CHEMOTAXIS AS A MODEL FOR SENSORY SYSTEMS

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Sensory systems in all biological species have certain similarities. Receptors are specifically designed to interact with certain stimuli and not with others. The signals from the stimulus are transmitted through a complex analyzing system to generate some type of behavioral response. The response in turn provides some survival value for the organism. The response of organisms to chemical stimuli is one such subclassification which is pervasive in living systems. Mammals are able to taste and smell a wide variety of compounds and are incapable of tasting or smelling others. Similarly insects show a specificity in their response to some compounds acting as pheromones and even bacteria respond to certain chemicals and not others.

The highly differentiated neurosystem of a higher species, the moderately intricate systems of insects and the presumable simple systems of bacteria represent an enormous span in complexity. Yet a fundamental act of faith of biochemists since the days of Buchner has been that the study of 'lower' forms of life can provide biochemical insight into the most complex species. Whether this will be true in sensory systems remains to be seen. In this article I shall discuss some current thinking on bacterial chemotaxis and some potential relations to higher sensory systems.

The Phenomenon and the sensing problem

Bacterial chemotaxis was discovered in the 1880's by Engelman [1] and Pfeffer [2] who showed that a capillary containing attractant caused bacteria in the external solution to migrate into the capillary in numbers far greater than mere chance. Pfeffer used meat extracts to indicate that the change in concentration of attractant was the key factor in the rate of movement of the bacteria. F. Dahlquist, and P. Lovely [3] designed an apparatus which could quantitate the average migration velocity of bacterial populations and found that the migration velocity in *Salmonella* was roughly proportional to the steepness of the gradient, not to the absolute concentration of the attractant. Similiar findings have been obtained by Adler and coworkers using a quantitative procedure based on the attraction of *E. coli* into a capillary tube [4]. These studies established that bacteria were capable of sensing ratios of concentrations over short distances, i.e. $(dc/c)dx$.

Biased random walk

Observations under the microscope show that *Salmonella* and *E. coli* appear to travel in roughly straight lines and then turn abruptly. Sometimes they appear to be tumbling head over tails for a brief period and then swim off in a new direction at random. These qualitative observations were converted to a quantitative analysis by Howard Berg and his associates [5,6] utilizing a tracking apparatus devised to follow bacteria in three-dimensional space. The findings were that: i) the length of the runs (distance between tumbles or 'twiddles') was Poissonian; ii) the angle of the turns averaged 62° with a Poissonian distribution; iii) the length of an average run was increased by travelling up a gradient of attractant; iv)

the length of an average run going down a gradient of attractant was about the same as normal. The results supported the assumption that bacteria migrate by a 'biased random walk' [6a], but they contradicted the widely held view that the migration followed a 'shock reaction,' i.e., increased tumbling on going down a gradient [6a]. In fact the movement depended on decreased tumbling on going up the attractant gradient, a conclusion also indicated by the experiments reported below.

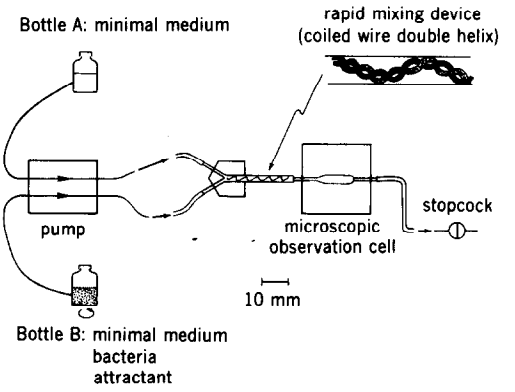

Fig. 1. Schematic illustration of temporal gradient apparatus. Attractant concentrations are: (*i*) Bottle B, C_i (≥ 0) (*ii*) bottle A, C_i' (>, = or < C_i) (*iii*) observation cell (as a result of stream mixing) C_f (>, =, or < C_i). Bacteria experience $C_i \rightarrow C_f$, and thus can be subjected to positive, zero, or negative temporal gradients as desired. Gradient is given by $\Delta C/\Delta t$, where $\Delta C = C_f - C_i$ and Δt is mixing time.

