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PREFACE

Volume 4 of the *International Review of Neurobiology* is now before you. The editors have continued to solicit reviews from each discipline which deals with the working of the nervous system. This is to them *neurobiology* in its broadest sense. Cross-fertilization of ideas is of utmost importance in any constructive approach to the many unsolved problems in this field. Techniques which may be known to the behavioral scientist may be clarified by the refined techniques of the anatomist working with tissue cultures. Any drug which has a selective locus of action may be profitably used by research workers in any discipline. Advances in basic biochemistry, psychopharmacology, or biophysics thus may give rise to useful developments in the clinical field, but mature judgement is required to select, from the vast detail of basic information, those specific bits which may apply to human disease. The review authors are supplying this mature judgement.

The popularity of this approach is indicated by the necessity to publish an additional volume. This has been accomplished by the acceptance of two groups of manuscripts in the year 1962 with deadlines of June 1 and October 1 for Volumes 5 and 6, respectively. Hereafter the annual deadline for receipt of manuscripts will be October 1 of each year; Volume 7 will have a deadline of October 1, 1963.

These reviews and summaries ordinarily are written by invitation. The editors, however, will be happy to review unsolicited manuscripts if these are submitted in complete or outline form.

CARL C. PFEIFFER
JOHN R. SMYTHIES

Spring 1962

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THE NATURE OF SPREADING DEPRESSION IN NEURAL NETWORKS

By Sidney Ochs

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I. Introduction

Since the discovery of spreading depression (SD) by Leão (1944a), it has been intensively studied in a number of laboratories. Part of the fascination of SD is the slowness of its spread through the cortex. The rate of 2 to 6 mm/min has seemed too slow to be readily accounted for on the basis of known types of nervous conduction. Spreading depression was included by Roitbak (1955) in his book on electrophysiology of the cerebral cortex; Marshall's (1959) review of spreading depression is a comprehensive guide to the subject through 1958. Since then, new developments have clarified some long-standing conflicts concerning the basic cellular mechanisms involved. Spreading depression appears to be a neuronal reaction of the apical dendrites of pyramidal cells that is triggered by a component released from depressed neurons which in turn excites contiguous neurons. In the depressed cells an increased permeability occurs with a shift of electrolytes and water into the apical dendrites. Concomitant metabolic changes are found, in part connected with energetic requirements for reversion to the normal state. (We shall be concerned later with the evidence for this summary statement.) Fundamental aspects of SD will be dealt with in Sections II and III. Emphasis will be placed on cellular mechanisms as follows: factors in propagation, Section IV; molecular changes within cells, Section V; and events occurring between parts of the cortex (particularly with respect to the closely related phenomenon, spreading convulsion), Section VI.

Some special difficulties are associated with the study of SD. The phenomenon is of long duration, and the long-lasting changes induced in the tissues are difficult to handle experimentally. It is not surprising that SD has been unrecognized or variously described. Sloan and Jasper (1950) compared SD with suppression which was believed to originate from special suppressor strip areas (Fulton, 1955), and they concluded that suppression and SD are the same phenomenon. Difficulty in confirming the presence of suppressor strips has convinced most experimenters that suppressions of cortical activity are SD's and not suppression elicited from special suppressor-strip regions (Marshall, 1959). To those familiar with the phenomenon, the literature contains records which may represent unrecognized instances of SD. For this reason alone when working

on the cerebral cortex it is important to be alert to recognize SD when it appears. Empirical precautions against cortical damage probably have their basis in an obscure awareness of the prolonged changes in excitability produced by SD's. Some part of the difficulty experienced in recognizing SD is related to differences in the readiness with which it may be elicited in the various species. Of the common experimental animals it is regularly evoked in the rat and the rabbit, and has been observed in mouse, guinea pig, porcupine, marmoset, pigeon, sloth, macaque, and possibly also in man. A relationship between the computed movement of the cortical disturbance underlying a migrain escotoma and the slow rate of movement of SD was noted by Milner (1958) and the similarity between the rate of SD movement and that of Jacksonian March was also pointed out (Marshall, 1959).

Recently, SD has been used along with conditioned responses to study the higher functions of the cortex and to give another index of the effect of SD on cortical processes. The use of SD applied to studies of behavior has opened a new area of research. Localization of learning traces in one cortical hemisphere and even to parts of a cerebral cortex is accomplished with the aid of SD. These investigations and related ones concerning the functional connection of the hemispheres to one another and to subcortical structures will be discussed in Section VII.

