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Published in:
Brain Research

Publication date:
1990

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Quistorff, B., C. Haselgrove, J., Bashford, C. L., H. Barlow, C., Chan'ce, B., & Mayevsky, A. (1990). Time resolved 3-dimensional recording of redox ratio during spreading depression in gerbil brain. *Brain Research*, 506(1), 109-114.

Time resolved 3-dimensional recording of redox ratio during spreading depression in Gerbil brain

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(Accepted 6 June 1989)

Key words: Spreading depression; Mitochondria; Redox; Imaging; Spectroscopy; Nicotinamide adenine dinucleotide; Flavoprotein; Fluorescence

Optical fluorescence and reflectance measurements have been used to map the distribution of metabolic states in three dimensions in the gerbil brain with a spatial resolution of 200 μm and a time resolution of 4–6 s. In Mongolian gerbils anesthetized with pentobarbital, the redox states of the nicotinamide adenine dinucleotide (NADH) and flavoprotein components of the electron transport chain exhibit two distinct phases during the wave of spreading depression: (1) a transient period of oxidation and (2) a prolonged period of reduction, during which the cytochromes are reduced, and the hemoglobin is predominantly in the deoxy form. These data are interpreted as indicating that the energy demand placed on the gerbil brain during such spreading depression wave is sufficient to drive the brain temporarily hypoxic.

INTRODUCTION

Spreading depression (SD) of Leao¹⁵ introduces profound changes in cortical DC potential¹⁸, membrane potential⁶, extracellular potassium^{9,24} and blood flow^{9,18,31,32}. These changes spread across the surface of the cerebral cortex at a speed of 2–4 mm/min from the site of initiation of SD. Mitochondrial metabolism during spreading depression has been followed by monitoring optical signals from different components of the respiratory chain^{20,24,29}, showing activation of the ATP utilization. Using the terminology of Chance and Williams⁵ this represents a state 4–3 transition. Indeed, increased glucose metabolism^{8,30} has been shown to occur during SD. Nonetheless, there is considerable evidence which suggests that SD is accompanied by anaerobic glycolysis: increased lactate, decreased pH, and increased glucose consumption^{7,8,12,30}. Anaerobic glycolysis represents state 5 of mitochondria.

Previous techniques used for following cerebral energy metabolism are restricted in either their spatial or temporal resolution. Optical measurements *in vivo* can be recorded in two dimensions but are restricted to the surface of the brain. Glucose uptake measurements have excellent spatial resolution (of the order of a few microns) but have usually required a 45 min uptake

time³⁰ causing the transient signals to be minimized. Lactate measurements can be recorded from selected volumes of the order of $0.4 \times 0.4 \times 1.0 \text{ mm}^3$. Recent developments in the instrumentation for optical measurements²⁸ now allow us to examine the 3-dimensional distribution of mitochondrial redox states throughout brain which have been freeze-trapped²⁷ during the propagation of a wave of spreading depression. The present study constitutes a spatial analysis of the various phases of the freeze-trapped SD wave in terms of mitochondrial redox state. Since the speed of progression of the SD is measured, the spatial analysis also serves as a temporal analysis of the SD wave.

METHODS

Animals

Male adult Mongolian gerbils (60–80 g) from Tumblebrook farms (MA, U.S.A.) were anesthetized by an intraperitoneal injection of pentobarbital (30 mg/kg) and mounted to a head-holder. The animals were not paralyzed or ventilated. The skull was exposed, and a 3 mm diameter region of the parietal bone of one hemisphere was removed exposing the dura. About 30 min elapsed between the time the animals were anesthetized and the initiation of a single cycle of an SD, which was elicited by one of two methods. (i) The brain was injured mechanically by inserting a 27 gauge needle ($< 0.4 \text{ mm}$ diameter) 2 mm into the brain. There was no bleeding beyond the bore made by the needle. (ii) A drop of 0.5 M KCl saline solution was applied epidurally to the exposed surface of the brain.

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Insertion of the needle initiates SD along a column through the cortex; if the insertion was deep, it would also initiate SD in deeper parts of the brain (see Results). The application of KCl initiated SD from a small area at the surface. There was no other observable difference in the results of these two methods. The metabolism of the brain was arrested by 'funnel freezing'²⁷ at a given time (10 s to 60 min) after the initiation of the SD. Funnel freezing avoids ischemic artefacts because the blood supply to the lower unfrozen regions of the brain continues until frozen at the same time as the metabolism. A funnel with an opening 15–20 mm wide was placed over the exposed skull, the junction sealed with vaseline, and liquid nitrogen poured into the funnel. Freezing was continued for about 30–60 s, during which time the animal continued breathing. When breathing stopped, the whole animal was immersed in liquid nitrogen. The frozen animal was decapitated, and the head mounted in the 3-D scanning instrument²⁸.

