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(72) Inventeurs/Inventors:
HEINEMANN, STEPHEN F., US;
BOULTER, JAMES R., US;
HOLLMANN, MICHAEL, US;
BETTLER, BERNHARD, US;
JENSEN, JAN E., US

(73) Propriétaire/Owner:
THE SALK INSTITUTE FOR BIOLOGICAL STUDIES,
US

(54) Titre : COMPOSITIONS ET METHODES POUR LE RECEPTEUR DU GLUTAMATE

(54) Title: GLUTAMATE RECEPTOR COMPOSITIONS AND METHODS

(57) Abrégé/Abstract:

The present invention discloses novel DNAs that encode proteins having electrophysiological and pharmacological properties characteristic of glutamate receptors. The glutamate receptors are exemplified by proteins encoded by representative cDNA clones GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7, fragments thereof, and functional combinations of these glutamate receptor proteins and/or fragments. DNA sequences from the cDNA clones for GluR1, GluR2, GluR3, GluR4 and GluR5 are especially useful as probes, thus enabling those skilled in the art to identify, without undue experimentation, other members of the L-glutamate receptor family.

(74) **Agent:** MARKS & CLERK

ABSTRACT

The present invention discloses novel DNAs that encode proteins having electrophysiological and pharmacological properties characteristic of glutamate receptors. The glutamate receptors are exemplified by proteins encoded by representative cDNA clones GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7, fragments thereof, and functional combinations of these glutamate receptor proteins and/or fragments. DNA sequences from the cDNA clones for GluR1, GluR2, GluR3, GluR4 and GluR5 are especially useful as probes, thus enabling those skilled in the art to identify, without undue experimentation, other members of the L-glutamate receptor family.

GLUTAMATE RECEPTOR COMPOSITIONS AND METHODS

Field of the Invention

5 The present invention relates to a family of
novel DNA sequences and encoded receptors proteins that
comprise the glutamate neurotransmitter system. The
invention also relates to methods for making the
glutamate receptors and for using the receptor proteins
10 in assays designed to identify and characterize
glutamate agonists and antagonists.

Background of the Invention

 The amino acid L-glutamate is a major
15 excitatory neurotransmitter in the mammalian central
nervous system. Anatomical, biochemical and
electrophysiological analyses suggest that glutamatergic
systems are involved in a broad array of neuronal
processes, including fast excitatory synaptic
20 transmission, regulation of neurotransmitter release,
long-term potentiation, learning and memory,
developmental synaptic plasticity, hypoxic-ischemic
damage and neuronal cell death, epileptiform seizures,
as well as the pathogenesis of several neurodegenerative
25 disorders. See generally, Monaghan et al., *Ann. Rev.*
Pharmacol. Toxicol. 29, 365-402 (1989). This extensive
repertoire of functions, especially those related to
learning, neurotoxicity and neuropathology, has
stimulated recent attempts to describe and define the
30 mechanisms through which glutamate exerts its effects.

 Currently, glutamate receptor classification
schemes are based on pharmacological criteria which
serve to define five receptor subtypes or classes:
those activated by N-methyl-D-aspartic acid (NMDA),
35 kainic acid (KA), α -amino-3-hydroxy-5-methyl-
isoxazole-4-propionic acid (AMPA, formerly called the
quisqualic acid or QUIS receptor), 2-amino-4-

phosphonobutyric acid (AP4 or APB), and 1-amino-cyclopentyl-1,3-dicarboxylic acid (ACPD). The effects of glutamate are mediated primarily through interactions with cation-selective, ionotropic receptors (Foster and Fagg, *Brain Res. Rev.* 7, 103-164 (1984); Strange, *Biochem. J.* 249, 309-318 (1988)). An exception is the ACPD receptor subtype which has the properties of a metabotropic receptor. This class of glutamate receptors alters synaptic physiology via GTP-binding proteins and the second messengers diacylglycerol and inositol 1,4,5-triphosphate (Gundersen, et al., *Proc. R. Soc. London Ser. B* 221, 127 (1984); Sladeczek, et al., *Nature* 317, 717 (1985); Nicoletti, et al., *J. Neurosci.* 6, 1905 (1986); Sugiyama, et al., *Nature* 325, 531 (1987)).

The electrophysiological and pharmacological properties of the glutamate receptors have been studied extensively and are now well established. See for example, Foster and Fagg, *Brain Res. Rev.* 7, 103 (1984); Cotman et al., *Trends Neurosci.* 10, 263 (1987); Mayer and Westbrook, *Prog. Neurobiol.* 28, 197 (1987); Watkins and Olvermann, *Trends Neurosci.* 10, 265 (1987); and Blair et al., *Science* 242, 577 (1988). This is in contrast to their biochemical characteristics and structure at the molecular level, which, until the teaching of the present invention, remained largely unknown.

Definitions

In the present specification and claims, reference will be made to phrases and terms of art which are expressly defined for use herein as follows:

As used herein, glutamate receptors refer to neurotransmitter receptor proteins that are activated by L-glutamate and related compounds. These receptor proteins are classified on the basis of their "pharmacology". Currently there are five classes of receptors: those activated by N-methyl-D-aspartate

(NMDA), kainic acid (KA), α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA, formerly called the quisqualic acid or QUIS receptor), 2-amino-4-phosphonobutyric acid (AP4 or APB), and 1-amino-cyclopentyl-1,3-dicarboxylic acid (ACPD).

As used herein, NMDA means N-methyl-D-aspartate, which is a ligand (agonist) for the NMAD glutamate receptor subtype.

As used herein, AMPA means α -amino-3-hydroxy-5-methyl-isoxazole-4-propionate, which is a ligand (agonist) for the AMPA glutamate receptor subtype. The AMPA receptor was formerly called the QUIS (quisqualate) receptor.

As used herein, QUIS means quisqualic acid or quisqualate, which is a ligand (agonist) for the pharmacologically defined receptor subtype previously referred to as the QUIS (quisqualate) receptor. The receptor previously referred to as the QUIS receptor is now referred to as the AMPA receptor.

As used herein, KA means kainic acid or kainate, which is a ligand (agonist) for the kainate glutamate receptor subtype.

As used herein, APB means 2-amino-4-phosphonobutyrate, which is a ligand (agonist) for the APB glutamate receptor subtype. The acronym AP4 is also sometimes used to refer to the 2-amino-4-phosphonobutyrate glutamate receptor subtype.

As used herein, ACPD means 1-amino-cyclopentyl-1,3-dicarboxylic acid, which is a ligand (agonist) for the ACPD glutamate receptor subtype.

As used herein, when used as a modifier of glutamate receptor protein(s) of the present invention, the phrase "having electrophysiological and pharmacological properties characteristic of a glutamate receptor" means that the neuronal signal(s) generated by receptor protein in response to glutamate or glutamate-like ligands will be comparable to those of known

glutamate receptors. See also, the definition for "functional", which is given below.

As used herein, homomeric means receptors comprised of only one type of subunit.

5 As used herein, heteromeric means receptors comprised of more than one type of subunit.

As used herein, functional, when used as a modifier of glutamate receptor protein(s) of the present invention, means that binding of glutamate (or
10 glutamate-like) ligand to receptor protein(s) causes membrane "ion channels" to open. This allows ions to move across the membrane, which in turn depolarizes the cell and generates a neuronal signal. Stated another way, functional means that a neuronal signal is
15 generated as a consequence of ligand binding to receptor protein(s).

As used herein, GABA means γ -aminobutyric acid.

20 As used herein, γ -DGG means γ -D-glutamyl-glycine.

As used herein, GAMS means γ -D-glutamylamino-methyl-sulphonate.

As used herein, PDA means 2,3-cis-piperidine dicarboxylic acid.

25 As used herein, GDEE means glutamate diethyl-ester.

As used herein, TMDs mean transmembrane domains.

30 As used herein, APV means 2-amino-5-phospho-novaleric acid.

As used herein, CPP means 3-(2-carboxypiperazin-4-yl)propyl-1-phosphate.

As used herein, nAChRs mean neuronal nicotinic acetylcholine receptors.

35 As used herein, CNS means central nervous system.

As used herein, PNS means peripheral nervous system.

As used herein, an agonist binding subunit is a receptor subunit that contains a binding site for a pharmacological compound which, upon binding, activates the receptor.

As used herein, a non-agonist binding subunit is a receptor subunit that does not bind agonists.

As used herein, the term antagonist refers to a substance that interferes with receptor function. Antagonists are of two types: competitive and non-competitive. A competitive antagonist (also known as a competitive blocker) competes with an agonist for overlapping binding sites. A non-competitive antagonist or blocker inactivates the functioning of the receptor by binding to a site on the receptor other than the agonist binding site.

As used herein, GluR1 refers to the cDNA clone which encodes a single glutamate receptor subunit protein of the same name having a M_r = ca. 99,769 daltons. GluR1 was the first glutamate receptor subunit encoding cDNA to be isolated; it was previously referred to as GluR-K1. GluR-K1 has been renamed glutamate receptor subunit gene 1 or, more simply, GluR1. Additional glutamate receptor subunits or subunit related genes are called GluR2, GluR3, GluR4, GluR5, GluR6, GluR7 and so forth. GluR1 cDNA has been deposited with the American Type Culture Collection; it has been accorded ATCC No. 68134.

As used herein, GluR2 refers to a cDNA clone which encodes a single glutamate receptor subunit protein of the same name having a M_r = ca. 96,400 daltons. GluR2 cDNA has been deposited with the American Type Culture Collection; it has been accorded ATCC No. 68132.

As used herein, GluR3 refers to a cDNA clone which encodes a single glutamate receptor subunit

protein of the same name having a M_r = ca. 98,000 daltons. GluR3 cDNA has been deposited with the American Type Culture Collection; it has been accorded ATCC No. 68133.

5 As used herein, GluR4 refers to a cDNA clone which encodes a single protein of the same name having a M_r = ca. 98,500. GluR4 cDNA has been deposited with the American Type Culture Collection; it has been accorded ATCC No. 68375.

10 As used herein, GluR5 refers to a GluR5 cDNA clone which encodes a single protein of the same name having a M_r = ca. 100,000. GluR5 cDNA (as GluR5-1) has been deposited with the American Type Culture Collection; it has been accorded ATCC No. 68374. (There
15 are two length variants of GluR5 cDNA, referred to herein as GluR5-1 and GluR5-2. Translation of the GluR5 cDNAs predicts a single long open reading frame of 920 amino acids. The difference between GluR5-1 and GluR5-2 DNA derives from an insertion of 45 nucleotides (15
20 amino acids) in the GluR5-1 DNA which does not interrupt this reading frame. The 15 amino acid insertion in the GluR5-1 receptor protein is unique among the receptor proteins disclosed herein; thus the shorter GluR5-2 variant is the counterpart of the GluR1, GluR2, GluR3,
25 GluR4, GluR6 and GluR7 subunits.)

 As used herein, GluR6 refers to a cDNA clone which encodes a single protein of the same name having a M_r = ca. 100,000.

30 As used herein, GluR7 refers to a cDNA clone which encodes a single protein of the same name. GluR7 encoding fragments 35-T3 and U35 are shown in Figure 12.

 As used herein, GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7 are each used interchangeably to refer to genes, cDNA clones and the glutamate receptor
35 proteins they encode.

 Use of the phrase "substantial sequence homology" in the present specification and claims means

that the DNA, RNA or amino acid sequences which have slight and non-consequential sequence variations from the actual sequences disclosed and claimed herein are considered to be equivalent to the sequences of the present invention, and as such are within the scope of the appended claims. In this regard, "slight and non-consequential sequence variations" mean that "homologous" sequences (i.e., the sequences that have substantial sequence homology with the DNA, RNA, or proteins disclosed and claimed herein) will be functionally equivalent to the sequences disclosed and claimed in the present invention. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein.

Use of the phrase "substantially pure" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been separated from their *in vivo* cellular environment by means of human effort; as a result of this separation and purification, the substantially pure DNAs, RNAs, polypeptides and proteins are useful in ways that the non-separated, impure DNAs, RNAs, polypeptides or proteins are not.

The amino acids which comprise the various amino acid sequences appearing herein may be identified according to the following three-letter or one-letter abbreviations:

	Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
5	L-Alanine	Ala	A
	L-Arginine	Arg	R
	L-Asparagine	Asn	N
	L-Aspartic Acid	Asp	D
	L-Cysteine	Cys	C
10	L-Glutamine	Gln	Q
	L-Glutamic Acid	Glu	E
	L-Glycine	Gly	G
	L-Histidine	His	H
	L-Isoleucine	Ile	I
15	L-Leucine	Leu	L
	L-Lysine	Lys	K
	L-Methionine	Met	M
	L-Phenylalanine	Phe	F
	L-Proline	Pro	P
20	L-Serine	Ser	S
	L-Threonine	Thr	T
	L-Tryptophan	Trp	W
	L-Tyrosine	Tyr	Y
	L-Valine	Val	V

25

The nucleotides which comprise the various nucleotide sequences appearing herein have their usual single-letter designations (A, G, T, C or U) used routinely in the art.

30

As used herein, the words "protein", "peptide" and polypeptide" are considered to be equivalent terms and are used interchangeably.

As used herein, bp means base pairs. Kbp means kilo base pairs, or base pairs times 1000.

35

As used herein, all temperatures are given in degrees Centigrade unless indicated otherwise.

Deposits

cDNA clones encoding representative glutamate receptor protein subunits of the present invention have been deposited with the American Type Culture
5 Collection, Rockville, Maryland, U.S.A. (ATCC) to provide those skilled in the art with the best mode of making and using the probes necessary to identify additional members of the L-glutamate family of genes. The deposits have been made under the terms of the
10 Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the cloned DNA sequences are and will be available to industrial property offices and
15 other persons legally entitled to receive them under the terms of the Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or inter-
national organizations in which this application, or an
20 application claiming priority of this application, is filed or in which any patent granted on any such application is granted.

The ATCC Deposit Numbers and Deposit Dates for the deposited representative cDNA clones are as follows:

25	GluR1 Clone	ATCC No. 68134, October 19, 1989
	GluR2 Clone	ATCC No. 68132, October 19, 1989
	GluR3 Clone	ATCC No. 68133, October 19, 1989
	GluR4 Clone	ATCC No. 68375, August 2, 1990
	GluR5 Clone	ATCC No. 68374, August 2, 1990

30

Summary of the Invention

The present invention discloses a family of novel glutamate receptor proteins and DNA sequences that
35 encode them. The glutamate receptors of the invention have electrophysiological and pharmacological properties characteristic of glutamate receptors of the central

nervous system. The glutamate receptors of the present invention are exemplified by cation-selective ion channel-type proteins encoded by cDNA clones GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7. In addition to being useful for the production of glutamate receptor proteins, these cDNAs are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate additional proteins in the glutamate receptor family.

10 The novel functional glutamate receptors of the present invention can be assembled from either individual GluR subunits (homomeric) or from combinations of subunit polypeptides (heteromeric). GluR1, GluR2, GluR3, GluR4 and GluR5 are examples of presently preferred subunits for forming homomeric receptors, while the combinations of GluR1, GluR2, GluR3, GluR4 and GluR5 are examples of presently preferred subunits for forming heteromeric receptors.

20 In addition to disclosing novel DNAs and proteins the present invention also comprises methods for making glutamate receptors and using them to identify and characterize agonists and antagonists of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as glutamate receptors.

DRAWINGSBrief Description of the Drawings

The following is a brief description of the drawings.

The drawings comprise 10 Figures, of which:

Figure 1 is a drawing that shows the nucleotide and deduced amino acid sequence of the clone GluR1.

10 Figure 2 (a and b) comprises two sequence homology analyses. Figure 2a compares the extracellular region located between amino acid residues 89 and 106 of GluR1 with the "Cys-Cys-loop" region found in all other ligand-gated ion channels, showing sequence homology. Figure 2b compares the putative TMD II region of GluR1 with hypothetical TMD II regions of other ligand-gated ion channels, suggesting protein sequence conservation.

Figure 3 is a drawing that shows the nucleotide and deduced amino acid sequence of the clone GluR2.

Figure 4 is a drawing that shows the nucleotide and deduced amino acid sequence of the clone GluR3.

Figure 5 is a drawing which shows the alignment of deduced amino acid sequences for the GluR1, GluR2, GluR3, GluR4 and GluR5 (GluR5-1) subunits of the glutamate receptor gene family.

Figure 6 (A and B) is comprised of two graphs which compare current responses measured in *Xenopus* oocytes injected with individual GluR1, GluR2 and GluR3 subunit RNAs (Figure 6A) or rat brain hippocampus poly(A)⁺ RNA (Figure 6B).

10 Figure 7 (A and B) is comprised of two graphs which compare current responses measured in *Xenopus* oocytes injected with combinations of GluR1, GluR2 and GluR3 RNAs.

Figure 8 is comprised of three graphs which illustrate the dependence of the current responses to 100mM KA upon the membrane potential.

Figure 9 is a drawing that shows the nucleotide and deduced amino acid sequence of the clone GluR4.

20 Figure 10 is a drawing that shows the nucleotide and deduced amino acid sequence of the cDNA clone encoding glutamate receptor subunit GluR5-1.

Figure 11 is a drawing that shows the nucleotide and deduced amino acid sequence of the clone GluR6.

Figure 12 (A and B) is a drawing that shows the nucleotide and deduced amino acid sequence of fragments 35-T3 and U35 from the clone GluR7.

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Detailed Description of the Drawings

Figure 1. The nucleotide and deduced amino acid sequence of the clone pGluR1. Nucleotides are numbered in 5'- to 3'-terminal direction, starting with the first nucleotide of the codon for the putative amino-terminal residue of the mature protein. Cleavage of the assumed signal peptide is predicted (von Heijne, *Nucl. Acids Res.* 14, 4683 (1986)) to occur at position

1. Nucleotides located upstream of the predicted 5'-terminal base have been assigned negative numbers. Nucleotides -1 to -54 encode a putative signal peptide (von Heijne, *supra*), and bases -55 through -251 represent a 5'-untranslated region. At the 3'-terminus of the clone 71 nucleotides of untranslated sequence were found. The deduced amino acid sequence for GluR1 is shown above the nucleotide sequence, the numbering starting with the first residue of the mature protein.

10 Possible extracellular N-glycosylation sites are marked with an asterisk above the amino acid residue. The region marked by two arrows (amino acids 89-103) bears some resemblance to the ligand-gated ion channel signature postulated by some workers (Grenningloh et al., *Nature* 330, 25 (1987); Barnard et al., *TINS* 10, 502 (1987)).

Figure 1 Methods. The insert cDNA of the bacteriophage clone λ ZAPII-GluR-K1 was cut out with *Eco*RI/*Xho*I, blunt-ended and subcloned into the *Sma*I-site

20 of the bacteriophage vector M13mp19 (Messing et al., *Proc. Natl. Acad. Sci. USA*, 74, 3642 (1977)), yielding clones with both orientations of the cDNA. Overlapping deleted subclones were constructed for each strand orientation using the Cyclone™ kit from USB.

Single-strand sequencing by the dideoxynucleotide chain-termination method (Sanger et al., *Proc. Natl. Acad. Sci. USA*, 74, 5463 (1977)), was carried out with all 45 subclones, and additionally, 10 oligonucleotide primers were synthesized to facilitate sequencing across

30 areas where compressions or gaps were encountered in the sequences derived from the deletion subclones. Complete sequences for both strands were thus obtained. IntelliGenetics software packages (IntelliGenetics version 5.0, and PC/Gene™) were used for analyzing sequences.

Figure 2a. Comparison of the extracellular region located between amino acid residues 89 and 106 of GluR1 with the "Cys-Cys-loop" region found in all other

ligand-gated ion channels, showing sequence homology. Sequences of nAChR subunits $\alpha 1$, $\beta 1$, γ , δ are from mouse muscle (Heinemann et al., in: *Molecular Neurobiology: Recombinant DNA Approaches* (ed. Heinemann, S. & Patrick, J.) 45-96 (Plenum Press, New York, 1987)), those of nAChR subunits $\alpha 2$, $\alpha 3$, $\alpha 4$, $\beta 2$, $\beta 3$, and $\beta 4$ are from rat brain (Deneris et al., *J. Biol. Chem.* 264, 6268 (1989); Duvoisin et al., *Neuron* 3, 487 (1989)). GABA_A subunits α and β are from calf brain (Barnard et al., *Trends Neurosci.* 10, 502 (1987)). GlyR 48k is the $M_r = 48$ kDa subunit of the glycine receptor from rat brain (Grenningloh et al., *Nature* 328, 215-220 (1987)). Boxed amino acid residues are found at identical positions in GluR1 as well as in at least one of the other receptor sequences. One gap has been introduced arbitrarily.

Figure 2b. Comparison of the putative TMD II region of GluR1 with hypothetical TMD II regions of other ligand-gated ion channels (Barnard et al., *Trends Neurosci.* 10, 502 (1987); Deneris et al., *J. Biol. Chem.* 264, 6268 (1989); Duvoisin et al., *Neuron* 3, 487 (1989); Grenningloh et al., *Nature* 328, 215 (1987); Heinemann et al., in: *Molecular Neurobiology: Recombinant DNA Approaches* (ed. Heinemann, S. & Patrick, J.) 45-96 (Plenum Press, New York, 1987))1987), suggesting protein sequence conservation.

Figure 3. Nucleotide and deduced amino acid sequence of cDNA clone 1RB14 encoding the rat glutamate receptor subunit gene GluR2. The nucleotides are numbered in the 5' to 3' direction, starting with the first nucleotide in the codon corresponding to the putative amino-terminal residue in the mature protein. Nucleotides extending 5' of base 1 are designated with negative numbers and include the region encoding the putative signal peptide and 5'-untranslated region. There are five potential N-linked glycosylation sites located at Asn-235, Asn-349, Asn-385, Asn-392, and

Asn-835. The calculated molecular mass of the mature, non-glycosylated form of the GluR2 protein is 96,400 daltons. The asterisk indicates a translation termination codon.

Figure 4. Nucleotide and deduced amino acid sequences of cDNA clone lRB312 encoding the rat glutamate receptor subunit gene GluR3. The nucleotides are numbered in the 5' to 3' direction, starting with the first nucleotide in the codon corresponding to the putative amino-terminal residue in the mature protein. Nucleotides extending 5' of base 1 are designated with negative numbers and include the region encoding the putative signal peptide and 5'-untranslated region. There are five potential N-linked glycosylation sites located at Asn-35, Asn-238, Asn-352, Asn-387, and Asn-394. The calculated molecular mass of the mature, non-glycosylated form of the GluR3 protein is 98,000 daltons. The asterisk indicates a translation termination codon.

Figure 5. Alignment of deduced amino acid sequences for the rat glutamate receptor subunits. Identical residues in all compared sequences are boxed, spaces were introduced to maximize homology. Predicted signal peptides and four proposed membrane spanning regions (MSR I-IV) are indicated. Two arrowheads (▼) delimit a region in GluR1 proposed (Hollman, et al., Nature 342, 643 (1989)) to correspond to the 'Cys-Cys' loop found in other ligand-gated ion channel subunits (Barnard, et al., Trends Neurosci. 10, 502 (1987)). The hatched line denotes the insertion of 15 amino acid residues found in the GluR5-1, but not in the GluR5-2 protein.

Figure 6. Comparison of current responses measured in *Xenopus* oocytes injected with individual GluR1, GluR2 and GluR3 subunit RNAs or rat brain hippocampus poly(A)⁺ RNA. (A) Responses of oocytes to 100mM KA measured 3 days after injection of individual

GluR1 (2ng), GluR2 (10ng) or GluR3 (2ng) RNA. The insert shows examples of voltage recording traces obtained from such oocytes except that the GluR2 response was obtained 5 days after injection of 25ng RNA. (B) Responses of oocytes to the indicated agonists measured 3 days after injection with GluR1 (2ng) or GluR3 (2ng) RNA or adult rat brain hippocampus poly(A)⁺ RNA (ca. 50ng). All values are normalized to the response obtained with 100mM KA and are presented as the mean $\pm$ S.E.M. with $n \geq 3$ for all measurements. All oocytes were voltage-clamped to -70mV and recordings were performed as described by Hollmann, et al., *Nature* 342, 643 (1989).

Figure 7. Comparison of current responses measured in *Xenopus* oocytes injected with combinations of GluR1, GluR2 and GluR3 RNAs. (A) Responses of oocytes to 100mM KA measured 3 days after injection of 2ng RNA for each of the indicated GluR subunits. The open columns represent the sum of the responses measured in oocytes expressing the individual GluR subunit RNAs, while the stippled columns show the measured amplitudes after co-expression of the GluR subunit RNAs in individual oocytes. (B) Responses of oocytes to the indicated agonists measured 3 days after injection of 2ng RNA for each of the indicated GluR subunits or 50ng rat brain hippocampus poly(A)⁺ RNA. Values have been normalized to the response obtained with 100mM KA and are presented as the mean $\pm$ S.E.M. with $n \geq 3$ for all measurements. All oocytes were clamped to -70mV and recordings performed as described by Hollmann, et al., *Nature* 341, 643 (1989).

Figure 8. The dependence of the current responses to 100mM KA upon the membrane potential. (A) Data obtained from oocytes injected with rat brain hippocampus poly(A)⁺ RNA (50 ng, Solid Square ■), GluR1 (Open Square □) or GluR3 RNA (Open circle, O); (B) GluR1 plus GluR2 RNAs (Solid Square ■) or GluR1 plus

GluR3 RNAs (Open Square □); and (C) GluR2 plus GluR3 RNAs (Solid Square ■) or all three GluR subunit RNAs (Open Square □). Recordings were made from oocytes 3 days after injection of 2ng RNA for each GluR subunit. Voltages were stepped by 10mV between -150mV and +50mV and all values are normalized to the response measured at -70mV.

Figure 10. Nucleotide and amino acid sequence of a single cDNA clone (λRB20) encoding the rat glutamate receptor subunit GluR5-1. The deduced amino acid sequence is shown above the nucleotide sequence. Cleavage of the assumed signal peptide is predicted at amino acid position 1 (von Heijne, *Nucl. Acids Res.* 14, 4683 (1986)). This cleavage site is after a proline residue, which is atypical. Amino acids encoding the signal peptide were assigned negative numbers. Nucleotides are numbered in the 5' to 3' direction, beginning with the first residue of the codon for the putative amino-terminal residue of the mature protein.

20 The nucleotides on the 5' side of amino acid residue 1 are indicated by negative numbers. Stop codons flanking the open reading frame (ORF) are denoted with 'END', the polyadenylation signals are underlined. Sites of potential N-linked glycosylation are indicated by asterisks (*). Two arrowheads (▼) delimit the insertion of 45 nucleotides between nucleotides 1113 and 1159 found in λRB20, but not in 12 of the 29 cDNA clones isolated.

Description of the Invention

The present invention discloses a family of glutamate receptors and novel DNA sequences that encode these receptors.

More specifically, in one aspect, the present invention comprises substantially pure functional proteins, or functional fragments thereof, or functional combinations of these proteins and/or fragments wherein the proteins or fragments or functional combinations have electrophysiological and pharmacological properties characteristic of a glutamate receptor.

10

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In another aspect, the invention comprises substantially pure functional proteins, or functional fragments thereof, or functional combinations of these proteins and/or fragments, wherein the proteins or
5 functional fragments or combinations have electrophysiological and pharmacological properties characteristic of a glutamate receptor subtype selected from the group consisting of N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA),
10 kainate (KA) and 2-amino-4-phosphonobutyrate (APB).

In another aspect, the invention comprises substantially pure functional proteins, or functional fragments thereof, or functional combinations of these proteins and/or fragments, wherein the proteins or
15 functional fragments have electrophysiological and pharmacological properties characteristic of KA and/or AMPA glutamate receptor subtypes.

In still another aspect, the invention comprises substantially pure proteins, or fragments
20 thereof, or functional combinations of the proteins and/or the fragments, wherein the proteins or fragments or functional combinations have electrophysiological and pharmacological properties of a glutamate receptor and are encoded by DNA having at least about 40% homology
25 with at least one member of the group consisting of GluR1 DNA, GluR2 DNA, GluR3 DNA, GluR4 DNA, GluR5 DNA, GluR6 DNA and GluR7 DNA.

