BRIEF COMMUNICATION

MEMBRANE CURRENTS FOLLOWING ACTIVITY IN CANINE CARDIAC PURKINJE FIBERS

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ABSTRACT Recent experiments in canine Pukinje fibers (Gadsby and Cranefield, 1979) have shown that following a period of sodium loading in K^+-free solution a slowly decaying outward current is observed. This current has been attributed to the activity of the electrogenic Na^+-K^+ exchange pump. In the present paper we show that similar slowly decaying outward currents are observed following prolonged periods of overdrive with action potentials or with brief depolarizing voltage clamp pulses. The dependence of the prolonged outward current on the duration and frequency of the preceding period of overdrive and on the potential following overdrive is reported. We also present results which indicate that a large portion of this current can be induced by phasic Na^+ loading through the fast-inward channel.

Cardiac Purkinje fibers exposed to K^+-free solution exhibit a rise in internal $[Na^+]$ (Ellis, 1977). On return to solutions of normal $[K^+]$, a decaying outward current is observed for periods up to 5 min (Gadsby and Cranefield, 1979; Eisner and Lederer, 1980). The dependence of this outward current on the preceding period of Na^+ loading led investigators to suggest the involvement of an electrogenic Na^+-K^+ exchange pump, driven by the rise in internal $[Na^+]$ (Gadsby and Cranefield, 1979; Eisner and Lederer, 1980). Similar experiments studying the effects of either low temperature (Glitsch et al., 1978) or exposure to cardiac glycosides (Daut and Rüdel, 1980) have also provided evidence for electrogenic Na^+ transport in both atrial and ventricular muscle.

If an elevation of $[Na^+]_i$ can increase the activity of the Na^+-K^+ exchange pump causing an outward membrane current, then sustained periods of high frequency stimulation, which also increase $[Na]_i$ (Cohen and Fozzard, 1979), would be expected to induce this current. Transient hyperpolarization of the membrane potential following periods of rapid stimulation led Vassalle (1970) to suggest that an increase in internal $[Na^+]$ initiated by high frequency stimulation might be important in suppressing pacemaker activity due to the outward electrogenic current generated. We report here the results of experiments designed to examine the properties of the membrane current following variable duration and frequencies of rapid stimulation and in the presence of tetrodotoxin (TTX).

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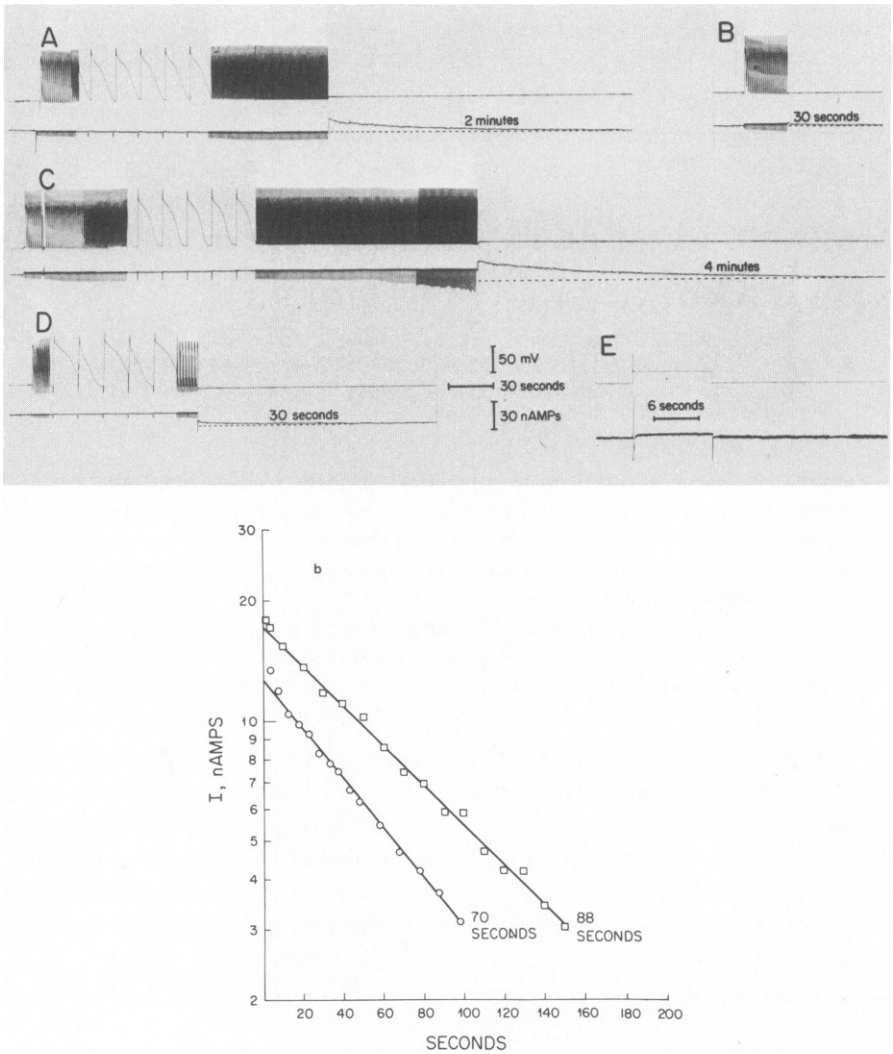

