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FURTHERING PHARMACOLOGICAL AND PHYSIOLOGICAL ASSESSMENT OF THE GLUTAMATERGIC RECEPTORS AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

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ABSTRACT OF THESIS

FURTHERING PHARMACOLOGICAL AND PHYSIOLOGICAL ASSESSMENT OF THE GLUTAMATERGIC RECEPTORS AT THE *DROSOPHILA* NEUROMUSCULAR JUNCTION

Drosophila larval neuromuscular junctions (NMJs) serve as a model for synaptic physiology. The molecular sequence of the postsynaptic glutamate receptors has been described; however, the pharmacological profile has not been fully elucidated. Despite the postsynaptic molecular sequence used to classify the receptors as a kainate subtype, they do not respond pharmacologically as such. Kainate does not depolarize the muscle, but dampens evoked EPSP amplitudes. Quantal responses show a decreased amplitude and area under the voltage curve indicative of reduced postsynaptic receptor sensitivity to glutamate transmission. ATPA, a kainate receptor agonist, did not mimic kainate's action. The metabotropic glutamate receptor agonist t-ACPD had no effect. Domoic acid, a quisqualate receptor antagonist, blocks the postsynaptic receptors without depolarizing the muscle, which supports the presence of quisqualate subtype receptors. The results suggest a direct postsynaptic action of kainate due to partial antagonist action on the quisqualate receptors. There does not appear to be presynaptic auto-regulation via a kainate receptor subtype or a metabotropic

auto-receptor. A complete pharmacological profiling of the known receptor subtypes at this NMJ has not yet occurred; however, this study aids in furthering the ongoing investigations to provide a clearer picture of pharmacokinetic profile and specificity of the receptor subtypes.

KEY WORDS: Synaptic facilitation, NCX, calcium, *Drosophila* larval heart

Junyoung Lee

August 2, 2009

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OF THE GLUTAMATERGIC RECEPTORS AT THE *DROSOPHILA*
NEUROMUSCULAR JUNCTION

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Thesis

Junyoung Lee

The Graduate School

University of Kentucky

2009

FURTHERING PHARMACOLOGICAL AND PHYSIOLOGICAL ASSESSMENT
OF THE GLUTAMATERGIC RECEPTORS AT THE *DROSOPHILA*
NEUROMUSCULAR JUNCTION

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master in the
College of Arts and Sciences at the
University of Kentucky
By

Junyoung Lee

Lexington, Kentucky

Director: Dr. Robin Lewis Cooper, Associate Professor

2009

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ACKNOWLEDGMENTS

I came to the University of Kentucky from South Korea two years ago. I am in active duty as a captain in the Korean Army. I was sent here by the Korean Army to learn Biology and experimental methods to address how the nervous system functions. When I began pursuing this masters program, I had little knowledge of the biological field. When I was a student at the Korean Military Academy I focused on Chemistry and Military Science. The time I spent in the biology department was stressful and compounded with frustration, as the language was new to me and the courses moved along very rapidly. So I feel extremely happy and lucky to have made it through the program. It was not a miracle that I survived in this foreign country, as I had very helpful people around me. It is a great pleasure to have a chance to be aquatinted with them.

The first person I appreciate is Dr. Robin L Cooper who is my mentor. I emphatically state that he is one of the best professors I have experienced. He has helped me a lot to feel comfortable both inside and outside the lab. His patience with me was nothing short of miraculous and he even never lost smile on his face. 설레임과 두려움을 안고 미국으로 온 저에게 항상 웃는 얼굴로 학업 및 생활하는데 불편함이 없도록 직접 보살펴 주시고, 많이 바쁜 와중에도 저의 엉뚱한 질문도 그냥 넘기는 것 없이 자상하게 해주시는 모습을 보면서 남몰래 눈물을 흘린 적도 있었습니다. In fact, it is impossible for me to relay of all gratitude and respect in this letter. 이 곳 렉싱턴에서의 교수님과 함께했던 시간은 소중한 추억으로 간직되어 평생 잊지 못할 것입니다. 쿠퍼 교수님 그리고 사모님 감사합니다.

I appreciate my committee members, Dr. Doug Harrison and Dr. Bruce O'Hara for their expertise and intellectual conversations over the years.

I also acknowledge all professors at the chemistry department in Korea Military Academy. They always led cadets and their juniors by taking the initiative. What I had seen and learned from them became the cornerstones of my life and the motives of studying here. Among them, I should especially thank Dr. IL-Woo Yang, Dr. Nam-Taek Lee and Dr. Woo-Young Chung for willingly writing letters of recommendation and for encouragement to me. Also, I could not have been happier when I learned that Dr. Woo-Young Chung and his family were spending a sabbatical year in Lexington, KY. 지금도 공부를 하다가 '교수님이 안 계셨으면 어떻게 되었을까?'하고 생각을 하곤 합니다. 표현이 서툴러 교수님께 감사하다는 말씀도 못드렸습니다. 제가 느끼고 배운 것들을 소중히 간직하여 베풀고 실천하면서 살도록 항상 노력하겠습니다. 감사합니다.

I had a pleasurable time with my lab colleagues, Sonya Bierbower and Mohati Desai. I can surely say you guys are my family. You guys helped me a lot to get through the hard times and to become familiar to the American society. Time being with you guys was always awesome. I should not forget a lot of new words I learned from Sonya. Also, I appreciate many other graduate and undergraduate students that worked in the lab over the past 2 years.

Appreciation is expressed to Korean Army Headquarters that supported my tuition and fees. I did not have to worry about financial matters while studying. Their support was a great advantage to me to focus on research and curriculum.

마지막으로, 제 인생에서 가장 중요하고 많은 부분을 차지하는 부모님께 감사드립니다. 자식들 뒷바라지 하느라 많은 것을 희생하신 우리 부모님. 아버지, 어머니의 존재 자체만으로도 저에게는 그 무엇보다 큰 힘이 되었습니다. 이제는 제가 아버지, 어머니의 든든한 기둥이 되어 얼굴에 항상 미소가 끊이지 않도록 노력하겠습니다. 사랑해요~♡.

p.s. 본문에 구체적으로 적지는 않았지만, 우리 사랑하는 누님, 이윤환 중대장님, 군 선배님 및 후배, 백두산 부대와 승리부대에서의 동생들, 그리고 켄터키 대학에서 물심양면으로 많은 도움을 주신 형님, 누님들께도 감사드립니다.

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Chapter One

Background of the *Drosophila* neuromuscular junction and research goals

The nervous system in animals is used to convey information of the surrounding external and internal environment, as well as to react to it. In the majority of cases, the electrical signals neurons use are relayed to target cells by chemical communication. When this occurs for close targets, the site of communication is referred to as a synapse. The chemical released from the presynaptic neuron is referred to as transmitter. This transmitter enters into the synaptic cleft and then binds to receptors on a target cell. The target cell can be a neuron, a muscle or an endocrine cell or even a combination of cell types. The post-synaptic receptors determine if the target cell will be excited or inhibited. These determine actions by the receptor itself, being a ligand-gated ion channel or by initiation of a cellular cascade to influence ion channels (Berridge, 1998; Miyazaki, 1995). Receptors that mediate secondary cascades are referred to as metabotropic receptors, whereas receptors that act as a receptor mediated ion channel are termed ionotropic receptors.

The neurotransmitter molecules are stored in vesicles within the presynaptic nerve terminal. These vesicles fuse to the presynaptic membrane with evoked stimulation because of the rise in $[Ca^{2+}]_i$ (Sudhof, 2004). The $[Ca^{2+}]_i$ is generally very low compared to that outside the neuron ($[Ca^{2+}]_o$ is 10 mM). The entry of Ca^{2+} into the nerve terminal causes the mobilization of both the reserve and the readily releasable pools of vesicles (Rosenmund and Stevens, 1996; Kuromi and Kidokoro, 1998).

The whole process of vesicle fusion must be finely regulated as not to over or under excite the target cells or else the information will not be correctly conveyed. Thus, there are various levels of excitation and inhibition to regulate nerve terminals, such as ion pumps, ion exchanges and auto-receptors for negative feedback. Because of the difficulty in accessibility of postsynaptic dendrites and the wide complexity of the central nervous system in general, most of our understanding in synaptic properties at a vesicle level (i.e., quantal level) has been gained by studying accessible and relatively simple preparations, such as neuromuscular junctions (NMJ) in invertebrates and amphibians (Bennett and Pettigrew, 1975; Nudell and Grinnell, 1983; Wilkinson and Lunin, 1994; Wilkinson et al., 1992). The frog, locust and crayfish neuromuscular junctions were initially used because of the relatively ease for accessibility and viability in relatively minimal physiological salines (Fatt and Katz, 1953a, b; Dudel, 1965, 1981; Dudel et al., 1983).

More recently the *Drosophila melanogaster* has become a model for synaptic transmission at the larval NMJ. This is because a known genome that can be manipulated for altered protein expression to examine mechanistic cellular functions of synapses (Atwood et al., 1993; Betz et al. 1993; Jan and Jan 1976; Kurdyak et al., 1994; Li et al., 2002; Li and Cooper, 2001; Pawlu et al., 2004; Ruffner et al., 1999; Sigrist et al., 2002, 2003; Stewart et al., 1994, 1996). A recent review in Ca^{2+} regulation which influences synaptic transmission in the preparation has appeared (Desai-Shah and Cooper, 2009).

Since the larval *Drosophila* NMJ is becoming a synaptic model of choice recently for many reasons, it is important to understand the pharmacological and physiological profiles of this synapse. The *Drosophila* NMJ consists of excitatory motor neurons and muscle. Recent studies described which postsynaptic receptor subtypes are expressed in muscle. There are five different subunits, which fall into kainate/AMPA type glutamate receptors based on molecular sequence homology (Betz et al., 1993; Völkner et al., 2000; Marrus et al., 2004; Qin et al., 2005; Guerrero et al., 2005). However, in an experiment investigating a pharmacological sensitivity on the postsynaptic receptors for intact 3rd instar *Drosophila* NMJ, kainate did not depolarize the muscle but reduced the EPSP amplitude. Also, AMPA did not alter the EPSP amplitude nor alter the resting membrane potential (Bhatt and Cooper, 2005). Thus, the postsynaptic receptors at the *Drosophila* NMJ should not be classified based on a pharmacological profiling nomenclature as a kainate/AMPA subtype of glutamate receptors. Based on my investigations and the previous study (Bhatt and Cooper, 2005), kainate appears to be blocking the glutamate activities at the larval *Drosophila* NMJ.

To date, the pharmacological assessment of this NMJ has not been tackled carefully. No one has even addressed if the motor nerve terminals may possess glutamate auto-receptors as noted to occur in other arthropod species (Schramm and Dudel, 1997). Thus, I choose to address these issues in my studies.

In this research project, I first support the idea that the postsynaptic receptors at the *Drosophila* NMJ are not of a kainate subtype of glutamate receptors and that application of kainate reduces the evoked EPSP amplitude. I approached these studies by use of pharmacological and physiological investigations. Specifically I addressed the spontaneous vesicular postsynaptic responses at the 3rd instar NMJ using domoic acid and glutamate. Domoic acid is a potent agonist of the kainate subtype of glutamate receptors in vertebrates. I also addressed how exogenous application of kainate could be causing the reduction of the EPSP amplitudes.

The hypotheses I am testing is based on preliminary findings at this NMJ and combined reports on other glutamatergic synapses in various animal

models. The hypotheses addresses why kainate produces a reduction in the EPSP amplitude. My hypotheses are as following;

- 1) Kainate has a direct postsynaptic action as an antagonist.
- 2) Kainate has a direct presynaptic action reducing glutamate release, possibly through activating glutamatergic autoreceptors on the nerve terminals.

Addressing these aims is very important in understanding processes in synaptic regulation at the neuromuscular junction of this model preparation. Several disorders are known for altered synaptic function in the vertebrate brain. In order to understand these disorders better, one can use invertebrate models such as *Drosophila*, which can serve as good electrophysiological and genetic models to study synaptic regulation on a fundamental level.

In order to elucidate my hypotheses about kainate's action at the *Drosophila* NMJ, I conducted several experiments as described in detail within Chapter Two. In short, I examined the action of kainate on spontaneous fusion of vesicles with exposure to kainate. This is diagrammatically shown in **Figure 1-1** with kainate either having an action on the presynaptic terminal or having an action on the postsynaptic receptors as to alter receptivity to glutamate. Since $[Ca^{2+}]_i$ is tightly regulated within the nerve terminal for rapid transmission, if kainate had a presynaptic action via alteration in the $[Ca^{2+}]_i$, I would predict that short-term facilitation or depression, known to occur at this synaptic preparation, would be greatly affected. Thus, I used a four-pulse stimulation train and measured the facilitation index before and during application of kainate at various concentrations of kainate as well as $[Ca^{2+}]_o$. Since transmission is so sensitive to $[Ca^{2+}]_o$ altering the concentration with various concentration of kainate exposure might unmask any subtle effects of kainate. This general model to address this experimental paradigm is shown in **Figure 1-2**. In addition to the action of kainate other pharmacological agents were examined to help in describing the pharmacological profiles of the putative presynaptic and known postsynaptic glutamatergic receptor subtypes. The working models of action for ATPA (**Figure 1-3**) and t-ACPD (**Figure 1-4**) are shown for action on evoked transmission.

In addition, the reports which characterized the molecular sequence of the glutamate receptors at the *Drosophila* NMJ to the human's kainate receptor subunits, did not provide the statistical similarities between them. The paper published in 2005 by Qin et al., designated some amino acid sequences as similar even though they appear to be far from being similar. Besides, in this earlier study only five amino acid sequences of the postsynaptic receptor subunits at the *Drosophila* NMJ were examined although there are around 30 different glutamate receptor genes in *Drosophila* genome (Littleton and Ganetzky,

2000). Thus, I show statistical similarities of the aligned sequences (**Figure 1-5a,b**) and provide a phylogeny tree with the known 36 predicted amino acid sequences of the glutamate receptors in *Drosophila* genome (**Table 1-1**).

Model of a Neuromuscular Junction

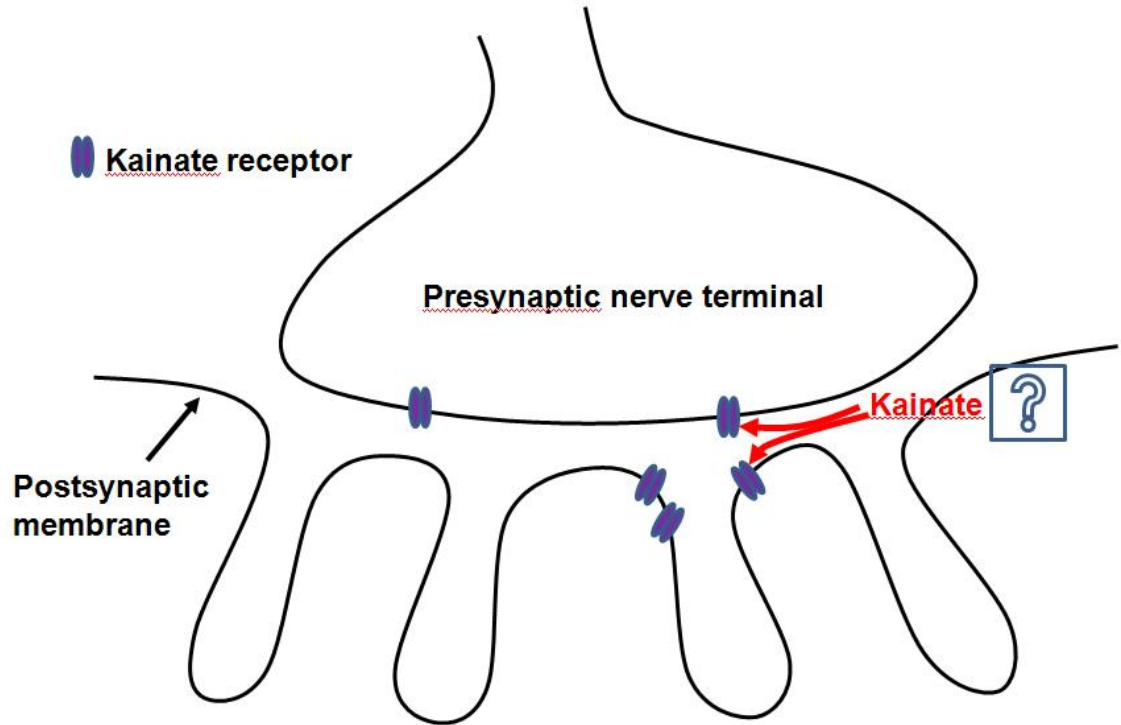


Figure 1-1. Schematic model of predicted kainate action at the *Drosophila* neuromuscular junction, which is the site of communication from the axon terminal of motorneuron to the muscle. Kainate is added into the synaptic cleft without neural stimulation to measure any possible direct effect on either the presynaptic nerve terminal or on postsynaptic sensitivity. Spontaneous responses are involved in one vesicle fusion events. If kainate has an effect presynaptically then one would expect an alteration in the frequency of spontaneous response. If there was an affect on the postsynaptic receptors then one would expect a change in the amplitudes or shape of the spontaneous events. The frequency and amplitude of spontaneous responses before and during exposure to kainate are compared.

Presynaptic nerve terminal

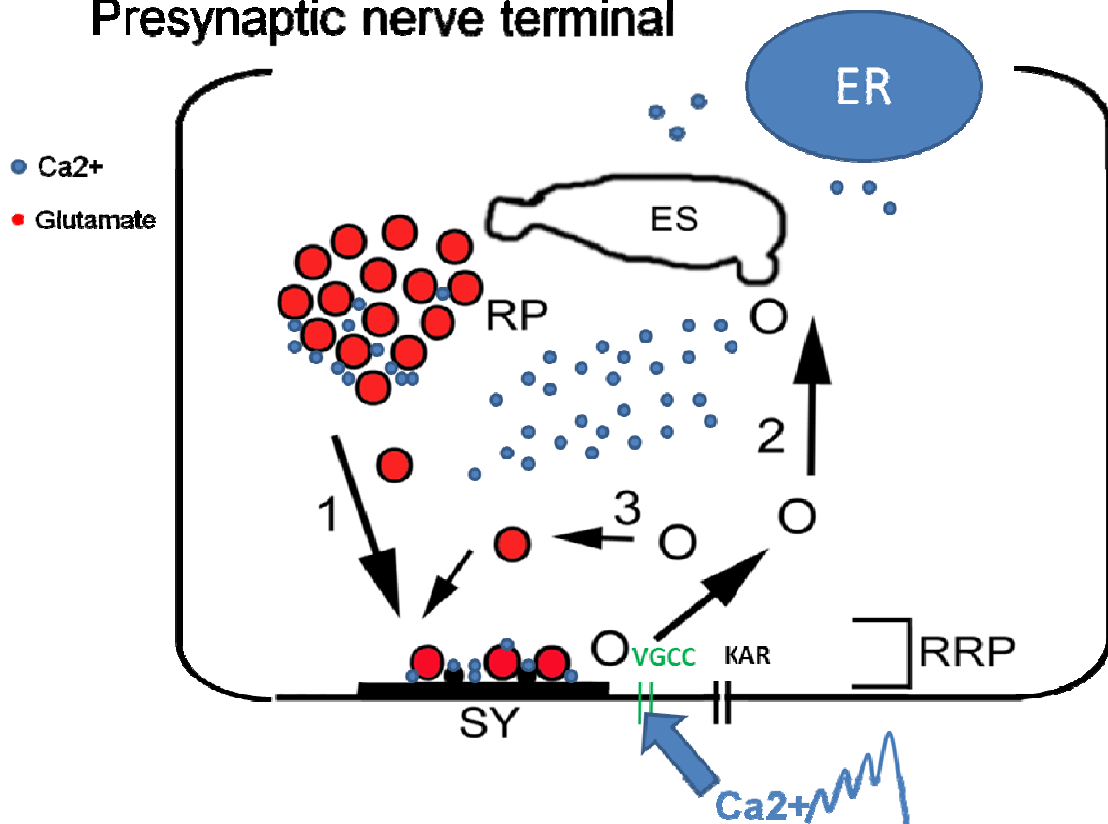


Figure 1- 2. Schematic model of facilitation effect on presynaptic nerve terminal. Ca^{2+} plays a critical role in vesicle fusion in the presynaptic nerve terminal. $[\text{Ca}^{2+}]$ in the presynaptic nerve terminal can be changed at each pulse of induced facilitation. Four-pulses with 20Hz is induced in the presynaptic nerve terminal, which may alter the dynamics affecting several factors involved in vesicle fusion inside the cell. This presynaptic activity at each pulse will have a slightly different condition. If there is a kainate effect on Ca^{2+} dynamics it would likely alter the vesicle fusion events during facilitation. Facilitation Index(FI) (4^{th} pulse amplitude / 1^{st} pulse amplitude -1) is measured before and during kainate exposure to determine if there is a presynaptic action of kainate. (RP: reserve pool, RRP: readily releasable pool, ER: intracellular Ca^{2+} storage, ES: endosome, VGCC: voltage-gated Ca^{2+} channel, KAR: kainate receptor)

Model of a Neuromuscular Junction

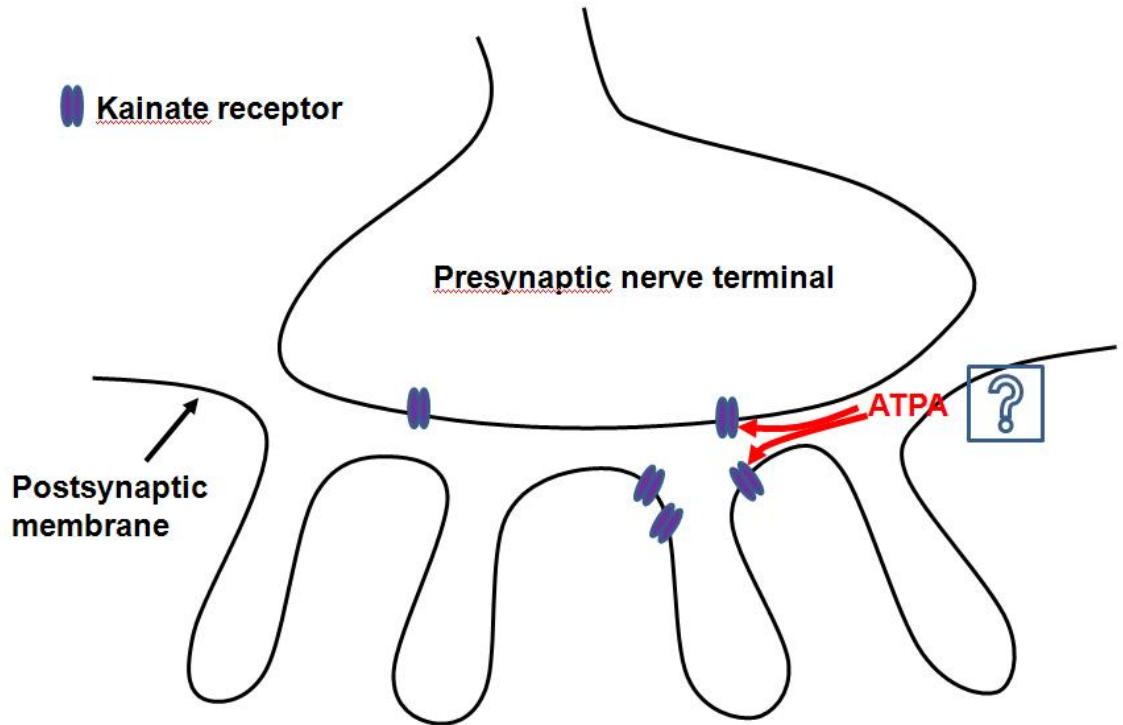


Figure 1- 3. Schematic model of predicted ATPA action at *Drosophila* NMJ. (RS)-2-Amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA) is a kainate receptor agonist. If ATPA has an action either presynaptically or postsynaptically it would alter the frequency or amplitude of spontaneous events and the size of evoked responses. Excitatory postsynaptic potential (EPSP) amplitudes and membrane potential in the muscle are measured with the bath application of ATPA to compare if they are affected.

Model of a Neuromuscular Junction

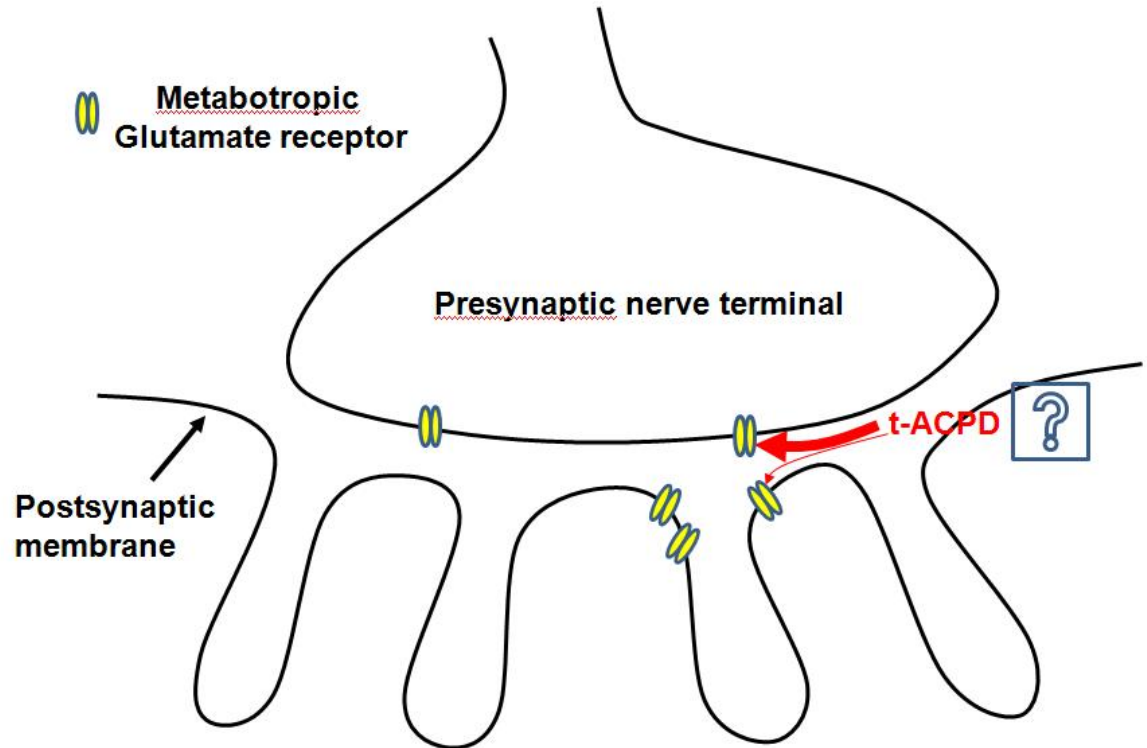


Figure 1- 4. Schematic model of predicted t-ACPD action at *Drosophila* NMJ. trans-(±)-1-Amino-1,3-cyclopentanedicarboxylic acid monohydrate (t-ACPD) is a well-known metabotropic glutamate receptor agonist. If t-ACPD has an action either presynaptically or postsynaptically it would alter the amplitude of evoked response. Excitatory postsynaptic potential (EPSP) amplitudes and membrane potential in the evoked response are measured with the bath application of t-ACPD to see if there is a t-ACPD action through the metabotropic glutamate receptors. In the figure, narrow arrow is described to show the action of t-ACPD on the postsynaptic receptor because of a small possibility of a postsynaptic action on receptors at the *Drosophila* NMJ. The receptors are known as ionotropic glutamate receptors.

GluR-IIA -----MRLCPVVIYAFIIIIIGFLEGIIALGGDDRNEITVG----AIFYENEKEIELSFDQAFREVN--NMKFSELRFVTIKRYMPTNDSFLLQQ
 GluR-IIB -----MHGLQFLVLLALAIASGANEDTLVITVG----AIFYENEKEIELSFDQAFREVN--NMKFSELRFVTIKRYMPTNDSFLLQQ
 GluR-IIC MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIG----AFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVTMQE
 GluR-IID -----MHFCWIS---LIILSLSRVQAQFYGGNAYEASSGQSIRLG----LITDDATDRIRQTFEHAISVVN----NELGVPLVGETEQVAYGNSVQAF
 GluR-IIE -----MFFNHFVILWSLFSIHISVNWAQYENFGG-YDNYQSLESVIPG----LLTDQNTQMNIVFDHAIDVAN----QEVGTSLSLKEEVNYGDAYQSYG
 GluR-5 -----MEHGTLAQPLWTRDTSWALLYFLCYILPQTAP-QVLRIGGIFETVENEFPVNVVEELAFKFAVTSINRNRRLMPNTTLLTYDIQRINLFDSEASR

ITCELI SNGVAAIFG PSSKAASDIVAQIANATGIPHI EYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI I KSN-YEWRTFTLIYETPEG--LARLQDLMNIQA
 ITCELI SNGVAAIFG PSSKAASDIVAQIANATGIPHI EYDLKQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSH--LIRLQHILAWKQ
 HACDLIDNGVAAIFG PSSKAASDIVALVCNSTGIPHI EFDISDEGIQAEPNHQMTLNLYPQAQAILSKAYADIVQNF--GWRKFTIVYDADDARAAARLQDLLQLRE
 QLCRLMQSGVAVFGPAARHTASHLLNACDSKDIPIFIYPHLSWGSNPDG-----FNLHPSPEDIANALYDIVNQF--EWSRFIFCYESA EY--LKILDHLMTRYG
 KLCRMLLETGIAGVFGPSSRHTAVHLMSICDAMDIPHIYSYMS--ENAEG-----FNLHPPADLAKALYSLITEF--NWTRFIFLYESA EY--LNILNELTTMLG
 RACDQLALGVAALFGPSSHSSVSAVQSICNALEVPHIQTRWKHPSVDNKDL---FYINLYPDYAAISR AILDVLVLY--NWKTVTVVYEDSTG--LIRLQELIKAPS

LNSDYVKLRNLADYADDYRILWKETDETFHEQRI I LDCEPKTLKEL LKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYN--EDFKANITSVRLKVVDANPFERKKTR
 LHKAGIKMQEFE-RGDDYRILWKRINN-AREK FVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNLDTHLSGIDGFYS--RDFTVAVAAVRI RTYVPPP VHDEIDV
 VHNDVVRVRFH-KDDDFRVMWKSIRG---ERRVVLDCPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNI TAARLLVNPDP PPSLPG
 IKGPV I KVMRYDLNLNGNYKSVLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYI I GNLNLTFTDLEEYKYS----EANITGIRMFSPDQEEVRLMEK
 KSGTVITVLR YDMQLNGNYKQVLRVRKSV DNRIVVVGSSETMPEFLNQAQQVG I INEDYKYI I GNLD FHSFDLEEYKYS----EANITGLRLFSPEKMAVKELLMK
 RYNIKIKIRQLPSGNK-DAKPLLKEMKKGKEFYVIFDCSHETA AEILKQILFMGMTEYYHYFFTLLDLFALDLELYRS----GVNMTGFRLLNIDNPHVSSII EK

LT-KVDQILGN-----QTMLPIL IYDAV VLFASSARNVIAAMQP-FHPP----NRHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEY GQRIHFNL
 FDNSVDTRFS-----SLGSQLVYDSIVL FYN-ALLEISQRPG-FYIP----NFSCG-RGFWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNL
 YVTQRDNIVYES---SDPPRTLIHDLI HDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRTWAMGRYLARLMKGTSG-VNNTNFRTSILQFDEDEGQRITFNI
 LHQELGESEP---VNSGSTFITMEMALTYDAVRVIAETTKHLPYQPOM-----LNCSEHDNVQPDGSTFRNYMR--SLEIKEKTITGRIYFEGNVRKGF TFDV
 LGYPTDQDE---FRNGSCPI TVEMALTYDAVQLFAQTLK NLPFKPMP-----QNCQRTESVRDDGSSFKNYMR--TLRLTDRLLTGPIYFEGNVRKGYHLDV
 WSMERLQAPRPETGLLDGMMTTEAALMYDAVYMVVAIASHRASQLTVS-----SLQCHRHKPWR-LGPRFMNLIK--EARWDGLTGHITFNKTNGLRKDFDLDI

Figure 1-5a. Sequence alignment with predicted amino acid sequences of the postsynaptic receptor subunits at *Drosophila* NMJ and human's kainate receptor subunits. Compared subunits are shown on the left of the first row in aligned sequences. Identical and similar amino acids matched are colored by red and putative extracellular domains are shaded by yellow, respectively. ClustalW2 is used for sequence analysis.