In still another aspect, the invention comprises substantially pure proteins, or fragments
30 thereof, or functional combinations of the proteins and/or the fragments, wherein the proteins or fragments or functional combinations have electrophysiological and pharmacological properties of a glutamate receptor and have at least about 40% amino acid homology with at
35 least one member of the group consisting of GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11) and GluR7 (Fig. 12).

In another aspect, the invention comprises substantially pure protein selected from the group of proteins consisting of GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7, and combinations thereof wherein
5 said combinations are functional as glutamate receptor(s).

In another aspect, the invention comprises a substantially pure proteins having M_r s of about 99,769 (GluR1), 94,400 (GluR2), 98,000 (GluR3), 98,500 (GluR4),
10 100,000 (GluR5) and 100,000 (GluR6) that form ion channels which possess the electrophysiological and pharmacological properties characteristic of glutamate receptors of the KA and/or AMPA subtypes.

In yet another aspect the invention comprises
15 substantially pure functional proteins having substantial sequence homology with the substantially pure functional proteins of the invention.

The present invention also includes substantially pure DNA encoding functional proteins, or
20 functional fragments thereof, having electrophysiological and pharmacological properties characteristic of glutamate receptors.

In another aspect, the invention comprises substantially pure DNA encoding functional proteins or
25 functional fragments having electrophysiological and pharmacological properties characteristic of a glutamate receptor subtype selected from the group consisting of N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate (KA) and
30 2-amino-4-phosphonobutyrate (APB).

Still further the invention comprises DNA encoding KA and/or AMPA glutamate receptors.

In another aspect, the invention comprises substantially pure DNA selected from the group
35 consisting of GluR1 DNA, GluR2 DNA, GluR3 DNA, GluR4 DNA, GluR5 DNA, GluR6 DNA and GluR7 DNA.

Still further the invention comprises substantially pure DNA having at least about 40% homology with at least one member of the group consisting of GluR1 DNA, GluR2 DNA, GluR3 DNA, GluR4
5 DNA, GluR5 DNA, GluR6 DNA, and GluR7 DNA.

In another related aspect, the invention comprises DNA encoding proteins having at least about 40% homology with at least one member of the group consisting of GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3
10 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11), and GluR7 (Fig. 12).

The invention also comprises substantially pure DNA encoding substantially pure proteins having M_r s of about 99,769 (GluR1), 96,400 (GluR2), 98,000 (GluR3),
15 98,500 (GluR4), 100,000 (GluR5) and 100,000 (GluR6) that form ion channels which possess the electrophysiological and pharmacological properties characteristic of a glutamate receptor of the KA and/or AMPA subtypes.

The invention further comprises substantially
20 pure DNA that is functionally equivalent to any of the substantially pure DNA of the invention wherein functionally equivalent means that the substantially pure DNA will encode proteins, or functional fragments thereof, which will form ion channel(s) in response to
25 ligands for glutamate receptors.

In another aspect, the invention comprises substantially pure sense or antisense mRNA transcribed from the DNAs of the invention wherein the DNAs encode substantially pure functional proteins that have
30 electrophysiological and pharmacological properties characteristic of a glutamate receptor.

In another aspect, the invention comprises cDNA clones that encode the amino acid sequences of functional peptides having electrophysiological and
35 pharmacological properties characteristic of a glutamate receptor. Representative clones have been deposited with the American Type Culture Collection to provide to

those skilled in the art necessary starting materials (i.e., probes) to identify and isolate additional members of the glutamate receptor family. The representative cDNA clones include: GluR1 (ATCC
5 No. 68134), GluR2 (ATCC No. 68132), GluR3 (ATCC No. 68133), GluR4 (ATCC No. 68375), and GluR5 (ATCC No. 65374). Either the full length cDNA clones or fragments thereof can be used as probes. When fragments are used
10 as probes, preferably the DNA sequences will be from the carboxyl encoding portion of the DNA, and most preferably will include ion channel encoding portions of the DNA sequences.

In another aspect, the invention comprises functional peptide fragments, and functional
15 combinations thereof, encoded by the DNAs of the invention. Such functional peptide fragments can be produced by those skilled in the art, without undue experimentation, by eliminating some or all of the amino acids in the sequence not essential for the peptide to
20 function as a glutamate receptor. A determination of the amino acids that are essential for glutamate receptor function is made, for example, by systematic digestion of the DNAs encoding the peptides and/or by the introduction of deletions into the DNAs. The
25 modified (e.g., deleted or digested) DNAs are expressed, for example by transcribing the DNA and then introducing the resulting mRNA into *Xenopus* oocytes, where translation of the mRNAs will occur. Functional analysis of the proteins thus expressed in the oocytes
30 is accomplished by exposing the oocytes to ligands known to bind to and functionally activate glutamate receptors, and then monitoring the oocytes to see if the expressed fragments form ion channel(s). If ion channel(s) are detected, the fragments are functional as
35 glutamate receptors.

In still another aspect, the invention comprises cells transformed with DNAs of the invention.

In still another aspect, the invention comprises *Xenopus* oocytes to which mRNA of the invention has been introduced, e.g., by injection.

Still further, the invention comprises novel
5 glutamate receptors made by expression of DNA sequences of the invention, or translation of the corresponding mRNAs. Such novel receptors include the individual GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7
10 receptors, fragments thereof plus functional combinations of the receptors or fragments.

Still further, the invention comprises DNA, RNA and proteins that are functionally equivalent to the DNAs, RNAs and proteins of the present invention. Such functionally equivalent DNAs, RNAs and proteins will
15 function in substantially the same manner as the DNAs, RNAs and proteins of the invention.

In addition to DNA, RNA and protein compositions of matter, the invention comprises methods. A first method of the invention is a method for
20 identifying DNA that is homologous to DNA known to encode glutamate receptor protein(s). This method comprises contacting an "unknown" or test sample of DNA with a glutamate receptor DNA probe (e.g., GluR1, GluR2, GluR3, GluR4, GluR5, GluR6, GluR7, etc.) under low
25 and/or high stringency hybridization conditions, and then identifying as glutamate receptor homologous DNA that "unknown" or test DNA which hybridizes with the glutamate probe DNA.

A second method of the invention is directed to
30 identifying functional glutamate receptors (i.e., glutamate receptors that form ion channels). This method comprises contacting proteins, preferably in an oocyte expression system, with at least one ligand known to activate glutamate receptors, measuring ion channel
35 response to the ligand(s), and identifying as functional glutamate receptor(s) those proteins which exhibit an ion channel response as a consequence of the contact.

A third method of the invention is a method for determining whether a substance is a functional ligand for glutamate receptor protein. According to this method, proteins known to function as glutamate
5 receptors are contacted with an "unknown" or test substance, the ion channel activity of the known glutamate receptor is monitored subsequent to the contact with the "unknown" or test substance, and those substances which effect an ion channel response in the
10 known glutamate receptor(s) are identified as functional ligands for receptor proteins.

Turning now to some of the specific DNAs of the invention, cDNA clone GluR1 was isolated from a rat forebrain cDNA library by screening for expression of
15 kainate-gated ion channels in *Xenopus* oocytes. An insert from clone GluR1 was used as a probe to screen cDNA brain libraries (first under low-stringency hybridization conditions and then under higher stringency conditions) in order to find cDNA clones that
20 encode other members of the glutamate receptor family. Use of the GluR1 probe cDNA led to the identification and isolation of the GluR2 and GluR3 clones. A probe from GluR2 was used to identify and isolate clones GluR4 and GluR5, and GluR5 was used to isolate clones for
25 GluR6 and GluR7.

cDNA clone GluR1 encodes a functional glutamate receptor subunit which consists of a single protein of about $M_r = 99,769$, before any post-translational modifications. This protein forms an ion channel which
30 possesses the electrophysiological and pharmacological properties of KA and AMPA receptors.

The proteins encoded by the GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 and GluR7 genes exhibit considerable inter-subunit amino acid sequence identity.
35 See Table 2. These proteins have been found to form distinct homomeric and heteromeric KA/AMPA-sensitive ion channels in *Xenopus* oocytes. For example, single

protein subunits of glutamate receptors, GluR1, GluR2, GluR3, GluR4 and GluR5 are sufficient to form homomeric functional receptor ion-channel complexes activated by KA, AMPA, QUIS but not by NMDA and APB. While GluR2
5 subunits can form functional homomeric complexes, this subunit more efficiently assembles receptor-ion channel complexes in heteromeric combination with GluR1 or GluR3 subunits.

The GluR5 protein forms in *Xenopus* oocytes a
10 homomeric ion channel which is weakly responsive to glutamate but not to N-methyl-D-aspartate, kainate, quisqualate and 2-amino-4-phosphonobutyrate. The fact that oocytes expressing GluR5 are responsive to L-glutamate but not to KA, quisqualate or AMPA may
15 indicate that this protein can participate in the formation of receptors with a different pharmacological profile than the KA/AMPA subunits. This suggestion is supported by *in situ* hybridization data.

During embryonic and postnatal development the
20 GluR5 gene is expressed in subsets of neuronal cells in the CNS and PNS and the spatial and temporal expression pattern of the GluR5 gene is largely overlapping with the KA/AMPA subunit GluR4. However, in adult brains, the GluR5 gene is expressed in a pattern distinct from
25 those of the KA/AMPA subunit genes, consistent with the suggestion that GluR5 represents a subtype of glutamate receptors different from the KA/AMPA receptors.

Without further elaboration, it is believed that one of ordinary skill in the art can, using the
30 preceding description, and the following Examples and Detailed Descriptions of the Drawings, utilize the present invention to its fullest extent. The material disclosed in the examples, unless otherwise indicated, is disclosed for illustrative purposes and therefore
35 should not be construed as being limiting in any way of the appended claims.

ExamplesExample 1Production of Brain cDNA Libraries

5 Poly(A)⁺ RNA is purified by the guanidine
thiocyanate-CsCl method (Chirgwin et al., *Biochem.* 18,
5294 (1979) from various regions of the brain, eg.,
mammalian brain, or a suitable cell line, eg., the NCB-
20 cell line. The purified RNA is used as a template to
10 prepare double strand cDNA. A poly-dT primer linked to
a Xho I restriction site is used as a primer to prime
the Moloney reverse transcriptase for the synthesis of
the first strand using 5-methyl dCTP instead of cCTP as
a precursor. RNase H and DNA polymerase I are added to
15 complete the second strand. The cDNA is blunt-ended
with T4 DNA polymerase which increases the chance of
making a full-length cDNA. Eco RI adapters are ligated
to the blunt-end and the ends are kinased. The cDNA is
then digested with the Xho I restriction enzyme. This
20 enzyme cannot cleave hemimethylated DNA so it only
cleaves the un-methylated Xho I restriction site that
had been attached to the dT-primer at the 3' end of the
mRNA. The resulting double stranded cDNA has a Xho I
restriction site at the 3' end of the mRNA and an Eco RI
25 site at the 5' end. This cDNA is then placed into an
appropriate vector, such as λZAP vector, which is part
of the λZAP-cDNATM cloning system from Statagene. When
the λZAP vector is used, the cDNA is placed into the
vector so that the 5' end of the mRNA is near the lacZ
30 promoter. (The λZAP vector has a T3 RNA polymerase
promoter at one end of the cDNA insert and a T7 RNA
polymerase promoter at the other end which makes it
possible to synthesize either sense or antisense RNA for
further experiments, including expression in oocytes.

Example 2Low and High Stringency Hybridization

cdNA libraries are preferably made with the λ ZAP-cDNA system described in Example 1. A hybridization probe is preferably made from DNA obtained from Glu1, GluR2, GluR3, GluR4, GluR5, GluR6 or GluR7 clones or another suitable clone or source. The DNA is labeled by the random prime method, preferably using the Amersham MultiprimeTM DNA labeling kit. Preferably, low stringency hybridization conditions are used, at least at first. A suitable hybridization solution is as: (1 M NaCl, 50 mM Tris [pH 8.0], 0.5% SDS, 0.1% sodium pyrophosphate, 100 mg/ml denatured herring sperm DNA, 0.1% [w/v] each of FicollTM, polyvinylpyrrolidone and bovine serum albumin). For low stringency screening, a temperature of 50° C. is preferable, and library filters are washed in 2 X SSPE (1 X SSPE is 180 mM NaCl, 9 mM Na₂HPO₄, 0.9 mM NaH₂PO₄ and 1 mM EDTA, pH 7.4) at room temperature and exposed to Kodak XAR-5TM film at -70° C. For high stringency screening, the temperature is adjusted to 65° C. and the filters are washed at this temperature in 0.2X SSPE containing 0.5% sodium dodecyl sulfate. Filters are exposed to Kodak XAR-5TM film with Cronex QuantaTM II/III intensifying screens at -70° C. for 18-48 hr.

Under these low-stringency hybridization conditions, about one in two thousand brain cdNA clones show some hybridization to the probe made from the GluR1 cdNA insert.

Example 3Analysis of Clones Identified by Hybridization

At least two different approaches can be used to analyze clones that are identified by low-stringency hybridization screening. One approach is to pick positive λ ZAP clones and pool them into mixtures of about 100 clones. mRNA is made *in vitro* from these pools of λ ZAP clones and the mRNA is injected into oocytes in order to test the ability of the mRNA to direct synthesis of functional glutamate receptors. If a positive clone is found, the individual λ ZAP cDNA clone is isolated by subdividing the pool until the functional clone is isolated. (See Example 5, below, for a discussion of how this approach was used to isolate the GluR1 clone.) A second approach that can be used to evaluate positive clones is to analyze each insert individually. Although this is tedious, the "individual clone" approach has the advantage that initially it does not require functional expression. When the "individual clone" approach is used, each clone is plaque purified and the cDNA insert is analyzed individually. This is facilitated by the fact that, at least in the λ ZAP cDNA system, the cDNA is cloned into a cassette flanked by the bacteriophage fl origin of replication. The cDNA is contained within a pBluescriptTM plasmid which can be rescued from the λ ZAP bacteriophage by helper infection. (Once this is done the cDNA is in the small pBluescriptTM plasmid which is much easier to work with than the much larger λ bacteriophage.) Sense or antisense RNA is made from the cDNA insert in the pBluescriptTM plasmid using either the T3 (sense) or T7 (anti-sense) promoter.

The cDNA insert is also preferably mapped with restriction enzymes. For example, the cDNA inserts are cut with frequent cutting restriction enzymes. The resulting fragments are size fractionated on a gel and the fragments transferred to a filter for Southern blot

analysis. The filters are hybridized with a probe made from known glutamate receptors, eg., GluR1, GluR2, GluR3, GluR4, GluR5, GluR6 or GluR7. The hybridizing fragments from each clone are subcloned into the single-stranded vector M13, (mp18 or mp19), and the fragment
5 sequenced. DNA sequencing is performed using the dideoxynucleotide chain termination method of Sanger et al., *Proc. Natl. Acad. Sci. USA* 74, 5463 (1977), or an automatic sequencer such as one manufactured by Applied
10 Biosystems. The sequence is preferably analyzed by computer using software such as the programs developed by Intelligenetics, Staden and the University of Wisconsin.

mRNA made from full-length or nearly full-length clones are expressed in the oocyte system and the functional properties of the new receptors are characterized. If a clone is not functional when expressed by itself, it is tested in the presence of mRNA made from other candidate clones.

20

Example 4

Expression Cloning and Assay in Xenopus Oocytes

This assay is an adaptation of the assay of Masu et al., *Nature* 329, 836 (1987). It depends upon
25 the fact that when foreign mRNA is injected into *Xenopus* oocytes, the mRNA is translated into functional protein.

DNA templates, either a λ ZAP cDNA preparation or a plasmid containing the cDNA to be tested, are cut downstream from the cDNA insert with a restriction
30 enzyme. The post-restriction digest is digested with Proteinase K and then extracted with two phenol:chloroform (1:1) extractions. The DNA template is then ethanol precipitated. The template is mixed with either T3 RNA polymerase, to make sense strand, or T7 RNA
35 polymerase, to make anti-sense strand, plus rATP, rCTP, rGTP, rUTP, and RNase inhibitor. Simultaneously, the RNA transcripts are capped with a 7meGpppG cap.

Throughout the procedure gloves are worn and the water is treated with diethylpyrocarbonate to inactivate RNases.

5 The *in vitro* synthesized mRNA transcripts are injected into *Xenopus* oocytes. 50 nl of an RNA solution, 0.2-0.6 mg/ml, is injected into the stage V oocytes. The injected oocytes are incubated at 16° C. in Barth's medium for 2-7 days before they are analyzed for the presence of functional receptors.

10 Voltage recordings are made by penetrating the oocyte with a micro-electrode filled with 3M KCL and connected to the bridge circuit of an appropriate voltage clamp unit, eg., the Dagan 8500 voltage clamp unit. Voltage clamp studies are preferably carried out
15 with two electrodes, a voltage electrode filled with 3M KCl and a current electrode filled with 0.25 M CsCl, 0.25 M CsF and 50 mM EGTA. (See Example 6, below, for a discussion of results of recordings from oocytes injected with RNA from GluR1 cDNA coding for a KA/AMPA
20 glutamate receptor.)

Before injection the oocytes are prepared by taking ovaries from anesthetized adult female *Xenopus*. The ovary tissue is treated with collagenase, 2 mg/ml, for two hours and then the ovarian epithelium and
25 follicular cells are dissected away.

Example 5

Expression Cloning of the GluR1 Receptor

Xenopus oocytes were injected with poly(A)⁺ RNA
30 isolated from rat forebrain. 2-10 days later, the oocytes were tested electrophysiologically for their ability to respond to selective agonists for glutamate receptor subtypes. Both glutamate and quisqualate remove induced depolarizations displaying a biphasic
35 pattern composed of a fast acting, smooth, presumably ligand-gated ion channel response, and a longer lasting, fluctuating, probably second-messenger mediated

response. NMDA and KA elicited smooth responses with fast onsets. APB gave no response.

A directional cDNA library (λ ZAPII RTB1; complexity: 8×10^5 elements), consisting of 18 independent sublibraries of 44,000 clones each, was constructed from this poly(A)⁺ RNA using the bacteriophage expression vector λ ZAPII. A pool of *in vitro* transcripts, comprised of transcripts made separately from all 18 amplified sublibraries, was injected into oocytes. Small depolarizations (1-3 mV) were seen in voltage recordings from oocytes challenged with 100 μ M kainate 10 days after injection. No responses to NMDA or quisqualate were detected. Neither uninjected oocytes nor water-injected oocytes showed any responses to glutamate receptor agonists. Subsequently, pools of 44,000 clones (= the single sublibraries), 4000, 400 and 40 clones were tested. In each of these tests at least one pool responded to KA. The following criteria were used throughout the screening procedure to assure that the very small responses observed initially were not recording artifacts: (a) responses in a given oocyte were reproducible, (b) responses were fast (within one second of agonist application), (c) responses were readily reversible upon superfusion of the oocyte with control Ringer solution, (d) 10 μ M domoate gave a response similar to the one elicited by 100 μ M kainate. The pools yielding the largest responses at each stage were selected for further subdivision. The clones in the final positive pool of 40 clones were analyzed for their insert size, and the 12 clones with the largest inserts (all > 2 kb) were tested individually for their ability to direct the synthesis of a functional kainate receptor. Only one clone, carrying a 3.0 kb insert, was found to elicit kainate responses and was named λ ZAPII-GluR1.

Example 6Electrophysiological and Pharmacological
Characterization of the GluR1 Clone

The plasmid pGluR1 was subsequently rescued
5 from bacteriophage λ ZAPII-GluR1, and upon transcription
in vitro sense RNA but not antisense RNA induced kainate
responses when injected into oocytes. As little as 10pg
of GluR1 sense transcript under voltage-clamp conditions
(-70mV holding potential) gave detectable responses to
10 100 μ M KA.

In order to rule out the possibility that GluR1
codes for a transcription factor, oocytes injected with
pGluR1 in vitro transcripts were kept in medium
supplemented with 50 μ g/ml actinomycin D to inhibit
15 endogenous transcription. These oocytes exhibited the
same responses to kainate as those kept in control
medium. Therefore, injection-induced transcription from
the oocyte genome seems unlikely to contribute to the
observed responses.

20 L-glutamate evoked much smaller responses than
did KA, and even at 1mM elicited only 50% of the
depolarization seen with 30 μ M KA. This is consistent
with previous reports that glutamate is only a weak
agonist for the KA receptor subtype (Monaghan *et al.*,
25 *Nature* 306, 176 (1983)). Other glutamate receptor
agonists such as NMDA, quisqualate and L-aspartate
evoked no responses when applied at 150 μ M, 10 μ M, and
100 μ M, respectively. Unrelated neurotransmitter
receptor agonists such as glycine, GABA_A, serotonin and
30 nicotine also failed to evoke responses, even when
tested at concentrations as high as 1mM. Glycine did
not potentiate the KA response. Dose-response curves
for KA and domoate were recorded and EC₅₀ values of 39 μ M
and 1.8 μ M, respectively, were derived. The average
35 reversal potential extrapolated from current responses
to 10 μ M kainate obtained at a series of holding
potentials between 0 and -130mV was 10mV. Responses to

KA did not desensitize, not even after prolonged (up to 10 min) superfusion with high concentrations (100 μ M) of the agonist. These findings are also consistent with earlier reports from experiments with KA receptors expressed from total poly(A)⁺ RNA (Verdoorn & Dingledine, *Molecular Pharmacology* 34, 298 (1988)). Similarly, the pharmacological profile of GluR1 (see Table 1), as revealed by the inhibiting properties of various known glutamate receptor antagonists, is consistent with previous reports for KA receptors in systems where total poly(A)⁺ RNA was used as a source of kainate receptor message (Hirono et al., *Neurosci. Res.* 6, 106 (1988); Lerma et al., *Proc. Natl. Acad. Sci. USA* 86, 2083 (1989)). Of all the compounds tested (each at 1mM), the broad specificity glutamate receptor antagonist kynurenic acid clearly was the most potent inhibitor of the kainate-evoked depolarizations in oocytes injected with GluR1 RNA synthesized in vitro. To a lesser extent, γ -D-glutamylglycine (γ -DGG), reported to preferentially block KA and NMDA receptors but not quisqualate receptors (Davies and Watkins, *Brain Res.* 206, 172 (1981)), inhibited kainate responses, as did γ -D-glutamylaminomethyl-sulphonate (GAMS), which reportedly prefers KA and AMPA receptors (Fagg, *Trends Neurosci.* 8, 207 (1985)). Similarly, 2,3-cis-piperidine dicarboxylic acid (PDA), which is known to block all subtypes of glutamate receptors (Foster and Fagg, *Brain Res. Rev.* 7, 103 (1984)), blocked the GluR1 response. Glutamate diethylester (GDEE), which is thought to preferentially inhibit AMPA-type glutamate receptors (Foster and Fagg, *Brain Res. Rev.* 7, 103 (1984)), did not block the response significantly, but instead displayed weak agonist properties. The NMDA receptor antagonists 2-amino-5-phosphonovaleric acid (APV) and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphate (CPP), as well as NMDA itself, slightly inhibited the KA

responses, thus acting as weak antagonists. They did not show any agonist properties.

Taken together, the electrophysiological properties observed in oocytes injected with GluR1 transcript, as well as the observed pharmacological properties, indicate that GluR1 represents a functional KA receptor indistinguishable from the one observed in oocytes injected with total poly(A)⁺ RNA. Thus, the single protein subunit encoded by GluR1 is sufficient to form an active receptor-ion channel complex.

Example 7

Sequencing and Primary Structure of GluR1

The cDNA insert of the plasmid pGluR1 was subcloned into M13mp19 and sequenced (Fig. 1). An open reading frame of 2721 bp was found within the total length of 2992 bp. Thus, the predicted protein consists of 889 amino acids, with a calculated M_r of about 99,769. The deduced protein sequence contains a putative signal peptide of 18 amino acids at its N-terminus, with a predicted cleavage site that conforms to empirical rules (von Heijne, *Nucl. Acids Res.* 14, 4683 (1986)). The N-terminus of the protein therefore is expected to be located extracellularly. There are 6 possible N-glycosylation sites present in the presumed extracellular domain. Sequence comparisons with other sequenced ligand-gated ion channels, the nicotinic acetylcholine receptors, GABA_A receptors and glycine receptors, reveals little over-all homology.

All ligand-gated ion channel subunits sequenced previous to this disclosure have a conserved extracellular region characterized by 2 cysteine residues spaced 14 amino acids apart from each other, with conserved proline and aspartate residues located 8 and 10 amino acids, respectively, downstream from the first of these two cysteines (Barnard et al., *Trends Neurosci.* 10, 502 (1987)). This hypothetical signature

for neurotransmitter receptor-channel complexes is poorly conserved in the protein encoded by GluR1. The proline and aspartate residues are present, but the first cysteine residue is located only 7 residues upstream from the proline residue, and the second cysteine residue is absent.

A hydropathy plot analysis of GluR1 revealed several regions which are candidates for transmembrane domains (TMDs). The region between amino acids 455 and 810 was notable because its hydropathy profile resembled that seen in the other ligand-gated ion channels: three closely spaced putative TMDs which are separated by ca. 175 amino acid residues from a fourth putative TMD which is located close to the C-terminus of the protein. Within this region, the following four transmembrane regions are assigned in GluR1: TMD I, located between amino acid residues 463 and 480, TMD II between residues 521 and 539, TMD III between residues 596 and 614, and TMD IV between residues 788 and 808.

20

Example 8

Isolation and Characterization of GluR2 and GluR3

cDNA clones encoding the GluR2 and GluR3 genes were isolated from an adult rat forebrain library using a low-stringency hybridization screening protocol (see Example 2) and a radiolabeled fragment of the GluR1 cDNA as probe. Figures 3 and 4 show the nucleotide and derived amino acid sequence of clones λ RB14 (GluR2) and λ RB312 (GluR3). The calculated molecular weights for the mature, non-glycosylated forms of GluR2 and GluR3 are 96,400 daltons (862 amino acids) and 98,000 daltons (866 amino acids), respectively. Potential N-linked glycosylation sites occur in the GluR2 protein at Asn-235, Asn-349, Asn-385, Asn-392, and Asn-835 and in the GluR3 protein at Asn-35, Asn-238, Asn-352, Asn-387, and Asn-394. Like GluR1, the hydrophobicity profile for both GluR2 and GluR3 reveals five strongly hydrophobic

regions: one such domain is located at the amino terminus of each protein and has characteristics of a signal peptide, while four additional hydrophobic regions probably form membrane-spanning helices (MSR I-
5 IV) and are located in the carboxy-terminal half of each polypeptide.

Figure 5 is an alignment of deduced amino acid sequences for the proteins encoded by the GluR1, GluR2 and GluR3 (and GluR4 and GluR5) genes. As the aligned
10 sequences demonstrate, there is significant sequence identity between GluR1 and both GluR2 (70%) and GluR3 (69%) as well as between GluR2 and GluR3 (74%). (Also see Table 2). The sequence identity is most pronounced in the carboxy-terminal half of each protein.

15

Example 9

Electrophysiological Comparison: GluR1, GluR2 and GluR3

The strong sequence similarity between the proteins encoded by GluR1, GluR2 and GluR3 suggested
20 that, as with GluR1, the GluR2 and GluR3 proteins might function as homomeric, kainate-sensitive ion channels in *Xenopus* oocytes. To test this hypothesis, oocytes were injected with *in vitro* synthesized RNA transcripts derived from individual cDNA clones. Figure 6A shows
25 that both GluR2 and GluR3 injected oocytes depolarize in response to bath application of 100 μ M KA.

The amplitudes of the KA responses were not equivalent for the three glutamate receptor subunits: with equal amounts of injected RNA (2 ng), responses in
30 GluR3 RNA-injected oocytes were invariably larger than GluR1 responses. KA-invoked depolarizations in GluR2-injected oocytes were the weakest and could only be detected in oocytes injected with much larger amounts of RNA (10 to 25 ng).

