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ELECTROPHYSIOLOGICAL EVIDENCE FOR PUSH-PULL INTERACTIONS IN THE INNER RETINA OF TURTLE*

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The responses of the inner retinal neurons of turtle to light spots of sizes were studied in an attempt to reveal characteristics that may reflect possible interactions of the neural circuits underlying the center and surround responses. For the ON-OFF cells, the responses were also analyzed to observe whether interference or augmentation of these responses occur.

The intracellular recordings revealed several such interactions, observed either in the form of altered spike activity or as changes in the transiency of the light responses. The ON-responding amacrine cell presented in this study became more sustained, while for the ON-OFF amacrine cells larger light spots tended to make the responses more transient and both the ON and OFF components became more pronounced. The spiking activity of the OFF-type ganglion cell shifted in relation to the light stimulus and the number of spikes observed upon presentation of larger spots increased.

We suggest that the surround circuits activated by increasing light spots may substantially influence and reorganize not only the overall center-surround balance, but also the center response of the cells. Although it cannot be excluded that intrinsic membrane properties also influence these processes to some extent, it is more likely that lateral inhibition and disinhibitory mechanisms play the leading role in this process.

Keywords: Amacrine cells – ganglion cells – ON/OFF response – center-surround – lateral inhibition

INTRODUCTION

Vision is the sense that most prominently influences the reactions of most vertebrates to environmental clues. It is not surprising, therefore, that substantial efforts have been directed to unraveling the neural circuits that underlie visual information processing. This article is dedicated to József Hámori on the occasion of his birthday. During his scientific career, among many other problems, Prof. Hámori has devoted his attention to those portions of the central nervous system of mammals where this process occurs: the subcortical relay stations, and particularly the lateral geniculate nucleus [14, 20, 22], the visual cortex [12, 23, 25] and the retina [21]. He has made lasting contributions to the understanding of the anatomy and the functions of all

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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these structures. He has always been interested in how inhibition carves and strengthens the essential elements of the visual signals transmitted to the brain [10, 11, 14, 19]. Most recently, he turned to studies of the retinal networks, where inhibitory pathways exert a particularly powerful influence on signal processing [8].

Two basic electrophysiological features of the functional organization in vertebrate retinal neurons have been described: the ON/OFF light response properties [15] and the antagonistic center-surround organization of the receptive fields [4]. While the former feature seems to be mostly created in the outer retina and can be related to the type of glutamate receptor that is present on the bipolar cell dendrites and their axonal arborization pattern in the inner plexiform layer (for a review, see [24]), the latter is thought to be generated mainly in the inner retina by the activity of the amacrine cells [5, 7]. Moreover, active propagation of the signal appears to be required in order to maintain a regular center-surround organization [27]. The amacrine cells are the third-order interneurons of the vertebrate retina occurring in 20–30 types, depending on the species [18]. Most of them seem to contain gamma-aminobutyric acid (GABA) or glycine as a transmitter; thus they are supposed to be inhibitory in nature. In amphibians and reptiles, they are particularly strongly represented among the retinal cell types [17, 26, 29]. This is because the retinal circuits of lower vertebrates process more elements of the visual information than do those of mammals, possibly because of the lack of a large visual cortex in their brain [8]. From this regard, turtles have been especially well investigated. The anatomy and physiology of the various cell types [2, 17] and the quantitative aspects of the synaptology of the inner plexiform layer [13] have all been studied in some detail. With this background knowledge we set out to study the light responses of inner retinal neurons, with particular attention to the effect of increasing size of the stimulating white light. We hoped to reveal characteristics that may reflect possible interactions of the neural circuits underlying the center and surround responses. For the ON-OFF cells, we planned to analyze the responses in order to establish whether interference or augmentation occurs.

MATERIALS AND METHODS

Adult turtles *(Pseudemys scripta elegans)* of either sex were used for this study. The animals were sacrificed by decapitation, and under normal room light the eye was enucleated and hemisected and the lens was removed. Four radial slices were made and the resultant flattened eyecup was pinned to the bottom of a wax chamber. The chamber was mounted within a light-tight Faraday cage and the vitreal surface was superfused with Ringer solution (21–23 °C). The composition of the Ringer solution was 100 mM NaCl; 20.5 mM $NaHCO_3$; 2.5 mM KCl; 1.2 mM $MgCl_2$; 1.8 mM $CaCl_2$; 5 mM glucose. Solutions were gassed continuously with 95% O_2 plus 5% CO_2 so as to maintain a pH of 7.4.

