MICROELECTRODE STUDY OF SPREADING DEPRESSION (SD) IN FROG RETINA —GENERAL OBSERVATIONS OF FIELD POTENTIAL ASSOCIATED WITH SD—

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Abstract Using microelectrodes, the field potential change associated with SD (SDP) was recorded from frog retinas conditioned with Cl^- -free Ringer's. In such retinas, SDP was induced by light or chemical agents such as glutamate, aspartate and K^+ . These chemicals, when applied iontophoretically, produced a local graded response which eventually triggered SDP. A potential similar to the local response to chemicals was often discerned on the rising phase of SDPs produced by light or occurring spontaneously. The SDP was maximal across an innermost retinal layer 50 μm or less in thickness with the intraretinal polarity predominantly negative, indicating that the major sink of SDP is in the inner plexiform layer. The influence of SDP on the receptor potential was relatively small, but the other components completely disappeared at the beginning of SDP, recovering gradually thereafter. Concomitantly, a strong depolarization occurred in the ganglion cells. Stimulation of the optic nerve could induce SDP, but nerve impulse activity is not important for SDP because tetrodotoxin was unable to prevent SDP due to light or chemical agents.

Leão's spreading depression (SD) (LEÃO, 1944) of nervous tissue is characterized by a focal burst of neuronal activity followed by profound neuronal depression. This pathologically high neuronal activity followed by depression slowly spreads to neighboring regions. In the meantime, first the original site and then the neighboring regions gradually recover their normal functional state. SD is also accompanied by a number of other signs that include changes in field potential and tissue impedance (MARSHALL, 1959; OCHS, 1962), transparency (VAN HARREVELD and FIFKOVA, 1970), and light scattering (MARTINS-FERREIRA and CASTRO,

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1966) but the sign and amplitude of these changes are functions of the recording methods and the particular tissue under investigation. LEÃO (1944) originally found SD in the cortex and GOURAS (1958) described SD accompanied by field potential and optical changes in the amphibian retina. Recently, three other groups have contributed substantially to our knowledge of SD in the retina. MARTINS-FERREIRA and his colleagues (1966, 1971, 1974) have investigated optical changes during SD and recently described a circling SD. VAN HARREVELD and FIFKOVA (1970, 1971, 1972) have shown that glutamate is released from the retina during SD and studied the effects of chemical agents applied to the retina as measured by the optical changes they produce, and HANAWA and his colleagues (1967a, 1967b, 1968) have produced a series of studies in which they developed a reliable retinal preparation for the study of SD based on their observation that SD can be easily and reproducibly induced when the retina is exposed to low Cl^- Ringer's.

Although the underlying pathology of SD is unknown, there have been a number of observations that give information about a possible mechanism. GRAFSTEIN (1956) was the first to suggest that the neural overactivity followed by depression and recovery of SD may be triggered by the release of potassium ion by active neurons. VAN HARREVELD and FIFKOVA (1970, 1971, 1972) extended this idea by showing that glutamate is released from the retina during SD and they suggest that glutamate is the first link in the chain of events that cause SD. Nevertheless, there is no information regarding exactly where within the retina the SD actually is initiated and there is also no information regarding the distribution of K^+ within the retina, and changes in K^+ during both normal activity and SD. Lastly, while the changes in neuronal activity have been investigated, there is no information regarding the intracellularly recorded activity of the retinal glial cells, Müller cells.

Our investigations attack these unknown points and will be described in two parts. The second part (MORI *et al.*, 1976) will deal with the potassium ion changes within the retina and the relationship of Müller cell activity to SD. In the first part we describe our investigations of the field potential associated with SD. Using depth recording of the field potential we localize the initiation of SD in the inner plexiform layer and the relation of several chemical agents to the initiation of SD is detailed.

METHODS

Frogs, *Rana pipiens*, of about 10 cm in length, measured from the tip of the nose to the bottom of the spine, were used. Since frogs supplied during winter did not give well-reproducible SDs, most of our experiments were performed during the rest of the year.