35 The data in Figure 6B show that, in addition to KA, oocytes injected with GluR1 or GluR3 RNA also respond to QUIS (10 μ M), AMPA (50 μ M), glutamate (GLU, 100

μM). No detectable responses were obtained with NMDA ($30\mu\text{M}$ plus $10\mu\text{M}$ glycine) or APB ($50\mu\text{M}$). Responses obtained from oocytes injected with GluR2 RNA were too small for reproducible quantitation and were, therefore, excluded from the analysis. For GluR1-injected oocytes the responses to AMPA and QUIS were typically 35-40% of the maximal KA response, while for GluR3 they were about 10% of the KA response. Relative to KA, the response of GluR1 to domoic acid (DOM, $10\mu\text{M}$) is about 6-fold greater than that seen for GluR3. Taken together these data demonstrate that receptors assembled from GluR1 or GluR3 subunits are pharmacologically distinct. Furthermore, the observation that homomeric GluR1 and GluR3 receptors respond to both QUIS and AMPA, albeit with reduced efficiencies, provides direct evidence that KA, QUIS and AMPA can bind to the same glutamate receptor polypeptide.

Figure 6B also shows that the pharmacological profile of oocytes injected with individual GluR1 or GluR3 subunit RNA is significantly different than that seen in oocytes injected with rat brain hippocampus poly(A)⁺ RNA. This finding suggests that the responses seen in oocytes injected with hippocampus RNA are mediated by heteromeric glutamate receptors assembled from various combinations of GluR1, GluR2 and GluR3 subunit polypeptides. This suggestion is supported by the fact that all three GluR subunit genes are actively transcribed in the hippocampus.

30

Example 10

Pharmacological Comparison of GluR1, GluR2 and GluR3

This example addresses the question of whether glutamate receptors assembled from mixtures of the proteins encoded by the GluR1, GluR2 and GluR3 subunit genes have pharmacological properties significantly different from each other or from those observed for single subunit receptors.

A comparison of the data in Figure 6B and 7B suggests that, for the agonists tested, there are few substantial differences in the pharmacology. However, the responses to QUIS, AMPA and GLU are, relative to
5 GluR1, significantly reduced in the oocytes expressing the subunit combinations. Except for the NMDA response, the overall agonist profiles for oocytes injected with GluR subunit combinations are more similar to oocytes containing hippocampus poly(A)⁺ RNA than to those
10 injected with either GluR1 or GluR3 subunit RNA alone.

Example 11

Comparison of KA-Activated Currents

Recorded from GluR1, GluR2 and GluR3

15 Figure 7A compares KA-activated currents recorded from oocytes injected with mixtures of GluR1, GluR2 and GluR3 subunit (stippled columns) with the summed currents measured for the individual subunits (blank columns). The principle finding is a significant
20 potentiation of KA-evoked currents in oocytes coexpressing GluR1 plus GluR2 subunits (approximately 4-fold increase over the summed responses for singly-injected oocytes) or GluR2 plus GluR3 subunits (approximately 2-fold increase). Injection of all three
25 subunit RNAs results in an average 2.5 fold-increase in KA-evoked currents.

These results support the conclusion that in oocytes individual glutamate receptor subunit polypeptides do not behave in a simple independent
30 fashion but rather interact with each other. It is probable that such interaction results in the generation of heteromeric glutamate receptors with properties which are distinct from the receptors comprised of solitary glutamate receptor subunits.

Example 12Current-Voltage Relationships for KA-Evoked Responses

This example examines the current-voltage (I/V) relationships for KA-evoked responses measured in oocytes injected with individual GluR subunits, combinations thereof or hippocampus poly(A)⁺ RNA.

The I/V data are illustrated in Figure 8 where oocytes injected with individual glutamate receptor subunit RNAs are shown in panel A, oocytes injected with combinations of subunits are shown in panels B and C, and for purposes of comparison, oocytes expressing hippocampus poly(A)⁺ RNA are also shown in panel A. The KA responses measured in oocytes injected with brain poly(A)⁺ RNA show an approximately linear I/V relationship with a reversal potential of about -10mV. This result is in marked contrast to the I/V curves obtained for oocytes injected with single GluR1 or GluR3 subunit RNA. The GluR1 and GluR3 I/V curves show strong inward rectification and reversal potentials near -40mV. From these data it is clear that the KA-sensitive receptors present in oocytes injected with hippocampus RNA are different from those assembled by oocytes injected with GluR1 or GluR3 subunit RNAs.

In Figure 8B the I/V curve for the GluR1 plus GluR2 combination is noticeably different from that observed for the GluR1 subunit alone. Oocytes injected with this pair of RNAs show a nearly linear I/V plot and have a reversal potential of approximately -10mV. This plot is strikingly similar to that seen with hippocampus RNA-injected oocytes (panel A). In contrast, the I/V curve for the GluR1 plus GluR3 combination is only marginally different from those measured in oocytes expressing the individual subunits. Figure 8C shows some inward rectification in the I/V curve for the GluR2 plus GluR3 subunit combination, as well as a reversal potential somewhat more negative (-20mV) than those determined for GluR1 plus GluR2 or hippocampus RNA I/V

plots (-10mV). When all three glutamate receptor subunit RNAs are combined in a single oocyte, the resulting I/V curve approximates that seen for the GluR1 plus GluR2 combination in both reversal potential and slope; however, the responses with the three subunits show a pronounced inward rectification not observed with GluR1 plus GluR2.

Example 13

Distribution of GluR1, GluR2 and GluR3 mRNA In The Mammalian Central Nervous System

The hypothesis that proteins encoded by the GluR1, GluR2 and GluR3 genes assemble to form heteromeric glutamate receptors *in vivo* can be disproved by a showing that the individual subunit genes are transcribed in different neuroanatomical loci. Therefore, the distribution of GluR1, GluR2 and GluR3 RNAs in the adult rat brain was examined using radiolabeled anti-sense RNA probes and *in situ* hybridization histochemistry essentially as described by Deneris, et al., *J. Biol. Chem.* 264, 6268 (1989). The results indicated that the hybridization patterns obtained with the GluR1, GluR2 and GluR3 probes were nearly identical, with the strongest hybridization seen in the CA1-CA3 regions of the hippocampus and the dentate gyrus. High-resolution analysis of these areas suggests that the hybridization signal originates in the pyramidal cell layer of regions CA1-CA3 and the granule cell layer of the dentate gyrus. Somewhat weaker hybridization of all three probes was seen in the piriform cortex, caudate-putamen, amygdala, and hypothalamus. Low levels of hybridization were detected in the thalamus, with little or no signal observed in fiber tracts. While differential hybridization was seen in the medial habenula and neocortex, the overall patterns of expression for the GluR1, GluR2 and GluR3 subunit genes showed substantial concordance.

Example 14Isolation of cDNAs for GluR4 and GluR5

Using a fragment of the GluR2 cDNA
(nucleotides 1793-2240) as probe and a low stringency
5 hybridization protocol, several GluR4 and R5 clones were
isolated from a rat forebrain library. Sequence
analysis demonstrated that none of the cDNA clones
contained an entire open reading frame. Northern blots
with mRNA from different adult rat brain tissues
10 indicated that the GluR4 and GluR5 transcripts were most
abundant in the cerebellum. Consequently, the partial
GluR4 and GluR5 cDNA clones were used as probes under
high stringency screening conditions to isolate cDNAs
encoding large open reading frames from an adult rat
15 cerebellum cDNA library.

Of the cDNA clones thus isolated, two GluR4-
related clones (λ CER112 and λ CER121B) encoded only
portions of the GluR4 gene but possessed sufficient
overlap to engineer a full-length, expressible construct
20 in the pBS SK (+) vector (StratageneTM Cloning Systems).
The nucleotide sequence of this GluR4 construct,
designated pK45, was determined and the deduced amino
acid sequence is presented in Figure 9.

Among the 29 GluR5-related cDNAs isolated from
25 the cerebellum library, three clones, specified λ RB12,
 λ RB15 and λ RB20, were identified which encode an
identical large open reading frame. The sequence of
cDNA clone λ RB20 (GluR5-1) is shown in Figure 10. λ RB15
and λ RB12 are shorter than λ RB20 at the 5' end. The
30 λ RB20 cDNA consists of a 187 bp 5' untranslated region,
a continuous open reading frame of 2760 bp, and a 3'
untranslated region of 303 bp. The 5' untranslated
region ends with the sequence AAGATGG which is
characteristic of a translational start site.

35 Three additional cDNA clones originally
isolated from the forebrain library were also examined.
The sequences of these cDNAs are identical to λ RB20 in

the predicted translation initiation site region. Sequence analysis revealed that two variants of the GluR5 cDNA are represented in the forebrain and the cerebellum libraries. This heterogeneity derives from the insertion of 45 nucleotides found in λ RB20 and 17 of the 29 GluR5-related cDNA clones isolated (Figure 10). This insertion does not interrupt the open reading frame. Furthermore, consensus splice donor and acceptor sites are absent (Breathnach and Chambon, *Ann. Rev. Biochem.* 50, 349-383 (1981)), which suggests that the insertion does not arise from an unspliced intron and is, most likely, the result of an alternative splice event. Nucleotide sequence analysis indicates that the two GluR5 variants are otherwise identical.

No cDNA clone was found for the shorter splice variant encoding the entire open reading frame. Therefore a clone (λ RB Δ 20) was constructed that is missing the 45 nucleotide insertion found in λ RB20 (GluR5-1) but is otherwise identical to that clone. The shorter splice variant clone (λ RB Δ 20) is referred to as GluR5-2. Both λ RB20 and λ RB Δ 20 were used in *Xenopus* oocyte expression experiments (see Example 16) and the variant proteins encoded were named GluR5-1 and GluR5-2, respectively.

The GluR4 mRNA was detected on Northern blots of cerebellum RNA as a 4.5 kb species. The smaller size mRNA may represent splice variants. Northern blot analysis also indicated that the major GluR5 mRNA has a size of 6 kilobases.

30

Example 15

Structural Features of GluR5 cDNA and Protein

Translation of the cDNA nucleotide sequence for GluR5-1 predicts a single long open reading frame of 920 amino acid residues (Figure 10). The GluR5 sequence has overall amino acid sequence identity with each of the KA/AMPA subunits (Figure 5, and Table 2). The 15

35

amino acid insertion in GluR5-1 is unique among the proteins listed, thus the shorter GluR5-2 variant is the counterpart to the KA/AMPA subunits characterized. Table 2 shows that GluR5 is thus far the most dissimilar glutamate receptor subunit identified and the comparison of GluR5 with the KA/AMPA subunits highlights the most conserved sequence elements (Figure 5). Within other ligand-gated ion channel families, the neuronal acetylcholine receptor, the GABA_A receptor and the glycine receptor families, the N-terminal extracellular domain is most conserved while the C-terminal sequences diverge between the membrane-spanning regions (MSR) III and IV. In the glutamate receptor subunit gene family, in contrast, the regions N-terminal of the proposed MSR I (Hollmann, et al., *Nature* 342, 643 (1989)), have only 17% identity and are less similar than the regions C-terminal of MSR I which have 45% identity. The 'Cys-Cys loop', a signature for ligand-gated neurotransmitter receptor channel complexes (Barnard, et al., *Trends Neurosci.* 10, 502 (1987)) is not conserved in the glutamate receptor subunit family (Figure 5). The C-terminal half of glutamate receptor subunits are suggested to be involved in channel formation and contain the postulated membrane spanning helices (MSR I-IV; Figure 5) (Hollmann, et al., *Nature* 342, 643 (1989)). The presumed MSR III is the most conserved continuous sequence, with only one conservative amino acid exchange (Val to Ile) in the GluR5 protein (Figure 5). As mentioned above, in other ligand-gated channel families the segment between MSR III and IV is divergent in length and sequence. In the glutamate receptor subunit family the similarity in this postulated segment is high (48%) and only GluR5 exhibits a sequence length variation. The KA/AMPA receptors and the GluR5 protein are generally divergent C-terminal of the proposed MSR IV.

The hydrophobicity plot for GluR5 is similar to those of the KA/AMPA receptors, suggesting a conserved secondary structure in the proposed ion channel forming portion of the protein. However, the N-terminal half of the GluR5 hydrophobicity plot is unusual. In this region, GluR5, as compared to the KA/AMPA subunits, is more hydrophobic and contains several segments that could span the membrane. Based on algorithms that search for membrane-associated helices, four (Rao and Argos, *Biochim. Biophys. Acta* 869, 197-214 (1986)) or seven (Eisenberg, et al., *J. Mol. Biol.* 179, 125-142 (1984)) putative transmembrane regions can be assigned to GluR5.

A comparison of the C-terminal regions of all five glutamate receptor subunits with the frog (Gregor, et al., *Nature* 342, 689 (1989)) and chicken (Wada, et al., *Nature* 342, 684 (1989)) KA-binding proteins demonstrates a similar extent of sequence conservation (35-40% amino acid identity). A FASTATM search (Pearson and Lipman, *Proc. Natl. Acad. Sci.* 85, 2444 (1988)) of the GenBank, EMBL and SWISS-PROT databases with the GluR5 sequence uncovered no significant similarities to other proteins.

25

Example 16

Electrophysiological Properties of GluR5 mRNA

Injected Oocytes Exposed to L-Glutamate

In vitro synthesized GluR5-1 and GluR5-2 cRNAs were individually expressed in *Xenopus* oocytes. With either cRNA, the glutamate receptor agonists KA (100 μ M), AMPA (50 μ M), quisqualate (10 μ M), APB (100 μ M) and NMDA (100 μ M, applied with 10 μ M glycine) did not elicit membrane depolarizations in cRNA injected oocytes. However, weak membrane depolarizations induced by L-glutamate (100 μ M) were recorded in oocytes injected with GluR5-1 cRNA (maximal depolarization 3.5 mV) and GluR5-2 cRNA (maximal depolarization 4.5 mV).

Significantly stronger membrane depolarizations were not found in response to L-glutamate in oocytes co-injected with GluR5-1 and GluR5-2 cRNA as compared to oocytes injected with GluR5-2 cRNA alone. For any particular
5 oocyte injected with GluR5-1 or GluR5-2 cRNA, the depolarizations were reproducible, showed fast onset, and were slowly (within 5 min) reversed when agonist superfusion was switched to buffer superfusion. Neither
10 un-injected oocytes nor water-injected oocytes showed a response to the glutamate receptor agonists tested. Responses to L-glutamate were recorded in 7 (out of 7) oocytes for GluR5-1 (membrane depolarization 2.29 ± 0.26 mV s.e.m) and 29 (out of 33) oocytes for GluR5-2 (2.27 ± 0.19 mV s.e.m).

15

Example 17

Distribution of GluR4 and GluR5 mRNA In the Developing Central and Peripheral Nervous Systems

For the developmental study of GluR4 and R5
20 gene expression, sections of mice from embryonic day 10 (E10) through postnatal day 21 (P21) were analyzed using *in situ* hybridization and histochemistry.

In the entire CNS, a diffuse expression of the GluR4 and R5 genes was detected at E10. These first
25 hybridization signals originate from postmitotic neurons. This is best demonstrated in the myelencephalon at E12. The ependymal layer is facing the neural canal and contains dividing neuroblasts. No hybridization was detectable in these cells. The
30 postmitotic cells are located in the exterior part of the neural tube and express both genes. Later in development transcripts for GluR5 and, to a lesser extent, GluR4 were particularly pronounced in areas where neurons differentiate and assemble into nuclei.
35 These temporal changes in the hybridization pattern were best observed for GluR5 in the primary sensory nuclei of the medulla oblongata and the nuclei of the pons which

hybridized more intensely than surrounding structures
At E14, GluR5 gene expression was particularly intense
in several discrete brain nuclei, whereas GluR4 gene
expression was detectable over the entire rostral and
5 caudal parts of the brain. During postnatal
development, the spatial distribution of GluR4 gene
transcripts did not change but usually smaller amounts
of mRNA were detectable than at late embryonic stages.
In contrast, GluR5 gene expression appeared to become
10 more restricted spatially during development and
transcript levels were down-regulated. Extreme changes
in the temporal GluR5 hybridization pattern were
apparent in the cerebellar cortex. Until P12, high
GluR5 transcript levels were detected in the granular
15 and Purkinje cell layer. Later, the intensity of
hybridization signals in the granular cell layer was
reduced relative to the Purkinje cell layer and starting
at P14, only a faint hybridization signal was detected
in the granular cell layer. In general, those regions
20 of the brain that exhibited a dense labeling during
embryonic development also had detectable transcript
levels in adults. In P21 animals the highest GluR4
transcript levels were observed in the cell layers of
the olfactory bulb, the hippocampus, the cerebellum and
25 the retina. In the retina, strong hybridization was
found in the ganglion cell layer and in the amacrine
cells of the inner nuclear layer. No expression was
detected in Muller cells. For GluR5, the strongest
hybridization signals at P21 were found in the olfactory
30 bulb, the amygdala, the colliculi and some hypothalamic
nuclei.

In the developing peripheral nervous system
(PNS), the hybridization assays showed that the GluR4
and R5 genes are expressed to varying degrees in the
35 cranial ganglia (e.g., trigeminal ganglion, acoustic
ganglia), dorsal root ganglia and the mural ganglia of
the intestinal organs. Comparable to the CNS,

transcripts in the PNS are detected by E10 for GluR4 and by E11 for GluR5. During development, hybridization signals for GluR4 continuously increase until early postnatal stages and then persist with similar intensity in adults. Hybridization signals for GluR5 increase up to E16 and remain with comparable intensity in later developmental stages. In postnatal animals, the dorsal root ganglia (GluR5) and the mural ganglia of the intestinal organs (GluR4 and GluR5) exhibit higher levels of hybridization than the CNS. High resolution autoradiography in the dorsal root ganglia demonstrates hybridization of the GluR5 probe over neuronal cells whereas satellite cells are unlabeled.

15

Example 18Distribution of GluR4 and GluR5 mRNA in the
Adult Mammalian (Rat) Brain

The distribution of the GluR4 and GluR5 mRNA transcripts in the adult CNS was studied by *in situ* hybridization. In the forebrain region, high levels of GluR4 transcripts were detected in the CA1 and the dentate gyrus of the hippocampus, in the medial habenula and particularly in the reticular thalamic nucleus. The hippocampus showed only weak expression of the GluR5 gene and no transcripts were detected in the medial habenula. The GluR5 hybridization signal was intense in the cingulate and piriform cortex, several hypothalamic nuclei, the amygdala and the lateral septum. In the cerebellum, the hybridization patterns for GluR4 and R5 probes were overlapping but distinct. Both probes were detected at high levels in the Purkinje cell layer. In the granular cell layer the GluR4 probe produced strong labeling, while GluR5 probe labeling was weak.

Example 19Isolation of GluR6 and GluR7

cdNA clones encoding the GluR6 and GluR7 genes were isolated from a adult rat forebrain library using a low-stringency hybridization screening protocol (see Example 2) and a radiolabeled fragment of about 1.2 kbp (nucleotides 705-2048) of the GluR5 cDNA as probe. The selected clones were identified by restriction digest map and sequencing. Figure 11 shows the nucleotide and derived amino acid sequence of the GluR6 clone; Figure 12 shows the same for fragments 35-T3 (Fig. 12 A) and U35 (Fig. 12 B) from the GluR7 clone.

Example 20GluR-Related Assays

The GluR cDNAs, mRNAs, proteins and functional fragments thereof, are useful in various assays designed to identify and characterize L-glutamate receptors and ligands. For example, the cDNAs are useful as probes to identify additional members of the glutamate receptor gene family. mRNAs transcribed from the DNAs of the invention are especially useful in assays designed to identify and characterize both functional receptors and ligands. This use is especially important for the identification and design of compounds that can affect L-glutamate receptor function.

In an assay for identifying and characterizing functional receptors, mRNA is transcribed from DNAs of the invention (either full length or fragments thereof produced by deletions, substitutions, synthesis, etc.) and then translated to produce GluR proteins. In preferred form, the mRNAs are translated in oocytes, preferably *Xenopus* oocytes. The expressed glutamate receptor proteins are then exposed to ligands known to functionally bind to and activate glutamate receptors. The electrophysiological characteristics of the glutamate receptor proteins are measured, and those that

form functional ion channels are concluded to be functional glutamate receptor.

In a related assay designed to identify functional ligands for glutamate receptors, proteins known to functionally bind to glutamate receptor activating compound(s) are contacted with at least one "unknown" or test compound whose ability to induce glutamate receptors to form ion channels is sought to be determined. The electrophysiological properties of the glutamate receptors are measured following exposure to the test compound(s), and those that can effect an ion channel response are concluded to be functional ligands for glutamate receptors.

Tables

5 Table 1 Pharmacology of the glutamate receptor encoded by GluR1: properties of various glutamate receptor antagonists as measured in oocytes injected with GluR1 *in vitro* RNA

10	compound tested	compound alone (%) ^a	compound plus kainate (%) ^b
15	kainate (agonist control)	100.0	100.0
	Kynurenic acid	3.4 ± 0.2	9.6 ± 1.3
	γ-DGG	-0.1 ± 0.5	30.8 ± 1.1
	GAMS	1.0 ± 0.6	30.7 ± 1.1
20	GDEE	24.8 ± 5.7	97.8 ± 6.6
	PDA	2.5 ± 0.7	31.3 ± 2.0
	APV	3.1 ± 1.4	73.7 ± 3.2
	CPP	9.1 ± 0.7	78.8 ± 0.9

25

a = % of the response evoked by 30μM kainate immediately before drug application

b = % of the response seen with 30μM kainate alone immediately before application of drug/kainate mixture

30

Oocytes had been injected with 1.25ng of GluR1 *in vitro* sense RNA 3 days prior to the recording. The oocytes were voltage-clamped at -70mV, and the test compounds (all at 1mM, except kainate, which was 30μM) applied by rapid superfusion, with 5 min intervals between drugs. Peak currents were recorded, and each number represents the average of 3 recordings from 3 different oocytes, ± SEM. The 100% current response corresponds to 40 - 200nA, depending on the oocyte.

35

40

5 Table 2. The percent amino acid sequence identity among pairwise combinations of members of the glutamate receptor subunit gene family.

10		GluR1	GluR2	GluR3	GluR4	GluR5
15	GluR1	100	70	69	68	40
	GluR2		100	73	72	40
	GluR3			100	73	41
	GluR4				100	41
	GluR5					100

20
25 The sequences were compared using sequence analysis software from the University of Wisconsin Genetics Computer Group (Devereux, et al., *Nucl. Acids Res.* 12, 387 (1984)). The percent sequence identity between paired sequences was calculated by dividing the number of aligned positions with identical amino acids by the total number of aligned positions in the shortest of the sequences examined and
30 multiplying the quotient by 100.

35 Specification Summary

From the foregoing description, one of ordinary skill in the art can understand that the present invention discloses substantially pure DNA segments encoding a family of glutamate receptor subunit
40 proteins. The glutamate receptors of the invention are proteins, or functional fragments thereof, that have electrophysiological and pharmacological properties characteristic of glutamate receptors, including glutamate receptor subtypes selected from the group
45 consisting of N-methyl-D-aspartate (NMDA), AMPA or quisqualate, kainate (KA) and 2-amino-4-phosphonobutyrate (APB). The new glutamate receptors of the present invention are exemplified by proteins encoded by cDNA clones GluR1, GluR2, GluR3, GluR4 and GluR5, GluR6 and
50 GluR7 and functional combinations thereof, as well as functional peptide fragments encoded thereby. Both the

glutamate receptor genes and proteins of the present invention are useful for drug screening. In addition, the cDNAs from GluR1-GluR7 can be used as probes, by those skilled in the art, without undue experimentation, to identify and isolate other new and novel glutamate receptors. Such other new and novel glutamate receptors are expressly within the scope of the present invention.

Without departing from the spirit and scope of this invention, one of ordinary skill can make various changes and modifications to the invention to adapt it to various usages and conditions. As such, these changes and modifications are properly, equitably, and intended to be, within the full range of equivalence of the following claims.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. One or more substantially pure proteins, fragments, or combinations thereof, wherein said proteins, fragments, or combinations are encoded by DNA having a minimum of about 50% overall nucleotide sequence identity with at least one member of the group consisting of GluR1 DNA (Fig. 1), GluR2 DNA (Fig. 3), GluR3 DNA (Fig. 4), GluR4 DNA (Fig. 9), GluR5 DNA (Fig. 10), GluR6 DNA (Fig. 11) and GluR7 DNA (Fig. 12), and wherein said proteins, fragments, or combinations have electrophysiological and pharmacological properties characteristic of a glutamate receptor.
2. One or more substantially pure proteins, fragments, or combinations thereof, wherein said proteins, fragments, or combinations have a minimum of about 50% amino acid sequence identity with at least one member of the group consisting of GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11) and GluR7 (Fig. 12), and wherein said proteins, fragments, or combinations have electrophysiological and pharmacological properties characteristic of a glutamate receptor.
3. One or more substantially pure proteins, fragments, or combinations thereof, according to claim 1 or 2, wherein said proteins, fragments, or combinations have electrophysiological and pharmacological properties characteristic of at least one glutamate receptor subtype selected from the group consisting of N-methyl-D-aspartate (NMDA) subtype, α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) subtype, kainate (KA) subtype, and 2-amino-4-phosphonobutyrate (APE) subtype.

4. A homodimer of any one of the proteins, fragments, or combinations thereof, as defined in claim 1, 2 or 3, wherein said homodimer has electrophysiological and pharmacological properties characteristic of a glutamate receptor.
5. A heterodimer or heteromultimer of any two or more of the proteins, fragments, or combinations thereof, as defined in claim 1, 2, or 3, wherein said heterodimer or heteromultimer has electrophysiological and pharmacological properties characteristic of a glutamate receptor.
6. Substantially pure nucleic acid probe capable of hybridizing with at least one polynucleotide selected from the group consisting of GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11), and GluR7 (Fig. 12) in a solution containing 1M NaCl, 50mM Tris (pH 8.0), 0.5% sodium dodecyl sulfate, 0.1% sodium pyrophosphate, 100 mg/ml denatured herring sperm DNA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin at 50°C.
7. Substantially pure DNA capable of hybridizing with the complement of at least one polynucleotide selected from GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11), and GluR7 (Fig. 12) in a solution containing 1M NaCl, 50mM Tris (pH 8.0), 0.5% sodium dodecyl sulfate, 0.1% sodium pyrophosphate, 100 mg/ml denatured herring sperm DNA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin at 50°C,

wherein said DNA encodes a protein having electrophysiological and pharmacological properties of at least one glutamate receptor subtype selected from the N-methyl-D-aspartate

(NMDA) subtype, the α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) subtype, the kainate (KA) subtype, or the 2-amino-4-phosphonobutyrate (APE) subtype.

8. DNA according to claim 7, wherein said glutamate receptor subtypes are of the KA or AMPA subtype.

9. A nucleic acid probe comprising an ion channel encoding portion of the DNA as defined in claim 6, 7 or 8.

10. The nucleic acid probe according to claim 9, wherein said probe has a minimum of about 20 base pairs.

11. The nucleic acid probe according to claim 9, wherein said probe is from the carboxyl-encoding end of the DNA sequence.

12. The RNA transcribed from the DNA as defined in claim 6, 7 or 8.

13. Isolated mRNA complementary to the DNA as defined in claim 6, 7 or 8.

14. Genetically engineered oocytes expressing the mRNA as defined in claim 12 or 13.

15. DNA according to claim 6, 7 or 8, encoding a protein having substantially the same amino acid sequence as GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11) or GluR7 (Fig. 12).

16. A nucleic acid probe comprising an ion channel encoding portion of the DNA as defined in claim 15.
17. Isolated mRNA complementary to the DNA as defined in claim 15.
18. Genetically engineered oocytes expressing the mRNA as defined in claim 17.
19. DNA according to claim 6, 7 or 8, wherein said DNA has substantially the same sequence as GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11) or GluR7 (Fig. 12).
20. A nucleic acid probe comprising an ion channel encoding portion of the DNA as defined in claim 19.
21. Isolated mRNA complementary to the DNA as defined in claim 19.
22. Genetically engineered oocytes expressing the mRNA as defined in claim 21.
23. A method for identifying DNA encoding glutamate receptor proteins, the method comprising:
 - (a) contacting DNA with a probe as defined in any one of claims 9, 10, 11, 16 and 20, wherein said contacting is carried out under hybridization conditions; and
 - (b) identifying one or more DNA's which display a substantial degree of hybridization to said probe.

24. A method for identifying a protein capable of functioning as a glutamate receptor, the method comprising:

- (a) contacting said proteins with at least one ligand known to activate a glutamate receptor selected from GluR1 (Fig. 1), GluR2 (Fig. 3), GluR3 (Fig. 4), GluR4 (Fig. 9), GluR5 (Fig. 10), GluR6 (Fig. 11) or GluR7 (Fig. 12);
- (b) measuring ion channel response to said at least one ligand; and
- (c) identifying that protein which exhibits an ion channel response as a functional glutamate receptor.

