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(54) Title: A PROTEIN THAT CAN MANIPULATE THE CELL MEMBRANE POTENTIAL IN RESPONSE TO ELECTROMAGNETIC FIELD (EMF) STIMULATION, AND METHODS OF USE

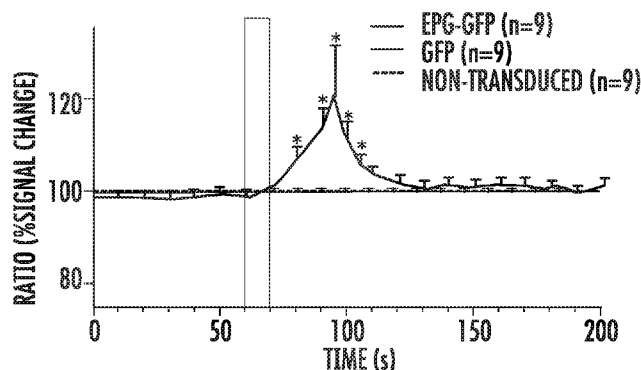


FIG. 7C

(57) Abstract: Disclosed herein is the identification of an ion channel protein that is can be remotely activated by non-invasive electromagnetic fields and termed the electromagnetic perceptive gene (EPG) gene. Also provided herein is an isolated nucleic acid encoding the EPG, cDNA for the EPG, and an amino acid sequence encoding the EPG. Methods of isolation of the nucleic acid, protein, polypeptides and methods of making antibodies to the EPG are also provided. The use of the EPG in cells or populations of cells for modulation of cell function and excitability are also provided.

A PROTEIN THAT CAN MANIPULATE THE CELL MEMBRANE POTENTIAL IN
RESPONSE TO ELECTROMAGNETIC FIELD (EMF) STIMULATION, AND METHODS
OF USE.

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/126,745, filed on March 2, 2015, which is hereby incorporated by reference for all purposes as if fully set forth herein.

STATEMENT OF GOVERNMENTAL INTEREST

[0002] This invention was made with government support under grant nos. RO1NS079288 and PO1DK072084, awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED
ELECTRONICALLY

[0003] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 9, 2014, is named P12648_ST25.txt and is 2,784 bytes in size.

BACKGROUND OF THE INVENTION

[0004] Major advances in molecular and synthetic biology have revolutionized the capability to control cell excitability in living organisms. Yet, the majority of the technologies available today that manipulate cellular function in a cell- and spatio-temporal-specific manner demand the use of optics (Nat. Neurosci., 8, 1263 (Sep, 2005)), drugs (*Neuron* 63, 27 (Jul 16, 2009)) or radio-wave heating (*Science* 336, 604 (May 4, 2012)). However, controlling cellular function by inducing electromagnetic fields that penetrate deep tissue non-invasively has yet to be explored. While it is known that various aquatic species use the Earth's magnetic field and electric fields (ELMF) for orientation, navigation and detection of prey and predators (*Nature* 199, 88 (Jul 6, 1963)), the cellular mechanism by which the encoding of the electromagnetic fields is carried out remains unknown. The

Kryptopterus bicirrhys, a fresh water fish, contains an ampullary organ dedicated to sense ELMF and evidence suggests that induction of ELMF resulted in immediate calcium influx in the electroreceptors cells that reside in the ampullary organ (*Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 130, 607 (Oct, 2001)). Thus, it is plausible that the electroreceptors cells of the *Kryptopterus bicirrhys* express proteins (membrane ion channels, transporters or co-receptors) that are sensitive to changes in ELMF. Fundamentally, identification of an ion channel that is remotely activated by non-invasive ELMF could complement the growing arsenal of technologies dedicated for external control of cellular activity *in vivo*.

SUMMARY OF THE INVENTION

[0005] In accordance with an embodiment, the present invention provides an isolated polynucleotide encoding the electromagnetic perceptive gene (EPG).

[0006] In accordance with another embodiment, the present invention provides an isolated polynucleotide encoding the EPG peptide, having the sequence of SEQ ID NO: 1.

[0007] In accordance with an embodiment, the present invention provides isolated polynucleotides encoding the EPG peptide comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0008] In accordance with an embodiment, the present invention provides a cDNA encoding the EPG.

[0009] In accordance with another embodiment, the present invention provides a cDNA encoding the EPG, having the sequence of SEQ ID NO: 2.

[0010] In accordance with an embodiment, the present invention provides isolated polynucleotides encoding a cDNA of the EPG comprising the sequence of SEQ ID NO: 2, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0011] In accordance with an embodiment, the present invention provides a recombinant expression vector comprising isolated polynucleotides encoding the EPG comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional

homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0012] In accordance with an embodiment, the present invention provides an isolated host cell comprising a recombinant expression vector comprising isolated polynucleotides encoding the EPG comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0013] In accordance with an embodiment, the present invention provides an isolated or purified polypeptide comprising the EPG peptide sequence of SEQ ID NO: 3, b) a functional fragment of a); c) a functional homolog of a) or b) or a functional fragment thereof; and d) a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0014] In accordance with an embodiment, the present invention provides an antibody, or antigen binding portion thereof, which specifically binds to a polypeptide comprising the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; and a fusion polypeptide comprising an amino acid sequence of any of the above.

[0015] In accordance with yet another embodiment, the present invention provides a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; and a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, or a host cell or population of cells comprising the EPG said isolated nucleic acid, or an antibody to said EPG peptide or antigen binding portion thereof, and a pharmaceutically acceptable carrier.

[0016] In accordance with another embodiment, the present invention provides a method of modulating an ion channel or receptor in a cell or population of cells by non-invasive ELMF comprising administering to the cell or population of cells a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; and a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, and a pharmaceutically acceptable carrier.

[0017] In accordance with another embodiment, the present invention provides a method of modulating a disease or condition associated with ion channel function in a subject by non-invasive ELMF comprising administering to the subject a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; or a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1A-1C shows *K. bicirrhys* swim away in response to ELMF. The TMS coil was placed on the right side of the fish tank and induced pulses at a rate of 50 Hz and 30% power for 5s. Before the stimulation was applied, (a) fish were scattered in the tank. During stimulation, (b) all the fish swam away from the stimulation source that was located on the right. When stimulation was over (c) the fish swam again to all directions. Fish were rewarded at the end of the trial.

[0019] Figures 2A-2G illustrate the EPG expressed in *X. laevis* oocytes responds to ELMF stimulation. (A-D) *X. laevis* oocyte membrane currents at different constant voltages. Short line to the left marks zero current. (A) Water-injected control oocyte without stimulation. (B) Water-injected oocyte with 50 Hz stimulation; no effect on membrane currents. (C) EPG-expressing oocyte exhibited greater voltage-dependent currents than control. (D) EPG-expressing oocyte with 50 Hz stimulation resulted in a downward shift in current at each voltage compared to the unstimulated conditions. (E) Current-voltage (I-V) relation from the oocytes in A-D. EPG-expressing oocyte with no stimulation (pink) shows increased voltage-dependent currents. 50 Hz stimulation shifted EPG's reversal potential to the right about 10 mV (red). Inactivation of currents was not observed with EPG with or without 50 Hz stimulation. The water-injected unstimulated oocyte showed little endogenous membrane current (light blue), and there is no evident change in current amplitude or reversal potential with 50 Hz stimulation (dark blue). (F) Population data of the current difference (ΔI) with and without stimulation in EPG-expressing (red) and control (blue) oocytes (mean $\pm$ sem). ΔI is pronounced for EPG compared to control, with a greater difference at

positive voltages. The difference in ΔI at each voltage between EPG and control is statistically significant (* $p < 0.05$, unpaired t test). (G) EPG I-V relation with 10 mM Ca^{2+} ND96 (gray). ND96 is in blue. EPG currents decreased after increasing extracellular calcium concentration suggesting that calcium may play a role in EPG function.

[0020] Figure 3 is a hydropathy plot which predicts transmembrane domains in the amino acid sequence of the EPG of the present invention. “DAS” transmembrane prediction algorithm score (y-axis) and distribution of transmembrane spanning domains are aligned relative to amino acid position (x-axis). Putative transmembrane domains (yellow). The “strict” cutoff (blue) is the algorithm default. The “loose” cutoff (red) was selected based on the relationship between the quality score and DAS score threshold for TM and non-TM proteins averaged over large data sets. The “loose” cutoff was selected as less stringent while still excluding false positives.

[0021] Figures 4A-4I show that EPG expressed in HEK293T cells responds to ELMF stimulation. (4A-4D) Representative time- course current recordings at various constant voltages (4A, top) using whole-cell patch-clamp in non- transduced and EPG-expressing HEK293T cells. (4A) Control current traces (bottom) with no stimulation. ‘0’ and short line on the left mark zero current. (4B) TMS stimulation did not affect membrane current in non-transduced HEK293T cells. (4C) EPG current traces with no stimulation. EPG exhibits greater voltage-dependent current compared to endogenous levels in control. (4D) TMS stimulation decreased the current amplitude at each voltage in EPG-expressing HEK293T cells compared to unstimulated conditions. (4E-4G) Population I-V relations (mean $\pm$ sem) from (4E) EPG-expressing HEK293T cells with (red) and without (pink) stimulation (* $p < 0.05$, paired, Student’s t test), (4F) non-transduced HEK293T cells with (dark blue) or without (light blue) stimulation, and (4G) GFP-expressing HEK293T cells with (dark green) and without (light green) stimulation. (4H) EPG-expressing HEK293T cells in Ringers (pink) vs. gluconate (gray). Current decreased when chloride in Ringers is replaced by gluconate. (4I) EPG- expressing HEK293T cells in Ringers (pink) vs. NMDG (gray). Current decreased when sodium in Ringers is replaced by NMDG (* $p < 0.05$, paired, Student’s t test).

[0022] Figure 5 is voltage-clamp data of 293T cells expressing EPG, depicting an I-V relationship.

[0023] Figure 6 is voltage-clamp data, depicting EPG’s I-V relationship in NMDG which shows that $V_{\text{rev,NMDG,expt}}$ shifts to the right and closer to zero in reference to $V_{\text{rev,Ringers,expt}} = -30.82 \text{ mV}$.

[0024] Figures 7A-7D depict wireless activation of EPG in neurons induces significant increases in $[Ca^{2+}]_i$. a) Primary cortical mixed neuron and glia cultures were transduced with viral constructs for EPG tagged with mCherry under the CamKII promoter, and GCaMP6 expression. Static magnetic field was applied for 10 s (gray bar). Significant increases in $[Ca^{2+}]_i$ compared to baseline values were measured only in neurons expressing EPG-GFP (* $p < 0.0005$, Student's *t* test). b) Calcium spikes raster plot of the EPG and GCaMP6 expressing neurons. c) Primary cortical mixed neuron and glia cultures were transduced with either AAV expressing the EPG tagged with GFP or AAV expressing GFP only, under the CamKII promoter. Non-transduced cells were also used as a control. Cells were loaded with fura-2 calcium indicator dye before the experiment. A 20 Hz alternating magnetic field was applied for 10 s (gray bar). Significant increases in $[Ca^{2+}]_i$ compared to baseline values were measured in only in neurons expressing EPG-GFP (* $p < 0.0005$, Student's *t* test). d) Examples of neurons loaded with fura-2 that were GFP-positive i.e., expressing EPG, are indicated by the yellow arrows, and an example of a GFP-negative neuron is indicated by the orange arrow.

[0025] Figure 8 depicts Expression of EPG in the rat brain is cell-specific and region-specific. Top: In order to get a widespread expression, pAAV2-CaMKIIprom::-EPG-IRES-hrGFP was injected into the sub-ventricular zone of P1 rats. Immunofluorescence was performed two weeks after injection (P15). Results demonstrate that EPG (indicated by GFP) was successfully expressed in excitatory neurons in the primary somatosensory cortex (S1) (indicated by CaMKII). Scale bar= 10 μ m. Bottom: Stereotaxic injection of pAAV2-CaMKIIprom::-EPG-IRES-hrGFP into the adult rat's right hippocampus, resulted in neuronal-specific (indicated by CaMKII, red) and region-specific expression of EPG (indicated by GFP, green). The contralateral, un-injected, hippocampus (left) exhibits only CaMKII expression, indicative of excitatory cells.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Recently, efforts to remote control protein production in vivo using radio-frequencies (RF) and magnetic field heating have been made. For example, a mammalian temperature-gated calcium channel (TRPV1) coupled to calcium-dependent insulin gene transcription was bioengineered to be activated by RF. While this novel technology can

provide means to remote control cell activity in vivo, it requires enough energy deposit to heat the tissue.

