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Mechanism of Behavior

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Abstract

Behavior of bacteria. Behavior of fruit flies. Decision-making.

Keywords: Organisms; Eukaryote *Drosophila*; Bacteria; Chemotaxis in Bacteria; Biology; Metabolism; Genetics; Neurobiology; Psychology; Biochemistry; Neuroscience

Behavior of Bacteria

History

Anton van Leeuwenhoek, in Holland. improved the microscope and then discovered bacteria and their motility, His life: October 24, 1632-August 26, 1723.

The German botanist Theodor Engelmann in Utrecht, Holland, and later at the University of Berlin, discovered in 1881 that bacteria are attracted to oxygen, and he discovered that bacteria are attracted to light. He used this bacterial oxygen response to demonstrate that green plants, including algae, give off oxygen during photosynthesis. For details see Engelmann [1], Gerhart Drews [2] & Howard Berg [3] part 1 (Figure 1).

Wilhelm Pfeffer, in (Figure 2) Tuebingen, Germany, discovered in [4] that bacteria are attracted and repelled by various chemicals, this was named "chemotaxis" by him. He studied this in 1884, [5,6] by placing into a suspension of motile bacteria a tube containing complex attractant (the leg of a fly or a piece of meat or meat extract or potato sap or tryptone or peptone) or repellent (inorganic acids or inorganic salts or alcohol). With attractants he observed that the bacteria accumulated around the mouth of the tube and after a while also inside, and with repellents he observed that the bacteria moved away. Chemotaxis was studied by Pfeffer in a mainly qualitative and subjective way since rather few defined chemicals were known by then. This is reviewed by Jacqes Loeb [7], in "Forced Movements, Tropisms, and Animal Conduct". Roderick Clayton at Cal Tech studied phototaxis in photosynthetic bacteria from [8].

Our studies on the behavior of bacteria

In order for us to identify stimuli in *E. coli*, it was first necessary to determine the conditions needed for optimal motility and the conditions needed for chemotaxis. The behavior of bacteria was now studied in the following way, see Adler, "A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis", [9]. That paperdescribes an objective method for measuring chemotaxis in bacteria. The procedure was made quantitative by measuring how many bacteria accumulate inside a capillary tube containing the stimulus by plating the contents of the capillary tube and then counting colonies the next day. At the start of this work (1960) I got *E. coli* from Hatch Echols upstairs. It just happened to be a strain that required L-methionine for growth. That's how I discovered that methionine plays a central role in bacterial chemotaxis! (Otherwise, it might have taken many years to find out that methionine is needed for bacterial chemotaxis.) (Figures 3-10).

Figure 11 shows the key figure from the very first summary of the mechanism of bacterial chemotaxis [10]. It formed the basis for all the subsequent reports by many other scientists who study the behavior of bacteria.

The biochemical mechanism for chemotaxis in bacteria is presented here (Figure 12):

As indicated in that Figure, bacteria sense attractants and repellents by means of sensory chemotaxis proteins, called methyl-accepting chemotaxis proteins (MCP). These send the sensed information onward by means of excitation, which then informs the flagella to respond. Then MCP by its methylation brings about adaptation to stop the excitation process (Figure 13).

MCP can be methylated or demethylated to effect adaptation or deadaptation. This involves use of L-methionine (as S-adenosyl-L-methionine) for methylation, or production of methanol for demethylation (Figures 14 & 15).

Figure 1: Theodore Wilhelm Engelmann in Utrecht, Holland, 1872. His life: November 14, 1843-May 20, 1909.

Figure 2: Wilhelm Pfeffer, in Tuebingen, Germany. His life: March 9, 1845-January 31, 1920. See below about Pfeffer's study of bacterial chemotaxis.

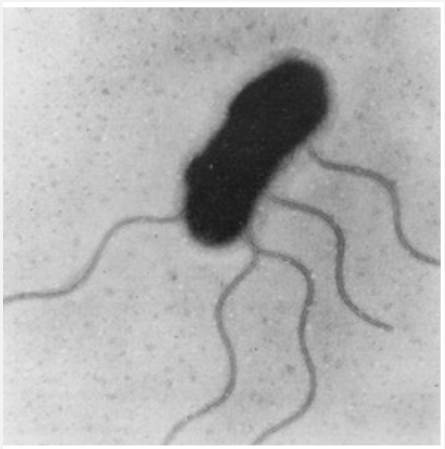

Figure 3: Electron micrograph of *Escherichia coli*, the bacterium used in this research. *E. coli* is about 1.5 micrometers long. Note the flagella, which serve for motility. This photograph was taken by Dr. Alan P. MacKenzie [20].

