Structural determinants and mechanism of action of a GluN2C-selective NMDA receptor positive allosteric modulator

Alpa Khatri, Pieter B. Burger, Sharon A. Swanger, Kasper B. Hansen, Sommer Zimmerman, Erkan Karakas, Dennis C. Liotta, Hiro Furukawa, James. P. Snyder, Stephen F. Traynelis

Journal: Molecular Pharmacology

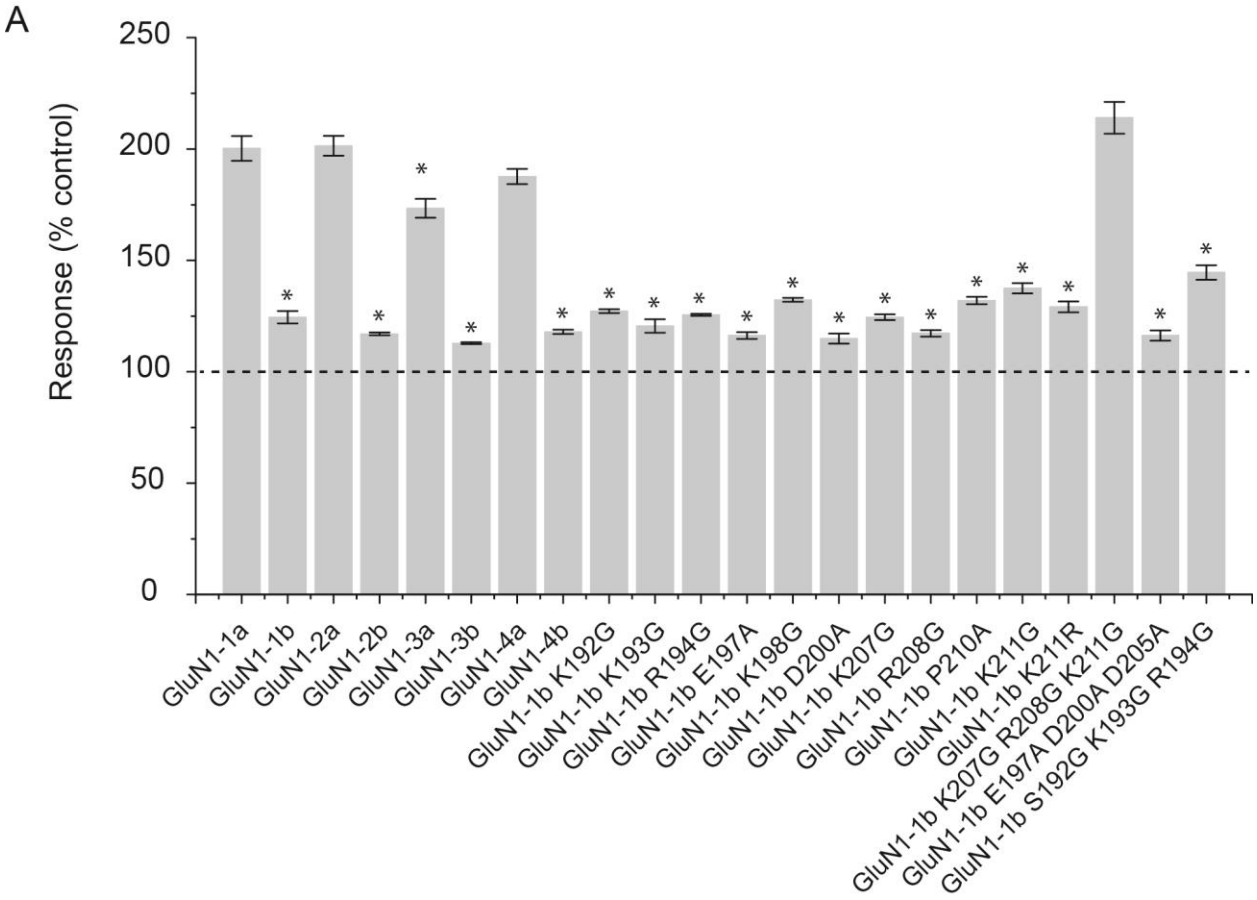
Emory University, Pharmacology Department: AK, SAS, SFT

