Mukhtasimova et al., http://www.jgp.org/cgi/content/full/jgp.201611584/DC1

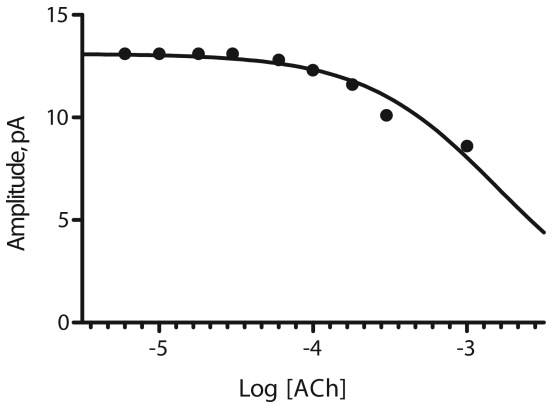


Figure S1. **Single channel current amplitude versus ACh concentration.** Current amplitudes were determined from the fit of the sum of Gaussian functions to all-points histograms from raw data obtained at the indicated concentrations; the difference between the means of the baseline and open channel currents is displayed. The same recordings used for kinetic analysis were analyzed. The smooth curve is a fit of the function for single site inhibition with an IC_{50} of 1.6 mM.

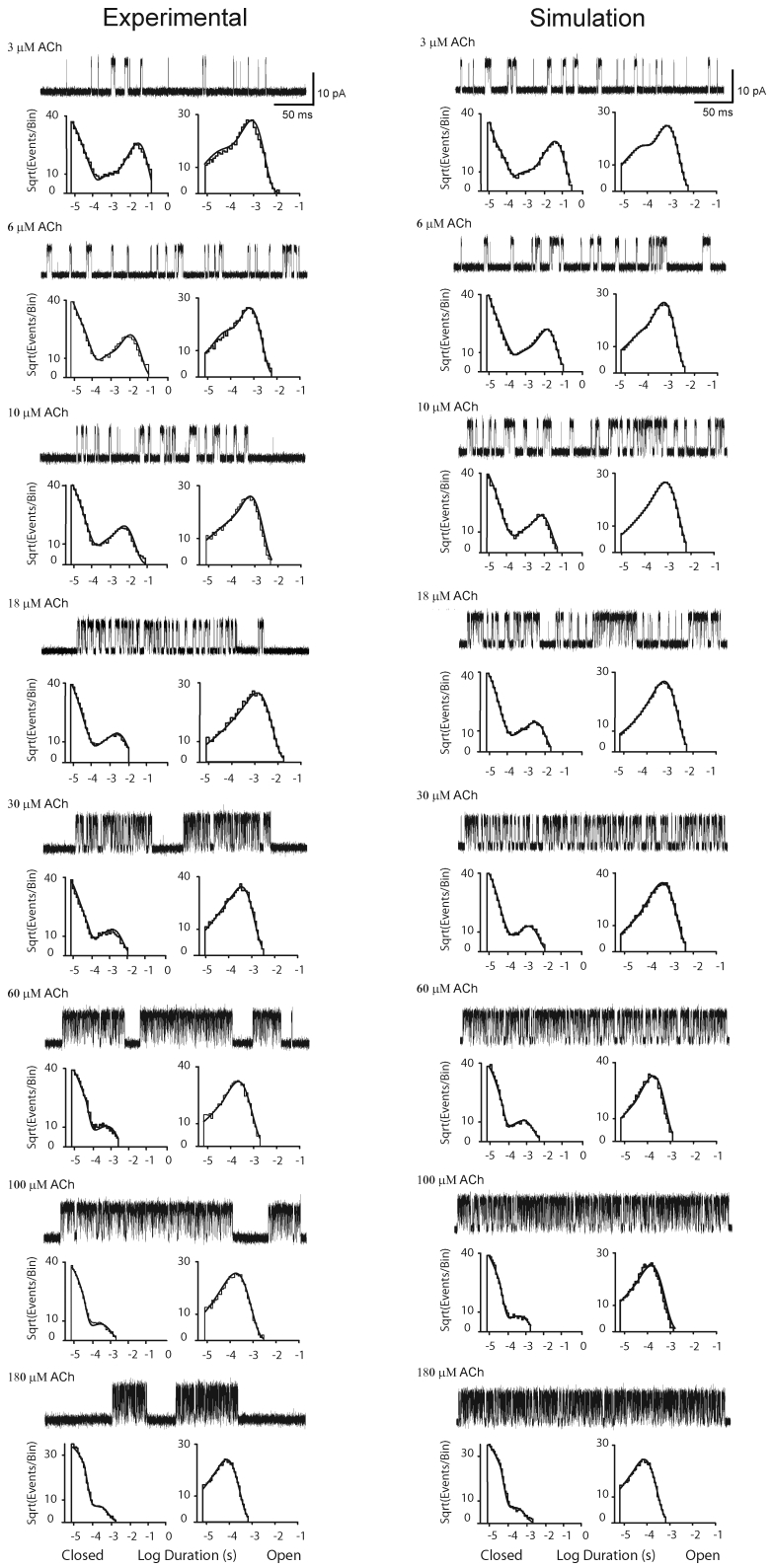


Figure S2. **Analysis of experimental and simulated single channel currents for the adult human AChR activated by ACh.** Data for all ACh concentrations used in the global fit are shown. For each of the indicated ACh concentrations, events from a single channel are displayed at a bandwidth of 25 kHz, and the corresponding closed and open time histograms are displayed with the global fit of Scheme 1 to the data overlaid. Rate constants from the global fit are presented in Table 1.

