Selective Functional Vulnerability of Cortical Neurons Following Transient MCA-Occlusion in the Cat

G. Rosner, Ph.D., R. Graf, Ph.D., K. Kataoka, M.D., and W.-D. Heiss, M.D.

SUMMARY  Simultaneous recordings of several cortical neurons were obtained before, during and after transient 15 min occlusion of the middle cerebral artery in cats. With the use of a multiple electrode array consisting of 4-7 platinum/iridium microelectrodes, the cortical pericellular blood flow was concurrently measured by means of the hydrogen clearance technique. Hydrogen clearance measurements revealed a homogeneous blood flow distribution throughout all phases of the experiment in the area covered by the different microelectrodes. Considering only the results of experiments with low residual blood flow during ischemia (< 0.1 ml/g/min), single unit activity ceased immediately after occlusion and remained so during the ischemic period. The recovery time of action potentials after reperfusion ranged from 10 min to 3 hours depending on the examined neuron. Lower values for discharge rates of the individual cells were generally observed after recollection, although some units exhibited temporarily an even higher spike frequency. Furthermore, the spike form usually changed in that the hyperpolarizing afterpotentials were enlarged after recirculation. However, some cells with a nearly unchanged spike form were found as well. The results indicate that the recovery of cell function largely depends on the individual neuron which supports the idea of a selective functional vulnerability of cortical neurons in response to ischemia.

Methods

Animal Preparation

Six adult cats of either sex weighing 2 to 3 kg underwent acute terminal experiments. Anesthesia was initiated with i.m. injection of ketamine hydrochloride (25 mg/kg). After tracheotomy and immobilization with d-tubocurarine (0.5 mg/kg), the animals were artificially ventilated with a mixture of 70% nitrous oxide and 30% oxygen. The left femoral vein was cannulated. A continuous intravenous infusion (3 ml/hr) was maintained during the following surgery and throughout the experiment. The infused isotonic saline solution contained supplementary pentobarbital sodium (1 mg/kg/hr) to maintain anesthesia and a mixture of gallamine triethiodide (5 mg/kg/hr) and d-tubocurarine (0.5 mg/kg/hr) to maintain paralysis. Additionally, the left femoral artery was cannulated for continuous monitoring of arterial blood pressure. Deep body temperature was kept constant at about 37.5°C by means of a temperature controlled blanket. Systemic arterial blood pressure, pO2, pCO2, and pH were controlled and maintained within the range found in awake cats.

The head of each cat was fixed in a stereotaxic frame and the left middle cerebral artery (MCA) was exposed by the transorbital route. A small hook made out of an 18-gauge needle was passed around the proximal segment of the artery. After fixation of the hook with dental cement, the extended optic foramen was sealed with gelfoam and the orbit was filled with epoxy. The needle was kept closed to prevent leakage of cerebrospinal fluid until the artery was occluded. An angiographic wire was then inserted into the needle and driven towards the artery with a microdrive to reversibly occlude the vessel.

A craniotomy about 5 mm in diameter was performed in the left parietal bone above the middle ectosylvian gyrus. The dura was carefully removed under a microscope and the exposed cortex was covered with warm mineral oil.

DIFFERENCES IN THE SUSCEPTIBILITY TO ISCHEMIA among different regions in the brain, among various types of cells and even among neurons of the same brain region have already been established by Spielmeyer and Scholz. Recent investigations have shown that hippocampal neurons are particularly susceptible to transient ischemia developing irreversible loss of spontaneous single unit activity and cellular death after a "maturation period." Similar patterns of damage were found in related pathological conditions, e.g. hypoxia, epileptic states, and hypoglycemia.

The different ischemic vulnerability of various cells in a brain region could be caused either by a heterogeneity of microcirculation supplying neighbouring cells at sub- or suprathreshold levels, or by the properties of the individual cells affecting their susceptibility to ischemic damage. The following experiments were designed to determine the importance of these two factors: With a special multiple microelectrode array, spontaneous activity of several cortical neurons was recorded and related to the pericellular flow determined simultaneously with each microelectrode. By means of this approach, pericellular flow and cellular activity of several locations within small cortical regions were observed before and during short ischemic periods and during active reperfusion.

