Short Isoforms of the Cold Receptor TRPM8 Inhibit Channel Gating by Mimicking Heat Action rather than Chemical Inhibitors

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SUPPLEMENTAL DATA

FIGURE LEGENDS

FIGURE S1. **The 1D dwell-time distributions for TRPM8 co-expressed with sM8-6 are described by the sums of 2 open (top) and 5 closed (bottom) exponential components.** Compare to Figs. 1 C, 2 B, and 3 B for differences with WT TRPM8 data and inhibition by BCTC and clotrimazole.

FIGURE S2. **The 7-state kinetic scheme for TRPM8 shown in Figure 4 predicts the main features of the gating of TRPM8 co-expressed with sM8-6 isoforms.** *A* and *B*, the 1D histograms are for experimental data for sM8-6 isoforms from Fig. 1 C and the continuous lines are the predicted distributions from the 7-state model in Fig. 4. *C*, the 2D dwell-time distribution predicted by the kinetic scheme captures the major features of the experimental distributions (compare to Fig. 1 D, middle). The most likely rate constants were obtained by fitting the 2D experimental dwell-time distribution and were then used to simulate 100,000 open and closed intervals which were analyzed in the same way the experimental data were analyzed to generate the predicted 1D distributions in *A* and *B* and the predicted 2D dwell-time distributions in *C*. Experimental and simulated distributions were normalized to have the same numbers of events.

FIGURE S3. **The 7-state kinetic scheme shown in Figure 4 predicts the main features of the gating in the absence and presence of BCTC.** *A*, The 1D dwell-time histograms are for experimental data from Fig. 2 B and the continuous lines are the predicted distributions for the 7-state model in Fig. 4. *B*, the predicted 2D dwell-time distributions capture the major features of the experimental distributions (compare to Fig. 2 C). The most likely rate constants and predictions were obtained as described in the legend to Fig. S2.

FIGURE S4. **The 7-state kinetic scheme shown in Figure 4 predicts the main features of the gating in the absence and presence of clotrimazole.** *A*, the 1D dwell-time histograms are for experimental data from Fig. 3 B and the continuous lines are the predicted distributions for the 7-state model in Fig. 4. *B*, the predicted 2D dwell-time distributions capture the major features of the experimental distributions (compare to Fig. 3 C). The most likely rate constants and predictions were obtained as described in the legend to Fig. S2.

TABLE S1

Effects of sM8-6 isoforms on 1D exponential components

	WT TRPM8 (RT)[a]		sM8-6 (RT)[b]		WT TRPM8 (30°C)[c]	
Exp.	τ (ms)	Area	τ (ms)	Area	τ (ms)	Area
EC1	0.23 ± 0.01	0.44 ± 0.02	0.23 ± 0.09	0.14 ± 0.02**	0.26 ± 0.02	0.20 ± 0.03**
EC2	1.13 ± 0.13	0.37 ± 0.02	1.87 ± 0.49	0.31 ± 0.04	1.11 ± 0.09	0.31 ± 0.03
EC3	4.41 ± 1.05	0.16 ± 0.02	13.4 ± 4.65	0.18 ± 0.04	15.6 ± 2.99**	0.19 ± 0.04
EC4	19.2 ± 4.12	0.025 ± 0.006	48.1 ± 7.60*	0.27 ± 0.05**	67.6 ± 17.5**	0.25 ± 0.05**
EC5	2428 ± 896	0.00035 ± 0.00008	93.2 ± 16.5**	0.10 ± 0.03**	735 ± 223*	0.072 ± 0.042**
EO1	0.87 ± 0.08	0.56 ± 0.04	0.41 ± 0.06**	0.83 ± 0.08*	0.29 ± 0.03**	0.77 ± 0.03**
EO2	2.27 ± 0.27	0.44 ± 0.04	1.22 ± 0.22*	0.17 ± 0.08*	0.77 ± 0.09**	0.23 ± 0.03**

[a and c] Means ± SEM for 2 open (EO1–EO2) and 5 closed (EC1–EC5) exponential components from 8 (RT, control, two left columns) and 13 single-channel patches (30°C, two right columns). Data from (15). These mean values should not be used to calculate P_o and mean open and mean closed time. Rather separate values would need to be calculated for each channel and then averaged.

