TITLE:

Molecular recognition at the neuromuscular synapse

RUNNING TITLE:

ACh vs. choline affinity

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SUPPLEMENTARY INFORMATION

Table S1: Energy estimates for aromatic mutants

Construct	Ligand	E_2 ± SEM	E_0[a] ± SEM	G_B ± SEM, kcal/mol	ΔG_B, kcal/mol
WT	ACh	25.4[b]	7.4E-7	-5.1	
	TMA	2.54[b]	7.4E-7	-4.5	
	Cho	0.046[b]	7.4E-7	-3.3	
	4OHB[c]	0.71	7.4E-7	-4.1	
	PTMA[c]	0.29	7.4E-7	-3.8	
	3OHP[c]	0.15	7.4E-7	-3.6	
αW149F	ACh	1.13[b] ± 0.10	2.3E-6 ± 3.E-7	-3.9 ± 0.04	1.3
	TMA	0.75 ± 0.06	2.3E-6 ± 3.E-7	-3.8 ± 0.04	0.7
	Cho	0.04 ± 0.004	2.3E-6 ± 3.E-7	-2.9 ± 0.04	0.4
αW149A	ACh	0.07[b] ± 0.003	5.5E-6 ± 3.E-8	-2.8 ± 0.01	2.4
	TMA	0.004 ± 0.001	5.5E-6 ± 3.E-8	-1.9 ± 0.06	2.5
	Cho	0.003 ± 0.001	5.5E-6 ± 3.E-8	-1.8 ± 0.07	1.5
αY190F	ACh	0.02[b] ± 0.002	4.1E-7 ± 7.E-8	-3.2 ± 0.06	2.0
	TMA	1.4E-3 ± 2.E-4	4.1E-7 ± 7.E-8	-2.4 ± 0.07	2.1
	Cho	4.5E-5 ± 6.E-6	4.1E-7 ± 7.E-8	-1.4 ± 0.06	1.9
	4OHB	1.5E-4 ± 7.E-6	4.1E-7 ± 7.E-8	-1.7 ± 0.05	2.3
	PTMA	2.0E-4 ± 6.E-6	4.1E-7 ± 7.E-8	-1.8 ± 0.05	2.0
	3OHP	8.1E-5 ± 2.E-5	4.1E-7 ± 7.E-8	-1.6 ± 0.07	2.1
αY190A	ACh	5.3E-5[b] ± 3.E-6	6.3E-7 ± 6.E-8	-1.3 ± 0.03	3.8
	TMA	1.7E-5 ± 9.E-7	6.3E-7 ± 6.E-8	-1.0 ± 0.03	3.5
	Cho	7.3E-6 ± 8.E-7	6.3E-7 ± 6.E-8	-0.7 ± 0.04	2.6
αY198F	ACh	14.02[b] ± 6.20	6.1E-7 ± 9.E-8	-5.0 ± 0.12	0.1
	TMA	0.71 ± 0.19	6.1E-7 ± 9.E-8	-4.1 ± 0.08	0.3
	Cho	0.013 ± 0.003	6.1E-7 ± 9.E-8	-2.9 ± 0.08	0.3
	4OHB	0.122 ± 0.015	6.1E-7 ± 9.E-8	-3.6 ± 0.05	0.5
	3OHP	0.043 ± 0.003	6.1E-7 ± 9.E-8	-3.3 ± 0.05	0.3
αY198A	ACh	0.027[b] ± 0.009	8.3E-7 ± 1.E-7	-3.0 ± 0.11	2.1
	TMA	0.003 ± 3.E-4	8.3E-7 ± 1.E-7	-2.4 ± 0.04	2.1
	Cho	5.0E-4 ± 1.E-4	8.3E-7 ± 1.E-7	-1.9 ± 0.10	1.4
αK145A	ACh	0.55 ± 0.18	4.0E-7 ± 8.E-8	-4.2 ± 0.10	1.0
	TMA	0.02 ± 0.001	4.0E-7 ± 8.E-8	-3.2 ± 0.06	1.3
	Cho	4.7E-4 ± 8.E-5	4.0E-7 ± 8.E-8	-2.1 ± 0.07	1.2
αK145Q	Cho	0.002 ± 2.E-4	1.3E-6 ± 5.E-7	-2.2 ± 0.11	1.1
αK145A+ Y190F	ACh	0.08 ± 0.02	1.5E-6 ± 2.E-8	-3.2 ± 0.07	1.9
	TMA	0.002 ± 5.E-4	1.5E-6 ± 2.E-8	-2.2 ± 0.05	2.2
	Cho	6.5E-5 ± 2.E-5	1.5E-6 ± 2.E-8	-1.1 ± 0.07	2.1

