Spreading Depression of Activity in Amphibian Retina

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ABSTRACT

GOURAS, PETER. Spreading depression of activity in amphibian retina. Am. J. Physiol. 195(1): 28-32. 1958.—Microelectrode studies on excised amphibian retina have uncovered a phenomenon of spreading retinal depression which in many respects resembles Leão’s spreading cortical depression. The phenomenon is characterized by a spontaneous ‘milky’ wave that periodically marches across the surface of the retina and lasts for 2–3 minutes at any one point. There is a concomitant and sudden negative shift in the resting retinal potential of 1–2 mv which gradually returns to its previous level in 3–6 minutes. As the wave front approaches a microelectrode recording ganglion cell activity, there is a marked increase in its spontaneous firing climaxing in an intense discharge as the wave engulfs the electrode. A profound depression follows so that even the most intense photic stimulation becomes unable to elicit a local response. As the color change disappears and the d.c. level returns, the local area of retina becomes re-exitable. Similar changes occur in the local electroretinogram, which always returns slightly before the ganglion cell activity. The process occurs spontaneously but can also be induced by trauma, electrical current, or the application of KCl. It happens less often in bright light or with oxygenation, although neither of these factors can prevent its occurrence. It appears to be entirely reversible.

Spreading depression has become a well established phenomenon of cortical electrophysiology ever since its initial description by Leão in 1944 (1). The process is characterized by an expanding wave of depression of spontaneous electrical activity which travels across the cerebral cortex at the rate of 2–3 mm/min. and lasts for 2–6 minutes at any one point. There is a concomitant unresponsiveness to electrical stimulation and a marked shift in the resting cortical potential. Grafstein (2) has recently shown that the same phenomenon can occur in the isolated cerebral cortex of the cat and is frequently accompanied by increased unitary activity at the beginning of the wave of depression. The entire process is reversible. It occurs spontaneously but can be induced by either weak mechanical or electrical stimulation (1) or the application of KCl, CaCl₂ or strychnine (3, 4). Its spontaneous occurrence is facilitated by dehydration (5), cooling (6), or deterioration (7), suggesting that it is a pathologic condition.

So far the description of this phenomenon has been confined to mammalian cerebral cortex, but the analogy between it and a process found in the excised retina suggests a common relationship. This report presents observations made on a spreading electrical depression, noticed during microelectrode studies of toad retina, and discusses similarities between it and Leão’s phenomenon. Similar observations were made by Hartline during his original work on single optic nerve fiber preparations of frog retina (personal communication).

METHODS

The electrical activity at the anterior retinal surface of the eyes of the toad, Bufo marinus, was studied. The isolated posterior, hemispheric segment containing most of the retina, choroid and sclera as well as the intraorbital segment of the optic nerve was dissected away

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from the anterior portion of the eye along the equator. This preparation was placed in a circular receptacle, enclosed in a Lucite chamber containing amphibian Ringer's solution, so that the posterior scleral surface contacted the electrolyte while the anterior retinal surface remained exposed. Oxygen could be bubbled up through the solution leaving from an adjustable opening at the top of the chamber through which both the microelectrode and the stimulating light entered. The chamber was fixed to a mechanical stage permitting three dimensional movement. The entire retinal surface could be viewed through a dissecting microscope at magnifications of 6-40X. Illumination was provided by a tungsten filament lamp with a retinal brightness of 500 foot candles which could also be used for diffuse stimulation. Local stimulation was obtained by focusing a fluorescent spot, from the tube face of a cathode ray oscilloscope, on the retinal surface. At the sharpest focus the retinal light spot had a diameter of 300 micra. The spectral composition of the fluorescent light from this P-II phosphor has a band extending from 4000 to 6000 Å with a single peak at 4600 Å. This light source could be conveniently triggered by the recording system in a rhythmic manner being ‘on’ and ‘off’ for equal periods of usually 5 seconds. The microelectrodes were both insulated and noninsulated tungsten wire, electrochemically sharpened to tips, 3-5 micra in diameter (8). Larger chlorided silver electrodes were used to measure d.c. potentials occurring without changes in the level of illumination. The electrodes were held in a mechanically driven micromanipulator. The electrical activity was measured between the microelectrode and the posterior scleral surface which was led to ground through either a platinum or chlorided silver electrode. Low frequency changes were recorded by a direct writing chopper type d.c. amplifier while the high frequency activity was led through an RC coupled variable filter (40 kc-2 cps) preamplifier to both a loud speaker and a cathode ray oscilloscope from which the signals were photographed. The preparation was kept at 20-25°C in a room that was dark except for the stimulating light. Each retina was studied from 4 to 6 hours after dissection.