From the Max-Planck-Institut für neurologische Forschung, KölN, Federal Republic of Germany.

Address correspondence to: Professor Dr. W.-D. Heiss, Max-Planck-Institut für neurologische Forschung, Österheimer Strasse 200, 5000 Köln 91 (Merheim), Federal Republic of Germany.

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Measurement of Neuronal Activity and Cerebral Blood Flow

Single unit activity and cerebral blood flow were measured simultaneously by means of a floating multiple electrode especially designed for this study. It consisted of 7 single platinum/iridium microelectrodes which were inserted into the single barrels of a multi-barrel glass micropipette with a diameter of about 1.5 mm. Consequently, the interelectrode distance ranged from 300 to 600 μm. In vertical direction, the electrode tips were almost on one level so that recordings could be performed in 1–2 cortical layers. The micropipette was fixed with soft wax to a heating device within a Plexiglass holder which was attached to the stereotaxic microdrive. When the electrodes had been advanced into the desired location for recording, the wax was melted by the heater thus releasing the electrode and permitting it to float. Using this multiple electrode array the spontaneous electrical activity of 4–7 single cortical neurons was recorded extracellularly for more than 5 hours.

After amplification and high pass filtering (100 Hz), the spikes were observed on storage oscilloscopes, plotted online by a fiberglass recorder and stored on a multichannel magnetic tape for later analysis. The neuronal discharge rate was recorded in 10 sec intervals on a multichannel recorder after window discrimination and counter/voltage conversion. Wave form analysis of the spikes was performed with an averaging program (50 sweeps, sample rate 13 kHz) on a MINC computer (Digital Equipment Corp.). In some instances, (e.g. experiment shown in fig. 1), the background multiunit activity was recorded using a second window discriminator for separation from the background noise level and the single unit action potentials.

To measure the pericellular blood flow with the hydrogen clearance technique, a voltage of 400 mV was applied to each of the electrodes and compensated by bridge circuits for recording on a polygraph or a tape recorder after amplification and low-pass filtering (0.1 Hz). For measuring the H₂ clearance curve, 5–10% H₂ were added to the inhaled gas mixture until an adequate saturation was observed on the polygraph. Then, the clearance curve was recorded and later plotted on semilogarithmic paper for calculation of the cerebral blood flow within the first minutes of the desaturation. The blood flow was calculated according to the equation

$$\text{CBF} = \lambda (0.693 \times \frac{T}{2}) \text{ ml/g/min}$$

where $T/2$ is the time in minutes required for the clearance curve to decrease by one half. The brain volume recorded with the H₂ clearance using a microelectrode was estimated to have a diameter of about 50 μm. The mean CBF of the investigated cortical area was calculated from measurements with each microelectrode of a multiple electrode array.

After preparation of the animals, the electrode array was advanced into the middle ectosylvian gyrus by means of a nanostep motor drive until spikes were obtained simultaneously on at least 4 electrodes. Then the electrode array was made to float. Control measurements of CBF were obtained; then, the MCA was occluded for 15 min and the residual CBF was measured. After reopening the artery, the recovery of CBF and neuronal activity was observed for about 4 hours. To facilitate the evaluation of a possible differential behavior of concurrently recorded single cell activity in response to ischemia, we only selected experiments with critical ischemia for the present study. Ischemia was considered to be critical, when the mean CBF was below 0.1 ml/g/min after MCA occlusion.

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Time course of single unit activity (shaded bars) and multiunit activity (black bars) and of pericellular blood flow as recorded simultaneously with 6 microelectrodes during a single experiment with a 15 min occlusion of the middle cerebral artery.
more, experiments were excluded when arterial blood pressure, $p_{O_2}$, $p_{CO_2}$, and brain temperature changed by more than 10%.

