Two mechanisms can favour block of clustered sodium channel openings

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The cardiac sodium channel $Na_v1.5$ can reopen repeatedly during a single depolarization. In the long-QT-syndrome variant R1623Q, initial openings and openings within a burst had the same reported mean duration, raising the question of whether a blocker could distinguish them by their timing rather than their length.[1] Here we examine a two-step binding mechanism proposed for BT-01, in which a drug-bound channel remains conductive before its pore becomes blocked. With the number of openings, total open time and total elapsed time held constant, clustering four of five openings increased the calculated suppression of sodium charge from 3.88% to 14.02%. This preference was not specific to the proposed mechanism: residual pore block alone also favoured closely spaced openings. The two mechanisms were distinguishable, however, in the time course of a second opening. Residual block reduced current at reopening, whereas retained conductive binding accelerated the subsequent development of block. Allowing opening durations to vary weakened burst selectivity, and neither burst protocol reached the specified 50% charge-suppression target. These results identify a measurement that can test the proposed mechanism without treating preferential burst inhibition as evidence that the mechanism exists.

Opening history as a pharmacological variable

A blocker that suppresses late sodium entry without compromising the peak current of a subsequent beat would need to distinguish between different patterns of activity in the same channel. Single-channel recordings of $Na_v1.5$-R1623Q illustrate why opening duration alone may be insufficient. At −20 mV, the mutant channel burst more readily than the wild-type channel, yet its initial openings and openings within bursts both had a reported mean duration of 1.7 ms. A separate study of GS967 illustrates a complementary difficulty: preferential inhibition of late sodium current under tonic conditions coexisted with substantial use-dependent inhibition of peak current during repeated stimulation. Selectivity measured under one stimulation protocol therefore need not imply preservation of current under another. [1,2]

We asked whether a conducting, drug-bound intermediate could confer sensitivity to the interval between openings. The proposed sequence was $U \rightarrow A \rightarrow B$, where U is unbound, A is drug-bound but fully conductive, and B is pore-blocked. Both transitions occurred only while the channel gate was open. During closure, either bound state could return to U. We assigned A a longer lifetime than B, so that a channel could recover its ability to conduct while retaining a drug molecule capable of rapidly blocking the pore on reopening.

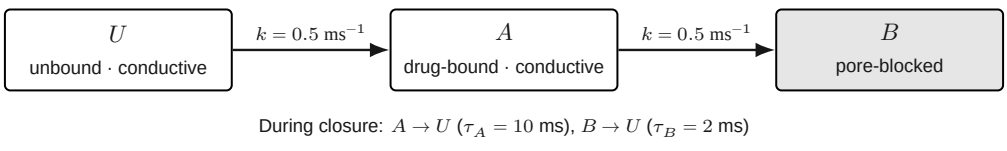


Figure 1 | Proposed two-step state mechanism and recovery kinetics. Transitions to A and B occur only while the gate is open.

BT-01 denotes the candidate mechanism under study. The rate constants were neither measured for the compound nor

inferred from its structure. The calculations therefore address two questions about the mechanism—not the efficacy of the molecule: how much charge can it suppress, and what observation would distinguish retained conductive binding from incomplete recovery of an already blocked pore?

Clustering increases block at fixed total open time

We first prescribed five openings of 1 ms each. Because every opening carried the same unitary current in the absence of block, each train offered the same opportunity for sodium entry. The outcome was the fraction of that charge prevented over the *entire train*, not the fraction of channels blocked at its end.

Table 1 | Effect of opening history on sodium charge suppression

Opening protocol	Successive closed intervals (ms)	Total elapsed time (ms)	Charge suppressed (%)
One uninterrupted 5-ms opening	None	5	34.78
Five 1-ms openings, closely spaced	1, 1, 1, 1	9	19.42
Five 1-ms openings, first four clustered	1, 1, 1, 77	85	14.02
Five 1-ms openings, evenly spaced	20, 20, 20, 20	85	3.88

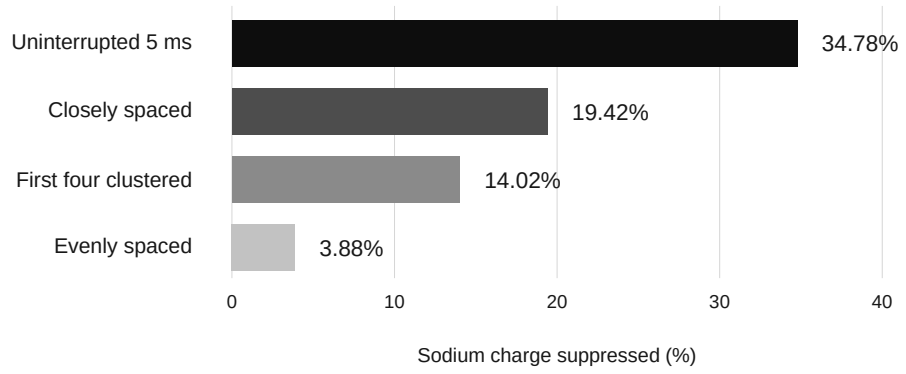


Figure 2 | Charge suppression by opening protocol. Exact values correspond to Table 1.

