SUPPLEMENTAL DATA

The energetic consequences of loop 9 gating motions in acetylcholine receptor-channels

Archana Jha, Shaweta Gupta, Shoshanna N. Zucker and Anthony Auerbach
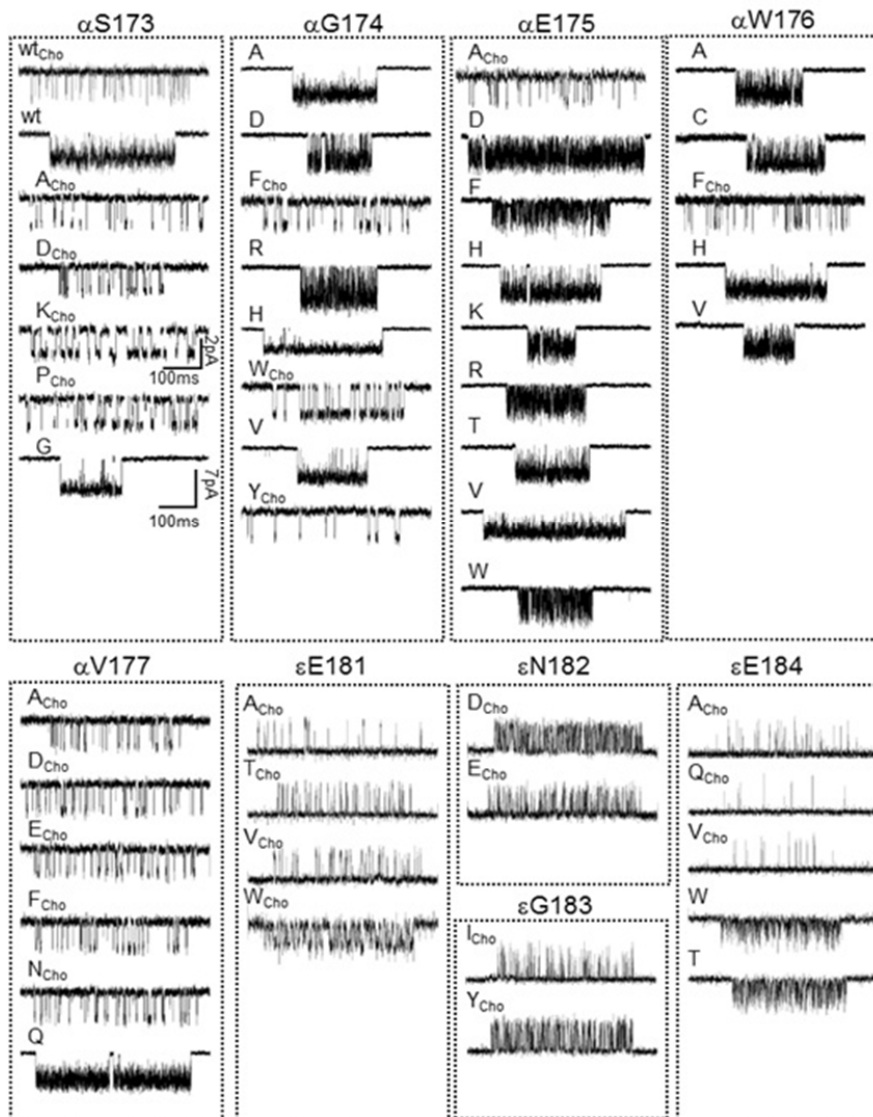Department of Physiology and Biophysics

