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<b>(54) Title:</b> DNA ENCODING A HUMAN 5-HT <sub>1E</sub> RECEPTOR AND USES THEREOF  <b>(57) Abstract</b> <p>This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor, an isolated protein which is a human 5-HT<sub>1E</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor, mammalian cells comprising such vectors, antibodies directed to the human 5-HT<sub>1E</sub> receptor, nucleic acid probes useful for detecting nucleic acid encoding human 5-HT<sub>1E</sub> receptors, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human 5-HT<sub>1E</sub> receptor, pharmaceutical compounds related to human 5-HT<sub>1E</sub> receptors, and nonhuman transgenic animals which express DNA encoding a normal or a mutant human 5-HT<sub>1E</sub> receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving the human 5-HT<sub>1E</sub> receptor.</p>		

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**DNA ENCODING A HUMAN 5-HT<sub>1E</sub> RECEPTOR AND USES THEREOF****Background of the Invention**

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Since the purification of a pressor substance in blood serum termed serotonin (Rapport et al., 1947) and later identified as 5-hydroxytryptamine (5-HT) (Rapport, 1949), there has been a plethora of reports demonstrating that this indoleamine not only plays a role in the functioning of peripheral tissues but, indeed, performs a key role in the brain as a neurotransmitter. Certainly, the anatomical localization of serotonin and serotonergic neurons in both the peripheral and central nervous systems supports its role in such diverse physiologic and behavioral functions as pain perception, sleep, aggression, sexual activity, hormone secretion, thermoregulation, motor activity, cardiovascular function, food intake and renal regulation (For review see Green, 1985; Osborne and Hamon, 1988; Sanders-Bush, 1988; Peroutka, 1991). Taken together, it appears that serotonin plays an important role in homeostasis and in modulating responsiveness to environmental stimuli. Accordingly, studies demonstrating that abnormalities in the serotonergic system may be associated with disease states has created a drug development effort towards agents which may selectively modulate the function of serotonin (Glennon, 1990).

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In relation to the characterization of physiologic or biochemical responses resulting from the release of serotonin are simultaneous investigations examining the receptor sites responsible for the actions elicited by

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the indoleamine transmitter. Following early in vitro pharmacological assays describing the existence of two different serotonin receptors, designated as D and M, in the guinea pig ileum (Gaddum and Picarelli, 1957), the advent of receptor binding technique in the 1970's has brought to light during the last decade the diversity of 5-HT receptors existing in both the brain and peripheral tissues. Thus, although the concept of D and M receptors has not been invalidated, serotonin receptors not fitting either category have been identified using radioligand methods. To date using this technique, there appears to be four classes of serotonin receptors found in the brain: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub> and, 5-HT<sub>4</sub> (Peroutka, 1991). Furthermore, 5-HT<sub>1</sub> sites have been subclassified as: 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub> (Hamon et al., 1990) and 5-HT<sub>1E</sub> (Leonhardt et al., 1989). Although a detailed characterization of the 5-HT<sub>1E</sub> binding site is lacking, extensive pharmacologic, biochemical and functional properties have clearly shown that the other four subtypes of 5-HT<sub>1</sub> sites are receptors according to classical criteria. Interestingly, the 5-HT<sub>1E</sub> binding site was first observed in human cortical tissue using [<sup>3</sup>H]5-HT as the radioligand probe in the presence of 5-carboxyamidotryptamine and mesulergine to mask other members of the 5-HT<sub>1</sub> receptor class. The affinity constants of the nine drugs tested indicated a unique pharmacological profile. In particular, the low affinity of 5-CT and ergotamine seemed to clearly discriminate the pharmacologically defined 5-HT<sub>1D</sub> site from that of this novel serotonergic site. Importantly, it was demonstrated that 5-HT<sub>1E</sub> sites are saturable and exist in a density consistent with other known neurotransmitter receptors. Furthermore, this site appeared to interact with a GTP-binding protein. Overall, the data provided a framework suggesting that the 5-HT<sub>1E</sub> binding site may

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represent a functional receptor.

During the last few years, the field of molecular biology has provided an important facet to receptor research by cloning these proteins and allowing more precise characterizations in isolated systems (Hartig et al., 1990). This has been accomplished for the 5-HT<sub>1A</sub> (Fargin et al., 1988), 5-HT<sub>1C</sub> (Julius et al., 1988), 5-HT<sub>1D</sub> (Branchek et al., 1990) and 5-HT<sub>2</sub> receptors (Pritchett et al., 1988). Thus, there is no doubt that these binding sites represent "true" functional receptors. Indeed, the pharmacological characterization of serotonin receptors involved in various physiological or biochemical functions is a key component of drug development for the serotonergic system. As one can deduce from the diversity of serotonin binding sites, many targets are available for advancement in selective drug design. The coupling of molecular biological methods to pharmacological characterization particularly for cloned human receptors will open new avenues for pharmaceutical development which have not been previously explored.

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Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor.

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This invention also provides an isolated protein which is a human 5-HT<sub>1E</sub> receptor.

This invention provides a vector comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor.

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This invention also provides vectors such as plasmids comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding the 5-HT<sub>1E</sub> receptor as to permit expression thereof.

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This invention provides a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor.

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This invention provides a method for determining whether a ligand not known to be capable of binding to a human 5-HT<sub>1E</sub> receptor can bind to a human 5-HT<sub>1E</sub> receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1E</sub> receptor with the ligand under conditions permitting binding of ligands known to bind to a 5-HT<sub>1E</sub> receptor, detecting the presence of any of the ligand bound to a human 5-HT<sub>1E</sub> receptor, and thereby determining whether the ligand binds to a human 5-HT<sub>1E</sub> receptor.

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This invention also provides a method for determining whether a ligand not known to be capable of binding to the human 5-HT<sub>1E</sub> receptor can functionally activate its activity or prevent the action of a ligand which does so. This comprises contacting a mammalian cell comprising an isolated DNA molecule which encodes a human 5-HT<sub>1E</sub> receptor with the ligand under conditions permitting the activation or blockade of a functional response, detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1E</sub> receptor functional output.

This invention further provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1E</sub> receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human 5-HT<sub>1E</sub> receptor.

This invention also provides a method of screening drugs to identify drugs which interact with, and activate or block the activation of, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting the mammalian cell comprising an isolated DNA molecule encoding and expressing a human 5-HT<sub>1E</sub> receptor with a plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with, and activate or block the activation of, a human 5-HT<sub>1E</sub> receptor.

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This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor.

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This invention also provides a method of detecting expression of the 5-HT<sub>1E</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1E</sub> receptor which comprises obtaining total mRNA from the  
10 cell and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor under  
15 hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the 5-HT<sub>1E</sub> receptor by the cell.

This invention provides an antisense oligonucleotide  
20 having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT<sub>1E</sub> receptor so as to prevent translation of the mRNA molecule.

25 This invention provides an antibody directed to a human 5-HT<sub>1E</sub> receptor.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor. This  
30 invention also provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT<sub>1E</sub> receptor. This invention further  
35 provides a transgenic nonhuman mammal whose genome

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comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1E</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a 5-HT<sub>1E</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1E</sub> receptor thereby reducing its translation.

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This invention provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a transgenic nonhuman animal whose levels of human 5-HT<sub>1E</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1E</sub> receptor expression.

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This invention also provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT<sub>1E</sub> receptor.

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This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1E</sub> receptor allele which comprises: a. obtaining DNA of subjects suffering from the disorder; b. performing a restriction digest of the DNA with a panel of restriction enzymes; c. electrophoretically separating the resulting DNA fragments on a sizing gel; d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>1E</sub> receptor and labelled with a detectable marker; e. detecting labelled bands which have hybridized to the DNA encoding a human 5-HT<sub>1E</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing DNA obtained for diagnosis by steps a-e; and g. comparing the

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unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

5 This invention provides a method of preparing the isolated 5-HT<sub>1E</sub> receptor which comprises inducing cells to express 5-HT<sub>1E</sub> receptor, recovering the receptor from the resulting cells and purifying the receptor so recovered.

10 This invention also provides a method of preparing the isolated 5-HT<sub>1E</sub> receptor which comprises inserting nucleic acid encoding 5-HT<sub>1E</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

15 This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a receptor so as to prevent translation of the mRNA molecule.

20 This invention also provides a transgenic nonhuman mammal expressing DNA encoding a receptor.

25 This invention further provides a transgenic nonhuman mammal expressing DNA encoding a receptor so mutated as to be incapable of normal receptor activity, and not expressing native receptor.

30 This invention also provides a method of determining the physiological effects of expressing varying levels of a

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receptor which comprises producing a transgenic nonhuman animal whose levels of receptor expression are varied by use of an inducible promoter which regulates receptor expression.

5 This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of the receptor.

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This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA  
15 encoding the receptor and which hybridizes to mRNA encoding the receptor thereby preventing its translation.

This invention provides a method for determining whether  
20 a ligand not known to be capable of binding to a receptor can bind to a receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the receptor with the ligand under conditions permitting binding of ligands known to bind to a  
25 receptor, detecting the presence of any of the ligand bound to the receptor, and thereby determining whether the ligand binds to the receptor.

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**Brief Description of the Figures**

Figure 1. Nucleotide and deduced amino acid sequence of gene 5-HT<sub>1E</sub>. (Seq. I.D. Nos. 1 and 2).

5 Numbers above the nucleotide sequence indicate nucleotide position. DNA sequence was determined by the chain termination method of Sanger, et al., on denatured double-stranded plasmid templates using the enzyme Sequenase. Deduced amino acid sequence (single letter  
10 code) of a long open reading frame is shown.

Figure 2. Comparison of the human 5-HT<sub>1E</sub> receptor primary structures with other serotonin receptors. (Seq. I.D. Nos. 3 - 5HT<sub>1A</sub>; 4 - 5HT<sub>1C</sub>; 5 - 5HT<sub>1Dα</sub>; 6 - 5HT<sub>1Dβ</sub>; 7 - 5HT<sub>2</sub>)

15 Amino acid sequences (single letter code) are aligned to optimize homology. The putative transmembrane spanning domains are indicated by stars and identified by Roman numerals (TM I-VII).

20 Figure 3. Respective pKi values of various drugs for the inhibition of [<sup>3</sup>H]5-HT specific binding to the cloned 5-HT<sub>1E</sub> receptor and human cortical membrane preparations containing the native 5-HT<sub>1E</sub> receptor.

25 Values for the native membrane preparation are taken from Leonhardt et al., 1989. "r" is the correlation coefficient between pKi values calculated for the two  
30 receptor preparations and clearly indicates the similarity in binding profile.

35 Figure 4. 5-HT concentration-effect curve for the inhibition of forskolin-stimulated CAMP formation in Y1 cells transfected with the 5-HT<sub>1E</sub> receptor.

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Values are expressed as a percentage of forskolin(Fsk)-stimulated cAMP production.

Figure 5. 5-HT<sub>1E</sub> mRNA localization in the human brain.

5 cDNA was reverse transcribed from total RNA (5  $\mu$ g) using  
random hexamers (500 pmoles). One microgram of cDNA was  
subjected to 30 cycles of PCR amplification using 5'- and  
3'-primers directed to the third cytoplasmic loop of the  
5HT1E receptor gene. Amplified fragments were subjected  
10 to Southern blot analysis using an end-labeled  
oligonucleotide probe which was internal to the PCR  
primers. (+) control consisted of 5HT1E recombinant  
plasmid; (-) control consisted of all cDNA and PCR  
reagents without the addition of cDNA template.  
15 Intensity of signal is depicted as the number of plus  
signs as defined in the figure key.

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### Detailed Description of the Invention

As used herein, the 5-HT receptor family is defined as the group of mammalian proteins that function as  
5 receptors for serotonin. A 5-HT receptor subfamily is defined as a subset of proteins belonging to the 5-HT receptor family which are encoded by genes which exhibit  
10 homology of greater than 72% or higher with each other in their deduced amino acid sequences within presumed transmembrane regions (linearly contiguous stretches of  
hydrophobic amino acids, bordered by charged or polar amino acids, that are long enough to form secondary  
15 protein structures that span a lipid bilayer). Four human 5-HT receptor subfamilies can be distinguished based on the information presently available: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub> (Peroutka, 1991). The 5-HT<sub>2</sub> receptor subfamily contains the human 5-HT<sub>2</sub> receptor. Although no  
20 other human members of this family have been described, the rat 5-HT<sub>2</sub> receptor (Pritchett, et al. 1988; Julius, et al. Proc. Natl. Acad. Sci. USA 87:928-932, 1990) and the rat 5HT<sub>1C</sub> receptor (Julius, et al. 1988) constitute a  
rat 5-HT receptor subfamily. The 5-HT<sub>1</sub> subfamily has been  
25 subdivided further as: 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub> (Hamon et al., 1990) and 5-HT<sub>1E</sub> (Leonhardt et al., 1989). The 5-HT<sub>1A</sub> subfamily contains the human 5-HT<sub>1A</sub> receptor, also known as G-21 (Fargin, et al. 1988). The 5-HT<sub>1D</sub>  
30 receptor subfamily contains two members, the 5-HT<sub>1D-1</sub> receptor (also termed 5-HT<sub>10a</sub>) and the 5-HT<sub>1D-2</sub> receptor (also termed 5-HT<sub>10b</sub>). The 5-HT<sub>1E</sub> subfamily contains the human 5-HT<sub>1E</sub> receptor (also termed clone hp75d). Although  
this definition differs from the pharmacological  
35 definition used earlier, there is significant overlap between the present definition and the pharmacological definition. Members of the 5-HT<sub>1E</sub> receptor subfamily so

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described include the 5-HT<sub>1E</sub> receptor and any other receptors which have a greater than 72% homology to the DNA and amino acid sequence shown in Figure 1 according to the definition of "subfamily". This invention relates to the discovery of the first member of the human 5-HT<sub>1E</sub> receptor subfamily.

This invention provides an isolated nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor. As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is, a molecule in a form which does not occur in nature. Such a receptor is by definition a member of the 5-HT<sub>1E</sub> receptor subfamily. Therefore, any receptor which meets the defining criteria given above is a human 5-HT<sub>1E</sub> receptor. One means of isolating a human 5-HT<sub>1E</sub> receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from the human receptor gene 5-HT<sub>1E</sub> are particularly useful probes for this purpose. DNA and cDNA molecules which encode human 5-HT<sub>1E</sub> receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a human 5-HT<sub>1E</sub> receptor. Such molecules may have coding sequences substantially the same as the coding sequence shown in Figure 1. The DNA molecule of Figure 1 encodes

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the sequence of the human 5-HT<sub>1E</sub> receptor gene.

This invention further provides a cDNA molecule of encoding a human 5-HT<sub>1E</sub> receptor having a coding sequence substantially the same as the coding sequence shown in  
5 Figure 1. This molecule is obtained by the means described above.

This invention also provides an isolated protein which is a human 5-HT<sub>1E</sub> receptor. As used herein, the term  
10 "isolated protein means a protein molecule free of other cellular components. An example of such protein is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 which is a human 5-HT<sub>1E</sub> receptor. One means for obtaining  
15 isolated 5-HT<sub>1E</sub> receptor is to express DNA encoding the receptor in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been  
20 expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

25 This invention provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA encoding a human 5-HT<sub>1E</sub> receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia,  
30 Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction  
35 enzyme to create complementary ends on both molecules

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which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available. A specific example of such plasmids is a plasmid comprising cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone hp75d (Seq. I.D. No. 1).

