7
Optimal Control

To find out whether *E. coli* really knows what it is doing, Ed Purcell and I thought hard about the theory of chemoreception—I was the straight man—and concluded that its cells can sense temporal gradients about as well as any other device of similar size could possibly do (Berg and Purcell, 1977). And then my students and I looked more closely at how changes in tumble probability actually depend on the concentrations of attractants or repellents.

Time Resolution

To do this, we needed to stimulate cells in a known way and record responses on a time scale smaller than 1 second. This is hard to do by adding chemicals and mixing. Also, the problem is complicated by the fact that the response is stochastic: the probability of tumbling changes, but intervals between tumbles remain exponentially distributed. So one needs lots of data.

In recent work (e.g., Jasuja et al., 1999), ultraviolet light is used to cleave a photosensitive molecule. One of the fragments released is a chemical attractant (e.g., the amino acid aspartate). This allows one to generate concentration jumps on the millisecond time scale. We chose, instead, to use iontophoretic pipettes, developed earlier by others to stimulate receptors at the neuromuscular junction. This allows one to generate pulses as well as jumps, but on a somewhat longer time scale. The limit is the time required for a small molecule to diffuse from the tip of the pipette to a cell a few micrometers away, about 20 msec. Our target was either a tethered cell, fixed to glass by a single flagellar filament, or a filamentous cell linked via a single flagellum to an inert marker, as shown in Fig. 7.1. The first setup was used to probe the chemotactic response at the level of a single flagellar motor (Block et al., 1982) and the second to learn how signals are transmitted

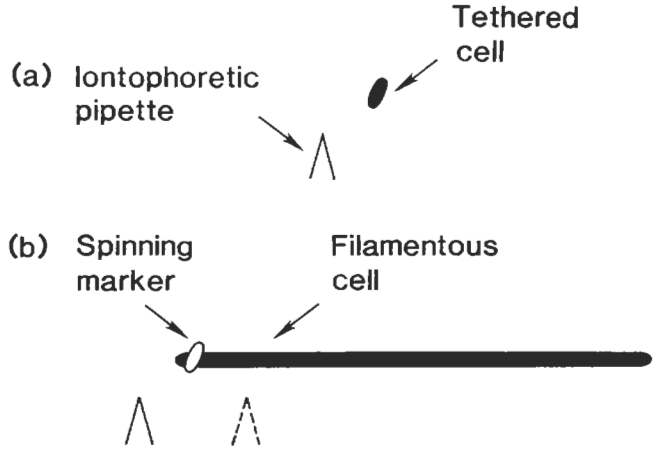

FIGURE 7.1. Stimulation with iontophoretic pipettes. (a) The tip of a pipette near a cell tethered to glass, such as the cell in Fig. 5.1. (b) The tip of a pipette either off one end or along the body of a filamentous cell linked by a single flagellum to an inert marker. This linkage was made via an abnormally long hook, called a polyhook, to polyhooks of a cell of normal size that had been treated with a chemical fixative (glutaraldehyde). Filamentous cells were obtained by growing normal cells in the presence of an antibiotic similar to penicillin (cephalexin) or by using mutants defective in septation. Such cells have a single cytoplasmic compartment.

intracellularly from the receptors to the flagella (Segall et al., 1985). The pipette was filled with a solution similar to the medium in which the cells were suspended containing, in addition, an attractant [e.g., aspartate (Fig. 3.1) or its nonmetabolizable analog α-methylaspartate]. At neutral pH, either amino acid has a net charge of -1, so it is expelled from the pipette when the electrical potential difference between the inside and the outside of the pipette is negative.

Impulse Responses

One can learn a great deal about a mechanical system by exciting it with a brief pulse. If, for example, you kick a sign post, it will wobble back and forth at a frequency that depends on its stiffness and mass and relax back to its initial quiescent state with a time

constant that depends on the rate at which mechanical energy is dissipated. You will get essentially the same result whether you wear a boot or a tennis shoe. If the system is linear, that is, if the way it responds to a new stimulus does not depend on how it is responding to past stimuli, the response to the impulse allows one to predict the response to any stimulus. Decompose the stimulus of interest into a sequence of impulsive stimuli of different magnitudes, weight the corresponding impulse responses by these magnitudes, and add them up.