FIGURE 1 (*a*) Membrane currents recorded in response to 2 min (*A*), 30 s (*B*), and 4 min (*C*) trains of overdrive. Following recovery from the 4-min train, a 30-s train (*D*) was again applied. The interstimulus interval was 700 ms. The voltage clamp potential following the period of stimulation was −76 mV. An 11-s depolarizing pulse from −76 to −54 mV is also shown (*E*). In each set of records the upper trace is voltage and the lower trace current. The dashed line in the current record indicates the steady-state level of membrane current at −76 mV. In *A*, *C*, and *D* the horizontal gain of the strip chart recorder was briefly expanded by 25× to reveal the action potential shape during the train. The alternating action potentials in *D* are due to the action potential duration initially exceeding the interstimulus interval. This also occurs during the first 30 s in *A*, *B*, and *C*. With continued stimulation the action potential shortens and each stimulus evokes the same response (*A* and *C*, expanded horizontal gain). The temperature was 35.8°C. (*b*) A semillogarithmic plot of $I(t)$-$I_{\text{steady-state}}$ against time following the 2-min (○) and 4-min (□) trains. The current flowing following the 11-s voltage clamp pulse and the two 30-s trains was too small in magnitude (i.e., <3 nA) to accurately resolve and therefore has not been plotted.

Experiments were performed on canine Purkinje fibers of small radius (<0.15 mm) which had been cut to lengths of <1.5 mm. Recent investigations (Cohen, et al., 1979; Cohen and Falk, 1980) have shown the suitability of this preparation for voltage clamp studies. The experimental set up was similar to that of Cohen et al. (1976). The preparations were bathed in Tyrode solution containing 140 mM NaCl, 4 mM KCl, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 2 mM $CaCl_2$, and 2 mM $MgCl_2$. A 95% O_2-5% CO_2 gas mixture was used to oxygenate the solution. The pH of the Tyrode in the tissue bath was 7.38. The temperature was maintained to within ±0.2°C of the value indicated in each of the figure legends.

The magnitude of the slowly decaying outward current depends on the duration of the previous period of overdrive. This is shown in Fig. 1 *a*. Stimuli were applied to a Purkinje fiber for 30 s, 2 min, and 4 min. After recovery from the 4-min train, the fiber was again overdriven for 30 s. The interstimulus interval for all trains was 700 ms. After each period of stimulation the preparation was voltage clamped to −76 mV. The outward current decays rapidly after both 30-s trains, but lasts several minutes and is much larger following the 2- and 4-min trains. A depolarizing pulse from −76 to −54 mV for 11 s is also shown. in Fig. 1 *b* the current following the 2- and 4-min trains is plotted on semilogarithmic coordinates against time following cessation of stimulation. The decaying currents for the 2- and 4-min trains can