Temporal sensing of gradients

The next question is 'How do the bacteria detect the gradient to alter the tumbling frequency?' In the detection of ratios bacteria are like higher species, e.g., man detects ratios of light intensities, but the small size of the bacterium posed a special problem. An average *E. coli* or *Salmonela* cell is only 2 μm in length. If spatial sensing, i.e., signals from receptors at the 'heads' and 'tails' of bacteria, is used to detect the gradients of the type described above, an analytical accuracy of 1 part in 10^4 is required. Statistical fluctuations are greater than that. This problem was recognized many years ago by Haldane among others, and some type of time-averaging seemed logical. A time dependent mechanism has been found in phototactic organisms [7]. It remained to determine whether spatial or temporal mechanisms apply to chemotactic bacteria.

To distinguish between spatial sensing and temporal sensing mechanisms, R. Macnab devised a temporal gradient apparatus shown in fig. 1 [8]. This apparatus uses a rapid mixing device to plunge the bacteria from one uniform concentration of attractant (C_i) into a final uniform concentration (C_f) where they are observed microscopically. Control experiments established that neither the mixing apparatus nor the absolute concentration of attractant affected the motility pattern, e.g., they swim similarly in uniform distributions of 0.5×10^{-5} M, or 2×10^{-5} M attractant. If the bacteria use instantaneous spatial sensors at their 'heads' and 'tails' all they 'see' after mixing is a uniform distribution of attractant. They should thus swim normally. If on the other hand, bacteria operate by a temporal sensing mechanism and their 'memory' span is greater than the mixing time of the instrument, they should show an altered motility pattern. The latter behaviour was observed.

Bacteria subjected to a decrease in concentration of attractant swam erratically and tumbled more frequently than normal immediately after mixing; those subjected to an increase in concentration swam more smoothly and tumbled far less frequently. In both cases the initial altered pattern gradually 'relaxed' back to normal swimming. This is exactly the pattern to be expected if the bacteria compared their past environment (C_i) with their present environment (C_f) by some type of temporal sensing or 'memory' mechanism. As the memory fades over time, they return to normal. This mechanism implies that bacteria detect attractant gradients by making temporal comparisons as they travel through space. In travelling up a gradient the comparison ($C_f > C_i$) tells them to tumble less frequently so they travel further. If they travel down a spatial gradient of attractant ($C_f < C_i$) they tumble more often and travel less far. The net effect is for movement towards higher concentrations of attractant by use of the 'memory' device.

Gradients of repellent caused a similar behavior with a precisely inverse pattern, i.e., the bacteria