[0027] In accordance with one or more embodiments, the present inventors provide methods and compositions that provide mammalian cells the ability to express EPG peptide and using a commercially available TMS system, remotely manipulate cell excitability and function. The present discovery of the EPG peptide as a putative channel responsive to ELMF allows the application of remote controlling or modulation cellular activity both in the central nervous system and other non-neuronal systems related to ion channel function, including, for example, the heart, smooth and skeletal muscles, and glial cells.

[0028] In accordance with an embodiment, the present invention provides an isolated polynucleotide encoding the electromagnetic perceptive gene (EPG). The nucleic acid sequence of the EPG is

ATGAAGTGTGTACTTTTGGGATTCGCAGCAGTGATCGGATTCTTCGCGATCGCGG
AGTCTCTTACCTGTAACACATGCTCAGTGAGTCTGATTGGAATATGTCTGAATCC
CGCAACAGCGACTTGCTCCACCAACACATCCGTCTGCACCACAGGAAGAGCCAG
TTTCACGGGCGTCCTCGGCTTCCTGGGCTTCAACTCCCAGGGCTGCACGGAGGGA
GCTCAGTGTAATGGCACCGTGTCCGGGTCCATCCTGGGTGCGTCGTACACGGTCA
CTCAAACCTGCTGCAGCACAAACAAGTCAACCCCGTGACCAGCGGCGCCTCCT
ACGTCCAGATCTCCGTCAGCGCGGCCCTGAGCGCCGCCCTGCTGGCCTGCGTCTG
GGGCCAGTCCGTCTAC (SEQ ID NO: 1).

[0029] In accordance with another embodiment, the present invention provides an isolated polynucleotide encoding the EPG peptide, having the sequence of SEQ ID NO: 1.

[0030] In accordance with an embodiment, the present invention provides isolated polynucleotides encoding the EPG peptide comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0031] “Substantially identical” used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

[0032] In accordance with some embodiments, the isolated polynucleotides encoding functional fragments or functional homologs of the EPG peptide can be substantially identical to the sequence of SEQ ID NO: 1.

[0033] The term "nucleic acid" as used herein, includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means an isolated or purified polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In some embodiments, the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0034] Preferably, the nucleic acids of the invention are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0035] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N⁶-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-

methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0036] The nucleic acid can comprise any nucleotide sequence which encodes any of the TCRs, polypeptides, or proteins, or functional portions or functional variants thereof. For example, the nucleic acid can comprise a nucleotide sequence comprising SEQ ID NO: 1, or 2. The nucleotide sequence alternatively can comprise a nucleotide sequence which is degenerate to SEQ ID NOS: 1, or 2, or which comprises a nucleotide sequence comprising a nucleotide sequence degenerate to SEQ ID NO: 1 and a nucleotide sequence degenerate to SEQ ID NO: 2. Preferably, the nucleic acid comprises a nucleotide sequence comprising SEQ ID NO: 1, or 2, or a nucleotide sequence which is degenerate thereto.

[0037] The invention also provides substituted nucleic acid sequences which encode any of encoding the EPG peptide comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0038] In accordance with an embodiment, the present invention provides a cDNA encoding the EPG. As used herein, the term "cDNA" means an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

[0039] In an embodiment, the cDNA for the EPG is

TACTTCACACATGAAAACCCTAAGCGTCGTCAGCTAAGCCTAAGAAGCGCTAGCGCC
TCAGAGAATGGACATTGTGTACGAGTCACTCAGACTAACCTTATACAGACTTAGG
GCGTTGTCGCTGAACGAGGTGTTGTGTAGGCAGACGTGGTGTCTTCTCGGTCA
AAGTGCCCGCAGGAGCCGAAGGACCCGAAGTTGAGGGTCCCGACGTGCCTCCCT
CGAGTCACATTACCGTGGCACAGGCCAGGTAGGACCCACGCAGCATGTGCCAG

TGAGTTTGGACGACGTCGTGTTTGTGACGTTGGGGCACTGGTCGCCGCGGAGGA
TGCAGGTCTAGAGGCAGTCGCGCCGGGACTCGCGGCGGGACGACCGGACGCAGA
CCCCGGTCAGGCAGATG (SEQ ID NO: 2).

[0040] In accordance with an embodiment, the present invention provides a cDNA encoding the EPG.

[0041] In accordance with another embodiment, the present invention provides a cDNA encoding the EPG, having the sequence of SEQ ID NO: 2.

[0042] In accordance with an embodiment, the present invention provides isolated polynucleotides encoding a cDNA of the EPG comprising the sequence of SEQ ID NO: 2, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0043] In accordance with some embodiments, the isolated polynucleotides encoding functional fragments or functional homologs of the cDNA of the EPG peptide can be substantially identical to the sequence of SEQ ID NO: 2.

[0044] The term "isolated" as used herein means having been removed from its natural environment. The term "purified" as used herein means having been increased in purity, wherein "purity" is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, can be greater than 60%, 70% or 80%, or can be 100%.

[0045] In accordance with another embodiment, the present invention provides a cDNA encoding the EPG, having the sequence of SEQ ID NO: 2.

[0046] In accordance with an embodiment, the present invention provides a recombinant expression vector comprising a polynucleotide sequence encoding the EPG.

[0047] The recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEI, 2 μ plasmid, λ , SV40, bovine papilloma virus, and the like.

[0048] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be

introduced, as appropriate and taking into consideration whether the vector is DNA or RNA based.

[0049] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, puromycin resistance genes and ampicillin resistance genes. In accordance with an embodiment, the expression vector can be pCR2.1 TOPO vector (Invitrogen), for example.

[0050] The term “primer” refers to an oligonucleotide, whether natural or synthetic, capable of acting as a point of initiation of DNA synthesis under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand is induced, i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization (i.e., DNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. A primer is preferably a single-stranded oligodeoxyribonucleotide. The appropriate length of a primer depends on the intended use of the primer but typically ranges from about 10 to about 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to specifically hybridize with a template. When primer pairs are referred to herein, the pair is meant to include one forward primer which is capable of hybridizing to the sense strand of a double-stranded target nucleic acid (the “sense primer”) and one reverse primer which is capable of hybridizing to the antisense strand of a double-stranded target nucleic acid (the “antisense primer”).

[0051] “Probe” refers to an oligonucleotide which binds through complementary base pairing to a sub-sequence of a target nucleic acid. A primer may be a probe. It will be understood by one of skill in the art that probes will typically substantially bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are typically directly labeled (e.g., with isotopes or fluorescent moieties) or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the target, by Southern blot for example.

[0052] The selection of promoters, e.g., strong, weak, inducible, cell or tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, the tet-on promoter, or the ubiquitin C promoter, for example.

[0053] In accordance with some embodiments, the choice of promoter can be used to selectively transfect a particular subpopulation of host cells with the EPG. For use in neuronal cells, promoters such as human synapsin I (SYN), mouse calcium/calmodulin-dependent protein kinase II (CaMKII), rat tubulin alpha I (Ta1), rat neuron-specific enolase (NSE) and human platelet-derived growth factor-beta chain (PDGF) promoters can be used. For other applications, such as cardiac tissues, promoters such as cardiac muscle-specific alpha myosin heavy chain (MHC) gene promoter, desmin (Des), myosin light chain 2 (MLC-2) and cardiac troponin C (cTnC) can be used.

[0054] In accordance with an embodiment, the present invention provides an isolated host cell comprising a recombinant expression vector comprising isolated polynucleotides encoding the EPG comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).

[0055] Another embodiment of the invention further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5 α *E. coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a eukaryotic cell. More preferably, the host cell is a neuronal cell, for example, an excitatory neuronal cell. In other embodiments, the host cell can be a cardiac myocyte. Most preferably, the host cell is a human cell.

[0056] In accordance with an embodiment, the present invention provides an isolated or purified polypeptide comprising a functional portion of the EPG. The amino acid sequence of the EPG is

MKCVLLGFAAVIGFFAIAESLTCNTCSVSLIGICLNPATATCSTNTSVCTTGRASFTGV
LGFLGFNSQGCTEGAQCNGTVSGSILGASYTVTQTCCSTNNCNPVTSGASYVQISVSA
ALS AALLACVWGQSVY (SEQ ID NO: 3).

[0057] The term, “amino acid” includes the residues of the natural α -amino acids (e.g., Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Lys, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in D or L form, as well as β -amino acids, synthetic and non-natural amino acids. Many types of amino acid residues are useful in the polypeptides and the invention is not limited to natural, genetically-encoded amino acids. Examples of amino acids that can be utilized in the peptides described herein can be found, for example, in Fasman, 1989, CRC Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Inc., and the reference cited therein. Another source of a wide array of amino acid residues is provided by the website of RSP Amino Acids LLC.

[0058] Reference herein to “derivatives” includes parts, fragments and portions of the inventive EPG. A derivative also includes a single or multiple amino acid substitution, deletion and/or addition. Homologues include functionally, structurally or stereochemically similar peptides to EPG. All such homologues are contemplated by the present invention.

[0059] Analogs and mimetics include molecules which include molecules which contain non-naturally occurring amino acids or which do not contain amino acids but nevertheless behave functionally the same as the peptide. Natural product screening is one useful strategy for identifying analogs and mimetics.

[0060] Examples of incorporating non-natural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, omithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A partial list of known non-natural amino acid contemplated herein is shown in Table 1.

[0061] Table 1: Non-natural Amino Acids

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino-a-methylbutyrate	Mgab	L-N-methylarginine	Nmarg

aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
aminoisobutyric acid	Aib	L-N-methylaspartic acid	Nmasp
aminonorbonyl-carboxylate	Norb	L-N-methylcysteine	Nmcys
cyclohexylalanine		L-N-methylglutamine	Nmgln
cyclopentylalanine		L-N-methylglutamic acid	Nmglu
D-alanine	Dal	Chexa L-N-methylhistidine	Nmhis
D-arginine	Darg	L-N-methylisoleucine	Nmile
D-aspartic acid	Dasp	L-N-methylleucine	Nmleu
D-cysteine	Dcys	L-N-methyllysine	Nmlys
D-glutamine	Dgln	L-N-methylmethionine	Nmmet
D-glutamic acid	Dglu	L-N-methylnorleucine	Nmnle
D-histidine	Dhis	L-N-methylnorvaline	Nmnva
D-isoleucine	Dile	L-N-methylornithine	Nmorn
D-leucine	Dleu	L-N-methylphenylalanine	Nmphe
D-lysine	Dlys	L-N-methylproline	Nmpro
D-methionine	Dmet	L-N-methylserine	Nmser
D-ornithine	Dorn	L-N-methylthreonine	Nmthr
D-phenylalanine	Dphe	L-N-methyltryptophan	Nmtrp
D-proline	Dpro	L-N-methyltyrosine	Nmtyr
D-serine	Dser	L-N-methylvaline	Nmval
D-threonine	Dthr	L-N-methylethylglycine	Nmetg
D-tryptophan	Dtrp	L-N-methyl-t-butylglycine	Nmtbug
D-tyrosine	Dtyr	L-norleucine	Nle
D-valine	Dval	L-norvaline	Nva
D- α -methylalanine	Dmala	α -methyl-aminoisobutyrate	Maib
D- α -methylarginine	Dmarg	α -methyl- γ -aminobutyrate	Mgab
D- α -methylasparagine	Dmasn	α -methylcyclohexylalanine	Mchexa
D- α -methylaspartate	Dmasp	α -methylcyclopentylalanine	Mcpen
D- α -methylcysteine	Dmcys	α -methyl- α -naphthylalanine	Manap
D- α -methylglutamine	Dmgln	α -methylpenicillamine	Mpen
D- α -methylhistidine	Dmhis	N-(4-aminobutyl)glycine	Nglu
D- α -methylisoleucine	Dmile	N-(2-aminoethyl)glycine	Naeg
D- α -methylleucine	Dmleu	N-(3-aminopropyl)glycine	Norn
D- α -methyllysine	Dmlys	N-amino- α -methylbutyrate	Nmaabu
D- α -methylmethionine	Dnmet	α -naphthylalanine	Anap
D- α -methylornithine	Dmorn	N-benzylglycine	Nphe
D- α -methylphenylalanine	Dmphe	N-(2-carbamylethyl)glycine	Ngln
D- α -methylproline	Dmpro	N-(carbamylnmethyl)glycine	Nasn
D- α -methylserine	Dmser	N-(2-carboxyethyl)glycine	Nglu
D- α -methylthreonine	Dmthr	N-(carboxymethyl)glycine	Nasp
D- α -methyltryptophan	Dmtrp	N-cyclobutylglycine	Ncbut
D- α -methyltyrosine	Dmtyr	N-cycloheptylglycine	Nchep
D- α -methylvaline	Dmval	N-cyclohexylglycine	Nchex
D-N-methylalanine	Dnmala	N-cyclodecylglycine	Ncdec
D-N-methylarginine	Dnmarg	N-cyclododecylglycine	Ncdod
D-N-methylasparagine	Dnmasn	N-cyclooctylglycine	Ncoct
D-N-methylaspartate	Dnmasp	N-cyclopropylglycine	Ncpro
D-N-methylcysteine	Dnmcys	N-cycloundecylglycine	Ncund
D-N-methylglutamine	Dnmgln	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylglutamate	Dnmglu	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylhistidine	Dnmhis	N-(3-guanidinopropyl)glycine	Narg
D-N-methylisoleucine	Dnmile	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylleucine	Dnmleu	N-(hydroxyethyl)glycine	Nser
D-N-methyllysine	Dnmlys	N-(imidazolethyl)glycine	Nhis
		N-(3-indolylethyl)glycine	Nhtrp
		N-methyl- γ -aminobutyrate	Nmgabu

N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ -aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- α -methylalanine	Mala
L- α -methylarginine	Marg	L- α -methylasparagine	Masn
L- α -methylaspartate	Masp	L- α -methyl-t-butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbonylmethyl)glycine		carbonylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
ethylamino)cyclopropane			

[0062] Analogs of the subject peptides contemplated herein include modifications to side chains, incorporation of non-natural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptide molecule or their analogs.

