# Hodgkin-Huxley-Katz Prize Lecture: Genetic and Pharmacological Control of Glutamate Receptor Channel through a Highly Conserved Gating Motif

Riley E. Perszyk[1], Scott J. Myers[1,2], Hongjie Yuan[1,2], Alasdair J. Gibb[3], Hiro Furukawa[4], Alexander I. Sobolevsky[5], Stephen F. Traynelis[1,2]

[1] Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA 30322, USA

[2] Center for Functional Evaluation of Rare Variants (CFERV), Emory University School of Medicine, Atlanta, GA 30322, USA

[3] Department of Neuroscience, Physiology & Pharmacology, University College London, London, UK

[4] WM Keck Structural Biology Laboratory, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, NY 11724, USA

[5] Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA

Corresponding Author:

Dr. Stephen F. Traynelis

Department of Pharmacology and Chemical Biology

Emory University School of Medicine

Rollins Research Center

1510 Clifton Road

Atlanta, GA 30322-3090, USA

Phone: 1-404-727-0357

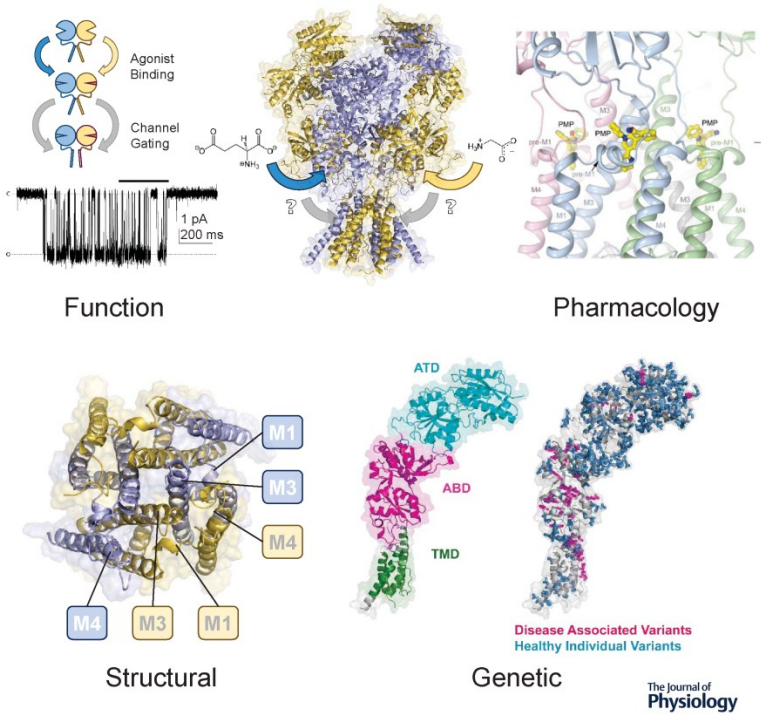
E-mail: strayne@emory.edu

This is an Accepted Article that has been peer-reviewed and approved for publication in The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP278086.

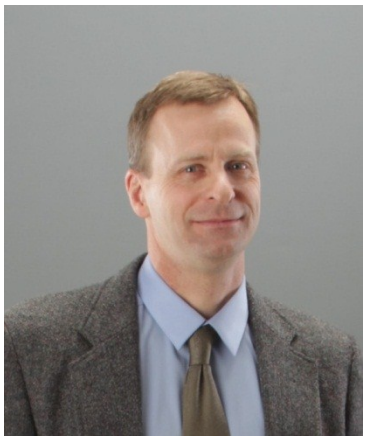
**Abstract**

Glutamate receptors are essential ligand-gated ion channels in the central nervous system that mediate excitatory synaptic transmission in response to the release of glutamate from presynaptic terminals. The structural and biophysical basis underlying the function of these receptors has been studied for decades by a wide range of approaches. However recent structural, pharmacological, and genetic studies have provided new insight into the regions of this protein that are critical determinants of receptor function. Lack of variation in specific areas of the protein amino acid sequences in the human population has defined three regions in each receptor subunit that are under selective pressure, which has focused research efforts and driven new hypotheses. In addition, these three closely positioned elements reside near a cavity that is shown by multiple studies to be a likely site of action for allosteric modulators, one of which is currently in use as an FDA-approved anticonvulsant. These structural elements are capable of controlling gating of the pore, and appear to permit some modulators bound within the cavity to also alter permeation properties. This creates a new precedent whereby features of the channel pore can be modulated by exogenous drugs that bind outside the pore. The convergence of structural, genetic, biophysical, and pharmacological approaches is a powerful means to gain insight into the complex biological processes defined by neurotransmitter receptor function.

The understand of ionotropic glutamate receptors is accelerated by using diverse experiments methods and data.



**Riley E. Perszyk** obtained a BS of Mechanical Engineering from Georgia Tech (Highest Honors) and PhD in 2018 from Emory University. He studies the molecular action of NMDA receptor allosteric modulators and their utility in neuronal tissue. He is currently developing engineering solutions to automate traditional electrophysiology experiments in a collaborative postdoctoral fellowship between Georgia Tech and Emory University.



**Dr. Stephen Traynelis** is a Professor of Pharmacology and Chemical Biology at Emory University. He received a BS from West Virginia University in Chemistry (1984, summa cum laude) and a Ph.D. in Pharmacology from the University of North Carolina (1988). He completed postdoctoral fellowships at University College London and the Salk Institute. Dr. Traynelis is an AAAS Fellow, former editor-in-chief of Molecular Pharmacology, recipient of a NIH Javits Award and R35 Research Program Award, director of the Center for Functional Evaluation of Rare Variants, and a co-founder of multiple startup companies.

## Introduction

Glutamate receptors are ligand-gated ion channels that mediate excitatory synaptic transmission throughout the brain and spinal cord, as well as serving other roles in development (Traynelis *et al.*, 2010; Paoletti *et al.*, 2013). This receptor family can be subdivided on the basis of genetic, structural, and pharmacological properties into at least three subfamilies; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and N-methyl-D-aspartate (NMDA). Additional families exist in non-human animals and plants (Gangwar *et al.*, 2019; Alfieri *et al.*, 2020; Mayer, 2020). All human subfamilies serve unique and essential roles in neuronal circuit function. The members of this receptor family are multimeric protein complexes with modular architecture (Figure 1a), composed of four subunits, each comprising four domains connected to each other by linker segments that lack secondary structural constraints. The four subunits each contribute a series of transmembrane elements that assemble together to form a cation-selective pore, which resembles an inverted potassium channel (Hansen *et al.*, 2017). Each subunit also contains two extracellular domains that adopt the shape of a bi-lobed clamshell. One domain (agonist binding domain, ABD, in Figure 1a) binds the co-agonists glutamate (GluN2) and glycine or D-serine (GluN1) within NMDA receptors, whereas in AMPA and kainate receptors, glutamate is bound to the ABD in all four subunits. These bilobed ABDs dimerize in all glutamate receptors, at least in some of their gating states (Naur *et al.*, 2007; Sobolevsky *et al.*, 2009; Karakas & Furukawa, 2014; Meyerson *et al.*, 2016; Burada *et al.*, 2020). The other clamshell domain encoded by the residues comprising the amino terminus resides distal to the membrane, and forms an assembly and modulatory control element of the receptor, as well as an important site of drug action (Figure 1a) (Ayalon & Stern-Bach, 2001; Gielen *et al.*, 2009; Yuan *et al.*, 2009; Karakas *et al.*, 2011; Watson *et al.*, 2017; Esmenjaud *et al.*, 2019; Regan *et al.*, 2019). The relative positioning of these two bilobed domains within the complex varies across the receptor family, which drives unique structural features of subfamilies as well as unique functional attributes. The multiple domains within each subunit and the multimeric nature of the tetrameric assembly of subunits create numerous protein-protein interfaces that can harbor modulator binding sites, including the region between the ABD and transmembrane domain (Figure 1b).

**Recent structural data illuminate key features in channel gating**

Structural advances in the last decade have provided dozens of crystallographic and cryo-EM structures for NMDA, AMPA, kainate and delta receptors (Greger *et al.*, 2017; Mayer, 2017; Zhu & Gouaux, 2017; Twomey & Sobolevsky, 2018; Chen & Gouaux, 2019; Greger & Mayer, 2019; MacLean *et al.*, 2019; Twomey *et al.*, 2019; Wang & Furukawa, 2019; Burada *et al.*, 2020), which also became templates for molecular modelling (Mollerud *et al.*, 2017; Paramo *et al.*, 2017). These data have ushered in a new era that has allowed close examination of the link between structure and function of these large, multimeric proteins. Evaluation of the structure-function relationship in light of structural data has led to the appreciation of elements critical for function that interact with the pore lining M3 helix (including the highly-conserved SYTANLAAF) and the agonist binding domain. While the resolution of the structural data for the extracellular domains has allowed detailed atomic characteristics to be elucidated (Karakas & Furukawa, 2014; Lee *et al.*, 2014; Tajima *et al.*, 2016; Twomey & Sobolevsky, 2018), the resolution for the linkers connecting the extracellular domains to each other and to transmembrane domains has been typically lower. For the majority of published structures, the lack of clear density for these linkers prevented modeling of amino acid side chains or entire sections of the polypeptide (Tajima *et al.*, 2016). However, pulling together information across a wide range of structural studies highlights a key element that is present in all glutamate receptors: a short two-turn helix (pre-M1) that lies parallel to the membrane and is in van der Waals contact with the pore-lining M3 helix from the same subunit (Figure 1b,d,e). Residues comprising this short helical element are conserved across species (Sobolevsky *et al.*, 2009; Karakas & Furukawa, 2014; Lee *et al.*, 2014). In addition, another short flexible linker connects the membrane-proximal lobe of the agonist binding domain to the M4 transmembrane helix, and multiple studies suggest both the pre-M4 linker and M4 transmembrane helix are important for function (Ren *et al.*, 2003; Yuan *et al.*, 2014; Amin *et al.*, 2017; Platzer *et al.*, 2017). The pre-M4 linker is closely positioned to the pre-M1 and M3 helices from the adjacent subunit (e.g. (Sobolevsky *et al.*, 2009; Talukder *et al.*, 2010)), and these three elements have been implicated in glutamate receptor gating and correspondingly proposed to comprise a gating

triad, shown in Figure 2a,b (Alsaloum *et al.*, 2016; Chen *et al.*, 2017; Yelshanskaya *et al.*, 2017; Gibb *et al.*, 2018; Amin *et al.*, 2020); see also (Sobolevsky *et al.*, 2009; Twomey & Sobolevsky, 2018).