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Figure 4: Another electron micrograph of *E. coli* bacterium. Flagella are two to four times the length of the cell. They are used for motility. Part of fimbraea, also called pili, are shown on the right end. They are used by bacteria for colonizing hosts. About them [25].

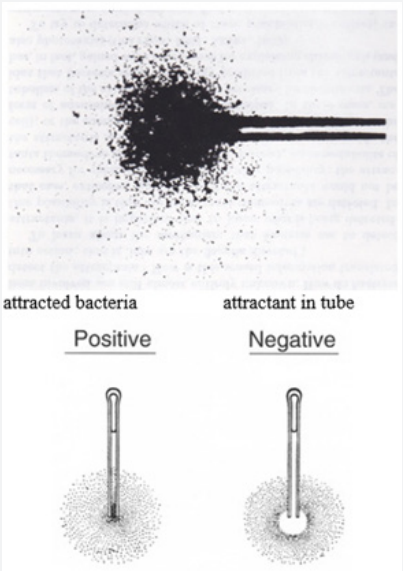

Figure 5: Positive chemotaxis by bacteria moving into a capillary tube containing attractant and negative chemotaxis by bacteria moving away from a capillary tube containing repellent. Bottom half by Wilhem Pfeffer, 1884.

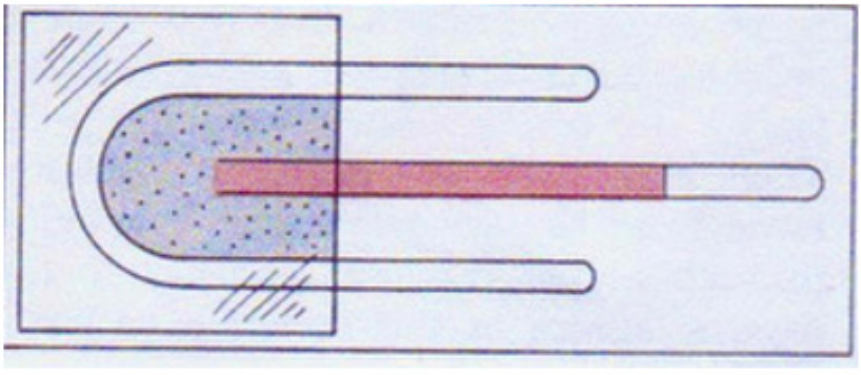

Figure 6: The apparatus used in the research here to study behavior of bacteria. On a glass slide was placed a U-tube, then a bacterial suspension shown in blue was added, then a capillary tube of attractant or repellent was inserted (in red). This was covered with a cover slip. It was then incubated usually for 1 hour, then the number of bacteria in the capillary tube was measured by plating and counting colonies the next day. Adler, Scientific American [24].

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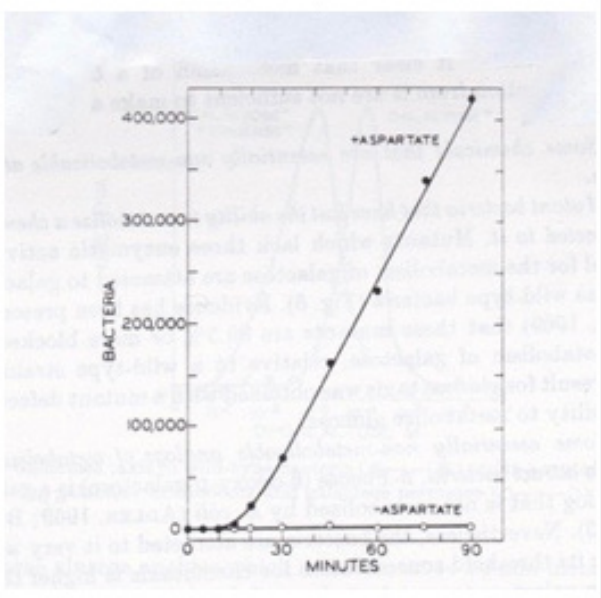

Figure 7: The assay of positive chemotaxis: attraction of *E. coli* bacteria to chemical in a capillary tube, then plating the contents of the capillary tube and counting the number of colonies the next day. In this case the attractant was 10-3M L-aspartate [17].

