Magnetoencephalography of Focal Cerebral Ischemia in Rats

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Background and Purpose: The purpose of this study was to use magnetoencephalography to record magnetic field changes in the brain during middle cerebral artery occlusion.

Methods: A direct-current electrocorticogram (two channels) and a direct-current magnetoencephalogram (seven channels) were simultaneously recorded from five rats subjected to middle cerebral artery occlusion for 1-2 hours.

Results: Direct-current electrocorticographic and direct-current magnetoencephalographic signal deflections were observed after the onset of middle cerebral artery occlusion and occurred repeatedly throughout the ischemic period, with a mean±SD time interval of 12±5 minutes. A one-to-one correspondence of the electrocorticographic and magnetoencephalographic signal deflections was demonstrated.

Conclusions: Direct-current magnetoencephalography can provide a new noninvasive technique for studying depolarization and/or spreading depression in focal cerebral ischemia. (Stroke 1992;23:1299-1303)

KEY WORDS • arterial occlusive diseases • cerebral ischemia • electroencephalography • rats

Spread depression is a wave of electrical depolarization propagating from a site in the brain at which the depolarization was triggered. 1 Spread depression can be evoked by electrical, chemical (e.g., KCl, excitatory amino acid), or mechanical (e.g., puncture) stimuli.2 Pathophysiological stimuli, such as cerebral ischemia3 4 and hypoxia,5 have also been shown to evoke disturbances in ionic homeostasis and may cause spreading depression in the brain. To identify spreading depression, spatially separated electrodes have to be invasively placed directly on the cortex. This makes it impractical for clinical application. To our knowledge, there have been no reports on noninvasive measurements of direct current (DC) electrical disturbances in the brain after cerebral ischemia.

Magnetoencephalography (MEG) may provide an alternative technique for monitoring changes in brain electrical activity. MEG measures, noninvasively, the magnetic field generated by an electric current in the brain. The MEG signal is independent of the tissues between the MEG probe and the source of the electric current (e.g., current dipole),6 while electrical signals are commonly distorted when passing through the tissue layers between the source and the measuring electrode. Thus, MEG provides a more direct evaluation of the signal source than do electrical measurements.

We have previously reported the application of the DC-MEG technique to record anoxic depolarization7 and spreading depression induced by the cortical application of KCl in rat brain. This finding was confirmed by simultaneously recording DC electrocorticographic (ECoG) and DC-MEG signals. These data demonstrated a one-to-one correspondence between DC-ECoG and DC-MEG signals. In the present study we report, for the first time, simultaneous measurement of DC-MEG and DC-ECoG signals in the brains of rats subjected to middle cerebral artery (MCA) occlusion.

Materials and Methods

Five male Wistar rats weighing 270-320 g were used. The animals were fasted overnight before surgery but allowed free access to water. Anesthesia was induced with 40-50 mg/kg i.p. sodium pentobarbital. A femoral artery and vein were cannulated for blood gas sampling and supplemental drug/fluid infusion.

The fur over the rat's head was shaved, and the skull was exposed. Three burr holes 1.5-2.0 mm in diameter were made in the skull for the placement of the DC-ECoG electrodes. The dura was left intact. The Burr holes were located in the right hemisphere 1.5 mm anterior, 3 mm lateral to the bregma and 3 mm posterior, 3 mm lateral to the bregma and in the left hemisphere 1.5 mm anterior, 3 mm lateral to the bregma (Figure 1).

Ag-AgCl electrodes 0.2 mm in diameter were used to record the DC-ECoG. New electrodes were made for each experiment. They were kept in saline with the connectors joined to each other until use. The electrodes were slid into the epidural space under the skull through the burr holes. The electrodes and the burr
holes were then sealed with dental acrylic cement. The two electrodes in the right hemisphere (A and B) served as the active electrodes. The reference electrode was placed in the opposite hemisphere for maximum voltage gradient. The rat was grounded through an electrode placed in the nose (Figure 1).

The MCA was occluded by blocking blood flow into the artery with an intraluminal suture introduced through the extracranial internal carotid artery (ICA).

The right common carotid artery was exposed, and the ICA was carefully dissected free distally. A 4-0 nylon surgical suture, with its tip rounded in an open flame, was introduced into the external carotid artery lumen through a sheath placed in the artery. The suture was then gently advanced into the ICA lumen until it was 2-3 mm away from blocking the MCA.