[b] Mean values from 5 single-channel patches (RT) with co-expression of sM8-6 isoforms.

[* or **] Significance according to the Mann-Whitney test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S2

Effects of BCTC on 1D exponential components

	0 μM		1 μM		10 μM	
Exp.	τ (ms)	Area	τ (ms)	Area	τ (ms)	Area
EC1	0.12 ± 0.01	0.40 ± 0.02	0.12 ± 0.01	0.33 ± 0.01*	0.14 ± 0.02	0.32 ± 0.05*
EC2	0.95 ± 0.12	0.32 ± 0.03	1.29 ± 0.20	0.31 ± 0.02	1.23 ± 0.25	0.27 ± 0.04
EC3	6.07 ± 1.00	0.23 ± 0.02	11.5 ± 3.95	0.21 ± 0.01	20.3 ± 5.83	0.19 ± 0.05
EC4	25.8 ± 5.59	0.056 ± 0.009	60.5 ± 20.6**	0.15 ± 0.02	152 ± 67**	0.19 ± 0.05
EC5	6440 ± 2334	0.0005 ± 0.0001	3819 ± 1191	0.0054 ± 0.0026**	5432 ± 3194	0.029 ± 0.017**
EO1	0.30 ± 0.02	0.47 ± 0.03	0.22 ± 0.03	0.53 ± 0.07	0.25 ± 0.03	0.73 ± 0.08
EO2	0.84 ± 0.05	0.53 ± 0.03	2.77 ± 2.23	0.47 ± 0.07	1.10 ± 0.49	0.27 ± 0.08

Means ± SEM for 2 open (EO1–EO2) and 5 closed (EC1–EC5) exponential components at RT from 10 single-channel patches with increasing concentrations of BCTC (from left to right). These mean values should not be used to calculate P_o and mean open and mean closed time. Rather separate values would need to be calculated for each channel and then averaged.

* or ** Significance according to the non-parametric ANOVA test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S3

Effects of clotrimazole on 1D exponential components

	0 μM		1 μM		10 μM	
Exp.	τ (ms)	Area	τ (ms)	Area	τ (ms)	Area
EC1	0.10 ± 0.01	0.46 ± 0.01	0.11 ± 0.01	0.40 ± 0.03**	0.18 ± 0.02	0.36 ± 0.03**
EC2	0.77 ± 0.08	0.28 ± 0.02	0.73 ± 0.06*	0.27 ± 0.02	1.35 ± 0.32*	0.28 ± 0.02
EC3	4.33 ± 0.33	0.21 ± 0.01	3.87 ± 0.32	0.22 ± 0.02	13.5 ± 5.63	0.19 ± 0.02
EC4	27.6 ± 5.66	0.054 ± 0.010	34.2 ± 5.22*	0.12 ± 0.02*	139 ± 49*	0.16 ± 0.04*
EC5	7123 ± 4538	0.0005 ± 0.0001	6770 ± 2653	0.0048 ± 0.0042*	10540 ± 3863	0.0096 ± 0.0056*
EO1	0.29 ± 0.02	0.42 ± 0.05	0.31 ± 0.03	0.59 ± 0.08	0.30 ± 0.03	0.65 ± 0.06
EO2	0.90 ± 0.05	0.58 ± 0.05	0.95 ± 0.13	0.41 ± 0.08	0.84 ± 0.08	0.35 ± 0.06

Means ± SEM for 2 open (EO1–EO2) and 5 closed (EC1–EC5) exponential components at RT from 6 single-channel patches with increasing concentrations of clotrimazole (from left to right). These mean values should not be used to calculate P_o and mean open and mean closed time. Rather separate values would need to be calculated for each channel and then averaged.

