Epilepsy Genetics Update 2020

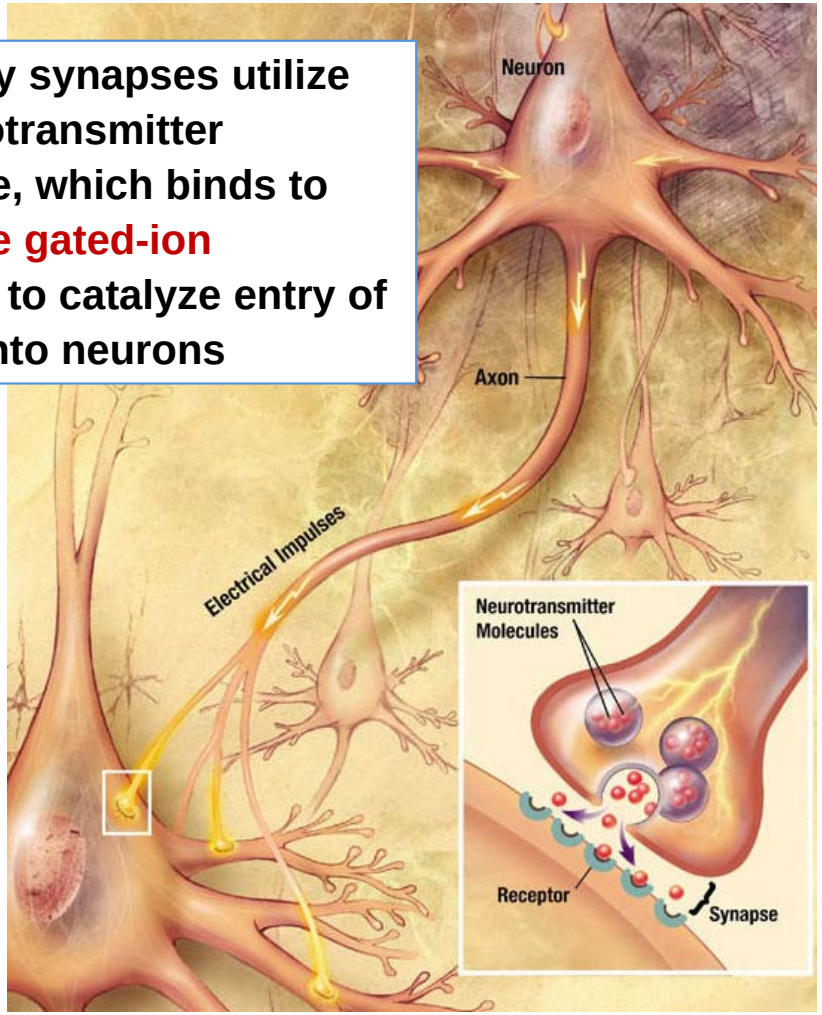
Molecular Studies of Genes Associated with Epilepsies:

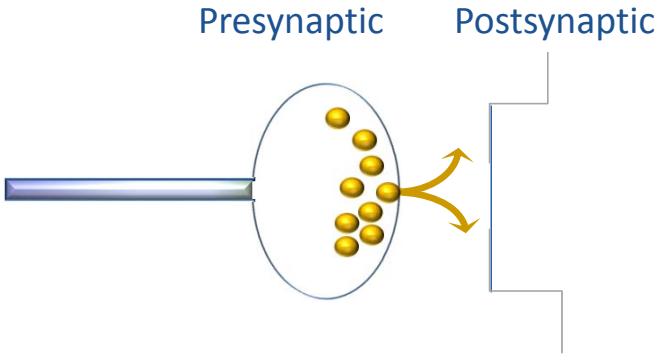
GRIN gene family (NMDA receptors)

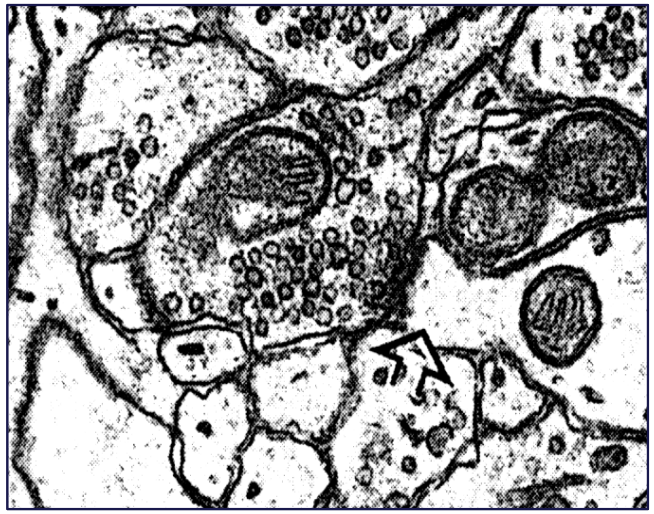
Stephen Traynelis

Center for Functional Evaluation of Rare Variants (CFERV)
Dept of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA

Excitatory synapses utilize the neurotransmitter glutamate, which binds to glutamate gated-ion channels to catalyze entry of cations into neurons







Glutamate receptor subtypes

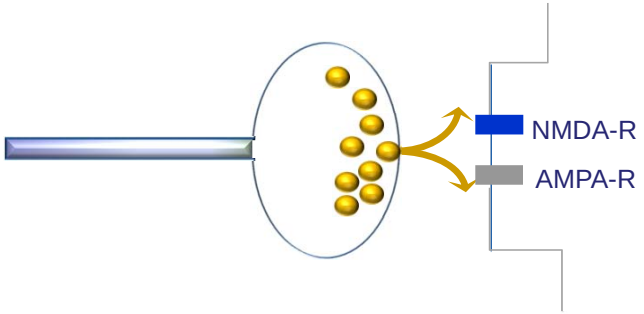
AMPA, kainate, delta, **NMDA**, and metabotropic

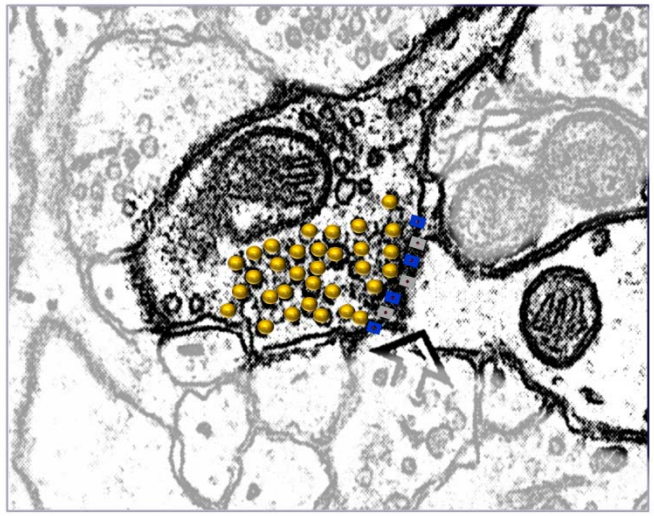
NMDA receptors are encoded by 5 genes

GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D

NMDA receptors are present in all neurons and are important for:

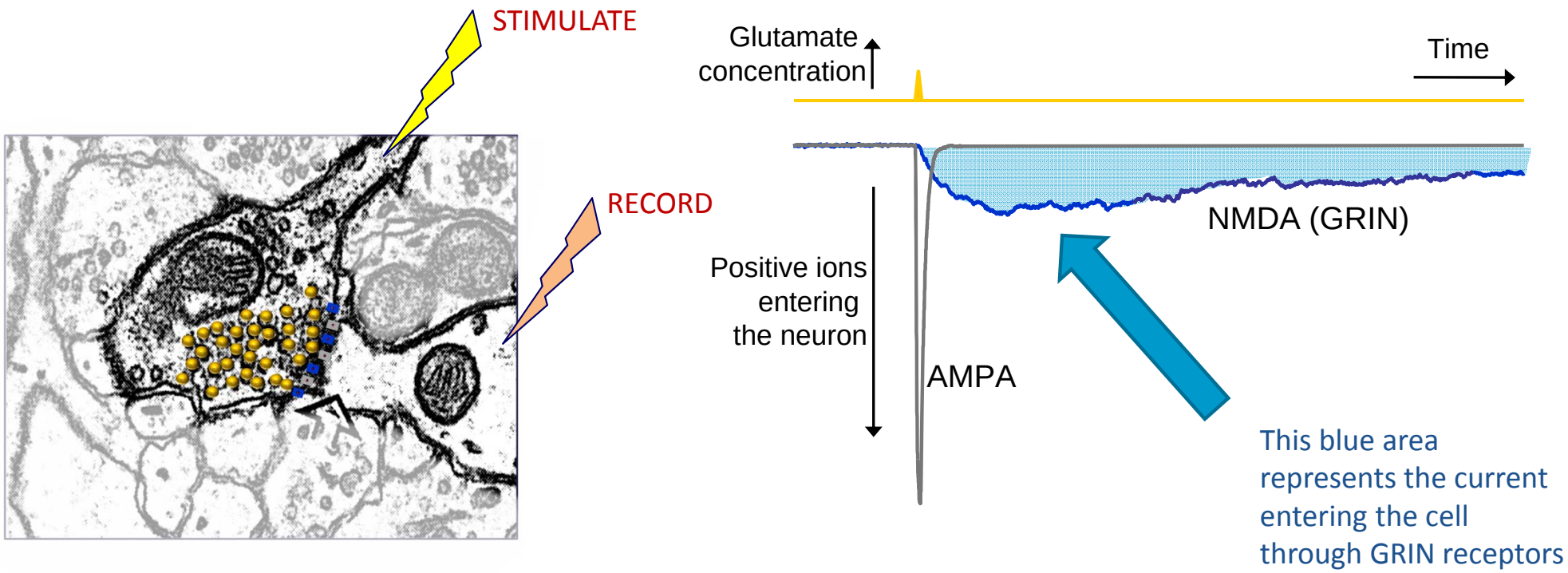
Excitatory synaptic transmission
Plasticity, learning, memory
Neuronal development





Stimulation of a pre-synaptic neuron while recording from a post-synaptic neuron reveals an excitatory synaptic current flowing through glutamate receptors

NMDA receptors mediate a slow inward current carried by Na^+ and Ca^{2+}



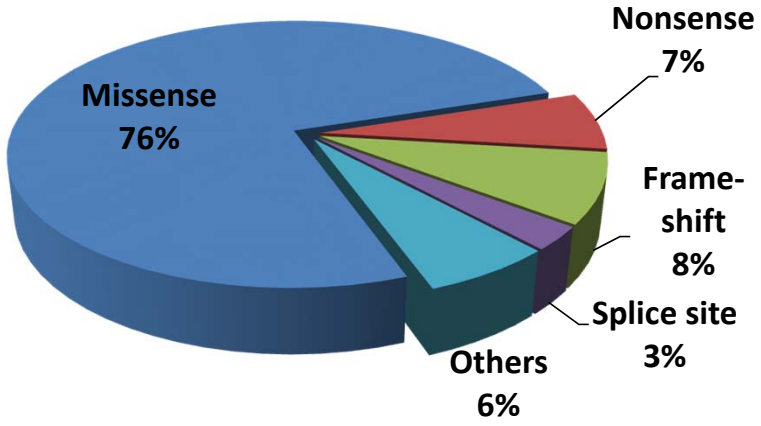
NMDA receptors are encoded by 5 GRIN genes that show fewer than expected naturally occurring variation

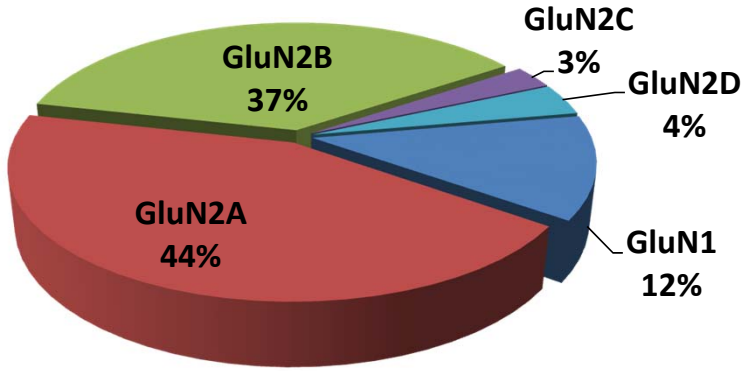
HGNC gene	Residual Variation Intolerance (%tile)
GRIN2B	1.09
GRIN2A	1.17
GRIN2D	4.56
GRIN1	4.67
GRIN3A	67.5
GRIN2C	81.7

Petrovski et al.,2013
genic-intolerance.org
gnomAD database

A large number of *GRIN* variants have been identified in patients (~700 positions described in the Literature, ClinVar)

	Missense	Nonsense	Frameshift	Splice	Other	Total
***GRIN1*, GluN1**	80	4	1	0	0	**85**
***GRIN2A*, GluN2A**	202	21	32	13	29	**297**
***GRIN2B*, GluN2B**	192	21	19	6	14	**252**
***GRIN2C*, GluN2C**	13	1	5	0	0	**19**
***GRIN2D*, GluN2D**	26	0	0	0	0	**26**
Total	**513**	**47**	**57**	**19**	**43**	**679**





Numbers of patients with *GRIN* variants

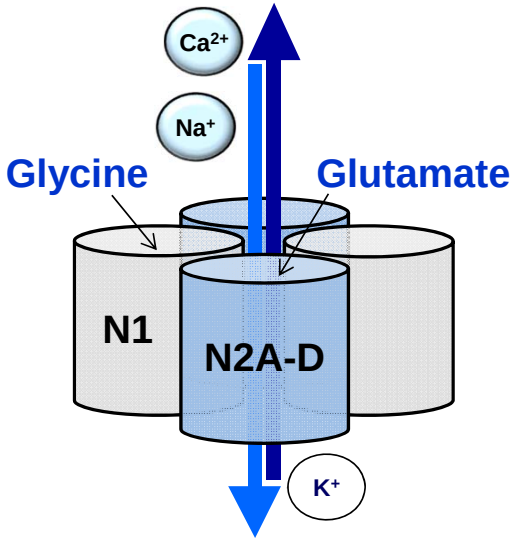
	Total	ID	EPI	ASD	SCZ/BP	ADHD	MD
***GRIN1*, GluN1**	**85**	**35**	**25**	**4**	**20**	**3**	**0**
***GRIN2A*, GluN2A**	**297**	**184**	**198**	**19**	**27**	**5**	**5**
***GRIN2B*, GluN2B**	**252**	**185**	**99**	**33**	**10**	**5**	**2**
***GRIN2C*, GluN2C**	**19**	**4**	**1**	**8**	**0**	**7**	**0**
***GRIN2D*, GluN2D**	**26**	**12**	**12**	**5**	**0**	**8**	**0**
Total	**679**	**420**	**335**	**69**	**57**	**28**	**7**

Number of patients with GRIN Variants in 2018 (Lemke, *Brain*, 2020)

