ramp depolarizations and synaptic blockade by substitution of sulfate for chloride ions in the saline. In both of these tests, only continuous trains of action potentials, and no bursts of action potentials, occurred (Friesen *et al.*, 1978). Therefore, the central neuronal oscillator for leech swimming appears not to rely upon endogenously bursting neurons, but rather it appears to be a network oscillator. It will be important, however, for workers to substantiate this claim further by additional experimental tests.

A DIGRESSION ON NEURONAL MODELING

Before proceeding with the description of the central neuronal circuit responsible for the alternating dorsal–ventral rhythm in leech swimming, it will be useful to consider some hypothetical circuit configurations that would be capable of producing such a rhythm. One reason this is useful is that the central oscillator circuit for leech swimming, as for several other rhythms, is very complex and difficult to understand without guiding principles. These principles have been developed through the theoretical study of hypothetical circuits (Wilson, 1966; Lewis, 1968). This approach is often called theoretical NEURONAL MODELING or CIRCUIT MODELING. This process, in its simplest form, involves simply drawing on paper a model consisting of a group of neurons that are interconnected by a particular configuration of excitatory and/or inhibitory synapses. One then attempts to follow through the sequence of neural activities expected to occur in each of the cells of the model, based upon their synaptic connections. In the present discussion, we will begin with this approach and will then consider briefly some more sophisticated modeling approaches employing electronic and computer techniques.

What kinds of model circuits could produce a rhythmic alternation of bursts of action potentials such as occur in the excitatory motor neurons to the dorsal and ventral muscles of the swimming leech? One such model consists of DELAYED EXCITATION between two neurons (Wilson, 1966). For instance, in Figure 9A, imagine that cell 1 receives, from a presynaptic neuron not shown, a brief train of EPSPs that produces in cell 1 a brief burst of action potentials. Once this initial event had occurred, rhythmically alternating bursts could be sustained by means of only the synaptic interactions shown between cells 1 and 2. The initial burst in cell 1, after a delay somehow built into the circuit, would cause in cell 2 a brief depolarization and a consequent brief burst of action potentials. This burst in turn would cause, after another delay, a depolarization and consequently a burst in cell 1, and so forth. The mechanism of the delay is not specified in this model but presumably could consist of a polysynaptic chain of neurons through which the excitatory signals between cells 1 and 2 pass. Such a system of mutual delayed excitation is thought to play a contributing role in controlling the rhythmic wingbeats in locust flight (Burrows, 1973) but has not yet been found to underlie any other rhythmic behavior.

The second model (Figure 9B) consists of two neurons interconnected not by excitation but by MUTUAL INHIBITION, or, as it is more often called, RECIPROCAL INHIBITION[3] (Wilson, 1966). In some forms of this model, each cell also

[3] This term should not be confused with reciprocal innervation (Chapter 8).