G_B is the binding energy, $G_B = -0.59\ln\sqrt{(E_2/E_0)}$. αW149, αY190, and αY198 in the AChR mouse α subunit correspond to TrpB, TyrC1, and TyrC2 in AChBP.

[a] E_0 measurements of the aromatic residue mutants were previously published (Purohit & Auerbach, 2010), corrected here for a E_0^{wt} value of 7.4×10^{-7} (Nayak *et al.*, 2012).

[b] Previously published E_2 measurements (Jadey *et al.*, 2011; Purohit *et al.*, 2012)

[c] 3OHP, 3-hydroxypropyltrimethylammonium; PMTA, propyltrimethylammonium; 4OHB, 4-hydroxybutyltrimethylammonium. Previously published E_2 measurements (Bruhova *et al.*, 2013).

Table S2: Observed and corrected rate/equilibrium constants

Mutant	Agonist	f_2	b_2	E_2^{obs}	bkgd	E_2^{corr}	n
αW149F	TMA	1215 ± 107	1190 ± 204	1.02 ± 0.09	1.35[ab]	0.755±0.063	3
	Cho	769 ± 104	1214 ± 67	0.63 ± 0.05	14.2[ac]	0.045±0.004	3
αW149A	TMA	154 ± 25	2850 ± 208	0.05 ± 0.01	14.2[ac]	0.004±0.001	4
	Cho	799 ± 36	2490 ± 506	0.32 ± 0.08	120[acd]	0.003±0.001	3
αY190F	TMA	554 ± 55	3325 ± 587	0.17 ± 0.03	120[acd]	1.4E-3±2.E-4	3
	PTMA	1451 ± 51	3576 ± 22	0.41 ± 0.01	2085[acde]	2.0E-4±6.E-6	3
	4OHB	1390 ± 276	4550 ± 723	0.31 ± 0.01	2085[acde]	1.5E-4±7.E-6	3
	3OHP	878 ± 143	5228 ± 729	0.17 ± 0.03	2085[acde]	8.1E-5±2.E-5	4
	Cho	468 ± 47	5070 ± 195	0.09 ± 0.01	2085[acde]	4.5E-5±6.E-6	4
αY190A	TMA	639 ± 38	2762 ± 84	0.23 ± 0.01	1.3E+4[abf]	1.7E-5±9.E-7	3
	Cho	462 ± 19	4572 ± 556	0.10 ± 0.01	1.3E+4[abf]	7.3E-6±9.E-7	4
αY198F	TMA	762 ± 177	1347 ± 269	0.57 ± 0.15	0.8[ag]	0.709±0.188	4
	4OHB	474 ± 15	2882 ± 677	0.16 ± 0.02	1.35[ab]	0.122±0.015	3
	3OHP	1169 ± 210	1918 ± 307	0.61 ± 0.04	14.2[ac]	0.043±0.003	2
	Cho	317 ± 72	1784 ± 290	0.18 ± 0.04	14.2[ac]	0.013±0.003	8
αY198A	TMA	761 ± 164	2207 ± 63	0.34 ± 0.03	120[acd]	0.003±3.E-4	4
	Cho	259 ± 89	4343 ± 336	0.06 ± 0.02	120[acd]	5.0E-4±1.E-4	4
αK145A	ACh	320 ± 142	7231 ± 805	0.04 ± 0.01	0.08[a]	0.55±0.18	4
	TMA	412 ± 5	1533 ± 321	0.27 ± 0.04	14.2[ac]	0.02±0.001	3
	Cho	318 ± 33	5661 ± 368	0.06 ± 0.01	120[acd]	4.7E-4±8.E-5	3
	None[*]	879 ± 198	10446 ± 1332	0.08 ± 0.02	2.1E+5[acdh]	4.0E-7±8.E-8	3
αK145Q	Cho	132 ± 4	4795 ± 643	0.03 ± 0.002	14.2[ac]	0.002±2.E-4	3
	None[*]	1395 ± 395	6066 ± 939	0.26 ± 0.08	2.1E+5[acdh]	1.3E-6±5.E-7	3
αK145A +Y190F	ACh	2020 ± 252	1748 ± 346	1.16 ± 0.28	14.2[ac]	0.08±0.02	4
	TMA	389 ± 14	654 ± 228	0.60 ± 0.11	241[abc]	0.002±5.E-4	3
	Cho	70 ± 22	9046 ± 1308	0.01 ± 0.002	120[acd]	6.5E-5±2.E-5	3
	None[*]	1246 ± 80	4067 ± 320	0.31 ± 0.004	2.1E+5[acdh]	1.5E-6±2.E-8	3

