Supplemental material

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An equilibrium binding model for noncompetitive AMPA receptor antagonists

The model in Fig. 3 C assumes that allosteric modulators can bind to four sites in the closed form of the receptor and two in the open form. This assumption is based on the differences between the open and closed structures of the receptor protein, in which two of the four sites are occluded in the open form (Fig. 3, C and D). Using simple equilibrium equations without cooperativity of binding and assuming values for the open/closed equilibrium (K_1) and the binding of modulator to the open (K_3) and closed (K_2) states, it is possible to predict the fraction of channels in the open state as a function of free modulator concentration (M), assuming that all glutamate binding sites are occupied as expected at 5 mM glutamate, using the following equation:

$$\text{Fraction open} = \frac{1 + \frac{2M}{K_3} + \frac{M^2}{K_3^2}}{1 + \frac{2M}{K_3} + \frac{M^2}{K_3^2} + K_1\left(1 + \frac{4M}{K_2} + \frac{6M^2}{K_2^2} + \frac{4M^3}{K_2^3} + \frac{M^4}{K_2^4}\right)}. \quad (1)$$

The "solver" routine in Microsoft Excel was used to fit the whole-cell data for GYKI-52 to this equation, and a reasonable fit was obtained (Fig. 3 F). This demonstrates that, despite an interaction with the open state, increasing the concentration of GYKI-52 can result in full inhibition. The single-channel data, particularly for GYKI-52, show a dramatic effect of the drug even at concentrations (10–20 µM) where only modest inhibition is observed. Using the three equilibrium constants determined from the whole-cell data and the model in Fig. 3 E, the fraction of receptors in the different, individual open states (RL_4^*, RL_4M^*, and $RL_4M_2^*$) can be predicted as a function of modulator concentration (Fig. 4 F, red, green, and blue lines). The three curves are generated using the denominator in the equation above with the following numerators: 1 for RL_4^*, $2M/K_3$ for RL_4M^*, and M^2/K_3^2 for $RL_4M_2^*$. Based on this, we would expect that any given single channel that is recorded in the range of 10–20 µM would be bound to one or two GYKI-52 molecules, and that it would be unlikely to observe a channel without GYKI-52 bound.

Table S1. **Control data mean time constant values; time constant values are sorted by occupancy pattern**

Pattern # LL/event	C_c	C_b	C_a	O_{1a}	O_{1b}	O_{1c}	O_{2a}	O_{2b}	O_{3a}	O_{3b}	O_{4a}	O_{4b}
P4	48 ± 26	6.1 ± 2.0	1.4 ± 0.50	1.8 ± 0.67	8.5 ± 0.09	30	0.94 ± 0.12	5.4 ± 2.4	1.6 ± 0.62	6.6 ± 0.78	5.5 ± 98	22 ± 3.8
3.7 ± 0.65	0.045 ± 0.069	0.45 ± 0.34	0.50 ± 0.412	0.74 ± 0.068	0.20 ± 0.028	0.10	0.59 ± 0.30	0.41± 0.30	0.46 ± 0.42	0.27 ± 0.14	0.77 ± 0.059	0.22 ± 0.064
P3	68 ± 41	7.5 ± 1.9	1.1	0.92 ± 0.42	5.9 ± 1.6	33 ± 12	0.78 ± 0.92	11 ± 3.0	6.2 ± 4.8	17 ± 5.2	3.8 ± 0.86	16 ± 3.9
3.3 ± 0.49	0.10 ± 0.069	0.51 ± 0.35	0.35	0.60 ± 0.068	0.36 ± 0.007	0.091 ± 0.011	0.40 ± 0.45	0.50 ± 0.45	0.67 ± 0.034	0.30 ± 0.001	0.91 ± 0.016	0.087 ± 0.017
P2	25 ± 5.6	6.1 ± 2.1	1.8 ± 1.5	1.3 ± 0.2	8.2 ± 3.4	50	2.1 ± 1.0	12 ± 5.3	2.7 ± 0.2	14 ± 2.9	2.4 ± 0.4	9.8 ± 2.0
3.9 ± 0.22	0.034 ± 0.012	0.19 ± 0.058	0.77 ± 0.079	0.70 ± 0.094	0.26 ± 0.078	0.007	0.67 ± 0.021	0.32 ± 0.022	0.82 ± 0.15	0.17 ± 0.16	0.90 ± 0.058	0.086 ± 0.059
P1	35 ± 6.8	8.9 ± 2.0	3.3	7.3 ± 1.5	48 ± 16	–	5.1 ± 1.7	36 ± 12	3.2 ± 1.1	18 ± 11	4.2 ± 2.1	49 ± 38
3.2 ± 0.54	0.14 ± 0.13	0.61 ± 0.39	0.24	0.81 ± 0.052	0.19 ± 0.052		0.85 ± 0.077	0.15 ± 0.076	0.75 ± 0.19	0.25 ± 0.19	0.93 ± 0.043	0.071 ± 0.040