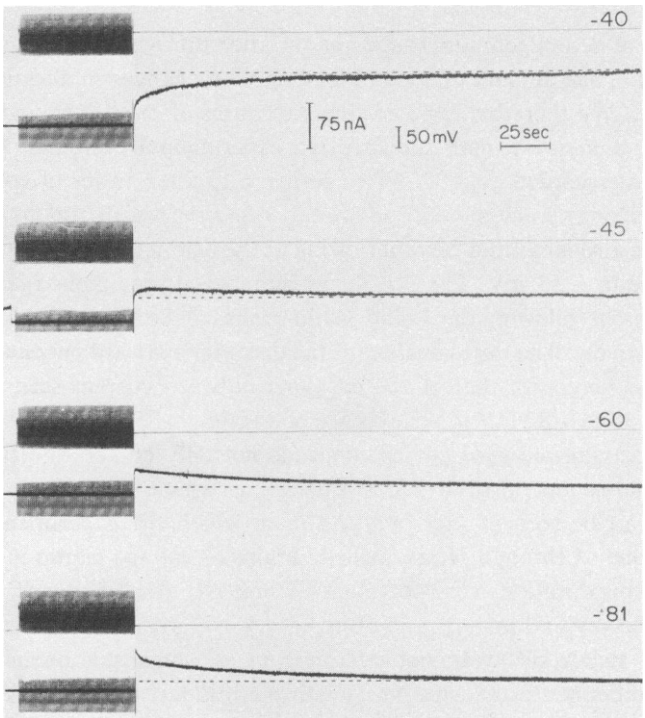

FIGURE 2 The voltage dependence of the activity induced current. In each set of records the upper trace is voltage and the lower trace current. The number at the right of each set of records is the potential to which the membrane was clamped following a 1-min train. The interstimulus interval was 500 ms. The dashed lines indicate the steady-state level of membrane current at each potential. The current following activity becomes more inward with time at −81 and −60 mV. It is biphasic at −45 mV and is increasingly outward at −40 mV. Possible interpretations are discussed in the text. The temperature was 39.0°C.

be fit by time constants of 72 and 88 s, respectively. The exponential fits to the slowly decaying outward current can be extrapolated to time zero to estimate the total magnitude (I_o) of the activity-induced current. There are several assumptions involved in this procedure, most notably the independence of the activity induced current from other membrane currents at early times, and the exponentiality of the activity induced current throughout its time course. Neither of these assumptions is necessarily correct. Nevertheless, these results as well as those from six additional preparations (15 trains from six fibers clamped at six different holding potentials) suggest a qualitative dependence of the current magnitude on the duration of the preceding period of overdrive.

The results of an experiment investigating the voltage dependence of the decaying outward current are shown in Fig. 2. Purkinje fibers were stimulated at a 700-ms interstimulus interval for a period of 60 s and then voltage clamped to −81, −60, −45, and −40 mV. At −81 and −60 mV, decaying outward currents are observed. At −45 mV the current is biphasic; it initially becomes more outward, and then more inward at later times. At −40 mV the current reverses direction and becomes more outward with time. In four additional experiments on the voltage dependence of the activity induced current, the current reversed sign twice at −20 mV and twice failed to reverse sign at potentials as positive as 0 mV. In the two experiments in which reversal of the current was not observed a biphasic current was recorded positive to −40 mV.

The alteration of action potential shape and duration during the stimulus trains (see Fig. 1 *a*) makes control of the amount of sodium loading with changes in duration or frequency of the train difficult. We therefore exposed fibers to trains of brief depolarizing voltage clamp pulses to achieve somewhat more standardized experimental protocols. Fig. 3 *a* shows the membrane current recorded at −82 mV in response to three trains of voltage clamp pulses (20, 60, and 180 s long). Each pulse, from −82 to −38 mV, was of 200 ms duration, delivered every 400 ms. We also measured current flowing at the holding potential (−82 mV) following a single 4-s pulse to −38 mV. Fig. 3 *b* shows semilogarithmic plots against time for these currents. The current following the 1- and 3-min trains can be fitted with time constants of 51 s and 72 s, respectively. The time constant of the decaying outward current following the 20-s train correlates closely with that of the decaying outward current seen upon stepping the potential from −38 to −82 mV in the absence of a train.