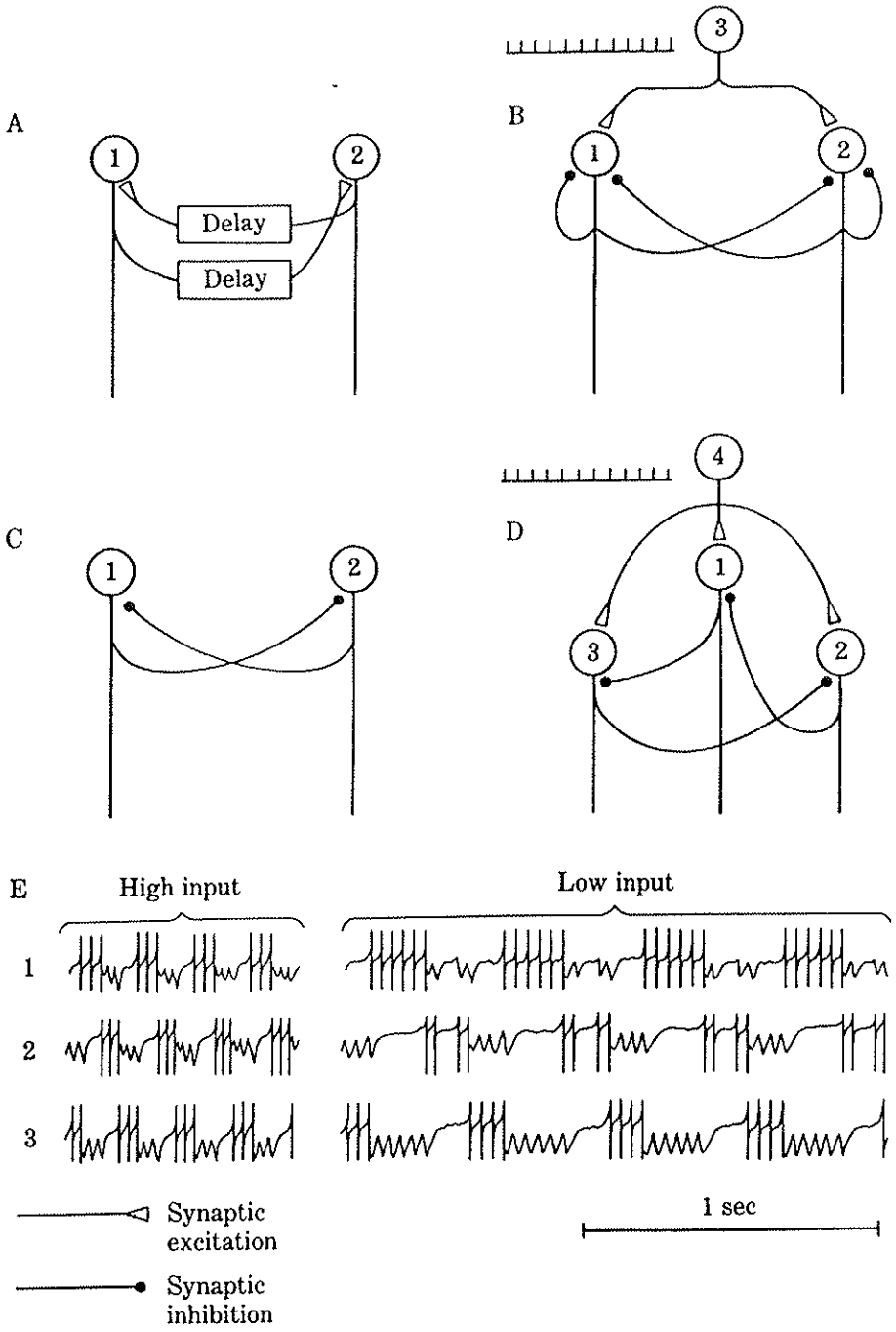

FIGURE 9. Theoretical modeling of rhythmic motor outputs. A. Delayed excitation. B. Reciprocal inhibition plus self-inhibition and shared excitation from a third neuron. C. Reciprocal inhibiton. D. Cyclic inhibition plus shared excitation from a fourth neuron. E. Impulses and simulated IPSPs recorded from three neuromimes connected according to the pattern of synapses among the neurons 1, 2, and 3 of D. When a high level of excitatory input was provided from a fourth neuron (simulating neuron 4 of D), the neuromimes produced short cycle periods. When a low level of excitatory input was provided, long cycle periods occurred. The phase was nearly constant, in spite of large changes in cycle period; phase of 2 with respect to 1 was 0.35 during short cycle periods and 0.45 during long cycle periods. (E after Friesen and Stent, 1977.)

inhibits itself, and both cells are excited by a third neuron (cell 3 in Figure 9B) that gives not brief bursts of action potentials but rather a continuous train. Imagine, for instance, that cell 3 of this model begins at some moment to give a regular train of action potentials, which then continues uninterruptedly. The onset of these action potentials would evoke a simultaneous depolarization of cells 1 and 2, each toward its threshold transmembrane voltage. By random chance, either cell 1 or cell 2 would reach its threshold before the other and at that moment would begin to give action potentials. Assume that cell 1 is the first to reach threshold. The synaptic inhibition from cell 1 to cell 2 would now prevent cell 2 from reaching its threshold and thus giving action potentials. Therefore, cell 1 alone gives action potentials. Soon, however, the inhibition by cell 1 upon itself would terminate its own train of action potentials. When this happens, cell 2 is thus freed from inhibition and can now become active. In fact, two separate factors could contribute to activate cell 2 at this moment. First, the continuing excitation from cell 3 is now unopposed by synaptic inhibition from cell 1. Second, as the inhibition from cell 1 ends, a phenomenon called postinhibitory rebound would contribute to the excitation in cell 2. A postinhibitory rebound, seen very commonly in neurons, is a brief burst of action potentials at the end of a period of inhibition.[4] When cell 2 begins to give its action potentials, these inhibit cell 1, maintaining its silence. But next, when cell 2 turns itself off by its self inhibition, cell 1 becomes active, and the whole cycle repeats itself.

