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Supplemental information

Agonist efficiency from concentration-response curves: Structural implications and applications

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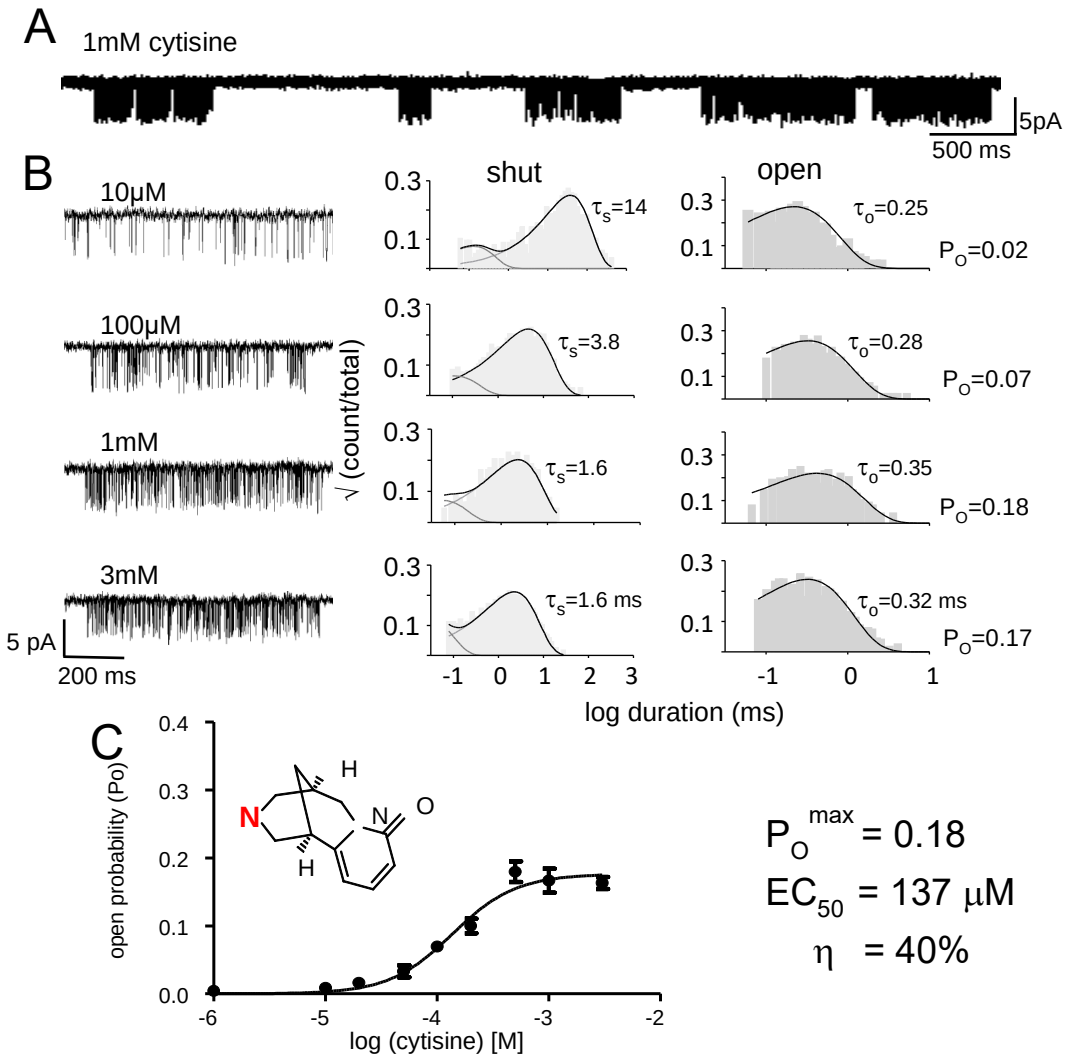


Figure S1. Constructing a CRC from single-channel currents (agonist, cytisine). A. Low-resolution view of cell-attached, single-channel currents showing clusters of openings and closing arising from a single AChR separated by long silent periods in which all AChRs in the patch are desensitized (open down). B. High-resolution views of example clusters at different cytisine concentrations and corresponding intra-cluster interval-duration histograms. The predominant shut and open interval durations (τ_s and τ_o) were used to calculate an open-channel probability (P_O) at each agonist concentration. C. CRC fitted by Eq. 1 to estimate EC_{50} and P_O^{max} (symbols, mean±s.e.m.). Efficiency (η) was calculated by using Eqs. 2-5.

