## 'Test Flash' Interaction in the Electroretinographic Response of the Compound Eye

The progressive increase in flicker response amplitude as the separation of the recurrent flashes increases has been related to the existence of a 'neural suppression' factor, which decayed exponentially in proportion to the period of darkness separating successive flashes[1]. An alternative explanation can be given in terms of linear response superposition[2].

On the other hand, some data exist which suggest the possibility that the response to light stimulus is affected, in the sense of 'facilitation', by a stimulus immediately preceding. The ommatidial nerve fibre discharge of the compound eye of Limulus is increased after brief subliminal light stimuli[3]: the ERG of vertebrates presents phenomena of 'differentiation velocity', consisting in increased rates of rise and fall of ERG components caused by persisting effects of prior illumination[4].

A brief flash of high intensity is capable of inducing in the compound eye of *Ligia occidentalis* Dana (Crustacea: Isopoda) a 'state of facilitation' in the mechanisms responsible for ERG which persist for more than 2 min. Such 'facilitation' manifests itself with greater amplitude and increased rates of rise and fall of the components of the electroretinographic response at the second flash in comparison with the response to the first[5].

We have further examined the question by experimenting on the compound eye of *Schistocerca gregaria* FORSK.[6]. (Orthoptera: Acridoidea).

*Materials and methods.* The experiments, about 40 of them, were performed on 20 adult animals. The electroretinographic recording of the illumination potential of the compound eye in situ (the intact animal was fastened to a small platform with plasticine) or surgically isolated and with exposition of ommatidial region, or still more reduced to a thin 'slab' containing receptors and with subtotal removal of the ganglionic components, immersed in HOYLE's physiological saline[7] (for technical details connected with the setting-up of the preparations, see [8]), was carried out in the conventional way through stainless steel electrodes (having a dc resistance of a few thousand ohms) connected with a dual beam cathode-ray oscilloscope (Tektronix 502 A: ac amplifier). Some experiments were performed substituting NaCl in physiological saline with choline chloride, or adding nicotine $10^{-5}M$, ACh $10^{-3}M$ or ACh $10^{-3}M$ with eserine sulphate 0.01% to the physiological solution in which the preparation was immersed. The light stimulus was provided by flashes of intense 'white' light of short duration (130 joule, about 1 msec: photographic strobe lamp) and transmitted through a rod of perspex and focused on the preparation with quartz lenses. Opportune reductions of light intensity were obtained with grey filters; the interposition of an interferential filter (Schott & Gen., Mainz) supplied an almost monochromatic light stimulus.

Stimuli of longer duration (10, 20 and 40 msec) were provided by an incandescent light bulb fed by stabilized dc with manually operated shutter in the light path. Photocells (vacuum and selenium barrier layer) monitored the characteristics of the stimulus and the intensity of any background illumination.

*Results.* The ERG of the compound eye of *Schistocerca* contains a predominant monophasic cornea-negative component (component 1 of RUCK[9]), in which initial phase, the presence of a cornea-positive oscillation (component 3 of RUCK) is often noted. The ERG of *Schistocerca* belongs to the 'Tachycines type'[10].

In the dark-adapted compound eye (room illumination $< 0.1$ lux) the electroretinographic response to the first flash of light has distinctly less amplitude, the latency is much longer and the rate of rise of the negative wave of the ERG is lower than the corresponding values relating to the second flash, as long as the interval between flashes does not exceed 2 min. This can be observed both in the compound eye in situ and in the one isolated, as well as in the compound eye isolated with exposition of receptors (Figure 1) and in single slabs with almost total removal of the ganglionic components; this phenomenon of 'facilitation' is present also with 10, 20 and 40 msec flashes.

With regard to the rate of rise, this decreases exponentially with the increase of the time interval ($\Delta t$) between the first and the second flash; the mathematical expression which represents the phenomenon can be given as:

$$y = a \exp(-k\Delta t) + b$$

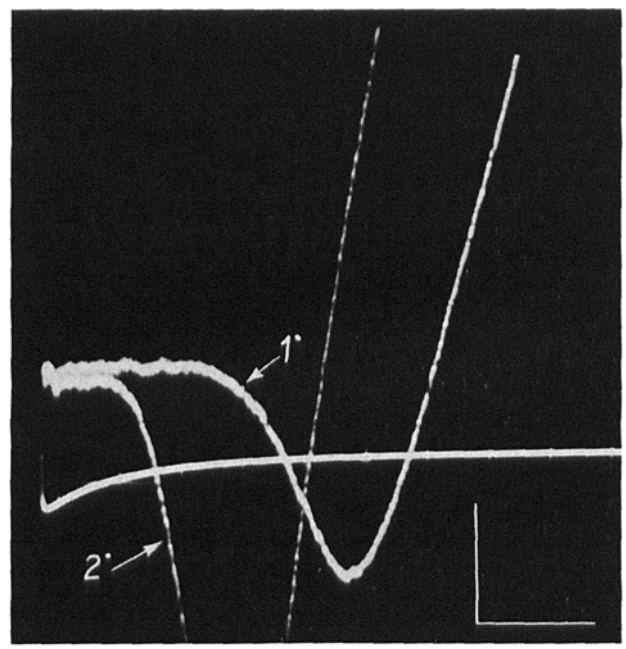


Fig. 1. Compound eye of *Schistocerca*, dark adaptation. Record showing the difference between the electroretinographic responses (superimposed) to first and second flashes. Interval between the flashes 5 sec. Calibrations: 200 $\mu$V and 10 msec; negativity downwards. The trace in the lower part of the record indicates the beginning of the stimulus.