The last two protocols isolate the effect of timing. Both contained five identical openings and 80 ms of closure within an 85-ms observation interval. Moving most of the closed time into the final gap increased charge suppression by a factor of 3.6. Thus, neither the opening count, the total open time nor the mean closed interval was sufficient to predict inhibition.

In the closely spaced train, suppression rose from 3.27% during the first opening to 30.24% during the fifth. The train as a whole nevertheless lost only 19.42% of its charge. This difference matters for interpreting an apparently strong late response: the blocker cannot recover charge that passed during earlier openings.

The uninterrupted opening establishes a stronger limit. In the specified scheme, a closure can erase progress towards block but cannot advance it. Starting from U, no partition of exactly 5 ms of open time can therefore suppress more charge than a continuous 5-ms opening. That upper bound was 34.78%, below the 50% design target. For these kinetics, no rearrangement of the five milliseconds can make up the difference.

Residual pore block also favours short gaps

To determine which state carried the effect of one opening into the next, we selectively reset A, B or both to U at each closure. These interventions left the kinetics during openings unchanged.

Table 2 | Charge suppression after selective removal of state persistence

State retained across closures	1-ms gaps (%)	20-ms gaps (%)
Neither state	3.27	3.27
Conducting bound state A only	8.21	3.88
Blocked state B only	10.07	3.27
Both states	19.42	3.88

Retaining B alone increased suppression from 3.27% with long gaps to 10.07% with short gaps, despite complete loss of A at every closure. Preferential inhibition of closely spaced openings therefore demonstrates that *some* effect persists between openings, but does not establish that the persistent state conducts.

The effects of A and B were not additive. For short gaps, retaining both produced 4.40 percentage points more suppression than the sum of their separate increments above baseline. The reason follows from the sequence of events: A retained after one opening can become B during the next, and that newly formed B can persist into a third. Resetting either state interrupts this sequence.

Variable opening durations weaken apparent selectivity

A train of identical 1-ms openings excludes long isolated events. We therefore repeated the calculation with independent, exponentially distributed opening durations of mean 1 ms. The expected total open time remained 5 ms, but individual trains could be shorter or longer. We calculated the ratio of expected charge lost to expected drug-free charge, so that each opening contributed in proportion to the charge it could carry.

Table 3 | Effect of opening-duration variability at fixed mean open time

Distribution of opening durations	Suppression, 1-ms gaps (%)	Suppression, 20-ms gaps (%)	Short-gap/long-gap ratio
Fixed at 1 ms	19.42	3.88	5.01
Exponential, mean 1 ms	25.86	11.67	2.22

Variability increased suppression in both trains, but the larger proportional change occurred in the dispersed train. An occasional long opening carries more charge and gives an initially unbound channel time to complete both binding steps before it closes.

The size of this effect can be obtained without simulating a train. For a single opening of exponentially distributed duration with mean τ, starting from U, the fraction of expected charge suppressed is

$$S_{Q,\mathrm{fresh}} = \left(\frac{k\tau}{1 + k\tau}\right)^2, \tag{1}$$

where k is the common rate constant for the two transitions. At $k = 0.5$ ms^{-1} and $\tau = 1$ ms, this gives $1/9$, or 11.11%. Thus, almost all of the 11.67% suppression in the dispersed train can be accounted for without invoking persistence between openings. Matching mean open time alone is not an adequate control when comparing burst selectivity.

The onset and development of block distinguish the mechanisms

The charge integral combines two physically distinct losses: current that is absent as soon as the channel reopens, and current that disappears *during* the new opening. Separating these losses yields a direct test of the proposed mechanism.

Let a_g and b_g be the fractions in states A and B immediately after a closed interval of duration g. Let $b_2(t)$ be the blocked fraction at time t during the second opening. Two relations describe its initial behaviour:

$$b_2(0) = b_g, \qquad \left.\frac{\mathrm{d}b_2}{\mathrm{d}t}\right|_{t=0} = k\,a_g. \tag{2}$$

The first relation measures residual pore block. The second follows because only channels already in A can enter B to first order in time. A channel starting in U must complete two transitions, so its probability of becoming blocked initially grows quadratically rather than linearly. Under the specified kinetics, residual B alone therefore produces an immediate current deficit but no initial slope of newly developing block. Retained A produces the converse: it conducts at reopening but gives rise to block with a non-zero initial slope.