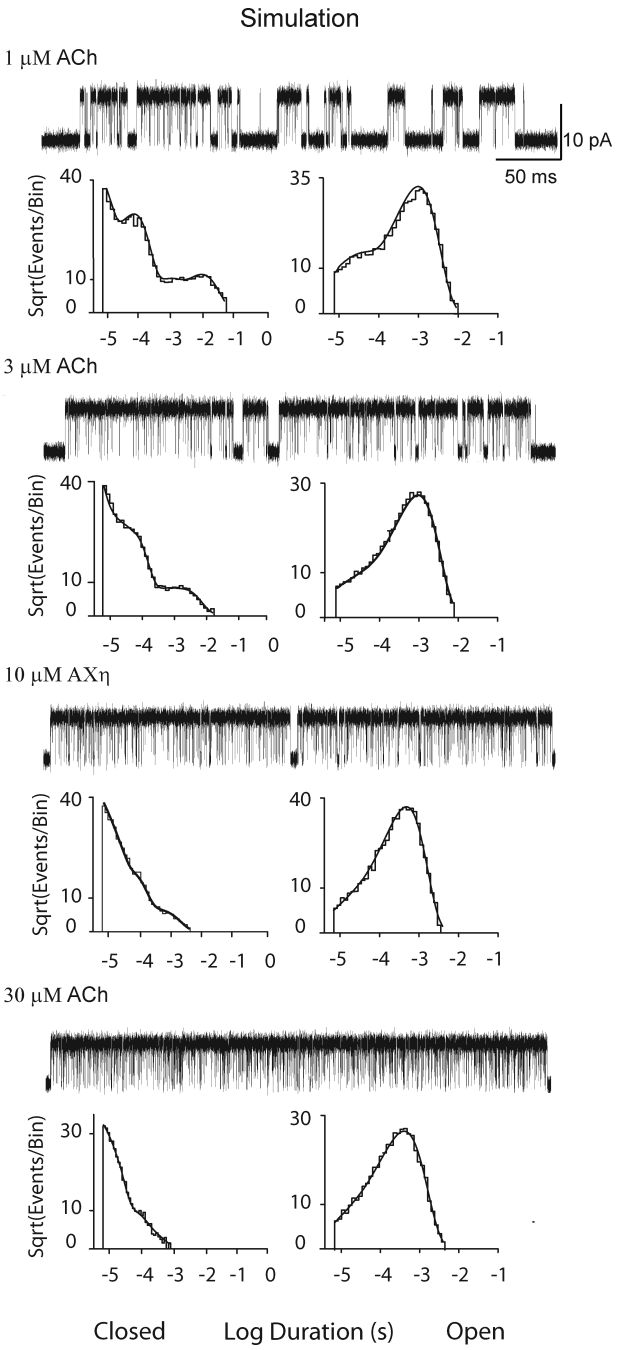


Figure S3. **Analysis of simulated single channel currents for the αG153S mutant AChR activated by ACh.** The corresponding experimental data are shown in Fig. 6. For each of the indicated ACh concentrations, events from a single channel are displayed at a bandwidth of 25 kHz, and the corresponding closed and open time histograms are displayed with the global fit of Scheme 1 to the data overlaid. Rate constants from the global fit are presented in Table 1.