The same is true for biochemical systems. If you kick the aspartate receptor by loading it up with ligand for a fraction of a second, the reactions set in motion by that change will play themselves out until the cell returns to its initial quiescent state. In practice, this takes about 4 seconds (Fig. 7.2). The impulse response for *E. coli* is biphasic. The probability that the motor spins counterclockwise rises from the baseline soon after the onset of the pulse, reaches

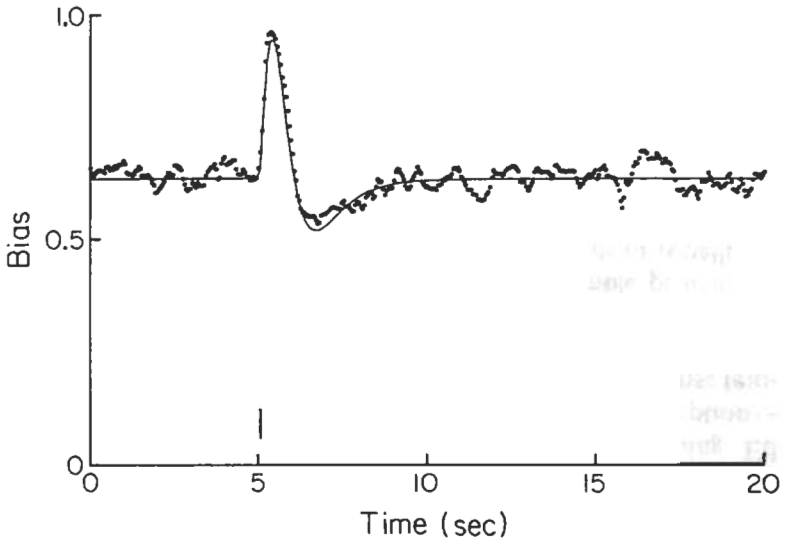

FIGURE 7.2. Impulse response of wild-type *E. coli* cells. The probability that a cell spins counterclockwise (the bias) is plotted as a function of time; the smooth curve is a fit to a sum of exponentials. Pulses of aspartate or α-methylaspartate were applied beginning at 5.06 seconds (vertical bar). The graph was constructed from 378 trials comprising 7566 flagellar reversals obtained with 17 cells. (From Segall et al., 1986, Fig. 1).

a maximum about 0.4 second later, crosses the baseline 1 second after the pulse, reaches a minimum at 1.5 second, and finally returns to the baseline at about 4 seconds. The areas of the positive and negative lobes of the response are equal (Segall et al., 1986).

From this analysis, it follows that wild-type cells exposed to stimuli in the physiological range (stimuli that do not saturate the response) make short-term temporal comparisons extending 4 seconds into the past. Stimuli received during the past second are given a positive weighting, and stimuli received during the 3 seconds before that are given a negative weighting, and the cells respond to the difference. The cells count molecules over a substantial time span—this improves the precision of the count—and then ask (within the time limit set by rotational brownian movement) whether the concentration is going up or down. This provides an optimum solution to the measurement problem, a solution that is matched to the constraints imposed by the physics discussed in Chapter 6. Simpler strategies, for example, one in which a cell sets its tumbling probability on the basis of measurements of the local concentration, do not work (Schnitzer et al., 1990).

The impulse response for a negative pulse (one that lowers the concentration of an attractant or raises the concentration of a repellent) is similar to the response shown in Fig. 7.2, except that it is of opposite sign (Block et al., 1982). Experiments with cells exposed to ramps of either sign indicate that thresholds for positive stimuli are small, while those for negative stimuli are large (Block et al., 1983). However, once these thresholds are crossed, equal increments in ramp rate generate equal increments in rotational bias, until the ramps are so steep that saturation occurs. Thus, if a cell has fully adapted, small negative stimuli are ignored. Evidently, this is why cells fail to respond when swimming down spatial gradients of attractants or when exposed to attractants destroyed enzymatically (see Chapter 4).