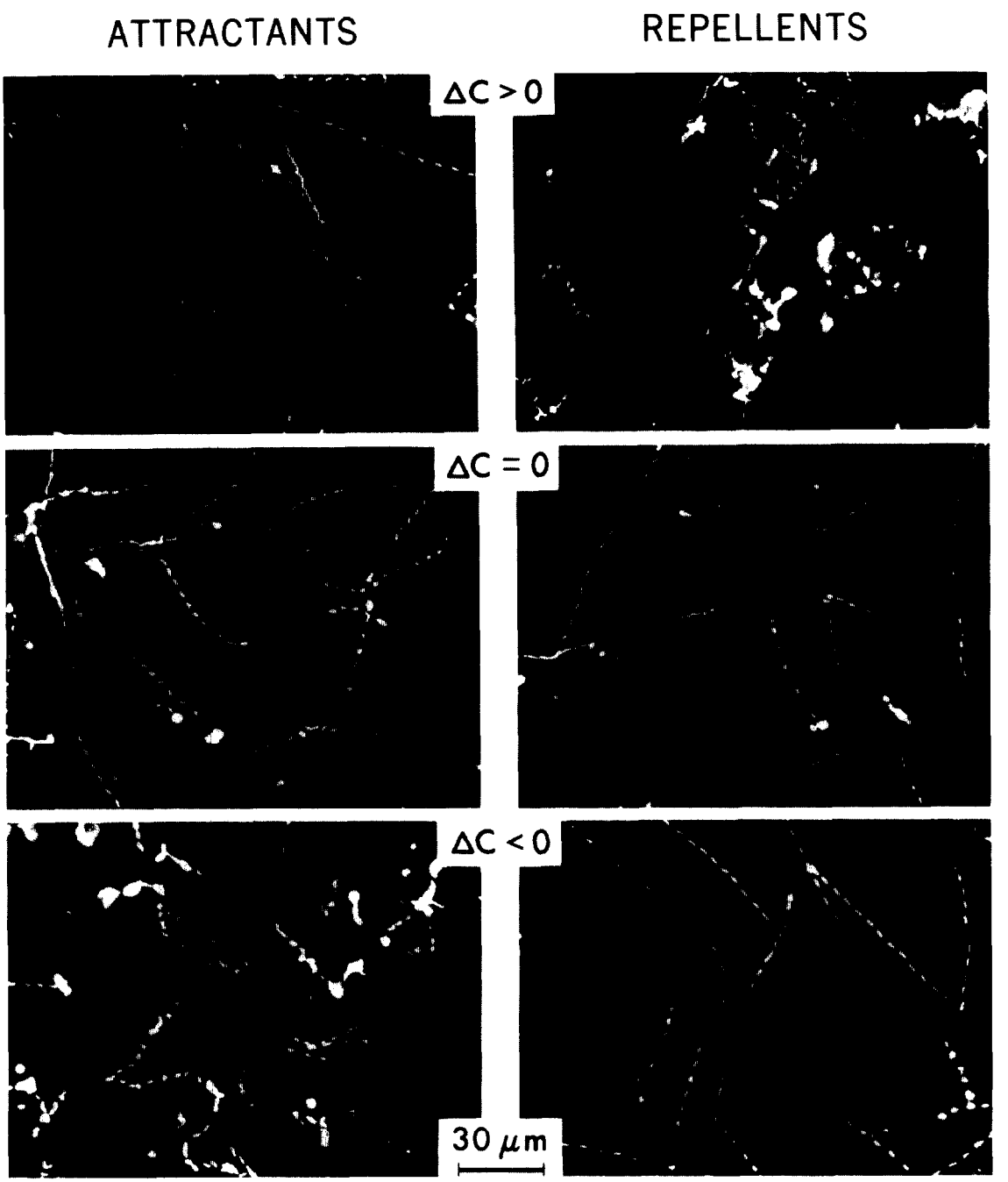

Fig. 2. Motility tracks of *Salmonella* in the interval 2–7 sec after subjection to temporal gradients of attractants and repellents. Photographs taken in darkfield with stroboscopic illumination operating at 5 pulses per second. Left hand side: Top: serine increase from 0 to 7.5×10^{-5} M. Middle: no change in serine (control). Bottom: serine decrease from 10^{-3} M to 2.4×10^{-4} M. Right hand side: Top: phenol increase from zero to 7.5×10^{-4} M. Middle: control, no change in concentration. Bottom: phenol decrease from 3×10^{-4} to 7.5×10^{-5} M. The 'smooth' response to favorable gradients (top left, bottom right) and the tumbling response to unfavorable gradients (top right, bottom left) relax back to the normal control patterns as time goes on.

tumbled more frequently on going to higher concentrations ($C_f > C_i$) and tumbled less frequently on going to lower concentrations ($C_f < C_i$) [9]. Some photographs of these experiments are shown in fig. 2.

These results fitted excellently with the tracking studies with one possible exception: our studies indicated a time dependent effect in both negative and positive gradients. Berg's tracking studies showed a quantitative alteration from the normal pattern in the positive direction only. Actually there is no conflict since the temporal studies showed an assymetry in the response also. Large positive concentration jumps give long relaxation times in the range of many minutes for steep gradients ($C_f >> C_i$) whereas large negative jumps ($C_f << C_i$) gave much shorter responses of the order of seconds. Thus the temporal studies are consistent with a more pronounced effect in the positive direction. Apparently the motility pattern is altered by a temporal sensing device which can enhance or suppress tumbling, but suppression of tumbling during movement in favorable directions is more significant quantitatively than the enhanced tumbling in unfavorable directions.

Receptor proteins

The temporal comparisons presumably require receptors to detect the external stimulus. Evidence for specific receptors in bacterial chemotaxis were first obtained by Julius Adler and his coworkers on *E. coli* with metabolic studies, competition and genetic techniques [10]. Mutants were found which failed to respond to a known attractant, e.g., galactose, while still responding to other attractants, e.g., serine. Competition and inhibition experiments extended these studies so that today specific receptors for attractants galactose, glucose, ribose, aspartate, and serine and for repellents phenol, isoleucine, indole and acetate are known [11,12]. Moreover, Hazelbauer and Adler [13] showed that a mutant lacking the galactose binding protein of galactose transport [14,15] failed to be attracted to galactose whereas the wild type was attracted.

