Regional Differences in Propagation of Spreading Cortical Depression in the Rabbit.* (22243)

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Leão(1) reported that in the rabbit spreading depression (SD) invaded the entire exposed surface of the stimulated hemisphere with the single exception of a small region medial to the parasagittal sulcus. This region appeared to be coextensive with area Rsg β of M. Rose(2), also with area 29d of Brodman(3) and area u of Droogleever-Fortuyn (4). Evidence has been presented by J. E. Rose and Woolsey(5) that this region is part of the rabbit's cingular area, the rest of which is situated on the medial aspect of the hemisphere.

In the present paper that part of the cingular area which is situated on the dorsal surface of the hemisphere will be indicated by "Cg." The dorso-lateral aspect routinely exposed, exclusive of Cg, will be called the "convexity" of the hemisphere. The invasion of Cg by SD was investigated, using as a criterion the slow potential change (SPC) which accompanies this phenomenon, in addition to the changes in the electrocorticogram (ECG) used by Leão. Marshall and his coworkers have shown, mostly in cats and monkeys in which SD is less easily obtained than in rabbits(6,7), that the production of this phenomenon can be facilitated in several ways (8-13). After having confirmed Leão's finding that SD does not normally propagate into Cg, it was attempted to make this area exhibit SD by application of the procedures described by Marshall *et al.*

Method. Rabbits were used exclusively. Cannulation of the trachea, jugular vein and femoral artery, and exposure of the cortex were performed under ether narcosis. During recording, the preparation was immobilized with Squibb's Intocostrin (about 5 units/kg bodyweight/hour) and maintained on artificial respiration. Blood pressure was recorded from the femoral artery when necessary. The electrodes were placed as shown in Fig. 1A. Electrode 1 was used for stimulation. Bipolar ECGs were led off from the convexity of the hemisphere and from Cg with electrode pairs 2-3 and 4-5, respectively. Slow potential changes with respect to an indifferent electrode contacting the pinna were recorded from electrodes 3 and 5. Recording and stimulating electrodes were of the silver-silver chloride type mounted on springs and resting lightly on the cortex. Electrocorticograms were recorded with an Offner electroencephalograph, slow potential changes were fed into d.c. amplifiers(14) and recorded on photographic paper with d'Arsonval-type galvanometers. Galvanic stimuli (3 to 5 volts), lasting for 2-3 seconds, were used.

Results. Spreading Depression in the Dorsal Part of the Cingular Area (Cg). The first stimulations elicit on the convexity of the

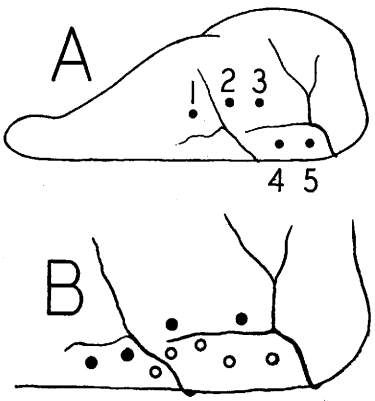

FIG. 1. A, a dorsal view of rabbit's hemisphere. Electrode placements are indicated by dots. Electrode 1 was used for stimulation. From electrodes 2 and 3 (on "convexity" of the hemisphere) and from electrodes 4 and 5 (on cingular area) bipolar electrocorticograms were led off. Slow potential changes were recorded from electrodes 3 and 5. B shows on a larger scale the cingular area on dorsal aspect of hemisphere. Electrode placements indicated by dots yielded typical slow potential changes, those indicated by open circles did not.