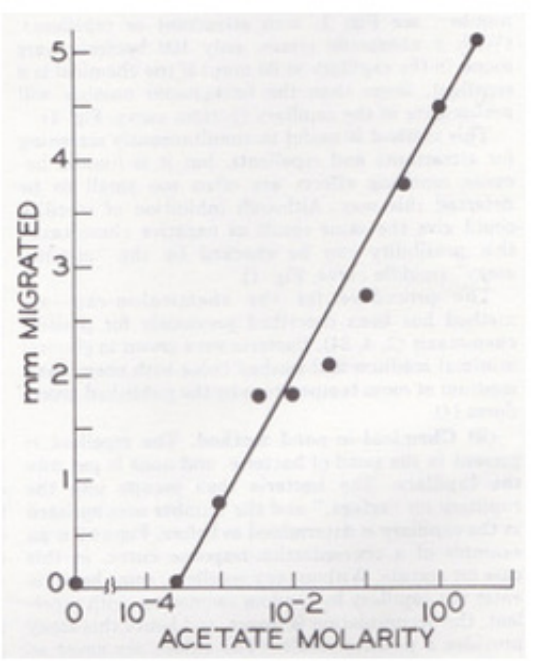

Figure 8: The assay of negative chemotaxis: repulsion of *E. coli* bacteria by a repellent. The bacteria migrate away from repellent in the capillary tube, an amount that depends on the concentration of repellent in the capillary tube. Mutants lacking negative chemotaxis remain in the tube. In this case the repellent was acetate [22].

Of the various stimuli, some are attractive, and others are repulsive. What the organism does depends on the intensity of each stimulus. When there is more attractant than repellent, the organism will be attracted and when there is more repellent than attractant, the organism will be repelled. We were interested in learning the mechanism that an organism uses to decide what to do.

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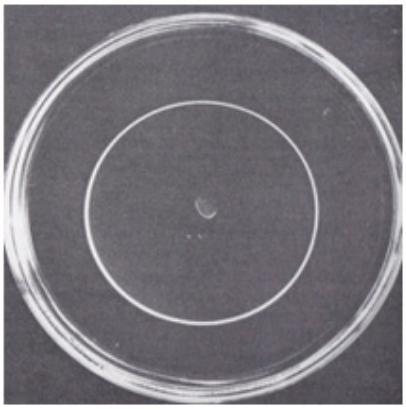

Figure 9: Positive chemotaxis studied as a visible ring of *E. coli* bacteria pursuing attractant. The bacteria are deposited in the center, they consume the attractant there, then they multiply and follow the gradient they create; thus, they make an expanding ring at the edge of the attractant-rich zone. The furthest point of consumption is where the ring is just now. Mutants lacking positive chemotaxis can be found in the center. The attractant in this case was D-galactose [17,23].

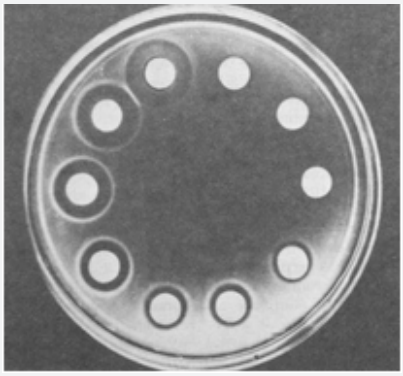

Figure 10: Negative chemotaxis studied as a visible repulsion. Repellent is placed at increasing concentrations in plugs around the plate, from zero to 3M. Higher concentrations show repulsion by a ring of *E. coli* bacteria. Mutants lacking negative chemotaxis remain in the plug [22].

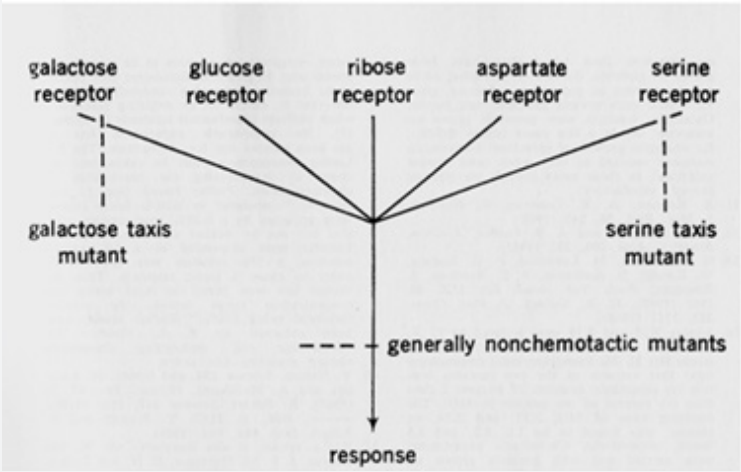

Figure 11: Mechanism of bacterial behavior. Specific mutants fail in a specific response owing to a receptor defect; for example, the galactose taxis mutant is deficient in the galactose receptor and the serine taxis mutant is deficient in the serine receptor. By contrast, generally nonchemotaxis mutants fail in all the responses owing to a defect in the final common pathway (Adler [21], Chemoreceptors in Bacteria, Science; & Adler [13] My Life with Nature, Annual Review of Biochemistry).