After about two hours dark adaptation, the eye was removed from the animal in dim light, cut along the equator, and the posterior part of the eye was cut in half

Fig. 1. Schematic illustration of experimental arrangements.

across the optic disk. The retina in either half of the eye was then detached from the pigment epithelium and placed on a small piece of filter paper. The filter paper with the retina on it was mounted on another piece of filter paper, which covered a small hole (4 mm dia.) in the experimental chamber and made contact with the solution underneath (Fig. 1). An excess of Ringer's solution or tissue fluid over and around the preparation permits physical spreading of electrical potentials and thus obscures the site of their origin in the retina. These effects were minimized by mounting the chamber slightly tilted so that a negative pressure was exerted through the hole, draining off any excess fluid.

The chamber, which was made of acrylic resin plate, had two compartments, main and accessory. The main compartment was filled, enough to touch the filter paper, with a solution which was either the control Ringer's or the Cl^- -free Ringer's as defined below. The control Ringer's was a modified Conway's solution (HANAWA *et al.*, 1967a) with the following composition in mM: NaCl, 72.0; KCl, 2.5; MgSO_4 , 1.2; Na_2SO_4 , 0.6; NaHCO_3 , 25.0; Na_2HPO_4 , 2.3; NaH_2PO_4 , 0.7; calcium gluconate, 0.9; glucose, 26.0. For the Cl^- free Ringer's, NaCl and KCl in the control Ringer's were replaced with Na_2SO_4 and K_2SO_4 , and an appropriate amount of sucrose was added to maintain the isotonicity.

The accessory compartment contained 2 M KCl. The two compartments were connected by an agar bridge consisting of 3% agar in control Ringer's. An Ag-AgCl electrode in the accessory compartment served as the reference electrode.

The light stimulus for SD was usually diffuse light over the retina, of a duration of 0.3 or 1.0 sec, and a moderate intensity, evoking a b-wave about 80% of the maxi-

Table 1. Thickness of retinal layers of *Rana pipiens*.
Mean and standard deviation in μm from
10 sections.

Total	Nerve fiber layer	Gangl. cell layer	Inner plex. layer	Inner nucl. layer	Outer plex. layer	Outer nucl. layer	Rod & cone layer
225	8 ± 3	6 ± 1	59 ± 6	44 ± 5	5 ± 2	16 ± 2	87 ± 11

mal amplitude. The light intensity was not critical, however, since weaker light was still able to induce SD.

Our index of SD was the characteristic potential change accompanying it which will subsequently be called SDP. For recording SDP, micropipette electrodes having a tip diameter of 1–2 μm and filled with control Ringer's were usually used. For simultaneous recording of SDP from two sites, a pair of such electrodes were set on a holder which was originally designed for coaxial microelectrodes (TOMITA and TORIHAMA, 1956). The holder allows independent manipulation of each electrode. The depth of an electrode in the retina was determined from the reading of a micrometer gauge. Table 1 may serve to estimate from the depth reading an approximate location of the electrode in retinas 225 μm in thickness. The data in the table were obtained from measurements of relative thickness of each layer in 10 sections from sampled retinas at random from our stock.

Several kinds of chemicals were injected iontophoretically into the retina to test their effectiveness as a stimulant for SD. For this type of experiment, either a pair of micropipette electrodes set close to each other, or more often, a double-barreled microelectrode was utilized, with one pipette filled with a chemical and the other with the control Ringer's. The micropipettes, whether single or double-barreled, were made from capillary-filled glass tubing (KANEKO, 1973) which makes them easily back-filled with solutions.

Two unity-gain probes (Model M701, W-P Instruments Inc.) were used for simultaneous recording from two pipettes. Their outputs were fed into a conventional type of two-channel dc amplifiers and oscilloscope (Tektronix RM502A) and then to a tape recorder (Hewlett-Packard 3960). The responses stored in the tape were reproduced by an ink-writing recorder (Hewlett-Packard 7402A). Iontophoretic injections of chemicals were made by pulses of current from a programmable pulse generator combined with stimulus isolator (Model DS-1 and PC-3, W-P Instruments, Inc.).