[0063] Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH₄; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH₄.

[0064] The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

[0065] The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

[0066] Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

[0067] Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

[0068] Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate. Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

[0069] In accordance with an embodiment, the isolated or purified polypeptides, and proteins of the invention (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

[0070] When the isolated or purified polypeptides and proteins of the invention (including functional portions and functional variants) are in the form of a salt, preferably, the

polypeptides are in the form of a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, *p*-toluenesulphonic acid.

[0071] The isolated or purified polypeptides, and/or proteins of the invention (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Patent No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 2007. Further, some of the polypeptides, and proteins of the invention (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a mouse, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the polypeptides, and/or proteins described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the inventive polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

[0072] Included in the scope of the invention are conjugates, e.g., bioconjugates, comprising any of the inventive polypeptides, or proteins (including any of the functional portions or variants thereof), nucleic acids, recombinant expression vectors, host cells, populations of host cells, or antibodies, or antigen binding portions thereof. Conjugates, as well as methods of synthesizing conjugates in general, are known in the art (See, for instance, Hudecz, F., *Methods Mol. Biol.* 298: 209-223 (2005) and Kirin et al., *Inorg. Chem.* 44(15): 5405-5415 (2005)).

[0073] Also provided by an embodiment of the invention is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a myocyte cell), which does not comprise any of the recombinant expression vectors, or a cell other than a myocyte, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a pre-adipocyte cell, a neuronal cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0074] The host referred to in the inventive methods can be any host. Preferably, the host is a mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Lagomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovine (cows) and Swine (pigs) or of the order Perissodactyla, including Equine (horses). It is most preferred that the mammals are of the order Primates, Ceboidea, or Simiiformes (monkeys) or of the order Anthropoidea (humans and apes). An especially preferred mammal is the human.

[0075] In accordance with an embodiment, the present invention provides an antibody, or antigen binding portion thereof, which specifically binds to a polypeptide comprising the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; and a fusion polypeptide comprising an amino acid sequence of any of the above.

[0076] In one embodiment, the antibody, or antigen binding portion thereof, binds to an epitope or peptide fragment which contains any of the mutant amino acids which differ from the wild-type proteins. The antibody can be any type of immunoglobulin that is known in the art. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring

antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form. Also, the antibody can have any level of affinity or avidity for the mutated portion of the EPG protein or peptide fragments thereof of the present invention, such that there is minimal cross-reaction with other peptides or proteins.

[0077] Methods of testing antibodies for the ability to bind to any functional portion of any of the EPG protein or isolated or purified peptide fragments thereof are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *infra*, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0078] Suitable methods of making antibodies are known in the art. For instance, standard hybridoma methods are described in, e.g., Köhler and Milstein, *Eur. J. Immunol.*, 5, 511-519 (1976), Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988), and C.A. Janeway et al. (eds.), *Immunobiology*, 5th Ed., Garland Publishing, New York, NY (2001)). Alternatively, other methods, such as EBV-hybridoma methods (Haskard and Archer, *J. Immunol. Methods*, 74(2), 361-67 (1984), and Roder et al., *Methods Enzymol.*, 121, 140-67 (1986)), and bacteriophage vector expression systems (see, e.g., Huse et al., *Science*, 246, 1275-81 (1989)) are known in the art. Further, methods of producing antibodies in non-human animals are described in, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0079] Phage display furthermore can be used to generate the antibody of the invention. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular biology and recombinant DNA techniques (see, e.g., Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001)). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete or partial antibody is reconstituted comprising the selected variable domain. Nucleic acid sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that antibodies having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Janeway et al., *supra*, Huse et al., *supra*, and U.S. Patent 6,265,150).

[0080] Antibodies can be produced by transgenic mice that are transgenic for specific heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., *supra*.

[0081] Methods for generating humanized antibodies are well known in the art and are described in detail in, for example, Janeway et al., *supra*, U.S. Patents 5,225,539, 5,585,089 and 5,693,761, European Patent No. 0239400 B1, and United Kingdom Patent No. 2188638. Humanized antibodies can also be generated using the antibody resurfacing technology described in U.S. Patent 5,639,641 and Pedersen et al., *J. Mol. Biol.*, 235, 959-973 (1994).

[0082] Also, the antibody, or antigen binding portion thereof, can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0083] The polypeptides, proteins, (including functional portions and functional variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), can be isolated and/or purified. The term "isolated" as used herein means having been removed from its natural environment. The term "purified" as used herein means having been increased in purity, wherein "purity" is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, can be greater than 60%, 70% or 80%, or can be 100%.

[0084] In accordance with yet another embodiment, the present invention provides a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; or a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, or a host cell or population of cells comprising the EPG said isolated nucleic acid, or an antibody to said EPG peptide or antigen binding portion thereof, and a pharmaceutically acceptable carrier.

[0085] In accordance with another embodiment, the present invention provides a method of modulating an ion channel or receptor in a cell or population of cells by non-invasive ELMF comprising administering to the cell or population of cells a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a

functional fragment thereof; or a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof,, and a pharmaceutically acceptable carrier.

[0086] The choice of carrier will be determined in part by the particular EPG protein, as well as by the particular method used to administer the EPG protein. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal and interperitoneal administration are exemplary and are in no way limiting. More than one route can be used to administer the EPG protein, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0087] 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.

[0088] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Trissel, 15th ed., pages 622-630 (2009)).

[0089] For purposes of the invention, the amount or dose of the recombinant expression vector comprising isolated polynucleotides encoding the EPG comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c) administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject over a reasonable time frame. The dose will be determined by the efficacy of the particular vector and the condition of a human, as well as the body weight of a human to be treated.

[0090] The dose of the recombinant expression vector comprising the EPG also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular vector. Typically, the attending physician will decide the dosage of the vector with which to treat each individual patient, taking into

consideration a variety of factors, such as age, body weight, general health, diet, sex, vaccine protein to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the vector can be about 1×10^6 to about 1×10^9 TU/ml.

[0091] With respect to the inventive method of detecting any of the EPG protein or nucleic acid molecules in a host, the sample of cells of the host can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction.

[0092] For purposes of the inventive detecting method, the contacting can take place *in vitro* or *in vivo* with respect to the host. Preferably, the contacting is *in vitro*.

[0093] As used herein, the term “treatment,” or “modulation” is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. The term “treatment,” can also mean prolonging survival as compared to expected survival if not receiving treatment. The term “treatment,” is an intervention performed with the intention of preventing the development of a disorder or altering the pathology of a disorder. Accordingly, the term “treatment,” refers to both therapeutic treatment and prophylactic or preventative measures.

[0094] In some embodiment, the term “modulation” means the ability of remote ELMF to affect ion channels in a host cell or population of cells, which can also affect different functions for the cell. In some embodiments, the disease or disorder is modulated by exposing the subject or population of cells to a suitable strength ELMF for a sufficient time to activate the ion channels in the transfected cells in the subject. Technologies that are capable to induce ELMF are, but are not limited to: non-invasive brain stimulation techniques such as transcranial magnetic stimulation (TMS).

[0095] In some embodiments the present invention provides a library of EPGs activated by specific-ELMF ranges that can be applicable to different biological systems and activated by different technologies emitting ELMF.

[0096] In addition, EPG can be expressed under different promoters enabling cell-specific targeting *in vivo*. Thus, for basic science research, EPG technology can provide an exciting and valuable tool for studying neural activity at the network, cellular, and molecular

levels. When applied to animal models, these channels can facilitate greater understanding of the role of different signaling pathways in disease pathophysiology which could be translated into changes in clinical strategies and yield novel assays for treatment development.

[0097] Recent measurements of stimulus-evoked changes in intracellular Ca^{2+} using Fura-2 in the ampullary electroreceptor organ show that a transduction current depolarizes the apical membrane leading to stimulation of presynaptic Ca^{2+} channels and activation of the synapse. Without being held to any particular theory, the present invention suggests a mechanism of transduction whereby ELMF shuts off a chloride channel. This would be expected to depolarize the cells thereby leading to hyperexcitability of electroreceptor cells and the stimulation of presynaptic Ca^{2+} channels as observed in the ampullary organ. Thus the present invention provides a novel mechanism of signal transduction that can provide a new technology to control cell-specific excitability in live organisms.

[0098] For example, in an embodiment, vectors encoding the EPG peptide can be used to transfect neuronal cells in vivo to modulate ion channels in neurons involved in conduction related diseases such as epilepsy, Parkinson's disease, Huntington's Disease, and other disorders of the brain, such as, for example, anxiety, depression and other disorders. ELMF can then be directed to the brains of the patient externally to modulate the ion channels of the transfected neurons. In another embodiment, vectors encoding the EPG peptide can be used to transfect cardiac myocytes that have arrhythmias and their conduction can be modulated externally through the use of directed ELMF.

EXAMPLES

[0099] All animal procedures were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and approved by the Johns Hopkins University Animal Care and Use Committee.

[00100] Construction of the cDNA library.

[00101] Total mRNA was extracted from freshly dissected anal fins of 80 anesthetized glass catfish using the FastTrack 2.0 mRNA Isolation kit (Life Technologies). The cDNA library was constructed in pDONR222 using the CloneMiner II cDNA Library Construction kit (Life Technologies). The final cDNA library was cloned into pcDNA-DEST40 by LR recombination, transformed into One Shot TOP10

[0100] Chemically Competent cells (Life Technologies), and stored as glycerol stocks in 500 μL aliquots at -80°C . The cDNA sub-libraries were constructed by replica plating. A 500

μ L glycerol stock of the total cDNA library was added to 5 mL LB (Quality Biological) containing 100 mg/mL Ampicillin (Sigma- Aldrich) and shaken at 225 rpm, 37°C for 1 hour. The total volume was evenly plated on ten 10-cm Ampicillin plates (Quality Biological) and incubated overnight at 37°C. The following day, the ten plates were replica plated to a second set of ten plates using nitrocellulose membranes and each nitrocellulose membrane was submerged in a flask containing 50 mL LB. The plates and flasks were incubated overnight at 37°C, cDNA sub-libraries were purified from the flasks' inoculums, and glycerol stocks were prepared.

[0101] cRNA Production.

[0102] Sub-library and individual cRNAs were transcribed using PmeI-digestion and the mMESSAGE mMACHINE T7 ULTRA kit (Life Technologies). After transcription, the poly (A) tailing reaction and DNase I treatment were performed according to the manufacturer's instructions. The cRNA was purified by either phenol:chloroform extraction followed by isopropanol precipitation or LiCl precipitation, and then dissolved in RNase-free water.

[0103] Oocyte microinjection and two-electrode voltage clamp.

