Initial Conditions and the Kinetics of the Sodium Conductance in *Myxicola* Giant Axons

I. Effects on the Time-Course of the Sodium Conductance

R. HAHIN and L. GOLDMAN

From the Department of Physiology, School of Medicine, University of Maryland, Baltimore, Maryland 21201. Dr. Hahin's present address is Department of Physiology and Biophysics, School of Basic Health Sciences, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794.

**ABSTRACT** The effects of conditioning polarizations, ranging from −150 to 0 mV and of durations from 50 μs to 30 ms, on the time-course of $G_{Na}$ during test steps in potential were studied in *Myxicola* giant axons. Beyond the effects of conditioning polarizations on the amplitude of $G_{Na}$, the only effect was to produce a translation of $G_{Na}(t)$ along the time axis without a change in shape. For depolarizing conditioning potentials, Hodgkin-Huxley kinetics predict time shifts about threefold greater than found experimentally, whereas the predictions of the coupled model of Goldman (1975. *Biophys. J.* 15:119-136) were in approximate agreement with our experiments. The time shifts developed over an exponential time-course as the conditioning pulse duration was increased. The time constant of development of the time shift was considerably faster than, and showed the opposite dependency on potential from, the values predicted by both models. It had a mean $Q_{10}$ of 1/2.50. This fast activation process cannot account for the observed rise time behavior of $G_{Na}$, suggesting that there is an additional activation process. All results are consistent with the idea that the gating structure displays more than three states, with state intermediate between rest and conducting.

**INTRODUCTION**

The behavior of the Na conductance ($G_{Na}$) in a variety of nerve, muscle, and cardiac preparations differs significantly from the predictions of Hodgkin-Huxley kinetics (Hodgkin and Huxley, 1952). These differences are not such that they can be accounted for by simple parameter adjustments of the Hodgkin-Huxley model. It must be that the gating machinery governing the changes in $G_{Na}$ is organized differently than suggested by a literal interpretation of the Hodgkin-Huxley formalism. This literature has been reviewed by Goldman (1976).

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There is evidence that one such difference is that $G_{Na}$ activation and inactivation are coupled into a multistate sequence rather than proceeding independently. (a) Measured steady-state inactivation depends on the degree of activation during the test pulse used, with greater test pulse activation producing less apparent inactivation (Hoyt and Adelman, 1970; Goldman and Schaaf, 1972; Connor, 1976; Rudy cited in Goldman, 1976), as predicted by coupled models (Hoyt, 1968; Goldman, 1975; Rawlings and Neumann, 1976). (b) There is a delay in the development of inactivation (Armstrong, 1970; Goldman and Schaaf, 1972; Peganov, 1973; Schaaf and Davis, 1975; Campbell and Hille, 1976; Bezanilla and Armstrong, 1977) with more slowly developing activation producing a longer delay in inactivation development as expected for a sequential process. (c) $G_{Na}$ during steps in potential may shut down entirely or nearly so (Goldman and Schaaf, 1973; Rudy cited in Goldman, 1976) even when an immediately following, more depolarizing step produces a substantial Na current, and the rate of this shut down $\tau_a$ may be many times faster than the rate of development of inactivation measured with the two-pulse method, $\tau_c$. (Goldman and Schaaf, 1973; Oxford and Pooler, 1975; Connor, 1976; Moore cited in Goldman, 1976). The inactivated state, then, actually loads less extensively than indicated by the amount of the shut down of $G_{Na}$ during the first potential step. These observations cannot be accounted for by a model in which the activation and inactivation variables are independent and appear as a product, no matter what the number of states assumed for either process. The "inactivation" variable would have to increase in value again during the second step; i.e., it must itself be coupled to an activation process. Similarly, Chandler and Meves (1970) found that the steady-state inactivation curve constructed from the two-pulse method at small depolarizations and the steady-state $G_{Na}$ for large depolarizations was not monotonic. This also implied that the inactivation that apparently developed could be reversed on a further depolarization, and they accounted for this by adding an additional (now coupled) activation process to the inactivation variable. (d) The asymmetrical displacement current associated with the opening of the Na gates (Armstrong and Bezanilla, 1973; Keynes and Rojas, 1974; Meves, 1974) may be inactivated with conditioning polarizations, and this effect is abolished on internal perfusion with pronase (Bezanilla and Armstrong, 1974; Armstrong and Bezanilla, 1974) which selectively destroys inactivation (Armstrong et al., 1973), consistent with the view that there is no separate potential sensor for the inactivation gate. Similarly, when gating charge displacement is compared at the onset and termination of a depolarizing step, the charge displacement at termination becomes smaller than that at onset as the step duration is increased (Armstrong and Bezanilla, 1976; Bezanilla and Armstrong, 1976; Meves and Vogel, 1977). This has been called charge immobilization.

