Supporting Information

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SI Materials and Methods

Site-Directed Mutagenesis. Mutant cDNAs were constructed using the QuikChange Site-Directed Mutagenesis kit (Stratagene) and then confirmed by sequencing the entire coding region (1). BOSC 23 cells, modified HEK 293 cells, were transfected by calcium phosphate precipitation as described previously (2). The ratios for coexpression of two different subunits are expressed in weight. Expression of human α7 in mammalian cells lines requires cotransfection with the intracellular chaperone Ric-3 (3). Ric-3 and α7 cDNAs were cotransfected at a ratio of 12:1 (wt:wt), with the total α7 cDNA ranging from ∼0.4–1 μg for a 35-mm culture dish (1, 4). All transfections were carried out for about 12 h in DMEM with 10% FBS and were terminated by exchanging the medium. Cells were used for single-channel recordings 2–4 d after transfection. To facilitate identification of transfected cells, a separate plasmid encoding green fluorescent protein was included in all transfections.

Single-Channel Recordings. Recordings were obtained in the cell-attached patch configuration essentially as described previously (1). Patch pipettes were pulled from glass capillary tubes and coated with Sylgard (Dow Corning). Single-channel currents were digitized at 5- to 10-μs intervals, and low-pass filtered to 10 kHz using an Axopatch 200 B patch-clamp amplifier (Molecular Devices). To dissect amplitude classes, the membrane potential was −70 mV for α7 and −120 mV for α7–5HT$_3$A receptors (5). Bursts of channel openings were identified as a series of closely separated openings preceded and followed by closings longer than a critical duration, which was taken as the point of intersection between the first and second briefest components in the closed-time histogram for bursts of α7 (∼300–500 μs), second and third closed components for bursts of α7TSLMF (∼1–2 ms), second and third closed components for bursts of α7 in the presence of 5-HI (∼1–3 ms), and second and third closed components for bursts of α7–5HT$_3$A (∼2–5 ms).

Statistics. Experimental data are shown as mean ± SD. Statistical comparisons were done using the Student t test or one-way ANOVA with Bonferroni's multiple comparison post test. A level of $P < 0.05$ was considered significant.

Single-Channel Simulations. Simulations of single-channel events were performed using the QuB suite (www.qub.buffalo.edu; State University of New York, Buffalo). For the simulations, we used a kinetic model that describes the two open components observed experimentally. Open-channel amplitude was fixed at 10 ± 0.6 pA.

1. Bouzat C, Bartos M, Corradi J, Sine SM (2008) The interface between extracellular and transmembrane domains of homomeric Cys-loop receptors governs open-channel lifetime and rate of desensitization. *J Neurosci* 28(31):7808–7819.
2. Bouzat C, Bren N, Sine SM (1994) Structural basis of the different gating kinetics of fetal and adult acetylcholine receptors. *Neuron* 13(6):1395–1402.
3. Williams ME, et al. (2005) Ric-3 promotes functional expression of the nicotinic acetylcholine receptor alpha7 subunit in mammalian cells. *J Biol Chem* 280(2): 1257–1263.
4. daCosta CJ, Free CR, Corradi J, Bouzat C, Sine SM (2011) Single-channel and structural foundations of neuronal α7 acetylcholine receptor potentiation. *J Neurosci* 31(39): 13870–13879.
5. Rayes D, De Rosa MJ, Sine SM, Bouzat C (2009) Number and locations of agonist binding sites required to activate homomeric Cys-loop receptors. *J Neurosci* 29(18): 6022–6032.

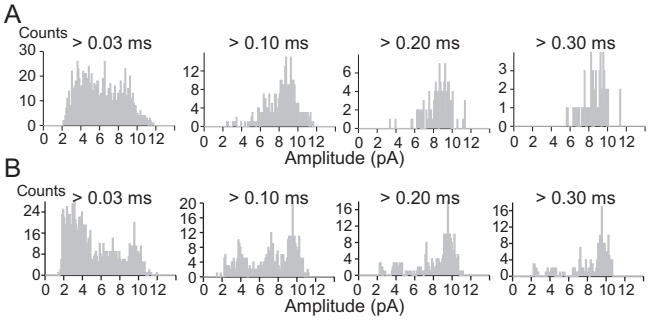

Fig. S1. Amplitude histograms for human α7 and α7TSLMF. Single-channel currents from α7 (*A*) or α7TSLMF (*B*) were recorded at −70 mV membrane potential. Typical amplitude histograms constructed for opening events longer than 0.03, 0.1, 0.2, or 0.3 ms are shown. The proportion of submaximal amplitude openings is systematically reduced as the minimal duration for openings included in the amplitude histogram is increased; and the majority of openings longer than 0.3 ms show maximal amplitude, suggesting that briefer events cannot be fully resolved. The percentage of total opening events is reduced to 23%, 7%, and 4% (α7) and to 45%, 28%, and 22% (α7TSLMF) in histograms restricted to opening events longer than 0.10, 0.20, and 0.30 ms, respectively. Although most openings are grouped in the highest amplitude class when the histograms are restricted to events longer than 0.2–0.3 ms, the reduced number of opening events for α7 makes the application of the electrically fingerprinting strategy technically unfeasible.

