RECORDING OF ACTION POTENTIALS FROM SINGLE CELLS IN THE FROG RETINA

Ken-ichi Naka*, Sono Inoma, Yoji Kosugi and Chih-Wen Tong**

Department of Physiology, School of Medicine, Keio University, Shinjuku-ku Tokyo

The recent work of Tomita and his collaborators14) proved definitely that the ERG is a mass response and in no way represents the activity of the individual retinal elements. In order to proceed the research in more analytical way, it is desirable to know how the individual cells respond to photic stimulation of the retina. The only paper that reports recording of responses intracellularly is the one by Wiesel16) who worked on the cat’s unopened eye. The slow action potential, or the S-potential, from the fish retina by Svætichin and others7, 8, 9, 10) has been considered to be intracellular, but there are several pieces of evidence that make one hesitate to be convinced of it12, 15). This paper will deal with results of our preliminary experiment in which responses in the frog retina were explored with superfine microelectrodes.

METHOD

The opened eye of the bullfrog, Rana catesbiana, and occasionally of Japanese toad, Bufo vulgaris, was used as material. The vitreous humor in the eye was drained off as far as possible with the aid of pieces of ‘Kleenex’ tissue. Micropipettes were inserted vertically from the anterior surface of the retina through the internal limiting membrane. The retina was stimulated either by small spot of light (140 to 280 micra in diameter) or by diffuse light over the retina. In some cases, monochromatic lights of 440 mμ and 610 mμ obtained through interference filters were used for illumination. The amplifier and recording system were the same as described elsewhere14).

The micropipette used was of a resistance more than 30 megohms when measured in 3M-KCl solution. The glass material was SB-24 (Shibata Kagaku, Tokyo) and 3M-KCl solution was filled by boiling.

Received for publication January 7, 1960.
*Present address: Department of Biology, Faculty of Science, Kyushu University, Fukuoka.
**中研一，研究そのの，小杉要治，董志文
RESULT

Responses recorded from within the frog retina may be classified into two groups, *i.e.* responses with spike potentials which are recorded from superficial layers and a slow potential with no spike potential from a deeper layer in the retina. The deepest layer producing spike potentials and the layer producing only the slow potential were distinctly separated by a distance of about 40 micra. It was difficult to locate responses in definite retinal layers from readings of the micrometer gauge, since it was probable 1) that the retina decreases its thickness during experiment due to decrease in water content by evaporation and 2) that the insertion of the electrode causes deformation of the retina. However, the relative depths of the layers from which different types of responses were obtained were consistent, suggesting that each type of response represents the activity in a certain retinal layer.

(1) Spike Potentials

Spike potentials observed most frequently so far can be grouped into three types according to their wave form and to relative depth from which they were obtained; the first or fast type, the second or slower type, and the third type with spikes superimposed on an ERG-like slow potential. All these spike potentials were of the amplitude of 20 to 60 mV and obtained after recording of a resting potential more than 20 mV.

Spike potentials of the fast type were characterised by its appearance at the most superficial retinal layer and by not being accompanied by any distinct pre- and after-potential. The receptive field was usually in a retinal region at a distance from the recording electrode, indicating that the response was from the axon of an optic ganglion cell.

Response from a little deeper layer was spike potentials of the slower type. The spike potentials of this type often showed two deflection points on their rising phase, especially when the cell was deteriorating. Blocking of the spikes occurred at either one of the two deflection points. In Fig. 1b, for instance, blocking is observed at the level of the first deflection point leaving a small prepotential. The spike potential obtained at a faster sweep (Fig. 1c) shows a wave form similar to that of the motoneuron (1). The receptive field of this type of response was relatively large but restricted to the retinal region under the recording electrode. It was very common that the spike potentials of this type discharged spontaneously, due probably to injury, and this made it difficult to determine whether the response was accompanied by a slow potential change or not.

Spike potentials of the third type were of the deepest origin among the three and they were accompanied by an ERG-like slow potential. As shown in Fig. 2, the initial hyperpolarization and later depolarization at ‘on’ correspond to the a- and b-wave, and the depolarization at ‘off’ to the d-wave in the ERG. Usually, the slow potential remained even after the spike potentials disappeared. Firing of the spike potentials appeared to be related to slow potential changes toward
depolarization which correspond to the b- and d-wave, suggesting that they are the generator potential for the spike potentials of this type. The receptive field was rather small, having a diameter of some 400 to 500 micra when explored by a light spot of 140 micra. The time course of each spike potential was relatively fast, starting with a small prepotential. Blocking could occur between the prepotential and spike (Fig. 2a).

![Image](image_url)

**Fig. 1.** Spike potentials of the second type, discharging spontaneously. Blocking of an impulse spike at the first deflection point is seen in b. In c is shown a single spike potential with two conspicuous deflection points on its rising phase.

In this and all subsequent records downward arrow indicates ‘on’ and upward arrow ‘off’ of illumination.