The activation and inactivation variables cannot appear as a product; they must appear as a sum (Goldman, 1975). If they are interpreted as the probability of occupancy of discrete states, then there are difficulties if this sum of exponentials description of $G_{Na}$ is taken as indicating independent activation and inactivation, inasmuch as one of the variables must be negative at some potentials. The simplest view, then, is that activation and inactivation are
coupled. The gating machinery could then be a structure which, on a change in the applied field, progresses sequentially through some series of states, including both activation and inactivation, but the details of its organization remained unclear.

To approach this problem, Goldman (1975) presented a model in which \( G_{Na} \) was proportional to the fifth power of a second order variable, \( \nu \). This is one kind of the simplest limiting-case assumption in that the second order variable will allow only three states for the gating unit (rest, conducting, and inactivated), the minimum number for a coupled model. With only three states, five subunits were required. This model quantitatively accounted for the kinetic data then available (Goldman, 1975). However, \( \nu \) cannot be interpreted as representing a simple three-discrete-state gating unit. Systematic computations involving some 20,000 combinations of input values, covering the experimentally determined range of parameters for the \( \nu^5 \) model, failed to produce a set of rate constants governing the transitions between states that were all real and positive, as is required (Goldman and Hahin, 1975).

The three-state model is inadequate. Inasmuch as the \( \nu \) variable is a generalized linear variable, and the number of independent and identical subunits was forced by the experimental records (Goldman, 1975), these results indicate that no three-state model, with first order transitions between the states and no subunit interactions, can be found which will account for the experimental results. By first order transitions we mean that the rate of transition out of any state \( i \) into any state \( j \) is just proportional to the occupancy of \( i \). Either there are more than three states or the channel kinetics are nonlinear or both. These results suggested that the experimental description of \( G_{Na} \) was far from complete.

We present here observations on the effects of conditioning polarizations of various amplitudes and durations on the time-course, and not just the peak amplitude, of \( G_{Na} \) in *Myxicola* axons during fixed test steps in potential. Observations on the decay of \( G_{Na} \) on resetting the membrane potential after test steps are presented in the following paper. The aim of these experiments was to provide a more complete experimental description of \( G_{Na} \) kinetics of *Myxicola*, in order to gain more insight into how the gating machinery is organized. The results suggest that there are at least two, and very likely three, processes in the activation of \( G_{Na} \), consistent with recent results on the Na gating current (Meves, 1974; Armstrong and Bezanilla, 1975; Bezanilla and Armstrong, 1975; Meves and Vogel, 1977), indicating that at least part of the difficulty with the three-state model is that it assumes too few states, and call into question, to a degree, the idea that the gating machinery is built up of independent and identical subunits.

Preliminary reports of some of these results have been made (Goldman and Hahin, 1977).

**METHODS**

*Myxicola* were obtained from Maritime Biological Laboratories, Deer Island, New Brunswick, Canada. Methods for preparing and voltage clamping the axons were as in Binstock and Goldman (1969). Compensated feedback (Hodgkin et al., 1952) to reduce
the series resistance, $R_s$, was used throughout. This will still leave some residual uncompensated $R_s$ (Goldman and Schauf, 1972). To reduce this error even further, the currents in all experiments were reduced by adding tetrodotoxin (TTX, Calbiochem, San Diego, Calif. or Sigma Chemical Co., St. Louis, Mo.) to the bathing medium, so that the product of maximum membrane current and $R_s$, during any experiment, was generally $\geq 3$ mV.