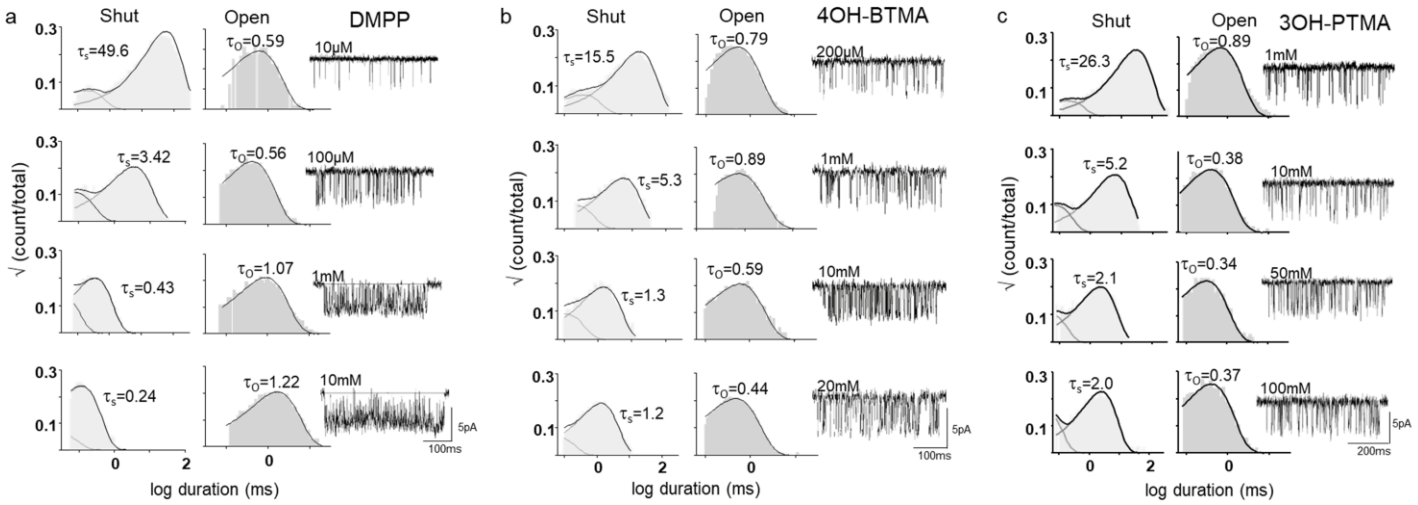


Figure S2. Activation of adult-type AChRs by agonists (open in down). Agonist abbreviations in Materials and Methods. Interval-duration histograms and an example cluster. The predominant intra-cluster shut and open interval durations (τ_s and τ_o) are in ms. CRCs are shown in Fig. 4A.