f_2, diliganded opening rate constant (s^{-1}); b_2, diliganded closing rate constant (s^{-1}); E_2^{obs}, measured diliganded gating equilibrium constant ($=f_2/b_2$); bkgd, the net fold-change in E_0 of the background; E_2^{corr}, diliganded gating equilibrium constant corrected to the reference condition (-100 mV, wt; $= E_2^{obs}$/bkgd); n, number of patches.

Background perturbations: [a] V_m=+100 mV; [b] εS450A, [c] εL269F, [d] εE181W, [e] δV269L, [f] α(D97A+Y127F), [g] εS450W, [h] δV269A (for individual fold-changes in E_0 see Table S3).

[*] None represents unliganded gating $E_0=f_0/b_0$, specifically for αK145A and Q mutants.

Table S3: Effects of mutations on E_0.

Perturbation	Location	$E_0^{mt}/E_0^{wt,\,-0.1V}$	Reference
αD97A	Loop A	168.5	(Chakrapani *et al.*, 2003)
αY127F	ECD	59	(Purohit *et al.*, 2007)
βL262A	M2	372	(Purohit *et al.*, 2013)
βL262M	M2	68.2	(Purohit *et al.*, 2013)
δW57A	ECD	0.5	(Bafna *et al.*, 2009)
δW57R	ECD	0.7	(Bafna *et al.*, 2009)
δV269L	M2	14	(Cymes *et al.*, 2002)
δP123R	ECD	0.22	(Gupta *et al.*, 2013)
εP121R	ECD	0.09	(Gupta *et al.*, 2013)
εE181T	Loop 9	2.2	(Jha *et al.*, 2012)
εE181W+εL269F	Loop 9	1492	(Jha *et al.*, 2012)
εL269F	M2	179	(Jha *et al.*, 2009)
εS450A	M4	17	(Mitra *et al.*, 2004)
εS450W	M4	9.9	(Mitra *et al.*, 2004)
+100mV	TMD	0.08	(Nayak *et al.*, 2012)

ECD: Extracellular domain, TMD, transmembrane domain; M2, M4: transmembrane segments 2 and 4.

Table S4: Single site AChRs with transmitter binding site mutations

Mutant site	Agonist	$E_0^{\text{bkgd, -0.1V}}$	$E_1^{\text{bkgd, -0.1V}}$	G_{B1}, kcal/mol	ΔG_{B1}, kcal/mol
Wt αδ	Cho			-3.5	
	Cho			-2.7	
Wt αε	TMA			-4.2	
	TMA			-3.8	
αW149A αδ	Cho	0.02[a]	0.69	-2.1	1.4
	TMA	0.02[a]	0.47	-1.8	2.4
αW149A αε	Cho	0.09[b]	0.78	-1.3	1.4
	TMA	0.09[b]	0.86	-1.3	2.5
αY198A αδ	Cho	0.03[c]	1.88	-2.2	1.3
	TMA	0.01[a]	0.25	-2.3	1.9
αY198A αε	Cho	0.34[d]	2.68	-1.2	1.5

WT Cho and TMA G_{B1} were previously published (Nayak *et al.*, 2014).
Along with the transmitter binding site mutant (αW149A or αY198A), the E_0 and E_1 were measured on a mutant construct containing the following background mutations:
[a]εP121R+εL269F+εE181T+βL262M; [b] δP123R+δW57R+εL269F+εE181W+βL262M; [c]εP121R+εL269F+εE181W+βL262A; [d] δP123R+δW57A+εL269F+εE181W+βL262A.

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