Nonstationary Noise Analysis of M Currents Simulated and Recorded in PC12 Cells

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Villarroel, Alvaro. Nonstationary noise analysis of M currents simulated and recorded in PC12 cells. J. Neurophysiol. 77: 2131–2138, 1997. M current relaxations recorded in PC12 cells were subjected to nonstationary noise analysis (NSNA) to obtain estimates of single-channel current (i), channel number (N), and open probability (Po) for the channels responsible for M current. The analysis was constrained such that N and single-channel conductance were the same at two potentials. The relation between variance and current indicated that the fraction of channels open was 0.58 ± 0.06 (mean ± SD) and 0.05 ± 0.04 (mean ± SD; n = 9) at −33 and −63 mV, respectively. The single M channel conductance was 4.0 pS, and a density of 1 functional M channel per 4 µm² was estimated. Monte Carlo simulations of a two-state model of M channels were used to obtain sets of simulated macroscopic M currents that were subjected to the same NSNA procedure so as to evaluate the accuracy of M channel parameters obtained with this method. The influence of current rundown and filter frequency on estimates of i, N, and Po were evaluated. The single-channel parameters estimated from the simulations differed by <10% from actual values at any level of current rundown, N, or Po. The dispersion in the estimation of N and Po increased as Po decreased. Decreasing filter frequency caused an underestimation of i, paralleled by an overestimation of N. The estimation of Po was relatively immune to the filter frequency, especially for data simulated with Po = 0.77.

INTRODUCTION

At a fixed membrane voltage the current level oscillates around a mean value because of the stochastic opening and closing of ion channels. If conditions that may modulate the activity of channels, such as intracellular calcium, are kept constant, the amplitude of those oscillations is a reflection of the single-channel current (i) and the number of channels (N) available in the membrane. Thus, from the relation between variance and mean current, it is possible to estimate the elemental properties of active channels. Sigworth (1980) introduced nonstationary noise analysis (NSNA) to study the properties of the voltage-dependent sodium channel. The method consists of obtaining the mean and variance of the current evoked in response to identical voltage steps; from the relation between mean and variance, an estimation of i and probability of the channel being open (Po) can be made. Although a direct measurement of the elemental properties can be achieved by single-channel recording, when several channels with similar ion selectivity are present in the membrane it can be difficult to recognize the channel of interest without a previous knowledge of some basic properties. This is particularly true for potassium channels, which present a striking diversity. NSNA represents a relatively simple method of estimating the single-channel conductance and Po at a given potential.

The purpose of this paper was to estimate the elementary properties of the M channels by NSNA. The M current is a noninactivating voltage-dependent potassium current that activates over several tens of milliseconds, thereby repolarizing the membrane potential and contributing to spike frequency accommodation. The suppression of M current by muscarine causes an increase of cell input resistance and excitability (reviewed in Adams et al. 1986).

The M current runs down over time under experimental conditions, affecting the amplitude of current fluctuation. In addition, the amplitude and time course of the noise is affected by the filter frequency employed for data acquisition. It is not known how current rundown and filter frequency may affect the estimation of channel properties by NSNA. Monte Carlo simulations were performed in the first part of this paper to evaluate the influence of current rundown and filtering in the estimation of i, Po, and N by NSNA. In addition, the influence of Po and N on their own estimates was also evaluated. In the second part, the M current in rat PC12 cells was subjected to NSNA.

METHODS

NSNA

In a homogeneous population of channels in which each channel can exist only in two states (conducting and nonconducting), the mean current (I) can be described according to the binomial distribution, and the following expression relating variance (σ²) and i (Sigworth 1980) can be obtained

\[ \sigma^2 = N \cdot P_o \cdot (1 - P_o) \cdot i^2 \]  (1)

where N is the total number of channels available, Po is the probability of the channel being open, and i is the single-channel current.