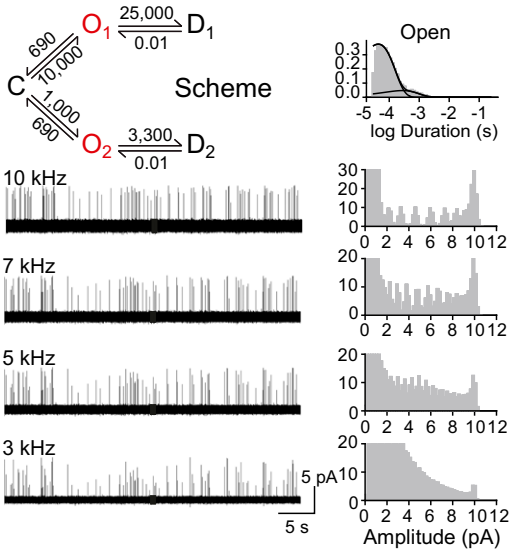

Fig. S2. Simulation of α7 single-channel currents. Single-channel currents were simulated using QUB software (www.qub.buffalo.edu; QUB Suite, State University of New York, Buffalo) on the basis of the shown scheme at an acquisition frequency of 100 kHz and then filtered at different bandwidths. The original channel amplitude was set to 10 pA. The resulting open time distribution (for 10-pA amplitude events) (*Top Right*) and all-point histograms for each condition (*Bottom Right*) are shown. To confirm that under our experimental condition multiple amplitudes are an artifact resulting from the brief duration of opening events, we simulated single-channel data on the basis of a very simple activation scheme that contains one closed state, two open states, and two desensitized states, connected from each open state. Opening rates were chosen on the basis of the relative areas of fast and slow open components experimentally detected. Desensitization rates were fixed to account for the mean duration of the open components because fast desensitization may govern open-channel lifetime (1). Amplitude was set to 10 pA, and channels were simulated at an acquisition frequency of 100 kHz. After simulation, the data were digitally filtered at different frequencies. Simulated channel activity and open time distributions closely reproduce the experimental ones (compare with data in Fig. 2). Remarkably, the simulated channels show nonuniform amplitudes, and the proportion of 10-pA channels is systematically reduced with increasing filtering. Thus, the simulations support that brief open durations may contribute significantly to the multiple open-channel amplitudes due to the lack of full resolution, and validate the use of PAMs or mutations that, by increasing open channel lifetime, may lead to uniform amplitudes, thus allowing the application of the electrical fingerprinting strategy.

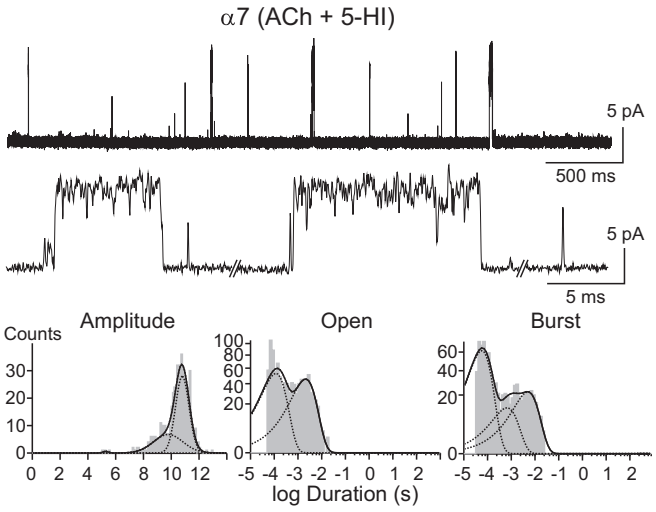

Fig. S3. Potentiation of α7 by 5-HI. Single-channel currents activated by 100 μM ACh in the presence of 2 mM 5-HI. Representative amplitude and open and burst duration histograms are shown. Membrane potential: −70 mV. Filter: 9 kHz. Amplitude histograms were constructed considering only events longer than 0.3 ms. The mean amplitude is identical to that of clusters of α7 potentiated by PNU-120596 (4) and to the maximal mean amplitudes detected for α7 wild-type and quintuple mutant receptors (Fig. 3), thus indicating that 2 mM 5-HI does not affect α7 conductance.