[0104] Sub-library and individual cRNAs were screened by two-electrode voltage clamp (TEVC). Stage V/VI oocytes harvested from *Xenopus laevis* as reported previously (*Proc Natl Acad Sci U S A* 110, E5016 (Dec 17, 2013)) were injected with 10 to 200 ng of cRNA and maintained at 16°C in ND97 solution (in mM): 96 NaCl, 2 KCl, 1.8 CaCl₂·2H₂O, 1 MgCl₂·6H₂O, 5 HEPES, pH 7.5 /NaOH). Control oocytes were injected with 50 nL water and incubated in ND97. Three days post-injection, TEVC was performed (Clampex 9.2) by impaling two electrodes (WPI) filled with 3 M KCl with a resistance < 1 M Ω . Recordings were low-pass filtered at 300 Hz. Oocytes were held at -40 mV for 232 ms then voltage-clamped between -100 and 40 mV in 20 mV steps lasting 1.6 ms each and then returned to -40 mV for 230 ms (Oocyte Clamp OC-725A, Warner Instruments). Recordings were made in various bath solutions with and without ELMF stimulation (50 Hz, 10 V_{pp}, Agilent 33220A LXI-certified 20 MHz function/arbitrary waveform generator). The stimulation field electrodes were submerged into the oocyte bath chamber approximately 2 cm apart. All experiments were conducted at room temperature (20-23°C). The standard physiological external solution, ND96, contained (in mM) 96 NaCl, 2 KCl, 1.8 CaCl₂·2H₂O, 1 mM MgCl₂·6H₂O, 5 mM HEPES, 2.5 mM Na-pyruvate, pH 7.5/NaOH. Calcium selectivity (10 mM Ca²⁺ ND96) was tested by decreasing NaCl to 85.7 mM and increasing CaCl₂·2H₂O to 10 mM. Offline data analysis was performed on custom MATLAB software

(MathWorks). For a single recording, current traces at various voltages were each fit to a fourth degree polynomial between 0.25 s and 1.82 s, and the peak of each fitted curve was used to generate the current-voltage (I-V) relation. ΔI -voltage relations were determined by taking the difference in the I-V relations with and without stimulation in each oocyte and then averaging across all oocytes within a population.

[0105] Cloning EPG into mammalian expression vectors.

[0106] The open reading frame of EPG was cloned into pcDNA3.1D/V5-His-TOPO (Life Technologies) to tag EPG for antibody detection in biochemistry assays (final plasmid: pcDNA3.1-EPG-V5-His). Briefly, primers for directional cloning containing the KOZAK sequence and start codon were used to PCR amplify the EPG gene and clone into the vector, according to product instructions. cRNA was amplified from this plasmid to verify conserved EPG function. To virally express EPG in brain slices, EPG was digested out from pcDNA3.1-EPG-V5-His and ligated into the multi-cloning site of pAAV- IRES-hrGFP (Agilent) using the BamHI and XhoI sites.

[0107] Adeno-associated virus (AAV) production.

[0108] High-titer AAVs were produced using the AAV Helper-Free System (Agilent) and concentrated using the AAV Purification Maxi kit (Biomiga). For virus titer ($\sim 1 \times 10^6 - 1 \times 10^9$ TU/mL), 10-fold serial dilutions of virus were transduced into HEK293T cells plated at 70-80% confluence in 24-well plates and green fluorescent cells were counted 4 days post-infection. Un-concentrated, low-titer virus was produced by following the same transfection procedure as in high-titer virus. 72-hours post-transfection, cells and supernatant were collected and freeze-thawed three times, centrifuged at 4000 rpm at 4°C for 15 minutes, filtered in a 0.45 μ m filter flask (Millipore), and stored as 1 mL aliquots in -80°C.

[0109] Patch clamp of mammalian cells.

[0110] HEK293T cells (from Il Min) plated on 12 mm circular cover glasses (Fisher) in 3.5 cm tissue culture dishes (BD Falcon) were either left non-transduced or transduced with un-concentrated, low-titer AAV virus bearing EPG (pAAV-EPG-FLAG-IRES-hrGFP) or GFP only (pAAV-IRES-hrGFP) and recorded from 2-3 days post-infection. Whole-cell currents with and without TMS stimulation were acquired from fluorescent EPG-expressing cells, fluorescent GFP-only cells, or non-transduced cells using borosilicate patch pipettes of 4-7 M Ω and an Axon MultiClamp 700B (Molecular Devices, Inc.) voltage-clamp amplifier. Voltage-clamp pulses were generated and data captured using a Digidata 1440 interfaced to a computer running the pClamp 10.3 software (Axon Instruments, Inc.). Currents were filtered

at 10 kHz and sampled at 20 kHz. Holding potential was -80 mV for 250 ms and voltage was clamped from -80 to 60 mV in 10 mV steps for 1 s then returned to holding at -80 mV for 200 ms. Standard Ringer's solution was used (in mM): 140 NaCl, 4 CsCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, 10 glucose (pH 7.4/NaOH). For ion substitution experiments, chloride in Ringers was replaced with equimolar Na-gluconate, or sodium in Ringers was replaced with equimolar NMDG. Pipettes were filled with (in mM) 148 CsCl, 2 MgCl₂, 0.5 CaCl₂, 5 EGTA, 10 Na-HEPES (pH 7.3/NaOH). Experiments were rejected if there were large leak currents. ELMF was delivered via TMS (Magstim Rapid2, Jali medical). All experiments were performed at ambient temperature (20-23°C). Offline data analysis was performed on custom MATLAB software (MathWorks). Briefly, current traces were filtered by robust local regression (window = 23) and curve fitting was performed with a seventh degree polynomial between 0.358 s and 1.022 s. I-V relations were generated by taking the peak of each fitted trace at each voltage.

[0111] Immunocytochemistry of HEK293T cells.

[0112] HEK293T cells were plated at a density of 5×10^4 cells/well in a 24-well plate and transfected with 0.5 μ g each of pcDNA3.1-EPG-V5-His or pAAV-EPG-FLAG-IRES-hrGFP. Control cells remained non-transfected. Cells were incubated at 30 °C for 3 days post-transfection. Briefly, cells were fixed in 4% PFA for 30 min, blocked in 5% BSA in PBS for 1 hour, incubated in 1:1000 anti-mouse anti-V5 (Life Technologies) or anti-rabbit anti-FLAG (Abcam) overnight at 4 °C, and incubated in 1:1000 Alexa 488 or Alexa 594 for 1 hour. The nucleus was stained with DAPI (25 ng/mL, Sigma-Aldrich) for 10 minutes. Images were acquired on the Nikon C1si True Spectral Imaging Confocal Laser Scanning Microscope (100X oil). All experiments were performed at ambient temperature (20-23 °C) unless stated otherwise.

[0113] Stereotaxic AAV injection in the rat brain.

[0114] Cryo-anesthesia was used during stereotaxic injection of 5 μ l AAV virus encoding EPG or GFP into the lateral ventricle of P1-2 Sprague-Dawley (Harlan) rats ($n=12$ in each group) (from bregma, anterior-posterior, 0.8 mm, medial-lateral, 2.0 mm, dorsal-ventral, 1.8 mm). Control rats were injected with AAV virus encoding GFP only ($n=12$). Another group of adult (180 g) rats were anesthetized with isofluorane and stereotaxically injected with 5 μ l of AAV virus encoding for EPG ($n=6$) or GFP ($n=6$) in the somatosensory cortex (from bregma, anterior-posterior, 0 mm, medial-lateral, 3.8 mm, dorsal-ventral, 0.7 mm).

[0115] Immunohistochemistry of brain slices.

[0116] Rats were sacrificed and perfused with 4% PFA 3-4 weeks after AAV stereotaxic injections. Free floating 30 μ m thick (unless indicated otherwise) coronal brain sections were immunostained with nuclear staining (DAPI), anti-rabbit anti-FLAG (indicative of EPG expression, 1:1000, Abcam), and anti-mouse anti-NeuN (neuronal marker, 1:1000, Millipore), followed by a one hour incubation with anti-rabbit Alexa 488 (1:1000, Life Technologies) and anti-mouse Alexa 594 (1:1000, Life Technologies), or with anti-mouse Alexa 488 (1:1000, Life Technologies) and anti-rabbit Alexa 594 (1:1000, Life Technologies). Images were acquired on the Nikon C1si True Spectral Imaging Confocal Laser Scanning Microscope (100X oil unless indicated otherwise).

[0117] Statistics.

[0118] Results and figures show the average $\pm$ standard error of mean (SEM). The Student's paired *t* test was used when appropriate (unless stated otherwise).

EXAMPLE 1

[0119] *K. bicirrhys* is responsive to ELMF.

[0120] The *K. bicirrhys* is known to respond to electromagnetic fields. We have tested the fish behavioral response to ELMF. Fourteen *K. bicirrhys* were housed in a 30-gallon tank that was kept at 25 °C. For induction of ELMF, a transcranial magnetic stimulation (TMS) system, with a maximum magnetic field of 2.0 Tesla and equipped with a 70 mm remote control coil, was placed on the side of the tank. During the experiments, the sides of the tank were covered. A behavioral session consisted of 8-10 single trials, including control trials, in which the TMS was placed on the side of the tank with only an audio-recording of the sound delivered. The trials were considered successful trials if the fish swam away from the ELMF, and the fish were rewarded with frozen bloodworms. Figure 1 shows the fish position prior to ELMF stimulation and their position in the tank 1 s after the induction of ELMF. During control trials, fish were indifferent to the sound.

EXAMPLE 2

[0121] To identify and characterize the putative ELMF-sensitive proteins, expression cloning in *Xenopus laevis* (*X. laevis*) oocytes was used (Nature 389, 816 (Oct 23, 1997), J Biol Chem 268, 17 (Jan 5, 1993)). We surgically isolated the anal fin containing the

electroreceptor organs (J Morphol 128, 291 (Jul, 1969)) from 80 anesthetized *Kryptopterus bicirrhus* and extracted the total mRNA from which a cDNA library was constructed. cDNA sub-libraries were screened by two-electrode voltage-clamp (TEVC) in *X. laevis* oocytes (Science 296, 2395 (2002), Cell 134, 1019 (2008)) for altered current responses to ELMF stimulation that was delivered to the oocyte bath solutions via a function generator. Current was recorded at constant voltages from -100 mV to 40 mV in 20 mV steps (Fig. 2A, top) with or without 50 Hz, 10 Vp-p stimulation. One of the sub-libraries exhibited increased, voltage-dependent membrane current in physiological (ND96) and sodium-free (NMDG) solutions. Hence, this sub-library's 44 cDNA clones were amplified and purified for further screening. The clones were sequenced and potential genes were identified using NCBI's BLAST. All putative genes were compared to the GenBank database, and candidate open reading frames of each putative gene were translated and compared to the protein database. The clones were further divided into smaller sub-libraries and current response was tested by TEVC, which led to the identification of a single open reading frame encoding a protein of 148 amino acids (~15 kDa) and displaying the constitutively increased outwardly rectifying current, which we termed electromagnetic perceptive gene (EPG).

EXAMPLE 2

[0122] Hydrophobicity analysis of the EPG sequence was performed using the "DAS" transmembrane prediction algorithm (Fig. 3, library size 32, 'trusted' evaluation). Based on the "loose cutoff" (Fig. 3, red line), the result reveals four hydrophobic transmembrane-spanning regions, characteristic of a transmembrane protein. A BLAST database search (blastn, tblastn, and blastp) revealed that the EPG sequence is uncharacterized with no similarity to any known characterized gene, suggesting that EPG is a novel channel responsive to ELMF.

EXAMPLE 3

[0123] The open reading frame of EPG was cloned into an expression vector, and mRNA was amplified and injected into *X. laevis* oocytes for TEVC. EPG conducted more current at all voltages (Figs. 2C and 2D) compared to the water-injected control oocytes (Figs. 2A and 2B). In the EPG-expressing oocyte, 50 Hz stimulation caused a downward shift in the

voltage-dependent current conduction (Fig. 2D) compared to the unstimulated condition (Fig. 2C). An example of the current-voltage (I-V) relations (Fig. 2E) demonstrates that the control oocyte with (Fig. 2E, dark blue) and without stimulation (Fig. 2E, light blue) has minimal endogenous current. In contrast, the EPG-expressing oocyte without stimulation (Fig. 2E, pink) conducts whole-cell currents with a robust dual inward/outward rectification in a voltage-dependent manner (current conductance varies according to voltage) and is not voltage-gated. Stimulation of the EPG-expressing oocyte (Fig. 2E, red) resulted in a 10 mV rightward shift in the reversal potential, demonstrating that EPG is sensitive to changes in ELMF. Further inspection of the average current difference (ΔI) between 50 Hz stimulation and without stimulation at all voltages was performed for control (Fig. 2F, blue) (n=31) and EPG-expressing (Fig. 2F, red) oocytes (n=12). While stimulation did not affect ΔI in control oocytes, it significantly altered the conductance across the voltage range in EPG-expressing oocytes (Fig. 2F) (Student's t-test, $p < 0.05$). Higher frequencies stimulation of 100 and 150 Hz produced similar results. Temperature measurements that were performed by placing a temperature probe in the bath solution showed that stimulation did not induce changes in temperature (22.6 ± 0.2 °C).