Fig 1S

Fig. 1S. Example currents from mutant loop 9 residues. Currents were from AChRs activated by 500 μM ACh unless noted by the subscript Cho (20 mM choline).
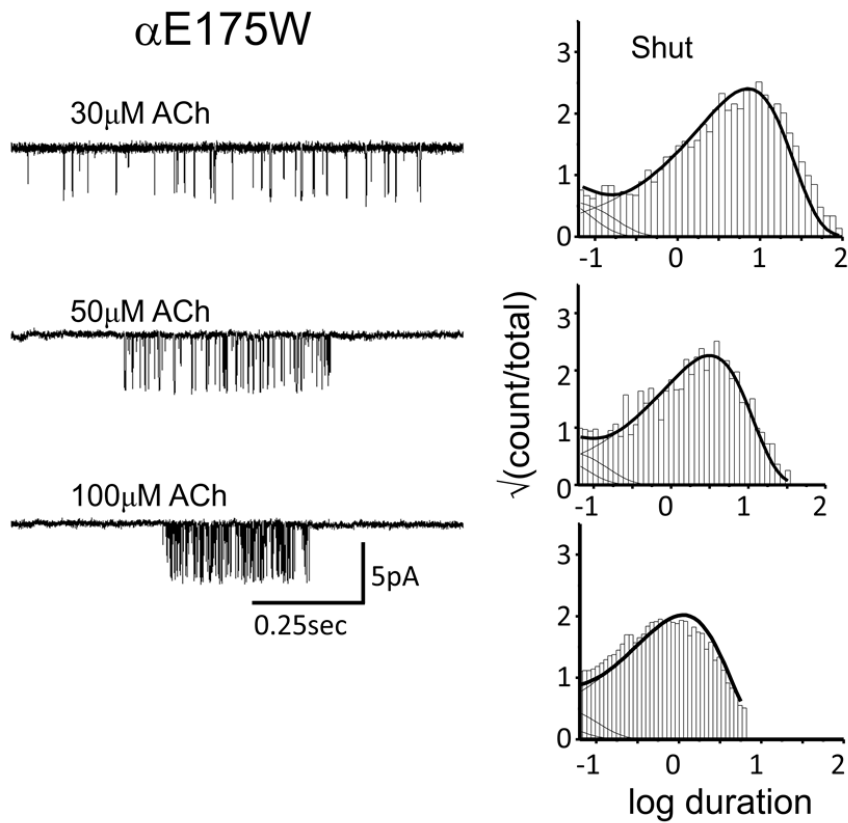
Fig 2S

Fig. 2S. Estimating K_d for the αL9 mutant αE175W. Left, current clusters elicited by different ACh concentrations. Right, corresponding shut-interval duration histograms. Solid lines were calculated from a global fit of the interval durations using a kinetic scheme with two equivalent binding sites (see Methods). The association and dissociation rate constant are: $k_+=143\pm8$ $\mu M^{-1}s^{-1}$ and $k_-=17,433\pm1004$ s^{-1} (mean$\pm$s.d; $K_d=123$ μM). The values for wt AChRs activated by ACh are: $k_+=167\pm8$ $\mu M^{-1}s^{-1}$ and $k_-=24,745\pm1004$ s^{-1} ($K_d=148$ μM)(Chakrapani *et al.*, 2004)

Fig 3S

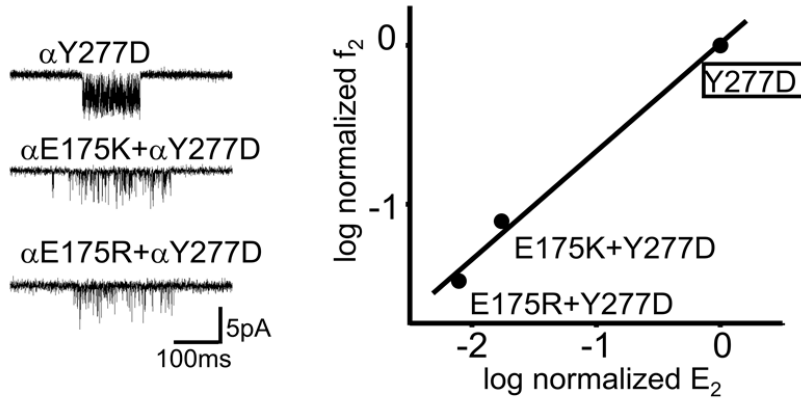Fig. 3S. Kinetic analysis of αY277D (in αM3; see Fig. 1B), on different αE175 backgrounds. Left, example current clusters. Right, R-E plot. The Φ-value of αY277 (Cadugan & Auerbach, 2007) was not influenced by two αE175 side chains.