25. Use of proteins, fragments, or combinations thereof, as defined in claim 1, 2 or 3, for screening a collection of compounds to determine those compounds which bind to a glutamate receptor.

26. A bioassay for evaluating whether a compound is an agonist for a receptor protein as defined in claim 1, 2 or 3, the bioassay comprising:

- (a) culturing cells containing DNA which expresses said receptor protein, wherein said culturing is carried out in the presence of at least one compound whose ability to induce the ion channel activity of said receptor protein is sought to be determined; and thereafter
- (b) monitoring said cells for changes in ion channel activity as a result of the presence of said compound.

27. A bioassay for evaluating whether test compounds are antagonists for a receptor protein as defined in claim 1, 2 or 3, the bioassay comprising:

- (a) culturing cells containing DNA which expresses said receptor protein, wherein said culturing is carried out in the presence of:

- i. increasing concentrations of a test compound whose ability to inhibit the ion channel activity of said receptor protein is sought to be determined; and
 - ii. a fixed concentration of at least one agonist for said receptor protein; and thereafter
- (b) monitoring in said cells changes in ion channel activity.
28. Use of at least one protein, fragment, or combination thereof, as defined in claim 1, 2 or 3, for the preparation of a medicament for the modification of the ion channel activity of glutamate receptors.
29. A method for identifying a DNA encoding glutamate receptor protein, the method comprising:
- (a) contacting DNA with the probe DNA as defined in any one of claims 9, 10, 11, 16 and 20, wherein said contacting is carried out under hybridization conditions; and
 - (b) identifying a DNA which displays a substantial degree of hybridization to said probe.

GluR1

AATTCGGCACGAGCTCGGC
-240

TCCCCTTCCAAGAGAAACAAGAGAAACCTCACGGAAGGAAGGGAGGAAGGAAAGAAGCA
-220 -200 -180

AGCAAGGAACTGCAGGAAGAAAAGAGCCGGCAGAGCATCAAGAAGAATCGAAGGGAGGGG
-160 -140 -120

AGGGAAGACCAAATCTATGGTTGGACCAGGGCTTCTTTTCGCCAATGTAAAAAGGAAT
-100 -80 -60

-10

Met Pro Tyr Ile Phe Ala Phe Phe Cys Thr Gly Phe Leu Gly Ala
ATG CCG TAC ATC TTT GCC TTT TTC TGC ACC GGT TTT CTA GGT GCG
-40 -20

-1 1 10

Val Val Gly Ala Asn Phe Pro Asn Asn Ile Gln Ile Gly Gly Leu
GTT GTG GGT GCC AAT TTC CCC AAC AAT ATC CAG ATA GGG GGG TTA
1 20

20

Phe Pro Asn Gln Gln Ser Gln Glu His Ala Ala Phe Arg Phe Ala
TTT CCA AAC CAA CAA TCA CAG GAA CAT GCG GCT TTT AGG TTT GCT
40 60 80

30 40

Leu Ser Gln Leu Thr Glu Pro Pro Lys Leu Leu Pro Gln Ile Asp
TTG TCA CAA CTC ACG GAG CCC CCC AAG CTG CTT CCC CAG ATC GAT
100 120

*

50

Ile Val Asn Ile Ser Asp Thr Phe Glu Met Thr Tyr Arg Phe Cys
ATT GTG AAC ATC AGC GAC ACG TTT GAG ATG ACT TAC CGT TTC TGT
140 160

60 70

Ser Gln Phe Ser Lys Gly Val Tyr Ala Ile Phe Gly Phe Tyr Glu
TCC CAG TTC TCC AAA GGA GTC TAT GCC ATC TTT GGA TTT TAT GAA
180 200

80

Arg Arg Thr Val Asn Met Leu Thr Ser Phe Cys Gly Ala Leu His
CGA AGG ACT GTC AAC ATG CTG ACC TCC TTC TGT GGG GCC CTC CAT
220 240 260

90 100

Val Cys Phe Ile Thr Pro Ser Phe Pro Val Asp Thr Ser Asn Gln
GTG TGC TTC ATT ACT CCA AGT TTT CCT GTT GAC ACA TCC AAT CAA
280 300

FIG. 1-1

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Phe Val Leu Gln Leu Arg Pro Glu Leu Gln Glu Ala Leu Ile Ser
 TTT GTC CTT CAG CTA CGC CCG GAA CTA CAG GAA GCT CTC ATT AGC
 110 320 340

Ile Ile Asp His Tyr Lys Trp Gln Thr Phe Val Tyr Ile Tyr Asp
 ATT ATC GAC CAT TAC AAG TGG CAA ACC TTT GTC TAC ATT TAT GAT
 120 130 360 380

Ala Asp Arg Gly Leu Ser Val Leu Gln Arg Val Leu Asp Thr Ala
 GCT GAC CGG GGC CTG TCA GTC CTG CAG AGA GTC TTG GAT ACA GCC
 400 420 440

Ala Glu Lys Asn Trp Gln Val Thr Ala Val Asn Ile Leu Thr Thr
 GCA GAG AAG AAC TGG CAG GTA ACG GCT GTC AAC ATT CTG ACA ACC
 150 160 460 480

Thr Glu Glu Gly Tyr Arg Met Leu Phe Gln Asp Leu Glu Lys Lys
 ACC GAG GAA GGA TAC CGG ATG CTC TTT CAG GAC CTG GAG AAG AAA
 170 500 520

Lys Glu Arg Leu Val Val Val Asp Cys Glu Ser Glu Arg Leu Asn
 AAG GAG AGG CTG GTG GTG GTT GAC TGT GAA TCA GAA CGC CTC AAC
 180 190 540 560

Ala Ile Leu Gly Gln Ile Val Lys Leu Glu Lys Asn Gly Ile Gly
 GCC ATC CTG GGC CAG ATC GTG AAG CTA GAA AAG AAT GGC ATC GGC
 580 600 620

Tyr His Tyr Ile Leu Ala Asn Leu Gly Phe Met Asp Ile Asp Leu
 TAC CAC TAC ATC CTC GCC AAT CTG GGC TTC ATG GAC ATT GAC TTA
 210 220 640 660

Asn Lys Phe Lys Glu Ser Gly Arg Asn Val Thr Gly Phe Gln Leu
 AAT AAG TTC AAG GAG AGC GGA CGC AAT GTG ACA GGT TTC CAG CTG
 230 * 680 700

Val Asn Tyr Thr Asp Thr Ile Pro Ala Arg Ile Met Gln Gln Trp
 GTG AAC TAC ACA GAC ACG ATC CCA GCC AGA ATC ATG CAG CAA TGG
 240 250 720 740

Arg Thr Ser Asp Ser Arg Asp His Thr Arg Val Asp Trp Lys Arg
 AGG ACA AGT GAC TCC CGA GAC CAT ACC AGG GTG GAC TGG AAG AGG
 760 780 800

Pro Lys Tyr Thr Ser Ala Leu Thr Tyr Asp Gly Val Lys Val Met
 CCA AAG TAC ACT TCT GCT CTC ACC TAT GAT GGT GTC AAG GTG ATG
 270 280 820 840

FIG. 1-2

Handwritten signature/initials

GluR1

Ala Glu Ala Phe Gln Ser Leu Arg Arg Gln Arg Ile Asp Ile Ser
 GCT GAG GCC TTC CAA AGC CTG CGG AGG CAG AGG ATT GAC ATA TCC
 290 860 880

Arg Arg Gly Asn Ala Gly Asp Cys Leu Ala Asn Pro Ala Val Pro
 CGC CGG GGG AAT GCT GGG GAC TGT CTG GCT AAC CCA GCT GTG CCC
 300 900 920 310

Trp Gly Gln Gly Ile Asp Ile Gln Arg Ala Leu Gln Gln Val Arg
 TGG GGT CAA GGG ATC GAC ATC CAG AGA GCC CTG CAG CAG GTG CGC
 940 960 980

Phe Glu Gly Leu Thr Gly Asn Val Gln Phe Asn Glu Lys Gly Arg
 TTC GAA GGT TTG ACA GGA AAT GTG CAG TTC AAC GAG AAA GGG CGC
 330 1000 1020 340

Arg Thr Asn Tyr Thr Leu His Val Ile Glu Met Lys His Asp Gly
 CGG ACC AAT TAC ACC CTC CAC GTG ATC GAA ATG AAA CAT GAT GGA
 350 1040 1060

Ile Arg Lys Ile Gly Tyr Trp Asn Glu Asp Asp Lys Phe Val Pro
 ATC CGA AAG ATT GGT TAC TGG AAT GAA GAC GAT AAA TTT GTC CCC
 360 1080 1100 370

Ala Ala Thr Asp Ala Gln Ala Gly Gly Asp Asn Ser Ser Val Gln
 GCA GCC ACC GAC GCT CAG GCT GGA GGG GAC AAC TCA AGC GTC CAG
 1120 1140 1160

Asn Arg Thr Tyr Ile Val Thr Thr Ile Leu Glu Asp Pro Tyr Val
 AAT AGG ACC TAC ATC GTC ACT ACT ATC CTC GAA GAT CCT TAC GTG
 390 1180 1200 400

Met Leu Lys Lys Asn Ala Asn Gln Phe Glu Gly Asn Asp Arg Tyr
 ATG CTT AAA AAG AAT GCC AAC CAG TTT GAG GGC AAT GAC CGC TAT
 410 1220 1240

Glu Gly Tyr Cys Val Glu Leu Ala Ala Glu Ile Ala Lys His Val
 GAG GGC TAC TGT GTG GAG CTG GCT GCA GAG ATC GCC AAG CAC GTG
 420 1260 1280 430

Gly Tyr Ser Tyr Arg Leu Glu Ile Val Ser Asp Gly Lys Tyr Gly
 GGC TAC TCC TAC CGA CTT GAG ATT GTC AGC GAC GGC AAA TAT GGA
 1300 1320 1340

FIG. 1-3

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GluR1

Ala	Arg	Asp	Pro	Asp	Thr	Lys	Ala	Trp	Asn	Gly	Met	Val	Gly	Glu
GCC	CGG	GAT	CCC	GAC	ACA	AAG	GCT	TGG	AAT	GGC	ATG	GTG	GGA	GAA
		450				1360						460		
Leu	Val	Tyr	Gly	Arg	Ala	Asp	Val	Ala	Val	Ala	Pro	Leu	Thr	Ile
CTG	GTC	TAT	GGA	AGA	GCA	GAC	GTG	GCT	GTG	GCT	CCC	TTG	ACC	ATA
			1400							1420				
Thr	Leu	Val	Arg	Glu	Glu	Val	Ile	Asp	Phe	Ser	Lys	Pro	Phe	Met
ACC	TTG	GTC	CGG	GAG	GAA	GTC	ATC	GAC	TTC	TCC	AAG	CCA	TTC	ATG
		480						1460				490		
Ser	Leu	Gly	Ile	Ser	Ile	Met	Ile	Lys	Lys	Pro	Gln	Lys	Ser	Lys
AGT	TTG	GGA	ATC	TCC	ATT	ATG	ATT	AAG	AAG	CCA	CAG	AAG	TCC	AAG
		1480				1500								1520
Pro	Gly	Val	Phe	Ser	Phe	Leu	Asp	Pro	Leu	Ala	Tyr	Glu	Ile	Trp
CCA	GGT	GTC	TTC	TCC	TTT	CTT	GAC	CCT	TTG	GCC	TAT	GAG	ATC	TGG
		510				1540						520		
Met	Cys	Ile	Val	Phe	Ala	Tyr	Ile	Gly	Val	Ser	Val	Val	Leu	Phe
ATG	TGT	ATA	GTG	TTT	GCC	TAC	ATT	GGA	GTG	AGC	GTC	GTC	CTC	TTC
			1580							1600				
Leu	Val	Ser	Arg	Phe	Ser	Pro	Tyr	Glu	Trp	His	Ser	Glu	Glu	Phe
CTG	GTC	AGC	CGT	TTC	AGC	CCC	TAC	GAA	TGG	CAC	AGC	GAA	GAG	TTT
		540						1640				550		
Glu	Glu	Gly	Arg	Asp	Gln	Thr	Thr	Ser	Asp	Gln	Ser	Asn	Glu	Phe
GAA	GAG	GGA	CGA	GAC	CAG	ACA	ACC	AGT	GAC	CAG	TCA	AAT	GAG	TTT
		1660					1680						1700	
Gly	Ile	Phe	Asn	Ser	Leu	Trp	Phe	Ser	Leu	Gly	Ala	Phe	Met	Gln
GGC	ATA	TTC	AAC	AGC	CTG	TGG	TTC	TCC	CTG	GGG	GCC	TTC	ATG	CAG
		570				1720						580		
Gln	Gly	Cys	Asp	Ile	Ser	Pro	Arg	Ser	Leu	Ser	Gly	Arg	Ile	Val
CAA	GGA	TGT	GAC	ATT	TCC	CCC	AGG	TCC	CTG	TCC	GGA	CGC	ATC	GTC
			1760							1780				
Gly	Gly	Val	Trp	Trp	Phe	Phe	Thr	Leu	Ile	Ile	Ile	Ser	Ser	Tyr
GGC	GGC	GTC	TGG	TGG	TTC	TTC	ACT	TTG	ATC	ATC	ATC	TCC	TCG	TAC
		600						1820				610		
Thr	Ala	Asn	Leu	Ala	Ala	Phe	Leu	Thr	Val	Glu	Arg	Met	Val	Ser
ACA	GCC	AAC	CTG	GCT	GCC	TTC	CTG	ACT	GTG	GAG	AGG	ATG	GTG	TCT
			1840				1860						1880	

FIG. 1-4

Handwritten signature

GluR1

630
 Pro Ile Glu Ser Ala Glu Asp Leu Ala Lys Gln Thr Glu Ile Ala
 CCC ATT GAG AGT GCA GAG GAC CTG GCA AAG CAG ACG GAA ATT GCT
 1900 1920

650
 Tyr Gly Thr Leu Glu Ala Gly Ser Thr Lys Glu Phe Phe Arg Arg
 TAT GGG ACA TTG GAA GCA GGC TCC ACT AAG GAG TTC TTC AGG AGA
 1940 1960

660
 Ser Lys Ile Ala Val Phe Glu Lys Met Trp Thr Tyr Met Lys Ser
 TCT AAA ATC GCT GTG TTT GAG AAG ATG TGG ACA TAC ATG AAG TCT
 1980 2000

680
 Ala Glu Pro Ser Val Phe Val Arg Thr Thr Glu Glu Gly Met Ile
 GCA GAA CCA TCC GTG TTT GTT CGG ACC ACA GAG GAA GGC ATG ATC
 2020 2040 2060

690
 Arg Val Arg Lys Ser Lys Gly Lys Tyr Ala Tyr Leu Leu Glu Ser
 AGA GTG AGA AAA TCT AAA GGC AAA TAC GCC TAC CTC CTG GAG TCC
 2080 2100

710
 Thr Met Asn Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met
 ACC ATG AAT GAG TAT ATT GAG CAA CGA AAG CCC TGT GAC ACC ATG
 2120 2140

720
 Lys Val Gly Gly Asn Leu Asp Ser Lys Gly Tyr Gly Ile Ala Thr
 AAA GTG GGA GGT AAC TTG GAT TCC AAA GGC TAT GGC ATT GCG ACA
 2160 2180

740
 Pro Lys Gly Ser Ala Leu Arg Asn Pro Val Asn Leu Ala Val Leu
 CCC AAG GGG TCC GCC CTG AGA AAT CCA GTA AAC CTG GCA GTG TTA
 2200 2220 2240

750
 Lys Leu Asn Glu Gln Gly Leu Leu Asp Lys Leu Lys Asn Lys Trp
 AAA CTG AAC GAG CAG GGG CTT TTG GAC AAA TTG AAA AAC AAA TGG
 2260 2280

770
 Trp Tyr Asp Lys Gly Glu Cys Gly Thr Gly Gly Gly Asp Ser Lys
 TGG TAC GAC AAG GGC GAG TGC GGC ACG GGG GGA GGT GAC TCC AAG
 2300 2320

780
 Asp Lys Thr Ser Ala Leu Ser Leu Ser Asn Val Ala Gly Val Phe
 GAC AAG ACC AGC GCT TTG AGC CTC AGC AAT GTG GCA GGC GTG TTC
 2340 2360

790

FIG. 1-5

GluR1

Tyr Ile Leu Ile Gly Gly Leu Gly 800
TAC ATC CTG ATT GGA GGG CTG GGA CTG GCC ATG CTG GTT GCC TTA
2380 2400 2420

Ile Glu Phe 810
ATC GAG TTC TGC TAC Lys Ser Arg Ser Glu Ser Lys Arg 820
2440 2460 Met Lys
ATG AAG CGG ATG AAG

Gly Phe Cys Leu Ile Pro Gln 830
GGT TTC TGT TTG ATC CCA CAG CAA TCC ATC Asn Glu Ala Ile Arg
2480 2500 AAT GAA GCC ATA CGG

Thr Ser Thr 840
ACA TCG ACC CTC CCC CGG AAC AGT GGG GCA GGA GCC Ser Gly Gly
2520 2540 AGC GGA GGA

Gly Gly Ser Gly Glu Asn Gly Arg 860
GGC GGC AGT GGA GAG AAT GGC CGG GTG GTC Ser Gln Asp Phe Pro
2560 2580 2600

Lys Ser Met 870
AAG TCC ATG CAA TCC Ile Pro Cys Met Ser His Ser Ser Gly Met
2620 2640 ATT CCC TGC ATG AGT CAC AGT TCA GGG ATG

Pro Leu Gly Ala Thr Gly Leu 890
CCC TTG GGA GCC ACA GGA TTG TAA CTGGAGCAGACAGGAAACCCTTGGGG
2660 2680

AGCAGGCTCAGGCTTCCACAGCCCCATCCCAAGCCCTTCAGTGCC
2700 2720 2740

FIG. 1-6

Travis Clark

FIG. 2a

Glur-K1	..	C	F	I	T	P	S	-	F	P	V	D	T	S	N	Q	..
nAChR- α 1	..	C	E	I	I	V	T	H	F	P	F	D	E	Q	N	C	..
nAChR- α 2	..	C	S	I	D	V	T	F	F	P	F	D	Q	Q	N	C	..
nAChR- α 3	..	C	K	I	D	V	T	Y	F	P	F	D	Y	Q	N	C	..
nAChR- α 4	..	C	S	I	D	V	T	F	F	P	F	D	Q	Q	N	C	..
nAChR- β 1	..	C	S	I	Q	V	T	Y	F	P	F	D	W	Q	N	C	..
nAChR- β 2	..	C	K	I	E	V	K	H	F	P	F	D	Q	Q	N	C	..
nAChR- β 3	..	C	T	M	D	V	T	F	F	P	F	D	R	Q	N	C	..
nAChR- β 4	..	C	K	I	E	V	K	H	F	P	F	D	Q	Q	N	C	..
nAChR- γ	..	C	P	I	A	V	T	Y	F	P	F	D	W	Q	N	C	..
nAChR- δ	..	C	P	I	S	V	T	Y	F	P	F	D	W	Q	N	C	..
GABA- α	..	C	P	M	H	L	E	D	F	P	M	D	A	H	A	C	..
GABA- β	..	C	M	M	D	L	R	R	Y	P	L	D	E	Q	N	C	..
GlyR 48k	..	C	P	M	D	L	K	N	F	P	M	D	V	Q	T	C	..

FIG. 2b

Glur-K1	..	Y	E	I	W	M	-	C	I	V	F	A	Y	I	G	V	S	V	V	L	F	L	V	S	R	F	S	P	..
nAChR- α 1	..	G	E	K	M	T	L	S	I	-	S	V	L	L	S	L	T	V	F	L	L	V	I	V	E	L	I	P	..
nAChR- α 2	..	G	E	K	I	T	L	C	I	-	S	V	L	L	S	L	T	V	F	L	L	L	I	T	E	I	I	P	..
nAChR- α 3	..	G	E	K	V	T	L	C	I	-	S	V	L	L	S	L	T	V	F	L	L	V	I	T	E	T	I	P	..
nAChR- α 4	..	G	E	K	V	T	L	C	I	-	S	V	L	L	S	L	T	V	F	L	L	L	I	T	E	I	I	P	..
nAChR- β 1	..	G	E	K	M	G	L	S	I	-	F	A	L	L	T	L	T	V	F	L	L	L	L	A	D	K	V	P	..
nAChR- β 2	..	G	E	K	M	T	L	C	I	-	S	V	L	L	A	L	T	V	F	L	L	L	I	S	K	I	V	P	..
nAChR- β 3	..	G	E	K	L	S	L	S	T	-	S	V	L	V	S	L	T	V	F	L	L	V	I	E	E	I	I	P	..
nAChR- β 4	..	G	E	K	M	T	L	C	I	-	S	V	L	L	A	L	T	F	F	L	L	L	I	S	K	I	V	P	..
nAChR- γ	..	G	Q	K	C	T	V	A	T	-	N	V	L	L	A	Q	T	V	F	L	F	L	V	A	K	K	V	P	..
nAChR- δ	..	G	E	K	T	S	V	A	I	-	S	V	L	L	A	Q	S	V	F	L	L	L	I	S	K	R	L	P	..
GABA- α	..	N	R	E	S	V	P	A	R	-	T	V	F	G	V	T	T	V	L	T	M	T	T	L	S	I	S	A	..
GABA- β	..	N	Y	D	A	S	A	A	R	V	-	A	L	G	I	T	T	V	L	T	M	T	T	I	S	T	H	L	..
GlyR 48k	..	N	M	D	A	A	P	A	R	V	-	G	L	G	I	T	T	V	L	T	M	T	T	Q	S	S	G	S	..

Handwritten signature: Charles A. Clark

GluR2

GAATTCGGCACGAGGTGC
-370ATGGGAGGGTGCTGATATTCCCAGACACCAGGACTACAGCGGCAGCTCAGCTAAAACT
-350 -330 -310GCATTCAGCCAGTCCTCGGGACTTCGGGAGCAGGGACAGGACGCAAGGCATCAACAGCCA
-290 -270 -250CCAGCTACAACCTGGGAAATAAGGATTCTTCTGCCTTCACTTCGTGTTTTTAGCAGCTCC
-230 -210 -190TTGCTAAATATCGACCTCACAATGCAGAGGATCTAATTTGCTGAGGAAAACAGTCAAAGA
-170 -150 -130AGGAAGAGGAAGAAAGGGAAACGAGGGGATATTTTGTGGATGCTCTACTTTTCTTGGAA
-110 -90 -70

Met	Gln	Lys	Ile	Met	His	Ile	Ser	Val	Leu	Leu	Ser	Pro	Val	Leu
ATG	CAA	AAG	ATT	ATG	CAT	ATT	TCT	GTC	CTC	CTT	TCT	CCT	GTT	TTA
				-50							-30			

Trp	Gly	Leu	Ile	Phe	Gly	Val	Ser	Ser	Asn	Ser	Ile	Gln	Ile	Gly
TGG	GGA	CTG	ATT	TTT	GGT	GTC	TCT	TCT	AAC	AGC	ATA	CAG	ATA	GGG
		-10			1	1			10					

Gly	Leu	Phe	Pro	Arg	Gly	Ala	Asp	Gln	Glu	Tyr	Ser	Ala	Phe	Arg
GGG	CTA	TTT	CCA	AGG	GGC	GCT	GAT	CAA	GAA	TAC	AGT	GCA	TTT	CGG
30							50							70

Val	Gly	Met	Val	Gln	Phe	Ser	Thr	Ser	Glu	Phe	Arg	Leu	Thr	Pro
GTA	GGG	ATG	GTT	CAG	TTT	TCC	ACT	TCG	GAG	TTC	AGA	CTG	ACA	CCC
					30							110		

His	Ile	Asp	Asn	Leu	Glu	Val	Ala	Asn	Ser	Phe	Ala	Val	Thr	Asn
CAT	ATC	GAC	AAT	TTG	GAG	GTA	GCC	AAC	AGT	TTC	GCA	GTC	ACC	AAT
			130							50				

Ala	Phe	Cys	Ser	Gln	Phe	Ser	Arg	Gly	Val	Tyr	Ala	Ile	Phe	Gly
GCT	TTC	TGC	TCC	CAG	TTT	TCA	AGA	GGA	GTC	TAC	GCA	ATT	TTT	GGA
		170			60				190					

Phe	Tyr	Asp	Lys	Lys	Ser	Val	Asn	Thr	Ile	Thr	Ser	Phe	Cys	Gly
TTT	TAT	GAC	AAG	AAG	TCT	GTA	AAT	ACC	ATC	ACA	TCA	TTC	TGT	GGG
210						230				80			250	

FIG. 3-1

Thanks & Clerk

GluR2

Thr Leu His Val Ser Phe Ile Thr Pro Ser Phe Pro Thr Asp Gly
 ACA CTC CAT GTG TCC TTC ATC ACA CCT AGC TTC CCA ACA GAT GGC
 90 270 290

Thr His Pro Phe Val Ile Gln Met Arg Pro Asp Leu Lys Gly Ala
 ACA CAT CCA TTT GTC ATC CAG ATG CGA CCT GAC CTC AAA GGA GCA
 100 310 110 330

Leu Leu Ser Leu Ile Glu Tyr Tyr Gln Trp Asp Lys Phe Ala Tyr
 CTC CTT AGC TTG ATT GAG TAC TAC CAA TGG GAC AAG TTC GCA TAC
 120 350 370

Leu Tyr Asp Ser Asp Arg Gly Leu Ser Thr Leu Gln Ala Val Leu
 CTC TAT GAC AGT GAC AGA GGC TTA TCA ACA CTG CAA GCT GTT CTG
 130 390 140 410 430

Asp Ser Ala Ala Glu Lys Lys Trp Gln Val Thr Ala Ile Asn Val
 GAT TCT GCT GCA GAG AAG AAG TGG CAG GTG ACT GCT ATC AAT GTG
 150 450 470

Gly Asn Ile Asn Asn Asp Lys Lys Asp Glu Thr Tyr Arg Ser Leu
 GGG AAC ATC AAC AAT GAC AAG AAA GAT GAG ACC TAC AGA TCG CTC
 160 490 170 510

Phe Gln Asp Leu Glu Leu Lys Lys Glu Arg Arg Val Ile Leu Asp
 TTT CAA GAT CTG GAG TTA AAA AAA GAA CGG CGT GTA ATC CTG GAC
 180 530 550

Cys Glu Arg Asp Lys Val Asn Asp Ile Val Asp Gln Val Ile Thr
 TGT GAA AGG GAT AAA GTA AAT GAC ATT GTG GAC CAG GTT ATT ACC
 190 570 200 590 610

Ile Gly Lys His Val Lys Gly Tyr His Tyr Ile Ile Ala Asn Leu
 ATT GGA AAA CAT GTT AAA GGG TAC CAT TAT ATC ATT GCA AAT CTG
 210 630 650

Gly Phe Thr Asp Gly Asp Leu Leu Lys Ile Gln Phe Gly Gly Ala
 GGA TTC ACT GAT GGG GAC CTG CTG AAA ATT CAG TTT GGA GGA GCA
 220 670 230 690

Asn Val Ser Gly Phe Gln Ile Val Asp Tyr Asp Asp Ser Leu Val
 AAT GTC TCT GGA TTT CAG ATT GTA GAC TAC GAC GAT TCC CTG GTG
 240 710 730

FIG. 3-2

2071879

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GluR2

250 Ser Lys Phe Ile Glu Arg Trp Ser Thr Leu 260 Glu Glu Lys Glu Tyr
 TCT AAA TTT ATA GAA AGA TGG TCA ACA CTG GAA GAG AAA GAA TAC
 750 770 790

Pro Gly Ala His Thr 270 Ala Thr Ile Lys Tyr Thr Ser Ala Leu Thr
 CCT GGA GCA CAC ACA GCG ACA ATT AAG TAT ACT TCG GCC CTG ACG
 810 830

280 Tyr Asp Ala Val Gln Val Met Thr Glu Ala 290 Phe Arg Asn Leu Arg
 TAT GAT GCT GTC CAA GTG ATG ACT GAA GCA TTC CGT AAC CTT CGG
 850 870