Results

Cerebral Blood Flow

The CBF recordings obtained from measurements with all electrodes used in the different experiments are summarized in table 1, column A. Before the occlusion, the mean CBF in the middle ectosylvian gyrus amounted to 0.62 ml/g/min. After reopening the MCA, the previously ischemic area was immediately recirculated, and hyperperfusion occurred lasting for about 30 min; thereafter, hyperperfusion developed slowly and persisted throughout the observation period (up to 250 min). The differences between the CBF values obtained during the different periods of the experiments were highly significant ($p < 0.001$; two way analysis of variance, mixed model).

The CBF measurements obtained with the single microelectrodes of the used multielectrode arrays did not differ significantly during all stages of the experiments ($p > 0.50$). Further support for the homogeneity of CBF in the small cortical area covered by the multielectrode array can be derived from the calculation of the differences among flow values measured with the different electrodes. The mean of these differences amounted to nearly zero (table 1, column B). Thus, it can be concluded that the blood flow in the tissue containing the investigated neurons was homogeneous under all conditions.

Neuronal Activity

Spontaneous activity of most single units was immediately and persistingly abolished during MCA occlusion. Only in a few units a brief episode of increased activity appeared during the first seconds after clamping the artery. Simultaneously with the cessation of single unit activity the ECoG was reduced in amplitude and became silent thereafter.

After the arterial clamp was removed, the previously ischemic areas were reperfused and spike activity returned in 34 out of 35 neurons. Various patterns of spike recovery could be distinguished with respect to time course and discharge rate. Fig 1 shows the time course of the reappearance of single unit activity (shaded bars) and background multiunit activity (black bars) on 6 electrodes in a single experiment. On E$_1$-E$_6$, single unit activity above the pre-ischemic trigger level recovered between the 50th and 100th min of recirculation. However, as apparent from the multiunit recordings, some cellular activity returned earlier on all channels but E$_6$. On E$_6$, a neuron with low amplitude and discharge rate was observed before ischemia. Thus, it was not possible to evaluate multi-unit activity on this channel. After ischemia, the neuron on E$_6$ exhibited a higher firing rate. As can be seen also in figure 1, CBF measured with the different electrodes was homogeneously distributed within the investigated brain region despite the varying experimental conditions with normoperfusion, ischemia, posts ischemic hyper- and hypoperfusion.

The recovery time of cellular activity after ischemia as evaluated from all experiments is summarized in figure 2 which shows the first reoccurrence of single unit activity on the different electrodes after reopening the MCA. It is demonstrated in this diagram that some cells recovered after a few minutes of recirculation while others regained their spike generating capacity as long as 3 hours after ischemia. However, as indicated in the multiunit recordings and in the simultaneously plotted electrocorticograms, some electrophysiological activity was often reestablished within the investigated brain regions before the spontaneous firing of the recorded single neurons returned.

The analysis of the activity of several single neurons in a small brain region stressed the finding of a variable recovery of cell function despite homogeneous blood supply. The mean firing rates (spikes/10 sec) of the different single cells recorded in one experiment are shown in figure 3. On all channels, the rates of spontaneous activity of the single cells were diminished after ischemia. Only during a brief period, higher discharge rates were recorded on electrode E$_6$. To give a further

