Exploring the Evolution of Ionotropic Glutamate Receptors in Metazoa

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1 Background

Ionotropic glutamate receptors (iGluRs): ligand-gated ion channels that activate upon binding to excitatory neurotransmitters, predominantly Glutamate.

iGluRs mediate most excitatory neurotransmission in both vertebrates and invertebrates and play an important role in many physiological processes.

Analysis based on sequence similarity and pharmacology proposes a classification of iGluRs into four subfamilies, including AKDF (AMPA, Kainate, Delta and Phi), NMDA, Epsilon and Lambda (1).

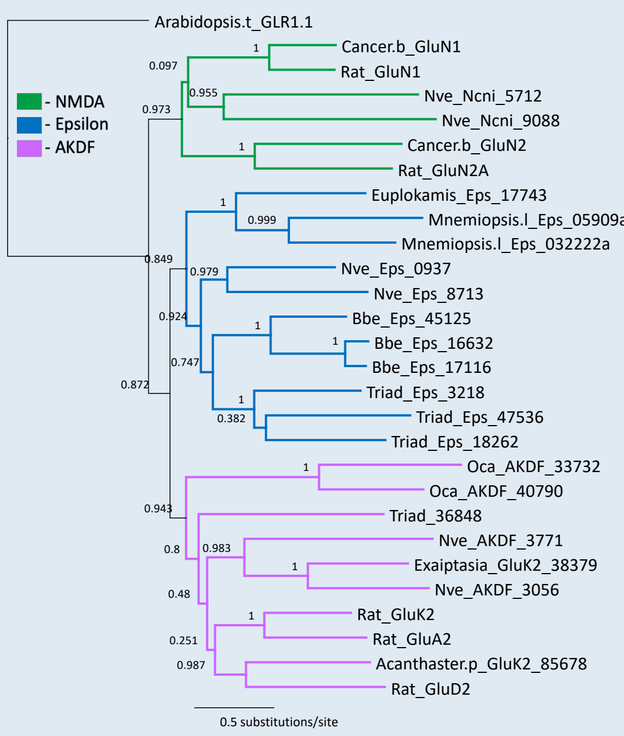
The ligand selectivity and potency of iGluRs is dictated by certain residues lining the agonist binding pocket in iGluR subunits.

Previous structural data suggest that ligand recognition is determined by residues in positions 674, 676, and 725 (nr based on Rat_GluA2), and that residues in positions 499, 501, and 726 influence ligand potency.

Due to lack of functional verification of the structural data, we explored how specific residues within the agonist binding pocket influence the binding efficacy of agonists by using molecular mutagenesis and TEVC recordings.

2 Evolutionary and structural background of iGluRs

Phylogeny of selected metazoan iGluRs



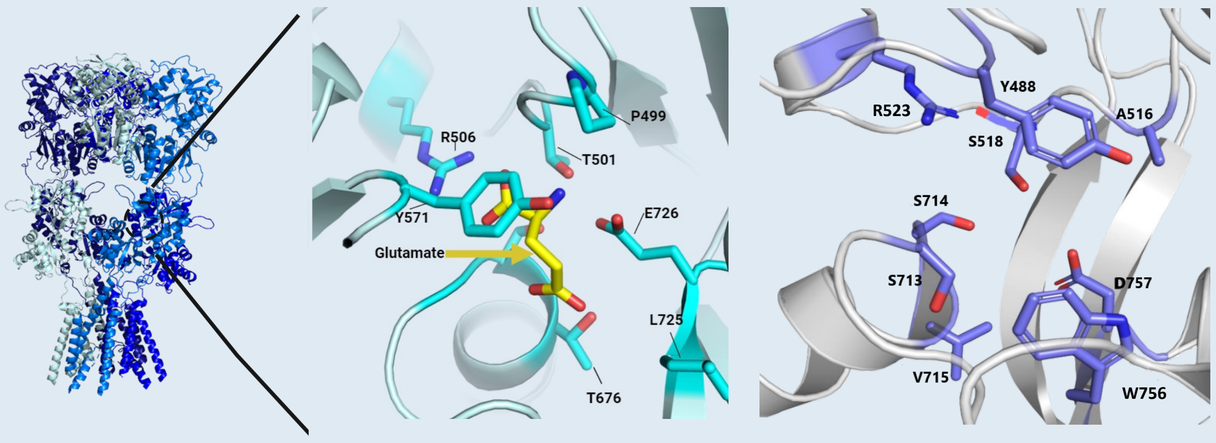
Multiple sequence alignment of iGluR residues involved in in ligand-binding