Lys Gln Arg Ile Glu 300 Ile Ser Arg Arg Gly Asn Ala Gly Asp Cys
 AAG CAG AGG ATT GAA ATA TCC CGG AGA GGA AAT GCA GGG GAT TGT
 890 910

310 Leu Ala Asn Pro Ala Val Pro Trp Gly Gln 320 Gly Val Glu Ile Glu
 TTG GCC AAC CCA GCT GTG CCC TGG GGA CAA GGG GTC GAA ATA GAA
 930 950 970

Arg Ala Leu Lys Gln 330 Val Gln Val Glu Gly Leu Ser Gly Asn Ile
 AGG GCC CTC AAG CAG GTT CAA GTT GAA GGC CTC TCT GGA AAT ATA
 990 1010

340 Lys Phe Asp Gln Asn Gly Lys Arg Ile Asn 350 Tyr Thr Ile Asn Ile
 AAG TTT GAC CAG AAT GGA AAA CGA ATA AAC TAC ACA ATT AAC ATC
 1030 1050

Met Glu Leu Lys Thr 360 Asn Gly Pro Arg Lys Ile Gly Tyr Trp Ser
 ATG GAG CTC AAA ACA AAT GGA CCC CGG AAG ATT GGG TAC TGG AGT
 1070 1090

370 Glu Val Asp Lys Met Val Val Thr Leu Thr 380 Glu Leu Pro Ser Gly
 GAA GTG GAT AAA ATG GTT GTC ACC CTA ACT GAG CTC CCA TCA GGA
 1130 1150

Asn Asp Thr Ser Gly 390 Leu Glu Asn Lys Thr Val Val Val Thr Thr
 AAT GAC ACG TCT GGG CTT GAA AAC AAA ACT GTG GTG GTC ACC ACA
 1170 1190

400 Ile Leu Glu Ser Pro Tyr Val Met Met Lys 410 Lys Asn His Glu Met
 ATA TTG GAA TCT CCA TAT GTT ATG ATG AAG AAA AAT CAT GAA ATG
 1210 1230

FIG. 3-3

GluR2

420
 Leu Glu Gly Asn Glu Arg Tyr Glu Gly Tyr Cys Val Asp Leu Ala
 CTT GAA GGG AAT GAG CGT TAC GAG GGC TAC TGT GTT GAC TTA GCT
 1250 1270

430 440
 Ala Glu Ile Ala Lys His Cys Gly Phe Lys Tyr Lys Leu Thr Ile
 GCA GAA ATT GCC AAA CAC TGT GGG TTC AAG TAC AAG CTG ACT ATT
 1310 1330

450
 Val Gly Asp Gly Lys Tyr Gly Ala Arg Asp Ala Asp Thr Lys Ile
 GTT GGG GAT GGC AAG TAT GGG GCC AGG GAT GCC GAC ACC AAA ATT
 1350 1370

460 470
 Trp Asn Gly Met Val Gly Glu Leu Val Tyr Gly Lys Ala Asp Ile
 TGG AAT GGT ATG GTT GGA GAG CTT GTC TAC GGG AAA GCT GAC ATT
 1390 1410

480
 Ala Ile Ala Pro Leu Thr Ile Thr Leu Val Arg Glu Glu Val Ile
 GCA ATT GCT CCA TTA ACT ATC ACT CTC GTG AGA GAA GAG GTG ATT
 1430 1450

490 500
 Asp Phe Ser Lys Pro Phe Met Ser Leu Gly Ile Ser Ile Met Ile
 GAC TTC TCC AAG CCC TTC ATG AGT CTT GGA ATC TCT ATC ATG ATC
 1490 1510

510
 Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe Ser Phe Leu Asp
 AAG AAG CCT CAG AAG TCC AAA CCA GGA GTG TTT TCC TTT CTT GAT
 1530 1550

520 530
 Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe Ala Tyr Ile
 CCT TTA GCC TAT GAG ATC TGG ATG TGC ATT GTG TTT GCC TAC ATT
 1570 1590

540
 Gly Val Ser Val Val Leu Phe Leu Val Ser Arg Phe Ser Pro Tyr
 GGG GTC AGT GTA GTT TTA TTC CTG GTC AGC AGA TTT AGC CCC TAC
 1610 1630

550 560
 Glu Trp His Thr Glu Glu Phe Glu Asp Gly Arg Glu Thr Gln Ser
 GAG TGG CAC ACT GAG GAA TTT GAA GAT GGA AGA GAA ACA CAA AGT
 1670 1690

570
 Ser Glu Ser Thr Asn Glu Phe Gly Ile Phe Asn Ser Leu Trp Phe
 AGT GAA TCA ACT AAT GAA TTT GGG ATT TTT AAT AGT CTC TGG TTT
 1710 1730

FIG. 3-4

Handwritten signature: Mark A. Clark

GluR2

580 Ser Leu Gly Ala Phe Met Arg Gln Gly Cys 590 Ile Ser Pro Arg
 TCC TTG GGT GCC TTT ATG CGG CAG GGA TGC GAT ATT TCG CCA AGA
 1750 1770

Ser Leu Ser Gly Arg 600 Ile Val Gly Gly Val Trp Trp Phe Phe Thr
 TCC CTC TCT GGG CGC ATT GTT GGA GGT GTG TGG TGG TTC TTT ACC
 1790 1810

610 Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn 620 Leu Ala Ala Phe Leu
 CTG ATC ATA ATC TCC TCC TAC ACG GCT AAC TTA GCT GCC TTC CTG
 1850 1870

Thr Val Glu Arg Met 630 Val Ser Pro Ile Glu Ser Ala Glu Asp Leu
 ACT GTA GAG AGG ATG GTG TCT CCC ATC GAA AGT GCT GAG GAT CTG
 1890 1910

640 Ser Lys Gln Thr Glu Ile Ala Tyr Gly Thr 650 Leu Asp Ser Gly Ser
 TCT AAG CAA ACA GAA ATT GCT TAT GGA ACA TTA GAC TCT GGC TCC
 1930 1950

Thr Lys Glu Phe Phe 660 Arg Arg Ser Lys Ile Ala Val Phe Asp Lys
 ACT AAA GAG TTT TTC AGG AGA TCT AAA ATC GCA GTG TTT GAT AAA
 1970 1990

670 Met Trp Thr Tyr Met Arg Ser Ala Glu Pro 680 Ser Val Phe Val Arg
 ATG TGG ACT TAT ATG AGG AGT GCA GAG CCC TCT GTG TTT GTG AGG
 2030 2050

Thr Thr Ala Glu Gly 690 Val Ala Arg Val Arg Lys Ser Lys Gly Lys
 ACT ACC GCA GAA GGA GTA GCC AGA GTC CGG AAA TCC AAA GGA AAG
 2070 2090

700 Tyr Ala Tyr Leu Leu Glu Ser Thr Met Asn 710 Glu Tyr Ile Glu Gln
 TAT GCC TAC TTG CTG GAG TCC ACA ATG AAC GAG TAC ATC GAG CAG
 2110 2130

Arg Lys Pro Cys Asp 720 Thr Met Lys Val Gly Gly Asn Leu Asp Ser
 AGG AAG CCT TGT GAC ACC ATG AAA GTG GGA GGA AAC TTG GAT TCC
 2150 2170

730 Lys Gly Tyr Gly Ile Ala Thr Pro Lys Gly Ser Ser Leu Gly Asn
 AAA GGC TAC GGC ATC GCC ACA CCT AAA GGA TCC TCA TTA GGA AAT
 2210 2230

FIG. 3-5

Handwritten signature: Mark & Clark

GluR2

Ala Val Asn Leu Ala Val⁷⁵⁰ Leu Lys Leu Asn Glu Gln Gly Leu Leu
GCG GTT AAC CTC GCA GTA²²⁵⁰ CTA AAA CTG AAT GAA CAA GGC CTG TTG²²⁷⁰

760 Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys⁷⁷⁰ Gly Glu Cys Gly
GAC AAA TTG AAA AAC AAA TGG TGG TAC GAC AAA²³¹⁰ GGA GAG TGC GGC

780 Ser Gly Gly Gly Asp Ser Lys Glu Lys Thr Ser Ala Leu Ser Leu
AGC GGG GGA GGT GAT TCC AAG GAA AAG ACC AGT GCC CTC AGT CTG²³⁵⁰

790 Ser Asn Val Ala Gly Val Phe Tyr Ile Leu⁸⁰⁰ Val Gly Gly Leu Gly
AGC AAC GTT GCT GGA GTA TTC TAC ATC CTT GTC GGG GGC CTT GGT²⁴¹⁰

810 Leu Ala Met Leu Val Ala Leu Ile Glu Phe Cys Tyr Lys Ser Arg
TTG GCA ATG CTG GTG GCT TTG ATT GAG TTC TGT TAC AAG TCA AGG²⁴⁵⁰

820 Ala Glu Ala Lys Arg Met Lys Val Ala Lys⁸³⁰ Asn Pro Gln Asn Ile
GCC GAG GCG AAA CGA ATG AAG GTG GCA AAG AAT CCA CAG AAT ATT²⁴⁹⁰

840 Asn Pro Ser Ser Ser Gln Asn Ser Gln Asn Phe Ala Thr Tyr Lys
AAC CCA TCT TCC TCG CAG AAT TCC CAG AAT TTT GCA ACT TAT AAG²⁵¹⁰

850 Glu Gly Tyr Asn Val Tyr Gly Ile Glu Ser⁸⁶⁰ Val Lys Ile
GAA GGT TAC AAC GTA TAT GGC ATC GAG AGT GTT AAA ATT TAG GGA²⁵⁷⁰ GGA²⁵⁹⁰

TGACCTTGAGTGATGTCATGAGGAGCAAGGCAAGGCTGTCAATTACAGGAAGTACTGGA²⁶¹⁰
GAAAATGGACGTGTTATGACTCCAGAATTTCCCAAAGCAGTGCATGCTGTCCCTTACGTG²⁶³⁰
AGTCCTGGCATGGGAATGAATGTCAGTGTGACTGATCTCTCGTGATTGATAGGAACCTT²⁶⁵⁰
CTGAGTGCCTTACACAATGGTTTCCTTGTGTGTTTATTGTCAAAGTGGTGAGAGGCATCC²⁶⁹⁰
GATATCTTGAAGGCTTTTCTTTTCAGCCAAGAATTCTTAAGTATGTGGAGTTCACCTTGA²⁷¹⁰
ATTGTAAGGAAAGATAAATTACAAACAGAGCATCATTTTCTACTCCGATATCAGAGGAAG²⁷³⁰
CGTGGTGGACATGCACAGCTAACATGGAAATACTATCATTTAACTGAAGTCTTTGTACA²⁷⁵⁰
GACAACAAACCCGTTTCCGCAGCCACTATTGTTAGTCTCTTGATTTCATAATGACTTAAGC²⁷⁷⁰
ACACTTGACATCAACTGCATCAAGATGTGACCTGTTTTATAAAAAAAAAAAAAAAAAA²⁸¹⁰
²⁸³⁰
²⁸⁵⁰
²⁸⁷⁰
²⁸⁹⁰
²⁹¹⁰
²⁹³⁰
²⁹⁵⁰
²⁹⁷⁰
²⁹⁹⁰
³⁰³⁰
³⁰⁵⁰
³⁰⁷⁰
³⁰⁹⁰
³¹¹⁰

FIG. 3-6

Thinks a Clerk

5'..... GCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
 -220 -200

CTCTTCTCTCAGAAATCGCTTTGGGGAACCCAGCTTGCAGCCAATGAACCCGCCTTCCAG
-180 -160 -140

ATTGGTGTGAAGACAGAAGTGAGCTTCGTTTTAGGCGTCAAGCAGCCAGGCAGAAGAAA
-120 -100 -80

Met Gly Gln Ser Val Leu Arg Ala Val Phe Phe Leu Val Leu Gly
ATG GGG CAA AGC GTG CTC CGG GCG GTC TTC TTT TTA GTC CTG GGG

Leu Leu Gly His Ser His ⁻¹Gly ¹Gly Phe Pro Asn Thr Ile Ser Ile
CTT TTG GGT CAT TCT CAC GGA GGA TTC CCC AAC ACC ATC AGC ATA
-20 1 20

10
 Gly Gly Leu Phe Met Arg Asn Thr Val Gln Glu His Ser Ala Phe
 GGT GGA CTT TTC ATG AGA AAC ACG GTT CAG GAG CAC AGC GCT TTC
 20
 40
 60

Arg Phe Ala Val Gln Leu Tyr Asn Thr Asn Gln Asn Thr Thr Glu
CGC TTT GCT GTG CAG TTA TAC AAC ACC AAC CAG AAC ACC ACT GAG

40
 Lys Pro Phe His Leu Asn Tyr His Val Asp His 50
 AAG CCC TTC CAT TTG AAT TAC CAC GTA GAC CAC TTG GAT TCC TCC
 120
 140
 160

Asn Ser Phe Ser Val Thr Asn Ala Phe Cys Ser Gln Phe Ser Arg
AAT AGT TTT TCT GTG ACT AAT GCT TTC TGC TCC CAG TTC TCC AGA

70
 Gly Val Tyr Ala Ile Phe Gly Phe Tyr Asp Gln Met Ser Met Asn
 GGG GTG TAT GCT ATC TTT GGA TTC TAT GAC CAG ATG TCA ATG AAC
 220 240

Thr Leu Thr Ser Phe Cys Gly Ala Leu His Thr Ser Phe Val Thr
ACC CTG ACC TCC TTC TGT GGG GCC CTG CAC ACA TCT TTT GTC ACA
260 280

Pro 100 Phe Pro Thr Asp Ala Asp Val Gln Phe 110 Val Ile Gln Met
CCT AGC TTT CCC ACT GAT GCA GAT GTG CAG TTT GTC ATC CAG ATG
300 320 340

Arg Pro Ala Leu Lys Gly Ala Ile Leu Ser Leu Leu Ser Tyr Tyr
CGC CCA GCC TTG AAG GGT GCC ATT CTG AGT CTT CTC AGT TAC TAC

FIG. 4-1

Wanda White

GluR3

130
 Lys Trp Glu Lys Phe Val Tyr Leu Tyr Asp Thr 140
 AAG TGG GAG AAG TTT GTG TAC CTC TAT GAC ACA GAA CGA GGG TTT
 400 420

150
 Ser Val Leu Gln Ala Ile Met Glu Ala Ala Val Gln Asn Asn Trp
 TCT GTC CTA CAA GCA ATT ATG GAG GCA GCA GTG CAA AAC AAC TGG
 440 460

160
 Gln Val Thr Ala Arg Ser Val Gly Asn Ile Lys 170
 CAA GTG ACA GCA AGG TCT GTG GGA AAC ATA AAG GAC GTC CAG GAA
 480 500 520

180
 Phe Arg Arg Ile Ile Glu Glu Met Asp Arg Arg Gln Glu Lys Arg
 TTC AGA CGC ATC ATT GAA GAA ATG GAC AGA AGG CAG GAA AAA CGA
 540 560

190
 Tyr Leu Ile Asp Cys Glu Val Glu Arg Ile Asn 200
 TAC TTG ATT GAC TGT GAA GTC GAA AGG ATT AAC ACA ATT TTG GAA
 580 600

210
 Gln Val Val Ile Leu Gly Lys His Ser Arg Gly Tyr His Tyr Met
 CAG GTT GTG ATC CTG GGG AAG CAT TCA AGA GGC TAT CAC TAC ATG
 620 640

220
 Leu Ala Asn Leu Gly Phe Thr Asp Ile Leu Leu 230
 CTT GCT AAC CTG GGT TTT ACT GAC ATT TTA CTG GAA AGA GTC ATG
 660 680 700

240
 His Gly Gly Ala Asn Ile Thr Gly Phe Gln Ile Val Asn Asn Glu
 CAT GGG GGA GCC AAC ATT ACA GGT TTC CAG ATT GTC AAC AAT GAA
 720 740

250
 Asn Pro Met Val Gln Gln Phe Ile Gln Arg Trp 260
 AAC CCA ATG GTT CAG CAG TTC ATA CAG CGC TGG Val Arg Leu Asp
 760 780

270
 Glu Arg Glu Phe Pro Glu Ala Lys Asn Ala Pro Leu Lys Tyr Thr
 GAA AGG GAA TTC CCT GAA GCC AAG AAT GCA CCA CTG AAG TAT ACA
 800 820

280
 Ser Ala Leu Thr His Asp Ala Ile Leu Val Ile 290
 TCT GCG CTG ACA CAT GAC GCA ATA TTG GTC ATA GCA GAA GCC TTC
 840 860 880

FIG. 4-2

Brink & Clark

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GluR3

Arg Tyr Leu Arg Arg Gln 300 Arg Val Asp Val Ser Arg Arg Gly Ser
CGA TAC CTG AGG AGA CAG AGA GTG GAT GTC TCC CGC AGA GGC AGT
900 920

Ala Gly Asp Cys Leu Ala Asn Pro Ala Val Pro 320 Trp Ser Gln Gly
GCT GGA GAC TGC TTA GCA AAT CCT GCT GTG CCC TGG AGT CAA GGA
940 960

Ile Asp Ile Glu Arg Ala 330 Leu Lys Met Val Gln Val Gln Gly Met
ATT GAT ATT GAG AGA GCT CTG AAA ATG GTG CAA GTA CAA GGA ATG
980 1000

Thr Gly Asn Ile Gln Phe Asp Thr Tyr Gly Arg 350 Arg Thr Asn Tyr
ACT GGA AAC ATC CAA TTT GAC ACT TAT GGA CGT AGG ACA AAT TAT
1020 1040 1060

Thr Ile Asp Val Tyr Glu 360 Met Lys Val Ser Gly Ser Arg Lys Ala
ACC ATT GAT GTC TAT GAA ATG AAA GTC TCG GGT TCT CGA AAA GCT
1080 1100

Gly Tyr Trp Asn Gly Tyr Glu Arg Phe Val Pro 380 Phe Ser Asp Gln
GGT TAC TGG AAC GAA TAT GAA AGG TTT GTG CCC TTC TCA GAT CAA
1120 1140

Gln Ile Ser Asn Asp Ser 390 Ser Ser Glu Asn Arg Thr Ile Val
CAA ATC AGC AAT GAC AGC TCA TCC TCA GAG AAC CGG ACC ATT GTA
1160 1180

Val Thr Thr Ile Leu Glu Ser Pro Tyr Val Met 410 Tyr Lys Lys Asn
GTG ACT ACC ATT CTG GAA TCA CCA TAT GTG ATG TAT AAA AAG AAT
1200 1220 1240

His Glu Gln Leu Glu Gly 420 Asn Glu Arg Tyr Glu Gly Tyr Cys Val
CAT GAG CAG CTG GAA GGA AAT GAG CGC TAT GAA GGC TAC TGT GTT
1260 1280

Asp Leu Ala Tyr Glu Ile Ala Lys His Val Arg 440 Ile Lys Tyr Lys
GAT TTA GCC TAT GAA ATA GCC AAA CAC GTA AGG ATC AAA TAC AAA
1300 1320

Leu Ser Ile Val Gly Asp 450 Gly Lys Tyr Gly Ala Arg Asp Pro Glu
TTG TCC ATT GTC GGT GAT GGG AAA TAT GGC GCC AGA GAT CCA GAG
1340 1360

Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu 470 Val Tyr Gly Arg
ACT AAA ATA TGG AAT GGC ATG GTT GGG GAA CTT GTC TAT GGA AGA
1380 1400 1420

FIG. 4-3

17/51

GluR3

Ala Asp Ile Ala Val Ala Pro Leu Thr Ile Thr Leu Val Arg Glu
 GCT GAT ATA GCT GTT GCT CCA CTC ACT ATA ACA TTG GTC CGT GAA
 480 1440 1460

Glu Val Ile Asp Phe Ser Asn Ala Phe Met Ser Leu Gly Ile Ser
 GAA GTC ATA GAT TTC TCA AAC GCA TTT ATG AGC CTG GGA ATC TCC
 490 1480 500 1500

Ile Met Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe Ser
 ATC ATG ATA AAG AAG CCT CAG AAA TCA AAG CCA GGC GTC TTT TCA
 510 1520 1540

Phe Leu Asp Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe
 TTC CTG GAT CCT TTG GCT TAT GAA ATC TGG ATG TGC ATT GTC TTC
 520 1560 530 1600

Ala Tyr Ile Gly Val Ser Val Val Leu Phe Leu Val Ser Arg Phe
 GCT TAC ATT GGA GTC AGT GTA GTT CTC TTC CTA GTC AGC AGA TTT
 540 1620 1640

Ser Pro Tyr Glu Trp His Leu Glu Asp Asn Asn Glu Glu Pro Arg
 AGC CCT TAT GAA TGG CAC TTG GAA GAC AAC AAT GAA GAA CCT CGT
 550 1660 560 1680

Asp Pro Gln Ser Pro Pro Asp Pro Pro Asn Glu Phe Gly Ile Phe
 GAC CCA CAA AGC CCT CCT GAT CCT CCC AAT GAA TTT GGA ATA TTT
 570 1700 1720

Asn Ser Leu Trp Phe Ser Leu Gly Ala Phe Met Gln Gln Gly Cys
 AAC AGT CTT TGG TTT TCC TTG GGT GCT TTC ATG CAG CAA GGA TGT
 580 1740 590 1760 1780

Asp Ile Ser Pro Arg Ser Leu Ser Gly Arg Ile Val Gly Gly Val
 GAT ATT TCT CCA AGA TCA CTT TCT GGG CGC ATT GTT GGA GGG GTT
 600 1800 1820

Trp Trp Phe Phe Thr Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn
 TGG TGG TTC TTC ACC CTG ATC ATA ATC TCT TCC TAC ACT GCA AAC
 610 1840 620 1860

Leu Ala Ala Phe Leu Thr Val Glu Arg Met Val Ser Pro Ile Glu
 CTT GCT GCT TTC CTG ACT GTG GAG AGG ATG GTG TCC CCT ATA GAG
 630 1880 1900

FIG. 4-4

GluR3

640
Ser Ala Glu Asp Leu Ala Lys Gln Thr Glu Ile Ala Tyr Gly Thr
AGC GCT GAA GAC TTA GCC AAG CAG ACT GAA ATT GCA TAT GGG ACC
1920 1940 1960

660
Leu Asp Ser Gly Ser Thr Lys Glu Phe Phe Arg Arg Ser Lys Ile
CTG GAC TCT GGT TCA ACA AAA GAA TTT TTC AGA CGA TCC AAA ATT
1980 2000

670
Ala Val Tyr Glu Lys Met Trp Ser Tyr Met Lys Ser Ala Glu Pro
GCT GTG TAT GAG AAA ATG TGG TCT TAC ATG AAA TCC GCA GAG CCA
2020 2040

690
Ser Val Phe Thr Lys Thr Thr Ala Asp Gly Val Ala Arg Val Arg
TCT GTG TTT ACC AAA ACA ACA GCT GAC GGG GTA GCC CGA GTT CGG
2060 2080

700
Lys Ser Lys Gly Lys Phe Ala Phe Leu Leu Glu Ser Thr Met Asn
AAG TCC AAG GGA AAG TTC GCC TTC CTG CTG GAG TCG ACC ATG AAC
2100 2120 2140

720
Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met Lys Val Gly
GAG TAC ATT GAG CAG AGA AAG CCG TGC GAT ACG ATG AAA GTT GGT
2160 2180

730
Gly Asn Leu Asp Ser Lys Gly Tyr Gly Val Ala Thr Pro Lys Gly
GGA AAT CTG GAT TCC AAA GGC TAT GGT GTG GCA ACC CCT AAA GGC
2200 2220

740
Ser Ala Leu Gly Asn Ala Val Asn Leu Ala Val Leu Lys Leu Asn
TCA GCA TTA GGA AAT GCT GTT AAC CTG GCA GTA TTA AAA CTG AAT
2240 2260

760
Glu Gln Gly Leu Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp
GAG CAA GGC CTC TTG GAC AAA TTG AAA AAC AAA TGG TGG TAC GAC
2280 2300 2320

780
Lys Gly Glu Cys Gly Ser Gly Gly Gly Asp Ser Lys Asp Lys Thr
AAA GGA GAG TGC GGC AGC GGG GGC GGT GAC TCC AAG GAC AAG ACC
2340 2360

790
Ser Ala Leu Ser Leu Ser Asn Val Ala Gly Val Phe Tyr Ile Leu
AGT GCT CTA AGC CTG AGC AAT GTG GCA GGC GTG TTC TAT ATA CTT
2380 2400

FIG. 4-5

Handwritten signature

GluR3

Val Gly Gly Leu Gly Leu Ala Met Met Val Ala Leu Ile Glu Phe
 GTC GGA GGT CTG GGC CTG GCC ATG ATG GTG GCT TTG ATA GAA TTC
 2420 2440

Cys Tyr Lys Ser Arg Ala Glu Ser Lys Arg Met Lys Leu Thr Lys
 TGT TAC AAA TCA CGG GCA GAG TCC AAA CGC ATG AAA CTC ACA AAG
 2460 2480 2500

Asn Thr Gln Asn Phe Lys Pro Ala Pro Ala Thr Asn Thr Gln Asn
 AAC ACC CAA AAC TTT AAG CCT GCT CCT GCC ACC AAC ACT CAG AAT
 2520 2540

Tyr Ala Thr Tyr Arg Glu Gly Tyr Asn Val Tyr Gly Thr Glu Ser
 TAC GCT ACA TAC AGA GAA GGC TAC AAC GTG TAT GGA ACA GAA AGT
 2560 2580

Val Lys Ile
 GTT AAG ATC TAG GGATCCCTTCCCACCAGAAGCATGCAATGAGAGGAAATCACTG
 2600 2620 2640

AAAACGTGGCTGCTTCAAGGATCCTGAGCCGGATTTCCTCTCCCTGGTGTCTGGGCAT
 2660 2680 2700

GACACGAATATTGCTGATGGTGCAATGACCTTTCAATAGGAAAACTGATTTTTTTTTTCC
 2720 2740 2760

TTCAGTGCCTTATGGAACACTCTGAGACTTGCGACAATGCAAACCATCATTGAAATCT
 2780 2800 2820

TTTTGCTTTGCTTGAAAAAAAAAAAAAAAA 3'
 2840

FIG. 4-6

Thanks a Clerk

[illegible]

Hubert Clark

FIG. 5-2

E	H	A	A	A	F	R	F	A	L	S	O	L	T	E	P	P	K	L	L	P	Q	I	D	I	V	N	I	S	D	T	F	E	M	T	Y	R	F	C	S	GluR1	
E	Y	S	A	A	F	R	V	G	H	V	Q	F	S	T	S	F	F	R	L	T	P	H	I	D	N	L	E	V	A	N	S	F	A	V	T	N	A	F	C	S	GluR2
E	H	S	A	F	R	F	A	V	Q	L	Y	N	T	N	Q	T	T	E	K	P	F	H	L	L	L	L	D	S	S	S	F	S	V	T	H	A	F	C	S	GluR3	
E	Y	T	A	F	R	L	A	I	F	L	H	N	T	S	P	H	A	S	E	A	P	F	H	L	L	L	E	T	A	N	S	F	A	V	T	H	A	F	C	S	GluR4
V	H	V	E	E	L	A	F	K	F	A	V	T	S	I	N	R	R	T	L	M	P	N	T	L	L	L	I	N	L	F	D	S	F	E	A	S	R	A	C	D	GluR5

E	L	Q	E	A	L	I	S	I	I	D	H	Y	K	Y	Q	M	Q	T	F	V	Y	I	Y	D	A	D	R	G	L	S	V	L	Q	R	V	L	D	T	A	A	E	K	N	W	GluR1
D	L	K	G	A	L	L	S	L	I	E	Y	Y	Q	Y	Q	M	D	K	F	A	Y	L	Y	D	S	D	R	G	L	S	T	L	Q	A	V	L	D	S	A	A	E	K	K	W	GluR2
A	L	K	G	A	I	L	S	L	L	S	Y	Y	K	Y	K	M	E	K	F	V	Y	L	Y	D	T	E	R	G	L	S	V	L	Q	A	I	H	E	A	A	V	Q	N	W	GluR3	
S	L	R	G	A	L	L	S	L	L	D	H	Y	E	Y	E	M	N	C	F	V	F	L	Y	D	T	D	R	G	L	S	I	L	Q	A	I	H	E	K	A	C	Q	N	W	GluR4	
L	Y	P	D	Y	A	A	I	S	R	A	V	L	D	L	V	L	Y	N	K	T	V	V	Y	E	D	S	T	G	L	I	R	L	Q	E	L	I	K	A	P	S	R	Y	N	I	GluR5