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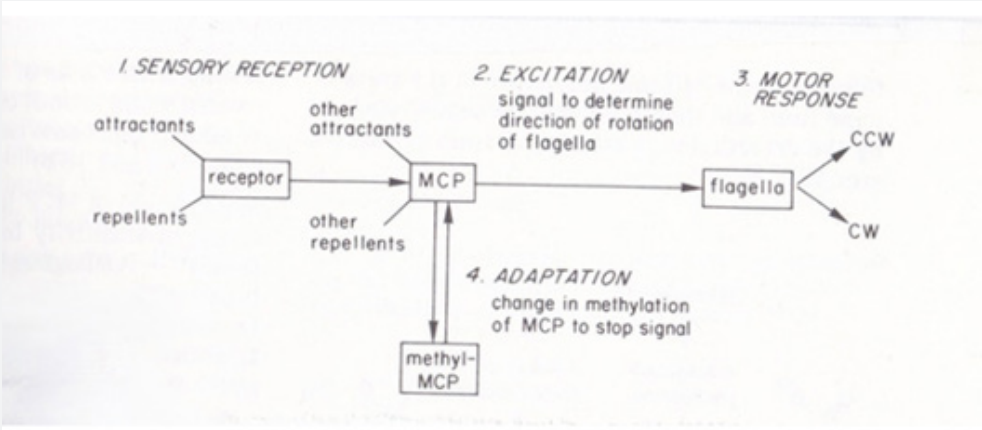

Figure 12: The mechanism of bacterial chemotaxis. From Adler, "How Motile Bacteria are Attracted and Repelled by Chemicals: An Approach to Neurobiology". Lecture held on the occasion of the receipt of the Otto-Warburg-Medaille [18].

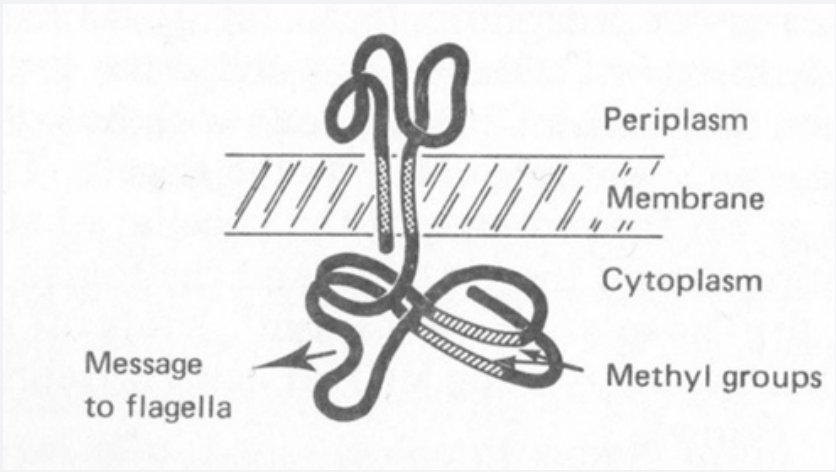

Figure 13: **Methyl groups in MCP. Andrew Russo and Daniel Koshland, "Separation of Signal Transduction and Adaptation". Science [19].**

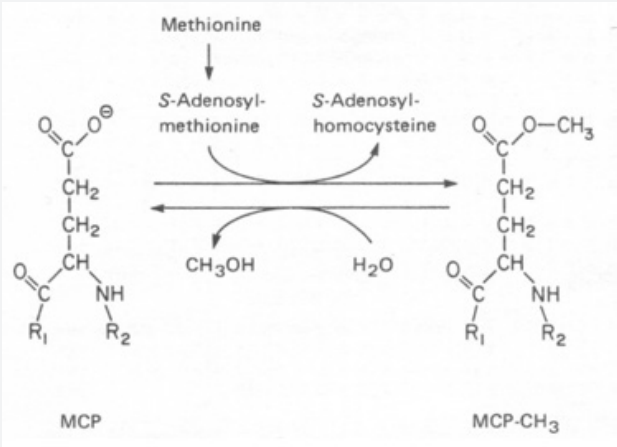

Figure 14: Methylation and demethylation of glutamate residues of MCP. From Adler, "How Motile Bacteria are Attracted and Repelled by Chemicals: An Approach to Neurobiology". Lecture held on the occasion of the receipt of the Otto-Warburg-Medaille [18].