Fig 4S

Fig. 4S. Example currents from multiply-mutated AChRs (E-T interface). The clusters for these mutant combinations showed heterogeneous kinetic properties.
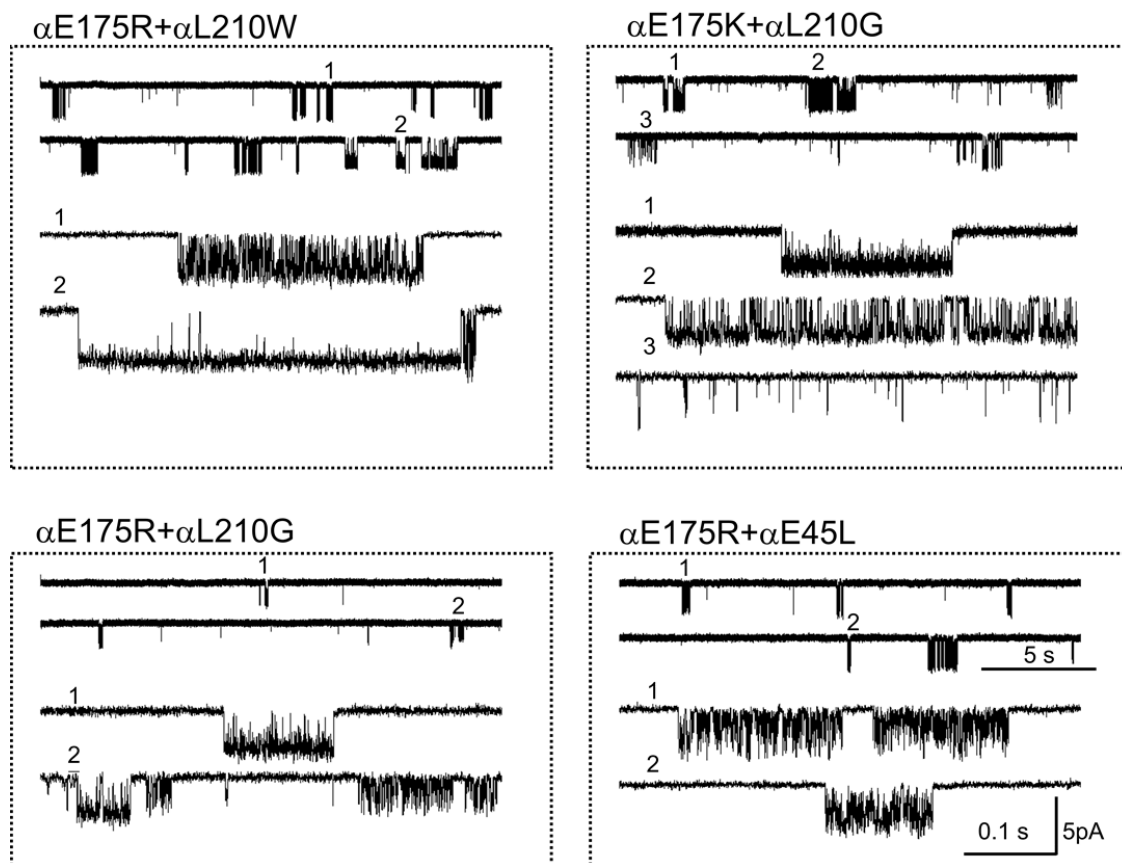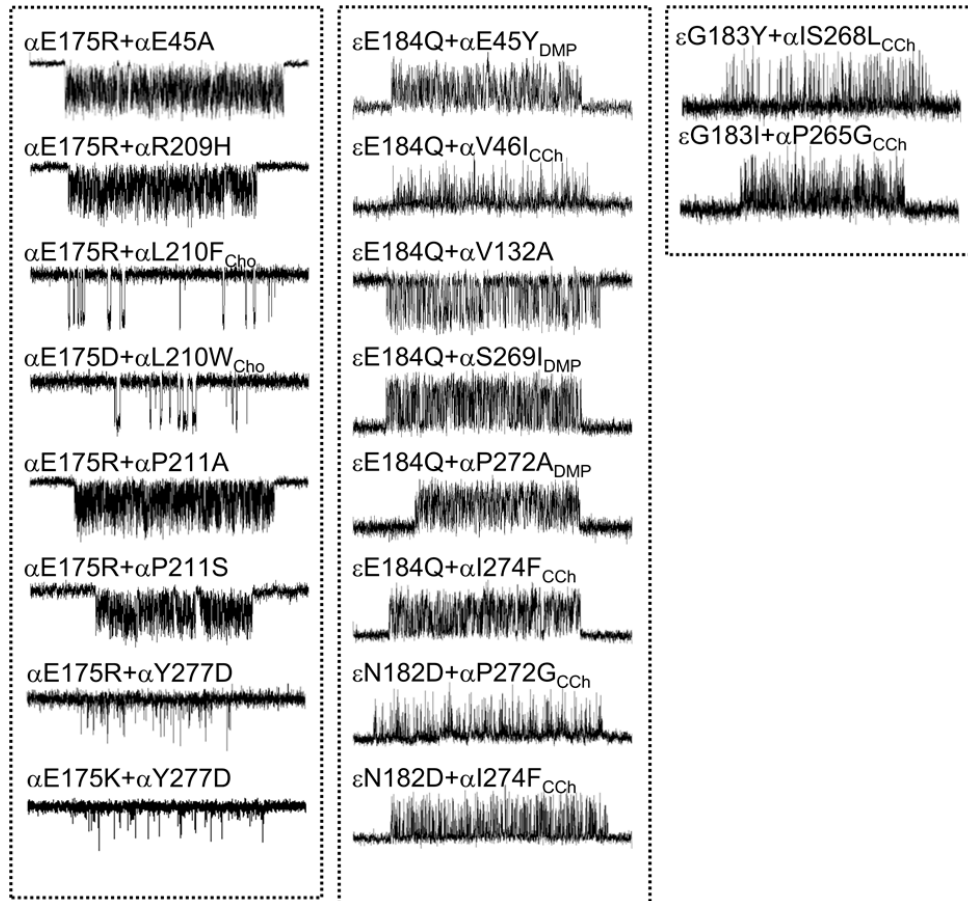
Fig 5S