<p>| Table 1 Mean Cerebral Blood Flow Evaluated from Measurements with all Electrodes in the Different Experiments (A), and Mean of the Differences between CBF Values Obtained with the Different Microelectrodes of a Multiple Electrode Array during the Single Experiments (B). Shown are the Means during Different Phases of the Ongoing Experiments |
|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean CBF (ml/g/min)</th>
<th>Mean dCBF (ml/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia</td>
<td>0.62 ± 0.16</td>
<td>-0.001 ± 0.041</td>
</tr>
<tr>
<td>After ischemia</td>
<td>0-10</td>
<td>1.04 ± 0.18</td>
</tr>
<tr>
<td>10-30</td>
<td>0.76 ± 0.26</td>
<td>0.002 ± 0.103</td>
</tr>
<tr>
<td>30-60</td>
<td>0.49 ± 0.11</td>
<td>0.003 ± 0.076</td>
</tr>
<tr>
<td>60-100</td>
<td>0.41 ± 0.11</td>
<td>0.004 ± 0.071</td>
</tr>
<tr>
<td>100-150</td>
<td>0.43 ± 0.13</td>
<td>-0.007 ± 0.062</td>
</tr>
<tr>
<td>150-250</td>
<td>0.39 ± 0.09</td>
<td>-0.004 ± 0.087</td>
</tr>
</tbody>
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Figure 2. Recovery time of single unit activity after a 15 min occlusion of the middle cerebral artery (pooled data of all experiments).
impression of the diversity of spike recovery in different cells, short episodes of original recordings from different phases of a single experiment were plotted in figure 4. The pre-ischemic recordings (see channels E₁ and E₅) document rather heterogeneous discharge patterns whereas the activity becomes more regular after ischemia. The reduced background activity after ischemia should be particularly noticed. Regular discharge patterns as can be seen on channels E₄ (200th min) and E₅ (100th min) were characteristic for about 30% of the observed cells after ischemia. The episodes with regular discharge lasted from a few seconds to several minutes. The general pattern (observed in 29 of 35 cells) can be described as a cessation of firing during ischemia and a reduction of the mean firing rate during the recovery period as compared to the pre-ischemic control. One nearly silent neuron with rare spontaneous spiking during the pre-ischemic normoperfusion exhibited more frequent activity during reperfusion as shown in figure 1 (E₃) and 5 cells produced brief episodes of higher discharge rates after ischemia.

The wave forms of the extracellularly recorded action potentials before and after the ischemic episode were investigated as another parameter of interest. As shown in figure 5, the duration of the averaged spike was considerably increased by the ischemic insult in most instances. In few cells (n = 4), however, the post-ischemic action potentials were comparable to those observed before the transient MCA occlusion.

Discussion

Methodological Considerations

Information on neurons recovering from an ischemic insult is more reliable than data concerning nonrecovering cells which actually may have been viable but lost from the recording electrode, or may have recovered later after recordings were terminated. On the other hand, morphological studies investigate the problem using another approach which only concerns morphologically damaged or necrotic cells and cannot yield any information on the function of apparently undamaged neurons. To provide a good chance for the recovery of single cell activity within time periods permitting reliable continuous extracellular recordings, a short ischemic attack by occluding the MCA for 15 min was chosen for our study.

The specially designed multiple electrode array permitted the recording from several neurons in a small brain region and additionally demonstrated that flow changed homogeneously within the investigated cortical tissue. However, certain disadvantages must be taken into account: the small distance between the tips of the electrodes caused a relatively high pressure on the brain surface necessary for the insertion of the array, and the resulting traumatization of brain tissue was probably considerably higher than that which would have resulted by using a single microelectrode. In contrast to other multi-electrode techniques, a floating device was used. With this device, simultaneous 5-hour recordings of several single units were obtained in control experiments without major pattern alterations. For the investigation of neuronal function during ischemia this technique is of special interest because brain movements caused by circulation and respiration and also by the interruption of blood supply and recirculation can be counterbalanced. Additionally, effects of traumatization on single unit activity were not observed in long pre-ischemic control measurements.

The low impedance (1-2 MΩ) of the microelectrodes facilitated the measurement of CBF with the hydrogen clearance technique but made it more difficult to record spikes with high amplitude. Thus, window discrimination was necessary to determine single unit activity. The analysis of neuronal activity was additionally complicated by amplitude alterations occurring during the post-ischemic recovery of single units. The question as to whether such alterations were caused by minor displacements of the electrodes or by changes of the electrophysiological properties of the cells was not answered.
cell cannot be answered. In general, post-ischemic spikes with amplitudes above the pre-ischemic trigger level were accepted as indication for recovered neurons. The analysis of the waveform of the action potential was used as a sensitive tool for proving selective functional changes in individual cells. It must be kept in mind, however, that the effects of ischemia and post-ischemic reperfusion on resting membrane potentials, on amplitude and time course of the action potentials and after-potentials can only be quantified by continuous intracellular recordings which are extremely difficult to perform in the cat's cortex for a long duration with changing experimental conditions.