Mean time constants (τ in ms) ± SD for states (top line) and their fractional areas ± SD (bottom line) are shown in each cell.

Table S2. **Control data mean equilibrium constant values; equilibrium constants (mean ± SD) for control data patterns**

Pattern #	$K_{Cc\text{-}Cb}$	$K_{Cb\text{-}Ca}$	$K_{Ca\text{-}O1a}$	$K_{O1a\text{-}O1b}$	$K_{O1b\text{-}O1c}$	$K_{O1a\text{-}O2a}$	$K_{O2a\text{-}O2b}$	$K_{O2a\text{-}O3a}$	$K_{O3a\text{-}O3b}$	K_{O3a_O4a}	$K_{O4a\text{-}O4b}$
P4	2.1 ± 1.7	2.2 ± 0.51	5.0 ± 2.0	1.1 ± 0.82	–	1.6 ± 1.3	2.6 ± 3.6	18 ± 27	0.89 ± 0.63	1.6 ± 0.80	0.25 ± 0.20
P3	1.6 ± 0.04	1.2 ± 0.52	2.8 ± 1.4	1.4 ± 0.40	0.77 ± 0.15	1.7 ± 1.8	7.8 ± 9.2	14 ± 17	0.41 ± 0.27	0.29 ± 0.34	0.18 ± 0.06
P2	2.2 ± 0.67	15 ± 11	1.7 ± 0.97	1.7 ± 0.64	–	5.5 ± 6.3	0.64 ± 0.19	0.51 ± 0.31	0.89 ± 0.91	0.08 ± 0.02	0.39 ± 0.26
P1	3.0 ± 3.1	5.1 ± 4.5	4.8 ± 4.3	0.68 ± 0.22	–	0.53 ± 0.18	0.48 ± 0.28	0.22 ± 0.06	0.47 ± 0.30	0.29 ± 0.19	0.29 ± 0.42

K_{eq} constants (mean ± SD) for transitions (top line) are shown in each cell.

Table S3. **GYKI-52 data; time constants observed in the presence of GYKI-52**

Pattern # LL/event	C_c	C_b	C_a	O_{1a}	O_{1b}	O_{2a}	O_{2b}	O_{3a}	O_{3b}	O_{4a}
P4	23	4.0	0.46	–	–	–	–	–	–	22
3.5	0.28	0.48	0.24							1.0
P3	250	17	0.71	–	–	–	–	9.6	21	–
3.5	0.004	0.69	0.30					0.67	0.33	
P2	24 ± 0.78	3.9 ± 0.70	0.35 ± 0.13	–	–	3.3 ± 0.38	30 ± 19	–	–	–
3.8 ± 0.31	0.18 ± 0.055	0.44 ± 0.060	0.43 ± 0.001			0.14 ± 0.11	0.87 ± 0.11			
P1	38 ± 10	5.4 ± 0.39	1.0 ± 0.11	4.1 ± 0.17	34 ± 18	–	–	–	–	–
3.53 ± 0.034	0.039 ± 0.036	0.380 ± 0.11	0.58 ± 0.073	0.13 ± 0.001	0.78 ± 0.13					