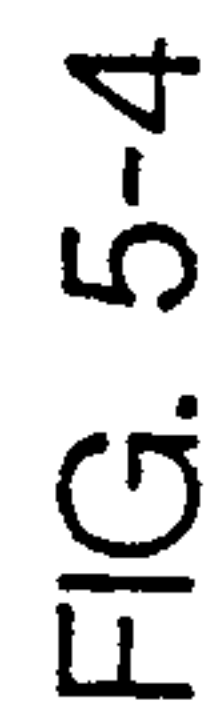
X	L	E	K	N	G	I	C	Y	H	Y	I	L	A	N	L	G	F	H	D	I	D	L	N	K	F	K	E	S	G	R	A	N	V	T	G	F	Q	L	V	N	Y	T	D	T	I	P	A	R	I	GluR1
T	I	G	K	H	V	K	G	Y	H	Y	I	I	A	N	L	G	F	T	D	G	D	L	L	K	I	Q	F	G	C	A	N	V	S	T	G	F	Q	I	V	D	Y	D	S	L	V	S	K	F	GluR2	
I	L	G	K	H	S	R	G	Y	H	Y	H	L	A	N	L	G	F	T	D	I	L	L	E	R	V	H	G	C	A	N	I	T	G	F	Q	I	V	N	H	E	N	T	P	H	V	Q	Q	F	GluR3	
S	V	G	K	H	V	K	G	Y	H	Y	I	I	A	N	L	G	F	K	D	I	S	L	L	E	R	F	I	H	G	C	A	N	V	T	G	F	Q	L	V	D	F	N	T	P	H	V	T	K	L	GluR4
F	H	G	H	M	T	E	Y	Y	H	Y	F	F	T	I	L	D	L	F	A	L	D	L	E	L	E	Y	R	Y	S	G	V	N	H	T	G	F	R	L	L	N	I	D	N	P	H	V	S	S	I	GluR5

G	N	A	G	D	C	L	A	N	P	A	V	P	M	G	O	G	I	D	I	Q	R	A	L	Q	Q	V	R	F	E	G	L	T	G	N	V	Q	F	H	K	R	R	I	N	Y	T	GluR1			
G	N	A	G	D	C	L	A	N	P	A	V	P	M	G	O	G	V	E	I	E	R	A	L	K	Q	V	Q	V	E	C	L	S	G	N	I	K	F	D	Q	N	G	K	R	I	N	Y	T	GluR2	
G	S	A	G	D	C	L	A	N	P	A	V	P	M	S	O	C	I	D	I	E	R	A	L	K	H	V	Q	V	Q	G	H	T	G	N	I	Q	F	D	T	Y	G	R	R	T	N	Y	T	GluR3	
G	N	A	G	D	C	L	A	N	P	A	V	P	M	G	Q	C	I	D	H	E	R	T	L	K	Q	V	R	I	Q	C	L	T	G	N	V	Q	F	D	H	Y	G	R	R	V	N	Y	T	GluR4	
L	T	V	S	S	L	Q	C	H	R	H	K	P	N	R	L	G	P	R	F	H	N	L	I	K	E	A	R	W	D	C	L	T	G	R	I	T	F	N	K	T	D	G	L	R	K	D	F	D	GluR5

H	S	S	V	Q	N	R	T	Y	I	V	T	T	I	L	E	D	P	Y	V	H	L	K	K	N	A	N	O	F	E	G	N	D	R	Y	E	G	Y	C	V	E	L	A	A	E	I	A	K	H	GluR1	
T	S	G	L	E	N	K	T	T	V	V	T	T	I	L	E	S	P	Y	V	H	H	Y	K	K	N	H	E	H	L	E	G	N	E	R	Y	E	G	Y	C	V	D	L	A	A	E	I	A	K	H	GluR2
S	S	S	S	E	N	R	T	I	V	V	T	T	I	L	E	S	P	Y	V	H	Y	K	K	N	H	E	Q	L	E	G	N	E	R	Y	E	G	Y	C	V	D	L	A	A	E	I	A	K	H	GluR3	
T	A	A	I	E	N	R	T	V	V	V	T	T	I	H	E	S	P	Y	V	H	Y	K	K	N	H	E	H	F	E	G	N	D	R	Y	E	G	Y	C	V	D	L	A	S	E	A	K	H	GluR4		
T	D	S	L	A	N	R	T	L	I	V	T	T	I	L	E	E	P	Y	V	H	Y	K	K	S	D	K	P	L	Y	C	G	N	D	R	F	E	G	Y	C	L	L	K	E	L	S	N	I	GluR5		

Mark & Clark

FIG. 5-3



Book Club

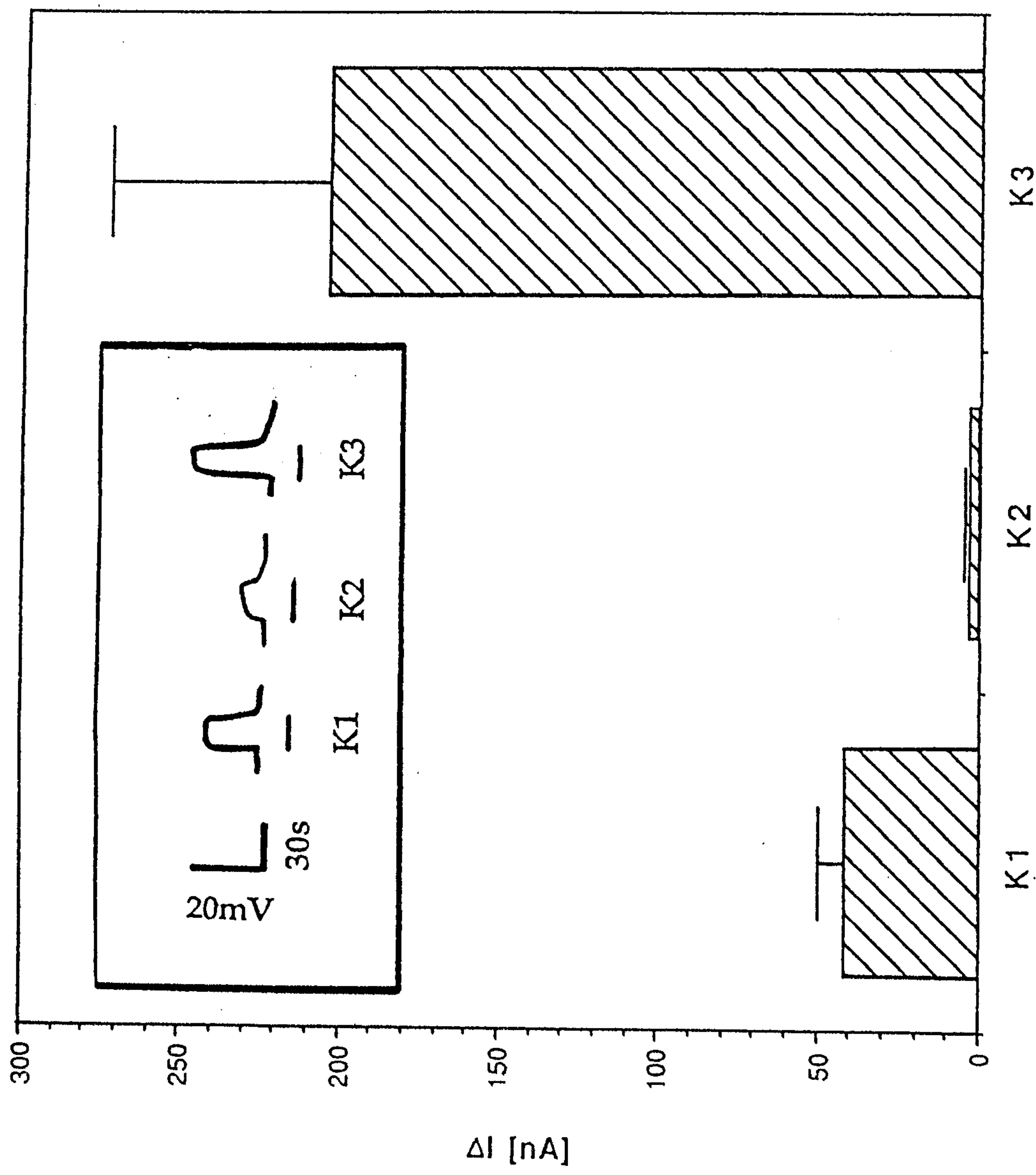


FIG. 6-1

Handwritten signature

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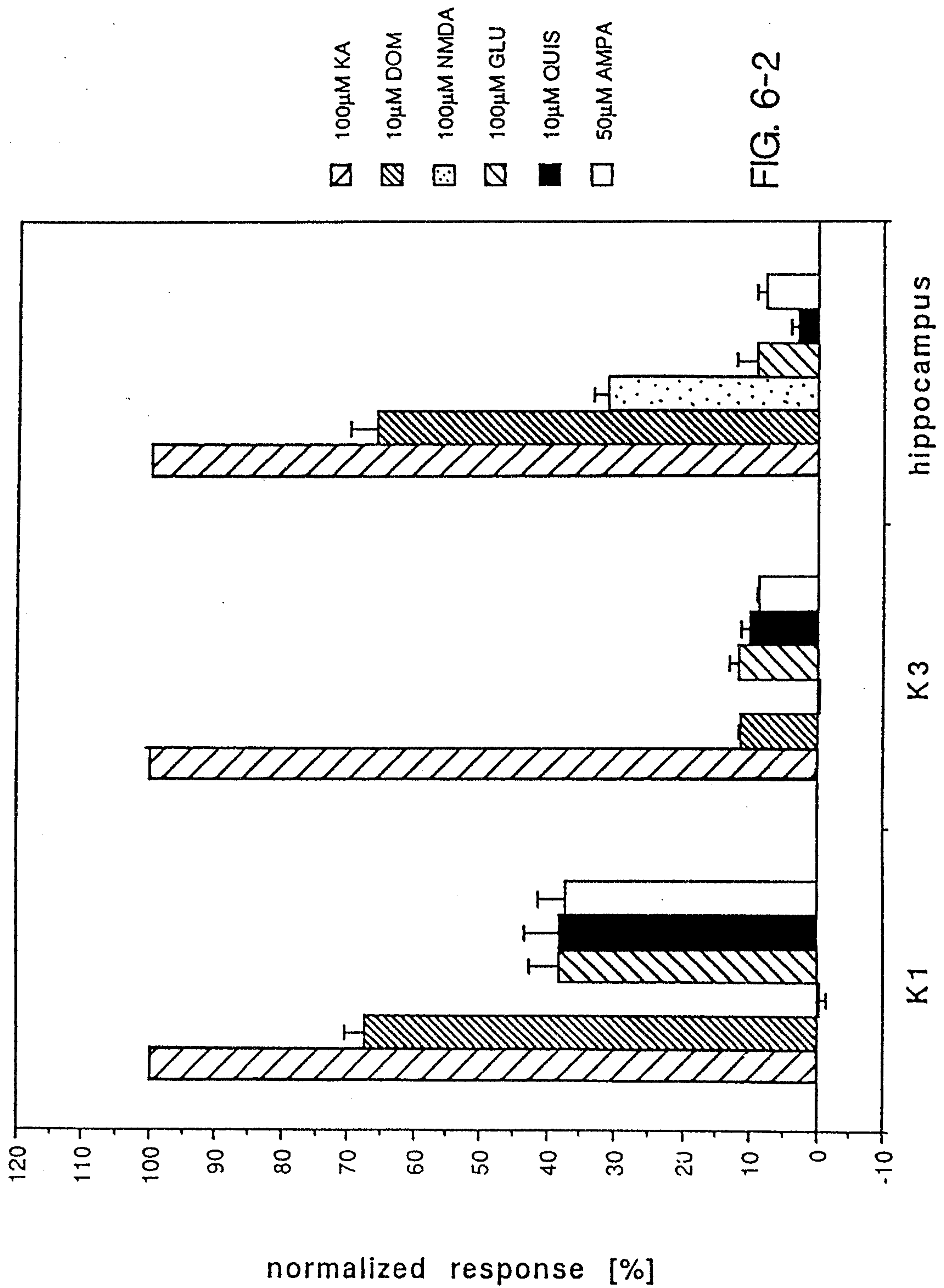


FIG. 6-2

Handwritten signature

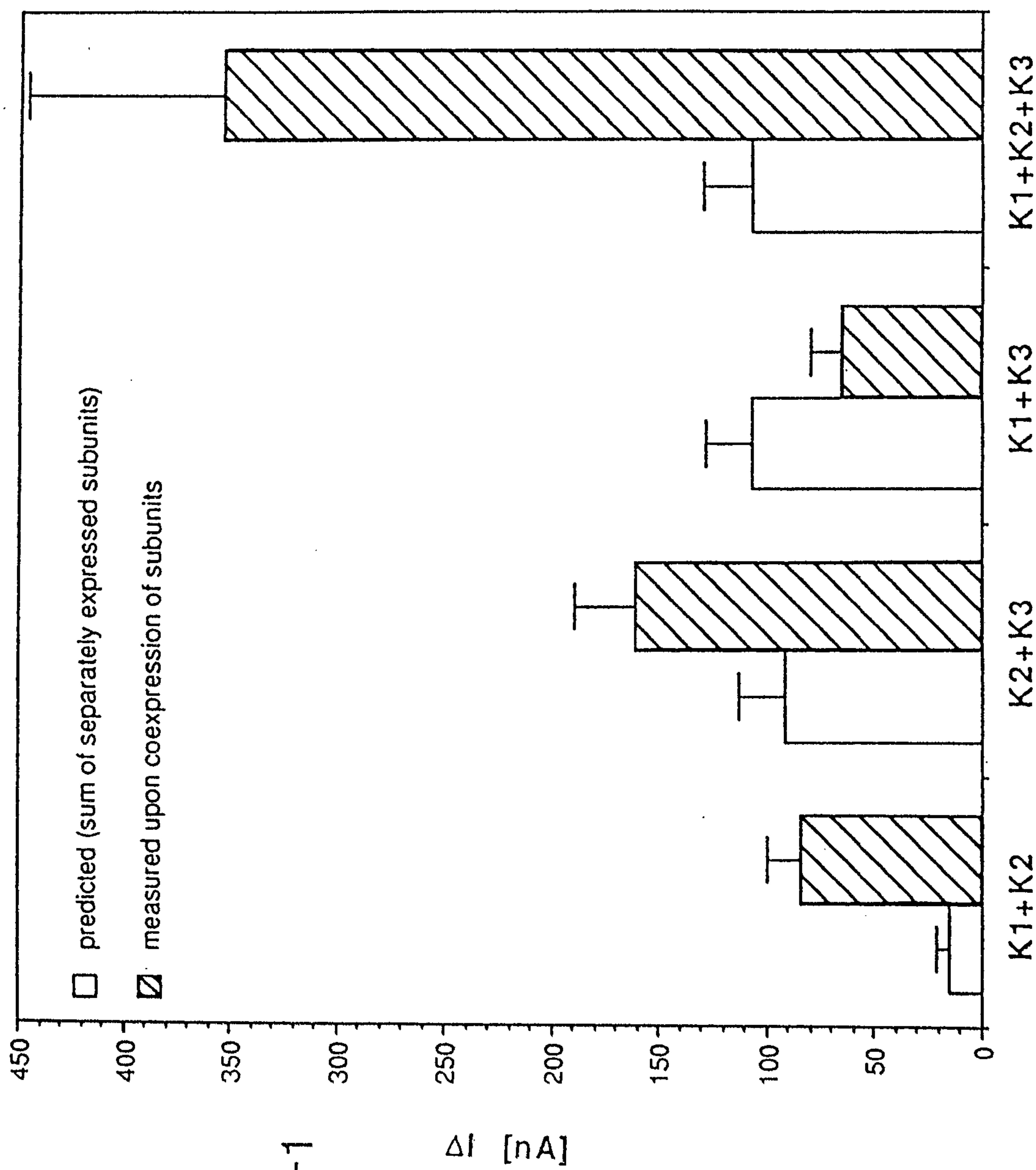


FIG. 7-1

Frank & Clark

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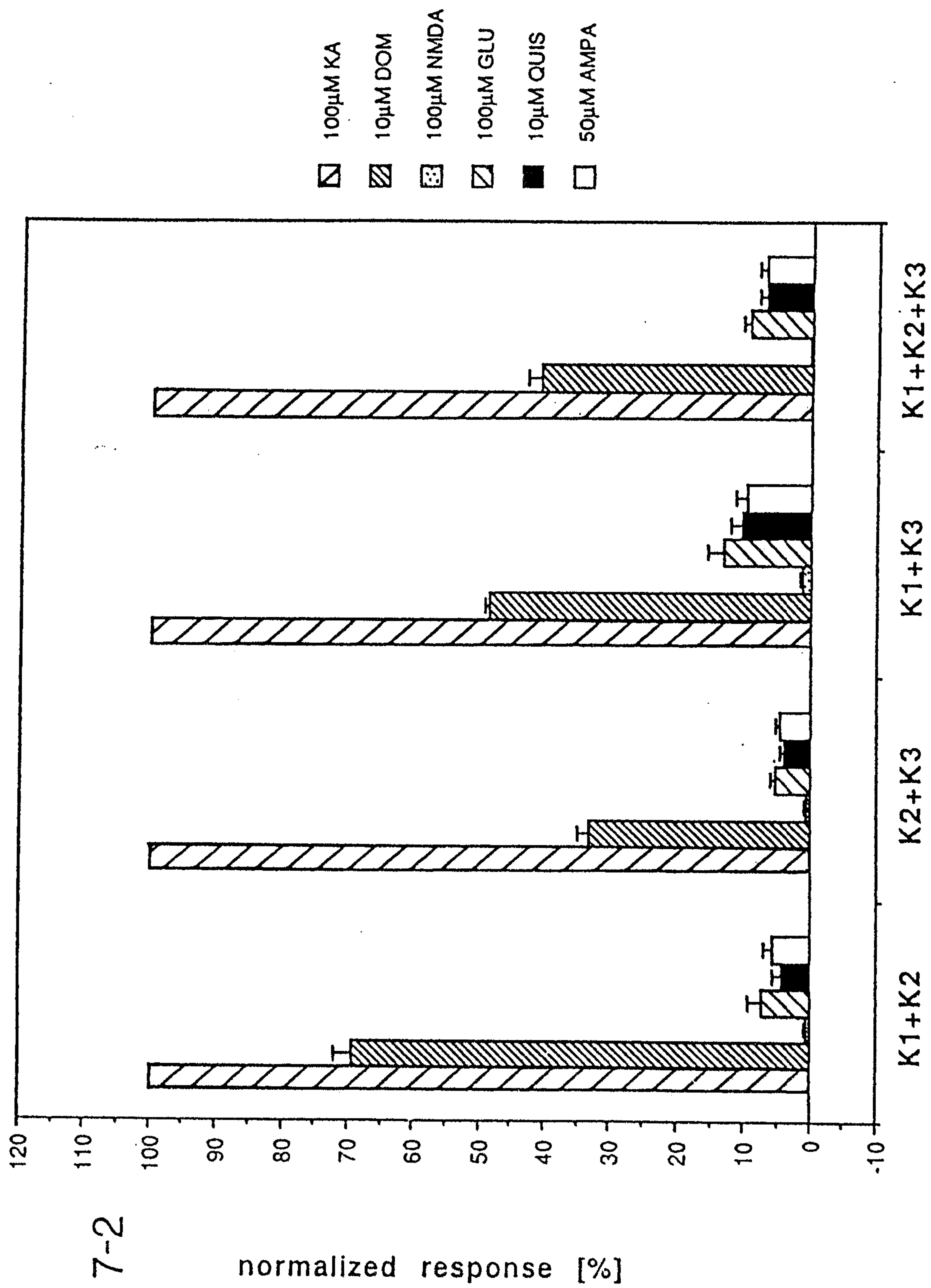


FIG. 7-2

Reade & Clark

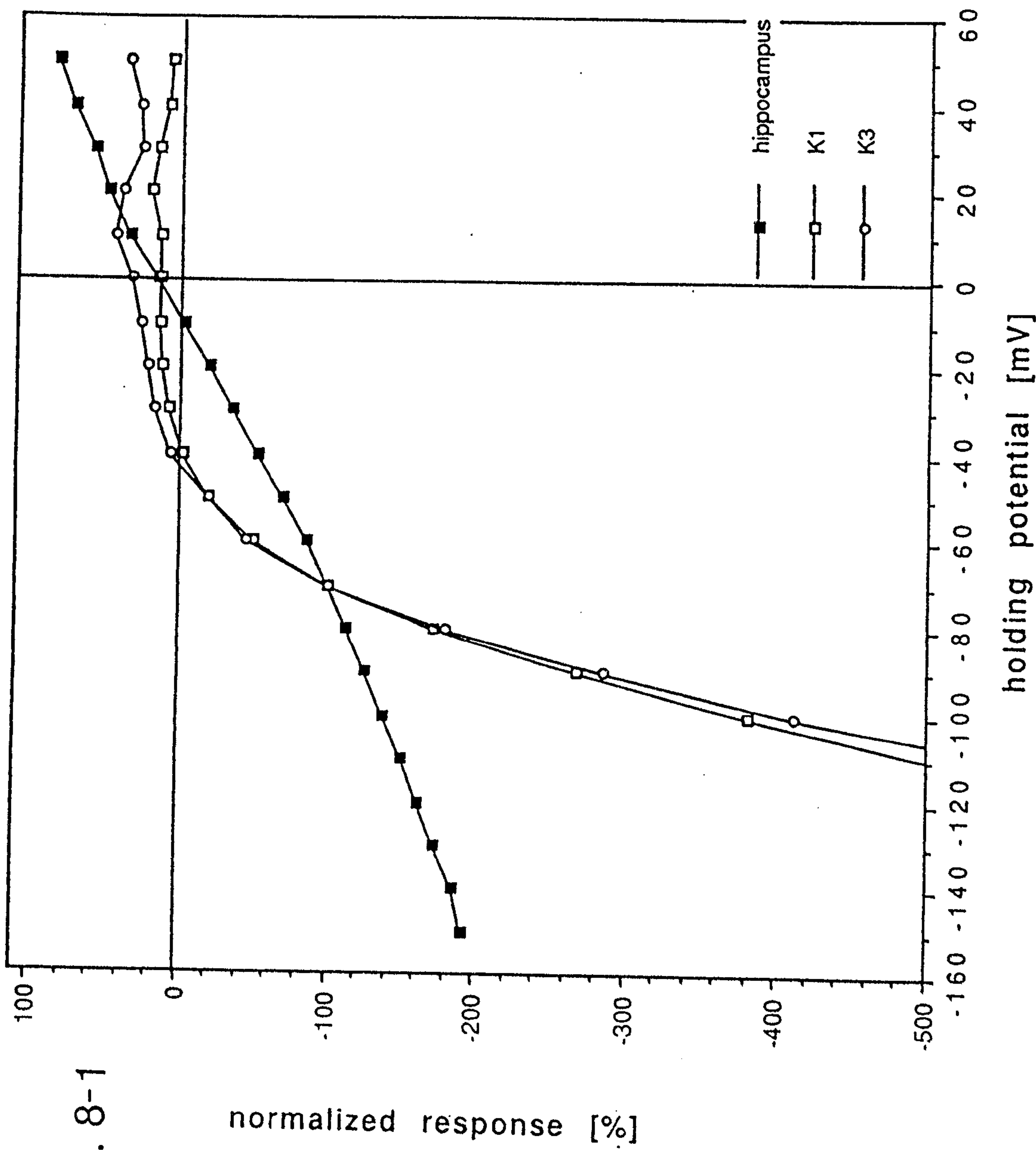


FIG. 8-1

Reada & Chub

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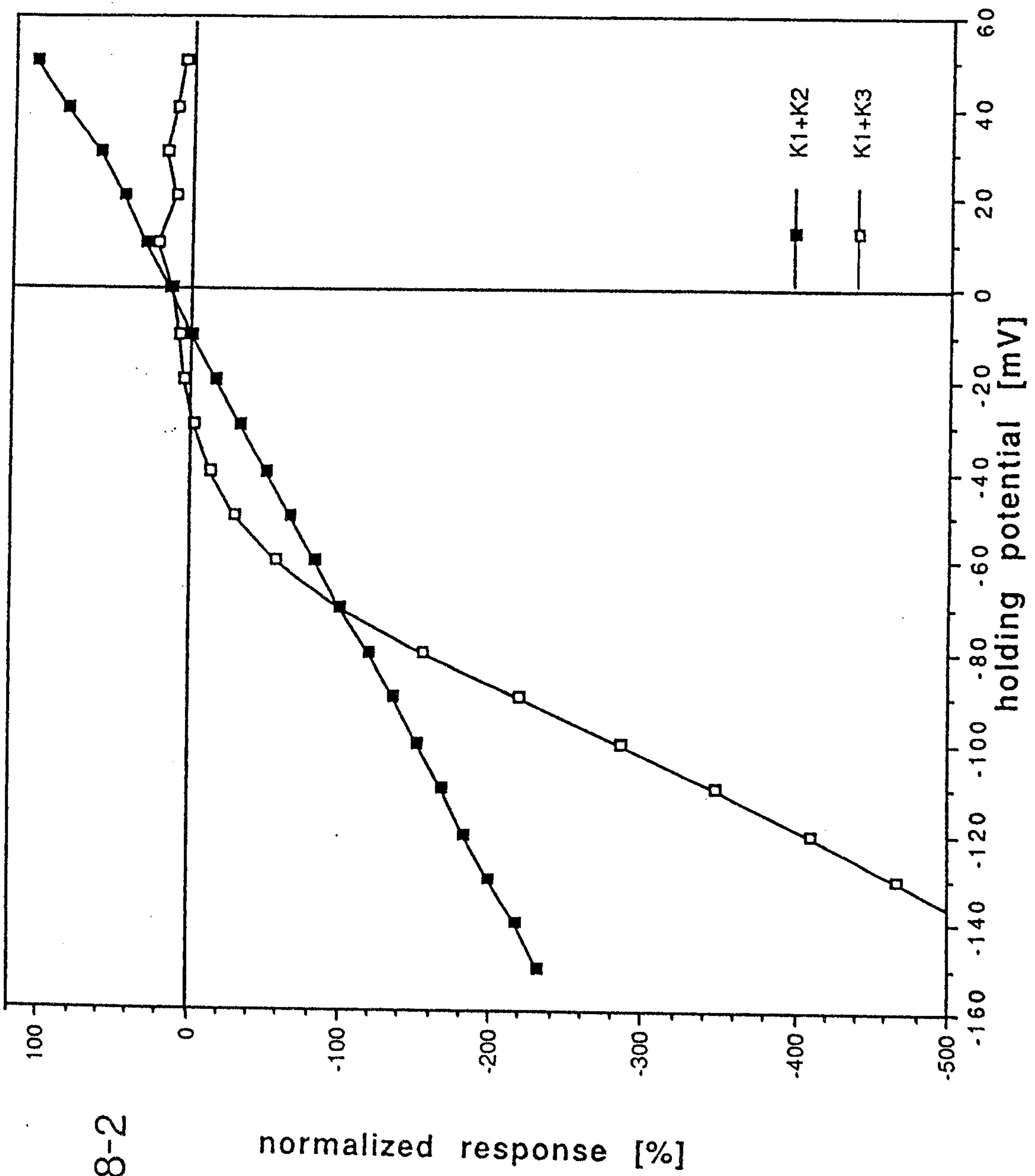
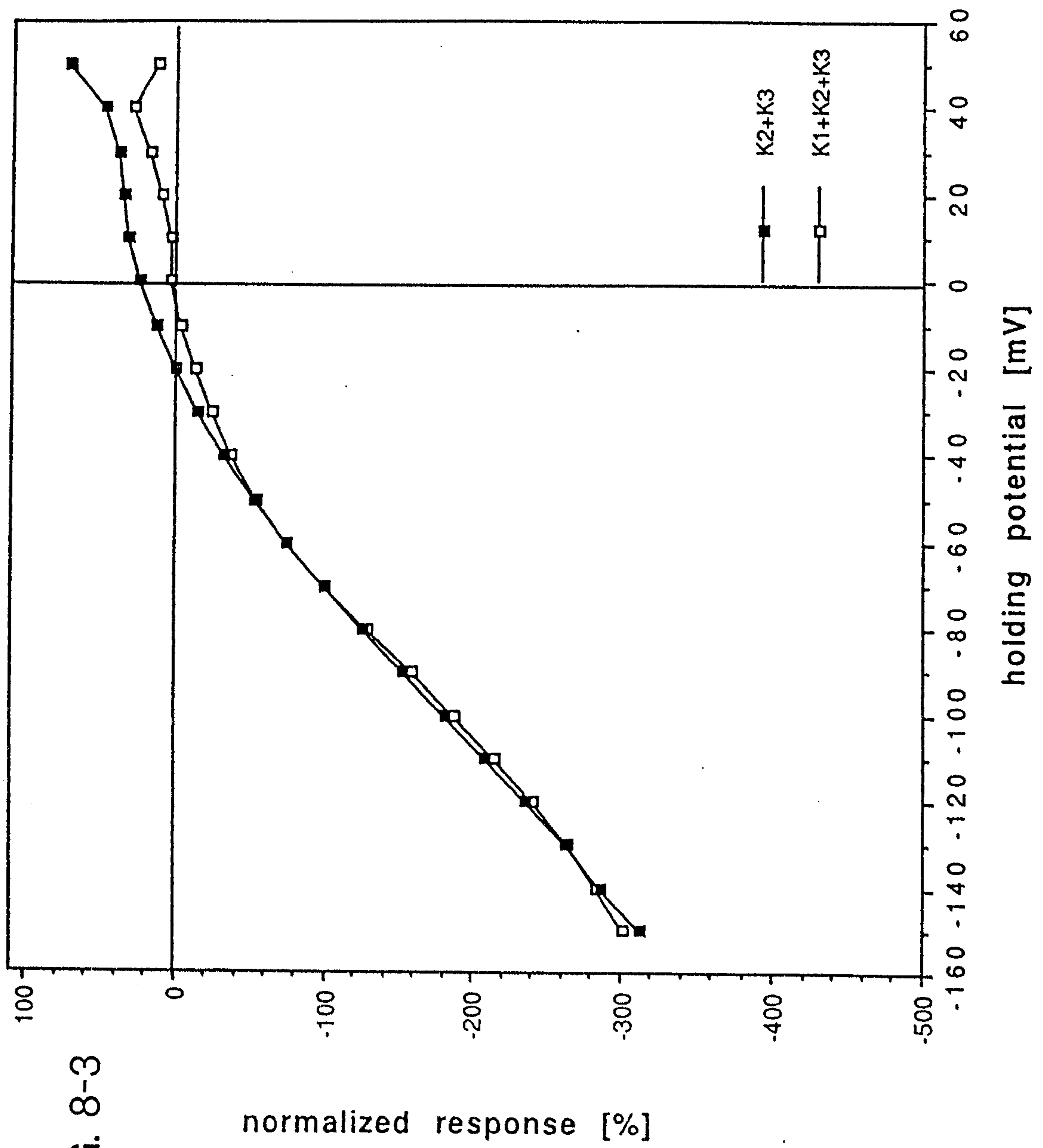