Intracellular recordings were obtained by using aluminosilicate micropipets filled with 4 M potassium acetate (resistance 250–700 MΩ).

A one-channel optical system was used to provide a white light stimulus; intensity was 13.86 log quanta/cm^2/s, and its duration 500 ms, presented twice within an interval of 5500 ms. The employed spots were 690 μm (spot 1), 1150 μm (spot 2), 2450 μm (spot 3), 3750 μm (spot 4) in diameter.

The light-evoked responses were amplified conventionally by an Axoclamp-2B amplifier (Axon Instruments) and digitized by a Digidata 1320A (Axon Instruments). Data acquisition was carried out with the Axoscope 8.0 program.

RESULTS

Intracellular recordings revealed that both the ON/OFF properties and the kinetics of the responses elicited by white light spots of increasing size but with the same intensity differed considerably. More than 100 cells were tested in the experiment, about half of them showing some, change in response properties. In 13 cases, these changes were unequivocal. Effects were observed on both ON-responding (n = 5) and OFF-responding (n = 2) cells of the inner retina. Whereas the ON and OFF cells responded by changing their kinetics, the ON-OFF cells (n = 6) exhibited a more sophisticated alteration in their physiological properties.

Analysis of ON cells

Some of the neurons (n = 1) responded to the onset of the smallest diameter light stimulus (d = 690 μm) with transient hyperpolarization (Fig. 1a). Substantial increase of the spot size (d = 3750 μm) resulted in a significant change in the response. A plateau phase appeared which lasted with small decrements while the lights were on (Fig. 1b). The interstimulus noise was small, in the range of ±2 mV around the dark resting membrane potential level. No other apparent changes in response properties could be observed. Thus this cell changed its transient light response to a sustained response upon increase of the stimulus size. Further increase of the size of the stimulating light spot was without effect. The response of this cell did not match any of the response types described by Ammermüller and Kolb [2, 3].

Another ON amacrine cell (n = 4) encountered during these experiments responded to the smallest spot size (d = 690 μm) with sustained depolarization without action potentials (Fig. 2a). On the basis of its light response, we tentatively identified this cell type as the A28 cell of Ammermüller and Kolb [2]. When a light spot somewhat larger than the first spot was presented (d = 1150 μm), the initial response of the cell developed faster and grew bigger (Fig. 2b). We also noted that the initial spike-like response was followed by a sustained plateau, and at lights-off a second spike-like component appeared. After this, the membrane potential returned to the dark resting level without delay. A substantial further increase of the spot size (d = 3750 μm) caused a prominent shift in the course of the light response (Fig. 2c). First, a fast and transient depolarization occurred, after which the membrane potential returned to the

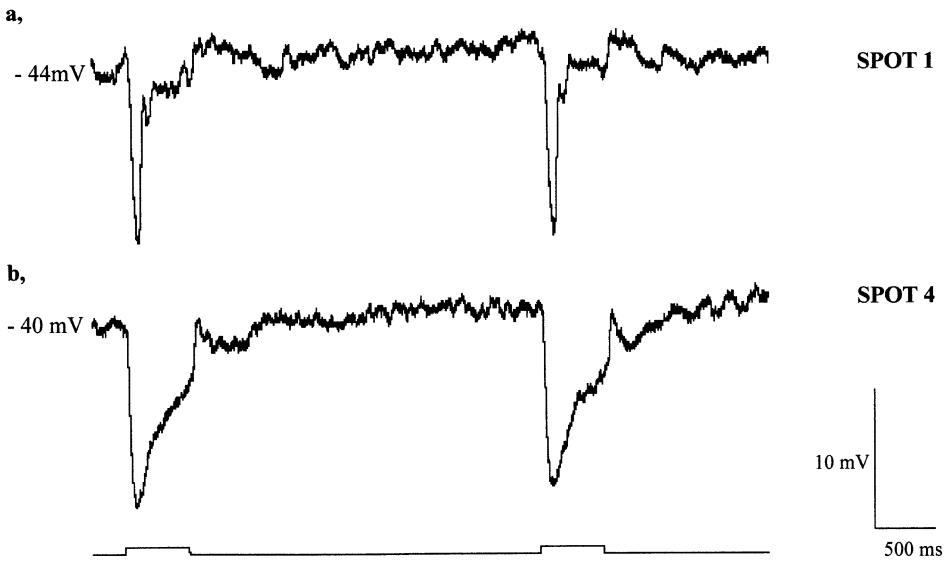

Fig. 1. Light responses of a hyperpolarizing ON amacrine cell to spots of different diameters (a, spot 1; b, spot 4)