The slowly decaying outward current depends not only on the duration of the train of voltage clamp pulses but also on the frequency at which the voltage clamp pulses are delivered. This can be seen in Figs. 4 *a* and *b*, in which the experimental protocols were applied in the order *A* through *H*, as labeled. Trains of voltage clamp pulses, from −81 to −39 mV, of 200 ms duration every 400 ms for 1 min (*A*) and 2.5 min (*C*) were followed by trains of 100 ms every 200 ms (*D*) and 50 ms every 100 ms (*F*) for 2.5 min. Each of these trains elicited a slowly decaying outward current of increasing magnitude. To test the possibility of significant steady-state Na^+ loading at the test potential (Attwell et al., 1979; Kass, et al., 1976) depolarizing voltage clamp pulses to −39 mV for 2.5 min were applied following recovery from each of these trains (*B, E, G*). Decaying outward currents induced by these pulses were of much smaller magnitude and decayed more rapidly than those seen following the trains. Immediately following protocols *F* and *G*, the fiber was perfused with 2.3×10^{-5} M TTX. Pulses of 50 ms duration every 100 ms for 2.5 min were then applied

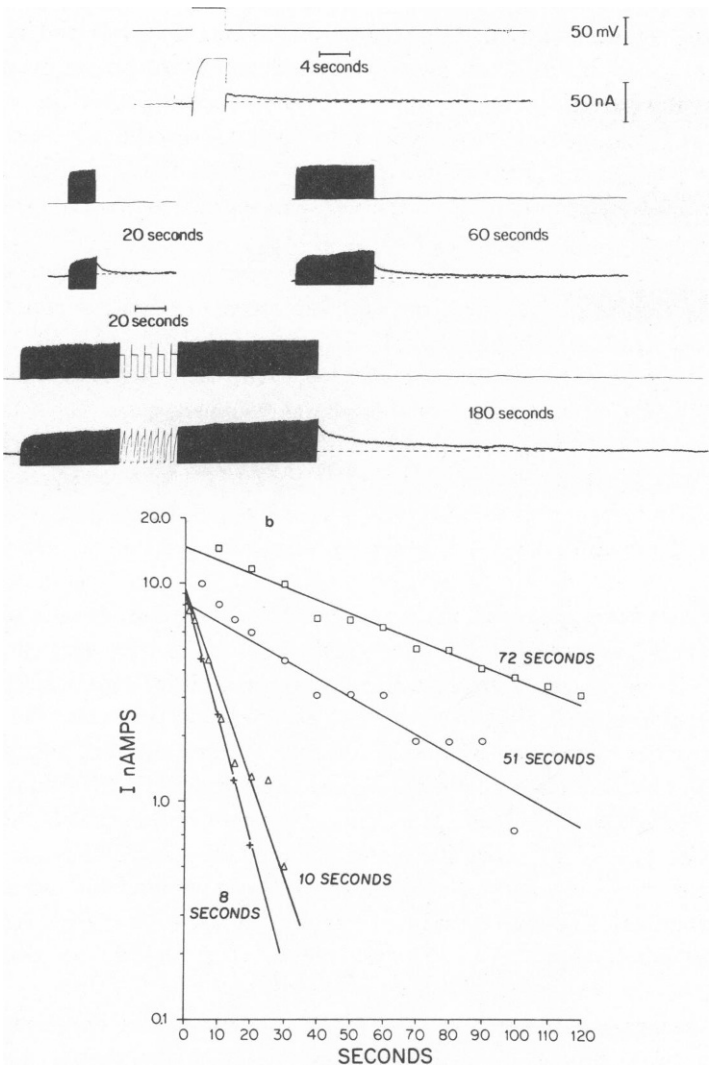

FIGURE 3 (*a*) Membrane currents recorded in response to: (1) a 4-s voltage clamp pulse from −82 to −38 mV (upper center); (2) repetitive voltage clamp pulses of 200 ms duration applied every 400 ms for 20, 60, and 180 s between the same potentials. In each set of records the upper trace is voltage and the lower trace current. Following both the 4-s pulse and the 20-s train the decay to steady-state current (indicated by the dashed lines) is rapid, while following the 60- and 180-s trains a slowly decaying outward current is observed. In the upper set of records the current goes off scale during the pulse because a high gain was needed to observe the tail current. In the lower set of records the horizontal gain of the strip chart recorder was briefly expanded by 25× to demonstrate the clamp pulses during the train. The current in the bottom record steadily grows more outward during the 3-min train. This may reflect increasing electrogenic pump stimulation as Na^+ loading proceeds. The temperature was 38.5°C. (*b*) A semilogarithmic plot of $I(t)$-$I_{steady\text{-}state}$ for the date of (*a*). The 20-s train (+) decays with nearly the same time constant as the single pulse (△), while the 1-min (●) and 3-min (□) trains have sequelae that decay much more slowly. △ – *V* step from −38 to −82 mV in absence of a train.