Fluorescence measurements

The mitochondrial redox state of the tissue was measured using a low-temperature scanning fluorometer. Light from a mercury arc source passes through a filter (alternately 366 and 450 nm) and is guided onto the surface of the brain by a light guide. The emitted light is picked up by a second arm of the light guide and guided through a filter (alternately 450 and 550 nm) to a photomultiplier. The experimental and theoretical principles of this technique have been described in detail in earlier papers^{4,28}. The metabolic state of the frozen brain was studied by recording fluorescence of signals from intrinsic fluorochromes. The two signals were: (i) NADH, which, when excited at a wavelength of 366 nm emits light with a peak intensity of about 450 nm. The intensity of the fluorescence from the reduced form of the nucleotide, NADH, is correlated to the redox state of the mitochondria. We call this the pyridine nucleotide fluorescence (PN). The oxidized form, NAD, has little absorption or fluorescence at those wavelengths. (ii) Flavoproteins, when excited with light of 460 nm fluoresce with a peak intensity near 540 nm. In contrast to the NAD-NADH fluorescence, the flavoproteins fluoresce more strongly in the oxidized form. We call this the flavoprotein fluorescence (FP).

For fluorescence measurements such as these in which the light is scattered and then re-emitted from the incident surface there is no uniquely defined light path. We do not therefore attempt to measure an absolute value for the fluorescence efficiency. Instead, we study the distribution of relative fluorescence across the tissue, which we record as the dimensionless digitized output of the photomultiplier.

As measured in the present experiments, the fluorescent signals originate mainly from the mitochondria^{11,26}. Regions of the tissue in which the mitochondrial electron-transport chain are oxidized will have increased flavoprotein fluorescence (FP) and decreased NADH fluorescence (PN)²⁰. However, the fluorescent signals will also be governed by the concentration of mitochondria (and hence

of the enzymes), and will be affected by local blood concentration and oxygenation state since the fluorescent signals are absorbed strongly by hemoglobin. Such hemoglobin and mitochondrial concentration-related changes will be similar for both the NADH and flavoprotein signals, so the ratio of these two signals at each point of the tissue provides a measure of the redox state which is relatively independent of local mitochondrial, and hemoglobin concentrations⁴. We used a modified form of this ratio $MR = FP/(FP + PN)$ chosen to keep the ratio within known bounds (0–1.0).

The distribution of fluorescence signals in 3 dimensions throughout the brain was recorded using an on line scanner to scan a series of planes at different depths through the brain. Successive surfaces were generated by grinding away the previous surface of the frozen brain. Typically, the scan of each plane consists of an array of 80×80 points, $200 \mu\text{m}$ apart. Each measurement represents a volume of tissue about $120' \times 120 \mu\text{m}$ in the plane of the surface and $50 \mu\text{m}$ deep. In all the images we show here we use our usual convention that the high signals are displayed white and the low ones dark.

Scanning reflectance spectroscopy

Reflectance spectra of the 500–650 nm region of specific regions on the freeze-trapped brains were made using a Johnson Foundation double beam spectrophotometer³ modified to record reflectance spectra, as described by Bashford et al.¹.

RESULTS

Spatial distribution of mitochondrial fluorescence

Fig. 1 shows the FP, PN and MR fluorescence distributions in a typical horizontal section through a brain frozen 35 s after the initiation of SD with KCl. The dominant feature is the circular region about 6 mm in diameter in the right hemisphere exhibiting decreased flavoprotein signal, increased NADH (PN), and decreased ratio. All these changes indicate that the mitochondria in this region are reduced.

The maximum extent of the reduction of FP and PN during the reduced period of SD was compared to that in the frozen cortex of control and completely ischemic gerbils. At the point of maximum reduction during SD, the FP signal decreased by $24 \pm 5\%$ (mean $\pm$ S.D.) while the PN signal increased $74 \pm 20\%$ the ratio MR decreased by $40 \pm 6\%$. Within experimental error, these

Fig. 1. FP, PN, and MR images of one section of a brain frozen 35 s after initiation of spreading depression. The plane scanned is parallel to the cortical surface and about $1400 \mu\text{m}$ below it. Each image covers an area $1.3 \times 1.3 \text{ cm}$.