Holding potentials under voltage clamp were always the natural resting membrane potential which generally ranged from $-65$ to $-75$ mV. The protocols in these experiments called for brief steps in potential. In most cases whether they were combinations of pre- and test or test and postpulses, the pair of pulses together lasted $< 15$ ms. In the few cases where longer prepulses were used, steps in potential still were applied for only a few tens of ms. With these procedures there should be little chance of difficulties arising from polarization of the axial wire. In all experiments 20 s were allowed between pulses so that the effects of slow inactivation (Goldman and Schauf, 1972; Rudy, 1975) could dissipate.

Na currents ($I_{Na}$) were extracted by repeating the pulse schedules in enough TTX ($10^{-6}$ M) to block $I_{Na}$ completely and then by making a point-by-point subtraction of the two sets of records in time. Photographic records of the oscilloscope screen were enlarged and traced onto graph paper. The tracings were digitized with a Sumagraphics (Fairfield, Conn.) NH-2-20 digitizer, which records x- and y-coordinates on punched cards. Subtractions were then done on the IBM 370 at the Health Sciences Computer Center at University of Maryland. The computer also scaled the corrected $I_{Na}$ records during each test pulse so that the peak $I_{Na}$ matched that of the control (without a conditioning pulse) for each clamp run. The corrected and scaled $I_{Na}$ records were then plotted and the effects of conditioning pulses were determined by overlaying a plot of a similarly corrected control record without a conditioning pulse. Samples of such processed records are shown in Fig. 2 A and D.

Artificial sea water (ASW) had the following composition: 430 mM Na, 10 mM K, 10 mM Ca, 50 mM Mg, 560 mM Cl, 5 mM tris (hydroxymethyl) aminomethane, pH 8.0 ± 0.1. Temperature was 5 ± 1°C. All potentials are reported as absolute membrane potential (inside minus outside) and have been corrected for liquid junction potentials according to the values of Cole and Moore (1960 a) which have been shown to be also suitable for *Myxicola* (Binstock and Goldman, 1971).

**RESULTS**

*Shift of the $G_{Na}$ along the Time Axis*

In this section we describe our experiments on the effects of conditioning polarizations on the time-course of $G_{Na}$ during test steps in potential. Digitized records of $I_{Na}$ during the test step (corrected for leak and K currents as described in Methods) were all scaled to have the same peak amplitude for any given clamp run. During a clamp run the conditioning and test potentials were fixed and the duration of the conditioning step was varied. Examples of current records for a test step of 20 mV and a conditioning step to $-100$ for durations of 0, 200, 400, and 1,000 $\mu$s are shown in Fig. 1. Typical corrected and scaled digitized records (now as $G_{Na}$) are shown in Fig. 2.

Fig. 2 A shows a plot of such digitized responses for a test step to 20 and a conditioning step to $-50$ mV. The conditioning step durations were 1,000 (left), 400 (middle), and 0 (right) $\mu$s. The early part of the record, even without a
conditioning pulse, has been lost owing to the settling time of the clamp with $R_s$
compensation. The start of the test steps for all three records have been made
to coincide in Fig. 2 A. In Fig. 2 D, the 400- and 1,000-μs records have been
shifted by 70 and 110 μs, respectively. The three records are now seen to
superimpose over their whole time-course; i.e., the depolarizing conditioning
step shifted $G_{Na}(t)$ along the time axis without a change in shape. Hyperpolariz-
ing conditioning steps produced a similar translation along the time axis, but
now in the opposite direction so as to result in increased delay in the rise of $G_{Na}$.
These results are similar to the shift of $G_K$ along the time axis (Cole and Moore,
1960 b; Goldman and Schauf, 1973). Time shifts of the rise of $G_{Na}$ have also been
reported for squid (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1976;
Rojas and Rudy, 1976) and node (Neumcke et al., 1976) but only as a response
to hyperpolarizing conditioning pulses.
Conditioning steps to potentials of −150 to 0 mV were examined, with
durations ranging from 50 μs up to 30 ms for hyperpolarizing and small
depolarizing steps. For larger depolarizing conditioning steps, durations beyond

\[ m = m_o + (m_o - m_w)e^{-\Delta t} + \Delta t m_w, \]  