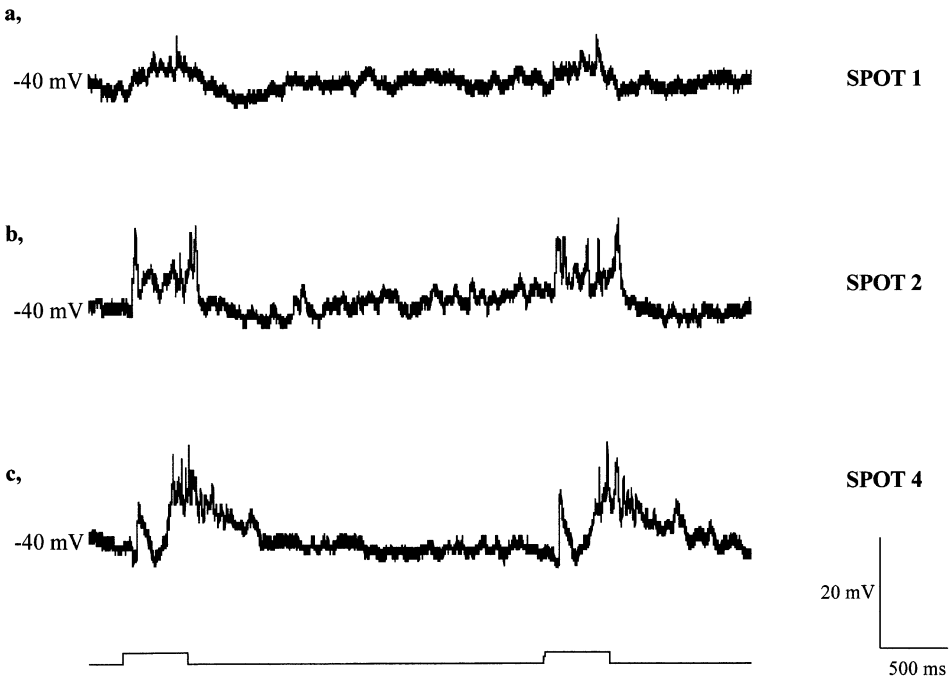

Fig. 2. Light-evoked response changes of ON depolarizing amacrine cell induced by different spot sizes (a, spot 1; b, spot 2; c, spot 4)

dark resting level momentarily, to give way to another depolarization which began before lights-off. This second depolarization was sustained and the membrane potential returned to the dark resting level only after 500 ms. A further increase of the spot size did not change the response characteristics.

Responses of OFF cells

In response to a small diameter white light spot (d = 1150 μm), an OFF ganglion cell produced multiple spikes after marked depolarization at lights-off. After a short series of discharges the membrane potential fell back quickly to the dark resting level (Fig. 3a). When a large spot (d = 3750 μm) was presented, the initial depolarizing step was still observed but, spikes were not fired by the cell (Fig. 3b). The membrane potential returned to the resting level for a brief interval, just to give rise to a long, sustained depolarization crowned by a long series of action potentials. This spike train was generated between two adjacent light stimuli. Increase of the spot size further resulted in a decrease in the frequency, but not in the duration of the interstimulus spike train (not illustrated). Only two such cells were encountered in our sample and this cell type did not match any of those described by Ammermüller and Kolb [2, 3], merely resembled the G18 cell slightly.

Changes in ON-OFF responses

An amacrine cell reacting to both lights-on and lights-off exibited prounced transient depolarizations without spiking activity in response to small (d = 690 μm) spots (Fig.