(Figure 1-5a cont.)

EIYKPTVNEPMMVWTPDN---GIKKRLLN---LELESAGTTQDFSEQRKVYTVVTHYEEPYFMMKEDHE--NFRGREKEYEGYAVDLISKLSSELMEDFYEFMIVNGN
 EVYNPIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWRREEPEGVHYEGNERFEGYAVDLIYMLAQECKFDNFEPVRDN
 EVYDPLDGIGIAIWDPGR-----QITQLNVDVKAQKKMIYRVATRIGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQ
 IELQTSGLVKVGTWEEGK-----DFEFQR-PPQAVNFNDIDDGSLVNKTFIVLISVATKPYASLVESID--TLIGNNQFQGYGVDLIKELADKLGFNFTFRDGGND
 IELQPSGIVKVGTWDEDR-----QYRQRLAPTTAQFDSVDN-SLANKTFIILLVSPNKPYAQLVETK--QLEGNSQYEGYGVDLIKELADKLGFNFTFVNGND
 ISLKEEGTEKIGIWNSNS-----GLNMTD---SNKDKSSNITDSLANNRTLIVTT-ILEEPYVMYRKSDK--PLYGNDRFEGYCLDLLKELSNILGFIYDVKLVPDG

TM1

--GKYNPETKQWDGIIRKLI DHHAQIGVCDLTITQMRRSVVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPF AVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNP
 KYGSYDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDFTVPFMQLGISILSYKEPPPKADIYAFLNPNYNAEVLWFVMIAMMITAFALIFTGRIDQYEDQPV
 QYQGLDKETKQWNGIIGEIINND AHMIGICDLTITQARKTAVDFTVPFMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMVTFKLTIVARISKMDWENPH
 -YGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIAILYKPKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEDNPNY
 -YGSYNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMNLGIAILYLKPKATPELFTFMDPFSSEVWVFLGFSFLGVSLSFFILGRLSPSEWDNPNY
 -KYGAQNDKGEWNGMVKELIDHRADLAVAPLTITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPH

TM2

TM3

PAIQDPDEL ENIWNVNNSTWLMVGSIMQQGCDILPRGPHMRILTGMWVFFALMMLSTYTANLAAFLT SNKWQSSIKSLQDLIEQD-KVHFGSMRGGSTSLFFSESDN
 ENVNREMERQNIWHL SNALWLVLSMLNQGCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQN-KVQFGTIRGGATSVYFSESDN
 PCNRDPEVL ENQWR IHNTGWLTVASIMTAGCDILPRSPQVRMFEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQK-KVKFGTIYGGSTYNLLADSNE
 PCIEEPEEL ENQFTINNSLWFTTGALLQQGSEIAPKALSTRTISAIWWFFTLIMVSSYTANLAAFLT IENPTSPINSVKDLADNKDDVYQAKRTGSTRNFFSTSEE
 PCIEEPEEL ENQFTLGNSIWFTTGALLQQGSEIGPKALSTRTVASFWWFFTLIVSSYTANLAAFLTIEKPQSLINSVDDLADNKDGVYGAKKTGSTRNFFMSTAE
 PCNPDSDVVENNFTLLNSFWFGVGMALMQGSELMPKALSTRIVGGIWWFFTLIISSYTANLAAFLTVERMESPID SADDLAKQT-KIEYGAVRDGSTMTFFKSKSI

TDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLEMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSIQLSERGELQKMKNKWWKNHNV
 TDNRMAWNKMLSFKPDFAFTKNNEEGVDRVKLSKGTYAFLMETTNLQYYVQRNCELTQIGESFGEKHYGIAVPLNADFRSNLSVGIILRLSERGELFKLRNKWFNSNES
 TVYRLAFNLMNNDP SAYTKDNL EGVDRVRKNRGDMFLMETTTLEYHREQNCDLRSVGEKFGEKHYAIAVPFGAEYRSNLSVAIILKLSERGELYDLKQKWWKNPNA
 PIYIKMNEYLNA-HP EMLMENNQQGVDKVKS-GTKYAFLMESTSIEFN TVREC NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQQV LARLKNKWWNEVGA
 ERYKMKMNF MSE-NPQYLTEDNMEGVNRVKTN-THYAFLMESTSIEYNTKREC NLKKGIDALDEKGYGIAMRKDWPHRGKFNALLELQEQQVLEKMKKNKWWNEVGT
 STYEKMWAFMSSRQQTALVRNSDEGIQRVLT--TDYALLMESTSIEYVTQRNCNL TQIGGLIDSKGYGVGTPIGSPYRDKITIAIILQLQEGLHMMKEKWWRGNG-

(Figure 1-5a cont.)

TM4

-TCDS-YHEVDGDELSIIELGGVFLVLAGVVLIGVILGIFEFLWNVQNVAVEERVTPWQAFKAELIFALKFWVRKKPMRISS--SSDKSSRRSS-----GSRRSSK
-TCDSNVPTIDDGQFDMDSVGGLFVVLIVGVVVLVIGVAEFLWHVQRISVKEKIPMLALKAEFYFVIRFWLTRKPLHTYR--QSRDSTSTGYSSLEQITSASSAK
-SCFEEDPDATPDMTFEELRGIFYTLYAGILIAFLIGITEFLVYVQQVALEERLTFKD AFKKEIRFVLCVWNNRKP I VAGTP I SSVRTPRRSLDKSLDRTPKSSR
GVCSAKSDDDGPSELGVDNLSGIYVVLVIGSIISIIISILWCYFVYKKAKNYEVPFCDALAEFRIVIRFSENERPLKSAQSIYSRSRNSSQSIESLKT DSEENMP
GICATKEDAPDATPLDMNNLEGVFFVLLVGSCCALLYGIISWVLFVMKKAHHRVPLRDALKEEFQFVIDFN NYVRVLKNSAS IYSRSRQSSMSVASVAQESQ----
---CPEEDNKEASALGVENIGGIFIVLAAGLVLVSVFVAIGEF IYKSRKNNDIEQCLSFNAIMEELGISLKNQKKIK--KKSRTKGKSSFTSILTCHQRRTQRKETVA

EKSRSKTVS-----
K K K K T R R I E K-----
R V V I G R S S E E M R E M A Q G S G S S S G S N N A G R G E K E A R V
V E D-----

(Figure 1-5a cont.)

GluR-IIA -----MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVG----AIFYENEKEIELSFDQAFREVN--NMKFSELRFVTIKRYMPTNDSFLLQQ
GluR-II B -----MHGLQFLVLLALAIASGANEDTLV IKIG----AIFFDTEMKLADAFSAALEEVN--AIN-PALKLDAIKRYVTVDSSIVLQD
GluR-II C MKKELSGNSSARWPQMFGGNSREAGMQRILLGCMWSAFFMCRSRGQQINIG----AFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDG SVTMQE
GluR-II D -----MHFCWIS---LIILSLSRVQAQFYGGNAYEASSGQSIRLG----LITDDATDRIRQTFEHAISVVN----NELGVPLVGETEQVAYGNSVQAF A
GluR-II E -----MFFNHFVILWSLFSIHSVNWAQYENFGG-YDNYQSLESVPIG----LLTDQNTQMNIVFDHAIDVAN----QEVGTSLSLKEEVNYGDAYQSYG
GluR-6 -----MKIIFPILSNPVFRRTVKLLLLCLLWIG--YSQGTTHVLRFGGIFEYVESGPMGAEEELAFRFVANTINRNR TLLPNTTLLTYDTQKINLYDSFEASK

ITCELSINGVAAIFGPPSSKAASDIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI I KSN-YEWRTFTLIYETPEG--LARLQDLMNIQA
ISCDLIGSGVAAIFGPPSSKTNSDIVEVLCNMTGIPHLQFDWHPPQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSH--LIRLQHILAWKQ
HACDLIDNGVAAIFGPPSSKAASDIVALVCNSTGIPHIEFDISDEGIQAEPNHQMTLNLYPQAQAILSKAYADIVQNF--GWRKFTIVYDADDARAAARLQDLLQLRE
QLCRLMQSGVGAVFGPAARHTASHLLNACDSKDIPIFIYP-----HLSWGSNPDGFNLHPSPEDIANALYDIVNQF--EWSRFIFCYESA EY--LKILDHLMTRYG
KLCRML ETGIAGVFGPSSRHTAVHLSICDAMDIPHIYS-----YMS--ENAEGFNLHPPADLAKALYSLITEF--NWTRFIFLYESA EY--LNILNELTTMLG
KACDQLSLGVAAIFGPPSHSSANAVQSICNALGVPHIQTRWK--HQVSDNKDSFYVSLYPDFSSLSRAILDLVQFF--KWKTVTVVYDDSTG--LIRLQELIKAPS

LNSDYVKLRNLADYADDYRILWKETDETFHEQRI I LDCEPKTLKELLKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYN--EDFKANITSVRLKVV DANPFERKKTR
LHKAGIKMQEFE-RGDDYRILWKRINN-AREK FVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNL DTHLSGIDGFYS--RDFTVAVAAVRI RTYVPPP VHDEIDV
VHNDVVRVRFKH-KDDDFRVMWKSIRG---ERRVVLDCPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAA RLLVNPDP PYPYSLPYG
IKGPV I KVMRYDLNLNGNYKSVLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYIIIGNLNLHTFDLEEYKYS----EANITGIRMFSPDQEEVRDLMEK
KSGTVITVLR YDMQLNGNYKQVLRVRVKSVDNRIVVVG SSETMPEFLNQAQQVGI INEDYKYIIIGNLDFHSFDLEEYKYS----EANITGLRLFSPEKMAVKELLMK
RYNLR LKIRQLPADTK-DAKPLLKEMKR GKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDL FALDVEPYRYS----GVNMTGFRILNTENTQVSSII EK

LT-KVDQILGN-----QTMLPIL IYDAVVLFASSARNVIAAMQP-FHPP----NRHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEY GQRIHFNLEIYK
FDNSVDTRFS-----SLGSQLVYDSIVL FYN-ALLEISQRPG-FYIP----NFSCG-RGFWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYN
YVTQRDNIVYESSDPPTLIHDLI HDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRTWAMGRYLARLMKGTSG-VNNTNFRTSILQFDEDGQRITFNIEVYD
LHQELGESEP-----VNSGSTFITMEMALTYDAVRVIAETTKHLPYQP-----QMLNC SERHDNVQPDGSTFRNYMRSLEIKEKTITGRIYFEGNVRKGF TFDVIE
LGYPTDQDE-----FRNGSCPITVEMALTYDAVQLFAQTLKNLPFKP-----MPQNC SQRTE SVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGNVRKGYHLDVIE
WSMERLQAPPK---PDSGLLDGFM TTTDAALMYDAHVVSVAVQFPQMT-----VSSLQCNRHK PWR-FGTRFMSLIKEAHWEGLTGRITFNKTNGLRTDFDL DVIS

PTVNEPMMVWTPDN---GIKKRLLN----LELESAGTTQDFSEQRKVYTVVTHYEEPYFMMKEDHE--NFRGREKYEGYAVDLISKLS ELMEDYEFMIVNGN--GK
PIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKDFDNFEPVRDNKYGS
PLDGIGIAIWDPRG-----QITQLNVDVKAQKKMIYRVATRIGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQOY GK
LQTSGLVKVGTWEE---GKDFEFQR-PPQAVNFNDIDDGSLVNKTFIVLISVATKPYASLVESID--TLIGNNQFQGYGVDLIKELADKLGFNFTFRDGGN-DYGS
LQPSGIVKVG TWDE---DRQYRPQRLAPTQAQFDSVDN-SLANKTFI ILLSVPNKPYAQLVETYK--QLEGNSQYEGYGVDLIKELADKLGFNFTFVNGGN-DYGS
LKEEGLEKIGTWDP---ASGLNMTE---SQKGK PANITDSL SNRSLIVTTILEEPYVLFK KSDK--PLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGA

(Figure 1-5a cont.)

YNPETKQWDGIIRKLI DHHAQIGVCDLTITQMRRSVVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNPAPIQ
YDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDFTVPFMQLGISILSYKEPPPKADIYAFLNPNYNAEVWLFVMIAMMITAFALIFTGRIDQYEWDPVENVN
LDKETKQWNGIIGEIINND AHMGICDLTITQARKTAVDFTVPFMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMVTFLLKTIVARISKMDWENPHPCNR
FNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIAILYVVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIE
YNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMNLGIAILYLKPKATPELFTFMDPFSEVWVWFLGFSFLGVSLSFFILGRLSPSEWDNPYPCIE
QDDANGQWNGMVRELI DHKADLAVAPLAITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMIYILLAYLGVSCVLFVIARFSPYEWYNPHPCNP

TM1

TM2

TM3

DPDELENIWNVNNSTWLMVGSIMQQGCDILPRGPHMRILTGMWVFFALMMLSTYTANLAAFLTSNKWQSSIKSLQDLIEQD-KVHFGSMRGGSTSLFFSESNDDTYQ
REMERQNIWHLSNALWLVLGSMNLNQGCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQN-KVQFGTIRGGATSVYFSESNDDTNR
DPEVLENQWRIHNTGWLTVASIMTAGCDILPRSPQVRMFEEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQK-KVKFGTIYGGSTYNLLADSNETVYR
EPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRTISAIWVFFTLIMVSSYTANLAAFLTIENPTSPINSVKDLADNKDDVQYGAKRTGSTRNFFSTSEPIYI
EPEELENQFTLGNSIWFTTGALLQQGSEIAPKALSTRTVASFVWVFFTLIVSSYTANLAAFLTIEKPQSLINSVDDLADNKDGVYGAKKTGSTRNFFMFTAERYK
DSDVVENNFLLNSFWFGVGLMQQGSELMPKALSTRIVGGIWWVFFTLIISSYTANLAAFLTVERMESPIDSADDLAKQT-KIEYGAVEDGATMTFFKKSISTYD

13

RAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSIQLSERGELQKMKNKWKNHNV-TCD
MAWNKMLSFKPDFAFTKNNEEGVDRVKLSKGTYAFLMETTNLQYYVQRNCEL TQIGESFGEKHYGIAVPLNADFRSNLSVGIILRLSERGELFKLRNKWFNSNES-TCD
LAFNLMNNDPSAYTKDNLEGVDRVRKNRGDYMFLETTTLEYHREQNCDLRSVGEKFGKHYAIAVPFGAEYRSNLSVAIILKLSERGELYDLKQKWWKNPNA-SCF
KMNEYLNAHPE-MLMENNQQGVDKVKS-GTKYAFLMESTSIEFNTVREC NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQGV LARLKNKWWNEVGAGVCS
KMNKFMSENPO-YLTEDNMEGVNRVKTN-THYAFLMESTSIEYNTKREC NLKIGDALDEKGYGIAMRKDWPHRGKFNALLELQEQGVLEKMKNKWWNEVGTGICA
KMWAFMSSRRQSVLVKSNEEGIQRVLT--SDYAFLMESTTIEFVTQRNCNL TQIGGLIDSKGYGVGTPMGSPYRDKITIAIILQLQEEGKLHMMKEKWWRGNG---C

TM4

S-YHEVDGDELSIIELGGVFLVLAGGV LIGVILGIFEF LWNVQNVAVEERVTPWQAFKAELIFALKFWVRKKPMRISS--SSDKSSSRSS-----GSRRSSKEKSR
SNVPTIDDGQFDMSVGGFLVVLIVGVVVLVIGVAEFLWHVQRI SVKEKIPMLALKAEFYFVIRFWLTRKPLHTYR--QSRDSTSTGYSSLEQITSASSAKKKK
EEDPDATPDMTFEELRGIFTYL YAGILIAFLIGITEFLVYVQVVALEERLTFKDAFKKEIRFVLCVWNNRKP I VAGTPISSVRTTPRRSLDKSLDRTPKSSRRVVI
AKSDDDGPSELGVDNLSGIYVVLVIGSIIISIIISILWCYFVYKKAKNYEVPCDALAEFRIVIRFSENERPLKSAQSIYSRSRNSQSIESLKT DSEENMPVED-
TKEDAPDATPLDMNLEGVFFVLLVGSCCALLYGIIISWVLFVMMKKAHHRVPLRDALKEEFQFVIDFNYYVRVLKNSASIYSRSRQSSMSVASVAQESQ-----
PEEESKEASALGVQNIIGGIFIVLAAGLVLSVFVA VGEFLYKSKNAQ-----LEKESSIWLVPYPHPDVT-----

(Figure 1-5a cont.)

SKTVS-----
TRRIEK-----
GRSSEEMREMAQSGSSSGSNNAGRGEKEARV

(Figure 1-5a cont.)

GluR-IIA -----MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVG----AIFYENEKEIELSFDQAFREVN--NMKFSELRFVTIKRYMPTNDSFLLQQ
GluR-IIB -----MHGLQFLVLLALAIASGANEDTLV IKIG----AIFFDTEMKLADAFSAALEEVN--AIN-PALKLDAIKRYVTVDSSIVLQD
GluR-IIC MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIG----AFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDG SVTMQE
GluR-IID -----MHFCWIS---LIILSLSRVQAQFYGGNAYEASSGQSIRLG----LITDDATDRIRQTFEHAISVVN----NELGVPLVGETEQVAYGNSVQAF
GluR-IIE -----MFFNHFVILWSLFSIHSVNWAQYENFGG-YDNYQSLESVPIG----LLTDQNTQMNIVFDHAIDVAN----QEVGTSLSLKEEVNYGDAYQSYG
GluR-6 -----MKIIFPILSNPVFRRTVKLLLLCLLWIG--YSQGTTHVLRFGGIFEYVESGPMGAEEELAFRFVANTINRNRTLLPNTTLLTYDTQKINLYDSFEASK

ITCELSINGVAAIFGPPSSKAASDIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKS N-YEWRTFTLIYETPEG--LARLQDLMNIQA
ISCDLIGSGVAAIFGPPSSKTNSDIVEVLCNMTGIPHLQFDWHPPQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSH--LIRLQHILAWKQ
HACDLIDNGVAAIFGPPSSKAASDIVALVCNSTGIPHIEFDISDEGIQAEPNHQMTLNLYPQAQAILSKAYADIVQNF--GWRKFTIVYDADDARAAARLQDLLQLRE
QLCRLMQSGVAVFGPAARHTASHLLNACDSKDIPIFIYP-----HLSWGSNPDGFNLHPSPEDIANALYDIVNQF--EWSRFIFCYESA EY--LKILDHLMTRYG
KLCRMLLETGIAGVFGPSSRHTAVHLMSICDAMDIPHIYS-----YMS--ENAEGFNLHPPADLAKALYSLITEF--NWTRFIFLYESA EY--LNILNELTTMLG
KACDQLSLGVAAIFGPPSHSSANAVQSICNALGVPHIQTRWK--HQVSDNKDSFYVSLYPDFSSLSRAILDLVQFF--KWKTVTVVYDDSTG--LIRLQELIKAPS

LNSDYVKLRNLADYADDYRILWKETDETFHEQRI IILDCEPKTLKELLKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYN--EDFKANITSVRLKVVDANPFERKKTR
LHKAGIKMQEFE-RGDDYRILWKRINN-AREK FVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNLDTHLSGIDGFYS--RDFTVAVAAVRI RTYVPPP VHDEIDV
VHNDVVRVRFKH-KDDDFRVMWKSIRG---ERRVVLDCPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAAARLLVNPDP PYPYSLPYG
IKGPV IKVMRYDLNLNGNYKSVLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYIIIGNLNLHTFDLEEYKYS----EANITGIRMFSPDQEEVRDLMEK
KSGTVITVLRDYMQLNGNYKQVLRVRVRSV DNRIVVVGSETMPEFLNQAQQVGI INEDYKYIIIGNLDFHSFDLEEYKYS----EANITGLRLFSPEKMAVKELLMK
RYNLR LKIRQLPADTK-DAKPLLKEMKRKGEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFDLVEPYRYS----GVNMTGFRILNTENTQVSSII EK

LT-KVDQILGN-----QTMLPIL IYDAVVLFASSARNVIAAMQP-FHPP----NRHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEYGQRIHFNLEIYK
FDNSVDTRFS-----SLGSQLVYDSIVL FYN-ALLEISQRPG-FYIP----NFSCG-RGFWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYN
YVTQRDNIVYESSDPPRTLIHDLI HDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRTWAMGRYLARLMKGTSG-VNNTNFRTSILQFDEDGQRITFNIEVYD
LHQELGESEP-----VNSGSTFITMEMALTYDAVRVIAETTKHLPYQP-----QMLNCSERHDNVQPDGSTFRNYMRSLEIKEKTITGRIYFEGNVRKGF TFDVIE
LGYPTDQDE-----FRNGSCPITVEMALTYDAVQLFAQTLKNL PFKP-----MPQNCQSRTESVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGNVRKGYHLDVIE
WSMERLQAPPK---PDSGLLDGFM TTTDAALMYDAHVVSVAVQFPQMT-----VSSLQCNRHK PWR-FGTRFMSLIKEAHWEGLTGRITFNKTNGLRTDFDL DVIS

PTVNEPMMVWTPDN---GIKKRLLN-----LELESAGTTQDFSEQRKVYTVVTHYEEPYFMMKEDHE--NFRGREKYEGYAVDLISKLSLMEFDYEFMIVNGN--GK
PIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKDFDNFEPVRDNKYGS
PLDGIGIAIWDPRG-----QITQLNVDVKAQKKMIYRVATRIGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQOYQK
LQTSGLVKVGTWEE---GKDFEFQR-PPQAVNFNDIDDGSLVNKTFIVLISVATKPYASLVESID--TLIGNNQFQGYGVDLIKELADKLGFNFTFRDGGN-DYGS
LQPSGIVKVG TWDE---DRQYRPQRLAPTQAQFDSVDN-SLANKTFI ILLSVPNKPYAQLVETYK--QLEGNSQYEGYGVDLIKELADKLGFNFTFVNGGN-DYGS
LKEEGLEKIGTWDP---ASGLNMTE---SQKGK PANITDSL SNRSLIVTTILEEPYVLFK KSDK--PLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGA

(Figure 1-5a cont.)

TM1

YNPETKQWDGIIRKLLIDHHAQIGVCDLTITQMRRSVVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNPPIQ
YDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDFTVPFMQLGISILSYKEPPPADIYAFLNPYNAEVLVFMAMIATAFALIFTGRIDQYEDQPVENVN
LDKETKQWNGIIGEIIINNDAHMGICDLTITQARKTAVDFTVPFMQLGVSILAYKSPHVEKTLDAYLAPFGGEEVWIWILISVFMVTLKTIVARISKMDWENPHPCNR
FNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIAIILYVYPKQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIE
YNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMNLGIAIILYLPKQKATPELFTFMDPFSSEVWVFLGFSFLGVSLSFFILGRLSPSEWDNPYPCIE
QDDANGQWNGMVRELI DHKADLAVAPLAITYVREKVIDFSKPFMTLGISILYRKPNGTNPVGF SFLNPLSPDIWMIYILLAYLGVSCVLFVIARFSPYEWYNPHPCNP

TM2

TM3

DPDELENIWNVNSTWLMVGSIMQQGCDILPRGPHMRILTGMWVFFALMMLSTYTANLAAFLTSNKWQSSIKSLQDLIEQD-KVHFQSMRGGSTSLFFSESNDTDYQ
REMERQNIWHLSNALWLVLGSMNLNQGCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQN-KVQFGTIRGGATSVYFSESNDTDNR
DPEVLENQWRIHNTGWLTVASIMTAGCDILPRSPQVRMFEEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQK-KVKFGTIYGGSTYNLLADSNETVYR
EPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRITISAIWVFFTLIMVSSYTANLAAFLTIENPTSPINSVKDLADNKDDVYQAKRTGSTRNFFSTSEPIYI
EPEELENQFTLGNSIWFTTGALLQQGSEIGPKALSTRITVASFVWVFFTLIVVSSYTANLAAFLTIEKPQSLINSVDDLADNKDGVVYGAKKTGSTRNFFMFTAERYK
DSDVVENNFTLLNSFWFGVGMALMQGSELMPKALSTRIVGGIWWVFFTLIISSYTANLAAFLTVERMESPIDSADDLAKQT-KIEYGAVEDGATMTFFKKSISTYD

16

RAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRNL SVS ILQLSERGELQKMKNKWWKNHNV-TCD
MAWNKMLSFKPDFAFTKNNEEGVDRVKLSKGTYAFLMETTNLQYYVQRNCELTQIGESFGEKHYGIAVPLNADFRSNLSVGIILRLSERGELFKLRNKWFNSNES-TCD
LAFNLMNNDPDSAYTKDNLEGVDRVRKNRGDYMFLMETTTLEYHREQNCDLRSVGEKFGEKHYAIAVPFGAEYRSNLSVAIILKLSERGELYDLKQKWWKNPNA-SCF
KMNEYLNAHPE-MLMENNQQGVDKVKS-GTKYAFLMESTSIEFNTVREC NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQQV LARLKNKWWNEVGAGVCS
KMNKFMSENPO-YLTDENMEGVNRVKTN-THYAFLMESTSIEYNTKREC NLKIGDALDEKGYGIAMRKDWPHRGKFNALLELQEQQVLEKMKNKWWNEVGTGICA
KMWAFMSSRRQSVLVKSNEEGIQRVLT--SDYAFLMESTTIEFVTQRNCNL TQIGGLIDSKGYGVGTPMGSPYRDKITIAIILQLQEGLHMMKEKWWRGNG---C

TM4

S-YHEVDGDELSIIELGGVFLVLAGGVLLIGVILGIFEFLLWNVQNVAVEERVTPWQAFKAELIFALKFWVRKPKMRIS--SSDKSSSRSS-----GSRRSSKEKSR
SNVPTIDDGQFDMSVGGFLVVLIVGVVGLVIGVAEFLWHVQRI SVKEKIPMLALKAEFYFVIRFWLTRKPLHTYR--QSRDSTSTGYSSLEQITSASSAKKKK
EEPDPDATPDMTFEELRGIFYTLIYAGILIAFLIGITEFLVYVQVVALEERLTFKDAFKKEIRFVLCVWNNRKP IVAGTPISSVRTTPRRSLDKSLDRTPKSSRRVVI
AKSDDDGPSELGVDNLSGIYVVLVIGSIIISIIISILWCYFVYKAKNYEVPFCDALAEFRIVIRFSENERPLKSAQSIYSRSRNSQSIESLKT DSEENMPVED-
TKEDAPDATPLDMNLEGVFFVLLVGSCCALLYGIIISWVLFVMMKKAHHRVPLRDALKEEFQFVIDFNYYVRV LKNSASIYSRSRQSSMSVASVAQESQ-----
PEEESKEASALGVQNIIGGIFIVLAAGLVLSVFVA VGEFLYKSKNAQ-----LEKESSIWLVPYHPDVT-----

(Figure 1-5a cont.)

SKTVS-----
TRRIEK-----
GRSSEEMREMAQSGSSSGSNNAGRGEKEARV

(Figure 1-5a cont.)