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This invention also provides vectors comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human 5-HT<sub>1E</sub> receptor as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human 5-HT<sub>1E</sub> receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by

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methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the receptor. Certain uses for such cells are described in more detail below.

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This invention further provides a plasmid adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell which comprises a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor and the regulatory elements  
10 necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human 5-HT<sub>1E</sub> receptor as to permit expression thereof. Some plasmids adapted for expression in a mammalian cell are pSVL (available from Pharmacia,  
15 Piscataway, NJ) and pcEXV-3 (Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)). A specific example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences  
20 substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pcEXV-hp75d and deposited under ATCC Accession No. 75138. Those skilled in the art will readily  
25 appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA of encoding human 5-HT<sub>1E</sub> receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to  
30 contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

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The deposit discussed supra, and the other deposits discussed herein, were made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor, the protein encoded thereby is expressed on the cell surface, and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human 5-HT<sub>1E</sub> receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, for example, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk<sup>+</sup> cells, Y1 cells, etc. A particular example of an Ltk<sup>+</sup> cell is a cell designated 5-HT<sub>1E</sub>-7 and deposited under ATCC Accession No. CRL 10913 and comprises the plasmid designated pcEXV-hp75d. Another example is the murine adrenal Y1 cell line designated Y-5-HT<sub>1E</sub> and deposited under ATCC Accession No. CRL 10914. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these 5-HT<sub>1E</sub> receptors may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding either human 5-HT<sub>1E</sub> receptor:

This invention provides a method for determining whether

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a ligand not known to be capable of binding to a human 5-HT<sub>1E</sub> receptor can bind to a human 5-HT<sub>1E</sub> receptor which comprises contacting a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor, the protein encoded thereby is expressed on the cell surface, with the ligand under conditions permitting binding of ligands known to bind to the 5-HT<sub>1E</sub> receptor, detecting the presence of any of the ligand bound to the 5-HT<sub>1E</sub> receptor, and thereby determining whether the ligand binds to the 5-HT<sub>1E</sub> receptor. This invention also provides a method for determining whether a ligand not known to be capable of binding to the human 5-HT<sub>1E</sub> receptor can functionally activate its activity or prevent the action of a ligand which does so. This comprises contacting a mammalian cell comprising an isolated DNA molecule which encodes a human 5-HT<sub>1E</sub> receptor with the ligand under conditions permitting the activation or blockade of a functional response, detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1E</sub> receptor functional output. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1 preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk<sup>-</sup> cell, in particular the Ltk<sup>-</sup> cell designated 5-HT<sub>1E</sub>-7. Another example of a non-neuronal mammalian cell to be used for functional assays is a Y1 murine adrenal cell, specifically the Y1 cell designated Y-5-HT<sub>1E</sub>. The preferred method for determining whether a ligand is capable of binding to the human 5-HT<sub>1E</sub> receptor comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of 5-HT or G-protein coupled receptor, thus will only express

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such a receptor if it is transfected into the cell) expressing a 5-HT<sub>1E</sub> receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with, in vivo binding of the ligands to a 5-HT<sub>1E</sub> receptor, detecting the presence of any of the ligand being tested bound to the 5-HT<sub>1E</sub> receptor on the surface of the cell, and thereby determining whether the ligand binds to, activates or prevents the activation of the 5-HT<sub>1E</sub> receptor. This response system is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a host system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of human 5-HT<sub>1E</sub> receptors with ligands as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the

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natural functions of the human 5-HT<sub>1E</sub> receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at the human 5-HT<sub>1E</sub> receptor sites.

5 This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human 5-HT<sub>1E</sub> receptor  
10 on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human 5-HT<sub>1E</sub> receptor. This invention also provides a method of screening drugs to  
15 identify drugs which interact with, and activate or block the activation of, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting the mammalian cell comprising an isolated DNA molecule encoding and expressing a human 5-HT<sub>1E</sub> receptor with a  
20 plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with , and activate or block the  
25 activation of, a human 5-HT<sub>1E</sub> receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an Ltk<sup>-</sup> cell, in  
30 particular the Ltk<sup>-</sup> cell designated 5-HT<sub>1E</sub>-7. Another example of a non-neuronal mammalian cell to be used for functional assays is a Y1 murine adrenal cell, specifically the Y1 cell designated Y-5-HT<sub>1E</sub>. Drug  
35 candidates are identified by choosing chemical compounds

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which bind with high affinity to the expressed 5-HT<sub>1E</sub> receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by  
5 identifying compounds which bind with high affinity to one particular 5-HT<sub>1E</sub> receptor subtype but do not bind with high affinity to any other serotonin receptor subtype or to any other known receptor site. Because selective, high affinity compounds interact primarily  
10 with the target 5-HT<sub>1E</sub> receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a pharmaceutical composition comprising a drug identified by the method described  
15 above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion,  
20 and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action  
25 for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available,  
30 in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

This invention provides a nucleic acid probe comprising  
35 a nucleic acid molecule of at least 15 nucleotides

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capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor, for example with a coding sequence included within the sequence shown in Figure 1. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human 5-HT<sub>1E</sub> receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding 5-HT<sub>1E</sub> receptor is altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes human 5-HT<sub>1E</sub> receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. An example of such DNA molecule is shown in Figure 1. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA

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molecule which encodes human 5-HT<sub>1E</sub> receptor of are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction. Synthesized oligonucleotides as described may also be used to determine the cellular localization of the mRNA produced by the 5-HT<sub>1E</sub> gene by in situ hybridization. An example of such an oligonucleotide is: GATGGTACACTGGCTGGGGGGTGGGCTGAGTTGACGGTGGCT (Seq. I.D. No. 8).

This invention also provides a method of detecting expression of a 5-HT<sub>1E</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1E</sub> receptor which comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>1E</sub> receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the 5-HT<sub>1E</sub> receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. In one possible means of performing this method, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting.

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However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

5 This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT<sub>1E</sub> receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a sequence capable of binding specifically with any  
10 sequences of the cDNA molecule whose sequence is shown in Figure 1. As used herein, the phrase "binding specifically" means the ability of a nucleic acid sequence to recognize a nucleic acid sequence complementary to its own and to form double-helical  
15 segments through hydrogen bonding between complementary base pairs. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

20 This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide described above effective to reduce expression of a human 5-HT<sub>1E</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding a human 5-HT<sub>1E</sub> receptor in  
25 the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable  
30 hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The  
35 structure may be part of a protein known to bind a cell-

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type specific receptor, for example an insulin molecule, which would target pancreatic cells. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figure 1 may be used as the oligonucleotides of the pharmaceutical composition.

5

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a 5-HT<sub>1E</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to

10 reduce expression of the 5-HT<sub>1E</sub> receptor by the subject.

This invention further provides a method of treating an abnormal condition related to 5-HT<sub>1E</sub> receptor activity which comprises administering to a subject an amount of

15 the pharmaceutical composition described above effective to reduce expression of the 5-HT<sub>1E</sub> receptor by the subject. Several examples of such abnormal conditions are dementia, Parkinson's disease, feeding disorders, pathological anxiety, schizophrenia, or a migraine

20 headache.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures

25 are designed to bind to mRNA encoding the 5-HT<sub>1E</sub> receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of 5-HT<sub>1E</sub> receptor genes in patients. This invention provides a means to therapeutically alter levels of expression of human 5-HT<sub>1E</sub>

30 receptors by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures

35 designed to recognize and selectively bind to mRNA, are

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constructed to be complementary to portions of the nucleotide sequences shown in Figure 1 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g. by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequence shown in Figure 1 by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNase I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense

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oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of 5-HT<sub>1E</sub> receptors.

This invention provides an antibody directed to the human 5-HT<sub>1E</sub> receptor, for example a monoclonal antibody directed to an epitope of a human 5-HT<sub>1E</sub> receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1E</sub> receptor included in the amino acid sequence shown in Figure 1 (Seq. I.D. No. 2). Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 1 will bind to a surface epitope of a human 5-HT<sub>1E</sub> receptor, as described. Antibodies directed to human 5-HT<sub>1E</sub> receptors may be

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serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as  
5 NIH3T3 cells or Ltk<sup>-</sup> cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequence shown in Figure 1 (Seq. I.D. No. 2). As a still further alternative, DNA,  
10 such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human 5-HT<sub>1E</sub> receptors encoded by the isolated DNA, or to inhibit the function of the  
15 receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the  
20 human 5-HT<sub>1E</sub> receptor effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human 5-HT<sub>1E</sub> receptor present on the surface of a cell and having an amino acid  
25 sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1E</sub> receptor included in the amino acid sequence shown in Figure 1 is useful for this purpose.

This invention also provides a method of treating  
30 abnormalities which are alleviated by reduction of expression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a subject an amount of the

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pharmaceutical composition described above effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor and thereby alleviate abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of 5-HT<sub>1E</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor and thereby alleviate the abnormal condition. Some examples of abnormal conditions are dementia, Parkinson's disease, feeding disorders, pathological anxiety, schizophrenia, and a migraine headache.

This invention provides a method of detecting the presence of a 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the human 5-HT<sub>1E</sub> receptor, under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby the presence of the human 5-HT<sub>1E</sub> receptor on the surface of the cell. Such a method is useful for determining whether a given cell is defective in expression of 5-HT<sub>1E</sub> receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

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This invention provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor so mutated as to be incapable of normal receptor activity, and not  
5 expressing native 5-HT<sub>1E</sub> receptor. This invention also provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1E</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a  
10 5-HT<sub>1E</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1E</sub> receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or  
15 restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequence shown in Figure 1. An example of a transgenic animal is a transgenic mouse. Exam~~pl~~es of tissue specificity-  
20 determining regions are the metallothionein promoter (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promoter (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).  
25

Animal model systems which elucidate the physiological and behavioral roles of human 5-HT<sub>1E</sub> receptors are produced by creating transgenic animals in which the  
30 expression of a 5-HT<sub>1E</sub> receptor is either increased or decreased, or the amino acid sequence of the expressed 5-HT<sub>1E</sub> receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of DNA encoding a  
35 human 5-HT<sub>1E</sub> receptor or homologous animal versions of



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fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

15

Since the normal action of receptor-specific drugs is to activate or to inhibit the receptor, the transgenic animal model systems described above are useful for testing the biological activity of drugs directed against these 5-HT<sub>1E</sub> receptors even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit these 5-HT<sub>1E</sub> receptors by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant 5-HT<sub>1E</sub> receptors in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against these 5-HT<sub>1E</sub> receptors are evaluated before such drugs become available. The transgenic animals which over or under produce the 5-HT<sub>1E</sub> receptor indicate by their physiological state whether over or under production of the 5-HT<sub>1E</sub> receptor is therapeutically useful. It is therefore useful to evaluate drug action based on the

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transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake, and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less receptor by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses receptor is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to 5-HT<sub>1E</sub> receptor is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the 5-HT<sub>1E</sub> receptor is achieved therapeutically either by producing agonist or antagonist drugs directed against these 5-HT<sub>1E</sub> receptors or by any method which increases or decreases the expression of these 5-HT<sub>1E</sub> receptors in man.

This invention provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a transgenic nonhuman animal whose levels of human 5-HT<sub>1E</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1E</sub> receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT<sub>1E</sub> receptor. Such animals may be produced by introducing different amounts of DNA encoding a human 5-HT<sub>1E</sub> receptor into the oocytes from

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which the transgenic animals are developed.

This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor comprising  
5 administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human 5-HT<sub>1E</sub> receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic  
10 nonhuman mammal as a result of overexpression of a human 5-HT<sub>1E</sub> receptor. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of DNA molecules are DNA or cDNA molecules having a  
15 coding sequence substantially the same as the coding sequence shown in Figure 1.

This invention provides a pharmaceutical composition comprising an amount of the substance described supra  
20 effective to alleviate the abnormalities resulting from overexpression of 5-HT<sub>1E</sub> receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the  
25 abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting  
30 from overexpression of a human 5-HT<sub>1E</sub> receptor.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor  
35 comprising administering the substance to the transgenic

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nonhuman mammal described above which expresses only nonfunctional human 5-HT<sub>1E</sub> receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human  
5 5-HT<sub>1E</sub> receptor.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of  
10 5-HT<sub>1E</sub> receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a subject  
15 an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1E</sub> receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes;  
20 25 c. electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>1E</sub> receptor and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human  
30 5-HT<sub>1E</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA  
35 obtained for diagnosis by steps a-e; and g) comparing

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the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human 5-HT<sub>1E</sub> receptor allele.

This invention provides a method of preparing the isolated 5-HT<sub>1E</sub> receptor which comprises inducing cells to express 5-HT<sub>1E</sub> receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered. An example of an isolated 5-HT<sub>1E</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example, serotonin or another substance which is known to bind to the receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains receptor activity or binds anti-receptor antibodies.

This invention provides a method of preparing the isolated 5-HT<sub>1E</sub> receptor which comprises inserting nucleic acid encoding 5-HT<sub>1E</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated 5-HT<sub>1E</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1. This method for

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preparing 5-HT<sub>1E</sub> receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding 5-HT<sub>1E</sub> receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. 5-HT<sub>1E</sub> receptor is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

10 This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a receptor so as to prevent translation of the mRNA molecule.

15

This invention also provides a transgenic nonhuman mammal expressing DNA encoding a receptor.

20 This invention further provides a transgenic nonhuman mammal expressing DNA encoding a receptor so mutated as to be incapable of normal receptor activity, and not expressing native receptor.

25 This invention provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a transgenic nonhuman animal whose levels of receptor expression are varied by use of an inducible promoter which regulates receptor expression.

30

This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of

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the receptor.

This invention further provides transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a receptor so placed as to be transcribed  
5 into antisense mRNA which is complementary to mRNA encoding the receptor and which hybridizes to mRNA encoding the receptor thereby preventing its translation.

10 This invention provides a method for determining whether a ligand not known to be capable of binding to a receptor can bind to a receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the receptor with the ligand under conditions  
15 permitting binding of ligands known to bind to a receptor, detecting the presence of any of the ligand bound to the receptor, and thereby determining whether the ligand binds to the receptor.

20 Applicants have identified individual receptor subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific receptor subtypes provide  
25 effective new therapies with minimal side effects.

This invention identifies for the first time a new receptor protein, its amino acid sequence, and its human  
30 gene. Furthermore, this invention describes a previously unrecognized group of receptors within the definition of a 5-HT<sub>1E</sub> receptor. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic  
35 assays for this new receptor protein, its associated mRNA

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molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule, or its associated  
5 genomic DNA.

Specifically, this invention relates to the first isolation of a human cDNA and genomic clone encoding a 5-HT<sub>1E</sub> receptor. A new human gene for the receptor  
10 identified herein as 5-HT<sub>1E</sub> has been identified and characterized, and a series of related cDNA and genomic clones have been isolated. In addition, the human 5-HT<sub>1E</sub> receptor has been expressed in Ltk<sup>+</sup> cells and Y1 cells by transfecting the cells with the plasmid pcEXV-hp75d. The  
15 pharmacological binding properties of the protein encoded have been determined, and these binding properties classify this protein as a serotonin 5-HT<sub>1E</sub> receptor. Mammalian cell lines expressing this human 5-HT<sub>1E</sub> receptor at the cell surface have been constructed, thus  
20 establishing the first well-defined, cultured cell lines with which to study this 5-HT<sub>1E</sub> receptor.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled  
25 in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

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**MATERIALS and METHODS**

**Cloning and Sequencing:** A human placental genomic library (Stratagene) was screened using oligonucleotides derived from the human 5-HT<sub>1Dβ</sub> receptor gene (U.S. Serial No. 520,716), as a probe. Overlapping oligomers complementary to the 5-HT<sub>1Dβ</sub> sequence in transmembrane domains III, V and VI were labeled with <sup>32</sup>P-dATP and <sup>32</sup>P-dCTP by synthesis with the large fragment of DNA Polymerase (Maniatis et al., 1982). Hybridization was performed at 40°C in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 1X Denhardt's (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), and 200 µg/ml of sonicated salmon sperm DNA. The filters were washed at 40°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate (SDS) and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen. Lambda phage hybridizing to the probe were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Maniatis et al., 1982). For subcloning and further Southern blot analysis DNA was inserted into pUC18 (Pharmacia, Piscataway, N.J.). Nucleotide sequence analysis was done by the Sanger dideoxy nucleotide chain-termination method (Sanger 1977) on denatured double-stranded plasmid templates using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

**Expression:** The entire coding region of clone hp75d was cloned into the eukaryotic expression vector pcEXV-3 (Miller, 1986). Stable cell lines were obtained by cotransfection with the plasmid pcEXV-3 (containing the 5-HT<sub>1E</sub> receptor gene) and the plasmid pGccos3neo

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(containing the aminoglycoside transferase gene) into Ltk<sup>+</sup> cells and Y1 cells using calcium phosphate (reagents obtained from Specialty Media, Lavallette, NJ). The cells were grown in a controlled environment (37° C, 5% CO<sub>2</sub>) as monolayers in Dulbecco's modified Eagle medium (Gibco, Grand Island, N.Y.) or Flo Medium Specialty Media, Inc.,  
5 Lavallette, NJ) containing 25 mM glucose and supplemented with 10% bovine calf serum, 100 U/ml penicillin G and 100 µg/ml streptomycin sulfate. Stable clones were then selected for resistance to the antibiotic G-418 and  
10 harvested membranes were screened for their ability to bind [<sup>3</sup>H]serotonin.

Detection of 5-HT<sub>1c</sub> Receptor mRNA in Brain Using PCR:

Total RNA from human brain tissue was extracted using the  
15 RNAsol protocol as described by the manufacturer. cDNA was prepared from 5 µg of total RNA with random hexanucleotide primers (500 pmoles) using Superscript reverse transcriptase (BRL, MD.) in 50 mM Tris-HCL pH8.3 buffer containing 40u RNasin, 2.5 mM MgCl<sub>2</sub>, 50 mM KCL and  
20 1mM dNTPs, at 42°C for 1 hr. The reaction was stopped by heating at 95°C for 5 min. and chilled on ice. After terminating the reaction, RNase H (2u) was added and incubated at 37°C for 20 min. An aliquot (1 µg) of the first strand cDNA was diluted (1:5) into a 50 µl PCR  
25 reaction mixture (200 µM dNTPs final concentration) containing 1.25 U of Taq polymerase (Cetus Corp., CA) in the buffer supplied by the manufacturer, and 1 µM of 5' primer = 5' TACCACGCGGCCAAGAGCCTTTACCA 3' (Seq. I.D. No. 9) and 3' primer = 5'TGGTGCTAGAGATCTGCTGACGTTC 3' (Seq.  
30 I.D. No. 10), oligonucleotides derived from the third cytoplasmic loop region. The PCR amplification reaction was carried out on a Perkin Elmer Cetus thermal cycler by first a 5 min. incubation at 95°C followed by 30 rounds  
35 of the following cycle: 2 min. at 94°C, 2 min. at 42°C,

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3 min. at 72°C, with a 3" extension, followed at the end by a 15 min. incubation at 72°C. In order to control for the amplification of DNA (carried over during the RNA extraction), control PCR reactions were run in parallel with RNA diluted in the same manner as the cDNA samples.

5 If necessary, RNA samples were pretreated with RNase-free DNase to eliminate any contaminating DNA. Positive controls were included in all experiments, consisting of plasmid containing 5HT1E receptor gene sequences. The products of the PCR amplification were separated by

10 electrophoresis in 1.5% agarose. Amplified fragments were identified by blotting the gel to nitrocellulose and probing with an oligonucleotide internal to the 5'- and 3'-primers

(5'GAGAAGTCAGACACACAGAAAGTCTGTGTAAGTTTTACAACCTGC 3')

15 (Seq. I.D. No. 11) and end-labeled with [ $\alpha$ -<sup>32</sup>P]ATP using T<sub>4</sub> polynucleotide kinase. Hybridization was performed at 40°C in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1X Denhardt's (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), and 200 µg/ml of sonicated salmon sperm DNA. The filters were washed at 50°C in 0.1X SSC

20 containing 0.1% sodium dodecyl sulfate and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen.

25

Membrane Preparation: Membranes were prepared from transfected Ltk<sup>-</sup> cells which were grown to 100% confluency. The cells were washed twice with phosphate-buffered saline, scraped from the culture dishes into 5

30 ml of ice-cold phosphate-buffered saline, and centrifuged at 200 x g for 5 min at 4°C. The pellet was resuspended in 2.5 ml of ice-cold Tris buffer (20 mM Tris -HCl, pH

35 7.4 at 23°C, 5 mM EDTA) and homogenized by a Wheaton

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tissue grinder. The lysate was subsequently centrifuged at 200 x g for 5 min at 4°C to pellet large fragments which were discarded. The supernatant was collected and centrifuged at 40,000 x g for 20 min at 4°C. The pellet resulting from this centrifugation was washed once in  
5 ice-cold Tris buffer and finally resuspended in a final buffer containing 50 mM Tris-HCl and 0.5 mM EDTA, pH 7.4 at 23°C. Membrane preparations were kept on ice and utilized within two hours for the radioligand binding assays. Protein concentrations were determined by the  
10 method of Bradford (1976) using bovine serum albumin as the standard.

Radioligand Binding: [<sup>3</sup>H]5HT binding was performed using slight modifications of the 5-HT<sub>1E</sub> assay conditions  
15 reported by Leonhardt et al. (1989) with the omission of masking ligands. Radioligand binding studies were achieved at 37° C in a total volume of 250 μl of buffer (50 mM Tris, 10 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 10 μM pargyline, 0.1 % ascorbate, pH 7.4 at 37° C) in 96 well microtiter  
20 plates. Saturation studies were conducted using [<sup>3</sup>H]5-HT at 12 different concentrations ranging from 0.5 nM to 100 nM. Displacement studies were performed using 4.5-5.5 nM [<sup>3</sup>H]5-HT. The binding profile of drugs in competition  
25 experiments was accomplished using 10-12 concentrations of compound. Incubation times were 30 min for both saturation and displacement studies based upon initial investigations which determined equilibrium binding conditions. Nonspecific binding was defined in the  
30 presence of 10 μM 5-HT. Binding was initiated by the addition of 50 μl membrane homogenates (10-20 μg). The reaction was terminated by rapid filtration through presoaked (0.5% polyethyleneimine) filters using 48R Cell Brandel Harvester (Gaithersburg, MD). Subsequently,  
35 filters were washed for 5 seconds with ice cold buffer

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(50 mM Tris HCL, pH 7.4 at 4° C), dried and placed into vials containing 2.5 ml of Rradi-Safe (Beckman, Fullerton, CA) and radioactivity was measured using a Beckman LS 5000TA liquid scintillation counter. The efficiency of counting of [<sup>3</sup>H]5HT averaged between 45-50%.

5 Binding data was analyzed by computer-assisted nonlinear regression analysis (Accufit and Accucomp, Lunden Software, Chagrin Falls, OH). IC<sub>50</sub> values were converted to Ki values using the Cheng-Prusoff equation (1973). All experiments were performed in triplicate.

10

#### Measurement of cAMP Formation:

Transfected Y1 cells (expression level = 688 fmol/ mg of protein) were incubated in DMEM, 5mM theophylline, 10mM Hepes (4-[2-Hydroxyethyl]-1-piperazineethanesulfonic acid), 10μM pargyline, for 20 minutes at 37°C, 5% CO<sub>2</sub>. A 5-HT dose-effect curve was then conducted by adding 12 different final concentrations of drug ranging from 10μM to 0.1 nM, followed immediately by the addition of forskolin (10 μM). Subsequently, the cells were incubated for an additional 10 minutes at 37°C, 5% CO<sub>2</sub>. The media was aspirated and the reaction terminated by the addition of 100mM HCl. The plates were stored at 4°C for 15 minutes and centrifuged for 5 minutes (500 x g at 4°C ) to pellet cellular debris. Aliquots of the supernatant fraction were then stored at -20°C prior to assessment of cAMP formation by radioimmunoassay (cAMP Radioimmunoassay kit, Advanced Magnetics, Cambridge, MA).

#### 30 DETECTION OF 5-HT<sub>1E</sub> RECEPTOR mRNA IN BRAIN USING IN SITU HYBRIDIZATION HISTOCHEMISTRY

Labeling of 5-HT<sub>1E</sub> receptor oligo probe with digoxigenin-11-dUTP: The 5-HT<sub>1E</sub> receptor oligo probe (45 mer sequence: GATGGTACACTGGCTGGGGGGTGGGCTGAGTTGACGGTGGCT)

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(Seq. I.D. No. 12) was synthesized in Molecular Biology Department of Neurogenetic Corp. The 3' end tailing reaction was as follows: To a sterile 1.5 ml Eppendorf tube add (1) 8  $\mu$ l of 5-HT<sub>1E</sub> receptor oligo probe (520 ng, denatured at 95°C, then chilled on ice); (2) 8  $\mu$ l of 5X tailing buffer (BRL); (3) 5  $\mu$ l of digoxigenin-11-dUTP (Boehringer); (4) 4  $\mu$ l of dATP at 1/50 dilution; (5) 7  $\mu$ l of terminal transferase (TdT, BRL); (6) 7  $\mu$ l of distilled water. The above reaction mixture was incubated at 37°C for 5 min. The tailed oligo probe was purified by ethanol precipitation, vacuum dried and reconstituted in 40  $\mu$ l of distilled water.

Tissue Preparation: Guinea pig brains were dissected and frozen, first in methylbutane, then on dry ice. Brain sections were cut at 11  $\mu$ m on a cryostat, thaw-mounted on gelatin-coated slides, and stored at -80°C. On the day of the experiment, the brain sections were quickly brought to room temperature using a cool air stream, and fixed in 3% paraformaldehyde made up in 0.1 M PBS containing 0.02% diethylpyrocarbonate (DEPC). After rinsed in 0.1M PBS and 2X SSC, the tissue sections were dehydrated in graded ethanol and air dried.

Prehybridization: All the tissue sections were pre-incubated with hybridization buffer in a humid chamber at 25°C for 1 hr. Twenty ml of the hybridization buffer contained (1). 10 ml of 100% formamide; (2) 4 ml of 20xSSC; (3) 0.4 ml of 50X Denhardt's; (4) 1.0 ml of 10 mg/ml salmon sperm DNA; (5) 0.5 ml of 10 mg/ml yeast tRNA; (6) 4 ml of Dextran sulfate.

Hybridization: The digoxigenin labeled 5-HT<sub>1E</sub> oligo probe (40  $\mu$ l) was diluted in 1 ml of hybridization buffer. Each tissue section was then covered with 0.1 ml of the

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hybridization buffer with probe and incubated at 40°C for 82 hr. After incubation all the sections were washed in 2X SSC (25°C, 1 hr), in 1X SSC (25°C, 1 hr), finally in 0.5X SSC, first 37°C 30 min, then 25°C 30 min.

Immunological Detection: Following the post-hybridization washes slides were rinsed in Buffer #1 (100mM Tris-HCl; 150mM NaCl; pH 7.5) for 1 min, then incubated with 2% normal sheep serum plus 0.3% triton X-100 in Buffer #1 for 30 min. After this preparation the sections were incubated with anti-digoxigenin conjugated with alkaline phosphatase at dilution 1:500 in Buffer #1 containing 1% normal sheep serum and 0.3% triton X-100 at RT for 1.5 hr, then at 4°C overnight. The next day the tissue sections were rinsed in Buffer #1 for 10 min, then in Buffer #2 (100 mM Tris-HCl; 100 mM NaCl; 50 mM MgCl<sub>2</sub>; pH9.5) for 10 min. The chromagen solution was prepared immediately before the colorimetric reaction: i.e. to 10 ml of Buffer #2, (1) 45 µl of 4-nitro blue tetrazolium chloride (NBT, 75 mg/ml in 70% dimethylformamide); (2) 35 µl of 5-bromo-4-chloro-3-indolyl-phosphate (x-phosphate, 50 mg/ml in 100% dimethylformamide); and (3) 2.5 mg of levamisole were added. In order to carry out the colorimetric reaction sections were incubated with the chromogen solution in a humid, light-tight box overnight. The chromogen reaction was halted by rinsing the slides in Buffer #3 (10 mM Tris-HCl; 1 mM EDTA; pH 8.0). The sections were then rinsed in PBS, covered with Aquamount, and examined under a light microscope.

Drugs: [<sup>3</sup>H]5-HT (specific activity = 28 Ci/mmole) was obtained from New England Nuclear, Boston, MA. All other chemicals were obtained from commercial sources and were of the highest grade purity available.

## RESULTS

### Isolation of a Genomic Clone Encoding a 5HT<sub>1E</sub> Receptor

A human genomic placental library was screened with  
5 oligonucleotide probes derived from transmembrane domains  
III, V and VI of the 5-HT<sub>10β</sub> receptor gene. The  
hybridization of these probes with the library was  
performed at low stringency and the result was the  
appearance of several hundred positive signals.  
10 Subsequently, approximately 350 of these clones were  
purified and categorized into various groups, based upon  
which of the three probes were responsible for the  
hybridization signal associated with a given clone. One  
group of clones exhibited hybridization signals with both  
15 transmembrane domain probes III and V. A number of these  
clones were subject to Southern blot analysis and  
determined to be identical or overlapping clones. A  
representative of this group, hp75d, was further  
characterized by nucleic acid sequence analysis and  
20 encoded what appeared to be a new serotonin receptor  
based upon its deduced amino acid sequence.

### Nucleotide Sequence and Deduced Amino Acid Sequence of hp75d

25 DNA sequence information obtained from clone hp75d is  
shown in Figure 1. An open reading frame extending from  
an ATG start codon at position 1 to a stop codon at  
position 1095 can encode a protein 365 amino acids in  
length, having a relative molecular mass (M<sub>r</sub>) of 41,633.  
30 A comparison of this protein sequence with previously  
characterized neurotransmitter receptors indicates that  
hp75d encodes a receptor which is a new member of a  
family of molecules which span the lipid bilayer seven  
35 times and couple to guanine nucleotide regulatory

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proteins (the G protein-coupled receptor family). A variety of structural features which are invariant in this family were present including the aspartic acid residues of transmembrane regions II and III, the DRV sequence at the end of transmembrane region III, and the  
5 conserved proline residues of transmembrane regions IV, V, VI and VII (Hartig et al. and references therein), were present in clone hp75d. A comparison of the transmembrane homology of hp75d to the other cloned serotonin receptors is shown in Figure 2 and exhibits the  
10 following order of identity: 5-HT<sub>10a</sub> (65%), 5-HT<sub>10b</sub> (64%), 5-HT<sub>1a</sub> (52%), 5-HT<sub>1c</sub> (40%) and 5-HT<sub>2</sub> (39%).

#### Receptor Expression in Transfected Mammalian Cells

Saturation analysis of membranes prepared from stably  
15 transfected Ltk<sup>-</sup> cells demonstrated that the receptor expressed was saturable and of high affinity. Scatchard plot analysis by non-linear regression revealed a Kd of  $10.3 \pm 1.2$  nM (mean  $\pm$  S.E.M., n=7) and a Bmax consistent with a high level of expression,  $10.9 \pm 2.6$  picomoles /  
20 mg of protein (mean  $\pm$  S.E.M., n=7). The percent specific binding determined at the measured Kd value for [<sup>3</sup>H]5-HT was greater than 85% of total binding. Furthermore, evidence that the receptor is coupled to a G-protein was  
25 demonstrated by the ability of Gpp(NH)p, a non-hydrolyzable analog of GTP, to inhibit the specific binding of [<sup>3</sup>H]5-HT ( $IC_{50} = 1885 \pm 556$ ,  $n_H = 0.87 \pm 0.04$ ,  $I_{max} = 26.4 \pm 5.6\%$ ).

30 Pharmacological analysis of the receptor was accomplished by testing the ability of drugs from different chemical classes to displace [<sup>3</sup>H]5-HT specific binding (Table 1). Of the compounds investigated, 5-HT and 5-hydroxylated tryptamine derivatives possessed the highest affinity  
35 which according to the classification system of Peroutka

Table 1.  $K_i$  (nM) values of various drugs for the inhibition of [ $^3\text{H}$ ]5-HT specific binding to clonal 5-HT<sub>1E</sub> cell membranes. Binding assays were performed with 4.5-5.5 nM of [ $^3\text{H}$ ]5-HT and 10-12 different concentrations of each inhibitory drug.  $K_i$  values were calculated from the  $\text{IC}_{50}$  values using the Cheng-Prusoff equation. Each value is the mean  $\pm$  S.E.M. of 3-4 independent determinations.

	COMPOUND	$K_i$ (nM)
5	5-HT	10.9 $\pm$ 1.0
	Lysergol	42.8 $\pm$ 5.3
	Ergonovine	87.7 $\pm$ 7.6
10	Methylergonovine	89.4 $\pm$ 4.2
	$\alpha$ -Methyl-5-HT	121 $\pm$ 13
	Methiothepin	194 $\pm$ 4
	1-Naphthylpiperazine	207 $\pm$ 69
	Methysergide	228 $\pm$ 16
15	Oxymetazoline	419 $\pm$ 49
	5-Methoxy-N,N-DMT	528 $\pm$ 32
	Ergotamine	599 $\pm$ 39
	2-Methyl-5-HT	817 $\pm$ 203
20	Yohimbine	1270 $\pm$ 233
	Sumatriptan	2520 $\pm$ 135
	Tryptamine	2559 $\pm$ 827
	DOI	2970 $\pm$ 592
25	5-Methoxytryptamine	3151 $\pm$ 1041
	8-OH-DPAT	3333 $\pm$ 310
	Rauwolscine	3434 $\pm$ 102
	Spiperone	5051 $\pm$ 689
	TFMPP	6293 $\pm$ 259
30	5-CT	7875 $\pm$ 284
	Ketanserin	>10,000
	Pindolol	>10,000
	Zacopride	>10,000
35	LY-165163 (PAPP)	>10,000
	DP-5-CT	>10,000

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and Snyder (1979) makes this site a member of the 5-HT<sub>1</sub> class. Interestingly, 5-CT possessed low affinity and, thus, discriminates this receptor from that of the 5-HT<sub>10</sub> receptor as well as other members of this class. Various ergoline compounds also bound with high affinity including agents which have potent hallucinogenic activity. Excluding methiothepin and 1-naphthylpiperazine (K<sub>i</sub> values = 194 and 207 nM, respectively), piperazine derivatives had low affinity and displayed K<sub>i</sub> values greater than 700 nM. Furthermore, rauwolfia alkaloids and serotonergic agents that possess high affinity for various subtypes of receptor within the serotonin family including ketanserin (5-HT<sub>2</sub>), 8-OH-DPAT (5-HT<sub>1A</sub>), DOI (5-HT<sub>1C</sub>/5-HT<sub>2</sub>), spiperone (5-HT<sub>1A</sub>/5-HT<sub>2</sub>), pindolol (5-HT<sub>1A</sub>/5-HT<sub>1B</sub>) and zacopride (5-HT<sub>3</sub>) had very poor affinity. In all cases, the Hill Coefficients did not differ significantly from unity. Taken together, the pharmacological profile of the 5-HT<sub>1E</sub> receptor is unique and contrasts to that of other known serotonin receptors. Accordingly, the probability of developing selective drugs for this receptor subtype is increased. Additional supporting evidence that the 5-HT<sub>1E</sub> receptor is functionally coupled to a G-protein was obtained by testing the ability of 5-HT to inhibit forskolin-stimulated cAMP production in Y1 cells transfected with the 5-HT<sub>1E</sub> receptor. Figure 4 demonstrates that the endogenous indoleamine, 5-HT, produced a concentration-related decrease in forskolin-stimulated cAMP production with an EC<sub>50</sub> of 23.1 nM. The maximum inhibition of cAMP production was 32%.

The tissue distribution of 5HT<sub>1E</sub> receptor mRNA was detected using PCR technology on cDNA from tissue-derived total RNA. The mRNA localization in human brain tissues demonstrates the presence of 5HT<sub>1E</sub> in: frontal cortex,

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cerebellar cortex, temporal cortex, choroid plexus, hippocampus, brain stem and cortex. We have not identified an area of the brain which does not contain 5HT<sub>1E</sub>. We conclude that the 5HT<sub>1E</sub> mRNA is abundant in human brain. These findings suggest that the 5HT<sub>1E</sub> receptor is not restricted to any one region of the brain and possibly cell type, but is expressed in numerous neuronal cell groups in many distinct regions of the human brain, as has been described for 5HT<sub>1C</sub>.

10 The presence of 5HT<sub>1E</sub> receptor mRNA in various regions of the human brain suggest that 5HT<sub>1E</sub> may modulate a number of the central actions attributed to serotonin. The abundance of 5HT<sub>1E</sub> receptors (mRNA) in the hippocampus may affect mood, behavior and hallucinogenesis. A greater understanding of possible physiological roles of this receptor subtype may be realized by the development of more specific 5HT<sub>1E</sub> receptor drugs as well as physiological manipulations of 5HT<sub>1E</sub> mRNAs/receptors.

20 The cellular localization of 5-HT<sub>1E</sub> receptor mRNA was detected with digoxigenin-11-dUTP labeled oligo probes employing in situ hybridization (ISHH) technology. Digoxigenin-11-dUTP labeled oxytocin (OT) oligo probes were used as a positive control for the experiment since the distribution of OT neurons in the central nervous system is well known. OT cells were intensely stained in the Guinea Pig's hypothalamus. Tissue sections pre-treated with RNase A were used as a negative control.

30 Guinea pig brain sections were examined under a light microscope. 5-HT<sub>1E</sub> receptor mRNA was found in cells located in the frontal cortex, piriform cortex, hippocampus (CA1, CA2 and CA3), lateral septal nucleus, triangular septal nucleus, septofimbrial nucleus and the basal ganglia (caudate-putamen and globus pallidus). It

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was detected in the amygdaloid complex, the bed nucleus stria terminalis and the hypothalamic area including anterior hypothalamus, periventricular nucleus, paraventricular nucleus (magnocellular and parvocellular populations), supraoptic nucleus which seemed to include  
5 both vasopressin and oxytocin cell populations, and the lateral hypothalamus. The 1E receptor mRNA was also detected in the thalamic area including anteroventral thalamic, anterodorsal thalamic, mediodorsal thalamic, ventrolateral thalamic, reticular thalamic paracentral  
10 thalamic, paratenial thalamic nuclei and the nucleus stria medullaris. Control sections pre-treated with RNase A did not exhibit any staining pattern.

#### Experimental Discussion

15 The deduced amino acid sequence of hp75d was analyzed to uncover relationships between it and the other cloned serotonin receptor sequences. Although the homology within the membrane spanning domains was greatest with the 5-HT<sub>10α</sub> receptor (Fig. 2), the nature of this newly  
20 cloned receptor could not be clearly predicted. The rationale for this ambiguity is the interpretation of the transmembrane domain homology (approximately 65%) to the 5-HT<sub>10α</sub> and 5-HT<sub>10β</sub> receptor subfamily. Closely related members of a "subfamily" of serotonin receptors (i.e.  
25 "subtypes") generally share a common transmitter and also have similar pharmacological profiles and physiological roles (for example, 5-HT<sub>2</sub> and 5-HT<sub>1c</sub> or 5-HT<sub>10α</sub> and 5-HT<sub>10β</sub>). Such "subtypes" display an amino acid identity of  
30 approximately 75-80% in their transmembrane domains. Serotonin receptors which are not members of the same "subfamily", but are members of the serotonin "family" (in which the receptors use the same neurotransmitter; i.e. 5-HT<sub>2</sub> and 5-HT<sub>10α</sub>) generally show much lower  
35 transmembrane homology (approximately 45%). Such

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transmembrane amino acid homologies can, therefore, give insight into the relationship between receptors and be used as predictors of receptor pharmacology. According to this type of analysis, although the newly cloned receptor appears to be more related to the 5-HT<sub>1D</sub> subfamily, it is likely to be in a subfamily distinct from all the other serotonin receptors.

The present pharmacological evidence substantiates the existence of a novel serotonin receptor in the human brain as first suggested by Leonhardt et al. (1989). Comparison of the pharmacological profile observed in native cortical membranes to that revealed for the cloned 5-HT<sub>1E</sub> receptor yielded a correlation coefficient of 0.987 (Fig. 3). Indeed, there were some differences in measured affinity constants but the relative values of selected drugs performed in both studies was similar. In this regard, a close examination of the Scatchard plot analysis performed in the study by Leonhardt et al. (1989) reveals that the reported K<sub>d</sub> value may have been underestimated since the radioligand concentrations did not exceed 10 nM. In order to have accurately determined the dissociation constant, the radioligand concentration should have been extended to at least 50 nM or 10 times the estimated K<sub>d</sub> (Yamamura et al., 1985). The initial study, however, was limited in tissue supply and lacked a comprehensive pharmacological characterization. The cloning of the 5-HT<sub>1E</sub> site will now allow more extensive investigations into the nature of this unique receptor.

The structure-activity relationships observed in the present study suggest that there are important requirements for high affinity binding to the 5-HT<sub>1E</sub> receptor. Substitution or removal of the 5-hydroxy group on serotonin decreases the affinity 300 fold for the

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receptor (egs., tryptamine, 5-methoxytryptamine and 5-carboxyamidotryptamine). Additionally, 2-methylation and  $\alpha$ -methylation of 5-HT essentially abolishes its affinity for the 5-HT<sub>1E</sub> site. In contrast to these substitutions, N,N -dimethylation of the aliphatic side chain of the indole ring increases the affinity approximately 20 fold (unpublished observations). Basic structural requirements of the ergoline derivatives demonstrate that N-methylation of the indole ring decreases affinity as does bulky substitutions. Furthermore, piperazine derivatives are not bound at high affinity.

Notably, the application of the human 5-HT<sub>1E</sub> receptor clone to pharmaceutical research can lead to new drug design and development. The localization of this receptor in the cerebral cortex (Leonhardt et al., 1989) and parts of the basal ganglia such as the putamen and globus pallidus (Lowther et al., 1991) suggests a putative link to limbic, cognitive and/or motor function (Nieuwenhuys et al., 1988; Kandel and Schwartz, 1985) and, thus, may be involved in such abnormal conditions as dementia, Parkinson's disease, feeding disorders, anxiety and schizophrenia. Notably, the ergot compounds that possess affinity for this site have been demonstrated to affect these type of behaviors in humans as well as animals (Wilkinson and Dourish, 1991). In relation to this, it appears that the 5-HT<sub>1E</sub> binding site is also present in rat and bovine brain (Leonhardt et al., 1989) as well as guinea-pig, rabbit and dog where the data was initially interpreted as evidence for subtypes of the 5-HT<sub>1D</sub> receptor (Middlemiss, 1990). Nonetheless, it must be taken into consideration that this novel site can possibly lead to selective drug therapy devoid of side effects. In regard to this, serotonin uptake blockers are effective in treating neuropsychiatric disorders such

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as depression and obsessive-compulsive illness (Blier et al., 1987; Asberg et al., 1986; Insel et al., 1985). However, these agents have side effects and, in fact, the mechanism of action for these compounds are not linked to any particular serotonergic receptor. The possibility  
5 that agents selective for the 5-HT<sub>1E</sub> receptor may have clinical utility as antidepressants, for example, without the side effects attributed to current treatment modalities can have significant implications for drug therapy. Furthermore, it should be noted that ergoline  
10 derivatives have had clinical usefulness as drugs capable of relieving migraines and, thus, the involvement of the 5-HT<sub>1E</sub> receptor in this disorder deserves future attention. Ultimately, in depth investigations into the localization of the 5-HT<sub>1E</sub> receptor in brain and  
15 peripheral tissue will target new sites that can lead to specific functional roles for this serotonergic receptor.

Another consideration for therapeutic application of this site may be related to the treatment of feeding disorders  
20 such as obesity, bulimia nervosa and/or anorexia nervosa. The involvement of serotonin and feeding behavior has received much attention during the last decade. It is now known that many of the identified and well-  
25 characterized serotonergic receptors are capable of modulating feeding (Blundell and Lawton, 1990). Notably, serotonin uptake blockers which have been used to treat feeding disorders act nonselectively and as such have  
side-effect potential (Jimerson et al., 1990). Although  
30 many different serotonergic receptors are involved in feeding, the search for the one site that can be exploited for selective drug development has yet to be found. There is no doubt that interest exists in finding  
drugs that interact with the serotonin system for the  
35 treatment of feeding disorders (Cooper, 1989).

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Thus, the pharmacological profile of the cloned human 5-HT<sub>1E</sub> receptor is unique and contrasts to other known serotonergic receptors. The utility of this site expressed in a cellular system and, thus, isolated for study will create excellent opportunities in drug development directed towards a novel serotonergic receptor that may have wide-range implications for drug therapy. Indeed, the potential therapeutic applications may extend to neuropsychiatric disorders including depression, anxiety, schizophrenia, dementia and obsessive-compulsive illness as well as obesity and migraine. The localization of 5-HT<sub>1E</sub> receptor mRNA by in situ hybridization makes it possible to predict its physiological and pathological functions. 5-HT<sub>1E</sub> receptor mRNA is detected in the limbic structures, such as the hippocampus, septal nuclei, piriform cortex (olfactory system), amygdaloid complex and the bed nucleus stria terminalis. The olfactory system sends afferent fibers to the hippocampus through the subiculum, and to the amygdaloid complex (Kupfermann, 1985). In turn the outputs of the hippocampus project to the septal area and hypothalamus while the amygdaloid complex projects to the hypothalamus via the stria terminalis (Kupfermann, 1985). The involvement of the limbic system in emotional behavior (e.g., fear, pleasure, sexual activities) and memory is well known (Kupfermann, 1985). Therefore, the finding of 5-HT<sub>1E</sub> receptor mRNA in these structures indicates a potential role in neuropsychiatric disorders such as depression, obsessive-compulsive illness, anxiety, schizophrenia and dementia.