iGluR genes		Ligand Binding Domain Segments						
	Rat_GluA2	*471*	*499*	*506*	*674*	*725*	*753*
NMDA	Cancer.b_GluN1	QFG	APLTINPERAQV		ATVKGSSVD	IWDS	GYG
	Rat_GluN1	KFG	APLTINNERAQY		ATVKQSSVD	IWDS	GFG
	Cancer.b_GluN2	FWG	TSIKINSEREEV		ATTLHGNTD	LYDA	GYG
	Rat_GluN2A	KHG	GSLTINEERSEV		GTVPNGSTE	IYDA	GYG
Epsilon	Nve_Eps_0937	NFG	TSLTISPERQKV		GTVLNSQPQ	IWDS	GYG
	Nve_Eps_8713	QFG	APLTISSEGQTV		GTLMNSQLQ	ISDR	GYG
	Bbe_Eps_45125	NFG	ATLTITAQREEA		GARGSGATE	ISDN	GYG
	Bbe_Eps_17116	QYG	AIMSITAQRQAD		GAISTYSSW	FTDS	GYG
	Triad_Eps_47536	QYG	VPLSITAELQEH		GVLKEGSTN	IADS	GHG
	Triad_Eps_18262	**SYG**	**GAFSISVSRAGE**		**GTVSGSSVH**	**LWDA**	**GYA**
AKDF	Triad_36848	IFG	APLSITSFRQSV		GTVIDSEAM	IWYY	GFG
	Exaiptasia_AKDF_38379	AYG	APITIYATRESV		GVLKGGAID	LTEQ	SYG
	Nve_AKDF_3056	SYG	GPITITAEREEV		GVQEGGSLF	LTDQ	SYA
	Rat_GluK2	KYG	APLAITYVREKV		GAVEDGATM	LMES	GYG
	Rat_GluA2	**KYG**	**APLTITLVREEV**		**GTLDSGSTK**	**LLES**	**GYG**
	Rat_GluD2	KYG	SALTITPDRENV		GTVLDSAVY	VWDA	GYG

Genes of interest

	Rat_GluA2	*499*	*506*	*674*	*725*	Selectivity
Cancer.b_GluN1		APLTINPERAQV		ATVKGSSVD	IWDS	Glycine
Rat_GluN1		APLTINNERAQY		ATVKQSSVD	IWDS	Glycine, D-Serine
Cancer.b_GluN2		TSIKINSEREEV		ATTLHGNTD	LYDA	Glutamate
Rat_GluN2A		GSLTINEERSEV		GTVPNGSTE	IYDA	Glutamate
Nve_Eps_8713		APLTISSEGQTV		GTLMNSQLQ	ISDR	unknown
Bbe_Eps_45125		ATLTITAQREEA		GARGSGATE	ISDN	Glutamate
Triad_Eps_47536		VPLSITAELQEH		GVLKEGSTN	IADS	unknown
Triad_Eps_18262		**GAFSISVSRAGE**		**GTVSGSSVH**	**LWDA**	**Glycine, D-Serine**
Nve_AKDF_3056		GPITITAEREEV		GVQEGGSLF	LTDQ	Glutamate, Aspartate
Rat_GluK2		APLAITYVREKV		GAVEDGATM	LMES	Glutamate
Rat_GluA2		**APLTITLVREEV**		**GTLDSGSTK**	**LLES**	**Glutamate**
Rat_GluD2		SALTITPDRENV		GTVLDSAVY	VWDA	Glycine, D-Serine

Homology models of iGluR agonist binding pocket



Rat_GluA2 tetramer (4)