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where $y$ is the rate of rise in $\mu$V/msec; $a$ and $k$ represent 2 constants (numerical value: 131.86 and 0.0719 respectively); $b$ is the value of the minimum asymptotic rate for intervals around 2 min; $\Delta t$ being the interval of time between the 2 flashes.

The expression $a$ exp $(-k\Delta t)$ would represent the factor of 'facilitation' which tends towards zero with the increase of $\Delta t$.

The 'facilitation' between the first and second flash is practically no longer present between the second and successive ones and so the electroretinographic responses from the second onwards appear almost identical. Such 'facilitation' disappears if the compound eye is under light adaptation conditions (Figure 3).

Substituting NaCl in the physiological saline with choline chloride, the phenomenon is still evident though with electroretinographic responses very much diminished.

The phenomenon of 'facilitation' is moreover evident in the spectral range 400-568 nm, disappears around 585 nm, and is completely absent using higher wave-lengths (the compound eye of *Schistocerca* presents a spectral sensitivity which extends from 400 to 679 nm; sensitivity to near UV has not been studied).

As the surgical removal of the ganglionic parts of the compound eye in slabs containing mostly receptors cannot be considered complete there is still the possibility that the effect observed comes about through the remaining nervous elements.

Adding nicotine $10^{-5}M$ (nicotine blocks sinapses in insects[11]) to the physiological saline in which the eye was immersed, the above described phenomenon is still clearly evident. It is also present adding ACh $10^{-3}M$ or ACh $10^{-3}M$ with eserine sulphate 0.01% to the solution (these drugs, as is known, increase the spontaneous activity of the nervous system in insects[12]). These experiments, not being conclusive, lead us to suppose that the retinal ganglionic elements are not the decisive factors in the manifestation of the phenomenon described.

*Conclusion.* The persistence of the phenomenon of 'facilitation' in slabs which are almost without ganglionic elements, or in preparations of the compound eye with synaptic nicotine block, or again in preparations with increase of the spontaneous activity of the C.N.S. by ACh and eserine, leads us to think that this happens at photoreceptor cell level.

Changes have recently been described in the latency and response amplitude of the retinal action potentials recorded from single cells in the drone compound eye, after 'off' of a steady light[13]. According to these results, the most conspicuous effect of light adaptation is a decrease in the latency, while the rising phase of the response becomes steeper. The authors claimed, for the interpretation of these facts, the existence of a membrane feedback system, which reduces the potential level and prevents overloading of the cell by a large step input.

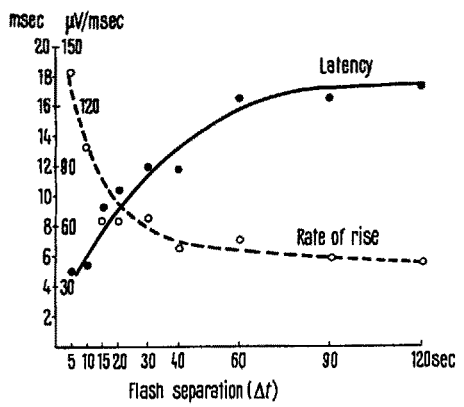


Fig. 2. Latency (msec) and rate of rise ($\mu$V/msec) of the second electroretinographic response of the dark adapted compound eye plotted as a function of the interval between the first and the second flash ($\Delta t$ sec).

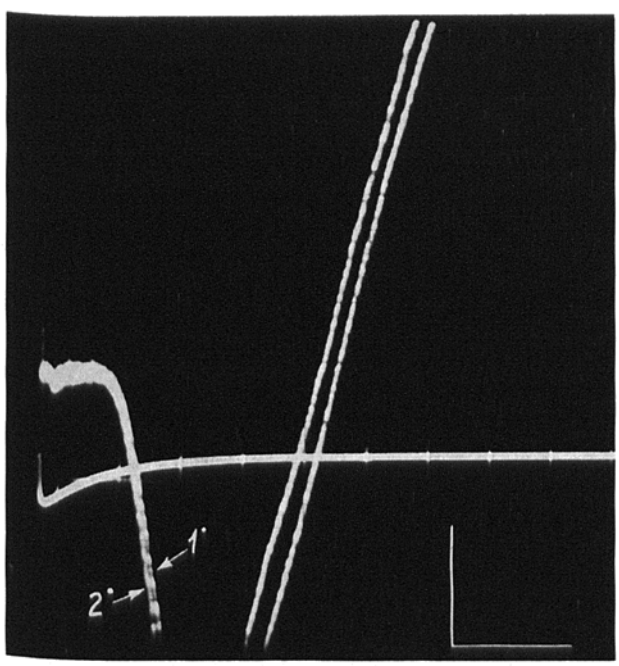


Fig. 3. The 2 stimulus flashes were superimposed to the background illumination (1000 lux; flash separation 5 sec): the 'facilitation' disappears completely. Other explanations see Figure 1.

*Riassunto.* L'occhio composto di *Schistocerca gregaria* Forsk., adattato al buio, è sede di un effetto «facilitatore» in seguito al primo stimolo luminoso; infatti la risposta elettroretinografica al secondo stimolo – purchè l'intervallo fra i due non superi i due minuti – presenta una latenza più breve e una ampiezza e una velocità di salita del potenziale più grandi. L'effetto descritto si verifica, con tutta probabilità, a livello delle cellule fotorecettrici.

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