The rat was transferred to a magnetically shielded room and then paralyzed with 1 mg/kg i.v. d-tubocurarine and mechanically ventilated with room air. A commercial videotape eraser was used to demagnetize the animal, minimizing the interference from magnetic particles carried by it. Blood gases were sampled before the start of the experiment and adjusted to normal ranges. The rat's head was supported by a nonmagnetic stereotaxic device to minimize any possible movement during MEG recording. The rectal temperature of the animal was kept at 36.5±1°C by means of warm water bags. Additional sodium pentobarbital and d-tubocurarine were administered through the venous cannula when necessary.

A seven-channel Neuromagnetometer (model 607, BTI, San Diego, Calif.) was used to record the DC magnetic field (0–50 Hz) of the rat brain. The probe assembly was positioned vertically over the animal's head. The central detector coil was centered 20 mm over the brain. The other six detector coils were centered every 60° on a circle of 21.5 mm radius around the central coil and tilted 7° to the vertical axis of the central coil (Figure 1). (These dimensions were selected to optimize measurements from the human head.) The DC-MEG signals from all seven coils were digitized at a rate of 130 Hz using an HP-694A multiprogrammer (Hewlett-Packard Co., Palo Alto, Calif.) and stored on disks for further analysis. The DC-ECOg signal was amplified and recorded using a P-18 DC amplifier and pen plotter (Grass Instrument Co., Quincy, Mass.).

Results

Autopsies performed immediately after the experiments revealed that the MCA was occluded in all five rats.

Baseline ECoG and MEG signals recorded at all channels before the onset of MCA occlusion were relatively stable (≤400 fT/min). The average DC-ECOg baseline showed no detectable change during the 10–30-minute period. After the onset of MCA occlusion, relatively sharp deflections of the ECOg and MEG signals were repetitively observed. These deflections usually lasted 1–2 minutes.

Figure 2 shows typical simultaneous recordings of the seven MEG channels and the two ECoG channels (A and B) before and during MCA occlusion. Occasional disturbances in the MEG signals were detected. The disturbances were readily attributed to random movements of the rat or environmental magnetic noise. As noted on the ECoG channels, multiple electrical depolarizations were detected. All electrical depolarizations were reflected in changes in the MEG signals. The pattern of signals in each MEG channel differed. Deflections corresponding to a particular depolarization event also differed among the seven MEG channels.

This is illustrated in detail in Figure 3, which shows an enlarged view of the MEG signals from each channel superimposed on the channel detector's location with respect to the rat's head. The MEG signal deflection observed in a specific channel was monophasic (either upward or downward) or multiphasic, depending on the location of the detector.

Maximum signal deflection amplitudes ranged from several hundred to 4,000 fT for DC-MEG and from 1 to 2 mV for DC-ECOg, depending on the location of the MEG or ECOg channel detector. The signal deflections were smaller in some MEG channels than in others. These differences may be attributed to a particular
FIGURE 2. Representative plot of direct-current magnetoencephalographic (MEG) and direct-current electrocorticographic signals from rat before and during middle cerebral artery (MCA) occlusion. Artifacts in recording could be identified as movement artifact (M) and system noise (S). Data acquisition was interrupted during time periods T1 and T2 for advancing suture to block MCA (onset of occlusion) and further drug administration, respectively. Arrows along time scale correspond to electrical depolarization. MEG channels are presented in horizontal rows for ease of comparison with Figure 3.

FIGURE 3. Representative plot of direct-current magnetoencephalogram (MEG) deflection patterns from rat (different from that in Figure 2) at each of seven MEG detector coil locations. Circles numbered 1-7 are MEG coil locations above rat's head. Note differences in signal deflection phase patterns and amplitudes among channels.

detector's distance from and orientation to the source of the electric current. Signal deflections were always clearly observed in MEG channel 1, the detector of which was set directly over the brain. Slow changes of the DC-MEG baseline signal other than signal deflection patterns were observed. The MEG signal gradually increased, decreased, or fluctuated after the onset of MCA occlusion. This relatively slow magnetic field variation was most prominent during the first 20–30 minutes and then gradually became less significant (Figure 2). The directions of these slow changes varied among the MEG channels. Corresponding slow changes of the DC-ECoG baseline signal, other than the signal deflection patterns, were not observed.