II. Phenomenological Aspects of Spreading Depression

A. EEG Changes

As first shown by Leão (1944a), mechanical, chemical, or electrical stimulation of the exposed cortex may induce SD. The appearance of SD is best shown with a series of bipolar recording electrodes set at successively greater distances from the stimulated site (Fig. 1). After stimulation, a latency occurs lasting a few minutes, and then the EEG is seen to gradually decrease in amplitude in the closest pair of recording electrodes and to remain depressed in amplitude for several minutes. The movement of SD in the cortex is observed by the successive decline of activity first in the electrode pair closest to the point stimulated to give SD; then with a delay corresponding to its slow rate of progression, there is a diminution in the next channel's record of EEG activity, etc. Within 3 or 4 minutes the ampli-

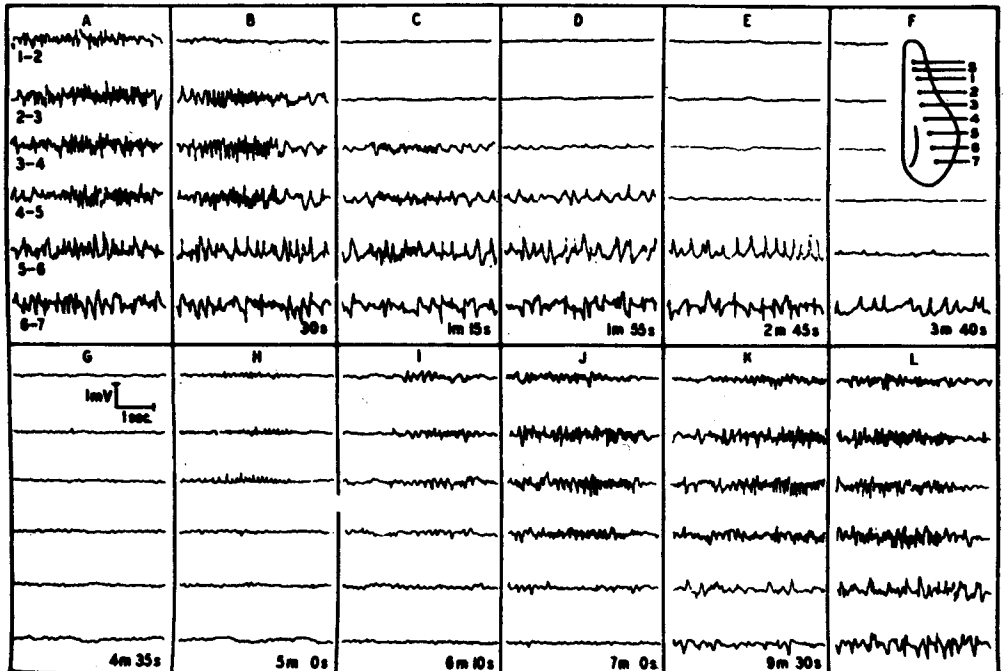

FIG. 1. Spread of depression. In the inset to the right the rabbit's hemisphere is shown with recording electrodes placed in line on its pia-arachnoid surface. S refers to the electrodes used to excite SD, and the electrode numbers shown on the brain correspond with the connections for each channel of EEG shown in different rows. Times in minutes and seconds shown in each lettered column of EEG samples. Soon after control EEG and stimulation (A), the EEG is depressed first in channel 1–2 (B), later in channel 2–3 further outward (C), etc. Recovery occurs gradually in the same order. Calibration in column G, vertical line 1 mv, horizontal line 1 sec. (From Leão, 1944a.)

tudes of the EEG gradually recover from their depression to attain control levels. Often, several large spike discharges are seen at the onset and during the depressed phase. These convulsive-like spikes occasionally may be so prominent as to replace the depression usually seen (see Section VI, B).

The spread is roughly similar to the outward ripples of a water wave, although irregularities are found at the advancing edge. If monopolar instead of bipolar electrodes are used, too wide an area is encompassed and the narrow region of passing depression may be missed (van Harreveld and Stamm, 1951). For this reason attempts to record SD by scalp electrodes through the skull have failed to show passage of SD in the underlying brain.