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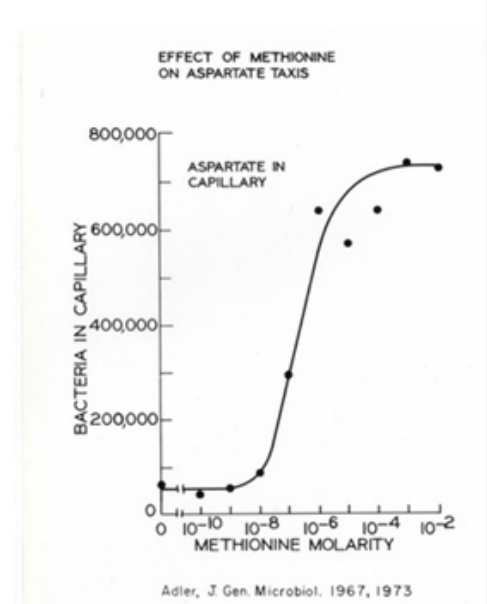

Figure 15: The effect of L-methionine concentration on methylation of MCP.

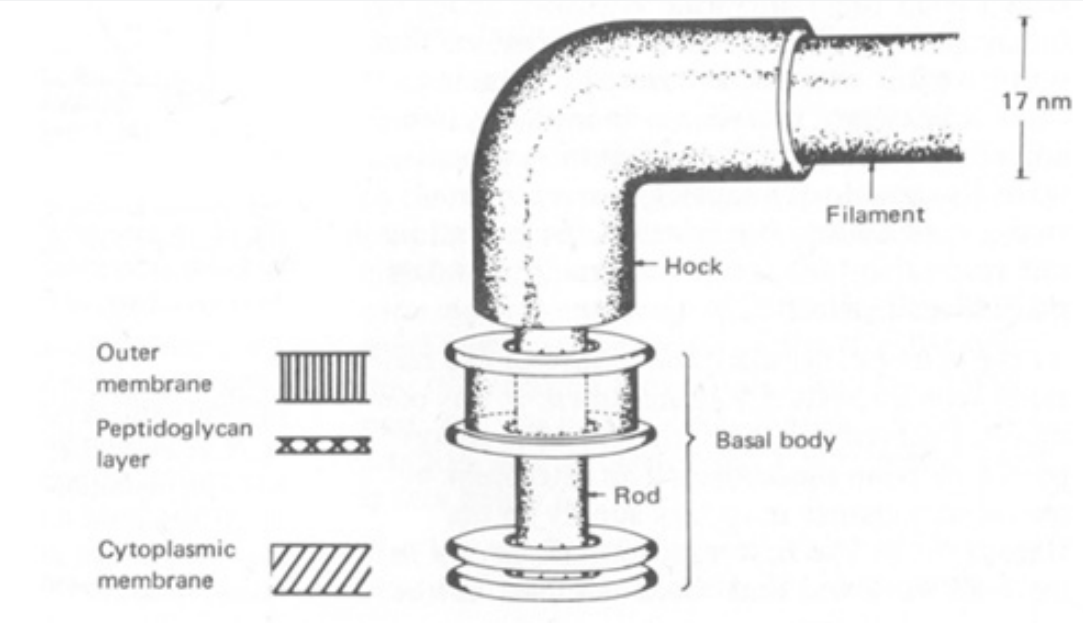

Figure 16: The flagellum and its base in *E. coli,* DePamphilis & Adler, [12]; see copy of the original electron microscope picture on page 55 of Adler "My Life with Nature" [13].

A powerful way to study behavior is to isolate and describe mutants that don't have normal behavior owing to defects in genes that control behavior. This is neurogenetics, also called behavioral genetics, see a review by Maxwell Cowan, Donald Harte & Eric Kandel [11]. That approach was first used in fruit flies (Margaret Bastock, Seymour Benzer, William Bak, Martin Heisenberg, and David Suzuki). It has been carried out in bacteria (Julius Adler, Sandy Parkinson, Gerald Hazelbauer, Joseph Falke, Mel Simon, Ann Stock, Jeff Stock and others), in nematodes (Sydney Brenner and others), in zebra fish (John Kuwada and others), and in mice

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(Richard Sidman, Pasko Ragik, Jacqueline Crawly and others). *E. coli* bacteria are attracted and repelled by a variety of different stimuli and for many of them mutants have been obtained. The response to stimuli takes place in the flagellar mechanism (Figure 16). The complex structure at the base of the flagella of *E. coli* was first described by Melvin DePamphilis & Adler [12]. Since 1971 other scientists have successfully produced a very much more complicated view of the bacterial flagellum as demonstrated in a figure by Fabienne Chevance and Kelly Hughes, 2008. See that figure on page 56 of Adler "My Life with Nature" [13]. Also see figures by Shin-Ichi Aizawa et al. [14] & David Blair [15].

Videos about the behavior of bacteria

Videos of the behavior of bacteria have been described by Adler with music by David Adler: go to "Google, The Behavior of Bacteria – You Tube, 7.5 minutes of behavior", to see and hear them. Here is a summary of a few of those videos (Figure 17). For an excellent review of behavior of bacteria see Howard Berg's "Marvels of Bacterial Behavior" in Proceedings of the American Philosophical Society 2006.