Data from the six recordings fitted with a linear (C–O) reaction mechanism were grouped by open conductance level. Time constant (τ in ms) values (above) and areas for each (below) are reported for each state. Time constant data for multilevel channel behavior in P3b and P2b are not included. Linear–branched models fit these data with LL/event statistics of 3.50 and 2.99, respectively.

Table S4. **GYKI-52 data; equilibrium constants (mean ± SD) for GYKI-52 data**

Pattern	$K_{Cd\text{-}Cc}$	$K_{Cc\text{-}Cb}$	$KC_{b\text{-}Ca}$	$K_{Ca\text{-}O1a}$	$K_{O1a\text{-}O1b}$	$K_{Ca\text{-}O2a}$	$K_{O2a\text{-}O2b}$	$K_{O2b\text{-}O2c}$	$K_{Ca\text{-}O3a}$	$K_{O3a\text{-}O3b}$	$K_{Ca\text{-}O4a}$	$K_{O4a\text{-}O4b}$
P4	7.0	0.89	0.39	–	–	**–**	–		**–**	–	**1.6 ± 0.80**	–
P3	0.50 ± 0.51	6.7 ± 7.7	0.21 ± 0.02	–	–	**–**	–	–	**9.8 ± 5.0**	0.33 ± 0.26	–	–
P2	–	1. 2	0.56	–	–	**2.3**	0.77	–	–	–	–	–
P1	0.14 ± 0.19	2.5 ± 1.7	1.1 ± 0.07	11.7 ± 0.21	1.2 ± 0.07	**–**	–		–	–	–	–
Group	$K_{Cd\text{-}Cc}$	$K_{Cc\text{-}Cb}$	$K_{Cb\text{-}Ca}$	$K_{Ca\text{-}O1a}$	$K_{O1a\text{-}O1b}$	$K_{Ca\text{-}O2a}$	$K_{O2a\text{-}O2b}$	$K_{Ca\text{-}O2b}$	$K_{O2a\text{-}O3a}$	$K_{O3a\text{-}O3b}$	$K_{O3a\text{-}O4a}$	$K_{O4a\text{-}O4b}$
Multi-level O_3>O_2	0.98	1.3	0.57	–	–	0.41	6.7	**2.9**	14.7	0.05	–	–
Multi-level O_3>O_4>O_2	–	1.0	0.46	–	–	0.41	2.9	–	7.8	1.1	0.64	0.09

K_{eq} values for the two channels that exhibited more than one open level are shown individually.