It is also possible for two neurons connected by reciprocal inhibition to give alternating bursts of action potentials even if they do not have self inhibition or continuous input from a third neuron (Figure 9C). All that is required is that each neuron produce a brief postinhibitory rebound of substantial magnitude (Perkel and Mulloney, 1974). For instance, in Figure 9C, suppose that cell 1 is depolarized briefly (for instance, by an initial, brief excitatory input from another neuron not shown) so that it gives a brief burst of action potentials. During this burst, cell 2 would receive inhibition from cell 1. As soon as the burst in cell 1 terminates (which would occur when the initial brief excitatory input to it terminates), cell 2 would give its postinhibitory rebound. If this rebound were sufficiently large, it would produce in cell 2 a substantial burst of action potential. During this burst, cell 1 would be inhibited. But when the burst in cell 2 terminates (which occurs when its postinhibitory rebound terminates), cell 1 would give its own postinhibitory rebound and consequently a burst of action potentials. The whole cycle would then repeat itself.

As will be shown later in this chapter, not all central neuronal oscillators produce BIPHASIC rhythms—alternating bursts in just two groups of neurons. Rather some oscillators produce POLYPHASIC rhythms in which each of three or more neurons is activated at its own time within the cycle period. We consider

[4] Postinhibitory rebound is thought to result from the lowering of the threshold voltage of a cell in response to prolonged inhibition. That is, the neuron now needs only to be depolarized to a voltage very slightly above the resting potential in order to elicit action potentials. In fact, the threshold can actually become as low as the resting potential itself. In such a case, when the inhibiting hyperpolarization terminates and the transmembrane potential suddenly returns to the resting potential, action potentials result. Very soon thereafter, the threshold voltage returns to its normal value and the action potentials cease.

here one model capable of producing a TRIPHASIC rhythm, in which each of three neurons is activated in the sequence 1 - 2 - 3 - 1 - 2 - 3 - . . . (Friesen and Stent, 1977). This sequence of activity can be produced by three neurons, each of which inhibits the one *preceding* in the sequence (Figure 9D). This pattern of connectivity is called CYCLIC INHIBITION. (In the present model, a fourth neuron provides continuous, nonrhythmic excitatory input to all three cells, analogous to cell 3 of Figure 9B. This fourth neuron is not essential, however.) In this model, when cell 1 begins producing action potentials, it would inhibit cell 3, which would thus become silent. This inhibition of cell 3 would allow cell 2 to recover from the inhibition that it had just been receiving from cell 3, before cell 3 was turned off. Thus, cell 2, now free from inhibition, can respond to excitation from cell 4 and to its own postinhibitory rebound by producing a burst of action potentials. These action potentials in cell 2 would silence cell 1 through inhibition. But this silence of cell 1 now releases cell 3 from inhibition. Cell 3 would thus give a burst of action potentials. This entire sequence, then, would consist of a burst of action potentials in cell 1, then in cell 2, then cell 3. The cycle would continue in the same sequence 1-2-3-1-2-3- . . . .

To summarize, then, Figure 9 shows one model based upon delayed reciprocal excitation, two models based upon reciprocal inhibition, and one model based upon cyclic inhibition. In the next several sections of this chapter, we will consider the complex central neuronal circuit for leech swimming, with these theoretical circuits as our guide.