Fig. 2. 2-D images of the ratio MR on sections of the cortex below the surface of gerbil brains frozen at different times after initiation of SD in the right hemisphere. Higher values of MR (corresponding to more oxidized tissue) are displayed as lighter tones, lower levels darker. Field of view of each image is 1.2×1.2 cm.

changes are the same as the changes in signals recorded from brains frozen in control ischemic animals²³. Thus it seems as if the wave of SD results in the electron carriers of the low-potential end of the mitochondrial electron-transport chain adopting fully reduced condition similar to that found in an anoxic or ischemic cortex.

The 'redox' image (FP/(FP+PN)) shows clearly that the region of reduced tissue is surrounded by an annulus 0.5 mm wide of tissue in which the mitochondria are more oxidized (white) than in the control hemisphere. While this response can be seen clearly as the bright annulus in the flavoprotein image, the NADH image does not show clearly a dark annulus expected for oxidized mitochondrial pyridine nucleotides. Chance has shown that the NADH and the flavoproteins are very tightly coupled in perfused liver and pigeon heart mitochondria⁴, and there is no evidence to the contrary for brain. Therefore the effect we see is probably due to hemodynamic effects. A decrease in blood volume in this region will increase both NADH and flavoprotein signals and will enhance the flavoprotein increase, while hiding an oxidative decrease in the NADH.

Time course of SD in the plane of the cortex

These images can be interpreted in terms of a radial wave for which a transient period of oxidation is followed by a prolonged period of reduction. Images of the MR ratio in the plane of the cortex of gerbils frozen at different times after initiation of SD (Fig. 2) show that the wave spreads radially from the point of trauma. The velocity is between 2–3 mm/min, in excellent agreement with other measurements^{2,7}. (Note that the waves of SD in the images of Fig. 2 were initiated by KCl over a 3 mm wide region at zero time.) Following the passage of the oxidative-reductive wavefront across the experimental hemisphere of the brain, the hemisphere remains reduced for several minutes, and finally reaches a redox state indistinguishable from the control hemisphere after 10–20 min. Since we know the velocity of the wave, we may use a spatial analysis of the wave front to determine the

time course. With a velocity 2–3 mm/min, the spatial resolution of $200 \mu\text{m}$ corresponds to a temporal resolution of 4–6 s. The time course of the changes of FP, PN, and MR during spreading depression is shown in Fig. 3. The wavefront of SD consists of a transient period of about 10 s during which the mitochondrial redox state is oxidized, followed by a period of 20–30 s of reduction.

SD in three dimensions

We investigated the spread of the wave of SD through the brain by scanning a series of planes. Fig. 4 shows MR images from such a set of a brain in which SD was initiated using a needle. The images show clearly that the wavefront spreads down through the cortex as well as parallel to the surface. The hippocampus is also reduced as a consequence of the wave of SD initiated there by the needle. The needle was inserted at a slight angle to the center plane of the animal, so that the point of initiation is closer to the mid plane at deeper levels. When SD is initiated by 0.5 M KCl applied epidurally the wave

Fig. 3. Plot of FP, PN fluorescence value, and of the ratio MR across a brain frozen 45 s after initiation of SD. The values of FP and PN are the results of digitizing the output of a photomultiplier, and are proportional to the fluorescence intensity. The SD was initiated at the right of this plot. The wave front of SD moves to the left.

Fig. 4. 2-D images of the redox ratio MR at different depths in a gerbil brain frozen 45 s after initiation of SD using a needle. Field of view of each image is 1.3×1.3 cm.

spreads throughout the cortex but does not pass to morphologically distinct regions such as the hippocampus.