Fig. 5S. Example currents from multiply-mutated AChRs (E-T and P-C interfaces). The clusters for these mutant combinations did not show heterogeneous kinetic properties. Currents were from AChRs activated by 500 μM ACh unless noted by the subscript Cho (20 mM choline), CCh (100 mM carbamylcholine) and DMP (100 mM dimethyl pyrrolidinium).

Table 1S : Rate and equilibrium constants

Position	f_2^{obs} (s^{-1})	b_2^{obs} (s^{-1})	f_2^{corr} (s^{-1})	b_2^{corr} (s^{-1})	b_2^* (s^{-1})	i_0 (pA)	i_B (pA)	E_2^{corr}	Agonist	Fold-change in E_2	n
wt$_{Cho}$[a]	120	834		2583	2100	7	2	0.046	Cho	1.00	
wt$_{Ach}$[b]	48000	-		1700	1000	7	5	28.2	ACh	1.00	
αS173A	82(4)	374(20)		1428	1012	6.3	1.65	0.057	Cho	1.24	3
αS173D	228(0.4)	549(50)		1983	1879	6.5	1.8	0.12	Cho	2.49	2
αS173G	28939(1302)	838(100)		1220	1127	6.7	4.6	23.7	ACh	0.84	3
αS173K	74(9)	115(7)		399	440	6.6	1.9	0.19	Cho	4.10	2
αS173P	104(1)	251(16)		879	1097	6.3	1.8	0.12	Cho	2.60	3
αG174A	25094(2770)	1433(274)		1874	1674	6.8	5.2	13.4	ACh	0.47	3
αG174D	1867(228)	885(111)		1062	931	5.2	4.3	1.75	ACh	0.062	4
αG174F	101(17)	271(13)		1070	608	7.5	1.9	0.09	Cho	1.95	3
αG174H	26578(1440)	975(264)		1099	794	5.3	4.7	24.18	ACh	0.85	3
αG174R	10420(1039)	1282(51)		1910	1882	7.3	4.9	5.45	ACh	0.19	3
αG174W	99(26)	195(48)		871	641	6.7	1.5	0.11	Cho	2.47	3
αG174V	24495(1296)	985(152)		1174	1115	6.2	5.2	20.86	ACh	0.74	3
αG174Y	32(1.8)	398(40)		2044	1527	7.5	1.46	0.016	Cho	0.35	3
αE175A	75(16)	674(65)		2152	2087	5.1	1.6	0.03	Cho	0.75	3
αE175C[c]	-	-		-	-	-	-	-	ACh	-	3
αE175D	15331(618)	3746(736)		4263	3240	7	6.15	3.59	ACh	0.13	3
αE175F[c]	-	-		-	-	-	-	-	ACh	-	3
αE175H	13716(1015)	1399(184)		2072	1965	7.1	4.8	6.6	ACh	0.23	3
αE175K	10014(1219)	3215(889)		5296	4723	6.9	4.2	1.89	ACh	0.07	2
αE175Q[c]	-	-		-		-	-	-	ACh	-	3
αE175R	7676 (948)	6405(391)		7419	7706	7	6.04	1.03	ACh	0.036	3
αE175R$_{Hyb}$	16982	2815		4582		7	4.3	3.7	ACh	0.13	3
αE175T	16437 (677)	713(84)		1283	776	7.2	4.0	12.8	ACh	0.45	3
αE175V	19581	2365		2819	1124	6.2	5.2	6.9	ACh	0.25	1
αE175W	4750(870)	4608(259)		5642	5781	6	4.9	0.84	ACh	0.029	3
αE175Y[c]	-	-		-	-	-	-	-	ACh	-	3
αW176H	25366(743)	1562(134)		1952	2073	6	4.8	12.9	ACh	0.46	3
αW176F	157(25)	1109(58)		3716	4052	6.7	2	0.04	Cho	0.92	2
αW176C	12151(785)	1586(247)		2241	2606	6.5	4.6	5.4	ACh	0.19	3
αW176A	8573(869)	1728(111)		2298	2480	6.5	4.9	3.73	ACh	0.13	3