To reduce extraneous noise, the headstage was installed within a Faraday cage, and the flow of extracellular medium was broken at several points to reduce the antenna provided by the flow system. Another source of excess fluctuations is thermal noise. The extra current variance of thermal origin is given by (Hille 1992)

\[ \sigma^2 = 4 \cdot k \cdot T \cdot B/R \]  (2)

where k is Boltzmann’s constant, R is the resistance, T is the absolute temperature in degrees Kelvin, and B is the recording bandwidth in hertz. The thermal noise has not been considered because it was estimated to be very small. Recording in the frequency range of 1–200 Hz with the use of a recording electrode with a 1-MΩ resistance, the variance due to thermal fluctuation will be 3.3 pA² at 20°C. Additional noise arises from the combination of
access resistance and capacitance. Above 1 kHz, the current variance is proportional to the access resistance and the squared capacitance (Sigworth 1985). The input resistance of PC12 cells varied from 1–2 MΩ at a holding potential of ~70 mV (with the voltage-dependent M channels closed) to ≥30 MΩ at ~30 mV (with 1 nA of current was passing through the activated M channels). The contribution of instrumental noise was measured with the use of model cells with different resistors and capacitors. It was found to be <4 pA² with a 1-MΩ resistor, a worst case scenario. Thus the changes in noise due to the variance in resistance were <5% of the variance at ~30 mV (see Fig. 4D).

The mean and variance were determined from local means and local variances of pairs of records. The set of local means and variances was averaged to obtain the final mean and variance. Groups of two records were used to minimize the extra variance arising from current rundown. Current rundown is defined here as the sum of the variance at each time point divided by the number of the mean duration in state A. The “variance index” was calculated for each pair of records as the variance divided by the number of the mean duration in state A and adding 1 / 4 acquisition frequency with the use of the SMOOTH algorithm of ASYST. 

\[ P_s, N, i \] follow the relation 

\[ P_s = I(N - i) \]  

(3)

where I is the mean current. Combining Eq. 1 and 3 and adding an arbitrary constant K to account for variance unrelated to the relaxation, the following equation is obtained

\[ a^2 = I \cdot i - P_s N + K \]  

(4)

With the use of this relation, N and i were estimated simultaneously for both activation and deactivation current relaxations.

The fit was constrained such that

1) N was the same at both potentials;  
2) the conductance was the same at both potentials;  
3) the constant K was ≥0 (i.e., it cannot be a negative variance).

Thus, four free parameters were fitted: N, i, \( K_{\text{adding}} \), and \( K_{\text{jump}} \) (the fit was improved by the use of 2 equations and 4 unknowns instead of 1 equation and 3 unknowns).

**Simulations**

Monte Carlo simulations of channel activity were carried out with programs written in ASYST, which has a random number generator that yields ~4.3 × 10³ different numbers. Macrophase currents were calculated by adding together N single-channel simulations. For each single-channel simulation, the initial state was chosen at random, with \( P_s \) given by

\[ P_s = O(\bar{D} + \bar{C}) \]  

(5)

where \( \bar{D} \) is the mean open duration and \( \bar{C} \) is the mean closed duration. The duration in each state (\( t \)) was determined by

\[ t = -\ln (D \cdot P) \]  

(6)

where D is the chosen mean duration of a given state and P is a random number between 0.0 and 1.0. The value \( t \) was rounded up to the next integer, because rounding down produced a slow component in the macroscopic current. The \( t \) elements on a “single-channel array” were given a value of 1 if the channel was conducting or 0 if it was nonconducting. At time \( t + 1 \) the state switched, and the duration of this state was determined as before. The simulation was carried out until the sum of partial durations exceeded the chosen duration of the record. Voltage pulses were simulated by changing the values of the mean durations. The final current was calculated by multiplying the macroscopic current by \( i \) at each voltage.

The mean open and closed durations at the step voltage (~60 mV) were always 125 and 2,500 ms, respectively. At the holding potential (~40 mV) the mean open duration was varied from 1,000 to 250 ms, and the mean closed duration varied from 300 to 1,500 ms.

**Three-state channel simulation**

In a channel following a linear three-state model

\[ [A_1] \xrightarrow{\alpha_1} [A_2] \xrightarrow{\beta_1} [A_3] \]  

(7)

the mean duration in state \( A_1 \) is \( 1/\alpha_1 \), that in state \( A_2 \) is \( 1/(\alpha_2 + \beta_1) \), and that in state \( A_3 \) is \( 1/\beta_1 \). The steady-state probability (\( P_s \)) in each state is given by (Rodiguin and Rodiguina 1964)

\[ P_s = K_s/(K_s + K_1 + K_2) \]  

(8)

where

\[ K_1 = \beta_1 \times \beta_2, \quad K_2 = \alpha_1 \times \beta_2, \quad \text{and} \quad K_3 = \alpha_1 \times \alpha_2 \]  

(9)

Three-state channels were simulated as described for two-state channels. In the simulations one state had zero conductance. The simulation was restricted so that the the probability of entering state 1 or state 3 from state 2 was the same.