FIGURE 1. Tracings of the time-course of $I_{Na}$ for a test potential of 20 mV and a
hyperpolarizing conditioning potential of −100 mV. Conditioning durations were
0, 200, 400, and 1,000 μs. The start of the conditioning steps have all been made to
coincide. The dashed line indicates zero current. Scale: 300 μA/cm^2, 200 μs.

1–3 ms could not be used as the inactivation, and more importantly, the
activation developing during the conditioning step made resolution of the rise
of $G_{Na}$ difficult if not impossible. Over this range, beyond the effects of
conditioning potentials on the amplitude of $G_{Na}$ which have already been
described in *Myxicola* (Goldman and Schauf, 1972; Schauf and Davis, 1975),
conditioning pulses always produced a simple translation of $G_{Na}(t)$ along the
time axis without a change in the wave form. There were no other effects.

Time shifts are predicted by a wide variety of kinetic models. Consider, for
example, the first order (two-state) activation variable of Hodgkin and Huxley,
$m$. For a depolarizing test step in potential rising instantaneously at $t = 0$ from
some fixed reference potential, and with a depolarizing conditioning step
smaller than the test step

\[ m = m_o + (m_o - m_w)e^{-\Delta t} + \Delta t m_w, \]
the same as that at the end of the conditioning step. Such a duration can always be found inasmuch as first order variables are monotonic and continuous across a step in potential. Hence, changes in initial conditions should shift \( m(t) \) along the time axis by some \( \Delta t \). The same result can be obtained with hyperpolarizing prepulses, but now with the term in the exponential given by \( -(t - \Delta t) / \tau_m \), because \( m \) at the end of any hyperpolarizing prepulse must lie between zero and \( m_o \). An equivalent result was obtained by Cole and Moore (1960 b), by Neumcke et al. (1976) and was apparently evident to Frankenhaeuser and Hodgkin (1957).

Clearly, \( m^2(t) \) will shift just as \( m(t) \). We have also simulated time shifts from the full Hodgkin-Huxley equations (i.e., from \( m^2(t) \) as modified for \( Myxicola \) (Goldman and Schauf, 1973). Over a range of depolarizing conditioning potentials and durations comparable to the experimentally studied range, the simulated \( \Delta t \)'s and those computed from \( m \) alone agree very precisely. Such simulated

![Figure 2](image)

**Figure 2.** Time-course of \( G_{Na}(t) \) during test steps in potential after various conditioning potential durations. The conditioning potential was \(-30 \text{ mV}\), and the test potential was \(20 \text{ mV}\) in A and D, and \(17 \text{ mV}\) elsewhere. Durations were 900, 300, and 0 \(\mu\text{s}\) (left to right) in part C and 1,000, 400, and 0 \(\mu\text{s}\) elsewhere. Peak \( G_{Na} \) in each part was scaled to match that of the unconditioned curve. Parts A and D are experimental results, parts B and E have been computed from a coupled kinetic model, and parts C and F from Hodgkin-Huxley kinetics. For further details see text.

results are shown in Fig. 2 C for a conditioning potential of \(-30\) and a test of \(17\) mV. Conditioning durations were 900 (left), 300 (middle), and 0 \(\mu\text{s}\) (right). These curves have been scaled as were experimental records. In Fig. 2 F, the three records are seen to superimpose over their whole time-course when shifted appropriately along the time axis.