Reflectance measurements

We complemented these fluorescence studies with reflectance spectroscopy to evaluate the state of reduction of mitochondrial cytochromes¹. Reflectance spectra

in the wavelength range of 500–650 nm were recorded from 4 different regions of a brain frozen 40 s after the initiation of SD (see Fig. 5A): the regions chosen were 1, the most reduced area of the SD wave, and control areas on the ipsi- and contralateral sides of the brain locations 2, 3 and 4. The spatial resolution of the spectrophotometer was 3 mm, which was not sufficient to record the initial oxidative phase of the wave. Difference spectra

Fig. 5. A: fluorescence images from a brain during SD. 4 areas are indicated; the region of tissue reduction during SD and 3 control areas. B: difference reflection spectra from different regions of a gerbil brain frozen during SD, (a) wave of SD — control on ipsilateral hemisphere; (b) two control areas, both on the contralateral hemisphere; (c) wave of SD — control region at equivalent position on contralateral hemisphere; (d) difference between control areas at similar positions on ipsi- and contralateral hemispheres. Dotted line, difference spectrum of deoxy-oxyhemoglobin.

between the various regions are shown in Fig. 5B and are compared with the difference spectrum of reduced oxy-hemoglobin obtained from frozen gerbil blood. Traces a and c both compare the region of SD with control areas and have the form of the difference spectrum for hemoglobin on which are superimposed peaks at 550 and 605 nm of reduced cytochromes *aa3* and *c*. Thus, relative to the control regions, the reduced part of the wave of SD is characterized by increased concentration of reduced cytochromes *aa3* and *c*, of reduced pyridine nucleotide and flavoprotein, and of deoxyhemoglobin. Traces b and d compare the differences between the various control regions of the brain: trace b shows that two regions of the same hemisphere are very similar, while trace d, which compares control areas on the two hemispheres, seems to be a mixture of the oxyhemoglobin and deoxyhemoglobin traces. This curve indicates that there is no difference in the redox state of the cytochromes between the unaffected areas of the two hemisphere of the brain. However, the data suggest that there are hemodynamic effects of decreased blood volume and decreased hemoglobin oxygenation between the control areas of the two hemispheres. This may reflect different hemodynamic conditions in the exposed, and the non-exposed hemispheres

DISCUSSION

The effect of the cortical spreading depression (SD) of Leao in terms of the redox state of the brain mitochondria has been studied in 3 dimensions using a computer linked light-guide scanner to look at the mitochondrial redox state. By using funnel freezing to quench the metabolism of the brains at different periods after the initiation of SD, it has been possible to study the propagation of the wave in 3 dimensions with a spatial resolution of 200 μm . The wave of SD is seen to travel through the cortex with a speed of 2–3 mm/min in agreement with the speed determined from other measurements². The wave of SD easily spreads through the part of the brain in which it is initiated, but does not readily cross distinct morphological boundaries; a wave of SD initiated into the cortex will not cross into the hippocampus, although it may be initiated there *ab initio* by a deeply inserted needle. We have not yet seen the SD spread from one hemisphere of a gerbil to the other as Leao reported in the rabbit¹⁷, or as Csiba et al. reported for the rat⁷.

Studies of extracellular potassium ion concentration^{9,24} show that the wave of SD is accompanied by a large increase in the extracellular potassium, which must then be pumped back into the cells. Glucose consumption is increased significantly^{8,30}. Such a work load on the cell

would be expected to raise the ADP level in the cell and switch the mitochondria from their resting state to the more active state 3 of Chance and Williams⁵ with a concomitant oxidation of the NADH and flavoproteins²⁴. Rosenthal and Somjen²⁹, and Mayevsky et al.^{21,24}, do observe what they term a state 4–3 transition at this time, while Haselgrove et al.¹⁰ report that the electrical changes precede the changes in redox levels.

The first effect of SD that we see on the mitochondria of brain is an increase in the oxidative state accompanied by a drop in blood volume. Such decreases in brain blood volume have also been reported by Mayevsky^{20,22}. It is not yet clear what hemodynamic effects give rise to this phenomenon. Van Harreveld and Stamm³² deduced that there is a vasoconstriction of pial vessels early in the course of SD, although Wahl et al.³³ could see no such effect by direct observation. Gjedde et al.⁸ measured a slight decrease in blood flow at this stage although Lauritzen did not¹⁴. It is possible that the spatial resolution of Lauritzen's technique (5–15 mm) was too large to detect a transient change occurring in a 1/2 mm-wide annulus such as we see. Nonetheless, regardless of the cause of the initial hemodynamic effects, the ratio data show clearly and consistently that the initial response to SD is that the brain tissue does become oxidized.