In studies on *Salmonella,* R. Aksamit in our laboratory has isolated and purified a ribose binding protein as the receptor for ribose chemotaxis [16]. The protein has been isolated in pure form ($\sim 10^4$ molecules per bacteria) from wild type bacteria and has been shown to be completely absent in a mutant which fails to chemotax to ribose [17]. The mutant is normal in response to other classes of attractants and can be reverted to wild type in which chemotaxis to ribose and the protein are restored. The specificity of the binding properties of purified protein agree precisely with the specificity of the chemotactic response. The actual binding equilibrium constants for ribose (10^{-7} M) and allose, a structural analog of ribose, (10^{-4} M) agree quantitatively with the optima for the chemotactic response. Thus, a correlation between the properties of the purified receptor protein and the behavioral response of a living species has been obtained in this sensory system.

The transmission system

Once the sensory signal has been induced by the receptor it must be analyzed and transmitted to the flagella. One clue in regard to the processing system has been obtained from genetic studies. Armstrong et al. [18] isolated three different types of general chemotactic mutants, i.e. mutants which did not chemotax to any attractant, and S. Parkinson has found a fourth in *E. coli* which blocks chemotaxis to some attractants [19]. D. Aswad has recently found four similar mutants in *Salmonella* [20]. There are undoubtedly other gene products involved in chemotaxis, but these results indicate that there are four proteins that are essential for transmission of information from receptor to flagella.

A second clue to the general chemotactic process was obtained when it was found by Adler and Dahl that a methionine auxotroph of *E. coli* did not give the chemotactic response to normal attractants and was found to swim more smoothly than normal wild types [21]. Study of this mutant on a special tracking apparatus [22] allowed D. Aswad to conclude that a methionine auxotroph of *Salmonella* did not tumble at all, at least in the period of time in which an individual bacteria could be tracked, e.g., 5 min [23]. Further studies by Armstrong [24], and by Aswad [25] have indicated that methionine may be acting through one of its metabolic products, *S*-adenosylmethionine. Careful study of the methionine effect has indicated that *S*-adenosylmethionine is not

the compound which has direct control of tumbling, but that it is required for the maintenance of the system, i.e., a certain minimum level of methionine or a product is needed for the organism to have the capacity to tumble [23].

A third clue has been obtained by R. Macnab's finding that high intensity light can generate tumbling by the bacteria [26]. Furthermore, this tumble generating phenomenon has the action spectrum of a flavin. Pursuing this effect further B. Taylor and B. Howlett [27] have concluded that perturbation of the electron flow of the electron transport system can generate or suppress the tumbling phenomenon. This does not mean that the electron transport machinery is necessary for chemotaxis, only that its perturbation can alter the level of some compounds which can generate tumbling.

A further clue has been obtained by the finding that repellents work through the same mechanism as attractants. Superimposing a gradient of repellent on a gradient of attractant gives a predictable effect provided they are treated as an algebraic sum [9]. Thus a gradient of attractant which by itself would cause bacteria to swim upwards and an equally strong gradient of repellent which by itself would cause bacteria to swim downwards will essentially nullify each other if superimposed. Reversing the gradient of repellent will cause reinforcement. This suggests that the effects of repellents and attractants are integrated as an algebraic sum possibly through a common chemical compound. Such an integration of inhibitory and excitatory effects is undoubtedly occurring in neurons also.

These studies do not yet reveal a clear biochemical pathway, but they, like studies on higher systems, reveal a machinery for processing information from receptors and delivering it to the next element involved in the behavioral response. Simple hypotheses have been developed to explain the time-dependent memory process in biochemical terms, e.g. the time-dependent conformation changes and diffusion barriers in membranes [8]. At the moment the data do not allow a precise choice between alternatives each of which is too simplistic for a final mechanism. Nevertheless a beginning has been made.

Useful memory

From the preceding discussion it appears that bacterial chemotaxis operates from receptors through some biochemical signalling system. If so the length of the bacterial memory can be controlled by the kinetic parameters controlling the rise and fall of a chemical compound which controls the tumbling process. Rapid return of this compound to normal levels would suggest a short memory. Slow return would give a long memory time. It is worthwhile to ask 'what length memory would be optimal for the bacteria?'