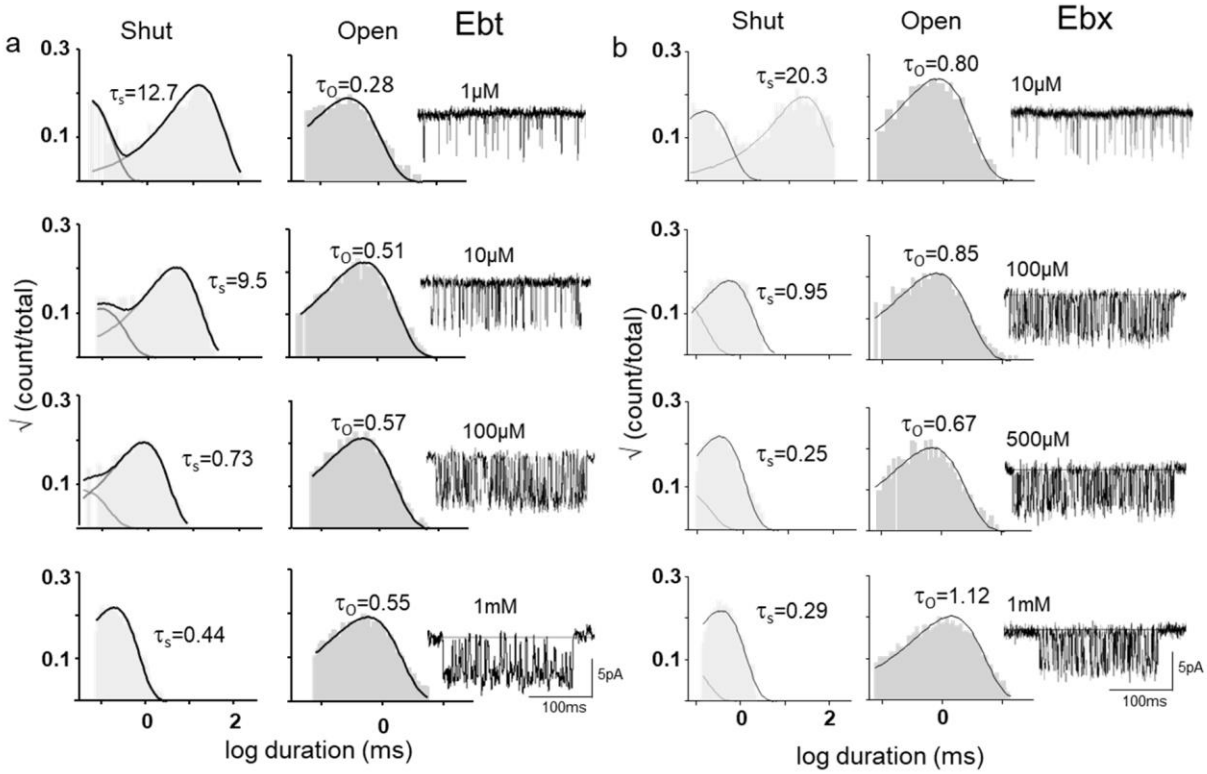


Figure S3. Activation of adult-type AChR in the presence of Ebt (epibatidine) and Ebx (epiboxidine). Time constants are ms. CRCs are shown in Fig. 4B.