RESULTS

Light-induced SDP in retinas mounted receptor side up

When the retina was mounted receptor side up so that the preparation exposed to Cl^- free Ringer's on the vitreous side (see METHODS), the b-wave response to light soon began to increase in amplitude, often up to 5 mV in an hour. The b-wave also was markedly prolonged in both rising and falling phases. Light became effective for inducing SD in 10 to 20 min. After the first SDP, it was consistently induced by light applied at an interval of 8 to 15 min for more than 2 hr. The upper three tracings in Fig. 3 show typical SDPs recorded with a microelectrode on the receptor surface. They start with a large negative shift followed by a small but long-lasting positive wave. The duration of the negative wave is about 1 min, and that of the positive wave 3–4 min. A light stimulus failed to evoke an SDP for the following several minutes. Also, light applied at any point before the

negative peak of SDP failed to elicit a b-wave. The b-wave recovered only gradually thereafter throughout the refractory period of SD. These observations were similar to those reported by HANAWA *et al.* (1967a, 1967b, 1968).

When a pair of electrodes were set a short distance apart on the retina and the site of one electrode was illuminated by a small spot of light, each electrode recorded an SDP but of different latencies. In an experiment with two electrodes set 1 mm apart, the difference between the latencies was 20 sec, indicating that the SDP-wave propagated at a slow speed of some 3 mm/min. Very often, however, the two electrodes recorded virtually simultaneous SDPs. In such cases, SD must have started at the same time over a large area around the light spot, probably responding to scattered light as well. For better control of the location of SD initiation in our later experiments, we preferred the method of microinjection of chemicals.

The SDP which was initiated by light at or close to the recording site, as indicated by short latencies, showed a rapid rising phase, but often with two wavelets (as discerned by notches) as shown in Fig. 2A. Wavelet-1 corresponds to the b-wave. Wavelet-2, which has no counterpart in ERG components, precedes the main SDP-wave. SDPs originating at a distance show a slow rising phase with no discernable wavelet on it (Fig. 2C). The fluctuation following the light flash in Fig. 2C corresponds to local ERG waves which failed to initiate an SDP at the recording site. As obvious from the long latency, the SDP in Fig. 2C was initiated at a distance and propagated to the recording site. In some animals obtained in winter, immature or abortive SDPs, which did not spread over the retina, were recorded sporadically among mature SDPs, as exemplified in Fig. 2A'. The two wavelets still show up but the second is smaller in amplitude compared with the one in the mature SDP.

Fig. 2. Sample SDPs carrying wavelets (1, 2) on rising phase, recorded from retina mounted receptor side up and with electrode on receptor surface. A and A'; light-induced mature and immature SDPs initiated at or near recording site. B and B'; mature and immature SDPs which occurred spontaneously at or near recording site. C; light-induced SDP initiated at distance from and propagated to recording site. Arrows show points of time at which flash of diffuse light was applied. For further explanation see text.

When the preparation was kept long in darkness, SDPs occurred spontaneously. They often were preceded by irregularities in the baseline which made their occurrence predictable. The spontaneous SDP resembled in every respect the light-induced SDP but lacked wavelet-1, as shown in the mature SDP in Fig. 2B, and the immature SDP in Fig. 2B'.

Figure 3 shows an example of depth recording of SDP induced by brief diffuse

Fig. 3. Depth recording of light-induced SDP from the retina mounted receptor side up. Upper tracings are SDPs from receptor surface; lower tracings are those recorded simultaneously with upper tracings by intraretinal electrode advanced to depth of 100 μm , 200 μm , and 225 μm . Arrows show light stimuli for initiation of SD.

light. Recording was made simultaneously by an electrode at the receptor surface (upper tracings) and an intraretinal electrode advanced to depths of 100 μm , 200 μm , and 225 μm (lower tracings). SDPs from various depths are similar in shape but not in amplitude. The intraretinal SDP at 100 μm is nearly identical in both shape and amplitude with the surface SDP. With further advancement of the intraretinal electrode, the amplitude of SDP gradually increases with the depth until it reaches a maximum at a depth corresponding to the inner plexiform layer. The intraretinal electrode has almost reached the inner limiting membrane at the depth of 225 μm .

Fig. 4. SDP induced by first flash of light and ERG waves in response to subsequent flashes repeated at 12 sec intervals. A; recording from receptor surface. B; differential recording across receptor layer, with one electrode on receptor surface and other at 100 μm depth. Initial and secondary slow positive deflections could be due to inaccurate alignment of micropipettes. Responses to flashes of light in later phase of tracings A and B are reproduced in inset on expanded scale. Calibration: 5 mV for A and 2 mV for B. Time: 1 min for both A and B; 12 sec for tracings in inset.