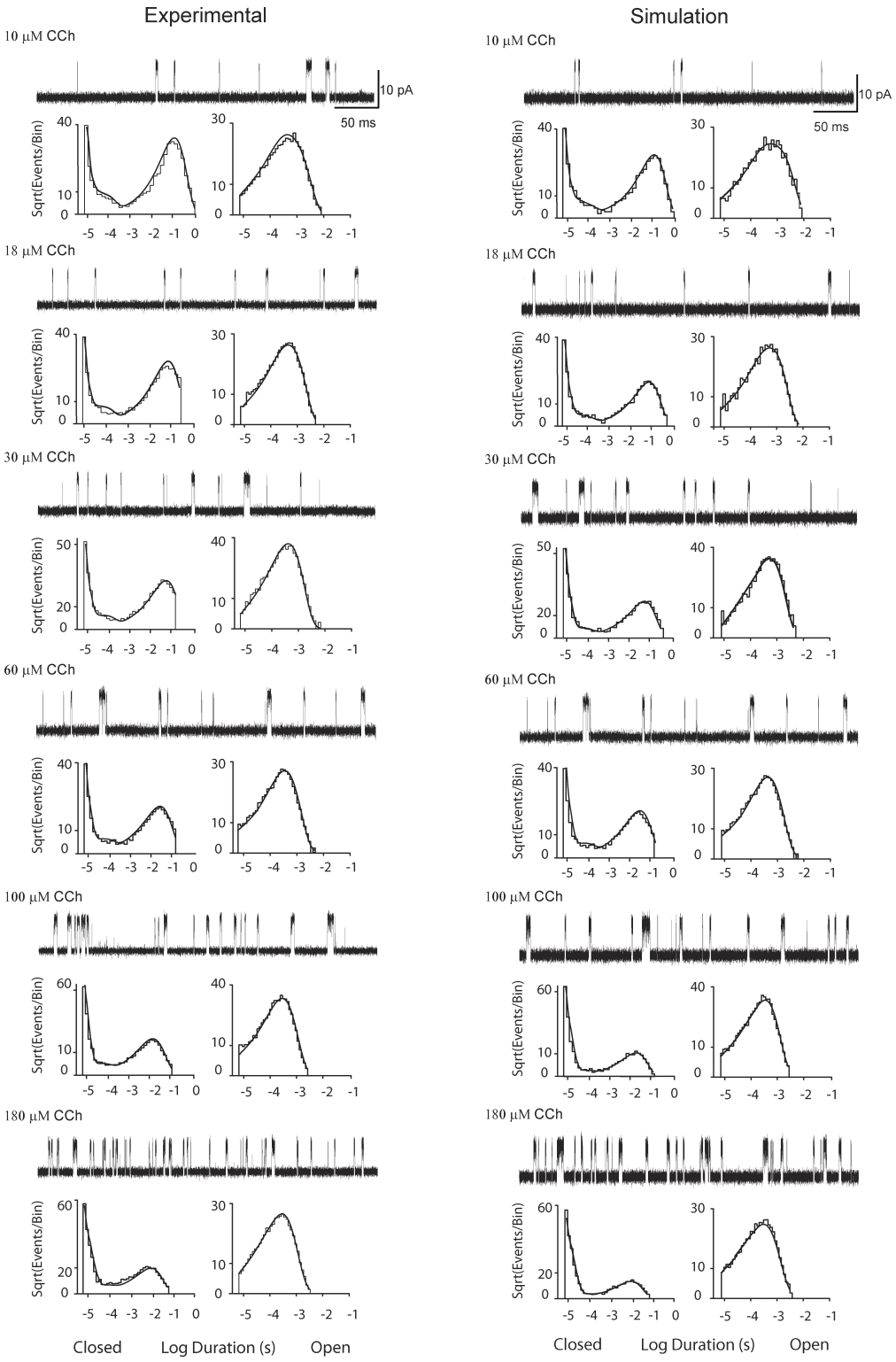


Figure S4. **Analysis of experimental and simulated single channel currents for the adult human AChR activated by CCh.** Data for all CCh concentrations used for the global fit are shown. For each of the indicated CCh concentrations, events from a single channel are displayed at a bandwidth of 25 kHz, and the corresponding closed and open time histograms are displayed with the global fit of Scheme 1 to the data overlaid. Rate constants from the global fit are presented in Table 2.