* or ** Significance according to the non-parametric ANOVA test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S4

Rate constants (s^{-1}) for 7-state scheme with increasing BCTC

	Control (0 μM)	1 μM	10 μM
Transition	2D	2D	2D
O1-C3	2720 ± 108	3791 ± 499	3073 ± 699
C3-O1	10192 ± 1080	9612 ± 1716	7627 ± 2313
O2-C4	5071 ± 301	8942 ± 1222	12668 ± 4399
C4-O2	535 ± 77	2512 ± 1376	4310 ± 1292
C3-C4	4916 ± 247	4852 ± 761	4435 ± 852
C4-C3	983 ± 96	981 ± 189	1265 ± 450
C4-C5	733 ± 128	1806 ± 817	2841 ± 892
C5-C4	490 ± 106	327 ± 49	398 ± 138
C5-C6	66 ± 16	116 ± 22	165 ± 36
C6-C5	89 ± 19	59 ± 10*	43 ± 15*
C6-C7	0.73 ± 0.16	0.96 ± 0.26	1.46 ± 1.10
C7-C6	0.41 ± 0.13	1.26 ± 0.75	8.42 ± 7.86

Means ± SEM for rate constants from the 7-state scheme with 2 open and 5 closed states at RT fitted separately to 10 single-channel patches and increasing concentrations of BCTC (from left to right).
* or ** Significance according to the non-parametric ANOVA test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S5

Rate constants (s^{-1}) for 7-state scheme with increasing clotrimazole

	Control (0 μM)	1 μM	10 μM
Transition	2D	2D	2D
O1-C3	3263 ± 276	3385 ± 114*	1718 ± 519*
C3-O1	13673 ± 928	12824 ± 1180	8443 ± 3817
O2-C4	5769 ± 702	6173 ± 564	5138 ± 468
C4-O2	507 ± 82	574 ± 75	4943 ± 1578
C3-C4	4444 ± 251	4910 ± 421	2635 ± 685
C4-C3	955 ± 63	820 ± 37	1346 ± 366
C4-C5	770 ± 131	794 ± 54	3389 ± 876
C5-C4	462 ± 70	408 ± 52	320 ± 62
C5-C6	59 ± 15	107 ± 25	138 ± 16
C6-C5	60 ± 11	47 ± 5	32 ± 5
C6-C7	0.85 ± 0.23	0.21 ± 0.06	1.30 ± 0.90
C7-C6	0.57 ± 0.21	0.16 ± 0.06	0.71 ± 0.33

Means ± SEM for rate constants from the 7-state scheme with 2 open and 5 closed states at RT fitted separately to 6 single-channel patches and increasing concentrations of clotrimazole (from left to right).

* or ** Significance according to the non-parametric ANOVA test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S6

Rate constants (s^{-1}) for 7-state scheme with sM8-6 isoforms

	WT TRPM8 (RT)[a]	sM8-6 (RT)[b]	WT TRPM8 (30°C)[c]
Transition	2D	2D	2D
O1-C3	888 ± 103	1708 ± 360	3007 ± 405**
C3-O1	4125 ± 634	1683 ± 283*	3096 ± 647
O2-C4	1874 ± 176	4955 ± 859**	6179 ± 635**
C4-O2	2679 ± 551	1816 ± 781	1400 ± 370
C3-C4	2775 ± 308	1270 ± 643	1582 ± 119**
C4-C3	1623 ± 309	183 ± 112**	490 ± 144**
C4-C5	2133 ± 601	1777 ± 1370	1168 ± 312
C5-C4	906 ± 172	356 ± 214*	237 ± 87**
C5-C6	64 ± 10	197 ± 123	99 ± 57
C6-C5	121 ± 20	59 ± 20	47 ± 13**
C6-C7	2.30 ± 0.91	1.89 ± 0.51	2.20 ± 0.67
C7-C6	3.64 ± 2.55	16 ± 3*	5.73 ± 2.34

[a or c] Means ± SEM for rate constants from 7-state scheme fitted separately to 8 (RT, control, left column) and 13 single-channel patches (30°C, right column). Data from (15).

[b] Mean values from 5 single-channel patches at RT with co-expressed sM8-6 isoforms (middle column).

[* and **] Significance according to the Mann-Whitney test (*, $P < 0.05$; **, $P < 0.01$).

TABLE S7

Number of rate constants moved in the same direction by each of the compared modulators

Modulators	Number	[a]P (cumulative)
sM8-6 vs. 30°C	12/12	0.00024
BCTC vs. CTZ	10/12	0.0194
sM8-6 vs. CTZ	6/12	0.613
sM8-6 vs. BCTC	8/12	0.194
30°C vs. CTZ	6/12	0.613
30°C vs. BCTC	8/12	0.193

[a] Probability (cumulative) of observing the indicated number or more of the rate constants (out of 12) moving in the same direction for each of the indicated modulators (see Materials and Methods). sM8-6 inhibition is most like 30°C inhibition, and BCTC inhibition is most like clotrimazole (CTZ) inhibition in terms of the directions the various modulators move the rate constants.