The observation that the intraretinal SDP at 100 μm is almost identical in shape and amplitude with the SDP from the receptor surface suggests that there is little, if any, contribution of receptors to SDP. This was tested by light flashes repeated at 12 sec intervals with the result shown in Fig. 4. The upper tracing A obtained from the receptor surface shows an SDP induced by the first flash, superimposed on small fluctuations which are responses to subsequent flashes. The lower tracing B shows potentials across the receptor layer, obtained by differential recording from the receptor surface and from 100 μm depth. The fluctuations in tracing B, each representing the receptor potential superimposed on the slow PIII of FABER (1969), continue to occur throughout the SDP, though there is a slight decrease in amplitude during an early phase of SDP. In contrast, the b-wave, which is manifested as negative spikes in the later phase of tracing A, is completely lost at the beginning of SDP. In tracing B, no trace of b-wave is seen even in the later phase. This is shown more clearly by responses to two flashes of light in the later phase, reproduced in the inset of Fig. 4 on an expanded time scale. Since the b-wave originates in layers proximal to the receptor layer, the absence of a b-wave in tracing B is a confirmation of satisfactory isolation of potentials in the receptor layer.

At depths where the maximal SDPs were obtained the intraretinal electrode often detected impulse spikes of ganglion cells in response to light and also at the beginning of SDP. Figure 5 shows our quasi-intracellular recording from a single ganglion cell. A burst of impulse spikes appears concomitant with the onset of SDP. The spikes show a rapid dwindling to nothing at the very initial phase of SDP. This suggests that the ganglion cell is depolarized far beyond physiological

Fig. 5. Quasi-intracellular recording of impulse spikes in single ganglion cell during SD induced by light. Duration of light is shown by elevation of uppermost line. A; intense burst of impulse spikes at start of SDP on slow (upper left A), and expanded (lower A) time scale. B; spontaneously occurring impulse spikes of small size and their modulation by light recorded 3 min after A (upper right B), and the wave form of the impulse spikes at onset of light on expanded time scale (lower B).

ranges. In about a minute the cell begins to show spontaneous activity and also responsiveness to light with small spikes of longer duration. Figure 5B was recorded 3 min after the initiation of SD showing slow recovery of the spike which fully recovered its former height and duration within 6 min. This confirms earlier observations concerning SD in the retina (GOURAS, 1958; MATSUMURA, 1968) and the cortex (HIGASHIDA *et al.*, 1974; MORLOCK *et al.*, 1964).

Light-induced SDP in retinas mounted vitreal side up

Figure 6 shows an example of depth recording from the vitreous side of the retina. The upper tracings are taken at the surface. They start with a positive transient followed by a long-lasting negativity. Intraretinally, the positive transient which usually persists though with smaller amplitude, is disrupted by a negativity much earlier and more abruptly than in the surface SDP. In preparations in which the layer of vitreous humor or Ringer's covering the retina was deeper, the disruption of the initial positive deflection by the negativity in the surface SDP was more prolonged and less abrupt, whereas this effect was small in the intraretinal SDP.

Fig. 6. Depth recording of light-induced SDP from retina mounted vitreal side up. Upper tracings are SDPs from vitreal surface, lower tracings are those recorded by intraretinal electrode at depths of 50 μm and 100 μm . Arrows show light stimuli for initiation of SD. In lefthand tracings responses to flashes of light repeated at 10 sec intervals are superimposed on SDP.

The amplitude of the negative wave of SDP diminishes rapidly as the electrode is advanced into the retina, as shown by the two tracing in Fig. 6 obtained at depths 50 μm , and 100 μm . The largest negative wave was usually at depths slightly less than 50 μm from the inner limiting membrane. When impulse spikes of ganglion cells were being recorded, the burst of impulse spikes associated with SD occurred concomitant with the negative deflection of the intraretinal SDP.

In retinas mounted vitreal side up, it took longer for the Cl^- -free Ringer's to allow the induction of SD by light. SDPs occurring with a long latency were more frequent in retinas mounted vitreal rather than receptor side up. However, when light happened to initiate SD at the recording site, the negative deflection of intraretinal SDP started with no positive transient, and both the surface and intraretinal SDPs resembled those elicited by chemical agents injected iontophoretically from a micropipette (see Fig. 10). Our impression is that the retina exposed to Cl^- -free Ringer's at the receptor surface is less susceptible to SD. For this reason, all the subsequent experiments, unless otherwise noted, were performed on retinas mounted receptor side up.

