

The 80th Anniversary of Cortical Spreading Depression of Leao: A Major Component in Experimental and Clinical Neuropathology

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Abstract

Understanding of the mechanisms behind brain neuropathology is a major factor in diagnosis as well as treatment of patients. The discovery of the reversible event of cortical spreading depression (CSD) in conjunction with epileptic activity led a new era in better understanding of brain pathology. Eighty years ago, A.A.P. Leao discovered this unique response, of the cerebral cortex of animals, to mechanical, chemical or electrical stimulation. A single CSD wave that propagate in the rate of 3-6 mm/minute includes electrical, ionic, circulatory and metabolic responses. The metabolic changes include activation of oxygen consumption as evaluated by monitoring of mitochondrial NADH redox state. It was found that CSD could be developed spontaneously after various perturbations such as moderate hypoxia or ischemia, epileptic activity, hyperbaric oxygenation, head injury and brain retraction. Our conclusion is that better clinical monitoring of the brain that will detect the appearance of CSD will improve patient's care in critical situations.

INTRODUCTION

In 1942 Rosenblueth and Cannon (Harvard Medical School) published in the American J. of Physiology ^[1], a significant paper entitled "Cortical Responses to Electric Stimulation" of the monkey motor cortex (experimental epilepsy). In 1941-2, a young Brazilian graduate student, Aristides Leao, joined the team at Harvard Medical School and in studying experimental epilepsy in rabbits he found an unexpected result that suggested a new brain pathophysiological event that was unknown before. He identified a decrease of the excitability spreading in increasing circles around the initial focus, a phenomenon he named "Spreading Depression" (CSD). In 1943 he submitted his Ph.D. thesis entitled "Spreading Depression of activity in the cerebral cortex" ^[2]. He published 3 papers based on his dissertation ^[3-5] and soon after this phenomenon was named "Cortical Spreading Depression of Leao". In addition, Leao also found that "The depression and tonic-clonic activity of experimental cortical epilepsy seem to be closely related" ^[3]. From the early studies of CSD by Leao and his collaborators in the 1940's it was suggested that CSD was also related to the availability of oxygen in the brain ^[2-4].

Thirty year after the discovery of CSD, in 1974, a book summarizing the large number of studies and findings was published by Bures et al ^[6]. Until then, only 2 electrical parameters, presented in Figure 1 ^[3,5] were monitored continuously in real time, namely, brain EEG (Fig. 1A) and also, the Slow Potential Changes presented in Figure 1B (SPC). The involvement of increased blood supply to the cerebral cortex during CSD waves was described in 1944 by Leao (Fig. 2), therefore it was clear that the CSD event includes a significant increase in oxygen consumption

[7]. The first direct indications that CSD leads to stimulation of mitochondrial function (increased oxygen consumption) was published in 1973 namely 40 years after its discovery. In two publications [8,9] it was shown that mitochondrial redox state became more oxidized as monitored by the autofluorescence of mitochondrial NADH in real time. In 1982, Mayevsky and Chance demonstrated the propagation of the mitochondrial responses to CSD waves in the rat brain using a 4-channel fluorometer [10]. Moderate decrease in oxygen supply to the brain, induced by hypoxia or partial ischemia, may lead to the development of cortical spreading depression wave [11]. After studying the phenomena of CSD, Epilepsy, Hypoxia and Ischemia for the last 50 years and publishing more than 100 papers, we found and concluded that CSD is a major component in brain pathophysiology as presented in Figure 3.

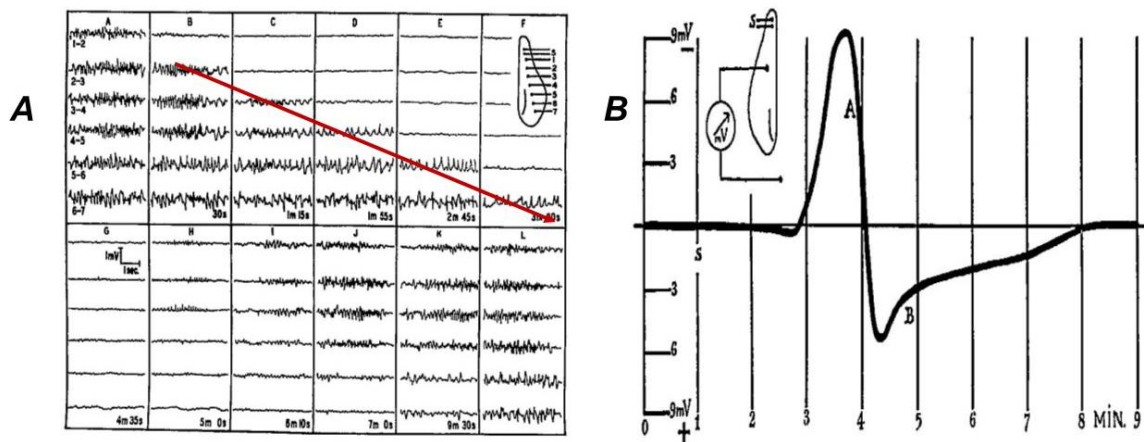


Figure 1A: Gradual spread of depression. Electrodes arranged as shown in the inset. A: Before stimulation L. 7 min after K. In this and the following figures, when a strip is timed, that time indicates the interval from the end of the stimulation period. Upward excursions in the records denote negativity of the more frontal electrode (lower number) with respect to the more occipital electrode (higher number). Unless otherwise mentioned, the stimuli were induction shocks at "tetanizing" frequency, applied for 3 to 5 sec. through electrodes S. [3]. B: A representative experiment on the slow voltage variation accompanying the spreading depression of activity. The curve was drawn from voltage readings taken with 5 second intervals. In this and the other two figures, an upward deflection denotes negativity of the cortex, with respect to the extra cortical reference electrode. Electrodes arranged as shown in the inset (s: stimulating electrodes). Stimulation, 5 seconds of "tetanizing" current from an induction coil, delivered at the time marked S. In this representative curve is indicated the time of occurrence (from A to B) of the specific electrical activity which often develops during the depression of the "spontaneous" activity. American Physiological Society, reprinted with permission [5].

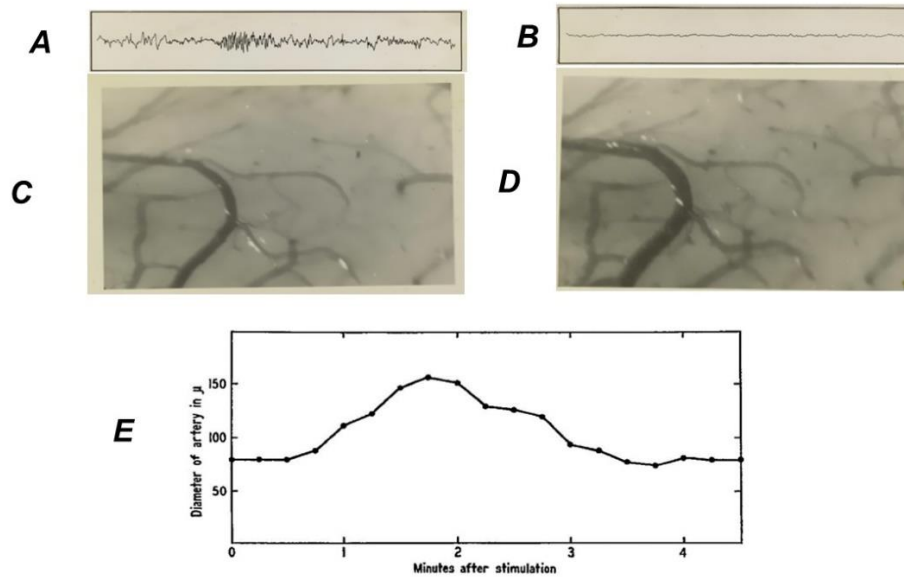


Figure 2: EEG activity recorded in the brain before (A) and during (B) the CSD wave. C+D - Photomicrograph of the pial vessels in the brain. C - Control before stimulation. The large vessel is an artery, of about 120 μ diameter. D - The wave of depression has reached the region. The diameter of the artery is approximately doubled during the wave of CSD [4]. E - Graph showing the time course and magnitude of arterial vasodilatation attending a depression. American Physiological Society, reprinted with permission [4].