Largely coincident with the availability of this structural data, multiple studies have provided a clear picture for gating in NMDA and AMPA receptors, which, given their structural similarities, operate by surprisingly distinct mechanisms (Banke & Traynelis, 2003; Popescu & Auerbach, 2003; Popescu *et al.*, 2004; Erreger *et al.*, 2005a; Erreger *et al.*, 2005b; Poon *et al.*, 2010; Kristensen *et al.*, 2011; Poon *et al.*, 2011; Gibb *et al.*, 2018). Single channel analysis and kinetic modelling indicate that following binding of the agonists, the receptors clearly undergo at least two additional rate-limiting conformational changes that precede explosive opening of the pore (Figure 2c), which yields step changes in current as the channel opens and closes (Iacobucci & Popescu, 2018). This is supported by structural data of AMPA receptors, one of such intermediate conformations is likely represented by the structures with partially closed agonist-bound LBDs and non-conducting closed channel pore (Durr *et al.*, 2014; Yelshanskaya *et al.*, 2014) and named a pre-active state in the framework of a simplistic gating model (Twomey & Sobolevsky, 2018). Evidence from NMDAR receptors where the ABDs have been cross-linked shut produces channels that still open and close with millisecond time scale closed times suggesting that pre-gating steps are being traversed independent of opening and closing of the agonist binding domain (Kussius & Popescu, 2010). Moreover, multiple studies that perturb the flexible linkers between the bilobed clamshell and transmembrane domains, in particular the linkers involved in the gating triad, reveal they can significantly impact channel function and the brief closed times that appear to represent pre-gating conformation changes (Talukder & Wollmuth, 2011; Ladislav *et al.*, 2018). Linker region cysteine mutants possess differing cross-linker modification rates, and the effects of these mutations in the absence and presence of agonist or antagonist implies activation-dependent movement (Beck *et al.*, 1999; Sobolevsky *et al.*, 2002; Talukder *et al.*, 2010; Salussolia *et al.*, 2011). Furthermore, smFRET experiments show that fluorescently labeled linkers (GluN2A Pre-M1) transition between multiple energy states in the apo- and agonist-bound states (Dolino *et al.*, 2017). All of these studies raise the possibility that rearrangement of linker elements could contribute to the two kinetically distinct pregating steps (Figure 2C).

Channel shut times could reflect the time needed for random movement of multiple side chains or the polypeptide backbone into a position (i.e. a state) that can decrease the energy for channel opening. These conformational changes may involve repositioning of pre-M1 or pre-M4 residues, including an aromatic residue that could perturb a network of aromatic interactions within the transmembrane domains (Chen *et al.*, 2017; Gibb *et al.*, 2018; McDaniel *et al.*, 2020). Based on channel closed times, two structurally distinct changes occur with different energies, which may reflect the cooperative yet unique gating triads, or perhaps larger structural elements such as a gating ring (Tajima *et al.*, 2016). Regardless of the precise nature of the intra-protein motion that underlies these two rate-limiting steps, it is clear that advances in our understanding will come from the convergence of structural data with functional and mechanistic data.

**Genetic studies highlight critical residues involved in gating**

Technological advances have yielded dramatic cost reduction for DNA sequencing, which has enabled an unprecedented increase in the amount of genetic data available for both patient populations and healthy individuals (Heinzen *et al.*, 2015). Evaluation of whole exome sequencing information has led to the proposal that regional variation in the number of *de novo* and inherited variants can provide insight to identify intolerant domains (Figure 3a) (Traynelis *et al.*, 2017). For example, for the GluN2A subunit within NMDA receptors, there are virtually no variants within the M3 helix, the pre-M1 helix, and portions of the pre-M4 /M4 region (see Figure 3b) (Swanger *et al.*, 2016; Ogden *et al.*, 2017; Strehlow *et al.*, 2019). That is, these regions are not only conserved across the animal kingdom, but appear to be necessary for human health. Consistent with this idea, there is a significant number of *de novo* missense variants within these regions in subunits from both the NMDA receptors (Figure 4a,b) and the AMPA receptors in patients with a wide range of neurological and neuropsychiatric indications (Firth *et al.*, 2009; Hamdan *et al.*, 2011; de Ligt *et al.*, 2012; Epi *et al.*, 2013; Freunscht *et al.*, 2013; Lemke *et al.*, 2013; Lesca *et al.*, 2013; Redin *et al.*, 2014; Yuan *et al.*, 2014; Farwell *et al.*, 2015; Ohba *et al.*, 2015; Helbig *et al.*, 2016; Kobayashi *et al.*, 2016; Lemke *et al.*, 2016; Li *et al.*, 2016; Lucariello *et al.*, 2016; Swanger *et al.*, 2016; Chen *et al.*, 2017; Ogden *et al.*, 2017; Platzer *et al.*, 2017; Tan *et al.*, 2017; Amin *et al.*, 2018; Fedele

*et al.*, 2018; Fernandez-Marmiesse *et al.*, 2018; Fry *et al.*, 2018; Vyklicky *et al.*, 2018; XiangWei *et al.*, 2018; Strehlow *et al.*, 2019). These two unbiased data sets (structural data and genetic data) provide perhaps the strongest rationale that the pre-M1, M3 and pre-M4/M4 regions play a critical and essential role in receptor function. Moreover, structural data reveal that they are perfectly positioned to cooperate with multiple elements in the receptor that control the transition from the closed to the open state, residing within 5 Angstroms of one another (Figure 4c) (Karakas & Furukawa, 2014; Lee *et al.*, 2014; Twomey & Sobolevsky, 2018). In AMPA receptors, cryo-EM data indicates two M3 helices reorient as the receptor transitions to the open state that results in different configurations of the pre-M1 and the M3 helix at their site of contact, emphasizing their close cooperative nature (Twomey *et al.*, 2017; Twomey & Sobolevsky, 2018).

The invariant nature in the general population of these three motifs suggest that the side chains within these regions are essential to allow the channel to remain closed under resting physiological conditions while also capable of responding to the energy provided by the binding of glutamate to open the channel with the appropriate level of activation. Almost any departure, even minor, from the side chains of conserved amino acids in these regions leads to either an increase or decrease in channel function, in some cases dramatically. Variants in the M3 region overwhelmingly produce a gain-of-function that results in a greater receptor response (Kohda *et al.*, 2000; Kashiwagi *et al.*, 2002; Yuan *et al.*, 2005; Sobolevsky *et al.*, 2007; Chang & Kuo, 2008; XiangWei *et al.*, 2019; Amador *et al.*, 2020). Some variants show a dramatic increase in open probability and reductions in sensitivity to endogenous allosteric modulators (Yuan *et al.*, 2014; Chen *et al.*, 2017; Fedele *et al.*, 2018; Li *et al.*, 2019), such that the channel pore is open almost all the time while the agonist is bound. Other gain-of-function variants can reduce voltage-dependent block of NMDA receptors (Endele *et al.*, 2010; Fedele *et al.*, 2018; Li *et al.*, 2019; Marwick *et al.*, 2019a; Marwick *et al.*, 2019b), and prolong the deactivation time course of both NMDA and AMPA receptors, which keeps the channel open for longer and allows for increased cation influx and likely contributes to clinical features displayed by patients (Yuan *et al.*, 2014; Swanger *et al.*, 2016; Chen *et al.*, 2017; Ogden *et al.*, 2017; Amin *et al.*, 2018). Surprisingly, different substitutions of specific residues yield a gain of function for every mutation tested (Yuan *et al.*, 2014; Ogden *et al.*, 2017), suggesting that these residues are necessary to maintain

channel closure under resting conditions and a precise level of activation following agonist domain closure. There are additional consequences of altering the wild type sequence in that many variants in this region also perturb eventual surface expression, suggesting protein synthesis quality control mechanisms may detect aberrant function or assembly of channels (Swanger *et al.*, 2016; Addis *et al.*, 2017; Ogden *et al.*, 2017; Fernandez-Marmiesse *et al.*, 2018; Vyklicky *et al.*, 2018; Li *et al.*, 2019; XiangWei *et al.*, 2019).

**Structural determinants of allosteric modulation overlap regions of genetic invariance**

The past decade has been exceptionally productive in terms of the development of novel allosteric modulators of glutamate receptor function. Multiple classes of subunit-selective and non-selective positive and negative allosteric modulators have been described for NMDA receptors (Strong *et al.*, 2014; Hackos & Hanson, 2017; Burnell *et al.*, 2019). These include modulators that act at the heterodimer interface between the glutamate and glycine binding domains (Bettini *et al.*, 2010; Hackos *et al.*, 2016; Yi *et al.*, 2016), the interface between the ATD and the glutamate binding domain (Khatri *et al.*, 2014; Kaiser *et al.*, 2018), the heterodimer interface between the ATDs (Karakas *et al.*, 2011; Mony *et al.*, 2011; Karakas & Furukawa, 2014; Lee *et al.*, 2014; Stroebel *et al.*, 2016; Regan *et al.*, 2019), and the cavity that lies behind the pre-M1 region and adjacent to the M3 helix (Ogden & Traynelis, 2013; Wang *et al.*, 2017; Perszyk *et al.*, 2018; Perszyk *et al.*, 2020). In addition, many new modulators for AMPA receptors have been developed, with multiple examples acting at two of these 4 sites mentioned above. There are multiple examples of AMPA modulators that act at the dimer interface between agonist binding domains (Sun *et al.*, 2002; Jin *et al.*, 2005; Ahmed & Oswald, 2010; Krintel *et al.*, 2013) that can modulate both deactivation and desensitization. There is also a series of structurally diverse compounds that bind in contact with the AMPA receptor pre-M1 (Balannik *et al.*, 2005; Yelshanskaya *et al.*, 2016) (see Figure 1b,c).

Remarkably, the modulators that have structural determinants overlapping with the gating triad are highly diverse in their action, including both positive and negative allosteric modulation. Some of these modulators have additional actions on key channel properties. Interestingly, while the experimentally determined poses for AMPA receptor modulators

appear to occupy similar space, the modulator chemical scaffolds are largely distinct, raising intriguing questions about the mechanistic basis for their actions. Functional data support the competition of positive and negative NMDA receptor modulators at overlapping sites (Perszyk *et al.*, 2018), suggesting that future atomic level information about related, competing modulators could be instructive in terms of how to enhance or decrease receptor function.