The hypothalamus regulates the body adjustments to the external and internal environments. It can control hunger (the ventromedial nucleus and the lateral

-57-

hypothalamus), endocrine functions (e.g. the supraoptic nucleus, the paraventricular nucleus and the periventricular nucleus), affective (emotional) behavior (the ventromedial and dorsomedial nuclei) and the activity of the visceral nervous system (the anterior hypothalamus) (Diamond et al., 1985). The discovery of 5-HT<sub>1E</sub> receptor mRNA in these nuclei indicates physiological and pathological roles of this receptor subtype in cardiovascular, gastrointestinal, endocrine, neurological and psychiatric systems.

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The thalamus is a relay station where the sensory and motor-related pathways passing up the brain stem synapse before proceeding on to the cerebral cortex for more elaborate integration and analysis. The 5-HT<sub>1E</sub> receptor mRNA was found in the anterior thalamic nucleus which receives the input from the mammillothalamic tract and sends fibers to the cingulate gyrus. Thus, this nucleus is an important part of the circuit connecting the hypothalamus, the thalamus, and the limbic lobe (Diamond et al., 1985). The 5-HT<sub>1E</sub> receptor mRNA was also found in the mediodorsal thalamic nucleus which is involved in emotional behavior and in the ventral lateral thalamic nucleus which connects the basal ganglia and premotor area. Furthermore, 5-HT<sub>1E</sub> receptor mRNA was found in the nucleus reticularis thalami through which pass most of the thalamocortical and corticothalamic fibers. The axons of corticothalamic and thalamocortical neurons provide collaterals for synapse with reticular cells, exerting a facilitatory effect, while the axons of the GABA-rich reticularis cells project back on the specific thalamic neurons, continuously modulating (by inhibition) the ascending flow of thalamocortical impulses (Diamond et al., 1985). The reticularis neurons play a

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-58-

significant role in the interactions between the thalamus and the frontal cortex. In addition, the 5-HT<sub>1E</sub> receptor mRNA was also visualized in the basal ganglia including the caudate-putamen and globus pallidus both of which control motor activity (Alheid et al., 1990). These  
5 findings indicate important roles of the 5-HT<sub>1E</sub> receptors in emotional behavior, sensation (e.g., pain) and motor activity.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Weinshank, Richard L.  
Branchek, Theresa  
Hartig, Paul R.
- (ii) TITLE OF INVENTION: DNA ENCODING A HUMAN 5-HT1E RECEPTOR AND  
USES THEREOF
- (iii) NUMBER OF SEQUENCES: 12
- 10 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Cooper & Dunham  
(B) STREET: 30 Rockefeller Plaza  
(C) CITY: New York  
(D) STATE: New York  
(E) COUNTRY: U.S.A.  
(F) ZIP: 10112
- 15 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 20 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: White, John P.  
(B) REGISTRATION NUMBER: 28,678  
(C) REFERENCE/DOCKET NUMBER: 1795/39317
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 212-977-9550  
(B) TELEFAX: 212-664-0525  
(C) TELEX: 422523 COOP UI
- 25 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2463 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: both  
(D) TOPOLOGY: linear
- 30 (ii) MOLECULE TYPE: cDNA
- 35

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: human placental genomic
  - (B) CLONE: hp75d
- (viii) POSITION IN GENOME:
  - (C) UNITS: bp
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 736..1830
- (ix) FEATURE:
  - (A) NAME/KEY: mat\_peptide
  - (B) LOCATION: 736..1830
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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   ACCACATGCT CTCACTCACC AGTGGAGTTG AACAAATGAGA ACACATGGCA CAGGGCCGGG      180
   AACATGACAC ACCAGGGCCT GTTGGGGGGT GGAGGGCTAG GGGAGGGATG GCATTAGGAG      240
   AAGTACCTAA TGTAGATGAT TGGTTGTTGG GTGCAGCAAA CCACCATGGC ACATGTATAC      300
20 CTATGTAGCA AACCTGCAAG TTCTGCACAT GTATCCCAGG ACTTAAAGTA TAATTTAAAA      360
   AAAAACAGTT TGAAAACCTC CCTGAAGTAA AAAAAGTATC CTTTGAGGAA CAATGTAACG      420
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   AACTATTTTC ATGTTTTTCCA GAAAAGTGT GGCTTTCTCA TTCATTAACC AATAGCATAA      540
   TATTTTCCAG GAACCTTCAC TCAGAAGAAA TGCTGTGGCC CTTCCCTTTA CCAACAGAAA      600
25 ATGGAACACA AGAGACCACA TAGCTGAACA AATTATAGCC TCCTTACAAG TGAGAAACCT      660
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   TGAAACAAGG GAAAC ATG AAC ATC ACA AAC TGT ACC ACA GAG GCC AGC ATG      771
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30 GCT ATA AGA CCC AAG ACC ATC ACT GAG AAG ATG CTC ATT TGC ATG ACT      819

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	Thr Thr Glu Phe Glu Lys Phe His Ala Ser Ile Arg Ile Pro Pro Phe	
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	AAATATCTTT ATTTGCAGAA TTTCTTATTA CTTATAAATT AAATACCTGA TAATGCCCTC	2240
	CATGGCATT AATCTGAGAT TATGGCTCTA TCTGCCGTACA TATCCAGTG GGAATTGCAT	2300
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30	AAAGTCGGGG GCTGTAGCAC TGTGACTACG TAGCCTATCA TTTCCAGGTAA AAATAGTACA	2420
35		

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2463

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 365 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 20 25 30

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 35 40 45

Thr Thr Lys Lys Leu His Gln Pro Ala Asn Tyr Leu Ile Cys Ser Leu  
 50 55 60

Ala Val Thr Asp Leu Leu Val Ala Val Leu Val Met Pro Leu Ser Ile  
 65 70 75 80

Ile Tyr Ile Val Met Asp Arg Trp Lys Leu Gly Tyr Phe Leu Cys Glu  
 85 90 95

Val Trp Leu Ser Val Asp Met Thr Cys Cys Thr Cys Ser Ile Leu His  
 100 105 110

Leu Cys Val Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asn Ala Ile  
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Glu Tyr Ala Arg Lys Arg Thr Ala Lys Arg Ala Ala Leu Met Ile Leu  
 130 135 140

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 145 150 155 160

Arg Ser His Arg Arg Leu Ser Pro Pro Pro Ser Gln Cys Thr Ile Gln  
 165 170 175

His Asp His Val Ile Tyr Thr Ile Tyr Ser Thr Leu Gly Ala Phe Tyr  
 180 185 190

Ile Pro Leu Thr Leu Ile Leu Ile Leu Tyr Tyr Arg Ile Tyr His Ala  
 195 200 205

Ala Lys Ser Leu Tyr Gln Lys Arg Gly Ser Ser Arg His Leu Ser Asn

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	210		215		220														
	Arg	Ser	Thr	Asp	Ser	Gln	Asn	Ser	Phe	Ala	Ser	Cys	Lys	Leu	Thr	Gln			
	225					230					235					240			
5	Thr	Phe	Cys	Val	Ser	Asp	Phe	Ser	Thr	Ser	Asp	Pro	Thr	Thr	Glu	Phe			
					245					250					255				
	Glu	Lys	Phe	His	Ala	Ser	Ile	Arg	Ile	Pro	Pro	Phe	Asp	Asn	Asp	Leu			
				260					265					270					
	Asp	His	Pro	Gly	Glu	Arg	Gln	Gln	Ile	Ser	Ser	Thr	Arg	Glu	Arg	Lys			
			275					280					285						
10	Ala	Ala	Arg	Ile	Leu	Gly	Leu	Ile	Leu	Gly	Ala	Phe	Ile	Leu	Ser	Trp			
		290					295					300							
	Leu	Pro	Phe	Phe	Ile	Lys	Glu	Leu	Ile	Val	Gly	Leu	Ser	Ile	Tyr	Thr			
	305					310					315					320			
	Val	Ser	Ser	Glu	Val	Ala	Asp	Phe	Leu	Thr	Trp	Leu	Gly	Tyr	Val	Asn			
					325					330					335				
	Ser	Leu	Ile	Asn	Pro	Leu	Leu	Tyr	Thr	Ser	Phe	Asn	Glu	Asp	Phe	Lys			
15				340					345					350					
	Leu	Ala	Phe	Lys	Lys	Leu	Ile	Arg	Cys	Arg	Glu	His	Thr						
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 422 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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30	Ala	Pro	Phe	Glu	Thr	Gly	Gly	Asn	Thr	Thr	Gly	Ile	Ser	Asp	Val	Thr			
				20					25					30					

35

5 Val Ser Tyr Gln Val Ile Thr Ser Leu Leu Leu Gly Thr Leu Ile Phe  
 35 40 45  
 Cys Ala Val Leu Gly Asn Ala Cys Val Val Ala Ala Ile Ala Leu Glu  
 50 55 60  
 Arg Ser Leu Gln Asn Val Ala Asn Tyr Leu Ile Gly Ser Leu Ala Val  
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 Thr Asp Leu Met Val Ser Val Leu Val Leu Pro Met Ala Ala Leu Tyr  
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 Gln Val Leu Asn Lys Trp Thr Leu Gly Gln Val Thr Cys Asp Leu Phe  
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 Thr Pro Glu Asp Arg Ser Asp Pro Asp Ala Cys Thr Ile Ser Lys Asp  
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 195 200 205  
 Leu Leu Met Leu Val Leu Tyr Gly Arg Ile Phe Arg Ala Ala Arg Phe  
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 Arg Ile Arg Lys Thr Val Lys Lys Val Glu Lys Thr Gly Ala Asp Thr  
 225 230 235 240  
 Arg His Gly Ala Ser Pro Ala Pro Gln Pro Lys Lys Ser Val Asn Gly  
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 305 310 315 320  
 35

Phe Glu Arg Lys Asn Glu Arg Asn Ala Glu Ala Lys Arg Lys Met Ala  
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 340 345 350  
 5 Thr Phe Ile Leu Cys Trp Leu Pro Phe Phe Ile Val Ala Leu Val Leu  
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 370 375 380  
 Ile Asn Trp Leu Gly Tyr Ser Asn Ser Leu Leu Asn Pro Val Ile Tyr  
 385 390 395 400  
 10 Ala Tyr Phe Asn Lys Asp Phe Gln Asn Ala Phe Lys Lys Ile Ile Lys  
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 460 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown  
 15

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20 (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Asn Leu Gly Asn Ala Val Arg Ser Leu Leu Met His Leu Ile  
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 25 Gly Leu Leu Val Trp Gln Phe Asp Ile Ser Ile Ser Pro Val Ala Ala  
 20 25 30  
 Ile Val Thr Asp Thr Phe Asn Ser Ser Asp Gly Gly Arg Leu Phe Gln  
 35 40 45  
 Phe Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Val Ile  
 50 55 60  
 30 Ile Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val Ser

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	65				70					75				80		
	Met	Glu	Lys	Lys	Leu	His	Asn	Ala	Thr	Asn	Tyr	Phe	Leu	Met	Ser	Leu
					85					90					95	
5	Ala	Ile	Ala	Asp	Met	Leu	Val	Gly	Leu	Leu	Val	Met	Pro	Leu	Ser	Leu
				100					105					110		
	Leu	Ala	Ile	Leu	Tyr	Asp	Tyr	Val	Trp	Pro	Leu	Pro	Arg	Tyr	Leu	Cys
			115					120					125			
	Pro	Val	Trp	Ile	Ser	Leu	Asp	Val	Leu	Phe	Ser	Thr	Ala	Ser	Ile	Met
			130				135					140				
10	His	Leu	Cys	Ala	Ile	Ser	Leu	Asp	Arg	Tyr	Val	Ala	Ile	Arg	Asn	Pro
	145					150					155				160	
	Ile	Glu	His	Ser	Arg	Phe	Asn	Ser	Arg	Thr	Lys	Ala	Ile	Met	Lys	Ile
					165					170					175	
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				180					185					190		
	Ile	Gly	Leu	Arg	Asp	Glu	Ser	Lys	Val	Phe	Val	Asn	Asn	Thr	Thr	Cys
			195					200					205			
15	Val	Leu	Asn	Asp	Pro	Asn	Phe	Val	Leu	Ile	Gly	Ser	Phe	Val	Ala	Phe
			210				215					220				
	Phe	Ile	Pro	Leu	Thr	Ile	Met	Val	Ile	Thr	Tyr	Phe	Leu	Thr	Ile	Tyr
	225					230					235				240	
	Val	Leu	Arg	Arg	Gln	Thr	Leu	Met	Leu	Leu	Arg	Gly	His	Thr	Glu	Glu
					245					250					255	
20	Glu	Leu	Ala	Asn	Met	Ser	Leu	Asn	Phe	Leu	Asn	Cys	Cys	Cys	Lys	Lys
				260					265					270		
	Asn	Gly	Gly	Glu	Glu	Glu	Asn	Ala	Pro	Asn	Pro	Asn	Pro	Asp	Gln	Lys
			275					280					285			
	Pro	Arg	Arg	Lys	Lys	Lys	Glu	Lys	Arg	Pro	Arg	Gly	Thr	Met	Gln	Ala
							295					300				
25	Ile	Asn	Asn	Glu	Lys	Lys	Ala	Ser	Lys	Val	Leu	Gly	Ile	Val	Phe	Phe
	305					310					315				320	
	Val	Phe	Leu	Ile	Met	Trp	Cys	Pro	Phe	Phe	Ile	Thr	Asn	Ile	Leu	Ser
					325					330					335	
	Val	Leu	Cys	Gly	Lys	Ala	Cys	Asn	Gln	Lys	Leu	Met	Glu	Lys	Leu	Leu
				340					345					350		
30	Asn	Val	Phe	Val	Trp	Ile	Gly	Tyr	Val	Cys	Ser	Gly	Ile	Asn	Pro	Leu

355 360 365

Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Lys Tyr  
 370 375 380

Leu Arg Cys Asp Tyr Lys Pro Asp Lys Lys Pro Pro Val Arg Gln Ile  
 385 390 395 400

5 Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn  
 405 410 415

Ile Tyr Arg His Thr Asn Glu Arg Val Ala Arg Lys Ala Asn Asp Pro  
 420 425 430

Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn  
 435 440 445

10 Pro Ser Asn Val Val Ser Glu Arg Ile Ser Ser Val  
 450 455 460

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 375 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
 