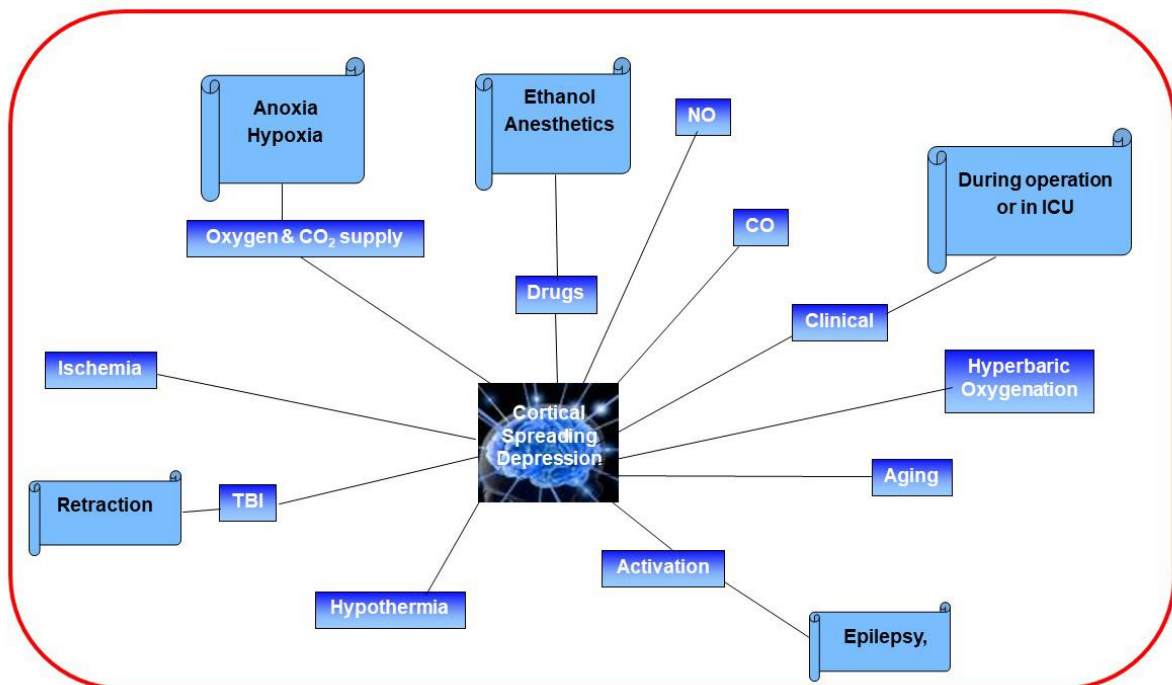
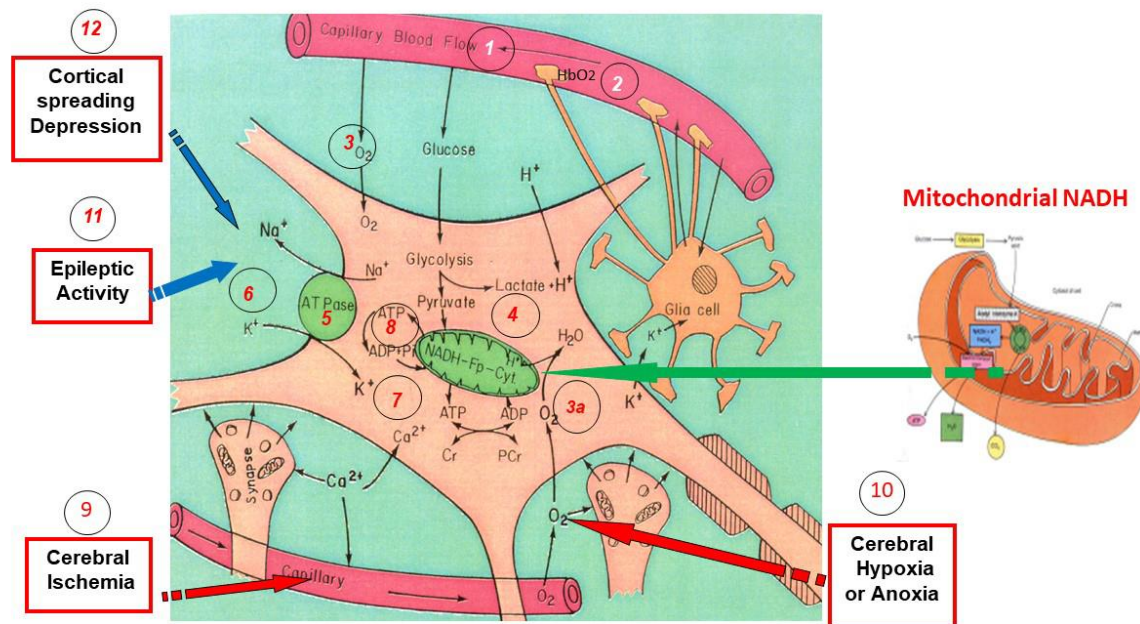


Figure 3: The effects of various experimental and clinical factors (tested in our studies) on the responses to Cortical Spreading Depression- CSD.

We selected 3 basic pathophysiological events presented in Figure 4 (labeled as 9,10,11 and 12) that are closely related to CSD. Our current understanding is that moderate Hypoxia or Ischemia

may lead to the development of CSD and also will affect the responses to CSD that may develop by various factors. The 4 neuropathological events shown in Figure 4 are divided into 2 groups according to their relationship to various physiological and biochemical parameters monitored *in vivo*. The first two factors (9 and 10), namely, systemic hypoxia and cerebral ischemia (red arrow) have two types of interactions with CSD, namely, they are decreasing the availability of oxygen therefore the response to CSD is affected. Also, the two events, hypoxia and ischemia may lead to the development of CSD. The other two perturbations shown are epileptic activity (11) and CSD - cortical spreading depression (12) that induced cortical depolarization (blue arrows). All four perturbations are affecting the same type of processes in the brain such as CBF, mitochondrial NADH redox state (green arrow), electrical activities and ionic homeostasis but in the different direction as seen in the scheme. Under hypoxia and ischemia, the production of ATP by the mitochondria is decreased and becomes a limiting factor in the system. Due to the inhibition of the ionic pumps, the ionic homeostasis is disrupted and hypoxic or ischemic cortical depolarization is developed. The end points of this process are changes in the electrical activities recorded as a depression of the ECoG and a negative shift in the DC steady potential. In addition, due to the depolarization event the extracellular potassium is elevated. If these situations (9 and 10) persist for more than a few minutes, irreversible damage to the cortex may develop.



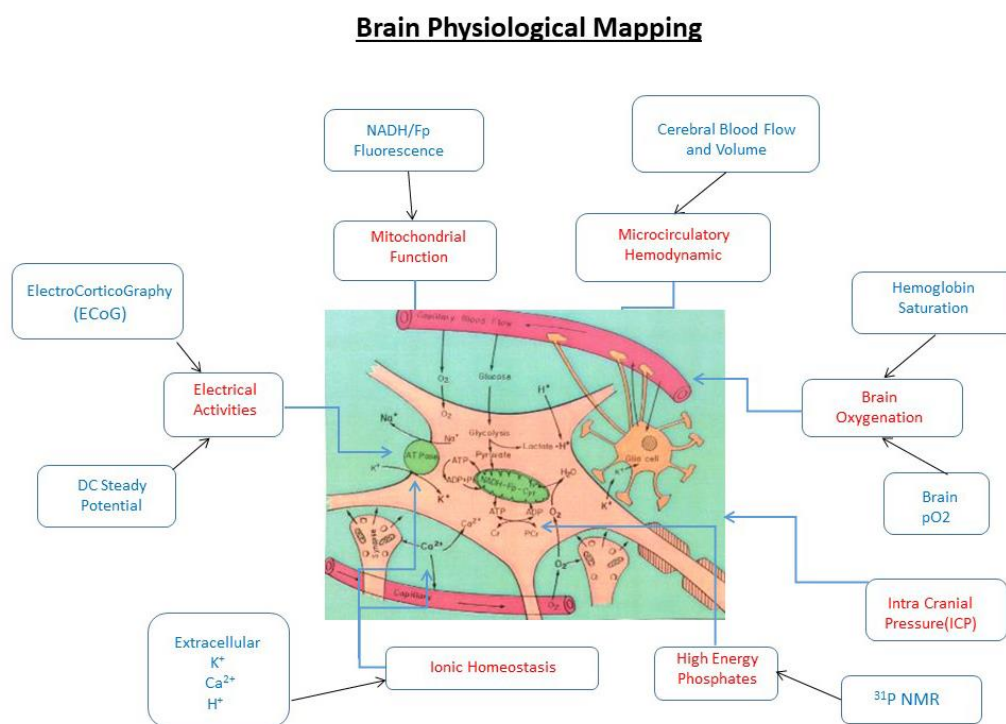


Figure 5: Schematic presentation of the concept “Brain physiological mapping”. All the presented techniques were developed in our laboratory [12].

METHODS

During the years 1973-2003 various NADH monitoring systems that were used in studying CSD were developed in our laboratory. During those years we developed and used 10 different monitoring systems that were used in studying the effects of CSD on the metabolic, hemodynamic, ionic and electrical activities in the brain *in vivo*, in real time. Figure 5 presents in a schematic way the various parameters (blue letters) that were monitored in our laboratory during the years.

Fiber Optic Based Fluorometer/Reflectometer

In order to enable the monitoring of brain NADH fluorescence in anaesthetized or unanaesthetized animals, a flexible means was needed to connect the fluorometer with the tested brain. This was achieved in 1972, when UV transmitting quartz fibers became available (Schott Jena Glass, Germany). We have used the light-guide-based fluorometer for *in vivo* monitoring of the brain [9,13] subjected to anoxia or CSD. The historical development of light-guide-based fluorometry-reflectometry was described previously [14-16]. Figure 6 shows the principles of the NADH (A) monitoring using the fluorescence of the reduced form (NADH) and not the oxidized form (NAD) as seen in part B. The fluorescence spectrum of the NADH is seen in part C. The response of the NADH spectrum to anoxia (nitrogen atmosphere) is presented in part D. Figure 7 presents the metabolic states of the mitochondria evaluated *in vitro* (part A) and the brain *in vivo* (part C) measured by the time sharing fluorometer/reflectometer [17] shown in part B of Figure 7.

