SPREADING DEPRESSION RESULTING FROM CORTICAL PUNCTURES

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Abstract. In acute experiments on cats under deep Nembutal anesthesia punctures of the cortex caused a prolonged negative shift of its surface potential (up to 15 mv, 5.5 min), which was followed by a prolonged positive shift. During these shifts of the potential, both components of the direct response were depressed. The changes in potential and the depression of electric activity spread over the cortex at a mean rate of 65 μm/sec. The phenomenon arose when the punctures are made with a pipet 20 μm and above in diameter. A puncture of the surface structures was critical; passage of the pipet through the middle and deep layers was ineffective. It is assumed that when cortical punctures are made the SD is caused by K⁺ ions which leak out of the destroyed glial structures.

INTRODUCTION

Spreading depression (SD) may be evoked in the cerebral cortex not only by chemical and electric stimulation (Leão 1944), but also by mechanical stimulation, for example, by pressure (Zachar and Zacharova 1961). In our experiments SD often appeared when we punctured the cortex; the punctures may be graduated by inserting pipets of various diameters to various depths, the time of making the puncture being noted in the record. We obtained facts that are of some interest in connection with the question of the SD mechanism.
METHODS

The study was conducted on 52 cats under deep Nembutal anesthesia (80 mg/kg); the cortex was exposed, its temperature was about 30°C. the respiration was normal. The recording electrodes were Ag-AgCl; the indifferent electrode was placed on the skull, the active—usually on the suprasylvian gyrus. Direct cortical responses were evoked by bipolar stimulation, stimuli being chosen of such intensity as clearly to manifest the second slow component (slow negativity). The potentials were amplified by a d-c amplifier and were fed to a KCII-4 self-recording potentiometer; the recording was made on paper. The cortical punctures were made with the aid of a microscrew with glass pipets, their point 2–80 μm in diameter. The pipets were placed with the aid of a microscope; the depth to which they were inserted was determined by the readings of a microscrew. The appearance of a characteristic prolonged shift of the potential of the cortical surface associated with depression of the direct responses served as the SD index.

RESULTS

Usually the cortical punctures did not cause a shift of the potential immediately after the operation, but began to produce it in 3–5 hr. In 14 preparations the cortical punctures caused no SD, most of these cases

![Fig. 1. Negative shifts of the cortical surface potential resulting from cortical punctures. Designations in the diagram: 1, puncturing pipet; 2, recording electrode; 3, stimulating electrodes. A, 4 hr after operation; arrow indicates moment of first cortical puncture (2.5 mm from the recording electrode); direct responses were evoked with a rhythm of 0.2/sec all through the recording time; depression of the responses during the shift of potential is seen. B, 1.5 hr later, cerebral edema; third puncture is made (2 mm from the recording electrode). Upward deflection, negativity of the active electrode. Calibration: 1 min, 1 mv.](image-url)
occurring in March. The amplitude of the negative shift (NS) amounted to 15 mv and lasted 5.5 min (Fig. 1); it was often preceded by a momentary positive deviation, the end of the NS being usually followed by a positive shift whose amplitude was smaller than that of the NS (Fig. 2). The NS was followed by a depression of the direct responses (Fig. 1).

Fig. 2. Effect of injury to the superficial and deep layers of the cortex. Puncturing pipet 50 μm in diameter. First arrow, puncture at a distance of 1 mm from the recording electrode, depth of insertion, 1,000 μm; after the puncture the pipet was not removed from the cortex. Second arrow, further insertion of the pipet to a depth of 2,000 μm. Third arrow, new puncture made next to the first to a depth of 1,100 μm; touching the cortical surface with pipet on the 18th min (cross). Calibration, 1 mv. Time in minutes.