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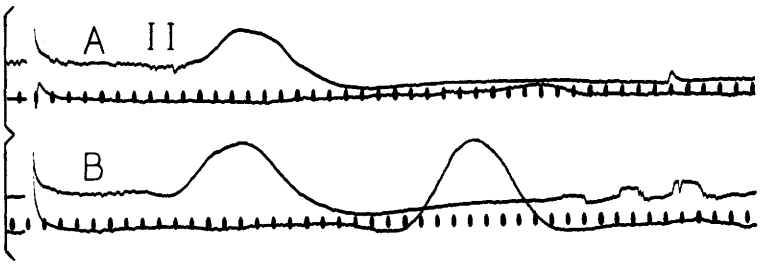

FIG. 2. Two slow potential change records, A and B. In each the upper trace is from convexity of hemispheres, lower trace from cingular area. A was made 33 min. and B 43 min. after start of infusion of 90% sucrose solution. Time marks indicate 10 sec intervals. The first 5 mV calibration line is for the upper, the second for the lower trace of each record.

hemisphere marked and often complete depressions of the spontaneous ECG accompanied by well developed slow potential changes (SPC). As the number of stimulations increases, depression tends to be replaced more and more by convulsoid patterns (1,6,15). The invasion of Cg by spreading depression (SD) was examined in 36 preparations. In 35 of them no typical SD or SPC invaded Cg. In most cases there was not the slightest indication of a slow potential change. In 9 preparations, however, a slight deflection was present in the SPC record from Cg. This small deflection appeared 1½ to 3 minutes later than the SPC recorded from the convexity of the hemisphere with electrode 3. Fig. 2A shows the relation in magnitude of the SPC recorded from the convexity and the small deflection recorded from Cg. A few spikes were sometimes observed in the ECG from Cg when the depression on the convexity of the hemisphere had been replaced by convulsoid activity. This spike activity, when present, appeared considerably later in Cg than on the convexity. Only in one preparation there was recorded from Cg a SPC comparable in size with that obtained from the convexity of the hemisphere. It was accompanied by a marked depression of the ECG. An attempt to delineate the region which does not produce the usual symptoms of SD is hampered by the long intervals between stimulations which are necessitated by the long refractory period of SD. Ten minute intervals were used in this investigation. Fig. 1B shows the results of an attempt to outline this region. It would appear that the limits of the unresponsive region correspond with that part of the area cingularis of J. E. Rose and Woolsey which is situated on the dorsal aspect of the hemisphere. In a few experiments the frontal boundary of the unresponsive area was traced as far as feasible on the medial aspect of the hemisphere. This boundary also appeared to coincide with that of Cg.

Effects of exposure of the cortex. In 7 preparations records made 2 to 3 hours after exposure of the cortex were not markedly different from the traces obtained shortly after removal of the dura. They showed clearcut SD and SPC in the traces recorded from the cortical convexity, no typical SD or SPC in Cg. Since long exposure of the cortex facilitates SD in monkeys(11) and cats(6,7) it was attempted to elicit this phenomenon in Cg of the rabbit in the same way. Two experiments were performed. In one, typical SDs and SPCs developed in Cg 8½ hours after exposure. They were present for two hours, after which the experiment was terminated. The other preparation examined for a similar period of time did not show a clearcut SPC in Cg.

Effects of intravenous administration of concentrated sugar solutions. Marshall(9) reported that dehydration of the cortex by slow infusion of a 90% sucrose solution facilitated the production of SD in cats. In the present experiments sucrose solution (90%) was infused through an inlying cannula into the jugular vein at a rate of 1-2 cc/min., causing a moderate blood pressure rise, profuse diuresis and in one preparation marked ascites. The brain shrank visibly. Three preparations were used, none of which exhib-