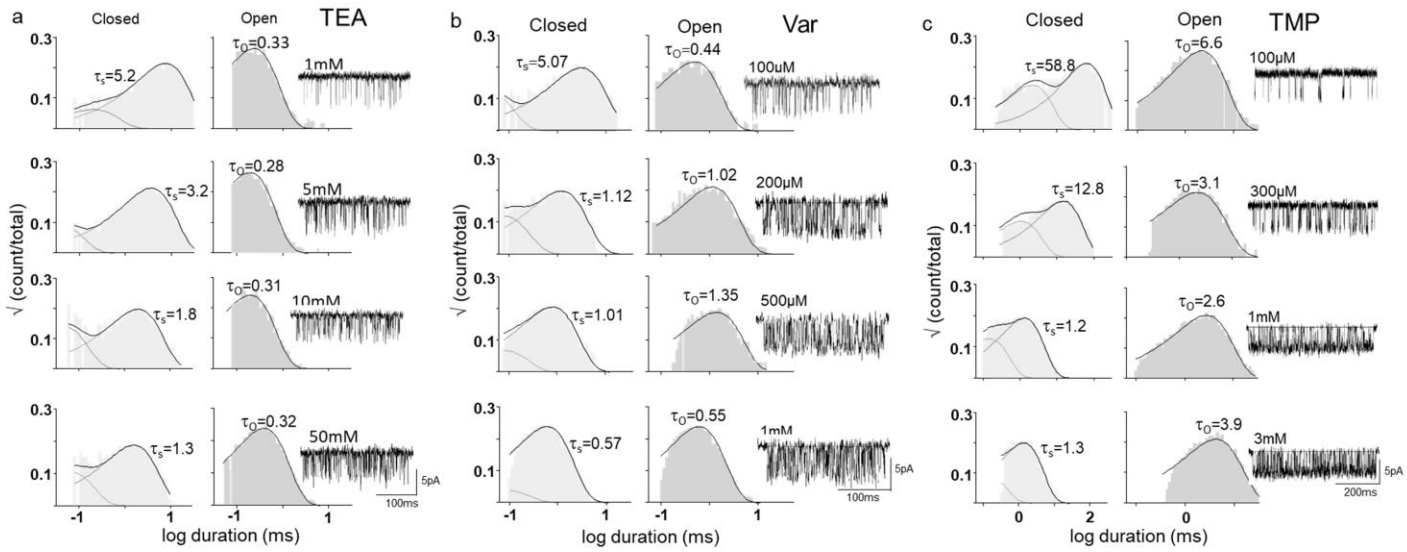


Figure S4. Activation of adult-type AChR by TEA (tetraethylammonium), Var (varenicline) and TMP (tetramethylphosphonium). Time constants are ms. CRCs are shown in Fig. 4C.

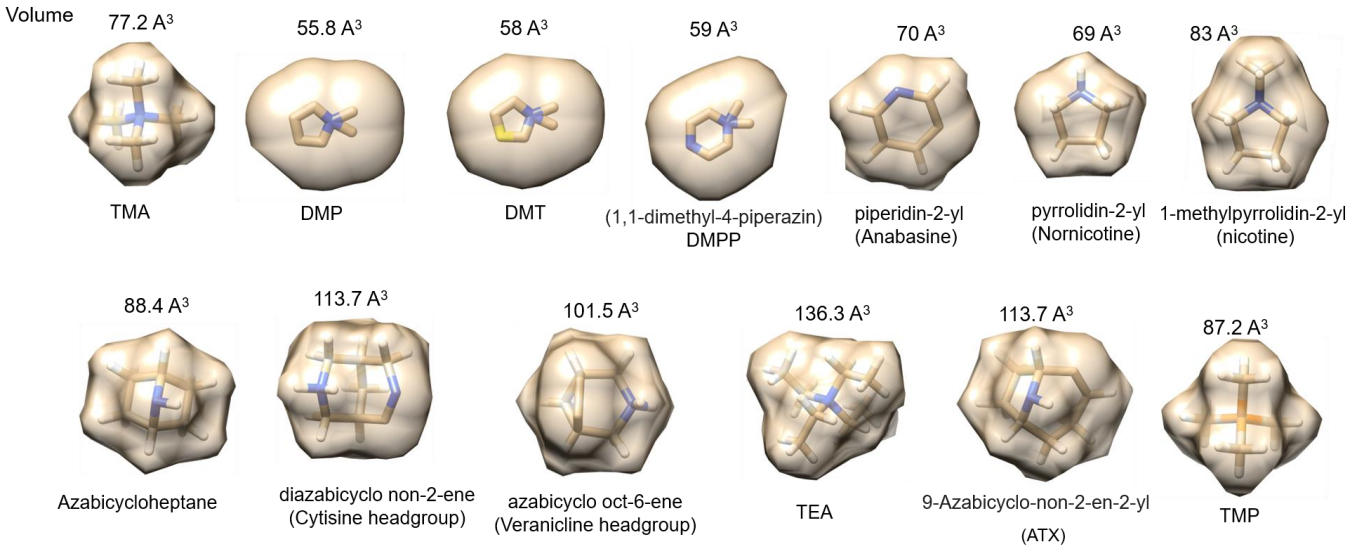
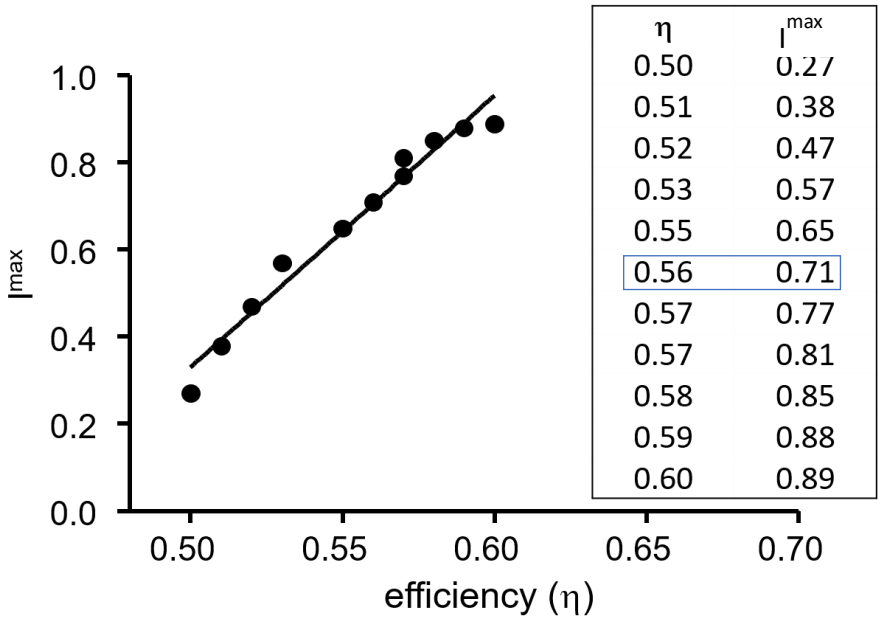