B. Steady Potential Changes

A DC amplifier and appropriate nonpolarizable electrodes are used to record the steady potential (SP) of the brain. This DC potential is related to the polarization state of the uppermost part of the apical dendrites of pyramidal cells with relation to lower parts of the cell (Gerard and Libet, 1940; O'Leary and Goldring, 1959). The SP of the cortex shows a characteristic variation when SD occupies the cortex under such an electrode (Leão, 1947). When SD enters an area of recording, the surface becomes negative in potential, and after 1 to 2 minutes the negative phase is succeeded by a smaller and longer-lasting positive phase before a return to the original level of resting potential (Fig. 2). The negative phase of the SP change is relatively large, with voltages of 5 to 15 mv usually recorded. Variants may occur in the pattern of depolarization. A prolonged negativity may be found that looks like a double negative phase, or the positive phase may be as great as, or even occasionally greater than, the negative phase. The most frequent type found is the pattern shown in Figs. 2 and 4. The relatively large amplitude of the negative swing indicates that many of the apical dendrites are synchronously depolarized. The SP records the activity of the apical dendrites found in great concentration in the molecular layer of the cortex. The negative variation of the SP likely reflects a differential depolarization greater in the upper apical dendritic portions than in the lower dendritic and somatic portions of pyramidal cells. The smaller positive phase which occurs after the negative phase may be a positive electrogenesis of the apical dendrites, but more likely it

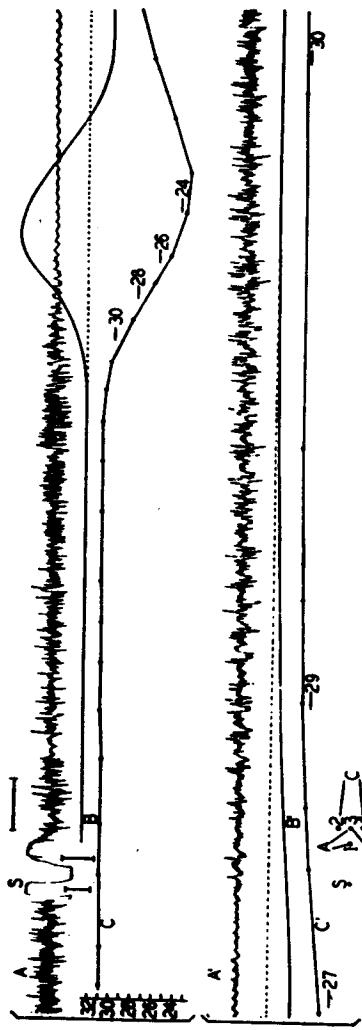

FIG. 2. EEG, SP, and impedance change during SD. Traces from above down are: (A) the EEG, (B) steady potential (SP), (C) electrical conductance of the cortex. The insert shows the electrodes arrangement. At S the cortex is stimulated' and after a latency of several minutes the EEG is diminished. SP changes in negative direction and at the same time conductance decreases. Return to control levels is indicated in the continuation of traces A', B', C' below. The negative variation of the SP is typically followed by a smaller positive variation shown below the dotted line with a slow return. Horizontal line 10 sec. Left vertical line 1 mv EEG, right vertical line 5 mv SP. (From van Harreveld and Ochs, 1957.)

reflects the presence of depolarization from the deeper layers of the cortex. Using semimicroelectrodes and recording SP changes from different depths within the cortex, Leão (1951) found that the negative phase appears at successively later times when recording from deeper parts of the cortex. There is, therefore, a slow movement down into the depth of the cortex, which stops when the white matter is reached.

C. Cortical Responses

Cortical responses evoked in a primary sensory area of the cortex as well as responses elicited by direct stimulation of the surface [direct cortical response (DCR)] are both depressed during SD. The decrease in DCR's during SD is shown in Figs. 12–14. The sensorily evoked responses are typically diphasic with a slow positive phase followed by a slow negative phase. The first of the smaller spikelike fast waves seen inscribed on the positive phase is due to activity of the entering afferent fibers ending within the cortex; the later fast waves are possibly due to intracortical cell discharge (Bishop and Clare, 1953). As SD invades an area of the cortex giving rise to an evoked response, the slow waves and then the later fast waves become depressed. Meanwhile the first fast wave representing activity in the afferent fibers is only briefly depressed. The significance of this temporary depression of the first fast wave will be discussed in Section IV. The slow negative component of the sensorily evoked response represents a discharge of the apical dendrites, and this part of the response is most deeply depressed. The simple negative-wave DCR is also a response of apical dendrites and is depressed by SD (Grafstein, 1956a; Ochs, 1958).