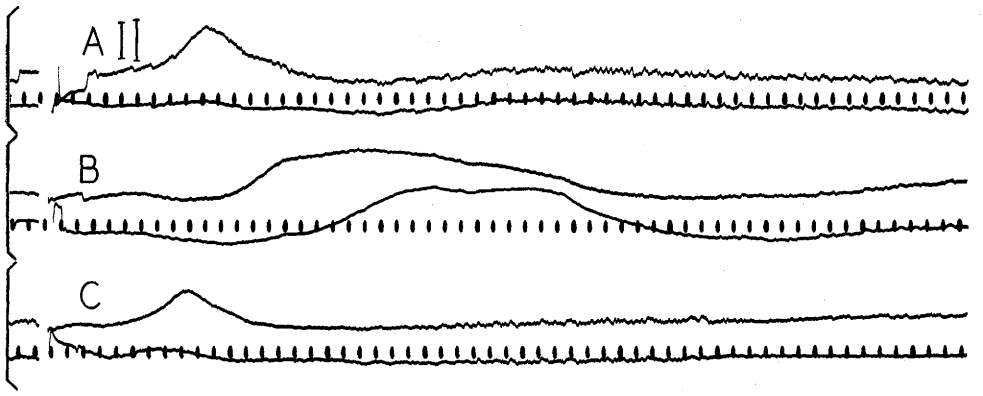

FIG. 3. Three slow potential change records, A, B and C. In each the upper trace is from convexity of hemisphere, the lower from cingular area. Record A was taken while cortex was covered with mineral oil near body temperature, B while cooled oil passed over cortex, and C again while covered by oil near body temperature. Time marks indicate 10 sec intervals. First 5 mV calibration line is for the upper, second for the lower trace of each record.

ited a typical SPC or SD in Cg before infusion. However, after 43, 50 and 53 min. of infusion a large SPC was recorded from Cg, accompanied by an incomplete but definite depression of the ECG. Fig. 2, A and B are SPC records produced by stimulations 33 and 43 minutes after the start of sugar infusion in one of these experiments. The upper trace of record A shows the well developed SPC led off from the convexity of the hemisphere. The lower trace recorded from Cg shows the small deflection mentioned above. Record B, taken 10 minutes later shows large SPCs of both the convexity and Cg, separated by a time interval of about 2 minutes. The duration of the depressions of the ECG recorded from the convexity of the hemisphere increased considerably during dehydration. Also, the height and duration of the SPC led off from the convexity were increased.

Effects of cooling of the cortex. Cooling the cortex was found by Marshall, Essig and Dubroff(10), to facilitate the production of SD. In the present experiments a skin well was formed over the exposed cortex by sewing the edges of the skin wound to a steel ring suspended a few cm. above the skull. The well was filled with oil near body temperature. Then cold oil (about 10°C) was passed through the well until a steady temperature of the outflowing oil was reached (17-20°C). Next the well was filled with warm oil again. After each change in oil temperature the cortex was stimulated. Fig. 3 is taken from such an experiment. Record A shows SPC traces from the convexity of the cortex (upper trace), and Cg (lower trace) respectively, while oil near body temperature filled the well. Record B was taken when the outflowing oil had reached a temperature of 18°C; and record C was made after a subsequent change to warm oil. Under oil near body temperature there is no obvious deflection in the SPC record from Cg, but after cooling there develops a large SPC of long duration which starts about 1 minute after the SPC recorded from the convexity. The SPC recorded from the convexity during cooling shows an increase in height and especially in duration (Fig. 3B, upper trace), as compared with the SPC led off from the same location under warm oil. Cooling increased the duration of the depression of the ECG from the convexity as much as 2 to 3 times, and had a tendency to decrease the convulsoid activity when present.

Effects of treating the cortex with isotonic sucrose solution. Essig and Marshall(8) reported that in cats and monkeys the production of SD is facilitated by treatment of the cortex with an isotonic sucrose solution. In the present experiments a solution of 10% sucrose in distilled water was made to flow at a rate of 10 to 12 cc/min. through the skin well described above. Since the solution has a high specific resistance, recording of ECG

and SPC can be continued during this procedure. Six experiments were successfully completed. Before application of the sugar solution typical spreading depressions were recorded from the convexity of the hemisphere but in none of the preparations from Cg. In all instances a sizeable SPC and definite depression of the electrocorticogram were recorded from this area after the cortex had been covered for some time (10 to 20 min.) by the sugar solution. The first stimulation after application of the sugar solution produced in each case a depression of the ECG of unusually long duration. Subsequent SD were of shorter duration and after 40 to 50 minutes of continued application of the sugar solution the depression was approximately of pre-treatment length. The SPC recorded from the convexity tended to be somewhat larger and of longer duration during the sugar application. In three experiments the sugar solution was replaced by Ringer's solution for about 10 minutes. The subsequent cortical stimulation caused a SD which was not propagated into Cg.