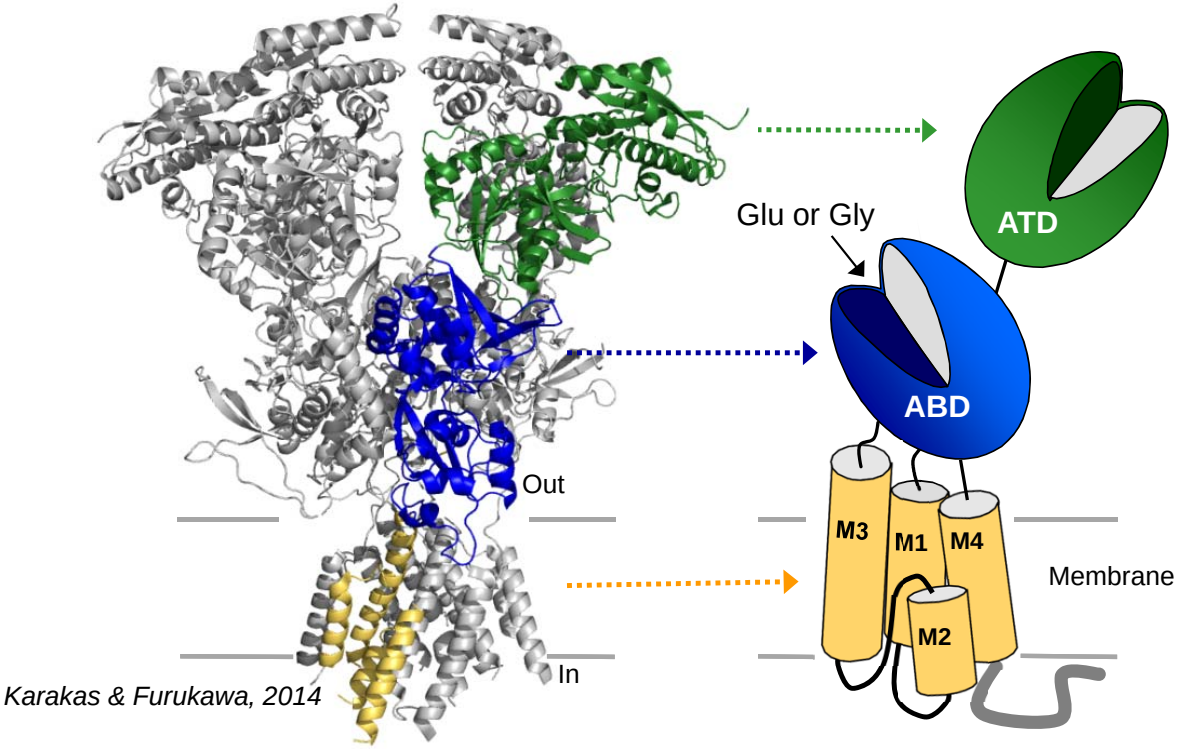
GRIN1	5.45 / 100,000 births	(207 / yr)
GRIN2A	3.23 / 100,000 births	(122 / yr)
GRIN2B	5.91 / 100,000 births	(224 / yr)
GRIN2D	4.65 / 100,000 births	(175 / yr)

Depending on life span, there could be 5,000-20,000 patients with GRIN variants in the US

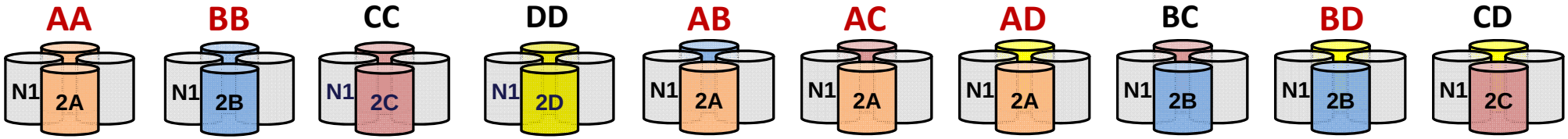
NMDA receptors are tetrameric assemblies of GluN1 and GluN2



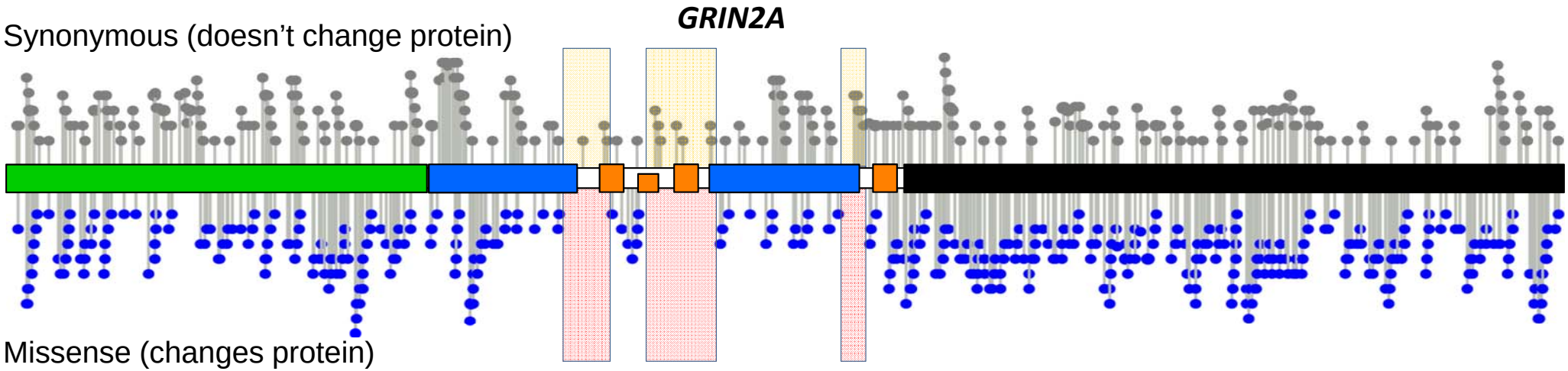
There are 4 GluN2 subunits (A-D)



Karakas & Furukawa, 2014



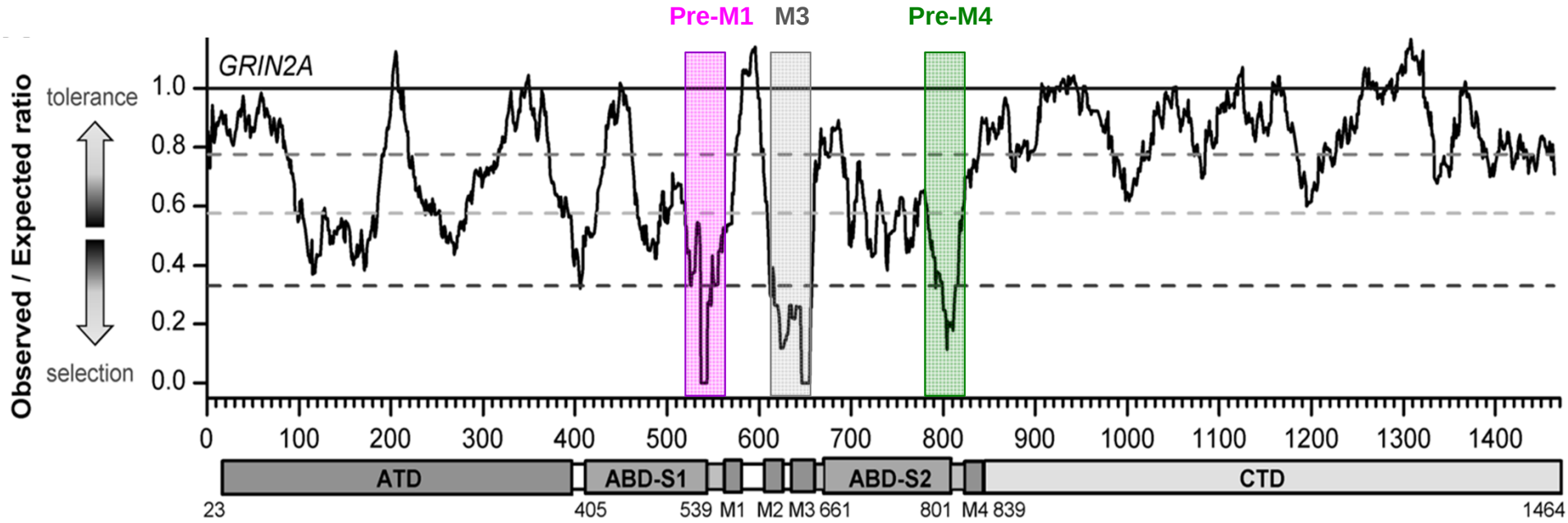
The ratio of synonymous to non-synonymous variants identifies vulnerable regions



Swanger et al., 2016

The pre-M1 helix, conserved M3 gating motif, and pre-M4 linker are invariant in the healthy population

