/22607; WO2005/072364; and WO/1999/011764; and Viral Vectors for Gene Therapy: Methods and Protocols, ed. Machida, Humana Press, 2003; Samulski et al., (1989) J. Virology 63, 3822 ; Xiao et al., (1998) J. Virology 72, 2224 ; lnoue et al., (1998) J. Virol. 72, 7024. Methods of producing pseudotyped AAV vectors have also been reported (*e.g.*, WO 00/28004), as well as various modifications or formulations of AAV vectors, to reduce their immunogenicity upon in vivo administration (*see e.g.*, WO 01/23001; WO 00/73316; WO 04/1 12727; WO 05/005610; WO 99/06562).

G. Pharmaceutical compositions

[0174] Also provided are pharmaceutical preparations, including pharmaceutical preparations of vector and pharmaceutical preparations of binding agent. Pharmaceutical preparations include the subject polynucleotide (RNA or DNA) encoding an engineered receptor, vector carrying a polynucleotide (RNA or DNA) encoding a subject engineered receptor, or binding agent present in a pharmaceutically acceptable vehicle. "Pharmaceutically acceptable vehicles" may be vehicles approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, such as humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the disclosure is formulated for administration to a mammal. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a mammal, the compounds and compositions of the disclosure and pharmaceutically acceptable vehicles, excipients, or diluents may be sterile. In some instances, an aqueous medium is employed as a vehicle when the compound of the disclosure is administered intravenously, such as water, saline solutions, and aqueous dextrose and glycerol solutions.

[0175] Pharmaceutical compositions can take the form of capsules, tablets, pills, pellets, lozenges, powders, granules, syrups, elixirs, solutions, suspensions, emulsions, suppositories, or sustained-release formulations thereof, or any other form suitable for administration to a mammal. In some instances, the pharmaceutical compositions are formulated for administration in accordance with routine procedures as a pharmaceutical composition adapted for oral or intravenous administration to humans. Examples of suitable pharmaceutical vehicles and methods for formulation thereof are described in Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro ed., Mack Publishing Co. Easton, Pa., 19th ed., 1995, Chapters 86, 87, 88, 91, and 92, incorporated herein by reference.

[0176] The choice of excipient will be determined in part by the particular vector, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present disclosure.

[0177] For example, the vector may 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.

[0178] As another example, the vector may be formulated into a preparation suitable for oral administration, including (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, or saline; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can include the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles including the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are described herein.

[0179] As another example, the subject formulations of the present disclosure can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They may also be formulated as pharmaceuticals for non-pressured preparations such as for use in a nebulizer or an atomizer.

[0180] In some embodiments, formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions

and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0181] Formulations suitable for topical administration may be presented as creams, gels, pastes, or foams, containing, in addition to the active ingredient, such carriers as are appropriate. In some embodiments the topical formulation contains one or more components selected from a structuring agent, a thickener or gelling agent, and an emollient or lubricant. Frequently employed structuring agents include long chain alcohols, such as stearyl alcohol, and glyceryl ethers or esters and oligo(ethylene oxide) ethers or esters thereof. Thickeners and gelling agents include, for example, polymers of acrylic or methacrylic acid and esters thereof, polyacrylamides, and naturally occurring thickeners such as agar, carrageenan, gelatin, and guar gum. Examples of emollients include triglyceride esters, fatty acid esters and amides, waxes such as beeswax, spermaceti, or carnauba wax, phospholipids such as lecithin, and sterols and fatty acid esters thereof. The topical formulations may further include other components, *e.g.*, astringents, fragrances, pigments, skin penetration enhancing agents, sunscreens (i.e., sunblocking agents), etc.

[0182] A compound of the disclosure may be formulated for topical administration. The vehicle for topical application may be in one of various forms, *e.g.* a lotion, cream, gel, ointment, stick, spray, or paste. They may contain various types of carriers, including, but not limited to, solutions, aerosols, emulsions, gels, and liposomes. The carrier may be formulated, for example, as an emulsion, having an oil-in-water or water-in-oil base. Suitable hydrophobic (oily) components employed in emulsions include, for example, vegetable oils, animal fats and oils, synthetic hydrocarbons, and esters and alcohols thereof, including polyesters, as well as organopolysiloxane oils. Such emulsions also include an emulsifier and/or surfactant, *e.g.* a nonionic surfactant to disperse and suspend the discontinuous phase within the continuous phase.

[0183] Suppository formulations are also provided by mixing with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams.

[0184] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may include

the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[0185] 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 compounds of the present disclosure calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present disclosure depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[0186] Dose levels can vary as a function of the specific compound, the nature of the delivery vehicle, and the like. Desired dosages for a given compound are readily determinable by a variety of means.

[0187] The dose administered to an animal, particularly a human, in the context of the present disclosure should be sufficient to effect a prophylactic or therapeutic response in the animal over a reasonable time frame, *e.g.*, as described in greater detail below. Dosage will depend on a variety of factors including the strength of the particular compound employed, the condition of the animal, and the body weight of the animal, as well as the severity of the illness and the stage of the disease. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound.

[0188] In pharmaceutical dosage forms, the ASC inducer compounds may be administered in the form of a free base, 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.

G. Clinical Applications and Methods of Treatment

[0189] The compositions and methods disclosed herein can be utilized to treat a neurological disease or disorder. In some aspects of the disclosure, a method of treating a neurological disease or disorder in a subject is provided, the method comprising the introducing an engineered receptor into a neuronal cell and providing a ligand that activates the engineered receptor in an effective amount to control the activity of the cell, thereby relieving pain in the

subject. In some aspects, vectors or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder.

[0190] In some cases, the methods and compositions of the disclosure are utilized to treat epilepsy. Compositions described herein may be used to prevent or control epileptic seizures. Epileptic seizures may be classified as tonic-clonic, tonic, clonic, myoclonic, absence or atonic seizures. In some cases, the compositions and methods herein may prevent or reduce the number of epileptic seizures experienced by a subject by about 5%, about 10%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99% or 100%.

[0191] In some cases, the methods and compositions of the disclosure are utilized to treat an eating disorder. An eating disorder may be a mental disorder defined by abnormal eating behaviors that negatively affect a subject's physical or mental health. In some cases, the eating disorder is anorexia nervosa. In other cases, the eating disorder is bulimia nervosa. In some cases, the eating disorder is pica, rumination disorder, avoidant/restrictive food intake disorder, binge eating disorder (BED), other specified feeding and eating disorder (OSFED), compulsive overeating, diabulimia, orthorexia nervosa, selective eating disorder, drunkorexia, pregorexia, or Gourmand syndrome. In some cases, the composition includes a G-protein coupled receptor that increases or decreases the production of one or more molecules associated with an eating disorder. In other cases, the composition includes a ligand-gated ion channel that alters the production of one or more molecules associated with an eating disorder. The one or more molecules associated with an eating disorder may include, without limitation, a molecule of the hypothalamus-pituitary-adrenal (HPA) axis, including vasopressin, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), cortisol, epinephrine, or norepinephrine; as well as serotonin, dopamine, neuropeptide Y, leptin, or ghrelin.

[0192] In some cases, the compositions and methods are utilized to treat post-traumatic stress disorder (PTSD), gastroesophageal reflex disease (GERD), addiction (*e.g.*, alcohol, drugs), anxiety, depression, memory loss, dementia, sleep apnea, stroke, urinary incontinence, narcolepsy, essential tremor, movement disorder, atrial fibrillation, cancer (*e.g.*, brain tumors), Parkinson's disease, or Alzheimer's disease. Other non-limiting examples of neurological diseases or disorders that can be treated by the compositions and methods herein include: Abulia, Agraphia, Alcoholism,

Alexia, Aneurysm, Amaurosis fugax, Amnesia, Amyotrophic lateral sclerosis (ALS), Angelman syndrome, Aphasia, Apraxia, Arachnoiditis, Arnold-Chiari malformation, Asperger syndrome, Ataxia, Ataxia-telangiectasia, Attention deficit hyperactivity disorder, Auditory processing disorder, Autism spectrum, Bipolar disorder, Bell's palsy, Brachial plexus injury, Brain damage, Brain injury, Brain tumor, Canavan disease, Capgras delusion, Carpal tunnel syndrome, Causalgia, Central pain syndrome, Central pontine myelinolysis, Centronuclear myopathy, Cephalic disorder, Cerebral aneurysm, Cerebral arteriosclerosis, Cerebral atrophy, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Cerebral gigantism, Cerebral palsy, Cerebral vasculitis, Cervical spinal stenosis, Charcot-Marie-Tooth disease, Chiari malformation, Chorea, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic pain, Coffin–Lowry syndrome, Coma, Complex regional pain syndrome, Compression neuropathy, Congenital facial diplegia, Corticobasal degeneration, Cranial arteritis, Craniosynostosis, Creutzfeldt-Jakob disease, Cumulative trauma disorders, Cushing's syndrome, Cyclothymic disorder, Cytomegalic inclusion body disease (CIBD), Cytomegalovirus Infection, Dandy-Walker syndrome, Dawson disease, De Morsier's syndrome, Dejerine-Klumpke palsy, Dejerine-Sottas disease, Delayed sleep phase syndrome, Dementia, Dermatomyositis, Developmental coordination disorder, Diabetic neuropathy, Diffuse sclerosis, Diplopia, Down syndrome, Dravet syndrome, Duchenne muscular dystrophy, Dysarthria, Dysautonomia, Dyscalculia, Dysgraphia, Dyskinesia, Dyslexia, Dystonia, Empty sella syndrome, Encephalitis, Encephalocele, Encephalotrigeminal angiomatosis, Encopresis, Enuresis, Epilepsy, Epilepsy-intellectual disability in females, Erb's palsy, Erythromelalgia, Exploding head syndrome, Fabry's disease, Fahr's syndrome, Fainting, Familial spastic paralysis, Febrile seizures, Fisher syndrome, Friedreich's ataxia, Fibromyalgia, Foville's syndrome, Fetal alcohol syndrome, Fragile X syndrome, Fragile X-associated tremor/ataxia syndrome (FXTAS), Gaucher's disease, Generalized epilepsy with febrile seizures plus, Gerstmann's syndrome, Giant cell arteritis, Giant cell inclusion disease, Globoid Cell Leukodystrophy, Gray matter heterotopia, Guillain-Barré syndrome, Generalized anxiety disorder, HTLV-1 associated myelopathy, Hallervorden-Spatz disease, Head injury, Headache, Hemifacial Spasm, Hereditary Spastic Paraplegia, Heredopathia atactica polyneuritiformis, Herpes zoster oticus, Herpes zoster, Hirayama syndrome, Hirschsprung's disease, Holmes-Adie syndrome, Holoprosencephaly, Huntington's disease, Hydranencephaly, Hydrocephalus, Hypercortisolism, Hypoxia, Immune-Mediated

encephalomyelitis, Inclusion body myositis, Incontinentia pigmenti, Infantile Refsum disease, Infantile spasms, Inflammatory myopathy, Intracranial cyst, Intracranial hypertension, Isodicentric 15, Joubert syndrome, Karak syndrome, Kearns-Sayre syndrome, Kinsbourne syndrome, Kleine-Levin Syndrome, Klippel Feil syndrome, Krabbe disease, Lafora disease, Lambert-Eaton myasthenic syndrome, Landau-Kleffner syndrome, Lateral medullary (Wallenberg) syndrome, Learning disabilities, Leigh's disease, Lennox-Gastaut syndrome, Lesch-Nyhan syndrome, Leukodystrophy, Leukoencephalopathy with vanishing white matter, Lewy body dementia, Lissencephaly, Locked-In syndrome, Lumbar disc disease, Lumbar spinal stenosis, Lyme disease - Neurological Sequelae, Machado-Joseph disease (Spinocerebellar ataxia type 3), Macrencephaly, Macropsia, Mal de debarquement, Megalencephalic leukoencephalopathy with subcortical cysts, Megalencephaly, Melkersson-Rosenthal syndrome, Menieres disease, Meningitis, Menkes disease, Metachromatic leukodystrophy, Microcephaly, Micropsia, Migraine, Miller Fisher syndrome, Mini-stroke (transient ischemic attack), Misophonia, Mitochondrial myopathy, Mobius syndrome, Monomelic amyotrophy, Motor skills disorder, Moyamoya disease, Mucopolysaccharidoses, Multi-infarct dementia, Multifocal motor neuropathy, Multiple sclerosis, Multiple system atrophy, Muscular dystrophy, Myalgic encephalomyelitis, Myasthenia gravis, Myelinoclastic diffuse sclerosis, Myoclonic Encephalopathy of infants, Myoclonus, Myopathy, Myotubular myopathy, Myotonia congenita, Narcolepsy, Neuro-Behçet's disease, Neurofibromatosis, Neuroleptic malignant syndrome, Neurological manifestations of AIDS, Neurological sequelae of lupus, Neuromyotonia, Neuronal ceroid lipofuscinosis, Neuronal migration disorders, Neuropathy, Neurosis, Niemann-Pick disease, Non-24-hour sleep–wake disorder, Nonverbal learning disorder, O'Sullivan-McLeod syndrome, Occipital Neuralgia, Occult Spinal Dysraphism Sequence, Ohtahara syndrome, Olivopontocerebellar atrophy, Opsoclonus myoclonus syndrome, Optic neuritis, Orthostatic Hypotension, Otosclerosis, Overuse syndrome, Palinopsia, Paresthesia, Parkinson's disease, Paramyotonia Congenita, Paraneoplastic diseases, Paroxysmal attacks, Parry-Romberg syndrome, PANDAS, Pelizaeus-Merzbacher disease, Periodic Paralyses, Peripheral neuropathy, Pervasive developmental disorders, Photic sneeze reflex, Phytanic acid storage disease, Pick's disease, Pinched nerve, Pituitary tumors, PMG, Polyneuropathy, Polio, Polymicrogyria, Polymyositis, Porencephaly, Post-Polio syndrome, Postherpetic Neuralgia (PHN), Postural Hypotension, Prader-Willi syndrome, Primary Lateral Sclerosis, Prion diseases, Progressive hemifacial atrophy, Progressive multifocal

leukoencephalopathy, Progressive Supranuclear Palsy, Prosopagnosia, Pseudotumor cerebri, Quadrantanopia, Quadriplegia, Rabies, Radiculopathy, Ramsay Hunt syndrome type I, Ramsay Hunt syndrome type II, Ramsay Hunt syndrome type III, Rasmussen encephalitis, Reflex neurovascular dystrophy, Refsum disease, REM sleep behavior disorder, Repetitive stress injury, Restless legs syndrome, Retrovirus-associated myelopathy, Rett syndrome, Reye's syndrome, Rhythmic Movement Disorder, Romberg syndrome, Saint Vitus dance, Sandhoff disease, Schilder's disease, Schizencephaly, Sensory processing disorder, Septo-optic dysplasia, Shaken baby syndrome, Shingles, Shy-Drager syndrome, Sjögren's syndrome, Sleep apnea, Sleeping sickness, Snatiation, Sotos syndrome, Spasticity, Spina bifida, Spinal cord injury, Spinal cord tumors, Spinal muscular atrophy, Spinal and bulbar muscular atrophy, Spinocerebellar ataxia, Split-brain, Steele-Richardson-Olszewski syndrome, Stiff-person syndrome, Stroke, Sturge-Weber syndrome, Stuttering, Subacute sclerosing panencephalitis, Subcortical arteriosclerotic encephalopathy, Superficial siderosis, Sydenham's chorea, Syncope, Synesthesia, Syringomyelia, Tarsal tunnel syndrome, Tardive dyskinesia, Tardive dysphrenia, Tarlov cyst, Tay-Sachs disease, Temporal arteritis, Temporal lobe epilepsy, Tetanus, Tethered spinal cord syndrome, Thomsen disease, Thoracic outlet syndrome, Tic Douloureux, Todd's paralysis, Tourette syndrome, Toxic encephalopathy, Transient ischemic attack, Transmissible spongiform encephalopathies, Transverse myelitis, Traumatic brain injury, Tremor, Trichotillomania, Trigeminal neuralgia, Tropical spastic paraparesis, Trypanosomiasis, Tuberous sclerosis, Unverricht-Lundborg disease, Von Hippel-Lindau disease (VHL), Viliuisk Encephalomyelitis (VE), Wallenberg's syndrome, West syndrome, Whiplash, Williams syndrome, Wilson's disease, or Zellweger syndrome.

[0193] In some cases, the compositions and methods disclosed herein can be used to treat brain cancer or brain tumors. Non-limiting examples of brain cancers or tumors that may be amenable to treatment with vectors and compositions described herein include: gliomas including anaplastic astrocytoma (grade III glioma), astrocytoma (grade II glioma), brainstem glioma, ependymoma, ganglioglioma, ganglioneuroma, glioblastoma (grade IV glioma), glioma, juvenile pilocytic astrocytoma (JPA), low-grade astrocytoma (LGA), medullablastoma, mixed glioma, oligodendroglioma, optic nerve glioma, pilocytic astrocytoma (grade I glioma), and primitive neuroectodermal (PNET); skull base tumors including acoustic neuroma (vestibular schwannoma), acromegaly, adenoma, chondrosarcoma, chordoma, craniopharyngioma, epidermoid tumor, glomus jugulare tumor, infratentorial meningioma, meningioma, pituitary adenoma, pituitary

tumor, Rathke's cleft cyst; metastatic cancer including brain metastasis, metastatic brain tumor; other brain tumors including brain cyst, choroid plexus papilloma, CNS lymphoma, colloid cyst, cystic tumor, dermoid tumor, germinoma, lymphoma, nasal carcinoma, naso-pharyngeal tumor, pineal tumor, pineoblastoma, pineocytoma, supratentorial meningioma, and vascular tumor; spinal cord tumors including astrocytoma, ependymoma, meningioma, and schwannoma.

[0194] The present disclosure contemplates, in part, compositions and methods for controlling, managing, preventing, or treating pain in a subject. "Pain" refers to an uncomfortable feeling and/or an unpleasant sensation in the body of a subject. Feelings of pain can range from mild and occasional to severe and constant. Pain can be classified as acute pain or chronic pain. Pain can be nociceptive pain (i.e., pain caused by tissue damage), neuropathic pain or psychogenic pain. In some cases, the pain is caused by or associated with a disease (*e.g.*, cancer, arthritis, diabetes). In other cases, the pain is caused by injury (*e.g.*, sports injury, trauma). Non-limiting examples of pain that are amenable to treatment with the compositions and methods herein include: neuropathic pain including peripheral neuropathy, diabetic neuropathy, post herpetic neuralgia, trigeminal neuralgia, back pain, neuropathy associated with cancer, neuropathy associated with HIV/AIDS, phantom limb pain, carpal tunnel syndrome, central post-stroke pain, pain associated with chronic alcoholism, hypothyroidism, uremia, pain associated with multiple sclerosis, pain associated with spinal cord injury, pain associated with Parkinson's disease, epilepsy, osteoarthritic pain, rheumatoid arthritic pain, visceral pain, and pain associated with vitamin deficiency; and nociceptive pain including pain associated with central nervous system trauma, strains/sprains, and burns; myocardial infarction, acute pancreatitis, post-operative pain, posttraumatic pain, renal colic, pain associated with cancer, pain associated with fibromyalgia, pain associated with carpal tunnel syndrome, and back pain.

[0195] The compositions and methods herein may be utilized to ameliorate a level of pain in a subject. In some cases, a level of pain in a subject is ameliorated by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100%. A level of pain in a subject can be assessed by a variety of methods. In some cases, a level of pain is assessed by self-reporting (i.e., a human subject expresses a verbal report of the level of pain he/she is experiencing). In some cases, a level of pain is assessed by behavioral indicators of pain, for example, facial expressions, limb

movements, vocalization, restlessness and guarding. These types of assessments may be useful for example when a subject is unable to self-report (*e.g.*, an infant, an unconscious subject, a non-human subject). A level of pain may be assessed after treatment with a composition of the disclosure as compared to the level of pain the subject was experiencing prior to treatment with the composition.

[0196] In various embodiments, a method for controlling, managing, preventing, or treating pain in a subject comprises administering to the subject an effective amount of an engineered receptor contemplated herein. Without wishing to be bound by any particular theory, the present disclosure contemplates using the vectors disclosed herein to modulate neuronal activity to alleviate pain in the subject.

[0197] In various embodiments, a vector encoding an engineered receptor that activates or depolarizes neuronal cells is administered to (or introduced into) one or more neuronal cells that decrease pain sensation, *e.g.,* inhibitory interneurons. In the presence of ligand the neuronal cell expressing the engineered receptor, is activated and decreases the sensitivity to pain potentiating the analgesic effect of stimulating these neuronal cells.

[0198] In various embodiments, a vector encoding an engineered receptor that deactivates or hyperpolarizes neuronal cells is administered to (or introduced into) one or more neuronal cells that increase pain sensation or sensitivity to pain, *e.g.,* nociceptor, peripheral sensory neurons, C-fibers, Aδ fibers, Aβ fibers, DRG neurons, TGG neurons, and the like. In the presence of ligand the neuronal cell expressing the engineered receptor, is deactivated and decreases the sensitivity to pain and potentiating an analgesic effect.

[0199] Targeting expression of an engineered receptor to a sub- population of nociceptors can be achieved by one or more of: selection of the vector (*e.g.*, AAV1, AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV5, AAV5(Y436+693+719F), AAV6, AAV6 (VP3 variant Y705F/Y731F/T492V), AAV-7m8, AAV8, AAV8(Y733F), AAV9, AAV9 (VP3 variant Y731F), AAV10(Y733F), and AAV-ShH10); selection of a promoter; and delivery means.

[0200] In particular embodiments, the compositions and methods contemplated herein are effective in reducing pain. Illustrative examples of pain that are amenable to treatment with the vectors, compositions, and methods contemplated herein, include but are not limited to acute pain, chronic pain, neuropathic pain, nociceptive pain, allodynia, inflammatory pain, inflammatory

hyperalgesia, neuropathies, neuralgia, diabetic neuropathy, human immunodeficiency virus-related neuropathy, nerve injury, rheumatoid arthritic pain, osteoarthritic pain, burns, back pain, eye pain, visceral pain, cancer pain (*e.g.*, bone cancer pain), dental pain, headache, migraine, carpal tunnel syndrome, fibromyalgia, neuritis, sciatica, pelvic hypersensitivity, pelvic pain, post herpetic neuralgia, post-operative pain, post stroke pain, and menstrual pain.

[0201] Pain can be classified as acute or chronic. "Acute pain" refers to pain that begins suddenly and is usually sharp in quality. Acute pain might be mild and last just a moment, or it might be severe and last for weeks or months. In most cases, acute pain does not last longer than three months, and it disappears when the underlying cause of pain has been treated or has healed. Unrelieved acute pain, however, may lead to chronic pain. "Chronic pain" refers to ongoing or recurrent pain, lasting beyond the usual course of acute illness or injury or lasting for more than three to six months, and which adversely affects the individual's well-being. In particular embodiments, the term "chronic pain" refers to pain that continues when it should not. Chronic pain can be nociceptive pain or neuropathic pain.

[0202] In particular embodiments, the compositions and methods contemplated herein are effective in reducing acute pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing chronic pain.

[0203] Clinical pain is present when discomfort and abnormal sensitivity feature among the patient's symptoms. Individuals can present with various pain symptoms. Such symptoms include: 1) spontaneous pain which may be dull, burning, or stabbing; 2) exaggerated pain responses to noxious stimuli (hyperalgesia); and 3) pain produced by normally innocuous stimuli (allodynia-Meyer et al., 1994, Textbook of Pain, 13-44). Although patients suffering from various forms of acute and chronic pain may have similar symptoms, the underlying mechanisms may be different and may, therefore, require different treatment strategies. Pain can also therefore be divided into a number of different subtypes according to differing pathophysiology, including nociceptive pain, inflammatory pain, and neuropathic pain.

[0204] In particular embodiments, the compositions and methods contemplated herein are effective in reducing nociceptive pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing inflammatory pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing neuropathic pain.

[0205] Nociceptive pain is induced by tissue injury or by intense stimuli with the potential to cause injury. Moderate to severe acute nociceptive pain is a prominent feature of pain from central nervous system trauma, strains/sprains, burns, myocardial infarction and acute pancreatitis, post-operative pain (pain following any type of surgical procedure), posttraumatic pain, renal colic, cancer pain and back pain. Cancer pain may be chronic pain such as tumor related pain (*e.g.*, bone pain, headache, facial pain or visceral pain) or pain associated with cancer therapy (*e.g.*, post chemotherapy syndrome, chronic postsurgical pain syndrome or post radiation syndrome). Cancer pain may also occur in response to chemotherapy, immunotherapy, hormonal therapy or radiotherapy. Back pain may be due to herniated or ruptured intervertebral discs or abnormalities of the lumber facet joints, sacroiliac joints, paraspinal muscles or the posterior longitudinal ligament. Back pain may resolve naturally but in some patients, where it lasts over 12 weeks, it becomes a chronic condition which can be particularly debilitating.

[0206] Neuropathic pain can be defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system. Etiologies of neuropathic pain include, *e.g.*, peripheral neuropathy, diabetic neuropathy, post herpetic neuralgia, trigeminal neuralgia, back pain, cancer neuropathy, HIV neuropathy, phantom limb pain, carpal tunnel syndrome, central post-stroke pain and pain associated with chronic alcoholism, hypothyroidism, uremia, multiple sclerosis, spinal cord injury, Parkinson's disease, epilepsy, and vitamin deficiency.

[0207] Neuropathic pain can be related to a pain disorder, a term referring to a disease, disorder or condition associated with or caused by pain. Illustrative examples of pain disorders include arthritis, allodynia, a typical trigeminal neuralgia, trigeminal neuralgia, somatoform disorder, hypoesthesis, hypealgesia, neuralgia, neuritis, neurogenic pain, analgesia, anesthesia dolorosa, causlagia, sciatic nerve pain disorder, degenerative joint disorder, fibromyalgia, visceral disease, chronic pain disorders, migraine/headache pain, chronic fatigue syndrome, complex regional pain syndrome, neurodystrophy, plantar fasciitis or pain associated with cancer.

[0208] The inflammatory process is a complex series of biochemical and cellular events, activated in response to tissue injury or the presence of foreign substances, which results in swelling and pain. Arthritic pain is a common inflammatory pain.

[0209] Other types of pain that are amenable to treatment with the vectors, compositions, and methods contemplated herein, include but are not limited to pain resulting from musculoskeletal disorders, including myalgia, fibromyalgia, spondylitis, sero-negative (non-

rheumatoid) arthropathies, non-articular rheumatism, dystrophinopathy, glycogenolysis, polymyositis and pyomyositis; heart and vascular pain, including pain caused by angina, myocardical infarction, mitral stenosis, pericarditis, Raynaud's phenomenon, scleredoma and skeletal muscle ischemia; head pain, such as migraine (including migraine with aura and migraine without aura), cluster headache, tension-type headache mixed headache and headache associated with vascular disorders; and orofacial pain, including dental pain, otic pain, burning mouth syndrome, and temporomandibular myofascial pain.

[0210] The effective amount of the compositions and methods contemplated herein to reduce the amount of pain experienced by a human subject can be determined using a variety of pain scales. Patient self-reporting can be used to assess whether pain is reduced; see, *e.g.*, Katz and Melzack (1999) Surg. Clin. North Am. 79:231. Alternatively, an observational pain scale can be used. The LANSS Pain Scale can be used to assess whether pain is reduced; see, *e.g.*, Bennett (2001) Pain 92:147. A visual analog pain scale can be used; see, *e.g.*, Schmader (2002) Clin. J. Pain 18:350. The Likert pain scale can be used; *e.g.*, where 0 is no pain, 5 is moderate pain, and 10 is the worst pain possible. Self -report pain scales for children include, *e.g.*, Faces Pain Scale; Wong-Baker FACES Pain Rating Scale; and Colored Analog Scale. Self-report pain scales for adults include, *e.g.*, Visual Analog Scale; Verbal Numerical Rating Scale; Verbal Descriptor Scale; and Brief Pain Inventory. Pain measurement scales include, *e.g.*, Alder Hey Triage Pain Score (Stewart et al. (2004) Arch. Dis. Child. 89:625); Behavioral Pain Scale (Payen et al. (2001) Critical Care Medicine 29:2258); Brief Pain Inventory (Cleeland and Ryan (1994) Ann. Acad. Med. Singapore 23: 129); Checklist of Nonverbal Pain Indicators (Feldt (2000) Pain Manag. Nurs. 1 : 13); Critical-Care Pain Observation Tool (Gelinas et al. (2006) Am. J. Crit. Care 15:420); COMFORT scale (Ambuel et al. (1992) J. Pediatric Psychol. 17:95); Dallas Pain Questionnaire (Ozguler et al. (2002) Spine 27:1783); Dolorimeter Pain Index (Hardy et al. (1952) Pain Sensations and Reactions Baltimore: The Williams & Wilkins Co.); Faces Pain Scale - Revised (Hicks et al. (2001) Pain 93:173); Face Legs Activity Cry Consolability Scale; McGill Pain Questionnaire (Melzack (1975) Pain 1 :277); Descriptor Differential Scale (Gracely and Kwilosz (1988) Pain 35:279); Numerical 1 1 point Box (Jensen et al. (1989) Clin. J. Pain 5: 153); Numeric Rating Scale (Hartrick et al. (2003) Pain Pract. 3:310); Wong-Baker FACES Pain Rating Scale; and Visual Analog Scale (Huskisson (1982) J. Rheumatol. 9:768).