GluR-IIA -----MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVGAIFYE--NEKEIELSFDQAFREVN--NMKFSSELRFVVTIKRYMPTNDSFLLQOI
GluR-II B -----MHGLQFLVLLALAIASGANEDTLV IKIGAIFFD---TEMKLADAFSAALEEVN--AIN-PALKLDAIKRYVTVDDSIVLQDI
GluR-II C MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIGAFFYD---DELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVTMQEH
GluR-II D -----MHFCWIS---LIILSLSRVQAQFYGGNAYEASSGQSIRLGLITDD---ATDRIRQTFEHAISVVN----NELGVPLVGETEQVAYGNSVQAFQAQ
GluR-II E -----MFFNFHVFILWSLFSIHSVNWAQYENFGG-YDNYQSLESVP IGLLTDQ---NTEQMNIVFDHAIDVAN----QEVGTSLSLKEEVNYGDAYQSYGK
KA1 -----MPRVSAPLVLLPAWLVMVACSPHSLRIAAILDDPMECSRGERLSITLAKNRINRAPERLGKAKVEVDIFELLRDSEYETAET

TCELI SNGVAAIFGPSSK-AASDIVAQIANATGIPHI EYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKS N-YEWRTFTLIYETPEG--LARLQDLMNIQA
SCDLIGSGVAAIFGPSSK-TNSDIVEVLCNMTGIPHLQFDWHPQQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTI AYERSSH--LIRLQHILAWKQ
ACDLIDNGVAAIFGPSSK-AASDIVALVCNSTGIPHI EFDISDEGIQA EKNHQMTLNLYP AQAILSKAYADIVQNF--GWRKFTIVYDADDARAAARLQDLLQLRE
LCRLMQSGVAVFGPAAR-HTASHLLNACDSKDIPIIYPHLSWGSNPDG-----FNLHPSPEDIANALYDIVNQF--EWSRFIFCYESA EY--LKILDHLMTRYG
LCRML ETGIAGVFGPSSR-HTAVHLMSICDAMDIPHIYSYMS--ENAEG-----FNLHHPADLAKALYSLITEF--NWTRFI FLYESA EY--LNILNELTTMLG
MCQILPKGVAVLGPSSSPASSI ISNICGEKEVPHFKVAPEEFVKFQFQR--FTTLNLHPSNTDISVAVAGILNFF--NCTTACLICAKAEC--LLNLEKLLRQFL

LNSDYVKLRNLAD-YADDYRILWKETDET FHEQRI IILDCEPKTLKELKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYN--EDFKANITSVRLKVV DANPFERKKT
LHKAGIKMQEFE--RGDDYRILWKRINN-AREKFVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNL DTHLSGIDGFYS--RDFTVAVA AVRIRTYVPPPVHDEID
VHNDVVRVRKFH--KDDDFRVMWKSIRG--ERRVVLDCPEPMLVELLNSSTEFGLTGQYNHIFLTNLE TYTDHLEELAADNETFAVNI TAARLLVNPDP PPYSLPY
IKGPVIKVMRYDLNLNGNYKSVLRRIRK-SEDSRIVVVGSTTGVAELLRQAQQVGI MNEDYTYIIIGNLNLHTFDLE EYKYS---EANITGIRMFSPDQEEVRDLME
KSGTVITVLR YDMQLNGNYKQVLRVRK-SVDNRIVVVGSSSETMPEFLNQAQQVGI INEDYKYIIIGNLDFHSFDLE EYKYS---EANITGLR LFSPEKMAVKELLM
ISKDTLSVRMLDD--TRDPTLLKEIRD-DKTATII I HANASMSHTILLKAAELGMVSAYTYI FTNLEFSLQRMDSLVDD---RVN I LGFSIFNQSHAFFQEFQAQ

RLT-KVDQILGN-----QTMLPIL IYDAVVLFASSARNVIAAMQP-FHPP---NRHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEY GQRIHFNLEI
VFDNSVDTRFS-----SLGSQLVYDSIVL FYN-ALLEISQRPG-FYIP---NFSCG-RGFWQPGPRLVEQMKQITPKMKVPPFKTQRLQINADGQREDFNLEV
GYVTQRDNIVYESSD-PPRTL IHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRTWAMGRYLARLMKGTSG-VNNTNFRTSILQFDEDGQRITFNIEV
KLHQELGESEPVNSGSTFITMEMALTYDAVRVIAETTKHLPYQPOM-----LNC SERHDNVQPDGSTFRNYMR--SLEIKEKTITGRIYFEGNVRKGF TFDVIE
KLGYP TDQDE-FRNGSCPI TVEMALTYDAVQLFAQTLK NLPFKPMP-----QNC SQRTESVRDDGSSFKNYMR--TLRLTDRLLTGPIYFEGNVRKGYHLDVIE
SLNQSWQENCDHVPFTG-PALSSALLFD AVYAVVTAVQELNRSQEIG-----VKPLSCGSAQI WQHGTSLMNYLR--MVELEGLTGHI EFNSKGQRSNYALKILQ

YKPTVNEPMMVWTPDN---GIKKRLLN---LELESAGTTQDFSEQRKVYTVVTHYE-EPYFMMKEDHE--NFRGREKYEGYAVDLISKLS ELM EFDYEFMIVNGN-
YNPIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQNKEDFSQKPIRYTVATRVG-KPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKFDNFEPVRDNK
YDPLDGIGIAIWDPRG-----QITQLNV DVKAQKKMIYRVATRIG-PPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGF EYVFPVADQQ
LQTSGLVKVGTWEEGK-----DFEFQR-PPQAVNFNDIDDGSLVNKTFIVLISVATKPYASLVESID--TLIGNNQFQGYGVDLIKELADKLG FNFTFR-DGGND
LQPSGIVKVG TWEDER-----QYRPQRLAPTTAQFDSVDN-SLANKTFI ILLSVPNKPYAQLVET YK--QLEGNSQYEGYGVDLIKELADKLG FNFTFV-NGGND
FTRNGFRQIGQWHVAEG-----LSMDSHLYASNISDTL FNTTLVVTIILEN PYLMLKGNHQ--EMEGNDRYEGFCVDMLKELAEILRFNYKIRLVGDGV

(Figure 1-5a cont.)

TM1

-GKYNPETKQWDGIIRKLIDHHAQIGVCDLTITQMRRSVVDFTVPPMQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNP
YGSYDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDFTVPPMQLGISILSYKEPPPKADIYAFLNPNYAEVWLVFVMIAMMITAFALIFTGRIDQYEWDPVE
YGKLDKETKQWNGIIGEIINNDAHMGICDLTITQARKTAVDFTVPPMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMVTFMLKTIVARISKMDWENPH
YGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIAILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYP
YGSYNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMNLGIAILYKPKATPELFTFMDPFSSEVWVWFLGFSFLGVSLSFFILGRLSPSEWDNPYP
YGVPEANG-TWTGMVGELIARKADLAVAGLTITAEREKVIDFSKPFMTLGISILYRVHMGRKPGYFSFLDPFSPGVWLFMLLAYLAVSCVLFVVARLTPYEWYSPHP

TM2

TM3

AIQ-DPDELENIWNVNSTWLMVGSIMQQGCDILPRGPHMRLTGMWVWFALMMLSTYTANLAAFLTSNKWQSSIKSLQDLIE-QDKVHFGSMRGGSTSLFFSESN
NVN-REMERQNIWHLSNALWLVLGSMNLNQGCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVD-QNKVQFGTIRGGATSVYFSESN
CNR-DPEVLENQWRIHNTGWLTVASIMTAGCDILPRSPQVRMFEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLA-QKKVKFGTIYGGSTYNLLADSNE
CIE-EPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRTISAIWWFFTLIMVSSYTANLAAFLT I ENPTSPINSVKDLADNKDDVYGAKRTGSTRNFFSTSEE
CIE-EPEELENQFTLGN I WFTTGALLQQGSEIGPKALSTRTVASFVWFFTLIVVSSYTANLAAFLTIEKPQSLINSVDDLADNKDGVVYGAKKTGSTRNFFMSTSAE
CAQGRCNLLVNQYSLGNSLWFPVGGFMQQGSTIAPRALSTRCSVGVWVAF T L I I S S Y T A N L A A F L T V Q R M D V P I E S V D D L A D - Q T A I E Y G T I H G G S S M T F F Q N S R Y

19

TDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSIQLSERGELQKMKNKWWKNHNV
TDNRMAWKNMLSFKPDAFTKNNEEGVDRVKLSKGTYAFLMETTNLQYYVQRNCEL T Q I G E S F G E K H Y G I A V P L N A D F R S N L S V G I L R L S E R G E L F K L R N K W F N S N E S
TVYRLAFNLMNNDPSAYTKDNLEGVDRVRKNRGDYMFLMETTTLEYHREQNCDLRSVGEKFGEKHYAIAVPFGAEYRSNLSVA I L K L S E R G E L Y D L K Q K W W K N P N A
PIYIKMNEYLNA-HPEMLMENNQGVDKVKS-GTKYAFLMESTSIEFNTVREC NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQQVLARLKNKWWNEVGA
ERYKMMNKF MSE-NPQYLTEDNMEGVNRVKTN-THYAFLMESTSIEYNTKREC NLK K I G D A L D E K G Y G I A M R K D W P H R G K F N N A L L E L Q E Q G V L E K M K N K W W N E V G T
QTYQRMWNYMYSKQPSVFKVSTEEGIARVLS--NYAFLLESTMNEYRQRNCNL T Q I G G L L D T K G Y G I G M P V G S V F R D E F D L A I L Q L Q E N N R L E I L K R K W W E G G K C

TM4

-TCDS-YHEVDGDELSIIELGGVFLVLAGGVILIGVILGIFEFLWNVQNVAVEERVTPWQAFKAELIFALKFWVRKPKMRIS--SSDKSSSRSS-----GSRRSSK
-TCDSNVPTIDDGQFDMSVGGLFVVLIVGVVGLVIGVAEFLWHVQRI SVKEKIPMLALKAEFYFVIRFWLTRKPLHTYR--QSRDSTSTGYSSLEQITSASSAK
-SCFEEDPDATPDMTFEELRGIFYTLYAGILIAFLIGITEFLVYVQVVALEERLTFKDAFKKEIRFVLCVWNNRKPPIVAGTPISSVRTTPRRSLDKSLDRTPKSSR
GVCSAKSDDDGPSELGVDNLSGIYVVLVIGSIIISIIISILWCYFVYKAKNYEVPFCDA LAE E F R I V I R F S E N E R P L K S A Q S I Y S R S R N S S Q S I E S L K T D S E E N M P
GICATKEDAPDATPLDMNLEGVFFVLLVGSCCALLYGIIISWVLFVMMKKAHHRVPLRDALKEEFQFVIDFNYYRVLKNAS I Y S R S R Q S S M S V A S V A Q E S Q - - - -
----PKEEDHRAKGLGMENIGGIFVVLICGLIVAI F M A M L E F L W T L R - H S E A T E V S V C Q E M V T E L R S I I L C Q D S I H P R R R R A A V P P P R P P I P E E R R P R G T A T L S N G K

(Figure 1-5a cont.)

EKSRSKTVS-----
KKKKTRRIEK-----
RVVIGRSSEEMREMAQSGSSSGSNNAGRGEKEARV-----
VED-----

LCGAGEPDQLAQRLAQEAALVARGCTHIRVCPERRFQGLRARPPARSEESLEWEKTTNSSEPE

(Figure 1-5a cont.)

GluR-IIA -----MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVGAIFYENEKE---IELSFDQAFREVN--NMKFSSELRFVVTIKRYMPTNDSFLLQOI
GluR-IIB -----MHGLQFLVLLALAIASGANEDTLV**IKIGAIFFD**TEMK---LADAFSAALEEVN--AIN-PALKLDAIKRYVTVDDSIVLQDI
GluR-IIC MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQ**INIGAFFYD**DELE---LEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVTMQEH
GluR-IID -----MHFCWIS---LIILSLSRVQAQFYGGNAYEASSGQS**IRLGLITD**DATDR---IRQTFEHAISVVN----NELGVPLVGETEQVAYGNSVQAFQAQ
GluR-IIE -----MFFNFHVFILWSLFSIHISVNWAQYENFGG-YDNYQSLES**VP IGLITD**QNTAQ---MNI VFDHAIDVAN----QEVGTSLSLKEEVNYGDAYQSYGK
KA2 -----MPAELLLLLIVAFASPSCQVLS**SLRMAA**ILDQTVCGRGERLALALAREQ**INGI**IEVPAKARVEVDIFELQRDSQYETTTD

TCELISNGVAA**IFGPSSK**-AASDIVAQIANAT**GIPHI**EYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFE**II**KSN-YEWRTFTLIYETPEG--LAR**LQD**LMNIQA
SCDLIGSGVAA**IFGPSSK**-TNSDIVEVLCNMT**GIPHL**QFDWHPQQSNRERMNHQLTVNVAPMEL**FLS**AAFSD**IL**ASKTFDWKSFT**IA**YERSSSH--LIR**LQH**ILAWKQ
ACDLIDNGVAA**IFGPSSK**-AASDIVALVCNST**GIPHI**EFDISDEGIQAEPNHQMTLNLYPAQAI**LS**KAYAD**IV**QNF--GWRKFT**IV**YDADDARAAAR**LQD**LLQLRE
LCRLMQSGV**AVFGPAAR**-HTASHLLNACDSK**IPFI**YPHLSWGSNPDG-----FNLHPS**PEDIAN**ALYD**IV**NQF--EWSRF**IFC**YESAEY--LK**ILD**HLMTRYG
LCR**MLETGI**AG**VFGPSSR**-HTAVHLMSICDAMD**IPHI**YSYMS--ENAEG-----FNLH**PHPADLAK**ALYSL**ITE**F--NWTR**IFL**YESAEY--LN**ILN**ELTTMLG
MC**QIL**PKGV**SVLGPSS**SPAS**ASTV**SHICGEKE**IPHI**KVGPE--ETPRLQYLR**FASVSLY**PSNED**VSLAVSRIL**KSF--NYPS**ASLI**CAKAEC--LLR**LEEL**VRGFL

LNSDY**VKL**RNLAD-YADDYR**ILWK**ETDET**FHEQR**I**ILD**DCEPKTLKEL**LKVS**IDFKLQGPFRNW**FLTH**LDTHNSGLRDIYN--EDFKAN**ITSVRL**KVV DANPFERKKT
LHKAG**IKM**QEFE--RGDDYR**ILWK**RINN-AREKF**VLLD**CPSDILVDV**INAS**IGYNMTGSFNHL**FLTN**LDTHLSG**IDG**FYS--RDFTV**VAAVR**IRTYVPPPVHDEID
VHND**VVR**VRKFH--KDDDFR**VMWKS**IRG--ERR**VVLD**DCEPNMLVEL**LNS**ST**EFG**LTGQYNH**IFLT**NLETYTDH**LEELA**ADNETF**AVNITA**ARLLVNPDP**PPY**SLPY
IKGPV**IKV**MRYDLNLNG**NYKSVL**RRIRK-SEDSR**IVV**VGSTTG**VAE**LL**RQAQ**QV**GI**MNEDYTY**II**GNLNLHTFD**LEEY**KYS----EAN**ITG**IRMFSPDQ**EEV**RD**LME**
KSGT**VIT**VLRYDMQLNG**NYKQV**LRVRK-SVDNR**IVV**VGSS**ETMPEFL**N**QAQ**QV**GI**INEDYKY**II**GNLDFHSFD**LEEY**KYS----EAN**ITG**LR**LF**SPEKMAVK**ELLM**
ISK**ETLS**VRMLDD--SRDPT**LLKE**IRD-DKVST**II**IDANASISHL**ILRKA**SEL**GMTSA**FYKY**ILTT**MD**FPI**LHL**DG**IVED----SSN**ILG**FS**MFNT**SHPFY**PEF**VR

RLT-KVDQILGN-----Q**TMLP**IL**IYDA**VV**LF**ASSARNVIAAMQP-FHPP----NRHCGSSSP**WMLGAF**IV**NEMK**TISEDDVEPHFKTENMKLDEY**GQRI**HF**NLEI**
VFDNSVDTRFS-----**SLGSQ**LVY**DSI**VL**FYN**-ALLEISQRPG-FYIP----NFSCG-RGF**WQPG**RL**VEQ**M**KQIT**PKMVKPPFKTQRLQINAD**GQ**RED**FNLE**V
GYVTQRDNIVYESSD-PPR**TLI**HDL**IHDAL**QL**FAQ**SWRNASFFYPDRMVVPRITCDFAASGGRT**WAMGRYLARL**M**KGT**SG-VNNTNFRTSILQFDE**DGQR**IT**FNIE**V
KLHQELGESEPVNSGST**IT**MEMALTY**DAVR**VIA**ETT**KHLPYQ**PQM**-----LNC**SER**HDNVQ**PDG**ST**FRNYMR**--SLEIKEKTITGRIYFEGNVR**KG**FTFD**VIE**
KLGYPTDQDE-FRNGSC**IT**MEMALTY**DAVQ**L**FAQ**TLK**NLP**FK**PMP**-----Q**NC**SQ**RTES**VRDD**GSS**F**KNYMR**--TLRLTDRL**LTGPI**YFEGNVR**KGY**HL**DVIE**
SLNMSWRENCEASTYLG-PAL**SAAL**MF**DAVH**VVVSAVRELNR**SQEIG**-----V**KPL**ACTSANI**WPHGT**SLM**NYLR**-----MVEYDGLTGRV**EFNSK**G**QRT**NY**TLRI**

YKPT--VNEPMM**VW**TPDN---GIKKRLLN----LELESAGTT**QDF**SEQRKVY**TV**VTHYE-EPYFMMKEDHE--NFRGREKY**YEGYAVD**L**ISK**LSELME**FDYEF**MIVNG
YNPI--IDRVTH**IN**K**EFQ**LVD**FEK**LRENST**QALKQ**KRLQ**NKED**FSQKPIRY**TV**ATRVG-KPYFSWREEPEGVHYEGNER**FEGYAVD**L**IYMLA**Q**ECK**FD**FN**FEPVRD
YDPL--DGIGIA**IWD**PRG-----QITQLNV**DVKA**Q**KMIYRV**ATRIG-PPYFSYNETARELNLT**GNALYQGYAVD**L**IDAIA**RHV**GFEYV**FVPVAD
LQTS--GLVKVGT**WEEGK**-----D**FEF**QR-PPQAVNFND**IDDG**SLV**NKTFI**VLISVAT**KPYA**SLVESID--TLIG**NNQFQGYGVD**L**IKELAD**K**LGFN**FTFR-DGG
LQPS--GIVKVGT**W**DEDR-----QYR**PQ**RLAP**TTA**Q**FD**SV**DN**-SLANK**TFI**ILLSVP**NPYA**QLVETYK--Q**LE**GN**SQYEGYGVD**L**IKELAD**K**LGFN**FT**FFV**-NGG
LEKSRQGHREIG**VW**YSNR-----TLAMNAT**TL**DINLS**Q**TLANK**TLV**VT**TI**LEN**PYV**MRRPN**FQ**--ALS**GNERFEGFCVDMLRELA**ELL**RFR**YRL**RL**VED

(Figure 1-5a cont.)

TM1

N--GKYNPETKQWDGIIRKLIDHHAQIGVCDLTITQMRRSVVDFTVPPMQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLP
NKYGSYDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDFTVPPMQLGISILSYKEPPPKADIYAFLNPNYAEVWLFVMIAMMITAFALIFTGRIDQYEWDP
QQYGKLDKETKQWNGIIGEIINNDAHMGICDLTITQARKTAVDFTVPPMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMVTFMKTIVARISKMDWENP
NDYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIAILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNP
NDYGSYNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMNLGIAILYKPKATPELFTFMDPFSSEVWVWFLGFSFLGVSLSFFILGRLSPSEWDNP
GLYGAPENGPNG-SWTGMVGELINRKADLAVAFAFTITAEREKVIDFSKPFMTLGISILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVFLAARLSPYEWYNP

TM2

TM3

NPAIQD-PDELENIWNVNSTWLMVGSIMQQGCDILPRGPHMRLTGMWVWFALMMLSTYTANLAAFLTSTNKWQSSIKSLQDLIE-QDKVHFGSMRGGSTSLFFSES
VENVNR-EMERQNIWHLSNALWLVLGSMNLNQGCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVD-QNKVQFGTIRGGATSVYFSES
HPCNRD-PEVLENQWRIHNTGWLTVASIMTAGCDILPRSPQVRMFEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSA-QKKVKFGTIYGGSTYNLLADS
YPCIEE-PEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRTISAIWWFFTLIMVSSYTANLAAFLT I ENPTSPINSVKDLADNKDDVYQAKRTGSTRNFFSTS
YPCIEE-PEELENQFTLGNSIWFTTGALLQQGSEIGPKALSTRTVASFVWFFTLIVVSSYTANLAAFLTIEKPQSLINSVDDLADNKDGVVYGAKKTGSTRNFFMSTS
HPCLRARPHILENQYTLGNSLWFPVGGFMQQGSEIMPRALSTRCVSGVWVWAFTLIIISSYTANLAAFLTIVQRMEVPVESADDLAD-QTNIEYGTIHAGSTMFTFFQNS

22 NDTDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSIQLSERGELQKMKNKWWKNH
NDTDNRMAWKNMLSFKPDAFTKNNEEGVDRVKLSKGTYAFLMETTNLQYYVQRNCELTIQIGESFGEKHYGIAVPLNADFRSNLSVGIILRLSERGELFKLRNKWFNSN
NETVYRLAFNLMNNDPSAYTKDNLEGVDRVRKNRGDYMFLEMTTLEHYHREQNCDLRSVGEKFGKHYAIAVPFGAEYRSNLSVAIILKLSERGELYDLKQKWWKNP
EPIYIKMNEYLNA-HPEMLMENNQGVDKVKS-GTKYAFLMESTSIEFNTVREC�LTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQGVLARLKNKWWNEV
AEERYKMMNKF MSE-NPQYLTEDNMEGVNRVKTN-THYAFLMESTSIEYNTKREC�LKKIGDALDEKGYGIAMRKDWPHRGKFNNALLELQEQGVLEKMKKNWWNEV
RYQTYQRMWNYMQSKQPSVFKSTE EGIARVLS--RYAFLLESTMNEYHRLNCNLTIQIGLLDTKGYGIGMPLGSPFRDEITLAILQLQENNRLEILKRWEGG

TM4

NV-TCDS-YHEVDGDELSIIEELGGVFLVLVAGGVILIGVILGIFEFLWNVQNVAVEERVTPWQAFKAELIFALKFWVRKPKMRIS--SSDKSSRRSS-----GSRRS
ES-TCDSNVPTIDDGQFDMSVGGFLVVLIVGVVGLVIGVAEFLWHVQRI SVKEKIPMLALKAEFYFVIRFWLTRKPLHTYR--QSRDSTSTGYSSLEQITSASS
NA-SCFEEDPDATPDMTFEELRGIFYTLYAGILIAFLIGITEFLVYVQVVALEERLTFKDAFKKEIRFVLCVWNNRKPIVAGTPISSVRTTPRRSLDKSLDRTPKS
GAGVCSAKSDDDGPSELGVDNLSGIYVVLVIGSIIISIIISILWCYFVYKAKNYEVPFCDALEEFIRVIRFSENERPLKSAQSIYSRSRNSQSIESLKTDEEN
GTGICATKEDAPDATPLDMNLEGVFFVLLVGSCCALLYGIIISWVLFVMMKKAHHRVPLRDALKEEFQFVIDFNYYRVLKNASIIYSRSRQSSMSVASVAQESQ--
RC----PKEEDHRAKGLGMENIGGIFIVLICGLIIAVFVAVMEFIWSTRRS AESEEVSVQCQEMQLQELRHAVSCRKTSR SRRRRRPPGSPRALLSLRAVREMRLSNGK

(Figure 1-5a cont.)

SKEKSRSKTVS-----
AKKKKKTRRIEK-----
SRRVVIGRSSEEMREMAQSGSSSGSNNAGRGEKEARV-----
MPVED-----

LYSAGAGGDAGSAHGQPQLLDDPGPPSGARPAAPTPTCTHVRVCQECRRIQALRASGAGAPRGLGVPAEATSPPRPRPGPAGPRELAEHE

GluR-IIA
GluR-5

-----MRLCPVVIYAFIIIIIGFLEGIIALGGDDRNEITVGAIIFYENEKE-----IELSFDQAFREVNN--MKFSELRFVVTIKRYMPTNDSFLLQQITCELI SNGVAAI
MEHGTL LLAQPGLWTRDTSWALLYFLCYILPQTAPQVLRIGGIFETVENE PVNVEELAFKFAVTSINRNRTLMPNTTLLTYDIQRINLFD SFEASRRACDQLALGVAAL

FGPSSKAASDIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKSNEYEWRTFTLIYETPEGLARLQDLMN IQALNSDYVKLRNLADYAD
FGPSHSSSVSAVQSI CNALEVPHIQTRWKHP SVDNK--DLFYINLYPDYAAISRAILDVLVY-YNWKT VTVVYEDSTGLIRLQELIKAPSRYNIKIKIRQLPSGNK

DYRILWKETDETTFHEQRIILDCEPKTLKELLKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYNEDFKANITSVRLKVVD----ANPFERKKTRLTKVDQILGNQTM L
DAKPLLKEMKK-GKEFYVIFDCSHETA AEILKQILFMGMMTEYYHYFFTLDLFDALDLELYR--YSGVNMTGFRLLNIDNPHVSSIIEKWSMERLQAPRPETGLLD

PILYDAVVLFASSARNVIAAMQP--FHPPNRHCSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEYGQRIHFNLEIYKPTVNEPMMVWTPDNGIKRLLNL
GMMTTEAALMYDAVYMVAIASHRASQLTVSSLQCHRHKPWRLGPRFMNLIKEARWDGLTGHI TFN--KTNGLRKDFDLDIISLKEEGTEKIGIWNSNSGLNMTDSNK

ELESAGTTQDFSEQRKVYTVVTHYEOPYFMMKEDHENFRGREKYEGYAVDLISKLS ELMEDYEFMIVNGNGKYNP--ETKQWDGIIRKLIDHHAQIGVCDLTITQM
DKSSN---ITDSLNRTLIVTTILEEPPYVMYRKSDKPLYGNDRFEGYCLDLLKELSNILGFIYDVKLVP-DGKYGAQNDKGEWNGMVKELIDHRADLAVAPLTITYV

24

RRSVVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPF AVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNPAIQDPDELENIWNVNNSTWLMVGSIMQQGCDILPR
REKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPHPCNPDSDDVVENNFTLLNSFWFGV GALMQQGSSEMPK

GPHMRILTGMWFFALMMLSTYTANLAAFLT SNKWQSSIKSLQDLIEQDKVHFGSMRGGSTSLFFSESNDTDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYA
ALSTRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPID SADDLAKQTKIEYGAVRDGTMTFFKKSISTYEKMWAFMSSRQQTALVRNSDEGIQ RVLTT--DYA

FLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSI LQLSERGELQKMKNKWKNNHVTCD SYHEVDGDELSIIE LGGVFLVLVLAGGVLIIGVILGI
LLMESTSIEYVTQRNCNLTQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQE EGKLMHMKKWRGN--GCPEEDNKEASALGVENIGGIFIVLAAGLVLSV FVAI

FEFLWNVQNVAVEERVTPWQAFKAELIFALKFVWRKKPMRISSSSDKSSRRSSGSRSSKEKSRSKTVS
GEFYIKSRKNNDIEQCLSFNAIMEELGISLKNQKIKKKSRTK GKSSFTSILTCHQRRTQRKETVA-----

Figure 1-5b. Sequence alignment with predicted amino acid sequences of the postsynaptic receptor subunits at *Drosophila* NMJ (GluRIIA, GluRIIB, GluRIIC, GluRIID and GluRIIE) and human's kainate receptor subunits (GluR5, GluR6, GluR7, KA1 and KA2). Sequence comparisons are achieved to investigate the similarities of the each subunit of *Drosophila* NMJ and human. Since homologous sequences are different lengths, gaps are inserted to gain the best match. Clustal W2 is used for sequence analysis.

(Figure 1-5b cont.)

GluR-II B
GluR-5

MHGLQFLVLLALAIASG-----ANEDTLVIKIGAIFFDTEMKLAD----AFSAALEEVN---AINPALKLDAIKRYVTVDSDIVLQDISCDLIGSGVAAI
MEHGTLQAQPLWTRDTSWALLYFLCYILPQTAPQVLRIGGIFETVENEPVNVEELAFKFAVTSINRNRITLMPNTTLYTDIQRINLFDSEASRRACDQLALGVAAL

FGPSSKTNSDIVEVLCNMTGIPHLQFDWHPQOSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSSHLIRLQHILAWKQLHKAGIKMQEFERGD
FGPSHSSSVSAVQSICNALEVPHIQTRWKHPSVDNK--DLFYINLYPDYAAISRAILDLVLY--YNWKTVTVVYEDSTGLIRLQELIKAPSRYNIKIKIRQLPSGN

-DYRILWKRINNAREKFVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNLDTHLSGIDGF-YSRDFTVAVAAVRIRTYVPPPVHDEIDVFDNSVDTRFSS-LGSQL
KDAKPLLKEMKKGKEFYVIFDCSHETAAEILKQILFMGMMTEYYHYFFTLLDLFALDLELYRYSGVNMTGFRLNIDNPHVSSIEKWSMERLQAPPRPETGLLDGM

VYDSIVLFYNALLEISQRPG---FYIPNFSCGRG-FWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYNPIIDRVTHIWNKEFQLVDFEKLREN
MTTEAALMYDAVYMVAIASHRASQLTVSSLQCHRHKPWRLGPRFMNLKEARWDGLTGHITFN----KTNGLRKDFDLDIISLKEEGTEKIG-----IWNSNSGLN

STQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKFDNFEPVRDNKYGSYDANTDEWDGIIRQLIDNNAQIGIC
MTDSNKDKSSNITDSLNR--TLIVTTILEEPYVMYRKSdk--PLYGNDRFEGYCLDLLKELSNILGFIDVVKLVPDGKYGAQND-KGEWNGMVKELIDHRADLAVA

25

DLTITQARRSVVDFTVPFMQLGISILSYKEPPPKADIYAFLNPNYAEVWLFVMIAMMITAFALIFTGRIDQYEWDPVENVNREMERQNIWHLSNALWLVLGSMLNQ
PLTITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPHPCNPDSVVENFTLLNSFWFGVGMALMQQ

TM1

TM2

TM3

GCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQNKVQFGTIRGGATSVYFSESDTDNRMAWNKMLSFKPDFAFTKNNEEGVDRVK
GSELMPKALSTRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPIDSADDLAKQTKIEYGAVRDGSTMTEFFKSKISTYKMWAFMSSRQQTALVRNSDEGIQRLV

TM4

LSKGTYAFLMETTNLQYYVQRNCELQIGESFGEKHYGIAVPLNADFRSNLSVGILRLSERGELFKLRNKWFNSNESTCDSNVPTIDDGQFDMDSVGGLFVVLIVGV
TTD--YALLMESTSIEYVTQRNCNLQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQEEGKLHMMKEKWWRGNGCPEEDNK---EASALGVENIGGIFIVLAAGL

VVGLVIGVAEFLWHVQRISVKEKIPMLALKAIFYFVIRFWLTRKPLHTYRQSRDSTSTGYSSLEQITSASSAKKKKTRRIEK
VLSVFVAIGEFIYKSRKNNDIEQCLSFNAIMEELGISLK-----NQQKIKKKSRTKGKSSFTSILTCHQRRTQRKETVA-

(Figure 1-5b cont.)

GluR-IIc MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQIN-IGAFFYDDELE---LEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSSVTMQ
GluR-5 -----MEHGTLLAQPGLWTRDTSWALLYFLCYILPQTAPQVLRIGGIFETVENEVPVVEELAFKFAVTSINRNRTLMPNTTLLTYDIQRINLFDSSFEAS

EHACDLIDNGVAAIFGPSSKAASDIVALVCNSTGIPHIEFDISDEGIQAEKPNHQMTLNLYPAQAAILSKAYADIVQNFGRKFTIVYDADDARAAARLQDLLQLREV
RRACDQLALGVAALFGPSHSSSVSAVQSIICNALEVPHIQTRWKHPSVDNKD---LFYINLYPDYAAISRAILDVLVYVYEDSTG--LIRLQELIKAPSR

HNDVVRVRKFKH-DDDFRVMWKSIRGER--RVVLDCPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAARLLVNPDPYPYSLPYGYV
YNIKIKIRQLPSGNKDAKPLLEKMKKGKEFYVIFDCSHETAAEILKQILFMGMMTEYYHYFFTTLDLFDLLELYRYS---GVNMTGFRLLNIDNPHVSSIIEKWS

TQRDNIVYESSDPRTLIHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRTWAMGRYLARLMKGTSGVNNTNFRFRTSILQFDEEDGQRITFNIEVYDPLD
MER---LQAPRPETGLLDGMMTEAALMYDAVYVMAIAS--HRASQLTVSSLQCHRHKPWRLGPRFMNLKEAR---WDGLTGHITFNKTNGLRKDFDLDIISLKE

G--IGIAIWDPRGQITQLNVDVKAQKKMIYRVATR-----IGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQYQGLDKETKQWNGI
EGTEKIGIWNNSGLNMTDSNKKSSNITDSLANTLIVTTILEEPYVMYRKS DKPL--YGNDRFEGYCLDLLKELSNILGFIDVKLVDPDGKYG-AQNDKGEWNGM

26

IGEIINNDAHMGICDLTITQARKTAVDFTVPFMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMFLKTIIVARISKMDWENPHPCNRDPEVLENQWRI
VKELIDHRADLAVAPLTITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPHPCNPDSVVENNFTL

TM1

TM2

TM3

HNTGWLTVASIMTAGCDILPRSPQVRMFEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQKKVKFGTIYGGSTYNLLADSNETVYRLAFNLMNDDPS
LNSFWFGVGMALMQQGSSELMPKALSTRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPIDSADDLAKQTKIEYGAVRDGSTMTEFFKSKISTYEKMWAFMSSRQT

AYTKDNLEGVDRVRKNRGDYMFLETTTLEYHREQNCDLRSVGEKFGKEHYAIAVPFGAEYRSNLSVAAILKLSERGELYDLKQKWWKPNASCFEEDPDPDATPDMFT
ALVRNSDEGIQRVLT--DYALLMESTSIEYVTQRNCNLTQIGGLIDSKGYGVGTPIGSPYRDKITIAAILQLQEEGKLHMMKEKWWRG--NGCPEEDNKEAS-ALGV

TM4

EELRGIFYTLYAGILIAFLIGITEFLVYVQVALEERLTFKDAFKKEIRFVLCVWNNRKPVAGTPISSVRTTPRRSLDKSLDRTPKSSRRVVIGRSSEEMREMAQG
ENIGGIFIVLAAGLVLSVFAIGEFIYKSRKNNDIEQCLSFNAIMEELGISLKNQKIK-----KKSRTKGKSSFTSILTCHQRTQRKETVA-----

SGSSSGSNNAGRGEKEARV

(Figure 1-5b cont.)