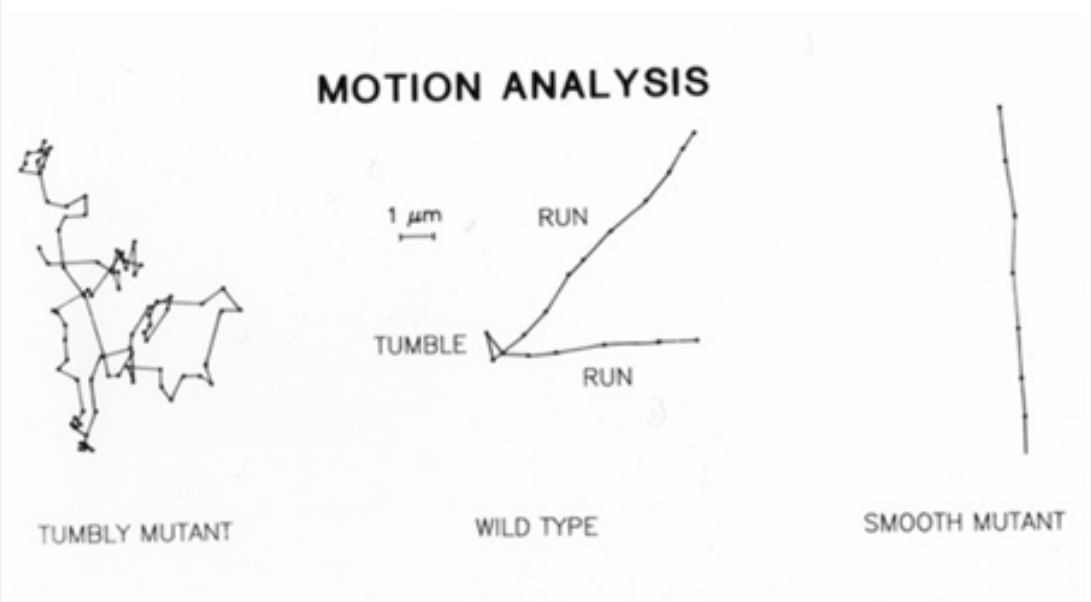

Figure 17: Examples of videos of bacterial behavior.

Behavior of Fruit Flies

Next is described the behavior of *Drosophila*, the fruit fly, which is a eukaryote (bacteria are prokaryotes).

The assay used here to study the behavior of fruit flies

The behavior of *Drosophila* fruit flies was studied here by use of the following method (Figure 18).

The mechanism of behavior of fruit flies

Fruit flies are attracted by many stimuli and repelled by many stimuli. Next, as an example, is the use of this assay to show flies attracted to light. Mutants that fail to be attracted to light have been obtained and the result for one of these is shown here (Figure 19).

Decision Making

"Decision-making has all the secrets of everything: who we are, what we do, how we navigate the world." "How do I decide? The brain with David Eagleman" [16].

Decision making in bacteria

What will a motile bacterium do if confronted simultaneously with a gradient of attractant and a gradient of repellent? In this "conflict" situation a bacterium must "decide" whether to pursue the attractant or flee from the repellent. Already in [5.6] Pfeffer reportedthat the relative strength of the two gradients determines whether attraction or repulsion will occur. He determined this microscopically by observing the entrance of bacteria into a tube containing both attractant (meat extract) and repellent (inorganic acids) at various concentrations.

We have confirmed and extended Pfeffer's report. First, *Escherichia coli* were exposed to a capillary tube containing only attractant or only repellent, and then after an hour the number of bacteria that had entered the capillary tube was determined by plating its contents. For attractant we used L-aspartate, a chemical that is beneficial because it can be readily metabolized, and for

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repellent we used L-valine, a chemical that harms by inhibiting the growth of *E. coli*. Then after that, attractant and repellent were used together. At a low concentration (10-6M) of L-aspartate, bacteria fail to be attracted to L-aspartate when there is a high concentration (10-1M) of L-valine (Figure 20) [17-25].