Two 1-ms openings separated by 10 ms illustrate the difference. Only **0.061%** of channels remained blocked at the onset of the second opening. By its end, **12.46%** were blocked, compared with **9.02%** at the end of a fresh opening. Holding the initial residual block fixed and removing only the retained A reduced the endpoint by **3.38 percentage points**. These are instantaneous blocked fractions, not integrated charge losses.

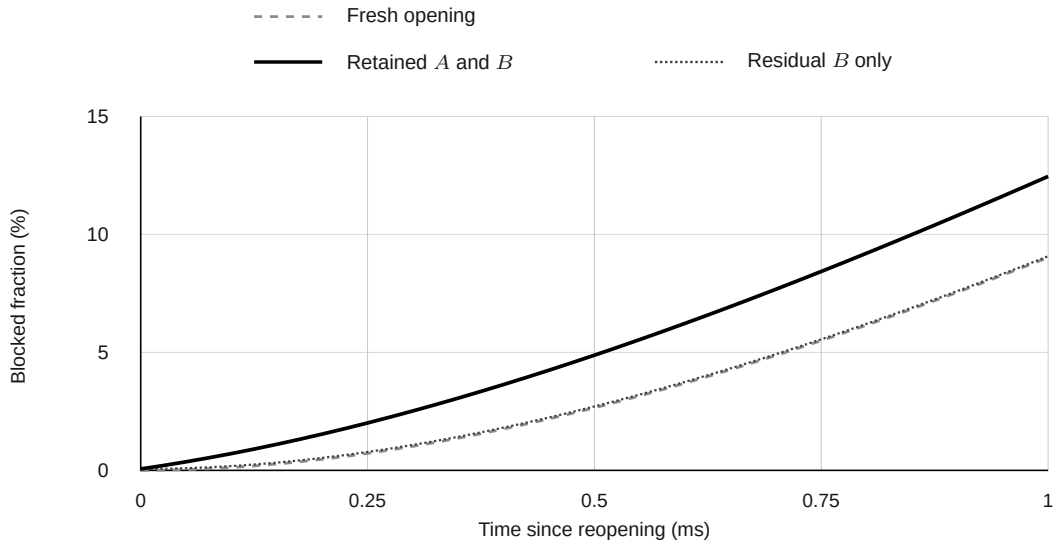


Figure 3 | Predicted development of pore block during the second 1-ms opening after a 10-ms gap. Curves are evaluations of the stated interval equations.

The discriminating interval depends on the recovery kinetics. As illustrative measurement criteria, we required residual block at onset to be below 0.1% and the endpoint contribution from retained A to be at least 2 percentage points. With lifetimes of 10 ms for A and 2 ms for B, both criteria were met over gaps of approximately 9–15 ms. If the lifetime of B was increased to 10 ms, reducing residual block below 0.1% required approximately 45 ms, by which time the signal from A was too small. The 9–15-ms interval is thus a prediction conditional on the assumed lifetimes, not a validated property of BT-01.

Discussion

The main result is a distinction between **selectivity and mechanism**. At fixed total open time and elapsed time, the placement of closures changed sodium charge suppression more than threefold. Yet two different processes produced the preference for short gaps: persistence of an already blocked pore and persistence of a bound drug that did not yet obstruct conduction. Measuring integrated charge established the timing preference; it did not establish its cause.

A more informative experiment would resolve the onset and subsequent time course of inhibition across several recovery intervals, with drug-free recovery measured under the same conditions. The decisive comparison is not simply whether the second response is smaller than the first. It is whether inhibition develops faster during the second response **after accounting for block already present at its onset**. Even this pattern would require controls for native gating. In R1623Q channels, for example, lidocaine was reported to enhance closed-state inactivation

without changing the single-channel open-time distribution. A macroscopic current deficit therefore need not reflect pore obstruction, and the beginning of a voltage step cannot be equated with the instant at which every channel opens. [3]

The calculations also define what the proposed mechanism did not achieve. Neither burst protocol reached 50% charge suppression. Allowing opening durations to vary substantially increased inhibition of dispersed openings. The analysis prescribed gating histories rather than predicting them: it did not calculate recovery over a cardiac cycle, ventricular peak current, action-potential duration or the QT interval. It therefore provides no evidence that BT-01 preserves the next heartbeat or prevents arrhythmia. Its experimentally testable prediction is narrower: **after pre-existing block has largely recovered, does a previous opening still accelerate block during the next one?**