[0211] In particular embodiments, a method of relieving pain in a subject is provided, the method comprising introducing an engineered receptor into a neuronal cell and controlling the activity of the cell by providing an effective amount of a ligand that activates the engineered receptor, thereby relieving pain in the subject. The method provides significant analgesia for pain without off-target effects, such as general central nervous system depression. In certain embodiments, the method provides a 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more reduction in the neuropathic pain in a subject compared to an untreated subject. In some embodiments, the method comprises the step of measuring pain in the subject before and after the administration of the binding agent, wherein the pain in the subject is reduced 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more. In such instances, the measuring may occur 4 hours or more after administration of the binding agent, e.g. 8 hours 12 hours, 16 hours, 24 hours, 36 hours, 48 hours, 3 days, or 4 days or more after administration of the binding agent.

[0212] In particular embodiments, the vectors contemplated herein are administered or introduced into one or more neuronal cells. The neuronal cells may be the same type of neuronal cells, or a mixed population of different types of neuronal cells. In one embodiment, the neuronal cell is a nociceptor or peripheral sensory neuron. Illustrative examples of sensory neurons include, but are not limited to, dorsal root ganglion (DRG) neurons and trigeminal ganglion (TGG) neurons. In one embodiment, the neuronal cell is an inhibitory interneuron involved in the neuronal pain circuit.

[0213] In some cases, a vector encoding an engineered receptor is administered to a subject in need thereof. Non-limiting examples of methods of administration include subcutaneous administration, intravenous administration, intramuscular administration, intradermal administration, intraperitoneal administration, oral administration, infusion, intracranial administration, intrathecal administration, intranasal administration, intraganglionic administration, intraspinal administration, cisterna magna administration and intraneural administration. In some cases, administration can involve injection of a liquid formulation of the vector. In other cases, administration can involve oral delivery of a solid formulation of the vector. In some cases, the oral formulation can be administered with food. In particular embodiments, a vector is parenterally, intravenously, intramuscularly, intraperitoneally, intrathecally,

intraneurally, intraganglionicly, intraspinally, or intraventricularly administered to a subject in order to introduce the vector into one or more neuronal cells. In various embodiments, the vector is rAAV.

[0214] In one embodiment, AAV is administered to sensory neuron or nociceptor, *e.g.*, DRG neurons, TGG neurons, etc. by intrathecal (IT) or intraganglionic (IG) administration. The IT route delivers AAV to the cerebrospinal fluid (CSF). This route of administration may be suitable for the treatment of *e.g.*, chronic pain or other peripheral nervous system (PNS) or central nervous system (CNS) indications. In animals, IT administration has been achieved by inserting an IT catheter through the *cisterna magna* and advancing it caudally to the lumbar level. In humans, IT delivery can be easily performed by lumbar puncture (LP), a routine bedside procedure with excellent safety profile.

[0215] In a particular case, a vector may be administered to a subject by intraganglionic administration. Intraganglionic administration may involve an injection directly into one or more ganglia. The IG route may deliver AAV directly into the DRG or TGG parenchyma. In animals, IG administration to the DRG is performed by an open neurosurgical procedure that is not desirable in humans because it would require a complicated and invasive procedure. In humans, a minimally invasive, CT imaging-guided technique to safely target the DRG can be used. A customized needle assembly for convection enhanced delivery (CED) can be used to deliver AAV into the DRG parenchyma. In a non-limiting example, a vector of the disclosure may be delivered to one or more dorsal root ganglia and/or trigeminal ganglia for the treatment of chronic pain. In another non-limiting example, a vector of the disclosure may be delivered to the nodose ganglion (vagus nerve) to treat epilepsy.

[0216] In yet another particular case, a vector may be administered to the subject by intracranial administration (i.e., directly into the brain). In non-limiting examples of intracranial administration, a vector of the disclosure may be delivered into the cortex of the brain to treat *e.g.*, an epileptic seizure focus, into the paraventricular hypothalamus to treat *e.g.*, a satiety disorder, or into the amygdala central nucleus to treat *e.g.*, a satiety disorder. In another particular case, a vector may be administered to a subject by intraneural injection (i.e., directly into a nerve). The nerve may be selected based on the indication to be treated, for example, injection into the sciatic nerve to treat chronic pain or injection into the vagal nerve to treat epilepsy or a satiety disorder. In yet

another particular case, a vector may be administered to a subject by subcutaneous injection, for example, into the sensory nerve terminals to treat chronic pain.

[0217] A vector dose may be expressed as the number of vector genome units delivered to a subject. A "vector genome unit" as used herein refers to the number of individual vector genomes administered in a dose. The size of an individual vector genome will generally depend on the type of viral vector used. Vector genomes of the disclosure may be from about 1.0 kilobase, 1.5 kilobases, 2.0 kilobases, 2.5 kilobases, 3.0 kilobases, 3.5 kilobases, 4.0 kilobases, 4.5 kilobases, 5.0 kilobases, 5.5 kilobases, 6.0 kilobases, 6.5 kilobases, 7.0 kilobases, 7.5 kilobases, 8.0 kilobases, 8.5 kilobases, 9.0 kilobases, 9.5 kilobases, 10.0 kilobases, to more than 10.0 kilobases. Therefore, a single vector genome may include up to or greater than 10,000 base pairs of nucleotides. In some cases, a vector dose may be about 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, 1×10^{11}, 2×10^{11}, 3×10^{11}, 4×10^{11}, 5×10^{11}, 6×10^{11}, 7×10^{11}, 8×10^{11}, 9×10^{11}, 1×10^{12}, 2×10^{12}, 3×10^{12}, 4×10^{12}, 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 1×10^{13}, 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13}, 6×10^{13}, 7×10^{13}, 8×10^{13}, 9×10^{13}, 1×10^{14}, 2×10^{14}, 3×10^{14}, 4×10^{14}, 5×10^{14}, 6×10^{14}, 7×10^{14}, 8×10^{14}, 9×10^{14}, 1×10^{15}, 2×10^{15}, 3×10^{15}, 4×10^{15}, 5×10^{15}, 6×10^{15}, 7×10^{15}, 8×10^{15}, 9×10^{15}, 1×10^{16}, 2×10^{16}, 3×10^{16}, 4×10^{16}, 5×10^{16}, 6×10^{16}, 7×10^{16}, 8×10^{16}, 9×10^{16}, 1×10^{17}, 2×10^{17}, 3×10^{17}, 4×10^{17}, 5×10^{17}, 6×10^{17}, 7×10^{17}, 8×10^{17}, 9×10^{17}, 1×10^{18}, 2×10^{18}, 3×10^{18}, 4×10^{18}, 5×10^{18}, 6×10^{18}, 7×10^{18}, 8×10^{18}, 9×10^{18}, 1×10^{19}, 2×10^{19}, 3×10^{19}, 4×10^{19}, 5×10^{19}, 6×10^{19}, 7×10^{19}, 8×10^{19}, 9×10^{19}, 1×10^{20}, 2×10^{20}, 3×10^{20}, 4×10^{20}, 5×10^{20}, 6×10^{20}, 7×10^{20}, 8×10^{20}, 9×10^{20} or more vector genome units.

[0218] In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about 1×10^9 genome particles/mL, at least about 1×10^{10} genome particles/mL, at least about 5×10^{10} genome particles/mL, at least about 1×10^{11} genome particles/mL, at least about 5×10^{11} genome particles/mL, at least about 1×10^{12} genome particles/mL, at least about 5×10^{12} genome particles/mL, at least about 6×10^{12} genome particles/mL, at least about 7×10^{12} genome particles/mL, at least about 8×10^{12} genome particles/mL, at least about 9×10^{12} genome particles/mL, at least about 10×10^{12} genome

particles/mL, at least about 15 x 10^{12} genome particles/mL, at least about 20 x 10^{12} genome particles/mL, at least about 25 x 10^{12} genome particles/mL, at least about 50 x 10^{12} genome particles/mL, or at least about 100 x 10^{12} genome particles/mL. The terms "genome particles (gp)" or "genome equivalents" or "genome copies" (gc) as used in reference to a viral titer, refer to the number of virions containing the recombinant AAV DNA genome, regardless of infectivity or functionality. The number of genome particles in a particular vector preparation can be measured by well understood methods in the art, for example, quantitative PCR of genomic DNA or for example, in Clark et al. (1999) Hum. Gene Ther., 10:1031-1039; Veldwijk et al. (2002) Mol. Ther., 6:272-278.

[0219] A vector of the disclosure may be administered in a volume of fluid. In some cases, a vector may be administered in a volume of about 0.1mL, 0.2mL, 0.3mL, 0.4mL, 0.5mL, 0.6mL, 0.7mL, 0.8mL, 0.9mL, 1.0mL, 2.0mL, 3.0mL, 4.0mL, 5.0mL, 6.0mL, 7.0mL, 8.0mL, 9.0mL, 10.0mL, 11.0mL, 12.0mL, 13.0mL, 14.0mL, 15.0mL, 16.0mL, 17.0mL, 18.0mL, 19.0mL, 20.0mL or greater than 20.0mL. In some cases, a vector dose may be expressed as a concentration or titer of vector administered to a subject. In this case, a vector dose may be expressed as the number of vector genome units per volume (i.e., genome units/volume).

[0220] In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about 5 x 10^{9} infectious units/mL, at least about 6 x 10^{9} infectious units/mL, at least about 7 x 10^{9} infectious units/mL, at least about 8 x 10^{9} infectious units/mL, at least about 9 x 10^{9} infectious units/mL, at least about 1 x 10^{10} infectious units/mL, at least about 1.5 x 10^{10} infectious units/mL, at least about 2 x 10^{10} infectious units/mL, at least about 2.5 x 10^{10} infectious units/mL, at least about 5 x 10^{10} infectious units/mL, at least about 1 x 10^{11} infectious units/mL, at least about 2.5 x 10^{11} infectious units/mL, at least about 5 x 10^{11} infectious units/mL, at least about 1 x 10^{12} infectious units/mL, at least about 2.5 x 10^{12} infectious units/mL, at least about 5 x 10^{12} infectious units/mL, at least about 1 x 10^{13} infectious units/mL, at least about 5 x 10^{13} infectious units/mL, at least about 1 x 10^{14} infectious units/mL. The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant AAV vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example, in McLaughlin et al. (1988) J. Virol., 62:1963-1973.

[0221] In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about 5 x 10^{10} transducing units/mL, at least about 1 x 10^{11} transducing units/mL, at least about 2.5 x 10^{11} transducing units/mL, at least about 5 x 10^{11} transducing units/mL, at least about 1 x 10^{12} transducing units/mL, at least about 2.5 x 10^{12} transducing units/mL, at least about 5 x 10^{12} transducing units/mL, at least about 1 x 10^{13} transducing units/mL, at least about 5 x 10^{13} transducing units/mL, at least about 1 x 10^{14} transducing units/mL. The term "transducing unit" (tu)" as used in reference to a viral titer, refers to the number of infectious recombinant AAV vector particles that result in the production of a functional transgene product as measured in functional assays such as described in, for example, in Xiao et al. (1997) Exp. Neurobiol., 144:113-124; or in Fisher et al. (1996) J. Virol., 70:520-532 (LFU assay).

[0222] The vector dose will generally be determined by the route of administration. In a particular example, an intraganglionic injection may include from about 1 x 10^{9} to about 1 x 10^{13} vector genomes in a volume from about 0.1mL to about 1.0mL. In another particular case, an intrathecal injection may include from about 1 x 10^{10} to about 1 x 10^{15} vector genomes in a volume from about 1.0 mL to about 12.0 mL. In yet another particular case, an intracranial injection may include from about 1 x 10^{9} to about 1 x 10^{13} vector genomes in a volume from about 0.1 mL to about 1.0 mL. In another particular case, an intraneural injection may include from about 1 x 10^{9} to about 1 x 10^{13} vector genomes in a volume from about 0.1 mL to about 1.0 mL. In another particular example, an intraspinal injection may include from about 1 x 10^{9} to about 1 x 10^{13} vector genomes in a volume from about 0.1 mL to about 1.0 mL. In yet another particular case, a cisterna magna infusion may include from about 5 x 10^{9} to about 5 x 10^{13} vector genomes in a volume from about 0.5 mL to about 5.0 mL. In yet another particular case, a subcutaneous injection may include from about 1 x 10^{9} to about 1 x 10^{13} vector genomes in a volume from about 0.1mL to about 1.0mL.

[0223] In some cases, a vector is delivered to a subject by infusion. A vector dose delivered to a subject by infusion can be measured as a vector infusion rate. Non-limiting examples of vector infusion rates include: 1-10 μL/min for intraganglionic, intraspinal, intracranial or intraneural administration; and 10-1000 μL/min for intrathecal or cisterna magna administration. In some cases, the vector is delivered to a subject by MRI-guided Convection Enhanced Delivery (CED). This technique enables increased viral spread and transduction distributed throughout large volumes of the brain, as well as reduces reflux of the vector along the needle path.

[0224] In various embodiments, a method is provided comprising administering a vector encoding a engineered receptor, that deactivates or hyperpolarizes neuronal cells, to one or more neuronal cells that increase pain sensation or sensitivity to pain, and administering a ligand that specifically binds the neuronal cell expressing the engineered receptor to the subject, thereby deactivating the cell, decreasing the sensitivity to pain and potentiating an analgesic effect.

[0225] In various embodiments, a method is provided comprising administering a vector encoding a engineered receptor, that activates or polarizes neuronal cells, to one or more neuronal cells that decrease pain sensation or sensitivity to pain, and administering a ligand that specifically binds the neuronal cell expressing the engineered receptor to the subject, thereby activating the cell, decreasing the sensitivity to pain and potentiating an analgesic effect.

[0226] Formulations of ligands may be administered to a subject by various routes. Non-limiting examples of methods of administration include subcutaneous administration, intravenous administration, intramuscular administration, transdermal administration, intradermal administration, intraperitoneal administration, oral administration, infusion, intracranial administration, intrathecal administration, intranasal administration, intraganglionic administration, and intraneural administration. In some cases, administration can involve injection of a liquid formulation of the ligand. In other cases, administration can involve oral delivery of a solid formulation of the ligand. In a particular case, a ligand is administered by oral administration (*e.g.*, a pill, tablet, capsule and the like). In some cases, the oral composition can be administered with food. In another particular case, a ligand is administered by intrathecal injection (i.e., into the subarachnoid space of the spinal cord) for delivery to the cerebrospinal fluid (CSF) of the subject. In another particular case, a ligand is administered topically (*e.g.*, dermal patch, cream, lotion, ointment and the like).

[0227] The dosages of the ligands administered to a subject are not subject to absolute limits, but will depend on the nature of the composition and its active ingredients and its unwanted side effects (*e.g.,* immune response against the antibody), the subject being treated and the type of condition being treated and the manner of administration. Generally, the dose will be a therapeutically effective amount, such as an amount sufficient to achieve a desired biological effect, for example an amount that is effective to decrease or attenuate the level of pain experienced by the subject. In particular embodiments, the dose can also be a prophylactic amount or an

effective amount. A therapeutically effective amount of ligand may depend on the route of administration, the indication being treated, and/or the ligand selected for use.

[0228] In one embodiment, the ligand is first administered to the subject prior to administration of the vector. A therapeutically effective amount of ligand may be administered to a subject at some time after delivery of a vector. Generally, after delivery of a vector, there will be a period of time required for one or more cells of the subject to generate a protein (i.e., engineered receptor) encoded by the vector. During this period of time, administration of a ligand to the subject may not be beneficial to the subject. In this situation, it may be suitable to administer the ligand after an amount of engineered receptor has been produced by one or more cells of the subject.

[0229] In one embodiment, the ligand is first administered to the subject at about the same time that the vector is administered to the subject.

[0230] In one embodiment, the ligand is first administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, or 12 hours, days, weeks, months, or years after administration of the vector to the subject. In some cases, a therapeutically effective amount of a ligand may be administered to a subject at least one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days or more than 30 days after delivery of the vector. In a particular example, a therapeutically effective amount of a ligand is administered to a subject at least one week after delivery of a vector. In a further example, the therapeutically effective amount of ligand is administered to the subject daily for at least three consecutive days.

[0231] A therapeutically effective amount or dose of a ligand of the disclosure can be expressed as mg or μg of the ligand per kg of subject body mass. In some instances, a therapeutically effective amount of a ligand may be about 0.001 μg/kg, about 0.005 μg/kg, about 0.01 μg/kg, about 0.05 μg/kg, about 0.1 μg/kg, about 0.5 μg/kg, about 1 μg/kg, about 2 μg/kg, about 3 μg/kg, about 4 μg/kg, about 5 μg/kg, about 6 μg/kg, about 7 μg/kg, about 8 μg/kg, about 9 μg/kg, about 10 μg/kg, about 20 μg/kg, about 30 μg/kg, about 40 μg/kg, about 50 μg/kg, about 60 μg/kg, about 70 μg/kg, about 80 μg/kg, about 90 μg/kg, about 100 μg/kg, about 120 μg/kg, about 140 μg/kg, about 160 μg/kg, about 180 μg/kg, about 200 μg/kg, about 220 μg/kg, about 240 μg/kg, about 260 μg/kg, about 280 μg/kg, about 300 μg/kg, about 320 μg/kg, about 340 μg/kg,

about 360 μg/kg, about 380 μg/kg, about 400 μg/kg, about 420 μg/kg, about 440 μg/kg, about 460 μg/kg, about 480 μg/kg, about 500 μg/kg, about 520 μg/kg, about 540 μg/kg, about 560 μg/kg, about 580 μg/kg, about 600 μg/kg, about 620 μg/kg, about 640 μg/kg, about 660 μg/kg, about 680 μg/kg, about 700 μg/kg, about 720 μg/kg, about 740 μg/kg, about 760 μg/kg, about 780 μg/kg, about 800 μg/kg, about 820 μg/kg, about 840 μg/kg, about 860 μg/kg, about 880 μg/kg, about 900 μg/kg, about 920 μg/kg, about 940 μg/kg, about 960 μg/kg, about 980 μg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, or greater than 10 mg/kg.

[0232] In particular embodiments, the dose of ligand administered to a subject is at least about 0.001 micrograms per kilogram (μg/kg), at least about 0.005 μg/kg, at least about 0.01 μg/kg, at least about 0.05 μg/kg, at least about 0.1 μg/kg, at least about 0.5 μg/kg, 0.001 milligrams per kilogram (mg/kg), at least about 0.005 mg/kg, at least about 0.01 mg/kg, at least about 0.05 mg/kg, at least about 0.1 mg/kg, at least about 0.5 mg/kg , at least about 1 mg/kg, at least about 2 mg/kg, at least about 3 mg/kg, at least about 4 mg/kg, at least about 5 mg/kg, at least about 5 mg/kg, at least about 6 mg/kg, at least about 7 mg/kg, at least about 8 mg/kg, at least about 8 mg/kg, at least about 9 mg/kg, or at least about 10 or more mg/kg.

[0233] In particular embodiments, the dose of ligand administered to a subject is at least about 0.001 μg/kg to at least about 10 mg/kg, at least about 0.01 μg/kg to at least about 10 mg/kg, at least about 0.1 μg/kg to at least about 10 mg/kg, at least about 1 μg/kg to at least about 10 mg/kg, at least about 0.01 mg/kg to at least about 10 mg/kg, at least about 0.1 mg/kg to at least about 10 mg/kg, or at least about 1 mg/kg to at least about 10 mg/kg, or any intervening range thereof.

[0234] In some aspects, a therapeutically effective amount of a ligand can be expressed as a molar concentration (i.e., M or mol/L). In some cases, a therapeutically effective amount of a ligand can be about 1nM, 2nM, 3nM, 4nM, 5nM, 6nM, 7nM, 8nM, 9nM, 10nM, 20nM, 30nM, 40nM, 50nM, 60nM, 70nM, 80nM, 90nM, 100nM, 200nM, 300nM, 400nM, 500nM, 600nM, 700nM, 800nM, 900nM, 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM, 100mM, 200mM, 300mM, 400mM, 500mM, 600mM, 700mM, 800mM, 900mM, 1000mM or greater.

[0235] A therapeutically effective amount of a ligand can be administered once or more than once each day. In some cases, a therapeutically effective amount of a ligand is administered

as needed (*e.g.*, when pain relief is needed). The ligand may be administered serially (*e.g.*, every day without a break for the duration of the treatment regimen). In some cases, the treatment regimen can be less than a week, a week, two weeks, three weeks, a month, or greater than a month. In some cases, a therapeutically effective amount of a ligand is administered for a day, at least two consecutive days, at least three consecutive days, at least four consecutive days, at least five consecutive days, at least six consecutive days, at least seven consecutive days, at least eight consecutive days, at least nine consecutive days, at least ten consecutive days, or at least greater than ten consecutive days. In a particular case, a therapeutically effective amount of a ligand is administered for three consecutive days. In some cases, a therapeutically effective amount of a ligand can be administered one time per week, two times per week, three times per week, four times per week, five times per week, six times per week, seven times per week, eight times per week, nine times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, 14 times per week, 15 times per week, 16 times per week, 17 times per week, 18 times per week, 19 times per week, 20 times per week, 25 times per week, 30 times per week, 35 times per week, 40 times per week, or greater than 40 times per week. In some cases, a therapeutically effective amount of a ligand can be administered one time per day, two times per day, three times per day, four times per day, five times per day, six times per day, seven times per day, eight times per day, nine times per day, 10 times per day, or greater than 10 times per day. In some cases, a therapeutically effective amount of a ligand is administered at least every hour, at least every two hours, at least every three hours, at least every four hours, at least every five hours, at least every six hours, at least every seven hours, at least every eight hours, at least every nine hours, at least every 10 hours, at least every 11 hours, at least every 12 hours, at least every 13 hours, at least every 14 hours, at least every 15 hours, at least every 16 hours, at least every 17 hours, at least every 18 hours, at least every 19 hours, at least every 20 hours, at least every 21 hours, at least every 22 hours, at least every 23 hours, or at least every day. The dose of ligand may be administered to the subject continuously, or 1, 2, 3, 4, or 5 times a day; 1, 2, 3, 4, 5, 6, or 7 times a week, 1, 2, 3, or 4 times a month, once every 2, 3, 4, 5, or 6 months, or once a year, or at even longer intervals. The duration of treatment can last a day, 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 7, 8, 9, 10, or 11 months, 1, 2, 3, 4, 5, or more years, or longer.

[0236] A subject treated by methods and compositions disclosed herein can be a human, or can be a non-human animal. The term “treat” and its grammatical equivalents used herein

generally refer to the use of a composition or method to reduce, eliminate, or prevent symptoms of a disease and includes achieving a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant slowing the progression of, halting the progression of, reversing the progression of, or eradication or amelioration of the symptoms of the disorder or condition being treated. A prophylactic benefit of treatment includes reducing the risk of a condition, retarding the progress of a condition, or decreasing the likelihood of occurrence of a condition.

[0237] Non-limiting examples of non-human animals include a non-human primate, a livestock animal, a domestic pet, and a laboratory animal. For example, a non-human animal can be an ape (*e.g.*, a chimpanzee, a baboon, a gorilla, or an orangutan), an old world monkey (*e.g.*, a rhesus monkey), a new world monkey, a dog, a cat, a bison, a camel, a cow, a deer, a pig, a donkey, a horse, a mule, a lama, a sheep, a goat, a buffalo, a reindeer, a yak, a mouse, a rat, a rabbit, or any other non-human animal. The compositions and methods as described herein are amenable to the treatment of a veterinary animal. A veterinary animal can include, without limitation, a dog, a cat, a horse, a cow, a sheep, a mouse, a rat, a guinea pig, a hamster, a rabbit, a snake, a turtle, and a lizard. In some aspects, contacting the tissue or cell population with a composition comprises administering the composition to a cell population or subject. In some embodiments, administration occurs *in vitro*, for example by adding the composition to a cell culture system. In some aspects, administration occurs *in vivo*, for example by administration through a particular route. Wherein more than one composition is to be administered, the compositions may be administered via the same route at the same time (*e.g.*, on the same day), or via the same route at different times. Alternatively, the compositions may be administered via different routes at the same time (*e.g.*, on the same day) or via different routes at different times.

[0238] The number of times a composition is administered to an subject in need thereof depends on the discretion of a medical professional, the disorder, the severity of the disorder, and the subject's response to the formulation. In some aspects, administration of a composition occurs at least once. In further aspects, administration occurs more than once, for example 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times in a given period. The dosage of each administration and/or frequency of administrations may be adjusted as necessary based on the patient's condition and physiologically responses.

[0239] In some embodiments, compositions may be administered a sufficient amount of times to achieve a desired physiologic effect or improvement in a subject's condition. In the case

wherein the subject's condition does not improve, upon the doctor's discretion the composition may be administered chronically, that is, for an extended period of time, including throughout the duration of the subject's life in order to ameliorate or otherwise control or limit the symptoms of the subject's disease or condition. In the case wherein the subject's status does improve, upon the doctor's discretion the composition may administered continuously; alternatively, the dose of drug being administered may be temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, and 365 days. The dose reduction during a drug holiday may be from 10%- 100%, including by way of example only 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, and 100%.

[0240] Where compositions are administered more than once, each administration may be performed by the same actor and/or in the same geographical location. Alternatively, each administration may be performed by a different actor and/or in a different geographical location.

[0241] With regard to human and veterinary treatment, the amount of a particular agent(s) that is administered may be dependent on a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific agent(s) employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific agent(s) employed; the duration of the treatment; drugs used in combination or coincidental with the specific agent(s) employed; the judgment of the prescribing physician or veterinarian; and like factors known in the medical and veterinary arts. Similarly, the effective concentration of a given composition may be dependent on a variety of factors including the age, sex, weight, genetic status, and overall health of the patient or subject.

EXAMPLES

Example 1. Discovery and Characterization of Engineered Receptors

[0242] Chimeric LGIC receptors comprising the ligand binding domain from the α7-nAChR and the chloride-conducting ion pore domain from the human GlyR1α were engineered and assessed for their responsiveness to acetylcholine, nicotine, and several small molecule agonist

ligands. Amino acid substitutions were then generated in these "parental" chimeric receptors and the mutants retested for responsiveness to these ligands.

[0243] Differences in potency (EC_{50}) and current flow (Imax) were observed, both among the engineered chimeric receptors and between the chimeric receptors and the wild type α7-nAChR, suggesting that the domains that contribute to the extracellular domain of the engineered receptor play an influential role in channel properties. Amino acid substitutions in these engineered channels further altered the responsiveness of the channel, in some cases differentially for different ligands. These results indicate that a toolbox of LGIC receptors can be engineered that have unique channel properties, providing an avenue for the development of therapeutics that can be used to control the activity of neurons in disease.

Materials and Methods

[0244] **Receptor construction**: Individual parental chimera receptors were cloned into pcDNA3.1(+) (Invitrogen) using BamHI and EcoRI sites by standard molecular biology techniques. Amino acid substitutions were introduced by site-directed mutagenesis.

[0245] **Plate Reader YFP Quench Assay**: Lenti-X 293T cells (LX293T, Clontech) were maintained in DMEM containing 10% FBS and 1% penicillin/streptomycin (Invitrogen). For plate reader assays, LX293T cells were infected with a lentivirus to create cells that stably express a mutant YFP (H148Q/I152L) reporter, which displays enhanced sensitivity to anions Two days before assay, cells were split at a density of 20,000 cells/well in a 96-well tissue culture plate coated with poly-d lysine (Thermo Scientific). The next day, the cells were transiently transfected with 0.1 μg of DNA per well using standard Fugene protocol (Promega). On the day of assay, cells were washed 2 times in 1X extracellular solution (1X ECS: 140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, 10 mM glucose, pH 7.2, mOsms 300). After the last wash, 100 μL of 1X ECS was added to the wells and the plate was incubated at 37°C for 30 min. While incubating the plate, the drugs were diluted to a 2X concentration in 1X ECS-NaI (same components as 1X ECS except the 140 mM NaCl was replaced by 140 mM NaI). The plates were then read on a Flexstation3 (Molecular Devices). Each well, 8 wells at a time, of the plate is read for 2 min using a Flexstation3 (Molecular Devices) as follows: 1) a baseline YFP fluorescence is read for 17 sec, 2) 100 μL of ligand is added, and 3) the changes in YFP fluorescence are then measured every 1.3 sec for the remaining time.