Met Ser Pro Leu Asn Gln Ser Ala Glu Gly Leu Pro Gln Glu Ala Ser  
 1 5 10 15

Asn Arg Ser Leu Asn Ala Thr Glu Thr Ser Glu Ala Trp Asp Pro Arg  
 20 25 30

Thr Leu Gln Ala Leu Lys Ile Ser Leu Pro Val Leu Leu Ser Val Ile  
 35 40 45

Thr Leu Ala Thr Val Leu Ser Asn Ala Phe Val Leu Thr Thr Ile Leu  
 50 55 60

Leu Thr Arg Lys Leu His Thr Pro Ala Asn Tyr Leu Ile Gly Ser Leu  
 65 70 75 80

Ala Thr Thr Asp Leu Leu Val Ser Ile Leu Val Met Pro Ile Ser Met  
 85 90 95  
 Ala Tyr Thr Ile Thr His Thr Trp Asn Phe Gly Gln Ile Leu Cys Asp  
 100 105 110  
 5 Ile Trp Leu Ser Ser Asp Ile Thr Cys Cys Thr Ala Ser Ile Leu His  
 115 120 125  
 Leu Cys Val Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asp Ala Leu  
 130 135 140  
 Glu Tyr Ser Lys Arg Arg Thr Ala Gly His Ala Ala Thr Met Ile Ala  
 145 150 155 160  
 10 Ile Val Trp Ala Ile Ser Ile Cys Ile Ser Ile Pro Pro Leu Phe Trp  
 165 170 175  
 Arg Gln Glu Lys Ala Gln Glu Glu Met Ser Asp Cys Leu Val Asn Thr  
 180 185 190  
 Ser Gln Ile Ser Tyr Thr Ile Tyr Ser Thr Cys Gly Ala Phe Tyr Ile  
 195 200 205  
 15 Pro Ser Val Leu Leu Ile Ile Leu Tyr Gly Arg Ile Tyr Arg Ala Ala  
 210 215 220  
 Arg Asn Arg Ile Leu Asn Pro Pro Ser Leu Ser Gly Lys Arg Phe Thr  
 225 230 235 240  
 Thr Ala His Leu Ile Thr Gly Ser Ala Gly Ser Val Cys Ser Leu Asn  
 245 250 255  
 20 Ser Ser Leu His Glu Gly His Ser His Ser Ala Gly Ser Pro Leu Phe  
 260 265 270  
 Phe Asn His Val Lys Ile Lys Leu Ala Asp Ser Ala Leu Glu Arg Lys  
 275 280 285  
 Arg Ile Ser Ala Ala Arg Glu Arg Lys Ala Thr Lys Ile Leu Gly Ile  
 290 295 300  
 25 Ile Leu Gly Ala Phe Ile Ile Cys Trp Leu Pro Phe Phe Val Val Ser  
 305 310 315 320  
 Leu Val Leu Pro Ile Cys Arg Asp Ser Cys Trp Ile His Pro Gly Leu  
 325 330 335  
 Phe Asp Phe Phe Thr Trp Leu Gly Tyr Leu Asn Ser Leu Ile Asn Pro  
 340 345 350  
 30 Ile Ile Tyr Thr Val Phe Asn Glu Glu Phe Arg Gln Ala Phe Gln Lys  
 355 360 365  
 35

Ile Val Pro Phe Arg Lys Ala  
 370 375

(2) INFORMATION FOR SEQ ID NO:6:

- 5 (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 398 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- 10 (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Glu Pro Gly Ala Gln Cys Ala Pro Pro Ala Pro Ala Gly Ser  
 1 5 10 15  
 Glu Thr Trp Val Pro Gln Ala Asn Leu Ser Ser Ala Pro Ser Gln Asn  
 20 25 30  
 Cys Ser Ala Lys Asp Tyr Ile Tyr Gln Asp Ser Ile Ser Leu Pro Trp  
 35 40 45  
 Lys Val Leu Leu Val Met Leu Leu Ala Leu Ile Thr Leu Ala Thr Thr  
 50 55 60  
 20 Leu Ser Asn Ala Phe Val Ile Ala Thr Val Tyr Arg Thr Arg Lys Leu  
 65 70 75 80  
 His Thr Pro Ala Asn Tyr Leu Ile Ala Ser Leu Asp Val Thr Asp Leu  
 85 90 95  
 Leu Val Ser Ile Leu Val Ile Pro Ile Ser Thr Met Tyr Thr Val Thr  
 100 105 110  
 25 Asp Arg Trp Thr Leu Ser Gln Val Val Cys Asp Phe Trp Leu Ser Ser  
 115 120 125  
 Asp Ile Thr Cys Cys Thr Ala Ser Ile Leu His Leu Cys Val Ile Ala  
 130 135 140  
 Leu Asp Arg Tyr Trp Ala Ile Thr Asp Ala Val Glu Tyr Ser Ala Lys  
 145 150 155 160  
 30 Arg Thr Pro Lys Arg Ala Ala Val Met Ile Ala Leu Val Trp Val Phe

					165					170					175	
	Ser	Ile	Ser	Ile	Ser	Leu	Pro	Pro	Phe	Phe	Trp	Arg	Gln	Ala	Lys	Ala
					180				185					190		
	Glu	Glu	Glu	Val	Ser	Glu	Cys	Val	Val	Asn	Thr	Asp	His	Ile	Leu	Tyr
			195					200					205			
5	Thr	Val	Tyr	Ser	Thr	Val	Gly	Ala	Phe	Tyr	Phe	Pro	Thr	Leu	Leu	Leu
		210					215					220				
	Ile	Ala	Leu	Tyr	Gly	Arg	Ile	Tyr	Val	Glu	Ala	Arg	Ser	Arg	Ile	Leu
	225					230					235					240
	Lys	Gln	Thr	Pro	Asn	Arg	Thr	Gly	Lys	Arg	Leu	Thr	Arg	Ala	Gln	Leu
					245					250					255	
10	Ile	Thr	Asp	Ser	Pro	Gly	Ser	Thr	Ser	Ser	Val	Thr	Ser	Ile	Asn	Ser
				260					265					270		
	Arg	Val	Pro	Asp	Val	Pro	Ser	Glu	Ser	Gly	Ser	Pro	Val	Tyr	Val	Asn
			275					280					285			
	Gln	Val	Lys	Val	Arg	Val	Ser	Asp	Ala	Leu	Leu	Glu	Lys	Lys	Lys	Leu
	290						295					300				
15	Met	Ala	Ala	Arg	Glu	Arg	Lys	Ala	Thr	Lys	Thr	Leu	Gly	Ile	Ile	Leu
	305					310					315					320
	Gly	Ala	Phe	Ile	Val	Cys	Trp	Leu	Pro	Phe	Phe	Ile	Ile	Ser	Leu	Val
				325						330					335	
	Met	Pro	Ile	Cys	Lys	Asp	Ala	Cys	Trp	Phe	His	Leu	Ala	Ile	Phe	Asp
				340					345					350		
20	Phe	Phe	Thr	Trp	Leu	Gly	Tyr	Leu	Asn	Ser	Leu	Ile	Asn	Pro	Ile	Ile
			355					360					365			
	Tyr	Thr	Met	Ser	Asn	Glu	Asp	Phe	Lys	Gln	Ala	Phe	His	Lys	Leu	Ile
	370					375						380				
	Arg	Leu	Ser	Ala	Gln	Val	Asp	Leu	Pro	Phe	Ala	Val	Gly	Pro		
	385					390					395					

25 (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 471 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

30

35

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	Met	Asp	Ile	Leu	Cys	Glu	Glu	Asn	Thr	Ser	Leu	Ser	Ser	Thr	Thr	Asn
	1				5					10					15	
	Ser	Leu	Met	Gln	Leu	Asn	Asp	Asp	Thr	Arg	Leu	Tyr	Ser	Asn	Asp	Phe
				20					25					30		
10	Asn	Ser	Gly	Glu	Ala	Asn	Thr	Ser	Asp	Ala	Phe	Asn	Trp	Thr	Val	Asp
			35					40					45			
	Ser	Glu	Asn	Arg	Thr	Asn	Leu	Ser	Cys	Glu	Gly	Cys	Leu	Ser	Pro	Ser
		50					55					60				
	Cys	Leu	Ser	Leu	Leu	His	Leu	Gln	Glu	Lys	Asn	Trp	Ser	Ala	Leu	Leu
	65					70					75					80
15	Thr	Ala	Val	Val	Ile	Ile	Leu	Thr	Ile	Ala	Gly	Asn	Ile	Leu	Val	Ile
					85					90					95	
	Met	Ala	Val	Ser	Leu	Glu	Lys	Lys	Leu	Gln	Asn	Ala	Thr	Asn	Tyr	Phe
				100					105					110		
	Leu	Met	Ser	Leu	Ala	Ile	Ala	Asp	Met	Leu	Leu	Gly	Phe	Leu	Val	Met
			115					120					125			
20	Pro	Val	Ser	Met	Leu	Thr	Ile	Leu	Tyr	Gly	Tyr	Arg	Trp	Pro	Leu	Pro
		130					135						140			
	Ser	Lys	Leu	Cys	Ala	Val	Trp	Ile	Tyr	Leu	Asp	Val	Leu	Phe	Ser	Thr
	145					150					155					160
	Ala	Ser	Ile	Met	His	Leu	Cys	Ala	Ile	Ser	Leu	Asp	Arg	Tyr	Val	Ala
				165						170					175	
25	Ile	Gln	Asn	Pro	Ile	His	His	Ser	Arg	Phe	Asn	Ser	Arg	Thr	Lys	Ala
				180					185					190		
	Phe	Leu	Lys	Ile	Ile	Ala	Val	Trp	Thr	Ile	Ser	Val	Gly	Ile	Ser	Met
			195					200					205			
	Pro	Ile	Pro	Val	Phe	Gly	Leu	Gln	Asp	Asp	Ser	Lys	Val	Phe	Lys	Glu
		210					215					220				
30	Gly	Ser	Cys	Leu	Leu	Ala	Asp	Asp	Asn	Phe	Val	Leu	Ile	Gly	Ser	Phe
	225					230					235					240

35

Val Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Phe Leu  
 245 250 255

Thr Ile Lys Ser Leu Gln Lys Glu Ala Thr Leu Cys Val Ser Asp Leu  
 260 265 270

5 Gly Thr Arg Ala Lys Leu Ala Ser Phe Ser Phe Leu Pro Gln Ser Ser  
 275 280 285

Leu Ser Ser Glu Lys Leu Phe Gln Arg Ser Ile His Arg Glu Pro Gly  
 290 295 300

Ser Tyr Thr Gly Arg Arg Thr Met Gln Ser Ile Ser Asn Glu Gln Lys  
 305 310 315 320

10 Ala Cys Lys Val Leu Gly Ile Val Phe Phe Leu Phe Val Val Met Trp  
 325 330 335

Cys Pro Phe Phe Ile Thr Asn Ile Met Ala Val Ile Cys Lys Glu Ser  
 340 345 350

Cys Asn Glu Asp Val Ile Gly Ala Leu Leu Asn Val Phe Val Trp Ile  
 355 360 365

15 Gly Tyr Leu Ser Ser Ala Val Asn Pro Leu Val Tyr Thr Leu Phe Asn  
 370 375 380

Lys Thr Tyr Arg Ser Ala Phe Ser Arg Tyr Ile Gln Cys Gln Tyr Lys  
 385 390 395 400

Glu Asn Lys Lys Pro Leu Gln Leu Ile Leu Val Asn Thr Ile Pro Ala  
 405 410 415

20 Leu Ala Tyr Lys Ser Ser Gln Leu Gln Met Gly Gln Lys Lys Asn Ser  
 420 425 430

Lys Gln Asp Ala Lys Thr Thr Asp Asn Asp Cys Ser Met Val Ala Leu  
 435 440 445

Gly Lys Gln His Ser Glu Glu Ala Ser Lys Asp Asn Ser Asp Gly Val  
 450 455 460

25 Asn Glu Lys Val Ser Cys Val  
 465 470

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 5 (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- 10 GATGGTACAC TGGCTGGGGG GTGGGCTGAG TTGACGGTGG CT 42
- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
15 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
20 (A) ORGANISM: oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
- TACCACGCGG CCAAGAGCCT TTACCA 26
- (2) INFORMATION FOR SEQ ID NO:10:
- 25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 30 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: oligonucleotide
- 35

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
TGGTGCTAGA GATCTGCTGA CGTTC 25

(2) INFORMATION FOR SEQ ID NO:11:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:  
GAGAAGTCAG ACACACAGAA AGTCTGTGTA AGTTTACAA CTTGC 45

(2) INFORMATION FOR SEQ ID NO:12:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: OLIGONUCLEOTIDE

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
GATGGTACAC TGGCTGGGGG GTGGGCTGAG TTGACGGTGG CT 42

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What is claimed is:

1. An isolated nucleic acid molecule encoding a human  
5- $HT_{1E}$  receptor.  
5
2. An isolated nucleic acid molecule of claim 1,  
wherein the nucleic acid molecule is a DNA molecule.
3. An isolated DNA molecule of claim 2, wherein the DNA  
10 molecule is a cDNA molecule encoding a human 5- $HT_{1E}$   
receptor.
4. An isolated human 5- $HT_{1E}$  receptor protein.
- 15 5. A vector comprising the DNA molecule of claim 2.
6. A plasmid comprising the vector of claim 5.
7. A vector of claim 5 adapted for expression in a  
20 bacterial cell which comprises the regulatory  
elements necessary for expression of the DNA in the  
bacterial cell so located relative to the DNA  
encoding the 5- $HT_{1E}$  receptor as to permit expression  
thereof.  
25
8. A vector of claim 5 adapted for expression in a  
yeast cell which comprises the regulatory elements  
necessary for expression of the DNA in the yeast  
cell so located relative to the DNA encoding the 5-  
30  $HT_{1E}$  receptor as to permit expression thereof.
9. A vector of claim 5 adapted for expression in a  
mammalian cell which comprises the regulatory  
elements necessary for expression of the DNA in the  
35

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mammalian cell so located relative to the DNA encoding the 5-HT<sub>1E</sub> receptor as to permit expression thereof.

- 5 10. A plasmid of claim 6 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the 5-HT<sub>1E</sub> receptor as to permit expression thereof.
- 10 11. A plasmid comprising the cDNA molecule of claim 3 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the cDNA molecule as to permit expression thereof, designated pcEXV-hp75d (ATCC Accession No. 75138).
- 15 12. A mammalian cell comprising the plasmid of claim 6.
- 20 13. An Ltk<sup>-</sup> cell comprising the plasmid of claim 6.
14. A Y1 cell comprising the plasmid of claim 6.
- 25 15. An Ltk<sup>-</sup> cell comprising the plasmid of claim 11, designated 5-HT<sub>1E</sub>-7 (ATCC Accession No. CRL 10913).
16. A Y1 cell comprising the plasmid of claim 11 designated Y-5-HT<sub>1E</sub> (ATCC Accession No. CRL 10914).
- 30 17. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule
- 35

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encoding a human 5-HT<sub>1E</sub> receptor.

18. The nucleic acid probe of claim 17 wherein the nucleic acid is DNA .
- 5 19. An antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human 5-HT<sub>1E</sub> receptor so as to prevent translation of the mRNA molecule.
- 10 20. An antisense oligonucleotide having a sequence capable of binding specifically to the cDNA molecule of claim 3.
- 15 21. An antisense oligonucleotide of claim 19 comprising chemical analogues of nucleotides.
22. An antibody directed to a human 5-HT<sub>1E</sub> receptor.
- 20 23. A monoclonal antibody directed to an epitope of a human 5-HT<sub>1E</sub> receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>1E</sub> receptor.
- 25 24. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 19 effective to reduce expression of a human 5-HT<sub>1E</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding a human 5-HT<sub>1E</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.
- 30 25. A pharmaceutical composition of claim 24, wherein
- 35

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the oligonucleotide is coupled to a substance which inactivates mRNA.

- 5
26. A pharmaceutical composition of claim 25, wherein the substance which inactivates mRNA is a ribozyme.
- 10
27. A pharmaceutical composition of claim 24, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.
- 15
28. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor and a pharmaceutically acceptable carrier.
- 20
29. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of 5-HT<sub>1E</sub> receptor and a pharmaceutically acceptable carrier.
- 25
30. A pharmaceutical composition which comprises an amount of the antibody of claim 22 effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor and a pharmaceutically acceptable carrier.
- 30
31. A transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor.
- 35
32. A transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>1E</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing

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native 5-HT<sub>1E</sub> receptor.

33. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human 5-HT<sub>1E</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a 5-HT<sub>1E</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>1E</sub> receptor thereby reducing its translation.
34. The transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the DNA encoding a human 5-HT<sub>1E</sub> receptor additionally comprises an inducible promoter.
35. The transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the DNA encoding a human 5-HT<sub>1E</sub> receptor additionally comprises tissue specific regulatory elements.
36. A transgenic nonhuman mammal of any of claims 31, 32 or 33, wherein the transgenic nonhuman mammal is a mouse.
37. A method for determining whether a ligand not known to be capable of binding to a human 5-HT<sub>1E</sub> receptor can bind to a human 5-HT<sub>1E</sub> receptor which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1E</sub> receptor with the ligand under conditions permitting binding of ligands known to bind to a 5-HT<sub>1E</sub> receptor, detecting the presence of any of the ligand bound to a human 5-HT<sub>1E</sub> receptor, and thereby determining whether the ligand binds to a human 5-HT<sub>1E</sub> receptor.

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38. A method for determining whether a ligand not known to be capable of binding to the human 5-HT<sub>1E</sub> receptor can functionally activate receptor activity or prevent the action of a ligand which does so comprising contacting a mammalian cell of claim 12 with the ligand under conditions permitting the activation or blockade of a functional response, and detecting by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the ligand activates or prevents the activation of the human 5-HT<sub>1E</sub> receptor functional output.
39. The method of claim 37 or 38 wherein the mammalian cell is nonneuronal in origin.
40. A method of claim 39, wherein the mammalian cell nonneuronal in origin is an Ltk<sup>c</sup> cell.
41. A method of claim 39, wherein the mammalian cell nonneuronal in origin is a Y1 cell.
42. A ligand detected by the method of claim 37 or 38.
43. A method of screening drugs to identify drugs which specifically interact with, and bind to, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human 5-HT<sub>1E</sub> receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human 5-HT<sub>1E</sub> receptor.
44. A method of screening drugs to identify drugs which

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- 5 interact with, and activate or block the activation of, the human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting the mammalian cell of claim 12 with a plurality of drugs, determining those drugs which activate or block the activation of the receptor in the mammalian cell using a bioassay such as a second messenger assays, and thereby identifying drugs which specifically interact with, and activate or block the activation of, a human 5-HT<sub>1E</sub> receptor.
- 10 45. The method of claim 43 or 44 wherein the mammalian cell is nonneuronal in origin.
- 15 46. The method of claim 45 wherein the mammalian cell nonneuronal in origin is an Ltk<sup>-</sup> cell.
47. The method of claim 45 wherein the mammalian cell nonneuronal in origin is a Y1 cell.
- 20 48. A pharmaceutical composition comprising a drug identified by the method of claim 43 or 44 and a pharmaceutically acceptable carrier.
- 25 49. A method of detecting expression of the 5-HT<sub>1E</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>1E</sub> receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 17 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the 5-HT<sub>1E</sub> receptor by the cell.
- 30 50. A method of treating abnormalities in a subject,
- 35

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wherein the abnormality is alleviated by the reduced expression of a 5-HT<sub>1E</sub> receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 24 effective to reduce expression of the 5-HT<sub>1E</sub> receptor by the subject.

5

51. A method of treating an abnormal condition related to an excess of 5-HT<sub>1E</sub> receptor activity which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 24 effective to reduce expression of the 5-HT<sub>1E</sub> receptor in the subject.
52. The method of claim 51 wherein the abnormal condition is dementia.
53. The method of claim 51 wherein the abnormal condition Parkinson's disease.
54. The method of claim 51 wherein the abnormal condition is a feeding disorder.
55. The method of claim 51 wherein the abnormal condition is pathological anxiety.
56. The method of claim 51 wherein the abnormal condition is schizophrenia.
57. The method of claim 51 wherein the abnormal condition is a migraine headache.
58. A method of treating abnormalities which are alleviated by reduction of expression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a

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subject an amount of the pharmaceutical composition of claim 30 effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor and thereby alleviate abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor.

5

59. A method of treating an abnormal condition related to an excess of 5-HT<sub>1E</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 30 effective to block binding of naturally occurring ligands to the 5-HT<sub>1E</sub> receptor and thereby alleviate the abnormal condition.

10

60. The method of claim 59 wherein the abnormal condition is dementia.

15

61. The method of claim 59 wherein the abnormal condition is Parkinson's disease.

20

62. The method of claim 59 wherein the abnormal condition is a feeding disorder.

63. The method of claim 59 wherein the abnormal condition is a pathological anxiety.

25

64. The method of claim 59 wherein the abnormal condition is schizophrenia.

30

65. The method of claim 59 wherein the abnormal condition is a migraine headache.

66. A method of detecting the presence of a human 5-HT<sub>1E</sub> receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 22

35

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under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a human 5-HT<sub>1E</sub> receptor on the surface of the cell.

5

67. A method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a transgenic nonhuman mammal whose levels of human 5-HT<sub>1E</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>1E</sub> receptor expression.

10

68. A method of determining the physiological effects of expressing varying levels of human 5-HT<sub>1E</sub> receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of human 5-HT<sub>1E</sub> receptor.

15

69. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor comprising administering a substance to the transgenic nonhuman mammal of claim 31 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human 5-HT<sub>1E</sub> receptor.

20

70. A method for treating the abnormalities resulting from overexpression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition of claim 28 effective to alleviate the abnormalities resulting from

30

35

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overexpression of a human 5-HT<sub>1E</sub> receptor.

71. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor comprising  
5 administering the substance to the transgenic nonhuman mammal of either of claims 32 or 33 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of  
10 underexpression of a human 5-HT<sub>1E</sub> receptor.
72. A method for treating the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor which comprises administering to a subject an amount of  
15 the pharmaceutical composition of claim 29 effective to alleviate the abnormalities resulting from underexpression of a human 5-HT<sub>1E</sub> receptor.
73. A method for diagnosing a predisposition to a disorder associated with the expression of a specific human 5-HT<sub>1E</sub> receptor allele which comprises:  
20
- a. obtaining DNA of subjects suffering from the disorder;  
25
  - b. performing a restriction digest of the DNA with a panel of restriction enzymes;
  - c. electrophoretically separating the resulting DNA fragments on a sizing gel;  
30
  - d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing  
35

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to DNA encoding a human 5-HT<sub>1E</sub> receptor and labelled with a detectable marker;

- 5 e. detecting labelled bands which have hybridized to the DNA encoding a human 5-HT<sub>1E</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- 10 f. preparing DNA obtained for diagnosis by steps a-e; and
- 15 g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.
- 20 74. The method of claim 73 wherein a disorder associated with the expression of a specific human 5-HT<sub>1E</sub> receptor allele is diagnosed.
- 25 75. A method of preparing the isolated 5-HT<sub>1E</sub> receptor of claim 4 which comprises:
- 30 a. inducing cells to express 5-HT<sub>1E</sub> receptor;
- b. recovering the receptor from the resulting cells; and
- c. purifying the receptor so recovered.
- 35 76. A method of preparing the isolated 5-HT<sub>1E</sub> receptor of

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claim 4 which comprises:

- a. inserting nucleic acid encoding 5-HT<sub>1E</sub> receptor in a suitable vector;
  - 5 b. inserting the resulting vector in a suitable host cell;
  - c. recovering the receptor produced by the resulting cell; and
  - 10 d. purifying the receptor so recovered.
- 15
- 20
- 25
- 30
- 35



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TGAAACAAGGGAAACATGAACATCACAACTGTACCACAGAGGCCAGCATGGCTATAAGA

M N I T N C T T E A S M A I R

50

70

90

CCCAAGACCATCACTGAGAAGATGCTCATTGTCATGACTCTGGTGGTCATCACCACCCTC

P K T I T E K M L I C M T L V V I T T L

110

130

150

ACCACGTTGCTGAACTTGGCTGTGATCATGGCTATTGGCACCACCAAGAAGCTCCACCAG

T T L L N L A V I M A I G T T K K L H Q

170

190

210

CCTGCCAACTACCTAATCTGTTCTCTGGCCGTGACGGACCTCCTGGTGGCAGTGCTCGTC

P A N Y L I C S L A V T D L L V A V L V

230

250

270

ATGCCCTGAGCATCATCTACATTGTGATGGATCGCTGGAAGCTTGGGTACTTCCTCTGT

M P L S I I Y I V M D R W K L G Y F L C

290

310

330

GAGGTGTGGCTGAGTGTGGACATGACCTGCTGCACCTGCTCCATCCTCCACCTCTGTGTC

E V W L S V D M T C C T C S I L H L C V

350

370

390

ATTGCCCTGGACAGGTACTGGGCCATACCAATGCTATTGAATACGCCAGGAAGAGGACG

I A L D R Y W A I T N A I E Y A R K R T

410

430

450

GCCAAGAGGGCCGCGCTGATGATCCTTACCGTCTGGACCATCTCCATTTTCATCTCCATG

A K R A A L M I L T V W T I S I F I S M

470

490

510

CCCCCTCTGTTCTGGAGAAGCCACCGCCGCTAAGCCCTCCCCCTAGTCAGTGCACCATC

P P L F W R S H R R L S P P P S Q C T I

530

550

570

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CAGCACGACCATGTTATCTACACCATTTACTCCACGCTGGGTGCGTTTTATATCCCCTTG

Q H D H V I Y T I Y S T L G A F Y I P L

590

610

630

ACTTTGATACTGATTCTCTATTACCGGATTTACCACGGCCAAGAGCCTTTACCAGAAA

T L I L I L Y Y R I Y H A A K S L Y Q K

650

670

690

AGGGGATCAAGTCGGCACTTAAGCAACAGAAGCACAGATAGCCAGAATTCTTTTGCAAGT

R G S S R H L S N R S T D S Q N S F A S

710

730

750

TGTAAACTTACACAGACTTTCTGTGTGTCTGACTTCTCCACCTCAGACCCTACCACAGAG

C K L T Q T F C V S D F S T S D P T T E

770

790

810

TTTGAAAAGTTCCATGCCTCCATCAGGATCCCCCCTTCGACAATGATCTAGATCACCCA

F E K F H A S I R I P P F D N D L D H P

830

850

870

GGAGAACGTCAGCAGATCTCTAGCACCAGGGAACGGAAGGCAGCACGCATCCTGGGGCTG

G E R Q Q I S S T R E R K A A R I L G L

890

910

930

ATTCTGGGTGCATTCATTTTATCCTGGCTGCCATTTTTCATCAAAGAGTTGATTGTGGGT

I L G A F I L S W L P F F I K E L I V G

950

970

990

CTGAGCATCTACACCGTGTCCCTCGGAAGTGGCCGACTTTCTGACGTGGCTCGGTTATGTG

L S I Y T V S S E V A D F L T W L G Y V

1010

1030

1050

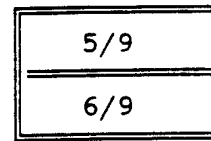
AATTCTCTGATCAACCCTCTGCTCTATACGAGTTTTAATGAAGACTTTAAGCTGGCTTTT

N S L I N P L L Y T S F N E D F K L A F



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FIGURE 2

Figure 2



	1					50
5HT <sub>1c</sub>	M.....	.....	...VNLGNAV	RSLLMHLIGL	LVWQFDISIS	
5HT <sub>2</sub>	MDILCEENTS	LSSTTNSLMQ	LNDDTRLYSN	DFNSGEANTS	DAFNWTVDSE	
5HT <sub>10a</sub>	M.....	.....	.....	.....SPLN	QSAEGLPQEA	
5HT <sub>10b</sub>	M.....	.....	.....EE	PGAQCAPPAP	AGSETWVPQA	
5HT <sub>1E</sub>	M.....	.....	.....	.....	.....	
5HT <sub>1A</sub>	M.....	.....	.....	.....	.....DVLSPGQGN	
TM Region	.....	.....	.....	.....	.....	
	51					100
5HT <sub>1c</sub>	PVAAIVTDTF	NSSDGGRLFQ	FPDGVQNWPA	LSIVVIIIMT	IGGNILVIMA	
5HT <sub>2</sub>	NRTNLSCEGC	LSPSCLSLH	LQE..KNWSA	LLTAVVIILT	IAGNILVIMA	
5HT <sub>10a</sub>	...SNRSLNA	TETSEAWDPR	TLQALKISLP	VLLSVITLAT	VLSNAFVLTT	
5HT <sub>10b</sub>	NLSSAPSQNC	SAKDYYQDS	ISLPWKVLLV	MLLALITLAT	TLSNAFVIAT	
5HT <sub>1E</sub>	.....NITNC	TTEASMAIRP	KTITEKMLIC	MTLVVITTLT	TLLNLAVIMA	
5HT <sub>1A</sub>	NTTSPAPFE	TGGNTTGISD	VTVSYQVITS	LLLGTLIFCA	VLGNACVVA	
TM Region	.....	.....	.....*****	*****..	I..*****	
	101					150
5HT <sub>1c</sub>	VSMEKKLHNA	TNYFLMSLAI	ADMLVGLLVM	PLSLLAILYD	YVWPLPRYLC	
5HT <sub>2</sub>	VSLEKKLQNA	TNYFLMSLAI	ADMLLGFLVM	PVSMLTILYG	YRWPLPSKLC	
5HT <sub>10a</sub>	ILLTRKLHTP	ANYLIGSLAT	TDLLVSILVM	PISMAYTITH	.TWNFGQILC	
5HT <sub>10b</sub>	VYRTRKLHTP	ANYLIASLDV	TDLLVSILVI	PISTMYTVD	.RWTL SQVVC	
5HT <sub>1E</sub>	IGTTKKLHQP	ANYLICSLAV	TDLLVAVLVM	PLSIIYIVMD	.RWKLG YFLC	
5HT <sub>1A</sub>	IALERSLQNV	ANYLIGSLAV	TDLMVSVLVL	PMAALYQVLN	.KWTLGQVTC	
TM Region	*.....	.....*****	***..II..*	*****..	.....	
	151					200
5HT <sub>1c</sub>	PVWISLDVLF	STASIMHLCA	ISLDRYVAIR	NPIEHSRFNS	RTKAIMKIAI	
5HT <sub>2</sub>	AVWIYLDVLF	STASIMHLCA	ISLDRYVAIQ	NPIHHSRFNS	RTKAFKIIA	
5HT <sub>10a</sub>	DIWLSSDITC	CTASILHLCV	IALDRYWAIT	DALEYSKRRT	AGHAATMIAI	
5HT <sub>10b</sub>	DFWLSSDITC	CTASILHLCV	IALDRYWAIT	DAVEYSAKRT	PKRAAVMIAL	
5HT <sub>1E</sub>	EVWLSVDMTC	CTCSILHLCV	IALDRYWAIT	NAIEYARKRT	AKRAALMILT	
5HT <sub>1A</sub>	DLFIALDVLC	CTSSILHLCV	IALDRYWAIT	DPIDYVNKRT	PRRAALISL	
TM Region	.*****.	.III..****	***.....	.....	.....****	
	201					250
5HT <sub>1c</sub>	VWAISIGVSV	PIPVIGLRDE	SKVFNNTTC	VLNDPNFVLI	GSFVAFFIPL	
5HT <sub>2</sub>	VWTISVGISM	PIPVFGLQDD	SKVF.KEGSC	LLADDNFVLI	GSFVSFFIPL	
5HT <sub>10a</sub>	VWAISICISI	PPLFWR.QEK	AQEEMSDCLV	NTSQISYTIY	STCGAFYIPS	
5HT <sub>10b</sub>	VWVFSISISL	PPFFWR.QAK	AEEEVSECVV	NTDHILYTVY	STVGAFYFPT	
5HT <sub>1E</sub>	VWTISIFISM	PPLFWRSHRR	LSPPPSQCTI	QHDHVIYTIY	STLGAFYIPL	
5HT <sub>1A</sub>	TWLIGFLISI	PPMLGWRTPE	DRSDPDACTI	SKDH.GYTIY	STFGAFYIPL	
TM Region	****..IV..	*****..	.....	.....****	*****..V..	
	251					300
5HT <sub>1c</sub>	TIMVITY...	.....	.....	.....	.....	
5HT <sub>2</sub>	TIMVITY...	.....	.....	.....	.....	
5HT <sub>10a</sub>	VLLIILYGRI	YRAAR.....	.....	.....	.....	
5HT <sub>10b</sub>	LLLIALYGRI	YVEAR.....	.....	.....	.....	
5HT <sub>1E</sub>	TLILILYYRI	YHAAKSLYQK	R.....	.....	.....	
5HT <sub>1A</sub>	LLMLVLYGRI	FRAARFRIK	TVKKVEKTGA	DTRHGASPAP	QPKKSVNGES	
TM Region	*****..	.....	.....	.....	.....	