Methods

State kinetics and interval propagation

Opening and closure refer to prescribed channel-gate states, not to observed current: a gate-open channel in B carries no current. During a gate-open interval, the occupancy probabilities u, a, b, with $u + a + b = 1$, obeyed

$$\frac{\mathrm{d}u}{\mathrm{d}t} = -ku, \qquad \frac{\mathrm{d}a}{\mathrm{d}t} = ku - ka, \qquad \frac{\mathrm{d}b}{\mathrm{d}t} = ka. \tag{3}$$

Both transitions had the effective first-order rate constant $k = 0.5$ ms^{-1}. No reverse transition occurred during an opening. For starting occupancies u_0, a_0, b_0, the exact interval solution was

$$\begin{aligned} u(t) &= u_0 e^{-kt}, \\ a(t) &= (a_0 + kt u_0) e^{-kt}, \\ b(t) &= 1 - u(t) - a(t). \end{aligned} \tag{4}$$

During a subsequent closed interval of duration g, no new binding or block occurred. If a_{end} and b_{end} denote occupancies at the end of the preceding opening, the starting occupancies for the next opening were

$$\begin{aligned} a_g &= a_{\text{end}} e^{-g/\tau_A}, \\ b_g &= b_{\text{end}} e^{-g/\tau_B}, \\ u_g &= 1 - a_g - b_g, \end{aligned} \tag{5}$$

with $\tau_A = 10$ ms and $\tau_B = 2$ ms unless otherwise specified. Every train began at $(u, a, b) = (1, 0, 0)$. The 1-ms benchmark was chosen to isolate timing effects; it was not a fit to the 1.7-ms mean reported for R1623Q. [1]

Charge suppression and exact averaging

For an opening of duration T, define the equivalent blocked time $L(T) = \int_0^T b(t)\,\mathrm{d}t$. Integration gives

$$\begin{aligned} L(T) = T &- \frac{(u_0 + a_0)(1 - e^{-kT})}{k} \\ &- \frac{u_0[1 - (1 + kT)e^{-kT}]}{k}. \end{aligned} \tag{6}$$

The unitary current amplitude was constant and identical in U and A, so it cancelled from the charge ratio. For prescribed durations T_j, the outcome was $S_Q = \sum_j L_j / \sum_j T_j$. For random durations, the outcome was instead

$$S_Q = \frac{\sum_j \mathbb{E}[L_j]}{\sum_j \mathbb{E}[T_j]}, \tag{7}$$

not the mean of individual opening or train percentages.

For independent exponential durations with mean τ, setting $z = k\tau$ gives

$$\mathbb{E}[e^{-kT}] = \frac{1}{1+z}, \qquad \mathbb{E}[kTe^{-kT}] = \frac{z}{(1+z)^2}. \tag{8}$$

These expectations were substituted into the interval updates. In this case $\mathbb{E}[L(T)] = \tau\,\mathbb{E}[b(T)]$, because the survival probability of an opening at time t is τ times its closing-time density. All reported values are evaluations of the stated equations, rounded for display. They have no Monte Carlo sampling error; their biological uncertainty lies in the choice of kinetics and opening histories.

Paired-opening comparison

For an initially unbound channel, let $b_{\text{fresh}}(t) = 1 - (1+kt)e^{-kt}$. At time t after reopening, the excess blocked fraction relative to a fresh opening is

$$b_2(t) - b_{\text{fresh}}(t) = \underbrace{(1+kt)e^{-kt}b_g}_{\substack{\text{contribution from}\\ \text{residual block}}} + \underbrace{kt\,e^{-kt}a_g}_{\substack{\text{contribution from retained}\\ \text{conductive binding}}}. \tag{9}$$

The endpoint contribution attributed to A was the second term evaluated at $t = 1$ ms. The illustrative gap window was obtained by imposing $b_g < 0.001$ and $kt\,e^{-kt}a_g \geq 0.02$ at that endpoint. Neither threshold was treated as an experimentally established detection limit.

Data availability. No new biological recordings or compound-specific binding measurements were generated. The opening schedules, rate constants, interval equations and numerical outcomes needed to reproduce the calculations are provided in the text and tables.

References

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3. Kambouris, N. G. *et al.* A revised view of cardiac sodium channel “blockade” in the long-QT syndrome. *J. Clin. Invest.* **105**, 1133–1140 (2000). `https://pubmed.ncbi.nlm.nih.gov/10772658/`