EXAMPLE 4

[0124] To determine EPG's ion selectivity, we performed TEVC under different external ionic conditions. Calcium selectivity was tested by increasing calcium in EPG-expressing *X. laevis* oocytes (n=12). Compared to currents in physiological solution (ND96), Current traces in high calcium remained level for the duration of each clamped voltage and did not exhibit a transient increase in current, suggesting that EPG is not an IP3-induced current. It remains to be determined whether the calcium replacement results are attributed to ion selectivity or to the complex role calcium plays in multiple cellular and physiological processes.

EXAMPLE 5

[0125] To test EPG response to ELMF in a mammalian system, Human Embryonic Kidney 293T cells (HEK293T) were transduced with an adeno-associated virus, pAAV-EPG-FLAG-IRES-hrGFP, and whole-cell patch clamp was performed in physiological Ringers solution at room temperature. ELMF was induced by transcranial magnetic stimulation

(TMS) with a maximum magnetic field of 2.0 Tesla equipped with a 70 mm remote control coil that was placed approximately 20 cm from the cells and delivered pulses at a rate of 19 Hz and 33% power for a total of 25 s. Representative current traces of an individual non-transduced HEK293T cell (Figs. 4A and 4B) and the group I-V relations (n=11; Fig. 4F) demonstrate that the non-transduced HEK293T cells are not affected by stimulation. HEK293T cells that were transduced with AAV bearing only GFP were also unresponsive to stimulation (n=13; Fig. 4G). On the other hand, and consistent with the results observed in the EPG-expressing *X. laevis* oocytes, EPG-expressing HEK293T (n=14) exhibited greater voltage-dependent current (Fig. 4C) compared to non-transduced cells, with stimulation inducing significant decreases in current amplitude in these cells (Fig. 4D). The group I-V relations of EPG-expressing cells demonstrate significant current decreases across all voltages (Fig. 4E). The ion selectivity of EPG in HEK293T cells was tested by substituting chloride with gluconate and sodium with NMDG. The results suggest that EPG is selective for chloride (Fig. 4H) and sodium (Fig. 4I). To determine EPG's permeability for chloride and sodium, we calculated the expected reversal potential shift based on the Goldman-Hodgkin-Katz equation. The results provide convincing evidence that EPG is highly permeable for chloride and minimally permeable for sodium.

[0126] EPG's reversal potential calculation

From voltage-clamp data of 293T cells expressing EPG, we have an I-V relationship (Figure 7).

[0127] The I-V relationship is fit to a 2nd order polynomial curve

$$y = 0.0051x^2 + 1.5275x + 42.233 \quad \text{Eqn (1).}$$

[0128] We calculate $V_{\text{rev,Ringers}}$ where $I = 0$ pA from the 2nd order polynomial curve fit (Eqn 1). Using the quadratic formula,

$$x = \frac{-b \pm \sqrt{4ac}}{2a} \quad \text{Eqn (2)}$$

we solve for x and select -30.82 mV as the best predicted solution for $V_{\text{rev,Ringers,expt}}$, since it best matches the experimental results (Fig. 7).

[0129] Next, we solve for the permeability ratio of Na^+ to Cl^- ($p_{\text{Na}}/p_{\text{Cl}}$) using the known intracellular and extracellular ionic concentrations of sodium and chloride in Ringers (Tables 2 and 3) and the Goldman-Hodgkin-Katz (GHK) equation (Eqn 3).

[0130] TABLE 2: Internal Solution

<i>Chemical</i>	<i>mM</i>
CsCl	148
MgCl ₂	2
CaCl ₂	0.5
EGTA	5
Na-HEPES	10
MgATP	2

[0131] TABLE 3: External Solutions

Ringers		NMDG	
<i>Chemical</i>	<i>mM</i>	<i>Chemical</i>	<i>mM</i>
NaCl	140	NMDG	140
CsCl	5	CsCl	5
CaCl ₂	2	CaCl ₂	2
MgCl ₂	1	MgCl ₂	1
HEPES	10	HEPES	10
Glucose	10	Glucose	10
---	---	HCl*	130

*NOTE: in NMDG about 10 mM more HCl is added to pH to 7.4.

[0132] The GHK equation can be used to calculate the equilibrium membrane potential when multiple ions contribute to the membrane potential (V_m) and can be used to calculate the reversal potential.

$$V_m = \frac{RT}{F} \ln \left(\frac{p_K [K^+]_o + p_{Na} [Na^+]_o + p_{Cl} [Cl^-]_i}{p_K [K^+]_i + p_{Na} [Na^+]_i + p_{Cl} [Cl^-]_o} \right) \quad \text{Eqn (3)}$$

RT/F at room temperature (23 °C) is

$$\frac{RT}{F} = \frac{\frac{8.314 \text{ J}}{\text{K} \cdot \text{mol}} \cdot (273.15 \text{ °C} + 23 \text{ °C})}{96485 \text{ C/mol}} \cdot \frac{1000 \text{ mV}}{\frac{\text{J}}{\text{C}}} = 25.5 \text{ mV} \quad \text{Eqn (4)}$$

so, the populated GHK is

$$V_{rev, \text{Ringers, expt}} = -30.82 \text{ mV} = 25.5 \text{ mV} \cdot \ln \left[\frac{140 \text{ mM} \cdot p_{Na} + 153 \text{ mM} \cdot p_{Cl}}{10 \text{ mM} \cdot p_{Na} + 151 \text{ mM} \cdot p_{Cl}} \right] \quad \text{Eqn (5)}$$

We solve for pNa/pCl

$$-\frac{30.82}{25.5} = \ln \left[\frac{140 \text{ mM} \cdot p_{Na} + 153 \text{ mM} \cdot p_{Cl}}{10 \text{ mM} \cdot p_{Na} + 151 \text{ mM} \cdot p_{Cl}} \right] \quad \text{Eqn (6)}$$

$$e^{\frac{-30.82}{25.5}} = \frac{140 \text{ mM} \cdot pNa + 153 \text{ mM} \cdot pCl}{10 \text{ mM} \cdot pNa + 151 \text{ mM} \cdot pCl} \quad \text{Eqn (7)}$$

$$0.29887 \cdot (10 \text{ mM} \cdot pNa + 151 \text{ mM} \cdot pCl) = 140 \text{ mM} \cdot pNa + 153 \text{ mM} \cdot pCl \quad \text{Eqn (8)}$$

$$(0.29887 \cdot 10 \cdot pNa) - 140 \cdot pNa = 153 \cdot pCl - (0.29887 \cdot 151 \cdot pCl) \quad \text{Eqn (9)}$$

$$\frac{pNa}{pCl} = \frac{153 - (0.29887 \cdot 151)}{(0.29887 \cdot 10) - 140} = -0.7873 \quad \text{Eqn (10)}$$

$$pNa = -0.7873 \cdot pCl. \quad \text{Eqn (11)}$$

We substitute the calculated pNa/pCl ratio (Eqn 11) into the GHK (Eqn 3) along with the different external concentrations for NMDG (no external Na⁺, Tables 2 and 3) to calculate $V_{rev,NMDG,GHK}$ for EPG.

$$V_m = V_{rev,NMDG,GHK} = 25.5 \text{ mV} \cdot \ln \left[\frac{0 \text{ mM} \cdot pNa + 153 \text{ mM} \cdot pCl}{10 \text{ mM} \cdot pNa + 151 \text{ mM} \cdot pCl} \right] \quad \text{Eqn (12)}$$

$$V_{rev,NMDG,GHK} = 25.5 \text{ mV} \cdot \ln \left[\frac{153 \text{ mM} \cdot pCl}{10 \text{ mM} \cdot (-0.7873 \cdot pCl) + 151 \text{ mM} \cdot pCl} \right] \quad \text{Eqn (13)}$$

$$V_{rev,NMDG,GHK} = 1.701 \text{ mV} \quad \text{Eqn (14)}$$

[0133] These results show that given $V_{rev,Ringers,expt} = -30.92 \text{ mV}$, the GHK predicts $V_{rev,NMDG,GHK} = 1.701 \text{ mV}$ (Eqn 14), indicating that EPG's reversal potential shifts closer to 0 mV in NMDG.

[0134] This makes sense in two ways. First, from voltage-clamp data, EPG's I-V relationship in NMDG (Fig. 8) shows that $V_{rev,NMDG,expt}$ shifts to the right and closer to zero in reference to $V_{rev,Ringers,expt} = -30.82 \text{ mV}$.

[0135] Quantitatively, fitting the data (Fig. 3) to a 2nd order polynomial curve and solving for x when $I = 0$ pA gives us $x = V_{\text{rev,NMDG,expt}} = -18.7032$ mV, which is indeed a shift to the right and closer to zero, compared to $V_{\text{rev,Ringers,expt}} = -30.82$ mV.

[0136] Second, the Nernst potential (equilibrium potential for a single ion) for Cl^- is

$$E_{\text{Cl}} = 25.5 \text{ mV} * \ln\left(\frac{153\text{mM}}{151\text{mM}}\right) = 0.335 \text{ mV} \quad \text{Eqn (15)}$$

which is close to zero. Nernst potential for Na^+ in NMDG is undefined since $\ln(x)$ is undefined at $x \leq 0$ and the small electrochemical gradient should minimally affect $V_{\text{rev,NMDG,expt}}$. Therefore, to account for $V_{\text{rev,NMDG,expt}}$ shifting closer to zero, E_{Cl} (Eqn 15) suggests that EPG must be highly permeable to Cl^- , therefore causing the shift closer to E_{Cl} (Eqn 15). However, the fact that there was a reversal potential shift in the absence of Na^+ (in NMDG), means EPG must be permeable to Na^+ as well, albeit small.

[0137] According to the Nernst potential for Na^+ in Ringers (Eqn 16), we show that EPG is minimally permeable to sodium.

$$E_{\text{Na,Ringers}} = 25.5 \text{ mV} * \ln\left(\frac{140\text{mM}}{10\text{mM}}\right) = 67.345 \text{ mV} \quad \text{Eqn (16)}$$

$V_{\text{rev,Ringers,expt}}$ (-30.82 mV) is far from $E_{\text{Na,Ringers}}$ (Eqn 16) and closer to E_{Cl} (Eqn 15), suggesting that $V_{\text{rev,Ringers,expt}}$ is more influenced by E_{Cl} (Eqn 15) than by $E_{\text{Na,Ringers}}$ (Eqn 16) and therefore leads us to conclude that EPG is more permeable for Cl^- compared to Na^+ .

[0138] In conclusion, our voltage-clamp data and these calculations show that EPG is selective for chloride and sodium but highly permeable to Cl^- and minimally permeable to Na^+ .

EXAMPLE 6

[0139] Confocal microscopy of HEK293T cells transduced with EPG fused to a V5 epitope shows that EPG is targeted to the periphery of the cells compared to controls (data not shown). HEK293T cells transfected with pcDNA3.1-EPG-V5-His showed membrane targeting of EPG. 3D orthogonal planes further demonstrated that EPG was targeted towards the periphery of the cells. HEK293T cells transfected with pAAV-EPG-FLAG-IRES-hrGFP showed that EPG is targeted towards the periphery of the cells. EPG was expressed in the

pyramidal neurons with apparent membrane targeting in the pAAV-EPG-FLAG-IRES-hrGFP virus infected brain. EPG appears to target the membrane of a single pyramidal neuron's apical dendrite (data not shown). Furthermore, stereotaxic injections of pAAV-EPG-FLAG-IRES-hrGFP and pAAV-IRES-hrGFP were performed into the sub-ventricular zone of P1-2 rats (n=12 per group) and into the somatosensory cortex of adult rats (n=6 per group).

[0140] EPG is not endogenously expressed in mammalian cells. There was no visible green fluorescence (indicative of EPG) is detected in control HEK293T cells nor was there is no visible green (indicative of GFP) or red fluorescence (indicative of EPG) in control HEK293T cells. Immunohistochemistry for EPG expression was performed 3-4 weeks after virus delivery to P1-P2 rat brain. EPG was undetectable in a control brain. The brains were transduced with EPG AAV virus as above but stained with only secondary antibodies and no primary antibodies. Brains from a naïve rats show no EPG expression. EPG is undetectable in a control, untransduced brain (data not shown). The subsequent immunohistochemistry shows that EPG can be expressed in cortical neurons suggesting that EPG, a putative membrane-targeted chloride channel, can be readily expressed in the rodent nervous system for future manipulation of neuronal function (data not shown).