A total of 32 groups of simultaneously detected DC-ECoG and DC-MEG deflections were observed in the five rats subjected to 1–2 hours of MCA occlusion. The first group of deflections was usually observed within 1–10 minutes after the onset of MCA occlusion. Repetitive occurrence of MEG and ECoG signal deflections was observed in each animal throughout the 1–2 hours of occlusion, with a mean±SD interval of 12±5 minutes between deflections. Maximum DC-ECoG deflections measured at the two cortical sites occurred with a time difference ranging from −70 to 418 (mean±SD, 73±47) seconds; electrode A's deflection was observed first. To compare the temporal relation between DC-MEG and DC-ECoG signal deflections, we chose MEG channel 1 and ECoG channel B. Maximum signal deflection at MEG channel 4 occurred 37±45 seconds after that at ECoG channel B (Figure 1).

Discussion

The present study provides the first successful simultaneous DC-MEG and DC-ECoG measurements of rat brain depolarization during MCA occlusion. The non-invasively recorded DC-MEG signal deflections corresponded to the invasively measured DC-ECoG signal deflections, suggesting that the brain depolarization induced by MCA occlusion is associated with an electric current, which gives rise to a magnetic field detected by MEG.

The time difference between maximum DC-ECoG deflections observed at different cortical positions indicates that MCA occlusion elicited spreading depression and that the propagating wave front passed the electrodes at different times.\(^1,2\) MCA occlusion causes a local decrease of the brain's oxygen and energy supply, which subsequently evokes a disturbance of cerebral ionic homeostasis. The accumulation of extracellular potassium during cerebral ischemia is attributed as the source of spreading depression.\(^4,5\) In cortical spreading depression, the ionic disturbance travels across the brain cortex at a speed of 2–7 mm/min.\(^6\) This spreading ionic disturbance is equivalent to a slowly moving electric current source in the tissue, causing a magnetic field detected by MEG.

Simultaneous measurements of DC-MEG and DC-ECoG have been conducted in KCl-induced cortical
spreading depression in rabbits and rats and in reversible anoxic brain depolarization in rats. A temporal correspondence between DC-MEG and DC-ECoG signal deflections was observed in all these studies, as well as in the present study, indicating that brain depolarization and/or spreading depression are closely associated with electrical current sources in tissue.

The source of the observed DC-MEG signal deflections might not be the same in anoxia-, KCl-, or ischemia-induced spreading depression; anoxia-induced brain depolarization is a global effect, with electric currents likely induced as a result of inhomogeneous tissue impedance, and the application of KCl to the cortical surface induces spreading depression by causing an initial focal depolarization, which then spreads over the cortical surface as a propagating wave, thus causing magnetic field changes. The initiating source of the electrical disturbances detected after MCA occlusion is unknown. These disturbances, which lead to both the DC-ECoG and the DC-MEG signals after MCA occlusion, are not necessarily localized and may conceivably arise from multiple sites within the ischemic or penumbral volumes. The different mechanisms of generating DC-MEG signals may contribute to the differences in MEG signal deflection patterns.

Maximum DC-ECoG signal deflections at the two cortical locations occurred 73 ±47 seconds apart, suggesting that the DC-ECoG signal deflections are attributed to a propagating electrical disturbance. Calculating the speed of the spreading depression requires an exact knowledge of the locations of the measuring electrodes and the location of the initial depolarization. We are unable to estimate the speed of spreading depression due to our lack of knowledge of both the location of the initial depolarization during MCA occlusion and the direction of the wave propagation. The wide range of times of maximum deflection observed between the two ECoG electrodes (~70 to 418 seconds) and variations of the signal deflection patterns in different MEG channels may be explained by one or more of the following assumptions: 1) spreading depression induced by MCA occlusion may be initiated at different locations; 2) the path of propagation of each spreading depression may vary, and because MCA occlusion would result in a large ischemic volume, it is possible that specific pathways in cerebral tissue might be depolarized and thus alter the propagation pathway of the spreading depression; and 3) tissue conductivity may vary during MCA occlusion, thus changing the speed of spreading depression.