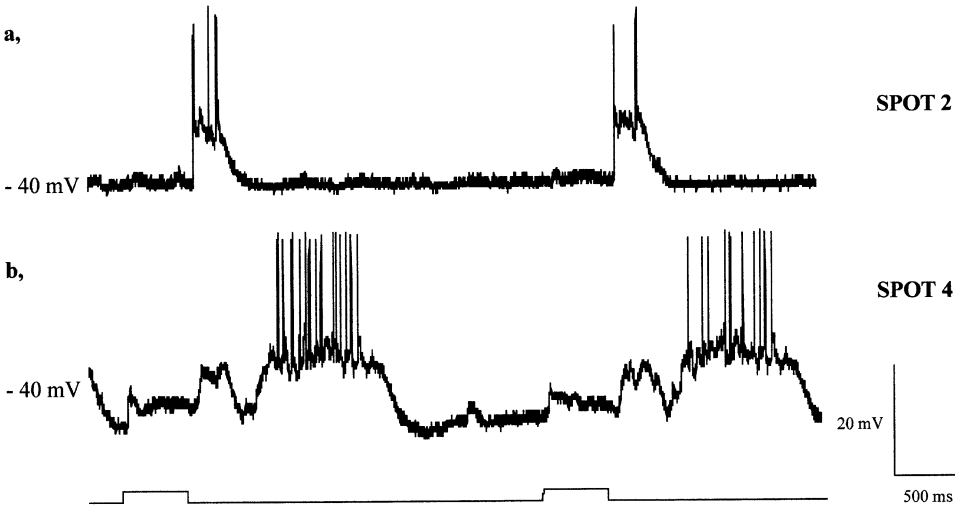

Fig. 3. Effects of increasing spot sizes on the light responses of an OFF ganglion cell (a, spot 2; b, spot 4)

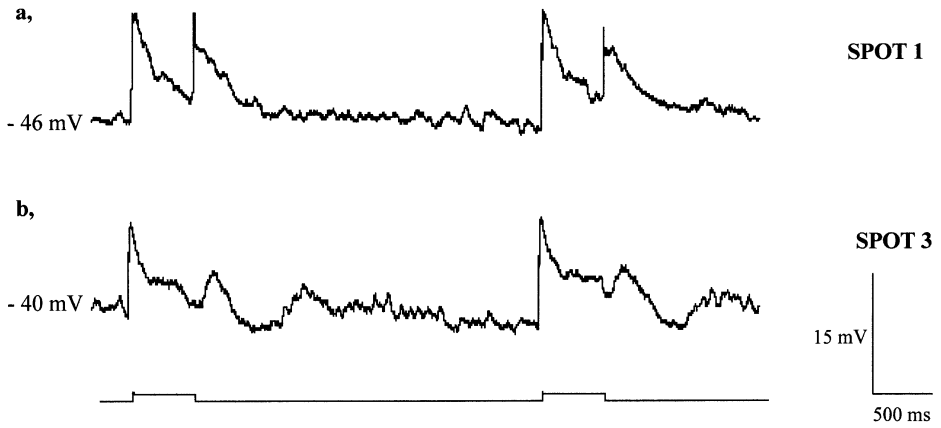

Fig. 4. Light responses of an ON-OFF amacrine cell to different diameter spots (a, spot 1; b, spot 3)

4a). This response type most closely resembled that of the A30 cell identified by Ammermüller and Kolb [3]. On increase of the spot diameter by about 4 times (d = 2450 μm), the ON part of the response became sustained, reaching a plateau at two-fifths of the stimulus length. At lights-off the membrane potential fell back to the resting level briefly; then, with a short (~100 ms) latency, a transient depolarization developed (Fig. 4b). By the next light stimulus the membrane potential had returned to the normal dark level. A further increase of spot size did not cause changes in the response character.

DISCUSSION

Center-surround properties of the inner retinal neurons are created by lateral inhibitory processes mediated by amacrine cells [7]. Changing physiological responses to increasing spot sizes have been noted [2, 3], but the physiological significance and the possible underlying circuit have not been clarified. The results of this study however indicate that excitation of the neurons which create the inhibitory surround may be involved in forming these circuits unique to the different neuron types. Only latency changes, but not waveform alterations, could be evoked when contrast steps were applied with the spot diameter constant [6]. An ON-OFF response interaction was recently described as a function of intracellular Ca^{2+} concentration [1]. In this case, however, only the depolarizing OFF component changed, and time constraints were also observed. The interactions we have described in this study must therefore stem from the structure of the inhibitory surround circuits.