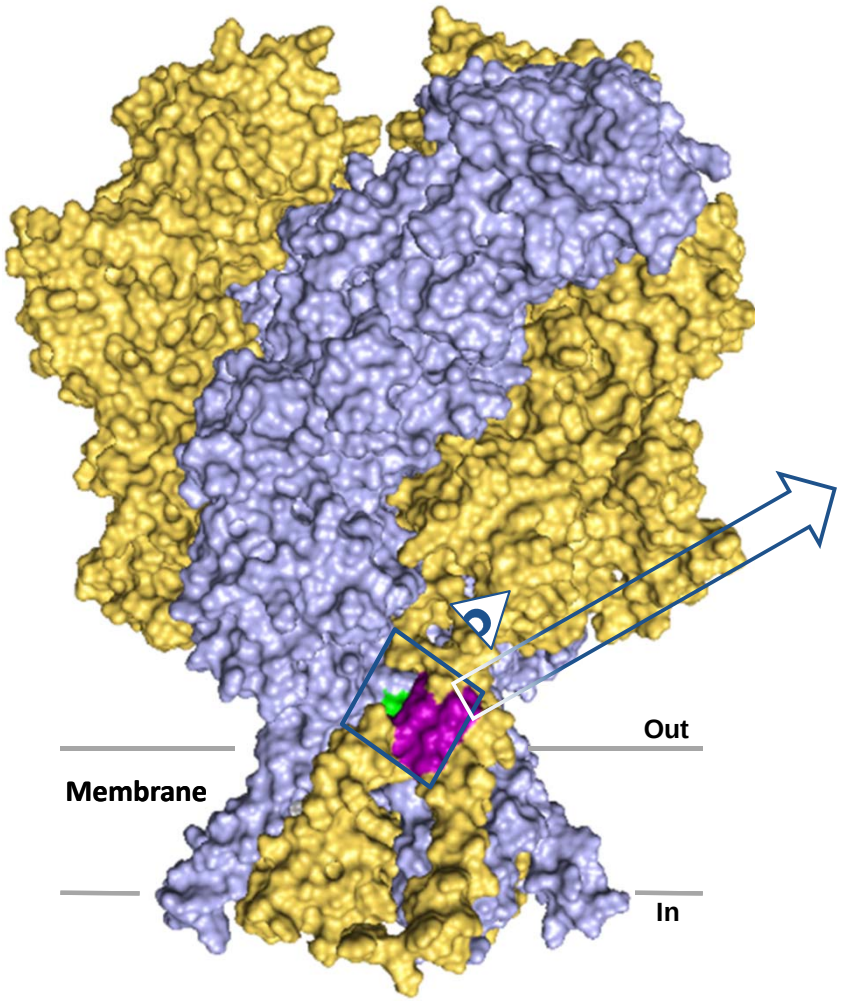
There are fewer variants than expected in these regions, which suggests purifying selection and crucial roles



http://mtr-viewer.mdhs.unimelb.edu.au

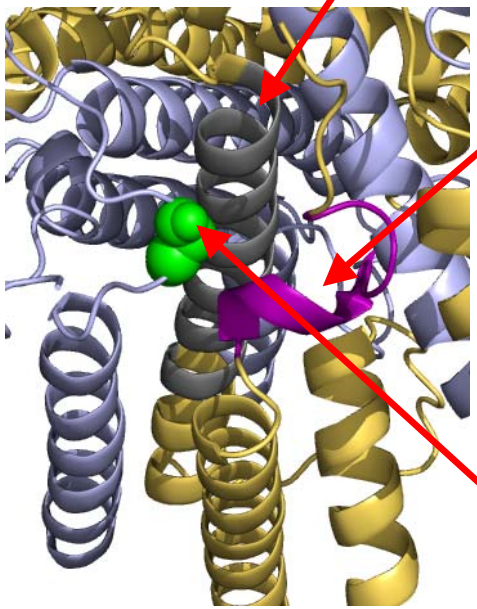
Ogden, Petrovski et al., 2017, Traynelis et al., 2018

Intolerant regions are a locus for disease-associated variants



Perszyk et al., 2020

"Gating Triad" comprises ~2% of protein and contains ~25% of the disease variants



GluN1 GluN2

M3/SYTANLAAF Variants

GluN1-A637V	GluN1-F654C	GluN2A-L649V	GluN2B-A652G
GluN1-M641I	GluN1-L655Q	GluN2A-F652V	GluN2B-I655F
GluN1-I642L	GluN2A-S632F	GluN2A-M653I	GluN2D-V667I
GluN1-I643V	GluN2A-W634X	GluN2A-M653V	GluN2D-L670F
GluN1-V644M	GluN2A-A635T	GluN2A-I654T	GluN2D-A675T
GluN1-A645S	GluN2A-A638V	GluN2B-A636P	GluN2D-A678D
GluN1-Y647C	GluN2A-L642R	GluN2B-A636V	GluN2D-M681I
GluN1-Y647S	GluN2A-A643D	GluN2B-A639V	GluN2D-M681L
GluN1-N650I	GluN2A-S644G	GluN2B-Y646C	
GluN1-N650K	GluN2A-T646A	GluN2B-N649T	
GluN1-A652V	GluN2A-T646R	GluN2B-N649S	
GluN1-A653G	GluN2A-N648S	GluN2B-A652P	

Pre-M1 Variants

GluN2A-A548P	GluN1-R548Q
GluN2A-E551K	GluN1-S549R
GluN2A-P552R	GluN1-L551P
GluN2A-S554R	GluN1-D552E
GluN2A-S556F	GluN1-Q556X
GluN2B-R540H	GluN1-P557R
GluN2B-S541R	GluN1-P557L
GluN2B-F550S	GluN1-Q559R
GluN2B-P553L	GluN1-S560dup
GluN2B-P553T	GluN2A-S547del
GluN2B-S555I	GluN2A-A548T
GluN2D-S573F	

Pre-M4/M4 Variants

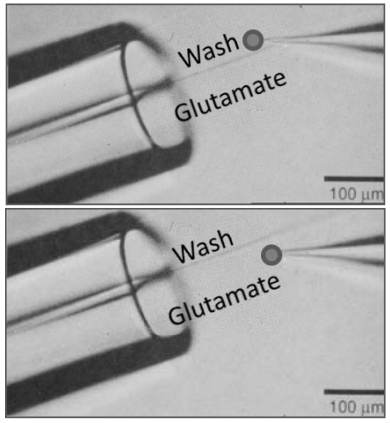
GluN1-P805L	GluN1-G827R	GluN2A-A818E	GluN2B-A819T
GluN1-A806E	GluN2A-S809R	GluN2B-E807K	GluN2B-G820A
GluN1-A806V	GluN2A-L812M	GluN2B-V808I	GluN2B-G820E
GluN1-T807I	GluN2A-I814T	GluN2B-S810N	GluN2B-G820V
GluN1-A814D	GluN2A-D815E	GluN2B-S810R	GluN2B-G820R
GluN1-G815R	GluN2A-M817V	GluN2B-N817S	GluN2B-M824R
GluN1-G815V	GluN2A-M817T	GluN2B-M818T	GluN2B-L825V
GluN1-F817L	GluN2A-M817R	GluN2B-M818L	GluN2B-G826E
GluN1-M818L	GluN2A-A818T	GluN2B-M818R	

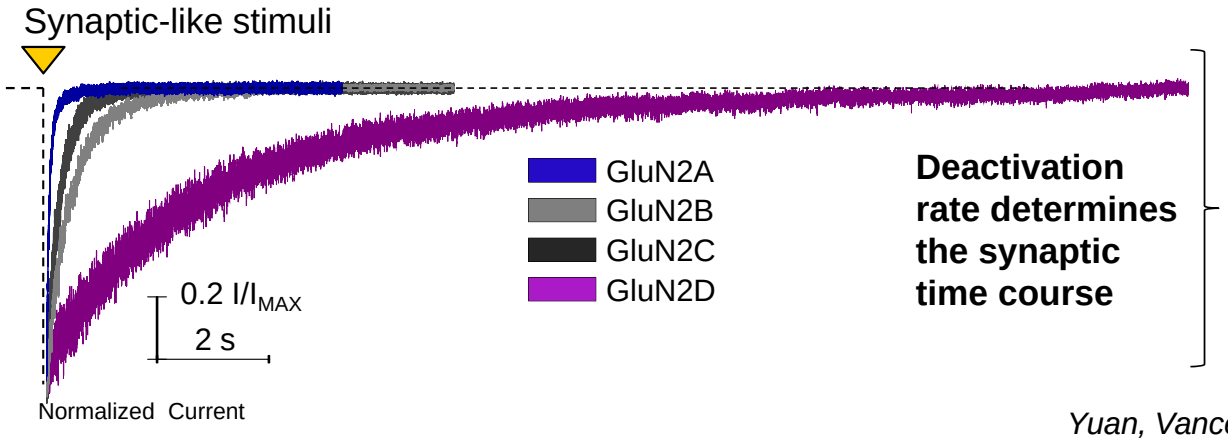
Center for Functional Evaluation of Rare Variants (CFERV)