**Color Change.** The surface of the normal toad retina appeared a dark brown when viewed by reflected light. As early as 30 minutes after dissection a grayish or ‘milky’ wave might be seen spontaneously originating at a point on the cut peripheral edge of the retina and spreading concentrically at a rate of \( r \pm 0.5 \text{ mm/min} \). There was a sharp junction between the normal retina and the wave front appearing as an intense brown line. The grayish change imperceptibly blended into the normal retina 2-3 mm behind the advancing wave front. Sometimes traversing the entire retina, other times only reaching the optic disc, this wave would eventually disappear only to be followed by another spontaneous one, 20-30 minutes later. The photomicrographs of figure 1 demonstrate such a wave passing over the retina and appearing as the faint gray shadow moving from left to right past the optic disc, which is seen in the lower left hand corner of each photograph. Its spontaneous occurrence was more frequent in the dark than in the light adapted retina. The interval between its spontaneous appearance was 39 ± 10 minutes in the light adapted and 26 ± 9 minutes in the dark adapted retina. It occurred later and less often during oxygenation and most frequently as deterioration progressed. The phenomenon could be induced by weak mechanical or electrical stimulation or by the gentle application of minute amounts of hypertonic KCl. A wave, initiated at the center of the retina, would spread radially in all directions from its site of origin. If the anterior layers of the retina were dissected away from the pigment epithelium at the wave front’s junction with normal

**Fig. 1.** Photomicrographs of a wave of depression passing the optic disc. Very dark area in the upper left corner is the result of the photo flash. Larger gray shadow, reaching down to the optic disc in the lower left corner, is the wave of spreading depression. Magnification is 11X.
retina, no color change could be seen in the corresponding region of the pigment cell layer, suggesting that the 'milkiness' depended upon changes in either the junction between pigment cells and photoreceptors, or in more anteriorly placed structures.

**Ganglion Cell Activity.** Barlow (9) has shown that in the frog the action potentials recorded from the anterior retinal surface are the result of discharges of ganglion cell somata. In contradistinction to in vivo cat retina (10), spontaneous ganglion cell activity is very infrequent in excised amphibian retina. Barlow, however, did observe spontaneous, rhythmic activity but stated that it was not the index of a normal retina. This rhythmic discharge of ganglion cells was associated with a slow oscillating potential similar to those observed by Adrian (11) in the optic ganglion of Dysticus. This activity was most frequent during dark adaptation although it might also occur during illumination. At times this spontaneous activity increased to a climax, then stopping abruptly, left the preparation inexcitable (9). In the case of the toad retina a similar phenomenon was found but it was always associated with a concomitant color change. As this 'milky' wave approached a microelectrode recording photically induced ganglion cell discharges there was a progressive increase in the spontaneous background activity. If the wave front reached the microelectrode, there was a sudden high frequency discharge (200-300/sec.) lasting 5-10 seconds and then profound depression, so that even the most intense stimulation failed to elicit a response. As the color change gradually disappeared the responsiveness of the ganglion cells returned, although spontaneous activity remained absent. The period of complete ganglion cell depression lasted for 3-5 minutes (910 ± 70 sec.), outlasting the concomitant color change, as shown in figure 2.

**D.C. Changes.** Contemporaneous with the wave front of the depression and the intense ganglion cell discharge there was an abrupt negative shift in the steady potential recorded at the anterior retinal surface, (fig. 2). The resting d.c. potential gradually returned to its previous level as the depression subsided. The amplitude of this d.c. shift varied in a constant manner with the recording electrode. The average change recorded with large chlorided silver electrodes was 1.5 ± 1 mv whereas changes of only 0.4 ± 1 mv were recorded with tungsten microelectrodes. This variation might reflect differences in the impedances of these electrodes to low frequency changes as well as differences in the amount of shunting resistance at the electrode-tissue junction. The duration of the d.c. shift varied from 2 to 4 minutes (148 ± 80 sec.). Its configuration always appeared as in figure 2, with the intense spike discharge occurring on the initial falling phase of the d.c. shift. There were no significant differences in the amplitude of this d.c. change between light and dark adapted retinas and positive components were not found either preceding or following the negative wave. These would have been undetectable, however, if they had been one-tenth of the magnitude of the negative shift as is usually the case in the cortical phenomenon.