The anterior front of the NS was steeper than the posterior. The configuration of the NS peak was very variable; the shifts lasting more than 2.5 min usually being double-humped, those lasting 1 min and less were single-humped. The character of the NS varied preparation to preparation, also, the NS in a given preparation changed in the course of the experiment and depended on the functional state of the cortex (Fig. 1 AB). The puncture causing a NS was in some cases followed by repeated shifts — one or a series of them — immediately after the first, or after an indefinite period (5-15 min).

In the experiments with cortical punctures made by pipets of various diameters it was found that the diameter of 20 μm is critical; punctures by pipets 20 μm in diameter usually failed to cause the SD, but pipets 30-40 μm in diameter usually produced the SD. Cortical punctures less than 1,000 μm deep were as effective in causing the SD as punctures to the entire depth.

If the pipet was briskly inserted to the full depth of the cortex a long time after a superficial puncture had caused a NS of large am-
plitude, it either evoked no NS at all or produced a very weak shift against the background in which the direct cortical responses were not noticeably depressed. On the other hand a new puncture side by side with the first caused a NS again (Fig. 2). In some cases a NS appeared when a puncture was made at a distance of not more than 1 mm from the recording electrode. When punctures were made at a distance of 1–5 mm the probability of recording an NS is high, however, a NS could also be recorded at a distance of up to 18 mm. If a distant puncture failed to cause a NS at the recording electrode it could be evoked after a NS was produced by a nearby puncture. In several cases a NS was caused by a puncture of the adjacent gyrus, although usually the NSs were not recorded from a puncture of the adjacent gyrus even when the distances between the puncture and the recording electrode were very small.

The rate of spread of the NS was calculated on the basis of the distance between the puncture and the recording electrode and the time from the moment the puncture was made to the beginning of the shift; it ranged from 40 \( \mu \text{m/sec} \) to 120 \( \mu \text{m/sec} \), the mean rate (determined on 27 preparations) amounting to 65 \( \mu \text{m/sec} \). In a given preparation the rate remained nearly constant and no change in the rate was observed to depend on the distance of the spread. An indication of the time required by the local process underlying the origin of NS and SD to develop at the point of the puncture was furnished by the results of the experiments with the punctures in the immediate proximity to the location of the stimulating electrode. After such a puncture the direct responses could no longer be evoked because of the development of SD at the site of stimulation. Thus in one experiment a stimulus applied 7 sec after the puncture was made failed to evoke the slow component of the direct response. In cases of punctures made at a distance of 0.5 mm from the recording electrode the shift could begin with a latent period of less than 3 sec.

Unlike the prolonged shift of the cortical surface potential which, as was mentioned, was usually caused only a long time after the operation, both components of the direct response were, as a rule, evoked immediately after the opening of the dura mater. During this period cortical punctures did not cause depression of the direct cortical response even when they were made in close proximity to the recording electrode. The puncture could cause profuse bleeding, but the responses did not change. If, however, the puncture caused a shift of the cortical potential, the direct response was depressed when the wave of the potential reached the area of electrode location. Figure 3 shows a record that illustrates this phenomenon. Apparently depression of slow negativi-
Fig. 3. Depression of direct responses during prolonged shift of the cortical potential. To evoke direct responses single stimuli were applied at intervals of 1 min and longer. Puncturing pipet, 50 μm; moment of puncture indicated by arrow. The puncture was made at a distance of 1 mm from the recording electrode; depth of insertion, 1,000 μm. Voltage calibration, 1 mv. Time in minutes.

ty started at the beginning of the negative phase of the shift and continued during its positive phase. A complete restoration of slow negativity occurred in 25 min, both phases of the shift of the potential lasting just as long. But depression of the direct responses may last longer than the shift of the potential on the cortical surface. The mean duration of the restoration for slow negativity was 17 min (this value was obtained on the basis of 74 experiments). The time of slow negativity restoration varied between 2 min, when its depression was incomplete — with a small shift amplitude, and 60 min, when the cortex was in a poor functional state — cortical edema; sometimes complete restoration did not take place in these cases.