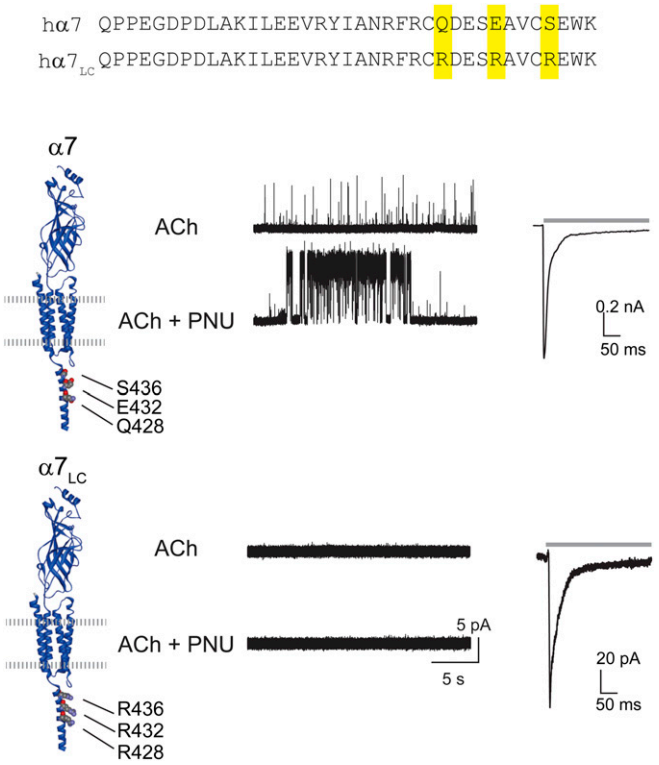

Fig. S4. Low-conductance form of human α7. Arginine residues located in the cytoplasmic linker spanning the M3 and M4 domains are responsible for the low conductance of 5-HT_3A receptors (1–3). The comparison of amino acid sequence shows that negative and noncharged residues are present at equivalent positions in α7, which shows high conductance (*Top*). The figure shows homology model of a single α7 subunit based on the Torpedo acetylcholine receptor structure (PDB ID code: 2BG9), with the triple mutation that leads to the low-conductance form of α7 ($\alpha7_{LC}$). Due to the low conductance, single channels are not detected in cell-attached patches in the presence of 100 μM ACh ($n = 10$) and even in the presence of 100 μM ACh plus 1 μM PNU ($n = 5$) (*Middle*). (*Right*) Although smaller in amplitude, macroscopic responses of $\alpha7_{LC}$ to 1 mM ACh show fast onset and desensitization, identical to those of wild-type α7 (see the different scales for representative currents).

1. Rayes D, De Rosa MJ, Sine SM, Bouzat C (2009) Number and locations of agonist binding sites required to activate homomeric Cys-loop receptors. *J Neurosci* 29(18):6022–6032.
2. Rayes D, Spitzmaul G, Sine SM, Bouzat C (2005) Single-channel kinetic analysis of chimeric alpha7-5HT3A receptors. *Mol Pharmacol* 68(5):1475–1483.
3. Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ, Peters JA (2003) A cytoplasmic region determines single-channel conductance in 5-HT3 receptors. *Nature* 424(6946):321–324.