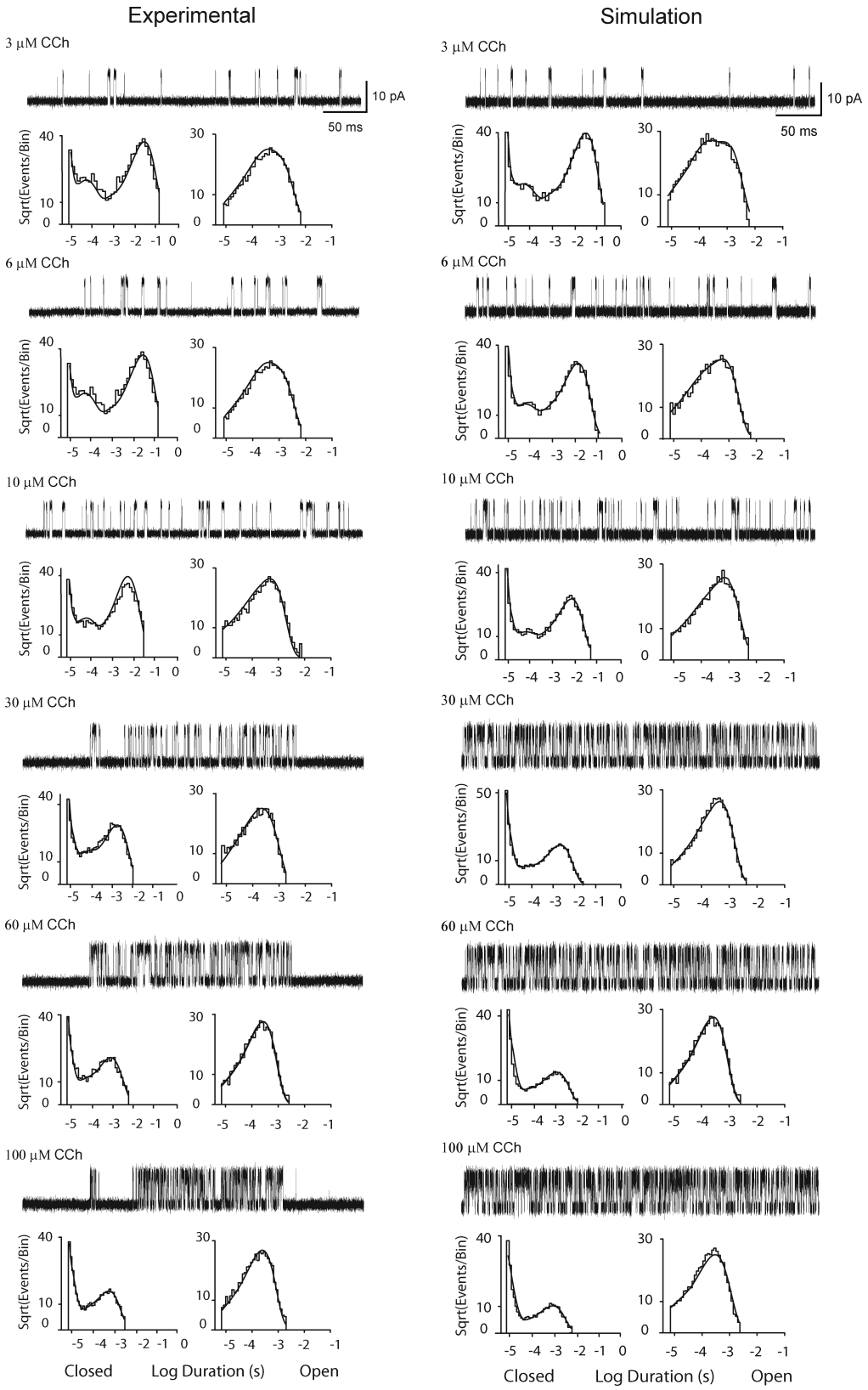


Figure S5. **Analysis of experimental and simulated single channel currents for the αG153S mutant AChR activated by CCh.** Data for all CCh concentrations used for the global fit are shown. For each of the indicated CCh concentrations, events from a single channel are displayed at a bandwidth of 25 kHz, and the corresponding closed and open time histograms are displayed with the global fit of Scheme 1 to the data overlaid. Rate constants from the global fit are presented in Table 2.

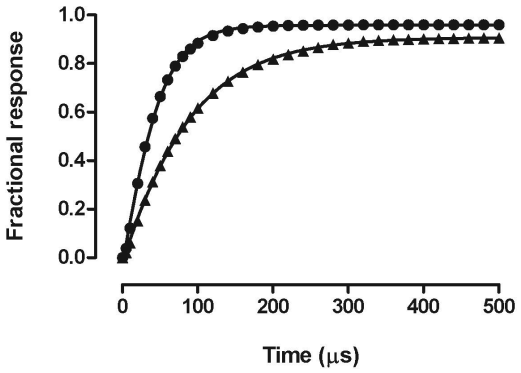
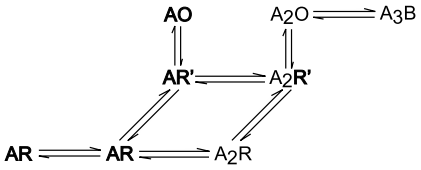
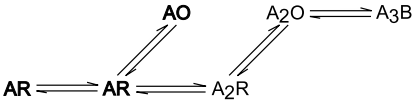


Figure S6. **Predicted macroscopic current as a function of time after a step increase of the ACh concentration for the wild-type AChR.** Filled triangles were generated from Scheme 2 and the fitted rate constants: p_{+2} = 19,200 s^{-1}, p_{-2} = 86,000 s^{-1}, β_2 = 125,000 s^{-1}, α_2 = 2,100 s^{-1} (see Table 2). Filled circles were generated by Scheme 2 and rate constants that yield a mean burst duration of 3.3 ms obtained in the presence of calcium: p_{+2} = 37,000 s^{-1}, p_{-2} = 44,000 s^{-1}, β_2 = 125,000 s^{-1}, α_2 = 2,100 s^{-1}. A sigmoid function was fitted to each time course. The 20–80% rise times are 110 µs for the triangles and 50 µs for the circles.