The transient oxidative phase of the wave of SD is followed by a prolonged period of reduction in which the mitochondria may be nearly completely reduced for a period of 30–40 s. During this period of maximal reduction, the reflectance spectra indicate that the cytochromes *aa3* and *c* are largely reduced, and the hemoglobin is deoxygenated. The simultaneous reduction of the cytochromes and the NADH/flavoprotein components of the mitochondria is typical of the mitochondria being in state 5: viz., the degree of oxidation is limited by the supply of oxygen. Presumably, the energy demands on the brain cells are so high that the mitochondria are required to function at a rate well in excess of the available oxygen supply, even though this second phase of SD is associated with a two-fold increase in blood flow^{8,9}. Indeed, previous workers^{7,8} have demonstrated that SD is accompanied by anaerobic glycolysis.

Previous studies by Mayevsky^{20,22} have shown that a lightly anesthetized or awake animal responds to SD with only an oxidative phase, but that more heavily anesthetized animals do show a biphasic (oxidized-reduced) response. Indeed the other studies which have found an anaerobic response using different techniques have used animals with similar anesthetic states to ours^{7,8}. It is possible that the deep pentobarbital anesthesia²⁵ may in part be responsible for the inability of the oxygen supply to keep up with the mitochondrial demand.

The third and final phase of the wave of SD is a slow recovery lasting from 5 to 20 min during which the redox state of the whole cortex slowly returns to that of the control hemisphere recovering from lactate acidosis. This recovery time is similar to that reported by Leao¹⁶ for the recovery of the spontaneous electrical activity in the rabbit.

The present studies indicate that in the anesthetized animal, the largest change in signal is that of a reduction of the redox state of the mitochondria putting them in state 4 or 5. Since the redox state of the NAD-NADH and of flavoproteins is similar in states 4 and 5, the

present data indicated that a resting, anesthetized brain may be in a more oxidized state much closer to state 3 than state 4 (say state 3.3). The same conclusion was reached by Mayevsky¹⁹ when working with awake animals. The fluorescence and reflectance data may then be all interpreted if the effect of SD is to increase energy output enormously. The mitochondria which start in state 3.3 move to state 3 by the increased levels of ADP, then go to state 5 when the supply of oxygen can not keep up with the demand, and finally return to their resting state.

Acknowledgements. Supported in part by NIH Grant NS22881

REFERENCES

- 1 Bashford, C.L., Barlow, C.G., Chance, C., Haselgrove, J.C. and Sorge, J.R., Optical measurements of oxygen delivery and consumption in gerbil cerebral cortex, *Am. J. Physiol.*, 242 (1982) C265-271.
- 2 Bures, J., Buresova, O. and Krivanek, J., *The mechanism and applications of Leao's spreading depression of electroencephalographic activity*, Academic Press, New York, 1974, pp 69-70.
- 3 Chance, B. and Graham, N., A rapid scanning dual wavelength spectrophotometer, *Rev. Sci. Instr.*, 42 (1975) 941-945.
- 4 Chance, B., Schoener, B., Oshino, R., Itshak, F. and Nakase, Y., Oxidation-reduction ratio studies in freeze-trapped samples, *J. Biol. Chem.*, 254 (1979) 4764-4771.
- 5 Chance, B. and Williams, G.R., Respiratory enzymes in oxidative phosphorylation, *J. Biol. Chem.*, 217 (1955) 409-427.
- 6 Collewijn, H. and Van Harreveld, A., Membrane potential of cerebral cortical cells during spreading depression and asphyxia, *Exp. Neurol.*, 156 (1966) 425-436.
- 7 Csiba, L., Paschen, W. and Mies, G., Regional changes in tissue pH and glucose content during cortical spreading depression in rat brain, *Brain Research*, 336 (1985) 167-170.
- 8 Gjedde, A., Hansen, A.J. and Quistorff, B., Blood-brain glucose transfer in spreading depression, *J. Neurochem.*, 37(4) (1981) 807-812.
- 9 Hansen, A.J., Quistorff, B. and Gjedde, A., Relationship between local changes in cortical blood flow and extracellular K during spreading depression, *Acta. Physiol. Scand.*, 109 (1980) 1-6.
- 10 Haselgrove, J., Barlow, C., Eleff, E., Chance, B. and Leboard, S., Correlation of electrical signals and mitochondrial redox state during spreading depression. In A.G.B. Kovach, M. Kessler and I.A. Silver (Eds.), *Adv. Physiol. Sci. Vol. 25, Oxygen Transport to Tissue*, Pergamon, 1980, p. 25.
- 11 Hassinen, I. and Chance, B., Oxidation-reduction properties of the mitochondrial flavoprotein chain, *Biochem. Biophys. Res. Commun.*, 31 (1968) 895-900.
- 12 Krivanek, J., Some metabolic changes accompanying Leao's spreading cortical depression in the rat, *J. Neurochemistry*, 6 (1961) 183-189.
- 13 Krivanek, J. and Fikova, E., The value of ultramicro-analysis of lactic acid in tracing the penetration of Leao's cortical spreading depression to subcortical areas, *J. Neurol. Sci.*, 2 (1965) 385-392.
- 14 Lauritzen, M., Regional cerebral blood flow during cortical spreading depression in rat brain: increased reactive hyperfusion in low-flow states, *Acta Neurol. Scand.*, 75 (1987) 1-8.
- 15 Leao, A.A.P., Spreading depression of activity in the cerebral cortex, *J. Neurophysiol.*, 7 (1944) 359-390.
- 16 Leao, A.A.P., Further observations on the spreading depression of activity in the cerebral cortex, *J. Neurophysiol.*, 10 (1947) 409-414.
- 17 Leao, A.A.P. and Morison, R.S., Propagation of spreading cortical depression, *J. Neurophysiol.*, 8 (1945) 33-45.
- 18 Marshall, W.H., Spreading cortical depression of Leao, *Physiol. Rev.*, 39 (1959) 239-279.
- 19 Mayevsky, A., Brain energy metabolism of the conscious rat exposed to various physiological and pathological situations, *Brain Research*, 113 (1976) 327-338.
- 20 Mayevsky, A. and Chance, B., Intracellular oxidation-reduction state measured in situ by a multi-channel fiber-optic surface fluorometer, *Science*, 217 (1982) 537-540.
- 21 Mayevsky, A. and Chance, B., Metabolic responses of the awake cerebral cortex to anoxia, hypoxia, spreading depression, and epileptiform activity, *Brain Research*, 98 (1975) 149-165.
- 22 Mayevsky, A., Leboard, S. and Chance, B., The interrelation between brain PO_2 and NADH oxidation-reduction state in the gerbil, *J. Neurosci. Res.*, 5 (1980) 173-182.
- 23 Mayevsky, A., Kaplan, H., Haveri, J., Haselgrove, J. and Chance, B., Three-dimensional metabolic mapping of the freeze-trapped brain: effects of ischemia in the mongolian gerbil, *Brain Research*, 367 (1986) 63-72.
- 24 Mayevsky, A., Zenthan, T. and Chance, B., Measurements of extracellular potassium, ECoG, and pyridine nucleotide levels during cortical spreading depression in rats, *Brain Research*, 76 (1974) 347-349.
- 25 Nilsson, B. and Siesjö, B., The effect of phenobarbitone anaesthesia on blood flow and oxygen consumption in the brain, *Acta. Anaesth. Scand. Suppl.*, 57 (1975) 18-24.
- 26 O'Connor, M.J., Welsh, F., Komarnicky, L., Davis, T., Stevens, J., Lewis, D. and Herman, C., Oxygen and physiological function. In F.F. Jobsis (Ed.), *Professional Information Library*, Dallas, Texas, 1976, pp. 90-99.
- 27 Ponten, U., Ratcheson, R.A., Salford, L.G. and Siesjö, B., Optimal freezing conditions for cerebral metabolites in rats, *J. Neurochem.*, 21 (1973) 1127-1138.
- 28 Quistorff, B., Haselgrove, J.C. and Chance, B., High spatial resolution read-out of 3-D-metabolic organ structure. An automated, low temperature redox ratio scanning instrument, *Anal. Biochem.*, 148 (1985) 389-400.
- 29 Rosenthal, M. and Somjen, G., Spreading depression, sustained potential shifts, and metabolic activity of cerebral cortex of cats, *J. Neurophysiol.*, 36 (1973) 739-749.
- 30 Shinohara, M., Dollinger, B., Brown, G., Rappaport, S. and Sokoloff, L., Cerebral glucose utilization: local changes during and after recovery from spreading cortical depression, *Science*, 203 (1979) 188-190.
- 31 Van Harreveld, A. and Ochs, S., Electrical and vascular concomitants of spreading depression, *Am. J. Physiol.*, 189 (1957) 159-166.
- 32 Van Harreveld, A. and Stamm, J.S., Vascular concomitants of spreading cortical depression, *J. Neurophysiol.*, 15 (1952) 487-496.
- 33 Wahl, M., Lauritzen, M. and Schilling, L., Change of cerebrovascular reactivity after cortical spreading depression in cats and rats, *Brain Research*, 411 (1987) 72-80.