While there has been exciting progress on the identification of multiple drug scaffolds that can selectively modulate glutamate receptors, there has been incomplete functional analysis of how these modulators operate, with little information available at the single channel level. A recent mechanistic study revealed surprising effects for a positive allosteric modulator of NMDA receptors (Perszyk *et al.*, 2020). EU1622-14 enhances the activity of all NMDA receptors, but to differential degrees in terms of effects on channel open probability and the glutamate deactivation time course. Remarkably, single channel studies revealed that this compound reduces single channel conductance, and ion substitution experiments as well as $Ca^{2+}$-imaging studies suggest that the modulator can alter the relative permeability of monovalent to divalent ions. The ability of a modulator to alter the permeation properties of the pore has not been described previously for NMDA receptors. The effects of EU1622-14 (Perszyk *et al.*, 2020) appear to arise from its actions within the gating triad that must in some way alter the configuration of the open pore or change the relative occupancy of allowed open states of the channel, each of which has different properties. These pharmacological effects can be replicated in part by various point mutations at the gating-triad (Kohda *et al.*, 2000; Li *et al.*, 2016; Ogden *et al.*, 2017; XiangWei *et al.*, 2019), in particular point mutations and deletions in the M3 helix can both decrease conductance and increase open probability (Ladislav *et al.*, 2018). Additionally, this pharmacological action is in agreement with a study that shows that activation of photo-switchable unnatural amino acids introduced to perturb the gating triad can constrain the pore to reduce channel conductance (Klippenstein *et al.*, 2017). Thus, for NMDA receptors, perturbation of the gating triad by pharmacological agents can not only impact gating, but the properties of the pore. For AMPA receptors, a shift in subunit-dependent gating occurs with increasing agonist occupancy of the four subunits, and the number of active subunits determines the relative conductance level. AMPA receptor modulators acting near the

gating triad reduce the activation of each subunit after binding, referred to as coupling efficiency (Kristensen *et al.*, 2011; Poon *et al.*, 2011), which reduces the proportion of high conductance levels, an observation demonstrated for the anti-epileptic drug perampanel (Yuan *et al.*, 2019) (Figure 1b,c). These properties are not universal for modulators that bind in close proximity to the gating triad, and it will be important to determine how they can be tuned through medicinal chemistry, perhaps for therapeutic gain.

Together, these structural, genetic, and pharmacological advances emphasize the power of considering different modalities to drive insight into protein function. We predict that the combination of structural information with genetic information will not only illuminate function across many protein families, but will also advance both the development of pharmacological probes and drugs. As mechanistic and structural data flesh out our understanding of how proteins respond to genetic and pharmacological perturbations, new insight will be gleaned on how proteins mediate their precise and complex tasks.

**References** (Endnote)

Addis L, Virdee JK, Vidler LR, Collier DA, Pal DK & Ursu D. (2017). Epilepsy-associated GRIN2A mutations reduce NMDA receptor trafficking and agonist potency - molecular profiling and functional rescue. *Sci Rep* **7,** 66.

Ahmed AH & Oswald RE. (2010). Piracetam defines a new binding site for allosteric modulators of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. *J Med Chem* **53,** 2197-203.

Alfieri A, Doccula FG, Pederzoli R, Grenzi M, Bonza MC, Luoni L, Candeo A, Romano Armada N, Barbiroli A, Valentini G, Schneider TR, Bassi A, Bolognesi M, Nardini M & Costa A. (2020). The structural bases for agonist diversity in an Arabidopsis thaliana glutamate receptor-like channel. *Proc Natl Acad Sci U S A* **117,** 752-760.

Alsaloum M, Kazi R, Gan Q, Amin J & Wollmuth LP. (2016). A Molecular Determinant of Subtype-Specific Desensitization in Ionotropic Glutamate Receptors. *J Neurosci* **36,** 2617-22.

Amador A, Bostick CD, Olson H, Peters J, Camp CR, Krizay D, Chen W, Han W, Tang W, Kanber A, Kim S, Teoh JJ, Petri S, Paek H, Kim A, Lutz CM, Yang M, Myers SJ, Bhattacharya S, Yuan H, Goldstein DB, Poduri A, Boland MJ, Traynelis SF & Frankel WN. (2020). Modeling and treating GRIN2A developmental and epileptic encephalopathy in mice. *Brain* **In Press,** DOI: 10.1093/brain/awaa147.

Amin JB, Leng X, Gochman A, Zhou HX & Wollmuth LP. (2018). A conserved glycine harboring disease-associated mutations permits NMDA receptor slow deactivation and high Ca(2+) permeability. *Nat Commun* **9,** 3748.

Amin JB, Moody GR & Wollmuth LP. (2020). From bedside-to-bench: What disease-associated variants are teaching us about the NMDA receptor. *J Physiol* **In Press,** DOI: 10.1113/JP278705.

Amin JB, Salussolia CL, Chan K, Regan MC, Dai J, Zhou H-X, Furukawa H, Bowen ME & Wollmuth LP. (2017). Divergent roles of a peripheral transmembrane segment in AMPA and NMDA receptors. *J Gen Physiol* **149,** 661-680.

Ayalon G & Stern-Bach Y. (2001). Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. *Neuron* **31,** 103-13.

Balannik V, Menniti FS, Paternain AV, Lerma J & Stern-Bach Y. (2005). Molecular mechanism of AMPA receptor noncompetitive antagonism. *Neuron* **48,** 279-88.

Banke TG & Traynelis SF. (2003). Activation of NR1/NR2B NMDA receptors. *Nat Neurosci* **6,** 144-52.

Beck C, Wollmuth LP, Seeburg PH, Sakmann B & Kuner T. (1999). NMDAR Channel Segments Forming the Extracellular Vestibule Inferred from the Accessibility of Substituted Cysteines. *Neuron* **22,** 559-570.

Bettini E, Sava A, Griffante C, Carignani C, Buson A, Capelli AM, Negri M, Andreetta F, Senar-Sancho SA, Guiral L & Cardullo F. (2010). Identification and characterization of novel NMDA receptor antagonists selective for NR2A- over NR2B-containing receptors. *J Pharmacol Exp Ther* **335,** 636-44.

Burada AP, Vinnakota R & Kumar J. (2020). Cryo-EM structures of the ionotropic glutamate receptor GluD1 reveal a non-swapped architecture. *Nature Structural & Molecular Biology* **27,** 84-91.

Burnell ES, Irvine M, Fang G, Sapkota K, Jane DE & Monaghan DT. (2019). Positive and Negative Allosteric Modulators of N-Methyl-d-aspartate (NMDA) Receptors: Structure-Activity Relationships and Mechanisms of Action. *J Med Chem* **62,** 3-23.

Chang HR & Kuo CC. (2008). The activation gate and gating mechanism of the NMDA receptor. *J Neurosci* **28,** 1546-56.

Chen S & Gouaux E. (2019). Structure and mechanism of AMPA receptor - auxiliary protein complexes. *Curr Opin Struct Biol* **54,** 104-111.

Chen W, Tankovic A, Burger PB, Kusumoto H, Traynelis SF & Yuan H. (2017). Functional Evaluation of a De Novo GRIN2A Mutation Identified in a Patient with Profound Global Developmental Delay and Refractory Epilepsy. *Mol Pharmacol* **91,** 317-330.

de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA & Vissers LE. (2012). Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* **367,** 1921-9.

Dolino DM, Chatterjee S, MacLean DM, Flatebo C, Bishop LDC, Shaikh SA, Landes CF & Jayaraman V. (2017). The structure-energy landscape of NMDA receptor gating. *Nat Chem Biol* **13,** 1232-1238.

Durr KL, Chen L, Stein RA, De Zorzi R, Folea IM, Walz T, McHaourab HS & Gouaux E. (2014). Structure and dynamics of AMPA receptor GluA2 in resting, pre-open, and desensitized states. *Cell* **158,** 778-792.

Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I, Milh M, Kortüm F, Fritsch A & Pientka FK. (2010). Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. *Nat Genet* **42,** 1021.

Epi KC, Epilepsy Phenome/Genome P, Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeeli Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaitis CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Berkovic SF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Dlugos D, Epstein MP, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glauser T, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, Kuzniecky R, Lowenstein DH, McGuire SM, Motika PV, Novotny EJ, Ottman R, Paolicchi JM, Parent JM, Park K, Poduri A, Scheffer IE, Shellhaas RA, Sherr EH, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widdess-Walsh P & Winawer MR. (2013). De novo mutations in epileptic encephalopathies. *Nature* **501,** 217-21.

Erreger K, Dravid SM, Banke TG, Wyllie DJ & Traynelis SF. (2005a). Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *J Physiol* **563,** 345-58.

Erreger K, Geballe MT, Dravid SM, Snyder JP, Wyllie DJ & Traynelis SF. (2005b). Mechanism of partial agonism at NMDA receptors for a conformationally restricted glutamate analog. *J Neurosci* **25,** 7858-7866.

Esmenjaud JB, Stroebel D, Chan K, Grand T, David M, Wollmuth LP, Taly A & Paoletti P. (2019). An inter-dimer allosteric switch controls NMDA receptor activity. *Embo J* **38**.

Farwell KD, Shahmirzadi L, El-Khechen D, Powis Z, Chao EC, Tippin Davis B, Baxter RM, Zeng W, Mroske C, Parra MC, Gandomi SK, Lu I, Li X, Lu H, Lu HM, Salvador D, Ruble D, Lao M, Fischbach S, Wen J, Lee S, Elliott A, Dunlop CL & Tang S. (2015). Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med* **17,** 578-86.

Fedele L, Newcombe J, Topf M, Gibb A, Harvey RJ & Smart TG. (2018). Disease-associated missense mutations in GluN2B subunit alter NMDA receptor ligand binding and ion channel properties. *Nat Commun* **9,** 957.

Fernandez-Marmiesse A, Kusumoto H, Rekarte S, Roca I, Zhang J, Myers SJ, Traynelis SF, Couce ML, Gutierrez-Solana L & Yuan H. (2018). A novel missense mutation in GRIN2A causes a nonepileptic neurodevelopmental disorder. *Mov Disord* **33,** 992-999.

Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM & Carter NP. (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *Am J Hum Genet* **84,** 524-33.

Freunscht I, Popp B, Blank R, Endele S, Moog U, Petri H, Prott EC, Reis A, Rubo J, Zabel B, Ze nker M, Hebebrand J & Wieczorek D. (2013). Behavioral phenotype in five individuals with de novo mutations within the GRIN2B gene. *Behav Brain Funct* **9,** 20.

Fry AE, Fawcett KA, Zelnik N, Yuan H, Thompson BAN, Shemer-Meiri L, Cushion TD, Mugalaasi H, Sims D, Stoodley N, Chung SK, Rees MI, Patel CV, Brueton LA, Layet V, Giuliano F, Kerr MP, Banne E, Meiner V, Lerman-Sagie T, Helbig KL, Kofman LH, Knight KM, Chen W, Kannan V, Hu C, Kusumoto H, Zhang J, Swanger SA, Shaulsky GH, Mirzaa GM, Muir AM, Mefford HC, Dobyns WB, Mackenzie AB, Mullins JGL, Lemke JR, Bahi-Buisson N, Traynelis SF, Iago HF & Pilz DT. (2018). De novo mutations in GRIN1 cause extensive bilateral polymicrogyria. *Brain* **141,** 698-712.

Gangwar SP, Green M & Sobolevsky AI. (2019). Structure of the Glutamate-Like Receptor GLR3.2 ligand-binding domain. *bioRxiv***,** DOI: 10.1101/2019.12.23.887497.

Gibb AJ, Ogden KK, McDaniel MJ, Vance KM, Kell SA, Butch C, Burger P, Liotta DC & Traynelis SF. (2018). A structurally derived model of subunit-dependent NMDA receptor function. *J Physiol* **596,** 4057-4089.

Gielen M, Retchless BS, Mony L, Johnson JW & Paoletti P. (2009). Mechanism of differential control of NMDA receptor activity by NR2 subunits. *Nature* **459,** 703-707.

Greger IH & Mayer ML. (2019). Structural biology of glutamate receptor ion channels: towards an understanding of mechanism. *Curr Opin Struct Biol* **57,** 185-195.

Greger IH, Watson JF & Cull-Candy SG. (2017). Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. *Neuron* **94,** 713-730.

Hackos DH & Hanson JE. (2017). Diverse modes of NMDA receptor positive allosteric modulation: Mechanisms and consequences. *Neuropharmacology* **112,** 34-45.

Hackos DH, Lupardus PJ, Grand T, Chen Y, Wang TM, Reynen P, Gustafson A, Wallweber HJ, Volgraf M, Sellers BD, Schwarz JB, Paoletti P, Sheng M, Zhou Q & Hanson JE. (2016). Positive Allosteric Modulators of GluN2A-Containing NMDARs with Distinct Modes of Action and Impacts on Circuit Function. *Neuron* **89,** 983-99.

Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, Spiegelman D, Dobrzeniecka S, Piton A, Tomitori H, Daoud H, Massicotte C, Henrion E, Diallo O, Group SD, Shekarabi M, Marineau C, Shevell M, Maranda B, Mitchell G, Nadeau A, D'Anjou G, Vanasse M, Srour M, Lafreniere RG, Drapeau P, Lacaille JC, Kim E, Lee JR, Igarashi K, Huganir RL, Rouleau GA & Michaud JL. (2011). Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. *Am J Hum Genet* **88,** 306-16.

Hansen KB, Yi F, Perszyk RE, Menniti FS & Traynelis SF. (2017). NMDA Receptors in the Central Nervous System. *Methods Mol Biol* **1677,** 1-80.

Heinzen EL, Neale BM, Traynelis SF, Allen AS & Goldstein DB. (2015). The genetics of neuropsychiatric diseases: looking in and beyond the exome. *Annu Rev Neurosci* **38,** 47-68.

Helbig KL, Farwell Hagman KD, Shinde DN, Mroske C, Powis Z, Li S, Tang S & Helbig I. (2016). Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet Med* **18,** 898-905.

Iacobucci GJ & Popescu GK. (2018). Kinetic models for activation and modulation of NMDA receptor subtypes. *Curr Opin Physiol* **2,** 114-122.

Jin R, Clark S, Weeks AM, Dudman JT, Gouaux E & Partin KM. (2005). Mechanism of positive allosteric modulators acting on AMPA receptors. *J Neurosci* **25,** 9027-36.

Kaiser TM, Kell SA, Kusumoto H, Shaulsky G, Bhattacharya S, Epplin MP, Strong KL, Miller EJ, Cox BD, Menaldino DS, Liotta DC, Traynelis SF & Burger PB. (2018). The Bioactive Protein-Ligand Conformation of GluN2C-Selective Positive Allosteric Modulators Bound to the NMDA Receptor. *Mol Pharmacol* **93,** 141-156.

Karakas E & Furukawa H. (2014). Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science* **344,** 992-7.

Karakas E, Simorowski N & Furukawa H. (2011). Subunit arrangement and phenylethanolamine binding in GluN1/GluN2B NMDA receptors. *Nature* **475,** 249-U170.

Kashiwagi K, Masuko T, Nguyen CD, Kuno T, Tanaka I, Igarashi K & Williams K. (2002). Channel blockers acting at N-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. *Mol Pharmacol* **61,** 533-45.

Khatri A, Burger PB, Swanger SA, Hansen KB, Zimmerman S, Karakas E, Liotta DC, Furukawa H, Snyder JP & Traynelis SF. (2014). Structural determinants and mechanism of action of a GluN2C-selective NMDA receptor positive allosteric modulator. *Mol Pharmacol* **86,** 548-60.

Klippenstein V, Hoppmann C, Ye S, Wang L & Paoletti P. (2017). Optocontrol of glutamate receptor activity by single side-chain photoisomerization. *eLife* **6,** e25808.

Kobayashi Y, Tohyama J, Kato M, Akasaka N, Magara S, Kawashima H, Ohashi T, Shiraishi H, Nakashima M, Saitsu H & Matsumoto N. (2016). High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. *Brain Dev* **38,** 285-92.

Kohda K, Wang Y & Yuzaki M. (2000). Mutation of a glutamate receptor motif reveals its role in gating and delta2 receptor channel properties. *Nat Neurosci* **3,** 315-22.

Krintel C, Harpsoe K, Zachariassen LG, Peters D, Frydenvang K, Pickering DS, Gajhede M & Kastrup JS. (2013). Structural analysis of the positive AMPA receptor modulators CX516 and Me-CX516 in complex with the GluA2 ligand-binding domain. *Acta Crystallogr D Biol Crystallogr* **69,** 1645-52.

Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, Johnson RC, Huganir R & Traynelis SF. (2011). Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat Neurosci* **14,** 727-35.

Kussius CL & Popescu GK. (2010). NMDA receptors with locked glutamate-binding clefts open with high efficacy. *J Neurosci* **30,** 12474-12479.

Ladislav M, Cerny J, Krusek J, Horak M, Balik A & Vyklicky L. (2018). The LILI Motif of M3-S2 Linkers Is a Component of the NMDA Receptor Channel Gate. *Front Mol Neurosci* **11**.

Lee CH, Lu W, Michel JC, Goehring A, Du J, Song X & Gouaux E. (2014). NMDA receptor structures reveal subunit arrangement and pore architecture. *Nature* **511,** 191-7.

Lemke JR, Geider K, Helbig KL, Heyne HO, Schutz H, Hentschel J, Courage C, Depienne C, Nava C, Heron D, Moller RS, Hjalgrim H, Lal D, Neubauer BA, Nurnberg P, Thiele H, Kurlemann G, Arnold GL, Bhambhani V, Bartholdi D, Pedurupillay CR, Misceo D, Frengen E, Stromme P, Dlugos DJ, Doherty ES, Bijlsma EK, Ruivenkamp CA, Hoffer MJ, Goldstein A, Rajan DS, Narayanan V, Ramsey K, Belnap N, Schrauwen I, Richholt R, Koeleman BP, Sa J, Mendonca C, de Kovel CG, Weckhuysen S, Hardies K, De Jonghe P, De Meirleir L, Milh M, Badens C, Lebrun M, Busa T, Francannet C, Piton A, Riesch E, Biskup S, Vogt H, Dorn T, Helbig I, Michaud JL, Laube B & Syrbe S. (2016). Delineating the GRIN1 phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. *Neurology* **86,** 2171-8.

Lemke JR, Lal D, Reinthaler EM, Steiner I, Nothnagel M, Alber M, Geider K, Laube B, Schwake M, Finsterwalder K, Franke A, Schilhabel M, Jahn JA, Muhle H, Boor R, Van Paesschen W, Caraballo R, Fejerman N, Weckhuysen S, De Jonghe P, Larsen J, Moller RS, Hjalgrim H, Addis L, Tang S, Hughes E, Pal DK, Veri K, Vaher U, Talvik T, Dimova P, Guerrero Lopez R, Serratosa JM, Linnankivi T, Lehesjoki AE, Ruf S, Wolff M, Buerki S, Wohlrab G, Kroell J, Datta AN, Fiedler B, Kurlemann G, Kluger G, Hahn A, Haberlandt DE, Kutzer C, Sperner J, Becker F, Weber YG, Feucht M, Steinbock H, Neophythou B, Ronen GM, Gruber-Sedlmayr U, Geldner J, Harvey RJ, Hoffmann P, Herms S, Altmuller J, Toliat MR, Thiele H, Nurnberg P, Wilhelm C, Stephani U, Helbig I, Lerche H, Zimprich F, Neubauer BA, Biskup S & von Spiczak S. (2013). Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. *Nat Genet* **45,** 1067-72.

Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, Salmi M, Tsintsadze T, Addis L, Motte J, Wright S, Tsintsadze V, Michel A, Doummar D, Lascelles K, Strug L, Waters P, de

Bellescize J, Vrielynck P, de Saint Martin A, Ville D, Ryvlin P, Arzimanoglou A, Hirsch E, Vincent A, Pal D, Burnashev N, Sanlaville D & Szepetowski P. (2013). GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet* **45,** 1061-6.