EXAMPLE 7

[0141] Wireless activation of EPG induces neuronal excitability.

[0142] We then sought to determine whether remote activation of EPG has the potential to modulate neuronal function. Cortical neurons obtained from E15-E16 mouse embryos were co-cultured with cortical glia obtained from P0-P2 mouse pups. We used calcium imaging as a mean to visualize neuronal responses using both exogenously applied calcium indicators (Fura-2) and genetically encoded calcium indicators (GCaMP6).

[0143] For imaging of Fura-2 ratio changes, cell cultures were transduced with a viral construct containing the EPG under the excitatory neuron-specific promoter, Ca^{2+} /calmodulin-dependent protein kinase II (pAAV2-CaMKIIprom::-EPG-IRES-hrGFP). Cultured cells were loaded with the calcium indicator fura-2/AM, and an Inverted Olympus microscope with a dual condenser illumination column was used to image the percent change in the fura-2 ratio at 340/380 nm excitation.

[0144] For imaging of GCaMP6, cultured cells were transduced with a viral constructs containing the EPG under CaMKII with a red fluorescence protein (pAAV2-CaMKIIprom::-EPG-IRES-mCherry) and GCaMP6 (AAV5-Syn-GCaMP6s-WPRE-SV40).

[0145] We tested how application of static and alternating magnetic fields for 10 s induced intracellular calcium changes. Static magnets were positioned 15 mm from the neuronal culture and induced a field of 15 mT. Alternating magnetic field was induced by a TMS system that delivered pulses at a rate of 20 Hz and induced a field of 250 mT. Neurons were considered responsive if they showed significant increases in intracellular calcium concentration $[Ca^{2+}]_i$ during the stimulus, and were GFP-positive (which was indicative of EPG expression in Fura-2 experiments), or mCherry-positive and GFP-positive (which were indicative of EPG and GCaMP6 expression, respectively, in GCaMP experiments).

[0146] Figure 7 demonstrates that both static and alternating magnetic fields induced significant changes in intracellular calcium concentration: Static magnetic stimulation induced $11.3 \pm 6\%$ increase in $[Ca^{2+}]_i$ in EPG-and GCaMP6 expressing neurons ($n=15$; $p<0.0005$) with an average delay (time to peak) of 2.3 ± 0.2 s. Neurons that expressed only GCaMP were not responsive to stimulation. Static magnets also produced similar neuronal responses when we used Fura-2 as the imaging probe. Alternating magnetic field induced $21 \pm 9\%$ increase in $[Ca^{2+}]_i$ in EPG-expressing neurons ($n=9$; $p<0.0005$) with an average delay (time to peak) of 28.3 ± 2.7 s, which were measured with Fura-2. Neurons that expressed only GFP or un-transduced neurons were not responsive to stimulation. Therefore, it appears that alternating magnetic field induced a response with a longer delay compared to the static magnets. This could be due to several reasons such as stimulation properties (magnetic vs. electromagnetic), distance from the magnet, field strength, frequency etc.

EXAMPLE 8

[0147] EPG can be expressed in the rodent brain.

[0148] In order to achieve a neuronal-specific and a widespread expression of the EPG, pAAV2-CaMKIIprom::-EPG-IRES-hrGFP and pAAV2-IRES-hrGFP (for control) were stereotaxically injected into the sub-ventricular zone of P1 rats ($n=24$). In order to achieve a neuronal-specific and a region-specific expression of the EPG, stereotaxic injection into the somatosensory cortex ($n=6$) and hippocampus ($n=12$) of adult rats were performed. The

subsequent immunohistochemistry (Fig. 8) demonstrates that EPG can be expressed in a specific sub-population of neurons, and in specific regions of the brain.

[0149] In addition, the present inventors set up a system to visualize magnetic stimulation induced GCaMP changes in acute brain slices expressing EPG (data not shown). Therefore, it is contemplated that the EPG technology of the present invention can be readily expressed in the rodent nervous system for future manipulation of neuronal function, and that GCaMP imaging can be used to detect neuronal responses associated with magnetic stimulation.

[0150] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0151] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0152] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by

applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims:

1. An isolated polynucleotide encoding the electromagnetic perceptive gene (EPG) gene.
2. The isolated polynucleotide of claim 1, having the sequence of SEQ ID NO: 1.
3. An isolated polynucleotide of claim 2, having at least 60 % sequence identity to SEQ ID NO: 1.
4. An isolated polynucleotide encoding the EPG peptide comprising the sequence of SEQ ID NO: 1, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).
5. A cDNA encoding the EPG of claim 1.
6. The cDNA of claim 5, having the sequence of SEQ ID NO: 2.
7. A cDNA of claim 5, having at least 60 % sequence identity to SEQ ID NO: 2.
8. An isolated polynucleotide encoding a cDNA of the EPG comprising the sequence of SEQ ID NO: 2, b) encoding a functional fragment of a); c) encoding a functional homolog of a) or b) or encoding a functional fragment thereof; and d) encoding a fusion polypeptide comprising an amino acid sequence of any of a) to c).
9. An isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid of any of claims 1 to 8.
10. A recombinant expression vector comprising the polynucleotide sequence of any of claims 1 to 8.
11. An isolated host cell comprising the recombinant expression vector of claim 10.
12. The isolated host cell of claim 11, wherein the cell is a neuron or a myocyte.
13. A population of cells comprising at least one host cell of either of claims 11 or 12.
14. An isolated or purified electromagnetic perceptive gene (EPG) peptide.

15. The isolated or purified polypeptide of claims 14, comprising the amino acid sequence of SEQ ID NO: 3, or a functional portion thereof.

16. An isolated or purified polypeptide comprising the EPG peptide sequence of SEQ ID NO: 3, b) a functional fragment of a); c) a functional homolog of a) or b) or a functional fragment thereof; and d) a fusion polypeptide comprising an amino acid sequence of any of a) to c).

17. An antibody, or antigen binding portion thereof, which specifically binds to a portion of the EPG peptide of any of claims 14 to 16.

18. A pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; or a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, or a host cell or population of cells comprising the EPG said isolated nucleic acid, or an antibody to said EPG peptide or antigen binding portion thereof, and a pharmaceutically acceptable carrier. \

19. A method of modulating an ion channel or receptor in a cell or population of cells by non-invasive ELMF comprising administering to the cell or population of cells a pharmaceutical composition comprising an isolated nucleic acid sequence encoding the EPG peptide or a functional fragment of the EPG peptide; a functional homolog of the EPG peptide or a functional fragment thereof; or a fusion polypeptide, or a recombinant expression vector comprising the EPG isolated nucleic acid, or an EPG peptide or functional portion thereof, and a pharmaceutically acceptable carrier.

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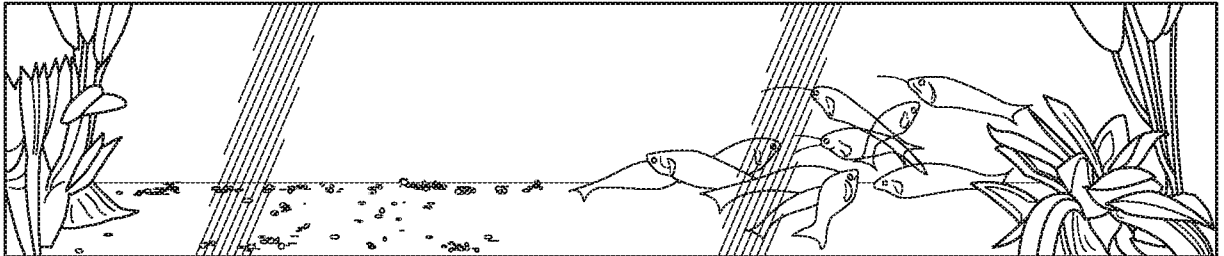


FIG. 1A

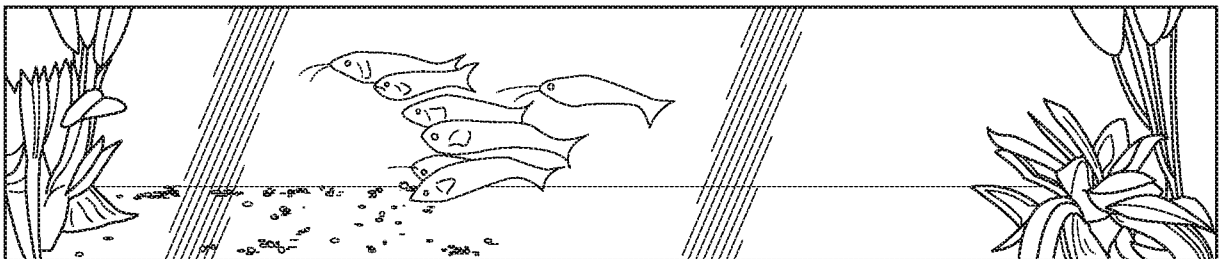


FIG. 1B

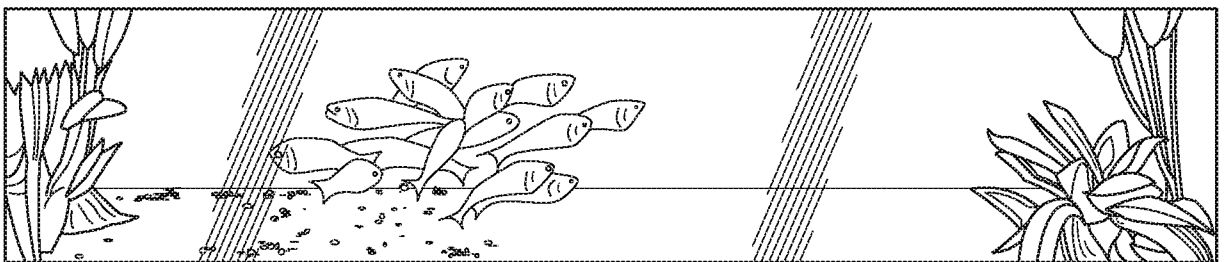
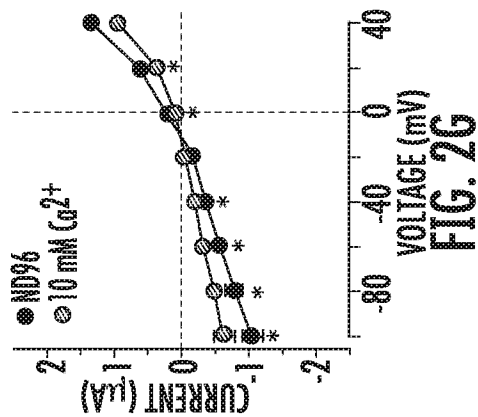
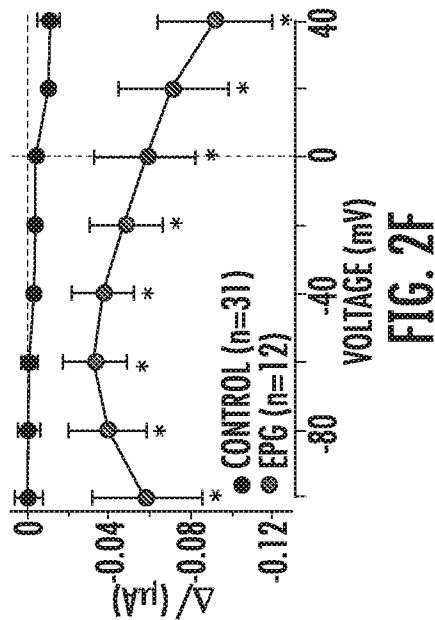
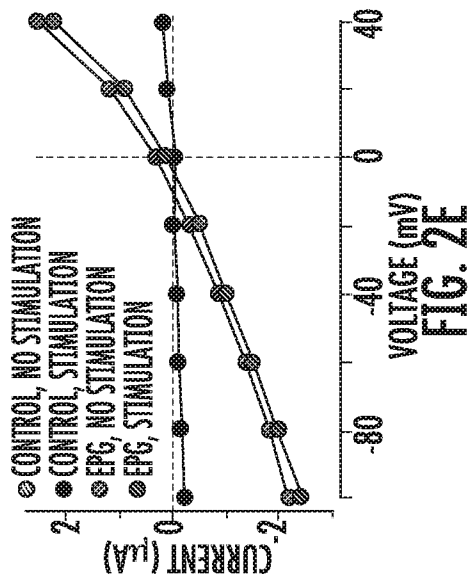
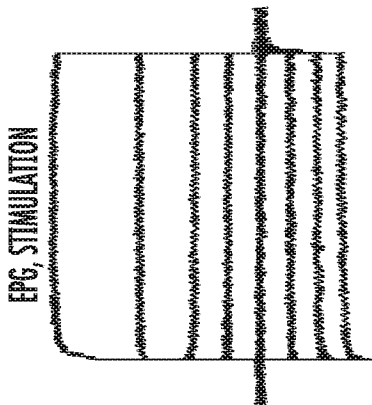
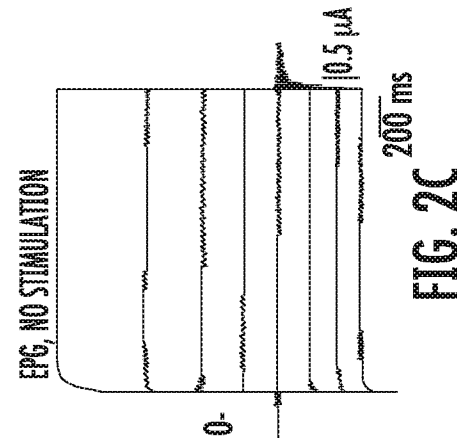
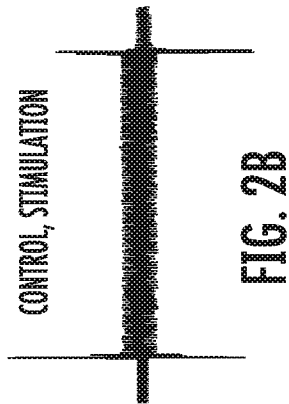
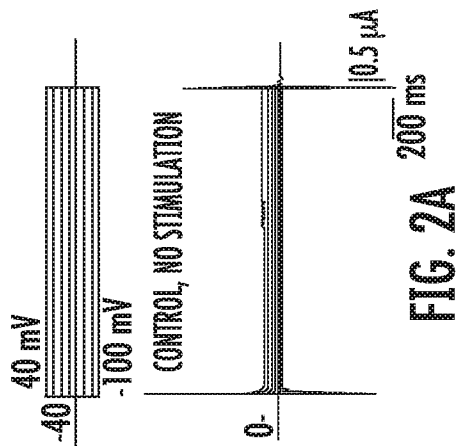