D. Electrical Impedance

Electrical impedance measurements of the functioning cortex had not been accorded much interest until Leão and Martins Ferreira (1953) showed a characteristic increase in the electrical impedance of the cortex occupied by SD. The increase of impedance found during SD is plotted as the inversely related decrease in conductance in the example given in Fig. 2. The measurements were recorded via electrodes placed on the surface of the brain using a bridge technique (van Harreveld and Ochs, 1956, 1957). The bridge was balanced to a null using a frequency of 1000 cycles/sec, and

changes in impedance during SD or cortical asphyxiation were measured by rebalance to the null or by the use of a rectifier and pen-recording technique. Fundamental work on measurements of cells in a conducting medium (Cole, 1940; Cole and Curtis, 1944) forms the basis for the interpretation of resistivity measurements and changes of resistivity of the cortex during SD. The cell membrane is characterized by resistive and capacitative elements. In a cell suspension at 1000 cycles/sec, the measuring current passes mainly between the cells via the intercellular medium. This path is resistive, and the degree of resistivity is related to the electrolyte concentration of the intercellular space and its comparative volume with respect to the total volume of the tissue. Increasing the volume of the cells in the suspension relative to the whole volume decreases the intercellular compartment and the path available to current flow. Therefore, the resistance measured is greater. We shall refer to the evidence in more detail in Section V, but briefly state, the 17% increase in electrical resistance observed during SD is due to the entrance of NaCl and water from the intercellular compartment into the cells. It is the loss of electrolytes from the intercellular compartment that results in rise of resistivity. Another technique of impedance measurement was developed by Freygang and Landau (1955). A pulse of current was applied to the cortex via a surface electrode with the indifferent electrode placed elsewhere, or impedance was measured with current pulses passed through a pair of semi-microelectrodes inserted across a small thickness of cortex. They found a 10–20% increase resistance during SD, which was most marked in the upper cortical layers. Under normal conditions the resistivity measured 220 ohm-cm, a value consistent with an intercellular compartment size of 25–30% (Section V, C).

E. Concomitant Vascular Changes

Leão (1944b) observed that the pial surface vessels undergo a marked dilation during SD, and he suggested that vessels are the medium of propagation of SD. A decreased amount of O_2 during SD was found in blood samples taken from the sagittal sinus and by microspectrophotometic measurements of the surface (Leão and Morison, 1945). This evidence seemed to favor the concept of vessels as the medium of propagation. Volume changes of the cortex during the passage of a wave of SD also seemed to support the idea that vessels were involved (van Harreveld and Stamm, 1952). Such

volume changes, recorded optically by means of a small mirror placed on the surface, indicated vasoconstriction and at the same time showed a decrease in O_2 pressure. A possible asphyxial change brought about by the constricted vessels was suggested. However, direct examination of the intracortical vessel showed that vasoconstriction was too brief to play a causal role (van Harreveld and Ochs, 1957). The intracortical vessels and changes in their diameter could be seen by means of a technique of fast-freeze and substitution-fixation. Ethyl alcohol cooled almost to its freezing point with liquid air was poured over the exposed surface to quick-freeze the brain; the frozen brain was then removed and placed in ethyl alcohol at −20°C. Substitution-fixation takes place over a period of 3 days with the alcohol slowly entering the solid tissue, and as it enters a region the ice dissolves. As the alcohol passes deeper into the frozen tissue, cells are fixed without dislocative movements of water. By this means cell contents and their relations to one another retain their original positions. The tissue can then be handled with the usual histological techniques. A benzedine stain was used to stain blood cells in the vessels and thereby show variations in the diameter of the blood vessels of the cortex. The vessels appear in these sections as vertical stalks with smaller branches to the side supplying the parenchyma. With an SD wave caught in passage, a brief period of vessel constriction developed in the rabbit cortex simultaneously with the SP change, followed by a vasodilation in its wake. Only vasodilation accompanies SD in the cat cortex. Evidence for a dilation during SD was found by Freygang *et al.* (1954) and by Sonnenschein and Walker (1956) using a heated thermocouple inserted into the brain. A surface thermocouple technique was used by Bureš ová (1957b) in her studies of vascular changes during SD, and she also found an increased blood flow. The vasodilation occurred 75 ± 15 seconds after the onset of the SP change of SD which is further evidence that the blood flow increase is a concomitant event and not a causal factor.

F. Subcortical Effects

When SD moves through the cortex, other parts of the brain neuronally connected with the cortex are secondarily involved. Winokur *et al.* (1950) recorded spontaneous activity from various thalamic nuclei and found alterations of EEG activity in those parts of the thalamus connected to the cortical region undergoing SD.