GluR-IIID ---MHFCWISLIILSLSRVQAQFYGGNAYEASSGQSIRLG----LITDDATDRIRQTFEHAISVVNN----ELGVPLVGETEQVAYGNSVQAFACLRLMQSGVGAV
GluR-5 MEHGTLAQPGLWTRDTSWALLYFLCYILPQTAPQVLRIGGIFETVENEVPVVEELAFKFAVTSINRNRTLMPNTTLLTYDIQRINLFDSEASRRACDQLALGVAAL

FGPAARHTASHLLNACDSKDIPFIYPHLSWGSNPDG----FNLHSPEDIANALYDIVNQFEWSRFIFCYESA EYLKILDHLMTRYGIKGPVIKVMRYDLNLNGNYK
FGPSHSSSVSAVQSI CNALEVPHIQTRWKHPSVDNKDLFYINLYPDYAAISRAILDLVLYNWKTVT VVYEDSTGLIRLQELIKAPSRYNIKIKIRQLPS-GNKDAK

SVLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYIIGNLNLHTFDLEEKYSEANITGIRMFSPDQEEVRDLMEKHLHQELGESEPVNSG---STFITME
PLLKEMKKGKEFYVIFDCSHETA AEILKQILFMGMMTEYYHYFFTLLDLFALDLELYRYSGVNMTGFRLLNIDNPHVSSIEKWSMERLQAPRPETGLLDGMMTTE

MALTYDAVRVIAETTKHLPYQPQLNCSERHDNVQPDGSTFRNYMRSLEIKEKTITGRIYFEGNVRKGF TFDVIELQTSGLVKVGTWEEGKDFEFQRPPQAVNFNDI
AALMYDAVYMVAIASHRASQLTVSSLQCHRHKPWR-LGPRFMNLIKEARWDGLTGHITFNKTNGLRKDFDLDIISLKEEGTEKIGIWNNSGLNMTDSNKDKSSNIT

DDGSLVNKTFIVLISVATKPYASLVESIDTLIGNNQFGYGVDLIKELADKLGFNFTFRDGGNDYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFS
D--SLANRTLIVTT-ILEEPYVMYRKS DKPLYGNDRFEGYCLDLLKELSNILGFIDVKLVDPDGKYGAQNDKGEWNGMVKELIDHRADLAVAPLTITYVREKVIDFS

27

TM1

TM2

IPFMNLGIAILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIEEPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRITIS
KPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPHPCNPDSVVENNFLLNSFWFGVGMALMQGSELMPKALSTRIVG

TM3

AIWFFFTLIMVSSYTANLAAFLT IENPTSPINSVKDLADNKDDVQYAKRTGSTRNFFSTSEPIYIKMNEYLN-AHPEMLMENNQQGVDKVKS GTKYAFLMESTSI
GIWFFFTLIISSYTANLAAFLTVERMESPID SADDLAKQT-KIEYGAVRDGSTM TFFKSKISTYEKMWAFMSSRQQTALVRNSDEGIQRVLT-TDYALLMESTSI

TM4

EFNTVRECNLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQV LARLKNKWWNEVGAGVCSAKSDDDGPSSELGVDNLSGIYVVLVIGSIIISIIISILWCYF
EYVTQRNCNL TQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQEEGKLHMMKEKWWRGNC----PEEDNKEASALGVENIGGIFIVLAAGLVLSVFVAIGEFIYK

VYKKAKNYEVPFCDALAEFRIVIRFSENERPLKSAQSIYSRSRNSSQSIESLKT DSEENMPVED
SRKNNDIEQCLSFNAIMEELGISLKNQKKIK--KSRTKGKSSFTSILTCHQRRTQRKETVA---

(Figure 1-5b cont.)

GluR-IIe MFFNHFVILWLSFSIHISVNWAQYENFGGYDNYQSLESVPIG----LLTDQNT EQMNI VFDHAI DVAN----QEVGTSLSLKEEVNYGDAYQSYGKLCRMLLETGIA
GluR-5 --MEHGHTLLAQPLWTRDTSWALLYFLCYILPQTAPQVLRIGGIFETVENE PVNVEELAFKFAVTSINRNRTLMPNTTLLTYDIQRINLFDSFEASRRACDQLALGVA

GVFGPSSRHTAVHLSICDAMDIPHIYSYMSENAEG-----FNLHHPADLAKALYSLITEFNWTRFIFLYESA EYLNILNELTTMLGKSGTVITVLR YDMQLNGN
ALFGPSHSSSVSAVQSICNALEVPHIQTRWKHPSVDNKDLFYINLYPDYAAISRAILDVLYYNWKTVTTVVYEDSTGLIRLQELIKAPSRYNIKIKIRQLPSGNK-D

YKQVLRVRKSV DNRIVVVGSS ETMP EFLNQAQQVGI INEDYKYI IGNLDFHSFDLEEKYSEANITGLR LFSPEKMAVKELLMKLGYP TDQDEF RNGS----CPIT
AKPLLKEMKKGKEFYVIFDCSHETA AEILKQILFMGMMTEYYHYFFTLLDLFALDLELYRYSGVNMTGFRLLNIDNPHVSSIEKWSMERLQAPRPETGLLDGMMT

VEMALTYDAVQLFAQTLKNLPFKPMPQNC SQRTE SVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGN--VRKGYHLDVIELQPSGIVKVG TWEDRQYRPQRLAPTTA
TEAALMYDAVYMVAIASHRASQLTVSSLQCHRHKPWR-LGPRFMNLIKEARWDG--LTGHI TFNKTNGLRKDFDLDIISLKEEGTEKIGIWNNSGLN--MTDSNKD

QFDSVDNSLANKTFFIILLVSPNKPYAQLVET YKQLEGNSQYEGYGVDLIKELADKLGFNFTFVNGGNDYGSY NKSTNESTGMLREIMTGRADLAITDLTIT SEREQA
KSSNITDSLANRTLIVTT-ILEEPYVMYRKS DKPLYGNDRFEGYCLDLLKELSNILGFIDVKLV PDGKYGAQNDKGEWNGMVKELIDHRADLAVAPLTITYVREKV

LDFTIPFMNLGIAILYLKPKATPELFTFMDPFSEEVWFLGFSFLGVLSFFILGRLSPSEWDNPYPCIEEPEELENQFTLGNSIWFTTGALLQQGSEIGPKALST
IDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYVLLACLGVSCVLFVIARFTPYEWYNPHPCNPDSVVENNFLLNSFWFGV GALMQGSELMPKALST

RTVASFWWFFTLIVSSYTANLAAFLTIEKQSLINSVDDLADNKDGVVYGAKKTGSTRNFFMTSAEERYKMKMNFMS ENPQYLTEDNMEGVNRVKTNTHYAFLMES
RIVGGIWWFFTLIISSYTANLAAFLTVERMESPID SADDLAKQTK-IEYGAVRDGSTM TFFKSKISTYEKMWAFMSSRQQTALVRNSDEGIQRVLT TDYALLMES

TSIEYNTKREC NLKKIGDALDEKGYGIAMRKDWP HRGKFNNALLELQE QGVLEKMKNKWNEVGTGICATKEDAPDATPLDMNNLEGVFFVLLV GSCCALLYGIISW
TSIEYVTQRNCNL TQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQEEGKLHMMKEKWWRGNG---CPEEDNKEASALGVENIGGIFIVLAAGLVLSVFVAIGEF

VLFVMKKAHHRVPLRDALKEEFQFVIDFN NYVRVLKNSASIYSRSRQSSMSVASVAQESQ--
IYKSRKNNDIEQCLSFNAIMEELGISLKNQKKIKKKSRTKGKSSFTSILTCHQRRTQRKETVA

28

TM1

TM2

TM3

TM4

(Figure 1-5b cont.)

GluR-IIA
GluR-6

----MRLCPVVIYAFIIIGFLEGIIALGGDRNEITVGAIFYENE-----KEIELSFDQAFREVNNMKFSELRFVTIKRYMPTNDSFLLQITCELISNGVAAIF
MKIIFPILSNPVFRRTVKLLLCLLWIGYSQGTTHVLRFGGIFEYVESGPMGAEEELAFRFVAVNTINRNRTLLPNTTLYDTQKINLYDSFEASKKACDQLSLGVAAIF

GPSSKAASDIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKSNEYEWRTFTLIYETPEGLARLQDLMNIQALNSDYVKLRNLADYADD
GPHSSSANAVQSI CNALGVPHIQTRWKHQVSDNK---DSFYVSLYPDFSSLSRAILDVLFQ-FKWKTVTVVYDDSTGLIRLQELIKAPSRYNLRRLKIRQLPADTKD

YRILWKETDETFHEQRIILDCEPKTLKELLKVSIDFKLQGPFRNWFLTHLDTHNGLRDIYNEDFKAN-ITSVRLKVVVDANPFERKKTRLTKVDQILGNQTMPLILI
AKPLLKEMKR-GKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFDALDVEPYRYSVGNMTGFRILNTEENTQVSSIEKWSMERLQAPPKPDGSLLDGFM

YDAVVLFASSARNVIAAMQPFHPPNR---HCGSSSPWMLGAFIVNEMKTISEDDVEPHFKTENMKLDEYGQRIHFNLEIYKPTVNEPMMVWTPDNGIKRLLNLELE
TTDAALMYDAHVVSVAVQFPQMTVSSLQCNRHKPWRFGTRFMSLIKEAHWEGLTGRITFN--KTNGLRDTDFLDVLSLKEEGLEKIGTWDPASGLNMTES-QKGK

SAGTTQDFSEQRKVYTVVTHYEOPYFMMKEDHENFRGREKYEYAVDLISKLSLMEFDYEFMIVNGN--GKYNPETKQWDGIIRKLIIDHHAQIGVCDLTITQMRRS
PANITDLSLN--RSLIVTTILEEPPYVLFKKSDDKPLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGAQDDANGQWNGMVRELIDHKADLAVAPLAITYVREK

29

TM1 TM2

VVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNPAIQDPDELENIWNVNNSTWLMVGSIMQQGCDILPRGPH
VIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMIYLLAYLGVSCVLFVIARFSPYEWYNPHPCNPDSDVVENNFLLNSFWFGVGLMQQGSELMPKALS

TM3

MRILTMWVWFALMMLSTYTANLAAFLTSTNKWQSSIKSLQDLIEQDKVHFGSMRGGSTSLFFSESDTDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLM
TRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPIDSAADLAKQTKIEYGAVEDGATMTFFKSKISTYDKMFAFMSSRRQSVLVKSNEEGIQRVLTS--DYAFLM

TM4

ETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSIQLSERGELQKMKNKWKNHNVTCDSYHEVDGDELSIELGGVFLVLAGGVILIGVILGIFEF
ESTTIEFVTQRNCNLTQIGGLIDSKGYGVGTPMGSPYRDKITIAILQLQEEGKLHMMKEKWWRGN--GCPEEESKEASALGVQNIIGGIFIVLAGLVLSVAVGEF

LWNVQNVAVEERVTPWQAFKAELIFALKFWVRKKPMRISSSSDKSSRRSSGSRSSKEKSRKTVS
LYKSKNAQLE-----KESSIWLVPYPHPDTV-----

(Figure 1-5b cont.)

GluR-IIB -----MHGLQFLVLLALAIASGANEDTLVIKIGAIFFDTEMKLABAFSAALEEVN-----AINPALKLDAIKRYVTVDDSIVLQDISCDLIGSGVAAIF
GluR-6 MKIIFPILSNPVFRRTVKLLLCLLWIGYSQGTTHVLRFGGIFEYVESGPMGAEEELAFRFVAVNTINRNRLLPNTTLYDTPKINLYDSFEASKKACDQLSLGVAAIF

GPSSKTNSDIVEVLCNMTGIPHLQFDWHPQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSSSLIRLQHILAWKQLHKAGIKMQEFERF-D
GPSHSSSANAVQSICNALGVPHIQTRWKHQVSD-----NKDSFYVSLYPDFSSLSRAILDVQFFKWKTVTVYDDSTGLIRLQELIKAPSRYNLRKIRQLPADTK

DYRILWKRINNAREKFVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNDLTHLSGIDGFYSRDFTVAVAAVR--IRTYVPPPVHDEIDVFDNSVDTRFSSLGSQLV
DAKPLLKEMKRGKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFDLVEPYRYSGVNMGTGFRILNTENTQVSSII EKWSMERLQAPPKPDSGLLDGFM

YDSIVLIFYNALLEIS----QRPGFYIPNFSCGRG-FWQPGPRLVEQMKQITPKMKPPFKTQRLQINADGQREDFNLEVYNPIIDRVTHIWNKEFQLVDFEKLRENS
TTDAALMYDAHVVSVAVQFPQMTVSSLQCNRHKPRWFGTRFMSLIKEAHWEGLTGRITFN----KTNGLRTDFDLVLSLKEEGLEKIG-----TWDPASGLNM

TQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKFDNFEPVRDNKYGSYDANTDEWDGIIRQLIDNNAQIGICD
TESQKGPANITDLSNR--SLIVTTILEEPYVLFKKSDK--PLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGAQDDANGQWNGMVRELIDHKADLAVAP

30

LTITQARRSVVDFTVPFQMLGISILSYKEPPPADIYAFLNPNYAEVWLFVMIAMMITAFALIFTGRIDQYEDQPVENVNREMERQNIWHLSNALWLVLSMNLQG
LAITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYILLAYLGVSCVLFVIARFSPYEWYNPHPCNPDSVVENFTLLNSFWFGVGLMQG

TM1

TM2

CDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQNKVQFGTIRGGATSVYFSESNDTDNRMAWNKMLSFKPDAFTKNNEEGVDRVKL
SELMPKALSTRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPIDSAADLAKQTKIEYGAVEDGATMTFFKKSKI STYDKMWAFFMSRRQSVLVKSNEEGIQRVLT

TM3

SKGTYAFLMETTNLQYVQRNCELTQIGESFGEKHYGIAVPLNADFRSNLSVGIILRLSERGELFKLRNKWFNSNESTCDSNPVTIDDGQFDMDSVGGLFVVLIVGVV
SD--YAFLMESTTIEFVTQRNCLTQIGGLIDSKGYGVGTPMGSPYRDKITIAILQLQEEGLHMMKEKWRRNGCPEEE---SKEASALGVQNIIGGIFIVLAAGLV

TM4

VGLVIGVAEFLWHVQRISVKEKIPPMALKAEFYFVIRFWLTRKPLHTYRQSRDSTSTGYSSLEQITSASSAKKKKKTRRIEK
LSVFVAVGEFLYKSKNAQLKESSIWLVPYHPDVT-----

(Figure 1-5b cont.)

GluR-IIc
GluR-6

MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQIN--IGAFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVMTQEHA
-----MKIIFPILSNPVFRRTVKLLLCLLWIGYSQGTTHVLRFGGIFEYVESGPMGAEELELAFRAVNTINRNRLLPNTTLLTYDTQKINLYDSFEASKKA

CDLIDNGVAAIFGPSSKAASDIVALVCNSTGIPHIEFDISDEGIQAEPNHQMTLNLPAQAAILSKAYADIVQNFGRKFTIVYDADDARAAARLQDLLQLREVHND
CDQLSLGVAAIFGPHSSANAVQSI CNALGVPHIQTRWKH--QVSDNKDSFYVSLYDFSSLSRAILDVQFFKWKTVTVVYDDSTG--LIRLQELIKAPSRYNL

VVRVRKFHKD-DDFRVMWKSIRGER--RVVLDCEPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAARLLVNPDPYSLPYGYVTQR
RLKIRQLPADTKDAKPLLKEMKRGKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFDLVEPYRYS---GVNMTGFRILNTENTQVSSIEKWSMER

DNIVYESSDPRTLIHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGGRWAMGRYLARLMKGTSGVNNTNFRSILQFDEGQRITFNIEVYDPLDG--
---LQAPPKDSGLLDGFMTTDAALMYDAVHVVSVAVQQFPQMT--VSSLQCNRHKPRWFG---TRFMSLIKEAHWEGLTGRITFNKTNGLRDTDFDLVDVLSLKEEGL

IGIAIWDPRGQITQLNVDVKAQKKMIYRVATR-----IGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQQYQKLDKETKQWNGIIGE
EKIGTWDPASGLNMTESQKGPANITDSLSNRSLIVTTILEEPYVLFKKSD--KPLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGAQDDANGQWNGMVR

31

INNDAHMIGICDLTITQARKTAVDFTVPFQGLGVSILAYKSPHVEKTLDAYLAPFGGEVWIIWILISVFMVFLKTIVARISKMDWENPHPCNRDPEVLENQWRIHNT
LIDHKADLAVAPLAITYVREKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMIYLLAYLGVSCVLFVIARFSPYEWYNPHPCNPDSDVVENNFLLNS

TM1

TM2

TM3

GWLTVASIMTAGCDILPRSPQVRMFEATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQKKVKFGTIYGGSTYNLLADSNETVYRLAFNLMNDDPSAYT
FWFGV GALMQQGSSELPKALSTRIVGGIWWFFTLIIISYTANLAAFLTVERMESPIDSAADLAKQTKIEYGAVEDGATMTFFKSKISTYDKMWAFMSSRRQSVLV

KDNLEGVDRVRKNRGDYMFLEMTTLEHYHREQNCDLRSVGEKFGKHYAIAVPFGAEYRSNLSVAAILKLSERGELYDLKQKWKPNPNASCFEEDPDATPDMTFEEL
KSNEEGIQRVLTS--DYAFLMESTTIEFVTQRNCNLQIGGLIDSKGYGVGTMPGSPYRDKITIAILQLQEEGLHMMKEKWR---GNGCPEESKEASALGVQNI

TM4

RGIFYTLYAGILIAFLIGITEFLVYVQQVALEERLTFKDAFKKEIRFVLCVWNNRKPVAGTPISSVTRTPRRSLDKSLDRTPKSSRRVVIGRSSEEMREMAQSGS
GGIFIVLAAGLVLSVFAVGEFLYKSKNAQLEKE-----SSIWLVPPYHPDTV-----

SSGSNNAGRGEKEARV

(Figure 1-5b cont.)

GluR-IIID --MHFCWISLIILSLSRVQAQFYGGNAYEASSGQSIRLG----LITDDATDRIRQTFEHAI SVVNN----ELGVPLVGETEQVAYGNSVQAFQALCRLMQSGVGAVF
GluR-6 MKIIFPILSNPVFRRRTVKLLLLCLLWIGYSQGTTHVLRFGGIFEYVESGPMGAEELAFRFVAVNTINRNRTLLPNTTLLTYDTQKINLYDSFEASKKACDQLSLGVAAIF

GPAARHTASHLLNACDSKDIPFIYPHLSWGSNPDG----FNLHPSPEDIANALYDIVNQFEWSRFIFCYESA EYLKILDHLMTRYGIKGPVIKVMRYDLNLNGNYKS
GPSHSSSANAVQSI CNALGVPHIQTRWKHQVSDNKDSFYVSLYPDFSSLSRAILDLVQFFKWKTVT VVYDDSTGLIRLQELIKAPSRYNLR LKIRQLPADTK-DAKP

VLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYIIGNLNLHTFDLEEKYSEANITGIRMFSPDQEEVRDLMEKHLHQELGESEPVNSG---STFITMEM
LLKEMKRGKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFALDVEPYRYSGVNMTGFRILN TENTQVSSII EKWSMERLQAPPKPD SGLLDGFMTTDA

ALTYDAVRVIAETTKHLPYQPQMLNCSERHDNVQPDGSTFRNYMRSLEIKEKTITGRIYFEGN--VRKGF TFDVIELQTSGLVKVGTWEEGKDFEFQRPPQAVNFND
ALMYDAHVVSVAVQQFP---QMTVSSLQCNRHKPWRFGTRFMSLIKEAHWEGLTGRITFNKTNGLR TDFDL DVI SLKEEGLEKIGTWD PASGLNMTESQK GK PANI

IDDGSLVNKT FIVLISVATKPYASLVESIDTLIGNNQFQGYGVDLIKELADKLGFNFTFRDGGN-DYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVID
TDS---LSNRSLIVTTILEEPYVLFKKS DKPLYGNDRFEGYCIDLLRELSTILGFTYEIRLVEDGKYGAQDDANGQWNGMVRELIDHKADLAVAPLAITYVREKVID

32

FSIPFMNLGIAILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIEEPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRIT
FSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMYILLAYLGVSCVLFVIARFSPYEWYNPHPCNPDSVVENNF TLLNSFWFGV GALMQGSELMPKALSTRIT

TM1

TM2

TM3

ISAIWFFFTLIMVSSYTANLAAFLT IENPTSPINSVKDLADNKDDVQYGA KRTGSTRNFFSTSEPIYIKMNEYLN-AHPEMLMENNQQGVDKVKS GTKYAFLMEST
VGGIWWFFTLIISSYTANLAAFLTVERMESPID SADDLAKQT-KIEYGAVEDGATMTFFKSKISTYDKM WAFMSSRRQSVLVKSNEEGIQRVLT-SDYAFLMEST

TM4

SIEFNTVRECNLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQE QV LARLKNKWWNEVGAGVCSAKSDDDGPSELGVDNLSGIYVVLVIGSIIISIIISILCWC
TIEFVTQRNCNL TQIGGLIDSKGYGVGTPMGSPYRDKITIAILQLQE EGK LHMKEKWWRGNGCPEEESKE----ASALGVQNI GGIFIVLAAGLVLSVFVAVGEFL

YFVYKAKKNYEVPFC DALAEFRIVIRFSENERP LKSAQSIYSRSRNSSQSI ESKTDSEENMPVED
YKSKKNAQ-----LEKESIWLVPYPYHPDTV-----

(Figure 1-5b cont.)

GluR-IIe -----MFFNHFVILWSLFSIHISVNWAQYENFGGYDNYQSLESVPIGLLTDQNTQMNIVFDHAIDVANQEVGTSLSLKEEVNYGDAYQSYGKLCRMLLET
GluR-6 MKIIFPILSNPVFRRTVKLLLCLLWIGYSQGTTHVLRFGG--IFEYVESGPMGAEELAFRFVNTINRNRTLTPN----TTLTYDTQKINLYDSFEASKKACDQLSL

GIAGVFGPSSRHTAVHLMSCDAMDIPHIYSYMSENAEG-----FNLHHPADLAKALYSLITEFNWTRFIFLYESAEYLNILNELTTMLGKSGTVITVLRYSYDMQL
GVAAIFGPPSHSSANAVQSI CNALGVPHIQTRWKHQVSDNKDSFYVSLYPDFSSLSRAILDLVQFFKWKTVTIVYDDSTGLIRLQELIKAPSRYNLRKIRQLPADT

NGNYKQVLRVRKSVNDRIVVVGSSSETMPEFLNQAQQVGIINEDYKYIIGNLDFHSFDLEEKYSEANITGLRFLSPEKMAVKELLMKLGYPDQDEFNRNGS----C
K-DAKPLLKEMKRGKEFHVIFDCSHEMAAGILKQALAMGMMTEYYHYIFTTLDLFDLVEPYRYSVGNMTGFRILNNTENTQVSSII EKWSMERLQAPPKPDSSGLLDG

PITVEMALTYDAVQLFAQTLKLNLPFKPMPQNCQRTE SVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGN--VRKGYHLDVIELQPSGIVKVGTDWEDRQYRPQRLAP
FMTTDAALMYDAHVVSVAVQFPQMTVSSLQCNRHKPPWR-FGTRFMSLIKEAHWEG--LTGRITFNKTNGLRDTDFDLVLSLKEEGLEKIGTWD PASGLN--MTES

TTAQFDSVDNSLANKTFIILLVSPNKPYAQLVETKQLEGN SQYEGYGVDLIKELADKLGFNFTFVNGGN-DYGSYNKSTNESTGMLREIMTGRADLAITDLTITSE
QKGGPANITDLSNRSIVTT-ILEEPYVLFKKS DKPLYGNDRFEGYCIDLLRELSTILGFTYERLVEDGKYGAQDDANGQWNGMVRELIDHKADLAVAPLAITYV

33

REQALDFTIPFMNLGIAILYLKPKATPELFTFMDPFSEEVWVFLGFSFLGVLSFFILGRLSPSEWDNPYPCIEEPELENQFTLGNSIWFTTGALLQQGSEIGPK
REKVIDFSKPFMTLGISILYRKPNGTNPGVFSFLNPLSPDIWMIYLLAYLGVSCVLFVIARFSPYEWYNPHPCNPDSVVENNFLLNSFWFGVGVGALMQGSELMPK

TM1

TM2

TM3

ALSTRIVASFVWFVFTLIVSSYTANLAAFLTIEKQSLINSVDDLADNKDGVVYGAKKTGSTRNFFMTSAEERYKMKMNFMSENPQ-YLTEDNMEGVNRVKTNTHYA
ALSTRIVGGIWWFVFTLIISSYTANLAAFLTVERMESPIDSDLLAKQTK-IEYGAVEDGATMTFFKSKISTYDKMWFMSRRQSVLVKSNEEGIQRVLT-SDYA

TM4

FLMESTSIEYNTKRECNLKKIGDALDEKGYGIAMRKDWPHRGKFNNALLELQEQVLEKMKNKWNEVGTGICATKEDAPDATPLDMNNLEGVFFVLLVGSCCALLY
FLMESTTIEFVTQRNCNLTQIGGLIDSKGYGVGTPMGSPYRDKITIAILQLQEEGKLHMMKEKWWRGNG----CPEEESKEASALGVQNIIGGIFIVLAAG-----

GIISWVLFVMMKAHHYRVPLRDALKEEFQFVIDFNYYVRVLKNSASIYSRSRQSSMSVASVAQESQ
--LVLSVFFVAVGEFLYKSKKNAQLEKEESSIWLVPYPHPDTV-----

(Figure 1-5b cont.)

GluR-IIA ---MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVGAIFYENE-----KEIELSFDQAFREVNNMKFSELRFVTIKRYMPTNDSFLLQQITCELISNGVA
GluR-7 MTAPWRRLRSLVWEYWAGLLVCAFWIPDSRGMPHVIRIGGIFYADGPNAQVMNAEEHAFRFSANI INRNRTLPLNTTLTYDIQRIHFHDSFEATKKACDQLALGVV

AIFGSSKAASDIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKSNEYWRTFTLIYETPEGLARLQDLMNIQALNSDYVKLRNLADY
AIFGPSQGSCTNAVQSI CNALEVPHIQLRWKHHPLDNK--DTFYVNLYPDYASLSHAILDLVQY-LKWRSATVVYDDSTGLIRLQELIMAPSRYNIRLKIQLPID

ADDYRILWKETDETFFHEQRIILDCEPKTLKELLKVSIDFKLQGFNRWFLTHLDTHNSGLRDIYNEDFKAN---ITSVRLKVV DANPFERKKTRLTKVDQILGNQTM
SDDSRPLLKEMKR-GREFRIIFDCSHTMAAQILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYSGVNLTGFRILNVDNPHVSAIVEKWSMERLQAAPRSESGLLD

LPILIIDAVVLFASSARNVIAAMQPFHPPN-RHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEYQRIHFNLEIYKPTVN--EPMVWTPDNGIKKRLI
GVMMTDAALLYDAVHIVSVCYQRAPQMTVNSLQCHRHKAWRFGRFMNFIKEAQWEGLTGRIVFN---KTSGLRTDFDLDIISLKEDGLEKGVWVSPADGLN---I

NLELESAGTTQDFSEQRKVYTVVTHYEOPYFMMKEDHENFRGREKYEGYAVDLISKLSLMEFDYEFMIVNGNGKYNP--ETKQWDGIIRKLI DHHAQIGVCDLTIT
TEVAKGRGPNVTDSLTNRSLIVTTVLEEPFVMFRKSDRTLYGNDRFEGYCIDLLKELAHILGFSYERLVE-DGKYGAQDDKGQWNGMVKELIDHKADLAVAPLTIT

34

QMRRSVVDFTVPFMQLGISILHYKSPPEPKNQFAFLEPFAVEVVIYMIFAQLIMTLAFVFIARLSYREWLPNPAIQDPDELENIWNVNNSTWLMVGSIMQQGCDIL
HVREKAIDFSKPFMTLGVSI LYRKPNGTNPVSVFSLNPLSPDIWMYVLLAYLGVSCVLFVIARFSPYEWYDAHPCNPGSEVVENNFTLLNSFWFGMGLMQGSELM

PRGPHMRILTGMWFFALMMLSTYTANLAAFLT SNKWQSSIKSLQDLIEQDKVHFGSMRGGSTSLFFSESNDTDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGG
PKALSTRIIGGIWFFTLIISSYTANLAAFLTVERMESPIDSAADLAKQTKIEYGAVKDGATMTFFKKSKI STFEKMWAFMSSK-PSALVKNNEEGIQRALT--AD

YAFLMETTSLTYNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSI LQLSERGELQKMKNKWKNNHVTCD SYHEVDGDELSI IELGGVFLVLAGGVLI GVIL
YALLMESTTIEYVTQRNCNLTQIGGLIDSKGYGIGTPMGSPYRDKITIAILQLQEEDKLHIMKEKWRGRS--GCPEENKEASALGIQKIGGIFIVLAAGLVLSVLV

GIFEFLLWVQNVAVEERVTPWQAFKAELIFALKFVWRKKPMRISSSSDKSSSRSSSGSRRSSKEKSRSKTVS
AVGEFVYKLRKTAEREQVS-----LRAWSLHRMGNGDSR-----

TM1

TM2

TM3

TM4

(Figure 1-5b cont.)