SDP by chemical agents

a. Glutamate and aspartate. Glutamate or aspartate was locally applied from pipette filled with 125 mM solutions of these chemicals as sodium salts. The amount of chemical injected was estimated from the amount (intensity $\times$ duration) of current applied through the pipette. Both of these amino acids, whether L- or D-type, were found to act as invariable stimulants of SD, and did not differ much in effectiveness. Injection in the proximal retina was always effective, while injection in the distal retina often failed to induce SD under the same conditions.

Figure 7 shows the result of aspartate injection at the depth of 150 μ m from the receptor surface. One barrel of a double-barreled microelectrode was used for the injection, while the other barrel recorded the change in potential (tracing B). Effects were also recorded simultaneously by another electrode at the receptor surface (tracing A). As seen in the figure, a negative potential is produced by aspartate, and as the amount applied is increased the amplitude of the negative potential also gradually increases. Beyond a certain amount of aspartate, a mature SDP is induced.

Fig. 7. Effects of iontophoretic injection of D-aspartate in increasing amounts, controlled by increasing time of injection from 0.1 sec to 1.0 sec with current fixed at -840 nA. Aspartate is injected from one barrel of double-barreled electrode at 150 μ m from receptor surface and effect is recorded by other barrel (tracing B) and also by single pipette electrode on receptor surface (tracing A). Mature SDP is triggered by 1.0 sec injection.

Figure 8 shows the effect of glutamate applied iontophoretically (370 nA $\times$ 0.2 sec) at 30 sec intervals. Figure 8A and B form a continuous record. SDPs triggered by local glutamate response are seen in this example to occur regularly once every 10 min. Whenever an SDP starts, subsequent local glutamate responses are completely lost during the initial half of the negative phase of SDP. The response recovers quickly towards the peak of the positive phase of SDP, but then is suppressed slightly again before it returns to the resting phase. The behavior of the local glutamate response is similar to that of wavelet-2 shown in Fig. 2. This is explicit in Fig. 8C where part of the tracing in Fig. 8A is reproduced on an expanded time scale. Flashes of light are interposed between glutamate injections in the latter half of Fig. 8B. In contrast to the glutamate response, the response to light recovers gradually in the course of SDP. The electrode, if sufficiently close to a ganglion cell, recorded a burst of impulses from that cell at the onset of each local response to chemical as well as to light stimuli.

Fig. 8. Effects of repetitive iontophoretic injections of L-glutamate of fixed amount (-370 nA $\times 0.2$ sec) at 30 sec intervals from one barrel of double-barreled electrode at depth $150\text{ }\mu\text{m}$ from receptor surface. Two SDPs triggered by glutamate response are shown in tracings A and B which make continuous record. In the latter half of tracing B flashes of light are interposed between glutamate injections. Tracing C is reproduction of part of tracing A on expanded time scale to show SDP starting on top of glutamate response.

The effect of continuous glutamate injection on the local response to intermittent glutamate injections and light flashes is shown in Fig. 9. The arrangements for this experiment are shown on the inset. One pipette of a double-barreled microelectrode is used for both continuous glutamate injection (-80 nA for 7 min) and intermittent injections (-230 nA $\times 0.2$ sec). An SDP is induced at the onset of continuous glutamate injection (Fig. 9B). Figure 9A shows its detection at a point 1 mm from the site of injection. The time lag between the two SDPs gives a value of 2 mm/min for the spreading velocity of the SDP.

A sustained negativity is produced in the retina by continuous glutamate injection. However, the current for the continuous injection produces an artifact

Fig. 9. Effects of continuous glutamate injection with current of -80 nA on local response to periodic glutamate injections and flashes of light, recorded with arrangement of electrodes shown on right. Electrodes A and B, separated laterally by 1 mm , are at depths $50\text{ }\mu\text{m}$ and $150\text{ }\mu\text{m}$ from receptor surface respectively. For further explanation see text.

which shifts the baseline in the negative direction and makes it difficult to estimate the real magnitude of the sustained negativity due to glutamate. It should be noted that in Fig. 9B the tracing between the onset and termination of current for continuous glutamate injection was parallel-shifted upward in an attempt to cancel the artifact. The criterion for the rather arbitrary size of this shift was to make the two SDPs in A and B similarly proportioned. The response to intermittent glutamate injections and the response to light flashes also are strongly suppressed throughout the period of continuous glutamate injection. Similar experiments but with weaker current for continuous glutamate injection gave similar results.