FIG. 8-2

Mark & Clark



Heath & Clark

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GluR4

10 30 50
GAATTCGGGTAATGGAGTGTACGCAAAATCCTCTGTCTGTGGACTCGCACCAGAGCCTCCC

70 90 110
AGAAAACCTGGGCGATCTGCGCCATCGTCTTCAATGCCTCTCTGAAAAGCCTTTAGCAAG

130 150 170
ACTGAGAGAAAGAGAAAAGAGAGCGCGCCAGAGAGAGGAGCAAAGAAGATGAGGATTATT
MetArgIleIle

190 210 230
TGCAGGCAGATTGTCTTGTTGTTTTCTGGATTTTGGGGACTCGCCATGGGAGCCTTTCCA
CysArgGlnIleValLeuLeuPheSerGlyPheTrpGlyLeuAlaMetGlyAlaPhePro

250 270 290
AGCAGCGTTCAAATAGGTGGTCTCTTCATCCGAAACACAGACCAGGAATACACTGCTTTT
SerSerValGlnIleGlyGlyLeuPheIleArgAsnThrAspGlnGluTyrThrAlaPhe

310 330 350
AGACTGGCAATCTTTCTTCATAACACCAGCCCCAATGCATCGGAAGCTCCTTTCAATTTG
ArgLeuAlaIlePheLeuHisAsnThrSerProAsnAlaSerGluAlaProPheAsnLeu

370 390 410
GTACCTCATGTGGACAACATTGAGACTGCCAACAGTTTTGCTGTGACAAACGCCTTCTGT
ValProHisValAspAsnIleGluThrAlaAsnSerPheAlaValThrAsnAlaPheCys

430 450 470
TCCAGTATTCTAGAGGGGTGTTTGCCATTTTTGGACTCTATGACAAGAGATCCGTGCAT
SerGlnTyrSerArgGlyValPheAlaIlePheGlyLeuTyrAspLysArgSerValHis

490 510 530
ACCTTGACCTCGTTCTGCAGGCGTCTGCACATCTCTCATCACACCAAGCTTTCCCACT
ThrLeuThrSerPheCysArgArgLeuHisIleSerLeuIleThrProSerPheProThr

550 570 590
GAAGGGGAGAGCCAGTTTGTGCTGCAGCTAAGACCTTCACTGAGAGGTGCACTCCTGAGC
GluGlyGluSerGlnPheValLeuGlnLeuArgProSerLeuArgGlyAlaLeuLeuSer

610 630 650
CTCCTGGATCACTATGAGTGGAAGTGTTCGTCTTCCTGTATGATACAGACAGGGGGTAT
LeuLeuAspHisTyrGluTrpAsnCysPheValPheLeuTyrAspThrAspArgGlyTyr

670 690 710
TCAATACTTCAAGCTATAATGGAAAAAGCAGGACAAAATGGATGGCATGTCAGTGCAATA
SerIleLeuGlnAlaIleMetGluLysAlaGlyGlnAsnGlyTrpHisValSerAlaIle

730 750 770
TGTGTGGAAAATTTTAATGATGTCAGCTACAGGCAACTGCTAGAAGAGCTTGACAGAAGA
CysValGluAsnPheAsnAspValSerTyrArgGlnLeuLeuGluGluLeuAspArgArg

790 810 830
CAAGAGAAGAAATTTGTGATAGATTGTGAGATAGAGAGGCTTCAAAACATTTTAGAACAA
GlnGluLysLysPheValIleAspCysGluIleGluArgLeuGlnAsnIleLeuGluGln

850 870 890
ATTGTGAGTGTGGGAAGCATGTCAAAGGCTACCATTATATCATCGCAAATTTGGGTTTC
IleValSerValGlyLysHisValLysGlyTyrHisTyrIleIleAlaAsnLeuGlyPhe

FIG. 9-1

Marks & Clerk

2071879

32 / 51

GluR4

910 930 950
AAGGATATTTCTCTTGAGAGATTTATACATGGAGGAGCAAATGTAACAGGATTCCAGTTG
LysAspIleSerLeuGluArgPheIleHisglyGlyAlaAsnValThrGlyPheGlnLeu

970 990 1010
GTAGATTTTAATACACCCATGGTAACCAAATAATGGATCGGTGGAAGAACTAGATCAG
ValAspPheAsnThrProMetValThrLysLeuMetAspArgTrpLysLysLeuAspGln

1030 1050 1070
AGAGAATATCCAGGTTCTGAAACACCTCCAAAGTACACCTCTGCTCTCACTTATGATGGA
ArgGluTyrProGlySerGluThrProProLysTyrThrSerAlaLeuThrTyrAspGly

1090 1110 1130
GTCCTGGTGATGGCTGAACTTTCCGAAGTCTCAGAAGACAGAAAATTGATATTTCAAGG
ValLeuValMetAlaGluThrPheArgSerLeuArgArgGlnLysIleAspIleSerArg

1150 1170 1190
AGAGGAAATGCTGGGGACTGTCTGGCAAACCCTGCTGCTCCCTGGGGCCAGGGAATTGAC
ArgGlyAsnAlaGlyAspCysLeuAlaAsnProAlaAlaProTrpGlyGlnGlyIleAsp

1210 1230 1250
ATGGAGAGGACACTGAAGCAGGTTCTGAATTCAAGGGCTGACTGGGAATGTTCAATTTGAC
MetGluArgThrLeuLysGlnValArgIleGlnGlyLeuThrGlyAsnValGlnPheAsp

1270 1290 1310
CATTATGGACGTAGAGTTAATTACACAATGGATGTGTTTGAACATAAAAGCACAGGACCT
HisTyrGlyArgArgValAsnTyrThrMetAspValPheGluLeuLysSerThrGlyPro

1330 1350 1370
CGAAAGGTTGGCTACTGGAATGATATGGATAAATTAGTCTTGATTCAAGATATGCCTACT
ArgLysValGlyTyrTrpAsnAspMetAspLysLeuValLeuIleGlnAspMetProThr

1390 1410 1430
CTGGGCAATGACACAGCAGCTATTGAGAACAGAACAGTGGTTGTAACCACAATTATGGAA
LeuGlyAsnAspThrAlaAlaIleGluAsnArgThrValValValThrThrIleMetGlu

1450 1470 1490
TCTCCCTATGTTATGTACAAGAAAAATCATGAAATGTTTGAAGGAAATGACAAGTACGAA
SerProTyrValMetTyrLysLysAsnHisGluMetPheGluGlyAsnAspLysTyrGlu

1510 1530 1550
GGCTACTGTGTAGATCTGGCATCGGAAAGTGCAAACATATTGGTATCAAATATAAAATT
GlyTyrCysValAspLeuAlaSerGluSerAlaLysHisIleGlyIleLysTyrLysIle

1570 1590 1610
GCCATTGTTCTGATGGAAAATATGGAGCAAGGGACGCAGACACTAAGATCTGGAATGGG
AlaIleValProAspGlyLysTyrGlyAlaArgAspAlaAspThrLysIleTrpAsnGly

1630 1650 1670
ATGGTAGGAGAGCTTGTGTATGGGAAAGCAGAGATTGCTATTGCCCTCTGACAATCACA
MetValGlyGluLeuValTyrGlyLysAlaGluIleAlaIleAlaProLeuThrIleThr

1690 1710 1730
TTGGTTCGAGAGGAAGTCATCGATTTTCTAAGCCTTTTATGAGTTTAGGCATCTCTATC
LeuValArgGluGluValIleAspPheSerLysProPheMetSerLeuGlyIleSerIle

FIG. 9-2

1750 1770 1790
 ATGATCAAAAACCTCAGAAATCTAAACCAGGAGTCTTTTCCTTCTTGGACCCTCTGGCC
 MetIleLysLysProGlnLysSerLysProGlyValPheSerPheLeuAspProLeuAla

1810 1830 1850
 TATGAGATCTGGATGTGCATAGTGTTCATACATTGGTGTGAGTGTGGTCTTGTTCTTA
 TyrGluIleTrpMetCysIleValPheAlaTyrIleGlyValSerValValLeuPheLeu

1870 1890 1910
 GTCAGTAGGTTTAGCCCATATGAGTGGCACACAGAAGAACCTGAGGATGGGAAGGAAGGA
 ValSerArgPheSerProTyrGluTrpHisThrGluGluProGluAspGlyLysGluGly

1930 1950 1970
 CCCAGTGACCAGCCTCCCAATGAATTTGGCATCTTTAACAGCCTTTGGTTTTCCCTGGGT
 ProSerAspGlnProProAsnGluPheglyIlePheAsnSerLeuTrpPheSerLeuGly

1990 2010 2030
 GCCTTTATGCAACAAGGATGTGACATTTACCCAGATCCCTGTCAGGTCGGATTGTTGGA
 AlaPheMetGlnGlnGlyCysAspIleSerProArgSerLeuSerGlyArgIleValGly

2050 2070 2090
 GGCCTGTGGTGGTTCTTCACACTCATCATTATATCGTCCTACACTGCTAATCTGGCTGCA
 GlyValTrpTrpPhePheThrLeuIleIleIleSerSerTyrThrAlaAsnLeuAlaAla

2110 2130 2150
 TTCCTTACTGTGGAGAGAATGGTCTCCCCCATAGAAAGTGCAGAAGACCTGGCCAAACAA
 PheLeuThrValGluArgMetValSerProIleGluSerAlaGluAspLeuAlaLysGln

2170 2190 2210
 ACAGAAATTGCCTATGGAACACTTGATTCTGGGTCAACAAAAGAATTCTTCAGAAGATCA
 ThrGluIleAlaTyrGlyThrLeuAspSerGlySerThrLysGluPhePheArgArgSer

2230 2250 2270
 AAAATAGCAGTGTATGAAAAGATGTGGACCTACATGCGATCGGCAGAGCCGTCTGTGTTC
 LysIleAlaValTyrGluLysMetTrpThrTyrMetArgSerAlaGluProSerValPhe

2290 2310 2330
 ACTAGAACTACAGCTGAGGGCGTGGCTCGTGTCCGCAAGTCCAAGGGCAAATTTGCCTTT
 ThrArgThrThrAlaGluGlyValAlaArgValArgLysSerLysGlyLysPheAlaPhe

2350 2370 2390
 CTCCTGGAGTCCACGATGAATGAATACATTGAGCAGCGAAAGCCCTGTGACACGATGAAA
 LeuLeuGluSerThrMetAsnGluTyrIleGluGlnArgLysProCysAspThrMetLys

2410 2430 2450
 GTGGGAGGAAACCTGGATTCCAAAGGCTATGGTGTAGCAACGCCCAAGGGTTCCTCATT
 ValGlyGlyAsnLeuAspSerLysGlyTyrGlyValAlaThrProLysGlySerSerLeu

2470 2490 2510
 AGAACTCCTGTAAACCTTGCCGTTTTGAACTCAGTGAGGCAGGCGTCTTAGACAAGCTG
 ArgThrProValAsnLeuAlaValLeuLysLeuSerGluAlaGlyValLeuAspLysLeu

2530 2550 2570
 AAAAACAAATGGTGGTACGATAAAGGTGAATGTGGACCCAAGGACTCGGGAAGCAAGGAC
 LysAsnLysTrpTrpTyrAspLysGlyGluCysGlyProLysAspSerGlySerLysAsp

FIG. 9-3

Frank & Clark

GluR4

2590 2610 2630
AAGACGAGTGCCTTGAGCCTGAGCAACGTAGCAGGCGTCTTCTACATTCTGGTTGGCGGC
LysThrSerAlaLeuSerLeuSerAsnValAlaGlyValPheTyrIleLeuValGlyGly

2650 2670 2690
CTGGGCTTGGCAATGCTGGTGGCTTTGATAGAGTTCTGTTACAAGTCCAGGGCAGAGGCG
LeuGlyLeuAlaMetLeuValAlaLeuIleGluPheCysTyrLysSerArgAlaGluAla

2710 2730 2750
AAGAGAATGAAGCTGACTTTTTCCGAAGCCATAAGAAACAAAGCCAGGTTATCCATCACT
LysArgMetLysLeuThrPheSerGluAlaIleArgAsnLysAlaArgLeuSerIleThr

2770 2790 2810
GGGAGTGTGGGAGAAAACGGCCGTGTGCTTACCCCTGACTGCCCAAGGCCGTACACACA
GlySerValGlyGluAsnGlyArgValLeuThrProAspCysProLysAlaValHisThr

2830 2850 2870
GGAAGTGAATTAGACAAAGTTCGGGATTGGCTGTCATTGCATCGGACCTACCATAAAAA
GlyThrAlaIleArgGlnSerSerGlyLeuAlaValIleAlaSerAspLeuProEnd

2890 2910 2930
CCAAAAAATAATTGAGTGCCTTAATCAAAGTGTGTTGGTGACTGACTGAAACGCAGCCC

2950 2970 2990
TGAGGGAAAGGCCAAGAGTGGGTCTTGACTAAATCCATCCTTTGGCTTGAGAGCAGAAGA

3010 3030
GCATACTGAGTGCCGGACAGAACCAGTGCATAATTTTCAG

FIG. 9-4

Mark & Clark

GIUR5

GCTAGGAAGCCGCTTCACGTCCCCAC
-260

GCTTGTTCCCTCCACCTCGCTCTCTGAGAGCAGAGCGCGGTGTGCAGACTCGGAGCATTCGGGAGGATGAGGCG
-240 -220 -200 -180

GGGACCCAGCCCAAGTTGGGTGCATCTTGCGGGCGTGAGGCCACAACTGGGTTTCGGCATGAATTAAGAAAGCTTGAAAG
-160 -140 -120 -100
END

-30 Met Glu Arg Ser Thr Val Leu Ile Gln Pro Gly Leu Trp Thr Arg Asp Thr Ser Trp Thr
ATG GAG CGC AGC ACA GTC CTT ATC CAA CCC GGG CTC TGG ACC AGG GAC ACC AGC TGG ACA
-80 -60 -40

-10 Leu Tyr Phe Leu Cys Tyr Ile Leu Pro Gln Thr Ser Pro Gln Val Leu Arg Ile Gly
CTC CTC TAT TTC CTG TGC TAC ATC CTC CTC CCT CCT CCT CAA GTG CTC AGG ATC GGA
-20 -1 -1 1 10

Gly Ile Phe Glu Thr Val Glu Asn Glu Pro Val Asn Val Glu Glu Leu Ala Phe Lys Phe
GGG ATT TTT GAA ACT GTG GAA AAT GAA CCT CCT GGT AAT AAT GTT GAA TTA GCT TTC AAG TTT
40 60 80 30

Ala Val Thr Ser Ile Asn Arg Asn Arg Thr Met Pro Asn Thr Thr Leu Thr Tyr Asp
GCA GTC ACC AGT ATT AAC CGA AAC CGA ACC ACC ATG CCC AAT ACC ACA TTA ACC TAT TAT GAC
100 120 140 50
*

Handwritten signature

FIG. 10-1

Ile ATC	Gln CAG	Arg AGA	Ile ATT	Asn AAT	Leu CTT	Phe TTT	Ser AGT	60 Phe TTT	Glu GAA	Ala GCC	Ser TCC	Arg CGA	Arg AGA	Ala GCA	Cys TGC	Asp GAC	Gln CAG	70 Leu CTG
Ala GCT	Leu CTC	Gly GGG	Val GTG	Ala GCC	Ala GCA	Leu CTC	Phe TTC	80 Pro CCT	Ser TCC	His CAC	Ser TCC	Ser TCC	Val GTC	Val GTC	Ser AGT	Ala GCT	Val GTA	90 Gln CAG
Ser TCT	Ile ATT	Cys TGC	Asn AAT	Ala GCT	Leu CTG	Glu GAA	Val GTT	100 His CAC	Ile ATT	Gln CAG	Thr ACT	Arg CGC	Trp TGG	Lys AAA	His CAC	Pro CCT	Ser TCC	110 Val GTG
Asp GAC	Ser AGC	Arg AGA	Asp GAC	Leu CTA	Phe TTT	Tyr TAT	Ile ATC	120 Leu CTC	Tyr TAC	Pro CCG	Asp GAC	Tyr TAT	Ala GCG	Ala GCT	Ile ATC	Ser AGC	Arg AGG	130 Ala GCG
Val GTC	Leu CTG	Asp GAT	Leu TTG	Val GTC	Leu CTC	Tyr TAT	Tyr TAC	140 Irp TGG	Lys AAA	Thr ACA	Val GTG	Thr ACG	Val GTG	Val GTG	Tyr TAT	Glu GAA	Asp GAT	150 Ser AGC
Thr ACA	Gly GGT	Leu CTA	Ile ATT	Arg CGT	Leu CTG	Gln CAA	Glu GAG	160 Ile ATC	Lys AAA	Ala GCT	Pro CCC	Ser TCC	Arg AGA	Tyr TAC	Asn AAC	Ile ATT	Lys AAA	170 Ile ATC
Lys AAA	Ile ATC	Arg CGC	Gln CAG	Leu CTT	Pro CCC	Pro CCT	Ala GCG	180 Lys AAA	Asp GAC	Ala GCC	Lys AAA	Pro CCT	Leu CTG	Leu CTC	Lys AAG	Glu GAG	Met ATG	190 Lys AAG

FIG. 10-2

Books & Quik

Glur5

Lys AAA	Ser AGC	Lys AAA	Glu GAG	Phe TTC	Tyr TAT	Val GTG	Ile ATA	Phe TTT	Asp GAT	Cys TGT	Ser TCG	His CAC	Glu GAA	Thr ACA	Ala GCT	Ala GCG	Glu GAA	Ile ATT	Leu CTT
										200									
										600									
Lys AAG	Gln CAG	Ile ATT	Leu TTG	Phe TTC	Met ATG	Gly GGC	Met ATG	Met ATG	Thr ACT	Glu GAA	Tyr TAT	Tyr TAT	His CAC	Tyr TAC	Phe TTC	Phe TTC	Thr ACA	Thr ACC	Leu CTG
										220									
										660									
Asp GAC	Leu TTG	Phe TTT	Ala GCT	Leu TTA	Asp GAT	Leu CTG	Glu GAA	Leu CTC	Tyr TAT	Arg AGG	Tyr TAC	Ser AGC	Gly GGT	Val GTA	Asn AAT	Met ATG	Thr ACT	Gly GGA	Phe TTT
										240									
										720									
Arg CGG	Leu TTG	Leu CTG	Asn AAT	Ile ATT	Asp GAC	Asn AAC	Pro CCT	His CAC	Val GTG	Ser TCA	Ser TCC	Ile ATC	Ile ATT	Glu GAG	Lys AAG	Trp TGG	Ser TCC	Met ATG	Glu GAG
										260									
										780									
Arg AGG	Leu TTG	Gln CAG	Ala GCC	Pro CCG	Pro CCC	Arg AGA	Pro CCC	Glu GAG	Thr ACT	Gly GGT	Leu CTT	Leu CTG	Asp GAT	Gly GGC	Met ATG	Met ATG	Thr ACA	Thr ACT	Glu GAA
										280									
										840									
Ala GCA	Ala GCG	Leu CTG	Met ATG	Tyr TAC	Asp GAT	Ala GCT	Val GTG	Tyr TAC	Met ATG	Val GTA	Ala GCC	Ile ATT	Ala GCG	Ser TCC	His CAC	Arg CGT	Ala GCC	Ser TCT	Gln CAG
										300									
										900									
Leu CTG	Thr ACC	Val GTC	Ser AGC	Ser TCC	Leu CTG	Gln CAG	Cys TGC	His CAT	Arg CGA	His CAT	Lys AAG	Pro CCA	Trp TGG	Arg CGC	Leu CTT	Gly GGA	Pro CCC	Arg AGA	Phe TTT
										320									
										960									

FIG. 10-3

GluR5

Met Asn Leu Ile Lys Glu Ala Arg Trp Asp 340
 ATG AAC CTC ATC AAA GAG GCT CGG TGG GAC 1020
 Gly Leu Thr Gly Arg Ile Thr Phe Asn * 350
 GGC TTG ACT GGG CGG ATC ACC TTC AAT AAG 1040
 Thr Asp Gly Leu Arg Lys Asp Phe Asp Leu 360
 ACC GAT GGC TTG AGA AAG GAT TTT GAC CTG 1080
 Asp Ile Ile Ser Leu Lys Glu Gly Thr 370
 GAT GAT GGC TTG AGA AAG GAT TTT GAC CTG 1100
 Glu Lys Ala Ser Gly Glu Val Ser Lys His 380
 GAA AAG GCC TCT GGT GAA GTG TCT AAA CAC 1140
 Trp Asn Ser Asn Ser Gly Leu Asn Met Thr 400
 TGG AAC TCC AAC AGT GGT GGC CTG AAC ATG 1200
 Thr Asp Ser Leu Ala Asn Arg Thr Leu Ile 420
 ACG GAC TCG CTG GCT AAC Asn Arg ACA CTC 1260
 Met Tyr Arg Lys Ser Asp Lys Pro Leu Tyr 440
 ATG TAC AGG AAA TCC GAT AAG CCC TTG TAT 1320
 Asp Leu Leu Lys Glu Leu Ser Asn Ile Leu 460
 GAT CTG CTG AAA GAA CTG CTG TCC AAT ATC 1380
 Leu Tyr Asp Val Lys Leu Val Cys 470
 GGT TTT CTT TAC GAT GTT AAA CTG GTT CCT 1400

FIG. 10-4

M. A. Clark

Glur5

Asp Gly Lys Tyr Gly Ala Gln Asn Asp Lys	480	Gly Glu Trp Asn Gly Met Val Lys Glu	490
GAT GGC AAA TAT GGA GCA AAT GAC AAA	1420	GGG GAA TGG AAT GGG ATG GTA AAA GAA	Leu CTC
Ile Asp His Arg Ala Asp GAC CTG GCA GTG	500	Pro Leu Thr Ile Thr Tyr Val Arg Glu	510
ATC GAC CAC AGA GCT GAC GAC CTG GCA GTG	1480	CCT CTC ACC ATC ACA TAC GTA CGG GAG	Lys AAA
Val Ile Asp Phe Ser Lys Pro Phe Met Thr	520	Leu Gly Ile Ser Ile Leu Tyr Arg Lys	530
GTC ATT GAC TTC TCC TCC AAG CCC TTC ATG	1540	CTG GGC ATT AGC ATC CTT TAC CGG AAG	Pro CCC
* Asn Gly Thr Asn Pro Gly Val Phe Ser Phe	540	Leu Asn Pro Leu Ser Pro Asp Ile Trp	550
AAT GGA ACC AAC CCG GGT GTC TTC TCC TTC	1600	CTC AAC CCC CTA TCT CCG GAC ATT TGG	Met ATG
Tyr Val Leu Leu Ala Cys Leu Gly Val Ser	560	Cys Val Leu Phe Val Ile Ala Arg Phe	570
TAC GTG CTG CTC GGC TGC CTA GGA GTC AGT	1660	TGT GTA CTG TTT GTG ATT GCG AGG TTC	Thr ACA
Pro Tyr Glu Trp Tyr Asn Pro His Pro Cys	580	Asn Pro Asp Ser Asp Val Val Glu Asn	* Asn
CCC TAC GAG TGG TAT AAC CCC CAC CCA TGC	1720	AAC CCC GAC TCA GAC GTG GTG GAA AAC	AAT
Phe Thr Leu Leu Asn Ser Phe Trp Phe	600	Val Gly Ala Leu Met Gln Gln Gly Ser	610
TTC ACT TTG CTA AAT AGT TTC TGG TTT	1780	GTT GGA GCT CTC ATG CAG CAA GGA TCA	Glu GAG

FIG. 10-5

Handwritten signature

GIUR5

Leu Met Pro Lys Ala Leu Ser Thr Arg Ile 620 Val Gly Gly Ile Trp Trp Phe Phe Thr 630
CTG ATG CCC AAG GCT CTA TCG ACC AGA ATA 1860 GTT GGA GGA ATA TGG TGG TTT TTC ACC CTA
1840
Ile Ile Ile Ser Ser Tyr Thr Ala Asn Leu 640 Ala Ala Phe Leu Thr Val Glu Arg Met 650
ATC ATC ATT TCA TCC TAC ACG GCC AAC CTG GCT GCC TTC TTG ACG GTA GAA AGA ATG GAA
1900 1920 1940
Ser Pro Ile Asp Ser Ala Asp Asp Leu Ala 660 Lys Gln Thr Lys Ile Glu Tyr Gly Ala Val 670
TCC CCC ATC GAT TCC GCA GAC GAT CTG GCC 1980 AAA CAA ACC ACC AAG ATA GAA TAT GGG GCA GTC
1960
Arg Asp Gly Ser Thr Met Thr Phe Phe 680 Lys Ser Lys Ile Ser Thr Tyr Glu Lys Met 690
AGA GAT GGC TCG ACG ATG ACC TTC TTC AAG 2040 AAA TCA AAG ATC TCC ACC TAT GAG AAA ATG
2020
Trp Ala Phe Met Ser Ser Arg Gln Gln Ser 700 Ala Leu Val Lys Asn Ser Asp Glu Gly Ile 710
TGG GCT TTC ATG AGC AGT AGA CAG CAG 2100 GCA CTG GTT AAA AAC AGT GAC GAG GGG ATC
2080
Gln Arg Val Leu Thr Thr Asp Tyr Ala Leu 720 Leu Met Glu Ser Thr Ser Ile Glu Tyr Val 730
CAA AGG GTG CTC ACC ACC GAC GAC TAC GCA CTG 2160 CTG ATG ATG GAG TCC ACC AGC ATT GAG TAT GTG
2140
Thr Gln Arg Asn Cys Asn Leu Thr Gln Ile 740 Gly Gly Leu Ile Asp Ser Lys Gly Tyr Gly 750
ACG CAG AGG AAC TGC AAC CTC ACT CAG 2220 GGG GGC CTC ATA GAC TCC AAA GGC TAT GGA
2200

