BRIEF COMMUNICATION

DIGITAL OPERATIONS CAN BE DECEPTIVE

SQUID GIANT AXON MEMBRANE

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ABSTRACT Action potentials produced by current clamp stimuli have been used to voltage-clamp axons, an analogue, and computers. The final currents reproduced the original stimulus except during the action potential, when fluctuations of $> \pm 20\%$ were found. But all of these operations were digitalized. When the original action potential from an analogue was recorded directly on magnetic tape and played back as the voltage clamp, the original stimulus was reproduced within experimental error. Five collaborators and I have shown that the digital transients can accumulate to surprising values.

In my 30-year search for tests of the adequacy of a voltage clamp, I was impressed by the idea of M. E. and R. J. Starzak (1976, 1978), who applied a short pulse to a squid axon in their space and current clamp, converted the output action potential, similar to Fig. 1 *A*, into digital form, and stored it in the memory. They then applied this stored action potential to the axon in voltage clamp and obtained a current like that shown in Fig. 1 *B* instead of retrieving the original stimulus. They got a similar result with a Lettvin membrane analogue (MetaMetrics Corp., Carlisle, Mass.), which H. M. Fishman confirmed. As a last resort, I persuaded R. FitzHugh to compute such a circular experiment with the Hodgkin-Huxley (1952) axon equations using NIH's modeling program MLAB—with the surprising result shown in Fig. 1 *B*. This was confirmed with constant time integration intervals by M. Wathey and it was found that the peak amplitude of the "hash" current decreased in proportion to the integration interval to suggest that, with patience, in the limit, the Starzak idea might be valid.

As I was puzzling over the fact that axon, analogue, and computer gave very similar hash patterns for the final output, J. W. Moore suggested that the answer might lie in the digital operations. So Fishman tape-recorded the current clamp output action potential directly from a stimulus applied to a Lettvin analogue, Fig. 2 *A*. When this tape record was applied directly, as a voltage clamp, the current output reproduced the original stimulus, Fig. 2 *B*, except for a 5-kHz band pass distortion of the tape, and with no indication of the now probable digital hash.

Although real axons need to be tested and may present other difficulties, I think the message is clear: digital operations may be fast, convenient and elegant but with anything as unstable as a squid axon membrane or its equations the transients can accumulate alarmingly and an analogue may be much safer.

I have hoped to start with a voltage clamp current and to test the output in a current clamp

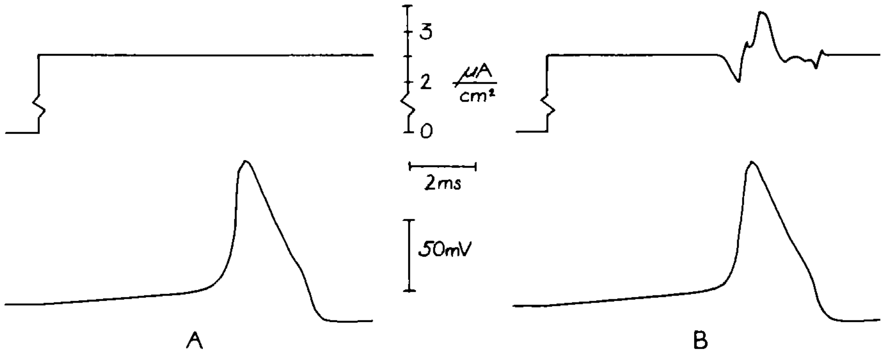

FIGURE 1 Responses of a computer programmed for the Hodgkin-Huxley axon membrane. (*A*) Above, the step stimulus applied in current clamp gives the action potential, below. (*B*) Below, the stored action potential from *A* is applied to the membrane as a voltage clamp giving the membrane current, above. (R. FitzHugh.)

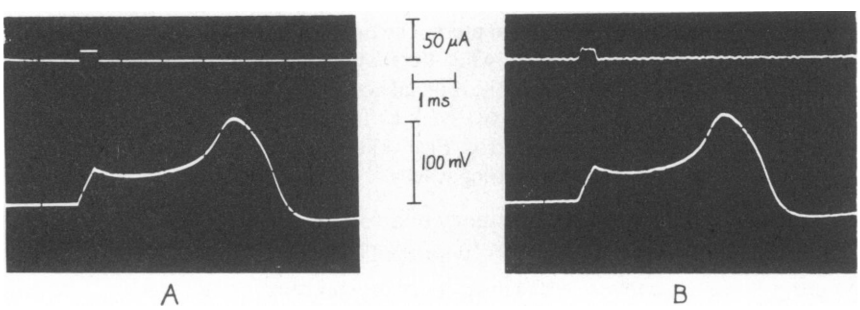

FIGURE 2 Responses of a Lettvin analogue of Hodgkin-Huxley axon membrane. (*A*) Above, applied pulse stimulus in current clamp giving the action potential response, below. (*B*) Below, the magnetic tape record from *A* applied as a voltage clamp to give the current, above. (H. M. Fishman.)

as more convenient experimentally. Three groups working this approach have so far failed to agree.

I am very indebted to all of the named individuals for their valuable unpublished cooperation. Each of the five is a co-author of his part.

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