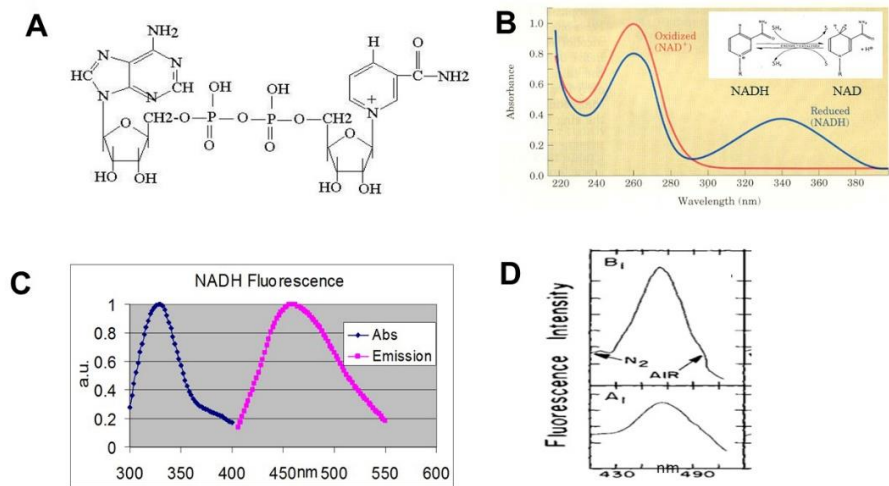


Figure 6: : A - Structures of NAD⁺. The nicotineamide group (broken ring) is the functional part of both molecules, i.e. the portion of molecules where oxidation and reduction take place (see arrow). B - Absorption spectra of NAD⁺ and NADH in solution. C - Absorption and emission spectra of NADH illuminated by UV light [15,18] . D - The emission spectra of rat cerebral cortex under aerobic conditions (lower trace) and under anoxic conditions (upper trace).

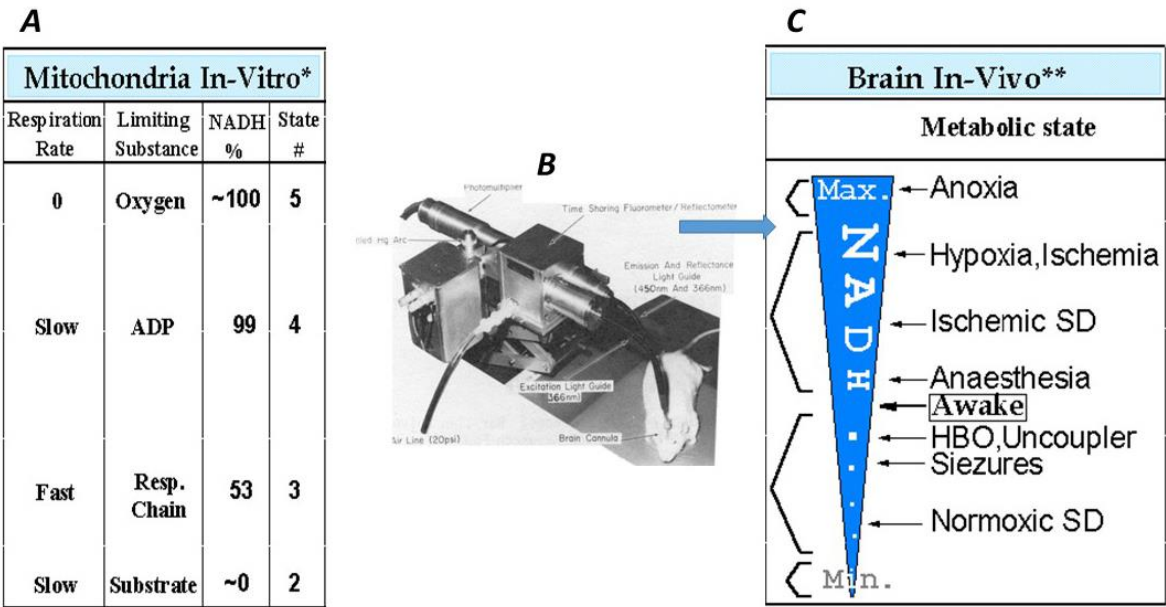


Figure 7: Comparison between mitochondrial metabolic state (*in vitro*), defined by Chance & Williams in 1955 (A) and responses of the *in vivo* brain to changes in O₂ supply and demand induced by brain activation described by Mayevsky (B and C) [19].

Monitoring of NADH, pO₂, Extracellular K⁺, DC and ECoG

The normal functioning brain requires a continuous supply of blood in order to obtain glucose as well as oxygen. Any change in the oxygenation of the tissue will result in an impairment of the normal function. Most of the oxygen taken up by the brain is consumed by the mitochondria in order to supply the large amount of ATP needed. A major part of the ATP is used by the

ATPase system to keep the neurons in the polarized state. Figure 4 shows in a schematic way the interrelation between the energy production system and the pumping activity going on in the membranes. In order to understand the connection between various events occurring in the brain *in vivo*, one has to measure as many parameters as possible from the same site.

Using surface probes, we monitored the metabolic, ionic and electrical activities from a small area of a rat or gerbil brain (Figure 8A and 8B). The metabolic state and activity was evaluated by monitoring partial pressure of oxygen (pO_2) as well as the intramitochondrial NADH redox state by surface fluorometry. Extracellular K^+ activity was measured by a surface valinomycin electrode. For electrical activities we measure DC potential and ECoG. Using the multiprobe approach we exposed the animal to anoxia, ischemia and CSD and recorded the various responses from the brain. All these electrodes were held in a multiprobe assembly described in detail elsewhere [20-22].

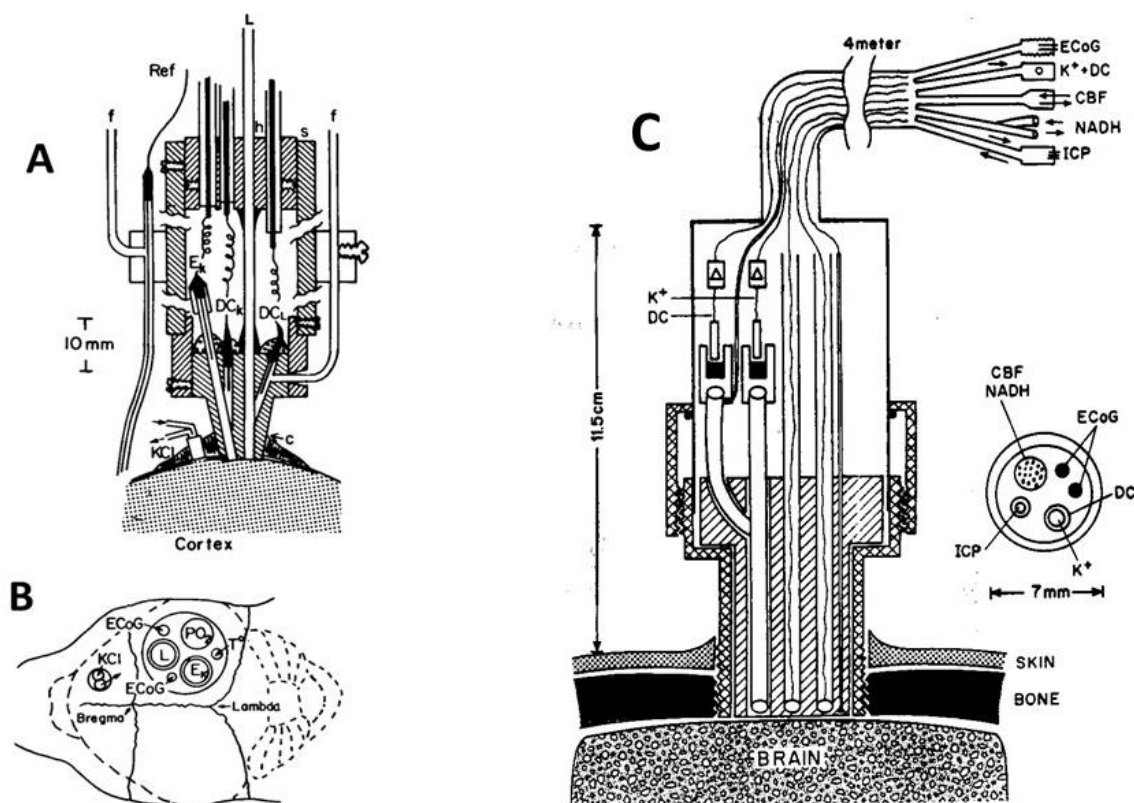


Figure 8A: Scheme of the multiprobe assembly (A) and its location on the gerbil brain (B). All details are in the text [23]. C - The multiprobe assembly (MPA) used for monitoring the brain of head-injured patients. The MPA is connected to the brain via a special holder screwed into the skull. ICP, intracranial pressure probe; CBF, NADH, fiber optic light guide probe to measure local cerebral blood flow, (CBF) and mitochondrial redox state (NADH); K^+ , DC, extracellular K^+ mini surface-electrode surrounded by a DC steady potential monitoring space; ECoG, bipolar electrocortical electrodes

Until 1994 it was not clear whether CSD could be developed and recorded in the human brain. Our group developed a unique monitoring system to be used in head injured patients treated in the neurosurgical ICU. Figure 8C shows the new MultiParametric Assembly- MPA, developed for the continuous monitoring of head injured patients in the ICU [24].