![Image](image_url)

**Fig. 2.** Two successive recordings of spike potentials of the third type superimposed on an ERG-like slow potential. In a, the spike potential at ‘on’ failed to fire leaving a small prepotential.

Upper part of spikes was off the screen.
Fig. 3. (Left) Spontaneous spike discharges on top of rhythmic slow potential changes of about 10 mV. Illumination caused hyperpolarization and inhibition of discharge.

Fig. 4. (Right) Slow negative potential recorded from a layer deeper than those giving spike potentials.

While the above three types of spike potentials were most commonly obtained from within the frog retina, there were also spike potentials which appeared not to belong to any of them. In an example illustrated in Fig. 3, spike potentials discharged spontaneously in groups, superimposed on a rhythmic slow potential changes of an amplitude of about 10 mV. Illumination caused a sustained hyperpolarization with inhibition of the spike activity.

(II) Slow Potential

When an electrode was inserted about 40 micra deeper than the layer producing the third type spike activity, a negative potential similar to the S-potential in the fish retina was often recorded in response to illumination. It ranged from 5 to 30 mV in amplitude (Fig. 4), and usually appeared after recording of a negative resting potential of more than 10 mV. Further minute movements of the electrode caused a decrease in amplitude of the response as well as the resting potential but these changes were only gradual. The receptive field was usually small, and in some cases the amplitude of the response was fairly independent of the area illuminated when the light spot on the retina was larger than 140 micra in diameter. Though the spectral sensitivity of the response has not yet been studied extensively, the slow potential in few cases was found to reverse its
polarity towards the red region of the spectrum in a test with successive illuminations by lights of 440 m\(\mu\) and 610 m\(\mu\). From the layers deeper than that producing the slow potential, no electrical response was discernible.

**DISCUSSION**

1) The intracellular recording of spike potentials from the vertebrate retina has not been successful except **WIESEL**\(^\text{16}\)** who recorded spike potentials intracellularly from the ganglion cells in the cat’s unopened eye. The difficulty seems to be largely due to the smallness of the retinal cells which are usually less than 10 micra in diameter. In the course of the present study, following points were found very important for intracellular recording from the frog retina. 1) The ordinary superfine micropipettes were not fine enough for the present purpose. Only those pipettes that showed a very slender taper near the tip could penetrate into the cells. 2) Introduction of the micropipettes from its anterior surface gave a good result, while our attempt with micropipettes inserted from the receptor side of the isolated retina was not very successful. 3) The vitreous humor hampered the insertion of micropipettes and it was necessary to remove it as far as possible.

2) Spike potentials in single optic nerve fibers were first recorded by **HARTLINE**\(^\text{5}\)** with his microdissection technique and later by **GRANIT** and **SVAETICHIN**\(^\text{4}\)** with metal microelectrodes. The spike potentials from the anterior surface of the retina were fast spike potentials either from the optic nerve fibers or from the optic ganglion cells. According to **KUFFLER**\(^\text{6}\)**, the spike potentials extracellularly obtained from the optic ganglion cells are diphasic, showing often blocking of conduction leaving a small prepotential. In view of the fact that the spike potentials of our second type were preceded by a prepotential, they might have been recorded from the ganglion cells. It is worthy of note that the spike potentials of the third type are fired on top of the fluctuations corresponding to b- and d-wave. This agrees with the classical notion by **GRANIT**\(^\text{2, 3}\)** that the b- and d-wave are associated with excitatory process, while the a-wave with inhibitory.

3) The slow potential recorded from a deepest layer in the frog retina showed a wave form very similar to the S-potential in the fish\(^{7,8,9,10,11}\). **TOMITA et al.**\(^\text{13}\)** reports that the layer producing spike potentials in the fish retina is about 50 micra anterior to the layer giving the S-potential. This agrees with our result in the frog retina in which the layers responding with spike activities and with the slow potential are separated by a distance of about 40 micra. However, there are several differences between the two materials: 1) The slow potential from the fish retina shows an enormous area effect\(^\text{12}\), while in the frog the area effect appeared to be less. 2) The slow potential from the fish retina could be recorded even with a micro-electrode of the tip diameters up to 5 micra\(^\text{11}\), while the slow potential from the frog retina was obtainable only by the use of a very fine microelectrode. The question of whether the two potentials are the same in nature or not remains
unsolved. The slow potential from the frog retina must have been recorded from a very small structure, because the ordinary superfine microelectrode has been unable to detect it.

**SUMMARY**

1) Methods for obtaining big action potentials from the frog retina were studied.
2) From superficial layers of the retina several types of spike potentials were recorded intracellularly. The spike potentials obtained from the deepest layer were superimposed on an ERG-like slow potential.
3) From a depth about 40 micra posterior to the layers giving the spike activity, a large negative potential resembling the S-potential in the fish retina was recorded.

The authors wish to express their most sincere appreciation to Prof. T. TOMITA of Keio University for his direction of the experiment, valuable criticisms and for reading the manuscript. The authors' thanks are also due to the staff of his laboratory for their kind support and encouragement throughout the experiment.

**REFERENCES**