[0246] **Electrophysiology**: For HEK293T studies, cDNAs encoding ion channels were cloned into pcDNA3.1 using standard recombination techniques. HEK293T cells from Clontech (Lenti-X™ 293T Cell Line) were cultured in DMEM supplemented with 10% FBS and 1% Pen/Strep to 40-50% confluence using standard cell culture protocols, transfected with ion channel plasmids at a concentration of 18 μg per 15 cm dish using Fugene 6, and grown for an additional 24 hours. Cells were then assayed on a medium throughput electrophysiological system (IonFluxHT and/or Mercury, Fluxion Biosciences) in which dose-response relationships may be assessed through a microfluidics-based platform for establishing whole cell configurations. Ensemble plates were primed with extracellular buffer (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.2 with NaOH, mOsm 310), intracellular buffer (145 mM CsCl, 2 mM CaCl2, 2 mM MgCl2, 10 mM HEPES, and 10 mM EGTA, pH 7.2 with CsOH, mOsm 305), as adapted from Lynagh and Lynch, and test compounds (stocks prepared fresh) diluted in extracellular buffer. Cells were then released from the plate with Accutase, centrifuged, resuspended in extracellular buffer, and loaded into Ensemble plates. Cells were then subjected to a standard protocol for priming, trapping, breaking, and establishing whole cell configuration, with cells held at -60 mV throughout the recording. After recording baseline, progressive doses of test compounds were applied using the IonFlux software to assess dose response relationships. Data were then analyzed off-line using custom Python scripts to convert data to .csv format, re-plot traces, and apply QC measurements to reject unstable recordings (i.e. thresholding based on access resistance and/or standard deviation in baseline, as well as artifact rejection). Peak currents were then calculated and population data were fit using a 4-parameter logistic equation as described by the Hill Equation

[0247] For Xenopus oocyte studies, injections of cDNAs encoding for ion channels at a final concentration of 0.04 μg/μL were performed using a proprietary automated injection device and receptor expression examined at least two days later. Oocytes were poked with two electrodes and their membrane potential maintained at -80 mV throughout the experiment. All recordings were performed at 18oC and cells were superfused with OR2 medium containing in mM: NaCl 82.5, KCl 2.5, HEPES 5, CaCl2.2H2O 1.8, MgCl2.6H2O 1, pH 7.4. Recordings progressed if oocytes had sufficiently low holding current, in which case they were then subjected to ligand or test compound application (5-10s wash intervals), with 100s of wash in between doses. Currents evoked by acetylcholine or other agonists were recorded using an automated process equipped

with standard two electrode voltage clamp configuration (TEVC). Data were captured and analyzed using HiQScreen proprietary data acquisition and analysis software running under Matlab (Mathworks Inc.). Acetylcholine was prepared as a concentrated stock solution (10-1 M) in water and then diluted in the recording medium to obtain the desired test concentration. Nicotine was diluted in water as stock solution (10-1 M), kept frozen and diluted immediately prior to the experiment at the desired concentration. Compounds were prepared as stock solution (10-1M) in OR2 and then diluted in the recording medium to obtain the desired test concentration. Stock solution for Compound-1 was made in OR2 at pH 5 adjusted with HCl. To assess that dilution at the final concentration of this stock solution introduced no significant changes, the pH of the final solution was confirmed using a colorimetric assay (Galietta L.J.V., et al. 2001. FEBS Letters 499: 220-224. Kruger W., et al. (2005). Neuroscience Letters 380: 340-345.)

Results

[0248] To generate LGICs that conduct anion current following exposure to non-native small molecule agonists of the human "α7-nAChR", fusion proteins were engineered that comprise the chloride-conducting ion pore domain of the human GlyRα1 subunit fused to the ligand binding domain of α7-nAChR. In some fusion proteins, the β1-β2 loop sequence and/or Cys loop sequence of α7-nAChR was replaced with the corresponding sequence from GlyRα1 (see schematics in Fig. 4). In other fusion proteins, the site of the junction between the 3' sequence of the extracellular domain and the 5' sequence of the ion pore domain (the "pre-M1 linker") was shifted. In yet other fusion proteins, the extracellular loop sequence between transmembrane domain 2 and transmembrane domain 3 of the GlyRα1 ion pore domain (the "M1-M2 linker") was replaced with a corresponding M1-M3 linker sequence from the α7-nAChR. The response of these engineered chimeric LGICs to a range of doses of the native ligand, acetylcholine, was assessed by electrophysiology following application of the acetylcholine for 1 second. Different EC_{50}s, Imax values, and desensitization kinetics were observed for each channel (Fig. 3), suggesting that including in the ligand binding domain of the channel heterologous sequences that correspond to extracellular regions of the protein that contributes to the ion pore domain of the chimera will alter the properties of the ion channel.

[0249] To better understand the impact of the heterologous domains that had been inserted into the channels, the ligand-dependent response profiles of wild type α7-nAChR and various

engineered chimeric LGIC receptors to acetylcholine as well as to non-native ligands TC-6987, AZD-0328, and Facinicline/RG3487 were assessed. Receptors were transiently expressed in HEK291T cells and currents were measured on an automated patch clamp system (Fluxion Biosciences) following 1 second addition of drug. Inclusion of a GlyRα1 Cys-loop sequence in the extracellular domain increased the potency of all small molecules on the engineered receptor (leftward shift in EC_{50}) (*e.g.* SEQ ID NO:35, Fig. 4C vs SEQ ID NO:16, Fig. 4B). Increasing the length of the heterologous Cys loop sequence modestly shifted the EC_{50} rightward for all compounds but increased Imax values significantly (SEQ ID NO:33, Fig. 4D vs SEQ ID NO:35, Fig. 4C), indicated a modest decrease in potency but increase in conductance overall. Inclusion of both the Cys-loop and β1-β2 loop sequences of GlyRα1 in the extracellular domain reduced the sensitivity by about 1 log from that observed with the full Cys-loop sequence alone, but further increased conductance approximately 2-fold and slowed the rate of deactivation of the channel (SEQ ID NO:41, Fig. 4E vs. SEQ ID NO:33, Fig 4D). These results were confirmed in a second model system in which the receptors were expressed in *Xenopus* oocytes and currents were measured on a manual patch clamp system following 10s applications of increasing doses of compound (Fig. 5).

[0250] Overall, SEQ ID NO:33 is approximately as sensitive to acetylcholine, ABT-126 and TC-6987 as wild type α7-nAChR, with TC-6987 showing partial agonist activity on SEQ ID NO:33 similar to wild type. SEQ ID NO:33 is approximate 2-fold less sensitive to nicotine and approximately 3-fold and 10-fold more sensitive to AZD-0328 and Facinicline/RG3487, respectively, than wild type. SEQ ID NO:41, further comprising the β1-β2 loop domain from GlyRα1, is comparably sensitive to ABT-126, TC-6987, and AZD-0328 as wild type α7-nAChR but less sensitive to acetylcholine and nicotine, with the EC_{50}s for acetylcholine and nicotine on SEQ ID NO:41 being approximately 4-fold and 7-fold larger (right shifted) than for wild type. While SEQ ID NO:41 is less sensitive to Facinicline/RG3487 than SEQ ID NO:33, it is still more sensitive to Facinicline/RG3487 than wild type, with the EC_{50} being approximately 2-fold lower (left shifted) (Fig. 5).

[0251] To further tune the responsiveness of the engineered LGIC receptors to native and non-native ligands, the binding pocket of each ligand in the alpha7-nAChR was modeled and the amino acid residues that form the binding pocket were mapped. Libraries of mutant chimeric LGICs were then generated, each mutant chimeric LGIC comprising substitutions in one or more

amino acids of the binding pocket. To screen these mutant LGICs for those having novel response profiles to ligands, an anion reporter assay was developed to assess the function of channels in a higher-throughput format. In this assay, cells expressing a YFP reporter whose fluorescence is quenched in the presence of anion are transfected with DNA encoding the channel of interest. Upon exposure to ligand, channels that are activated will flux anion, resulting in a dose-dependent quench of the YFP that can be detected on a plate reader. Fig. 6 depicts the responses of four engineered chimeric LGIC receptors (SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:41) to increasing concentrations of acetylcholine as measured by the quench in fluorescence of YFP over time. These quench plots demonstrate comparability between the electrophysiology and anion reporter approaches. Fig. 7 provides quench plots of the engineered chimeric LGIC receptors SEQ ID NO:33 and SEQ ID NO:41 to either acetylcholine (ACH) or AZD-0328. The parental chimeras respond differently to similar concentrations of different ligands.

[0252] Libraries of mutant chimeric LGIC receptors based on the parental chimera SEQ ID NO:33 were screened for responsiveness to acetylcholine and AZD-0328 using the anion reporter plate reader assay. FIG. 8 provides heat maps of the percent quench of YFP fluorescence following stimulation by various doses of either acetylcholine or AZD-0328. Many amino acid substitutions either reduced responsiveness to both ligands (Fig. 8A-F, rightward shift in EC_{50}) or increased responsiveness to ligand (Fig. 8I, leftward shift in EC_{50}) suggesting an overlap in the amino acids critical for binding and activation by both compounds. However, mutants were also identified that exhibit a leftward shift in response to acetylcholine but a rightward shift in response to AZD-0328 (Fig. 8G), or that exhibit a far rightward shift to acetylcholine as compared to the parental chimera but no significant change in response to AZD-0328 (Fig. 8H), demonstrating the ability of a single mutation to decouple responses to ligands.

[0253] Dose response curves were created for several mutants of interest to better characterize the EC_{50} of different ligands (Fig. 9). Substitutions L131S, L131T, and S172D in SEQ ID NO:33 resulted in a 2 log increase or more in EC_{50} for acetylcholine but only a slight increase (1 log or less, in some cases, less than 0.5 log) in EC_{50} for AZD-0328 as compared to that of unmutated SEQ ID NO:33 (Fig. 9). Substitution of W77M produced no shift in responsiveness to acetylcholine, but produced a significant increase (> 2 log) in EC_{50} to AZD-0328. These dose response curves confirmed that substitution of certain amino acid residues can dramatically alter the EC_{50} of select ligands from that observed on the parental chimera.

[0254] To confirm the EC_{50}s determined by plate reader as well as develop a better understanding of maximum current flow, the L131S, L131T, S172D and W77M mutations were assayed using electrophysiology. Substitution of L131S caused a significant right-ward shift (about 2 log, 80 - 100-fold) in the EC_{50} of acetylcholine relative to parental chimera SEQ ID NO:33, while the EC_{50} for AZD-0328 is only 2-fold right-ward shifted (FIG. 10A). Substitution of residue 131 with a T instead had an even greater impact, resulting in a right-ward shift in EC_{50} of ~2.5 log on acetylcholine and ~1 log on AZD-0328 (FIG. 10D). Substitution of S172D also caused a significant right-ward shift (about 2-log, or 58-fold) in the EC_{50} of acetylcholine relative to the parental chimera, while the EC_{50} for AZD-0328 on S172D is only 2-fold right-ward shifted. Substitution of W77M causes a moderate left-ward shift (2-fold) in the EC_{50} of acetylcholine relative to the parental chimera, while the EC_{50} for AZD-0328 on W77M is significantly right-ward shifted (>2.5 log). These results provide electrophysiological confirmation of the decoupling of acetylcholine and non-native ligand responses with single point mutations observed by plate reader.

Example 2. Assessing the efficacy of engineered receptors to treat disease in animal models

[0255] Chimeric LGIC receptors comprising the ligand binding domain from the α7-nAChR and the chloride-conducting ion pore domain from the human GlyRα1 subunit are assessed for their efficacy in providing analgesia in an rat model of neuropathic pain following administration of a small molecule ligand.

Materials and Methods

[0256] **AAV vector production:** Eight expression cassettes are constructed using standard molecular biology techniques. All contain a human synapsin-1 (hSYN) promoter linked to engineered chimeric receptors comprising a ligand binding domain of α7-nAChR and a Cys-loop domain and ion pore domain of GlyRα1. Their design is as follows:

(a) Chimera 1: no additional changes.

(b) Chimera 2: similar to 1, but also comprises an amino acids substitution from FIG. 8H that renders the engineered receptor less responsive to acetylcholine than either the wild type receptor or parental chimeric receptor from which it was derived, but maintains the responsiveness of the receptor to non-native ligand AZD-0328.

(c) Chimera 3: similar to 1, but also comprises an amino acid substitution from FIG 8A-F that renders the engineered receptor less responsive to both acetylcholine and non-native ligand than either the wild type receptor or parental chimeric receptor from which it was derived.

(d) Chimera 4: similar to , but also comprises an amino acid substitution from FIG. 8I that restores the EC50 of non-native ligand AZD-0328 and Facinicline to about wild type receptor levels.

(e) Chimera 5: same as 1, but also comprises a β1-2 loop domain from GlyRα1.

(f) Chimera 6: same as 2, but also comprises a β1-2 loop domain from GlyRα1.

(g) Chimera 7: same as 3, but also comprises a β1-2 loop domain from GlyRα1.

(h) Chimera 8: same as 4, but also comprises a β1-2 loop domain from GlyRα1.

[0257] These cassettes are subcloned into AAV bacmids, purified, transfected into Sf9 insect cells to produce recombinant bacuvlovirus, and then amplified. Sf9 cells are double infected using the amplified recombinant baculovirus containing the hSYN- α7-nAChR/GlyRα1 cassettes and another recombinant baculovirus containing the Rep and AAV6(Y705+731F+T492V) Cap genes to produce recombinant AAV vectors. The viral vectors are purified, viral titer determined using qPCR, and SDS-PAGE used to verify the purity of AAV vectors.

[0258] **Behavioral experiments and pain models:** To produce mechanical hypersensitivity in a model that mimics a neuropathic pain condition, the spared nerve injury (SNI) model (a validated model of mechanical allodynia) is used (Shields et al., 2003, The Journal of Pain, 4, 465-470). This model is produced by the sectioning of the common peroneal and the sural nerves and isolating the tibial branch. Mechanical withdrawal threshold is assessed by placing rats on an elevated wire-mesh grid and stimulating the plantar surface of the hind paw with von Frey filaments.

[0259] **AAV injection into the spinal cord of rats:** A dorsal hemilaminectomy is made at the level of the lumbar enlargement to expose two segments (about 1.5–2 mm) of lumbar spinal cord, after which the dura mater is incised and reflected. The viral solution is loaded into a glass micropipette (prefilled with mineral oil). The micropipette is connected to a manual micro-injector mounted on a stereotactic apparatus. The viral solution is targeted to the dorsal horn (left side). Along the rostro-caudal axis within the exposed region, 6 injections of 240 nl each are performed,

in an equidistant linear fashion. After each injection, 1 min of resting time is observed and then the muscle layer is sutured, the skin closed with staples, and the animals were allowed to recover with heated-pad before they were returned to their home cages. Animals are perfused for histological analysis after the last behavior test.

[0260] AAV intraganglionic injections into the dorsal root ganglion (DRG) of rats: The injection is performed with a borosilicate glass capillary (0.78/1 mm internal/external diameters) pulled to a fine point, attached by polyethylene tubing (0.4/0.8 mm internal/ external diameters) to a syringe mounted in a microinjection pump. The needle is mounted on an extended arm of a stereotaxic frame swung to the outside (used to hold and manipulate the needle only). Tubing, syringe, and needle are all filled with water. One microliter air is taken up into the needle followed by 3 μL of the viral vector solution. The needle is loaded separately with this volume for each injection. Animals are anesthetized prior to surgery. Following an incision along the dorsal midline, the L4 and L5 DRG are exposed by removal of the lateral processes of the vertebrae. The epineurium lying over the DRG is opened, and the glass needle inserted into the ganglion, to a depth of 400 μm from the surface of the exposed ganglion. After a 3-minute delay to allow sealing of the tissue around the glass capillary tip, 1.1 μL virus solution was injected at a rate of 0.2 μL/minute. After a further delay of 2 minutes, the needle is removed. The L4 ganglion is injected first followed by the L5 ganglion. The muscles overlying the spinal cord are loosely sutured together with a 5-0 suture and the wound closed. Animals are allowed to recover at 37 °C and received postoperative analgesia.

[0261] AAV intrathecal injections in rats: Rats are first anesthetized and then placed vertically with their head fixed in a stereotaxic frame. An incision is made in the base of the neck to expose the groove in the nuchal crest. An incision is made (1-2 mm) in the cisternal membrane to a depth such that cerebrospinal fluid leaks out. A 4 cm 32 G intrathecal catheter is then slowly inserted in the direction of the lumbar spinal cord and skin is closed by suture around the catheter. The rats are then allowed to recover. Rats are then anesthetized and the vector (6 μL) is administered. The catheter is flushed with 6 μL of PBS and then removed and rats allowed to recover.

Results

[0262] This SNI model is produced by the sectioning of the common peroneal and the sural nerves and isolating the tibial branch of the rat. The up-down method of Chaplan & Yaksh is used to determine mechanical thresholds before the injection of the AAV.hSYN-α7-nAChR/GlyRα1 into the spinal cord, DRG, or intrathecal space. Three weeks after unilateral vector injection, animals are tested again to verify that their mechanical withdrawal thresholds do not change. Motor coordination is also tested before and after injection, using an accelerating rotarod (Stoelting, USA) at a maximum speed of 33 rpm. The duration that the rat spends on the rotarod is recorded, with a cut-off at 300 sec. Each rat goes through three training trials and is tested two hours later. The animals receiving Chimera 1 and Chimera 5 appear to experience some analgesia even in the absence of synthetic ligand, suggesting that naturally occurring levels of acetylcholine may be activating the chimeric receptor. The remaining animals in the study show no change in mechanical withdrawal or motor coordination resulting from the injection of the AAV.

[0263] Subsequently, half of the rats in each chimera cohort are administered a single IP injection of AZD-0328 or Facinicline and mechanical thresholds tested using the up-down method at 1, 2, 5, 7, and 13 days post IP injection. When tested at high dose of ligand, a complete reversal of the mechanical hypersensitivity is observed in all rats. When tested at low dose, rats receiving Chimera 1, 2, 4, 5, 6, and 8 experience a complete reversal of the mechanical hypersensitivity, while rats receiving Chimera 3 and 7 (comprising the substitution that reduces responsiveness to non-native ligand but not the substitution that suppresses that effect) experience only a partial reversal of hypersensitivity. There is no change on the contralateral side, i.e. AZD-0328 has no measurable effect in the absence of vector. The reversal lasted for at least 2 days. On the third day, when the thresholds has returned to post-injury baseline, AZD-0328 is again injected IP and again a recovery to non-injury baseline thresholds is observed. These animals are followed for 48 hours and the thresholds remained at baseline. Animals are then perfused for histology.

Example 3. Treatment of a patient suffering from chronic pain

[0264] In a non-limiting example, a patient suffering from chronic radicular pain is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV.hSYN- α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into one or more dorsal root ganglia (i.e., intraganglionic convection-enhanced delivery into lumbar,

cervical, or thoracic DRGs). In this example, the AAV vector encodes the α7-nAChR/GlyRα1 chimera under the control of the human Synapsin-1 (SYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally as needed (i.e., during a pain episode).

Example 4. Treatment of a patient suffering from chronic pain

[0265] In a non-limiting example, a patient suffering from chronic craniofacial pain (*e.g.* trigeminal neuralgia or termporomandibular joint dysfunction) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV.hSYN-α7-nAChR/GlyRα1 in a volume of 0.150 mL delivered directly into the trigeminal ganglion (i.e., intraganglionic convection enhanced delivery). In this example, the AAV vector encodes the α7-nAChR/GlyRα1 chimera under the control of the human Synapsin-1 (SYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally as needed (i.e., during a pain episode).

Example 5. Treatment of a patient suffering from obesity

[0266] In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV. Ghrelin-α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the gastric branch of the vagus nerve (i.e., intraneural). In this example, the AAV vector encodes the engineered receptor under the control of the human Ghrelin promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for excess weight loss (i.e. for apetitite suppression).

Example 6. Treatment of a patient suffering from obesity

[0267] In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-TRPV1- α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the dorsal root ganglia innervating the pancreas (i.e., intragangionic). In this example, the AAV vector

encodes the engineered receptor under the control of the human TRPV1 promoter for selective neuronal expression in nociceptors. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for excess weight loss.

Example 7. Treatment of a patient suffering from obesity

[0268] In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-SIM1- α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the paraventricular nucleus (PVH) in the hypothalamus (i.e., intracranial, convection enhanced delivery). In this example, the AAV vector encodes the engineered channel under the control of the human Single-Minded Family BHLH Transcription Factor 1 (SIM1) promoter for selective neuronal expression in pro-opiomelanocortin (POMC) neurons and ultimately stimulation of the anorexigenic pathway. Two weeks post-injection, the patient returns to the clinic for a prescription forAZD-0328. The patient self-administers 0.15 mg/kg AZD-0328 orally daily for excess weight loss (i.e. for apetitite suppression).

Example 8. Treatment of a patient suffering from PTSD

[0269] In a non-limiting example, a patient suffering from post-traumatic stress disorder (PTSD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-hSYN1- α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the C6 stellate ganglion, (i.e., intraganglionic). In this example, the AAV vector encodes the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.15 mg/kg AZD-0328 orally daily for PTSD symptoms (i.e. for anxiety).

Example 9. Treatment of a patient suffering from depression

[0270] In a non-limiting example, a patient suffering from treatment-resistant depression (TRD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-hSYN1- α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the vagus nerve, (i.e., intraneural). In this example, the AAV vector encodes

the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for depression symptoms.

Example 10. Treatment of a patient suffering from GERD

[0271] In a non-limiting example, a patient suffering from gastroesophageal reflux disease (GERD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-hSYN1-α7-nAChR/GlyRα1 or AAV-CAG-α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into the lower esophageal sphincter (LES) vagus nerve and myenteric plexus (i.e., intraneural) or smooth muscle (intramuscular), respectively. In this example, the AAV vector encodes the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression or the CAG promoter for expression in LES myocytes. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.15 mg/kg AD-0328 orally daily for symptoms of GERD (i.e. acid reflux).

Example 11. Treatment of a patient suffering from epilepsy

[0272] In a non-limiting example, a patient suffering from seizures associated with epilepsy is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-CamKIIα-α7-nAChR/GlyRα1 in a volume of 1.0 mL delivered directly into a pre-determined seizure focus such as the motor cortex (i.e., intracranial). In this example, the AAV vector encodes the engineered receptor under the control of the human Calcium/calmodulin-dependent protein kinase II α (CamKIIα) promoter for selective neuronal expression in excitatory neurons. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for epileptic symptoms (i.e. seizures).

Example 12. Treatment of a patient suffering from a movement disorder

[0273] In a non-limiting example, a patient suffering from a movement disorder (*e.g.* Parkinsonian tremor) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with 10^{13} vector genomes of AAV-CamKIIα-α7-nAChR/GlyRα1 in a

volume of 1.0 mL delivered directly into the subthalamic nucleus (i.e., intracranial STN). In this example, the AAV vector encodes the engineered receptor under the control of the human Calcium/calmodulin-dependent protein kinase II α (CamKIIα) promoter for selective neuronal expression in excitatory neurons. Two weeks post-injection, the patient returns to the clinic for a prescription forAZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for movement disorder symptoms (i.e. tremor).

[0274] The preceding merely illustrates the principles of the disclosure. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the disclosure and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the disclosure and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the disclosure as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present disclosure, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present disclosure is embodied by the appended claims.

CLAIMS

1. An engineered receptor, wherein the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor and comprises

(a) a ligand binding domain derived from a first wild type Cys-loop LGIC receptor,

(b) a Cys-loop domain from a second wild-type Cys-loop LGIC receptor; and

(c) an ion pore domain derived from the second wild type Cys-loop LGIC receptor.

2. The engineered receptor according to claim 1, wherein the receptor further comprises a β1-2 loop domain from the second wild type Cys-loop LGIC receptor.

3. The engineered receptor according to claim 1 or 2, wherein the first and the second wild type Cys-loop LGIC receptor are different.

4. The engineered receptor according to any one of claims 1-3, wherein the first wild type Cys-loop LGIC receptor is the human α7 nicotinic acetylcholine receptor (α7-nAChR, SEQ ID NO:4).

5. The engineered receptor according to claim 3 or 4 wherein the second wild type Cys-loop LGIC receptor is the human Glycine receptor α1 subunit (GlyRα1, SEQ ID NO:2).

6. The engineered receptor according to claim 5, wherein the Cys-loop domain comprises amino acids 166-180 of SEQ ID NO:2.

7. The engineered receptor according to claim 5, wherein the Cys-loop domain comprises amino acids 166-172 of SEQ ID NO:2.

8. The engineered receptor according to claim 5, wherein the β1-2 loop domain comprises amino acids 81-84 of SEQ ID NO:2.

9. The engineered receptor according to any one of claims 1-8 wherein the ligand binding domain comprises one or more amino acid substitutions a residue corresponding to a residue of

α7-nAChR selected from the group consisting of W77, Y94, R101, W108, Y115, T128, N129, V130, L131, Q139, L141, Y151, S170, W171, S172, S188, Y190, Y210, C212, C213 and Y217.

10. The engineered receptor according to claim 9, wherein the one or more amino acid substitutions is a loss of function mutation that decreases the potency of acetylcholine on the receptor while substantially maintaining the potency of a non-native ligand on the receptor.

11. The engineered receptor according to claim 10, wherein the substitution is selected from a substitution corresponding to L131S, L131T, L131D, or S172D of α7-nAChR.

12. The engineered receptor according to claim 10, wherein the non-native ligand is selected from AZD-0328, TC6987, ABT-126 and Facinicline/RG3487.

13. The engineered receptor according to claim 9, wherein the one or more amino acid substitutions is a gain of function mutation that increases the potency of acetylcholine on the receptor.

14. The engineered receptor according to claim 13, wherein the substitution is selected from a substitution corresponding to L131N, L141W, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S172C, S188I, S188V, S188F, S188M, S188Q, S188T, S188P or S188W of α7-nAChR.

15. A polynucleotide encoding the engineered receptor from any one of claims 1-14.

16. A vector comprising the polynucleotide of claim 15.

17. The vector of claim 16, wherein the vector is a viral vector selected from the group consisting of an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, and a herpes simplex-1 viral vector (HSV-1).

18. The viral vector according to claim 17, wherein the AAV vector is AAV5 or a variant thereof, AAV6 or a variant thereof or AAV9 or a variant thereof.

19. A pharmaceutical composition comprising a polynucleotide according to claim 15 or the vector according to any one of claims 16-18 and a pharmaceutically acceptable vehicle.

20. A method of ameliorating a neurological disorder in a subject in need thereof comprising:

administering to the subject a polynucleotide encoding an engineered ligand-gated ion channel (LGIC) receptor according to any one of claims 1-15, and

administering to the subject a binding agent, wherein the binding agent acts as an agonist of the engineered LGIC receptor.

21. The method according to claim 20, wherein the polynucleotide is administered to the subject by a non-viral method.

22. The method according to claim 21, wherein the non-viral method is lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection.

23. The method according to claim 20, wherein the polynucleotide is delivered to the subject in a viral vector.

24. The method according to claim 23, wherein the viral vector is an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, or a herpes simplex-1 viral vector (HSV-1).

25. The method according to claim 24, wherein the AAV vector is AAV5 or a variant thereof, AAV6 or a variant thereof or AAV9 or a variant thereof .

26. The method according to any one of claims 20-25, wherein the engineered receptor is operably linked to a promoter that is active in an excitable cell.

27. The method according to claim 26, wherein the excitable cell is a neuron or a myocyte.

28. The method according to claim 27, wherein the neuron is a dorsal root ganglion, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

29. The method according to any one of claims 20-28, wherein the subject suffers from pain, a seizure disorder, a movement disorder, an eating disorder, a spinal cord injury, neurogenic bladder, a spasticity disorder, or pruritus.

30. The method according to any one of claims 20-28, wherein the subject suffers from Alzheimer's disease, Parkinson's disease, post-traumatic stress disorder (PTSD), gastroesophageal reflux disease (GERD), addiction, anxiety, depression, memory loss, dementia, sleep apnea, stroke, narcolepsy, urinary incontinence, essential tremor, movement disorder, or atrial fibrillation.

31. The method according to any one of claims 20-30, wherein the binding agent is selected from the group consisting of AZD0328, ABT-126, TC6987, or Facinicline/RG3487.

32. The method according to any one of claims 20-31, wherein the polynucleotide is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna.

33. The method according to any one of claims 20-31, wherein the binding agent is administered orally, subcutaneously, topically, or intravenously.