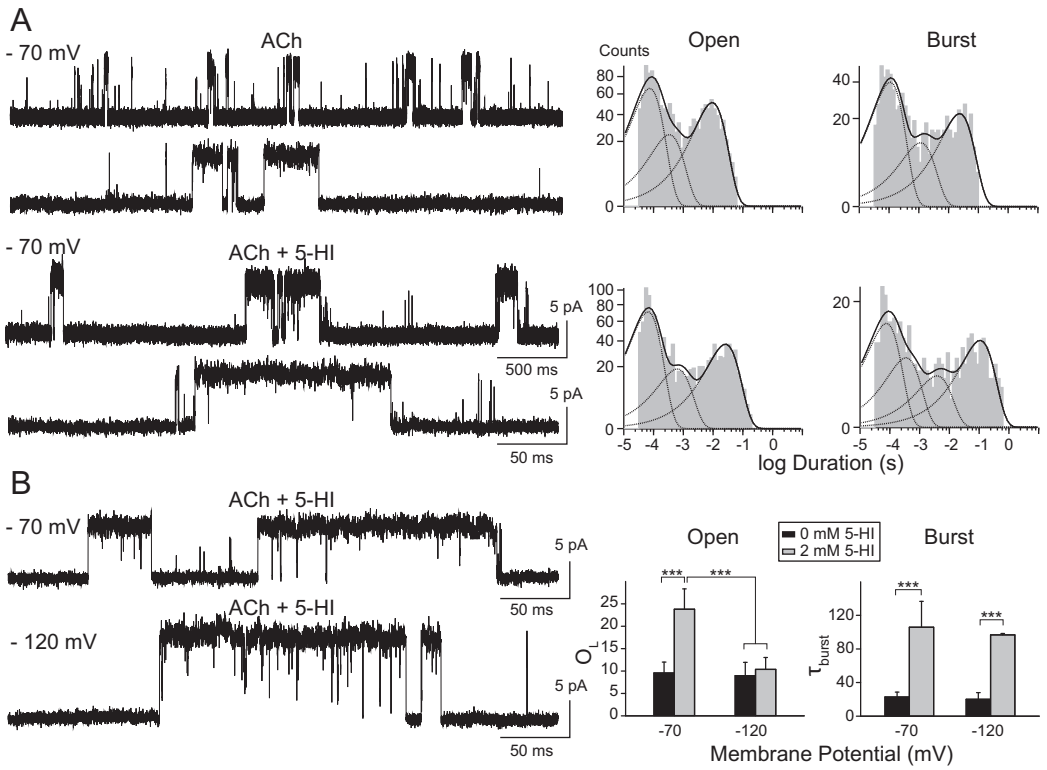

Fig. S5. Potentiation of $\alpha7$–$5HT_3A$ chimeric receptors by 5-HI. Single-channel recordings from cells transfected with the high-conductance form (HC) of $\alpha7$–$5HT_3A$ (1) activated by 1 mM ACh. (*A*) Single-channel currents of $\alpha7$–$5HT_3A_{HC}$ in the absence (*Upper*) or presence (*Lower*) of 2 mM 5-HI (membrane potential: −70 mV). Currents are shown at two different time scales with the corresponding open and burst duration histograms (*Right*). (*B*) 5-HI produces open-channel block of $\alpha7$–$5HT_3A_{HC}$ chimera, which is evidenced by a significant decrease in the mean open time at −120 mV with respect to that at −70 mV (left bar chart). In contrast, the burst duration is not affected between −70 and −120 mV (right bar chart). Data are shown as mean ± SD; ***$P < 0.001$ (Student *t* test).

1. Rayes D, Spitzmaul G, Sine SM, Bouzat C (2005) Single-channel kinetic analysis of chimeric alpha7-5HT3A receptors. *Mol Pharmacol* 68(5):1475–1483.

Table S1. Open and burst durations of wild-type and mutant α7

Condition (subunit ratio)	ACh, μM	O_L, ms (area)	τ_{burst}, ms (area)	*n*
α7	100	0.28 ± 0.09 (0.19 ± 0.02)	0.36 ± 0.08 (0.18 ± 0.12)	3
α7	500	0.23 ± 0.04 (0.05 ± 0.03)	0.24 ± 0.04 (0.10 ± 0.02)	4
(*) α7 + α7-Y188T (1:1)	500	0.18 ± 0.10 (0.04 ± 0.02)	0.27 ± 0.15 (0.06 ± 0.02)	3
(*) α7 + α7-Y188T (1:3)	500	0.19 ± 0.02 (0.05 ± 0.02)	0.23 ± 0.02 (0.12 ± 0.08)	4
α7TSLMF	100	0.90 ± 0.36 (0.18 ± 0.07)	1.60 ± 0.60 (0.12 ± 0.05)	7
α7 (2 mM 5-HI)	100	2.10 ± 0.20 (0.45 ± 0.19)	5.10 ± 0.60 (0.32 ± 0.20)	6

The table shows the mean ± SD values and the number of patches (*n*) for each condition. O_L corresponds to the longest duration component obtained from the corresponding open time histogram, and τ_{burst} is the mean burst duration. (*), Assuming identical expression of both subunits, the binomial distribution predicts that 15% (1:1 wt:wt ratio) and 39% (1:3 ratio) of the receptors contain only one functional binding site, and about 3% (1:1 ratio) and less than 0.1% (1:3 ratio), five functional binding sites.