Figure S5. Agonist head-group volume. Not shown: the head-groups of ACh, CCh, choline, 3OH-P and 4OH-B are the same as TMA, and those of Ebt and Ebx are the same as azabicycloheptane.



η	I^{max}
0.50	0.27
0.51	0.38
0.52	0.47
0.53	0.57
0.55	0.65
0.56	0.71
0.57	0.77
0.57	0.81
0.58	0.85
0.59	0.88
0.60	0.89

Figure S6. Using efficiency (η) to calculate I^{max} from EC_{50} (from whole cell CRCs normalized to a maximum of 1; Table 2, right) (see Fig. 8B). Agonist, TMA; experimental EC_{50}, 1.3 mM; $E_o=5.2x10^{-7}$. From non-normalized CRCs, the true I^{max}= 0.70 (Table 2, left). I^{max} calculated by using Eq. 9 is very sensitive to η, making it possible to estimate an agonist's efficiency by matching its calculated and experimental (non-normalized) efficacies.

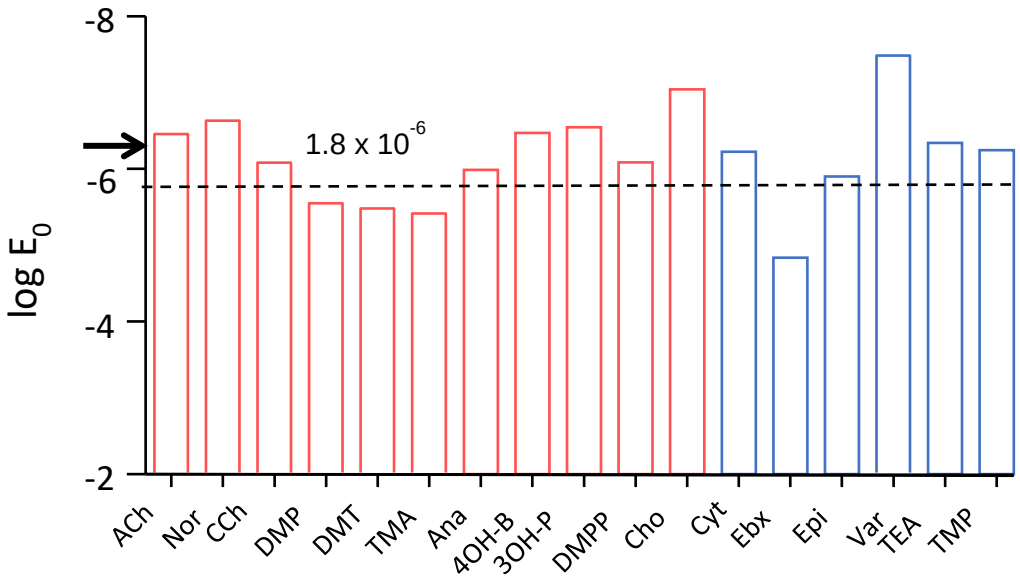


Figure S7. Unliganded gating equilibrium constant (E_o) estimated from CRC parameters. The assumed η values were either 52% (red) or 41% (blue). The calculated mean E_o of 1.8 x 10^{-6} (dashed line) is approximately the same regardless of η and reasonably close to the E_o value estimated by using other methods, 7.4 x 10^{-7} (arrow).