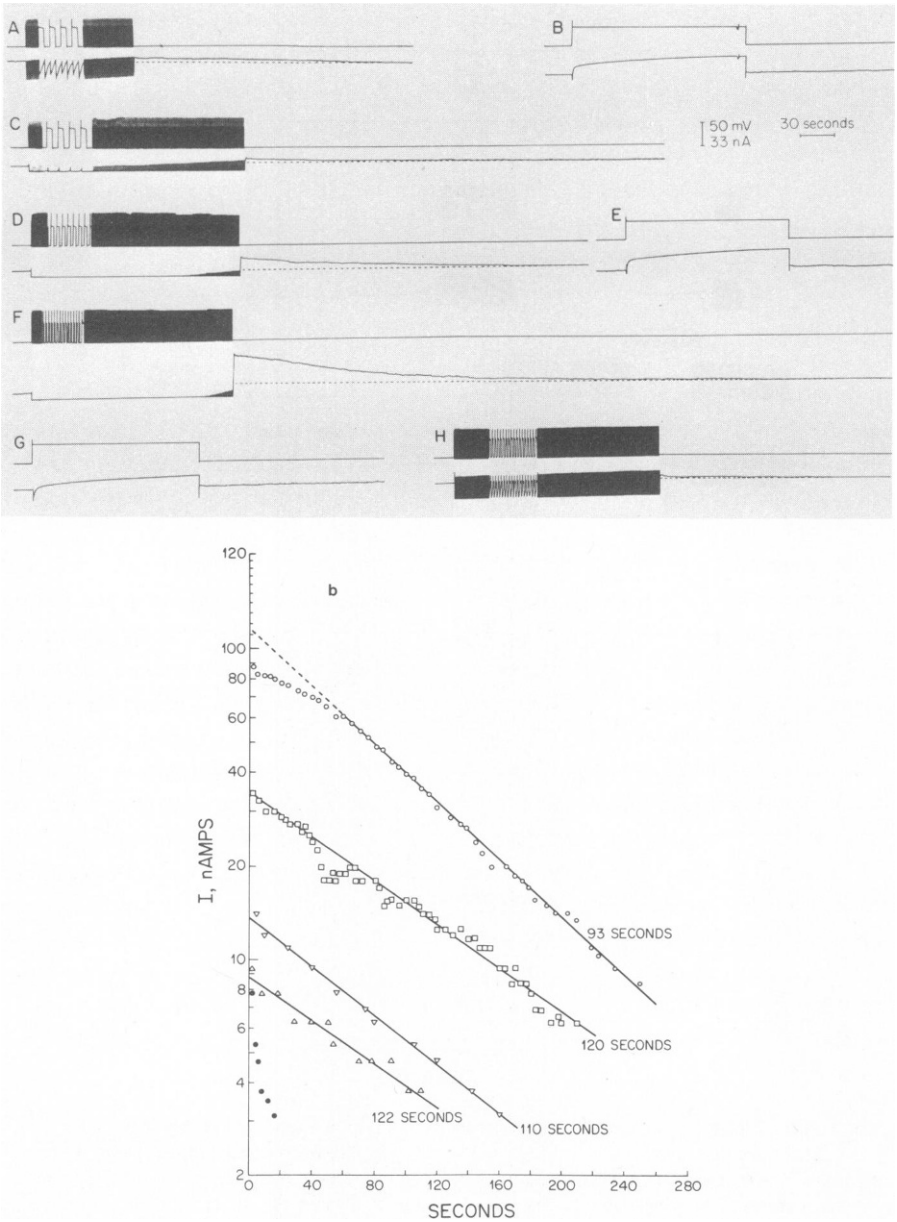

FIGURE 4 (*a*) The frequency dependence of the activity induced current and the effect of TTX. Experimental protocols were applied in the order *A* through *H*: Voltage clamp pulses of 200 ms duration delivered every 400 ms for 1 min (*A*) and 2.5 min (*C*); clamp pulses of 100 ms duration delivered every 200 ms for 2.5 min (*D*); clamp pulses of 50 ms duration delivered every 100 ms for 2.5 min in Tyrode solution (*F*) and upon addition of 2.3×10^{-5} M TTX (*H*); single 2.5 min depolarizing voltage clamp pulses (*B, E, G*). All voltage clamp steps were from −81 to −39 mV. In each set of records the upper trace is voltage and the lower trace current. The dashed lines indicate the steady-state current level at −81 mV. In *A, C, D, F,* and *H* the horizontal gain of the strip chart recorder was briefly expanded by 25× to