An example of glutamate-induced SDP in the retina mounted vitreous side up is shown in Fig. 10. The tracings A and B show the surface and intraretinal (50 μm from the vitreal surface) SDPs, recorded simultaneously. The intraretinal electrode was of the double-barreled type, and glutamate injection was made from one of the barrels. The negative deflection of intraretinal SDP starts immediately after injection with no positive transient. The surface SDP shows a sharp positive deflection which drops quickly to a negativity. The bottom tracing B-A, obtained by subtraction of A from B, represents the potential difference between the vitreal surface and a point at a depth of 50 μm . As might reasonably be expected, tracing B-A resembles the intraretinal SDP obtained from retinas mounted receptor side up (*cf.* Fig. 3).

Fig. 10. Simultaneous recording of glutamate-induced SDP by electrode at vitreal surface (tracing A) and by one barrel of double-barreled electrode at depth 50 μm from the inner limiting membrane (tracing B). Glutamate is injected from other barrel. Tracing B-A is result of subtraction of tracing A from B, showing SDP occurring across innermost retinal layer 50 μm in thickness. The vertical lines at the start of SDP are artifacts of current for glutamate injections ($-260 \text{ nA} \times 0.5 \text{ sec}$).

b. Other chemicals. Exploratory tests were made of the action of the following two other amino acids and of potassium.

Pyroglutamate, which has been tested in spinal neurons with no effect (CURTIS and WATKINS, 1960), had no stimulus effect for SD and failed to produce any local response.

DL-Homocysteic acid, on the other hand, acted as a stronger stimulant for SD

than glutamate or aspartate as in the central nervous system. In addition, it gave a more prolonged local negative response. An observation demonstrating the stronger effect of homocysteic acid is that pipettes filled with 125 mM of this chemical were found inadequate, because they caused, without current, a negative drift of the baseline due to leakage of the chemical out of the pipette tip. In order to avoid this effect, the solution for pipettes was changed from 125 mM to 12.5 mM.

Potassium was found to be a much weaker stimulant for SD. Pipettes were filled with 2 M KCl to allow enough current to initiate SD. Both local negative response and SDP elicited by potassium resembled those elicited by glutamate and aspartate.

SDP caused by antidromic impulses in the optic nerve

In the retina exposed to Cl^- -free Ringer's, SDP was evoked by antidromic impulses in the optic nerve. Figure 11B shows such an SDP evoked by stimulation of the optic nerve at a rate of 50/sec. Recording was made with a pipette located in the ganglion cell layer. Stimulation was continued for about 2 sec before the initiation of SDP. The stimulus effect peaked at the rate of 50/sec, and diminished above and below that frequency. Figure 11A shows a control SDP induced by light.

Fig. 11. SDPs induced by photic stimulation (upper tracing) and by stimulation of optic nerve at rate of 50 per sec for about 2 sec (lower tracing), recorded by electrode in the ganglion cell layer. The train of antidromic impulses appears in lower tracing as small negative deflection with thickening of the trace.

SDP under the effect of tetrodotoxin

When the retina was exposed to Cl^- -free Ringer's containing 10–20 $\mu\text{g}/\text{ml}$ tetrodotoxin, all the impulse activity was lost, but still light and glutamate were able to evoke SDPs similar in shape and amplitude to those in the absence of tetrodotoxin.