34. The method according to any one of claims 20-33, wherein the subject is a human.

Fig. 1

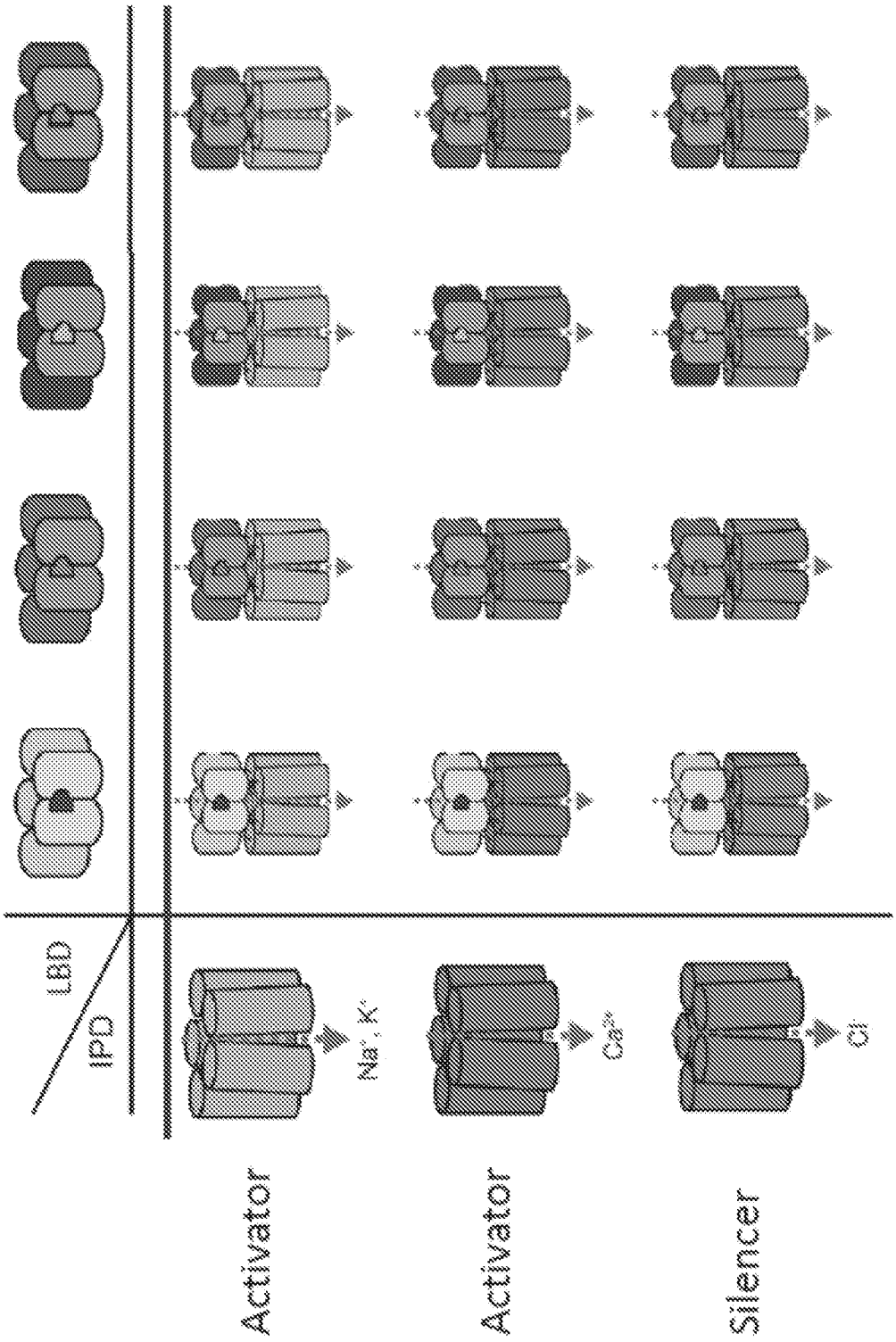

GlyR
GABA$_A$
5HT$_3$
nAChR
LBD
IPD
Activator
Activator
Silencer
Na^+, K^+
Ca^{2+}
Cl^-

Fig. 2

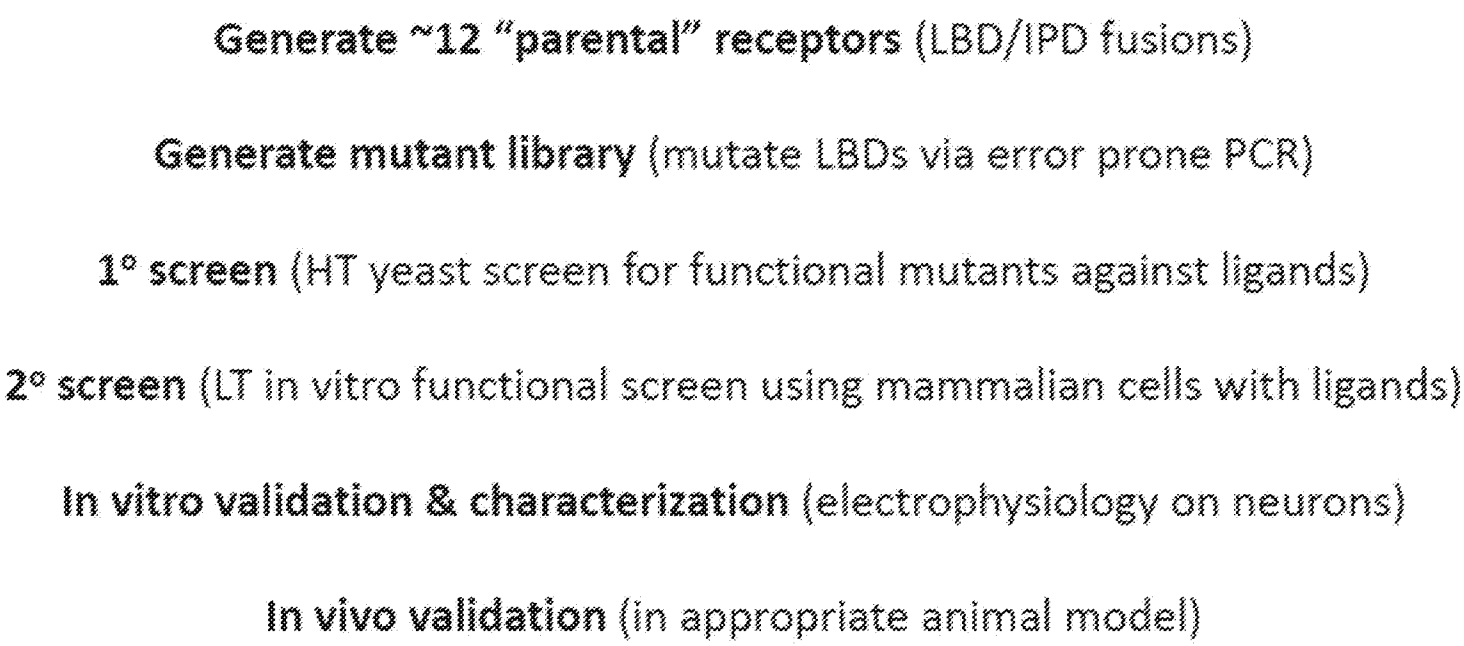

Generate ~12 "parental" receptors (LBD/IPD fusions)
Generate mutant library (mutate LBDs via error prone PCR)
1° screen (HT yeast screen for functional mutants against ligands)
2° screen (LT in vitro functional screen using mammalian cells with ligands)
In vitro validation & characterization (electrophysiology on neurons)
In vivo validation (in appropriate animal model)

Fig. 3

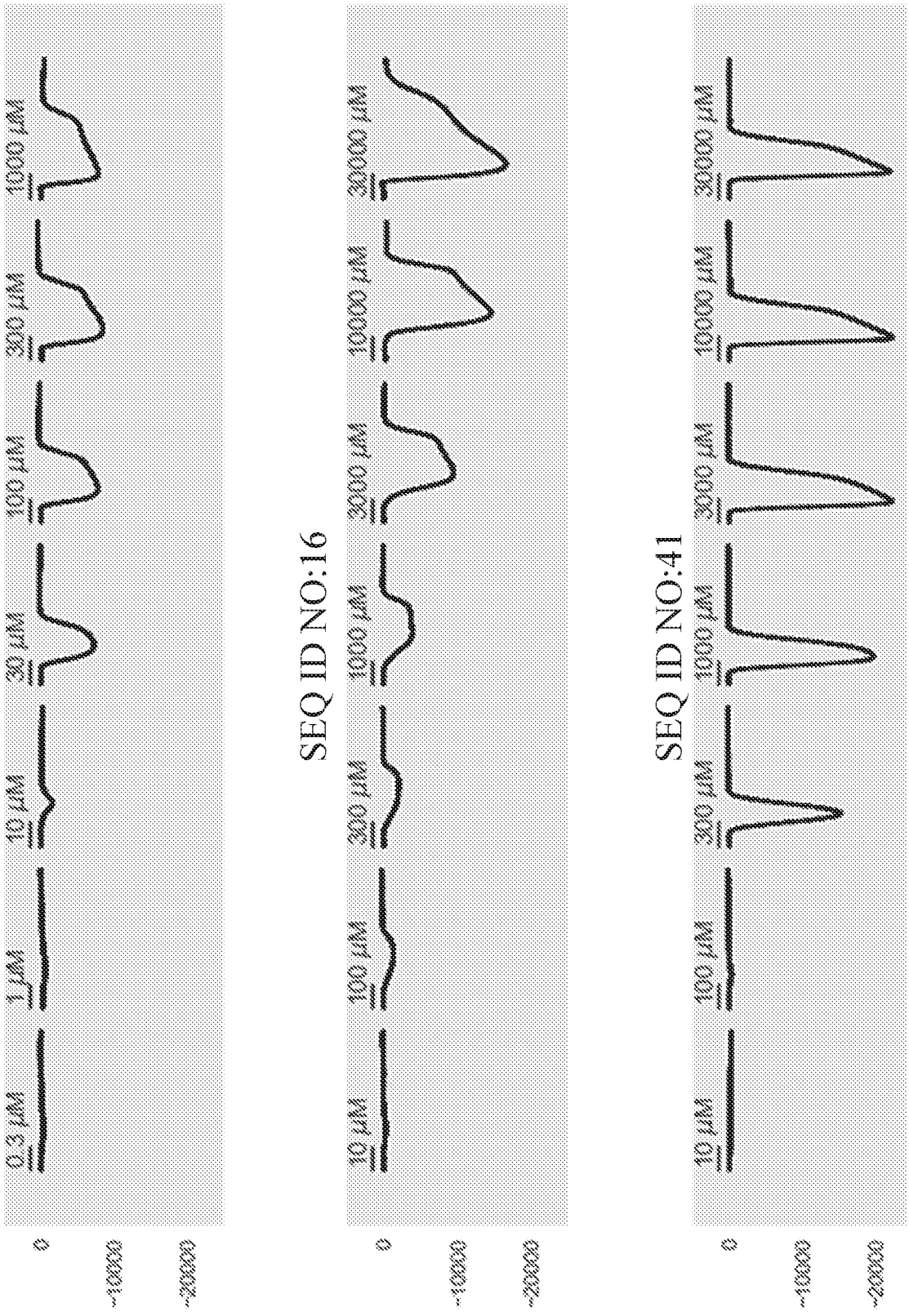

SEQ ID NO:34
0.3 μM
1 μM
10 μM
30 μM
100 μM
300 μM
1000 μM
SEQ ID NO:16
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
30000 μM
SEQ ID NO:41
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
30000 μM
0
-10000
-20000
Current (pA)
Ligand Dose

Fig. 4A

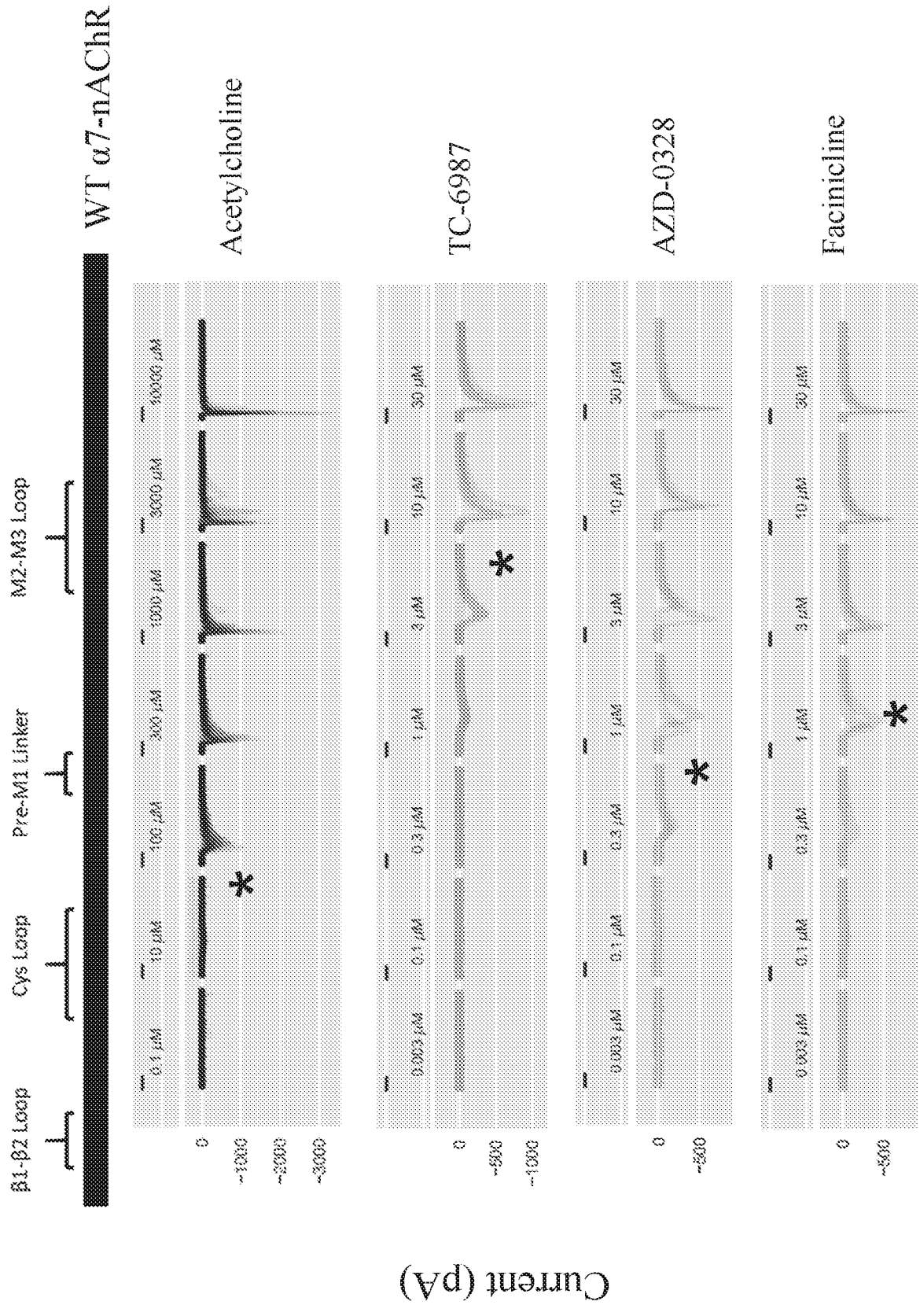
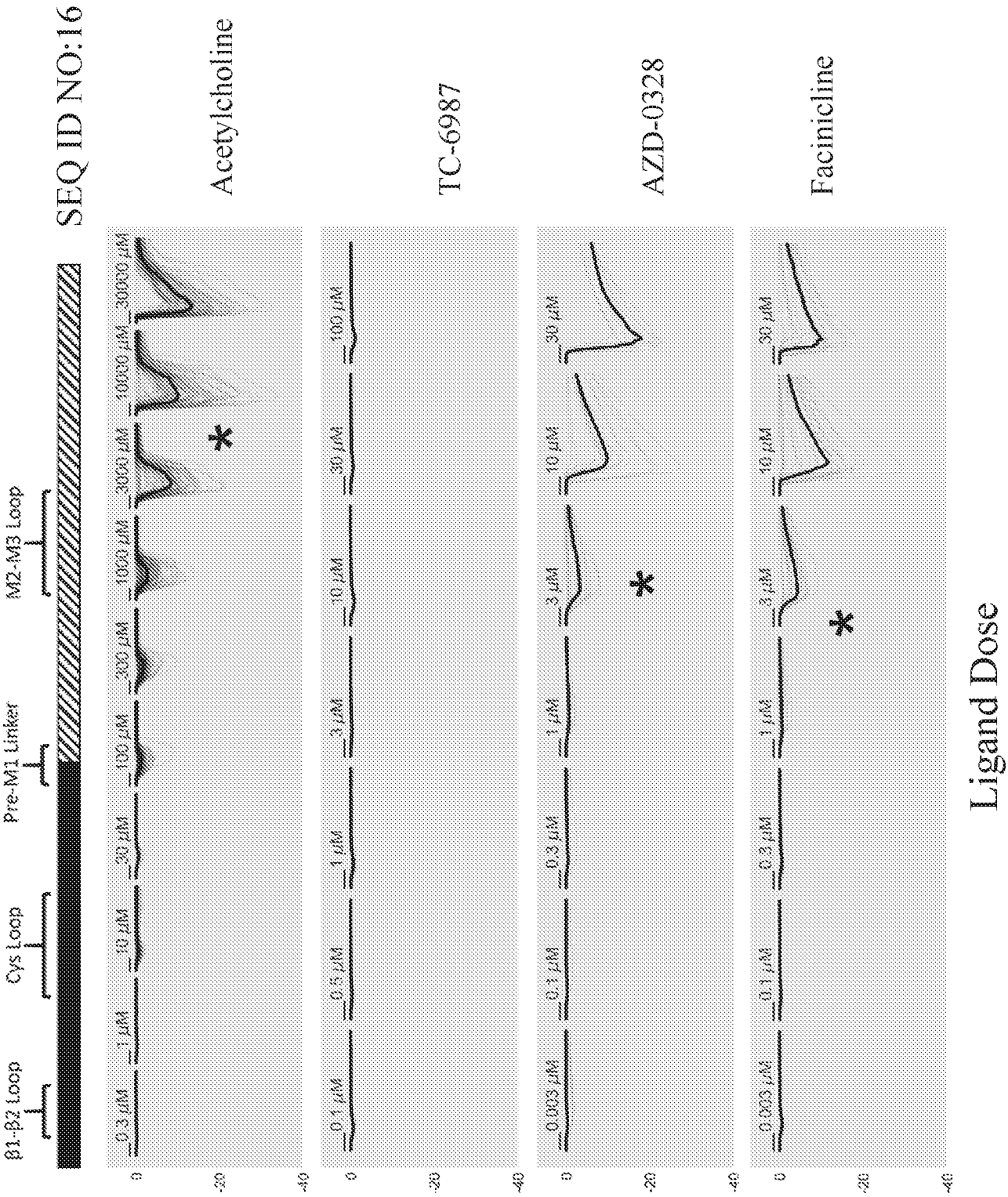

WT α7-nAChR
β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
Acetylcholine
TC-6987
AZD-0328
Fasiniciline
Current (pA)
Ligand Dose

Fig. 4B


SEQ ID NO:16
β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
Acetylcholine
TC-6987
AZD-0328
Facinicline
0.3 μM
1 μM
10 μM
30 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
30000 μM
0.1 μM
0.5 μM
3 μM
0.003 μM
0.03 μM
0.3 μM
0
-20
-40
*
Current (pA)
Ligand Dose

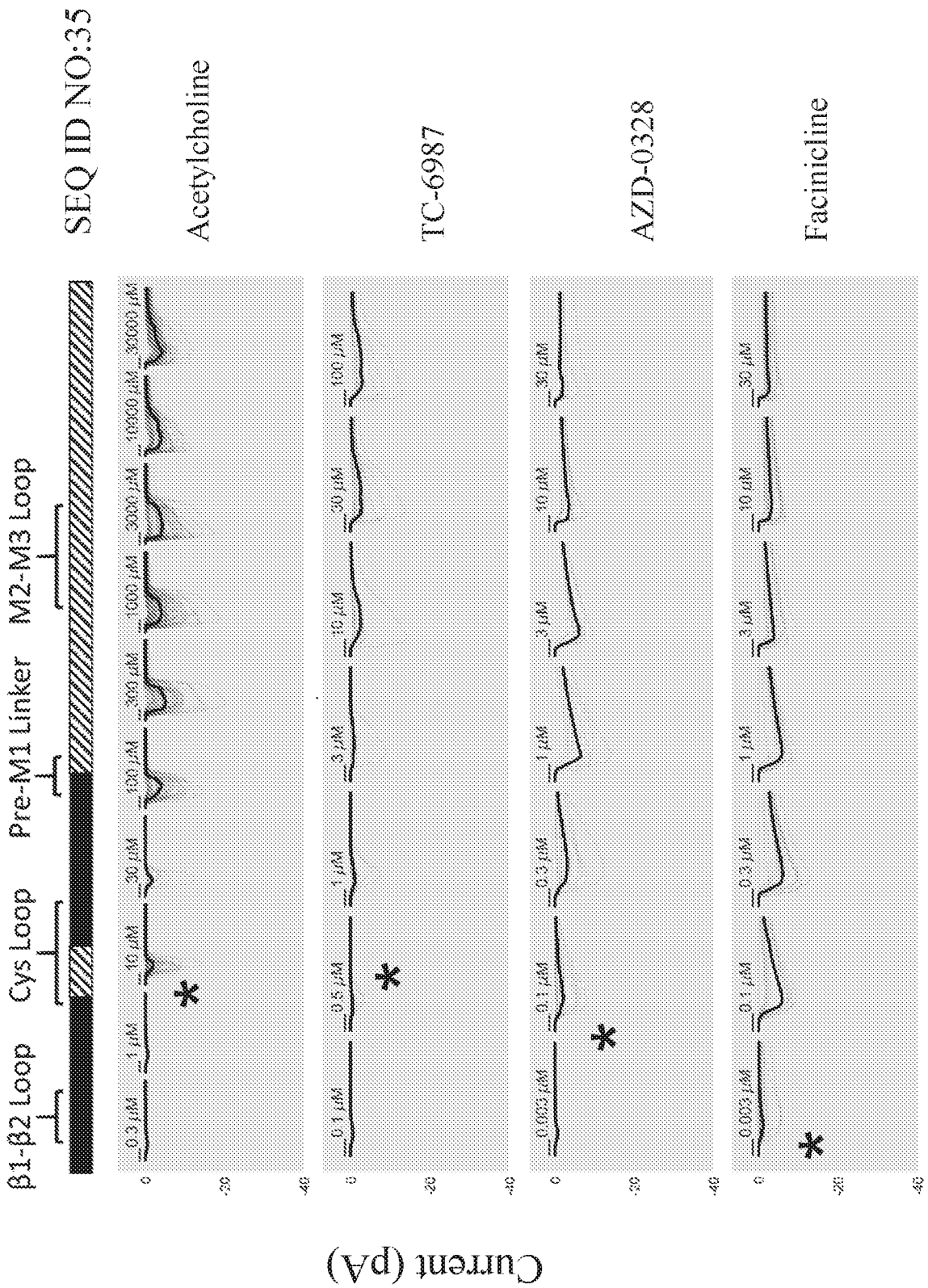

SEQ ID NO:35
β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
Acetylcholine
TC-6987
AZD-0328
Facinicline
Current (pA)
Ligand Dose

Fig. 4C

Fig. 4D

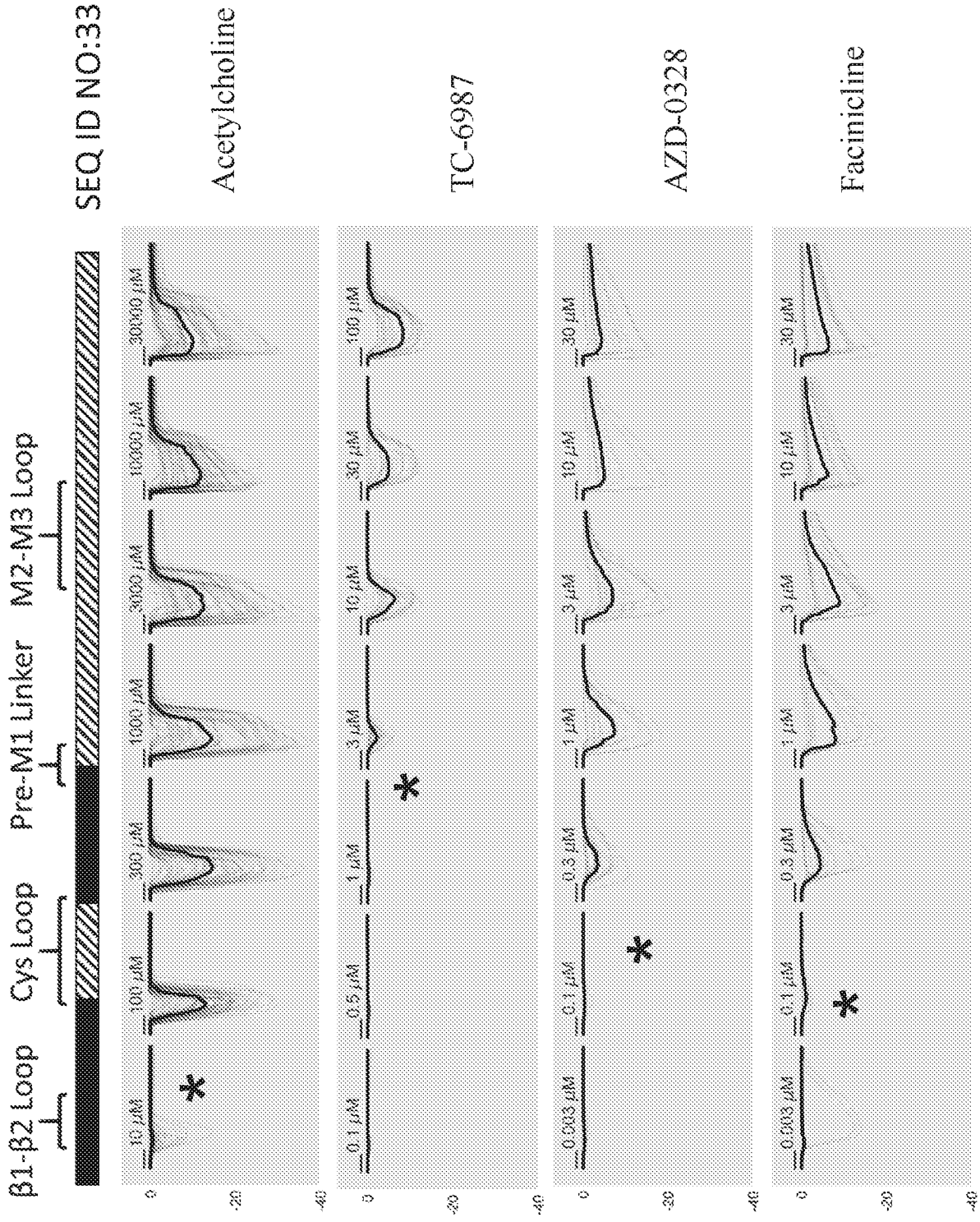

β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
SEQ ID NO:33
Acetylcholine
TC-6987
AZD-0328
Facinicline
Current (pA)
Ligand Dose

Fig. 4E

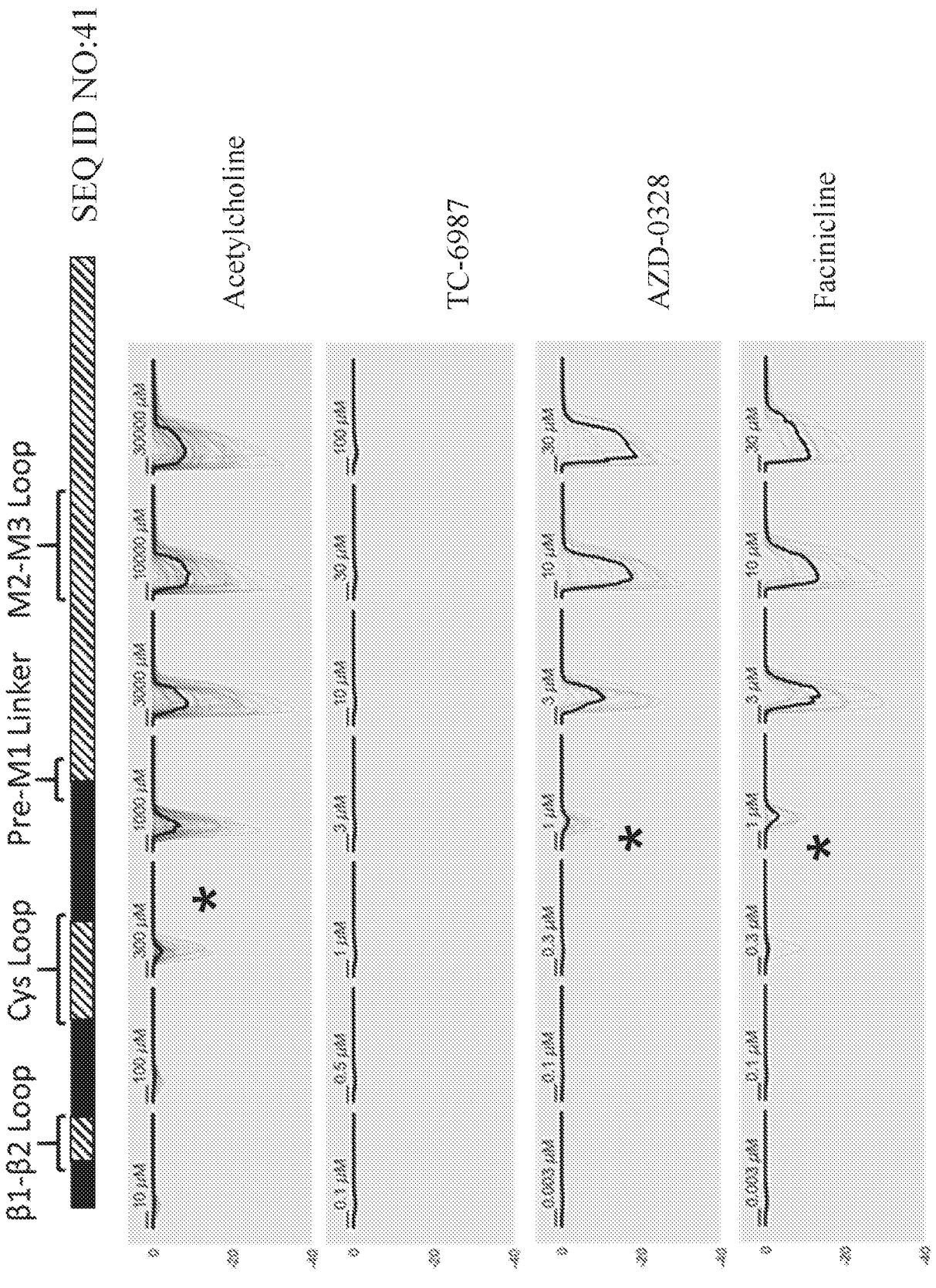

SEQ ID NO:41
β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
Acetylcholine
TC-6987
AZD-0328
Facinicline
Current (pA)
Ligand Dose