GluR-IIb
GluR-7

-----MHGLQFLVLLALAIASGANEDT----LVIKIGAIFFDTEMKLDADFSAALEEVNAIN-----PALKLDAIKRYVTVDDSIVLQDISCDLIGSGVA
MTAPWRRRLRSLVWEYWAGLLVCAFWIPDSRGMPhVIRIGGIFEYADGPNAQVMNAEEHAFRFSANI INRNRTLLPNTTLLTYDIQRIFHDSFEATKKACDQLALGVV

AIFGPPSKTNSDIVEVLCNMTGIPHLQFDWHPQQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSSHLIRLQHILAWKQLHKAGIKMQEFER
AIFGPSQGSCTNAVQSICNALEVPHIQLRWKHHPLDNK--DTFYVNLYPDYASLSHAILDVQ--YLKWRSATVVYDDSTGLIRLQELIMAPSRYNIRLKIRQLPI

-GDDYRILWKRINNAREKFVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNLDTLHSGIDGFYSRDFTVAVAAVRIPTYVPPVHDEIDVFDNS---VDTRFSSL
DSDDSRPLLKEMKRGREFRIIFDCSHTMAAQILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYSG--VNLTGFRILNVDNPHVSAIVEKWSMERLQAAPRSEGL

GSQLVYDSIVLFYNALLEIS---QRPGFYIPNFSCGRG-FWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYNPIIDRVTHIWNKEFQLVDFEK
LDGVMMTDAALLYDAVHIVSVCYQRAPQMTVNSLQCHRHKAWRFGGRFMNFIKEAQWEGLTGRIVFN---KTSGLRTDFDLDIISLKEDGLEKVG-----VWSPA

LRENSTQALKQKRLQNKEDFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKDFDNFEPVRDNKYGSYDANTDEWDGIIRQLIDNNAQ
DGLNITEVAKGRGPNVTDLSLTNR--SLIVTTVLEEPFVMFRKSDR--TLYGNDRFEGYCIDLLKELAHILGFSYEIRLVEDGKYGAQDD-KGQWNGMVKELIDHKAD

35

TM1

TM2

IGICDLTITQARRSVVDFTVPPMQLGISILSYKEPPPADIYAFLNPNYNAEVWLFVMIAMMITAFALIFTGRIDQYEWDPVENVNREMERQNIWHLSNALWLVLGS
LAVAPLTITHVREKAIDFSKPFMTLGVSIYRKPNGTNPVSFSLNPLSPDIWMYVLLAYLGVSCVLFVIARFSPYEWYDAHPCNPGSEVVENNFTLLNSFWFGMGS

TM3

MLNQCDLLPRGLPMRLLTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQNKVQFGTIRGGATSVYFSESNDTDNRMAWNKMLSFKPDAFTKNNEEGV
LMQQGSELMPKALSTRIIGGIWFFTLIIISSYTANLAAFLTVERMESPIDSADDLAKQTKIEYGAVKDGATMTFFKSKISTFEKMW-AFMSSKPSALVKNNEEGI

DRVKLSKGTYAFLMETTNLQYYVQRNCELQIGESFGEKHYGIAVPLNADFRSNLSVGILRLSERGELFKLRNKWFNSNESTCDNSVPTIDDGQFDMDSVGGLFVVV
QALATAD--YALLMESTTIEYVTQRNCNLQIGGLIDSKGYGIGTPMGSPYRDKITIAILQLQEEDKLHIMKEKWWRGSGCPEEENK---EASALGIQKIGGIFIVL

TM4

IVGVVVGLVIGVAEFLWHVQRISVKEKIPMLALKAIFYFVIRFWLTRKPLHTYRQSRDSTSTGYSSLEQITSASSAKKKKTRRIEK
AAGLVLSVLVAVGEFVYKLRKTAEREQVS-----LRAWSLHRMGNDSR-----

(Figure 1-5b cont.)

GluR-IIc MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIGAFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSSVTMQEHACD
GluR-7 MTAPWRRRLRSLVWEYWAG-----LLVCAFWIPDSRGMPHVIRIGGIFEYADGPNQVMNAEEHAFRFSANI INNRNRTLLPNTTLLTYDIQRIHFHDSFEATKKACD

LIDNGVAAIFGPSKAASDIVALVCNSTGIPHIEFDISDEGIQAEPNHQMTLNLYPQAAILSKAYADIVQNFGRKFTIVYDADDARAAARLQDLLQLREVHNDVV
QLALGVVAIFGPSQGSCTNAVQSICNALEVPHIQLRWKHHPLDNK--DTFYVNLYPDYASLSHAILDVQYKWRSATVVYDDSTG--LIRLQELIMAPSRYNIRL

RVRKFHKD-DDFRVMWKSIRGER--RVVLDCEPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAARLLVNPDPYSLPYGYVTQRDN
KIRQLPIDSDSRPLLKEMKRGREFRIIFDCSHTMAAQILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYS---GVNLTGFRILNVDNPHVSAIVEKWSMER--

IVYESSDPRTLIHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDFAASGRTWAMGRYLARLMKGTSGVNNTNFRTSILQFDEDGQRITFNIEVYDPLDG--IG
-LQAAPRSEGLLDGVMMTDAALLYDAVHIVSVCYQR--APQMTVNSLQCHRHKAWRFG---GRFMNFIKEAQWEGLTGRIVFNKTSGLRDTDFDLDIISLKEDGLEK

IAIWDPRGQITQLNVDVKAQKKMIYRVATR-----IGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQQYGKLDKETKQWNGIIGEI
VGVWSPADGLNITEVAKGRGPNVTDLSLTNRSLIVTTVLEEPFVMFRKSDRTL--YGNDRFEGYCIDLLKELAHILGFSYEIRLVEDGKYGAQD-DKGQWNGMVKELI

36

NNDAHMIGICDLTITQARKTAVDFTVPPMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVVFMTFLKTIVARISKMDWENPHPCNRDPEVLENQWRIHNTGW
DHKADLAVAPLTITHVREKAIDFSKPFMTLGVSIYRKPNGTNPVSFSLNPLSPDIWMYVLLAYLGVSCVLFVIARFSPYEWYDAHPCNPGSEVVENNFTLLNSFW

TM1

TM2 TM3
LTVASIMTAGCDILPRSPQVRMF EATWWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDL SAQKKVKFGTIYGGSTYNLLADSNETVYRLAFNLMNDDPSAYTKD
FGMGLMQQGSSELMPKALSTRIIGGIWFFTLIIISSYTANLAAFLTVERMESPIDSADDLAKQTKIEYGAVKDGATMTFFKKSISTFEKMWAFMSSK-PSALVKN

NLEGVDRVRKNRGDYMFLMETTTLEYHREQNCDLRSVGEKFGEKHAIYAVPFGAEYRSNLSVAAILKLSERGELYDLKQKWKPNPNASC FEEPDPDATPDMT FEELRG
NEEGIQRALT--ADYALLMESTTIEYVTQRNCNLTQIGGLIDSKGYGIGTPMGSPYRDKITIAAILQLQEEDKLHIMKEKWWRG--SGCPEENKEAS-ALGIQKIGG

TM4

IFYTLYAGILIAFLIGITEFLVYVQVALEERLTFKDAFKKEIRFVLCVWNNRKPPIVAGTPISSVRTTPRRSLDKSLDRTPKSSRRVVIGRSSEEMREMAQSGSSS
IFIVLAAGLVLSVLVAVGEFVYKLRKTAEREQVSLR-----AWSLHRMGNGDSR-----

GSNNAGRGEKEARV

(Figure 1-5b cont.)

GluR-IIID MHFCWISLIILSLSRVQAQFYGGNAYEASSGQ--SIRLG---LITDDATDRIRQTFEHAISVVNNELG-----VPLVGETEQVAYGNSVQAFQAQLCRLMQSGVG
GluR-7 MTAPWRRRLRSLVWEYWAGLLVCAFWIPDSRGMPHVIRIGGIFEYADGPNAQVMNAEEHAFRFSANI INRNRTLLPNTTLYTDIQRIFHDSFEATKKACDQLALGVV

AVFGPAARHTASHLLNACDSKDIPFIYPHLSWGSNPDG----FNLHPSPEDIANALYDIVNQFEWSRFIFCYESA EYLKILDHLMTRYGIKGPVIKVMRYDLNLNGN
AIFGPSQGSC TNAVQSI CNALEVPHIQLRWKHHPLDNKDTFYVNLYPDYASLSHA ILDLVQY LKWR SATVVYDDSTGLIRLQELIMAPSRYNIRLKI RQLPIDSD-D

YKSVLRRIRKSEDSRIVVVGSTTGVAELLRQAQQVGIMNEDYTYIIIGNLNLHTFDLEEKYSEANITGIRMFSPDQEEVRDLMEKHLHQELGESEPVNSG---STFIT
SRPLLKEMKRGREFRIIFDCSHTMAAQILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYSGVNLTGFRILNVDNPHVSAIVEKWSMERLQAAPRSESGLLDGVM

MEMALTYDAVRVIAETTKHLPYQPQLNCSERHDNVQPDGSTFRNYMRSLEIKEKTIITGRIYFEGNVRKGF TFDVIELQTSGLVKVGTWEEGKDFEFQRPPQAVNFN
TDAALLYDAVHIVSVCYQRAPQMTVNSLQCHRHKAWRFGG-RFMNF I KEAQWEGLTGRIVFNKTSGLRTDFDLDI ISLKEDGLEKVGWVSPADGLNI TEVAKGRGPN

DIDDGSLVNKTFIVLISVATKPYASLVESIDTLIGNNQFGYGVDLIKELADKLGFNFTFRDGGNDYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVID
VTDS---LTNRSLIVTTVLEEPFVMFRKSDRTLYGNDRFEGYCIDLLKELAHILGFSYEIRLVEDGKYGAQDDKGQWNGMVKELIDHKADLAVAPLTI THVREKAID

37

FSIPFMNLGIAILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIEEPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRIT
FSKPFMTLGVSI LYRKPNGTNPVSVFSFLNPLSPDIWMYVLLAYLGVSCVLFVIARFSPYEWYDAHPCNPGSEVVENNFTLLNSFWFGMGLMQGSELMPKALSTRIT

ISAIWFFFTLIMVSSYTANLAAFLT IENPTSPINSVKDLADNKDDVQYAKRTGSTRNFFSTSEPIYIKMNEYLNAPPEMLMENNQQGVDKVKS GTKYAFLMESTS
IGGIWFFFTLIISSYTANLAAFLTVERMESPID SADDLAKQT-KIEYGAVKDGATMTFFKSKISTFEKMWAFMSSKPSALVKNNEEGIQ RALT-ADYALLMESTT

IEFNTVREC NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQE QGVLARLKNKWWNEVGAGVCSAKSDDDGPS ELGVDNLSGIYVVLVIGSIIISIIISILCWCY
IEYVTQRNCNLTQIGGLIDSKGYGIGTPMGSPYRDKITIAILQLQEEDKLHIMKEKWWRGSGC----PEEENKEASALGIQKIGGIFIVLAAGLVLVAVGGEFVY

FVYKKAKNYEVPFCDALAEFRIVIRFSENERPLKSAQSIYSRSRNS SQSIESLKT DSEENMPVED
KLRKTAE-----REQVSLRAWSLHRMGNGDSR-----

(Figure 1-5b cont.)

GluR-IIe
GluR-7

---MFFNHFVILWSLFSIHISVNWAQYENFGGYDNYQS---LESVPIGLLLTDQNTTEQMNIVFDHAIDVANQEVG---TSLTSLKEEVNYGDAYQSYGKLCRMLLETGIA
MTAPWRRRLRSLVWEYWAGLLVCAFWIPDSRGMPHVIRIGGIFHEYADGPNAQVMNAEEHAFRFSANIINRNRTLLPNTTLLTYDIQRIFHDSFEATKKACDQLALGVV

GVFGPSSRHTAVHLMISICDAMDIPHIYSYMSENAEG-----FNLHPHPADLAKALYSLITEFNWTRFIFLYESAEYLNILNELTTMLGKSGTVITVLRVDMQLNGN
AIFGPSQGSCTNVQSIICNALEVPHIQLRWKHPLDNKDTFYVNLVDPDYASLSHAILDVLYLQKWSATVVYDDSTGLIRLQELIMAPSRYNIRLQIRQLPIDSD-D

YKQVLRVRVKSVDNRIVVVGSSSETMPEFLNQAQQVGIINEDYKYIIGNLDFHSFDLEEKYSEANITGLRFLSPEKMAVKELLMKLGYPDQDEFNRNGS----CPIT
SRPLLKEMKRGREFRIIFDCSHTMAAQILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYSGVNLTGFRILNVDNPHVSAIVEKWSMERLQAAPRSESGLLDGVMM

VEMALTYDAVQLFAQTLKNLPFKPMQNCQRTESVRDDGSSFKNYMRTRLRLTDRLLTGPIYFEGN--VRKGYHLDVIELQPSGIVKVGTVWEDRQYRPQRLAPTTA
TDAALLYDAVHIVSVCYQRAPQMTVNSLQCHRHKAWRFGG-RFMNFIKEAQWEG--LTGRIVFNKTSGLRDTDFDLDIISLKEDGLEKVGWVSPADGLNITEVAK--G

QFDSVDNSLANKTFIILLVSPNKPYAQLVETKQLEGNQYEGYGVDLIKELADKLGFNFTFVNGGNDYGSYNKSTNESTGMLREIMTGRADLAITDLTITSEREQA
RGPVNTDSLNTNRSLIVTT-VLEEFVVMFRKSDRTLYGNDRFEGYCIDLLKELAHILGFSYEIRLVEDGKYGAQDDKGQWNGMVKELIDHKADLAVAPLTIHVREKA

38

TM1

TM2

LDFTIPFMNLGIAILYLKPKATPELFTFMDPFSEEVWVFLGFSFLGVLSFFILGRLSPSEWDNPYPCIEEPEELENQFTLGNSIWFTTGALLQQGSEIGPKALST
IDFSKPFMTLGVSIYLRKPNGTNPSVFSFLNPLSPDIWMYVLLAYLGVSCVLFVIARFSPYEWYDAHPCNPGSEVVENNFTLLNSFWFGMGSMLQQGSELMPKALST

TM3

RTVASFWWFFTLIVSSYTANLAAFLTIEKPSQLINSVDDLADNKDGVVYGAKKTGSTRNFFMTSAEERYKKMNKFMSENPQYLTEDNMEGVNRVKTNTHYAFLMES
RIIGGIWWFFTLIISSYTANLAAFLTVERMESPIDSDDLAKQTK-IEYGAVKDGATMTFFKSKISTFEKMWAFMSSKPSALVKNNEEGIQRALT-ADYALLMES

TM4

TSIEYNTKRECNLKKIGDALDEKGYGIAMRKDWPHRGKFNALLELQEQGVLEKMKNKWNEVGTGICATKEDAPDATPLDMNNLEGVFFVLLVGGSCALLYGIISW
TTIEYVTQRNCNLTQIGGLIDSKGYGIGTPMGSPYRDKITIAILQLQEEDKLHIMKEKWWRGSG---CPEEENKEASALGIQKIGGIFIVLAAGLVLSVLVAVGEF

VLFVMMKKAHHYRVPLRDALKEEFQFVIDFNYYVRLKNSASIYRSRQSSMSVASVAQESQ
VYKLRKTAEREQVSLR-----AWSLHRMGNGDSR-----

(Figure 1-5b cont.)

GluR-IIA
KA1

MRLCPVVIYAFIIIIIGFLEGI IALGGDDRNEITVGAI FYENEKE - IELSFDAQAFREVNMMKFSELRFVTIKRYMPTNDSFLLQQITCELI SNGVAAIFGPSSK - AAS
---MPRV SAPLVLLPAWLVMVACSPHSLRIAAILDDPMECSRGERLSITLAKNRINRAPERLGKAKVEVDIFELLRDSEYETAETMCQILPKGVVAVLGPSSSPASS

DIVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKSNEYWRFTFLIYETPEGLARLQDL MNIQALNSDYVKLRNLADYADDYRILWKET
SII SNICGEKEVPHFKVAP -- EEFVKFQFQRF TTNLHPSNTDISVAVAGILN - FFNCTTACLICAKAECLLNLEKLLRQFLISKDTLSVRMLDDTRDPTPLKEIR

DETFHEQRIILDCEPKTLKELLKVSIDFKLQGPFRNWFLTHLDTHNSGLRDIYNEDFKAN --- ITSVRLKVV DANPFERKKTRLTKVDQILGNQTMLPILYDAVVL
DD -- KTATII I HANASMSHTILLKAAELGMVSAYYTYIFTNLEFSLQRMDSLVDNRVNLGFSIFNQSHAFFQEFASLNSQSWQENCDHVPFTGPALSSALLFDVAV

FASSARNVIAAMQPFHPPNRHCGSSSPWMLGAFIVNEMKTI SEDDVEPHFKTENMKLDEYQQR I HFNLEIYKPTVN -- EPMMVWTPDNGIKKRLLNLELESAGTTQD
AVVTAVQELNRSQEIGVKPLSCGSAQIWQHGTSLMNYLRMVELEGLTGHI EFN ---- SKQRSNYALKILQFTRNGFRQIGQWHVAEGLS --- MDSHLYASNISDT

FSEQRKVYTVVTHYEOPYFMMKEDHENFRGREKYEGYAVDLISKLSELMEFDYEFMIVNGNGKYNPETK - QWDGIIRKLI DHHAQIGVCDLTITQMRRSVVDFTVPF
LFN -- TTLVVTI LENPYLMLKGNHQEMEGNDRYEGFCVDMLKELAEILRFNYKIRLVGDGVYGVPEANGTWTGMVGELIARKADLAVAGLTITAEKVIDFSKPF

39

TM1 TM2

MQLGISILHYKSPPEPKNQFAFLEPFAVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNP AIQ - DPDELENIWNVNNSTWLMVGSIMQQGCDILPRGPHMRILTGM
MTLGISILYRVHMGRKPGYFSLDPFSPGVWLFMLLAYLAVSCVLFVVARLTPYEWYSPHPCAGRCNLLVNQYSLGNSLWFPVGGFMQQGSTIAPRALSTRCVSGV

TM3

WWFFALMMLSTYTANLAAFLTSNKWQSSIKSLQDLIEQDKVHFGSMRGGSTSLFFSESNDTDYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAFLETTSLTY
WWAFTLIIISSYTANLAAFLTVQRMDVPIESVDDLADQTAIEYGTIHGSSMTFFQNSRYQTYQRMWNYMYSKQPSVFKSTEELIARVLSN -- YAFLESTMNEY

TM4

NIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSI LQLSERGELQKMKNKWKNHNVTCD SYHEVDGDELSI IELGGVFLVLAGGVILIGVILGIFEFLWNVQN -
YRQRNCNL TQIGGLD TKGYGIGMPVGSVFRDEFDLAILQLQENNRLEILKRKWE GGGKCPKEEDHRAKG -- LGMENIGGIFVVLICGLIVAIFMAMLEFLWTLRHS

-----VAVEERVTPWQAFKAELIFALKFWVRKKPMRISSSSDK-----SSSRRSSGSSRRSSKEKSRS
EATEVSVCQEMVTELRSIILCQDSIHPRRRAAVPPRPP IPEERRPRGTATLSNGKLCGAGEPDQLAQRLAQEAALVARGCTHIRVCPECRRFQGLRARSPARSE

KTVS-----
ESLEWEKTTNSSEPE

(Figure 1-5b cont.)

GluR-IIB
KA1

MHGLQFLVLLALAIASGANEDTLV**IKIGAIFFD**-TEMKLADAFSAALEEVNAINPALKLD**AIKRYVTVDDSI**VLQDIS-----**CDLIGSGVAAIFGPSSK**-TNSDIV
MPRV**SAPLVLLPAWLVMVACSPHSLRIAAILDDPMECSRGERLSITLAKNRINRAPERL**GKAKVEVD**IFELLRDSEYETAETMCQILPKGVVAVLGPSSSPASSII**

EVLCNMTG**I**PHL**QFDWHPQQSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTI**AYERS**SHLIRLQHILAWKQLHKAGIKMQEFERGGDYRILWKRINNA**
SNICGEKEVP--**HFKVAPEEFVKFQFQRF**TT**LNHLHPSNTDISVAVAGILN**--FFNCT**TACLICAKAECLLNLEKLLRQFLISKDTLSVRMLDDTRDPTLLKEIRDD**

REKFVLLDCPSDILVDVINASIGYNMTGSFNHL**FLTNLDTHLSGIDGFYSR**-----**DFTVAVAAVRIRTYVPPPVHDEIDVFDNSVDTRFSSLSQLVYDSIVLFYN**
KTAT**IIIHANASMSHTILLKAAELGMVSAYTYIFTNLEFSLQRMDSLVDDR**VN**ILGFSIFNQSHAFFQEF**AQ**SLNQS**WQ**ENC**DHV**PFTGPALSSALLF**DAVYAVVT

ALLEISQRPGFY**IPNFSCG**-RGFWQ**PGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYNPIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQ**NKE
AVQELNRSQE**IGVKPLSCGSAQIQHGTSLMNYLR**-----**MVELEGLTGHI**EFNSKG**QRSNYALKILQFTRNGFRQIG**--**QWHVAEGLSMDSHLYASNISDTL**FN--

D**FSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKFDNFEPVRDNKYGSYDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDF**
-----**TTLVVTTILENPYMLKG**--**NHQEMEGNDRYEGFCVDMLKELAEILRFNYKIRLVGDGVYGVPEAN**-GTWTGMVGELIARKADLAVAGLTIT**AEREKVIDF**

40

TV**PFMQLGISILSYKEPPP**KAD**IYAFLNPNYNAEVWLFVMIAMMITAFALIFTGRIDQYEWDPVENVN**-REMER**QNIWHL**SNAL**WLV**LG**SMLNQGCDLLPRGLPMRL**
SK**PFMTLGISILYRVHMGRKPGYFSFLDPFSPGVWLFMLLAYLAVSCVLF**LVAR**LTPYEWYSPHPCAQGR**CNLL**VNQYSLGNSLWFPVGGFMQQGSTIAPRALSTRC**

TM1

TM2

LTAF**WWIFALLISQTYIAKLA**AFITSSKIAGDIGSLHDLVDQNK**VQFGTIRGGATSVYFSESNDTDNRMAWNKMLSFKPDAFTKNNEEGVDRVKLSKGT**YAFLMETT
VSGV**WVAFTLII**ISSY**TANLAAFLTVQRMDVPIESVDDLADQTAIEYGTIHGSSMTFFQNSRYQTYQRMWNYMYSKQPSV**FVK**STEEGIARVLSN**--**YAFLEST**

TM3

NLQ**YVVQRNCELTQIGESFGEKHYGIAVPLNADFRSNLSVGILRLSERGELFKLRNKWFNSNESTCDSNVPTIDDGQFDMD**SVGGLF**VVLI**VG**VVGLVIGVAEFLW**
MNE**YYRQRNCNLTQIGGLD**TKGYG**IGMPVGSVFRDEFDLAILQLQENNRLEILKRK**W**WEGGKCPKEEDHRAKGLG**--**MENIGGIFVVLICGLIV**AIM**AMLEFLW**

TM4

H**VQR**-----**ISVKEKIP**PMLAL**KAEFYFVIRFWLTRKPLHTYRQ**--SRD**STSTGYSSLEQITSASSAKKKKTRRIEK**-----
TL**RHSEATEVSVQEMVTE**LR**SII**LCQDS**IHPRRRRAAVPPRPPIPEERRPRGTATLSNGKLCGAGEPDQLAQR**LAQ**EALVARGCTHIRVCPECRRFQGLRARPS**

PAR**SEESLEWEKTTNSSEPE**

(Figure 1-5b cont.)

GluR-IIC
KA1 -MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIGAFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVTMQEHAC
MPRVSAPLVLLPAWLVMVACSP-----HSLRIAAILDDPMECS-RGERLSI-----TLAKNRINRAPERLG-KAKVEVDIFELLR-----DSEYETAETMC

DLIDNGVAAIFGPSSK-AASDIVALVCNSTGIPHIEFDISDEGIQAEPNHQMTLNLYPQAAILSKAYADIVQNFGWRKFTIVYDADDARAAARLQDLLQLREVHND
QILPKGVAVLGPSSSPASSIISNICGEKEVP--HFKVAPEEFVKFQFQRF'TTLNLHPSNTDISVAVAGILNFFNCTTACLICAK--AECLLNLEKLLRQFLISKD

VVRVRKFKHDDDFRVMWKSIRGER--RVVLDCPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNITAARLLVNPDPYPYSLPYGYVTQRD
TLSVRMLDDTRDPTLLKEIRDDKTATIIIHANASMSHTILLKAAELGMVSAYTYIFTNLEFSLQRMDSLVDDRNVILGFSIFNQSHAFFQEFASLQNSWQENCD

NIVYESSDPPRTLIHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDAASGGRTWAMGRYLARLMKGTSGVNNTNFRTSILQFDEGQRITFNIQVYDPLDGIGI
HVPFTGPALSSALLFDVAVVAVTAVQELNR-----SQEIGVKPLSCGSAQIWQHGTSLMNYLR-MVELEGLTGHI EFNSKGQRSNYALKILQFTRNGFRQIGQWHV

AIWDPRGQITQLNVDVKAQKKMIYRVATRIGPPYFSYNETARELNLTGNALYQGYAVDLIDAIARHVGFEYVFPVADQQYKGLDKETKQWNGIIGEIINNDAMGI
AEGLSMDSHLYASNISDTLFTNTLVVTTILENPLYMLKGNHQEME--GNDRYEGFCVDMLKELAEILRFNYKIRLVGDGVYG-VPEANGTWTGMVGEIARKADLAV

41

CDLTITQARKTAVDFTVPFMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIIWILISVFMVTLKTIVARISKMDWENPHPCNR-DPEVLENQWRIHNTGWLTVASIM
AGLTITAREKVIDFSKPFMTLGISILYRVHMGRKPGYFSFLDPFSPGVWLFMLLAYLAVSCVLFVARLTPYEWYSPHPCAQGRCNLLVNQYSLGNSLWFPVGGFM

TM1

TM2

TM3

TAGCDILPRSPQVRMFEATWWIFAIIIANSYTANLAAFLTSSKMEGSIANLKDLAQQKVKFGTIYGGSTYNLLADSNETVYRLAFNLMNDDPSAYTKDNLEGVDR
QQGSTIAPRALSTRCVSGVWVAFTLIIISSYTANLAAFLTQVRMDVPIESVDDLADQTAIEYGTIHGGSSMTFFQNSRYQTYQRMWNYMYSKQPSVFKSTEELIAR

VRKNRGDYMFLMETTTLEYHREQNCDLRSVGEKFGKHYAIAVPFGAEYRSNLSVAAILKLSERGELYDLKQKWKPNPNASCFEEDPDATPDMTFEELRGIFYTLYA
VLN--SNYAFLLLESTMNEYRQRNCNLTQIGLLDTKGYGIGMPVGSVFRDEFDLAILQLQENNRLEILKRKWWEG--GKCPKEED-HRAKGLGMENIGGIFVVLIC

TM4

GILIAFLIGITEFLVYVQVALEERLTFKDAFKKEIRFVLC---VWNNRKPVAGTPISSVRTTPRRSLDKSLDR-----TPKSSRRVVIIGRSSEEMRE
GLIVAIFMAMLEFLWTLRHSEATEVSVQCQEMVTELRSIILCQDSIHPRRRRAAVPPRPPPIPEERRPRGTATLSNGKLCGAGEPDQLAQLAQEAALVARGCTHIRV

MAQGSQSSSGSNNAGRGEKEARV-----
CPECRRFQGLRARSPARSEESLEWEKTTNSSEPE

(Figure 1-5b cont.)

GluR-IIID
KA1

MHFCWISLIILSLSRVQAQFYGGNAYEASSGQSIRLGLITDDATDRIR-----QTFEHAISVVNNELG-VPLVGETEQVAYGNSVQAFQAQLCRLMQSGVGVAVFGPA
-----MPRVSAPLVLLPAWLVMVACSPHSLRIAAILDDPMECSRGERLSITLAKNRINRAPERLGGKAKVEVDIFELLRDSEYETAETMCQILPKGVVAVLGP

ARHTASHLLN-ACDSKDIPIYPHLSWGS----NPDGFNLHPSPEDIANALYDIVNQFEWSRFIFCYESAELYKILDHLMTRYGIKGPVIKVMRYDLNLNGNYKSV
SSPASSIIISNICGEKEVPHFKVAPEEFVKFQFRFTTLNLHPSNTDISVAVAGILNFFNCTTACLICAKAECLLNLEKLLRQFLISKDTLSVRMLDDTRDP--TPL

LRRIRKSEDSRIVVVGSTTGVAELLRQAQVQVIMNEDYTYIIGNLNLHTFDLEEKYSEANITGIRMFSPDQEEVRDLMEKHLHQELGE---SEPVNSGSTFITMEMA
LKEIRDDKTATIIIHANASMSHTILLKAAELGMVSAYTYIFTNLEFSLQRMDSLVDVDRVNLGFSIFNQSHAFFQEFQSLNQSWEQNCNDHVPFTGPAALSSALLFD

LTYDAVRVIAETTKHLPYQPQMLNCSEHNDVQPDGSTFRNYMRSLEIKEKTIIGRIYFEGN-VRKGFTFDVIELQTSGLVKVGTWEEGKDFEFQRPQAVNFNDID
AVYAVVTAVQELNRSQEIGVKPLSCGS--AQIWQHGTSLMNYLRMVELEG--LTGHI EFNSKGQRSNYALKILQFTRNGFRQIGQWHVAEGLSMDSHLYASNISDT-

DGSLVNKTFIVLISVATKPYASLVESIDTLIGNNQFQYGVDLIKELADKLGFNFTFRDGGNDYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSI
----LFNTTLVVTTILENPLYMLKGNHQEMEGNDRYEGFCVDMLKELAEILRFNYKIRLVGDGVYGVPEANGTWTGMVGELIARKADLAVAGLTITAEREKVIDFSK

42

TM1

TM2

PFMNLGIAILYVVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNPYPCIE-EPEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRTIS
PFMTLGISILYRVHMGRKPGYFSFLDPFSPGVWLFMLLAVLAVSCVFLVVARLTPYEWYSPHPCAQGRCNLLVNQYSLGNSLWFPVGGFMQQGSTIAPRALSTRCVS

TM3

AIWWFFTLIMVSSYTANLAAFLTIENPTSPINSVKDLADNKDDVQYGAKRTGSTRNFFSTSEPIYIKMNEYLN-AHPEMLMENNQQGVDKVKSGETKYAFLMESTSI
GVVWAFTLIISSYTANLAAFLTVQRMDVPIESVDDLADQT-AIEYGTIHGSSMTFFQNSRYQTYQRMWNYMYSKQPSVFKSTEELIARVLN-SNYAFLLLESTMN

TM4

EFNTVRECNLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQVGLARLKNKWNNEVGAGVCSAKSDDGDPSELGVDNLSGIYVVLVIGSIIISIIISILWCYF
EYYRQRNCNLTQIGLLDTKGYGIGMPVGSVFRDEFDLAILQLQENNRLEILKRKWEEG---KCPKEEDHRAKGLGMENIGGIFVVLICGLIVAIIFMAMLEFLWT

VYKAKNYEVPFCDALAEFRIVIRFSENERPLKSAQSIYS-----RNRNS
LR-HSEATEVSVQCQEMVTELRSIILCQDSIHPRRRRAAVPPRPPIPEERRPRGTATLSNGKLCGAGEPDQLAQRLAQEAALVARGCTHIRVCPERRFQGLRARPS

-SQSIESLKT DSEENMPVED

PARSEESLEWEKTTNSSEPE

(Figure 1-5b cont.)