Li D, Yuan H, Ortiz-Gonzalez XR, Marsh ED, Tian L, McCormick EM, Kosobucki GJ, Chen W, Schulien AJ, Chiavacci R, Tankovic A, Naase C, Brueckner F, von Stulpnagel-Steinbeis C, Hu C, Kusumoto H, Hedrich UB, Elsen G, Hortnagel K, Aizenman E, Lemke JR, Hakonarson H, Traynelis SF & Falk MJ. (2016). GRIN2D Recurrent De Novo Dominant Mutation Causes a Severe Epileptic Encephalopathy Treatable with NMDA Receptor Channel Blockers. *Am J Hum Genet* **99,** 802-816.

Li J, Zhang J, Tang W, Mizu RK, Kusumoto H, XiangWei W, Xu Y, Chen W, Amin JB, Hu C, Kannan V, Keller SR, Wilcox WR, Lemke JR, Myers SJ, Swanger SA, Wollmuth LP, Petrovski S, Traynelis SF & Yuan H. (2019). De novo GRIN variants in NMDA receptor M2 channel pore-forming loop are associated with neurological diseases. *Hum Mutat* **40,** 2393-2413.

Lucariello M, Vidal E, Vidal S, Saez M, Roa L, Huertas D, Pineda M, Dalfo E, Dopazo J, Jurado P, Armstrong J & Esteller M. (2016). Whole exome sequencing of Rett syndrome-like patients reveals the mutational diversity of the clinical phenotype. *Hum Genet* **135,** 1343-1354.

MacLean DM, Durham RJ & Jayaraman V. (2019). Mapping the Conformational Landscape of Glutamate Receptors Using Single Molecule FRET. *Trends Neurosci* **42,** 128-139.

Marwick KFM, Hansen KB, Skehel PA, Hardingham GE & Wyllie DJA. (2019a). Functional assessment of triheteromeric NMDA receptors containing a human variant associated with epilepsy. *J Physiol* **597,** 1691-1704.

Marwick KFM, Skehel PA, Hardingham GE & Wyllie DJA. (2019b). The human NMDA receptor GluN2A(N615K) variant influences channel blocker potency. *Pharmacol Res Perspect* **7,** e00495.

Mayer ML. (2017). The Challenge of Interpreting Glutamate-Receptor Ion-Channel Structures. *Biophys J* **113,** 2143-2151.

Mayer ML. (2020). Glutamate receptors from diverse animal species exhibit unexpected structural and functional diversity. *J Physiol* **In Press,** DOI: 10.1113/JP279026.

McDaniel MJ, Ogden KK, Kell SA, Burger PB, Liotta DC & Traynelis SF. (2020). NMDA receptor channel gating control by the pre-M1 helix. *J Gen Physiol* **152**.

Meyerson JR, Chittori S, Merk A, Rao P, Han TH, Serpe M, Mayer ML & Subramaniam S. (2016). Structural basis of kainate subtype glutamate receptor desensitization. *Nature* **537,** 567-571.

Mollerud S, Frydenvang K, Pickering DS & Kastrup JS. (2017). Lessons from crystal structures of kainate receptors. *Neuropharmacology* **112,** 16-28.

Mony L, Zhu S, Carvalho S & Paoletti P. (2011). Molecular basis of positive allosteric modulation of GluN2B NMDA receptors by polyamines. *Embo J* **30,** 3134-46.

Naur P, Hansen KB, Kristensen AS, Dravid SM, Pickering DS, Olsen L, Vestergaard B, Egebjerg J, Gajhede M, Traynelis SF & Kastrup JS. (2007). Ionotropic glutamate-like receptor delta2 binds D-serine and glycine. *Proc Natl Acad Sci U S A* **104,** 14116-21.

Ogden KK, Chen W, Swanger SA, McDaniel MJ, Fan LZ, Hu C, Tankovic A, Kusumoto H, Kosobucki GJ, Schulien AJ, Su Z, Pecha J, Bhattacharya S, Petrovski S, Cohen AE, Aizenman E, Traynelis SF & Yuan H. (2017). Molecular Mechanism of Disease-Associated Mutations in the Pre-M1 Helix of NMDA Receptors and Potential Rescue Pharmacology. *PLoS Genet* **13,** e1006536.

Ogden KK & Traynelis SF. (2013). Contribution of the M1 transmembrane helix and pre-M1 region to positive allosteric modulation and gating of N-methyl-D-aspartate receptors. *Mol Pharmacol* **83,** 1045-56.

Ohba C, Shiina M, Tohyama J, Haginoya K, Lerman-Sagie T, Okamoto N, Blumkin L, Lev D, Mukaida S, Nozaki F, Uematsu M, Onuma A, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Tanaka F, Kato M, Ogata K, Saitsu H & Matsumoto N. (2015). GRIN1 mutations cause encephalopathy with infantile-onset epilepsy, and hyperkinetic and stereotyped movement disorders. *Epilepsia* **56,** 841-8.

Paoletti P, Bellone C & Zhou Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature reviews Neuroscience* **14,** 383-400.

Paramo T, Brown P, Musgaard M, Bowie D & Biggin PC. (2017). Functional Validation of Heteromeric Kainate Receptor Models. *Biophys J* **113,** 2173-2177.

Perszyk R, Katzman BM, Kusumoto H, Kell SA, Epplin MP, Tahirovic YA, Moore RL, Menaldino D, Burger P, Liotta DC & Traynelis SF. (2018). An NMDAR positive and negative allosteric modulator series share a binding site and are interconverted by methyl groups. *eLife* **7,** e34711.

Perszyk RE, Swanger SA, Shelley C, Khatri A, Fernandez-Cuervo G, Epplin MP, Zhang J, Le P, Bülow P, Garnier-Amblard E, Gangireddy PKR, Bassell GJ, Yuan H, Menaldino DS, Liotta DC, Liebeskind LS & Traynelis SF. (2020). Biased modulators of NMDA receptors control channel opening and ion selectivity. *Nature Chemical Biology* **16,** 188-196.

Platzer K, Yuan H, Schutz H, Winschel A, Chen W, Hu C, Kusumoto H, Heyne HO, Helbig KL, Tang S, Willing MC, Tinkle BT, Adams DJ, Depienne C, Keren B, Mignot C, Frengen E, Stromme P,

Biskup S, Docker D, Strom TM, Mefford HC, Myers CT, Muir AM, LaCroix A, Sadleir L, Scheffer IE, Brilstra E, van Haelst MM, van der Smagt JJ, Bok LA, Moller RS, Jensen UB, Millichap JJ, Berg AT, Goldberg EM, De Bie I, Fox S, Major P, Jones JR, Zackai EH, Abou Jamra R, Rolfs A, Leventer RJ, Lawson JA, Roscioli T, Jansen FE, Ranza E, Korff CM, Lehesjoki AE, Courage C, Linnankivi T, Smith DR, Stanley C, Mintz M, McKnight D, Decker A, Tan WH, Tarnopolsky MA, Brady LI, Wolff M, Dondit L, Pedro HF, Parisotto SE, Jones KL, Patel AD, Franz DN, Vanzo R, Marco E, Ranells JD, Di Donato N, Dobyns WB, Laube B, Traynelis SF & Lemke JR. (2017). GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. *J Med Genet* **54,** 460-470.

Poon K, Ahmed AH, Nowak LM & Oswald RE. (2011). Mechanisms of modal activation of GluA3 receptors. *Mol Pharmacol* **80,** 49-59.

Poon K, Nowak LM & Oswald RE. (2010). Characterizing single-channel behavior of GluA3 receptors. *Biophys J* **99,** 1437-46.

Popescu G & Auerbach A. (2003). Modal gating of NMDA receptors and the shape of their synaptic response. *Nat Neurosci* **6,** 476-83.

Popescu G, Robert A, Howe JR & Auerbach A. (2004). Reaction mechanism determines NMDA receptor response to repetitive stimulation. *Nature* **430,** 790-793.

Redin C, Gerard B, Lauer J, Herenger Y, Muller J, Quartier A, Masurel-Paulet A, Willems M, Lesca G, El-Chehadeh S, Le Gras S, Vicaire S, Philipps M, Dumas M, Geoffroy V, Feger C, Haumesser N, Alembik Y, Barth M, Bonneau D, Colin E, Dollfus H, Doray B, Delrue MA, Drouin-Garraud V, Flori E, Fradin M, Francannet C, Goldenberg A, Lumbroso S, Mathieu-Dramard M, Martin-Coignard D, Lacombe D, Morin G, Polge A, Sukno S, Thauvin-Robinet C, Thevenon J, Doco-Fenzy M, Genevieve D, Sarda P, Edery P, Isidor B, Jost B, Olivier-Faivre L, Mandel JL & Piton A. (2014). Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. *J Med Genet* **51,** 724-36.

Regan MC, Zhu Z, Yuan H, Myers SJ, Menaldino DS, Tahirovic YA, Liotta DC, Traynelis SF & Furukawa H. (2019). Structural elements of a pH-sensitive inhibitor binding site in NMDA receptors. *Nat Commun* **10,** 321.

Ren H, Honse Y, Karp BJ, Lipsky RH & Peoples RW. (2003). A Site in the Fourth Membrane-associated Domain of the N-Methyl-d-aspartate Receptor Regulates Desensitization and Ion Channel Gating. *Journal of Biological Chemistry* **278,** 276-283.

Salussolia CL, Prodromou ML, Borker P & Wollmuth LP. (2011). Arrangement of Subunits in Functional NMDA Receptors. *J Neurosci* **31,** 11295-11304.

Sobolevsky AI, Beck C & Wollmuth LP. (2002). Molecular Rearrangements of the Extracellular Vestibule in NMDAR Channels during Gating. *Neuron* **33,** 75-85.

Sobolevsky AI, Prodromou ML, Yelshansky MV & Wollmuth LP. (2007). Subunit-specific contribution of pore-forming domains to NMDA receptor channel structure and gating. *J Gen Physiol* **129,** 509-25.

Sobolevsky AI, Rosconi MP & Gouaux E. (2009). X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* **462,** 745-56.

Strehlow V, Heyne HO, Vlaskamp DRM, Marwick KFM, Rudolf G, de Bellescize J, Biskup S, Brilstra EH, Brouwer OF, Callenbach PMC, Hentschel J, Hirsch E, Kind PC, Mignot C, Platzer K, Rump P, Skehel PA, Wyllie DJA, Hardingham GE, van Ravenswaaij-Arts CMA, Lesca G, Lemke JR & group GAs. (2019). GRIN2A-related disorders: genotype and functional consequence predict phenotype. *Brain* **142,** 80-92.