Fig. 4F

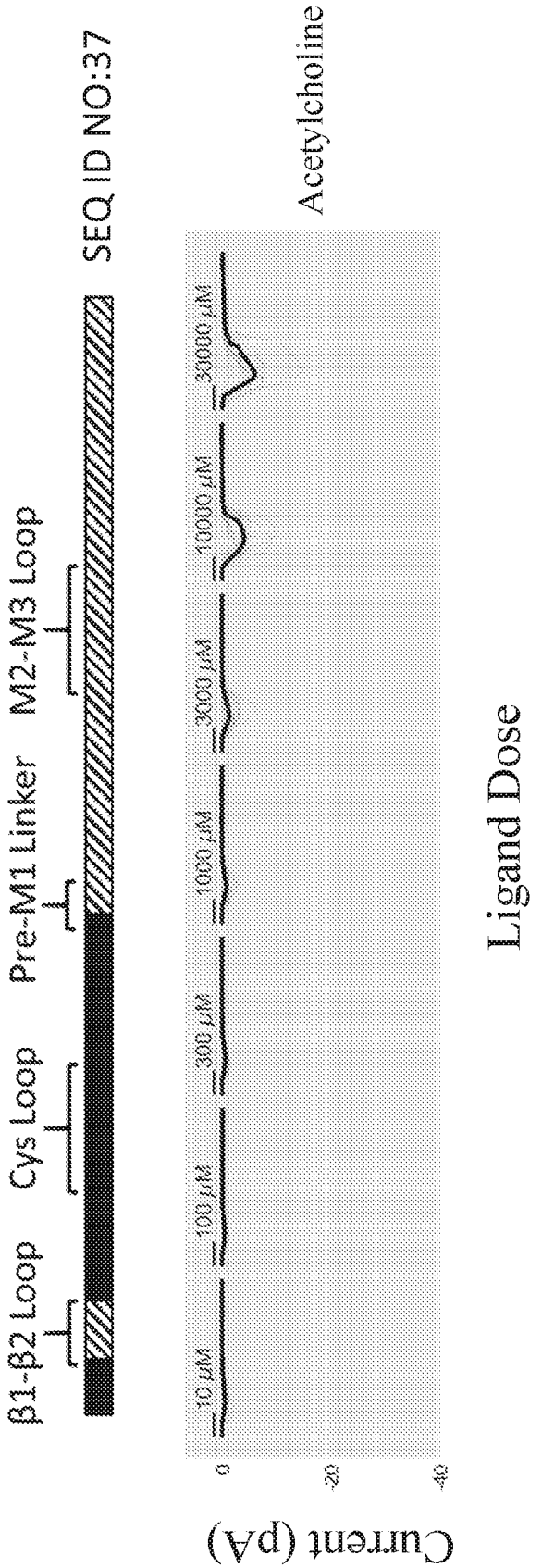

SEQ ID NO:37
β1-β2 Loop
Cys Loop
Pre-M1 Linker
M2-M3 Loop
Acetylcholine
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
30000 μM
0
-20
-40
Current (pA)
Ligand Dose

Fig. 5A

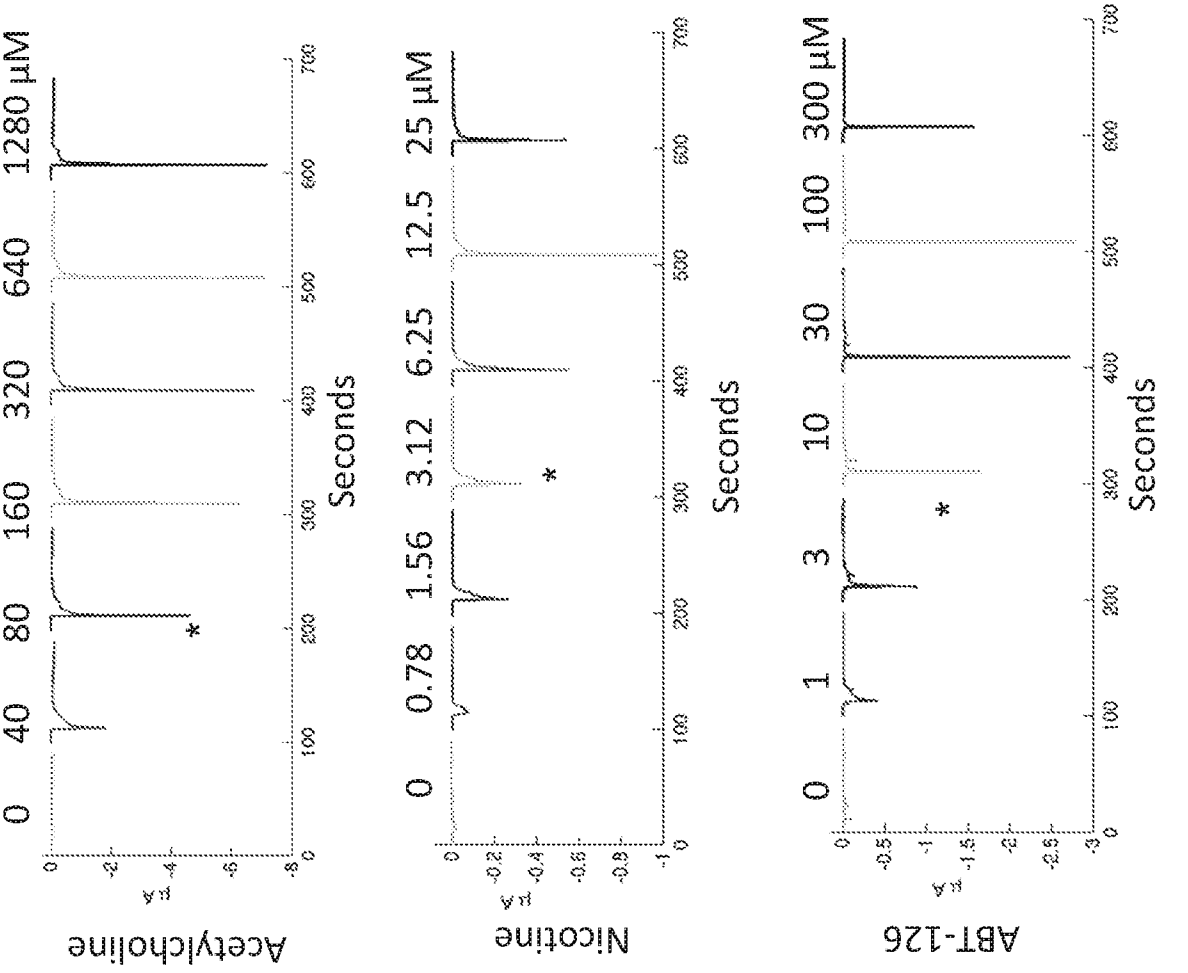

Acetylcholine
Nicotine
ABT-126
0 40 80 160 320 640 1280 µM
0 0.78 1.56 3.12 6.25 12.5 25 µM
0 1 3 10 30 100 300 µM
Seconds
Normalized Currents
Log [Acetylcholine] µM
Log [Nicotine] µM
Log [ABT-126] µM

Fig. 5B

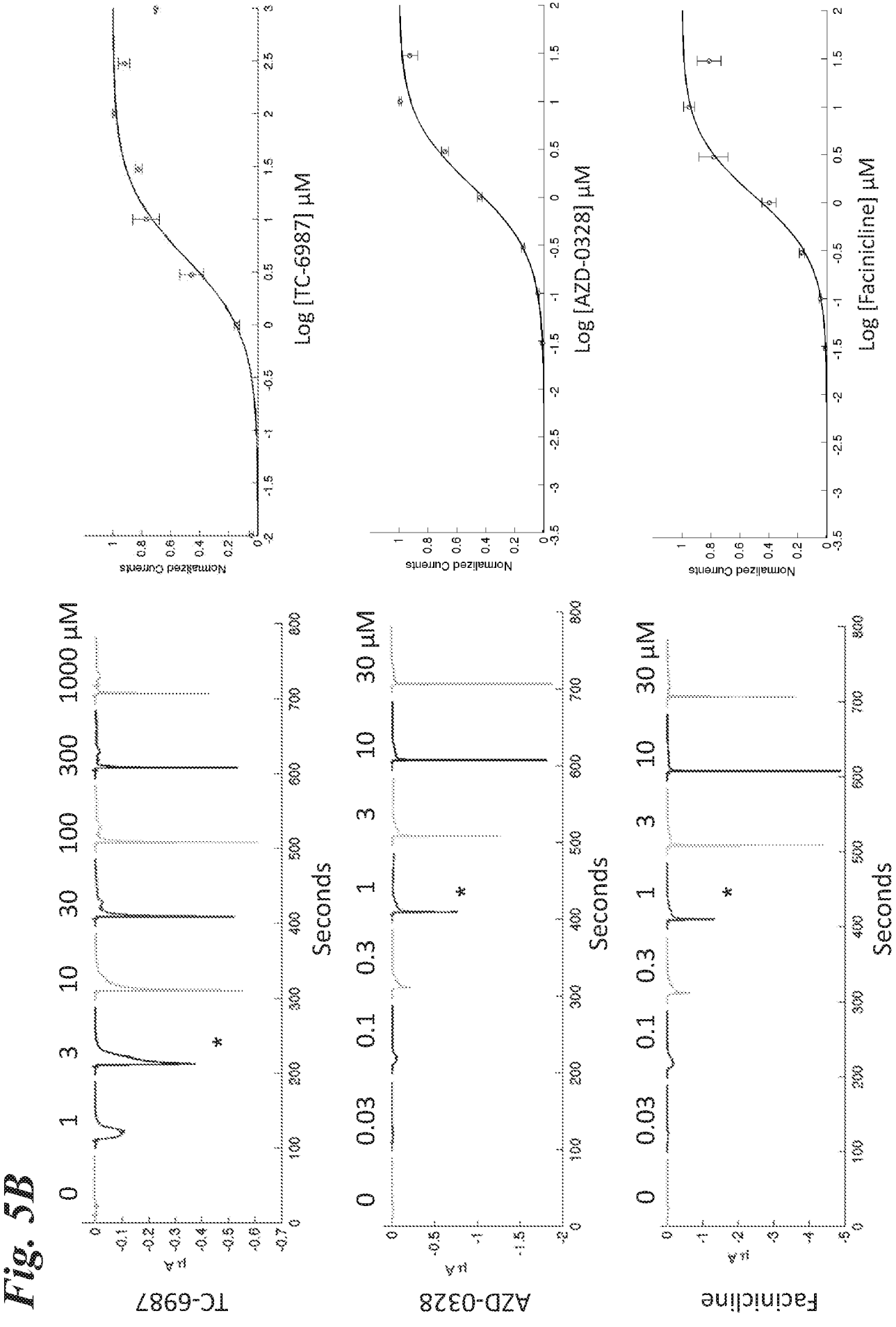

TC-6987
AZD-0328
Facinicline
0
0.03
0.1
0.3
1
3
10
30
100
300
1000 µM
Seconds
Normalized Currents
Log [TC-6987] µM
Log [AZD-0328] µM
Log [Facinicline] µM

Fig. 5C

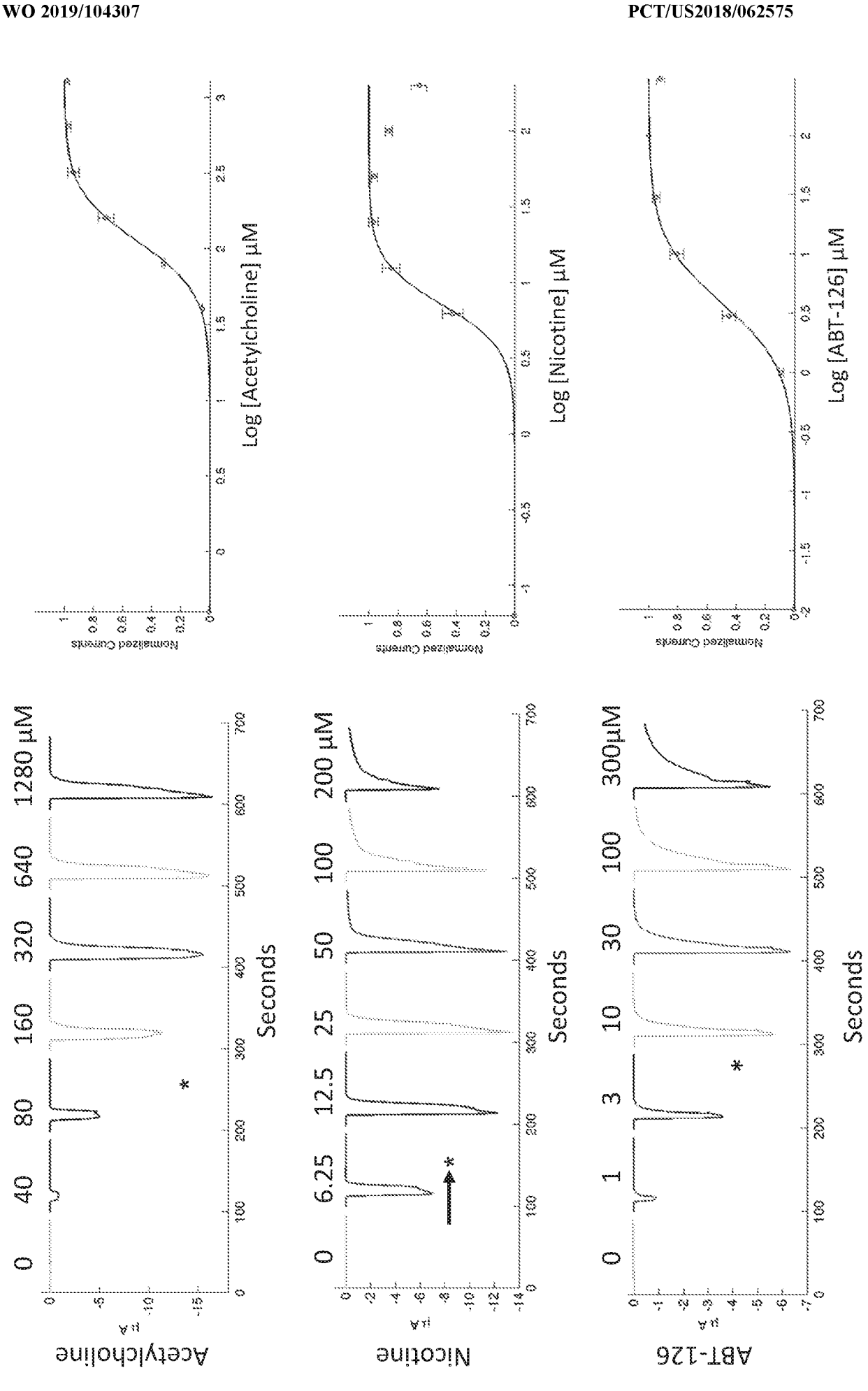

Acetylcholine
Nicotine
ABT-126
0 40 80 160 320 640 1280 µM
0 6.25 12.5 25 50 100 200 µM
0 1 3 10 30 100 300µM
Seconds
Normalized Currents
Log [Acetylcholine] µM
Log [Nicotine] µM
Log [ABT-126] µM

Fig. 5D

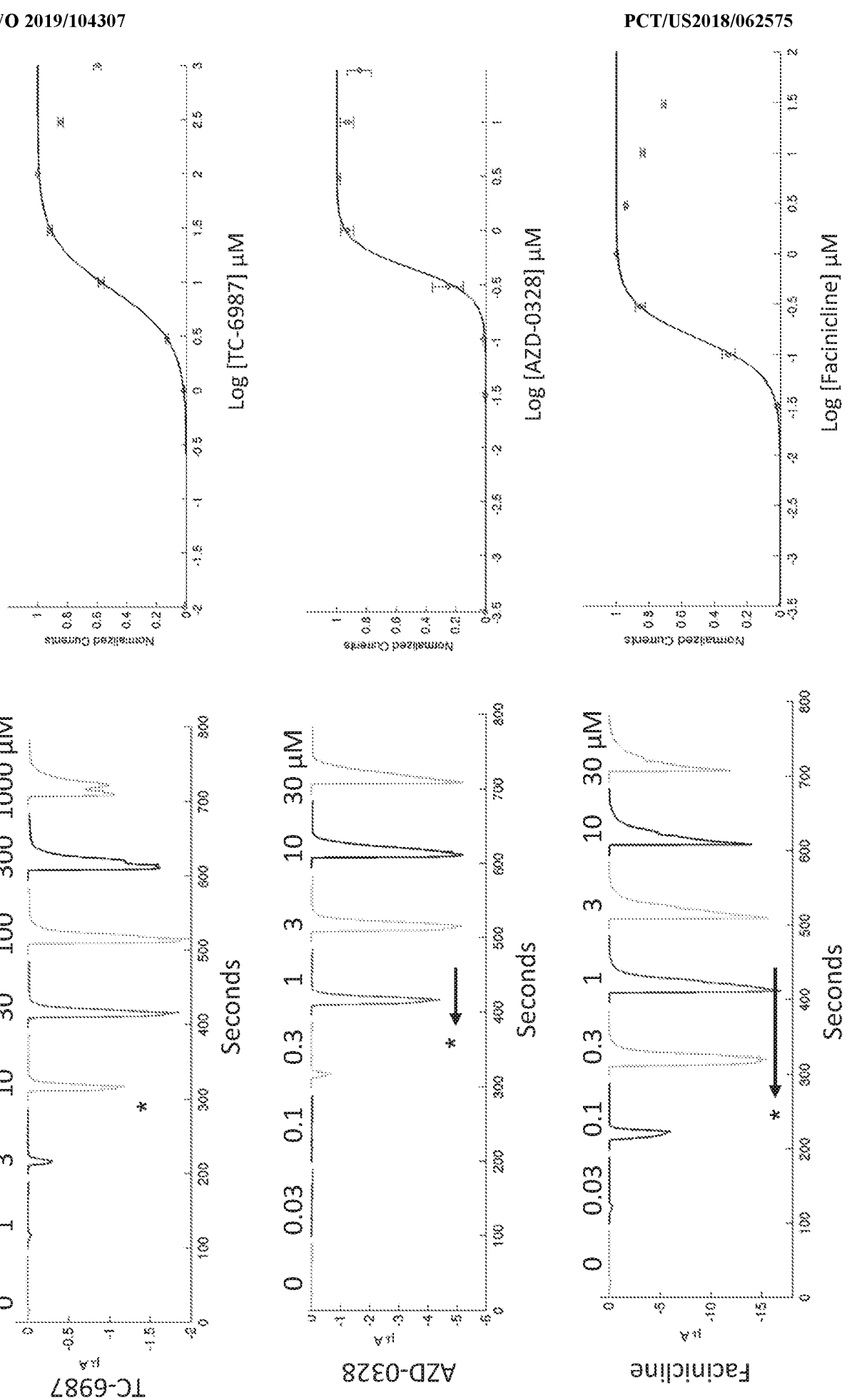

TC-6987
AZD-0328
Facinicline
0 0.03 0.1 0.3 1 3 10 30 μM
Seconds
Normalized Currents
Log [TC-6987] μM
Log [AZD-0328] μM
Log [Facinicline] μM

Fig. 5E

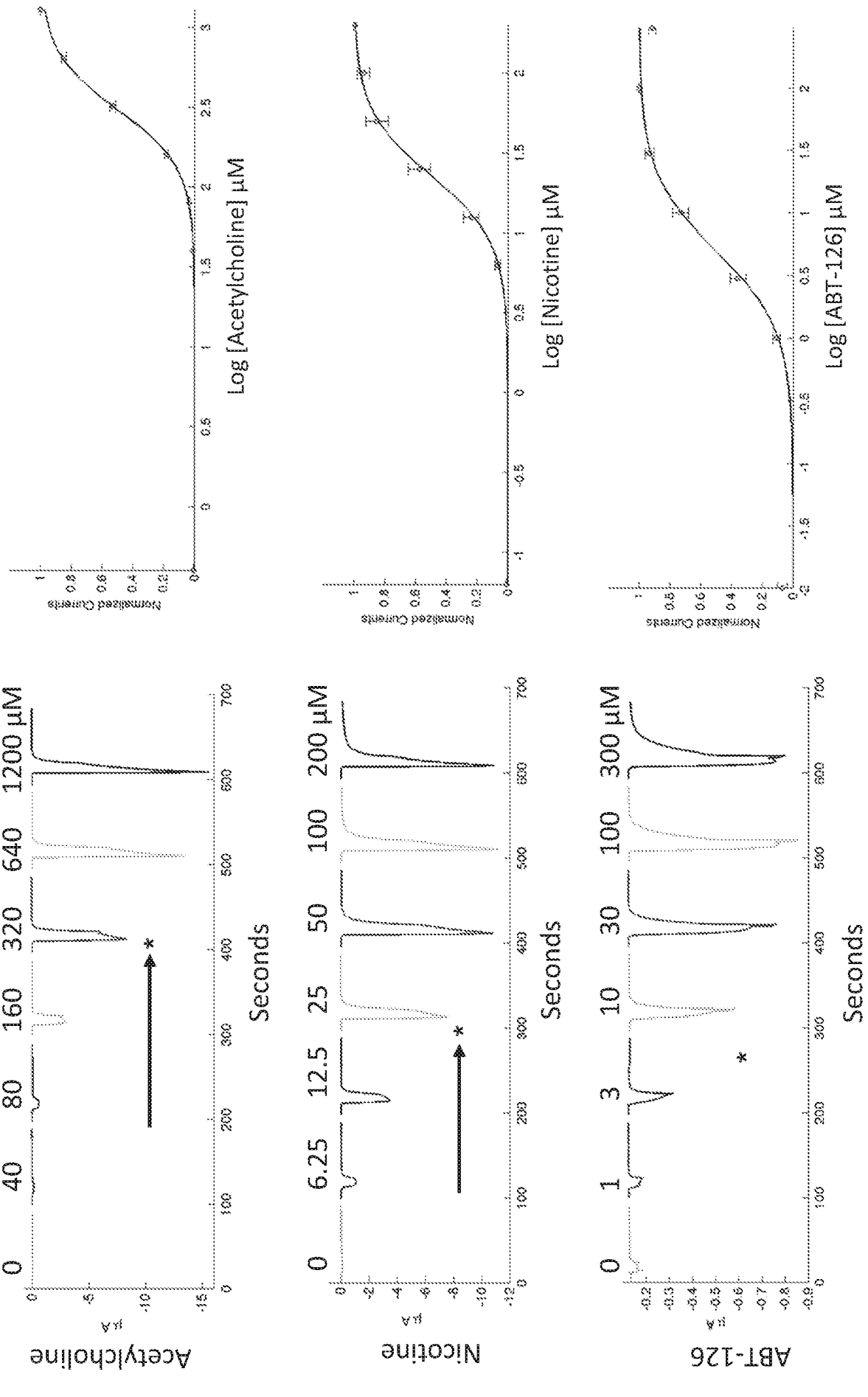

Acetylcholine
Nicotine
ABT-126
Seconds
Log [Acetylcholine] µM
Log [Nicotine] µM
Log [ABT-126] µM
Normalized Currents
0
40
80
160
320
640
1200 µM
6.25
12.5
25
50
100
200 µM
1
3
10
30
100
300 µM

Fig. 5F

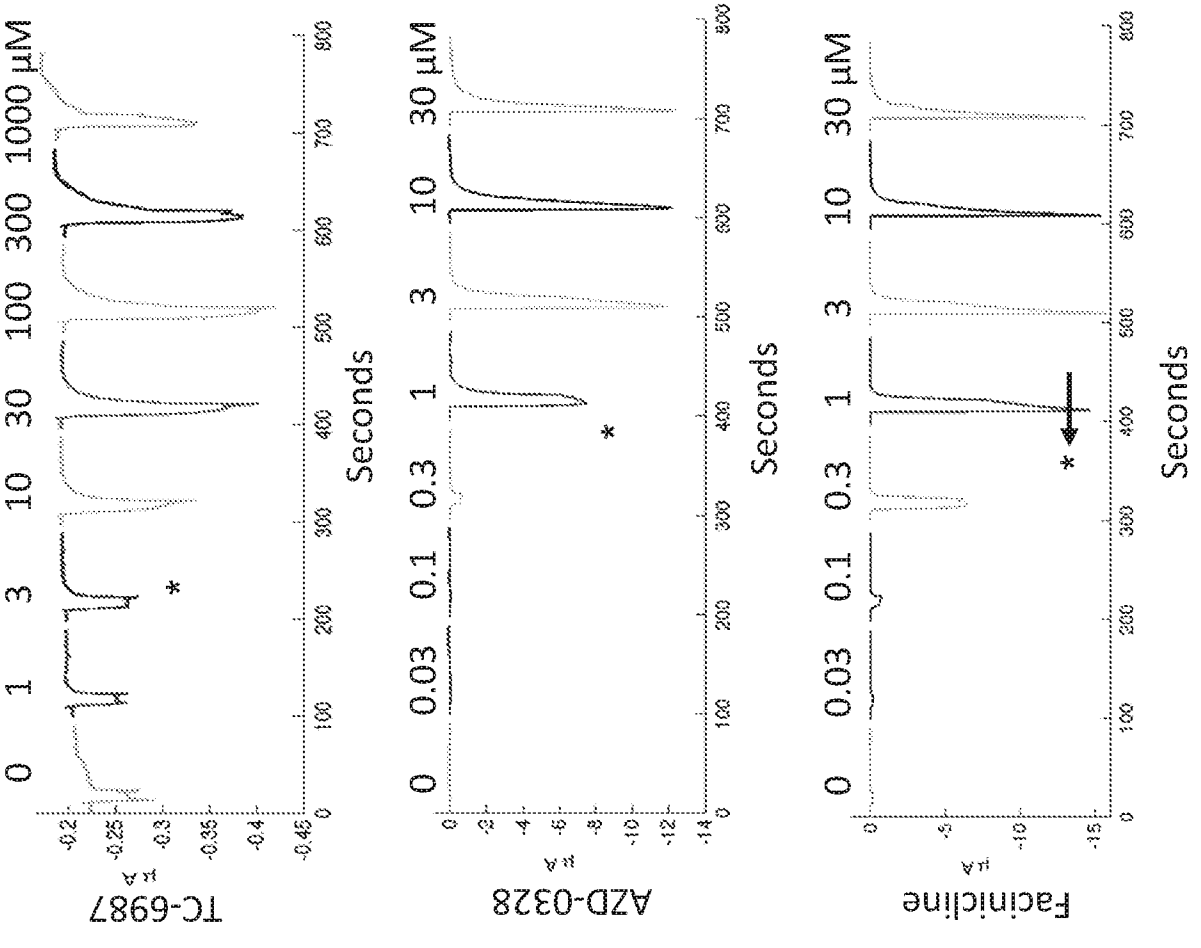
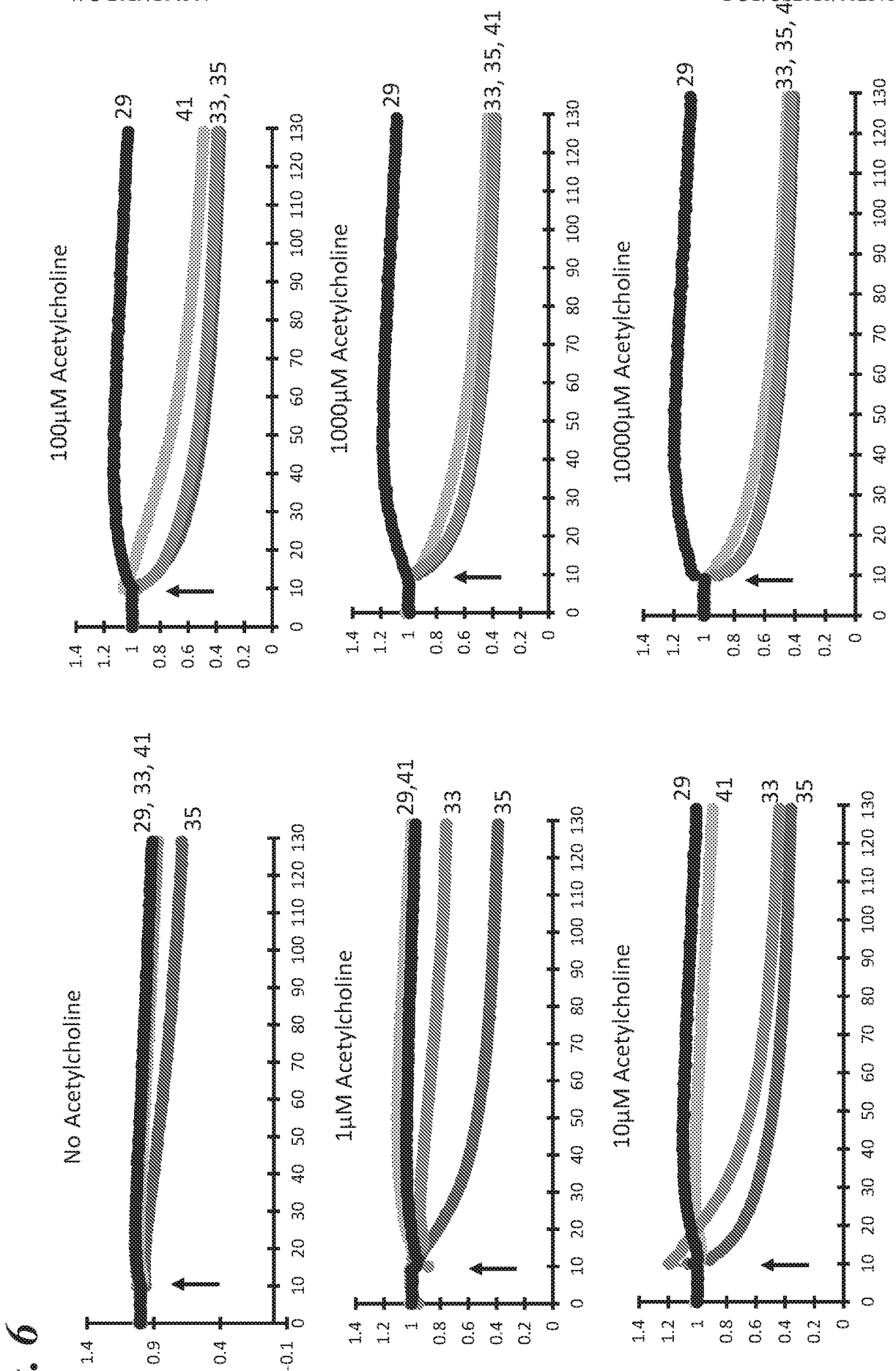

