Inhomogeneous propagation of cortical spreading depression—detection by electro- and magnetoencephalography in rats

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Abstract

Spreading depression (SD) propagates in cortical regions that are different in their morphological and functional characteristics. We tested whether the propagation pattern of spreading depression was different between parts of the cortex. In six adult rats, we recorded the ECoG by a 4×4 electrode array that covered parts of the frontal, parietal cortex and the cingulate cortex. Simultaneously a 16-channel magnetoencephalogram was recorded to characterize the development and direction of intracortical ion movements accompanying this phenomenon. Spreading depression was initiated by occipital application of 0.3 molar KCl solution. Depolarization was observed, at first, at lateral cortical regions and then at medial cortical regions. Thereafter, the propagation velocity increased in medial cortical regions and was faster than in lateral regions. Negative potential shifts were detected by all electrodes, but the depolarization reached a maximum over lateral and caudal cortical regions. The recorded magnetic fields indicated the same orientation of currents underlying these fields, which was perpendicular to the wave front and points away from the depolarization region. Overall, the data indicated that propagation patterns of spreading depression differed between parts of the cortex and, thus, propagation was inhomogeneous. This propagation was accompanied by strong currents parallel to the cortical surface.

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1. Introduction

Spreading depression (SD) is characterized by a slow moving suppression of neuronal activity that propagates from a focal point, extending across different cortical regions. It is associated with changes of the DC potential [6]. This phenomenon may be related to migraine aura [4,22], hypoxia [39], cerebral ischemia [32], trauma [37,43], intracerebral hemorrhage [27] and tolerance to cerebral hypoperfusion [34] and thus seems to be involved in disturbances, as well as protection, of brain function.

Although the mechanisms of SD initiation and propagation are not precisely known, it is evident that both neurons and astrocytes are involved. The contribution of both cell types differs. For example, when neurons depolarize during SD, extracellular concentrations of potassium and glutamate increase, and astrocytes are involved in the control of this extracellular environment. It is suggested that reduced potassium uptake by astrocytes favors the initiation of SD [20]. Thus, it is supposed that communicative interactions between astrocytes and neurons in intact brain tissue,
particularly in the neocortex, are a prerequisite for SD initiation and propagation.

There is extensive knowledge of SD with regard to differing types of brain tissue [11], various species [2,3,6,38] and also in humans [40], which all differ considerably concerning cytoarchitecture. If the cytoarchitecture does not change considerably in the direction of propagation, then the wave front extends outwards in a circular formation. This phenomenon may be seen in the retina of the eye [9,24], which consists of five cell layers. Cell density varies tangentially to the retinal surface only in the layer comprising cone and rod photoreceptors. In the retina, the two-dimensional propagation of a SD wave appears as an expanding circle, with its center at the site of stimulation. If artificial inhomogeneity has been induced in the retina, e.g., by electric stimulation [16] or chemical manipulation [8], a complex pattern of SD wave propagation results.

The cerebral cortex is not as homogeneous as the retina is. It comprises regions which differ in phylogenetic age and cytoarchitecture, consisting of allo-, periallo-, proiso- and isocortex. In these regions the initiation, amplitude and propagation of SD are differently expressed. The isocortex is the phylogenetically youngest cerebral tissue. Nevertheless, it is inhomogeneous with respect to cytoarchitecture and function. The somatosensory and motor cortices, which correspond to the frontal and parietal cortices in rats, comprise, together with the occipital cortex, the main areas of the isocortex. The somatosensory cortex is characterized by the presence of a granular layer IV, whereas the principal feature of the motor cortex is the lack of a distinct internal granular layer that marks layer IV in the sensory cortex [47]. As of yet, the question of whether differences in the cytoarchitecture of the isocortex influences SD propagation has not been investigated.