Effect of Ringer's Solution with 10 times normal potassium concentration. Treatment of the cortex with Ringer's solution containing more than the normal amount of potassium was also found to facilitate SD by Marshall *et al.*(12,13). In the experiments to be reported, KCl was added to normal Ringer's solution to make the K concentration 10 times normal. The solution was made to flow through the skin well at a rate of 10 to 12 cc/min. This treatment of the cortex caused spontaneous convulsive activity which, as was observed previously(16,17) can, and did act as stimulus for "spontaneous" SDs. By limiting the time of exposure in 2 experiments to 10 and 8 minutes, it was possible to avoid the convulsive activity so far that regular spreading depressions could be elicited. These preparations, which before the application of the K solution did not show a typical SPC or SD in Cg, did so after the cortex had been treated. After K treatment the magnitude of the SPC and the length of SD recorded from the convexity of the hemisphere were not increased. The cortex of the preparation with the 8 min. exposure was stimulated at 10 min. intervals following removal of the K solution. Only the first 2 stimuli produced typical SPCs in Cg; later stimuli although eliciting large SPCs on the convexity of the hemisphere failed to produce SPCs in Cg. The facilitation of the SD caused by exposure to the K solution thus seemed to be spontaneously reversible.

TABLE I. Latent Periods of Asphyxial Potentials from Convexity of Hemisphere and from Cingular Area (Cg).

No.	Convexity	Cg	Difference
1	310	460	150
2	125	230	105
3	130	270	140
4	140	280	140
5	120	160	40
6	80	85	5

Length of time, in sec between onset of asphyxia and development of the asphyxial potential. In preparations 1 to 5 the cingular area did not exhibit spreading depression; in preparation 6 it did.

Asphyxial potentials from the dorsal part of the cingular area. From 2 to 5 minutes after cortical asphyxiation a potential variation can be recorded from the cortex of similar magnitude and general appearance as the SPC accompanying the SD. Evidence has been advanced for the thesis that this asphyxial potential is related to the SPC accompanying SD(18-20). Since Cg is not normally invaded by the SD it was of interest to compare the asphyxial potential from the cortex on the convexity with the effects of asphyxiation of Cg. Six experiments were successfully completed. In 5 of them the SD did not invade Cg; the 6th experiment was the one, mentioned above, in which SPCs and depressions were recorded from this area. In all experiments an asphyxial potential was elicited both from the convexity and from Cg. However, in the 5 cases in which the SD had not invaded Cg the asphyxial potential developed from 40 to 150 sec. later in this area (Table I). In the experiment in which Cg exhibited SD the asphyxial potentials developed almost simultaneously.

Discussion. It is of interest that the limits of the area which is not normally invaded by SD correspond as far as investigated with the boundaries of the cingular area(5). There

thus seems to exist a relation between the presence or absence of SD and the anatomy of the cortex as given by cytoarchitectonic criteria.

On occasion a small deflection in the SPC record from Cg was observed. Since Cg is a strip of cortex only a few mm wide, electrodes placed on it are in general quite close to its lateral boundary in the parasagittal sulcus. Immediately lateral to the sulcus typical SDs and SPCs can be recorded. The distances involved are so small that the possibility has to be considered that the electrodes on Cg are affected by the physical spread of electrical phenomena occurring in the cortex immediately lateral to the sulcus. The time interval between the typical SD and SPC recorded from the hemispheral convexity and the atypical phenomena from Cg is commensurate with the time of propagation of the SD from the electrodes on the convexity to the cortex immediately lateral to the parasagittal sulcus. This explanation is consistent with experiments in which the propagation of SD was halted by a cortical cut(21); a small deflection similar to the one observed in the above experiments was recorded immediately across the cut on the non-stimulated side.