RESULTS

Figure 9 shows the responses of mitochondria in vivo and the EEG to repeated cycles of CSD. As seen a clear oxidation of the mitochondrial NADH was correlated to the depression of the electrical activity. In order to demonstrate the dependency of the recovery from CSD on the availability of oxygen the following experiment was performed. Figure 10 shows the effect of oxygen limitation on the recovery of the brain from the CSD event. In this study we used the multiparametric monitoring system (MPA) as seen in Figure 8A and 8B. This system enabled us to monitor simultaneously and continuously the brain oxygen level, mitochondrial NADH redox state, extracellular level of K^+ and brain EEG. The responses to a control wave of CSD (induced by KCl solution) is shown in the left side of Figure 10 (red elliptic line). As seen, after topical application of KCl a CSD wave was developed as recorded. Extracellular K^+ level increased, and NADH shows an oxidation cycle (decreased CF). After few minutes, a second CSD was developed and the same responses were recorded. In the middle of the 2nd response, the supply of oxygen was decreased by exposing the rat to 4% oxygen (Line A, hypoxia). This limitation in oxygen supply led to the inhibition of the recovery phase from the CSD wave. Only after the normal supply of oxygen (line B, 100% O_2) the recovery from the CSD was recorded. This experiment show very clearly that the recovery from CSD wave is depended on the normal supply of oxygen to the brain.

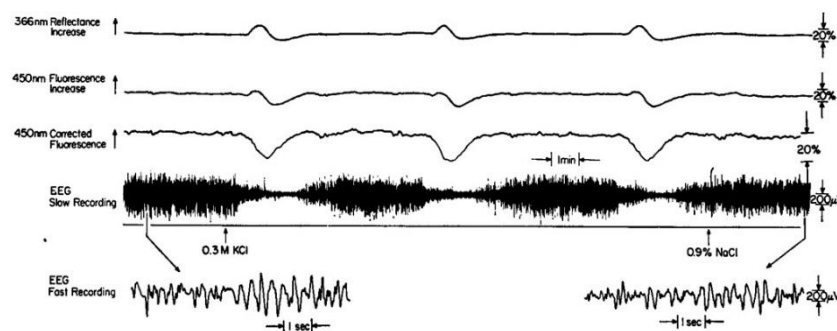


Figure 9: The response of the brain to the application of 0.3 M KCl solution, epidurally. Three cycles of oxidation of NADH were developed. The arrow direction shows an increase in the optical signals. Note that the third trace is on an expanded scale. Time proceeds from left to right [25].

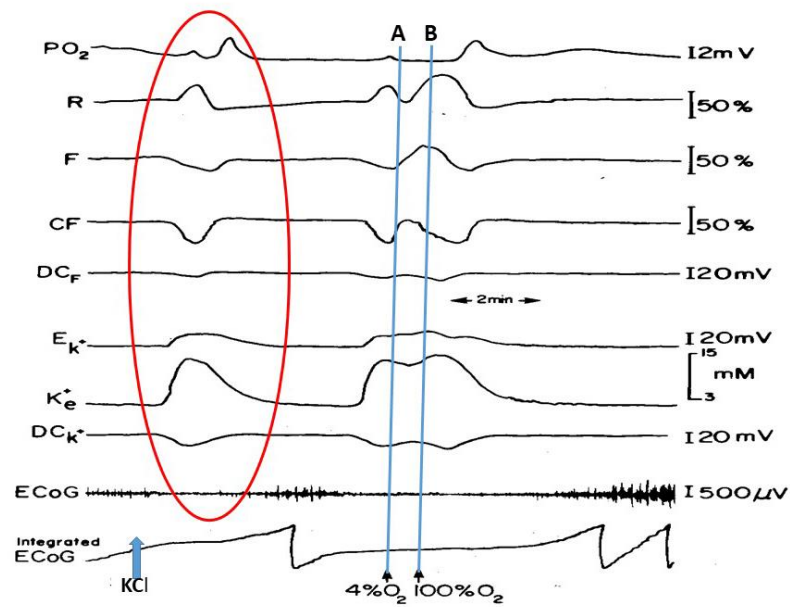


Figure 10: The effects of hypoxia (4% O_2) on the metabolic, ionic and electrical responses to spreading depression developed spontaneously [21].

We selected 3 basic pathophysiological events that are closely related to CSD. Our current understanding is that moderate Hypoxia or Ischemia may lead to the development of CSD and also will affect the responses to CSD that may develop by various factors. The 4 neuropathological events shown in Figure 4 are divided into 2 groups according to their relationship to various physiological and biochemical parameters monitored *in vivo*. The first two factors (9 and 10), namely, systemic hypoxia and cerebral ischemia have two types of interactions with CSD, namely, they are decreasing the availability of oxygen therefore the response to CSD is affected. Also, the two events, hypoxia and ischemia may lead to the development of CSD. The other two perturbations shown are epileptic activity (11) and CSD - cortical spreading depression (12) that induced cortical depolarization (blue arrows). All four perturbations are affecting the same type of processes in the brain such as CBF, mitochondrial NADH redox state (green arrow), electrical activities and ionic homeostasis but in the different direction as seen in the scheme. Under hypoxia and ischemia, the production of ATP by the mitochondria is decreased and becomes a limiting factor in the system. Due to the inhibition of the ionic pumps, the ionic homeostasis is disrupted and hypoxic or ischemic cortical depolarization is developed. The end points of this process are changes in the electrical activities recorded as a depression of the ECoG and a negative shift in the DC steady potential. In addition, due to the depolarization event the extracellular potassium is elevated. If these situations (9 and 10) persist for more than a few minutes, irreversible damage to the cortex may develop.

When the brain is exposed to epileptic activity or CSD (Fig. 4), the activation of the brain occurs and energy consumption is increased (blue arrows in Fig. 4). The coupling between the two pathological events is clear, and manifested by the electrical activity (ECoG) as well as extracellular K^+ levels and NADH redox state. During the epileptic stage, extracellular potassium increased and started to recover to the baseline, but then a larger elevation was recorded. There is a clear correlation between the various parameters during epilepsy and CSD. The decrease in NADH was smaller during the first stage, followed by an oxidation cycle typical for CSD.

Figure 5 illustrates the initiation of CSD wave during the period of anoxia (Fig. 11A) and ischemia (Fig. 11B) and the responses to the CSD wave started about one minute after the recovery from the anoxia or the ischemia Figure 11A shows a response to a 1-min anoxia induced by nitrogen respiration. Within 20 seconds, extracellular potassium (K^+_e) concentration started to change as the log of K^+ activity increased gradually. K^+_e returned to baseline level very shortly after room air was reintroduced. At the same time, NADH levels (third trace from top) responded as previously described and reached an elevated level under anoxic conditions. The typical decrease of the reflected light is recorded in the upper trace. The ECoG activity became severely depressed during complete anoxia. Very shortly after full recovery of K^+_e and NADH to their initial levels, a steep increase of K^+_e , which peaked at 91 meg/l, was measured, while the surface potential showed a wave whose negative shift reached 8.5 mV. The late response of the K^+_e is identical to that recorded during CSD wave. As was shown in Figures 11A, anoxia induced changes in K^+_e levels as well as in the oxidation state of the brain [26]. The initial effect of anoxia is a small increase of K^+_e due to the lack of available energy (as shown in the increase of NADH levels) with depolarization of a large population of neurons. This elevated level of K^+_e leads to varied responses that depend upon the subject; differing responses were even obtained in two hemispheres of the same subject.

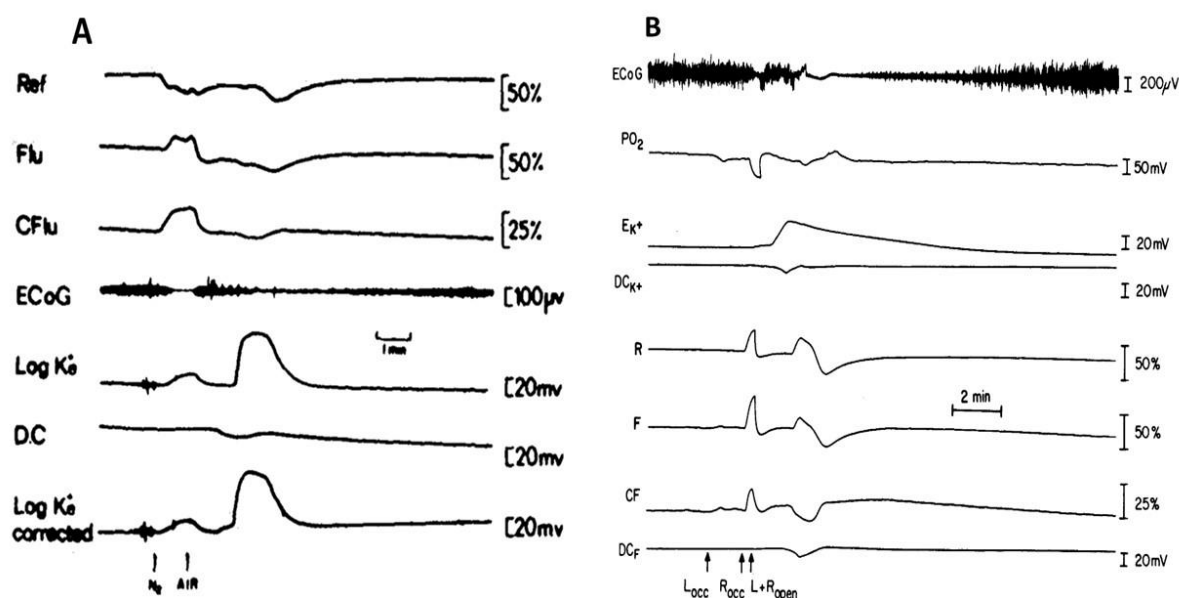


Figure 11: A - Effects of anoxia on mitochondrial NADH, ECoG, Extracellular potassium and DC steady potential in the gerbil brain [26]. B - Responses to unilateral and bilateral carotid occlusion in the gerbil brain [23].

When we exposed the gerbil brain to ischemia by occlusion of the carotid arteries (Fig. 11B) we monitored the parameters from the right hemisphere. When the contralateral carotid artery was occluded (L_{occ}), very small transient changes were recorded in the pO_2 and NADH [23]. Only after inducing complete ischemia (R_{occ}), pO_2 dropped to 0 and NADH increased to its maximal level. As a result, a spontaneous CSD cycle was initiated and the type of responses recorded were similar to those described in Figure 11A (after anoxia). These results confirm again our previous data about the oxidation of NADH when an extra pumping of K^+ is stimulated by CSD [26].

The coupling between epileptic activity and CSD is presented in Figure 12A. When Metrazol was applied topically on the right hemisphere of the brain, a clear epileptic EEG activity was recorded (line 1) in the right hemisphere. At the end of the epileptic EEG activity, a clear maximal depression of the EEG activity was recorded as seen in line 2. At this stage, the response of the mitochondrial NADH was very similar to the typical response to CSD as seen in figure 9. In part B of figure 12 another type of response of the brain to topical application of Metrazol is presented. In this case the epileptic activity was not followed by a CSD wave as was presented in part A of Figure 12.

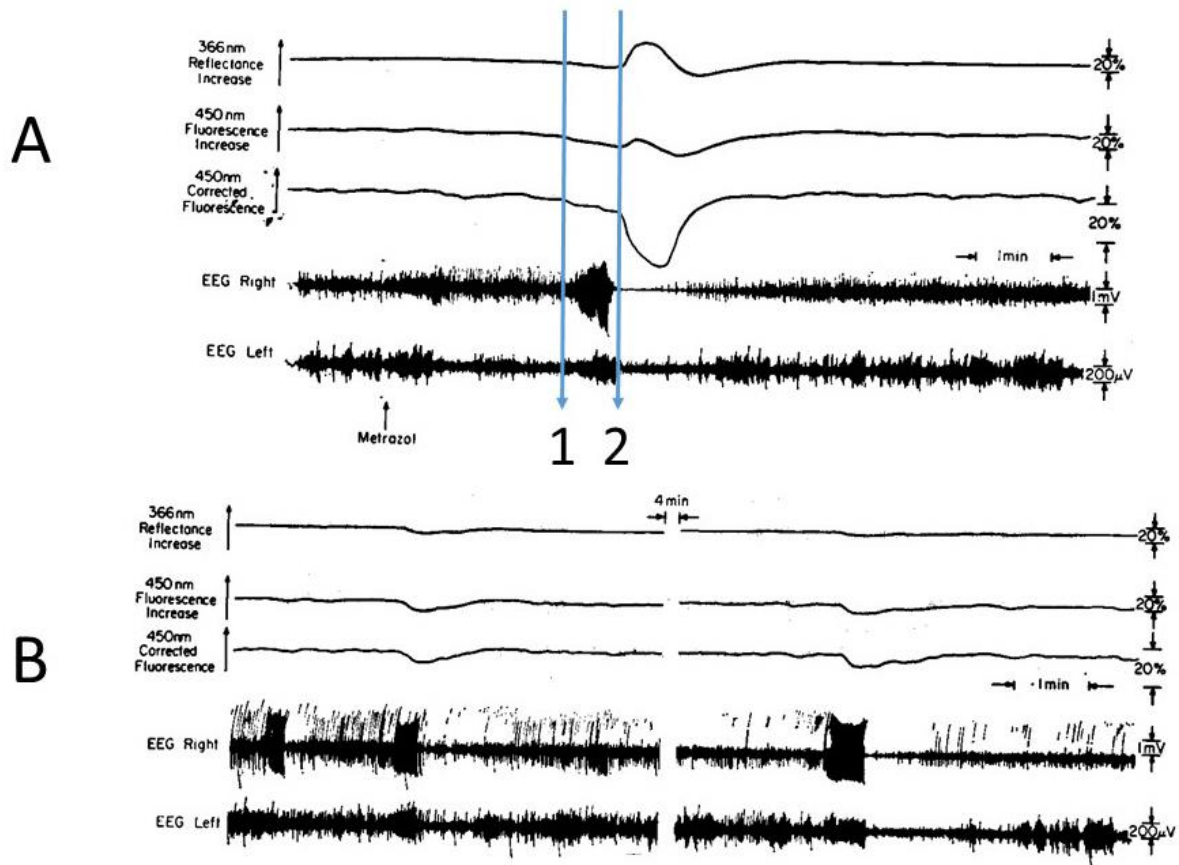


Figure 12: The effects of Metrazol on brain NADH fluorescence, 366 nm reflectance and the EEG of the two hemispheres. Note that the amplitude of the EEG calibration in the right hemisphere is 1 mV. The typical response is shown in part A. Part B shows another type of response to Metrazol applied 10 min before the record was taken [17].

The progression of seizure activity in a special strain of gerbils, developing epileptic effects as a result of monotonic noise is presented in Figure 13 [27,28]. As seen, the exposure of the awake gerbil to noise resulted in the development of epileptic activity followed by a CSD wave. The coupling between the two pathological events is clear, and manifested by the electrical activity (ECoG) as well as extracellular K^+ levels and NADH redox state. During the epileptic stage, extracellular potassium increased and started to recover to the baseline, but then a larger elevation in extracellular potassium was recorded. There is a clear correlation between the various parameters during epilepsy and CSD. The decrease in NADH was smaller during the first stage, followed by an oxidation cycle typical for CSD. The same coupling between epileptic activity and CSD is presented in Figure 14 [22]. The rat was exposed to hyperbaric oxygen environment (5ATA) in a special design chamber. Few minutes after reaching the 5ATA level an epileptic EEG activity was noticed as seen in line A. Also, a clear CSD like wave was noticed (line B) in the various monitored parameters (EEG, extracellular potassium level and mitochondrial NADH).

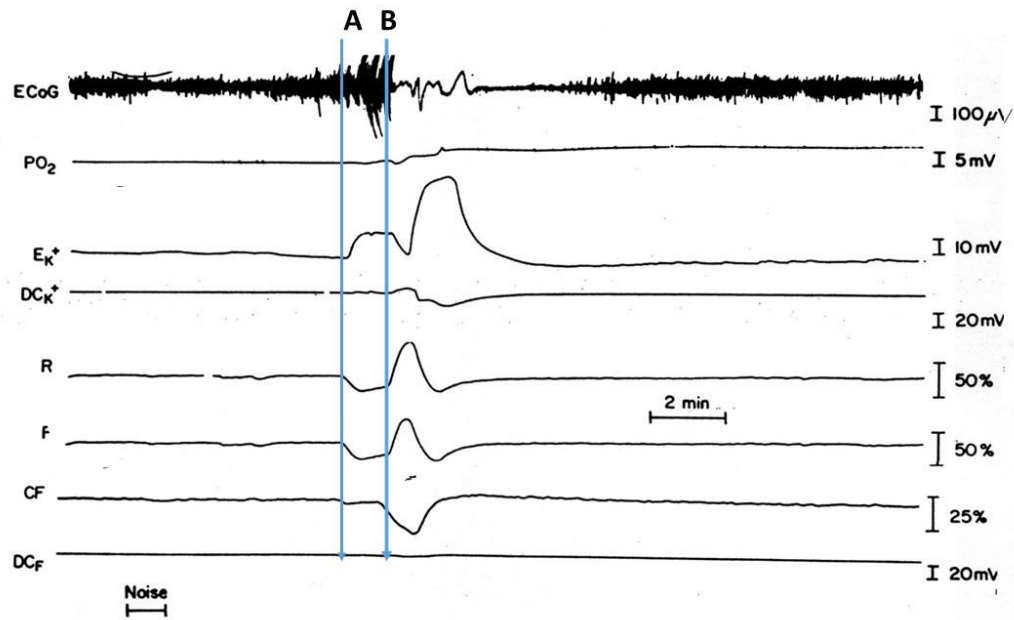


Figure 13: The development of epileptic activity followed by cortical spreading depression (CSD) in the brain of a seizure-prone gerbil. ECoG, electrocorticogram; PO₂, partial pressure of O₂ in the tissue; EK⁺, DCK⁺, extracellular potassium and DC steady potential measured around the K⁺ electrode; R, F, and CF, reflectance, NADH fluorescence, and corrected NADH fluorescence [28].

