Effects of Sequential Stimuli on *Halobacterium salinarium* Photobehavior

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ABSTRACT We analyzed the motor photoresponses of *Halobacterium salinarium* to different test stimuli applied after a first photophobic response produced by a step-down of red-orange light (prestimulus). We observed that pulses given with a suitable delay after the prestimulus produced unusual responses. Pulses of blue, green, or red-orange light, each eliciting no response when applied alone, produced a secondary photophobic response when applied several seconds after the prestimulus; the same occurred with a negative blue pulse (rapid shut-off and turning on of a blue light). Conversely, no secondary photophobic response was observed when the test stimulus was a step (a step-up for red-orange light, a step-down for blue light) of the same wavelength and intensity. When the delay was varied, different results were obtained with different wavelengths; red-orange pulses were typically effective in producing a secondary photophobic response, even with a delay of 2 s, whereas the response to a blue pulse was suppressed when the test stimulus was applied within 5 s after the prestimulus. The secondary photophobic response to pulses was abolished by reducing the intensity of the prestimulus without affecting the primary photophobic response. These results, some of which were previously reported in the literature as inverse effects, must be produced by a facilitating mechanism depending on the prestimulus itself, the occurrence of reversals being per se ineffective. The fact that red-orange test stimuli are facilitated even at the shortest delay, whereas those of different wavelengths become effective only after several seconds, suggests that the putative mechanism of the facilitating effect is specific for different signaling pathways.

INTRODUCTION

The general motile behavior of *Halobacterium salinarium* (formerly called *H. halobium*) consists of straight runs separated by reversals in the direction of motion (see Petracchi et al., 1994, and references therein). The frequency of these reversals—typically in the 0.02–0.2 Hz range for unstimulated cells—is affected by light intensity changes; repellent light stimuli increase the reversal frequency (photophobic response), whereas the opposite occurs with attractant light stimuli. A photophobic response normally occurs when a red-orange light is turned off or a blue or blue-green light is turned on; long-wavelength pulses act as attractant stimuli, whereas short-wavelength pulses work as repellent stimuli. Repellent stimuli, however, do not elicit a response when delivered within a period of about 0.5 s after a reversal, the so-called refractory period.

The photosensory apparatus of *H. salinarium* has been shown to consist of two pigments, the molecular, spectroscopic, and functional properties of which have been investigated by several groups; in particular, sensory rhodopsin I (SR-I), absorbing in the red-orange band, is converted into a signaling intermediate (SR_{373}) that also mediates the responses to blue light (Spudich and Bogomolni, 1984). Responses to blue-green light are mediated by a second sensory pigment, phoborhodopsin or sensory rhodopsin II (SR-II) (Takahashi et al., 1985, 1990; Spudich et al., 1986; Wolff et al., 1986; Marwan and Oesterhelt, 1987).

Under particular experimental conditions, however, the occurrence of so-called inverse or paradoxical responses (i.e., photophobic responses to attractant stimuli or depression of reversals after a repellent stimulus) has been reported. Hildebrand and Schimz (1987) claimed that inverse responses could occur within several seconds after a spontaneous reversal for both attractant and repellent stimuli. However, their data were obtained by measuring the response through the average interval after the stimulus. The use of the average interval can be deceptive (Krohs, 1994), particularly when the stimulus is a light step-down applied while the sample is still adapting to the onset of the illumination, as occurred in the experiments of Hildebrand and Schimz (reported by Krohs, 1994). By monitoring single cells, Krohs never found inverse responses to any attractant light stimulus delivered after a spontaneous reversal (1994). Nevertheless, a paradoxical response to a red-orange pulse after a burst of stimulated reversals had also been observed by McCain et al. (1987) under experimental conditions not subject to the aforementioned criticism.

Inverse effects can be considered as a signature of nonlinear oscillators. In fact, the hypothesis that a nonlinear oscillator drives the spontaneous behavior of *H. salinarium* was proposed by Schimz and Hildebrand (1985), whereas all other groups working on *H. salinarium* assumed that a stochastic process determines the occurrence of spontaneous reversals (McCain et al., 1987; Marwan and Oesterhelt, 1987).

We therefore carried out several sets of experiments aimed to systematically investigate the kind of stimuli (test stimuli) that could give rise to unusual responses in *H. salinarium*. If they were produced by a nonlinear endogenous oscillator, they should be observed with every sublim-

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inal or weak stimulus delivered at suitable delays, but the usual behavior should be restored by increasing the stimulus strength. Therefore, we varied the following features of the stimulation pattern: 1) the intensity and the wavelength of the test stimuli; 2) the shape of the test stimuli (pulses versus steps) applied after a peak of reversals elicited by a photophobic prestimulus; 3) the delay between the prestimulus and the test stimuli; 4) the intensity of the prestimulus.

MATERIALS AND METHODS

Cell culture

Experiments were carried out on the Fl×15 mutant strain of *H. salinarium* (BR^-, HR^-, $SR\text{-}I^+$, $SR\text{-}II^+$). Cells were grown under the standard conditions described by Spudich and Spudich (1982). Two-day-old cultures were used in experiments with green-light stimulation, and a narrow-band interference filter (see below, Stimulation Modes) was adopted to ensure that only SR-II was stimulated. Three-day-old cultures, which did not respond to green-light stimulation, were used in all other experiments.

Microscope and stimulation set-up

The scheme of the experimental set-up is shown in Fig. 1. The sample under the microscope was observed in dark field with infrared illumination using a 715-nm long-pass filter (RG 715; Schott, Germany); a video camera mounted on the microscope was used to monitor the sample and to send data to a PIP-1024B video digitizer board (Matrox Electronic Systems) in a 486 PC operating under DOS. Two quartz-iodine lamps were used to stimulate the sample, one for the prestimulus and the other for the test stimulus (see below). To focus the stimulation light on the sample, a first beam splitter conveyed the stimulation light through the dark-field condenser, and a second beam splitter was used to combine the light coming from the two lamps. Measured absolute intensities of nonattenuated stimulation lights (I_{max}) falling on the sample were, in photons cm^{-2} s^{-1}: 3.0×10^{17} for red-orange light, 1.4×10^{16} for blue light, and 7.2×10^{16} for green light.

We measured that, in the absence of applied light stimuli, the microscope field illumination yielded at 700 ± 25 nm a light intensity of 0.22 mW, corresponding to about 10^{16} photons cm^{-2} s^{-1}, which can account for the observed responses to blue pulses in the absence of red-orange background.

Motion analysis program

Experiments were monitored by a motion analysis system using a deeply modified version of a program written by D. P. Häder and K. Vogel. The basic algorithm used to identify objects that could be cells is described by Häder and Vogel (1991). Briefly, this algorithm picks up objects above a given gray threshold level displaying a suitable shape factor and determines the mass center coordinates. The identification of the object coordinates occurs during the reading of the video digitizer board; this results in a variation of the time required to read a frame, depending on the number of cells in that frame. In different experiments the time required to read a frame was between 0.3 and 0.5 s; within a single experiment, the variation was no more than ±5%.

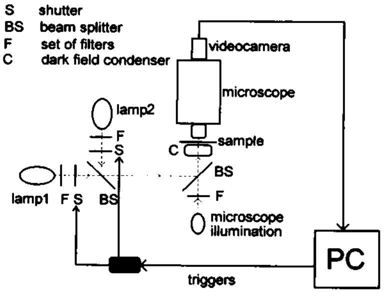

FIGURE 1 Schematic view of the experimental set-up.

In our modified version, the program delivers the stimuli and acquires the coordinates of cells. Typically, an experiment is carried out in several successive runs separated by readaptation intervals, during which no data are acquired. During a run, the program delivers the stimuli, acquires the data, and stores them frame by frame as a series of coordinates. After each run (i.e., during the readaptation time), the program analyses the data and yields the reconstruction of the cell trajectories. Thus the analysis is off-line, but the result of the experiment is soon available, just at the end of each run. The program then identifies the frames at which the reversals have occurred. In performing this task, the program can make some errors that affect the absolute value of the spontaneous frequency of reversals. The error is usually to introduce false reversals, but very seldom does the program miss true reversals. Thus, the program is most suitable for detecting photophobic responses to light stimuli, although the depression of the reversal frequency can also be clearly monitored.

Usually, 10 runs were performed to build an experimental figure, but sometimes up to 30 runs were collected. The repetition time between runs in different experiments was from 45 to 80 s, including the adaptation time, but was fixed within the same experiment.

Stimulation modes

Several kinds of stimuli were used. Red-orange, blue, and green lights were obtained with the long-pass OG570 Schott filter, and with the K40 and K50 Balzer band-pass interference filters with a bandwidth of ±25 nm and peaks at 400 and 500 nm, respectively. Unless otherwise stated, a prestimulus consisting of the shut-off of a red-orange light was always introduced as a conditioning stimulus producing a photophobic response; this light was switched on at the start of the readaptation interval. After a variable delay from the prestimulus, one of the following patterns of test stimuli was delivered: I) a "negative pulse" of blue light, produced by rapidly shutting off and turning on a blue light that was on from the onset of the run; II) either a step-up of red-orange light or a step-down of blue light; III) a pulse of blue or green light; IV)

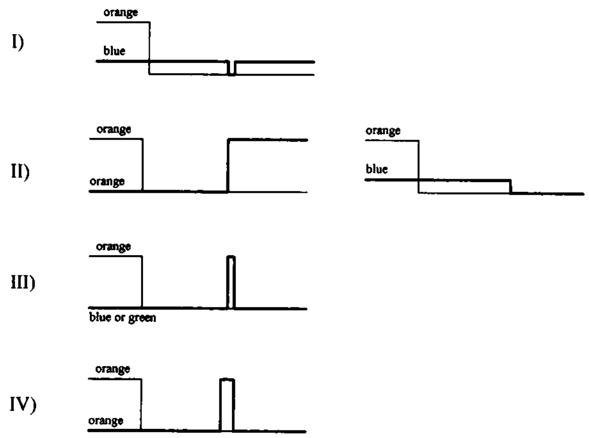

FIGURE 2 Diagram illustrating the stimulation modes used in this paper. Thin lines indicate the prestimulus, whereas thick lines indicate the test stimulus. This varies in different experiments and is of four types: I) a "negative pulse" of blue light; II) either a step up of red-orange light or a step down of blue light; III) a pulse of blue or green light; IV) a pulse of red-orange light.

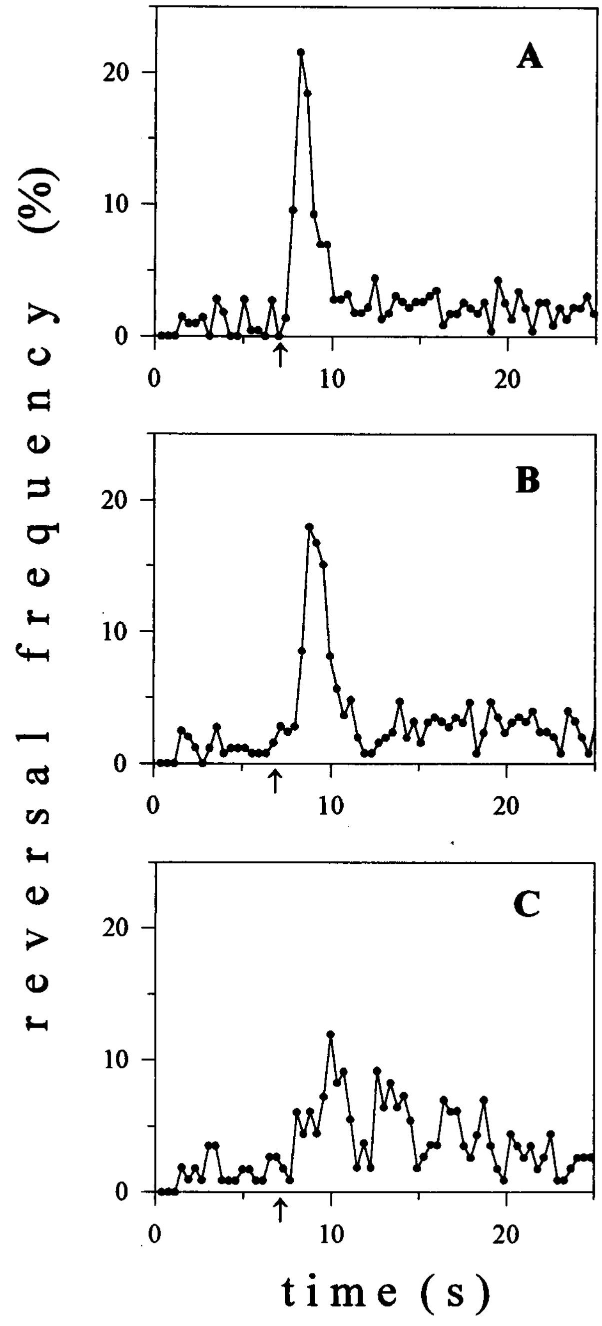

FIGURE 3 Responses to a red-orange step-down observed in different samples. The arrow marks the time at which the step was applied. A) Sustained plateau following the induced reversal peak. B) Presence of a trough between the peak and the plateau. C) Damped oscillations of reversal episodes. In this figure and in the following, the rate of data collection was around 2 frames/s, and the reversal frequency for each frame was calculated from the ratio between the number of reversals and the number of cells in that frame. Average cell numbers per frame were 252 in *A*, 216 in *B*, and 111 in *C*.

a pulse of red-orange light. To monitor the effect of the test stimulus alone, control experiments were performed in which the red-orange light was not turned on at the start of the readaptation interval and the prestimulus was then omitted. The various patterns of stimulation are schematically summarized in Fig. 2.

RESULTS AND DISCUSSION

The typical features of responses to the prestimulus alone, obtained by switching off a red-orange light, are depicted in Fig. 3. Three types of response were observed in different cell populations. In the first type, a peak of reversals is seen just after the red-orange step-down, followed by a sustained plateau lasting about 25 s, probably caused by multiple reversal events in some cells. In the second type, a trough or depression, similar to that reported by McCain et al. (1987), is observed between the peak and the plateau. This depression may be simply a statistical effect (Petracchi et al., 1994), because many cells respond at the same time and thus are also synchronized with respect to their refractory period. This interpretation, however, cannot account for the third type of response, where switching off the red light induced several episodes of reversals, resulting in a series of damped peaks. This type of behavior is reminiscent of the "secondary response" reported by Krohs (1995), although it was observed by a different monitoring technique. We did

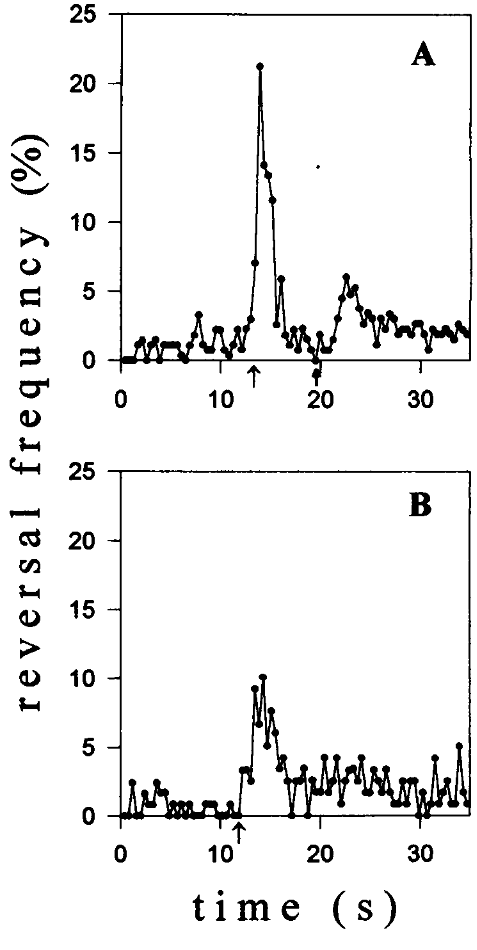

FIGURE 4 (*A*) Response to a negative blue pulse (thick arrow) given 7 s after the prestimulus (thin arrow). (*B*) Response to the prestimulus alone. The test stimulus was obtained by maintaining a blue light on at 10% of I_{max} and switching it off and on (time interval: 1.5 s); the thick arrow indicates the time at which the blue light was switched off. Average cell numbers per frame were 265 in *A* and 117 in *B*.

not attempt to investigate the cause of this difference in behavior, which seems to be independent of culture age. The experiments reported below were performed only on samples displaying the first two kinds of responses to the prestimulus.

We started our tests by considering that, if the paradoxical effect observed with red-orange pulses by McCain et al. (1987) were a true inverse effect, it should occur also with blue or green light.

In a first set of experiments, carried out on cells from 3-day-old cultures, we investigated the effect of stimulation mode I (negative blue pulse; a blue light at 10% of $I_{\max}$ was on from the onset of the runs). The expected effect is normally a depression of the reversal frequency, but the stimulus we used was so weak that no observable effect took place when it was delivered alone (data not shown); when delivered after the prestimulus, however, this test stimulus elicited a reversal peak (Fig. 4). This could be called an inverse effect. We must notice that the response to the prestimulus alone is much less than the same response in the presence of blue light (see below).

In a second set of experiments on 3-day-old cells, we looked for unusual effects using a step-down of blue light as the test stimulus (stimulation mode II); as in the preceding set of experiments, a blue light was on from the onset of the runs. Fig. 5 shows the results obtained by using several blue-light intensities. Here and in Fig. 4, the percentage of cells responding to the prestimulus is affected by the presence of the blue background; at 10% of $I_{\max}$ the response to the prestimulus is enhanced, whereas at 100% of $I_{\max}$, it completely disappears (Fig. 5 *D*). No reversal episodes were observed after the test stimulus at any blue-light intensity. This is evidence against the idea that step stimuli act as pulse stimuli, but also disfavors the interpretation of these unusual responses as inverse effects.

A third set of experiments was carried out using green or blue pulses on 2-day-old or 3-day-old samples, respectively (stimulation mode III). A subliminal pulse is able to elicit reversals after the red-orange photophobic prestimulus. The response to a weak green pulse delivered 7.5 s after the prestimulus is reported in Fig. 6 *A*, and Fig. 6 *B* shows the absence of any effect of the same green pulse when delivered alone. Similar results were obtained with a blue pulse (Fig. 6, *C* and *D*). In these experiments, the duration of pulses was purposely selected to give no response when delivered alone. Although this pattern of stimulation never produced an intense peak of reversals, the responses reported in Fig. 6 are consistent and reproducible. These results cannot be interpreted as inverse effects: a subliminal stimulus triggers the usually expected response when given at a suitable delay from the photophobic prestimulus.

In a fourth set of experiments we exploited stimulation mode IV, i.e., red-orange pulses, as test stimuli on 3-day-old

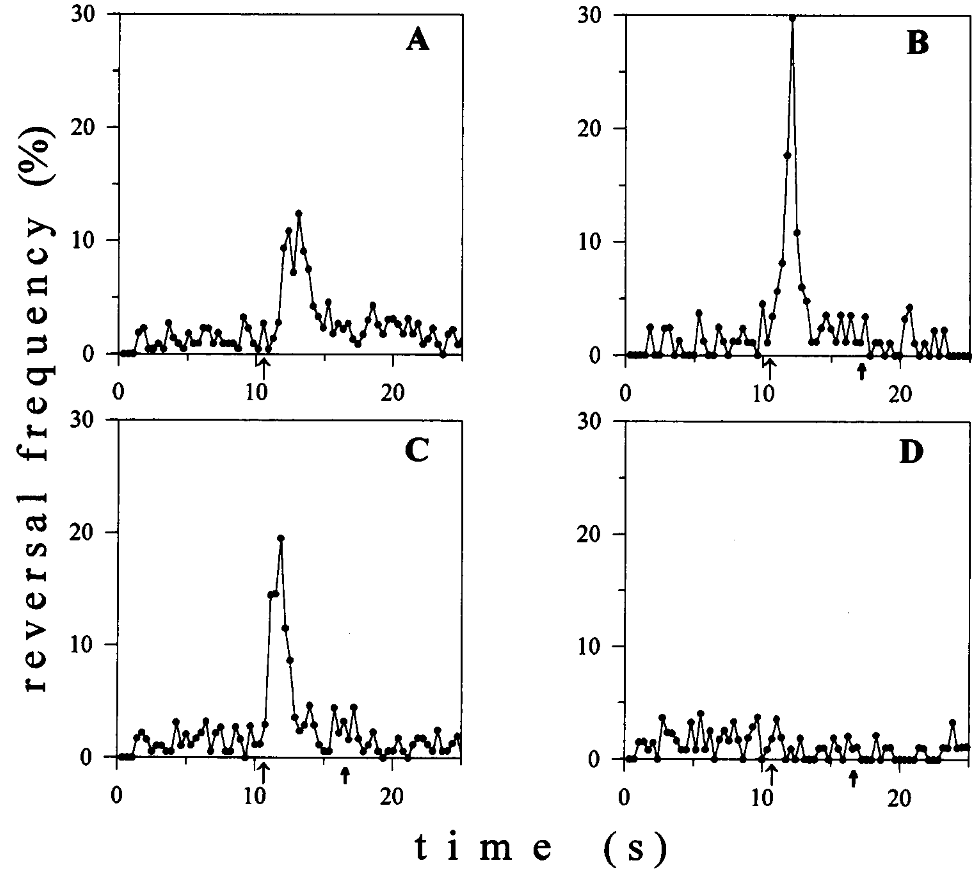

FIGURE 5 Effect of applying a blue-light step-down 7 s after the prestimulus (thin arrows). The response to the prestimulus alone is shown in *A*. In *B*–*D*, blue lights of relative intensities 4%, 10%, and 100% were switched off at the time marked by the thick arrows. The only effect of the blue light is to influence the prestimulus response, which completely disappears in *D*. Average cell numbers per frame were 215 in *A*, 84 in *B*, 172 in *C*, and 40 in *D*.

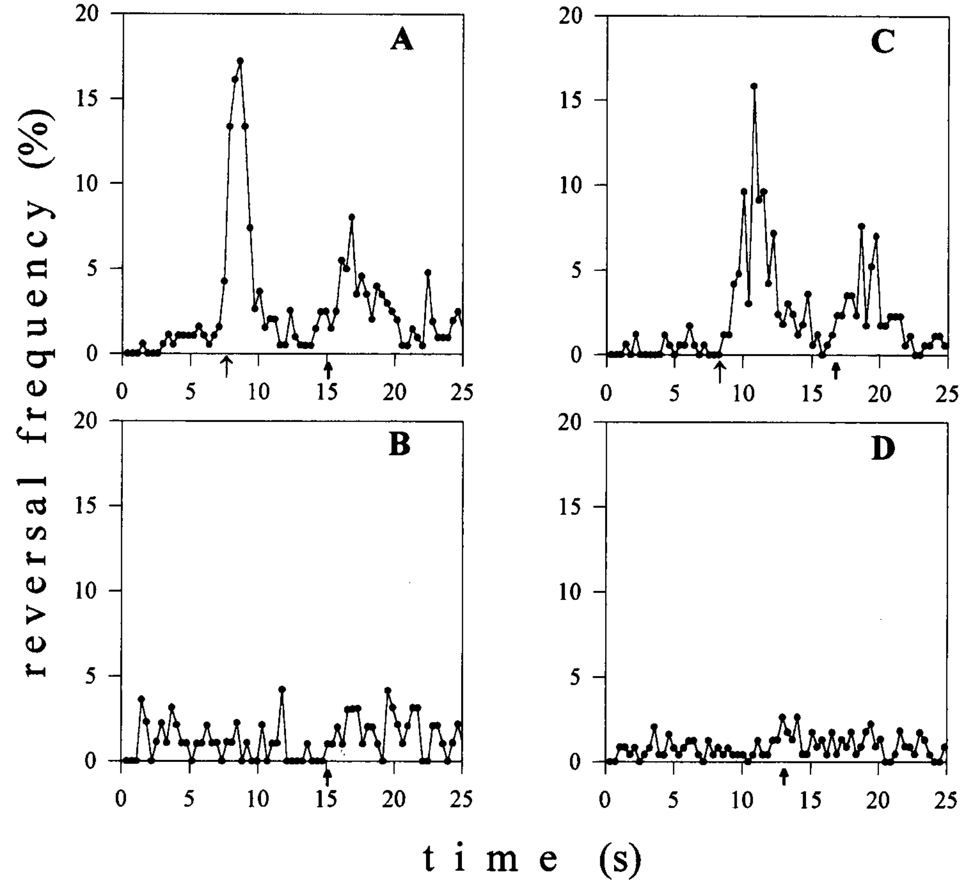

FIGURE 6 Responses to subliminal green (*A*) or blue (*C*) pulses delivered after a red-orange step-down. *B* and *D* show the sample behavior when either pulse was delivered in the absence of the prestimulus. Thin arrows mark the prestimulus timing; thick arrows show the time at which test stimuli were delivered. Pulse duration was 0.8 s in *A* and *B*, and 0.2 s in *C* and *D*. Average cell numbers per frame were 190 in *A*, 90 in *B*, 168 in *C*, and 225 in *D*.

cells. Fig. 7 shows experiments similar to those described by McCain et al. (1987). We probed the time interval over which the photophobic response to the test stimulus could occur by performing a sweep on the delays. In the experiment reported in Fig. 7 this interval lasted nearly 10 s; in other experiments, it lasted up to 25 s. In Fig. 7, *A–E*, the test stimulus was applied with increasing delays from the prestimulus; when delivered alone, the test stimulus did not produce any effect (Fig. 7 *F*). In Fig. 7, *B–E*, a depression in reversal frequency is consistently observed immediately after the test stimulus, followed by a peak of reversals. In Fig. 7 *A*, where the test stimulus is applied after a very short delay (2 s) after the prestimulus, the overall percentage of reversals in the first peak is lower than in the corresponding peak in Fig. 7 *B* (57% versus 83%); altogether, in the two peaks of Fig. 7 *A*, 90% of the monitored cells are reversing at least once.

In experiments similar to those described above, we observed that the duration of the pulse per se had no effect on this kind of response, provided that the total energy of the pulse is kept constant. When a suitably filtered electronic flash, lasting 0.6 ms, was used as the test stimulus, the response could not be distinguished from that shown in Fig. 7 (data not shown).

In other experiments stimulation mode II (red-orange step-up) was used as a test stimulus with different light intensities ranging from 3% to 100% $I_{\max}$. Systematically, no photophobic responses to this test stimulus were observed (results not shown). It therefore appears that the only kind of stimulus that can produce unusual effects implies the presence of both a rising and a falling phase of the stimulus, as already pointed out. Consequently, we shall consider all of these unusual responses as being due to some facilitating (or enhancing) conditions existing after a stimulated reversal episode.

Are these unusual effects correlated with the burst of stimulated reversals or with the stimulus itself? The first correlation is inherent in the nonlinear oscillator model, which at this point was clearly ruled out. But the question itself was still worth considering.

To approach this question we analyzed the response to a test stimulus after a weak step-down (from 10% to 0% instead of 100% to 0%) of the red-orange prestimulus light. In Fig. 8, the responses in the presence of a prestimulus obtained by switching off the full-intensity red-orange light are compared with the responses after a prestimulus obtained by switching off the same light attenuated by a neutral density filter with 10% transmittance. It is possible to observe that the primary (photophobic) response is almost the same in both cases, but the response to the test stimulus is abolished in the second case. This means that the unusual effect is a poststimulus effect, and there is no correlation with the burst of

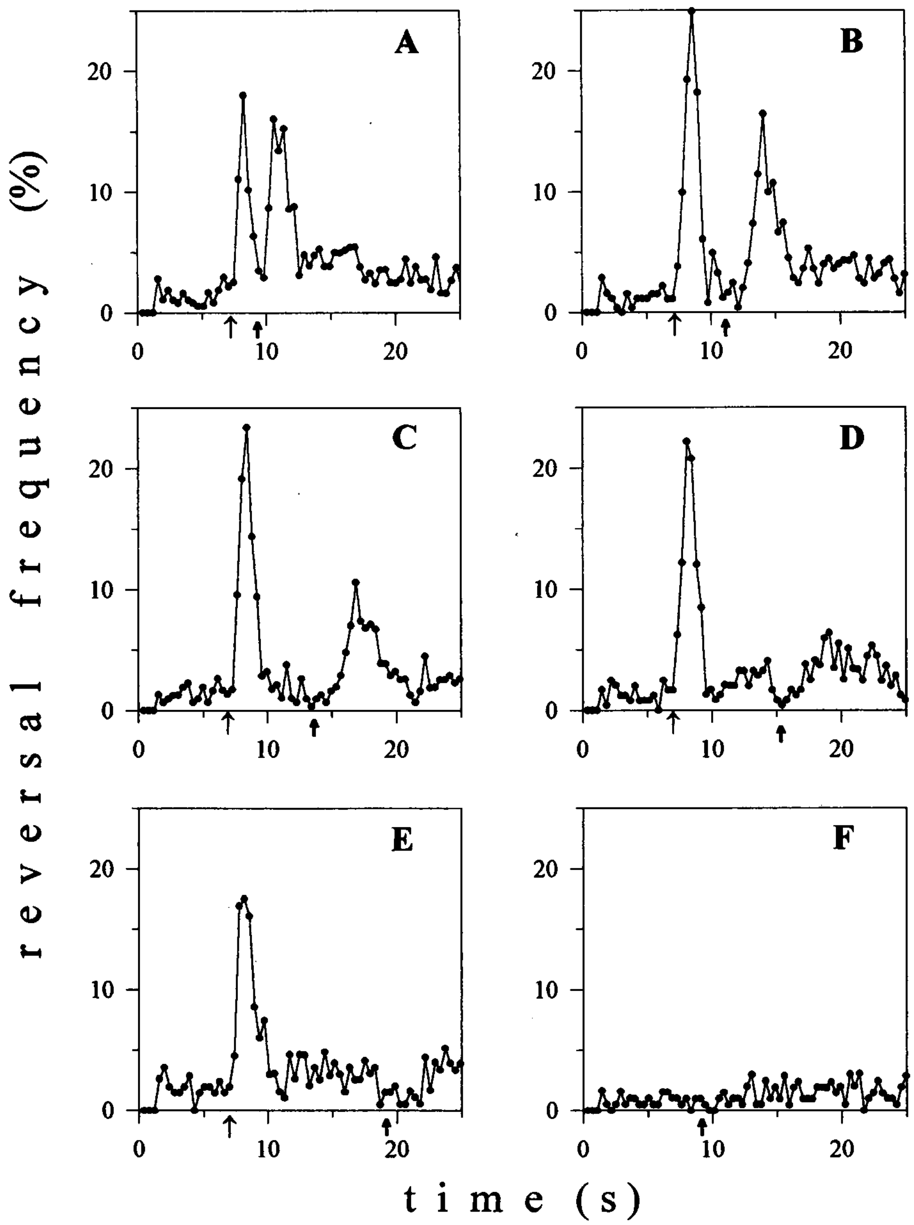

FIGURE 7 Effect of varying the delay between the prestimulus (thin arrow) and a test stimulus consisting of a red-orange pulse (duration 0.8 s). (*A–E*) test stimulus (thick arrow) delivered after 2 s, 4 s, 6 s, 8 s, and 12 s, respectively. (*F*) Test stimulus delivered in the absence of the prestimulus. Average cell numbers per frame were 352 in *A*, 242 in *B*, 296 in *C*, 233 in *D*, 182 in *E*, and 196 in *F*.

stimulated reversals, the dependence really being on the prestimulus.

Fig. 9 finally adds a last piece of evidence for the existence of a poststimulus effect: the effect of a blue pulse of 0.8 s, effective when delivered alone (Fig. 9 *B*), is suppressed when it is delivered just a few seconds after the prestimulus (Fig. 9 *A*). We emphasize that this result is not in contrast with that of Fig. 6 *C*, where a blue pulse of 0.2 s was given 9 s after the prestimulus. Moreover, as shown in Fig. 9 *C*, this suppression does not occur if a red-orange pulse is applied as test stimulus after the same delay, as also shown by the experiments in Fig. 7.

CONCLUDING REMARKS

The idea of a self-sustained nonlinear oscillator driving the motor of flagella in *H. salinarium*, as first proposed by Schimz and Hildebrand (1985), is difficult to reconcile with some existing data (Lucia et al., 1992; Krohs, 1994, 1995). One of the relevant points of this hypothetical model was that it could predict inverse effects. Indeed, unusual effects that could be interpreted as inverse effects were observed by McCain et al. (1987).

The results reported herein show that these unusual effects are clearly different from true inverse (or paradoxical) effects; they are rather to be interpreted as an enhancement of the reactivity of the cells after a red-orange step-down. In particular, this enhancement or facilitation—always producing a secondary photophobic response when pulses (i.e., two-phase stimuli) of various wavelengths are applied after the prestimulus—never occurs when a step-down of blue light or a step-up of red-orange light is applied as the test stimulus after the prestimulus. The facilitation should there-

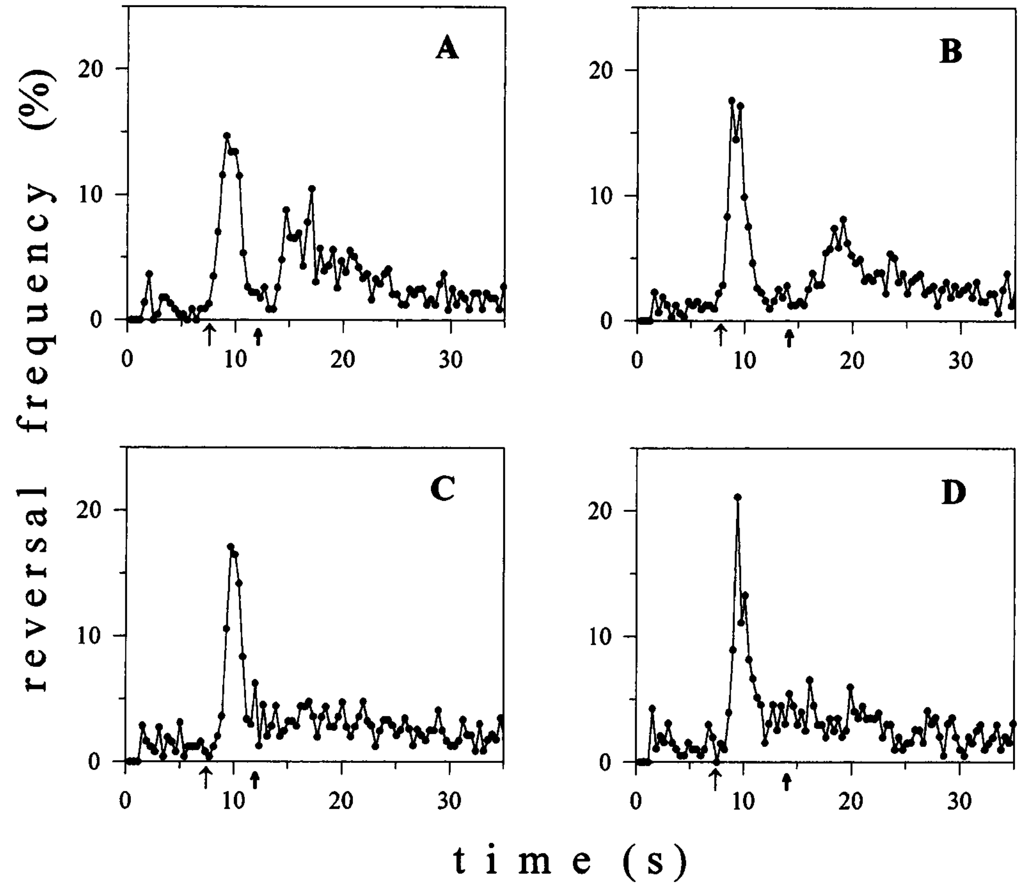

FIGURE 8 The response to a red-orange pulse (thick arrow) after the prestimulus (thin arrow) depends on the prestimulus strength. (*A* and *B*) The test stimulus is applied 4 s and 6 s, respectively, after a red-orange step-down from 100% I_{max} to zero. (*C* and *D*) Same as in *A* and *B*, but the step-down is from 10% I_{max} to zero. Pulse duration was 0.8 s; average cell numbers per frame were 225 in *A*, 310 in *B*, 239 in *C*, and 195 in *D*.

fore occur on the falling phase of a red-orange pulse and on the raising phase of a blue or green pulse. The time resolution of the two phases is irrelevant, as documented by experiments with a photographic flash.

The experiments reported in Fig. 9 are worthy of a more detailed comment: they offer evidence that specific interactions occur between different pathways of signal transduction. A red-orange prestimulus (impinging on SR_{587}) positively influences a subsequent red-orange stimulus, whereas it suppresses for several seconds the response to a blue stimulus (impinging on SR_{373}); however, more than 5 s after the red-orange prestimulus, a facilitation of the blue-light-mediated response is observed. The same time-dependent suppression and facilitation effects occur with green stimuli, impinging on SR-II. An attempt to interpret these results in terms of signal transduction system(s) is as follows. To account for the transient nature of the response, it has been proposed (McCain et al., 1987) that an activated photoreceptor produces, through different kinetic constants, two antagonist substances (X and Y). The results presented herein suggest that these substances are specific to different signaling states. The main effect of these substances would be to produce a photoresponse, but secondary effects could be postulated, such as cross-inhibition on an antagonistic pathway. This could account for the inhibition of the red-orange step-down on blue pulses, but not for its eventual facilitating effect on the same type of stimulus. However, a delayed positive feedback could account for the facilitation of a second red-orange pulse. Of course, *ad hoc* hypotheses of this kind might be variously formulated, and in our opinion they add little to the understanding of the photosensory transduction in *H. salinarium*, whose biochemical characterization is still incomplete.

Schematically, the present knowledge comprises 1) the photocycles of both SR-I and SR-II and their signaling states; 2) the release of fumarate in the cytoplasm when photophobic responses occur; 3) the occurrence of an unbalanced methylation/demethylation process as a light stimulus is turned off and on. Fumarate probably acts near the end of the transduction chain and presumably has nothing to do with the above-reported effects. The reversible methylation of phototransducer(s) (Alam et al., 1989; Spudich et al., 1989) might play a role in these phenomena, but different methylation sites with different effects should be postulated; moreover, the increased demethylation observed when a light stimulus is turned on and off should be inter-

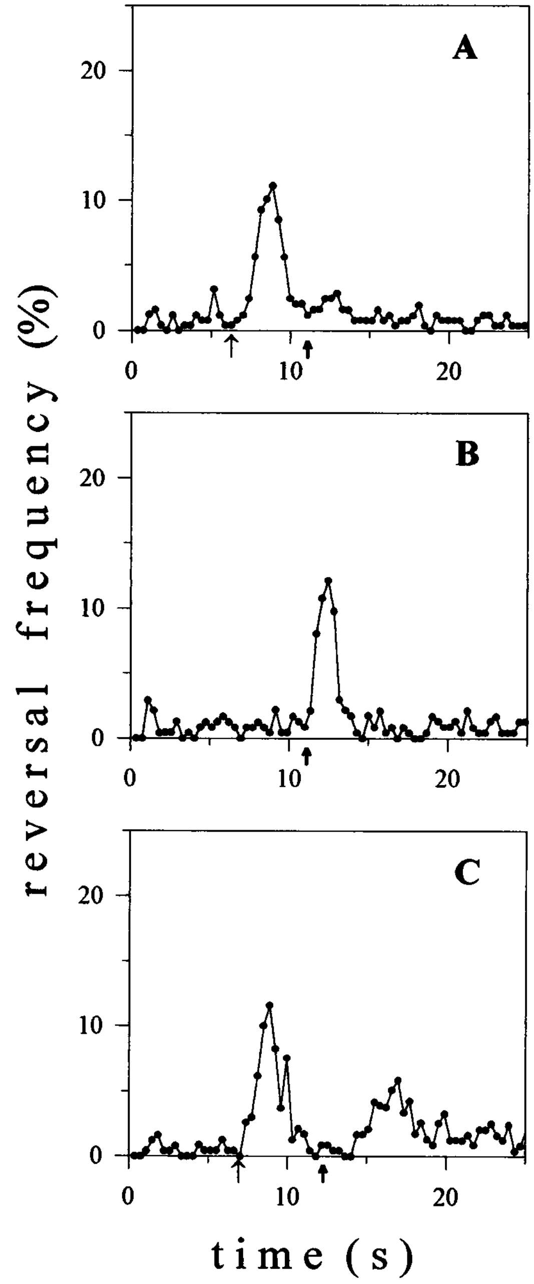

FIGURE 9 The response to a blue pulse (thick arrow) after the prestimulus (thin arrow) is suppressed when the test stimulus is applied close to the prestimulus. (*A*) Blue pulse delivered 5 s after the prestimulus. (*B*) Blue pulse applied alone. (*C*) The test stimulus (thick arrow) is here a red-orange pulse delivered 5 s after the prestimulus. For both types of pulse the duration was 0.8 s; average cell numbers per frame were 251 in *A*, 235 in *B*, and 242 in *C*.

preted differently in terms of sensory transduction, but no experimental evidence is available to support this idea.

A possible way to investigate whether these poststimulus effects take place at the level of the photoreceptor itself could be the study of phototaxis mutant behavior. It has recently been reported (Olson et al., 1995) that a single amino acid substitution (D201N) in the SR-I opsin (SopI) produces altered phototaxis signals—both red-orange and blue pulses elicit reversals. These authors suggest that the behavior of this mutant (Fl×15Δ*sopI/D201Ntr*) could be connected with the observed unusual response of a strain endowed with wild-type SR-I to an orange pulse delivered after an orange step-down (our results and McCain et al., 1987). It would therefore be interesting to record the photoresponses of the Fl×15Δ*sopI/D201Ntr* mutant to sequential stimuli.

However, all of the interpretations discussed above are to a large extent speculative, and at the present state of our knowledge this is necessarily so. What is clearly shown by the data reported here is the existence of specific interactions between different pathways starting from different signaling states. In particular, it appears that some sort of inhibition is exerted for a short time on other signaling pathways by the red-orange step-down eliciting the primary photophobic response, whereas this does not occur if the test stimulus is a pulse of the same wavelength. This point obviously deserves a deeper analysis, which will constitute the subject of a forthcoming paper.

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REFERENCES

Alam, M., M. Lebert, D. Oesterhelt, and G. L. Halzelbauer. 1989. Methyl-accepting taxis proteins in *Halobacterium halobium*. *EMBO J.* 8:631–639.

Häder, D. P., and K. Vogel. 1991. Simultaneous tracking of flagellates in real time by image analysis. *J. Math. Biol.* 30:63–72.

Hildebrand, E., and A. Schimz. 1987. Role of the response oscillator in inverse response of *Halobacterium halobium* to weak light stimuli. *J. Bacteriol.* 169:254–259.

Krohs, U. 1994. Sensitivity of *Halobacterium salinarium* to attractant light stimuli does not change periodically. *FEBS Lett.* 351:133–139.

Krohs, U. 1995. Damped oscillations in photosensory transduction of *Halobacterium salinarium* induced by repellent light stimuli. *J. Bacteriol.* 177:3067–3070.

Marwan, W., and D. Oesterhelt. 1987. Signal formation in the halobacterial photophobic response mediated by a fourth retinal protein (P_{480}). *J. Mol. Biol.* 195:333–342.

McCain, A., L. A. Amici, and J. L. Spudich. 1987. Kinetically resolved states of the flagellar motor switch and modulation of the switch by sensory rhodopsin I. *J. Bacteriol.* 169:4750–4758.

Olson, K. D., X.-N. Zhang, and J. L. Spudich. 1995. Residue replacements of buried aspartyl and related residues in sensory rhodopsin: D201N produces inverted phototaxis signals. *Proc. Natl. Acad. Sci. USA.* 92: 3185–3189.

Petracchi, D., S. Lucia, and G. Cercignani. 1994. Photobehaviour of *Halobacterium halobium*: proposed models for signal transduction and motor switching. *J. Photochem. Photobiol. B Biol.* 24:75–99.

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

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.

Spudich, E. N., S. A. Sundberg, D. Manor, and J. L. Spudich. 1986. Properties of a second sensory receptor protein in *Halobacterium halobium* phototaxis. *Proteins.* 1:239–246.

Spudich, E. N., T. Takahashi, and J. L. Spudich. 1989. Sensory rhodopsins I and II modulate a methylation/demethylation system in *Halobacterium halobium* phototaxis. *Proc. Natl. Acad. Sci. USA.* 86:7746–7750.

Spudich, J. L., and R. A. Bogomolni. 1984. The mechanism of colour discrimination by a bacterial sensory rhodopsin. *Nature.* 312: 509–513.

Takahashi, T., H. Tomioka, N. Kamo, and Y. Kobatake. 1985. A photosystem other than PS370 also mediates the negative phototaxis of *Halobacterium halobium. FEMS Microbiol. Lett.* 28:161–164.

Takahashi, T., B. Yan, P. Mazur, F. Derguini, K. Nakanishi, and J. L. Spudich. 1990. Color regulation in the archaebacterial phototaxis receptor phoborhodopsin (sensory rhodopsin II). *Biochemistry.* 29: 8467–8474.

Wolff, E. K., R. A. Bogomolni, P. Scherrer, B. Hess, and W. Stoeckenius. 1986. Color discrimination in halobacteria: spectroscopic characterization of a second sensory receptor covering the blue-green region of the spectrum. *Proc. Natl. Acad. Sci. USA.* 83:7272–7276.