# Integration of Photosensory Signals in *Halobacterium halobium*

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Stimulation of *Halobacterium halobium* through its sensory photosystems, PS 370 and PS 565, leads either to a prolonged or to a shortened interval between two reversals of the swimming direction of the cell, the attractant or repellent response. Stimuli are integrated to yield the same response regardless through which photosystem they are given. Simultaneously elicited attractant and repellent signals cancel each other at any time during a reversal interval, even in the period of refractoriness shortly after a reversal, when the cell is insensitive to repellent stimuli. Successively applied stimuli are less completely integrated. The net response depends on the moment of stimulation during the interval, on the sequence of stimuli, and on the delay between them. Integration of successively applied effective stimuli (after refractoriness) is to a great extent explained in terms of a cellular oscillator (A. Schimz and E. Hildebrand, Nature [London] 317:641–643, 1985) which is changed in opposite directions by attractant and repellent signals. Some conclusions on the shape of the oscillator after its disturbance by a stimulus can be made. Integration of signals during refractoriness leads us to postulate an additional step before the oscillator in the sensory pathway. Cancelling of simultaneous opposite signals is thought to proceed at this integrator. It also takes part in the integration of successively evoked signals. At this step signals rapidly decline within 10 ms, and the total life time (at least of repellent signals) does not exceed 1.2 s.

*Halobacterium halobium* has polar flagella and can swim in either direction along its long axis. Owing to a change of flagellar rotation from clockwise to counterclockwise or vice versa (1), the cells reverse their swimming direction about every 10 s. Successive reversal intervals are, on average, equally long, i.e., the cells have no preferred swimming direction (6, 9).

The organism has two retinal-dependent sensory photosystems of different spectral sensitivity, PS 370 and PS 565, through which its behavior can be controlled (2, 3, 12). It is still an open question whether two distinct proteins (7) or two states of a single molecule in a photochemical cycle (14) act as the receptors. Other retinal pigments and carotenoids contribute to photosensing (2, 11, 16). Light stimuli alter the interval between two reversals of the swimming direction. A light increase in the yellow-green range delays the next reversal and is therefore regarded as an attractant stimulus, while a light increase in the blue-UV range advances the next reversal and is regarded as a repellent stimulus. A light decrease in the yellow-green range, on the other hand, i.e., removal of attractant light, acts as a repellent stimulus, while a light decrease in the blue-UV range, i.e., removal of repellent light, acts as an attractant stimulus (3, 4, 15). Consequently, we define a stimulus-induced increase of the interval length as an attractant response and the decrease as a repellent response.

The frequency distribution of interval lengths is log normal (6). The responsiveness of the cells depends on the moment during an interval at which the stimulus is applied, i.e., the magnitude of the response increases and decreases in a sawtooth-shaped manner (9). The periodicity of spontaneous reversals can be entrained by rhythmic light pulses within a certain frequency range (10). These are criteria which indicate that the rhythmic swimming behavior is based on a cellular oscillator. Light stimuli are thought to change transiently the level of a regulatory substance and thereby shift

the phase of the oscillator (9). The cells respond only to a change in light intensity and quickly adapt to constant light conditions. Reversible methylation of membrane proteins has been found in halobacteria (8) and is thought to be involved in this kind of adaptation, as in eubacteria. A light stimulus alters the length of one interval only. This immediate return to the prestimulus behavioral pattern is probably caused by specific properties of the oscillator (9).

It has been reported that *H. halobium* can generally integrate stimuli applied to both photosystems (15). This paper deals with the integration of attractant and repellent stimuli applied simultaneously or successively at different moments during a reversal interval. The results indicate that at least two steps in the sensory pathway are involved in signal integration. Furthermore, they help to answer the question of how sensory signals change the shape of the oscillator.

## MATERIALS AND METHODS

*H. halobium* R1 was used for experiments. Culture conditions were as described previously (5). To study photosensory activity, single cells from the stationary growth phase were observed in a suspension through a microscope connected to a video-system. Stimulating light of 565- and 370-nm wavelengths was provided by a 200-W Hg lamp and a 150-W Xe lamp, respectively, each connected to a monochromator (M4QIII, Zeiss; and high intensity grating monochromator, vis. grating, Bausch & Lomb, Inc., Rochester, N.Y.). The light beams were combined by a 50% half mirror from quartz and directed through the objective of the microscope by means of an incident light illuminator. Stimuli were applied at different times after a spontaneous reversal had been observed. Delay times between a reversal and the onset of the stimulus and between two successive stimuli were selected with an electronic timer which triggers the opening or closing of electronically driven shutters. The stimulus was either a stepwise increase of light from zero to

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TABLE 1. Integration of simultaneously applied attractant and repellent stimuli through PS 370 and PS 565 of *H. halobium*[a]

| Stimulus | | Response interval length (mean ± SEM [s]) (*n*) |
|---|---|---|
| Kind and wavelength (nm) | Onset after reversal (s) | |
| None | | 11.5 ± 1.0 (60) |
| Attractant | | |
| 370 | 2 | 16.8 ± 1.3 (20) |
| 565 | 0.2 | 14.6 ± 0.8 (20) |
| 565 | 2 | 17.2 ± 1.6 (20) |
| Attractant + attractant | | |
| 370 + 370 | 2 | 23.6 ± 0.9 (100) |
| 565 + 565 | 2 | 21.5 ± 1.3 (100) |
| 370 + 565 | 2 | 23.0 ± 1.0 (100) |
| Repellent | | |
| 370 | 0.2 | 12.3 ± 1.5 (20) |
| 370 | 2 | 8.1 ± 0.9 (20) |
| 565 | 2 | 8.7 ± 0.9 (20) |
| Repellent + repellent | | |
| 370 + 370 | 2 | 6.6 ± 0.6 (20) |
| 565 + 565 | 2 | 6.7 ± 0.4 (40) |
| 370 + 565 | 2 | 6.6 ± 0.6 (20) |
| Attractant + repellent | | |
| 565 (a) + 370 (r) | 0.2 | 12.0 ± 1.2 (20) |
| | 2 | 10.6 ± 1.7 (20) |
| | 4 | 12.0 ± 1.6 (20) |
| 565 (r) + 370 (a) | 0.2 | 11.2 ± 0.9 (20) |
| | 2 | 11.9 ± 1.2 (20) |

[a] Stimulation of PS 370; Stepwise increase (repellent) or decrease (attractant) of light; PS 565; stepwise increase (attractant) or decrease (repellent) of light. The interval length between the reversal before and after stimulation was taken as a measure for the magnitude of the response.

a given fluence rate or a decrease to zero. Stimuli were adjusted to yield equal behavioral responses at 370 and 565 nm. The fluence rate at 370 nm was in the range of $3 \times 10^{12}$ to $1 \times 10^{13}$ photons $mm^{-2}$ $s^{-1}$; at 565 nm there were $1.3 \times 10^{14}$ to $5.2 \times 10^{14}$ photons $mm^{-2}$ $s^{-1}$. Background light for observation was white light >350 nm (250 $\mu W$ $mm^{-2}$). Temperature was 22 to 24°C. The interval length between the reversal before and after stimulation was taken as a measure for the magnitude of the response.

## RESULTS

**Simultaneously applied stimuli.** To study quantitatively the ability of *H. halobium* to integrate different kinds of light stimuli, we adjusted the stimulus strength for both photosystems, PS 370 and PS 565, so as to yield equal behavioral responses. This was necessary because cells are about 40 times as sensitive at 370 nm as at 565 nm under the conditions used throughout (3).

An attractant stimulus at 565 nm (light increase) and an equally effective attractant stimulus at 370 nm (light decrease) were integrated by the cell to yield the same response as stimulation of either photosystem with the twofold fluence rate, i.e., the interval length was increased to the same extent (Table 1). Similarly, simultaneous stimulation by a repellent stimulus at 565 nm (light decrease) and a

repellent stimulus at 370 nm (light increase) shortened the interval to the same extent as a repellent stimulus of the twofold fluence rate at either wavelength. Repellent and attractant stimuli cancelled each other (Table 1). The same results were obtained with the mutant strain Flx3 (13) which lacks bacteriorhodopsin and halorhodopsin (data not shown).

While attractant stimuli are effective immediately after a reversal, the cell is absolutely refractory to repellent stimuli for about 500 ms after a reversal (9). (This time refers to the first observable movement of the cell in the new direction. The actual refractory period with respect to the flagellar reversal which, according to our method could not be observed yet, will be longer by a constant amount.) We found that opposite stimuli cancel each other at any time, even during the period of refractoriness to repellents (Table 1). This shows that repellent stimuli are also recognized during that period.

**Successively applied stimuli. (i) Repellent stimuli.** A first repellent stimulus was applied 2 s after a spontaneous reversal, when repellent stimuli are fully effective (9). A second repellent stimulus of the same strength was given with increasing delay as long as the cell had not responded to the first stimulus (Fig. 1A). The resulting response was stronger than upon the first stimulus alone. No significant difference was found whether stimuli of the same wavelength were given or a stimulus at 565 nm was followed by a stimulus at 370 nm.

The shape of the curve is to a great extent determined by the increasing delay which is included in the measured interval. The response latency, i.e., the time between the onset of the second stimulus and the following reversal, which in this case better illustrates the responsiveness, shows that the strength of the response changed with increasing delay between the stimuli. It first decreased and, having reached a minimum at a delay of 0.5 to 1.0 s, again increased.

In another series the first repellent stimulus was given during the period of absolute refractoriness (Fig. 1B). Up to a delay of 1.2 s the resulting interval was shorter than that upon the second stimulus alone. At longer delays the first stimulus did not influence the response any more. Both curves first decreased as a result of increasing sensitivity during relative refractoriness, and thereafter the interval length increased owing to the increasing delay time, as in Fig. 1A.

**(ii) Attractant stimuli.** The first stimulus was given 2 s after a reversal, when its effect is maximal (9), and the second one was given with increasing delay. The resulting interval was longer than upon the first stimulus alone. The response was the same whether stimuli of the same wavelength were applied or a stimulus at 565 nm was followed by a stimulus at 370 nm (Fig. 2A). The magnitude of the response changed with increasing delay between the stimuli and reached a maximum when the stimuli were 2 s apart.

The time to reach the maximum in responsiveness slightly depended on the time at which the first stimulus was applied (Fig. 2B). It was longest when the first stimulus was given 2 s after a reversal and was shortened to 1.5 or 1 s when the first stimulus was given earlier or later. In all cases the overall response to the two stimuli became maximally 3-4s longer than to the respective first stimulus alone.

**(iii) Attractant and repellent stimuli.** When an attractant and a repellent stimulus were given with a delay of only 20 ms they no longer canceled each other (Fig. 3A). The magnitude of the response depended on the time after a

reversal at which the stimuli were delivered and on the sequence of the two stimuli. During the period of absolute refractoriness to repellent stimuli, the response was an attractant response but weaker than with the attractant stimulus alone (Fig. 3A). When the two stimuli were applied after the period of absolute refractoriness, the net response became a repellent response, which, however, was always weaker than with the repellent stimulus alone. The influence of the attractant stimulus was larger when it preceded than when it followed the repellent stimulus. Similar results were obtained when the delay between the two stimuli was raised to 500 ms (Fig. 3B). Whenever the repellent stimulus came after the refractory period, the net response was a repellent response.

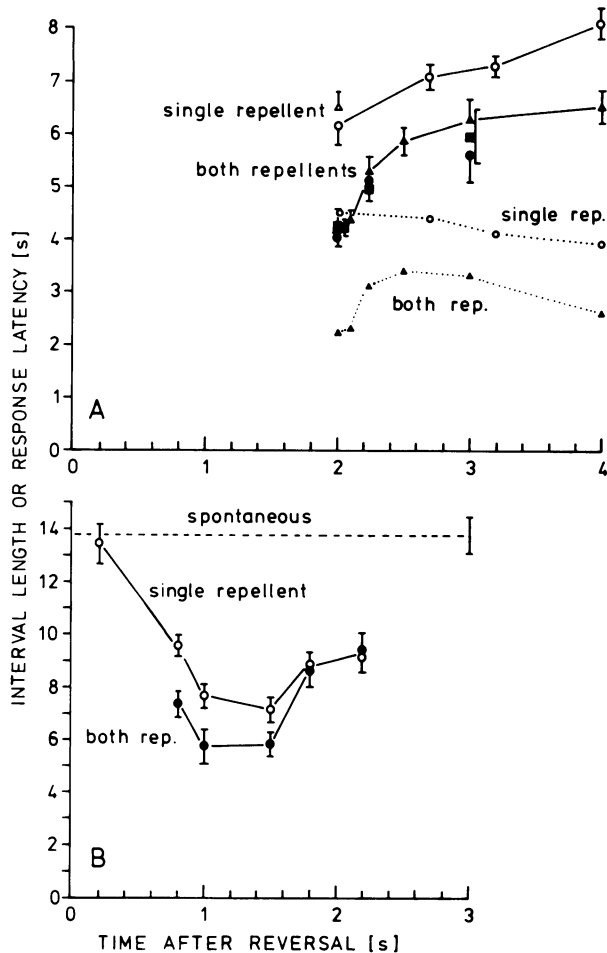To test whether the sequence of like stimuli also has an



FIG. 1. Integration of two successively applied repellent stimuli. (A) The first stimulus was applied 2 s after a spontaneous reversal had been observed, and the second stimulus followed with increasing delay times. Solid lines show interval length. Symbols: ●, both stimuli were an increase of light intensity at 370 nm; ▲-▲, both stimuli were a decrease of light at 565 nm; ■, first stimulus at 565 nm, second stimulus at 370 nm. Each point is the mean ± standard error of the mean (SEM) of 20 measurements. Dotted lines show response latency (time between stimulus and subsequent reversal) calculated from the solid curves. Symbol: ▲-▲, both stimuli, latency with respect to the second. (B) The first stimulus was applied 200 ms after a reversal, and the second stimulus followed after various delay times. Both stimuli were at 370 nm. Each point is the mean ± SEM of 20 measurements.
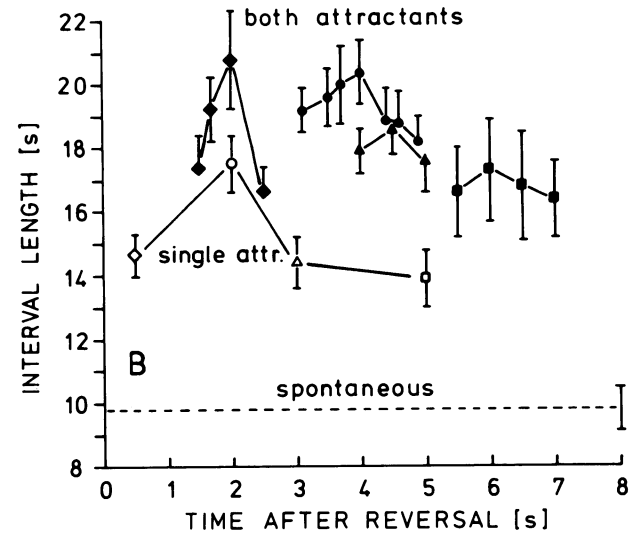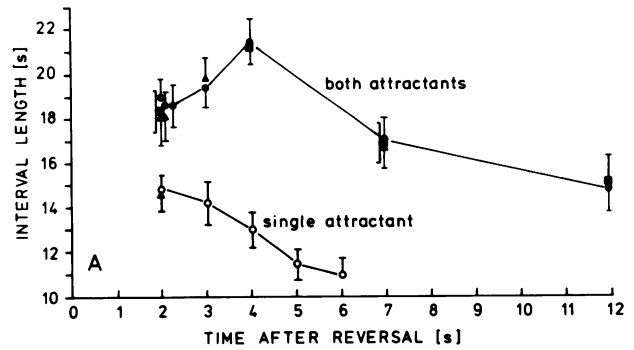


FIG. 2. Integration of two successively applied attractant stimuli. (A) The first stimulus was given 2 s after a reversal. The second stimulus was delivered after various delay times. Symbols indicate wavelengths as described in legend to Fig. 1A. Each point is the mean ± SEM of 20 measurements. (B) The first stimulus was applied 0.5 s (◆), 2 s (●), 3 s (▲), or 5 s (■) after a reversal; the second stimulus followed with increasing delay times. Stimuli were light decrease at 370 nm.

influence on the response, we applied two successive repellent stimuli of different fluence rate. It appeared that the stimulus given first had the stronger influence (Table 2).

In the following series of experiments the first stimulus was given at a fixed time after a reversal, and the second stimulus followed with increasing delay.

*Sequence: repellent-attractant.* A repellent stimulus given during the period of absolute refractoriness weakened the response to the following attractant stimulus up to a delay of about 1.2 s between the two stimuli (Fig. 4A). At longer delay times the interval length became the same as with the attractant stimulus alone delivered at the corresponding time.

When the repellent stimulus was delivered 550 ms after a reversal, i.e., during the period of relative refractoriness, it weakened the effect of the following attractant stimulus during the whole interval. The curve fairly well paralleled that obtained with the attractant stimulus alone (Fig. 4B).

When the repellent stimulus was given 2 s after a reversal, when the cells are fully responsive to repellent stimuli, the net response was always a repellent response, although weaker than with the repellent stimulus alone (Fig. 4C). In
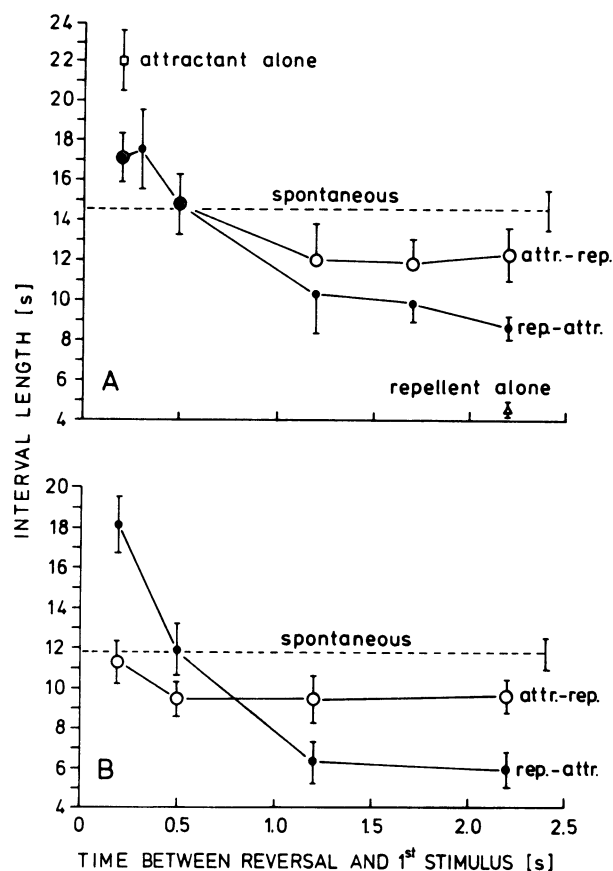
FIG. 3. Integration of successively applied attractant and repellent stimuli at different moments during a reversal interval. Stimuli were light increase or decrease at 370 nm, applied at given times after a spontaneous reversal. The delay between the stimuli was constant (in panel A, 20 ms; in panel B, 500 ms). Each point is the mean ± SEM of 20 measurements.

this case the interval length first decreased, went through a small maximum at a delay of 1.2 s, and then further decreased.

*Sequence: attractant-repellent.* An attractant stimulus

TABLE 2. Integration of two like stimuli of different intensities which were successively applied in alternating sequence

| 1st stimulus[a] | 2nd stimulus[a] | Response interval length (mean ± SEM [s]) ($n = 20$) |
|---|---|---|
| None | None | 11.4 ± 0.9 |
| Repellent (1× I) | None | 7.5 ± 0.6 |
| Repellent (2× I) | None | 5.6 ± 0.5 |
| Repellent (4× I) | None | 5.0 ± 0.3 |
| Repellent (1× I) | Repellent (2× I) | 5.1 ± 0.4 |
| Repellent (2× I) | Repellent (1× I) | 5.0 ± 0.4 |
| Repellent (1× I) | Repellent (4× I) | 4.7 ± 0.2 |
| Repellent (4× I) | Repellent (1× I) | 4.1 ± 0.2 |
| Repellent (1× I) | Repellent (8× I) | 4.0 ± 0.1 |
| Repellent (8× I) | Repellent (1× I) | 3.4 ± 0.1 |
| Attractant (1× I) | Attractant (4× I) | 21.1 ± 1.1 |
| Attractant (4× I) | Attractant (1× I) | 24.8 ± 1.6 |

[a] Stimuli, Change of fluence rate at 370 nm; I = 3 × $10^{12}$ photons mm$^{-2}$ s$^{-1}$. The first stimulus was applied 1.5 s after a spontaneous reversal had been observed, and the second stimulus was applied 20 ms later.

was given 200 ms after a reversal. As long as the following repellent stimulus fell into the period of absolute refractoriness, the net response was a weak attractant response as expected (Fig. 5A). With increasing delay, while the cell recovered from refractoriness (cf. Fig. 1B), the influence of the repellent stimulus became dominating. When the delay times became longer than the spontaneous interval, the response, of course, became an attractant response again, but it always remained weaker than with the attractant stimulus alone.

The same results were obtained whether the attractant and repellent stimulus were of the same wavelength or an attractant stimulus at 565 nm was followed by a repellent stimulus at 370 nm. The response latency with respect to the repellent stimulus became progressively shorter with increasing delay, but it always remained longer than with a repellent stimulus alone that was delivered at the corresponding time (Fig. 5A). The decrease of the response latency had about the same rate whether the repellent stimulus was given alone or after an attractant stimulus.

In another experiment, the stimulation program was started 2 s after a reversal so that the repellent stimulus came after the period of refractoriness. In this case both the interval length and the response latency with respect to the
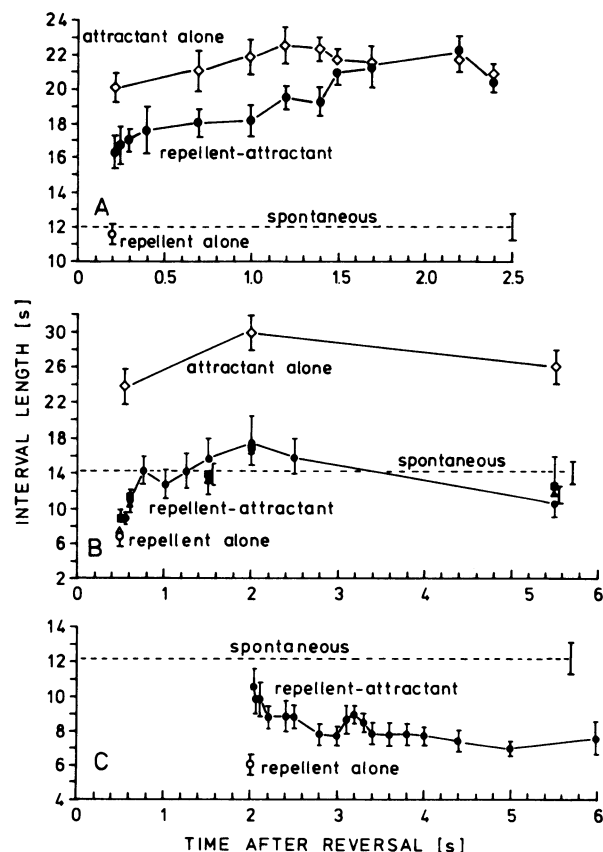


FIG. 4. Integration of a repellent stimulus and an attractant stimulus following with increasing delay times. (A) The first stimulus (repellent) was given within the period of absolute refractoriness, 200 ms after a spontaneous reversal. Both stimuli were at 370 nm. Each point is the mean ± SEM of 30 measurements. (B) The first stimulus (repellent) was applied 550 ms after a spontaneous reversal. Symbols indicate wavelengths as described in the legend to Fig. 1A. (C) The first stimulus (repellent) was applied 2 s after a reversal. Both stimuli were at 370 nm.

repellent stimulus first increased to reach the highest values at a delay of about 2 s and then decreased (Fig. 5B).

## DISCUSSION

Several experiments presented (Table 1; Fig. 1A, 2A, 4B, and 5A) show that there is no difference in the strength of the behavioral response of *H. halobium* whether in a particular stimulation program attractant or repellent stimuli or both are given through one of the two photosystems or through both. Therefore, it seems most probable that cellular signals generated by the two photosystems are of the same nature, but opposite in sign according to the direction of light changes, and that they are processed by a common link in the sensory transduction pathway. This implies that the cell cannot discriminate between light increase (decrease) in the yellow-green range and light decrease (increase) in the blue-UV range.

Light pulses (opposite steplike stimuli of the like wavelength through one photosystem) yield the same response as two successive steps of the same sign but of different wavelength through both photosystems. This indicates that the response to light pulses is the result of integration of the attractant and repellent steps rather than of a summation over the pulse duration, which had been concluded from previous experiments (3). It appears that with the sequence repellent-attractant the opposite signals are integrated in a way such that the law of reciprocity is apparently obeyed. At



FIG. 6. Oscillator model: assumed time-course of the oscillating regulatory substance. The heavy line illustrates the shape of free-running oscillation (from reference 9). The light line, derived from Fig. 2A, shows the change induced by an attractant stimulus; the dotted line, derived from Fig. 4C, shows the change upon a repellent stimulus. $R_s$, Spontaneous reversal; $R_r$, reversal after repellent stimulus; $R_a$, reversal after attractant stimulus. The dashed line indicates the critical concentration ($C_{crit.}$) of the regulator, at which a reversal is induced.
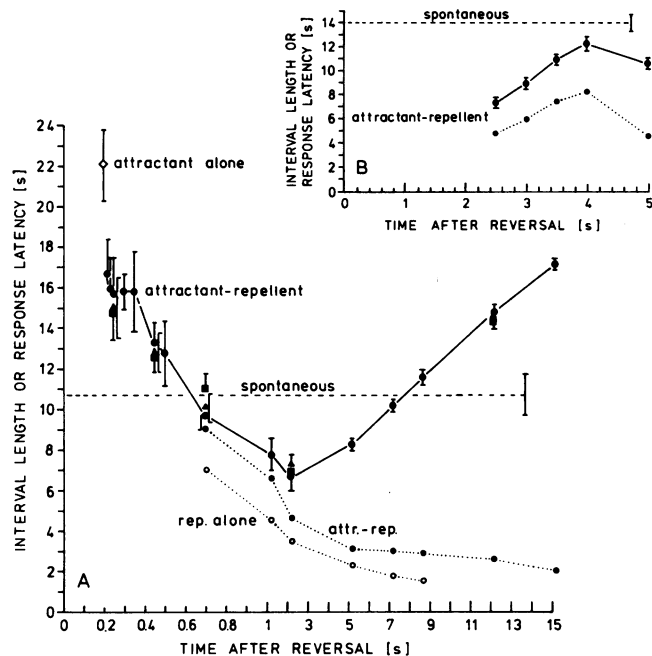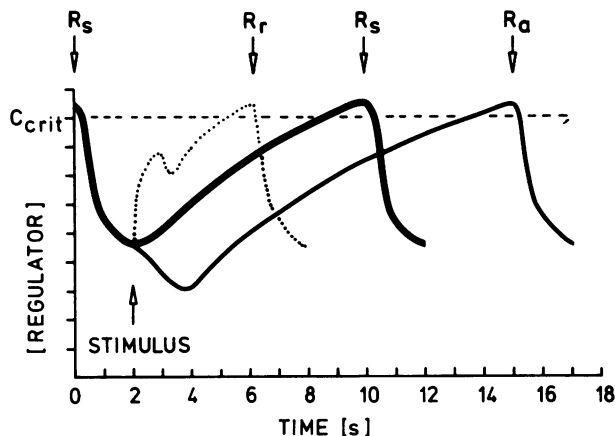


FIG. 5. Integration of an attractant stimulus and a repellent stimulus following with increasing delay times. (A) The first stimulus (attractant) was applied 200 ms after a spontaneous reversal, and the second stimulus (repellent) was applied after various delay times. Solid line, Interval length. Symbols indicate wavelengths as described in the legend to Fig. 1A. Each point is the mean ± SEM of 20 measurements. Dotted lines, Response latency (time between onset of repellent stimulus and subsequent reversal). Note the change in scale of the abscissa. (B) The first stimulus (attractant) was applied 2 s after a reversal, and the repellent stimulus followed with increasing delay. Both stimuli were at 370 nm. Solid line, Interval length; dotted line, response latency with respect to the repellent stimulus.

present it cannot be ruled out that with pulses shorter than 10 ms reciprocity actually holds.

Our results are consistent with the oscillator hypothesis (9). Moreover, they answer several important questions of sensory signal processing in *H. halobium*.

**Signal generation during refractoriness.** During the period of absolute refractoriness a repellent stimulus by itself does not change the spontaneous reversal interval (9). The present results, however, show that even during that period a repellent stimulus cancels the effect of a simultaneously applied attractant stimulus (Table 1). For a certain period it also influences the response to a following effective stimulus (Fig. 1B and 4A). This indicates that repellent stimuli are also recognized at any time and that cellular signals are generated and integrated.

**Involvement of a photochemical cycle of the receptor.** It has been proposed that slow rhodopsin, $sR_{587}$, is the receptor for yellow-green light and that an intermediate of its photocycle, $S_{373}$, is the UV receptor (14). Therefore, one could ask whether opposite stimuli could be integrated at the receptor level.

In a sequence of attractant-repellent, the second stimulus at 370 nm would meet the photochemical cycle in a different state depending on whether the first stimulus was a decrease at 370 nm or an increase at 565 nm. The data in Fig. 5A, however, show that the resulting response does not depend on the wavelength of the first stimulus. This was also found with other combinations of attractant and repellent stimuli (Fig. 1A, 2A, and 4B) and in corresponding experiments with the mutant strain Flx3 (data not shown). These results seem to rule out the possibility that the integration phenomena described here are accomplished by a photochemical cycle. The white background light used throughout would probably reduce the effect of such a cycle if there is any. We therefore have to postulate other sites for signal integration.

**Integration at the oscillator level.** Our oscillator model (9) says that a repellent stimulus should raise the concentration of a regulatory substance so that the critical level to induce a reversal is reached earlier. An attractant stimulus, on the
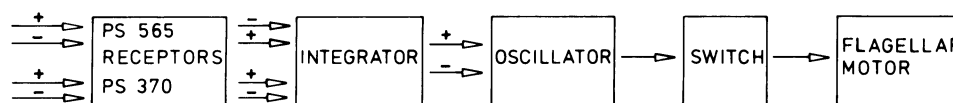
FIG. 7. Scheme of functional elements assumed in the photosensory pathway. Upon changes in fluence rate (+ or −) receptors generate repellent (+) or attractant (−) signals (the sign is chosen arbitrarily), which are handled by a common integrator. The output of the integrator is a + or − signal which changes the oscillator in opposite directions. Integration of these signals at the oscillator either advances (+) or delays (−) the time when the critical level is reached to activate the switch, which in turn induces a flagellar reversal.

other hand, should lower the level of the oscillator to delay the next reversal. An attractant stimulus should be more effective the farther away the oscillator is from the critical level at the moment of stimulation. The opposite is to be expected for a repellent stimulus. If successively applied stimuli are integrated at the oscillator, we would predict that the net response depends on the shape of oscillation induced by the first stimulus. Figure 2A shows that two attractant stimuli lead to the maximal response when they are 2 s apart. Consequently, when the second stimulus is a repellent one, its counteracting effect is smallest 2 s after the attractant stimulus (Fig. 5B). Our results are consistent with the idea that the signal generated by the second stimulus is added to the instantaneous state of the oscillator. The shape of the response curves (Fig. 2 and 5B) allows us to reconstruct the shape of oscillation after an attractant stimulus (Fig. 6). A comparison of the response latencies in Fig. 5A leads to the conclusion that the final increase of the oscillator has the same rate in the undisturbed state and after an attractant stimulus.

Similar considerations can be made if the first stimulus is an effective repellent one. The results of integration with a following attractant stimulus (Fig. 4C) indicate that owing to a repellent stimulus the oscillator first rapidly increases and then transiently decreases again before it finally rises toward the critical level (Fig. 6). The intermediate minimum of the oscillator can also be derived from the response latency in Fig. 1A. At higher stimulus strength the oscillator may reach the critical level directly without passing the intermediate dip.

During the period of relative refractoriness the cellular signal evoked by a repellent stimulus influences the oscillator only to a small extent (Fig. 4B). Since the response curve roughly parallels that of free-running oscillation monitored with a single attractant stimulus, we assume that under these conditions the repellent stimulus raises the level of the oscillator but does not disturb its shape very much. The results in Fig. 3 and Table 2 show that the cell can recognize the sequence of successively applied stimuli when there is a delay of only 20 ms between them. This ability needs a biological clock and is most probably provided by the oscillator.

**Integration before the oscillator.** According to the oscillator model, it seems reasonable to assume that a stimulus which does not change the spontaneous interval does not influence the oscillator. We therefore conclude that a repellent stimulus given during the refractory period can influence the response to a second effective stimulus only in a step before the oscillator in the sensory pathway.

The phenomenon of absolute refractoriness allows us to study the function of the proposed integrator element separately. During the period of absolute refractoriness a repellent stimulus cancels the effect of a simultaneously applied attractant stimulus (Table 1). A delay of 10 ms between the stimuli is sufficient to cause a response. The repellent stimulus weakens the effect of a following attractant stimulus

(Fig. 4A) and enhances the effect of a following repellent stimulus (Fig. 1B) up to a delay of 1.2 s. This kind of signal integration is ascribed to the integrator element. We conclude from our data that the repellent signal rapidly declines within 10 ms and that its total life time does not exceed 1.2 s.

In summary, we postulate at least two successive elements for signal integration in the photosensory pathway, the integrator and the oscillator (Fig. 7). The integrator is involved only at delay times up to 1.2 s between stimuli; at longer delays integration takes place merely at the oscillator.

**Dominance of the repellent signal.** In a sequence of opposite stimuli of equal strength, the effect of the repellent stimulus always dominates (Fig. 3 and 4C). Since opposite stimuli cancel each other when applied simultaneously, this cannot be due to different processing of the signals by the integrator. Rather, we have to assume that repellent signals alter the level of the oscillator faster or to a greater extent or both than attractant stimuli do (Fig. 6).

**Refractoriness may be an inherent property of the oscillator.** Since repellent signals generated during refractoriness are processed by the integrator, the phenomenon of refractoriness must be related to a later step. Obviously it is not the switch which is refractory, because reversals can occur at least every 4 s (10), while refractoriness is also observed with stimuli which would evoke the reversal at a later time. Our results make it most probable that during the refractory period a repellent signal by itself cannot change the oscillator. We assume that this is a property of the oscillator which occurs shortly after it has crossed the critical level. A decrease of the oscillator level owing to an attractant signal does not cause a second period of refractoriness during the interval. Already at 20 ms after an attractant stimulus a following repellent stimulus leads to a net repellent response (Fig. 3A). This indicates that it is not simply the decrease of the oscillator which causes refractoriness, but rather its behavior near the critical level.

## LITERATURE CITED

1. **Alam, M., and D. Oesterhelt.** 1984. Morphology, function and isolation of halobacterial flagella. J. Mol. Biol. **176:**459–475.
2. **Dencher, N. A., and E. Hildebrand.** 1979. Sensory transduction in *Halobacterium halobium*: retinal protein pigment controls UV-induced behavioral response. Z. Naturforsch. **34c:**841–847.
3. **Hildebrand, E., and N. Dencher.** 1975. Two photosystems controlling behavioural responses of *Halobacterium halobium*. Nature (London) **257:**46–48.
4. **Hildebrand, E., and A. Schimz.** 1983. Photosensory behavior of a bacteriorhodopsin-deficient mutant, ET-15, of *Halobacterium halobium*. Photochem. Photobiol. **37:**581–584.

5. Hildebrand, E., and A. Schimz. 1983. Consecutive formation of sensory photosystems in growing *Halobacterium halobium*. Photochem. Photobiol. **38**:593–597.

6. Hildebrand, E., and A. Schimz. 1985. Behavioral pattern and its photosensory control in *Halobacterium halobium*. p. 129–142. *In* M. Eisenbach and M. Balaban (ed.), Sensing and response in microorganisms. Elsevier Publishing Co., Amsterdam.

7. Hildebrand, E., and A. Schimz. 1985. Sensory transduction in *Halobacterium*, p. 93–111. *In* G. Colombetti and P.-S. Song (ed.), Sensory perception and transduction in aneural organisms. Plenum Publishing Corp., New York.

8. Schimz, A. 1982. Localization of the methylation system involved in sensory behavior of *Halobacterium halobium* and its dependence on calcium. FEBS Lett. **139**:283–286.

9. Schimz, A., and E. Hildebrand. 1985. Response regulation and sensory control in *Halobacterium halobium* based on an oscillator. Nature (London) **317**:641–643.

10. Schimz, A., and E. Hildebrand. 1986. Entrainment and temperature dependence of the response oscillator in *Halobacterium halobium*. J. Bacteriol. **166**:689–692.

11. Schimz, A., W. Sperling, P. Ermann, H. J. Bestmann, and E.

Hildebrand. 1983. Substitution of retinal by analogues in retinal pigments of *Halobacterium halobium*. Contribution of bacteriorhodopsin and halorhodopsin to photosensory activity. Photochem. Photobiol. **38**:417–423.

12. Sperling, W., and A. Schimz. 1980. Photosensory retinal pigments in *Halobacterium halobium*. Biophys. Struct. Mech. **6**:165–169.

13. Spudich, E. N., and J. L. Spudich. 1982. Control of transmembrane ion fluxes to select halorhodopsin deficient and other energy-transduction mutants of *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA **79**:4308–4312.

14. Spudich, J. L., and R. A. Bogomolni. 1984. Mechanism of colour discrimination by a bacterial sensory rhodopsin. Nature (London) **312**:509–513.

15. Spudich, J. L., and W. Stoeckenius. 1979. Photosensory and chemosensory behavior of *Halobacterium halobium*. Photobiochem. Photobiophys. **1**:43–53.

16. Takahashi, T., H. Tomioka, N. Kamo, and Y. Kobatake. 1985. A photosystem other than PS 370 also mediates the negative phototaxis of *Halobacterium halobium*. FEMS Microbiol. Lett. **28**:161–164.