In this discussion of theoretical modeling, we simply examined visually the sequence of events expected of each of our paper-and-pencil models. A more powerful approach, commonly employed in research on neuronal modeling, is to verify the details of a model's activity pattern by means of either electronic or computer techniques. For instance, one can represent a single neuron of a model by an electronic component called a NEUROMIME (Lewis, 1968). A neuromime produces an output consisting of voltage impulses that resemble action potentials. These impulses are produced whenever the neuromime receives as an input a sufficiently high rate of small positive voltage signals that resemble summating EPSPs. The stimulating effect of these model EPSPs in producing output impulses can be counteracted by the simultaneous input to the neuromime of small negative voltage signals, resembling IPSPs. Each output action potential of a neuromime can be made to generate a single EPSP, or an IPSP, in another neuromime to which it is connected. Thus, by connecting a group of neuromimes in a pattern simulating a particular configuration of neural connections, one can determine the temporal pattern of impulses, or action potentials, that each neuron so connected would produce. Each neuromime can be independently manipulated by the experimenter to vary the sizes and time courses of the EPSPs and IPSPs, as well as the threshold voltage for producing impulses.

Model circuits with as many as eight neuromimes, representing eight different neurons, have been tested in this way (Friesen and Stent, 1977). In fact, the activity patterns of each model shown in Figure 9 have been studied in detail using neuromimes or computers (Wilson, 1966; Perkel and Mulloney, 1974; Dagan *et al.*, 1975; Friesen and Stent, 1977). For instance, as Figure 9E shows, the impulses and IPSPs of each of three neuromimes interconnected according to the

cyclic inhibition model of Figure 9D reveal the expected triphasic rhythm. Moreover, by varying the amount of maintained excitatory input to each of the three neuromimes from a fourth neuromime (cell 4 in Figure 9D), additional useful information was obtained; the added excitation led to shorter cycle periods, while the phase of any one cell with respect to any other remained nearly constant. These details could not have been revealed simply by using paper-and-pencil models; rather, the use of neuromimes or a computer was essential.

MOTOR NEURONS AND THEIR SYNAPTIC CONNECTIONS IN LEECH SWIMMING

We will return now to the analysis of the neural circuit for leech swimming. Which neurons of the ganglia of a leech constitute its central oscillator for swimming? The search was begun with the motor neurons that innervate the dorsal and ventral muscles of the body wall, whose rhythmic contractions cause the swimming movements. Of course, these motor neurons were expected to show rhythmically alternating bursts of action potentials, which give rise to the swimming contractions. But is the rhythm of these neurons produced by their *own* synaptic interconnections, or are these cells merely *following* rhythmic signals from some presynaptic neurons? The initial strategy for answering this question was to determine the network of synaptic connections among the motor neurons and to see whether this network had a configuration that appeared capable by itself of producing rhythmic activity.

The cell bodies of many leech motor neurons, as well as some other cells, can be seen through a dissecting microscope in the living, translucent ganglia. Most of the cell bodies have constant positions relative to each other, a condition that permits individual neuronal identification (Figure 10). When making intracellular recordings from neurons of a ganglion, the first task is to determine whether a given impaled cell is a motor neuron, and if so, what muscles it innervates and what effect it has on these muscles. Figure 11 shows simultaneous recordings made intracellularly from a central cell body, extracellulary from a peripheral nerve, and intracellularly from a fiber (that is, a cell) of a dorsal muscle. The recording was made from a leech, part of whose body was pinned in place, but the rest of which was free to make rhythmic swimming movements. The impaled neuron shows rhythmic depolarizations and bursts of action potentials. Each of these action potentials is followed after a brief, fixed interval by an action potential in the peripheral nerve and then by a depolarizing, junctional potential in the muscle fiber. This is a slow muscle (Chapter 8), which is known (from other experiments) to contract in response to small depolarizations such as these. Thus, the physiological evidence of Figure 11 suggests that the cell body impaled is that of a motor neuron. Some cells physiologically identified in this manner have also been stained intracellularly and show an axon exiting the ganglion through a peripheral nerve, a finding that supports the physiological identification as a motor neuron (Muller and McMahan, 1976). By making repeated recordings of this type, investigators have found the cell bodies of many motor neurons, and the muscular targets of these neurons have been identified (Ort *et al.*, 1974). These neurons include four sets of motor neurons of interest to this discussion: