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The Minimum Volume of Depolarized Neural Tissue Required for Triggering Cortical Spreading Depression in Rat

T. MATSUURA* and J. BUREŠ

Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

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Summary. Cortical spreading depression (CSD) was evoked in anaesthetized rats by intracortical microinjection of $3.4 \cdot 10^{-8}$ mol KCl (the single injection threshold T_1). With two simultaneous injections at 1 mm tip separation 59% T_1 had to be applied to each point to elicit CSD. For interfocal distances 2, 3 and 4 mm the double injection thresholds $T_{1,2}$ were 65%, 74% and 97% of T_1 respectively. The spatial summation effect was still significant at 3 mm and undetectable at 4 mm tip separation. Recording electrodes placed 1—3 mm from the point of injection detected local slow potential changes which attained with subthreshold KCl injections 16% of the maximum CSD negativity at the 1.5 mm distance. The threshold amount of KCl required to trigger CSD at different intervals (30—480 sec) after initial injection of 0.8 T_1, exponentially increased with time from 28.2% T_1 at 30 sec to 88.9% at 480 sec with the slope 16.8% T_1 for a twofold increase of the interinjection interval. Quantitative analysis of results based on equations describing diffusion from an instantaneous point source indicates that the critical volume of depolarization is reached 61 sec after injection. According to the value of the diffusion coefficient of potassium the critical K^+ concentration lies between 45 and 12 mequ/l and is reached at a distance of 600—950 μ from the injection.

Key Words: Spreading depression — Slow potentials — Diffusion in brain tissue — Potassium chloride

Introduction

Although the mechanism of Leao's (1944) spreading depression (SD) is satisfactorily explained by the neurohumoral hypothesis (Grafstein, 1956, 1963; Marshall, 1959; Ochs, 1962; Brinley, 1963; Van Harreveld, 1966) according to which potassium and perhaps also glutamate ions mediate the underlying depolarization of neurons, less attention has been paid so far to the initiation of this phenomenon. SD is a sulfsustained autoregenerative process which spreads reliably through the entire volume of cortical grey matter in rats. Once the spreading stage is reached, the propagation depends on the density of somatodendritic membranes, on the amount of the transmitter released from a unit surface of these membranes, on the rate of propagation of the transmitter and on the firing level of depolarization in adjacent neurons. Other factors of varying importance are concentration

* Visiting scientist from Department of Physiology, Gifu University, School of Medicine, Gifu City, Japan.

of inactive elements (glial cells, myelinated fibers) and the geometry and organization of neurons. Reliable spreading is usually attained when the advancing front of the depolarization wave releases such amount of the transmitting agent that its concentration around the intact adjacent neurons exceeds their firing threshold.

Whereas the above considerations apply to a fully developed SD wave, it is intuitively obvious that depolarization of a single neuron does not raise the extracellular potassium concentration enough to depolarize the adjacent nerve cells and to start SD. Similarly, depolarization of hundreds or even thousands of neurons remains subthreshold. The aim of the present paper is to estimate the minimum volume of depolarized cortex required for triggering SD. Since most of the stimuli used for SD elicitation generate complex spatial gradients, the extent and slope of which is not known, it is difficult to infer the threshold volume of depolarization from the knowledge of the threshold stimulus alone. To overcome this difficulty the technique of spatial or temporal summation of two subthreshold stimuli was used to determine the range of stimulus interaction and to derive from the experimental data the size of the critical volume.

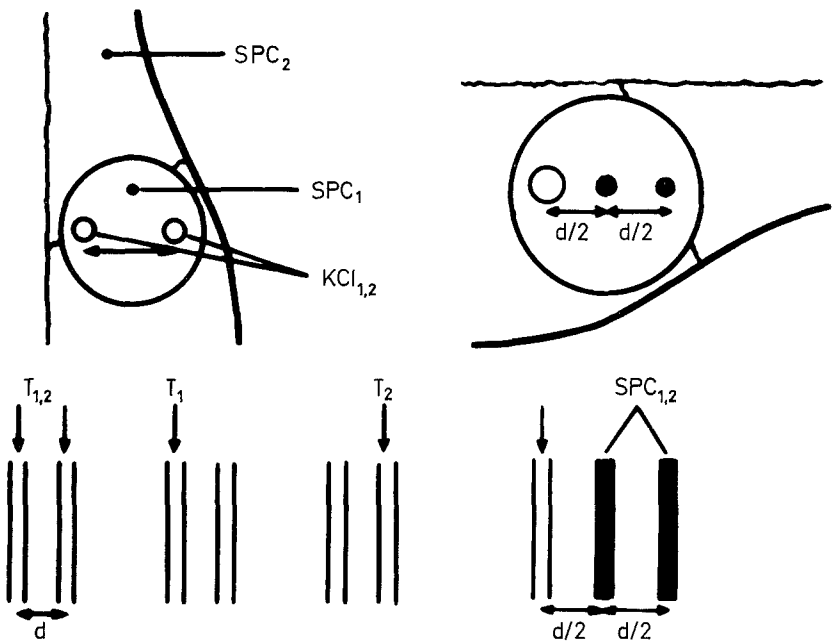

Fig. 1. *Distribution of the injecting cannulae (KCl) and recording electrodes (SPC) on the skull.* For details see text

Methods

Experiments were performed in 91 adult rats anesthetized with Allobarbital Spofa (40 mg/kg). A large trephine opening (5 mm in diameter) was made over the frontoparietal cortex and a small opening (2 mm in diameter) over the frontal cortex (Fig. 1A). The animal was then fixed in the stereotaxic apparatus, the microdrives of which were used for moving both the recording electrodes and the injecting capillaries.

In the spatial summation experiments 2 glass capillaries (200 μ external diameter) were glued together in parallel at distances from 0.7 to 4.0 mm. The capillaries were oriented perpen-

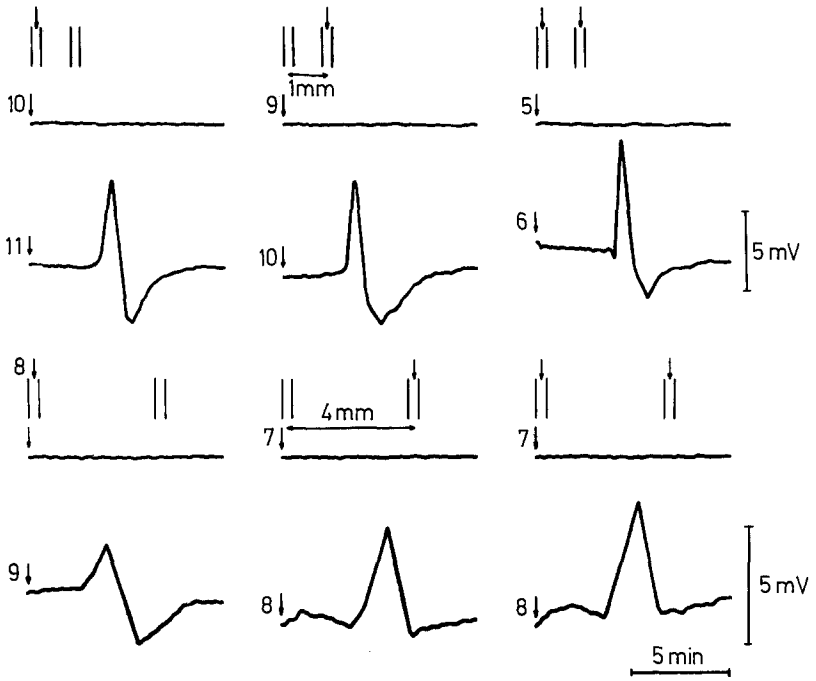

Fig. 2. *Typical experiments illustrating the technique of threshold estimation with the interfocal distance 1 mm (above) and 4 mm (below).* The T_1, T_2 and $T_{1,2}$ conditions are indicated in the inset diagrams. Numbers denote the injected K^+ quantities in arbitrary units. The effect of the highest subthreshold injection is shown in the first row, the threshold effect in the second row. Calibration 5 mV, 5 min

dicular to the cortical surface and inserted through the large trephine opening to a depth of 1 mm. The upper end of each capillary was connected to a polyethylene tubing (internal diameter 0.28 mm) filled with KCl solution. The amount of injected solution was proportional to the length of the tubing which was slowly squeezed between two motor driven rollers. At first 6% KCl was injected through each capillary separately to determine the single microinjection thresholds T_1 and T_2. An ascending series of KCl injections was applied at 5 min intervals until a SD wave was evoked. The increments were approximately 10% of the threshold volume. After T_1 and T_2 had been established, the 2 microinjections were simultaneously applied in order to assess the interaction between two adjacent foci. The double microinjection threshold ($T_{1,2}$) was determined in the same way as the T_1 and T_2 threshold. In different animals the distance d between the two capillaries was varied from 0.7 to 4.0 mm (Fig. 1A). The presence of spreading depression was detected by slow potential recording from the exposed cortical surface at two points 1 and 4 mm distant from the plane of injection (Fig. 1A). Cotton wicks soaked with saline connected the brain with calomel cell electrodes and with the cathode follower inputs of a 2 channel recording millivoltmeter.

The local slow potential change in the region of subthreshold KCl effect was recorded with an assembly of 3 parallel capillaries (200 μ external diameter) two of which were filled with saline and connected through cotton wicks with calomel cell electrodes. The third capillary of the same size was connected with polyethylene tubing (0.5 mm internal diameter) to the microinjection device. The electrode assembly was inserted 1 mm below the cortical surface in the same way as before and 2% KCl was injected in just infraliminal quantities. The distance d between the injecting capillary and the remote recording electrode was varied from 2—4 mm whereas the near electrode was always in the middle between the injection site and the far recording point (Fig. 1B).

Table 1. *Spatial summation of two subthreshold CSD foci at the inter-injection distances d*

d (mm)	n	T_1	T_2	$0.5(T_1+T_2)$	$T_{1,2}$	$\frac{100\ T_{1,2}}{0.5(T_1+T_2)}$
0.7—1.4	28	10.3 ± 1.0	10.2 ± 1.1	10.2 ± 0.6	6.0 ± 0.6	59%
2.0—2.1	19	13.2 ± 1.2	13.3 ± 1.4	13.2 ± 0.6	8.6 ± 0.7	65%
3.0	11	13.0 ± 1.4	11.8 ± 1.0	12.4 ± 0.9	9.0 ± 0.8	74%
4.0	12	9.6 ± 0.8	11.3 ± 1.2	10.5 ± 0.5	10.2 ± 0.6	97%

The thresholds are expressed in arbitrary units corresponding to $3.3 \cdot 10^{-9}$ mol K^+.

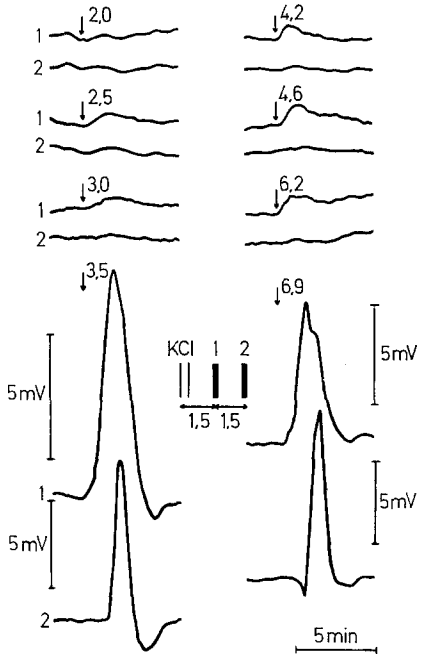

Fig. 3. *Examples of the slow potential changes recorded with the near (1) and remote (2) capillary electrodes in the vicinity of the injection.* Arrangement of electrodes is schematically shown in the inset diagram. Numbers indicate the injected quantities of KCl in arbitrary units. Calibration 5 mV, 5 min. Note the prepotential preceding the full size slow potential at the near electrode

In the temporal summation experiments an injecting capillary of the above size was inserted 1 mm into the cortex together with the recording capillary placed approximately 3 mm from the point of injection. The threshold volume (T_1) of 1.5% KCl was determined at first. Then 50 or 80% of T_1 was injected and at various time intervals ranging from 30 sec to 480 sec, 1.5% KCl was applied again. The minimum KCl dose T_s required to evoke SD with this second injection was determined and compared with T_1. A 10 min interval followed each pair of injections and at least 30 min intervals followed each SD wave.

Results

1. The spatial summation of two simultaneously applied microinjections of KCl.

Typical experiments, in which the distances between the microinjections were 1 mm and 4 mm respectively are illustrated by Fig. 2. With the 1 mm distance the T_1 and T_2 were 10 and 11 arbitrary volume units, while the $T_{1,2}$ was 6 units.

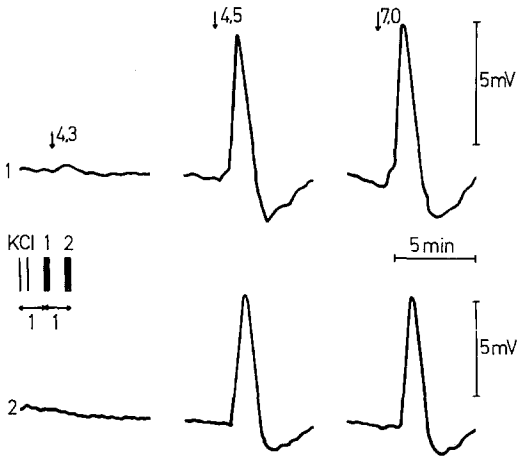

Fig. 4. *Example of the slow potential changes recorded in the vicinity of the KCl injection.* Same description as in Fig. 3. Note the absence of local slow potential change with the subthreshold dosage and of the prepotentials with suprathreshold injections at the 2 mm distance

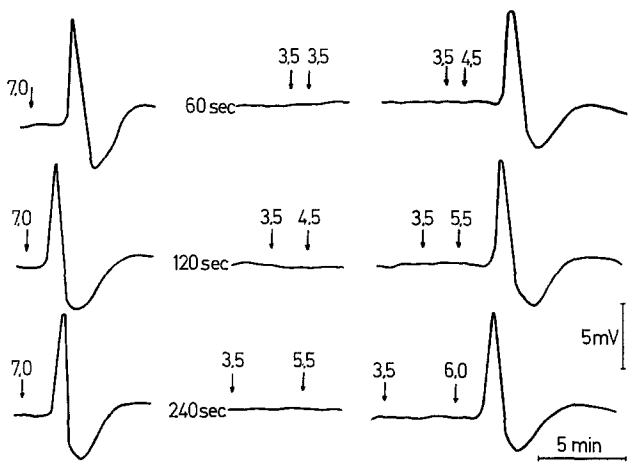

Fig. 5. *Typical experiments illustrating the temporal summation with the first injection equal to 0.5 T_1.* Left — the T_1 threshold. Middle — the highest subthreshold second injection. Right — the T_s threshold. The intervals between injections are indicated in seconds, the numbers denote the injected KCl quantities in arbitrary units. Calibration 5 mV

With the distance of 4 mm, $T_{1,2}$ was practically equal to T_1 or T_2 indicating that there was no interaction between the two subthreshold foci of this separation.

The degree of interaction can be expressed by the ratio $T_{1,2}/T_1$ or $T_{1,2}/T_2$. As shown in Table 1 this ratio was close to 50% with the 1 mm distance and approached 100% with 4 mm. The interaction was strong up to 2 mm and still significant at 3 mm but no interaction was observed at the 4 mm distance.

2. The local slow potential change recorded from the region of the subthreshold KCl effect.

With KCl dosages closely approaching threshold local slow potential changes could be recorded at a distance of 1 or 1.5 mm from the point of injection. Figure 3

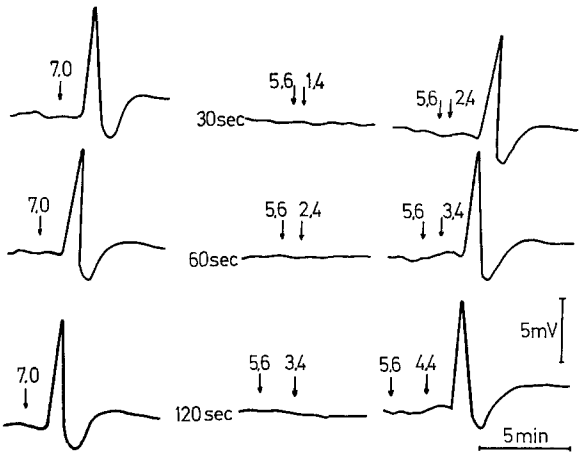

Fig. 6. *Typical experiment illustrating temporal summation with the first injection equal to 0.8 T_1.* Same description as in Fig. 5

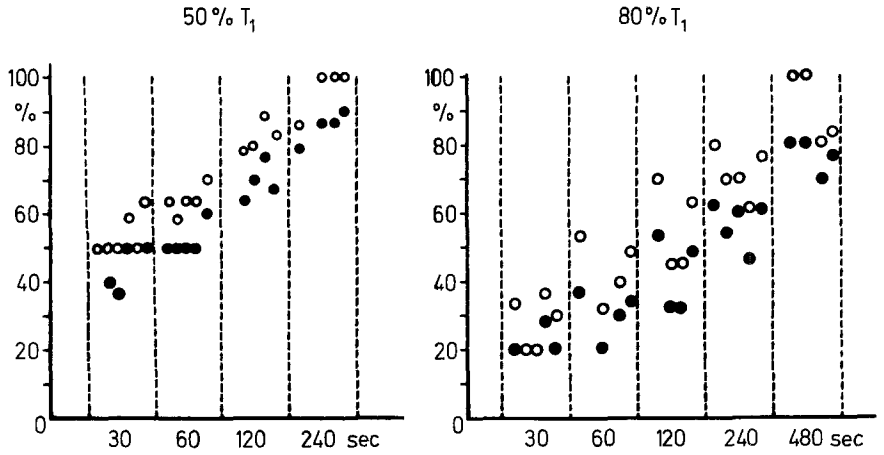

Fig. 7. *Temporal summation of two subthreshold injections of KCl.* Summary of results. Ordinate — amount of KCl applied with the second injection expressed in percentages of T_1. Abscissa — interval between injections. Black dots corresponds to the highest second injection which did not elicit CSD, circles indicate the threshold values of T_s. Points corresponding to one experiment lie on the same ordinate

shows a typical experiment with the injection-recording distance of 1.5 and 3.0 mm. The average maximum amplitude of the subthreshold slow potential was only 16% of the negative SPC recorded from the same point during fully developed SD. On the other hand at distances of 2 mm or more no local changes were observed (Fig. 4). The increment of potassium concentration is not detectable beyond the range of 2 mm from the point of subthreshold injection.

3. Temporal summation of two subthreshold microinjections of KCl.

A typical experiment in which the first injection was equal to 50% of the T_1 threshold is illustrated by Fig. 5. Since T_1 was 7.0 arbitrary units in this case, 3.5 units were applied at first. After 60 sec the minimum supplementary dosage

T_S required to evoke SD was 4.5 units. In the same way the T_S values for the time intervals of 120 and 240 sec were 5.5 and 6.0 units respectively. Figure 6 shows a similar example when 80% of T_1 was used with the first injection. The results of all experiments are summarized in Fig. 7 in which the T_S values are expressed in percentages of T_1 for the different intervals from 30 to 480 sec. The mean values

Table 2. *Temporal summation of two subthreshold microinjections applied to the same point with an interval t*

t (sec)	First injection 0.5 T_1 n	First injection 0.5 T_1 $\frac{100\ T_S}{T_1}$	First injection 0.8 T_1 n	First injection 0.8 T_1 $\frac{100\ T_S}{T_1}$
30	6	53.8 ± 2.7	5	28.2 ± 3.9
60	5	64.4 ± 1.9	5	38.8 ± 6.5
120	4	82.7 ± 2.6	5	55.8 ± 7.2
240	5	93.0 ± 4.9	5	71.6 ± 3.6
480	—	—	5	88.9 ± 5.1

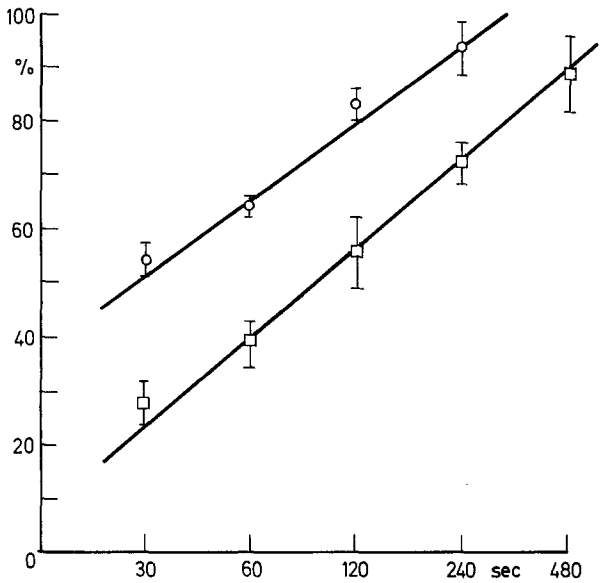

Fig. 8. *Temporal summation of two subthreshold injections of KCl:* average values of T_S when the first injection was 0.5 T_1 (dots) or 0.8 T_1 (crosses). Ordinate: T_S expressed in percentages of T_1. Abscissa: time between injections in a logarithmic scale. The regression lines were computed from the experimental data

of these percentages are given in Table 2 and in Fig. 8 which shows at the same time the computed regression lines for both sets of data. T_S increases as a function of the time interval between the two injections and the summation effect persists the longer, the higher was the dose applied at first.

Figure 9 shows how the effect of the first application decays with time. The two upper curves were obtained by subtracting the average values of T_S from

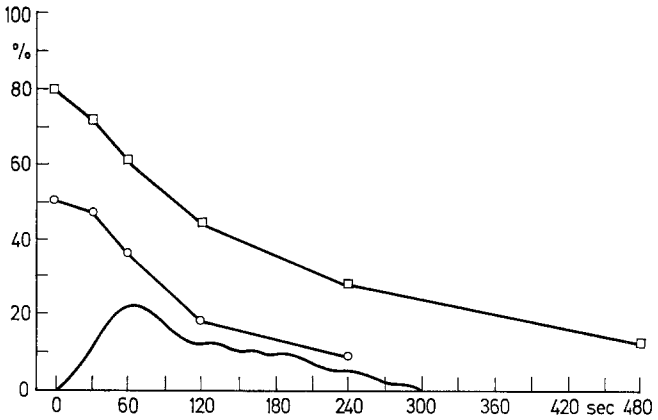

Fig. 9. *Decay of the subthreshold CSD focus established with 0.5 T_1 (dots) or 0.8 T_1 (crosses).* Ordinate: the (T_1—T_s) difference in percentages of T_1. Abscissa: interval between injections. The lower curve is the local slow potential change recorded at a distance of 1 mm from the injection

100%. The lower curve is an example of the local slow potential change recorded at the 1.5 mm distance from the KCl injection. The shape of all three curves is roughly similar in their descending portions.

Discussion

The results of the present paper indicate that approximately 1 mm^3 of cortical tissue must be depolarized in order to trigger a SD wave. This estimate is in good agreement with the value obtained with a different approach by Zachar and Zacharová (1963). Using mechanical stimuli (metal rod falling onto the exposed cortical surface) these authors examined the dependence of the SD threshold (erg/mm^2) on the size of the stimulated area (mm^2) and found steep increase of threshold when the surface hit by the falling body decreased below 0.5 mm^2. Since the impact affected the whole thickness of cerebral cortex (1.5—2.0 mm) and the mechanical deformation exceeded the diameter of the circular stimulus rod, the stimulated volume was approximately 1.0 mm^3.

Other experimental evidence supported the above conclusion (Zachar and Zacharová, 1961a). With the impact area 1.5 mm in diameter (1.76 mm^2) the threshold was 720 erg/mm^2. The relative refractory phase lasted 15 min when examined with the threshold stimulus, but could be shortened to 5 min, when the testing stimulus was increased 2.5 times. The authors suggested that 5 min after the conditioning stimulus enough elements had already recovered to start a new SD wave. Thus the difference between the threshold stimulus and the maximum stimulus expresses the ratio of the neurons activated by the threshold stimulus to the total neuronal population in the stimulated area. With the stimulus rod diameter 0.75 mm (0.44 mm^2) the threshold increased to 1630 erg/mm^2. The duration of the relative refractory period remained unchanged but the refractoriness could not be shortened even with stimulus intensities up to 6000 erg/mm^2. In this case the threshold stimulus was equal to the maximal stimulus, which means that

practically all active elements in the stimulated area had to be depolarized in order to trigger SD.

Whereas mechanical stimuli affect all stimulated elements at once and their action is limited to the area of stimulation, chemical stimuli used in the present study can be considered as an instantaneous point source which simultaneously expands in space and decays in time. The maximum concentration is reached immediately after injection, when the droplet of the injected fluid forms an artefficial extracellular space around the tip of the injecting capillary. In water at 37 °C potassium ions spread from a point source according to the equation (Curtis, 1964)

$$C = \frac{10^3 Q \exp \frac{-d^2}{4Dt\ 10^8}}{8(\pi\ Dt)^{1.5}} \tag{1}$$

where D is the diffusion coefficient (cm^2/sec) of potassium and C the K^+ concentration (mol/l) at the distance d (μ) from the point of application of a quantity Q (mol) of potassium after an interval t (sec) following injection. In every point adjacent to the injection site the potassium concentration first increases, reaches a maximum and then decays. The maximum concentration C_{max} is reached after time t (sec) which depends only on the distance d (μ) and on the diffusion coefficient D (cm^2/sec) according to equation

$$T = \frac{d^2}{6D\ 10^8} \tag{2}$$

After substituting T into equation (1) we obtain

$$C_{max} = \frac{10^3 Q \exp(-1.5)}{8(\pi\ DT)^{1.5}} \tag{3}$$

For the experimentally established single injection threshold $Q = 3.4 \cdot 10^{-8}$ mol K^+, C_{max} attains 30 mequ/l and 10 mequ/l after 13 and 27 sec at distances 445 and 641 μ respectively. Assuming the K^+ threshold within this range the minimum volume of tissue required for triggering SD would be approximately 0.5 mm^3. The real volume is larger, however, because the injected KCl is distributed mainly to the extracellular space forming about 25 % of the total tissue volume (Van Harreveld, 1966). This is the same as if the K^+ dosage is increased four times, i.e. to $13.6 \cdot 10^{-8}$ mol. In water the maximal concentrations of 30 and 10 mequ/l would then be reached after 33.2 and 69.0 sec at 705 and 1020 μ respectively. The time necessary to reach C_{max} is longer however, since roundabout diffusion pathways make D_K in brain approximately 2.5 times lower than in water. The use of this correction factor for longer distances was recently justified in experiments with microelectrophoretic application of glutamate by Herz, Ziegelgänsberger and Färber (1969). The maximal concentrations at the respective critical distances would then be reached after 83 and 172 sec respectively but remain otherwise unchanged, since the increase of T is compensated by the decrease of D_K.

The subliminal depolarization extends far beyond the supraliminal range. Assuming that the extracellular fluid has the same K^+ content as the cerebrospinal fluid (about 3 mequ/l — Van Harreveld, 1966) a subthreshold KCl injection

can double the K^+ content at a distance of 1500 μ from the injection site. This computed value is well compatible with the observation that the local depolarization recorded at this distance attains 16% of the maximum CSD depolarization. Assuming that the neuronal membrane is completely depolarized at the height of CSD, the 16% would correspond to the membrane depolarization by approximately 15 mV which could be brought about by a slightly less than two-fold increase of extracellular K^+.

Potassium ions released from the depolarized neurons add to the injected amount of KCl. Assuming that there are approximately 3.10^4 cm^2 membranes in 1 cm^3 of cortical tissue and that about 4.10^{-10} mol/cm^2 K^+ is released from the intracellular compartment during the initial phase of depolarization (Grafstein, 1963), this additional amount of endogenous potassium is 2.10^{-8} mol for the 705 μ radius and 6.10^{-8} for the 1060 μ radius. The ratio of released K^+ to the injected K^+ rapidly increases with the diameter of the depolarized region until the reaction becomes self maintained. Whereas with single subthreshold injections contribution of the released K^+ is not of critical importance, with simultaneous injections the K^+ release can be potentiated in the zone of subliminal interaction. This may account for the summation observed at the 3 mm distance. The shape of the computed isoconcentration lines deviates from the usual spherical form: the circular contours are elongated towards each other until they assume the form of a lemniscata or of an ellipse caved in the region of the short axis. The tissue lying at a given distance from the concave portions of the isoconcentration lines is evidently more affected by the released K^+ than the tissue lying at the same distance from the convex portions.

Whereas the spatial summation of two subthreshold foci is complicated by the geometry of interaction, temporal summation is easier to interpret because the K^+ ions are applied to a single point. From the time course of the temporal summation it is possible to establish the time t_c during which the critical concentration C_c is reached at the critical distance d_c from the injection. As follows from the experimental data and the computed regression line, 90.5 sec after injection of 0.8 Q_t it is necessary to apply 0.5 Q_t in order to trigger SD. Since with 0.5 Q_t the concentrations are decreased to 50 % at all distances, triggering SD under the above conditions indicates that the concentration reached at the critical distance d_c after an interval t_c following the injection of 0.5 Q_t is equal to the concentration persisting at this distance (t_c + 90.5) sec after injection of 0.8 Q_t. After substituting into equation (1) we obtain

$$\frac{10^3\ 0.5\ Q_t \exp \frac{-d_c^2}{4Dt_c 10^8}}{8(\pi\ Dt_c)^{1.5}} = \frac{10^3\ 0.8\ Q_t \exp \frac{-d_c^2}{4D(t_c+90.5)10^8}}{8(\pi\ D)^{1.5} \cdot (t_c+90.5)^{1.5}} \tag{4}$$

Since it can be surmised that d_c corresponds to the maximum concentration which can be attained at this distance by the second injection, we can substitute for it from equation (2). Equation (4) then becomes

$$\frac{10^3\ 0.5\ Q_t \exp \frac{-t_c 6D\ 10^8}{4Dt_c 10^8}}{8(\pi\ Dt_c)^{1.5}} = \frac{10^3\ 0.8\ Q_t \exp \frac{-t_c 6D\ 10^8}{4D(t_c+90.5)10^8}}{8(\pi\ D)^{1.5}\ (t_c+90.5)^{1.5}} \tag{5}$$

and after simplifying

$$\frac{0.5 \exp(-1.5)}{t_c^{1.5}} = \frac{0.8 \exp \frac{-6t_c}{4(t_c+90.5)}}{(t_c+90.5)^{1.5}} \quad (6)$$