Fig. 2 B and E show a similar computation for the coupled model of Goldman (1975). The model is

\[
G_{Na} = \overline{G_{Na}^c} \nu, \tag{2}
\]

where

\[
\nu + (a + b)\nu + ab(\nu - \nu_m) = 0, \tag{3}
\]

with \( a, b, \) and \( \nu_m \) being functions of membrane potential only. For a step in potential rising instantaneously at \( t = 0 \), a solution to Eq. 3 may be obtained:
\[ \nu = \nu_\infty \left( \frac{\nu(0) + b(\nu_0 - \nu_\infty)}{a - b} \right) e^{-at} + \left[ \frac{\nu(0) + a(\nu_0 - \nu_\infty)}{a - b} \right] e^{-bt}, \]  

where \( \nu_0 \) is the initial value of the variable; \( \nu(0) \) is the initial velocity (i.e., the derivative of \( \nu \) at \( t = 0 \)), and \( t \) is time. For the simulations of Fig. 2, the test and conditioning pulses are the same as in Fig. 2 C with the durations now 1,000, 400, and 0 \( \mu \)s. Again the three records can be made to superimpose, and both models then predict simple time shifts as a response to conditioning polarizations.

The experimentally determined time shifts presented below have been compared to the predictions of both independent (m\(^3\)h) and coupled (v\(^A\)) kinetic models. Within a wide variety of schemes, it is not the presence of a time shift that is of significance; i.e., a delay produced by conditioning hyperpolarization does not necessarily indicate an additional state or process. To assess the significance of the time shift one needs quantitative comparisons between model predictions and experiment (Cole and Moore, 1960 b).

STABLE-STATE TIME SHIFTS Fig. 3 shows the steady-state value of the time shift, \( \Delta \tau_e \), as a function of conditioning and test pulse potentials ranging from -10 to 30 mV. Each point indicates a single time shift observation on a single axon. For the hyperpolarizing side these may be taken as true steady-state values. \( \Delta \tau \) for a 500-\( \mu \)s conditioning pulse was not different from that for a 30-ms pulse (Fig. 8 B). For depolarizing conditioning pulses,
these are probably only quasi-steady-state values inasmuch as there seems to be an additional slower relaxation that is only partially resolved in these experiments (see below). The plotted values are the maximum measured $\Delta t$ for the indicated test and conditioning potentials.

For hyperpolarizing prepulses $\Delta t_{-}$ reaches a saturating value of $\sim 50$ $\mu$s and is independent of test pulse potential over this range. For depolarizing prepulses, $\Delta t_{+}$ increases over the whole range examined, and there is an effect of the test pulse, with lower test pulse potentials producing a somewhat greater shift.

The above results can not be compared directly to those of Keynes and Rojas (1976), on squid axons. They found their time shift values for hyperpolarized holding potentials to be strongly dependent on test pulse potentials over this same range, and saw no sign of a saturation with conditioning potential. They presented no results with depolarizing prepulses. However, the squid and Myxicola experiments have been done differently in two respects. First, the $\Delta t_{-}$ values reported by Keynes and Rojas are the corrections needed to make Hodgkin-Huxley kinetics fit their experimental records, whereas we have reported the actual shift from unconditioned test pulses. Second, they used changes in the holding potential whereas we used relatively brief conditioning pulses. Neumcke et al. (1976), in the node, also reported $\Delta t_{+}$ corrections needed to make Hodgkin-Huxley kinetics fit their experimental records. Their results were much like those of Keynes and Rojas when compared over the same range of potentials except that there was now no dependency on test pulse potential beyond $\sim 0$ mV. They used 50 ms conditioning pulses at 10°C which was much longer than the longest we used at 5°C.

The $\Delta t_{-}$ data for test potentials of 20 mV only are replotted as the points in Fig. 4. The dashed curve in Fig. 4 is the $\Delta t_{-}$ prediction from Hodgkin-Huxley kinetics. It is substantially greater than the observed values at every potential. This is interesting, there being a discrepancy between $m^{h}$ kinetics and the experiment which does not involve inactivation. The solid curve in Fig. 4 is the prediction from $v^{k}$ kinetics. This curve does not fit the data either, although the agreement is considerably better than for Hodgkin-Huxley kinetics, and the coupled model may be said to have a degree of rough predictive value.