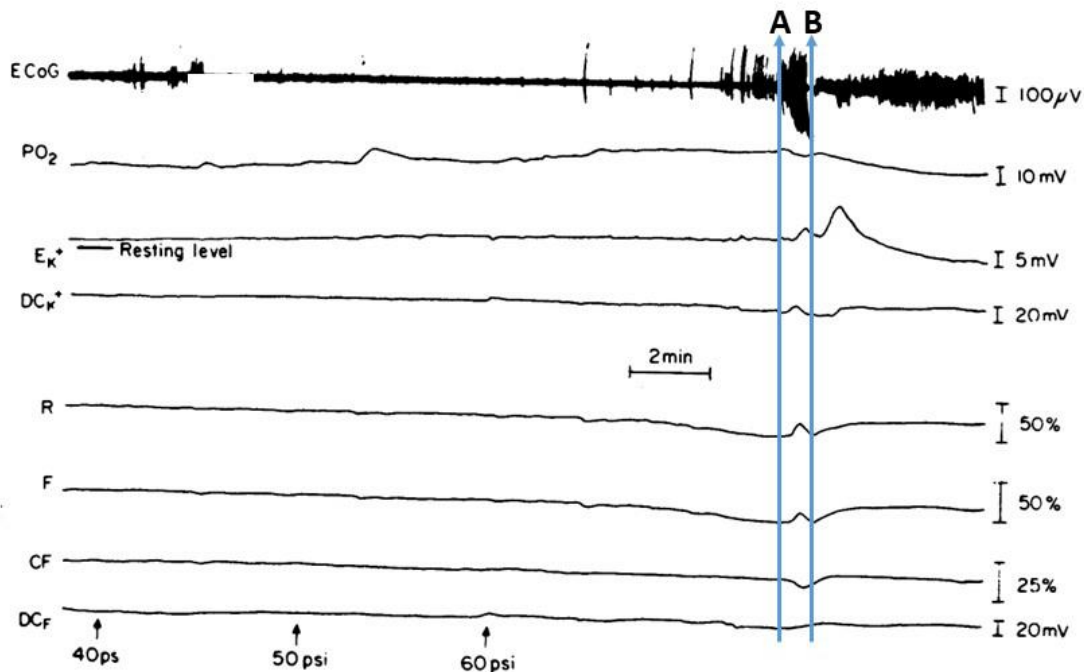


Figure 14: Effect of hyperbaric oxygenation on the various parameters measured in the awake rat inside the hyperbaric chamber. Pressure reached 60 psi (5 A T A) and first tonic-clonic episode is shown. Abbreviations as in Figure 13 [22].

Multiparametric Monitoring of Neurosurgical Patients

The new era in monitoring of mitochondrial function in patients started in 1990, when our team developed a unique multiparametric monitoring system that included the measurement of NADH fluorescence, using a light-guide-based device [29]. This system was initially applied to monitor neurosurgical patients undergoing brain surgery. In the first half of the 1990s [24,30,31], we were able to monitor the brain of comatose patients in the ICU and described, for the first time, the development of CSD-like responses, including marked NADH responses that were correlated to the microcirculatory blood flow. A modified multiparametric monitoring system was applied for NADH measurement during neurosurgical procedures in the operating room [32-35]. Based on the well-developed MultiProbe Assembly (MPA) used for animal experiments [36] a new MPA was developed and applied to patients monitored in the neurosurgical operation rooms and ICU. This device allowed us to monitor, in real-time, the hemodynamic, metabolic ionic and electrical activities in the brain of comatose patients. All details regarding the technology and the clinical setup appear in our published papers [24] and [16]. Figure 8C shows the longitudinal section of the MPA (measuring 8 parameters) used in the neurosurgical operating room and ICU.

The concept behind the development was that with more parameters monitored, a better diagnosis of the situation will be as presented. The envisioned system was to monitor 8 parameters from the brain (right side) in addition to the various systemic parameters monitored in every patient hospitalized (left side). The long term vision was that all parameters monitored by the 2 monitoring systems will be integrated in the same data bank and an expert system will be developed for a better diagnosis for the patients. The patient who developed CSD cycles showed signs of brain deterioration leading to brain death.

In one of the monitored patients we recorded CSD cycles that were very similar to those measured in the rat model as seen in Figure 15. This patient exhibited repetitive CSD cycles every 20-30 min, although at various times during the monitoring period, CSD cycles were recorded at shorter or longer intervals. The first five cycles recorded in this patient were very similar, i.e., NADH was oxidized while blood flow increased significantly (as in Fig. 15). However, in all the other cycles recorded later in the same patient (starting 4.5h after the CSD cycle shown in Fig. 15), the metabolic and hemodynamic responses were different (Fig. 16D) from the initial cycles [33]. The leakage of potassium and negative shift of the DC potential were very similar in all CSD cycles, whereas the NADH oxidation cycle was replaced by a biphasic cycle comprised mainly of a phase of increased NADH followed by a small oxidation phase. The compensation of blood flow and volume was also reversed at this time. The monophasic increase in CBF and CBV (Fig. 15 and 16D.) was replaced by an initial decrease followed by a smaller increase in flow and volume (Fig. 16D).

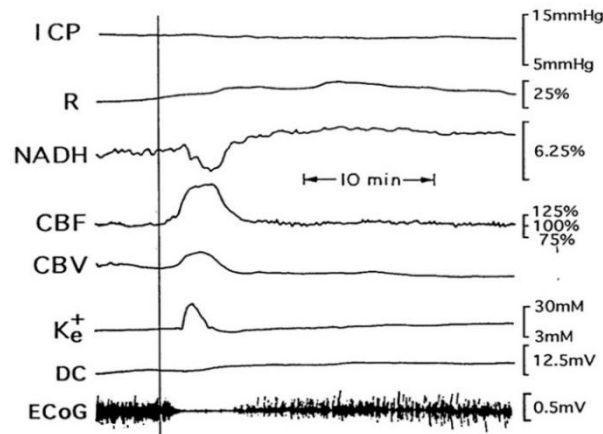


Figure 15: The responses of the brain to a wave of cortical spreading depression developed spontaneously in a severely head injured patient. ICP, intracranial pressure; R, NADH, 366 nm reflectance and 450 nm corrected fluorescence; CBF, CBV, cerebral blood flow and volume measured by laser Doppler flowmetry; K_e^+ , DC, extracellular potassium levels and the DC steady potential measured around the K^+ electrode; ECoG, electrocorticogram [24].

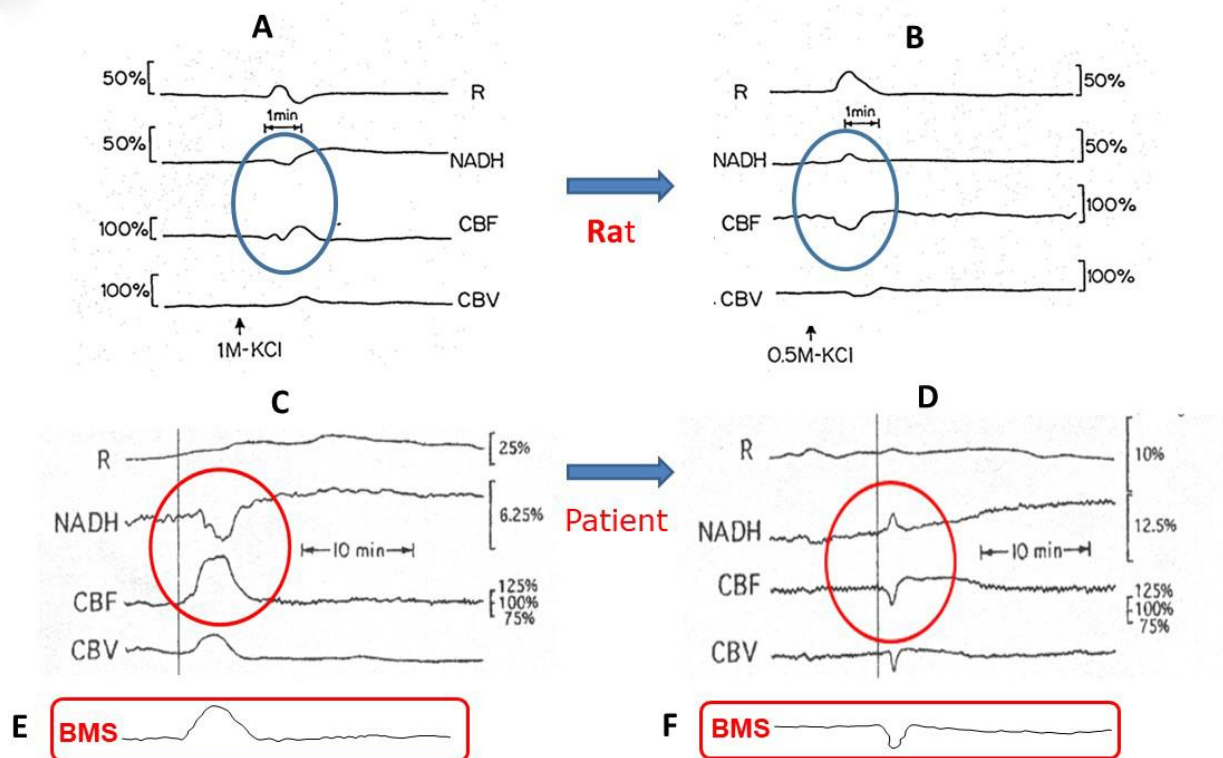


Figure 16: The responses to cortical spreading depression (CSD) measured in normoxic (A) and ischemic brain (B) in the rat model. Parts C and D were measured in head injured patients, E, F – Brain Metabolic Score (BMS) calculated for the normoxic (E) and partial ischemic (F) human brain responses to CSD. TBF – Cerebral Blood Flow, R- Reflectance, NADH – mitochondrial NADH redox state.