DISCUSSION

Conditioning of the retina for SD. It has been shown that in the cortex the initiation of SD, whether occurring spontaneously or caused by stimulation, is faci-

litated by conditioning the cortex with one of several treatments such as dehydration (MARSHALL, 1950), cooling (MARSHALL *et al.*, 1951), deterioration (MARSHALL and ESSIG, 1951), high potassium (MORLOCK *et al.*, 1964), and so forth. This applies also to the retina. If the retina is preserved in perfectly normal conditions, photic, mechanical or electrical stimulation usually fails to elicit SD (HANAWA *et al.*, 1967b). In isolated frog retinas, not intentionally conditioned, we have not encountered a single case of SD induced by antidromic impulses in the optic nerve. In our present experiment, the retina was conditioned for SD by exposure to Cl^- -free Ringer's. MILLER and DACHEUX (1973) reported that in the rabbit retina the lack of Cl^- ions in the bathing solution leads to a dramatic change in the electrical activity, such as the loss of b-wave and a change in the discharge pattern of ganglion cells. However, in the frog retina exposed to Cl^- -free Ringer's, the b-wave was enhanced instead of suppressed. The discrepancy might depend on the species used, but another cause could be differences in method: In the rabbit the retina was perfused by Cl^- -free solution, while in our experiment with the frog the retina was exposed to Cl^- -free solution only on one side. According to our unpublished observation, using a Cl^- -sensitive microelectrode, the extracellular space of such retinas still contained Cl^- ions in concentrations up to 20 mM.

Localization of SDP. Our depth recording revealed that the negative deflection of SDP is largest at a depth a little less than 50 μm from the inner limiting membrane. This depth corresponds to the inner plexiform layer, where ganglion cells synapse with bipolar and amacrine cells. Evidently the ganglion cells contribute to SDP. As shown in the present work and also by others (GOURAS, 1958; MATSUMURA, 1968), the ganglion cells when invaded by SD depolarize strongly, and display an intense initial burst of impulse discharge followed by profound depression of activity. It is probable that the ganglion cells depolarize at their dendrites in the inner plexiform layer, making this region a current sink. The ganglion cells can also depolarize as a consequence of vigorous nervous (impulse) activity. The SDP induced by antidromic impulses in the optic nerve could be the result of such depolarization. However, since SD is still induced by chemical and photic stimulation in retinas deprived of nervous activity by tetrodotoxin, the nervous activity appears to be of secondary importance for the initiation of SD. This point will be further discussed in a following paper (MORI *et al.*, 1976) on the basis of additional data.

It should be noted that SDPs recorded across the innermost retinal layers of only 50 μm or less in thickness can exceed 30 mV. The potential seems to be too large to be attributed solely to the ganglion cell depolarization. Evidence will be provided in a following paper (MORI *et al.*, 1976) that the Müller cell makes a significant contribution to the generation of SDP.

MARTINS-FERREIRA and CASTRO (1966, 1971) have observed in the chick retina a marked increase in scattering of light in the inner plexiform layer during SD. VAN HARREVELD and FIFKOVA (1970, 1971, 1972), also working in the chick retina, have observed an increase in transparency of the retina during SD and postulated

it to be a consequence of an increase in sodium permeability of the plasma membrane. The observations of the above two research groups are somewhat confusing since they appear to be dealing with just the opposite optical phenomena, but nevertheless, it is worthwhile to note that the site of the maximal optical change in the retina agrees with the site of the maximal SDP determined by us and that the strong depolarization of ganglion cells during SD should be associated with an increase in sodium permeability in the inner plexiform layer.

Comparison of SDPs from the opposite retinal surfaces. It is well known from ERG studies that a potential recorded at one retinal surface resembles the mirror image of the potential from the other retinal surface. Comparison of the wave form of SDP recorded at the receptor surface (upper tracings in Fig. 3) with that recorded at the vitreous surface (upper tracings in Figs. 6 and 10) never conforms to this generalization. An account for this will be given in the following paper (MORI *et al.*, 1976).

Amino acids and SDP. VAN HARREVELD and FIFKOVA (1971) have studied effects of several amino acids on SD in the chick retina. In their study the retina was superfused by the fluid containing each amino acid in various concentrations, and its effect was evaluated from the transparency changes of the retina which they used as the index of SD. The amino acids used in the present experiment were sampled from those used by Van Harrevel and Fifkova and their potency as a stimulant for SD was tested by iontophoretic application in the frog retina. Despite the difference in material and method, the results were in general agreement; both glutamate and aspartate proved to be strong stimulants for SDP and DL-homocysteic acid showed a much stronger action than the other two, while pyloglutamate showed no action for the initiation of SDP. Except for pyloglutamate, these amino acids when applied iontophoretically produce a graded local response (see Fig. 8) which corresponds to wavelet-2 in the photically induced SDP. The local response appears to trigger an SDP by a yet unknown mechanism.

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