GluR-IIe
KA1

MFFNHFVILWLSLFSIHISVNWAQYENFGGYDNYQSLESVPIGLLTDQNTSEQMNIIVFDH-AIDVANQEVGTSLSLKEEVNYG-DAYQSYGKLCRMLLETGIAGVFGPS
-----MPRVSAPLVLLPAWLVMVACSPHSLRIAAILDDPMECSRGERLSITLAKNRINRAPERLGKAKVEVDIFELLRDSEYETAETMCQILPKGVVAVLGPS

SRHTAVHLMS-ICDAMDIPHIYS-----YMSENAEGFNLHPHPADLAKALYSLITEFNWTRFIFLYESA EYLNILNELTTMLGKSGTVITVLR YDMQLNGNYKQV
SSPASSIISNICGEKEVPHFKVAPEEFVKFQFRFTTLNLHPSNTDISVAVAGILNFFNCTTACLICAKAECLLNLEKLLRQFLISKDTLSVRMLDDTRDP--TPL

LRRVRKSVDNRIVVVGSSSETMPEFLNQAQQVGIINEDYKYIIGNLDFHSFDLEEKYSEANITGLR LFSPEKMAVKELLMKLGYP TDQDEF RNGSCPITVEMALTYD
LKEIRDDKTATIIIHANASMSHTILLKAAELGMVSAYTYIFTNLEFSLQRMSLVDDR VNILGFSIFNQSHAFFQEF AQSLNQSWQENCDHVPFTGPALSSALLFD

AVQLFAQTLK NLPFKPMP--QNCSQRTE SVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGN-VRKGYHLDVIELQPSGIVKVG TWDEDRQYRPQRLAPT TAQFDSVDN
AVYAVVTAVQELNRSQEIGVKPLSCGSAQIWQHGTSLMNYLRMVELEG--LTGHI EFNSKGQRSNYALKILQFTRNGFRQIGQWH-----VAEGLSMDSHLYASNIS

SLANKTFIILLVSPNKPYAQLVET YKQLEGNSQYEGYGVDLIKELADKLGFNFTFVNGGNDYGSYNKSTNESTGMLREIMTGRADLAITDLTIT SEREQALDFTIPF
DTLFTNTLVVTTILEN PYLMLKGNHQEMEGNDRYEGFCVDMLKELAEILRFNYKIRLVGDGVYGVPEANGTWTGMVGELIARKADLAVAGLTIT AEREKVIDFSKPF

43

TM1

TM2

MNLGIAILYLKPKATPELFTFMDPFSEEVVWFLGFSFLGVLSFFILGR LSPSEWDNPYPCIE-EPEELENQFTLGNSIWFTTGALLQQGSEIGPKALSTR TVASF
MTLGISILYRVHMGRKPGYFSFLDPFSPGVWLFMLLAYLAVSCVLFVVARLTPYEWYSPHPCAQGR CNLLVNQYSLGNSLWFPVGGFMQQGSTIAPRALSTR CVSGV

TM3

WWFFTLIVSSYTANLAAFLTIEKPSLINSVDDLADNKDGVVYGA KKTGSTRNFFMTSAEERYKKMNKFMS-ENPQYLTEDNMEGVNRVKTNTHYAFLMESTSIEY
WWAFTLIIISSYTANLAAFLTVQRMDVPIESVDDLADQT-AIEYGTIHGSSMTFFQNSRYQTYQRMWNYMYSKQPSV FVKSTEEG IARVLN-SNYAFLLESTMNEY

TM4

NTKREC NLKKIGDALDEKGYGIAMRKDWPHRGKFNNALLELEQGVLEKMKNKWNEVGTGICATKEDAPDATPLDMNNLEGVFFVLLVGS CCALLYGIIISWVLFVM
YRQRNCNLTQIGLLDTKGYGIGMPVGSVFRDEFDLAILQLQENNRLEILKRKWE EGG---KCPKEEDHRAKGLGMENIGGIFVVLICGLIV AIFMAML-EFLWTL

KKAHHYRVPLRDALKEEFQFVIDFN NYVRVLKNSASIYSR-----SRQSSMSVASVAQESQ-----
RHSEATEVSVCQEMVTELSIILCQDSIHPRRRAAVPPRPPIPEERRPRGTATLSNGKLCGAGEPDQLAQRLAQEAALVARGCTHIRVCPECRRFQGLRARPSPA

RSEESLEWEKTTNSSEPE

(Figure 1-5b cont.)

GluR-IIA MRLCPVVIYAFIIIIIGFLEGIIALGGDDRNEITVGAIIFYENEKEIELSFDQAFREVNMMKFSELRFVTIKRYMPTNDSFLLQOITCELI SNGVAAIFGPSSK-AASD
KA2 ---MPAELLLLLLIVAFASPSCQVLSLLRMAAILDDQTVCGRGERLALALAREQINGIIIEVPAKARVEVDIFELQORDSQYETTTMCQILPKGVVSVLGPSSSPASAS

IVAQIANATGIPHIEYDLKLEATRQEQLNHQMSINVAPSLSVLSRAYFEI IKSNEYEWRTFTLIYETPEGLARLQDLMNIQALNSDYVKLRNLADYADDIRILWKETD
TVSHICGEKEIPHIKVGPP--EETPRLQYLRFAVSLSLYPSNEDVSLAVSRILKS-FNYPSASLI CAKAECLLRLEELVRGFLISKETLSVRMLDDSRDPTPLLKEIRD

ETFHEQRIILDCEPKTLKELLKVSIDFKLQGFNRWFLTHLDTHNSGLRDIYNED-----FKANITSVRLKVV DANPFERKKTRLTKVDQILGNQTMPLI LIYDAVV
D--KVSTIIIDANASISHLILRKASELGMTSAFYKYILTTMDFPILHLDGIVEDSSNILGFSMFNTSHPFYPEFVRS LNMSWRENCEASTYLG-PALSAALMFDVAVH

LFASSARNVIAAMQPFHPPNRHCGSSSPWMLGAFIVNEMKTI SEDDVPHFKTENMKLDEYQRIHFNLEIYKPTVN--EPMVWTPDNGIKRLLNLELESAGTTQ
VVVSAVR-ELNRSQEI GVKPLACTSANI WPHGTSLMNYLRMVEYDGLT-----GRVEFN SKGQRTNYTLRILEKSRQGHREIGVWYSN-----RTLAMNATTL DINL

DFSEQRKVYTVVTHYE EYPYFMMKEDHENFRGREKYEGYAVDLISKLS ELMEDYEFMIVNGNGKYNPETK-QWDGIIRKLI DHHAQIGVCDLTTITQMRRSVVDFTVP
SQTLANKT LVVTTILENPIYVMRPNFQALSGNERFEGFCVDMLRELAELLRFYRRLRLVEDGLYGAPENGSWTGMV GELINRKADLAVA AFTTITAEREKVIDFSKP

44

TM1

TM2

FMQLGISILHYKSPPEPKNQFAFLEPF AVEVWIYMIFAQLIMTLAFVFIARLSYREWLPNPAIQD-PDELENIWNVNNSTWLMVGSIMQQGCDILPRGPHMRILT G
FMTLGISILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVLF LAARLSPYEWYNPHPCLRARPHILENQYTLGNSLWFPVGGFMQQGSEIMPRALSTR CVSG

TM3

MWWF FALMMLSTYTANLAAFLT SNKWQSSIKSLQDLIEQDKVHFGSMRGGSTSLFFSESNDDTYQRAWNQMKDFNPSAFTSTNKEGVARVRKEKGGYAF LMETTSLT
VWVAFTLIIISSYTANLAAFLT VQRMEVPVESADDLADQTNIEYGTIHAGSTMTFFQNSRYQTYQRMWNYMQSKQPSV FVKSTE EGIARVLSNR--YAF LLESTMNE

TM4

YNIERNCDLTQIGEQIGEKHYGLAVPLGSDYRTNLSVSI LQLSERGELQMKNKWKNHNVTCD SYHEVDGDELSI IELGGVFLVLAGGV LIGVILGIFEF LWNVQN
YHRRLNCNLTQIGGLLDTKGYGIGMPLGSPFRDEITLAILQLQENNRLEILKRKWE GGRCPKEEDHRAKG--LGMENIGGIFIVLICGLIIAVFVAVMEFIWSTR

VAVEERVTPWQAFKAELIFALKFWVRKPP-----MRISSSSDKS
SAESEEVSVCEMLQELRHAVSCRKTSR SRRRRRPGGPSRALLSLRAVREMRLSNGKLYSAGAGGDAGSAHGGPQRL LDDPGPPSGARPAAPT PCTHVRVCQECRRI

SSRRSSGSRSSKEKSRSKTVS-----
QALRASGAGAPPRLGLVPAEATSPPRPRPGPAGPRELAEHE

(Figure 1-5b cont.)

GluR-IB
KA2

MHGLQFLVLLALAIASGANEDTLVIKIGAIFFDTEMKLAD--AFSAALEEVN--AINPALKLDAIKRYVTVDDSIVLQDISCDLIGSGVAAIFGPSSK-TNSDIV
-MPAELLLLLLIVAFASPCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIIEVPAKARVEVDIFELQRDSQYETTTMCQILPKGVSVLGPSSSPASASTV

EVLGNMTGIPHLQFDWHPQOSNRERMNHQLTVNVAPMELFLSAAFSDILASKTFDWKSFTIAYERSHILRLQHILAWKQLHKAGIKMQEFERGGDYRILWKRINNA
SHICGEKEIPHIKVG--PEETPRLQYLRFAVSLSYPSNEDVSLAVSRILKS--FNYPSASLIIKAKAECLLRLEELVRGFLISKETLSVRMLDDSRDPTLLKEIRDD

REKFVLLDCPSDILVDVINASIGYNMTGSFNHLFLTNLDT--HLSGIDGFYSRDFTVAVAAVRIRTYVPPPVDHEIDVFDNSVDTRFS--SLGSQLVYDSIVLFYN
KVSTIIIDANASISHLILRKASELGMTSAFYKYILTTMDFPILHLDGIVEDSSNILGFSMFNTSHPFYPEFVRSLSNMSWRENCEASTYLGPAALSALMFDVAHVVS

ALLEISQRPGFYIPNFSCGRG-FWQPGPRLVEQMKQITPKMVKPPFKTQRLQINADGQREDFNLEVYNPIIDRVTHIWNKEFQLVDFEKLRENSTQALKQKRLQNK
AVRELNRSQEIGVKPLACTSANIWPHTSLMNYLR-----MVEYDGLTGRVEFNKSGQRTNYTLRILEKSRQGHREIG-----VWYSNRTLAMNATTLDINL

DFSQKPIRYTVATRVGKPYFSWREEPEGVHYEGNERFEGYAVDLIYMLAQECKDFNFEPVRDNKYGSYDANTDEWDGIIRQLIDNNAQIGICDLTITQARRSVVDF
SQTLANCTLVVTILENPIYVMR--PNFQALSNGERFEGFCVDMLRELAELLRFYRLRLVEDGLYGAPEN-GSWTGMVGELINRKADLAVAAFTITAREKVIDF

45

TM1

TM2

TVPFMQLGISILSYKEPPPKADIYAFLNPNYNAEVWLFVMIAMMITAFALIFTGRIDQYEWDPVENVN-REMERQNIWHLSNALWLVLSMLNQGCDLLPRGLPMRL
SKPFMTLGISILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVLFLLAARLSPYEWYNPHCLRARPHILENQYTLGNSLWFPVGGFMQQGSEIMPRALSTRC

TM3

LTAFWWIFALLISQTYIAKLAAFITSSKIAGDIGSLHDLVDQNKVQFGTIRGGATSVYFSESNDTDNRMAWNKMLSFKPDAFTKNNEEGVDRVKLSKGTYAFLMETT
VSGVWWAFTLIIISSYTANLAAFLTVQRMEVPVESADDLADQTNIEYGTIHAGSTMTFFQNSRYQTYQRMWNYMQSKQPSVFKSTEEGIARVLNSR--YAFLEST

TM4

NLQYYVQRNCELTQIGESFGEKHYGIAVPLNADFRSNLSVGILRLSERGELFKLRNKWFNSNESTCDSNVPTIDDGQFDMDSVGGLFVVLIIVGVVGLVIGVAEFLW
MNEYHRRLNCLNTQIGGLDGTKYGIIMPLGSPFRDEITLAILQLQENNRLEILKRKWEEGRCPEEDHRAKGLG--MENIGGIFIVLICGLIIAVFVAVMEFIW

HVQRI SVKEKIPPLALKAEFYFVIRFWLTRKP-----LHTYRQSRDSTSTGYSS-----LEQITSASSAKKKK
STRRSAESSEEVSVCEMLQELRHAVSCRKTSRSTRRRRPPGSPRALLSLRAVREMRLSNGKLYSAGAGGDAGSAHGGPQRLDDPGPPSGARPAAPTCTHVRVCQE

TRRIEK-----
CRRIQALRASGAGAPRGLGVPAEATSPPRPRPGPAGPRELAEHE

(Figure 1-5b cont.)

GluR-IIc KA2 MKKELSGNSSARWPQMFGGNSREAGMWQRILLGCMWSAFFMCRSRGQQINIGAFFYDDELELEKEFMTVVNAINGPESEQTMRFYPLIKRLKPEDGSVMTQEHACD
-----MPAELLLLLLIVAFASPSCQVLSSLRMAAILDDQTVCG-RGERL-----ALALAREQINGIIEVP-AKARVEVDIFELQR-----DSQYETTTDMCQ

LIDNGVAAIFGPSSK-AASDIVALVCNSTGIPHIEFDISDEGIQAEKPNHQMTLNLYPQAAILSKAYADIVQNFGRKFTIVYDADDARAAARLQDLLQLREVHNDV
ILPKGVSVLGPSSSPASASTVSHICGEKEIPHIKVGPP--EETPRLQYLRFAVSLYPSNEDVSLAVSRILKSFNYPSASLI CAK--AECLLRLEELVRGFLISKET

VRVRKFHKDDDFRVMWKSIRGERR--VVLDCPEPNMLVELLNSSTEFGLTGQYNHIFLTNLETYTDHLEELAADNETFAVNI TAARLLVNPDP PPYSLPYGYVTQRDN
LSVRMLDDSRDPTLLKEIRDDKVSTIIIDANASISHLILRKASELGMTSAFYKYILT TMDFPILHLDGIVED---SSNILGFSMFNTSHPFYPEFVRSLSNMSWRE

IVYESSDPPRTL IHDLIHDALQLFAQSWRNASFFYPDRMVVPRITCDF AASGGRTWAMGRYLARLMKGTSGVNNTNFRSILQFDEGQRITFNIEVYDPLDG--IG
NCEASTYLGPA LSAALMFDAVHVVS AVR--ELNRSQEIGVKPLACTSAN----I WPHGTSLMNYLR----MVEYDGLTGRVEFN SKGQRTNYTLRILEKSRQGHRE

IAIWDPRGQITQLNVDVKAQKKMIYRVATRIGPPYFSYNETARELN---LTGNALYQGYAVDLIDAIARHVGFEYVFPVADQQY GKLDKETKQWNGIIGEIIINDDA
IGVWYSNRTLAMNATTL DINLSQTLANKTLVVTTILENYPVMRRPNFQALSGNERFEGFCVDMLRELAELLRFRYRLRLVEDGLYGAPEN-GSWTGMV GELINRKA

46

HMGICDLTITQARKTAVDFTV PFMQLGVSILAYKSPHVEKTLDAYLAPFGGEVWIWILISVFMVTLKTIVARISKMDWENPHPCNRD-PEVLENQWR IHNTGWLTV
DLAVAAFTITAEAREKVIDFSKPFMTLGISILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVLF LAARLSPYEWYNPHPCLRARPHILENQYTLGNSLWFPV

TM1

TM2

TM3

ASIMTAGCDILPRSPQVRMFEATWIFAI I IANSYTANLAAFLTSSKMEGSIANLKDLSAQKKVKFGTIYGGSTYNLLADSNETVYRLAFNLMNDDPSAYTKDNLE
GGFMQQGSEIMPRALSTRCVSGVWVAFTLIIISSYTANLAAFLT VQRMEVPVESADDLADQTNIEYGTIHAGSTMTFFQNSRYQTYQRMWNYMQSKQPSV FVKSTEE

GVDRVRKNRGDYMFLETTTLEYHREQNCDLRSVGEKFGKHYAIAVPFGAEYRSNLSVA I LKLSERGELYDLKQKWKPNPNASC FEEDPDATPDMTFEELRGIFY
GIARVLNSR--YAFLL ESTMNEYHRLNCNL TQIGLLDTKGYGIGMPLGSPFRDEITLAILQLQENNRLEILKRKWWEG--GRCPKEED-HRAKGLGMENIGGIFI

TM4

TLYAGILIAFLIGITEFLVYVQVALEERLTFKDAFKKEIRFVLCVWNNRKP-----IVAGTPISSVRTTTPRRSLDKS---
VLICGLIIAVFVAVMEFIWSTRRSAESEEVSVCEMLQELRHAVSCRKTSR SRRRRRPPGGPSRALLSLRAVREMRLSNGKLYSAGAGGDAGSAHGGPQRLLDDPGPP

LDRTPKSSRRVIGRSS EEMREMAQSGSSSGSNNAGRGEKEARV-----
SGARPAAPTCTHVRVCQE CRRIQALRASGAGAPPRGLGVP AEATSPPRPRPGPAGPRELAEHE

(Figure 1-5b cont.)

GluR-IIID MHFCWISLIILSLSRVQAQFYGGNAYEASSGQSIRLGLITDDATDRIRQTFEHAISVNNELGVPLVGETE QVAYGNSVQAFALCRLMQSGVAVFGPAAR-HTAS
KA2 MPAELLLLLLIVAFASPSCQVLSLRLMAAILDDQTVCGRGERLALALAREQINGIIEVPAKAR---VEVDIFELQRDSQYETTTDTCQILPKGVSVLGPSSSPASAS

HLLNACDSKDIPFIYPHLSWGS-----NPDGFNLHPSPEDIANALYDIVNQFEWSRFIFCYESA EYLKILDHLMTRYGIKGPVIKVMRYDLNLNGNYKSVLRRIRKS
TVSHICGEKEIPHIKVGP EETPRLQYLRFASVSLYPSNEDVSLAVSRILKSFNYPSASL ICAKAECLLRLEELVRGFLISKETLSVRMLDDSRDP--TPLLKEIRDD

EDSRIVVVGSTTGVAELLRQAQVGMNEDYTYIIGNLNLHTFDLEEKYSEANITGIRMFSPDQEEVRDLMEKHLQELGESEPVNSGSTFITMEMALTYDAVRVIA
KVSTIIIDANASISHLILRKASELGMTSAFYKYILTTMDFPILHLDGIVEDSSNILGFSMFNTSHPFYPEFVRSNLNMSWREN-CEASTYLGPALSAALMFDAVHVVV

ETTKHLPYQP--QMLNCSERHDNVQPDGSTFRNYMRSLEIKEKTIITGRIYFEGN-VRKGFTFDVIELQTSGLVKVGTWEEGKDFEFQRPPQAVNFNDIDDGSLVNKT
SAVRELNRSQEIGVKPLACTSANIWPHGTSLMNYLRMVEYDG--LTGRVEFN SKGQRTNYTLRILEKSRQG HREIGVWYSNR TLAMNATTL D INLSQT----LANK

FIVLISVATKPYASLVESIDTLIGNNQFQYGVDLIKELADKLGFNFTFRDGGNDYGSFNKTTNSTSGMLKEIVEGRADLAITDLTITSEREEVIDFSIPFMNLGIA
TLVVTTILENPNYVMRRPNFQALSGNERFEGFCVDMLRELAELLRFRYRLRLVEDGLYGAPENGSWTGMVGELINRKADLAVAAFTITAEREKVIDFSKPFMTLGIS

47

TM1

TM2

ILYVKPQKAPPALFSFMDPFSSEVWLYLGIAYLGVSLCFFIIGRLSPIEWDNYPYPCIEE-PEELENQFTINNSLWFTTGALLQQGSEIAPKALSTRTISAIWWFFTL
ILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVLF LAARLSPYEWYNPHPCLRARPHILENQYTLGNLWFPVGGFMQQGSEIMPRALSTRCVSGVWWAFTL

TM3

IMVSSYTANLAAFLT IENPTSPINSVKDLADNKDDVQYAKRTGSTRNFFSTSEEP IYIKMNEYLN-AHPEMLMENNQQGVDKVKS GTKYAFLMESTSIEFN TVREC
IIISSYTANLAAFLTVQRMEVPVESADDLADQTN-IEYGTIHAGSTM TFFQNSRYQTYQRMWNYMQSKQPSV FVKSTEEGIARVLN-SRYAF LLESTMNEYHRR LNC

TM4

NLTKVGDPLDEKGYGIAMVKNWPYRDKFNKALLELQEQV LARLKNKWNNEVGAGVCSAKSDDDG PSELGVDNL SGIYVVLVIGSIIISIIISILCWCFVYK KAKNY
NLTQIGGLD TKGYGIGMPLGSPFRDEITLAILQLQENNRLEILKRKWWEGG---RCPKEEDHRAKGLGMENIGGIFIVLICGLIIAVFVAVMEFIWSTRR SAESE

EVPFCDALAEFRIVIRFSENER-----PLKSAQSIYSRSRNSSQSIESLKT
EVSVCQEMLQELRHAVSCRKTSR SRRRRRPGGPSRALLSLRAVREMRLSNGKLYSAGAGGDAGSAHG GPQRLDDPGPPSGARPAAPT PCTHVRVCQECRR IQALRA

DSE-----ENMPVED-----
SGAGAPPRGLGVP AEATSPPRPRPGPAGPRELAEHE

(Figure 1-5b cont.)

GluR-IIe KA2 MFFNHFVILWSLFSIHSVNWAQYENFGGYDNYQSLESVPIGLLTDQNTSEQMNI VFDHAIDVANQEVGTSLSLKEEVNYGDAYQSYGKLCRMLETGIAGVFGPSSR
-MPAELLLLLLIVAFASPCQVLSLRLMAAILDDQTVCGRGERLALALAREQINGIIIEVPAKAR--VEVDIFELQKRS---QYETTDTCQILPKGVSVLGPSSS

-HTAVHLMSICDAMDIPHIYSYMSEN-----AEGFNLHPHPADLAKALYSLITEFNWTRFIIFLYESA EYLNILNELTTMLGKSGTVITVRLYDMQLNGNYKQVLR
PASASTVSHICGEKEIPHIKVGPEETPRLQYLRFASVSLYPSNEDVSLAVSRILKSFNYPSASLI CAKAECLLRLEELVRGFLISKETLSVRMLDDSRDP--TPLLK

RVRKSVNDRIVVVGSSSETMPEFLNQAQVGIINEDYKYIIIGNLDFHSFDLEEKYSEANITGLRFLSPEKMAVKELLMKLGYPDQDEFNRNGSCPITVEMALTYDAV
EIRDDKVSTIIIDANASISHLILRKASELGMTSAFYKYILTTMDFPILHLDGIVEDSSNILGFSMFNTSHPFYPEFVRS LNMSWRENCEASTYLGPALSAALMFDVAV

QLFAQTLKKNLPFKPMP--QNCSQRTESVRDDGSSFKNYMRTLRLTDRLLTGPIYFEGN-VRKGYHLDVIELQPSGIVKVGTDWEDRQYRPQRLAPTTAQFDSVDNSL
HVVS AVRELNRSQEIGVKPLACTSANIWPHTSLMNYLRMVEYDG--LTGRVEFN SKGQRTNYTLRILEKSRQGHREIGVWYSN---RTLAMNATTL D IN-LSQTL

ANKTFIILLVSPNKPYAQLVETKQLEGN SQYEGYGVDLIKELADKLGFNFTFVNGGNDYGSYNKSTNESTGMLREIMTGRADLAITDLTITSEREQALDFTIPFMM
ANKTLVVTTILEN-PYVMRRPNFQALSGNERFEGFCVDMLRELAELFRYRLRLVEDGLYGAPENGSWGTGMV GELINRKADLAVAAFTITAREKVIDFSKPFMT

48

TM1

TM2

LGIAILYLKPKATPELFTFMDPFSEEVVWFLGFSFLGVLSFFILGR LSPSEWDNYPYPCIEE-PEELENQFTLGNSIWFTTGALLQQGSEIGPKALSTRTVASFVW
LGISILYRVHMGRKPGYFSFLDPFSPAVWLFMLLAYLAVSCVLF LAARLSPYEWYNPHPCLRARPHILENQYTLGNSLWFPVGGFMQQGSEIMPRALSTRCVSGVWW

TM3

FFTLIVSSYTANLAAFLTIEKPSLINSVDDLADNKDGVVYGA KKTGSTRNFFMTSAEERYKMKNFMS-ENPQYLTEDNMEGVNRVKTNTHYAFLMESTSIEYNT
AFTLIISSYTANLAAFLTVQRMEVPVESADLDLADQTN-IEYGTIHAGSTMTFFQNSRYQTYQRMWNYMQSKQPSV FVKSTEEG IARVLN-SRYAFLESTMNEYHR

TM4

KRECNLKKIGDALDEKGYGIAMRKDWPHRGKFNNALLELQEQVLEKMKNKWNEVGTGICATKEDAPDATPLDMNNLEGVFFVLLVGS CCALLYGIIISWVLFVMMK
RLNCNLTIQIGLLDTKGYGIGMPLGSPFRDEITLAILQLQENNRLEILKRKWE GEG---RCPKEEDHRAKGLGMENIGGIFIVLICGLIIAVFVAVMEFIWSTRRS

AHHYRVPLRDALKEEFQFVIDFN NYVRVLKN-----SASIYSR-----SRQSSMSVASVAQESQ---
AESEEVSVQEMLQELRHAVSCRKTSR SRRRRRPGGPSRALLSLRAVREMRLSNGKLYSAGAGGDAGSAHG GPQRLDDPGPPSGARPAAPTCTHVRVCQECRRIQ

ALRASGAGAPPRGLGVPAEATSPPRPRPGPAGPRELAEHE

Subunit name	Protein ID	Gene ID	Subunit name	Protein ID	Gene ID	Subunit name	Protein ID	Gene ID
GluR-IIA	NP 523484	33788	CG 3814	NP 610824 NP 725236	36418	CG 14586	NP 649013	39983
GluR-IIB	NP 523485	33789	CG 33513	NP 001014716 NP 001014715 NP 001014714	31107	CG 14585	NP 649012	39982
GluR-IIC (GluRIII)	NP 608557 NP 722670	33275	CG 2902	NP 730940	40665	CG15732	NP 572795	32189
GluR-IID (=kaiRIA)	NP 651982	44483	CG 30379	NP 724626	246578	CG 6185	NP 648455	39269
GluR-IIIE	NP 001036733	318623	GluR I	NP 476855	38742	CG 15324	NP 572411	31692
CG 11155	NP 651941 NP 726648 NP 726649	43822	GluR IB	NP 524755	44484	CG 11775	NP 649833	41052
CG 9935	NP 651931 NP 001036250 NP 001036251	43806	CG 17274	NP 650923	42471	CG 10633	NP 647962	38616
Clumsy (=CG 8681)	NP 523610 NP 001036373	35394	CG 31718	NP 723585	318912	CG 10101	NP 649720	40891
CG 5621	NP 650927 NP 001036735	42476	CG 2657	NP 001097043	33157	CG 15627	NP 608863	33683
CG 3822	NP 650925	42473	CG 7385	NP 649176	40198	CG 8533	NP 649148 NP 730452 NP 730453	40157
CG 32704	NP 727328	31867	CG 14076	NP 649074	40065	CG 17275	NP 650924	42472
CG 3798	NP 725237 NP 725238 NP 523722 NP 725239 NP 725241 NP 725240	36419	CG 33492 (=CG 17587)	NP 995744	2768714	CG 17152	NP 648548	39378

Table 1-1. Glutamate receptor genes in *Drosophila* genome. 36 different glutamate receptors genes are found in NCBI database (National Center for Biotechnology Information).

Chapter Two

Furthering Pharmacological and Physiological Assessment of the Glutamatergic Receptors at the *Drosophila* Neuromuscular Junction

INTRODUCTION

The function and pharmacology of glutamate receptors in general are of prime interest since they are the major excitatory receptor type used within the mammalian central nervous system (CNS). There are extensive investigations into the pharmacology, physiology, molecular structure and regulation in expression as well as turnover in mammalian preparations of glutamate receptors (Dingledine et al., 1999; Hollmann and Hainemann, 1994; Lipsky and Goldman, 2003; Mayer, 2005; Pauly et al., 2005; Seeburg, 1993; Thiagarajan et al., 2005). Earlier studies on glutamate receptors at the neuromuscular junction of insects and crustaceans paved the way to dissecting the functional aspects of these receptors within vertebrates (Anderson et al. 1976; Cull-Candy and Parker, 1983; Gratton et al., 1981; Jan and Jan, 1976; Patlak et al., 1979; Shinozaki and Ishida, 1981; Shinozaki and Shibuya, 1974). Still today, invertebrate models serve a vital role in describing regulation and developmental aspects of glutamate receptor function (Featherstone et al., 2005; Guerrero et al., 2005; Littleton and Ganetzky, 2000; Logsdon et al., 2006; Pawlu et al., 2004; Rasse et al., 2005). Considering that *Drosophila melanogaster* is a true model organism with a known genome and used for successful rapid induction of mutations in various studies of development and synaptic mechanisms, it is important to understand the pharmacological and physiological function within this model synaptic neuromuscular preparation (Atwood et al., 1993; Betz et al. 1993; Jan and Jan 1976; Kurdyak et al., 1994; Li et al., 2002; Li and Cooper, 2001; Pawlu et al., 2004; Ruffner et al., 1999; Sigrist et al., 2002, 2003; Stewart et al., 1994, 1996).

The molecular sequence of the glutamate receptors on the *Drosophila* skeletal muscle has been described; however, to date there are few detailed investigations on the pharmacological profile of the intact larval *Drosophila* NMJ (Chang and Kidokoro, 1996; Chang et al., 1994; Delgado et al., 1989; Schuster et al., 1991; Zhang et al., 1999). Glutamate receptors are defined as NMDA (N-methyl-D-aspartic acid) and non-NMDA subtypes (i.e. AMPA, kainate and δ) based on their pharmacological sensitivity activating the receptors. The molecular sequences of the glutamate receptors for *Drosophila* NMJs were performed on the body wall muscles in larvae by isolating the skeletal muscle and the body wall (skeletal muscles attach to the cuticle body wall). The total mRNA was extracted from this muscle tissue and probed against an "affymetrix *Drosophila* genechip". A number of *Drosophila* glutamate receptor subunits were found to be present. The sequences isolated were identified by comparison to whole larval DNA known sequences for glutamate receptors. The ones identified

are GluRIIA, GluRIIB, GluRIIC and GluRIID. GluRIIE was later isolated when real-time PCR was used to follow up on the amplified mRNA of the four receptor subtypes in quantification of expression levels. The expression profile of the five glutamate receptor subunits has been conducted in body wall muscle by in situ hybridization for embryos and larvae (Guerrero et al., 2005; Marrus et al., 2004).