Rat GluA2 agonist binding pocket

Trichoplax Epsilon^{18262} iGluR agonist binding pocket

3 Experimental approach to simulate evolution of iGluRs

Gene variants created:

Sequencing result of the gene variants electrophysiologically tested:

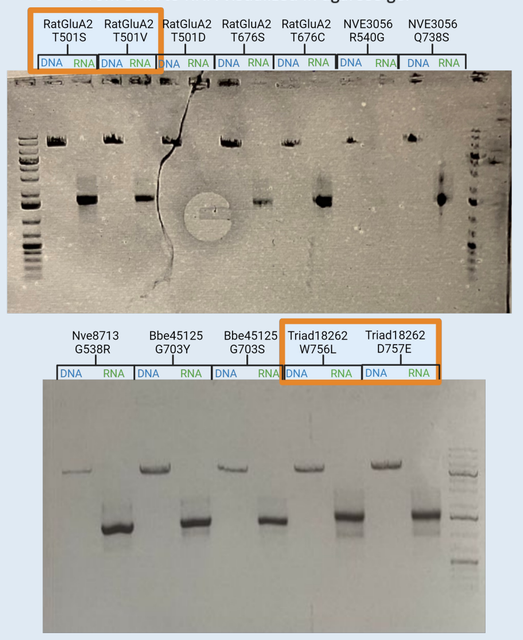
Rat (AMPA)
Rat_GluA2_T501S → CCATTAAGTATCACT
Rat_GluA2_T501V → CCATTAGTTATCACT
Rat_GluA2_T501C ----------
Rat_GluA2_T501D ----------
Rat_GluA2_T676S ----------
Rat_GluA2_T676C ----------
Rat_GluA2_T676D ----------

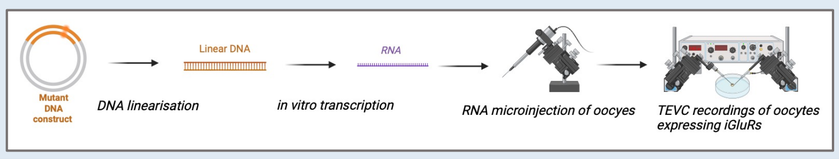
Nematostella(Epsilon)
NVE3056_R540G ----------
NVE3056_Q738S ----------
Nve8713_G538R ----------

Trichoplax(Epsilon)
Tad47536_L512R ----------
Triad18262_F517L ----------
Triad18262_S713G ----------
Triad18262_V715T ----------
Triad18262_S713G_V715T ----------
Triad18262_W756L → TTTCTGTTGGATGCA
Triad18262_W756T ----------
Triad18262_D757E → TGTGGGAAGCAGCTT

Branchiostoma(Epsilon)
Bbe45125_G703Y ----------
Bbe45125_G703S ----------

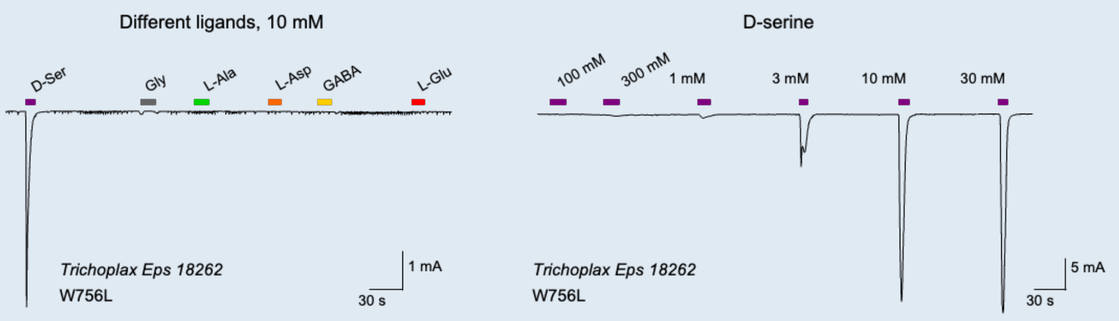
From DNA to RNA visualized in agarose gel