DISCUSSION

It was found that the responses to cortical spreading depression (CSD) in normoxic and ischemic brain are different [37]. Rosenthal & Martel [38] showed that ischemia affected the response to CSD, namely, instead of NADH oxidation, an increase in NADH was recorded. Almost 20 years ago we reported, for the first time, on the development of CSD in the brain of a comatose patient [24]. In this patient, the typical "oxidation cycle" of NADH was replaced by a "reduction cycle" due to a decrease in O₂ compensation capabilities when the patient shows signs of brain deterioration due to high intracranial pressure (ICP) levels. Figure 16 presents the responses to SD in a rat model (A and B) and in patients at the lower part (C and D). When we induced SD in the animal study, we were sure about the oxygen supply to the animal. The blood supply to the brain decreased between part A and B. The shift in response to CSD from the normoxic (A) to the ischemic (B) state is clear as discussed before [37]. When we move to patients' monitoring, we want to use the NADH trace as an indicator to the pathophysiological state of the patient or the brain in this example. As seen in the lower part, the two responses developed in the neurosurgical patients (C and D) are not the same. There are similarities between A and C as well as between B and D. Therefore, we concluded that the brain of the patient in state C was probably normoxic while in D the brain was partially ischemic. Indeed, this patient was deteriorating in time and finally brain death was determined [24]. This example demonstrates that the basic pathophysiological events developed in patients present very similar results as compared to those monitored in animal models.

In order to enable the users of a multiparametric monitoring system we decided to develop a new index or score that will represent the functional state of the brain in animal studies as well as in patients monitoring. The calculated Brain Metabolic Score - BMS traces, measured from the patient shown in part 16C and 16D are presented in part 16E and 16F and the difference in the responses to CSD is very obvious. The initial response measured in the patient (part 16C) is very similar to the responses measured in rat shown in Figure 16A. The same similarity is seen in the BMS responses shown in part F and part B and D in Figure 16.

Figure 17 presents the concept of oxygen balance or homeostasis in the brain which represents the balance between oxygen demand and oxygen supply. From our studies we can conclude that real time monitoring the responses of mitochondrial NADH redox state in the brain is a good indicator of its oxygen balance.

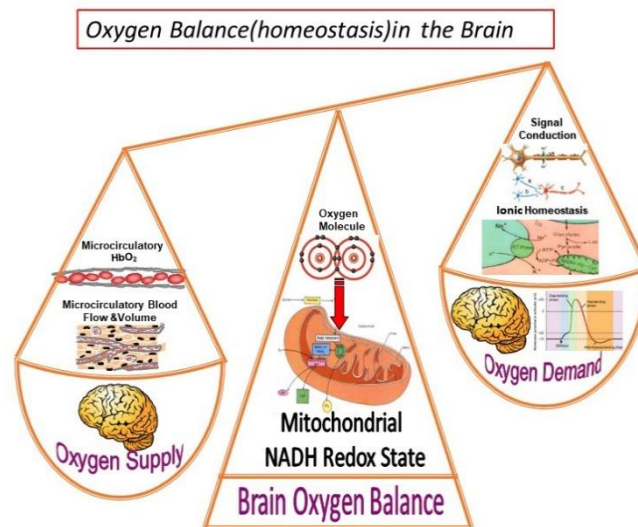


Figure 17: Schematic presentation of brain oxygen balance evaluated by oxygen supply and demand. Oxygen supply could be evaluated by monitoring brain blood flow (CBF), brain blood volume (CBV), hemoglobin oxygenation (HbO₂) and brain tissue oxygen partial pressure. Oxygen demand includes Ionic Homeostasis, Signal Conduction and Biochemical synthesis. Mitochondrial NADH redox state served as an indicator for brain oxygen balance or brain oxygen homeostasis [39].

References

- 1 Rosenblueth, A. and Cannon, W. B. (1942) Cortical responses to electric stimulation. *Am. J. Physiol.* **135**, 690-744
- 2 Leão, A. A. P. (1943) Spreading depression of activity in the cerebral cortex. Thesis, Harvard University, USA
- 3 Leão, A. A. P. (1944) Spreading depression of activity in cerebral cortex. *J. Neurophysiol.* **7**, 359-390
- 4 Leão, A. A. P. (1944) Pial circulation and spreading depression of activity in the cerebral cortex. *J. Neurophysiol.* **7**, 391-396
- 5 Leão, A. A. P. (1947) Further observations on the spreading depression of activity in the cerebral cortex. *J. Neurophysiol.* **10**, 409-414
- 6 Bures, J., Buresova, O. and Krivanek, J. (1974) The Mechanism and Applications of Leão's Spreading Depression of Electroencephalographic Activity. Academic, New York
- 7 Mayevsky, A. and Weiss, H. R. (1991) Cerebral blood flow and oxygen consumption in cortical spreading depression. *J. CBF Metab.* **11**, 829-836
- 8 Rosenthal, M. and Somjen, G. (1973) Spreading depression, sustained potential shifts, and metabolic activity of cerebral cortex of cats. *J. Neurophysiol.* **36**, 739-749
- 9 Mayevsky, A. and Chance, B. (1973) A new long-term method for the measurement of NADH fluorescence in intact rat brain with implanted cannula. (Bicher, H. I. and Bruley, D. F., eds.). pp. 239-244, Plenum Press, New York
- 10 Mayevsky, A. and Chance, B. (1982) Intracellular oxidation-reduction state measured *in situ* by a multichannel fiber-optic surface fluorometer. *Science.* **217**, 537-540

- 11 Mayevsky, A. and Sonn, J. (2022) Cortical Spreading Depression of Leao, "From Mitochondrial Function to Brain Metabolic Score (BMS)". Springer International Publishing Switzerland
- 12 Mayevsky, A., Sonn, J. and Barbiro-Michaely, E. (2013) Physiological mapping of brain functions *in vivo*: Surface monitoring of hemodynamic metabolic ionic and electrical activities in real-time. *J. Neurosci. Neuroeng.* **2**, 150-177
- 13 Chance, B., Oshino, N., Sugano, T. and Mayevsky, A. (1973) Basic principles of tissue oxygen determination from mitochondrial signals. *Oxygen Transport to Tissue, Adv. Exp. Med. Biol.* **37A**, 277-292
- 14 Mayevsky, A. and Chance, B. (2007) Oxidation-reduction states of NADH *in vivo*: from animals to clinical use. *Mitochondrion.* **7**, 330-339
- 15 Mayevsky, A. and Barbiro-Michaely, E. (2013) Shedding light on mitochondrial function by real time monitoring of NADH fluorescence: I. Basic methodology and animal studies. *J. Clin. Monit. Comp.* **27**, 1-34
- 16 Mayevsky, A. and Barbiro-Michaely, E. (2013) Shedding light on mitochondrial function by real time monitoring of NADH fluorescence: II: Human studies. *J. Clin. Monit. Comp.* **27**, 125-145
- 17 Mayevsky, A. and Chance, B. (1975) Metabolic responses of the awake cerebral cortex to anoxia hypoxia spreading depression and epileptiform activity. *Brain Res.* **98**, 149-165
- 18 Mayevsky, A. and Meirovithz, E. (2020) Oxygen Homeostasis and Mitochondrial Function in COVID-19 and ARDS Patients: The Ultimate Vital Signs. *J. Biosci. Biomed. Eng.* **1**, 1-24
- 19 Mayevsky, A., Kutai-Asis, H. and Tolmasov, M. (2020) Mitochondrial function and brain Metabolic Score (BMS) in ischemic Stroke: Evaluation of "neuroprotectants" safety and efficacy. *Mitochondrion.* **50**, 170-194
- 20 Friedli, C. M., Sclarsky, D. S. and Mayevsky, A. (1982) Multiprobe monitoring of ionic, metabolic, and electrical activities in the awake brain. *Am. J. Physiol.* **243**, R462-469
- 21 Mayevsky, A., Zarchin, N., Yoles, E. and Tannenbaum, B. (1986) Oxygen supply to the brain in hypoxic and hyperoxic conditions. In *Advances in the Biosciences. "O₂-Transport in Red Blood Cells"* (Nicolau, C., ed.). pp. 119-132, Pergamon Press
- 22 Mayevsky, A. (1983) Multiparameter monitoring of the awake brain under hyperbaric oxygenation. *J. Appl. Physiol.* **54**, 740-748
- 23 Mayevsky, A. and Sclarsky, D. S. (1983) Correlation of brain NADH redox state, K⁺, PO₂ and electrical activity during hypoxia, ischemia and spreading depression. *Adv. Exp. Med. Biol.* **159**, 129-141
- 24 Mayevsky, A., Doron, A., Manor, T., Meilin, S., Zarchin, N. and Ouaknine, G. E. (1996) Cortical spreading depression recorded from the human brain using a multiparametric monitoring system. *Brain Res.* **740**, 268-274
- 25 Mayevsky, A. and Chance, B. (1974) Repetitive patterns of metabolic changes during cortical spreading depression of the awake rat. *Brain Res.* **65**, 529-533
- 26 Mayevsky, A., Crowe, W. and Mela, L. (1980) The interrelation between brain oxidative metabolism and extracellular potassium in the unanesthetized gerbil. *Neurol. Res.* **1**, 213-226
- 27 Mayevsky, A., Kaplan, H., Haveri, J., Haselgrove, J. and Chance, B. (1986) Three-dimensional metabolic mapping of the freeze-trapped brain: Effects of ischemia on the Mongolian gerbil. *Brain Res.* **367**, 63-72