**TIME CONSTANT OF DEVELOPMENT OF THE SHIFT**

Experiments were done with conditioning steps of different durations, and their effect on the magnitude of $\Delta t$ were observed. $\Delta t$ developed over an exponential time-course. Fig. 5 shows $\Delta t$ corrected for its steady-state value plotted logarithmically vs. the duration of the conditioning pulse. Typical results for a hyperpolarizing step to $-110$ mV (Fig. 5 B) and for several depolarizing steps to $-25, -20, -15$, and $-10$ mV (Fig. 5 A) are illustrated. The points are reasonably well described by straight lines. Here, and in every case, when the lines are extrapolated back to $t = 0$, they cross through the steady state $\Delta t$ values, i.e., there is no sign of any additional, faster relaxations from these data. As will be shown in the following paper, with experiments on the $I_{Na}$ tails, there does seem to be a faster process present in the activation of the $G_{Na}$ not seen in these experiments.

Values for the time constant of development of $\Delta t$, $\tau_{\text{shift}}$, are shown in Fig. 6 as a function of conditioning potential and for several different test potentials.
There is no clear effect of the test potential. For hyperpolarizing conditioning pulses $\tau_{\text{shift}}$ reaches a saturated value of about 100 $\mu$s. For depolarizing conditioning pulses $\tau_{\text{shift}}$ rises throughout the range studied.

The dashed curve in Fig. 6 is the $\tau_{\text{shift}}$ prediction from Hodgkin-Huxley kinetics. Unlike the experiments, $\Delta t$ does not develop over a simple exponential time-course in Hodgkin-Huxley kinetics. As time shifts computed from $m^3h$ and from $m$ are the same, this may be illustrated by Eq. 5:

$$\frac{-\Delta t}{\tau_m} = \log_e \left[ \frac{m_{e^+} - m_\infty + (m_0 - m_{e^+})e^{-t/\tau_m}}{m_0 - m_\infty} \right],$$

where terms subscripted with a $\epsilon$ refer to the conditioning pulse. Eq. 5 is not a simple exponential. However, at every potential semilog plots of $\Delta t$ vs. $t_c$ had an initial linear region, which was fairly extensive at small depolarizations. This was taken as the most reasonable comparison with experiment. The dashed curve differs substantially from the experimental points. $\tau_{\text{shift}}$ is very much faster than predicted by Hodgkin-Huxley kinetics.

The solid curve is the $\tau_{\text{shift}}$ prediction from the coupled model. In this model $\Delta t$ vs. $t_c$ is given by the sum of two exponentials which are reasonably well separated (Fig. 7), the time constants differing by 10-20-fold. The faster of the
two time constants is plotted in Fig. 6, whereas the peak $\Delta t$ at small and the 1.5 ms value for large depolarizations were taken as the most reasonable comparison with experiment in Fig. 4. Decomposing $G_{Na}(t)$ into the sum of exponentials (Eq. 4) as compared to the product (as with $m^3h$), produces a faster time constant of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Difference between the time shift ($\Delta t$) and its steady-state value $\Delta t_{ss}$ as a function of conditioning duration. Conditioning potentials were (♦) -25, (○) -20, (□) -15, and (●) -10 mV in part A, and -110 mV in part B.}
\end{figure}

activation. Correspondingly, the solid curve lies below the dashed in Fig. 6. However, the coupled model still does not predict the $\tau_{shift}$ values. The predicted values are much too slow, especially at small depolarizations. There is then, a process in the activation of $G_{Na}$ that is very much faster than had been supposed.

Another discrepancy between both models and experiment is that the experimental $\tau_{shift}$ values increase with potential although both models predict that they should decrease. The slowing of $\tau_{shift}$ with depolarizations is surprising as the time-to-peak of $G_{Na}$ decreases steeply with increasing potential. $\tau_{shift}$ then is both too fast and shows the wrong dependency on potential to account for the
observed times to peak of $G_{Na}$ (2-3 ms at -40 mV). Both these observations suggest that there must be yet another process in the activation of $G_{Na}$ which will provide for its very slow rise at small depolarizations and for a decrease in the rise time with increasing potential.