With the RT-PCR and the genomic profiling the sequence indicates that there are 5 glutamate receptor subunits (DGluR-IIA, DGluR-IIB, DGluR-IIC, DGluR-IID, DGluR-IIE) expressed in skeletal muscle and all of them are ionotropic receptors. In comparing the molecular sequence of these 5 muscle-expressed glutamate receptors with vertebrates, they showed the most similarity to the vertebrate kainate/AMPA receptor subtypes; therefore, they have been defined as a kainate receptor subtype without pharmacological examination (Betz et al., 1993; Guerrero et al., 2005; Marrus et al., 2004; Qin et al., 2005; Völkner et al., 2000). However, a recent physiological and pharmacological study demonstrates that the responses are primarily of a quisqualate subtype and not a kainate or an AMPA subtype since the muscle is not depolarized by kainate or AMPA at concentrations as high as 1mM (Bhatt and Cooper, 2005). In fact, it appears that kainate might block the glutamate receptors since the excitatory postsynaptic potential (EPSP) is reduced in the presence of kainate (on average by 40% for 1mM and 500 μ M but about 20% for 100 μ M). The reduction in the EPSP amplitude occurred without any significant change in resting membrane potential; thus, kainate did not depolarize the muscle as observed for application of glutamate or quisqualate (Bhatt and Cooper, 2005).

In addition, the procedures used to identify the molecular sequence of the glutamate receptors at the NMJ, by homogenizing the skeletal muscle and body wall, did not allow for assessment for potential presynaptic glutamate receptors. In the past procedure, the CNS was removed and thus the cell bodies of the motor neurons were not incorporated in the assay. Considering that there are presynaptic glutamatergic receptors for neurons in the vertebrate CNS (Park et al., 2006) and at crayfish NMJs (Schramm and Dudel, 1997), there may well be pre-synaptic autoreceptors present at the *Drosophila* NMJ. Crayfish NMJs show many similarities to the larval *Drosophila* NMJs (Atwood and Cooper, 1995, 1996) which is not so surprising since they are both arthropods. However, mixed results for the glutamatergic autoreceptors were obtained at the crayfish NMJ in that the responses depended on the depolarized state of the nerve terminal for the facilitatory action of exogenously applied glutamate and for the metabotropic agonist t-ACPD (Schramm and Dudel, 1997). In addition, some preparations showed a depression of presynaptic release while yet other preparations exhibited excitation at the same concentration of t-ACPD. Regardless of the response at the crayfish NMJ, there appears to be one or more types of presynaptic, glutamatergic receptors for the crayfish preparation (Schramm and Dudel, 1997). The earlier observation of the rapid reduction in the EPSP by kainate at the *Drosophila* NMJ is most likely due to one or both of the following factors: (1) antagonist action on the postsynaptic glutamate receptors; (2) reducing the number of presynaptic vesicular fusion events. It is possible both mechanisms might occur. Direct blocking action on postsynaptic receptors is a

possibility for kainate as many known agonists can be antagonists for subtypes within a receptor family (Chang and Weiss, 2002; Eberle et al., 2001; Hruby et al., 2003). This is likely as a part of the extracellular receptor domains for glutamate receptors must be similar in order to bind glutamate. However, at the 3rd instar *Drosophila* NMJ the binding of kainate does not transduce activation of the glutamate ligand gated ion channels (Bhatt and Cooper, 2005; Dudel et al. 1992; Heckmann and Dudel, 1997). There is also precedence for kainate, a potent agonist for some vertebrate metabotropic receptors, to act presynaptically in reducing evoked transmitter release via autoreceptors on the presynaptic terminals (Chittajallu et al., 1996; Frerking et al., 2001; Kamiya and Ozawa, 1998; McLarnon and Quastel, 1988; Park et al., 2006). Kainate is known to bind metabotropic glutamate receptors (Zhang et al., 1999) and have a presynaptic action in altering transmitter release from presynaptic terminals in vertebrates (Kamiya and Ozawa, 2000; Kamiya et al., 2002; Park et al., 2006). In rats, kainate and the kainate receptor agonist, ATPA, act presynaptically in reducing glutamate release from primary sensory neurons (Kerchner et al., 2001). However, in the rat neocortex, kainate receptors have been demonstrated to have both facilitatory as well as inhibitory actions due to dose dependent effects. The mechanism proposed for the biphasic effect is that low doses excite the presynaptic terminal while high doses have an inhibitory effect on the postsynaptic target (Campbell et al., 2007). Thus, depending on the type of preparation and dosage, kainate might excite or inhibit the presynaptic terminal. The evidence suggests a mechanism of action via kainate-sensitive glutamatergic autoreceptors through a metabotropic action within the presynaptic terminals or by direct action on the presynaptic voltage-gated Ca²⁺ channels (see reviews Lerma, 2003 and Nicoll et al., 2000). In addition, the actions of glutamate at newly hatched *Drosophila* larvae revealed presynaptic actions in enhancing the occurrences of spontaneous events without any alteration in the amplitude of the quantal currents (Zhang et al., 1999). The mechanisms suggested for this phenomenon are that presynaptic autoreceptors are present which enhances synaptic transmission. In addition, the pharmacological agonist (1S, 3S)-ACPD for mGluR mimicked the effect of glutamate (Zhang et al., 1999). This helps to establish that autoreceptors at this NMJ are likely in the 3rd instar and that there maybe developmental differentiation in presynaptic receptor subtypes to account for the depressing action of kainate in the 3rd instar.

The purpose of this study is to test for these possibilities. I have approached this by accessing the characteristics of single quanta (i.e., peak amplitude) and frequency of spontaneous events with intracellular measures of miniature EPSPs (mEPSPs) from the whole muscle. I also examined further pharmacological profiles of the *Drosophila* NMJ to build knowledge of this model synaptic preparation.

METHODS

Electrophysiology and staging of flies

All the electrophysiology and staging of larvae are routine procedures and have been described previously. The dissection technique was previously reported (Ball et al., 2003; Campos-Ortega and Hartenstein, 1985; Li et al., 2002). The physiological saline is HL3 (in mM): 1.0 CaCl₂ 2H₂O, 20 MgCl₂, 70 NaCl, 5 KCl, 10NaHCO₃, 5 trehalose, 115 sucrose, 5 BES (N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid) and adjusted to a pH of 7.2 (Stewart et al., 1994). All experiments were performed at room temperature (20-21°C). The recording techniques have been previously described for intracellular EPSP measures (Dasari and Cooper, 2004; Sparks et al., 2004; Stewart et al., 1994) and extracellular focal quantal recordings (Cooper et al., 1995a; Harrison and Cooper, 2003). The compound amplitude of the excitatory postsynaptic potentials (EPSP) elicited by Is and Ib motor nerve terminals in segment 3 of muscle m6 were monitored as in Kurdyak et al., (1994) and Ruffner et al., (1999). The identified m6 muscle was used in each preparation. Only preparations with a resting membrane potential of -50mV or greater were used and the HL3 dissection medium was completely replaced by HL3 media containing the pharmacological compounds.

Quantal analysis

All quantal analysis of intracellular whole muscle recordings were as described previously (Cooper et al., 1995b). The quantal analysis of measuring spontaneous frequency and characteristics of the quantal shape are standard approaches.

Pharmacology

In an earlier report (Bhatt and Cooper, 2005), actions of NMDA, AMPA, L-glutamate, kainate, quisqualic acid, NBQX, AP5, and DNQX, with regard to synaptic transmission and direct effects on the muscle fibers, was examined. This study showed that kainate reduced evoked EPSPs. The same procedures for exchanging the bathing medium with pharmacological agents were used. The compound trans-(±)-1-Amino-1,3-cyclopentanedicarboxylic acid monohydrate (t-ACPD) is a known mGluR agonist (Holscher et al., 1997) and has been shown to have some effect on newly hatched *Drosophila* larvae (Zhang et al. 1999). I examined the actions of ATPA, a known agonist of the kainate receptor in vertebrates, and 1, 3-ACPD on synaptic responses. The ATPA, domoic acid and the trans-(±)-1-Amino-1,3-cyclopentanedicarboxylic acid monohydrate (t-ACPD) were obtained from Sigma

Statistical analysis

A non-parametric analysis (Wilcoxon rank sum) or a parametric Student's *t*-test were used for assigning a significance at a $p < 0.05$ depending on the appropriate experimental design as explained in the RESULTS section.

Sequence Comparisons

All of the glutamate receptor amino acid sequences used are annotated in National Center for Biotechnology Information (NCBI) database. There are 36

different genes encoding 36 different kinds of glutamate receptor subunits in *Drosophila melanogaster*. Among them, five subunits consisting of postsynaptic receptors were compared to five different kainate type glutamate receptor subunits in *Homo sapiens*. Both whole protein and extracellular domain sequences of each subunit were aligned to show the similarity and calculate the statistical matches between two species, respectively. The *bl2seq* in NCBI and Clustal W were used to align two different protein sequences. In Clustal W the default settings were used except ITERATION to alignment and NUMERITER were set to 3.

RESULTS

In measuring the evoked EPSPs and spontaneous quantal events a 3rd instar larvae is filleted so that each segment is observed. By stimulating a selected segmental nerve, only one nerve root is recruited which is important since the muscle fibers are electrically coupled between segments. Various identified muscles with the rather simplistic innervation profiles are shown in **Figure 2-1A**. In these studies, I utilized muscle 6 (m6) because of the well characterized innervation as depicted in **Figure 2-1B** (Atwood et al., 1993; Kurdyak et al., 1994; Li et al., 2002). The synaptic properties of the Ib and Is motor nerve terminals have been previously described (Atwood et al., 1993; Kurdyak et al., 1994; Li et al., 2002). I obtained similar measures as earlier reported. I did ensure to observe all three sized EPSP events (Ib, Is, and Ib+Is) in order to subsequently monitor the compound Ib+Is EPSP. The resting membrane potential was also monitored and only preparations which did not appear damaged or with a resting membrane potential more negative than -50mV, in normal physiological saline, were used for assessing the effects of various pharmacological agents.

Application of a saline containing kainate (1mM) rapidly reduces the EPSP amplitude and the effect can be partially reversed by extensive exchanging the bathing media back to normal saline (**Figure 2-2A**). The extent in the reduction of the EPSP amplitude is dose-dependent, with an almost 50% reduction at 1mM (**Figure 2-2B**). All three concentrations produced a significant reduction in the EPSP amplitudes (n=6 for each concentration; p<0.05 Wilcoxon rank sum non-parametric). There is no significant effect on the resting membrane potential on m6 for any of the three concentrations tested (**Figure 2-2C**). However when considering sham control experiments of exchanging the bathing medium with saline and providing the same time period in which EPSP measure were made there is run down in the EPSP amplitude by 13.5% (**Figure 2-2D**). The individual values are shown next to the mean value. The sham procedures produced a significant effect in reducing the EPSP responses (n=6; p<0.05 Wilcoxon rank sum non-parametric). The mean value was not subtracted from experimental conditions with pharmacological agents in the remaining results, but this effect should be considered. Thus, exposure to 500µM and 1mM kainate produced a significant difference from sham control, whereas 100µM did not (p<0.05 Student's *t*-test).

In examining the effects of the AMPA and kainate receptor agonist, domoic acid (10 μ m and 1mM), the postsynaptic receptor sensitivity to spontaneous quantal responses and the frequency of spontaneous events were assessed. It was readily apparent that domoic acid at 1mM rapidly reduced the amplitude of spontaneous mEPSP quantal events (**Figure 2-3A**) as well as the frequency of occurrences (**Figure 2-3B**). Domoic acid at 10 μ M or 1 mM did not produce an effect on the resting membrane potential (**Figure 2-3C**). Glutamate at 10mM produced similar effects in reducing the mEPSP amplitude and frequency but this was compounded by the rapid depolarization that glutamate produced ($p < 0.05$; **Figure 2-3C**). The differences in the AMPA and kainate receptor agonist and glutamate are that, domoic acid did not significantly depolarize the muscle; however, the mEPSPs were still affected. The combination of domoic acid (1mM) and glutamate (10mM) produced a reduced depolarization of the muscle and as expected, a reduced mEPSP amplitude and frequency occurred (**Figure 2-3B, C**). These results suggest domoic acid is an antagonist to the postsynaptic glutamate receptors. The effect on the reduced frequency of the mEPSPs is due to the gradual reduction in the mEPSP amplitude, such that they are not discernable from noise in the baseline and thus are not detected to monitor their frequency.

Similar results were obtained for domoic acid as for kainate. The mEPSP amplitudes and the frequency are reduced (**Figure 2-4A, B**). Also the resting membrane potential is not significantly altered. In examining subtle effects on single quantal events the shape of the quantal events were examined and significant effects in rise time or decay were measured (data not shown). The amplitude and area (i.e., area under the curve) of the mEPSPs both decreased in a dose-dependent manner for 1mM and 500 μ M (**Figure 2-4C**). With exposure to kainate, the smaller mEPSPs appeared to fade into the background noise while the previously larger mEPSPs events were now observed as smaller mEPSPs.

Since the postsynaptic glutamate receptors on the muscle appear to bind kainate, I expected the known agonist of kainate receptors, ATPA, to act similarly, despite knowing that the receptors are not a kainate subtype. ATPA at 1mM did not reduce the evoked EPSPs (**Figure 2-5A, B**) nor did ATPA have any effect on the resting membrane potential (**Figure 2-5C**). In fact, some preparations increased in the EPSP amplitude while others decreased, thus producing no net significant effect.

If there are subtle effects of kainate in depressing presynaptic vesicular docking or altering $[Ca^{2+}]_i$ dynamics, one would expect that facilitation or the short-term depression (i.e., negative facilitation) index might detect such an effect. A short four pulse train of 20Hz produces the characteristic response known for this preparation, as shown in **Figure 2-6A**. The initial EPSP is normally used as a relative reference to the 4th EPSP in the train. It has been previously demonstrated that 1mM $[Ca^{2+}]_o$ will produce a marked depression in the response train where as 500 μ M $[Ca^{2+}]_o$ produces less depression and sometimes a slight facilitation. Thus, both 1mM and 500 μ M $[Ca^{2+}]_o$ were examined with 1mM and 500 μ M kainate to unmask any ceiling effect in the depression by 1mM $[Ca^{2+}]_o$ exposure. The 1st and 4th EPSPs rapidly attenuate in amplitude upon exposure to 1mM kainate and 0.5mM $[Ca^{2+}]_o$ (**Figure 2-6B**). The EPSPs also

show a large reduction to kainate when bathed with 1mM $[Ca^{2+}]_o$ (**Figure 2-6C**). However, no significant differences, by parametric analysis (Student's *t*-test), in the facilitation index are observed among the four conditions for exposure to kainate as determined by the difference in the median FI values. The difference is measured from saline to exposure of kainate within each preparation (n=5; 500 μ M kainate-1mM $[Ca^{2+}]_o$; 1mM kainate-1mM $[Ca^{2+}]_o$; 500 μ M kainate-500 μ M $[Ca^{2+}]_o$; 1mM kainate-500 μ M $[Ca^{2+}]_o$; **Figure 2-6D**). If the effect to exposure of kainate (1mM) with 1mM $[Ca^{2+}]_o$ is analyzed by non-parametric statistics, then five out five preparations showed an increase in the mean FI ($p < 0.05$, Wilcoxon Rank Sum). However, two preparations showed only small increases of 10 and 12 %. Thus, I do not feel confident in stating that there is a significant increase in the mean FI with kainate by non-parametric analysis. On the other hand, box-whisker plots of the FI values before and after exposure to kainate, for each preparation, in the various paradigms revealed a trend again related to the same data set of kainate (1mM) with 1mM $[Ca^{2+}]_o$ (**Figure 2-7**). The box-whisker plots represent the median, 25th, 75th, and range. One can observe that the variability in the distributions is larger for the kainate (1mM) with 1mM $[Ca^{2+}]_o$ as compared to the same preparations before the exposure. In the other paradigms the variability is about the same for saline and kainate exposure. To further examine this variability in FI, the standard deviation for each preparation before and after exposure to kainate was examined. Only the kainate (1mM) with 1mM $[Ca^{2+}]_o$ showed a consistent trend (five out of five; $p < 0.05$, Wilcoxon Rank Sum Test; also by Student's *t*-test $p < 0.05$) with an increase in the variability while exposed to kainate as compared to the saline baseline. The changes in the individual preparations for this paradigm are shown in **Figure 2-8**.

In order to further investigate possible presynaptic, metabotropic glutamate receptor action on the motor nerve terminals, the common metabotropic glutamate receptor agonist trans-1-aminocyclopentane-1, 3-dicarboxylic acid (t-ACPD) was used. Potentially, an increase or a decrease in the evoked EPSP would be possible at this insect NMJ since at the crayfish NMJ (also an arthropod species) an effect was reported (Schramm and Dudel, 1997). However, no significant alterations on evoked EPSPs amplitudes (**Figure 2-9A, B**) or on the resting membrane potential of the muscle occurred during exposure to t-ACPD (500 μ M) at the *Drosophila* NMJ. In comparison to the sham saline control (**Figure 2-2D**), the distributions in EPSP amplitudes over lap; thus, the slight reduction in EPSP amplitudes with t-ACPD are not significant.

Discussion

In this study I showed, in a concentration-dependant manner, that kainate rapidly reduced the amplitude of evoked EPSPs as well as the spontaneous quantal events. The lack in any response of t-ACPD and ATPA as well as a lack in these compounds mimicking kainate's action suggest that there is not a presynaptic mechanism of a kainate-like or metabotropic autoreceptors on these motor nerve terminals. However, the kainate receptor agonist, domoic acid, did follow the actions of kainate in reducing the evoked EPSPs. I suggest this action

is due to domoic acid and kainate acting as an antagonist on the postsynaptic quisqualate receptors present on muscle and that no presynaptic contribution can be proposed for the rapid reduced amplitude in the evoked EPSP and quantal responses. The reduced frequency in the spontaneous quantal responses in the presence of kainate or domoic acid appears to be due to the responses decreasing in size to a point that they are not detectable from the noise or that the postsynaptic receptors are fully blocked.

The responses observed at the crayfish NMJ by Schramm and Dudel (1997) for analogous types of NMJs as I used in the *Drosophila* larval NMJ (Atwood and Cooper, 1995, 1996) must be different in regards to presynaptic autoreceptors. In their study, t-ACPD and glutamate itself produced varied responses in mean quantal content. Some preparations they reported showed an enhancement while others a decrease in evoked postsynaptic events. They concluded that there may be differential regulation in the type and density of the metabotropic presynaptic autoreceptors to account for variation among preparations and effects to t-ACPD and glutamate. There are methodological differences in stimulating the nerve terminals. In my studies, I elicited action potentials within the nerve to result in Ca^{2+} influx and vesicular fusion. However, Schramm and Dudel used depolarization pulses delivered over the terminal, through the focal electrode, to directly open voltage-gated channels. Seal stability with the focal electrode over the NMJ, longer than normal action potential depolarization pulses and tweaking the current pulses to a fine degree in order to balance a percentage of activating voltage dependent ion channels, would appear to induce potentially more experimental variability among preparations than inducing a standard action potential within the nerve for evoking synaptic transmission. The large field effect of a current pulse, to induce presynaptic depolarization, through a macropatch electrode could have an effect on the postsynaptic glutamate receptors such that the receptor interaction with pharmacological agents or even glutamate itself might be altered. To resolve these issues in the crayfish would require selectively stimulating the motor nerve and repeating the study.

As for domoic acid, the results suggest kainate is an antagonist to the postsynaptic glutamate receptors in a dose-dependent manner. It appears kainate can behave as an antagonist but not ATPA for these postsynaptic muscle receptors. In addition, since ATPA did not produce any significant alteration in the evoked EPSP amplitude, there does not appear to be any pre- or post-synaptic action of ATPA. Quisqualate has been suggested to activate ionotropic as well as metabotropic glutamate receptors just as glutamate does itself, which suggests quisqualate is a non-selective glutamate receptor agonist (Schoepp et al., 1990). However, AMPA, which is significantly similar to quisqualate in structure and is a weak kainate receptor agonist, cannot activate nor even bind to homomeric GluR6 kainate receptor (Egebjerg et al., 1991). Such differences may be induced by steric hindrance near the ligand binding domain. In fact, AMPA did not change the EPSP amplitude in my studies, but kainate resulted in reduced amplitude by acting as an antagonist. Therefore, there seems to be other factors besides steric hindrance. It would seem likely that a ligand binding site of kainate

at the *Drosophila* NMJ would be highly conserved to the vertebrate kainate receptor, since kainate is blocking evoked responses; however, the similarity of extracellular domains are not very high. Recent studies suggested that the properties of ionotropic glutamate receptor subunits can be changed by mutating the amino acid at the second transmembrane segment which forms the lining of the pore region (Bochet et al., 1991; Curutchet et al., 1992; Verdoorn et al., 1991). For the functional muscle-expressed glutamate receptor GluR-IIC, GluR-IID, GluR-IIE and either GluR-IIA or GluR-IIB is essential for function. Therefore, only based on the ligand binding domain tetrameric structure was suggested. The correct subunit stoichiometry is not known. The receptor can be pentameric or higher such as (IIA)(IIB)(IIC)(IID)(IIE) or (IIA)(IIA)(IIC)(IID)(IIE) to be functional. Also, glutamate receptor complexes which contain either GluR-IIA or GluR-IIB have opposite physiological properties by showing slow and fast desensitization, respectively (DiAntonio et al., 1999). Thus, as constituted receptor subunits are put together it is likely that the receptor is functionally changed. There could also be mixed subunit combinations along the nerve terminal among the many synaptic sites, since the terminal is developmental different in age along its length (Atwood and Cooper, 1995; He et al., 2009; Li et al., 2002) and function (Bogdanik et al., 2004; Guerrero et al., 2005; Marrus et al., 2004; Liebl and Featherstone, 2005;). Developmental alteration in kainate receptors are known in vertebrates (Lee et al., 2001). Thus, variability in whole muscle responses to pharmacological agents might be masked, but if older and younger regions of the terminals were monitored with focal macropatch electrodes larger differences in the pharmacological profiles and sensitivities might be observed.

It was proposed that the five subunits consisting of postsynaptic receptors in *Drosophila* NMJ are somewhat similar in homology to kainate receptors in vertebrates and thus, they were termed kainate-like (Betz et al., 1993; Guerrero et al., 2005; Marrus et al., 2004; Qin et al., 2005; Völkner et al., 2000). In comparing the postsynaptic receptor molecular sequence of *Drosophila* NMJ with a kainate receptor in *Homo sapiens*, which contains the highest homology in the vertebrates, each subunit shows a moderate similarity between two species. Extracellular domains of the receptors are not significantly higher in similarity compared to total sequence (**Figure 2-10**). In a phylogeny tree with 36 glutamate receptor protein sequences in *Drosophila melanogaster* and human's NMDA, kainate and AMPA receptors, the five subunits of postsynaptic receptors in *Drosophila* NMJ appear to be in a different subgroup to even the closest of the vertebrate kainate/AMPA type receptors (**Figure 2-11**).

There are multiple examples of pre- and/or post-synaptic action of kainate in enhancing or suppressing synaptic transmission in vertebrate neuronal circuits (Grabauskas et al., 2007; Hegarty et al., 2007; Kamiya and Ozawa, 2000; Pinheiro and Mulle, 2008; Rozas et al., 2003; Schoepp, 2002); and it has been reported earlier that kainate blocked synaptic transmission at the *Drosophila* NMJ (Chang and Kidokoro, 1996; McLarnon and Quastel, 1988). However, the earlier studies with *Drosophila* did use Schneider's *Drosophila* medium which is known to have a depressing action on synaptic transmission itself (Ball et al., 2003; Stewart et al., 1994). Mechanistically I do not know how kainate is blocking

transmission, but it appears likely that kainate acts as an antagonist in this preparation. Possible kainate could act on the receptor to desensitize it to glutamate or in addition act as a non-competitive antagonist. Quisqualate receptors are known to be prone to desensitization at the crayfish NMJ (Tour et al., 2000) as well as other glutamate receptors (Sun et al., 2002).

The results with kainate at different concentrations and the two concentrations of bathing $[Ca^{2+}]_o$ is interesting since only the one condition of 1mM kainate and 1mM $[Ca^{2+}]_o$ produced a significant trend in both an increase in the median FI value as well variability in the responses as compared to baseline saline exposure (**Figures 2-7** and **2-8**). As stated earlier the increase in median values is small in some preparations so I am cautious in bearing too much emphasis on this result; however, the increase in variability when exposed to kainate is of interest. If kainate is acting as a competitive antagonist then it is feasible to speculate with each pulse in the stimulus train the glutamate is resulting in a greater displacement of the kainate and possibly resulting in more competitive-competition. As the glutamate is being taken back up in the nerve terminal by the glutamate transporters, kainate's action can dominate producing very variable responses on the amplitudes of the EPSPs throughout the four pulse train. Considering when saline is exchanged for saline containing kainate, the 1st EPSP within the train is dampened significantly by the antagonistic action and during the evoked release of glutamate then the kainate is displaced would possible explain the slight, but consistent, increase in the median FI values as well for this experimental paradigm. This also follows for the concept that when the $[Ca^{2+}]_o$ is reduced to 0.5mM, the evoked amount of glutamate is not as pronounced so less displacement of the kainate would occur, which might explain why there is not as change on FI or on the median as well as the variability in the FI (**Figure 2-7**). I am not aware of reports addressing pharmacological examination of competitive antagonists during pulse trains to account for fluctuation in facilitation measures to compare my findings. More studies are warranted to examine this tentative working model in competitive antagonism during synaptic facilitation/depression to explain the mechanistic details behind these findings.

Since I have not been able to detect any presynaptic autoreceptor function at these NMJs, it would be interesting to know if these motor neurons produce mRNA for any glutamate receptor subtype. There has yet to be investigation of possible presynaptic actions at other NMJs in the larvae or adult of *Drosophila*. The subset of junctions that I examined may not be indicative of all the *Drosophila* NMJs, so further investigations are warranted.

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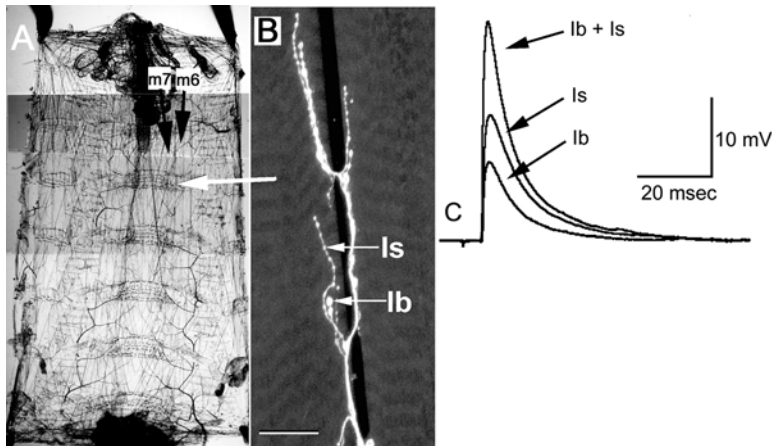


Figure 2-1: The dissected 3rd instar *Drosophila* larva preparation. **(A)** The preparation is pinned at the four corners to keep the preparation taut. The ventral abdominal muscle m6 is used in this study. The segmental nerves are stimulated by placing the nerve into a suction electrode and recruiting the Is and Ib motor neurons. **(B)** The terminals of Ib and Is on m6 and m7 are readily observed after treatment with fluorescently tagged anti-HRP antibody (Scale: 50 μ m). **(C)** Elicited excitatory postsynaptic potentials (EPSPs) in m6 are measured with an intracellular recording. Representative individual responses from the Ib and Is motor axons as well as the composite Ib and Is response are shown for a early 3rd instar.