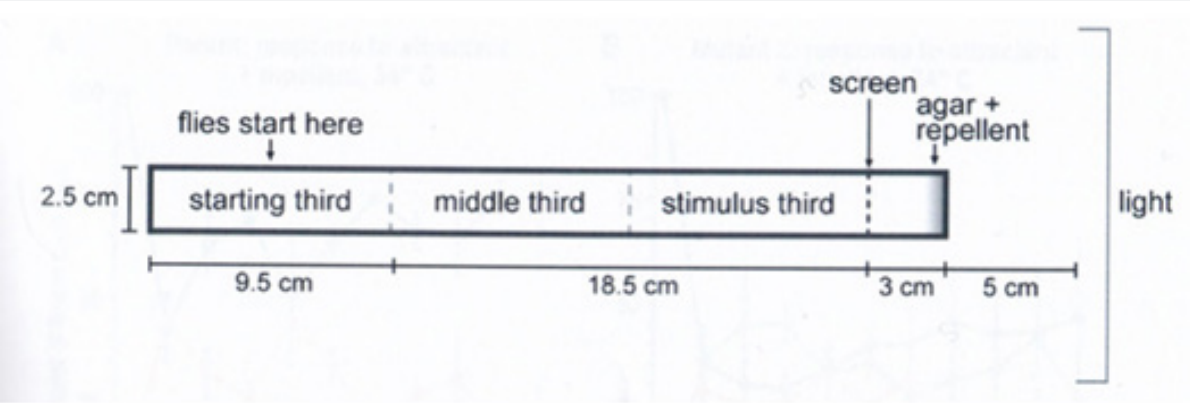

Figure 18: How to measure response to attractant or repellent. Attractant or repellent is used separately, or here in this case both attractant (light) and repellent (eugenol) are used together.

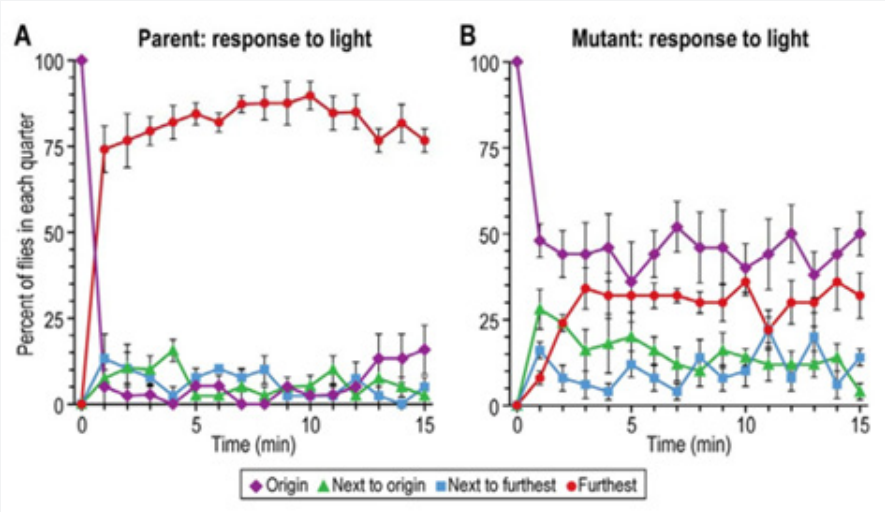

Figure 19: Attraction of fruit flies to light and failure of attraction to light in a mutant.

The conclusion of this: motile bacteria presented simultaneously with both attractant and repellent respond to whichever is present in the more effective concentration. Apparently, bacteria have a processing mechanism that compares opposing signals from the chemoreceptors, sums these signals up, and then communicates this to the flagella.

Decision-Making and its mutants in fruit flies

Here is a report on decisions made by fruit flies. Mutants of this are then studied for getting at the mechanism involved in decision-making. Fruit flies were placed at one end of a tube and attractant (light) plus overpowering repellent (eugenol) were placed at the other end. The result is described here (Figure 21). That Figure shows that the parent flies stay put because they are repelled by more repellent than attractant coming at them. But mutants in decision-making can't tell this, so the mutants move randomly over the whole tube. In this way decision-making mutants were obtained.

Another way for measuring flies making a decision: At right, attractant light plus attractant temperature, at left flies start out with repulsive chemical (benzaldehyde) and repulsive high temperature; the flies move to the attractive end. But mutants in decision-making can't tell this, so the mutants move randomly over the whole tube. In this way additional decision-making mutants were obtained (Figure 22). This mutant 2 is missing RNA

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splicing and RNA helicase, so those activities would be a part of this mechanism (Figure 23).

Finally, the big question now is: How does the decision-making mechanism function? The mutants in decision making will help to answer this. See also Adler [26] "A search for The Boss: The thing inside each organism that is in charge", including Figure 4 on the role of The Boss, in Anatomy Physiology & Biochemistry International Journal; and see (2023 in press) "Every organism has its own The Boss. Behavior of bacteria compared to behavior of eukaryotes" in Anatomy Physiology & Biochemistry International Journal.