Figure S1

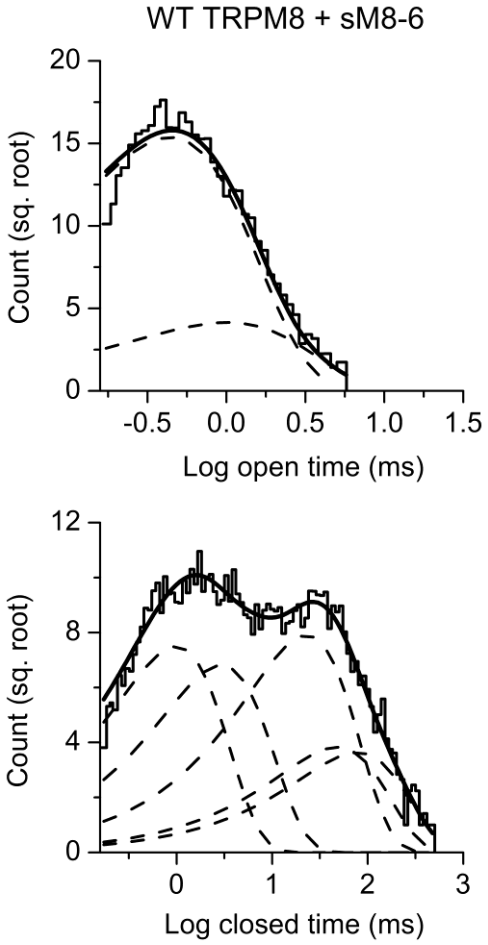

WT TRPM8 + sM8-6
Count (sq. root)
Log open time (ms)
Count (sq. root)
Log closed time (ms)

Figure S2

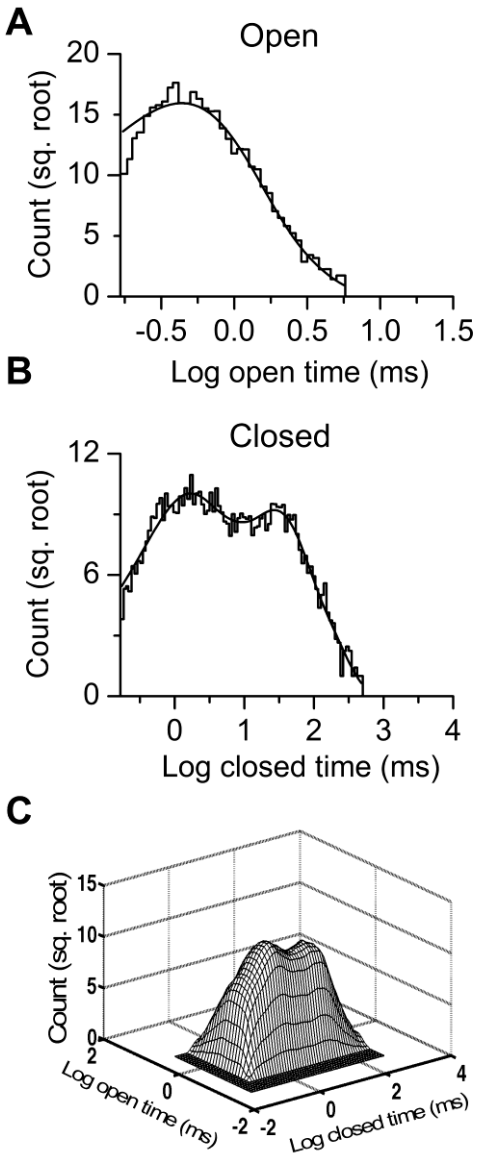

A
Open
Count (sq. root)
20
15
10
5
0
-0.5
0.0
0.5
1.0
1.5
Log open time (ms)
B
Closed
Count (sq. root)
12
6
0
0
1
2
3
4
Log closed time (ms)
C
Count (sq. root)
15
10
5
0
2
0
-2
Log open time (ms)
-2
0
2
4
Log closed time (ms)