Since the bacteria are moving through space with occasional tumbling followed by movement in a new direction, an integrating apparatus operating over very long time intervals would be useless or valueless. The signal generated by a positive gradient to make the bacteria swim for longer intervals between tumbles might be transmitted at a time when the bacterium had actually changed direction and was swimming down the gradient. (cf. fig. 3). A short memory would obviate this difficulty, but would not allow the bacterium to move long distances with respect to its body length before the memory had lapsed. This

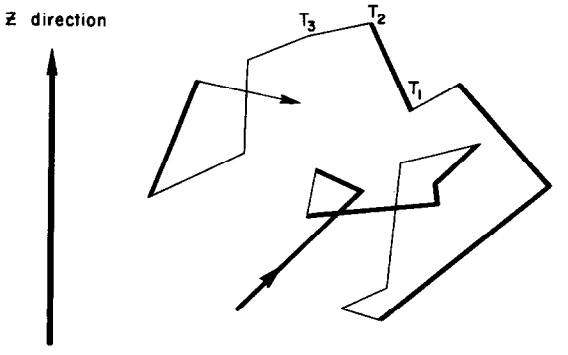

Fig. 3. Idealized trajectory of a swimming bacterium. It consists of straight line segments (runs) interrupted by discontinuous changes in direction (turns or tumbles). The practical difficulty of defining tumbles unambiguously is exemplified by the events shown. T_1 and T_2 clearly qualify as tumbles, but T_3 represents a small deflection which at some stage will require arbitrary definition. The concept of *persistence* in the z direction is illustrated by drawing +z segments with heavy lines. Persistence numbers from the beginning of the path shown are 2, 3, 2, etc. in the +z direction and 1, 4, 1, etc. in the −z direction.

would create exactly the same analytical problem as a spatial comparison, so that a very short memory time would have little advantage from an analytical viewpoint over a spatial separation of receptors over a body length.

To analyze this problem the question of persistence time was developed theoretically and experimentally. Persistence time is the length of time the bacterium spends going up a gradient (or down a gradient) before making a turn which changes the sign of its direction. A tracking device was developed in our laboratory [2] with several different features. In it a stable gradient was made which varied concentration in the z direction without any changes in the x and y directions. Thus a one dimensional gradient was provided, vastly simplifying the mathematical analysis. After analyzing 2400 run lengths of *Salmonella* in such gradients a persistence time between 1 and 10 sec was obtained [28]. The bacterium travels at a velocity of approximately 30 μm/sec and a 10 sec persistence time would for example allow it to travel $(30 \times 10)/2 \approx 150$ body lengths during an average persistence time. In other words its useful memory allows it to extend its body length between 15–150-fold and thereby reduce its analytical problem. Occasionally the memory signal from a positive feedback will be delivered when the bacterium is going in an unfavorable direction and vice versa, but statistically the correlation will be good. This time response seems to be a reasonable compromise between too long and too short a memory. The same demands may well be made on neuron firing times.

Relationship of sensory responses of bacteria to higher organisms

To state with certainty that there is an identity between a bacterial sensing system and a higher neural system is obviously premature, but there are significant points of similarity. The bacterial receptor specificities, e.g., positive for ribose, negative for deoxyribose and glucose, correspond to those of higher organisms and are characteristic of the specificity we identify with protein molecules. A processing system exists which analyzes the initial stimulus and then transmits the signal to the motor apparatus. In the case of the bacterial system the stimulus involves the rate of change of attractant (or repellent) concentration and the motor response involves the inhibition (or generation) of tumbling. The regulation of the tumbling frequency allows the bacterium to select its movement in favorable directions for its nutritional or survival needs. Thus a formal analogy exists between the sensing to motility system of bacteria and those of higher species which can also control movement in response to stimuli.

The bacterium has a 'useful memory' span in the sense that the decay time of the biochemical system is optimized for the behavioral response for which it is designed. Higher neurons have similar selection devices based on useful forgetfulness as well as useful memory. Our brain is not designed to remember everything we see, but rather to filter trivia from needed long term memory. Bacteria also apparently have the ability to integrate several types of sensory responses. Repellents and attractants can be analyzed so that an algebraic discrimination is achieved. It is too much to claim bacteria show wisdom, but they are capable of evaluating a net effect between inhibitory and excitatory events, a requirement also of higher organisms.

The detailed biochemistry of bacterial sensory mechanisms is only at the stages of infancy. Yet again the analogies suggest themselves. Some chemical compound or compounds appear to be involved in the transmission of the signal from receptors to flagells. Like a membrane depolarization the flagella signal must be somewhat abrupt; yet it can be enhanced or depressed by biochemical manipulation. Only time will tell how complete are the biochemical analogies to the higher system. Certainly the design of an analytical system which can detect better than a change of one part in 100 and amplify it to alter flagella function in such a purposeful manner will have relevance to many biochemical systems.

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