Four types of interactions have been presented here and all of them seem to require more than one type of interneuron. Indeed, 37 amacrine cell types have been identified in the turtle retina [3, 17], providing a morphological basis for these interactions.

Disinhibitory circuits may be more frequent and more important in the functioning of the inner retina than previously thought [28]. Furthermore, it is known that amacrine-amacrine synapses are more frequent in turtle than in mammals [13]. Since close to 90% of the amacrine cells in turtle contain GABA or glycine [9, 16], it is reasonable to suppose that most of the synapses formed in the inner plexiform layer in turtle are inhibitory and many of them are disinhibitory.

With the roles of disinhibitory mechanisms in mind, we have attempted to identify the possible circuits that may underlie the changes in the response to increasing spot sizes. In the case of the first cell described above a transient hyperpolarizing ON response changed to a sustained response. This subtle change, however, can be borne out by a relatively elaborate circuitry (Fig. 5). It should involve an ON bipolar cell that should synapse with a bistratified amacrine cell, which could disinhibit the recorded amacrine cell. To produce a transient response, the ON bipolar cell must have a short negative feed-back circuit from a narrow-field amacrine cell. By shining a large spot on the retinal surface, we could activate another ON bipolar cell type which could excite an ON amacrine cell. This amacrine cell in turn should be able to inhibit the amacrine cell which provides a negative feed-back to the first bipolar cell thus making its response elongated.

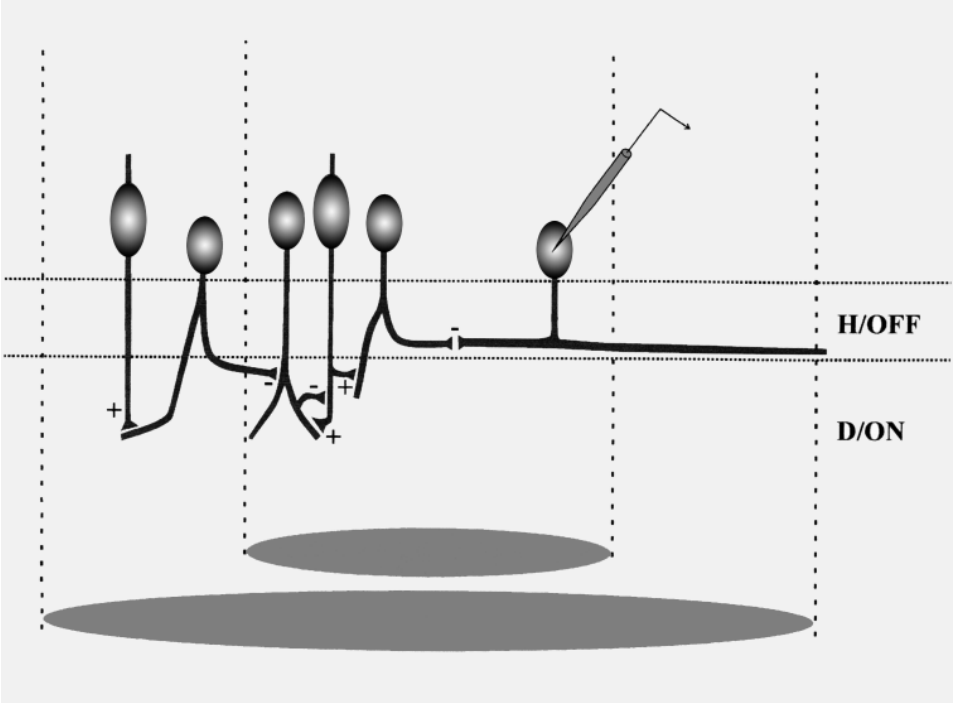

Fig. 5. Possible neuronal interactions elongating the response of the cell recorded in Fig. 1

The second cell type was an amacrine cell (A28) [2, 3]. It was encountered more frequently than the other cells presented in this study and perhaps also underwent the most marked changes in response to spot size increase. The initial response can be explained by a simple input from an ON bipolar cell, to which our cell responded with a slow sustained depolarization (Fig. 6). When the spot size is increased slightly, it is sufficient for another bipolar cell to join in, which could activate an inhibitory amacrine cell to truncate the response of the recorded cell. To enhance the light-off component, a third circuit, involving a wide-field bistratified amacrine cell producing a disinhibitory effect on the recorded cell could result in a more pronounced and sharp OFF response (Fig. 6).