Table S1. **Comparison of schemes with and without primed states**

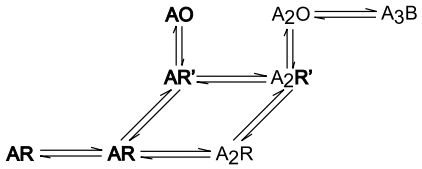


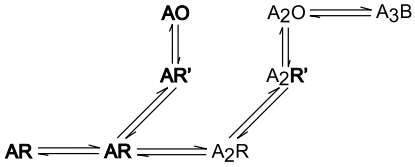


Wild type/ACh	LL = 1,940,170	LL = 1,938,987
n = 250,278	SIC = −1,940,077	SIC = −1,938,925
αG153S/ACh	LL = 969,095	LL = 967,534
n = 123,444	SIC = −969,007	SIC = −967,475
Wild type/CCh	LL = 604,855	LL = 604,520
n = 97,985	SIC = −604,769	SIC = −604,446
αG153S/CCh	LL = 424,253	LL = 423,911
n = 60,419	SIC = −424,170	SIC = −423,856

The Schwartz information criterion (SIC), used to compare nonnested schemes, is given by SIC= −LL + FP/2*lnN, where LL is the log likelihood, FP is the number of free parameters, and N is the number of open and closed dwell times (Koehler and Murphree, 1988; Shelley et al., 2010). The scheme with the smallest SIC value has the highest rank.

Table S2. **Comparison of cyclic and noncyclic schemes**





Wild type/ACh	LL = 1,940,170	LL = 1,939,864	LLR = 5.72 P < 0.001
αG153S/ACh	LL = 969,095	LL = 968,614	LLR = 6.17 P < 0.001
Wild type/CCh	LL = 604,855	LL = 604,834	LLR = 3.04 P < 0.02
αG153S/CCh	LL = 424,253	LL = 424,235	LLR = 2.89 P < 0.02

Comparison of the two schemes was done using the likelihood ratio test (Materials and methods).

Table S3. **Effect of noise on event detection in simulated data**

[ACh]	Total events simulated	Events above threshold after adding rise time	Events above threshold after adding rise time, baseline, and open channel noise
3 μM	21,563	10,000	10,657
6 μM	23,227	10,000	10,937
10 μM	23,370	10,000	11,044
18 μM	21,771	10,000	10,639
30 μM	21,841	10,000	10,701
60 μM	22,159	10,000	10,793
100 μM	21,785	10,000	10,661
180 μM	22,377	10,000	10,699

Stochastic events for each ACh concentration were simulated using the rate constants in Table 1 derived from a direct fit of Scheme 1 to the experimental single channel dwell times for the wild-type AChR activated by ACh, as described in Materials and methods. Dwell times were then sampled at intervals of 0.2 μs, subjected to the effective Fc corresponding to a 25-kHz digital Gaussian filter, and detected before and after adding noise.

Table S4. **Results from simulation followed by kinetic fitting**

Receptor/Agonist	Input	Output
Wild type/ACh		
Experimental β_2 and α_2	190,000/3,140	320,000/6,900
Simulated β_2 and α_2	78,000/1,304	96,000/1,507
	100,000/1,850	150,000/2,486
	130,000/2,425	220,000/3,625
	125,000/2,100	200,000/3,180
αG153S/ACh		
Experimental β_2 and α_2	240,000/3,600	440,000/13,500
Simulated β_2 and α_2	150,000/2,370	250,000/3,700
Wild type/CCh		
Experimental β_2 and α_2	310,000/6,200	330,000/10,700
Simulated β_2 and α_2	100,000/1,900	310,000/6,700
αG153S/CCh		
Experimental β_2 and α_2	330,000/6,400	340,000/9,910
Simulated β_2 and α_2	130,000/2,570	320,000/6,700

For each receptor/agonist combination, the indicated input values of β_2 and α_2 are used to simulate stochastic dwell time sequences, followed by application of the experimental sampling interval, effective filter bandwidth, and baseline and open channel noise. Units of the rate constants are s^{-1}. The output values of β_2 and α_2 were obtained after detection of open and closed intervals for the same range of agonist concentrations used experimentally, followed by maximum likelihood fitting of the global data (see Results section).

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