FIG. 1C



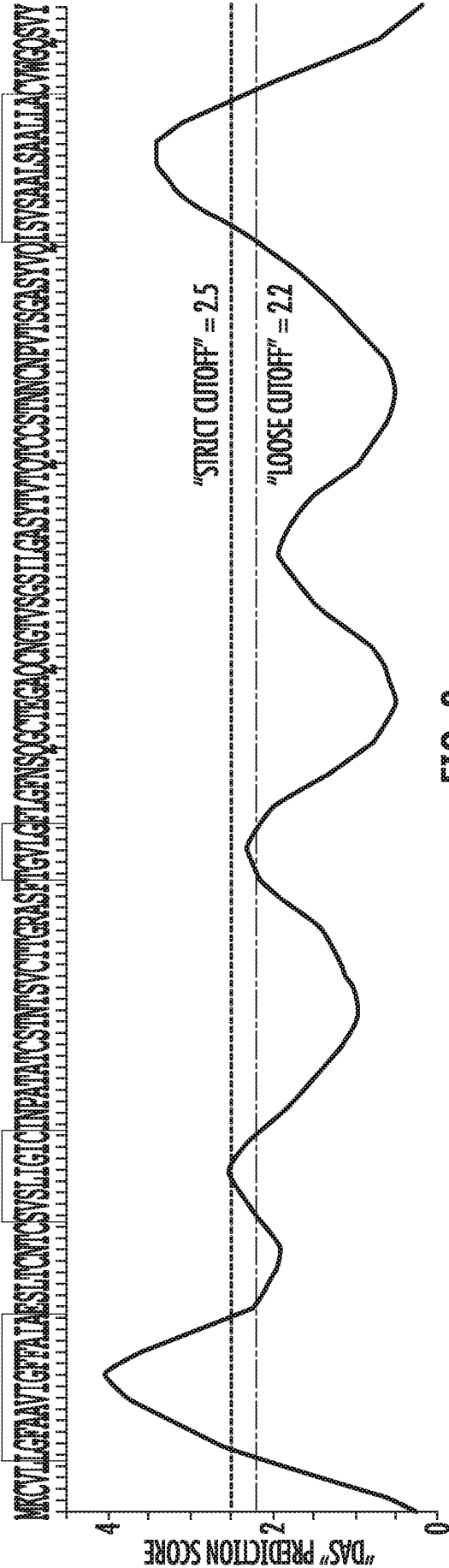


FIG. 3

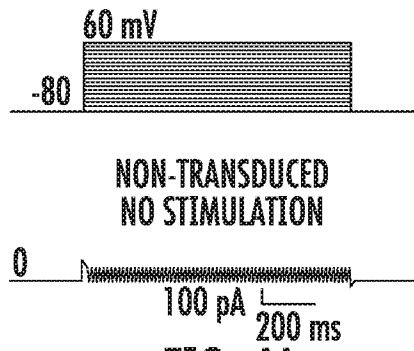


FIG. 4A

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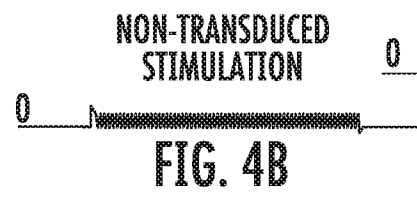


FIG. 4B

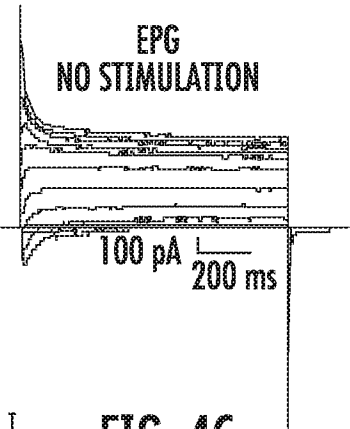


FIG. 4C

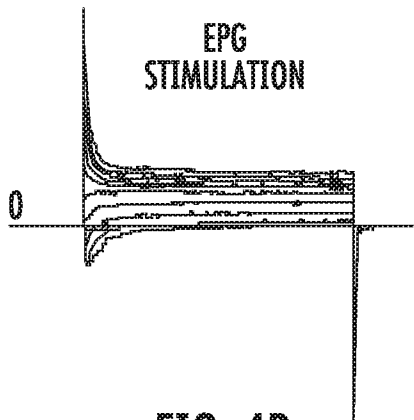


FIG. 4D

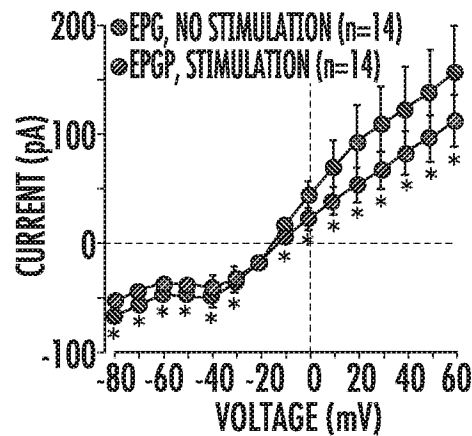


FIG. 4E

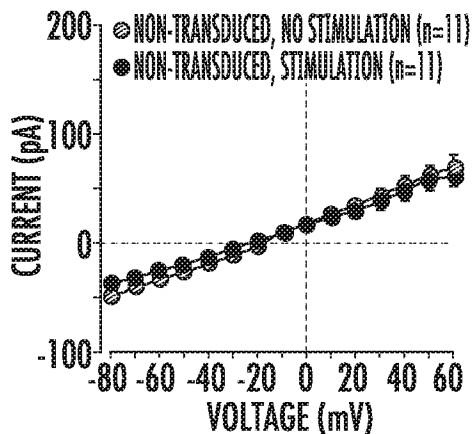


FIG. 4F

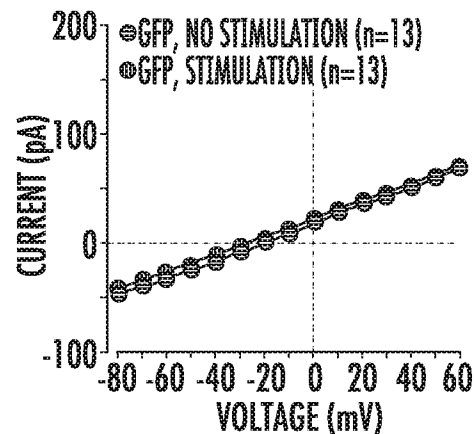


FIG. 4G

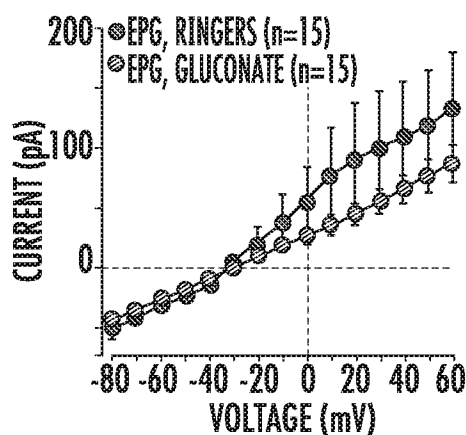


FIG. 4H

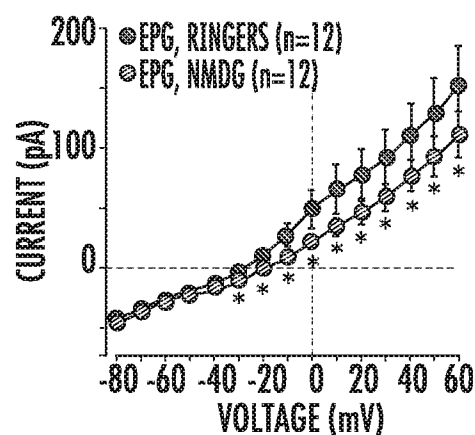


FIG. 4I

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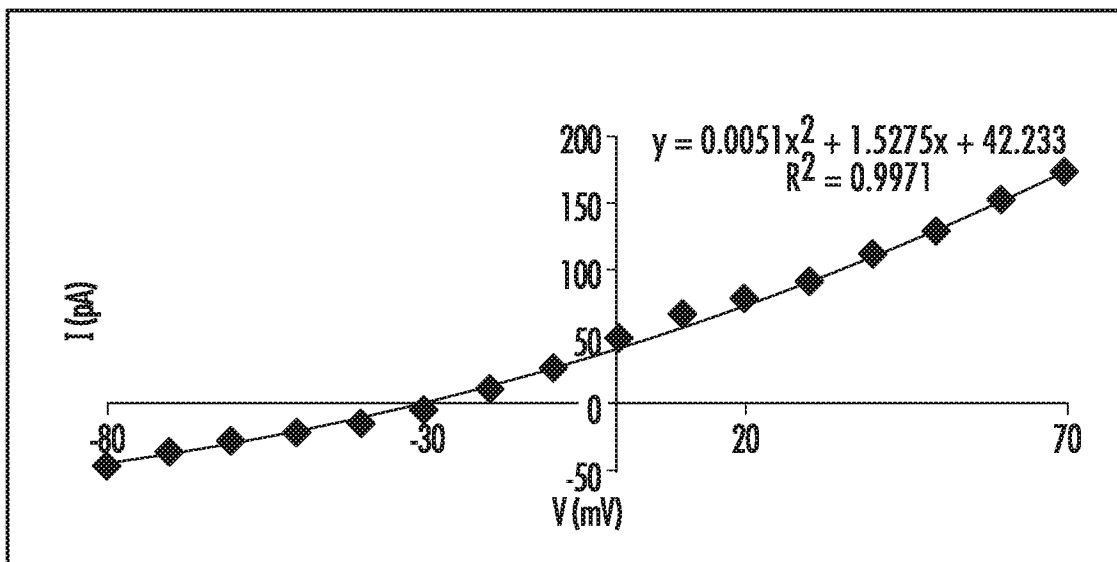