TC-6987
AZD-0328
Facinicline
0 0.03 0.1 0.3 1 3 10 30 μM
Seconds
Normalized Currents
Log [TC-6987] μM
Log [AZD-0328] μM
Log [Facinicline] μM

Fig. 6


No Acetylcholine
29, 33, 41
35
1µM Acetylcholine
29,41
33
35
10µM Acetylcholine
29
41
33
35
100µM Acetylcholine
29
41
33, 35
1000µM Acetylcholine
29
33, 35, 41
10000µM Acetylcholine
29
33, 35, 41
SEQ ID NO:29
SEQ ID NO:33
SEQ ID NO:35
SEQ ID NO:41

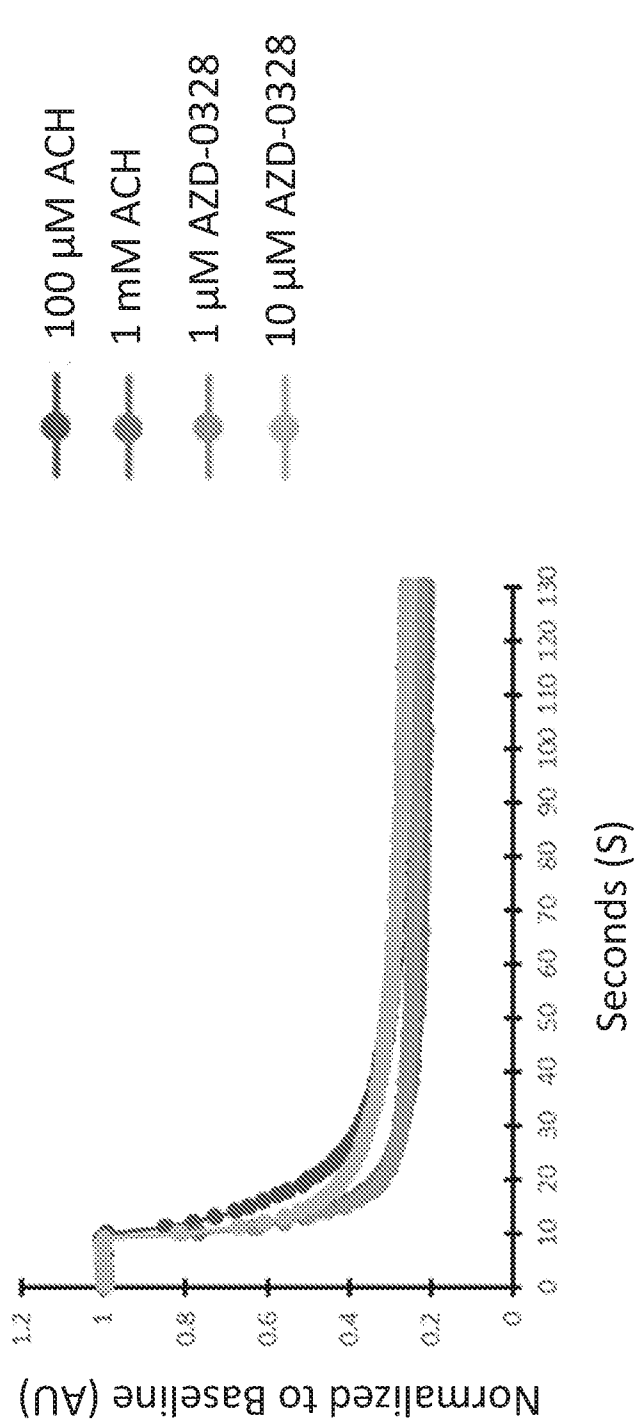

100 µM ACH
1 mM ACH
1 µM AZD-0328
10 µM AZD-0328
Normalized to Baseline (AU)
Seconds (S)
0
0.2
0.4
0.6
0.8
1
1.2
0 10 20 30 40 50 60 70 80 90 100 110 120 130

Fig. 7A

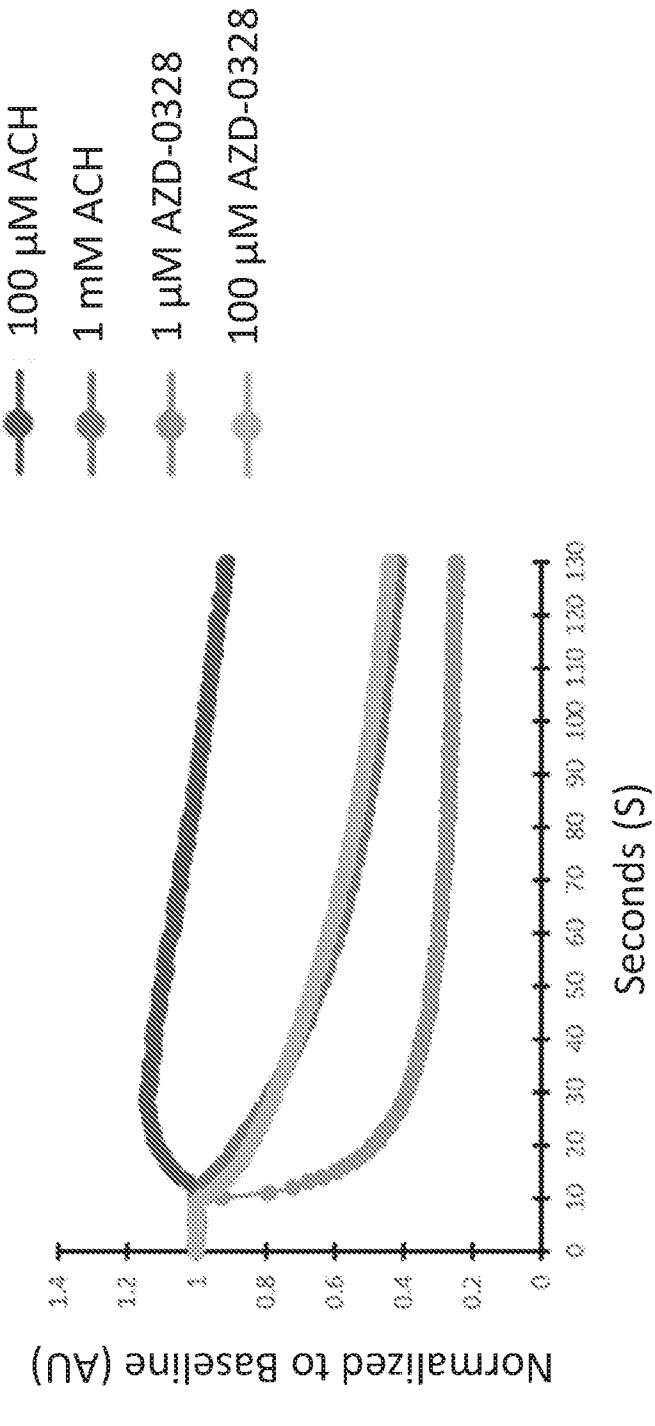
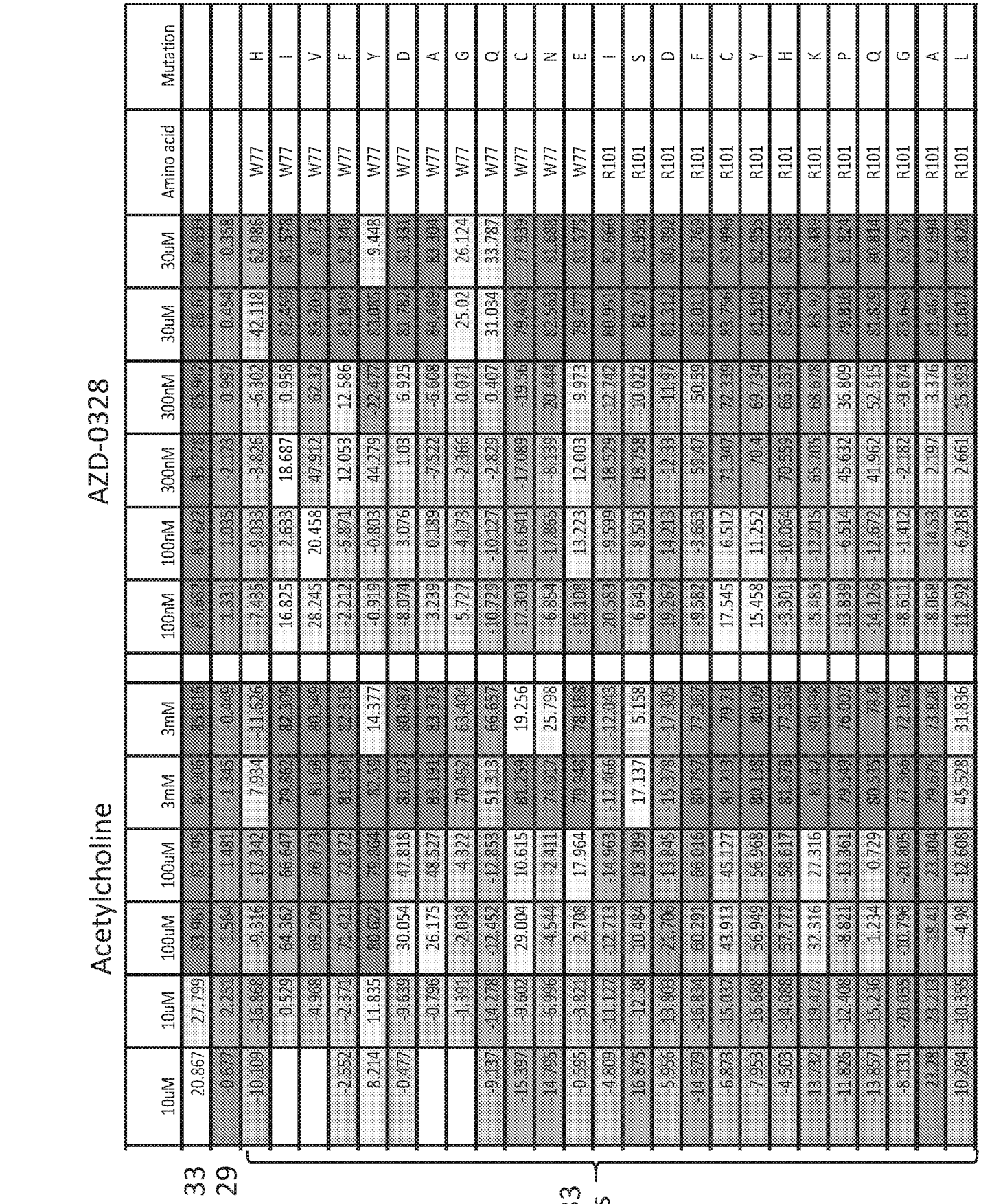

100 µM ACH
1 mM ACH
1 µM AZD-0328
100 µM AZD-0328
Normalized to Baseline (AU)
Seconds (S)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0 10 20 30 40 50 60 70 80 90 100 110 120 130

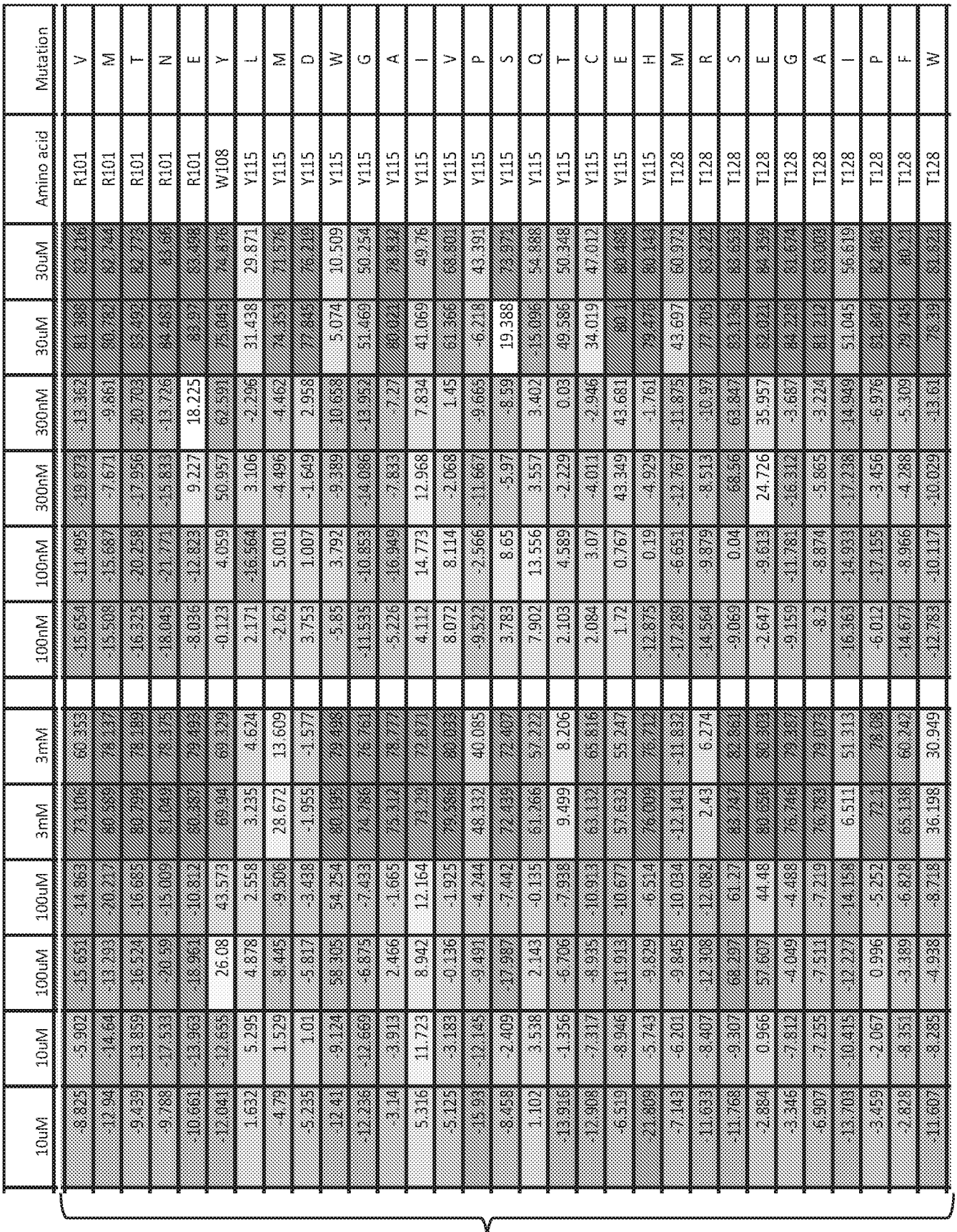
Fig. 7B

Fig. 84



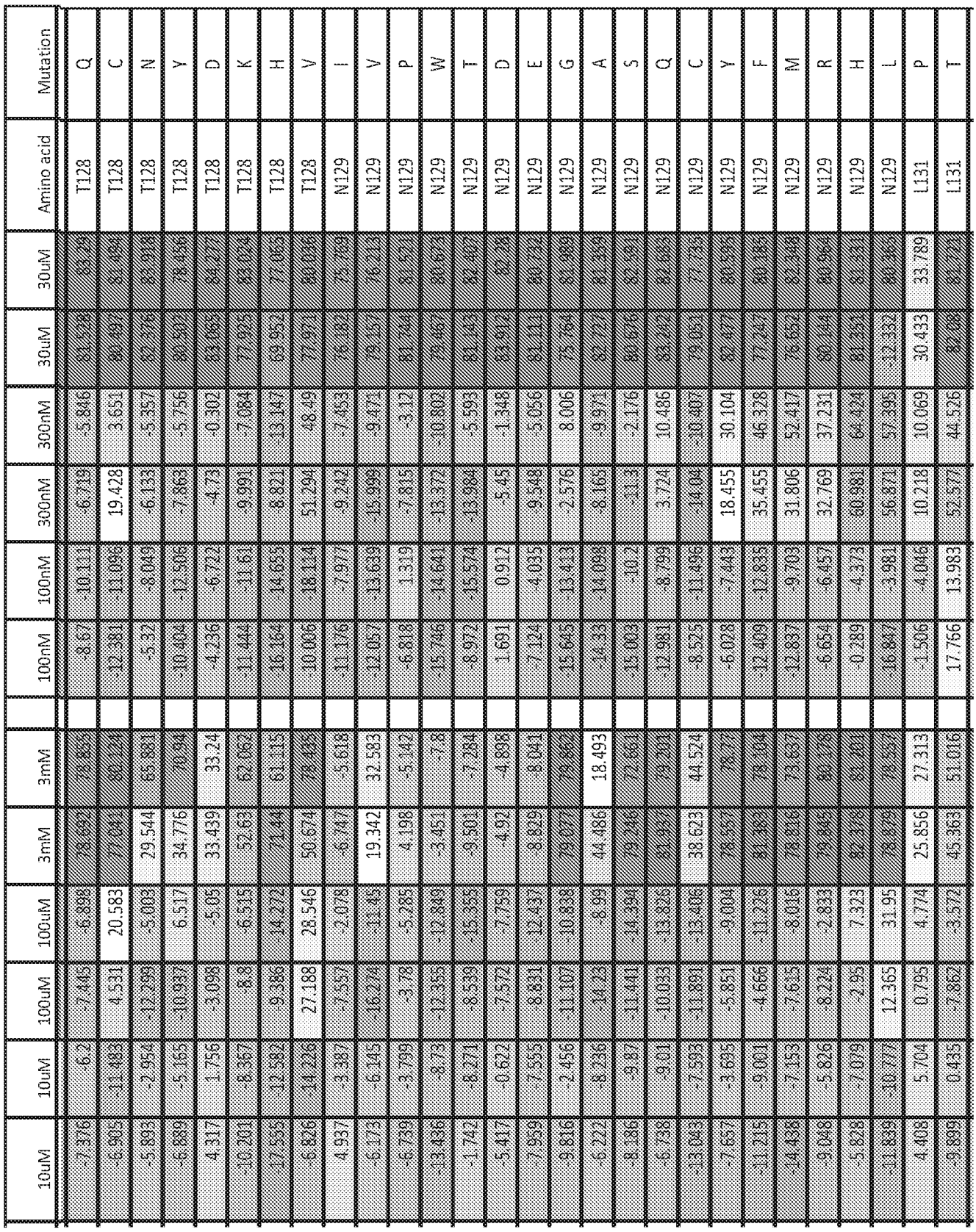
	Acetylcholine						AZD-0328						
	10uM	10uM	100uM	100uM	3mM	3mM	100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
SEQ ID 33	20.867	27.799	83.861	92.199	84.906	85.018	83.852	83.852	85.221	85.941	86.07	86.336		
SEQ ID 29	0.671	2.251	1.564	1.491	-1.383	-0.449	1.331	1.035	2.123	0.997	0.454	-0.338		
SEQ ID 33 variants	-10.109	-16.868	-9.316	-17.392	7.934	-11.626	-7.435	-9.033	-3.826	-6.302	42.118	62.986	W77	H
		0.529	64.362	66.647	79.662	80.189	16.825	2.633	18.687	0.958	82.453	83.578	W77	I
		-4.968	69.209	76.777	81.598	80.549	28.245	20.458	47.912	62.32	83.201	81.73	W77	V
		-2.552	-2.371	72.431	72.823	81.254	82.115	-2.212	-5.871	12.053	12.586	82.601	82.349	W77	F
		8.214	11.835	80.622	78.644	82.631	14.377	-0.919	-0.803	44.279	22.877	23.053	9.448	W77	Y
		-0.477	-9.639	30.054	47.818	81.057	80.389	-8.074	3.076	1.03	6.925	81.781	81.534	W77	D
			-0.796	26.175	48.527	81.491	69.372	3.239	0.189	7.522	6.608	82.463	82.368	W77	A
			-1.391	-2.038	4.322	70.452	63.404	5.727	-4.173	-2.366	0.071	25.02	26.124	W77	G
			-9.137	-14.776	-12.452	-12.853	51.313	66.657	-10.729	-10.177	-2.829	0.407	31.034	33.787	W77	Q
			-15.397	-9.802	29.004	10.615	81.256	19.256	-17.383	-16.641	-17.869	-19.56	79.453	77.339	W77	C
			-14.795	-6.996	-4.544	-2.411	74.917	25.798	-6.854	-17.865	-8.139	-20.444	82.563	81.882	W77	N
			-0.595	-3.671	2.708	17.964	79.662	78.189	-15.188	13.223	12.003	9.973	79.477	81.575	W77	E
			-4.809	-11.127	-12.713	-14.963	-12.466	-12.043	-20.363	-9.599	-18.529	-12.742	80.951	82.668	R101	I
			-16.873	-12.38	-10.484	-18.349	17.137	5.158	-6.845	-8.503	-18.259	-10.022	82.271	83.946	R101	S
			-5.956	-13.803	-21.706	-13.845	-15.378	-17.309	-19.267	-14.213	-12.33	-11.97	81.322	80.932	R101	D
			-14.579	-16.834	60.291	66.016	80.751	77.367	-9.582	-3.663	59.47	50.59	82.011	81.769	R101	F
			-6.873	-15.033	43.913	45.127	81.773	79.24	17.545	6.512	72.307	72.339	83.754	84.196	R101	C
			7.953	16.688	56.949	56.968	80.139	80.89	15.458	11.252	70.4	69.734	82.939	82.855	R101	Y
			-4.503	-14.088	57.777	58.617	81.578	77.559	-3.301	-10.064	70.559	66.357	83.294	83.196	R101	H
			-13.732	-19.477	32.316	27.316	81.43	80.499	-5.485	-12.315	65.709	68.678	83.93	82.489	R101	K
			11.839	12.408	8.821	13.363	74.159	76.012	13.833	-6.514	45.632	36.809	79.811	81.824	R101	P
			-13.857	-15.236	1.234	0.729	80.336	78.81	14.128	12.672	41.962	52.515	82.291	81.854	R101	Q
			-8.131	-20.555	-10.796	-20.805	77.369	77.169	-8.611	-1.412	-2.182	-9.674	81.643	82.178	R101	G
			23.223	23.213	-3.41	23.388	76.272	73.829	-8.088	-14.53	2.197	3.376	82.451	83.691	R101	A
			-10.204	-10.355	-4.98	-12.608	45.528	31.836	-13.252	-6.216	2.661	-15.393	83.177	81.342	R101	L

