Determination of Ultramicroelectrode Tip Position in the Retina in Relation to S Potential

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In a previous paper, this author presented histological evidences that the S potential originated in the secondary neuronal layer of the retina (Mitarai, 1958). This finding is in general agreement with the results obtained independently by other investigators (MacNichol, Macpherson, and Svaetichin, 1957; Tomita, Murakami, Sato, and Hashimoto, 1959; Oikawa, Ogawa, and Motokawa, 1959). The present paper gives more detailed information on the localization of the typical response. Due to the limitations of the histological methods so far used for the localization of the microelectrode tip in the retina, the accurate origin of the S potential has not yet been determined. The work to be described is a further attempt to show the origin of the S potential by the use of an improved lithium carmine method developed by the author (Mitarai, 1958). In this paper a detailed description is given of this improved method and its limitations.

METHODS

Microelectrodes filled with a lithium carmine solution were used for the recording and for local staining of the tissue. The lithium carmine was prepared by adding the carmine to a saturated lithium carbonate solution; in a similar manner as when preparing the solution which is frequently used for supravital staining. Lithium carbonate is an alkaline powder and will dissolve in water but not in alcohol, while the carmine is soluble in basic solutions but not in acid ones. Further, the lithium carmine is soluble in water but not in alcohol, nor in Bouin's solution. This is important since the retina has to pass through these solutions during the process of fixation. Thus, the characteristics of the stain served for the purpose of this study.

Particular attention was given to the preparation of a clear solution of the stain by filtrating it every time before it was poured into the pipette, since

when the solution was stored particles were formed in it which would hinder the electrophoretic transport through the microelectrode tip. For this reason the ultramicropipettes, which had a tip diameter of less than $0.5\ \mu$, were filled with the stain solution only a moment before the experiment started. The pipettes showed an electrical resistance of 20 to 40 meg Ω and served well as recording electrodes.

The lithium carmine is charged positively, and therefore moves towards the negative pole during the electrophoretic action. The amount of current which is needed to remove a small amount of the stain from the ultramicroelectrode could be estimated when the lithium carmine electrode was inserted into a Ringer gelatin through which a direct current was passed. It was found that a spot of stain around the electrode tip could be produced in less than 1 second by a current between about 2 and 4 μa .

The recording of the S potential was carried out on the isolated fish retina. The eye of the *Lutianidae sp.* was used to obtain the luminosity type of response, and that of the *Centropomidae sp.* for the chromatic response. The techniques used for recording and for the preparation of the retina were based on the original description by Svaetichin (1953).

After the recording of a typical S potential, the lithium carmine was introduced into the retina by the electrophoretic method mentioned above. About ten spots were marked inside 1 cm.² of the retina, after which the retina was fixed in the Bouin's solution, and serial paraffin sections were cut having a thickness of 7 to 10 μ . The specimen was left unstained in order to avoid the washing out or movement of the lithium carmine. The retina had a yellowish color due to the picric acid of the Bouin's solution, and the red spots, which corresponded to the position of the electrode tip, showed up clearly against the background color.

RESULTS

Large luminosity responses were easily picked up from the isolated retinas of the *Lutianidae sp.* and the *Centropomidae sp.*, whereas the chromatic responses were not found in the retinas of the former. Hence, the *Lutianidae sp.* was used for determining the origin of the L type of responses and the *Centropomidae sp.* for the chromatic responses. The lithium carmine appeared in the histological sections in two different states. Sometimes a grouping of small particles was seen and this was considered an artifact caused by mechanical movements during the procedures of washing, dehydration, fixation, and manipulation of the tissue. In other cases, a homogenous staining of a whole cell was observed, appearing as a real tissue staining in histological sense. As the stain particles in the first case (considered as artifact) could be found

widely distributed within the retinal structures and even outside the retina, only the second type of spots were considered for the purpose of these experiments.

A large number of sections were studied, and the second type of spot, showing a homogenous cell staining, was exclusively observed among the bipolar cells. The two micrographs (Fig. 1) which are reproduced in this paper, show typical stained spots corresponding to bipolar cells.

It is concluded from these findings that both the luminosity and the chromatic responses originate in the bipolar cells.

DISCUSSION

On the basis of previous experiments the origin of the S potential has been assumed to be: (a) the horizontal cells for luminosity, and the bipolar cells for the chromatic response (MacNichol, Macpherson, and Svaetichin, 1957); (b) the horizontal and the amacrine cells (Mitarai, 1958); (c) the bipolar cell layer and the inner plexiform layer (Tomita, 1959); and (d) the horizontal cells (Oikawa, Ogawa, and Motokawa, 1959).

The methods for localizing the recording position of the microelectrode, as used by the above mentioned authors, made possible an approximate location of the retinal cell layer from which the S potential originated. However, the techniques were not accurate enough to determine the origin of the response to a single cell. The improved lithium carmine method used in the present study proved to be suitable for the staining of small spots in the retina without causing any microscopically visible harmful effects to the tissue.

According to the description of MacNichol, Macpherson, and Svaetichin (1957), the chromatic responses were recorded 20 to 30 μ deeper than the luminosity responses, when the electrode was introduced from the receptor side into the retina.

In the present study, the colored spots observed in the sections of the retina obtained from the *Centropomidae* sp., which gave the chromatic responses, were located in a region further away from the receptor layer than the spots seen in the sections obtained from the *Lutianidae* sp., which gave the luminosity responses. In these experiments, however, two species of fish were used for determining each different type of response, and therefore, it is possible that the difference observed in the depth of the origin of the responses is due to species differences in the organization of the retina.

On the basis of the present experiments it was not possible to decide whether the S potential represents an intracellular recording (Svaetichin, 1953; Mitarai, 1958; Oikawa *et al.*, 1959) or not (Tomita *et al.*, 1959).

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FIGURE 1. Micrographs of retinas of Centropomidae *sp.* (left side) and Lutianidae *sp.* (right side) showing the red spots of lithium carmine introduced electrophoretically from the tip of the recording microelectrode. The position of the spot is indicated by the arrow. Scale: 10μ .