FIG. 5

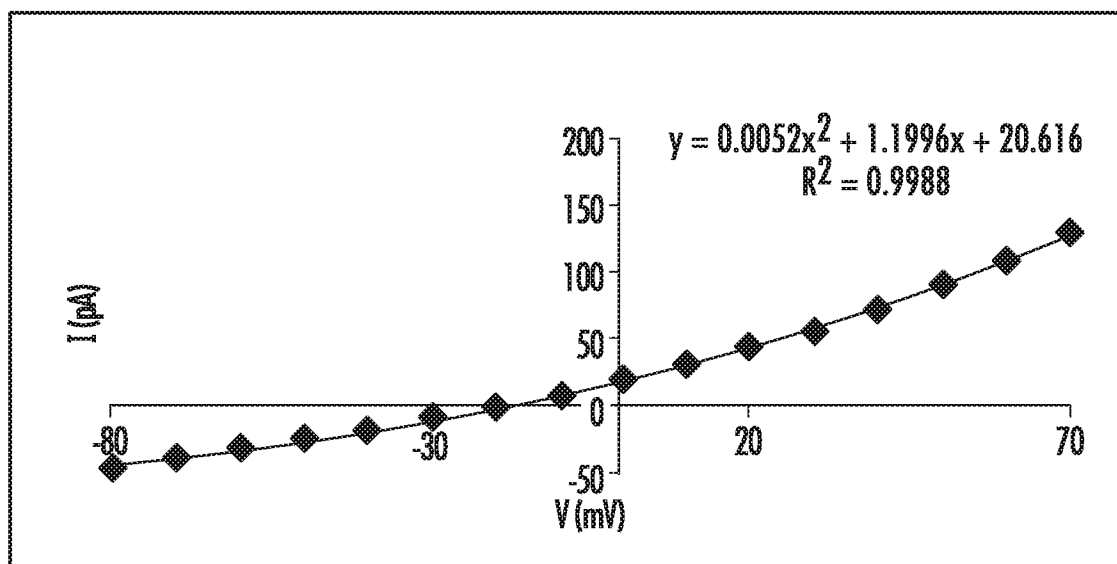
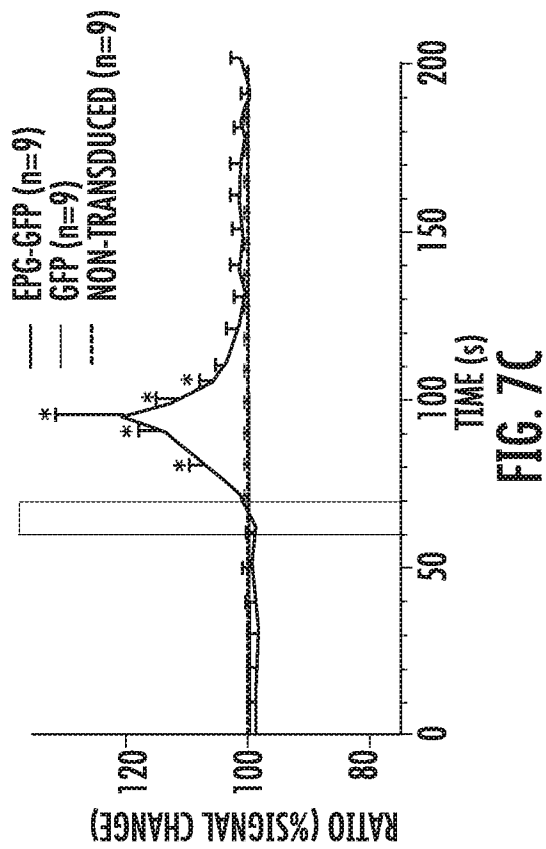
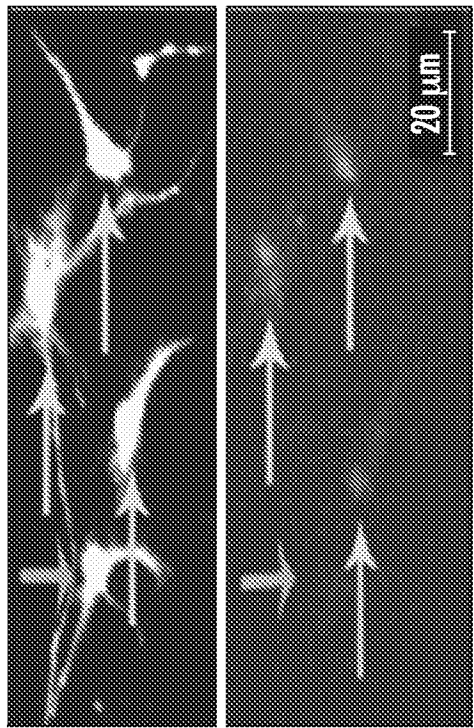
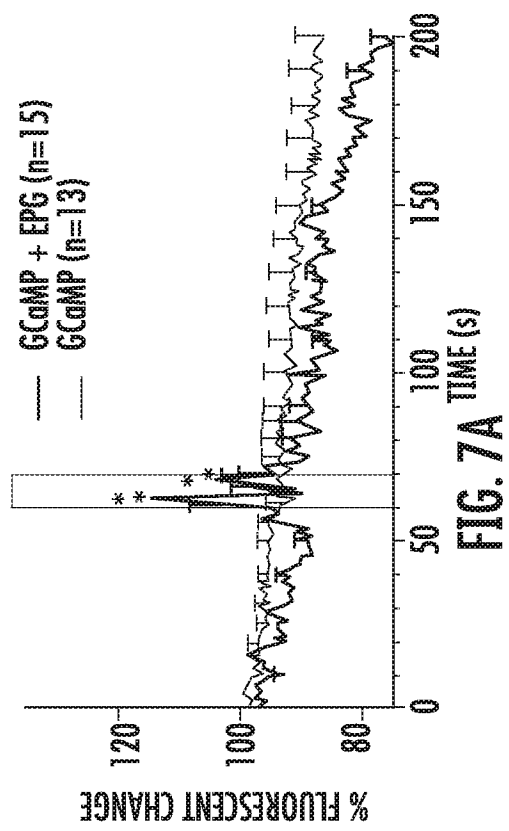
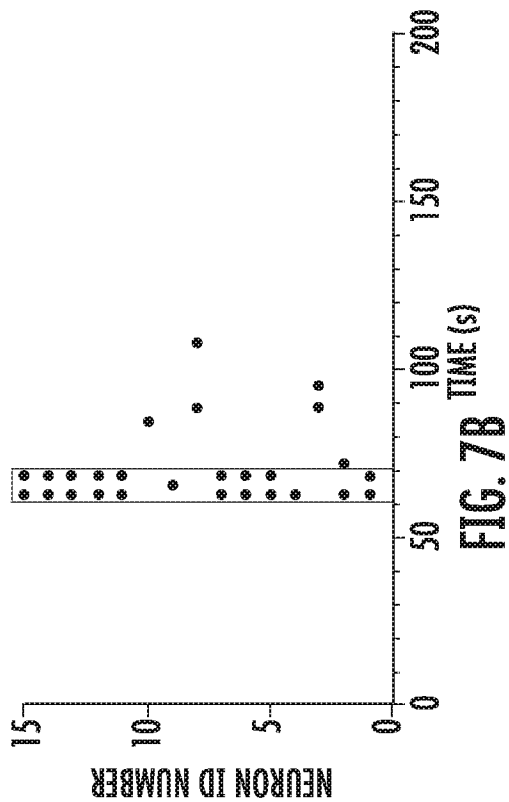
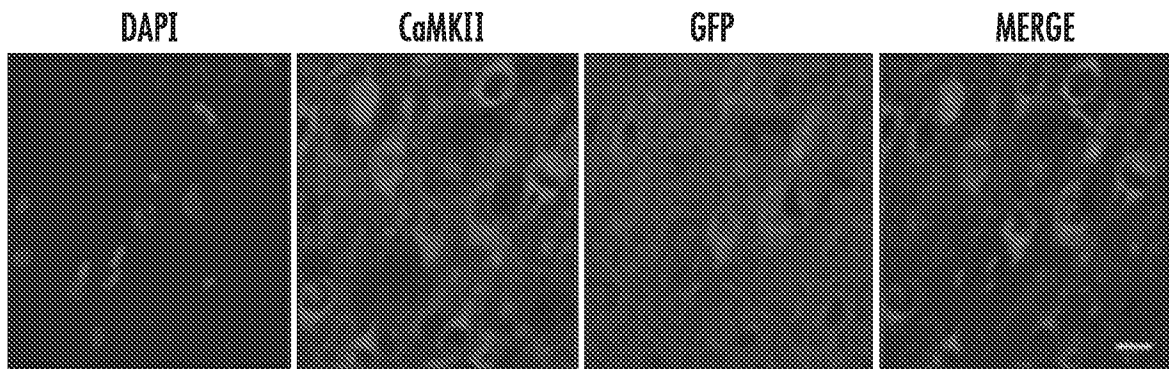


FIG. 6



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SOMATOSENSORY CORTEX



HIPPOCAMPUS

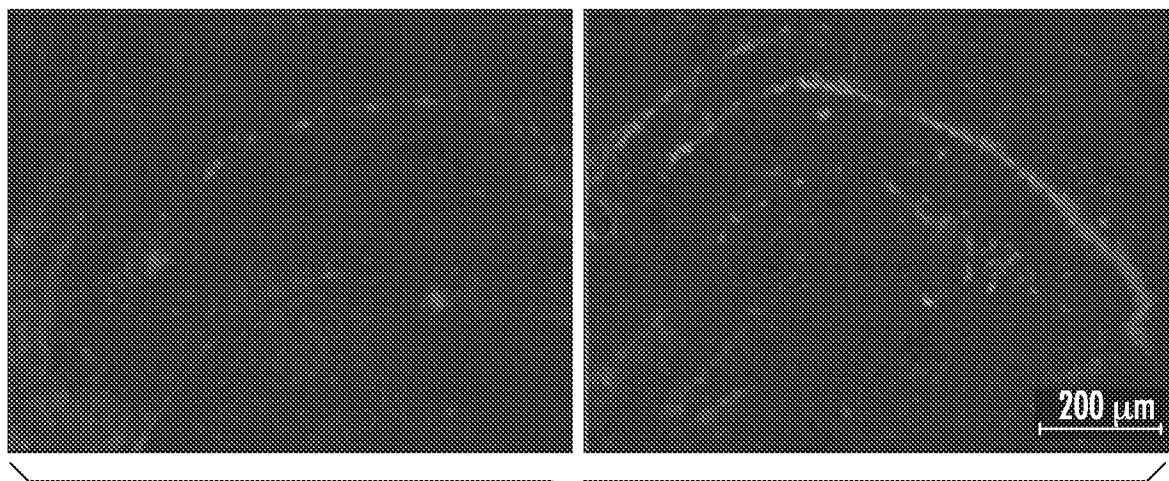


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/020862

A. CLASSIFICATION OF SUBJECT MATTER (see extra sheet) 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) C12N 15/11, 15/63, 15/10, C07K 14/705, 16/00, A61K 48/00, 38/16 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CIPO, DEPATISNET, DWPI, EAPATIS, EMBL, EPO-Internal, ESP@CE, ESP@CENET, KIPRIS, PAJ, PubMed, SCIEDIRECT, SIPO, USPTO, WIPO, GoogleScholar, PatSearch (RUPTO internal)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	WU R.Y. et al. «The effect of 50 Hz magnetic field on GCSmRNA expression in lymphoma B cell by mRNA differential display», J. Cell Biochem., 2000, Vol.79, no.3, p.460-470, abstract	1, 5, 9, 14 17-19 2-4, 6-8, 15-16
X Y	OLIVARES-BANUELOS T. et al. «Differentiation of chromaffin cells elicited by ELF MF modifies gene expression pattern», Cell Biol Int., 2004, Vol.28, no.4, p.273-279	1,5, 9, 14 17-19
X Y	WO 2004/009781 A2 (BIOMED SOLUTIONS LLC et al.) 29.01.2004, p.6, line 10 - p.11, line 13, p.22, line 31-p.23, line 7	1, 9, 10,11, 12-14 17-19
☒ 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 document 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 30 June 2016 (30.06.2016)		Date of mailing of the international search report 07 July 2016 (07.07.2016)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhevskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37		Authorized officer S.I'chenko Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/020862

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HARLOW ED et al. «Antibodies: A Laboratory Manual», Cold Spring Harbor Publications, New York, 1988, p. 1-742, especially p.148-172	17
Y	GOODMAN R. et al. «Insights Into Electromagnetic Interaction Mechanisms», JOURNAL OF CELLULAR PHYSIOLOGY, 2002, Vol.192, p.16-22	18, 19

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.

PCT/US 2016/020862

C12N 15/11 (2006.01)
C12N 15/63 (2006.01)
C07K 14/705 (2006.01)
C07K 16/00 (2006.01)
A61K 48/00 (2006.01)
A61K 38/16 (2006.01)