The third cell is a ganglion cell which could not be found in the catalogue of Ammermüller and Kolb [2, 3]; it seems to have a less complicated center-surround circuit. The cell responds with a burst of spikes at lights-off, and this pattern remains unchanged. However, a subtle alteration in the spike onset (500 ms delay) and an elongated spike activity (spike number per light stimulus triples) suggest a refined surround circuitry which involves disinhibition. A hypothetical circuit for this cell (Fig. 7) may be that, for small spots only the OFF bipolar cell that drives the light response of the ganglion cell is active and dominates the electrical activity of the OFF ganglion cell. It also activates an OFF amacrine cell, but this cell has no direct

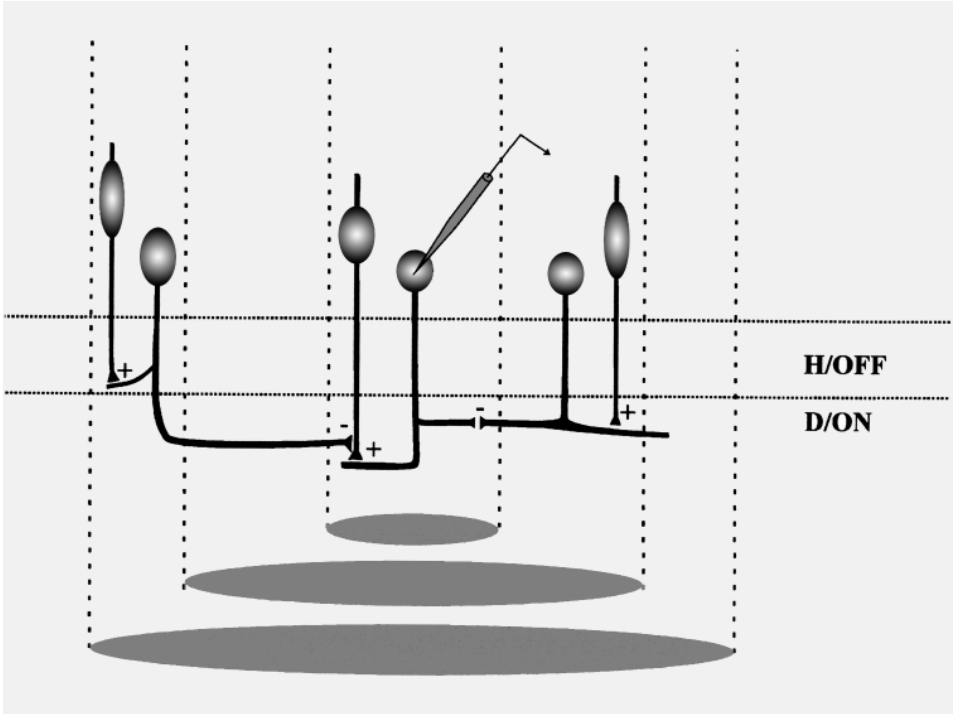

Fig. 6. Presumed circuit making sustained ON responses more transient and ON-OFF in character