Table S1. ACh efficiency after mutation of a binding site residue (aromatics and εP121).

	K_{dC} (mM)	K_{dO} (μM)	log K_{dC}	log K_{dO}	η
WT	0.166	0.028	-3.78	-7.55	*0.50*
αY93W	2.01	1.20	-2.70	-5.92	*0.54*
H	3.20	2.90	-2.49	-5.54	*0.55*
A	1.21	1.30	-2.92	-5.89	*0.50*
F	2.59	5.80	-2.59	-5.24	*0.51*
S	6.24	14.0	-2.20	-4.85	*0.55*
αW149Y	2.41	3.00	-2.62	-5.52	*0.53*
F	12.8	19.0	-1.89	-4.72	*0.60*
A	28.8	260.0	-1.54	-3.59	*0.57*
αY190F	3.60	16.0	-2.44	-4.80	*0.49*
W	6.46	56.0	-2.19	-4.25	*0.48*
A	16.5	1900	-1.78	-2.72	*0.35*
αY198F	0.23	0.053	-3.64	-7.28	*0.50*
H	5.70	9.10	-2.24	-5.04	*0.55*
W	0.61	0.88	-3.21	-6.06	*0.47*
S	3.90	12.0	-2.41	-4.92	*0.51*
T	9.20	38.0	-2.04	-4.42	*0.54*
L	4.10	21.0	-2.39	-4.68	*0.49*
A	7.50	41.0	-2.12	-4.39	*0.52*
εP121L	0.72	2.20	-3.14	-5.66	*0.44*
Y	1.27	3.00	-2.90	-5.52	*0.48*
G	1.00	1.20	-3.00	-5.92	*0.49*

Equilibrium dissociation constants (K_d) of ACh to resting (C) and active (O) conformations of the binding site (Fig. 1) estimated by kinetic modeling (1). Efficiency (η) is 1-logK_{dC}/logK_{dO}.

Table S2. Efficiency after mutation os αG153.

	Ligand	E_2	E_0	K_{dC}	K_{dO}	log K_{dC}	log K_{dO}	η
αG153	Cho	0.05	7.4E-07	4.0E-03	1.5E-05	-2.40	-4.81	***0.50***
S	Cho	0.63	1.9E-05	3.7E-04	2.0E-06	-3.43	-5.69	***0.40***
A	Cho	1.18	4.2E-05	2.9E-04	1.7E-06	-3.54	-5.77	***0.39***
P	Cho	1.1	4.8E-05	1.5E-04	1.0E-06	-3.81	-5.99	***0.36***
K	Cho	3	1.4E-04	2.6E-04	1.8E-06	-3.59	-5.75	***0.38***
αG153	DMP	0.4	7.4E-07	2.1E-03	2.9E-06	-2.68	-5.54	***0.52***
S	DMP	6.14	1.9E-05	1.8E-04	3.2E-07	-3.74	-6.50	***0.42***
A	DMP	12.2	4.2E-05	1.9E-04	3.6E-07	-3.71	-6.44	***0.42***
P	DMP	13.8	4.8E-05	2.0E-04	3.8E-07	-3.69	-6.42	***0.43***
K	DMP	20.6	1.4E-04	2.3E-04	5.9E-07	-3.64	-6.23	***0.41***
αG153	TMA	2.5	7.4E-07	8.1E-04	4.4E-07	-3.09	-6.36	***0.51***
S	TMA	25.8	1.9E-05	8.1E-05	7.0E-08	-4.09	-7.16	***0.43***
A	TMA	285	4.2E-05	3.9E-05	1.5E-08	-4.41	-7.82	***0.44***
P	TMA	179	4.8E-05	1.0E-04	5.2E-08	-4.00	-7.29	***0.45***
K	TMA	627	1.4E-04	1.7E-05	8.1E-09	-4.76	-8.09	***0.41***
αG153	Nicotine	0.87	7.4E-07	1.0E-03	9.2E-07	-3.00	-6.04	***0.50***
S	Nicotine	12.34	1.9E-05	9.2E-05	1.1E-07	-4.04	-6.94	***0.42***
A	Nicotine	17.7	4.2E-05	1.1E-04	1.8E-07	-3.94	-6.76	***0.42***
P	Nicotine	12.5	4.8E-05	3.8E-05	7.4E-08	-4.42	-7.13	***0.38***
K	Nicotine	549	1.4E-04	1.2E-07	6.1E-11	-6.92	-10.22	***0.32***
E	Nicotine	32.05	4.5E-05	2.6E-05	4.0E-09	-4.59	-8.40	***0.45***
R	Nicotine	23.64	3.8E-05	3.2E-05	5.7E-09	-4.49	-8.25	***0.45***

Equilibrium dissociation constants (K_d) of 4 agonists to resting (C) and active (O) conformations (Fig. 1) estimated by kinetic modeling (2). Efficiency (η) is 1-logK_{dC}/logK_{dO}.

Table S3. EC_{50} from efficacy given η and E_0 for analogues of choline.

Input values				Calculated values					
Ligand	**E_2**	**η**	**E_0**	**P_O^{max}**	**log E_0**	**log E_2**	**log E_2-logE_0**	**K_{dC} (mM)**	**EC_{50} (mM)**
TMA	2.54	0.52	5.2E-7	0.72	-6.28	0.40	6.69	0.82	0.49
ETMA	0.25	0.52	5.2E-7	0.20	-6.28	-0.60	5.68	2.39	2.86
PTMA	0.29	0.52	5.2E-7	0.22	-6.28	-0.54	5.75	2.61	2.61
BTMA	2.44	0.52	5.2E-7	0.71	-6.28	0.39	6.67	0.83	0.51
Cho	0.05	0.52	5.2E-7	0.05	-6.28	-1.30	4.98	5.01	6.84
3OH-PT	0.15	0.52	5.2E-7	0.13	-6.28	-0.82	5.46	3.02	3.85
4OH-B	0.71	0.52	5.2E-7	0.42	-6.28	-0.15	6.14	1.47	1.42
Cl Cho	0.19	0.52	5.2E-7	0.16	-6.28	-0.72	5.56	2.71	3.37
2OH-P	0.02	0.52	5.2E-7	0.02	-6.28	-1.70	4.59	7.65	10.7
Cholamine (pH 9.0)	0.04	0.52	5.2E-7	0.04	-6.28	-1.40	4.89	5.56	7.63

Using η and E_0 to calculate EC_{50} and K_{dC} (Eq. 7) from E_2 (3). TMA (tetramethyl ammonium), ETMA (ethyltrimethyl ammonium), PTMA (propyltrimethyl ammonium), BTMA (butyltrimethyl ammonium), Cho (choline), 3OH-PTMA (3-hydroxypropyl trimethyl ammonium), 4OH-BTMA (4-hydroxybutyl trimethyl ammonium), Cl Cho (2-chloroethyl trimethyl ammonium), 2OH-PTMA (2-hydroxypropyl trimethyl ammonium), cholamine (2-aminoethyl trimethyl ammonium).

References:

1. Purohit P, Bruhova I, Gupta S, Auerbach A. Catch-and-Hold Activation of Muscle Acetylcholine Receptors Having Transmitter Binding Site Mutations. Biophysical Journal. 2014;107(1):88-99.
2. Jadey S, Purohit P, Auerbach A. Action of nicotine and analogs on acetylcholine receptors having mutations of transmitter-binding site residue alphaG153. J Gen Physiol. 2013;141(1):95-104.
3. Bruhova I, Auerbach A. Molecular recognition at cholinergic synapses: acetylcholine versus choline. J Physiol. 2017;595(4):1253-61.