When after repeated stimulation the depression on the convexity of the hemisphere had been replaced by a convulsoid pattern, spike activity was sometimes observed in Cg. It seems possible that this spike activity was propagated actively from the cortex lateral of the parasagittal sulcus into Cg, since it is known that such convulsoid patterns are propagated to cortical areas not primarily affected(22). On the other hand, spread of a typical SPC into Cg is visualized as a change of this area, which makes it possible to be invaded by SD.

The 5 procedures which tend to facilitate the propagation of the SD in Cg produced changes in SD and SPC recorded from the convexity of the hemisphere which varied a great deal. Intravenous sucrose (90%) administration produced a gradual increase in the duration of the depression of cortical activity long before SD was propagated into Cg. Cooling caused a 100 to 200% lengthening of the depression. Treatment with isotonic sucrose solution produced an immediate prolongation of the depression, decreasing with the duration of the sugar application. The Ringer's solution with increased potassium concentration was without apparent effect on the length of the depression. Treatment of the cortex with isotonic sucrose solution, the intravenous administration of 90% sucrose, and especially cooling, increased and prolonged the SPC. The 5 procedures which facilitate the propagation of the SPC in Cg may not do so in the same manner. In the propagation of SD there may be involved a chain of events which can be facilitated at several points. For instance, it is conceivable that dehydration by bringing individual cortical elements closer together enhances the effect of one element on another. Or, if depolarization of neuronal elements is a necessary step in the propagation of SD(21) then this may be facilitated by the treatment with greater than normal potassium concentrations. If it be assumed that the 5 procedures facilitate via the same mechanism, then it follows from the varied effects of these procedures on the SPC of the convexity that this mechanism is not that underlying the changes in size, shape or duration of the SPC.

It was observed that the asphyxial potential develops later in Cg than in the cortex on the convexity of the hemisphere (in those preparations in which Cg is not invaded by SD). Assuming that the SPC accompanying the SD and the asphyxial potential are based on the same mechanism (18-20), then both this time difference and the fact that SD does not normally invade Cg may be indicative of the greater difficulty with which this mechanism is set in motion in Cg as compared with the cortex on the convexity.

Summary. 1. Of 36 rabbits consistently exhibiting spreading depressions and slow potential changes in response to galvanic stimulation of the cortex, only one exhibited propagation of these phenomena into that part of the area cingularis which is situated on the dorsal aspect of the hemisphere. Occasional small deflections recorded from this area were interpreted as evidence of physical

spread of typical slow potential changes in nearby cortex. 2. Where previously absent, typical slow potential changes and spreading depressions were produced in the cingular area by a) prolonged exposure of the cortex, b) intravenous infusion of concentrated sucrose solution, c) cooling of the cortex, d) treatment of the cortex with isotonic sucrose solution, and e) with Ringer's solution containing 10 times the normal amount of potassium. 3. Asphyxial potentials were recorded both from the cingular area and from the cortex on the convexity of the hemisphere. However, the latent period of this potential from the cingular area was markedly longer than that recorded from the convexity.

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Enzyme Studies in Muscular Dystrophy. II. Muscle Dipeptidase Activity and Vitamin E-Deficiency.* (22244)

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Vit. E-deficiency in the rabbit and other species results in characteristic muscular wasting and weakness. In previous investigations, on a number of tissues of the deficient rabbit, we found the cathepsin activity, *in vitro*, to be markedly increased in muscle, but unchanged in the other tissues(1). To investigate further the metabolism of protein in this form of muscular dystrophy, we have studied the activity of two previously characterized peptidase systems of muscle, glycyl-l-leucine and glycylglycine dipeptidase(2-4).

Methods. Vit. E-deficiency was induced in young rabbits by maintaining them on Diet 11 of Goettsch and Pappenheimer(5) treated with 1% ethereal $FeCl_3$. Control animals received the untreated diet supplemented with 25 mg % dl-α-tocopherol. After the appearance of symptoms of Stage II(6) of muscular

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