and Na activation is illustrated by the data of Fig. 3. The open circles are the experimental I_{Na} (t) values during the conditioning pulse from the same experiment shown in Fig. 2 (bottom). The solid curve has been computed according to the sequential coupled scheme:

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \rightleftharpoons E$$

with A the resting, B and C activated but not conducting, D the conducting and E the inactivated state. I_{Na} (t) is then given by

$$I_{Na} = I_{Na_\infty} - A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} - A_3 e^{-t/\tau_3} + A_4 e^{-t/\tau_4}.$$

For the solid curve of Fig. 3, τ_4 is taken as the measured τ_h from the current record, and τ_3 and τ_2 are just the same τ_{delay3} and τ_{delay2} values that were fitted to the inactivation time-course in the lower part of Fig. 2. τ_1 was not resolved and so was not included in the reconstruction, leaving the first few 100 μs of $I_{Na}(t)$ undescribed. However, for the rest of the I_{Na} time-course the fit is quite reasonable, clearly establishing the detailed correspondence between Na activation and the inactivation delay.

Several interpretations of these data are possible, but the simplest seems to be that at least some fraction of Na channels in *Myxicola* must conduct before they can inactivate.

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SINGLE CHANNEL ANALYSIS OF VOLTAGE-SENSITIVE K^+ CHANNELS IN CULTURED PURKINJE NEURONS

D. L. GRUOL

A. V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California 92138

Recent data suggest that ionic mechanisms intrinsic to the cell membrane, in addition to neuronal circuitry, play an important role in generating and regulating the electrical activity of vertebrate central nervous system (CNS) neurons. For example, the Purkinje neurons (PN) of the cerebellum are characterized by a unique firing pattern comprised of simple and complex spikes in addition to a series of pauses (1). Intracellular voltage recordings from the PN revealed that voltage-sensitive, pacemaker-like activity plays a major role in generating the firing pattern characteristic of this CNS neuronal type (2–5). The intrinsic ionic mechanisms responsible for this activity appear to be localized to both the somal and dendritic membranes, and both regions exhibit spike electrogenesis.

To identify and characterize the ion channel population mediating the intrinsic activity of PN and to facilitate studies using intracellular voltage recording and the extracellular patch clamp technique, a cultured cerebellar model system was developed (4, 5). The cultured PN can be clearly identified on a morphological basis and both the somal and dendritic regions are easily accessible for electrophysiological analysis. Electrical activity characteristic of PN in vivo is demonstrated in the cultured PN (Fig. 1). This activity is significantly altered by K^+ channel blockers, suggesting that K^+ channels play a major role in generating or regulating the intrinsic activity of the PN's.[1] Experiments in progress using the "gigaohm seal" patch clamp technique are directed towards characterizing the somal and dendritic K^+ channel population of PN and identifying the factors (voltage, ions, chemicals) which regulate their activity.

[1]D. L. Gruol. Unpublished results.