Stroebel D, Buhl DL, Knafels JD, Chanda PK, Green M, Sciabola S, Mony L, Paoletti P & Pandit J. (2016). A Novel Binding Mode Reveals Two Distinct Classes of NMDA Receptor GluN2B-selective Antagonists. *Mol Pharmacol* **89,** 541-51.

Strong KL, Jing Y, Prosser AR, Traynelis SF & Liotta DC. (2014). NMDA receptor modulators: an updated patent review (2013-2014). *Expert Opin Ther Pat* **24,** 1349-66.

Sun Y, Olson R, Horning M, Armstrong N, Mayer M & Gouaux E. (2002). Mechanism of glutamate receptor desensitization. *Nature* **417,** 245-253.

Swanger SA, Chen W, Wells G, Burger PB, Tankovic A, Bhattacharya S, Strong KL, Hu C, Kusumoto H, Zhang J, Adams DR, Millichap JJ, Petrovski S, Traynelis SF & Yuan H. (2016). Mechanistic Insight into NMDA Receptor Dysregulation by Rare Variants in the GluN2A and GluN2B Agonist Binding Domains. *Am J Hum Genet* **99,** 1261-1280.

Tajima N, Karakas E, Grant T, Simorowski N, Diaz-Avalos R, Grigorieff N & Furukawa H. (2016). Activation of NMDA receptors and the mechanism of inhibition by ifenprodil. *Nature* **534,** 63-8.

Talukder I, Borker P & Wollmuth LP. (2010). Specific sites within the ligand-binding domain and ion channel linkers modulate NMDA receptor gating. *J Neurosci* **30,** 11792-804.

Talukder I & Wollmuth LP. (2011). Local constraints in either the GluN1 or GluN2 subunit equally impair NMDA receptor pore opening. *J Gen Physiol* **138,** 179-194.

Tan TY, Dillon OJ, Stark Z, Schofield D, Alam K, Shrestha R, Chong B, Phelan D, Brett GR, Creed E, Jarmolowicz A, Yap P, Walsh M, Downie L, Amor DJ, Savarirayan R, McGillivray G, Yeung A, Peters H, Robertson SJ, Robinson AJ, Macciocca I, Sadedin S, Bell K, Oshlack A, Georgeson P, Thorne N, Gaff C & White SM. (2017). Diagnostic Impact and Cost-effectiveness of Whole-

Exome Sequencing for Ambulant Children With Suspected Monogenic Conditions. *JAMA Pediatr* **171,** 855-862.

Traynelis J, Silk M, Wang Q, Berkovic SF, Liu L, Ascher DB, Balding DJ & Petrovski S. (2017). Optimizing genomic medicine in epilepsy through a gene-customized approach to missense variant interpretation. *Genome Res* **27,** 1715-1729.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ & Dingledine R. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* **62,** 405-96.

Twomey EC & Sobolevsky AI. (2018). Structural Mechanisms of Gating in Ionotropic Glutamate Receptors. *Biochemistry* **57,** 267-276.

Twomey EC, Yelshanskaya MV, Grassucci RA, Frank J & Sobolevsky AI. (2017). Channel opening and gating mechanism in AMPA-subtype glutamate receptors. *Nature* **549,** 60-65.

Twomey EC, Yelshanskaya MV & Sobolevsky AI. (2019). Structural and functional insights into transmembrane AMPA receptor regulatory protein complexes. *J Gen Physiol* **151,** 1347-1356.

Vyklicky V, Krausova B, Cerny J, Ladislav M, Smejkalova T, Kysilov B, Korinek M, Danacikova S, Horak M, Chodounska H, Kudova E & Vyklicky L. (2018). Surface Expression, Function, and Pharmacology of Disease-Associated Mutations in the Membrane Domain of the Human GluN2B Subunit. *Front Mol Neurosci* **11,** 110.

Wang JX & Furukawa H. (2019). Dissecting diverse functions of NMDA receptors by structural biology. *Curr Opin Struct Biol* **54,** 34-42.

Wang TM, Brown BM, Deng L, Sellers BD, Lupardus PJ, Wallweber HJA, Gustafson A, Wong E, Volgraf M, Schwarz JB, Hackos DH & Hanson JE. (2017). A novel NMDA receptor positive allosteric modulator that acts via the transmembrane domain. *Neuropharmacology* **121,** 204-218.

Watson JF, Ho H & Greger IH. (2017). Synaptic transmission and plasticity require AMPA receptor anchoring via its N-terminal domain. *eLife* **6**.

XiangWei W, Jiang Y & Yuan H. (2018). De novo mutations and rare variants occurring in NMDA receptors. *Curr Opin Physiol* **2,** 27-35.

XiangWei W, Kannan V, Xu Y, Kosobucki GJ, Schulien AJ, Kusumoto H, Moufawad El Achkar C, Bhattacharya S, Lesca G, Nguyen S, Helbig KL, Cuisset JM, Fenger CD, Marjanovic D, Schuler E, Wu Y, Bao X, Zhang Y, Dirkx N, Schoonjans AS, Syrbe S, Myers SJ, Poduri A, Aizenman E, Traynelis SF, Lemke JR, Yuan H & Jiang Y. (2019). Heterogeneous clinical and functional

features of GRIN2D-related developmental and epileptic encephalopathy. *Brain* **142,** 3009-3027.

Yelshanskaya MV, Li M & Sobolevsky AI. (2014). Structure of an agonist-bound ionotropic glutamate receptor. *Science* **345,** 1070-4.

Yelshanskaya MV, Mesbahi-Vasey S, Kurnikova MG & Sobolevsky AI. (2017). Role of the Ion Channel Extracellular Collar in AMPA Receptor Gating. *Sci Rep* **7,** 1050.

Yelshanskaya MV, Singh AK, Sampson JM, Narangoda C, Kurnikova M & Sobolevsky AI. (2016). Structural Bases of Noncompetitive Inhibition of AMPA-Subtype Ionotropic Glutamate Receptors by Antiepileptic Drugs. *Neuron* **91,** 1305-15.

Yi F, Mou TC, Dorsett KN, Volkmann RA, Menniti FS, Sprang SR & Hansen KB. (2016). Structural Basis for Negative Allosteric Modulation of GluN2A-Containing NMDA Receptors. *Neuron* **91,** 1316-29.

Yuan CL, Shi EY, Srinivasan J, Ptak CP, Oswald RE & Nowak LM. (2019). Modulation of AMPA Receptor Gating by the Anticonvulsant Drug, Perampanel. *ACS Medicinal Chemistry Letters* **10,** 237-242.

Yuan H, Erreger K, Dravid SM & Traynelis SF. (2005). Conserved structural and functional control of N-methyl-D-aspartate receptor gating by transmembrane domain M3. *Journal of Biological Chemistry* **280,** 29708-16.

Yuan H, Hansen KB, Vance KM, Ogden KK & Traynelis SF. (2009). Control of NMDA Receptor Function by the NR2 Subunit Amino-Terminal Domain. *J Neurosci* **29,** 12045-12058.

Yuan H, Hansen KB, Zhang J, Pierson TM, Markello TC, Fajardo KV, Holloman CM, Golas G, Adams DR, Boerkoel CF, Gahl WA & Traynelis SF. (2014). Functional analysis of a de novo GRIN2A missense mutation associated with early-onset epileptic encephalopathy. *Nat Commun* **5,** 3251.

Zhu S & Gouaux E. (2017). Structure and symmetry inform gating principles of ionotropic glutamate receptors. *Neuropharmacology* **112,** 11-15.

**Additional information**

*Competing interests*

SFT is a PI on research grants from Biogen and Janssen to Emory University, is a paid consultant for Janssen, is a member of the SAB for Sage Therapeutics, GRIN2B Foundation, and CureGRIN Foundation, is co-founder of NeurOp Inc, and receives licensing fees and royalties for software. SFT is a co-inventor on Emory University-owned Intellectual Property that includes allosteric modulators of glutamate receptor function. HY is a PI on research grant from Sage Therapeutics to Emory University. HF is a PI on a research grant from Allergan. The opinions expressed in this paper are those of the author(s) and do not necessarily reflect the views of the funding agency.

*Author contributions*

REP, SJM, HY, AJG, HF, AIS, and SFT contributed to the conception or design of the work and drafting the work or revising it critically for important intellectual content.

*Funding*

This work was supported by the NIH (R35 NS111619 to SFT; R01 HD082373 to HY; R01 CA206573, R01 NS083660, and R01 NS107253 to AIS; and R01 NS111745 and RO1 MH085926 to HF), NSF (1818213 to AIS), Austin's purpose (to SFT and HF), and by the University Research Committee (URC; #00085889 to HY) from Emory University.

*Acknowledgements*

We thank Gabriela Popescu for insightful comments on the manuscript.