Emory University, Chemistry Department: SZ, PB, DCL, JPS

University of Montana, Dept of Biomedical and Pharmaceutical Sciences, and Center for Biomolecular Structure and Dynamics: KBH

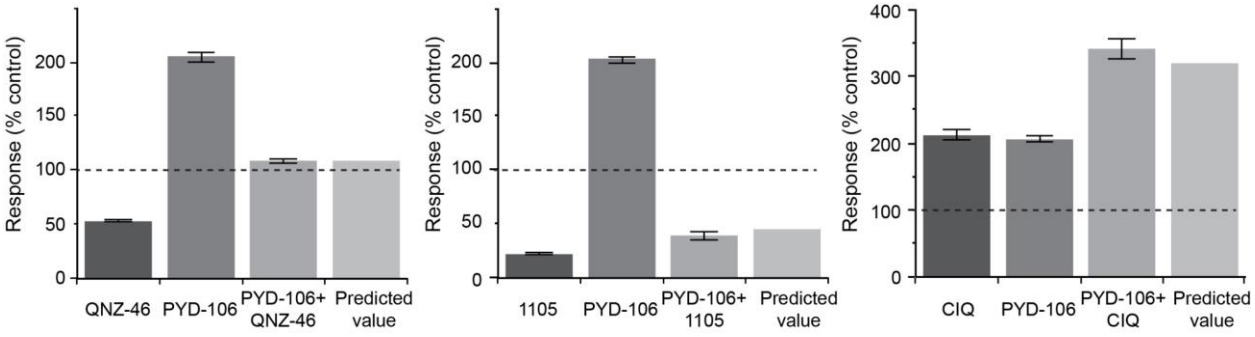
Cold Spring Harbor Labs: EK, HF

Supplemental Figure S1.



Supplemental Figure S1. Evaluation of the effects of individual GluN1-1b exon-5 mutations on enhancement of the responses of GluN1-1b/GluN2C expressed in *Xenopus* oocytes to 100 μM glutamate and 30 μM glycine by 100 μM PYD-1. The response shown is the ratio of current obtained in the presence and absence of PYD-1, and is shown as a percent of control recorded in the same oocyte. For all responses, n = 4-18 oocytes. * $p < 0.05$ paired *t*-test or ANOVA against control experiments with wild-type receptor.

Supplemental Figure S2.



Supplemental Figure S2. (A-C) PYD-106 was co-applied with QNZ-46 (2C/D inhibitor), DQP-1105 (2C/D inhibitor), and CIQ (2C/D positive allosteric modulator) to determine if PYD-106 shares structural determinants of action with these modulators. The effects of co-applying PYD-106 can be calculated assuming its actions are independent of modulation by QNZ-46, DQP-1105, or CIQ (given as the predicted value). The control response in the absence of PYD is shown as a dashed line, and is taken as 100%. Co-application of PYD-106 should enhance the response in QNZ-46 to 108% of drug-free control, in DQP-1105 inhibition to 46% of drug-free control, and in CIQ to 318% of drug-free control. The observed values were 108 ± 2.3% of control for QNZ-46, 39 ± 3.5% of control for DQP-1105, and 340 ± 14.3% of control for CIQ. The predicted values for QNZ-46 and CIQ co-modulation were within 6% of observed values ($p < 0.05$), consistent with independent sites of action. The predicted co-modulation of DQP-1105 was 5.5% higher than that observed ($p < 0.05$), raising the possibility that QNZ-46 and PYD-106 may share some downstream structural determinants (for all n = 4-7 oocytes, one way ANOVA, Tukey's post hoc).

Supplemental Table S1.

Chimera	GluN2A	GluN2C
GluN2A	1462	0
GluN2C	0	1238
2A (2C ATD)	391-end	1-400
2A (2C ATD, L0)	405-end	1-414
2A (2C ATD, L0, S1)	538-end	1-535
2A (2CL0, S1, M123, S2)	1-390, 803-end	401-813
2A (2C S2)	1-656, 814-end	654-811
2C (2A L0, S1, M123, S2)	383-813	1-330, 812-end
2C (2A ATD)	1-382	330-end
2C (2A L0)	391-404	1-388, 401-end
2C (2A L0, S1)	391-539	1-388, 538-end
2C (2A S1)	405-539	1-401, 538-end
2C (2A S2)	661-801	1-658, 800 to end

The amino acid compositions of the chimeras are given as the GluN2A and GluN2D amino acids included in the chimera (See Figure 6).