**Cell culture and electrophysiology**

PC12 cells were grown in 9% CO₂ at 37°C in Dulbecco’s Modified Eagle Medium supplemented with 5% fetal calf serum (Hazelton), 10% horse serum (Hyclone), and 100 μg/ml streptomycin and 100 U/ml penicillin. Fused cells were used because the M current was more stable than in normal cells under the conditions employed. At least 1 day before recording, cells were fused with 50% polyethylene glycol 1500 (Villarroel et al. 1989). Briefly, cells were grown at high density in 100-mm plastic petri dishes, and treated with polyethylene glycol for 80 s to induce fusion. Fused cells were separated from nonfused cells with a 9–30% serum gradient. Cells were plated in 35-mm plastic petri dishes and treated with 50 ng/ml nerve growth factor for ~1 day.

Whole cell currents were recorded in continuous mode at room temperature (22–25°C) with 3-MΩ soft glass electrodes with the use of an EPC7 amplifier, acquired at 200–400 Hz, and filtered with an eight-pole Bessel filter at one half the acquisition frequency. The access resistance (~5 MΩ) was compensated by 70–80%. Cells were continuously perfused at 1 ml/min with a calcium-free solution that consisted of (in mM) 140 N-methyl-d-glucamine-aspartate, 2 MnCl₂, 1 MgCl₂, 3 KCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, and 10 glucose, pH 7.5. The electrode solution was composed of (in mM) 125 potassium aspartate, 10 Kbis-(o-aminophenoxy)-N,N,N’,N’-tetraacetic acid, 4 CaCl₂ (~75 mM free calcium), 1.5 MgCl₂, 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, and 1 Na₂ATP, pH 7.0. Sodium currents were suppressed by replacing sodium with N-methyl-d-glucamine-aspartate, chloride currents were suppressed by replacing most chloride with aspartate, calcium currents were suppressed by replacing calcium with the calcium channel blocker manganese, and calcium-activated potassium currents were eliminated by suppressing calcium currents and efficiently chelating intracellular calcium. The M current was further
isolated by holding the membrane at depolarized potentials at which other voltage-dependent potassium channels inactivate.

Cell capacitance was estimated by imposing a series of 10-mV hyperpolarizing triangular pulses (Palti and Adelman 1969) after having blocked potassium currents with quinidine (1 mM) or another potassium channel blocker (tetraethylammonium or barium), or with a holding voltage of −70 mV, at which there was little contribution of voltage-dependent channels. The input resistance of the cells was 1–2 GΩ.

**RESULTS AND DISCUSSION**

Figure 1A illustrates the result of a simulation consisting of 5,000 channels of 3.0 pS (with the use of a reversal potential of −100 mV) with \( P_o = 0.555 \) at the holding potential of −40 mV and \( P_o = 0.0476 \) at the jump potential of −60 mV. The variance plotted against mean current follows a parabolic function (Fig. 1B). The quadratic dependence of the variance on \( i \) (see Eq. 1) produces more noise in the variance at the holding potential, at which the single current is bigger than at the jump potential. The deviation of the estimated relation from the theoretical relation (plotting Eq. 4 with known \( N \) and \( i \)) was very small (compare · · · and solid line in Fig. 1B), suggesting that the method used is dependable.

**Influence of current rundown**

Many currents, including the M current, often run down over time. The influence of this phenomenon on the estimation of the three parameters (\( N, P_o, \) and \( i \)) was evaluated by simulating it as a reduction of \( N \) between subsequent jumps. The effects of five rundown levels were evaluated under four different “holding” \( P_o \) values with the use of the same “step” \( P_o \) of 0.046, and four \( N \) values, yielding a total of 80 conditions. One hundred records were analyzed pairwise for each condition. The result of the simulation was fitted to Eq. 4, fixing \( K = 0 \) and estimating \( N \) and \( i \). Equation 3 was used to estimate \( P_o \). The estimated values were normalized by dividing them by the value employed to generate the simulation and multiplying by 100. The normalized estimations of the three parameters under the different conditions are plotted in Fig. 2.

The estimations of \( i \) (□) were very close to the original values under every condition studied. The maximum error under any conditions was <10%. On the other hand, the estimation of \( N \) (○) and \( P_o \) (△) deteriorated significantly as the original holding \( P_o \) decreased. This result is to be expected, because as the value of \( P_o \) is reduced, there is less of a curvature of a parabola available to fit with Eq. 4, increasing the fitting error.

The original \( N \) (ranging from 5,000 to 625) did not appear to have a major influence in the estimation of the parameters. Current rundown tended to cause an underestimation of \( N \), especially when the original \( N \) was lower. However, for original holding \( P_o \) values ≥0.55, \( N \) was underestimated by <30% when the rundown level was 0.5% per jump (Fig. 2, A and B). At this rundown level the current will decrease by 39% after 100 jumps. The error in the estimation of \( N \) was as high as 33% and 44% when the original holding \( P_o \) was 0.55 and 0.33, respectively (Fig. 2, B and C). However, in general, the error was <15% at these \( P_o \) values. When a holding \( P_o \) of 0.14 was used to generate the data, the error was as high as 50%. The direction and magnitude of the errors at this low \( P_o \) were unrelated to \( N \) or to extent of current rundown (Fig. 2D).

The errors in the estimation of \( P_o \) were a mirror image of the errors in the estimation of \( N \), but of lesser magnitude in general. Notably, the estimation of the holding \( P_o \) was not affected by any current rundown level when the original \( P_o \) was between 0.77 and 0.33 (Fig. 2, A–C), except when the original \( N \) was low (625). The error in this set of data, however, increased as the original holding \( P_o \) decreased. The range of values estimated was 100 ± 1% at 0.77, 100 ± 10% at 0.55, and 100 ± 20% at 0.33. At \( P_o = 0.14 \) the error was as high as 89%. In addition, the SD for the estimations of \( P_o \) and \( N \) for each group of four simulations increased as \( P_o \) decreased.

**Influence of filter frequency**

Filtration will reduce the amplitude of the fluctuations. The influence of data filtering was studied by digitally low-pass filtering the data at different frequencies, with the use of the SMOOTH routine of ASYST (Fig. 3). The time constants of the simulated current relaxations, which were acquired at 400 Hz, varied from 120 to 250 ms. As expected,
the estimation of $i$ decreased as the filter frequency was reduced (Fig. 3A).

Excessive filtering tended to cause an overestimation of $N$ (Fig. 3B) that paralleled the underestimation of the single-channel conductance, such that the estimation of the probability was affected to a lesser extent. This was particularly true at higher original $P_o$ values (Fig. 3C). With an original holding $P_o$ of 0.77, the estimation of $P_o$ was immune to the extent of filtering, even at a filter frequency as low as 20 Hz.

**Analysis of simulated three-state channel**

To determine whether NSNA can be applied to channels with more than one conducting state, Monte Carlo simulations of channels with two conducting states and one nonconducting state were carried out. The voltage steps could be simulated by changing the mean duration of at least one state, whether or not this was in combination with changes in the mean duration of other states. The analysis was restricted to changes in the mean duration of the three states. The mean durations of two states were reduced during the simulated voltage step, whereas the duration of the third state was increased. The ratio of $i$ of the two conducting states was either 1, 2, 4, or 10. For a given set of conditions (mean open times and $i$), simulations were performed in which one of the three states was nonconducting. Each simulation of macroscopic current arising from 5,000 channels was performed four times. The set of mean durations employed in the simulations is indicated in Table 1.

The variance and mean was obtained as above, and the data were fitted to Eq. 4, fixing $K = 0$. The time course of the variance could not be fitted to Eq. 4 for the results of some simulations. All of the simulations that could not be fitted had in common that the state with the longest duration was conducting. Note that in these examples the mean duration of this state increased during the voltage step, whereas the mean duration of the other state decreased.

The results of condition 1 could not be fitted when the $i$ of state 3 (the state with the longest main duration) was half of any of the remaining two states. The same was true for condition 2 and state 2, condition 3 and state 3, and condition 4 and state 2 (not shown). The fit was better when the $i$ of the state with the longest main duration was twice or the same as the other conducting state (not shown). Thus the time course of the variance of a three-state channel cannot always be described by the equations derived assuming that the binomial distribution applies. NSNA may serve as a diagnosis for channels with at least two open states whose mean durations change in opposing directions in response to a stimulus, such as voltage.

For the rest of the conditions, the results could be fitted well to Eq. 4 (not shown). The estimated $i$ was a value between the original $i$ values of the two conducting states (Table 2). The value obtained depended on the mean duration and $i$ in a nonlinear fashion.
The influence of low-pass filtering was studied for the set of conditions indicated in Table 2. The results were similar to those obtained for the two-state channel simulations (see above and Fig. 3).

In summary, these results indicate that NSNA is a very dependable method for the estimation of \(i\), and for, to a lesser extent, the estimation of \(P_o\) for two-state channels. The dispersion in the estimation of \(N\) was greater than the dispersion for the estimation of the other two parameters, especially when the original \(P_o\) was low. It should be stressed, however, that the introduction of the constant in Eq. 4 will cause an overestimation of \(P_o\) and an underestimation of \(N\) if the channel can exist in a closed state with

| TABLE 1. Mean durations used for the simulations of three-state channels |
|-------------------------|--------|--------|--------|
|                         | State 1 | State 2 | State 3 |
| Condition 1             |        |        |        |
| Holding                 | 375    | 375    | 750    |
| Step                    | 50     | 50     | 2,500  |
| Condition 2             |        |        |        |
| Holding                 | 375    | 750    | 375    |
| Step                    | 50     | 2,500  | 50     |
| Condition 3             |        |        |        |
| Holding                 | 450    | 225    | 750    |
| Step                    | 70     | 35     | 2,500  |
| Condition 4             |        |        |        |
| Holding                 | 450    | 750    | 225    |
| Step                    | 70     | 2,500  | 35     |

All values are in ms.

| TABLE 2. Original and fitted values for the single-channel current |
|-------------------------|--------|--------|--------|
|                         | State 1 | State 2 | State 3 |
| Condition 1             |        |        |        |
| 1                       | 1      | 0      | 0.98 ± 0.190 |
| 1                       | 0.5    | 0      | 0.86 ± 0.230 |
| 1                       | 0.25   | 0      | 0.87 ± 0.020 |
| 1                       | 0.1    | 0      | 0.92 ± 0.027 |
| 0.5                     | 1      | 0      | 0.98 ± 0.008 |
| 0.25                    | 1      | 0      | 0.82 ± 0.007 |
| 0.1                     | 1      | 0      | 0.90 ± 0.002 |
| Condition 2             |        |        |        |
| 1                       | 0      | 1      | 0.98 ± 0.024 |
| 1                       | 0      | 0.5    | 0.79 ± 0.035 |
| 1                       | 0      | 0.25   | 0.81 ± 0.052 |
| 1                       | 0      | 0.1    | 0.90 ± 0.070 |
| 0.5                     | 0      | 1      | 0.85 ± 0.021 |
| 0.25                    | 0      | 1      | 0.87 ± 0.024 |
| 0.1                     | 0      | 1      | 0.93 ± 0.026 |
| Condition 3             |        |        |        |
| 1                       | 1      | 0      | 1.03 ± 0.020 |
| 1                       | 0.5    | 0      | 0.94 ± 0.007 |
| 1                       | 0.25   | 0      | 0.95 ± 0.009 |
| 1                       | 0.1    | 0      | 0.98 ± 0.013 |
| 0.5                     | 1      | 0      | 0.74 ± 0.019 |
| 0.25                    | 1      | 0      | 0.72 ± 0.019 |
| 0.1                     | 1      | 0      | 0.82 ± 0.019 |
| Condition 4             |        |        |        |
| 1                       | 0      | 1      | 1.01 ± 0.019 |
| 1                       | 0      | 0.5    | 0.87 ± 0.032 |
| 1                       | 0      | 0.25   | 0.88 ± 0.051 |
| 1                       | 0      | 0.1    | 0.93 ± 0.060 |
| 0.5                     | 0      | 1      | 0.81 ± 0.021 |
| 0.25                    | 0      | 1      | 0.83 ± 0.024 |
| 0.1                     | 0      | 1      | 0.91 ± 0.024 |

All values are in pA; fitted values are means ± SD. \(n = 4\). Values are from simulations of 3-state channels using mean open durations indicated in Table 1.
very long duration. This is because the mean and variance associated with the transitions to this state will be very slow and will change very little during the voltage steps included in the analysis. The variance time course due to the entrance into and exit from this state will appear to be uncorrelated with the current time course. In other words, a constant variance will be observed during the portion of time analyzed, and included in the term $K$ of Eq. 4.

**NSNA in PC12 cells**

The current relaxations evoked in response to hyperpolarizing voltage steps from a depolarized voltage are completely suppressed in PC12 cells dialyzed with guanosine 5’-O-(2-thiodiphosphate) (GTP-γ-S) after treatment with bradykinin (Villarroel 1996), suggesting that the M current arises from the activity of a homogeneous population of channels. The instantaneous current-voltage relationship is linear (Villarroel et al. 1989), indicating that the M channels do not rectify in the voltage range studied. To estimate the conductance of the channels underlying the current relaxations, the nonstationary fluctuations of the whole cell currents (Sigworth 1980) were examined. Previous attempts to apply NSNA to the study of the M current failed, presumably because of contamination by other currents (Bosma et al. 1990). Therefore care has been taken to eliminate sodium, chloride, calcium, and calcium-activated currents (see METHODS).

Figure 4 shows the final variance, which was the averaged local variances calculated from pairs of records (see METHODS) associated with the current relaxations at a holding potential of $-33 \text{ mV}$ and a jump potential of $-63 \text{ mV}$. When the hyperpolarizing voltage pulse was imposed, there was an instantaneous decrease in current and variance, due to the reduction in the driving force of potassium. During the first 50 ms of the voltage jump, the variance was modified very little, and subsequently receded as the mean current subsided. On repolarization, the variance increased much faster than the mean current, reaching a maximum value while the mean current was still increasing. The current relaxations and the associated variance were suppressed with the potassium channel blockers quinidine ($1 \text{ mM}$, Fig. 4B), tetraethylammonium ($20 \text{ mM}$), and barium ($4 \text{ mM}$; not shown), indicating that the variance originated from the gating of potassium channels underlying the current relaxations.

The results of fitting the variance and mean current in this experiment to Eq. 4 are shown in Fig. 4, C and D. The fit was constrained such that $N$ and the single-channel conductance were the same at both the holding and step potentials (see METHODS). Both activation (Fig. 4C) and deactivation (Fig. 4D) showed a parabolic relation.

The average single-channel conductance was 4 pS (Table 3), which represents a weighted mean of the different conductance levels (Sigworth 1980). This value is in close agreement with the 3 pS estimated by stationary fluctuation analysis (SFA) (Neher et al. 1988) in NG108-15 neuroblastoma cells. Similar values have been estimated in bull...
frog sympathetic neurons by SFA (Marrion et al. 1992). A lower value (1–2 pS) was estimated by SFA in rat sympathetic neurons (Owen et al. 1990). More recently, several reports identifying M channels directly in patches from rat and frog sympathetic neurons have appeared (Marrion et al. 1992; Owen et al. 1990; Selyanko et al. 1992). There are, however, substantial discrepancies between the single M channels recorded in frog (Marrion 1993) and rat sympathetic neurons (Selyanko et al. 1992; Stansfeld et al. 1993). In rat neurons, a slow inactivating component emerged in cell-attached and inside-out patches (Stansfeld et al. 1993), whereas in patches from frog neurons this component was not observed (Marrion 1993). The single-channel conductance in symmetrical potassium in frog neurons is fairly consistent with the values obtained by fluctuation analysis (Marrion 1993). However, the main conductance recorded in cell-attached patches from rat neurons is 7 pS (Stansfeld et al. 1993), almost twice the 4 pS estimated here. The reason for this discrepancy is not clear. Species and tissue differences could account for it, although rat PC12 cells and sympathetic neurons have the same developmental origin. The analysis filtering at 200 Hz will underestimate the conductance if there is a significant contribution of conducting states with brief mean open times (<5 ms). However, the absence of a measurable fast component in the current relaxations indicates that the contributions to the current of such states, should they exist, would not be significant. The possibility that the procedure for seal formation may have contributed to these differences must be considered. For instance, Marrion (1993) reported that seals were formed with very little or no suction, presumably causing little stress to the membrane and structures under the patch.

The relation between variance and current was parabolic and showed an inflection as the current increased in both activating and deactivating relaxations (Fig. 4, C and D), indicating that $P_o$ at the holding potential was $>0.5$. The final $P_o$ was estimated to be 0.56 at $-33$ mV and 0.04 at $-63$ mV. By estimating $N$ and $i$, the macroscopic currents were converted to probabilities with the use of the relation in Eq. 3 as an average. In a channel with two states, the dependence of $P_o$ on voltage could be described by a Boltzmann relation

$$P_o = \frac{1}{1 + e^{-\frac{V_{1/2} - V_m}{S}}}$$

where $V_{1/2}$ is the voltage at which $P_o$ is 0.5, $V_m$ is the membrane potential, and $S$ is the slope factor. Table 3 shows a summary of experiments. The half-activation value was $-36$ mV (Fig. 5), in close agreement with the value expected from the dependence of the relaxation time constant on voltage (Adams et al. 1982). Furthermore, the simulations indicated that the estimated value of $P_o$ differed from the true value by $<10\%$. However, the estimated value of $P_o$ differs significantly from the value of 0.1 at $-30$ mV estimated by SFA in rat neurons reported by Owen et al. (1990). The use of manganese in the experiments reported here may have contributed to this disparity. It has been shown that 2 mM Mn can shift the inactivation current-voltage relation in voltage-dependent potassium currents up to $+15$ mV (Mayer and Sugiya 1988; Villarroel 1993; Villarroel and Schwarz 1996). However, the voltage shift necessary to account for the difference in the results is $-21$ mV, that is, it goes in

\[
\begin{array}{ccccccc}
\text{Cell} & V_{1/2}, \text{mV} & \text{Slope, mV} & \text{Density, Channels/pF} & \text{Conductance, pS} & \text{Number of Jumps} & \text{Rundown, %} \\
M1912B1 & -34.0 & 8.0 & 2.4 & 4.2 & 100 & 0.06 \\
M925B3 & -38.8 & 7.0 & 19.0 & 3.7 & 374 & 0.06 \\
M927A1 & -38.4 & 9.2 & 19.3 & 3.4 & 191 & 0.24 \\
M929E1 & -34.8 & 6.8 & 20.3 & 3.5 & 302 & 0.06 \\
M003B2 & -33.2 & 7.6 & 16.4 & 4.6 & 293 & 0.18 \\
M006B1 & -37.5 & 13.5 & 4.2 & 4.2 & 248 & 0.18 \\
M006B3 & -36.3 & 11.7 & 4.0 & 4.0 & 128 & 0.24 \\
M006B4 & -35.3 & 11.9 & 4.3 & 4.3 & 79 & 0.23 \\
M006B5 & -37.6 & 10.1 & 19.5 & 4.4 & 117 & 0.30 \\
M006B6 & -37.6 & 10.1 & 66.2 & 3.8 & 201 & 0.15 \\
M009B1 & -37.6 & 10.1 & 26.8 & 3.4 & 125 & 0.18 \\
\text{Mean} & -36.2 \pm 2.0 & 9.6 \pm 2.4 & 26.8 \pm 17.7 & 3.97 \pm 4.0 \\
\end{array}
\]

\[
\text{Mean values are means } \pm \text{ SD. NSNA, nonstationary noise analysis; } V_{1/2}, \text{ voltage at which channel open probability is 0.5. Only these experiments met the criteria for analysis described in METHODS in a group of 26 experiments.}
\]

\[
\text{FIG. 5. Relation between } P_o \text{ and voltage. Boltzmann relation constructed from the probabilities derived by nonstationary noise analysis (NSNA) (see Table 3). Each symbol represents a different cell. Continuous line: average, with voltage at which } P_o = 0.5 \left(V\_{1/2}\right) = -36 \text{ mV and slope factor (S) } = 9.6 \text{ mV, corresponding to the movement of 2.4 charges.}
\]

\[
\text{TABLE 3. Summary of parameters obtained by NSNA}
\]
the opposite direction. The extracellular medium employed in the study by Owen included 2 mM calcium, and the intracellular solution did not effectively chelate calcium [0.5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid was included in the electrode solution], opening the possibility that calcium-activated channels contributed significantly to the fluctuations, affecting the estimation of $P_{a}$. The density in PC12 cells was $\sim 25$ channels per picofarad, corresponding (assuming 1 $\mu$F/cm$^2$) to 1 channel per 4 $\mu$m$^2$. In contrast, the density of $N$-methyl-d-aspartate channels is 4 times higher (Casado et al. 1996). With the use of 3- to 5-M$\Omega$ electrodes, Casado et al. (1996) succeeded $>80\%$ of the time in recording $N$-methyl-d-aspartate channel activity. If the M channel is homogeneously distributed, similar patches will contain functional M channels $>20\%$ of the time.

In conclusion, the M channels of PC12 cells have a conductance near 4 pS, and they are open 50% of the time at voltages around $-36$ mV. Monte Carlo simulations confirmed the accuracy of our estimates, provided M channels behave as a two-state channel.

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