Measurement of the two-dimensional pattern of electric potentials using ECoG with a spatial resolution high enough to differentiate between cortical regions is appropriate to describe a SD wave front properly. However, by this method, only data from regions covered by electrodes are obtained. Additional information is needed, which may be acquired by the detection of intracortical ion movements, which can only be detected by magnetoencephalography (MEG). Ion-sensitive electrodes show a fast increase of the potassium ion concentration within the extracellular space during SD [21,30]. This high-potassium ion concentration depolarizes astrocytes and neuronal cells. Potassium ions are taken up by astrocytes and sodium ions by neuronal cells at the SD wave front, causing intracellular concentration gradients [13,29]. Ion gradients are responsible for ion movements that are accompanied by small magnetic fields [10,13,14]. Experiments in rats [7], rabbits [2,15], in turtles [33] and, recently, in piglets [3] have shown, indeed, that these SD-related magnetic fields can be detected by highly sensitive superconducting instruments. Thus, a magnetic field can provide a global estimation of the direction of intracortical currents and is most sensitive to those currents directed tangentially to the cortical surface.

In the present study, we describe the two-dimensional pattern of SD propagation induced by focal potassium application to the rat cerebral cortex. For this purpose, simultaneous recordings of the electric potential and the magnetic field were made to assess the spatial orientation of the depolarization wave front in conjunction with the intracortical currents.

2. Materials and methods

2.1. Animal preparation

Six rats were used for simultaneous 16-channel ECoG and 16-channel MEG (magnetoencephalogram) recordings. Animals were initially anaesthetized by halothane (1.5 vol.%). After all surgical procedures were completed, anesthesia was continued by i.v. α-chloralose (10 mg/kg) and urethan (30 mg/kg). Rats were breathing spontaneously, and O₂/N₂O (1:2) was applied. All incision areas and pressure points were locally anesthetized with 2% Xylocitin. A catheter was placed in the femoral artery for continuous blood pressure monitoring. Blood gases were tested hourly and rectal temperature was recorded continuously.

Craniootomy was performed in a rectangular area of 33 mm² (5.5×6 mm; AP: −3.8 to 2.2, lateral: 5 mm) over the right hemisphere. To avoid heat loss that influences propagation velocity [44], evaporation and gas exchange with the environment, the exposed cortical area was covered by a silicon strip containing the recording electrodes.

A small cap was inserted into a burr hole (diameter 3 mm; the center was located at bregma −7.5 mm and lateral 3.5 mm) to allow the application of KCl (0.3 molar) from outside the shielded chamber using a push–pull system. At first, the cap and part of the feeding tube were filled with physiological saline solution separated from the KCl solution in the more distant tube by a bleb. Thus, 5 s after starting the push–pull system, potassium started to come into contact with the cortical surface. The dura mater remained intact, except for a small incision within the center of the burr hole (Fig. 1A). To avoid movement artifacts, rats were fixed in a plastic head holder using ear bars.

The experimental procedures were approved by the Animal Care and Use Committee of Thuringia and were in conformity with the Guiding Principles for Research Involving Animals and Human Beings.

The push–pull system was used to avoid overflow of flush solution outside the cap and development of high pressure on the cortical surface (Fig. 1C). SD was initiated by rinsing the cortical surface (bregma: −7.5 mm; lateral: 3.5 mm) with KCl for 1 min. Immediately after rinsing the cortex, the cap was filled again with physiological saline solution by the push–pull system. This was repeated in each animal three or four times after an interval of half an hour.
Additionally, in one sham rat, the same procedure was performed with normal saline solution. In pilot experiments, we have shown that perfusion of the plastic cap, on its own, did not produce any measurable magnetic signal.

2.2. Electrocorticography

The electric potential was measured by a grid of $4 \times 4$ electrodes (Fig. 1A) that was placed over the right hemisphere, covering an area $1$ to $-2.75$ mm from the bregma and lateral $0.5$ to $4.25$ mm [35,48] (interelectrode distances 1.25 mm; diameter of Ag/AgCl electrodes: 0.25 mm). The location of the electrodes in relation to the cortex was measured with an accuracy of 0.1 mm using a stereotactic system. They were placed over the frontal (agranular cortex), parietal (granular cortex) and cingulate cortices (regio retrosplenialis agranularis–proisocortex). The reference electrode was placed over the nasal bone. Electrodes were prepared as described by Bures et al. [5]. In brief, the electrodes were stored 12 h before the recording in physiological saline solution within a dark chamber to ensure stable electrochemical gradients. ECoG and MEG recordings were filtered (0–15 Hz), digitized with 40 Hz and stored on hard disk.

Because the onset of DC deflection is difficult to determine, we calculated the first derivative of the low pass filtered (0.05 Hz) electric signal (Fig. 2B). In doing so, the depolarization is transformed into a sequence of negative values, the first of which was defined as the beginning. Using this latency of the beginning at different electrodes and the distance between these electrodes, we calculated the mean propagation velocity. To describe the propagation pattern of the DC potential, we determined the temporal and spatial sequence of activity appearing at the electrodes and counted the number of electrodes at which the negative deflection had already been detected.

To compare SD propagation in a homogeneous medium and in the rat cerebral cortex, a simplified model of

![Fig. 1](image1.png)

![Fig. 2](image2.png)
propagation of electric activity was developed. This model consisted of uniformly activated cortical tissue, which expanded from the stimulation point with a circular wave front on the cortical surface at a constant velocity. The cortical surface in this model was approximated as a plane area that nearly corresponds to the geometry of the investigated cortical area in the rat. The distance between the electrodes and the region of SD initiation was taken into account. For each electrode, the start of depolarization was calculated assuming constant spreading velocity. This was calculated for spreading velocities between 2.0 and 5.0 mm/min. We also measured how frequently the electrodes detect the arrival of depolarization. The patterns of depolarization measured in the experiments, and the simulated data for each period, were compared statistically by means of $\chi^2$ test of homogeneity.

2.3. Magnetoencephalography

The MEG was recorded simultaneously with the ECoG using a 16-channel measurement system developed for animal research [31]. The system consists of 16 antennae (first-order asymmetric gradiometers) adapted to detect magnetic sources in the immediate vicinity (sensitivity 20–25 fT/√Hz). The distance between the antennae to the brain surface was about 6 mm and thus ensured high signal strength at the antennae. The diameter of a single antenna was 6.7 mm. An array of $4 \times 4$ antennae enables us to

![Fig. 3](image1.png)

Fig. 3. (A) Spatial distribution of maximal amplitudes of the negative depolarizations. Highest amplitudes were observed over lateral and caudal cortical regions near the location of SD initiation (mean ± S.E.M.). (B) Typical 16-channel ECoG traces of SD. The dashed line in each frame indicates the time point of the maximal negative depolarization at E1. The latency measured at the other recording sites is longer with increasing distances to E1.

![Fig. 4](image2.png)

Fig. 4. (A) Electrode array and percentage of electrodes showing the DC potential at particular time points (in s). The amount of filling of the circle encodes the percentage of DC deflections in individual electrodes and thus gives an impression of the spatial propagation of SD. At first, depolarization arrived at E1 and propagates thereafter in the mediorostral direction. (B) Illustration of the propagation of depolarization in an individual trace (increment: 3 s; arrow: caudorostral propagation direction). Sometimes, the wave front is distorted mainly over median cortical regions. (C) Propagation of depolarization wave front resulting from the simulation (increment: 3 s; propagation velocity: 2.2 mm/min; arrow: caudorostral propagation direction).
measure the magnetic field with high spatial resolution and sensitivity. The right hemisphere was centered below the system (Fig. 1B).

Measurements were performed within a magnetically shielded chamber (Amuneal, USA). Additionally, active compensation of low-frequency external magnetic fields was made with a separate system. This system detected the magnetic field in the chamber at a distance of 50 cm from the animal. This magnetic signal was used to generate a current outside the chamber, which compensates for the low-frequency magnetic field from the environment. Employing this active compensation, we achieved a noise attenuation in the frequency band <1 Hz of more than 25 dB [36].

The measurement of the magnetic signal started 1 min before KCl application, and the duration of recording was between 15 and 30 min.

On the basis of the spatial distribution of the magnetic field, we estimated the main direction of the intracortical current parallel to the cortical surface. The main current orientation was presumed to be perpendicular to the line between the positive and negative magnetic field maximum.

All values were expressed as mean±S.E.M. Statistical comparisons were made using the Matched Pairs Signed Rank Test according to Wilcoxon.

3. Results

Cortical perfusion with KCl for 1 min reliably elicited SD. Seventeen SD episodes were obtained in five animals. No changes of the electric potential or the magnetic field were observed when the cortical surface was rinsed with normal saline solution in the sham animal. The following sections will describe the electric potentials and magnetic fields associated with SD propagation.

Fig. 5. (A) Characteristic MEG traces of SD (A1–A16: antennae 1–16). The curve below trace A4 indicates the ECoG recorded simultaneously (E2: electrode 2). (B) Two-dimensional magnetic field pattern during the propagation of SD across the right hemisphere (increment: 250 fT). The antennae layout is as in Fig. 1B. The magnetic field maps during which the electric potential is depicted in Fig. 3B are marked by black frames (plus-magnetic field was directed out of the brain, minus-magnetic field was directed into the head, arrow-global intracortical current direction tangential to the cortical surface).
3.1. Electric potential

Application of KCl to the cortex was followed by a sequence of slow negative and positive potential shifts at the cortical surface. These potential shifts were highly reproducible in their temporal pattern (Fig. 2A).

Negative potential shifts were measured by electrodes at all recording sites. The depolarization reached a maximum over the lateral and caudal cortical regions (E1 or E2), with an amplitude of $-3 \pm 1.5 \, \text{mV}$ on average (Fig. 3A). Positive potentials peaked over the same cortical regions, with an amplitude of $1.8 \pm 0.6 \, \text{mV}$. Comparing site and maximal amplitudes of negative and positive potential at all electrodes, a significant correlation ($r=0.78$, $p<0.01$) can be observed.

The mean distance between the SD initiation site and E1 was 5.6 mm, and between the SD initiation site and E4, 6.4 mm. In all cases, the negative deflection was observed at E1 first, which was closest to the stimulation site, but subsequently, it was observed at all other recording sites. Both the onset of depolarization and the negative peak latency differed between electrodes (Fig. 3B). The onset in channel E1 was at $175 \pm 28$ s. The period of depolarization was followed by a positive potential shift.

Fig. 4A shows that the negative potential was observed in the initial period (up to 24 s in Fig. 4A) in lateral cortical regions and thereafter in medial cortical regions. Subsequently, the propagation velocity increased in the medial cortical regions and was significantly faster than in the lateral regions $[10.9 \pm 2.8 \, \text{s/mm} \quad (5.50 \, \text{mm/min}) \quad \text{vs.} \quad 21.2 \pm 2.2 \, \text{s/mm} \quad (2.83 \, \text{mm/min}); \quad p<0.01]$. Thus, the latency of depolarization between the medial and lateral cortical regions decreased with SD propagation to the rostral–cortical regions. An individual propagation pattern in Fig. 4B illustrates this result.

Fig. 4B shows the measured propagation profile of an SD with respect to location and time, and Fig. 4C shows theoretical values calculated for the model. In the theoretical model, the electrodes E1 and E2 were the first to detect the depolarization (Fig. 4C). When depolarization arrived at the second electrode row, the caudal region was completely depolarized. The differences between the pattern of measured and of simulated propagation reached significance between 7 and 24 s after stimulation ($p<0.025$).

3.2. Magnetic field

The magnetic field was analyzed in 15 artifact-free recordings from 17 SDs detected by ECoG. Typical traces are shown in Fig. 5A. In the channel exhibiting the maximal amplitude of the magnetic signal, the onset of the SD-related magnetic field changes was observed at $35 \pm 2$ s after rinsing the occipital cortex. The maximum of the magnetic field changes was reached at $80 \pm 6$ s. Thereafter, magnetic field changes gradually declined.

The initial main field shifts were positive (i.e., the magnetic field was directed away from the head) on the left side caudal regions, and field shifts were negative (i.e., the magnetic field was directed into the head) at antennae positioned over the right rostral side (Fig. 5B). This corresponds to a global intracortical current tangential to the cortical surface directed from the right caudal to the left rostral side (arrows in Fig. 5B). Thereafter, the orientation of the magnetic field and, thus, the direction of the intracortical current changed. A positive magnetic field was located on the left side, and a negative magnetic field over the right side. This corresponds to a current directed from caudal to rostral. This magnetic field configuration persisted during the appearance of slow positive potentials. Strong intracortical currents are perpendicular to the depolarization wave front and are directed away from it.

4. Discussion

Two main results were obtained in this study: (i) SD propagation is inhomogeneous tangential to the cortical surface, and (ii) SD is accompanied by strong currents parallel to the cortical surface and oriented in the direction of the depolarization wave.

The electric potential was recorded from cortical regions that are different in function and morphology. The frontal cortical region differs from other granular cortical regions, such as the occipital cortex, with respect to SD. Leao [23] demonstrated high susceptibility for the initiation of SD in the frontal cortex in studies using rabbits. Electrodes 11 and 12 and 14–16 in the present study were located over this area and detected an increase of SD propagation velocity. Additionally, in rabbits, there is a cortical region not invaded by SD, namely, the regio retrosplenialis agranularis [15]. In rats, it has been shown that, although the SD wave is not completely stopped in this region, its propagation through this area is considerably reduced [11]. Electrodes 4 and 8 in our recordings were located over this area and may thus have detected such a delayed propagation (electrodes 4 and 8). As demonstrated by the simulations, the result of the inhomogeneous spread of depolarization cannot be explained by an interindividually different, but rather a homogeneous, propagation velocity. Thus, the different features of SD seem to be related to differences in the cortical areas.

Some of the caudal and medial electrodes were located over the dorsal part of the cingulate cortex, the regio retrosplenialis agranularis. This region is characterized by a ganglionic layer of predominantly pyramidal neurons (V), a relatively undifferentiated external pyramidal layer (III), a granular layer (II) and a clearly differentiable but very thin layer IV [46]. Lateral to the cingulate cortex is a small caudal part of the frontal cortex abuts, which is involved in motor function and is characterized by an agranular morphology of the cortical lamination caused by a less
developed layer IV than in other isocortical regions. Most laterally located are parietal cortical regions that receive sensory inputs from the hind and fore limbs and comprise a well-developed lamina granularis (lamina IV). Thus, the principal difference between the frontal and parietal regions is the development of the lamina IV [47]. The lamina consists mainly of densely packed small pyramidal neurons and is characterized by the lowest glia–neuron ratio in comparison with other cortical layers [12]. Thus, neurons are more abundant. Therefore, ascending apical dendrites of pyramidal neurons from layer V are surrounded, to a higher extent, by neurons then in the frontal region, in which the lamina IV forms a very thin layer. Thus, the glia–neuron ratio, especially in the middle cortical depth and the spatial relationship between cellular elements, differs between the frontal and parietal cortical regions.

From the results published by Strong et al. [42], it can be assumed that the higher the frequency of glia cells, the less probable the appearance of SD. However, there are also contrasting results. SD is easy to initiate in the stratum radiatum of the C1 region of the dorsal hippocampus [19], where the frequency of glia cells is much higher in comparison with stratum pyramidale [17]. Similarly, Gardiner-Medwin et al. [15] demonstrated that SD does not invade regio retrospleniais agranularis in rabbits. In rats, however, SD propagation only slows down in this region [11]. Cytoarchitecture in this region of both species differs, with a lower frequency of neurons in the lamina IV in rats than in rabbits [46]. Thus, a higher frequency of glial cells or a lower frequency of neurons is not, in general, related to reduced propagation. We assume that the spatial relation of glia and neurons, in particular, with respect to the apical dendrites of pyramidal cells, is of importance.