Fig. 8B



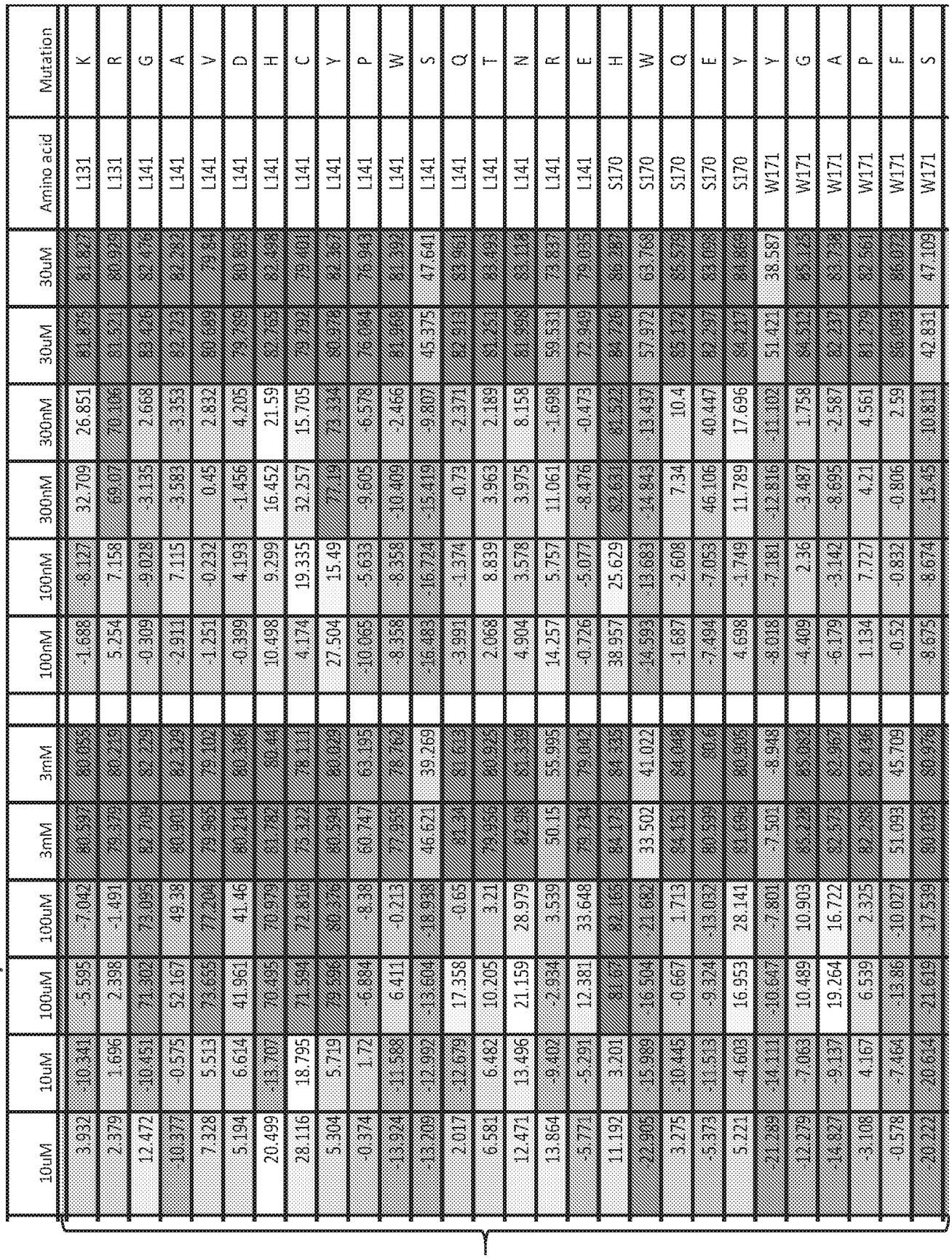
SEQ ID 33 variants	Acetylcholine						AZD-0328						Amino acid	Mutation
	10uM	10uM	100uM	100uM	3mM	3mM	100nM	100nM	300nM	300nM	30uM	30uM		
1	-8.823	-5.902	-15.651	-14.863	73.106	60.353	-15.654	-11.493	-19.872	-13.362	41.363	42.216	R101	V
2	-12.783	-14.64	-13.283	-20.777	81.569	78.777	-15.508	-15.667	-7.671	-9.861	59.753	52.754	R101	M
3	-9.439	-13.859	-16.524	-16.683	80.799	78.189	-16.325	-20.253	-17.956	-20.703	65.193	82.773	R101	T
4	-9.783	-17.533	-20.59	-15.009	81.449	78.122	-18.045	-21.771	-15.833	-13.726	84.467	84.98	R101	N
5	-10.661	-13.963	-18.961	-10.812	70.857	73.443	-8.036	-12.823	9.227	18.225	68.97	63.446	R101	E
6	-12.041	-12.835	26.08	43.573	69.94	68.329	-0.123	4.059	50.957	62.591	75.863	74.876	W108	Y
7	1.632	5.295	4.878	2.558	3.235	4.624	2.171	-16.564	3.106	-2.296	31.438	29.871	Y115	L
8	4.79	1.529	8.445	-9.508	28.672	13.609	-2.62	5.001	4.496	4.462	74.333	71.576	Y115	M
9	-5.235	1.01	-5.817	-3.438	-1.955	-1.577	3.753	1.007	-1.649	2.958	77.848	76.219	Y115	D
10	12.81	-9.124	58.305	54.234	81.497	74.486	-5.85	3.792	-9.389	10.658	5.074	10.509	Y115	W
11	-12.236	-12.669	-6.875	-7.433	74.788	74.253	-10.535	-10.853	-14.086	-13.962	51.469	50.254	Y115	G
12	-3.14	3.913	2.466	1.665	75.112	73.777	-5.226	-16.949	-7.833	-7.27	80.022	73.632	Y115	A
13	5.316	11.723	8.942	12.164	74.29	72.871	4.112	14.773	12.968	7.834	41.069	49.76	Y115	I
14	-5.123	-3.183	-0.136	-1.925	75.269	68.652	8.072	8.114	-2.068	1.45	61.366	68.801	Y115	V
15	-15.93	-13.145	-3.491	-4.244	48.332	40.085	-9.523	-2.566	13.667	-9.663	-6.218	43.391	Y115	P
16	-8.458	-2.409	-17.987	-7.442	72.439	72.467	3.783	8.65	-5.97	8.59	19.388	28.372	Y115	S
17	1.102	3.538	2.143	-0.135	61.368	57.232	7.902	13.556	3.557	3.402	45.896	54.888	Y115	Q
18	-13.916	-1.356	-6.706	-7.938	9.499	8.206	2.103	4.589	-2.729	0.03	49.586	50.348	Y115	T
19	-12.908	-7.117	-9.935	-10.963	63.132	63.816	2.084	3.07	-4.011	-2.946	34.019	47.012	Y115	C
20	-9.519	8.946	-11.913	-10.672	57.8332	55.2247	1.72	0.767	43.349	43.681	80.1	80.432	Y115	E
21	-12.809	-5.743	8.829	-6.514	75.009	72.777	12.875	0.19	-4.929	1.761	76.277	80.247	Y115	H
22	-7.149	-6.201	-9.805	-10.034	-12.141	11.833	17.289	-6.651	-12.787	-11.875	43.697	60.373	T128	M
23	11.633	8.407	12.308	12.083	2.43	6.274	14.564	9.879	8.513	10.97	77.785	83.722	T128	R
24	-11.765	-9.307	68.287	61.27	83.727	81.553	-9.089	0.04	66.36	68.847	23.159	85.533	T128	S
25	-2.884	0.966	57.607	44.48	80.238	80.222	-2.647	-9.613	24.726	35.957	72.011	80.559	T128	E
26	-3.346	-7.112	-4.049	-4.498	75.442	75.447	-9.159	-11.781	28.312	-3.687	64.223	71.671	T128	G
27	-9.605	-7.255	-7.511	-7.239	76.283	74.072	-8.3	-8.874	-5.865	-3.224	81.733	85.304	T128	A
28	-13.203	-10.415	-12.327	-10.168	6.511	51.313	16.363	-14.931	-17.228	-14.949	51.045	56.619	T128	I
29	-3.459	-2.067	0.996	-5.252	72.1	72.49	-6.012	-17.159	-3.456	-6.976	61.947	70.462	T128	P
30	-7.823	-8.351	-3.309	-6.828	65.139	69.342	-14.877	-8.966	-4.268	-5.309	73.943	80.17	T128	F
31	-11.607	-8.285	-4.938	-8.748	36.198	30.949	-12.783	-10.117	-10.009	-13.61	72.591	81.224	T128	W

Fig. 8C



	Acetylcholine						AZD-0328							
SEQ ID 33 variants	10uM	10uM	100uM	100uM	3mM	3mM	100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
	-7.376	-6.2	-7.445	-6.899	18.692	28.853	-8.67	-10.111	-5.846	-6.739	31.528	33.04	T128	Q
	-6.905	-11.483	4.531	20.583	71.041	69.224	-12.381	-13.096	3.651	19.428	80.491	81.484	T128	C
	-5.893	-2.954	12.269	-5.009	29.544	63.983	-5.32	-8.049	-5.357	-6.133	57.776	82.918	T128	N
	-6.889	-5.165	-10.937	6.517	34.776	70.98	-10.404	-12.509	-5.756	-7.863	36.567	78.456	T128	Y
	4.317	1.756	-3.098	-5.08	33.439	33.24	-4.236	-6.722	-0.302	-4.73	63.563	84.477	T128	D
	-10.201	-8.367	-6.8	-6.515	52.63	62.062	-11.444	-11.61	-7.064	-9.991	77.925	83.624	T128	K
	-12.555	-12.583	-9.386	-14.272	71.44	61.115	-16.164	-14.659	-13.147	-8.921	69.954	77.065	T128	H
	-6.826	-14.226	27.188	28.546	50.674	78.433	-10.006	18.134	48.49	51.294	77.921	81.034	T128	V
	4.937	-3.387	-7.557	-2.078	-6.747	-5.618	-11.176	-7.977	-7.453	-9.242	63.183	75.539	N129	I
	-6.173	-6.145	16.274	11.45	19.342	32.583	-13.057	13.639	15.999	-9.473	78.157	78.212	N129	V
	-6.739	-3.799	-3.78	-5.239	4.198	-5.142	-6.818	1.319	-7.816	-3.12	81.744	83.331	N129	P
	-13.436	-8.173	-12.355	-12.849	-3.451	-7.8	-15.746	-14.641	-13.372	-10.893	77.557	80.475	N129	W
	-1.742	-8.271	-8.539	-15.353	-9.501	-7.284	-8.972	-15.574	-13.984	-5.593	81.453	82.402	N129	T
	-5.417	-0.622	-7.572	-7.759	-4.92	-4.898	1.691	0.912	-5.45	-1.345	53.991	82.26	N129	D
	-7.959	-7.555	-8.831	-12.437	-8.829	-8.041	-7.124	-4.035	-9.348	-3.056	81.111	80.737	N129	E
	-9.816	-2.456	-11.107	-10.838	29.077	74.669	-15.645	-13.433	2.576	8.006	75.764	81.969	N129	G
	-5.222	-8.236	-14.33	-8.99	44.486	18.493	-14.33	-14.088	-8.165	-9.873	82.777	82.369	N129	A
	-8.386	-9.87	-11.441	-14.994	49.246	72.669	-15.023	-10.3	-13.3	-2.176	81.541	82.591	N129	S
	-5.738	-9.01	-10.033	-13.826	41.557	69.319	-13.981	-8.799	3.724	10.486	83.721	81.345	N129	Q
	-13.043	-7.593	-11.891	-13.488	38.623	44.524	-8.525	-11.496	-14.04	-10.407	79.869	77.233	N129	C
	-7.657	-3.695	-5.851	-9.004	43.557	76.72	-6.028	-7.443	18.455	30.104	82.477	83.635	N129	Y
	-11.215	-9.001	-4.666	-11.226	81.283	79.104	-13.469	-12.839	35.455	46.328	77.247	80.183	N129	F
	-14.638	-7.153	-7.615	-8.016	78.316	73.637	-12.837	-9.703	31.806	52.417	76.552	82.448	N129	M
	-9.048	-5.826	-8.214	-2.833	28.445	80.728	-6.654	-6.457	32.769	37.231	80.114	80.864	N129	R
	-5.828	-7.079	2.95	7.323	82.746	81.491	-0.289	-4.373	60.881	64.824	84.231	82.211	N129	H
	-11.839	-10.772	12.365	31.95	78.279	81.659	-16.847	-3.981	56.871	57.395	82.382	80.265	N129	L
	4.408	5.704	0.795	4.774	25.856	27.313	1.506	-4.046	10.218	10.069	30.433	33.789	L131	P
	-9.899	0.455	-7.862	-3.572	45.363	51.016	17.766	13.983	52.577	44.526	82.68	80.771	L131	T

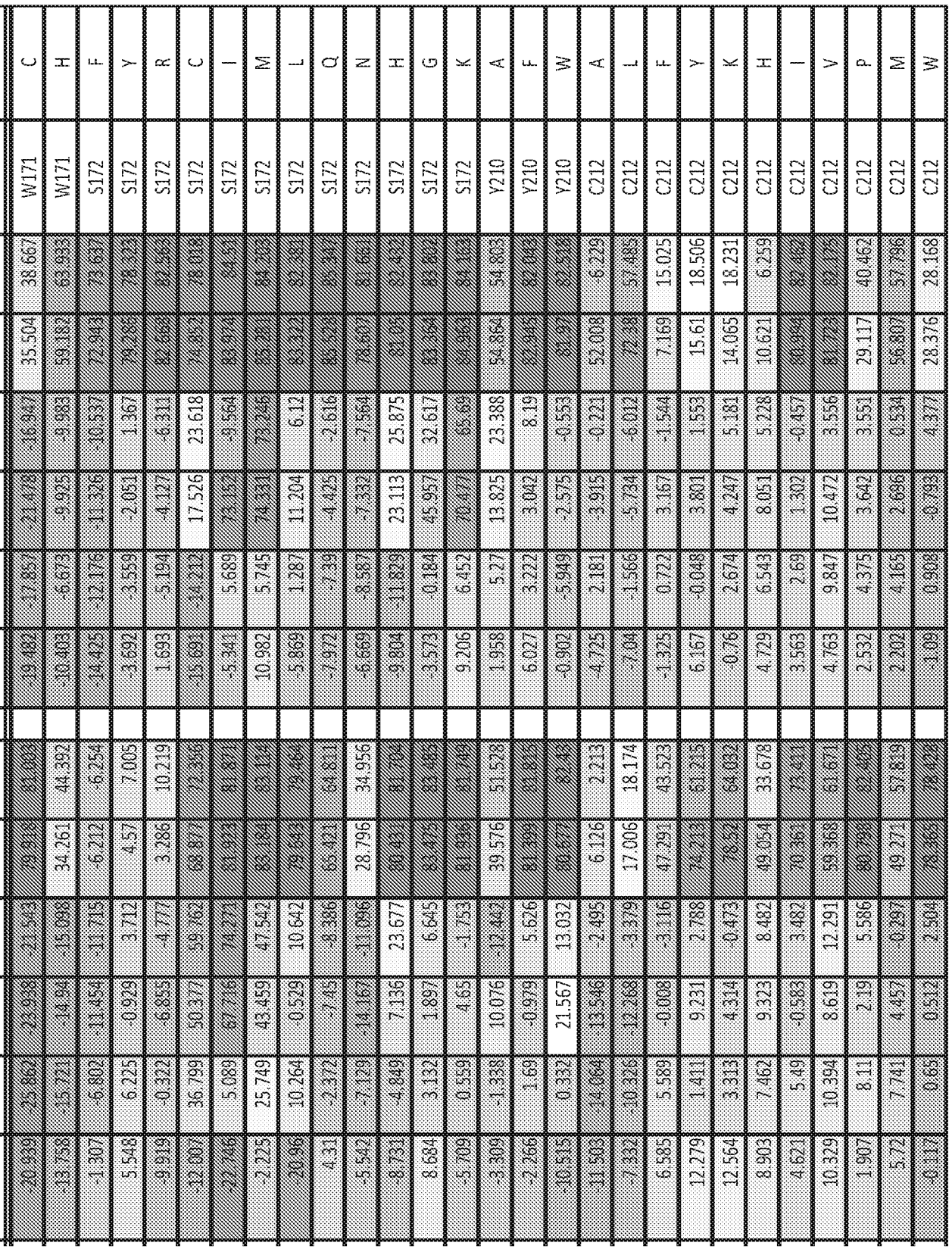
Fig. 8D



Acetylcholine						AZD-0328							
10uM	10uM	100uM	100uM	3mM	3mM	100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
3.932	-10.341	-5.595	-7.042	86.557	88.055	-1.688	-8.127	32.709	26.851	81.675	81.322	L131	K
2.379	1.696	2.398	-1.491	78.322	80.226	5.254	7.158	69.07	70.163	71.521	80.228	L131	R
12.472	-10.451	71.402	73.095	82.729	82.729	-0.309	-9.028	-3.135	2.668	83.196	82.419	L141	G
-10.377	-0.575	52.167	49.38	82.829	82.829	-2.911	7.115	-3.583	-3.353	82.723	82.283	L141	A
7.328	5.513	73.635	77.284	29.102	29.102	-1.251	-0.232	0.45	2.832	80.589	23.84	L141	V
5.194	6.614	41.961	41.46	80.214	80.394	-0.399	4.193	-1.456	4.205	79.299	80.633	L141	D
20.499	-13.707	70.495	70.879	82.232	80.44	10.498	9.299	16.452	21.59	82.765	82.438	L141	H
28.116	18.795	11.594	12.816	75.321	78.111	4.174	19.335	32.257	15.705	19.791	29.401	L141	C
5.304	5.719	79.536	80.736	80.394	80.139	27.504	15.49	77.19	73.334	80.376	82.352	L141	Y
-0.374	1.72	-6.884	-8.38	60.747	63.195	-10.065	-5.633	-9.605	6.578	76.694	76.443	L141	P
13.324	-11.588	6.411	-0.213	77.333	78.762	8.358	-8.358	-10.409	2.466	81.768	82.317	L141	W
-13.209	-12.992	-13.684	-18.938	46.621	39.269	-16.483	-16.724	-15.419	-9.807	45.375	47.641	L141	S
2.017	-12.679	17.358	-0.65	81.24	82.243	-3.991	-1.374	-0.73	-2.371	82.111	83.511	L141	Q
6.581	6.482	10.205	3.21	79.859	80.955	2.068	8.839	3.963	2.189	81.474	81.493	L141	T
12.471	13.496	21.159	28.979	82.69	81.739	4.904	3.578	3.975	8.158	81.966	82.116	L141	N
13.864	-9.402	-2.934	3.539	50.15	55.995	14.257	5.757	11.061	-1.698	69.831	73.837	L141	R
-5.771	-5.291	12.381	33.648	79.734	79.042	-0.726	-5.077	-8.476	-0.473	72.349	79.033	L141	E
11.192	3.201	81.67	82.165	84.177	84.133	38.957	25.629	27.841	6.521	84.726	85.047	S170	H
22.906	15.989	16.904	21.682	33.502	41.022	-14.893	-13.883	-14.843	-13.437	57.972	63.763	S170	W
3.275	-10.445	-0.667	1.713	84.253	84.044	-1.687	-2.608	7.34	10.4	83.734	83.739	S170	Q
-5.373	-11.513	-9.324	-13.032	80.539	80.4	-7.494	-7.083	46.106	40.447	82.787	83.089	S170	E
5.221	-4.603	16.953	28.141	83.696	80.903	4.698	-1.749	11.789	17.696	84.413	84.449	S170	Y
-21.289	-14.111	-10.647	-7.801	-7.503	-8.948	-8.018	-7.181	-12.816	-11.303	51.421	38.587	W171	Y
13.279	7.063	10.489	10.903	85.204	85.052	4.409	2.36	3.487	1.758	84.512	84.722	W171	G
-14.827	-9.137	19.264	16.722	81.521	81.557	-6.179	-3.142	-8.695	-2.587	82.331	82.566	W171	A
-3.108	4.167	6.539	2.325	81.293	81.736	1.134	7.727	4.21	4.561	81.779	81.591	W171	P
-0.578	-7.461	13.86	-10.027	51.089	45.709	-0.52	-0.832	-0.806	2.59	82.553	82.442	W171	F
-20.222	-20.614	21.619	-17.539	80.415	80.176	-8.675	-8.674	-15.45	-10.811	42.831	47.109	W171	S

SEQ ID 33 variants

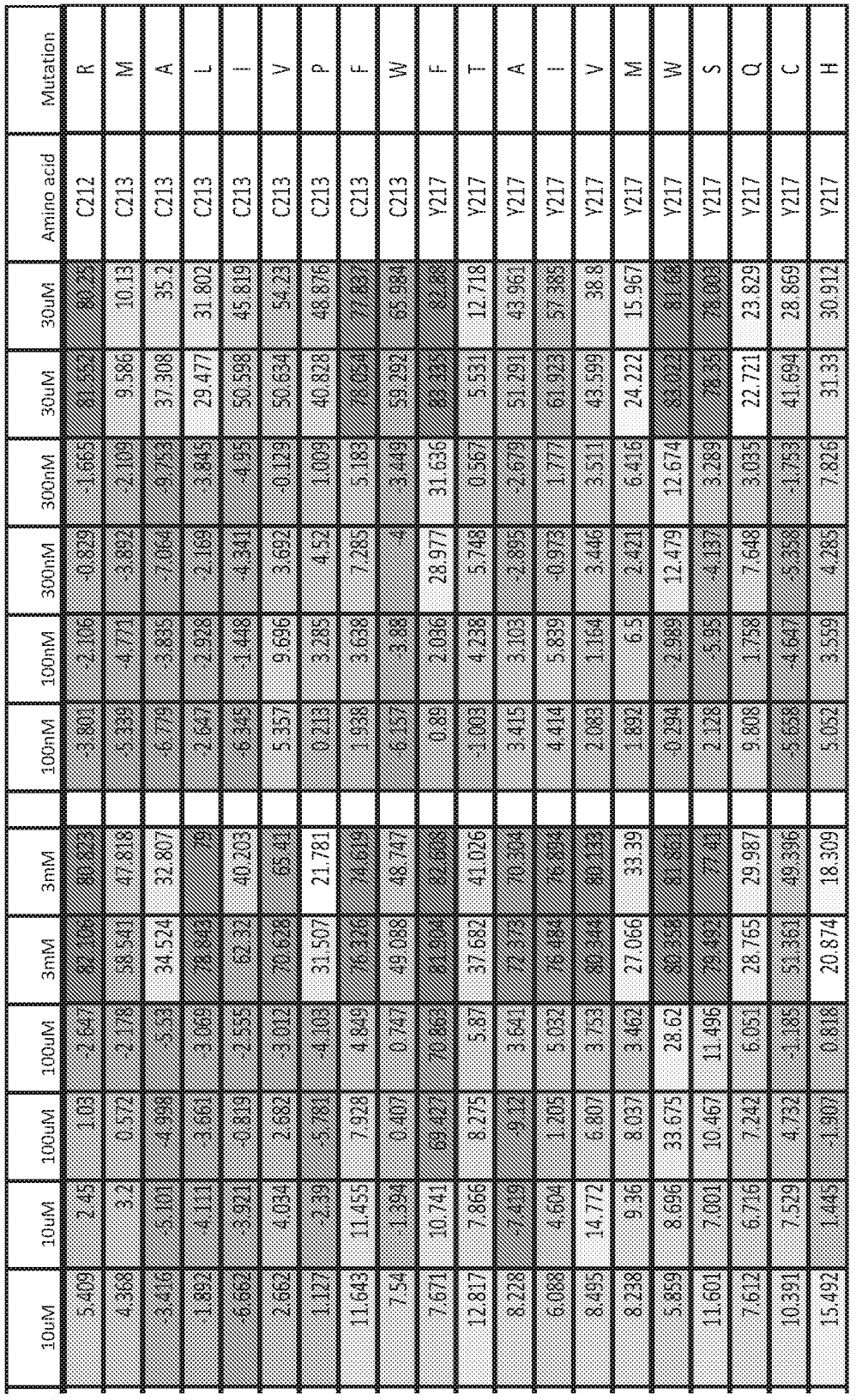
Fig. 8E



Acetylcholine							AZD-0328						
10uM	10uM	100uM	100uM	3mM	3mM		100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
-20.439	25.862	-23.938	22.543	49.911	43.402		19.483	-17.857	-21.178	-16.847	35.504	38.667	W171	C
-13.758	-15.721	-14.84	-15.098	34.261	44.392		-10.403	-6.673	-9.925	-9.583	59.182	63.833	W171	H
-1.307	-6.802	-11.454	-11.715	-6.212	-6.254		-14.425	-12.176	-11.326	-10.537	72.943	73.637	S172	F
5.548	6.225	-0.929	3.712	4.57	7.005		-3.692	-3.559	-2.051	1.367	73.786	76.323	S172	Y
-9.919	-0.322	-6.855	-4.777	3.286	10.219		1.693	-5.194	-4.127	-6.311	82.468	82.265	S172	R
-12.007	36.799	50.377	59.762	68.877	72.356		-15.691	-14.219	17.526	23.618	74.353	78.018	S172	C
-22.746	5.089	67.716	74.271	81.993	81.871		-5.341	5.689	22.162	-9.564	83.514	84.533	S172	I
-2.225	25.749	43.459	47.542	83.124	83.122		10.982	5.745	74.331	72.249	85.251	84.259	S172	M
-26.389	10.264	-0.529	10.642	79.541	79.462		-5.869	1.287	11.204	6.12	83.744	82.383	S172	L
-4.31	-2.372	-7.45	-3.386	69.421	64.811		-7.972	-7.39	-4.425	-2.616	81.728	80.281	S172	Q
-5.542	-7.129	-14.167	-11.096	28.796	34.956		-6.669	-8.587	-7.332	-7.564	78.607	81.669	S172	N
-8.731	-4.849	7.136	23.677	82.423	81.784		-9.804	-11.839	23.113	25.875	82.095	82.442	S172	H
8.684	3.132	1.897	6.645	83.493	83.483		-3.573	-0.184	45.957	32.617	83.364	83.607	S172	G
-5.709	0.559	4.65	-1.753	81.993	83.749		9.206	6.452	70.477	65.69	84.353	84.123	S172	K
-3.309	-1.338	10.076	-12.447	39.576	51.528		1.958	5.27	13.825	23.388	54.864	54.803	Y210	A
-2.266	1.69	-0.979	5.626	81.469	81.822		6.027	3.222	3.042	8.19	82.448	82.048	Y210	F
-10.515	0.332	21.567	13.032	78.677	82.48		-0.902	-5.949	-2.575	-0.553	81.97	82.516	Y210	W
-11.503	-14.064	-13.946	-2.495	6.126	2.213		-4.725	2.181	3.915	-0.221	52.008	-6.229	C212	A
-7.332	-10.326	-12.868	-3.379	17.006	18.174		-7.04	-1.566	-5.734	-6.012	72.38	57.485	C212	L
6.585	5.589	-0.008	-3.116	47.291	43.523		-1.325	0.722	3.167	-1.544	7.169	15.025	C212	F
12.279	1.411	9.231	2.788	78.213	83.215		6.167	-0.048	3.801	1.553	15.61	18.506	C212	Y
12.564	3.313	4.314	-0.473	78.52	84.032		-0.76	2.674	4.247	5.181	14.065	18.231	C212	K
8.903	7.462	9.323	8.482	49.054	33.678		4.729	6.543	8.051	5.228	10.621	6.259	C212	H
4.621	5.49	-0.583	3.482	70.367	73.431		3.563	2.69	1.302	-0.457	80.998	82.462	C212	I
10.329	10.394	8.619	12.291	83.368	83.671		4.763	9.847	10.472	3.556	84.723	83.175	C212	V
1.907	8.11	2.19	5.586	80.233	82.485		2.532	4.375	3.642	3.551	29.117	40.462	C212	P
5.72	7.741	4.457	0.397	49.271	57.839		2.202	4.165	2.696	0.534	56.803	57.786	C212	M
-0.117	0.65	0.512	2.504	74.641	78.428		-1.09	0.908	-0.793	4.377	28.376	28.168	C212	W

SEQ ID 33 variants

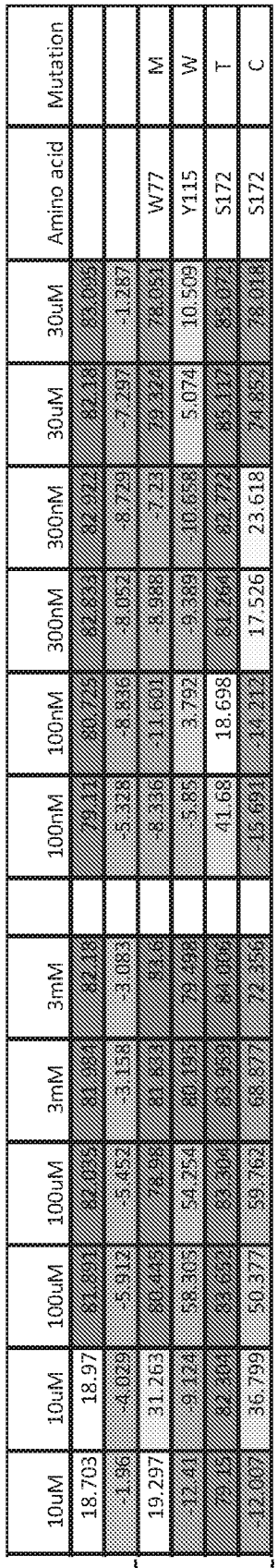
Fig. 8F



SEQ ID 33 variants

Acetylcholine							AZD-0328							
10uM	10uM	100uM	100uM	3mM	3mM		100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
5.409	2.45	1.03	-2.647	42.224	80.223		-3.801	-2.106	-0.829	-1.665	29.353	80.22	C212	R
4.368	3.2	0.572	-3.178	58.541	47.819		5.339	4.771	3.892	2.109	9.586	10.13	C213	M
-3.416	-5.101	-4.998	-5.33	34.524	32.807		-6.779	-3.835	-7.064	-9.753	37.308	35.2	C213	A
1.882	-4.111	-3.661	-3.069	74.443	74		2.647	2.928	2.169	-3.845	29.477	31.802	C213	L
6.662	-3.981	-0.819	-2.555	62.32	40.203		-6.345	-1.448	-4.341	-4.35	50.598	45.819	C213	I
2.662	4.034	2.682	-3.012	76.628	65.43		5.357	9.696	3.692	-0.139	50.634	54.23	C213	V
1.127	-2.39	-5.781	-4.103	31.507	21.781		0.213	3.285	4.52	1.009	40.828	48.876	C213	P
11.643	11.455	7.928	4.849	76.326	74.614		1.938	3.638	7.285	5.183	70.754	71.457	C213	F
7.54	1.394	0.407	0.747	49.088	48.747		6.157	3.38	4	3.449	59.392	65.984	C213	W
7.671	10.741	69.427	70.863	74.854	72.668		0.89	2.036	28.977	31.636	68.233	72.48	Y217	F
12.817	7.866	8.275	5.87	37.682	41.026		1.003	4.238	5.748	0.567	5.531	12.718	Y217	T
8.228	7.449	9.132	3.641	72.373	70.304		3.415	3.103	-2.895	-2.879	51.291	43.961	Y217	A
6.088	4.604	1.205	5.032	76.184	76.814		4.414	5.839	-0.973	1.777	61.923	57.385	Y217	I
8.495	14.772	6.807	3.753	80.144	80.223		2.083	1.164	3.446	3.511	43.599	38.8	Y217	V
8.238	9.36	8.037	3.462	27.066	33.39		1.892	6.5	2.421	6.416	24.222	15.967	Y217	M
5.859	8.696	33.675	28.62	80.334	81.861		6.294	2.988	12.479	12.674	83.173	81.138	Y217	W
11.601	7.001	10.467	11.496	79.492	77.44		2.128	5.95	4.137	3.289	78.33	78.003	Y217	S
7.612	6.726	7.242	6.051	28.765	29.987		9.808	1.758	7.648	3.035	22.721	23.829	Y217	Q
10.391	7.529	4.732	-1.185	51.363	49.396		5.658	-4.647	-5.358	-1.753	41.694	28.869	Y217	C
15.492	1.445	-1.907	0.818	20.874	18.309		5.052	3.559	4.285	7.826	31.33	30.912	Y217	H

