"Prebumps": Evidence for Double-Hits at Functional Subunits in a Rhabdomeric Photoreceptor

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In fly photoreceptors the latency of "bumps" due to single photon absorption is in the mean 60–70 (± 20) ms (10 °C). With increasing stimulus intensities first the amplitude of the receptor potential increases without shortening of the latency; with still higher intensities the latency is reduced also. The first reduction occurs if neighboured microvilli are simultaneously hit by one light quantum each or if individual microvilli are hit by two quanta. Such "double hits" lead to a discrete reduction in latency which is manifested as a "prebump".

The receptor potential of rhabdomeric photoreceptors is considered as a superposition of individual "bumps", whereby each "bump" is caused by absorption of a single light quantum [1]. A difficulty of this concept arises from the fact that the latency of the receptor potential decreases with increasing stimulus intensity. Actually, if short flashes (1/e-time 2 ms) are used as stimuli, the latency is strictly related to the number of absorbed and efficient quanta, that is also to the amplitude of the response (Fig. 1). Variation of latency of single bumps, induced by very weak stimuli, is rather small, ± 20 ms about a mean of 60–70 ms at 10 °C. The receptor response to slightly higher intensities (10–30 absorbed quanta per flash) can be approximated by the superimposition of these statistically distributed "bumps". These synthesized signals are similar in latency, peak time and duration. It is impossible, however, to synthesize the receptor response to higher intensities by this summation technique since the latency of no single bump is ever as small as 5–7 ms, which is the range of latency exhibited by responses at saturating intensities. Hence an acceleration of the system must be due to simultaneous multiple absorptions of quanta in the rhabdomere.

We determined the number of absorbed quanta at which the first reduction in latency can be detected. It occurs if 30 to 300 quanta are simultaneously absorbed per rhabdomere. These numbers have been estimated on the basis of visual pigment photometry as well as on " bump" counts. 30 to 300 absorbed quanta, respectively, correspond to a mean distance of absorbed quanta in the rhabdomere of 7 to 0.7 µm, if we take the length of the rhabdomere of 200 µm into account. This seems to indicate that the reduction in latency is due to an interaction between excited centers over considerable distances (200 to 20 rows of microvilli). However, we have to keep in mind that the actual excited centers are not distributed equally but according to the Poisson statistic, which allows to calculate the following numbers.

Within each rhabdomere we have approximately 10^8 rhodopsin molecules in about 10^5 microvilli. In order to get on the average one double hit at one of the rhodopsin molecules we need more than 10^4 absorbed quanta. In order to get an average of one double hit at individual microvilli we need 440 quanta absorbed. Double hits at directly neighboured microvilli are expected already if only 125 quanta in the mean are absorbed. It is assumed in these calculations that the rhabdomere is homogeneously illuminated, that it has a length of 200 µm and that it is constant in thickness over its length.

Double hits at individual rhodopsin molecules can be excluded as a cause for the reduction in latency since 10^4 quanta certainly have not been absorbed when the first shortening of the latency can be observed. According to our data it is most likely, that double hits at neighboured microvilli are just sufficient to reduce the latency. However, we cannot yet exclude with certainty that double hits at individual microvilli are necessary. In order to discrim-

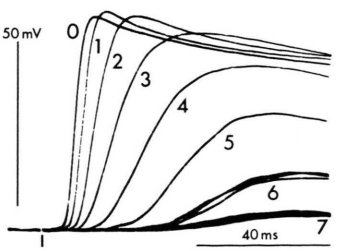

Fig. 1. Intracellularly recorded receptor potentials in response to blue flashes of increasing intensities (vertical bar indicates the onset of the flash, 1/e-time = 2 ms). The latency of the response, at different intensities varies between 6 and 40 ms (*Calliphora* ♀, mutant, chalky, 10 °C). Numbers indicate the negative logarithm of the relative stimulus intensities. Three traces have been superimposed at $I = 10^{-7}$ and $I = 10^{-6}$, respectively.

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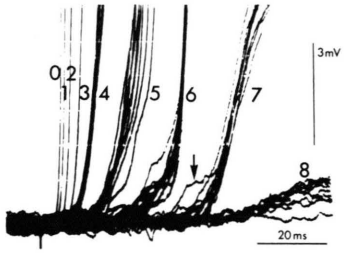

Fig. 2. Receptor potentials to flashes of increasing intensity recorded at a high gain in order to demonstrate the occurence of "prebumps". At relative intensities of 10^{-4} to 10^{-8} 10 traces have been superimposed, at $I = 10^{-3}$ two traces. Responses to stimuli at 10^{-2} to 10^{0} have been recorded once each.

inate between the two cases, we have to determine the number of absorbed quanta to a factor better than 3.5. Since also still several parameters as mentioned above affect the estimate of the distance between excited spots, not yet taken into account, we need more quantitative data for a final decision.

In any case, irrespective of whether the reduction in latency is due to double hits at neighboured or at individual microvilli, we predict that its shortening occurs in a discrete manner, since double hits are qualitatively different from single hits.

The discrete shortening is demonstrated in Fig. 2. As can be seen at a relative intensity of 10^{-8} individual "bumps" can be recorded. At $I = 10^{-7}$ the amplitude of the response is increased. However, the latency remains constant in 9 of 10 traces. In one trace, in addition to the normal response, a "prebump" (arrow) clearly can be seen. This "prebump" has a shorter latency, and an amplitude of approximately one millivolt, similar to the single "bumps". It is qualitatively different from the single "bump" by its steep rising phase (less than 10 ms compared to more than 20 ms). According to our interpretation this "prebump" is due to a double hit at either neighboured or at individual microvilli. Increasing the intensity by a further factor of 10 to $I = 10^{-6}$ shortens the latency since now many double hits occur, and, in addition, again a class of still faster "prebumps" can be descriminated. Calculation shows that these "prebumps" should be due to "triple hits". The further reduction in latency with still increasing intensity is interpreted as being due to higher order multiple hits.

The results show that the reduction in latency is caused by double hits either at neighboured or at single microvilli. To distinguish between these two cases seems to be of special interest since it allows to discriminate between different models of the functional organisation of rhabdomeric photoreceptors. In both cases the system of channels that change the membrane permeability must be within the microvilli or in close proximity. As can be seen in Fig. 2 the latency of individual "bumps" is rather long, 40 ms. Visual pigment transition in the fly is rather fast (500 µs at 10 °C, [2]). Since diffusion can be excluded as the reason the long latency can be considered to be due to the time needed for biochemical reactions necessary to trigger the channel system. *E.g.* an enzyme could become activated that produces a "transmitter", which has to overcome a threshold concentration in order to produce a "bump".

If double hits at neighboured microvilli cause a "prebump" then the shortening of the latency can be interpreted as being due to an increased production of a transmitter, originating from two enzyme centers, which therefore reaches the threshold concentration earlier. One and the same channel system in this case should be accessible from neighboured microvilli. The fine structure of the rhabdomere suggests that the channel system could be localised at the "bubble" located at the basis of the microvilli [3]. If double hits at individual microvilli create a "prebump", the shortening of the latency could be interpreted as being due to the fact that the microvillus is activated not only from one but from two active centers, whereby its time of action is shortened.

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