(record *H*). In the presence of TTX the train induced only a very small rapidly decaying outward current (compare records *F* and *H*). Fig. 4 *b* shows semiexponential plots of the decaying outward currents following each of the trains in Fig. 4 *a*.

In summary, slowly decaying membrane currents are observed following trains of high frequency stimulation or repetitive voltage clamp pulses. The magnitude of this current increases with both the length and the frequency of stimulation.

Furthermore, experiments with TTX indicate that, over the voltage range examined, phasic Na^+ loading through the fast inward channel induces a large proportion of the slowly decaying outward current. This is supported by the additional findings that (*a*) changings the pulsing protocol from 50 ms every 100 ms to 10 ms every 100 ms has only a small effect on the magnitude of the decaying outward current, and (*b*) altering the holding potential from −80 to −55 mV where most of the Na^+ current is inactivated eliminates most of the slowly decaying outward current.

The origin of the apparent current reversal seen at depolarized potentials is at present not known but could result from: (*a*) steady-state sodium loading at depolarized potentials, (*b*) potassium accumulation or depletion at depolarized potentials, (*c*) contamination of the activity induced current by a time and voltage dependent current (e.g., I_x), or (*d*) a complicated dependence of the activity induced current on potential. Eisner and Lederer (1980) have presented data showing reversal of a similar current following periods of Na^+ loading in 0 mM $[K^+]_B$ in sheep Purkinje fibers. They have suggested that depletion of K^+ in the narrow spaces of the sheep Purkinje preparation might cause this reversal. Although this hypothesis is attractive, further investigation is needed to rule out the alternative possibilities.

The activity induced current may play a major role in normal cardiac electrophysiology. It can account for the slowing of pacemaker activity following rapid stimulation rates (Vasalle, 1970) and for the associated suppression of extrasystoles which otherwise would be expected to occur during these lengthened diastolic intervals. The importance of the fast Na^+ channel in the Na^+ loading process provides a possible explanation for the greater susceptibility to overdrive suppression encountered in Purkinje preparations in which action potentials are initiated from normal diastolic potentials, as compared to those in which action potentials are initiated from partially depolarized levels (Dangman and Hoffman, personal communication; Ferrier and Rosenthal, 1980).

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demostrate the clamp pulses during the train. A slowly decaying outward current is seen following the trains in Tyrode solution (*A, C, D, F*). Both the 2.5-min depolarizing clamp pulses (*B, E, G*) and the train of pulses in the presence of TTX (*H*) elicit a smaller magnitude and more rapidly decaying outward current. The temperature was 35.5°C. The contrast was heightened graphically for clarity. (*b*) Semilogarithmic plots of $I(t)$-$I_{\text{steady-state}}$ against time for the current following the trains in *a* (i.e., protocols *A, C, D, F,* and *H*). The slowly decaying outward current which clearly increases in magnitude with both the frequency of voltage clamp pulses and with the train duration is removed by addition of 2.3×10^{-5} M TTX. The currents following the 2.5-min voltage clamp pulses were too small in magnitude to accurately resolve (<3.0 nA) and were therefore not plotted. The number beside each current plot is the calculated time constant, assuming an exponential decay. It can be seen that the early time course of the current following the 4-min train is slowed relative to later times. This is seen occasionally after long, high frequency trains and may be due to a reduced pump rate caused by extracellular K^+ depletion. (△) 200-ms pulse/400 ms for 1 min; (▽) 200-ms pulse/400 ms for 2.5 min; (□) 100-ms pulse/200 ms for 2.5 min; (○) 50-ms pulse/100 ms for 2.5 min; (●) 50-ms pulse/100 ms for 2.5 min plus 2.3×10^{-5} M TTX.

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