αW176V	13371(701)	2254(236)		3255	1722	6.0	4.1	5.05	ACh	0.15	2
αV177A	135(9)	832(223)		3432	3262	6.6	1.6	0.04	Cho	0.87	4
αV177D	178(1.5)	942(102)		3706	2565	6.3	1.6	0.048	Cho	1.0	2
αV177E	95(21)	866(174)		3983	1516	6.9	1.5	0.024	Cho	0.52	3
αV177F	112	779		2548	2119	7	2.1	0.044	Cho	0.95	1
αV177N	112(19)	809(24)		3438	2859	7	1.7	0.03	Cho	0.71	3
αV177Q	34468(2806)	702(73)		974	1195	6.9	5	35.38	ACh	1.25	3
εE181A[d]	15	1312	11.5	820				0.01	Cho	0.3	1
εE181T[d]	180(55)	2073(36)	138	1295				0.11	Cho	2.2	2
εE181V[d]	289(120)	1216(66)	222	760				0.29	Cho	6.1	2
εE181W[d]	1021(9)	1352	785	845				0.93	Cho	19.4	2
εN182D[d]	1898	1730	1460	912				1.60	Cho	33.3	3
εN182E[d]	717	3233(14)	551	2020				0.27	Cho	5.7	3
εG183I[d]	494(30)	4249(1035)	380	2655				0.14	Cho	3.0	3
εG183Y[d]	1121	3264	862	2040				0.42	Cho	8.8	1
εE184W	1040(158)	9386(285)	800	5866				0.11	ACh	0.004	2
εE184T	3431	9869	2639	6168				0.34	ACh	0.012	1
εE184Q[d]	22	4677	17	2923				0.01	Cho	0.12	1
εE184A[d]	47(11)	3670(371)	36	2294				0.02	Cho	0.3	3
εE184V[d]	16	4496	12	2810				0.004	Cho	0.09	1

f_2^{obs} is the observed forward, channel-opening rate constant; b_2^{obs} is the observed backward, channel-closing rate constant; f_2^{corr} is the opening rate constant corrected for the background mutation; b_2^{corr} is the closing rate constant corrected for channel block (see Methods); b_2^* is the diliganded closing rate constant measured at a low agonist concentration; i_0 is the single-channel current amplitude at a low agonist concentration (no block); i_B is the observed channel current amplitude at a high agonist concentration (with block); E_2^{corr} is the corrected diliganded gating equilibrium constant ($=f_2^{corr}/b_2^{corr}$), which is for -70 mV, 23 °C; the agonist was either acetylcholine (ACh) or choline (Cho); n , the number of patches. Values are mean (±SE).a , from (Chakrapani *et al.*, 2003) and b, from (Mitra *et al.*, 2005). c, rate constants not estimated because of heterogenous cluster kinetics. d, corrected for the background condition (+70 mV, αV283W) (Cadugan & Auerbach, 2007).