	301				350
5HT <sub>1C</sub>	.....FLTIY	VLRROTMLLL	RG.HTEEELA	NMSLNFLNCC	CKKNGGEEEN
5HT <sub>2</sub>	.....FLTIK	SLQKEATLCV	SDLGTRAKLA	..SFSFLPQS	SLSSEKLFQR
5HT <sub>10a</sub>	...NRIL.NP	PSLSGKRFTT	AHLITGSAG.	..SVCSL...	..NSSLHEGH
5HT <sub>10b</sub>	...SRILKQT	PNRTGKRLTR	AQLITDSPGS	TSSVTSI...	..NSRVPDVP
5HT <sub>1E</sub>	.GSSRHLSNR	STDSQNSFAS	CKLTQTFCVS	DFSTSDP...	..TTEFEKFKH
5HT <sub>1A</sub>	GSRNWRLGVE	SKAGGALCAN	GAVRQGDDGA	ALEVIEVHRV	GNSKEHLPLP
TM Region	.....	.....	.....	.....	.....

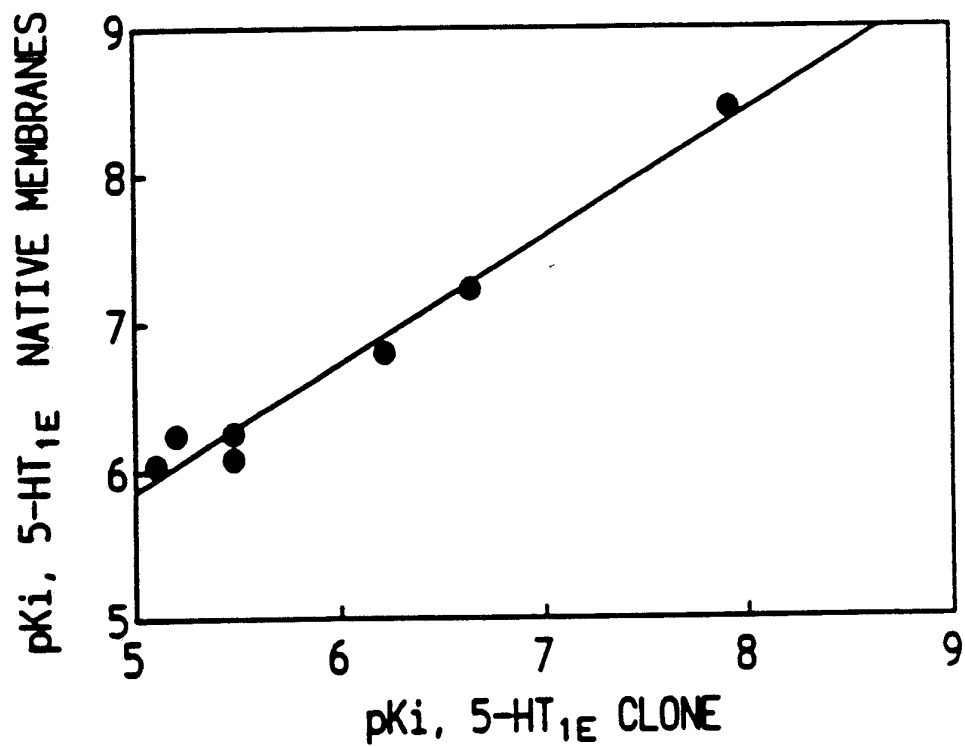
	351				400
5HT <sub>1C</sub>	APNPNPDQKP	RR...KKKEK	RPRGTMQAIN	NEKKASKVLG	IVFFVFLIMW
5HT <sub>2</sub>	SIHREPGSYT	GR...R....	....TMQIS	NEQKACKVLG	IVFFLVVMW
5HT <sub>10a</sub>	SHSAGSPLFF	NHVKIKLADS	ALERKRISAA	RERKATKILG	IILGAFIICW
5HT <sub>10b</sub>	SES.GSPVYV	NQVKVRVSDA	LLEKKLMAA	RERKATKTLG	IILGAFIVCW
5HT <sub>1E</sub>	A.SIRIPPF	NDL.....DH	PERQOISST	RERKAARILG	LILGAFILSW
5HT <sub>1A</sub>	SEAGPTPCAP	ASFERKNERN	AEAKRKMAA	RERKTVKTLG	IIMGTFILCW
TM Region	.....	.....	.....	.....**	*****..V

	401				450
5HT <sub>1C</sub>	CPFFITNILS	VLCGKACNQK	LMEKLLNVFV	WIGYVCSGIN	PLVYTLFNKI
5HT <sub>2</sub>	CPFFITNIMA	VICKESCNE	VIGALLNVFV	WIGYLSSAVN	PLVYTLFNKT
5HT <sub>10a</sub>	LPFFVVSIVL	PICRDSCWIH	P..GLDFDFT	WLGYNLSLIN	PIIYTVFNEE
5HT <sub>10b</sub>	LPFFIISLVM	PICKDACWFH	L..AIFDFT	WLGYNLSLIN	PIIYTMSNED
5HT <sub>1E</sub>	LPFFIKELIV	GLSIYTVSSE	V..A..DFLT	WLGYNLSLIN	PLLYTSFNED
5HT <sub>1A</sub>	LPFFIVALVL	PFCESSCHMP	T..LLGAIIN	WLGYSNSLLN	PVIYAYFNKD
TM Region	I..*****	*.....	..*****	**..VII..*	*****...

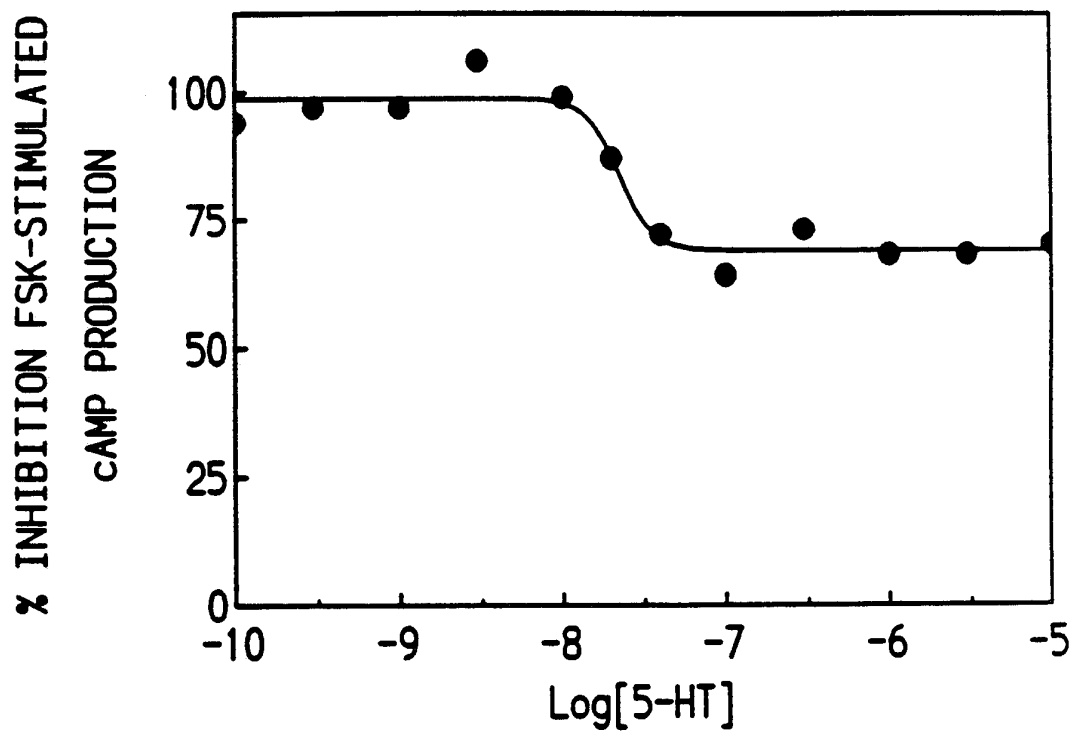
	451				500
5HT <sub>1C</sub>	YRRAFASKYLR	CDYKPKKP.	PVRQIPRVAA	TALSGRELNV	NIYRHTNERV
5HT <sub>2</sub>	YRSAFSRYIQ	CQYKENKKPL	QLILVNTIPA	LAYKSSQLQM	GQKKNKQDA
5HT <sub>10a</sub>	FRQAFQKIVP	FRKA.....	.....	.....	.....
5HT <sub>10b</sub>	FKQAFHKLIR	LSAQVDLPFA	VGP.....	.....	.....
5HT <sub>1E</sub>	FKLAFKKLIR	CREHT.....	.....	.....	.....
5HT <sub>1A</sub>	FQNAFKKIIK	CLFCRQ*...	.....	.....	.....
TM Region	.....	.....	.....	.....	.....

	501			536
5HT <sub>1C</sub>	ARKANDPEPG	IEMQVENLEL	PVNPSNVVSE	RISSV*
5HT <sub>2</sub>	KTTDNDCSMV	ALGKQHSEEA	SKDNSDGVNE	KVSCV*
5HT <sub>10a</sub>	.....	.....	.....	.....
5HT <sub>10b</sub>	.....	.....	.....	.....
5HT <sub>1E</sub>	.....	.....	.....	.....
5HT <sub>1A</sub>	.....	.....	.....	.....
TM Region	.....	.....	.....	.....

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FIGURE 3



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FIGURE 4



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FIGURE 5

SUMMARY OF HUMAN BRAIN TISSUE DISTRIBUTION FOR 5-HT<sub>1E</sub> mRNA

Brain Tissues	5-HT <sub>1E</sub>		
	Experiment 1	Experiment 2	Experiment 3
Frontal Cortex	+	+	+
Cerebellar Cortex	++	+	++
Temporal Cortex	+	++	+++
Choroid Plexus	+++	++	+++
Hippocampus	+++	++	+++
Brain Stem	+	++	+++
Cortex	++	++	+++
(+) Control	+++	+++	+++
(-) Control	-	-	-

Figure Key:

+++ = dark

++ = light

+ = present but faint

- = absent

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/10377

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C07H 21/00; C12N 5/00, 15/00 A61K 49/00  
US CL : 536/27; 435/41, 172.1, 240.2; 424/9; 800/2  
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)  
U.S. : 536/27; 435/41, 172.1, 240.2; 424/9; 800/2

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

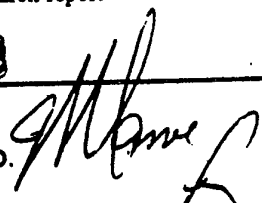
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS, DIALOG  
search terms: serotonin receptors, recombinant

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Neurochemistry, Volume 53(2), issued 1989, S. Leonhardt et al., "Detection of a novel serotonin receptor subtype (5-HT1E) in human brain: interaction with a GTP binding protein", pages 465-471, entire document.	1-3,5-16,31-36,67-69, 71,75,76
Y	Nature, Volume 329, issued 29 October 1987; Y. Masu et al., "cDNA cloning of bovine substance-K receptor through oocyte expression system", pages 836-838, entire document.	1-3,5-16,31-36,67-69,71,75,76
Y	US, A, 4,675,285 (Clark et al.) 23 June 1987, entire document.	1-3,5-16,31-36,67-69,71,75,76
Y	Science, Volume 240, issued 10 June 1988, R. Jaenisch, "Transgenic Animals", pages 1468-1474, entire document.	31-36,67-69,71
Y	Bio/Technology, Volume 6, issued February 1988, J.P. Simons et al., "Gene transfer into sheep", pages 179-183, entire document.	31-36,67-69,71
Y	Nature, volume 315, issued 20 June 1985, R.E. Hammer et al., "Production of transgenic rabbits, sheep and pigs by microinjection", pages 680-683, entire document.	31-36,67-69,71

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

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be part of particular relevance	"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
"E" earlier document published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 16 February 1993	Date of mailing of the international search report 26 FEB 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer LORRAINE M. SPECTOR, PH.D.  Telephone No. (703) 308-0196

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

- I. Claims 1-3, 5-16, 31-36, 67-69, 71, 75, 76 drawn to nucleic acid species, a method of producing a receptor protein, and transgenic animals comprising the nucleic acids, Class 536, subclass 27, Class 435, subclasses 41, 172.1, and 240.2, and Class 800, subclass 2.
- II. Claim 4, drawn to an isolated receptor protein, Class 530, subclass 350.
- III. Claims 17-21, and 24-29, drawn to oligonucleotides and pharmaceutical compositions, Class 536, subclass 27, Class 514, subclass 44, and various other classifications, dependent upon species.
- IV. Claims 22, 23, 30, drawn to antibodies to human 5HT1E receptor and composition thereof, Class 530, subclass 387 and Class 424, subclass 85.8.
- V. Claims 37-41 and 43-47, drawn to methods for identification and characterization of ligands and methods of screening drugs for activity, Class 436, subclass 501.
- VI. Claims 42 and 48, drawn to ligands to human 5HT-1E receptor and a pharmaceutical composition, Classification dependent upon species.
- VII. Claim 49, drawn to a method of detecting gene expression, Class 435, subclass 6.
- VIII. Claims 50-57, drawn to methods of treatment using oligonucleotides, Class 514, subclass 44.
- IX. Claims 58-66, drawn to methods of treatment using antibodies and a method of detecting 5-HT1E receptors, Class 424, subclass 85.8 and Class 436, subclass 501.
- X. Claims 70 and 72, drawn to a method of treating 5-HT1E receptor expression abnormalities, Classification dependent upon species.
- XI. Claims 73 and 74, drawn to diagnostic methods, Classification dependent upon species.

The products of Groups I-IV and VI and processes of Groups V and VII-XI are separate and distinct inventions in that each is defined by a different nucleotide sequence and different expression properties and functions involving different method steps and results as well as different products made and used, all of which constitute the special technical features which define the contribution of each invention. Since these special technical features are not shared by each product and/or process and since the common features do not establish an advance over the prior art, the invention of Groups I-XI do not share a technical relationship and do not form a single inventive concept within the meaning of Rule 13.2.