**Local Electoretinogram.** Similar changes occurred in the local low frequency responses to focal illumination during a wave of spreading depression. In figure 3 the typical responses to dim, local illumination of the retina at the foot of the recording microelectrode are shown at both 'on' and 'off' while rhythmic stimulation was maintained. The usual responses to focal illumination were quite characteristic and predominantly negative at both 'on' and 'off'. Ganglion cell ac-
FIG. 3. Oscillograms of responses to dim, local illumination which is 'on' and 'off' for equal periods of 5 sec. At left is time before, during (0-3 min.) and after a wave of spreading depression. Frequency response is 1 kc to .2 cps. Calibration is 125 msec. horizontally and 50 µv vertically. Negativity is up.

tivity could be seen especially at the top of the negative waves. As the wave front engulfed the recording electrode both the low frequency and spike responses disappeared. The depression again lasted for 3 minutes, after which time the photically induced activity gradually returned. At first the low frequency potentials returned with a lower amplitude, longer latency and no evidence of spike activity. Slowly the amplitude of these local potentials increased as their latencies correspondingly decreased. When the slow waves finally reached their previous level the spikes would reappear, again at the summit of the negative deflections, as shown in figure 3.

The familiar electroretinogram produced by diffuse illumination was also affected by a wave of spreading depression but the effect varied not only with its configuration and position but also with the ratio of depressed to undepressed retina. A wave of depression spreading concentrically from the foot of the recording electrode tended to cause a decrease in the positive component of the 'on' response (b-wave) and an increase in the positive component of the 'off' response (d-wave) as it progressed peripherally. The changes were most pronounced when the wave front was 0.5-4.0 mm from the microelectrode's tip. As it spread further into the periphery the electroretinogram gradually returned to its previous amplitude.

DISCUSSION

The significance of this phenomenon is of importance to retinal electrophysiologists because sporadic, spontaneous activity and depressed retinas can often be of distressing concern. It is of even more interest, however, in its close resemblance to the spreading cortical depression of Leão. Both phenomena are characterized by waves of depression of electrical activity which travel relatively slowly over laminated neuronal structures. They occur spontaneously but can be induced by the use of similar agents. They both have an associated d.c. shift which in the case of the retina is always accompanied by an intense burst of unit activity. Both processes appear to be pathophysiological. In the cortex spreading depression is more frequent in exposed, aging preparations (7) and is more easily elicited in isolated rather than intact cortex (2). In the case of excised retina, its spontaneous appearance occurs 30 minutes or more after dissection and with increasing frequency as deterioration progresses. There are, however, noticeable differences between this retinal process and Leão's cortical phenomenon. The depression spreads more slowly in the retina at 1 mm/min. whereas it usually travels two or three times as fast in the cortex. Such differences might be expected between poikilothermic and normothermic tissue assuming a Q_{10} ≈ 2 or 3. The d.c. shifts in the cerebral cortex are in the order of 10-15 µv and are preceded and followed by smaller positive components. In the retina the negative shift is much smaller, and no positive components are detectable. Intense unitary activity always precedes spreading retinal depression although it may not be an essential component of Leão's cortical depression (W. H. Marshall, personal communication). The
retinal phenomenon has a marked color change which is not at all similar to the vasomotor changes described by Leão (12) and others (13) in the intact brain. This characteristic may be the result of either the well known photomechanical responses of the pigment epithelium and photoreceptors (14) or changes in the photosensitive pigments. There is, in addition, evidence that the retinal process may be photosensitive.

Only cautious speculations can be made as to the origin and the mechanism of propagation of this wave. Its slow rate and completeness of spread to contiguous areas suggests a non-synaptic and possibly a humoral diffusion. The mechanism, Grafstein (2) has hypothesized to be responsible for spreading depression in isolated cortex may also be responsible here, namely, that the synchronous release of K+ by discharging layers of cells spreads to excite adjacent regions, and the process is thus propagated in a chain-like manner.

The interesting fact that intense unit activity always precedes the depression indicates the epileptiform nature of this phenomenon. The slow spread of discharging sheets of nerve cells with subsequent profound depression and the whole process appearing to be triggered in a rather cyclic manner by a focal point of injury, is very suggestive of the suspected pathophysiology of Jacksonian epilepsy.

REFERENCES