Supplementary Table S2: Off-target responses for PYD-106

Receptor	100 – 100 ($I_{Test(PYD-106)} / I_{control}$) (%)	n
Serotonin receptor 5-HT1A	9.1	4
Serotonin receptor 5-HT1B	26.1	4
Serotonin receptor 5-HT1D	-11.3	4
Serotonin receptor 5-HT1E	11.2	4
Serotonin receptor 5-HT2A	-3.7	4
Serotonin receptor 5-HT2B	40.8	4
Serotonin receptor 5-HT2C	-29.7	4
Serotonin receptor 5-HT3	-9	4
Serotonin receptor 5-HT5A	1.2	4
Serotonin receptor 5-HT6	17.8	4
Serotonin receptor 5-HT7	11.8	4
Adrenergic receptor α1A	-19.3	4
Adrenergic receptor α1B	21	4
Adrenergic receptor α1D	0.6	4
Adrenergic receptor α2A	25.5	4
Adrenergic receptor α2B	11	4
Adrenergic receptor α2C	51.4	4
Adrenergic receptor β2	2.1	4
Adrenergic receptor β3	-9.9	4
Benzodiazepine site in Rat Brain	1.8	4
Dopamine receptor D1	-0.1	4
Dopamine receptor D2	20.7	4
Dopamine receptor D3	-1.1	4
Dopamine receptor D4	21.6	4
Dopamine receptor D5	19.2	4
Opioid receptor (δ)	-1.2	4
Opioid receptor (κ)	76.8	4
Opioid receptor (μ)	4.5	4
Histamine receptor H1	4.7	4
Histamine receptor H2	-16.6	4
Histamine receptor H3	14	4
Muscarinic receptor M1	-14.2	4
Muscarinic receptor M2	-18	4
Muscarinic receptor M3	-10.7	4
Muscarinic receptor M4	1.1	4
Muscarinic receptor M5	-1.9	4
Sigma receptor 1	31.9	4
Sigma receptor 2	-14.2	4
Peripheral benzodiazepine receptor	-2	4
Norepinephrine transporter	-18.6	4
Dopamine transporter	52.6	4
Serotonin transporter	18.6	4

PYD-106 (10 μM) was screened for binding to 42 receptors, transporters, or ion channels in primary radioligand-binding assays; the percent inhibition of binding is shown. Targets at which the primary screen showed more than 50% inhibition of binding were subject to determination of Ki; both the adrenergic α2C receptor and dopamine transporter had a Ki of greater than 10 μM whereas the κ-Opioid receptor (KOR) had a Ki of 6 μM. Data is from the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-025C. The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill (Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA).

Supplemental Table S3.

	$I_{TEST}/ I_{CONTROL}$ (%)						
# [a]	$GluN2C_{C1}$*+$GluN2C_{C2}$* [b]			$GluN2A_{C1}$+$GluN2C_{C2}$* [b]			N
PYD-1	213	±	11.1	92	±	1.4	4
PYD-100	212	±	4.8	86	±	1.3	4
PYD-115	146	±	7.0	96	±	1.7	4
PYD-156	156	±	6.4	92	±	2.5	4
PYD-84	246	±	3.6	88	±	1.8	4
PYD-103	170	±	4.1	96	±	2.5	4
PYD-102	160	±	2.3	92	±	0.9	4
PYD-106	231	±	4.0	93	±	1.5	4
PYD-72	212	±	5.2	93	±	1.3	4
PYD-62	194	±	2.4	89	±	1.0	4
PYD-69	156	±	1.9	89	±	1.2	4
PYD-119	245	±	5.2	94	±	3.5	4
PYD-65	225	±	0.6	85	±	0.7	4
PYD-118	149	±	0.8	90	±	0.7	4
PYD-116	207	±	9.0	79	±	1.0	4
PYD-117	197	±	1.3	85	±	2.6	4
PYD-111	177	±	2.9	97	±	2.3	4
PYD-112	170	±	1.8	85	±	0.9	4
PYD-113	149	±	1.1	87	±	0.5	4

[a] Corresponding number in Zimmerman et al 2014.

[b] Responses as percent of control are shown for 100 μM of the indicated PYD analogue co-applied with 100 μM glutamate and 30 μM glycine.