If one looks at these data in the frequency domain, one finds that the sensory system behaves as a bandpass filter, with its response maximally tuned to frequencies of a few tenths of a Hz, approximately equal to those encountered when cells move up and down in a spatial gradient, as shown in Fig. 7.3. Thus, *E. coli* has matched its sensory system to the signals that it needs to analyze.

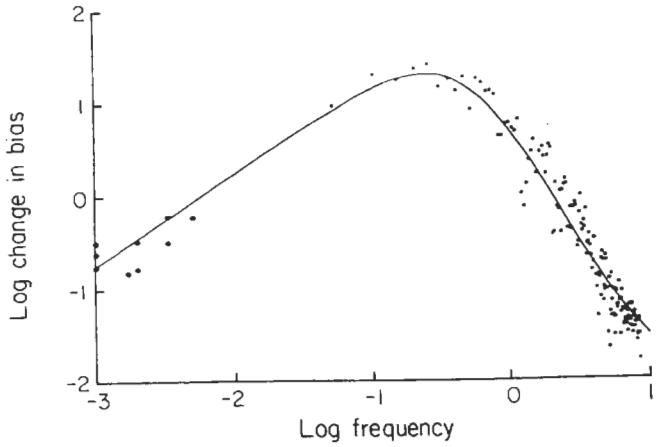

FIGURE 7.3. Impulse and ramp data viewed in the frequency domain. The change in bias resulting from variations in the concentration of an attractant (α-methylasparate) at different frequencies are plotted in a log-log scale. Data at the left were obtained from exponentiated sine-wave stimuli, while data at the right were obtained from the Fourier transform of the impulse response. The smooth line is a similar transform of the smooth curve of Fig. 7.2. (From Segall et al., 1986, Fig. 3B.)

Simulations of the Biased Random Walk

Given the impulse response, it is possible to simulate the biased random walk. Pick a run velocity and let the cell move by displacing it, say, every 0.01 second. Weight the recent and more distant past using the impulse response in the manner described earlier. If the output of this computation is negative, that is, if the concentration has been decreasing for some time, ignore the result and determine whether the cell should tumble by picking at random from an exponential distribution with a mean of 1 second. If the output of this computation is positive, determine whether the cell should tumble by picking at random from an exponential distribution with a larger mean (one with an exponent decreased in linear proportion to that output). If the cell is tumbling, determine whether it should run by picking from an exponential distribution with a mean of 0.1 second. In either case, if a new run is called for, pick the change in angle from the old to the new run at random from a distribution peaked in the forward direction (Berg

and Brown, 1972, Fig. 3). Finally, add the effects of brownian rotation by giving the cell a small kick in angle every iteration. When watching such tracks evolve on a computer screen, one gets the impression of a bloodhound following a scent. The cell sniffs about (with the bias close to the baseline), picks up the spoor, and then howls up the gradient. Eventually, rotational brownian motion carries it off the track, and it is forced to sniff about again. Most of the progress up the gradient appears to occur in long runs.

Intracellular Signaling

Experiments of the sort sketched in Fig. 7.1b were used to study the range of the intracellular signal that couples the receptors to the flagella (Segall et al., 1985). Stimuli delivered at one end of a filamentous cell did not affect the response at the other end. There was no evidence for long-range signaling, as would be expected, for example, were the receptors to signal the flagella by changing membrane potential. Motors near the pipette responded, whereas those far away did not. The response of a given motor decreased with distance, but it did so less sharply when the pipette was moved along the cell surface (to the right in Fig. 7.1b) than when it was moved out into the external medium (to the left in Fig. 7.1b). This implies that there is an internal signal, but that its range is short (only a few micrometers). The data could not be fit by models in which the receptor simply releases or binds a small molecule or in which a receptor-attractant complex diffuses to the flagellar motor. However, they could be fit by a model in which the signal is a ligand or a small protein that is activated by the receptor and inactivated as it diffuses through the cytoplasm. As we will see in Chapter 9, this molecule proved to be a small protein, CheY, which is active when phosphorylated and inactive when not.

References

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