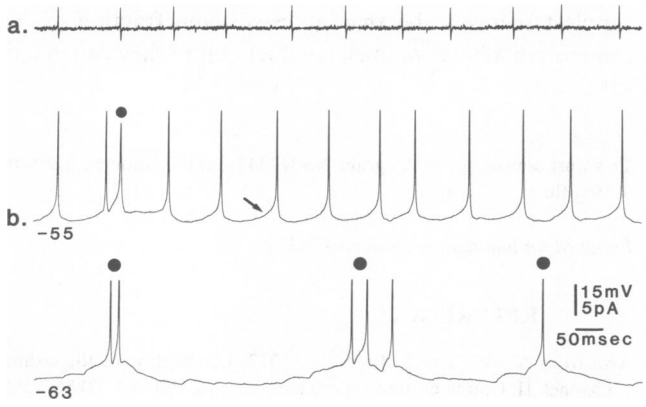

FIGURE 1 A cell-attached patch recording (trace *a*) and intracellular voltage recording (obtained after rupturing the patch membrane; trace *b*) from a cultured PN demonstrating a firing pattern characteristic of this CNS neuronal type. Single channel activity is not evident in the patch recording but action potentials occurring in extra-patch regions can be observed due to RC-coupling. The pattern of activity recorded extracellularly is reflected in the intracellular recording. Action potentials marked with dots in trace *b* probably represent synaptically evoked activity because they occur at irregular intervals and are still present after the membrane potential is hyperpolarized. The synaptically evoked activity is superimposed on a regular firing pattern generated by pacemaker potentials (arrows). The pacemaker activity is only observed at membrane potentials ranging from −40 to −60 mV. The action potential amplitude is not fully reproduced. The bath saline contained NaCl, 137 mM; KCl, 5 mM; KH_2PO_4, 0.4 mM; $NaHCO_3$, 4.2 mM; $Na_2HPO_4 7H_2O$, 0.34 mM; Glucose, 10 mM; $CaCl_2$, 2.2 mM; $MgSO_4 7H_2O$, 2 mM; HEPES Buffer-NaOH (pH 7.4), 10 mM. Micropipettes (2–5 M Ω resistances) were filled with the bath saline or a high K^+ saline containing NaCl, 16 mM; KCl, 144 mM; $MgCl_2$, 2 mM; Glucose, 10 mM; $CaCl_2$, 0.002 mM; EGTA, 0.04 mM. Experiments were preformed at room temperature. Cultures of PN were prepared from 20-d old rat embryos and maintained for 12 wk or longer before use.

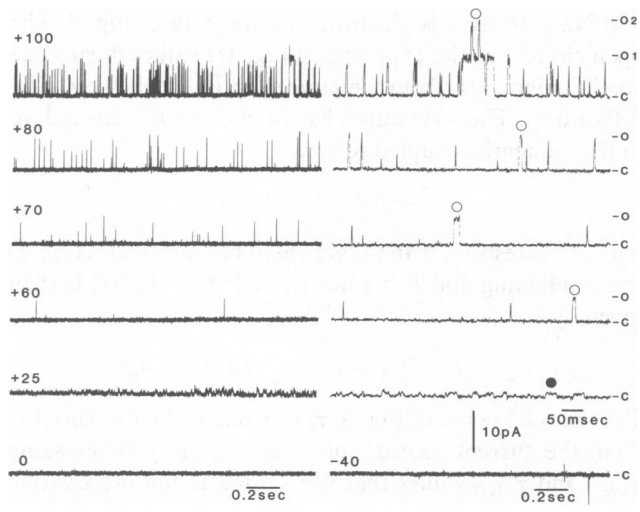

FIGURE 2 Single channel recordings from a cell attached patch on the somal region of a PN. Patch electrodes were filled with saline. Numbers to the left of each trace indicate the amount of applied polarization, positive values denoting depolarizations. Traces at the right (except for the bottom right trace) show the channel activity for each potential on an expanded time base. Channel activity was infrequent at zero applied voltage and at hyperpolarized membrane potentials (bottom trace). Depolarization of the patch membrane activated the outward currents mediated by the two channel types: activity mediated by the smaller channel (•) is evident in the trace representing activity evoked by a 25 mV depolarization, and the activity mediated by the larger channel (o) is evident at more depolarized potentials. *o* = open state; *c* = closed state; 01, 02 indicates the simultaneous opening of two channels.

RESULTS

Single channel recordings were obtained from cell-attached patches on the somal and dendritic regions of cultured PN using normal or high K^+ saline filled micropipettes. The potential of the patch membrane was varied by applying voltage steps. The single-channel conductance and reversal potential for the currents were obtained from the relationship between unitary current amplitude and membrane potential. Channel activity was infrequent at 0 applied polarization and at hyperpolarizing potentials, but depolarization of the patch membrane revealed a variety of inward and outward channel activity in all patches studied (n = 40). At least two channel types mediating outward current events could be distinguished (Fig. 2). Both channel types were activated at patch potentials depolarized from rest (~ −50 mV), demonstrated flickering in the open state and were largely K^+ selective.

One channel type was activated by depolarizations of 10–20 mV and had a single channel conductance ~35 pS; the frequency of channel openings decreased during maintained depolarization and as larger depolarizations were applied. The second channel type was activated at depolarizations >40 mV and had a single channel conductance ~100 pS; the frequency of channel openings and the open channel duration increased with larger patch depolarizations. Both channel types were also observed in cell-attached recordings from the dendritic region of the PN and were often associated with spontaneous action potentials recorded by the extracellular patch pipette due to RC coupling (8). Most patches studied contained both channel types including several of the larger type.

With saline-filled electrodes, the extrapolated reversal potential for the unitary currents was near resting membrane potential for both channel types. When the electrodes contained a high K^+/normal Cl^- saline, the reversal potentials shifted to a depolarized membrane potential, suggesting that both channels are largely K^+ selective. Under this condition the conductance of the larger channel was significantly increased and both channels were active at zero applied polarization (Fig. 3).