Equation (6) is satisfied by $t_c = 61$ sec which is independent of Q_t and D. To this value of t_c corresponds in aqueous solution at 37°C $d_c = 957\ \mu$ for $Q_t = 3.4 \cdot 10^{-8}$ mol and $C_c = 2.93$ mequ/l. As pointed out by Krnjević and Mitchell (1960) diffusion of a substance in a multicompartment system involves tortuitous pathways. When the boundaries of the extracellular space are considered as spherical, longer distances have to be multiplied by $\pi/2$ and the diffusion coefficient has to be decreased by $(\pi/2)^2$, i.e. approximately by 2.5. For $D_K = 1.0 \cdot 10^{-5}$ cm²/sec, d_c decreases to 605 μ and C_c increases to 11.6 mequ/l. The real concentration would be about 4 times higher, however, since K^+ diffuses into the extracellular space forming approximately 25% of the total cortical volume. The resulting K^+ concentration of 45 mequ/l is probably higher than the actual threshold, the discrepancy being due to an underestimation of D_K which is equal to $1.0 \cdot 10^{-5}$ cm²/sec only close to the injection site. When the critical distance is approached more and more K^+ ions are released from neurons, the K^+ diffusion proceeds over shorter distances and is further enhanced by electrotonic spread and impulse conduction. Because of the combined action of all these factors D_K increases and may even exceed the value typical for aqueous solution. The critical distance and concentration should thus be sought between 600 and 950 μ and between 12 and 45 mequ/l.

Temporal summation of 2 mechanical stimuli was studied by Zachar and Zacharová (1951) with rather different results. The suprathreshold stimulus applied within 1 min after a subthreshold stimulus did not elicit CSD in nearly 50% cases. This finding is not unexpected when we take into account that the two stimuli were applied to almost identical neuronal populations and that the second one did not release, therefore, significant amount of additional potassium. The partial refractoriness disappears after 2 min because of reabsorption of the released potassium into the intracellular compartment. Comparison of the temporal summation of mechanical and chemical stimuli supports the conclusion that potassium released from cells does not contribute much to the effect of subthreshold potassium injections. The potassium ions applied with the second injection following the 0.8 Q_t stimulus mainly diffuse across an already depolarized region, from which no additional potassium ions are liberated. Potassium is released only at the periphery where the summation gradient exceeds the firing level of depolarization in the yet unaffected neurons. Although the second stimulus is deprived of a substantial part of the endogenous K^+ contribution the results indicate that its effectiveness is not significantly decreased.

References

Brinley, F.J.: Ion fluxes in the central nervous system. Int. Rev. Neurobiol. **5**, 183—242 (1963).

Curtis, D.R.: Microelectrophoresis. In: Nastuk, W.L. (Ed.): Physical techniques in biological research, Vol. V. — Electrophysiological Methods. Part A, pp. 144—192. New York-London: Academic Press 1964.

Grafstein, B.: Mechanism of spreading cortical depression. J. Neurophysiol. **19**, 154—171 (1956).
— Neuronal release of potassium during spreading depression. In: Brazier, M.A.B. (Ed.): Brain function, vol. 1. Berkeley: University of California Press 1963.
Herz, A., Ziegelgänsberger, W., Färber, G.: Microelectrophoretic studies concerning the spread of glutamic acid and GABA in brain tissue. Exp. Brain Res. **9**, 221—235 (1969).
Krnjević, K., Mitchell, J.F.: Diffusion of acetylcholine in agar gels and in the isolated rat diaphragm. J. Physiol. (Lond.) **153**, 562—572 (1960).
Leao, A.A.P.: Spreading depression of activity in the cerebral cortex. J. Neurophysiol. **7**, 359—390 (1944).
Marshall, W.H.: Spreading cortical depression of Leao. Physiol. Rev. **39**, 239—279 (1959).
Ochs, S.: The nature of spreading depression in neural networks. Int. Rev. Neurobiol. **4**, 1—69 (1962).
Van Harreveld, A.: Brain tissue electrolytes. Washington, D.C.: Butterworths 1966.
Zachar, J., Zacharová, D.: The refractory phase of Leaos spreading cortical depression. Physiol. bohemoslov. **10**, 341—348 (1961a).
— — Subthreshold changes at the site of initiation of spreading cortical depression by mechanical stimuli. EEG clin. Neurophysiol. **13**, 896—904 (1961b).
— — Initiation of spreading cortical depression. Bratislava: Publishing House of the Slovak Academy of Sciences 1963.

Dr. J. Bureš
Institute of Physiology
Czechoslovak Academy of Sciences
Budějovická 1083
Praha 4 — Krč (Czechoslovakia)