Though spreading depressions have been reported during focal cerebral ischemia in rats, there are no reports of periodic depolarizations as found in the present study. Our observation of periodic spreading depressions after the induction of ischemia is, however, similar to that observed after the focal application of KCl to the cerebral cortex. The difference between the spreading depression signals observed in our study and those in other studies of focal ischemia may possibly be attributed to the method of inducing focal ischemia. We employed a method in which a suture is inserted into the ICA to block the MCA. Thus, the skull and brain are not surgically violated. In contrast, occluding the MCA by bipolar coagulation via a craniectomy, the method commonly used in ECoG measurement of spreading depression in focal cerebral ischemia, may cause direct cortical tissue damage and electrical dysfunction, which will likely affect subsequent brain electrical activity.

The brain depolarization signals observed in this study using DC-ECoG electrodes were comparable to those reported by other researchers. However, the DC-ECoG signal deflection amplitudes that we observed were lower, partially as a result of using epidural rather than subdural electrodes. The use of epidurally placed wire electrodes was not optimum for measuring DC-ECoG but was imposed by the spatial limitation of the experimental setup. The amplitude of the electric signal deflection may be improved by placing the reference electrode in a more remote location (such as neck muscle). Nevertheless, relative deflections of the DC potential recorded epidurally reflect relative electric potential changes of the cerebral cortex and thus can be used for temporal comparison with the DC-MEG signal.

Relatively slow components in the DC-MEG signal deflections were observed, compared with the DC-ECoG signal deflections. This may be due to the sampling volume of the MEG detector coils, which are of the dimension of the rat brain and, hence, sample magnetic fields from an extended region of the brain rather than from a very localized region as the electrodes do. Thus, magnetic field shifts are detected during the entire time the wave of spreading depression is within range of the MEG detector coil. Interpretation of the full complexities of the MEG waveform awaits future experiments.

The magnetometer used in the current experiment is specifically designed for human use, and its detector array is fixed in a geometry suited for the human head. It is therefore impossible for us to localize the current sources in the present experiment. Given the size of a rat brain and the geometry of the MEG detectors, it is not unreasonable for MEG signal deflections to occur nearly simultaneously, with large variations in their phase patterns and absolute amplitudes. It is expected that spatial localization will be improved when the DC-MEG technique is applied to a larger subject such as a human.

The present study was not intended to quantify the electromagnetic response of brain subjected to an ischemic attack. Rather, our intention was to explore whether the noninvasive MEG technique could be used to detect physiological disturbances during cerebral ischemia, given the system sensitivity and signal strength. The results from this study suggest that MEG may provide a new noninvasive technique for studying the electrophysiology of the brain during MCA occlusion.

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References

Chen et al have completed an interesting study on the slow (direct current [DC]) shifts of spreading depression induced by middle cerebral artery occlusion in an animal model, comparing recordings of the new, experimental, noninvasive technique of magnetoencephalography (MEG) with the established, validated, invasive technique of electrocorticography (ECoG). These authors are some of the few who are working in the field of DC-MEG and have approached the problem systematically. As the authors are aware, DC-MEG changes in the brain are large for epileptic seizures. If DC-MEG could be used to monitor or detect cerebral ischemia, the authors believe that DC-MEG eventually might have important clinical applications in migraine and transient ischemic attacks.

The study design benefits from direct correlation of spontaneous activity in DC-MEG and DC-ECoG. The authors have used a state-of-the-art MEG system in a magnetically shielded room to eliminate the large magnetic artifacts from the environment. Middle cerebral artery occlusion was confirmed by pathological analysis.

The authors report two new findings: periodic (12-minute) depolarization shifts in MEG and ECoG and slow variations in the MEG signal without a counterpart in ECoG. The authors report very large signals that might be used as markers for acute ischemia. The authors give reasons why their methodology should detect these particular signals despite their not having been reported previously in the literature. The authors are sensitive to the fact that there must be more work in this field to confirm their findings.

The periodic (12-minute) depolarization shifts in ECoG could be confirmed immediately in many laboratories. Confirmation of the slow variations in the MEG signal without a counterpart in ECoG will require testing by a sensitive magnetometer, likely in a shielded, or at least low-noise, environment. This is likely to require more time because there are relatively few laboratories that have the capability or experience to perform such sophisticated DC-MEG/DC-ECoG comparisons.

It is important to confirm these results because there could be potential diagnostic value of detecting such slow shifts in human cerebrovascular diseases.

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