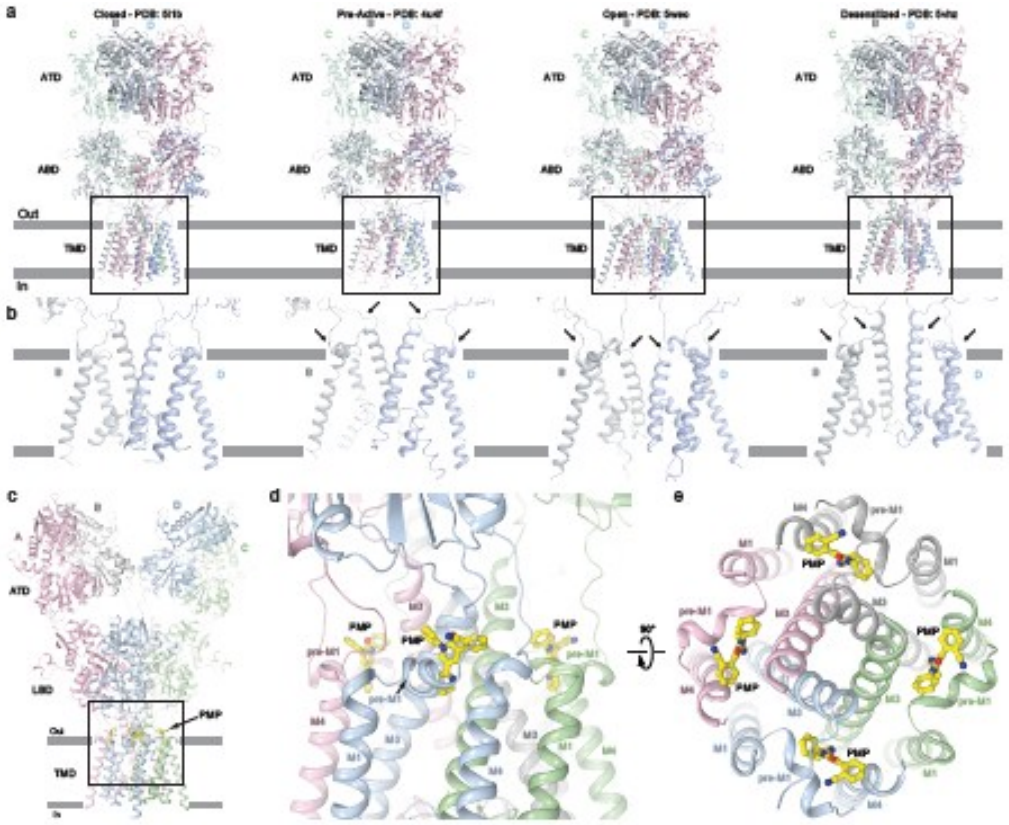



**Figure 1**. **AMPA receptor gating conformations and binding of perampanel (PMP). a**) Structures of the GluA2 AMPA receptor in different conformations (PDB entries 5l1b, 4u4f, 5weo, 5vhz) are shown in ribbon representation and viewed parallel to the membrane, with four subunits colored differently and the layers of the amino-terminal domain (ATD), the agonist-binding domain (ABD), and the transmembrane domain (TMD) labeled. The rectangles indicates the region of the structure viewed more closely in **b**. **b**) A closer view of the TMD (receptor subunits B and D) for the structures in **a** to highlight the gating transitions indicated by the arrows. **c**) Crystal structure of GluA2 (PDB entry 5L1F), with perampanel shown in sticks (yellow). The rectangle indicates the region of the structure viewed more closely in **d** and **e**. **d**) An expanded view of the perampanel binding sites, at the outermost end of the transmembrane domain and adjacent to the linkers connecting the LBD and the TMD. **e**) A top-down view of perampanel binding sites in the pockets formed by pre-M1, M3, and pre-M4/M4.

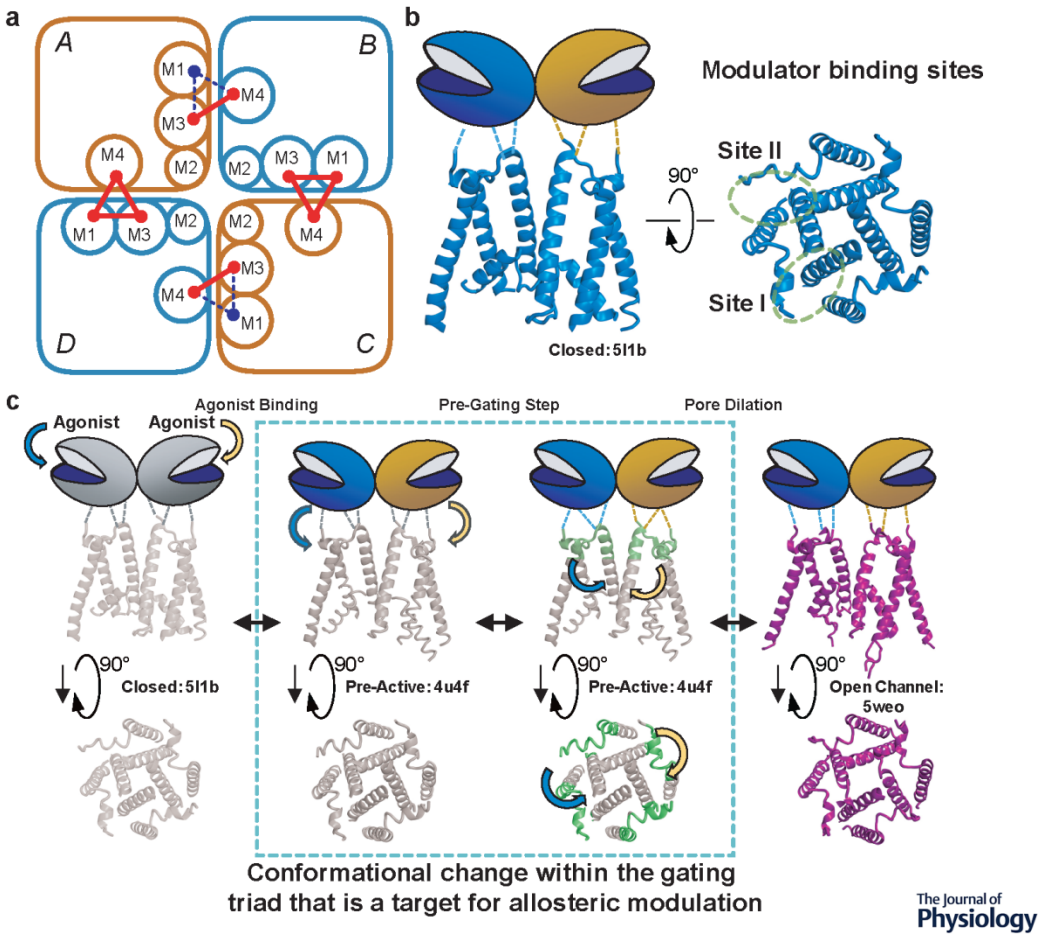
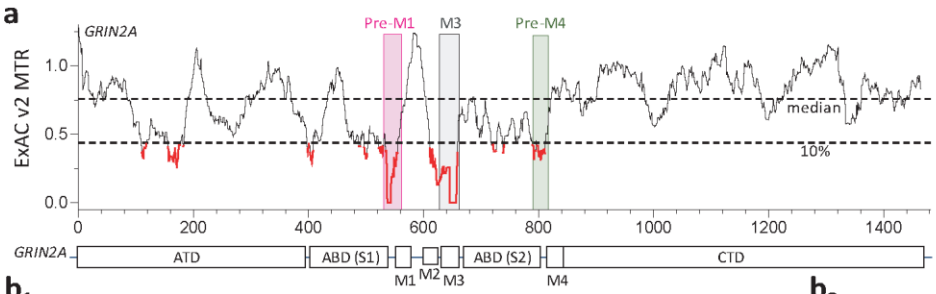



**Figure 2. A model of subunit-dependent conformational changes for a tetrameric glutamate receptor. a**) Carton illustrating the unique gating triads that exist between heteromeric glutamate receptor assembled with two asymmetrical subunits in alternating sequence. **b**) Cartoon illustrating only the ligand binding domain, M3 transmembrane helices, and pre-M1 helix for four subunits. Two potentially distinct modulator binding pockets are shown to the right. The evidence that sites are, indeed, not identical is supported by, in AMPA receptors, asymmetrical motions during gating and, in NMDA receptors, due to the fact that they are composed of GluN1 and GluN2 subunits. **c**) Illustration of hypothesized steps (arrows) within each subunit starting with agonist binding to the bi-lobed agonist binding domain that involves domain closure (*left*), followed by a conformational change in a linker between the agonist binding domain and the transmembrane domain that is distinct of domain closure (*center*), followed by opening of the pore (*right*). The conformational changes within the gating triads that are a target for allosteric modulation and a site of ' disease-causing variation. *Panel a* was adapted from Gibb et al., 2018, J Physiol, 596(17):4057-4089 with permission)




**b$_1$**

Pre-M1

| Subunit | # | | | | | | | | | | | | | | | | | | | #V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GluN2A | 540 | S | N | G | T | V | - | S | P | S | A | F | L | E | P | F | S | A | S | V | 1 |
| GluN2B | 541 | S | N | G | T | V | - | S | P | S | A | F | L | E | P | F | S | A | D | V | 0 |
| GluN2C | 538 | S | N | G | T | V | - | S | P | S | A | F | L | E | P | Y | S | P | A | V | 7 |
| GluN2D | 568 | S | N | G | T | V | - | S | P | S | A | F | L | E | P | Y | S | P | A | V | 4 |
| GluN1 | 545 | E | I | P | - | R | S | T | L | D | S | F | M | Q | P | F | Q | S | T | L | 2 |
| GluA1 | 521 | P | Q | K | S | K | P | G | V | F | S | F | L | D | P | L | A | Y | E | I | 6 |
| GluA2 | 528 | P | Q | K | S | K | P | G | V | F | S | F | L | D | P | L | A | Y | E | I | 0 |
| GluA3 | 537 | P | Q | K | S | K | P | G | V | F | S | F | L | D | P | L | A | Y | E | I | 3 |
| GluA4 | 529 | P | Q | K | S | K | P | G | V | F | S | F | L | D | P | L | A | Y | E | I | 4 |
| GluK1 | 560 | P | N | G | T | N | P | G | V | F | S | F | L | N | P | L | S | P | D | I | 10 |
| GluK2 | 545 | P | N | G | T | N | P | G | V | F | S | F | L | N | P | L | S | P | D | I | 10 |
| GluK3 | 547 | P | N | G | T | N | P | S | V | F | S | F | L | N | P | L | S | P | D | V | 8 |
| GluK4 | 529 | H | M | G | R | K | P | G | Y | F | S | F | L | D | P | F | S | P | G | V | 11 |
| GluK5 | 528 | H | M | G | R | K | P | G | Y | F | S | F | L | D | P | F | S | P | A | S | 8 |
| #Human Var: | | 8 | 8 | 2 | 9 | 5 | 3 | 2 | 11 | 0 | 1 | 1 | 3 | 3 | 1 | 1 | 3 | 5 | 5 | 3 | 74 |

**b$_2$**

| Subunit | #V |
|---|---|
| GluN2B | 0 |
| GluA2 | 0 |
| GluN2A | 1 |
| GluN1 | 2 |
| GluA3 | 3 |
| GluN2D | 4 |
| GluA4 | 4 |
| GluA1 | 6 |
| GluN2C | 7 |
| GluK3 | 8 |
| GluK5 | 8 |
| GluK1 | 10 |
| GluK2 | 10 |
| GluK4 | 11 |
| | 74 |