Fig. 8G

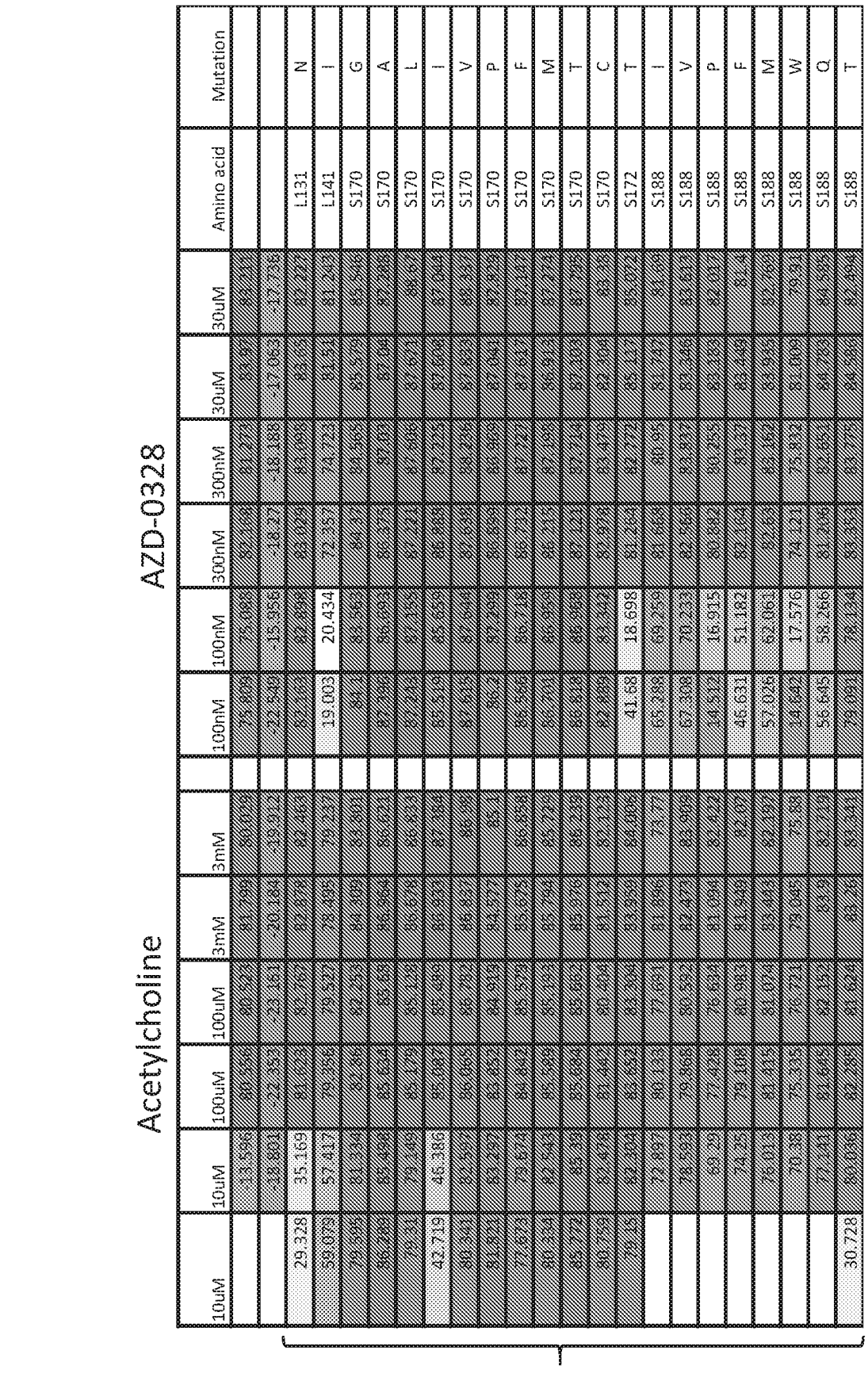


	Acetylcholine							AZD-0328							
	10uM	10uM	100uM	100uM	3mM	3mM		100nM	100nM	300nM	300nM	30uM	30uM	30uM	Amino acid	Mutation
SEQ ID 33	18.703	18.97	81.774	82.089	81.364	82.15		73.11	80.729	82.122	84.122	82.19	83.159		
SEQ ID 29	1.36	4.029	5.312	5.452	3.158	3.083		5.338	8.836	8.052	8.729	7.297	1.287		
SEQ ID 33 variants	19.297	31.263	80.469	74.96	81.611	84.16		84.336	81.601	83.968	77.23	73.344	78.091	W77	M
	12.41	9.124	58.305	54.254	80.163	79.359		5.85	3.792	9.389	10.658	5.074	10.509	Y115	W
	72.15	82.304	83.107	83.104	83.423	84.016		41.68	18.698	81.264	82.772	85.117	85.077	S172	T
	12.007	36.799	50.377	59.762	68.877	72.356		16.691	14.212	17.526	23.618	74.852	79.018	S172	C

Fig. 8H

	Acetylcholine							AZD-0328							
	10uM	10uM	100uM	100uM	3mM	3mM		100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
SEQ ID 33	13.204	23.203	82.695	81.723	81.736	82.007		82.057	81.236	82.236	82.236	81.135	82.16		
SEQ ID 29	-8.106	-4.64	7.936	5.991	6.085	8.164		10.757	7.838	11.334	9.063	8.169	12.078		
SEQ ID 33 variants	-7.125	-3.087	-7.362	-3.152	68.506	68.092		42.897	44.726	69.607	71.676	81.261	81.569	L131	S
	3.999	0.435	7.863	3.572	45.363	51.016		17.766	13.983	52.577	44.526	82.09	81.721	L131	T
	6.046	7.12	22.528	16.578	80.094	83.979		67.254	68.242	78.511	76.476	80.4	77.784	L131	D
	1.864	-0.236	3.597	4.693	80.257	80.272		63.241	68.78	81.673	82.724	81.58	81.659	S172	D

Fig. 8I



Acetylcholine								AZD-0328							
10uM	10uM	100uM	100uM	3mM	3mM		100nM	100nM	300nM	300nM	30uM	30uM	Amino acid	Mutation
	-13.596	86.226	86.223	81.799	86.175		75.408	75.408	82.175	81.772	86.119	86.119		
	-18.801	-22.353	-23.161	-20.184	-18.912		-22.549	-19.358	-18.22	-18.188	-17.053	-17.038		
29.328	35.169	81.773	82.769	82.174	82.243		82.774	82.766	83.129	83.169	82.174	82.227	L131	N
38.079	57.417	79.238	78.327	78.435	79.227		19.003	20.434	72.237	74.223	81.241	81.243	L141	I
72.38	81.744	82.136	82.256	82.569	82.601		82.11	82.651	82.11	82.561	85.578	85.516	S170	G
86.283	85.564	82.854	82.86	86.564	86.624		87.736	86.091	86.371	87.801	87.194	87.266	S170	A
79.11	79.149	85.179	85.179	86.611	86.611		82.113	82.113	82.668	82.611	82.611	86.6	S170	L
42.719	46.386	86.496	86.496	86.511	86.544		86.511	86.511	86.496	86.496	86.494	86.494	S170	I
80.331	82.574	86.786	86.786	86.786	86.786		82.113	82.113	86.786	86.786	86.786	86.786	S170	V
83.223	83.223	85.457	84.929	84.577	85.1		85.577	85.577	85.457	85.457	85.457	85.457	S170	P
77.673	78.634	84.647	85.578	85.633	86.658		85.556	86.744	86.731	87.611	87.611	87.167	S170	F
80.353	82.754	86.786	86.786	86.754	86.774		82.754	86.754	82.754	86.754	86.754	86.754	S170	M
85.777	85.78	85.684	85.684	85.978	86.778		85.978	85.978	85.978	85.978	85.978	85.978	S170	T
80.773	82.456	82.456	82.456	82.456	82.456		82.456	82.456	82.456	82.456	82.456	82.456	S170	C
79.18	82.894	82.853	81.894	82.894	84.086		41.68	18.698	82.893	82.772	85.107	85.087	S172	T
	72.697	83.173	77.691	83.691	83.775		65.398	69.223	81.356	80.93	83.178	83.178	S188	I
	78.593	79.869	80.532	82.471	83.969		67.308	70.233	82.564	81.817	83.346	83.611	S188	V
	59.229	72.624	73.634	81.554	82.162		14.512	16.915	80.182	80.251	82.169	82.037	S188	P
	74.255	79.108	78.963	81.409	84.007		46.631	51.182	82.104	83.227	83.449	8.14	S188	F
	76.013	83.011	81.004	82.401	82.561		57.026	62.061	82.233	82.662	84.561	82.569	S188	M
	71.38	72.339	76.721	79.049	75.85		14.643	17.576	74.121	75.832	83.069	79.81	S188	W
	77.243	82.844	82.192	84.1	82.771		56.645	58.266	81.318	82.253	84.725	84.595	S188	Q
30.728	80.086	82.286	81.624	83.344	83.341		73.061	78.134	85.352	85.772	86.586	82.494	S188	T

Fig. 9A

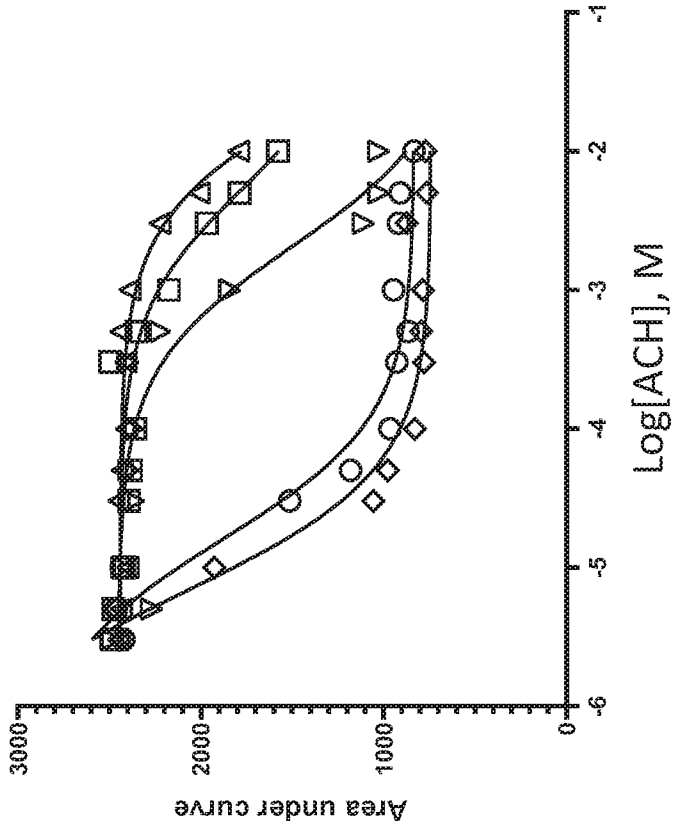
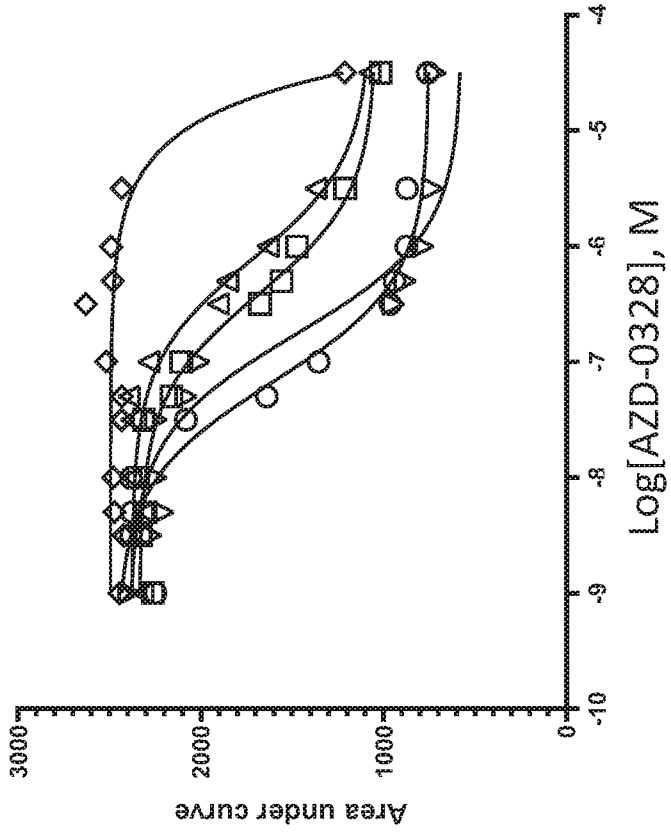

Area under curve
3000
2000
1000
0
-6
-5
-4
-3
-2
-1
Log[ACH], M

Fig. 9B


Area under curve
3000
2000
1000
0
-10
-9
-8
-7
-6
-5
-4
Log[AZD-0328], M

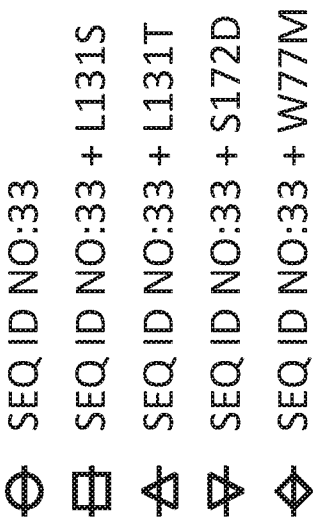

SEQ ID NO:33
SEQ ID NO:33 + L131S
SEQ ID NO:33 + L131T
SEQ ID NO:33 + S172D
SEQ ID NO:33 + W77M

Fig. 10A

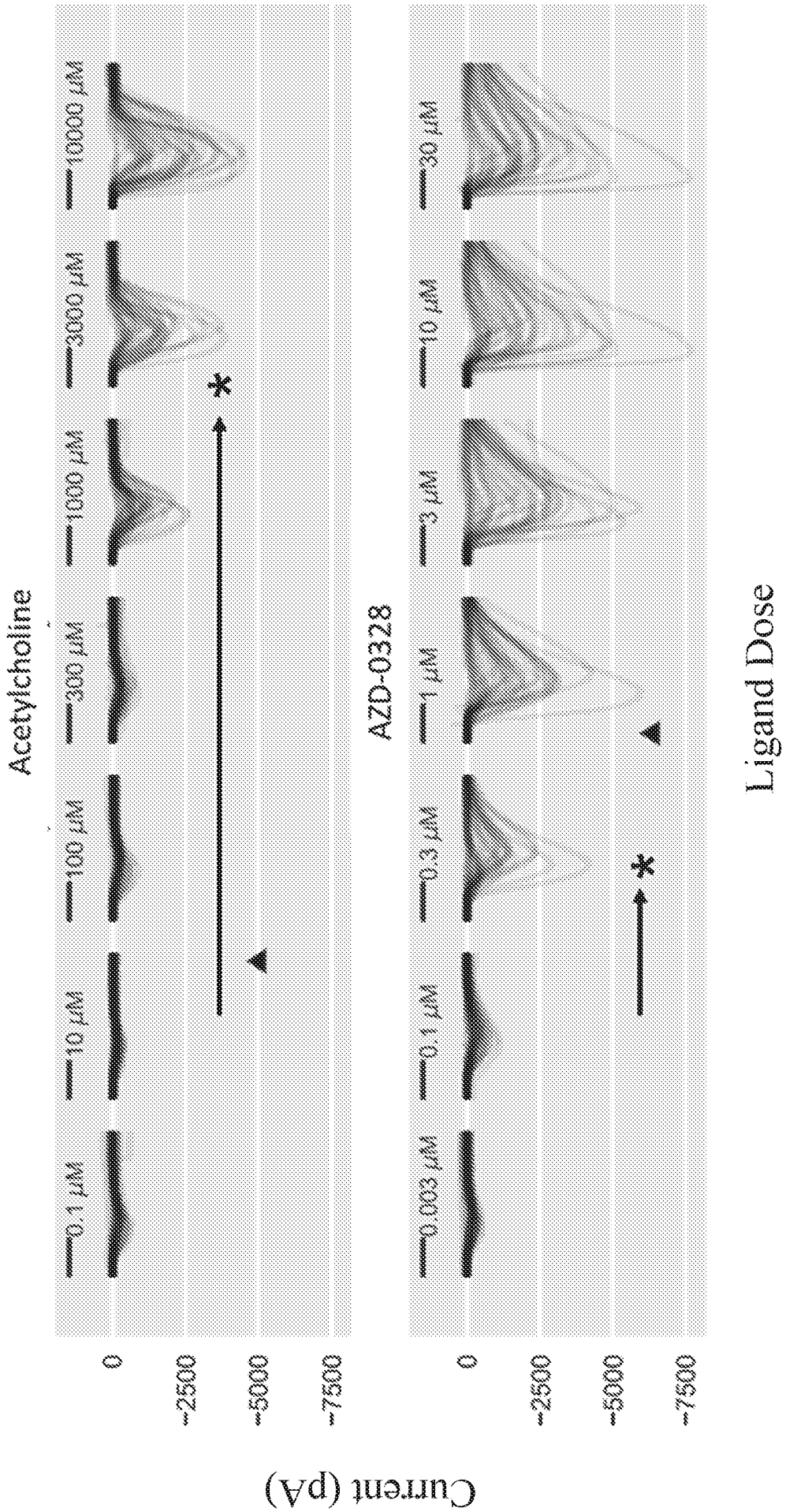

Acetylcholine
0.1 μM
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
AZD-0328
0.003 μM
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
0
-2500
-5000
-7500
Current (pA)
Ligand Dose

Fig. 10B

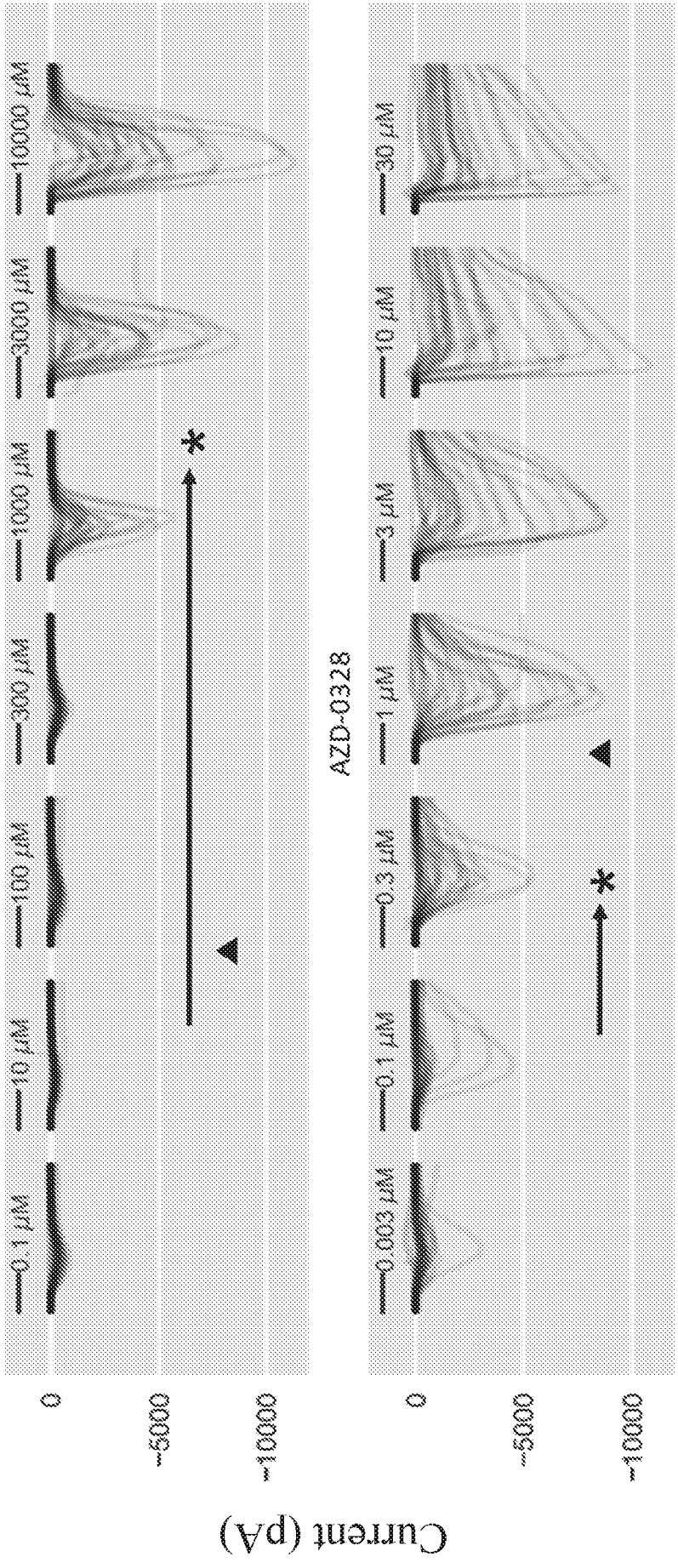

Acetylcholine
0.1 μM
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
AZD-0328
0.003 μM
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
0
-5000
-10000
Current (pA)
Ligand Dose

Fig. 10C

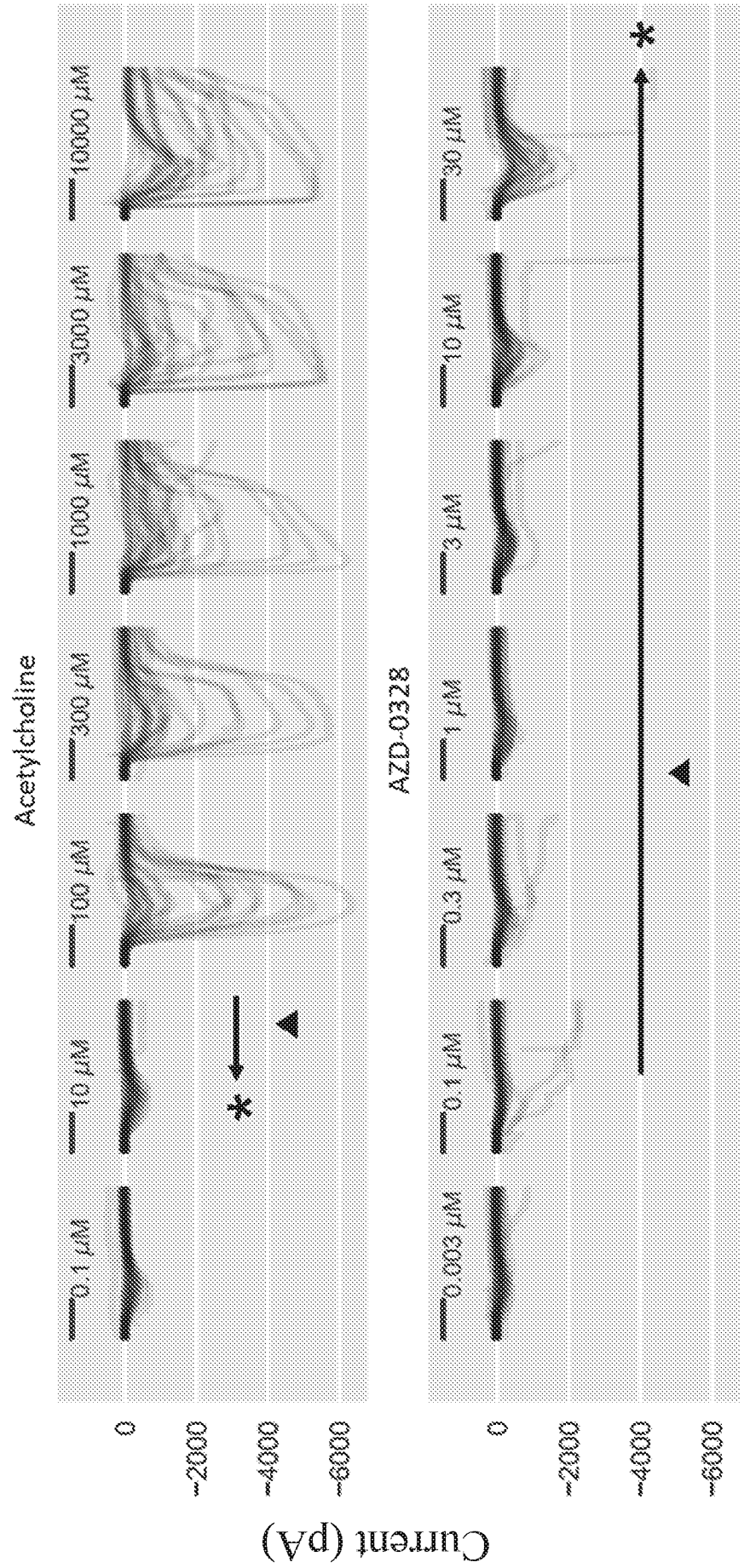
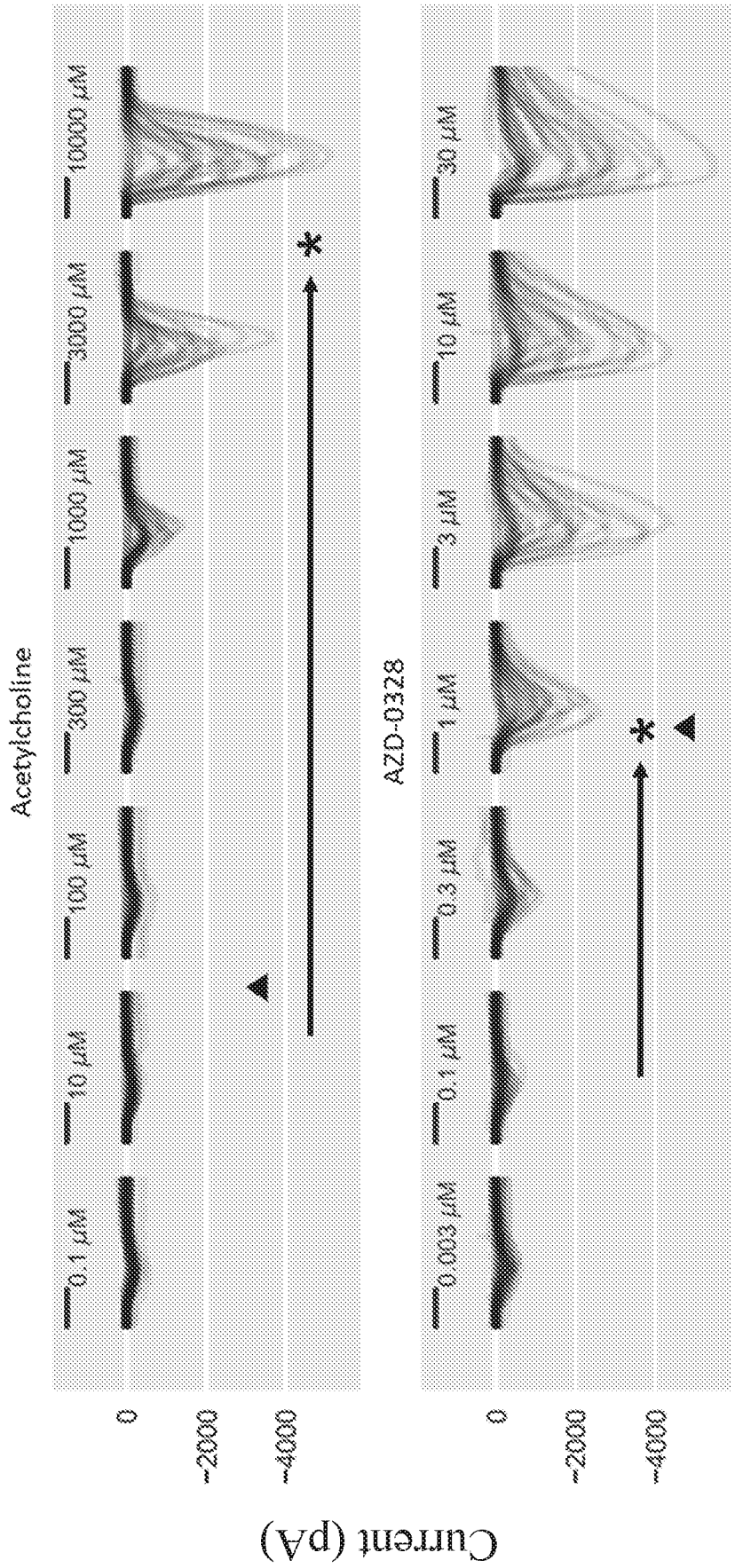

Acetylcholine
AZD-0328
0.1 μM
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
0.003 μM
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
0
−2000
−4000
−6000
Current (pA)
Ligand Dose

Fig. 10D


Acetylcholine
0.1 μM
10 μM
100 μM
300 μM
1000 μM
3000 μM
10000 μM
AZD-0328
0.003 μM
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
0
-2000
-4000
Current (pA)
Ligand Dose

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/62575

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00, A61K 38/16, A61K 38/17 (2019.01)
CPC - A61K 38/164, A61K 38/177, C07K 14/705

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 2017/0190758 A1 (UNIVERSITY OF PITTSBURGH - OF THE COMMONWELTH SYSTEM OF HIGHER EDUCATION) 06 July 2017 (06.07.2017) abstract; claims 1, 7; Fig 8B; para [0007]; [0012]; [0019]; [0022]; [0041]; [0076]; [0133]-[0136]; SEQ ID NOs: 32 and 35.	1-3
A	US 2010/0130420 A1 (HOWARD HUGHES MEDICAL INSTITUTE) 27 May 2010 (27.05.2010) abstract; para [0007]-[0014].	1-3

☐ 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
17 January 2019	11 FEB 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	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 18/62575

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.: 4-34
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.