DISCUSSION

During SD the neuron membranes are depolarized to +8 mv and the glial membranes to —10 mv; the maximum depolarization of glia occurs at the height of NS (Higashida et al. 1974). An important role in the appearance of SD is played by an increase in [K+]0 — in the event SD is produced by application of a concentrated KCl solution K+ ions are introduced into the cortex from without, but if SD is caused by electric stimulation of the cortex, K+ ions leak out of the excited neural elements and accumulate in intercellular clefts. By means of a K+ selective microelectrode it was found that during NS [K+]0 reaches 60 mM (Vyskocil et al. 1972). One of the arguments in favor of the leading role of K+ ions is that the amplitude of glial depolarization during NS depends on the initial level of the glial membrane potential; this indi-
cates that during SD glial depolarization was caused by ions which determine the glial membrane potential, i.e., $K^+$ ions (Higashida et al. 1971).

It may be supposed that the reason for SD when the cortex is punctured is that $K^+$ ions pour out of destroyed cortical elements and that the critical diameter of 20 $\mu$m corresponds to the critical mass of the destroyed elements. It turns out, however, that apart from this factor, it is important that the elements which are destroyed should be in the surface layers of the cortex, destruction of the same or larger mass in middle or deep layers being ineffective.

It follows that, to cause SD by a cortical puncture, it is necessary to destroy a certain critical mass of elements on the surface of the cortex. The connection between SD and the surface layers of the cortex is indicated by the fact that superficial incisions of the cortex prevented its spread (Leão 1944). There also are indications of the involvement of the pia-arachnoid mechanism (Marshall 1950), but apparently damage to the vessels is not critical, the vascular reaction in SD being a secondary phenomenon (Ochs 1962). Our findings that the destruction of a large mass of elements during the passage of the pipet through the middle and deep layers does not result in SD warrant the assumption that the determining factor is not related to the destruction of neural elements. Electron microscopy shows that the cortical surface is a membrane formed by astroglia (Ramsey 1965); the superficial marginal glial membrane is up to 15 $\mu$m thick and is composed of lamellae derived from astrocytes (Haug 1971); the glial processes form connections with each other (Haug 1971). It may be assumed therefore that the phenomena arising from cortical punctures are related to destruction of the aforesaid glial layer, and that when a puncture 20 $\mu$m in diameter is made, $K^+$ ions leak out in such amounts that a critical depolarization of the elements surrounding this site occurs with a subsequent spread of NS. It has been established that SD and NS may be caused by application of KCl to the part of the cortex poisoned by tetrodotoxin, i.e., without participation of the impulse activity of the neurons (see Bureš et al. 1974). Phenomena similar to SD were produced in a tissue culture by various stimuli, including mechanical, the shift of the potential taking place also in the culture containing only glial cells (Walker and Hild 1972).

The slow component of the direct cortical response (slow negativity) manifests depolarization of glial cells (Castellucci and Goldring 1970, Roitbak and Fanardjian 1973) initiated by the action of $K^+$ ions being liberated from excited neural elements (Ransom and Goldring 1973). Glial depolarization in response to stimulation is the less intense the lower the glial cell potential, and at a certain value of the potential —
under 30 mv — stimulation of the cortex does not evoke an electrical response in the glial membrane (Grossman et al. 1969, Roitbak and Fanardjian 1973). As was stated, SD is accompanied by a sharp depolarization of neurons and glial cells; this explains the depression of both components of the direct cortical response during a prolonged shift of the cortical potential in SD.

The foregoing facts indicate that the SD phenomenon may often appear in experiments with insertion of electrodes 40–60 μm in diameter into the cortex. This possibility must be taken into consideration even in microelectrode studies. This is also indicated by a number of facts obtained in experiments on tissue culture (Walker and Hild 1972). The NS resulting from cortical punctures may be restricted to an area with a 1-mm radius and thus remain unnoticed.

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