- In 2014 the number of new variants identified by sequencing outnumbered those with functional data by over **20:1**
- Functional data was rarely comprehensive and not comparable between papers
- CFERV's goal is to provide **comprehensive, comparable data** on all relevant variants in the glutamate receptor family in a standardized assay format
- This allows **clinical stratification** and development of mechanistic hypotheses, and informs selection of variants for development of animal models
- CFERV obtains variants from the **literature**, from **ClinVar**, from **patients**, from **clinical collaborators**, as well as from **gnomAD**

GluN2 controls multiple distinct functional properties

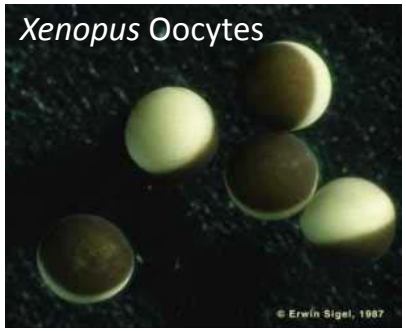
	GluN1/GluN2A	GluN1/GluN2D
Sensitivity to neuro-transmitter, endogenous modulators	• Glutamate EC_{50} **5.4** µM • Glycine EC_{50} **1.1** µM • Inhibited by **0.02-0.1** µM Zn^{2+}	• Glutamate EC_{50} **0.5** µM • Glycine EC_{50} **0.2** µM • Inhibited by **20-40** µM Zn^{2+}
Current flow into cell	• P_{OPEN} ~ **0.5** • Larger conductance • Higher Ca^{2+} permeability • More Mg^{2+} sensitive	• P_{OPEN} ~ **0.01** • Smaller conductance • Lower Ca^{2+} permeability • Less Mg^{2+} sensitive
Signal timing	• Deactivation fast **50 ms**	• Deactivation slow **5000 ms**

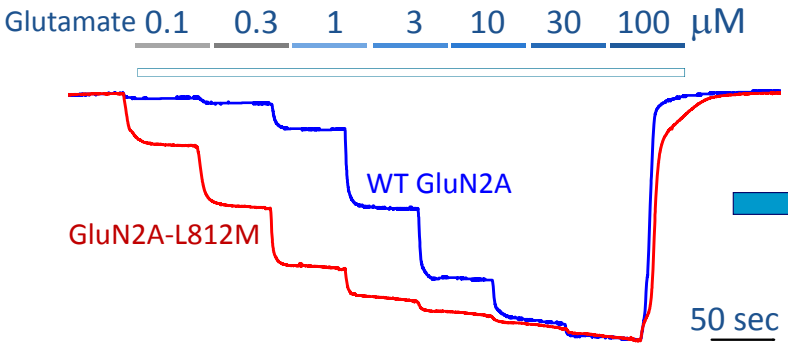


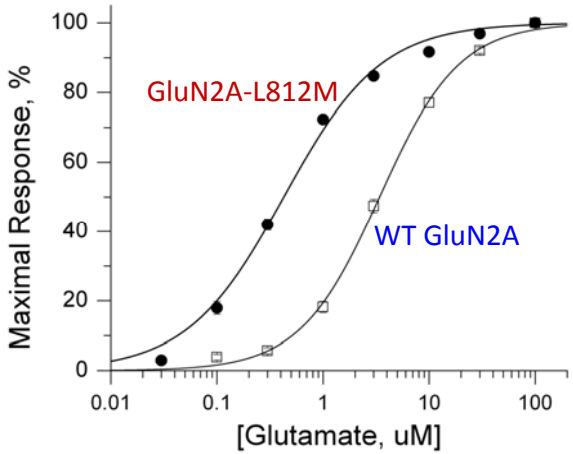


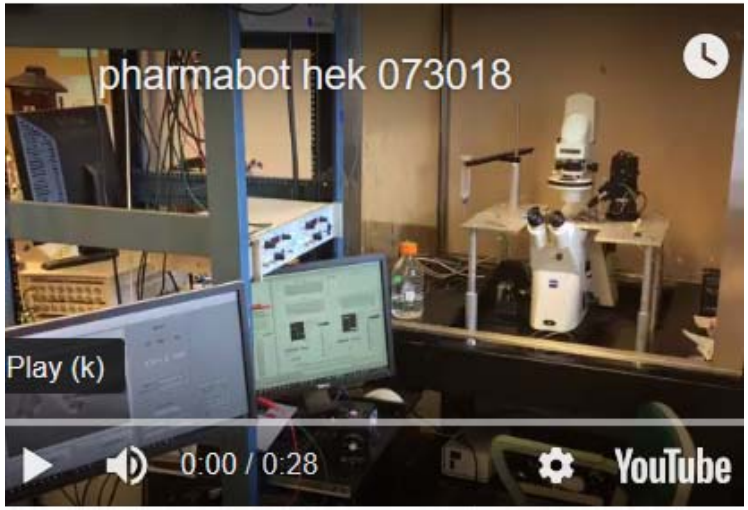
Yuan, Vance, Traynelis

CFERV performs functional analysis of up to 8 parameters for *GRIN* variants









Robotic patch clamp
lifts cells in front of a rapid application system to apply synaptic-like concentrations
Craig Forest, GaTech

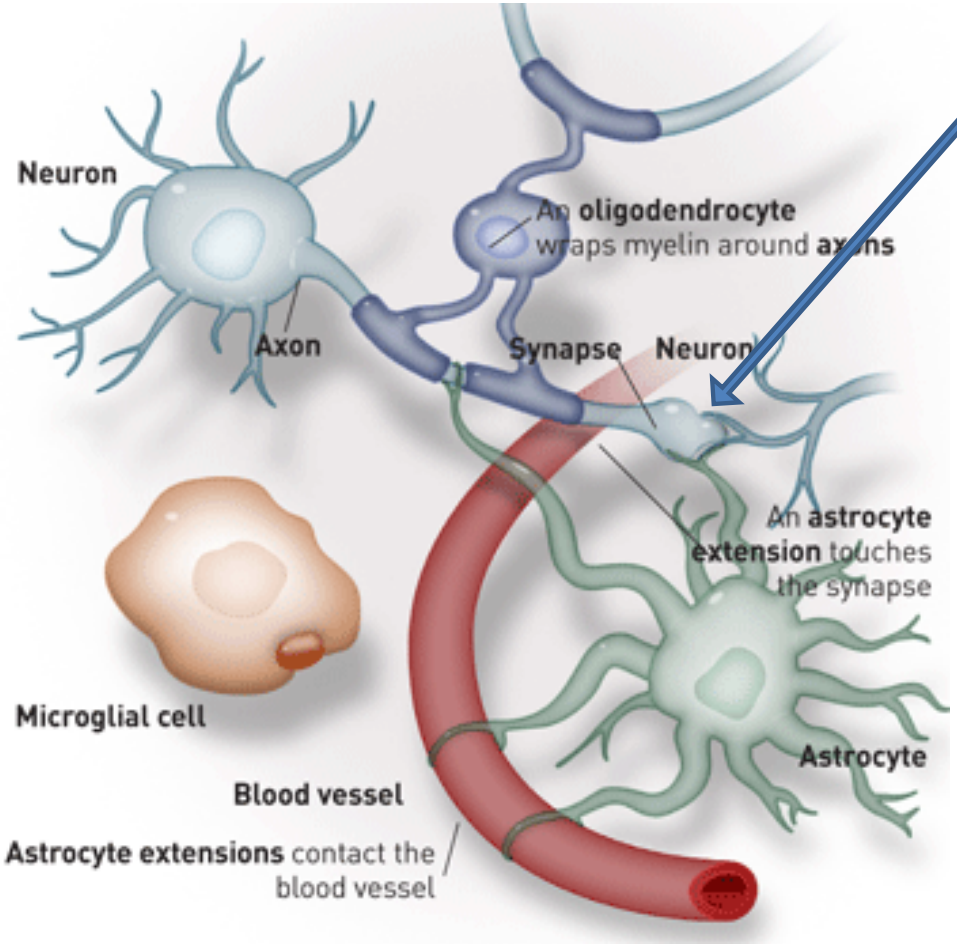
Gene	# Missense Variants with Full or Partial Data Set
GRIN1	84
GRIN2A	159
GRIN2B	151
GRIN2C	3
GRIN2D	12
Total	409