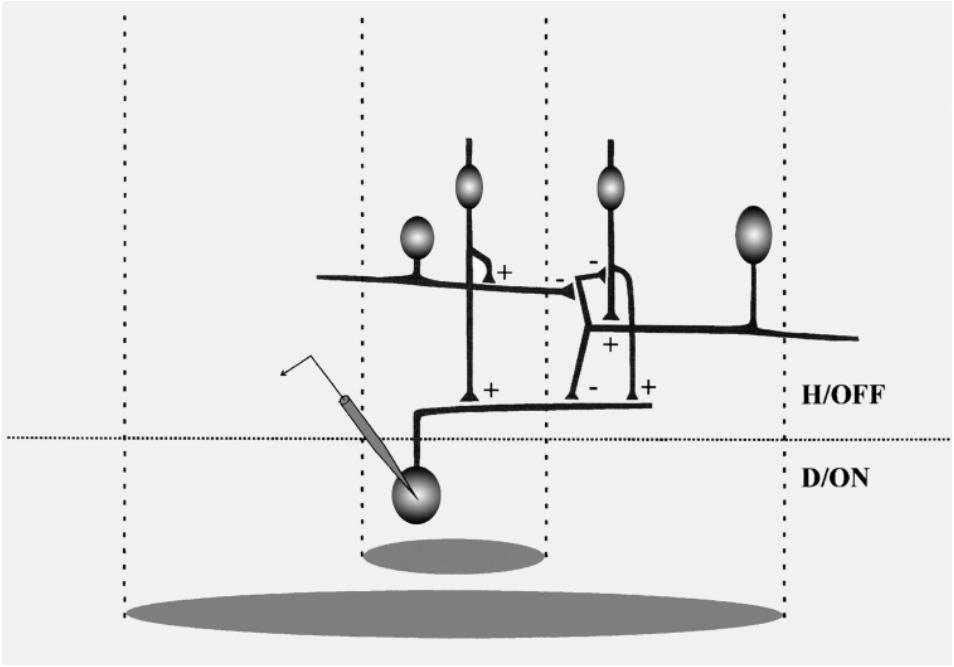

Fig. 7. Hypothetical center-surround circuit of the OFF ganglion cell recorded in Fig. 3

connection to the ganglion cell which we record from, and therefore its activity has no direct influence on it.

In the fourth case, though the change in response is subtle, the underlying circuit seems to be the most sophisticated of all the cases described in this paper (Fig. 8). It may involve both ON and OFF bipolar cell inputs to the recorded cell, as well as negative feed-back and negative feed-forward circuits which converge on the bipolar cell terminals. It supposes an enormous concentration of information-processing power at the bipolar cell terminal which at the morphological level is manifested as serial synapses. Such structures have been described in the inner plexiform layer of the turtle retina [13] and have recently been analysed in the rabbit retina from this point of view [28]. The conclusions in both reports cited above agree with our experimental data as far as the importance of negative feed-back and feed-forward and also disinhibitiory processes are concerned.

As a general conclusion, we suggest that surround circuits activated by increasing light spots may substantially influence and reorganize not only the overall center-surround balance, but also the center response of the cells. Although it cannot be excluded that intrinsic membrane properties too influence these processes to some degree [1], it is more likely that lateral inhibition plays the leading role in this process [7].

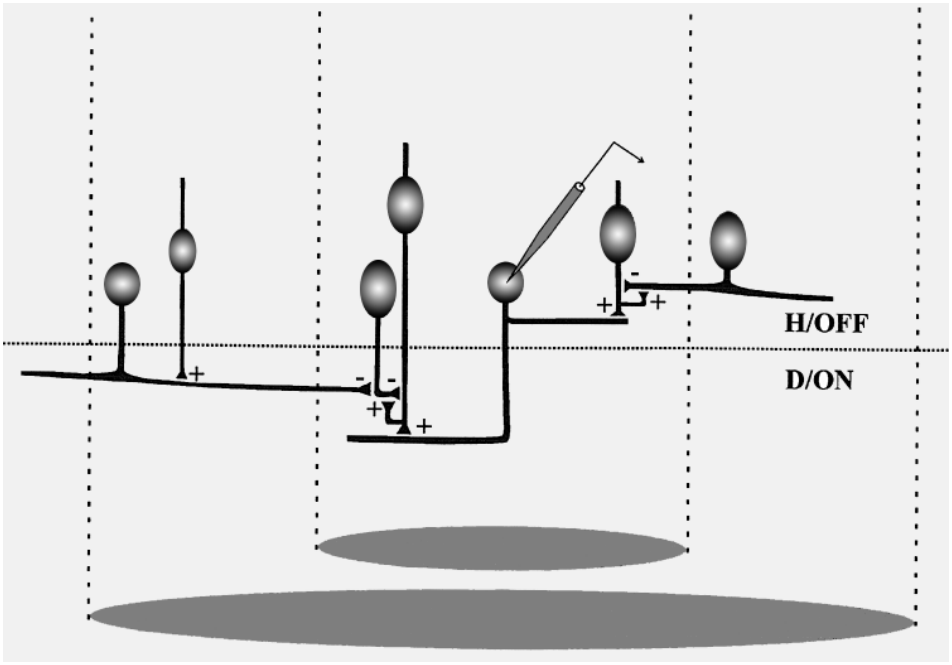

Fig. 8. A circuit diagram that describes how ON-OFF responses may be less prominently shaped but more sustained

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