- 28 Mayevsky, A. and Rogatsky, G. (2007) Mitochondrial function *in vivo* evaluated by NADH fluorescence: From animal models to human studies. *Am. J. Physiol. Cell Physiol.* **292**, C615-C640
- 29 Mayevsky, A., Flamm, E. S., Pennie, W. and Chance, B. (1991) A fiber optic based multiprobes system for intraoperative monitoring of brain functions. *SPIE.* **1431**, 303-313
- 30 Mayevsky, A., Meilin, S., Manor, T., Ornstein, E., Zarchin, N. and Sonn, J. (1998) Multiparametric monitoring of brain oxygen balance under experimental and clinical conditions. *Neurol. Res.* **20**, S76-S80
- 31 Meilin, S., Mendelman, A., Sonn, J., Manor, T., Zarchin, N. and Mayevsky, A. (1999) Metabolic and hemodynamic oscillations monitored optically in the brain exposed to various pathological states. *Adv. Exp. Med. Biol.* **471**, 141-146
- 32 Mayevsky, A., Doron, A., Meilin, S., Manor, T., Ornstein, E. and Ouaknine, G. E. (1999) Brain viability and function analyzer: multiparametric real-time monitoring in neurosurgical patients. *Acta Neurochir. Suppl (Wien.)*. **75**, 63-66
- 33 Mayevsky, A., Manor, T., Meilin, S., Doron, A. and Ouaknine, G. E. (1998) Real-time multiparametric monitoring of the injured human cerebral cortex - a new approach. In *Acta Neurochir. Suppl (Wien.)* (Marmarou, R. and Bullock, R., eds.). pp. 78-81, ICPX
- 34 Mayevsky, A., Manor, T., Meilin, S., Razon, N. and Ouaknine, G. E. (2001) Multiparametric monitoring of tissue vitality in clinical situations. *SPIE.* **4255**, 33-39
- 35 Mayevsky, A., Ornstein, E., Meilin, S., Razon, N. and Ouaknine, G. E. (2002) The evaluation of brain CBF and mitochondrial function by a fiber optic tissue spectroscope in neurosurgical patients. *Acta Neurochir. Suppl.* **81**, 367-371
- 36 Mayevsky, A., Meilin, S., Rogatsky, G. G., Zarchin, N. and Sonn, J. (1995) Multiparametric monitoring of the awake brain exposed to carbon monoxide. *J. Appl. Physiol.* **78**, 1188-1196
- 37 Mayevsky, A. (1978) Ischemia in the brain: The effects of carotid artery ligation and decapitation on the energy state of the awake and anesthetized rat. *Brain Res.* **140**, 217-230
- 38 Rosenthal, M. and Martel, D. L. (1979) Ischemia-induced alterations in oxidative "recovery" metabolism after spreading cortical depression *in situ*. *Exp. Neurol.* **63**, 367-378
- 39 Mayevsky, A., Meirovithz, E., Livnat, A., Tolmasov, M., Kutai-Asis, H. and Mandelbaum, M. (2020) Urethral Tissue Metabolic Score (TMS) as a Surrogate Marker of Brain Oxygen Balance in Stroke, ARDS and Critical Care. *EC Emergency Medicine and Critical care.* **SI.01**, 05-40

LEGENDS TO FIGURES

Figure 1A: Gradual spread of depression. Electrodes arranged as shown in the inset. A: Before stimulation L. 7 min after K. In this and the following figures, when a strip is timed, that time indicates the interval from the end of the stimulation period. Upward excursions in the records denote negativity of the more frontal electrode (lower number) with respect to the more occipital electrode (higher number). Unless otherwise mentioned, the stimuli were induction shocks at "tetanizing" frequency, applied for 3 to 5 sec. through electrodes S [3]. B: A representative experiment on the slow voltage variation accompanying the spreading depression of activity. The curve was drawn from voltage readings taken with 5 second intervals. In this and the other two figures, an upward deflection denotes negativity of the cortex, with respect to the extra cortical reference electrode. Electrodes arranged as shown in the inset (s: stimulating electrodes). Stimulation, 5 seconds of "tetanizing" current from an induction coil, delivered at the time marked S. In this representative curve is indicated the time

of occurrence (from A to B) of the specific electrical activity which often develops during the depression of the "spontaneous" activity. American Physiological Society, reprinted with permission [5].

Figure 2: EEG activity recorded in the brain before (A) and during (B) the CSD wave. C+D - Photomicrograph of the pial vessels in the brain. C - Control before stimulation. The large vessel is an artery, of about 120 μ diameter. D - The wave of depression has reached the region. The diameter of the artery is approximately doubled during the wave of CSD [4]. E - Graph showing the time course and magnitude of arterial vasodilatation attending a depression. American Physiological Society, reprinted with permission [4].

Figure 3: The effects of various experimental and clinical factors (tested in our studies) on the responses to Cortical Spreading Depression- CSD.

Figure 4: Schematic presentation of the "basic building stones" of a typical cerebral cortex tissue. The four pathological states (9-12) are related directly or indirectly to the cortical spreading depression.

Figure 5: Schematic presentation of the concept "Brain physiological mapping". All the presented techniques were developed in our laboratory [12].

Figure 6: A - Structures of NAD⁺. The nicotinamide group (broken ring) is the functional part of both molecules, i.e. the portion of molecules where oxidation and reduction take place (see arrow). B - Absorption spectra of NAD⁺ and NADH in solution. C - Absorption and emission spectra of NADH illuminated by UV light [15,18]. D - The emission spectra of rat cerebral cortex under aerobic conditions (lower trace) and under anoxic conditions (upper trace).

Figure 7: Comparison between mitochondrial metabolic state (*in vitro*), defined by Chance & Williams in 1955 (A) and responses of the *in vivo* brain to changes in O₂ supply and demand induced by brain activation described by Mayevsky (B and C) [19].

Figure 8: Scheme of the multiprobe assembly (A) and its location on the gerbil brain (B). All details are in the text [23]. C - The multiprobe assembly (MPA) used for monitoring the brain of head-injured patients. The MPA is connected to the brain via a special holder screwed into the skull. ICP, intracranial pressure probe; CBF, NADH, fiber optic light guide probe to measure local cerebral blood flow, (CBF) and mitochondrial redox state (NADH); K⁺, DC, extracellular K⁺ mini surface-electrode surrounded by a DC steady potential monitoring space; ECoG, bipolar electrocortical electrodes.

Figure 9: The response of the brain to the application of 0.3 M KCl solution, epidurally. Three cycles of oxidation of NADH were developed. The arrow direction shows an increase in the optical signals. Note that the third trace is on an expanded scale. Time proceeds from left to right [25].

Figure 10: The effects of hypoxia (4% O₂) on the metabolic, ionic and electrical responses to spreading depression developed spontaneously [21].

Figure 11: A - Effects of anoxia on mitochondrial NADH, ECoG, Extracellular potassium and DC steady potential in the gerbil brain [26]. B - Responses to unilateral and bilateral carotid occlusion in the gerbil brain [23].

Figure 12: The effects of Metrazol on brain NADH fluorescence, 366 nm reflectance and the EEG of the two hemispheres. Note that the amplitude of the EEG calibration in the right hemisphere is 1 mV. The typical response is shown in part A. Part B shows another type of response to Metrazol applied 10 min before the record was taken [17].

Figure 13: The development of epileptic activity followed by cortical spreading depression (CSD) in the brain of a seizure-prone gerbil. ECoG, electrocorticogram; PO_2 , partial pressure of O_2 in the tissue; EK^+ , DCK^+ , extracellular potassium and DC steady potential measured around the K^+ electrode; R, F, and CF, reflectance, NADH fluorescence, and corrected NADH fluorescence [28].

Figure 14: Effect of hyperbaric oxygenation on the various parameters measured in the awake rat inside the hyperbaric chamber. Pressure reached 60 psi (5 A T A) and first tonic-clonic episode is shown. Abbreviations as in Figure 13 [22].

Figure 15: The responses of the brain to a wave of cortical spreading depression developed spontaneously in a severely head injured patient. ICP, intracranial pressure; R, NADH, 366 nm reflectance and 450 nm corrected fluorescence; CBF, CBV, cerebral blood flow and volume measured by laser Doppler flowmetry; K^+_e , DC, extracellular potassium levels and the DC steady potential measured around the K^+ electrode; ECoG, electrocorticogram [24].

Figure 16: The responses to cortical spreading depression (CSD) measured in normoxic (A) and ischemic brain (B) in the rat model. Parts C and D were measured in head injured patients, E, F – Brain Metabolic Score (BMS) calculated for the normoxic (E) and partial ischemic (F) human brain responses to CSD. TBF – Cerebral Blood Flow, R- Reflectance, NADH – mitochondrial NADH redox state.

Figure 17: Schematic presentation of brain oxygen balance evaluated by oxygen supply and demand. Oxygen supply could be evaluated by monitoring brain blood flow (CBF), brain blood volume (CBV), hemoglobin oxygenation (HbO_2) and brain tissue oxygen partial pressure. Oxygen demand includes Ionic Homeostasis, Signal Conduction and Biochemical synthesis. Mitochondrial NADH redox state served as an indicator for brain oxygen balance or brain oxygen homeostasis [39].