TABLE 2S: Coupling energies

Construct	f_2^{obs} (s^{-1})	b_2^{obs} (s^{-1})	f_2^{corr} (s^{-1})*	b_2^{corr} (s^{-1})*	E_2^{corr}	E$_2$ fold change observed	E$_2$ fold change predicted	Agonist	ΔΔG (kcal/mol)	n
αE175R+αE45A	8786(414)	7789(1517)		12981	0.67	0.023	.0036	ACh	-1.09	2
αE175R+αE45L[a]	-	-		-	-	-	-	ACh	-	3
αE175R+αR209H	8819(952)	8455(15)		11778	0.75	0.026	0.029	ACh	+0.053	2
αE175K+αL210G[a]	-	-		-	-	-	-	ACh	-	4
αE175R+αL210G[a]	-	-		-	-	-	-	ACh	-	3
αE175R+αL210F	41(4)	287(71)		913	.045	0.97	0.22	Cho	-0.87	2
αE175D+αL210W	77(9)	382(19)		1216	0.063	1.37	1.39	Cho	-	2
αE175R+αL210W[a]	-	-		-	-	-	-		-	
αE175R+αP211A	3149(190)	1684(145)		3185	0.98	0.035	.012	ACh	-0.65	3
αE175R+αP211S	4236	1552		3104	1.36	0.05	.05	ACh	-	1
βK46E	13982(474)	2856(645)		3702	3.8	0.13		ACh		
αE175R+βK46E	883(207)	19795(2892)		32992	.027	0.00095	.0048	ACh	+0.96	3
αE175R+αY277D	364(83)	17381(803)		30417	.012	0.00042	.0018	ACh	+0.90	3
αE175K+αY277D	869(63)	19287(856)		32929	0.026	0.00092	.0035	ACh	+0.81	3
εE184Q+αE45Y[b]	1927(264)	2588(381)	2698	863	3.13	8.24	7.58	DMP	-0. 05	2
εE184Q+αV46I[b]	1799 (182)	13160(641)	2519	4387	0.57	0.11	0.06	CCh	-0.36	2
εE184Q+αV132A	709(89)	1356(186)		2034	0.35	0.012	0.016	ACh	+0.47	2
εE184Q+αS269I[b]	1839(390)	1692(221)	2575	564	4.6	0.86	2.6	DMP	+0.65	2
εE184Q+αP272A[b]	4861(412)	1381(83)	6805	460	14.8	2.8	5.3	DMP	+0.38	2
εE184Q+αI274F[c]	4075 (216)	1683(131)	1132	2475	0.46	0.086	0.0062	CCh	-1.56	2
εN182D+αP272G[b]	1095	12376	1521	4175	0.36	0.068	0.125	CCh	+0.36	1
εN182D+αI274F[b]	1543(42)	6065(826)	2143	2046	1.05	0.20	0.25	CCh	+0.13	2
εG183I+αP265G[b]	3082(286)	11689(647)	4281	3943	1.08	0.20	0.07	CCh	-1.7	2
εG183Y+αS268L[b]	842(16)	5811(734)	1169	1960	0.6	0.11	0.18	CCh	+0.3	2

See Table 1S legend. E_2 fold change: observed, E_2^{mut}/E_2^{wt}; predicted, product of E_2^{corr} for each mutant pair; $\Delta\Delta G$, coupling energy is calculated as described in the Methods. Agonists were acetylcholine (ACh), choline (Cho), carbamylcholine (CCh) or dimethyl pyrrolidinium (DMP) (Jadey *et al.*, 2011). a, not estimated because of heterogeneous kinetics; b, expressed on the βT464I background (Mitra *et al.*, 2005); c, expressed on the β(L269D+T464F) background (Jadey,S.V., Purohit,P. and Auerbach,A. 2009.Biophysical Society.Abstr.862-Pos.)

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