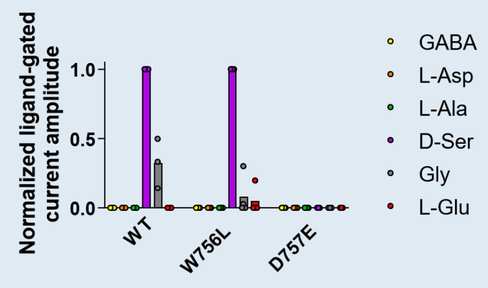




4 Electrophysiological recordings of Trichoplax and Rat iGluRs expressed in *Xenopus laevis* oocytes

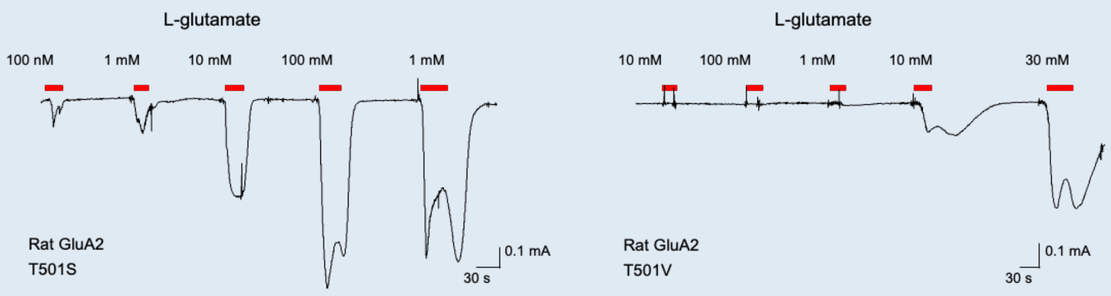
Ligand-gated current responses in oocytes expressing mutant W756L *Trichoplax* Epsilon^{18262} iGluRs

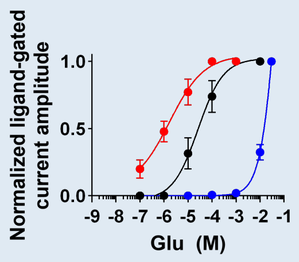




Change in *Trichoplax* Epsilon^{18262} iGluR function through amino acid substitution

The current response recorded in oocytes expressing mutant T501S and T501V Rat GluA2 AKDF iGluR when exposed to L-glutamate





Summary of the expression by iGluR channels with different amino acids substituting at T501 position

5 Conclusion

Due to the increase in T501S iGluR expression and decrease in T501V iGluR expression when compared to the wildtype T501, the results confirm that polar interactions between T501 (or equivalent) and the ligand amine group are important for ligand sensitivity.

Among the more divergent residues in position equivalent to 725 in Rat GluA2, the W/L difference between glycine-binding and glutamate-binding receptors does not seem to determine ligand selectivity, although it may contribute to ligand potency.

References:
1. Ramos-Vicente, D., Grant, S. G. N., & Bayés, À. (2021). Metazoan evolutionand diversity of glutamate receptors and their auxiliary subunits. *Neuropharmacology*, *195*, 108640.
2. Ramos-Vicente, D., Ji, J., Gratacòs-Batlle, E., Gou, G., Reig-Viader, R., Luís, J., Burguera, D., Navas-Perez, E., García-Fernández, J., Fuentes-Prior, P., Escriva, H., Roher, N., Soto, D., & Bayés, À. (2018). Metazoan evolution of glutamate receptors reveals unreported phylogenetic groups and divergent lineage-specific events. eLife, 7, e35774.
3. Sandra, S., Oksana, S., Yuhong, W., Hassan, Y. H., & Timothy, L. (2023). Constitutive activity of ionotropic glutamate receptors via a hydrophobic plug in the ligand-binding domain. bioRxiv, 2023.2008.2003.551817.
4. Rat GluA2 tetramer - PDB:3KG2, Rat GluA2/Glu binding pocket - PDB:1FTJ, Triad^{18262} binding pocket - AlphaFold Model

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