Table S5. **GYKI-53 data; time constants in GYKI-53 by occupancy pattern**

Pattern # LL/event	C_c	C_b	C_a	O_{1a}	O_{1b}	O_{2a}	O_{2b}	O_{3a}	O_{3b}	O_{4a}	O_{4b}
P4	–	30 ± 1.1	7.6 ± 0.52	0.59 ± 0.06	4.1 ± 0.98	0.60 ± 0.59	–	1.0 ± 0.13	5.0 ± 0.11	6.8 ± 1.6	–
3.2 ± 0.13		0.34 ± 0.045	0.56 ± 0.057	0.65 ± 0.21	0.40 ± 0.14	0.98 ± 0.002		0.75 ± 0.076	0.22 ± 0.12	0.93 ± 0.056	
P3	65 ± 24	12 ± 63	1.7 ± 1.6	0.98 ± 0.75	4.8 ± 1.3	1.4 ± 0.70	23 ± 9.7	4.5 ± 3.6	12 ± 1.1	4.1 ± 4.2	8.9 ± 6.1
3.5 ± 0.13	0.15 ± 0.14	0.57 ± 0.32	0.25 ± 0.23	0.92 ± 0.059	0.066 ± 0.036	0.95 ± 0.024	0.017 ± 0.016	0.67 ± 0.49	0.46 ± 0.44	0.92 ± 0.036	0.092 ± 0.10
P2	72	17 ± 0.87	3.6 ± 1.3	1.2 ± 0.04	10 ± 4.5	2.5	23 ± 7.2	2.9	15 ± 2.3	–	–
3.1 ± 0.64	0.003	0.19 ± 0.023	0.79 ± 0.008	0.78 ± 0.066	0.29 ± 0.087	0.29	0.85 ± 0.22	0.42	0.56 ± 0.62		
P1	170 ± 21	23 ± 8.1	3.9 ± 0.40	5.5 ± 3.5	32 ± 20	5.7 ± 4.1	35 ± 29	1.7	120	–	–
3.0 ± 0.40	0.13 ± 0.15	0.071 ± 0.013	0.82 ± 0.17	0.65 ± 0.37	0.35 ± 0.37	0.78 ± 0.10	0.22 ± 0.10	0.93	0.068		

Mean time constants (τ in ms) ± SD for states (top line) and their fractional areas ± SD (bottom line) are shown in each cell.

Table S6. **GYKI-53 data; equilibrium constants in GYKI-53 by occupancy pattern and model**

Loop Models	$K_{Cc\text{-}Cb}$	$K_{Cb\text{-}Ca}$	$K_{Ca\text{-}O1a}$	$K_{O1a\text{-}O1b}$	$K_{C1\text{-}O2a}$	$K_{O1a\text{-}O2a}$	$K_{O2a\text{-}O2b}$	$K_{C1\text{-}O3a}$	$K_{O1a\text{-}O3a}$	$K_{O2a\text{-}O3a}$	$K_{O3a\text{-}O3b}$	$K_{O1a\text{-}O4a}$	$K_{O3a\text{-}O4a}$	$K_{O4a\text{-}O4b}$
P4 2-loop	–	2.0	0.13	–	–	0.98	–	–	**2.6**	2.7	–	**16**	5.3	_
P4 1-loop	–	1.9	0.18	–	–	0.28	–	–	**1.3**	–	0.32	–	5.5	–
P3 2-loop	1.3	0.91	0.08	0.13	–	0.89	0.39	**2.7**	**33**	37	0.43	**–**	0.17	0.09
P3 1-loop	0.42	0.2	0.25	0.15	**0.06**	0.25	4.4	–	–	28	–	–	0.07	–
P2 1-loop	–	2.9	0.53	0.82	**13**	–	–	–	–	0.13	–	**–**	–	–
Linear Models	$K_{Cc\text{-}Cb}$	$K_{Cb\text{-}Ca}$	$K_{Ca\text{-}O1a}$	$K_{O1a\text{-}O1b}$	$K_{O1b\text{-}O1c}$	$K_{O1a\text{-}O2a}$	$K_{O2a\text{-}O2b}$	$K_{O2a\text{-}O3a}$	$K_{O3a\text{-}O3b}$	$K_{O3a\text{-}O4a}$				
P2 n = 3	4.8 ± 8.4	1.7 ± 0.29	0.55 ± 0.26	2.6 ± 0.81	0.53 ± 0.66	20 ± 7.6	0.90 ± 0.78	0.23 ± 0.21	0.20 ± 0.18	0.20 ± 0.18	–	–	–	–
P1 n = 2	0.34 ± 0.48	1.9 ± 0.14	3.5 ± 0.57	–	–	0.53 ± 0.02	0.30± 0.42	–	–	–	–	–	–	–

K_{eq} values for transitions in linear and in the 1- and 2-loop models were applied for P3- and P4-like files and one P2-record fit by a 1-loop model. Loop transitions are indicated in bold. Mean ± SD reported for linear models.