Interaction between astrocytes and neurons is a prerequisite for SD initiation and propagation in brain tissue [25]. The lowest threshold to ignite SD by extracellular potassium application is near the proximal part of the apical dendrites, in comparison with soma, basal dendrites or axons. The regulation of extracellular potassium concentration is ensured by astrocytes. As shown by simulations, reduced potassium uptake by astrocytes decreases the threshold for ignition of SD [20]. Indeed, SD is modified if the normal function of astrocytes is altered, as shown by a recent investigation of mice with astrocyte-directed inactivation of connexin-43 [45]. It is supposed that a partial uncoupling of astrocytes by blocking connexin-43 reduces the capacity of extracellular environment regulation. Modification of gap junction permeability and, thus, coupling of the cells has a dose-dependent effect [29]. A low concentration of heptanol increases SD propagation, whereas a high concentration blocks SD. Naturally occurring gap junctions differ concerning permeability. Gap junctions containing connexin-32 have a conductivity of about 120 pS, whereas those containing connexin-43 have a lower conductivity of about 60–90 pS [41]. Recent investigations have revealed abundant connexin-32 in the rat occipital cortex compared with the parietal and frontal cortex areas, whereas the density of connexin-43 was highest in the frontal cortex [28]. As mentioned above, reduced coupling between astrocytes increases the propagation of SD. We suppose that lower cell coupling in the frontal cortical regions may contribute to the higher propagation of SD.

Previously, SD was described as an all-or-nothing process of involved cells [20]. In the present study, the amplitude of depolarization and the subsequent positive potential differed between cortical regions. Both negative and positive potential decreased with increasing distance to the initiation site. The spatial pattern for both decrements was similar. This observation confirms the results of Amemori et al. [1] who described that wave amplitudes gradually decline, and it is also in agreement with the gradual decrease of magnetic field changes seen during the propagation of SD in the rostral direction in the present study. Somjen has proposed that, once SD has been initiated, the membrane potential will reach the same extent of depolarization in each affected neuron. However, the extracellular voltage shift observed in this study may decrease if fewer neurons contribute to the SD, although the affected neurons do fully depolarize [39]. Thus, our data may indicate that the greater the distance is between the SD initiation site and the recording site, the fewer the neurons that are involved in a SD.

The propagation of SD within the cortex of the rat brain causes magnetic signals that can be measured early after cortical KCl application. The MEG is 6–10 times more sensitive to tangential currents in the cortex than to radial currents [18,26]. Thus, the relatively strong magnetic fields (in the order of pT) observed in this study are most likely due to currents oriented tangentially to the cortical surface. Recent investigations have found strong currents oriented perpendicularly to the cortical surface. However, in this paper, these intense tangential currents have been described for the first time. Intracellular current flow is, in part, glial current associated with potassium clearance. The tangential forward and backward currents accompanying SD are similar and, for the most part, cancel each other out in their magnetic effects. Simulations recently carried out show that SD is initiated if the net membrane current turns inward in the apical dendrites near the soma [20]. Simultaneously occurring current sources are detected in the distal dendritic tree. Because dendrites are, in part, oriented tangentially to the cortical surface, the intracellular currents are directed tangentially as well. We assume that the magnetic field is partly caused by currents in asymmetrically depolarized dendritic trees at the front of the SD wave.

In this investigation, differences between the MEG and ECoG latencies were observed, which were related to the methods used. The MEG detected signals from the whole of the hemisphere, and the ECoG, only from the area near to the electrode. Therefore, the first changes in the MEG signal corresponded to the beginning of SD in the occipital cortical region. Thereafter, SD propagated in a rostral direction and
reached the electrode array. It was at this point that the ECoG signal would begin to indicate strong depolarization.

In summary, simultaneous noninvasive recordings of the electric and magnetic fields with high spatial resolution has enabled us to determine the topological pattern of SD induced by focal potassium application. In rats, the SD propagation tangential to the cortical surface appears to depend on the region involved. Propagation is faster in regions that encompass five cell layers in comparison with those with six layers. Moreover, the further the SD wave propagates, the smaller the magnitude of depolarization and of ion movements. Most likely, the number of cells involved decreases in an SD the greater the distance is between initiation and recording site. Thus, an increase of propagation velocity does not necessarily correspond to an increase in the number of involved cells. The spread of depolarization is accompanied by strong currents oriented parallel to the cortical surface and directed away from the wave front. Further investigations are necessary to determine the physiological importance of these currents.

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