M3

| Subunit | # | | | | | | | | | | | | | | | | | | | #V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GluN2A | 629 | I | M | V | S | V | W | A | F | F | A | V | I | F | L | A | S | Y | T | 1 |
| GluN2B | 630 | I | M | V | S | V | W | A | F | F | A | V | I | F | L | A | S | Y | T | 1 |
| GluN2C | 627 | I | M | V | L | V | W | A | F | F | A | V | I | F | L | A | S | Y | T | 7 |
| GluN2D | 657 | I | M | V | L | V | W | A | F | F | A | V | I | F | L | A | S | Y | T | 2 |
| GluN1 | 631 | I | L | G | M | V | W | A | G | F | A | M | I | I | V | A | S | Y | T | 0 |
| GluA1 | 614 | I | V | G | G | V | W | W | F | F | T | L | I | I | I | S | S | Y | T | 2 |
| GluA2 | 621 | I | V | G | G | V | W | W | F | F | T | L | I | I | I | S | S | Y | T | 0 |
| GluA3 | 632 | I | V | G | G | V | W | W | F | F | T | L | I | I | I | S | S | Y | T | 2 |
| GluA4 | 622 | I | V | G | G | V | W | W | F | F | T | L | I | I | I | S | S | Y | T | 6 |
| GluK1 | 650 | I | V | G | G | I | W | W | F | F | T | L | I | I | I | S | S | Y | T | 4 |
| GluK2 | 635 | I | V | G | G | I | W | W | F | F | T | L | I | I | I | S | S | Y | T | 6 |
| GluK3 | 637 | I | I | G | G | I | W | W | F | F | T | L | I | I | I | S | S | Y | T | 5 |
| GluK4 | 620 | C | V | S | G | V | W | W | A | F | T | L | I | I | I | S | S | Y | T | 9 |
| GluK5 | 619 | C | V | S | G | V | W | W | A | F | T | L | I | I | I | S | S | Y | T | 4 |
| #Human Var: | | 2 | 4 | 2 | 1 | 3 | 1 | 1 | 4 | 0 | 3 | 1 | 4 | 2 | 2 | 1 | 2 | 3 | 5 | 0 |

| Subunit | #V |
|---|---|
| GluN1 | 0 |
| GluA2 | 0 |
| GluN2A | 1 |
| GluN2B | 1 |
| GluN2D | 2 |
| GluA1 | 2 |
| GluA3 | 2 |
| GluK1 | 4 |
| GluK5 | 4 |
| GluK3 | 5 |
| GluA4 | 6 |
| GluK2 | 6 |
| GluN2C | 7 |
| GluK4 | 9 |
| | 49 |

Pre-M4/M4

| Subunit | # | | | | | | | | | | | | | | | | | | | #V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GluN2A | 802 | N | E | K | - | - | N | E | V | M | S | S | Q | L | D | I | D | N | M | 4 |
| GluN2B | 803 | N | E | K | - | - | N | E | V | M | S | S | Q | L | D | I | D | N | M | 2 |
| GluN2C | 800 | N | E | K | - | - | N | E | V | M | S | S | K | L | D | I | D | N | M | 12 |
| GluN2D | 830 | N | D | K | - | - | I | E | V | M | S | S | K | L | D | I | D | N | M | 5 |
| GluN1 | 800 | S | R | - | - | - | - | S | N | A | P | A | T | L | T | F | E | N | M | 1 |
| GluA1 | 789 | S | G | G | G | D | S | K | D | K | T | S | A | L | S | L | S | N | V | 4 |
| GluA2 | 796 | A | K | D | S | G | S | K | E | K | T | S | A | L | S | L | S | N | V | 7 |
| GluA3 | 807 | A | K | D | S | G | S | K | D | K | T | S | A | L | S | L | S | N | V | 2 |
| GluA4 | 797 | P | K | D | S | G | S | K | D | K | T | S | A | L | S | L | S | N | V | 9 |
| GluK1 | 821 | E | E | - | - | - | D | N | K | E | A | S | A | L | G | V | E | N | I | 9 |
| GluK2 | 806 | E | E | - | - | - | E | S | K | E | A | S | A | L | G | V | Q | N | I | 4 |
| GluK3 | 807 | E | E | - | - | - | E | N | K | E | A | S | A | L | G | I | Q | K | I | 8 |
| GluK4 | 791 | K | E | - | - | - | E | D | H | R | A | K | G | L | G | M | E | N | I | 9 |
| GluK5 | 790 | K | E | - | - | - | E | D | H | R | A | K | G | L | G | M | E | N | I | 10 |
| #Human Var: | | 6 | 5 | 4 | 1 | 1 | 2 | 4 | 4 | 5 | 4 | 3 | 6 | 1 | 3 | 4 | 3 | 2 | 2 | 2 |

| Subunit | #V |
|---|---|
| GluN1 | 1 |
| GluN2B | 2 |
| GluA3 | 2 |
| GluN2A | 4 |
| GluA1 | 4 |
| GluK2 | 4 |
| GluN2D | 5 |
| GluA2 | 7 |
| GluK3 | 8 |
| GluA4 | 9 |
| GluK1 | 9 |
| GluK4 | 9 |
| GluK5 | 10 |
| GluN2C | 12 |
| | 86 |

The Journal of Physiology

**Figure 3**. **Intolerance of the gating triad. a**) The Missense Tolerance Ratio (MTR) plot for *GRIN2A*, based on ExAC (the Exome Aggregation Consortium browser, version 2). The gating-triad regions (Pre-M1, M3, and Pre-M4/M4, highlighted in magenta, grey, and green, respectively) are some of the most intolerant portions of *GRIN2A*. **b**) Comparison of conservation from homologous proteins and scarcity of naturally-occurring variants in a human population-based genomic database. **b$_1$**) Sequence homology of the pre-M1, M3,

and pre-M4/M4 regions across all iGluR subunits. The final column (#V) is the cumulative number of human variants across the subdomain for each receptor subunit reported in ExAC (version 2, accessed 01/24/202), and bottom row (#Human Var) is the cumulative number of human variants in ExAC found at the aligned position, different from WT, across all iGluRs. **$b_2$**) The iGluR subunits are rearranged based on the cumulative number of ExAC variants in each subdomain sequence. Sequence alignment by Clustal Omega using translated open reading frames from these human reference sequences: GluN2A, NM_000833.4; GluN2B, NM_000834.4; GluN2C, NM_000835.4; GluN2D, NM_000836.2; GluN1, NM_007327.3; GluA1, NM_000827.3; GluA2, NM_000826.3; GluA3, NM_007325.4; GluA4, NM_000829.3; GluK1, NM_000830.4; GluK2, NM_021956.4; GluK3, NM_000831.3; GluK4, NM_014619.4; GluK5, NM_002088.4.

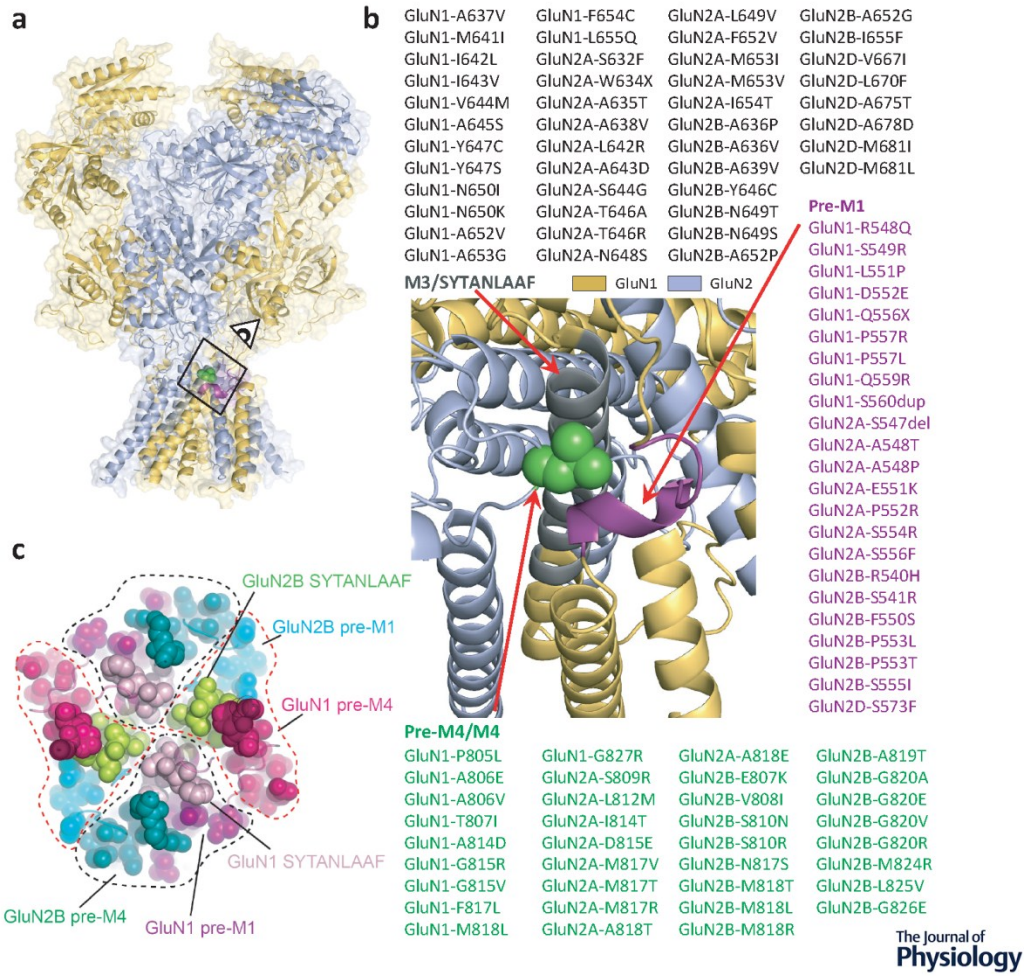



**Figure 4**. **Orientation of the gating-triad components and list of disease-causing variants found in humans from the peer-reviewed literature or ClinVar. a**) Full cartoon-ribbon structure of the NMDAR. **b**) View of one gating-triad instance (this view point is depicted in **a** by the eye and rotated box) and a list of the associated variants of the M3 helix are listed in grey, Pre-M1 variants are in magenta, and Pre-M4/M4 variants are in green. **c**) A space-filling representation of the gating triad showing their close contact with each other (viewed looking down at the pore from the extracellular side). See Supplemental Table 1 for PubMed ID and ClinVar references.