Determination of functional properties for variants yields complex data sets

Receptor	Pheno-type	Glutamate EC_{50},μM (n)	Glycine EC_{50},μM (n)	tau_w ms (n)	Amplitude, peak, pA/pF (n)	Glutamate EC_{50}	Glycine EC_{50}	Mg^{2+} IC_{50}	pH, %	Zn^{2+} IC_{50}	Zn^{2+} min %
WT GluN2A	--	3.4 ± 0.11 (51)	1.2 ± 0.05 (76)	51 ±2.5 (46)	136 ± 19 (41)	--	--	--	--	--	--
GluN2A-V452M	SCZ	1.0 ± 0.13(10)*	1.1 ± 0.11 (12)	70 ± 6.4 (7)	152 ± 55 (6)	3.3	0	0	0	0	0
GluN2A-G483R	Epi	54 ± 6.3 (10)*	1.5 ± 0.12 (16)	20 ± 3.0 (10)*	30 ± 11 (10)*	-12.5	0	0	1.5	-3.3	0
GluN2A-R504W	Epi	2.8 ± 0.18 (6)	0.96 ± 0.04 (4)	89 ± 8.7 (12)*	59 ± 13 (14)	0	0	0	1.3	1.9	2.4
GluN2A-V506A	Epi	2.0 ± 0.18 (6)*	1.1 ± 0.11 (12)	81 ± 13 (7)	67 ± 34 (7)	2.3	0	0	1.2	--	--
GluN2A-K669N	Epi	1.1 ± 0.49 (6)*	0.31 ± 0.05 (10)*	239 ± 30 (13)*	143 ± 40 (11)	4.7	4.9	0	0	0	-1.9
GluN2A-V685G	Epi	270 ± 11 (10)*	1.5 ± 0.14 (6)	24 ± 2.7 (10)*	7.1 ± 3.1 (8)*	-126	0	--	-1.2	0	0
GluN2A-I694T	Epi	9.8 ± 0.37 (12)*	0.94 ± 0.13 (9)	45 ± 3.7 (11)	57 ± 15 (8)*	-2.9	0	0	0	-3.2	0
GluN2A-P699S	Epi	2.2 ± 0.33 (6)*	1.3 ± 0.23 (8)	52 ± 4.7 (7)	98 ± 51 (7)	2.4	0	0	0	-1.7	-1.8
GluN2A-M705V	Epi	5.7 ± 0.14 (8)*	1.0 ± 0.15 (8)	57 ± 9.1 (6)	34 ± 19 (5)	-1.4	0	2	-2.1	0	0
GluN2A-A716T	Epi	20 ± 1.9 (10)*	1.3 ± 0.09 (11)	32 ± 3.6 (13)*	63 ± 15 (14)	-6.6	0	0	0	0	-1.4
GluN2A-A727T	Epi	5.1 ± 0.37 (10)*	1.4 ± 0.09 (8)	50 ± 4.2 (6)	83 ± 24 (6)	-1.7	0	0	0	0	0
GluN2A-D731N	Epi	6418 ± 278 (7)*	1.5 ± 0.26 (10)	---#	0.22 ± 0.16 (5)*	-2913	0	0	-1.6	-5.3	0
GluN2A-V734L	Epi	5.1 ± 0.80 (8)*	1.3 ± 0.10 (12)	30 ± 2.3 (11)*	69 ± 24 (12)	-1.5	0	0	0	0	0
GluN2A-K772E	Epi	4.8 ± 0.17 (10)*	1.3 ± 0.09 (10)	47 ± 5.6 (13)	55 ± 22 (13)*	-1.6	0	0	-1.2	0	0
WT GluN2B	--	1.5 ± 0.07 (57)	0.38 ± 0.03 (47)	570 ± 23 (35)*	41 ± 5.0 (31)*	--	--	--	--	--	--
GluN2B-E413G	ID	79 ± 5.3 (12)*	0.32 ± 0.02 (8)	20 ± 1.3 (9)*	3.3 ± 1.3 (8)*	-57	0	0	0	--	--
GluN2B-C456Y	ID	0.39 ± 0.03 (14)*	1.0 ± 0.05 (7)*	---#	0.03 ± 0.01 (6)*	3	-3.1	0	3.4	--	--
GluN2B-C461F	Epi	169 ± 9.0 (14)*	0.15 ± 0.007 (8)*	28 ± 1.8 (6)*	4.2 ± 1.0 (6)*	-95	1.7	0	2.5	--	--
GluN2B-R696H	ID	0.33 ± 0.07 (8)*	0.44 ± 0.01 (7)	2079 ± 165 (8)*	10 ± 3.6 (7)*	12.2	0	0	--	--	--

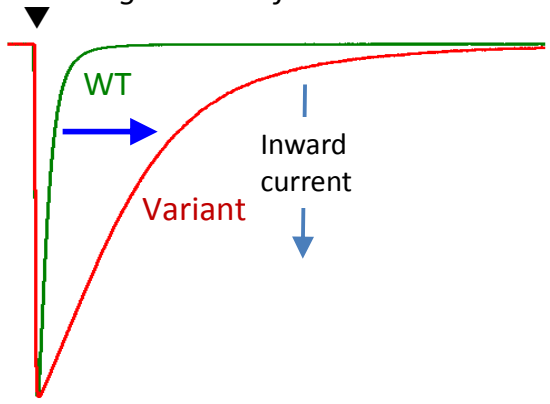
Swanger et al., 2016

Both specific and net functional consequences contribute to mechanism



Synaptic charge transfer

1 mM glutamate for ~ 1-3 ms



Amplitude is controlled by:

Agonist EC_{50}
Surface expression
Open probability
Sensitivity to Mg^{2+} + Endogenous modulators

Time course controlled by:

Deactivation time course and glutamate unbinding

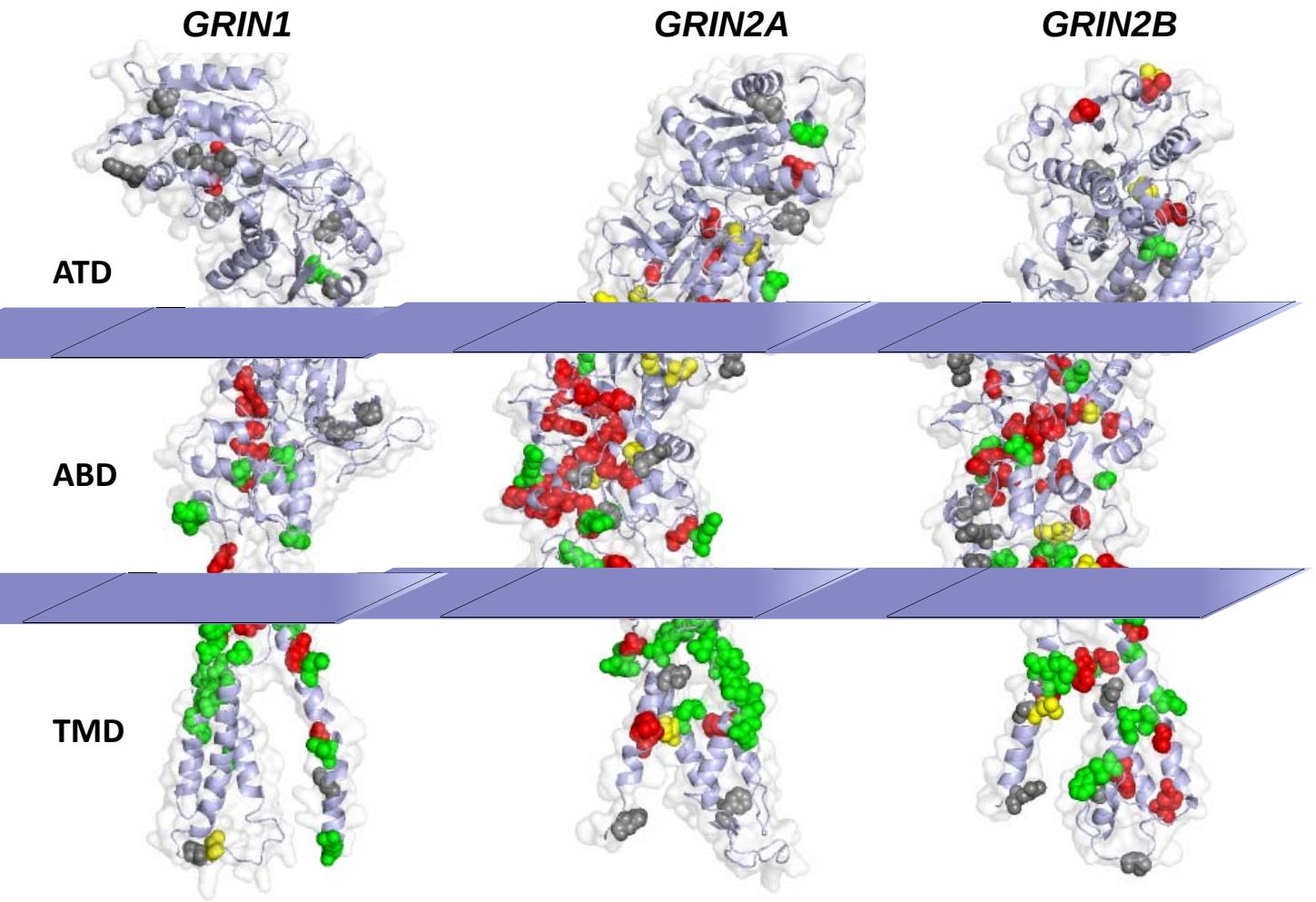
Swanger et al., 2016

Estimating variant effects on synaptic and non-synaptic function

	Relative Synaptic charge transfer	Synaptic Result	Relative Non-synaptic charge transfer	Non-synaptic Result
GluN2A-V452M	**1.4**	Gain of Function	**3.7**	Gain of Function
GluN2A-G483R	**8.5E-02**	Loss of Function	**1.6E-02**	Loss of Function
GluN2A-R504W	**0.26**	Loss of Function	**0.19**	Loss of Function
GluN2A-V506A	**2.0**	Gain of Function	**2.4**	Gain of Function
GluN2A-K669N	**1.2**	--	**0.87**	--
GluN2A-V685G	**4.2E-02**	Loss of Function	**1.5E-03**	Loss of Function
GluN2A-I694T	**0.35**	Loss of Function	**0.15**	Loss of Function
GluN2A-P699S	**0.76**	--	**1.3**	--
GluN2A-M705V	**0.28**	Loss of Function	**0.17**	Loss of Function
GluN2A-A716T	**0.08**	Loss of Function	**0.02**	Loss of Function
GluN2A-A727T	**0.32**	Loss of Function	**0.24**	Loss of Function
GluN2A-D731N	**1.3E-02**	Loss of Function	**5.1E-05**	Loss of Function
GluN2A-V734L	**0.49**	Loss of Function	**0.61**	Loss of Function
GluN2A-K772E	**5.7E-02**	Loss of Function	**4.8E-02**	Loss of Function
GluN2B-E413G	**3.4E-03**	Loss of Function	**2.0E-03**	Loss of Function
GluN2B-C456Y	**0.06**	Loss of Function	**0.21**	Loss of Function
GluN2B-C461F	**5.0E-03**	Loss of Function	**1.0E-03**	Loss of Function
GluN2B-R696H	**1.9**	Gain of Function	**2.4**	Gain of Function

Swanger et al., 2016

Functional effects vary by domain



	Gain of function
	Loss of Function
	Uncertain
	No Effect

ATD N=41 Variants
22% LOF
12% GOF
66% uncertain, no effect

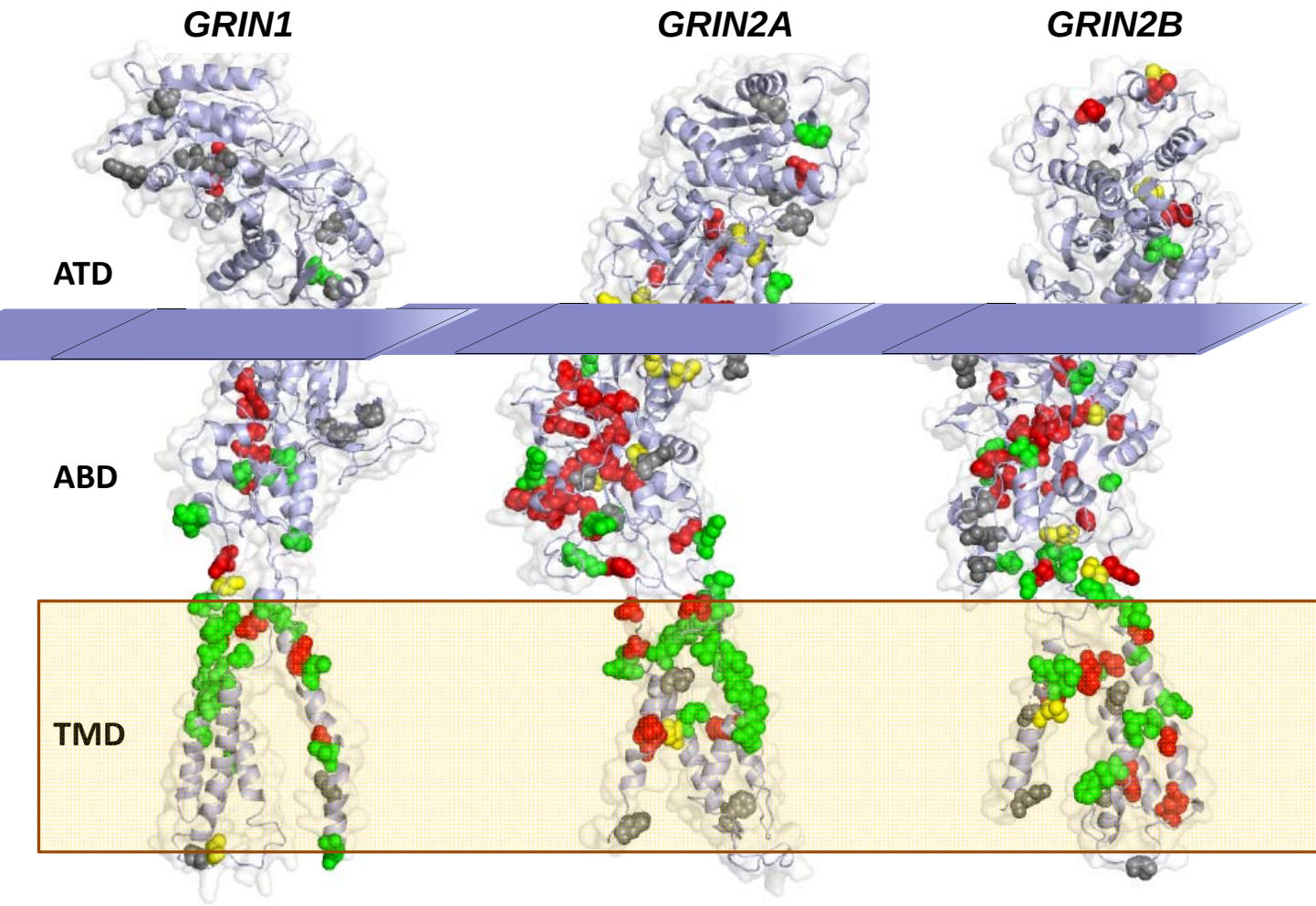
ABD N=88 Variants
56% LOF
12% GOF
32% uncertain, no effect