FIG. 10-6

Handwritten signature

GLUR5

Val Gly Thr Pro Ile Gly Ser Pro Tyr Arg 760
 GTG GGG ACG CCT ATC GGC TCC CCT TAC CGG 2260
 Asp Lys Ile Thr Ile Ala Ile Leu Gln 770
 GAT AAA ATT ACG ATT GCC ATT CTT CAA CTG 2300
 Gln Glu Glu Gly Lys Leu His Met Met Lys 780
 CAA GAA GAA GGG AAG CTT CAT ATG ATG AAA 2320
 Glu Lys Trp Trp Arg Gly Asn Gly Cys 790
 GAG AAG TGG TGG AGG GGG AAT GGC TGC CCT 2360
 Glu Glu Asp Ser Lys Glu Ala Ser Ala Leu 800
 GAA GAA GAC AGT AAG AAG GAA GCC AGT GCT CTG 2380
 Gly Val Glu Asn Ile Gly Gly Ile Phe 810
 GGA GTG GAA AAT ATC GGC GGC ATC TTC ATT 2420
 Val Leu Ala Ala Gly Leu Val Leu Ser Val 820
 GTT CTG GCT GCA GGA CTC GTG CTT TCT GTG 2440
 Phe Val Ala Ile Gly Glu Phe Leu Tyr 830
 TTT GTA GCC ATT GGA GAA TTT TTA TAC AAA 2480
 Ser Arg Lys Asn Asn Asp Val Glu Gln Cys 840
 TCA CGG AAG AAC AAT GAC GTT GAG CAG TGT 2500
 Leu Ser Phe Asn Ala Ile Met Glu Glu 850
 CTC TCT TTC AAT GCC ATC ATG GAA GAG CTG 2540
 Gly Ile Ser Leu Lys Asn Gln Lys Lys Leu 860
 GGA ATA TCC CTC AAG AAT CAG AAA AAA TTA 2560
 Ser Phe Thr Ser Ile Leu Thr Cys His Gln 880
 TCT TTC ACA AGT ATC CTT ACT TGT CAC CAG 2620
 Arg Arg Thr Gln Arg Lys Glu Thr Val Ala 890
 AGA CGA ACT CAG AGA AAA GAG ACA GTG GCG 2660

FIG. 10-7

GluR5

End
TGA TCAAAGAACACACCTGTAGAAGAAAAGCCACACGTCGCTGCACATATTTGGAGGACAGATTTTCAGAGGAC 2680 2700 2720 2740
TATGTCCTTTATCCATAACCCAGTCGTGGACAGAGGGGAAGAAATGCACAATTTTTAAAGCTCACATAGATATTACTTGA 2760 2800 2820
GAAGTGAAACTGATTCTTTTCAGATGAATTTGTATGCACACTTATTTTGAATTTTCCATTTCCTCCGATAAATTGCT 2840 2880 2900
ATGTGTGCTTTCTAAATAATAACAAGCGGACTTTGTTTTTCATAAAAAAAAAAAAA 2920 2940 2960

FIG. 10-8

Heads & Clerk

GluR6

AAT TCG GGC TCG CAA GGG CTT CGC AGG CTG GAC ATT GTG CTT GCT GGA TTT TTC CCG GAT
- 300 -2 80 -26 0

GCT CCC GGA CTA ACA TGG ATG TCC CAC CAT CCC TTG CAG TGG AAG CTT GCT CCT TGG CGC
- 240 -2 20 -20 0

AGT GAG AGT GAA GAA CAT GCA GCG ACT GCT AAT GGG TTT GGG AAG CGG AGA CTC CTT CCT
- 180 -1 60 -14 0

CTT TCT GTG ACC ATG CCG TGA TTG TGT CTG CGG CCA CTA CTC CAC GCA TCT TCC TTC TnG
- 120 -1 00 -8 0

TCC AAG CCC GGA GCC TAA CGC TAG ATC GGG GAA AGT GGG TGC CGC GCG AGG CAC GGA
-60 - 40 -2 0

Met Lys Ile Ile Ser Pro Val Leu Ser Asn Leu Val Phe Ser Arg Ser Ile Lys
AAC ATC ATG AAG ATT ATT TCC CCA GTT TTA AGT AAT CTA GTC TTC AGT CGC TCC ATT AAA
0 20 4 0

Val Leu Leu Cys Leu Trp Ile Gly Tyr Ser Gln Gly Thr Thr His Val Leu Arg Phe
GTC CTG CTC TGC TGA TTA TTG TGG ATC GGA TAT TCG CAA GGA ACC ACA CAT CAT GTG TTA AGA TTC
60 80 10 0

Gly Gly Ile Phe Glu Tyr Val Glu Ser Gly Pro Met Gly Ala Glu Glu Leu Ala Phe Arg
GGT ATA TTT GAA TAT GTG GAA TCT GGC CCC ATG GGA GAA GAA CTT GCA TTC AGA
120 1 40 16 0

Phe Ala Val Asn Thr Ile Asn Arg Asn Arg Thr Leu Leu Pro Asn Thr Thr Leu Thr Tyr
TTT GCT GTG AAT ACC ATC AAC AGA AAC AGG ACT TTG CTG CCC AAC ACC ACT TTA ACT TAT
180 2 00 22 0

FIG. 11-1

FIG. 11-2

GluR6

Asp Thr Gln Lys Ile Asn Leu Tyr Asp Ser Phe Glu Ala Ser Lys Lys Ala Cys Asp Gln
GAT ACT CAG AAG ATC AAT CTC TAT TAT GAC AGT TTT GAA GCA TCT AAG AAA GCT TGT GAT CAG
240 2 60 28 0

Leu Ser Gly Val Ala Ala Ile Phe Gly Pro Ser His Ser Ser Ser Ala Asn Ala Val
CTG TCT CTT GGG GTG GCT GCT ATC TTC TTC GGC CCT TCA TCA TCA TCA GCC AAT GCT GTG
300 3 20 34 0

Gln Ser Ile Cys Asn Ala Leu Gly Val Pro His Ile Gln Thr Arg Trp Lys His Gln Val
CAG TCC ATC ATC TGC AAT GCT GCT CTG GGG GTT CCC CAC CAC ATA CAG ACC CGC TGG AAG CAC CAG GTG
360 3 80 40 0

Ser Asp Asn Lys Asp Ser Phe Tyr Val Ser Leu Tyr Pro Asp Phe Ser Ser Arg Ser Arg
TCA GAC AAC AAG GAT TCC TTC TAC TAC CTC AGT AGT CTC TAC CCA GAC GAC TTC TCT TCC CTG AGC CGC
420 4 40 46 0

Ala Ile Leu Asp Leu Val Gln Phe Phe Lys Trp Lys Thr Val Val Tyr Asp Asp
GCC ATC TTG GAT TTG GTG CAG TTT TTT AAG TGG AAA ACT GTC ACA GTT GTG TAT GAC GAC
480 5 00 52 0

Ser Thr Gly Leu Ile Arg Leu Gln Glu Leu Ile Lys Ala Pro Ser Arg Tyr Asn Leu Arg
AGC ACT GGT CTC ATT CGC TTG CAA GAG CTC ATC AAA GCT CCA TCG AGG TAC AAT CTT CGT
540 5 60 58 0

Leu Lys Ile Arg Gln Leu Pro Ala Asp Thr Lys Asp Ala Lys Pro Leu Leu Lys Glu Met
CTT AAA ATT CGT CAG CAG CTG CCA GCT GAT ACC ACC AAA GAT GCA AAA CCT TTG CTG AAG GAG ATG
600 6 20 64 0

Lys Arg Gly Lys Glu Phe His Val Ile Phe Asp Cys Ser His Glu Met Ala Ala Gly Ile
AAA AGA GGC AAG GAG TTC CAC GTG ATC TTC GAC TGC AGC CAT GAG ATG GCA GCA GGC ATT
660 6 80 70 0

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FIG. 11-3
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Leu	Lys	Gln	Ala	Leu	Ala	Met	Gly	Met	Thr	Glu	Tyr	Tyr	His	Tyr	Ile	Phe	Thr	Thr
TTA	AAA	CAG	GCA	TTA	GCT	ATG	GGA	ATG	ACA	GAA	TAC	TAT	CAC	TAT	ATA	TTT	ACA	ACT
720							7 40						76 0					
Leu	Asp	Leu	Phe	Ala	Leu	Asp	Val	Glu	Pro	Tyr	Arg	Tyr	Ser	Gly	Val	Asn	Met	Gly
CTG	GAC	CTC	TTT	GCT	CTT	GAC	GTG	GAG	CCC	TAC	AGA	TAC	AGT	GGC	GTA	AAT	ATG	GGG
780							8 00							82 0				
Phe	Arg	Ile	Leu	Asn	Thr	Glu	Asn	Thr	Gln	Val	Ser	Ser	Ile	Ile	Glu	Lys	Trp	Met
TTC	AGG	ATA	CTA	AAT	ACA	GAG	AAT	ACC	CAA	GTC	TCC	TCC	ATC	ATC	GAA	AAG	TGG	TCT
840							8 60							88 0				ATG
Glu	Arg	Leu	Gln	Ala	Pro	Pro	Lys	Pro	Asp	Ser	Gly	Leu	Leu	Asp	Gly	Phe	Met	Thr
GAA	CGG	TTA	CAG	GCG	CCT	CCA	AAA	CCT	GAC	TCA	GGT	TTG	CTG	GAT	GGA	TTT	ATG	ACT
900							9 20							94 0				
Asp	Ala	Ala	Leu	Met	Tyr	Asp	Ala	Val	His	Val	Val	Ser	Val	Ala	Val	Gln	Phe	Pro
GAT	GCT	GCT	CTG	ATG	TAT	GAT	GCA	GTG	CAC	GTT	GTG	TCT	GTG	GCT	GTC	CAA	CAG	TTT
960							9 80							100 0				CCC
Gln	Met	Thr	Val	Ser	Ser	Leu	Gln	Cys	Asn	Arg	His	Lys	Pro	Trp	Arg	Phe	Gly	Arg
CAG	ATG	ACA	GTC	AGC	TCC	TTG	CAA	TGC	AAT	CGA	CAC	AAA	CCC	TGG	CGC	TTT	GGG	ACC
1 020							10 40							106 0				CGC
Phe	Met	Ser	Leu	Ile	Lys	Glu	Ala	His	Trp	Glu	Gly	Leu	Thr	Gly	Arg	Ile	Thr	Asn
TTC	ATG	AGT	CTA	ATT	AAA	GAG	GCT	CAC	TGG	GAA	GGT	CTC	ACA	GGC	AGA	ATA	ACA	TTT
1 080							11 00							112 0				AAC
Lys	Thr	Asn	Gly	Leu	Arg	Thr	Asp	Phe	Asp	Leu	Asp	Val	Ile	Ser	Leu	Lys	Glu	Gly
AAA	ACC	AAT	GGA	TTA	CGG	ACA	GAT	TTT	GAT	TTG	GAT	GTG	ATC	AGT	CTC	AAG	GAA	GGT
1 140							11 60							118 0				

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FIG. 11-4

GluR6

Leu	Glu	Lys	Ile	Gly	Thr	Trp	Asp	Pro	Ser	Ser	Gly	Leu	Asn	Met	Thr	Glu	Ser	Gln	Lys
CTG	GAG	AAG	ATT	GGA	ACT	TGG	GAT	CCG	TCC	AGT	GGC	CTG	AAT	ATG	ACA	GAA	AGT	CAG	AAA
1	200						12	20						124	0				
Gly	Lys	Pro	Ala	Asn	Ile	Thr	Asp	Ser	Leu	Ser	Asn	Arg	Ser	Leu	Ile	Val	Thr	Thr	Ile
GGA	AAG	CCA	GCA	AAT	ATC	ACA	GAC	TCA	TTG	TCT	AAT	CGT	TCT	TTG	ATT	GTT	ACC	ACC	ATT
1	260						12	80						130	0				
Leu	Glu	Glu	Pro	Tyr	Val	Leu	Phe	Lys	Lys	Ser	Asp	Lys	Pro	Leu	Tyr	Gly	Asn	Asp	Arg
TTG	GAA	GAA	CCG	TAT	GTT	CTG	TTT	AAG	AAG	TCT	GAC	AAA	CCA	CTC	TAT	GGG	AAT	GAT	CGA
1	320						13	40						136	0				
Phe	Glu	Gly	Tyr	Cys	Ile	Asp	Leu	Leu	Arg	Glu	Leu	Ser	Thr	Ile	Leu	Gly	Phe	Thr	Tyr
TTT	GAA	GGC	TAC	TGT	ATT	GAT	CTC	CTA	CGA	GAG	TTA	TCT	ACA	ATC	CTT	GGC	TTT	ACA	TAT
1	380						14	00						142	0				
Glu	Ile	Arg	Leu	Val	Glu	Asp	Gly	Lys	Tyr	Gly	Ala	Gln	Asp	Asp	Val	Asn	Gly	Gln	Trp
GAG	ATT	AGG	CTT	GTG	GAG	GAT	GGG	AAA	TAT	GGA	GCC	CAG	GAT	GAT	GTG	AAC	GGA	CAA	TGG
1	440						14	60						148	0				
Asn	Gly	Met	Val	Arg	Glu	Leu	Ile	Asp	His	Lys	Ala	Asp	Leu	Ala	Val	Ala	Pro	Leu	Ala
AAT	GGA	ATG	GTT	CGT	GAA	CTA	ATC	GAT	CAT	AAA	GCT	GAC	CTT	GCA	GTT	GCT	CCA	CTG	GCT
1	500						15	20						154	0				
Ile	Thr	Tyr	Val	Arg	Glu	Lys	Val	Ile	Asp	Phe	Ser	Lys	Pro	Phe	Met	Thr	Leu	Gly	Ile
ATA	ACC	TAT	GTT	CGT	GAG	AAG	GTC	ATC	GAC	TTT	TCA	AAG	CCG	TTT	ATG	ACA	CTT	GGA	ATA
1	560						15	80						160	0				
Ser	Ile	Leu	Tyr	Arg	Lys	Pro	Asn	Gly	Thr	Asn	Pro	Gly	Val	Phe	Ser	Phe	Leu	Asn	Pro
AGT	ATT	TTG	TAC	CGC	AAG	CCC	AAT	GGT	ACA	AAC	CCA	GGC	GTC	TTC	TCC	TTC	CTG	AAT	CCT
1	620						16	40						166	0				

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FIG. 11-5

GluR6

Leu	Ser	Pro	Asp	Ile	Trp	Met	Tyr	Val	Leu	Leu	Ala	Cys	Leu	Gly	Val	Ser	Cys	Val	Leu
CTC	TCC	CCT	GAT	ATC	TGG	ATG	TAT	GTT	CTG	CTG	GCT	TGC	TTG	GGT	GTC	AGT	TGT	GTG	CTC
1	680				17	00								172	0				
Phe	Val	Ile	Ala	Arg	Phe	Ser	Pro	Tyr	Glu	Trp	Tyr	Asn	Pro	His	Pro	Cys	Asn	Pro	Asp
TTT	GTC	ATA	GCC	AGG	TTT	AGT	CCC	TAT	GAG	TGG	TAT	AAC	CCA	CAC	CCT	TGC	AAC	CCT	GAC
1	740				17	60								178	0				
Ser	Asp	Val	Val	Glu	Asn	Asn	Phe	Thr	Leu	Leu	Asn	Ser	Phe	Trp	Phe	Gly	Val	Gly	Ala
TCA	GAC	GTG	GTG	GAA	AAC	AAT	TTT	ACC	TTG	CTA	AAT	AGT	TTC	TGG	TTT	GGA	GTT	GGA	GCT
1	800				18	20								184	0				
Leu	Met	Arg	Gln	Gly	Ser	Glu	Leu	Met	Pro	Lys	Ala	Leu	Ser	Thr	Arg	Ile	Val	Gly	Gly
CTC	ATG	CGG	CAA	GGT	TCT	GAG	CTC	ATG	CCC	AAA	GCA	CTC	TCC	ACC	AGA	ATA	GTG	GGA	GGC
1	860				18	80								190	0				
Ile	Trp	Trp	Phe	Phe	Thr	Leu	Ile	Ile	Ile	Ser	Ser	Tyr	Thr	Ala	Asn	Leu	Ala	Ala	Phe
ATT	TGG	TGG	TTT	TTC	ACA	CTT	ATC	ATC	ATT	TCT	TCG	TAT	ACC	GCT	AAC	CTA	GCC	GCC	TTT
1	920				19	40								196	0				
Leu	Thr	Val	Glu	Arg	Met	Glu	Ser	Pro	Ile	Asp	Ser	Ala	Asp	Asp	Leu	Ala	Lys	Gln	Thr
CTG	ACT	GTG	GAA	CGC	ATG	GAG	TCG	CCC	ATT	GAC	TCT	GCT	GAC	GAT	TTA	GCT	AAG	CAA	ACC
1	980				20	00								202	0				
Ile	Val	Glu	Tyr	Gly	Ala	Val	Glu	Asp	Gly	Ala	Thr	Met	Thr	Phe	Phe	Lys	Lys	Ser	Lys
AAAG	ATA	GAG	TAT	GGA	GCA	GTG	GAG	GAC	GGC	GCA	ACC	ATG	ACG	TTT	TTT	AAG	AAA	TCA	AAA
2	040				20	60								208	0				
Ile	Ser	Thr	Tyr	Asp	Lys	Met	Trp	Ala	Phe	Met	Ser	Ser	Arg	Arg	Gln	Ser	Val	Leu	Val
TAT	TCA	ACG	TAT	GAT	AAA	ATG	TGG	GCG	TTT	ATG	AGC	AGC	AGG	AGA	CAG	TCT	GTG	CTT	GTC
2	100				21	20								214	0				

FIG. 11-6

GluR6																			
Lys	Ser	Asn	Glu	Lys	Gly	Ile	Gln	Gln	Val	Leu	Thr	Ser	Asp	Tyr	Ala	Phe	Leu	Met	Glu
AAA	AGC	AAT	GAG	AAA	GGG	ATC	CAA	CAA	GTC	CTC	ACC	TCG	GAT	TAT	GCT	TTC	TTA	ATG	GAG
2	160				21	80								220	0				
Ser	Thr	Thr	Ile	Glu	Phe	Val	Thr	Gln	Arg	Asn	Cys	Asn	Leu	Thr	Gln	Ile	Gly	Gly	Leu
TCA	ACA	ACC	ATC	GAG	TTT	GTT	ACA	CAG	CGG	AAC	TGT	AAC	CTC	ACG	CAG	ATT	GGC	GGC	CTT
2	220				22	40								226	0				
Ile	Asp	Ser	Lys	Gly	Tyr	Gly	Val	Gly	Thr	Pro	Met	Gly	Ser	Pro	Tyr	Arg	Asp	Lys	Ile
ATA	GAC	TCC	AAA	GGC	TAT	GGC	GTT	GGC	ACT	CCT	ATG	GGC	TCT	CCA	TAT	CGA	GAC	AAA	ATC
2	280					23	00							232	0				
Thr	Ile	Ala	Ile	Leu	Gln	Leu	Gln	Glu	Glu	Gly	Lys	Leu	His	Met	Met	Lys	Glu	Lys	Trp
ACC	ATA	GCA	ATT	CTT	CAG	CTG	CAG	GAG	GAA	GGC	AAG	CTG	CAC	ATG	ATG	AAG	GAG	AAA	TGG
2	340				23	60								238	0				
Trp	Arg	Gly	Asn	Gly	Cys	Pro	Glu	Glu	Glu	Ser	Lys	Glu	Ala	Ser	Ala	Leu	Gly	Val	Gln
TGG	CGG	GGC	AAT	GGC	TGC	CCA	GAG	GAG	GAG	AGC	AAA	GAG	GCC	AGT	GCT	CTG	GGG	GTG	CAG
2	400					24	20							244	0				
Asn	Ile	Gly	Gly	Ile	Phe	Ile	Val	Leu	Ala	Ala	Gly	Leu	Val	Leu	Ser	Val	Phe	Val	Ala
AAT	ATT	GGT	GGT	ATC	TTC	ATT	GTC	CTG	GCA	GCC	GGC	TTG	GTG	CTC	TCA	GTT	TTT	GTG	GCA
2	460					24	80							250	0				
Val	Gly	Glu	Phe	Leu	Tyr	Lys	Ser	Lys	Lys	Asn	Xxx	Gln	Leu	Glu	Lys	Arg	Ser	Phe	Cys
GTG	GGA	GAG	TTT	TTA	TAC	AAA	TCC	AAA	AAA	AAC	nnt	CAA	TTG	GAA	AAG	AGG	TCC	TTC	TGT
2	520					25	40							256	0				
Ser	Ala	Met	Val	Glu	Glu	Leu	Arg	Met	Ser	Leu	Lys	Ser	Gln	Arg	Arg	Ser	Asn	Ile	Ser
AGC	GCT	ATG	GTG	GAA	GAG	CTG	AGA	ATG	TCC	CTG	AAG	TCC	CAG	CGT	CGC	TCA	AAC	ATA	AGC
2	580					26	00							262	0				

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GluR6

His Ser Pro Ser Tyr Cys Glu Asn Arg Arg Val Ile Asn Met His Thr Phe Asn Asp Arg
CAC AGC CCC AGT TAT TTT TTT TTT CCA AAC AAT TTA GCG AGA ATG TTT CCT GTG GAA ATA TGC AAC
2 640 26 60 27 20 27 80 286 0 268 0

Arg Cys Arg End
AGG TGC AGG TAA AGA AAC CAT GGC ATG AAG CTG GAG GCC AAT CAC CCA GCA CAA ACT GTC
2 700 27 20 27 80 286 0 274 0

GTC TTT TTT TTT TTT TTT CCA AAC AAT TTA GCG AGA ATG TTT CCT GTG GAA ATA TGC AAC
2 760 27 80 286 0 280 0

CTG TGC AAA ATA AAA TGA GTT CCT CAT GCC GCT GTG TCT ATG AAC TAG AGA CTC TGT GAT
2 820 28 40 286 0 286 0

CTA AGC AGT TTC AGT AGT GAT CAG ACT TGA TTT ACA AGC ACC GTG GAT CAA CCA AGT TAC ACG
2 880 29 00 292 0 292 0

GGG TTA CAC TGT TTA TCA TAG GTT CCT CCC TTC CTT TGA GTG AAG TGT TAC ATG CAA ATG
2 940 29 60 298 0 298 0

TTG TGG CTG GTT TCA AAT GCA GTC CAG GAG AAA TGC AGT CCA GGG AGA AAC TGC TGG TTC
3 000 30 20 304 0 304 0

CTT CTG AAG CTC AGC TGT CGT CAG GAG ATG GAA TGC CGG TGC CCA AAA GGG TAA CCA ATA
3 060 30 80 310 0 310 0

AAA ATG CCA TAA AAA TTT TAA AAA AAT GCG TGA GAT CGG CAA AAA TTA TAG TGT TAC AAG
3 120 31 40 316 0 316 0

AAA CAG TCA GTC CCT GGT CAC CAA CAC AAT AGA GGT GAT AAT GTT ACT AGC CCC CCG AAT
3 180 32 00 322 0 322 0

FIG. 11-7 51

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Glur7

Gly Ile Arg Leu Val Glu Asp Gly Lys Tyr Gly Ala Gln Asp Lys Gly Gln Trp Asn
GGG ATC CGG CTG 10 GTC GAA GAC GGC AAG TAC GGG GCA CAG GAC GAC AAG GGC CAG TGG AAC 50

Gly Met Val Lys Glu Leu Ile Asp His Lys Ala Asp Leu Ala Val Ala Pro Leu Thr Ile
GGC ATG GTC AAG 70 GAA CTC ATT GAC CAC CAC AAA GCA GAT CTG GCT GGT GCT CCC CTG ACC ATC 110

Thr His Val Arg Glu Lys Ala Ile Asp Phe Ser Lys Pro Phe Met Thr Leu Gly Val Ser
ACC CAT GTC CGA 130 GAG AAG GCC ATT GAC TCT TCT AAG CCT TTT ATG ACC CTC GGA GTG AGC 170

Ile Leu Tyr Arg Lys Pro Asn Gly Thr Asn Pro Ser Val Phe Ser Phe Leu Asn Pro Leu
ATC TTA TAT CGA 190 AAA CCC AAT GGC ACC ACC AAC CCC AGT GTC TTC TCC TTC CTC AAC CCC CTG 230

Ser Pro Asp Ile Trp Met Tyr Val Leu Leu Ala Tyr Leu Gly Val Ser Cys Val Leu Phe
TCC CCA GAC ATC 250 TGG ATG TAC GTG CTA CTC GCC TAC TAC GGT GTC AGC TGT GTC CTC TTC 290

Val Ile Ala Arg Phe Ser Pro Tyr Glu Trp Tyr Asp Pro His Pro Cys Asn Pro Gly Ser
GTC ATT GCC AGA 310 TTC AGC CCT TAT GAA TGG TAT GAT GAT CCC CAC CCC TGC AAC CCC GGC TCT 350

Glu Val Val Glu Asn Asn Phe Thr Leu Leu Asn Ser Phe Trp Phe Gly Met Gly Ser Leu
GAG GTG GTG GAG AAT AAC TTC ACG CTG CTC AAC AGC TTC TGG TTT GGA ATG GGC TCC CTG 390 410

FIG. 12-1

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GluR7

Met Gln Gln Gly Ser Glu Leu Met Pro Lys Ala Leu Ser Thr Arg Ile Ile Gly Gly Ile
 ATG CAA CAA GGA TCT GAA CTG ATG CCC AAA GCT CTG TCT ACC CGC ATC ATT GGC GGC ATC
 430 450 470

Trp Trp Phe Phe Thr Leu Ile Ile Ser Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu
 TGG TGG TTC TTC ACC CTT ATT ATC ATC TCC TCC TAC ACG GCC GCT GCT GCC TTC CTG
 490 510 530

Thr Val Glu Arg Met Glu Ser Pro Ile Asp Ser Ala Asp Asp Leu Ala Lys Gln Thr Lys
 ACC GTG GAG CGC ATG GAG TCA CCC ATC GAC TCT TCT GAT GAC CTG GCC AAG CAG ACC AAA
 550 570 590

Ile Glu Tyr Gly Ala Val Lys Asp Gly Ala Thr Met Thr Phe Phe Lys Lys Ser Lys Ile
 ATA GAG TAC GGT GCT GTC AAG GAT GGG GCC ACC ATG ACC TTC TTC AAG AAA TCC AAG ATC
 610 630 650

Ser Thr Phe Glu Lys Met Trp Ala Phe Met Ser Ser Lys Pro Ser Ala Leu Val Lys Asn
 TCC ACC TTT GAG AAG ATG ATG TGG GCC TTC ATG AGC AGC AAG CCC TCG GCT CTG GTG AAG AAC
 670 690 710

Asn Glu Glu Gly Ile Gln Arg Thr Leu Thr Ala Asp Tyr Ala Leu Leu Met Glu Ser Thr
 AAT GAG GAG GGC ATC CAG CGG ACA CTC ACA GCT GAC GAC TAC GCT CTG CTC ATG GAG TCC ACG
 730 750 770

Thr Ile Glu Tyr Ile Thr Gln Arg Asn Cys Asn Leu Thr Gln Ile
 ACC ATA GAG TAC ATC ACA CAA AGG AAC TGC AAT CTC ACC CAG ATT
 790 810

FIG. 12-2

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