Table S2. Open and burst durations from receptors containing different number of wild-type and low-conductance α7 subunits

Schemes for receptors of different amplitude classes					
α7 TSLMF + α7 TSLMF$_{LC}$					
Amplitude class, pA	2.3 ± 0.2	3.7 ± 0.3	6.0 ± 0.3	8.1 ± 0.2	10.0 ± 0.2
O_L, ms	0.92 ± 0.10	0.71 ± 0.12	0.74 ± 0.231	0.82 ± 0.26	0.92 ± 0.24
τ_{burst} , ms	1.00 ± 0.32	1.15 ± 0.51	1.38 ± 0.87	1.20 ± 0.40	1.24 ± 0.31
n	5	5	6	6	6
α7 + α7$_{LC}$ + 5-HI					
Amplitude class, pA	nm	4.4 ± 0.2	6.4 ± 0.2	8.3 ± 0.4	10.1 ± 0.3
O_L, ms	nm	1.50 ± 0.30	1.80 ± 0.50	2.40 ± 0.50	2.40 ± 0.80
τ_{burst}, ms	nm	5.40 ± 1.70	5.00 ± 1.30	5.50 ± 1.40	6.00 ± 1.60
n	7	9	5	6	3

Single channels were recorded in the presence of 100 μM ACh from cells expressing the subunit combination shown. For α7 and α7$_{LC}$, 2 mM 5-HI was also present in the pipette solution. Amplitude classes are expressed in pA (−70 mV). The table shows the mean ± SD values for *n* different recordings for each condition. O_L corresponds to the longest duration component obtained from the corresponding open time histogram, and τ_{burst} corresponds to the mean duration of bursts. Nonstatistically significant differences are observed in the mean values among amplitude classes for the same condition. nm, not measured. In the schemes, high- and low-conductance subunits are shown as black and gray circles, respectively. The arrows indicate functional binding sites.

Table S3. Channel lifetime of α7 channels carrying one or five functional binding sites

Schemes for receptors with different number of binding sites	No. of binding sites: 5 	No. of binding sites: 1 
Subunits	α7TSLMF + α7TSLMF$_{LC}$	α7TSLMF-Y188T + α7TSLMF$_{LC}$
O_L, ms	0.82 ± 0.26	0.72 ± 0.10
τ_{burst}, ms	1.20 ± 0.40	1.10 ± 0.55
n	6	8
Subunits	α7 + α7$_{LC}$ + 5-HI	α7-Y188T + α7$_{LC}$ + 5-HI
O_L , ms	2.30 ± 0.50	2.20 ± 0.70
τ_{burst}, ms	5.40 ± 1.40	5.10 ± 1.00
n	12	7

Single channels were recorded from cells transfected with the low-conductance forms of α7TSLMF or α7 subunits together with the wild-type conductance subunit carrying (1 functional binding site) or not (5 functional binding sites) the Y188T mutation. The 8-pA channels recorded from cells transfected with α7TSLMF and α7TSLMF$_{LC}$ or α7 and α7$_{LC}$ combinations contain five functional binding sites whereas those from cells transfected with α7TSLMF-Y188T and α7TSLMF$_{LC}$ or α7-Y188T and α7$_{LC}$ contain only one functional binding site. O_L corresponds to the longest duration component, and τ_{burst} corresponds to the mean burst duration. Data are expressed as the mean ± SD of *n* different recordings for each condition. ACh, 100 μM; 5-HI, 2 mM. In the schemes, LC subunits are shown in gray, and Y188T mutation is represented by a small black circle. The arrows indicate functional binding sites.

Table S4. Open and burst duration of α7 channels containing different number of functional binding sites activated by ACh and potentiated by 5-HI

Schemes for receptors with different number of binding sites	No. of binding sites				
	1	2	3	4	5
					
O_L, ms	2.20 ± 0.70	1.90 ± 0.43	1.80 ± 0.57	2.48 ± 0.70	2.70 ± 0.30
τ_{burst}, ms	5.10 ± 1.00	4.70 ± 0.95	5.50 ± 2.00	6.26 ± 1.40	6.20 ± 1.80
n	7	9	5	6	4

We measured single-channel activity from cells transfected with α7-Y188T and $\alpha7_{LC}$ or the reverse combination, α7 and $\alpha7_{LC}$-Y188T, to obtain pentameric arrangements containing different numbers of active binding sites. The table shows the mean ± SD of the duration of the slowest open component (O_L) and burst (τ_{burst}). ACh, 100 μM; 5-HI, 2 mM. *n*, Number of patches for each condition. LC subunits are shown in gray, and Y188T mutation is shown by a small black circle. The arrows indicate functional binding sites. Nonstatistically significant differences are observed in the mean values among amplitude classes.