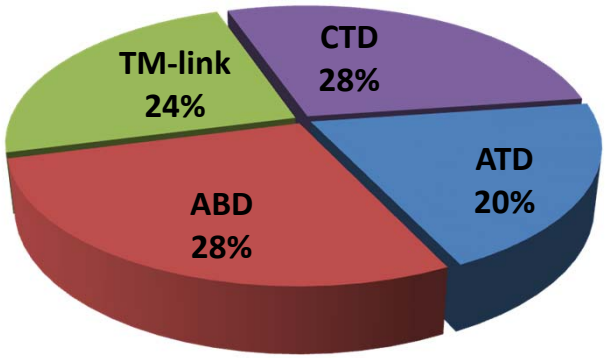
TMD N=106 Variants
24% LOF
62% GOF
14% uncertain, no effect

CTD N=52 Variants, **6% LOF**, **10% GOF**, 84% uncertain, no effect

2019 CFERV, unpublished

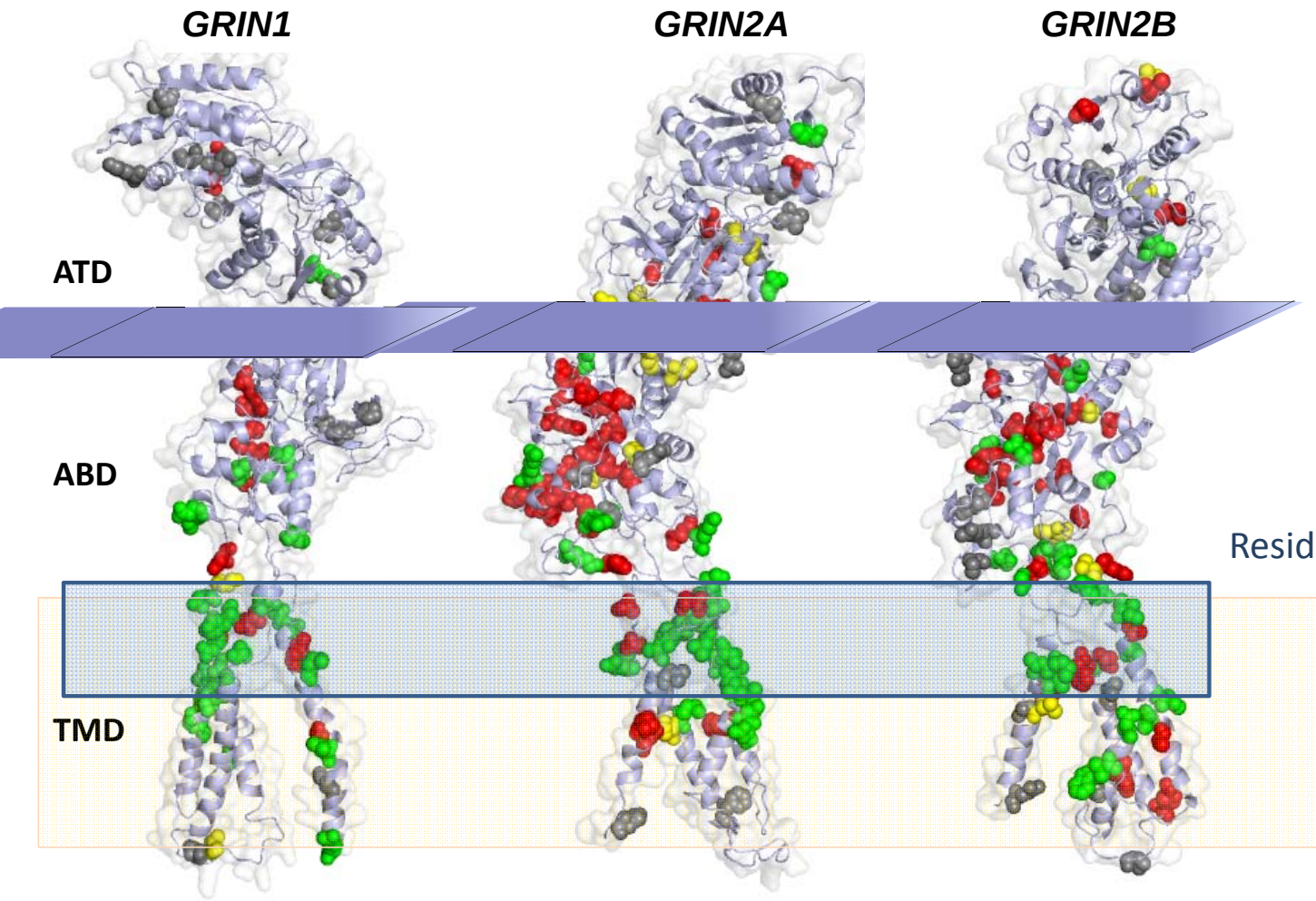
Functional effects vary by domain

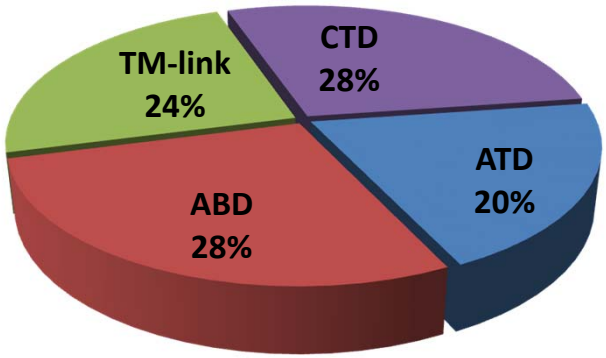




Most severe clinical phenotype
Strehlow et al., 2019

Functional effects vary by domain





Residues in the “Gating Triad”

Summary

1. Functional and structural analyses of variants allows stratification of patients, which is essential for precision medicine approaches

2. We established CFERV to provide functional data world-wide for all GRIN variants.

Results are posted pre-publication to a publicly accessible server

3. Enrollment of GRIN patients in registries (UC-Denver, Leipzig) that track natural history and response to treatments is essential.

Future Directions

1. We are studying mice harboring *GRIN* variants to study mechanisms that contribute to patient phenotype and evaluate genetic and pharmacological treatment strategies.

2. Ten mouse lines exist or are being made

***GRIN2A-S644G**, GRIN2A-P552R, **GRIN2A-KO**, GRIN2D-V667I*

We are collaborating with Wayne Frankel and Ann Poduri

GluN2B-E413G

We are working on this transgenic line

GluN2B-M818T

We are currently making this transgenic line

GluN2A-V685G, GluN2B-S1413L

*GRIN1-Q536R, **GRIN1-Y647C**, GRIN1-L655Q*

Acknowledgements, Support, Disclosure

Laboratory members

Yuchen Xu
Rui Song
Lingling Xie
Riley Perszyk
Chad Camp
Tue Banke
Jing Zhang
Sukhan Kim
James Allen
Scott Myers
Chris Sinon

Collaborators (Other)

Tim Benke (UC Denver)
Johannes Lemke (Leipzig)
Yuwu Jiang (First Peking)
Ann Poduri (Harvard)
Wayne Frankel (Columbia)
Elias Aizenman (Pitt)
Katherine Roche (NIH)
Lonnie Wollmuth (Stoneybrook)
Alasdair Gibb (UCL)
Tim Lovenberg (Janssen)

Collaborators (Emory)

Hongjie Yuan (Pharmacology)
Sooky Koh (Pediatrics)
Andy Jenkins (Anesthesiology)
Dennis Liotta (Chemistry)
Pieter Burger (Chemistry)
Dave Menaldino (Chemistry)

Funding:

NIH-NINDS
CURE
Austin's Purpose Fnd
CureGRIN Fnd

Disclosure (2020):

NeurOp (co-founder)
Sage, Eumentis (SAB)
Janssen (grant, consult)
Allergan (grant)
Biogen (grant)