Figure S3

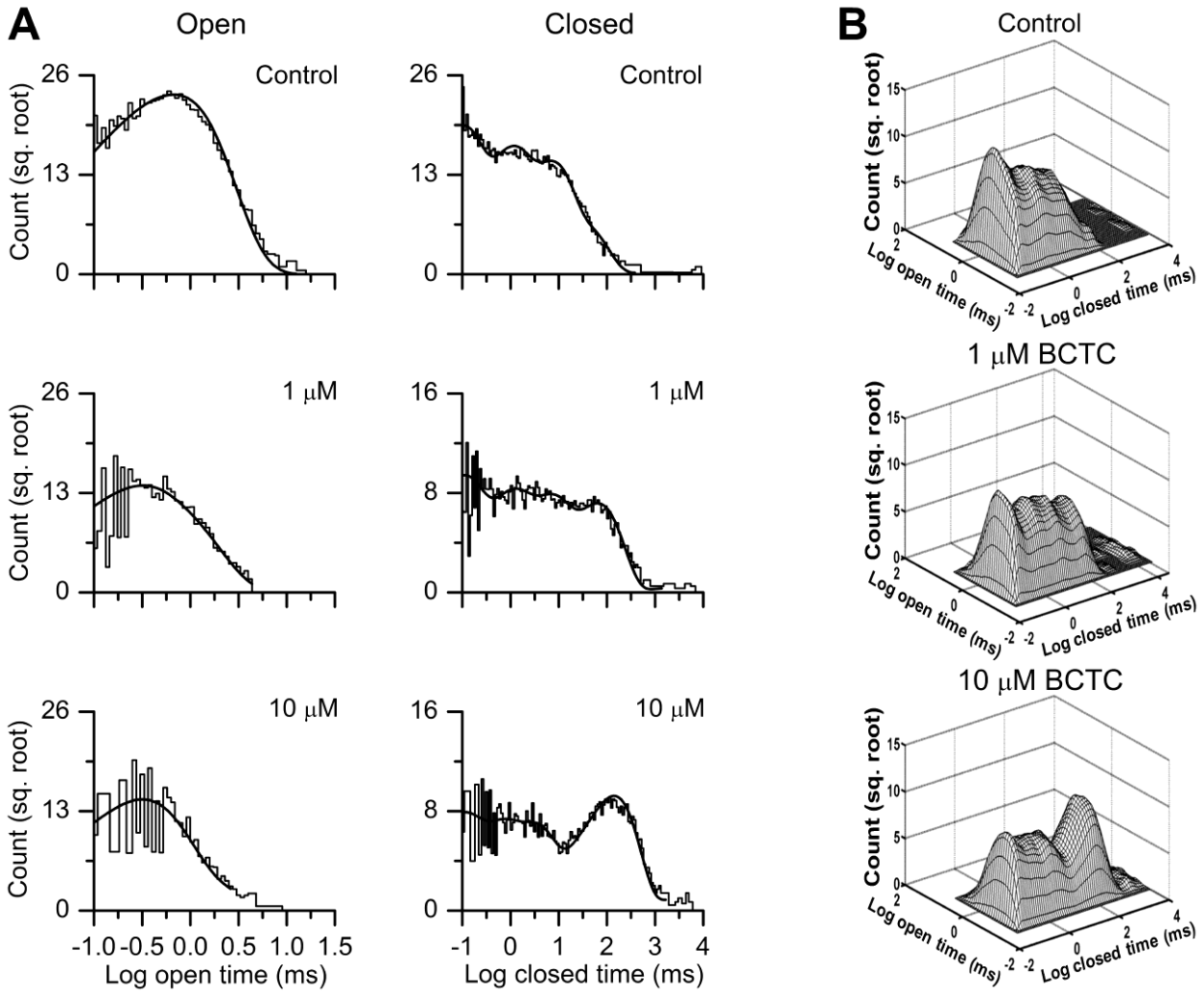
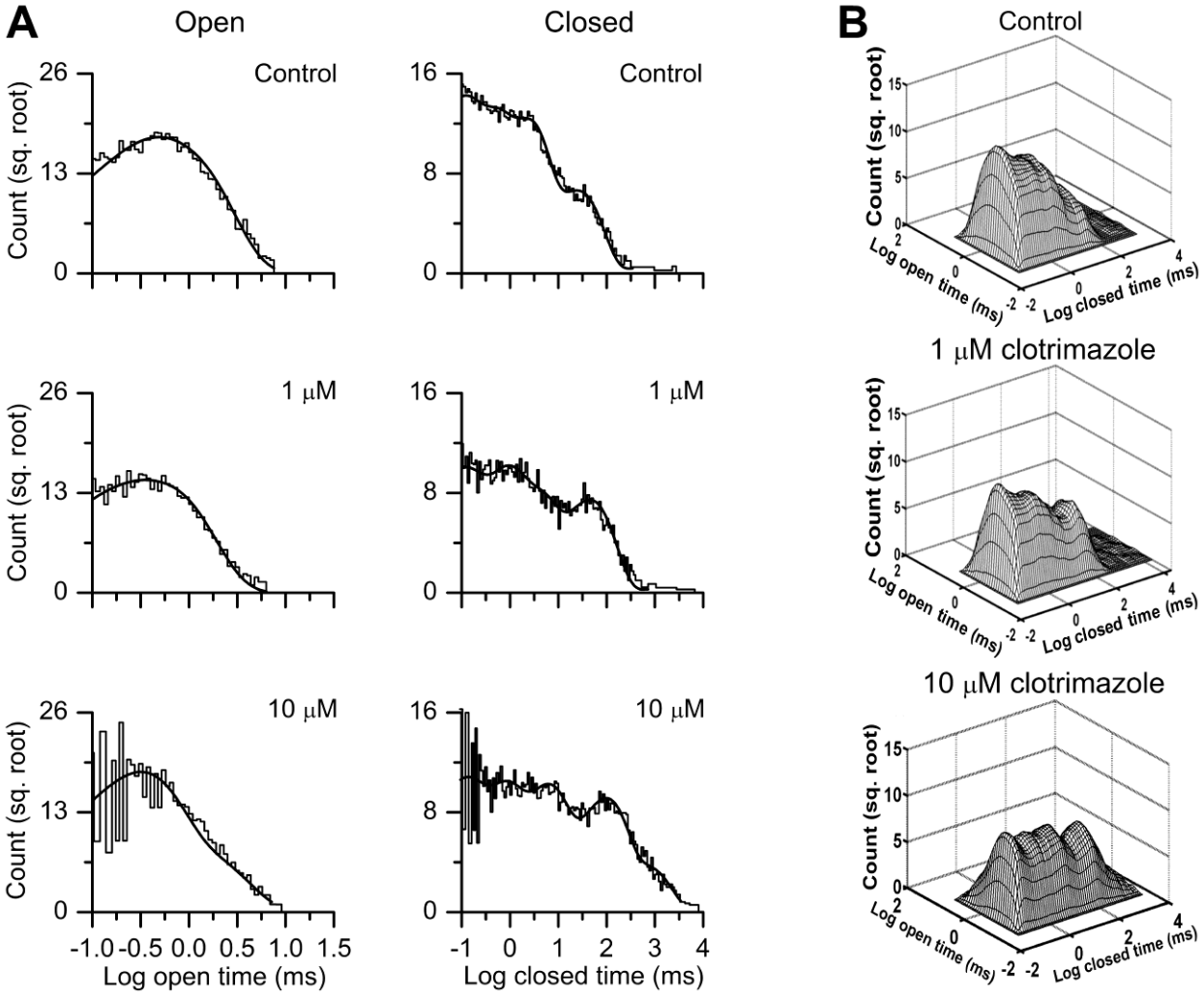

A
Open
Closed
Control
Control
1 μM
1 μM
10 μM
10 μM
Count (sq. root)
Log open time (ms)
Log closed time (ms)
B
Control
1 μM BCTC
10 μM BCTC
Count (sq. root)
Log open time (ms)
Log closed time (ms)

Figure S4


A
Open
Closed
Control
Control
1 μM
1 μM
10 μM
10 μM
Count (sq. root)
Log open time (ms)
Log closed time (ms)
B
Control
1 μM clotrimazole
10 μM clotrimazole
Count (sq. root)
Log open time (ms)
Log closed time (ms)