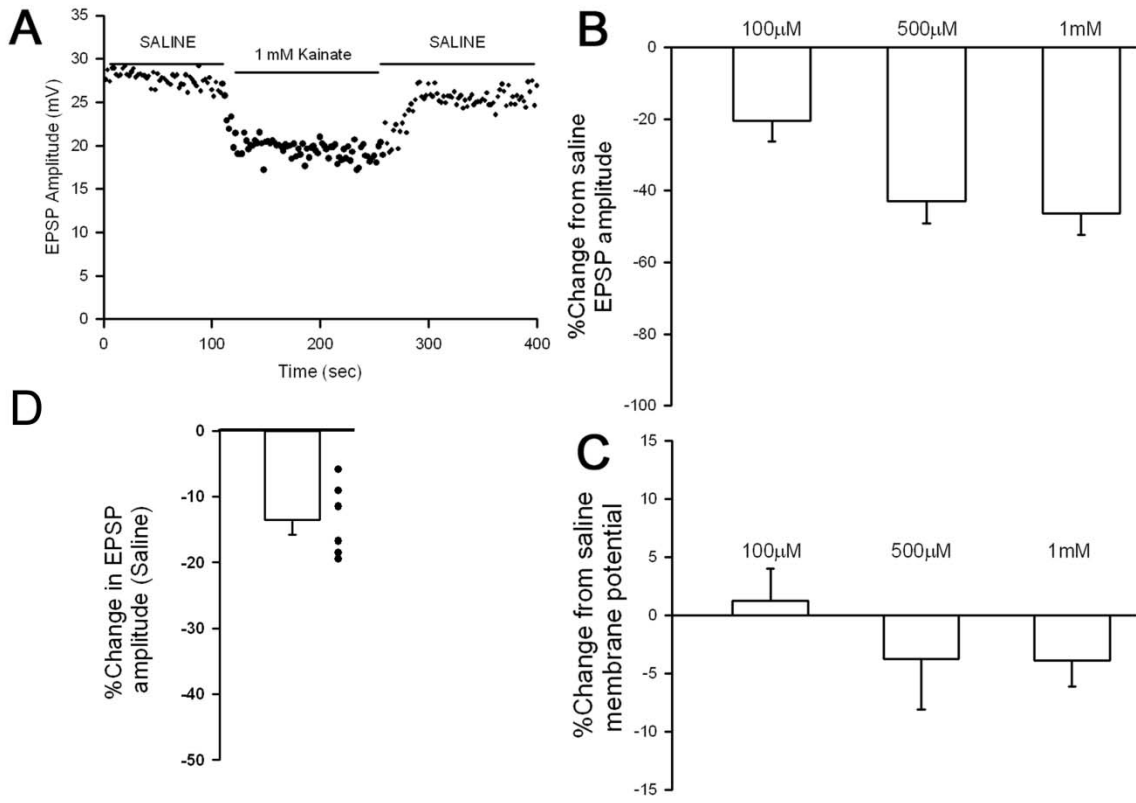


Figure 2-2: The effect of kainate on the evoked EPSP amplitudes. **(A)** Application of 1mM kainate reduces the EPSP rapidly and the effect can be partially washed out. **(B)** The dose-response effect in damping the EPSP amplitude. For all concentrations of kainate the EPSP amplitudes are reduced. **(C)** The dose-response effects on the resting membrane potential for m6. The resting membrane potentials do not appear to be changed. With the maintaining resting membrane potential, reduced EPSP amplitudes are induced by the antagonistic action of kainate. **(D)** Sham control for exchanging the saline bath and time revealed a 13.5% reduction in the EPSP amplitude. When compared to sham control, both 500μM and 1mM still show significantly reduced EPSPs (around 46% decrease)

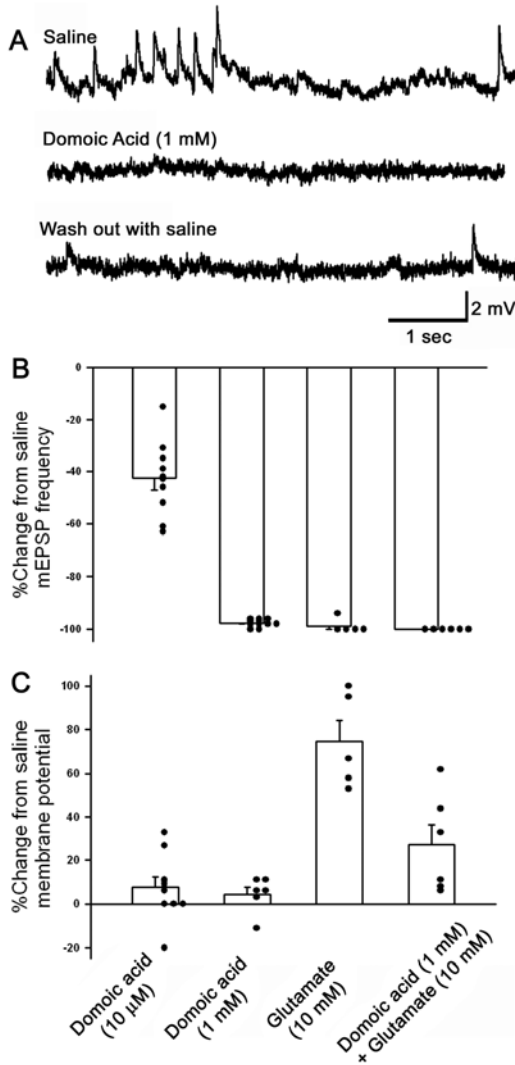


Figure 2-3: Effects of domoic acid (agonist for AMPA and kainate receptors) at the NMJ. Domoic acid is a potent kainate receptor agonist. **(A)** Spontaneous or mEPSPs (miniature EPSPs) are reduced in amplitude upon exposure to 1mM of domoic acid. With washing out the domoic acid, the spontaneous responses gradually come up again. **(B)** The frequency of mEPSPs is reduced by domoic acid in a dose-dependent manner. Glutamate mimics the effect. **(C)** Domoic acid did not produce a consistent effect on the resting membrane potential; however glutamate produced a drastic depolarization fitting for agonistic action. Domoic acid attenuates the effect of glutamate on membrane depolarization. So, domoic acid appears to be blocking the glutamate action on the muscle.

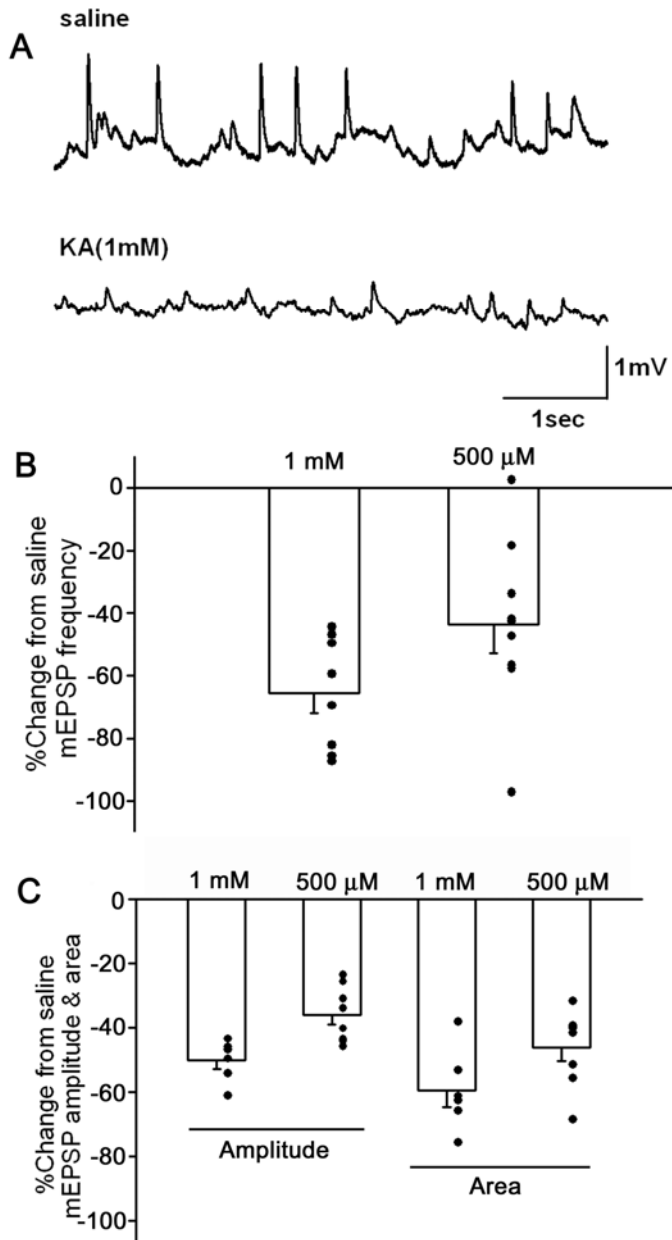


Figure 2-4: Effects of kainate (1 and 0.5 mM) on mEPSPs properties. **(A)** Spontaneous or mEPSPs (miniature EPSPs) are reduced in amplitude upon exposure. **(B)** The frequency of mEPSPs is reduced by domoic acid in a dose-dependent manner, as well as the **(C)** amplitude and area of the mEPSPs. Kainate seems to be acting presynaptically as well as postsynaptically. However, the problem of this measuring is that the mEPSPs can be buried within the noise as the amplitude of mEPSPs are reduced.

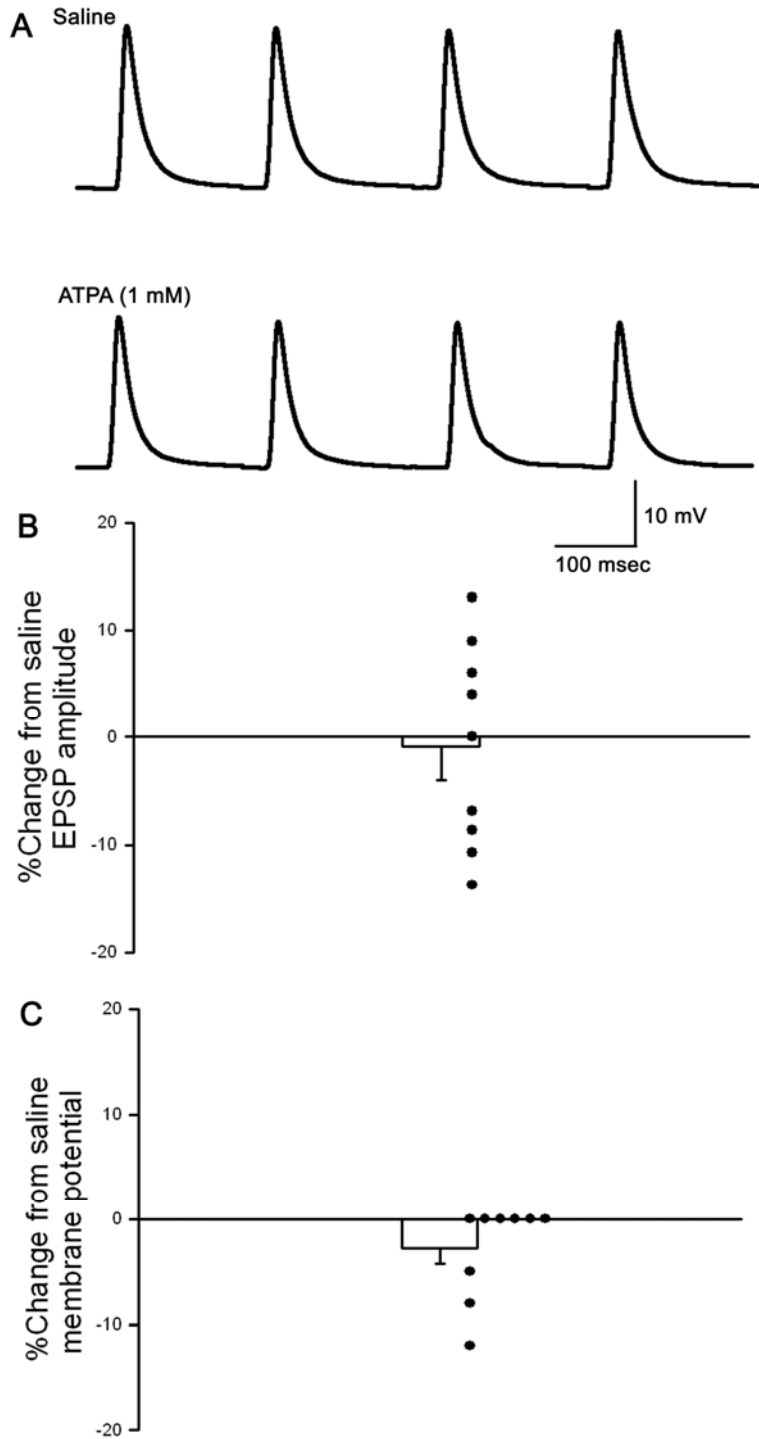


Figure 2-5: Effects of ATPA (1 mM; a selective agonist of vertebrate GluR5, kainate receptors) on the amplitude of evoked EPSPs (**A**), (**B**) as well as the resting membrane potential of the muscle (**C**). ATPA does not appear to alter the EPSP amplitude or the resting membrane potential.

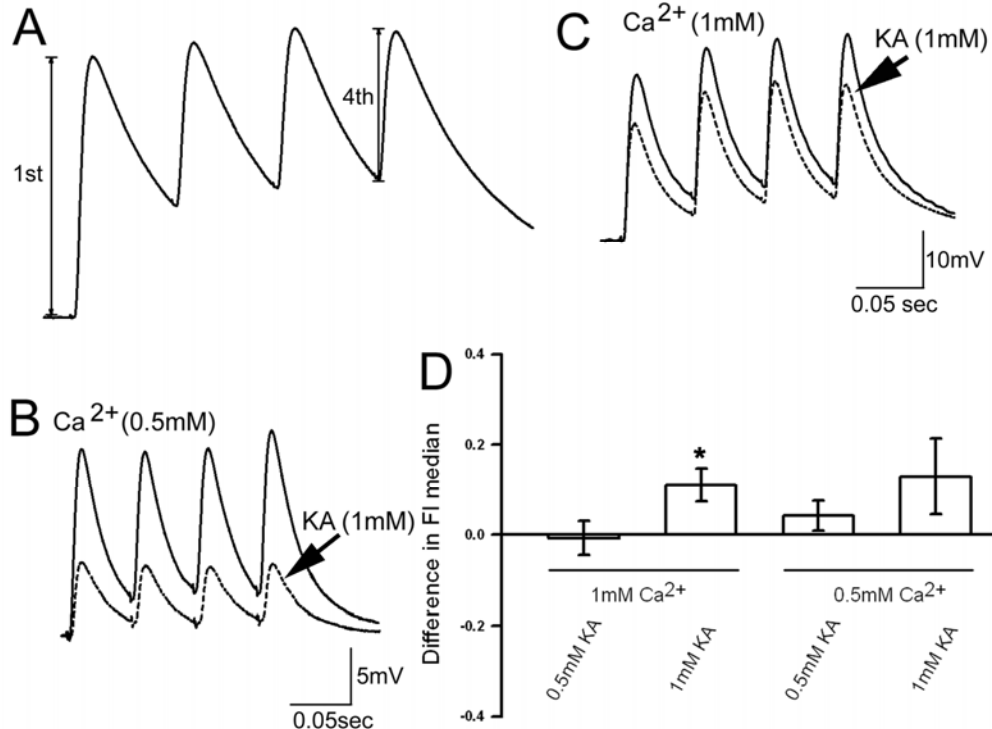


Figure 2-6: Effects of kainate on short-term depression (i.e., negative facilitation) with varied $[Ca^{2+}]_o$. (A) A four pulse stimulus train delivered at 20Hz results in an initial large EPSP followed by depressed responses. The procedure for measuring EPSP amplitudes is indicated. Facilitation index (FI) is then calculated from these measures. (B) A typical response for the effect of kainate (KA; 1 mM) on the pulse train in the presence of 0.5 mM $[Ca^{2+}]_o$. (C) A typical response for the effect of KA (1 mM) on the pulse train in the presence of 1 mM $[Ca^{2+}]_o$. (D) The difference in facilitation index (FI) is shown for both 1mM and 0.5mM KA with either 1mM or 0.5mM $[Ca^{2+}]_o$. The differences in the median values are obtained from the difference in FI saline to exposure of KA within each preparation. (n=5 for each paradigm; * $p < 0.05$ Wilcoxon Rank Sum Test for the effect of KA as compared to saline).

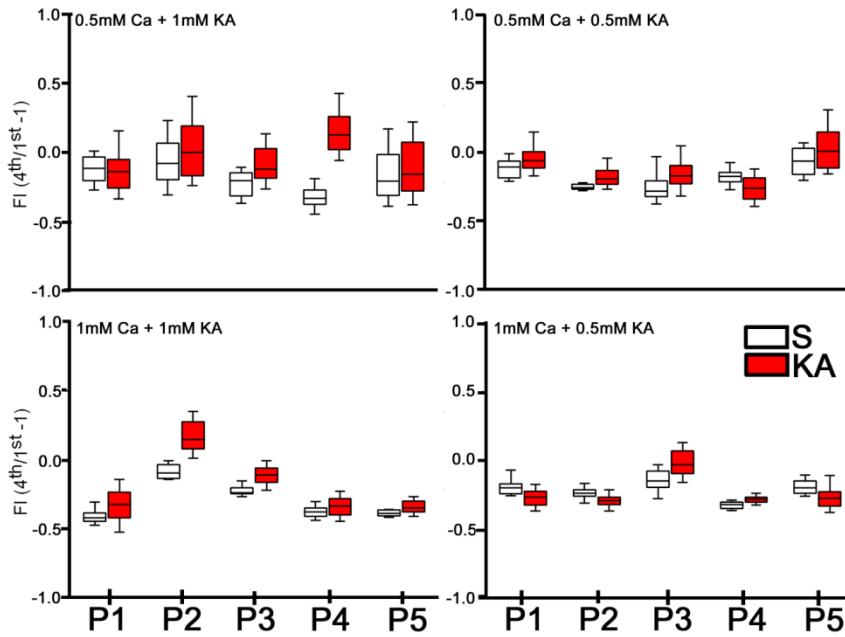


Figure 2-7: Effects of kainate (KA) and calcium on FI. Box and whisker plots for FI in various conditions are shown as before and after exposure to kainate, for each of the five preparations in the four experimental conditions. The plots show the median, 25th, 75th percentiles and range. In almost all preparations, median values of FI index seem to be the same before and after exposure to kainate. So, kainate does not appear to have a presynaptic action. With 1mM Ca²⁺ and 1mM kainate, both median and variation of FI index are increased in five out of five preparations after exposure to kainate. However, after changing the [kainate] into 0.5mM, neither median nor variation of facilitation index shows a consistent increase in values. So, kainate seems to be acting as a competitive antagonist.

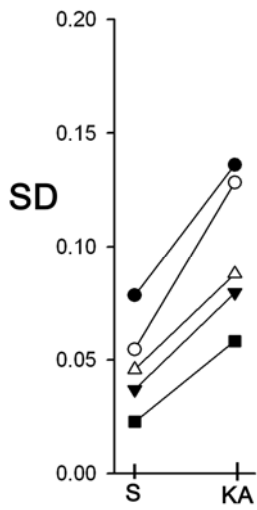


Figure 2-8: The standard deviation (SD) for each preparation before and during exposure to kainate (1mM) with 1mM $[Ca^{2+}]_o$ produced a consistent trend in increasing in the variability among the data sets while exposed to kainate (KA) as compared the saline (S) baseline ($p < 0.05$, Wilcoxon Rank Sum Test; also $p < 0.5$ Student's t -test).

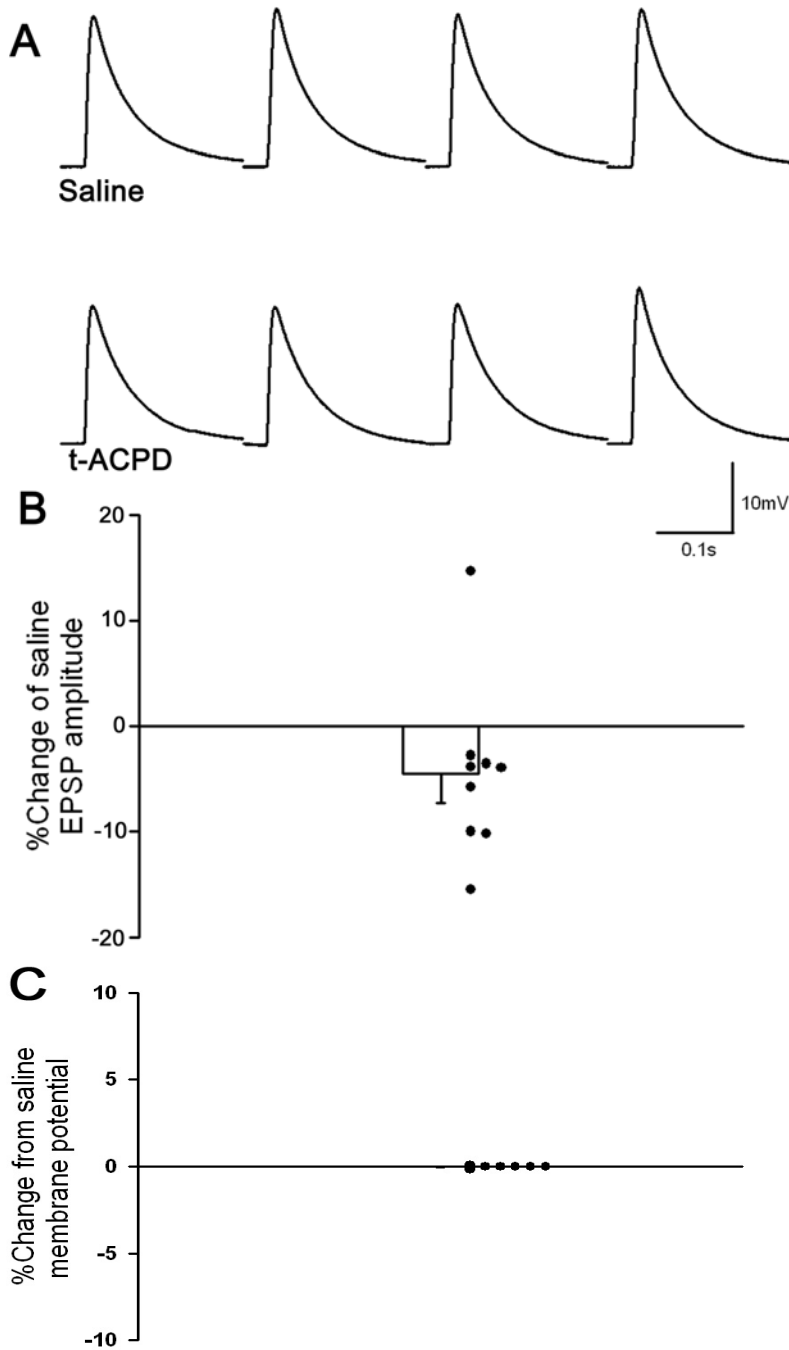


Figure 2-9: Action of t-ACPD on evoked EPSPs and resting membrane potential. **(A)** Evoked EPSPs in saline and during exposures to t-ACPD (50 μ M). **(B)** The mean percent change in the amplitude of the evoked EPSPs with exposure to t-ACPD. **(C)** The mean percent change in the resting membrane potential with exposure to t-ACPD. t-ACPD does not appear to have an action on the postsynaptic muscle or the glutamate receptors.

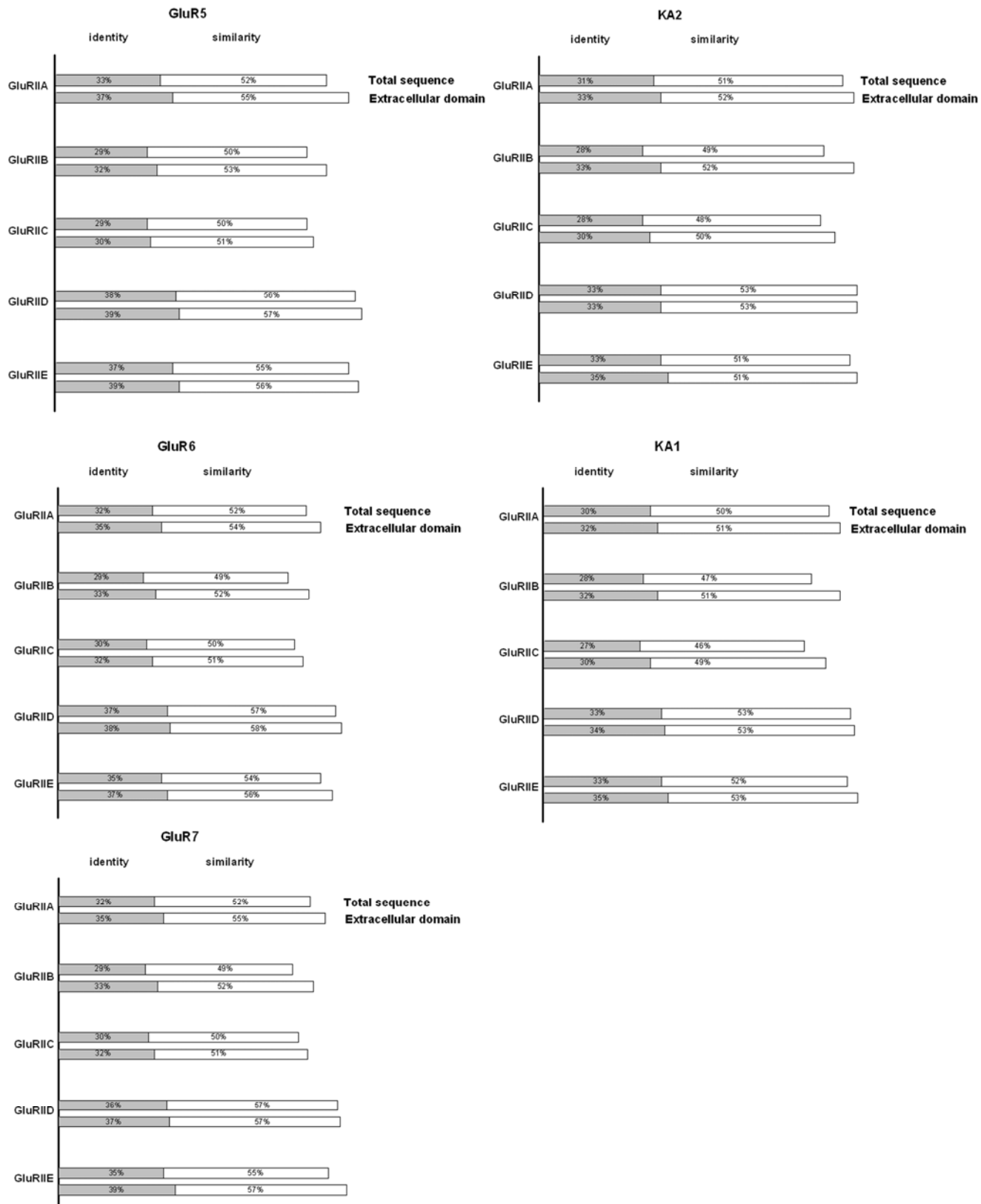


Figure 2-10: Sequence comparison of five postsynaptic receptor subunits identified from *Drosophila* muscle mRNA to the five known human kainate receptor subunits. Each two sequences were compared by using a blastP alignment. The top bar is a comparison for the total protein sequence. The bottom bar is a comparison of the putative extracellular domains among the proteins. The gray area is the percentage of identical sequence and the white are the percent in similarity based on the blastP (NCBI) algorithm. The five human receptor subunits used are: (A) GluR5; (B) GluR6; (C) GluR7; (D) KA1; and (E) KA2.

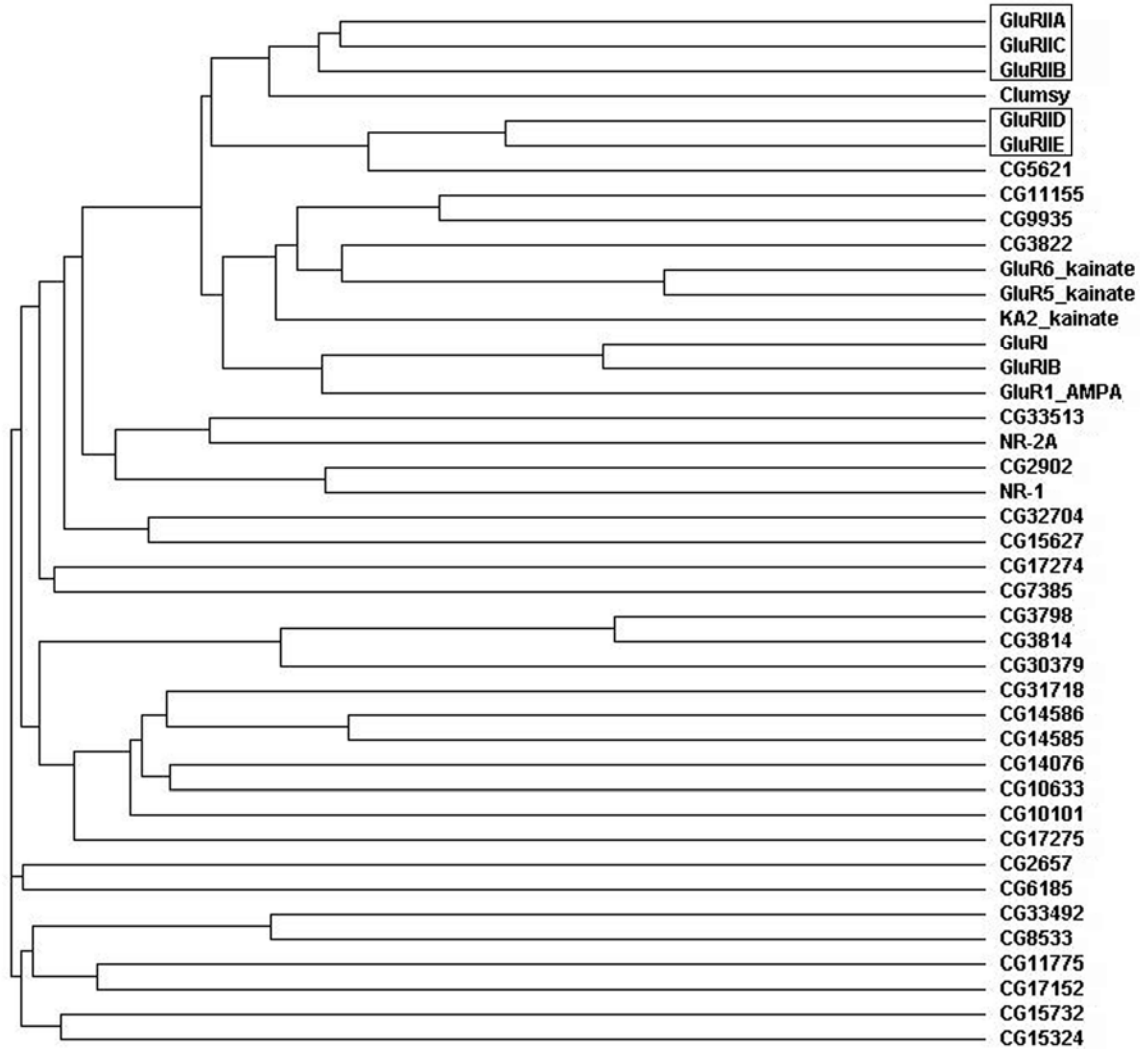


Figure 2-11: Phylogeny tree comparing all known putative 36 ionotropic glutamate receptor subunits in the *Drosophila* genome with human glutamate receptors (kainate receptor subunits GluR5, GluR6 and KA2; AMPA receptor subunit GluR1; NMDA receptor subunits NR-1 and NR-2). The comparison was performed by ClustalW2 (EMBL-EBI).

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Abstracts

1. Lee, J.Y., Chung, W.Y., Logsdon, S., Johnstone, A. F. M., Cooper, R.L. (2008) The regulation of synaptic vesicle pools within motor nerve

terminals. Society for Integrative and Comparative Biology. Annual meeting. January 2-6, 2008, San Antonio, TX.

2. Lee, J.Y., Chung, W.Y., Logsdon, S., Johnstone, A. F. M., Cooper, R.L. (2008) The regulation of synaptic vesicle pools within motor nerve terminals. Annual meeting of the BlueGrass Chapter of the Society for Neuroscience. March 12, 2008. Univ. of KY.

3. Lee, J.-Y., Bhatt, D., Bhatt, D., Chung, W.-Y., Lee, N.-T. and Cooper, R.L. (2008) Pre- & Post-synaptic Actions of Kainate: Negative Feedback at Glutamate-ergic Nerve Terminals. Annual meeting of Society for Neuroscience. Washington, DC.

4. Lee, J.-Y., Bhatt, D., Bhatt, D., Chung, W.-Y., Lee, N.-T. and Cooper, R.L. (2008) Pre- & Post-synaptic Actions of Kainate: Negative Feedback at Glutamatergic Nerve Terminals. Kentucky Academy of Sciences. Annual meeting. November 1, 2008, Lexington, KY (Univ. of KY campus).

5. Lee, J.-Y., Bhatt, D., Bhatt, D., Chung, W.-Y., Lee, N.-T. and Cooper, R.L. (2009) Pre- & Post-synaptic Actions of Kainate: Negative Feedback at Glutamate-ergic Nerve Terminals. Society for Integrative and Comparative Biology. Annual meeting. January 3-7, 2008, Boston, MA.

Outreaching

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1. 2008. Judged at Central KY Regional Science and Engineering Fair.

Held at the Univ of KY

2. 2008 & 2009 Judged at Glendover elementary school.

3. 2008 & 2009 Judged at Morton middle school.