$\tau_{shift}$ is not a direct measure of an activation time constant, but a function of it. $\tau_m$ in Hodgkin-Huxley kinetics and the activation $\tau$ in the coupled model are less than their respective predicted $\tau_{shift}$ values at every potential. Hence, the underlying time constants studied in these time shift experiments may be faster than the values shown in Fig. 6.

**TEMPERATURE DEPENDENCY** $Q_{10}$'s were measured over the range 5-10°C in three axons with a conditioning step to -25 mV and a test step to 20 mV. $\tau_{shift}$ had a mean $Q_{10}$ of 1/2.50 which agrees well with the value reported for other kinetic properties of $G_{Na}$ (Schauf, 1973). The steady-state $\Delta \tau$ had a mean $Q_{10}$ of
1/1.68. This temperature dependency may arise from the fact that there seems not to be a true steady state.

**SLOW EFFECTS** Fig. 7 shows \( \Delta t \) vs. \( t_c \) computed from the coupled model for several conditioning potentials as indicated. At small depolarizations \( \Delta t \) is reduced again at long conditioning duration, whereas for larger depolarizations \( \Delta t \) is increased further. The slow reduction of \( \Delta t \) was interesting in that no similar effect was predicted by Hodgkin-Huxley kinetics, for which \( \Delta t \) vs. \( t_c \) is monotonic as is suggested by examination of Eq. 5, and confirmed by simulations with the full Hodgkin-Huxley equations. The slow reduction in \( \Delta t \) is then a differential prediction between Hodgkin-Huxley and coupled kinetics, and we looked for such effects experimentally.

Fig. 8 B shows \( \Delta t \) vs. \( t_c \) values for an axon with a hyperpolarizing conditioning step to \(-93 \) mV and a test step to \(7 \) mV. Once the \( \Delta t \) value was reached (by a \( t_c \) of \(500 \) ms), extending the conditioning pulse duration to \(30 \) ms was without additional effect. This was the case in each of four axons where conditioning hyperpolarizations of \(20 \) ms or more were applied. Conditioning hyperpolarization never produced any slow effects over the whole range of durations examined.

Fig. 8 A shows \( \Delta t \) vs. \( t_c \) values for a different axon with a depolarizing conditioning step to \(-41 \) mV and a test step to \(20 \) mV. There is an initial rapid rise in \( \Delta t \) to a quasi-steady state followed by a small but clear decline. Similar effects were seen in five out of seven axons. Detecting the slow decline in \( \Delta t \) was difficult, because for large depolarizing steps where \( \Delta t \) was large long prepulses could not be applied, and small conditioning steps produced small \( \Delta t \) values. We are inclined to think that the slow effect seen in Fig. 8 A is real as it is reproducible. However, a definitive answer on such small effects will have to
await more accurate measurements such as could be obtained with completely automatic on-line digitizing of current records.

**DISCUSSION**

Over the range examined, conditioning polarizations produced a translation of the normalized $G_{Na}(t)$ along the time axis without a change in shape. Effects of conditioning pulses on the time-course of inactivation were reported, however, for myelinated nerve fibers (Frankenhaeuser, 1963), and for $I_{Na}$ in cooled Purkinje fibers (Dudel and Rüdel, 1970). This is probably because inactivation in these preparations seems to proceed in two sequential steps (Dudel and Rüdel, 1970; Chiu, 1976), and different initial conditions change the relative weights of the two exponential terms. In *Myxicola* inactivation is always reasonably well described as a single exponential. Any contributions from a second process must be relatively minor.

![Graph](image)

**Figure 8.** Time shift of $G_{Na}$ as a function of the duration of the conditioning pulse. Test and conditioning potentials were 20 and $-41$ mV in A, and 7 and $-95$ mV in B, respectively.

The experimental $\tau_{act}$ values cannot be accounted for by either Hodgkin-Huxley kinetics or the coupled kinetic model of Goldman (1975). There is a process in the activation of $G_{Na}$ which is faster than either model predicts. This fast process is, however, not sufficient to account for the observed activation kinetics of $G_{Na}$, suggesting that there is an additional activation process. One possibility, therefore, is that the difficulties with the three-state model could be overcome with an additional state between rest and conducting. This idea is supported by the results of the following paper.

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