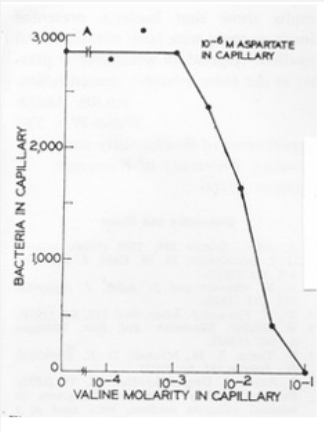

Figure 20: Effect of the repellent L-valine on the accumulation of *E. coli* in a capillary tube containing also an attractant, 10-6M L-aspartate. (Figure 1A) [17].

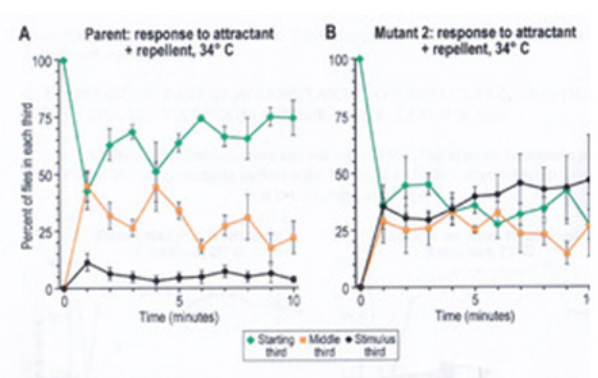

Figure 21: Response to attractant plus overpowering repellent. Attractant plus overpowering repellent were placed at one end of the tube and bacteria were placed at the other end. A. Parent. B. One of the mutants obtained.

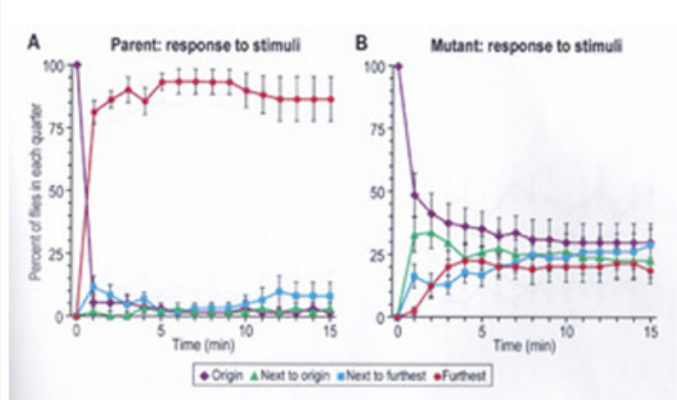

Figure 22: Response to stimuli. A. parent, response to the four stimuli. B. mutant, response to the four stimuli. At one end were flies with repellent benzaldehyde plus repellent high temperature, at other end were attractive light plus attractive temperature.

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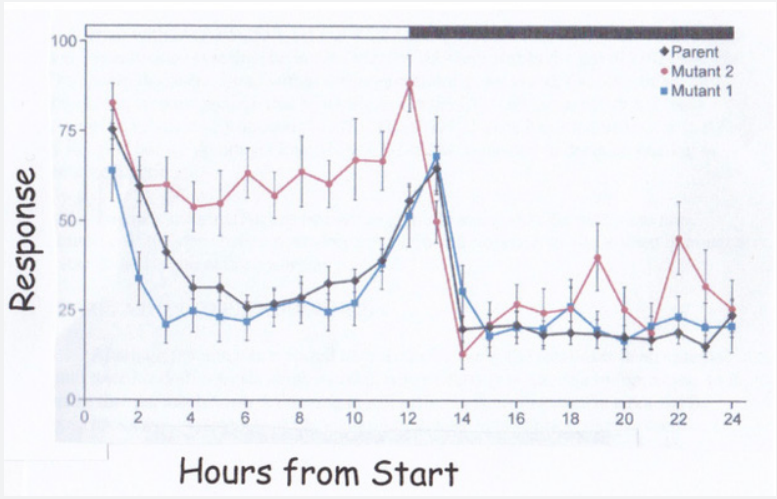

Figure 23: Parent response (black) in sleep-wake; mutant responses (red and blue) in sleep-wake. The left half is` light, the right half is dark. Unlike the parent Mutant 2 showed high activity throughout the day and Mutant 1 was less active than the parent at the start of the day. The procedure and parental response of C. Pfeiffenberger et al. were employed.

Acknowledgement

I am highly thankful to the National Institutes of Health for support of the research carried out in my laboratory over many years. I am most grateful to The Camille and Henry Dreyfus Foundation for six years of grants in support of my undergraduate research program on *Drosophila* fruit flies. Lar Vang has been an associate research specialist here. I am sorry to report his death, due to pancreatic cancer, I am most sad. Robert Kreber, a research specialist in Barry Ganetzky's laboratory, has helped us greatly in studies of the genetics of our mutants. I thank Barry Ganetzky for teaching me about fruit flies. I am very thankful to Laura Vanderploeg for the beautiful artwork.

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