Control CBF, Ischemia, and Post-ischemic Reperfusion

The main disadvantage of the hydrogen clearance method as carried out with micro-electrodes is its inaccuracy at low flow rates, the determination of which is of special importance in studies of ischemic states. During severe ischemic conditions, hydrogen saturation becomes too slow and reflects diffusion rather than flow; clearance curves cannot be obtained within a 15 min period and CBF cannot be determined reliably. Therefore, all MCA occlusions causing the CBF to drop below 0.1 ml/g/min — a value which could be determined with acceptable accuracy — were defined as states of severe ischemia and only experiments in which flow below this level was maintained during the ischemic period were used for further analysis.

As found previously, recovery of neuronal function was not related to the state of reperfusion as long as the flow was above the functional threshold.

It was on purpose of the present investigation to examine the flow distribution within the small cortical area covered by the microelectrode array during various experimental steps, namely during ischemic and post-ischemic reperfusion. No significant differences between the electrodes were observed with respect to control flow values and the various induced or spontaneously occurring changes of perfusion. The homogeneous distribution of flow in this small cortical region during the various experimental stages indicated that each of the single neurons simultaneously recorded in an individual experiment had an almost identical history of pericellular flow. With ischemic insults of such short duration neither a non-reflow phenomenon nor heterogeneity of perfusion were observed in the investigated volumes of brain tissue. Such mechanisms are of importance only when larger tissue compartments or various locations of the brain are studied.

Spontaneous Activity as an Indicator of Function

The activity of a single cell may be considered as one part of a complex network; only the intact circuitry guarantees the normal brain function. The immediate effects of MCA occlusion on single unit activity, therefore, correspond well to the immediate onset of neurologic defects when blood supply is interrupted in the MCA model: activity ceases within 20-60 sec; sometimes, brief bursting activity indicates transient increased excitability of a single cell. The persistence of abolition of cellular activity throughout the experiment reflects the completeness of interruption of blood supply or the maintenance of residual flow below the functional threshold during the ischemic episode. After the 15 min ischemia all but one cell regained their ability to generate action potentials, and a permanent damage of cell function observed previously with short lasting ischemia in a few examples was only observed in one example of our study. Conclusions about long time effects of short lasting ischemic episodes on neuronal activity cannot be drawn from our experiments. The altered cellular function documented in the altered firing pattern during the first hours of reperfusion is certainly accompanied by disturbed neurologic function. Later on, cortical function may never the less fully recover.

The recovery time of single cell activity after ischemia covered a wide range with an even distribution between 10 and 150 min reperfusion. The multiunit and ECoG recordings, however, indicated that many cells recover faster after the 15 min MCA occlusion. A substantial part of the activity observed in the background multiunit discharge may originate from smaller
neurons which escape individual recording due to their limited potential fields. The variable delay of recovery of spontaneous discharge and the various post-ischemic firing patterns including longer lasting regular as well as bursting activity reflect impaired cellular function of different severity in the simultaneously recorded neurons: During the post-ischemic observation period, most of the cells fire at a lower frequency than before MCA occlusion, while only a few spike at a higher rate; the regular firing pattern was found in about 30% of the single units. Such changes of spontaneous neuronal discharge patterns, however, may be influenced by a variety of factors causing inhibition or excitation of the synaptic input to the investigated cell. Additionally, the wave form analysis of single unit action potentials provided some evidence for selective changes of membrane properties in individual neurons: the hyperpolarizing afterpotentials were enlarged after ischemia in most cells as could be expected from studies in asphyxia, while some neurons produced nearly unchanged action potentials.

In conclusion, our results provide evidence for a different functional tolerance of individual cortical neurons to short lasting ischemia. The causes of this selective vulnerability must be sought in intrinsic properties of individual cells which remain poorly understood. In our experiments, effects with regard to inhomogeneous post-ischemic reperfusion could be ruled out.