Outward currents similar to those mediated by both channel types were also observed in outside-out patches from the somal region. This channel activity was completely blocked by tetraethylammonium bromide (TEA) applied to the external surface, supporting the identity of the channels as a K^+ selective channels. The characteristics of the activity mediated by the larger channel suggest it may be a Ca^{++}-activated K^+ channel similar to that described in nonneuronal (6–8) and peripheral neuronal

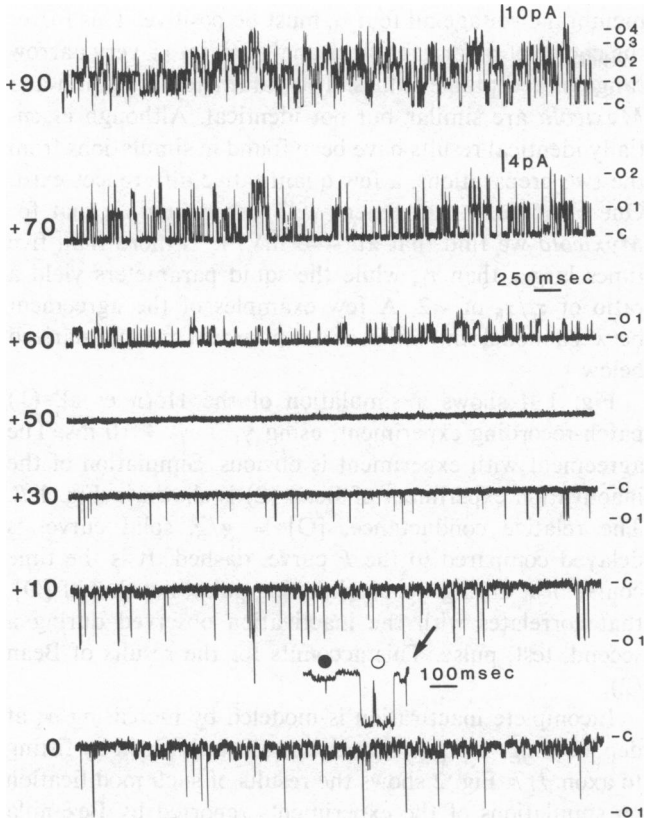

FIGURE 3 Single channel recordings from a cell attached patch on the somal region of a PN. The electrode contained high K^+ saline. Under this condition, channel activity was observed at zero applied polarization, and currents were inward. Currents mediated by both the large (○) and small (●) channel types could be identified. Note that the frequency of small channel openings decreased with depolarization while the frequency of large channel openings increased. Inset shows the currents at an expanded time base. The patch contained several channels of the larger type (single channel conductance = 250 pS).

(9) tissue. The Ca^{++} sensitivity of the K^+ channels in PN is presently under investigation.

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A MODEL OF THE SODIUM CHANNEL

ROSALIE C. HOYT
Department of Physics, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

We suggest that the opening and closing of sodium channels involves, at each channel site, two membrane components: (*a*) a single two-state gate system, G, with open (O) or closed (C) states; (*b*) a collection of coupled systems, E, each of which can exist in one of three states. The fraction of E systems in the "active" state (A) determines the probability, P_A, that the G system is in its (O) state.

$$C \underset{\gamma_2(A)}{\overset{\gamma_1(A)}{\rightleftharpoons}} O \qquad (G)$$

$$R \underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}} A \underset{\alpha_4}{\overset{\alpha_3}{\rightleftharpoons}} I \qquad (E)$$

$$P(A) = \gamma_1(A)/[\gamma_1(A) + \gamma_2(A)] \qquad (I)$$

The rates, α_i, of the E systems are assumed to depend on the local electric field and thus on the membrane voltage, and are chosen to give the best fits to experimental clamp data. The rates of the G system are assumed to be independent of the voltage and to depend on the fraction of the E systems in the neighborhood of G that are in the (A) state. The form of the probability function P_A is not specified a priori, but is chosen to give best fits to experimental data. Scheme (E) has been restricted to three states since this is the maximum number that allows the α_i to be determined empirically from single step clamps with no ad hoc assumptions.

Simulations have revealed no clear discrepancies between prediction and experiment. Asymmetry currents