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References
Angiotensin II Decreases Mortality Rate in Gerbils with Unilateral Carotid Ligation

LEONARDO A. FERNANDEZ, M.D., DENNIS D. SPENCER, M.D., AND THEODORE KACZMAR, JR., M.D.

SUMMARY Evidence indicates that after vascular occlusion, infusion of angiotensin II restores blood supply to ischemic tissues by stimulating the development of collateral circulation through a mechanism independent of the mechanical effects of increased blood pressure. To test this effect in focal cerebral ischemia, angiotensin II was intravenously administered for four hours to gerbils immediately after unilateral carotid ligation. Three different pressor doses, 50, 250, and 500 ng/kg/min, were used, and mortality rate was evaluated at 1 and 2 days after vascular occlusion. Two additional groups similarly prepared were infused either with saline or with the pressor agent metaraminol. There was a significant inverse relationship between the infusion dose of angiotensin II and mortality: the greater the infusion dose of angiotensin II, the lower the mortality rate. Infusion of metaraminol, at the dose chosen to mimic the pressor effect of the highest angiotensin II dose, yielded a mortality rate which was statistically indistinguishable from that obtained with saline infusion. It is concluded that the mortality rate after unilateral carotid occlusion is significantly reduced by intravenous administration of angiotensin II through mechanisms unrelated to its hypertensive action. Evidence suggests that this may occur by the enhancement of the development of collateral circulation and therefore the reduction of the severity of brain ischemia.

CEREBROVASCULAR DISEASE caused by restriction of blood flow to the brain through normal vascular channels is a common clinical occurrence. Attempts have been made to protect the central nervous system from ischemia using pharmacological or surgical procedures or by elimination or inhibition of putative harmful products generated following the impairment of blood supply. The effectiveness of these therapeutic measures remains speculative. The removal of the causative factors of brain ischemia is still the treatment of choice. This is not often accomplished after a sudden ischemic event at which time increasing blood flow to the ischemic areas is critical. Therefore, the enhancement of the development of collateral circulation would be, under these circumstances, the optimum palliative mechanism to maintain the necessary blood supply to the brain. Stimulation of the development of collateral circulation to ischemic areas could represent a potential therapeutic tool in the treatment of the ischemia of the central nervous system.

Collateral circulation was defined by Liebow as a secondary system of vessels which develops to substitute for major vascular pathways. There are at least two phases during collateral circulation development. Immediately after vascular occlusion, there is a rapid enhancement in function of previously present “in reserve” vascular pathways which is followed, as time proceeds, by the formation of new vessels. Several factors of neural, mechanical and chemical origin have been proposed as stimuli in the development of collateral circulation.

In the kidney, the renin-angiotensin system has been demonstrated to act as a stimulus for the development of collateral circulation. After induction of renal ischemia, the endogenous renin-angiotensin system or the administration of angiotensin II have been shown to rapidly restore blood flow to the affected area. Evidence indicates that this action is unrelated to the mechanical effect of the increased blood pressure produced by angiotensin II. Renin or the renin-angiotensin cascade is not exclusively present in the kidney but is found also in other organs, including the central nervous system. Its role to the ischemic kidney, serving as a protective mechanism against focal ischemia by promoting the development of collateral circulation, suggests that a similar function might be applied to the ischemic brain. If increased levels of circulating angiotensin II have an effect in the ischemic brain similar to that seen in the kidney, then infusion of this peptide should result in an increased survival rate after focal cerebral ischemia.

The present experiments were designed to study the effect of exogenous administration of angiotensin II to gerbils with focal brain ischemia. It is known that the

From the Department of Surgery (Neurosurgery), Yale University School of Medicine, New Haven, Connecticut.

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Address correspondence to: Leonardo A. Fernandez, M.D., Section of Neurosurgery, Yale University, 333 Cedar Street, New Haven, Connecticut 06510.

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Selective functional vulnerability of cortical neurons following transient MCA-occlusion in the cat.

G Rosner, R Graf, K Kataoka and W D Heiss

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